UNIVERSITAT DE BARCELONA

Synthesis and development of bioactive compounds: soluble epoxide hydrolase inhibitors and antiviral molecules

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SYNTHESIS AND DEVELOPMENT OF BIOACTIVE COMPOUNDS: SOLUBLE EPOXIDE HYDROLASE INHIBITORS AND ANTIVIRAL MOLECULES

JUAN MARTÍN LÓPEZ 2022

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PROGRAMA DE DOCTORAT DE QUÍMICA ORGÀNICA

SYNTHESIS AND DEVELOPMENT OF BIOACTIVE COMPOUNDS: SOLUBLE EPOXIDE HYDROLASE INHIBITORS AND ANTIVIRAL MOLECULES

Memòria presentada per Juan Martín López per optar al títol de doctor per la Universitat de Barcelona

Director i tutor, Dr. Santiago Vázquez Cruz

Doctorand, Juan Martín López

A Óscar Lozano Ventura,

porque da coraje que la gente tan buena se vaya tan pronto.

Acknowledgement

First of all, I would like to thank Santi Vázquez for his search for funding to carry out this doctorate, without which this thesis would not be possible.

Second, to the great human and scientific team that works in the Pharmaceutical Chemistry Unit of the Faculty of Pharmacy. To all my colleagues, professors and professionals who make research possible every day.

Thirdly, to my partner Cristela Martínez, for standing with me these years and coming to Barcelona to live with me when our life could be simpler and more comfortable in our motherland.

Finally, to my family, who have always been there. Logical because it would not make much sense for them to stop talking to me just because I am doing a PhD…

And a special dedication to my future niece, still in gestation but who will surely make the whole family happy.

Summary

The present Thesis is divided in three chapters:

1. Design and synthesis of new soluble epoxide hydrolase inhibitors (sEHI)

The synthesis of new soluble epoxide hydrolase inhibitors based on ureas as the main pharmacophore, a benzohomoadamantane unit and different arylpiperidine groups. The aim was to broaden the spectrum of inhibitors previously developed by the group, taking as starting point the Astellas pharmaceutical product AS2586114. Twelve new urea-based sEHIs were synthesized and evaluated. Two of them were selected for further *in vitro* studies.

The synthesis of new sEHI derived from the substitution of the urea group by and amide group, in order to extend the Markush formula of a patent that the group filled in two years ago and, on the other hand, to improve the solubility of the urea-containing sEHI. Twelve novel amide-based sEHI were synthesized and evaluated. One of them was selected for further *in vitro* studies demonstrating potent anti-inflammatory properties.

2. Design and synthesis of new anti-influenza virus molecules.

The development of new antiviral molecules against the influenza A virus H1/N1 subtype targeting the hemagglutinin of the virus, continuing previous research by our group. This part of the Thesis greatly improved and completed previous SAR studies on this kind of hemagglutinin inhibitors.

3. Design and synthesis of new anti-coronavirus molecules.

The development of new antiviral molecules against coronavirus 229E, continuing group's research in the field carrying out a more complete SAR study.

Abbreviation list

AD: Alzheimer Disease

APAU: 1-(1-Acetyl-piperidin-4-yl)-3-adamantan-1-yl-urea

ARA: Arachidonic Acid

AUDA: 2-(3-adamantan-1-ylureido)dodecanoic acid

BBB: Blood Brain Barrier

cAMP: Cyclic Adenosine Monophosphate

c-AUCB: 4-[[cis-4-[[(tricyclo[3.3.1.13,7]dec-1-ylamino)carbonyl]amino]cyclohexyl]oxy]-benzoic acid

CM: Convoluted Membrane

CoV: Coronavirus

COX: Cyclooxygenase

CPE: Cytopathic effect

CPU: N-cyclohexyl-N′-(3-phenylpropyl)urea

CYP: Cytochrome P

DAST: Diethylaminosulfur Trifluoride

DCM: Dichloromethane

DCU: *N,N′*-dicyclohexyl-urea

DHET: Dihydroxyeicosatrienoic acid

DiHFA: Dihydroxy-fatty acid

DIPEA: *N,N*-Diisopropiletilamina

DMAP: Dimethylaminopyridine

DMF: Dimethylformamide

DMPK: Drug Metabolism and Pharmacokinetics

DMS: Double-Membrane Spherules

DMSO: Dimethyl Sulfoxide

DMV: Double-Membrane Vesicles

DNA: Deoxyribonucleic Acid

EDC: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

EET: Epoxyeicosatrienoic Acid

EpFA: Epoxy-Fatty Acid

ERGIC: Endoplasmic-Reticulum–Golgi Intermediate Compartment

EtOAc: Ethyl Acetate

FA: Fatty Acid

FIV: Feline Infectious Peritonitis

GABA: γ-Aminobutyric Acid

GPCR: G Protein-Coupled Receptor

HA: Hemagglutinin

HAIs: Hemagglutinin Inhibitors

HATU: Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium

HCoV: Human Coronavirus

HE: Hemagglutinin Esterase

hERG: Human Ether-à-go-go-Related Gene

HIV: Human Immunodeficiency Virus

HOBt: Hydroxybenzotriazole

IKK: I κB kinase

IL: Interleukin

iNOS: Inducible Nitric Oxide Synthase

LHS: Left-Hand side

LKT: Leukotriene

LOX: Lipoxygenase

LPS: lipopolysaccharide

MD: Molecular dynamics

MDCK: Madin-Darby Canine Kidney cell

MERS: Middle East Respiratory Syndrome

mRNA: Messenger Ribonucleic Acid

NA: Neuraminidase

NADPH: Nicotinamide Adenine Dinucleotide Phosphate

NAI: Neuraminidase inhibitors

NF-κB: Nuclear Factor κB

NMR: Nuclear Magnetic Resonance

NO: Nitric Oxide

NSAID: Nonsteroidal Anti-Inflammatory Drug

PAMPA: Parallel Artificial Membrane Permeability Assay

PG: Prostaglandin

PI: Propidium Iodide

PPAR: Peroxisome proliferator-activated receptor

RdRp: RNA-dependent RNA polymerase

RHS: Right-Hand side

RNA: Ribonucleic Acid

RT: Room Temperature

RTC: Replication and Transcription Complex

SAR: Structure-Activity Relationship

SARS: Severe Acute Respiratory Syndrome

sEH: Soluble Epoxide Hydrolase

sEHI: Soluble Epoxide Hydrolase Inhibitor

SEM: Standard Error Mean

Sn2:   Bimolecular Nucleophilic Substitution

SnAr: Aromatic Nucleophilic Substitution

t-AUCB: 4-[[*t*-4-[[(tricyclo[3.3.1.1^{3,7}]dec-1-ylamino)carbonyl]amino]cyclohexyl]oxy]benzoic acid

TBHQ: *tert*-Butylhydroquinone

TEA: Triethylamine

THF: Tetrahydrofuran

TNF-α: Tumor Necrosis Factor α

TPAU: 1-trifluoromethoxyphenyl-3-(1-acetylpiperidin-4-yl)urea

TPPU: *N*-[1-(1-Oxopropyl)-4-piperidinyl]-*N'*-[4-(trifluoromethoxy)phenyl]urea

VCAM-1: Vascular Cell Adhesion Molecule 1

vRNA: Viral Ribonucleic Acid

vRNP: Viral Ribonucleoprotein

Index

1. Design and synthesis of new soluble epoxide hydrolase inhibitors (sEHI)

1.1. Introduction

1.1.1. Pathways of arachidonic acid metabolism

The arachidonic acid (ARA) cascade is one of the main metabolic pathways that can be found in the organism. ARA is an ω-6, 20-carbon polyunsaturated fatty acid that is released from membrane phospholipids of cells when they are activated upon various stimuli.[1] The metabolism of ARA is widely studied and follows three main pathways. In the first two pathways, ARA is transformed, through the action of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, in prostaglandins (PGs) and thromboxanes, for COX, and leukotrienes (LKTs), for LOX, which are lipid chemical mediators with pro-inflammatory properties. Both enzymes are well-known drug targets against inflammation (Fig. 1) and there are already a large number of drugs in the market that target them.[2] The third branch has been less studied: the cytochrome P450 (CYP450) cascade. This cascade generates physiologically important eicosanoids such as epoxyeicosatrienoid acids (EETs).[3] These EETs are formed after epoxidation of ARA by CYP2C and CYP2J enzymes and, in opposition with PGs and LKTs, they exert an anti-inflammatory role.[4] No enzyme involved in this pathway has been pharmaceutically targeted yet, therefore, the research in this field is of great importance to the scientific community. Thus, the first chapter of the present thesis is going to focus on it.

[1] Harizi, H.; Corcuff, J. B.; Gualde, N. *Trends Mol. Med*. **2008**, *14*, 461-469.

[2] Funk, C. D. *Science* **2001**, *294*, 1871-1875.

[3] Zeldin, D. C. *J. Biol. Chem.* **2001**, *276*, 36059-36062.

[4] Node, K.; Huo, Y.; Ruan, X.; Yang, B.; Spiecker, M.; Ley, K.; Zeldin, D. C.; Liao, J. K. *Science* **1999**, *285*, 1276-1279.
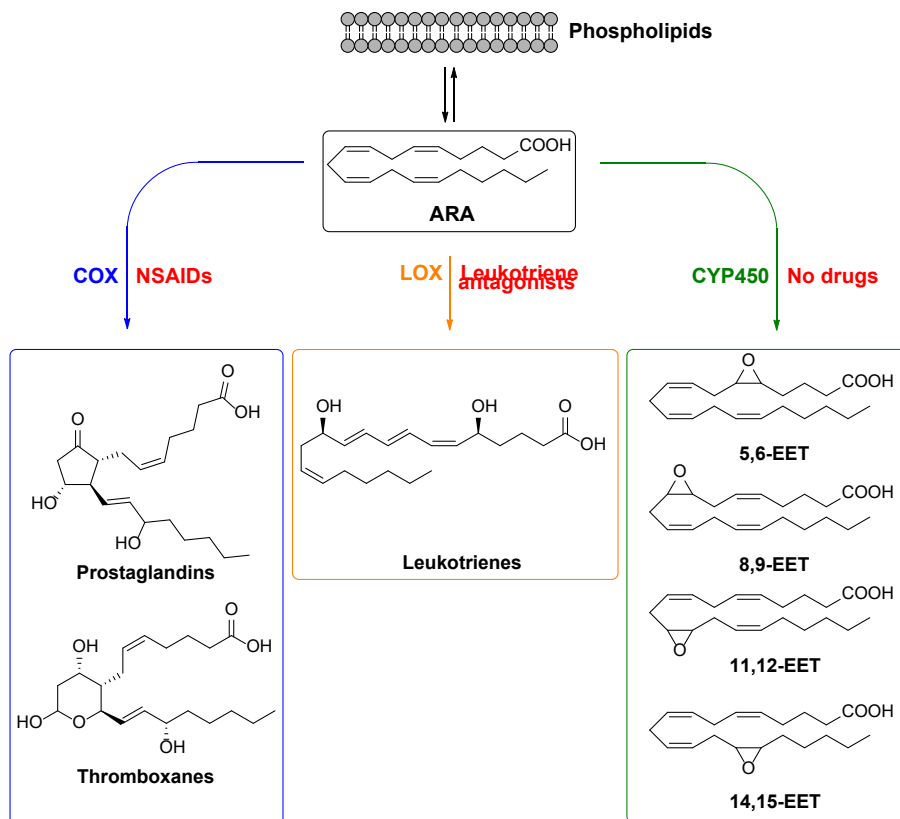
Fig. 1. The three metabolic pathways of ARA: the COX (blue), LOX (orange) and CYP450 (green) enzymatic routes. COX-1 and COX-2 nonsteroidal anti-inflammatory drugs (NSAIDs) inhibitors are used for the treatment of inflammation and pain (red). LOX antagonists are indicated in allergic and asthmatic states (red). There are no drugs in the market acting on the CYP450 pathway (red).

1.1.2. Biological relevance of EETs

The EETs are signaling molecules formed within various types of cells, mainly found in the endothelium, through the action of the enzymes CYP450. These signaling molecules are both autocrine (acting in the same cell that produces them) and paracrine (acting in the nearby cells). These eicosanoids are generally short-lived, being rapidly hydrolyzed from epoxides to the corresponding diols species, dihydroxyeicosatrienoic acids (DHETs), which are less biologically active or completely inactive. The family of enzymes responsible for this transformation are the epoxide hydrolases. There are four

different enzymes in this family: microsomal epoxide hydrolase, soluble epoxide hydrolase (sEH) and the more recently discovered epoxide hydrolase 3 and epoxide hydrolase 4. All these enzymes are structurally closely related isozymes. Among all of them, the sEH, an enzyme that belongs to the α/β hydrolase family which is widely distributed in all mammalian cells, has the most important role in the metabolization of the EETs formed in the ARA cascade.

As already mentioned above, EETs are potent anti-inflammatory and also vasodilatory agents.[5] The anti-inflammatory effect seems to be carried out through the resolution of inflammation rather than preventing it *via* inhibition of the nuclear factor κB (NF-κB), decreasing the expression of inducible COX-2 and activation of subfamilies of nuclear peroxisome proliferator-activated receptors (PPARs). On the other hand, the vasodilatory effect is related with the ability of EETs to lower blood pressure by both stimulating arterial vasorelaxation and inhibiting the kidney's retention of salts and water, decreasing the intravascular blood volume.

Apart from the anti-inflammatory and vasodilatory roles, EETs are thought to be involved in other beneficial effects such as analgesic, anti-platelet aggregation, anti-hypertensive, pro-angiogenic and fibrinolytic and anti-apoptotic effects.[6,7] In short, EETs are involved in any disease in which inflammation is a key factor. Besides, some other metabolic events are mediated by EETs. Control of insulin release and modulation of insulin sensitivity[8,9] are good examples of the importance of these chemical mediators. To sum up, EETs are considered important chemical mediators as they interact with a great number of proteins to generate a wholesome set of effects, however, the different cascade processes derived from the action of EETs are still unrevealed for specific effects. Until now, no EET receptor has been identified yet. A schematic representation of the potential mechanism that EETs

[5] Morisseau, C.; Hammock, B. D. *Annu. Rev. Pharmacol. Toxicol.* **2013**, *53*, 37-58.
[6] El-Sherbeni, A. A.; El-Kadi, A. O. S. *Arch. Toxicol.* **2014**, *88*, 2013-2032.
[7] Duflot, T.; Roche, C.; Lamoureux, F.; Guerrot, D.; Bellien, J. *Expert Opin. Drug Discov.* **2014**, *9*, 1-15.
[8] Falck, J. R.; Manna, S.; Moltz, J.; Chacos, N.; Capdevila, J. *Biochem. Biophys. Res. Commun.* **1983**, *114*, 743-749.
[9] Skepner, J. E.; Shelly, L. D.; Ji, C.; Reidich, B.; Luo, Y. *Prostaglandins Other Lipid Mediat*. **2011**, *94*, 3-8.

perform for the cardiovascular, anti-inflammatory and analgesic effects is shown in figure 2.[10]
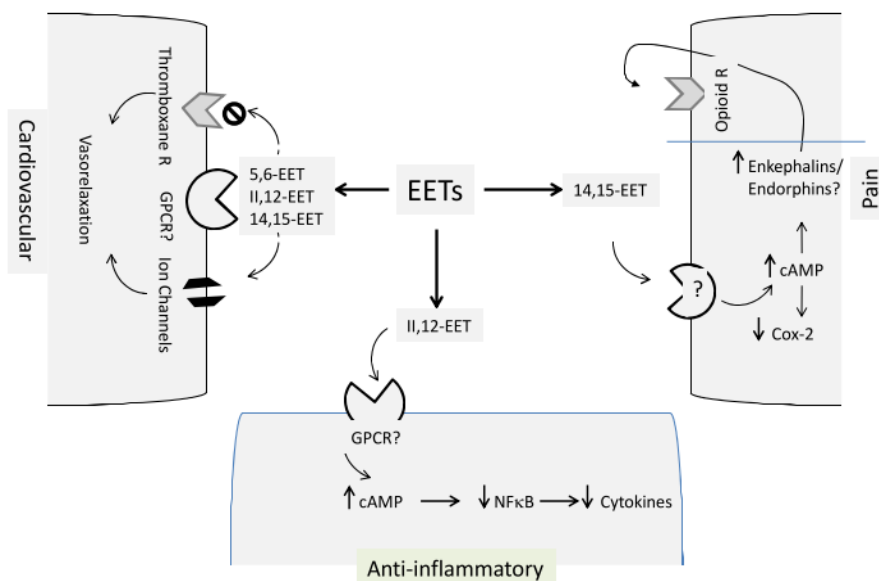
Fig. 2. Possible mode of action of EETs in cardiovascular system, inflammation and pain. GPCR: G protein-coupled receptor; cAMP: cyclic adenosine monophosphate. Figure taken from Elena Valverde's thesis.[11]

EETs, as short-lived molecules, are rapidly hydrolyzed by the enzyme sEH facilitating the addition of water to the epoxide group of EETs.

1.1.3. Soluble Epoxide Hydrolase (sEH)

Soluble epoxide hydrolase (sEH) is a bifunctional enzyme member of the epoxide hydrolase family that can be found in the cytosol and peroxisomes of all organs and tissues, but at different levels. Liver, followed by kidney, heart,

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[10] Pillarisetti, S. *Inflamm. Allergy - Drug Targets* **2012**, *11*, 143-158.
[11] Valverde, E. Ph. D. Dissertation Exploring adamantane-like scaffolds for a wide range of therapeutic targets. *University of Barcelona*, **2020**.

lung and brain are the organs where sEH carries out the highest specific activity.

Regarding its structure, this enzyme consists of a homodimer formed by two ~62 kDa monomeric subunits separated by a short proline-rich linker arranged in an anti-parallel fashion (Fig. 3).[12] The sEH performs two different activities in two separate domains: the *C*-terminal epoxide hydrolase activity (soluble epoxide hydrolase) and the *N*-terminal phosphatase activity (lipid-phosphate phosphatase). The latter activity is beyond this thesis so the focus will be put on the former activity; the soluble epoxide hydrolase activity.
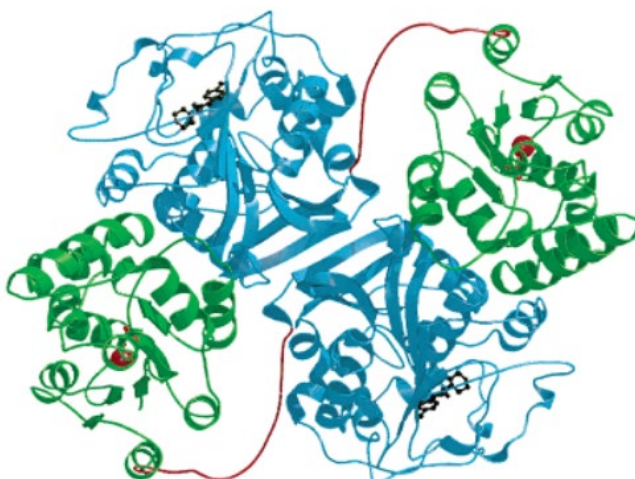
Fig. 3. X-Ray structure of the human sEH dimer. Green: *N*-terminal domains. Blue: *C*-terminal domains. PDB code: 1S80. Figure taken from reference 11.

sEH acts by binding specific epoxides and converting them to the corresponding DHETs through the addition of a molecule of water (Fig. 4). The resulting diol group improves the solubility of the molecule making it more easily excreted by the organism.

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[12] Gomez, G. A.; Morisseau, C.; Hammock, B. D.; Christianson, D. W. *Biochemistry* **2004**, *43*, 4716–4723.
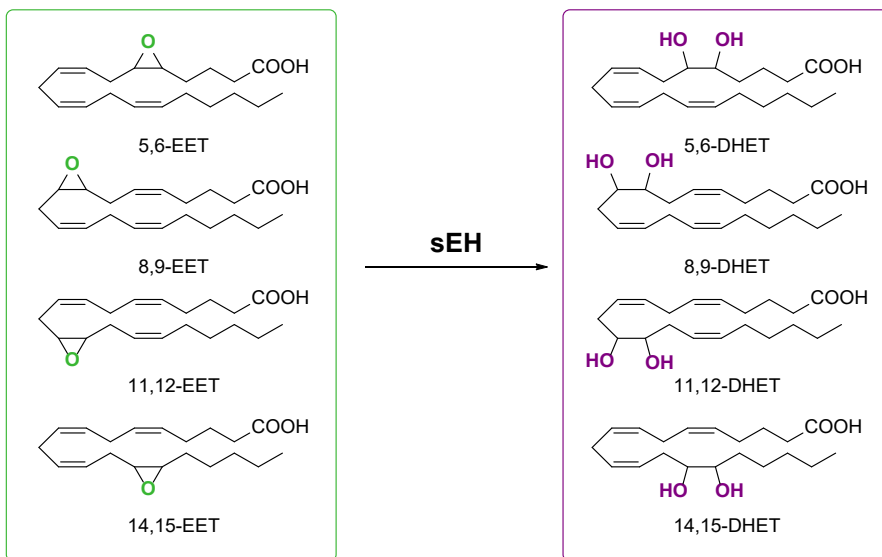
Fig. 4. Transformation of EETs to DHETs through sEH.

As it can be easily seen, this soluble epoxide hydrolase activity has EETs as the main substrates so regulating sEH will exert a direct control of the amount of EETs present within the cell. This fact has made that sEH has emerged as a promising therapeutic target for the treatment of multiple conditions given that its inhibition stabilizes the levels of EETs for the maintenance of the cellular homeostasis. As EETs have different roles in other metabolic routes and are present in other metabolic processes, their metabolization and concentration is controlled by several mechanisms. This fact avoids the appearance of serious secondary effects since there are no great variations in the concentration of EETs within the cell even if their metabolic route is altered.

1.1.4. Soluble Epoxide Hydrolase Inhibition

During the last years, many research groups have shown interest in the inhibition of sEH. Putting together the fact that this pathway has not been widely studied yet and the huge therapeutical potential derived from its multifunctional nature, the inhibition of sEH has recently become of great interest.

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Understanding the mechanism of action of sEH is a prerequisite for the development of potent and selective inhibitors. In the last few years, the catalytic mechanism of the sEH has been described and the key residues involved in the hydrolysis are well defined. X-ray crystallographic data has revealed that the catalytic triad consist of two tyrosine residues (Tyr381 and Tyr465) which act as hydrogen bond donors to assist epoxide-ring opening by the attack *via* an S_N2-type reaction of an aspartic acid residue (Asp333) previously deprotonated, forming a hydroxyl alkyl-enzyme intermediate (Fig. 5). Then, after different proton shifts, a basic water molecule activated by a histidine residue (Hys523) attacks the intermediate transforming the initial epoxide group into a diol product.[13]
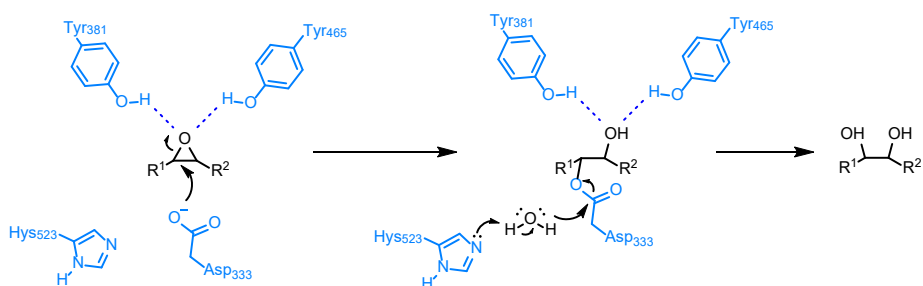
Fig. 5. Schematic representation of the epoxide-ring polarization by the two tyrosine residues and the opening of the ring by the attack of the aspartic acid residue.

The catalytic pocket of sEH is in turn formed by two hydrophobic pockets arranged in an "L"-shape that are not in contact with the aqueous medium (Fig. 6). On the left-hand side (LHS) of the enzyme, the largest pocket that represents the long part of this "L" can be found. On the other side, the right-hand side (RHS), another but smaller hydrophobic pocket that corresponds to the short part of "L"-shape is found. In the middle, joining both hydrophobic pockets, we find the catalytic triad. Finally, it is important to

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[13] Gomez, G. A.; Morisseau, C.; Hammock, B. D.; Christianson, D. W. *Protein Sci.* **2006**, *15*, 58–64.

mention that each branch features residues involved in specific interactions such as Van der Walls and hydrogen bond interactions and π-stacking.[14]
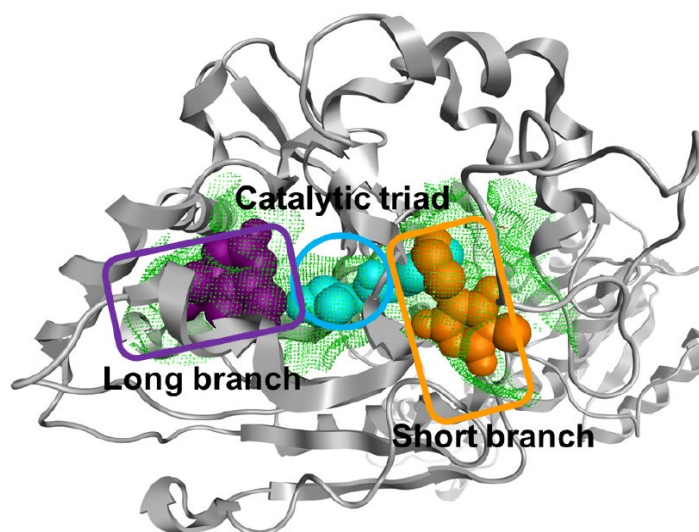
Fig. 6. Overview of the catalytic pocket, with representative fragments bound to each site (catalytic triad: cyan; long branch: purple; short branch: orange). Figure taken from reference 13.

Since the first studies performed 30 years ago, the value of the inhibition of sEH both *in vitro* and in *in vivo* disease models have been demonstrated. This thesis will focus on the anti-inflammatory role of this inhibition but a short description of some other different roles in which this enzyme is implied will be disclosed.

1.1.4.1. Effects of sEH inhibitors (sEHI) on neuropathic pain

Neuropathic pain is a recurrent pain in many disease states for which there is still no fully satisfactory pharmacological treatment in the market. Despite the intensive efforts in understanding the mechanisms behind

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[14] Amano, Y.; Yamaguchi, T.; Tanabe, E. *Bioorg. Med. Chem.* **2014**, *22*, 2427-2434.

neuropathic pain, no great successes have been attained in this field yet. However, sEH has recently emerged as a non-channel and non-neurotransmitter target for neuropathic pain.[15] As inhibition of sEH has demonstrated a reduction of inflammation in some rodent models, it was expected that the inhibition of sEH also reduces neuropathic pain. Unsurprisingly, this effect has been observed in multiple animal models of pain in which sEH inhibitors and EETs reduced pain perception.[16,17] The mechanism of this pain relief has been confirmed to imply both GABAergic and opioid pathways, as well as PPARs.[18]

1.1.4.2. sEH and cardiovascular disease

EETs are putative endothelium-derived hyperpolarizing factors which act increasing the open-state frequency of calcium ion channels. This fact results in a vasodilatation of vascular smooth muscle through a cAMP/protein kinase A-dependent mechanism.[19] As EETs are involved in vasodilatory properties, sEH was suggested to have a role in blood pressure regulation. EETs also regulate indirectly the blood pressure by promoting natriuresis.[20] This hypothesis has been confirmed using inhibitors of sEH, leading to an increase of the EETs' levels which, in the end, resulted in a reduction of blood pressure in hypertensive rodent models.[21]

[15] Hammock, B. D.; Wagner, K.; Inceoglu, B. *Pain Manag.* **2011**, *1*, 383-386.

[16] Wagner, K.; Inceoglu, B.; Dong, H.; Yang, J.; Hwang, S. H.; Jones, P.; Morisseau, C.; Hammock, B. D. *Eur. J. Pharmacol.* **2013**, *700*, 93-101.

[17] Sing, K.; Lee, S.; Morisseau, C.; Fu, S. H.; Yang, J.; Wang, P.; Ulu, A.; Mate, C. A.; Nguyen, L. V.; Wul, H.; Hammock, B. D. *J. Med. Chem.* **2014**, *57*, 7016-7030.

[18] Pillarisetti, S.; Khanna, I. *Drug Discov. Today* **2015**, *20*, 1382-90*.*

[19] Fleming, I.; Rueben, A.; Popp, R.; Fisslthaler, B.; Schrodt, S.; Sander, A.; Haendeler, J.; Falck, J. R.; Morisseau, C.; Hammock, B. D.; Busse, R. *Arterioscler. Thromb. Vasc. Biol.* **2007**, *27*, 2612-2618.

[20] Imig, J. D. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2004**, *287*, R3-5

[21] Loch, D.; Hoey, A.; Morisseau, C.; Hammock, B. O.; Brown, L. *Cell Biochem. Biophys.* **2007**, *47*, 87-98.

1.1.4.3. Role of sEH in the development of diabetes and metabolic syndrome. Involvement of ER stress

EETs display a significant role in the coordination of many metabolic processes and their pathophysiology of the endocrine system in relation to glucose homeostasis. Whilst EETs and sEHI do not alter significantly the levels of insulin and glucose in healthy patients, in some diabetes models have proven efficacy in the regulation of glycemic states.[22]

1.1.4.4. Regulation of inflammation by sEHI

As previously described above, EETs are signaling molecules that belong to the ARA cascade. In this cascade three different pathways are found and all of them are related with the inflammatory response of the body against a stimulus.

The anti-inflammatory role of EETs has been widely demonstrated through different strategies including overexpression of CYP450, deletion of sEH in animal models, use of sEHI or directly monitoring EET effects.[23,24,25] Moreover, reducing the activation of NF-κB, a protein complex that controls transcription of DNA, is a crucial event in the anti-inflammatory activity of EETs, specially of 11,12-EET and 14,15-EET. This reduction in the activity of NF-κB seems to follow three complementary cellular mechanisms (Fig. 7): i) decrease of the activation of NF-κB induced by tumor necrosis factor-α (TNF-α); ii) increase of PPAR-γ activity; iii) reduction of the prostaglandin E_2 (PGE$_2$).[26]

[22] Luo, P.; Chang, H. H.; Zhou, Y.; Zhang, S.; Hwang, S. H.; Morisseau, C.; Wang, C. Y.; Inscho, E. W.; Hammock, B. D.; Wang, M.H. *J. Pharmacol. Exp. Ther.* **2010**, *334*, 430-438.

[23] Thomson, S. J.; Askari, A.; Bishop-Bailey, D. *Int. J. Vasc. Med*. **2012**, 2012:605101.

[24] Schmelzer, K. R.; Kubala, L.; Newman, J. W.; Kim, I.; Eiserich, J. P.; Hammock, B. D. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 9772-9777.

[25] Deng, Y.; Edin, M. L.; Theken, K. N.; Schuck, R. N.; Flake, G. P.; Kannon, M. A.; DeGraff, L. M.; Falck, J. R.; Zeldin, D. C.; Lee, C. R. *FASEB J.* **2011**, *25*, 703-713.

[26] Liu, Y.; Zhang, Y.; Schmelzer, K.; Fang, X.; Zhu, Y.; Spector, A. A.; Gill, S.; Morisseau, C.; Hammock, B. D.; Shyy, J. Y. J. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 16747-16752.

Fig. 7. Action of epoxy-fatty acids (EpFAs) in the inflammatory process. PLA2: phospholipase A2. FAs: fatty acids. DiHFAs: dihydroxy-fatty acids. IKK: I κB kinase. iNOS: inducible nitric oxide synthase. VCAM-1: vascular cell adhesion molecule 1. IL: interleukin. NO: nitric oxide. Figure taken from Elena Valverde's thesis.[11]

In addition, it is also known that the agents that elevate cAMP within the cell are known to be anti-inflammatory, also by inhibiting NF-κB transcription complex.[27] Thus, the activation of GPCR proteins and the elevation of the concentration of cAMP is another major mechanism behind the anti-inflammatory effects of EETs.

To sum up, it can be considered that sEH is an important emerging therapeutic target in a great number of diseases in which inflammation is a common underlying cause, as Alzheimer disease (AD).

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[27] Ollivier, V.; Parry, G. C. N.; Cobb, R. R.; de Prost, D.; Mackman, N. *J. Biol. Chem.* **1996**, *271*, 20828-20835.

1.1.4.5.   sEHI and Alzheimer's disease

AD is a multifactorial disease in which neuroinflammation has an important role.[28] This inflammation is caused mainly by two factors: amyloid beta aggregation, a protein which is deposited in senile plaques and causes direct toxic effects on neurons, and uncontrolled phosphorylation of the TAU protein, preventing its normal function and facilitating its auto-aggregation. As sEH plays an important role in inflammatory processes, it is logical to think that its inhibition can result in an improvement of the inflammatory process and therefore in an improvement for Alzheimer's patients.

Of note, our group and others have found strong evidence that sEH is overexpressed in the astrocytes of the brains of Alzheimer's patients and in the 5xFAD amyloid beta mouse model of AD.[29,30] It is demonstrated that long-term administration of TPPU, a known sEHI, to the 5xFAD mouse model reversed microglia and astrocyte reactivity and immune pathway dysregulation. Through this mechanism, amyloid beta pathology is reduced and the synaptic integrity and cognitive function are improved.

The reduction of neuroinflammation, neuronal death and oxidative stress in Alzheimer's disease are the possible mechanisms by which sEHI could help alleviate the symptoms this disease. In either case, the molecular mechanism by which sEH inhibition may help alleviate Alzheimer's symptoms is still unknown.

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[28] Heneka, M. T.; Carson, M. J.; El Khoury, J.; Landreth, G. E.; Brosseron, F.; Feinstein, D. L.; Jacobs, A. H.; Wyss-Coray, T.; Vitorica, J.; Ransohoff, R. M.; Herrup, K.; Frautschy, S. A.; Finsen, B.; Brown, G. C.; Verkhratsky, A.; Yamanaka, K.; Koistinaho, J.; Latz, E.; Halle, A.; Petzold, G. C.; Town, T.; Morgan, D.; Shinohara, M. L.; Perry, V. H.; Holmes, C.; Bazan, N. G.; Brooks, D. J.; Hunot, S.; Joseph, B.; Deigendesch, N.; Garaschuk, O.; Boddeke, E.; Dinarello, C. A.; Breitner, J. C.; Cole, G. M.; Golenbock, D. T.; Kummer, M. P. *Lancet Neurol*. **2015** Apr;14(4):388-405.

[29] Ghosh, A.; Comerota, M. M.; Wan, D. B.; Chen, F. D.; Propson, N. E.; Hwang, S.H.; Hammock, B.D.; Zheng, H. *Sci. Transl. Med.* **2020** Dec 9;12(573):eabb1206.

[30] Grinan-Ferre, C.; Codony, S.; Pujol, E.; Yang, J.; Leiva, R.; Escolano, C.; Puigoriol-Illamola, D.; Loza, M. I.; Brea, J.; Morisseau, C.; Hammock, B. D.; Vazquez, S.; Pallas, M.; Galdeano, C. *Neurotherapeutics* **2020**. 17, 1825–1835.

1.1.5. Previous work on sEHI

Finally, it is important to talk about the previous work that has been made on sEHI during the last decades in other research groups and within our group.

1.1.5.1. Previous work on sEHI in other research groups

Trying to emulate the epoxide functional group present in EETs, the first selective sEH inhibitors reported were chalcone oxides such as 4-phenylchalcone oxide (Fig. 8). However, they are quite unstable and slowly hydrolyzed by sEH, so the residence time was quite short.[31]

4-phenylchalcone oxide
human sEH $IC_{50} = 200$ nM
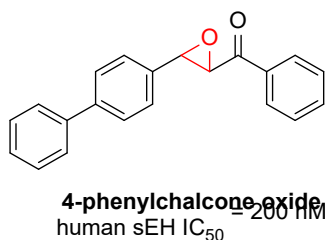
Fig. 8. Structure and inhibitory potency of 4-phenylchalcone oxide. IC_{50}: concentration producing 50% inhibition of sEH.

The first potent and stable inhibitors were reported in 1999 but, in this case, the main pharmacophore was changed from the epoxide to urea. This urea group suits very well in the catalytic pocket; while the oxygen atom acts as a hydrogen-bond acceptor for Tyr381 and Tyr465 present in the catalytic triad, the N-H of the urea group acts as a hydrogen-bond donor of the third amino acid of the catalytic triad, the Asp333.

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[31] Mullin, C. A.; Hammock, B. D. *Arch. Biochem. Biophys* **1982**, 216, 423–439.

This urea group shows a high potency and its discovery led to a great number of new urea compounds designed as inhibitors of sEH, improving the bioavailability of the epoxide-based molecules.[32]

The first two urea-based inhibitors synthetized and tested against sEH were *N,N'*-dicyclohexyl-urea (DCU) and *N*-cyclohexyl-*N'*-(3-phenylpropyl)urea (CPU) (Fig. 9). They both have moderate to good potency against human sEH with IC$_{50}$ values in the submicromolar range. Despite DCU was tested in hypertensive animal models performing successfully an antihypertensive effect and rising the EETs concentration,[33] the high melting point and low water solubility affected its oral availability and bioavailability leading to unacceptable pharmacokinetic properties.

N,N'-dicyclohexylurea (DCU)
human sEH IC$_{50}$ = 160 nM

N-cyclohexyl-*N'*-(3-cyclohexylpropyl)urea (CPU)
human sEH IC$_{50}$ = 290 nM
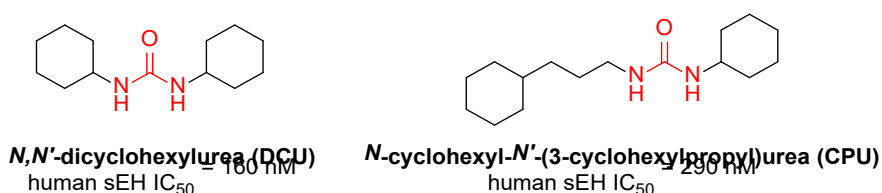
Fig. 9. Structures and inhibitory potencies of DCU and CPU. IC$_{50}$: concentration producing 50% inhibition of sEH.

Attending to the sEH catalytic "L"-shaped pocket described above, the large side of the hydrophobic pocket in the LHS of the enzyme could accommodate bigger hydrophobic moieties than these of DCU and CPU, resulting in better hydrophobic interactions. For the short pocket in the RHS of the enzyme, a shorter hydrophobic moiety ended by a hydrophilic group could be fitted, directing the hydrophilic group towards the water media that surrounds the enzyme.

From this new approach emerged a new family of adamantane-based ureas in which a voluminous hydrophobic moiety was present in the LHS of the

[32] Morisseau, C.; Hammock, B. D. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, 96, 8849–8854.
[33] Yu, Z.; Xu, F.; Morisseau, C.; Hammock, B. D.; Zeldin, D. C.; Kroetz, D. L. *Circ. Res*. **2000**, 87,992–998.

molecule, leaving a flexible chain in the RHS of the molecule. The first inhibitor bearing this two moieties was 2-(3-adamantan-1-ylureido)dodecanoic acid (AUDA) and its prodrug, AUDA-nBE (Fig. 10). The introduction of a flexible chain improved both handicaps of DCU, the limited water solubility and the high melting point, while maintaining the potency against sEH.[34] However, AUDA and AUDA-nBE were very sensitive to β-oxidation so their metabolization and excretion was very fast. limiting its potential application in the clinic.[35]

AUDA

R = H
human sEH IC_{50} = 100 nM

R = *n*-Bu
human sEH IC_{50} = 7 nM
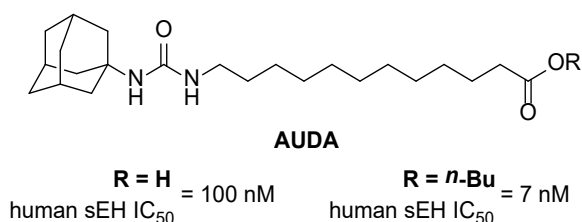
Fig. 10. Structures and inhibitory potencies of AUDA and AUDA-nBE. IC_{50}: concentration producing 50% inhibition of sEH.

On the basis of structure-activity relationship (SAR) analysis of more than 300 of urea-based inhibitors, seeking for an improvement in the physical properties while reducing metabolism problems, new polar pharmacophores were incorporated into the right-hand side (RHS) of the urea. As a result of these new discoveries, the group of Prof. B. D. Hammock afforded a general structure for the future design of new sEHI (Fig. 11).[36]

[34] Morisseau, C.; Goodrow, M. H.; Newman, J. W.; Wheelock, C. E.; Dowdy, D. L.; Hammock, B. D. *Biochem. Pharmacol*. **2002**, 63, 1599−1608.

[35] Duflot, T.; Guerrot, D.; Bellien, J. *Expert Opin. Drug Discovery* **2014**, 9, 229−243.

[36] McElroy, N. R.; Jurs, P. C.; Morisseau, C.; Hammock, B. D. *J. Med. Chem*. **2003**, 46, 1066−1080.
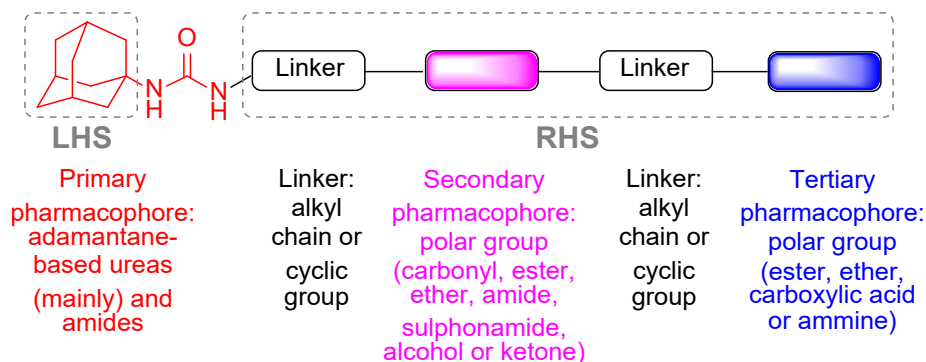
Fig. 11. Representation of general structure for the design of improved adamantane-based inhibitors. RHS: Right-hand side. LHS: Left-hand side.[37]

This new general structure consists of a urea or amide central moiety with at least one N-H free-bond (necessary to make hydrogen bonds) linked to a voluminous hydrophobic moiety (adamantane) as primary pharmacophore. A secondary pharmacophore with slightly polar functional groups (ester, carbonyl, sufonamide, etc.) linked with a carbon chain to the urea in order to improve the solubility and pharmacokinetic properties of the molecule and finally, a tertiary pharmacophore formed by a high polar functional group.

Linkers between the urea, the second and the third pharmacophore might be flexible alkyl chains or restricted cyclic structures. Interestingly, cyclic rigid structures resulted in an improvement of the oral bioavailability of the compound and in a reduction of the possibility of suffering β-oxidation, very common in flexible alkyl chains.[38]

With these findings in hand, many new sEHI were synthetized according to the general structure of Hammock's group. APAU, TPAU, *c*-AUCB and *t*-AUCB were synthetized using saturated rings as linkers between the first and the second pharmacophore (Fig. 12). Piperidine for the first two examples,

[37] Shen, H. C.; Hammock, B. D. *J. Med. Chem.* **2012**, *55*, 1789–1808.

[38] Jones, P. D.; Tsai, H. J.; Do, Z. N.; Morisseau, C.; Hammock, B. D. Bioorg. *Med. Chem. Lett*. **2006**, *16*, 5212–5216.

cyclohexane in the latter two. All of them improved the pharmacokinetics profile of their predecessors.[39]
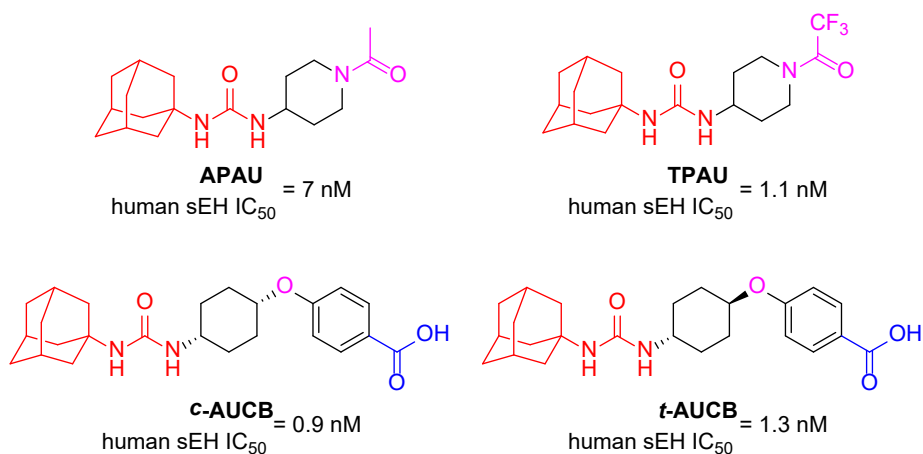
Fig. 12. Structures and inhibitory potencies of APAU, TPAU, *c*-AUCB and *t*-AUCB. IC$_{50}$: concentration producing 50% inhibition of sEH.

Among all of them, the compound APAU is the most important since it laid the foundations for the future development of other sEHIs, as well as being the first sEHI to reach the clinical phase worldwide.[40] A second highly relevant compound was *t*-AUCB, a compound with a good pharmacokinetic profile, very stable against human microsomes and great potency as sEHI. In addition, *t*-AUCB displays some important biological effects such as anti-inflammatory activities, as well as protective effects against cardiovascular and liver diseases.[41,42,43] The crystal structure of *t*-AUCB is shown in figure 13.

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[39] Hwang, S. H.; Tsai, H. J.; Liu, J. Y.; Morisseau, C.; Hammock, B. D. *J. Med. Chem*. **2007**, *50*, 3825–3840.

[40] https://clinicaltrials.gov/ct2/show/NCT00847899 (accessed on June 2022).

[41] Li, J.; Tang, C.; Li, L.; Li, R.; Fan, Y. *J. Exp. Clin. Cancer Res*. **2016**, *35*, 61.

[42] Shen, L.; Peng, H. C.; Zhao, S. P.; Xu, D. Y. *Biol. Chem*. **2014**, *395*, 443–451.

[43] Roche, C.; Guerrot, D.; Harouki, N.; Duflot, T.; Besnier, M.; Remy-Jouet, I.; Renet, S.; Dumesnil, A.; Lejeune, A.; Morisseau, C.; Richard, V.; Bellien, J. *Prostaglandins Other Lipid Mediat*. **2015**, *120*, 148–154.
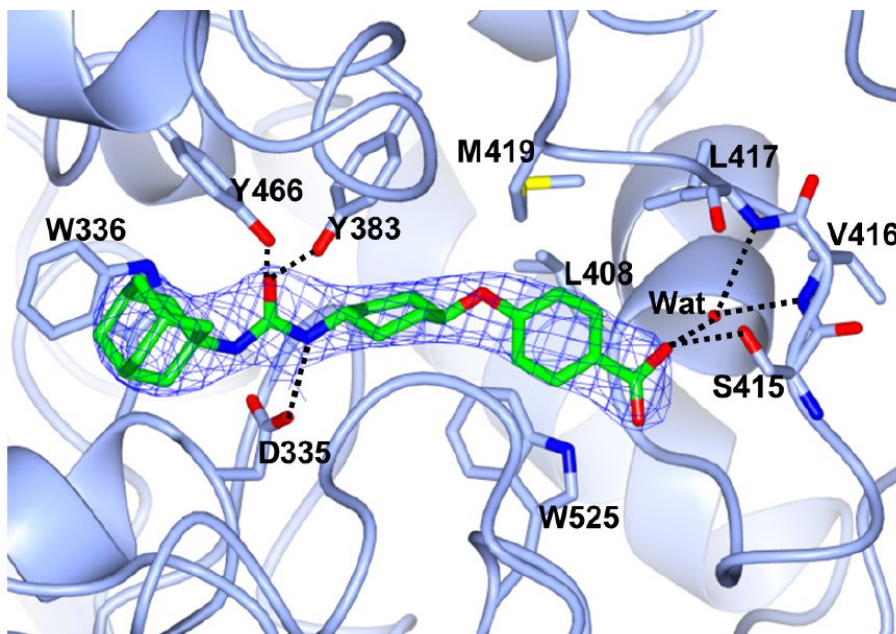
Fig. 13. Crystal structure of *t*-AUCB (green) bound to sEH catalytic pocket. PDB code: 3WKE. Figure taken from reference 13.

1.1.5.2.   Previous work on sEHI in our research group

During the last years, the group of Dr. Santiago Vázquez has focused its research on the synthesis of bioactive compounds derived from adamantane-like polycyclic moieties. Many different adamantane-like scaffolds such as oxa-derivatives, ring-expanded and ring contracted and other related compounds have been designed, synthesized and evaluated in several different targets (Fig. 14).
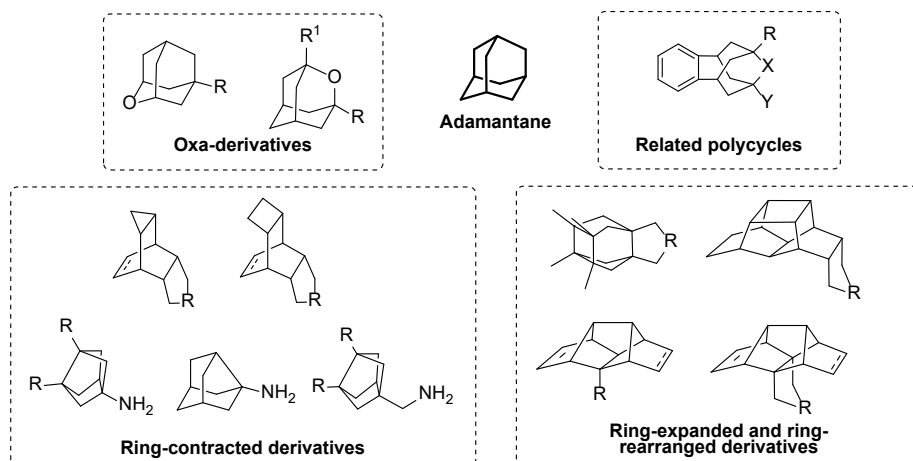
Fig. 14. Some of the polycyclic scaffolds synthesized and used by the group of Dr. Santiago Vázquez for the design of bioactive compounds.

Some of the targets in which the different polycyclic compounds have been tested on are the M2 channel of the influenza A virus or the human NMDA channel.[44,45,46,47,48,49,50,51] More recently, these polycyclic scaffolds have been

[44] Duque, M. D.; Ma, C.; Torres, E.; Wang, J.; Naesens, L.; Juárez-Jiménez, J.; Camps, P.; Luque, F. J.; DeGrado, W. F.; Lamb, R. A.; Pinto, L. H.; Vázquez, S. *J. Med. Chem.* **2011**, *54*, 2646-2657.

[45] Rey-Carrizo, M.; Torres, E.; Ma, C.; Barniol-Xicota, M.; Wang, J.; Wu, Y.; Naesens, L.; Degrado, W. F.; Lamb, R. A.; Pinto, L. H.; Vázquez, S. *J. Med. Chem.* **2013**, *56*, 9265-9274.

[46] Torres, E.; Leiva, R.; Gazzarrini, S.; Rey-Carrizo, M.; Frigolé-Vivas, M.; Moroni, A.; Naesens, L.; Vázquez, S. *ACS Med. Chem. Lett.* **2014**, *5*, 831-836.

[47] Rey-Carrizo, M.; Gazzarrini, S.; Llabrés, S.; Frigolé-Vivas, M.; Juárez-Jiménez, J.; Font-Bardia, M.; Naesens, L.; Moroni, A.; Luque, F. J.; Vázquez, S.; Santiago, V. *Eur. J. Med. Chem.* **2015**, *96*, 318-329.

[48] Camps, P.; Duque, M. D.; Vázquez, S.; Naesens, L.; De Clercq, E.; Sureda, F. X.; López-Querol, M.; Camins, A.; Pallàs, M.; Prathalingam, S. R.; Kelly, J. M.; Romero, V.; Ivorra, D.; Cortés, D. *Bioorg. Med. Chem.* **2008**, *16*, 9925-9936.

[49] Duque, M. D.; Camps, P.; Profire, L.; Montaner, S.; Vázquez, S.; Sureda, F. X.; Mallol, J.; López-Querol, M.; Naesens, L.; De Clercq, E.; Prathalingam, S. R.; Kelly, J. M. *Bioorg. Med. Chem.* **2009**, *17*, 3198-3206.

[50] Torres, E.; Duque, M. D.; López-Querol, M.; Taylor, M. C.; Naesens, L.; Ma, C.; Pinto, L. H.; Sureda, F. X.; Kelly, J. M.; Vázquez, S. *Bioorg. Med. Chem.* **2012**, *20*, 942-948.

[51] Valverde, E.; Sureda, F. X.; Vázquez, S. *Bioorg. Med. Chem.* **2014**, *22*, 2678-2683.

explored in other targets such as the human P2X7 channel and the 11β-HSD1 enzyme.[52,53,54]

Importantly, the aforementioned results revealed that the adamantane moiety is not always the best choice for satisfactorily filling the hydrophobic space on a given biological target.

After the extensive work carried out by groups both at the university and in the industry to obtain a drug candidate as sEHI, very few compounds have reached clinical trials and, what is more, none of them have reached the approval of the different agencies to be marketable. This fact demonstrates that obtaining sEHI is a complex and a challenging process. This extra difficulty is mainly due to the great limitations that the sEHIs usually present like the high lipophilicity, their low metabolic stability and low water solubility. Taking into account that the adamantane moiety presents these poor physicochemical properties, the group of Dr. Santiago Vázquez hypothesized that replacing the adamantane nucleus by other polycyclic structures may result in compounds with improved profiles, maintaining the high potency characteristic of the urea-based compounds.

Therefore, during the thesis of Dr. Elena Valverde, our group started a new research program aimed to the discovery of new sEHI bearing adamantane-like scaffolds as LHS moieties. Many novel sEHI were synthesized containing these scaffolds together with selected units taken from the RHS of previously disclosed sEHI. Several parameters were evaluated on these new molecules; inhibitory potency, microsomal stability, solubility, permeability, melting points, etc. Among them, the 2-oxaadamantane analogs presented improved water solubility, lower melting points and higher membrane permeability trough Caco-2 cells. On the other hand, the potency was slightly reduced (Table 1).

[52] Barniol-Xicota, M.; Kwak, S. H.; Lee, S. D.; Caseley, E.; Valverde, E.; Jiang, L. H.; Kim, Y. C.; Vázquez, S. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 759-763.

[53] Leiva, R.; Seira, C.; McBride, A.; Binnie, M.; Luque, F. J.; Bidon-Chanal, A.; Webster, S. P.; Vázquez, S. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 4250-4253.

[54] Valverde, E.; Seira, C.; McBride, A.; Binnie, M.; Luque, F. J.; Webster, S. P.; Bidon-Chanal, A.; Vázquez, S. *Bioorg. Med. Chem.* **2015**, *23*, 7607-7617.
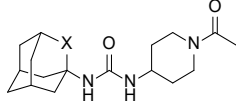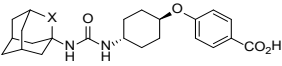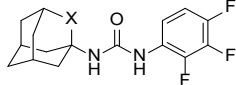
Compounds	X	hsEH IC$_{50}$ (nM)	Solu. (µM)	Mp (ºC)	Permeability (Caco-2) App (nm/s) A→B B→A		ER
APAU	CH$_2$	8.0	24	202-204	2.2	141.2	64.5
1	O	29.9	59	172-173	22.4	94.5	4.3
t-AUCB	CH$_2$	0.5	25	250-255	1.9	210.3	111
2	O	9.0	>100	255-257	1.4	75.5	55.4
3	CH$_2$	7.7	16	216-219	6.7	4.6	0.7
4	O	21.3	27	196-198	168	151.0	0.9

Table 1. Human sEH IC$_{50}$, solubility, melting point and permeability values for known adamantane inhibitors APAU and *t*-AUCB, compound **3**, and their oxygen-bearing analogs **1**, **2** and **4**.

Despite the improvement achieved with these compounds, the group was still far from a molecule whose pharmacologic profile was interesting enough to be tested further in animals and could really be a drug candidate in the future. As Elena's thesis finished, Sandra Codony started her PhD. continuing this research line in order to get a drug candidate that could improve the pharmacokinetics parameters substantially.

In view of the significant improvement of 2-oxaadamantane nucleus, more compounds bearing this LHS moiety were synthetized and evaluated.[55] A screening cascade was made allowing the group to select a suitable candidate for *in vivo* proof of concept studies. Finally, sulfonamide **5** was the selected compound, as it presented the best balance between potency,

[55] Codony, S.; Valverde, E.; Leiva, R; Vázquez-Carrera, M.; Vázquez, S. *University of Barcelona* **2017**, WO2017017048.

solubility and drug metabolism and pharmacokinetics (DMPK) profile (Fig. 15).[56]

5

human sEH IC$_{50}$ = 197 nM
murine sEH IC$_{50}$ = 79 nM
Mic. Stability (human) (remanent % 1 h) = 73
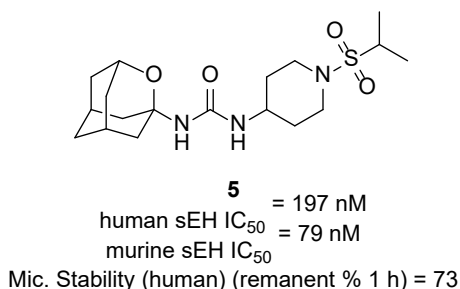
Fig. 15. Structure, inhibitory potency and microsomal stability of compound **5**. IC$_{50}$: concentration producing 50% inhibition of sEH. Mic. Stability: remanent compound after 1 h incubation in microsomal cells.

The *in vivo* evaluation was performed in a murine model of acute pancreatitis, a disease in which inflammation has a key role. The evaluation was relatively successful since the treatment diminished the overexpression of ER stress and inflammation markers induced by cerulein. However, when the group met several prospective investors and pharmaceutical companies, their feedback was that the inhibitory potency should still be improved to the low nanomolar range. Indeed, computational studies showed that the oxygen of the oxaadamantane group provided the molecule with a rigidity that prevented its proper orientation within the hydrophobic pocket of the sEH.[56]

With these new findings, and taking into account the size of the hydrophobic pocket of sEH, the optimal size of the hydrophobic unit of the sEHIs was explored in order to evaluate the impact in potency of more suitable lipophilic moieties. More analogs of APAU and *t*-AUCB were synthetized where the LHS of the molecule was substituted by other polycyclic units of different sizes; ring-contracted analogs such as noradamantane and larger analogs

[56] Codony, S.; Pujol, E.; Pizarro, J.; Feixas, F.; Valverde, E.; Loza, M. I.; Brea, J. M.; Saez, E.; Oyarzabal, J.; Pineda-Lucena, A.; Pérez, B.; Pérez, C.; Rodríguez-Franco, M. I.; Leiva, R.; Osuna, S.; Morisseau, C.; Hammock, B. D.; Vázquez-Carrera, M.; Vázquez, S. *J. Med. Chem*. **2020**, 63, 9237–9257.

such as diamantane.[57] Overall, it seems clear that the hydrophobic pocket of sEH can accommodate larger moieties, improving the potency as the ring size is expanded (Fig. 16). Nevertheless, the low solubility and the high metabolic liability of these new derivatives made them unsuitable for their application in medicinal chemistry.

6
human sEH IC$_{50}$ = 21.7 nM

APAU
human sEH IC$_{50}$ = 8.0 nM

7
human sEH IC$_{50}$ = 3.4 nM
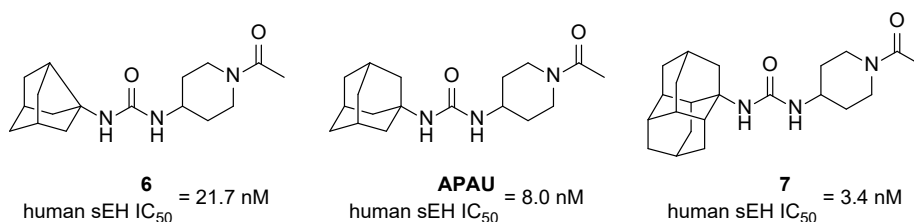
Fig. 16. Inhibitory potency increase as ring is expanded in different sEHIs. IC$_{50}$: concentration producing 50% inhibition of sEH.

In parallel with the Thesis of E. Valverde, Hammock's group discovered that the replacement of the adamantane nucleus of its clinical candidate APAU by a substituted phenyl ring and the modification of the third pharmacophore resulted in compounds TPPU[58] and EC5026[59] (Fig. 17). Both compounds presented improved drug-like properties over their adamantane-compounds counterparts (e.g., APAU). This fact, and our discovery that the LHS of the sEH can accommodate lipophilic units large than adamantane, made the group to consider that the fusion of a phenyl ring with an adamantane scaffold could present the advantages of drug-like properties of phenyl rings and the ability to fully occupy the pocket of sEH in a more efficient manner.

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[57] Codony, S.; Valverde, E.; Leiva, R.; Brea, J.; Loza, M. I.; Morisseau, C.; Hammock, B. D.; Vázquez, S. *Bioorg. Med. Chem.* **2019**, *27*, 115078.

[58] Rose, T. E.; Morisseau, C.; Liu, J. Y.; Inceoglu, B.; Hammock, B. D. *J. Med. Chem*. **2010**, 53, 7067–7075.

[59] Lee, K. S. S.; Ng, J. C.; Yang, J.; Hwang, S. H.; Morisseau, C.; Wagner, K.; Hammock, B. D. *Bioorg. Med. Chem.* **2020**, *28*, 115735.
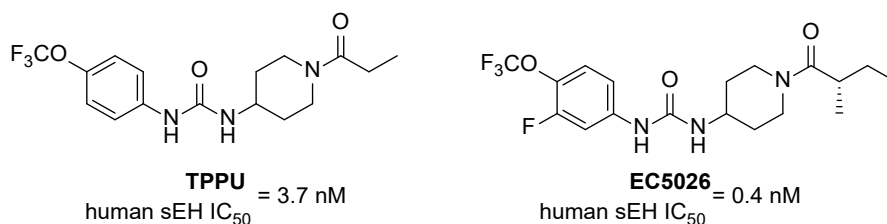
Fig. 17. Structure and inhibitory potency of TPPU and EC5026. IC_{50}: concentration producing 50% inhibition of sEH.

This fusion generated a novel large lipophilic nucleus called benzohomoadamantane ring (Fig. 18). Besides, different substitutions in the X position together with different substitutions in the aromatic ring by electron donating and/or electron withdrawing groups could lead to a larger number of benzohomoadamantane-based ureas with a great margin of modification.
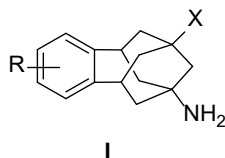
Fig. 18. General structure **I** of the benzohomoadamantane scaffold.

From this new approach, many new compounds were synthesized and evaluated for its pharmacological parameters in the group, resulting in different candidates which really afforded great expectations for their evaluation in *in vivo* studies. This is the case of compound **8**, a molecule that is endowed with high potency, great stability and good solubility parameters (Fig. 19). Compound **8**, as compound **5**, was evaluated in a cerulein-induced acute pancreatitis mice model, significantly reducing pancreatic damage.[60]

[60] Codony, S.; Calvó-Tusell, C.; Valverde, E.; Osuna, S.; Morisseau, C.; Loza, M. I.; Brea, J.; Pérez, C.; Rodríguez-Franco, M. I.; Pizarro-Delgado, J.; Corpas, R.; Griñán-Ferré, C.; Pallàs, M.; Sanfeliu, C.; Vázquez-Carrera, M.; Hammock, B. D.; Feixas, F; Vázquez, S. *J Med Chem*. **2021**, *64*, 5429-5446.

Molecular dynamics simulations were also performed in this work, indicating that sEH reshapes the active site pocket to stabilize the aromatic ring of the benzohomoadamantane scaffold, indicating that this big group fills efficiently the catalytic pocket.

8
human sEH IC$_{50}$ = 0.4 nM
murine sEH IC$_{50}$ = 0.4 nM
Mic. stability (human)
(remanent % 1 h) = 89%
CYP 2C19 (% inh at 10 μM) = 2.2%

Fig. 19. Structure, inhibitory potency, microsomal stability and cytochrome inhibition of compound **8**. IC$_{50}$: concentration producing 50% inhibition of sEH. Mic. Stability: remanent compound after 1 h incubation in microsomal cells. CYP 2C19 inhibition: Percentage of cytochrome 2C19 inhibition when the compound is at 10 M.

Continuing with the group's work on this new benzohomoadamantane-based scaffold, new benzohomoadamantane-based piperidine derivatives, analogs of the clinical candidates AR9281 and EC5026, were synthesized. The *in vitro* profiling of these new sEHIs, where solubility, cytotoxicity, metabolic stability, etc. were evaluated, allowed to select two suitable candidates for *in vivo* efficacy studies (Fig. 20). This time, the selected murine model for the *in vivo* evaluation was the capsaicin induced murine model of allodynia, a predictive model of efficacy in neuropathic pain. The administration of compounds **9** and **10** reduced pain in a dose-dependent manner and outperformed AS2586114, a well-known sEHI developed by the Japanese pharmaceutical company Astellas (see below). Moreover, compound **10** was selected and tested in another murine model: the cyclophosphamide-induced

murine model of cystitis, where the robust analgesic effect was again revealed.[61]

9
human IC$_{50}$ = 0.4 nM
murine IC$_{50}$ = 1.0 nM
Mic. stability (human)
(remanent % 1 h) = 47%
CYP 2C19 (% inh at 10 μM) = 38%

10
human IC$_{50}$ = 0.4 nM
murine IC$_{50}$ = 0.5 nM
Mic. stability (human)
(remanent % 1 h) = 66%
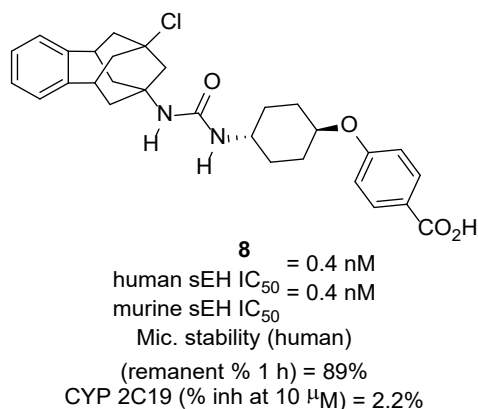CYP 2C19 (% inh at 10 μM) = 32%
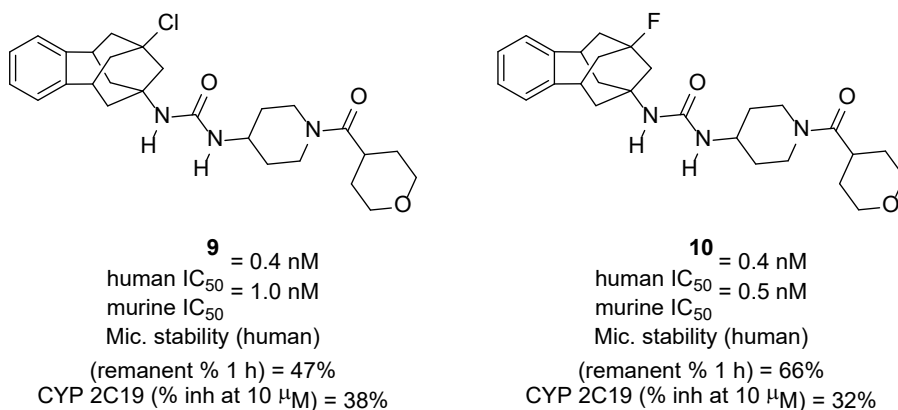
Fig. 20. Structure, inhibitory potency, microsomal stability and cytochrome inhibition of **9** and **10**. IC$_{50}$: concentration producing 50% inhibition of sEH. Mic. Stability: remanent compound after 1 h incubation in microsomal cells. CYP 2C19 inhibition: Percentage of cytochrome 2C19 inhibition when the compound is at 10 M.

In conclusion, the benzohomoadamantane moiety emerges as a suitable hydrophobic scaffold for the design of novel sEHIs, opening a whole range of applications of these new sEHIs in the pain and likely other fields.

[61] Codony, S.; Entrena, J. M.; Calvó-Tusell, C.; Jora, B.; González-Cano, R.; Osuna, S.; Corpas, R.; Morisseau, C.; Pérez, B.; Barniol-Xicota, M.; Loza, M. I.; Pérez, C.; Rodríguez-Franco, M. I.; Martínez, A. L.; Pallàs, M.; Sanfeliu, C.; Verhelst, S. H. L.; Feixas, F.; Brea, J.; Cobos, E. J.; Hammock, B. D.; Vázquez, S. *J Med Chem*. **2022**, accepted but not yet published.

1.2. Objetives

The latest results obtained during Dr. Sandra Codony's thesis[62] and part of Beatrice Jora's thesis are quite promising. However, despite having developed molecules that greatly improved the existing sEHI, there is still room for improvement in terms of solubility and microsomal stability. That is why throughout this thesis two lines of research were followed with the aim of trying to improve these two DMPK parameters.

1.2.1. *N*-arylpiperidinyl and aryl-oxycyclohexyl ureas

In this first line, the objective was the synthesis of new ureas using the benzohomoadamantane scaffold in the LHS and *N*-arylpiperidines or aryloxycyclohexanes as the second and third pharmacophores for the RHS. The aim was to broaden the spectrum of previously developed products, taking as a starting point AS2586114, a sEHI discovered and preclinically developed by Astellas Pharma[63] (Fig. 21), as well as compound **8** (Fig. 19). The inhibitory potency and pharmacokinetic properties of both the Astellas product and compound **8** have already been proven both *in vitro* and *in vivo*.[57,64]

AS2586114
human IC$_{50}$ = 0.4 nM
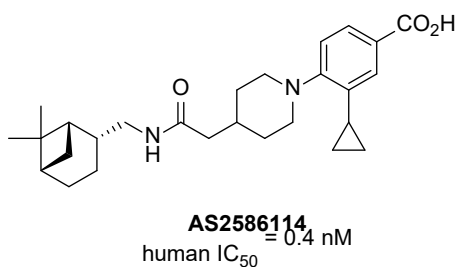
Fig. 21. Structure and inhibitory potency of AS2586114.

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[62] Codony, S. Ph. D. Dissertation. From the design to the in vivo evaluation of novel soluble epoxide hydrolase inhibitors. *University of Barcelona*, **2020**.

[63] Miura, M.; Sato, I.; Kiyohara, H.; Yokoyama, K.; Takakura, K.; Terada, H. Yamaguchi, T.; Amano, Y. *Astellas Pharma Inc* **2011**, JP2011016743A.

[64] Ma, M.; Ren, Q.; Fujita, Y.; Ishima, T.; Zhang, J. C.; Hashimoto, K. *Pharmacol Biochem Behav.* **2013**, Sep;110:98-103.

The use of this previous knowledge by Astellas and the application of the knowledge previously acquired by the group during Sandra's and Beatrice's thesis supposed the opening of a whole new field for the improvement of the benzohomoadamantane compounds in which the group works. To date, very few *N*-arylpiperidinyl or aryloxycyclohexyl ureas have been synthesized within the group's line, so a wide spectrum of new compounds was to be done. The general structures **II** and **III** followed for both urea-based derivatives are exposed in figure 22.
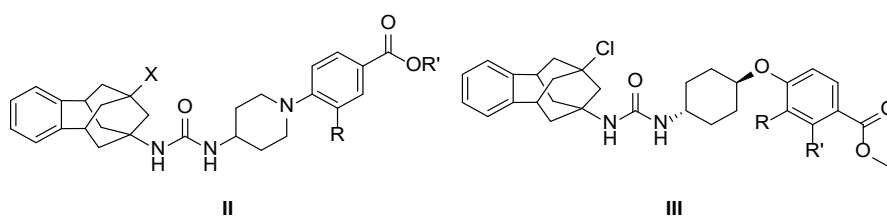

Fig. 22. General structure **II** (*N*-arylpiperidines) and **III** (aryloxycyclohexyls) of the urea-based sEHIs.

1.2.2. Arylpiperidinyl and sulfone-piperidinyl amides

In this second line of research, the substitution of the urea group by an amide group was carried out with a double objective; on the one hand, to extend the Markus formula of the patent[65] that the group already had in progress and, on the other hand, to improve the solubility of the urea compounds. To address one of the main handicaps that the group faced throughout the investigation, the compounds solubility problems, it was thought to replace the urea group with an amide group. It is well known that the urea functional group is prone to solubility problems and typically exhibits relatively high melting points compared to its amide counterparts. As it has already been explained throughout the introduction, Hammock's group presented a SAR[37] in which not

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[65] Codony, S.; Turcu, A.; Valverde, E.; Leiva, R. Galdeano, C. M.; Vázquez, S. *University of Barcelona* **2019**, WO2019243414.

only ureas were used as main pharmacophores, but also amides, and they showed to also have a high affinity for the active center of the sEH.

Inspired by both the Astellas product (Fig. 21) and the compound **5** (Fig. 15) of the 2-oxaadamantane derivatives synthesized by Dr. Codony in the first part of her thesis, a series of compounds with amides as the main pharmacophore were designed, synthesized and evaluated following general structure **IV** (Fig. 23).
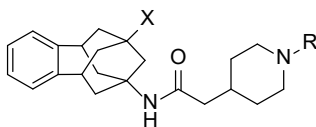

Fig. 23. General structure **IV** for the amide-based sEHIs.

1.4. Results and discussion

### 1.4.1. *N*-arylpiperidinyl and aryloxycyclohexyl ureas as sEHIs

1.4.1.1. Design and synthesis of *N*-arylpiperidinyl and aryloxycyclohexyl ureas

The design of the new sEHI series was performed keeping the benzohomoadamantane ring developed during Sandra Codony's thesis (Fig. 18). For the first *N*-arylpiperidinyl inhibitors, the aromatic ring of the benzohomoadamantane nucleus was preserved without any type of substituent on the aromatic ring, while different substituents were added to the X position of the nucleus. On the other hand, the RHS of the molecule, inspired by the Astellas compound AS2586114, was modified with different substituents in the *meta*-position of the aromatic ring, following general structure **II** (Fig. 22).

The synthesis of the *N*-arylpiperidinyl urea derivatives was convergently done. On the one hand, the LHS of the molecule corresponding to the benzohomoadamantanamine nucleus, with the relevant substituent in position X, was synthesized following the synthetic route previously described by the group (Scheme 1).[66,67] On the other side, the amine corresponding to the RHS of the compound was separately synthesized in order to, through an isocyanate formation reaction, condense both building blocks forming the final urea.

[66] Duque, M. D.; Camps, P.; Torres, E.; Valverde, E.; Sureda, F. X.; López-Querol, M.; Camins, A.; Prathalingam, S. R.; Kelly, J. M.; Vázquez, S. *Bioorg. Med. Chem*. **2010**, 18, 46-57.
[67] Valverde, E.; Sureda, F. X.; Vázquez, S. *Bioorg. Med. Chem*. **2014**, 22, 2678-2683.
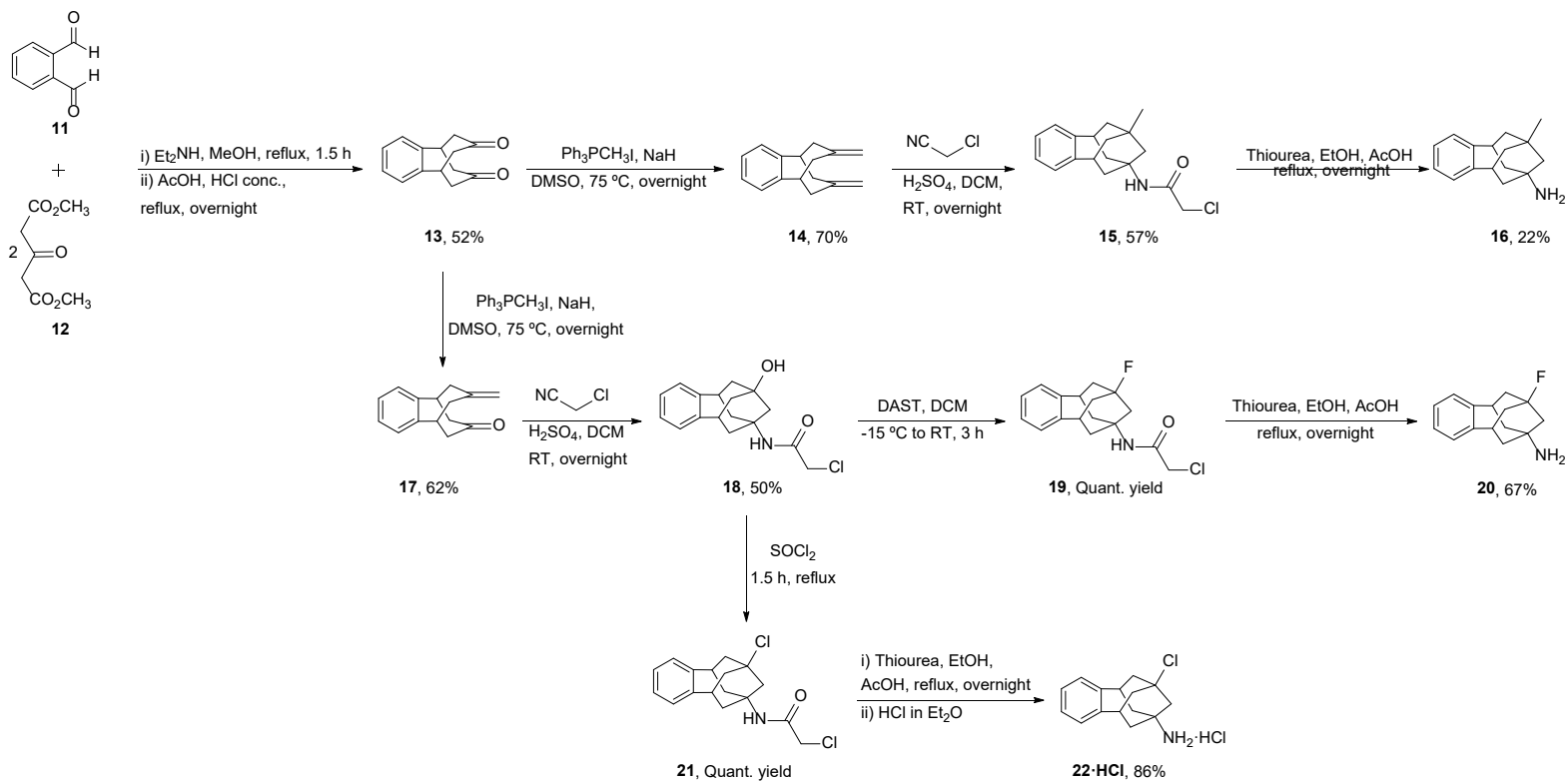
**Scheme 1**. Synthetic route for amines **16**, **20** and **22** (X = Me, F and Cl, respectively).

The synthesis of the benzohomoadamantane nucleus starts from *o*-phthaldehyde and dimethyl 3-oxopentanedioate. A Weiss multicomponent condensation followed by hydrolysis and decarboxylation was carried out to give the diketone product **13** in a moderate yield.[68] Subsequently, the resulting product was dehydrated using toluene in a Dean-Stark apparatus, an important step before performing a Wittig reaction on the diketone. Depending on the desired final product, and by controlling the number of equivalents of methyltriphenylphosphonium iodide, a mono or double olefination was carried out. If a methyl group was desired in the X position, a double olefination was performed to give diene **14**. For any other X position substitution, a mono-olefination was done to give enone **17**. In both cases, the next step consisted of a transannular Prins-Ritter reaction using 2-chloroacetonitrile as the nucleophile, leading to chloroacetamides **15** and **18** in moderate yield.

Deprotection of product **15** using thiourea in an acidic medium with EtOH as solvent gave the benzohomoadamantane amine **16** in low yield.

On the other hand, for chloroacetamide **18**, its hydroxyl group was replaced by either a fluorine or a chlorine atom using DAST or $SOCl_2$, furnishing compounds **19** and **21**, respectively, in quantitative yields. Then, as abovementioned, a transannuar Prins-Ritter reaction followed by deprotection with thiourea yielded products **20** and **22** respectively in good yields.

Regarding the synthesis of the different amines belonging to the RHS of the *N*-arylpiperidine compounds, they were obtained following the routes of scheme 2.

[68] Föshlisch, B.; Dukek, U.; Graessle, I.; Novotny, B.; Schupp, E.; Schwaiger, G.; Widmann, E. *Liebigs Ann*. *Chem.* **1973**, *11*, 1839-1850.
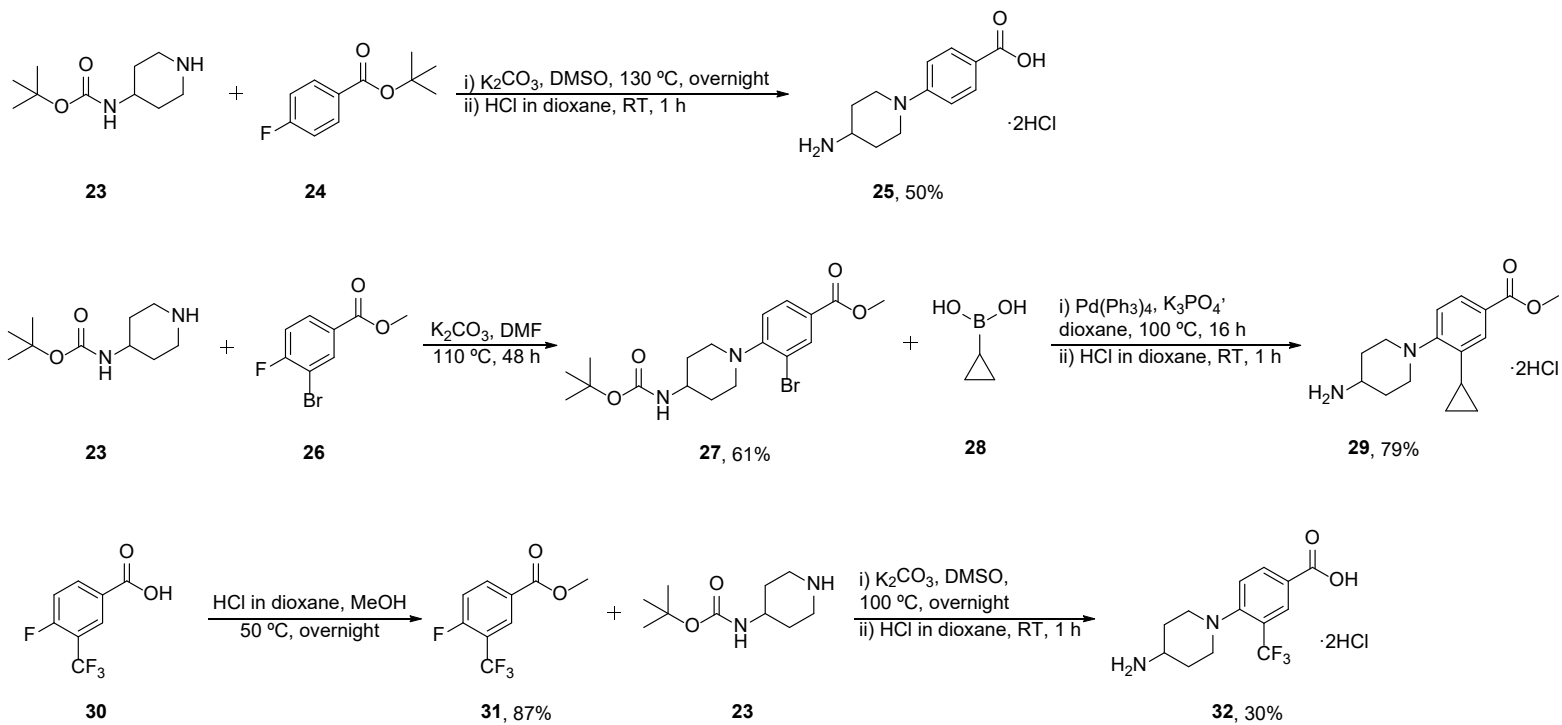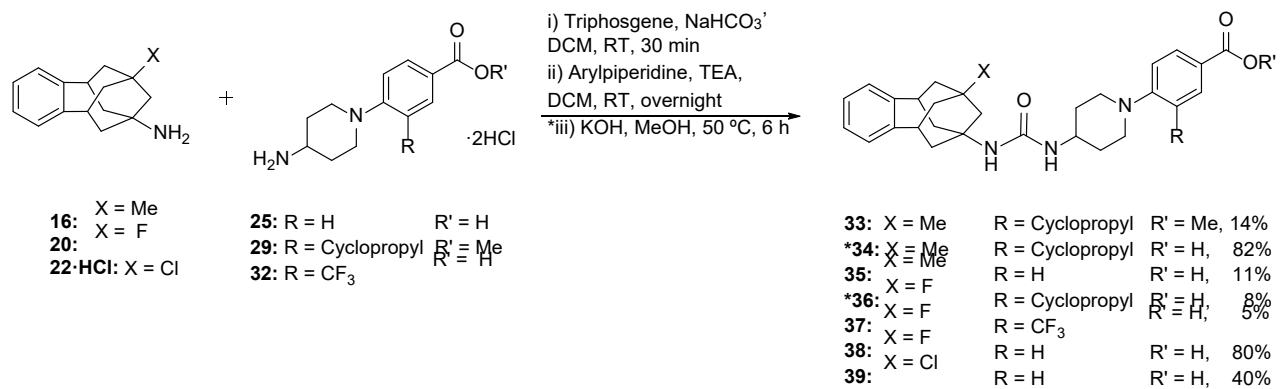
Scheme 2. Synthetic route for the different *N*-arylpiperidinyl-4-amines for the RHS.

Compound **25** was synthesized *via* the direct reaction of *tert*-butyl piperidin-4-ylcarbamate with *tert*-butyl 4-fluorobenzoate through a S$_N$Ar type reaction. After that, both the Boc-group and *tert*-butyl ester group were hydrolyzed using HCl in dioxane yielding aminoacid **25** as its dihydrochloride, in a good overall yield.

A similar approach was followed with compound **29**. *Tert*-butyl piperidin-4-ylcarbamate reacted with methyl 3-bromo-4-fluorobenzoate **26** to afford molecule **27** via a S$_N$Ar reaction. Subsequently, reaction with cyclopropylboronic acid via a Suzuki coupling afforded **29** in good yield.

For the latter compound, the first step was an esterification reaction with MeOH in the presence of an acid. Then, ester **31** reacted with *tert*-butyl piperidin-4-ylcarbamate followed by hydrolysis to afford compound **32** in low yield.

In order to join both parts of the final molecules, the benzohomoadamantane amines were treated with triphosgene to form their corresponding isocyanates. Then, the corresponding *N*-arylpiperidinyl-4-amines of the RHS was condensed with these isocyanates furnishing the different *N*-arylpiperidinyl urea derivatives (Scheme 3). Compounds **33**, **35**, **37**, **38** and **39** were obtained by direct reaction in very low yields. On the other hand, compounds **34** and **36** were obtained by hydrolysis with KOH in methanol of their corresponding methyl esters.

i) Triphosgene, NaHCO₃'
DCM, RT, 30 min
ii) Arylpiperidine, TEA,
DCM, RT, overnight
*iii) KOH, MeOH, 50 ºC, 6 h

16: X = Me
20: X = F
22·HCl: X = Cl

25: R = H   R' = H
29: R = Cyclopropyl R' = Me
32: R = CF₃   R' = H

33: X = Me   R = Cyclopropyl R' = Me, 14%
*34: X = Me R = Cyclopropyl R' = H, 82%
35: X = Me   R = H   R' = H, 11%
*36: X = F   R = Cyclopropyl R' = H, 8%
37: X = F   R = CF₃   R' = H, 5%
38: X = F   R = H   R' = H, 80%
**39:** X = Cl   R = H   R' = H, 40%

Scheme 3. Synthesis of *N*-arylpiperidinyl urea derivatives. *Reaction made only to the esters (R' = Me) to obtain compounds **34** and **36**.

In another vein, five aryloxycyclohexyl sEHIs were synthesized following the same approach as the previous compounds but modifying the RHS of the molecule. This time, the 4-aminopiperidine group was substituted by a *trans*-4-aminocyclohexan-1-ol unit, maintaining the aryl group with the acid (or ester) group in *para*-position while making substitutions in either the *meta* or the *ortho* positions of the aromatic ring.

The first step to synthesize the new RHS of the compounds was the esterification with MeOH in acid medium of the different benzoic acid derivatives. Then, a S_NAr reaction was performed with NaH in DMF to obtain from low to moderate yields compounds **49**-**52** (Scheme 4). After that, the coupling with the benzohomoadamantane amine was carried out as in the previous series of ureas, furnishing compounds **53**-**56** in low yields.

As it can be seen, for this series of derivatives, the group X of the benzohomoadamantane amine was set to chlorine in all cases. This is due to the fact that the derivatives with a chlorine atom in that position gave the best *in vitro* results during the group previous work evaluations,[60] so that position was decided to be kept constant in all compounds. Interestingly, after obtaining derivatives **53**-**56**, the four esters were intended to be hydrolyzed to form the corresponding carboxylic acids. Surprisingly, in all cases, the molecules did not undergo hydrolysis, but they fragmented at the benzyl ether position, splitting up the molecule in two alcohols. Likely, the poor electronic density of the aromatic ring favors the attack of the hydroxyl anion to the *ortho* position, leading to a S_NAr that may led to the two observed fragments.
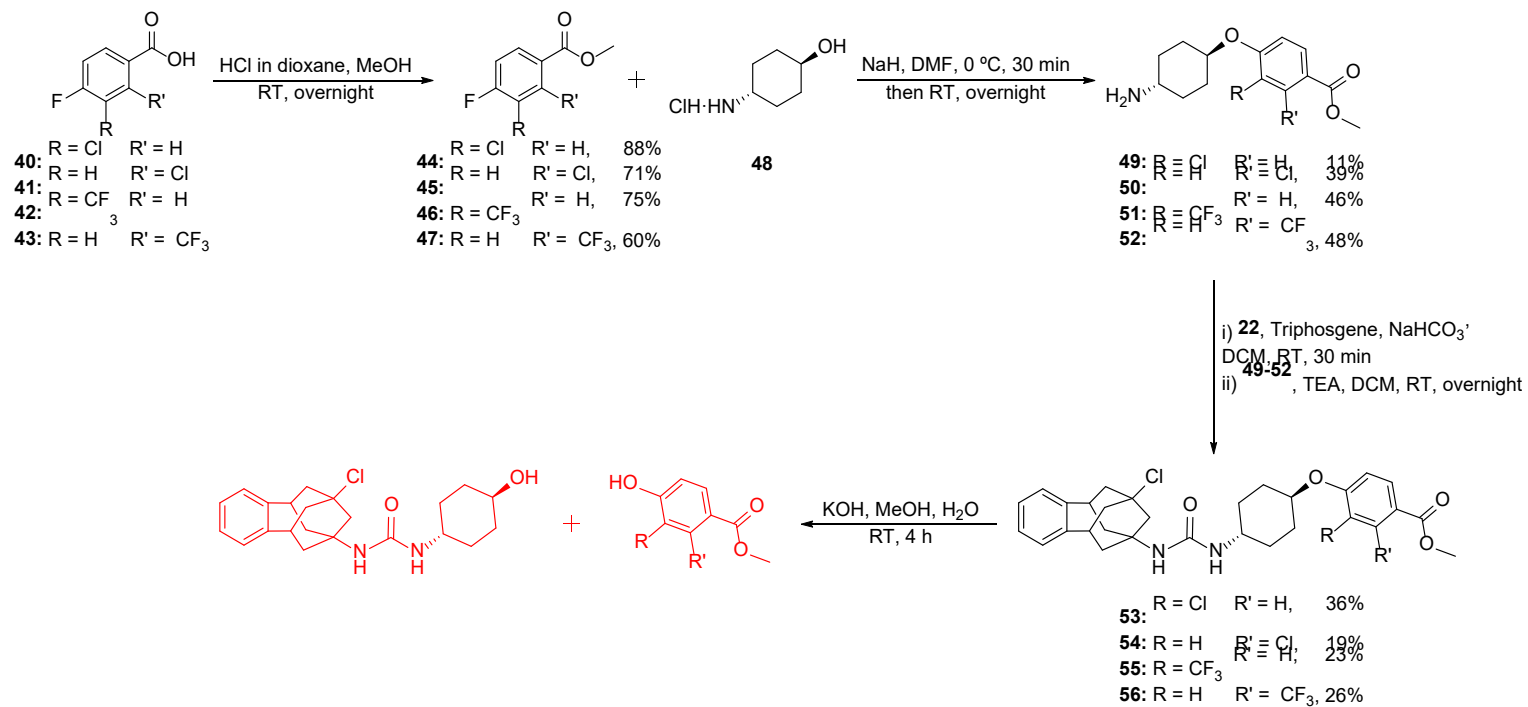
Scheme 4. Synthetic route to the ureas **53**-**56** and unsuccessful deprotection of the methyl esters.

Starting materials:
- **40:** R = Cl   R' = H
- **41:** R = H   R' = Cl
- **42:** R = CF₃   R' = H
- **43:** R = H   R' = CF₃

After HCl in dioxane, MeOH, RT, overnight:
- **44:** R = Cl   R' = H,   88%
- **45:** R = H   R' = Cl,   71%
- **46:** R = CF₃   R' = H,   75%
- **47:** R = H   R' = CF₃, 60%

Compound **48**

After NaH, DMF, 0 °C, 30 min then RT, overnight:
- **49:** R = Cl   R' = H,   11%
- **50:** R = H   R' = Cl,   39%
- **51:** R = CF₃   R' = H,   46%
- **52:** R = H   R' = CF₃, 48%

i) **22**, Triphosgene, NaHCO₃, DCM, RT, 30 min
ii) **49-52**, TEA, DCM, RT, overnight

- **53:** R = Cl   R' = H,   36%
- **54:** R = H   R' = Cl,   19%
- **55:** R = CF₃   R' = H,   23%
- **56:** R = H   R' = CF₃, 26%

KOH, MeOH, H₂O, RT, 4 h

57

Taking into account the interesting biological activity of compound **8**,[60] we envisaged the synthesis of its *cis*-stereoisomer. As before, following a convergent synthesis, the RHS of the molecule was synthesized starting from *cis*-4-aminocyclohexanol, **57**, and 4-fluorobenzonitrile, **58**, through an S$_N$Ar-type reaction, using the previously conditions, that is, NaH in DMF as solvent (Scheme 5). Subsequently, compound **59** was linked to the benzohomoadamantane amine nucleus through the formation of an urea as in all previous cases. Lastly, the nitrile group was hydrolyzed under harsh conditions by heating in strong basic medium to give compound **61** in a medium overall yield.
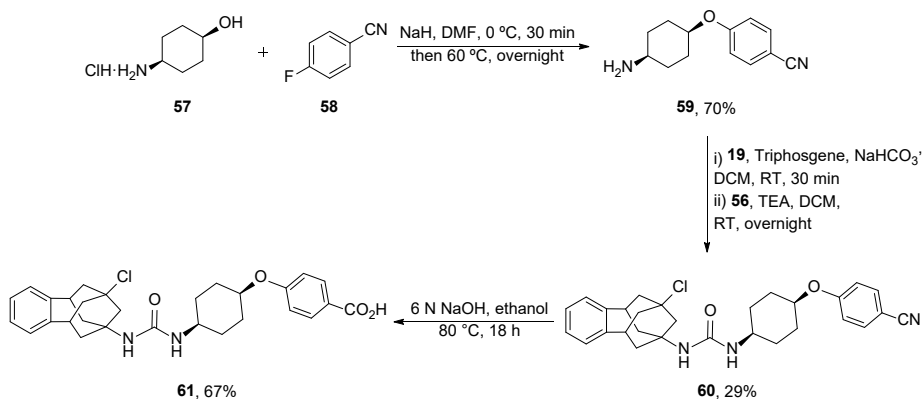
Scheme 5. Synthesis of urea **61**.

1.4.1.2. sEH inhibition and pharmacokinetic profile of *N*-arylpiperidinyl and aryloxycyclohexyl ureas

All the compounds were tested in a large battery of *in vitro* assays by many different laboratories. These evaluations include: potency as human and murine sEHIs, tested by Dr. Christophe Morisseau, from the group of Bruce D. Hammock at the UCD; microsomal stability (human, mouse and rat species), hERG inhibition, CYP inhibition, solubility and permeability through Caco-2 cells, performed by the group of Prof. M. Isabel Loza and Prof. José M. Brea of the Drug Screening Platform/Biofarma Research Group of the University of Santiago de Compostela (Spain); PAMPA-BBB performed by Prof. Belén Pérez

of the Autonomous University of Barcelona (Spain); and finally, cytotoxicity in SH-SY5Y cells evaluated by Drs Coral Sanfeliu and Rubén Corpas of the Institute of Biomedical Research of Barcelona (CSIC).

Inspired in the good results of the Astellas compound AS2586114 (Fig. 21), the cyclopropyl group in *meta*-position of the aromatic ring was kept and a methyl group was chosen as the substituent in position X of the benzohomoadamantane nucleus. From this first combination compounds **33** and **34** were obtained. Despite the high inhibitory potency of both compounds, they showed unacceptable poor microsomal stability and they were discarded (Table 3). The X position, previously occupied by a methyl group, was then changed to a fluorine atom (compound **36**). Despite a significant increase in the microsomal stability, particularly in human microsomes, the values were still low in murine and rat microsomes. Roughly, a compound is suitable for further *in vitro* studies if the % of remaining compound after 1 hour in microsomes is higher than 50-60%. Therefore, compound **36** was also discarded. This improvement in microsomal stability seemed to indicate that a fluorine atom might be a better substituent than a methyl group at the X position of benzohomoadamantane. Next, and maintaining the fluorine atom in position X, the substitution of the cyclopropyl group in *meta*-position of the aromatic ring of the RHS by a CF_3 group was carried out (compound **37**), showing very similar results to compound **36**.

As this last result did not improve microsomal stability, it was thought to completely eliminate any substitution in *meta*-position of the aromatic ring of the RHS resulting in the compound **38**. As shown in table 2, in this case the stability improved drastically in human and murine microsomes, although the stability in rat microsomes still was very low. Anyway, taking into account its outstanding subnanomolar potency in both the human and murine enzymes and that our *in vivo* assays are carried out in murine models, we considered **38** as a promising excellent compound.

Due to this recent success and keeping the benzoic acid ring without any substitutions in *meta*-position, the X position of the

benzohomoadamantane nucleus was again explored resulting in two new derivatives: one with the methyl group, previously explored, and the other with a chlorine atom.

Although the compound with the methyl group in the X position (compound **35**) presented an acceptable microsomal stability, surpassing the results of **33** and **34**, it was again the chlorine-substituted compound, **39**, that was the more stable compounds. Thus, **38** and **39** were selected for further assays.

Cmpd	Inhibitory potency[1] IC_{50} (nM)		Microsomal stability[2] % remaining at 1 h		
	Human sEH	**Murine sEH**	**Human**	**Mouse**	**Rat**
33	0.4	0.4	6.5	0.0	0.0
34	0.7	3.4	0.1	0.0	0.0
35	0.4	0.4	63	44	0.5
36	0.4	0.4	47	18	12
37	0.4	0.4	46	30	13
38	**0.5**	**0.4**	**100**	**86**	**8**
39	**0.4**	**0.4**	**99**	**70**	**12**

Table 2. sEH inhibition and microsomal stability of new urea-based sEHI (**33-39**). [1]IC_{50} values are the average of three replicates. The fluorescent assay as performed here has a standard error between 10 and 20% suggesting that differences of two-fold or greater are significant. Because of the limitations of the assay, it is difficult to distinguish among potencies < 0.5 nM. [2]Percentage of remaining compound after 60 min of incubation with human, mouse and rat microsomes obtained from Tebu-Xenotech in the presence of NADPH at 37 °C.

These two compounds, **38** and **39**, together with 15 more compounds synthesized in the context of the PhD of Sandra Codony and Beatrice Jora, were evaluated in further several *in vitro* assays by Neuraly, a pharmaceutical company focused in neurodegenerative diseases. The company is interested in the acquisition of the patent that protects these benzohomoadamantane sEHIs. Neuraly carried out a series of *in vitro* assays in astrocytes and microglia cell cultures with the aim of thoroughly evaluating each of the 17 compounds. Unfortunately, after a few months of pharmacological and pharmacokinetic evaluations, neither compound **38** nor **39** were selected for *in vivo* evaluations due to their poor performance in some of the *in vitro* evaluations. However, among the 17 compounds sent, one of them was selected by Neuraly for *in vivo* studies due to its good performance during these *in vitro* tests: compound **8** (Fig. 17). The compound was evaluated by the group of M. Pallàs (UB) in the *in vivo* murine model 5xFAD, a transgenic murine model of Alzheimer's disease that shows neuroinflammation, beta-amyloid accumulation and TAU protein hyperphosphorylation. The results of the efficacy of compound **8** in the 5xFAD murine model were positive, but cannot be disclosed due to our agreement with Neuraly. Of note, it was observed that the drug carried out an almost complete passage through the BBB, easily reaching the brain. Although this result was very positive since passing through the BBB is essential for the treatment of the Alzheimer disease, the oral bioavailability of the compound was low.

For this last reason, the group is still investigating with the aim of improving this bioavailability. To continue with this work, the group thought of synthesizing new molecules inspired in compound **8** and the first step was to increase the lipophilicity of the molecule.

Computational studies carried out on the sEH[13] by the group of Prof. Silvia Osuna and Dr. Ferran Feixas, from the Universitat de Girona, showed that there is still unoccupied space around the aromatic ring of the RHS, so there was room for different substitutions in this ring. This fact motivated the group to add different EWGs in the aryl ring in order to fill this space more efficiently, while increasing lipophilicity. As these EWGs should be of small size, the group decided to add a chlorine atom or a CF_3 group in the *meta* or the *ortho* positions giving rise to four new sEHI derivatives **53**-**56**.

As previously mentioned, we were not able to successfully hydrolyse the methyl ester groups present in these four derivatives. Thus, acid derivatives could not be synthesized for direct comparison with **61** and the products were s ent for evaluation as ester derivatives. It was thought that metabolization inside the body by esterases may furnish the corresponding carboxylic acids, maintaining the inhibitory potency. At the moment of writing this Thesis, these four compounds are still under evaluation by Neuraly.

Finally, we decided to synthesize the *cis* derivative of the compound **8**, as it was known that both *c-AUCB* and *t-AUCB* are potent inhibitors of sEH (Figure 12). As it was expected, compound **61** was an outstanding inhibitor of both the human and murine sEH, but, once again, the compound showed a very poor rodent microsomal stability.

Cmpd	Inhibitory potency[1] IC$_{50}$ (nM)		Microsomal stability[2] % remaining at 1 h		
	Human sEH	**Murine sEH**	**Human**	**Mouse**	**Rat**
61	< 0.4	0.4	83	2	19

Table 3. sEH inhibition and microsomal stability of new urea-based sEHI (**61**). [1]IC$_{50}$ values are the average of three replicates. The fluorescent assay as performed here has a standard error between 10 and 20% suggesting that differences of two-fold or greater are significant. Because of the limitations of the assay, it is difficult to distinguish among potencies < 0.5 nM. [2]Percentage of remaining compound after 60 min of incubation with human, mouse and rat microsomes obtained from Tebu-Xenotech in the presence of NADPH at 37 °C.

1.4.2. *N*-Arylpiperidinyl and *N*-sulfonylpiperidinyl as sEHIs

1.4.2.1. Design and synthesis of *N*-arylpiperidinyl and *N*-sulfonylpiperidinyl amides

For the design of this line of research, were the main pharmacophore of the molecule, urea, is replaced by an amide, multiple factors and previous results obtained by the group were taken into account.

First, it is known that the amide group, like the urea group, is endowed with a high inhibitory capacity for sEH, as deduced from a study by Hammock's group.[37] In fact, there are currently some drug candidates as sEHI that present the amide group in their structure as the main pharmacophore, examples of which are the already mentioned Astellas candidate AS2586114 (Fig. 21) or the GSK clinical candidate GSK2256294 (Fig. 24).

GSK2256294
human IC_{50} = 0.7 nM
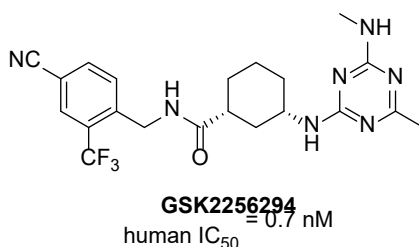
Fig. 24. Structure and inhibitory potency of GSK2256294.

Second, almost all the urea-based sEHI present quite serious solubility problems. This is relatively common for compounds bearing a urea functional group so, as the amide function significantly improves the physical properties compared to the corresponding ureas, its substitution by an amide group may improve the solubility while lowering the melting points on the same time.[69,70]

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[69] Sun, C. P.; Zhang, X. Y.; Morisseau, C.; Hwang, S. H.; Zhang, Z. J.; Hammock, B. D.; Ma, X. C. *J. Med. Chem*. **2021**, 64, 184-215.

[70] Kim, I. H.; Heirtzler, F. R.; Morisseau, C.; Nishi, K.; Tsai, H. J.; Hammock, B. D. *J. Med. Chem*. **2005**, 48, 3621-629.

For the synthesis of these new amide-based derivatives, the strategy followed was similar to the convergent strategy previously performed for the urea-type inhibitors.

On the one hand, the benzohomoadamantane scaffold with methyl, fluorine or chlorine group in the X position were synthesized following the same route previously described (Scheme 1). The nucleus with H and D in the X position were synthesized following Scheme 6. On the other hand, different derivatives of 2-(piperidin-4-yl)acetic acid were coupled to the benzohomoadamantane amine. In some cases, derivatization was performed after the coupling; in others, the RHS of the molecule was fully synthesized separately and then coupled to the benzohomoadamantane amine.

The substitution of the X position of the nucleus by H or D began with compound **18**, which was deprotected with thiourea. Next, compound **63** was treated with thionyl bromide to give the brominated derivative **64** in moderate yield. From this compound, two radical reactions were carried out separately with tributyltin hydride or tributyl deuteride to afford compounds **65** and **66**, respectively (Scheme 6).
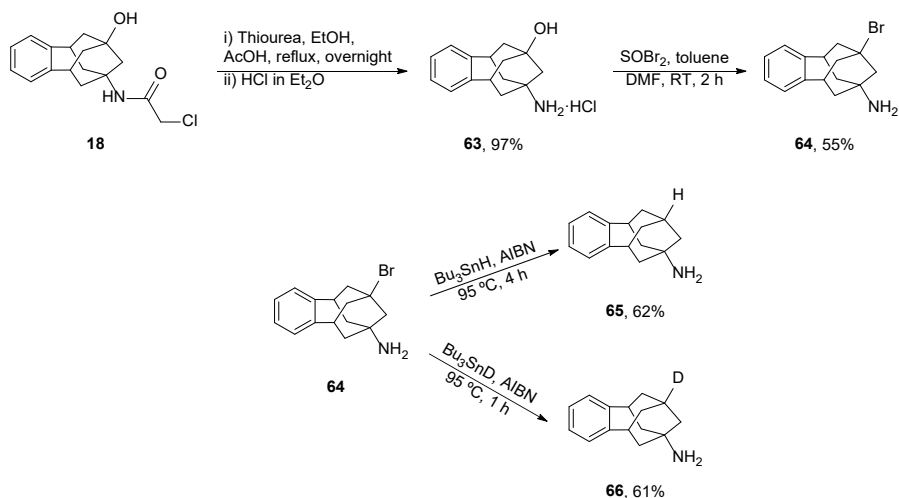
Scheme 6. Synthetic route for amines **65** and **66**.

For the synthesis of the first amide-based sEHIs, the different X substituted benzohomoadamantane amines were reacted with 2-(1-(*t*-butoxycarbonyl)piperidin-4-yl)acetic acid with HOBt or HATU as coupling agents followed by deprotection of boc-groups with HCl in dioxane to afford compounds **68-72**. After that, the four compounds were finally reacted with 2-propanesulfonyl chloride to obtain amides **73-77** in moderate to good overall yields (Scheme 7).
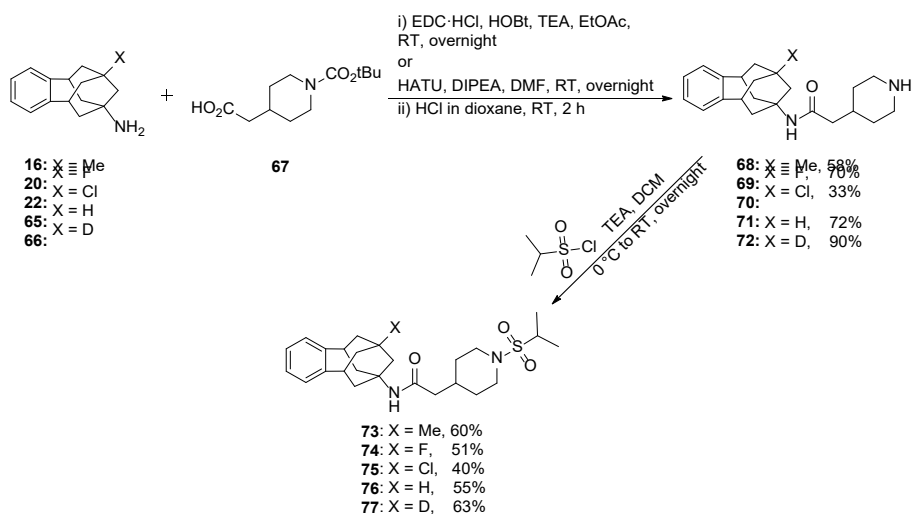
Scheme 7. Synthetic route to amides **73-77**. (Compounds **68** and **73** were synthesized by Sandra Codony during her thesis, but included here for completeness[62]).

Secondly, another pair of compounds were synthesized in this case bearing a benzyl group on the piperidine nitrogen. Both were made by direct coupling of scaffolds **16** and **20** with 2-(1-benzylpiperidin-4-yl)acetic acid **78** affording compounds **79** and **80** with moderate yield using HOBt or HATU as coupling agents, respectively (Scheme 8).
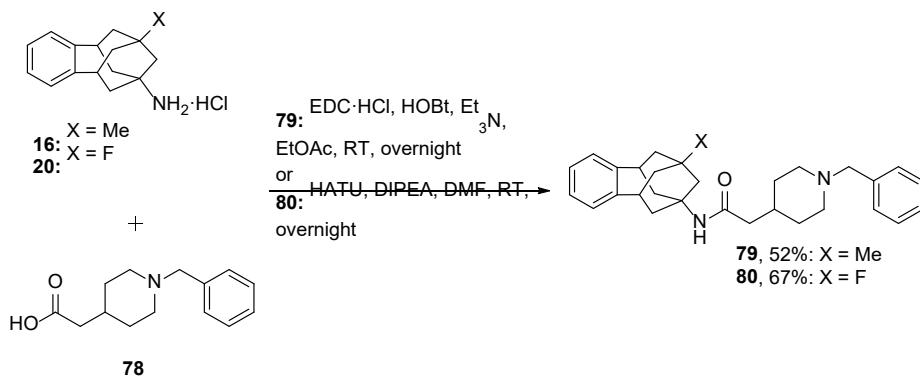
Scheme 8. Synthetic route to amides **79-80**. (Compound **79** was synthesized by Sandra Codony during her thesis, but included here for completeness[62]).

The third family of amides, including an *N*-arylpiperidine, was synthesized following the same path mentioned in the previous chapter. The different acids corresponding to the RHS were synthesized starting from 2-(1-(*t*-butoxycarbonyl)piperidin-4-yl)acetic acid, which was deprotected in acid medium to give 2-(piperidin-4-yl)acetic acid hydrochloride **82** (Scheme 9). Again, through S_NAr mechanisms, different substituents were added to the piperidine nitrogen, yielding the compounds **83**-**86** in moderate yields. The bromine of compound **83**, in turn, was replaced through a Suzuki reaction by a cyclopropyl group in low yield to afford **87**.

Then, by a HATU-assisted coupling, the previously synthesized RHS compounds were joined to give amides **88**-**92**. Compounds **88**-**90** were hydrolyzed subsequently in an acidic and basic medium to finally give rise to the final molecules, though in very low yields after both reactions. In four cases F was chosen as the substituent of the benzohomoadamantane nucleus, in one case it was Cl. The reason of this election will be explained in the next section.
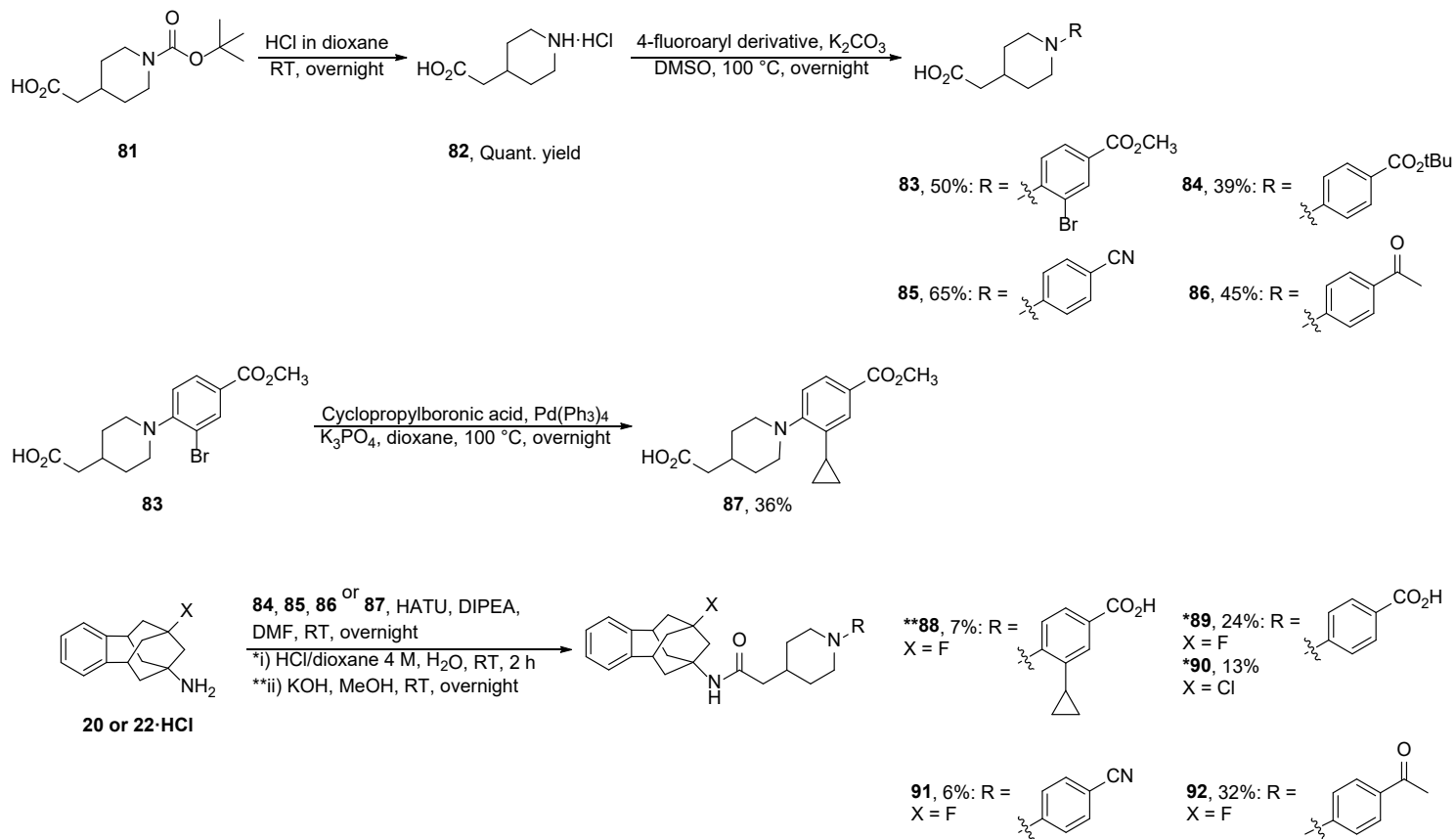
Scheme 9. Synthetic route of amides **88-92**. *Reaction made only to obtain compounds **89** and **90**. **Reaction made only to obtain compound **88**.

1.4.2.2.   sEH inhibition and pharmacokinetic profile of *N*-arylpiperidinyl and *N*-sulfonylpiperidinyl amides

All the compounds were tested in a large battery of *in vitro* assays by many different laboratories. These evaluations include: potency as human and murine sEHIs, tested by Dr. Christophe Morisseau, from the group of Bruce D. Hammock at the UCD; microsomal stability (human, mouse and rat species), hERG inhibition, CYP inhibition, solubility and permeability through Caco-2 cells, performed by the group of Prof. M. Isabel Loza and Prof. José M. Brea of the Drug Screening Platform/Biofarma Research Group of the University of Santiago de Compostela (Spain); PAMPA-BBB performed by Prof. Belén Pérez of the Autonomous University of Barcelona (Spain); and finally, cytotoxicity in SH-SY5Y cells, evaluated by Drs Coral Sanfeliu and Rubén Corpas of the Institute of Biomedical Research of Barcelona (CSIC).

It is important to remember here that inhibitory potency and microsomal stability are our two key parameters for choosing one compound over another and that these evaluations mark the line to follow in terms of chemical modifications made to the molecules.

Inspired by the good results obtained by compound **5** (Fig. 15), the first amides that were synthesized were benzohomoadamantane derivatives with different substituents in the X position but keeping the RHS identical to that of compound **5**. A complete evaluation of the X position was performed and compounds **73-77** arose from this first approach (Table 4).

Cmpd	Inhibitory potency[1] IC$_{50}$ (nM)		Microsomal stability[2] % remaining at 1 h		
	Human sEH	Murine sEH	Human	Mouse	Rat
73	30.2	0.4	0.0	0.0	ND
74	1.6	0.4	5.5	5.1	0.0
75	1.0	0.4	7.0	1.4	0.0
76	2.8	0.5	0.6	0.2	0.0
77	2.4	0.4	1.3	0.5	0.0
79	37.5	3.3	0.1	0.1	0.1
80	21.4	12.9	0.3	0.1	0.3
88	0.4	0.2	11	2	10
89	**0.4**	**0.7**	**60**	**39**	**51**
90	1.7	0.7	52	6	17
91	3.4	0.4	0.0	0.1	0.1
92	0.6	0.7	0.1	0.1	0.0

Table 4. sEH inhibition and microsomal stability of new amide-based sEHI (**70-89**). [1]IC$_{50}$ values are the average of three replicates. The fluorescent assay as performed here has a standard error between 10 and 20% suggesting that differences of two-fold or greater are significant. Because of the limitations of the assay, it is difficult to distinguish among potencies < 0.5 nM. [2]Percentage of remaining compound after 60 min of incubation with human, mouse and rat microsomes obtained from Tebu-Xenotech in the presence of NADPH at 37 °C.

As it can be seen, despite being very potent inhibitors, particularly at the murine enzyme, the microsomal stability of all of them was quite poor, so

no further evaluations were carried out on them. It should be noted that the compounds with a better microsomal stability were again those that present a halogenated substituent in the X position but, in all the products, the amount of remaining compound after 60 min incubation was below 10%. As compound **74** showed the highest human and murine microsomal stability, we selected the fluorinated derivative for the following studies keeping constant the LHS of the molecule.

With these results in hand, a new substitution on the nitrogen atom of the piperidine ring was performed. Compound **80** was synthesized and compared with another compound previously synthesized by Dr. Sandra Codony, **79**. In both cases, not only microsomal stability did not improve, but potency worsened, so the addition of the benzyl group to the piperidine was completely discarded (Table 4). Perhaps, the increased basicity of the nitrogen atom of the piperidine when moving from the sulfonamides to the benzylamines may account for the reduced potency.

After the unpromising results obtained for the first two batches of compounds, the group thought of reproducing the RHS of the Astellas compound molecule again and carrying out the amide derivatives of the urea compounds made in the first section of this chapter, furnishing molecules **88** and **89**. Gratifyingly, the biological results of these amides are quite similar to those of their ureas counterparts (Table 4). On the one hand, both amides showed very good potencies, on the other hand, the amide that does not have any extra substituents on the aromatic ring showed a better microsomal stability. In light of this result, the equivalent amide bearing a Cl atom in position X, **90**, was synthesized giving similar potency and human microsomal stability results (Table 4).

Finally, two new compounds were synthesized in order to compare the substitution of the acid group by more apolar groups in the aryl ring of the RHS. A nitrile group and a ketone were chosen to make these substitutions, giving compounds **91** and **92**. In both cases, despite the inhibitory potency was maintained, a pronounced worsening of the microsomal stability was observed, so both were discarded (Table 4).

Taking into account the results that have been analyzed throughout this chapter only compound **89** presented, in addition to a high inhibitory potency, an acceptable microsomal stability. This led the group to make further evaluations on this compound comparing with the reference compound TPPU[45] (Fig. 17). Firstly, a cytotoxic evaluation was performed in SH-SY5Y neuroblastoma cells by propidium iodide (PI) staining after 24 h of incubation. Neither **89** nor TPPU showed any cytotoxicity at the highest concentration tested (100 µM). Namely, the calculated percentages of cell death were similar to that of control treatment for both tested compounds, **89** and TPPU.

To enter the next analysis performed, it is important to explain that lipopolysaccharide (LPS) is a pro-inflammatory agent present in the bacterial wall that induces a phenotypic change in the macrophages and in the brain microglia, the cells considered as the main players of the innate immune system. These cells become reactive to fight the infection by increasing their phagocytic activity, releasing reactive oxygen species and inflammatory mediators, including nitric oxide, pro-inflammatory cytokines and eicosanoids. However, dysregulation in the immune response due to age or infections can lead to age-related ailments and progressive neurodegeneration.[71] Of note, the nitric oxide acts as a signaling molecule and its overproduction by the enzyme nitric acid synthase (iNOS) can produce deleterious inflammatory processes.[72] The evaluation of the anti-inflammatory activity of compound **89** was performed exploiting this capacity of the cell to increase the production of nitrogen oxide after a pro-inflammatory stimulus. For this purpose, microglial BV2 cells activated with pro-inflammatory LPS were used. As shown in figure 25, LPS treatment induced a significant increase in the levels of nitric oxide present in the medium. Gratifyingly, after co-incubation with compound **89** the pro-inflammatory effects of LPS were completely inhibited. Comparing the statistical means, we observed that both at 50 µM and at 100 µM the amount of nitric oxide present was reduced to levels indistinguishable from the control levels despite the presence of LPS. In contrast, the reference compound TPPU was

[71] Molina-Martínez, P.; Corpas, R.; García-Lara, E.; Cosín-Tomás, M.; Cristòfol, R.; Kaliman, P.; Solà, C.; Molinuevo, J. L.; Sánchez-Valle, R.; Antonell, A.; Lladó, A.; Sanfeliu, C. *Front. Aging Neurosci*. **2021**, 12, 622360.
[72] Yuste, J. E.; Tarragon, E.; Campuzano, C. M.; Ros-Bernal, F. *Front Cell Neurosci*. **2015**, 9, 322.

less effective, and only partially inhibited LPS-induced effects. TPPU at 100 µM only exerted a 50% reduction, which was significantly higher than control cells. Notably, statistical comparison of nitrite levels between both sEHIs showed significantly greater protection by compound **89** than by TPPU against inflammatory cell injury by 1 µg/ml of LPS.
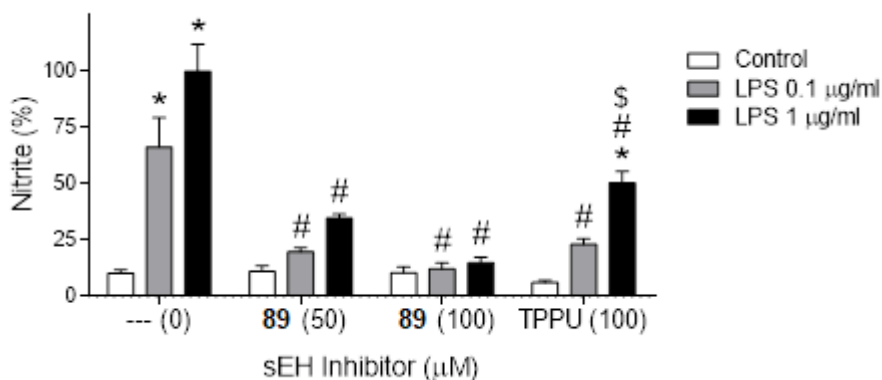
Figure 25. Anti-inflammatory effects of **89** in activated BV2 microglial cells. Nitrite levels in the culture media, that indicate nitric oxide generation induced by LPS, were decreased to cell resting levels by co-incubation with **89**. However, TPPU was less effective and lead to a partial decrease. Values are mean ±SEM of n = 10-15. Statistics: *P < 0.001 compared to the corresponding Control group without LPS; #P < 0.001 compared to the corresponding LPS group without anti-inflammatory agents; $P < 0.001 compared to the corresponding LPS concentration treated with **89**.

1.5. Conclusions

- A total of 12 urea-based inhibitors were synthesized, of which 8 were fully characterized and evaluated *in vitro*.

- All of them exhibited great potency but only **38** and **39** were endowed with high microsomal stability as well.

- These two compounds were tested *in vitro* by several collaborators at the USC, UAB, CSIC and also by the pharmaceutical company Neuraly, where many pharmacokinetic parameters were evaluated. Although **38** and **39** showed positive outcomes in most of these assays, other candidates had better results, so both compounds were discarded for *in vivo* evaluation.

- A total of 12 amide-based inhibitors were synthesized, fully characterized, and evaluated *in vitro*.

- All of them exhibited great potency but only two were endowed with moderate microsomal stability as well.

- Only compound **89** was tested *in vitro* for its anti-inflammatory potency exerting a completely diminution of iNOS concentration in microglial BV2 cells activated with pro-inflammatory LPS.

- Compound **89** showed significantly greater protection than the well-known sEHI TPPU against inflammatory cell injury.

2. Design and synthesis of new anti-influenza virus molecules

2.1. Introduction

2.1.1. Virus

A virus is a microscopic acellular infectious agent that can only replicate within the cells of other organisms. Viruses are made up of genes that contain nucleic acids that form long DNA or RNA molecules, surrounded by proteins. Upon infecting a cell, these genes "force" the host cell to synthesize viral nucleic acids and proteins in order to form new viruses that infect other cells to keep reproducing their life cycle.[73]

Viruses infect all kinds of organisms: animals, fungi, plants, protists, bacteria and archaea. They can be found in almost every ecosystem on Earth; indeed, they are the most abundant biological entity.[74] They are also the smallest biological entity, most about a hundred times smaller than bacteria, on the order of about 10 nanometers. They are made up of different three main parts: their genetic material, which carries hereditary information and can be DNA or RNA; a protein coat that protects these genes (capsid or head) and, in some, a lipid bilayer that surrounds them when they are outside the cell (viral envelope) (Fig. 26).[75] Some of them have other structural parts or proteins like tails, fivers, spikes, etc. Viruses vary in their shape from nearly perfect polyhedral, such as HIV virus, helical shape, such as tobacco mosaic virus, or more complex structures such as bacteriophages.[76]

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[73] Blanco, A. Química Biológica. *El Ateneo* **2012**, p. 128.
[74] Lawrence, C. M.; Menon, S.; Eilers, B. J. *J. Biol. Chem.* **2009**, *284*, 12599-603.
[75] https://www.sciencefacts.net/virus-structure.html. Accessed on 30 May 2022.
[76] Villarreal, L. P. Viruses and the Evolution of Life. *ASM Press* **2005**.
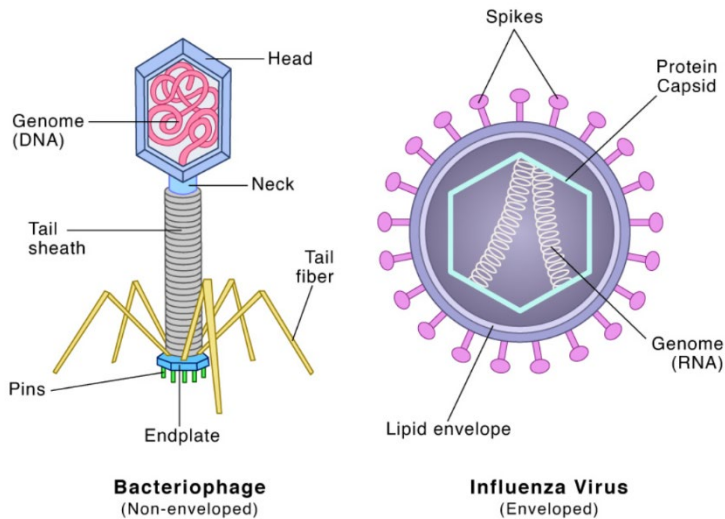
Fig. 26. General structure of different viruses. Figure taken from reference 75.

Viruses are spread in many different ways. Many of them need transmission vectors which are living organisms that carry them from one organism to another. There are others that do not require vectors: the influenza virus (O*rthomyxovirus*) and the common cold (*Rhinovirus* and *Orthocoronavirinae*) spread through the air via sneezing and coughing; *Noroviruses* are transmitted by the fecal-oral route, etc. Once the virus has infected the host, it develops its replicative cycle, the multiplication process of viruses. For this, they need to be introduced into cells where they will control their replicative mechanisms.[77] This cycle generally consists of the following phases:

Attachment: A specific binding between viral capsid proteins and specific receptors on the host cell surface.

Entry: After receptor adhesion, changes are inducted in the viral envelope protein resulting in fusion of the viral and cellular membranes.

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[77] Yin, J.; Redovich, J. *Microbiol. Mol. Biol. Rev.* **2018**, *82*, e00066-17.

Uncoating: The process in which the viral capsid is degraded by viral or host enzymes, thus releasing the nucleic acid and proteins of the viral genome into the host cell.

Transcription and translation: The process of copying a segment of DNA into messenger RNA (mRNA). For some RNA viruses, the viral RNA acts as mRNA which can be directly translated into protein products. For viruses with negative stranded RNA or DNA, transcription must occur before translation. The mRNA is used to instruct the host cell to make virus components taking advantage of the existing cell structures to replicate itself.

Virion assembly: Newly synthesized genome and proteins are assembled to form new virus particles. This may take place in the cytoplasm, nucleus or at the plasma membrane of the host cell.

Release: Mature viruses are released by either sudden rupture of the cell or gradual extrusion of enveloped viruses through the cell membrane.

2.1.2. Influenza A virus

Influenza A virus is a negative-sense, single-stranded and segmented RNA viruses. They are the only species of the genus *Alphainfluenzavirus* of the virus family *Orthomyxoviridae*, which causes influenza in birds and mammals. There is another virus from the same family that causes influenza, the influenza B virus. The influenza B virus only affects humans and seals, so it is much less dangerous due to its lower capacity for propagation and mutation. This work will be focused only on the influenza A virus.

Occasionally, wild birds infect domestic poultry, and this can cause an outbreak and give rise to human influenza pandemics.[78] This is the reason why the influenza virus has a fairly high dangerous potential and one of the main causes of pandemics worldwide. Therefore, developing new treatments as vaccines or anti-viral molecules is of great importance for the human healthcare worldwide.

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[78] Klenk, H.; Matrosovich, M.; Stech, J. Animal Viruses: Molecular Biology. *Caister Academic Press* **2008**.

Regarding its structure (Fig. 27)[79], influenza A virus presents the typical abovementioned viral structure. On the outside, the virus has three proteins with different functions; on the one hand, there is the hemagglutinin (HA). The globular head of this glycoprotein is responsible for cleaving to the sialic acid residues present in the receptors of the respiratory cells of the host organism.[80] After endocytosis, the HA stalk domain undergoes low pH-induced refolding, leading to the fusion of the viral envelope with the endosomal membrane thus introducing the virus into the cell *via* endocytosis (Fig. 29).[81] Hemagglutinin varies greatly between each virus subtypes and is one of the main reasons why obtaining a universal vaccine against the influenza virus is extremely difficult.

The second outer protein is the neuraminidase (NA). NA is a projected "mushroom" shaped tetramer whose main function is to break the molecular bond between HA and sialic acid. [82] This break has a triple function. On the one hand, when the new virions are formed in the cell, they try to go outside but remain attached to the cell membrane since HA remains bound to sialic acid. Because NA breaks the molecular bond, these virions can detach from the cell to continue infecting the host. In addition, the released virions are coated with sialic acid. The NA also helps pull this acid off the surface of the virion, preventing them from aggregating with each other. The third function is related to the entry of the virus into the respiratory system. The mucus of the respiratory system is rich in sialic acid, which makes the HA molecules (and therefore the virus) stick to it. The action of the NA breaks the union and releases the virus that can penetrate the respiratory tract.

[79] Jung, H. E.; Lee, H. K. *Viruses* **2020**, 12, 504.

[80] Russell, R. J.; Kerry, P. S.; Stevens, D. J.; Steinhauer, D. A.; Skehel, J. J. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 17736-41.

[81] Hamilton, B. S.; Whittaker, G. R.; Daniel, S. *Viruses* **2012**, *4*, 1144–1168.

[82] Palese, P.; Tobita, K.; Ueda, M. *Virology* **1974**, *61*, 397–410.
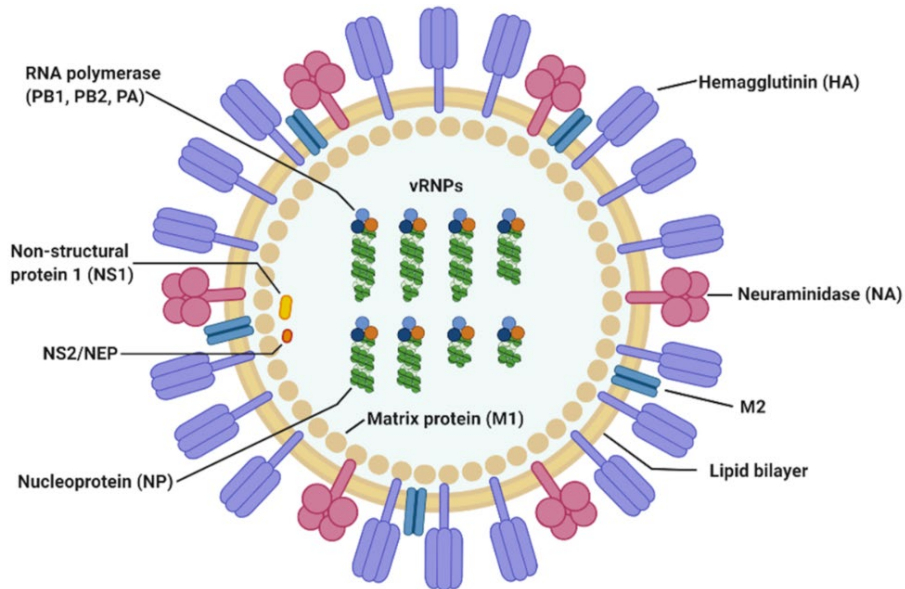
Fig. 27. Influenza A virus structure. Figure taken from reference 79.

Interestingly, to illustrate the importance of these two proteins, the different influenza A virus subtypes are labeled according to an H number from H1 to H18 (for the type of HA) and an N number from N1 to N11 (for the type of NA) (Fig. 28).[83] In turn, hemagglutinin is subdivided into two groups according to its structure: Group 1 for H1, H2, H5, H6, H8, H9, H11, H12, H13, H16, H17 and H18 and group 2 for H3, H4, H7, H10, H14, H15. This is especially relevant for antiviral design.

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[83] https://en.wikipedia.org/wiki/Influenza_A_virus. Accessed on 30 May 2022.
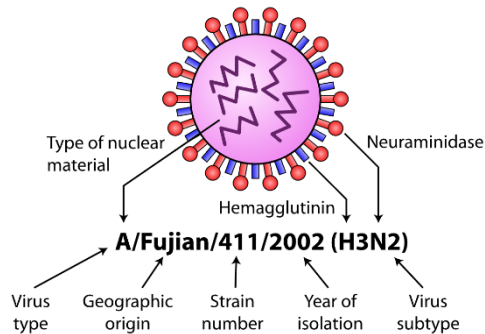
Fig. 28. Influenza A virus structure. Taken from reference 83.

The third protein presented in the virus cortex is the M2 proton channel. The M2 channel is a homotetramer formed of four identical helix-shaped units. This protein is an essential component of the viral envelope because of its ability to form a highly selective, pH-regulated, proton-conducting channel.[84] During virus entry, the M2 channel maintains pH across the viral envelope. After virus endocytosis, endosomal acidification takes place and M2 channel is opened. This activation of M2 channel brings protons into the virion core resulting in an acidification of the virus interior. This acidification weakens the electrostatic interaction between the matrix protein M1 and viral ribonucleoprotein (vRNP) complexes, releasing the uncoated vRNPs into the cytoplasm which is then imported to the nucleus to start viral replication (Fig. 29).[85]

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[84] Pielak, R. M.; Chou, J. J. *Biomembranes* **2011**, *1808*, 522–529.
[85] te Velthuis, A. J. W.; Fodor, E. *Nat. Rev. Microbiol.* **2016**, *14*, 479–493.
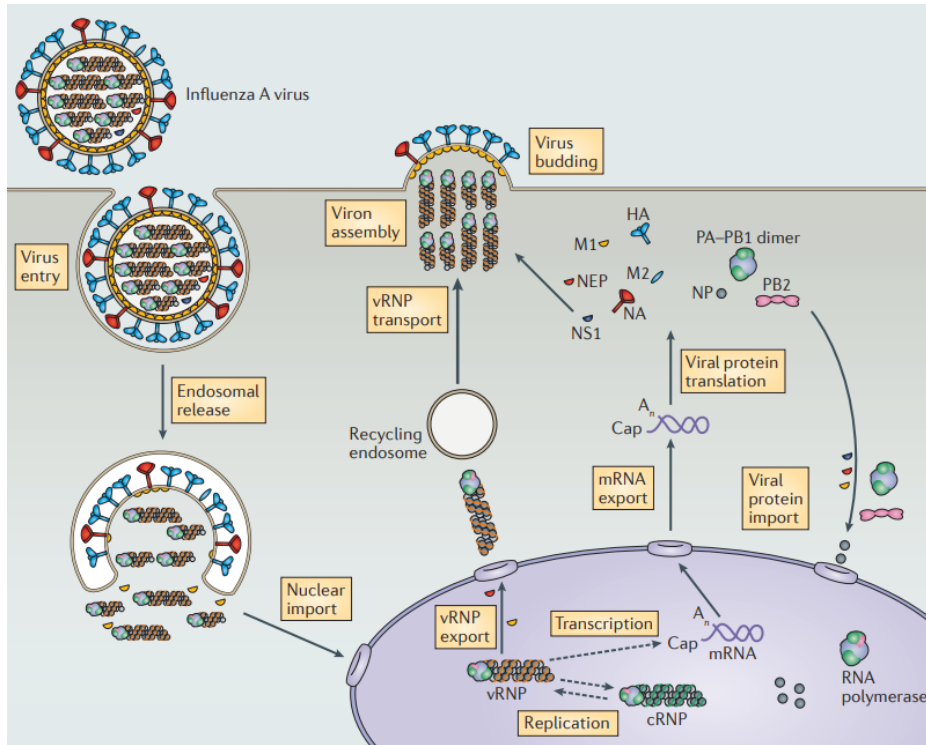
Fig. 29. Scheme of viral life cycle. Figure taken from reference 85.

It is also important to explain that influenza A virus has two non-structural proteins; NS1 and NS2, which have many different roles. NS1 seems to have a key role in suppression of host innate immunity, shutoff of host gene expression, apoptosis and viral replication. On the other hand, NS2 protein is involved in vRNP nuclear export and viral RNA transcription and replication.[86]

Regarding the virus genetic information, the influenza A virus genome consist of eight negative-sense, single-stranded viral RNA (vRNA) segments. These segments are numbered in order of decreasing length. Segments 1, 3, 4, and 5 encode only one protein per segment: the PB2, PA, HA and NP proteins. On segment 2, influenza A virus encodes the polymerase subunit PB1; in some subtypes of influenza A virus, this segment codes for the accessory protein PB1-F2 as well. Conversely, the NA protein is encoded in

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[86] Hao, W.; Wang, L.; Li, S. *Pathogens* **2020**, *9*, 812.

segment 6 while segment 7 codes for the M1 matrix protein and, by RNA splicing, this segment also expresses the M2 ion channel. Finally, segment 8 in influenza A virus is a single RNA segment from which they express the interferon-antagonist NS1 protein. Again, by mRNA splicing, the NEP/NS2 protein is also encoded in segment 8, a protein involved in vRNP export from the host cell nucleus.[87]

2.1.3. Therapeutic options against influenza A virus

Although the influenza A virus is present throughout the year in all latitudes of the world, it is usually manifested with greater virulence in the autumn and winter of each hemisphere, so flu can be considered as a seasonal illness. This seasonality is due to several causes: the high sensitivity of the virus to ultraviolet exposure from the sunlight, people spending more time in closed spaces, which facilitates its spread, school vacations that reduce contagion among children, who are one of the main vectors of the virus, etc.

Throughout history there have been several global pandemics caused by the influenza A virus, such as the pandemic of 1889-1890 with more than 1 million deaths,[88] the famous and misnamed Spanish flu of 1918-1919 with 20 to 40 million of deaths,[89] or the Asiatic pandemic of 1957-1958 with around 1 million deaths.[90]

With a high variability depending on the year, in Spain there are from a hundred deaths (2013) to more than 1,800 deaths (2018) according to the Statistic National Institute of Spain (INE).

The therapeutic treatments used to fight against the flu virus are: vaccines, as a preventive treatment, and antiviral drugs.

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[87] Bouvier, N. M.; Palese, P. *Vaccine* **2008**, 26 Suppl 4: D49-D53.
[88] Shally-Jensen, M. *Encyclopedia of Contemporary American Social Issues* **2010**.
[89] Pandemic Influenza Risk Management: WHO Interim Guidance. *World Health Organization* **2013**. p. 19.
[90] Echeverri, B. *Centro de Investigaciones Sociológicas* **1993**.

2.1.3.1. Vaccines

As previously mentioned, the virus has a high mutagenic capacity, so an effective vaccine against the virus must be developed every year before the seasonal flu. While their effectiveness varies for each seasonal flu, most provide modest to high protection against influenza. Sometimes, vaccines become completely ineffective before the end of the seasonal flu and many versions of them are developed twice a year, as the influenza virus rapidly changes.[91]

Most influenza vaccines are produced using egg proteins, although recombinant vaccines and attenuated virus vaccines are also widely used worldwide. In total, from 4 to 5 million vaccines are shot every year in Spain (INE).

2.1.3.2. Antiviral drugs

Antiviral molecules are drugs that are responsible for preventing the disease or fighting the infection once it has occurred. These molecules act in some of the phases of the life cycle of the virus, preventing it from continuing to multiply inside the body.

There is a good number of antiviral drugs on the market and they are classified according to their mechanism of action in:

Hemagglutinin inhibitors (HAIs): Umifenovir (Arbidol®) is the only example in the market (Russia and China only) which inhibit the activity of HA blocking the conformational change of it at a low pH and preventing virus endocytosis (Fig. 30).[92]

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[91] Vaccines against influenza: WHO position paper. *Weekly Epidemiological Record.* **2012**, *87*, 461–476.

[92] Leneva, I. A.; Russell, R. J.; Boriskin, Y. S.; Hay, A. J. *Antivir. Res.* **2009**, *81*, 132–140.
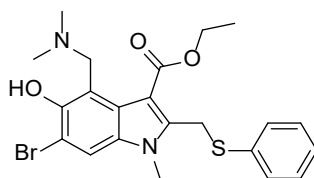
Fig. 30. Umifenovir structure.

Neuraminidase inhibitors (NAIs): These molecules inhibit the action of the viral neuraminidase enzyme, preventing the virus from leaving the cell once it is formed. These drugs act against both influenza A and B viruses. Among them we can find oseltamivir (Tamiflu®), which is taken orally, zanamivir (Relenza®), taken by inhalation, and peramivir (Rapivab®), administered intravenously (Fig. 31). Antiviral resistance and reduced susceptibility to NAIs among currently circulating influenza viruses is low, but this can change as the virus mutability is extremely high.

Oseltamivir   Zanamivir   Peramivir
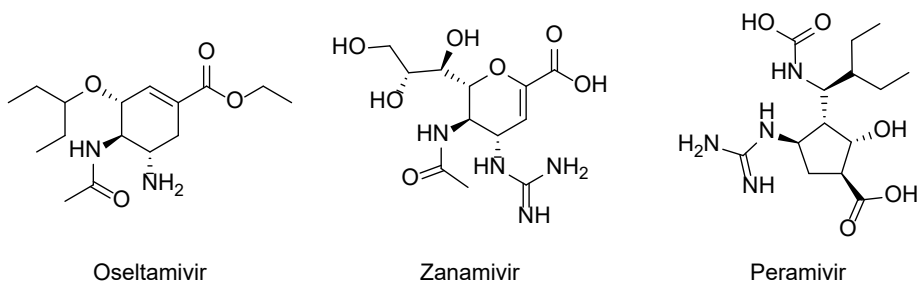
Fig. 31. Neuraminidase inhibitors' structures.

Endonuclease inhibitors: These molecules inhibit capsule-dependent endonuclease that interferes with viral RNA transcription, preventing virus reproduction. The main molecules of this family are baloxavir acid and its clinically approved prodrug, baloxavir marboxil (Xofluza®) (Fig. 32). For this class of drugs, that are active against influenza A and B viruses, viral resistance is low too.
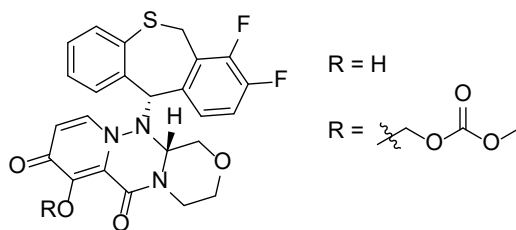
Fig. 32. Structures of baloxavir acid (R = H) and prodrug baloxavir marboxil (R = $CH_2OCO_2CH_3$).

M2 channel blockers: These inhibitors target the M2 ion channel protein of influenza A viruses and are known as adamantanes because of their chemical structure. Amantadine and rimantadine are antiviral drugs of this type (Fig. 33). Both acts only against the influenza A virus and, in addition, several subtypes such as A(H3N2) and A(H1N1) usually present a very high resistance against them due to mutations in this channel. Therefore, neither are recommended for antiviral treatment nor for chemoprophylaxis of the currently circulating influenza virus strains.

Amantadine   Rimantadine
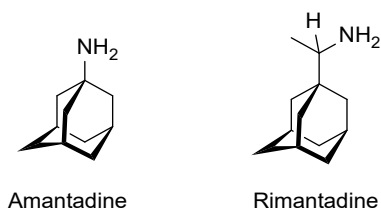
Fig. 33. M2 channel blockers' structures.

As already mentioned, the influenza viruses are endowed with high mutability due to the error-prone replication in their life cycle. The virus evolves as a quasispecies and widespread use of antiviral drugs can lead to resistant strains. Therefore, the development of new antiviral molecules is essential for the long-term to continue protecting ourselves from the flu.

2.1.4. Previous work on anti-influenza virus molecules in our research group

The Dr. Santiago Vázquez's group research on anti-influenza molecules has been focused in small molecules that act as HA inhibitors. During this research, inspired in the previously known HA inhibitors CL-385319[93] and RO5464466/RO5487624[94] (Fig. 34), two series of easily accessible small anilines were synthesized and evaluated as antiviral compounds.

CL-385319

R = H, RO5464466
R = Cl, RO5487624
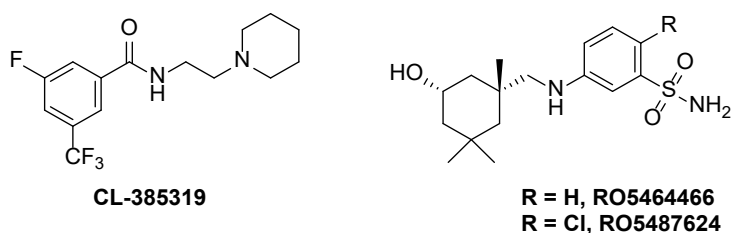
Fig. 34. CL-385319 and RO5464466/RO5487624 structures.

More than 20 anilines were evaluated. Many of them displayed activity against A/H1N1 influenza with EC_{50} values in the low micromolar range and very low cytotoxicity. Among them, aniline **93** (Fig. 35) was selected for further studies: influenza fusion, virus resistance, and NMR experiments demonstrated that **93** interferes with HA-mediated fusion by binding to the HA stalk and preventing its refolding triggered by the pH drop.[95]

[93] Plotch, S. J.; O'Hara, B.; Morin, J.; Palant, O.; LaRocque, J.; Bloom, J. D.; Lang, S. A.; DiGrandi, M. J.; Bradley, M.; Nilakantan, R.; Gluzman, Y. *J. Virology* **1999**, *73*, 140-151.

[94] Zhu, L.; Li, Y.; Li, S.; Li, H.; Qiu, Z. *PLOS ONE* **2011**, 6(12): e29120.

[95] Leiva, R.; Barniol-Xicota, M.; Codony, S.; Ginex, T.; Vanderlinden, E.; Montes, M.; Caffrey, M.; Luque, F. J.; Naesens, L.; Vázquez, S. *J. Med. Chem.* **2018**, *61*, 98-118.

CC$_{50}$ > 100 µM

H1N1/A/PR/8/34
Antiviral EC$_{50}$ (µM)
Visual CPE score = 4.6
MTS = 5.5

H1N1/A/Virginia/ATCC3/2009
Antiviral EC$_{50}$ (µM)
Visual CPE score = 1.7
MTS = 1.5
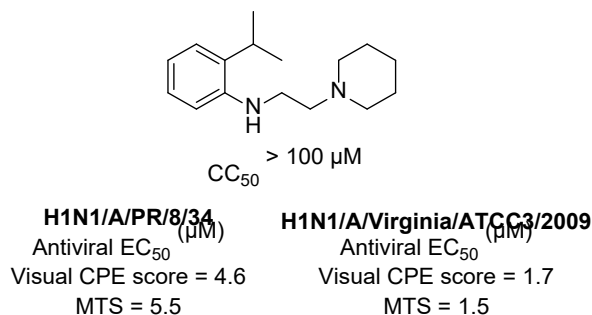
Fig. 35. Candidate **93** structure, antiviral activity against H1N1/A/PR/8/34 and H1N1/A/Virginia/ATCC3/2009 strains and cytotoxicity. MDCK cells: Madin-Darby canine kidney cells. CC$_{50}$: 50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. EC$_{50}$: 50% effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.

Molecular dynamics (MD) simulations suggest that candidate **93** is able to fill the same pocket than ligand *tert*-butylhydroquinone (TBHQ)[96] (Fig. 36), a well-known HA inhibitor, in the HAs of A/PR/8/34 and A/Virginia/ATCC3/ 2009. The "TBHQ pocket" seems to represent a common and particularly relevant site for small-molecule HA fusion inhibitors. Noteworthy, group-1 and group-2 HA subtypes present quite different "TBHQ pockets" in terms of polarity, so developing a small-molecule that could fit both is still a major challenge as **93** only target group 1 HA subtype.
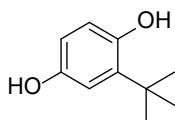
Fig. 36. Structure of *tert*-butylhydroquinone.

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[96] Bodian, D.; Yamasaki, R.; Buswell, R.; Stearns, J.; White, J.; Kuntz, I. *Biochemistry* **1993**, 32, 2967–2978.

In another vein, the previously mentioned HA inhibitor CL-385319 is able to inhibit the viral entry of some HA group 1 influenza subtypes (H1N1 and H5N1).[97] In 2012, Chinese researchers synthesized a few analogues (compounds **94**, **95** and **96**) endowed with potent antiviral activity.[98] Also, another Chinese group disclosed a series of sulfonamides with potent anti-influenza activity (compound **97**) (Scheme 10).[99]
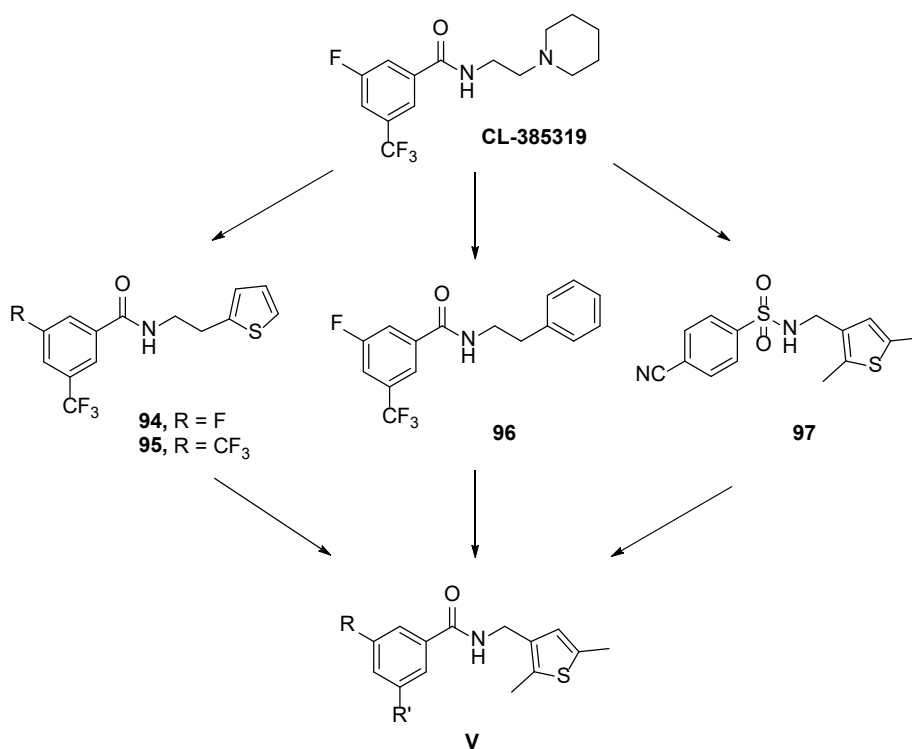
Scheme 10. Structure of different HA's inhibitors and the merging in a new general structure **V**.

[97] Liu, S.; Li, R.; Zhang, R.; Chan, C. C. S.; Xi, B.; Zhu, Z.; Yang, J.; Poon, V. K. M.; Zhou, J.; Chen, M.; Münch, J.; Kirchhoff, F.; Pleschka, S.; Haarmann, T.; Dietrich, U.; Pan, C.; Du, L.; Jiang, S.; Zheng, B. *Eur. J. Pharmacol.* **2011**, *660*, 460-467.
[98] Zhu, Z.; Li, R.; Xiao, G.; Chen, Z.; Yang, J.; Zhu, Q.; Liu, S. *Eur. J. Med. Chem.* **2012**, *57*, 211-216.
[99] Yu, Y.; Tazeem, Xu, T.; Du, L.; Jin, M.; Dong, C.; Zhou, H.-B.; Wu, S. *Med. Chem. Commun.* **2019**, *10*, 89-100.

Inspired in the structure of these molecules, a merging strategy was carried out resulting in the novel structure **V** (Scheme 10). The Vazquez' research group synthesized a new series of 13 benzamides following the general structure **V**, including mono- and di-substituted benzamides (Fig. 37), exploring the SAR of aromatic ring substitutions.
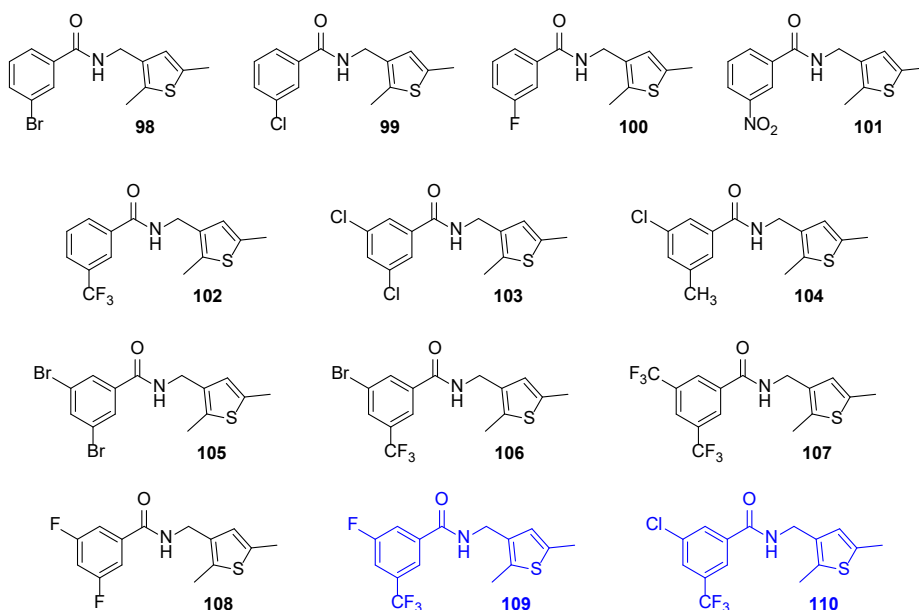
Fig. 37. Different benzamides mono- and di-substituted.

All the new compounds were tested in Leuven (Belgium), by the group of Prof. Lieve Naesens, for cytotoxicity and for antiviral activity. Regarding cytotoxicity, none of them displayed cytotoxicity. Regarding anti-influenza activity, the compounds were tested against three influenza subtypes: influenza H1N1/A/Ned/378/05, influenza H3N2/A/HK/7/87 and influenza B/Ned/537/05. As expected, none of the compounds was activity against influenza B. Also, the compounds were inactive against the H3N2 subtype. However, two of them, **109** and **110**, were very potent against the H1N1/A/Ned/378/05 subtype (Fig. 38).
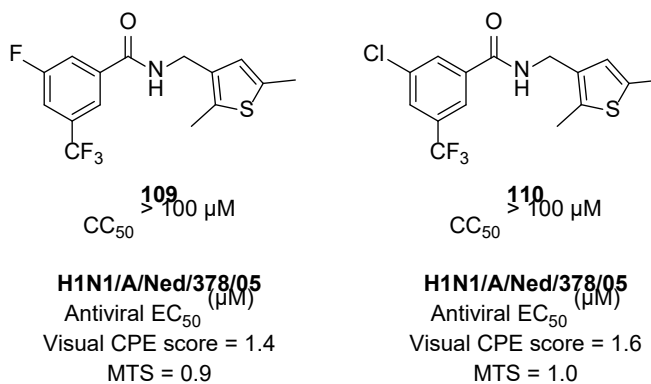
Fig. 38. Compounds **109** and **110** structure, antiviral activity in Influenza H1N1/A/Ned/378/05 strain and cytotoxicity. MDCK cells: Madin-Darby canine kidney cells. CC_{50}: 50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. EC_{50}: 50% effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.

2.2. Objectives

Currently, as it was mentioned in antiviral drugs section, influenza therapy relies mainly on three classes of antiviral drugs: NAIs, endonuclease inhibitors and M2 proton channel blockers.[100,101] The M2 inhibitors currently approved suffer from global virus resistance, making current treatments useless in few years and forcing scientific community to develop new chemical scaffolds. Resistance is also a growing issue for endonuclease inhibitors and NAIs.[102]

For all this, there is an urgent need for new drugs having higher barriers of resistance, superior efficacy and, importantly, targeting other proteins relevant in the viral life cycle.[103,104]

Taking these requirements into account for the development of new antiviral molecules, and after the promising results of HAIs **109** and **110** for influenza A virus H1N1 subtype, the present thesis continues with the group's research in this field carrying out a more complete SAR study in which not only aromatic substitutions were performed, but also other modifications in the molecule following general structure **VI** (Fig. 39). As previously, cytotoxicity and activity evaluations of all the new molecules were carried out by the group of Prof. L. Naesens, at the KU Leuven (Belgium).
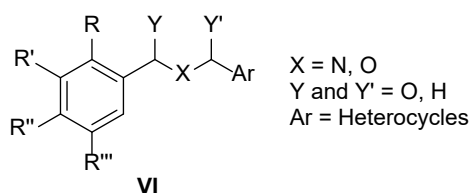
Fig. 39. General structure **VI** of the new HAIs.

[100] Laborda, P.; Wang, S. Y.; Voglmeir, J. *Molecules* **2016**, *21*, 1513.
[101] Wang, J.; Li, F.; Ma, C. *Biopolymers* **2015**, *104*, 291–309.
[102] Samson, M.; Pizzorno, A.; Abed, Y.; Boivin, G. *Antiviral Res*. **2013**, *98*, 174–185.
[103] Das, K.; Aramini, J.; Ma, L.; Krug, R.; Arnold, E. *Nat. Struct. Mol. Biol*. **2010**, *17*, 530–538.
[104] Hayden, F. G. N. *Viruses* **2013**, *7*(S1), 63–75.

2.3. Results and discussion

2.3.1. Synthesis of new anti-influenza virus molecules

Continuing with the general structure **V** proposed during the development of the previous HAIs, the Vázquez' group decided to carry out modifications along its entire structure to develop a more advanced SAR study. For this purpose, the general structure **VI** was proposed. This novel approximation presents modifications not only in the substituents of the benzoic acid ring, but also substitutions of different aromatic rings in the RHS and modifications in the bridge that joins both parts of the molecule (Fig. 37).

For the first derivatives, both the bridge and the thiophene ring were maintained, while substitutions in the benzoic acid ring of the LHS of the molecule were performed. The required (2,5-dimethylthiophen-3-yl)methanamine hydrochloride **112** was obtained starting from 2,5-dimethylthiophene-3-carboxylic acid **111** (Scheme 11). Transformation of the carboxylic acid group to an acyl chloride with thionyl chloride followed by treatment with aqueous ammonium hydroxide yielded 2,5-dimethylthiophene-3-carboxamide. This amide was then reduced with LiAlH$_4$ in anhydrous THF to the amine **112**.

i) Thionyl chloride, toluene, reflux, 2 h
ii) Ammonium hydroxyde 25%, DCM, RT, 1 h
iii) LiAlH$_4$, THF, 0 °C to reflux, 4 h
iv) HCl in Et$_2$O

111

112·HCl, 55%
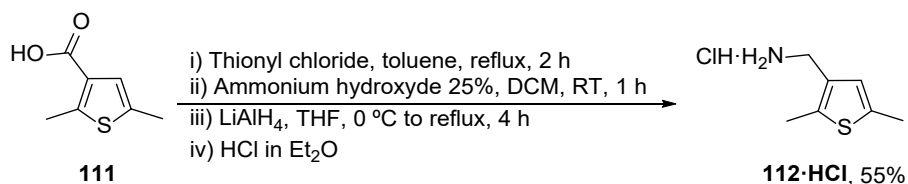
Scheme 11. Synthesis of (2,5-dimethylthiophen-3-yl)methanamine hydrochloride (not commercially available).

The next step in the synthesis of these derivatives was carried out coupling each benzoic acid derivative **113-117**, through the formation of the corresponding acyl chloride with thionyl chloride, with the previously formed

(2,5-dimethylthiophen-3-yl)methanamine hydrochloride **112**, affording compounds **118-122** in moderate yields (Scheme 12).
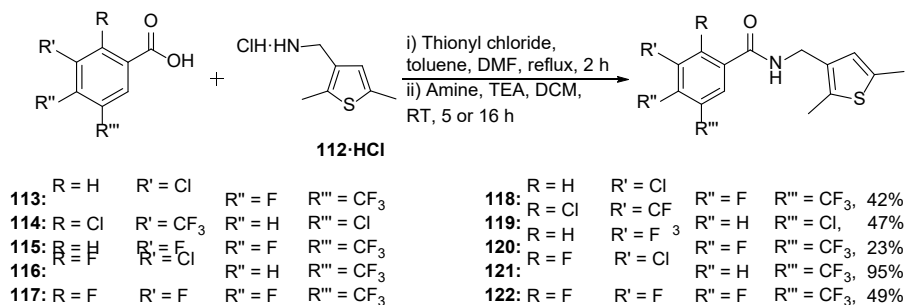
Scheme 12. Synthesis of the firsts HAIs **118-122**.

The second batch of molecules were made maintaining the substituents of the two compounds that had obtained the best results in the previous works of the group, compounds **109** and **110**. Different substitutions were carried out in the thiophene ring of the molecule (Scheme 14).

For this approach, we followed the same strategy than for the first batch of molecules, the first step was the synthesis of (2-methylthiophen-3-yl)methanamine hydrochloride and (5-methylthiophen-3-yl)methanamine hydrochloride (Scheme 13). First, the conversion of the corresponding carboxylic acids to the corresponding acyl chlorides with thionyl chloride was made. Then, these acyl chlorides were treated with aqueous ammonium hydroxide and the formed amides were reduced with $LiAlH_4$ in anhydrous THF to afford amines **125** and **126** in moderate and low overall yields, respectively.
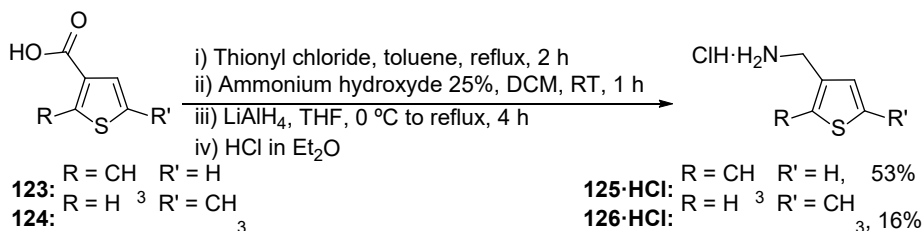
Scheme 13. Synthesis of (2-dimethylthiophen-3-yl)methanamine and (5-dimethylthiophen-3-yl)methanamine hydrochlorides (not commercially available).

The next reaction was again a coupling between the different heteroaromatic amines of the RHS of the molecule with the benzoic acids 3-fluoro-5-(trifluoromethyl)benzoic acid, **127**, and 3-chloro-5-(trifluoromethyl)benzoic acid, **136**, through the formation of the corresponding acyl chloride with thionyl chloride. Interestingly, coupling with 3-chloro-5-(trifluoromethyl)benzoyl chloride afforded moderate yields while coupling with 3-fluoro-5-(trifluoromethyl)benzoyl chloride afforded lower yields (Scheme 14).
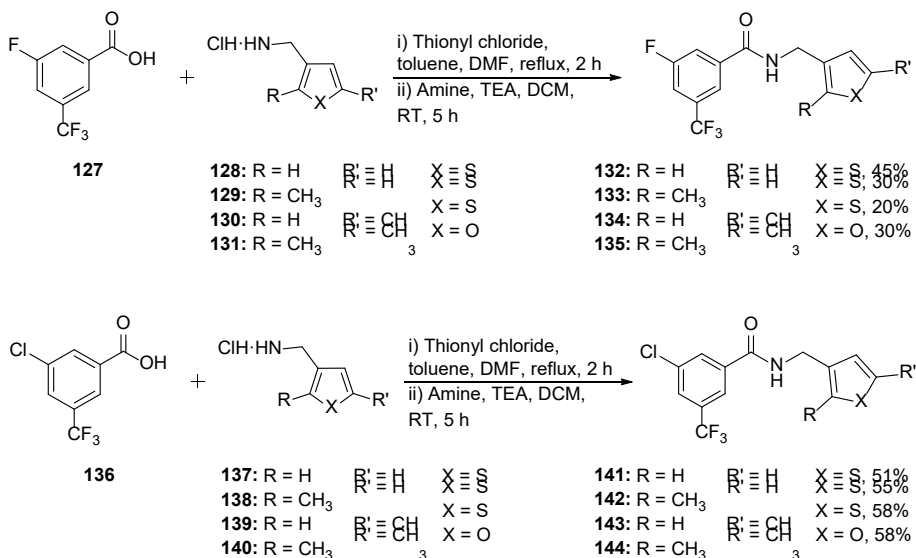
Scheme 14. Synthesis of HAIs **132-135** and **141-144** with variations in the thiophene/furane ring.

The third batch of molecules were synthesized in identical way as the previous batches. Benzoic acids **145**-**149** were reacted *via* acyl chloride formation with thiophene **112** to afford compounds **150**-**154**.
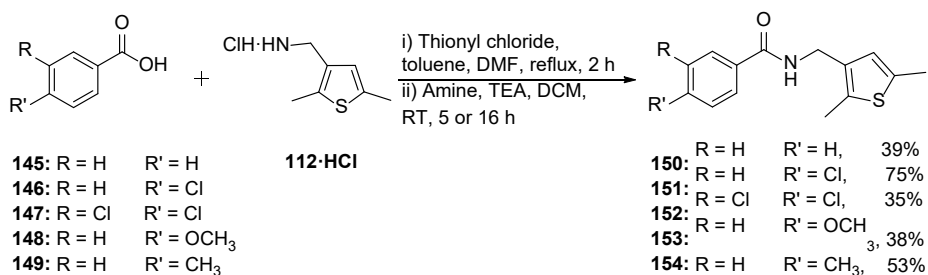
Scheme 15. Synthesis of the firsts HAIs **150-154**.

Keeping constant the LHS of the molecule as a 3-fluoro-5-(trifluoromethyl)aryl, and aiming to continue exploring the RHS of the molecule, different amine-aromatic heterocycles were coupled affording compounds **162-168** from good to moderate yields (Scheme 16).
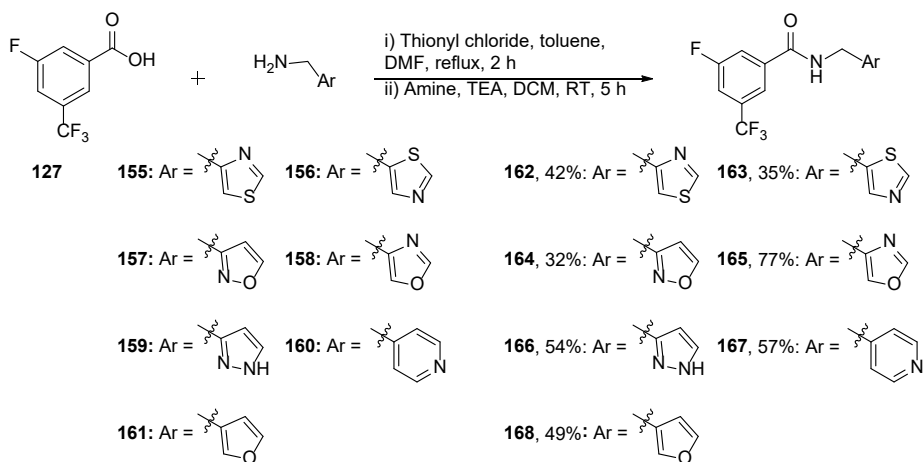
Scheme 16. Synthesis of HAIs **162-168** with variations in the RHS with different heterocycles.

For the last batch, modifications were made to the bridge that links both sides of the molecule. First, it was necessary to synthesize the alcohol-derivative of the 2,5-dimethylthiophene. To do this, 2,5-dimethylthiophene-3-carboxylic acid was reduced with LiAlH$_4$ in anhydrous THF to give (2,5-dimethylthiophen-3-yl)methanol (Scheme 17).
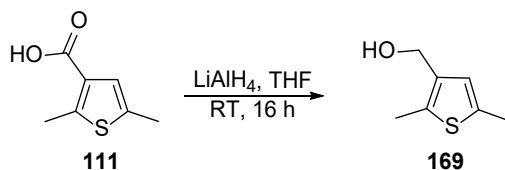
Scheme 17. Synthesis of (2,5-dimethylthiophen-3-yl)methanol (not commercially available).

Three derivatives were synthesized beginning with the ester bioisoter of compound **109**. A typical coupling reaction between benzoic acid **137** and alcohol **169** using EDC·HCl and DMAP in DCM was performed, affording ester **170** in moderate yield. Secondly, a S_N2 reaction between 1-(bromomethyl)-3-fluoro-5-(trifluoromethyl)benzene, **171**, and amine **112·HCl** afforded the secondary amine **172** in moderate yield. The last derivative, **174**, was also an amide but, in this case, the position of the carbonyl was next to the thiophene ring. Thus, **174** is a reversed amide of compound **109** (Scheme 18).
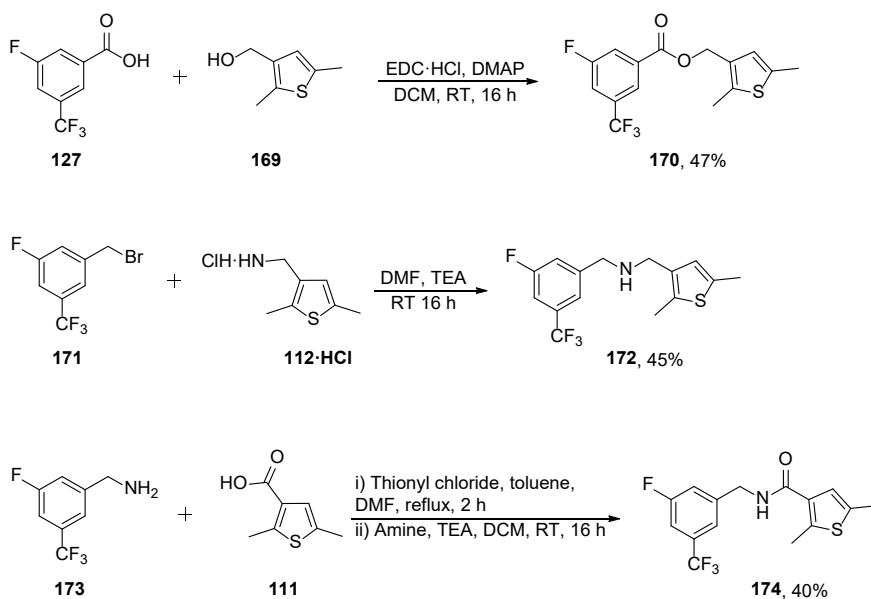
Scheme 18. Synthesis of new bridge derivatives.

2.3.2. Design, antiviral activity and cytotoxicity of new anti-influenza virus molecules

First of all, it is important to mention that all the compounds of the present chapter were evaluated for its inhibitory effect in the influenza A virus H1N1 subtype (PR/8/34-RG and Virginia/ATCC3/2009 strains), H3N2 subtype (A/HK/7/87 strain) and influenza B virus (Ned/537/05 strain). Due to the high mutability of the influenza virus and the time elapsed between the first tests of the group and the current Thesis, the viral strains where the compounds were tested are different, although very similar between them as the virus subtype remains the same. This fact is quite common in studies of influenza viruses.

The inhibitory effect of the compounds as well as their cytotoxicity were monitored by microscopic examination of the viral cytopathic effect (CPE) at 3 days post infection and confirmed by the colorimetric MTS cell viability assay.[105] This system is a complex system where many parameters are taken into account as the activity is evaluated *ex vivo*. This is the reason why compounds exerting inhibition in low micromolar values are considered as good antiviral molecules in contrast with the low nanomolar activity values required for other *in vitro* evaluations.

In order to compare the results obtained during the evaluations of these anti-influenza compounds, the antiviral activity and cytotoxicity of some approved drugs already in the market are disclosed in table 5. As discussed in the introduction, zanamivir and baloxavir acid are clinically approved anti-influenza drugs and both are endowed with high antiviral potency against PR/8/34 and PR/8/34-RG strain, while only baloxavir acid is also potent in the Virginia/ATCC3/2009 strain. In contrast, zanamivir is also active against influenza B virus, whereas baloxavir acid also inhibits H3N2 influenza A subtype of A/HK/7/87 strain but has moderate effect against influenza B virus. On the other hand, ribavirin is an anti-viral molecule considered inactive against influenza virus with values of one order of magnitude more than zanamivir and baloxavir acid.

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[105] Vanderlinden, E.; Göktas, F.; Cesur, Z.; Froeyen, M.; Reed, M. L.; Russell, C. J.; Cesur, N.; Naesens, L. *J. Virol.* **2010**, 84, 4277–4288.

Cmpd	Antiviral activity[1] (µM)								Cytotoxicity[2] (µM)	
	H1N1				H3N2		Influenza B		MDCK[3] cells	
	PR/8/34-RG		Virginia/ATCC3/2009		A/HK/7/87		Ned/537/05			
	EC_{50} (CPE)	EC_{50} (MTS)	EC_{50} (CPE)	EC_{50} (MTS)	EC_{50} (CPE)	EC_{50} (MTS)	EC_{50} (CPE)	EC_{50} (MTS)	MCC	CC_{50} (MTS)
Zanamavir	1.3	1.0	23.3	5.6	49.1	-	1.2	0.2	>100	>100
Baloxavir acid	1.4	1.5	1.5	0.8	1.0	-	8.1	8.5	>40	>40
Ribavirin	19.3	16.7	24.9	17.9	13.1	-	17.1	11.5	>100	>100

Table 5 Antiviral activity and cytotoxicity of zanamivir, baloxavir acid and ribavirin. [1]EC_{50}: 50% effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay. [2]CC_{50}: 50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. MCC: minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology. [3]MDCK cells: Madin-Darby canine kidney cells.

During the first SAR study in the group's previous work, the general structure **V** was followed (Scheme 10). The molecules that were synthesized presented only mono- or di-substitutions with electro withdrawing groups (EWGs) (Fig. 37), so there was still place for many other modifications along the molecule.

For the first batch, several derivatives with EWG substitutions, more than two in all cases, were made to extend the scope previously obtained in the previous work, widening the previous SAR study. This first batch afforded compounds **118-122**. Inhibitory effect and cytotoxicity were evaluated (Table 6).

Cmpd	Antiviral activity[1] (µM)								Cytotoxicity[2] (µM)	
	H1N1				H3N2		Influenza B		MDCK[3] cells	
	PR/8/34-RG		Virginia/ATCC3/2009		A/HK/7/87		Ned/537/05			
	EC_{50} (CPE)	EC_{50} (MTS)	EC_{50} (CPE)	EC_{50} (MTS)	EC_{50} (CPE)	EC_{50} (MTS)	EC_{50} (CPE)	EC_{50} (MTS)	MCC	CC_{50} (MTS)
118	1.6	0.2	2.3	0.2	>100	>16	>100	>100	>100	>100
119	10.4	5.8	5.7	>16	5.3	>16	>100	>100	>100	>100
120	0.7	0.2	0.5	0.2	>100	>16	>100	>100	>100	>100
121	1.6	2.8	>100	>100	>100	>100	>100	>100	>100	>100
122	>100	>100	>100	>100	>100	>100	>100	>100	11.1	>24

Table 6. Antiviral activity and cytotoxicity of compounds **118-122**. [1]EC_{50}: 50% effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay. [2]CC_{50}: 50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. MCC: minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology. [3]MDCK cells: Madin-Darby canine kidney cells.

As it can be seen in all cases, none of the compounds was active in the H3N2 subtype of influenza A or in influenza B. However, and following the line of the previous antivirals evaluated by the group, almost all of them were active against the two strains of influenza A virus, within the low micromolar range. It should be noted that compounds **118** and **120** exerted the highest inhibitory potency, so it seems that a triple substitution pattern on the aromatic ring, with two halogen atoms together with a CF_3 group in *meta* position improves the inhibitory capacity in both strains studied. On the other hand, compounds **119**, **121** and **122,** with substituents in *ortho* position, almost completely lost their inhibitory capacity regardless of the position or nature of the other substituents. Importantly, neither of them induced any toxicity in the cells evaluated.

The next modifications explored, keeping in this case the substitutions in the aromatic ring of compounds **109** and **110**, were changes in the thiophene ring of the RHS. Modifications in the position and number of the methyl groups together with the substitution of the sulfur atom by an oxygen atom were carried out. Compounds **132-135**, bearing fluorine and CF_3 in the aromatic ring, and **141-144**, bearing chlorine and CF_3, were synthesized (Table 7).

Cmpd	Antiviral activity[1] (µM)								Cytotoxicity[2] (µM)	
	H1N1				H3N2		Influenza B		MDCK[3] cells	
	PR/8/34-RG		Virginia/ATCC3/2009		A/HK/7/87		Ned/537/05			
	EC_{50} (CPE)	EC_{50} (MTS)	EC_{50} (CPE)	EC_{50} (MTS)	EC_{50} (CPE)	EC_{50} (MTS)	EC_{50} (CPE)	EC_{50} (MTS)	MCC	CC_{50} (MTS)
132	0.4	0.4	19.7	4.8	>100	>100	>100	>100	>100	>100
133	0.2	0.2	8.3	4.5	>100	>100	>100	>100	>100	>100
134	0.3	0.7	1.6	0.4	>100	>100	>100	>100	>100	67
135	0.5	0.5	3.6	0.5	>100	>100	>100	>100	>100	79
141	1.0	2.0	2.1	1.2	>100	>100	>100	>100	>100	70
142	0.1	0.1	4.0	0.3	>100	>100	>100	>100	>100	>100
143	0.5	0.3	1.5	0.2	>100	>100	>100	>100	>100	>100
144	0.2	0.2	>100	32	>100	>100	>100	>100	33	53

Table 7. Antiviral activity and cytotoxicity of compounds **132-135** and **141-144**. [1]EC_{50}: 50% effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay. [2]CC_{50}: 50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. MCC: minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology. [3]MDCK cells: Madin-Darby canine kidney cells.

Regarding Table 7, it can be seen how the substitution of sulfur for oxygen gave rise to cytotoxicity problems in compound **135**, so it was discarded together with compound **144** to avoid future complications. The activity of all the other compounds was similar, all of them in the order of low micromolar and only active in the two strains of influenza A. Adding or removing methyl groups, as well as their position in the ring, does not seem to affect activity at all. Of note, the best compounds, **142** and **143**, were more potent against H1N1 than clinical approved anti-influenza drugs zanamivir, baloxavir acid and ribavirin (see Table 5).

To better rationalize the results shown in the first batch of compounds, it was decided to make a Topliss[106] study with modifications in the LHS ring of the molecule. Besides, as was shown in the second batch, small changes in the thiophene ring of the RHS of the molecule seemed to have no effect in activity. This led the group to carry out also in this third batch of compounds a complete substitution of the thiophene ring by other heterocycles of different nature. Unfortunately, at the time of finishing the writing of this Thesis we are still waiting for these results.

To finish with the SAR study of these anti-influenza A compounds, three different modifications in the bridge were carried out. First, the amide was substituted by an ester group to explore if the nitrogen was essential for the activity. Secondly, an amine was placed in the middle of the bridge. The amine is a pH dependent functional group that could give some extra information about the nature of the pocket of the targeted protein, which is still unknown. The last substitution was done by reversing the place of the carbonyl of the amide. These compounds were the last set of products synthesized during this Thesis and, consequently, we are still waiting for the cytotoxicity and antiviral activity results.

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[106] Topliss, J. G. *J. Med. Chem.* **1977**, 20, 463-469.

2.4. Conclusions

- A total of 28 anti-influenza A virus molecules where synthesized. 13 of them were evaluated for their cytotoxicity and inhibitory activity against H1N1 subtype (PR and Virginia strains), H3N2 subtype (HK strain) and influenza B virus.

- 8 of them were active in the low micromolar range against both H1N1 strains.

- A triple substitution pattern on the aromatic ring with two halogen atoms and a CF_3 group in *meta* position improves the inhibitory capacity in both H1N1 strains.

- A quadruple substitution pattern destroys completely the activity.

- Adding or removing methyl groups in the thiophene ring does not seem to affect activity at all.

- We are still waiting for the results of the antiviral activity for several compounds. Once we receive them, a clearer picture of the SAR of this group of molecules will be obtained.

3. Design and synthesis of new anti-coronavirus molecules

3.1. Introduction

3.1.1. Coronavirus

Coronaviruses (*Orthocoronavirinae*) are a subfamily of positive single-stranded RNA viruses belonging to the *Coronaviridae* family. These include phylogenetically similar genogroups of viruses with an enveloped, helical-symmetric nucleocapsid (capsid plus RNA) whose virions can measure between 50 and 200 nm in diameter.

Coronaviruses (CoV) can infect birds and mammals, producing a series of respiratory and digestive diseases, many of them lethal, resulting in serious damage to poultry and livestock. They can also infect humans causing illnesses ranging from the common cold to more serious illnesses such as bronchitis, bronchiolitis, pneumonia, Middle East respiratory syndrome (MERS), severe acute respiratory syndrome (SARS), and COVID-19 among others. Most people are infected with these viruses at some point in their lives.[107]

They are subdivided in four different genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus* and *Deltacoronavirus,* but only in the former two human coronaviruses are present. The genus Alphacoronavirus includes as the most representative members the human coronaviruses 229E and NL63, both broadly used in research. The genus *Betacoronavirus* includes several subgroups. Among them, there are three particularly important for humans' coronaviruses: the *Embecovirus* subgroup, where the human CoV species OC43 and HKU1 can be found, the *Sarbecovirus* subgroup where SARS-CoV and SARS-CoV-2 are the most representative subspecies and the *Merbecovirus* subgroup that includes MERS-CoV. While the *Alphacoronavirus* and *Betacoronavirus* genera come from the genetic pool that has bats as its

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[107] Cui, J.; Li, F.; Shi, Z. L. *Nat. Rev. Microbiol.* **2018**, *17*, 181-192.

host, the genus *Gammacoronavirus* and *Deltacoronavirus* come from pigs and birds.[108,109,110]

Structurally, coronaviruses are very similar to the influenza virus. Its genetic material is a positive-sense single-stranded RNA, the largest within RNA viruses with genomes ranging from 26 to 32 kilonucleotides.[111] Three viral structural proteins E, S and M are found in the envelope. Glycoprotein S is a richly glycosylated high-molecular weight protein located on the outer part of the envelope membrane and is responsible for the bulging projections (peplomeres) that characterize these viruses, which they use as ligands in membrane fusion (similar to the HA of influenza viruses). The E and M proteins are the structural proteins that combined with the lipid bilayer make the shape of the viral envelope and maintain its size. Glycoprotein M (matrix protein) is a transmembrane molecule and is located in the inner part of the envelope. During initiate entry and infection, glycoprotein M participates in virus-induced cell fusion to release the nucleocapsid to the cytoplasm. The fourth structural protein, not located in the envelope, is the nucleocapsid protein (N) responsible for the helical symmetry of the capsid inside (Fig. 40).[112,113,114] For the case of *Embecovirus* subtype of *Betacoronaviruses*, there is another spike protein called hemagglutinin esterase (HE), shorter than the S protein but with a complementary function.[108]

[108] Woo, P. C. Y.; Huang, Y.; Lau, S. K. P.; Yuen, K. Y. *Viruses* **2010**, *2*, 1804-1820.

[109] Knipe, D. M.; Howley, P. M.; Griffin, M. A. *Fields virology* **2001**, pp. 1163-85.

[110] Woo, P. C. Y.; Lau, S. K. P.; Lam, C. S. F.; Lau, C. C. Y.; Tsang, A. K. L.; Lau, J. H. N.; Bai, R.; Teng, J. L. *J. Virology* **2012**, *86*, 3995-4008.

[111] Woo, P. C. Y.; Huang, Y.; Lau, S. K. P.; Yuen, K. Y. *Viruses* **2010**, *2*, 1804-1820.

[112] Lai, M. M.; Cavanagh, D. *Adv. Virus Res.* **1997**, *48*, 1–100.

[113] Liu, D. X.; Liang, J. Q.; Fung, T. S. *Encyclopedia of Virology* **2021**, 428–440.

[114] Belete, T. M. *Vacunas* **2020**, *21*, 121–128.
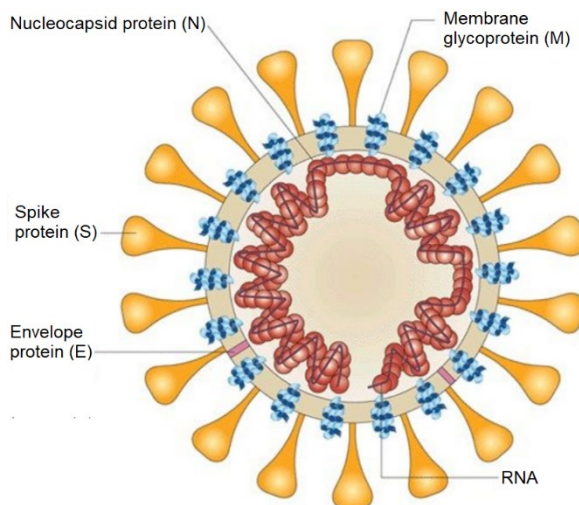
Fig. 40. Coronavirus structure. Figure taken from reference 114.

Apart from the proteins described above, which are part of the structure of the coronavirus, coronaviruses possess 16 non-structural proteins (nsp1 to nsp16) with very diverse functions. In most cases, these non-structural proteins actively participate in the viral replication cycle by forming the replication complex, encoding new proteins or synthesizing new vRNA. Most of them are essential for virus life-cycle and their deletion induce virus death, so targeting these proteins is of great importance for developing new anti-viral drugs.[113]

The life cycle of coronavirus follows the same cycle as other viruses, very similar to the influenza virus. Infection begins when the viral spike protein S attaches to its complementary host cell receptor (angiotensin ACE2). After attachment, a serine protease of the host cell cleaves and activates the receptor-attached spike protein. Depending on the host cell protease available, usually serine protease TMPRSS2, the virus will enter the cell by endocytosis or by direct fusion of the viral envelope with the host membrane.[115] In both cases, the strand of RNA is inserted into the cell and the capsid is abandoned (Fig. 41).

[115] Simmons, G.; Zmora, P.; Gierer, S.; Heurich, A.; Pöhlmann S. *Antiviral Res.* **2013**, *100*, 605–14.
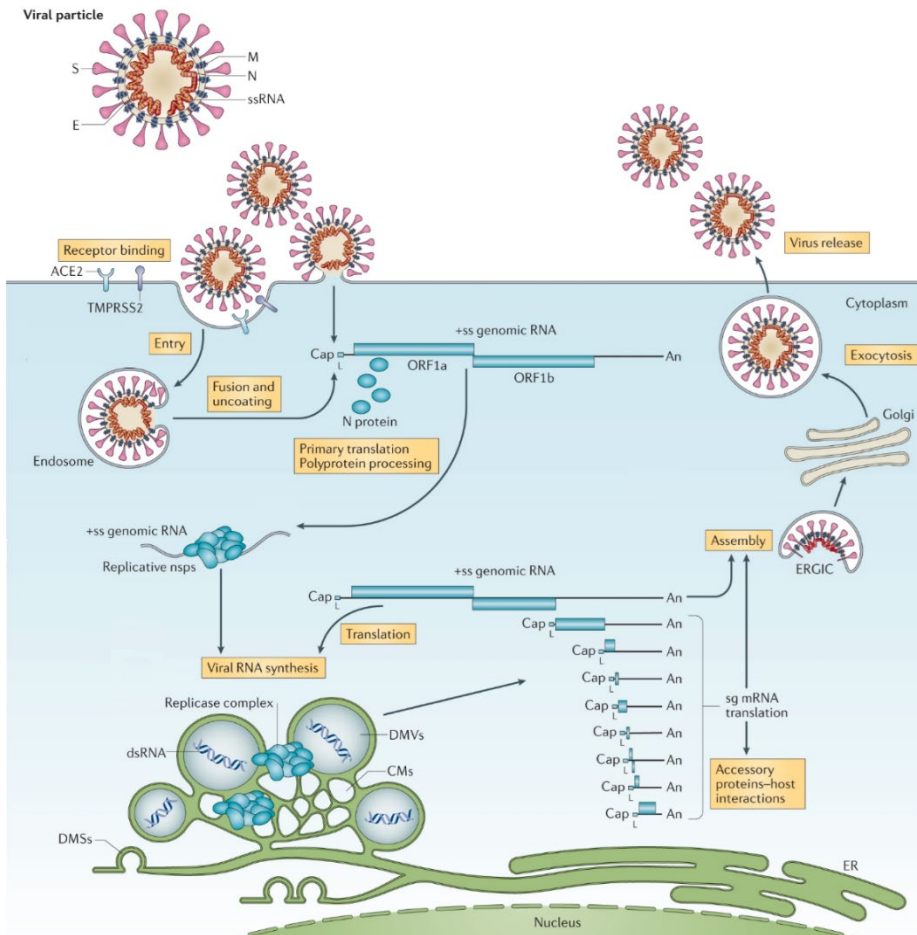
Fig. 41. Scheme of viral life cycle. Figure taken from reference 116.

The coronavirus RNA genome has a 5' methylated cap and a 3' polyadenylated tail, which allows RNA to attach directly to ribosomes for translation as mRNA, creating the reading frames ORF1a and ORF1b. From this, two polyproteins, pp1a and pp1ab, are synthesized and processed into the individual non-structural proteins (nsp1 to nsp16) that form the viral replication and transcription complex (RTC) such as RNA-dependent RNA polymerase (nsp12), RNA helicase (nsp13), exoribonuclease (nsp14) or endonuclease (nsp15) and other important proteins. This viral replication complex only

recognizes and produces viral RNA, allowing the viral genome to be transcribed into new copies of RNA using the machinery of the host cell (Fig. 41).[116,117]

To create a protective microenvironment for viral genomic RNA replication and transcription, some viral replication organelles are developed such as perinuclear double-membrane vesicles (DMVs), convoluted membranes (CMs) and small open double-membrane spherules (DMSs).

At this stage, as the host cell's cytoplasm is filled with viral proteins and RNA, the N protein helps to bind genomic RNA to achieve encapsidation of the viral genome.[118] The M protein integrates into the membrane of the endoplasmic reticulum, on the capsid side; and HE and S proteins cross the membrane of the endoplasmic reticulum, via the translocation protein, and position themselves on the opposite side, creating the endoplasmic-reticulum–Golgi intermediate compartment (ERGIC). Binding between the capsid and the M proteins, the reticulum membrane invaginates and buds forming the final virus form. This viral progeny is then encapsulated and transported by Golgi vesicles to the cell membrane to be finally externalized, *via* exocytosis, out of the cell.

3.1.2. Therapeutic options against coronavirus

Only seven coronaviruses are known to cause disease in humans. Coronaviruses 229E, NL63, OC43 and HKU1 cause about 15 to 30% of cases of the common cold. Serious lower respiratory tract infections, including bronchiolitis and pneumonia, can occur very rarely, especially in infants, the elderly, and immunocompromised persons.[119] The other three coronaviruses cause much more severe and sometimes deadly respiratory infections and have caused major outbreaks of deadly pneumonia in the XXI century: SARS-CoV (2003), MERS-CoV (2012) and SARS-CoV-2 (2019). These coronaviruses that cause serious respiratory infections are zoonotic pathogens, beginning in

[116] V'kovski, P.; Kratzel, A.; Steiner, S. *Nat. Rev. Microbiol.* **2021**, *19*, 155–170.

[117] Fehr, A.R.; Perlman, S. *Methods Mol. Biol.* **2015**, *1282*, 1–23.

[118] Hsieh, P. K.; Chang, S. C.; Huang, C. C.; Lee, T. T. *J. Virol.* **2005**, *79*, 13848–13855.

[119] Gaunt, E. R.; Hardie, A.; Claas, E. C. J.; Simmonds, P.; Templeton, K. E. *J. Clin. Microbiol.* **2010**, *48*, 2940–2947.

infected animals and spreading from animals to people.[120] Both SARS-CoV and MERS-CoV caused high mortality, so neither of the two viruses could spread easily due to the severe clinical condition of the affected patients.[117,121] However, the most recent outbreak caused by SARS-CoV-2 is still a major health problem for all the countries in the world, causing more than 6 million deaths worldwide.[122]

It is important to note that, as the first four types of coronaviruses cause mild infections, their treatment or prevention is practically unnecessary. However, and due to the similarity with other coronaviruses, their study can help to discover treatments not only against the three most aggressive coronaviruses, but for the incoming coronavirus outbreaks. In addition, working with these coronaviruses entails little risk for the scientists who manipulate them, which is why only a level 1 security laboratory is needed for their manipulation, making research easier and cheaper.[123]

At the present time, the therapeutic arsenal against coronaviruses is quite scarce. Thus, the pharmacopoeia has few effective antiviral molecules and proven vaccines. Also, some studies with monoclonal antibodies have been performed but these biochemical treatments are beyond this thesis.[124]

3.1.2.1.   Vaccines

There are currently very few vaccines to treat the different coronaviruses since these have a high mutagenic capacity that prevents the appearance of an effective long-termed vaccine.[125] To date, only vaccines against SARS-CoV-2 are approved and currently in the market, whereas some vaccines against SARS-CoV and MERS-CoV reached clinical trials (such as

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[120] Ye, Z. W.; Yuan, S.; Yuen, K. S.; Fung, S. Y.; Chan, C. P.; Jin, D. Y. *Int. J. Biol. Sci.* **2020**, *16*, 1686–1697.

[121] Zumla, A.; Hui, D.S.; Perlman, S. *Lancet* **2015**, *386*, 995–1007.

[122] ArcGIS. *Johns Hopkins University*, **2022**.

[123] Biosafety in Microbiological and Biomedical Laboratories. *U.S. Department of Health and Human Services* **2009**, pp. 30–59.

[124] Jiang, S.; Hillyer, C.; Du, L. *Trends Immunol.* **2020**, *41*, 355-359.

[125] Li, X.; Luk H. K. H.; Lau, S. K. P.; Woo, P. C. Y. *Reference Module in Biomedical Sciences* **2019**, ISBN 9780128012383.

BVRS-GamVac-Combi or VTP-500 (ChAdOx1) MERS-CoV),[126] none of them has ever been approved by any regulating organism.[127]

Due to the urgency in containing the outbreak caused by SARS-CoV-2 in 2019, a large number of companies and countries began to work against the clock to obtain vaccines that were effective for the treatment of COVID-19. These vaccines received the support of regulatory agencies and were approved more quickly than established for this type of treatment. Thus, a large number of vaccines with different mechanisms of action were developed.[128]

SARS-CoV-2 vaccines developed in 2020 involved nucleic acid technologies (nucleoside-modified mRNA and DNA), peptides, non-replicating viral vectors, recombinant proteins and the classicals live attenuated viruses and inactivated viruses.[129] The mass vaccination that was carried out in most countries of the world gave better results than expected within the scientific community, making the fight against COVID-19 an unprecedented milestone.[130]

3.1.2.2. Antiviral drugs

As of 2020 (date of the worldwide spread of the COVID-19 pandemic), all the antiviral molecules tested against different coronaviruses that are found to be effective *in vitro* (ex: ribavirine, hexachlorophene, nitazoxanide, protein TMPRSS2, etc.) do not present any efficacy *in vivo* or exhibited many secondary effects, making these molecules useless for their use in clinic.[131]

To date, a small number of compounds have been approved for use against SARS-CoV-2. As in the case of vaccines, regulatory agencies are rapidly approving some compounds against SARS-CoV-2 that are already known and used for other pathologies but have shown efficacy against this disease. As these molecules are already approved compounds, their

[126] https://www.precisionvaccinations.com/ Accessed on 29 May 2022
[127] Li, Y. D.; Chi, W. Y.; Su, J. H. C. *J. Biomed. Sci.* **2020**, *27*, 104.
[128] Francis, A. I.; Ghany, S.; Gilkes, T. *Postgrad. Med. J.* **2022**, 98:389-394.
[129] Le, T.T.; Cramer, J. P.; Chen, R.; Mayhew, S. *Nat. Rev. Drug Discovery* **2020**, *19*, 667–68.
[130] Mallapaty, S.; Callaway, E.; Kozlov, M.; Ledford, H.; Pickrell, J.; van Noorden, R. *Nature* **2021**, *600*, 580–583.
[131] Baudesson, C. Thèse de doctorat en Biologie cellulaire et moléculaire, Paris Est, **2019**.

pharmacokinetics and side effects are known, so their use against COVID-19 should not cause major complications. As for all the other antiviral molecules, these drugs can interfere with target proteins or RNA of the virus or with proteins or biological processes in the host that support the virus.[116]

Remdesivir (Veklury®), a broad-spectrum antiviral medication originally developed for treating infections caused by Ebola virus, was the first treatment for COVID-19 to be approved by the U.S. Food and Drug Administration (FDA) (Fig. 42).[132] Remdesivir is a prodrug which is activated when three molecules of phosphate are added to the LHS of the molecule. This active form acts as a nucleoside analog and inhibits the RNA-dependent RNA polymerase (RdRp) of different coronaviruses, including SARS-CoV-2.[133]
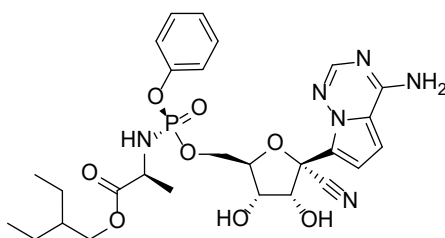
Fig. 42. Structure of remdesivir.

Currently, the main drug treatment used against SARS-CoV-2 is Paxlovid®, approved by FDA in December 2021.[134] This drug results from the combination of two active ingredients: nirmatrelvir and ritonavir (Fig. 43). Nirmatrelvir is a peptidomimetic inhibitor of the SARS-CoV-2 nsp5 protease, the main viral protease (also called 3C-like-protease, 3CL^{pro} or main protease, M^{pro}) reducing the ability of SARS-CoV-2 to multiply in the body. Ritonavir is used as a booster of other protease inhibitors, like nirmatrelvir, by inhibiting the enzyme that normally metabolizes protease inhibitors; cytochrome P450-3A4 (CYP3A4).

[132] *U.S. Food and Drug Administration* (FDA), **2020**.
[133] Kokic, G.; Hillen, H. S.; Tegunov, D. M. *Nat Commun* **2021**, 12, 279.
[134] *U.S. Food and Drug Administration* (FDA), **2021**.

This result in a sustained and prolonged action of nirmatrelvir, allowing it to stay longer in the body at levels that affect virus multiplication.[135,136,137]
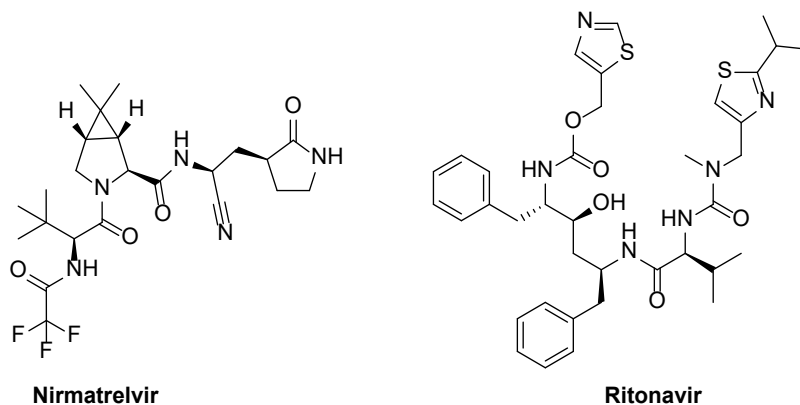
Fig. 43. Structures of nirmatrelvir and ritonavir.

Some other drugs have been studied or still are under study such as favipiravir, interferons, ivermectin, dutasteride, fluvoxamine, etc. Unfortunately, none of them has unequivocally proved to be clinically active.[138]

In the case of animals, coronaviruses are also of high interest, particularly because they cause infections in cats and pigs. Of note, GS-441524, a metabolite of remdesivir, is highly effective against the feline infectious peritonitis (FIV), a fatal disease caused by a coronavirus, and it is employed against FIV in a non-regulated used. It is also used as a reference compound to compare the inhibitory potency of new molecules under investigation (Fig. 44).[139]

[135] The Royal Australian College of General Practitioners (RACGP), 19 october **2021**.

[136] Owen, D. R.; Allerton, C. M.; Anderson, A. S.; Aschenbrenner, L.; Avery, M.; Berritt, S. *Science* **2021**, 374 (6575): 1586–1593.

[137] Zeldin, R. K.; Petruschke, R. A. *J. Antimicrob. Chemother.* **2004**, 53 (1): 4–9.

[138] Şimşek-Yavuz, S.; Komsuoğlu Çelikyurt, F. I. *Turk. J. Med. Sci.* **2021**, 51(SI-1), 3372–3390.

[139] Pedersen, N. C.; Perron, M.; Bannasch, M.; Montgomery, E.; Murakami, E.; Liepnieks, M.; Liu, H. *J. Feline Med. Surg.* **2019**, 21(4), 271–281.
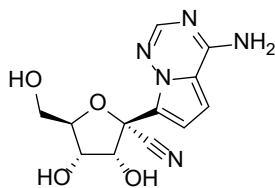
Fig. 44. GS-441524 structure.

3.1.3. Previous work on anti-coronavirus molecules in our research group

As it has already been mentioned in the section related with influenza, our group collaborates with a Belgian group led by Prof. Lieve Naesens, at the Rega Institute for Medical Research of the KU Leuven. Any compound with possible antiviral activity that is submitted there is routinely tested against different viruses, subtypes and strains, regardless of the virus that is initially targeted. Indeed, our group also send to Leuven compounds initially designed against non-viral targets. In this way, compounds that have activity against another type of virus (or even not designed at all as antiviral) for which they were designed can be discovered by serendipity.

This is the case for two families of compounds synthesized in the group. The first is compound **93** and its analogs (Fig. 35), mentioned in the previous chapter, the compound published in 2018 belonging to a series of inhibitors of the influenza H1N1 virus acting on hemagglutinin-mediated fusion in the "TBHQ pocket".[95] All the compounds of this family were evaluated against human CoV-229E (HCoV-229E), the coronavirus model that will be used for all the activity studies. Surprisingly, **93** exerted an activity of 24-33 μM against this coronavirus. Inspired in these findings, the group synthesized several analogs and evaluated them for their inhibitory capacity. This small SAR study found many other compounds that exhibited higher inhibitory potency against HCoV-229E than compound **93**. Some *meta*-substituted, *meta,meta*-disubstituted and *meta,para*-disubstituted derivatives were active in the low micromolar range against HCoV-229E. Some modifications in the size of the bridge and the piperidine ring were also performed. From all this, some clues were obtained: *meta,meta*-disubstituted derivatives had the best activity, fluorine substitutions

and ring contraction led to poor activities and bridge and ring expansion had no significant effect in activity. Among all, the best inhibitory potency was found with anilines **175** and **176**. It is also important to note that aniline **177** was endowed with the best inhibitory activity among the mono-*meta*-substituted anilines, which led the group to think that SF_5 group would be helpful in further studies. Finally, it is also important to underline that all of them were fully inactive against the influenza A virus (Fig. 45).

175
CC_50 > 100 µM
HCoV-229E (µM)
Antiviral EC_50
Visual CPE score = 7.9

176
CC_50 > 100 µM
HCoV-229E (µM)
Antiviral EC_50
Visual CPE score = 8.9

177
CC_50 > 100 µM
HCoV-229E (µM)
Antiviral EC_50
Visual CPE score = 10.0
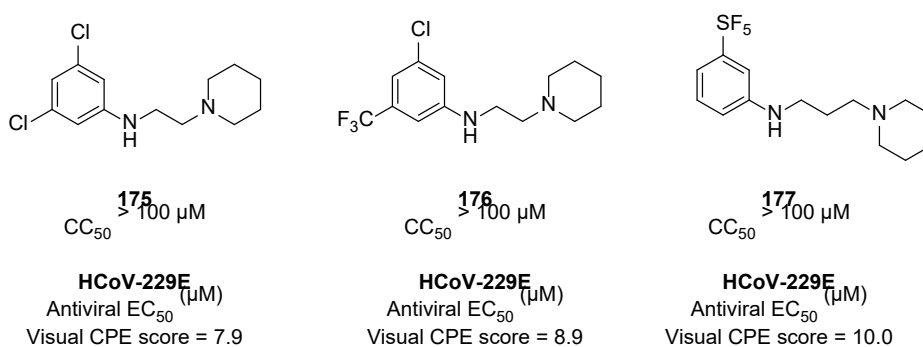
Fig. 45. Compounds **175**, **176** and **177** structure, antiviral activity in HCoV-229E and cytotoxicity in HEL cells. HEL cells: Erythroleukemia cells. CC_{50}: 50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. EC_{50}: 50% effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE.

In the second case, in the context of the Thesis of Dr. Eugènia Pujol[140], that partly revolved around the design, synthesis and evaluation of new drugs for the treatment of type 2 diabetes and non-alcoholic steatohepatitis (NASH), many diarylurea compounds were synthesized and evaluated in HCoV-229E. Among all of them, compound **178** was endowed with a high inhibitory activity against this coronavirus in the low-micromolar range, a potency considered very high for this type of test (Fig. 46).

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[140] Pujol, E. Ph. D. Urea-based compounds for a broad range of therapeutic applications. *University of Barcelona*, **2021**.
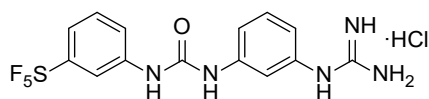
178
CC$_{50}$ > 100 µM

HCoV-229E
Antiviral EC$_{50}$ (µM)
Visual CPE score = 1.12
MTS = 1.17

Fig. 46. Compound **178** structure, antiviral activity in HCoV-229E and cytotoxicity in HEL cells. HEL cells: Erythroleukemia cells. CC$_{50}$: 50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. EC$_{50}$: 50% effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.

Importantly, from a very large batch of diarylureas synthesized by Dr. Pujol and tested at Leuven, this compound is the only one bearing a guanidine as the second pharmacophore. This group is not very common in pharmacology due to its high polarity, which makes it difficult to pass through cell membranes. However, there are two compounds that were tested against SARS-CoV-2 that have this functional group: nafamostat and camostat (Fig. 47).[141,142] Both compounds are inhibitors of the serine protease TMPRSS2, an enzyme present in the cell wall that is used by coronaviruses as an anchor point for their entry into the cell.[143,144]

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[141] Kawase, M.; Shirato, K.; van der Hoek, L.; Taguchi, F.; Matsuyama, S. *J. Virol.* **2012**, 86 (12): 6537–45.

[142] Wang, M.; Cao, R.; Zhang, L.; Yang, X.; Liu, J.; Xu, M. *Cell Res.* **2020**, 30 (3): 269–271.

[143] Rahman, N.; Basharat, Z.; Yousuf, M.; Castaldo, G.; Rastrelli, L.; Khan, H. *Molecules* **2020**, 25 (10): 2271.

[144] Huggins, D. J. *J. Mol. Graph. Model.* **2020**, 100, 107710.
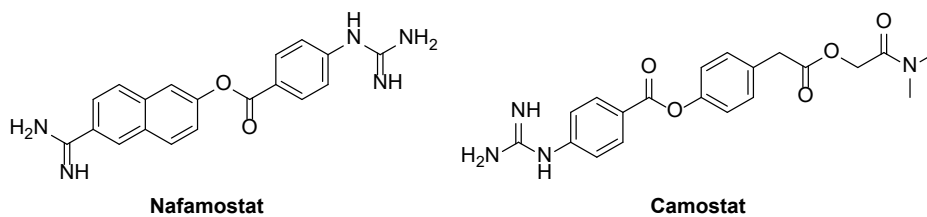
Fig. 47. Structures of nafamostat and camostat.

Taking into account the presence of the arylguanidine group in **178** and that the target of nafamostat and camostat is TMPRSS2, we thought that, reasonably, the target of **178** may be also TMPRSS2. For this reason, we sent **178** to the group of Prof. Mabel Loza and José M. Brea, at the Universidad de Santiago de Compostela, for being tested as a TMPRSS2 inhibitor. However, the assays revealed that **178** is not an inhibitor of TMPRSS2.

3.2. Objectives

After the interesting results obtained by serendipity for both the first family of arylpiperidine antivirals and the second family of diarylurea-guanidine, no more molecules of either family were synthesized. For this reason, no SAR study was carried out and no further studies were conducted to understand the mechanism of action of the compounds in HCoV-229E. Both lines of research remained paused at this stage until the present thesis, mostly performed under the impact of the global COVID-19 crisis.

Arylpiperidine molecules

For all the above, with the aim of developing a more complete SAR study. The first objective of this chapter was to make different modifications in the nature, number and position of the substituents of the aromatic ring. Also, modifications of both the size of the bridge and the size of the ring were performed in order to broaden the spectrum of compounds and reach better conclusions. Finally, substitution of the aniline by a phenol was carried out to see whether the nitrogen atom was important for the activity or not. All these changes are done following general structure **VII** (Fig. 48). After this, all the compounds were evaluated for their cytotoxicity and inhibitory potency in HCoV-229E.

$X = NH, O$
$n = 1$ or 2

VII
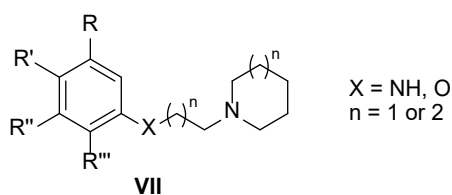
Fig. 48. General structure **VII** of the new arylpiperidine compounds.

Diarylurea-guanidine molecules

3.3. Results and discussion:

Arylpiperidine compounds as anti-HCoV-229E

3.3.1.1. Synthesis of arylpiperidine compounds as anti-HCoV-229E

Continuing with the structure of compounds **175-177** (Fig. 45), a new general structure **VII** was proposed to develop a more advanced SAR study. This novel approximation presents modifications not only in the substituents of the benzoic acid ring, but also in the nature of it. Phenol and anilines were synthesized with modifications in the size of both the bridge and the piperidine ring (Fig. 48).

For the first batch of derivatives, only modifications in the nature, number and position of substituents of the aromatic ring were performed. For this, different commercially available anilines, **180**-**191**, were reacted with 1-(2-chloroethyl)piperidine hydrochloride, **179**, *via* S_N2 using NaH as base in anh. DMF with catalytic amounts of KI at 70 ºC. After that, an excess of HCl was added to form the corresponding HCl salt furnishing final compounds **192**-**203** in low yields. Only in one case, and trying to find better conditions, aniline **179** was reacted using K_2CO_3 as base in DMF with KI at 90 ºC, affording very low yield (Scheme 19).

i) NaH, KI, DMF, 70 °C, 2 h
or
*K$_2$CO$_3$, KI, DMF, 90 °C, 24 h
ii) HCl in Et$_2$O

179·HCl

180: R = Cl	R' = CH$_3$	R" = H	R''' = H		**192:** R = Cl	R' = CH$_3$	R" = H	R''' = H,	11%
181: R = Cl	R' = F	R" = H	R''' = H		***193:** R = Cl	R' = F	R" = H	R''' = H,	8%
182: R = Cl	R' = OCH$_3$	R" = Cl	R''' = H		**194:** R = Cl	R' = OCH$_3$	R" = Cl	R''' = H,	29%
183: R = Cl	R' = CH$_3$	R" = Cl	R''' = H		**195:** R = Cl	R' = CH$_3$	R" = Cl	R''' = H,	27%
184: R = Cl	R' = Cl	R" = Cl	R''' = H		**196:** R = Cl	R' = Cl	R" = Cl	R''' = H,	37%
185:		R" = Cl	R''' = H		**197:**		R" = Cl	R''' = H,	19%
186: R = Cl	R' = Br	R" = Cl	R''' = H		**198:** R = Cl	R' = Br	R" = Cl	R''' = H,	14%
187: R = Cl	R' = H	R" = Cl	R''' = Cl		**199:** R = Cl	R' = H	R" = Cl	R''' = Cl,	52%
188:		R" = Cl	R''' = OCH$_3$		**200:**		R" = Cl	R''' = OCH$_3$,	17%
189: R = CF$_3$	R' = CH$_3$	R" = H	R''' = H		**201:** R = CF$_3$	R' = CH$_3$	R" = H	R''' = H,	13%
190: R = CF$_3$	R' = F	R" = H	R''' = H		**202:** R = CF$_3$	R' = F	R" = H	R''' = H,	9%
191: R = CF$_3$	R' = H	R" = CF$_3$	R''' = Cl		**203:** R = CF$_3$	R' = H	R" = CF$_3$	R''' = Cl,	38%

Scheme 19. Synthesis of arylpiperidines with variations in the aromatic ring **192**-**203**. *Reaction made only to synthesize compound **193**.

Secondly, following the same synthetic strategy, commercially available substituted phenols were reacted *via* S$_N$2 with 1-(2-chloroethyl)piperidine hydrochloride, **179**, using Cs$_2$CO$_3$ as base in DMF with catalytic amounts of KI. Compounds **209**-**213** were synthesized in moderate yields (Scheme 20).

i) Cs$_2$CO$_3$, KI, DMF, 90 °C, 24 h
ii) HCl in Et$_2$O

179·HCl

204: R = Cl	R' = H	R" = CF$_3$	R''' = H		**209:** R = Cl	R' = H	R" = CF$_3$	R''' = H, 75%
205: R = SF$_5$		R" = H	R''' = H		**210:** R = SF$_5$		R" = H	R''' = H, 42%
206: R = Cl	R' = Cl	R" = Cl	R''' = H		**211:** R = Cl	R' = Cl	R" = Cl	R''' = H, 68%
207: R = Cl	R' = NH$_2$	R" = Cl	R''' = H		**212:** R = Cl	R' = NH$_2$	R" = Cl	R''' = H, 36%
208:		R" = Cl	R''' = Cl		**213:**		R" = Cl	R''' = Cl, 63%
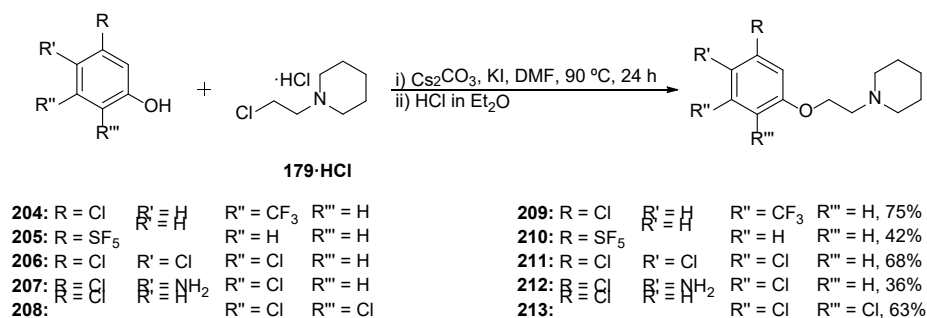
Scheme 20. Synthesis of arylpiperidines with variations in the aromatic ring **208**-**211**.

For the third and last group of compounds, the variations were made in the bridge and ring size. For this, the first step was to synthesize the non-commercially available 1-(3-chloropropyl)azepane, **216**. It was done in one step

via S_N2 reaction by heating at reflux a mixture of commercially available azepane, **214**, and 1-bromo-3-chloropropane, **215**, in THF using Na_2CO_3 as base (Scheme 21).
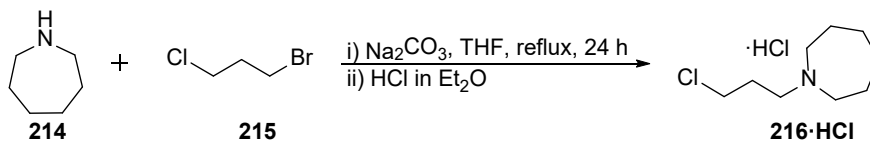
Scheme 21. Synthesis of 1-(3-chloropropyl)azepane hydrochloride **216**.

In this batch, the aryl substituents were kept fixed, choosing two *meta,meta*-disubstituted anilines and another with the SF_5 group in *meta*. The synthetic route was the same as the previous ones, a S_N2 type reaction with the commercially available 1-(3-chloropropyl)piperidine, **217**, and 1-(2-chloropropyl)azepane, **218**, together with the 1-(3-chloropropyl)azepane, **216**, synthesized previously (Scheme 22).

219: R = Cl	R' = Cl	X = N	
220: R = Cl	R' = CF_3	X = N	
221: R = Cl	R' = CF_3	X = O	
222: R = SF_5	R' = H	X = N	
223: R = SF_5	R' = H	X = O	

216·HCl:	n = 2 n' = 2
217·HCl:	n = 2 n' = 1
218·HCl:	n = 1 n' = 2

224: R = Cl	R' = Cl	n = 2	n' = 2	X = N, 33%
225: R = Cl	R' = CF_3	n = 1	n' = 2	X = N, 21%
226: R = Cl	R' = CF_3	n = 2	n' = 2	X = N, 31%
*227: R = Cl	R' = CF_3	n = 1	n' = 2	X = O, 49%
228: R = SF_5	R' = H	n = 1	n' = 2	X = N, 18%
229: R = SF_5	R' = H	n = 2	n' = 1	X = N, 38%
230: R = SF_5	R' = H	n = 2	n' = 2	X = N, 9%
*231: R = SF_5	R' = H	n = 1	n' = 2	X = O, 60%
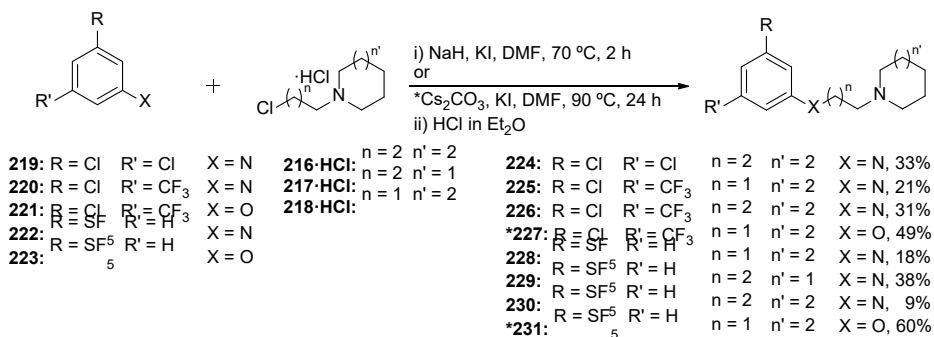
Scheme 22. Synthesis of arylpiperidine and arylazepane with variations in the aromatic ring **224**-**231**. *Reaction made only to synthesize compounds **227** and **231**.

3.3.1.2. Design, antiviral activity and cytotoxicity of arylpiperidine compounds as anti-HCoV-229E

Some conclusions were drawn from previous work by the group, but these were limited by the small size of the SAR study. For this reason, the first compounds made in this new study were more examples of substitutions in the aromatic ring of the molecule. All the molecules were evaluated against HCoV-229E for their inhibitory activity in the line of HEL cells, where cytotoxicity was also evaluated. They were compared with the anti-coronavirus inhibitory activity of GS-441524, a metabolite of anti-coronavirus drug remdesivir.

As can be extracted from table 8, the compounds with the best antiviral activity were **195**-**201**. With the exception of **201**, all the others were compounds with two chlorine atoms in the *meta,meta* positions. It seems that any other substitution does not add or subtract activity as long as both chlorines are held at those positions, e.g., **195** and **200** with donor substituents or **196** and **198** with acceptor substituents, possess similar activity. Also, comparing them with compound **175**, none of them improves the activity so adding more substituents seems to be indifferent.

Cmpd	Antiviral activity[1] (µM)		Cytotoxicity[2] (µM)	
	HCoV-229E		HEL[3] cells	
	EC_{50} (CPE)	EC_{50} (MTS)	MCC	CC_{50} (MTS)
GS-441524	3.0	2.5	>40	>40
192	19.2	13.6	>100	>100
193	23.1	15.1	>100	>100
194	16.3	10.4	>100	77
195	8.5	8.1	>100	>61
196	10.5	8.4	>100	56

197	9.3	7.3	37	37
198	10.6	8.4	70	51
199	9.1	7.9	70	60
200	9.5	8.8	>100	62
201	9.4	8.3	>100	65
202	19.6	12	>100	>100
203	23.1	19.2	>100	82

Table 8. Anti-HCoV-229E activity and cytotoxicity of compounds **192-203**. [1]EC$_{50}$: 50% effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay. MCC: minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology. [2]CC$_{50}$: 50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. [3]HEL cells: Erythroleukemia cells.

Once the importance of the double substitution in *meta,meta* by chlorine atoms was underlined, several examples were carried out substituting the anilines for phenols. The result in this case was similar to the previous one, that is, the compounds with chlorine atoms in *meta,meta* **211** and **213** had an analogous activity to the respective anilines **197** and **199**. The importance of the double substitution of chlorine was emphasized again, making substitution of one heteroatom for another irrelevant (Table 9).

Cmpd	Antiviral activity[1] (µM)		Cytotoxicity[2] (µM)	
	HCoV-229E		HEL[3] cells	
	EC_{50} (CPE)	EC_{50} (MTS)	MCC	CC_{50} (MTS)
209	12.7	6.9	>100	>100
210	14.2	7.5	>100	>100
211	9.3	6.1	>100	59
212	24.2	19.2	>100	62
213	8.7	6.2	>100	56

Table 9. Anti-HCoV-229E activity and cytotoxicity of compounds **209-213**. [1]EC_{50}: 50% effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay. MCC: minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology. [2]CC_{50}: 50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. [3]HEL cells: Erythroleukemia cells.

Lastly, several analogs were synthesized by modifying the size of the chain as well as the size of the ring. In turn, in some cases extra changes were made such as the substitution of the nitrogen atom for an oxygen atom or using the rather unusual SF_5 group in *meta,* the only mono-substituent that maintained the inhibitory activity of this family of compounds as was described previously. Again, no large changes in the inhibitory potency of either compound were seen by enlarging the bridge or ring size. Interestingly, and unexpectedly, the best compound that has been achieved if both the previous studies and this new work are taken into account was compound **231**, which does not have any chlorine in its aryl ring. Although it does not drastically improve potency, it was the most active against HCoV-229E and its cytotoxicity was null in both experiments (Table 10).

Cmpd	Antiviral activity[1] (µM)		Cytotoxicity[2] (µM)	
	HCoV-229E		HEL[3] cells	
	EC_{50} (CPE)	EC_{50} (MTS)	MCC	CC_{50} (MTS)
224	9.9	6.8	70	50
225	10.4	7.8	>100	61
226	8.9	7.3	>100	54
227	15.2	6.5	>100	>100
228	11.1	6.8	>100	60
229	15.5	10.7	>100	59
230	8.9	7.6	>100	60
231	6.1	5.1	>100	>100

Table 10. Anti-HCoV-229E activity and cytotoxicity of compounds **224**-**231**. [1]EC_{50}: 50% effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay. MCC: minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology. [2]CC_{50}: 50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. [3]HEL cells: Erythroleukemia cells.

Unfortunately, no great conclusions could be drawn from this study that could serve to continue with the development of an optimal molecule since the changes in activity were less than an order of magnitude among all molecules. For this reason, deeper pharmacological studies were not carried out to find out the target of these compounds, their DMPK properties, etc.

Conclusions

- A total of 25 arylpiperidine potential HCoV-229E inhibitors were synthesized and their potency and cytotoxicity were evaluated.

- They exhibited moderate potency against HCoV-229E but none of them ameliorated substantially the compounds previously synthesized by the group.

- The most active molecule, compound **231**, introduced an innovative pentafluorosulfanyl (SF_5) group.

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EXPERIMENTAL SECTION

General methods:

Commercially-available reagents and solvents were used without further purification unless stated otherwise. Preparative normal phase chromatography was performed on an Isolera Biotage (Biotage AB, Uppsala, Sweden) with pre-packed RediSep Rf silica gel cartridges. Thin-layer chromatography was performed with aluminum-backed sheets with silica gel 60 F254 (Merck, Darmstadt, Germany, ref 1.05554), and spots were visualized with UV light and/or 1% aqueous solution of $KMnO_4$. Melting points were determined in open capillary tubes with an MFB 595010M Gallenkamp. 400 MHz ^{1}H, 100.6 MHz ^{13}C and 376.5 MHz ^{19}F NMR spectra were recorded on a Varian Mercury 400 or on a Bruker 400 Avance III spectrometers. The chemical shifts are reported in ppm (scale) relative to internal tetramethylsilane, and coupling constants are reported in Hertz (Hz). Assignments given for the NMR spectra of the compounds have been carried out on the basis of HSQC experiments. IR spectra were run on Perkin-Elmer Spectrum RX I (Waltham, MA, USA) spectrophotometer. Absorption values are expressed as wave-numbers (cm^{-1}); only significant absorption bands are given. The elemental analyses were carried out in a Flash 1112 series Thermofinnigan elemental microanalyzator (A5) to determine C, H, N and S. HPLC/MS were determined with an HPLC Agilent 1260 Infinity II LC/MSD coupled to a photodiode array and mass spectrometer. Five microliters of sample 0.5 mg/ml in methanol:acetonitrile was injected using an Agilent Poroshell 120 EC-C18, 2.7 micrometers, 50 mm x 4.6 mm column at 40 °C. The mobile phase was a mixture of A = water with 0.05% formic acid and B = acetonitrile with 0.05% formic acid, with the method described as follows: flow 0.6 ml/min; gradient: 95% A−5% B to 100% B in 3 min, 100% B 3 min, from 100% B to 95% A−5% B in 1 min, 95% A−5% B 3 min. Purity is given as % of absorvance at 254 nm.Data from mass spectra were analyzed by electrospray ionization in positive and negative between 100 and 1000 Da. The analytical samples of all of the new compounds, which were subjected to pharmacological evaluation, possessed a purity of >95%, as evidenced by either their elemental analyses or their HPLC-MS.

13, synthesis of (5s,9s)-5,6,8,9-tetrahydro-7H-5,9-propanobenzo[7]annulene-7,11-dione
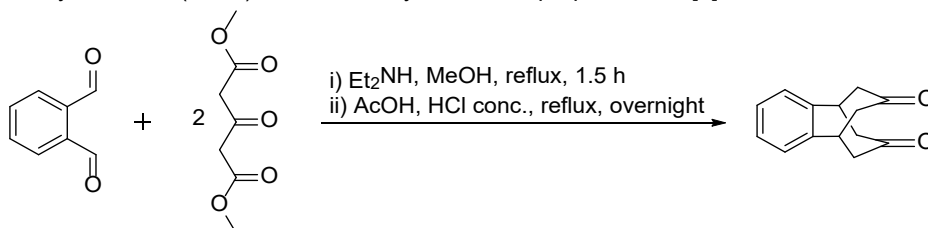
To a solution of *o*-phthaldialdehyde (20.00 g, 149.10 mmol) and dimethyl 1,3-acetonedicarboxylate (45.07 mL, 54.35 g, 313.12 mmol) in methanol (400 mL) was added diethylamine (2.0 mL, 1.41 g, 19.33 mmol) and the solution was heated at reflux for 1.5 h. After that time, solvent was concentrated until half of the initial volume and the mixture was cooled to 4 ºC overnight. The resulting precipitated was filtered *in vacuo* and washed with cold MeOH (2 x 100 mL) to give the intermediate tetraester as white crystals (49.92 g, 75% yield).

To a round bottom flask containing the intermediate tetraester prepared in the last step (49.92 g, 112 mmol), glacial acetic acid (300 mL) and conc. HCl (80 mL) were added and the mixture was heated at reflux overnight. Acids were removed under vacuo and Et$_2$O (200 mL) was added to the resulting solid. The mixture was stirred for 20 min and then cooled to 4 ºC overnight. The solid was filtered and washed with cold Et$_2$O (2 x 100 mL). The resulting solid was dehydrated by refluxing toluene (200 mL) in a Dean-Stark system overnight. Solvent evaporation afforded a beige solid (16.51 g, 52% yield).

1H NMR (400 MHz, CDCl$_3$) δ: 2.75 (dd, *J* = 15.4, 3.8 Hz, 4H), 2.89 (dd, *J* = 15.2, 4.8 Hz, 4H), 3.36 (p, *J* = 4.4 Hz, 2H), 7.33 (m, 4H).

14, synthesis of (5s,9s)-7,11-dimethylene-6,7,8,9-tetrahydro-5H-5,9-propanobenzo[7]annulene
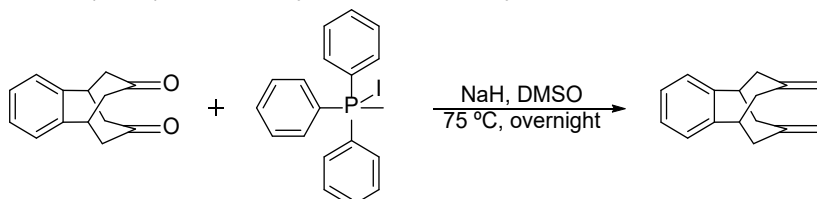
A suspension of sodium hydride 60% dispersion in mineral oil (3.51 g, 87.75 mmol) in anh. DMSO (170 mL) was heated to 75 ºC for 45 min under Ar atmosphere. After the reaction mixture was tempered, triphenylmethylphophonium iodide (37.00 g, 77.03 mmol, 80% purity) was added and the resulting yellow solution was stirred at room temperature for 20 min. Then, a suspension of (5s,9s)-5,6,8,9-tetrahydro-7H-5,9-propanobenzo[7]annulene-7,11-dione (7.84 g, 36.59 mmol) in anh. DMSO (20 mL) was added and the obtained solution was heated to 75 ºC overnight. The resulting black solution was allowed to cool to room temperature and poured into water (200 mL). A mixture of hexane/EtOAc 9/1 (150 mL) was added and the phases were separated. The aqueous phase was extracted with further hexane/EtOAc 9/1 (2 x 150 mL) and the combined organic phases were dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 5%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a colorless liquid (5.41 g, 70% yield).

1H NMR (400 MHz, CDCl$_3$) δ: 2.48 (dd, *J* = 14.0 Hz, *J'* = 5.1 Hz, 4H), 2.60 (dd, *J* = 14.0 Hz, *J'* = 4.2 Hz, 4H), 3.09 (p, *J* = 4.6 Hz, 2H), 4.67 (s, 4H), 7.07 (m, 2H), 7.12 (m, 2H).

15, synthesis of 2-chloro-*N*-(9-methyl-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)acetamide
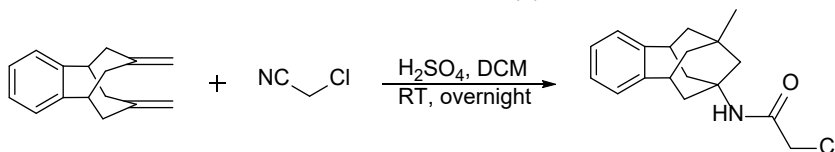
To a solution of (5s,9s)-7,11-dimethylene-6,7,8,9-tetrahydro-5H-5,9-propanobenzo[7]annulene (5.41 g, 25.72 mmol) in DCM (40 mL) was added chloroacetonitrile (1.79 mL, 2.14 g, 28.29 mmol) and the mixture was cooled to 0 ºC with an ice-bath. Then, conc. H$_2$SO$_4$ (2.06 mL, 3.78 g, 38.58 mmol) was added dropwise without reaching a temperature greater than 10 ºC. After the addition, the reaction mixture was allowed to reach room temperature and stirred overnight. Water (10 mL) was added and the mixture was stirred for 10 min more. Solvent was concentrated *in vacuo* and Na$_2$CO$_3$ sat. was added until pH = 7 followed by EtOAc (150 mL). The phases were separated and the aqueous phase was extracted again with EtOAc (100 mL). The combined organic phases were dried over Na$_2$SO$_4$ anh., filtered and evaporated *in vacuo* to afford a white solid that was used as such without further purification (4.42 g, 57% yield).

¹H NMR (400 MHz, CDCl$_3$) δ: 0.93 (s, 3H), 1.58 (m, 2H), 1.67 (dd, *J* = 13.8 Hz, *J'* = 5.7 Hz, 2H), 1.85 (s, 2H), 2.08 (m, 2H), 2.17 (dd, *J* = 12.2 Hz, *J'* = 5.7 Hz, 2H), 3.09 (t, *J* = 6.2 Hz, 2H), 3.93 (s, 2H), 6.30 (broad s, 1H), 7.06 (dq, *J* = 6.3 Hz, *J'* = 4.6 Hz, 4H).

16, synthesis of 9-methyl-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine
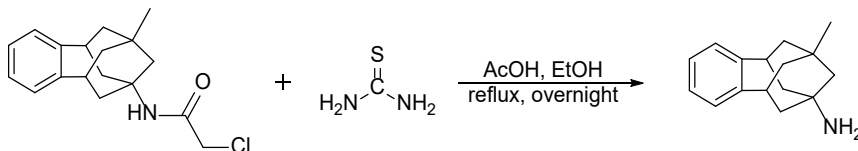
To a solution of 2-chloro-*N*-(9-methyl-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)acetamide (4.42 g, 14.55 mmol) in ethanol (100 mL) were added thiourea (1.33 g, 17.46 mmol) and glacial acetic acid (10.0 mL) and the mixture was heated at reflux overnight. The resulting suspension was then tempered to room temperature and concentrated *in vacuo*. Water (200 mL) was added and the pH adjusted to ~12 with NaOH 2 M. A mixture of EtOAc/MeOH 9/1 (200 mL) was added, phases were separated and the aqueous phase was extracted with further EtOAc/MeOH 9/1 (4 x 200 mL). The combined organic layers were dried over Na$_2$SO$_4$ anh., filtered and solvent was concentrated *in vacuo*.

The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 6%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a beige solid (720 mg, 22% yield).

¹H NMR (400 MHz, CD$_3$OD) δ: 0.92 (s, 3H), 1.42 (m, 2H), 1.46 (m, 1H), 1.49 (s, 1H), 1.57-1.68 (complex signal, 4H), 1.77 (m, 2H), 3.06 (t, *J* = 6.0 Hz, 2H), 7.03 (s, 4H).

17, synthesis of (5R,9S)-7-methylene-6,7,8,9-tetrahydro-5H-5,9-propanobenzo[7]annulen-11-one
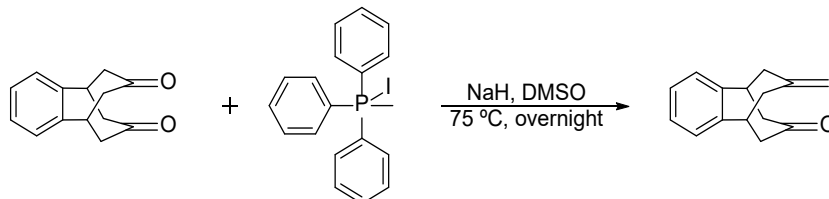
A suspension of sodium hydride 60% dispersion in mineral oil (3.70 g, 92.50 mmol) in anh. DMSO (350 mL) was heated to 75 ºC for 45 min under Ar atmosphere. After the reaction mixture was tempered, triphenylmethylphophonium iodide (42.08 g, 77.03 mmol, 74% purity) was added and the resulting yellow solution was stirred at room temperature for 20 min. Then, a suspension of (5s,9s)-5,6,8,9-tetrahydro-7H-5,9-propanobenzo[7]annulene-7,11-dione (16.50 g, 77.03 mmol) in anh. DMSO (50 mL) was added and the obtained solution was heated to 75 ºC overnight. The resulting black solution was allowed to cool to room temperature and poured into water (400 mL). A mixture of hexane/EtOAc 9/1 (200 mL) was added and the phases were separated. The aqueous phase was extracted with further hexane/EtOAc 9/1 (2 x 100 mL) and the combined organic phases were washed with brine, dried over Na_2SO_4 anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 30%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (10.09 g, 62% yield).

^{1}H NMR (400 MHz, CDCl$_3$) δ: 2.48 (d, *J* = 12.8 Hz, 1H), 2.60 (dd, *J* = 16.0 Hz, *J'* = 3.6 Hz, 1H), 2.68 (dd, *J* = 14.1 Hz, *J'* = 5.2 Hz, 2H), 2.88 (m, 2H), 3.18 (m, 2H), 4.96 (m, 2H), 7.19-7.24 (complex signal, 4H).

18, synthesis of 2-chloro-*N*-(9-hydroxy-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)acetamide
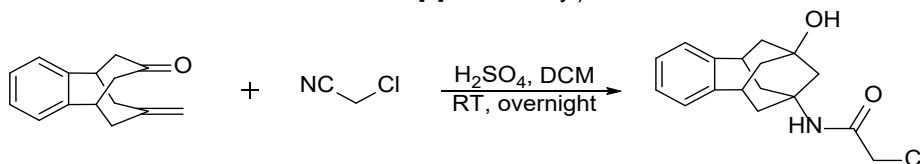
To a solution of of (5R,9S)-7-methylene-6,7,8,9-tetrahydro-5H-5,9-propanobenzo[7]annulen-11-one (10.09 g, 47.52 mmol) in DCM (75.0 mL) was added chloroacetonitrile (3.32 mL, 3.96 g, 52.27 mmol) and the mixture was cooled to 0 ºC with an ice-bath. Then, conc. H_2SO_4 (3.79 mL, 6.94 g, 71.28 mmol) was added dropwise without reaching a temperature greater than 10 ºC. After the addition, the reaction mixture was allowed to reach room temperature and stirred overnight. Water (20 mL) was added and the mixture was stirred for 10 min more. Solvent was concentrated *in vacuo* and NaHCO$_3$ sat. was added until pH = 7 followed by EtOAc (150 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 x 100 mL). The combined organic phases were dried over Na_2SO_4 anh., filtered and concentrated *in vacuo* to afford a beige solid that was used as such without further purification (7.25 g, 50% yield).

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.70 (m, 3H), 1.95 (m, 2H), 2.02-2.19 (complex signal, 5H), 3.20 (m, 2H), 3.95 (s, 2H), 7.10 (s, 4H).

19, synthesis of 2-chloro-*N*-(9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)acetamide
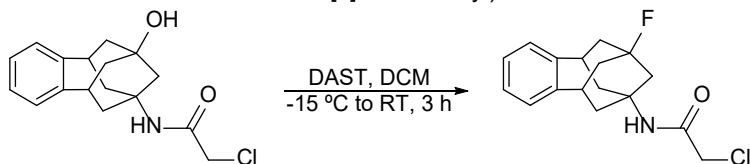
To a stirring solution of 2-chloro-*N*-(9-hydroxy-5,6,8,9,10,11-dimethanobenzo[9]annulen-7-yl)acetamide (4.22 g, 13.80 mmol) in anh. DCM (30.0 mL) at -15 °C, diethylaminosulfur trifluoride (DAST) (2.73 mL, 20.70 mmol) was added dropwise over 5 min. After addition was completed, the mixture was allowed to reach room temperature and it was stirred for 3 h. DCM (20 mL) and water (50 mL) were added to the mixture and layers were separated. The organic layer was washed again with brine (50 mL). The organic layer was then dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting reddish syrup crude was used as such without further purification. Stoichiometric yield (4.25 g, crude).

20, synthesis of 9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine
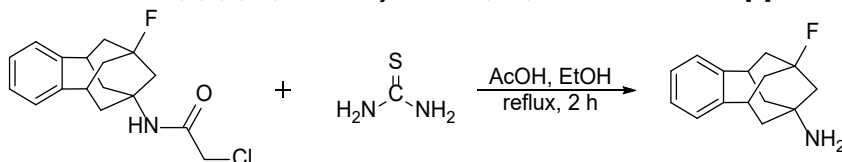
To a solution of chloroacetamide 2-chloro-*N*-(9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)acetamide (4.25 g, 13.80 mmol) in ethanol (80.0 mL) were added thiourea (1.26 g, 16.56 mmol) and glacial acetic acid (3.0 mL) and the mixture was heated at reflux for 2 h. Solvents were half concentrated *in vacuo* and water (100 mL) was added and the pH adjusted to ~12 with NaOH 2 M. The mixture was extracted with EtOAc (3 x 60 mL). The combined organic layers were dried over Na$_2$SO$_4$ anh., filtered and solvent was concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 8%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a beige solid (2.13 g, 67% yield).
¹H NMR (400 MHz, CD$_3$OD) δ: 1.79 (d, *J* = 13.0 Hz, 2H), 1.87 (m, 2H), 2.05 (m, 4H), 2.18 (m, 2H), 3.37 (m, 2H), 7.14 (m, 4H).

21, synthesis of 2-chloro-*N*-(9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)acetamide
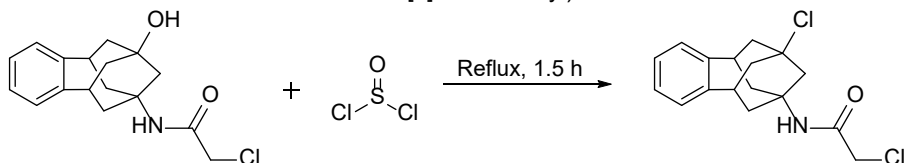
A solution of 2-chloro-*N*-(9-hydroxy-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)acetamide (500 mg, 1.64 mmol) in thionyl chloride (8.0 mL, 13.10 g, 110.15 mmol) was heated at reflux for 1.5 h. The reaction mixture was then cooled to 0 °C under ice-bath and water was added dropwise until no more HCl formation was observed. Na$_2$CO$_3$ was added until pH = 8 and then the aqueous layer was extracted with EtOAc (2 x 30 mL). Both organic layers were joined, dried over

Na$_2$SO$_4$ anh., filtered and evaporated *in vacuo* to afford a reddish syrup that was used as such without further purification. Stoichiometric yield (531 mg, crude).

22, synthesis of 9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine
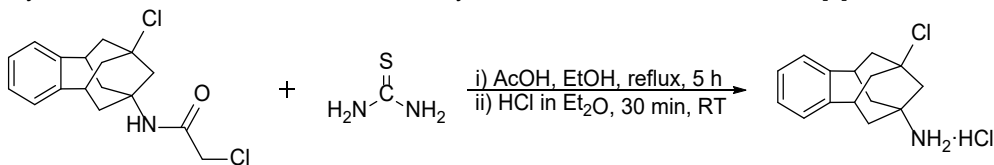
To a solution of chloroacetamide 2-chloro-*N*-(9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)acetamide (1.09 mg, 3.36 mmol) in ethanol (20.0 mL) were added thiourea (307 mg, 4.03 mmol) and glacial acetic acid (2.0 mL) and the mixture was heated at reflux for 5 h. The resulting suspension was then tempered to room temperature, water (50 mL) was added and the pH adjusted to ~12 with Na$_2$CO$_3$ sat. EtOAc (100 mL) was added, the phases were separated and the aqueous phase was extracted with further EtOAc (2 x 100 mL). The combined organic layers were dried over Na$_2$SO$_4$ anh., filtered and solvent was concentrated *in vacuo*.

HCl 2 M in Et$_2$O (4.0 mL) was added and the mixture was stirred for 30 min at room temperature. Then, the precipitate was filtered and washed with further Et$_2$O to afford an orange solid (820 mg, 86% yield).

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.86 (d, *J* = 13.0 Hz, 2H), 2.10 (m, 2H), 2.15 (d, *J* = 13.3 Hz, 2H), 2.32 (s, 2H), 2.32 (m, 2H), 3.34 (m, 2H), 7.08-7.19 (complex signal, 4H).

25, synthesis of 4-(4-aminopiperidin-1-yl)benzoic acid dihydrochloride
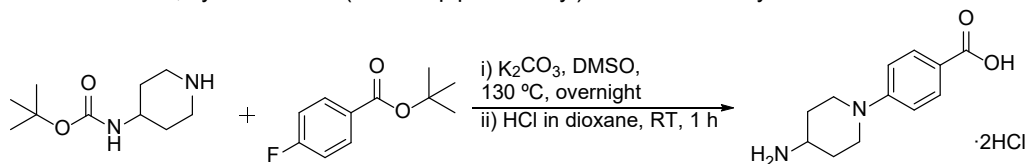
To a solution of tert-butyl piperidin-4-ylcarbamate (178 mg, 0.89 mmol) and tert-butyl 4-fluorobenzoate (145 mg, 0.74 mmol) in DMSO (1.5 mL) was added K$_2$CO$_3$ (184 mg, 1.33 mmol) and the mixture was stirred at 130 ºC overnight. EtOAc (15 mL) was added and the mixture was washed with Na$_2$CO$_3$ sat. (2 x 25 mL). The organic layer was dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 12%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid.

HCl 4 M in dioxane (2.0 mL, 8.00 mmol) with some drops of water were added to the solid and the mixture was stirred at room temperature overnight. Solvent was concentrated *in vacuo* to afford an orangish solid (107 mg, 50% yield).

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.87 (td, *J* = 12.3 Hz, *J'* = 3.9 Hz, 2H), 2.16 (d, *J* = 12.8 Hz, 2H), 3.20 (t, *J* = 13.1 Hz, 2H), 3.44 (m, 1H), 4.00 (d, *J* = 13.1 Hz, 2H), 7.22 (d, *J* = 8.6 Hz, 2H), 7.97 (d, *J* = 8.7 Hz, 2H).

27, synthesis of methyl 3-bromo-4-(4-((tert-butoxycarbonyl)amino)piperidin-1-yl)benzoate
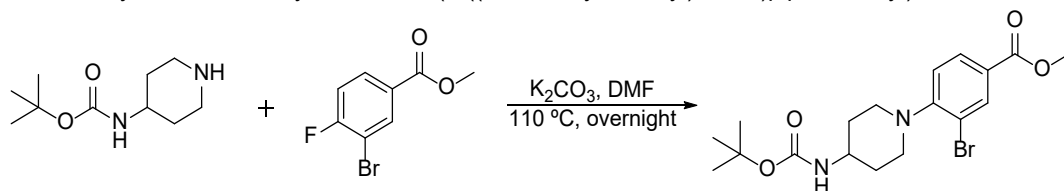
To a solution of tert-butyl piperidin-4-ylcarbamate (300 mg, 1.50 mmol) and methyl 3-bromo-4-fluorobenzoate (419 mg, 1.80 mmol) in anh. DMF (4.0 mL) was added K_2CO_3 (414 mg, 3.00 mmol) and the mixture was stirred at 110 ºC overnight. EtOAc (15 mL) was added and the mixture was washed with brine (2 x 25 mL). The organic layer was dried over Na_2SO_4 anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 10%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (377 mg, 61% yield).
¹H NMR (400 MHz, CDCl₃) δ: 1.46 (s, 9H), 1.64 (qd, *J* = 11.5 Hz, *J'* = 4.0 Hz, 2H), 2.07 (d, *J* = 10.9 Hz, 2H), 2.81 (t, *J* = 11.4 Hz, 2H), 3.42 (d, *J* = 11.9 Hz, 2H), 3.64 (broad s, 1H), 3.89 (s, 3H), 4.52 (broad s, 1H), 7.02 (d, *J* = 8.4 Hz, 1H), 7.91 (dd, *J* = 8.4 Hz, *J'* = 2.0 Hz, 1H), 8.21 (d, *J* = 2.0 Hz, 1H).

29, synthesis of methyl 4-(4-aminopiperidin-1-yl)-3-cyclopropylbenzoate dihydrochloride
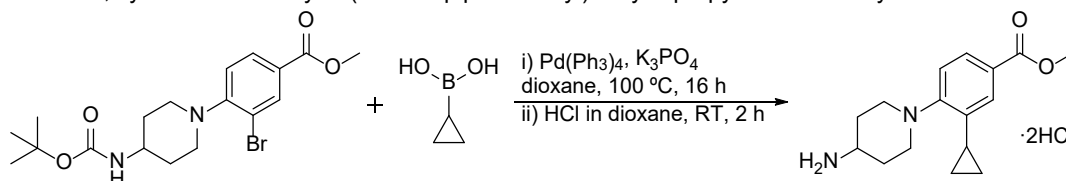
A suspension of methyl 3-bromo-4-(4-((tert-butoxycarbonyl)amino)piperidin-1-yl)benzoate (377 mg, 0.91 mmol), cyclopropylboronic acid (118 mg, 1.37 mmol) and K_3PO_4 (581 mg, 2.74 mmol) in 1,4-dioxane (10.0 mL) was degassed bubbling N_2 for 10 min. Then, tetrakis(triphenylphosphine)palladium(0) (104 mg, 0.09 mmol) was added and the mixture was heated at 100 ºC and stirred for 16 h. The mixture was filtered using a pad with Celite® and EtOAc as eluting agent. Solvents were concentrated *in vacuo* and water was added (20 mL) followed by EtOAc (20 mL) and the mixture was extracted. The organic layer was washed again with brine (20 mL). The organic layer was dried over Na_2SO_4 anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 20%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a beige solid (320 mg).
HCl 4 M in dioxane (2.0 mL, 8.00 mmol) was added to methyl 4-(4-((tert-butoxycarbonyl)amino)piperidin-1-yl)-3-cyclopropylbenzoate (320 mg, 0.85 mmol) and the mixture was stirred at room temperature for 2 h. The mixture was filtered and the solid was washed with Et₂O (2 x 5 mL). The solid was collected and dried under vacuum to afford a beige solid that was used as such without further purification (250 mg, 79% yield).
¹H NMR (400 MHz, DMSO-d6) δ: 0.71 (m, 2H), 1.06 (m, 2H), 1.75 (m, 2H), 1.99-2.11 (complex signal, 3H), 2.76 (t, *J* = 11.7 Hz, 2H), 3.18 (s, 1H), 3.41 (d, *J* = 12.3 Hz, 2H), 3.79 (s, 3H), 7.07 (d, *J* = 8.4 Hz, 1H), 7.35 (m, 1H), 7.71 (dd, *J* = 8.4 Hz, *J'* = 2.1 Hz, 1H), 8.17 (broad s, 2H).

31, synthesis of methyl 4-fluoro-3-(trifluoromethyl)benzoate
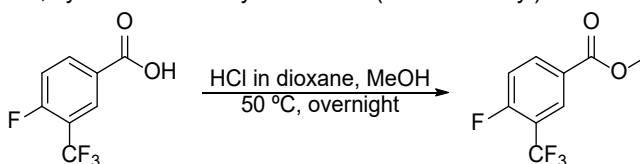
To a solution of tert-butyl piperidin-4-ylcarbamate (300 mg, 1.44 mmol) in MeOH (2.0 mL, 1.58 g, 49.44 mmol) was added HCl 4 M in dioxane (2.0 mL, 8.00 mmol) and the mixture was stirred at 50 ºC overnight. Solvent was concentrated *in vacuo* and EtOAc (15 mL) was added followed by NaHCO$_3$ sat. (20 mL) and the mixture was extracted. The aqueous layer was extracted again with EtOAc (15 mL). Both organic layers were joined, dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was used as such without further purification (280 mg, 87% yield).

32, synthesis of 4-(4-aminopiperidin-1-yl)-3-(trifluoromethyl)benzoic acid dihydrochloride
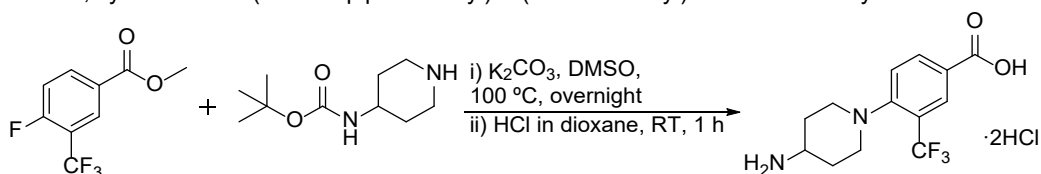
To a solution of tert-butyl piperidin-4-ylcarbamate (210 mg, 1.05 mmol) and methyl 4-fluoro-3-(trifluoromethyl)benzoate (280 mg, 1.26 mmol) in DMSO (4.0 mL) was added K$_2$CO$_3$ (435 mg, 3.13 mmol) and the mixture was stirred at 100 ºC overnight. Water was added (30 mL) and the mixture was extracted with EtOAc (20 mL). The organic layer was washed again with brine (20 mL). Then, it was dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 3%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (123 mg, 30% yield).
^{1}H NMR (400 MHz, CDCl$_3$) δ: 1.46 (s, 9H), 1.54-1.68 (complex signal, 3H), 2.05 (d, *J* = 12.5 Hz, 2H), 2.88 (t, *J* = 11.3 Hz, 2H), 3.23 (m, 2H), 3.62 (s, 1H), 4.52 (s, 1H), 7.29 (d, *J* = 8.6 Hz, 1H), 8.17 (m, 1H), 8.34 (d, *J* = 2.0 Hz, 1H).
HCl 4 M in dioxane (2.0 mL, 8.00 mmol) and dioxane (2.0 mL) were added to methyl 4-(4-((tert-butoxycarbonyl)amino)piperidin-1-yl)-3-(trifluoromethyl)benzoic acid (123 mg, 0.32 mmol) and the mixture was stirred at room temperature for 1 h. Solvent was concentrated *in vacuo* to afford a white solid that was used as such without further purification. Stoichiometric yield (115 mg, crude). Analysis data agrees with bibliography.

33, synthesis of methyl 3-cyclopropyl-4-(4-(3-(9-methyl-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)ureido)piperidin-1-yl)benzoate
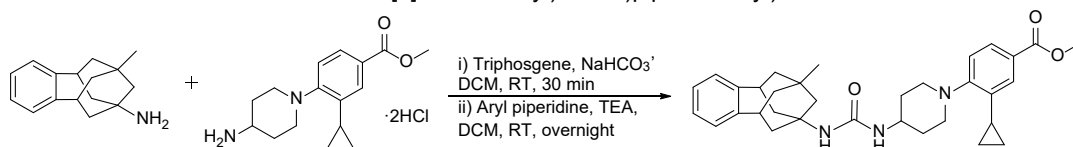
To a stirring biphasic mixture of DCM (8.0 mL), NaHCO$_3$ sat. (8.0 mL) and triphosgene (320 mg, 1.08 mmol) was added 9-methyl-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine

(246 mg, 1.08 mmol) portionwise. The mixture was then stirred at room temperature for 30 min. Phases were separated and the organic layer was dried over Na_2SO_4 anh., filtered and concentrated *in vacuo*. A suspension of methyl 4-(4-aminopiperidin-1-yl)-3-cyclopropylbenzoate dihidrochloride (250 mg, 0.72 mmol) and triethylamine (401 μL, 291 mg, 2.88 mmol) in DCM (4.0 mL) was added and the mixture was stirred at room temperature overnight. Solvent was concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 20%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (55 mg, 14% yield).

Mp: 123-124 ºC

IR (ATR) *v*: 3328, 2905, 2841, 1715, 1634, 1603, 1551, 1494, 1436, 1381, 1298, 1253, 1127, 1067, 984, 929, 832, 773, 758, 739, 631, 606, 569, 559 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 0.79 [m, 2H, 2'(3')-H$_{ax}$], 0.92 (s, 3H, C$\underline{H}_3$), 1.01 [m, 2H, 2'(3')-H$_{eq}$], 1.49-1.61 (complex signal, 4H, 3''(5'')-H$_{ax}$, 10'''(13''')-H$_{ax}$), 1.65 [m, 2H, 10'''(13''')-H$_{eq}$], 1.83 (s, 2H, 8'''-H), 1.97 [d, *J* = 12.8 Hz, 2H, 6'''(12''')-H$_{ax}$], 2.06 [m, 2H, 3''(5'')-H$_{eq}$], 2.10-2.20 [complex signal, 3H, 1'-H, 6'''(12''')-H$_{eq}$], 2.81 [m, 2H, 2''(6'')-H$_{ax}$], 3.07 [broad t, *J* = 6.2 Hz, 2H, 5'''(11''')-H], 3.37 [broad d, *J* = 12.4 Hz, 2H, 2''(6'')-H$_{eq}$], 3.69 (m, 1H, 4''-H), 3.86 (s, 3H, OC$\underline{H}_3$), 4.06-4.12 (complex signal, 2H, N$\underline{H}$CON$\underline{H}$), 6.96 (d, *J* = 8.4 Hz, 1H, 5-H), 7.02-7.09 [complex signal, 4H, 1'''(4''')-H, 2'''(3''')-H], 7.45 (d, *J* = 2.0 Hz, 1H, 2-H), 7.76 (dd, *J* = 8.4 Hz, *J'* = 2.1 Hz, 1H, 6-H).

^{13}C NMR (101 MHz, CDCl$_3$) δ: 9.6 [CH$_2$, C2'(3')], 11.5 (CH, C1'), 32.4 (CH$_3$, $\underline{C}$H$_3$), 33.6 [CH$_2$, C3''(5'')], 33.9 (C, C9'''), 40.1 [CH$_2$, C6'''(12''')], 41.3 [CH, C5'''(11''')], 41.4 [CH$_2$, C10'''(13''')], 47.4 [d, *J* = 9.8 Hz, CH, C4''], 48.2 (CH$_2$, C8'''), 51.1 [CH$_2$, C2''(6'')], 52.0 (CH$_3$, O$\underline{C}$H$_3$), 53.8 (d, *J* = 11.7 Hz, C, C7'''), 118.1 (CH, C5), 124.2 (C, C1), 125.6 (CH, C2), 126.4 [CH, C2'''(3''')], 127.8 (CH, C6), 128.1 [CH, C1'''(4''')], 136.9 (C, C3), 146.5 [C, C4'''a(11'''a)], 156.3 (C, C4), 156.6 (C, NH$\underline{C}$ONH), 167.4 (C, $\underline{C}$O$_2$CH$_3$).

Anal. calcd for C$_{33}$H$_{41}$N$_3$O$_3$: C 75.11, H 7.83, N 7.96. Calcd for C$_{33}$H$_{41}$N$_3$O$_3$ · 0.35 CH$_2$Cl$_2$: C 71.86, H 7.54, N 7.54. Found: C 71.96, H 7.52, N 7.36.

HRMS: Calcd for [C$_{33}$H$_{41}$N$_3$O$_3$+H]$^+$: 528.3225, found: 528.3221.

34, synthesis of 3-cyclopropyl-4-(4-(3-(9-methyl-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)ureido)piperidin-1-yl)benzoic acid
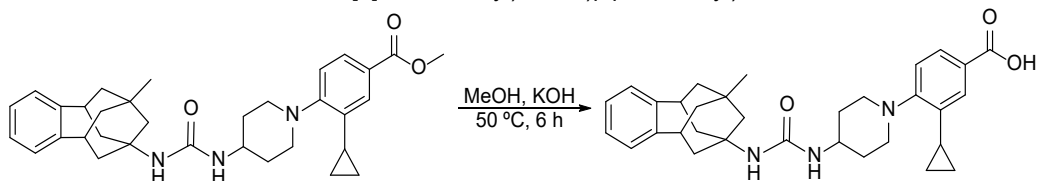
To a solution of methyl 3-cyclopropyl-4-(4-(3-(9-methyl-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)ureido)piperidin-1-yl)benzoate (30 mg, 0.06 mmol) in MeOH (0.8 mL) and water (0.2 mL) was added KOH (32 mg, 0.57 mmol) and the mixture was stirred at 50 ºC for 6 h. Water (5 mL) was added followed by HCl 2 M until pH = 3. The mixture was extracted with DCM (2 x 10 mL). Both organic layers were joined, dried over Na$_2$SO$_4$ anh., filtered and concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 70%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford an off-white solid (24 mg, 82% yield).

Mp: 199-200 ºC

IR (ATR) *v*: 3340, 2919, 2839, 1683, 1632, 1604, 1553, 1499, 1451, 1379, 1303, 1254, 1223, 1130, 1093, 1020, 934, 913, 831, 758, 717, 653, 630, 570 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 0.74 [m, 2H, 2'(3')-H$_{ax}$], 0.92 (s, 3H, C$\underline{H}_3$), 1.03 [m, 2H, 2'(3')-H$_{eq}$], 1.48 [d, *J* = 13.5 Hz, 2H, 10'''(13''')-H$_{ax}$], 1.58 [m, 2H, 3''(5'')-H$_{ax}$], 1.67 [m, 2H, 10'''(13''')-H$_{eq}$], 1.72 (s, 2H,

8'''-H), 1.98 [m, 2H, 3''(5'')-H$_{eq}$], 2.02-2.15 [complex signal, 4H, 6'''(12''')-H$_2$], 2.21 (m, 1H, 1'-H), 2.83 [m, 2H, 2''(6'')-H$_{ax}$], 3.05 [broad t, J = 6.1 Hz, 2H, 5'''(11''')-H], 3.36 [m, 2H, 2''(6'')-H$_{eq}$], 3.61 (m, 1H, 4''-H), 7.02-7.04 [complex signal, 4H, 1'''(4'')-H, 2''(3''')-H], 7.05 (d, J = 7.2 Hz, 1H, 5-H), 7.45 (s, 1H, 2-H), 7.75 (d, J = 7.2 Hz, 1H, 6-H).

^{13}**C NMR** (101 MHz, CD$_3$OD) δ: 9.9 [CH$_2$, C2'(3')], 12.2 (CH, C1'), 32.9 (CH$_3$, CH$_3$), 34.3 [CH$_2$, C3''(5'')], 34.6 (C, C9'''), 41.0 [CH$_2$, C6'''(12''')], 42.6 [CH, C5'''(11''') and CH$_2$, C10'''(13''')], 47.6 (CH, C4''), 49.3 (signal overlapped, CH$_2$, C8''), 52.0 [CH$_2$, C2''(6'')], 54.1 (C, C7'''), 119.1 (CH, C5), 126.5 (CH, C2), 127.3 [CH, C2'''(3''')], 128.9 [CH, C6 and CH, C1'''(4'')], 138.1 (C, C3), 147.7 [C, C4'''a(11'''a)], 158.0 (C, C4), 159.4 (C, NHCONH). The signal from C1 was not observed.

Anal. calcd for C$_{32}$H$_{39}$N$_3$O$_3$: C 74.82, H 7.65, N 8.18. Anal. calcd for C$_{32}$H$_{39}$N$_3$O$_3$ · 1 H$_2$O: C 72.29, H 7.77, N 7.9. Found: C 71.96, H 7.41, N 7.59.

HRMS: Calcd for [C$_{32}$H$_{39}$N$_3$O$_3$+H]$^+$: 514.3061, found: 514.3064.

35, synthesis of 4-(4-(3-(9-methyl-6,7,8,9,10,11-hexahydro-5H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)ureido)piperidin-1-yl)benzoic acid
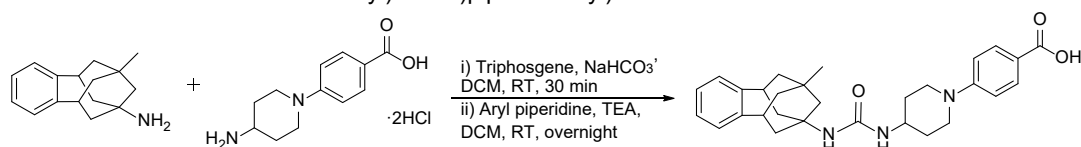
To a stirring biphasic mixture of DCM (4.0 mL), NaHCO$_3$ sat. (4.0 mL) and triphosgene (170 mg, 0.57 mmol) was added 9-methyl-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine (130 mg, 0.57 mmol) portionwise. The mixture was then stirred at room temperature for 30 min. Phases were separated and the organic layer was dried over Na$_2$SO$_4$ anh. and filtered. Solvent was concentrated *in vacuo* until 4 mL were left.

A suspension of 4-(4-aminopiperidin-1-yl)benzoic acid dihydrochloride (84 mg, 0.29 mmol) and triethylamine (299 μL, 217 mg, 2.15 mmol) in DCM (1.0 mL) was added to the previous solution and the mixture was stirred at room temperature overnight. Water (10 mL) was added to the mixture and layers were separated. The aqueous layer was extracted again with DCM (15 mL x 2). All the organic layers were joined, dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 2%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a beige solid (15 mg, 11% yield).

Mp: 254-255 ºC

IR (ATR) *v*: 3343, 2923, 2212, 1665, 1639, 1592, 1555, 1500, 1443, 1283, 1225, 1194, 1121, 1097, 999, 957, 915, 770, 758, 719, 622, 584, 569 cm^{-1}.

^{1}**H NMR** (400 MHz, CD$_3$OD) δ: 0.92 (s, 3H, CH$_3$), 1.36-1.54 [complex signal, 4H, 3'(5')-H$_{ax}$, 10''(13'')-H$_{ax}$], 1.65 [m, 2H, 10''(13'')-H$_{eq}$], 1.70 (s, 2H, 8''-H), 1.93 [m, 2H, 3'(5')-H$_{eq}$], 1.99-2.19 [complex signal, 4H, 6''(12'')-H$_2$], 2.98 [t, J = 11.0 Hz, 2H, 2'(6')-H$_{ax}$], 3.05 [broad t, J = 6.8 Hz, 2H, 5''(11'')-H], 3.63 (m, 1H, 4'-H), 3.81 [broad d, J = 13.2 Hz, 2H, 2'(6')-H$_{eq}$], 6.94 [d, J = 8.2 Hz, 2H, 3(5)-H], 7.03 [s, 4H, 1''(4'')-H, 2''(3'')-H], 7.85 [d, J = 8.5 Hz, 2H, 2(6)-H].

^{13}**C NMR** (101 MHz, CD$_3$OD) δ: 32.9 (CH$_3$, CH$_3$), 33.2 [CH$_2$, C3'(5')], 34.5 (C, C9''), 41.0 [CH$_2$, C6''(12'')], 42.6 [CH, C5''(11'') and CH$_2$, C10''(13'')], 47.7 (CH, C4'), 47.9 [CH$_2$, C2'(6')], 49.3 (signal overlapped, CH$_2$, C8''), 54.1 (C, C7''), 115.0 [CH, C3(5)], 127.3 [CH, C2''(3'')], 129.0 [CH, C1''(4'')], 132.6 [CH, C2(6)], 147.7 [C, C4''a(11''a)], 155.6 (C, C4), 159.4 (C, NHCONH). The signal from C1 and CO$_2$H was not observed.

Anal. calcd for C$_{29}$H$_{35}$N$_3$O$_3$: C 73.54, H 7.45, N 8.87. Anal. calcd for C$_{29}$H$_{35}$N$_3$O$_3$ · 1.75 H$_2$O · 0.2 CH$_4$O: C 68.56, H 7.74, N 8.21. Found: C 69.01, H 7.20, N 7.67.

HRMS: Calcd for $[C_{29}H_{35}N_3O_3+H]^+$: 474.2761, found: 474.2751.

36, synthesis of 3-cyclopropyl-4-(4-(3-(9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)ureido)piperidin-1-yl)benzoic acid
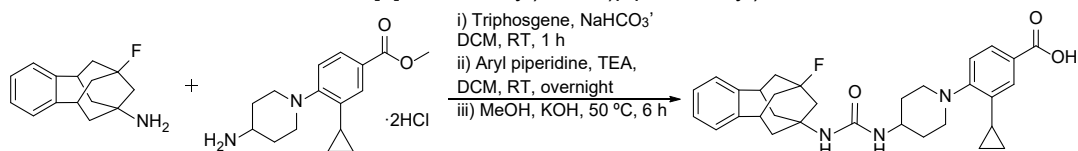
To a stirring biphasic mixture of DCM (3.0 mL), NaHCO₃ sat. (3.0 mL) and triphosgene (160 mg, 0.54 mmol) was added 9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine (125 mg, 0.54 mmol) portionwise. The mixture was then stirred at room temperature for 1 h. Phases were separated and the organic layer was dried over Na₂SO₄ anh., filtered and concentrated *in vacuo*. A suspension of 4-(4-aminopiperidin-1-yl)-3-cyclopropylbenzoate dihydrochloride (120 mg, 0.35 mmol) in DCM (3.0 mL) was added followed by triethylamine (151 μL, 109 mg, 1.08 mmol) and the mixture was stirred at room temperature overnight. Solvent was concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 3%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a yellowish solid (54 mg).

After that, to a solution of methyl 3-cyclopropyl-4-(4-(3-(9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)ureido)piperidin-1-yl)benzoate (54 mg, 0.10 mmol) in MeOH (0.8 mL) and water (0.2 mL) was added KOH (58 mg, 0.57 mmol) and the mixture was stirred at 50 ºC for 6 h. Water (5 mL) was added followed by HCl 2 M until pH = 3. The mixture was extracted with DCM (2 x 10 mL). Both organic layers were joined, dried over Na₂SO₄ anh., filtered and concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 65%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford an off-white solid (14 mg, 8% yield).

Mp: 166-167 ºC

IR (ATR) *v*: 3339, 2928, 2854, 1602, 1554, 1500, 1442, 1305, 1255, 1223, 1178, 1119, 1098, 1043, 1012, 916, 869, 760, 718, 652, 622, 570 cm⁻¹.

¹H NMR (400 MHz, CD₃OD) δ: 0.74 [m, 2H, 2'(3')-H$_{ax}$], 1.03 [m, 2H, 2'(3')-H$_{eq}$], 1.58 [m, 2H, 3''(5'')-H$_{ax}$], 1.82 [broad d, J = 12.0 Hz, 2H, 10'''(13''')-H$_{ax}$], 1.93-2.04 [complex signal, 4H, 3''(5'')-H$_{eq}$, 6'''(12''')-H$_{ax}$], 2.07-2.25 [complex signal, 7H, 1'-H, 6'''(12''')-H$_{eq}$, 8'''-H, 10'''(13''')-H$_{eq}$], 2.83 [t, J = 10.3 Hz, 2H, 2''(6'')-H$_{ax}$], 3.24 [broad t, J = 7.1 Hz, 2H, 5'''(11''')-H], 3.36 [m, 2H, 2''(6'')-H$_{eq}$], 3.62 (td, J = 10.2 Hz, J' = 4.9 Hz, 1H, 4''-H), 7.04 (d, J = 8.3 Hz, 2H, 5-H), 7.09 [s, 4H, 1'''(4''')-H, 2'''(3''')-H], 7.45 (d, J = 2.0 Hz, 1H, 2-H), 7.75 (dd, J = 8.3, 2.0 Hz, 1H, 6-H).

¹³C NMR (101 MHz, CD₃OD) δ: 9.9 [CH₂, C2'(3')], 12.2 (CH, C1'), 34.3 [CH₂, C3''(5'')], 40.5 [CH₂, C6'''(12''')], 41.1 [d, $^3J_{CF}$ = 13.2 Hz, CH, C5'''(11''')], 41.5 [d, $^2J_{CF}$ = 20.1 Hz, CH₂, C10'''(13''')], 47.6 (CH, C4''), 47.9 (d, $^2J_{CF}$ = 17.8 Hz, CH₂, C8'''), 52.0 [CH₂, C2''(6'')], 57.6 (d, $^3J_{CF}$ = 11.3 Hz, C, C7'''), 95.0 (d, $^1J_{CF}$ = 176.9 Hz, C, C9'''), 119.1 (CH, C5), 125.7 (C, C1), 126.5 (CH, C2), 127.9 [CH, C2'''(3''')], 129.0 (CH, C6), 129.1 [CH, C1'''(4''')], 138.2 (C, C3), 146.4 [C, C4'''a(11'''a)], 158.1 (C, C4), 159.2 (C, NH$\underline{C}$ONH), 170.3 (C, $\underline{C}$O₂H).

Anal. calcd for C₃₁H₃₆FN₃O₃: C 71.93, H 7.01, N 8.12. Anal. calcd for C₃₁H₃₆FN₃O₃ · 1.3 H₂O: C 68.82, H 7.19, N 7.77. Found: C 68.89, H 6.95, N 7.52.

HRMS: Calcd for $[C_{31}H_{36}FN_3O_3+H]^+$: 518.2813, found: 518.2806.

37, synthesis of 4-(4-(3-(9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)ureido)piperidin-1-yl)-3-(trifluoromethyl)benzoic acid
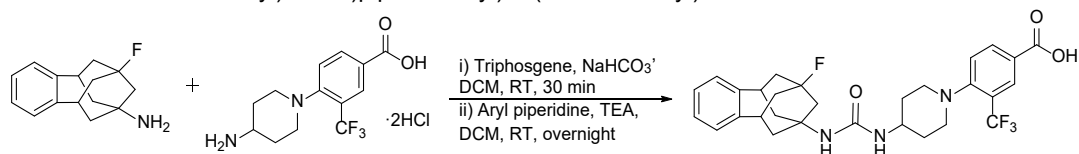
To a stirring biphasic mixture of DCM (4.0 mL), NaHCO$_3$ sat. (4.0 mL) and triphosgene (246 mg, 0.83 mmol) was added 9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine (191 mg, 0.83 mmol) portionwise. The mixture was then stirred at room temperature for 30 min. Phases were separated and the organic layer was dried over Na$_2$SO$_4$ anh., filtered and concentrated *in vacuo*. A suspension of 4-(4-aminopiperidin-1-yl)-3-(trifluoromethyl)benzoic acid dihydrochloride (200 mg, 0.55 mmol) in DCM (5.0 mL) was added followed by triethylamine (230 µL, 167 mg, 1.65 mmol) and the mixture was stirred at room temperature overnight. Solvent was concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 5%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a yellowish solid (16 mg, 5% yield).

Mp: 242-243 ºC

IR (ATR) *v*: 3339, 2925, 2851, 1608, 1554, 1500, 1452, 1306, 1252, 1223, 1176, 1119, 1097, 1044, 1012, 924, 868, 758, 719, 669, 622, 570 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.55 [qd, *J* = 10.7 Hz, *J'* = 3.7 Hz, 2H, 3'(5')-H$_{ax}$], 1.81 [broad d, *J* = 11.3 Hz, 2H, 10''(13'')-H$_{ax}$], 1.89-2.06 [complex signal, 4H, 3'(5')-H$_{eq}$, 6''(12'')-H$_{ax}$], 2.09-2.21 [complex signal, 6H, 6''(12'')-H$_{eq}$, 8''-H$_2$, 10''(13'')-H$_{eq}$], 2.89 [t, *J* = 10.2 Hz, 2H, 2'(6')-H$_{ax}$], 3.15 [m, 2H, 2'(6')-H$_{eq}$], 3.24 [broad t, *J* = 6.6 Hz, 2H, 5''(11'')-H], 3.59 (td, *J* = 10.5 Hz, *J'* = 5.3 Hz, 1H, 4'-H), 7.09 [s, 4H, 1'''(4''')-H, 2'''(3''')-H], 7.47 (d, *J* = 8.4 Hz, 1H, 5-H), 8.17 (dd, *J* = 8.4 Hz, *J'* = 1.9 Hz, 1H, 6-H), 8.23 (s, 1H, 2-H).

^{13}C NMR (101 MHz, CD$_3$OD) δ: 34.2 [CH$_2$, C3'(5')], 40.5 [CH$_2$, C6''(12'')], 41.1 [d, $^3J_{CF}$ = 13.2 Hz, CH, C5''(11'')], 41.4 [d, $^2J_{CF}$ = 20.1 Hz, CH$_2$, C10''(13'')], 47.4 (CH, C4'), 47.9 (d, *J* = 17.8 Hz, CH$_2$, C8''), 53.7 [CH$_2$, C2'(6')], 57.6 (d, $^3J_{CF}$ = 11.3 Hz, C, C7''), 95.0 (d, $^1J_{CF}$ = 176.9 Hz, C, C9''), 124.6 (CH, C5), 125.3 (q, $^1J_{CF}$ = 273.1 Hz, C, $\underline{C}$F$_3$), 126.6 (q, $^2J_{CF}$ = 29.3 Hz, C, C3), 127.9 [CH, C2''(3'')], 129.1 [CH, C1''(4'')], 130.2 (CH, C2), 135.4 (CH, C6), 146.4 [C, C4''a(11''a)], 158.1 (C, C4), 159.1 (C, NH$\underline{C}$ONH), 168.2 (C, $\underline{C}$O$_2$H). The signal from C1 was not observed.

Anal. calcd for C$_{29}$H$_{31}$F$_4$N$_3$O$_3$: C 63.84, H 5.73, N 7.70. Anal. calcd for C$_{29}$H$_{31}$F$_4$N$_3$O$_3$ · 0.55 C$_4$H$_{10}$O · 0.85 H$_2$O: C 62.29, H 6.40, N 6.98. Found: C 62.46, H 6.06, N 6.64.

HRMS: Calcd for [C$_{29}$H$_{31}$F$_4$N$_3$O$_3$+H]$^+$: 546.2374, found: 549.2363.

38, synthesis of 4-(4-(3-(9-fluoro-6,7,8,9,10,11-hexahydro-5H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)ureido)piperidin-1-yl)benzoic acid
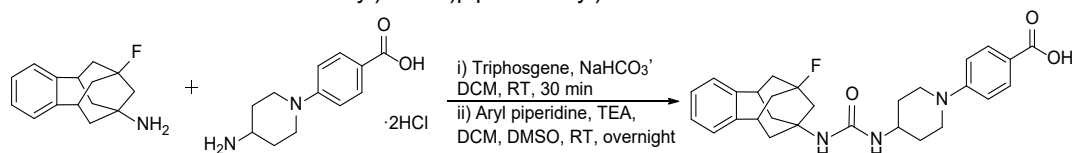
To a stirring biphasic mixture of DCM (3.0 mL), NaHCO$_3$ sat. (3.0 mL) and triphosgene (60 mg, 0.20 mmol) was added a suspension of 9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine (110 mg, 0.48 mmol) in DCM (1.0 mL). The mixture was then stirred at room temperature for 30 min. Phases were separated and the organic layer was dried over Na$_2$SO$_4$ anh., filtered and concentrated *in vacuo*.

DCM (3.0 mL) was added to the previous crude and then it was added onto a suspension of 4-(4-aminopiperidin-1-yl)benzoic acid dihydrochloride (154 mg, 0.70 mmol) and triethylamine (220 µL, 159 mg, 1.58 mmol) in DMSO (3.0 mL) and the mixture was stirred at room temperature overnight. Water (10 mL) was added and layers were separated. The aqueous layer was extracted again with EtOAc/MeOH 9/1 (15 mL x 2). All the organic layers were joined, dried over Na_2SO_4 anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 6%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a beige solid (157 mg, 80% yield).

Mp: 267-268 ºC

IR (ATR) *v*: 3334, 2928, 2851, 1675, 1602, 1555, 1520, 1306, 1224, 1183, 1120, 1098, 1045, 1010, 867, 771, 752, 719, 618, 570 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.44 [dtd, *J* = 12.6 Hz, *J'* = 10.7 Hz, *J''* = 3.8 Hz, 2H, 3'(5')-H$_{ax}$], 1.81 [d, *J* = 12.0 Hz, 2H, 10''(13'')-H$_{ax}$], 1.89-2.03 [complex signal, 4H, 3'(5')-H$_{eq}$, 6''(12'')-H$_{ax}$], 2.08-2.19 [complex signal, 6H, 6''(12'')-H$_{eq}$, 8''-H$_2$, 10''(13'')-H$_{eq}$], 2.99 [ddd, *J* = 13.5 Hz, *J'* = 11.1 Hz, *J''* = 2.8 Hz, 2H, 2'(6')-H$_{ax}$], 3.23 [broad t, *J* = 7.2 Hz, 2H, 5''(11'')-H], 3.65 (m, 1H, 4'-H), 3.80 [broad dt, *J* = 13.5, 4.0 Hz, 2H, 2'(6')-H$_{eq}$], 6.94 [d, *J* = 8.4 Hz, 2H, 3(5)-H], 7.09 [s, 4H, 1''(4'')-H, 2''(3'')-H], 7.85 [d, *J* = 8.6 Hz, 2H, 2(6)-H].

^{13}C NMR (101 MHz, CD$_3$OD) δ: 33.1 [CH$_2$, C3'(5')], 40.4 [CH$_2$, C6''(12'')], 41.1 [d, $^3J_{CF}$ = 13.2 Hz, CH, C5''(11'')], 41.4 [d, $^2J_{CF}$ = 20.0 Hz, CH$_2$, C10''(13'')], 47.8 (CH, C4' and CH$_2$, C8''), 47.9 [CH$_2$, C2'(6')], 57.6 (d, $^3J_{CF}$ = 11.2 Hz, C, C7''), 95.0 (d, $^1J_{CF}$ = 176.9 Hz, C, C9''), 115.0 [CH, C3(5)], 127.9 [CH, C2''(3'')], 129.1 [CH, C1''(4'')], 132.5 [CH, C2(6)], 146.4 [C, C4''a(11''a)], 155.6 (C, C4), 159.1 (C, NH<u>C</u>ONH), 170.5 (C, <u>C</u>O$_2$H). The signal from C1 was not observed.

Anal. calcd for C$_{28}$H$_{32}$FN$_3$O$_3$: C 70.42, H 6.75, N 8.80. Anal. calcd for C$_{28}$H$_{32}$FN$_3$O$_3$ · 1.4 H$_2$O: C 66.89, H 6.98, N 8.36. Found: C 66.76, H 6.61, N 8.00.

HRMS: Calcd for [C$_{28}$H$_{32}$FN$_3$O$_3$+H]$^+$: 478.2500, found: 478.2523.

39, synthesis of 4-(4-(3-(9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)ureido)piperidin-1-yl)benzoic acid
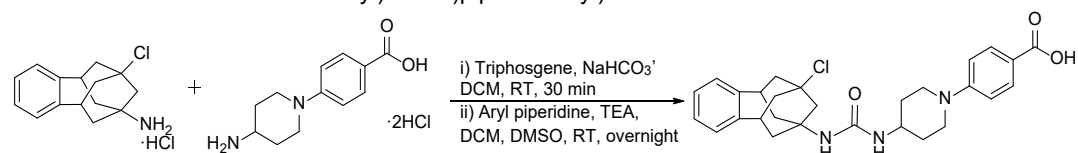
To a stirring biphasic mixture of DCM (2.5 mL), NaHCO$_3$ sat. (2.5 mL) and 9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine hydrochloride (110 mg, 0.39 mmol) was added triphosgene (80 mg, 0.27 mmol). The mixture was then stirred at room temperature for 30 min. DCM (10 mL) and brine (10 mL) were added subsequently and phases were separated. The organic layer was dried over Na$_2$SO$_4$ anh., filtered and concentrated *in vacuo*.

DCM (3.0 mL) was added to the previous crude and then it was added onto a suspension of 4-(4-aminopiperidin-1-yl)benzoic acid dihydrochloride (148 mg, 0.50 mmol) and triethylamine (210 µL, 153 mg, 1.51 mmol) in DMSO (3.0 mL) and the mixture was stirred at room temperature overnight. EtOAc (10 mL) and water (10 mL) were added followed by HCl 2 M until pH = 3. Layers were separated. The aqueous layer was extracted twice again with EtOAc (2 x 15 mL). All organic layers were joined, dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 2%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a brown solid (77 mg, 40% yield).

Mp: 226-227 ºC

IR (ATR) *v*: 2922, 2851, 1672, 1601, 1553, 1518, 1385, 1357, 1221, 1184, 1120, 1089, 1039, 802, 759, 698, 607, 553 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.44 [m, 2H, 3'(5')-H$_{ax}$], 1.90-2.00 [complex signal, 4H, 3'(5')-H$_{eq}$, 6''(12'')-H$_{ax}$], 2.07 [d, *J* = 13.4 Hz, 2H, 10''(13'')-H$_{ax}$], 2.20 [m, 2H, 6''(12'')-H$_{eq}$], 2.39 [m, 2H, 10''(13'')-H$_{eq}$], 2.43 (m, 2H, 8''-H), 3.00 [ddd, *J* = 13.5 Hz, *J'* = 11.2 Hz, *J''* = 2.8 Hz, 2H, 2'(6')-H$_{ax}$], 3.18 [ddd, *J* = 6.6 Hz, *J'* = 5.0 Hz, *J''* = 2.0 Hz, 2H, 5''(11'')-H], 3.65 (tt, *J* = 10.3 Hz, *J'* = 4.1 Hz, 4'-H), 3.81 [broad dt, *J* = 13.7 Hz, *J'* = 4.1 Hz, 2H, 2'(6')-H$_{eq}$], 6.94 [d, *J* = 9.1 Hz, 2H, 3(5)-H], 7.04-7.13 [complex signal, 4H, 1''(4'')-H, 2''(3'')-H], 7.85 [d, *J* = 9.1 Hz, 2H, 2(6)-H].

^{13}C NMR (101 MHz, CD$_3$OD) δ: 33.1 [CH$_2$, C3'(5')], 40.0 [CH$_2$, C6''(12'')], 42.7 [CH, C5''(11'')], 46.0 [CH$_2$, C10''(13'')], 47.8 (CH, C4'), 47.9 [CH$_2$, C2'(6')], 52.1 (CH$_2$, C8''), 56.3 (C, C7''), 70.5 (C, C9''), 114.9 [CH, C3(5)], 120.4 (C, C1), 127.9 [CH, C2''(3'')], 129.1 [CH, C1''(4'')], 132.5 [CH, C2(6)], 146.3 [C, C4''a(11''a)], 155.6 (C, NH$\underline{C}$ONH), 159.1 (C, C4), 170.3 (C, $\underline{C}$O$_2$H).

Anal. calcd for C$_{28}$H$_{32}$FN$_3$O$_3$: C 68.07, H 6.53, N 8.51. Anal. calcd for C$_{28}$H$_{32}$FN$_3$O$_3$ · 1.4 H$_2$O: C 66.89, H 6.98, N 8.36. Found: C 62.44, H 6.15, N 7.75.

HRMS: Calcd for [C$_{28}$H$_{32}$FN$_3$O$_3$-H]$^-$: 492.2059, found: 492.2057.

44, synthesis of methyl 3-chloro-4-fluorobenzoate
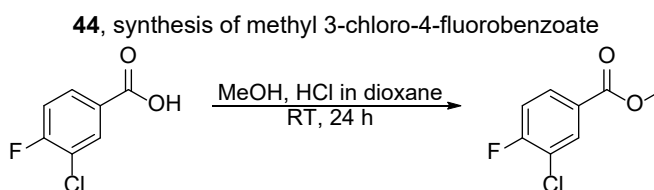
To a solution of 3-chloro-4-fluorobenzoic acid (500 mg, 2.86 mmol) in methanol (12.0 mL, 9.50 g, 296.63 mmol) was added HCl in dioxane 4 M (4.0 mL, 16.00 mmol). The mixture was stirred 24 h at room temperature. Solvent was concentrated *in vacuo*. EtOAc (20 mL) was added and the solution was washed with Na$_2$CO$_3$ sat. (3 x 30 mL). The organic layer was dried over Na$_2$SO$_4$ anh., filtered and solvent was concentrated *in vacuo* to afford a colourless oil that was used as such without further purification (475 mg, 88% yield).

45, synthesis of methyl 2-chloro-4-fluorobenzoate
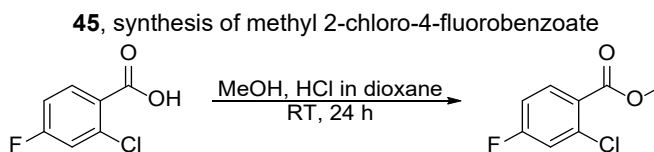
To a solution of 2-chloro-4-fluorobenzoic acid (500 mg, 2.86 mmol) in methanol (12.0 mL, 9.50 g, 296.63 mmol) was added HCl in dioxane 4 M (4.0 mL, 16.00 mmol). The mixture was stirred 24 h at room temperature. Solvent was concentrated *in vacuo*. EtOAc (20 mL) was added and the solution was washed with Na$_2$CO$_3$ sat. (3 x 30 mL). The organic layer was dried over Na$_2$SO$_4$ anh., filtered and solvent was concentrated *in vacuo* to afford a colourless oil that was used as such without further purification (352 mg, 71% yield).

46, synthesis of methyl 4-fluoro-3-(trifluoromethyl)benzoate
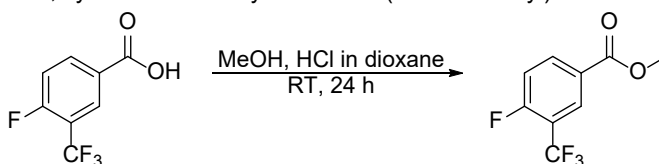
To a solution of 4-fluoro-3-(trifluoromethyl)benzoic acid (500 mg, 2.40 mmol) in methanol (12.0 mL, 9.50 g, 296.63 mmol) was added HCl in dioxane 4 M (4.0 mL, 16.00 mmol). The mixture was stirred 24 h at room temperature. Solvent was concentrated *in vacuo*. EtOAc (20 mL) was added and the solution was washed with Na_2CO_3 sat. (3 x 30 mL). The organic layer was dried over Na_2SO_4 anh., filtered and solvent was concentrated *in vacuo* to afford a colourless oil that was used as such without further purification (400 mg, 75% yield).

47, synthesis of methyl 4-fluoro-2-(trifluoromethyl)benzoate
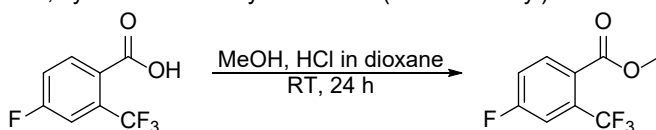
To a solution of 4-fluoro-2-(trifluoromethyl)benzoic acid (500 mg, 2.40 mmol) in methanol (12.0 mL, 9.50 g, 296.63 mmol) was added HCl in dioxane 4 M (4.0 mL, 16.00 mmol). The mixture was stirred 24 h at room temperature. Solvent was concentrated *in vacuo*. EtOAc (20 mL) was added and the solution was washed with Na_2CO_3 sat. (3 x 30 mL). The organic layer was dried over Na_2SO_4 anh., filtered and solvent was concentrated *in vacuo* to afford a colourless oil that was used as such without further purification (318 mg, 60% yield).

49, synthesis of methyl 4-(((1r,4r)-4-aminocyclohexyl)oxy)-3-chlorobenzoate
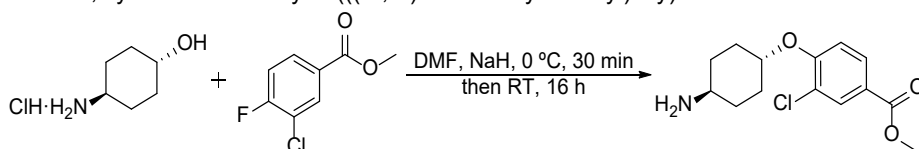
To a solution of trans-4-aminocyclohexanol hydrochloride (420 mg, 2.77 mmol) in anh. DMF (10.0 mL) under ice-bath was added NaH 60% dispersion in mineral oil (302 mg, 7.56 mmol) and the mixture was stirred for 30 min. After that time, methyl 3-chloro-4-fluorobenzoate (475 mg, 2.52 mmol) was added and then stirred at room temperature for 24 h. After that time, water was added until no more bubbling was observed. The resulting suspension was diluted with EtOAc (15 mL) and washed with Na_2CO_3 sat. (20 mL). The aqueous layer was extracted again with EtOAc (2 x 15 mL). All the organic layers were joined, dried over anh. Na_2SO_4, filtered and concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 8%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a colorless oil (88 mg, 11% yield). Spectroscopic data matched with those previously published.

50, synthesis of methyl 4-(((1r,4r)-4-aminocyclohexyl)oxy)-2-chlorobenzoate
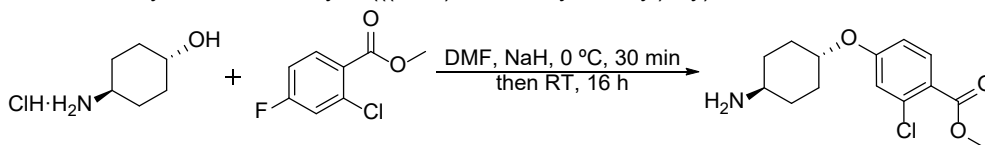
To a solution of trans-4-aminocyclohexanol hydrochloride (309 mg, 2.04 mmol) in anh. THF (12.0 mL) under ice-bath was added NaH 60% dispersion in mineral oil (245 mg, 6.12 mmol) and the mixture was stirred for 30 min. After that time, methyl 2-chloro-4-fluorobenzoate (350 mg, 1.86 mmol) was added and the reaction was stirred at room temperature for 16 h. After that time, water was added under ice bath until no more bubbling was observed. The resulting suspension was diluted with EtOAc (15 mL) and washed with Na_2CO_3 sat. (20 mL). The aqueous layer was extracted again with EtOAc (2 x 15 mL). All the organic layers were joined, dried over anh. Na_2SO_4, filtered and concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 8%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a yellowish oil (204 mg, 39% yield). Analysis data agrees with bibliography.

51, synthesis of methyl 4-(((1r,4r)-4-aminocyclohexyl)oxy)-3-(trifluoromethyl)benzoate
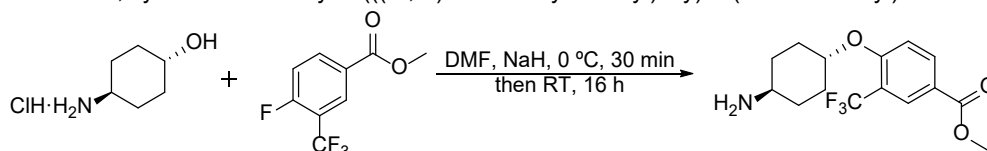
To a solution of trans-4-aminocyclohexanol hydrochloride (375 mg, 2.48 mmol) in anh. THF (12.0 mL) under ice-bath was added NaH 60% dispersion in mineral oil (297 mg, 7.43 mmol) and the mixture was stirred for 30 min. After that time, methyl 4-fluoro-3-(trifluoromethyl)benzoate (550 mg, 2.48 mmol) was added and then stirred at room temperature for 24 h. After that time, water was added until no more bubbling was observed. The resulting suspension was diluted with EtOAc (15 mL) and washed with Na_2CO_3 sat. (20 mL). The aqueous layer was extracted again with EtOAc (2 x 15 mL). All the organic layers were joined, dried over anh. Na_2SO_4, filtered and concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 8%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a colorless syrup (360 mg, 46% yield). Analysis data agrees with bibliography.

52, synthesis of methyl 4-(((1r,4r)-4-aminocyclohexyl)oxy)-2-(trifluoromethyl)benzoate
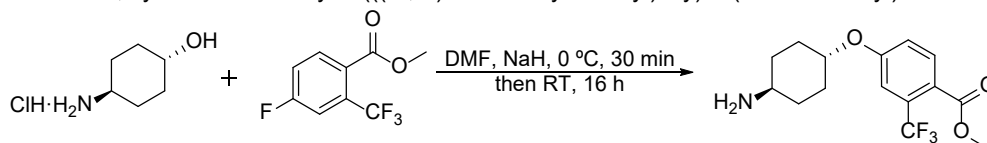
To a solution of trans-4-aminocyclohexanol hydrochloride (217 mg, 1.43 mmol) in anh. THF (8.0 mL) under ice-bath was added NaH 60% dispersion in mineral oil (172 mg, 4.29 mmol) and the mixture was stirred for 30 min. After that time, methyl 4-fluoro-2-(trifluoromethyl)benzoate (318 mg, 1.43 mmol) was added and then stirred at room temperature for 24 h. After that time, water was added until no more bubbling was observed. The resulting suspension was diluted with EtOAc (15 mL) and washed with Na_2CO_3 sat. (20 mL). The aqueous layer was extracted again with EtOAc (2 x 15 mL). All the organic

layers were joined, dried over anh. Na$_2$SO$_4$, filtered and concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 8%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford as a colorless syrup (220 mg, 48% yield). Analysis data agrees with bibliography.

53, synthesis of methyl 3-chloro-4-(((1r,4r)-4-(3-(9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)ureido)cyclohexyl)oxy)benzoate
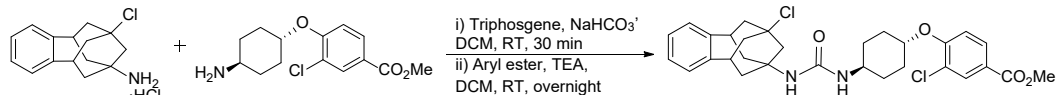
To a stirring biphasic mixture of DCM (3.0 mL), NaHCO$_3$ sat. (2.0 mL) and 9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine hydrochloride (114 mg, 0.40 mmol) was added triphosgene (46 mg, 0.16 mmol). The mixture was then stirred at room temperature for 30 min. DCM (5 mL) and brine (10 mL) were added subsequently and phases were separated. The organic layer was dried over Na$_2$SO$_4$ anh., filtered and concentrated *in vacuo*.

DCM (2.0 mL) was added to the previous crude and then it was added onto a solution of methyl 4-(((1r,4r)-4-aminocyclohexyl)oxy)-3-chlorobenzoate (88 mg, 0.31 mmol) and triethylamine (86 µL, 63 mg, 0.62 mmol) in DCM (2.0 mL) and the mixture was stirred at room temperature overnight. DCM (10 mL) was added and the mixture was washed with water (20 mL). The aqueous layer was extracted again with DCM (10 mL). Both organic layers were joined, dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 50%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (62 mg, 36% yield).

Mp: 221-222 ºC

IR (ATR) *v*: 3310, 2931, 2856, 1708, 1632, 1598, 1553, 1499, 1452, 1351, 1320, 1263, 1241, 1121, 1062, 1021, 937, 905, 806, 763, 708, 620 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 1.26 [m, 2H, 3'(5')-H$_{ax}$], 1.61 [m, 2H, 2'(6')-H$_{ax}$], 1.96 [d, *J* = 13.0 Hz, 2H, 6''(12'')-H$_{ax}$], 2.04-2.10 [complex signal, 4H, 2'(6')-H$_{eq}$, 3'(5')-H$_{eq}$], 2.16 [d, *J* = 13.1 Hz, 2H, 10''(13'')-H$_{ax}$], 2.23 [m, 2H, 6''(12'')-H$_{eq}$], 2.37 [m, 2H, 10''(13'')-H$_{eq}$], 2.50 (s, 2H, 8''-H), 3.16 [t, *J* = 6.4 Hz, 2H, 5''(11'')-H], 3.58 (dtd, *J* = 11.0, 7.5, 3.7 Hz, 1H, 4'-H), 3.96 (s, 3H, C$\underline{H}_3$), 4.21 (d, *J* = 7.4 Hz, 1H, piperidine-N$\underline{H}$), 4.26 (s, 1H, adamante-N$\underline{H}$), 4.88 (tt, *J* = 10.6 Hz, *J'* = 3.3 Hz, 1H, 1'-H), 6.93 (d, *J* = 8.7 Hz, 1H, 5-H), 7.05 [m, 2H, 1''(4'')-H], 7.11 [m, 2H, 2''(3'')-H], 7.91 (dd, *J* = 8.6 Hz, *J'* = 2.1 Hz, 1H, 6-H), 8.02 (d, *J* = 2.1 Hz, 1H, 2-H).

^{13}C NMR (101 MHz, CDCl$_3$) δ: 30.3 [CH$_2$, C2'(6')], 31.4 [CH$_2$, C3'(5')], 39.1 [CH$_2$, C6''(12'')], 41.4 [CH, C5''(11'')], 44.7 [CH$_2$, C10''(13'')], 48.1 (CH, C4'), 51.0 (CH$_2$, C8''), 55.9 (C, C7''), 56.5 (CH$_3$, $\underline{C}$H$_3$), 69.6 (C, C9''), 72.9 (CH, C1'), 111.3 (CH, C5), 122.6 (C, C3), 123.8 (C, C1), 127.0 [CH, C2''(3'')], 128.3 [CH, C1''(4'')], 130.0 (CH, C6), 131.7 (CH, C2), 144.9 [C, C4''a(11''a)], 156.1 (C, NH$\underline{C}$ONH), 158.8 (C, C4), 165.1 (C, $\underline{C}$O$_2$Me).

Anal. calcd for C$_{30}$H$_{34}$Cl$_2$N$_2$O$_4$: C 64.63, H 6.15, N 5.02. Found: C 64.33, H 6.07, N 4.80.

HRMS: Calcd for [C$_{29}$H$_{32}$Cl$_2$N$_2$O$_4$+H]$^+$: 557.1968, found: 557.1989.

54, synthesis of methyl 2-chloro-4-(((1r,4r)-4-(3-(9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)ureido)cyclohexyl)oxy)benzoate
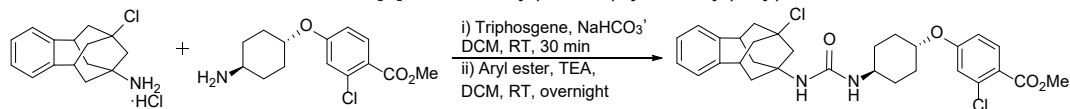
To a stirring biphasic mixture of DCM (8.0 mL), NaHCO₃ sat. (6.0 mL) and 9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine hydrochloride (205 mg, 0.72 mmol) was added triphosgene (85 mg, 0.29 mmol). The mixture was then stirred at room temperature for 30 min. DCM (5 mL) and brine (10 mL) were added subsequently and phases were separated. The organic layer was dried over Na₂SO₄ anh., filtered and concentrated *in vacuo*.

DCM (2.0 mL) was added to the previous crude and then it was added onto a solution of methyl 4-(((1r,4r)-4-aminocyclohexyl)oxy)-2-chlorobenzoate (204 mg, 0.72 mmol) and triethylamine (201 µL, 146 mg, 1.44 mmol) in DCM (2.0 mL) and the mixture was stirred at room temperature overnight. DCM (10 mL) was added and the mixture was washed with water (20 mL). The aqueous layer was extracted again with DCM (10 mL). Both organic layers were joined, dried over Na₂SO₄ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 45%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (78 mg, 19% yield).

Mp: 239-240 ºC

IR (ATR) *v*: 3308, 2935, 2857, 1697, 1642, 1600, 1560, 1494, 1452, 1261, 1234, 1120, 1023, 902, 807, 760, 611 cm⁻¹.

¹H NMR (400 MHz, CDCl₃) δ: 1.26 [m, 2H, 3'(5')-H_{ax}], 1.62 [m, 2H, 2'(6')-H_{ax}], 1.95 [d, *J* = 13.3 Hz, 2H, 6''(12'')-H_{ax}], 2.05-2.12 [complex signal, 4H, 2'(6')-H_{eq}, 3'(5')-H_{eq}], 2.15 [d, *J* = 13.1 Hz, 2H, 10''(13'')-H_{ax}], 2.23 [m, 2H, 6''(12'')-H_{eq}], 2.36 [m, 2H, 10''(13'')-H_{eq}], 2.48 (s, 2H, 8''-H), 3.15 [t, *J* = 6.5 Hz, 2H, 5''(11'')-H], 3.57 (m, 1H, 4'-H), 3.84 (s, 3H, C$\underline{H}_3$), 4.22 (d, *J* = 7.0 Hz, 1H, piperidine-N$\underline{H}$), 4.25 (s, 1H, adamante-N$\underline{H}$), 4.90 (tt, *J* = 10.7 Hz, *J'* = 4.0 Hz, 1H, 1'-H), 6.81 (dd, *J* = 8.8 Hz, *J'* = 2.5 Hz, 1H, 5-H), 6.95 (d, *J* = 2.5 Hz, 1H, 3-H), 7.05 [m, 2H, 1''(4'')-H], 7.11 [m, 2H, 2''(3'')-H], 7.82 (d, *J* = 8.8 Hz, 1H, 6-H).

¹³C NMR (101 MHz, CDCl₃) δ: 30.2 [CH₂, C2'(6')], 31.3 [CH₂, C3'(5')], 39.1 [CH₂, C6''(12'')], 41.4 [CH, C5''(11'')], 44.7 [CH₂, C10''(13'')], 48.1 (CH, C4'), 51.0 (CH₂, C8''), 55.9 (C, C7''), 55.9 (CH₃, $\underline{C}$H₃), 69.6 (C, C9''), 73.1 (CH, C1'), 112.7 (CH, C5), 116.5 (CH, C3), 122.1 (C, C1), 127.0 [CH, C2''(3'')], 128.2 [CH, C1''(4'')], 133.4 (CH, C6), 135.8 (C, C2), 144.9 [C, C4''a(11''a)], 156.2 (C, NH$\underline{C}$ONH), 162.6 (C, C4), 165.0 (C, $\underline{C}$O₂Me).

Anal. calcd for C₃₀H₃₄Cl₂N₂O₄: C 64.63, H 6.15, N 5.02. Found: C 64.79, H 6.13, N 4.80.

HRMS: Calcd for [C₂₉H₃₂Cl₂N₂O₄+H]⁺: 557.1968, found: 557.1983.

55, synthesis of methyl 4-(((1r,4r)-4-(3-(9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)ureido)cyclohexyl)oxy)-3-(trifluoromethyl)benzoate
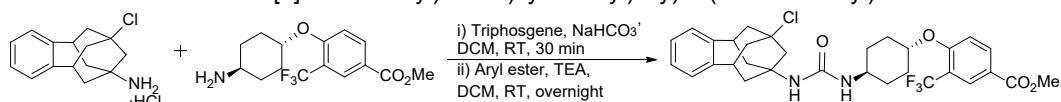
To a stirring biphasic mixture of DCM (6.0 mL), NaHCO₃ sat. (3.0 mL) and 9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine hydrochloride (161 mg, 0.57 mmol) was added triphosgene (67 mg, 0.23 mmol). The mixture was then stirred at room temperature for 30 min. DCM (5 mL) and brine (10 mL) were added subsequently and phases were separated. The organic layer was dried over Na₂SO₄ anh., filtered and concentrated *in vacuo*.

DCM (2.0 mL) was added to the previous crude and then it was added onto a solution of methyl 4-(((1r,4r)-4-aminocyclohexyl)oxy)-3-(trifluoromethyl)benzoate (180 mg, 0.57 mmol) and triethylamine (158 µL, 114 mg, 1.13 mmol) in DCM (4.0 mL) and the mixture was stirred at room temperature overnight. DCM (10 mL) was added and the mixture was washed with water (20 mL). The aqueous layer was extracted again with DCM (10 mL). Both organic layers were joined, dried over Na₂SO₄ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 50%). Fractions

containing the desired product were collected and concentrated *in vacuo* to afford a white solid (76 mg, 23% yield).

Mp: 221-222 ºC

IR (ATR) *v*: 3303, 2935, 1713, 1644, 1616, 1557, 1505, 1454, 1320, 1279, 1245, 1123, 1056, 1021, 996, 946, 807, 766, 674 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 1.26 [m, 2H, 3'(5')-H$_{ax}$], 1.62 [m, 2H, 2'(6')-H$_{ax}$], 1.96 [d, *J* = 13.0 Hz, 2H, 6''(12'')-H$_{ax}$], 2.05-2.11 [complex signal, 4H, 2'(6')-H$_{eq}$, 3'(5')-H$_{eq}$], 2.16 [d, *J* = 13.7 Hz, 2H, 10''(13'')-H$_{ax}$], 2.23 [m, 2H, 6''(12'')-H$_{eq}$], 2.37 [m, 2H, 10''(13'')-H$_{eq}$], 2.50 (s, 2H, 8''-H), 3.16 [t, *J* = 6.1 Hz, 2H, 5''(11'')-H], 3.58 (dtd, *J* = 11.1 Hz, *J'* = 7.5 Hz, *J''* = 3.8 Hz, 1H, 4'-H), 3.97 (s, 3H, C$\underline{H}_3$), 4.10 (d, *J* = 7.7 Hz, 1H, piperidine-N$\underline{H}$), 4.17 (s, 1H, adamante-N$\underline{H}$), 4.90 (tt, *J* = 10.8 Hz, *J'* = 3.8 Hz, 1H, 1'-H), 7.02 (d, *J* = 8.7 Hz, 1H, 5-H), 7.06 [m, 2H, 1''(4'')-H], 7.11 [m, 2H, 2'(3'')-H], 8.17 (dd, *J* = 8.7 Hz, *J'* = 2.1 Hz, 1H, 6-H), 8.23 (d, *J* = 2.1 Hz, 1H, 2-H).

^{13}C NMR (101 MHz, CDCl$_3$) δ: 30.3 [CH$_2$, C2'(6')], 31.4 [CH$_2$, C3'(5')], 39.1 [CH$_2$, C6''(12'')], 41.4 [CH, C5''(11'')], 44.6 [CH$_2$, C10''(13'')], 48.2 (CH, C4'), 51.0 (CH$_2$, C8''), 55.9 (C, C7''), 56.4 (CH$_3$, $\underline{C}$H$_3$), 69.6 (C, C9''), 73.0 (CH, C1'), 111.6 (CH, C5), 118.9 (q, $^2J_{CF}$ = 31.6 Hz, C, C3), 122.6 (C, C1), 123.3 (q, $^1J_{CF}$ = 272.9 Hz, C, $\underline{C}$F$_3$), 127.0 [CH, C2''(3'')], 128.3 [CH, C1''(4'')], 129.1 (q, $^3J_{CF}$ = 5.4 Hz, CH, C2), 135.3 (CH, C6), 144.9 [C, C4''a(11''a)], 156.1 (C, NH$\underline{C}$ONH), 161.1 (C, C4), 165.0 (C, $\underline{C}$O$_2$Me).

Anal. calcd for C$_{31}$H$_{34}$ClF$_3$N$_2$O$_4$: C 62.99, H 5.80, N 4.74. Found: C 63.67, H 5.69, N 4.09.

HRMS: Calcd for [C$_{31}$H$_{34}$ClF$_3$N$_2$O$_4$+H]$^+$: 591.2232, found: 591.2237.

56, synthesis of methyl 4-(((1r,4r)-4-(3-(9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)ureido)cyclohexyl)oxy)-2-(trifluoromethyl)benzoate
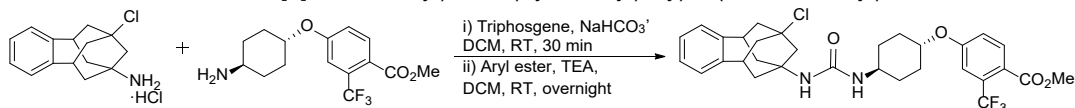
To a stirring biphasic mixture of DCM (10.0 mL), NaHCO$_3$ sat. (6.0 mL) and 9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine hydrochloride (216 mg, 0.76 mmol) was added triphosgene (91 mg, 0.31 mmol). The mixture was then stirred at room temperature for 30 min. DCM (5 mL) and brine (10 mL) were added subsequently and phases were separated. The organic layer was dried over Na$_2$SO$_4$ anh., filtered and concentrated *in vacuo*.

DCM (2.0 mL) was added to the previous crude and then it was added onto a solution of methyl 4-(((1r,4r)-4-aminocyclohexyl)oxy)-2-(trifluoromethyl)benzoate (220 mg, 0.69 mmol) and triethylamine (212 µL, 153 mg, 1.52 mmol) in DCM (2.0 mL) and the mixture was stirred at room temperature overnight. DCM (10 mL) was added and the mixture was washed with water (20 mL). The aqueous layer was extracted again with DCM (10 mL). Both organic layers were joined, dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 50%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (115 mg, 26% yield).

Mp: 221-222 ºC

IR (ATR) *v*: 3299, 2940, 2855, 1708, 1644, 1613, 1552, 1501, 1453, 1356, 1296, 1265, 1237, 1136, 1081, 1036, 993, 936, 900, 834, 807, 781, 763, 663, 628, 602 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 1.22 [m, 2H, 3'(5')-H$_{ax}$], 1.58 [m, 2H, 2'(6')-H$_{ax}$], 1.95 [d, *J* = 14.0 Hz, 2H, 6''(12'')-H$_{ax}$], 2.02-2.13 [complex signal, 4H, 2'(6')-H$_{eq}$, 3'(5')-H$_{eq}$], 2.15 (d, *J* = 13.7 Hz, 2H, 10''(13'')-H$_{ax}$], 2.21 [m, 2H, 6''(12'')-H$_{eq}$], 2.36 [m, 2H, 10''(13'')-H$_{eq}$], 2.48 (s, 2H, 8''-H), 3.15 [t, *J* = 6.5 Hz, 2H, 5''(11'')-H], 3.55 (dtt, *J* = 10.9 Hz, *J'* = 7.3 Hz, *J''* = 3.8 Hz, 1H, 4'-H), 3.88 (s, 3H, C$\underline{H}_3$), 4.21 (d, *J* = 7.6 Hz, 1H, piperidine-N$\underline{H}$), 4.27 (s, 1H, adamante-N$\underline{H}$), 4.89 (tt, *J* = 10.9 Hz, *J'* = 4.0 Hz, 1H, 1'-H), 7.02-

7.08 [complex signal, 3H, 5-H, 1''(4'')-H], 7.11 [m, 2H, 2''(3'')-H], 7.24 (d, J = 2.6 Hz, 1H, 3-H), 7.81 (dd, J = 8.6 Hz, J' = 0.7 Hz, 1H, 6-H).

^{13}C NMR (101 MHz, CDCl$_3$) δ: 29.9 [CH$_2$, C2'(6')], 31.3 [CH$_2$, C3'(5')], 39.1 [CH$_2$, C6''(12'')], 41.4 [CH, C5''(11'')], 44.6 [CH$_2$, C10''(13'')], 48.1 (CH, C4'), 51.0 (CH$_2$, C8''), 55.8 (C, C7''), 55.9 (CH$_3$, $\underline{C}$H$_3$), 69.6 (C, C9''), 73.7 (CH, C1'), 113.4 (q, $^3J_{CF}$ = 6.0 Hz, CH, C3), 116.1 (CH, C5), 123.2 (C, C1), 123.2 (q, $^1J_{CF}$ = 273.8 Hz, C, $\underline{C}$F$_3$), 127.0 [CH, C2''(3'')], 128.2 [CH, C1''(4'')], 130.9 (q, $^2J_{CF}$ = 32.5 Hz, C, C2), 133.1 (CH, C6), 144.9 [C, C4''a(11''a)], 156.2 (C, NH$\underline{C}$ONH), 161.8 (C, C4), 166.0 (C, $\underline{C}$O$_2$Me).

Anal. calcd for C$_{31}$H$_{34}$ClF$_3$N$_2$O$_4$: C 62.99, H 5.80, N 4.74. Found: C 63.45, H 5.78, N 4.67.

HRMS: Calcd for [C$_{31}$H$_{34}$ClF$_3$N$_2$O$_4$+H]+: 591.2232, found: 591.2225.

59, synthesis of 4-(((1s,4s)-4-aminocyclohexyl)oxy)benzonitrile
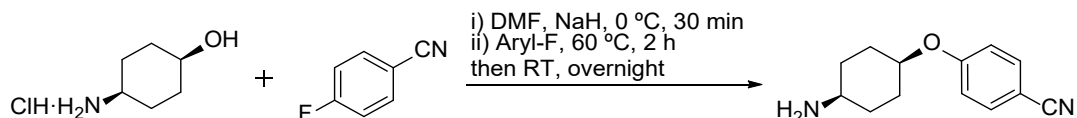
To a solution of cis-4-aminocyclohexanol hydrochloride (200 mg, 1.32 mmol) in anh. DMF (5.0 mL) under ice-bath was added NaH 60% dispersion in mineral oil (211 mg, 5.27 mmol) and the mixture was stirred for 30 min. After that time, 4-fluorobenzonitrile (192 mg, 1.58 mmol) was added and the reaction was heated to 60 ºC for 2 h and then stirred at room temperature for 24 h. After that time, water was added until no more bubbling was observed. The resulting suspension was diluted with EtOAc (20 mL) and washed with Na$_2$CO$_3$ sat. (2 x 30 mL). The organic layer was dried over anh. Na$_2$SO$_4$, filtered and concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 10%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a brownish solid (201 mg, 70% yield).

**60**, synthesis of 1-(9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)-3-((1s,4s)-4-(4-cyanophenoxy)cyclohexyl)urea
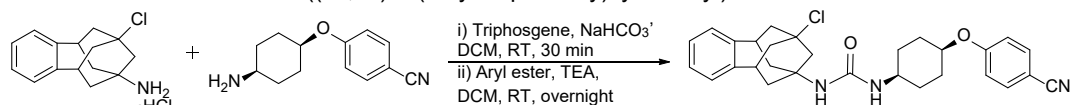
To a stirring biphasic mixture of DCM (5.0 mL), NaHCO$_3$ sat. (3.0 mL) and 9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine hydrochloride (131 mg, 0.46 mmol) was added triphosgene (55 mg, 0.18 mmol). The mixture was then stirred at room temperature for 30 min. DCM (5 mL) and brine (10 mL) were added subsequently and phases were separated. The organic layer was dried over Na$_2$SO$_4$ anh., filtered and concentrated *in vacuo*.

DCM (2.0 mL) was added to the previous crude and then it was added onto a suspension of 4-(((1s,4s)-4-aminocyclohexyl)oxy)benzonitrile (100 mg, 0.46 mmol) and triethylamine (64 µL, 47 mg, 0.46 mol) in DCM (3.0 mL) and the mixture was stirred at room temperature overnight. Solvent was concentrated *in vacuo* and EtOAc (10 mL) was added and the mixture was washed with water (2 x 20 mL). The organic layer was dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 50%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford an orangish solid (66 mg, 29% yield).

Mp: 127-128 ºC

IR (ATR) v: 3343, 2933, 2222, 1631, 1603, 1531, 1505, 1445, 1357, 1298, 1251, 1170, 1109, 1037, 947, 832, 804, 759, 698 cm^{-1}.

¹H NMR (400 MHz, CDCl₃) δ: 1.52 [m, 2H, 2'(6')-H$_{ax}$], 1.70 [m, 2H, 3'(5')-H$_{ax}$], 1.80 [m, 2H, 2'(6')-H$_{eq}$], 1.92-2.03 [complex signal, 4H, 6(12)-H$_{ax}$, 3'(5')-H$_{eq}$], 2.16 [dd, J = 13.3 Hz, J' = 6.0 Hz, 2H, 10(13)-H$_{ax}$], 2.24 [m, 2H, 6(12)-H$_{eq}$], 2.37 [m, 2H, 10(13)-H$_{eq}$], 2.50 (s, 2H, 8-H), 3.16 [broad t, J = 6.4 Hz, 2H, 5(11)-H], 3.62 (tdt, J = 10.8 Hz, J' = 7.7 Hz, J'' = 3.8 Hz, 1H, 1'-H), 4.10 [m, 2H, 3'(5')-H$_{eq}$], 4.53 (m, 1H, 4'-H), 6.91 [d, J = 9.0 Hz, 2H, 2''(6'')-H], 7.06 [m, 2H, 1(4)-H], 7.12 [m, 2H, 2(3)-H], 7.56 [d, J = 9.0 Hz, 2H, 3''(5'')-H].

¹³C NMR (101 MHz, CDCl₃) δ: 28.1 [CH₂, C2'(6')], 28.4 [CH₂, C3'(5')], 39.1 [CH₂, C6(12)], 41.4 [CH, C5(11)], 44.6 [CH₂, C10(13)], 47.8 (CH, C1'), 51.0 (CH₂, C8), 55.9 (C, C7), 69.6 (C, C9), 71.7 (CH, C4'), 103.8 (C, C4''), 116.3 [CH, C2''(6'')], 119.4 (C, $\underline{C}$N), 127.0 [CH, C2(3)], 128.3 [CH, C1(4)], 134.2 [CH, C3''(5'')], 144.9 [C, C4a(11a)], 155.9 (C, NH$\underline{C}$ONH), 161.0 (C, $\underline{C}$O).

HRMS: Calcd for [C₂₉H₃₂ClN₃O₂+H]⁺: 490.2256, found: 490.2251.

61, synthesis of 4-(((1s,4s)-4-(3-(9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)ureido)cyclohexyl)oxy)benzoic acid
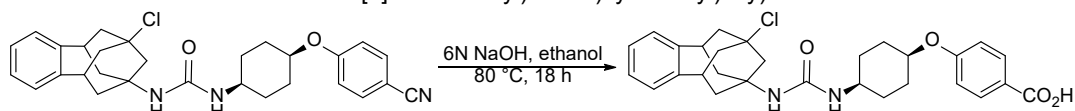
6 N NaOH solution (81 mg, 300 µL, 2.02 mmol) was added to a solution of 1-(9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)-3-((1s,4s)-4-(4-cyanophenoxy)cyclohexyl)urea (66 mg, 0.13 mmol) in ethanol (1.0 mL) and the reaction mixture was heated to 80 ºC for 16 h. Amberlite® 120 H+ was added until pH = 3 and the mixture was filtered using MeOH as eluting agent. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 3%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (45 mg, 67% yield).

Mp: 190-191 ºC

IR (ATR) *v*: 3335, 2931, 1678, 1604, 1531, 1451, 1355, 1297, 1248, 1167, 1100, 1063, 946, 849, 759, 698, 582 cm⁻¹.

¹H NMR (400 MHz, CDCl₃) δ:

¹³C NMR (101 MHz, CDCl₃) δ:

Anal. calcd for C₂₉H₃₃ClN₂O₄: C 68.43, H 6.53, N 5.50. Found: C 71.35, H 7.14, N 3.20.

HRMS: Calcd for [C₂₉H₃₃ClN₂O₄+H]⁺: 509.2202, found: 509.2204.

63, synthesis of 9-amino-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-ol
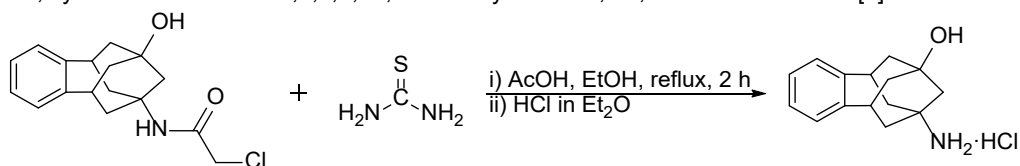
To a solution of chloroacetamide 2-chloro-*N*-(9-hydroxy-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)acetamide (1.0 g, 2.29 mmol) in ethanol (20.0 mL) were added thiourea (300 mg, 3.93 mmol) and glacial acetic acid (1.10 mL) and the mixture was heated at reflux for 2 h. The resulting suspension was then tempered to room temperature, water (30 mL) was added and the pH adjusted to ~12 with Na₂CO₃ sat. EtOAc (30 mL) was added, the phases were separated. The aqueous phase was extracted with further EtOAc/10% MeOH (3 x 20 mL). The combined organic layers were dried over Na₂SO₄ anh., filtered and solvent was concentrated *in vacuo*.

HCl 2 M in Et$_2$O (5.0 mL) was added to form its hydrochloride, followed by filtration of the solid to afford a pale-yellow solid (847 mg, 97% yield).

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.75 (m, 2H), 1.82 (d, *J* = 12.9 Hz, 2H), 1.89 (s, 2H), 1.90-2.09 (complex signal, 4H), 3.28 (m, 2H), 7.13 (s, 4H).

64, synthesis of 9-bromo-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine hydrochloride
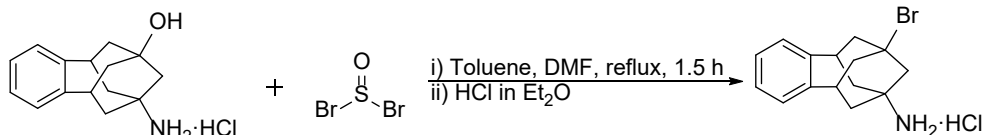
To a solution of 9-amino-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-ol hydrochloride (847 mg, 3.19 mmol) in toluene (25.0 mL) and few drops of DMF was added thionyl bromide (3.69 mL, 9.94 g, 47.80 mmol). The resulting orange suspension was stirred at room temperature for 1.5 h. The reaction mixture was then cooled to 0 ºC under ice-bath and water was added dropwise until no more HBr formation was observed. NaOH 2 M was added until pH = 12 and then the aqueous layer was extracted with EtOAc/10% MeOH (3 x 40 mL). All organic layers were joined, dried over Na$_2$SO$_4$ anh., filtered and solvent was concentrated *in vacuo*.

HCl 2 M in Et$_2$O (7.0 mL) was added to form its hydrochloride, followed by filtration of the solid to afford a pale-orange solid (574 mg, 55% yield).

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.90 (d, *J* = 12.9 Hz, 2H), 2.14 (m, 2H), 2.34 (d, *J* = 13.2 Hz, 2H), 2.55 (s, 2H), 2.64 (m, 2H), 3.28 (m, 2H), 7.10-7.19 (complex signal, 4H).

65, synthesis of 5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine
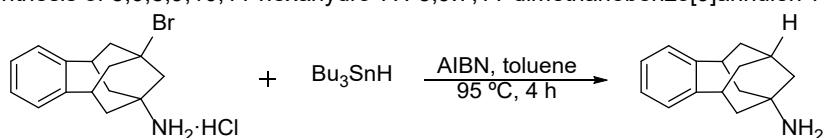
To a suspension of amine 9-bromo-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine hydrochloride (224 mg, 0.68 mmol) in dry and deoxygenated toluene (3.0 mL) under Ar atmosphere were added tri-*n*-butyltin hydride (331 µL, 358 mg, 1.23 mmol) and 2,2'-azobisisobutyronitrile (AIBN) (0.16 mg, 0.10 mmol). The resulting solution was heated to 95 ºC for 4 h adding more AIBN (0.16 mg, 0.10 mmol) each 60 min. Na$_2$CO$_3$ sat. was added until pH = 12 and then the aqueous layer was extracted with EtOAc/10% MeOH (3 x 15 mL). All organic layers were joined, dried over Na$_2$SO$_4$ anh., filtered and solvent was concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH/NH$_3$ 0.7 M in DCM from 0% to 4%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a red syrup (90 mg, 62% yield).

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.64-1.75 (complex signal, 6H), 1.85 (m, 2H), 1.94 (m, 2H), 2.32 (m, 1H), 3.03 (t, *J* = 6.2 Hz, 2H), 7.02 (s, 4H).

66, synthesis of 5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-9-d-7-amine
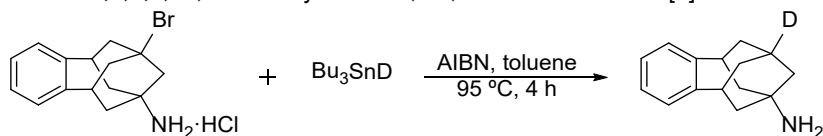
To a suspension of amine 9-bromo-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine hydrochlorie (250 mg, 0.76 mmol) in dry and deoxygenated toluene (3.0 mL) under Ar atmosphere were added tri-*n*-butyltin deuteride (370 µL, 400 mg, 1.37 mmol) and 2,2'-azobisisobutyronitrile (AIBN) (0.16 mg, 0.10 mmol). The resulting solution was heated to 95 ºC for 4 h adding more AIBN (0.16 mg, 0.10 mmol) each 60 min. Na_2CO_3 sat. was added until pH = 12 and then the aqueous layer was extracted with EtOAc (3 x 15 mL). All organic layers were joined, dried over Na_2SO_4 anh., filtered and solvent was concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of $MeOH/NH_3$ 0.7 M in DCM from 0% to 4%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a pale-yellow solid (100 mg, 61% yield).

Mp: 72-73 ºC

IR (ATR) *v*: 2906, 2838, 1653, 1560, 1490, 1439, 1352, 1115, 1047, 990, 933, 914, 874, 826, 755, 702, 610 cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD) δ: 1.64-1.74 (complex signal, 6H, 6(12)-H_{ax}, 8-H, 10(13)-H_{ax}), 1.85 [m, 2H, 6(12)-H_{eq}], 1.93 [m, 2H, 10(13)-H_{eq}], 3.03 [tt, *J* = 6.0, 1.5 Hz, 2H, 5(11)-H], 7.02 [s, 4H, 1(4)-H, 2(3)-H].

^{13}C NMR (101 MHz, CD_3OD) δ: 32.7 (t, J_{CD} = 20.0, CD, C9), 35.4 [CH_2, C6(12)], 42.9 [CH, C5(11)], 44.1 [CH_2, C10(13)], 45.0 (CH_2, C8), 48.8 (C, C7), 127.3 [CH, C2(3)], 129.0 [CH, C1(4)], 148.0 [C, C4a(C11a)].

Anal. calcd for $C_{15}H_{18}DN$: C 84.06, H 9.41, N 6.54. Calcd for $C_{15}H_{18}DN \cdot 0.1 H_2O$: C 83.36, H 8.49, N 6.48. Found: C 83.51, H 8.81, N 6.33.

HRMS: Calcd for $[C_{15}H_{18}DN+H]^+$: 215.1658, found: 215.1653.

68, synthesis of *N*-(9-methyl-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)-2-(piperidin-4-yl)acetamide
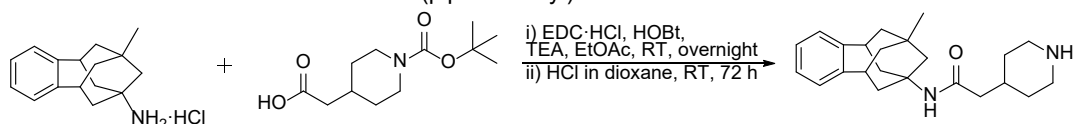
To a suspension of 9-methyl-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine hydrochloride (500 mg, 1.89 mmol) in EtOAc (5.0 mL), 2-(1-(t-butoxycarbonyl)piperidin-4-yl)acetic acid (461 mg, 1.89 mmol), HOBt (384 mg, 2.84 mmol), EDC·HCl (440 mg, 2.84 mmol) and triethylamine (1.06 mL, 767 mg, 7.58 mmol) were added. The mixture was stirred at room temperature overnight. Water (10 mL) and DCM (20 mL) were added to the resulting suspension and the two phases were separated. The organic phase was washed with sat. $NaHCO_3$ aqueous solution (10 mL), brine (10 ml), 2 M HCl solution (10 mL) and 2 M NaOH (10 mL), dried over Na_2SO_4 anh., filtered and concentrated under vacuum to afford a yellow solid (515 mg, 60% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.92 (s, 3H), 1.11 (dq, *J* = 4.4 Hz, *J'* = 11.6 Hz, 2H), 1.4 (s, 9H), 1.54 (d, *J* = 13.6 Hz, 2H), 1.63-1.68 (complex signal, 4H), 1.84 (s, 2H), 1.91 (m, 1H), 1.97 (s, 2H), 2.0 (d, *J* = 12.8 Hz, 2H), 2.14-2.18 (complex signal, 2H), 2.69 (t, *J* = 13.2 Hz, 2H), 3.06 (t, *J* = 6 Hz, 2H), 4.06 (s broad signal, 2H), 5.14 (s, 1H), 7.02-7.08 (complex signal, 4H).

To a solution of t-butyl 4-(2-((9-methyl-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)amino)-2-oxoethyl)piperidine-1-carboxylate (250 mg, 0.55 mmol) in

DCM (4.0 mL) was added 4 M HCl in 1,4-dioxane (0.5 ml, 2.00 mmol). The reaction mixture was stirred at room temperature for 72 h. Then, the solvent was evaporated under vacuum and the residue was dissolved in DCM (10 mL) and washed with 5 M NaOH solution, dried over Na$_2$SO$_4$ anh., filtered and concentrated under vacuum to afford a yellow solid (189 mg, 97% yield).

¹H-NMR (400 MHz, CDCl$_3$) δ: 0.91 (s, 3H), 1.12 (dq, J = 4 Hz, J' = 12.0 Hz, 2H), 1.53 (d, J = 13.2 Hz, 2H), 1.62-1.71 (complex signal, 4H), 1.84 (s, 2H), 1.88 (m, 1H), 1.95-2.01 (complex signal, 4H), 2.14-2.19 (complex signal, 2H), 2.6 (dt, J = 2.8 Hz, J' = 12.0 Hz, 2H), 3.00-3.07 (complex signal, 4H), 5.15 (s, 1H), 7.02-7.09 (complex signal, 4H).

69, synthesis of *N*-(9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)-2-(piperidin-4-yl)acetamide
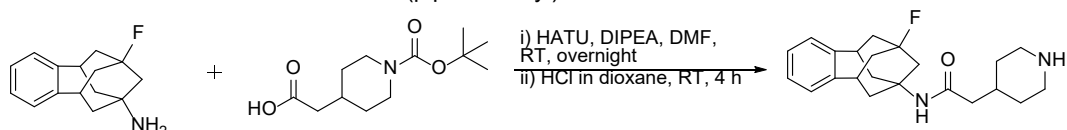
To a suspension of 9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethano-benzo[9]annulen-7-amine (173 mg, 0.75 mmol) in DMF (3.0 mL), 2-(1-(tert-butoxycarbonyl)piperidin-4-yl)acetic acid (218 mg, 0.90 mmol), HATU (430 mg, 1.13 mmol), and DIPEA (520 μL, 386 mg, 3.00 mmol) were added. The mixture was stirred at room temperature overnight. Solvent was concentrated *in vacuo* and EtOAc (10 mL) was added. The mixture was washed with NaHCO$_3$ sat. (2 x 30 mL). The organic layer was dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was used as such without further purification. Stoichiometric yield (342 mg).

¹H NMR (400 MHz, CDCl$_3$) δ: 1.10 (m, 2H), 1.44 (s, 9H), 1.47 (s, 2H), 1.66 (d, J = 13.5 Hz, 2H), 1.87-2.05 (complex signal, 6H), 2.09-2.24 (complex signal, 5H), 2.27 (d, J = 6.2 Hz, 2H), 2.68 (m, 2H), 3.23 (m, 2H), 5.23 (s, 1H), 7.05-7.15 (complex signal, 4H).

HCl 4 M in dioxane (2.0 mL, 8.00 mmol) and dioxane (2.0 mL) were added to tert-butyl 4-(2-((9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)amino)-2-oxoethyl)piperidine-1-carboxylate (341 mg, 0.75 mmol) and the mixture was stirred at room temperature for 4 h. NaHCO$_3$ sat. (20 mL) was added followed by EtOAc (15 mL) and the mixture was partitioned. The aqueous layer was extracted with EtOAc/MeOH 9/1 (2 x 15 mL). All organic layers were joined, dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH/NH$_3$ 0.7 M in DCM from 0% to 10%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a yellowish solid (186 mg, 70% yield).

¹H NMR (400 MHz, DMSO-d6) δ: 0.92-1.06 (complex signal, 2H), 1.50 (m, 2H), 1.72 (m, 2H), 1.90 (d, J = 7.2 Hz, 2H), 1.98-2.02 (complex signal, 4H), 2.06-2.13 (complex signal, 4H), 2.32-2.45 (m, 2H), 2.87 (m, 2H), 3.21 (s, 4H), 7.11 (s, 4H), 7.61 (s, 1H).

70, synthesis of *N*-(9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)-2-(piperidin-4-yl)acetamide
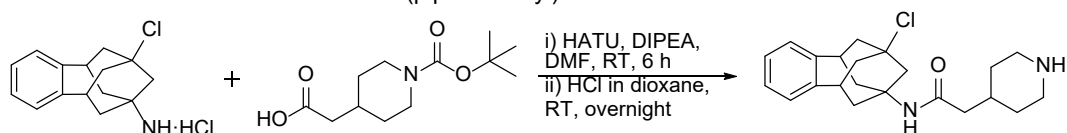
To a suspension of 9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine hydrochloride (178 mg, 0.63 mmol) in DMF (3.0 mL), 2-(1-(tert-butoxycarbonyl)piperidin-4-

yl)acetic acid (183 mg, 0.75 mmol), HATU (357 mg, 0.94 mmol), and DIPEA (439 µL, 326 mg, 2.52 mmol) were added. The mixture was stirred at room temperature for 6 h. Solvent was concentrated *in vacuo* and EtOAc (15 mL) was added. The mixture was washed with NaHCO$_3$ sat. (2 x 30 mL). The organic layer was dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was used as such without further purification. Stoichiometric yield (298 mg, crude).

¹H NMR (400 MHz, CDCl$_3$) δ: 1.44 (s, 9H), 1.69 (m, 2H), 1.91 (m, 1H), 1.95-2.01 (complex signal, 4H), 2.04 (s, 1H), 2.12-2.32 (complex signal, 6H), 2.38 (m, 2H), 2.53 (s, 1H), 2.70 (d, *J* = 12.1 Hz, 2H), 3.17 (t, *J* = 6.5 Hz, 2H), 4.07 (m, 2H), 5.21 (s, 1H), 7.04-7.15 (complex signal, 4H).

HCl 4 M in dioxane (2 mL) and dioxane (2 mL) were added to *tert*-butyl 4-(2-((9-chloro-5,6,8,9,10,11-hexahydro-7*H*-5,9:7,11-dimethanobenzo[9]annulen-7-yl)amino)-2-oxoethyl)piperidine-1-carboxylate (296 mg, 0.63 mmol) and the mixture was stirred at room temperature overnight. Saturated aqueous NaHCO$_3$ solution (20 mL) was added followed by EtOAc (15 mL) and the mixture was partitioned. The aqueous layer was further extracted with EtOAc/MeOH 9/1 (2 x 15 mL). All organic layers were joined, dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH/NH$_3$ 0.7 M in DCM from 0% to 10%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a reddish solid.

HCl 2 M in Et$_2$O (5.0 mL) was added to form its hydrochloride, followed by filtration of the solid to afford an orange solid (84 mg, 33% yield).

Mp: >300 ºC

IR (ATR) *v*: 3300, 2937, 1655, 1557, 1493, 1450, 1357, 1298, 1280, 1206, 1089, 930, 793, 765, 632, 614 cm⁻¹.

¹H NMR (400 MHz, CD$_3$OD) δ: 1.47 [m, 2H, 3''(5'')-H$_{ax}$], 1.90 [d, *J* = 14.1 Hz, 2H, 3''(5'')-H$_{eq}$], 1.99-2.09 [complex signal, 5H, 6'(12')-H$_{ax}$, 10'(13')-H$_{ax}$, 4''-H], 2.14 (d, *J* = 7.2 Hz, 2H, 2-H), 2.22 [dd, *J* = 13.2, 6.0 Hz, 2H, 6'(12')-H$_{eq}$], 2.38 [dd, *J* = 13.2, 6.0 Hz, 2H, 10'(13')-H$_{eq}$], 2.49 (s, 2H, 8'-H), 2.97 [t, *J* = 12.5 Hz, 2H, 2''(6'')-H$_{ax}$], 3.18 [broad t, *J* = 6.2 Hz, 2H, 5'(11')-H], 3.36 [d, *J* = 12.4 Hz, 2H, 2''(6'')-H$_{eq}$], 7.03-7.14 [complex signal, 4H, 1'(4')-H, 2'(3')-H].

¹³C NMR (101 MHz, CD$_3$OD) δ: 29.5 [CH$_2$, C3''(5'')], 32.7 (CH, C4''), 38.9 [CH$_2$, C6'(12')], 42.5 [CH, C5'(11')], 43.7 (CH$_2$, C2), 45.1 [CH$_2$, 2''(6'')], 45.9 [CH$_2$, C10'(13')], 50.9 (CH$_2$, C8'), 57.6 (C, C7'), 70.2 (C, C9'), 127.9 [CH, C2'(3')], 129.1 [CH, C1'(4')], 146.1 [C, C4a'(11a')], 173.1 (C, CO).

HRMS: Calcd for [C$_{22}$H$_{29}$ClN$_2$O+H]⁺: 373.2041, found: 373.2047.

71, synthesis of *N*-(5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)-2-(piperidin-4-yl)acetamide
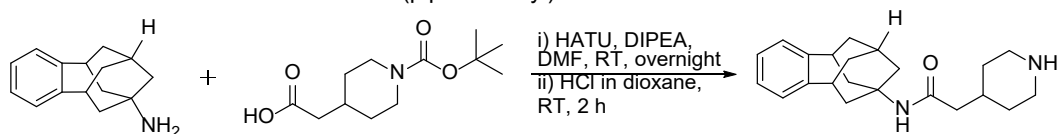
To a suspension of 5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine (90 mg, 0.42 mmol) in DMF (2.0 mL), 2-(1-(tert-butoxycarbonyl)piperidin-4-yl)acetic acid (123 mg, 0.51 mmol), HATU (239 mg, 0.63 mmol), and DIPEA (219 µL, 162 mg, 1.26 mmol) were added. The mixture was stirred at room temperature overnight. Solvent was concentrated *in vacuo* and EtOAc (10 mL) was added. The mixture was washed with NaHCO$_3$ sat. (2 x 30 mL). The organic layer was dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was used as such without further purification. Stoichiometric yield (184 mg).

HCl 4 M in dioxane (2.0 mL, 8.00 mmol) and dioxane (2.0 mL) were added to tert-butyl 4-(2-((5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)amino)-2-oxoethyl)piperidine-

1-carboxylate (185 mg, 0.42 mmol) and the mixture was stirred at room temperature for 2 h. Na_2CO_3 sat. was added until pH = 12 followed by EtOAc (15 mL) and the mixture was partitioned. The aqueous layer was extracted with EtOAc/MeOH 9/1 (2 x 10 mL). All organic layers were joined, dried over Na_2SO_4 anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH/NH$_3$ 0.7 M in DCM from 0% to 10%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford an orangish solid (108 mg, 72% yield).

Mp: 185-186 ºC

IR (ATR) *v*: 3301, 2907, 2855, 2349, 1653, 1556, 1450, 1356, 1320, 1280, 1137, 1046, 951, 929, 794, 765, 737, 660, 631, 616 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.20 [m, 2H, 3''(5'')-H$_{ax}$], 1.63-1.77 [complex signal, 4H, 10'(13')-H$_{ax}$, 3''(5'')-H$_{eq}$], 1.83 (m, 1H, 4''-H), 1.98 [m, 2H, 10'(13')-H$_{eq}$], 2.02 (d, *J* = 7.2 Hz, 2H, 8'-H), 2.06 (m, 2H, 2-H), 2.12-2.26 [complex signal, 4H, 6'(12')-H], 2.32 (m, 1H, 9'-H), 2.59 [m, 2H, 2''(6'')-H$_{ax}$], 2.98-3.08 [complex signal, 4H, 5'(11')-H, 2''(6'')-H$_{eq}$], 7.03 [s, 4H, 1'(4')-H, 2'(3')-H].

^{13}C NMR (101 MHz, CD$_3$OD) δ: 32.6 (CH, C9'), 33.1 [CH$_2$, C3''(5'')], 35.0 (CH, C4''), 35.7 [CH$_2$, C10'(13')], 40.4 [CH$_2$, C6'(12')], 41.5 (CH$_2$, C2), 42.6 [CH, C5'(11')], 45.2 (CH$_2$, C8'), 46.7 [CH$_2$, 2''(6'')], 53.8 (C, C7'), 127.3 [CH, C2'(3')], 129.0 [CH, C1'(4')], 148.0 [C, C4a'(11a')], 173.9 (C, CO).

HRMS: Calcd for [C$_{22}$H$_{30}$N$_2$O+H]$^+$: 339.2438, found: 339.2431.

72, synthesis of *N*-(5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl-9-d)-2-(piperidin-4-yl)acetamide
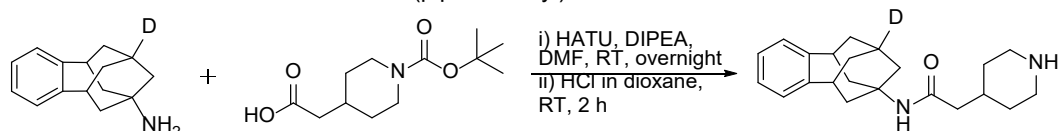
To a suspension of 5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-9-d-7-amine (90 mg, 0.42 mmol) in DMF (2.0 mL), 2-(1-(tert-butoxycarbonyl)piperidin-4-yl)acetic acid (123 mg, 0.51 mmol), HATU (239 mg, 0.63 mmol), and DIPEA (219 µL, 162 mg, 1.26 mmol) were added. The mixture was stirred at room temperature overnight. Solvent was concentrated *in vacuo* and EtOAc (10 mL) was added. The mixture was washed with NaHCO$_3$ sat. (2 x 30 mL). The organic layer was dried over Na_2SO_4 anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was used as such without further purification. Stoichiometric yield (184 mg).

HCl 4 M in dioxane (2.0 mL, 8.00 mmol) and dioxane (2.0 mL) were added to tert-butyl 4-(2-((5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl-9-d)amino)-2-oxoethyl)piperidine-1-carboxylate (185 mg, 0.42 mmol) and the mixture was stirred at room temperature for 2 h. Na_2CO_3 sat. was added until pH = 12 followed by EtOAc (15 mL) and the mixture was partitioned. The aqueous layer was extracted with EtOAc/MeOH 9/1 (2 x 15 mL). All organic layers were joined, dried over Na_2SO_4 anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH/NH$_3$ 0.7 M in DCM from 0% to 10%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (129 mg, 90% yield).

Mp: 188-189 ºC

IR (ATR) *v*: 3300, 2909, 2852, 2351, 1654, 1555, 1492, 1449, 1356, 1297, 1280, 1046, 928, 840, 794, 764, 737, 655, 616 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.24 [m, 2H, 3''(5'')-H$_{ax}$], 1.69-1.76 [complex signal, 4H, 10'(13')-H$_{ax}$, 3''(5'')-H$_{eq}$],1.86 (m, 1H, 4''-H), 1.99 [m, 2H, 10'(13')-H$_{eq}$], 2.02-2.07 (complex signal, 4H, 2-H, 8'-H),

2.19 [m, 4H, 6'(12')-H], 2.66 (dt, J = 12.6, 2.8 Hz, 2H, 2''(6'')-H$_{ax}$), 3.04 (m, 2H, , 5'(11')-H], 3.09 [dt, J = 12.6, 2.8 Hz, 2H, 2''(6'')-H$_{eq}$], 7.03 [s, 4H, 1'(4')-H, 2'(3')-H].

^{13}**C NMR** (101 MHz, CD$_3$OD) δ: 32.4 [CH$_2$, C3''(5'')], 32.5 (m, CD, C9'), 34.5 (CH, C4''), 35.6 [CH$_2$, C10'(13')], 40.4 [CH$_2$, C6'(12')], 41.4 (CH$_2$, C2), 42.6 [CH, C5'(11')], 44.9 (CH$_2$, C8'), 46.4 [CH$_2$, 2''(6'')], 53.8 (C, C7'), 127.3 [CH, C2'(3')], 129.0 [CH, C1'(4')], 148.0 [C, C4a'(11a')], 173.7 (C, CO).

HRMS: Calcd for [C$_{22}$H$_{29}$DN$_2$O+H]$^+$: 340.2500, found: 340.2494.

73, synthesis of 2-(1-(isopropylsulfonyl)piperidin-4-yl)-*N*-(9-methyl-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)acetamide
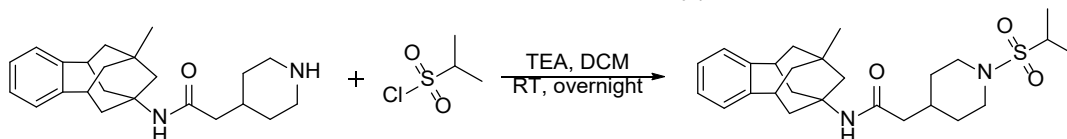
To a solution of *N*-(9-methyl-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)-2-(piperidin-4-yl)acetamide (185 mg, 0.52 mmol) and triethylamine (87 µL, 63 mg, 0.63 mmol) in DCM (5.0 mL) was added 2-propanesulfonyl chloride (58 µL, 74 mg, 0.52 mmol) under ice-bath. Then, the mixture was allowed to warm up to room temperature and stirred overnight. NaHCO$_3$ sat. (15 mL) was added followed by EtOAc (15 mL) and the mixture was partitioned. The aqueous layer was extracted again with EtOAc (2 x 15 mL). All organic layers were joined, dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 70%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (145 mg, 60% yield).

Mp: 172-173 ºC

IR (NaCl disk) v: 3365, 3319, 3059, 3018, 2916, 2852, 1648, 1536, 1493, 1452, 1361, 1323, 1309, 1265, 1190, 1167, 1138, 1091, 1044, 1011, 993, 945, 905, 881, 801, 759, 732, 702, 665 cm^{-1}.

^{1}**H NMR** (400 MHz, CDCl$_3$) δ: 0.91 (s, 3H, C9''-CH$_3$), 1.25 [dq, J = 12.0 Hz, J' = 4.0 Hz, 2H, 3'(5')-H$_{ax}$], 1.31 [d, J = 6.8 Hz, 6H, 2'''(3''')-H], 1.53 [d, J = 13.6 Hz, 2H, 10''(13'')-H$_{ax}$], 1.65 [dm, J = 13.6 Hz, 2H, 10''(13'')-H$_{eq}$], 1.74 [dm, J = 12.0 Hz, 2H, 3'(5')-H$_{eq}$], 1.82 (s, 2H, 8''-H$_2$), 1.93 (m, 1H, 4'-H), 1.97-2.04 [complex signal, 4H, 2-H$_2$, 6''(12'')-H$_{ax}$], 2.15 [dd, J = 11.6 Hz, J' = 6.0 Hz, 2H, 6''(12'')-H$_{eq}$], 2.85 [dt, J = 12.4 Hz, J' = 2.4 Hz, 2H, 2'(6')-H$_{ax}$], 3.06 [t, J = 6.0 Hz, 2H, 5''(11'')-H], 3.15 [hept, J = 6.8 Hz, 1H, 1'''-H], 3.80 [dt, J = 12.8 Hz, J' = 2.0 Hz, 2H, 2'(6')-H$_{eq}$], 5.22 (s, 1H, NH), 7.03 [m, 2H, 1''(4'')-H], 7.07 [m, 2H, 2''(3'')-H].

^{13}**C NMR** (101 MHz, CDCl$_3$) δ: 16.8 [CH$_3$, C2'''(3''')], 32.2 (CH$_3$, C9-CH$_3$), 32.3 [CH$_2$, C3'(5')], 33.1 (CH, C4'), 33.6 (C, C9''), 39.1 [CH$_2$, C6''(12'')], 41.0 [CH, C5''(11'')], 41.1 [CH$_2$, C10''(13'')], 44.4 (CH$_2$, C2), 46.5 [CH$_2$, C2'(6')], 47.2 (CH$_2$, C8'), 53.2 [CH, C1'''], 54.7 (C, C7'), 126.3 [CH, C2''(3'')], 128.0 [CH, C1''(4'')], 146.1 [C, C4a''(11a'')], 170.3 (C, CO).

Anal. calcd for C$_{26}$H$_{38}$N$_2$O$_3$S: C 68.09, H 8.35, N 6.11. Found: C 67.75 H 8.62, N 5.74.

HRMS: Calcd for [C$_{26}$H$_{38}$N$_2$O$_3$S+H]$^+$: 459.2676, found: 459.2675.

74, synthesis of *N*-(9-fluoro-6,7,8,9,10,11-hexahydro-5H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)-2-(1-(isopropylsulfonyl)piperidin-4-yl)acetamide
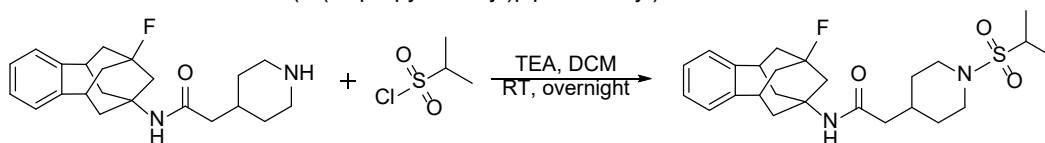
To a solution of *N*-(9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)-2-(piperidin-4-yl)acetamide (186 mg, 0.52 mmol) and triethylamine (87 µL, 63 mg, 0.63 mmol) in DCM (2.0 mL) was added 2-propanesulfonyl chloride (70 µL, 89 mg, 0.63 mmol) under ice-bath. Then, the mixture was allowed to warm up to room temperature and stirred overnight. NaHCO$_3$ sat. (15 mL) was added followed by EtOAc (15 mL) and the mixture was partitioned. The aqueous layer was extracted again with EtOAc (2 x 15 mL). All organic layers were joined, dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 70%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (123 mg, 51% yield).
Mp: 192-193 ºC

IR (ATR) *v*: 3342, 2914, 2855, 1663, 1536, 1449, 1316, 1138, 1045, 1017, 998, 953, 940, 865, 761, 753, 732, 652 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 1.24 [m, 2H, 3''(5'')-H$_{ax}$], 1.31 [d, *J* = 6.8 Hz, 6H, 2'''(3''')-H], 1.73 [m, 2H, 3''(5'')-H$_{eq}$], 1.88-2.04 [complex signal, 7H, 2-H, 6'(12')-H$_{ax}$, 10'(13')-H$_{ax}$, 4''-H], 2.10-2.23 [complex signal, 4H, 6'(12')-H$_{eq}$, 10'(13')-H$_{eq}$], 2.26 (d, *J* = 6.3 Hz, 2H, 8'-H), 2.85 [m, 2H, 2''(6'')-H$_{ax}$], 3.14 (h, *J* = 7.0 Hz, 1H, 1'''-H), 3.23 [m, 2H, 5'(11')-H], 3.80 [m, 2H, 2''(6'')-H$_{eq}$], 5.42 (broad s, 1H, NH), 7.07 [m, 2H, 1'(4')-H], 7.13 [m, 2H, 2'(3')-H].

^{13}C NMR (101 MHz, CDCl$_3$) δ: 16.9 [CH$_3$, C2'''(3''')], 32.4 [CH$_2$, C3''(5'')], 33.2 (CH, C4''), 38.7 [CH$_2$, C6'(12')], 39.6 [d, $^3J_{CF}$ = 13.3 Hz, CH, C5'(11')], 40.2 [d, $^2J_{CF}$ = 20.2 Hz, CH$_2$, C10'(13')], 44.3 (CH$_2$, C2), 46.1 (d, $^2J_{CF}$ = 18.5 Hz, CH$_2$, C8'), 46.7 [CH$_2$, C2''(6'')], 53.4 (CH, C1'''), 58.0 (d, $^3J_{CF}$ = 11.4 Hz, C, C7'), 94.2 (d, $^1J_{CF}$ = 177.6 Hz, C, C9'), 127.1 [CH, C2'(3')], 128.3 [CH, C1'(4')], 144.8 [C, C4a'(11a')], 170.6 (C, CO).

Anal. calcd for C$_{25}$H$_{35}$FN$_2$O$_3$S: C 64.91, H 7.63, N 6.06. Found: C 65.08, H 7.97, N 5.74.

HRMS: Calcd for [C$_{25}$H$_{35}$FN$_2$O$_3$S+H]$^+$: 463.2425, found: 463.2425.

75, synthesis of *N*-(9-chloro-6,7,8,9,10,11-hexahydro-5H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)-2-(1-(isopropylsulfonyl)piperidin-4-yl)acetamide
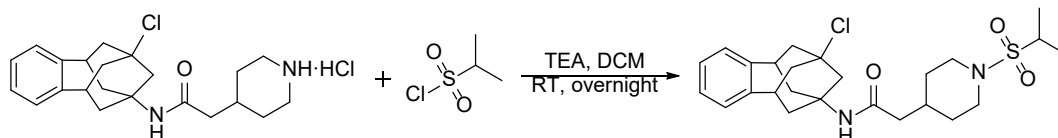
To a solution of *N*-(9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)-2-(piperidin-4-yl)acetamide hydrochloride (75 mg, 0.18 mmol) and triethylamine (100 µL, 73 mg, 0.72 mmol) in anh. DCM (1.0 mL) was added 2-propanesulfonyl chloride (31 µL, 39 mg, 0.27 mmol). Then, the mixture was stirred at room temperature overnight. NaHCO$_3$ sat. (15 mL) was added followed by EtOAc (10 mL) and the mixture was partitioned. The aqueous layer was extracted again with EtOAc (10 mL). Both organic layers were joined, dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 70%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a reddish solid (37 mg, 40% yield).
Mp: 163-164 ºC

IR (ATR) *v*: 3305, 2922, 2906, 2858, 1643, 1548, 1356, 1321, 1276, 1196, 1138, 1058, 1047, 952, 935, 799, 764, 738, 658 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.22 [m, 2H, 3''(5'')-H$_{ax}$], 1.29 [d, *J* = 6.8 Hz, 6H, 2'''(3''')-H], 1.72 [m, 2H, 3''(5'')-H$_{eq}$], 1.88 (m, 1H, 4-H''), 2.02-2.12 [complex signal, 6H, 2-H, 6'(12')-H$_{ax}$, 10'(13')-H$_{ax}$], 2.22 [m, 2H, 6'(12')-H$_{eq}$], 2.40 [m, 2H, 10'(13')-H$_{eq}$], 2.48 (s, 2H, 8'-H), 2.90 [m, 2H, 2''(6'')-H$_{ax}$], 3.19 [m, 2H,

5'(11')-H], 3.28 (p, J = 6.9 Hz, 1H, 1'''-H), 3.76 m, [2H, 2''(6'')-H$_{eq}$], 7.05-7.14 [complex signal, 4H, 1'(4')2'(3')-H].

¹³C NMR (101 MHz, CD₃OD) δ: 17.0 [CH₃, C2'''(3''')], 33.3 [CH₂, C3''(5'')], 34.6 (CH, C4''), 38.9 [CH₂, C6'(12')], 42.6 [CH, C5'(11')], 44.4 (CH₂, C2), 45.9 [CH₂, C10'(13')], 47.4 [CH₂, C2''(6'')], 51.0 (CH₂, C8'), 54.0 (CH, C1'''), 57.6 (C, C7'), 70.3 (C, C9'), 128.0 [CH, C2'(3')], 129.1 [CH, C1'(4')], 146.2 [C, C4a'(11a')], 173.8 (C, CO).

Anal. calcd for C₂₅H₃₅ClN₂O₃S: C 62.68, H 7.36, N 5.85. Found: C 62.63, H 7.31, N 5.68.

HRMS: Calcd for [C₂₅H₃₅ClN₂O₃S+H]⁺: 479.2130, found: 479.2143.

76, synthesis of *N*-(6,7,8,9,10,11-hexahydro-5H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)-2-(1-(isopropylsulfonyl)piperidin-4-yl)acetamide
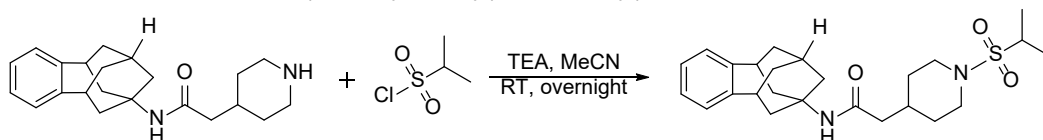
To a solution of *N*-(5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)-2-(piperidin-4-yl)acetamide (90 mg, 0.27 mmol) and triethylamine (150 µL, 109 mg, 1.08 mmol) in anh. MeCN (1.0 mL) was added 2-propanesulfonyl chloride (60 µL, 76 mg, 0.53 mmol). Then, the mixture was stirred at room temperature overnight. NaHCO₃ sat. (10 mL) was added followed by EtOAc (10 mL) and the mixture was partitioned. The aqueous layer was extracted again with EtOAc (10 mL). Both organic layers were joined, dried over Na₂SO₄ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 70%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (68 mg, 55% yield).

Mp: 136-137 ºC

IR (ATR) v: 3301, 2922, 2858, 1642, 1548, 1355, 1320, 1276, 1195, 1137, 1047, 951, 935, 799, 764, 738, 684, 657, 618 cm⁻¹.

¹H NMR (400 MHz, CD₃OD) δ: 1.24 [m, 2H, 3''(5'')-H$_{ax}$], 1.29 [d, J = 6.9 Hz, 6H, 2'''(3''')-H], 1.68-1.77 [complex signal, 4H, 10'(13')-H$_{ax}$, 3''(5'')-H$_{eq}$], 1.86 (m, 1H, 4''-H), 2.00 [m, 2H, 10'(13')-H$_{eq}$], 2.02-2.09 (complex signal, 4H, 2-H, 8'-H), 2.16 [m, 2H, 6'(12')-H$_{ax}$], 2.23 [m, 2H, 6'(12')-H$_{eq}$], 2.32 (m, 1H, 9'-H), 2.90 [m, 2H, 2''(6'')-H$_{ax}$], 3.04 [m, 2H, 5'(11')-H], 3.26 (p, J = 6.8 Hz, 1H, 1'''-H), 3.76 [m, 2H, 2''(6'')-H$_{eq}$], 7.03 [s, 4H, 1'(4')-H, 2'(3')-H].

¹³C NMR (101 MHz, CD₃OD) δ: 17.0 [CH₃, C2'''(3''')], 32.6 (CH, C9'), 33.3 [CH₂, C3''(5'')], 34.6 (CH, C4''), 35.7 [CH₂, C10'(13')], 40.4 [CH₂, C6'(12')], 41.4 (CH₂, C2), 42.6 [CH, C5'(11')], 44.5 (CH₂, C8'), 47.4 [CH₂, 2''(6'')], 53.9 (CH, 1'''C), 54.0 (C, C7'), 127.3 [CH, C2'(3')], 129.0 [CH, C1'(4')], 148.0 [C, C4a'(11a')], 173.6 (C, CO).

Anal. calcd for C₂₅H₃₆N₂O₃S: C 67.53, H 8.16, N 6.30. Calcd for C₂₅H₃₆N₂O₃S · 0.2 H₂O: C 66.99, H 8.19, N 6.25. Found: C 67.04, H 8.12, N 6.09.

HRMS: Calcd for [C₂₅H₃₆N₂O₃S+H]⁺: 445.2528, found: 445.2519.

77, synthesis of *N*-(5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl-9-d)-2-(1-(isopropylsulfonyl)piperidin-4-yl)acetamide
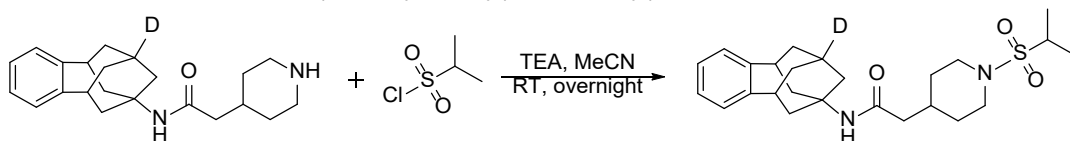
To a solution of *N*-(5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl-9-d)-2-(piperidin-4-yl)acetamide (111 mg, 0.33 mmol) and triethylamine (184 µL, 134 mg, 1.32 mmol) in anh. MeCN (1.0 mL) was added 2-propanesulfonyl chloride (73 µL, 93 mg, 0.65 mmol). Then, the mixture was stirred at room temperature overnight. NaHCO$_3$ sat. (20 mL) was added followed by EtOAc (15 mL) and the mixture was partitioned. The aqueous layer was extracted again with EtOAc (15 mL). Both organic layers were joined, dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 70%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (97 mg, 63% yield).

Mp: 138-139 ºC

IR (ATR) *v*: 3299, 2922, 2857, 1643, 1549, 1321, 1276, 1138, 1047, 952, 934, 764, 738, 656 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.22 [m, 2H, 3''(5'')-H$_{ax}$], 1.29 [d, *J* = 6.9 Hz, 6H, 2'''(3''')-H], 1.68-1.76 [complex signal, 4H, 10'(13')-H$_{ax}$, 3''(5'')-H$_{eq}$], 1.86 (m, 1H, 4''-H), 1.99 [m, 2H, 10'(13')-H$_{eq}$], 2.03-2.08 (complex signal, 4H, 2-H, 8'-H), 2.16 [m, 2H, 6'(12')-H$_{ax}$], 2.23 [m, 2H, 6'(12')-H$_{eq}$], 2.89 [m, 2H, 2''(6'')-H$_{ax}$], 3.03 [m, 2H, 5'(11')-H], 3.25 (p, *J* = 6.8 Hz, 1H, 1'''-H), 3.76 [m, 2H, 2''(6'')-H$_{eq}$], 7.03 [s, 4H, 1'(4')-H, 2'(3')-H].

^{13}C NMR (101 MHz, CD$_3$OD) δ: 17.0 [CH$_3$, C2'''(3''')], 32.1 (CD, C9'), 33.3 [CH$_2$, C3''(5'')], 34.6 (CH, C4''), 35.6 [CH$_2$, C10'(13')], 40.4 [CH$_2$, C6'(12')], 41.3 (CH$_2$, C2), 42.6 [CH, C5'(11')], 44.5 (CH$_2$, C8'), 47.4 [CH$_2$, 2''(6'')], 53.9 (CH, 1'''C), 54.0 (C, C7'), 127.3 [CH, C2'(3')], 129.0 [CH, C1'(4')], 148.0 [C, C4a'(11a')], 173.6 (C, CO).

Anal. calcd for C$_{25}$H$_{35}$DN$_2$O$_3$S: C 67.38, H 8.37, N 6.29. Calcd for C$_{25}$H$_{35}$DN$_2$O$_3$S · 0.3 H$_2$O: C 66.58, H 7.96, N 6.21. Found: C 66.68, H 8.05, N 6.10.

HRMS: Calcd for [C$_{25}$H$_{35}$DN$_2$O$_3$S+H]$^+$: 446.2589, found: 446.2582.

79, synthesis of 2-(1-benzylpiperidin-4-yl)-*N*-(9-methyl-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)acetamide
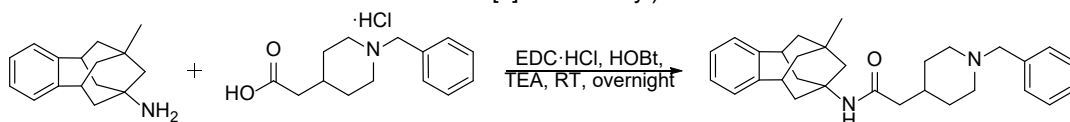
To a suspension of 9-methyl-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine hydrochloride (250 mg, 0.95 mmol) in EtOAc (5.0 mL), 2-(1-benzylpiperidin-4-yl)acetic acid hydrochloride (255 mg, 0.95 mmol), HOBt (192 mg, 1.42 mmol), EDC·HCl (220 mg, 1.42 mmol) and Et$_3$N (480 mg, 4.74 mmol) were added. The mixture was stirred at room temperature overnight. Water (10 mL) and DCM (10 mL) were added to the resulting suspension and the two phases were separated. The organic phase was washed with sat. NaHCO$_3$ aqueous solution (10 mL), brine (10 ml), dried over Na$_2$SO$_4$ anh., filtered and concentrated under vacuum to give a yellow gum (479 mg). The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 4%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a brown solid (280 mg, 67% yield).

Mp: 145-146 ºC

IR (NaCl disk) *v*: 3302, 3060, 3025, 2917, 2842, 2799, 2756, 1641, 1545, 1493, 1452, 1361, 1343, 1309, 1279, 1211, 1185, 1144, 1078, 1009, 974, 944, 917, 794, 757, 737, 698 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 0.91 (s, 3H, C9'-CH$_3$), 1.27 [dq, *J* = 12.4 Hz, *J'* = 3.6 Hz, 2H, 3''(5'')-H$_{ax}$], 1.53 [d, *J* = 13.2 Hz, 2H, 10'(13')-H$_{ax}$], 1.62-1.70 [complex signal, 4H, 3''(5'')-H$_{eq}$, 10'(13')-H$_{eq}$], 1.77 (m, 1H, 4''-H), 1.84 (s, 2H, 8'-H), 1.94-2.02 [complex signal, 6H, 6'(12')-H$_{ax}$, 2-H, 6''(2'')-H$_{ax}$], 2.16 [dd, *J* = 12 Hz, *J'* = 6 Hz, 2H, 6'(12')-H$_{eq}$], 2.86 [dt, *J* = 11.6 Hz, *J'* = 2.8 Hz, 2H, 2''(6'')-H$_{eq}$], 3.06 [t, *J* = 6 Hz, 2H, 5'(11')-H], 3.48 (s, 2H, benzyl-C$\underline{H}_2$), 5.20 (s, 1H, NH), 7.03 [m, 2H, 1'(4')-H], 7.06 [m, 2H, 2'(3')-H], 7.23 (m, 1H, 4'''-H), 7.27-7.32 [complex signal, 4H, 2'''(6''')-H, 3'''(5''')-H].

^{13}C NMR (101 MHz, CD$_3$OD) δ: 32.0 [CH$_2$, C3''(5'')], 32.2 (CH$_3$, C9'-CH$_3$), 33.4 (C, C9'), 33.5 (CH, C4''), 39.1 [CH2, C6'(12')], 40.9 (CH, C5'(11')], 41.1 [CH$_2$, C10'(13')], 44.9 (CH$_2$, C2), 47.1 (CH$_2$, C8'), 53.5 [CH$_2$, C2''(6'')], 54.4 (C, C7'), 63.3 (CH$_2$, benzyl-$\underline{C}$H$_2$), 126.2 [CH, C2'(3')], 126.9 (CH, C4''), 127.9 [CH, C1'(4')], 128.1 [CH, C3'''(5''')], 129.2 [CH, C2'''(6''')], 138.3 (C, C1'''), 146.1 [C, C4a'(11a')], 171.0 (C, NHCO).

HRMS: Calcd for [C$_{30}$H$_{38}$N$_2$O+H]$^+$: 443.3057; Found: 443.3061.

80, synthesis of 2-(1-benzylpiperidin-4-yl)-*N*-(9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)acetamide
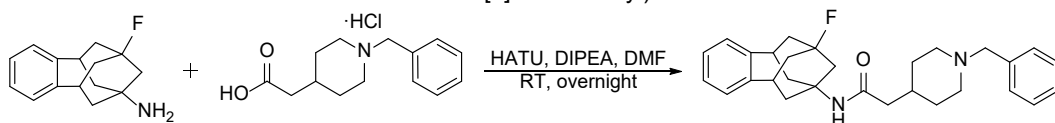
To a solution of 9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine (71 mg, 0.31 mmol) in DMF (2.0 mL), 2-(1-benzylpiperidin-4-yl)acetic acid hydrochloride (100 mg, 0.37 mmol), HATU (176 mg, 0.46 mmol), and DIPEA (160 μL, 119 mg, 0.92 mmol) were added. The mixture was stirred at room temperature overnight. EtOAc (7 mL) was added and the mixture was washed with NaHCO$_3$ sat. (2 x 20 mL). The organic layer was dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 5%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a brown solid (72 mg, 52% yield).

Mp: 164-165 ºC

IR (ATR) *v*: 3287, 2923, 2856, 2216, 1643, 1604, 1548, 1515, 1494, 1446, 1359, 1300, 1240, 1177, 1088, 999, 864, 842, 821, 763, 734, 697, 644, 570, 558 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.30 [m, 2H, 3''(5'')-H$_{ax}$], 1.68 [dm, *J* = 12.7 Hz, 2H, 3''(5'')-H$_{eq}$], 1.74 (m, 1H, 4''-H), 1.81 [broad d, *J* = 12.8 Hz, 2H, 10'(13')-H$_{ax}$], 2.04 (d, *J* = 7.1 Hz, 2H, 2-H), 2.06-2.17 [complex signal, 8H, 6'(12')-H$_2$, 10'(13')-H$_{eq}$, 2''(6'')-H$_{ax}$], 2.19 (d, $^2J_{HF}$ = 6.5 Hz, 2H, 8'-H$_2$), 2.94 [broad d, *J* = 12.1 Hz, 2H, 2''(6'')-H$_{eq}$], 3.23 [broad s, 2H, 5'(11')-H], 3.57 (s, 2H, benzyl-C$\underline{H}_2$), 7.06-7.10 [complex signal, 4H, 1'(4')-H, 2'(3')-H], 7.28 (m, 1H, 4'''-H), 7.31-7.36 [complex signal, 4H, 2'''(6''')-H, 3'''(5''')-H].

^{13}C NMR (101 MHz, CD$_3$OD) δ: 32.2 [CH$_2$, C3''(5'')], 34.5 (CH, C4''), 39.3 [CH$_2$, C6'(12')], 41.0 [d, $^3J_{CF}$ = 13.1 Hz, CH, C5'(11')], 41.4 [d, $^2J_{CF}$ = 20.1 Hz, CH$_2$, C10'(13')], 44.5 (CH$_2$, C2), 46.9 (d, $^2J_{CF}$ = 18.3 Hz, CH$_2$, C8'), 54.4 [CH$_2$, C2''(6'')], 58.8 (d, $^3J_{CF}$ = 11.2 Hz, C, C7'), 64.1 (CH$_2$, benzyl-$\underline{C}$H$_2$), 94.7 (d, $^1J_{CF}$ = 177.1 Hz, C, C9'), 127.9 [CH, C2'(3')], 128.7 (CH, C4'''), 129.2 [CH, C1'(4')], 129.4 [CH, C3'''(5''')], 131.0 [CH, C2'''(6''')], 137.5 (C, C1'''), 146.3 [C, C4a'(11a')], 174.1 (C, $\underline{C}$ONH).

Anal. calcd for C$_{29}$H$_{35}$FN$_2$O: C 77.99, H 7.90, N 6.27. Anal. calcd for C$_{29}$H$_{35}$FN$_2$O · 0.6 H$_2$O: C 76.15, H 7.98, N 6.12. Found: C 75.99, H 7.81, N 6.06.

HRMS: Calcd for [C$_{29}$H$_{35}$FN$_2$O+H]$^+$: 447.2806, found: 447.2804.

82, synthesis of 2-(piperidin-4-yl)acetic acid hydrochloride
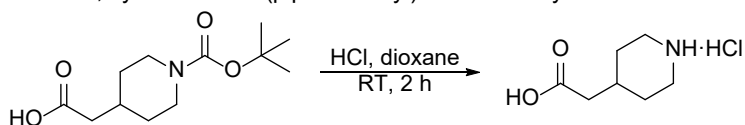
HCl 4 M in dioxane (8.0 mL, 32.00 mmol) and dioxane (8.0 mL) were added to 2-(1-(tert-butoxycarbonyl)piperidin-4-yl)acetic acid (2.0 g, 8.22 mmol) and the mixture was stirred at room temperature for 2 h. Solvents were concentrated *in vacuo*. The resulting white crude was used as such without further purification. Stoichiometric yield (1.47 g, crude).

83, synthesis of 2-(1-(2-bromo-4-(methoxycarbonyl)phenyl)piperidin-4-yl)acetic acid
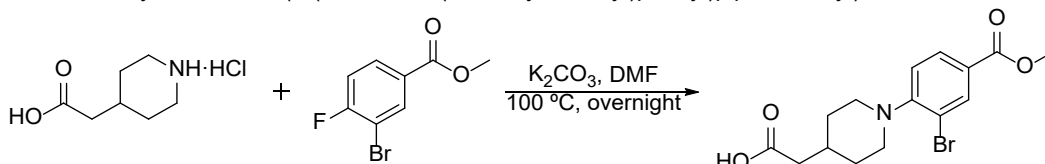
To a solution of 2-(piperidin-4-yl)acetic acid (200 mg, 1.11 mmol) and methyl 3-bromo-4-fluorobenzoate (311 mg, 1.34 mmol) in DMF (3.0 mL) was added K_2CO_3 (612 mg, 4.44 mmol) and the mixture was stirred at 100 ºC overnight. Water was added (20 mL) followed by HCl 1 M until pH = 4. The mixture was extracted with EtOAc (10 mL). The organic layer was washed again with water at pH = 4 (20 mL). Then, the organic layer was dried over Na_2SO_4 anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 2%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (200 mg, 50% yield).
¹H NMR (400 MHz, CDCl₃) δ: 1.51 (m, 2H), 1.85 (d, *J* = 13.0 Hz, 3H), 2.32 (d, *J* = 6.9 Hz, 2H), 2.71 (t, *J* = 11.7 Hz, 2H), 3.46 (d, *J* = 11.5 Hz, 2H), 3.88 (s, 3H), 7.01 (d, *J* = 8.5 Hz, 1H), 7.91 (dd, *J* = 8.4, 2.0 Hz, 1H), 8.21 (d, *J* = 2.0 Hz, 1H).

84, synthesis of 2-(1-(4-(tert-butoxycarbonyl)phenyl)piperidin-4-yl)acetic acid
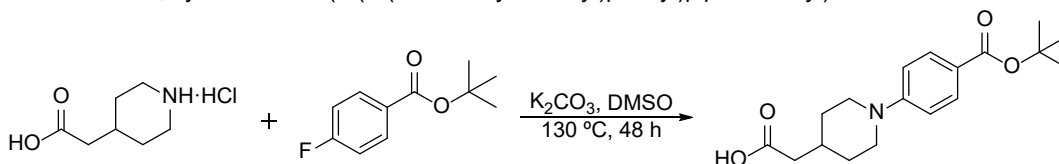
To a solution of 2-(piperidin-4-yl)acetic acid (369 mg, 2.06 mmol) and tert-butyl 4-fluorobenzoate (409 µL, 443 mg, 2.26 mmol) in DMSO (8.0 mL) was added K_2CO_3 (1.14 g, 8.22 mmol) and the mixture was stirred at 130 ºC for 48 h. Water was added (20 mL) followed by HCl 1 M until pH = 5. The mixture was extracted with EtOAc (20 mL). The organic layer was washed again with water at pH = 5 (20 mL). Then, the organic layer was dried over Na_2SO_4 anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 30%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (255 mg, 39% yield).
¹H NMR (400 MHz, CD₃OD) δ: 1.40 (qd, *J* = 12.4 Hz, *J'* = 4.0, 2H), 1.57 (s, 9H), 1.87 (d, *J* = 13.1, 2H), 2.02 (m, 1H), 2.33 (d, *J* = 7.0, 2H), 2.86 (td, *J* = 12.4 Hz, *J'* = 2.6, 2H), 3.84 (d, *J* = 12.4, 2H), 6.85 (d, *J* = 9.0, 2H), 7.86 (d, *J* = 9.0, 2H).

85, synthesis of 2-(1-(4-cyanophenyl)piperidin-4-yl)acetic acid
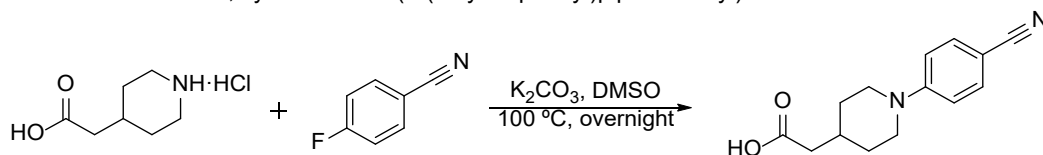
To a solution of 2-(piperidin-4-yl)acetic acid (800 mg, 4.45 mmol) and 4-fluorobenzonitrile (647 mg, 5.34 mmol) in DMSO (15.0 mL) was added K_2CO_3 (2.45 g, 17.76 mmol) and the mixture was stirred at 100 ºC overnight. Water was added (50 mL) followed by HCl 1 M until pH = 4. The mixture was extracted with EtOAc (30 mL). The organic layer was washed again with water at pH = 4 (50 mL). Then, the organic layer was dried over Na_2SO_4 anh., filtered and solvents were concentrated *in vacuo* to afford a beige solid that was used as such without further purification (708 mg, 65% yield).

¹H NMR (400 MHz, CD₃OD) δ: 1.33 (qd, *J* = 12.7, 4.0 Hz, 2H), 1.86 (m, 2H), 2.00 (m, 1H), 2.26 (d, *J* = 7.1 Hz, 2H), 2.89 (td, *J* = 12.7, 2.6 Hz, 2H), 3.94 (m, 2H), 6.98 (d, *J* = 9.1 Hz, 2H), 7.48 (d, *J* = 9.0 Hz, 2H).

86, synthesis of 2-(1-(4-acetylphenyl)piperidin-4-yl)acetic acid
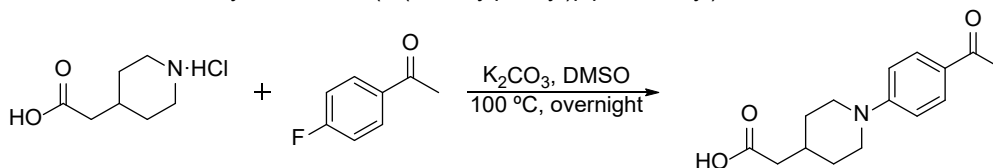
To a solution of 2-(piperidin-4-yl)acetic acid (200 mg, 1.11 mmol) and 4′-Fluoroacetophenone (163 µL, 185 mg, 1.34 mmol) in DMSO (3.0 mL) was added K_2CO_3 (612 mg, 4.44 mmol) and the mixture was stirred at 100 ºC overnight. Water was added (20 mL) followed by HCl 1 M until pH = 4. The mixture was extracted with EtOAc (20 mL). The organic layer was washed again with water at pH = 4 (20 mL). Then, the organic layer was dried over Na_2SO_4 anh., filtered and solvents were concentrated *in vacuo* to afford an orangish solid that was used as such without further purification (132 mg, 45% yield).

¹H NMR (400 MHz, CD₃OD) δ: 1.34 (qd, *J* = 12.3 Hz, *J'* = 4.0 Hz, 2H), 1.86 (m, 2H), 2.01 (m, 1H), 2.26 (d, *J* = 7.1 Hz, 2H), 2.49 (s, 3H), 2.91 (td, *J* = 12.8 Hz, *J'* = 2.6 Hz, 2H), 3.98 (m, 2H), 6.94 (d, *J* = 9.0 Hz, 2H), 7.86 (d, *J* = 9.0 Hz, 2H).

87, synthesis of 2-(1-(2-cyclopropyl-4-(methoxycarbonyl)phenyl)piperidin-4-yl)acetic acid
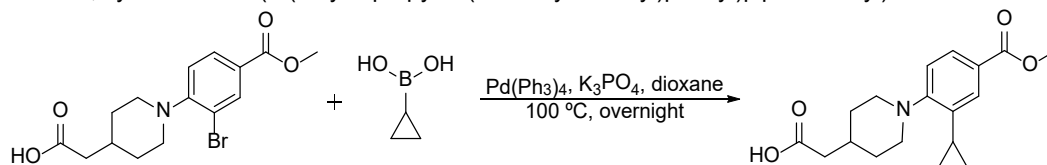
A suspension of 2-(1-(2-bromo-4-(methoxycarbonyl)phenyl)piperidin-4-yl)acetic acid (200 mg, 0.56 mmol), cyclopropylboronic acid (96 mg, 1.12 mmol) and K_3PO_4 (360 mg, 1.70 mmol) in dioxane (5.0 mL) was degassed bubbling with N_2 for 10 min. Then, tetrakis(triphenylphosphine)palladium(0) (65 mg, 0.06 mmol) was added and the mixture was heated at 100 ºC and stirred overnight. Water was added (20 mL) followed by HCl 1 M until pH = 4. The mixture was extracted with EtOAc (20 mL). The aqueous layer was extracted again with EtOAc (20 mL). Both organic layers were dried over Na_2SO_4 anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 2%). Fractions

containing the desired product were collected and concentrated *in vacuo* to afford a green solid (100 mg, 36% yield, 64% purity given by HPLC-UV).

¹H NMR (400 MHz, CDCl₃) δ: 0.79 [m, 2H, 2'''(3''')-H$_{ax}$], 1.02 [m, 2H, 2'''(3''')-H$_{eq}$], 1.52 [qd, J = 12.0 Hz, J' = 3.8 Hz, 2H, 3'(5')-H$_{ax}$], 1.89 [m, 2H, 3'(5')-H$_{ax}$], 1.99 (m, 1H, 4'-H), 2.13 (m, 1H, 1'''-H), 2.39 [d, J = 7.0 Hz, 2H, 3'(5')-H$_{eq}$], 2.73 [td, J = 12.0 Hz, J' = 2.2 Hz, 2'(6')-H$_{ax}$], 3.45 [d, J = 12.0 Hz, 2H, 2'(6')-H$_{eq}$], 3.86 (s, 3H, C$\underline{H}$₃), 6.97 (d, J = 8.4 Hz, 1H, 6''-H), 7.45 (d, J = 2.1 Hz, 1H, 3''-H), 7.78 (dd, J = 8.4 Hz, J' = 2.1 Hz, 1H, 5''-H).

88, synthesis of 3-cyclopropyl-4-(4-(2-((9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)amino)-2-oxoethyl)piperidin-1-yl)benzoic acid
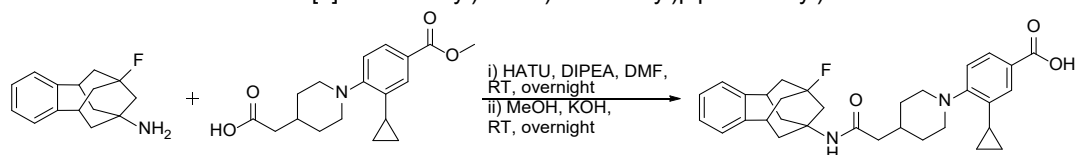
To a solution of 9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine (100 mg, 0.45 mmol) in DMF (2.0 mL), 2-(1-(2-cyclopropyl-4-(methoxycarbonyl)phenyl)piperidin-4-yl)acetic acid (171 mg, 0.54 mmol), HATU (257 mg, 0.68 mmol), and DIPEA (235 µL, 174 mg, 1.35 mmol) were added. The mixture was stirred at room temperature overnight. EtOAc (7 mL) was added and the mixture was washed with NaHCO₃ sat. (2 x 20 mL). The organic layer was dried over Na₂SO₄ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 30%). Fractions containing the desired product were collected and concentrated *in vacuo*.

MeOH (1.0 mL), KOH (116 mg, 2.07 mmol) and some drops of water were added and the mixture was stirred at 50 ºC for 4 h. Amberlite® 120 H⁺ was added until pH = 4 and the mixture was filtered using MeOH as eluting agent. Solvents were concentrated *in vacuo* to afford a white solid that was purified column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 3%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a reddish solid (16 mg, 7% yield).

Mp: 190-191 ºC

IR (ATR) *v*: 3289, 2921, 2856, 2214, 1644, 1604, 1549, 1515, 1444, 1360, 1300, 1239, 1177, 1116,1088, 1048, 1010, 998, 863, 843, 820, 759, 639, 570, 555 cm⁻¹.

¹H NMR (400 MHz, CD₃OD) δ: 0.73 [m, 2H, 2'(3')-H$_{ax}$], 1.02 [m, 2H, 2'(3')-H$_{eq}$], 1.47 [qd, J = 12.0 Hz, J' = 3.8 Hz, 2H, 3''(5'')-H$_{ax}$], 1.76-1.86 [complex signal, 4H, 3''(5'')-H$_{eq}$, 10''''(13'''')-H$_{ax}$], 1.90 (m, 1H, 4''-H), 2.06-2.21 [complex signal, 9H, 1'-H, 1'''-H, 6''''(12'''')-H₂, 10''''(13'''')-H$_{eq}$], 2.22 (d, $^2J_{HF}$ = 6.4 Hz, 2H, 8''''-H), 2.71 [td, J = 11.9 Hz, J' = 2.2 Hz, 2H, 2''(6'')-H$_{ax}$], 3.25 [broad t, J = 5.7 Hz, 2H, 5''''(11'''')-H], 3.43 [broad d, J = 12.1 Hz, 2H, 2''(6'')-H$_{eq}$], 7.03 (d, J = 8.4 Hz, 1H, 5-H), 7.10 [s, 4H, 1''''(4'''')-H, 2''''(3'''')-H], 7.44 (d, J = 2.0 Hz, 1H, 2-H), 7.75 (dd, J = 8.4 Hz, J' = 2.1 Hz, 1H, 6-H).

¹³C NMR (101 MHz, CD₃OD) δ: 9.9 [CH₂, C2'(3')], 12.2 (CH, C1'), 33.6 [CH₂, C3''(5'')], 35.0 (CH, C4''), 39.4 [CH₂, C6''''(12'''')], 41.0 [d, $^3J_{CF}$ = 13.2 Hz, CH, C5''''(11'''')], 41.4 [d, $^2J_{CF}$ = 20.1 Hz, CH₂, C10''''(13'''')], 44.9 (CH₂, C1'''), 46.9 (d, $^2J_{CF}$ = 18.3 Hz, CH₂, C8''''), 53.3 [CH₂, C2''(6'')], 58.8 (d, $^3J_{CF}$ = 11.3 Hz, C, C7''''), 94.8 (d, $^1J_{CF}$ = 177.2 Hz, C, C9''''), 119.1 (CH, C5), 125.6 (C, C1), 126.4 (CH, C2), 128.0 [CH, C2''''(3'''')], 129.0 (CH, C6), 129.2 [CH, C1''''(4'''')], 138.1 (C, C3), 146.3 [C, C4'''a(11'''a)], 158.3 (C, C4), 170.5 (C, $\underline{C}$ONH), 174.3 (C, $\underline{C}$O₂H).

Anal. calcd for C₃₂H₃₇FN₂O₃: C 74.39, H 7.22, N 5.42. Anal. calcd for C₃₂H₃₇FN₂O₃ · 0.35 H₂O · 0.6 C₄H₁₀O: C 72.82, H 7.76, N 4.94. Found: C 73.02, H 7.55, N 4.71.

HRMS: Calcd for [C₃₂H₃₇FN₂O₃+H]⁺: 517.2861, found: 517.2847.

89, synthesis of 4-(4-(2-((9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)amino)-2-oxoethyl)piperidin-1-yl)benzoic acid
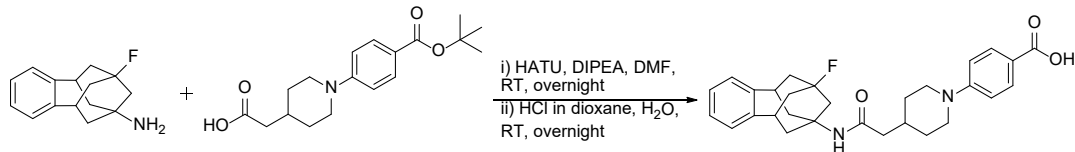
To a solution of 9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine (82 mg, 0.36 mmol) in DMF (2.0 mL), 2-(1-(4-(tert-butoxycarbonyl)phenyl)piperidin-4-yl)acetic acid (125 mg, 0.39 mmol), HATU (203 mg, 0.53 mmol), and DIPEA (124 µL, 92 mg, 0.71 mmol) were added. The mixture was stirred at room temperature overnight. EtOAc (15 mL) was added and the mixture was washed with brine (2 x 30 mL). The organic layer was dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 30%). Fractions containing the desired product were collected and concentrated *in vacuo*.

HCl 4 M in dioxane (2 mL) with some drops of water were added to the solid and the mixture was stirred at room temperature overnight. EtOAc (10 mL) was added and the mixture was washed with water acidified at pH = 4 with HCl 2 M (2 x 20 mL). The organic layer was dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 4%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford an off-white solid (40 mg, 24% yield).

Mp: 267-268 ºC

IR (ATR) *v*: 2923, 2853, 1665, 1634, 1601, 1519, 1431, 1418, 1318, 1283, 1211, 1189, 1089, 993, 827, 767, 697, 632, 553 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.34 [qd, *J* = 12.6 Hz, *J'* = 4.1 Hz, 2H, 3'(5')-H$_{ax}$], 1.76 [broad d, *J* = 12.1 Hz, 2H, 3'(5')-H$_{eq}$], 1.82 [broad d, *J* = 10.4 Hz, 2H, 10'''(13''')-H$_{ax}$], 1.95 (m, 1H, 4'-H), 2.05-2.13 [complex signal, 4H, 1''-H, 6'''(12''')-H$_{ax}$], 2.13-2.19 [complex signal, 4H, 6'''(12''')-H$_{eq}$, 10'''(13''')-H$_{ax}$], 2.21 (d, $^2J_{HF}$ = 6.4 Hz, 2H, 8'''-H), 2.84 [td, *J* = 12.7 Hz, *J'* = 2.6 Hz, 2H, 2'(6')-H$_{ax}$], 3.24 (broad s, 2H, 5'''(11''')-H], 3.92 [broad d, *J* = 13.3 Hz, 2H, 2'(6')-H$_{eq}$], 6.93 [d, *J* = 9.1 Hz, 2H, 3(5)-H], 7.07-7.13 [complex signal, 4H, 1'''(4''')-H, 2''(3''')-H], 7.85 [d, *J* = 9.1 Hz, 2H, 2(6)-H].

^{13}C NMR (101 MHz, CD$_3$OD) δ: 32.4 [CH$_2$, C3'(5')], 35.1 (CH, C4'), 39.3 [CH$_2$, C6'''(12''')], 41.0 [d, $^3J_{CF}$ = 13.1 Hz, CH, C5'''(11''')], 41.4 [d, $^2J_{CF}$ = 20.2 Hz, CH$_2$, C10'''(13''')], 44.7 (CH$_2$, C1''), 46.9 (d, $^2J_{CF}$ = 18.4 Hz, CH$_2$, C8'''), 49.1 [CH$_2$, C2''(6'')], 58.8 (d, $^3J_{CF}$ = 11.4 Hz, C, C7'''), 94.8 (d, $^1J_{CF}$ = 177.1 Hz, C, C9'''), 114.9 [CH, C3(5)], 120.1 (C, C1), 128.0 [CH, C2'''(3''')], 129.2 [CH, C1'''(4''')], 132.5 [CH, C2(6)], 146.3 [C, C4''a(11''a)], 155.9 (C, C4), 170.4 (C, C<u>C</u>ONH), 174.1 (C, <u>C</u>O$_2$H).

Anal. calcd for C$_{29}$H$_{33}$FN$_2$O$_3$: C 73.09, H 6.98, N 5.88. Anal. calcd for C$_{29}$H$_{33}$FN$_2$O$_3$ · 0.3 H$_2$O: C 72.27, H 7.03, N 5.81. Found: C 72.64, H 7.16, N 5.39.

HRMS: Calcd for [C$_{29}$H$_{33}$FN$_2$O$_3$-H]$^-$: 475.2402, found: 475.2400.

90, synthesis of 4-(4-(2-((9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)amino)-2-oxoethyl)piperidin-1-yl)benzoic acid
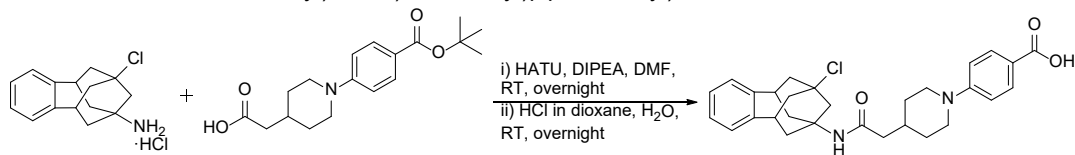
To a solution of 9-chloro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine hydrochloride (101 mg, 0.36 mmol) in DMF (2.0 mL), 2-(1-(4-(tert-butoxycarbonyl)phenyl)piperidin-4-yl)acetic acid (125 mg, 0.39 mmol), HATU (203 mg, 0.53 mmol), and DIPEA (124 µL, 92 mg, 0.71 mmol) were added. The mixture was stirred at room temperature overnight. EtOAc (15 mL) was added and the mixture was washed with brine (2 x 30 mL). The organic layer was dried over Na_2SO_4 anh., filtered and solvents were concentrated. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 25%). Fractions containing the desired product were collected and concentrated *in vacuo*.

HCl 4 M in dioxane (2.0 mL, 8.00 mmol) with some drops of water were added and the mixture was stirred at room temperature overnight. EtOAc (10 mL) was added and the mixture was washed with water acidified at pH = 4 with HCl 2 M (2 x 20 mL). The organic layer was dried over Na_2SO_4 anh., filtered and solvents were concentrated *in vacuo*. The crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 4%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a pink solid (23 mg, 13% yield).

Mp: 242-243 ºC

IR (ATR) *v*: 2920, 2855, 1664, 1638, 1600, 1518, 1415, 1391, 1357, 1283, 1230, 1184, 1110, 1082, 978, 947, 931, 900, 830, 800, 773, 762, 697, 645 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.35 [m, 2H, 3'(5')-H$_{ax}$], 1.77 [broad d, *J* = 12.9 Hz, 2H, 3'(5')-H$_{eq}$], 1.95 (m, 1H, 4'-H), 2.02-2.11 [complex signal, 6H, 1''-H, 6'''(12''')-H$_{ax}$, 10'''(13''')-H$_{ax}$], 2.23 [m, 2H, 6'''(12''')-H$_{eq}$], 2.40 [m, 2H, 10'''(13''')-H$_{eq}$], 2.49 (s, 2H, 8''-H), 2.85 [td, *J* = 12.6 Hz, *J'* = 2.6 Hz, 2H, 2'(6')-H$_{ax}$], 3.19 [broad t, *J* = 6.5 Hz, 2H, 5'''(11''')-H], 3.92 [broad d, *J* = 13.4 Hz, 2H, 2'(6')-H$_{eq}$], 6.93 [d, *J* = 9.1 Hz, 2H, 3(5)-H], 7.06-7.12 [complex signal, 4H, 1'''(4''')-H, 2''(3''')-H], 7.85 [d, *J* = 9.0 Hz, 2H, 2(6)-H].

^{13}C NMR (101 MHz, CD$_3$OD) δ: 32.4 [CH$_2$, C3'(5')], 35.1 (CH, C4'), 39.0 [CH$_2$, C6'''(12''')], 42.6 [CH, C5'''(11''')], 44.7 (CH$_2$, C1''), 45.9 [CH$_2$, C10'''(13''')], 49.1 [signal overlapped, CH$_2$, C2'(6')], 51.0 (CH$_2$, C8''), 57.6 (C, C7'''), 70.3 (C, C9'''), 114.9 [CH, C3(5)], 120.1 (C, C1), 128.0 [CH, C2'''(3''')], 129.1 [CH, C1'''(4''')], 132.5 [CH, C2(6)], 146.2 [C, C4'''a(11'''a)], 155.9 (C, C4), 170.4 (C, CONH), 174.1 (C, CO$_2$H).

Anal. calcd for C$_{29}$H$_{33}$ClN$_2$O$_3$: C 70.65, H 6.75, N 5.68. Anal. calcd for C$_{29}$H$_{33}$ClN$_2$O$_3$ · 0.45 H$_2$O · 0.2 C$_4$H$_8$O$_2$: C 69.00, H 6.90, N 5.40. Found: C 68.77, H 6.66, N 5.18.

HRMS: Calcd for [C$_{29}$H$_{33}$ClN$_2$O$_3$-H]$^-$: 491.2107, found: 491.2106.

91, synthesis of 2-(1-(4-cyanophenyl)piperidin-4-yl)-*N*-(9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)acetamide
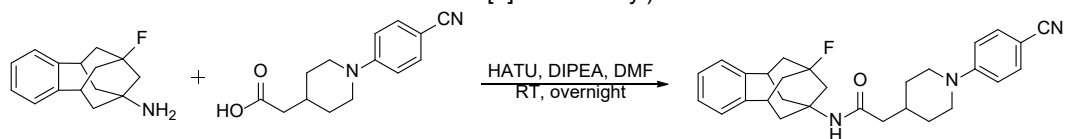
To a solution of 9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine (200 mg, 0.86 mmol) in DMF (4.0 mL), 2-(1-(4-cyanophenyl)piperidin-4-yl)acetic acid (252 mg, 1.03 mmol), HATU (490 mg, 1.29 mmol), and DIPEA (449 µL, 333 mg, 2.58 mmol) were added. The mixture was stirred at room temperature overnight. EtOAc (15 mL) was added and the mixture was washed with brine (2 x 30 mL). The organic layer was dried over Na_2SO_4 anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 45%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a pale-pink solid (23 mg, 6% yield).

Mp: 180-181 ºC

IR (ATR) *v*: 3317, 2926, 2857, 2212, 1645, 1603, 1515, 1445, 1359, 1306, 1239, 1177, 1112, 1010, 863, 819, 762, 732, 680, 645, 569, 560 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 1.29 [m, 2H, 3''(5'')-H$_{ax}$], 1.80 [broad d, *J* = 10.3 Hz, 2H, 3''(5'')-H$_{eq}$], 1.93 [m, 2H, 10'(13')-H$_{ax}$], 1.97-2.08 [complex signal, 5H, 2-H$_2$, 6'(12')-H$_{ax}$, 4''-H], 2.11-2.25 [complex signal, 4H, 6'(12')-H$_{eq}$, 10'(13')-H$_{eq}$], 2.28 (d, $^2J_{HF}$ = 6.3 Hz, 2H, 8'-H$_2$), 2.87 [td, *J* = 12.7 Hz, *J'* = 2.6 Hz, 2H, 2''(6'')-H$_{ax}$], 3.23 [m, 2H, 5'(11')-H], 3.82 [3.82 [broad dt, *J* = 12.9 Hz, *J'* =2.5 Hz, 2H, 2''(6'')-H$_{eq}$], 5.33 (broad s, 1H, NH), 6.83 [d, *J* = 9.0 Hz, 2H, 2'''(6''')-H], 7.08 [m, 2H, 1'(4')-H], 7.12 [m, 2H, 2'(3')-H], 7.45 [d, *J* = 9.0 Hz, 2H, 3'''(5''')-H].

^{13}C NMR (101 MHz, CDCl$_3$) δ: 31.3 [CH$_2$, C3''(5'')], 33.5 (CH, C4''), 38.7 [CH$_2$, C6'(12')], 39.6 [d, $^3J_{CF}$ = 13.2 Hz, CH, C5'(11')], 40.1 [d, $^2J_{CF}$ = 20.2 Hz, CH$_2$, C10'(13')], 44.4 (CH$_2$, C2), 46.0 (d, $^2J_{CF}$ = 18.4 Hz, CH$_2$, C8'), 47.9 [CH$_2$, C2''(6'')], 58.0 (d, $^3J_{CF}$ = 11.4 Hz, C, C7'), 94.2 (d, $^1J_{CF}$ = 177.6 Hz, C, C9'), 99.5 (C, <u>C</u>N), 114.4 [CH, C2'''(6''')], 120.3 (C, C4'''), 127.1 1 [CH, C2'(3')], 128.3 [CH, C1'(4')], 133.6 [CH, C3'''(5''')], 144.7 [C, C4a(11a)], 153.3 (C, C1'''), 170.7 (C, <u>C</u>ONH).

Anal. calcd for C$_{29}$H$_{32}$FN$_3$O: C 76.12, H 7.05, N 9.18. Anal. calcd for C$_{29}$H$_{32}$FN$_3$O · 1.7 H$_2$O: C 71.35, H 7.31, N 8.61. Found: C 70.78, H 6.67, N 8.17.

HRMS: Calcd for [C$_{29}$H$_{32}$FN$_3$O+H]$^+$: 458.2602, found: 458.2593.

92, synthesis of 2-(1-(4-acetylphenyl)piperidin-4-yl)-*N*-(9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-yl)acetamide
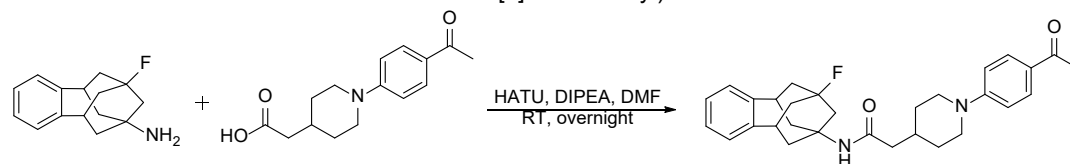
To a solution of 9-fluoro-5,6,8,9,10,11-hexahydro-7H-5,9:7,11-dimethanobenzo[9]annulen-7-amine (50 mg, 0.22 mmol) in DMF (1.0 mL), 2-(1-(4-acetylphenyl)piperidin-4-yl)acetic acid (68 mg, 0.26 mmol), HATU (123 mg, 0.32 mmol), and DIPEA (115 μL, 85 mg, 0.66 mmol) were added. The mixture was stirred at room temperature overnight. EtOAc (7 mL) was added and the mixture was washed with NaHCO$_3$ sat. (2 x 15 mL). The organic layer was dried over Na$_2$SO$_4$ anh., filtered and solvents were concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 60%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford an off-white solid (33 mg, 32% yield).

Mp: 229-230 ºC

IR (ATR) *v*: 3318, 2931, 2854, 1659, 1644, 1605, 1551, 1514, 1443, 1380, 1359, 1282, 1233, 1180, 1080, 1011, 998, 960, 865, 835, 818, 767, 632, 603, 593, 569 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 1.30 [m, 2H, 3''(5'')-H$_{ax}$], 1.79 [dm, *J* = 12.7 Hz, 2H, 3''(5'')-H$_{eq}$], 1.93 [m, 2H, 10'(13')-H$_{ax}$], 1.97-2.06 [complex signal, 5H, 2-H$_2$, 6'(12')-H$_{ax}$, 4''-H], 2.14-2.26 [complex signal, 4H, 6'(12')-H$_{eq}$, 10'(13')-H$_{eq}$], 2.29 (d, $^2J_{HF}$ = 6.4 Hz, 2H, 8'-H$_2$), 2.50 (s, 3H, CH$_3$), 2.88 [td, *J* = 12.7, 2.6 Hz, 2H, 2''(6'')-H$_{ax}$], 3.24 [m, 2H, 5'(11')-H], 3.87 [m, 2H, 2''(6'')-H$_{eq}$], 5.30 (broad s, 1H, NH), 6.84 [d, *J* = 9.0 Hz, 2H, 2'''(6''')-H], 7.08 [m, 2H, 1'(4')-H], 7.13 [m, 2H, 2'(3')-H], 7.84 [d, *J* = 9.0 Hz, 2H, 3'''(5''')-H].

^{13}C NMR (101 MHz, CDCl$_3$) δ: 26.2 (CH$_3$, <u>C</u>H$_3$), 31.4 [CH$_2$, C3''(5'')], 33.6 (CH, C4''), 38.7 [CH$_2$, C6'(12')], 39.6 [d, $^3J_{CF}$ = 13.3 Hz, CH, C5'(11')], 40.2 [d, $^2J_{CF}$ = 20.1 Hz, CH$_2$, C10'(13')], 44.6 (CH$_2$, C2), 46.1 (d, $^2J_{CF}$ = 18.4 Hz, CH$_2$, C8'), 48.0 [CH$_2$, C2''(6'')], 58.0 (d, $^3J_{CF}$ = 11.4 Hz, C, C7'), 94.2 (d, $^1J_{CF}$ = 177.6 Hz, C, C9'), 113.6 [CH, C2'''(6''')], 127.1 [CH, C2'(3')], 128.3 [CH, C1'(4')], 130.6 [CH, C3'''(5''')], 144.8 [C, C4a(11a)], 154.1 (C, C1'''), 170.8 (C, <u>C</u>ONH), 196.6 (C, <u>C</u>OCH$_3$). The signal from C4''' was not observed.

Anal. calcd for $C_{30}H_{35}FN_2O_2$: C 75.92, H 7.43, N 5.90. Anal. calcd for $C_{30}H_{35}FN_2O_2 \cdot 0.1\ H_2O$: C 75.21, H 7.47, N 5.85. Found: 75.34, H 7.31, N 5.69.

HRMS: Calcd for $[C_{30}H_{35}FN_2O_2+H]^+$: 475.2755, found: 475.2763.

112, synthesis of (2,5-dimethylthiophen-3-yl)methanamine

i) Thyonyl chloride, toluene, reflux, 2 h
ii) Ammonium hydroxyde 25%, DCM, RT, 1 h
iii) LiAlH$_4$, THF, 0 °C to reflux, 4 h
iv) HCl in Et$_2$O
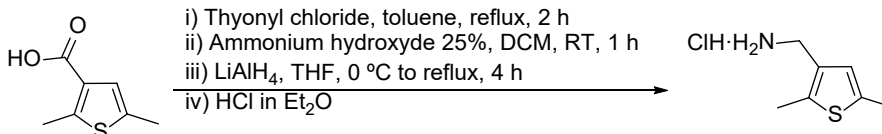
Thionyl chloride (2.32 mL, 3.81 g, 32.00 mmol) was added to a solution of 2,5-dimethylthiophen-3-carboxylic acid (1.0 g, 6.40 mmol) in anh. toluene (15.0 mL) and the complex mixture was kept under reflux for 2 h. After reaction completed, the solution was concentrated. Without further purification, the crude product 2,5-dimethylthiophen-3-carbonyl chloride was immediately used for the next step.

Ammonium hydroxide 25% aqueous solution (10.0 mL, 64.00 mmol) was added dropwise to a solution of 2,5-dimethylthiophen-3-carbonyl chloride in DCM (5.0 mL) at 0 °C and the mixture was allowed to warm up to room temperature and stirred for 1 h. Brine (30 mL) followed by DCM (20 mL) were added and the mixture was extracted. The aqueous layer was extracted again with DCM (2 x 20 mL). All the organic layers were joined, dried over Na$_2$SO$_4$ anh. and filtered to afford the crude product 2,5-dimethylthiophene-3-carboxamide that was used as such without further purification.

To a solution of 2,5-dimethylthiophene-3-carboxamide in anh. THF (10.0 mL) at 0 °C, LiAlH$_4$ 1 M in THF (32.0 mL, 32.00 mmol) was added dropwise. Then, the suspension was heated to reflux and stirred for 4 h.

The reaction suspension was quenched adding water dropwise under ice-bath until no more bubbling was observed. Then, Na$_2$SO$_4$ anh. was added and the mixture was filtered through a pad with Celite® using MeOH (3 x 30 mL) as eluting agent. Solvent was concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 7%). Fractions containing the desired product were collected and concentrated *in vacuo*.

An excess of HCl 2 M in Et$_2$O was added to the suspension of the amine in Et$_2$O (5.0 mL) to form its hydrochloride, followed by filtration of the solid to afford a brownish solid (620 mg, 55% yield).

^{1}H NMR (400 MHz, CD$_3$OD) δ: 2.40 (m, 3H), 2.41 (s, 3H), 3.97 (s, 2H), 6.71 (s, 1H).

118, synthesis of 3-chloro-*N*-((2,5-dimethylthiophen-3-yl)methyl)-4-fluoro-5-(trifluoromethyl)benzamide

i) Thyonyl chloride, toluene, DMF, reflux, 2 h
ii) Amine, TEA, DCM, RT, 5 h
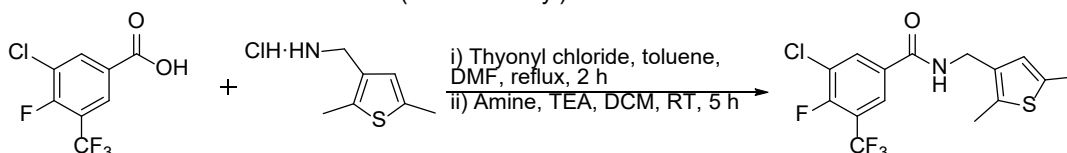
To a solution of 3-chloro-4-fluoro-5-(trifluoromethyl)benzoic acid (68 mg, 0.28 mmol) in anh. toluene (1.0 mL), thionyl chloride (101 µL, 166 mg, 1.40 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford 3-chloro-4-fluoro-5-(trifluoromethyl)benzoyl chloride as a brown oil.

To a suspension of (2,5-dimethylthiophen-3-yl)methanamine hydrochloride (50 mg, 0.28 mmol) and triethylamine (117 µL, 85 mg, 0.84 mmol) in anh. DCM (0.5 mL), a solution of the proper acyl chloride (73 mg, 0.28 mmol) in anh. DCM (0.5 mL) was slowly added. The mixture was kept under stirring at room temperature for 5 h. Water (15 mL) followed by EtOAc (15 mL) were added and the mixture was

extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 8%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (43 mg, 42% yield).

Mp: 133-134 ºC

IR (ATR) *v*: 3273, 1636, 1552, 1481, 1417, 1327, 1317, 1262, 1221, 1140, 1037, 919, 904, 833, 744, 716, 672, 626, 573 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 2.38 (m, 6H, 2'-C$\underline{H}_3$, 5'-C$\underline{H}_3$), 4.44 (dd, *J* = 5.3 Hz, *J'* = 2.0 Hz, 2H, C$\underline{H}_2$), 6.29 (broad s, 1H, N$\underline{H}$), 6.57 (s, 1H, 4'-H), 7.92 (dd, *J* = 5.9 Hz, *J'* = 2.2 Hz, 1H, 6-H), 8.04 (dd, *J* = 6.3 Hz, *J'* = 2.2 Hz, 1H, 2-H).

^{13}C NMR (101 MHz, CDCl$_3$) δ: 13.0 (C2'-$\underline{C}$H$_3$), 15.2 (C5'-$\underline{C}$H$_3$), 37.8 (CH$_2$, $\underline{C}$H$_2$), 120.2 (qd, $^2J_{CF}$ = 34.0 Hz, $^2J_{CF}$ = 13.0 Hz, C, C5), 121.8 (q, $^1J_{CF}$ = 273.3 Hz, C, $\underline{C}$F$_3$), 123.6 (d, $^2J_{CF}$ = 17.6 Hz, C, C3), 124.5 (m, CH, C6), 126.3 (CH, C4'), 131.4 (d, $^4J_{CF}$ = 4.6 Hz, C, C1), 132.5 (C, C3'), 133.5 (CH, C2), 134.4 (C, C2'), 136.9 (C, C5'), 157.3 (d, $^1J_{CF}$ = 264.0 Hz, C, C4), 163.80 (C, $\underline{C}$O).

Anal. calcd for C$_{15}$H$_{12}$ClF$_4$NOS: C 49.25, H 3.31, N 3.83. Found: C 49.22, H 3.29, N 3.72.

HRMS: Calcd for [C$_{15}$H$_{12}$ClF$_4$NOS+H]$^+$: 366.0337, found: 366.0335.

119, synthesis of 2,5-dichloro-*N*-((2,5-dimethylthiophen-3-yl)methyl)-3-(trifluoromethyl)benzamide
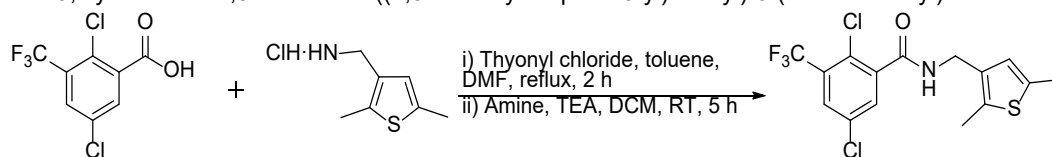
To a solution of 2,5-dichloro-3-(trifluoromethyl)benzoic acid (122 mg, 0.47 mmol) in anh. toluene (2.0 mL), thionyl chloride (170 µL, 279 mg, 2.35 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford 2,5-dichloro-3-(trifluoromethyl)benzoyl chloride as a brown oil.

To a suspension of (2,5-dimethylthiophen-3-yl)methanamine hydrochloride (100 mg, 0.56 mmol) and triethylamine (234 µL, 170 mg, 1.68 mmol) in anh. DCM (1.0 mL), a solution of the proper acyl chloride (130 mg, 0.47 mmol) in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at room temperature for 5 h. Water (15 mL) followed by EtOAc (15 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 5%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (85 mg, 47% yield).

Mp: 157-158 ºC

IR (ATR) *v*: 3268, 1646, 1544, 1482, 1429, 1316, 1289, 1258, 1172, 1142, 1051, 886, 830, 742, 711, 689, 830, 576 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 2.39 (m, 6H, 2'-C$\underline{H}_3$, 5'-C$\underline{H}_3$), 4.46 (d, *J* = 5.2 Hz, 2H, C$\underline{H}_2$), 6.03 (s, broad, 1H, N$\underline{H}$), 6.59 (s, 1H, 4'-H), 7.67 (d, *J* = 2.5 Hz, 1H, 2-H), 7.72 (d, *J* = 2.5 Hz, 1H, 4-H).

^{13}C NMR (101 MHz, CDCl$_3$) δ: 13.0 (C2'-$\underline{C}$H$_3$), 15.2 (C5'-$\underline{C}$H$_3$), 37.7 (CH$_2$, $\underline{C}$H$_2$), 121.9 (q, $^1J_{CF}$ = 274.2 Hz, C, $\underline{C}$F$_3$), 126.3 (CH, C4'), 127.5 (C, C6), 129.2 (q, $^3J_{CF}$ = 5.6 Hz, CH, C4), 130.9 (q, $^2J_{CF}$ = 32.0 Hz, C, C5), 132.1 (C, C3'), 132.6 (CH, C2), 133.5 (C, C3), 134.5 (C, C2'), 136.8 (C, C5'), 139.8 (C, C1), 164.3 (C, $\underline{C}$O).

Anal. calcd for C$_{15}$H$_{12}$Cl$_2$F$_3$NOS: C 47.13, H 3.16, N 3.66. Found: C 47.01, H 3.10, N 3.57.

HRMS: Calcd for [C$_{15}$H$_{12}$Cl$_2$F$_3$NOS+H]$^+$: 382.0042, found: 382.0047.

120, synthesis of *N*-((2,5-dimethylthiophen-3-yl)methyl)-3,4-difluoro-5-(trifluoromethyl)benzamide
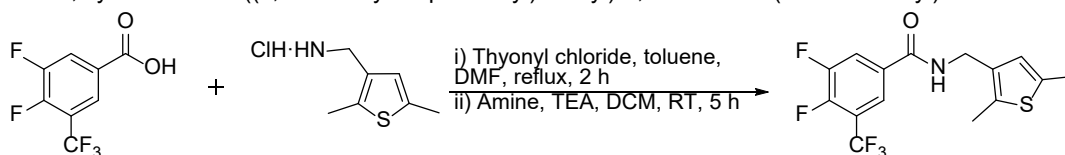
To a solution of 3,4-difluoro-5-(trifluoromethyl)benzoic acid (106 mg, 0.47 mmol) in anh. toluene (2.0 mL), thionyl chloride (170 µL, 279 mg, 2.35 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford 3,4-difluoro-5-(trifluoromethyl)benzoyl chloride as a brown oil.

To a suspension of (2,5-dimethylthiophen-3-yl)methanamine hydrochloride (100 mg, 0.56 mmol) and triethylamine (234 µL, 170 mg, 1.68 mmol) in anh. DCM (1.0 mL), a solution of the proper acyl chloride (115 mg, 0.47 mmol) in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at room temperature for 5 h. Water (15 mL) followed by EtOAc (15 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 9%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a beige solid (38 mg, 23% yield).

Mp: 130-131 ºC

IR (ATR) *v*: 3239, 1647, 1627, 1552, 1504, 1436, 1371, 1349, 1295, 1276, 1192, 1139, 1042, 1001, 942, 885, 838, 731, 674 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 2.38 (m, 6H, 2'-CH$_3$, 5'-CH$_3$), 4.44 (d, *J* = 5.2 Hz, 2H, CH$_2$), 6.34 (broad s, 1H, NH), 6.57 (s, 1H, 4'-H), 7.78 (m, 1H, 6-H), 7.85 (ddd, *J* = 9.6 Hz, *J'* = 6.9 Hz, *J''* = 2.2 Hz, 1H, 2-H).

^{13}C NMR (101 MHz, CDCl$_3$) δ: 12.9 (C2'-CH$_3$), 15.2 (C5'-CH$_3$), 37.8 (CH$_2$, CH$_2$), 120.5 ($^2J_{CF}$ = 18.8 Hz, CH, C2), 120.7 (m, CH, C6), 120.8 (qd, $^2J_{CF}$ = 33.9 Hz, $^2J_{CF}$ = 9.6 Hz, C, C5), 121.7 (qd, $^1J_{CF}$ = 272.9 Hz, $^4J_{CF}$ = 3.5 Hz, C, CF$_3$), 126.3 (CH, C4'), 131.4 (t, $^3J_{CF}$ = 4.8 Hz, C, C1), 132.5 (C, C3'), 134.4 (C, C2'), 136.9 (C, C5'), 150.2 (dd, $^1J_{CF}$ = 263.7 Hz, $^2J_{CF}$ = 13.7 Hz, C, C3), 150.8 (dd, $^1J_{CF}$ = 251.1 Hz, $^2J_{CF}$ = 9.6 Hz, C, C4), 163.8 (C, CO).

Anal. calcd for C$_{15}$H$_{12}$F$_5$NOS: C 51.57, H 3.46, N 4.01. Found: C 51.60, H 3.42, N 4.85.

HRMS: Calcd for [C$_{15}$H$_{12}$F$_5$NOS+H]$^+$: 350.0633, found: 350.0636.

121, synthesis of 3-chloro-*N*-((2,5-dimethylthiophen-3-yl)methyl)-2-fluoro-5-(trifluoromethyl)benzamide
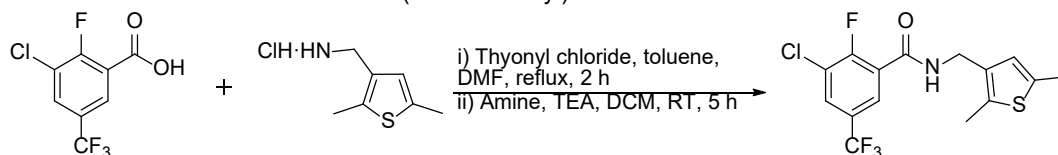
To a solution of 3-chloro-2-fluoro-5-(trifluoromethyl)benzoic acid (68 mg, 0.28 mmol) in anh. toluene (1.0 mL), thionyl chloride (101 µL, 166 mg, 1.40 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford 3-chloro-2-fluoro-5-(trifluoromethyl)benzoyl chloride as a brown oil.

To a suspension of (2,5-dimethylthiophen-3-yl)methanamine hydrochloride (50 mg, 0.28 mmol) and triethylamine (117 µL, 85 mg, 0.84 mmol) in anh. DCM (0.5 mL), a solution of the proper acyl chloride (73 mg, 0.28 mmol) in anh. DCM (0.5 mL) was slowly added. The mixture was kept under stirring at room temperature for 5 h. Water (15 mL) followed by EtOAc (15 mL) were added and the mixture was

extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 4%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (98 mg, 95% yield).

Mp: 118-119 ºC

IR (ATR) *v*: 3285, 2920, 1633, 1544, 1345, 1328, 1277, 1161, 1144, 1122, 1055, 896, 867, 767, 723, 661, 648, 578 cm⁻¹.

¹H NMR (400 MHz, CDCl₃) δ: 2.40 (m, 6H, 2'-C$\underline{H}_3$, 5'-C$\underline{H}_3$), 4.50 (d, *J* = 5.2 Hz, 2H, C$\underline{H}_2$), 6.59 (s, 1H, 4'-H), 6.68 (broad s, 1H, N$\underline{H}$), 7.80 (dd, *J* = 6.5 Hz, *J'* = 2.4 Hz, 1H, 4-H), 8.30 (dd, *J* = 6.2 Hz, *J'* = 2.5 Hz, 1H, 6-H).

¹³C NMR (101 MHz, CDCl₃) δ: 13.0 (C2'-$\underline{C}H_3$), 15.2 (C5'-$\underline{C}H_3$), 37.9 (CH₂, $\underline{C}H_2$), 122.8 (q, $^1J_{CF}$ = 272.6 Hz, C, $\underline{C}F_3$), 123.1 (d, $^2J_{CF}$ = 21.4 Hz, C, C1), 123.6 (d, $^2J_{CF}$ = 13.7 Hz, C, C3), 126.2 (CH, C4'), 128.1 (m, C, C5), 128.2 (p, $^3J_{CF}$ = 3.6 Hz, CH, C6), 130.8 (CH, C4), 132.4 (C, C3'), 134.3 (C, C2'), 136.8 (C, C5'), 157.8 (d, $^1J_{CF}$ = 253.7 Hz, C, C2), 160.8 (d, $^3J_{CF}$ = 3.5 Hz, C, $\underline{C}O$).

Anal. calcd for $C_{15}H_{12}ClF_4NOS$: C 49.25, H 3.31, N 3.83. Found: C 49.40, H 3.21, N 3.66.

HRMS: Calcd for $[C_{15}H_{12}ClF_4NOS+H]^+$: 366.0337, found: 366.0339.

122, synthesis of *N*-((2,5-dimethylthiophen-3-yl)methyl)-2,3,4-trifluoro-5-(trifluoromethyl)benzamide
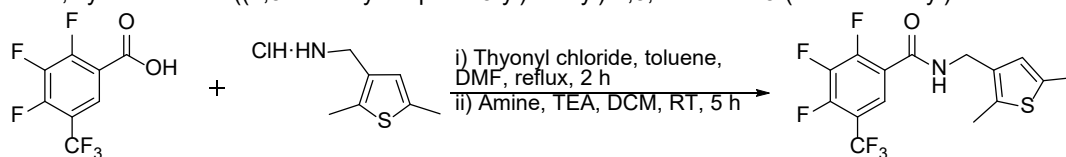
To a solution of 2,3,4-trifluoro-5-(trifluoromethyl)benzoic acid (150 mg, 0.61 mmol) in anh. toluene (4.0 mL), thionyl chloride (445 µL, 726 mg, 6.14 mmol) followed by some drops of DMF were added. The mixture was heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford 2,3,4-trifluoro-5-(trifluoromethyl)benzoyl chloride as a brown oil.

To a suspension of (2,5-dimethylthiophen-3-yl)methanamine hydrochloride (131 mg, 0.74 mmol) and triethylamine (411 µL, 298 mg, 2.95 mmol) in anh. DCM (2.0 mL), a solution of the proper acyl chloride (161 mg, 0.61 mmol) in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at room temperature for 5 h. Water (15 mL) followed by EtOAc (15 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 7%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (110 mg, 49% yield).

Mp: 109-110 ºC

IR (ATR) *v*: 3282, 2925, 1644, 1553, 1487, 1369, 1280, 1205, 1168, 1133, 1054, 994, 905, 721, 709, 672, 632, 574 cm⁻¹.

¹H NMR (400 MHz, CDCl₃) δ: 2.39 (m, 6H, 2'-C$\underline{H}_3$, 5'-C$\underline{H}_3$), 4.49 (d, *J* = 5.3 Hz, 2H, C$\underline{H}_2$), 6.58 (s, 1H, 4'-H), 6.60 (s, 1H, N$\underline{H}$), 8.23 (td, *J* = 7.5 Hz, *J'* = 1.7 Hz, 1H, 6-H).

¹³C NMR (101 MHz, CDCl₃) δ: 13.0 (C2'-$\underline{C}H_3$), 15.2 (C5'-$\underline{C}H_3$), 37.8 (CH₂, $\underline{C}H_2$), 116.8 (m, C, C5), 118.7 (d, $^2J_{CF}$ = 10.1 Hz, C, C1), 121.4 (q, $^1J_{CF}$ = 272.0 Hz, C, $\underline{C}F_3$), 123.9 (CH, C6), 126.2 (CH, C4'), 132.3 (C, C3'), 134.3 (C, C2'), 136.9 (C, C5'), 140.5 (ddd, $^1J_{CF}$ = 256.5 Hz, $^2J_{CF}$ = 17.5 Hz, $^3J_{CF}$ = 2.6 Hz, C, C2), 150.9 (dd, $^1J_{CF}$ = 265.7 Hz, $^2J_{CF}$ = 12.0 Hz, C, C3), 151.85 (ddd, $^1J_{CF}$ = 259.1 Hz, $^2J_{CF}$ = 11.0 Hz, $^3J_{CF}$ = 5.3 Hz, C, C4), 159.9 (C, $\underline{C}O$).

Anal. calcd for $C_{15}H_{11}F_6NOS$: C 49.05, H 3.02, N 3.81. Found: C 49.21, H 3.14, N 3.74.

HRMS: Calcd for $[C_{15}H_{11}F_6NOS+H]^+$: 368.0538, found: 368.0532.

125, synthesis of (2-methylthiophen-3-yl)methanamine

i) Thyonyl chloride, toluene, reflux, 2 h
ii) Ammonium hydroxyde 25%, DCM, RT, 1 h
iii) LiALH$_4$, THF, 0 ºC to reflux, 4 h
iv) HCl in Et$_2$O
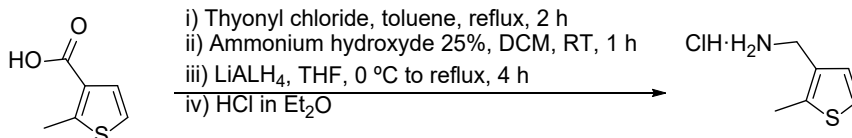
Thionyl chloride (6.96 mL, 11.42 g, 96.00 mmol) was added to a solution of 2-methylthiophen-3-carboxylic acid (1.36 g, 9.60 mmol) in anh. toluene (25.0 mL) followed by 20 drops of DMF and the mixture was kept under reflux for 2 h. After reaction completed, the solution was concentrated. Without further purification, the crude product 2-methylthiophen-3-carbonyl chloride was immediately used for the next step.

Ammonium hydroxide 25% aqueous solution (14.4 mL, 192.00 mmol) was added dropwise to a solution of 2-methylthiophen-3-carbonyl chloride in DCM (15 mL) at 0 °C and the mixture was allowed to warm up to room temperature and stirred for 1 h. Brine (40 mL) followed by DCM (30 mL) were added and the mixture was extracted. The aqueous layer was extracted again with DCM (2 x 30 mL). All the organic layers were joined, dried over Na$_2$SO$_4$ anh. and filtered to afford the crude product 2-methylthiophene-3-carboxamide that was used as such without further purification.

To a solution of 2-methylthiophene-3-carboxamide in anh. THF (40.0 mL) at 0 °C, LiAlH$_4$ (1.82 g, 48.00 mmol) was added portionwise. Then, the suspension was heated to reflux and stirred for 4 h.

The reaction suspension was quenched adding water dropwise under ice-bath until no more bubbling was observed. Then, Na$_2$SO$_4$ anh. was added and the mixture was filtered through a pad with Celite® using MeOH (3 x 50 mL) as eluting agent. Solvent was concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 1% to 4%). Fractions containing the desired product were collected and concentrated *in vacuo*.

An excess of HCl 2 M in Et$_2$O was added to the suspension of the amine in Et$_2$O (10.0 mL) to form its hydrochloride, followed by filtration of the solid to afford a beige solid (606 mg, 53% yield).

^{1}H NMR (400 MHz, CD$_3$OD) δ: 2.50 (s, 3H), 4.07 (s, 2H), 7.05 (d, *J* = 5.3 Hz, 1H), 7.25 (d, *J* = 5.3 Hz, 1H).

126, synthesis of (5-methylthiophen-3-yl)methanamine

i) Thyonyl chloride, toluene, reflux, 2 h
ii) Ammonium hydroxyde 25%, DCM, RT, 1 h
iii) LiALH$_4$, THF, 0 ºC to reflux, 4 h
iv) HCl in Et$_2$O
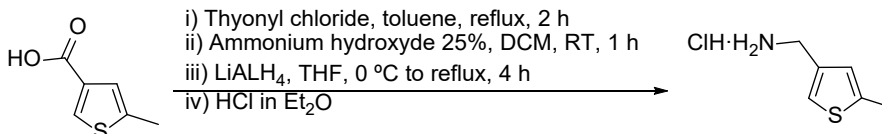
Thionyl chloride (3.48 mL, 5.71 g, 48.00 mmol) was added to a solution of 5-methylthiophen-3-carboxylic acid (680 mg, 4.80 mmol) in anh. toluene (12.0 mL) followed by 10 drops of DMF and the mixture was kept under reflux for 2 h. After reaction completed, the solution was concentrated. Without further purification, the crude product 5-methylthiophen-3-carbonyl chloride was immediately used for the next step.

Ammonium hydroxide 25% aqueous solution (7.2 mL, 96.00 mmol) was added dropwise to a solution of 5-methylthiophen-3-carbonyl chloride in DCM (7 mL) at 0 °C and the mixture was allowed to warm up to room temperature and stirred for 1 h. Brine (20 mL) followed by DCM (15 mL) were added and the mixture was extracted. The aqueous layer was extracted again with DCM (2 x 15 mL). All the

organic layers were joined, dried over Na_2SO_4 anh. and filtered to afford the crude product 5-methylthiophene-3-carboxamide that was used as such without further purification.

To a solution of 5-methylthiophene-3-carboxamide in anh. THF (20.0 mL) at 0 °C, $LiAlH_4$ (910 mg, 24.00 mmol) was added portionwise. Then, the suspension was heated to reflux and stirred for 4 h. The reaction suspension was quenched adding water dropwise under ice-bath until no more bubbling was observed. Then, Na_2SO_4 anh. was added and the mixture was filtered through a pad with Celite® using MeOH (3 x 25 mL) as eluting agent. Solvent was concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 1% to 5%). Fractions containing the desired product were collected and concentrated *in vacuo*.

An excess of HCl 2 M in Et_2O was added to the suspension of the amine in Et_2O (5.0 mL) to form its hydrochloride, followed by filtration of the solid to afford a beige solid (127 mg, 16% yield).

¹H NMR (400 MHz, CD_3OD) δ: 2.48 (d, J = 1.0 Hz, 3H), 4.05 (s, 2H), 6.87 (m, 1H), 7.26 (m, 1H).
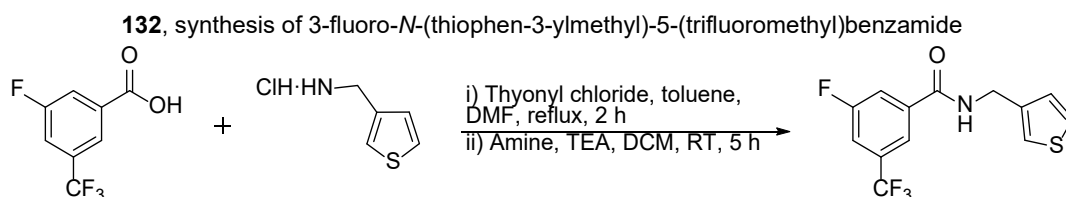
132, synthesis of 3-fluoro-*N*-(thiophen-3-ylmethyl)-5-(trifluoromethyl)benzamide

To a solution of 3-fluoro-5-(trifluoromethyl)benzoic acid (100 mg, 0.48 mmol) in anh. toluene (2.0 mL), thionyl chloride (349 μL, 572 mg, 4.81 mmol) followed by two drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford 3-fluoro-5-(trifluoromethyl)benzoyl chloride as a brown oil.

To a suspension of thiophen-3-ylmethanamine (65 mg, 0.58 mmol) and triethylamine (323 μL, 235 mg, 2.32 mmol). in anh. DCM (1.0 mL), a solution of the proper acyl chloride (109 mg, 0.48 mmol) in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at room temperature for 5 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 9%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford an off-white solid (65 mg, 45% yield).

Mp: 75-76 ºC

IR (ATR) *v*: 3231, 3103, 2938, 1642, 1603, 1550, 1469, 1447, 1368, 1348, 1291, 1251, 1219, 1169, 1130, 1096, 1046, 1010, 945, 925, 886, 784, 691, 635, 563 cm⁻¹.

¹H NMR (400 MHz, $CDCl_3$) δ: 4.61 (d, J = 5.7 Hz, 2H, C$\underline{H_2}$), 6.78 (broad s, 1H, N$\underline{H}$), 7.06 (dd, J = 4.9 Hz, J' = 1.3 Hz, 1H, 4'-H), 7.19 (m, 1H, 2'-H), 7.31 (dd, J = 5.0 Hz, J' = 3.0 Hz, 1H, 5'-H), 7.44 (d, J = 8.1 Hz, 1H, 4-H), 7.68 (dt, J = 8.7 Hz, J' = 2.0 Hz, 1H, 2-H), 7.81 (s, 1H, 6-H).

¹³C NMR (101 MHz, $CDCl_3$) δ: 39.6 (CH_2, $\underline{C}H_2$), 115.9 (dq, $^2J_{CF}$ = 24.6 Hz, $^3J_{CF}$ = 3.7 Hz, CH, C4), 118.1 (d, $^2J_{CF}$ = 22.8 Hz, CH, C2), 119.7 (p, $^3J_{CF}$ = 3.7 Hz, CH, C6), 123.0 (qd, $^1J_{CF}$ = 272.8 Hz, $^4J_{CF}$ = 2.9 Hz, C, $\underline{C}F_3$), 123.1 (CH, C2'), 126.9 (CH, C5'), 127.5 (CH, C4'), 133.1 (qd, $^2J_{CF}$ = 33.8 Hz, $^3J_{CF}$ = 7.7 Hz, C, C5), 137.8 (d, $^3J_{CF}$ = 6.9 Hz, C1), 138.2 (C, C3'), 162.6 (d, $^1J_{CF}$ = 251.1 Hz, C, C3), 164.8 (d, $^4J_{CF}$ = 2.3 Hz, C, $\underline{C}O$).

Anal. calcd for $C_{13}H_9F_4NOS$: C 51.49, H 2.99, N 4.62. Found: C 51.69, H 2.86, N 4.50.

HRMS: Calcd for $[C_{13}H_9F_4NOS-H]^-$: 302.0268, found: 302.0270.

133, synthesis of 3-fluoro-*N*-((2-methylthiophen-3-yl)methyl)-5-(trifluoromethyl)benzamide
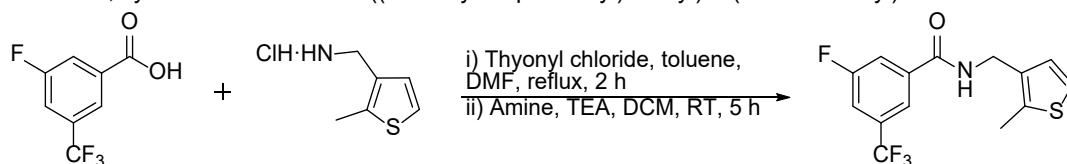
To a solution of 3-fluoro-5-(trifluoromethyl)benzoic acid (100 mg, 0.48 mmol) in anh. toluene (2.0 mL), thionyl chloride (349 µL, 572 mg, 4.81 mmol) followed by two drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford 3-fluoro-5-(trifluoromethyl)benzoyl chloride as a brown oil.

To a suspension of (2-methylthiophen-3-yl)methanamine hydrochloride (95 mg, 0.58 mmol) and triethylamine (323 µL, 235 mg, 2.32 mmol). in anh. DCM (1.0 mL), a solution of the proper acyl chloride (109 mg, 0.48 mmol) in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at room temperature for 5 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 10%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a beige solid (45 mg, 30% yield).

Mp: 109-110 ºC

IR (ATR) *v*: 3325, 3227, 3066, 2923, 1635, 1604, 1539, 1466, 1443, 1364, 1335, 1283, 1220, 1170, 1122, 1093, 1052, 895, 875, 775, 722, 689, 654 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 2.47 (s, 3H, 2'-C$\underline{H}_3$), 4.54 (d, *J* = 5.3 Hz, 2H, C$\underline{H}_2$), 6.44 (broad s, 1H, N$\underline{H}$), 6.94 (d, *J* = 5.3 Hz, 1H, 4'-H), 7.07 (d, *J* = 5.2 Hz, 1H, 5'-H), 7.45 (d, *J* = 8.1 Hz, 1H, 4-H), 7.68 (dt, *J* = 8.6 Hz, *J'* = 2.0 Hz, 1H, 2-H), 7.79 (s, 1H, 6-H).

^{13}C NMR (101 MHz, CDCl$_3$) δ: 13.1 (C2'-$\underline{C}$H$_3$), 37.6 (CH$_2$, $\underline{C}$H$_2$), 115.9 (dq, $^2J_{CF}$ = 24.6 Hz, $^3J_{CF}$ = 3.7 Hz, CH, C4), 118.1 (d, $^2J_{CF}$ = 22.8 Hz, CH, C2), 119.6 (p, $^3J_{CF}$ = 3.7 Hz, CH, C6), 122.5 (CH, C5'), 123.0 (qd, $^1J_{CF}$ = 272.7 Hz, $^4J_{CF}$ = 2.9 Hz, C, $\underline{C}$F$_3$), 128.5 (CH, C4'), 133.0 (C, C3'), 133.1 (qd, $^2J_{CF}$ = 33.9 Hz, $^3J_{CF}$ = 7.7 Hz, C, C5), 136.8 (C, C2'), 137.8 (d, $^3J_{CF}$ = 6.9 Hz, C1), 162.6 (d, $^1J_{CF}$ = 251.1 Hz, C, C3), 164.6 (d, $^4J_{CF}$ = 2.3 Hz, C, $\underline{C}$O).

Anal. calcd for C$_{14}$H$_{11}$F$_4$NOS: C 53.00, H 3.49, N 4.41. Found: C 53.05, H 3.41, N 4.34.

HRMS: Calcd for [C$_{14}$H$_{11}$F$_4$NOS-H]$^-$: 316.0425, found: 316.0431.

134, synthesis of 3-fluoro-*N*-((5-methylthiophen-3-yl)methyl)-5-(trifluoromethyl)benzamide
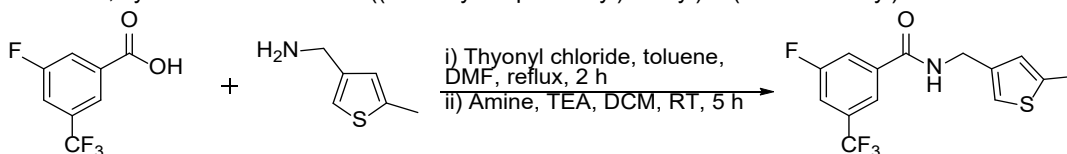
To a solution of 3-fluoro-5-(trifluoromethyl)benzoic acid (100 mg, 0.48 mmol) in anh. toluene (2.0 mL), thionyl chloride (174 µL, 285 mg, 2.40 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford 3-fluoro-5-(trifluoromethyl)benzoyl chloride as a brown oil.

To a suspension of (5-methylthiophen-3-yl)methanamine (73 mg, 0.58 mmol) and triethylamine (162 µL, 118 mg, 1.16 mmol) in anh. DCM (1.0 mL), a solution of the proper acyl chloride (109 mg, 0.48 mmol) in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at room temperature for 5 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was

extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 9%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a yellow solid (30 mg, 20% yield).
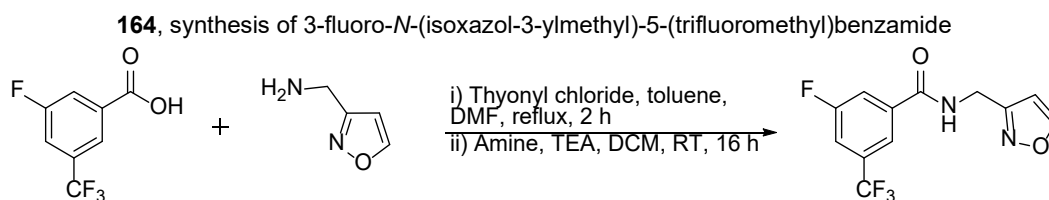
Mp: 86-87 ºC

IR (ATR) *v*: 3274, 1639, 1603, 1549, 1446, 1357, 1279, 1219, 1181, 1164, 1124, 1094, 1068, 1006, 952, 885, 835, 779, 710, 964, 626, 584 cm⁻¹.

¹H NMR (400 MHz, CDCl₃) δ: 2.45 (s, 3H, 5'-C$\underline{H}$₃), 4.53 (d, *J* = 5.5 Hz, 2H, C$\underline{H}$₂), 6.57 (broad s, 1H, N$\underline{H}$), 6.73 (s, 1H, 4'-H), 6.94 (s, 1H, 2'-H), 7.45 (dt, *J* = 8.3 Hz, *J'* = 1.9 Hz, 1H, 4-H), 7.70 (dt, *J* = 8.7 Hz, *J'* = 2.0 Hz, 1H, 2-H), 7.81 (s, 1H, 6-H).

¹³C NMR (101 MHz, CDCl₃) δ: 15.4 (C5'-$\underline{C}$H₃), 39.9 (CH₂, $\underline{C}$H₂), 115.9 (dq, $^2J_{CF}$ = 24.6 Hz, $^3J_{CF}$ = 3.7 Hz, CH, C4), 118.1 (d, $^2J_{CF}$ = 22.8 Hz, CH, C2), 119.6 (p, $^3J_{CF}$ = 3.7 Hz, CH, C6), 120.8 (CH, C2'), 123.0 (qd, $^1J_{CF}$ = 272.8 Hz, $^4J_{CF}$ = 2.9 Hz, C, $\underline{C}$F₃), 125.7 (CH, C4'), 133.1 (qd, $^2J_{CF}$ = 33.8 Hz, $^3J_{CF}$ = 7.7 Hz, C, C5), 137.9 (d, $^3J_{CF}$ = 6.9 Hz, C1), 138.0 (C, C3'), 141.5 (C, C5'), 162.6 (d, $^1J_{CF}$ = 251.1 Hz, C, C3), 164.7 (d, $^4J_{CF}$ = 2.3 Hz, C, $\underline{C}$O).

Anal. calcd for $C_{14}H_{11}F_4NOS$: C 52.99, H 3.49, N 4.41. Found: C 53.14, H 3.58, N 4.33.

HRMS: Calcd for $[C_{14}H_{11}F_4NOS+H]^+$: 318.0570, found: 318.0576.
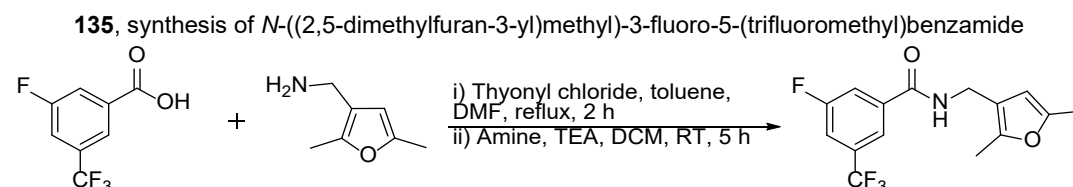
135, synthesis of *N*-((2,5-dimethylfuran-3-yl)methyl)-3-fluoro-5-(trifluoromethyl)benzamide

To a solution of 3-fluoro-5-(trifluoromethyl)benzoic acid (100 mg, 0.48 mmol) in anh. toluene (2.0 mL), thionyl chloride (349 µL, 572 mg, 4.81 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford 3-fluoro-5-(trifluoromethyl)benzoyl chloride as a brown oil.

To a suspension of (2,5-dimethylfuran-3-yl)methanamine (72 mg, 0.58 mmol) and triethylamine (323 µL, 235 mg, 2.32 mmol) in anh. DCM (1.0 mL), a solution of the proper acyl chloride (109 mg, 0.48 mmol) in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at room temperature for 5 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 6%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (46 mg, 30% yield).

Mp: 71-72 ºC

IR (ATR) *v*: 3312, 3095, 2921, 1647, 1604, 1557, 1443, 1348, 1294, 1249, 1174, 1142, 1094, 1038, 926, 887, 803, 753, 691, 621 cm⁻¹.

¹H NMR (400 MHz, CDCl₃) δ: 2.22 (s, 3H, 5'-C$\underline{H}$₃), 2.25 (s, 3H, 2'-C$\underline{H}$₃), 4.33 (d, *J* = 5.2 Hz, 2H, C$\underline{H}$₂), 5.89 (s, 1H, 4'-H), 6.35 (broad s, 1H, N$\underline{H}$), 7.44 (m, 1H, 4-H), 7.67 (dt, *J* = 8.7 Hz, *J'* = 2.1 Hz, 1H, 2-H), 7.78 (s, 1H, 6-H).

¹³C NMR (101 MHz, CDCl₃) δ: 11.6 (C2'-$\underline{C}$H₃), 13.5 (C5'-$\underline{C}$H₃), 35.7 (CH₂, $\underline{C}$H₂), 106.9 (CH, C4'), 115.8 (C, C3'), 115.8 (dq, $^2J_{CF}$ = 24.6 Hz, $^3J_{CF}$ = 3.8 Hz, CH, C4), 118.0 (d, $^2J_{CF}$ = 22.8 Hz, CH, C2), 119.6 (p, $^3J_{CF}$ = 3.7 Hz, CH, C6), 123.0 (qd, $^1J_{CF}$ = 272.8 Hz, $^4J_{CF}$ = 2.9 Hz, C, $\underline{C}$F₃), 133.1 (qd, $^2J_{CF}$ = 33.7

Hz, $^3J_{CF}$ = 7.6 Hz, C, C5), 137.9 (d, $^3J_{CF}$ = 6.8 Hz, C1), 147.7 (C, C2'), 150.5 (C, C5'), 162.6 (d, $^1J_{CF}$ = 251.0 Hz, C, C3), 164.7 ($^4J_{CF}$ = 2.3 Hz, C, <u>C</u>O).
Anal. calcd for $C_{15}H_{13}F_4NO_2$: C 57.15, H 4.16, N 4.44. Found: C 57.06, H 4.30, N 4.31.
HRMS: Calcd for $[C_{15}H_{13}F_4NO_2-H]^-$: 314.0810, found: 314.0819.
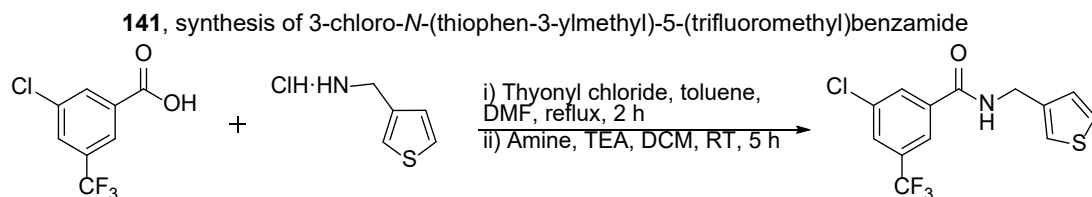
141, synthesis of 3-chloro-*N*-(thiophen-3-ylmethyl)-5-(trifluoromethyl)benzamide

To a solution of 3-chloro-5-(trifluoromethyl)benzoic acid (100 mg, 0.45 mmol) in anh. toluene (2.0 mL), thionyl chloride (323 μL, 530 mg, 4.45 mmol) followed by two drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford 3-chloro-5-(trifluoromethyl)benzoyl chloride as a brown oil.
To a suspension of thiophen-3-ylmethanamine (60 mg, 0.53 mmol) and triethylamine (149 μL, 108 mg, 1.07 mmol). in anh. DCM (1.0 mL), a solution of the proper acyl chloride (108 mg, 0.45 mmol) in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at room temperature for 5 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 6%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (72 mg, 51% yield).
Mp: 91-92 °C
IR (ATR) ν: 3246, 3091, 2927, 1635, 1549, 1429, 1349, 1324, 1284, 1175, 1124, 1050, 1004, 889, 826, 788, 738, 689, 637, 621 cm^{-1}.
^{1}H NMR (400 MHz, CDCl$_3$) δ: 4.60 (d, *J* = 5.6 Hz, 2H, C<u>H</u>$_2$), 6.84 (broad s, 1H, N<u>H</u>), 7.05 (dd, *J* = 4.9 Hz, *J'* = 1.3 Hz, 1H, 4'-H), 7.18 (m, 1H, 2'-H), 7.30 (dd, *J* = 5.0 Hz, *J'* = 3.0 Hz, 1H, 5'-H), 7.71 (m, 1H, 4-H), 7.90 (m, 1H, 6-H), 7.93 (t, *J* = 1.8 Hz, 1H, 2-H).
^{13}C NMR (101 MHz, CDCl$_3$) δ: 39.6 (CH$_2$, <u>C</u>H$_2$), 122.3 (q, $^3J_{CF}$ = 3.7 Hz, CH, C6), 123.0 (q, $^1J_{CF}$ = 273.2 Hz, C, <u>C</u>F$_3$), 123.0 (CH, C2'), 126.8 (CH, C5'), 127.4 (CH, C4'), 128.5 (q, $^3J_{CF}$ = 3.7 Hz, CH, C4), 130.8 (CH, C2), 132.7 (q, $^2J_{CF}$ = 33.6 Hz, C, C5), 135.7 (C, C3), 137.0 (C, C1), 138.2 (C, C3'), 164.8 (C, <u>C</u>O).
Anal. calcd for $C_{13}H_9ClF_3NOS$: C 48.84, H 2.84, N 4.38. Found: C 49.08, H 2.83, N 4.16.
HRMS: Calcd for $[C_{14}H_{11}ClF_3NOS-H]^-$: 317.9973, found: 317.9975.

142, synthesis of 3-chloro-*N*-((2-methylthiophen-3-yl)methyl)-5-(trifluoromethyl)benzamide
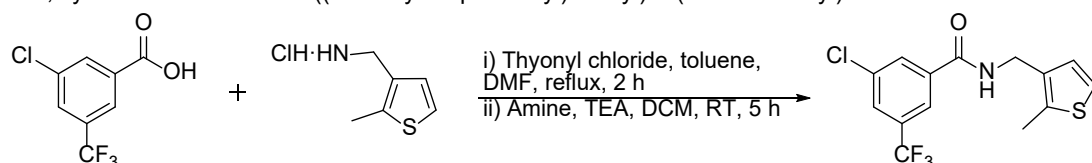
To a solution of 3-chloro-5-(trifluoromethyl)benzoic acid (100 mg, 0.45 mmol) in anh. toluene (2.0 mL), thionyl chloride (323 μL, 530 mg, 4.45 mmol) followed by two drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford 3-chloro-5-(trifluoromethyl)benzoyl chloride as a brown oil.

To a suspension of (2-methylthiophen-3-yl)methanamine hydrochloride (87 mg, 0.53 mmol) and triethylamine (298 μL, 217 mg, 2.14 mmol). in anh. DCM (1.0 mL), a solution of the proper acyl chloride (108 mg, 0.45 mmol) in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at room temperature for 5 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 9%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a beige solid (81 mg, 55% yield).

Mp: 117-118 ºC

IR (ATR) *v*: 3299, 3082, 2935, 1639, 1540, 1440, 1322, 1279, 1166, 1124, 887, 827, 770, 745, 721, 690, 658, 611 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 2.46 (s, 3H, 2'-CH$_3$), 4.53 (d, *J* = 5.3 Hz, 2H, CH$_2$), 6.55 (broad s, 1H, NH), 6.93 (d, *J* = 5.3 Hz, 1H, 4'-H), 7.06 (d, *J* = 5.2 Hz, 1H, 5'-H), 7.71 (m, 1H, 4-H), 7.89 (m, 1H, 6-H), 7.91 (t, *J* = 1.8 Hz, 1H, 2-H).

^{13}C NMR (101 MHz, CDCl$_3$) δ: 13.1 (C2'-CH$_3$), 37.6 (CH$_2$, CH$_2$), 122.3 (q, $^3J_{CF}$ = 3.7 Hz, CH, C6), 122.5 (CH, C5'), 123.0 (q, $^1J_{CF}$ = 273.1 Hz, C, CF$_3$), 128.4 (q, $^3J_{CF}$ = 3.8 Hz, CH, C4), 128.5 (CH, C4'), 130.8 (CH, C2), 132.7 (q, $^2J_{CF}$ = 33.6 Hz, C, C5), 133.0 (C, C3'), 135.7 (C, C3), 136.8 (C, C2'), 137.0 (C, C1), 164.7 (C, CO).

Anal. calcd for C$_{14}$H$_{11}$ClF$_3$NOS: C 50.38, H 3.32, N 4.20. Found: C 50.36, H 3.20, N 4.17.

HRMS: Calcd for [C$_{14}$H$_{11}$ClF$_3$NOS-H]$^-$: 332.0129, found: 332.0134.
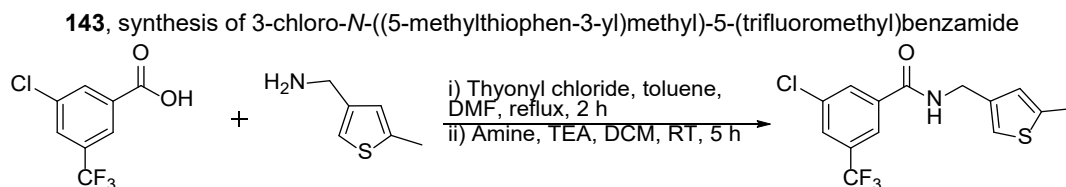
143, synthesis of 3-chloro-*N*-((5-methylthiophen-3-yl)methyl)-5-(trifluoromethyl)benzamide

To a solution of 3-chloro-5-(trifluoromethyl)benzoic acid (100 mg, 0.45 mmol) in anh. toluene (2.0 mL), thionyl chloride (163 μL, 268 mg, 2.25 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford 3-chloro-5-(trifluoromethyl)benzoyl chloride as a brown oil.

To a suspension of (5-methylthiophen-3-yl)methanamine (68 mg, 0.53 mmol) and triethylamine (148 μL, 107 mg, 1.06 mmol) in anh. DCM (1.0 mL), a solution of the proper acyl chloride (109 mg, 0.45 mmol) in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at room temperature for 5 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The aqueous layer was extracted again with DCM (2 x 10 mL). All the organic layers were joined, dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 4%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a pale-yellow solid (87 mg, 58% yield).

Mp: 130-131 ºC

IR (ATR) *v*: 3268, 1632, 1583, 1538, 1437, 1362, 1322, 1275, 1171, 1136, 1101, 1056, 886, 828, 756, 691, 648, 577, 560 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 2.44 (d, *J* = 1.1 Hz, 3H, 5'-CH$_3$), 4.51 (d, *J* = 5.6 Hz, 2H, CH$_2$), 6.71 (s, 1H, 4'-H), 6.75 (broad s, 1H, NH), 6.92 (s, 1H, 2'-H), 7.71 (s, 1H, 4-H), 7.91 (s, 1H, 6-H), 7.93 (s, 1H, 2-H).

^{13}**C NMR** (101 MHz, CDCl$_3$) δ: 15.4 (C5'-C$\underline{H}_3$), 39.8 (CH$_2$, C$\underline{H}_2$), 120.7 (CH, C2'), 122.3 (q, $^3J_{CF}$ = 3.7 Hz, CH, C6), 123.0 (q, $^1J_{CF}$ = 273.2 Hz, C, C$\underline{F}_3$), 125.6 (CH, C4'), 128.4 (q, $^3J_{CF}$ = 3.8 Hz, CH, C4), 130.8 (CH, C2), 132.7 (q, $^2J_{CF}$ = 33.6 Hz, C, C5), 135.7 (C, C3), 137.1 (C, C1), 138.0 (C, C3'), 141.4 (C, C5'), 164.7 (C, C$\underline{O}$).

Anal. calcd for C$_{14}$H$_{11}$ClF$_3$NOS: C 50.38, H 3.32, N 4.20. Found: C 50.50, H 3.26, N 4.03.

HRMS: Calcd for [C$_{14}$H$_{11}$ClF$_3$NOS+H]$^+$: 334.0275, found: 334.0282.
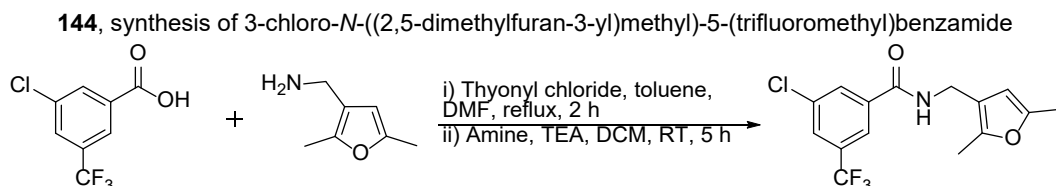
144, synthesis of 3-chloro-*N*-((2,5-dimethylfuran-3-yl)methyl)-5-(trifluoromethyl)benzamide

To a solution of 3-chloro-5-(trifluoromethyl)benzoic acid (100 mg, 0.45 mmol) in anh. toluene (2.0 mL), thionyl chloride (323 μL, 530 mg, 4.45 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford 3-chloro-5-(trifluoromethyl)benzoyl chloride as a brown oil.

To a solution of (2,5-dimethylfuran-3-yl)methanamine (67 mg, 0.53 mmol) and triethylamine (147 μL, 107 mg, 1.06 mmol) in anh. DCM (1.0 mL), a solution of the proper acyl chloride (109 mg, 0.45 mmol) in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at room temperature for 5 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na$_2$SO$_4$ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 6%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a pale-yellow solid (86 mg, 58% yield).

Mp: 88-89 ºC

IR (ATR) *v*: 3304, 3079, 2922, 2441, 1636, 1542, 1428, 1321, 1289, 1174, 1129, 1031, 924, 889, 794, 691, 624 cm^{-1}.

^{1}**H NMR** (400 MHz, CD$_3$OD) δ: 2.17 (s, 3H, 5'-C$\underline{H}_3$), 2.24 (s, 3H, 2'-C$\underline{H}_3$), 4.26 (s, 2H, C$\underline{H}_2$), 5.92 (s, 1H, 4'-H), 7.85 (m, 1H, 4-H), 8.06 (m, 1H, 6-H), 8.08 (m, 1H, 2-H).

^{13}**C NMR** (101 MHz, CD$_3$OD) δ: 11.4 (C2'-C$\underline{H}_3$), 13.3 (C5'-C$\underline{H}_3$), 35.9 (CH$_2$, C$\underline{H}_2$), 108.1 (CH, C4'), 117.8 (C, C3'), 123.7 (q, $^3J_{CF}$ = 3.9 Hz, CH, C6), 124.5 (q, $^1J_{CF}$ = 272.0 Hz, C, C$\underline{F}_3$), 129.1 (q, $^3J_{CF}$ = 3.8 Hz, CH, C4), 132.2 (CH, C2), 133.5 (q, $^2J_{CF}$ = 33.3 Hz, C, C5), 136.5 (C, C3), 138.7 (C, C1), 148.3 (C, C2'), 150.9 (C, C5'), 166.5 (C, C$\underline{O}$).

Anal. calcd for C$_{15}$H$_{13}$ClF$_3$NO$_2$: C 54.31, H 3.95, N 4.22. Found: C 54.29, H 4.09, N 4.03.

HRMS: Calcd for [C$_{15}$H$_{13}$ClF$_3$NO$_2$-H]$^-$: 330.0514, found: 330.0519.

150, synthesis of *N*-((2,5-dimethylthiophen-3-yl)methyl)benzamide
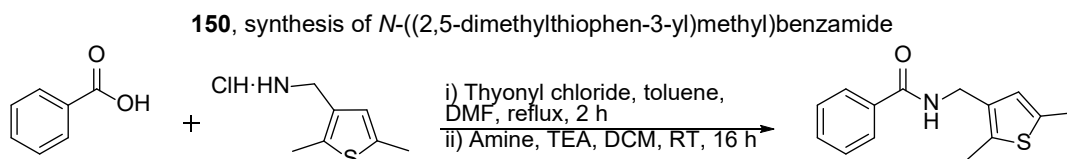
To a solution benzoic acid (41 mg, 0.34 mmol) in anh. toluene (1.0 mL), thionyl chloride (245 μL, 402 mg, 3.38 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford benzoyl chloride as a brown oil.

To a suspension of (2,5-dimethylthiophen-3-yl)methanamine hydrochloride (50 mg, 0.28 mmol) and triethylamine (118 μL, 85 mg, 0.84 mmol) in anh. DCM (1.0 mL), a solution of the proper acyl chloride

in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at room temperature for 16 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na₂SO₄ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 20%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (26 mg, 39% yield).

Mp: 98-99 °C

IR (ATR) *v*: 3237, 1627, 1536, 1490, 1356, 1307, 1253, 1210, 1141, 1038, 961, 931, 830, 807, 718, 694, 568 cm⁻¹.

¹H NMR (400 MHz, CDCl₃) δ: 2.38 (s, 6H, 2'-C$\underline{H}$₃, 5'-C$\underline{H}$₃), 4.46 (d, *J* = 5.2 Hz, 2H, C$\underline{H}$₂), 6.24 (broad s, 1H, N$\underline{H}$), 6.60 (m, 1H, 4'-H), 7.42 [m, 2H, 3(5)-H], 7.49 (m, 1H, 4-H), 7.77 [m, 2H, 2(6)-H].

¹³C NMR (101 MHz, CDCl₃) δ: 12.9 (C2'-$\underline{C}$H₃), 15.2 (C5'-$\underline{C}$H₃), 37.5 (CH₂, $\underline{C}$H₂), 126.5 (CH, C4'), 127.1 [CH, C2(6)], 128.7 [CH, C3(5)], 131.6 (CH, C4), 133.3 (C, C3'), 133.9 (C, C1), 134.5 (C, C2'), 136.5 (C, C5'), 167.3 (C, $\underline{C}$O).

Anal. calcd for C₁₄H₁₅NOS: C 68.54, H 6.16, N 5.71. Found: C 68.52, H 6.10, N 5.60.

HRMS: Calcd for [C₁₄H₁₅NOS+H]⁺: 246.0947, found: 246.0944.

151, synthesis of 4-chloro-*N*-((2,5-dimethylthiophen-3-yl)methyl)benzamide
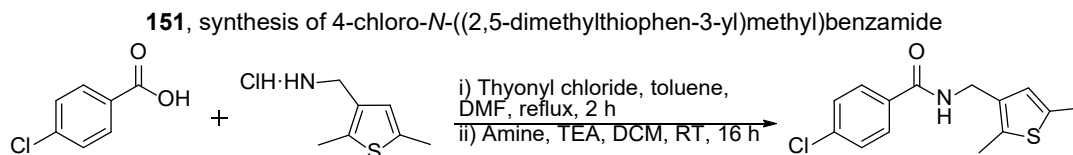
To a solution 4-chlorobenzoic acid (53 mg, 0.34 mmol) in anh. toluene (1.0 mL), thionyl chloride (245 µL, 402 mg, 3.38 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford benzoyl chloride as a brown oil.

To a suspension of (2,5-dimethylthiophen-3-yl)methanamine hydrochloride (50 mg, 0.28 mmol) and triethylamine (118 µL, 85 mg, 0.84 mmol) in anh. DCM (1.0 mL), a solution of the proper acyl chloride in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at room temperature for 16 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na₂SO₄ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 30%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford an off-white solid (59 mg, 75% yield).

Mp: 133-134 °C

IR (ATR) *v*: 3313, 2915, 1715, 1632, 1544, 1486, 1447, 1357, 1318, 1214, 1090, 1039, 1013, 851, 514, 759, 708, 635, 570 cm⁻¹.

¹H NMR (400 MHz, CDCl₃) δ: 2.37-2.39 (complex signal, 6H, 2'-C$\underline{H}$₃, 5'-C$\underline{H}$₃), 4.44 (d, *J* = 5.2 Hz, 2H, C$\underline{H}$₂), 6.19 (broad s, 1H, N$\underline{H}$), 6.58 (q, *J* = 1.1 Hz, 1H, 4'-H), 7.38 [d, *J* = 8.8 Hz, 2H, 3(5)-H], 7.70 [d, *J* = 8.8 Hz, 2H, 2(6)-H].

¹³C NMR (101 MHz, CDCl₃) δ: 13.0 (C2'-$\underline{C}$H₃), 15.2 (C5'-$\underline{C}$H₃), 37.5 (CH₂, $\underline{C}$H₂), 126.4 (CH, C4'), 128.5 [CH, C2(6)], 128.9 [CH, C3(5)], 132.9 (C, C1), 133.1 (C, C3'), 134.1 (C, C2'), 136.6 (C, C5'), 137.9 (C, C4), 166.2 (C, $\underline{C}$O).

Anal. calcd for C₁₄H₁₄ClNOS: C 60.10, H 5.04, N 5.01. Found: C 60.40, H 5.00, N 4.96.

HRMS: Calcd for [C₁₄H₁₄ClNOS+H]⁺: 280.0557, found: 280.0558.

152, synthesis of 3,4-dichloro-*N*-((2,5-dimethylthiophen-3-yl)methyl)benzamide
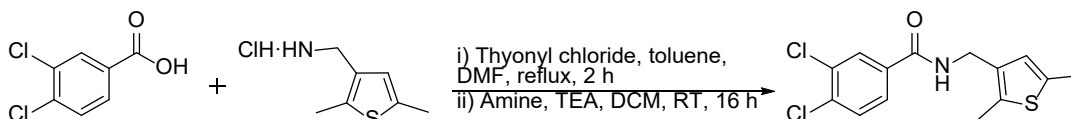
To a solution 3,4-dichlorobenzoic acid (90 mg, 0.47 mmol) in anh. toluene (1.0 mL), thionyl chloride (339 µL, 556 mg, 4.67 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford benzoyl chloride as a brown oil.

To a suspension of (2,5-dimethylthiophen-3-yl)methanamine hydrochloride (70 mg, 0.39 mmol) and triethylamine (164 µL, 118 mg, 1.17 mmol) in anh. DCM (1.0 mL), a solution of the proper acyl chloride in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at RT for 16 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 20%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a brownish solid (43 mg, 35% yield).

Mp: 102-103 ºC

IR (ATR) *v*: 3241, 2917, 1634, 1593, 1538, 1469, 1425, 1375, 1354, 1309, 1251, 1213, 1142, 1131, 1029, 971, 886, 858, 833, 760, 724, 683, 670, 567 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 2.38 (s, 3H, 2'-C$\underline{H}_3$), 2.39 (m, 3H, 5'-C$\underline{H}_3$), 4.43 (d, *J* = 5.2 Hz, 2H, C$\underline{H}_2$), 6.22 (broad s, 1H, N$\underline{H}$), 6.58 (d, *J* = 1.2 Hz, 1H, 4'-H), 7.48 (d, *J* = 8.3 Hz, 1H, 5-H), 7.58 (dd, *J* = 8.3 Hz, *J'* = 2.1 Hz, 1H, 6-H), 7.85 (d, *J* = 2.1 Hz, 1H, 2-H).

^{13}C NMR (101 MHz, CDCl$_3$) δ: 13.0 (C2'-$\underline{C}H_3$), 15.2 (C5'-$\underline{C}H_3$), 37.6 (CH$_2$, $\underline{C}H_2$), 126.3 (CH, C6), 126.4 (CH, C4'), 129.3 (CH, C2), 130.8 (CH, C5), 132.8 (C, C1), 133.2 (C, C3'), 134.2 (C, C2'), 134.3 (C, C3), 136.1 (C, C4), 136.7 (C, C5'), 165.1 (C, $\underline{C}$O).

Anal. calcd for C$_{14}$H$_{13}$Cl$_2$NOS: C 53.51, H 4.17, N 4.46. Found: C 53.61, H 4.08, N 4.35.

HRMS: Calcd for [C$_{14}$H$_{13}$Cl$_2$NOS+H]$^+$: 314.0168, found: 314.0165.

153, synthesis of *N*-((2,5-dimethylthiophen-3-yl)methyl)-4-methoxybenzamide
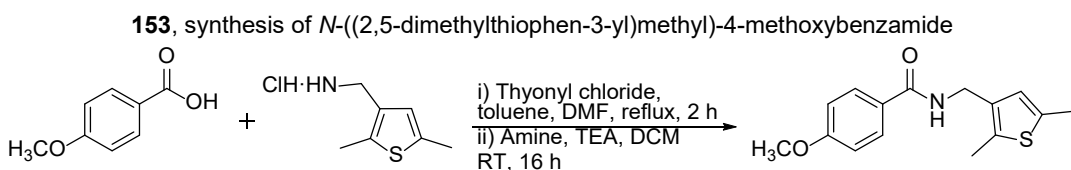
To a solution 4-methoxybenzoic acid (83 mg, 0.54 mmol) in anh. toluene (1.0 mL), thionyl chloride (391 µL, 641 mg, 5.39 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford benzoyl chloride as a two-phased liquid.

To a suspension of (2,5-dimethylthiophen-3-yl)methanamine hydrochloride (80 mg, 0.45 mmol) and triethylamine (211 µL, 152 mg, 1.50 mmol) in anh. DCM (1.0 mL), a solution of the proper acyl chloride in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at RT for 16 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 30%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a brownish solid (46 mg, 38% yield).

Mp: 117-118 ºC

IR (ATR) *v*: 3309, 2914, 2838, 1633, 1606, 1549, 1504, 1462, 1317, 1252, 1214, 1174, 1112, 1031, 974, 844, 825, 770, 722, 661, 632, 606 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 2.36-2.39 (complex signal, 6H, 2'-C$\underline{H}_3$, 5'-C$\underline{H}_3$), 3.83 (s, OC$\underline{H}_3$), 4.43 (d, *J* = 5.2 Hz, 2H, C$\underline{H}_2$), 6.17 (broad s, 1H, N$\underline{H}$), 6.60 (m, 1H, 4'-H), 6.90 [d, *J* = 8.8 Hz, 2H, 3(5)-H], 7.73 [d, *J* = 8.9 Hz, 2H, 2(6)-H].

^{13}C NMR (101 MHz, CDCl$_3$) δ: 12.9 (C2'-$\underline{C}$H$_3$), 15.2 (C5'-$\underline{C}$H$_3$), 37.4 (CH$_2$, $\underline{C}$H$_2$), 55.5 (CH$_3$, O$\underline{C}$H$_3$), 113.8 [CH, C3(5)], 126.6 (CH, C4'), 126.8 (C, C1), 128.9 [CH, C2(6)], 133.5 (C, C3'), 133.8 (C, C2'), 136.4 (C, C5'), 162.3 (C, C4), 166.8 (C, $\underline{C}$O).

Anal. calcd for C$_{15}$H$_{17}$NO$_2$S: C 65.43, H 6.22, N 5.09. Found: C 65.27, H 6.25, N 4.93.

HRMS: Calcd for [C$_{15}$H$_{17}$NO$_2$S+H]$^+$: 279.1053, found: 279.1059.

154, synthesis of *N*-((2,5-dimethylthiophen-3-yl)methyl)-4-methylbenzamide
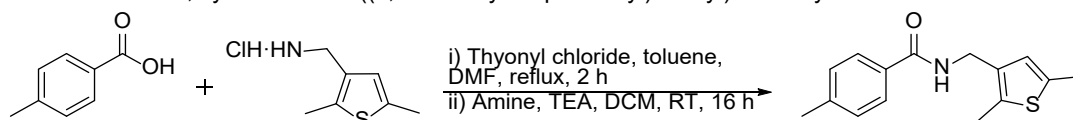
To a solution 4-methylbenzoic acid (46 mg, 0.34 mmol) in anh. toluene (1.0 mL), thionyl chloride (245 µL, 402 mg, 3.38 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford benzoyl chloride as a brown oil.

To a suspension of (2,5-dimethylthiophen-3-yl)methanamine hydrochloride (50 mg, 0.28 mmol) and triethylamine (118 µL, 85 mg, 0.84 mmol) in anh. DCM (1.0 mL), a solution of the proper acyl chloride in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at RT for 16 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na$_2$SO$_4$ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 20%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a beige solid (39 mg, 53% yield).

Mp: 132-133 ºC

IR (ATR) *v*: 277, 2917, 1631, 1612, 1532, 1501, 1348, 1292, 1279, 1211, 1187, 1140, 1118, 1062, 1021, 966, 907, 835, 752, 655, 632, 607, 571 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 2.38-2.38 (complex signal, 9H, 4-C$\underline{H}_3$, 2'-C$\underline{H}_3$, 5'-C$\underline{H}_3$), 4.44 (d, *J* = 5.2 Hz, 2H, C$\underline{H}_2$), 6.25 (broad s, 1H, N$\underline{H}$), 6.59 (q, *J* = 1.2 Hz, 1H, 4'-H), 7.21 [dd, *J* = 8.6, 0.7 Hz, 2H, 3(5)-H], 7.66 [d, *J* = 8.2 Hz, 2H, 2(6)-H].

^{13}C NMR (101 MHz, CDCl$_3$) δ: 12.9 (C2'-$\underline{C}$H$_3$), 15.2 (C5'-$\underline{C}$H$_3$), 21.5 (C4-$\underline{C}$H$_3$), 37.4 (CH$_2$, $\underline{C}$H$_2$), 126.5 (CH, C4'), 127.0 [CH, C2(6)], 129.3 [CH, C3(5)], 131.6 (C, C1), 133.5 (C, C3'), 133.8 (C, C2'), 136.4 (C, C5'), 142.0 (C, C4), 167.2 (C, $\underline{C}$O).

Anal. calcd for C$_{15}$H$_{17}$NOS: C 69.46, H 6.61, N 5.40. Found: C 69.24, H 6.55, N 5.28.

HRMS: Calcd for [C$_{15}$H$_{17}$NOS+H]$^+$: 260.1104, found: 260.1109.

162, synthesis of 3-fluoro-*N*-(thiazol-4-ylmethyl)-5-(trifluoromethyl)benzamide
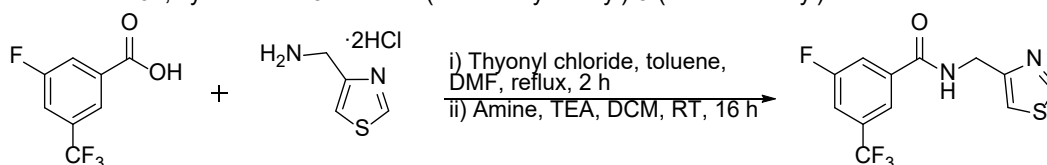
To a solution 3-fluoro-5-(trifluoromethyl)benzoic acid (100 mg, 0.48 mmol) in anh. toluene (2.0 mL), thionyl chloride (279 μL, 457 mg, 3.84 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford benzoyl chloride as a brown oil.

To a suspension of thiazol-4-ylmethanamine dihydrochloride (99 mg, 0.53 mmol) and triethylamine (268 μL, 194 mg, 1.92 mmol) in anh. DCM (1.0 mL), a solution of the proper acyl chloride in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at RT for 16 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 30%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a white solid (61 mg, 42% yield).

Mp: 98-99 ºC

IR (ATR) *v*: 3268, 3138, 3069, 1664, 1611, 1543, 1520, 1471, 1428, 1407, 1348, 1289, 1247, 1209, 1170, 1123, 1093, 1041, 1002, 943, 921, 882, 859, 825, 765, 750, 729, 689, 643, 584 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 4.77 (dd, J = 5.5 Hz, J' = 0.7 Hz, 2H, C$\underline{H}_2$), 7.31 (dt, J = 2.0 Hz, J' = 0.8 Hz, 1H, 2'-H), 7.40 (broad m, 1H, N$\underline{H}$), 7.43 (m, 1H, 4-H), 7.72 (dt, J = 8.7 Hz, J' = 2.1 Hz, 1H, 2-H), 7.84 (td, J = 1.6 Hz, J' = 0.8 Hz, 1H, 6-H), 8.77 (d, J = 2.0 Hz, 1H, 5'-H).

^{13}C NMR (101 MHz, CDCl$_3$) δ: 40.1 (CH$_2$, $\underline{C}$H$_2$), 115.9 (dq, $^2J_{CF}$ = 24.8 Hz, $^3J_{CF}$ = 3.7 Hz, CH, C4), 116.1 (CH, C2'), 118.2 (d, $^2J_{CF}$ = 22.7 Hz, CH, C2), 119.8 (p, $^3J_{CF}$ = 3.6 Hz, CH, C6), 123.0 (qd, $^1J_{CF}$ = 272.7 Hz, $^3J_{CF}$ = 2.8 Hz, C, $\underline{C}$F$_3$), 133.1 (qd, $^2J_{CF}$ = 33.9 Hz, $^3J_{CF}$ = 7.7 Hz, C, C5), 137.6 (d, $^3J_{CF}$ = 6.8 Hz, C, C1), 153.0 (C, C3'), 153.7 (CH, C5'), 162.6 (d, $^1J_{CF}$ = 251.0 Hz, C, C3), 164.8 (d, $^4J_{CF}$ = 2.4 Hz, C, $\underline{C}$O).

Anal. calcd for C$_{12}$H$_8$F$_4$N$_2$OS: C 47.37, H 2.65, N 9.21. Anal. calcd for C$_{12}$H$_8$F$_4$N$_2$OS · 0.3 C$_3$H$_6$O: C 47.65, H 2.80, N 9.03. Found: C 47.85, H 2.54, N 9.05.

HRMS: Calcd for [C$_{12}$H$_8$F$_4$N$_2$OS-H]$^-$: 303.0221, found: 303.0219.
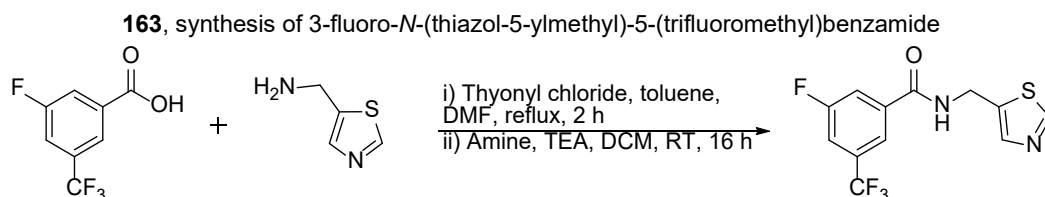
163, synthesis of 3-fluoro-*N*-(thiazol-5-ylmethyl)-5-(trifluoromethyl)benzamide

To a solution 3-fluoro-5-(trifluoromethyl)benzoic acid (100 mg, 0.48 mmol) in anh. toluene (2.0 mL), thionyl chloride (279 μL, 457 mg, 3.84 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford benzoyl chloride as a brown oil.

To a solution of thiazol-5-ylmethanamine (60 mg, 0.53 mmol) and triethylamine (268 μL, 194 mg, 1.92 mmol) in anh. DCM (1.0 mL), a solution of the proper acyl chloride in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at RT for 16 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 50%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a reddish syrup (51 mg, 35% yield).

IR (ATR) *v*: 3269, 3080, 1646, 1604, 1543, 1520, 1467, 1446, 1408, 1348, 1280, 1220, 1169, 1126, 1093, 1039, 1003, 926, 881, 797, 752, 692, 633, 601 cm^{-1}.

¹H NMR (400 MHz, CDCl₃) δ: 4.86 (dd, *J* = 5.9 Hz, *J'* = 0.9 Hz, 2H, C$\underline{H}_2$), 6.85 (broad m, 1H, N$\underline{H}$), 7.48 (dt, *J* = 8.0 Hz, *J'* = 1.7 Hz, 1H, 4-H), 7.72 (dt, *J* = 8.5 Hz, *J'* = 2.1 Hz, 1H, 2-H), 7.81 (d, *J* = 0.8 Hz, 1H, 4'-H), 7.82 (s, 1H, 6-H), 8.76 (s, 1H, 2'-H).

¹³C NMR (101 MHz, CDCl₃) δ: 36.3 (CH₂, $\underline{C}$H₂), 116.3 (dq, $^2J_{CF}$ = 24.4 Hz, $^3J_{CF}$ = 3.7 Hz, CH, C4), 118.2 (d, $^2J_{CF}$ = 23.0 Hz, CH, C2), 119.7 (p, $^3J_{CF}$ = 3.7 Hz, CH, C6), 122.9 (q, $^1J_{CF}$ = 272.7 Hz, C, $\underline{C}$F₃), 133.3 (qd, $^2J_{CF}$ = 34.0 Hz, $^3J_{CF}$ = 7.7 Hz, C, C5), 135.0 (C, C5'), 137.2 (d, $^3J_{CF}$ = 6.9 Hz, C, C1), 142.5 (CH, C4'), 154.1 (CH, C2'), 162.7 (d, $^1J_{CF}$ = 251.5 Hz, C, C3), 164.8 (C, $\underline{C}$O).

Anal. calcd for C₁₂H₈F₄N₂OS: C 47.37, H 2.65, N 9.21. Found: C 47.36, H 2.67, N 9.05.

HRMS: Calcd for [C₁₂H₈F₄N₂OS+H]⁺: 285.0304, found: 285.0312.

164, synthesis of 3-fluoro-*N*-(isoxazol-3-ylmethyl)-5-(trifluoromethyl)benzamide

To a solution 3-fluoro-5-(trifluoromethyl)benzoic acid (78 mg, 0.37 mmol) in anh. toluene (1.5 mL), thionyl chloride (272 µL, 446 mg, 3.75 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford benzoyl chloride as a brown oil.

To a solution of isoxazol-3-ylmethylamine (37 mg, 0.37 mmol) and triethylamine (209 µL, 152 mg, 1.50 mmol) in anh. DCM (1.0 mL), a solution of the proper acyl chloride in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at RT for 16 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na₂SO₄ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 20%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a brownish solid (34 mg, 32% yield).

Mp: 110-111 ºC

IR (ATR) *v*: 267, 3114, 1670, 1630, 1606, 1573, 1550, 1501, 1467, 1438, 1422, 1354, 1293, 1245, 1222, 1162, 1128, 1093, 1059, 1044, 1020, 1003, 938, 888, 862, 795, 713, 692, 651, 592, 555 cm⁻¹.

¹H NMR (400 MHz, CDCl₃) δ: 4.77 (d, *J* = 5.6 Hz, 2H, C$\underline{H}_2$), 6.43 (d, *J* = 1.7 Hz, 1H, 4'-H), 6.97 (broad s, 1H, N$\underline{H}$), 7.49 (m, 1H, 4-H), 7.74 (dt, *J* = 8.5 Hz, *J'* = 1.8 Hz, 1H, 2-H), 7.86 (s, 1H, 6-H), 8.41 (d, *J* = 1.6 Hz, 1H, 5'-H).

¹³C NMR (101 MHz, CDCl₃) δ: 36.3 (CH₂, $\underline{C}$H₂), 104.1 (CH, C4'), 116.3 (dq, $^2J_{CF}$ = 24.6 Hz, $^3J_{CF}$ = 3.8 Hz, CH, C4), 118.2 (d, $^2J_{CF}$ = 22.9 Hz, CH, C2), 119.8 (p, $^3J_{CF}$ = 3.8 Hz, CH, C6), 122.9 (q, $^1J_{CF}$ = 272.7 Hz, C, $\underline{C}$F₃), 133.3 (qd, $^2J_{CF}$ = 34.0 Hz, $^3J_{CF}$ = 7.7 Hz, C, C5), 137.2 (d, $^3J_{CF}$ = 6.9 Hz, C, C1), 159.4 (CH, C5'), 159.7 (C, C3'), 162.7 (d, $^1J_{CF}$ = 251.3 Hz, C, C3), 165.0 (C, $\underline{C}$O).

Anal. calcd for C₁₂H₈F₄N₂O₂: C 50.01, H 2.80, N 9.72. Found: C 50.16, H 2.64, N 9.55.

HRMS: Calcd for [C₁₂H₈F₄N₂O₂-H]⁻: 287.0449, found: 287.0446.
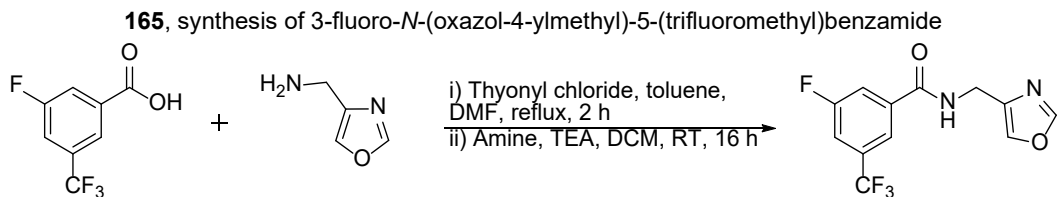
165, synthesis of 3-fluoro-*N*-(oxazol-4-ylmethyl)-5-(trifluoromethyl)benzamide

To a solution 3-fluoro-5-(trifluoromethyl)benzoic acid (100 mg, 0.48 mmol) in anh. toluene (2.0 mL), thionyl chloride (279 µL, 457 mg, 3.84 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford benzoyl chloride as a brown oil.

To a suspension of oxazol-4-ylmethanamine hydrochloride (71 mg, 0.53 mmol) and triethylamine (268 µL, 194 mg, 1.92 mmol) in anh. DCM (1.0 mL), a solution of the proper acyl chloride in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at RT for 16 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 40%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a yellowish solid (107 mg, 77% yield).

Mp: 99-100 ºC

IR (ATR) *v*: 3272, 3117, 1659, 1608, 1547, 1506, 1470, 1442, 1427, 1348, 1328, 1295, 1280, 1247, 1232, 1210, 1171, 1122, 1095, 1068, 1045, 1004, 968, 923, 916, 881, 859, 796, 774, 748, 690, 664, 621 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 4.56 (d, *J* = 5.1 Hz, 2H, C$\underline{H}_2$), 7.28 (broad s, 1H, N$\underline{H}$), 7.43 (m, 1H, 4-H), 7.68-7.72 (complex signal, 2H, 2-H, 2'-H), 7.82 (s, 1H, 6-H), 7.86 (s, 1H, 5'-H).

^{13}C NMR (101 MHz, CDCl$_3$) δ: 35.8 (CH$_2$, $\underline{C}$H$_2$), 116.0 (dq, $^2J_{CF}$ = 24.5 Hz, $^3J_{CF}$ = 3.9 Hz, CH, C4), 118.2 (d, $^2J_{CF}$ = 22.7 Hz, CH, C2), 119.8 (p, $^3J_{CF}$ = 3.7 Hz, CH, C6), 122.9 (qd, $^1J_{CF}$ = 273.0 Hz, $^3J_{CF}$ = 3.1 Hz, C, $\underline{C}$F$_3$), 133.1 (qd, $^2J_{CF}$ = 33.8 Hz, $^3J_{CF}$ = 7.7 Hz, C, C5), 136.4 (CH, C2'), 136.4 (CH, C3'), 137.5 (d, $^3J_{CF}$ = 6.9 Hz, C, C1), 151.6 (CH, C5'), 162.6 (d, $^1J_{CF}$ = 251.0 Hz, C, C3), 164.9 (d, $^4J_{CF}$ = 2.4 Hz, C, $\underline{C}$O).

Anal. calcd for C$_{12}$H$_8$F$_4$N$_2$O$_2$: C 50.01, H 2.80, N 9.72. Found: C 50.24, H 2.67, N 9.50.

HRMS: Calcd for [C$_{12}$H$_8$F$_4$N$_2$O$_2$-H]$^-$: 287.0449, found: 287.0445.
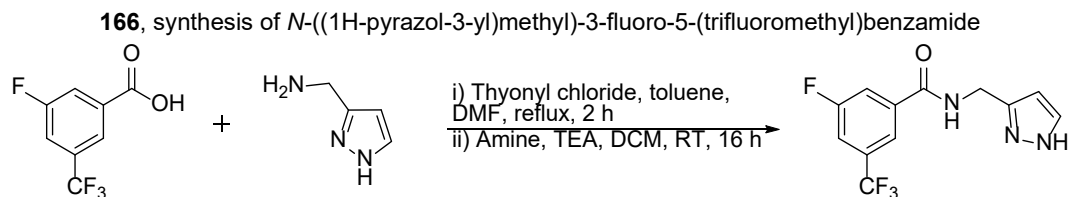
166, synthesis of *N*-((1H-pyrazol-3-yl)methyl)-3-fluoro-5-(trifluoromethyl)benzamide

To a solution of 3-fluoro-5-(trifluoromethyl)benzoic acid (100 mg, 0.48 mmol) in anh. toluene (2.0 mL), thionyl chloride (279 µL, 457 mg, 3.84 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford benzoyl chloride as a brown oil.

To a suspension of (1*H*-pyrazol-3-yl)methanamine (51 mg, 0.53 mmol) and triethylamine (201 µL, 146 mg, 1.44 mmol) in anh. DCM (1.0 mL), a solution of the proper acyl chloride in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at RT for 16 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the

resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 80%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a pale-yellow solid (75 mg, 54% yield).

Mp: 130-131 ºC

IR (ATR) *v*: 3197, 3069, 2945, 1645, 1599, 1556, 1471, 1441, 1428, 1343, 1297, 1240, 1212, 1174, 1131, 1096, 1051, 1035, 1002, 921, 692, 859, 790, 765, 748, 716, 664, 615 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 4.70 (d, *J* = 5.6 Hz, 2H, C$\underline{H}_2$), 6.30 (d, *J* = 2.2 Hz, 1H, 4'-H), 7.31 (broad s, 1H, N$\underline{H}$), 7.45 (m, 1H, 4-H), 7.54 (d, *J* = 2.2 Hz, 1H, 5'-H), 7.74 dt, *J* = 9.2 Hz, *J'* = 2.2 Hz, 1H, 2-H), 7.85 (td, *J* = 1.6 Hz, *J'* = 0.8 Hz, 1H, 6-H).

^{13}C NMR (101 MHz, CDCl$_3$) δ: 37.1 (CH$_2$, $\underline{C}$H$_2$), 104.5 (CH, C4'), 115.8 (dq, $^2J_{CF}$ = 24.5 Hz, $^3J_{CF}$ = 4.0 Hz, CH, C4), 118.1 (d, $^2J_{CF}$ = 22.9 Hz, CH, C2), 119.7 (m, CH, C6), 122.8 (qd, $^1J_{CF}$ = 270.5 Hz, $^3J_{CF}$ = 2.8 Hz, C, $\underline{C}$F$_3$), 132.3 (CH, C5'), 132.9 (qd, $^2J_{CF}$ = 33.9 Hz, $^3J_{CF}$ = 7.5 Hz, C, C5), 137.4 (d, $^3J_{CF}$ = 6.9 Hz, C, C1), 146.3 (C, C3'), 162.4 (d, $^1J_{CF}$ = 250.9 Hz, C, C3), 165.0 (d, *J* = 2.2 Hz, C, $\underline{C}$O).

Anal. calcd for C$_{12}$H$_9$F$_4$N$_3$O: C 50.18, H 3.16, N 14.63. Found: C 50.26, H 3.23, N 14.32.

HRMS: Calcd for [C$_{12}$H$_9$F$_4$N$_3$O-H]$^-$: 286.0609, found: 286.0605.
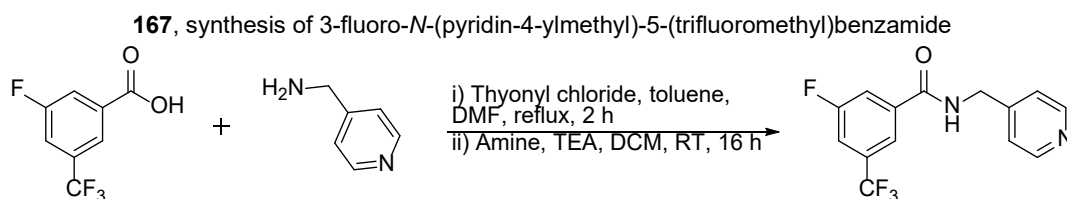
167, synthesis of 3-fluoro-*N*-(pyridin-4-ylmethyl)-5-(trifluoromethyl)benzamide

To a solution 3-fluoro-5-(trifluoromethyl)benzoic acid (100 mg, 0.48 mmol) in anh. toluene (2.0 mL), thionyl chloride (346 μL, 567 mg, 4.81 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford benzoyl chloride as a brown oil.

To a solution of pyridin-4-ylmethanamine (54 μL, 57 mg, 0.53 mmol) and triethylamine (268 μL, 194 mg, 1.92 mmol) in anh. DCM (1.0 mL), a solution of the proper acyl chloride in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at RT for 16 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na$_2$SO$_4$ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 65%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a pale-yellow syrup (81 mg, 57% yield).

IR (ATR) *v*: 3282, 3061, 1650, 1602, 1543, 1467, 1445, 1417, 1351, 1325, 1283, 1219, 1169, 1126, 1094, 1059, 1002, 928, 883, 799, 754, 691, 615 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 4.65 (d, *J* = 6.0 Hz, 2H, C$\underline{H}_2$), 7.12 (broad s, 1H, N$\underline{H}$), 7.23 [d, *J* = 6.1 Hz, 2H, 2'(6')-H], 7.49 (m, 1H, 4-H), 7.76 (dt, *J* = 8.6 Hz, *J'* = 2.1 Hz, 1H, 2-H), 7.86 (s, 1H, 6-H), 8.53 [d, *J* = 6.1 Hz, 2H, 3'(5')-H].

^{13}C NMR (101 MHz, CDCl$_3$) δ: 43.2 (CH$_2$, $\underline{C}$H$_2$), 116.3 (dq, $^2J_{CF}$ = 24.3 Hz, $^3J_{CF}$ = 3.7 Hz, CH, C4), 118.3 (d, $^2J_{CF}$ = 22.8 Hz, CH, C2), 119.7 (p, $^3J_{CF}$ = 4.0 Hz, CH, C6), 122.6 [CH, C2'(6')], 122.9 (qd, $^1J_{CF}$ = 272.9 Hz, $^3J_{CF}$ = 2.9 Hz, C, $\underline{C}$F$_3$), 133.3 (qd, $^2J_{CF}$ = 33.7 Hz, $^3J_{CF}$ = 7.6 Hz, C, C5), 137.3 (d, $^3J_{CF}$ = 6.7 Hz, C, C1), 146.9 (C, C1'), 150.2 [CH, C3'(5')], 162.7 (d, $^1J_{CF}$ = 251.5 Hz, C, C3), 165.2 (d, $^4J_{CF}$ = 2.4 Hz, C, $\underline{C}$O).

Anal. calcd for C$_{14}$H$_{10}$F$_4$N$_2$O: C 56.38, H 3.38, N 9.39. Found: C 55.35, H 3.41, N 9.08.

HRMS: Calcd for [C$_{14}$H$_{10}$F$_4$N$_2$O+H]$^+$: 299.0802, found: 299.0805.

168, synthesis of 3-fluoro-*N*-(furan-3-ylmethyl)-5-(trifluoromethyl)benzamide
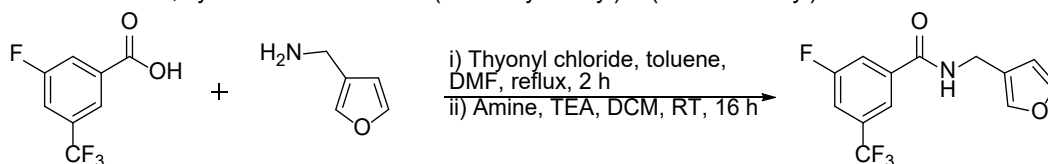
To a solution 3-fluoro-5-(trifluoromethyl)benzoic acid (100 mg, 0.48 mmol) in anh. toluene (2.0 mL), thionyl chloride (346 μL, 567 mg, 4.81 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford benzoyl chloride as a brown oil.

To a solution of furan-3-ylmethanamine (51 mg, 0.53 mmol) and triethylamine (268 μL, 194 mg, 1.92 mmol) in anh. DCM (1.0 mL), a solution of the proper acyl chloride in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at RT for 16 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 15%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a reddish syrup (67 mg, 49% yield).

IR (ATR) *v*: 3295, 3087, 1645, 1604, 1544, 1467, 1445, 1341, 1285, 1249, 1219, 1171, 1128, 1093, 1021, 967, 925, 884, 874, 768, 692, 619, 599 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 4.50 (d, *J* = 5.5 Hz, 2H, C$\underline{H}_2$), 6.38 (broad s, 1H, N$\underline{H}$), 6.43 (m, 1H, 4'-H), 7.42 (t, *J* = 1.7 Hz, 1H, 2'-H), 7.45-7.48 (complex signal, 2H, 4-H, 5'-H), 7.69 (dt, *J* = 8.6 Hz, *J'* = 2.0 Hz, 1H, 2-H), 7.79 (s, 1H, 6-H).

^{13}C NMR (101 MHz, CDCl$_3$) δ: 35.4 (CH$_2$, $\underline{C}$H$_2$), 110.4 (CH, C4'), 116.0 (dq, $^2J_{CF}$ = 24.4 Hz, $^3J_{CF}$ = 3.7 Hz, CH, C4), 118.1 (d, $^2J_{CF}$ = 22.9 Hz, CH, C2), 119.6 (p, $^3J_{CF}$ = 3.8 Hz, CH, C6), 121.6 (C, C3'), 123.0 (q, $^1J_{CF}$ = 271.5 Hz, C, $\underline{C}$F$_3$), 133.2 (qd, $^2J_{CF}$ = 33.9 Hz, $^3J_{CF}$ = 7.3 Hz, C, C5), 137.8 (d, $^3J_{CF}$ = 6.8 Hz, C, C1), 140.7 (CH, C5'), 143.9 (CH, C2'), 162.7 (d, $^1J_{CF}$ = 251.2 Hz, C, C3), 164.8 (C, $\underline{C}$O).

Anal. calcd for C$_{13}$H$_9$F$_4$NO$_2$: C 54.36, H 3.16, N 4.88. Anal. calcd for C$_{13}$H$_9$F$_4$NO$_2$ · H$_2$O: C 53.20, H 3.33, N 4.77. Found: C 53.06, H 3.15, N 4.67.

HRMS: Calcd for [C$_{13}$H$_9$F$_4$NO$_2$-H]$^-$: 286.0497, found: 286.0494.

169, synthesis of (2,5-dimethylthiophen-3-yl)methanol
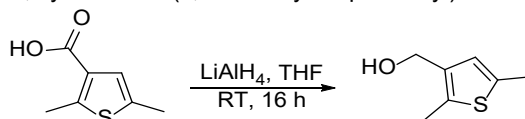
To a solution ethyl 2,5-dimethylthiophene-3-carboxylate (100 mg, 0.64 mmol) in anh. THF (3.0 mL) was added portinonwise LiAlH$_4$ (73 mg, 1.92 mmol) under ice-bath. The mixture was stirred at RT for 16 h. Under ice-bath, HCl 2 M (2.0 mL) was added and the mixture was stirred for 10 min more. Then, water (10 mL) followed by EtOAc (15 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na$_2$SO$_4$ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 15%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a colorless oil (88 mg, 97% yield). Spectroscopic data matched with those previously published.

170, synthesis of (2,5-dimethylthiophen-3-yl)methyl 3-fluoro-5-(trifluoromethyl)benzoate
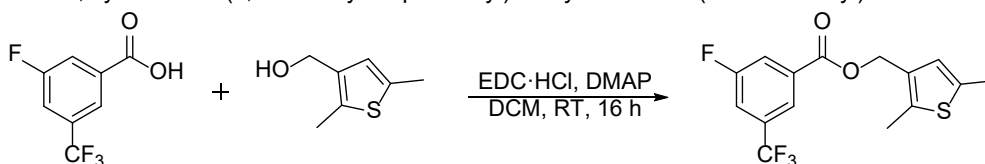
To a solution of (2,5-dimethylthiophen-3-yl)methanol (88 mg, 0.62 mmol), EDC·HCl (178 mg, 0.93 mmol) and DMAP (30 mg, 0.25 mmol) in anh. DCM (2.0 mL) was added 3-fluoro-5-(trifluoromethyl)benzoic acid (155 mg, 0.74 mmol) under Ar atmosphere and the mixture was stirred at RT for 16 h.

DCM (10 mL) was added and the mixture was washed with brine (2 x 20 mL). The organic layer was dried over Na_2SO_4 anh., filtered and concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 10%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a colorless oil (96 mg, 47% yield).

IR (ATR) v: 2923, 1727, 1606, 1453, 1363, 1347, 1249, 1238, 1202, 1171, 1131, 1103, 1091, 959, 906, 887, 828, 768, 692, 582 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 2.42 (m, 3H, 5'-CH$_3$), 2.46 (s, 3H, 2'-CH$_3$), 5.24 (s, 2H, CH$_2$), 6.69 (d, J = 1.2 Hz, 1H, 4'-H), 7.51 (dddd, J = 8.1 Hz, J' = 2.5 Hz, J'' = 1.6 Hz, J''' = 0.7 Hz, 1H, 4-H), 7.91 (ddd, J = 8.7 Hz, J' = 2.6 Hz, J'' = 1.4 Hz, 1H, 2-H), 8.11 (s, 1H, 6-H).

^{13}C NMR (101 MHz, CDCl$_3$) δ: 13.0 (C2'-CH$_3$), 15.2 (C5'-CH$_3$), 61.0 (CH$_2$, CH$_2$), 117.3 (dq, $^2J_{CF}$ = 24.6 Hz, $^3J_{CF}$ = 3.7 Hz, CH, C4), 120.2 (d, $^2J_{CF}$ = 23.0 Hz, CH, C2), 122.5 (p, $^3J_{CF}$ = 3.9 Hz, CH, C6), 123.0 (qd, $^1J_{CF}$ = 272.8 Hz, $^4J_{CF}$ = 2.9 Hz, C, CF$_3$), 126.9 (CH, C4'), 131.0 (C, C3'), 133.0 (qd, $^2J_{CF}$ = 33.9 Hz, $^3J_{CF}$ = 7.5 Hz, C, C5), 133.7 (d, $^3J_{CF}$ = 7.6 Hz, C1), 136.5 (C, C2'), 136.8 (C, C5'), 162.4 (d, $^1J_{CF}$ = 250.4 Hz, C, C3), 164.2 (d, $^4J_{CF}$ = 2.3 Hz, C, CO).

Anal. calcd for C$_{15}$H$_{12}$F$_4$O$_2$S: C 54.22, H 3.64. Found: C 54.44, H 3.54.

HRMS: Calcd for [C$_{15}$H$_{12}$F$_4$O$_2$S+Na]$^+$: 355.0386, found: 355.0389.

172, synthesis of 1-(2,5-dimethylthiophen-3-yl)-*N*-(3-fluoro-5-(trifluoromethyl)benzyl)methanamine
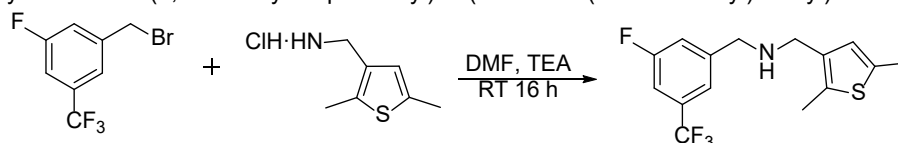
To a solution 1-(bromomethyl)-3-fluoro-5-(trifluoromethyl)benzene (50 mg, 0.19 mmol) and triethylamine (81 μL, 59 mg, 0.58 mmol) in DMF (1.0 mL) was added (2,5-dimethylthiophen-3-yl)methanamine hydrochloride (35 mg, 0.19 mmol) and the mixture was kept under stirring at RT for 16 h. Water (15 mL) followed by EtOAc (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 15%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a colorless oil (27 mg, 45% yield).

IR (ATR) v: 2921, 2860, 1605, 1452, 1342, 1227, 1166, 1125, 1091, 975, 869, 830, 761, 721, 698, 712 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 2.30 (s, 3H, 2'-CH$_3$), 2.40 (m, 3H, 5'-CH$_3$), 3.62 (s, 2H, thio-CH$_2$), 3.84 (s, 2H, aryl-CH$_2$), 6.59 (q, J = 1.2 Hz, 1H, 4'-H), 7.20 (m, 1H, 4-H), 7.29 (dddd, J = 9.4 Hz, J' = 2.6 Hz, J'' = 1.4 Hz, J''' = 0.7 Hz, 1H, 2-H), 7.41 (tq, J = 1.5 Hz, J' = 0.7 Hz, 1H, 6-H).

^{13}C NMR (101 MHz, CDCl$_3$) δ: 12.9 (C2'-C<u>H</u>$_3$), 15.2 (C5'-C<u>H</u>$_3$), 46.1 (CH$_2$, thio-C<u>H</u>$_2$), 52.3 (CH$_2$, aryl-C<u>H</u>$_2$), 111.4 (dq, $^2J_{CF}$ = 24.6 Hz, $^3J_{CF}$ = 3.8 Hz, CH, C4), 118.4 (d, $^2J_{CF}$ = 21.3 Hz, CH, C2), 120.6 (p, $^3J_{CF}$ = 3.6 Hz, CH, C6), 123.5 (qd, $^1J_{CF}$ = 272.5 Hz, $^3J_{CF}$ = 3.1 Hz, C, C<u>F</u>$_3$), 126.7 (CH, C4'), 132.4 (qd, $^2J_{CF}$ = 33.0 Hz, $^3J_{CF}$ = 8.1 Hz, C, C5), 132.9 (C, C3'), 135.4 (C, C2'),, 136.0 (C, C5'), 144.8 (d, $^3J_{CF}$ = 7.1 Hz, C, C1), 162.7 (d, $^1J_{CF}$ = 248.2 Hz, C, C3).

Anal. calcd for C$_{15}$H$_{15}$F$_4$NS: C 56.77, H 4.76, N 4.41. Found: C 56.85, H 4.82, N 4.63.

HRMS: Calcd for [C$_{15}$H$_{15}$F$_4$NS+H]$^+$: 318.0934, found: 318.0934.

174, synthesis of *N*-(3-fluoro-5-(trifluoromethyl)benzyl)-2,5-dimethylthiophene-3-carboxamide
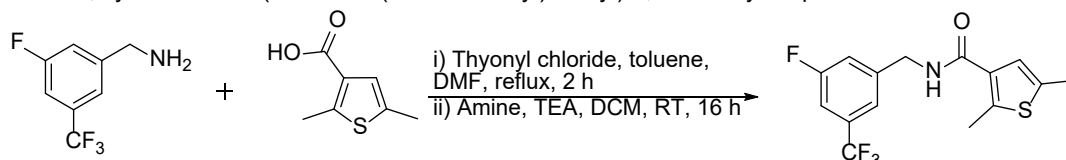
To a solution 2,5-dimethylthiophene-3-carboxylic acid (100 mg, 0.64 mmol) in anh. toluene (3.0 mL), thionyl chloride (279 µL, 457 mg, 3.84 mmol) followed by some drops of DMF were added. The mixture was then heated and refluxed for 2 h. Then, the solvent was evaporated *in vacuo* to afford benzoyl chloride as a brown oil.

To a suspension of (3-fluoro-5-(trifluoromethyl)phenyl)methanamine (148 mg, 0.77 mmol) and triethylamine (268 µL, 194 mg, 1.92 mmol) in anh. DCM (2.0 mL), a solution of the proper acyl chloride in anh. DCM (1.0 mL) was slowly added. The mixture was kept under stirring at RT for 16 h. Water (10 mL) followed by DCM (10 mL) were added and the mixture was extracted. The organic layer was washed again with brine (15 mL), then it was dried over Na$_2$SO$_4$ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 15%). Fractions containing the desired product were collected and concentrated *in vacuo* to afford a beige solid (85 mg, 40% yield).

Mp: 105-106 ºC

IR (ATR) *v*: 3293, 2925, 1634, 1609, 1563, 1515, 1454, 1424, 1351, 1341, 1315, 1280, 1229, 1169, 1120, 1084, 1041, 994, 973, 875, 852, 834, 785, 768, 716, 701, 690, 650, 601 cm^{-1}.

^{1}H NMR (400 MHz, CDCl$_3$) δ: 2.37 (s, 3H, 2'-C<u>H</u>$_3$), 2.63 (s, 3H, 5'-C<u>H</u>$_3$), 4.59 (d, *J* = 6.1 Hz, 2H, C<u>H</u>$_2$), 6.35 (broad s, 1H, N<u>H</u>), 6.76 (d, *J* = 1.2 Hz, 1H, 4'-H), 7.22 (m, 2H, 2-H, 4-H), 7.35 (s, 1H, 6-H).

^{13}C NMR (101 MHz, CDCl$_3$) δ: 15.0 (C2'-C<u>H</u>$_3$), 15.1 (C5'-C<u>H</u>$_3$), 42.8 (d, $^4J_{CF}$ = 1.7 Hz, CH$_2$, C<u>H</u>$_2$), 111.9 (dq, $^2J_{CF}$ = 24.6 Hz, $^3J_{CF}$ = 3.9 Hz, CH, C4), 118.1 (d, $^2J_{CF}$ = 21.9 Hz, CH, C2), 120.1 (m, CH, C6), 123.3 (qd, $^1J_{CF}$ = 272.5 Hz, $^3J_{CF}$ = 3.3 Hz, C, C<u>F</u>$_3$), 123.9 (CH, C4'), 130.6 (C, C3'), 132.9 (qd, $^2J_{CF}$ = 33.3 Hz, $^3J_{CF}$ = 8.2 Hz, C, C5), 136.5 (C, C5'), 142.9 (d, $^3J_{CF}$ = 7.2 Hz, C, C1), 143.8 (C, C2'), 162.8 (d, $^1J_{CF}$ = 249.4 Hz, C, C3), 164.7 (C, <u>C</u>O).

Anal. calcd for C$_{15}$H$_{13}$F$_4$NOS: C 54.38, H 3.95, N 4.23. Found: C 54.46, H 3.83, N 4.19.

HRMS: Calcd for [C$_{15}$H$_{13}$F$_4$NOS-H]$^-$: 330.0581, found: 330.0573.

192, synthesis of 3-chloro-4-methyl-*N*-(2-(piperidin-1-yl)ethyl)aniline hydrochloride
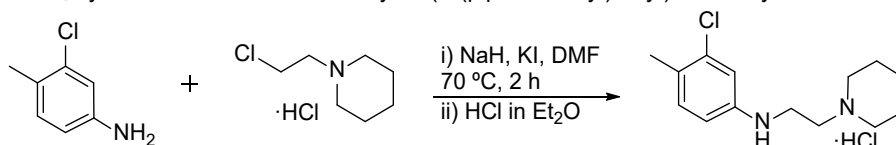
To a suspension of 3-chloro-4-methylaniline (100 mg, 0.71 mmol) in DMF (2.0 mL) was added NaH, 60% dispersion in mineral oil (85 mg, 2.12) and the mixture was stirred at room temperature for 20 min. Then, KI (21 mg, 0.13 mmol) followed by 1-(2-chloroethyl)piperidine hydrochloride (143 mg, 0.78 mmol) were added and the mixture was stirred at 70 ºC for 2 h.

EtOAc (8 mL) was added and the mixture was washed with Na_2CO_3 sat. (2 x 20 mL). The organic layer was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 2%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et_2O (1.0 mL) was added followed by HCl 2 M in Et_2O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford an orangish solid (23 mg, 11% yield).

Mp: 222-223 ºC

IR (ATR) v: 3258, 2939, 2632, 2547, 2028, 1607, 1513, 1488, 1545, 1310, 1249, 1197, 1095, 1015, 972, 857, 817, 764, 684, 585 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.54 (m, 1H, 4''-H$_{ax}$), 1.79-2.01 [complex signal, 5H, 3''(5'')-H$_2$, 4''-H$_{eq}$], 2.33 (s, 3H, C$\underline{H}_3$), 3.04 [td, J = 12.0 Hz, J' = 4.3 Hz, 2H, 2''(6'')-H$_{ax}$], 3.45 (t, J = 6.4 Hz, 2H, 2'-H), 3.63 [d, J = 12.3 Hz, 2H, 2''(6'')-H$_{eq}$], 3.74 (t, J = 6.4 Hz, 2H, 1'-H), 7.08 (dd, J = 8.2 Hz, J' = 2.3 Hz, 1H, 6-H), 7.28 (m, 1H, 2-H), 7.31 (m, 1H, 5-H).

^{13}C NMR (101 MHz, CD$_3$OD) δ: 19.4 (CH$_3$, $\underline{C}$H$_3$), 22.5 (CH$_2$, C4''), 24.1 [CH$_2$, C3''(5'')], 43.2 (CH$_2$, C1'), 54.9 [CH$_2$, C2''(6'')], 55.1 (CH$_2$, C2'), 117.9 (d, J = 3.5 Hz, CH, C6), 119.6 (CH, C2), 133.1 (m, C, C4), 133.2 (CH, C5), 136.2 (d, J = 8.2 Hz, C, C3), 141.3 (t, J = 10.9 Hz, C, C1).

Anal. calcd for C$_{14}$H$_{22}$Cl$_2$N$_2$: C 58.14, H 7.67, N 9.69. Anal. calcd for C$_{14}$H$_{22}$Cl$_2$N$_2$ · 1.5 H$_2$O: C 53.17, H 7.97, N 8.86. Found: C 52.69, H 7.41, N 8.57.

HRMS: Calcd for [C$_{14}$H$_{21}$ClN$_2$+H]$^+$: 253.1466, found: 253.1455.

193, synthesis of 3-chloro-4-fluoro-*N*-(2-(piperidin-1-yl)ethyl)aniline dihydrochloride
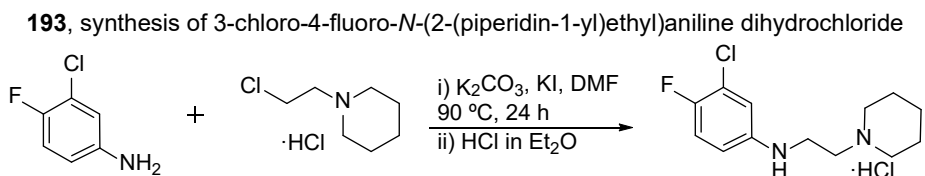
To a suspension of 3-chloro-4-fluoroaniline (100 mg, 0.69 mmol), K$_2$CO$_3$ (95 mg, 0.69 mmol) and KI (10 mg, 0.06 mmol) in DMF (2.0 mL) was added 1-(2-chloroethyl)piperidine hydrochloride (63 mg, 0.34 mmol) and the mixture was stirred at 90 ºC for 24 h.

After cooling down to room temperature, EtOAc (8 mL) was added and the mixture was washed with Na$_2$CO$_3$ sat. (2 x 20 mL). The organic layer was dried over Na$_2$SO$_4$ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 1.5%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et$_2$O (1.0 mL) was added followed by HCl 2 M in Et$_2$O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford an off-white solid (17 mg, 8% yield).

Mp: 221-222 ºC

IR (ATR) v: 3256, 2945, 2640, 2555, 2034, 1613, 1537, 1503, 1454, 1265, 1235, 1044, 973, 860, 808, 744, 703, 692, 605, 588, 572 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.54 (m, 1H, 4''-H$_{ax}$), 1.81-1.90 [complex signal, 3H, 3''(5'')-H$_{ax}$, 4''-H$_{eq}$], 1.95 [m, 2H, 3''(5'')-H$_{eq}$], 3.02 [td, J = 12.2 Hz, J' = 3.4 Hz, 2H, 2''(6'')-H$_{ax}$], 3.38 (t, J = 6.3 Hz, 2H, 2'-H), 3.58-3.65 [complex signal, 4H, 1'-H, 2''(6'')-H$_{eq}$], 6.90 (m, 1H, 6-H), 7.06 (m, 1H, 2-H), 7.14 (t, J = 8.9 Hz, 1H, 5-H).

^{13}C NMR (101 MHz, CD$_3$OD) δ: 22.6 (CH$_2$, C4''), 24.1 [CH$_2$, C3''(5'')], 41.4 (CH$_2$, C1'), 54.8 [CH$_2$, C2''(6'')], 56.0 (CH$_2$, C2'), 116.3 (d, $^3J_{CF}$ = 6.8 Hz, CH, C6), 117.8 (CH, C2), 118.3 (d, $^2J_{CF}$ = 22.4 Hz, CH, C5), 122.4 (d, $^2J_{CF}$ = 18.7 Hz, C, C3), 143.2 (C, C1), 154.1 (d, $^1J_{CF}$ = 240.4 Hz, C, C4).

Anal. calcd for C$_{13}$H$_{19}$Cl$_2$FN$_2$: C 47.36, H 6.12, N 8.50. Anal. calcd for C$_{13}$H$_{19}$Cl$_2$FN$_2$ · 1.5 H$_2$O: C 48.76, H 6.92, N 8.75. Found: C 48.14, H 6.22, N 8.45.

HRMS: Calcd for [C$_{13}$H$_{18}$ClFN$_2$+H]$^+$: 257.1215, found: 257.1211.

194, synthesis of 3,5-dichloro-4-methoxy-*N*-(2-(piperidin-1-yl)ethyl)aniline hydrochloride
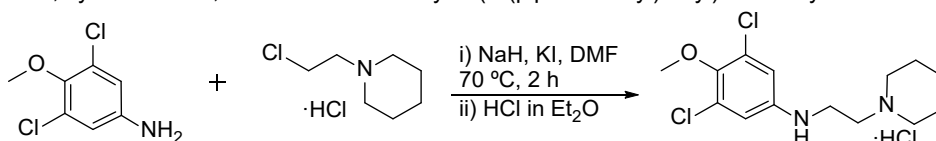
To a suspension of 3,5-dichloro-4-methoxyaniline (50 mg, 0.26 mmol) in DMF (2.0 mL) was added NaH, 60% dispersion in mineral oil (31 mg, 0.78) and the mixture was stirred at room temperature for 20 min. Then, KI (21 mg, 0.13 mmol) followed by 1-(2-chloroethyl)piperidine hydrochloride (53 mg, 0.29 mmol) were added and the mixture was stirred at 70 ºC for 2 h.

EtOAc (8 mL) was added and the mixture was washed with Na$_2$CO$_3$ sat. (2 x 20 mL). The organic layer was dried over Na$_2$SO$_4$ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 1.5%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et$_2$O (1.0 mL) was added followed by HCl 2 M in Et$_2$O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford an off-white solid (26 mg, 29% yield).
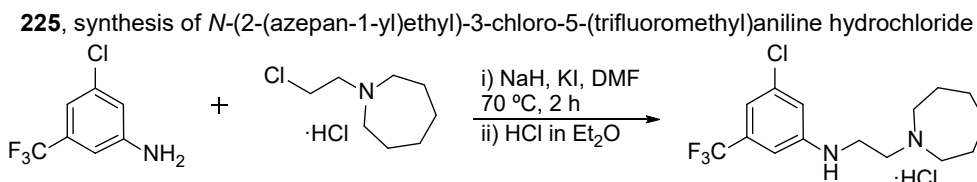
Mp: 169-170 ºC

IR (ATR) *v*: 3258, 2938, 2637, 2557, 2408, 2036, 1599, 1516, 1479, 1426, 1233, 1116, 1005, 977, 860, 807, 764, 635 cm^{-1}.

^{1}**H NMR** (400 MHz, CD$_3$OD) δ: 1.54 (m, 1H, 4''-H$_{ax}$), 1.79-1.89 [complex signal, 3H, 3''(5'')-H$_{ax}$, 4''-H$_{eq}$], 1.94 [m, 2H, 3''(5'')-H$_{eq}$], 3.01 [td, *J* = 12.2 Hz, *J'* = 3.5 Hz, 2H, 2''(6'')-H$_{ax}$], 3.30 (m, 2H, 2'-H), 3.52 (t, *J* = 6.2 Hz, 2H, 1'-H), 3.58 [d, *J* = 12.6 Hz, 2H, 2''(6'')-H$_{eq}$], 3.76 (s, 3H, C$\underline{H}_3$), 6.73 [s, 2H, 2(6)-H].

^{13}**C NMR** (101 MHz, CD$_3$OD) δ: 22.6 (CH$_2$, C4''), 24.1 [CH$_2$, C3''(5'')], 39.4 (CH$_2$, C1'), 54.6 [CH$_2$, C2''(6'')], 56.6 (CH$_2$, C2'), 61.2 (CH$_3$, $\underline{C}$H$_3$), 114.0 [CH, C2(6)], 130.7 [C, C3(5)], 144.8 (C, C1), 146.3 (C, C4).

Anal. calcd for C$_{14}$H$_{21}$Cl$_3$N$_2$O: C 49.50, H 6.23, N 8.25. Found: 49.29, H 6.32, N 7.99.

HRMS: Calcd for [C$_{14}$H$_{20}$Cl$_2$N$_2$O+H]$^+$: 303.1025, found: 303.1018.

195, synthesis of 3,5-dichloro-4-methyl-*N*-(2-(piperidin-1-yl)ethyl)aniline hydrochloride
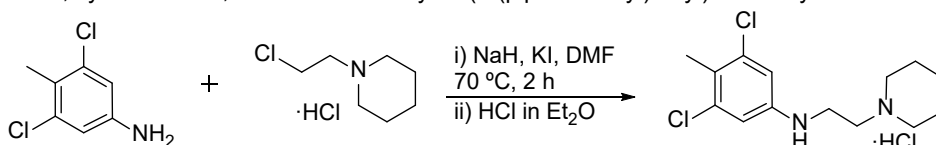
To a suspension of 3,5-dichloro-4-methylaniline (100 mg, 0.57 mmol) in DMF (2.0 mL) was added NaH, 60% dispersion in mineral oil (68 mg, 1.70) and the mixture was stirred at room temperature for 20 min. Then, KI (21 mg, 0.13 mmol) followed by 1-(2-chloroethyl)piperidine hydrochloride (115 mg, 0.62 mmol) were added and the mixture was stirred at 70 ºC for 2 h.

EtOAc (8 mL) was added and the mixture was washed with Na$_2$CO$_3$ sat. (2 x 20 mL). The organic layer was dried over Na$_2$SO$_4$ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude

was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 1.5%). Fractions containing the desired product were collected and concentrated *in vacuo*. Et$_2$O (1.0 mL) was added followed by HCl 2 M in Et$_2$O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford an off-white solid (49 mg, 27% yield).

Mp: 229-230 ºC

IR (ATR) *v*: 3270, 2942, 2632, 2547, 2405, 2025, 1605, 1518, 1463, 1453, 1312, 1256, 1126, 1075, 1057, 999, 974, 952, 849, 789, 690, 610 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.55 (m, 1H, 4''-H$_{ax}$), 1.79-1.91 [complex signal, 3H, 3''(5'')-H$_{ax}$, 4''-H$_{eq}$], 1.94 [m, 2H, 3''(5'')-H$_{eq}$], 2.31 (s, 3H, C$\underline{H}_3$), 3.01 [td, *J* = 12.1 Hz, *J'* = 3.7 Hz, 2H, 2''(6'')-H$_{ax}$], 3.33 (m, 2H, 2'-H), 3.55 (t, *J* = 6.3 Hz, 2H, 1'-H), 3.58 [d, *J* = 11.5 Hz, 2H, 2''(6'')-H$_{eq}$], 6.80 [s, 2H, 2(6)-H].

^{13}C NMR (101 MHz, CD$_3$OD) δ: 16.4 (CH$_3$, $\underline{C}$H$_3$), 22.6 (CH$_2$, C4''), 24.1 [CH$_2$, C3''(5'')], 39.6 (CH$_2$, C1'), 54.7 [CH$_2$, C2''(6'')], 56.4 (CH$_2$, C2'), 114.1 [CH, C2(6)], 124.1 (C, C4), 136.9 [C, C3(5)], 147.4 (C, C1).

Anal. calcd for C$_{14}$H$_{21}$Cl$_3$N$_2$: C 51.95, H 6.54, N 8.65. Anal. calcd for C$_{14}$H$_{21}$Cl$_3$N$_2$ · 0.55 H$_2$O: C 50.41, H 6.68, N 8.40. Found: C 50.12, H 6.33, N 8.13.

HRMS: Calcd for [C$_{14}$H$_{20}$Cl$_2$N$_2$+H]$^+$: 287.1076, found: 287.1072.

196, synthesis of 3,5-dichloro-4-fluoro-*N*-(2-(piperidin-1-yl)ethyl)aniline hydrochloride
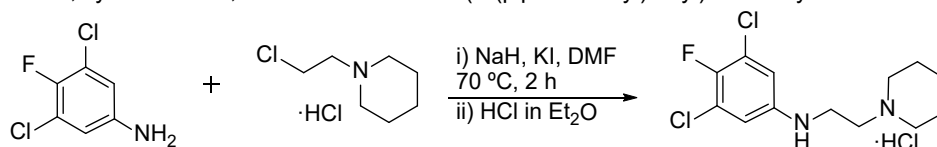
To a suspension of 3,5-dichloro-4-fluoroaniline (100 mg, 0.56 mmol) in DMF (2.0 mL) was added NaH, 60% dispersion in mineral oil (89 mg, 2.22) and the mixture was stirred at room temperature for 20 min. Then, KI (21 mg, 0.13 mmol) followed by 1-(2-chloroethyl)piperidine hydrochloride (133 mg, 0.72 mmol) were added and the mixture was stirred at 70 ºC for 2 h.

EtOAc (8 mL) was added and the mixture was washed with Na$_2$CO$_3$ sat. (2 x 20 mL). The organic layer was dried over Na$_2$SO$_4$ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 1.5%). Fractions containing the desired product were collected and concentrated *in vacuo*. Et$_2$O (1.0 mL) was added followed by HCl 2 M in Et$_2$O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford a beige solid (67 mg, 37% yield).

Mp: 218-219 ºC

IR (ATR) *v*: 3267, 2937, 2634, 2550, 1607, 1589, 1531, 1493, 1484, 1455, 1241, 1126, 1084, 1013, 976, 953, 846, 813, 791, 772, 688, 628, 585 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.54 (m, 1H, 4''-H$_{ax}$), 1.81-1.89 [complex signal, 3H, 3''(5'')-H$_{ax}$, 4''-H$_{eq}$], 1.94 [m, 2H, 3''(5'')-H$_{eq}$], 3.01 [td, *J* = 12.2 Hz, *J'* = 3.4 Hz, 2H, 2''(6'')-H$_{ax}$], 3.31 (m, 2H, 2'-H), 3.53 (t, *J* = 6.3 Hz, 2H, 1'-H), 3.58 [d, *J* = 11.5 Hz, 2H, 2''(6'')-H$_{eq}$], 6.76 [dd, *J* = 5.5 Hz, *J'* = 0.7 Hz, 2H, 2(6)-H].

^{13}C NMR (101 MHz, CD$_3$OD) δ: 22.6 (CH$_2$, C4''), 24.1 [CH$_2$, C3''(5'')], 39.4 (CH$_2$, C1'), 54.7 [CH$_2$, C2''(6'')], 56.5 (CH$_2$, C2'), 113.8 [CH, C2(6)], 123.4 [d, $^2J_{CF}$ = 18.2 Hz, C, C3(5)], 146.0 (C, C1), 148.0 (d, $^1J_{CF}$ = 238.1 Hz, C, C4).

Anal. calcd for C$_{13}$H$_{18}$Cl$_3$FN$_2$: C 47.66, H 5.54, N 8.55. Found: C 47.87, H 5.39, N 8.26.

HRMS: Calcd for [C$_{13}$H$_{17}$Cl$_2$FN$_2$+H]$^+$: 291.0826, found: 291.0820.

197, synthesis of 3,4,5-trichloro-*N*-(2-(piperidin-1-yl)ethyl)aniline hydrochloride
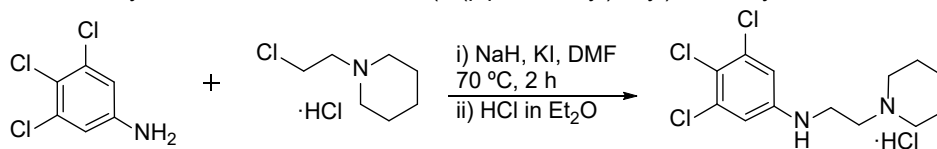
To a solution of 3,4,5-trichloroaniline (100 mg, 0.51 mmol) in DMF (2.0 mL) was added NaH, 60% dispersion in mineral oil (61 mg, 1.53) and the mixture was stirred at room temperature for 30 min. Then, KI (21 mg, 0.13 mmol) followed by 1-(2-chloroethyl)piperidine hydrochloride (112 mg, 0.61 mmol) were added and the mixture was stirred at 70 ºC for 2 h.

EtOAc (8 mL) was added and the mixture was washed with Na_2CO_3 sat. (2 x 20 mL). The organic layer was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 1.5%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et_2O (1.0 mL) was added followed by HCl 2 M in Et_2O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford an off-white solid (34 mg, 19% yield).

Mp: 228-229 ºC

IR (ATR) *v*: 3270, 2947, 2635, 2548, 2412, 2025, 1595, 1546, 1445, 1353, 1157, 1115, 970, 847, 811, 683, 612, 568 cm^{-1}.

^{1}H NMR (400 MHz, CD_3OD) δ: 1.54 (m, 1H, 4''-H_{ax}), 1.80-1.90 [complex signal, 3H, 3''(5'')-H_{ax}, 4''-H_{eq}], 1.95 [m, 2H, 3''(5'')-H_{eq}], 3.01 [td, *J* = 12.4 Hz, *J'* = 3.3 Hz, 2H, 2''(6'')-H_{ax}], 3.31 (m, 2H, 2'-H), 3.54 (t, *J* = 6.3 Hz, 2H, 1'-H), 3.58 [d, *J* = 11.9 Hz, 2H, 2''(6'')-H_{eq}], 6.84 [s, 2H, 2(6)-H].

^{13}C NMR (101 MHz, CD_3OD) δ: 22.6 (CH_2, C4''), 24.1 [CH_2, C3''(5'')], 38.9 (CH_2, C1'), 54.7 [CH_2, C2''(6'')], 56.5 (CH_2, C2'), 114.0 [CH, C2(6)], 119.1 (C, C4), 135.4 [C, C3(5)], 148.8 (C, C1).

Anal. calcd for $C_{13}H_{18}Cl_4N_2$: C 45.38, H 5.27, N 8.14. Found: C 45.37, H 5.34, N 7.86.

HRMS: Calcd for $[C_{13}H_{17}Cl_3N_2+H]^+$: 307.0530, found: 307.0527.

198, synthesis of 4-bromo-3,5-dichloro-*N*-(2-(piperidin-1-yl)ethyl)aniline hydrochloride
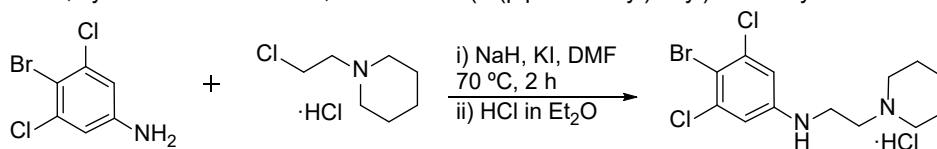
To a suspension of 4-bromo-3,5-dichloroaniline (100 mg, 0.42 mmol) in DMF (2.0 mL) was added NaH, 60% dispersion in mineral oil (66 mg, 1.66) and the mixture was stirred at room temperature for 20 min. Then, KI (21 mg, 0.13 mmol) followed by 1-(2-chloroethyl)piperidine hydrochloride (99 mg, 0.54 mmol) were added and the mixture was stirred at 70 ºC for 2 h.

EtOAc (8 mL) was added and the mixture was washed with Na_2CO_3 sat. (2 x 20 mL). The organic layer was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 1.5%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et_2O (1.0 mL) was added followed by HCl 2 M in Et_2O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford a beige solid (22 mg, 14% yield).
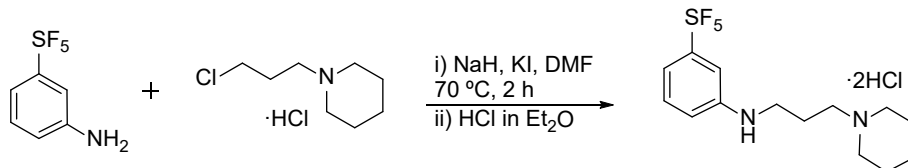
Mp: 215-216 ºC

IR (ATR) *v*: 3267, 2939, 2632, 2547, 2410, 2031, 1592, 1512, 1438, 1314, 1250, 1146, 973, 847, 809, 680, 609 cm^{-1}.

¹H NMR (400 MHz, CD₃OD) δ: 1.54 (m, 1H, 4''-H$_{ax}$), 1.77-1.91 [complex signal, 3H, 3''(5'')-H$_{ax}$, 4''-H$_{eq}$], 1.95 [m, 2H, 3''(5'')-H$_{eq}$], 3.01 [td, *J* = 12.5 Hz, *J'* = 3.5 Hz, 2H, 2''(6'')-H$_{ax}$], 3.30 (m, 2H, 2'-H), 3.54 (t, *J* = 6.3 Hz, 2H, 1'-H), 3.58 [d, *J* = 12.8 Hz, 2H, 2''(6'')-H$_{eq}$], 6.84 [s, 2H, 2(6)-H].

¹³C NMR (101 MHz, CD₃OD) δ: 22.6 (CH₂, C4''), 24.1 [CH₂, C3''(5'')], 38.9 (CH₂, C1'), 54.7 [CH₂, C2''(6'')], 56.4 (CH₂, C2'), 109.6 (C, C4), 113.9 [CH, C2(6)], 137.5 [C, C3(5)], 149.4 (C, C1).

Anal. calcd for C₁₃H₁₈BrCl₃N₂: C 40.19, H 4.67, N 7.21. Found: C 40.26, H 4.79, N 7.12.

HRMS: Calcd for [C₁₃H₁₇BrCl₂N₂+H]⁺: 351.0025, found: 351.0018.

199, synthesis of 2,3,5-trichloro-*N*-(2-(piperidin-1-yl)ethyl)aniline hydrochloride
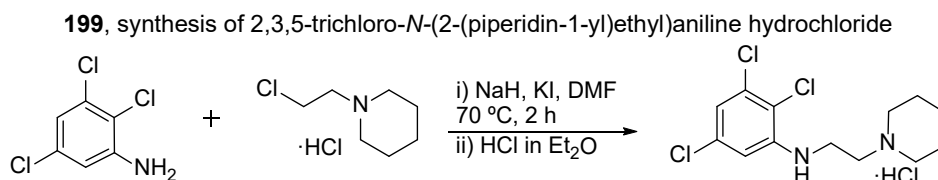
To a suspension of 2,3,5-trichloroaniline (75 mg, 0.38 mmol) in DMF (2.0 mL) was added NaH, 60% dispersion in mineral oil (61 mg, 1.53) and the mixture was stirred at room temperature for 20 min. Then, KI (21 mg, 0.13 mmol) followed by 1-(2-chloroethyl)piperidine hydrochloride (92 mg, 0.50 mmol) were added and the mixture was stirred at 70 ºC for 2 h.

EtOAc (8 mL) was added and the mixture was washed with Na₂CO₃ sat. (2 x 20 mL). The organic layer was dried over Na₂SO₄ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 1.5%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et₂O (1.0 mL) was added followed by HCl 2 M in Et₂O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford a white solid (68 mg, 52% yield).

Mp: 220-221 ºC

IR (ATR) *v*: 3418, 2952, 2484, 1938, 1905, 1576, 1463, 1418, 1279, 1109, 1041, 963, 846, 812, 650, 578, 557 cm⁻¹.

¹H NMR (400 MHz, CD₃OD) δ: 1.54 (m, 1H, 4''-H$_{ax}$), 1.80-1.91 [complex signal, 3H, 3''(5'')-H$_{ax}$, 4''-H$_{eq}$], 1.96 [m, 2H, 3''(5'')-H$_{eq}$], 3.03 [td, *J* = 12.3 Hz, *J'* = 3.3 Hz, 2H, 2''(6'')-H$_{ax}$], 3.34 (t, *J* = 6.4 Hz, 2H, 2'-H), 3.62 [d, *J* = 11.2 Hz, 2H, 2''(6'')-H$_{eq}$], 3.69 (t, *J* = 6.4 Hz, 2H, 1'-H), 6.82 (d, *J* = 2.2 Hz, 1H, 6-H), 6.88 (m, 1H, 4-H).

¹³C NMR (101 MHz, CD₃OD) δ: 22.6 (CH₂, C4''), 24.1 [CH₂, C3''(5'')], 39.0 (CH₂, C1'), 54.7 [CH₂, C2''(6'')], 56.2 (CH₂, C2'), 110.4 (CH, C6), 117.3 (C, C2), 118.8 (CH, C4), 134.7 (C, C5), 134.9 (C, C3), 146.9 (C, C1).

Anal. calcd for C₁₃H₁₈Cl₄N₂: C 45.38, H 5.27, N 8.14. Found: C 45.52, H 5.22, N 7.93.

HRMS: Calcd for [C₁₃H₁₇Cl₃N₂+H]⁺: 307.0530, found: 307.0525.

200, synthesis of 3,5-dichloro-2-methoxy-*N*-(2-(piperidin-1-yl)ethyl)aniline hydrochloride
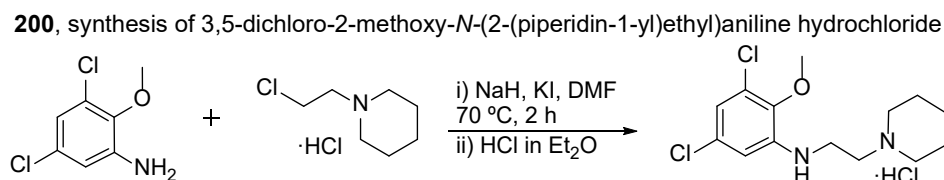
To a suspension of 3,5-dichloro-2-methoxyaniline (75 mg, 0.39 mmol) in DMF (2.0 mL) was added NaH, 60% dispersion in mineral oil (47 mg, 1.17) and the mixture was stirred at room temperature for 20 min. Then, KI (21 mg, 0.13 mmol) followed by 1-(2-chloroethyl)piperidine hydrochloride (79 mg, 0.43 mmol) were added and the mixture was stirred at 70 ºC for 2 h.

EtOAc (8 mL) was added and the mixture was washed with Na$_2$CO$_3$ sat. (2 x 20 mL). The organic layer was dried over Na$_2$SO$_4$ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 1.2%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et$_2$O (1.0 mL) was added followed by HCl 2 M in Et$_2$O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford a beige solid (22 mg, 17% yield).

Mp: 178-179 ºC

IR (ATR) *v*: 3412, 3272, 2953, 2618, 2495, 1979, 1584, 1503, 1445, 1423, 1406, 1302, 1272, 1219, 1170, 1087, 986, 969, 879, 838, 820, 760, 721, 593, 579, 559 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.55 (m, 1H, 4''-H$_{ax}$), 1.77-1.91 [complex signal, 3H, 3''(5'')-H$_{ax}$, 4''-H$_{eq}$], 1.97 [m, 2H, 3''(5'')-H$_{eq}$], 3.03 [td, *J* = 12.4 Hz, *J'* = 3.3 Hz, 2H, 2''(6'')-H$_{ax}$], 3.33 (m, 2H, 2H, 2'-H), 3.57-3.65 (complex signal, 4H, 1'-H, 2''(6'')-H$_{eq}$), 3.80 (s, 3H, C$\underline{H}_3$), 6.68-6.72 (complex signal, 2H, 2-H, 4-H).

^{13}C NMR (101 MHz, CD$_3$OD) δ: 22.6 (CH$_2$, C4''), 24.2 [CH$_2$, C3''(5'')], 38.9 (CH$_2$, C1'), 54.7 [CH$_2$, C2''(6'')], 56.5 (CH$_2$, C2'), 60.5 (CH$_3$, $\underline{C}$H$_3$), 110.5 (C, C6), 118.2 (C, C4), 129.0 (C, C5), 131.5 (C, C3), 143.4 (C, C1), 144.3 (C, C2).

Anal. calcd for C$_{14}$H$_{21}$Cl$_3$N$_2$O: C 49.50, H 6.23, N 8.25. Found: C 49.18, H 6.19, N 7.99.

HRMS: Calcd for [C$_{14}$H$_{21}$Cl$_2$N$_2$O]$^+$: 303.1025, found: 303.1028.

201, synthesis of 4-methyl-*N*-(2-(piperidin-1-yl)ethyl)-3-(trifluoromethyl)aniline dihydrochloride
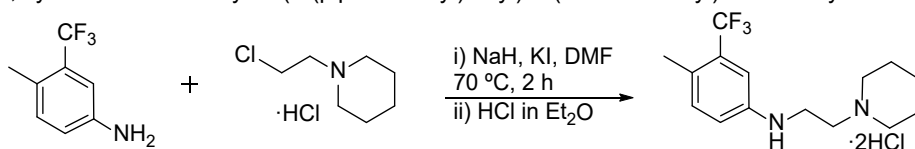
To a suspension of 4-methyl-3-(trifluoromethyl)aniline (100 mg, 0.57 mmol) in DMF (2.0 mL) was added NaH, 60% dispersion in mineral oil (69 mg, 1.71) and the mixture was stirred at room temperature for 20 min. Then, KI (21 mg, 0.13 mmol) followed by 1-(2-chloroethyl)piperidine hydrochloride (116 mg, 0.63 mmol) were added and the mixture was stirred at 70 ºC for 2 h.

EtOAc (8 mL) was added and the mixture was washed with Na$_2$CO$_3$ sat. (2 x 20 mL). The organic layer was dried over Na$_2$SO$_4$ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 3%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et$_2$O (1.0 mL) was added followed by HCl 2 M in Et$_2$O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford a beige solid (23 mg, 13% yield).
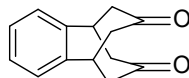
Mp: 205-206 ºC

IR (ATR) *v*: 3263, 2942, 2632, 2550, 2286, 1624, 1504, 1458, 1431, 1318, 1269, 1174, 1137, 1110, 1048, 974, 869, 834, 670 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.54 (m, 1H, 4''-H$_{ax}$), 1.85 (m, 1H, 4''-H$_{eq}$), 1.85-2.00 (complex signal, 4H, 3''(5'')-H$_2$), 2.43 (q, *J* = 1.8 Hz, 3H, C$\underline{H}_3$), 3.05 [td, *J* = 12.0 Hz, *J'* = 4.2 Hz, 2H, 2''(6'')-H$_{ax}$], 3.46 (t, *J* = 6.4 Hz, 2H, 2'-H), 3.64 [d, *J* = 11.9 Hz, 2H, 2''(6'')-H$_{eq}$], 3.77 (t, *J* = 6.4 Hz, 2H, 1'-H), 7.32 (dd, *J* = 8.4 Hz, *J'* = 2.5 Hz, 1H, 6-H), 7.38 (d, *J* = 8.3 Hz, 1H, 5-H), 7.45 (d, *J* = 2.4 Hz, 1H, 2-H).

^{13}C NMR (101 MHz, CD$_3$OD) δ: 18.7 (q, $^4J_{CF}$ = 2.1 Hz, CH$_3$, $\underline{C}$H$_3$), 22.6 (CH$_2$, C4''), 24.1 [CH$_2$, C3''(5'')], 42.9 (CH$_2$, C1'), 54.9 [CH$_2$, C2''(6'')], 55.3 (CH$_2$, C2'), 116.3 (q, $^3J_{CF}$ = 5.9 Hz, CH, C2), 121.9 (CH, C6), 125.6 (q, $^1J_{CF}$ = 273.1 Hz, C, $\underline{C}$F$_3$), 130.9 (q, $^2J_{CF}$ = 30.1 Hz, C, C3), 132.7 (C, C4), 134.7 (CH, C5), 141.2 (C, C1).

Anal. calcd for C$_{15}$H$_{23}$Cl$_2$F$_3$N$_2$: C 50.15, H 6.45, N 7.80. Found: C 50.47, H 6.38, N 7.65.

HRMS: Calcd for [C$_{15}$H$_{21}$F$_3$N$_2$+H]$^+$: 287.1730, found: 287.1720.

202, synthesis of 4-fluoro-*N*-(2-(piperidin-1-yl)ethyl)-3-(trifluoromethyl)aniline dihydrochloride
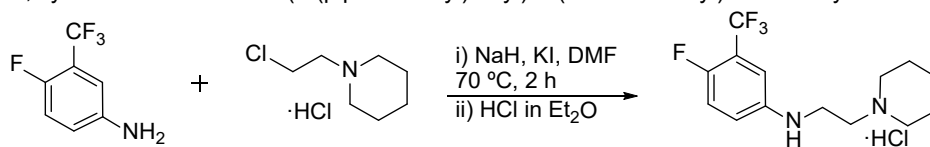
To a suspension of 4-fluoro-3-(trifluoromethyl)aniline (100 mg, 0.56 mmol) in DMF (2.0 mL) was added NaH, 60% dispersion in mineral oil (67 mg, 1.67) and the mixture was stirred at room temperature for 20 min. Then, KI (21 mg, 0.13 mmol) followed by 1-(2-chloroethyl)piperidine hydrochloride (113 mg, 0.61 mmol) were added and the mixture was stirred at 70 ºC for 2 h.

EtOAc (8 mL) was added and the mixture was washed with Na_2CO_3 sat. (2 x 20 mL). The organic layer was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 2%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et_2O (1.0 mL) was added followed by HCl 2 M in Et_2O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford a beige solid (16 mg, 9% yield).

Mp: 177-178 ºC

IR (ATR) *v*: 3261, 2934, 2636, 1545, 1506, 1432, 1329, 1292, 1236, 1161, 1122, 1049, 971, 871, 829, 730, 665, 579 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.56 (m, 1H, 4''-H$_{ax}$), 1.82-1.93 [complex signal, 3H, 3''(5'')-H$_{ax}$, 4''-H$_{eq}$], 1.97 [m, 2H, 3''(5'')-H$_{eq}$], 3.05 [td, *J* = 12.1 Hz, *J'* = 3.6 Hz, 2H, 2''(6'')-H$_{ax}$], 3.39 (t, *J* = 6.2 Hz, 2H, 2'-H), 3.59-3.66 [complex signal, 4H, 1'-H, 2''(6'')-H$_{eq}$], 7.09 (d, *J* = 4.7 Hz, 1H, 2-H), 7.11 (m, 1H, 6-H), 7.20 (m, 1H, 5-H).

^{13}C NMR (101 MHz, CD$_3$OD) δ: 22.6 (CH$_2$, C4''), 24.1 [CH$_2$, C3''(5'')], 40.5 (CH$_2$, C1'), 54.7 [CH$_2$, C2''(6'')], 56.3 (CH$_2$, C2'), 112.9 (d, $^3J_{CF}$ = 5.0 Hz, CH, C2), 118.9 (q, $^2J_{CF}$ = 22.0 Hz, CH, C5), 119.5 (dd, $^2J_{CF}$ = 32.6 Hz, $^2J_{CF}$ = 13.5 Hz, C, C3), 120.1 (d, $^3J_{CF}$ = 7.7 Hz, CH, C6), 124.2 (q, $^1J_{CF}$ = 271.1 Hz, C, CF$_3$), 144.2 (d, $^4J_{CF}$ = 2.3 Hz, C, C1), 154.4 (d, $^1J_{CF}$ = 245.5 Hz, C, C4).

Anal. calcd for C$_{14}$H$_{19}$ClF$_4$N$_2$: C 46.30, H 5.55, N 7.71. Anal. calcd for C$_{14}$H$_{19}$ClF$_4$N$_2$ · 1.5 H$_2$O: C 47.77, H 6.24, N 7.96. Found: C 47.21, H 5.64, N 7.70.

HRMS: Calcd for [C$_{14}$H$_{18}$F$_4$N$_2$+H]$^+$: 291.1479, found: 291.1468.

203, synthesis of 2-chloro-*N*-(2-(piperidin-1-yl)ethyl)-3,5-bis(trifluoromethyl)aniline hydrochloride
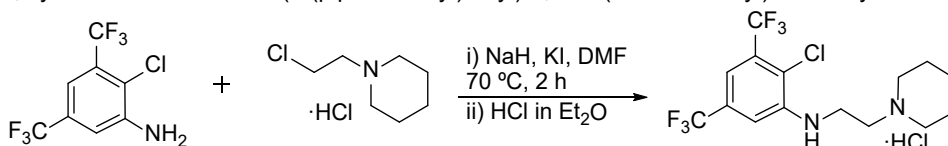
To a suspension of 2-chloro-3,5-bis(trifluoromethyl)aniline (100 mg, 0.38 mmol) in DMF (2.0 mL) was added NaH, 60% dispersion in mineral oil (46 mg, 1.14) and the mixture was stirred at room temperature for 20 min. Then, KI (21 mg, 0.13 mmol) followed by 1-(2-chloroethyl)piperidine hydrochloride (77 mg, 0.42 mmol) were added and the mixture was stirred at 70 ºC for 2 h.

EtOAc (8 mL) was added and the mixture was washed with Na_2CO_3 sat. (2 x 20 mL). The organic layer was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 15%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et_2O (1.0 mL) was added followed by HCl 2 M in Et_2O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford a white solid (60 mg, 38% yield).

Mp: 218-219 ºC

IR (ATR) v: 3364, 2957, 2453, 2403, 1900, 1600, 1517, 1485, 1450, 1379, 1280, 1202, 1167, 1118, 1038, 860, 728, 704, 627 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.56 (m, 1H, 4''-H$_{ax}$), 1.78-1.91 [complex signal, 3H, 3''(5'')-H$_{ax}$, 4''-H$_{eq}$], 1.95 [s, 2H, 3''(5'')-H$_{eq}$], 3.05 [m, 2H, 2''(6'')-H$_{ax}$], 3.39 (t, J = 6.4 Hz, 2H, 2'-H), 3.65 [m, 2H, 2''(6'')-H$_{eq}$], 3.79 (t, J = 6.4 Hz, 2H, 1'-H), 7.30 (s, 1H, 4-H), 7.32 (s, 1H, 6-H).

^{13}C NMR (101 MHz, CD$_3$OD) δ: 22.6 (CH$_2$, C4''), 24.2 [CH$_2$, C3''(5'')], 38.9 (CH$_2$, C1'), 54.8 [CH$_2$, C2''(6'')], 56.2 (CH$_2$, C2'), 111.8 (d, $^3J_{CF}$ = 3.8 Hz, CH, C6), 112.4 (m, CH, C4), 121.4 (C, C2), 124.0 (q, $^1J_{CF}$ = 272.8 Hz, C, C3-CF$_3$), 124.9 (q, $^1J_{CF}$ = 272.0 Hz, C, C5-CF$_3$), 130.8 (q, $^2J_{CF}$ = 31.4 Hz, C, C3), 131.4 (q, $^2J_{CF}$ = 33.1 Hz, C, C5), 147.1 (C, C1).

Anal. calcd for C$_{15}$H$_{18}$Cl$_2$F$_6$N$_2$: C 43.81, H 4.41, N 6.81. Found: C 43.87, H 4.44, N 6.60.

HRMS: Calcd for [C$_{15}$H$_{17}$ClF$_6$N$_2$+H]$^+$: 375.1057, found: 375.1043.

209, synthesis of 1-(2-(3-chloro-5-(trifluoromethyl)phenoxy)ethyl)piperidine hydrochloride
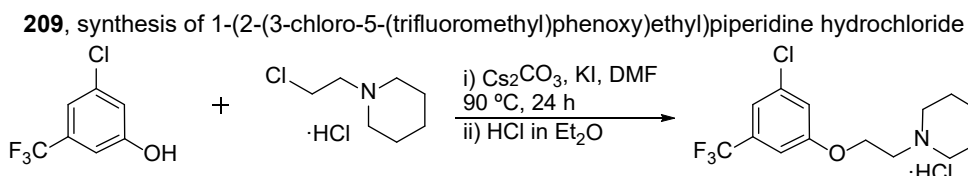
To a suspension of 3-chloro-5-(trifluoromethyl)phenol (100 mg, 0.51 mmol), Cs$_2$CO$_3$ (497 mg, 1.53 mmol) and KI (13 mg, 0.08 mmol) in DMF (2.0 mL) was added 1-(2-chloroethyl)piperidine hydrochloride (113 mg, 0.61 mmol) and the mixture was stirred at 90 ºC for 24 h.

After cooling down to room temperature, EtOAc (8 mL) was added and the mixture was washed with Na$_2$CO$_3$ sat. (2 x 20 mL). The organic layer was dried over Na$_2$SO$_4$ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 20%). Fractions containing the desired product were collected and concentrated *in vacuo*.

HCl 2 M in Et$_2$O (2.0 mL) was added to form its hydrochloride. After 20 min stirring the solvent was concentrated *in vacuo* to afford a white solid (132 mg, 75% yield).

Mp: 172-173 ºC

IR (ATR) v: 2940, 2471, 1590, 1453, 1328, 1307, 1270, 1229, 1173, 1113, 1098, 1019, 1002, 973, 908, 864, 850, 835, 702 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.58 (s, 1H, 4-H$_{ax}$), 1.75-195 [complex signal, 3H, 3(5)-H$_{ax}$, 4-H$_{eq}$], 1.94 [s, 2H, 3(5)-H$_{eq}$], 3.10 [s, 2H, 2(6)-H$_{ax}$], 3.60 (t, J = 4.8 Hz, 2H, 1'-H), 3.62 [s, 2H, 2(6)-H$_{eq}$], 4.48 (t, J = 4.9 Hz, 2H, 2'-H), 7.28 (ddd, J = 2.3 Hz, J' = 1.5 Hz, J'' = 0.7 Hz, 1H, 6''-H), 7.35 (td, J = 1.6 Hz, J' = 0.7 Hz, 1H, 4''-H), 7.37 (t, J = 2.1 Hz, 1H, 2''-H).

^{13}C NMR (101 MHz, CD$_3$OD) δ: 22.5 (CH$_2$, C4), 24.1 [CH$_2$, C3(5)], 54.9 [CH$_2$, C2(6)], 56.9 (CH$_2$, C1'), 63.9 (CH$_2$, C2'), 111.2 (q, $^3J_{CF}$ = 3.9 Hz, CH, C6''), 119.6 (q, $^3J_{CF}$ = 3.9 Hz, CH, C4''), 119.7 (CH, C2''), 124.6 (q, $^1J_{CF}$ = 282.1 Hz, C, CF$_3$), 134.0 (q, $^2J_{CF}$ = 33.1 Hz, C, C5''), 137.2 (C, C3''), 160.3 0 (C, C1'').

Anal. calcd for C$_{14}$H$_{18}$Cl$_2$F$_3$NO: C 48.85, H 5.27, N 4.07. Found: C 49.14, H 5.26, N 3.90.

HRMS: Calcd for [C$_{14}$H$_{17}$ClF$_3$NO+H]$^+$: 308.1032, found: 308.1024.

210, synthesis of 1-(2-(3-(pentafluoro-λ^6-sulfanyl)phenoxy)ethyl)piperidine hydrochloride
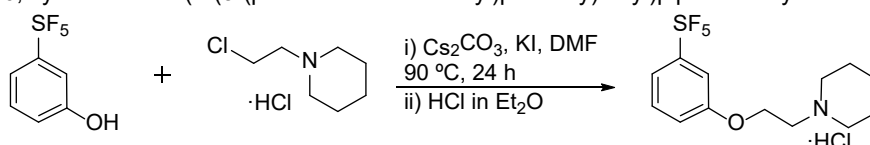
To a suspension of 3-(pentafluorothio)phenol (100 mg, 0.45 mmol), Cs_2CO_3 (440 mg, 1.35 mmol) and KI (12 mg, 0.07 mmol) in DMF (2.0 mL) was added 1-(2-chloroethyl)piperidine hydrochloride (99 mg, 0.54 mmol) and the mixture was stirred at 90 ºC for 24 h.

After cooling down to room temperature, EtOAc (8 mL) was added and the mixture was washed with Na_2CO_3 sat. (2 x 20 mL). The organic layer was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 30%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et_2O (1.0 mL) was added followed by HCl 2 M in Et_2O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford a white solid (70 mg, 42% yield).

Mp: 170-171 ºC

IR (ATR) v: 2948, 2614, 2514, 1609, 1493, 1455, 1434, 1409, 1326, 1244, 1172, 1113, 1070, 1017, 1002, 964, 904, 832, 791, 777, 682, 640, 593, 575 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.57 (m, 1H, 4-H$_{ax}$), 1.78-1.92 [complex signal, 3H, 3(5)-H$_{ax}$, 4-H$_{eq}$], 1.96 [s, 2H, 3(5)-H$_{eq}$], 3.11 [t, J = 11.6 Hz, 2H, 2(6)-H$_{ax}$], 3.62 (t, J = 4.9 Hz, 2H, 1'-H), 3.65 [s, 2H, 2(6)-H$_{eq}$], 4.47 (t, J = 5.0 Hz, 2H, 2'-H), 7.30 (dd, J = 8.1 Hz, J' = 1.8 Hz, 1H, 6''-H), 7.45-7.50 (complex signal, 2H, 2''-H, 4''-H), 7.54 (m, 1H, 5''-H).

^{13}C NMR (101 MHz, CD$_3$OD) δ: 22.5 (CH$_2$, C4), 24.1 [CH$_2$, C3(5)], 54.9 [CH$_2$, C2(6)], 57.0 (CH$_2$, C1'), 63.8 (CH$_2$, C2'), 114.4 (p, $^3J_{CF}$ = 4.8 Hz, CH, C2''), 119.0 (CH, C6''), 120.3 (p, $^3J_{CF}$ = 4.8 Hz, CH, C4''), 131.3 (CH, C5''), 155.7 (p, $^2J_{CF}$ = 17.2 Hz, C, C3''), 159.0 (C, C1'').

Anal. calcd for C$_{13}$H$_{19}$ClF$_5$NOS: C 42.45, H 5.21, N 3.81. Found: C 42.57, H 5.24, N 3.69.

HRMS: Calcd for [C$_{13}$H$_{18}$F$_5$NOS+H]$^+$: 332.1100, found: 332.1102.

211, synthesis of 1-(2-(3,4,5-trichlorophenoxy)ethyl)piperidine hydrochloride
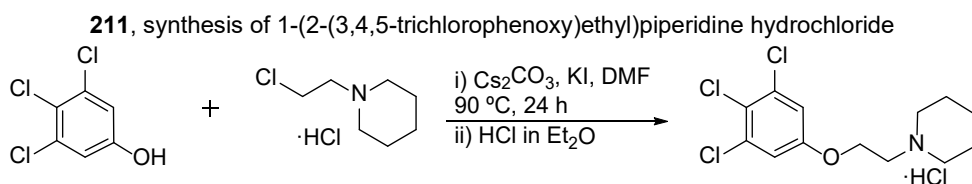
To a suspension of 3,4,5-trichlorophenol (75 mg, 0.38 mmol), Cs_2CO_3 (371 mg, 1.14 mmol) and KI (13 mg, 0.08 mmol) in DMF (2.0 mL) was added 1-(2-chloroethyl)piperidine hydrochloride (83 mg, 0.46 mmol) and the mixture was stirred at 90 ºC for 24 h.

After cooling down to room temperature, EtOAc (8 mL) was added and the mixture was washed with Na_2CO_3 sat. (2 x 20 mL). The organic layer was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 25%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et_2O (1.0 mL) was added followed by HCl 2 M in Et_2O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford a white solid (89 mg, 68% yield).

Mp: 198-199 ºC

IR (ATR) v: 3293, 2941, 1644, 1585, 1557, 1440, 1398, 1282, 1246, 1143, 1052, 982, 851, 825, 805, 677, 573 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.55 (m, 1H, 4-H$_{ax}$), 1.80-1.92 [complex signal, 3H, 3(5)-H$_{ax}$, 4-H$_{eq}$], 1.98 [m, 2H, 3(5)-H$_{eq}$], 3.09 [td, J = 12.3 Hz, J' = 3.4 Hz, 2H, 2(6)-H$_{ax}$], 3.59 (t, J = 4.9 Hz, 2H, 1'-H), 3.62 [d, J = 12.9 Hz, 2H, 2(6)-H$_{eq}$], 4.43 (t, J = 4.9 Hz, 2H, 2'-H), 7.25 [s, 2H, 2''(6'')-H].

^{13}C NMR (101 MHz, CD$_3$OD) δ: 22.5 (CH$_2$, C4), 24.1 [CH$_2$, C3(5)], 54.9 [CH$_2$, C2(6)], 56.8 (CH$_2$, C1'), 64.1 (CH$_2$, C2'), 117.0 [CH, C2''(6'')], 124.8 (C, C4''), 135.5 [C, C3''(5'')], 157.8 (C, C1'').

Anal. calcd for $C_{13}H_{17}Cl_4NO$: C 45.25, H 4.97, N 4.06. Found: C 45.23, H 4.89, N 3.84.
HRMS: Calcd for $[C_{13}H_{16}Cl_3NO+H]^+$: 308.0370, found: 308.0364.

212, synthesis of 2,6-dichloro-4-(2-(piperidin-1-yl)ethoxy)aniline dihydrochloride
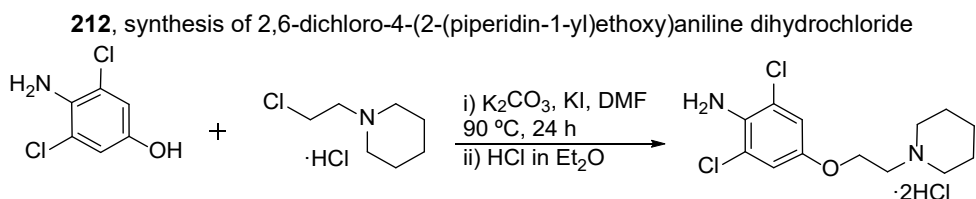
To a suspension of 4-amino-3,5-dichlorophenol (100 mg, 0.56 mmol), K_2CO_3 (85 mg, 0.62 mmol) and KI (13 mg, 0.08 mmol) in anh. DMF (2.0 mL) was added 1-(2-chloroethyl)piperidine hydrochloride (113 mg, 0.62 mmol) and the mixture was stirred at 90 ºC for 24 h.
After cooling down to room temperature, EtOAc (8 mL) was added and the mixture was washed with Na_2CO_3 sat. (2 x 20 mL). The organic layer was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 3%). Fractions containing the desired product were collected and concentrated *in vacuo*.
Et_2O (2.0 mL) was added to the resulting crude followed by HCl 2 M in Et_2O (2.0 mL) to form its hydrochloride. After 10 min stirring solvents were concentrated *in vacuo* to afford a reddish solid (66 mg, 36% yield).
Mp: 247-248 ºC
IR (ATR) *v*: 3352, 3196, 2952, 2641, 2542, 2048, 1594, 1562, 1479, 1405, 1225, 1199, 1056, 984, 949, 869, 845, 800, 618, 587 cm⁻¹.
¹H NMR (400 MHz, CD₃OD) δ: 1.58 (m, 1H, 4''-H$_{ax}$), 1.82-1.95 [complex signal, 3H, 3''(5'')-H$_{ax}$, 4''-H$_{eq}$], 2.01 [m, 2H, 3''(5'')-H$_{eq}$], 3.15 [td, *J* = 12.4 Hz, *J'* = 3.3 Hz, 2H, 2''(6'')-H$_{ax}$], 3.65 (t, *J* = 4.9 Hz, 2H, 2'-H), 3.78 [d, *J* = 12.3 Hz, 2H, 2''(6'')-H$_{eq}$], 4.40 (t, *J* = 4.8 Hz, 2H, 1'-H), 7.30 [s, 2H, 3(5)-H].
¹³C NMR (101 MHz, CD₃OD) δ: 22.6 (CH₂, C4''), 24.0 [CH₂, C3''(5'')], 54.8 [CH₂, C2''(6'')], 57.5 (CH₂, C2'), 68.0 (CH₂, C1'), 122.4 [CH, C3(5)], 131.1 [CH, C2(6)], 135.6 (CH, C1), 148.8 (C, C4).
Anal. calcd for $C_{13}H_{20}Cl_4N_2O$: C 43.12, H 5.57, N 7.74. Found: C 42.71, H 5.66, N 7.38.
HRMS: Calcd for $[C_{13}H_{18}Cl_2N_2O+H]^+$: 289.0869, found: 289.0860.

213, synthesis of 1-(2-(2,3,5-trichlorophenoxy)ethyl)piperidine hydrochloride
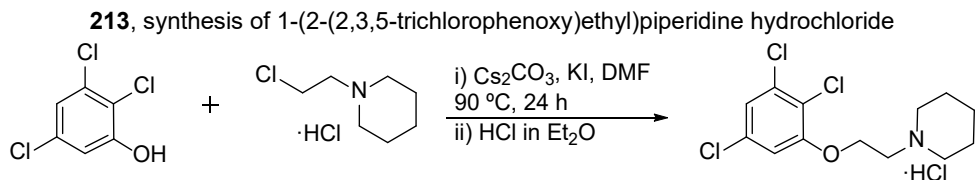
To a suspension of 2,3,5-trichlorophenol (50 mg, 0.25 mmol), Cs_2CO_3 (249 mg, 0.77 mmol) and KI (10 mg, 0.06 mmol) in DMF (1.0 mL) was added 1-(2-chloroethyl)piperidine hydrochloride (69 mg, 0.38 mmol) and the mixture was stirred at 90 ºC for 24 h.
After cooling down to room temperature, EtOAc (8 mL) was added and the mixture was washed with Na_2CO_3 sat. (2 x 20 mL). The organic layer was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 30%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et$_2$O (1.0 mL) was added followed by HCl 2 M in Et$_2$O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford a white solid (54 mg, 63% yield).

Mp: 241-242 ºC

IR (ATR) v: 2947, 2319, 1847, 1820, 1568, 1447, 1427, 1409, 1282, 1120, 1070, 1041, 981, 922, 854, 817, 668, 587, 561 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.56 (m, 1H, 4-H$_{ax}$), 1.80-1.94 [complex signal, 3H, 3(5)-H$_{ax}$, 4-H$_{eq}$], 1.98 [m, 2H, 3(5)-H$_{eq}$], 3.18 [td, J = 12.3 Hz, J' = 3.3 Hz, 2H, 2(6)-H$_{ax}$], 3.65 (t, J = 4.9 Hz, 2H, 1'-H), 3.72 [d, J = 12.8 Hz, 2H, 2(6)-H$_{eq}$], 4.53 (t, J = 4.9 Hz, 2H, 2'-H), 7.23 (d, J = 2.2 Hz, 1H, 6''-H), 7.29 (d, J = 2.2 Hz, 1H, 4''-H).

^{13}C NMR (101 MHz, CD$_3$OD) δ: 22.4 (CH$_2$, C4), 24.3 [CH$_2$, C3(5)], 55.5 [CH$_2$, C2(6)], 57.0 (CH$_2$, C1'), 65.9 (CH$_2$, C2'), 114.0 (CH, C6''), 121.5 (C, C2''), 124.0 (CH, C4''), 134.6 (C, C5''), 135.3 (C, C3''), 156.3 (C, C1'').

Anal. calcd for C$_{13}$H$_{17}$Cl$_4$NO: C 45.25, H 4.97, N 4.06. Found: C 45.55, H 5.20, N 3.88.

HRMS: Calcd for [C$_{13}$H$_{16}$Cl$_3$NO+H]$^+$: 308.0370, found: 308.0365.

216, synthesis of 1-(3-chloropropyl)azepane hydrochloride
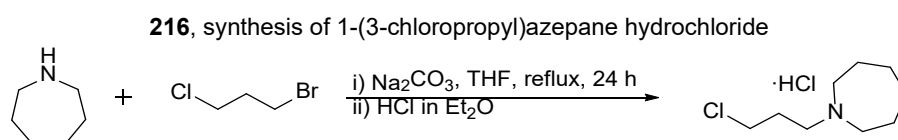
To a solution of 1-bromo-3-chloropropane (997 µL, 1.59 g, 10.08 mmol) in THF (12.0 mL) was added Na$_2$CO$_3$ (1.07 g, 10.08 mmol) and azepane (578 µL, 500 mg, 5.04 mmol). Then, the mixture was refluxed for 24 h. After that time the mixture was filtered and the solvent was concentrated *in vacuo*. The resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 4%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et$_2$O (5.0 mL) was added followed by HCl 2 M in Et$_2$O (5.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford a brownish solid (325 mg, 30% yield).

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.70-1.78 (complex signal, 4H), 1.84 (s, 2H), 2.02 (m, 1H), 2.24 (m, 1H), 2.60 (m, 1H), 3.23 (m, 1H), 3.31 (m, 2H), 3.48 (m, 1H), 3.63 (t, J = 5.2 Hz, 2H), 3.69 (t, J = 6.2 Hz, 1H), 4.28 (t, J = 8.3 Hz, 2H).

224, synthesis of *N*-(3-(azepan-1-yl)propyl)-3,5-dichloroaniline dihydrochloride
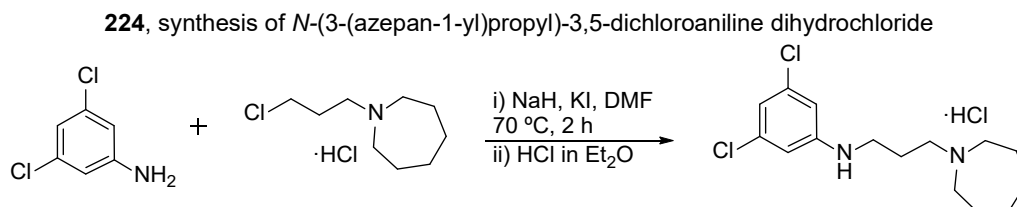
To a suspension of 3,5-dichloroaniline (92 mg, 0.57 mmol) in DMF (2.0 mL) was added NaH, 60% dispersion in mineral oil (90 mg, 2.26) and the mixture was stirred at room temperature for 20 min. Then, KI (21 mg, 0.13 mmol) followed by 1-(3-chloropropyl)azepane hydrochloride (80 mg, 0.38 mmol) were added and the mixture was stirred at 70 ºC for 2 h.

EtOAc (8 mL) was added and the mixture was washed with Na$_2$CO$_3$ sat. (2 x 20 mL). The organic layer was dried over Na$_2$SO$_4$ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 3.5%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et$_2$O (1.0 mL) was added followed by HCl 2 M in Et$_2$O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford a brownish solid (42 mg, 33% yield).

Mp: 215-216 °C

IR (ATR) v: 3271, 2937, 2707, 2667, 2616, 2517, 2127, 1867, 1588, 1555, 1484, 1459, 1434, 1383, 1321, 1286, 1107, 1048, 1025, 985, 920, 857, 800, 674, 618, 570 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.67-1.81 [complex signal, 4H, 4''(5'')-H$_2$], 1.86-2.01 [complex signal, 4H, 3''(6'')-H$_2$], 2.16 (m, 2H, 2'-H), 3.22 [ddd, J = 13.5 Hz, J' = 8.7 Hz, J'' = 2.8 Hz, 2H, 2''(7'')-H$_{ax}$], 3.26-3.36 (complex signal, 4H, 1'-H, 3'-H), 3.50 [ddd, J = 13.5 Hz, J' = 7.4 Hz, J' = 2.9 Hz, 2H, 2''(7'')-H$_{eq}$], 6.98 (s, 1H, 4-H), 6.99 [m, 2H, 2(6)-H].

^{13}C NMR (101 MHz, CD$_3$OD) δ: 24.1 (CH$_2$, C2'), 24.8 [CH$_2$, C3''(6'')], 27.3 [CH$_2$, C4''(5'')], 44.5 (CH$_2$, C1'), 56.1 [CH$_2$, C2''(7'')], 56.2 (CH$_2$, C3'), 115.9 [CH, C2(6)], 122.0 (CH, C4), 136.8 [d, J = 10.7 Hz, C3(5)], 147.07 (t, J = 12.4 Hz, C, C1).

Anal. calcd for C$_{15}$H$_{23}$Cl$_3$N$_2$: C 48.15, H 6.47, N 7.49. Anal. calcd for C$_{15}$H$_{23}$Cl$_3$N$_2$ · 1.4 H$_2$O: C 49.64, H 7.16, N 7.72. Found: C 48.95, H 6.40, N 7.43.

HRMS: Calcd for [C$_{15}$H$_{22}$Cl$_2$N$_2$+H]$^+$: 301.1233, found: 301.1223.

225, synthesis of *N*-(2-(azepan-1-yl)ethyl)-3-chloro-5-(trifluoromethyl)aniline hydrochloride

To a suspension of 3-chloro-5-(trifluoromethyl)aniline (100 mg, 0.51 mmol) in DMF (2.0 mL) was added NaH, 60% dispersion in mineral oil (82 mg, 2.04) and the mixture was stirred at room temperature for 20 min. Then, KI (21 mg, 0.13 mmol) followed by 1-(2-chloroethyl)azepane hydrochloride (111 mg, 0.56 mmol) were added and the mixture was stirred at 70 °C for 2 h.

EtOAc (8 mL) was added and the mixture was washed with Na$_2$CO$_3$ sat. (2 x 20 mL). The organic layer was dried over Na$_2$SO$_4$ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 1.5%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et$_2$O (1.0 mL) was added followed by HCl 2 M in Et$_2$O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford a reddish solid (39 mg, 21% yield).

Mp: 127-128 °C

IR (ATR) v: 3273, 2943, 2593, 2491, 1610, 1545, 1468, 1352, 1286, 1167, 1121, 1089, 988, 857, 827, 698 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.68-1.82 [complex signal, 4H, 4''(5'')-H$_2$], 1.87-2.02 [complex signal, 4H, 3''(6'')-H$_2$], 3.27 [m, 2H, 2''(7'')-H$_{ax}$], 3.40 (t, J = 6.3 Hz, 2H, 2'-H), 3.54 [ddd, J = 13.6 Hz, J' = 7.1 Hz, J'' = 3.2 Hz, 2H, 2''(7'')-H$_{eq}$], 3.60 (t, J = 6.3 Hz, 2H, 1'-H), 6.89 (m, 1H, 6-H), 6.90 (m, 1H, 4-H), 6.95 (t, J = 2.0 Hz, 1H, 2-H).

^{13}C NMR (101 MHz, CD$_3$OD) δ: 24.5 [CH$_2$, C3''(6'')], 27.6 [CH$_2$, C4''(5'')], 39.3 (CH$_2$, C1'), 56.2 [CH$_2$, C2''(7'')], 56.7 (CH$_2$, C2'), 109.0 (q, $^3J_{CF}$ = 3.9 Hz, CH, C6), 114.4 (q, $^3J_{CF}$ = 4.0 Hz, CH, C4), 116.2 (CH, C2), 125.0 (q, $^1J_{CF}$ = 271.9 Hz, C, CF$_3$), 133.9 (q, $^2J_{CF}$ = 32.4 Hz, C, C5), 137.0 (C, C3), 151.0 (C, C1).

Anal. calcd for C$_{15}$H$_{20}$Cl$_2$F$_3$N$_2$: C 50.43, H 5.93, N 7.84. Found: C 50.20, H 5.90, N 7.61.

HRMS: Calcd for [C$_{15}$H$_{19}$ClF$_3$N$_2$+H]$^+$: 321.1340, found: 321.1330.

226, synthesis of *N*-(3-(azepan-1-yl)propyl)-3-chloro-5-(trifluoromethyl)aniline dihydrochloride
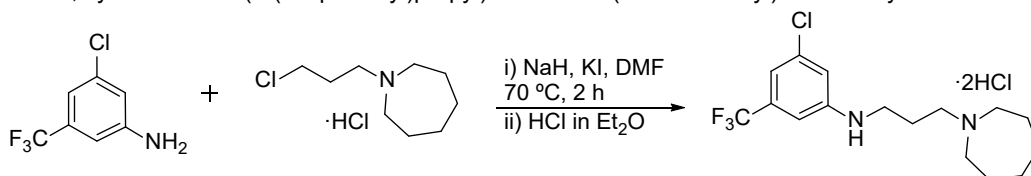
To a suspension of 3-chloro-5-(trifluoromethyl)aniline (111 mg, 0.57 mmol) in DMF (2.0 mL) was added NaH, 60% dispersion in mineral oil (90 mg, 2.26) and the mixture was stirred at room temperature for 20 min. Then, KI (21 mg, 0.13 mmol) followed by 1-(3-chloropropyl)azepane hydrochloride (80 mg, 0.38 mmol) were added and the mixture was stirred at 70 ºC for 2 h.

EtOAc (8 mL) was added and the mixture was washed with Na_2CO_3 sat. (2 x 20 mL). The organic layer was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 4%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et_2O (1.0 mL) was added followed by HCl 2 M in Et_2O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford a brownish solid (44 mg, 31% yield).

Mp: 186-187 ºC

IR (ATR) *v*: 2944, 2712, 2681, 2520, 2141, 1602, 1557, 1501, 1451, 1323, 1182, 1134, 1107, 1049, 989, 923, 881, 830, 703, 695, 645, 603 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.67-1.81 [complex signal, 4H, 4''(5'')-H$_2$], 1.86-2.01 [complex signal, 4H, 3''(6'')-H$_2$], 2.14 (m, 2H, 2'-H), 3.22 [ddd, *J* = 13.5 Hz, *J'* = 8.8 Hz, *J''* = 2.8 Hz, 2H, 2''(7'')-H$_{ax}$], 3.27-3.34 (complex signal, 4H, 1'-H, 3'-H), 3.50 [ddd, *J* = 13.5 Hz, *J'* = 7.4 Hz, *J''* = 2.9 Hz, 2H, 2''(7'')-H$_{eq}$], 7.02 (complex signal, 2H, 4-H, 6-H), 7.08 (t, *J* = 2.0 Hz, 1H, 2-H).

^{13}C NMR (101 MHz, CD$_3$OD) δ: 24.5 (CH$_2$, C2'), 24.8 [CH$_2$, C3''(6'')], 27.3 [CH$_2$, C4''(5'')], 42.9 (CH$_2$, C1'), 56.1 [CH$_2$, C2''(7'')], 56.5 (CH$_2$, C3'), 110.6 (m, CH, C6), 116.1 (m, CH, C4), 118.1 (CH, C2), 124.9 (q, $^1J_{CF}$ = 272.0 Hz, C, $\underline{C}$F$_3$), 134.0 (q, $^2J_{CF}$ = 32.5 Hz, C, C5), 137.0 (C, C3), 149.2 (C, C1).

Anal. calcd for C$_{16}$H$_{24}$Cl$_3$F$_3$N$_2$: C 47.13, H 5.93, N 6.87. Found: C 47.13, H 5.77, N 6.80.

HRMS: Calcd for [C$_{16}$H$_{22}$ClF$_3$N$_2$+H]$^+$: 335.1496, found: 335.1485.

227, synthesis of 1-(2-(3-chloro-5-(trifluoromethyl)phenoxy)ethyl)azepane hydrochloride
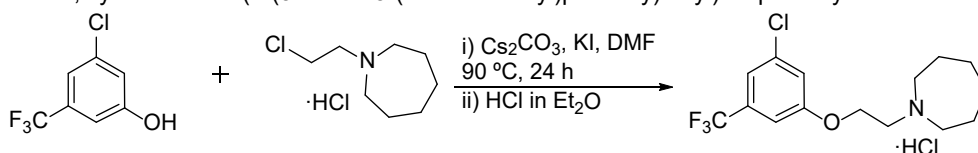
To a suspension of 3-chloro-5-(trifluoromethyl)phenol (50 mg, 0.25 mmol), Cs$_2$CO$_3$ (249 mg, 0.77 mmol) and KI (10 mg, 0.06 mmol) in DMF (1.0 mL) was added 1-(2-chloroethyl)azepane hydrochloride (76 mg, 0.38 mmol) and the mixture was stirred at 90 ºC for 24 h.

After cooling down to room temperature, EtOAc (8 mL) was added and the mixture was washed with Na$_2$CO$_3$ sat. (2 x 20 mL). The organic layer was dried over Na$_2$SO$_4$ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 25%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et$_2$O (1.0 mL) was added followed by HCl 2 M in Et$_2$O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford a white solid (44 mg, 49% yield).

Mp: 169-170 ºC

IR (ATR) v: 2934, 2509, 1965, 1587, 1452, 1330, 1165, 1129, 1091, 1051, 941, 916, 874, 862, 829, 696 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.77 [s, 4H, 4(5)-H$_2$], 1.97 [s, 4H, 3(6)-H$_2$], 3.38 [s, 2H, 2(7)-H$_{ax}$], 3.56 [s, 2H, 2(7)-H$_{eq}$], 3.67 (t, J = 4.9 Hz, 2H, 1'-H), 4.48 (t, J = 4.9 Hz, 2H, 2'-H), 7.29 (ddd, J = 2.3 Hz, J' = 1.5 Hz, J'' = 0.7 Hz, 1H, 6''-H), 7.35 (td, J = 1.6 Hz, J' = 0.7 Hz, 1H, 4''-H), 7.38 (t, J = 2.1 Hz, 1H, 2''-H).

^{13}C NMR (101 MHz, CD$_3$OD) δ: 24.3 [CH$_2$, C3(6)], 27.7 [CH$_2$, C4(5)], 56.5 [CH$_2$, C2(7)], 56.9 (CH$_2$, C1'), 64.3 (CH$_2$, C2'), 111.8 (q, $^3J_{CF}$ = 3.9 Hz, CH, C6''), 119.6 (q, $^3J_{CF}$ = 4.0 Hz, CH, C4''), 119.8 (CH, C2''), 124.6 (q, $^1J_{CF}$ = 272.0 Hz, C, $\underline{C}$F$_3$), 134.2 (q, $^2J_{CF}$ = 33.1 Hz, C, C5''), 137.2 (C, C3''), 160.3 (C, C1'').

Anal. calcd for C$_{15}$H$_{20}$Cl$_2$F$_3$NO: C 50.29, H 5.63, N 3.91. Found: C 50.22, H 5.57, N 3.80.

HRMS: Calcd for [C$_{15}$H$_{19}$ClF$_3$NO+H]$^+$: 322.1180, found: 322.1173.

228, synthesis of *N*-(2-(azepan-1-yl)ethyl)-3-(pentafluoro-λ^6-sulfanyl)aniline hydrochloride

To a suspension of 3-(pentafluoro-□^{6}-sulfanyl)aniline (100 mg, 0.46 mmol) in DMF (2.0 mL) was added NaH, 60% dispersion in mineral oil (73 mg, 1.83) and the mixture was stirred at room temperature for 20 min. Then, KI (21 mg, 0.13 mmol) followed by 1-(2-chloroethyl)azepane hydrochloride (99 mg, 0.50 mmol) were added and the mixture was stirred at 70 ºC for 2 h.

EtOAc (8 mL) was added and the mixture was washed with Na$_2$CO$_3$ sat. (2 x 20 mL). The organic layer was dried over Na$_2$SO$_4$ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 2%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et$_2$O (1.0 mL) was added followed by HCl 2 M in Et$_2$O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford a beige solid (32 mg, 18% yield).

Mp: 212-213 ºC

IR (ATR) v: 3270, 2951, 2871, 2395, 2046, 1610, 1499, 1480, 1457, 1363, 1301, 1154, 1071, 918, 826, 797, 778, 684, 643, 595, 566 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.68-1.82 [complex signal, 4H, 4''(5'')-H$_2$], 1.87-2.02 [complex signal, 4H, 3''(6'')-H$_2$], 3.27 [m, 2H, 2''(7'')-H$_{ax}$], 3.40 (t, J = 6.3 Hz, 2H, 2'-H), 3.54 [ddd, J = 13.5 Hz, J' = 7.1 Hz, J'' = 3.2 Hz, 2H, 2''(7'')-H$_{eq}$], 3.60 (t, J = 6.3 Hz, 2H, 1'-H), 6.92 (dd, J = 8.2 Hz, J' = 2.3 Hz, 1H, 6-H), 7.08 (ddd, J = 8.1 Hz, J' = 2.2 Hz, J'' = 0.8 Hz, 1H, 4-H), 7.11 (t, J = 2.2 Hz, 1H, 2-H), 7.32 (tt, J = 8.2 Hz, J' = 1.1 Hz, 1H, 5-H).

^{13}C NMR (101 MHz, CD$_3$OD) δ: 24.5 [CH$_2$, C3''(6'')], 27.6 [CH$_2$, C4''(5'')], 39.5 (CH$_2$, C1'), 56.2 [CH$_2$, C2''(7'')], 56.9 (CH$_2$, C2'), 111.6 (p, $^3J_{CF}$ = 4.8 Hz, CH, C2), 115.7 (p, $^3J_{CF}$ = 4.7 Hz, CH, C4), 116.4 (CH, C6), 130.7 (CH, C5), 149.6 (C, C1), 156.1 (p, $^2J_{CF}$ = 16.1 Hz, C, C3).

Anal. calcd for C$_{14}$H$_{22}$ClF$_5$N$_2$S: C 44.15, H 5.82, N 7.36. Found: C 44.13, H 5.74, N 7.20.

HRMS: Calcd for [C$_{14}$H$_{21}$F$_5$N$_2$S+H]$^+$: 345.1418, found: 345.1409.

229, synthesis of *N*-(3-(piperidin-1-yl)propyl)-3-(pentafluoro-*λ*⁶-sulfanyl)aniline dihydrochloride

To a suspension of 3-(pentafluoro-□⁶-sulfanyl)aniline (100 mg, 0.46 mmol) in DMF (2.0 mL) was added NaH, 60% dispersion in mineral oil (73 mg, 1.83) and the mixture was stirred at room temperature for 20 min. Then, KI (21 mg, 0.13 mmol) followed by 1-(3-chloropropyl)piperidine hydrochloride (99 mg, 0.50 mmol) were added and the mixture was stirred at 70 ºC for 2 h.

EtOAc (8 mL) was added and the mixture was washed with Na_2CO_3 sat. (2 x 20 mL). The organic layer was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 3%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et_2O (1.0 mL) was added followed by HCl 2 M in Et_2O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford a white solid (66 mg, 38% yield).

Mp: 197-198 ºC

IR (ATR) *v*: 3277, 3060, 2942, 2478, 1608, 1524, 1481, 1389, 1335, 1288, 1258, 1217, 1115, 895, 806, 688, 648, 595, 575 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.55 (m, 1H, 4''-H$_{ax}$), 1.77-1.90 [complex signal, 3H, 3''(5'')-H$_{ax}$, 4''-H$_{eq}$], 1.95 [m, 2H, 3''(5'')-H$_{eq}$], 2.22 (m, 2H, 2'-H), 2.97 [td, *J* = 12.6 Hz, *J'* = 3.4 Hz, 2H, 2''(6'')-H$_{ax}$], 3.25 (m, 2H, 3'-H), 3.44 (t, *J* = 7.2 Hz, 2H, 1'-H), 3.57 [d, *J* = 12.3 Hz, 2H, 2''(6'')-H$_{eq}$], 7.39 (dt, *J* = 7.3 Hz, *J'* = 2.1 Hz, 1H, 6-H), 7.50-7.58 (complex signal, 2H, 4-H, 5-H), 7.62 (m, 1H, 2-H).

^{13}C NMR (101 MHz, CD$_3$OD) δ: 22.7 (CH$_2$, C4''), 23.4 (CH$_2$, C2'), 24.2 [CH$_2$, C3''(5'')], 45.9 (CH$_2$, C1'), 54.5 [CH$_2$, C2''(6'')], 55.6 (CH$_2$, C3'), 116.6 (p, $^3J_{CF}$ = 4.5 Hz, CH, C2), 121.7 (p, $^3J_{CF}$ = 4.3 Hz, CH, C4), 122.2 (CH, C6), 131.6 (CH, C5), 143.8 (C, C1), 155.9 (p, $^2J_{CF}$ = 17.5 Hz, C, C3).

Anal. calcd for C$_{14}$H$_{23}$Cl$_2$F$_5$N$_2$S: C 40.30, H 5.56, N 6.71. Found: C 40.63, H 5.97, N 6.55.

HRMS: Calcd for [C$_{14}$H$_{21}$F$_5$N$_2$S+H]$^+$: 345.1418, found: 345.1413.

230, synthesis of *N*-(3-(azepan-1-yl)propyl)-3-(pentafluoro-*λ*⁶-sulfanyl)aniline dihydrochloride
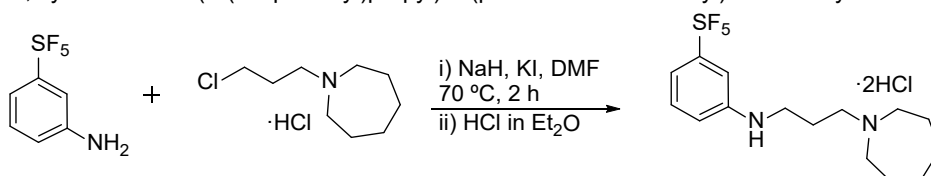
To a suspension of 3-(pentafluoro-□⁶-sulfanyl)aniline (103 mg, 0.47 mmol) in DMF (4.0 mL) was added NaH, 60% dispersion in mineral oil (38 mg, 0.94) and the mixture was stirred at room temperature for 20 min. Then, KI (21 mg, 0.13 mmol) followed by 1-(3-chloropropyl)azepane hydrochloride (50 mg, 0.24 mmol) were added and the mixture was stirred at 70 ºC for 2 h.

EtOAc (8 mL) was added and the mixture was washed with Na_2CO_3 sat. (2 x 20 mL). The organic layer was dried over Na_2SO_4 anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of MeOH in DCM from 0% to 5%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et_2O (1.0 mL) was added followed by HCl 2 M in Et_2O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford a beige solid (17 mg, 9% yield).

Mp: 187-188 ºC

IR (ATR) v: 3277, 2944, 2688, 2112, 1606, 1482, 1431, 1379, 1328, 1212, 1118, 894, 834, 803, 790, 686, 644, 596, 574 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.67-1.81 [complex signal, 4H, 4''(5'')-H$_2$], 1.83-2.04 [complex signal, 4H, 3''(6'')-H$_2$], 2.16 (m, 2H, 2'-H), 3.22 [ddd, J = 13.5 Hz, J' = 8.7 Hz, J'' = 2.7 Hz, 2H, 2''(7'')-H$_{ax}$], 3.29 (m, 2H, 3'-H), 3.38 (t, J = 7.0 Hz, 2H, 1'-H), 3.50 [ddd, J = 13.5 Hz, J' = 7.4 Hz, J'' = 2.9 Hz, 2H, 2''(7'')-H$_{eq}$], 7.22 (dd, J = 8.4 Hz, J' = 1.9 Hz, 1H, 6-H), 7.38 (ddd, J = 8.3 Hz, J' = 2.1 Hz, J'' = 0.9 Hz, 1H, 4-H), 7.43 (t, J = 2.1 Hz, 1H, 2-H), 7.47 (t, J = 8.2 Hz, 1H, 5-H).

^{13}C NMR (101 MHz, CD$_3$OD) δ: 24.2 (CH$_2$, C2'), 24.8 [CH$_2$, C3''(6'')], 27.3 [CH$_2$, C4''(5'')], 44.6 (CH$_2$, C1'), 56.1 [CH$_2$, C2''(7'')], 56.3 (CH$_2$, C3'), 114.9 (m, CH, C2), 119.7 (m, CH, C4), 120.4 (CH, C6), 131.3 (CH, C5), 145.7 (C, C1), 156.0 (p, $^2J_{CF}$ = 17.0 Hz, C, C3).

Anal. calcd for C$_{15}$H$_{25}$Cl$_2$F$_5$N$_2$S: C 41.77, H 5.84, N 6.49. Anal. calcd for C$_{15}$H$_{25}$Cl$_2$F$_5$N$_2$S · 0.2 H$_2$O: C 41.32, H 6.05, N 6.28. Found: C 41.30, H 6.00, N 6.22.

HRMS: Calcd for [C$_{15}$H$_{23}$F$_5$N$_2$S+H]$^+$: 359.1575, found: 359.1572.

231, synthesis of 1-(2-(3-(pentafluoro-λ^6-sulfanyl)phenoxy)ethyl)azepane hydrochloride
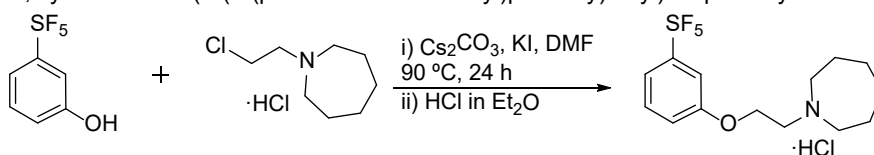
To a suspension of 3-(pentafluoro-$\square^6$-sulfanyl)phenol (50 mg, 0.23 mmol), Cs$_2$CO$_3$ (220 mg, 0.68 mmol) and KI (10 mg, 0.06 mmol) in DMF (1.0 mL) was added 1-(2-chloroethyl)azepane hydrochloride (67 mg, 0.34 mmol) and the mixture was stirred at 90 ºC for 24 h.

After cooling down to room temperature, EtOAc (8 mL) was added and the mixture was washed with Na$_2$CO$_3$ sat. (2 x 20 mL). The organic layer was dried over Na$_2$SO$_4$ anh. and filtered. Solvents were concentrated *in vacuo* and the resulting crude was purified by column chromatography in silica gel (using as eluent mixtures of EtOAc in hexane from 0% to 50%). Fractions containing the desired product were collected and concentrated *in vacuo*.

Et$_2$O (1.0 mL) was added followed by HCl 2 M in Et$_2$O (1.0 mL) to form its hydrochloride. After 10 min stirring the mixture was filtered to afford a white solid (53 mg, 60% yield).

Mp: 162-163 ºC

IR (ATR) v: 2943, 2520, 1993, 1602, 1491, 1328, 1257, 1114, 847, 828, 798, 683, 644, 597, 566 cm^{-1}.

^{1}H NMR (400 MHz, CD$_3$OD) δ: 1.77 [s, 4H, 4(5)-H$_2$], 1.96 [s, 4H, 3(6)-H$_2$], 3.37 [s, 2H, 2(7)-H$_{ax}$], 3.58 [s, 2H, 2(7)-H$_{eq}$], 3.67 (t, J = 4.9 Hz, 2H, 1'-H), 4.45 (t, J = 4.9 Hz, 2H, 2'-H), 7.30 (dd, J = 7.4 Hz, J' = 1.9 Hz, 1H, 6''-H), 7.48 (d, J = 2.3 Hz, 1H, 2''-H), 7.49 (m, 1H, 4''-H), 7.54 (m, 1H, 5''-H).

^{13}C NMR (101 MHz, CD$_3$OD) δ: 24.3 [CH$_2$, C3(6)], 27.7 [CH$_2$, C4(5)], 56.4 [CH$_2$, C2(7)], 57.0 (CH$_2$, C1'), 64.1 (CH$_2$, C2'), 114.4 (p, $^3J_{CF}$ = 4.7 Hz, CH, C2''), 119.0 (CH, C6''), 120.3 (p, $^3J_{CF}$ = 4.7 Hz, CH, C4''), 131.3 (CH, C5''), 155.8 (p, $^2J_{CF}$ = 17.3 Hz, C, C3''), 159.0 (C, C1'').

Anal. calcd for C$_{14}$H$_{21}$ClF$_5$NOS: C 44.04, H 5.54, N 3.67. Found: C 44.22, H 5.51, N 3.58.

HRMS: Calcd for [C$_{14}$H$_{20}$F$_5$NOS+H]$^+$: 346.1259, found: 346.1251.

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272

85

86

273

87

88

89

90

277

91

278

92

CIH·H₂N

112

118

119

120

121

284

122

CLH·H$_2$N

125

CLH·H$_2$N

126

132

133

134

135

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296

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153

154

¹H NMR peaks: 7.67, 7.66, 7.22, 7.21, 7.21, 7.20, 7.20, 7.20, 6.59, 6.59, 6.25, 6.24, 4.45, 4.44, 2.38, 2.38

Integrations: 2.01, 2.00, 0.95, 0.88, 2.06, 9.25

¹³C NMR peaks: 167.21, 141.97, 136.37, 133.79, 133.45, 131.64, 129.30, 127.04, 126.53, 37.40, 21.54, 15.19, 12.92, 0.11

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·2HCl

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8.11
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0.91
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2.06
0.99
0.98

f1 (ppm)

157.99
154.68
142.17
136.82
136.64
131.42
130.36
128.51
125.14
124.72
123.26
120.36
119.01
116.87

f1 (ppm)

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354

357