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Modification of natural flavonoids for their use as drugs

Modificació de flavonoides naturals per al seu ús com a medicaments

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Campus d'Excel·lència Internacional

Continua tot i que tots esperin que abandonis. No deixis que s'oxidi el ferro que hi ha en tu.

Teresa de Calcuta

Primerament voldria agrair al Dr. Xavier Ariza, tutor d'aquest treball, tant la seva dedicació com el bon tracte que m'ha donat durant aquests mesos, fet que m'ha motivat a posar tot el meu esforç en aquest projecte.

Als membres del grup de recerca, amb qui he compartit dia a dia la consecució d'aquest treball, gràcies per l'ajuda prestada. Especialment, a en Marc i el Roberto, companys de "batalla" amb una gran disposició i paciència.

Per descomptat, agrair als meus pares els seu suport incondicional i el orgull que mostren per mi, que sempre em motiva a esforçar-me al màxim i donar-ho tot de mi mateix. Tant en els bons com en els mals moments sempre he pogut comptar amb ells.

Per últim agrair a la meva parella, sense la qual no hagués pogut realitzar aquest treball, ja que m'ha donat el suport necessari per continuar endavant en els moments més difícils.

REPORT

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1. SUMMARY

Obesity is a new problem today due to the increased consumption of energy-dense foods along with a sedentary lifestyle. That is why some studies have been carried out to discover new anti-obesity drugs. It has been found that flavones, a type of molecule in the flavonoid family, are important components of many natural plants with important protective effects, such as antioxidant, anti-inflammatory activity against many diseases and the increase of fat burning by different ways depending on the specific molecule.

In this work, we have focused on the synthesis and characterization of five chrysin derivates: 5-hydroxy-7-methoxyflavone, 5,7-dimethoxyflavone, 6-bromo-5,7-dimethoxyflavone, 6,8-dibromo-5,7-hydroxyflavone and 6-benzyl-5,7-dimethoxyflavone. These compounds could be useful for the study of browning of fat cells because it is supposed that they allow to convert WAT (white adipose tissue) to BAT (brown adipose tissue) which could help in different diseases and upgrade the fat burning of our bodies.

Keywords: Chrysin, synthesis, drugs, flavones, browning, CPT1

2. RESUM

L'obesitat és un nou problema de l'actualitat degut a l'augment en el consum d'aliments densos en energia juntament amb un estil de vida sedentari. Per això s'han realitzat alguns estudis per descobrir nous fàrmacs en contra de l'obesitat. S'ha comprovat que les flavones, un tipus de molècula de la família dels flavonoides, són components importants de moltes plantes naturals amb efectes protectors importants com l'activitat antioxidant, antiinflamatòria contra moltes malalties i l'augment de la crema de greixos de diferents maneres segons la molècula específica.

En aquest treball, ens hem centrat en la síntesi i caracterització de cinc derivats de crisina: 5-hidroxi-7-metoxiflavona, 5,7-dimetoxiflavona, 6-bromo-5,7-dimetoxiflavona, 6,8-dibromo-5,7-hidroxiflavona i 6-benzil-5,7-dimetoxiflavona. Aquests compostos poden ser útils per a l'estudi del ´browning´ de les cèl·lules de greix ja que se suposa que permeten convertir WAT (teixit adipós blanc) en BAT (teixit adipós marró) el qual podria ajudar en diferents malalties i millorar la crema de greixos del nostre cos.

Paraules clau: Crisina, sintesis, medicaments, flavones, ´´browning´´, CPT1

3. INTRODUCTION

Overweight and obesity are defined as an abnormal or excessive fat accumulation that may impair health. Obesity occurs due to an imbalance between energy intake and expenditure. The consumption of energy dense foods coupled with a sedentary lifestyle predisposes individuals to the development of obesity. Drug discovery efforts towards anti-obesity drugs have not been very rewarding as many of the approved drugs cause serious adverse drug reactions. Therefore, it is of paramount importance to increase the focus on screening of natural compounds for the treatment of obesity which are perceived to show fewer and/or less severe adverse effects. Higher intake of dietary flavonoids has been associated with reduced body weight in a cohort of adults living in the Mediterranean area. (1)

3.1. FLAVONOIDS

Flavonoids are important components of many natural plants with important protective effects, such as antioxidant and anti-inflammatory activity against many diseases. Flavonoids are common food additives and healthy food supplements as well as active ingredients in Chinese herbal medicines. (2)

Flavonoids are classified into 12 major subclasses based on chemical structures, six of which, namely anthocyanidins, flavan-3-ols, flavonols, flavones, flavanones, and isoflavones are of dietary significance.

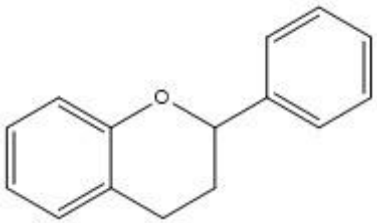


Figure 1. General Flavonoid

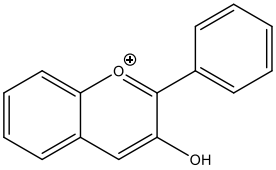
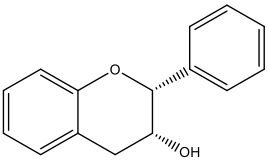
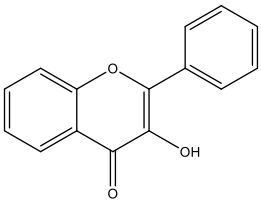
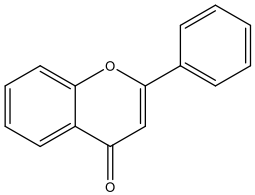
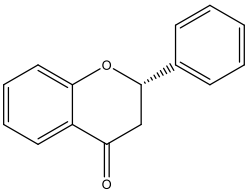
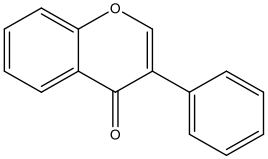
Flavonoid subclass	Examples	Food sources
 *Anthocyanidins*	Cyanidin, Delphinidin, Malvidin, Pelargonidin, Peonidin, Petunidin	Berries, Grapes, Wine
 *Flavan-3-ols*	(+)-Catechin, (-)-Epicatechin, (-)-Epigallocatechin, (+)-Gallocatechin Proanthocyanidins Theaflavins, Thearubigins	Apples, Berries, cocoa-based products, Teas, Wine
 *Flavonols*	Isorhamnetin, Kaempferol, Myricetin, Quercetin	Onions, Apples, Teas, Berries, Scallions, Kale, Broccoli,
 *Flavones*	Baicalein, Chrysin, Luteolin, Apigenin	Parlsey, Hot peppers, Thyme, Celery
 *Flavanones*	Naringenin, Hesperetin, Eriodictyol	Citrus fruits
 *Isoflavones*	Daidzein, Genistein, Glycitein, Biochanin A, Formononetin	Soybeans, Legumes

Table 1. Common Flavonoids

3.2. BENEFITS OF FLAVONOIDS

3.2.1 Weight management

Flavonoids are also associated with inflammation and weight loss. “Flavonoid content can relieve inflammation and decrease the levels of an appetite-suppressing hormone, leptin. (2)

3.2.2 Longevity

Flavonoid consumption was significantly associated with longevity. The researchers suggested flavonoid consumption could account for 25 percent of the observed difference in mortality rates from coronary heart disease and cancer. (2)

3.2.3 Cardiovascular disease

Their antioxidant and anti-inflammatory behaviors, flavonoids are associated with cardiovascular disease prevention. They may also improve the quality of blood vessel walls.

Several studies have found an association between higher flavonoid intake levels and lowered cardiovascular disease risk across various groups, including postmenopausal women, male smokers and middle-age men and women.

Various flavonoids, including quercetin, have shown to be effective at preventing platelet aggregation. Platelet aggregation is a known component in heart disease because it contributes to forming blood clots that can lead to strokes and other problems. (2)

3.2.4 Diabetes

A study published in 2013 in the journal Diabetic Medicine found that among men with type 2 diabetes, adding a flavonoid-rich spice mix to hamburger meat significantly improved their vascular function during subsequent hours. The spice mix included rosemary, garlic, ginger, black pepper and oregano — all spices that contain flavonoids. World’s Healthiest Foods notes that similar effects have been seen in studies of grape juice, chocolate, pomegranate juice and soy foods. (2)

3.2.5 Cancer prevention

The research in this area has produced mixed results. Animal studies have shown positive results when it comes to lung, mouth, stomach, colon, skin and other cancers, but human studies have yet to show consistently similar results. More research is needed.

The most promising studies to date regard breast and stomach cancer. A large study published in 2003 in the British Journal of Cancer found that women with higher levels of flavone intake were at a lower risk for developing breast cancer, while a study in Cancer Causes & Control found a correlation between kaempferol intakes and reduced gastric cancer risk. On the other hand, another study, published in the same journal, did not associate reduced gastric cancer risk with kaempferol but flavanones. (2)

3.2.6 Neurodegenerative disease prevention

Flavonoids' anti-inflammatory and antioxidant effects may help protect against neurodegenerative diseases like Alzheimer's and Parkinson's. In animal studies, flavonoid levels have been positively correlated with reduced risk of these diseases, but human studies have yielded inconclusive results. Flavonoids may also increase blood flow to the brain, improving cognitive function. (2)

3.3. BAICALIN AND MITOCHONDRIAL FATTY ACID B-OXIDATION (FAO)

Baicalin is a major flavonoid component from the herbal medicine *Scutellaria baicalensis* that has been shown to have an antisteatosis effect. Baicalin acts as a natural allosteric activator of carnitine palmitoyltransferase 1 (CPT1), the rate limiting enzyme of fatty acid β-oxidation (FAO). By directly binding to CPT1 and activating its activity to accelerate fatty acid degradation. (3)

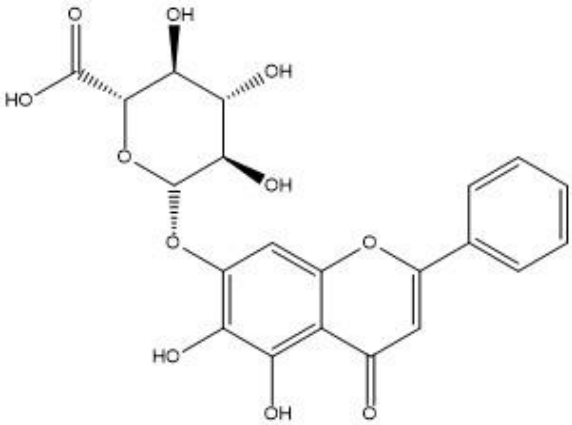


Figure 2. Baicalin

The major route for lipid expenditure is through mitochondrial fatty acid β-oxidation (FAO), an essential process in which free fatty acids are esterified with CoA, transported into the mitochondria matrix, and oxidized to generate acetyl-CoAs. The transport of long-chain acyl-CoA esters into the mitochondria matrix is mediated by the carnitine palmitoyl transferase (CPT) system that consists of three proteins: CPT1, acylcarnitine translocase, and CPT2. CPT1 is anchored on the mitochondrial outer membrane and is responsible for converting acyl-CoAs into acylcarnitine, which are shuttled across the mitochondria membranes by the translocase and converted back to acyl-CoAs by CPT2 inside mitochondria before entering β-oxidation. CPT1 is considered as the rate-limiting enzyme for FAO, and its inhibition by malonyl-CoA, the first committed intermediate for lipogenesis, serves as the key regulatory mechanism to maintain the balance of fatty acid metabolism. Genetic suppression of acetyl-CoA carboxylases (ACCs) to reduce the production of malonyl-CoA was shown to reverse diet-induced hepatic steatosis and insulin resistance, and over-expression of an active but malonyl-CoA–insensitive mutant of CPT1 was able to ameliorate insulin resistance in mice, both of which support the rationale of activating. (4,5)

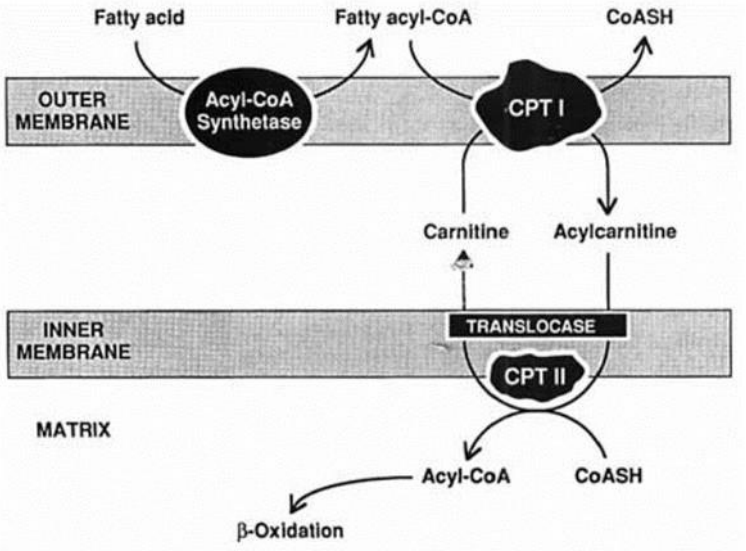


Figure 3. The mitochondrial CPT system

3.4. CHRYSIN AND BROWNING

Chrysin is a flavone found in propolis and many plants, predominantly in the *Passiflora* species. Chrysin has also been shown to inhibit the differentiation of adipocytes and induce the browning of white adipocytes in vitro. However, these effects have not been substantiated in animal models of obesity. The present study tested the hypothesis that chrysin can exert anti-obesity effect by inhibiting pancreatic lipase. (1)

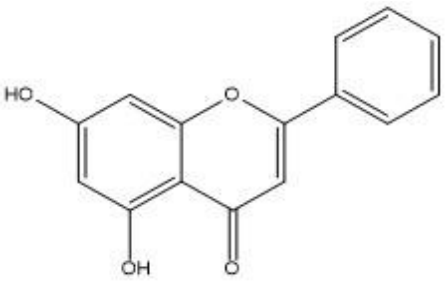


Figure 4. Chrysin

It is well known today that mammals have two types of adipose tissue, white adipose tissue (WAT) and brown adipose tissue (BAT). Whereas WAT is most common and found as subcutaneous tissue, around the abdomen, thighs, and waist, the BAT is found particularly as perivascular, epicardial, supra-adrenal, and supra-scapular tissue. The major difference between WAT and BAT is that BAT has more mitochondria and small fat droplets whereas WAT has a big fat droplet and less mitochondria. High accumulation of WAT leads to obesity.

WAT has two depots in body: visceral white adipose tissue (vWAT) and subcutaneous white adipose tissue (scWAT). Both type of WAT are very much involved in functions such as: lipid storage, hormone production, immune function, and local tissue architecture.

Brown adipose tissue (BAT) is specialized in energy expenditure and it is present in nearly all mammalian species. BAT has an important thermogenic function as a natural defense system against cold stress in newborn mammals and in adults. BAT has not only crucial role against hypothermia but also has a power to modulate the proton gradient by uncoupling cellular respiration from mitochondrial ATP synthesis.

Since the end of the 20th century researchers are trying to define the process of browning. The important landmark in this field of research was studies on animals as well as on humans exposed to cold conditions. In these types of studies, it was obvious that BAT activity in man was acutely cold induced and was stimulated via the sympathetic nervous system. Taking all that in consideration, recent studies on mice have reported that browning can be mediated by the emergence of UCP1-expressing cells in WAT not only by cold exposure or adrenergic stimulation but also by hormonal stimulation. (6)

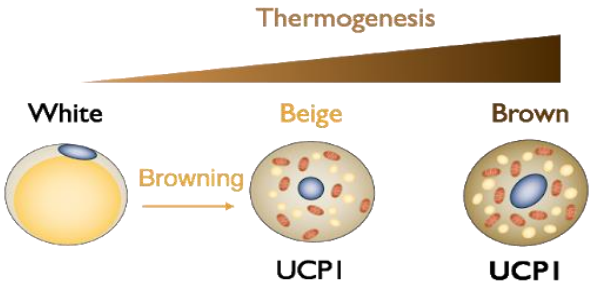


Figure 5. Browning of fat cell

4. OBJECTIVES

The main objective of this work is the preparation of chrysin derivates for their use in future biological studies, specifically in the investigation of new drugs for obesity.

The specific objectives were the synthesis and spectroscopic characterization of 5-hydroxy-7-methoxyflavone (**1**), 5,7-dimethoxyflavone (**2**), 6-bromo-5,7-dimethoxyflavone (**3**), 6,8-dibromo-5,7-hydroxyflavone (**4**) and 6-benzyl-5,7-dimethoxyflavone (**5**).

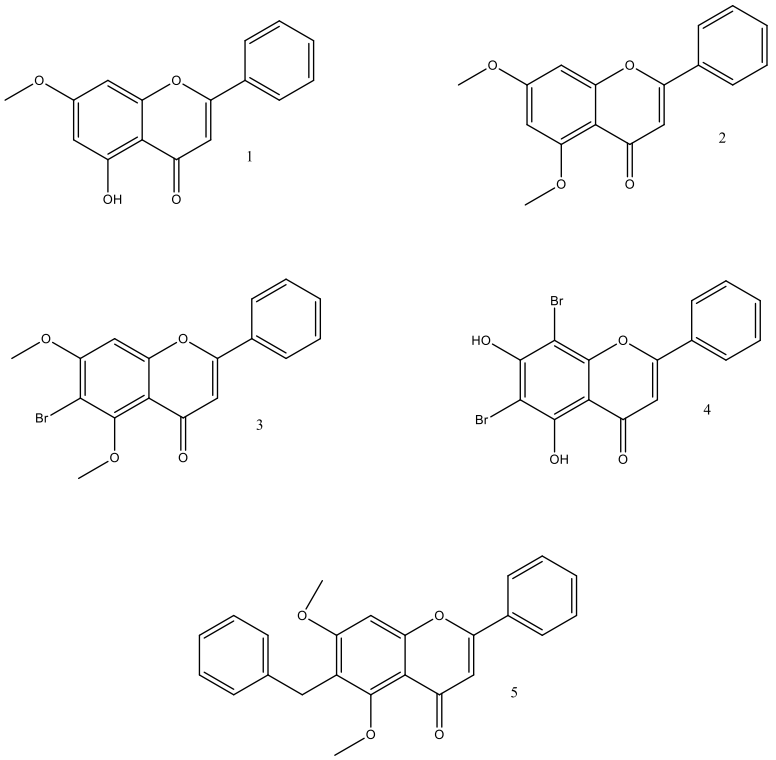


Figure 6. Molecules synthetized

5. EXPERIMENTAL SECTION

Firstly, a list of reagents and solvents and the methods used for the description of all compounds is shown in this section.

5.1. MATERIALS AND METHODS

5.1.1 REAGENTS AND SOLVENTS

Reagent/solvent	Supplier
Chrysin	TCI
Potassium Carbonate	Panreac
Dimethyl Sulfate	Aldrich
Ammonia	Scharlau
HCl (conc)	Scharlau
Anhydrous $MgSO_4$	J. Escuder
NBS	Aldrich
TBATB	TCI
$Pd(PPh_3)_4$	Aldrich
Benzyl zinc Bromide (0.5 M in THF)	Aldrich
Chloroform-d	Aldrich
Dimethyl sulfoxide-d_6	Aldrich
Anhydrous THF	-
Chloroform	-
DMF	Fluka
Acetone	Scharlau

Table 2. List of used reagents and solvents

5.1.2 METHODS AND INSTRUMENTATION

5.1.2.1. NUCLEAR MAGNETIC RESONANCE (NMR)

^{1}H and ^{13}C spectra data was collected with a Varian Mercury 400 spectrometer at 25 ºC. Tetramethylsilane was the intern reference compound (0 ppm). Chemical shift (δ) is always in ppm and coupling constants (*J*) in Hz. Solvent is indicated in each characterization.

5.1.2.2. Melting point

For the measures of the melting point, a SMP 10 instrument was used by placing the sample in a capillary tube.

5.1.2.3. Thin layer chromatography (TLC)

For all reactions this method was used when they finished, and sometimes for the control of the reaction advance. Analytical TLC Silica gel 60 F254 was used (Merck, 0.2 mm of thickness). Light (UV: 254 nm and 365 nm) was used as developer.

5.1.2.4. Column chromatography

This method was used to purify substances. Silica gel was used as stationary phase, and the elution was favored with pressure air. Eluent is specified in each case.

5.2. SYNTHESIS OF 5-HYDROXY-7-METHOXYFLAVONE

K_2CO_3 (1.630 g, 11.8 mmol) and $(CH_3O)_2SO_2$ (1.5 mL, 15.8 mmol) were added to a solution of chrysin (1.010 g, 3.95 mmol) in acetone (30 mL). The mixture was stirred for 1.5 h at 60 ºC. The mixture was quenched with NH_4OH (5 mL of 10% sol in water) and extracted with ethyl acetate (3 x 30 mL). The combined organic layers were washed with brine (30 mL) and dried over anhydrous sodium sulfate. After filtration and elimination of the solvent under reduced pressure the crude residue was purified by column chromatography (silica gel, hexane/ethyl acetate [80/20]) to afford 0.715 g (67 % yield) 5-hydroxy-7-methoxyflavone as a yellow powder.

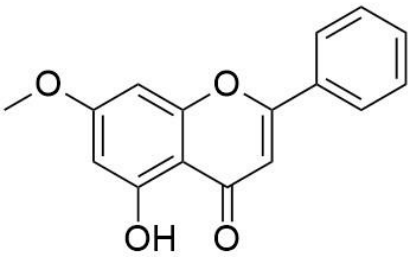


Figure 7. 5-hydroxy-7-methoxyflavone

^{1}H NMR (DMSO-d_6, 400 MHz) δ: 12.79 (1H, s, OH), 8.08-8.06 (2H, d, *J* = 8 Hz, $C^{2'}$-H, $C^{6'}$-H), 7.61-7.54 (3H, m, $C^{3'}$-H, $C^{4'}$-H, $C^{5'}$-H), 7.02 (1H, s, C^3-H), 6.79 (1H, d, J_{86} = 2 Hz, C^8-H), 6.39 (1H, d, J_{68} = 2 Hz, C^6-H), 3.86 (3H, s, CH_3).

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 182.5, 165.6, 164.0, 162.2, 157.8, 131.8, 131.3, 129.1, 126.3, 105.9, 105.7, 98.2, 92.7, 55.8.

Mp: 161-164 ºC

R_f: 0.60 (hexane/ethyl acetate [70/30])

5.3. SYNTHESIS OF 5,7-DIMETHOXYFLAVONE

With 4 mmol.

K_2CO K_2CO_3 (1.630 g, 11.8 mmol) and $(CH_3O)_2SO_2$ (1.5 mL, 15.8 mmol) were added to a solution of chrysin (1.010 g, 3.95 mmol) in acetone (30 mL). The mixture was stirred for 6 h at 60 ºC. The mixture was quenched with NH_4OH (5 mL of 10% sol in water). The mixture was extracted with ethyl acetate (3 x 30 mL). The combined organic layers were washed with brine (30 mL) and dried over anhydrous sodium sulfate. After filtration and elimination of the solvent under reduced pressure the crude residue was purified by crystallization to afford 0.697 g (63 % yield) of 5,7-dimethoxyflavone as a deep green powder.

^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.04-8.02 (2H, d, *J* = 8 Hz, $C^{2'}$-H, $C^{6'}$-H), 7.56-7.53 (3H, m, $C^{3'}$-H, $C^{4'}$-H, $C^{5'}$-H), 6.86 (1H, d, J_{86}= 2 Hz, C^{8}-H), 6.76 (1H, s, C^{3}-H), 6.50 (1H, d, J_{68} = 2 Hz, C^{6}-H), 3.89 (3H, s, CH_3), 3.82 (3H, s, CH_3).

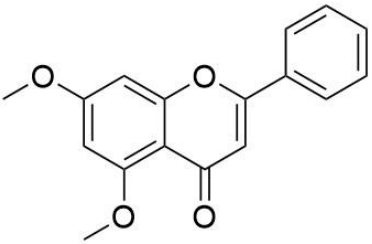


Figure 8. 5,7-dimethoxyflavone

^{13}C NMR ($CDCl_3$, 100 MHz) δ: 177.6, 164.0, 160.9, 160.6, 159.9, 131.5, 131.2, 128.9, 125.9,109.3, 109.1, 96.2, 92.9, 56.5, 55.8.

Mp: 141-144 °C

R_f: 0.625 (ethyl acetate)

With 20 mmol

K_2CO_3 (8.270 g, 59 mmol) and $(CH_3O)_2SO_2$ (7.5 mL, xxx mmol) were added to a solution of chrysin (5.010 g, 19.7 mmol) in acetone (150 mL). The mixture was stirred for 6 h at 60 °C. The mixture was quenched with NH_4OH (25 mL of 10% sol in water). The mixture was extracted with ethyl acetate (3 x 150 mL). The combined organic layers were washed with brine (150 mL) and dried over anhydrous sodium sulfate. After filtration and elimination of the solvent under reduced pressure to afford 7.039 g of 5,7-dimethoxyflavone as a deep green powder unpurified with a red insoluble solid.

5.4. SYNTHESIS OF 6-BROMO-5,7-DIMETHOXYFLAVONE

With NBS and 0.7 mmol

N-Bromosuccinimide (NBS, 0.142 g, 0.71 mmol) was added to a solution of 5,7-dimethoxyflavone (0.200 g, 0.71 mmol) in DMF (5 mL). The mixture was stirred for 2.5 h at 0 °C. The mixture was quenched with a cold 2M solution of HCl (5 mL). The mixture was extracted with ethyl acetate (3 x 10 mL) and dried over anhydrous sodium sulfate. After filtration and elimination of the solvent under reduced pressure the product was purified by crystallization to afford 0.060 g (23 % yield) of 6-bromo-5,7-dimethoxyflavone as a white powder.

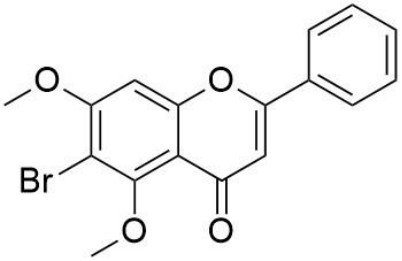


Figure 9. 6-bromo-5,7-dimethoxyflavone

^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.11-8.07 (2H, d, *J* = 8 Hz, $C^{2'}$-H, $C^{6'}$-H), 7.60-7.57 (3H, m, $C^{3'}$-H, $C^{4'}$-H, $C^{5'}$-H), 6.89 (1H, s, C^{8}-H), 6.76 (1H, s, C^{3}-H), 4.05 (3H, s, CH_3), 3.95 (3H, s, CH_3).

^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm): 177.4, 160.8, 160.4, 160.3, 155.3, 131.5, 131.1, 129.0, 126.2, 108.3, 92.2, 91.1, 56.6.
Mp: 244-247 °C
R_f: 0.50 (ethyl acetate)

With NBS and 5.3 mmol

N-Bromosuccinimida (NBS, 1.102 g, 5.325 mmol) was added to a solution of 5,7-dimethoxyflavone (1.500 g, 5.325 mmol) in DMF (37.5 mL). The mixture stirred for 2.5 h at 0 °C. The mixture was quenched with a cold 2M solution of HCl (37.5 mL). The mixture was extracted with ethyl acetate (3 x 60 mL) and dried over anhydrous sodium sulfate. After filtration and elimination of the solvent under reduced pressure the product was purified by column chromatography (silica gel, ethyl acetate) to afford 0.901 g (46 % yield) of 6-bromo-5,7-dimethoxyflavone as white powder. Additionally, a second product was separated 0.211g (9% yield) of 6,8-dibromo-5,7-dimethoxyflavone as a white powder.

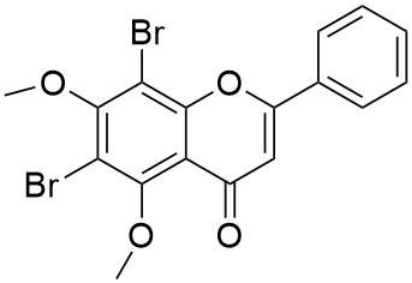


Figure 10. 6,8-dibromo-5,7-dimethoxyflavone

^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.11-8.07 (2H, d, *J* = 8 Hz, $C^{2'}$-H, $C^{6'}$-H), 7.60-7.57 (3H, m, $C^{3'}$-H, $C^{4'}$-H, $C^{5'}$-H), 6.89 (1H, s, C^{8}-H), 6.76 (1H, s, C^{3}-H), 4.05 (3H, s, CH_3), 3.95 (3H, s, CH_3).

^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm):
Mp: 229-233 °C
R_f: 0.65 (ethyl acetate)

With TBATB and 0.885 mmol

Tetrabutylammonium tribromide (TBATB) (0.840 g, 1.74 mmol) was added to a solution of 5,7-dimethoxyflavone (0.250 g, 0.885 mmol) in chloroform (7 mL). The mixture was stirred for 1.5 h. The mixture was diluted with water (7 mL). The mixture was extracted with ethyl acetate (3 x 10 mL). The combined organic layers were washed with brine (20 mL) and dried over anhydrous sodium sulfate. After filtration and elimination of the solvent under reduced pressure the crude residue was purified by column chromatography (silica gel, ethyl acetate) to afford 0.094 g (29 % yield) of 6-bromo-5,7-dimethoxyflavone as a white powder.

With TBATB and 5.31 mmol

Tetrabutylammonium tribromide (TBATB) (0.840 g, 1.74 mmol) was added to a solution of 5,7-dimethoxyflavone (0.250 g, 0.885 mmol) in chloroform (7 mL). The mixture was stirred for 1.5 h. The mixture was diluted with water (7 mL). The mixture was extracted with ethyl acetate (3 x 10 mL). The combined organic layers were washed with brine (20 mL) and dried over anhydrous sodium sulfate. After filtration and elimination of the solvent under reduced pressure to afford 0.806 g (42 % yield) of 6-bromo-5,7-dimethoxyflavone as a white powder.

5.5. SYNTHESIS OF 6,8-DIBROMO-5,7-HYDROXYFLAVONE

Tetrabutylammonium tribromide (TBATB) (1.660 g, 3.94 mmol) was added in one portion to a solution of chrysin (0.500 g, 1.97 mmol) in chloroform (7 mL). The mixture was stirred for 2.5 h. The mixture was diluted with water (20 mL). The mixture was extracted with ethyl acetate (3 x 20 mL) and dried over anhydrous sodium sulfate. After filtration and elimination of the solvent under reduced pressure the crude was purified by crystallization to afford 0.520 g (64% yield) of 6,8-dibromo-5,7-hydroxyflavone as a yellow solid.

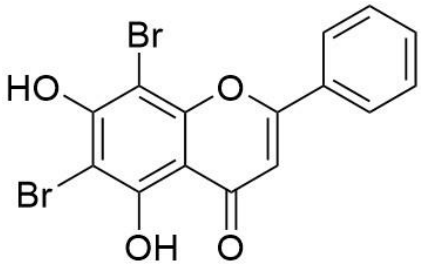


Figure 11. 6,8-dibromo-5,7-hydroxyflavone

^{1}H NMR (DMSO-d_6, 400 MHz) δ: 13.74 (2H, s, C^{5}-OH), 8.16-8.13 (2H, d, *J* = 8 Hz, $C^{2'}$-H, $C^{6'}$-H), 7.64-7.58 (3H, m, $C^{3'}$-H, $C^{4'}$-H, $C^{5'}$-H), 7.20 (1H, s, C^{3}-H).

^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm): 182.0, 163.9, 158.0, 157.5, 152.8, 132.9, 130.7, 129.7, 126.9, 105.6, 95.1, 89.0.
Mp: 268-273 ^{o}C
R_f: 0.275 (hexane/ethyl acetate [70/30])

5.6. SYNTHESIS OF 6-BENZYL-5,7-DIMETHOXYFLAVONE

With 0.1% of catalyst and 3h reaction

A solution of freshly prepared $Pd(PPh_3)_4$ (1.3 mg, 0.001 mmol) in anhydrous THF (0.5 mL) was added , to 6-bromo-5,7-dimethoxyflavone (0.362 g, 1.00 mmol) in dissolved in anhydrous THF (3 mL) under nitrogen atmosphere in a sealed vial. Then a commercial solution of benzylzinc bromide (0.5 M in THF, 3 mL, 1.50 mmol). The mixture was purged again with nitrogen before sealing the vial with a screwcap and heated up to 90 ^{o}C for 3h. The mixture was cooled down to room temperature, the reaction crude was filtered with ethyl acetate (20 mL) through celite directly onto a saturated aqueous solution of NH_4Cl (30 mL). The aqueous phase was extracted with ethyl acetate (20 mL). Then, the combined organic layers were dried over anhydrous sodium sulfate. After filtration and elimination of the solvent under reduced pressure the crude was purified by column chromatography (silica gel, ethyl acetate/hexane [50/50]) to give 0.038 g (10 % yield) of 6-benzyl-5,7-dimethoxyflavone as a white powder.

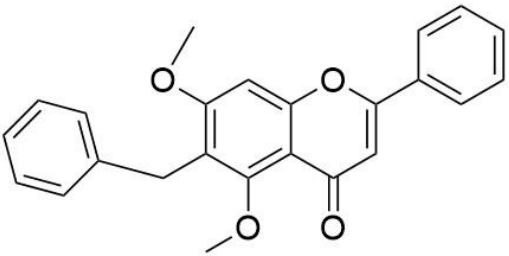


Figure 12. 6-benzyl-5,7-dimethoxyflavone

^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.88-7.86 (2H, d, *J* = 8 Hz, $C^{2'}$-H, $C^{6'}$-H), 7.57-7.47 (3H, m, $C^{3'}$-H, $C^{4'}$-H, $C^{5'}$-H), 7.25-7.18 (4H, m, Ph-CH_2), 7.12(1H, m, Ph-CH_2), 6.75 (1H, s, C^{8}-H), 6.73 (1H, s, C^{3}-H), 4.19 (2H, s, Ph-CH_2), 3.99 (3H, s, CH_3), 3.93 (3H, s, CH_3).

^{13}C NMR (DMSO-d_6, 100 MHz) δ: 178.8, 176.5, 161.8, 160.0, 159.7, 156.2, 140.8, 131.7, 131.5, 129.5, 128.8, 128.2, 126.3, 108.5, 108.4, 93.3, 56.8, 56.6, 28.4.

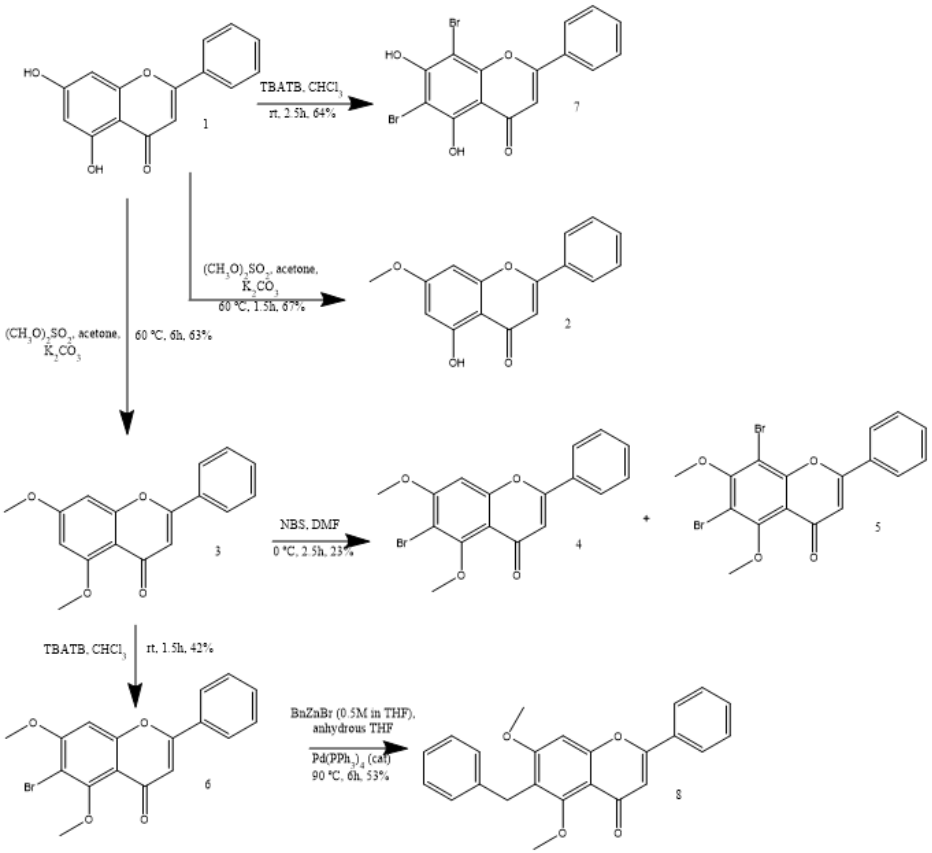
Mp: 145-149 ^{o}C R_f: 0.25 (ethyl acetate)

With 0.5% of catalyst and 6h reaction

A solution of freshly prepared $Pd(PPh_3)_4$ (1.3 mg, 0.005 mmol) in anhydrous THF (0.5 mL) was added , to 6-bromo-5,7-dimethoxyflavone (0.362 g, 1.00 mmol) in dissolved in anhydrous THF (3 mL) under nitrogen atmosphere in a sealed vial. Then a commercial solution of benzylzinc bromide (0.5 M in THF, 3 mL, 1.50 mmol). The mixture was purged again with nitrogen before sealing the vial with a screwcap and heated up to 90 ^{o}C for 6h. The crude was purified directly by column chromatography (silica gel, ethyl acetate) to give 0.199 g (53 % yield) of 6-benzyl-5,7-dimethoxyflavone as a white-yellow powder.

6. RESULTS AND DISCUSSIONS

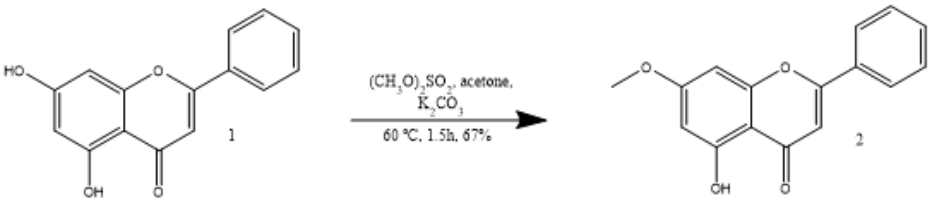
The reactions carried out and the corresponding results are treated in this section. Some reactions deserve a detailed explanation, but most of them are usual transformations that do not require further comments. All reactions were done following the literature [7], except for the product 8 that was based on a doctoral thesis. [8]



Scheme 1.

6.1. SYNTHESIS OF 5-HYDROXY-7-METHOXYFLAVONE

As shown in Scheme 2, the synthesis of the chrysin derivative **2**, 5-hydroxy-7-methoxyflavone, was planned through a nucleophilic substitution reaction (S_N2). To start the reaction chrysin was mixed with an excess of dimethyl sulfate in order to methylate the phenol.



Scheme 2.

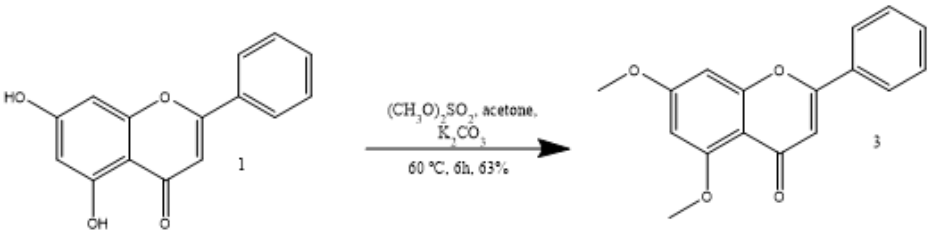
During the extraction some of the crude was impossible to solubilize, that could explain some losses during this process because some product got stuck on the walls of the separatory funnel.

When TLC (hexane/ethyl acetate 70:30) of the crude was carried out the result was as expected, it revealed a mix of two different products that could be easily separated by column chromatography. The problem found was, as earlier, the low solubility of the product that do not let work the column correctly. To avoid this problem the crude was previously silicated and then charged to the column. After the solvent was removed, 0.715 g of 5-hydroxy-7-methoxyflavone was recovered as yellow powder.

^{1}H NMR of the product **2** showed that the reaction took place. It was possible to see that one of the signals of hydroxyl had disappeared and a new signal at 3.86 ppm appeared, which belongs to the new methyl group added. ^{13}C NMR spectrum showed 14 signals as expected. This information matches the reference data. (7)

6.2. SYNTHESIS OF 5,7-DIMETHOXYFLAVONE

As shown in Scheme 3, the synthesis of the chrysin derivative **3**, 5,7-dimethoxyflavone, was planned through a nucleophilic substitution reaction (S_N2). To start the reaction chrysin was mixed with an excess of dimethyl sulfate in order to methylate the phenol, as the previous reaction. This reaction was repeated by scaling 5 times in order to obtain more product for be used at following reactions.



Scheme 3.

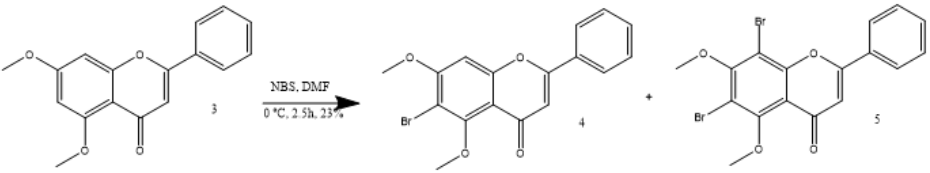
On the first reaction, when TLC (hexane/ethyl acetate 70:30) of the crude was carried out, the result was as expected. It revealed a pure product that do not need to be purified. After the solvent was removed 0.697 g of 5,7-dimethoxyflavone was recovered as deep green powder.

On the second reaction, when TLC (hexane/ethyl acetate 70:30) of the crude was carried out the result showed that a little portion of chrysin had not reacted yet. But then, when ^{1}H NMR of the crude was performed, it was shown that the crude was enough pure to follow up.

^{1}H NMR of the product **3** showed that the reaction took place. It was possible to see that the signals of hydroxyl had disappeared and new signals at 3.89 ppm and 3.82 ppm appeared, which belong to the new methyl groups added. ^{13}C NMR spectrum showed 15 signals as expected. This information matches the reference data. (7)

6.3. 6-BROMO-5,7-DIMETHOXYFLAVONE

As shown in Scheme 4 and 5, the synthesis of the chrysin derivative **4** and **6**, 6-bromo-5,7-dimethoxyflavone, was planned through a nucleophilic substitution reaction (S_N2). This reaction was done with two different bromine agents and then both were repeated by scaling 5 times in order to obtain more product for be used in the following reactions.



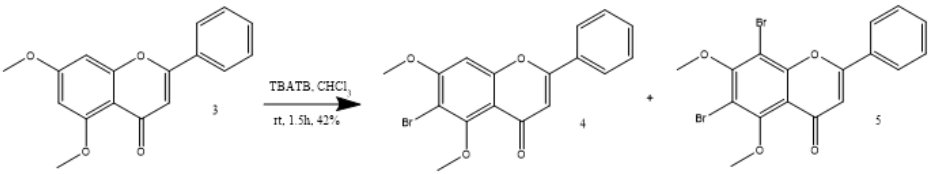
Scheme 4.

To start the first reaction of the product **4**, 5,7-dimethoxyflavone, was mixed with an equivalent of NBS in order to brominate the molecule.

On the first reaction, when TLC (ethyl acetate) of the crude was carried out, the result was as expected. It revealed a pure product that do not need to be purified. After the solvent was removed 0.209 g of 6-bromo-5,7-dimethoxyflavone was recovered as white powder.

Then, on the scale up, some differences were found. When TLC (ethyl acetate) of the crude was carried out, the result was four different spots. This crude was purified by column and the different fractions were analyzed by ^{1}H NMR. It revealed that we obtained some impurities, the product that was expected and the product of a double bromination. After the solvent was removed, 0.901 g of 6-bromo-5,7-dimethoxyflavone was recovered as white powder and 0.211 g of 6,8-dibromo-5,7-dimethoxyflavone was recovered as a white powder.

^{1}H NMR of the product **4** showed that the reaction took place. It was possible to detect that a signal of hydrogen had disappeared. ^{13}C NMR spectrum showed 13 signals as expected. This information matches the reference data. (7)



Scheme 5.

To start the second reaction of the product **6**, 5,7-dimethoxyflavone, was mixed with two equivalents of TBATB in order to brominate the molecule.

During the extraction, some of the crude was impossible to solubilize due to the formation of an emulsion between the phases. That could explain some losses during this process because some product got stuck on the walls of the separatory funnel.

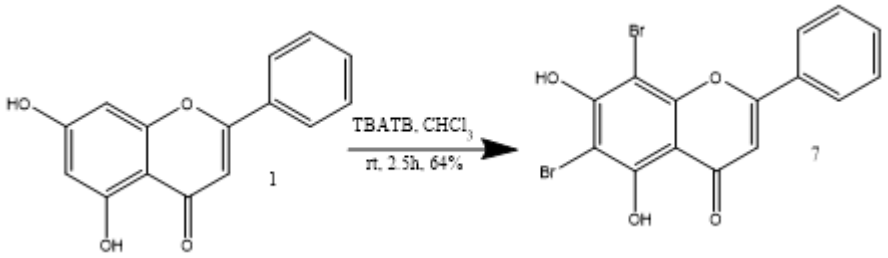
On the second reaction, when TLC (ethyl acetate) of the crude was carried out, the result showed that a little portion of reactive **3** had not reacted yet, and that there a mistake when the mass of TBATB was calculated. The crude of reaction was purified by column and after the solvent was removed, 0.094 g of 6-bromo-5,7-dimethoxyflavone was recovered as white powder.

Then, on the scale up, when TLC (ethyl acetate) of the crude was carried out, the result was as expected. It revealed a pure product that do not need to be purified. After the solvent was removed, 0.806 g of 6-bromo-5,7-dimethoxyflavone was recovered as white powder.

^{1}H NMR of the product **6** showed that the reaction took place. It was possible to detect that a signal of hydrogen had disappeared. ^{13}C NMR spectrum showed 15 signals as expected. This information matches the reference data. (7)

6.4. SYNTHESIS OF 6,8-DIBROMO-5,7-HYDROXYFLAVONE

As shown in Scheme 6, the synthesis of the chrysin derivative **7**, 6,8-dibromo-5,7-hydroxyflavone, was planned through a nucleophilic substitution reaction (S_N2). To start the reaction chrysin was mixed with an excess of dimethyl sulfate in order to methylate the phenol.



Scheme 6.

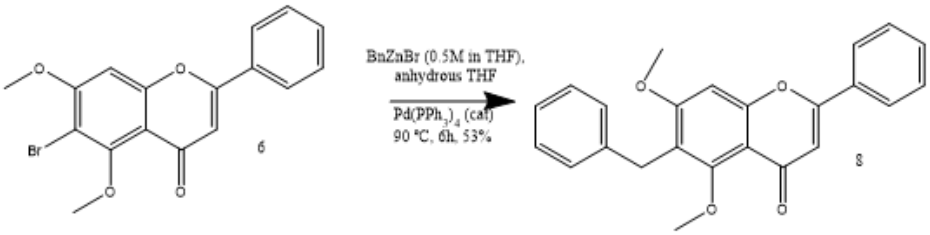
During the extraction some of the crude was impossible to solubilize, that could explain some losses during this process because some product got stuck on the walls of the separatory funnel.

When TLC (hexane/ethyl acetate 70:30) of the crude was carried out the result was as expected, it revealed a mix of two different products that could be separate easily by column chromatography. The problem found was, as before, the low solubility of the product that do not let the work column correctly. To avoid this problem the crude was previously silicate and then charged to the column. After the solvent was removed, 0.520 g of 6,8-dibromo-5,7-hydroxyflavone was recovered as yellow powder.

^{1}H NMR of the product **2** showed that the reaction took place. It was possible to detect that two signals of hydrogen had disappeared. ^{13}C NMR spectrum showed 12 signals as expected. This information matches the reference data. [7]

6.5. SYNTHESIS OF 6-BENZYL-5,7-DIMETHOXYFLAVONE

As shown in Scheme 7, the synthesis of the chrysin derivative **8**, 6-benzyl-5,7-dimethoxyflavone, was planned through the Negishi coupling, which is a transition metal catalyzed cross-coupling reaction.[8] This reaction was performed twice, the second time, the conditions were scaled up.



To start the reaction of the product **8**, 6-bromo-5,7-dimethoxyflavone, was mixed with benzylzinc bromide, then $Pd(PPh_3)_4$ was added as catalyzer. Finally, the mixture was purged with nitrogen and heated to start the reaction.

On the first reaction, when TLC (ethyl acetate) of the crude was carried out the result was not as expected, it revealed a mixture of products. The crude of reaction was purified by column chromatography and then a ^{1}H NMR of each fraction was done. It showed that the expected product **8** was obtained but mixed with the initial starting material **6** and the product of debromination **3**.

The yield of reaction was too low to separate well the products, so the reaction was repeated changing some conditions and work-up.

On the second reaction, when TLC (ethyl acetate) of the crude was carried out, the result was not as expected. It revealed a mixture of products. The crude of reaction was purified by column chromatography and then a ^{1}H NMR of each fraction was done. It showed that we got the expected product **8** was obtained but mixed with the initial starting material **6** and the product of debromination **3**. The changes worked well and now 0.199 g of 6-benzyl-5,7-dimethoxyflavone was recovered as white-yellow powder.

^{1}H NMR of the product **8** showed that the reaction took place. It was possible to detect new signals of the benzyl group, that appeared at 7.25-7.18 ppm and at 4.19 ppm. ^{13}C NMR spectrum showed 19 signals.

7. CONCLUSIONS

As expected, all compounds were obtained satisfactorily. However, the yield of some of the reactions was lower than expected due to the low solubility of these kind of products in the solvents used.

This work includes many well-established reactions of methylation and bromination. The use of known reactions allows us to understand better what happens in each case.

A less common reaction was also carried out: benzylation by Negishi reaction. In our hands, this reaction seems to be a useful tool to form a C–C bond. The importance of the absence of water in the reacting media was reflected on Negishi reaction for the preparation of 6-benzyl-5,7-dimethoxyflavone. This reaction involved sensible reagents, so it had to be carried out with the appropriate care, under N_2 atmosphere as it was performed in this work.

Due to the planned applications of the prepared compounds, this project will be useful to study the lipid expenditure by browning of fat cells, instead of what was initially thought, the activation of CPT1 enzyme.

8. REFERENCES AND NOTES

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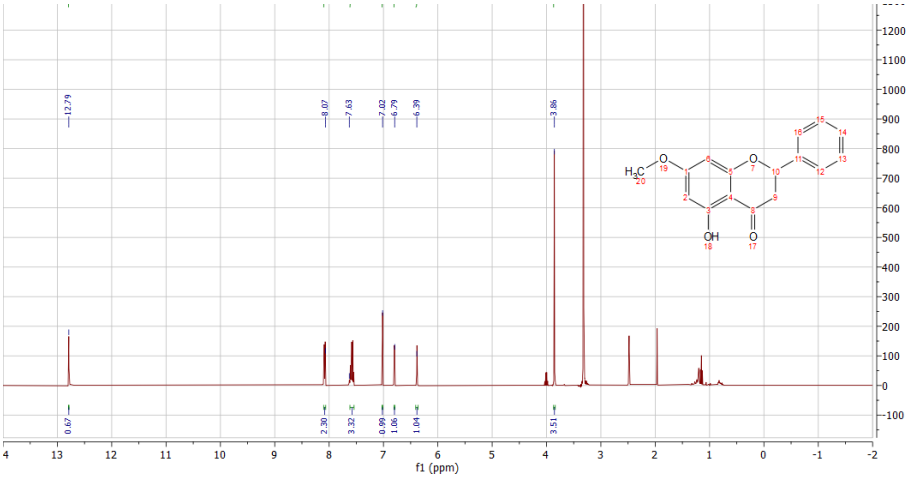
9. ACRONYMS

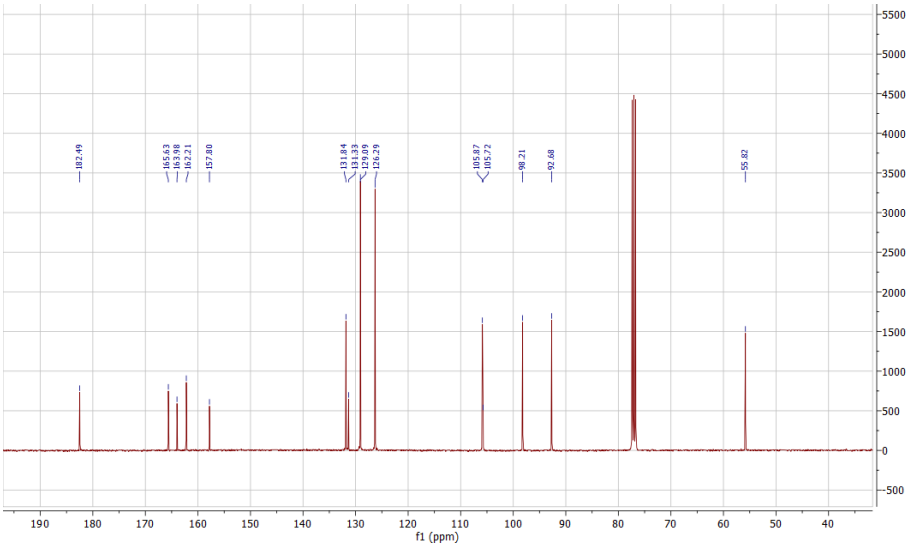
Abbreviation	Meaning
CPT	Carnitine Palmitoyltransferase
NMR	Nuclear Magnetic Resonance
TLC	Thin Layer Chromatography
r.t.	Room Temperature
DMSO	Dimethyl Sulfoxide
NBS	*N*-Bromosuccinimida
TBATB	Tetrabutylammonium tribromide

APPENDICES

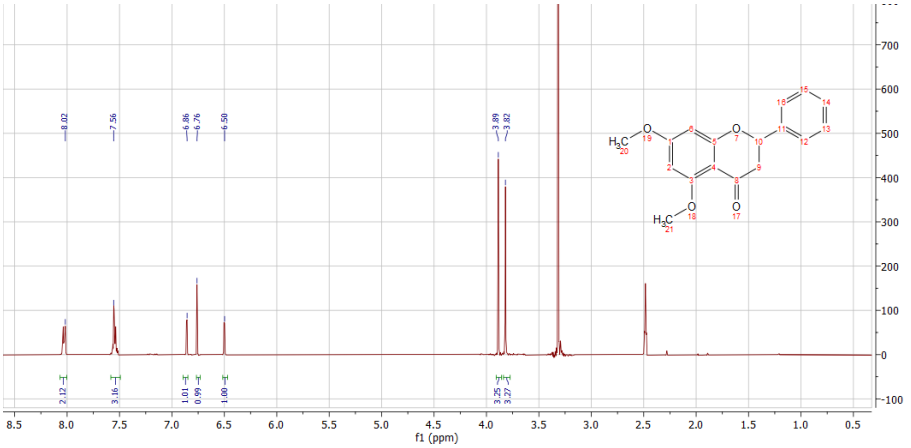
APPENDIX 1: ^{1}H AND ^{13}C NMR SPECTRA OF THE COMPOUNDS

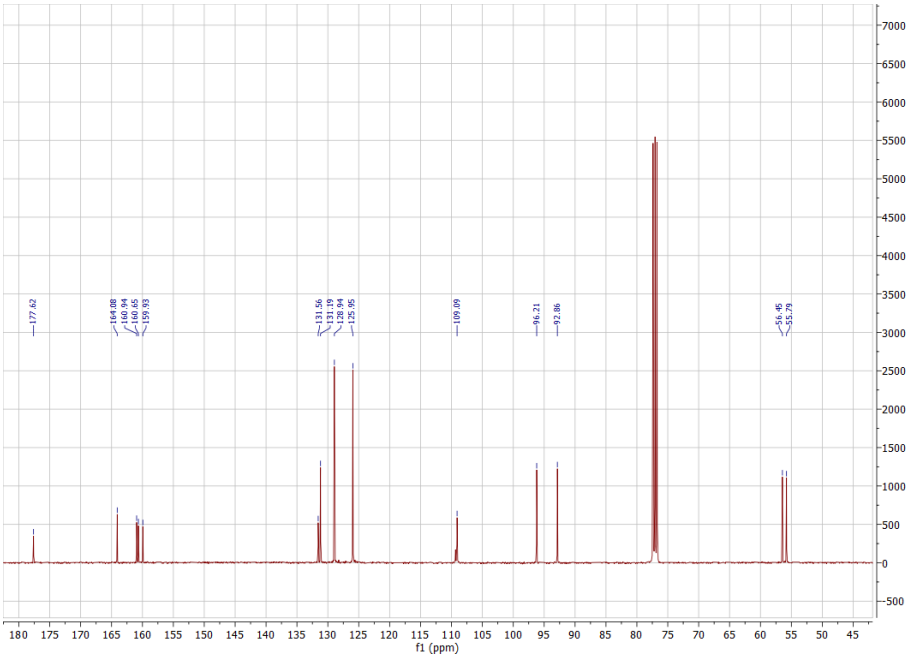
5-HYDROXY-7-METHOXYFLAVONE



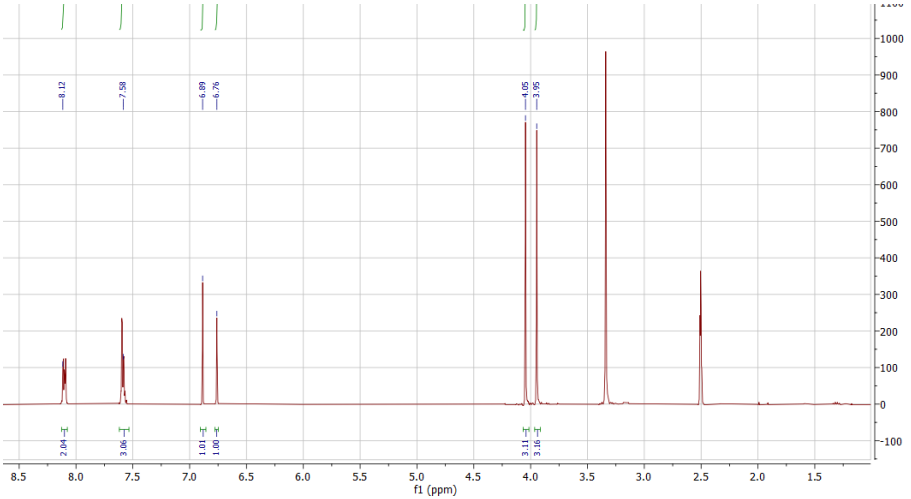


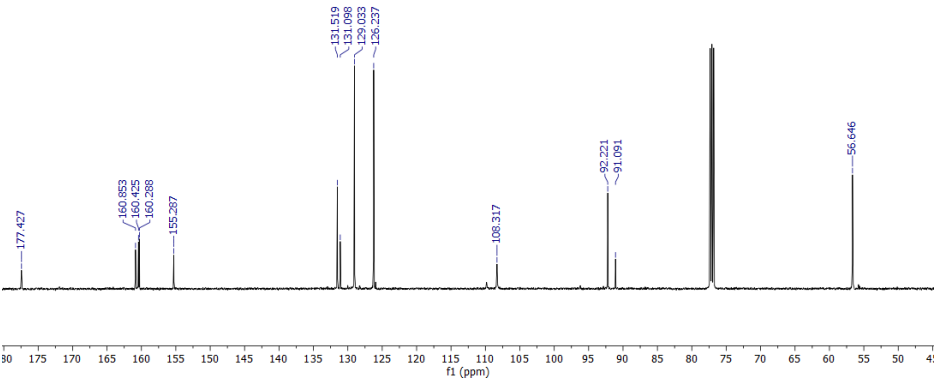
5,7-DIMETHOXYFLAVONE



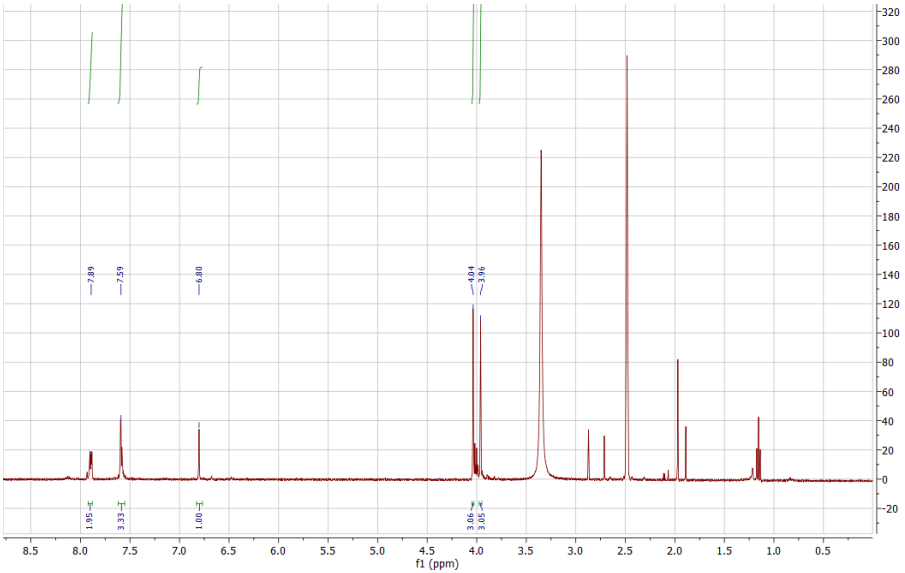


6-BROMO-5,7-DIMETHOXYFLAVONE

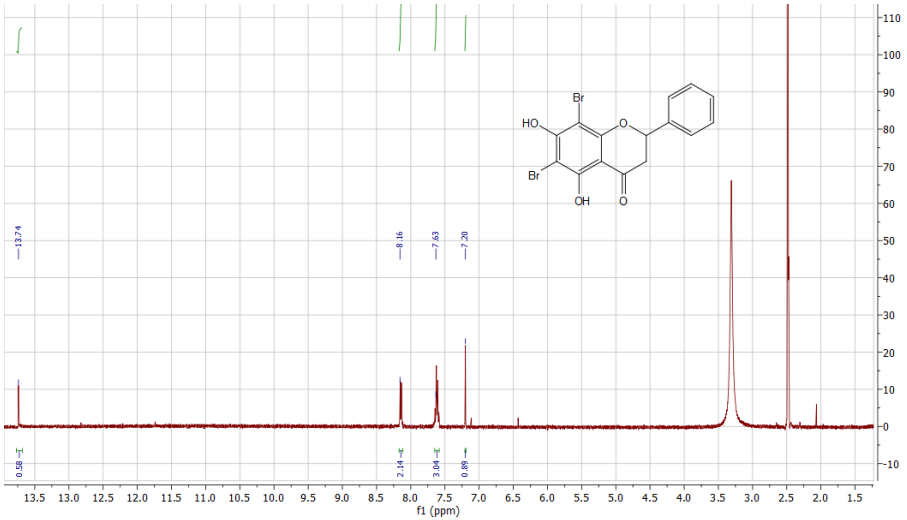


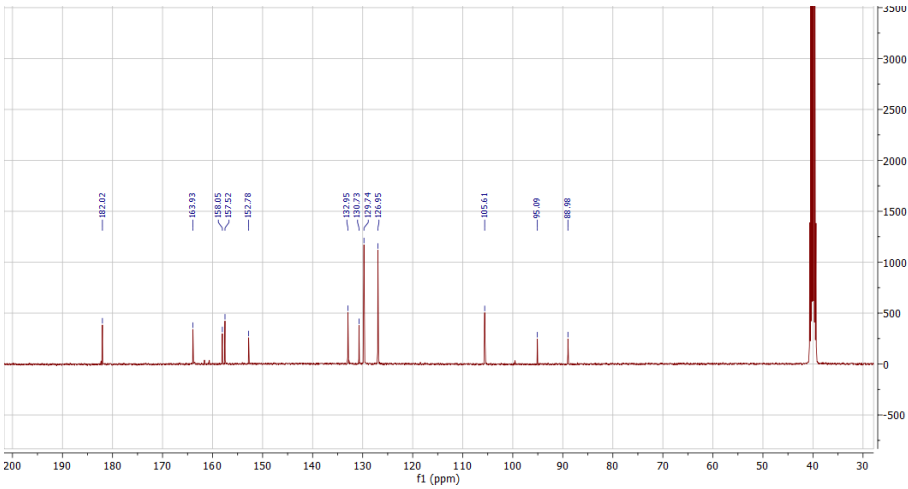


6,8-DIBROMO-5,7-DIMETHOXYFLAVON



6,8-DIBROMO-5,7-HYDROXYFLAVONE





6-BENZYL-5,7-DIMETHOXYFLAVONE