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Treball Final de Grau

Analogues synthesis of the GFP chromophore. Precursors of a new family of polymers without metallic components.

Síntesi d'anàlegs del cromòfor de la GFP. Precursors d'una nova família de polímers sense components metàl·lics.

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UNIVERSITAT DE BARCELONA

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Investigar es ver lo que todo el mundo ha visto, y pensar lo que nadie más ha pensado.

Albert Szent

En primer lloc voldria agrair la oportunitat d'haver pogut col·laborar en el grup d'investigació del Dr. López Calahorra, i la seva confiança mostrada en el treball diari.

També voldria donar les gràcies a l'Alexis, el que ha estat el meu professor i el meu company al laboratori. Gràcies per permetre'm sentir-me químic per primer cop i per deixar-me conèixer la química amb llibertat, tant les satisfaccions que aquesta pot arribar a aportar com les frustracions que provoca quan no s'aconsegueixen els resultats desitjats.

Donat que el científic, i en especial el químic, es un treballador d'equip, no m'agradaria personalitzar a l'hora de donar les gràcies.

Simplement agrair a tota la gent que ha format part del meu equip durant aquesta etapa, i, sobretot, per la que espero que sigui una companyia per tota la vida.

Gràcies per la vostra ajuda, la vostra paciència i per romandre al meu costat.

REPORT

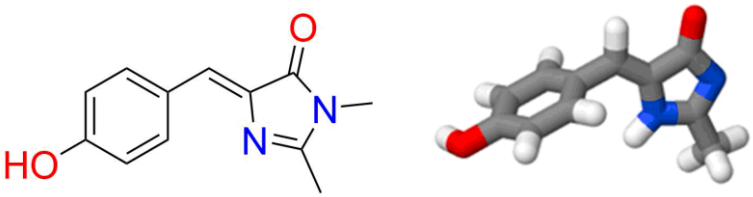
CONTENTS

1. SUMMARY

In this research study, the synthesis of a new set of organic molecules that present an analogous structure to the Green Fluorescent Protein's chromophore is described. This protein, has nowadays a paramount importance in several areas of scientific knowledge, because it is mainly used as a biological marker.

The analogous structures are formed by two aromatic rings bonded through an exocyclic carbon bridge. The first one is, in all the synthetized molecules, an electron-withdrawing heterocycle (imidazolone), whereas the electron delocalization in the other π-system depends on the different substituents (*R*), that have been selected.

In the specific case of GFP chromophore, the *R* substituent is an -OH group, located in the *para* position respect to the carbon bridge. Consequently, this second heterocycle forms an electron-donor system. As a result, the final structure of the GFP chromophore is 4-(p-hydroxybenzylidene) imidazolidin-5-one showed in the following figure.



p-HBDI

Left: Basic structure of p-HBDI. Right: p-HBDI rotation around the carbon bridge (C=C), generated with Molview.

Previous studies disclose that this chromophore, and some synthetized analogues, showed an unexpected magnetic behaviour, being non-diamagnetic molecules. This behaviour is due to the fact that there is a thermal equilibrium between the closed-shell singlet state and the excited triplet state, that is more stable when this type of molecule loss its planarity. This conformational equilibrium is called valence tautomerism.

The formation of a stable diradical in the triplet excited state, can be understood with the resonance stabilization of the two unpaired electrons found, each one, in both aromatic rings, through the carbon bridge.

The main objective of this study is to increase the existent family of this analogues with different substituents, in *para* position, to see the impact on the stable formation of the diradical triplet.

Subsequent studies will use these molecules to enable the anchoring in polymeric supports to check their magnetic response, and observe if a response amplification is produced by magnetic cooperativity between vicinal molecules. This fact could provide interesting advances in the development of new polymeric materials without metallic nor radical components in its structure.

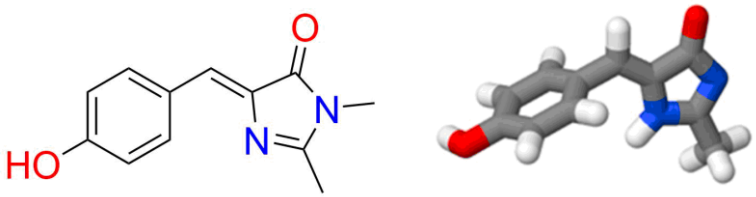
Keywords: diradical triplet, valence tautomerism, GFP, p-HBDI analogues, magnetic polymer, chromophore.

2. RESUM

En aquest estudi es descriu la síntesi d'un nou conjunt de molècules orgàniques que presenten una estructura anàloga al cromòfor de la GFP (Green Fluorescent Proteïn), la qual té una importància cabdal com a marcador biològic, i és objecte de diversos estudis de caire científic en l'actualitat.

L'estructura base d'aquests compostos consta de dos sistemes aromàtics units mitjançant un doble enllaç carbònic exocíclic. El primer dels anells és, en el cas de totes les molècules d'estudi, un heterocicle atractor o π-deficient (imidazolona), mentre que el segon sistema aromàtic és un heterocicle benzènic, la naturalesa química del qual, dependrà del substituent (*R*) que presenti aquest benzè.

En el cas específic del cromòfor de la GFP el substituent *R* és un grup -OH, en posició *para,* resultant aquest un heterocicle donador o π-excedent. L'estructura final del cromòfor de la GFP és 4-(p-hydroxybenzylidene) imidazolidin-5-one (p-HBDI) i es mostra a la següent figura.



p-HBDI

Esquerra: Estructura bàsiica de p-HBDI. Right: Rotació al enotrn del doble enllaç exocíclic (C=C), generat mitjançant Molview.

Estudis anteriors revelen que aquest cromòfor, i alguns anàlegs que s'han sintetitzat, presenten un comportament magnètic, diferent al convencional, tractant-se de molècules no diamagnètiques. Això es degut a l'existència d'un equilibri tèrmic entre l'estat fonamental de capes tancades i l'estat triplet excitat, més estable quan es produeix una pèrdua de planaritat en la molècula, equilibri conformacional conegut amb el nom de tautomeria de valència.

La formació d'un dirradical estable, corresponent al estat excitat triplet, pot deure's a l'estabilització per ressonància dels dos electrons desaparellats presents en cadascun dels anells aromàtics, a través del doble enllaç exocíclic.

L'objectiu del estudi és ampliar la família existent d'aquests anàlegs amb diferents substituents en posició para, per veure els efectes que tenen sobre l'estabilització per ressonància del triplet dirradicalari.

Estudis veniders possibilitaran l'ancoratge d'aquestes molècules a suports polimèrics de diversa naturalesa per tal de comprovar la diferent resposta magnètica dels anàlegs, i veure si es produeix una amplificació d'aquesta resposta per cooperativitat magnètica entre molècules veïnes, fet que podria ser de gran interès en l'estudi de nous materials polimèrics amb propietats magnètiques, amb la peculiaritat de no presentar àtoms metàl·lics en la seva estructura.

Paraules clau: triplet dirradicalari, tautomeria de València, GFP, anàlegs p-HBDI, polímer magnètic, cromòfor

3. INTRODUCTION

3.1. GREEN FLUORESCENT PROTEIN

Green Fluorescent Protein (GFP), is a protein that fluoresces in the green area of the visible spectrum and was discovered by Osamu Shimomura [(1)] as a companion protein to *Aequorin,* the chemiluminescent protein from *Aequorea Victoria* jellyfish. [(2)] [(3)] [(4)]

Therefore, the GFP is not a chemiluminescent protein that produces light itself through chemical reactions, instead of that, fluoresces after absorb more energetical light.

Shimomura soon published the absorption and emission spectrum of GFP, which presents a peak of emission at 509nm, and two peaks of excitement. The principal absorption band peaking at 395nm with a minor band having a maximum at 475nm. Excitation of wild-type GFP with UV light at 395nm produces fluorescence emission at 509nm, according to the Shimomura experiments. [(5)]

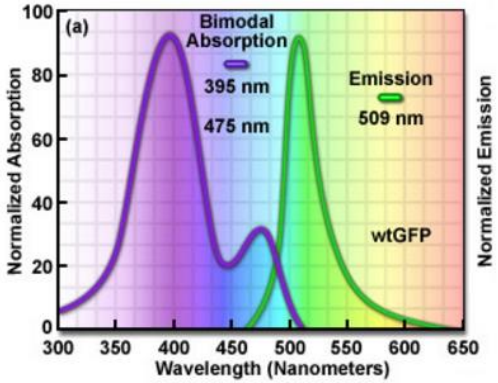


Figure 1: Emission-Absorption spectrum of GFP *(imatge taken from Zeiss et al, ref. 6)*

In addition, they noted that the chemiluminescence light of pure *Aequorin* was blue and peaked near 470nm, which was so close to one of the excitation peaks of GFP. Subsequent studies confirm that the GFP turn the blue light emission of *Aequorin* into the green glow of the intact cell and animals. [(7)] [(8)]

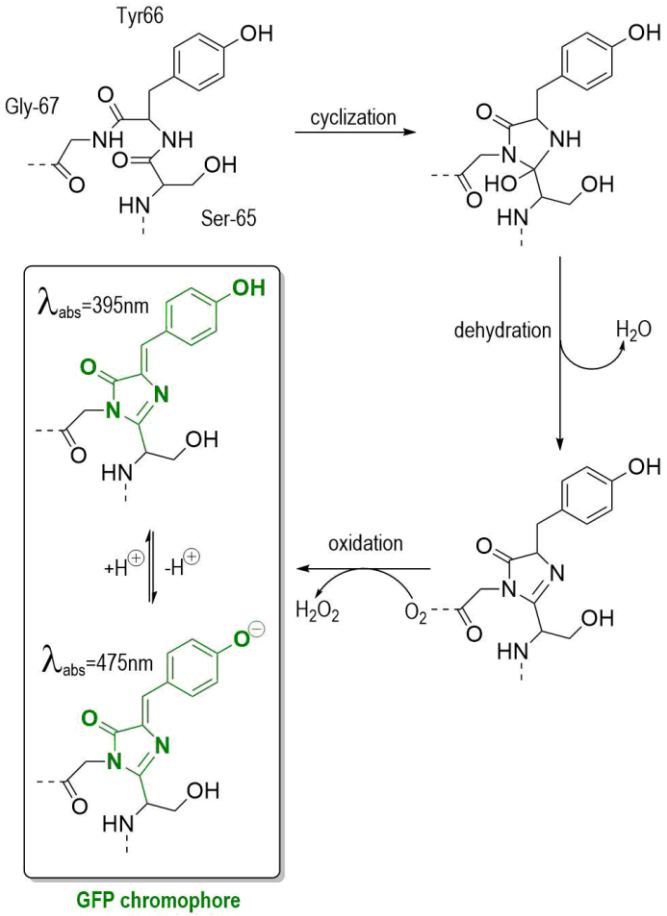


Figure 2: Natural formation of GFP chromophore's. Absorption wavelenghts of protonated and deprotonated p-HBDI.

Ward et al. [7] [8] observed that if they irradiate the protein with cyan-blue light at 480nm, it results a peak emission at maximum 503nm. This confirm the presence of two chemically distinct species in the wild GFP, the neutral form of GFP (OH substituent) and the anionic form, deprotonated (O^-). In conclusion, the origin of the bimodal absorption and dual emission phenomena is attributed to the existence of an equilibrium between this two protonation states of the chromophore (neutral and anionic). These two species exist in a ground-state equilibrium.

It's in 1979 and after other works that [5] proposed the structure of GFP chromophore and its spontaneous mechanism formation from a -Ser65-Tyr66-Gly67- tripeptide.

The complete structure of GFP was described by Tsien in successive studies.[9] The protein is made by 238 amino acid residues that form eleven beta chains, with six alpha helixes, which combination form a beta barrel, forming a structure similar than a cylinder. In the middle of this one, we find the chromophore, linked by a covalent bond to an alpha helix.

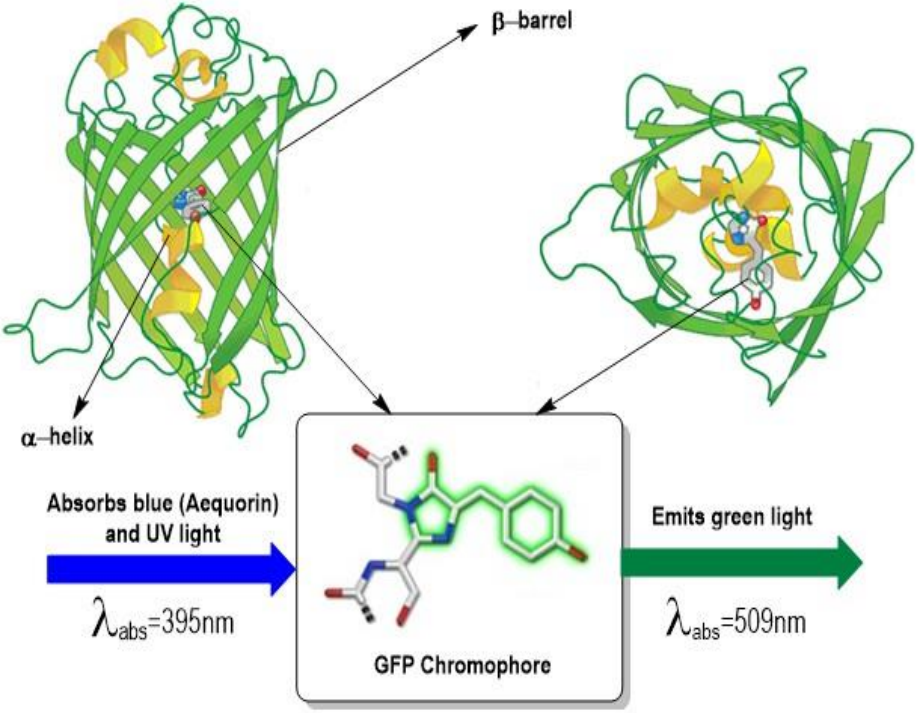


Figure 3: Complete structure of GFP showing the chromophore disposition inside of the beta barrel.

[10]Therefore, the GFP is unique among fluorescent proteins in that its fluorophore is not a separately synthesized prosthetic group, it's composed of modified amino acid residues within the polypeptide chain.

Prasher et al.[11] clone the gene and Inoue and Tsuji [12] demonstrate that expression of the gene in other organisms creates fluorescence. These two studies conclude that the GFP contains all the information necessary for the synthesis of the chromophore, without specific enzymes of the jellyfish needed. That combines with the idea that, the denaturized GFP and the isolated chromophore are poorly fluorescent outside of their protein shells.

Owing to that reason this protein is used nowadays in many scientific areas, particularly in medicine, genetic engineering, microbiology and biochemistry. As a consequence of all these characteristics, it is used as a marker of processes that were invisible in the past, and that would provide us large amounts of information about them. For instance, inter alia, processes involved in neurology development, how is the spreading of cancer cells in the human body, the development of Alzheimer's disease, or study the viral invasion of AIDS in the organism, and the different celerity in appearing his impacts. [13][14][15]

3.2. ANALOGUES OF GFP CHROMOPHORE

Besides all the applications mentioned above, the López Calahorra's research group has proved that the chromophore of the GFP has a triplet electronic state stable at room temperature, that's why we could expect a paramagnetic response of the solid formed after the anchorage of this type of molecule to the polymer resin.

Our aim is to extend the family of this type of molecule by changing the substituent in *para* position to the carbon bridge, or adding another type of substituents in *ortho* or *meta* positions, to see the effects of electron delocalization in these cases. To achieve our goal an alternative synthetic route is proposed, through the oxazolones synthesis. The oxazolones are derivatives of the oxazol group and the imidazolones, derivatives of the imidazole group.

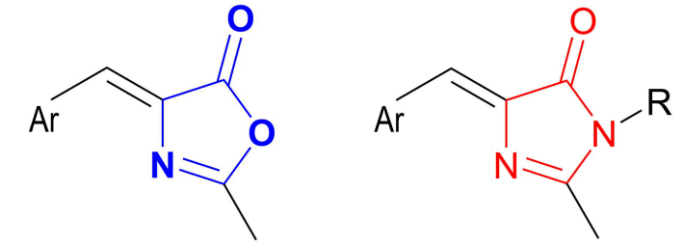


Figure 4: Oxazol group (blue) and Imidazole group (red)

3.2.1. Benzenic heterocycle substituents

The molecules described in this work, consist of an electron-withdrawing heterocycle (oxazolone or imidazolone) linked by an exocyclic carbon-carbon double bond to a substituted benzene, that could act as an attractor or donor heterocycle, according to the type of substituent used.

3.2.1.1. Oxazolones

A wide spectrum of oxazolones have been obtained for their subsequent conversion into discrete imidazolones, some of them have been synthesized in this same study, and their anchoring to a resin polymer in later studies, where their magnetic behaviour will be analysed.

To start, oxazolones have been obtained, with the benzene system monosubstiuted, focusing on the substitution in para, with resonance electron donor substituents, owing to the fact that is expected a similar magnetic behaviour of the GFP chromophore, achieving a good yield in all cases, as will be explained later.

- *Para*-substitution:
  - Electron donor substituents by resonance (π-excessive): -OH, -$N(Me)_2$, -$OCOCH_3$, -NH_2
  - Electron attractor/withdrawing substituents by resonance (π-deficient): -NO_2, -CN
  - Electron donors by resonance and electron attractors by strong inductive effect (halogens): -Br, -Cl
- *Ortho*-substitution: -Cl, -F
- *Meta*-substitution: -F

Different attempts have been made to acquire three oxazolones with a disubstituted benzene system, which only the **(13)** has been satisfactorily achieved.

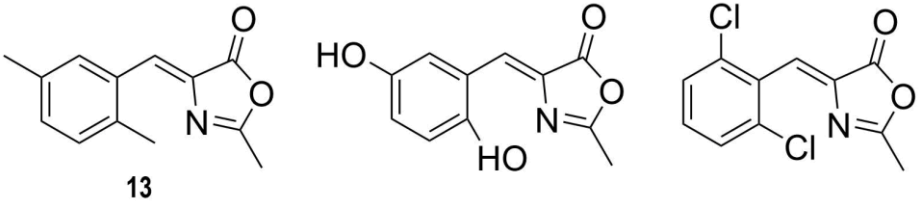


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Figure 5: Disubstituted oxazolone **(13).** (2,5-Dimethylbenzylidene)-2-methyloxazol-5-one

3.2.1.2. Imidazolones

Only substituted in para position, analogous to the substitution observed in our reference biomolecule, or without substituent (*R*).

- Electron donor substituents by resonance (π-excessive): -OH, -$N(Me)_2$, -Br, -Cl
- Electron attractor/withdrawing substituents by resonance (π-deficient): -NO_2, -CN

These groups have been selected for the following reasons:

-**OH**: The OH substituent was chosen because it is the substituent that presents the GFP chromophore.

-**N(Me)$_2$**: A disubstituted ammine was chosen because is an excellent electron donor group, similar as the OH group, and it will give electron density to the benzene ring, theoretically stabilizing the diradical.

-**NO$_2$**: The nitro group was picked because it has the opposite electronic behaviour respect the OH substituent. This substituent is an electron withdrawing group and therefore attracts electron density of the ring. We would know if that favour the formation of the diradical, in the triplet state.

-**CN**: The nitrile group was selected for the same reason than NO_2, with an additional interest on the future polymerization. The polymers are identified by IR, and the CN group has a very representative signal at 2100cm^{-1}.

-**Br/-Cl**: Halogens like bromine and chlorine were selected because of their special electron attraction by inductive effect in addition to their capacity to donate electronic charge with the pairs of electrons not bonded directly to the benzene ring.

Non-substituted benzene: In order to check if the substituent has a significant role with the stabilization of the electronic triplet state with two free radicals.

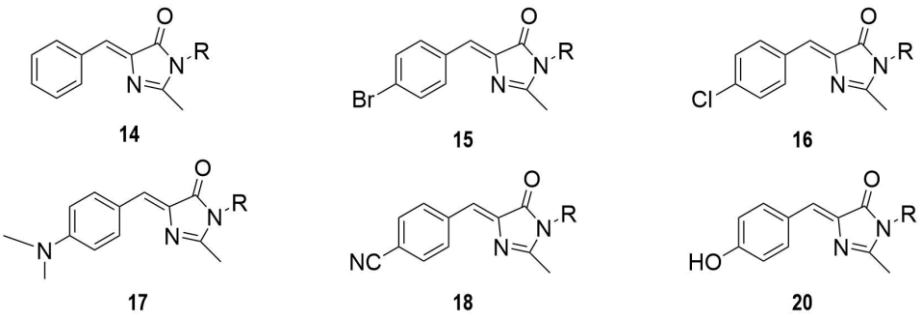


Figure 6: Imidazolones structures synthesized in this study.

3.3. CONFORMATIONAL EQUILIBRIUM OF THE GFP CHROMOPHORE

To explain the magnetism of p-HBDI analogues, demonstrated in several studies [16][17][18] previously is convenient stablish a theoretical model, in order to understand the special behaviour that p-HBDI adopts, to reach a magnetic response if an external magnetic field is applied.

It is thought that the phenomenon of GFP fluorescence, already explained, and the ability of this organic molecule to reach a diradical structure through a thermal equilibrium, are not related. Although, it could be the object of extensive study to corroborate that this is truly and the biological activity of p-HBDI is not intrinsically related to diradical conformation.

The mechanism that allows our natural product to adopt the thermally available triplet diradical structure is not described in the literature. Nevertheless, in the studies its magnetic response after photochemical excitation is demonstrated by carrying out RPEs.

In the Thesis of Carlos Heras [19] a theoretical model is presented explaining the behaviour of p-HBDI, supported in other studies about the valence tautomerism. [20][21] Then, an explanation is given for the conformational equilibrium that takes places on the GFP's chromophore.

The origin of the paramagnetic phenomenon in the solid state, is based on the double carbon bridge that are established between the two heterocycles.

The C=C free rotation allows to stabilize the unpaired electrons thanks to the conjugation extension in the aromatic rings, and the substituents that are able to stabilize this charge on their free orbitals.

At this point it is where relapses the interest of the synthesis that is carried out in this study to analyse if stable p-HBDI analogues could be afforded with a good yield.

Moreover, in subsequent studies will be tested if any analogue presents a greater magnetic response than our reference, which has a substituent -OH in *para* position.

If we considerate the double carbon bond like a hybrid between sp^2 and sp^3, it is possible to understand the double rotation that takes place around this bond in the molecule.

According to Weber's study [22] this fact is explained through a hula-twist motion (HT), a simultaneous movement around de C-C bond with intermediate hybridization.

When the rotation occurs, the push-pull system breaks up and thus fluorescence is not produced.

The major part of the scientific works around GFP, prefers to present this phenomenon as underlying, in the other two predominant phenomena, that are tentatively independents. The first one, widely explained is the fluorescence.

The fluorescence is manifested when an electron passes from a singlet excited state to the closed shell ground state.

This will occur when either of dihedral angles existing in the molecule, α or β will rotate (Figure 7). What's more this will be the only effect if the chromophore rotates on its the β angle.

The other conformational movement involving a loss of planarity in our system is related to the rotation of the dihedral angle α.

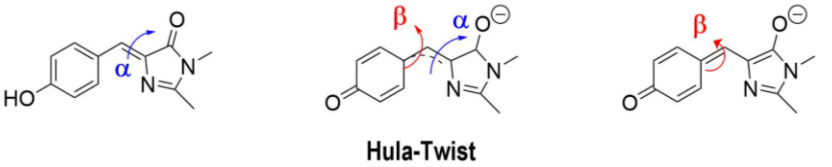
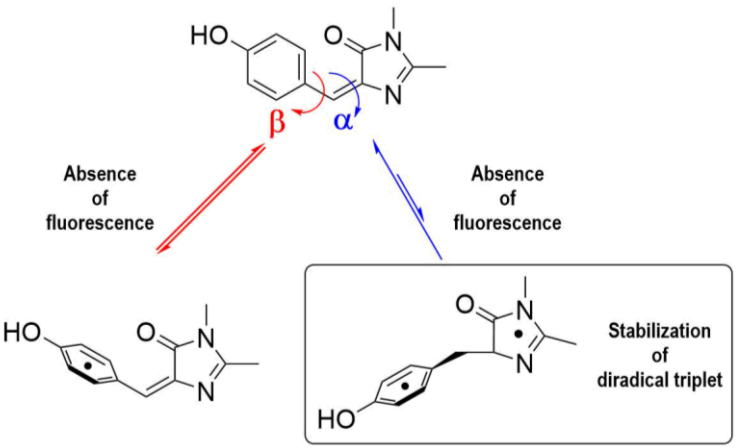


Figure 7: Different rotations allowed around de (C=C). Hula-Twist motion: simultaneous rotation around both bonds. α and β are the dihedral angles.

When there is a rotation through the carbon bridge via variation of the angle, involves a loss of planarity in our molecule, which causes the access to an open-shell system, the diradical triplet. The stabilization of the excited state is produced by this angle, when is more close to 90 degrees.



This conformational equilibrium is called valence tautomerism. [19] [23]

Figure 8: Conformational equilibriums of p-HBDI

Only a small percentage of the p-HBDI molecules will present valence tautomerism. Our intention is to synthesize similar species, analogues, that this conformational equilibrium is favoured, changing the substituent in *para* position.

In other words, due to the valence tautomerism, a transition towards the diradical triplet system T_1^{BR} will be produced from the ground state S_0 through a spin-crossover coupling.

Both conformational equilibrium happens in the neutral form and in the deprotonate form of p-HBDI. In the case of fluorescence, the response was greater in the deprotonate form, therefore, is logical to think that this form also could have a greater magnetic response. **(24)**

Valence tautomerism will be favoured in the deprotonate form, setting with more probability a bond with a hydrogen of other molecule of p-HBDI (making a hydrogen bridge), increasing the magnetic response in the polymer. **(24)**

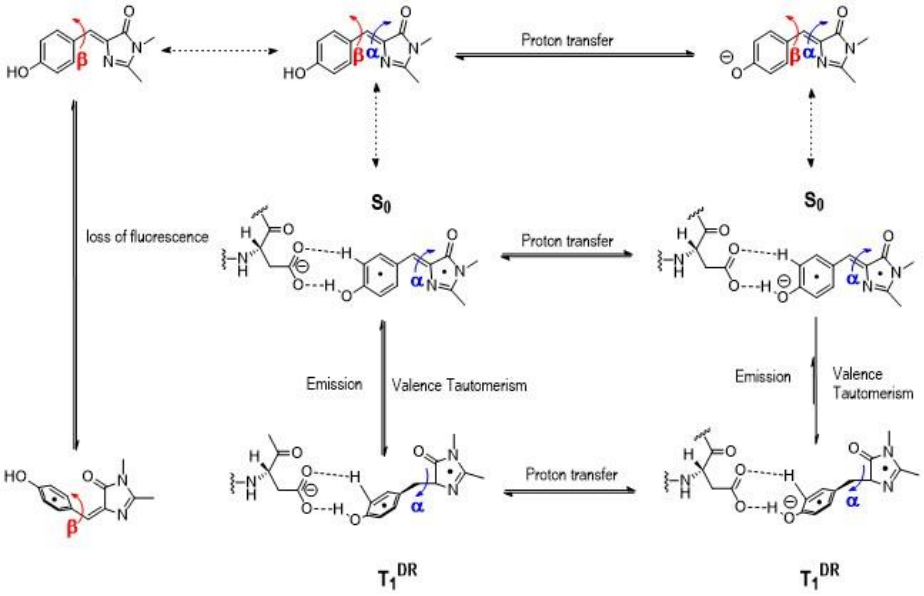


Figure 9: Scheme of diferent conformational equilibriums, acid-base and valence tautomerism on the p-HBDI molecule.

3.4. MAGNETIC BEHAVIOUR

The organic compounds have, in general, a disposition of their energetic levels similar than the schematic representation showed in the left of the Figure 10, in four levels.

From lowest to highest energy, we found first, with the minimum energy the ground state S_0 with a closed-shell disposition. This confer a diamagnetic behaviour to the vast majority of organic molecules.

After, the diradical states, $\mathbf{S_1^{DR}}$ and $\mathbf{T_1^{DR}}$, are found virtually degenerated, and the top of energy is associated with de **CT** level, which refers to the charge transfer.

López-Calahorra's research group had studied the apparent paramagnetic character of some organic heterocyclic molecules without any metallic atom existence, like our reference, the p-HBDI.

They have confirmed that these molecules present magnetism in solid state between 4 and 300ºK, obtaining an EPR with the typical signal of a triplet state at 3400.

As discussed in the previous section, the paramagnetic behaviour in solid state, is related with a push-pull system due to the breaking of the double bond, forming a conformational equilibrium between the S_0 (0º dihedral angle) and the T_1^{DR} (very stable when the dihedral angle is 90º). In addition, T_1^{DR} is stabilized by resonance through the exocyclic carbon bridge. In conclusion, if dihedral angle is 0º or close to this angle, the molecule is in a planar conformation, which favours the electronic fundamental state, S_0, as expected in most organic compounds, all electrons are spin paired in this electronic state which leads to a paramagnetic behaviour.

If we analyse the magnetic field generated by these molecules using an EPR, we would not have an apparent response.

According to the previously explained, if the dihedral angle is changing this ground state progressively increases in its energy being less stable. At one point of this rotation, the ground and excited state potential surfaces, may approach even cross each other in a conical intersection, leading to rapid deactivation of this ground state, S_0.

Near to 90º is the point where the diradical triplet and the diradicalarian singlet, with almost the same energy, are stabilized, although the triple state is always more stable.

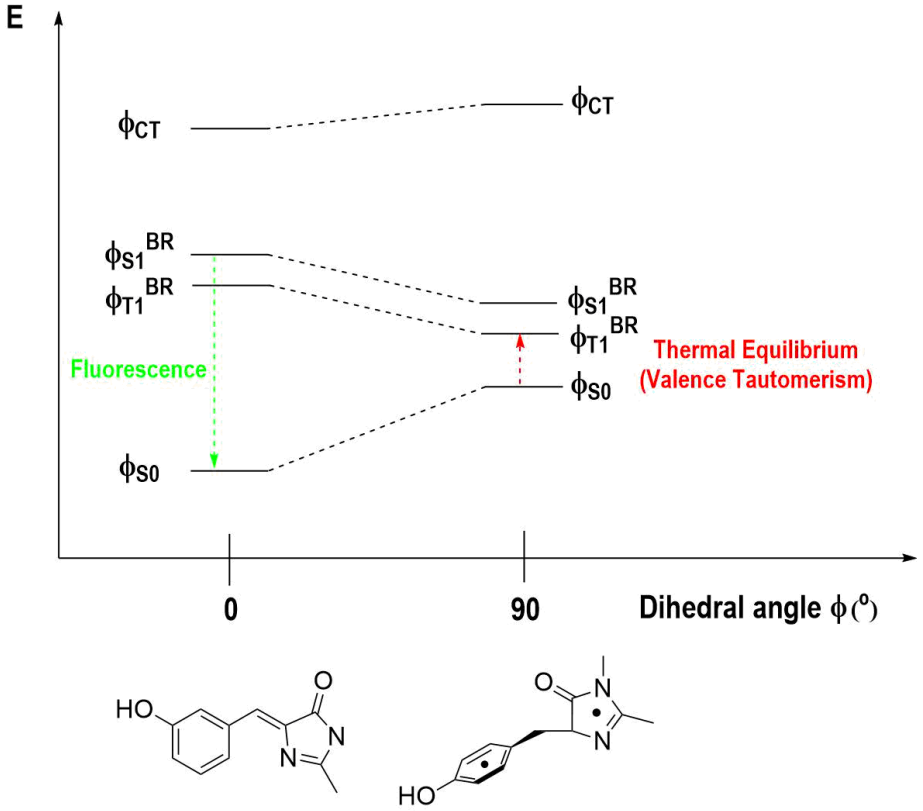


Figure 10: Schematically representation of the energetic states in basis of the change of rotation (dihedral angleΦ).

The formation and above all the stability of this kind of biradical molecules have two main causes.

On one hand, the rotation of the methylene group, lead to the loss of planarity in the molecule, this create a strongly decreased bond order in the diradical triplet, changing the exocyclic carbon atoms hybridization from sp^2 to sp^3. It is also added the effect that these two aromatic systems stabilize the diradical form with an electronic delocalization detailed in the Figure11.

To conclude, it should be noted that previous studies demonstrate that the magnetic response is amplified by anchoring it to a polymer. This, may be due to the interactions explained between neighbouring p-HBDI molecules. In this work, we will try to synthesize a broad spectrum of GFP analogues, in order to fin done or more molecules that show a paramagnetic response even greater that shown by this chromophore. This fact would be an interesting revelation for the creation of magnets without metallic atoms.

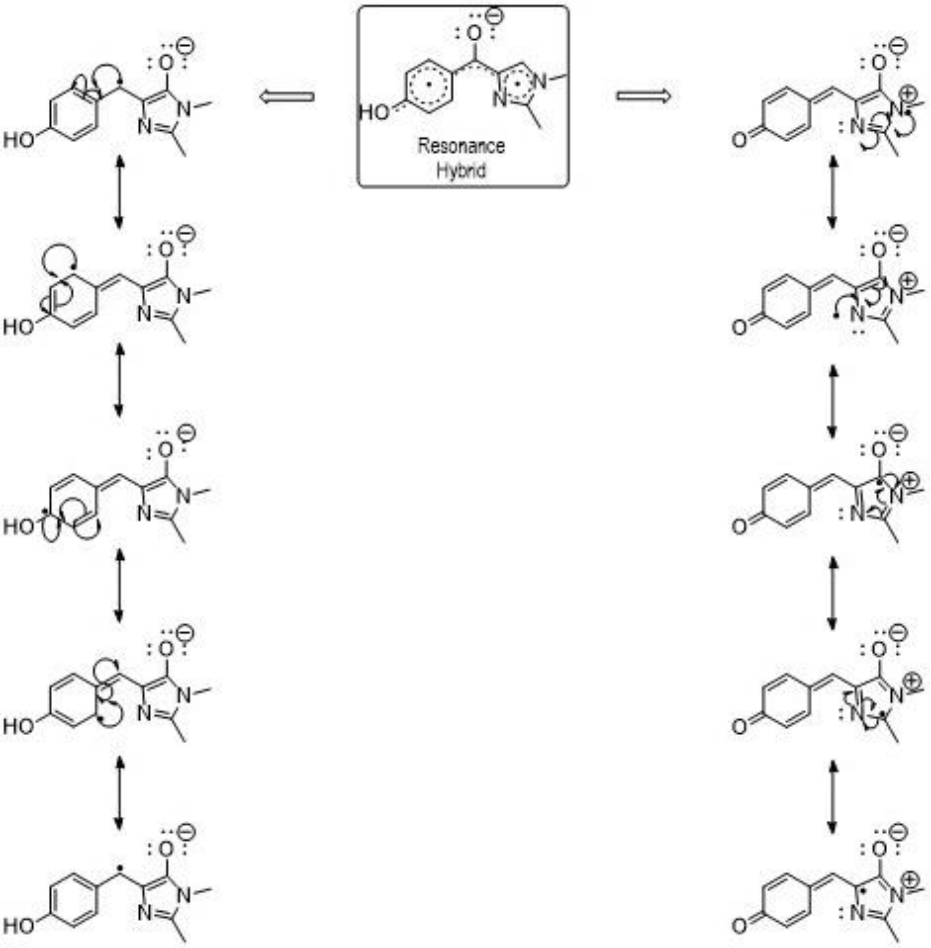


Figure 11: Stabilisation thanks to the electronic delocalization analysis of the two unpaired electrons between the aromatic systems in the biradical form that adopts the GFP chromophore.

4. OBJECTIVES

The main objectives of the project are:

- The production of high purity p-HBDI, through a replicable synthetic process to further works, with a good yield.
- To develop an effective synthetic route for a wide range de of imidazolones, with an analogous structure to p-HBDI, varying the benzene's heterocycle substituent.
- To produce a suitable functionalized imidazolone in order to anchorage it to a polymeric support.
- To check the stability conferred by the different substituents to the diradical triplet state and try to stablish a behaviour model of the imidazolones depending on the electron donating or electron withdrawing character of these substituents.
- To obtain oxazolones, with the same general structure as the imidazolones, (Aryl Heterocycle-Carbon Bridge-Electron withdrawing heterocycle), for its future magnetic analysis with the aim of establishing which is the minimum structural unit that produces this paramagnetic behaviour.

5. RESULTS AND DISCUSSION

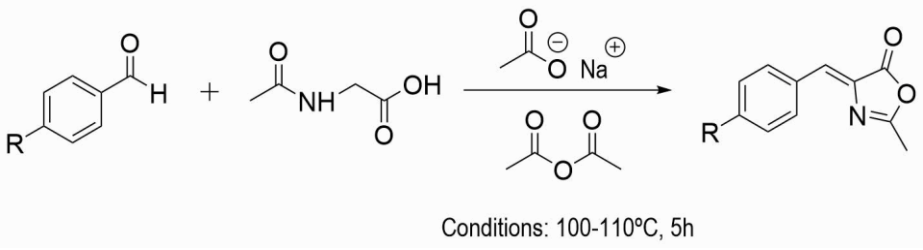
This section is divided into three parts. The first part explains the mechanisms used on the oxazolones synthesis. Subsequently, it is elucidated the conversion of oxazolones into imidazolones, which have an analogous structure to the studied biomolecule. The explanation of the complete GFP chromophore synthesis is the last section.

5.1. OXAZOLONES SYNTHESIS

Monosubstituted and disubstituted oxazolones have been synthesized for two principal reasons:

1. Oxazolones and imidazolones have a similar structure, complying with the necessary structural requirements to carry out the future study about the stabilization of the diradical triplet state.
   This structural unit is: Electron attractor or donor heterocycle, (depending on *R* of benzylidene)/Carbon Bridge/Electron-withdrawing heterocycle (oxazol).
2. The imidazolone synthesis from the previous oxazolone production is an effective method to insert a large number of substituents with different electronic behaviour.

Preparation of azlactones is produced by a condensation reaction, that follows the scheme 1:



Scheme 1

Arylaldehydes react with N-acetylglycine, in presence of sodium acetate (NaOAc) and anhydride acetic (Ac2O) utilized as solvent. The reaction is at reflux for 5h (100-110ºC). This reaction is known as Erlenmeyer azlactone reaction. The quantities are specified in the Experimental Section. The reaction is produced by the mechanism shown in Figure 12.

It is a Knoevenagel condensation, an organic reaction that is a modification of the aldol condensation. The Knoevenagel condensation consists on a nucleophilic addition of an active

hydrogen compound (N-acetylglicine) to a carbonyl group, followed by a dehydration reaction in which a molecule of water is removed. The obtained product is an α,β-unsaturated ketone, or what is the same, a conjugated enone. In this specific case, we have an esterification of our carboxylic acid (N-acetylglicine) that produces the coupling. Later, the steric disposition favours the formation of a stable heterocycle, resulting our final product, the oxazolones.

Most oxazolones are obtained with satisfactory yields by the procedure described and the data are shown on the following tables.

In these tables the substituents are ranked in decreasing order according to their ability to transform the benzene heterocycle into electron donor, a part from (1) and (2,) that was on the top list due to their importance to obtain our natural product, p-HBDI, in spite of it, (1) and (2) are also strong electron-donor heterocycles.

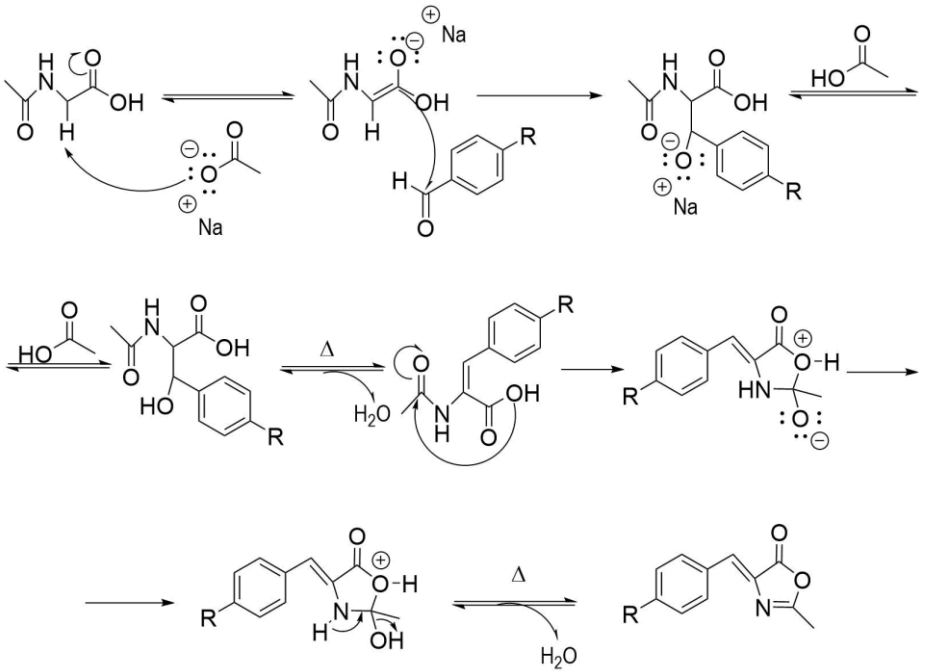


Figure 12: Mechanism of Erlenmeyer Azlactone reaction.

5.1.1 Monosubstituted Oxazolones

5.1.1.1. Para-substitution

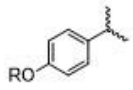
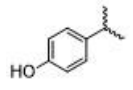
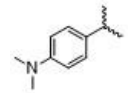
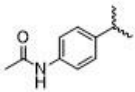
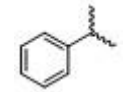
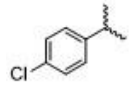
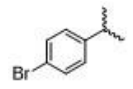
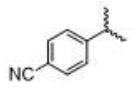
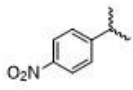
Entry	Aryl Substituent	**Product** (Yield)
1[a]		Product **1** (80%)
2		Product **2** (78%)
3		Product **3** (51%)
4		Product **4** (67%)
5		Product **5** (99%)
6		Product **6** (81%)
7[b]		Product **7** (77%)
8		Product **8** (84%)
9[a]		Product **9** (87%)

Table 1: Summary of data obtained during the synthesis of para-substituted oxazolones.

5.1.1.2. Different from para-substitution

Entry	Aryl Substituent	**Product** (Yield)
1		Product **10** (78%)
2		Product **11** (92%)
3		Product **12** (80%)

Table 2: Summary of data obtained during the synthesis of meta-substituted and ortho-substituted oxazolones.

5.1.2 Disubstituted Oxazolones

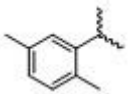
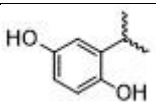
Entry	Aryl Substituent	**Product** (Yield)
1		Product **13** (33%)
2		Product **21** (N.D.P)
3		Product **22** (N.D.P)

Table 3: Summary of data obtained during the synthesis of disubstituted oxazolones.

It can be concluded that substituted oxazolones are synthesized with great yields without detect an effect of the different substituted positions nor the electronic nature of the substituents.

The steric effect is the only variable that generates low yields, and it is the reason for complications in the disubstituted oxazolones synthesis, forming undesired products.

5.1.3. Unexpected behaviour in the production of oxazolones

*5.1.3.1. Production of Oxazolone **(4)***

On the oxazolone synthesis produced with the substituent acetamide in para position, an orange solid which presents a heterogeneous appearance after filtration is obtained.

After carrying out several solubility tests with this product, it was decided to wash with hexane.

A HMRS is performed, confirming the main presence of **(4).**

The product was purified by a chromatography column, using AcOEt:Hexane (1:1) as eluent.

(4) is not the final desired molecule, because we are not interested in products with acetylated substituents, which could generate undesired secondary reactions when the imidazolone is anchored to the amine polymer, in an analogous case of the obtaining of **(1)** instead of the direct obtaining of **(2),** that is the desired oxazolone to produce p-HBDI.

5.1.3.2. Synthesis of 4-(4-aminobenzylidene)-2-methyloxazol-5-one depart from (4)

An acid hydrolysis is carried out with the objective of produce the open chained product to synthesize the desired oxazolone after with a cyclization, but the acidic medium explains the production of an ammonium salt in its place.

It is difficult to obtain the desired molecule. After analysing the structure of the experimentally obtained one after the ring-opening reaction of (3), it has a protonated amino group and a carboxylic acid group. It is not possible to establish a pH range in which this molecule has the amino group in the neutral form (-NH_2) and the carboxylic acid in his protonated form, because the carboxylic acid group (with a very low pKa, around 1) loses its proton before protonated amino group (NH_3^+) with a pka around 10.

It is concluded that for the synthesis of ,*4-(4-aminobenzylidene)-2-methyloxazol-5-one*, other synthetic route must be used, probably the simpler one through 4-aminobenzaldehyde, and an analogous reaction to the other oxazolones with de Erlenmeyer azlactone condensation.

*5.1.3.3. Synthesis of (2,5-Dimethylbenzylidene)-2-methyloxazol-5-one **(13)***

Initially an intense orange color is obtained which turns out to be a mixture of two products

The final product could be synthesized after carrying out a chromatography column using AcOEt as eluent.

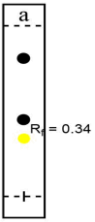
The sole presence of the desired product is confirmed by HRMS and COSY.

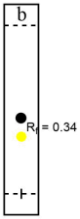
*5.1.3.4. Production of Oxazolone **(22)***

The **(22)** synthesis is tried by the condensation reaction explained previously. Nevertheless, in the mixture obtained, which is a brown oil, and according to the mass spectrum, the main product has acetylated one of the two alcohols, as in the case of the synthesis of p-HBDI precursor. In the TLC it is seen that there are three different compounds, the starting reagent with a high retention factor and two products with a similar Rf, probably the acetylate oxazolone and the desired product with two hydroxy groups, the diol.

A column is required to try to separate both products, so it is made using as eluent AcOEt:Hex (1:2).The TLC's of the product before and after the column are shown (Rf of desired product=0.34)

TLC (Before the column) **TLC (After the column)**





Unfortunately, the desired product cannot be obtained in an acceptable proportion, so purification is not carried out.

5.2. SYNTHESIS OF IMIDAZOLONES

After the oxazolones production, the synthesis of GFP chromophores analogues, known in general terms as imidazolones, is initiated.

This process has place through two approaches. The first one consists on a direct condensation of the oxazolone with primary amines in the presence of a base, but the results were unsatisfactory. Because of that, a second synthetic route was designed.

It was proposed a two-step synthetic route, through one ring opening reaction for the subsequent dehydrative cyclization.

5.2.1. First approach (Route A)

This synthetic route through the direct condensation of oxazolones with primary amines is widely described in [25] [26] [27] [28] and follows the figure 13 mechanism. Despite being the most used method, it does not present great experimental results, obtaining very low yields in the synthetized products, as it can be seen in the study of Lee et al. [29]

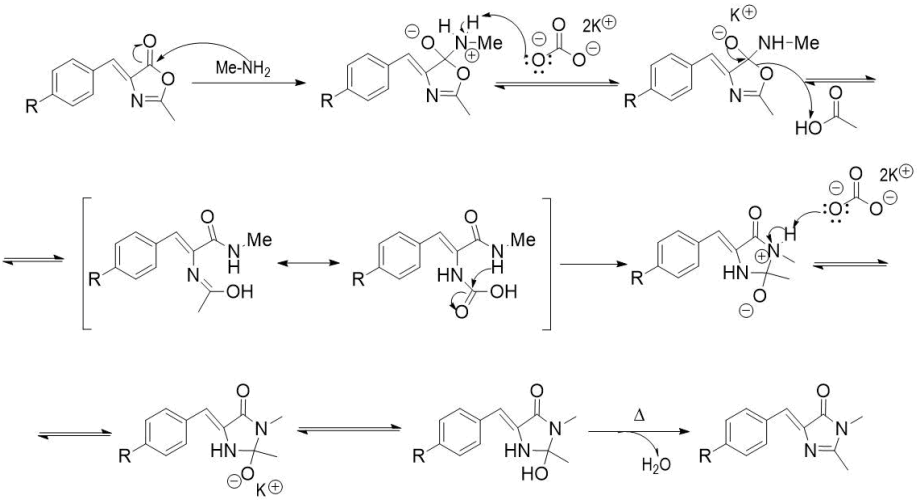
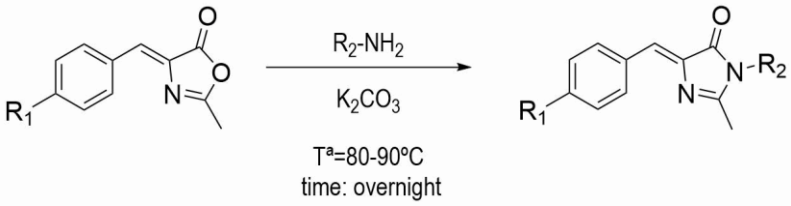
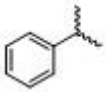
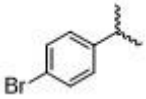
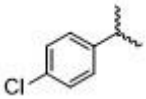
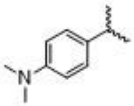
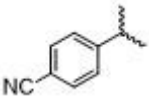
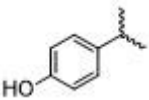
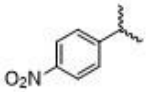


Figure 13: Mechanism of imidazolones formation from oxazolones with a direct condensation in presence of a primary ammine.

The amine behaves as a nucleophile attacking the carbonyl and opening the cycle previously acquired (oxazolone). The K_2CO_3 acting as a base, remove a proton of the amine, and causes the ring opening. After that, an intramolecular attack of the generated amide by the first nucleophilic attack, occurs, generating again a five-member stable ring, where the N of the amide replaces the O of the oxazol.



Scheme 2: General scheme of the transformation of oxazolone into imidazolone.

Entry	Aryl Substituent	R2	**Product** (Yield%)
1		Bn	**14** (33%)
		Me	N.D.P
2		Bn	**15a** (11%)
		Me	**15b** (7%)
3		Bn	**16** (9%)
		Me	N.D.P.
4		Bn	**17a** (21%)
		Me	**17b** (9%)
5		Bn	N.D.P.
		Me	N.D.P
6		Bn	**20a** (29%)
		Me	**20b** (20%)
7		Bn	N.D.P
		Me	N.D.P
8		Bn	N.D.P
		Me	N.D.P

a) NDP= Not desired product

Table 4: Summary of data acquired during the synthesis of imidazolones, through the Route A.

These conclusions can be deduced about the information of the Table 4.

In general, the first thing that can be observed is that the obtained 4-arylidene-5-imidazolinones, are produced with very low yields, even in the presence of an excess of a nucleophilic amine.

This synthetic route provides the obtaining, with poor yields, of imidazolones which have an aryl group able to act as an electron-donor. Imidazolones with strong electron-withdrawing substituents have not been obtained.

The best results are reached utilizing benzylamine than methylamine, as shown in previous studies. [27] [29] Knowing that methylamine is more nucleophilic than benzylamine, the most probable cause of the more positive results obtained with benzylamine is that, due to its steric impediment, it can prevent the competence generated by another nucleophile as the solvent EtOH with greater efficiency than methylamine. Also favouring the later intramolecular attack, without taking place undesired reactions.

5.2.1.1. Different behaviour to the general scheme to Route A:

5.2.1.1.1. Synthesis of 4-(4-Dimethylaminobenzylidene)-1-benzyl-2-methylimidazol-5-one **(17a)**

A solid is obtained through the condensation reaction with benzylamine, and after confirming by HMRS the presence of the expected molecular mass, in the RMN-H it is observed the appearance of two type of signals in the zone of aromatic compounds. At first it was thought that the product might have decomposed in the column, since previously, apparently, in the column only it was seen a product with smaller impurities.

Finally, it is deduced that it is because of the double bond rotation, that generates one isomer E and another one Z, obtaining the two products that cannot be separated.

5.2.1.1.2. Synthesis of 4-(4-Dimethylaminobenzylidene)-1-methyl-2-methylimidazol-5-one **(17b)**

In the compound **(17b)** preparation with benzylamine and the oxazolone **(3)**, it was seen that through acidifying the mother liquors with 10% HCl, two different phases were separated, being the upper one of an intense red colour. It remains cooling down and it had not seen precipitate, but a thin thicker oil layer is seen.

In view of this fact, and due to the colouring, reminding the generated product as oxazolone, it had been proceeding to its liquid-liquid extraction with AcOEt.

The organic phase was separated to be dried with $MgSO_4$, and it was filtered. The solvent present was removed under reduced pressure.

A chromatography column of the product has been done with AcOEt:Hexane (1:2) increasing progressively the polarity. After their realisation, an intense orange solid was obtained, changing finally to a purple colour when it was washed with Et_2O.

It has been confirmed by RMN^{1}H y HMRS that it was our desired product.

5.2.1.1.3. Synthesis of 4-(4-Cyanobenzylidene)-1-benzyl-2-methylimidazol-5-one **(18)**

An intense orange oil is obtained. The purification is carried out after observing by TLC three different spots and being one of them the starting product. By high HRMS it is confirmed that the desired product is present in the mixture.

A satisfactory separation is not obtained by doing the column with the most used eluent on the synthesis of imidazolones , AcOEt:Hexano (1:1).

5.2.1.1.4. Synthesis of 4-(2-Chlorobenzylidene)-1-benzyl-2-methylimidazol-5-one

It is obtained a pale purple oil. After proceeding with the work-up, a brown solid appears, which does not present an uniform aspect. A thin layer chromatography is carried out in which 3 spots can be observed, being 2 of them overlapped.

The characterization through HRMS appears to confirm that there has been no cyclization, but it has appeared an open chain product an a high molecular mass product, which indicates that there has been another cyclization because of having the substituent in the ortho position. Although the expected mass is found, it is in very low proportion and the purification of the product it is not carried out.

5.2.1.1.5. Synthesis of 4-(4-Nitrobenzylidene)-1-benzyl-2-methylimidazol-5-one **(17b)**

Solid is not obtained. In its place a very dense dark brown oil appears, which is similar in colour to the oxazolone that was synthesized previously to purification.

The compound purification is not performed because the spectrometric tests show the presence of several unknown compounds, and the desired one is not in the higher proportion.

5.2.2. Second approach (Route B)

With the results obtained after the first approximation, a second synthetic route to achieve the imidazolone is designed. The condensation process is divided into two independent processes, in order to increase the yield of each stage to the maximum.

Step 1: It consists on a ring opening reaction of oxazolones previously synthesized with a primary amine, in more excess proportion than in the first route and during more time reaction, with the objective of favouring as maximum as possible the nucleophilic attack of these compounds over the carbonyl of oxazol group. Open chain molecules, in general, 2-acylamino-3-arylacrylamides, are synthesized.

In general, after obtaining the reaction product (an oil), the solvent is eliminated under reduced pressure. Purification by chromatographic column it is initiated with AcOEt:Hexane (1:2); in order to eliminate the first impurities and polarity is increased up to AcOEt:Hexane (1:1).

Step 2: The dehydrative cyclization of this product is carried out, for obtaining the imidazolones. It is performed with two different bases, K_2CO_3 and pyridine, being with the last one the only that get successful results.

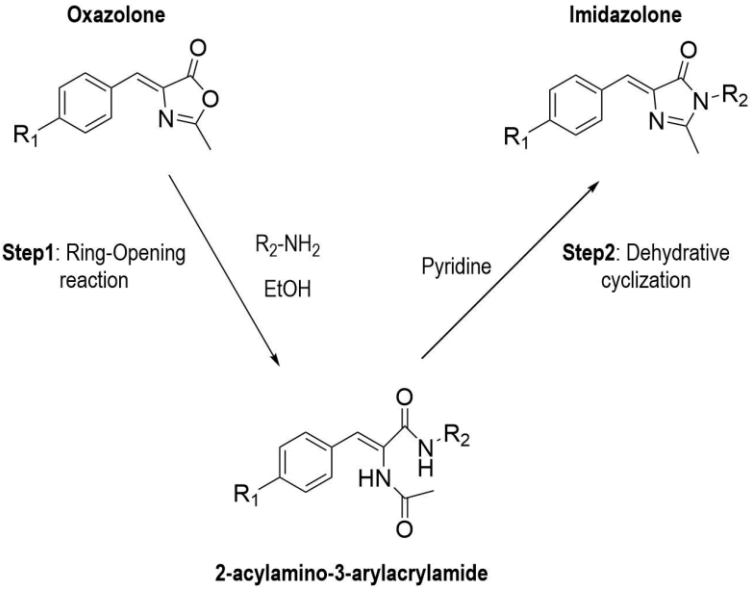
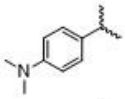
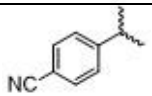
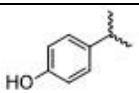


Figure 14: Scheme showing the two steps involved in the second approach of the imidazolones production (Route B)

Entry	R2	Aryl Substituent	Base	**Product** (Yield%)
1	Me		Pyr	**17b** (39%)
	Me		K_2CO_3	**17b** N.D.P
2	Me		Pyr	**18** (20%)
	Bn		K_2CO_3	**18b** N.D.P
3a	Bn		Pyr	**20a** (32%)
	Me		Pyr	**20b** (48%)

a) Only the best results of **(20)** production are showed, all the tests are in section **5.3.4.**

Table 5: Summary of data acquired during the synthesis of imidazolones, through the Route B

The next conclusions can be deduced about the data seen in this table.

The separation of the process in two stages allows for improving the results. Moreover, it increases the spectrum of usable chemical compounds to carry them out, especially in relation to the cyclization. Although in this study only two compounds are used, because of time constraints, being the pyridine which offers better results, it can be searched other bases that optimize the process.

This synthetic route allows for obtaining imidazolones with electron-withdrawing substituents, as CN^-, which could not be synthesized with Route A conditions.

Better results are obtained using methylamine than benzylamine, as seen in previous studies. [25] [29]The conversion is now enhanced with methylamine for two reasons. The first one is that acrylamide formation is favoured since methylamine is a better nucleophile than benzylamine. The second one is that methylamine facilitates the extraction of the amide proton, being more accessible due to its lower steric impediment, allowing for the cyclization.

Finally, despite dividing a process in two stages can increase the loss of product, the obtained products are purer, facilitating the work of purification and making the work-up not laborious.

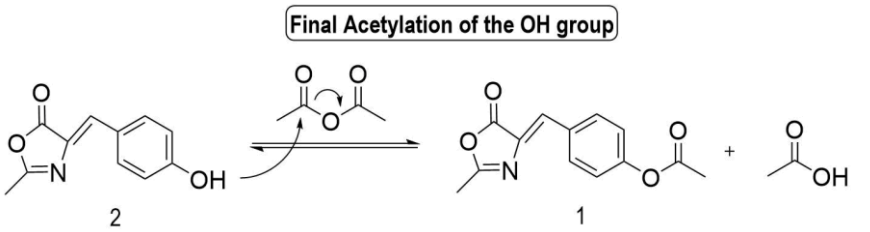
5.3. SYNTHESIS OF THE CORE GFP CHROMOPHORE (P-HBDI)

Although in part of the consulted bibliography [30] [31] [32] the synthesis of 4-(4-Hydroxyenzylidene)-1-benzyl-2-methylimidazol-5-one is detailed in two steps through a Erlenmeyer-Plöchl synthesis, for the subsequent reaction of **(2)** with methylamine in presence of

K_2CO_3 in a condensation reaction, the true is that in the first of them the acetylated product is obtained in the position "para", so it is not possible to undertake directly the condensation reaction, since the desired final product would not be obtained.

The p-HBDI synthesis proposed in this study is composed of 4 successive steps, through oxazolones formation, generating a great flexibility in the synthesis of compounds with different substituents and which will be subject of study in other works.

5.3.1. 1st step. General oxazolone generation. Synthesis of the acetylated oxazolone (1)



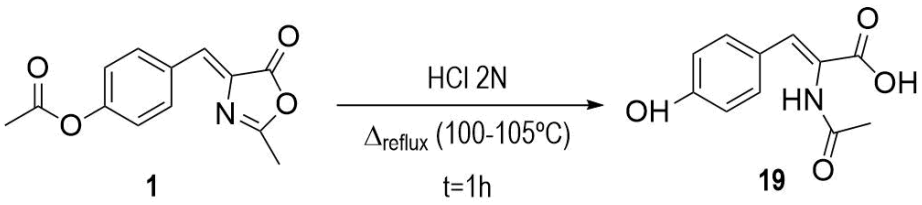
Scheme 3: Fiinal undesired step in the production of the oxazolone **(2)**

The first stage consists on the preparation of azlactone (oxazolone), through a condensation reaction from p-hydroxybenzaldehyde with N-acetylglycine, in the presence of sodium acetate (NaOAc) and Anhydride Acetic (Ac_2O) used as solvent, and reflux during 5h (100-110ºC), called Erlenmeyer azlactone reaction. The reaction has a general mechanism showed in Figure12 followed with a final stage where acetylation of the OH group is produced, to obtain a not desired product.

A brown crystalline solid is obtained. It is washed with a large amount of 1:1 mixture of Ethanol:H_2O, and that is the reason for being a little bit wet, so it is left two days drying up before calculating the final yield and going on the next reaction.

5.3.2. 2nd step. Obtaining 2-(Acetylamino)-3-(4-hydroxyphenyl)-2-propenoic acid (19).

It is carried out a ring-opening reaction through two consecutives acid-catalyzed hydrolysis taking place in both carbonyl groups, presents in the (molecule number) for obtaining the open chained acid **(19)**. In general, this type of reaction is a nucleophilic acyl substitution. A nucleophile, in our case, the water, displaces a leaving group, the ester, in an equilibrium process, the reverse of the Fischer esterification.



Scheme 4: Ring Opening reaction to obtain the open chained acid **(19)**

The simplified mechanism of reaction is specified in Figure **(15).** The carbonyl compound reacts with nucleophiles via an addition mechanism. Under acidic conditions, the carbonyl of our acyl compound is protonated, which activates it towards the nucleophilic attack. The nucleophile attacks the protonated carbonyl, forming a tetrahedral intermediate. A proton transfer is produced from the nucleophile to the leaving group, and this one collapses to eject the leaving group, giving a protonated carbonyl compound. The loss of a proton gives the substitution product. It is important to use a large excess of reactant (water), to favour the forward reaction, because of the fact that this nucleophile, and the leaving group has a similar pK_a. [33] .For the correct synthesis of the product **(19)** it is used HCl 0'2N, and it is warmed at reflux (100-105ºC) during 1 hour.

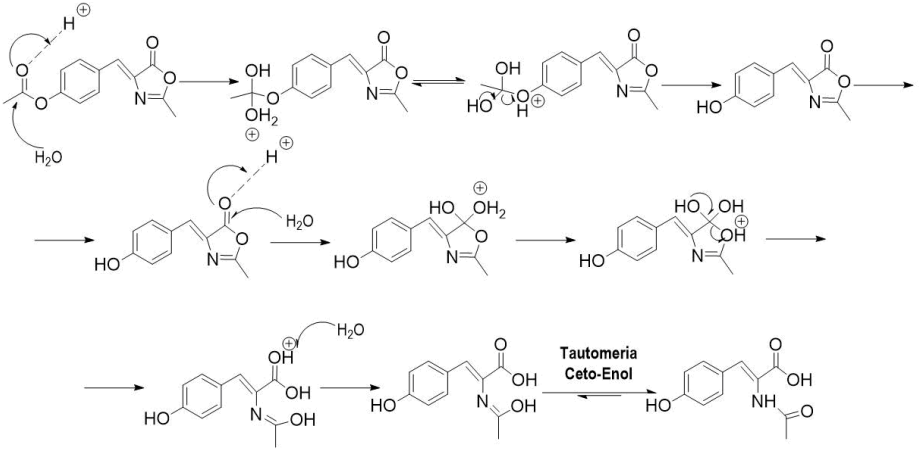


Figure 15: Mechanism of the 1st Ring Opening Reaction.

It is used HCl 0,2N and no more concentrated product in order to avoid affecting other places of the molecule and obtaining unintended subproducts.

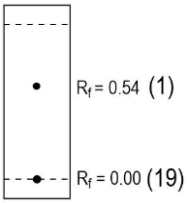
The work-up detailed in the experimental section is carried out in order to obtain an oily brown crude.

Before doing the characterization by the appropriate techniques, it can be deduced if the reaction has been carried out, cause since the oil gives off a characteristic smell to acetic acid, which is obtained as a subproduct during the hydrolysis.

An intense orange solid is obtained, changing to a purple colour when is washed with Et_2O.

It is confirmed by RMN^{1}H and HRMS that it is the desired product.

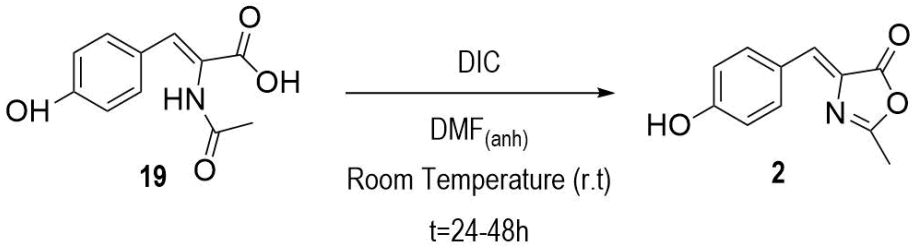
In the TLC it is observed that the oil contains two products: the desired product (Rf=0), and the starting product (Rf=0.54).



After this verification, and in order to obtain the pure product, it is carried out the by a chromatographic column, using as eluent AcOEt 9:1 MeOH with an increasing gradient of the polar fraction (MeOH).

5.3.3. 3rd step. Obtaining the desired oxazolone. Production of compound (2)

This step consists of the 4-arylidene-5-imidazolones production, through a dehydrative cyclization with DIC to obtain 4-arylidene-5-oxazolinones is novel in relation to other studies that work on the synthesis of these compounds.



Scheme 5: General scheme of the dehydrative cyclization with DIC

In this reaction DIC is the agent that induces the cyclization, through its coupling to the carboxylate, activating it and generating an intermediate O-acylurea, for the later formation of the diisopropylurea. In fact, this reaction, in general, is widely used for the generation of ureas and runs through the following mechanism:

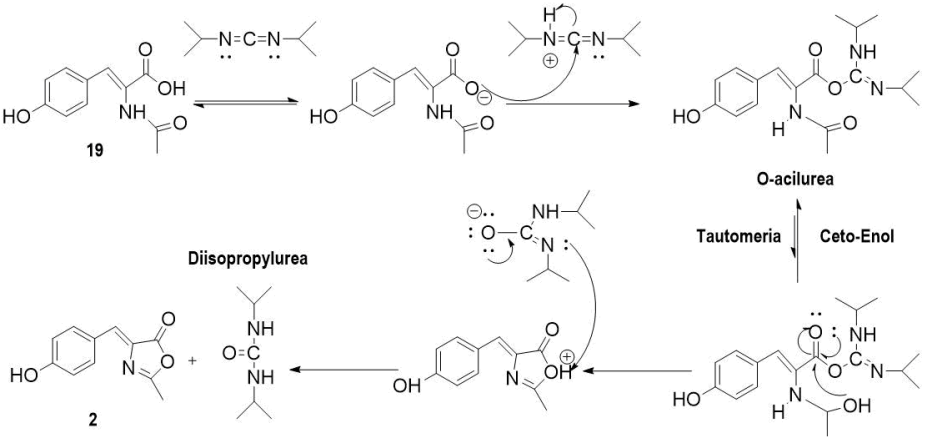


Figure 16: General mechanism to obtain ureas. Cyclization of the open chained acid to form the desired oxazolone (2).

Dimethyllformamide (DMF), a non-nucleophilic base, is used as solvent, since it dissolves correctly our reactive without competing with the desired reaction, as it occurs in the case of EtOH. If ethanol or another nucleophilic base were used as a solvent, they could react with the carbonyl group, generating undesired products. In addition DMF has a relatively low boiling point in comparison with other similar solvents, and that fact makes its elimination under reduced pressure possible. After the removal by the rotary evaporator of the majority of the solvent, a red wine coloured oil was obtained.

It is characterized by TLC (Rf=0.28) and NMR^{1}H.

In NMR^{1}H were observed the typical signals of diisopropylurea (The reaction was worked) and dimethylformamide. For that reason, the solvent is completely removed under reduced pressure and the purification is carried out by chromatographic column. After successive tests on TLC, it is chosen ACOEt:Hex (1:2) as eluent.

5.3.4. 4th step. Preparation of p-HBDI (20b) and the functionalized imidazolone (20a).

In this last step, through analogous reactions, were synthesized p-HBDI, the GFP core chromophore, and the product **(20b)** which presents a benzyl group instead of a methyl one linked to the amide nitrogen, as this functionalization will be necessary in further works to synthesize the polymers. The polymer resin contains this Bn group.

It has been tried to synthesize through two different routes, as it is seen on that diagram as a summary.

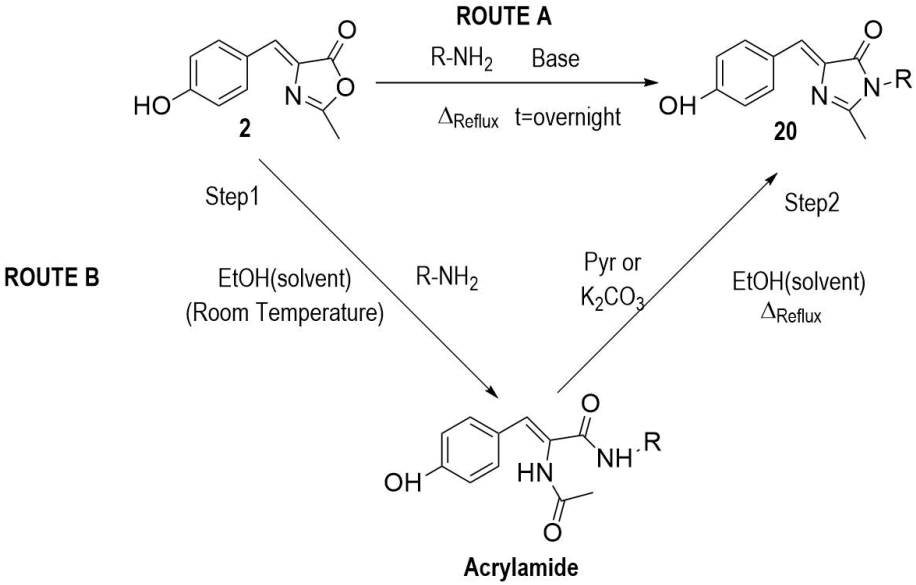


Figure 17: Scheme containing the two routes for obtaining imidazolones.

Route	$R\text{-}NH_2$	Base	Time [h]	Product (Yield%)
A	Bn	K_2CO_3	4	**20a** (29%)
			24	**20a** N.D.P
	Me		4	**20b** (20%)
			24	**20b** N.D.P
3a	Bn	Pyr	8	**20a** (32%)
	Me	Pyr	24	**20b** (48%)

Table 6: Summary of the **(20)** synthesis trough the transformation of the oxazolone **(2)**

The first attempts to obtain the desired products were made through the ROUTE A, but unfortunately very low yields were obtained, similar to what was experienced by Lee et al. [29]

Because of that and in order to increase the yield reaction, another alternative route was designed, ROUTE B, in which the product **(2)**, is converted into the acrylamide intermediate

(Step1), in order to obtain the final imidazolone **(20)** (Step2), by its reaction with a non-nucleophilic base.

By examining the data obtained, the conclusion is that the product is synthesized with greater yield, and with a high purity through the route B, especially using pyridine as a base in the last dehydrative cyclization.

Purification by chromatography column using ACOEt:Hex (1:1). Pale yellow crystalline solid.

6. EXPERIMENTAL SECTION

6.1. MATERIALS AND METHODS

6.1.1. Reagents and solvents

SUPPLIER	REAGENT OR SOLVENT
Acros	2-Fluorobenzaldehyde, 3-Fluorobenzaldehyde, 4-Acetamidobenzaldehyde, 4-Hydroxybenzaldehyde,
Aldrich	$CDCl_3$ d_6, DMSO d_6, Et_2O, $MeNH_2$, Benzaldehyde, , 2,5-Dymethylbenzaldehyde, 2,5-Dihydroxybenzaldehyde, 4-Bromobenzaldehyde, 4-Cyanobenzaldehyde, N-Acetylglycine,
Alfa Aesar	Pyridine
Fluka	Ac_2O, DMSO, HNO_3 (c)
Jescuder	$NaHCO_3$, NaCl, NaOH
Merck	Silica gel
Panreac	H_2SO_4 (c)
Probus	NaOAc
Scharlau	Acetone, AcOEt, CH_2Cl_2, HCl, HAc, HNO_3, Hexane, MeOH, THF
TCI	$BnNH_2$, 2-Chlorobenzaldehyde, 4-Nitrobenzaldehyde, 4-Chlorobenzaldehyde, 2,6-Dichlorobenzaldehyde

6.1.2. Instrumental

INSTRUMENT	SUPPLIER	MODEL
ESI-MS	Waters	Micromass ZQ
IR	Nicolet	6700 FT-IR
HRMS	Agilent Technologies	Agilent 1100 Trace DSQ
NMR	Varian-Inova	Mercury 400
Rotary Evaporator	IKA	RV 05 basic

6.1.2.1. Mass Spectroscopy

The High-Resolution Mass Spectra (HMRS) were obtained using an Agilent Technologies 1100 Trace DSQ, and the Electrospray Ionization Mass Spectra (ESI-MS) were obtained with a Micromass ZQ Waters, with a Q-HPLC Waters 2695. The spectroscopy conditions were: capillary voltage 4KV (positive), 3.5KV (negative); fragmentor 215V; gas temperature 325ºC, nebulizator gas N_2 15 psi; dried gas, N_2 at 7.0L7min, flow of EtOH at 200 µL/min.

6.1.2.2. Infrared Spectroscopy

IR spectra were taken using a Nicolet 6700 FTIR spectrometer. In the compounds description are only indicated the most characteristic peaks, wavenumbers in cm^{-1}.

6.1.2.3. Nuclear Magnetic Resonance Spectroscopy

1H NMR (400 MHz) and 13C NMR (400MHz) spectra were recorded at 25ºC on a Varian-Inova instrument in DMSO-d6 and CDCl3-d6, using TMS as internal standard. All chemical shifts (δ) are quoted in SI units (ppm), and all coupling constant (J) are expressed in Hz.

6.1.3. Chromatography

6.1.3.1. Thin-Layer Chromatography

TLC is a solid-liquid partitioning technique. It was carried in silica gel plates (F254 Merck). Plates were inspected under UV light. The visualization reagent used for colourless componds was KMnO4.

6.1.3.2. Column Chromatography

Column Chromatography is a solid-liquid phase-partitioning technique. The stationary solid phase used was silica gel. The mobil phase passing through the column that elutes the components was a mixture of various solvents, specified in each case, depending on the purified compound polarity.

6.2. SYNTHETIC PROCEDURES AND CHARACTERIZATION

6.2.1. Synthesis of oxazolones

6.2.1.1. General Procedure

The aryaldehydes (1.35eq) react with N-acetylglycine (1eq), in the presence of NaOAc (1.625eq) and Ac_2O used as a solvent. The reaction under reflux conditions during 5h at 100-110ºC of temperature.

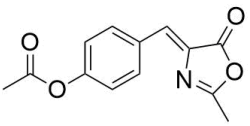
After carrying out this reaction, a general work-up is performed for all oxazolones. It consists on cooling down the crude to room temperature and pouring it into a beaker with a water-ice mixture, generating the appearance of the solid.

The solid is filtered by filter plate and it is washed with a mixture of cold ethanol and water in proportion 1:1.

6.2.1.1.1. Para-substituted oxazolones

6.2.1.1.1.1. Synthesis of 4-(4-Dymethylaminobenzylidene)-2-methyloxazol-5-one

4-Dymethylaminobenzaldehyde (3.327g, 23.98mmol), N-Acetylglycine (1.850g, 17,76mmol), NaOAc anh (2.322g, 27.74mmol) and Ac2O (20 ml) were mixed in a 250mL flask with a stirring bar. The reaction mixture was refluxed for 5h. The general work-up is followed. The resulting precipitate was washed with 4x15mL of 50%aq EtOH.



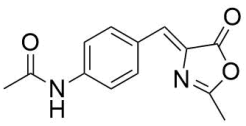
3

Intense red color solid. Yield: 51%. **IR** (KBr);(cm^{-1}): 3082,2895,2803,1758,1647,1578,1518,1432,1366,1309,1261,1230,1154,1125,935,897,815. **^{1}H NMR** (DMSO-d_6, 400 MHz); (ppm): δ 8.04-8.02 (d, *J*=9.2 Hz, 2H, Ar), 7.07 (s, 1H, CH_2), 6.79-6.76 (d, *J*=9.2Hz, 2H, Ar), 3.03 (s, 6H,N-Me), 2.34 (s, 1H, Me). **^{13}C NMR** (DMSO-d_6, 400 MHz); (ppm): δ 168.6 (C=O), 166.7 (C=N), 152.4(=C-N), 134.6(Ar), 134.3(Ar), 132.3(Ar), 132.1(Ar), 129.5 (C=), 120.8(Ar-C**H**), 26.7(CH_3), 15.7(CH_3). **HRMS (ESI)**: m/z calc. $C_{13}H_9NO_2^+$ [M+H]$^+$ 231.1128; found 231.1125.

6.2.1.1.1.2. Synthesis of 4-(4-Acetamidebenzylidene)-2-methyloxazol-5-one

This compound was prepared by the described general procedure. 4-Acetamidobenzaldehyde (1.881g, 11.53mmol), N-Acetylglycine (1.000g, 8.54 mmol), NaOAc anh (1.185, 14.45mmol) and Ac2O (20 ml) were mixed in a 250mL flask with a stirring bar. The reaction mixture was refluxed for 5h. The general work-up is followed. The resulting precipitate was washed with 6x10mL of 50%aq EtOH. After that is washed another time with 3x10mL of

Hexane. The reaction was purified by flash column chromatography using AcOEt:Hexane (1:1) to give the product (4).

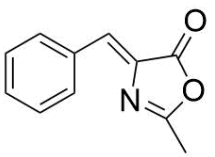


4

Brown solid. Yield: 67%. **TLC**: **IR** (KBr);(cm^{-1}): 3317,1699,1696,1662,1653,1635,1588,1507,1490,1253,1176,668,651,534
^{1}H NMR (DMSO-d_6, 400 MHz); (ppm): δ 8.12-8.10 (d, *J*=8.8 Hz, 2H, Ar), 7.70-7.68 (d, *J*=8.8Hz, 2H, Ar), 7.26 (s, 1H, CH_2), 7.13 (s, 1H, NH_2) 2.19 (s, 3H, O-Me), 1,99 (s, 3H, Me). **^{13}C NMR** (DMSO-d6, 400 MHz); (ppm): δ 168.2 (C=O), 166.7 (C=O), 161.9(C=N), 140.3(Ar), 136.5(Ar), 134.7(Ar), 132.9(Ar),132.7 (C=), 120.4 (Ar-C**H**) 26.6(O-CH_3), 16.2(CH3). **HRMS (ESI)**: m/z calc $C_{13}H_{13}N_2O_3^+$ $[M+H]^+$ 245.0921; found 245.0919.

6.2.1.1.1.3. Synthesis of 4-Benzylidene-2-methyloxazol-5-one

This oxazolone is synthesized through the general procedure before detailed. Benzaldehyde (4.74ml; 4.90g, 34.15mmol), N-Acetylglycine(4,00g, 25.30mmol), NaOAc anh (4,64g, 41.11mmol) and Ac2O (40 ml) were mixed in a 250ml flask with a stirring bar. The reaction mixture was refluxed for 5h. The general work-up is followed. The resulting precipitate was washed with small quantities of 50%aq EtOH.

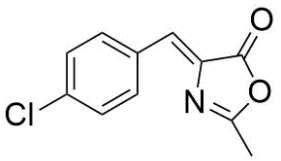


5

Intense yellow solid. Yield: 99%. **IR** (KBr);(cm^{-1}): 3228,1729,1695,1621,1490,1387,1275,1241,1104,898,651,532.
^{1}HNMR (DMSO-d_6, 400 MHz); (ppm): δ 8.12-8.10 (d, *J*=8.8 Hz, 2H, Ar), 7.70-7.68 (d, *J*=8.8Hz, 2H, Ar), 7.26 (s, 1H, CH2), 7.13 (s, 1H, NH_2) 2.19 (s, 3H, O-Me), 1,99 (s, 3H, Me). **^{13}C NMR** ($CDCl_3$-d_6, 400 MHz); (ppm): δ 165.9 (C=O), 162.1(C=N), 140.3(Ar), 137.3(Ar), 136.1(Ar), 133.9(Ar), 133.3(C=) 129.6 (Ar-C**H**), 15.8(CH_3). **HRMS (ESI)**: m/z calc $C_{11}H_{10}NO_2^+$ $[M+H]^+$ 188.0707; found 188.0707.

6.2.1.1.1.4. Synthesis of 4-(4-Chlorobenzylidene)-2-methyloxazol-5-one

The usual procedure for the synthesis of the oxazolones is followed. Mixing in a 250ml flask the appropriate quantities of 4-Chloroenzaldehyde (3.246g, 23.06mmol), N-Acetylglycine (2.008g, 17.08mmol), NaOAc anh (2.324g, 27.76mmol) and Ac2O (40 ml) were mixed in a 250ml flask stirring for 5h under reflux conditions. Temperature: 100-110ºC. After that the work-up is done, with the normal conditions. The resulting precipitate was washed with 3x15 of 50%aq EtOH and a little of hexane.

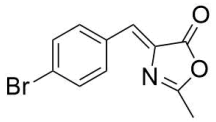


6

Yellow solid. Yield: 81%. **IR** (KBr);(cm^{-1}): 3084,1797,1770,1678,1657,1492,1423,1280,1256,1164,1086,910,898,865, 848,711,686,529. **^{1}H NMR** (DMSO-d_6, 400 MHz); (ppm): δ 8.19-8.17 (d, *J*=8.4 Hz, 2H, Ar), 7.57-7.55 (d, *J*=8.8Hz, 2H, Ar), 7.22 (s, 1H, CH2), 2.39 (s, 3H, Me). **^{13}C NMR** (DMSO-d_6, 400 MHz); (ppm): δ 167.6 (C=O), 166.6(C=N), 136.1(Ar), 133.9(Ar), 133.5(Ar), 132.4(Ar), 132.2(C=) 129.4 (Ar-C**H**), 16.1(CH_3). **HRMS (ESI)**: m/z calc $C_{11}H_9ClNO_2^+$ $[M+H]^+$ 222.0316; found 222.0318.

6.2.1.1.1.5. Synthesis of 4-(4-Bromobenzylidene)-2-methyloxazol-5-one

Achieved following the normal procedure explained at the top of the section. 4-Bromobenzaldehyde (4.323g, 11.69mmol), N-Acetylglycine (2.002g, 17.06mmol), NaOAc anh (2.332g, 27.72mmol) and Ac2O (40 ml) were mixed in continue stirring in a 250ml flask during 5 hours at 100-110ºC. After the general work-up appears an intense yellow solid washed with 5x5ml of $EtOH_{(aq)}$ 50% and 3x5ml of Hexane to obtain a pale yellow solid.

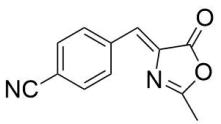


7

Yellow solid. Yield: 77%. **IR** (KBr);(cm^{-1}): 3099,1797,1774,1678,1600,1579,1484,1421,1256,1235,1183,1162,1050, 899,869,833,666,648,529. **^{1}HNMR** (DMSO-d_6, 400 MHz); (ppm): δ 8.20-8.18 (d, *J*=8.0 Hz, 2H, Ar), 7.51-7.57 (d, *J*=8.0Hz, 2H, Ar), 7.24 (s, 1H, Ar-C**H**), 2.37 (s, 3H, Me). **^{13}C NMR** ($CDCl_3$-d_6, 400 MHz); (ppm): δ 166.8 (C=O), 166.0(C=N), 135.4(Ar), 134.2(Ar), 133.9(Ar), 132.0(Ar), 128.1(C=H), 16.4(CH_3). **HRMS (ESI)**: m/z calc $C_{11}H_9BrNO_2^+$ $[M+H]^+$ 265.9815; found 265.9812.

6.2.1.1.1.6. Synthesis of 4-(4-Cyanobenzylidene)-2-methyloxazol-5-one

Cyanobenzaldehyde (3.06g 23.34mmol), N-Acetylglycine (2.002g 17.10mmol), NaOAc anh (2.321g 28.29mmol) and Ac2O (40 ml) were mixed in a 250mL flask with a stirring bar. The reaction mixture was refluxed for 5h. The general work-up is followed. The resulting precipitate was washed with 5x10mL of 50%aq EtOH.

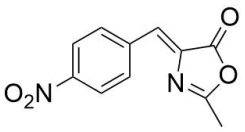


8

Yellow solid. Yield: 84%. **IR** (KBr);(cm^{-1}): 3094,3044,3007,2361,2332,2224,1656,1606,1594,1255,1180,1164,896,835, 848,692,669,573,553. **^{1}H NMR** (DMSO-d_6, 400 MHz); (ppm): δ 8.32-8.30 (d, *J*=8.4 Hz, 2H, Ar), 7.95-7.93 (d, *J*=8.4Hz, 2H, Ar), 7.28 (s, 1H, Ar-C**H**), 2.42 (s, 3H, Me) **^{13}C NMR** (DMSO-d_6, 400 MHz); (ppm): δ 168.5 (C=O), 166.9(C=N), 137.4(Ar), 135.2(Ar), 132.6(Ar), 132.1(Ar), 127.5(Ar) 126.9 (Ar), 118.5(C=) 112.4(C=H) 20.5(CN) 15.5(Me) **HRMS (ESI)**: m/z calc $C_{12}H_9N_2O_2^+$ $[M+H]^+$ 213.0659; found 213.0657.

6.2.1.1.1.7. Synthesis of 4-(4-Nitrobenzylidene)-2-methyloxazol-5-one

The usual procedure for the synthesis of the oxazolones is followed. 4-Nitrobenzaldehyde (4.188g 26.99mmol), N-Acetylglycine (2.802g 23.84mmol), NaOAc anh (2.832g 32.17mmol) and Ac2O (40 ml) were mixed in a 250ml flask with a stirring bar. The reaction mixture was refluxed for 5h. After cool down the oil to room temperature these one is transfered to a 600ml beaker with a water-ice mixture. A brown solid is appeared. Due to the fact that the solid didn't present a uniform appearance was washed with 8x5 ml of 50%aq EtOH. After solubility tests is washed with Et_2O and air dried. A light brown solid is achieved.



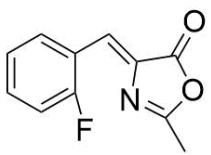
9

Light brown solid. Yield: 87%. **IR** (KBr);(cm^{-1}): 3106,3061,3011,2850,1790,1605,1515,1340,1267,1166,1108,853,763,675, 646. **^{1}H NMR** (DMSO-d_6, 400 MHz); (ppm): δ 8.40-8.38 (d, *J*=9.2 Hz, 2H, Ar), 8.32-8.30 (d, *J*=9.2Hz, 2H, Ar), 7.33 (s, 1H, Ar-C**H**), 2.43 (s, 3H, Me) **^{13}C NMR** (DMSO-d_6, 400 MHz); (ppm): δ 168.2 (C=O), 130.5(Ar), 130.4(Ar), 128.0(C=) 126.3(C=H),123.9(Ar),123.5(Ar),15.6(CH_3). **HRMS (ESI)**: m/z calc $C_{11}H_9N_2O_4^+$ $[M+H]^+$ 233.0552; found 233.0551.

6.2.1.1.2. Ortho-substituted oxazolones

6.2.1.1.2.1. Synthesis of 4-(2-Fluorobenzylidene)-2-methyloxazol-5-one

This compound was prepared following the described general procedure. 2-Fluorobenzaldehyde (4.96 ml 5.84g 25.12mmol), N-Acetylglycine (4.001 34.12mmol), NaOAc anh (2.245 26.97mmol) and Ac2O (40 ml) were mixed in a 250mL flask with a stirring bar. The reaction mixture was in reflux conditions (100-110ºC) for 5h. After that has been carried up the typical work up process of the oxazolones. The resulting precipitate was washed with 2x10mL of 50%aq EtOH and after with CH_2Cl_2.

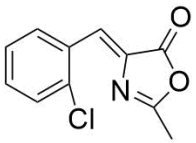


10

Light yellow solid. Yield: 78%. **IR** (KBr);(cm^{-1}): 3392,1793,1714,1657,1627,1528,1478,1227,892,793,751. **^{1}H NMR** (DMSO-d_6, 400 MHz); (ppm): δ 8.58 (td, *J*=8.0 *J*=1.6 Hz, 1H, Ar), 7.54 (qd, *J*=10.4Hz,*J*=1.6Hz 1H, Ar), 7.32 (qd, *J*=9.6 *J*=0.8 Hz, 2H, Ar) 7.14 (s, 1H, Ar-C**H**), 2.39 (s, 3H, Me) **^{13}C NMR** (DMSO-d_6, 400 MHz); (ppm): δ 167.6 (C=O), 131.5(Ar), 131.2(Ar), 129.9(Ar), 129.5(Ar), 126.0(C=) 124.1(C=H),15.7(CH_3). **HRMS (ESI)**: m/z calc $C_{11}H_9FNO_2^+$ $[M+H]^+$ 206.0612; found 206.0612.

6.2.1.1.2.2. Synthesis of 4-(2-Chlorobenzylidene)-2-methyloxazol-5-one

2-Chlorobenzaldehyde (5.3ml 6.61g 47.05mmol), N-Acetylglycine (4.000g 34.16mmol), NaOAc anh (4.646g 55.50mmol) and Ac2O (40 ml) were mixed in a 250mL flask with a stirring bar. The reaction mixture was refluxed for 5h. The general work-up is followed. The resulting precipitate was washed with 4x10mL of 50%aq EtOH.



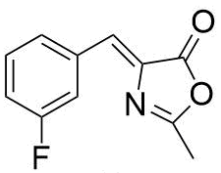
11

Intense yellow solid. Yield: 92%. **IR** (KBr);(cm^{-1}): 3327,1714,1627,1514,1432,1395,1280,1212,1111,1034,959,744,720.**^{1}HNMR** (DMSO-d_6, 400 MHz); (ppm): δ 8.61 (td, *J*=9.6 *J*=1.2 Hz, 1H, Ar), 7.54 (q_5d, *J*=12.8Hz,*J*=2Hz 1H, Ar), 7.47 (q_4d, *J*=10.8 *J*=1.2 Hz, 2H, Ar) 7.30 (s, 1H, Ar-C**H**), 2.38 (s, 3H, Me) **^{13}C NMR** (DMSO-d_6, 400 MHz); (ppm): δ 168.0 (C=O), 133.1(Ar), 132.7(Ar), 130.9(Ar), 130.3(Ar), 127.0(C=) 124.5(C=H),16.0(CH_3). **HRMS (ESI)**: m/z calc $C_{11}H_9ClNO_2^+$ $[M+H]^+$ 222.0316; found 222.0320.

6.2.1.1.3. Meta-substituted oxazolones

6.2.1.1.3.1. Synthesis of 4-(3-Fluorobenzylidene)-2-methyloxazol-5-one

The usual procedure for the synthesis of the oxazolones is followed. 3-Fluorobenzaldehyde (5 ml 5.51g 47.53mmol), N-Acetylglycine (4.003g 34.18mmol), NaOAc anh (4.660g 56.80mmol) and Ac2O (40 ml) were mixed in a 250mL flask with a stirring bar. Reflux (100-110ºC). General work-up. The product was washed with 3x10 of 50%aq EtOH.



12

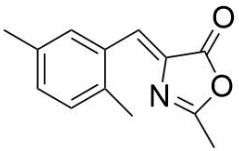
Light yellow solid. Yield: 90%. **IR** (KBr);(cm^{-1}): 3075,1787,1654,1600,1486,1438,1378,1252,1157,881,856,783,682,647. **^{1}HNMR** (DMSO-d_6, 400 MHz); (ppm): δ 7.99 (d, *J*=10.4, 1H, Ar), 7.87 (d, *J*=8.0Hz,1H, Ar), 7.49 (dd, *J*=22Hz *J*=4.8 Hz, 1H, Ar) 7.27 (td, *J*=8.4 *J*=2.4 Hz, 1H, Ar) 7.14 (s, 1H, Ar-C**H**), 2.36 (s, 3H, Me) **^{13}C NMR** (DMSO-d_6, 400 MHz); (ppm): δ 167.8 (C=O), 131.8(Ar), 131.5(Ar), 130.0(Ar), 129.6(Ar), 126.2(C=) 124.3(C=H),15.5(CH_3). **HRMS (ESI)**: m/z calc $C_{11}H_9FNO_2^+$ $[M+H]^+$ 206.0612; found 206.0612.

6.2.1.1.4. Disubstituted oxazolones

6.2.1.1.4.1. Synthesis of 4-(2,5-Dymethylbenzylidene)-2-methyloxazol-5-one

The compound (13) was the only disubstituted oxazolone that was obtained satisfactorily, the other two attempts showed the preferred production of double-condensation compounds, or did not cyclize after the ring was opened.

It was generated with the general procedure explained in point *(6.2.1.1)* with the following amounts of the different reagents. 2,5-Dymethylbenzaldehyde (4.033g 30.27mmol), N-Acetylglycine (4.002g 34.17mmol), NaOAc anh (4.579g 52.29mmol) and Ac2O (40 ml) mixed in a 250mL flask with the aid of magnetic stirring. The reaction was in reflux during 5 hours at approximately 100ºC. After the general work-up a purification by flash column chromatography is needed. Eluent(AcOEt100%).The light red solid produced was washed with Et_2O.



13

Orange solid. Yield: 33%. **IR** (KBr);(cm^{-1}): 3064,1767,1673,1655,1612,1592,1453,1379,1296,1259,1165,890,869,823, 650,621,562. **^{1}HNMR** (DMSO-d_6, 400 MHz); (ppm): δ 8.32 (s, 1H, Ar-C**H**), 7.24 (d, *J*=8.0Hz,1H, Ar), 7.19 (dd, *J*=12.0Hz *J*=1.6 Hz, 1H, Ar) 7.15 (d, *J*=8.0 Hz, 1H, Ar) 7.14 (s, 1H, Ar-C**H**), 2.40 (s, 3H, Me), 2.39 (s, 3H, Me), 2.30 (s, 3H, Me). **HRMS(ESI)**: m/z calc $C_{13}H_{14}NO_2^+$ $[M+H]^+$ 216.1019; found 216.1020.

6.2.2. Synthesis of imidazolones

The synthesis of imidazolones is carried out through two different routes, because the first one did not obtain the desired results.

6.2.2.1. First approach: Route A

6.2.2.1.1. General Procedure A

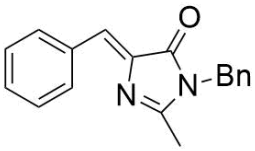
1.5eq of K_2CO_3 and a primary amine ($MeNH_{2\,(aq)}$ 6.5eq) or ($BnNH_2$ 5eq) were added to a solution of the adequate oxazolone solved in 120 ml of absolute EtOH. The reaction was disposed with magnetic stirring, and heated at reflux temperature “overnight” (at minimum 16-22h). After cooling to room temperature. It is washed with small volumees of absolute ethanol and the remaining white solid is filtrated (K_2CO_3, ethanol insoluble). The resulting solution was filtered, and the filtrate was collected. The excess of EtOH is eliminated under reduced pressure.

The mother liquors were neutralized with 10% HCl in several cases, and they were left cooling down. All the products were purified by flash column chromatography.

6.2.2.1.1.1. Synthesis of 4-Benzylidene-1-benzyl-2-methylimidazol-5-one

	Oxazolone	$BnNH_2$	K_2CO_3
Quantity [g]	3.93	9.87 (10ml)	2.95
Quantity [mmol]	14.18	92.18	21.34

Eluent used: AcOEt:Hex (1:2)



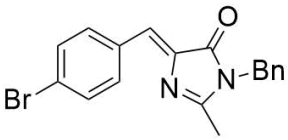
14

Yellow crystalline solid. Yield 33%. **TLC** (Rf=0.23) **IR** (KBr);(cm^{-1}): 3018,1665,1649,1550,1521,1478,1250,1212,1184,1145,878,851,622. **^{1}H NMR** ($CDCl_3$-d_6, 400 MHz); (ppm): δ 8.17 (d, *J*=7.2Hz, 2H), 7.32-7.40 (m, 3H), 7.25-7.31 (m, 5H) 7.21 (s, 1H, C=C**H**), 4.82 (s, 2H, CH_2), 2.29 (s,3H,CH_3) **^{13}C NMR** ($CDCl_3$-d_6, 400 MHz); (ppm): δ 170.2 (C=O), 163.1 (C=N), 138.7 (Ar), 136.5 (Ar), 134.3 (Ar), 132.2 (Ar), 130.2 (Ar), 129.3 (Ar) 129.0 (Ar) 128.2 (Ar) 127.8 (Ar) 44.0 16.2(CH_3). **HRMS (ESI)**: m/z calc. $C_{18}H_{17}N_2O^+$ $[M+H]^+$ 277.1336; found 277.1337.

6.2.2.1.1.2. Synthesis of 4-Bromobenzylidene-1-benzyl-2-methylimidazol-5-one

	Oxazolone	$BnNH_2$	K_2CO_3
Quantity [g]	1.503	3.94g (4ml)	1.17
Quantity [mmol]	5.65	36.73	8.48

Eluent used: AcOEt:Hex (1:2)



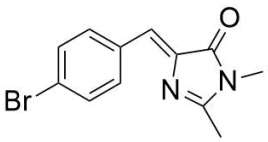
15A

Yellow crystalline solid. Yield 11%. **TLC** (Rf=0.13) **IR** (KBr);(cm^{-1}): 3015,2948,1745,1687,1644,1601,1551,1389,1223,1210,1167,1130, 931 854,813,785,761,697,645. **^{1}H NMR** ($CDCl_3$-d_6, 400 MHz); (ppm): δ 8.18 (d, *J*=8.0, 2Hz, 2H), 7.70 (d, *J*=8.4Hz,2H), 7.22-7.33 (m, 5H) 7.11 (s, 1H, C=C**H**), 4.80 (s, 2H, CH_2), 2.25 (s,3H,CH_3) **^{13}C NMR** ($CDCl_3$-d_6, 400 MHz); (ppm): δ 169.5 (C=O), 164.0 (C=N), 138.8 (Ar), 136.6 (Ar), 133.9 (Ar), 133.6 (Ar), 132.2 (Ar), 129.3 (Ar) 128.2 (Ar) 128.0 (Ar) 127.4 (Ar) 41.9 15.3(CH_3). **HRMS (ESI)**: m/z calc. $C_{18}H_{17}BrN_2O^+$ $[M+H]^+$ 356.0519; found 356.0520 .

6.2.2.1.1.3. Synthesis of 4-Bromobenzylidene-1-methyl-2-methylimidazol-5-one

	Oxazolone	$MeNH_2$	K_2CO_3
Quantity [g]	1.510	1.15 (1.28ml)	1.18
Quantity [mmol]	5.68	36.92	8.52

Eluent used: AcOEt:Hex (1:2)



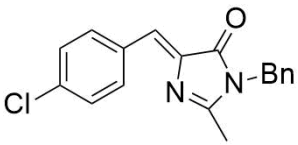
15B

Light brown solid. Yield 7%. **TLC** (Rf=0.15) **IR** (KBr);(cm-1): 3011,1740,1688,1613,1457,1289,1255,1191,1055,976,874,829,795,641 **^{1}H NMR** ($CDCl_3$-d_6, 400 MHz); (ppm): δ 8.00 (d, *J*=8.0, 2Hz, 2H), 7.51 (d, *J*=8.4Hz,2H), 7.01 (s, 1H, C=CH), 3.14 (s, 3H, NCH_3), 2.21 (s,3H,CH_3) **^{13}C NMR** ($CDCl_3$-d_6, 400 MHz); (ppm): δ 169.9 (C=O), 163.4 (C=N), 139.8 (Ar), 133.9 (CH), 133.6 (Ar), 132.1(CH), 126.0 (CH) 125.23(Ar) 28.4(CH_3) 15.5(CH_3). **HRMS** (ESI): m/z calc. $C_{18}H_{17}BrN_2O$+ [M+H]+ 279.0130; found 279.0129.

6.2.2.1.1.4. Synthesis of 4-Chlorobenzylidene-1-methyl-2-methylimidazol-5-one

	Oxazolone	$BnNH_2$	K_2CO_3
Quantity [g]	1.497	4.91 (5ml)	1.40
Quantity [mmol]	6.77	44.02	10.16

Eluent used: AcOEt:Hex (1:3)



16

Light orange solid. Yield 9%. **TLC** (Rf=0.13) **IR** (KBr);(cm^{-1}): 3085,1785,1753,1704,1644,1553,1289,1255,1103,1090,855,733 **^{1}H NMR** ($CDCl_3$-d_6, 400 MHz); (ppm): δ 8.05 (d, *J*=8.4Hz, 2H), 7.55-7.45(m,5h) 7.32 (d, *J*=7.2Hz,2H), 7.11 (s, 1H, C=C**H**), 4.70 (s, 2H, CH_2), 2.25 (s,3H,CH_3) **^{13}C NMR** ($CDCl_3$-d_6, 400 MHz); (ppm): δ 170.4 (C=O), 163.0 (C=N), 138.8 (Ar), 135.5 (Ar), 134.6 (Ar), 132.1(Ar), 130.0 (CH) 129.2(CH) 128.1(CH) 127.3(CH) 126.5 (CH) 42.7(CH_2) 16.0(CH_3). **HRMS (ESI)**: m/z calc. $C_{18}H_{16}ClN_2O^+$ [M+H]$^+$ 311.0943; found 311.0941.

6.2.2.2. Second approach: Route B

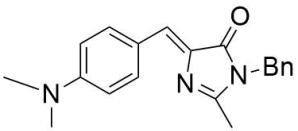
6.2.2.2.1. General Procedure B

1st step: Ring opening reaction with R-NH_2, EtOH (solvent) in room temperature. The quantities are specified in each case. After remove all the solvent under reduced pressure a purification by chromatographic column it is initiated with AcOEt:Hexane (1:2); in order to eliminate the first impurities and polarity is increased up to AcOEt:Hexane (1:1).

2nd step: It is performed a dehydrative cyclization, the best results were obtained with pyridine.

6.2.2.2.1.1. Synthesis of 4-(4-Dimethylaminobenzylidene)-1-benzyl-2-methylimidazol-5-one

	Acrylamide	Pyr	EtOH
Quantity [g or ml]	0.807	1.23 (1.1ml)	25ml
Quantity [mmol]	3.23	15.56	-

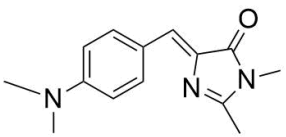


17A

Purple solid. Yield 21%. **TLC** (Rf=0.28) **IR** (KBr);(cm^{-1}): 3350,3297,3024,1684,1557,1525,1460,1381,1280,1240,1150,1083 ,865,772,641.**^{1}H NMR** ($CDCl_3$-d_6, 400 MHz); (ppm): δ 8.13-8.11 (d, *J*=8.4Hz, 2H), 7.48-7.35(m,5H) 7.00 (s, 1H, C=C**H**) 6.74-6.72 (d,2H,*J*=8.8Hz)4,87(s,2H,CH_2), 3.06(s, 6H, CH_3), 2.23 (s,3H,CH_3) **^{13}C NMR** ($CDCl_3$-d_6, 400 MHz); (ppm): δ 170.0(C=O), 164.2 (C=N), 153.2 (C=N) 138.2 (Ar), 134.7 (Ar), 133.9 (CH), 130.1(Ar), 129.2 (CH) 129.0(CH) 128.1(CH) 127.0(CH) 121.6 (CH) 42.9(CH_2) 39.9(CH_3) 16.0(CH_3). **HRMS (ESI)**: m/z calc. $C_{20}H_{22}N_3O^+$ $[M+H]^+$ 320.1757; found 320.1759.

6.2.2.2.1.2. Synthesis of 4-(4-Dimethylaminobenzylidene)-1-methyl-2-methylimidazol-5-one

	Acrylamide	Pyr	EtOH
Quantity [g or ml]	1.05	1.25 (1.12ml)	25ml
Quantity [mmol]	3.88	16.13	-



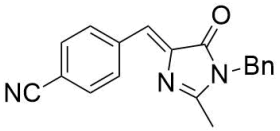
17B

Purple solid. Yield 39%. **TLC** (Rf=0.21) **IR** (KBr);(cm^{-1}): 3351,3244,3122,3003,1802,1667,1559,1479,1327,1310,1180,953 841,802..**^{1}H NMR** ($CDCl_3$-d_6, 400 MHz); (ppm): δ 8.08-8.06 (d, *J*=8.4Hz, 2H),7.04 (s, 1H, C=C**H**) 6.67-6.65 (d,2H,*J*=8.Hz) 3.16(s,3H,CH_3), 3.02(s, 6H, CH_3), 2.31 (s,3H,CH_3) **^{13}C NMR** ($CDCl_3$-d_6, 400 MHz); (ppm): δ 170.0 (C=O), 167.2 (C=N), 158.4 (C=N) 134.6m(Ar), 134.0(CH), 128.7(CH) 122.3(CH) 111.6 (CH) 40.2(CH_2) 26.8(CH_3) 15.4(CH_3). **HRMS (ESI)**: m/z calc. $C_{14}H_{18}N_3O^+$ $[M+H]^+$ 244.1447; found 244.1447.

6.2.2.2.1.3. Synthesis of 4-(4-Cyanobenzylidene)-1-methyl-2-methylimidazol-5-one

	Acrylamide	Pyr	EtOH
Quantity [g]	0.977	1.26 (1.15ml)	25ml
Quantity [mmol]	3.41	17.02	-

Eluent used different at the general procedure: AcOEt:Hex (1:1)



18

Orange crystalline solid. Yield 20%. **TLC** (Rf=0.27) **IR** (KBr);(cm^{-1}): 3018,2204,2102,1765,1709,1420,1284,1127,1040,852,733,619. **^{1}H NMR** ($CDCl_3$-d_6, 400 MHz); (ppm): δ 8.21 (d, *J*=8.4Hz, 2H), 7.61-7.59 (d,*J*=8.0Hz,2H) 7.41-7.35 (m, 3H), 7.27-7.25 (d, 2H, J=7.2Hz) 7.08 (s, 1H, C=C**H**), 4.81 (s, 2H, CH_2), 2.28 (s,3H,CH_3) **^{13}C NMR** ($CDCl_3$-d_6, 400 MHz); (ppm): δ 170.0 (C=O), 164.3 (C=N), 139.7 (Ar), 138.5 (Ar), 135.8 (Ar), 132.2 (CH), 130.1 (CH), 129.3 (CH) 128.2 (CH) 127.6 (Ar) 124.1 (Ar) 118.3 117.6 44.0(CH_2) 16.2(CH_3). **HRMS (ESI)**: m/z calc. $C_{19}H_{16}N_3O^+$ $[M+H]^+$ 302.1281; found 302.1280.

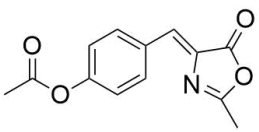
6.2.3. Procedure to synthetize the core GFP's chromophore

The synthesis of GFP's chromophore has been attempted in four steps.

6.2.3.1. Synthesis of 4-(4-Methylacetatebenzylidene)-2-methyloxazol-5-one

The usual procedure for the synthesis of the oxazolones is followed, but the desired product (2) is not obtained. This oxazolone (1) with the acetylated product is an undesired product, but expected at the initial study to obtain our final product, the p-HBDI (20).

4-Hydroxybenzaldehyde (13.2g, 54mmol), N-Acetylglycine (14.2g, 40mmol), NaOAc anh (10.6g, 65mmol) and Ac2O (50 ml) were mixed in a 250mL flask with a stirring bar. 5 hours in reflux conditions. Work-up general conditions of oxazolones is followed. A brown solid is obtained. This solid is washed with a plenty volume of the 50%aq EtOH.



1

Brown solid. Yield: 80%. **TLC:** (Rf=0.54) **IR** (KBr);(cm^{-1}): 3291,1761,1687,1630,1600,1524,1500,1412,1371,1296,1163 1016,916,868,835,790,674,534.**^{1}H NMR** (DMSO-d_6, 400 MHz); (ppm): δ 8.23-8.21 (d, *J*=8.8 Hz, 2H, Ar), 7.28-7.26 (d, *J*=8.8Hz, 2H, Ar), 7.23 (s, 1H, CH_2), 2.32 (s, 3H, Me), 2.31 (s, 3H, Me). **^{13}C NMR** (DMSO-d_6, 400 MHz); (ppm): δ 167.7 (C=O), 164.7 (C=O), 161.1(C=N), 134.9 (CH), 130.5 (CH=), 130.3 (C=) 127.4 (Ar), 124.4(Ar),116.7 (Ar-C**H**) 27.0(AcO-CH_3), 15.4 (CH_3). **HRMS (ESI)**: m/z calc $C_{13}H_{12}NO_4^+$ $[M+H]^+$ 246.0763; found 246.0761.

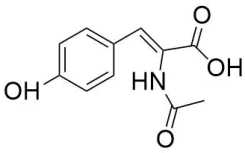
6.2.3.2. Synthesis of 2-(Acetylamino)-3-(4-hydroxyphenyl)-2-propenoic acid

The product number **(19)** is achieved after a ring-opening reaction through a double acid-catalyzed hydrolysis taking place in both carbonyl groups.

The reagents are found in the following quantities:

6.5g, 26.1mmol of the acetylated oxazolone **(1)** and 250ml of HCl (0.2N).

Purified by column chromatography AcOEt 9:1 MeOH with an increasing gradient of (MeOH).

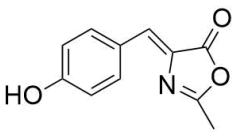


19

Red oil. Yield: 61%. **TLC:** (Rf=0.00)
^{1}H NMR (DMSO-d_6, 400 MHz); (ppm): δ 8.20-8.18 (d, *J*=8.8 Hz, 2H, Ar) 7.74-7.72 (d, *J*=9.6 Hz, 2H, Ar), 7.45-7.43 (d, *J*=8.8Hz, 1H, Ar), 7.37-7.35(m, 1H) 7.24(s,1H, C=C**H**) 7.17-7.19 (d, *J=8.0Hz,*1H, CH_2), 2.19 (s, 3H, Me). **HRMS (ESI)**: m/z calc $C_{11}H_{12}NO_4^+$ [M+H]$^+$ 222.2194; found 222.2198.

6.2.3.3. Synthesis of 4-(4-Hydroxylbenzylidene)-2-methyloxazol-5-one

The desired product was obtained through a reaction between the compound **(19)** (1eq) with N,N'-Diisopropylcarbodiimide (DIC) (3eq) and DMF anh used as a solvent in a one mouth flask of 250 mL and stirring with the aid of a magnetic stirrer. These products were allowed to react at room temperature for 48 hours. The DMF was removed under reduced pressure in a rotary evaporator. A dark red oil was the resulting product. After a TLC, it was considered to do a purification using column chromatography with AcOEt:Hex (1:2), to acquire the product (2).



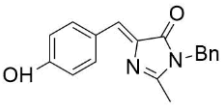
2

Yellow crystalline solid. Yield 78%. **TLC** (Rf=0.28) **IR** (KBr);(cm^{-1}): 3239,2358,2334,1732,1649,1600,1567,1533,1506,1432,1374,1279,1206, 1160,904,836,812.**^{1}H NMR** (DMSO-d_6, 400 MHz); (ppm): δ 10.35 (s, 1H, OH), 8.07-8.05 (d, *J*=8.8 Hz, 2H, Ar), 7.12 (s, 1H, CH_2) 6.88-6.86 (d, *J*=8.0Hz, 2H, Ar), 2.35 (s, 1H, Me). **^{13}C NMR** (DMSO-d_6, 400 MHz); (ppm): δ 168.2 (C=O), 164.3 (C=O), 160.7(C=N), 134.5 (CH), 129.5 (Ar), 129.1 (Ar-C**H**), 118.8(CH), 118.3 (C=) 16.4(CH_3). **HRMS (ESI)**: m/z calc. $C_{11}H_{10}NO_3^+$ [M+H]$^+$ 204.0655; found 204.0652.

6.2.3.4. Synthesis of p-HBDI and its functionalized analogue

6.2.3.4.1. Synthesis of 4-(4-Hydroxybenzylidene)-1-benzyl-2-methylimidazol-5-one

	Open acid form	Pyr	EtOH
Quantity [g or ml]	3.027	3.61 (3.28ml)	50ml
Quantity [mmol]	9.11	45.57	-

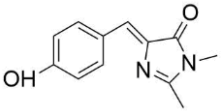


20A

Yellow crystalline solid. Yield 32%. **TLC** (Rf=0.25) **IR** (KBr);(cm^{-1}): 3019,1735,1701,1627,1603,1522,1504,1397,1204,1107,1046,895,717,674. **^{1}H NMR** (DMSO-d_6, 400 MHz); (ppm): δ 10.21 (s, 1H, OH, broad band), 8.09-8.07 (d, *J*=8.4 Hz, 2H, Ar),7.36-7.30 (m,3H), 7.24-7.22(d,*J*=8.0Hz,2H) ,7.01 (s, 1H, C=C**H**) 6.85-6.83 (d, *J*=8.4Hz, 2H, Ar),4.81 (s,2H,CH_2), 2.28 (s, 3H, Me). **^{13}C NMR** (DMSO-d_6, 400 MHz); (ppm): δ 170.5 (C=O), 162.3 (C=O), 160.1(C=N), 137.2 (CH), 136.6(CH) 136.2(CH) 133.9(CH) 129.5 (Ar), 129.1 (Ar-C**H**), 127.4(CH), 125.9 118.3 (C=) 41.7(CH_2) 16.1(CH_3). **HRMS (ESI)**: m/z calc. $C_{18}H_{17}N_2O_2^+$ $[M+H]^+$ 293.1286; found 293.1286

6.2.3.4.2. Synthesis of 4-(4-Hydroxybenzylidene)-1-methyl-2-methylimidazol-5-one

	Open acid form	Pyr	EtOH
Quantity [g or ml]	3.125	4.47 (4.07ml)	50ml
Quantity [mmol]	10.24	56.60	-



20B

Yellow crystalline solid. Yield 48%. **TLC** (Rf=0.20) **IR** (KBr);(cm^{-1}): 3018,1890,1703,1629,1603,1593,1400,1365,1276,1204,1128,1062,845, 709,672.**^{1}H NMR** (DMSO-d_6, 400 MHz); (ppm): δ 10.10 (s, 1H, OH, broad band), 8.07-8.05 (d, *J*=8.4 Hz, 2H, Ar), 6.99 (s, 1H, C=C**H**) 6.84-6.82 (d, *J*=8.0Hz, 2H, Ar), 3.06 (s, 3H, Me), 2.31(s,3H,CH_3). **^{13}C NMR** (DMSO-d_6, 400 MHz); (ppm): δ 170.2 (C=O), 163.3 (C=O), 160.1 (C=N), 135.9 (CH), 136.3(CH) 129.7 (Ar), 125.0 (Ar-C**H**), 119.1(CH) 117.0(CH) 26.6(CH_3) 16.4(CH_3). **HRMS (ESI)**: m/z calc. $C_{12}H_{13}N_2O_2^+$ $[M+H]^+$ 217.0977; found 217.0975

10. CONCLUSIONS

- The main objective of the work has been fulfilled due to the fact that p-HBDI, the core chromophore of the GFP has been successfully synthesized and it has been able to be functionalized with the benzyl substituent to carry out the subsequent anchorage on polymer resin and study its possible paramagnetic behaviour.

- A large spectrum of oxazolones has been synthetized by a simple reaction scheme and achieving a satisfactory yield, regardless of the electronic type of substituent. This oxazolones could be also used, in further works, to check if they present a phenomenon similar to the imidazolones, and stablishing a minimum structural unit that presents paramagnetism.

- Finally, it is possible to produce the imidazolones (p-HBDI analogs) through their synthesis from oxazolones, although the first synthetic route proposed (Route A) did not give the expected results.

- Because of the poor yields obtained with the Route A, an alternative synthetic route has been proposed, which allows to improve the yield of the products, as well as, to obtain also functionalized imidazolones with electron withdrawing substituents that important fact did not take place through the first route.

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12. ACRONYMS

Ac_2O: Acetic Anhydride
AcOEt: Ethyl Acetate
Bn: Benzyl
CDCl3: Deuterated chloroform
COSY: Correlation Spectroscopy
CT: Charge Transference
DIC: Diisopropylcarbodiimide
DMF: Dimethylformamide
DMSO: Dimethyl sulfoxide
EPR: Electron Paramagnetic Resonance
ESI-MS: Electrospray Ionization Mass Spectroscopy
EtOH: Ethanol
GFP: Green Fluorescent Protein
HRMS: High Resolution Mass spectrometry
Hz: Hertz
IR: Infrared
MeOH: Methanol
NaOAc: Sodium Acetate
NMR: Nuclear Magnetic Resonance
p-HBDI: p-Hydroxybenzylideneimidazolidin-5-one
ppm: parts per million
Psi: Pounds per square inch
Q-HPLC: Quadrupole-High-Performance Liquid Chromatography
Rf: Retention Factor
NMR: Nuclear Magnetic Resonance
S_0: Singlet ground state
S_1^{DR}: Diradical Singlet
SI: International System
T_1^{DR}: Diradical Triplet
TLC: Thin Layer Chrompatography
TMS: Tetramethylsilane
UV: Ultraviolet