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FACULDADE DE FARMÁCIA
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SYNTHESIS OF MARINE INSPIRED FLAVONES WITH POTENTIAL ANTIFOULING ACTIVITY

Relatório da UC Projeto I do Mestrado Integrado em Ciências Farmacêuticas

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Abstract

Both macro and microorganisms are part of the great community responsible for the process known as biofouling, which occurs when certain marine species attach to natural or artificial underwater surfaces and accumulate. As a result, several nautical problems have emerged and great investments have been made in order to discover new antifouling agents. However, even though many antifouling agents were in fact created, soon their harmful effects on the sea were exposed and the need to research for less toxic and environment-friendly compounds came forward.

In the light of previous studies concerning the biological activities of natural marine compounds, it is undeniable the nontoxic antifouling potential of some flavonoids, particularly glycosylated flavone sulfates such as Thalassiolins A-C. Moreover, 1,2,3-triazole rich molecules have gained much importance because of their excellent antimicrobial and antifouling properties, attributed to the triazole ring. So, the main goal of this project was the synthesis of flavone derivatives, inspired by the marine antifouling-acting compounds Thalassiolins A-C. To fulfill this, the flavone nucleus was firstly synthesized and then, a glycosyl moiety was incorporated, using 1,2,3-triazole ring as a linker between the flavone nucleus and the sugar. The synthesis of flavones **LuMe** and **LuNi** was accomplished by solvent-free direct thermal cyclocondensation of phloroglucinol and α-ketoesters. The following step in the synthetic process was the propargylation of the previously prepared flavone **LuMe** and, after that, a copper catalyzed alkyne-azide cycloaddition of the resulting product, originating **LuMeTGluAc**.

In the future, the process will have continuity and the glycosyl moiety present in **LuMeTGluAc** shall undergo deacetylation, followed by the sulfation of the remaining hydroxyl groups in the sugar moiety. Finished the whole multistep process, the resulting structure should combine the advantages of the flavone nucleus, 1,2,3-triazole ring and sulfated glycosyl moieties and potentially behave as a powerful antifouling agent.

Keywords: Antifouling activity, Glycosylated flavone sulfates, 1,2,3-Triazole.

Resumo

Macro e microrganismos fazem parte da grande comunidade responsável pelo processo conhecido por bioincrustação, o qual ocorre quando determinadas espécies marinhas se acumulam sobre superfícies subaquáticas, naturais ou artificiais. Como resultado, inúmeros problemas náuticos têm surgido e grandes investimentos foram já feitos, numa tentativa de se descobrirem novos agentes anti-incrustantes. No entanto, embora muitos agentes tenham sido já desenvolvidos, rapidamente os seus efeitos nocivos para o oceano foram expostos tendo ressurgido a necessidade de se procurarem novos compostos, menos tóxicos, e mais benignos para o meio ambiente.

À luz de estudos prévios acerca da atividade biológica de compostos marinhos de origem natural, é inegável o potencial anti-incrustante e não tóxico de alguns flavonoides, particularmente flavonas glicosiladas sulfatadas, como as Talassiolinas A-C. Por outro lado, o anel 1,2,3-triazol tem também demonstrado características favoráveis em relação a esta atividade e, nos últimos anos, moléculas ricas neste anel têm vindo a ganhar muita atenção devido às suas excelentes propriedades antimicrobianas e anti-incrustantes, atribuídas maioritariamente ao triazol. Assim, este projeto teve como principal objetivo a síntese de flavonas inspiradas nos flavonóides marinhos Talassiolinas A-C. Para cumprir este objetivo, o núcleo flavona foi inicialmente sintetizado e, em seguida, foi incorporada uma porção glicosilo, utilizando o anel 1,2,3-triazol para fazer a união entre o núcleo flavona e o açúcar. A síntese das flavonas **LuMe** e **LuNi** foi efetuada por ciclocondensação térmica direta e sem solvente de floroglucinol com α-cetoésteres. O passo seguinte no processo sintético foi a propargilação da flavona previamente preparada e, posteriormente, a cicloadição do alcino resultante com uma azida, catalisada pelo cobre, originando a **LuMeTGluAc**.

Futuramente, o processo terá continuidade com a desacetilação da fração glicosilo presente na **LuMeTGluAc**, seguida da sulfatação dos grupos hidroxilo do açúcar. Terminado todo o processo, a estrutura resultante deverá, idealmente, combinar as vantagens do núcleo flavona, do anel 1,2,3-triazol e do grupo glicosilo sulfatado e comportar-se, potencialmente, como um poderoso agente anti-incrustante.

Palavras-chave: Atividade anti-incrustante, Flavonas glicosiladas sulfatadas, 1,2,3-triazol
.

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Nesta secção, gostaria de expressar a minha gratidão para com todos aqueles que de alguma forma contribuíram para a realização deste projeto. Aproveito para deixar um agradecimento especial à:

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Abbreviations and symbols

^{13}C NMR	Carbon nuclear magnetic resonance
^{1}H NMR	Proton nuclear magnetic resonance
CC	Column Chromatography
CuAAC	Copper catalyzed azide-alkyne cycloaddition
d	doublet
dd	double doublet
FTIR	Fourier transform infrared spectroscopy
HBMC	Heteronuclear multiple bond correlation
HSQC	Heteronuclear single bond correlation
IR	Infrared spectroscopy
J	Coupling constant
m	multiplet
MAOS	Microwave assisted organic synthesis
MNP	Marine Natural Products
MW	Microwave
NCEs	New Chemical Entities
NMR	Nuclear Magnetic Resonance
s	singlet
TBAB	Tetra-*N*-butylammonium bromide
TLC	Thin-layer chromatography
TMS	Tetramethylsilane
δ	Chemical shift
δ_C	Carbon Chemical shift
δ_H	Proton Chemical shift
υ	Wavenumver (cm^{-1})
NBS	*N*-bromosuccinimide

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1. Introduction

Chemical substances from botanic, animal and microbial origin have been used to treat human diseases since the early days of society. The investigation of natural products as a source of novel human therapeutics reached its peak in the Western pharmaceutical industry in the 70s through 80s period. From this period until 2002, around 50% of the New Chemical Entities (NCEs) introduced in the market were from natural source or inspired by a natural pharmacophore. [1,2] At the moment, natural products continue to play a highly significant role in the drug discovery and development process. That occurs because, typically, they represent the most potent and specific ligands towards the wanted target, when compared to artificially designed molecules. It is that way because of the natural evolutionary selection, in which nature acted by doing its own high-throughput screening process, for the optimization of biologically active compounds. [3,4]

Among natural resources, Marine Natural Products (MNP) have been considered one of the most promising sources of bioactive compounds, with a steadily increase in the reported number of molecules from this origin. It does not appear as unexpected, since a considerable portion of the Earth's biodiversity, nearly 25% of the total number of species, compromise marine organisms. It is only natural that the first source to be explored by the ancient people were the terrestrial plants, much closer than any other source, but with the emergence of novel technological means of research, new horizons could be broaden. Since 1984, until the present days, a wide variety of marine compounds have been isolated from marine microorganisms, fungi, algae, sponges, mangroves and mollusks. These organisms have developed mechanisms to survive in extremely different and hostile environments compared with land, in terms of light, salinity and pressure. This ability reflects itself on the innumerable quantity of secondary metabolites, which they produce to defend themselves against predators, locate mates and to have advantage in the struggle for limited resources. [5,6,7]

Despite their historical value as a successful source of therapeutic agents, natural products have an inconvenient disadvantage. The vast majority of the most promising lead compounds, found in this source, are available only in extremely small amounts, leading to poor yields of extraction, which is a huge limiting factor to an industry that requires tones of active substance for the worldwide production and distribution of medicines. Organic synthesis arises as a solution to overcome this issue, allowing the production of those compounds, via chemical reactions, and also the

opportunity to make changes in the molecule, aiming the design of structure-related derivatives. [8]

1.1. Flavones

Chemically, flavones are a class of flavonoids based on the backbone of 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one) (**Figure 1**). [9,10]

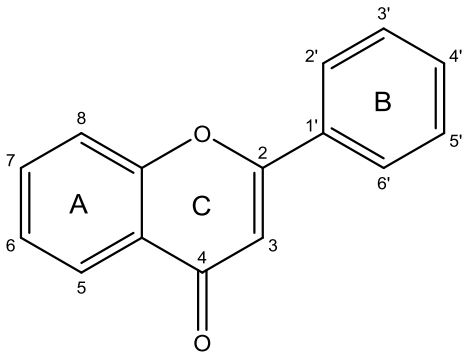


Figure 1: Basic structure of flavones and its numbering.

Flavones can be classified into several subgroups according to its substitution pattern. Natural flavones frequently possess a hydroxyl group at positions 5 and 7 in the A ring. On the other hand, the B-ring is commonly oxygenated in the 4'-, 3',4' or 3',4',5'-positions, as expected from their biosynthetic origin, based on the acetate-shikimate pathway. [5,11] Flavones can also occur as glycosides, namely as 7-*O*-glycosides, being D-glucose the most common sugar residue. However, other sugar moieties such as D-galactose, L-rhamnose and D-xylose can also be found. [12,13]

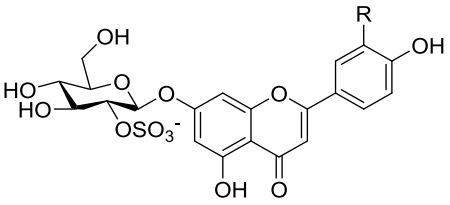
Flavones are considered one of the most prominent classes of flavonoids, well-known by their broad range of biological properties. In fact, these natural products have been reported has having, not only pharmacological activities (antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, antiallergic, antithrombotic, antitumor, hepatoprotective), but also antifouling activity, demonstrating their potential as lead compounds for pharmaceutical and chemical industries. [14,15,16,17,18,19,20]

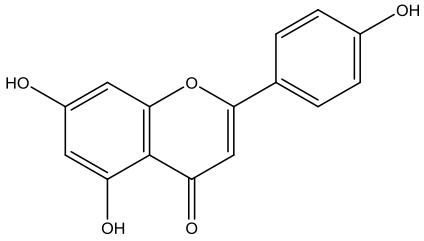
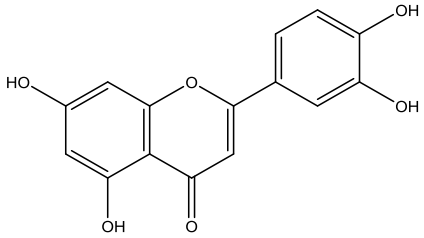
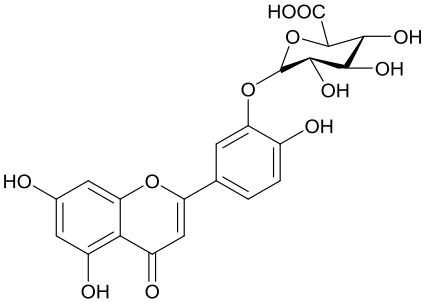
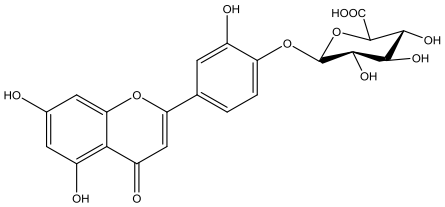
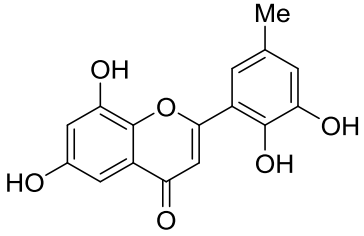
In general, flavonoids are secondary metabolites, that humans inadvertently consume in their daily diet. [21,22] Although many flavonoids with different substitution patterns have been exhaustively isolated from land, these secondary metabolites can also be found in marine environments, not only from plants (angiosperms, mangroves, seagrass, halophyte and algae), but also from mollusks, coral and fungi. [21]

1.1.1. Flavones as antifouling agents

Biofouling is the name of the process by which micro- and macroorganisms attach to natural or artificial underwater surfaces. The accumulation of this miscellaneous community is responsible for the development of several economic and environmental nautical problems and, as a result, great investments have been made to discover new antifouling agents. These compounds should be capable to inhibit the settlement process of biofouling species by interfering with mechanisms like ion channel transportation, energy producing enzymes, adhesive glands and adhesive production/release. Less specific, and therefore more toxic, mechanisms include the interference with neurotransmitters, proteins, arrangement of biofilm, induction of oxidative stress or acting as lethal toxins. [26,27,28,29] Although many antifouling agents were discovered, soon their harmful effects on the sea were exposed and the need to research for less toxic and environment-friendly compounds emerged. Once more, the search for natural solutions stood out and flavones came forward as a possible option. More specifically, MNP have recently been considered as one of the most promising sources of antifouling compounds. In fact, several marine macroorganisms are themselves capable to stay free from biofouling, and therefore, their secondary metabolites are believed to be the chemical defensive substances behind that protection. These MNP are easily biodegradable and leave no residue in the environment, thus have been considered as a potential source for environmentally friendly natural antifouling agents. In the past decades, many MNP with a variety of structural features, namely flavonoids, have been reported as nontoxic antifouling compounds. [30,31,32,33] Some examples of marine flavones with antifouling activity are presented in **Table 1**.

Table 1: Examples of marine flavones with antifouling activity.

Compound	Organism	Mechanism of action	Reference
 **R = OH, Luteolin-7-O-β-D-glucopyranosyl-2''-sulfate (Thalassiolin A) (1)** **R = OMe; Chrysoeriol-7-O-β-D-glucopyranosyl-2''-sulfate**	Seagrass *Thalassia testudinum*	Microbiological activity against the co-occurring protists *Schizochytrium aggregatum*	[23]

(Thalassiolin B) (2) **R = H; Apigenin-7-O-β-D-glucopyranosyl-2''-sulfate** **(Thalassiolin C) (3)**			
 **Apigenin (4)**	Seagrass *Enhalus acoroides*	Anti-larval settlement and antifeedant activity	[26]
 **Luteolin (5)**	Seagrass *Enhalus acoroides*	Anti-larval settlement activity	[26]
 **Luteolin-3'-glucuronide (6)**	Seagrass *Enhalus acoroides*	Antifeedant activity	[24]
 **Luteolin-4'-glucuronide (7)**	Seagrass *Enhalus acoroides*	Antifeedant activity against *Spodoptera litura* larvae. Antibacterial activity. Antilarval activity towards *Bugula neritina* larvae.	[24]
 **6,8,5',6'-tetrahydroxy-3'-methylflavone (8)**	Coral *Dichotella gemmacea*	Anti-larval settlement activity	[11]

1.1.2. Synthesis of flavones

It is possible to synthesize flavones throughout several different methods, starting from other flavonoids, like chalcones and flavanones, or from simpler precursors by means of condensation reactions.

1.1.2.1. Dehydrogenation of chalcones and flavanones

As mentioned before, this reaction allows the synthesis of flavones in a dehydrogenation process started with a chalcone or a flavanone. To illustrate the process, the synthesis of the flavone cirsimaritin will be used as example (**Figure 2**). The starting material - 2'-hydroxy-3',4',6'-trimethoxy-4-benzyloxychalcone - has to be previously brominated to form 2'-hydroxy-3',4',6'-trimethoxy-4-benzyloxy-α,β-dibromochalcone **(9)**. This compound is then boiled in methanol to produce 2'-hydroxy-β,3',4',6'-tetramethoxy-4-benzyloxy-α-bromochalcone **(10)**, which is lastly converted by a simultaneous process of thermal cyclization and partial demethylation, into 5,4'-dihydroxy-6,7-dimethoxyflavone, known as cirsimaritin **(11)**. [8]

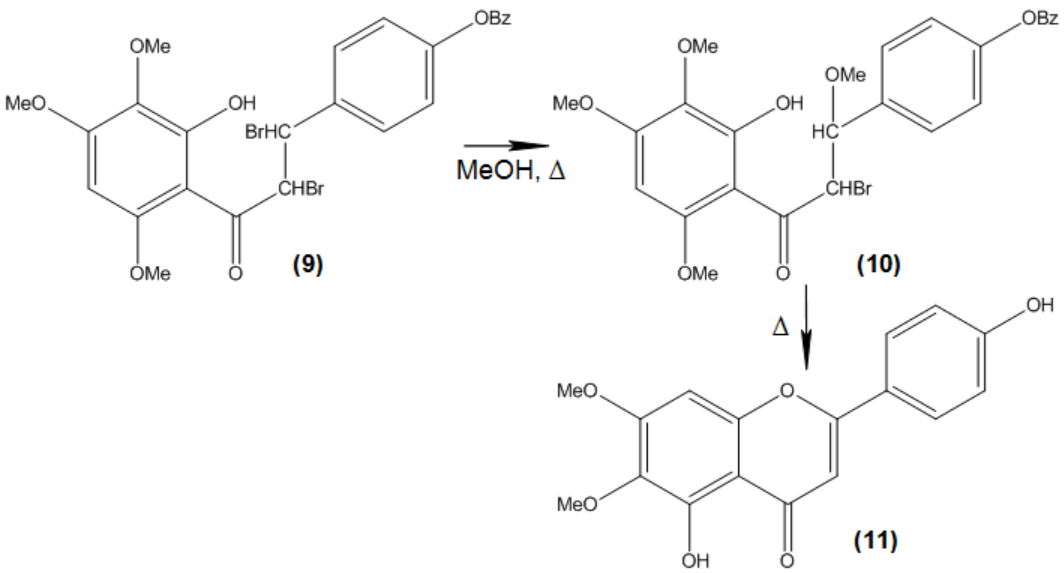


Figure 2: Synthesis of cirsimaritin **(11)** by dehydrogenation of a chalcone.

Other method to produce flavones is based on a benzoyl peroxide-catalyzed bromination of partially acetylated flavanones, in the presence of *N*-bromosuccinimide (NBS) in carbon tetrachloride, followed by acid hydrolysis. This reaction is shown bellow, using as example the synthesis of diosmetin **(13)** from the flavanone hesperetin **(12)** (**Figure 3**). [8]

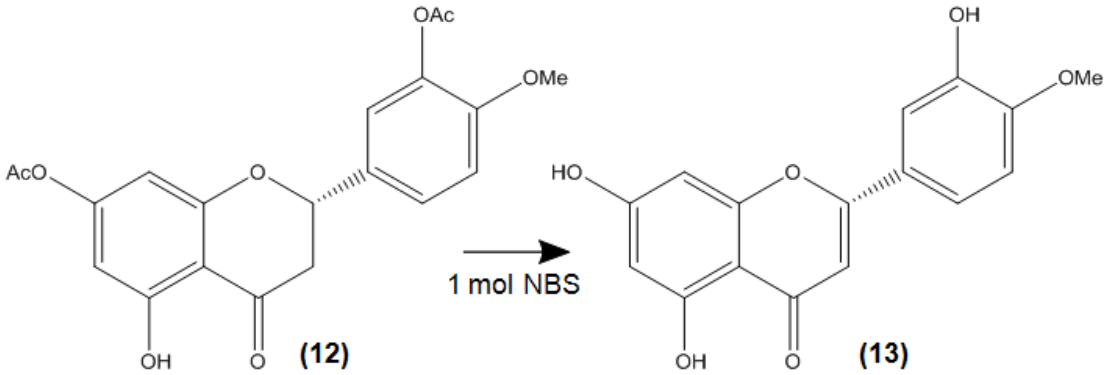


Figure 3: Synthesis of diosmetin **(13)** by dehydrogenation of hesperetin **(12)**.

1.1.2.2. Baker-Venkataraman rearrangement

The Baker-Venkataraman rearrangement is one of the most common reactions to prepare flavones and involves the conversion of an *o*-hydroxyacetophenone **(14)** into a phenolic ester **(15)**, by reacting with aromatic acid chlorides in pyridine. The phenolic ester then undergoes an intramolecular Claisen condensation, in the presence of a base. This process results in the development of a β-diketone (**Figure 4**) and the rearrangement occurs with the formation of an enolate **(15a)** followed by an intramolecular acyl transfer (**Figure 5**). The β-diketone **(16)** is then cyclized to a flavone **(17)**, under acidic conditions, namely by heating with concentrated sulfuric acid in glacial acetic acid (**Figure 4**). [8,18]

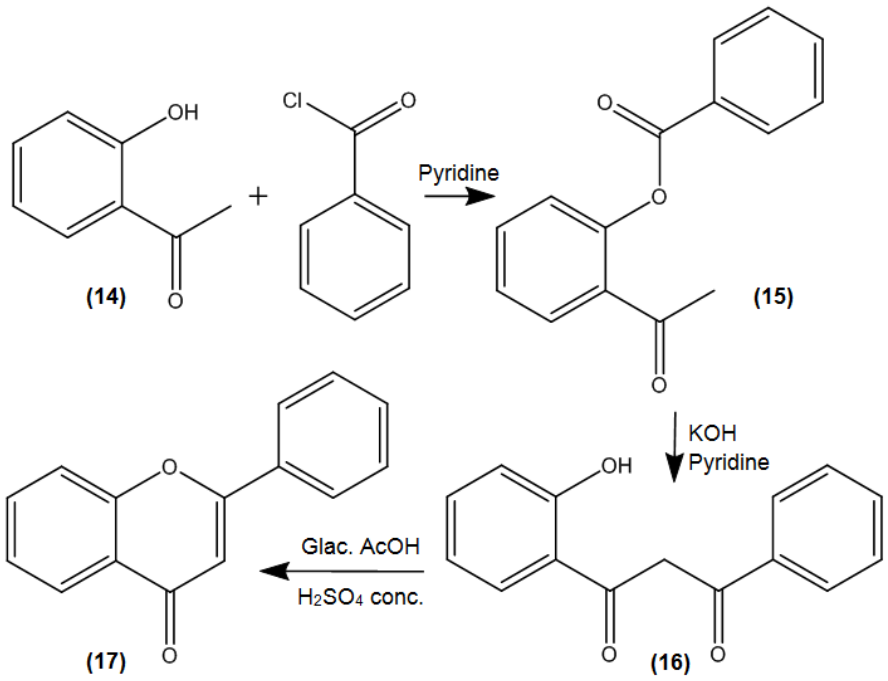


Figure 4: Synthesis of flavones via Baker-Venkataraman rearrangement.

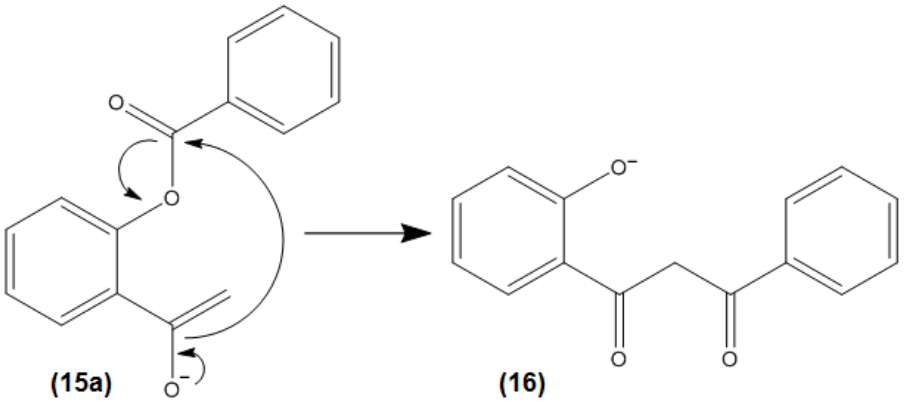


Figure 5: Baker-Venkataraman rearrangement.

1.1.2.3. Allan-Robinson condensation

The Allan-Robinson process allows the one step synthesis of flavones throughout the condensation of an *o*-hydroxyacetophenone **(17)** with an aromatic acid anhydride in the presence of sodium salts of the same acid. As a result of this reaction, a hemiketal intermediate **(18)** is formed and two products are created. Under basic conditions, the hemiketal originates a flavone **(19)**. Alternatively, it can also form a ω-benzoyl-*o*-hydroxyacetophenone **(20)**, which possesses an acidic proton, that will further react with acid anhydride to form a triketone **(21)** leading to hemiketal **(22)**, which undergoes dehydration to yield a 3-benzoyl-flavone **(23)** (**Figure 6**). [8,18]

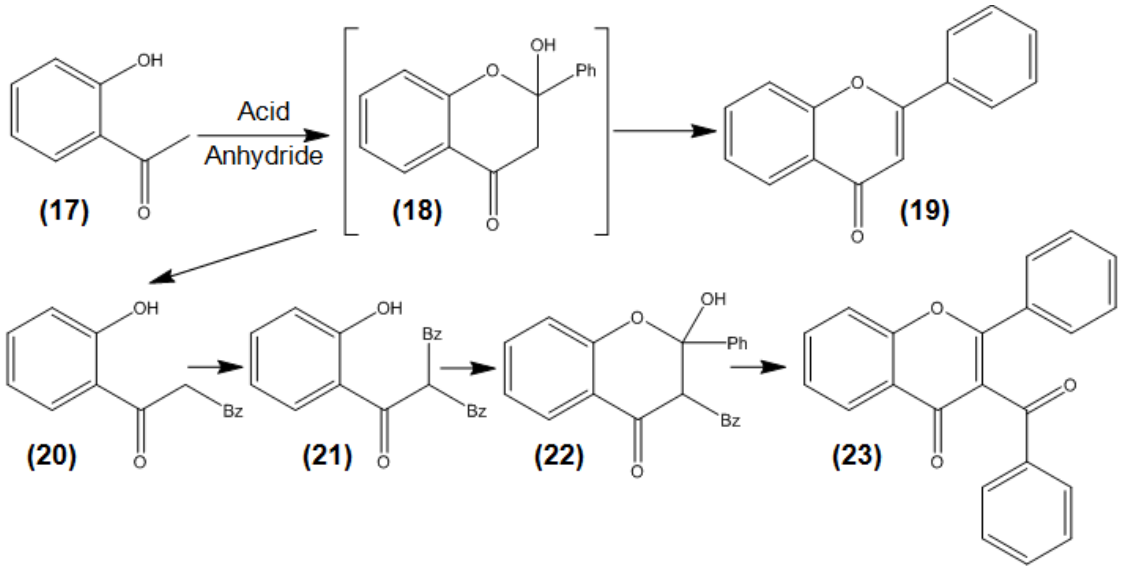


Figure 6: Synthesis of flavones and 3-benzoylflavones by Allan-Robinson condensation.

1.1.2.4. Solvent-Free synthesis of functionalized flavones by the Mentzer synthesis

This reaction was first reported by Mentzer et al. in 1946. [34,35] The synthesis of flavones is achieved from a solvent-free process in which phloroglucinol and β-ketoesters are heated at high temperature. [36] This reaction has been modified to occur under microwave irradiation. [36] This process has arisen as more advantageous, being faster, cleaner and high yielding when compared with the classical conditions. The principle of the method is a simple cycloaddition of an α-oxo ketene intermediate followed by an uncatalyzed thermal Fries rearrangement (**Figure 7**). [36] The first step to occur is the production, at high temperatures, of the α-oxo ketene intermediate **(24)** from the β-ketoester. Next, it will take place the cycloaddition of phoroglucinol **(25)** and the previously formed α-oxo ketene **(24)**, to give an ester **(26)**. Once the ester is formed, it should undergo the uncatalyzed thermal Fries rearrangement, in which there will be an increase in the polarity of the transition state. The last steps involve an hemiketal **(27)** and a dehydration process. [37]

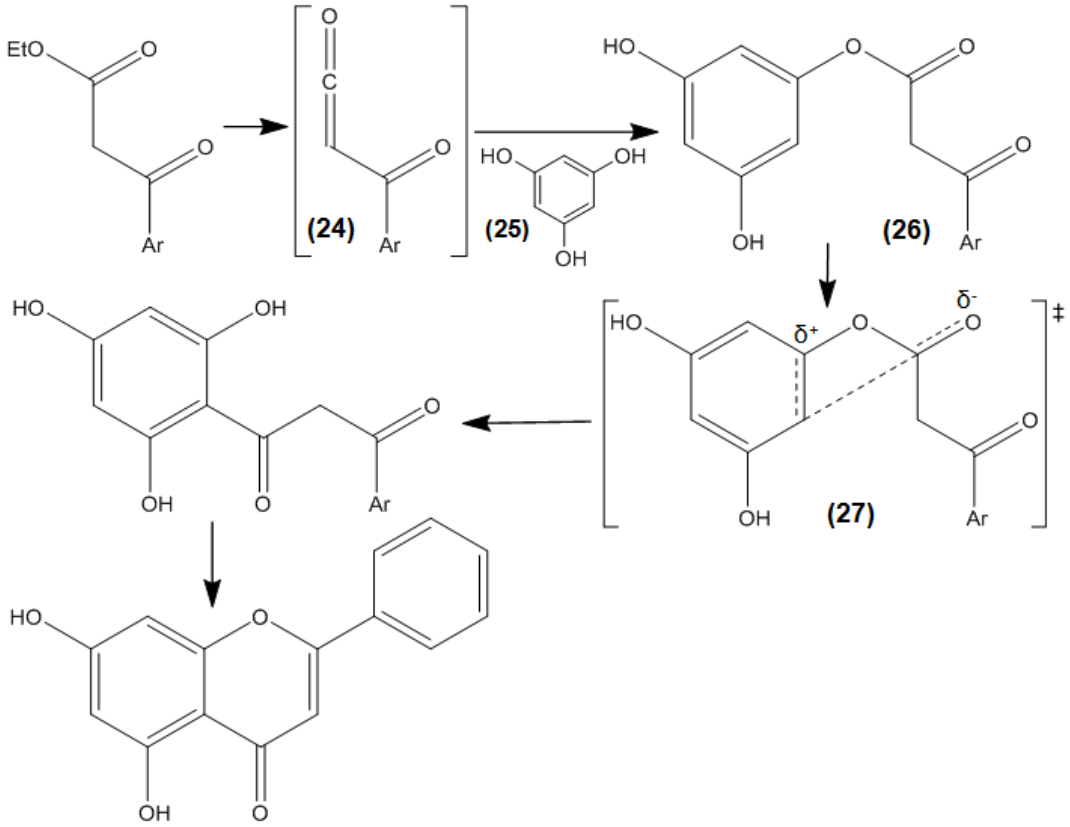


Figure 7: Synthesis of flavones throughout the Mentzer synthesis.

1.2. 1,2,3-Triazole

During the past few years, 1,2,3-triazole moiety (**Figure 8**) has been a target of great interest, both in pharmaceutical fields, where it has contributed for interesting biological activities, and in materials science. [38]

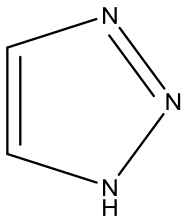


Figure 8: 1,2,3-triazole.

The 1,2,3-triazole core is able to form π-π interactions with aromatic ring structures and two of the nitrogen atoms, N2 and N3, can establish hydrogen bonding with hydrogen bond donors and even coordinate with metal ions. Additionally, 1,2,3-triazole represent a very stable structure, under physiological conditions. Because of this, diverse biological activities have been described for several compounds possessing this moiety, such as antibacterial, antifungal, antivirus and anticancer. [39,40,43]

Besides, the hydrogen bond making potential of the 1,2,3-triazole core makes it capable of acting as a biologically active site, which shields the material from various bacterial and fungal attacks. This particular set of skills allows it to be extremely useful to the improvement of polymeric materials, such as biomedical applications, corrosion protection, fabric coatings, anti-microbial coatings and biodegradable polymers. Has it was mentioned before, shipping industry has been facing problems with fouling of marine organisms on ship hulls. New tin-containing coating materials were developed in the 1970's, but they came up as harmful for marine environments and so, the next generation of coatings was designed replacing the tin with copper. However, the conversion of Cu^{2+} into Cu^{o} was greatly decreasing the resilience of the coat and so, in order to get a long-term antifouling action, it would be necessary the addition of other biocides, the so called boosters. As a result, after years of research, it was found that triazole based molecules had that ability to boost the antifouling nature of other already existing coatings, since these units coordinate with copper ions, stabilizing them and controlling their release. [41,42]

The first solid studies, concerning the 1,2,3-triazole synthesis, were carried out by Huisgen, in the 1960's, in which they promote the 1,3-dipolar cycloaddition reaction

between an alkyne and azide to give both 1,4- and 1,5-disubstituted-tiazole regioisomers (**Figure 9**). [38]

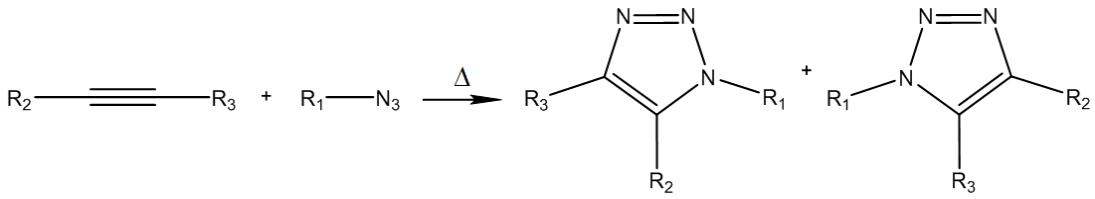


Figure 9: Huisgen's 1,3-dipolar cycloaddition.

To control the reaction's regioselectivity, multiple strategies were developed, including several using transition metals as catalysts. For instance, the copper catalyzed azide-alkyne cycloaddition (CuAAC), developed by Sharpless in 2002, allow the selective synthesis of the 1,4-regioisomer and is a well known example of click chemistry. Click chemistry describes a set of selective and powerful reactions that allow the quickly and reliably synthesis of substances, by joining small units together through heteroatom links. The process relies on advantageous characteristics including accessible reaction conditions, readily available reagents and starting materials, no solvents or green ones, like water, and simple purification processes. [38]

In the CuAAC reaction (**Figure 10**), the copper acts through a marked change in the reaction mechanism, ultimately improving the yields, considerably accelerating the cycloaddition reaction and allowing its execution at room temperature. The Cu(I) or Cu(II) salts, plus a reducing agent, are gathered in an organic solvent or in a mixture of water and *tert*-butyl alcohol, at room temperature. An *in situ* catalyst preparation takes place by reduction of Cu salts. [39,40]

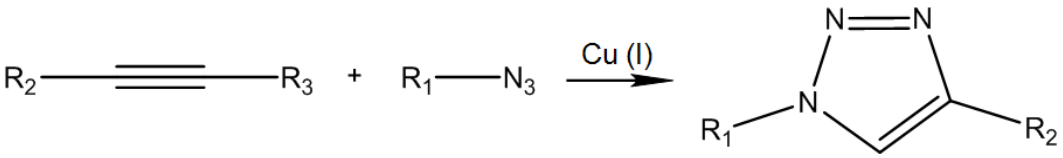


Figure 10: CuAAC reaction.

2. Aims and research plan

The main purpose of this research work was to obtain new flavones based on promising MNP and, therefore, leading to novel non-toxic antifouling agents. Inspired by the potential of marine natural flavonoids as antifouling agents, namely Thalassiolin A **(1)** (**Table 1**), a new sulfated glycosylated flavone, only found in the marine world, we aimed to synthesize structure related analogues. [23]

Taking into account the antimicrobial and antifouling properties that have been reported to several 1,2,3-triazole rich molecules, we also aimed to incorporate this core into potentially active antifouling structures related with Thalassiolins A-C. To fulfill this, we planned to synthesize the flavone nucleus and afterwards incorporate the sulfated glycosyl moiety, using 1,2,3-triazole ring as a linker between the flavone nucleus and the sugar moiety (**Figure 11**). [41,42]

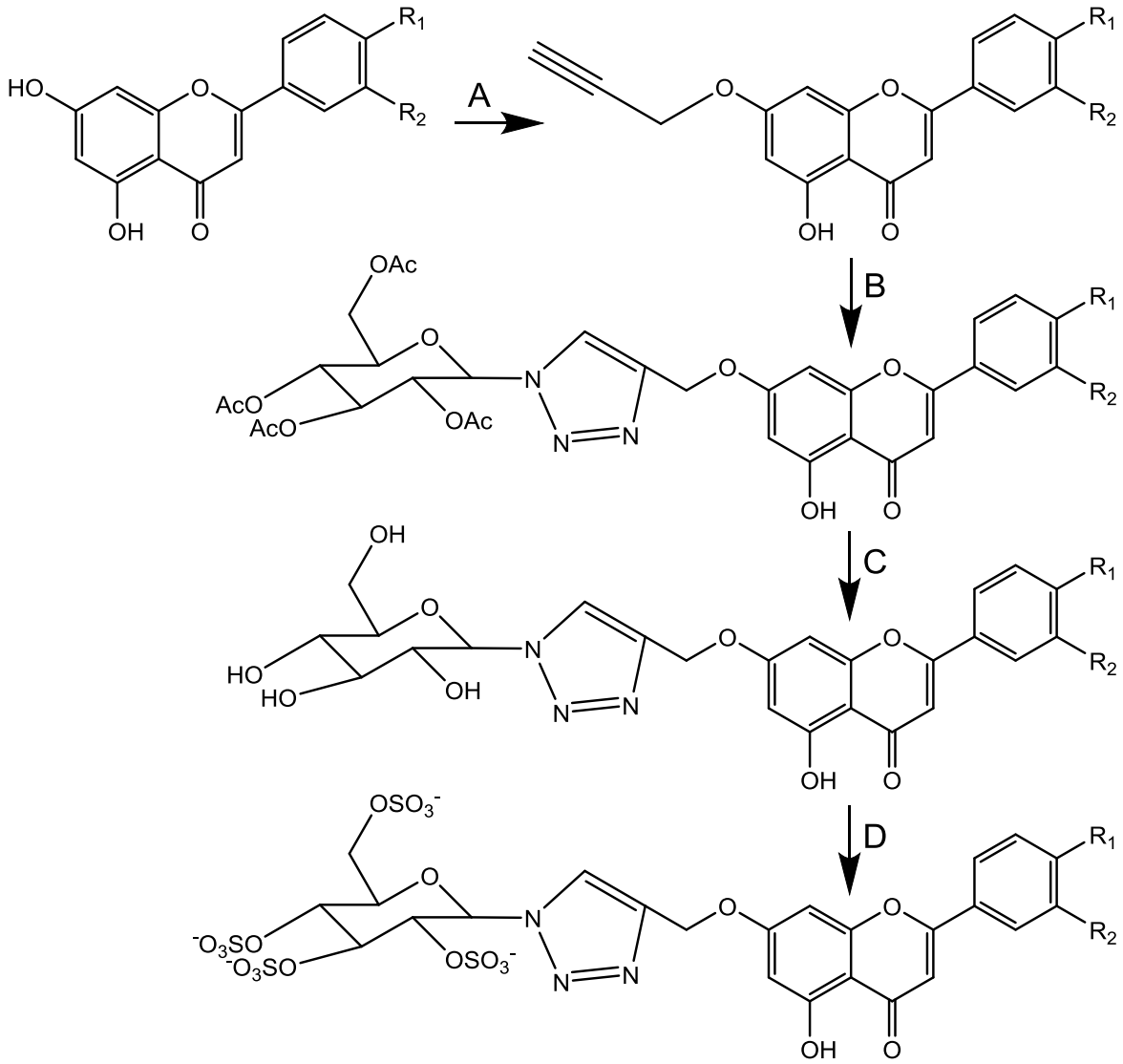


Figure 11: Research plan. A- Propargylation of the pre-synthesized flavones; B- Copper catalyzed alkyne-azide cycloaddition (CuAAC); C- Deacetylation; D- Sulfation.

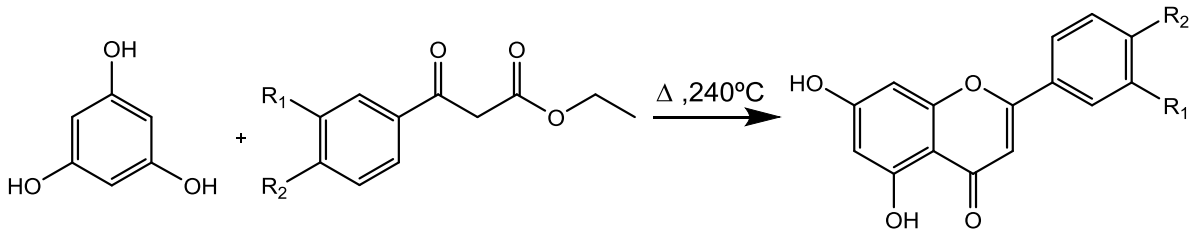
3. Results and Discussion

3.1. Synthesis

During the research period, two flavones (**LuNi** and **LuMe**) were synthesized. For **LuMe** a multistep process was carried out in order to synthesize a Thalassiolin A-C analogue with 1,2,3-triazole ring as a linker between the flavone nucleus and the sugar moiety, as illustrated in **Figure 11 (Page 11)**. The results obtained in the synthesis are discussed in the following sections.

3.1.1. Synthesis of Flavone derivatives LuMe and LuNi

Flavones **LuMe** and **LuNi** were synthesized by solvent-free direct thermal cyclocondensation of phloroglucinol and α-ketoesters, according to the procedure descrived by Mentzer et al. (**Figure 12**). [24,25]

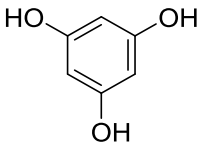
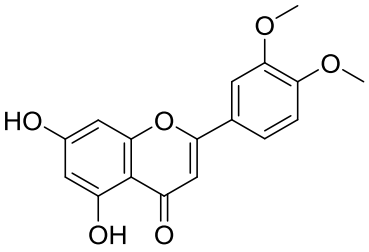
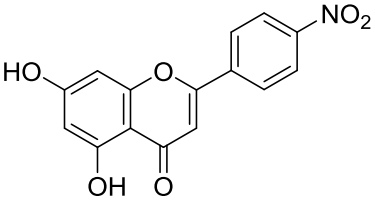


	LuMe	LuNi
R_1	$-OCH_3$	-H
R_2	$-OCH_3$	$-NO_2$

Figure 12: Synthesis of LuMe and LuNi.

Table 2 summarizes the reaction conditions and the results obtained in the synthesis of flavone derivatives **LuMe** and **LuNi**.

Table 2: General conditions for the synthesis of flavone derivatives.

Building block	Reaction conditions	Product	Yield
 Phloroglucinol	Ethyl 3,4-dimethoxybenzoylacetate, 1h	 **LuMe**	73.9%
	Ethyl 4-nitrobenzoylacetate, 50 min	 **LuNi**	15.5%

While **LuMe** was obtained with a good yield, the yield for **LuNi** synthesis was quite low. This could be explained since the reaction was not entirely completed and led to the formation of several by-products, which led to the need to purify the compound by flash column chromatography, followed by crystallization.

3.1.2. Synthesis of LuMeProp

The following step in the synthetic process was the propargylation of the previously prepared **LuMe**. The synthetic approach used for the synthesis of **LuMeProp** was based on the reaction of **LuMe** with 1 equivalent of propargyl bromide, in presence of anhydrous cesium carbonate, using TBAB (tetra-N-butylammonium bromide) as phase transfer catalyst (**Figure 13**).

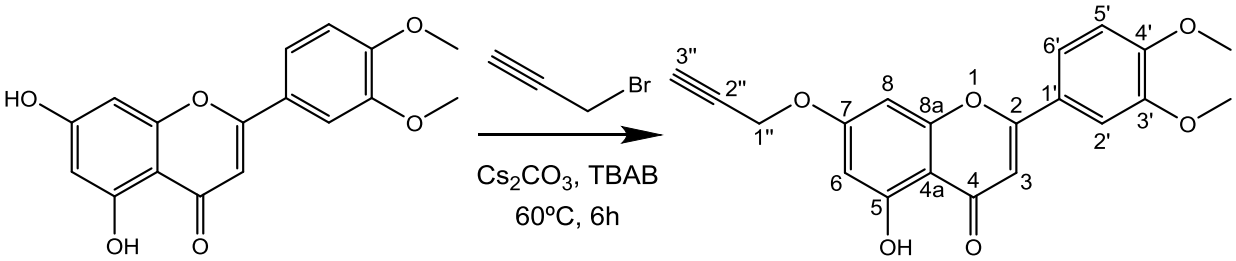


Figure 13: Synthesis of **LuMeProp**.

Since the hydroxyl group in position 5 is involved in an intramolecular hydrogen bond with the carbonyl group, only the 7-*O*-monosubstituted derivative was obtained with a 66% yield.

3.1.3. Synthesis of LuMeTGluAc

LuMeTGluAc was synthesized by copper catalyzed alkyne-azide cycloaddition (CuAAC). The reaction between the previously formed **LuMeProp** and 2,3,4,6-tetra-*O*-acetyl-β-glucopyranosyl azide was carried out in the presence of copper sulfate ($CuSO_4$) and sodium ascorbate, in water and tetrahydrofuran (1:2) at 80 ºC, under microwave irradiation, using MAOS methodology (**Figure 14**).

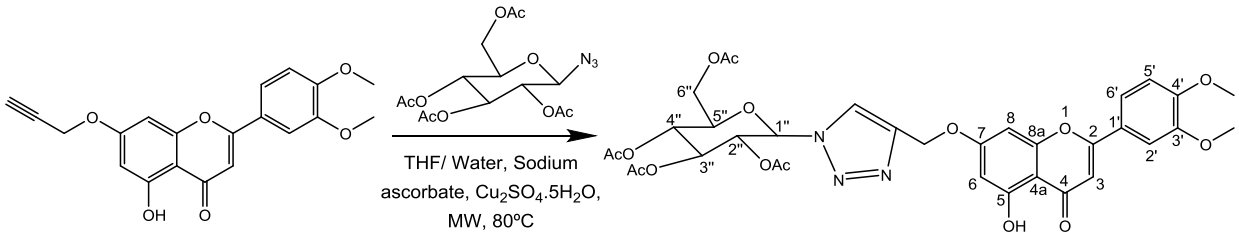


Figure 14: Synthesis of **LuMeTGluAc**.

After purification by flash column chromatography **LuMeTGluAc** was obtained with a 82% yield.

3.2. Structure Elucidation

The structure elucidation of all flavone derivatives was established on the basis of IR and NMR techniques. ^{13}C NMR assignments were determined by 2D heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) experiments.

The numbering concerning the NMR assignments of flavone derivatives **LuMe**, **LuNi**, **LuMeProp**, and **LuMeTGluAc** are presented in **Figure 15**.

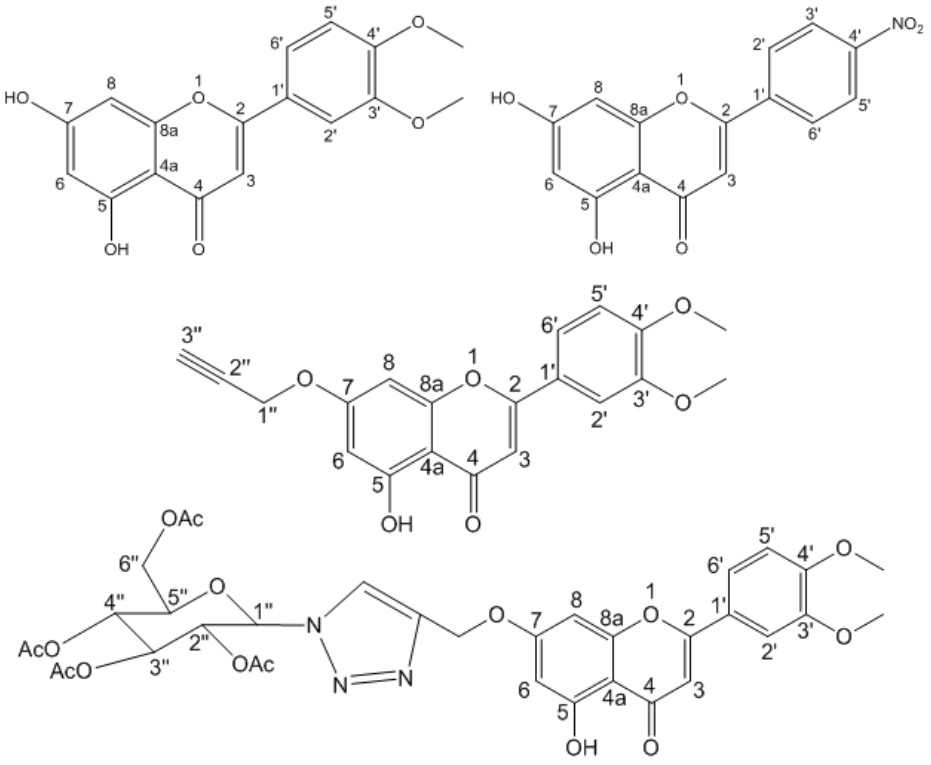


Figure 15: LuMe, **LuNi**, **LuMeProp** and **LuMeTGluAc** numbering, respectively.

3.2.1. Flavone derivatives, LuMe and LuNi

Analyzing the IR spectra of **LuMe** and **LuNi** it is possible to detect the presence of free hydroxyl groups, since a large band of stretching vibration at 3600-3300 cm^{-1} (OH) is present. Additionally, the present of bands at 1655-1653 cm^{-1} (α,β-unsaturated carbonyl group), 1599-1430 cm^{-1} (aromatic C=C) and 1274-1256 cm^{-1} (C-O) complies with the expected compounds. Finally, the presence of bands at 2950 cm^{-1} for **LuMe** suggests the presence of alkyl groups and of bands at 1519 cm^{-1} (N-O) and 1346 cm^{-1} (C-N) for **LuNi** suggests the presence of a nitro group (**Table 3**).

Table 3: IR data of **LuMe** and **LuNi.**

υ (cm^{-1})		
Groups	**LuMe**	**LuNi**
O-H	3600-3300	3600-3300
Aliphatic C-H	2950	-
C=O	1655	1653
Aromatic C=C	1581 1518 1504 1430	1599 1519 1457 1431
N-O	-	1519

C-N	-	1346
C-O	1256	1274

The ^{1}H and ^{13}C NMR data of flavone derivatives **LuMe** and **LuNi** are reported in **Table 4** and **Table 5**, respectively.

The ^{1}H NMR spectra of both flavone derivatives showed characteristic signals of a 5,7-dihydroxyphenyl A ring (OH-5: δ_H 12.92-12.53 s; H-6: δ_H 6.23-6.29 d; OH-7: δ_H 10.97-10.54 s; H-8: δ_H 6.55-6.49 d), and H-3 (δ_H 6.96-6.86 s).

For derivative **LuNi** the ^{1}H NMR spectra also showed two *orto* coupled doublets at δ_H 8.38 and δ_H 8.16, characteristic of a 4'-substituted B ring. Alternatively, for derivative **LuMe**, characteristic signals of a 3',4'-dimethoxy phenyl B ring (δ_C: 152.2, 149.0, 122.9, 120.1, 111.7, 109.4; 55.9 (OCH_3), 55.8 (OCH_3); δ_H: 7.68 dd, 7.56 d, 7.12 d, 3,87 s (OCH_3)) are observed in ^{1}H and ^{13}C NMR spectra.

The structure of these derivatives was confirmed by comparison with the ^{1}H and ^{13}C NMR data previously reported. [36]

Table 4: ^{1}H NMR data of flavone derivatives **LuMe** and **LuNi**.

	LuMe	**LuNi**
H-3	6.96 (s)	6.86 (s)
H-5	12.92 (s, OH)	12.53 (s, OH)
H-6	6.23 (d, J=2.1)	6.29 (d, J=2.0)
H-7	10.97 (s, OH)	10.54 (s, OH)
H-8	6.55 (d, J=2.1)	6.49 (d, J=2.0)
H-2'	7.56 (d, J=2.1)	8.38 (d, J=8.9)
H-3'	3.87 (s, OCH_3)	8.16 (d, J=8.9)
H-4'	3.87 (s, OCH_3)	-
H-5'	7.12 (d, J=8.7)	8.16 (d, J=8.9)
H-6'	7.68 (dd, J=8.7; J=2.1)	8.38 (d, J=8.9)

Values in parts per million (δ_H). Measured in DMSO-d_6 at 300.13 MHz. J values (Hz) are presented in parentheses.

Table 5: ^{13}C NMR data of flavone derivatives **LuMe** and **LuNi**.

	LuMe	**LuNi**
C-2	163.4	161.5
C-3	103.9	107.4
C-4	181.9	181.3
C-4a	103.8	104.2

C-5	161.4	160.3
C-6	98.9	99.3
C-7	164.3	164.6
C-8	94.2	93.9
C-8a	157.4	157.3
C-1'	122.9	136.7
C-2'	109.4	127.1
C-3'	149.0	123.7
C-4'	152.2	148.8
C-5'	111.7	123.7
C-6'	120.1	127.1
OCH_3	55.9	-

Values in parts per million (δ_C). Measured in DMSO-d_6 at 75.47 MHz.

3.2.2. Propargyl flavone derivative, LuMeProp

The IR data of **LuMeProp** derivative is in accordance with the predicted structure and it has similar characteristic signals to its precursor, **LuMe** (**Table 3**). Analyzing the IR spectrum of **LuMeProp** it is also possible to detect the presence of a band of stretching vibration at 3234 cm^{-1} (C≡C) which is expected for the alkyne moiety of this derivative (**Table 6**).

Table 6: IR data of **LuMeProp**.

Groups	**υ (cm^{-1})**
O-H	3600-3300
C≡C	3234
Aliphatic C-H	2963 2917 2849
C=O	1647
Aromatic C=C	1518 1504 1468 1453 1429
C-O	1261

The ^{1}H and ^{13}C NMR data of propargyl derivative **LuMeProp** are reported in **Table 7**.

As expected, the ^{1}H and ^{13}C NMR spectra of propargyl derivative **LuMeProp** are very similar to those of the precursor. However, no characteristic signal of HO-7 is observed in the spectrum ^{1}H NMR of **LuMeProp**, suggesting that the substitution occurred in this position. Alternatively, signals of two oxymethylenic protons at δ 4.76 d (J=2.0 Hz, H-1''), and one methynic proton at 3.61 s (H-3''), correlated in the HSQC spectrum with the ^{13}C NMR signals at δ_C 56.9 (C-1''), and δ_C 79.1 (C-3''), respectively, were observed, suggesting the propargylation of the hydroxyl group at C-7 (**Figure 16**).

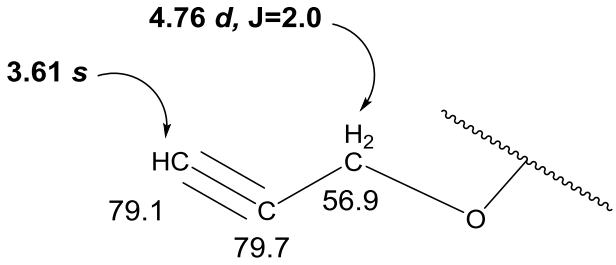


Figure 16: ^{1}H and ^{13}C NMR data for propargyl group of **LuMeProp**.

The position of the propargyl side chain was also evidenced by the correlations found in the HMBC as indicated in **Figure 17**.

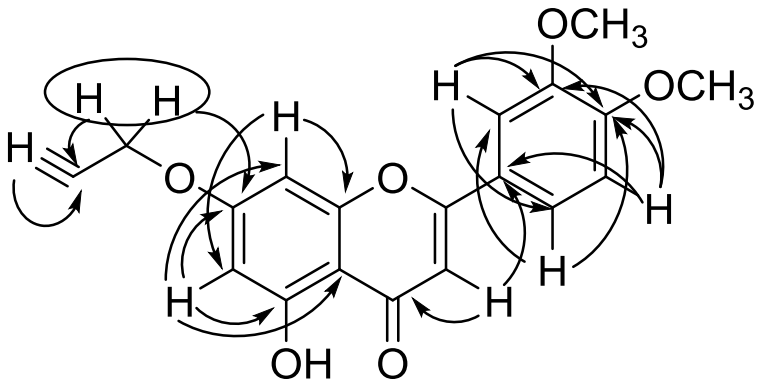


Figure 17: Main correlations observed in the HMBC spectrum of **LuMeProp**.

Table 7: ^{1}H and ^{13}C NMR data of propargyl flavone derivative **LuMeProp**.

	^{1}H	**^{13}C**
2	-	163.7
3	6.84 (s)	105.8
4	-	182.8
4a	-	104.8
5	12.74 (s, OH)	161.8
6	6.26 (d, J=2.0)	99.3
7	-	164.5
8	6.68 (d, J=2.0)	94.5
8a	-	157.8
1'	-	123.4
2'	7.39 (d, J=2.0)	110.1
3'	3.68 (s, OCH_3)	149.7
4'	3.69 (s, OCH_3)	152.9
5'	6.96 (d, J=8.5)	112.4
6'	7.53 (dd, J=8.5; 2.0)	120.9
1''	4.76 (d, J=2.0)	56.9
2''	-	79.7
3''	3.61 (s)	79.1

Values in parts per million (δ_H and δ_C). Measured in DMSO-d_6 at 500.15 MHz. J values (Hz) are presented in parentheses.

3.2.3. Flavone *O*-acetyl glycoside, LuMeTGluAc

The IR data of *O*-acetyl glycoside **LuMeTGluAc** derivative is in accordance with the predicted structure (**Table 8**). In addition to the bands expected for a flavone derivative observed for the precursor, the presence of a absorption band at 1322 cm^{-1} (C-N) is in accordance to the presence of a 1,2,3-triazole moiety and of a band at 1757 cm^{-1} (C=O) suggests the presence of a ester group.

Table 8: IR data of **LuMeTGluAc**.

Groups	**υ (cm^{-1})**
O-H	3600-3300
Aliphatic C-H	2963
C=O (Ester)	1757
C=O (Ketone)	1655

Aromatic C=C	1516 1502 1430
C-N	1322
C-O	1258 1226

The ^{1}H and ^{13}C NMR data of **LuMeTGluAc** are reported in **Table 9**. These spectra showed characteristic signals of the scaffold of the precursor **LuMeProp**. Beyond that, instead of characteristic signals of the propargyl group at C-7, signals of a triazole ring and an *O*-acetyl-β-glucopyranosyl group are observed (**Figure 18**).

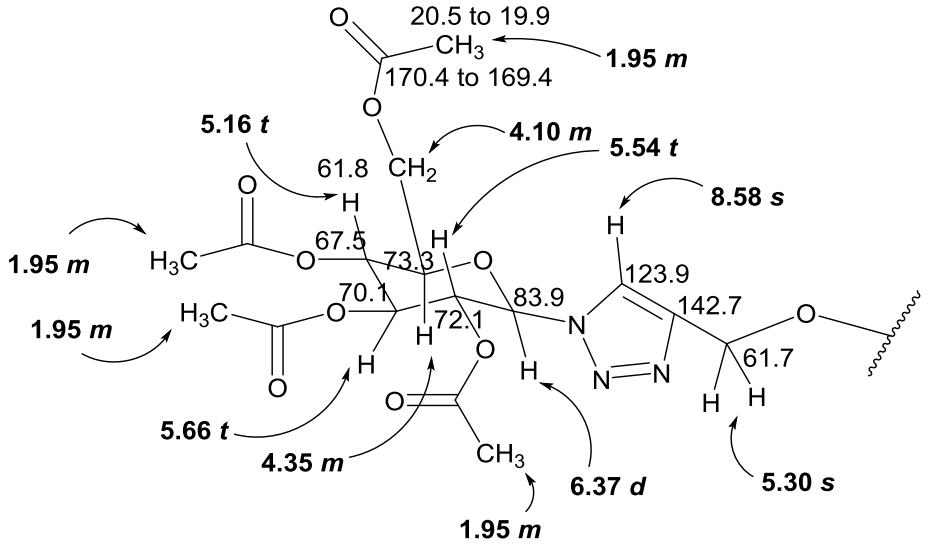


Figure 18: ^{1}H and ^{13}C NMR data of the *O*-acetyl-β-glucopyranosyl group of **LuMeTGluAc.**

The position of this group was evidence by the correlations found in the HMBC as indicated in **Figure 19**.

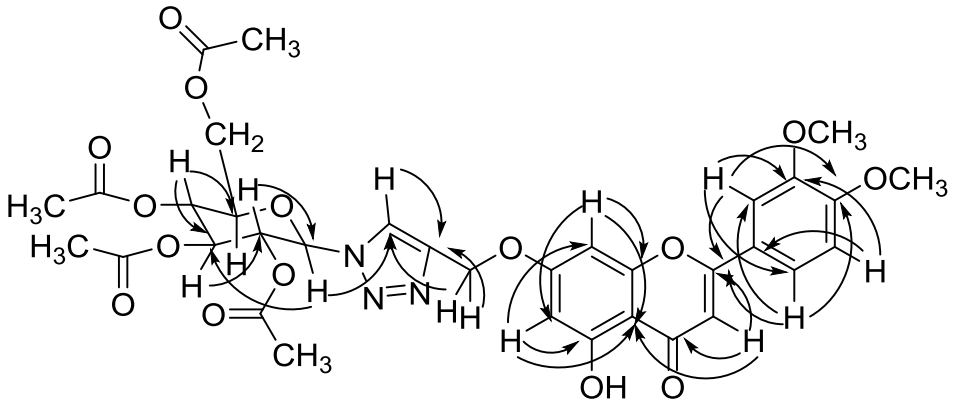


Figure 19: Main correlations observed in the HMBC spectrum of **LuMeTGluAc**.

Table 9: ^{1}H and ^{13}C NMR data of **LuMeTGluAc.**

	^{1}H	**^{13}C**
2	-	163.7
3	7.03 (s)	104.1
4	-	182.1
4a	-	105.0
5	12.90 (s, OH)	161.2
6	6.45 (d, J=2.0)	98.7
7	-	168.5
8	6.92 (d, J=2.0)	93.7
8a	-	157.2
1'	-	122.7
2'	7.57 (d, J=2.0)	109.5
3'	3.85 (s, OCH_3)	149.0
4'	3.85 (s, OCH_3)	152.3
5'	7.12 (d, J=8.5)	111.7
6'	7.69 (dd, J=8.5; 2.0)	120.2
Triazole	8.58 (s)	142.7 (C=CH) 123.9 (CH=C)
OCH_2	5.30 (s)	61.7
1''	6.37 (d, J=9.0)	83.9
2''	5.54 (t, J=9.5)	72.1
3''	5.66 (t, J=9.5)	70.1
4''	5.16 (t, J=9.8)	67.5
5''	4.35 (m)	73.3
6''	4.10 (m)	61.8
$COCH_3$	1.95 (m)	170.4 to 169.4 (C=O) 20.5 to 19.9 (OCH_3)

Values in parts per million (δ_H and δ_C). Measured in DMSO-d_6 at 500.15 MHz. J values (Hz) are presented in parentheses.

4. Experimental

4.1. General methods

MW reactions were performed using a glassware setup for atmospheric-pressure reactions and a 100 mL or 30 ml Teflon reactor (internal reaction temperature measurements with a fiber-optic probe sensor) and were carried out using an Ethos MicroSYNTH 1600 Microwave Labstation from Milestone.

All the reactions were monitored by TLC.

Purifications of compounds were carried out by flash column chromatography using Macherey-Nagel silica gel 60 (0.04-0.063 mm), and crystallization.

Melting points were obtained in a Köfler microscope and are uncorrected.

IR spectra were obtained in KBr microplate in a FTIR spectrometer Nicolet iS10 from Thermo Scientific with Smart OMNI-Transmisson accessory (Software OMNIC 8.3).

^{1}H and ^{13}C NMR spectra were taken in CDCl3 at r.t., on Bruker Avance 300 and 500 instruments (300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C). Chemical shifts are expressed in δ (ppm) values relative to TMS (tetramethylsilane), used as an internal reference; ^{13}C NMR assignments were made by 2D (HSQC and HMBC) NMR experiments (long-range ^{13}C-^{1}H coupling constants were optimized to 7 Hz).

Compounds were considered to be pure if, when using two different chromatographic conditions, a single spot appeared by TLC.

All commercially available reagents were purchased from Sigma Aldrich Co. Reagents and solvents were purified and dried according to the usual procedures. The following materials were synthesized and purified by the described procedures.

4.2. Synthesis of Flavone derivative LuMe

A mixture of phloroglucinol (175 mg, 1 mmol) and ethyl 3,4-dimethoxybenzoylacetate (700 mg, 2 mmol) was placed inside a muffle furnace and heated under set temperature of 240°C, for 60 min. The crude mixture was dissolved in 10% aq NaOH (20 mL) and washed with diethyl ether (2x20 mL). The product was obtained by precipitation, after adding concentrated 37% aq HCl. The solid was filtered under reduced pressure and washed with water. The chromatographic control was performed via TLC (silica gel; n-hexane: ethyl acetate, 5:5).

Luteolin 3',4'-dimethyl ether (LuMe). Yield: 74%; mp 280-281ºC; IR (kBr) υmax: 3600-3300, 2950, 1655, 1581-1430, 1256 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300.13 MHz): see **Table 4 (Page 16)**; ^{13}C NMR (DMSO-d_6, 75.47 MHz): see **Table 5 (Page 16)**.

4.3. Synthesis of Flavone derivative LuNi

A mixture of phloroglucinol (175 mg, 1 mmol) and ethyl 4-nitrobenzoylacetate (658 mg, 2 mmol) was placed inside a muffle furnace and heated under set temperature of 240°C, for 50 min. The crude mixture was dissolved in 10% aq NaOH (20 mL) and washed with diethyl ether (2x20 mL). The product was obtained by precipitation, after adding concentrated 37% aq HCl. The solid was filtered under reduced pressure and washed with water. The resulting yellow-orange solid was purified by flash CC (silica gel; n-hexane: ethyl acetate, 7:3). The chromatographic control was performed via TLC (silica gel; n-hexane: ethyl acetate, 5:5).

5,7-Dihydroxy-4'-nitroflavone (LuNi). Yield: 16%; mp 284-285ºC; IR (kBr) υmax: 3600-3300, 1653, 1599-1431, 1519, 1346, 1274 cm^{-1}; ^{1}H NMR (DMSO- d_6, 300.13 MHz): see **Table 4 (Page 16)**; ^{13}C NMR (DMSO-d_6, 75.47 MHz): see **Table 5 (Page 16)**.

4.4. Synthesis of Propargyl flavone derivative LuMeProp

To a solution of **LuMe** (200 mg, 0.64 mmol), cesium carbonate (Cs_2CO_3) (FLUKA 20959, 209 mg, 1 eq.) and tetra-*N*-butylammonium bromide (TBAB) (SIGMA-ALDRICH 86868, 206 mg, 1 eq.) in anhydrous acetone (20 mL), a solution of propargyl bromide (SIGMA-ALDRICH P51001, 80% wt% in toluene, 0.9 mL, 1 eq.) was added. The mixture was refluxed at 60 ºC during 6 h and filtered. Purification was carried out by flash column chromatography (silica gel; n-hexane: ethyl acetate, 8:2).

7-(prop-2-yn-yloxy)luteolin 3',4'-dimethyl ether (LuMeProp). Yield: 66%; mp 223ºC; IR (kBr) υmax: 3600-3300, 3234, 2963-2849, 1647, 1518-1429, 1261 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500.15 MHz): see **Table 7 (Page 19)**; ^{13}C NMR (DMSO-d_6, 500.15 MHz): see **Table 7 (Page 19)**.

4.5. Synthesis of Flavone O-acetyl glycosides LuMeTGluAc

To a solution of 7-(prop-2-yn-yloxy)luteolin 3',4'-dimethyl ether (**LuMeProp**) (100 mg, 0.28 mmol) and tetraacetate *β*-glucopyranosyl azide (105 mg, 0.28 mmol) in tetrahydrofuran (THF)/ water solvent mixture (2:1; 30 mL), sodium ascorbate ($C_6H_7NaO_6$) (Sigma A 7631, 222 mg, 1.12 mmol) and copper (II) sulfate pentahydrate

($Cu_2SO_4.5H_2O$) (Panreac 131270, 140 mg, 0.56 mmol) were added. The reaction was stirred for 30 min at 70ºC and under MW irradiation, at 500 W (2 cycles: 5 min to achieve 70ºC and 10 min for cooling). After cooling, the reaction mixture was filtered and concentrated under reduced pressure. The water suspension was extracted twice with ethyl acetate. The combined organic layers were dried out, using anhydrous sodium sulfate, filtered, concentrated under reduced pressure and then purified by flash column chromatography (silica gel; n-hexane: ethyl acetate, 5:5).

7-((2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-1H-1,2,3-triazole-4-yl)methoxy) luteolin 3',4'-dimethyl ether (LuMeTGluAc). Yield: 82%; mp 155 ºC; IR (kBr) υmax: 3600-3300, 2963, 1757, 1655, 1516-1430, 1322, 1258, 1226 cm^{-1}; ^1H NMR (DMSO-d_6, 500.15 MHz): see **Table 9 (Page 21)**; ^{13}C NMR (DMSO-d_6, 500.15 MHz): see **Table 9 (Page 21)**.

5. Conclusion

This research project allowed the synthesis and identification of four flavone derivatives, including two new flavones not reported before. Firstly, it was synthesized the flavone nucleus, and two flavones were originated, one with methoxy (**LuMe**) and another with a nitro substitution (**LuNi**). Afterwards, a sugar moiety was added, using 1,2,3-triazole ring as a linker, forming **LuMeTGluAc**. To fulfill the previous reaction, **LuMe** had to be previously propargylated, and so **LuMeProp** was created.

The synthesis of flavones **LuMe** and **LuNi** was accomplished by solvent-free direct thermal cyclocondensation of phloroglucinol and α-ketoesters. This process afforded **LuMe** in quite high yields, since no purification processes were required. However, for **LuNi** derivative, this method wasn't so productive and other by-products were produced. As a result, purification using flash CC was necessary and low yields were obtained. The propargylation of the previously prepared **LuMe**, as well as the copper catalyzed alkyne-azide cycloaddition of the resulting product were both achieved with considerably high yields.

Considering the aims proposed for this project, and in order to create structures inspired by the marine natural flavonoids Thalassiolins A-C, the process will have continuity and the glycosyl moiety present in **LuMeTGluAc** shall undergo deacetylation, followed by the sulfation of the remaining hydroxyl groups in the sugar moiety. Finished the whole multistep process, the resulting structure should combine the advantages of the flavone nucleus, 1,2,3-triazole ring and sulfated glycosyl moieties and potentially behave as a powerful antifouling agent.

Finally, the study of the antifouling activity of all synthesized compounds will be assessed and, for the most potent compounds, the mechanism of action associated with their antifouling activity will also be determined.

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