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FACULDADE DE FARMÁCIA
UNIVERSIDADE DO PORTO

SYNTHESIS AND STRUCTURE ELUCIDATION OF ENANTIOMERIC PURE CHIRAL DERIVATIVES OF 4'-METHYLCHRYSOERIOL

Relatório da UC Projeto I do Mestrado Integrado em Ciências Farmacêuticas

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2018/2019

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Synthesis and structure elucidation of enantiomeric pure chiral derivatives of 4'-methylchrysoeriol

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Abstract

Flavones are a group of compounds belonging to the vast class of flavonoids and characterized by remarkable biochemical and pharmacological actions.

Flavones can be synthesized through different methods, such as oxidative cyclization of chalcones, Baker-Venkataraman rearrangement, Allan-Robinson condensation, and Mentzer synthesis.

The importance of chirality is increasing in the drug development process as enantiomers of the same molecule can display different biological activities.

The main goal of this work was to obtain new chiral flavone derivatives based on chiral xanthone derivatives, which have shown an increased pharmacological effect.

Within this project, a flavone and four derivatives were synthesized in moderate to good yields. The starting flavone, **LuMe**, was obtained through the thermal cyclocondensation of phloroglucinol and ethyl 3,4-dimethoxybenzoylacetate. Following, a Williamson ether synthesis was applied, and **LuMe-Es** was obtained. Hydrolysis of the methyl ester derivative yielded the acid derivative **LuMe-Ac**. The last reactions performed were the coupling of **LuMeAc** with two enantiomers of tryptophan, providing the pure L and D enantiomers (**LuMe-LTrp** and **LuMe-DTrp**).

The structure elucidation of the synthesized compounds was established based on IR, ^{1}H and ^{13}C NMR, and HMBC and HSQC techniques.

Enantiomeric purity of the compounds was confirmed through HPLC, using a cellulose chiral column.

To the best of our knowledge these compounds have never been synthesized before.

Keywords: flavone; chirality; tryptophan; enantioselectivity; synthesis.

Resumo

As flavonas são um grupo de compostos que pertencem à vasta classe dos flavonóides e são caracterizadas por demonstrarem atividades bioquímicas e farmacológicas promissoras.

Sinteticamente, as flavonas podem ser obtidas por diversos métodos, tais como, ciclização oxidativa de calconas, rearranjo de Baker-Venkataraman, condensação de Allan-Robinson, e síntese de Mentzer.

A importância da quiralidade no processo de desenvolvimento de fármacos tem vindo a aumentar, uma vez que enantiómeros da mesma molécula podem manifestar diferentes atividades biológicas.

O objetivo principal deste projeto é a obtenção de novos derivados quirais de flavonas, baseados em derivados xantónicos quirais que demonstraram melhoria nos seus efeitos farmacológicos.

Neste projeto, uma flavona e quatro derivados foram sintetizados com rendimentos moderados a bons. A flavona inicial, **LuMe**, foi obtida através da ciclocondensação térmica do floroglucinol com o 3,4-dimetoxibenzoílacetato de etilo. De seguida, procedeu-se à aplicação de uma síntese de Williamson que levou à obtenção da **LuM-Es**. A hidrólise do ester de metilo deste derivado originou o derivado ácido **LuMe-Ac**. Por fim, reações de acoplamento entre a **LuMe-Ac** e os dois enantiómeros do triptofano levaram à obtenção dos enantiómeros L e D puros (**LuMe-LTrp** and **LuMe-DTrp**).

A elucidação estrutural dos compostos sintetizados foi conseguida através de métodos espetroscópicos, nomeadamente, espetrofotometria do infravermelho, ressonância magnética nuclear de ^{1}H e ^{13}C, e técnicas de HMBC e HSQC.

A pureza enantiomérica dos compostos foi confirmada por HPLC, usando uma coluna quiral de celulose.

Tanto quanto é do nosso conhecimento, estes compostos nunca tinham sido sintetizados anteriormente.

Palavras-chave: flavonas; quiralidade; triptofano; enantiosseletividade; síntese.

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Abbreviations and symbols

δ	Chemical shift
λ	Wavelength (nm)
$[\propto]_D^{25°C}$	Specific rotation (°)
^{13}C NMR	Carbon nuclear magnetic resonance
^{1}H NMR	Proton nuclear magnetic resonance
CC	Column Chromatography
CIIMAR	Centro Interdisciplinar de Investigação e Marinha Ambiental
d	Doublet
dd	Double doublet
FTIR	Fourier transform infrared spectroscopy
HMBC	Heteronuclear multiple bond correlation
HPLC	High Performance Liquid Chromatography
HSQC	Heteronuclear single bond correlation
IR	Infrared spectroscopy
J	Coupling constant
LC	Liquid chromatography
LQOF	Laboratório de Química Orgânica e Farmacêutica
m	Multiplet
m.p.	Melting point
NMR	Nuclear Magnetic Resonance
Rs	Chromatographic resolution factor
s	Singlet
t_0	Chromatographic dead time
td	Triple doublet
TLC	Thin-layer chromatography

t_R	Retention time
UV	Ultraviolet
υ	Wavenumber (cm^{-1})
Vis	Visible
α	Chromatographic separation factor
δ_C	Carbon Chemical shift
δ_H	Proton Chemical shift

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1. Introduction

1.1. Flavonoids

Flavonoids are a group of polyphenolic secondary metabolites with low molecular weight commonly produced by plants. Extensive research has been conducted for over a century, since they play important roles in a number of biological processes, exhibiting a broad range of effects on both prokaryotic and eukaryotic cells [1].

One of the most exuberant functions of flavonoids is the pigmentation of flowers, which in turn has the purpose of attracting pollinators, while also conferring protection to petals and other flower parts from ultraviolet (UV) irradiation and oxidative stress. There is a wide variety of subclasses of flavonoids, classified according to their chemical structure. Some are described as co-pigments and involved in hormone signaling, or disease resistance, others reduce the palatability and prevent plants from being eaten by herbivores. The synthesis of some flavonoids can be induced when plants are infected, injured or under low nutrients conditions.[2]

They widely exist in the roots, stems, leaves, flowers and fruits of higher plants, ferns and even in seaweeds. The production of these secondary metabolites in seaweeds can be related with their antioxidant effect, as it has been observed that reactive oxygen species (ROS) production in algae is stimulated by various environmental stresses, such as UV radiation, high salt concentrations, and the presence of heavy metals.[3] Some flavonoids can also inhibit the activity of enzymes, such as xanthine oxidase, cyclooxygenase, lipoxygenase and phosphoinositide 3-kinase, and have antitumor, antivirus and anti-inflammatory activity. Furthermore, potential treatment and prevention effects have been shown in degenerative diseases. Additionally, some subgroups of flavonoids possess potential application as weak hormones at treating menopausal syndrome.[4]

1.1.1. Structure and Classification of Flavonoids

The basic structure of flavonoids consists of two phenyl rings (A and B rings) linked by a three-carbon unit which can form an oxygenated heterocyclic ring (C ring). Based on functional groups present on the rings, differences in the generic structure of the C ring and the position at which the B ring is attached to the C ring, flavonoids are classified into six major subclasses, namely flavones (e.g., apigenin, luteolin), flavonols (e.g., quercetin, myricetin), flavanones (e.g., naringenin, hesperidin), catechins or flavanols (e.g., epicatechin, gallocatechin), anthocyanidins (e.g., cyanidin, pelargonidin), and isoflavones (e.g., genistein, daidzein). (Figure 1). The non-cyclization of the C3 fragment originates chalcones, with a different numbering system [5].

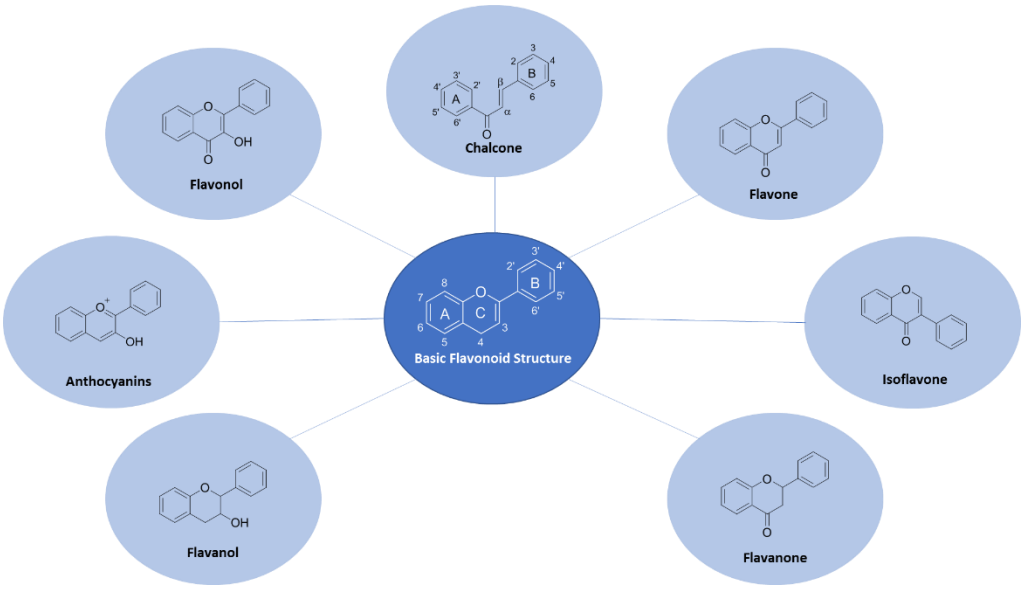


Figure 1 - General skeleton of flavonoids and subclasses

1.1.1.1. Flavones

The general structure of flavones is characterized by the presence of a keto group at the C4 position and a double bond between C2 and C3, with no substitution at the C3 position. This "key-skeleton" is responsible for the display of a remarkable biochemical and pharmacological actions, including antitumor, antimicrobial, antioxidant, and anti-inflammatory activities, as well as the potential to prevent the progression of neurodegenerative pathologies and to promote cognitive performance[6, 7].

The most abundant flavones in plants are luteolin (5,7,3′,4′-tetrahydroxyflavone) and apigenin (5,7,4′-trihydroxyflavone). In 90% of the cases, the A-ring is substituted by two phenolic hydroxyl group at C-5 and C-7. These hydroxyl groups can be free or etherified. The B-ring is most commonly substituted by a hydroxyl group, in C-4' (80% of cases), or C-3',4', being also isolated flavones with hydroxyl groups at C-3',4',5'.

Flavones occur in nature as aglycones, as well as glycosides and methylated derivatives. They are typically conjugated with glucose and other sugars as 7-*O*-glycosides. Nevertheless, flavones *C*-glycosides with sugar moieties at positions 6 and 8 can also be found in nature. D-Glucose is by far the most common sugar, but other sugar moieties such as D-apiose, D-mannose, L-arabinose, D-fucose, L-ramnose, D-xylose, D-galactose, and D-glucosamine have been reported [8].

1.1.1.1.1. Biosynthetic pathway

Chalcones are intermediate precursors of the other sub-classes of flavonoids (Figure 2).The A ring of chalcones is normally formed from sequential condensation of three molecules of malonyl coenzyme A (malonyl-CoA), synthesized *via* acetate pathway, being for this reason commonly hydroxylated at the 5′ and 7′ positions. The B ring comes from the shikimate pathway and is commonly 4′-, 3′,4′-, or 3′,4′,5′-hydroxylated. In this pathway, cinnamic acid is hydroxylated to *p*-coumaric acid by a P450 monooxygenase, cinnamate 4-hydroxylase (C4H). Coumaric acid is then converted to 4-coumaroyl coenzyme A (4-coumaroyl-CoA) by 4-coumaroyl: CoA ligase (4CL) Next, chalcone synthase (CHS) catalyzes condensation of 4-coumaroyl-CoA with three molecules of malonyl-CoA, in which tetrahydroxychalcone is formed [8, 9]. Chalcone is subsequently isomerized by the action of chalcone isomerase (CHI) into a flavanone. This enzyme is very efficient and highly specific to the chalcone substrate. Finally, flavones are synthesized by the introduction of a double bond between the C-2 and C-3 positions by two different enzymes known as flavone synthase I, and flavone synthase II. Flavone synthases have the unique characteristic of catalyze the conversion of equivalent substrates (flavanones) to identical products (flavones), by rather different mechanism [10-12].

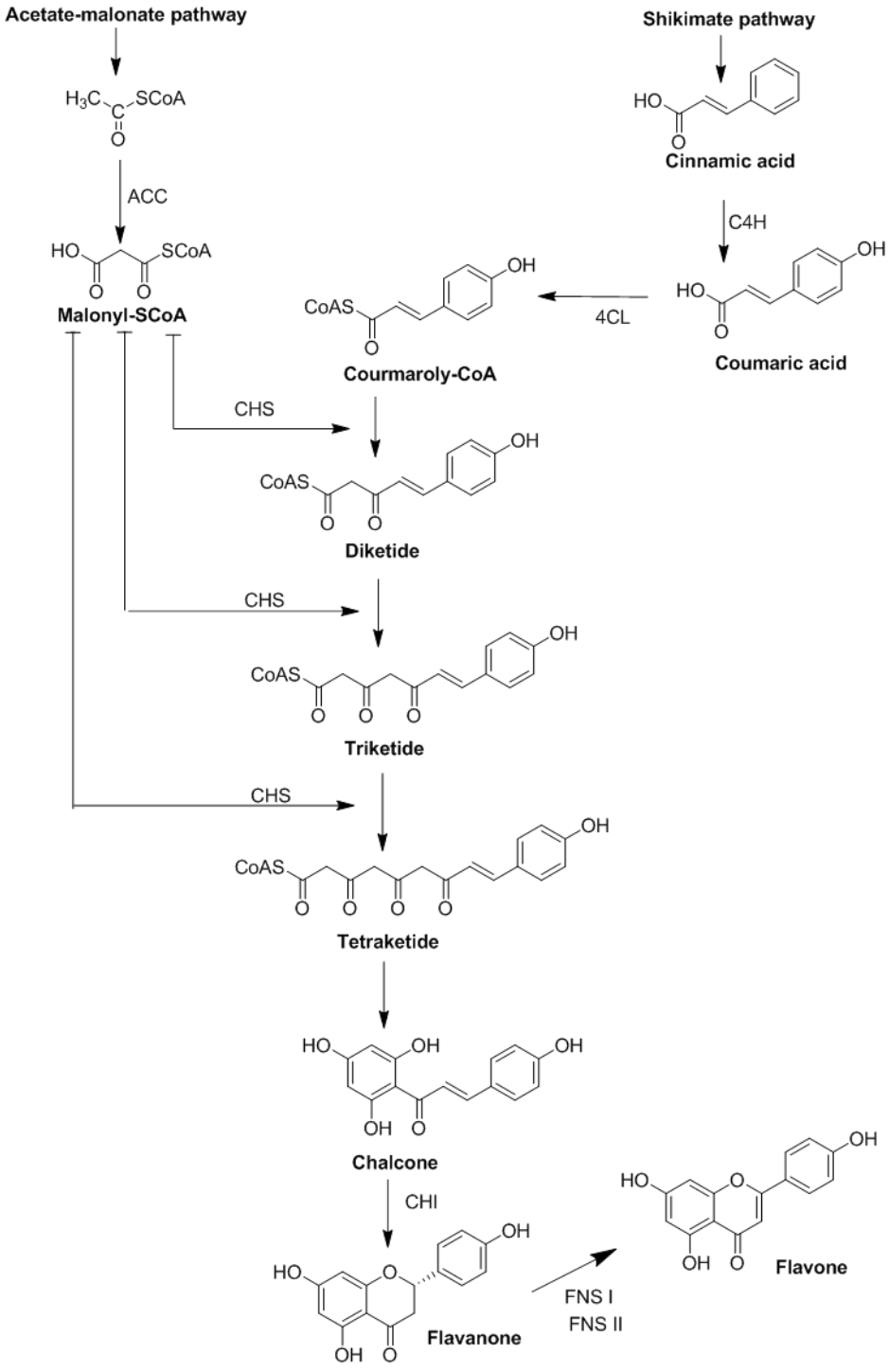


Figure 2 - Flavone biosynthseis. ACC - acetyl-CoA carboxylase, C4H -cinnamate 4-hydroxylate, 4CL- 4-coumaraye CoA-ligase, CHS- chalcone synthase, CHI- chalcone isomerase, FNS I and FNS II- Flavone synthase I and II.

1.1.1.1.2. Synthetic methods

Most of the naturally occurring compounds have the disadvantage of being available only in small amounts. The extraction of these active substances for industrial

production of medicines is not feasible, so organic synthesis is used in order to obtain the necessary quantities, allowing molecular modifications that are the most advantageous.

Regarding flavones, several synthetic methods have been developed and modified to get products of high yield and purity. Flavones can be prepared by different methods such as Claisen-Schmidt condensation reaction followed by oxidative cyclization, Baker-Venkataraman rearrangement, and Allan–Robinson condensation.

1.1.1.1.2.1. Oxidative cyclization of chalcones

Oxidative cyclization of 2'-hydroxychalcones constitutes an important route for the synthesis of flavones. Using this approach 2'-hydroxychalcones are firstly prepared upon condensation of appropriately substituted 2-hydroxy-acetophenones with various substituted benzaldehydes under basic conditions (Figure 3).

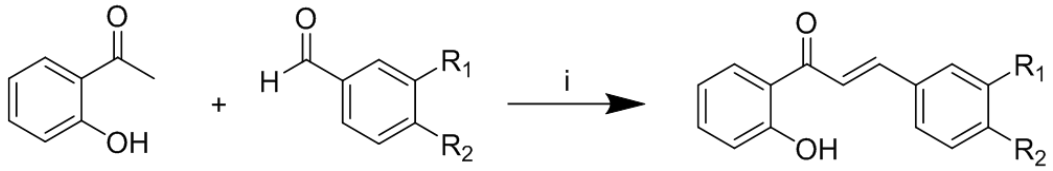


Figure 3 - Condensation of 2'-hydroxychalcone. Reaction conditions (i): NaOH/EtOH, HCl

For the oxidative cyclization many oxidizing agents such as I_2-DMSO, oxalic acid, $InBr_3$/$InCl_3$ $FeCl_3$, among others, have been reported in the literature. However, most of these methods are not very satisfactory due to drawbacks such as low yields, high reaction temperature, long reaction time and formation of a mixture of products containing flavones, flavanones and aurones. Therefore, some modifications of these methods emerged in the recent years. To illustrate the process of oxidative cyclization, the synthesis of flavones using NH_4I under solvent-free conditions is presented in Figure 4.

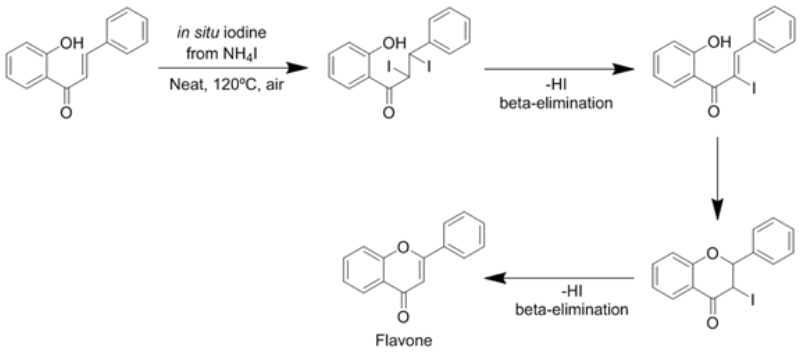


Figure 4 - Synthesis of flavones by oxidative cyclization of chalcones with NH4I

The mixture of 2′-hydroxychalcone and ammonium iodide was heated at 120 °C for 1 h. Ammonium iodide decomposes with iodine release when suspended in the humid air, promoting the cyclization and dehydrogenation of 2′-hydroxychalcone in the corresponding flavone.[13]

1.1.1.1.2.2. Baker-Venkataraman rearrangement

This method is of prime importance in the synthesis of flavones, and involves the conversion of *o*-hydroxyacetophenone into phenolic ester, that in the presence of a base, undergoes an intramolecular Claisen condensation to form β-diketone, which is cyclized to flavone by an acid-catalyzed cyclodehydration.

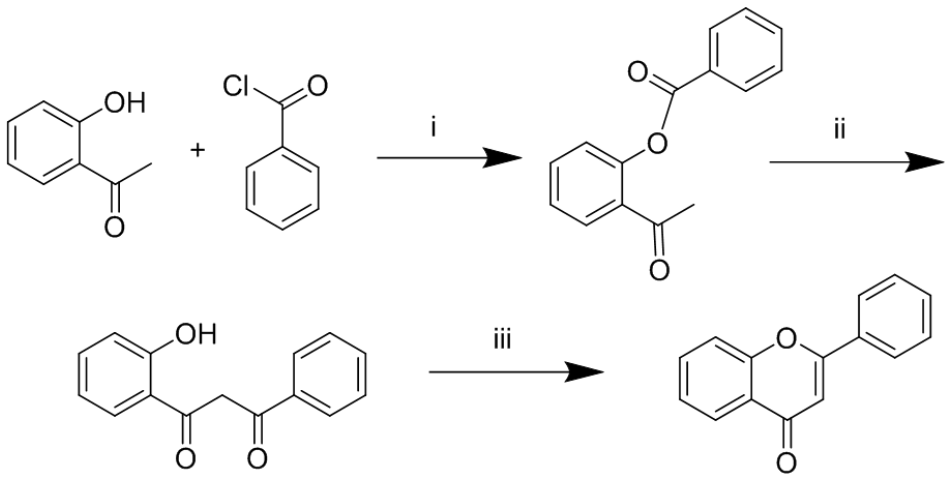


Figure 5 - Synthesis of flavones via β-diketone. Reaction conditions (i): Py; (ii): KOH, Py; (iii): AcOH glacial, H_2SO_4 conc.

Using the example (Figure 6) as an illustration of a typical reaction, base abstraction of one of the α-hydrogens of the aromatic ketone occurs, with the formation of enolate. Intramolecular attack of the enolate occurs onto the ester carbonyl to form the cyclic charged hemiacetal. More stable phenolate is formed, which is protonated during acid workup to give the 1,3-diketone Baker–Venkataraman product. To complete the construction of the flavone core, cyclodehydration is required. This was commonly afforded by treatment with strong acid, however many milder conditions have now been developed.[14]

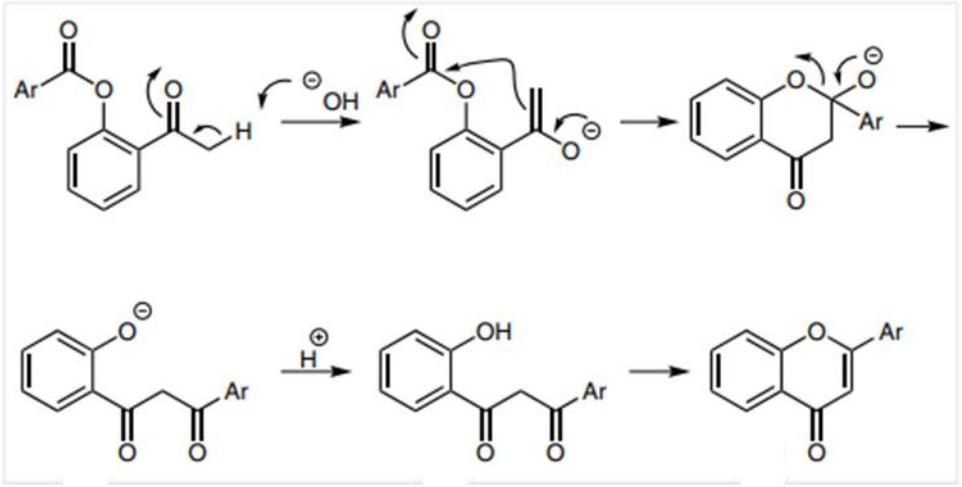


Figure 6 - The mechanism of the Baker–Venkataraman rearrangement

1.1.1.1.2.3. Allan-Robinson condensation

In this approach, flavones are obtained in one step by condensation of *o*-hydroxy-acetophenone with the anhydride of an aromatic acid, in the presence of the sodium salt of corresponding acid in anhydride (Figure 8). This reaction can also lead to the synthesis of isoflavones [15].

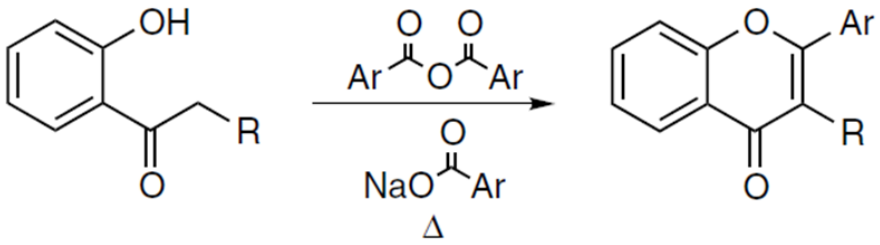


Figure 7 - Synthesis of flavones by Allan-Robinson condensation

1.1.1.1.2.4. Mentzer synthesis

Mentzer reported a two-component thermal cyclocondensation reaction between phenols and β-ketoesters to yield flavones under very harsh conditions (250 °C for long reaction times) and extended this method to a wide number or flavones [16, 17] The reaction is described as proceeding *via* formation of an intermediate β-diketone in a cycloaddition reaction of α-oxo ketene with phloroglucinol, followed by a thermal Fries rearrangement.[18]

The proposed mechanism of the two-component synthesis involves several key steps that starts with the activation of the β-ketoester to yield an α-oxo ketene intermediate. Addition of the phenol to the α-oxo ketene yielded a phenol ester intermediate, completing the "transesterification" step. Then the phenol ester (*O*-acylation product) underwent a base-promoted *ortho*-Fries rearrangement, followed by cyclization of the resulting 1,3-diaryl diketone (formation of the hemiketal). Final proton transfer and dehydration drove the reaction sequence forward (Figure 9).[19]

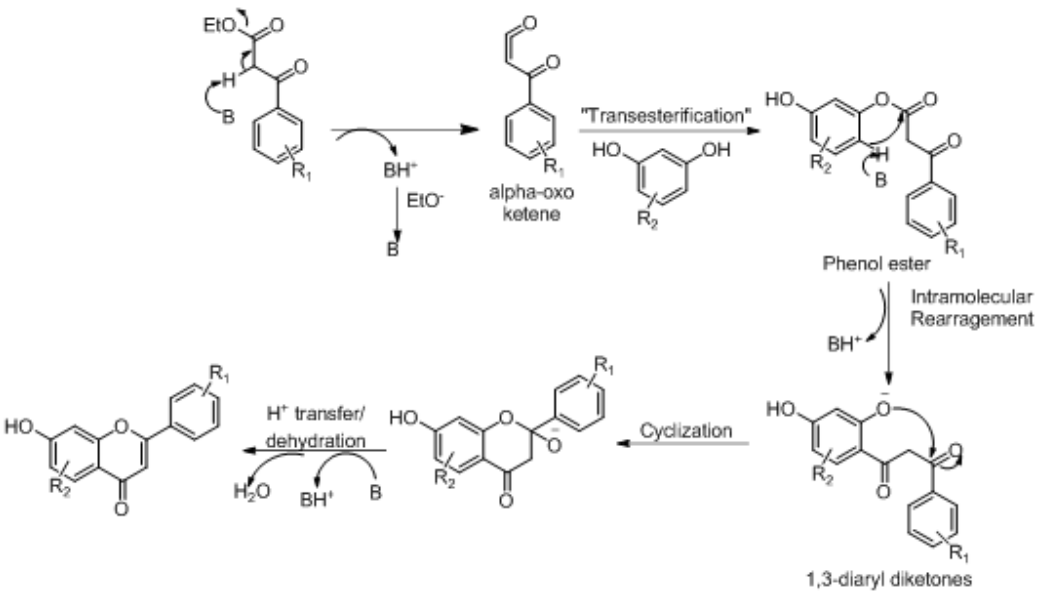


Figure 8 - Mentzer reaction mechanism

1.2. Chirality

Chirality research is a hot topic in chemistry and biology and has become critical in the quest for new drugs. This geometric property was discovered in 1848 by Louis Pasteur. In his meticulous experiments, he separated by hand the mirror-image forms of salts of tartaric acid, and saw that their solutions would rotate the plane of polarized light in opposite directions. [20] Subsequently, he made the first observation of biological enantioselectivity, when he noted that bacteria only ferment dextro-tartaric acid. In a similar way, as a result of the asymmetrical influence throughout evolution, the human body is a chiral environment, since proteins are constituted by L-amino acids, which implies that enzymes are also chiral. [21]

Based in the enantiodiscrimination of biological systems, the pharmacological and toxicological effects could be significantly different between enantiomeric drugs, even though they share an identical molecular formula, similar bonds between atoms and similar distance of bonds. They differ in spatial or three-dimensional arrangement, and one enantiomer can be responsible for the therapeutic activity while the other enantiomer can be inactive, possess lower activity, or can be an antagonist of the active enantiomer. [22]

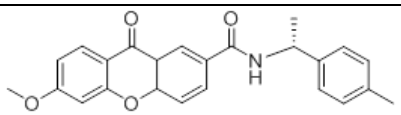
One of the most notable examples is that of DOPA or dihydroxy-3,4 phenylalanine (a precursor of dopamine) that is effective in the treatment of Parkinson disease. DOPA was use as a racemic mixture, however, the D-isomer is not only therapeutically ineffective, it is toxic and cause agranulocytosis, therefore, only L-DOPA form is currently used in therapeutics. [23]

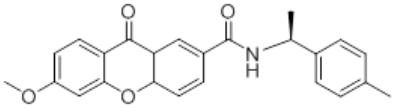
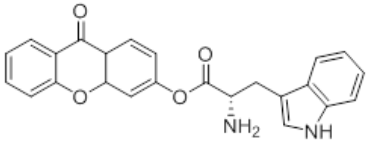
2. Aims

As result of the search of new bioactive chiral oxygenated heterocycles from LQOF/CIIMAR research group some chiral derivatives of xanthones (CDXs) with antitumor activity have been identified. Moreover, it was demonstrated that the effects on the growth of the human tumor cell lines was dependent not only on the nature and positions of substituents on the xanthonic scaffold, but also to the stereochemistry of the CDXs. Particularly, enantiomeric CDXs **XA** and **XB** exhibited high enantioselectivity for MCF-7 and NCI-H460 human tumor cell lines (Table 1).[24] Moreover, several natural and synthetic CDX have been reported for their promising antimicrobial activity. [25] Specifically, the tryptophan xanthone derivative **XC** have shown to have strong antimicrobial activity against several bacteria, such as *S. aureus*, *B. subtilis*, *E. coli* and *K. pneumonia*.

The main aim of this work was to obtain new potential bioactive chiral derivatives of flavones based on CDXs **XA**, **XB** and **XC**. This was accomplished through an enantioselective synthesis, in which commercially available chiral amino esters (L-tryptophan and D-tryptophan) were coupled to the flavone by their amine group.

*Table 1 - Biological activity of **XA**, **XB** and **XC**.*

Structure	Antitumor activity	Antimicrobial activity	ref
 XA	MCF-7: GI_{50}>150 μM NCI – H460: GI_{50}=85.88μM		24

 XB	MCF-7: GI_{50}=91.91 µM NCI – H460: GI_{50}=42.62µM		24
 XC		*S. aureus* (24 mm-25µg/mL); *B. substilis* (26 mm-25 µg/mL); *E. coli* (26 mm-25µg/mL); *K. pneumonia* (23 mm-25 µg/mL)	25

3. Results and Discussion

3.1. Synthesis

Throughout this project, one flavone (**LuMe**) was synthesized by a two-component single-step reaction (Mentzer synthesis). From **LuMe**, a multistep process was carried out in order to obtain both enantiomeric derivatives in high enantiomeric purity. The results are discussed in the following sections.

3.1.1. Synthesis of 4'-methylchrysoeriol (**LuMe**)

Flavone **LuMe** was synthesized by solvent-free direct thermal cyclocondensation of phloroglucinol and ethyl 3,4-dimethoxybenzoylacetate, according to the procedure described by Mentzer et al. (Figure 9).

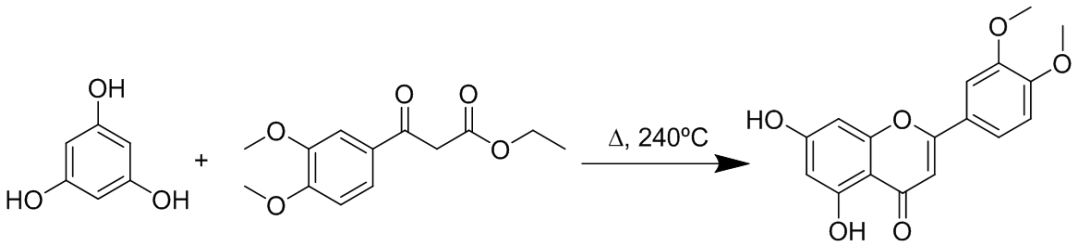


Figure 9 - Synthesis of **LuMe**

The reaction was monitored by TLC. As this reaction was not entirely complete and several by-products were obtained, the reaction mixture need to be submitted to purification by column chromatography, followed by crystallization, in order to obtain **LuMe** in a moderate yield (47%).

3.1.2 Synthesis of 7-(2-methoxy-2-oxoethoxy)-4'-methylchrysoeriol (**LuMe-Es**)

The following step in the synthetic process was the Williamson ether synthesis with methyl bromoacetate in alkaline medium, taking **LuMe** as starting material. After crystallization, **LuMe-Es** was obtained with a 50% yield, as a pale yellow solid.

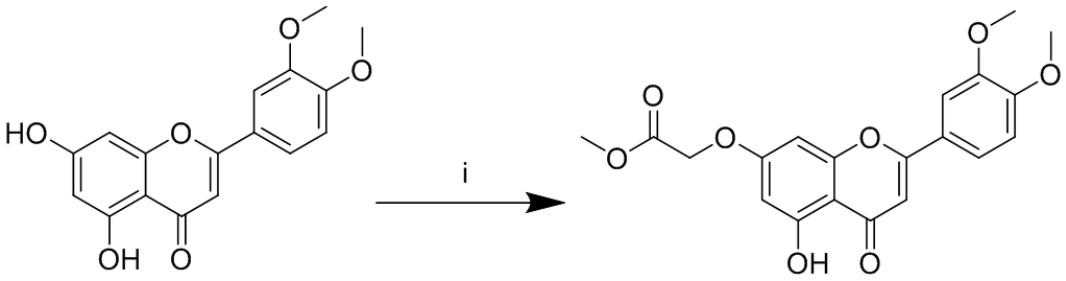


Figure 10 - Synthesis of **LuMe-Es**. Reaction conditions (i): $BrCH_2COOCH_3$, acetone, K_2CO_3, reflux.

3.1.3 Synthesis of 7-carboxymethoxy-4'-methylchrysoeriol (**LuMe-Ac**)

The metyl ester (**LuMe-Es**) was then hydrolyzed, without previous purification, providing **LuMe-Ac**, as a pink salmon solid, with a very good yield (74%).

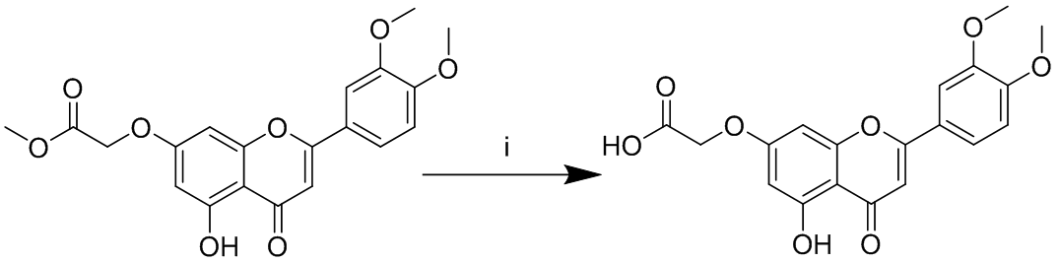


Figure 11 - Synthesis of **LuMe-Ac**. Reaction conditions (i): 5M NaOH, $CHCl_2$/MeOH; room temperature.

3.1.4 Synthesis of (*R*)-7-(2-((3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethoxy)-4'-methylchrysoeriol (**LuMe-DTrp**) and (*S*)-7-(2-((3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethoxy)-4'-methylchrysoeriol (**LuMe-LTrp**)

In this coupling reaction, the activation of the carboxylic acid group attached to the flavone scaffold was carried out using (1-Cyano-2-ethoxy-2-oxoethylidenaminooxy) dimethylamino-morpholino-carbenium hexafluorophosphate (COMU®), as a coupling reagent. L-Tryptophan methyl ester hydrochloride, and D-Tryptophan methyl ester hydrochloride were select as enantiomerically pure building blocks, for their non-interconversion and racemization capacity, and for having a primary amine as reactive group for the amide formation. The coupling reactions were performed in tetrahydrofuran (THF) using equivalent amounts of the carboxyflavone derivative substrate (**LuMe-Ac**), the commercial chiral block and COMU® at room temperature with 2 equivalents of triethylamine (TEA). The purification procedures include acid-base liquid-liquid extraction and crystallization.

The syntheses worked equally well with both chiral blocks, affording the desired flavone derivatives in excellent yields (80%, 88%), as a yellow solids.

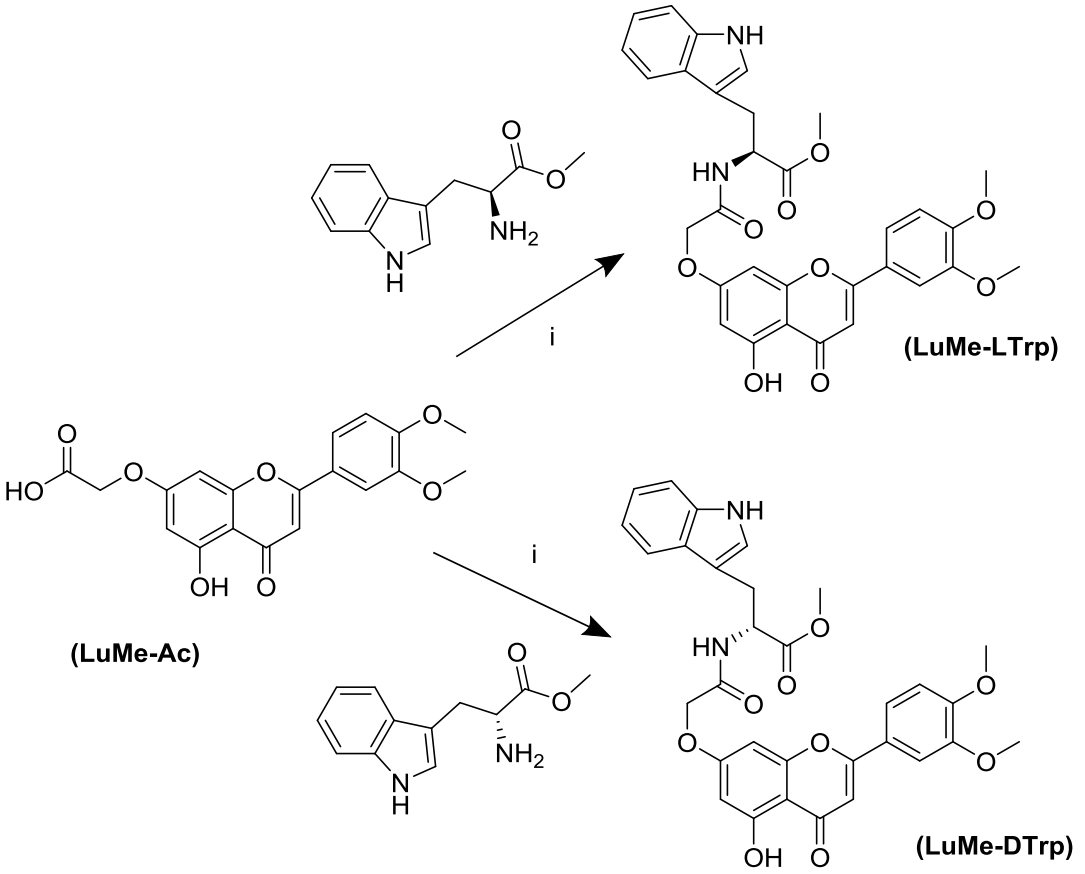


Figure 12 - Synthesis of **LuMe-LTrp**.and **LuMe-DTrp**. Reaction conditions (i): COMU®, THF, TEA, room temperature.

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**, **LuMe-Es**, **LuMe-Ac** and **LuMe-DTrp** and **LuMe-LTrp** are present in Figure 6

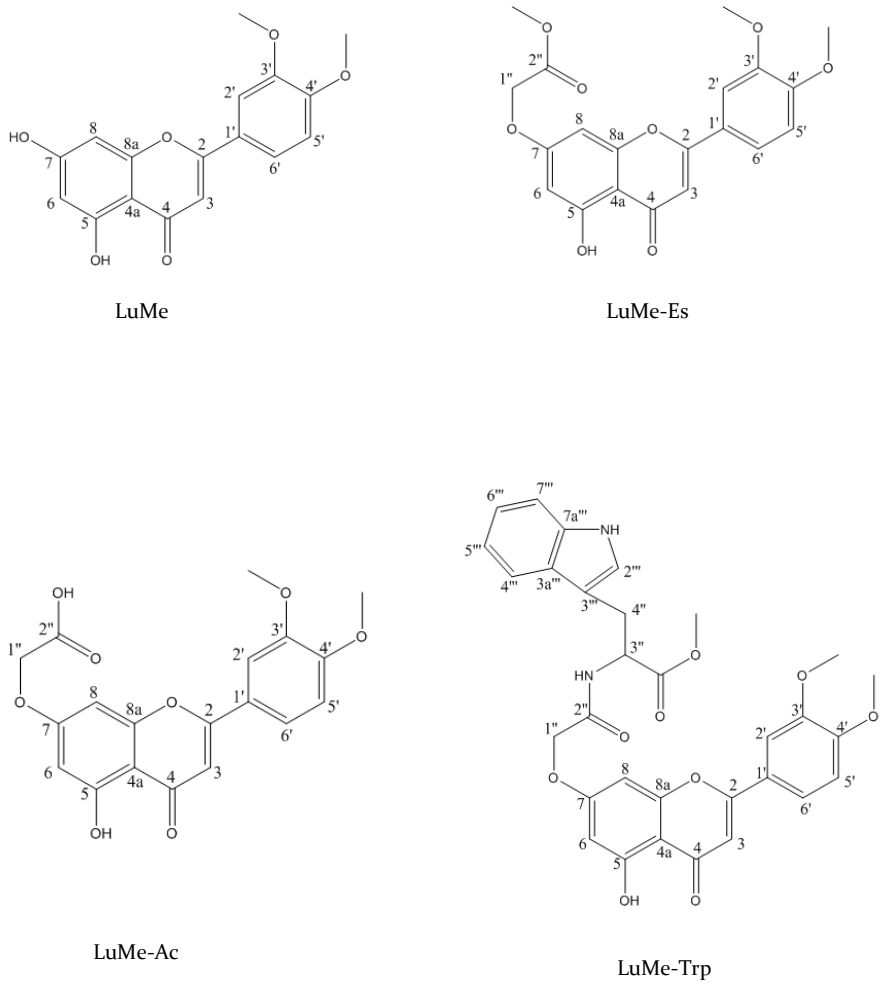


Figure 13 - Numbering of synthesized compounds concerning the NMR assignments

The IR data of flavone derivatives **LuMe**, **LuMe-Es**, **LuMe-Ac, LuMe-LTrp** and **LuMe-DTrp** are presented in **Table 2.**

Analyzing the IR spectra of all flavone derivatives, it is possible to detect the presence of free hydroxyl groups, due to the presence of a large band of stretching vibration at 3600-3300 cm^{-1} (OH). The presence 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. Additionally, bands between 2920 cm^{-1} and 2936 cm^{-1} suggest the presence of alkyl groups. The presence of bands at 1646-1647 cm^{-1}, 1534

cm^{-1}, and 3398 cm^{-1} in the spectra of **LuMe-LTrp** and **LuMe-DTrp** suggest that the coupling with amino acid was successfully accomplished.

***Tabel 2** - IR data of flavone derivatives.*

ν (cm^{-1})					
Groups	**LuMe**	**LuMe-Es**	**LuMe-Ac**	**LuMe-LTrp**	**LuMe-DTrp**
O-H	3600-3300	3600-3300	3600-3300	3600-3300	3600-3300
Aliphatic C-H	2920	2936	2920	2927	2927
C=O	1653	1665	1651	1668	1667
Aromatic C=C	1576 1520 1506 1429	1587 1519 1501 1421	1591 1559 1506 1433	1598 1521 1500 1436	1599 1521 1500 1437
C=O (ester)	-	1748	1754 (acid)	1740	1740
C-O	1254	1260	1263	1260	1260
-CO-NH-R	-	-	-	1647	1646
C=N	-	-	-	1534	1534
N-H	-	-	-	3398	3398

The ^{1}H NMR and ^{13}C NMR data for flavone derivatives are reported in **Table 3** and **4**, respectively.

The ^{1}H NMR spectra of **LuMe** showed characteristic signals of 5,7-dihydroxyphenyl A ring (OH-5: δ_H 12.86 s; H-6: δ_H 6.17 d; OH-7: δ_H 10.63 s; H-8: δ H 6.45 d), and H-3 (δ H 6.84 s). The ^{1}H NMR spectra also showed characteristic signals of a 3',4'-dimethoxy B ring ($\delta_{H:}$ 7.62 dd, 7.51 d, 7.06 d, 3.89 s, 3.88 s). The structure of **LuMe** derivatives was confirmed by comparison with the ^{1}H and ^{13}C NMR data previously reported. [16]

The structure elucidation of **LuMe-Es**, **LuMe-Ac**, **LuMe-LTrp** and **LuMe-DTrp** was accomplished by the comparison of the ^{1}H and ^{13}C NMR data with that of the precursor.

The ^{1}H and ^{13}C NMR data of **LuMe-Es** is similar to that of **LuMe**. Nevertheless, instead of a singlet at 10.63 (HO-7) observed in the ^{1}H NMR data of **LuMe**, the spectrum **LuMe-Es** revealed the presence of two singlets at 4.77 (H-1") and 3.73 (OCH_3) ppm,

confirming the introduction of the 2-methoxy-2-oxoethoxy side chain at C-7. The 13C NMR was in accordance to the performed molecular modification.

The ^{1}H and ^{13}C NMR data of **LuMe-Ac** is similar to that of **LuMe-ES**. Nevertheless, the singlet at 3.73 ppm in the ^{1}H NMR spectrum of **LuMe-Es** was not observed in the ^{1}H NMR spectrum of **LuMe-Ac**. The signal of the oxymethylic carbon at 51.8 ppm (OCH_3) detected in the ^{13}C NMR spectrum of **LuMe-ES** was also not observed in the ^{13}C NMR spectrum of **LuMe-Ac**. These data confirmed that the hydrolysis of **LuMe-ES** give rise **LuMe-Ac.**

Concerning compounds **LuMe-LTrp** and **LuMe-DTrp**, when comparing the ^{1}H and ^{13}C NMR data of both chiral products with those of **LuMe-Ac**, used as building block, characteristic signals of the introduction of the chiral moiety confirm the structure of compounds.

***Tabel 3** - ^{1}H NMR data for flavone derivatives*

	LuMe	**LuMe-Es**	**LuMe-Ac**	**LuMe-Trp**
H-3	6.84 (s)	6.71 (s)	7.04 (s)	7.05 (s)
H-5	12.86 (s, OH)	12.85 (s, OH)	12.92 (s, OH)	12.93 (s, OH)
H-6	6.17 (d, *J*=2.0)	6.28 (d, *J*=2.2)	6.38 (d, *J*=2.3)	6.39 (d, *J*=2.2)
H-7	10.63 (s, OH)	-	-	-
H-8	6.45 (d, *J*=2.1)	6.56 (d, *J*=2.2)	6.82 (d, *J*=2.3)	6.75 (d, *J*=2.2)
H-2′	7.51 (d, *J*=2.0)	7.40 (d, *J*=2.0)	7.58 (d, *J*=2.1)	7.58 (d, *J*=2.1)
H-3'	3.86 (OCH_3)	3.87 (OCH_3)	3.85 (OCH_3)	3.86 (OCH_3)
H-4'	3.89 (OCH_3)	3.89 (OCH_3)	3.88 (OCH_3)	3.88 (OCH_3)
H-5'	7.06 (d, *J*=8.6)	6.98 (d, *J*=8.5)	7.13 (d, *J*=8.7)	7.13 (d, *J*=8.7)
H-6'	7.62 (dd, *J*=8.5; *J*=2.0)	7.55 (dd, *J*=8.5; *J*=1.9)	7.71 (dd, *J*=8.5; *J*=2.1)	7.70 (dd, *J*=8.5; *J*=2.1)
H-1"	-	4.77 (s)	4.84 (s)	4.66-4.59 (m)
OCH_3 ester	-	3.73 (s)	-	3.62 (s)-
H-3"				4.66-4.59 (m)

H-1'''				10.90 (d, *J*=1.4, NH)
H-2'''				7.19 (d, *J*=2.3)
H-4'''				7.51 (d, *J*=7.8)
H-5'''				6.97 (td, *J*=7.9, *J*=1.1)
H-6'''				7.06 (td, *J*=7.8, *J*=1.1)
H-7'''				7.33 (d, *J*=7.9)
NH				8.49 (d, *J*=7.7)
Values in parts per million (δ_H). Measured in DMSO-d_6 at 300.13 MHz. *J* values (Hz) are presented in parentheses.				

Tabel 4 *- ^{13}C NMR data for flavone derivatives*

	LuMe	**LuMe-Es**	**LuMe-Ac**	**LuMe-Trp**
C-2	163.1	163.0	163.7	163.4
C-3	103.9	105.3	105.0	104.1
C-4 (C=O)	181.7	181.8	182.0	182.1
C-4a	103.8	104.0	104.0	105.1
C-5	161.5	161.4	161.2	161.1
C-6	98.8	98.1	98.5	98.6
C-7	164.1	163.6	163.7	163.7
C-8	93.8	93.0	93.5	93.6
C-8a	157.3	157.0	157.2	157.1
C-1'	123.1	122.8	122.7	122.7
C-2'	109.2	109.0	109.5	109.4
C-3'	149.0	148.8	149.0	149.0
C-4'	152.0	151.9	152.3	152.3
C-5'	111.3	111.1	111.7	111.7
C-6'	119.8	120.0	120.2	120.2
OCH_3	55.7, 55.5	55.7,55.5	55.9,55.8	55.9,55.8
C=O ester	-	168.0	-	170.0

OCH_3 ester	-	51.8	-	52.0
C-1"	-	64.7	64.9	66.9
C-3"				52.9
C-4"				26.8
C=O carbox.	-	-	169.5	-
C=O amide				167.0
C-2"'				123.8
C-3"'				109.2
C-3a"'				127.1
C-4"'				118.0
C-5"'				118.5
C-6"'				121.0
C-7"'				111.5
C-7a"'				136.1
Values in parts per million (δ_C). Measured in DMSO-d_6 at 75.47 MHz				

3.3. Enantiomeric purity determination*

**Work developed by Dra. Virgínia Gonçalves under the supervision of Prof. Elizabeth Tiritan*

The retention factor (k) was calculate using the equation ($k=[t_R-t_0]/t_0$). The separation factor (α) was calculated as ($\alpha=k_2/k_1$). The resolution factor (Rs) was calculated using the equation ($Rs=1.18*[t_{R2}-t_{R1}]/[½W_1+½W_2]$), where t_{R1} and t_{R2} are the retention times of the first and second enantiomers, respectively, and $½W_1$ and $½W_2$ are the corresponding peak width measured on the half height. The mathematical formula used for the calculation of the percentage of enantiomeric excess was used (%ee=100*[(S-R)/(S+R)]).

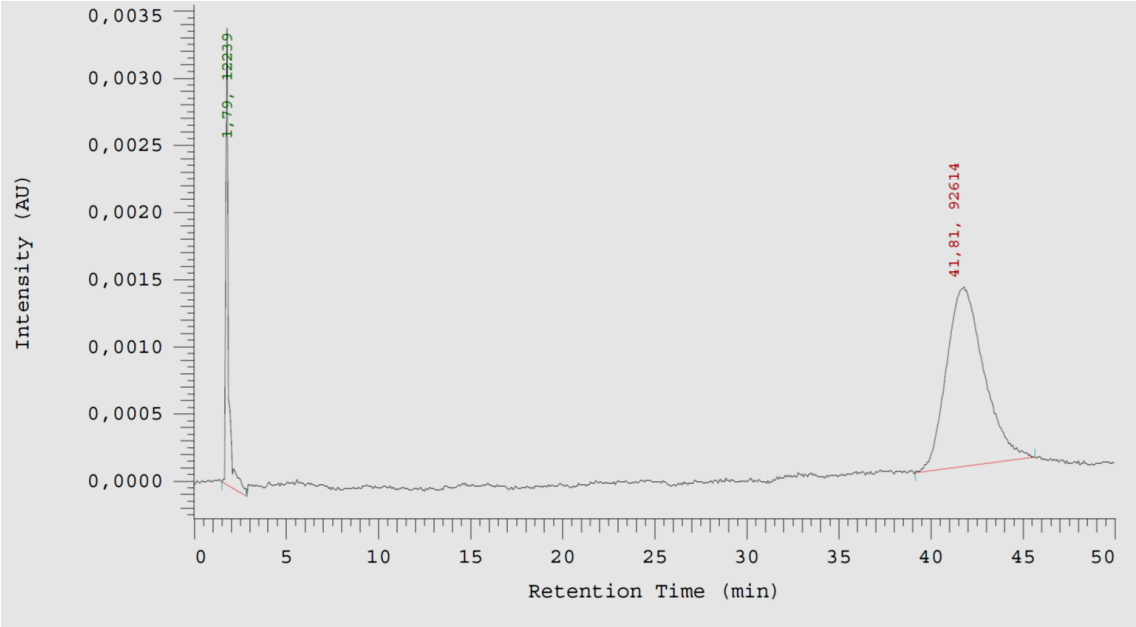


Figure 14 - Chromatogram from HPLC analysis of **LuMe-DTrp.**

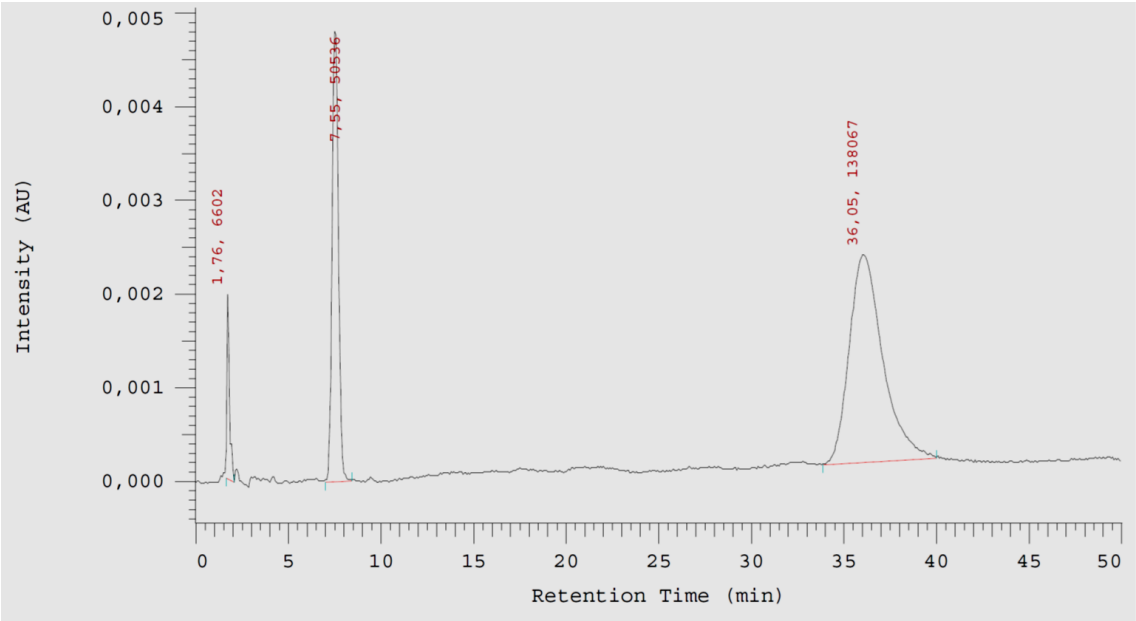


Figure 15 - Chromatogram from HPLC analysis of **LuMe-LTrp**

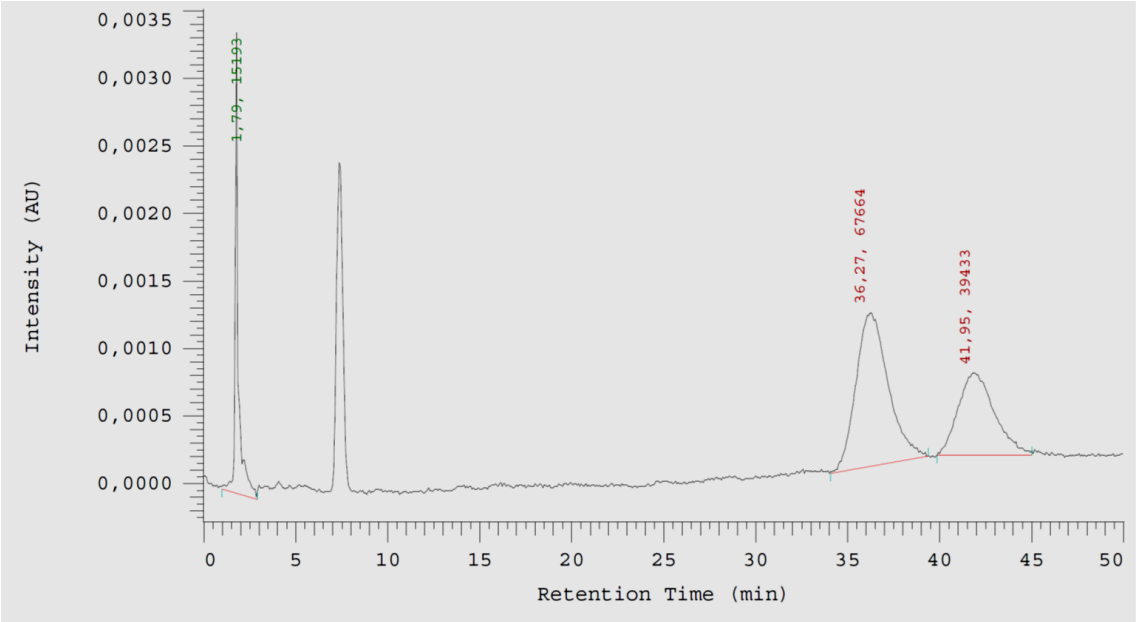


Figure 16 - Chromatogram from HPLC analysis of **LuMe-LTrp** and **LuMe-DTrp** mixture

Table 5 summarizes the HPLC determinations for the chiral derivatives synthesized. It can be observed that the **LuMe-DTrp** is more retained than **LuMe-LTrp**, and the enantiomeric ratio of the enantiomeric mixture is 26.36% (S).

Table 5 - Chromatographic parameters of the chiral derivatives.

	t_R (min)	k	α	Rs	%ee
LuMe-DTrp	41.81	22.36	-	-	-
LuMe-LTrp	36.00	19.11	-	-	-
LuMe-LTrp + LuMe-DTrp	36.27 41.95	19.26 22.43	1.16	1.74	26.36 (S)

4. Experimental

4.1. General methods

All reagents and solvents were purchased from Sigma Aldrich, and had no further purification process, HPLC solvents used were HPLC grade. Solvents were evaporated using rotary evaporator, from Büchi, under reduced pressure.

All reactions were monitored by TLC carried out on precoated plates with 0.2 mm of thickness using Merck silica gel 60 (GF_{254}). The UV light at 254 and 365 nm.

Purification of the synthesized compounds was carried out by column chromatography using Merck silica gel 60 (0.2-0.5 mm), liquid-liquid extraction and crystallization.

Melting points (m.p.) were obtained in a Köfler microscope and are uncorrected. Infrared (IR) spectra were obtained in KBr microplates in a Fourier transform infrared spectroscopy (FTIR) spectrometer Nicolet iS10 from Thermo Scientific with Smart OMNI-Transmisson accessory (Software OMNIC 8.3) (cm^{-1}).

Optical rotation measurements were carried out on a Bellinghom+Standeley, Ltd, modal ADP410 polarimeter using dichloromethane as solvent, and 10 cm cell.

NMR spectra were performed in University of Aveiro, Department of Chemistry, and were taken in DMSO-d_6 (Deutero GmbH) at room temperature on Bruker Avance 300 spectrometer (300.13 MHz for ^{1}H and 75.47 MHz for 13C). ^{13}C NMR assignments were made by bidimentional HSQC and HMBC experiments (long-range C, H coupling constants were optimized to 7 and 10 Hz) or by comparison with the assignments of similar molecules.

HPLC analyses for the studies of the enantiomeric excess were performed in the Laboratory of Applied Chemistry of Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde IINFACTS from Cooperativa de Ensino Superior Politécnico Universitário (CESPU). The HPLC analysis was performed on a LaChrom Merck Hitachi HPLC, equipped with a L-7100 pump, an L-7200 auto-injector, an L-7455 DAD and a D-7000 interface. The stationary phase was a Lux® 3µm Cellulose-2 (150x4,6mm) from Phenomenex and data analysis was performed using HPLC System Manager HSMD-7000 software, version 3.0. Ethanol (EtOH) (with 0.01% trifluoroacetic acid (TFA)) and *n*-hexane (*n*-Hex) (with 0.01% trifluoroacetic acid (TFA)) (50:50 *v*/*v*) were used as mobile phase, in a flow rate of 1,0mL/min, and the chromatograms were monitored by UV detection at λ=264nm. The sample injections (10 µL) were carried out on duplicates.

4.2. Synthesis of 4'-methylchrysoeriol (**LuMe**)

A mixture of phloroglucinol (175 mg, 1 mmol) and ethyl 3,4-dimethoxybenzoylacetate (700 mg, 2 mmol) was heated at 240 ˚C in heating mantle for 120 min. The crude mixture was dissolved in 10% aq NaOH (25 mL) and washed with diethyl ether (2x25 mL), and the product was precipitated by adding 37% aq HCl. The brown solid was filtered, wash with cool water, and vacuum-dried. The chromatographic control was performed by TLC (silica gel; *n*-hexane: ethyl acetate, 5:5 *v*/*v*).

Purification of the synthesized compound was carried out by column chromatography (silica gel; *n*-hexane: ethyl acetate in gradient) and crystallization in acetone.

4'-methylchrysoeriol (LuMe). Yield: 47%; mp 282-284˚C; IR (KBr): see Table 2; **^{1}H NMR** (DMSO-d_6, 300.13 MHz): see Table 3 **^{13}C NMR** (DMSO-d_6, 75.47 MHz): see Table 4.

4.3. Synthesis of 7-(2-methoxy-2-oxoethoxy)-4'-methylchrysoeriol (**LuMe-Es**)

LuMe (200 mg, 0.64 mmol) was dissolved in anhydrous acetone (10 mL) and K_2CO_3 (110 mg, 0.76 mmol) and methyl bromoacetate (73 µL, 0.76 mmol) were added. The mixture was kept under reflux and magnetic stirring for 24 h. Then, the mixture was concentrated under reduced pressure and dichloromethane was added to dissolve the compound and separat it from K_2CO_3 by filtration. Then the solution was concentrated under reduced pressure and n-hexane was added. The solid was separated by filtration and purified by crystallization in *n*-hexane. The chromatographic control was performed via TLC (silica gel; chloroform: methanol: acetic acid, 9:1:0.1 *v*/*v*).

7-(2-methoxy-2-oxoethoxy)-4'-methylchrysoeriol (LuMe-Es). Yield: 50%; mp 195-200˚C; IR (KBr): see table 2; **^{1}H NMR** (DMSO-d_6, 300.13 MHz): see Table 3, **^{13}C NMR** (DMSO-d_6, 75.47 MHz): see Table 4.

4.4. Synthesis of 7-carboxymethoxy-4'-methylchrysoeriol (**LuMe-Ac**)

LuMe-Es (100 mg, 0.26 mmol) was dissolved in dichloromethane/methanol (10 mL) and a solution of 5M NaOH (0.52 mL) was added. The mixture and methylbromoacetate (73 µL, 0.76 mmol) were added. The mixture was kept at room temperature and magnetic stirring for 5 h. Then, the dichloromethane and methanol were evaporated under reduced pressure and the suspension was washed with dichloromethane (2x25 mL). The aqueous phase was acidified with a solution of 5M HCl, resulting in the formation of a precipitate that was collected by filtration under reduced pressure. The pink salmon solid was washed with cool water and recrystallized from methanol. The chromatographic control was performed by TLC (silica gel; chloroform: methanol: acetic acid, 9:1:0.1 *v*/*v*).

7-carboxymethoxy-4'-methylchrysoeriol (LuMe-Ac). Yield: 49%; mp 265-269˚C; IR (KBr): see Table 2, **^{1}H NMR** (DMSO-d_6, 300.13 MHz): see Table 3, **^{13}C NMR** (DMSO-d_6, 75.47 MHz): see Table 4.

4.5. Synthesis of (*S*)-7-(2-((3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethoxy)-4'-methylchrysoeriol (**LuMe-LTrp**)

LuMe-Ac (139 mg, 0.37 mmol) was dissolved in THF (40 mL) and TEA (155 µL) was added. Following, the coupling reagent (COMU®, 1.2 eq mmol) was added and the solution stirred for about 15 min before the addition of the chiral reagent (L-tryptophan methyl ester hydrochloride, 1.7 eq). The mixture was kept at room temperature and magnetic stirring for 24 h. The reaction was followed by TLC (silica gel; chloroform: methanol: acetic acid, 9:1:0.1 *v*/*v*). After its completion, the solvent was evaporated under reduced pressure at 40˚C and the crude product was dissolved in dichloromethane. This solution was washed with a 5% HCl solution (2x15 mL), 5% NaHCO3 solution (2x15 mL) and water (3x20 mL). The organic layer was dried with anhydrous sodium sulfate, filtered and the solvent was evaporated under reduce pressure. The product was crystallized from methanol at low temperature and a yellow solid was obtained.

(*L*)-7-(2-((3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethoxy)4'-methylchrysoeriol (LuMe-LTrp) Yield: 88%; mp 214-216˚C; $[\propto]_D^{25℃}$ +23˚C (c=7,5x10^{-3} g/mL in dichloromethane); IR (KBr): see Table 2; **^{1}H NMR** (DMSO-d_6, 300.13 MHz): see Table 3, **^{13}C NMR** (DMSO-d_6, 75.47 MHz): see Table 4.

4.6. Synthesis of (*R*)-7-(2-((3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethoxy)-4'-methylchrysoeriol (**LuMe-DTrp**)

LuMe Ac (150 mg, 0.59 mmol) was dissolved in THF (40 mL) and TEA (155 µL) was added. Following, the coupling reagent (COMU®, 1.2 eq mmol) was added and the solution stirred for about 15 min before the addition of the chiral reagent (D-tryptophan methyl ester hydrochloride, 1.7 eq). The mixture was kept at room temperature and magnetic stirring for 24 h. The reaction was followed by TLC (silica gel; chloroform: methanol: acetic acid, 9:1:0.1 *v/v*). After its completion, the solvent was evaporated under reduced pressure at 40 ˚C and the crude product was dissolved in dichloromethane. This solution was washed with a 5% HCl solution (2x15 mL), 5% NaHCO3 solution (2x15 mL) and water (3x20 mL). The organic layer was dried with anhydrous sodium sulfate, filtered and the solvent was evaporated under reduce pressure. The product was crystallized from methanol at low temperature and a yellow solid was obtained.

(*R*)-7-(2-((3-(1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-yl)amino)-2-oxoethoxy)-4'-methylchrysoeriol (LuMe-DTrp) Yield: 80%; mp 209-210˚C; $[\propto]_D^{25℃}$ -24˚C (c=7,5x10^{-3} g/mL in dichloromethane); IR (KBr): see Table 2; **^{1}H NMR** (DMSO-d_6, 300.13 MHz): see Table 3, **^{13}C NMR** (DMSO-d_6, 75.47 MHz): see Table 4.

4.7. Enantiomeric purity determination

The normal phase mode LC evaluation to the determine enantiomeric purity was carried out using a mixture of EtOH and *n*-Hex as mobile phase, prepared in a volume/volume (50:50 *v/v*) relation with 0.01% TFA. Prior to the analysis an UV/Vis spectrum was collected to determine the λ of monitoring the LC (Figure 17).

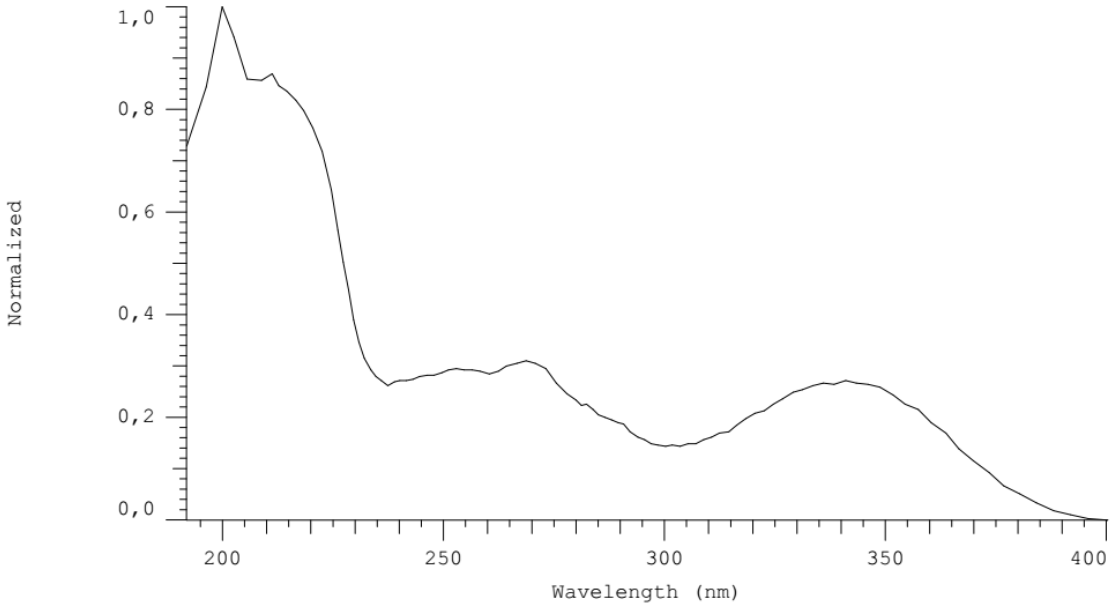


Figure 17- UV-Vis spectrum from **LuMe-LTrp** in EtOH.

The LC dead time (t_o) was considered to be equal to the peak of the solvent front and was taken from each particular run. The stock solutions of the chiral flavonic derivatives in EtOH at the concentration of 0.4mg/mL were prepared and working solutions of enantiomeric mixtures were prepared mixing equal aliquots of each enantiomer. The analyses were performed at room temperature.

The retention factor (k) was calculate using the equation ($k=[t_R-t_o]/t_o$). The separation factor (α) was calculated as ($\alpha=k_2/k_1$). The resolution factor (Rs) was calculated using the equation ($Rs=1.18*[t_{R2}-t_{R1}]/[½W_1+½W_2]$), where t_{R1} and t_{R2} are the retention times of the first and second enantiomers, respectively, and $½W_1$ and $½W_2$ are the corresponding peak width measured on the half height. The mathematical formula used for the calculation of the percentage of enantiomeric excess was used ($\%ee=100*[(S-R)/(S+R)]$).

5. Conclusions

This research project allowed the synthesis and identification of five flavone derivatives, including four new flavones not reported before. Firstly, it was synthesized the flavone nucleus (**LuMe**), afterwards, a ring A ester derivative was

coupled (**LuMe-Es**), and converted by hydrolyzed in an acid derivative (**LuMe-Ac**). Finally, the chiral derivatives (**LuMe-LTrp** and **LuMe-DTrp**) were created by coupling with the corresponding amino acids.

The synthesis of flavone **LuMe** was accomplished by solvent-free direct thermal cyclocondensation of phloroglucinol and α-ketoesters. **LuMe-Ac** and **LuMe-Es** were achieved with considerably good yields, without complex purification process.

Considering the aims proposed for this project, and in order to create structures inspired by previous works from our research group, the synthesis of two chiral flavonic derivatives from **LuMe** were obtained with quit high yields and enantiomerically pure.

Finally, the study of the biological activity (antitumor and antimicrobial) of all synthesized compounds will be determined in the future.

6. References

1. Benjamin M. Hariri, D. B. M., Bei Chen, Nithin D. Adappa, James N. Palmer, David W. Kennedy, Robert J. Lee, Plant flavones enhance antimicrobial activity of respiratory epithelial cell secretions against Pseudomonas aeruginosa. *PLoS One* **2017,** *12* (9), 1-18.

2. Umeo Takahama, T. O., Flavonoids and Some Other Phenolics as Substrates of Peroxidase: Physiological Significance of the Redox Reactions. *Journal of Plant Research* **2000,** *113* (3), 301-309.

3. Massoumeh Farasat, R.-A. K.-N., Seyed Mohammad Bagher Nabavi and Foroogh Namjooyan, Antioxidant Activity, Total Phenolics and Flavonoid Contents of some

Edible Green Seaweeds from Northern Coasts of the Persian Gulf. *Iranian Journal of Pharmaceutical Research* **2013,** *13* (1), 163-170.

4. A. N. Panche, A. D. D. a. S. R. C., Flavonoids: an overview. *Journal of Nutritional Science* **2016,** *5* (e47), 1-15.

5. Middleton Jr E, K. C., Theoharides TC, The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. *Pharmacological Reviews* **2001,** *52* (4), 673-751.

6. Manjinder Singh, M. K., Om Silakari, Flavones: An important scaffold for medicinal chemistry. *European Journal of Medicinal Chemistry* **2014,** *84*, 206-239.

7. Hanem M. Awad, H. I. A.-A., Mohamed A. Ibrahim, Eslam R. El-Sawy, Mohamed M. Abdalla, Flavones from Heavenly Blue as modulators of Alzheimer's amyloid-beta peptide (Aβ) production. *Medicinal Chemistry Research* **2018,** *27* (3), 768-776.

8. Andersen, O. M.; Markham, K. R., *Flavonoids: Chemistry, Biochemistry and Applications*. CRC Press: 2005.

9. CROFT, K. D., The Chemistry and Biological Effects of Flavonoids and Phenolic Acidsa. *Annals of the New York Academy of Sciences* **1998,** *854* (1), 435-442.

10. Leonard, E.; Yan, Y.; Lim, K. H.; Koffas, M. A. G., Investigation of Two Distinct Flavone Synthases for Plant-Specific Flavone Biosynthesis in Saccharomyces cerevisiae. *Applied and Environmental Microbiology* **2005,** *71* (12), 8241-8248.

11. Stefan Martens, A. M., Flavones and flavone synthases. *Phytochemistry* **2005,** *66* (20), 2399-2407.

12. Jiang, N.; Doseff, A. I.; Grotewold, E., Flavones: From Biosynthesis to Health Benefits. *Plants (Basel)* **2016,** *5* (2), 27.

13. Kulkarni, P. D. K., Dasharath & Varala, Ravi., Cyclization of 2'-hydroxychalcones to flavones using ammonium iodide as an iodine source - An eco-friendly approach. *Journal of the Serbian Chemical Society.* **2011,** *78* (7), 909-916.

14. Ameen, D.; Snape, T., *Mechanism and Application of Baker–Venkataraman O→C Acyl Migration Reactions*. 2014; Vol. 47.

15. Wang, Z., Allan-Robinson Condensation. In *Comprehensive Organic Name Reactions and Reagents*, 2010; pp 64-67.

16. Seijas, J. A.; Vázquez-Tato, M. P.; Carballido-Reboredo, R., Solvent-Free Synthesis of Functionalized Flavones under Microwave Irradiation. *The Journal of Organic Chemistry* **2005,** *70* (7), 2855-2858.

17. Wang, Z., Mentzer Pyrone Synthesis. In *Comprehensive Organic Name Reactions and Reagents*, 2010; pp 1901-1904.

18. Larhed, M.; Olofsson, K., *Microwave Methods in Organic Synthesis*. Springer Berlin Heidelberg: 2006.

19. Yuan Zhao, L. C., Qiang Sui, Feng Lin, Wen Jiang, Jianli Chen, Weigeng Lu, Qi Gao,, Facile synthesis of acacetin and its derivatives,. *Bioorganic & Medicinal Chemistry Letters,* **2016,** *26* (15), 3577-3580,.

20. Gal, J., Pasteur and the art of chirality. *Nature Chemistry* **2017,** *9*, 604.

21. Nerkar AG, L. K., Gadhave NA, Sawant SD, Chiral switches: A Review. *Journal of Pharmacy Research* **2011,** *4* (4), 1300-1303.

22. Tsuchiya, H.; Mizogami, M., The membrane interaction of drugs as one of mechanisms for their enantioselective effects. *Medical Hypotheses* **2012,** *79* (1), 65-67.

23. Nguyen, L. A.; He, H.; Pham-Huy, C., Chiral drugs: an overview. *Int J Biomed Sci* **2006,** *2* (2), 85-100.

24. Fernandes, C.; Masawang, K.; Tiritan, M. E.; Sousa, E.; de Lima, V.; Afonso, C.; Bousbaa, H.; Sudprasert, W.; Pedro, M.; Pinto, M. M., New chiral derivatives of xanthones: Synthesis and investigation of enantioselectivity as inhibitors of growth of human tumor cell lines. *Bioorganic & Medicinal Chemistry* **2014,** *22* (3), 1049-1062.

25. Araújo, J.; Fernandes, C.; Pinto, M.; Tiritan, M. E., Chiral Derivatives of Xanthones with Antimicrobial Activity. *Molecules* **2019,** *24* (2), 314.