KEREM BURAN

INSTITUTE OF HEALTH SCIENCES

PHARMACEUTICAL CHEMISTRY

PhD THESIS

2018

T.C.
YEDITEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

DESIGN, SYNTHESIS AND EVALUATION OF THE BIOLOGICAL ACTIVITY OF NOVEL COUMARIN DERIVATIVES

DOCTOR OF PHILOSOPHY THESIS

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SUPERVISOR

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İSTANBUL – 2018

THESIS APPROVAL FORM

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This study have approved as a Doctorate Thesis in regard to content and quality by the Jury.

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Member/Examiner:	Prof. Dr. Mert ÜLGEN Acıbadem University	

APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated 12/06/2018 and numbered 2018/10-02

Prof. Dr. Bayram YILMAZ
Director of Institute of Health Sciences

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Bu çalışma jurimiz tarafından kapsam ve kalite yönünden Doktora Tezi olarak kabul edilmiştir.

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ONAY

Bu tez Yeditepe Üniversitesi Lisansüstü Eğitim-Öğretim ve Sınav Yönetmeliğinin ilgili maddeleri uyarınca yukarıdaki jüri tarafından uygun görülmüş ve Enstitü Yönetim Kurulu'nun 12/06/2018 tarih ve 2018/10-02 sayılı kararı ile onaylanmıştır.

Prof. Dr. Bayram YILMAZ
Sağlık Bilimleri Enstitüsü Müdürü

BEYAN

Bu tezin kendi çalışmam olduğunu, planlamasından yazımına kadar hiçbir aşamasında etik dışı davranışımın olmadığını, tezdeki bütün bilgileri akademik ve etik kurallar içinde elde ettiğimi, tez çalışmasıyla elde edilmeyen bütün bilgi ve yorumlara kaynak gösterdiğimi ve bu kaynakları kaynaklar listesine aldığımı, tez çalışması ve yazımı sırasında patent ve telif haklarını ihlal edici bir davranışımın olmadığını beyan ederim.

07.06.2018

KEREM BURAN

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

07.06.2018

KEREM BURAN

To my mother..

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ABBREVIATIONS

2GT	2-Coumarate *O*-b-glucosyltransferase
AID	Adjuvant-induced disease
$AlCl_3$	Aluminium chloride
ATP	Adenosin-5`-triphosphate
C2'H	Coumaroyl-CoA 2'-hydroxylase
C2H	Cinnamate/coumarate 2-hydroxylase
C3H	Coumarate 3-hydroxylase
C4H	Cinnamic acid 4-hydroxylase
CA	Carbonic anhydrase enzyme
CAI	Carbonic anhydrase inhibitor
CCoAOMT	Caffeoyl-CoA O-methyltransferase
CDK2	Cyclin-dependent kinase 2 gene
CDKN1A	Cyclin-dependent kinase inhibitör 1A
CH_2Cl_2	Dichloromethane
$CHCl_3$	Chloroform
CK2	Casein kinase II
COX	Cyclooxygenase
CYP-450	Cytochrome P450
DBC	7-Diethylamino-3(2'-benzoxazolyl)coumarin
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DHPM	Dihydropyrimidinone
DMA	Dimethylacetamide
DNA	Deoxyribonucleic acid
DPPH	1,1-Diphenyl-2-picrylhydrazyl
FDA	Food and drug administration
G_1	Gap 1 phase
G_2	Gap 2 phase
HCT	Hydroxycinnamoyl-CoA

hPBMC	Human peripheral blood mononuclear cells
HSP90	Heat shock protein 90
hTERT	Human telomerase reverse transcriptase
IC50	The half maximal inhibitory concentration
IND	Indomethacin
IUPAC	International Union of Pure and Applied Chemistry
iNOS	Inducibel nitric oxide synthase
L-NAME	Nω-Nitro-L-arginine methyl ester hydrochloride
LC-MS	Liquid chromatography - Mass spectroscopy
LPS	Lipopolysaccharides
M	Mitosis phase
M.P.	Melting point
MAP Kinase	Mitogen-activated protein kinase
MCT	Monocarboxylate transporters
MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)
MW	Microwave
Mw	Molecular weigth
NBS	N-Bromosuccinimide
NF-κB	Nuclear factor kappa B
NMR	Nuclear magnetic resonance
NO	Nitric oxide
PAL	Phenyl alanine ammonia-lyase
PGE2	Prostaglandin E2
Ph	Phenyl
S	Synthases phase
SAR	Structure-activity relationship
SFKs	Src family of kinases
TAL	Tyrosine ammonia lysase
TFA	Trifluoroactetic acid
TLR	Toll-like receptors
TNFα	Tumor necrosis factor α

UV	Ultraviolet
VEGF	Vascular endothelial growth factor
WHO	World Health Organisation

ABSTRACT

BURAN, K. (2018). Design, Synthesis and Evaluation of the Biological Activity of Novel Coumarin Derivatives. Yeditepe University, Institute of Health Sciences, Department of Pharmaceutical Chemsitry, PhD Thesis, İstanbul.

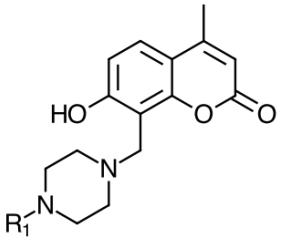
Coumarins, (2*H*-1-benzopiran-2-one) derivatives that can be isolated from several plants, have been reported for their anticoagulant, antimicrobial, anti-inflammatory or anticancer activity. Some of these structures are currently approved for the treatment of cardiovascular diseases (warfarin), as antibiotics (novobiocin or chlorobiocin) or as an anticancer drug (geiparvarin).

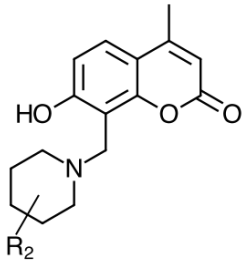
Given the great potential of this structure and the limited number of studies that focus on molecules that derive from the 8th carbon of the benzopiranone heterocycle, we synthesized in this project thirty eight coumarin derivatives (**2 - 50**), twenty two of them being novel, by substituting to the 8th carbon of the benzopiran ring some aromatic and aliphatically subtituted piperidine and piperazines. Synthesized derivatives were evaluated for their anti-inflammatory activity, analgesic activity and cytotoxicity.

Targeted molecules were synthesized in two steps. The first step consisted on the synthesis of the 7-hydroxy-4-methyl-chromen-2-one coumarin scaffold *via* the Pechmann reaction. In the second step, the 7-hydroxy-4-methyl-chromen-2-one coumarin scaffold, (compound **1**), was derivatized with aromatic and aliphatic substituted piperazine and piperidine groups. Structures of synthesized compounds were elucidated with ^{1}H-NMR, ^{13}C-NMR, LC-MS, FT-IR.

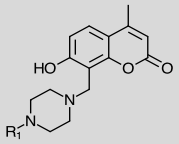
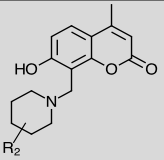
The anti-inflammatory and analgesic activities and cytotoxicity were performed *in vitro*. Cytotoxicity assays were carried out with the MTT test on RAW264.7 macrophage and MCF-7 breast cancer cells. The nitrite inhibition test was applied for evaluating the anti-inflammatory activity on RAW264.7 macrophage cells. Indomethacin (IND) and L-NAME were used as reference drugs for both anti-inflammatory and cytotoxicity tests. For the analgesic activity, PGE_2 production was monitored. According to the results, compound **11**, **23** and **31** revealed promising anti-inflammatory activity. Their results were better than the reference drugs and compound **11** was three times more active than IND. Additionally, compound **11** demonstrated some analgesic activity with low cytotoxicity.

Keywords: Coumarin, piperazine, piperidine, anti-inflammatory activity, analgesic activity, cytotoxicity.





R_1: **2 - 32** R_2: **33 - 50**

	
R_1= 2 - 32	**R_2= 33 - 50**

R_1 and R_2 Groups

1	Coumarin	**26**	-4-CF_3-Ph
2	-H	**27**	-3-CF_3-Ph
3	-CH_3	**28**	-*o*-F-Ph
4	-CH_2CH_3	**29**	-*m*-Cl-Ph
5	-CH_2CH_2OH	**30**	-Benzo[1,3]dioxol
6	-$CH_2CH{=}CH_2$	**31**	-Naphthalen
7	-CH_2CH_2Ph	**32**	-Pyridinyl
8	-CH_2CH_2CN	**33**	-
9	-COPh	**34**	-4-CH_3
10	-$CH_2COOHCH_2CH_3$	**35**	-2-CH_3
11	-$COOCH_2Ph$	**36**	-3,5-$(CH_3)_2$
12	-$COOCH_2CH_3$	**37**	-2-CH_2CH_3
13	-COH	**38**	-2-$(CH_2)_2CH3$
14	-$(CH_2)_3N(CH_3)_2$	**39**	-2-$(CH_2)_2OH$
15	-Ph	**40**	-2-CH_2OH
16	-*p*-Cl-Ph	**41**	-3-CH_2OH
17	-*p*-F-Ph	**42**	-2-$COOCH_2CH_3$
18	-*p*-Br-Ph	**43**	-2-CO; 3-COOEt
19	-*p*-CH_3-Ph	**44**	-2-CO; 3-COOH
20	-*p*-NO_2-Ph	**45**	-4-COOH
21	-*o*-CN- Ph	**46**	-3-COOH
22	-*p*-OCH_3-Ph	**47**	-4-Ph
23	-*p*-Cl-Bn	**48**	-4-OH-Ph
24	-*p*-OH-Ph	**49**	-4-Bn
25	-3,4-$(Cl)_2$-Ph	**50**	-4-Benzimidazol-2-one

ÖZET

BURAN, K. (2018). Yeni kumarin türevlerinin tasarlanması, sentezi ve biyolojik aktivitlerinin değerlendirilmesi. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Farmasötik Kimya ABD., Doktora Tezi, İstanbul.

Kumarinler (2*H*-1-benzopiran-2-on türevleri) birçok bitkiden izole edilebilen benzopiran yapısında moleküllerdir. Bu moleküller antikoagülant, antimikrobiyal, antienflamatuvar, antikanser gibi birçok biyolojik aktiviteye sahiptirler. Günümüzde, kumarin türevleri, kalp ve damar sistemi hastalıkları tedavisinde (varfarin), bakteriyel enfeksiyon (novobiyosin ve klorobiyosin) ve kanser tedavilerinde kullanılmaktadır.

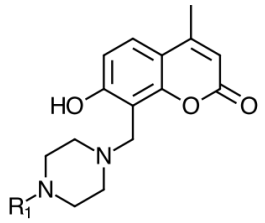
Bu çalışmada, biyolojik aktivite potansiyeli olan kumarin molekülünün (7-hidroksi-4-metil-kromen-2-on) çok fazla çalışma yapılmamış sekiz numaralı karbonundan alifatik ve aromatik gruplar içeren piperazin ve piperidin gruplarıyla türevlendirilmesiyle yirmi ikisi özgün otuz sekiz molekül sentezlenmiş ve bunların antienflamatuvar, analjezik aktivite ve sitotoksite çalışmaları yapılmıştır.

Hedeflenen moleküllerin sentezlenmesi iki basamak halinde gerçekleştirilmiştir. Birinci basamakta kumarin ana yapısı olan 7-hidroksi-4-metil-kromen-2-on, Pechman sentez yolağı kullanılarak sentezlenmiştir. Elde edilen kumarin bileşiği ikinci basamakta piperazin ve piperidin grupları kullanılarak sekiz numaralı pozisyonundan türevlendirilmiştir. Sentezlenen moleküllerin yapıları, ^{1}H-NMR, ^{13}C-NMR, LC-MS FT-IR ile aydınlatılmıştır.

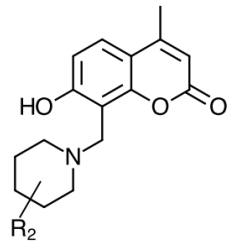
Sentezlenen yeni bileşiklerin antienflamatuvar, analjezik aktiviteleri ile sitotoksite çalışmaları *in vitro* olarak yapılmıştır. Sitotoksite çalışması MTT testiyle RAW264.7 ve MCF-7 kanser hücre hatlarında gerçekleştirilmiştir. Antienflamatuvar aktivite çalışmasında ise nitrit inhibisyon testi RAW264.7 makrofaj hücrelerinde yapılmıştır. Bu iki testte de referans ilaç olarak indometazin (IND) ve L-NAME kullanılmıştır. Analjezik aktivite testinde ise PGE_2 oluşum konsantrasyonları ELISA kiti kullanılarak incelenmiştir. Elde edilen sonuçlara göre, kumarin yapısının sekiz numaralı pozisyonun piperazin gruplarıyla türevlendirilmesi sonucunda elde edilen **11**, **23** ve **31** numaralı bileşiklerin antienflamtuvar aktivitelerinin oldukça yüksek olduğu görülmüştür. **11** numaralı bileşiğin antienflamatuvar aktivitesinin referans ilaç olan IND'den üç katı kadar olduğu tespit edilmiştir. Bunun yanı sıra, bileşik **11**'in analjezik aktivite gösterdiği ve

100µM dozunda uygunlandığında RAW 264.7 hücre hattında canlılığının %64 düzeyinde seyrettiği izlenmiştir.

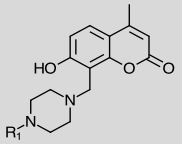
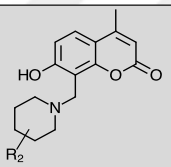
Anahtar Kelimeler: Kumarin, piperazin, piperidin, antienflamatuvar aktivite, analjezik aktivite, sitotoksite.



R_1: **2 - 32**



R_2: **33 - 50**

	
R_1= 2 - 32	**R_2= 33 - 50**

R_1 ve R_2 Grupları

1	Coumarin	**26**	-4-CF_3-Ph
2	-H	**27**	-3-CF_3-Ph
3	-CH_3	**28**	-*o*-F-Ph
4	-CH_2CH_3	**29**	-*m*-Cl-Ph
5	-CH_2CH_2OH	**30**	-Benzo[1,3]dioxol
6	-$CH_2CH{=}CH_2$	**31**	-Naphthalen
7	-CH_2CH_2Ph	**32**	-Pyridinyl
8	-CH_2CH_2CN	**33**	-
9	-COPh	**34**	-4-CH_3
10	-$CH_2COOHCH_2CH_3$	**35**	-2-CH_3
11	-$COOCH_2Ph$	**36**	-3,5-$(CH_3)_2$
12	-$COOCH_2CH_3$	**37**	-2-CH_2CH_3
13	-COH	**38**	-2-$(CH_2)_2CH3$
14	-$(CH_2)_3N(CH_3)_2$	**39**	-2-$(CH_2)_2OH$
15	-Ph	**40**	-2-CH_2OH
16	-*p*-Cl-Ph	**41**	-3-CH_2OH
17	-*p*-F-Ph	**42**	-2-$COOCH_2CH_3$
18	-*p*-Br-Ph	**43**	-2-CO; 3-COOEt
19	-*p*-CH_3-Ph	**44**	-2-CO; 3-COOH
20	-*p*-NO_2-Ph	**45**	-4-COOH
21	-*o*-CN- Ph	**46**	-3-COOH
22	-*p*-OCH_3-Ph	**47**	-4-Ph
23	-*p*-Cl-Bn	**48**	-4-OH-Ph
24	-*p*-OH-Ph	**49**	-4-Bn
25	-3,4-$(Cl)_2$-Ph	**50**	-4-Benzimidazol-2-one

1. INTRODUCTION AND AIM

Coumarins (2*H*-1-benzopyran-2-one) are compounds which can be obtained from many plants (Fig. 1.1). Since these compounds were first isolated from the seeds of *Coumarouna odorata Aube* (*Diptryx odorata*), they carry the name of coumarin [1]. In the literature, it is mentioned that coumarin derivatives show many biological activities such as anticoagulant [2, 3], antimicrobial [4], anti-inflammatory [5], anticancer [6], antidepressant [7]. This broad spectrum of activities has led this building block being a preferred ring for drug development. The fact that the coumarin ring is a common occurrence in drug development has led to the development of new synthesis methods. Coumarin scaffold can be synthesized *via* Perkin, Pechmann, Knovenagel, Wittig, Kostanecki-Robinson, and Reformatsky reactions [8].

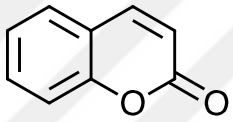
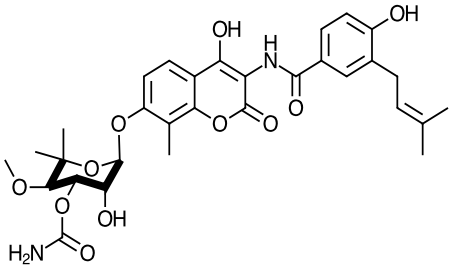
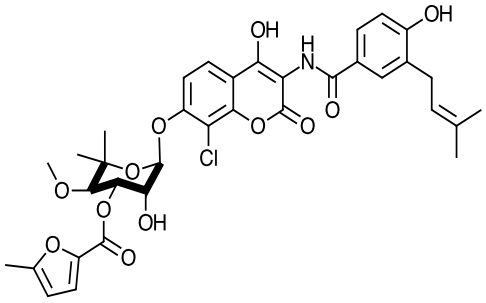


Figure 1.1. Structures of coumarin, isocoumarin, chroman, and chromone.

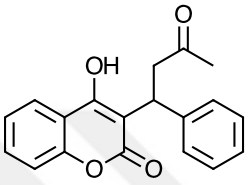
Today, there are many medicines on the market that contain the coumarin structure. Warfarin, for instance, is widely used as an anticoagulant in cardiovascular system diseases [8]. Another important derivative of the coumarin is used as an antibiotic. The most known coumarin-derived antibiotics are Novobiocin and Chlorobiocin. They are highly effective against *Gram*-positive bacteria by inhibiting the DNA gyrase enzyme [9, 10]. Geiparvarin is also isolated from plants containing the coumarin structure and many of its derivatives have been synthesized and exhibited high cytotoxic activity [6] (Fig. 1.2).



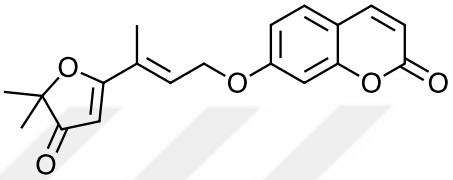
Novobiocin



Chlorobiocin



Warfarin



Geiparvarin

Figure 1.2. Coumarin derivatives used as drug.

Sandhu *et al*. reviewed in a publication published in 2014 coumarin derivatives where they classified molecules and their bioactivities according to the position from which the coumarin structure has been derivatized. The authors noticed that the molecules were mostly reported for their anticancer, anti-inflammatory, antioxidant and antiviral effects [11] (Fig. 1.3). The review also pointed out the importance of the derivatization from carbon 3 and 4 since the bioactive compounds were mainly obtained by some variations at these two positions. Yet, the derivatization from the 8th position was found to be limited and only very few references focus on the anti-inflammatory and anticancer activity of such coumarin derivatives.

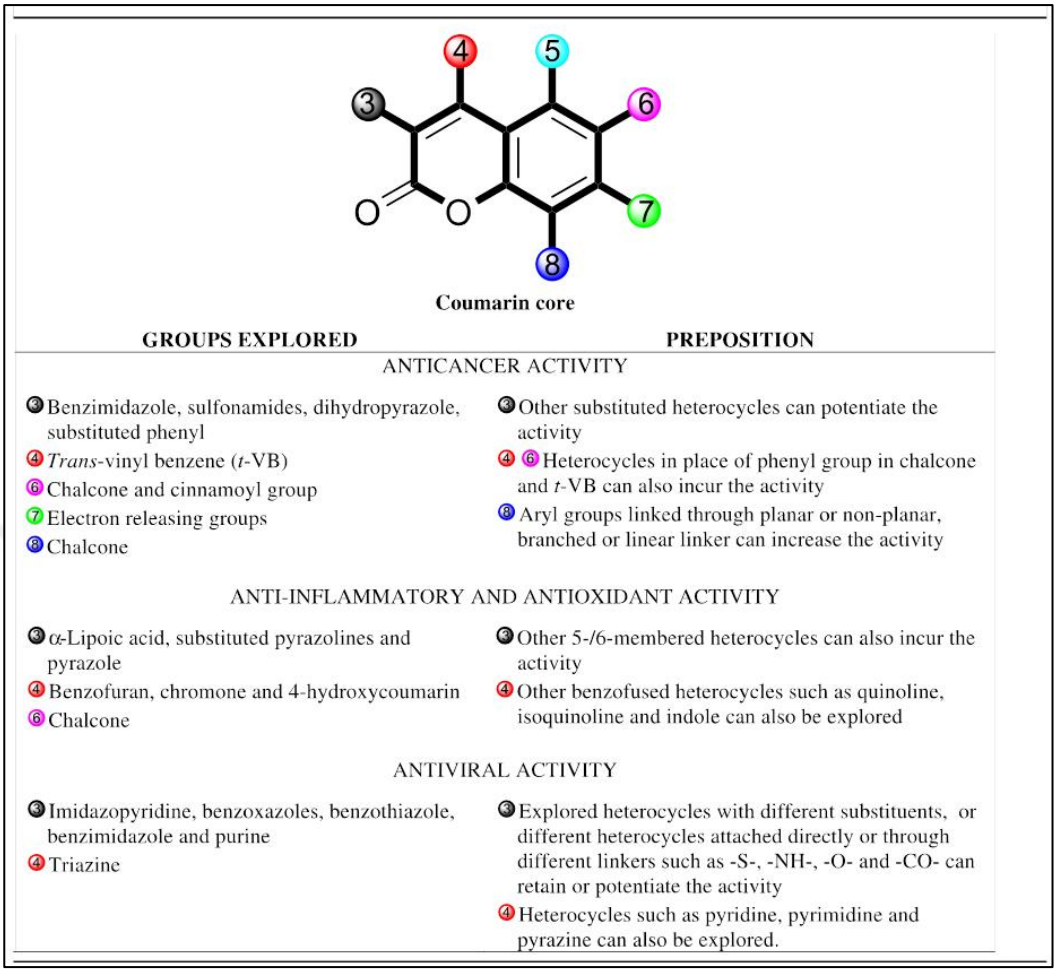


Figure 1.3. Correlation between derivatization position and biologic activity of coumarin derivatives.

One of these studies was carried out by Kontogiorgis and Hadjipavlou-Litina 2005 [5]. In this study, the 7-hydroxychromen-2-one structure was derivatized with amine groups at the 8th position and the antiinflammatory activities were investigated. The compound with the highest antiinflammatory activity has been shown to bear a piperazine at the 8th position of the coumarin scaffold (Fig. 1.4). No significant activity was observed for other synthesized amine derivatives.

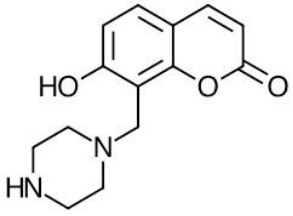


Figure 1.4. The most active coumarin compound

In 2013, Chand *et al*. developed a series of a series of 6- and 8-cinnamoylchromen-2-one to discover some new Src kinase enzyme inhibitors, Src kinase being involved in the development of metastatis. The authors investigated the anticancer activity on cancer cell lines and their results indicated that structures derivatized from the 8th position of the coumarin scaffold were less toxic than the others [12].

Jakubowski *et al*. published a study in 2017 where they isolated from *Diceratella elliptica* a coumarin molecule with a glycoside moiety at its 8th position (Fig. 1.5) that presented high cytotoxicity on hepatic HepG2, cervical HeLa, and colon HCT116 cancer cell lines [13].

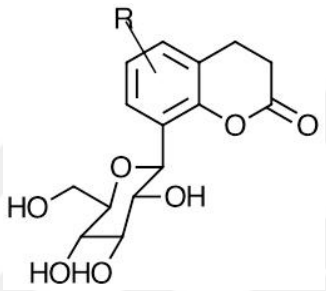


Figure 1.5. The coumarin compound isolated from Diceratella elliptica.

Since the 8th position of the coumarin ring is less studied than the other positions, and given the results published by Jakubowski et al. and Kontogiorgis and Hadjipavlou-Litina, we believe that unique and effective molecules can be developed by deriving the coumarin scaffold from this position.

In addition, in recent years, studies demonstrated that the molecular hybridization strategy in drug development that consists of combining two or more pharmacophores leads to improved bioactivity. Also an analysis of the structures of FDA-approved drugs in 2014 carried out by Vitaku *et al*. demonstrated that 72 of the investigated molecules contains a piperidine moiety and 59 have a piperazine heterocycle [14] (Fig. 1.6), structures that were already noticed for their remarkable bioactivity [15-17].

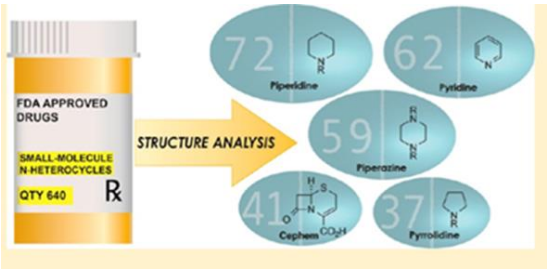
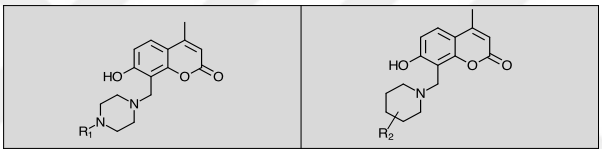


Figure 1.6. FDA approved drugs that contain nitrogen contained heterocyclic groups.

Thus, in this project, we aimed to synthesize some 8-substituted 7-hydroxy-4-methylchromen-2-one structures using aromatic and aliphatic substituted piperazines and piperidines (Table 1.1) and to furhter determine the bioactivity of the synthesized structures.

Table 1.1. Structures of targeted compounds.



R_1= 2 - 32	R_2= 33 - 50

R_1 and R_2 Groups

1	Coumarin	**26**	-4-CF_3-Ph
2	-H	**27**	-3-CF_3-Ph
3	-CH_3	**28**	-*o*-F-Ph
4	-CH_2CH_3	**29**	-*m*-Cl-Ph
5	-CH_2CH_2OH	**30**	-Benzo[1,3]dioxol
6	-CH_2CH=CH_2	**31**	-Naphthalen
7	-CH_2CH_2Ph	**32**	-Pyridinyl
8	-CH_2CH_2CN	**33**	-
9	-COPh	**34**	-4-CH_3
10	-$CH_2COOHCH_2CH_3$	**35**	-2-CH_3
11	-$COOCH_2Ph$	**36**	-3,5-$(CH_3)_2$
12	-$COOCH_2CH_3$	**37**	-2-CH_2CH_3
13	-COH	**38**	-2-$(CH_2)_2CH3$
14	-$(CH_2)_3N(CH_3)_2$	**39**	-2-$(CH_2)_2OH$
15	-Ph	**40**	-2-CH_2OH
16	-*p*-Cl-Ph	**41**	-3-CH_2OH
17	-*p*-F-Ph	**42**	-2-$COOCH_2CH_3$
18	-*p*-Br-Ph	**43**	-2-CO; 3-COOEt
19	-*p*-CH_3-Ph	**44**	-2-CO; 3-COOH
20	-*p*-NO_2-Ph	**45**	-4-COOH
21	-*o*-CN- Ph	**46**	-3-COOH
22	-*p*-OCH_3-Ph	**47**	-4-Ph
23	-*p*-Cl-Bn	**48**	-4-OH-Ph
24	-*p*-OH-Ph	**49**	-4-Bn
25	-3,4-$(Cl)_2$-Ph	**50**	-4-Benzimidazol-2-one

2. GENERAL INFORMATION

2.1. Coumarins

Coumarin is a heterocyclic compound, member of the benzopyrone chemical family. Its IUPAC name is 2*H*-1-benzopyran-2-one or chromen-2-one. In addition to coumarins, chroman and chromones belong to the same chemical family (Fig. 2.1). Basically, coumarins are composed of a fusion of a pyrone ring with a benzene molecule. Coumarins are conjugated compounds and they can be characterized with UV-spectrophotometry. In UV, their blue fluorescent is a distinct feature.

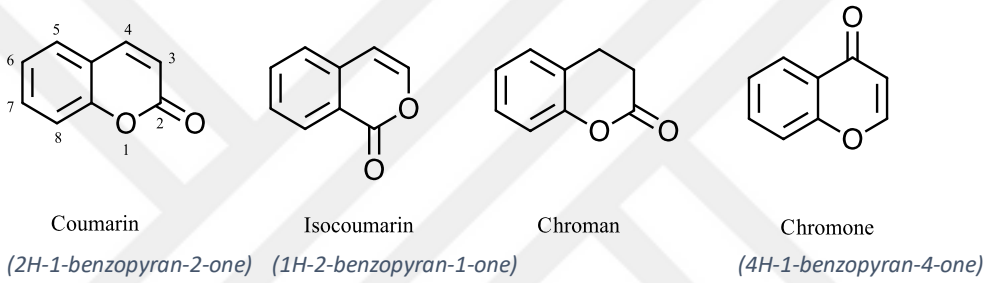


Figure 2.1. Structures of coumarins

Most of the coumarins are originated from natural sources. The first coumarin molecule was isolated from tonka bean (*Dipteryx odorata* Wild) by Vogel in 1820 [18] (Fig. 2.2). They can be found in several green plants and other organisms such as; clover leaf, microorganism [19] and animals [20, 21].

Figure 2.2. Dipteryx odorata Wild

Coumarin derivatives may have different pharmacological properties like; antimicrobial, anticancer, neuroprotective, antidepressant, anti-inflammatory, antioxidant, anticoagulant activities. Also, coumarins can be used as industrial additives in perfumes, cosmetics, and tobaccos [22, 23].

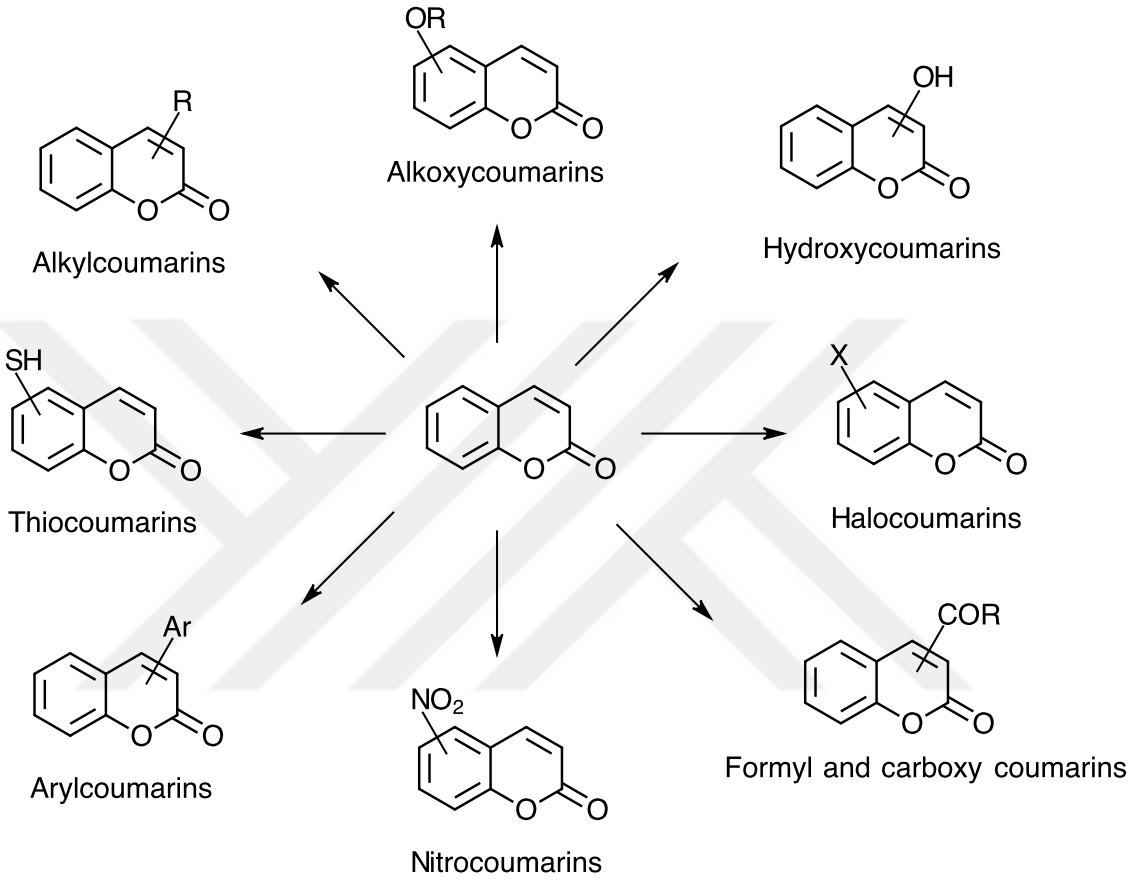
Coumarin molecules are divided into 4 subgroups according to their chemical structures [24]:

1) Simple 2*H*-chromen-2-ones
2) Furanocoumarins (2*H*-furochromen-2-ones)
3) Pyranocoumarins (2*H*-pyranochromen-2-ones)
4) Bis-and tris 2*H*-chromen-2-one

These different types of coumarins are isolated from different plants and can be obtained *via* various synthetic procedures. But, since our study is mainly focused on simple coumarins, the remaining 3 subgroups will not be further discussed.

2.1.1. Simple Coumarins: 2*H*-benzopyran-2-ones

Simple coumarins are composed of a benzene ring fused with a pyrone heterocycle, 2H- benzopyran-2-ones. This class of coumarins' general structure can be defined as hydroxylated, alkoxylated and alkylated coumarin scaffolds (Sch. 2.1).

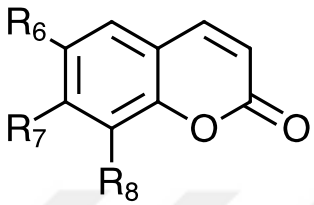


Scheme 2.1 Simple coumarins

Simple coumarins are widely spread in different plant species and can be isolated from more than 40 different plant families. About 1300 different coumarin derivatives were extracted from *Angiospermae*, *Monocotyledoneae* and *Dicotyledoneae* families. More than 100 coumarin species were isolated from *Araliales*, *Rutales*, *Asterales*, *Fabales*, *Oleales*, *Urticales*, *Thymelaeales, Apiaceae* (*Umbelliferae*), *Rutaceae*, *Asteraceae* (*Compositae*), *Fabaceae* (*Leguminosae*), *Oleaceae*, *Moraceae*, and *Thymelaeaceae* families [25]. Natural simple coumarins can be categorized according to the number of substituents: monosubstituted (Table 2.1), disubstituted (Table 2.2) and trisubstituted (Table 2.3) coumarins and so on. Plants can contain remarkable amount of coumarins in their different parts such as oil tubes of fruits, leaves, leaf tissue and seed coats. In some cases, more than one part can contain different amounts of a coumarin-

like molecule such as the *Angelica archangelica*, where seeds contain much more coumarins than fruits [26]. The amount of coumarin in a plant depends on seasonal conditions, age and environmental conditions [27]. Besides these extrinsic factors, intrinsic factors such as enzymes and genes are also crucial for the biosynthesis of coumarins.

Table 2.1. Natural monosubstituted coumarins



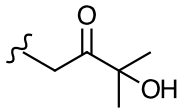
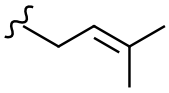
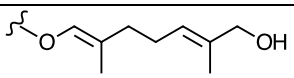
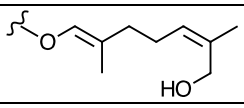
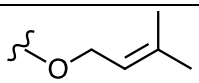
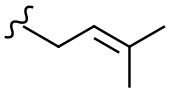
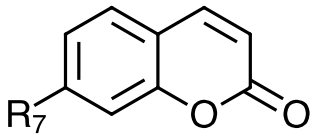
Compound	**R_6**	**R_7**	**R_8**	**Source**
Esculetin	OH	OH	H	*Alchemilla speciose* *Santolina oblongifolia* *Taraxacum formosanum*
Peucedanone		OH	H	*Angelica gigas*
Osthenol	H	OH		*Angelica koreana*
Artekeiskeanol A	OMe		H	*Artemisia keiskeana*
Artekeiskeanol B	OMe		H	*Artemisia keiskeana*
8-Methoxy-7-prenyloxycoumarin	H		OMe	*Artemisia carviforia*
Osthol	H	OMe		*Atractylodes ovata*
Scopoletin	OMe	OH	H	*Eurycoma harmandiana* *Guarea rhophalocarpa* *Xeromphis nilotica*

Table 2.2. Natural disubstituted coumarins



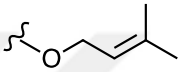
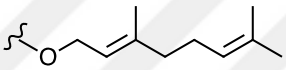
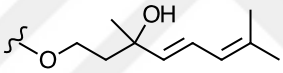
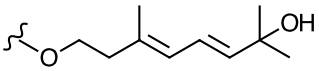
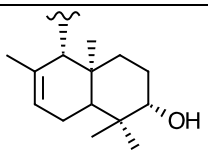
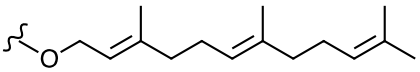
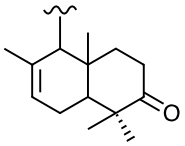
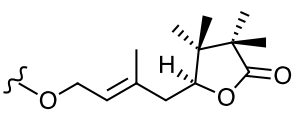
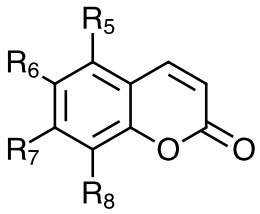
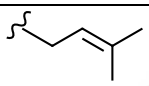
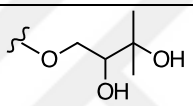
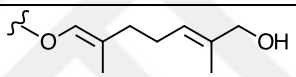
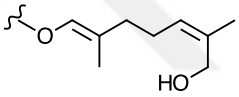
Compound	**R_7**	**Source**
Herniarine	OMe	*Artemisia dracunculus*
Ulopterol		*Baccharis pedunculata*
Aurapten		*Citrus hassaku Zanthoxylum schinifolium*
Ferulagol A		*Ferula ferulago*
Ferulagol B		*Ferula ferulago*
Feselol		*Ferula sumbul*
Umbelliprenin		*Peucedanum zenkeri*
Conferona		*Ferula sumbul*
3", 4"-Dihydrocapnolactone		*Micromelum minutum*

Table 2.3. Natural trisubstituted coumarins



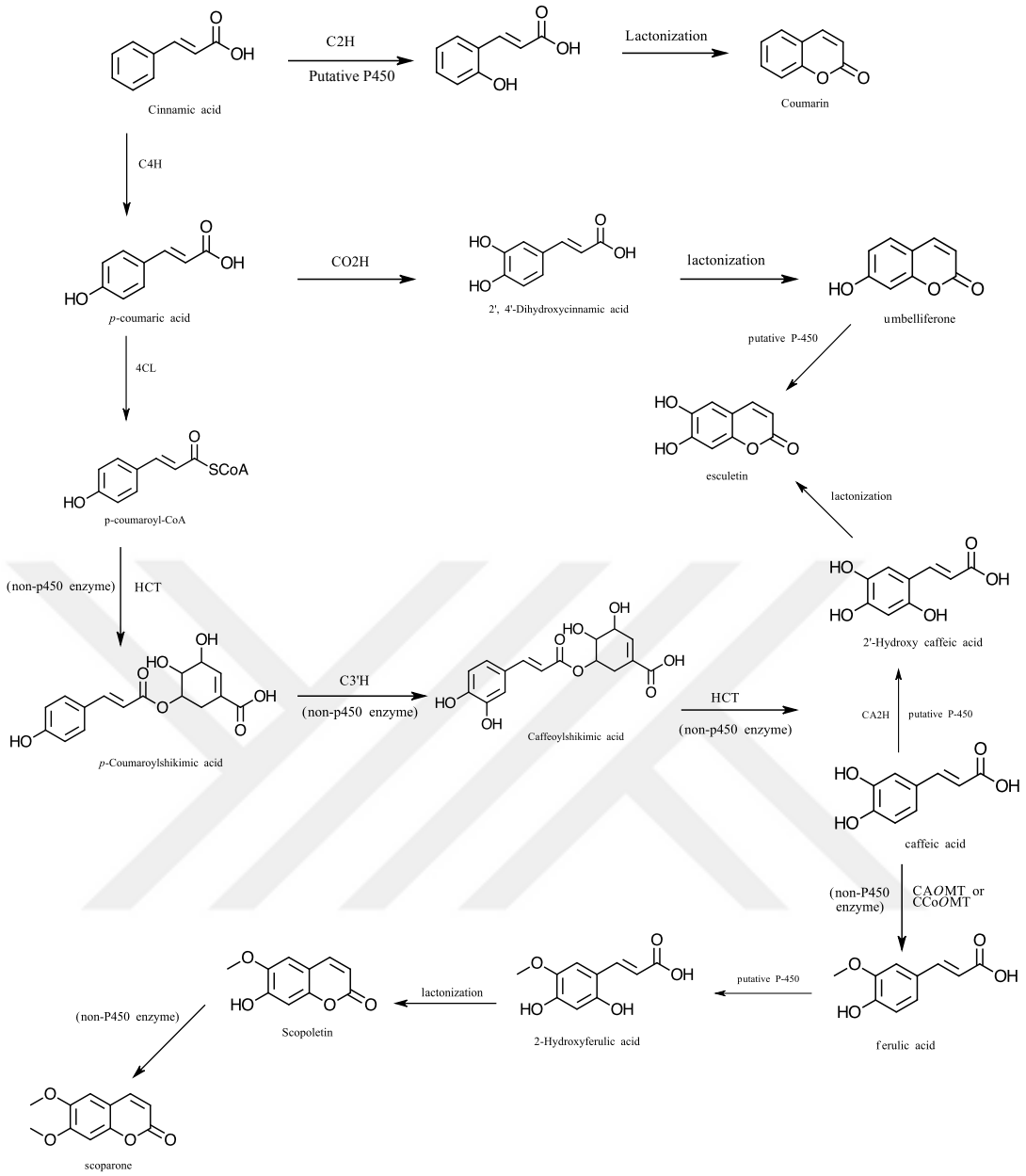
Compound	R_5	R_6	R_7	R_8	Source
Arteminin	OH	OMe	H	OMe	*Artemisia apiacea*
Isocedrelopsin Microfolicoumarin		OR R= H, Me	OMe	H	*Cedrelopsis grevei*
2', 3'-Dihydroxypuberulin	H	OMe		OMe	*Pterocaulun redolens*
Artekeiskeanol C	H	OMe		OMe	*Artemisia keiskeana*
Artekeiskeanol D	H	OMe		OMe	*Artemisia keiskeana*

2.1.2. The Biosynthesis of Simple Coumarins

2.1.2.1. Biosynthesis of Simple Coumarins in Plants

The investigation of the biosynthesis of a natural substance is a challenging and attractive area for scientists. After the isolation of coumarin, its biosynthetic pathway has been searched, and important parts were explored throughout the 60s and 70s [28]. These studies led to the discovery of the main enzymes and genes which are responsible for the biosynthesis of coumarin (Table 2.3). Coumarins are constructed by cyclization of cinnamic acid [29]. Generally, the biosynthesis of the coumarin derivatives follows the phenylpropanoid pathway [30]. In the phenylpropanoid pathway, coumarin biosynthesis starts with the ortho-hydroxylation of the cinnamic acid which is catalyzed by the P450 enzyme, also involved in the following lactonization reaction (Sch. 2.2). To investigate the location of the P-450 enzyme Gestetner *et al.* and Ranjeva *et al.* carried out studie on *Melilotus alba* and *Petunia hyrida* respectively. This study indicated that the P-450 enzyme was located in the chloroplast fraction of the plant [31, 32].

An important coumarin derivative is umbelliferone. The formation of umbelliferone starts from cinnamic acid. The *p*-hydroxylation of cinnamic acid is proceeded with cinnamate 4-hydroxylase (C4H) which is a CYP-450 type enzyme and belongs to the CYP73A family [33]. After that, the *o*-hydroxylation of coumaric acid occurres most probabaly via the CO2H enzyme. Lastly, the lactonization step is completed and umbelliferone is synthesized. Then, umbelliferone can be converted by an enzyme to esculetin and scopoletin (Sch. 2.2).

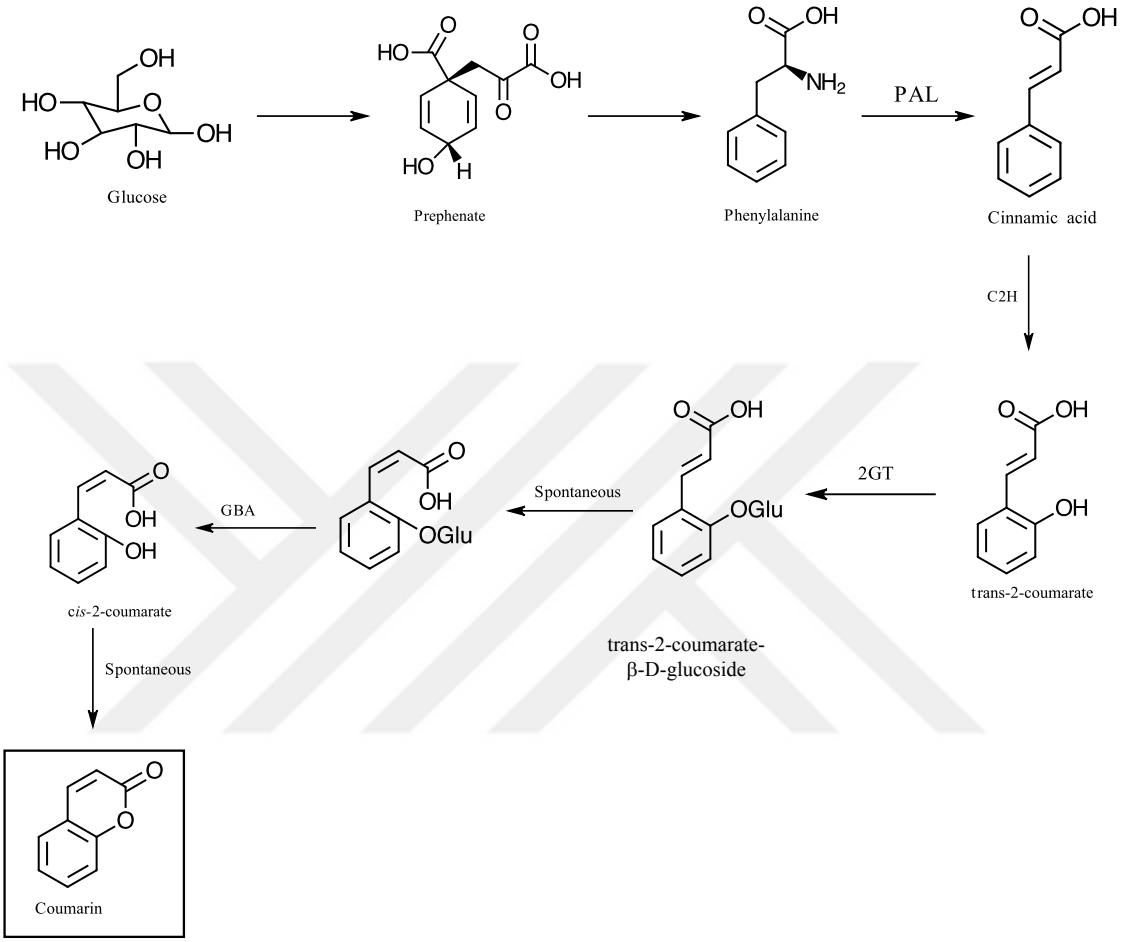


Scheme 2.2. Phenylpropanoid pathway for biosynthesis of coumarins.

C2H, cinnamic acid 2-hydroxylase; C4H cinnamic acid 4-hydroxylase; 4CL, 4-coumarate; CO2H, 4-coumaric acid 2-hydroxylase; HCT, hydroxycinnamoyl-transferase; CAOMT, caffeic acid O-methyltransferase; CCoAOMT, caffeoyl CoA O-methyltransferase; CA2H, caffeic acid 2-hydroxylase.

The other biosynthetic pathway to synthesize coumarin starts with glucose. Glucose is converted into prephenate which then gives phenylalanine. Phenylalanine is converted into cinnamic acid with PAL (phenylalanine ammonia-lyase) [34]. In the following step, C2H (cinnamate 2-hydroxylase) converts cinnamic acid to trans-2-coumarate [31]. A glucosyl group is attached to trans-2-coumarate with 2GT (2-

coumarate O-β-glucosyltransferase), before *cis-trans* isomerization. The trans isomer is spontaneously converted into the cis isomer and hydroxylation is catalyzed by GBA (β-glucosidase). In the last step, lactonization proceeds again spontaneously to give the coumarin molecule (Sch. 2.3).



Scheme 2.3. Biosynthetic pathway of coumarin in plants with starting from glucose.

Thus far, only plant-based biosynthetic pathways of coumarins were aforementioned, but plants are not the only source of coumarins. The other biosynthetic source for them is from microorganisms that synthesize coumarin derivatives in a different manner.

Table 2.4. Enzymes of coumarin and furocoumarins in their biosynthesis. Studies were performed *in vitro* or by precursor feeding assays. N.D: Not determined

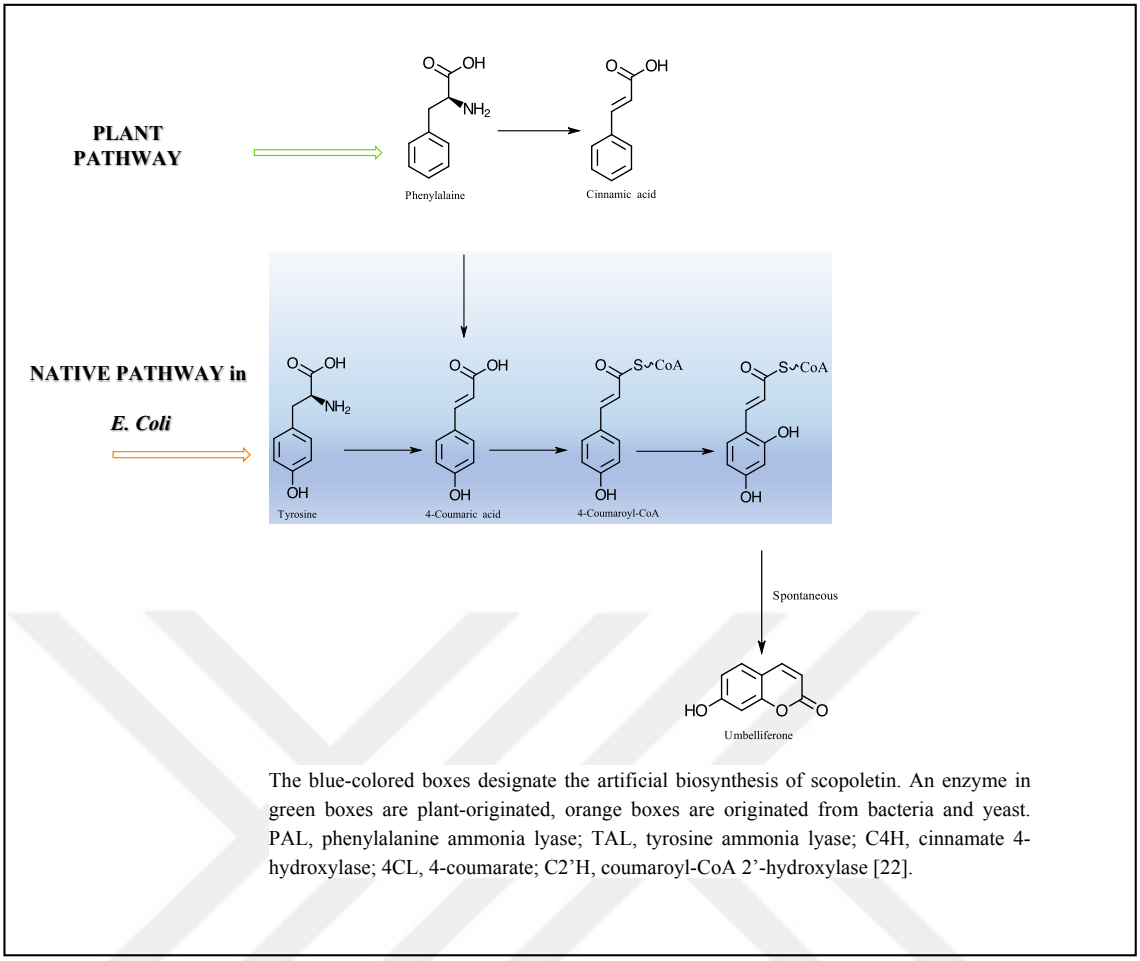
Enzyme	Substrate	Kinetic constant	Plant	Comments
Benzoic acid 2-hydroxylase	Benzoic acid	N.D.	*Nicotiana tabacum*	P450, soluble enzyme
Coumarate/Cinnamate/ Ferulate 2-hydroxylase	*p*-coumaric acid; Ferulic acid; Cinnamic acid	N.D.	*Hydrangea macrophylla*	Chloroplastic activity
Esculetin synthase	Umbelliferone	N.D.	*Cichorium intybus*	Precursor feeding studies only
Daphnetin synthase	Umbelliferone	N.D.	*Daphne mezereum*	Precursor feeding studies only
Umbelliferone 7-*O*-prenyltransferase	Umbelliferone	K_m Umbelliferone = 6.5 μM K_m DMAPP= 30 μM	*Amni majus*	P*mg* elicited cell cultures
Umbelliferone 6-*O*-prenyltransferase	Umbelliferone	N.D.	*Amni majus*	P*mg* elicited cell cultures
Umbelliferone 6-*O*-prenyltransferase	Umbelliferone	N.D.	*Ruta graveolens*	Plastidic enzyme
Marmesin synthase	Demethylsuberosin	K_m DMS= 10.3 μM K_m NADPH= 19.6 μM	Amni majus	P450, P*mg* elicited cell cultures optimal pH=7.5
Marmesin 5-hydroxylase	(+)-Marmesin	N.D.	*Ruta graveolens Ficus carica*	Precursor feeding studies only
Psoralen synthase	(+)-Marmesin	K_m NADPH= 52 μM	*Petroselinum crispum*	P450, optimal Ph=7-7.25
Angelicin synthase	(+)-Columbianetin	N.D.	*Heracleum mantegazzianum*	Precursor feeding studies only
Psoralen 5-monooxygenase	Psoralen	K_m Psoralen= 12 μM K_m NADPH= 200 μM	*Amni majus*	P450, P*mg* elicited cell cultures

2.1.2.2. Biosynthesis of Simple Coumarins in Bacteria

Coumarins' primary source are plants, but recent studies demonstrated that plants are not the only biosynthetic source for coumarins, since the biosynthesis of coumarin derivatives was shown to also be achieved by microorganisms sush as bacteria. This finding led to the development of artificial biosynthetic pathways where bacterial cells are manipulated and managed to produce efficiently coumarin molecules.

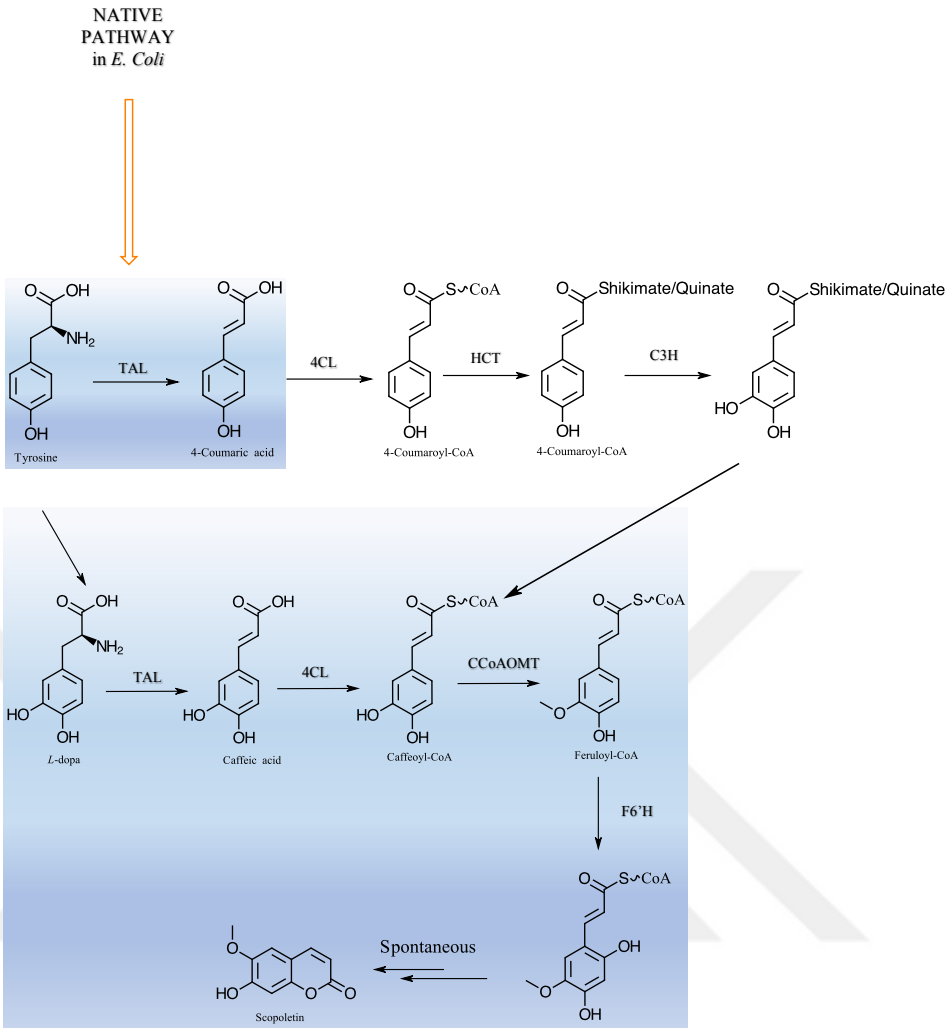
The pehylpropanoid pathway that involves the C2H, 2GT, and GBA enzymes cannot be used for such a purpose since the genes encoding for these enzymes have not been identified yet [35].

However, the Fe (II)- and 2-oxoglutarate-dependent dioxygenase (2OGD), feruloyl-CoA 6′-hydroxylase (F6′H1) isolated from *Arabidopsis thaliana* in 2008 by Kai *et al*. [36] and its homologs found in sweet potato and rue were shown to convert feruloyl-CoA into its *o*-hydroxylated derivative, a precursor of umbelliferone (Sch. 2.4) [37, 38]. This pathway has been adapted by Lin *et al*. into a novel biosynthetic pathway that microorganisms such as bacteria and yeast for the preparation of coumarin derivatives. In fact, in this pathway the F6′H1enzyme which was isolated from *Arabidopsis thaliana*, hydroxylated feruloyl-CoA specifically and produced 6′-hydroxyferuloyl-CoA and a sponataneous isomerization and lactonization process led to the synthesis of a simple coumarin, scopoletin. (Sch. 2.5) [35].



The blue-colored boxes designate the artificial biosynthesis of scopoletin. An enzyme in green boxes are plant-originated, orange boxes are originated from bacteria and yeast. PAL, phenylalanine ammonia lyase; TAL, tyrosine ammonia lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate; C2'H, coumaroyl-CoA 2'-hydroxylase [22].

Scheme 2.4. The proposed artificial biosynthetic pathway of Umbelliferone.



The blue-colored boxes designate the artificial biosynthesis of scopoletin. An enzyme in green boxes are plant-originated, orange boxes are originated from bacteria and yeast. PAL, phenylalanine ammonia lyase; TAL, tyrosine ammonia lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate; CoA ligase; C2'H, coumaroyl-CoA 2'-hydroxylase; HCT, hydroxycinnamoyl-CoA: shikimate/quinate hydroxycinnamoyltransferase; C3H, coumarate 3-hydroxylase; CCoAOMT, caffeoyl-CoA *O*-methyltransferase; F6'H, Feruloyl-CoA 6'-hydroxylase [22].

Scheme 2.5. The proposed artificial biosynthesis pathway of Scopoletin

Even if efficient biosynthetic pathways have been developed to get coumarin derivatives, these are not adapted for drug development as they allow neither high amount synthesis nor structure diversification. Therefore, several synthetic pathways have been developed to synthesize efficiently diverse coumarin derivatives in high amounts.

2.1.3. Synthetic Pathways for Simple Coumarins

A lot of coumarin derivatives have been isolated from the natural sources. Yet, to investigate their properties for drug development, it was necessary to develop more efficient ways to prepare them., The first synthetic coumarin was obtained in 1868 by the English chemist William Henry Perkin [39, 40] from o-hydroxybenzaldehyde and acetic acid. To date, several synthetic routes have been discovered to synthesize coumarin derivatives. These include *Perkin reaction*, *Pechmann condensation*, *Knoevengel reaction, Wittig reaction, Kostanecki-Robinson reaction, Reformatsky* reaction and also some other miscellaneous reactions.

2.1.3.1. The Perkin Reaction

The Perkin reaction was discovered by William Henry Perkin in 1868. In this reaction, cinnamic acid derivatives are synthesized from aromatic aldehydes and aliphatic carboxylic acid anhydrates or carboxylic derivatives in the presence of catalyst. Bases such as tertiary amines and pyridine are suitable catalysts for the Perkin reaction (Fig. 2.3).

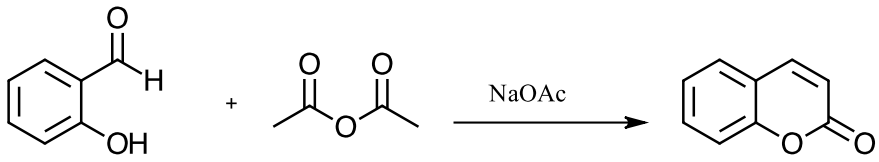
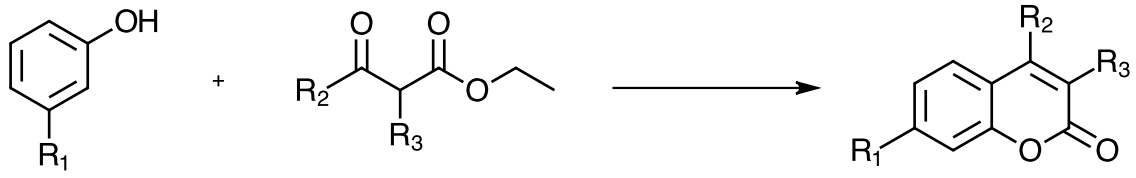


Figure 2.3. *Perkin reaction*

2.1.3.2. Pechmann Reaction

One of the most useful methods for the synthesis of coumarins is the *Pechmann condensation reaction*. In this reaction phenols and β-keto esters are used (Fig. 2.4). The reaction is catalyzed by strong Bronsted acids such as $AlCl_3$ which is used to catalyze

transesterification and the keto-enol tautomerization. Then, the coumarin skeleton is formed by a *Michael addition* and this causes the rearomatisation. When acetoacetic esters and their derivatives are used, this reaction is called a *Pechmann-Duisberg reaction* [6].



Mechanism

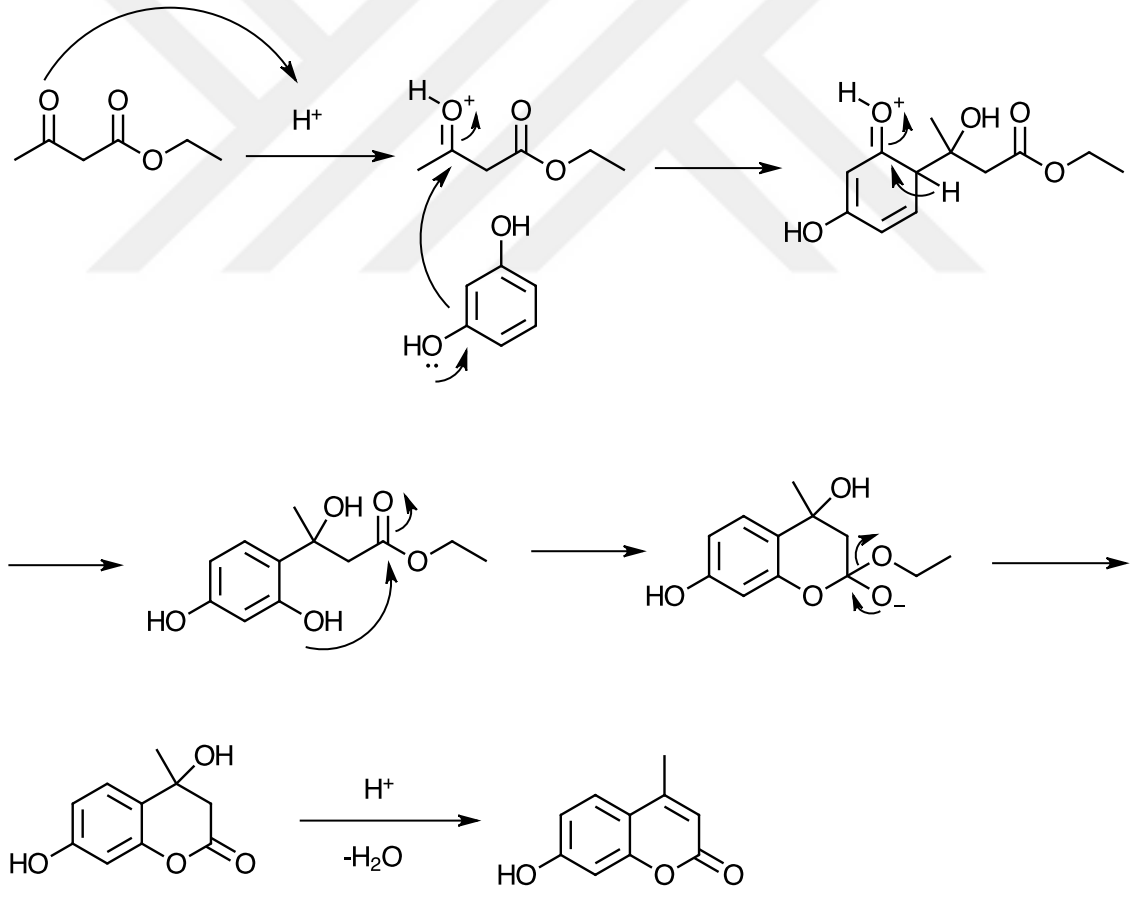


Figure 2.4. *Pechmann condensation reaction* and mechanism

To realize this reaction under mild conditions, more activated phenols like resorcinol should be used. Mild conditions are very useful for the synthesis of derivatives of umbelliferone with high yield (Fig. 2.5).

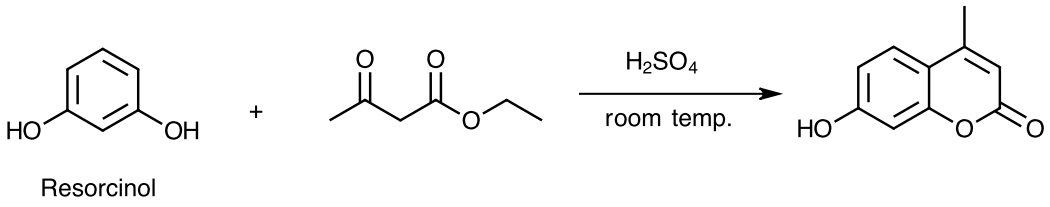


Figure 2.5. *Pechmann reaction* with activated phenols and H_2SO_4 as a catalyst.

To improve the yield and decrease the duration of reactions, coumarin synthesis can be proceeded with the different catalysts, such as samarium (III) nitrates hexahydrate ($Sm(NO_3)_3.6H_2O$) (Fig. 2.6) [41] or titanium (IV) chloride ($TiCl_4$) (Fig. 2.7) [42]. An advantage of $TiCl_4$ is that the reaction can occur in a solvent-free medium: the reaction does not need a solvent to process, therefore is compatible with green chemistry requirements.

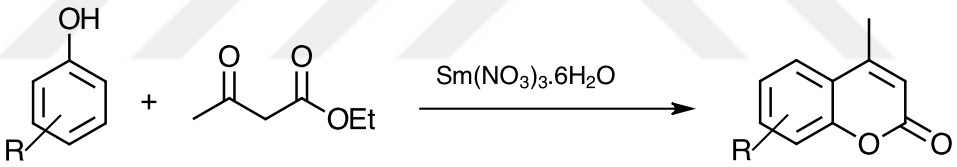


Figure 2.6. *Pechmann condensation* with catalyst Sm(NO3)3.6H2O.

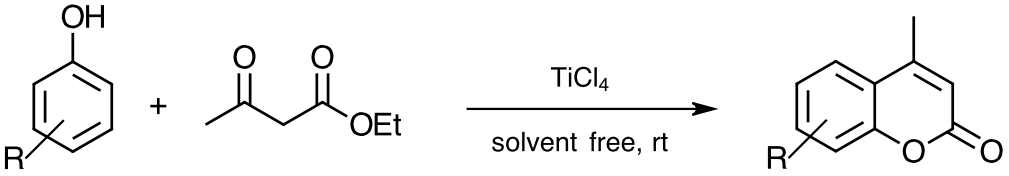


Figure 2.7. *Pechmann condensation* with catalyst $TiCl_4$.

Another solvent free Pechmann condensation method was developed with microwave supported one-pot coumarin synthesis. This reaction is also conducted in solvent-free medium with the green-friendly catalyst FeF_3 (Fig. 2.8) [43].

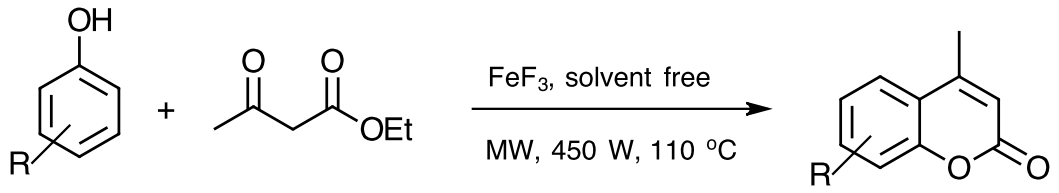


Figure 2.8. *Pechmann condensation* with catalyst FeF_3.

To get a better result with the Pechmann reaction, in addition to metal catalysts the Montmorillonite clay can also be used as a catalyst. The Montmorillonite clay is a clay catalyst that is used as a condensing agent to improve the yield of Pechmann reaction. This catalyst is impressive for the synthesis of 7-hydroxy-4-alkylcoumarins [44]. Other effective catalysts for the synthesis of the 7-hydroxy-4-alkylcoumarins are Nafion resin/silica nanocomposites and amberlyst 15 [45].

2.1.3.3. Knoevenagel Reaction

The Knoevenagel reaction is an organic synthesis reaction that was found by Emil Knoevenagel in the 1890's [46] (Fig. 2.9). The Knoevenagel reaction is a different type of aldol condensation reaction and C-C bond formation is a benefit of this reaction. It is used to get substituted olefins from an aldehyde or a ketone and active methylene group, the reaction being catalyzed by a basic catalyst, such as ammonia or amines. The goal of the catalyst is the deprotonation of the active C-H group. After that, a spontaneous dehydration occurs and leads to the unsaturated compound. Then, a condensation step occurs and the coumarin scaffold is synthesized.

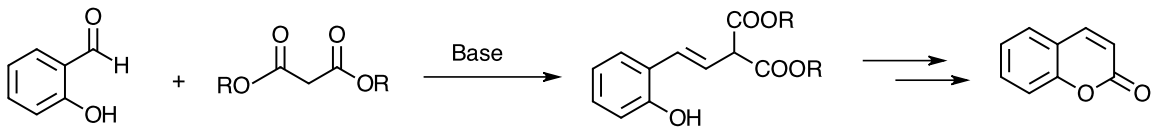


Figure 2.9. *Knoevenagel reaction*

The Knoevenagel reaction is one of the most valuable methods for the synthesis of different types of coumarins, such as coumarin-3-carboxylic acids, amino- and alkylaminocoumarins, 3-acetylcoumarins, 3-benzoylcoumarins crown ethers and so on [47, 48]. To develop better reaction conditions and improve the reaction yield, novel methods have been investigated. Using metal catalysts in the Knoevenagel reaction is one of the improvement for this purpose. For example, isofraxidin (7-hydroxy-6,8-dimethoxycoumarin) is a biologically active coumarin derivative that was synthesized using ZnO as a catalyst with Meldrum's acid. ZnO was used to get the olefinic Knoevenagel type compound. After condensation the 3- carboxylic acid substituted coumarin was observed, and Cu was used to remove the carboxylic acid group to get isofraxidin [49] (Fig. 2.10).

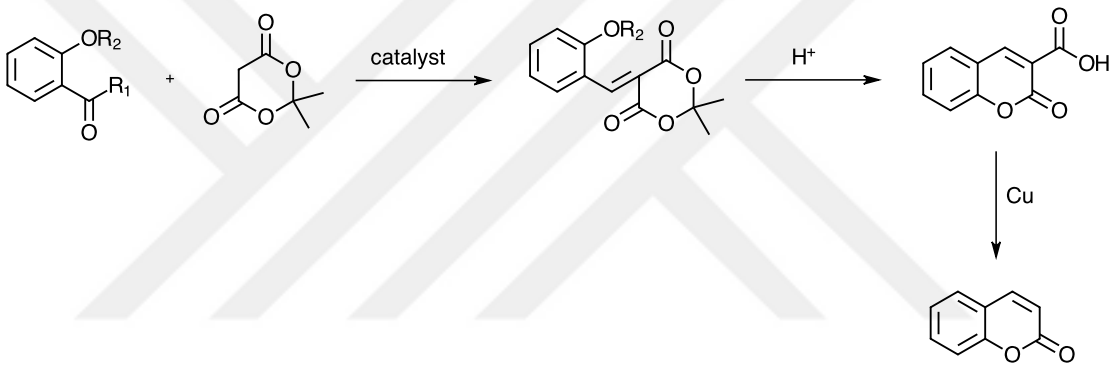


Figure 2.10. Synthesis of isofraxidin

The synthesis of coumarin-3-carboxylic acid derivatives is another way to derivatize the coumarins. To synthesize this type of coumarins, heterogeneous catalysts, like zeolites and clays, can be used [50]. Additionally, a solid phase synthesis is also used for the synthesis of substituted coumarin-3-carboxylic acids. Solid phase synthesis method was found by Knoevenagel in 1898, and malonic acid and *o*-hydroxyarylaldehydes were used for the preparation of coumarin scaffolds with mild conditions and high purity. According to the investigation done by Watson *et al.*, ethyl malonate bound to the Wang resin and *o*-hydroxyarylaldehydes were used for the synthesis of substituted coumarin-3-carboxylic acids (Fig. 2.11) [51].

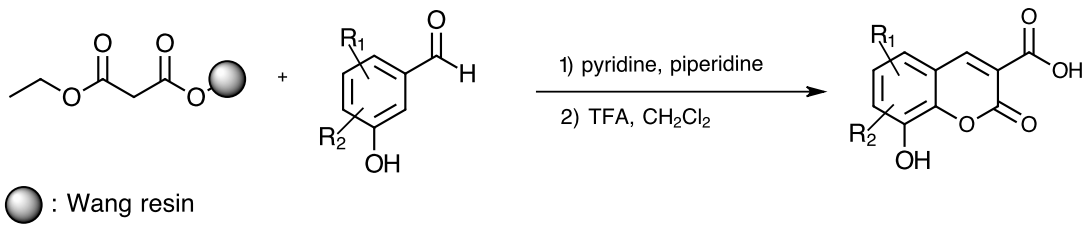


Figure 2.11. *The Knoevenagel synthesis* with Wang resin.

2.1.3.4. The Wittig Reaction

The Wittig reaction is a useful method to convert some primary and secondary alkyl halides and an aldehyde or ketone to get an olefin in the presence of triphenylphosphine and base (Fig. 2.12). In this reaction, first a phosphonium ylide and then zwitterion are obtained. Then, some rearrangements occur and the olefinic product is observed and finally to obtain the coumarin cycle, a final lactonization occurs. When a phosphine oxide carbanion or a phosphate carbanion are used instead of the ylide, the reaction is called Horner or Horner-Emmons-Wadsworth respectively.

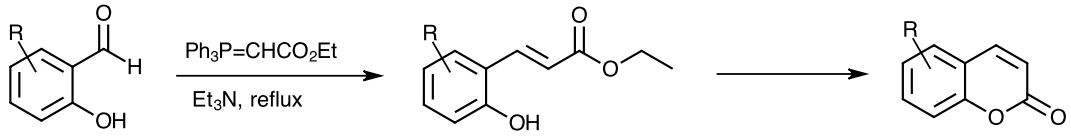


Figure 2.12. Coumarin synthesis *via the Wittig reaction*

While synthesizing coumarin derivatives *via the Wittig reaction*, different type of ylides have been used. In 2009, Upadhyay and *et al.* developed a novel one-pot intramolecular cyclization method using a triphenyl (α-carboxymethylene) phosphorene imidazolide as a ylide (Fig. 2.13). So, they found an efficient synthetic pathway to prepare several coumarins [52].

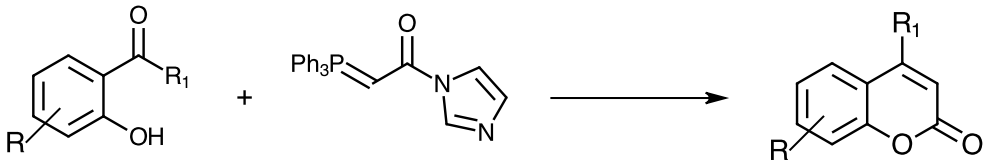


Figure 2.13. *Wittig reaction* with different ylides.

Another one-pot coumarin synthesis with *the Wittig reaction* is conducted with three components. The process contains bromination, the Wittig reaction, and cyclization parts. By using this method 3-bromocoumarin derivatives can be synthesized easily and efficiently (Fig. 2.14) [53].

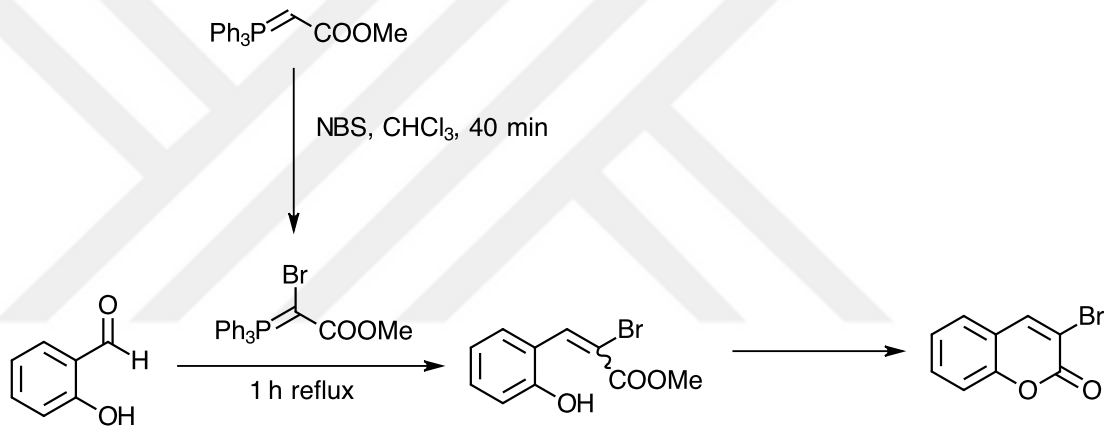


Figure 2.14. One-pot synthesis of 3-bromocoumarin.

2.1.3.5. Reformatsky Reaction

The conventional method of *the Reformatsky reaction* was found in 1887. The reaction uses Zn metal as a catalyst which catalyzes the formation of β-hydroxyalkanoates from ethyl α-haloacetates with aldehydes or ketones [54]. Although Zn is generally used for this reaction, different types of metal catalysts such as; chromium, samarium, and indium have also been used for the Reformatsky reaction (Fig. 2.15).

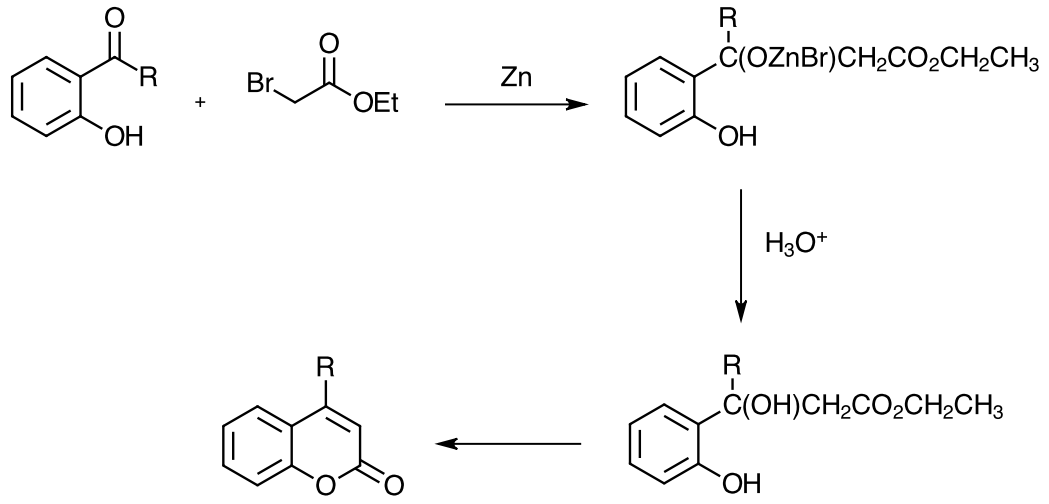


Figure 2.15. *Reformatsky reaction*

Indium promoted reactions differ from other metal-catalyzed Reformatsky reactions as they can proceed in aqueous medium, because hydroxyl groups do not reduce the organoindium reagent [55] and a chemoselective reaction occurs when addition to the aldehyde occurs [56].

2.1.3.5. Kostanecki-Robinson Reaction

The Kostanecki-Robinson reaction consists of synthesizing 3- and 4- substituted coumarins, by acylating *o*-hydroxyaryl ketones with aliphatic acid anhydrides (Fig. 2.16).

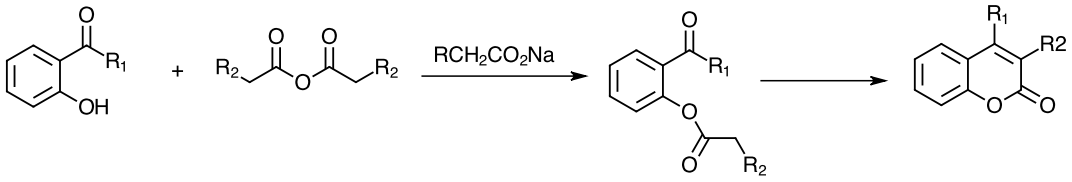


Figure 2.16. *Kostanecki-Robinson reaction*

Although this reaction is useful for the synthesis of 4-arylcoumarins derivatives, it has some limitations as it requires high amount of chemicals, hard reaction conditions, and provides poor yields. Therefore, Hwang *et al.* developed a novel reaction pathway that involves the Kostanecki condensation in the presence of DBU as a base. The advantage of this reaction is that it allows the synthesis of highly functionalized 4-arylcoumarins and one-pot synthesis of the 3,4-disubstituted coumarins.

2.1.3.6. Miscellaneous

Metal-catalyzed synthesis is an alternative synthetic method for synthesizing coumarins. Stereo- and regioselective synthesis of coumarins can be accomplished with palladium catalysts. This type of reactions are intramolecular reactions in which aryl halides and alkynes are used as starting materials (Fig. 2.17) [57].

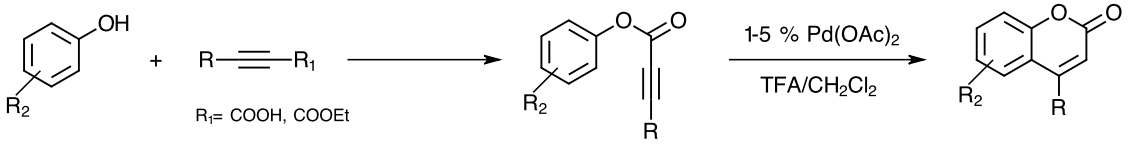


Figure 2.17. Synthesis of coumarins with palladium catalyst.

Another palladium-catalyzed coumarin synthesis can occur between aryl halides and alkenes through *the Heck coupling reaction*. The reaction has been described for 2-bromophenols and cinnamic acid esters to obtain 4-phenylcoumarin derivatives (Fig. 2.18).

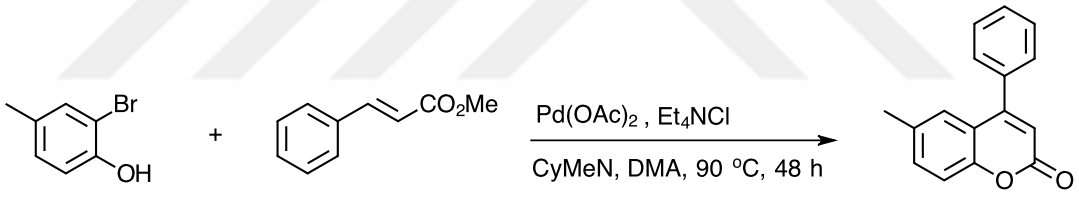


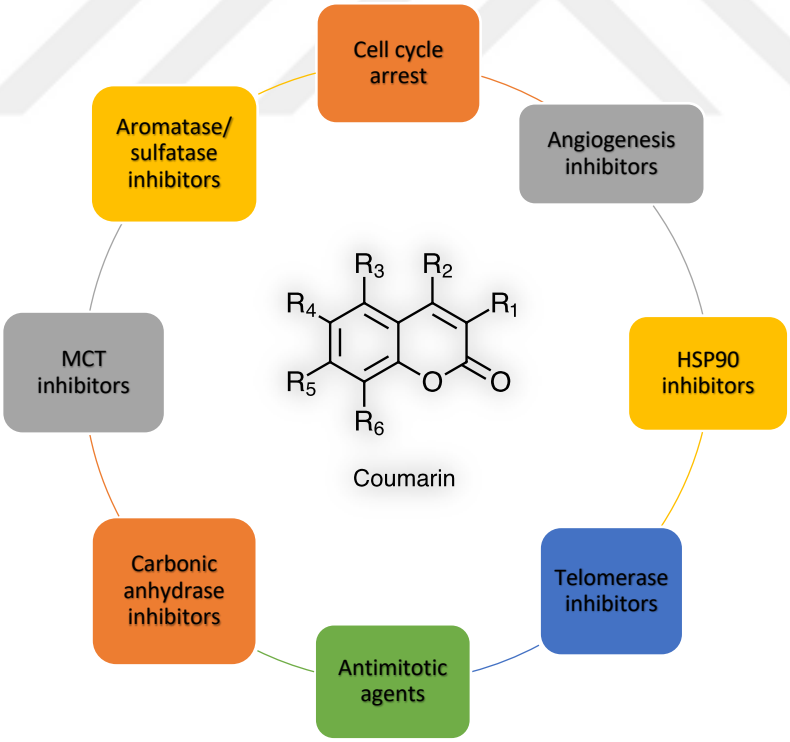
Figure 2.18. *The Heck coupling reaction*

2.1.4. Biological Activities of Simple Coumarins

Coumarins have been attractive molecules for the scientists, because of their broad range of biological activities. Prominent pharmacological activities are anticancer [58], anti-inflammatory [59, 60], antimicrobial, antiviral, antioxidant [61, 62], anticoagulant [63-65], enzyme inhibition [66] and cental nervous system [67] activities. In this thesis, only the anticancer and anti-inflammatory activities will be developed.

2.1.4.1. Anticancer Activity

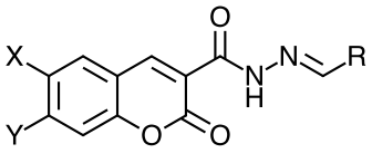
Cancer is termed for a broad range of desperate diseases in which cells proliferate abnormally and spread over other tissues without control. According to the World Health Organization (WHO), the primary reason of death is cancer in the world, and it is estimated that the number of cancer case will increase more than predicted in the next decade [68]. Different cancer treatment strategies have been investigated but chemotherapy remains the most useful treatment method for the moment. Therefore, the development of cytotoxic agents is still crucial. In this regard, coumarin derivatives are promising cytotoxic agents for cancer treatment and their anticancer bioactivity can be attributed to kinase inhibition, cell cycle arrest, angiogenesis inhibition, heat shock protein (HSP90) inhibition, telomerase inhibition, antimitotic activity, carbonic anhydrase inhibition, mono-carboxylate transporters inhibition, aromatase inhibition and sulfatase inhibition [69-71] (Sch. 2.6).



Scheme 2.6. Mechanism of action of coumarin derivatives as anticancer agent.

2.1.4.1.1. Kinase Inhibitors

Protein kinases are proteins that are responsible for the addition of a phosphate group from ATP to a protein, a process called phosphorylation. Kinases are responsible for various cell functions such as; cell signaling, cell division, cell growth, cell proliferation. So, activated forms of kinases prevent from apoptosis, promote angiogenesis in a lots of cancer types. Beside this, phosphorylation is also involved in the inflammatory processes. Therefore, inhibition of kinases is important for preventing from cancer and some other diseases and many coumarin derivatives were described to be kinase inhibitors. In 2014, Nasr *et al.* studied on coumarin compounds that showed activity against resistant pancreatic cells and Hep-G2 and CCRF which are drug fragile cells. They synthesized hydrazine-hydrozone substituted coumarin derivatives (Fig. 2.19). According to their results, neither coumarin nor hydrazine-hydrazone alone showed good biological activity, but hydrazine-hydrozone substituted coumarin derivatives demonstrated even better activity then the reference doxorubicin. To discover the action mechanism of these coumarin derivatives a caspase 3/7 assay was applied and it was observed that caspase 3/7 was activated by compounds **72.1** – **72.4** and these compounds were ultimately shown to induce apoptosis. Then the molecular targets were found *via* a microarray analysis. Compound **72.1** was described to exhibit its anticancer activity by inhibiting the cyclin-dependent kinase 1A (CDKN1A) and the cyclin-dependent kinase 2 (CDK2) [72]. Based on their SAR study, the authors noticed that coumarine hydrazine-hydrazone scaffold was essential for anticancer activity as previously mentioned. Concerning the R substituents; 2-pyrryl or 2-thienyl substituted coumarin hydrazine-hydrazone derivatives demonstrated the highest anticancer activity. The most potent compound was observed, when the X group was a bromine, the selectivity being attributed to the electronegativity of Br. For the Y substituent, it was shown tha this position does not have a high impact on the activity so, H atoms were used for this position.



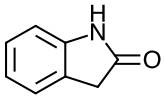
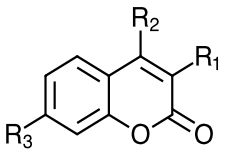
Compound	R	X	Y	IC_{50} (μM)		
				Hep-G2	Panc-1	CCRF
72.1		Br	H	3.6	6.5	5.15
72.2		Br	H	31.14	2.02	11.73
72.3		Br	H	4.16	12.61	9.02
72.4		Br	H	16.10	2.15	11.29

Figure 2.19. SAR study of hydrazine-hydrazone derivatives of coumarin [72].

Src kinases are found to be overexpressed in the several human tumors such as; colon, ovary, breast, lung, prostate, pancreas tumor cells [73, 74]. In 2011, Kathuria *et al*. developed six different series of anticancer molecules and investigated their Src kinase inhibitory activity [75]. In their study, they synthesized 3-alkyl-4-methyl-coumarins, pyranocoumarins, coumarin carboxamides, quaternary ammonium coumarins, 7-aminocoumarins and 4-aminocoumarins but observed a weak correlation between the antiproliferative activity and the Src kinase inhibitory activity. Synthesized compounds **75.5** and **75.6** with hexyl and decyl groups respectively showed poor antiproliferative activity while demonstrating high Src kinase inhibitory activity (Fig. 2.20).



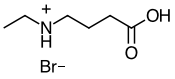
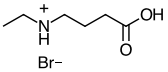
Compound	R_1	R_2	R_3	IC_{50} (μM)
75.5	C_6H_{13}	CH_3		36.5
75.6	$C_{10}H_{21}$	CH_3		21.6
75.7	$C_{10}H_{21}$	CH_3	NH_2	73.5
75.8	H	CH_3	NH_2	30.9

Figure 2.20. Quaternary ammonium derivatives and their SAR studies.

In 2012 Kini *et al*. investigated the anticancer property of coumarin derivatives. They targeted tyrosine kinase receptors and then, a docking study was performed. According to the docking study results, they synthesized benzothiazole substituted coumarin derivatives. Among all, compound **76.11** had the highest anticancer activity (Fig. 2.21). For this compound, the highest activity was observed when allyloxy methylene substituents were at 6 and 7 positions of the coumarin the activity being attributed to the hydrophobic property of these groups that was thaught to allow a better interaction with the receptor. The benzothiazole substituent was also shown to be important for binding the receptor, as responsible for binding the ATP-binding part of protein kinase [76].

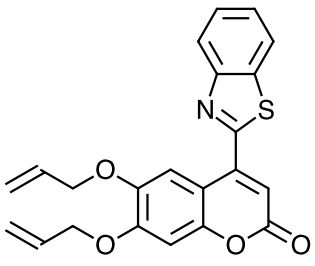
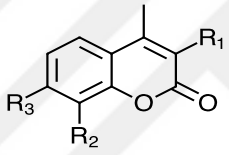
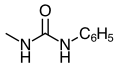
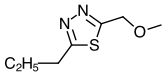
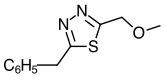
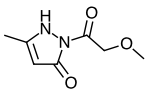


Figure 2.21. Compound **76.11**, benzothiazole derivative of coumarin.

In 2014, a docking study of coumarin derivatives was performed by El-Ansary *et al*. According to their results, fourteen novel coumarin derivatives, substituted-1*H*-benzopyran-2-ones, substituted amino-5*H*-pyrano[6,5-e]benzoxazol-5-ones and substituted-2,6-dihydropyrano[6,5-f]-1,4- benzoxazin-6-ones were synthesized. Synthesized compounds were tested on CCR-CEM, HL-60, HOP-92, NCI-H460, HCT-116 and SF-295 cancer cell lines. Results showed that a coumarin scaffold with a dihydropyrazole ring or thiadiazole ring shows promising anticancer activity (Fig. 2.22). After finding an anticancer activity, the target was identified. Docked compounds were shown to have similar binding properties with an inhibitor of CK2 (casein kinase II), which led to the hypothesis that these compounds may be CK2 inhibitors. Binding modes of compounds **77.11** and **77.12** are shown below and H-binding modes with CK2 were observed (Fig. 2.23) [77].



Compound	R_1	R_2	R_3
77.9	CH_3		OH
77.10	CH_3	H	
77.11	CH_3	H	
77.12	CH_3	H	

Test compound and growth inhibition %				
Cell lines	**77.9**	**77.10**	**77.11**	**77.12**
CCR-CEM	34.32	13.42	27.41	27.69
HL-60	41.78	18.20	19.42	28.91
HOP-92	38.58	28.67	NR	47.63
NCI-H460	27.42	NR	10.68	10.43
HCT-116	26.99	14.14	21.17	18.83
SF-295	45.07	27.34	50.46	39.73

Figure 2.22. Dihydropyrazole and thiadiazole substituted coumarins and their activity results.

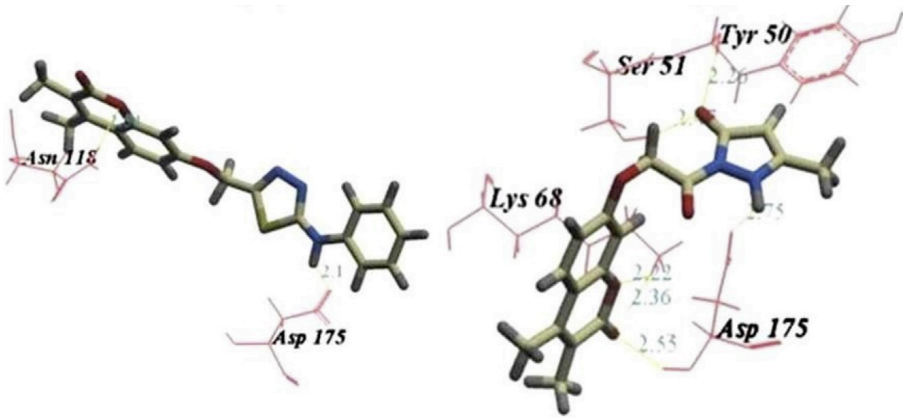
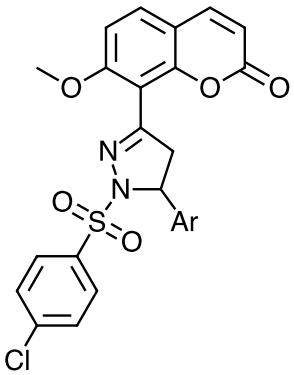


Figure 2.23. Binding modes of compound **77.11** and **77.12**.

Another study was focused on coumarin-pyrazoline structures. In 2013, Amin *et al.* synthesized coumarin-pyrazoline compounds substituted with phenylsulfonyl groups and a terminal sulfamoyl group. These compounds were tested for their anticancer activity on sixty different cancer cell lines. According to the results, compound **78.13** demonstrated the highest activity (Fig. 2.24). Compound **78.13** was substituted with a chlorophenyl sulfonyl at its first position, a phenyl moiety at its 5th position and a pyrazoline ring at its 8th position. This compound had high anticancer activity, especially against breast cancer (MCF) and colon cancer (HCT-116) and some PI3K protein kinase inhibitory activity [78].

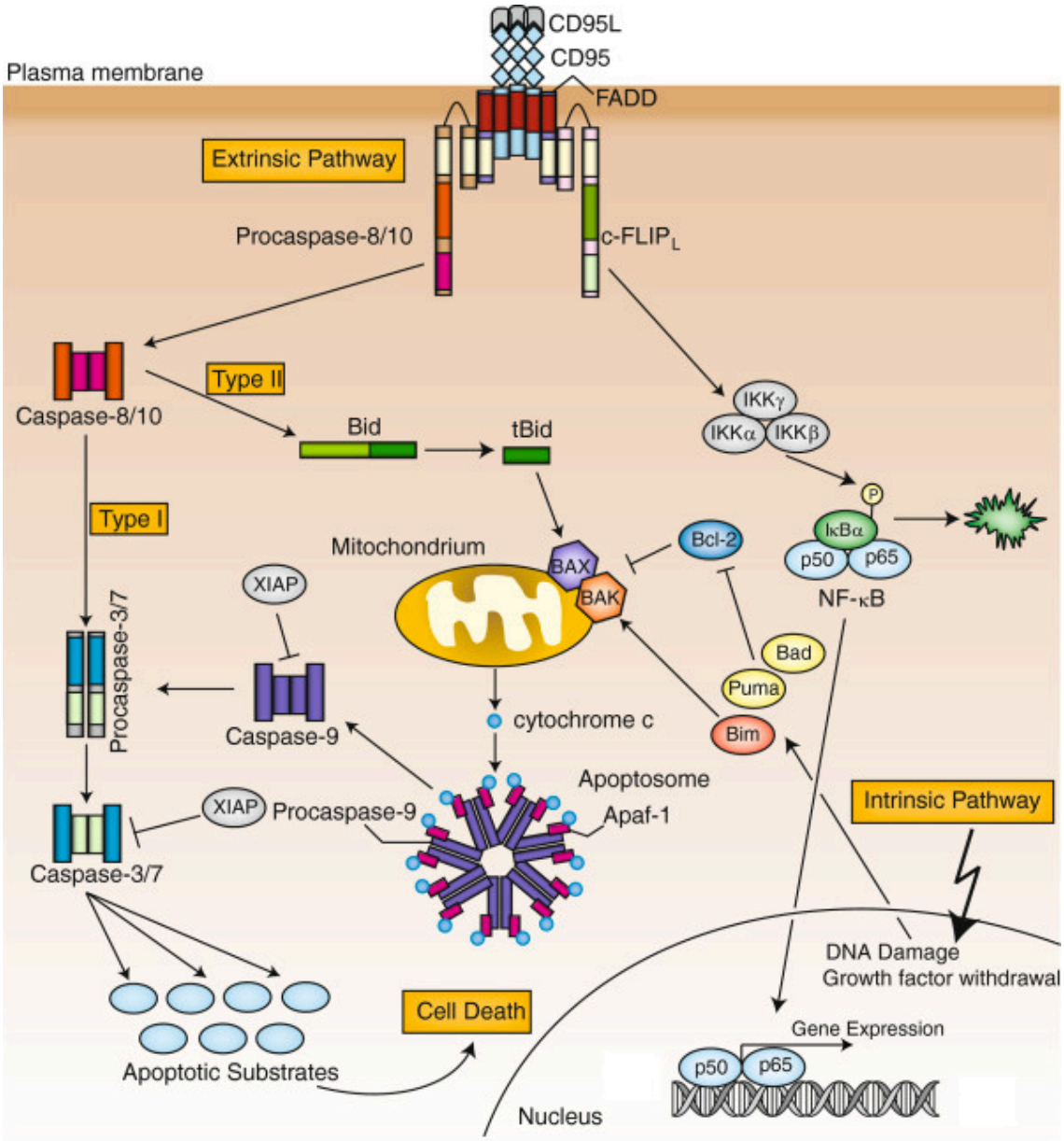


Compounds	Ar	R_1	IC_{50} (μM)
Doxorubicin	-	-	0.63
78.13	C_6H_6	Cl	0.01

Figure 2.24.Pyrazoline substituted coumarin and its activity result.

2.1.4.1.2. Cell Cycle Arrest

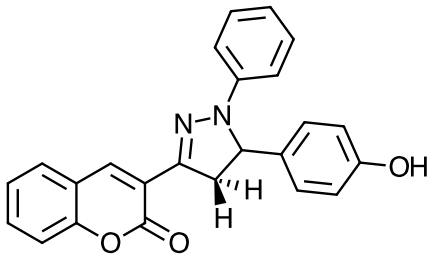
Apoptosis is a programmed cell death, which is essential for all cell types. It involves the development of multicellular organisms. This natural death mechanism is also used to treat different cancer types. So, inducing apoptosis is a powerful treatment method for cancers. For this purpose, several types of molecules have been developed to induce apoptosis. One of the promising compound family is coumarins. Coumarins are capable of stopping the cell cycle at the G0, G1, S or M phases. This cell cycle arrest induces apoptosis [79]. Coumarins induce apoptosis by a caspase-dependent manner and by changing the amounts of the Bcl-2 protein [70]. The release of cytochrome c from mitochondria decreases metalloproteinase amounts and activates the initiator of caspase-9, which further activivates caspases-3/7 to finally lead to cell death (Sch. 2.7).



Scheme 2.7. Apoptosis cascade

3-(4,5-Dihydro-1-phenyl-5-substituted phenyl-1*H* pyrazol-3-yl)-2*H*-chromen-2-one derivatives were synthesized by Kumar *et al* in 2013. The anticancer activity of these compounds was investigated and results showed that these compounds had anticancer activity against sixty different cancer cell lines. After that, they found the mechanism of action of these molecules. Molecules showed anticancer activity by inducing the arrest at the G1 phase. To understand the impact of coumarin scaffold on the anticancer activity, hydrolysis of the coumarin derivatives was prevented, until they reached to their target tissue. Then, only conserved coumarin derivatives induced cell cycle arrest. This means that anticancer activity basically depended on coumarin scaffold. Among all synthesized

compounds, compound **80.14** showed impressive anticancer activity against breast cancer, renal cancer and non-small cell lung cancer cell lines (Fig. 2.25) [80].



Compound	Mean growth %	Range of growth %	Most sensitive cell line	Growth of most sensitive cell lines
80.14	77.59	-68.37 to 78.46	MDA-MB-231/ATCC	-67.37
	77.59	-65.18 to 78.46	CAKI-1	-65.18
	77.59	-63.77 to 78.46	UO-31	-63.77
	77.59	-67.37 to 78.46	NCI-H522	-67.37

Figure 2.25. Anticancer activity test results of compound **80.14**.

Besides the conventional biologically active molecule development method, hybrid molecular development has been taken an attention. Hybrid molecule development is based on the combination of two active pharmacophore molecules to get high affinity, efficacy, and activity. In 2012, Sashindhara *et al*. constructed hybrid molecule by associating coumarin and monastrol as an anticancer agent. Monastrol is a dihydropyrimidinone (DHPM) structure which is a cell-permeable small molecule. Monastrol is known to block the bipolar mitotic spindle to induce mitotic arrest in mammalian cells (Fig. 2.26).

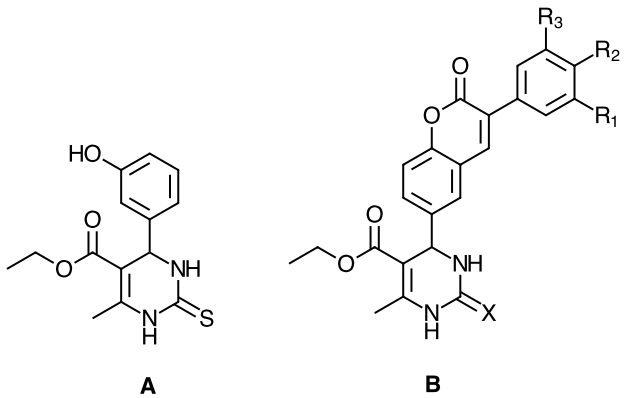
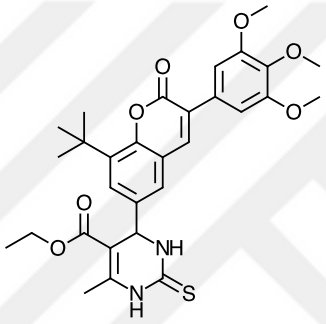


Figure 2.26. Structure of Monastrol and coumarin-monastrol hybrid molecule.

Compound **81.15**, a coumarin-monastrol hybrid molecule, showed promising anticancer activity against MCF-7, T47D and MDA-MB-231 cell lines (Fig. 2.27). Based on SAR studies, methoxy groups which were attached to the benzene ring were shown to affect the anticancer activity directly. Furthermore, the tertiary butyl group attached to the other benzene ring also had the high impact on the anticancer activity. To investigate the mechanism of action of compound **81.15** apoptotic studies were performed. According to the results of this study, compound **81.15** was shown to induce caspase-3 in primary and metastatic breast cancer cells. Moreover, cell cycle analyses were also performed on MCF-7 cell lines and compound **81.15** arrested the cell cycle at the G-1 phase. Additionally, cells at S and G2 phase were found to be reduced [81].



	IC_{50} (μM)								
Compound	**MCF-7**	**T47D**	**MDA-MB-231**	**DU-145**	**PC-3**	**A54**	**HepG2**	**NIH/3T3**	**Mouse skin fibroblast**
81.15	2.42	3.13	3.978	> 20	> 20	> 20	> 20	> 20	> 20

Figure 2.27. Coumarin-monastrol hybrid molecule and its IC_{50} values against cancer cell lines.

2.1.4.1.3. Angiogenesis Inhibitors

Angiogenesis is the growth of blood vessels from preexisting ones. That is a complicated and dynamic process and occurs with activation of endothelial cells during lifetime. Endothelial cells have an important role in angiogenesis because they are normally inactive but, in pathological conditions such as in chronic ischemia, cancer and rheumatoid arthritis, these cells depart from their neighbors and proliferate. As a result, new vessels are formed [82]. For vascular endothelial cells, several growth factors induce angiogenesis. Among them, vascular endothelial growth factor (VEGF) has a particular importance since it is a powerful angiogenic factor and mitogen specific for vascular endothelial [83]. To be activated, VEGF binds receptors like VEGFR-1, VEGFR-2. These

receptors are at the surface of endothelial cells [84]. In pathological angiogenesis, the receptors and VEGF play capital roles, therefore inhibiting the action of VEGF or its receptor become crucial for cancer treatment [85]. In recent years, many coumarin-based angiogenesis inhibitors have been synthesized. Scopoletin (6-methoxy-7-hydroxycoumarin) is one of the angiogenesis inhibitors that showed promising activity according to the study of R. Pan *et al*. who revealed that scopoletin prevented new blood vessels formation in the synovium of adjuvant-induced arthritis rats [86].

2.1.4.1.4. Hsp90 Inhibitors

Hsp90 is one of the crucial parts of a chaperone machine which is responsible for folding, stability, and activation of proteins. All these processes are operated by Hsp90 with conformational changes of proteins. Conformational changes are conducted by binding and hydrolysis of the ATP with the help of Hsp90 and co-chaperones [87]. Eight cancer hallmarks are directly relevant with Hsp90, therefore inhibition of the Hsp90 has become an important target for cancer treatment [88]. One of promising Hsp90 inhibitors are coumarins, which can bind directly Hsp90 and thus take down co-chaperone and client proteins and ultimately prevent cancer cells from proliferation.

In 2000, Neckers *et al.* investigated binding sides and properties of Hsp90. According to this study, both carboxyl- and amino- terminal of Hsp90 can interact with small molecules. They tried to inhibit Hsp90 with novobiocin, and it was observed that not only novobiocin but also clorobiocin and coumermycin showed inhibitory activity against Hsp90 (Fig. 2.28) [88, 89].

In 2013, Zhang *et al.* studied coumarin analogs, which were Hsp90 C-terminal inhibitors. They aimed to synthesize the already known Hsp90 C-terminal inhibitor with an extra 3′-amino group to the noviose moiety of the structure. According to the biological results, it was shown that the addition of the amino group to noviose increased the anticancer activity (**90.16** – **90.17**). Antiproliferative activity of 3-benzamido and 3-acetamino derivatives were compared and 3-benzamido substituted compounds were more active than 3-acetamino derivatives (Fig. 2.29) [90].

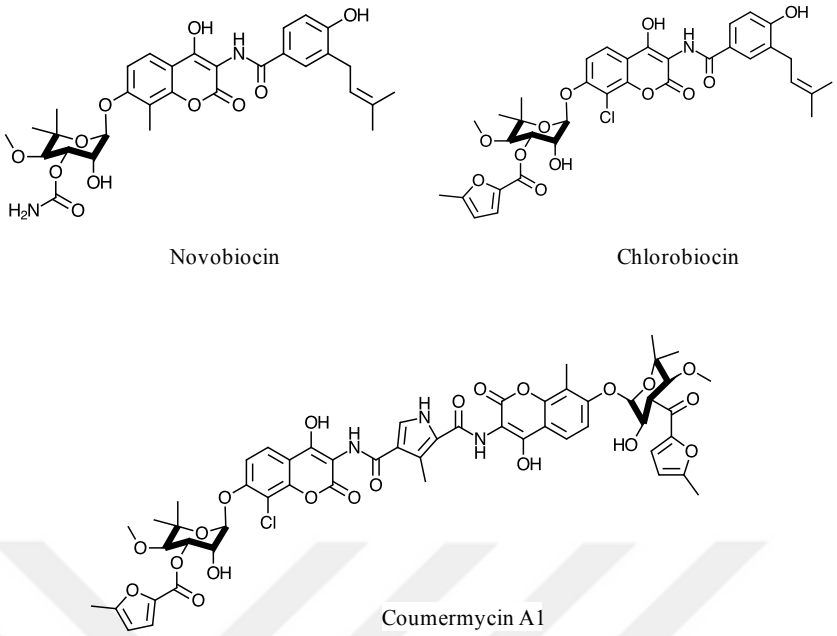
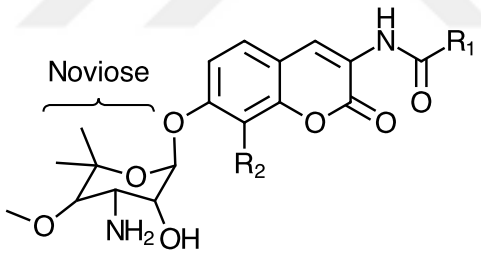


Figure 2.28. Structures of Novobiocin, Chlorobiocin and Coumermycin A1.



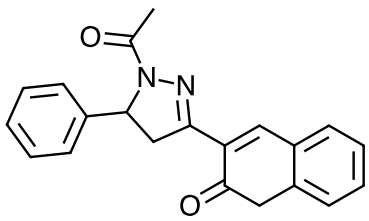
Compound	R1	R2	IC_{50} (μM)/L			
			A2780	HeLa	Ketr-3	MCF-7
90.16	Ph	Cl	2.83	5.35	14.6	13.84
90.17	Ph	Me	7.52	2.89	2.14	8.12

Figure 2.29. SAR study of coumarin derivatives containing the noviose group.

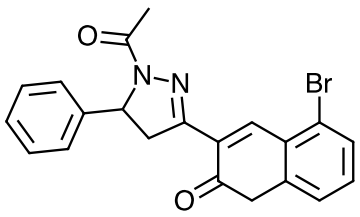
2.1.4.1.5. Telomerase Inhibitors

Telomerases are DNA-polymerases that produce telomeric DNA sequences. They provide cells unlimited proliferation. In normal human cells, telomeres shorten conversely, in cancer cells, they drag on by human telomerase reverse transcriptase (hTERT). Another hallmark of normal and tumor cells is the difference in the telomerase amount in cells. In normal cells, telomerase is not detected but, in cancer cells, it is overexpressed 80 to 95 % more when compared to normal ratios. Because of the crucial role of telomerases in cancer cell proliferation, they became an important target for cancer therapy. In tumor cells, inhibition of telomerase causes shortened telomere length and, accordingly apoptosis and cell aging is observed. To inhibit the telomerase activity, several strategies have been developed and these mainly are small molecule inhibitors, antisense oligonucleotides, immunotherapies and gene therapies [91]. Concerning small molecules, coumarins have been described for inhibiting telomerases.

In 2010, X.-H. Liu *et al.* designed and synthesized coumarin derivatives with 4,5-dihydropyrazole moieties. Synthesized compounds were investigated against SGC-7901 (human gastric cancer), A431 (human epidermoid carcinoma), and PC-3 (human prostate cancer) cells. According to the result, compound **92.18** showed high cytotoxicity against SGC-7901 (IC_{50}: 2.69 ± 0.60 lg/mL). Compound **92.18** and **92.19** (Fig. 2.30) exhibited promising telomerase inhibition activity (IC_{50}: 2.0 ± 0.07 and 1.8 ± 0.35 lM respectively) [92]. Then to enlighten the mechanism of action of the active structures, docking studies were performed on the 3DU6 telomerase.



92.18



92.19

Figure 2.30. Structure of 4,5-dihydropyrazole of coumarin, compound **92.18** and **92.19**.

The docking study revealed two capital hydrogen bonds (between peptidic bonds of the enzyme and the oxygen atoms involved in the lacton function of the active molecule) that were shown to be crucial for binding the enzyme (Fig. 2.31). Additionally, the 4,5-dihydropyrazole ring was found to interact with the hydrophobic region of the protein. Indeed the hydrophobic region surrounded with the Pro 201, Asp 202, Ser 203 and Ala 204 residues is known to be important for interaction and to control telomerase selectivity [92].

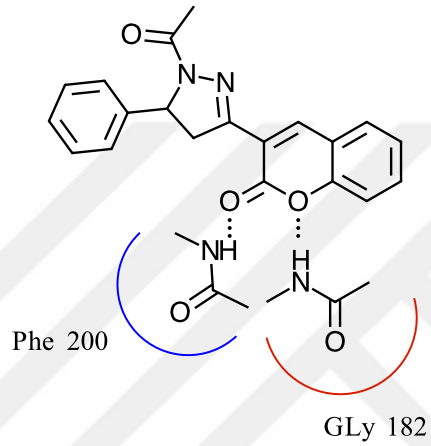
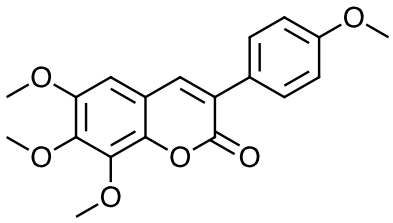


Figure 2.31. Hydrophobic binding mode of **92.18** in the ATP binding site of 3DU6.

2.1.4.1.6. Antimitotic Agents

Mitosis is a type of cell division that occurs both in normal and tumor cells. Parent cell is divided to give two identical daughter cells. Mitosis contains five phases: the prophase, prometaphase, metaphase, anaphase, and telophase. Each phase has a crucial role in the division and can be targeted in cancer treatment strategies [88]. Allylpolyalkoxybenzenes such as; myristicin and apiol are biologically active molecules which belong to the umbelliferae family. In 2013 Tsyganov *et al*. synthesized plant-based polyalkoxy-3(4-methoxyphenyl)coumarins and investigated their antimitotic activity. For the antimitotic activity investigation, sea urchin embryos were used and SAR studies demonstrated that the three methoxy groups at C5 - C6 - C7 carbons are required for optimum activity. Compound **93.20** showed the best antimitotic activity (Fig. 2.32). It

exhibited an antiproliferative activity that was related to its interaction with tubulin and/or microtubules [93].



Compound	Cleavage alterations	Cleavage arrest	Embryo spinning
93.20	0.2	4	>5

Figure 2.32. Biological activity result of polyalkoxy-3(4-methoxyphenyl)coumarin

Microtubules are capital for normal and tumor cell division and thereby the inhibition of microtubules have become an important issue in cancer therapy. Cell division requires the formation of the mitotic spindle and chromosomes are then separated by dynamic changes of microtubules [94]. Blocking microtubular dynamics results in a defect in chromosome segregation which stops cell division. Currently two classes of drugs are used in clinic as microtubule inhibitors: taxanes (paclitaxel and docetaxel) and *Vinca* alkaloids. Although these drugs are effective against cancer cells, they are insufficient against multidrug resistance cancer species [95]. To overcome this difficulty, S.-N. Kim *et al.* investigated the anticancer activity of the 7-diethylamino-3(2′-benzoxazolyl)coumarin molecule (DBC) especially on multidrug resistant cancer cells (Fig. 2.33). According to the results, DBC inhibited tubulin polymerization and induces G2/M phase arrest in normal and multidrug resistant cancer cells. Additionally, DBC acted on a concentration-dependent manner; at low concentration, DBC inhibited the cell cycle at the G2 phase and at high concentration mitotic arrest was observed [95].

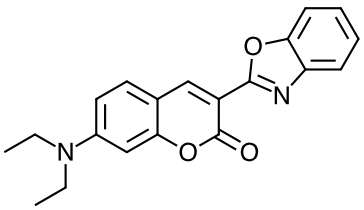


Figure 2.33. Structure of 7-diethylamino-3(2′-benzoxazolyl)coumarin (DBC)

2.1.4.1.7. Carbonic Anhydrase Inhibitors

Carbonic anhydrases (CAs) are a type of metalloenzymes that contain a zinc ion (Zn^{+2}) and are present in eukaryotes and prokaryotes. CA enzymes are necessary for the conversion of CO_2 into the bicarbonate ion in physiological and pathological processes (Fig. 2.34). For instance, in metabolizing tissues and lungs, they manage the transport of CO_2 and bicarbonate; they balance pH and CO_2 concentration, or control the secretion of electrolyte in tissues and organs; the ureagenesis, glucogenesis, lipogenesis; calcification and tumorigenicity ans thus are important targets for the treatment of glaucoma, obesity, epilepsy, and cancer. One hallmark of cancer is is hypoxia. It consists on an insufficient oxygen supply in tissues. Hypoxia leads to the expression of some genes like CAs. For instance, in many tumors such as gliomas/ependymomas [96], papillary/follicular carcinomas [96], uterine cervix [97, 98], breast [96, 99, 100] cancers CA IX expression is highly increased.

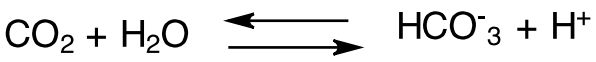


Figure 2.34. Conversion of carbondioxide to bicarbonate ion by carbonic anhydrase enzyme.

Recently, a screening of natural product extracts revealed a coumarin molecule as a novel CA inhibitor (CAI). 6-(1S-hydroxy-3-methylbutyl)-7- methoxy-2H-chromen-2-one, **101.21,** (Fig. 2.35) (CAI) isolated from *Leionema ellipticum* (*Rutaceae*) has been described to have different inhibitory properties when compared to other known CAIs since the isolated coumarin did not show any interaction with the zinc-binding domain [101]. Therefore, this molecule should have a different binding mechanism to be active.

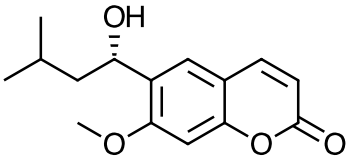


Figure 2.35. 6-(1S-hydroxy-3-methylbutyl)-7-methoxy-2H-chromen-2-one, **101.21**

In 2009, Maresca *et al.* studied on the 6-(1*S*-hydroxy-3-methylbutyl)-7- methoxy-2*H*-chromen-2-one molecule to determine its mechanism of action. They performed inhibition studies on thirteen different mammalian isoenzymes, namely CA I to XV (Fig. 2.37). According to their results, coumarins inhibited the CA enzymes after being hydrolyzed (Fig. 2.36) and the resulting metabolite was found to be responsible for the interaction with the enzyme cavity in a manner that was not observed before. [102].

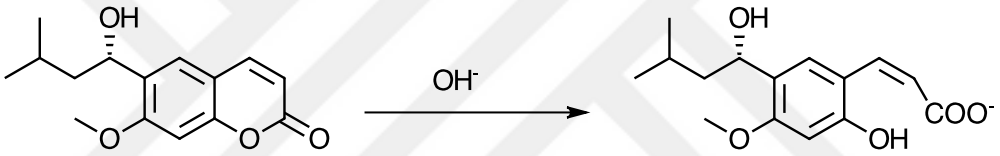
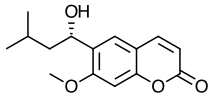
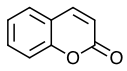


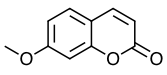
Figure 2.36. Hydrolysis of coumarin



102.21



102.22



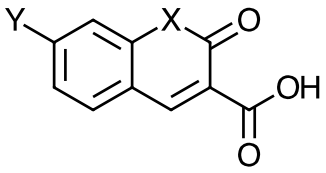
102.23

	K_I (µM)		
isozyme	**21**	**22**	**23**
hCA I	0.08	3.1	5.9
hCA II	0.06	9.2	0.07
hCA III	> 1000	> 1000	161
hCA IV	3.8	62.3	7.8
hCA VA	96.0	> 1000	645
hCA VB	17.7	578	48.6
hCA VI	35.7	> 1000	61.2
hCA VII	27.9	> 1000	9.1
hCA IX	54.5	> 1000	767
hCA XII	48.6	> 1000	167.4
hCA XIII	7.9	> 1000	6.0
hCA XIV	7.8	> 1000	9.7
hCA XV	93.1	> 1000	> 1000

Figure 2.37. Coumarin **102**.**21**, **22**, and **23** and their inhibition of mammalian isozymes CA I - XV assay with stopped-flow CO_2 hydration.

2.1.4.1.8. Monocarboxylate Transporters (MCT) Inhibitors

Tumor types can be divided into subgroups according to their metabolic features. Some tumors such as triple negative breast cancer and gliomas cannot be treated based on their metabolic behavior. Hypoxic and non-hypoxic feature of tumors have metabolic differences and therefore must be treated differently. As hypoxic tumors are resistant to standart therapies, targeted therapies should be developed and therefore the metabolic pathways of hypoxic tumor cells have to be analyzed. Monocarboxylate transporters (MCTs) were found to be involved in the metabolic pathways of hypoxic cells and therefore constitute an important target. Indeed, when lactate transportation is inhibited, cell function is not maintained [103]. As coumarins have already been described for inhibiting lactate transportation, in 2014, Draoui *et al*. studied on 7-aminocarboxycoumarins and investigated their lactate transport inhibitory activity. Based on their SAR study results, they showed that the coumarin scaffold was necessary to obtain a satisfactory inhibitory activity. The authors noticed that when coumarin's oxygen atom was replaced with a nitrogen, the inhibitory activity was lost. They also showed that the replacement of the acid group at the C3 position with an ester was leading to the loss of the bioacitivity. The other important substitution for the activity was found to be on the C7 of coumarin. While O-benzyl and substituted amine groups improved the activity, methyl, lactone or lactam groups were reported to decrease the inhibitory activity (Fig. 2.38) (Compound **103.24**) [103].



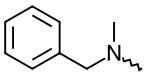
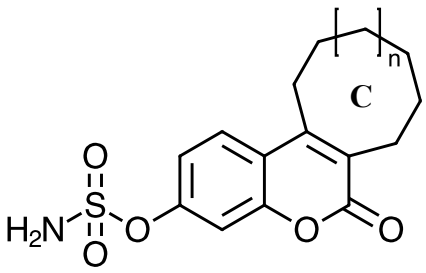
Compound	X	Y	Cytotoxicity (EC_{50} µM)	Lactate uptake inhibition (IC_{50} µM)
103.24	O		0.22	0.059

Figure 2.38. Compound **103.24** and its SAR study and activity results.

2.1.4.1.9. Aromatase and Sulfatase Inhibitors

Estrogens and other relevant hormones have a crucial role in the development of cancer and especially breast cancer. Sulfatase and aromatase enzymes are key proteins that are involved in the synthesis of estranes. Sulfatase is an enzyme that responsible for catalyzing the cleavage of sulfate esters while aromatases are involved in the obtention of the aromatic cycle during the biosynthesis of estrogens. Additionally, conversion of androgens to estrogen is also inhibited by aromatases. Therefore, inhibition of these enzymes has become an important target for the treatment and prevention of the devlopment of breast, endometrial and prostate cancer [8, 104, 105]. Inhibition of these enzymes can be achieved *via* coumarin derivatives.

In 2011, Woo *et al*. studied on the first steroid sulfatase inhibitors (STS) that were called irosustats which consisted of three fused-ring systems (Fig. 2.39). The SAR studies of the authors demonstrated that the length of the ring **C** affected the cytotoxicity of the irosustats. Potent compounds were observed with 9, 10 and 11 membered **C** rings. In those molecules, coumarin's C7 was derivatized with a sulfamate group. This group increased the activity of the molecule and when two sulfamate groups were attached to the C6 and C7 of coumarin, the cytotoxicity of irosustats was found to be decreased. Then, other alternatives were investigated for C6 and the best result was observed with compound **106.27** [106].

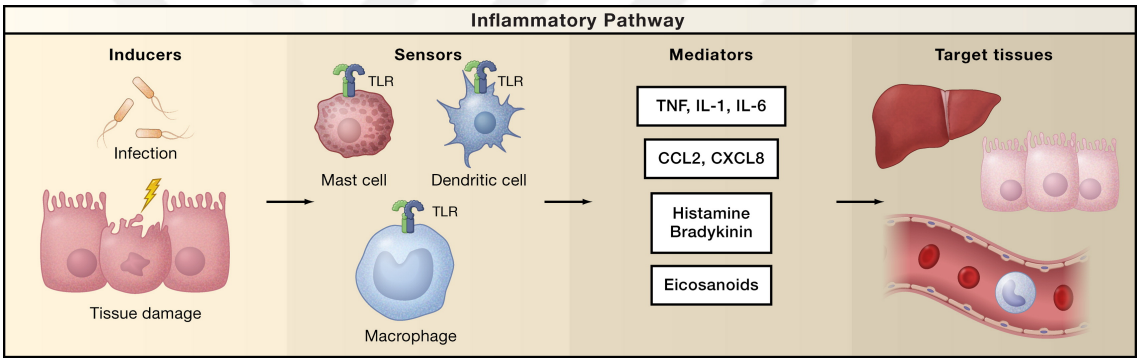


Compound	n	PM (IC_{50} nM)	JEG-3 (IC_{50} nM)
106.25	7	2.4	0.022
106.26	8	1	0.025
106.27	9	13	0.015

Figure 2.39. SAR study and activity results of Irosustats.

2.1.4.2. Anti-Inflammatory Activity

In literature, up to now several natural and synthetic coumarin molecules have been reported for potential anti-inflammatory activity but none led to the development of a marketed drug yet. Inflammation is a process that is a response of body's immune system against injury or foreign invaders like bacteria and viruses [60]. Inflammation is characterized by five important symptoms: redness, heat, swelling, pain and disturbance of function. Inflammatory pathways involve four major actors: inflammatory inducers, sensors, inflammatory mediators and target tissues (Fig. 2.40). Inflammation starts with inducers i.e. an infection which is then detected by sensors for instance Toll-like receptors (TLRs). Sensors affect sentinel cells (mast cells, dendritic cells, and macrophages), that produce mediators such as bioactive amines, chemokines, cytokines and eicosanoids. The main role of mediators is to adapt target tissues to inflammation conditions [107].



Adapted from *Crit Rev Biomed Eng* 2012; 40(4):295-312

Figure 2.40. Inflammatory pathway constituents.

The inflammation process can be blocked by inhibiting inducers, sensors, inflammatory mediators (plasma proteases) [108]. Also, the role of the mediators such as PGE2, leukotrienes (arachidonic acid metabolites), serotonin, histamine, nitric oxide, cytokines, and chemokines has been investigated for the development of effective anti-inflammatory strategies [109]. Up to now, a large number of heterocyclic compound derivatives have been synthesized for their anti-inflammatory activities and coumarin derivatives have been reported to reduce inflammation *via* different mechanisms (Fig. 2.41). In this chapter, coumarin derivatives are investigated according to their sources; natural (plant-based) and synthetic coumarins. Additionally, their SAR studies and anti-inflammatory activities are also discussed.

Coumarins		
Sensor Level	**Mediator Level**	**Protection**
MAP kinases	Prostaglandins inhibitor Leukotrienes inhibitor TNFα inhibitor iNOS inhibitor NF-κB inhibitor	Atherosclerosis Rheumatoid arthritis

Figure 2.41. Effects of Coumarins on inflammation.

2.1.4.2.1. Anti-Inflammatory Activity of Natural Coumarins

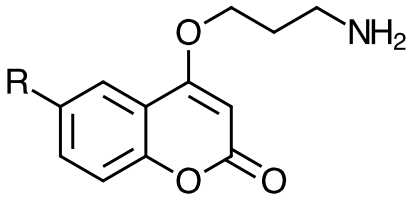
Plant-based coumarins are important phytonutrients. They are found in essential oils, fruits or green tea. The *Rutaceae* and *Umbelliferae* families are known for containing the highest amounts of coumarin-based molecules [60]. Several coumarin derivatives, which are extracted from plants, have been described for their anti-inflammatory activities. They either inhibit enzymes such as cyclooxygenase or lipoxygenase, decrease tissue edema or inhibit the occurrence of free radicals. Based on the anti-inflammatory effect of coumarin derivatives, a brief SAR model was generated. Attaching α,β-unsaturated carbonyl groups or hydroxyl group to the C8 position of the coumarin ring was shown to increase the activity. Subtitution of the C6 and C7 positions were also reported to be essential for the maintenance of bioactivity. Monosubstitution or disubstitution of these positions with oxygen containing groups were shown to be appropriate for the anti-inflammatory activity. An interesting finding was that the C4 position of all natural coumarins is unsubstituted. Finally, substitution of the C3 position with saturated long chains was also reported to be suitable for good activity [60]. Beside naturally occuring coumarins, a lot of synthetic coumarins have been investigated for their anti-inflammatory activity.

2.1.4.2.2. Anti-Inflammatory Activity of Synthetic Coumarins

After the discovery of the promising biological activity of natural coumarins,a considerable effort was put for the design and development of novel synthetic coumarin derivatives. Actually, over the past decade, many alkyls or aryl substituted coumarin derivatives were synthesized and evaluated for their anti-inflammatory activity.

In 2005, Jackson *et al.* synthesized ether substituted coumarin derivatives and evaluated their inhibitory activity against iNOS. iNOS is an enzyme which is responsible for releasing NO (nitric oxide). NO is a messenger molecule, which is synthesized by nitric oxide synthase (NOS), and is involved in several physiological and pathological processes and especially in inflammation. Therefore, iNOS constitutes an important target for the development of anti-inflammatory drugs. In their study, the authors developed C4 and C6-substitutes coumarins. Their SAR studies demonstrated that halo-substituted compounds at the C6 position had better activity than unsubstituted ones. When activities of halogenated coumarins (**110.28, 110.29, 110.30, 110.31)** were

compared, the chloro- substituted molecule (**110.28)** was found to exhibit the best activity (Fig. 2.42) [110].



Compound	R	IC_{50} µM	Target/Activity
110.28	Cl	0.061	
110.29	Br	0.143	iNOS
110.30	F	0.441	
110.31	CH_3	0.304	

Figure 2.42. Halogens and methyl derivatives of coumarin and their activities.

Lin *et al*. also studied the NO release inhibition ability of some 6-acyl-4-aryl/alkyl-5,7-dihydroxycoumarin derivatives. The compounds were evaluated on LPS-induced RAW 264.7 cells and the results indicated that the anti-inflammatory activity was observed at low micromolar levels for compound **111.32** (7.6 µM) (Fig. 2.43). Beside the anti-inflammatory activity, some derivatives of this study were also shown to have a role in protecting DNA against hydroxyl radicals. The most relevant antioxidant activity was again obtained with Compound **111.32** as it inhibited 50% of hydroxyl radicals at 100 µM [111].

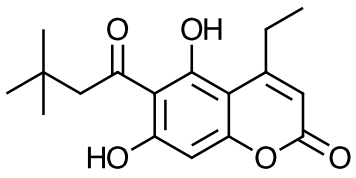
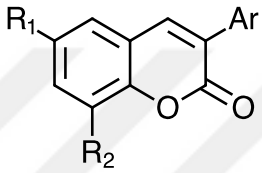


Figure 2.43. Compound **111.32**

In 2014, W. Pu *et al.* investigated the effect of substitution of the C3 position on the ability of coumarins to inhibit NO production. So, they synthesized thirty five

derivatives of 3-arylcoumarins and evaluated their bioactivity on LPS-induced mouse macrophage RAW 264.7 cells. The C3 substituted stuctures were also substituted at their C6-8 positions with halogens and methoxy groups. SAR studied indicatd that the aryl substitution at C3 was necessary for anti-inflammatory activity. The most potent compounds **112.33** and **112.34** were disubstituted at C6-8 positions. Compound **112.33** was substituted with a chlorine at both the C6 and C8 positions while **112.34** was substituted with a chlorine and methoxy group at the C6 and C8 positions respectively (Fig. 2.44) [112].



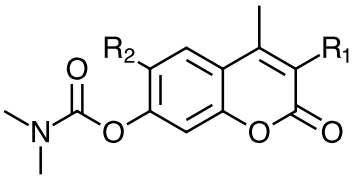
Compound	R1	R2	IC_{50} mg/L	Inhibition of NO (%)	Cell viability (%)
112.33	Cl	Br	2.7	76.9	119.17
112.34	Cl	OCH_3	2.5	72.28	116.42

Figure 2.44. Structure of compound **112.33**, **112.34** and their activity results.

TNF-α (tumor necrosis factor α) is another an important target for anti-inflammatory drugs. TNF-α is a proinflammatory cytokine. It is released from macrophages and monocytes as a response to inflammatory stimuli. During the inflammatory process, TNF-α induces the release of other cytokines or agents. Therefore, inhibiting TNF-α consititutes a crucial target to block the inflammation process.

In 2004, J.-F. Cheng *et al.* studied on coumarin-based TNF-α inhibitors. The coumarin scaffold was derivatized with carbamates at the C7 position and the *in vitro* activity assays were performed on human peripheral blood mononuclear cells (hPBMC). According to the SAR study, hydrophobic groups at C3 position gave better results, especially long alkyl groups were shown to increase the inhibitory activity. The highest activities were observed when a benzyl group was attached to the C3 position (compound **113.35**, **113.36**). The other important position was found to be the C6. When C6 was substituted with a bromine, iodine, and chlorine high TNF-α inhibitory activity was also

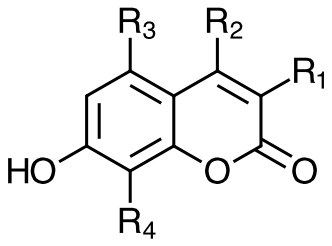
observed (compound **113.35**, **113.36**, **113.37**). The highest inhibition of TNF-α production was obtained with compound **113.37** (86% inhibition at 100 mg/kg sc) (Fig. 2.45) [113].



Compound	R_1	R_2	IC_{50} µM
113.35	Bn	Bromine	0.06
113.36	Bn	Iodine	0.06
113.37	Chlorine	3-Pyridinyl	0.45

Figure 2.45. Structures and activities of compound **113.35**, **113.36**, and **113.37**.

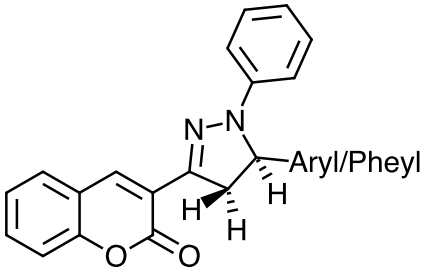
Umbelliferone, or 7-hydroxycoumarin, is one of the most known and biologically active metabolites of coumarin. To explore the novel active coumarin derivatives, Umbelliferone has been used as a starting compound. Based on this, Timonen *et al*. investigated 7-hydroxycoumarin derivatives and their anti-inflammatory activity. The anti-inflammatory study was focused on the inhibition of iNOS, COX-2, NO and IL-6 release. The authors demonstrated that 7-hydroxycoumarin derivatives inhibited IL-6 and NO release in a dose-dependent manner. Three compounds (**114.38, 114.39,** and **114.40)** exhibited satisfactory antiinflammatory activity on LPS-induced cells (Fig. 2.46). Indeed, when the applied concentration of **114.38** was increased to 100µM for J774 macrophages, LPS-induced IL-6 production was inhibited up to %92. SAR studies revealed that the phenyl ring located at the C4 position was important for the activity since when the phenyl ring was replaced with a prenyl group the activity of the compound was decreased (not shown in th figure). Also, It was shown that although some active compounds contained a methoxy group at the C8 position, this was not indispensable for activity [114].



Compound	R_1	R_2	R_3	R_4	% inhibition	Target/Dose
114.38	CH_3	CH_3	CH_3	CH_3	51.8	NO production (J774 macrophage, dose 10 μM)
114.39	H	Prenyl	H	OCH_3	57.0	
114.40	H	Phenyl	H	OCH_3	71.5	

Figure 2.46. Structure of compounds **114.38**, **114.39**, **114.40** and their % inhibition of NO production.

In last years, combining active compounds to take benefit of their synergistic activity has become a topic of great interest in medicinal chemistry. Pyrazole is a heterocycle that is found in the structure of many non-steroidal anti-inflammatory drugs as it is in, for instance, famprofazone, mefobutazone and celecoxib. In this perspective, there are some studies in which the coumarin scaffold has been combined with the pyrazole ring to discover highly active anti-inflammatory drugs. In 2009, S. Khode *et al*. synthesized and evaluated 5-(substituted)aryl-3-(3-coumarinyl)-1-phenyl-2-pyrazolines, some pyrazole substituted coumarin derivatives. SAR studies revealed that substitution of the 5th position of the pyrazoline ring with a chlorine, fluorine and methoxy groups increased the analgesic and anti-inflammatory activities according to assays performed on carrageenan-induced rat paw edema and adjuvant-induced arthritis (Fig. 2.47). Beside these findings, some compounds also showed high antipyretic activity in yeast-induced pyrexia model [115].



Compound	Aryl/Phenyl	Anti-inflammatory activity %inhibition after 2h	Anti-inflammatory activity % inhibition after 4h	Anti-inflammatory activity % inhibition after treatment (on day 19) (Adjuvant-induced arthritis model)
115.41	4-Cl-C $_6$H $_4$	39.2	64.7	39.1
115.42	2,4-(Cl) $_2$-C $_6$H $_3$	56.7	67.5	45.4
115.43	3-OMe-C $_6$H $_4$	38.3	61.5	20.9
115.44	4-F-C $_6$H $_4$	44.9	66.7	40.5

Figure 2.47. Structures of compound **115.41, 115.42, 115.43, 115.44** and their anti-inflammatory activities.

To develop novel anti-inflammatory drugs, in addition to pyrazole different types of heterocycles have also been evaluated. The isoxazole group is another heterocyclic ring that can be found in the structure of anti-inflammatory drugs. Therefore, isoxazole derivatized coumarin scaffolds have also been investigated. Accordingly, in 1998 Nicolaides *et al*. investigated 4-(1,2,4-oxadiazlyl)coumarin derivatives. Their anti-inflammatory activities were studied on the carrageenin-induced rat paw edema and by evaluating the LOX inhibition capacity. A great majority of synthesized compounds revealed promising anti-inflammatory activity (59.1 – 87.6%) on the rat paw edema and compound **116.45** (Fig. 2.48) demonstrated higher activity (64.6%) than reference indomethacin drug (53.3%) on the *in vitro* 12-LOX inhibition assay [116].

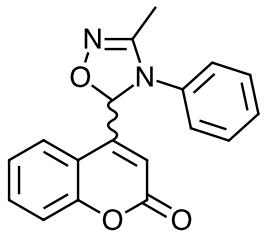
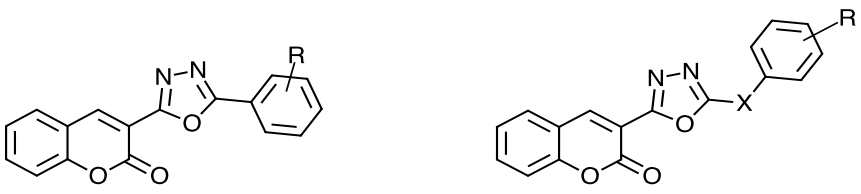


Figure 2.48. Compound **116.45**

In 2011, Akhter *et al*. synthesized derivatives of 3-(5-phenyl/phenylamino-[1,3,4]-oxadiazol-2-yl)-chromen-2-one and N-[5-(2-oxo-2*H*-chromen-3-yl)-

[1,3,4]oxadiazol-2-yl]-benzamide. All synthesized compounds were tested for their anti-inflammatory activity and it was observed that almost all showed high anti-inflammatory activity. The most active compound among them was **117.49** which showed %89 inhibitory activity on rat paw edema and COX-2 selectivity *in vitro* (COX-1 IC_{50}: 41.6 μM; COX-2 IC_{50}: 0.4 μM). Also some inhibition was also observed against 5-LOX and 15-LOX. Beside these activities, compound **117.49** revelaed some analgesic and antioxidant activity. SAR study showed that, to see the anti-inflammatory activity the coumarin scaffold had to be substituted with a 1,3,4 oxadiazole moiety at its C3 position. The 1,3,4 oxadiazole group was derivatized with a phenyl moiety at its 5th position. Unsubstituted phenyl derivative (**117.46**) showed % 80 inhibitory activity. Then, this phenyl group was further substituted with Cl (**117.47**), CH_3 (**117.48**) and the obtained inhibition ratios were 72 % and73% respectively. The most active compound **117.49** was a 2,4-$(Cl)_2$ substituted structure with 89% of inhibition (Fig. 2.49-A). As halogenation (Cl) of phenyl ring increased the activity and substitution with di-chloro moieties showed the highest activity, it was concluded that electron-withdrawing groups on the phenyl ring affected the activity positively. When the distance between the oxadiazole and phenyl ring was increased with linkers such as –NH- (**117.50**) and -NHCO- (**117.51**), activity was found to be decreased slightly (Fig. 2.49-B) [117].



117.46- R: H
117.47- R: Cl
117.48- R: CH_3
117.49- R: 2,4-$(Cl)_2$

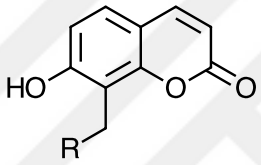
117.50- X: NH, R: 4-Cl
117.51- X: NHCO, R: 2-CH_3

A **B**

Figure 2.49. Structures of compound **117.46 -117.51**.

Mannich bases, also, have been reported for exhibiting some biological activities. Coumarin-based mannich bases are another important class of anti-inflammatory agents.

In 2005 for instance, Kontogiorgis and Hadjipavlou-Litina synthesized a series of coumarin mannich bases to investigate their anti-inflammatory activity and evaluating their enzyme inhibition capability in the arachidonic acid conversion pathway. They first constructed a novel coumarin scaffold using a 7-OH moiety, a functionnalization that active metabolites of natural coumarins actually present. 7-OH coumarins were then diversified with a series of amines. Synthesized compounds showed protective property against carrageenan-induced paw edema (CPE). The edema inhibition ratio range varied from 31.2% to 77.7 % for synthesized compounds while indomethacin, the reference drug, gave an inhibition of 47%. The most potent compound was identified to be **4.52,** the C8-piperazine substituted molecule (31.2%), while the least active compound was reported to be **4.58** (Fig. 2.50). Another potent compound was notice to be **4.53,** the morpholine substituted structure. Finallly, the authors also noticed that eventhough bearing an amino group compound **4.57** did not exhibit significant inhibition capacity.



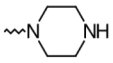
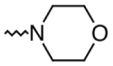
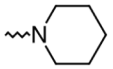
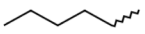
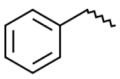
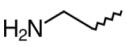
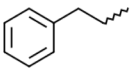
Compund	R	CPE% 0.01 mmol/kg body weight
4.52		77.7
4.53		75.7
4.54		47.4
4.55		52.7
4.56		47.5
4.57		56
4.58		31.2
Coumarin		30.2
Indomethacin		47
7-OH coumarin		NR

Figure 2.50. C8 derivated of coumarin compounds and their anti-inflammatory activities.

The promising activity observed for compounds **4.52** and **4.53** was first attributed to the hydrophilic nature of their substituents. Yet, since the lipophilic compound **4.55** also exhibited a satisfactory protection against CPE the authors concluded that lipohilicity could only partly explain the activity of the synthesized molecules. After being identified as the most active compounds, **4.52** and **4.53** were further evaluated on athe djuvant-induced disease (AID) as AID is a good model of rheumatoid arthritis. In this *in vivo* experiment, compound **4.52** and **4.53** were tested on rats and arthritis development was observed when animals were treated with these molecules. To investigate the role of 7-OH coumarin scaffold on anti-inflammatory activity, 7-OH coumarin was also tested on rats. The arthritic scores, cahexia and body weight loss were found to be as satisfying as those obtained with **4.52** and **4.53**, but some side effects were observed in the gastrointestinal route (hyperemia and ulcer), side effects that were not observed with **4.52** and **4.53** because of their acidity. According to this study, some minimum molecular requirements were established to have potent anti-inflammatory coumarin molecules: an unsubtituted hydroxyl group on C7 and an alicyclic (i.e. piperazines) or aliphatic amine on C8 [5].

2.2. Nitrogen Heterocycles

Nitrogen heterocycles, composed of three-, four-, five-, six-, seven- or eight-membered rings, are chemical structures that are found in 59% of U.S. FDA approved small-molecule drugs. Among the FDA approved 1994 drugs, 84% (910) have been found to contain at least one nitrogen atom and 59% have at least one nitrogen heterocycles (Fig. 2.51). So, nitrogen-containing heterocycles are of great interest for drug development [17].

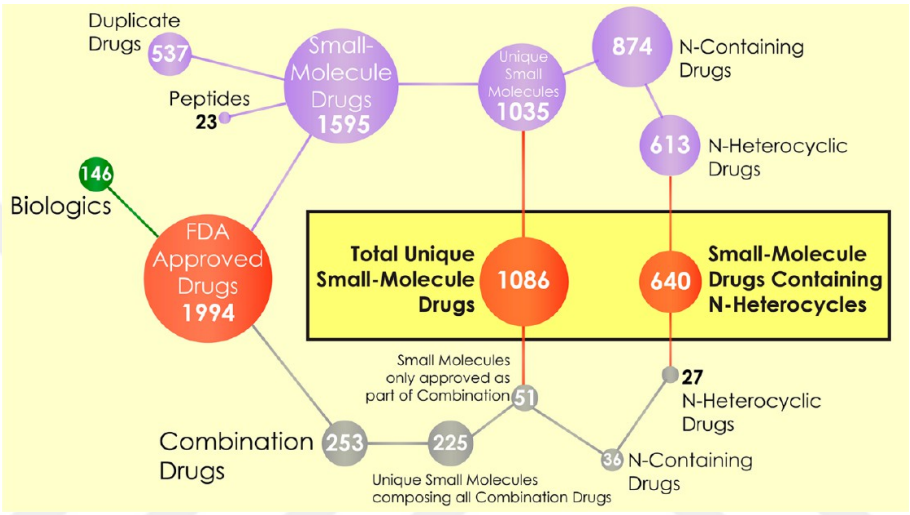


Figure 2.51. Analysis of the U.S. FDA approved drugs. Adapted from [16].

In the study published in 2014, FDA approved drugs were categorized according to the nature of the group that presented the nitrogen atom, such as molecules with only the nitrogen atom, with a heterocycle, with a fused or bicyclic structure. Results showed that the first three prevalent stubstrucutres are the piperidine, pyridine and piperazine heterocycles. These moieties are six-membered aromatic or nonaromatic heterocycles. The list continues with the cephem ring, pyrrolidine, thiazole, imidazole rings and so on (Fig 2.52). Based on this analysis, the most active or effective moieties are piperidines and piperazines. Molecules containing these heterocycles can be bioactive either viw this heterocylcle or their presence can increase the biological activity of an already active molecule [17]. The following two subsections are dedicated to these two heterocycles.

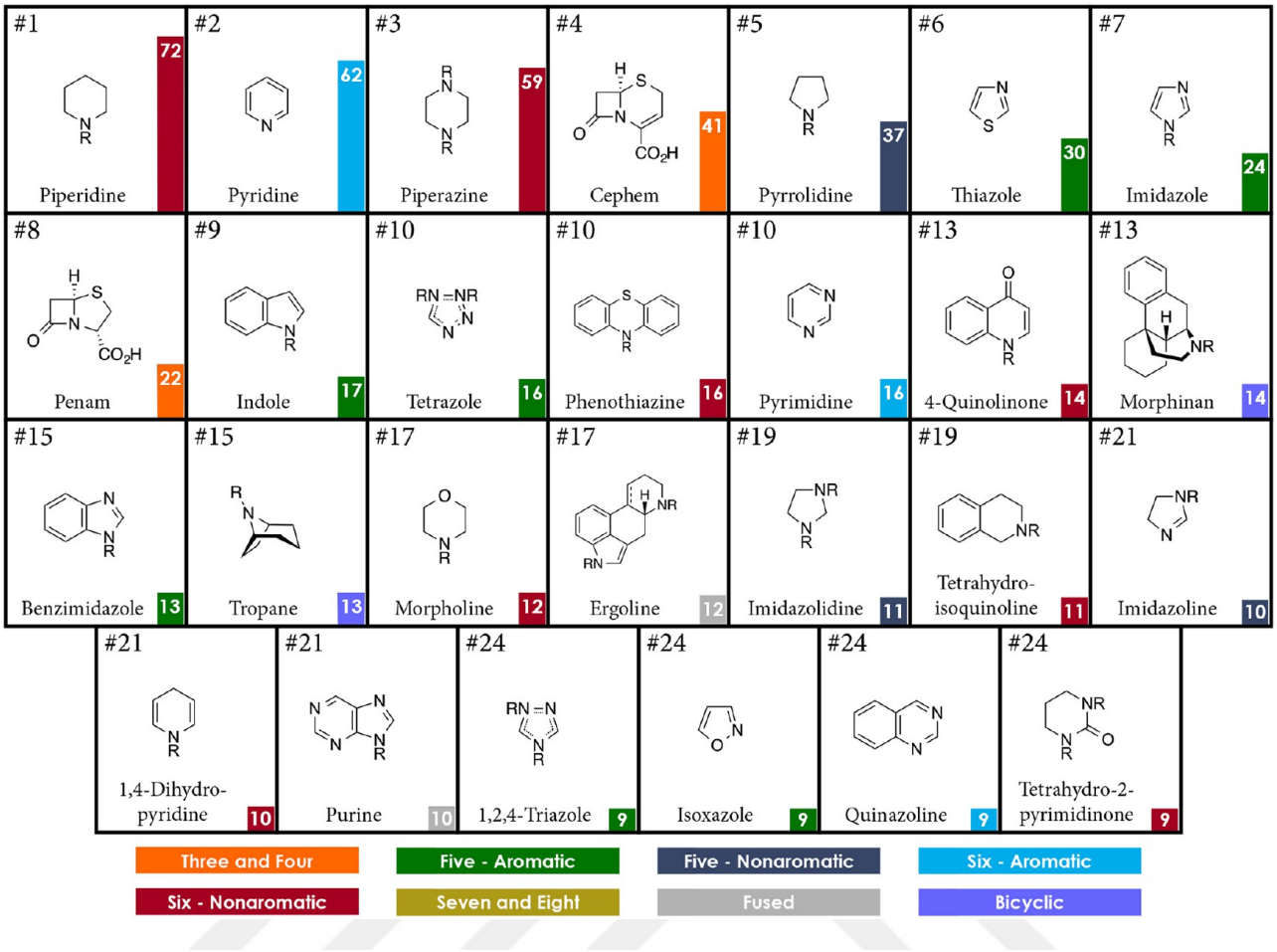


Figure 2.52. Nitrogen heterocycles that are involved in U.S. FDA approved drugs.

2.2.1. Piperidine

Piperidines, nitrogen-contained six-membered nonaromatic heterocycles, are the most common moiety found in FDA approved drugs. In the 72 piperidine-containing drugs the N1 and C4 positions of the ring have been reported to be crucial for bioactivity. The heterocycle is most commonly found among the antihistamine drugs as in loratadine, desloratidine, azatadine, ketotifen and cyproheptadiene. Another drug class that present carboxamide substituted piperidine scaffolds is local anesthetics [118]. Ropivacaine, mepivacaine, and bupivacaine and its pure stereoisomer levobupivacaine are examples of piperidine containing local anesthetics. Fentanyl is also another example of 1,4-disubstituted piperidine anesthetic. Antiviral agents are also piperidine-containing drugs. Nelfinavir, for instance, is an antiviral agent which presents a thioether, an amide and a fused ring system with a tetrasubstituted pipereridine in its structure. Paroxetine (antidepressant) and aminoglutethimide (anti-hormone) are also examples for piperidine-containing drugs (Fig 2.53) [17].

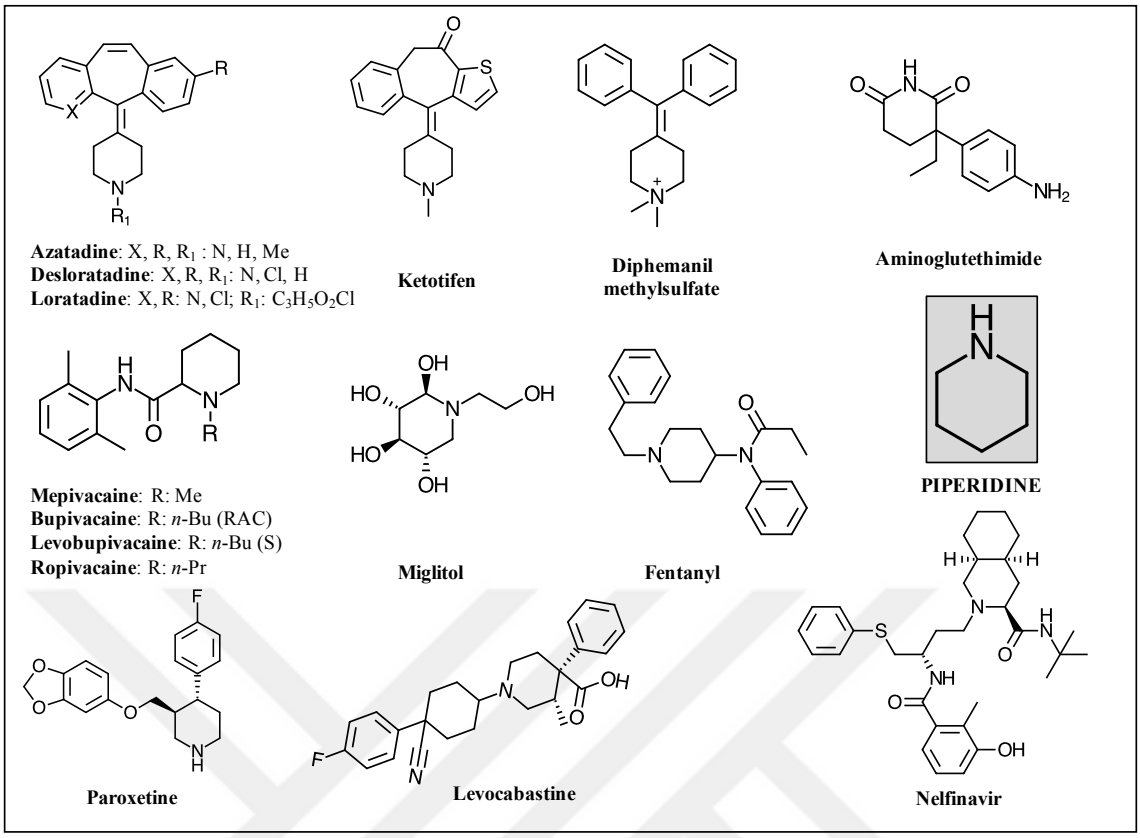


Figure 2.53. Piperidine-containing drugs.

2.2.2. Piperazine

Piperazine is a six-membered nonaromatic heterocycle which is found in 59 approved drug molecules. Piperazine is the basic component of the three drug classes; antibiotics (ofloxacin, levofloxacin), antihistamines (cyclizine, hydroxyzine, cetirizine, levocetirizine) and blood pressure regulators. 32% of the structures of these classes contain a piperazine scaffold and 10 of the 59 approved drugs are antibiotics. The most active sites of piperazines are the N1 and N4 positions. The approved blockbuster sildenafil drug (Viagra ™), which was fisrt developed as antihypertensive, also comprises a piperazine moiety (Fig. 2.54) [17].

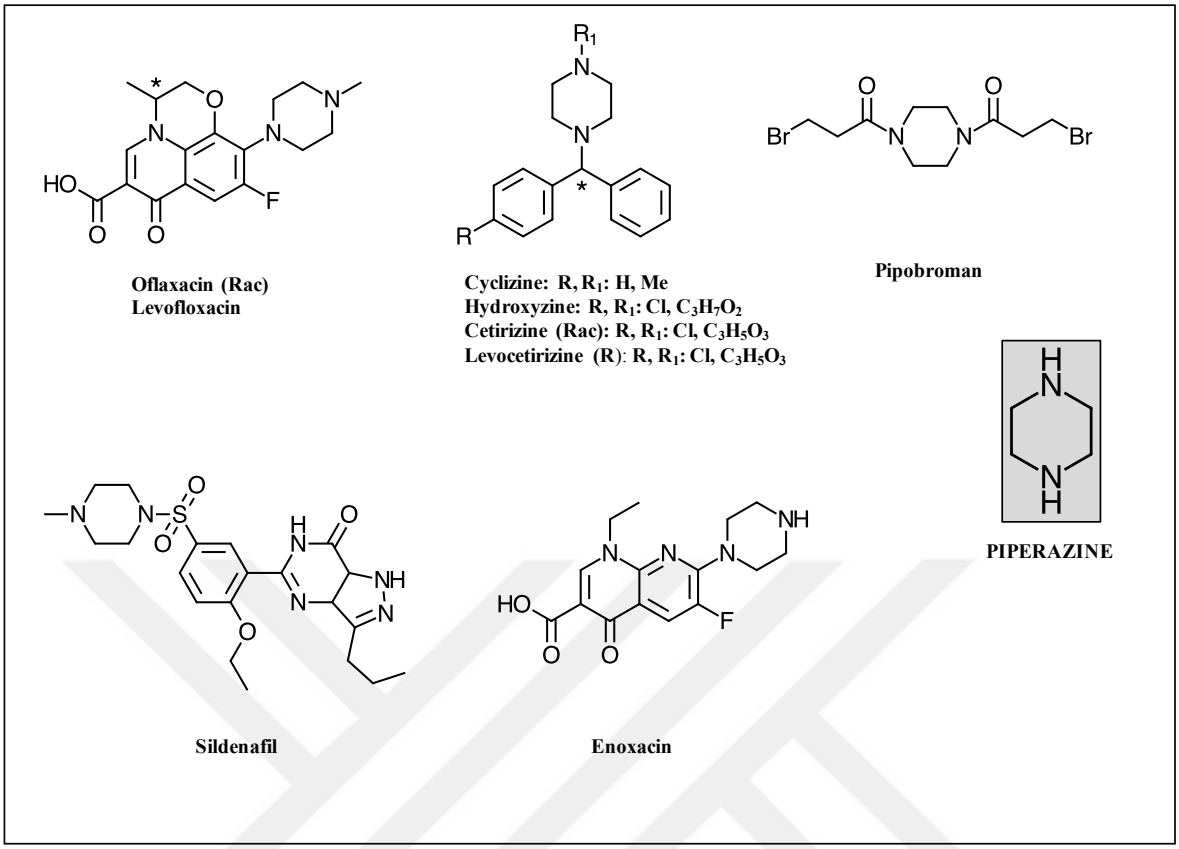


Figure 2.54. Piperazine-containing drugs.

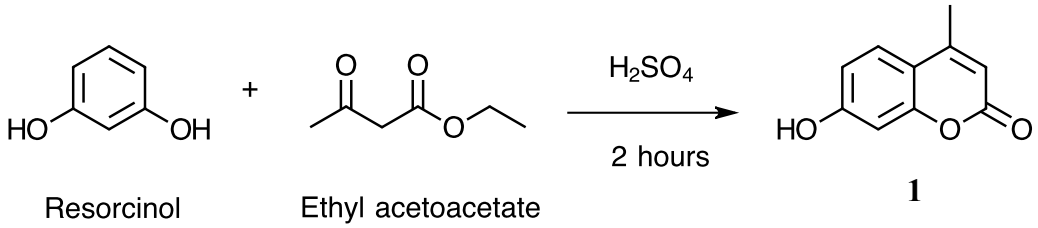
3. MATERIALS AND METHODS

3.1. Chemicals

In this study, ethylacetoacetate, sulphuric acid, N-methylpiperazine, 1-isopropylpiperazine, 1-(2-hydroxyethyl)piperazine, 2-aminoethylpiperazine, allylpiperazine, N-(4-chlorophenyl)piperazine, 1-(4-fluorophenyl)piperazine, 4-hydroxyphenylpiperazine, 1-(4-methylphenyl)piperazine, 1-(*p*-nitrophenyl)piperazine, 1-benzoylpiperazine hydrochloride, 1-(2-fluorophenyl)piperazine, 1-(3-chlorophenyl)piperazine, 1-(3-dimethylaminopropyl)piperazine, 4-phenylpiperidine, 4-hydroxy-4-phenylpiperidine, 4-piperidine-methanol, piperidine, 4-(2-oxo-1-benzimidazolinyl)piperidine, 1-(4-bromophenyl)piperazine, 1-phenylpiperazine, 3,5-dimethyl piperidine, 2-(2-hydroxyethyl)piperidine, 3-hydroxypiperidine, 2-piperidine methanol, 3-hydroxymethyl piperidine, 4-piperidine methanol, 1-Boc-piperidone, ethyl-1-piperazine carboxylate, ethyl piperizino acetate, 1-(2-phenylethyl)piperazine, benzyl-1-piperazine carboxylate, piperazine, N-phenylpiperazine, 1-(4-chlorophenyl)piperazine, 1-(2-cyanophenyl)piperazine, 1-(1-naphtylmethyl)piperazine, piperidineethyl pipecolinate, 2-propyl piperidine, 2-ethyl piperidine, 3-carboxy-2-piperidone, 1-Boc-piperidone, 1-piperazine carboxyaldehyde, nipecotic acid were purchased from Sigma-Aldrich. 1-Butylpiperazine, 1-(3,4-dichlorophenyl)piperazine, 3-piperizino propionitrile, 4-methylpiperidine, 2-methylpiperidine, 3-methylpiperidine, , piperidine-4-carboxylic acid, 3-ethoxy carbonyl 2-piperidone were purchased from Fluka. 1-(3-trifluorophenyl)piperazine monohydrate chloride, 1-(2-pyridyl)piperazine, 1-piperonyl piperazine were purchased from Alfa Aesar. 1-(4-methoxyphenyl)piperazine was purchased from ABCR GmbH. 4-benzyl piperidine was purchased from Acros. Resorcinol was purchased from Riedel-de Haën. Formaldehyde was purchased from J. T. Baker. Ethanol was purchased from Merck.

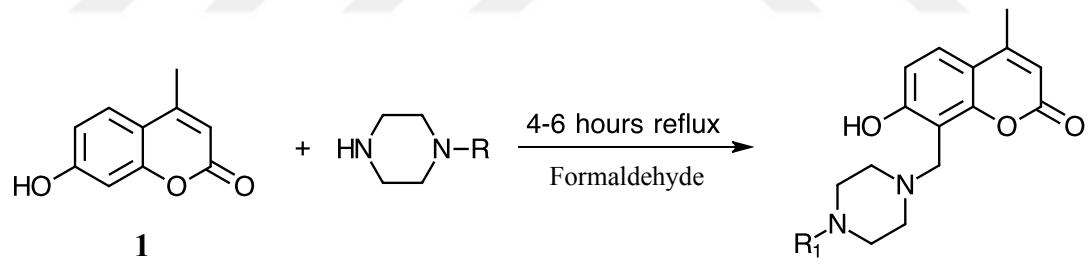
3.2. Methods of Synthesis

3.2.1. General Procudure A: Synthesis of Coumarin Scaffold



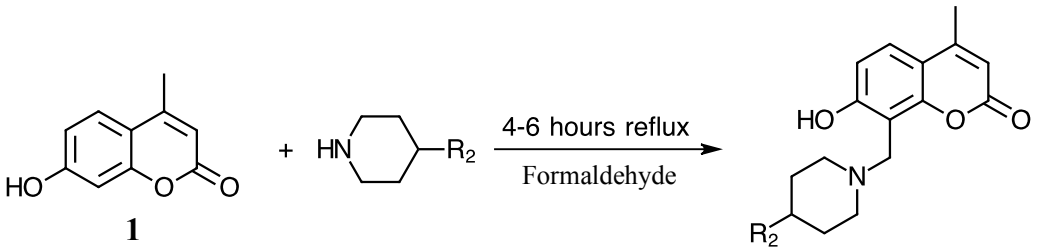
Resorcinol (1.22 eq) was dissolved in concentrated H_2SO_4 (20 mL) at 0 ^oC, then ethyl acetoacetate (1.0 eq) was slowly added and the mixture was stirred at 0-5 ^oC for 2 hours. At the end of the 2 hours, the reaction mixture was poured onto ice-water. Then the solid was washed with water and recrystallized from EtOH. Finally, pure white solid 7-Hydroxy-4-methyl-chromen-2-one was obtained [119].

3.2.2. General Procudure B: Synthesis of 7-hydroxy-4-methyl-8 (piperazines)chromen-2-ones



Compound **1** (7-Hydroxy-4-methyl-chromen-2-one; 1.0 eq) was dissolved in 95% EtOH (5 mL), then piperazine derivative(s) (R_1) (1.0 eq) and formaldehyde (0.2 mL) were added to the reaction medium. The reaction mixture was refluxed for 4-6 hours. At the end of the 4-6 hours, the reaction medium was cooled, then the solvent was evaporated in *vacuo*. Pale-yellow oils were obtained and further treated with a little amount of cool acetone. Then, the white solids were crystallized from acetone. [120].

3.2.3. Synthesis of 7-hydroxy-4-methyl-8-(piperidine)chromen-2-ones



Compound **1** (7-Hydroxy-4-methyl-chromen-2-one) was dissolved in 95% EtOH, then piperazine derivative(s) (R_2) and formaldehyde were added to the reaction medium. The reaction mixture was refluxed for 4-6 hours. At the end of the 4-6 hours, the reaction medium was cooled, then the solvent was evaporated in *vacuo*. Pale-yellow oils were obtained and further treated with a little amount of cool acetone. Then, the white solids were crystallized from acetone [120].

3.3. Analytical Methods

3.3.1. Melting Point Determination

Melting points of synthesized compounds were determined by using a Mettler Toledo FP62 capillary melting apparatus

3.3.2. Analysis of Thin Layer Chromatography

TLC Plates: TLC aluminum sheets 20x20 Silica gel 60 F_{254} (Merck)

Solvent Systems: Ethyl acetate:*n*-Hexane (50:50)

Dragging Process: TLC chamber was filled with the solvent system and kept for 1 hour for suitable saturation.

Spot determination: Spots of the synthesized compounds and their starting materials were determined by UV light (254/365 nm) and ninhydrine dye was used to visualize some piperazine and piperidine derivatives.

3.3.3. Spectrometric Methods

3.3.3.1. Fourier-Transform Infrared Spectroscopy (FT-IR)

Fourier-Transform Infrared Spectroscopy analyses were performed with a Perkin-Elmer Spectrum One series FT-IR apparatus (Version 5.0.1) and potassium bromide pellets were used. Spectrums were expressed in cm^{-1}.

3.3.3.2. ^{1}H-NMR

The ^{1}H-NMR spectra were recorded with a Bruker Spectrospin Avance DPX-400 (400 MHz), using tetramethylsilane (TMS) as the reference and dimethylsulfoxide (DMSO-d_6) and deuterated chloroform ($CDCl_3$) were used as a solvent. The chemical shifts were designated in parts per million (ppm).

3.3.3.3.^{13}C-NMR Spectra

The ^{13}C-NMR spectra were recorded with a Bruker Spectrospin Avance DPX-400 (400 MHz), using tetramethylsilane (TMS) as the reference and dimethylsulfoxide (DMSO-d_6) and deuterated chloroform ($CDCl_3$) were used as a solvent. The chemical shifts were designated in parts per million (ppm).

3.3.3.4. LC-MS Analyses

The LC-MS spectra were recorded with Waters Acquity UPLC. Photo Diode Detector, UPLC LG 500 nm, was used. Column was XBridge BEH C8 column. Mobile phase:
0-1 min: Acetonitrile : water : 1% formic acid = 9 : 90 : 1
1-2 min: Acetonitrile : water : 1% formic acid = 24 : 75 : 1
2-3 min: Acetonitrile : water : 1% formic acid = 0 : 99 : 1
3-12min: Acetonitrile : water : 1% formic acid = 9 : 90 : 1

3.4. Biological Activity Tests

Cell culture and viability

Cell viability was examined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Plated RAW264.7 cells ($2x10^5$ cells per mL) were exposed to 100 μM (highest applied concentration) of compounds dissolved in dimethyl

sulfoxide (DMSO). After 24 h incubation, MTT was added to all wells at 0.5 mg/mL of concentration and incubated an additional 2 h at 37 °C. After discarding the medium from plates, 100 µl of isopropanol was added to the wells. Absorbance of the MTT formazan was determined at 540 nm by a UV-spectrophotometric plate reader (Thermo Multiscan Spectrum, Finland). Viability was defined as the ratio (expressed as a percentage) of absorbance of the cells exposed to compounds to the cells treated with 0.5% DMSO (as control). All measurements were done in triplicates.

MCF-7 % Cell Viability Protocol

Cell viability was assayed by MTT (3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) test. MCF-7 human breast cancer cell line, plated on 48-well plates and was exposed to 10 µM of compounds prepared with dimethyl sulfoxide (DMSO) when cells reached 70-80% confluent. After 24 hours of incubation, the prepared MTT was applied at a concentration of 0.5 mg / mL and incubated for 2 hours at 37 ° C. Following incubation, 100 µL isopropanol was added to the wells and the absorption of MTT formazan was determined at 540 nm with a UV-spectrophotometric plate reader (Thermo Multiscan Spectrum, Finland). The absorbance of the cells exposed to the compounds for viability was defined as % viability by proportion to the viability of cells treated with 0.5% DMSO control. All measurements were done in triplicates.

Evaluation of Antiinflammatory Activity by Nitrite Assay

RAW264.7 macrophages cells (ATCC, USA) were maintained in DMEM (Gibco, England), supplemented with 10% FBS (Gibco, USA) and 1% streptomycin and penicillin (Gibco, USA) at 37°C in 5% CO_2. Antiinflammatory activity of the compounds were evaluated by measuring the stable nitric oxide (NO) metabolite, nitrite, with Griess assay (Kiemer and Vollmar, 1997). Briefly, RAW-264.7 cells were plated at the density of $2x10^5$ cell per mL in a 48 well-plate and incubated for 24 hours at 37°C in 5% CO_2. Plated cells were pre-treated with 100 µM (highest applied concentration) of compounds for 2 hours and then stimulated with 1 µg/mL of lipopolysaccharide (LPS) for additional 22 hours. The culture supernatant (50 µL) was mixed with Griess reagent [1% sulfanilamide and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride (Sigma, USA) in 5 % phosphoric acid (Mettler, Switzerland)] and incubated at room temperature for 10 min. The absorbance of the mixture was determined at 570 nm using a microplate reader (Multiskan Ascent, Finland). The amount of nitrite in the test samples was calculated

using sodium nitrite (Fluka Chemika, Germany) standard curve. As positive control, 100 µM of Indomethacin (Fluka Chemika, Germany) (dissolved in DMSO) and 100 µM of L-NAME (Nω-Nitro-L-arginine methyl ester hydrochloride) (prepared in DMEM) were used. Results are given as the ratio $[NO]_{treated\ cells}/[NO]_{untreated\ cells}$ and expressed as percentages. All measurements were done in triplicates.

Analgesic Activity

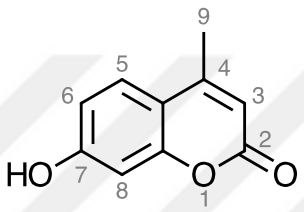
The analgesic activity was determined with the prostaglandin E2 ELISA Kit (Abcam, UK) according to the manufacturers instruction by using the supernatants of cells treated with LPS and compounds.

4. EXPERIMENTAL: SPECTRAL DATA

4.1. Coumarin Scaffold

7-Hydroxy-4-methylchromen-2-one (1)

(**CAS No:** 90-33-5)



Resorcinol (2.0 g, 0.018 mmol) and ethylacetoacetate (1.94 g, 0.015 mol), H_2SO_4 (5 mL) were reacted according to the general procedure A 3.2.1. The form of compound is white, powdered crystals.

$C_{10}H_8O_3$

Yield: 55%

Mw: 176.17 g/mol

MP: 188.8 ^{O}C

Rf: 0.49 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

FT-IR: (KBr), cm^{-1}: 3150-3050 (OH); 3011 (aromatic C-H); 2958 (aliphatic C-H); 1679 (C=O, lactone); 1599 (C=C, aromatic).

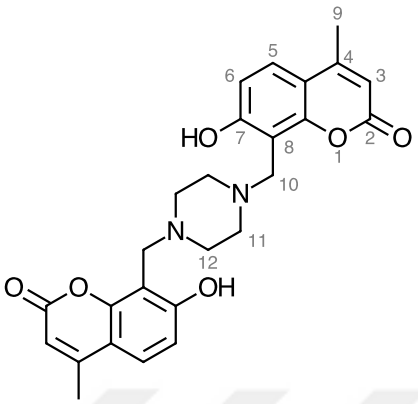
^{1}H NMR: (400 MHz, DMSO) δ 10.50 (s, 1H, OH); 7.55 (d, J = 8.7, 1H, H_5); 6.78 (dd, J_1 =8.7 Hz, J_2 = 2.4 Hz, 1H, H_6); 6.67 (d, J = 2.4 Hz, 1H, H_8); 6.08 (q, J = 1.2 Hz, 1H, H_3); 3.33 (d, J = 1.2 Hz 3H, H_9).

^{13}C NMR: (400 MHz, DMSO) δ 161.5 (C_2); 160.7, 155.2, 153.9, 127.0, 113.2, 112.4 ($C_{coumarin\text{-}aromatic}$); 110.6, 102.6 ($C_{3\text{-}4}$); 18.5 ($C_9$).

LC-MS: rt= 5.71 min., *m/z*, 178.13 $[M+H]^+$, Purity: 91%, UV (ACN/Water, λ_{max}): 320 nm.

4.2. Piperazine Derivatives (2-32)

1,4-bis[(7-hydroxy-4-methylcoumarin-8-yl)methyl] piperazine (2) [121]



Compound **1** (175 mg, 0.99 mmol), piperazine (1.71 mg, 0.99 mmol), formaldehyde (0.1 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{15}H_{18}N_2O_3$

Yield: 63% (171 mg, white solid)

Mw: 462.49 g/mol

Rf: 0.1 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

FT-IR: (KBr), cm^{-1}: 3200-3050 (OH); 3048 (aromatic C-H); 2958 (aliphatic C-H); 1722 (C=O, lactone); 1627 (C=C, alkene); 1602 (C=C, aromatic).

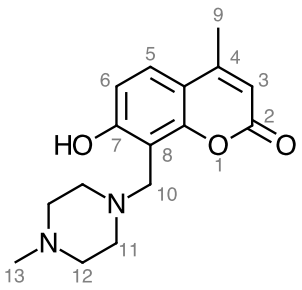
^{1}H NMR (400 MHz, $CDCl_3$) δ7.41 (t, J = 9.70 Hz, 2H, H_5); 6.77 (dd, J_1 = 8.70 Hz, J_2 = 15.3 Hz, 2H, H_6); 6.08 (d, J = 6.50 Hz, 2H, H_3); 4.08 (d, J = 15.80 Hz, 4H, H_{10}); 2.49 (s, 16H, $H_{11,12}$); 2.38 (s, 6H, H_9).

^{13}C NMR (400 MHz, $CDCl_3$) δ 162.5 (C_2); 161.2, 153.3, 152.4, 126.8, 113.3, 112.1 ($C_{coumarin\text{-}aromatic}$); 110.6, 107.6 ($C_{3,4}$); 53.8, 52.6 ($C_{11,12}$); 51.4 ($C_{10}$); 18.8 ($C_{10}$).

LC-MS: rt= 5.10 min., *m/z*, 463.42 $[M+H]^+$, Purity: 93%, UV (ACN/Water, λ_{max}): 320 nm.

7-Hydroxy-4-methyl-8-(4-methylpiperazin-1-ylmethyl)chromen-2-one (3)

(**CAS No:** 307535-36-0), [120, 122-124]



Compound **1** (250 mg, 1.42 mmol), N-methylpiperazine (142 mg, 1.42 mmol), formaldehyde (0.15 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2.

$C_{16}H_{20}N_2O_3$

Yield: 32%

Mw: 288.34 g/mol

MP: 131.8 ^{O}C

Rf: 0.1 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

FT-IR: (KBr), cm^{-1}: 3300-3100 (OH); 3071 (aromatic C-H), 1726 (C=O, lactone), 1609 (C=C, nonaromatic), 1580 (Ar C=C).

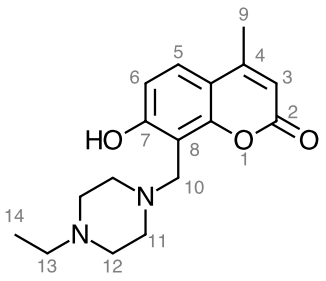
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.7 Hz, 1H, H_5); 6.75 (d, *J* = 8.7 Hz, 1H, H_6); 6.07 (q, *J* =1.1 Hz, 1H, H_3); 4.06 (s, 2H, H_{10}); 2.90 – 2.40 (m, 8H, $H_{11,12}$); 2.37 (d, *J* =1.1 Hz, 3H, H_9); 2.31 (s, 3H, H_{13}).

^{13}C NMR (101 MHz, $CDCl_3$) δ 162.4 (C_2); 161.2, 153.2, 152.5, 124.6, 113.3, 112.2 ($C_{coumarin\text{-}aromatic}$); 110.6, 107.5 ($C_{3\text{-}4}$); 54.7, 53.7 ($C_{11\text{-}12}$); 52.5 ($C_{10}$); 45.8 ($C_{13}$); 18.7 ($C_9$).

LC-MS: rt= 0.9 min., *m/z* 289.33 $[M+H]^+$. Purity: 92%, UV (ACN/Water, λ_{max},): 322nm.

8-(4-Ethylpiperazin-1-ylmethyl)-7-hydroxy-4-methylchromen-2-one (4)

(**CAS No:** 347914-58-3)



Compound **1** (350 mg, 1.98 mmol), ethylpiperazine (227 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{17}H_{22}N_2O_3$

Yield: 47%

Mw: 302.37g/mol

MP: 125.4 ^{O}C

Rf: 0.12 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

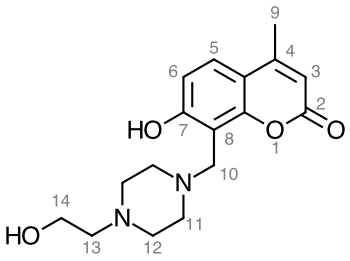
FT-IR: (KBr), cm^{-1}: 3250-3050 (OH); 3075 (aromatic C-H), 1720 (C=O, lactone), 1600 (C=C, nonaromatic), 1567 (C=C, aromatic).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, J = 8.7 Hz, 1H, H_5); 6.76 (d, J = 8.7 Hz, 1H, H_6); 6.07 (q, J =1.1 Hz, 1H, H_3); 4.07 (s, 2H, H_{10}); 3.77 – 2.50 (m, 8H, $H_{11,12}$); 2.44 (q, J = 7.2 Hz, 2H, C_{13}); 2.37 (d, J =1.1 Hz, 3H, H_9); 2.16 (s, 1H, OH); 1.08 (t, J = 7.2 Hz, 3H, H_{14}).

^{13}C NMR (101 MHz, $CDCl_3$) δ 162.5 (C_2), 161.2, 153.7, 153.3, 152.5, 124.6, 113.4 ($C_{coumarin\text{-}aromatic}$); 110.6, 107.5 ($C_{3,4}$); 58.9, 52.6 ($C_{11,12}$); 52.0 ($C_{10}$); 52.0 ($C_{13}$); 18.9($C_9$); 11.9 ($C_{14}$).

LC-MS: rt= 0.9 min., *m/z* 303.38 $[M+H]^+$, Purity: 95%, UV (ACN/Water, $\lambda_{max,}$): 322 nm.

7-Hydroxy-8-[4-(2-hydroxyethyl)piperazin-1-ylmethyl]-4-methylchromen-2-one (5) (**CAS No:** 294875-39-1)



Compound **1** (350 mg, 1.98 mmol), 1-(2-hydroxyethyl)piperazine (259 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{17}H_{22}N_2O_4$

Yield: 26%

Mw: 318.37g/mol

MP: 140 ^{O}C

Rf: 0.1 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

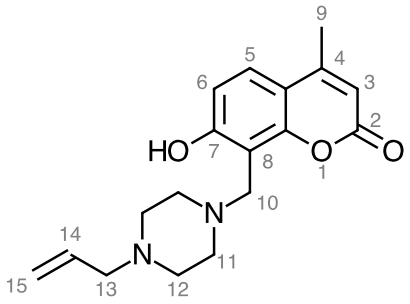
FT-IR: (KBr), cm^{-1}: 3100-3400 (OH); 3222 (aromatic C-H), 1719 (C=O, lactone), 1597 (C=C, nonaromatic), 1567 (C=C, aromatic).

^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.7 Hz, 1H, H_5); 6.76 (d, *J* = 8.7 Hz, 1H, H_6); 6.07 (q, *J* =1.1 Hz, 1H, H_3); 4.07 (s, 2H, H_{10}); 3.63 (t, *J* = 5.3 Hz, 2H, H_{14}); 3.58 – 2.55 (m, 8H, $H_{11,12}$); 2.58 (t, *J* = 5.3 Hz, 2H, H_{13}); 2.38 (d, *J* =1.1 Hz, 3H, H_9), 2.17 (s, 1H, OH).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 162.2 (C_2); 161.1, 153.2, 152.5, 124.6, 113.3, 112.3 ($C_{coumarin\text{-}aromatic}$); 110.7, 107.5 ($C_{3,4}$); 59.0, 57.8 ($C_{11,12}$); 53.7 ($C_{14}$); 52.6 ($C_{10}$); 52.5 ($C_{13}$); 18.8 ($C_9$).

LC-MS: rt= 0.91 min., *m/z* 319.37 $[M+H]^+$, Purity: 94%, UV (ACN/Water, $\lambda_{max,}$): 322 nm Purity: 97%.

8-(4-allylpiperazin-1-ylmethyl)-7-hydroxy-4-methylchromen-2-one (6) [125]



Compound **1** (350 mg, 1.98 mmol), allylpiperazine (250 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{18}H_{22}N_2O_3$

Yield: 50%

Mw: 314.38g/mol

MP: 107.5 ^{O}C

Rf: 0.14 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

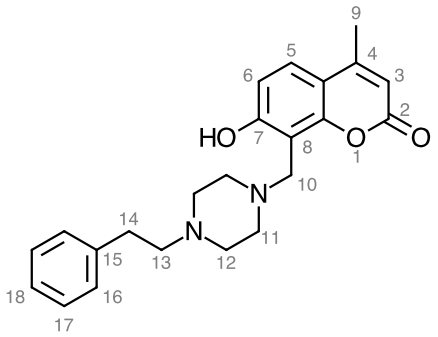
FT-IR: (KBr), cm^{-1}: 3100-3300 (OH); 3067 (aromatic C-H), 3008 (aliphatic C-H); 1728 (C=O, lactone), 1599 (C=C, nonaromatic), 1495 (C=C, aromatic).

^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.40 (d, J = 8.7 Hz, 1H, H_5); 6.75 (d, J = 8.7 Hz, 1H, H_6); 6.07 (d, J = 1.2 Hz, 1H, H_3); 5.84 (tt, J_1=5.8 Hz, J_2= 17.0 Hz, H_{14}); 5.18 (ddt, J_1= 1.5 Hz, J_2= 1.5 Hz, J_3= 17 Hz, 2H, H_{15}); 4.06 (s, 2H, H_{10}); 3.02 (dt, J_1 = 1.5 Hz, J_2 = 5.8 Hz, 2H, H_{13}); 2.98 – 2.40 (m, 8H, $H_{11,12}$); 2.37 (d, J = 1.2 Hz, 3H, H_9).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 162.4 (C_2); 161.2, 153.2, 152.4, 124.6, 113.3, 112.2 ($C_{coumarin\text{-}aromatic}$); 134.4, 118.4 ($C_{14,15}$); 110.6, 107.5 ($C_{3,4}$); 61.4 ($C_{13}$); 53.8, 52.5 ($C_{11,12}$); 52.5 ($C_{10}$); 18.8 ($C_9$).

LC-MS: rt= 0.91 min., *m/z* 319.37 $[M+H]^+$, Purity: 97%, UV (ACN/Water, $\lambda_{max,}$): 322 nm Purity: 95%.

7-Hydroxy-4-methyl-8-(4-phenethylpiperazin-1-ylmethyl)chromen-2-one (7)



Compound **1** (350 mg, 1.98 mmol), 1-(2-phenylethyl)piperazine (378 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{23}H_{26}N_2O_3$

Yield: 78%

Mw: 378.46 g/mol

MP: > 300 ^{O}C

Rf: 0.12 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

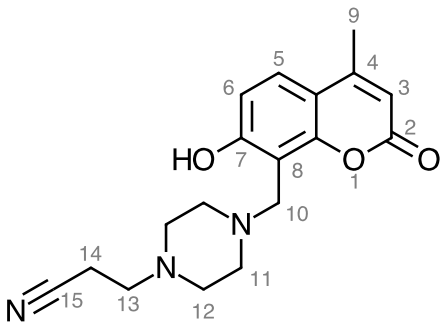
FT-IR: (KBr), cm^{-1}: 3300-3100 (OH); 3027 (aromatic C-H); 2952 (aliphatic C-H); 1706 (C=O, lactone); 1598 (C=C, aromatic).

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.25 (s, 1H, OH); 7.38 (d, *J* = 8.7 Hz, 1H, H_5); 7.26 (m, 2H, H_{16}); 7.17 (m, 3H, $H_{17,18}$); 6.74 (d, *J* = 8.7 Hz, 1H, H_6); 6.05 (q, *J* = 1.2 Hz, 1H, H_3); 4.04 (s, 2H, H_{10}); 2.90-2.79 (m, 12H, $H_{11,12,13,14}$); 2.35 (d, *J* = 1.14 Hz, 3H, H_9).

^{13}C NMR (400 MHz, $CDCl_3$) δ 162.4 (C_2); 161.1, 153.2, 152.5, 128.3, 113.3, 122.2 ($C_{Coumarin\text{-}aromatic}$); 140.0 ($C_{15}$); 128.6 ($C_{17}$); 126.1 ($C_{16}$); 124.6 ($C_{18}$); 110.6, 107.5 ($C_{3,4}$); 60.0 ($C_{13}$); 53.8, 52.7 ($C_{11,12}$); 52.5 ($C_{10}$); 33.5 ($C_{14}$); 18.8 ($C_9$).

LC-MS: rt= 4.80 min., *m/z* 379.21 $[M+H]^+$, Purity: 98%, UV (ACN/Water, λ_{max}): 322 nm, Purity: 97%.

3-[4-(7-Hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yl)methyl)-piperazin-1-yl]-propionitrile (8)



Compound **1** (350 mg, 1.98 mmol), 3-piperizinopropionitrile (276.5 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{18}H_{21}N_3O_3$

Yield: 35%

Mw: 327.38 g/mol

MP: 207.1 ^{O}C

Rf: 0.13 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

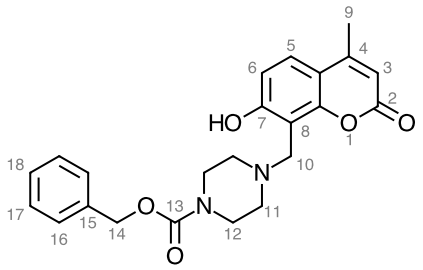
FT-IR: (KBr), cm^{-1}: 3350-3010 (OH); 3062 (aromatic C-H); 2950 (aliphatic C-H); 2249 (CN); 1711 (C=O, lactone), 1626 (C=C, nonaromatic); 1599 (C=C, aromatic).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, J_2 = 8.75 Hz, 1H, H_5); 6.73 (d, J_1 = 8.78 Hz, 1H, H_6); 6.03 (q, J = 1.25 Hz, 1H, H_3); 4.04 (s, 2H, H_{10}); 2.69 (t, J = 7.01 Hz, 2H, H_{13}); 3.00 – 2.40 (broad s, 8H, $H_{11,12}$); 2.49 (t, J = 7.01 Hz, 2H, H_{14}); 2.34 (d, J = 1.12 Hz, 3H, H_9).

^{13}C NMR (400 MHz, $CDCl_3$) δ 161.5 (C_2); 161.8, 160.9, 153.2, 124.8, 113.3, 112.4 ($C_{coumarin\text{-}aromatic}$); 118.5 ($C_{15}$); 110.7, 107.4 ($C_{3,4}$); 53.6, 53.0 ($C_{11,12}$); 52.2 ($C_{10}$); 18.7 ($C_9$), 15.9 ($C_{14}$).

LC-MS: rt= 2.93 min., *m/z* 328.44 $[M+H]^+$, Purity: 96%, UV (ACN/Water, λ_{max}): 322 nm, Purity: 93%.

4-(7-Hydroxy-4-methyl-2-oxo-2*H*-chromen-8-ylmethyl)-piperazine-1-carboxylic acid benzyl ester (11)



Compound **1** (350 mg, 1.98 mmol), benzyl 1-piperazine carboxylate (437.6 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{23}H_{24}N_2O_5$

Yield: 25%

Mw: 408.45 g/mol

MP: 94.4 ^{O}C

Rf: 0.25 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

FT-IR: (KBr), cm^{-1}: 3350-3150 (OH); 3030 (aromatic C-H); 2958 (aliphatic C-H); 1730 (C=O, lactone); 1626 (C=C, nonaromatic); 1600 (C=C, aromatic).

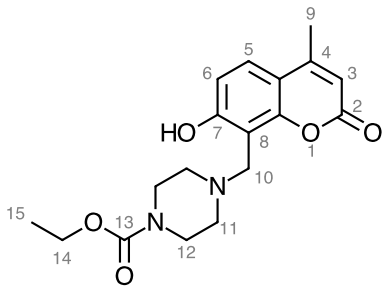
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, J = 8.72 Hz, 1H, H_5); 7.22 – 7.18 (m, 5H, $H_{15,16,17,18}$); 6.75 (d, J = 8.70 Hz, H_6); 6.04 (s, 1H, H_3); 4.92 (s, 2H, H_{14}); 4.01 (s, 2H, H_{10}); 3.57 – 2.58 (m, 8H, $H_{11,12}$); 2.38 (s, 3H, H_9).

^{13}C NMR (400 MHz, $CDCl_3$) δ 168.2 (C_{13}); 161.7 (C_2); 160.4, 156.3, 152.4, 122.8, 113.7, 111.4 ($C_{Coumarin\text{-}aromatic}$); 142.3 ($C_{15}$); 125.8 ($C_{16}$); 127.9 ($C_{17}$); 126.2 ($C_{18}$); 109.8, 107.1 ($C_{3,4}$); 68.9 ($C_{14}$); 55.7, 53.2 ($C_{11,12}$); 43.2 ($C_{10}$); 18.7

LC-MS: rt= 6.16 min., *m/z* 409.38 $[M+H]^+$, Purity: 94%; UV (ACN/Water, λ_{max}): 198 nm.

4-(7-Hydroxy-4-methyl-2-oxo-2*H*-chromen-8-ylmethyl)-piperazine-1-carboxylic acid ethyl ester (12)

(**CAS No:** 1024743-11-0), **[120]**



Compound **1** (350 mg, 1.98 mmol), ethyl 1-piperazine carboxylate (314 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{18}H_{22}N_2O_5$

Yield: 31%

Mw: 346.38 g/mol

MP: 137.3 ^{O}C

Rf: 0.22 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

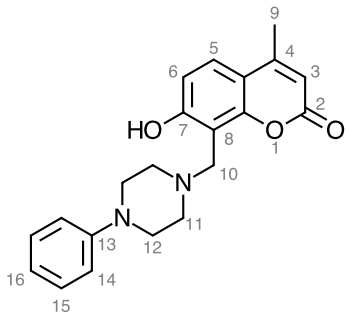
FT-IR: (KBr), cm^{-1}: 3300-3100 (OH); 3050 (aromatic C-H); 2987 (aliphatic C-H); 1725 (C=O, lactone); 1688 (C=O, amide); 1625 (C=C, nonaromatic); 1602 (C=C, aromatic).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, *J* = 8.71 Hz, 1H, H_5); 6.73 (d, *J* = 8.71 Hz, 1H, H_6); 6.04 (s, 1H, H_3); 4.12 (q, *J*=7.1 Hz, 2H, H_{14}); 4.03 (s, 2H, H_{10}); 3.53 (broad s, 4H, H_{12}); 2.57 (broad s, 4H, H_{11}); 2.35 (s, 3H, H_9); 1.22 (t, *J*=7.1 Hz, 3H, H_{15}).

^{13}C NMR (400 MHz, $CDCl_3$) δ 161.9 (C_2); 160.9, 155.2, 153.2, 124.8, 113.3, 112.4 ($C_{Coumarin\text{-}aromatic}$); 152.4 ($C_{13}$); 110.8, 107.3 ($C_{3,4}$); 61.6 ($C_{14}$); 53.7, 52.2 ($C_{11,12}$); 43.2 ($C_{10}$); 18.7 ($C_9$); 14.5 ($C_{15}$).

LC-MS: rt= 3.96 min., *m/z* 347.09 $[M+H]^+$, Purity: 95%, UV (ACN/Water, λ_{max}): 322 nm.

7-Hydroxy-4-methyl-8-(4-phenylpiperazin-1-ylmethyl)chromen-2-one (15)



Compound **1** (350 mg, 1.98 mmol), N-phenylpiperazine (322 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{21}H_{22}N_2O_3$

Yield: 53%

Mw: 350.41g/mol

MP: 260.5 ^{O}C

Rf: 0.33 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

FT-IR: (KBr), cm^{-1}: 3200-3000 (OH); 3047 (aromatic C-H); 2956 (aliphatic C-H); 1715 (C=O, lactone); 1626 (C=C, nonaromatic); 1599 (C=C, aromatic).

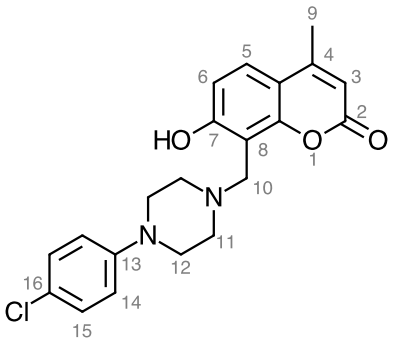
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, *J* = 8.7 Hz, 1H, H_5); 7.96-7.27 (m, 2H, H_{15}); 6.96-6.87 (m, 3H, $H_{14,16}$); 6.79 (d, *J* = 8.7 Hz, 1H, H_6); 6.09 (q, *J* = 1.10 Hz, 1H, H_3); 4.13 (s, 2H, H_{10}); 3.04 (broad s, 8H, $H_{11,12}$); 2.39 (d, *J* = 1.11 Hz, 3H, H_9).

^{13}C NMR (400 MHz, $CDCl_3$) δ 162.2 (C_2); 161.1, 153.2, 152.5, 129.1, 124.7, 113.3 ($C_{Coumarin\text{-}aromatic}$); 150.7 ($C_{13}$); 120.4 ($C_{15}$); 116.4 ($C_{16}$); 112.3, 110.7 ($C_{3,4}$); 107.5 ($C_{14}$); 53.8 ($C_{10}$); 52.6, 49.1 ($C_{11,12}$); 18.7 ($C_9$).

LC-MS: rt= 5.63 min., *m/z* 351.37 $[M+H]^+$. Purity: 97%, UV (ACN/Water, λ_{max}): 322 nm.

8-[4-(4-Chlorophenyl)piperazin-1-ylmethyl]-7-hydroxy-4-methylchromen-2-one (16)

(**CAS No:** 142529-58-6)



Compound **1** (350 mg, 1.98 mmol), N-(4-chlorophenyl)piperazine (390 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{21}H_{21}ClN_2O_3$

Yield: 64%

Mw: 384.85g/mol

MP: 199.7 ^{O}C

Rf: 0.35 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

FT-IR: (KBr), cm^{-1}: 3300-3050 (OH); 3043 (aromatic C-H); 2966 (aliphatic C-H); 1714 (C=O, lactone); 1625 (C=C, nonaromatic); 1599 (C=C, aromatic).

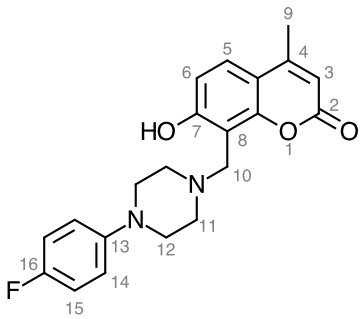
^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.43 (d, J = 8.75 Hz, 1H, H_5); 7.22 (d, J = 9.0 Hz, 2H, H_{15}); 6.84 (d, J = 9.0 Hz, 2H, H_{14}); 6.79 (d, J = 8.75 Hz, 1H, H_6); 6.10 (q, J = 1.2 Hz, 1H, H_3); 4.13 (s, 2H, H_{10}); 3.36 – 2.63 (br, 8H, $H_{11,12}$); 2.39 (d, J = 1.1 Hz, 3H, H_9).

^{13}C NMR: (101 MHz, $CDCl_3$) δ 162.2(C_2); 161.1, 153.3, 152.5, 125.2, 117.6, 110.8 ($C_{coumarin\text{-}aromatic}$); 149.3, 129.1, 124.8, 113.4, ($C_{phenyl\text{-}arom}$); 112.4, 107.4 ($C_{3,4}$); 53.7, 52.4 ($C_{11,12}$); 49.1 (C_{10}); 18.8 (C_9).

LC-MS: rt= 6.56 min., *m/z* 385.38 $[M+H]^+$, Purity: 96%, UV (ACN/Water, λ_{max}): 322 nm.

8-[(4-(4-Fluorophenyl)piperazin-1-yl)methyl]-7-hydroxy-4-methylchromen-2-one (17)

(**CAS No:** 1574411-13-4)



Compound **1** (350 mg, 1.98 mmol), 1-(4-fluorophenyl)piperazine (358 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{21}H_{21}FN_2O_3$

Yield: 66%

Mw: 368.40g/mol

MP: > 300 ^{O}C

Rf: 0.31 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

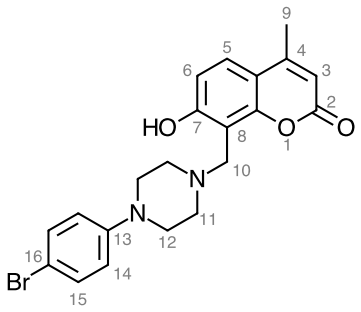
FT-IR: (KBr), cm^{-1}: 3250-3050 (OH); 3053 (aromatic C-H); 2989 (aliphatic C-H); 1718 (C=O, lactone); 1622 (C=C, nonaromatic); 1594 (C=C, aromatic).

^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.43 (d, J = 8.7 Hz, 1H, H_5); 7.01 – 6.92 (m, 2H, H_{15}); 6.91 – 6.86 (m, 2H, H_{14}); 6.78 (d, J = 8.7 Hz, 1H, H_6); 6.09 (q, J = 1.21 Hz, 1H, H_3); 4.13 (s, 2H, H_{10}); 3.29 – 2.65 (br, 8H, $H_{11,12}$); 2.39 (d, J = 1.18 Hz, 3H, H_9).

^{13}C NMR: (400 MHz, $CDCl_3$) δ 162.2 (C_2); 161.2, 153.3, 14.7, 118.3, 118.2, 115.7, 115.5, 113.4, 112.4 (C_{arom}); 110.8, 107.5 ($C_{3,4}$); 53.7, 52.6 ($C_{11,12}$); 50.1(C_{10}); 18.8 (C_9).

LC-MS: rt= 6.02 min., *m/z* 369.32 $[M+H]^+$, Purity: 98%, UV (ACN/Water, λ_{max}): 239 nm, 321 nm.

7-Hydroxy-8-[4-(bromophenyl)-piperazin-1-ylmethyl]-4-methylchromen-2-one (18)



Compound **1** (350 mg, 1.98 mmol), 1-(4-bromophenyl)piperazine (479 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{21}H_{21}BrN_2O_3$

Yield: 50%

Mw: 429.31 g/mol

MP: 221.1 ^{O}C

Rf: 0.5 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

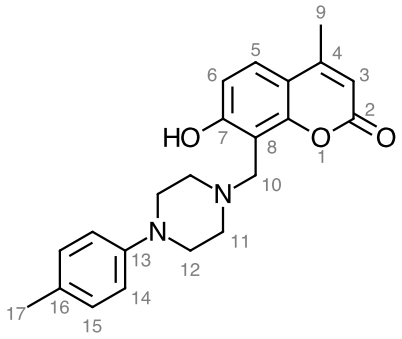
FT-IR: (KBr), cm^{-1}: 3350-3100 (OH); 3038 (aromatic C-H); 2965 (aliphatic C-H); 1702 (C=O, lactone); 1625 (C=C, nonaromatic); 1598 (C=C, aromatic).

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.58 (s, 1H, OH); 7.41 (d, *J* = 8.8 Hz, 1H, H_5); 7.32 (d, *J* = 8.8 Hz, 2H, H_{15}); 6.77 (d, *J* = 8.8 Hz, 3H, $H_{6,14}$); 6.07 (s, 1H, H_3); 4.10 (s, 2H, H_{10}); 3.21 (broad s, 4H, H_{12}); 2.72 (broad s, 4H, H_{11}); 2.37 (s, 3H, H_9).

^{13}C NMR: (400 MHz, $CDCl_3$) δ 162.1 (C_2); 161.0, 153.2, 152.5, 124.8, 113.3, 112.4 ($C_{Coumarin\text{-}aromatic}$); 149.7 ($C_{13}$); 131.9 ($C_{15}$); 117.9 ($C_{14}$); 112.5 ($C_{16}$); 110.7, 107.5 ($C_{3,4}$); 53.7, 52.4 ($C_{11,12}$); 48.8 ($C_{10}$); 18.8 ($C_9$).

LC-MS: rt= 6.60 min., *m/z* 432.24, 433.21 $[M+H]^+$, Purity: 96%, UV (ACN/Water, λ_{max}): 252 nm.

7-Hydroxy-4-methyl-8-(4-*p*-tolyl-piperazin-1-ylmethyl)chromen-2-one (19)



Compound **1** (350 mg, 1.98 mmol), 1-(4-methylphenyl)piperazine (350 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{22}H_{24}N_2O_3$

Yield: 60%

Mw: 364.44g/mol

MP: 264.7 ^{O}C

Rf: 0.33 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

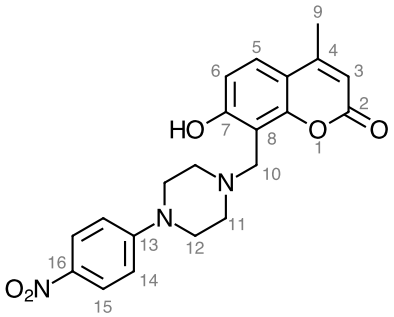
FT-IR: (KBr), cm^{-1}: 3200-3050 (OH); 3008 (aromatic C-H); 2962 (aliphatic C-H); 1703 (C=O, lactone); 1624 (C=C, nonaromatic); 1599 (C=C, aromatic).

^{1}H NMR: (400 MHz, DMSO) δ 7.54 (d, *J* = 8.8 Hz, 1H, H_5); 7.00 (d, *J* = 8.7 Hz, 2H, H_{15}); 6.82 (d, *J* = 8.7 Hz, 2H, H_{14}); 6.78 (d, *J* = 8.7 Hz, 1H, H_6); 6.12 (q, *J* = 1.2 Hz, 1H, H_3); 3.92 (s, 2H, H_{10}); 3.08 (t, *J*=4.5 Hz, 4H, H_{12}); 2.67 (t, *J*=4.5 Hz, 4H, H_{11}); 2.36 (d, *J* = 1.2 Hz, 3H, H_9); 2.17 (s, 3H, H_{17}).

^{13}C NMR: (400 MHz, DMSO-d_6) δ 162.2 (C_2); 160.5, 154.5, 153.2, 149.1, 129.8, 128.3, 116.2, 113.3 ($C_{aromatics}$); 110.1, 108.8 ($C_{3,4}$); 52.5, 49.1 ($C_{11,12}$); 40.5 (C_{10}); 20.3 (C_{17}); 18.8 (C_9).

LC-MS: rt= 6.37 min., *m/z* 365.22 $[M+H]^+$, Purity: 95%, UV (ACN/Water, λ_{max}): 322 nm.

7-Hydroxy-4-methyl-8[4-(4-nitrophenyl)-piperazin-1-ylmethyl]-chromen-2-one (20)



Compound **1** (350 mg, 1.98 mmol), 1-(*p*-nitrophenyl)piperazine (412 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is yellow, powdered crystals.

$C_{21}H_{21}N_3O_5$

Yield: 47%

Mw: 395.41g/mol

MP: 222 ^{O}C

Rf: 0.17 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

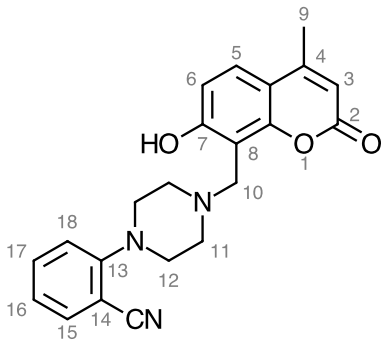
FT-IR: (KBr), cm^{-1}: 3300-3100 (OH); 3069 (aromatic C-H); 2995 (aliphatic C-H); 1722 (C=O, lactone); 1624 (C=C, nonaromatic); 1587, 1384 (NO_2).

^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.14 (d, *J* = 9.4 Hz, 1H, H_{15}); 7.45 (d, *J* = 8.76 Hz, 1H, H_5); 6.86 (d, *J* = 9.4 Hz, 2H, H_{14}); 6.80 (d, *J* = 8.76 Hz, 1H, H_6); 6.10 (q, *J* = 1.16 Hz, 1H, H_3); 4.14 (s, 2H, H_{10}); 3.64 – 3.40 (br, 4H, H_{12}); 2.92 – 2.72 (br, 4H, H_{11}); 2.40 (d, *J*= 1.13 Hz, 3H, H_9).

^{13}C NMR: (400 MHz, $CDCl_3$) δ 161.8 (C_2); 161.0, 154.5, 153.3, 125.8, 125.0, 113.4, 113.3 ($C_{aromatic}$); 10.9, 107.2 ($C_{3,4}$); 53.6, 52.0 ($C_{11,12}$); 46.9 (C_{10}); 18.8 (C_9).

LC-MS: rt= 5.93 min., *m/z* 396.40 $[M+H]^+$, Purity: 97%, UV (ACN/Water, λ_{max}): 222, 324, 379 nm.

2-[4-(7-Hydroxy-4-methyl-2-oxo-2*H*-chromen-8-ylmethyl)-piperazin-1-yl]-benzonitrile (21)



Compound **1** (256 mg, 1.45 mmol), 1-(2-cyanophenyl)piperazine (272 mg, 1.45 mmol), formaldehyde (0.15 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{22}H_{21}N_3O_3$

Yield: %28

Mw: 375.42g/mol

MP: > 300 ^{O}C

Rf: 0.23 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

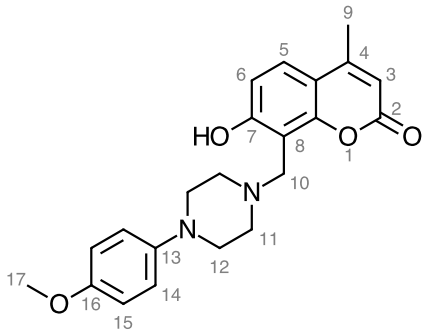
FT-IR: (KBr), cm^{-1}: 3250-3050 (OH); 3072 (aromatic C-H); 2978 (aliphatic C-H); 2217 (CN); 1725 (C=O, lactone); 1624 (C=C, nonaromatic); 1598 (C=C, aromatic).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (dd, J_1 = 1.38 Hz, J_2 = 7.68 Hz, 1H, H_{15}); 7.51 (ddd, J_1 = 1.6 Hz, J_2 = 7.5 Hz, J_3 = 8.3 Hz, 1H, H_{16}); 7.43 (d, J = 8.7 Hz, 1H, H_5); 7.06 (td, J_1 = 1.0, Hz, J_2 = 7.60 Hz, 1H, H_{17}), 7.01 (d, J= 7.0 Hz, 1H, H_{18}); 6.79 (d, J = 8.72 Hz, 1H, H_6), 6.10 (q, J = 1.21 Hz, 1H, H_3); 4.17 (s, 2H, H_{10}); 3.41 – 3.17 (br, 4H, H_{12}); 2.95 – 2.80 (br, 4H, H_{11}); 2.39 (d, J = 1.20 Hz, 3H, H_9).

^{13}C NMR (400 MHz, $CDCl_3$) δ 162.0 (C_2); 161.1, 154.9, 152.5, 124.8, 113.3, 112.5 ($C_{aromatic\text{-}coumarin}$); 153.2 ($C_{13}$), 134.4 ($C_{17}$), 133.9 ($C_{15}$),, 122.41 ($C_{16}$), 118.8 ($C_{18}$),, 106.4 ($C_{14}$); 118.1 (CN); 110.9, 107.5 ($C_{3,4}$); 53.7, 52.5 ($C_{11,12}$); 51.2 (C_{10}); 18.8 (C_9).

LC-MS: rt= 5.56 min., *m/z* 376.37 $[M+H]^+$, Purity: 96%, UV (ACN/Water, λ_{max}): 322 nm.

7-Hydroxy-8-[4-(4-methoxy-phenyl)-piperazin-1-ylmethyl]-4-methyl-chromen-2-one (22)



Compound **1** (350 mg, 1.98 mmol), 1-(4-methoxyphenyl)piperazine (382 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{22}H_{24}N_2O_4$

Yield: 62%

Mw: 380.44g/mol

MP: > 300 ^{O}C

Rf: 0.24 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

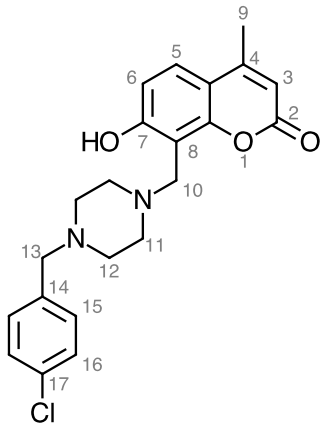
FT-IR: (KBr), cm^{-1}: 3300-3100 (OH); 3046 (aromatic C-H); 2947 (aliphatic C-H); 1715 (C=O, lactone); 1624 (C=C, nonaromatic); 1599 (C=C, aromatic).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, *J* = 8.74 Hz, 1H, H_5); 6.92-6.82 (m, 4H, H_{14+15}); 6.78 (d, *J* = 8.76 Hz, 1H, H_6); 6.09 (q, *J* = 1.18 Hz, 1H, H_3); 4.12 (s, 2H, H_{10}); 3,76 (s, 3H, H_{17}); 3.27 – 2.70 (br, 8H, $H_{11,12}$); 2.39 (d, *J* = 1.17 Hz, 3H, H_9).

^{13}C NMR (400 MHz, $CDCl_3$) δ 162.2 (C_2); 161.2, 154, 153, 145, 124, 112.3 ($C_{aromatic-coumarin}$); 152.5, 118.6, 114.5, 113.4 ($C_{13,14,15,16}$); 110.7, 107.5 ($C_{3,4}$); 55.5 (C_{17}); 53.8, 52.7 $C_{11,12}$); 50.6 (C_{10}); 18.8 (C_9).

LC-MS: rt= 5.41 min., *m/z* 381.22 $[M+H]^+$, Purity: 95%, UV (ACN/Water, λ_{max}): 200 nm.

8-[4-(4-Chlorobenzyl)-piperazin-1-ylmethyl]-7-hydroxy-4-methylchromen-2-one (23)



Compound **1** (350 mg, 1.98 mmol), 1-(4-chlorobenzyl)piperazine (419 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{22}H_{23}ClN_2O_3$

Yield: 38%

Mw: 398.88 g/mol

MP: 185.2^{O}C

Rf: 0.19 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

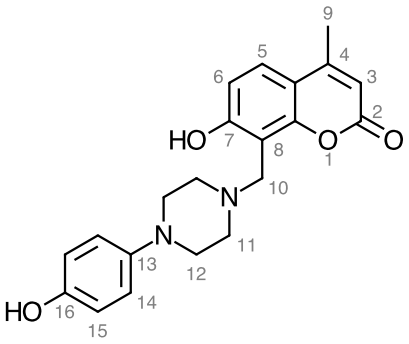
FT-IR: (KBr), cm^{-1}: 3250-3100 (OH); 3061 (aromatic C-H); 2951 (aliphatic C-H); 1716 (C=O, lactone); 1626 (C=C, nonaromatic); 1601 (C=C, aromatic).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 (d, *J* = 8.72 Hz, 1H, H_5); 7.29-7.23 (m, 4H, $H_{15,16}$); 6.74 (d, *J* = 8.76 Hz, 1H, H_6); 6.07 (q, *J* = 1.20 Hz, 1H, H_3); 4.06 (s, 2H, H_{10}); 3.49 (s, 2H, H_{13}); 3.05 – 2.46 (br, 8H, $H_{11,12}$); 2.37 (d, *J* = 1.19 Hz, 3H, H_9).

^{13}C NMR (400 MHz, $CDCl_3$): δ 162.4 (C_2); 161.2, 153.2, 152.5, 124.6, 113.3, 112.2 ($C_{aromatic\text{-}coumarin}$); 136.2 ($C_{14}$); 132.9 ($C_{17}$); 130.2 ($C_{15}$); 128.4 ($C_{16}$); 110.6, 107.5 ($C_{3,4}$); 61.8 ($C_{13}$); 53.8, 52.6 ($C_{11,12}$); 52.5 ($C_{10}$); 18.8 ($C_9$).

LC-MS: rt= 6.20 min., *m/z* 399.36 $[M+H]^+$, Purity: 97%, UV (ACN/Water, λ_{max}): 220, 322 nm.

7-Hydroxy-8-[4-(4-hydroxyphenyl)-piperazin-1-ylmethyl]-4-methylchromen-2-one (24)



Compound **1** (350 mg, 1.98 mmol), 4-hydroxyphenylpiperazine (354 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is grey, powdered crystals.

$C_{21}H_{22}N_2O_4$

Yield: 54%

Mw: 366.41g/mol

MP : 252.9 ^{O}C

Rf: 0.11 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

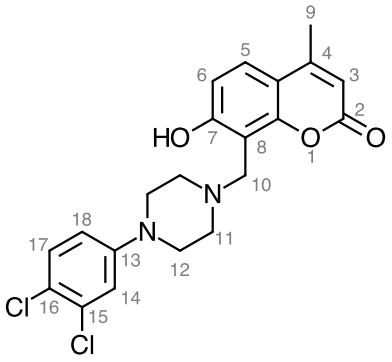
FT-IR: (KBr), cm^{-1}: 3350-3100 (OH); 3029 (aromatic C-H); 2944 (aliphatic C-H); 1699 (C=O, lactone); 1601 (C=C, aromatic).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.54 (d, *J* = 8.67 Hz, 1H, H_5); 6.79 – 6.75 (m, 3H, $H_{6,15}$); 6.64 – 6.60 (m, 2H, H_{14}); 6.13 (q, *J* = 1.14 Hz, 1H, H_3); 3.93 (s, 2H, H_{10}); 2.97 (s, 8H, $H_{11,12}$); 2.35 (d, *J* = 1.05 Hz, 3H, H_9).

^{13}C NMR: (400 MHz, DMSO-d_6) δ 161.8 (C_2); 160.5, 154.4, 153.0, 125.7, 113.2, 112.2 ($C_{coumarin\text{-}arom}$); 151.5, 150.9, 144.3, 122.8 ($C_{13,14,15,16}$); 110.4, 108.8 ($C_{3,4}$); 59.5, 52.7 ($C_{11,12}$); 52.1 (C_{10}); 18.7 (C_9).

LC-MS: rt= 1.53 min., *m/z* 367.30 $[M+H]^+$, Purity: 96%, UV (ACN/Water, λ_{max}): 200 nm.

8-[4-(3,4-Dichlorophenyl)piperazin-1-ylmethyl]-7-hydroxy-4-methylchromen-2-one (25)



Compound **1** (350 mg, 1.98 mmol), 1-(3,4-dichlorophenyl)piperazine (469 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{21}H_{20}Cl_2N_2O_3$

Yield: 68%

Mw: 419.30 g/mol

MP: 203 ^{O}C

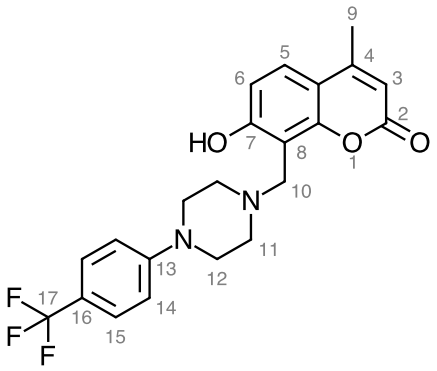
Rf: 0.3 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

FT-IR: (KBr), cm^{-1}: 3300-3100 (OH); 3051 (aromatic C-H); 2960 (aliphatic C-H); 1720 (C=O, lactone); 1628 (C=C, nonaromatic); 1601 (C=C, aromatic).

No NMR data, the product could not be dissolved in any deuterated solvent.

LC-MS: rt= 6.78 min., *m/z* 421.09 $[M+H]^+$, Purity: 95%, UV (ACN/Water, λ_{max}): 216 nm.

7-Hydroxy-4-methyl-8-[4-(4-trifluoromethylphenyl)-piperazin-1-ylmethyl]-chromen-2-one (26)



Compound **1** (153 mg, 0.87 mmol), 1-(4-trifluoromethylphenyl)piperazine (200 mg, 0.87 mmol), formaldehyde (0.09 mL) and ethanol (4 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{22}H_{21}F_3N_2O_3$

Yield: 36%

Mw: 418.41 g/mol

MP: 190.2 ^{O}C

Rf: 0.32 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

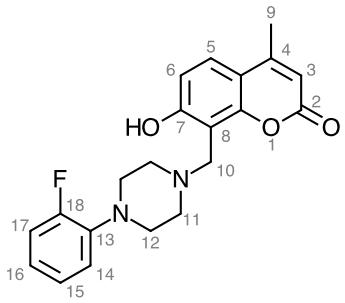
FT-IR: (KBr), cm^{-1}: 3350-3150 (OH); 3020 (aromatic C-H); 2945 (aliphatic C-H); 1723(C=O, lactone), 1615 (C=C, nonaromatic); 1602 (C=C, aromatic), 1342 (C-F).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, J = 7.2 Hz, 2H, H_{15}); 7.43 (dd, J_1 = 8.7 Hz, J_2 = 2.0 Hz, 1H, H_5); 6.93 (d, J = 7.4 Hz, 2H, H_{14}); 6.79 (dd, J_1 = 8.7 Hz, J_2 = 2.2 Hz, 1H, H_6); 6.09 (q, J = 1.0 Hz, 1H, H_3); 4.12 (s, 2H, H_{10}); 3.34 (broad s, 4H, H_{12}); 2.81 (broad s, 4H, H_{11}); 2.39 (d, J = 1.0 Hz, 3H, H_9).

^{13}C NMR (400 MHz, $CDCl_3$) δ 162.0 (C_2); 161.0, 153.2, 152.8, 113.3, 112.5 ($C_{Coumarin\text{-}aromatic}$); 152.5 ($C_{13}$); 126.4 (q, J_1 = 3.65 Hz, J_2 = 7.41 Hz, C_{17}); 124.8 (C_{15}); 114.9 (C_{14}); 110.8, 107.4 ($C_{3,4}$); 53.7, 52.3 ($C_{11,12}$); 47.9 (C_{10}); 18.7 (C_9).

LC-MS: rt= 6.76 min., *m/z* 420.21 $[M+H]^+$, Purity: 94%, UV (ACN/Water, λ_{max}): 322 nm.

8-[4-(2-Fluorophenyl)piperazin-1-ylmethyl]-7-hydroxy-4-methylchromen-2-one (28)



Compound **1** (350 mg, 1.98 mmol), 1-(2-fluorophenyl)piperazine (358 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{21}H_{21}FN_2O_3$

Yield: 76%

Mw: 368.40 g/mol

MP: 206 ^{O}C

Rf: 0.35 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

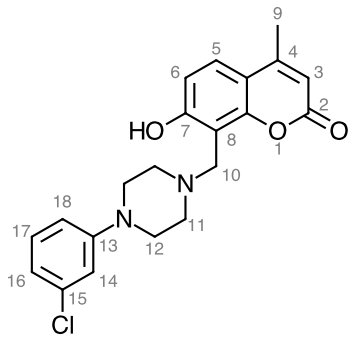
FT-IR: (KBr), cm^{-1}: 3300-3150 (OH); 3063 (aromatic C-H); 2986 (aliphatic C-H); 1706 (C=O, lactone); 1625 (C=C, nonaromatic); 1594 (C=C, aromatic).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.78 Hz, 1H, H_5); 7.06-6.90 (m, 4H, $H_{14,15,16,17}$); 6.77 (d, *J* = 8.79 Hz, 1H, H_6); 6.07 (q, *J* = 1.22 Hz, 1H, H_3); 4.11 (s, 2H, H_{10}); 3.16 (broad s, 4H, H_{11}); 2.82 (broad s, 4H, H_{12}); 2.37 (d, *J* = 1.24 Hz, 3H, H_9).

^{13}C NMR (400 MHz, $CDCl_3$) δ 162.2 (C_2); 161.0, 156.9, 154.4, 124.7, 113.3, 112.3 ($C_{coumarin\text{-}aromatic}$); 153.2, 152.5, 139.4, 122.9, 119.0, 116.3 ($C_{13,14,15,16,17,18}$); 110.7, 107.5 ($C_{3,4}$); 53.8, 52.6 ($C_{11,12}$); 50.2 (C_{10}); 18.7 (C_9).

LC-MS: rt= 6.17 min., *m/z* 369.19 $[M+H]^+$, Purity: 95%, UV (ACN/Water, λ_{max}):322 nm.

8-[4-(3-Chlorophenyl)piperazin-1-ylmethyl]-7-hydroxy-4-methylchromen-2-one (29)



Compound **1** (350 mg, 1.98 mmol), 1-(3-chlorophenyl)piperazine (391 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{21}H_{21}ClN_2O_3$

Yield: 15%

MP: >300 ^{O}C

Mw: 384.85 g/mol

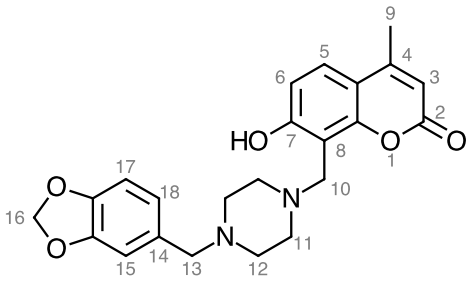
Rf: 0.2 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

FT-IR: (KBr), cm^{-1}: 3300-3100 (OH); 3010 (aromatic C-H); 2936 (aliphatic C-H); 1682 (C=O, lactone); 1595 (C=C, aromatic).

No NMR data, the product could not be dissolved in any deuterated solvent.

LC-MS: rt= 6.58 min., *m/z* 385.32; $[M+H]^+$, Purity: 94%, UV (ACN/Water, λ_{max}): 248 nm.

8-(4-Benzo[1,3]dioxol-5-ylmethyl-piperazin-1-ylmethyl)-7-hydroxy-4-methyl-chromen-2-one (30) [120]



Compound **1** (350 mg, 1.98 mmol), 1-piperonylpiperazine (437 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is brown, powdered crystals.

$C_{23}H_{24}N_2O_5$

Yield: 60%

Mw: 408.45 g/mol

MP: 151.3 ^{o}C

Rf: 0.14 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

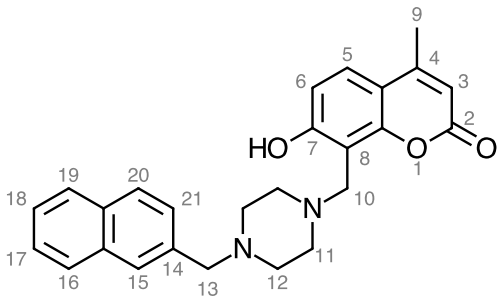
FT-IR: (KBr), cm^{-1}: 3250-3110 (OH); 3047 (aromatic C-H); 2950 (aliphatic C-H); 1718 (C=O, lactone); 1595 (C=C, aromatic).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.52 (s, 1H, OH); 7.37 (d, J = 8.7 Hz, 1H), 6.80-6.70 (m, 3H, $H_{6+15,17}$); 6.01 (broad s, 1H, H_{18}); 5.90 (q, 1H, J = 1.1 Hz, H_3); 4.02 (s, 2H, H_{10}); 3.41 (s, 2H, H_{13}); 2.52 (m, 8H, $H_{11,12}$); 2.34 (s, 3H, H_9).

^{13}C NMR (400 MHz, $CDCl_3$) δ 162.4 (C_2); 161.2, 153.3, 152.4, 124.5, 122.1, 112.1, 110.5 ($C_{Coumarin\text{-}aromatic}$); 147.6, 146.6 ($C_{phenyl}$); 131.4 ($C_{14}$); 122.1 ($C_{18}$); 113.3 ($C_{15}$); 109.3 ($C_{17}$); 107.8, 107.5 ($C_{3,4}$); 100.8 ($C_{16}$); 62.3 ($C_{13}$); 53.8, 52.5 ($C_{11,12}$); 52.4 ($C_{10}$); 18.7 ($C_9$).

LC-MS: rt= 9.81 min, *m/z* 409.38; $[M+H]^+$, Purity: 96%, UV (ACN/Water, λ_{max}): 220 nm, 321 nm

7-Hydroxy-4-methyl-8-(4-naphthalen-2-ylmethyl-piperazin-1-ylmethyl)-chromen-2-one (31)



Compound **1** (350 mg, 1.98 mmol), 1-(1-naphthylmethyl)piperazine (450 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is orange, powdered crystals.

$C_{26}H_{26}N_2O_3$

Yield: 37%

Mw: 414.49 g/mol

MP: 155.2 ^{O}C

Rf: 0.27 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

FT-IR: (KBr), cm^{-1}: 3350-3150 (OH); 3058 (aromatic C-H); 2946 (aliphatic C-H); 1704 (C=O, lactone), 1594 (C=C, aromatic).

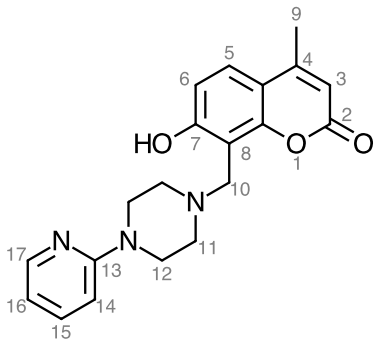
^{1}H NMR (400 MHz, $CDCl_3$) δ 9.64 (s, 1H, OH); 8.27 (d, *J* = 8.29 Hz, 1H, $H_{arom.}$); 7.84 (d, *J*= 7.80 Hz, 1H, H_5); 7.84-7.37 (m, 6H, H_{arom}) 6.76 (d, *J*= 7.80, 1H, H_6); 6.05 (q, *J*=1.1 Hz, 1H, H_3); 4.03 (s, 2H, H_{13}); 3.92 (s, 2H, H_{10}); 2.86 – 2.44 (broad s, 8H, $H_{11,12}$); 2.34 (s, 3H, H_9).

^{13}C NMR (400 MHz, $CDCl_3$) δ 162.5 (C_2); 161.2, 153.3, 152.5, 128.1, 113.3, 112.2 ($C_{Coumarin\text{-}aromatic}$); 133.8, 133.4, 132.4, 128.3, 127.4, 125.8, 125.7, 125.0, 124.6 ($C_{14\text{-}21}$); 110.5, 107.6 ($C_{3,4}$); 60.8 (C_{13}); 53.7, 52.8 ($C_{11,12}$); 52.6 (C_{10}); 18.7 (C_9).

LC-MS: rt= 6.53 min., *m/z* 415.42 $[M+H]^+$, Purity: 95%, UV (ACN/Water, λ_{max}): 223 nm, 321 nm.

7-Hydroxy-4-methyl-8-(4-pyridin-2-yl-piperazin-1-yl)-chromen-2-one (32)

(**CAS No:** 1401560-38-0)



Compound **1** (350 mg, 1.98 mmol), 1-(2-pyridyl)piperazine (324 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{20}H_{21}N_3O_3$

Yield: 52%

Mw: 351.40 g/mol

M.P.: 200.8 ^{O}C

Rf: 0.19 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

FT-IR: (KBr), cm^{-1}: 3370-3200 (OH); 3092 (aromatic C-H); 2990 (aliphatic C-H); 1699 (C=O, lactone), 1587 (C=C, aromatic).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.75 (s, 1H, OH); 8.16 (dd, J_1 = 1.56 Hz, J_2 = 5.33 Hz, 1H, H_{17}), 7.46 (m, 1H, H_{arom}); 7.40 (d, J = 8.75 Hz, 1H, H_5); 6.76 (d, J = 8.75 Hz, 1H, H_6); 6.64-6.61 (m, 2H, $H_{14,16}$); 6.05 (s, 1H, H_3); 4.08 (s, 2H, H_{10}); 3.63 (broad s, 4H, H_{12}); 2.73 (broad s, 4H, H_{11}); 2.35 (s, 3H, H_9).

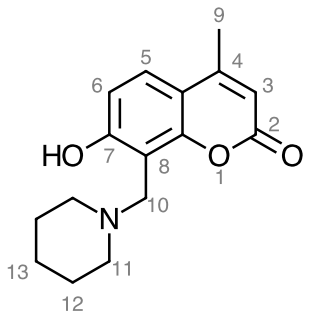
^{13}C NMR (400 MHz, $CDCl_3$) δ 162.1 (C_2); 161.0, 147.9, 137.5, 113.3, 107.4 ($C_{13,14,15,16,17}$); 159.0, 153.2, 152.5, 124.7, 112.3, 110.7 ($C_{coumarin\text{-}aromatic}$); 113.3, 107.1 ($C_{3,4}$); 53.8, 52.3 ($C_{11,12}$); 44.9 ($C_{10}$); 18.7 ($C_9$).

LC-MS: rt= 2.55 min., *m/z* 352.38 $[M+H]^+$, Purity: 97%, UV (ACN/Water, λ_{max}): 200 nm.

4.3. Piperidine Derivatives (33-50)

7-Hydroxy-4-methyl-8-piperidin-1-ylmethyl-chromen-2-one (33)

(**CAS No:** 10549-62-9), **[120, 126-129]**



Compound **1** (350 mg, 1.98 mmol), piperidine (169 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is yellow, powdered crystals.

$C_{16}H_{19}NO_3$

Yield: 50%

Mw: 273.33 g/mol

MP: 132.3 ^{O}C

Rf: 0.15 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

FT-IR: (KBr), cm^{-1}: 3300-3150 (OH); 3069 (aromatic C-H); 2983 (aliphatic C-H); 1721 (C=O, lactone); 1593 (C=C, aromatic).

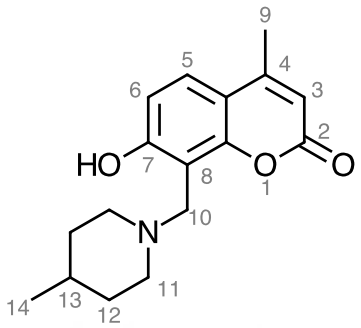
^{1}H NMR (400 MHz, $CDCl_3$) δ 12.3 (s, 1H, OH); 7.35 (d, J =8.75 Hz, 1H, H_5); 6.70 (dd, J_1 = 1.35 Hz, J_2 = 8.74 Hz, 1H, H_6); 6.01 (broad s, 1H, H_3); 3.97 (s, 2H, H_{10}); 2.67 – 2.10 (broad s, 7H, H_{11}); 2.33 (s, 3H, H_9); 1.63 (broad s, 6H, $H_{12,13}$).

^{13}C NMR (400 MHz, $CDCl_3$) δ 163.0 (C_2); 161.2, 153.3, 152.4, 124.4, 113.3, 111.9 ($C_{Coumarin\text{-}aromatic}$); 110.3, 107.7 ($C_{3,4}$); 54.6 ($C_{10}$); 53.8, 25.6, 23.6 ($C_{11,\ 12,\ 13}$); 18.7 ($C_9$).

LC-MS: rt= 3.26 min., *m/z* 274.03 $[M+H]^+$, Purity: 95%, UV (ACN/Water, λ_{max}): 322 nm

7-Hydroxy-4-methyl-8-(4-methyl-piperidin-1-ylmethyl)-chromen-2-one (34)

(**CAS No:** 307535-34-8)



Compound **1** (350 mg, 1.98 mmol), 4-methylpiperidine (197 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is yellow, powdered crystals.

$C_{17}H_{21}NO_3$

Yield: 66%

Mw: 287.35 g/mol

MP: 108.7 ^{O}C

Rf: 0.28 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

FT-IR: (KBr), cm^{-1}: 3400-3200 (OH); 3052 (aromatic C-H); 2974 (aliphatic C-H); 1723 (C=O, lactone), 1606 (C=C, aromatic).

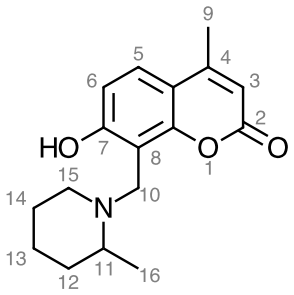
^{1}H NMR (400 MHz, $CDCl_3$) δ 11.64 (s, 1H, OH); 7.34 (d, *J* = 8.64 Hz, 1H, H_5); 6.69 (d, *J* = 8.64 Hz, 1H, H_6); 6.00 (s, 1H, H_3); 3.98 (s, 2H, H_{10}); 2.95, (d, *J* =10.17 Hz, 2H, H_{11}); 2.33 (s, 3H, H_9); 2.19 (t, *J* = 10.12 Hz, 2H, H_{11}); 1.67 (d, *J* = 12.96 Hz, 2H, H_{12}); 1.43 (broad s, 1H, H_{13}); 1.24 (m, 2H, H_{12}); 0.91 (d, *J* = 6.21 Hz, 3H, H_{14}).

^{13}C NMR (400 MHz, $CDCl_3$) δ 163.2 (C_2); 161.4, 153.5, 152.4, 124.5, 113.4, 111.8 ($C_{coumarin\text{-}aromatic}$); 110.2, 107.6 ($C_{3,4}$); 54.1 ($C_{10}$); 53.3 ($C_{11}$); 33.8 ($C_{12}$); 30.1 ($C_{13}$); 21.5 ($C_{14}$); 18.7 ($C_9$).

LC-MS: rt= 4.06 min., *m/z* 289.21 $[M+H]^+$, Purity: 98%, UV (ACN/Water, λ_{max}): 322 nm.

7-Hydroxy-4-methyl-8-(2-methyl-piperidin-1-ylmethyl)-chromen-2-one (35)

(**CAS No:** 370841-03-5)



Compound **1** (350 mg, 1.98 mmol), 2-methylpiperidine (197 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is yellow, powdered crystals.

$C_{17}H_{21}NO_3$

Yield: 48%

Mw: 287.35 g/mol

MP: 148.1 ^{O}C

Rf: 0.14 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

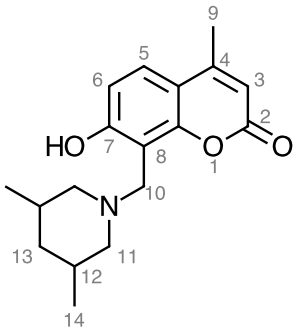
FT-IR: (KBr), cm^{-1}: 3370-3150 (OH); 3078 (aromatic C-H); 2962 (aliphatic C-H); 1711 (C=O, lactone); 1594 (C=C, aromatic).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.77 (s, 1H, OH); 7.33 (dd, J_1 = 8.7 Hz, J_2 = 1.4 Hz, 1H, H_6); 6.67 (dd, J_1 = 8.7 Hz, J_2 = 2.0 Hz, 1H, H_5); 6.00 (q, J = 0.7 Hz, 1H, H_3); 4.29 (d, J = 12.7 Hz, 1H, H_{10}); 3.89 (d, J = 15.7 Hz, 1H, H_{10}); 2.33 (s, 4H, $H_{9,11}$); 1.78 – 1.51 (m, 4H, $H_{14,15}$); 1.40 (broad s, 2H, H_{13}); 1.17-1.15 (m, 5H, $H_{12,16}$).

^{13}C NMR (400 MHz, $CDCl_3$) δ

LC-MS: rt= 3.90 min., *m/z* 288.07 $[M+H]^+$, Purity: 96%, UV (ACN/Water, λ_{max}): 322 nm.

8-(3,5-Dimethyl-piperidin-1-ylmethyl)- 7-hydroxy-4-methylchromen-2-one (36)



Compound **1** (350 mg, 1.98 mmol), 3,5-dimethylpiperidine (225 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{18}H_{23}NO_3$

Yield: 20%

Mw: 301.38 g/mol

M.P.: 134.9 ^{O}C

Rf: 0.33 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

FT-IR: (KBr), cm^{-1}: 3300-3100 (OH); 3012 (aromatic C-H); 2936 (aliphatic C-H); 1678 (C=O, lactone); 1598 (C=C, aromatic).

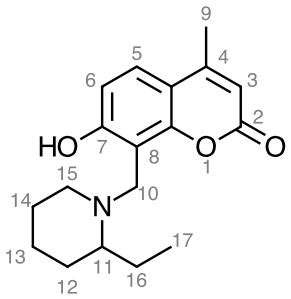
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (t, *J* = 8.4 Hz, 1H, H_5); 6.73 (t, *J* = 8.5 Hz, 1H, H_6); 6.04 (d, *J* = 7.2 Hz, 1H, H_3); 4.01 (d, *J* = 8.0 Hz, 2H, H_{10}); 2.92 (d, *J* = 7.1 Hz, 2H, H_{11}); 2.37 (d, *J* = 1.1 Hz, 1H, H_{13}); 2.35 (s, 2H, H_{11}); 1.72-1.69 (m, 5H, $H_{9,12}$); 0.90 – 0.83 (m, 6H, H_{14}); 0.61 (d, *J* = 8.2 Hz, 1H, H_{13}).

^{13}C NMR (400 MHz, $CDCl_3$) δ 163.1 (C_2); 161.3, 153.3, 124.4, 113.3, 112.0 ($C_{Coumarin\text{-}aromatic}$); 110.4, 107.8 ($C_{3,4}$); 60.4 ($C_{11}$); 54.2 ($C_{10}$); 41.4 ($C_{13}$); 31.1 ($C_{12}$); 19.2 ($C_{14}$); 18.7 ($C_9$).

LC-MS: rt= 5.14 min., *m/z* 302.06 $[M+H]^+$, Purity: 95%, UV (ACN/Water, λ_{max}): 322 nm.

8-(2-Ethylpiperidin-1-ylmethyl)- 7-hydroxy-4-methylchromen-2-one (37)

(**CAS No:** 307535-35-9)



Compound **1** (350 mg, 1.98 mmol), 2-ethylpiperidine (225 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is yellow, powdered crystals.

$C_{18}H_{23}NO_3$

Yield: 52%

Mw: 301.38 g/mol

MP: 164.5^{O}C

Rf: 0.15 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50)

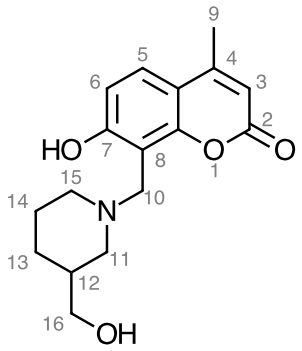
FT-IR: (KBr), cm^{-1}: 3300-3150 (OH); 3073 (aromatic C-H); 2937 (aliphatic C-H); 1715 (C=O, lactone); 1597 (C=C, aromatic).

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.47 (s, 1H, OH); 7.34 (dd, J_1 = 1.84 Hz, J_2 = 8.74 Hz, 1H, H_5); 6.68 (dd, J_1 = 2.30 Hz, J_2 = 8.74 Hz, 1H, H_6); 6.00 (d, J = 1.00 Hz, 1H, H_3); 4.31 (d, J = 15.72 Hz, 1H, H_{10}); 3.93 (d, J = 15.72 Hz, 1H, H_{10}); 2.35-2.30 (m, 4H, $H_{9,11}$); 1.84 – 1.48 (m, 8H, $H_{12,13,14,15}$); 1.44 – 1.17 (m, 2H, H_{16}); 0.90 (t, J_2 = 7.40 Hz, 3H, H_{17}).

^{13}C NMR (400 MHz, $CDCl_3$) δ 161.5 (C_2); 155.3, 153.5, 152.3, 125.6, 113.7, 111.5 ($C_{Coumarin\text{-}aromatic}$); 110.9, 103.1 ($C_{3,4}$); 58.2 ($C_{11}$); 50.6 ($C_{15}$); 29.6 ($C_{12}$); 24.7 ($C_{13}$); 18.7 ($C_9$); 18.6 ($C_{14}$); 9.7 ($C_{17}$).

LC-MS: rt= 2.13 min., *m/z* 302.31 $[M+H]^+$, Purity: 95%, UV (ACN/Water, λ_{max}): 322 nm.

7-Hydroxy-8-(3-hydroxymethylpiperidin-1-ylmethyl)-4-methylchromen-2-one (41)



Compound **1** (350 mg, 1.98 mmol), 3-hydroxymethylpiperidine (229 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{17}H_{21}NO_4$

Yield: 52%

Mw: 303.35 g/mol

MP: 189.3 ^{O}C

Rf: 0.11 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

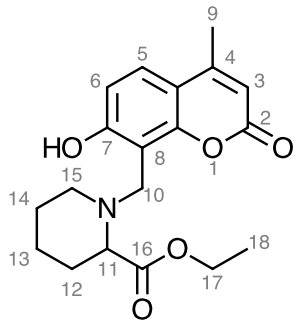
FT-IR: (KBr), cm^{-1}: 3250-3100 (OH); 3074 (aromatic C-H); 2924 (aliphatic C-H); 1705 (C=O); 1600 (C=C, aromatic).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, J_1 = 8.7 Hz, 1H, H_5); 6.70 (d, J_1 = 8.7 Hz, 1H, H_6); 6.01 (s, 1H, H_3); 4.00 (s, 2H, H_{10}); 3.57–3.46 (m, 2H, H_{16}); 2.96-2.94 (m, 2H, $H_{11,15}$); 2.33 (s, 3H, H_9); 1.94 – 1.54 (m, 3H, $H_{11,12,15}$); 1.28 – 1.00 (m, 4H, $H_{13,14}$).

^{13}C NMR (400 MHz, $CDCl_3$) δ 162.9 (C_2); 161.3, 153.4, 152.4, 124.5, 113.3, 112.0 ($C_{Coumarin\text{-}aromatic}$); 110.3, 107.6 ($C_{3,4}$); 65.3 ($C_{16}$); 56.3 ($C_{11}$); 54.5 ($C_{15}$); 53.6 ($C_{10}$); 29.6 ($C_{12}$); 26.4 ($C_{14}$); 24.5 ($C_{13}$); 18.7 ($C_9$).

LC-MS: rt= 2.78 min., *m/z* 304.07 $[M+H]^+$, Purity: 95%, UV (ACN/Water, λ_{max}): 322 nm.

1-(7-Hydroxy-4-methyl-2-oxo-2*H*-chromen-8-ylmethyl)-piperidine-2-carboxylic acid ethyl ester (42)



Compound **1** (350 mg, 1.98 mmol), ethylpipecolinate (312 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{19}H_{23}NO_5$

Yield: 40%

Mw: 345.39 g/mol

MP: 121.2 ^{O}C

Rf: 0.7 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

FT-IR: (KBr), cm^{-1}: 3330-3150 (OH); 3055 (aromatic C-H); 2973 (aliphatic C-H); 1727 (C=O, lactone); 1625 (C=C, nonaromatic); 1594 (C=C, aromatic).

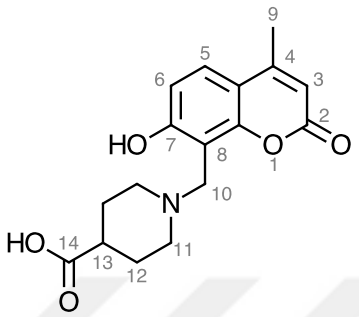
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (d, J = 8.7 Hz, 1H, H_5); 6.75 (d, J = 8.7 Hz, 1H, H_6); 6.03 (q, J = 1.18 Hz, 1H, H_3); 4.18 (qd, J=1.3 Hz, J=7.1 Hz, 2H, H_{17}); 4.04 (d, J_1 = 14.91 Hz, 2H, H_{10}); 3.94 (d, J_1 = 14.91 Hz, 2H, H_{10}); 3.15 (dd, J= 3.3Hz, J=9.5Hz, 1H, H15), 3.01 (td, J=4.0Hz, J=11.9 Hz, 1H, H15); 2.34 (d, J=1.1Hz, 3H, H9); 2.29 – 2.13 (m, 1H, H_{11}); 1.83 – 1.34 (m, 6H, $H_{12,13,14}$); 1.25 (t, J = 7.08 Hz, 3H, H_{18}).

^{13}C NMR (400 MHz, $CDCl_3$) δ 172.7 (C_{16}); 162.2 (C_2); 161.2, 153.3, 152.6, 124.4, 113.4, 112.1 ($C_{Coumarin\text{-}aromatic}$); 110.4, 107.8 ($C_{3,4}$); 65.1 ($C_{11}$); 61.1 ($C_{17}$); 51.9 ($C_{15}$); 50.9 ($C_{10}$); 29.6 ($C_{14}$); 24.6 ($C_{12}$); 22.6 ($C_{13}$); 18.7 ($C_9$); 14.1 ($C_{17}$).

LC-MS: rt= 6.13 min., *m/z* 346.33 $[M+H]^+$, Purity: 97%, UV (ACN/Water, λ_{max}): 203 nm.

1-(7-Hydroxy-4-methyl-2-oxo-2*H*-chromen-8-ylmethyl)-piperidine-4-carboxylic acid (45)

(**CAS No:** 2196259-94-4)



Compound **1** (350 mg, 1.98 mmol), piperidine-4-carboxylic acid (257 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{17}H_{19}NO_5$

Yield: 75%

Mw: 317.34 g/mol

MP: 170.5 ^{O}C

Rf: 0.1 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

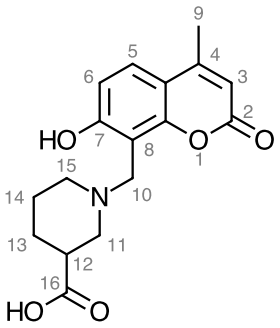
FT-IR: (KBr), cm^{-1}: 3200-3050 (OH); 2971 (aromatic C-H); 1776 (C=O, carboxylic acid); 1708 (C=O, lactone); 185 (C=C, aromatic).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.75 Hz, 1H, H_5); 6.71 (d, *J* = 8.74 Hz, 1H, H_6); 6.06 (s, 1H, H_3); 3.88 (s, 2H, H_{10}); 2.87-2.84 (m, 2H, H_{11}); 2.28-2.25 (m, 3H, H_{11}, H_{13}); 2.31 (s, 3H, H_9); 2.15-2.11 (m, 2H, H_{12}); 1.56-1.53 (m, 2H, H_{12}).

^{13}C NMR (400 MHz, $CDCl_3$) δ 176.1 (C_{14}); 162.4 (C_2); 160.3, 154.2, 152.8, 125.5, 113.2, 111.9 ($C_{Coumarin\text{-}aromatic}$); 110.1, 108.2 ($C_{3,4}$); 52.7 ($C_{10}$); 52.1, 40.0, 28.1 ($C_{11,12,13}$); 18.6 ($C_9$).

LC-MS: rt= 2.85 min., *m/z* 318.11 $[M+H]^+$, Purity: 95%, UV (ACN/Water, λ_{max}): 322 nm.

1-(7-Hydroxy-4-methyl-2-oxo-2*H*-chromen-8-ylmethyl)piperidine-3-carboxylic acid (46)



Compound **1** (350 mg, 1.98 mmol), nipecotic acid (257 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{17}H_{19}NO_5$

Yield: 68%

Mw: 317.34 g/mol

MP: > 300 ^{O}C

Rf: 1.1 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

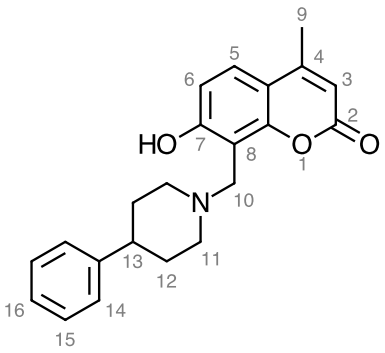
FT-IR: (KBr), cm^{-1}: 3331-3100 (OH); 3060 (aromatic C-H); 2953 (aliphatic C-H); 1728 (C=O, lactone); 1573 (C=C, aromatic).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.75 Hz, 1H, H_5); 6.70 (d, *J* = 8.74 Hz, 1H, H_6); 6.04 (s, 1H, H_3); 4.02 (s, 2H, H_{10}); 2.85-2.82 (m, 2H, H_{11}); 2.27-2.25 (m, 2H, H_{11}, H_{13}); 2.33 (s, 3H, H_9); 2.15-1.56 (m, 5H, $H_{12,14,15}$).

^{13}C NMR

LC-MS: rt= 3.04 min., *m/z* 318.18 $[M+H]^+$, Purity: 96%, UV (ACN/Water, λ_{max}): 322 nm.

7-Hydroxy-4-methyl-8-[(4-phenylpiperidin-1-yl)methyl]-chromen-2-one (47)



Compound **1** (350 mg, 1.98 mmol), 4-phenylpiperidine (320 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{22}H_{23}NO_3$

Yield: 64%

Mw: 349.42 g/mol

MP: 199.9 ^{O}C

Rf: 0.32 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

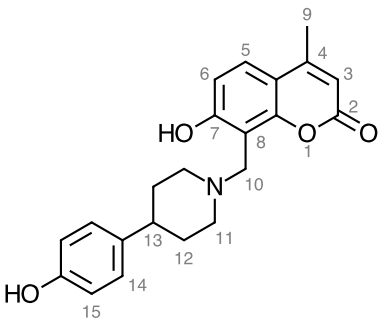
FT-IR: (KBr), cm^{-1}: 3300-3100 (OH); 3024 (aromatic C-H); 2939 (aliphatic C-H); 1704 (C=O, lactone), 1625 (C=C, nonaromatic); 1598 (C=C, aromatic).

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.4 (s, 1H, OH); 7.41 (d, *J* = 8.75 Hz, 1H, H_5); 7.27 (m, 5H, $H_{14,15,16}$); 6.77 (d, *J* = 8.85 Hz, 1H, H_6); 6.07 (d, *J* = 1.16 Hz, 1H, H_3); 4.09 (s, 2H, H_{10}); 3.15 (m, 2H, H_{11}); 2.61 (m, 1H, H_{13}); 2.40-2.34 (m, 2H, H_{11}); 2.37 (d, *J* = 1.13 Hz, 3H, H_9); 1.86 (m, 4H, H_{12}).

^{13}C NMR (400 MHz, $CDCl_3$) δ 162.8 (C_2); 161.2, 153.3, 128.5, 126.7, 113.4, 112.1 ($C_{coumarin\text{-}aromaric}$); 152.5, 126.4, 124.5 ($C_{14,15,16}$); 110, 107.8 ($C_{3,4}$); 54.2, 53.7 ($C_{11,12}$); 41.9 ($C_{10}$); 33.1 ($C_{13}$); 18.8 ($C_9$).

LC-MS: rt= 6.26 min., *m/z* 351.24 $[M+H]^+$, Purity: 95%, UV (ACN/Water, λ_{max}): 198 nm.

7-Hydroxy-8-[4-(4-hydroxyphenyl)-piperidin-1-ylmethyl]-4-methylchromen-2-one (48)



Compound **1** (350 mg, 1.98 mmol), 4-(4-hydroxyphenyl)piperidine (352 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{22}H_{23}NO_4$

Yield: 56%

Mw: 365.42 g/mol

MP: 200.9 ^{O}C

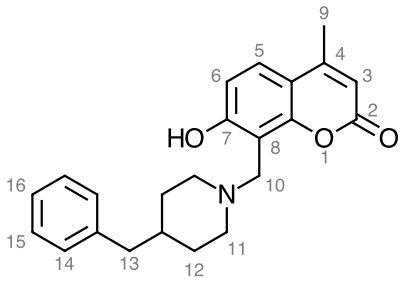
Rf: 0.31 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

FT-IR: (KBr), cm^{-1}: 3250-3100 (OH); 3024 (aromatic C-H); 2939 (aliphatic C-H); 1702 (C=O, lactone), 1587 (C=C, aromatic).

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.5 (s, 1H, OH); 7.40 (d, *J* = 8.76 Hz, 1H, H_5); 7.30 (d, *J* = 7.40 Hz, 1H, H_{14}); 7.31-7.17 (m, 4H, $H_{14,15}$); 6.76 (d, *J* = 8.67 Hz, 1H, H_6); 6.05 (s, 1H, H_3); 4.08 (d, *J* = 1.45 Hz, 2H, H_{10}); 3.13 (m, 2H, H_{12}); 2.60 (m, 1H, H_{13}); 2.36 (m, 5H, $H_{9,11}$); 1.87 (m, 4H, $H_{11,12}$).

^{13}C NMR (400 MHz, $CDCl_3$) δ 162.8 (C_2); 161.2, 153.2, 152.5, 124.5, 113.3, 112.1 ($C_{coumarin\text{-}aromatic}$); 145.1, 126.7, 126.4, 124.5 ($C_{14,15,16,17}$); 110.5, 107.8 ($C_{3\text{-}4}$); 76.8 (C_{11}); 54.2, 53.7 (C_{12}); 41.9 (C_{10}); 33.1 (C_{13}); 18.7 (C_9).

8-(4-Benzyl-piperidin-1-ylmethyl)-7-hydroxy-4-methylchromen-2-one (49)



Compound **1** (350 mg, 1.98 mmol), 4-benzylpiperidine (348 mg, 1.98 mmol), formaldehyde (0.2 mL) and ethanol (5 mL) were reacted according to the general procedure B 3.2.2. The form of compound is white, powdered crystals.

$C_{23}H_{25}NO_3$

Yield: 24%

Mw: 363.45 g/mol

MP: 119.1 ^{O}C

Rf: 0.18 (Solvent System: Ethyl acetate:*n*-Hexane = 50:50).

FT-IR: (KBr), cm^{-1}: 3002 (sp^2-CH); 2976 (sp^3-CH); 1729 (C=O); 1626 (C=C, alkene); 1602 (C=C, aromatic).

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.07 (s, 1H, OH); 7.38 (d, *J* = 8.70 Hz, 1H, H_5); 7.27 (t, *J* = 7.28 Hz, 2H, H_{15}); 7.18 (t, *J* = 7.10 Hz, 1H, H_{16}); 7.13 (d, *J* = 7.35 Hz, 2H, H_{14}); 6.74 (d, *J* = 8.73 Hz, 1H, H_6); 6.04 (s, 1H, H_3); 4.01 (s, 2H, H_{10}); 2.99 (d, *J* = 10.85 Hz, 2H, H_{11}); 2.55 (d, *J* = 6.94 Hz, 2H, H_{13}); 2.36 (s, 3H, H_9); 2.18 (d, *J* = 9.13 Hz, 2H, H_{12}); 1.72 (d, *J* = 13.25 Hz, 2H, H_{11}); 1.63 (broad s, 1H, H-piperidine); 1.35 (m, 2H, H_{12}).

^{13}C NMR (400 MHz, $CDCl_3$) δ 163.0 (C_2); 161.3, 153.4, 152.4, 128.2, 113.4, 112.0 ($C_{Coumarin\text{-}aromatic}$); 140.0, 129.0, 126.0, 124.4 ($C_{Phenylcarbons}$); 110.4, 107.7 ($C_{3,4}$); 54.2 (C_{11}); 53.2 (C_{10}); 42.9 (C_{13}); 37.3 ($C_{12'}$); 31.9 (C_{12}); 18.7 (C_9).

LC-MS: rt= 6.47 min., *m/z* 364.34 $[M+H]^+$, Purity: 96%, UV (ACN/Water, λ_{max}): 322 nm.

5. RESULTS AND DISCUSSION

In this study, novel piperazine and piperidine analogues of 7-hydroxy-4-methyl-chromen-2-one were synthesized and their anti-inflammatory, analgesic activities and cytotoxicity were evaluated for *in vitro*. ^{1}H-NMR, ^{13}C-NMR, FT-IR, UV-Vis and LC-MS were used for structure confirmation.

5.1. Chemistry

The retrosynthetic pathway analysis for targeted compounds is given below (Fig 5.1). The final compounds are composed of two main moieties: a coumarin scaffold and piperazine/ piperidine groups. The coumarin moiety was synthesized *via* the Pechmann reaction in the presence of H_2SO_4. Then, piperazine and piperidine groups were introduced at the 8th position of the coumarin ring using formaldehyde and ethanol.

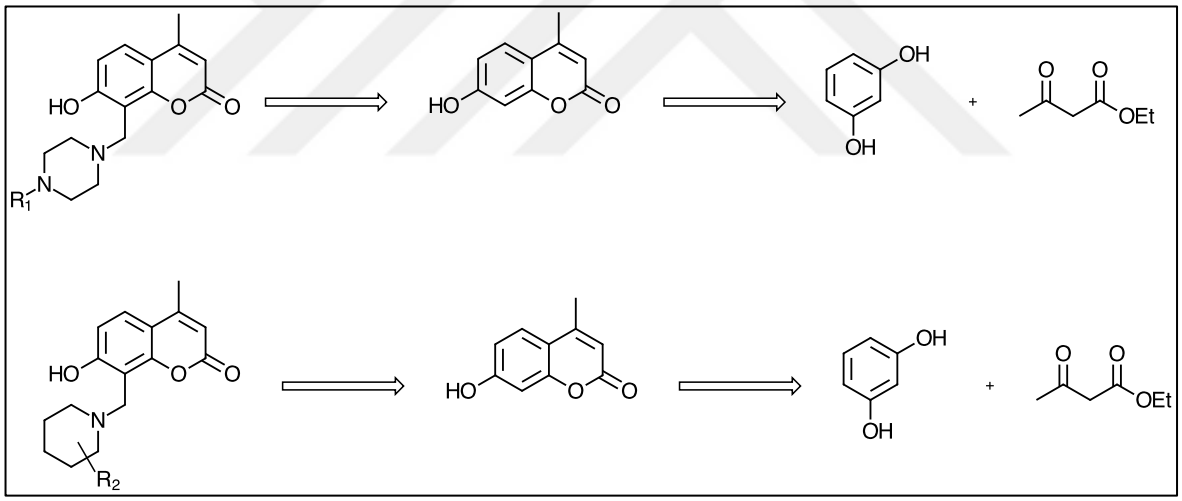


Figure 5.1 Retrosynthesis of piperazine/piperidine analogues

5.1.1. Step 1: Synthesis of 7-hydroxy-4-methylchromen-2-one (Compound 1)

The coumarin ring (7-hydroxy-4-methylchromen-2-one, compound **1**) was synthesized according to the Pechmann reaction. Resorcinol and ethyl acetoacetate were used as starting materials and reaction was conducted in acidic medium. The reaction and its mechanism are given below (Fig. 5.2 and Sch. 5.1).

STEP 1:

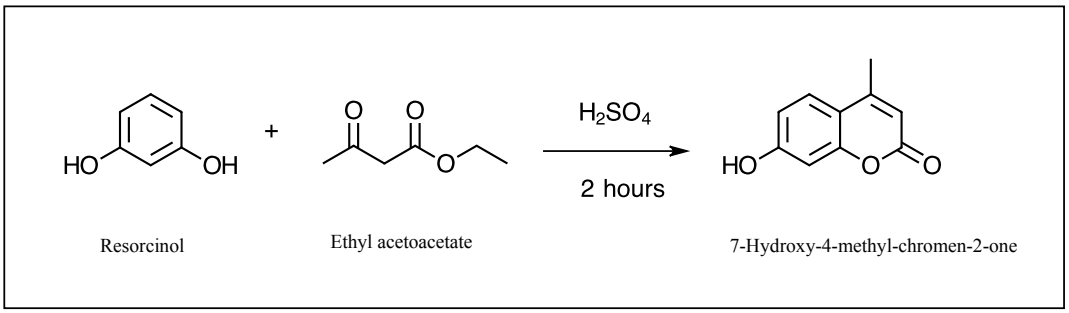
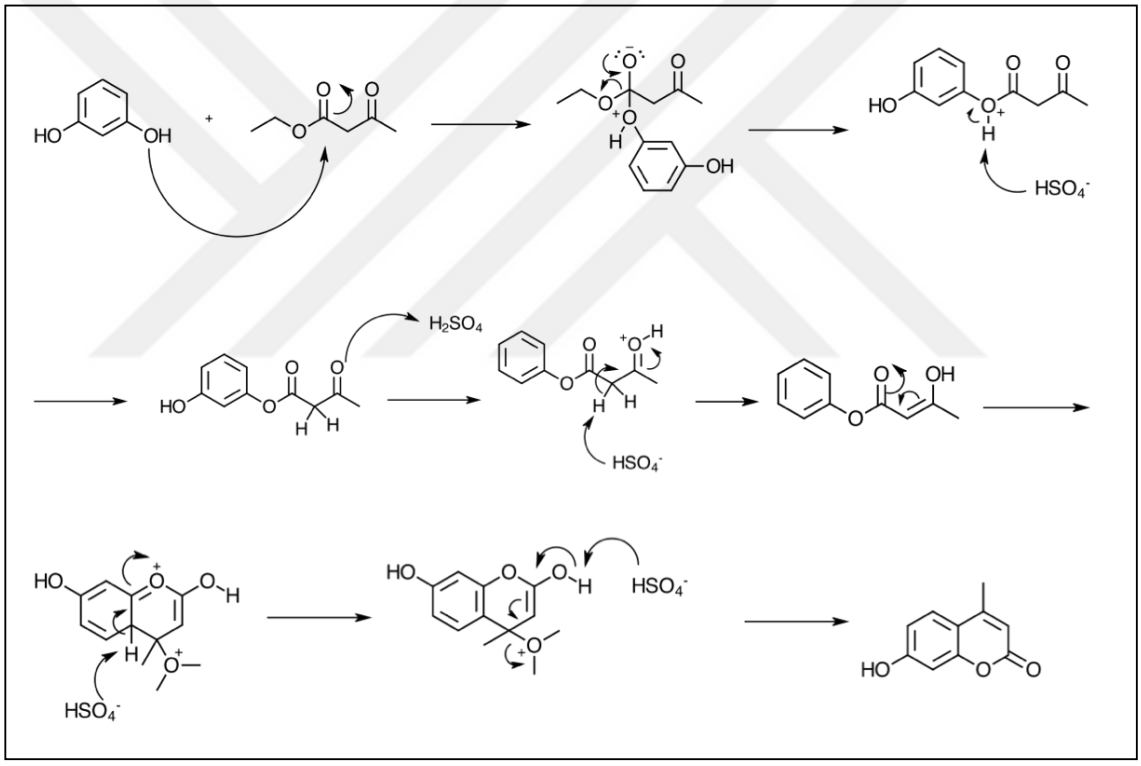


Figure 5.2. Synthesis of 7-hydroxy-4-methylchromen-2-one

MECHANISM:



Scheme 5.1. Reaction mechanism of 7-hydroxy-4-methyl-chromen-2-one

After its synthesis, compound **1** was characterized with ^{1}H-NMR, ^{13}C-NMR, LC-MS and FTIR (Fig. 5.3-5.6). According to the ^{1}H-NMR analysis, the aromatic $\mathbf{H_5}$ is observed as a doublet at 7.57ppm (J=8.7Hz). Its neighbouring proton $\mathbf{H_6}$ gives a doublet of doublet at 6.78 ppm (J_1=8.7Hz, J_2=2.4Hz) since in addition to the ortho coupling with $\mathbf{H_5}$, there is a meta coupling with $\mathbf{H_8}$. This finding is confirmed by the doublet with the same J=2.4 Hz coupling constant obtained at 6.68 ppm for the $\mathbf{H_8}$ proton. The quartet at

6.10 ppm (J=1.2Hz) corresponds to the **H_3** proton, which presents a typical a cis-allylic coupling with the methyl moiety bond to the cyclic double bond. Finally, the presence of the methyl is confirmed by the doublet obtained at 2.34 ppm (J=1.2 Hz) that integrates for three protons and the hydroxyl gives a signal for one proton at 10.5 ppm. All these findings confirm clearly the cyclization that leads to the coumarin ring (Fig. 5.3).

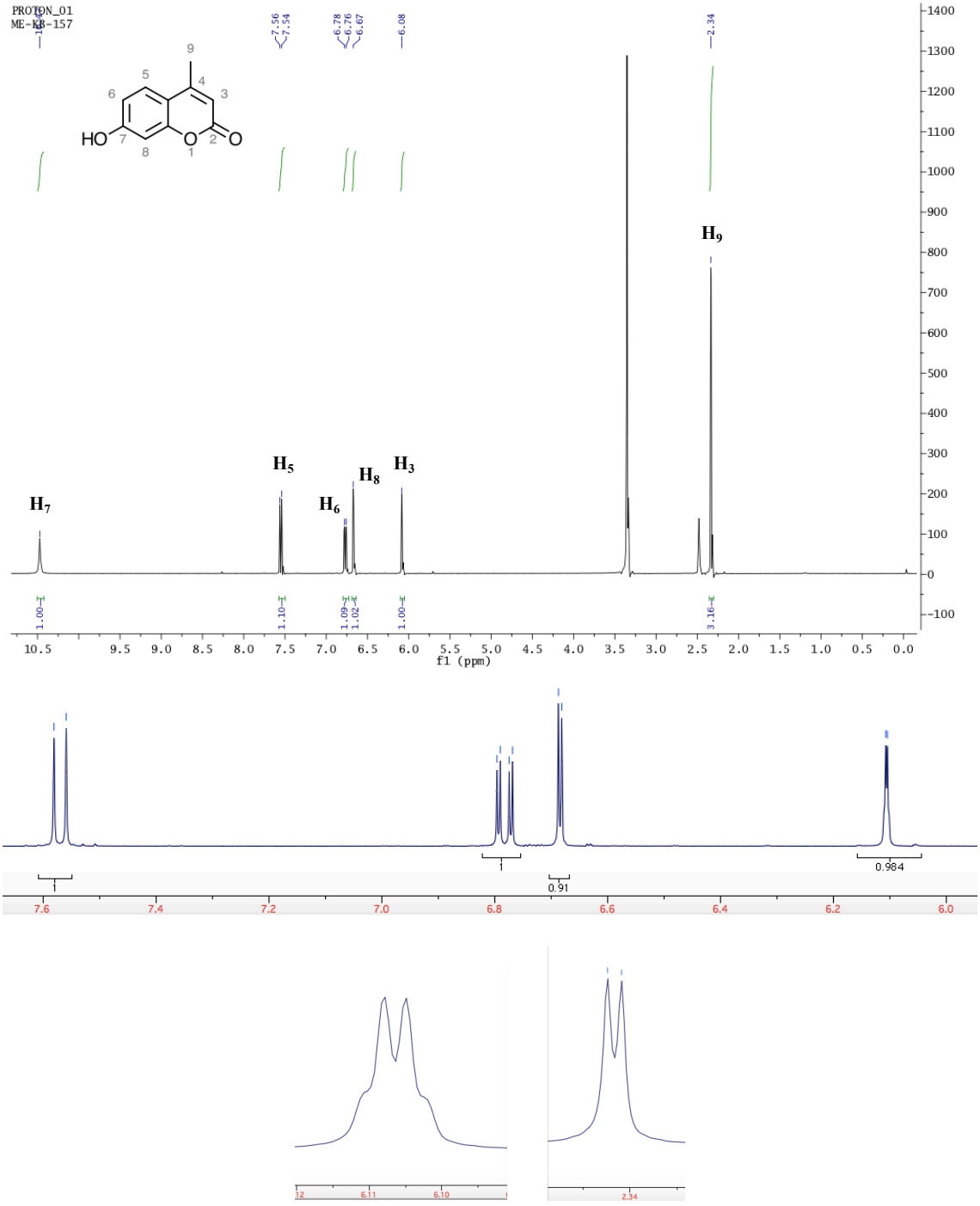


Figure 5.3. [1]H-NMR spectrum of compound **1**

The obtention of the coumarin scaffold was also validated by ^{13}C-NMR since the spectrum displayed a typical carbonyl signal at 161 ppm; a peak at 18 ppm for the methyl **C9** carbon and 8 signals that are typical of sp^2 hybridized carbons for the **C3**, **C4 and C5-C8** (aromatic carbons of coumarin) (Fig. 5.4).

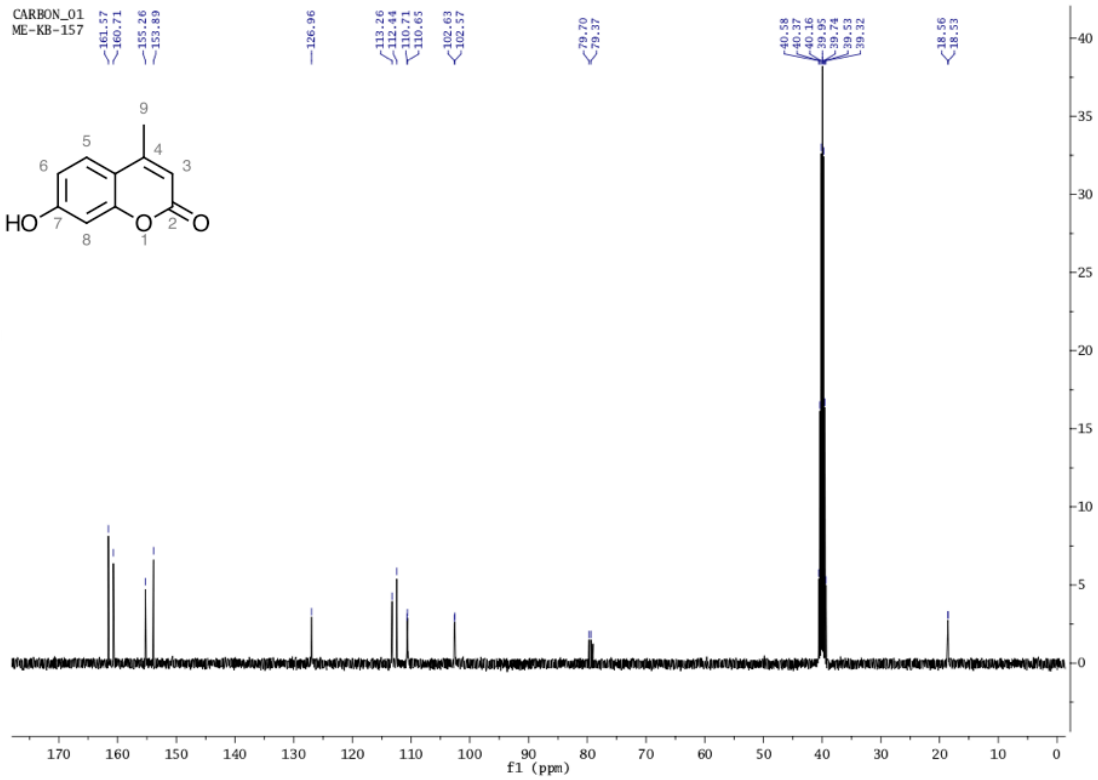


Figure 5.4. ^{13}C-NMR of compound **1**

The cyclization was also confirmed by the mass spectrum that exhibited a M+H peak at 176.8 (m/z) along with the molecule's isotopic peaks at 178.1 (m/z) and 179.1 (m/z) (Fig. 5.5). Finally the UV-Vis spectrum was compatible with literature as it exhibited an inflection at 220 nm, a minimum around 260 nm and a maximum at 320 nm [130] (Fig 5.6).

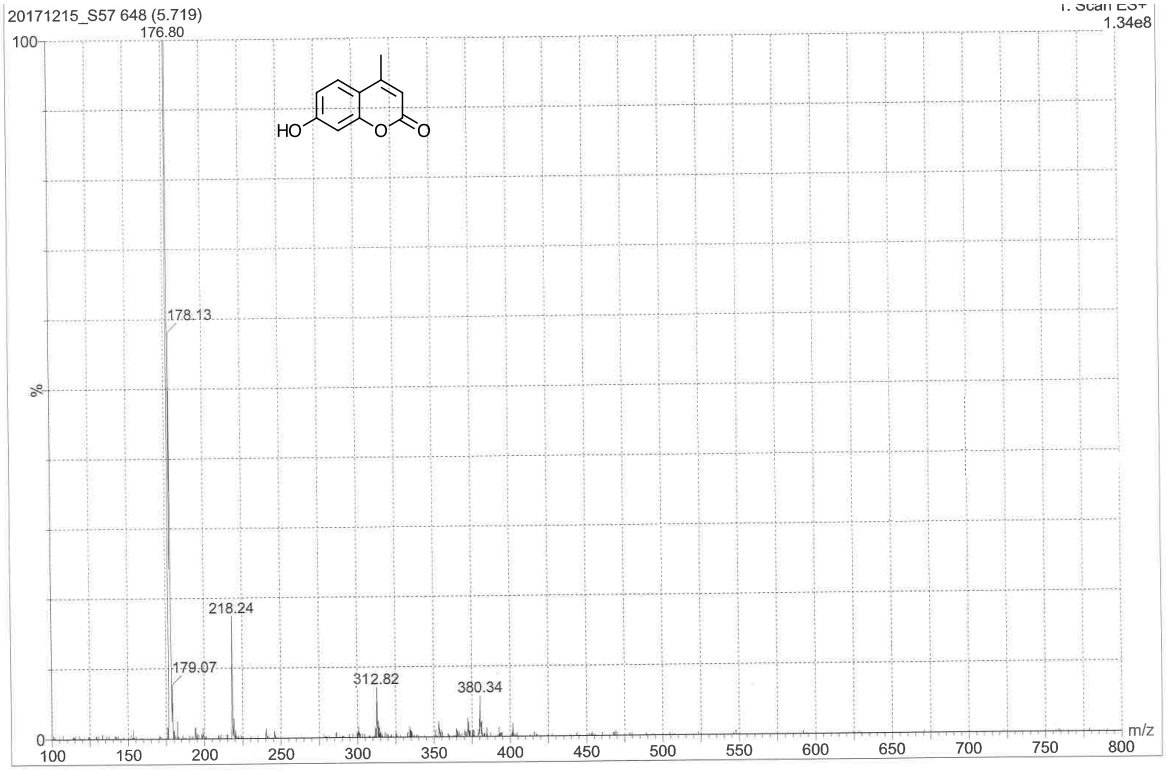


Figure 5.5. LC-MS spectrum of compound **1**

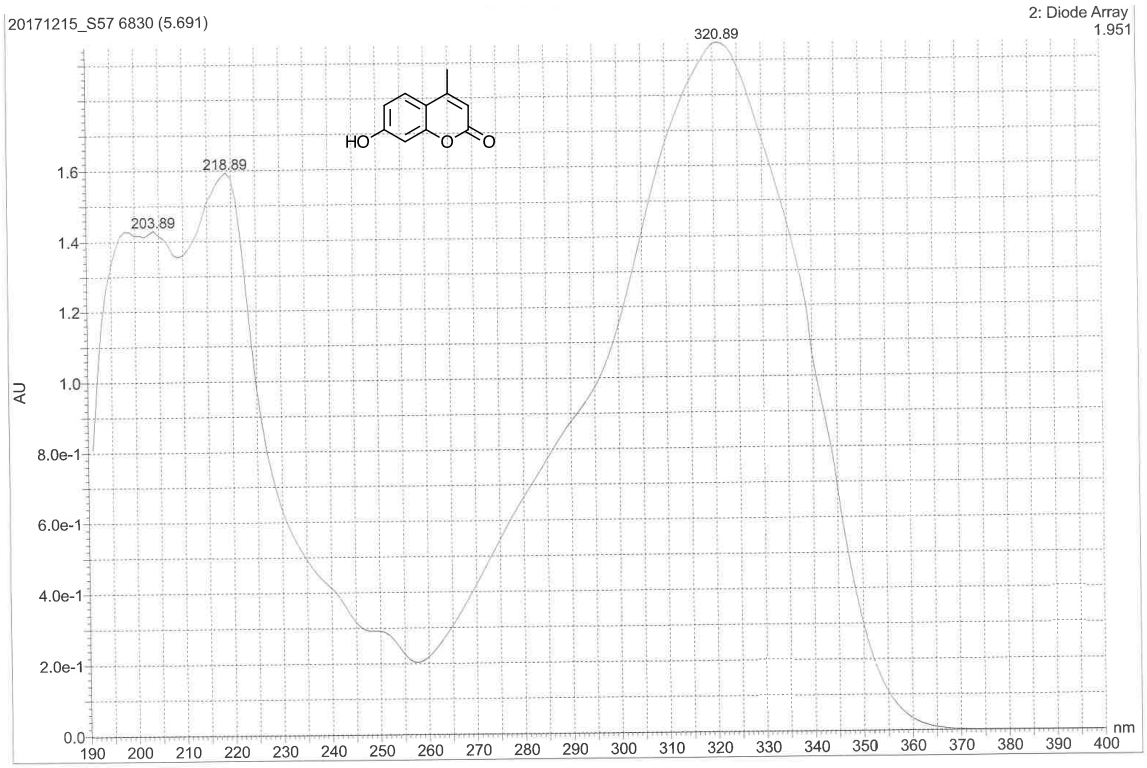


Figure 5.6. UV-Vis spectrum of compound **1**

5.1.2. Step 2: Synthesis of Piperazine and Piperidine Analogues

In the second step, the synthesized compound **1** was derivatized with a series of piperazine and piperidine molecules (Fig. 5.7). To generate targeted molecules formaldehyde was used for binding the heterocyclic moities at 8th position of compound **1**. The mechanism that is thought to take place is as follows. The step starts with the protonation of formaldehyde, which is followed by the nulceophilic attack of the nitrogen of the secondary amine function of either piperazine or piperidines. After proton exchanges, an iminium ion is generated and the attack of compound **1** on the electrophilic carbon allows the binding of the piperazine or piperidine heterocycle *via* a methylene linker at the 8th position of the coumarin ring (Sch. 5.2).

STEP 2:

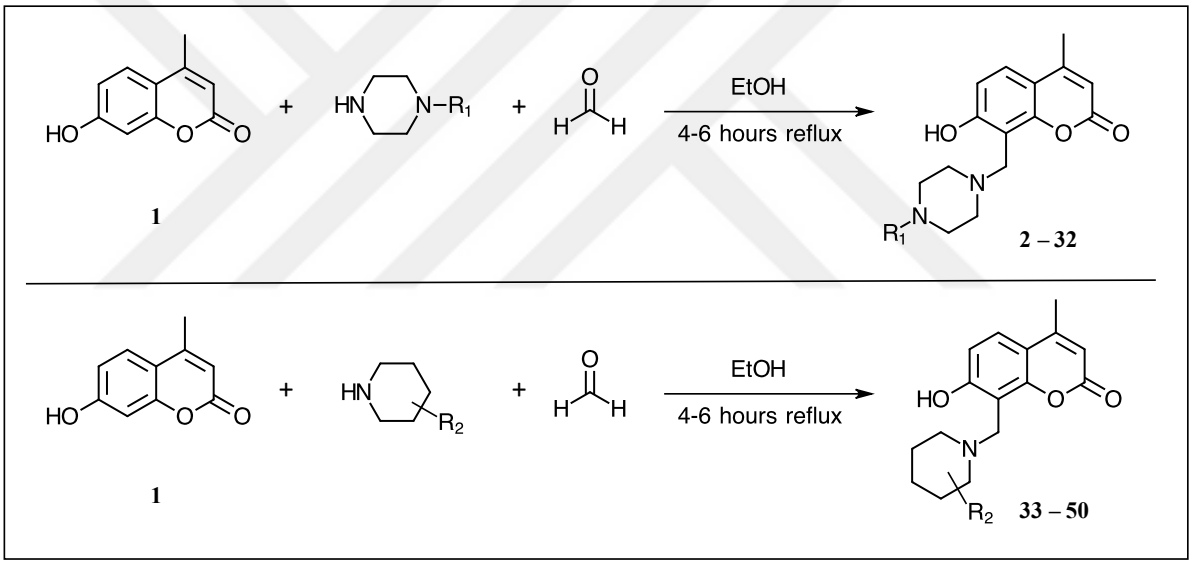
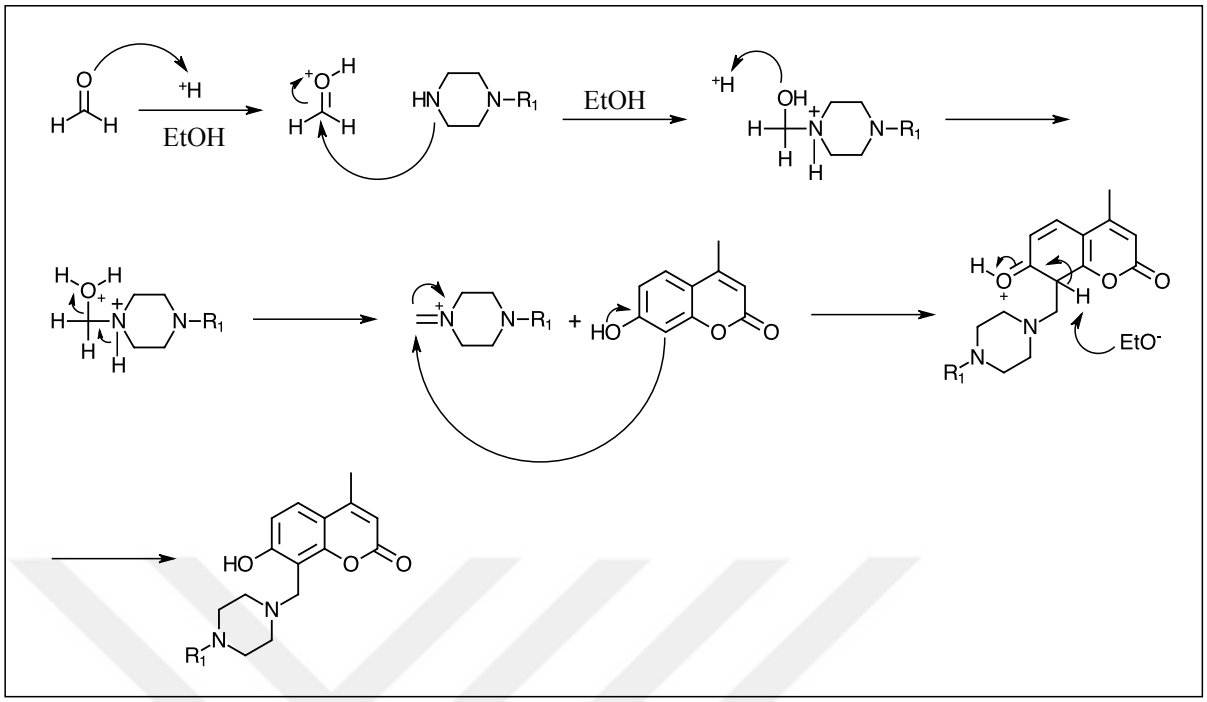


Figure 5.7. Synthesis of targeted compounds **2-50**

MECHANISM:



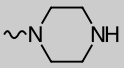
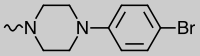
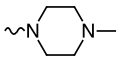
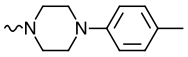
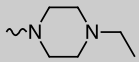
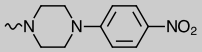
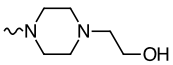
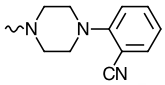
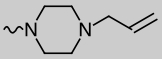
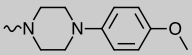
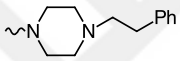
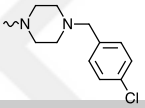
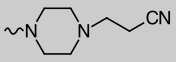
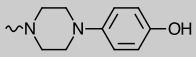
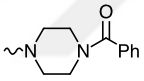
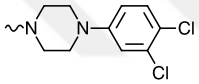
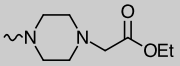
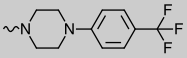
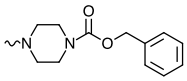
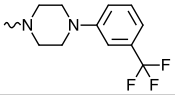
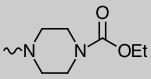
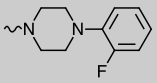
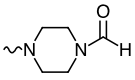
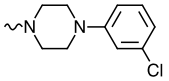
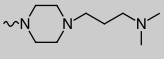
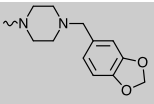
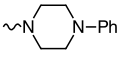
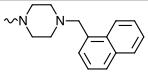
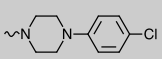
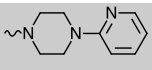
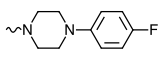
Scheme 5.2. General reaction mechanism of the compounds **2-50**.

In this study, a total of fifty coumarin derivatives (twenty two original structures) were aimed to be synthesized, thirty two of them being piperazine (fifteen original structures) and eighteen of them piperidine derivatives (seven original structures). After its synthesis each structure has been characterized with ^{1}H-NMR, ^{13}C-NMR, LC-MS and FT-IR.

Piperazine derivatives (2-32)

Thirty two different piperazines with various aliphatic and aromatic moieties substituted on the N4 atom of the heterocycle were used to generate this family of derivatives. Twenty six products were obtained with satisfactory to moderate yields (Table 5.1). Structure confirmation was achieved *via* spectroscopic methods as already mentioned.

Table 5.1. Piperazine derivatives yields

Compound		% Yield	Compound		% Yield
2		63	**18**		50
3		32	**19**		60
4		47	**20**		47
5		26	**21**		28
6		50	**22**		62
7		78	**23**		38
8		35	**24**		54
9		-	**25**		68
10		-	**26**		36
11		25	**27**		-
12		31	**28**		76
13		-	**29**		15
14		-	**30**		60
15		53	**31**		38
16		64	**32**		51
17		67			

The substitution of the coumarin heterocycle was easy to monitor *via* ^{1}H-NMR as a successful substitution gave a new singlet at around 4.0 ppm that integrated for 2 protons, corresponding to the methylene spacer between the coumarin and piperazine heterocycles. Yet, interpreting the signals of the piperazine cycle was not obvious as they can give multiplets or more commonly broad singlets due 8 non-equivalent protons that in addition to short-range correlation can present long-range correlations as mentioned in the literature [131]. An example of ^{1}H NMR spectrum for an aliphatic and an aromatic compound are given through the results obtained for compound **3** and compound **15** respectively. For compound **3**, signals attributable to the coumarin heterocyle are the doublet at 7.4 ppm, the doublet at 6.7 ppm, the quartet at 6.0 ppm and the doublet at 2.37 ppm that correspond to $\mathbf{H_5}$, $\mathbf{H_6}$, $\mathbf{H_3}$ and $\mathbf{H_9}$ respectively. Substitution is confirmed by the singlet obtained at 4.0 ppm that corresponds to the methylene linker ($\mathbf{H_{10}}$). The singlet at 2.31 ppm is the signal obtained for the N-methyl group ($\mathbf{H_{13}}$). Finally, the spectrum shows a very broad signal that integrates for 8 protons and this signal corresponds to the 8 protons of the piperazine cycle (Fig. 5.8).

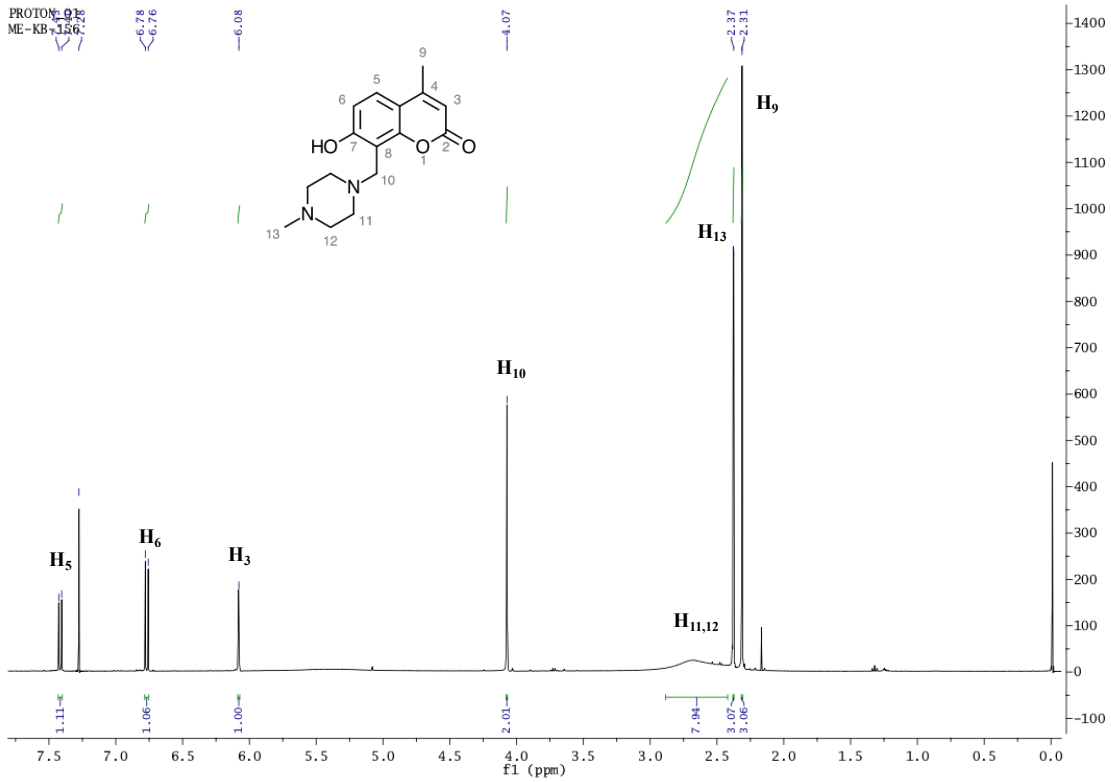


Figure 5.8. ^{1}H-NMR spectrum of compound **3**.

For compound **15**, in addition to the aromatic protons of coumarin found as doublets at 7.42 and 6.78 ppm, the downfield presents signals that correspond to the phenyl moiety.

A quartet at 6.1 ppm (J=1.2 Hz) corresponds to the vinylic proton of the coumarin cycle and the signal of the methylene that confirms the linkage is found at 4.10 ppm. The upfield present two broad singlets for the protons of the piperazine cycle in addition to the signal of the methyl group of the coumarin scaffold that appears at 2.37 ppm (Fig. 5.9).

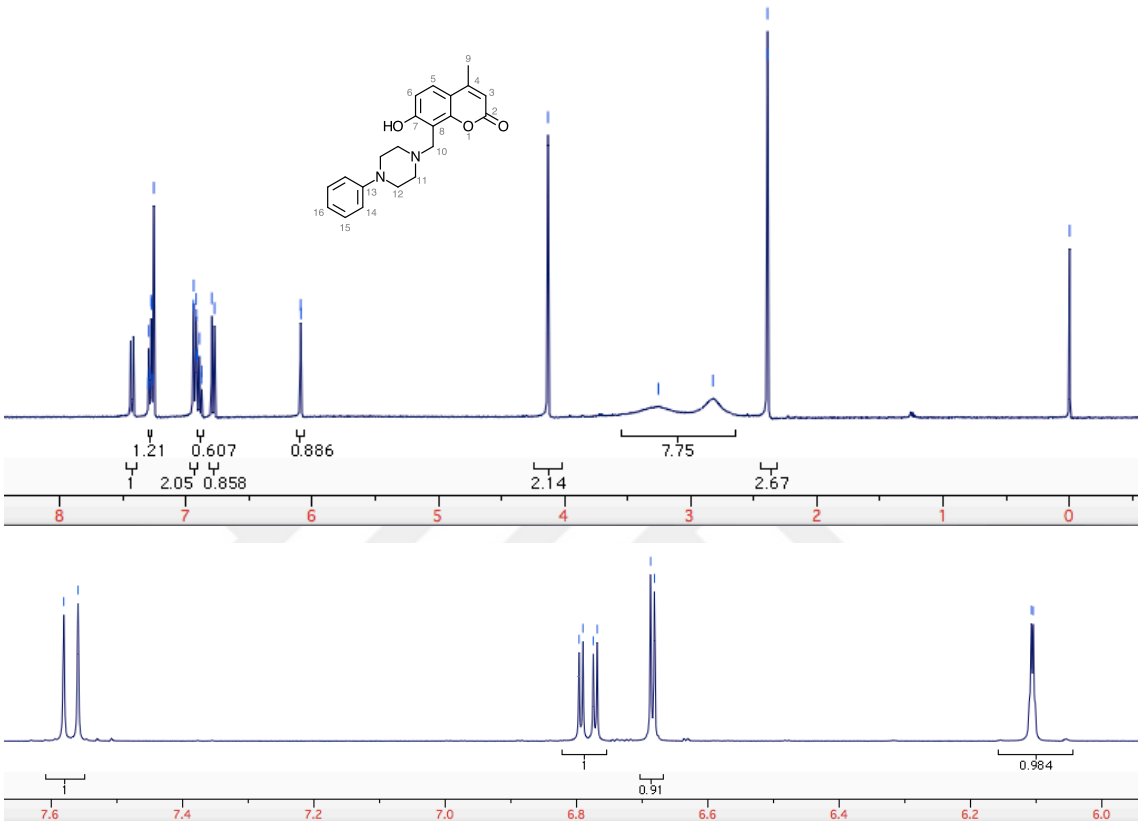


Figure 5.9. ^{1}H-NMR spectrum of compound **15**.

Structures of the generated products were also confirmed via ^{13}C NMR, FT-IR and LC-MS. Results obtained for compound **3** and **15** are given here for illustration.

To assign the signals of compound **3**, its ^{13}C NMR spectrum was compared to the one obtained with compound **1**. The analysis of both spectra shows that in addition to the signals obtained for the coumarin scaffold (compound **1**), there are extra peaks between 50 and 53 ppm that belong to piperazine ring (findings that are consistent with literature) and a signal at 45 ppm that is attributed to the methylene C (Fig. 5.10).

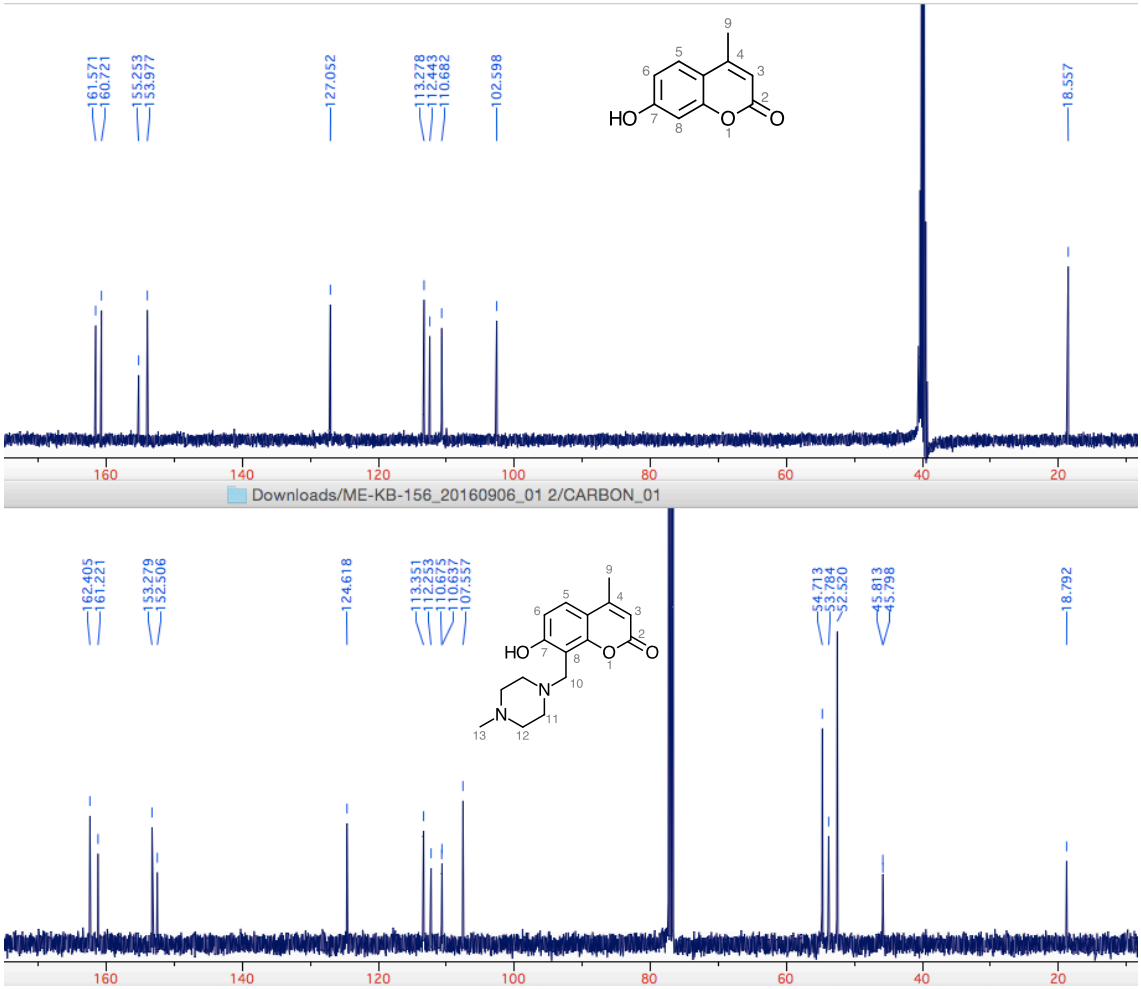


Figure 5.10. 13C-NMR spectrum of compound **1** (top) and compound **3** (bottom).

The spectra of compound **1**, **3** and **15** were plotted together and a similar result was obtained in this case too. The piperazine signals were obtained around 53 ppm whereas the methylene carbon gave a signal at 49 ppm. The aromatic carbons of the phenyl group gave peaks as expected in the 107-130 ppm range in addition to those obtained for the coumarin scaffold (Fig. 5.11).

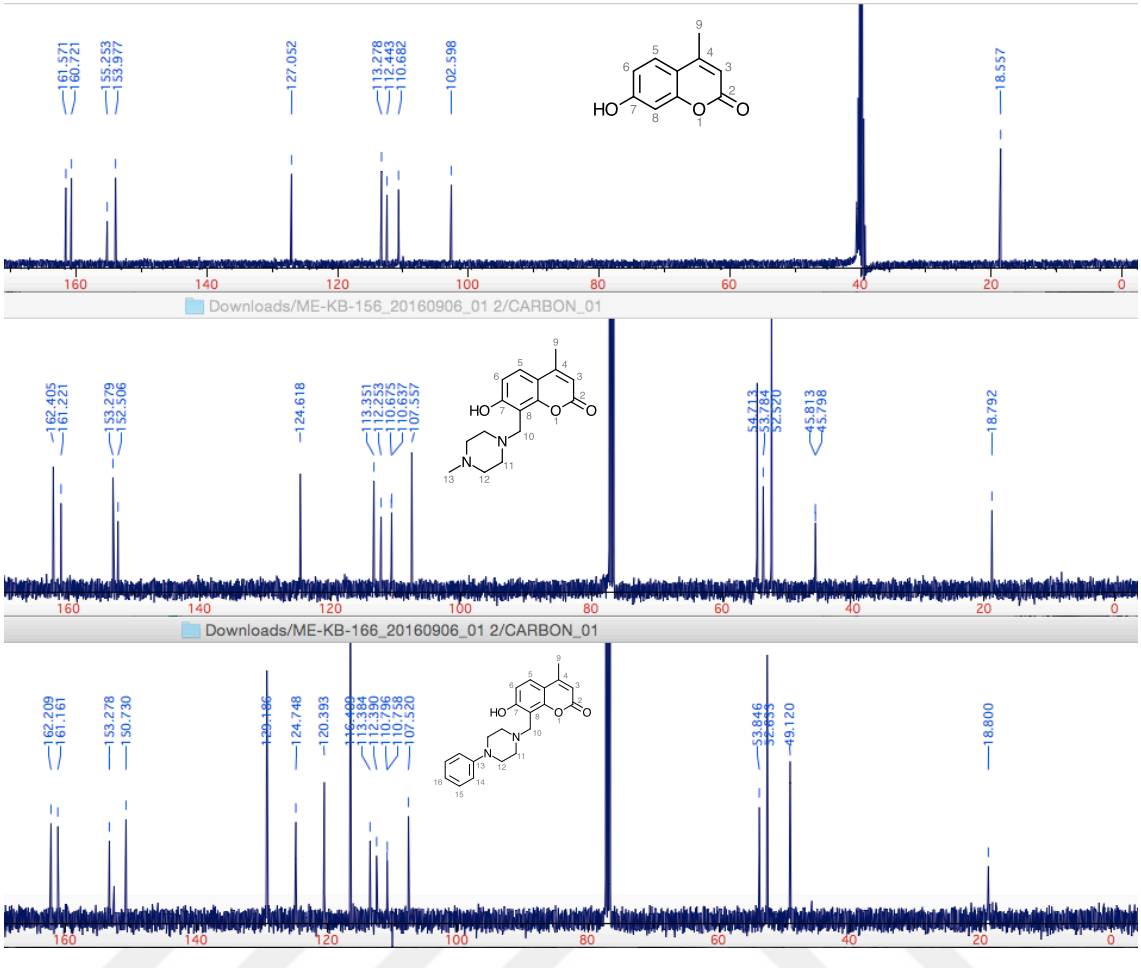


Figure 5.11. ^{13}C-NMR spectrum of compound **1** (top), compound **3** (middle) and compound **15** (bottom).

It is not obvious to determine the outcome of the reaction through the FT-IR analysis since neither the methylene nor the piperazine ring give characteristic vibrations on infrared spectroscopy.

Concerning the MS spectra of compound **3** and **15**, they point out the obtention of the expected products since the M+H peaks are observed at 289.33 (m/z) and 351.37 (m/z) respectively (Fig. 5.12, 5.13).

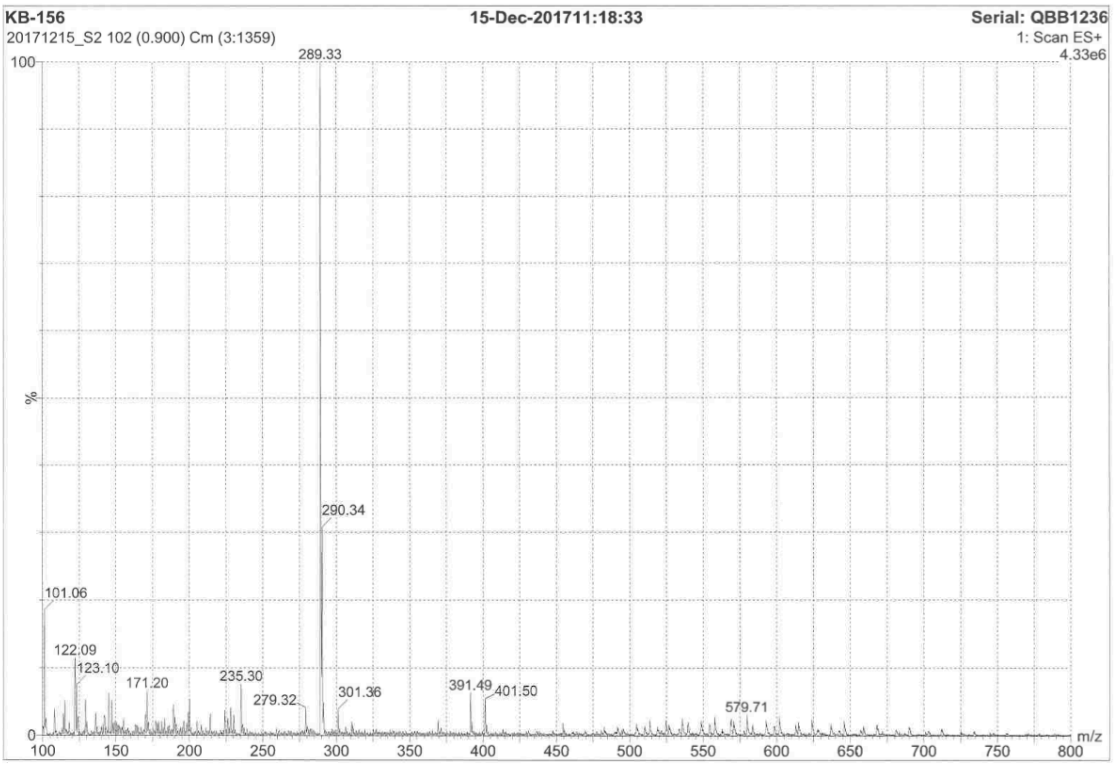


Figure 5.12. LC-MS spectrum of compound **3**.

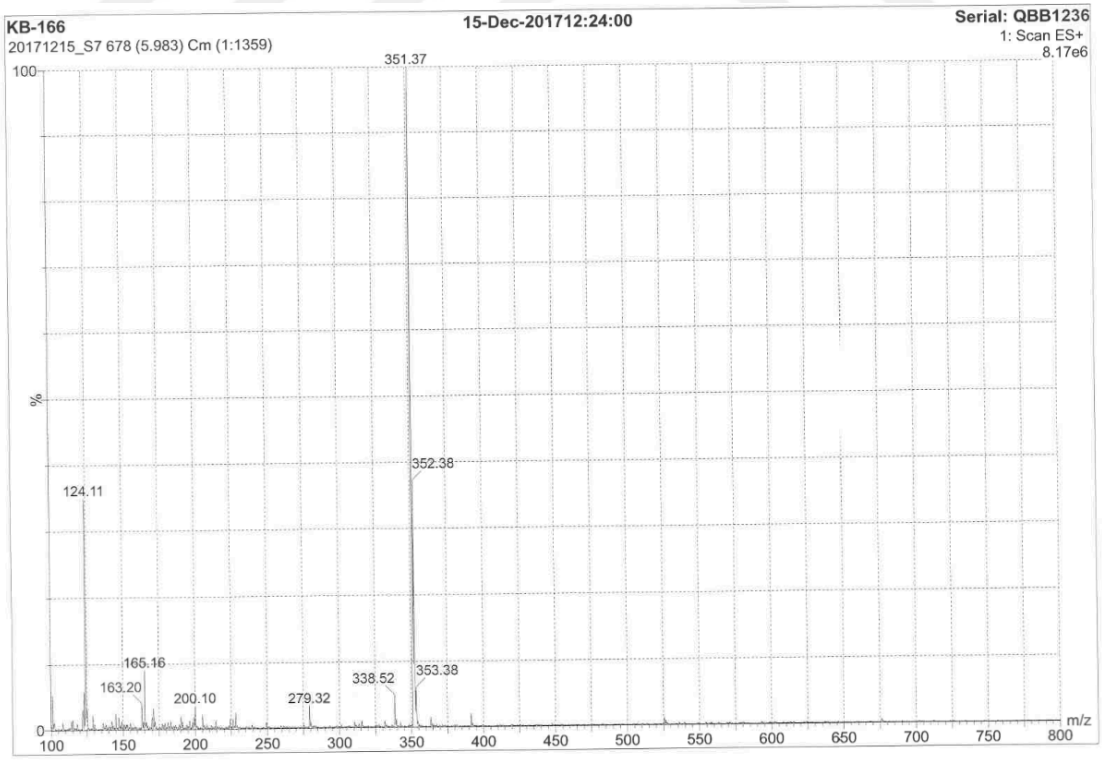
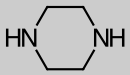
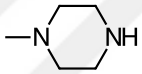
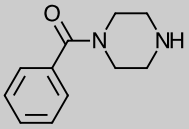
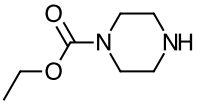


Figure 5.13. LC-MS spectrum of compound **15**

Compounds **9**, **10**, **13, 14** and **27** were not obtained as their ^{1}H NMR and LC-MS data only exhibited signals corresponding to the unsubtituted hydroxycoumarin **1** (Table 5.3.). The failure in the synthesis of these molecules can be partially explained by the acylation of the nitrogen for compounds **9** and **13**. Indeed, the presence of an electron-withdrawing group on the nitrogen decreases the basicity and hence the nucleophilicity of the second nitrogen which is necessary to generate the immium. This hypothesis can also explain the low yields obtained for compounds **11** and **12** (24 and 31% respectively). The experimentally determined pKa values of the corresponding piperazines (Table 5.2) that were found in the literature are given in the following table and strenghten this hypotothesis [132-135].

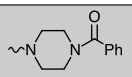
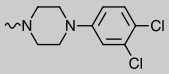
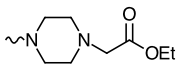
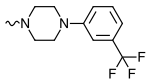
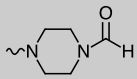
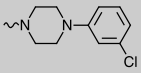
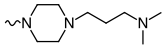
Table 5.2.Experimentally determined pKa values of some piperazines

Piperazine	pKa value
	9.81
	9.14
	7.78
	8.28

Concerning compound **14** the 5-atom-long subsituent may cause a steric hinderance around the nucleophilic nitrogen and thus not allow its attack on the protonated carbonyl of formaldehyde.

For compounds **25** and **29**, the obtained structures could not be dissolved in a suitable amount in any deuterated solvent to be analyzed in NMR. Their LC-MS yet gave the expected peaks presumably indicating that the piperazine moiety has been successfully grafted to the coumarin.

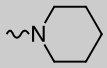
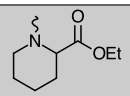
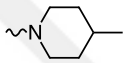
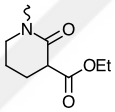
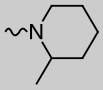
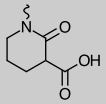
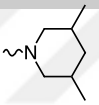
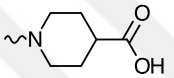
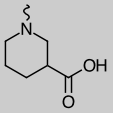
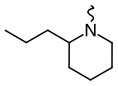
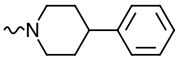
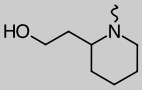
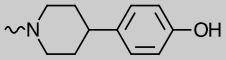
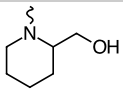
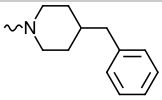
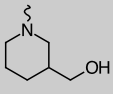
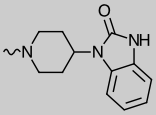
Table 5.3. Piperazine compounds not obtained in NMR

Compound		Compound	
9		**25**	
10		**27**	
13		**29**	
14			

Piperidine derivatives (33-50)

Eighteen different mono- or disubstituted piperidine molecules were used to generate the second family of compounds (Table 5.4). Twelve of the targeted molecules were successfully obtained with mostly satisfactory yields. Structure confirmation was again carried out through spectroscopic methods.

Table 5.4. Yields obtained for the eighteen piperidine derivatives

Compound		% Yield	Compound		% Yield
33		50	**42**		40
34		66	**43**		-
35		48	**44**		-
36		20	**45**		75
37		52	**46**		68
38		-	**47**		64
39		-	**48**		56
40		-	**49**		24
41		52	**50**		-

For this family of compounds, it was again possible to monitor the substitution of the coumarin heterocycle *via* the signals of the methylene spacer in ^{1}H-NMR as a successful

grafting resulted in detection of a new singlet at around 4.0 ppm that integrated for 2 protons. Interpreting the signals of the piperidine cycle were not obvious as the equatorial and axial protons of the cycle give different signals and can couple with each other. The ^{1}H NMR of compound **47** (the 4-phenyl) is presented in order to illustrate the results obtained for the piperidine derivatives.

The ^{1}H NMR spectrum of compound **47** exhibits in addition to the signals of coumarin, the typical singlet of the methylene spacer that confirms the linkage of the heterocyles. The piperidine signals have been identified according to data found in the literature [136, 137]. The multiplet at 3.13 - 3.16 ppm most probably belongs to the equatorial protons that are neighbouring the nitrogen atom ($\mathbf{H_{11}}$). The triplet of triplet that integrates for one proton corresponds to the proton of the substitution position ($\mathbf{H_{13}}$). The multiplet around 2.3 ppm that overlaps the doublet of the methyl group ($\mathbf{H_{9}}$) of the coumarin is attributed to axial protons neighbouring the N atom ($\mathbf{H_{11}}$) and the multiplet that integrates for 4 protons are attributed to $\mathbf{H_{12}}$, namely the remaining 4 protons of the piperidine cycle. The downfield also presents in addition to the doublets of the coumarin scaffold (7.40 and 6.77 ppm) 5 aromatic protons that are the proton of the substituted phenyl moiety (Fig. 5.14).

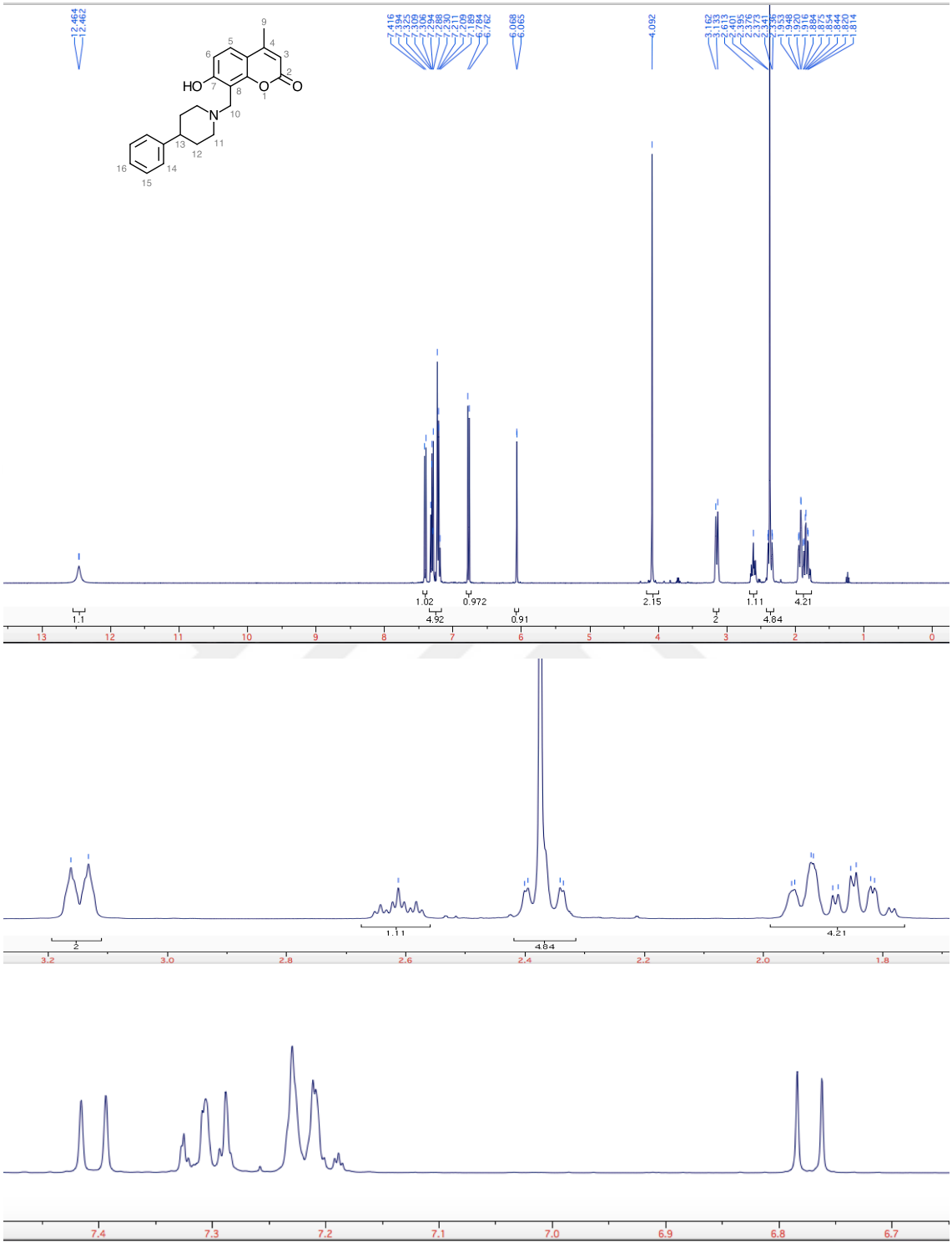


Figure 5.14. ^{1}H-NMR spectrum of compound **47**.

The ^{13}C NMR also confirms the obtention of the piperidine substituted coumarin since the methylene carbon appears at 41.9 ppm and the piperidine cycle gives signals that are

detected at 54, 53 and 33 ppm, shifts that are consistent with the literature [137] (Fig. 5.15).

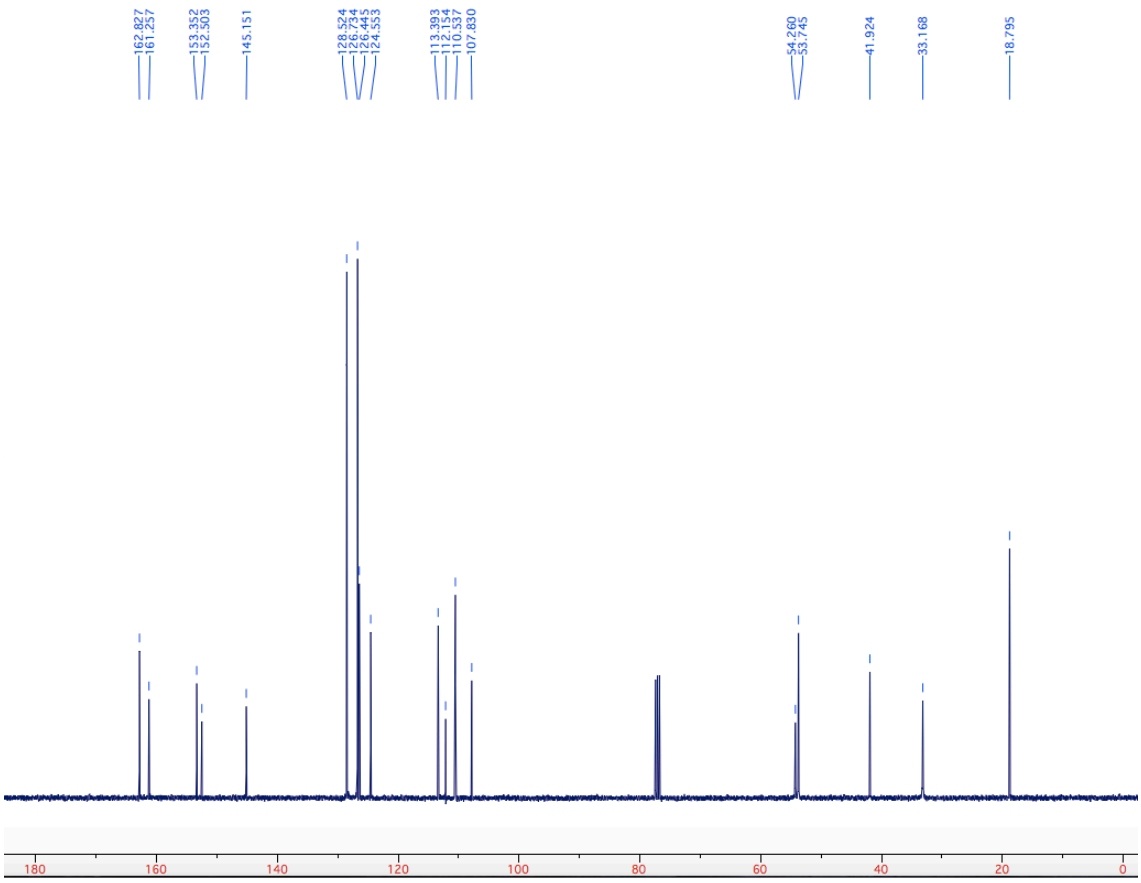
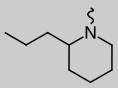
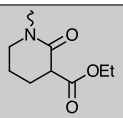
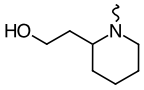
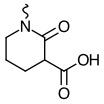
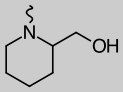
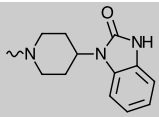


Figure 5.15. ^{13}C-NMR spectrum of compound **47**.

NMR and LC-MS results indicated that the synthesis of compounds **38**, **39**, **40**, **43**, **44** and **50** were not achieved (Table 5.5). For the first 4 the NMR data gave only the signals of the unsubstituted coumarin whereas for compound **50** a more complex spectrum was obtained. The piperidines that were used for the first 4 molecules were found to be substituted at their 2nd position, possibly generating a steric hinderance that does not allow the amine to attack successfully on the carbonyl of formaldehyde. In fact, the only molecules for which the piperidine is substituted at the 2nd position and that were successfully synthesized are compound **35**, **37** and **42** that are generated from piperidines substituted with a 2-methyl, 2-ethyl and 2-caboxylic acid ethyl ester respectively. These substituents are either small (for **35** and **37**) or should be rigid enough (**42**) to allow the approach of the electrophilic molecule. The failure concerning compound **50** should be due to some unexplained side reactions.

Table 5.5. Piperidine compounds not obtained in NMR

Compound		Compound	
38		**43**	
39		**44**	
40		**50**	

To sum up, 49 piperazine and piperidine substituted compounds were aimed to be obtained and finally 26 (15 novel) piperazine and 12 piperidine (7 novel) derived molecules were synthesized. The following section is devoted to the biological analysis of these compounds.

5.2. Biology

5.2.1. Cytotoxicity

The synthesized compounds were evaluated first for their cytotoxicity. The assay was performed *via* the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on non-carcinogenic RAW264.7 macrophage cells. Cells were treated with 100 μM of obtained molecules and viability was determined as the ratio of absorbance obtained for treated cells compared to the absorbance obtained for the cells treated with 0.5% DMSO, the solvent used to dissolve the molecules. Values were given as percentages (Table 5.6). The analysis of the data indicated that the synthesized structures are mostly not cytotoxic except for the *p*-chlorobenzyl substituted piperazine derivative **23** that demonstrated a viability of 36% when used at 100 μM. As cells were treated at very high concentrations and given that the viability did not drop under 10%, this molecule can still be considerated as only moderately cytotoxic.

Once results were obtained with healthy cells, some of the structures were also evaluated for their anticancer activity on MCF-7 breast cancer cells. Given the low cytotoxicity obtained with macrophages, the molecules were examined in this assay at a concentration of 10 μM. Again, for almost all of the investigated structures, no cytotoxicity was detected except for molecules **17** and **23** for which viability dropped to 37 and 23% respectively. Even if these values are not good enough to characterize a structure as an antiproliferative molecule, they are still significant and can allow molecules **17** and **23** to constitute starting points for the development of novel potent anticancer coumarin derivatives.

Table 5.6. Cytotoxicity results on RAW 264.7 macrophage and MCF-7 breast cancer cell lines

	Compound	R	RAW 264.7	MCF-7
Piperazines	**1**	-	78,9 ± 9,9	109 ± 1,2
	2	-	ND	ND
	3	CH_3	76,11 ± 11	102 ± 4,9
	4	CH_2CH_3	76 ± 11,2	101 ± 3,6
	5	CH_2CH_2OH	66 ± 6,6	106 ± 1,5
	6	CH_2=$CHCH_2$	70 ± 8,1	96 ± 6,9
	7	CH_2CH_2Ph	ND	ND
	8	CH_2CH_2CN	65 ± 8,9	ND
	11	$COOCH_2Ph$	64 ± 3,4	ND
	12	$COOCH_2CH_3$	66 ± 6	ND
	15	Ph	66 ± 4,3	116 ± 4,0
	16	*p*-Cl-Ph	87 ± 1,9	90 ± 4,4
	17	*p*-F-Ph	67 ± 13,6	37 ± 3,8
	18	*p*-Br-Ph	64 ± 9,5	ND
	19	*p*-CH_3-Ph	60 ± 8,3	85 ± 1,7
	20	*p*-NO_2-Ph	59 ± 2,3	102 ± 2,1
	21	*o*-CN-Ph	55 ± 6,9	93 ± 2,2
	22	*p*-OMe-Ph	64 ± 7,8	85 ± 1,7
	23	*p*-Cl-Bn	36 ± 5,1	23 ± 1,9
	24	*p*-OH-Ph	65 ± 7,7	ND
	25	3,4-$(Cl)_2$-Ph	77 ± 3,7	95 ± 3,9
	26	*p*-CF_3-Ph	ND	ND
	28	*o*-F-Ph	77 ± 11,8	ND
	30	Benzo[1.3]dioxol-5yl	65 ± 12,7	ND
	31	Naphthylmethyl	68 ± 5,2	ND
	32	Pyridin-2-yl	64 ± 11	ND
Piperidines	**33**	-	61 ±12,5	ND
	34	4-CH_3	67 ±5,4	ND
	35	2-CH_3	79 ± 10,7	ND
	36	3,5-$(CH_3)_2$	77 ± 6,4	ND
	37	2-CH_2CH_3	69 ± 13,6	ND
	41	3-CH_2OH	77 ± 6,1	ND
	42	2-$COOCH_2CH_3$	81 ± 2,9	ND
	45	4-COOH	57 ±3,1	ND
	46	3-COOH	ND	ND
	47	4-Ph	ND	ND
	48	4-(*p*-OH-Ph)	61 ±5,2	ND
	49	4-Bn	59 ± 10,7	ND

ND: Not determined

5.2.2. Anti-inflammatory Activity

5.2.2.1. The Nitrite Assay

The anti-inflammatory activities of the synthesized compounds were evaluated using the nitrite inhibition assay. Nitrite levels are significant inflammation indicators as they are known to be substantively increased in inflammatory tissues. RAW264.7 macrophages cells were treated with LPS to induce an inflammatory state and then the obtained compounds were added (100 μM) to the culture medium. As not soluble in DMSO, compounds **2**, **7**, **26**, **47** and **48** were excluded from the evaluation. Indomethacin (IND) (100 μM) and N^{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME) were used as reference drugs (100 μM) and the nitrite levels were determined in culture supernatant to be compared to the levels obtained for LPS induced cells treated with the solvent used to dissolve the molecules (0.5% DMSO+LPS). Results are given as the ratio $[NO]_{\text{treated cells}}/[NO]_{\text{untreated cells}}$ and expressed as percentages in the following table (Table 5.7).

Results indicated first of all that the unsubstituted coumarin molecule, compound **1**, does not show an anti-inflammatory activity. Data obtained with the piperazine structures substituted with aliphatic moieties (**2**-**12**) show that the small alkyl group such as methyl, ethyl, allyl, or hydroxy- or cyano- substituted alkyl group are also inactive. With compounds that have an acylated nitrogen, the activity has been found to be increased. Indeed, the ethyl carbamate derivative **12** gave a decrease of 11%. Also, 90% of decrease in nitrite levels was obtained when cells were treated with the benzyl carbamate substituted compound **11**. This compound actually gave the best activity among all the tested compounds being three times more potent then the reference indomethacin molecule.

Piperazines substituted with aromatic rings also gave satisfactory results. Even if the phenyl substituted piperazine derivative led to an inhibition in NO levels of only 14%, its substitution significantly increased the activity of the molecules. Substitution at the para- position of the phenyl ring was found to increase the bioactivity of the compounds since the *p*-Cl (**16**), *p*-F (**17**), *p*-Br (**18**), *p*-OCH_3 (**22**), *p*-OH (**24**) demonstrated better inhibition levels even if still moderate (30, 23, 23, 20, 17 and 47% respectively), the best activity being obtained with the *p*-NO_2 compound **20** (59%). The only exception to this trend was the *p*-CH_3 (**19**), structure for which no inhibition ability was determined.

Table 5.7. Antiinflammatory activities of the tested compounds.

	Compound	R	% Nitrite Reduction
Piperazines	**1**	-	-2,0 ± 10,4
	2	-	NR
	3	CH_3	-2,8 ± 8,3
	4	CH_2CH_3	7,1 ± 5,0
	5	CH_2CH_2OH	9,0 ± 4,2
	6	CH_2=$CHCH_2$	-1,6 ± 2,3
	7	CH_2CH_2Ph	NR
	8	CH_2CH_2CN	1,1 ± 1,0
	11	$COOCH_2Ph$	90,1 ± 8,7
	12	$COOCH_2CH_3$	11,5 ± 4,9
	15	Ph	13,9 ± 3,7
	16	*p*-Cl-Ph	21,6 ± 7,4
	17	*p*-F-Ph	30,0 ± 6,6
	18	*p*-Br-Ph	23,2 ± 11,2
	19	*p*-CH_3-Ph	1,6 ± 6,8
	20	*p*-NO_2-Ph	58,8 ± 8,4
	21	*o*-CN-Ph	20,2 ± 7,2
	22	*p*-OMe-Ph	17,3 ± 4,8
	23	*p*-Cl-Bn	83,2 ± 8,2
	24	*p*-OH-Ph	47,2 ± 2,8
	25	3,4-$(Cl)_2$-Ph	42,5 ± 4,2
	26	*p*-CF_3-Ph	NR
	28	*o*-F-Ph	2,1 ± 3,5
	30	Benzo[1.3]dioxol-5yl	26,0 ± 2,0
	31	Naphthylmethyl	73,0 ± 6,1
	32	Pyridin-2-yl	3,7 ± 2,2
Piperidines	**33**	-	-6,0 ± 3,4
	34	4-CH_3	-3,1 ± 7,4
	35	2-CH_3	17,1 ± 3,9
	36	3,5-$(CH_3)_2$	-4,1 ± 3,0
	37	2-CH_2CH_3	-0,9 ± 3,4
	41	3-CH_2OH	-0,7 ± 5,2
	42	2-$COOCH_2CH_3$	-22,6 ± 10,1
	45	4-COOH	5,7 ± 0,9
	46	3-COOH	-2,6 ± 6,9
	47	4-Ph	NR
	48	4-(*p*-OH-Ph)	NR
	49	4-Bn	38,5 ± 5,2
		IND + LPS	31,5 ± 6,8
		L-NAME + LPS	44,4 ± 6,2

NR: No result

Substitution at the ortho- position also gave moderate activities since compounds **21**, **28** and **29** led to 20, 2 and 24% of inhibition respectively. The electron-withdrawing or electron-attracting property of the substituted groups seemed to not affect the bioactivity since similar activities were obtained for the *p*-NO_2 and *p*-OH compounds **20** and **24** the first being a very effective electron-withdrawing whereas the second is a good electron donating group. Finally, the best results for this class of compounds were obtained for the p-chlorobenzyl (**23**) and naphtylmethyl (**31**) derivatives with 83 and 73% of inhibition respectively, suggesting that a methylene spacer could be necessary for the bioactivity to reach considerable levels. Concerning the piperidine compounds (**33**-**49**), data indicated that these structures do not present any anti-inflammatory as NO levels were found to remain unchanged if not increased when cells were treated with 100µM of these compounds.

To investigate if the detected bioactivities were dose-dependent or not, some of the compounds (**11**, **17**, **19**, **20**, **21**, **22**, **23**, **24**, **28**, **29**, **31**)were tested further in a range of concentration varying between 6.25 and 100 µM. Results are given in the following table where the compounds are clustered according to their activities: the most active compounds (**11**, **23**, **31**), compounds for which the inhibition percentages were about 50 % (**20**, **24**), compounds for which the inhibition percentages were below 30 % (**17**, **21**, **29**) and inactive compounds (**19**, **22**, **28**) (Table 5.8).

Table 5.8. Reduction% of nitrite concentration for different compound concentrations.

NO inhibition from 70 to 90%			
	11	**23**	**31**
100 µM	90,1 ± 8,7	83,2 ± 8,2	73,0 ± 6,1
50 µM	65,5 ± 6,4	26,7 ± 6,8	66,5 ± 4,5
25 µM	30,2 ± 10,0	-14,2 ± 2,7	50,3 ± 5,5
12.5 µM	16,9 ± 9,2		11,4 ± 7,8
6.25 µM	-16,1 ± 5,2		-15,1 ± 5,2

NO inhibition from 50 to 70%		
	20	**24**
100 µM	58,8 ± 8,4	47,2 ± 2,8
50 µM	49,4 ± 3,1	45,5 ± 2,0
25 µM	53,3 ± 7,5	
12.5 µM	56,2 ± 4,4	

NO inhibition from 20 to 50%		
	17	**21**
100 μM	30,0 ± 6,6	20,2 ± 7,2
50 μM	23,0 ± 4,9	2,1 ± 4,0
25 μM	8,5 ± 8,6	

Inactive Compounds			
	19	**22**	**28**
100 μM	1,6 ± 6,8	17,3 ± 4,8	2,1 ± 3,5
50 μM	11,9 ± 6,4	18,6 ± 4,0	0,3 ± 4,1

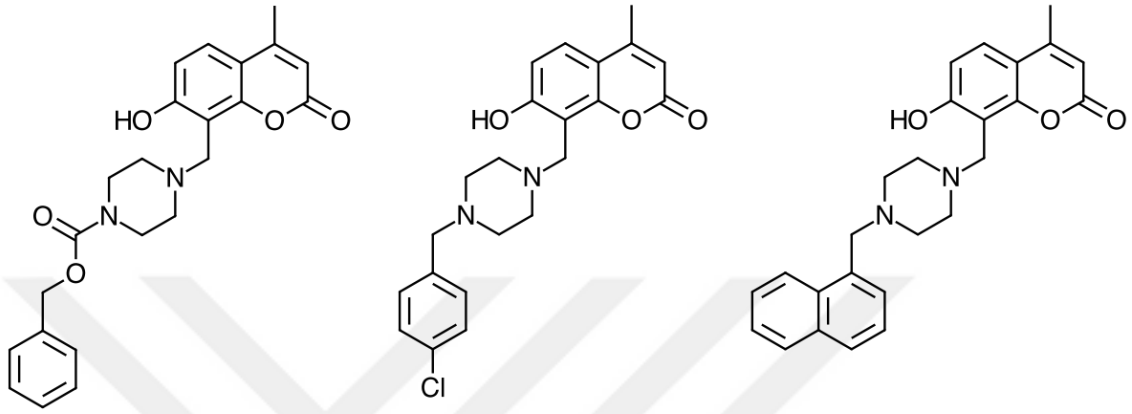
The most active compound was **11** and its inhibition ability dropped from 90.1 % to 65.5 % when a concentration of 50 μM was applied. Concentration was decreased up to 6.25 μM and **11** became inactive indicating that the anti-inflammatory activity of compound **11** is dose-dependent. Compound **23** followed the same pattern as **11**, but the activity of **23** was found to decrease sharply. Actually, when 100 μM of this compound was applied reduction in nitrite levels was of 83.2 % but, for 25 μM the ratio dropped to -14.2 %. Concerning the relationship between the activity of the compound **31** and its concentration – its activity profile was found to be a little different than the others since up to 25 μM, the nitrite reduction levels did not change significantly. But, below 25 μM compound **31** became inactive.

Results obtained for compounds **20** and **24** indicated that their inhibition capacity did not change according to the concentration applied since for these two molecules, the nitrite levels remained approximately the same for all the tested concentrations.

Concerning the moderately active compounds **17**, **21** and **29**, nitrite levels decreased as expected with the compound concentrations applied to cells, compound **29** being completely inactive at 50 μM.

The activity of compounds **22** and **28** did not change with the concentration as expected. Yet, interestingly, the anti-inflammatory activity of compound **19** increased when it was tested at 50μM. Some additional studies at lower concentrations can be carried out for this molecule to check if this unexpected change in the activity can be still observed.

As a result, our study showed that substituting the coumarin scaffold with a piperidine moiety or alkyl-substituted piperazines is not suitable for getting some anti-inflammatory activity, whereas the aryl-substituted piperazine heterocyclse seems to be appropriate for the development of novel coumarin substituted anti-inflammatory drugs (Fig. 5.16).



11 (90%) **23** (83%) **31** (73%)

Figure 5.16. Structures of the most active compounds: **11**, **23**, and **31**.

5.2.2.2. Monitoring of PGE_2 Production

Most of the anti-inflammatory compounds are involved in the arachidonic acid pathway. In this pathway, the inhibition of the synthesis of prostaglandin H_2 also stops the production of PGE_2 which is responsible for the sensation of pain. Therefore, compounds **11**, **20**, **24**, **25**, **31**, and **49** that demonstrated promising anti-inflammatory activity, were evaluated for their analgesic activity. The analgesic activity was evaluated with the prostaglandin E2 Elisa kit which analyzes the prostaglandin E2 (PGE_2) production. Compound **23** for which considerable anti-inflammatory activity was also determined was not included into this evaluation given its cytotoxicity. Indeed, molecules with a cytotoxicity that induces a viability smaller then 70% cannot give significant data since the amount of PGE2 production will be restricted by the cytotoxicity of the molecule. The obtained data are gathered in Table 5.9.

Table 5.9. Analgesic activity test results

Compound	PGE_2 Production (pg/mL)
11	2768,6
20	3418,9
24	2663,6
25	2830,7
31	3269,7
49	3215,8
%0.5 DMSO + LPS	3325,84
IND + LPS	265,38
L-NAME + LPS	3380,54

According to the inhibition of PGE_2 production test results, the amount of PGE_2 production was found to be decreased significantly (t-test, $p<0.1$) with compound **11**, **24** and **25**, but not as efficiently as the one obtained with the indomethacin reference drug. Compound **11** which had the highest anti-inflammatory activity revealed moderate analgesic activity (PGE_2 production 2768.6 pg/mL).

6. CONCLUSION

In conclusion, in this study we aimed to synthesize fourty nine coumarin derivatives substituted with piperizine and piperidine groups at the eighth position and thirty eight of them (twenty two being novel) were successfully obtained. Synthesized coumpounds were evaluated for their cytotoxicities and anti-inflammatory activities. The results indicated that the obtained molecules do not present any anticancer activity. Therefore, any correlation was not observed between anticancer activity and anti-inflammatory activities of coumarin derivatives in this study. Concerning the anti-inflammaroty activity, according to the NO inhibition assay results, piperazine derivatives were found to be much more active than the piperidines. The highest anti-inflammatory activities were observed for compounds **11**, **23** and **31** as they were 2 to 3 times more active than the reference indomethacin drug. Moreover, the activities of these compounds (**11**, **23**, **31**) were found to be dose-dependent. Beside compound **11** also led to a slight decrease in PGE_2 synthesis and low cytotoxicity. As a result, the substitution of the eighth position of coumarin molecule with some aryl substituted piperazine moieties was revealed to be suitable for the development of novel anti-inflammatory drugs.

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8.CURRICULUM VITAE

Kişisel Bilgiler

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Öğrenim Durumu

Derece	Alan	Mezun Olduğu Kurumun Adı	Mezuniyet Yılı
Doktora	Farmasötik Kimya	Yeditepe Üniversitesi	2018
Yüksek Lisans	Farmasötik Kimya	Yeditepe Üniversitesi	2013
Lisans	Kimya Bölümü	ODTÜ	2009
Lise	-		

[#] Başarılmış birden fazla sınav varsa(KPDS, ÜDS, TOEFL; EELTS vs), tüm sonuçlar yazılmalıdır

Bildiği Yabancı Dilleri	Yabancı Dil Sınav Notu (#)
YÖKDİL	90

İş Deneyimi (Sondan geçmişe doğru sıralayın)

Görevi	Kurum	Süre (Yıl - Yıl)
Araştırma Görevlisi	Yeditepe Üniversitesi	2011-2018
		-

Bilgisayar Bilgisi

Program	Kullanma becerisi
Microsof office	iyi

*Çok iyi, iyi, orta, zayıf olarak değerlendirin

Bilimsel Çalışmaları

SCI, SSCI, AHCI indekslerine giren dergilerde yayınlanan makaleler

1) The Cysteine Releasing Pattern of Some Antioxidant Thiazolidine-4-carboxylic acids. **Eur. J. Med. Chem.** 114, 337-344 **(2016).**
2) Evaluation of the mutagenic and genotoxic effects of the ALC67 thiazolidine compound in Salmonella strains and human lymphocytes in vitro. **Hum. Exp. Toxicol.** 35(10), 1108-1115 (**2016)**.
3) 3-Propionyl-thiazolidine-4-carboxylic acid ethyl esters: a family of antiproliferative thiazolidines. **Med.Chem.Commun.** 6, 90-93 **(2015)**.
4) Sol-Gel approaches for elaboration of polyol dehydrogenase-based bioelectrodes. **Z. Phys. Chem.** 227, 5, 667-689 **(2013)**.
5) Gold(I)/Zn(II) catalyzed tandem hydroamination/annulation reactions of 4-yne-nitrile. **Chem.Commun**. 46, 8032-8034 **(2010)**.

Diğer dergilerde yayınlanan makaleler

Uluslararası bilimsel toplantılarda sunulan ve bildiri kitabında (*Proceedings*) basılan bildiriler