T.C.
REPUBLIC OF TURKEY
HACETTEPE UNIVERSITY
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PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON SOME *PAPAVER* SPECIES IN TURKEY

Dr. MSc. Omer BAYAZEID

Program of Pharmacognosy
DOCTOR OF PHILOSOPHY THESIS

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Prof. Dr. Funda N. Yalçın

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Phytochemical and Pharmacological Studies on Different *Papaver* Species in Turkey

MSc. Pharm. Omer BAYAZEID

This study has been approved and accepted as a PhD dissertation in the program of Pharmacognosy by the examining committee, whose members are listed below on 14.07.2017.

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Date 14 Ağustos 2017

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- ○ **Tezimin/Raporumun……..tarihine kadar erişime açılmasını istemiyorum ancak kaynak gösterilmek şartıyla bir kısmı veya tamamının fotokopisinin alınmasını onaylıyorum**.

- ○ **Serbest Seçenek/Yazarın Seçimi**

14/08/2017

ETHICAL DECLARATION

In this thesis study, I declare that all the information and documents have been obtained in the base of the academic rules and all audio-visual and written information and results have been presented according to the rules of scientific ethics. I did not do any distortion in data set. In case of using other works, related studies have been fully cited in accordance with the scientific standards. I also declare that my thesis study is original except cited references. It was produced by myself in consultation with supervisor Prof. Dr. Funda Nuray Yalçın and written according to the rules of thesis writing of Hacettepe University Institute of Health Sciences.

Dr. MSc. Omer BAYAZEID

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Dr. Msc. Omer BAYAZEID

ABSTRACT

Omer BAYAZEID, Phytochemical and Pharmacological Studies on Some *Papaver* Species in Turkey. Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, Doctor of Philosophy Thesis, Ankara, 2017. *Papaver* species have been used for treating varieties of diseases including depression for centuries in Anatolia. We screened five *Papaver* species plant extracts for their antidepressant-like activity *in vivo* and their influence on BDNF expression in SH-SY5Y cells *in vitro*. According to the biological activity tests, the most active species *in vivo* and *in vitro P. lacerum* was chosen for the phytochemical analysis. One new 5-*O*-(6-*O*-*α*-rhamnopyranosyl-*β*-glucopyranosyl mevalonic acid (RGM) and one known Tyrosol-1-*O*-*β*-xylopyranosyl-(1→6)-*O*-*β*-glucopyranoside (TXG) glycosides were isolated from the active extract, which exhibited moderate effects on BDNF. To determine the metabolites related to BDNF expression, commercially available main alkaloids in *P. lacerum*, (roemerine and (+)-pronuciferine) were purchased and tested. Both roemerine, (+)-pronuciferine (20 and 10 μM) were significantly ($p< 0.0001$ - 0.001) active. LC-MS/MS analysis confirmed that roemerine and (+)-pronuciferine exist in the active *P. lacerum* extract. To identify possible effects of the isolated compounds TXG and RGM, *in silico* methods were used. *In silico* results showed that both compounds might exhibit anticancer activity. To confirm the *in silico* result, both compounds have been tested *in vitro* against HeLa cell line to evaluate their cytotoxicity activities using MTT assay. As a result both TXG and RGM were cytotoxic to HeLa cells with IC_{50} from 54 – 66.4 μM. roemerine, (+)-pronuciferine, TXG and RGM did not show any cytotoxicity to the normal cell line (L929 cells).

Keywords: Depression, BDNF, *in silico*, cytotoxicity.

Supporter Foundations: TÜBİTAK Ph.D. fellowship **(**2215), Hacettepe University Scientific Researches Coordination Unit (Project Number: 014 D11 301 003-734).

ÖZET

Omer BAYAZEID, Türkiye'deki Bazı *Papaver* Türleri Üzerinde Fitokimyasal ve Farmakolojik Çalışmalar, Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü Farmakognozi Programı Doktora Tezi, Ankara, 2017. *Papaver* türleri Anadolu'da yüzyıllar boyunca depresyon gibi bazı zihinsel hastalıkların tedavisinde kullanılmıştır. Beş *Papaver* türünün ekstrelerini antidepresan benzeri aktiviteleri *in vivo* ve BDNF ekspresyonu üzerindeki etkileri SH-SY5Y hücreleri üzerinde *in vitro* olarak incelenmiştir. *In vivo* ve *in vitro* biyolojik aktivite testlerine göre *P. lacerum*, en aktif bitki olarak bulunmuş ve fitokimyasal analizler için seçilmiştir. Aktif ekstreden bilinen bir glikozit [Tyrosol-1-*O*-*β*-ksilopiranozil-(1→6)-*O*-*β*-glukopiranozit (TXG)] ile birlikte yeni bir glikozit [5-*O*-(6-*O*-*α*-ramnopiranozil-*β*-glukopiranozil mevalonik asit (RGM)] izole edilmiştir. Her iki bileşik de BDNF üzerinde orta etkili bulunduğundan ekstredeki BDNF ekspresyonu ile ilişkili metabolitleri tespit etmek için *P. lacerum*'da bulunan ana alkaloidler (roemerin, (+)–pronusiferin) denenmiştir. Hem roemerin hem de (+)-pronusiferin (20 and 10 μM) SH-SY5Y hücrelerinde belirgin derecede ($p< 0.0001$ - 0.001) aktif bulunmuştur. LC-MS/MS analizleri, roemerin ve (+)-pronusiferinin *P. lacerum* ekstresinde bulunduğunu doğrulamıştır. TXG ve RGM bileşiklerinin olası biyolojik etkilerini tespit etmek için uygulanan *in silico* testler her iki bileşiğin de antikanser etkili olabileceğini göstermiştir. Her iki bileşik de sitotoksik etkilerini değerlendirmek için MTT yöntemiyle HeLa hücre dizisine karşı uygulanmıştır. TXG ve RGM'nin her ikisi de HeLa hücrelerine karşı 54–66.4 μM IC_{50} ile sitotoksik etki göstermiştir. TXG ve RGM, normal L929 hücre dizisine herhangi bir sitotoksisite göstermemiştir.

Anahtar Kelimeler: Depresyon, BDNF, *in silico*, sitotoksisite.

Destekleyen Kuruluşlar: TÜBİTAK Doktora (2215), Hacettepe Üniversitesi Bilimsel Araştırmalar Koordinasyon Birimi (Proje Numarası: 014 D11 301 003-734).

CONTENT

ABBREVIATIONS

5-HT	Serotonin
BDNF	Brain Derived Neurotrophic Factor
C-18	Reverse phase column
cAMP	Cyclic adenosine monophosphate
CNS	Central Nervous System
COSY	2D ^{1}H, ^{1}H homonuclear correlation spectroscopy
CP	ChemProt
CREB	cAMP response element binding
DA	Dopamine
DMEM	Dulbecco's Modified Eagle's Medium
DP	DRAR-CPI
ELISA	Enzyme-linked immunosorbent assay
EtOAc	Ethyl acetate
FBS	Fetal Bovine Serum
GABA	Gamma-Aminobutyric acid
HMBC	2D ^{1}H, ^{13}C Heteronuclear multiple-bond correlation spectroscopy
HSQC	2D ^{1}H, ^{13}C heteronuclear single quantum coherence
LC-MS/MS	Liquid chromatography tandem-mass spectrometry
LH-20	Sephadex column
MAO	Monoamine oxidase enzyme
MAOIs	Monoamine oxidase inhibitors
MAPK/ERK	Mitogen-activated protein kinase/extracellular signal-regulated protein kinase
mBDNF	Mature BDNF
MeOH	Methanol

MDD	Major Depressive Disorder
MOE	Molecular Modeling and Simulations program
MRG	5-*O*-(6-*O*-*α*-rhamnopyranosyl-*β*-glucopyranosyl) mevalonic acid
MTT	MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)
NE	Norepinephrine
NGF	Nerve Growth Factor
N-Methyl-D-aspartic acid	NMDA
NT-3	Neurotrophin-3
NT-4/5	Neurotrophin-4/5
P	(+)-Pronuciferine
PI3K	Phosphoinositide 3-kinase
P. lacerum	*Papaver lacerum*
PLCg	Phospholipase Cg
PM	PharmMapper
proBDNF	proBDNF
R	Roemerine
ROCS	Rapid Overlay of Chemical Structures
SEA	Similarity Ensemble Approach
Sect.	Section
Spec.	Species
SSRIs	Selective serotonin reuptake inhibitors
SW	SwissTarget
TCA	Tricyclic antidepressants
TNF-α	Tumor necrosis factor-α
TrkB	Tropomyosin receptor kinase B
TXG	Tyrosol-1-*O*-*β*-xylopyranosyl-(1→6)-*O*-*β*-glucopyranoside
VLC	Vacuum liquid chromatography
WHO	World Health Organization

TABLES

FIGURES

1. INTRODUCTION

Depression is a severe, highly prevalent and life-threatening psychiatric disorder caused by the cumulative influence of genetic factors and/or by persistent stress. It can affect the lifestyle of individuals, including work, quality of life and social functioning (1, 2). According to the World Health Organization, about 350 million people worldwide (WHO Organization, 2017) have been affected by depression disorders. Nearly 1 in 5 people will experience a major depressive episode at some point in their lives (2). Patients with depression show symptoms of depressed mood, irritability, difficulties in concentrating, anhedonia, feelings of worthlessness and abnormalities in appetite and sleep (3).

Depression is believed to occur due to a decrease in the level of neurotrophic growth factors such as brain-derived neurotrophic factor (BDNF) in the hippocampus. Neurotrophic factors play a key role in developing and maintaining structures of both the central and the peripheral nervous systems. They are involved in regulation of growth, development, differentiation, and survival of cell populations as well as their adaptation to environmental influences (4). Patients with depression are associated with a low level of the brain-derived neurotrophic factor. Most of the antidepressants used in medicine increase the level of BDNF in the brain and improve BDNF signaling in the prefrontal cortex and in the hippocampus (2). Some secondary plant-derived metabolites such as flavonoids were shown to increase BDNF levels *in vitro* (5).

Although various antidepressants are available in the market, there are still many cases that response poorly to available medications which lead to increase in recurrence rate (6). Chemical varieties in the plants can give a lead to pipeline drug and it is initial steps in new drug discovery.

There are so many different plants used in folkloric medicine for the treatment of mental disorders; especially *Hypericum perforatum*, *Lavandula* spec. and *Melissa officinalis*, which have been used worldwide to treat depression and related mental disorders (7-15). Additionally to these species: *Banxia Houpu*, *Coccinia grandis*,

Crocus sativum Datura metel are effectively used as sedative and antidepressant in Asia (13, 14, 16-18); *Sceletium* spp, *Lactuca virosa*, *Catha edulis*, *Boophone* spec. and *Olea europaea* have been used to treat central nervous system abnormalities in Africa and the Middle East, (19); *Solidago virgaaurea*, *Verbena officinalis, Leonurus cardiaca, Artemisia absinthium, Tanacetum parthenium* and *Rosmarinus officinalis* have been used effectively to treat anxiety and depression in Europe (15, 20, 21). *Justicia pectoralis*, *Ilex* spp and T*agetes* spp are reported to be used as stimulant and for anxiety cases in America (22). *Matricaria chamomilla*, *Ocimum basilicum, Punica granatum, Cretaegus aronica* and *Juglans regia* are used to treat insomnia and depression in Turkey (11, 23, 24).

There are more than 110 *Papaver* species distributed around the world, 35 of them grow naturally in Turkey and 10 of them are endemic. *Papaver* species have been used for the treatment of several diseases such as inflammation, diarrhea, and sleep disorders, as well as used as antidepressant in some regions of Anatolia. Furthermore, *Papaver rhoeas* is used as sedative and hypnotic in other countries too (25, 26). In Eastern part of Anatolia, aerial part of *Papaver rhoeas* is used as a sedative (27), while the fruits are used to treat depression (28). Infusions and/or syrups prepared from the aerial parts of *Papaver lateritium* is used as a calmative, antitussive, and hypnotic in Anatolia (29).

Papaver species are well known due to their isoquinoline alkaloids content, especially morphine, thebaine and codeine. More than 170 alkaloids have been reported from the members of the genus. Also, some flavonoids and other phenolic compounds have been isolated from several *Papaver* spec. (30-32). However, there are limited studies on their phytochemical content except for alkaloids and flavonoids.

In this study, it was planned to collect some *Papaver* species (*P. glaucum, P. macrostomum, P. rhoeas, P. lacerum and P. syriacum*) from different regions of Anatolia and screen them for their antidepressant activity *in vivo* and for their effects on BDNF expression *in vitro*. According to the preliminary results, the most active extract will be chosen for further phytochemical analysis. If the secondary metabolites isolated from the active extract do not display any effect comparable with

an active extract, *in silico* studies will be performed to identify their possible biological targets. Metabolites in the extract possessing antidepressant effect will be determined by using LC-MS/MS technique.

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2. GENERAL INFORMTION

2.1. Description of Plants

2.1.1. Family: *Papaveraceae*

Plant's base usually is woody, with clear or colored sap. Leaves are mostly an alternate, but occasionally they are estipulate, opposite, frequently abundantly divided. Sepals are usually 2 to 3, free and flurried. Petals are usually 4 to 6, free and marginally attached to the base. Flower's corolla is either zygomorphic or actinomorphic. Stamens are plentiful 4 to 6. Ovary is higher, syncarpons with 2 to 20 carpels. Seeds vary from 1 up to many. Fruit is a capsule, siliquiform or lomentum, nut. It consists of many genuses including; *Corydalis, Fumaria, Hypecoum, Papaver, Chelidonium, Roemeria and Glaucium* (33).

2.1.2 Genus: *Papaver* L.

There are more than 110 *Papaver* species distributed around the world, 35 of them naturally grow in Turkey and 10 of them are endemic.

- **Flower:** Panicles, solitary, panicles or in racemes, with separate petals.
- **Petals:** colored, from 4 to 6.
- **Stamens:** Several.
- **Filaments:** Linear or dilated.
- **Stigmas:** From 4 to 20 borne on sessile' disc above the placentas.
- **Capsule:** Under the stigmatic disc.
- **Syntypes:** F. 1909. *Papaveraceae – Hypecoideae- Papaveroideae*. *Pflanzenr*. 40 (IV.104): 288-386.

Papaver genus habitats throughout East Europe, Middle East, US and Turkey. Lifespan is annual, biennial and perennial herbs. *Papaver* species are divided into more than 10 sections (Argemonidium, Carinata, Glauca, Horrida, Meconella,

Miltantha, Oxytona, Papaver, Pilosa, and Rhoeadium). They are grouped according to their morphological characteristics (e.g., by capsule shape, markings and petal coloring). Additionally, species can be distinguished by chemical techniques and cytological. There are 35 *Papaver* species reported in the flora of Turkey belong to 8 sections including: *P. orientale, P. bracteatum, P. lasiothrix, P. paucifoliatum, P. spicatum, P. pilosum, P. apokrinomenon, P. strictum, P. lateritium, P. tauricola, P. acrochaetum, P.triniifolium, P. armeniacum, P. fugax, P. cylindricum, P. polychaetum, P. curvisacapum, P. somniferum, P. glaucum, P. glaucum, P. macrostomum, P. rhoeas, P. lacerum, P. commutatum, P. postii, P. syriacum, P. rhopalothece, P. dubium, P. arenarium, P. stylatum, P. clavatum, P. argemone, P. virchowii, P. hybridum and P.apulum* (33).

2.1.3. *Papaver* Species

***Papaver glaucum* Boiss. & Hausskn**

- **Section:** Papaver.
- **Leaves:** Triangular-oblong to linear-oblong segments.
- **Buds:** Ovoid, 2-3 cm.
- **Flower:** Petals red with black blotch at the base.
- **Capsule:** 1.5-2 cm.
- **Types:** N. Syria [Turkey **C7** Urfa] Tschermalik (Çermelik), *Haussknecht*.
- **Habitat:** Armenia, Mesopotamia, **B8** Erzurum, **C8** Mardin, Syrian dessert, Iran and Iraq (33).

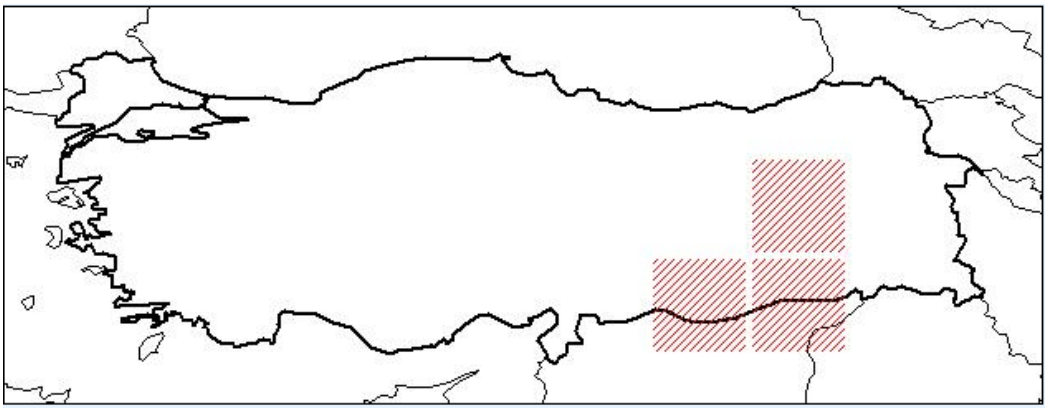


Figure 1. Distribution of *Papaver glaucum* in Turkey.

***Papaver macrostomum* Boiss. & Huet ex Boiss**

- **Section:** Carinatae.
- **Stem:** 30-45 cm erect, branched, spreading setose below.
- **Leaves:** Pinnatisect shape.
- **Flower:** 4-5 cm in diameter, petals broader than long, purple or crimson with or without black spot.
- **Capsule:** Elliptic-oblong, 1-1.5 cm.
- **Types:** [Turkey A8 Erzurum] Armenia, circa Tortum, ad vias, 1853, Huet (K!).
- **Habitate: A7** Gümüşane, **B4** Ankara, **B6** Malatya, **C4** Konya and **C5** Içel.
- **Lifespan:** Annual herb (33).

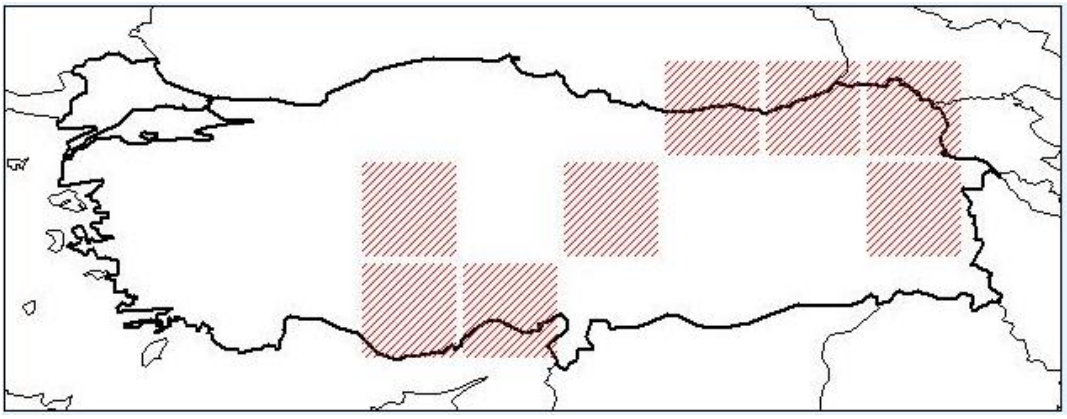


Figure 2. Distribution of *Papaver macrostomum* in Turkey.

***Papaver rhoeas* L.**

- **Section:** Rhoeadium.
- **Stem:** Erect up to 90 cm.
- **Leaves:** Variable, usually pinnatisect shape.
- **Flower:** Variable in size, petals red to crimson, rarely white, with or without blotch at the base.
- **Capsule:** Glabrous, globose, round at the base.
- **Type:** (Hb. Linn. 669/6!).
- **Habitate: A1** Tekirdağ, Çankaya, **A2** Istanbul, **A3** Bolu, **A5** Amasya, **A6** Samsun, **A7** Trabzon, **B3** Eskişehir, **B7** Elaziğ, **B8** Erzurum, **C2** Antalya, **C4** Içel, **C5** Seyhan and **C6** Gaziantep.
- **Lifespan:** Annual herb (33).

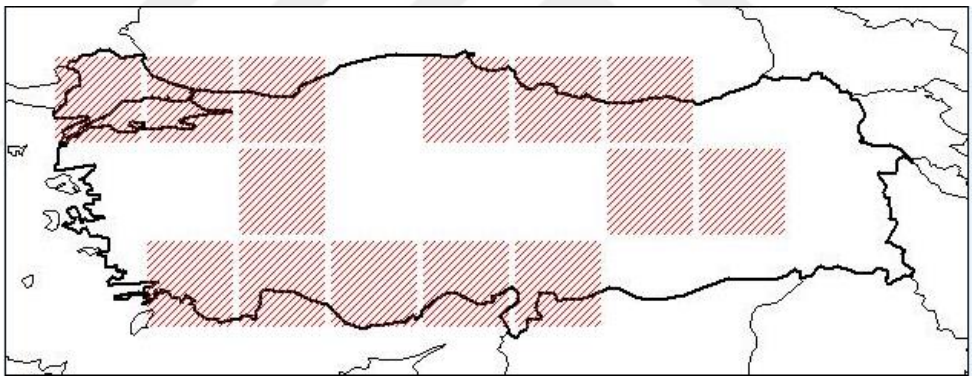


Figure 3. Distribution of *Papaver rhoeas* in Turkey.

***Papaver lacerum* Popov**

P. lacerum which is now treated as a synonyme of *P.dubium* subsp. *luevigatum* was accepted as a distinct species by Cullen (34).

- **Section:** Rhoeadium.
- **Stem:** Up to 40 cm with little branched.
- **Leaves:** Pinnatisect shape, the segment often dentate.
- **Buds:** Ellipsoid, 10-11 cm.
- **Flower:** Petals pale red, each with a dark spot at the base up to 15 mm.

- **Capsule:** Rounded at the base, subglobose up to 10 mm.
- **Syntypes:** Turkey [**A4**] Paphlagonia, Wilayet Kastambouli [Kastamonu], 1892,*Sintenis* 3702 (LE); Marsifoun A5.
- **Habitate: A1** Tekirdağ, Çanakkale, **A2** Bursa, **A3** Bakirlar**, B4** Ankara, **B7** Erzincan and **C5** Niğde.
- **Lifespan:** Annual herb (33).

Figure 4. *Papaver lacerum*, Kilis.

***Papaver syriacum* Boiss. & Blanche**

- **Section:** Rhoeadium.
- **Stem:** Up to 30 cm.
- **Leaves:** Pinnatifid shape, lower leaves much broader than others.
- **Flower:** Petals deep crimson.
- **Capsule:** Gradually tapered to the base.
- **Syntypes:** Lebanon, Beirout, Blanche.
- **Habitate: C6** Seyhan, Maraş, Hatay, Iskenderon and Adana.
- **Lifespan:** Annual herb (33).

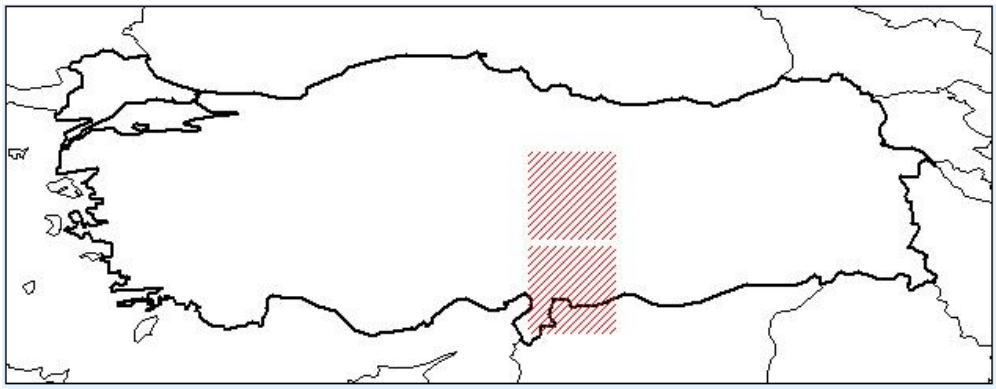


Figure 5. Distribution of *Papaver syriacum* in Turkey.

2.1.4. Key to *Papaver* Species (33)

1. Annuals, roots slender
 2. Stem leaves amplexicaul
  3. Capsule obovate-oblong, *c.* 1·5 x 0·5-0·75 cm; stigmas 6-7 **20. *gracile***
  3. Capsule globose to subglobose, (1·5-)2-5(-7) x 4-5 cm; stigmas (5-)8-18
   4. Leaves pinnatisect; capsule (1·5)2 cm; buds ovoid, 2-3 cm **19. *glaucum***
   4. Leaves serrate-dentate; capsule 5-7 cm or more; buds ovoid-oblong, 1·5-2 **18. *somniferum***
 2. Stem leaves sessile, but not amplexicaul
  5. Capsule glabrous; filaments undilated
   6. Stigmatic radii not keeled, the sinuses between them extending to *c.* 2/3 of the radius of the disc **21. *macrostomum***
   6. Stigmatic radii not keeled, the sinuses between them extending at most to 1/3 of the radius of the disc
    7. Capsule globose to subglobose, rounded at the base, length/breath ratio 1-2:1 **22. *rhoeas***
    8. Petals broader than long; peduncle usually hispid; terminal segment of the lower leaves longer than the laterals
    8. Petals longer than broad or as long as broad; peduncles adpressedsetose; terminal segment of lower leaves ± equalling the laterals
     9. Leaves and branches strict; petals pink or red, each with a black spot at the base **23. *lacerum***

2.2. Traditional Usage of Some *Papaver* Species

Papaver species have been used for treating several diseases worldwide including; inflammation, diarrhea, sleep disorders, cough, respiratory problems, headache, painful menstruation and inflammation of the stomach. Furthermore, it has been used as sedative, analgesia and used to t reduce withdrawal symptoms of the opioid addiction (35, 50). In the table below traditional usage of *Papaver* species in Turkey are listed.

Table 1. Traditional usage of some *Papaver* species in Turkey.

Species	Part use	Traditional usage
Papaver arenarium	Aerial	Antitussive and expectorant (36)
Papaver bracteatum	Stem	Abscess healing, wound healing (37)
Papaver dubium	Aerial	Expectorant and colds (36), cold and flu (38), Fungal infections and eczema (39)
	Flower	Colds and cough (27)
	Leaf	Food (40)
Papaver lacerum	Bud	Goiter (27)
Papaver lateritium	Aerial	Calmative, antitussive, bronchial and hypnotic (29)
Papaver macrostomum	Flower	Cough (27)
Papaver orientale	Seed	Laxative and sedative (27)
	Leaf	Asthma (27)
	Capsule	Food (28), children's fever (41)
Papaver rhoeas	Aerial	Sedative (27) Cold and Expectorant (42), sedative, soporific and coughing (43), tranquilizer, antitussive, sedative (38), insomnia and emollient (44)
	Fruit	Depression, pain killer and sedative (28)
	Aerial	
	Whole plant	Preventing abortion (43)
	Leaf	Rheumatism (43)
	Flower	Anemia (45)
Papaver somniferum	Flower, Capsule	Analgesic (43)

2.3. Biological Activity Studies on *Papaver* Species

Biological activitiy studies on *Papaver* species are listed below with the exclusion of *Papaver somniferum,* due to the extensive studies done on this species.

Table 2. Biological activities of *Papaver* species.

Species	Part used	Biological activity
Papaver argemones		Antibacterial (46)
Papaver bracteatum		Narcotics and analgesics, sedative and cytotoxic properties (47) Increase fertilization (48)
Papaver dubium		Antibacterial (46)
Papaver dubium subsplaevigatum	Aerial	Cytotoxic (34)
Papaver libanoticum	Aerial	Analgesic (49)
Papaver pseudocanescens		Antiviral effects against the replication of poliovirus 1 and human rhinovirus 14 (50)
Papaver radicatum		Coloring agent (51)
Papaver rhoeas	Aerial	Antibacterial (46, 52)
	Fruit	Antioxidant (53-55)
	Petals	Sedative effect (26) tonic (56)
	Plant	Anodyne, emmenagogue, emollient, expectorant, hypnotic, sedative (56) Scavenging activity (57) Pancreatic lipase inhibitory (58) Increases the quality of ovulated oocytes (59) Anti-influenza (60), Antidepressant (61)
	Root	Anti-ulcerogenic (62) (63) Decrease morphine withdrawal symptoms (64-66) Anti-inflammatory (67)

2.4. Phytochemical Studies on *Papaver* Species

Having the utmost level of phytochemical and botanical variability, *Papaver* genus contains several species with many subspecies and varieties yielding more than 170 alkaloids (68). In Tables 3-11 *Papaver* species alkaloids are listed according to their sections. In Table 12, phenolic compounds in *Papaver* species are listed.

2.4.1. Alkaloids

Oxytona Section

Table 3. Sect. Oxytona alkaloids.

Alkaloid	***P. bracteatum***	***P. orientale***	***P.pseudo-orientale***	***P. lasiothrix***
Simple isoquinoline 1				
Cotarnine 1a			(69)	
Cotarnoline 1b			(69)	
N-methylcorydaldine 1c	(70)			
Proaporphine 2				
Orientalinone 2a			(71)	
Aporphine 3				
Bracteoline 3a	(72)		(73)	
Isothebaine 3b	(72)	(74)	(74)	
Corytuberine 3c	(72)			
Magnoflorine 3d	(72)			
Orientinine 3e		(75)		
Orientine 3f		(75)		
Morphinane 4				
Thebaine *N*-oxide	(76)			(71)
Thebaine 4a	(77)	(74)	(74)	(71)
Oripavine 4b	(76)	(74)		
Neopine 4c	(76)			

Table 3. Sect. Oxytona alkaloids. (Continue)

Alkaloid	***P. bracteatum***	***P. orientale***	***P. pseudo-orientale***	***P. lasiothrix***
Promorphinane 5				
Salutadimerine 5a			(71)	(71)
Salutaridine 5b	(71)	(74)	(74)	(71)
Salutaridine *N*-oxide	(76)			(71)
Norsalutaridie			(69)	(71)
Secoberbine 6				
Macrataline 6a			(71)	
Macrantaldehyde 6b			(69)	
Macrantoridine 6c			(71)	
Narcotindiol 6d			(71)	
Narcotinediol 6e			(69)	
Papaveroxidine 6f			(69)	
Papaveroxine 6g			(69)	
Papaveroxinoline 6h			(69)	
Protoberberine 7				
Mecambridine 7a		(71)	(73)	(71)
Orientalidine 7b		(71)	(73)	(71)
N-methylpapaverberbine 7c			(69)	
Phthalideisoquinoline 8				
Narcotine 8a			(69)	
Narcotinehemiacetal 8b			(69)	
Narcotolinol 8c			(69)	
Rhoeadine & Papaverrubine 9				
Alpinine 9a			(71)	
Papaverrubine G, E ,D and C 9b	(72)			
Rhoeadine 9c	(72)			
Alpinigenine 9e	(72)	(74)	(74)	
Epialpinine	(72)			

Table 3. Sect. Oxytona alkaloids. (Continue)

Alkaloid	***P. bracteatum***	***P. orientale***	***P. pseudo-orientale***	***P. lasiothrix***
Protoberberine (Tetrahydroproto berberine) 10				
Coptisine 10 a	(72)			
Scoulerine 10b	(72)			
Palmatine 10 c	(72)			
Protopine 11				
Protopine 11a	(72)			
Simple tetrahydroisoquinoline 12				
Codine 12a	(72)			
Morphine 12b	(72)			
Others				
N-methylthebainium ydroxide	(72)			
Or1			(73)	
Nor-Methyl Or1			(73)	

Miltantha Section

Table 4. Sect. Miltantha alkaloids.

Alkaloid	***P. armeniacum***	***P. cylindricum***	***P. fugax***	***P. persicum***	***P. polychaetum***	***P. trinifoline***	***P. acrochaetum***
Proaporphine 2							
Mecambrine 2b	(71)		(78)	(71)		(79)	
N-methylcrotonosine 2c						(79)	
Pronuciferine 2d			(80)				
Aporphine 3							
Floripavidine 3g	(71)	(81)	(82)	(71)		(79)	
N-methylasimilobine 3h	(71)	(81)	(71)	(71)			
Nuciferine 3i						(79)	
Isocorydine 3j			(80)				
Roemerine 3k						(83)	
Remeroline 3l			(78)				
Remerine			(78)				
Isolaureline 3m			(78)				
Lirinidine 3n	(80)						
Morphinane 4							
Thebaine 4a	(83)	(81)	(83)			(79)	
N-methylthebaine		(69)					
Oripavine 4b		(81)					
Promorphinane 5							
Salutaridine 5b		(81)	(80)	(71)		(79)	
Amurine 5c						(71)	
Secoberbine 6							
Papaveroxine 6g			(71)				
Phthalideisoquinoline 8							
Narcotine 8a	(71)	(81)	(81)			(79)	
N-methylnarcotine		(69)					
Narceine 8d						(79)	
Narcotinehemiacetal 8d			(71)				
Rhoeadine & Papaverrubine 9							
Papaverrubine 9b	(84)		(84)			(79)	
Rhoeadine 9c	(81)	(81)	(81)	(71)		(79)	(85)

Table 4. Sect. Miltantha alkaloids. (Continue)

Alkaloid	***P. armeniacum***	***P. curviscapum***	***P. cylindricum***	***P. fugax***	***P. persicum***	***P. polychaetum***	***P. triniifoline***	***P. acrochaetum***
Rhoeagenine 9d	(80)				(71)		(71)	(85)
O-ethylrhoeagenine	(80)						(71)	
O-ethyloreogenine							(71)	
Alpinigenine 9e				(80)				
O-ethylalpinigenine				(80)				
Epiglaudine					(71)			
Glaucamine 9f				(80)	(71)			
Glaudine 9g	(80)			(80)	(71)			
O-ethyl-glucamine				(80)				
*O*reodine 9h	(71)			(80)	(71)		(79)	
*O*reogenine 9i				(80)	(71)		(71)	
Triniifoline 9j							(79)	
O-ethyltriniifoline							(79)	
Dubirheine 9k	(86)							
Protoberberine/Tetrahydroprotoberberine 10								
Mecambridine 7a					(71)			
Coptisine 10a							(79)	
Scoulerine 10b			(81)	(78)			(79)	
Palmatine 10c				(84)			(83)	
Berberine 10d		(71)				(71)		
Cheilanthifoline 10e			(81)	(78)			(79)	
Sinactine 10f					(71)		(79)	
N-methylsinactine							(71)	
Isocorypalmine 10g							(71)	
Protopine 11								
Protopine 11a	(84)	(71)		(84)			(79)	(85)

Table 4. Sect. Miltantha alkaloids. (Continue)

Alkaloid	***P. armeniacum***	***P. curviscapum***	***P. cylindricum***	***P. fugax***	***P. persicum***	***P. polychaetum***	***P. trinitifoline***	***P. acrochaetum***	***P. curviscapum***
Allocryptopine 11b		(71)							(85)
1-methoxy-allocryptopine		(71)							
1-methoxy-13-oxoallocryptopine 11c		(71)							
Cryptopine 11d	(80)								
Muramine 11e							(83)		
Simple tetrahydro isoquinoline 12									
Codine 12a				(87)					
Phenanthrene 13									
Argentinine 13				(82)					
Benzophenanthridine 14									
Sanguinarine 14a							(79)		
Oxysanguinarine 14b							(79)		
Benzylisoquinoline 15									
Armepavine 15a			(81)	(78)	(71)		(79)		
Norarmepavine							(83)		
Crykonisine 15b							(71)		
Miltanthaline 15c							(79)		
Miltanthoridine 15d							(88)		
Miltanthoridinone 15e							(88)		
Papaverine 15f	(71)		(81)				(79)		
Reticuline 15g				(78)					
Isopavine 16									
Amurensinine 16a				(80)	(71)				

Rhoeadium Section

Table 5. Sect. Rhoeadium alkaloids.

Alkaloid	*P. commutatum*	*P. dubium*	*P. dubium laevigatum*	*P. lacerum*	*P. rhoeas*	*P. rhopalothece*	*P. commutatum subsp. Fuxinum*	*P. stylatum*	*P. syriacum*
Proaporphine 2									
Mecambrine 2b		(34)	(71)	(89)					(90)
Pronuciferine 2d				(89)					
Aporphine 3									
N-methylasimilobine 3h				(89)	(91)				
Isocorydine 3j	(92)	(34)			(93)	(94)	(92)	(95)	
Roemerine 3k	(96)	(34)	(71)	(89)	(93)	(94)			
Corydine 3o		(34)							
Rhopalotine 3p						(94)			
Morphinane 4									
Thebaine 4a		(87)			(25)				(90)
Promorphinane 5									
Amurine 5c								(95)	
Phthalideisoquinoline 8									
Narcotine 8a					(25)	(94)			
Narceine 8d						(94)			
Noscapine 8e						(94)			
Rhoeadine & Papaverrubine 9									
Papaverrubine A 9b	(92)						(92)		(90)
Rhoeadine 9c	(71)			(71)	(93)				(90)
Rhoeagenine 9d	(92)	(97)			(93)		(92)		(90)
Dubirheine 9k		(86)							
Isorhoeadine 9l					(98)				(90)
Isorhoeagenine 9m	(86)				(99)				
Protoberberine/tetrahydroprotoberberine 10									

Table 5. Sect. Rhoeadium alkaloids. (Continue)

Alkaloid	*P. commutatum*	*P. dubium*	*P. dubium laeviqatum*	*P. rhoeas*	*P. rhopalothece*	*P. commutatum subsp. Euxinum*	*P. stylatum*	*P. syriacum*	*P. clavatum*
Coptisine 10a				(93)	(94)				
Tetrahydropseudocoptisine		(34)							
Berberine 10d		(34)	(71)	(93)	(94)		(95)	(90)	(95)
Cheilanthifoline 10e	(71)			(60)		(92)			
Sinactine 10f				(93)					
Thalifendine 10h		(34)							
Stylopine 10i		(34)		(91)				(90)	
Protopine 11									
Protopine 11a				(93)	(94)			(90)	
Allocryptopine 11b		(34)	(71)	(93)					
Cryptopine 11d					(94)				
Coulteropine 11f				(93)					
Couteropine					(71)				
Simple Tetrahydroisoquinoline 12									
Codine 12a		(87)							
Morphine 12b		(87)							
Benzylisoquinoline 15									
Papaverine 15f	(96)			(25)					
Isopavine 16									
Amurensinine 16a	(71)					(92)			
Phenanthridine 17									
Corysamine 17								(90)	
Tetrahydrobenzylisoquinoline 18									
Latericine 18a				(98)					
Isoquinoline 19									
Cularine 19					(94)				
Others									
Meconoquintupline							(95)		

Pilosa Section

Table 6. Sect. Pilosa alkaloids.

Alkaloid	***P.apokrinomenon***	***P. lateritium***	***P. pilosum***	***P. spicatum***	***P. strictum***
Proaporphine 2					
Mecambrine 2b			(100)		
Aporphine 3					
Roemerine 3k	(71)		(100)	(101)	(71)
N-methylglaucine 3q				(101)	(71)
Glaucine 3r	(71)		(100)	(101)	
Roemerefidine				(101)	(71)
N-methyltetrahydro palmatinium hydroxide		(71)			
Dehydroroemerine 3s	(71)		(100)	(71)	(71)
Dehydroglaucine 3t	(71)		(100)	(71)	(71)
Promorphinane 5					
Amurine 5c	(71)		(100)	(71)	(71)
Dihydronudaurine			(100)	(71)	(71)
Epiamurinine			(100)	(71)	(71)
Amurinine 5d			(100)	(71)	(71)
Secoberbine 6					
Macranthaline 6i		(71)			
Rhoeadine & Papaverrubine 9					
Rhoeadine 9c		(71)			
Rhoeagenine 9d		(71)			
Protoberberine/ Tetrahydroprotoberberine 10					
Mecambridine 7a		(71)			
Protopine 11					
Protopine 11a		(71)			
Cryptopine 11d		(71)			
Others					
N-methyllaurotetanine	(71)				(71)

Papaver Section

Table 7. Sect. Papaver alkaloids.

Alkaloid	***P. somniferum***	***P. glaucum***	***P. setigerum***
Simple isoquinoline 1			
Hydrocotarnine 1d			(98)
Aporphine 3			
Isothebaine 3b	—		
Corytuberine 3c		(102)	(103)
Magnoflorine 3d		(102)	(103)
Isocorydine 3j		(102)	
Roemerine 3k		(85)	
Roemerine N-oxide		(85)	
Corydine 3o		(102)	
Dehydroroemerine 3s		(85)	
Isoboldine 3u			(103)
Liriodenined 3v		(85)	
Morphinane 4			
Thebaine 4a	(104)	(87)	(103)
Oripavine 4b	(104)		
Phthalideisoquinoline 8			
Narcotine 8a	(104)		(104)
Narceine 8d	(104)		(104)
Noscapine 8e	(105)		(106)
Narcotoline 8f	(104)		(104)
Nornarceine	(104)		(104)
Corlumine 8g			(98)
Rhoeadine & Papaverrubine 9			
Papaverrubine 9b		(102)	
Rhoeadine 9c			(103)
Rhoeagenine 9d			(103)
Glaucamine 9f		(102)	

Table 7. Sect. Papaver alkaloids (Continue).

Alkaloid	***P. somniferum***	***P. glaucum***	***P. setigerum***
Glaudine 9g		(102)	
Epiglaudine		(86)	
N-methyl-14-*O*-demethyl-epiporphyroxine	(86)		
Protoberberine/ Tetrahydroprotoberberine 10			
Coptisine 10a		(102)	(103)
Scoulerine 10b			(103)
Stylopine 10i			(103)
Canadine 10j			(98)
Protopine 11			
Protopine 11a		(102)	(103)
Allocryptopine 11b		(102)	(98)
Cryptopine 11d		(102)	(103)
Simple tetrahydroisoquinoline 12			
Codine 12a	(104)	(87)	(103)
Morphine 12b	(104)	(87)	(103)
Benzophenanthridine 14			
Sanguinarine 14a		(102)	
Benzylisoquinoline 15			
Papaverine 15f			(103)
Setigerine 15h			(103)
Setigeridine 15i			(103)
Papaveraldine 15j			(106)
Tetrahydrobenzylisoquinoline 18			
Laudanosine 18b			(98)
Laudanine 18c			(98)
Others			
N-methylporphyroxine	(86)		

Argemonidium and Carinatae Sections

Table 8. Sect. Argemonidium and Carinatae alkaloids.

Alkaloid	*P. argemone*	*P. macrostomum*	*P. hybridum*	*P. alpinum*
Proaporphine 2				
Mecambrine 2b		(32)		
Aporphine 3				
Isocorydine 3j	(107)	(32)		
Magnoflorine 3d	(107)			
Rhoeadine & Papaverrubine 9				
Papaverrubine 9b	(107)	(108)		
Rhoeadine 9c	(107)	(108)		
Alpinigenine 9e				(109)
Glaucamine 9f			(74)	
Glaudine 9g			(74)	
Alpinine 9a				(109)
Protoberberine/ Tetrahydroprotoberberine 10				
Scoulerine 10b	(107)			
Cheilanthifoline 10e		(32)		
Coptisine 10a	(107)			
Protopine 11				
Protopine 11a	(107)	(108)		
Cryptopine 11d	(107)			
Allocryptopine 11b	(107)			
Benzylisoquinoline 15				
Macrostomine 15k		(108)		
Dehydronormacrostomine		(108)		
Sevanine 15l		(108)		
Isopavine 16				
Amurensinine 16a		(32)		
Amurensine 16b		(32)		-
Amuresine	(71)			
Tetrahydrobenzylisoquinoline 18				
Laudanosine 18c		(32)		
Spirobenzylisoquinoline 20				
Fumariline 20a	(71)			
Fumarophycine 20b	(71)			

Meconella and Californicum Sections

Table 9. Sect. Meconella and Californicum alkaloids.

Alkaloid	*P. kerneri*	*P. californicum*
Promorphinane 5		
Amurine 5c	(110)	
Nudaurine	(110)	
Protoberberine 7		
Mecambridine 7a	(110)	
Phthalideisoquinoline 8		
Noscapine 8e		(111)
Rhoeadine & Papaverrubine 9		
Papaverrubine 9b	(110)	
Alpinigenine 9e	(110)	
Epialpinine	(110)	
Protoberberine (Tetrahydroprotoberberine) 10		
Coptisine 10a	(110)	
Alborine 10k	(110)	
Protopine 11		
Protopine 11a	(110)	
Allocryptopine 11b	(110)	
Cryptopine 11d	(110)	
Muramine 11e	(110)	
Simple tetrahydroisoquinoline 12		
Codine 12a		(111)
Morphine 12b		(111)
14-methylnormorphine 12		(111)
Ignimorphinan		(111)
Benzylisoquinoline 15		
Papaverine 15f		(111)
Ignipapaverine		(111)
Isopavine 16		
Amurensinine 16a	(110)	
Amurensine 16b	(110)	
Others		
N-methyltetrahtdropalmatinium hydroxide	(110)	

Other *Papaver* Alkaloids (Part I)

Table 10. Other *Papaver* alkaloids (Part I).

Alkaloid	*P. nudicaule*	*P. tatricum*	*P. atlanticum*	*P.cf.stevenianu*	*P. rupifragum*	*P. degenii*	*P. tauricola*
Simple isoquinoline 1							
O-methylcorypaline						(112)	
Salsolidine						(112)	
Proaporphine 2							
Mecambrine 2b				(99)			(80)
Pronuciferine 2d							(80)
Amuronine	(97)						
Amuroline	(97)						
Aporphine 3							
Isothebaine 3b			(102)				
Corytuberine 3c		(110)	(102)	(99)	(113)		
Magnoflorine 3d			(102)				
Nuciferine 3i							(80)
Isocorydine 3j				(99)			
Roemerine 3k							(80)
Irinidine 3n?							(80)
Corydine 3o		(110)		(99)			
Nantenine							(80)
Mangoflorine					(113)		
Morphinane 4							
Thebaine 4a				(99)			(83)
O-methilflavinantine						(112)	
Promorphinane 5							
Nudaurine 5	(97)						
Amurine 5c	(114)						
8,14-dihydroamurine	(114)						
8,14-dihydro flavinantine	(115)						

Table 10. Other *Papaver* alkaloids (Part I). (Continue)

Alkaloid	*P. nudicaule*	*P. tatricum*	*P. atlanticum*	*P.cf.stevenianu*	*P. rupifragum*	*P. degenii*	*P. tauricola*
Protoberberine 7							
Protoberberine 7							(84)
Phthalideisoquinoline 8							
Narcotine 8a							(81)
Rhoeadine & Papaverrubine 9							
Papaverrubine 9b			(102)	(99)	(113)		(84)
Rhoeadine 9c			(102)		(113)		(116)
Rhoeagenine 9d			(102)		(113)		(116)
Glaudine 9g							(116)
Epiglaudine 9		(110)					(116)
Dubirheine 9k							(86)
Oreogenine 9i							(116)
Glaucamine 9f							(116)
Oreodine 9h							(116)
Isorhoeadine 9l					(113)		
Protoberberine/ Tetrahydroprotoberberine 10							
Scoulerine 10b			(102)	(99)			
Berberine 10d				(99)			
Coptisine 10a				(99)	(113)		
Stylopine 10i			(102)		(113)		
N-methyltetrahydro palmatiniumhydroxide		(110)					
Palmatine		(110)					
Protopine 11							
Protopine 11a	(97)	(110)	(102)	(99)	(113)	(112)	(80)
Allocryptopine 11b	(115)	(110)		(99)	(113)	(112)	

Table 10. Other *Papaver* alkaloids (Part I). (Continue)

Alkaloid	***P. nudicaule***	***P. tatricum***	***P. atlanticum***	***P.cf.stevenianu***	***P. rupifragum***	***P. degenii***	***P. tauricola***
Cryptopine 11d		(110)	(102)		(113)		(80)
Muramine 11e	(97)	(110)	(102)			(112)	
Criptopine						(112)	
Benzophenanthridine 14							
Sanguinarine 14a			(102)				
Benzylisoquinoline 15							
Armepavine 15a							(80)
Laudanosoline						(112)	
Codamine						(112)	
Tetrahydroescholamine						(112)	
Isopavine 16							
Amurensinine 16a	(114)	(110)				(112)	(116)
Amurensine 16b	(114)	(110)				(112)	
O-methylthalisopavine	(114)						
O-methylisopavine						(112)	
Phenanthridine 17							
Corysamine 17					(113)		
Isoquinoline 19							
Pseudoprotopine	(115)						
Dihydroamuronine	(115)						
Amurensinine N-oxide	(115)						
Morphinandienone 21							
Flavinantine	(114)						
Benzophenantridine 22							
Chelidonine						(112)	
Others							
13β-hydroxy-N-methyl stylopininum hydroxide			(102)				
N-methylcanadinium				(99)			
N-methylthebainium hydroxide				(99)			

Other *Papaver* Alkaloids (Part II)

Table 11. Other *Papaver* alkaloids (Part II).

Alkaloid	***P. croceum***	***P. arenarium***	***P. aculeatum***	***P.tenuifolium***	***P. pavoninum***	***P.pseudocanescens***	***P. albiforum***
Aporphine 3							
Corytuberine 3c					(107)		(99)
Magnoflorine 3d					(107)		
Isocorydine 3j					(107)		
Corydine 3o	(110)				(107)		
N-acetylanonaine			(117)				
Morphinane 4							
Thebaine 4a				(87)			(99)
Promorphinane 5							
Nudaurine 5	(110)						
Amurine 5c	(110)						
8,14-dihydroamurine						(118)	
8,14-dihydro flavinantine						(118)	
Protoberberine 7							
Mecambridine 7a						(118)	
Rhoeadine &Papaverrubine 9							
Papaverrubine 9b	(110)						(99)
Rhoeadine 9c							(99)
Protoberberine/Tetra hydroprotoberberine 10							
Alborine 10k						(118)	
Scoulerine 10b							(99)
Berberine 10d							(99)
Coptisine 10a					(107)		(99)
Mecambridine methohydroxide						(118)	

Table 11. Other *Papaver* alkaloids (Part II). (Continue)

Alkaloid Name	***P. croceum***	***P. arenarium***	***P. aculeatum***	***P.tenuifolium***	***P .pavoninum***	***P.pseudocanescens***	***P. albiflorum***
Protopine 11							
Protopine 11a					(107)		(99)
Allocryptopine 11b					(107)		(99)
Simple tetrahydroisoquinoline 12							
Morphine 12b				(87)			
Benzophenanthridine 14							
Oxysanguinarine 14b	(110)						
Benzylisoquinoline 15							
Macrostomine 15k		(96)					
Isopavine 16							
Amurensinine 16a						(118)	
Morphinandienone 21							
Flavinantine						(118)	
Others							
N^2-methyl-1,2,3,4-tetrahydro-β-carboline					(107)		
O-methylarmepavie						(118)	

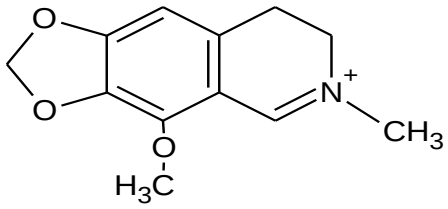
2.4.2. Phenolics

Table 12. Phenolic compounds present in *Papaver* species.

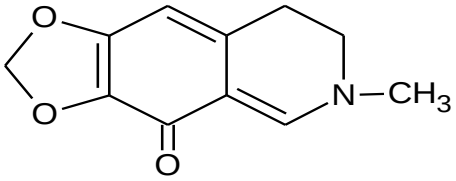
Compound	***P. somniferum***	***P. rhoeas***	***P. macrostomum***	***P. radicatum***	***P. nudicaule***	***P. californicum***
Isoquercitrin 1		(30)				
Astragalin 2		(30)		(51)		
Hyperoside 3		(30)				
Kaempferol 4	(31)	(30)				
Luteoline 5	(31)	(30)	(32)			
Hypolaetin 6		(30)				
Quercetin 7	(31)	(25)				
Apigenin 8	(31)					
Kaempferol-3-sophoroside		(59)			(119)	
Kaempferol-3-neohesophoroside		(59)				
Kaempferol-3-sambubioside		(59)				
Kaempferol-3-glucoside		(59)				
Quercetin-3-sophoroside		(59)				
Gossypitrin 9				(51)	(119)	
Herbacitrin 10				(51)		
Nudicaulins 11					(119)	
Myricetin 12						(111)
Kaempferol acetate						(111)
Malvidin-3-*O*-glucuronide						(111)
Isoquercetin		(25)				
Tricine 13			(32)			

2.5. Structures

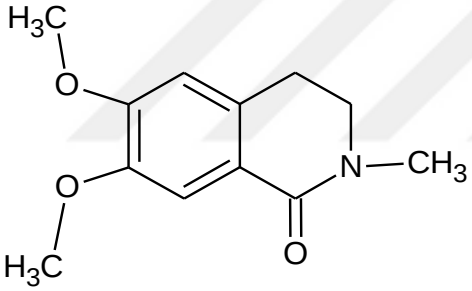
2.5.1. Simple Isoquinoline 1



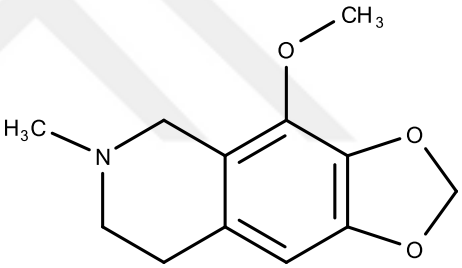
1a



1b

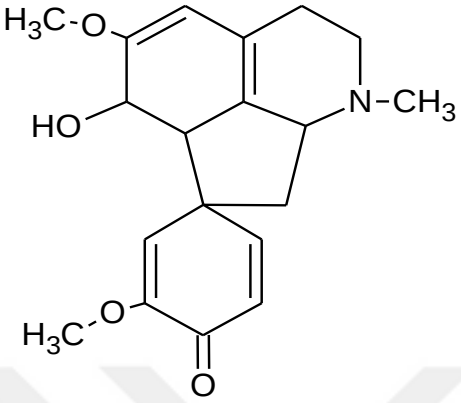


1c



1d

2.5.2. Proaporphine 2



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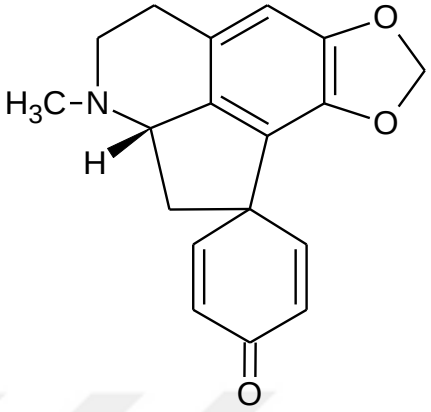

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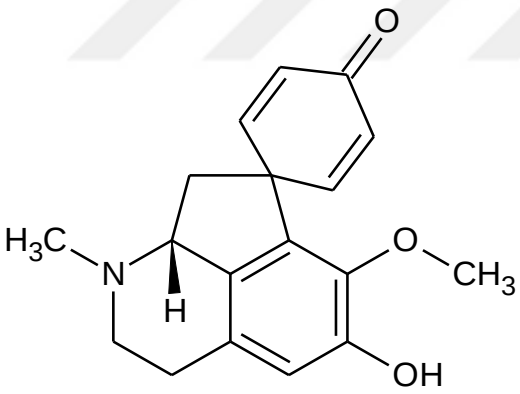

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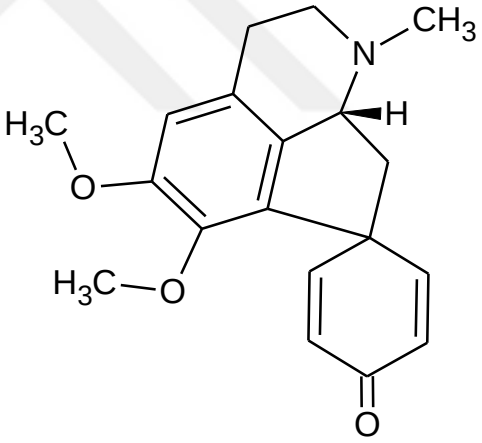
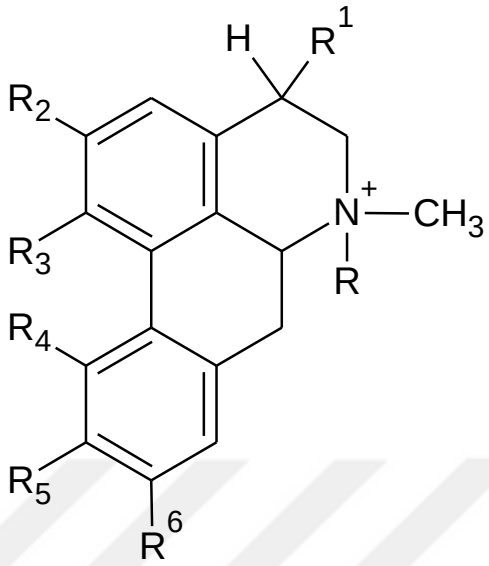
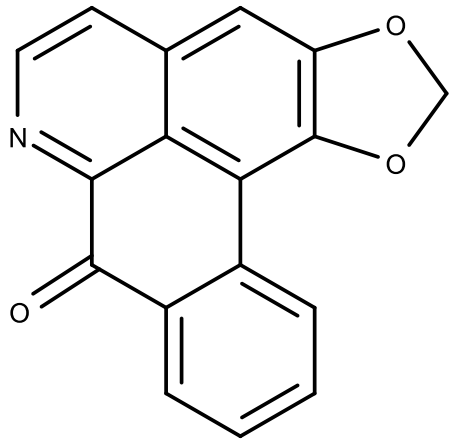


2d

2.5.3. Aporphine 3

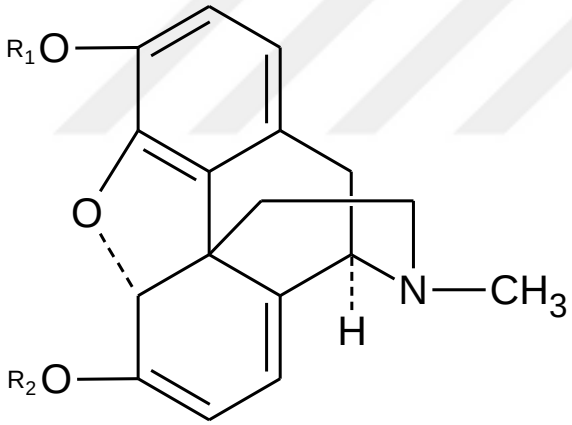


	R	R_1	R_2	R_3	R_4	R_5	R_6
3a	-	H	OCH_3	OH	H	OH	OCH_3
3b	-	H	OCH_3	OH	OCH_3	H	H
3c	-	H	OCH_3	OH	OH	OCH_3	H
3d	CH_3	H	OCH_3	OH	OH	OCH_3	H
3e	-	H	OCH_3	OH	H	H	OCH_3
3f	-	H	OCH_3	OCH_3	H	H	OCH_3
3g	-	H	O-Glu	OCH_3	H	H	H
3h	-	H	OH	OCH_3	H	H	H
3i	-	H	OCH_3	OCH_3	H	H	H
3j	-	H	OCH_3	OCH_3	OH	OCH_3	H
3k	-	H	O-CH_2-O		H	H	H
3l	-	H	O-CH_2-O		H	H	OH
3m	-	H	O-CH_2-O		H	H	OCH_3
3n	-	H	OCH_3	OH	H	H	H
3o	-	H	OCH_3	OH	OCH_3	OCH_3	H
3p	-	OH	OCH_3	OH	OCH_3	OCH_3	H
3q	CH3	H	OCH_3	OCH_3	H	OCH_3	OCH_3
3r	-	H	OCH_3	OCH_3	H	OCH_3	OCH_3
3s	-	H	O-CH_2-O		H	H	H
3t	-	H	OCH_3	OCH_3	H	OCH_3	OCH_3
3u	-	H	OCH_3	OH	H	OCH_3	OH

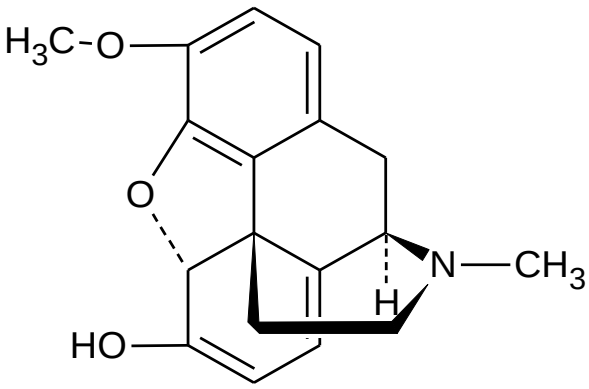


3v

2.5.4. Morphinane 4

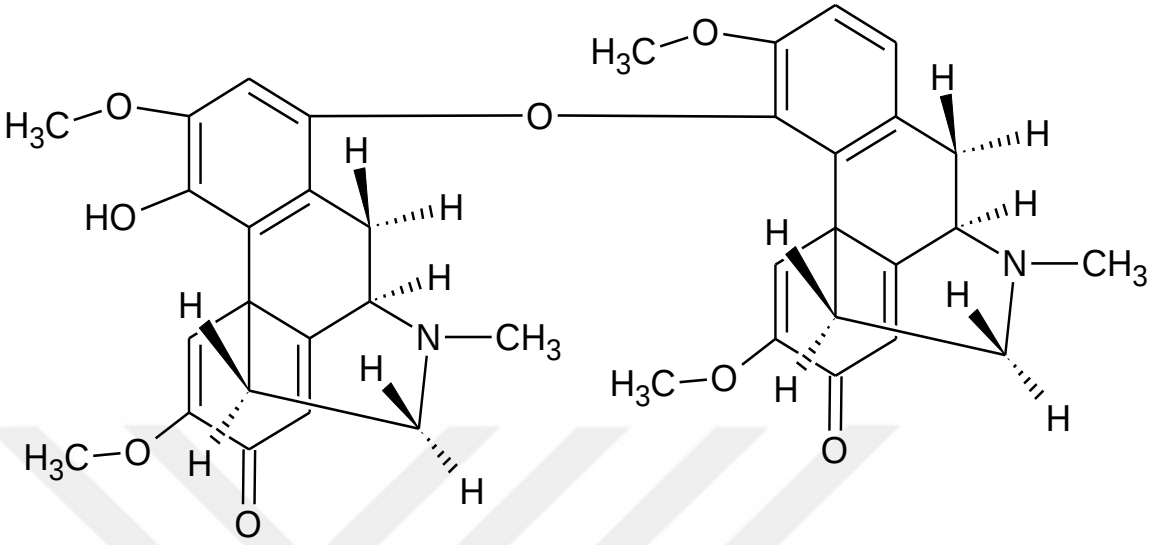


	R_1	R_2
4a	OCH_3	OCH_3
4b	OH	OCH_3

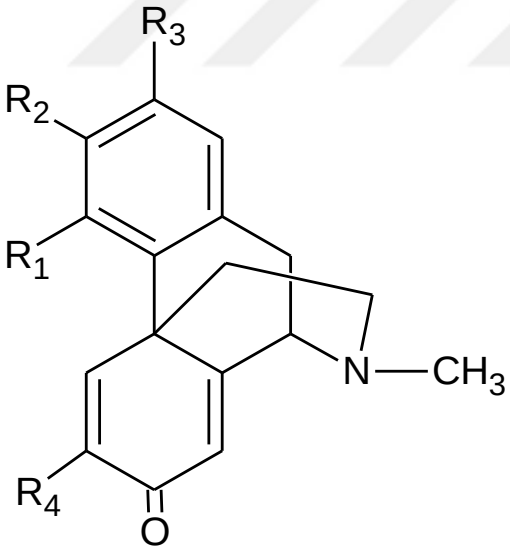


4c

2.5.5. Promorphinane 5

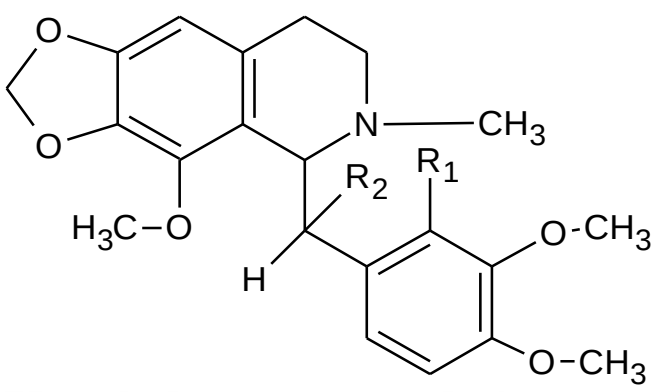


5a



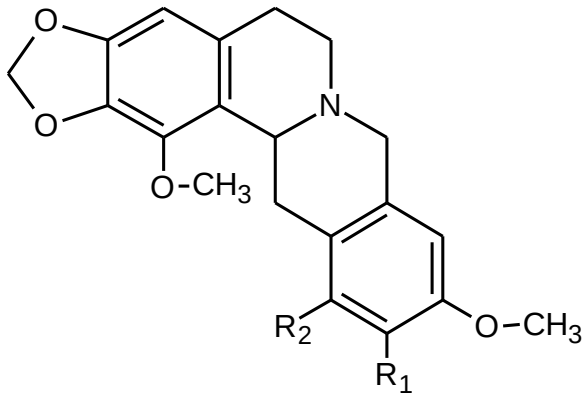
	R_1	R_2	R_3	R_4
5b	OH	OCH_3	H	OCH_3
5c	H	O-CH_2-O		OCH_3
5d	H	O-CH_2-O		OCH_3

2.5.6. Secoberbine 6

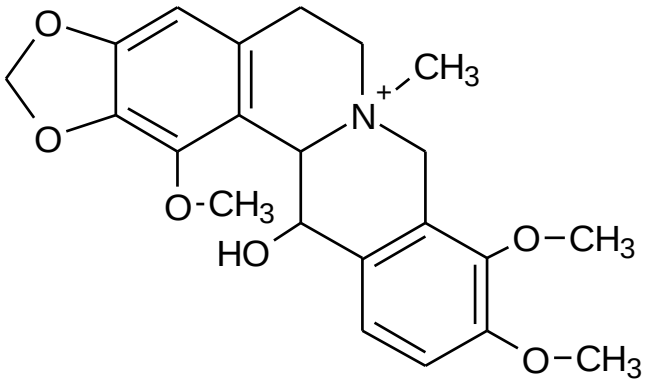


	R_1	R_2
6a	CH_2OH	H
6b	CHO	H
6c	COOH	H
6d	CH_2OH	OH
6e	CH_2OH	OH
6f	COOH	$OCOCH_3$
6g	CHO	$OCOCH_3$
6h	CH_2OH	$OCOCH_3$
6i	CH_2OH	OH

2.5.7. Protoberberine 7

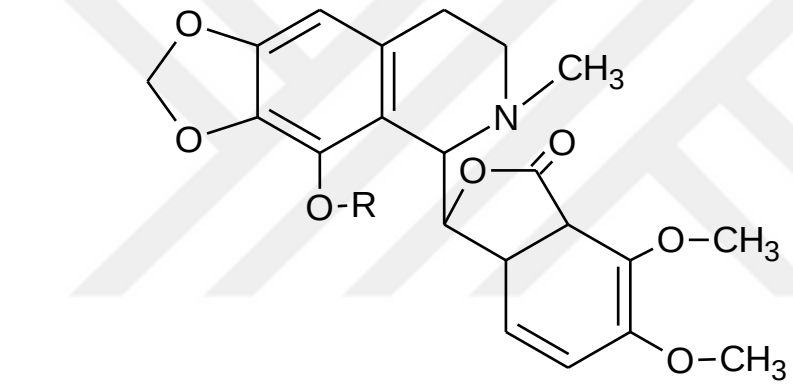


	R_1	R_2
7a	OCH_3	CH_2OH
7b	CH_2-O-CH_2	

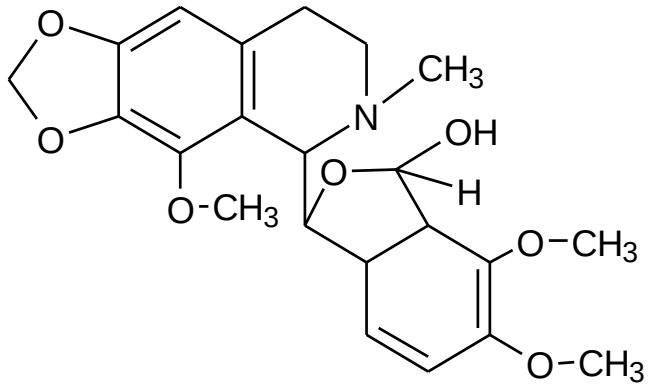


7c

2.5.8. Phthalideisoquinoline 8



	R
8a	OCH_3
8c	OH



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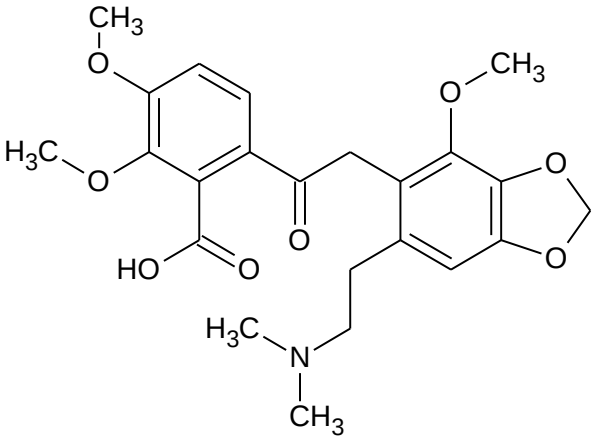

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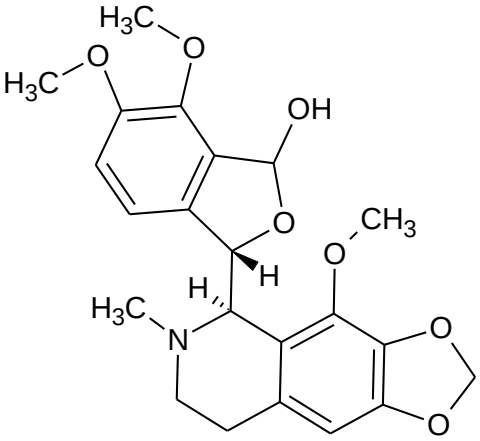

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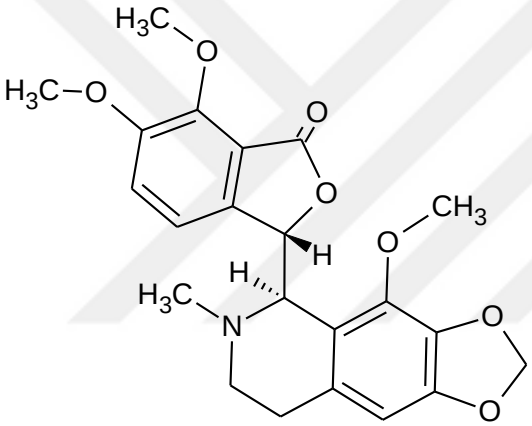

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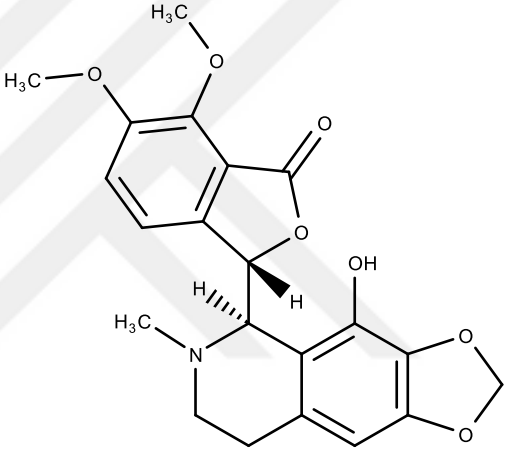

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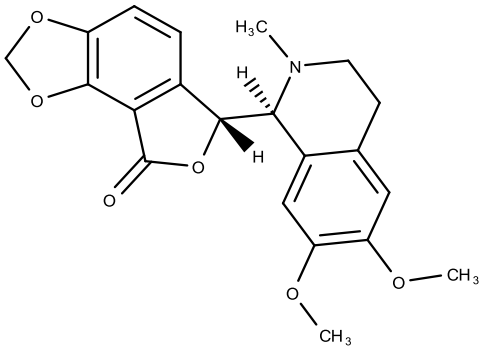
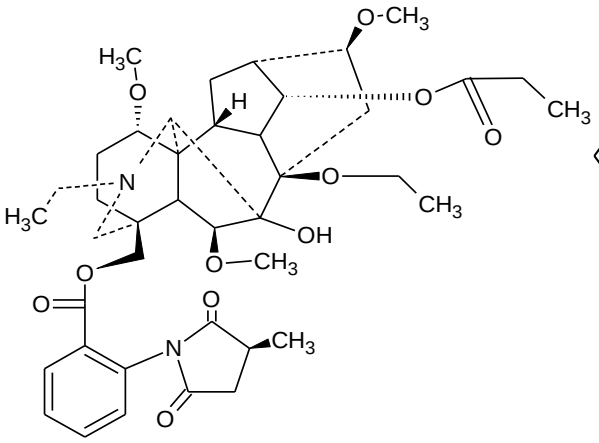
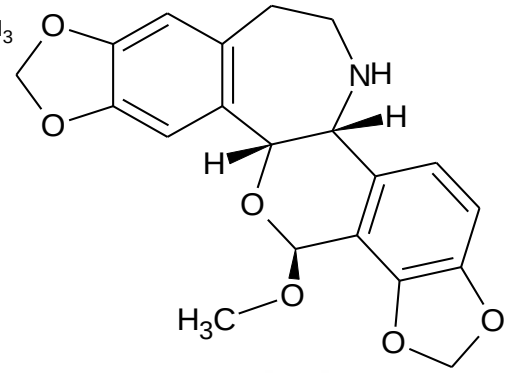


8g

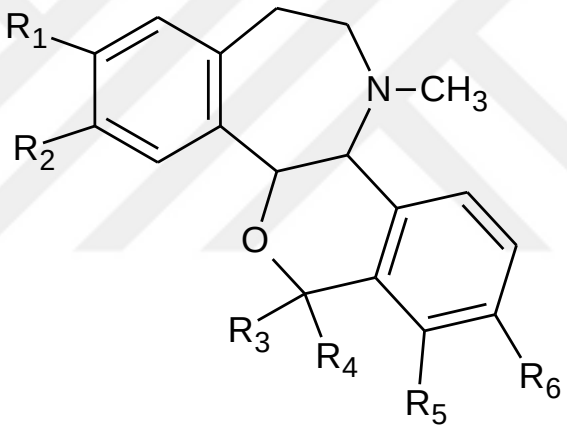
2.5.9. Rhoeadine 9



9a

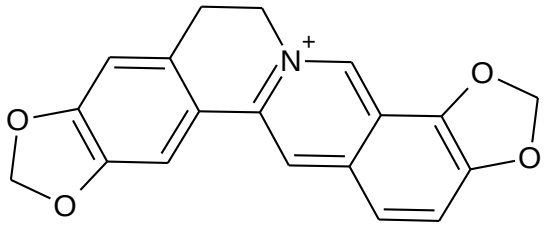


9b

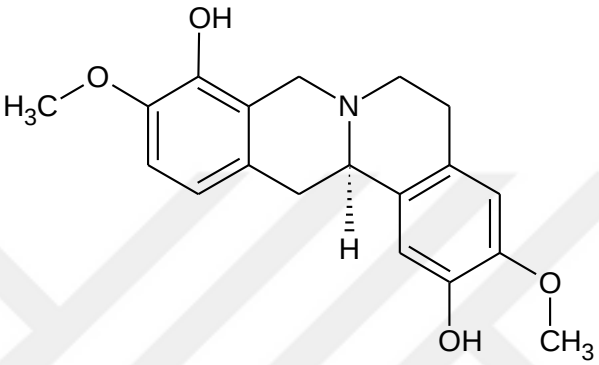


	R_1	R_2	R_3	R_4	R_5	R_6
9c	O-CH_2-O		H	OCH_3	O-CH_2-O	
9d	O-CH_2-O		H	OH	O-CH_2-O	
9e	OCH_3	OCH_3	H	OH	OCH_3	OCH_3
9f	OCH_3	OCH_3	OH	H	O-CH2-O	
9g	OCH_3	OCH_3	H	OCH_3	O-CH_2-O	
9h	OCH_3	OCH_3	OCH_3	H	O-CH_2-O	
9i	OCH_3	OCH_3	H	OH	O-CH_2-O	
9j	O-CH_2-O		H	OH	OCH_3	OCH_3
9K	O-CH_2-O		H	$OCH2CH_3$	O-CH_2-O	
9l	O-CH_2-O		H	OCH_3	O-CH_2-O	
9m	O-CH_2-O		H	OH	O-CH_2-O	

2.5.10. Protoberberine (Tetrahydroprotoberberine) 10



10a



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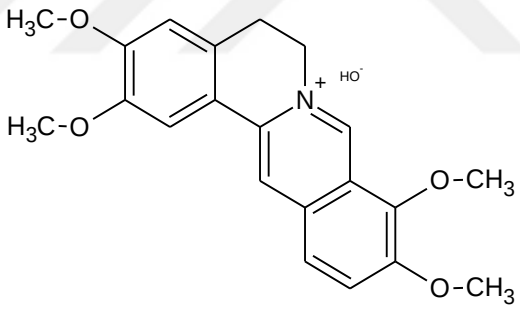
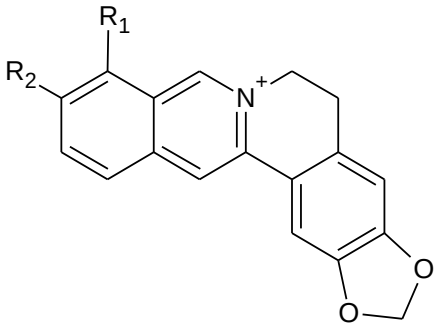
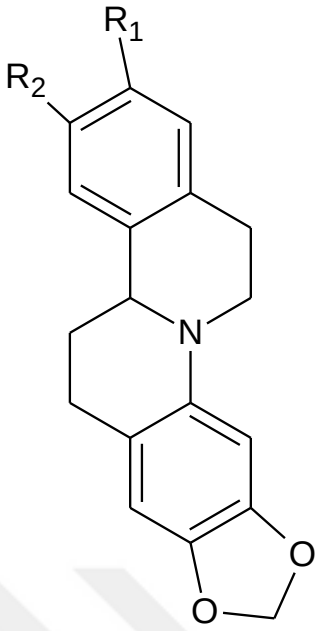
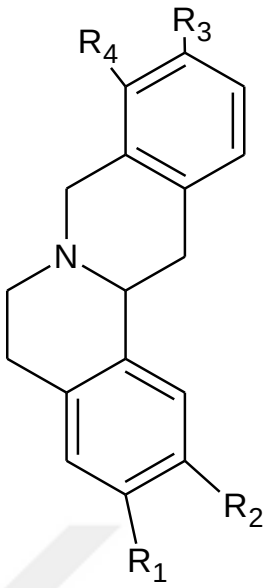


10c



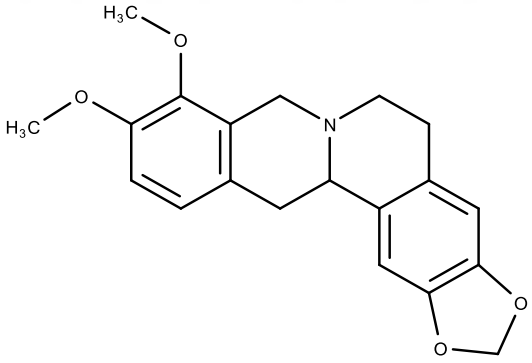
	R_1	R_2
10d	OCH3	OCH3
10h	OCH3	OH



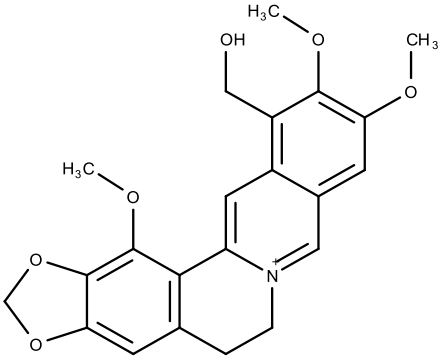


	R_1	R_2
10e	OCH_3	OH
10f	OCH_3	OCH_3

	R_1	R_2	R_3	R_4
10g	OCH_3	OH	OCH_3	OCH_3
10i	O-CH_2-O		O-CH_2-O	

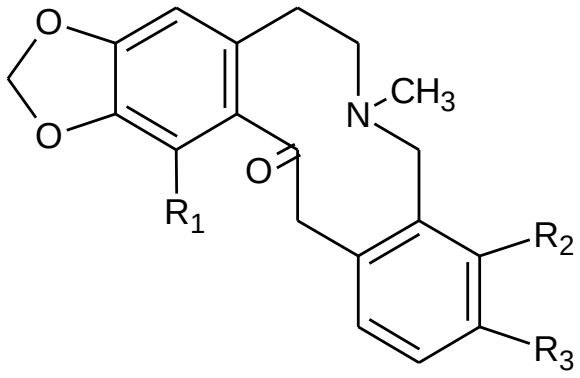


10j

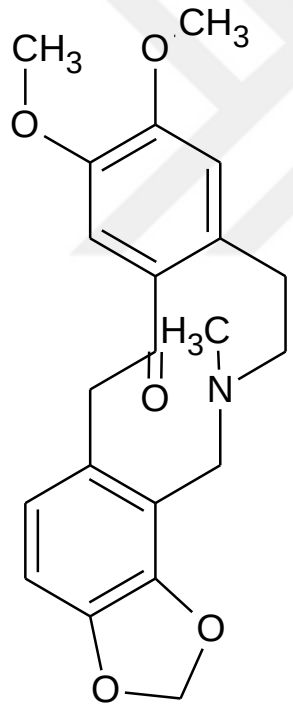


10k

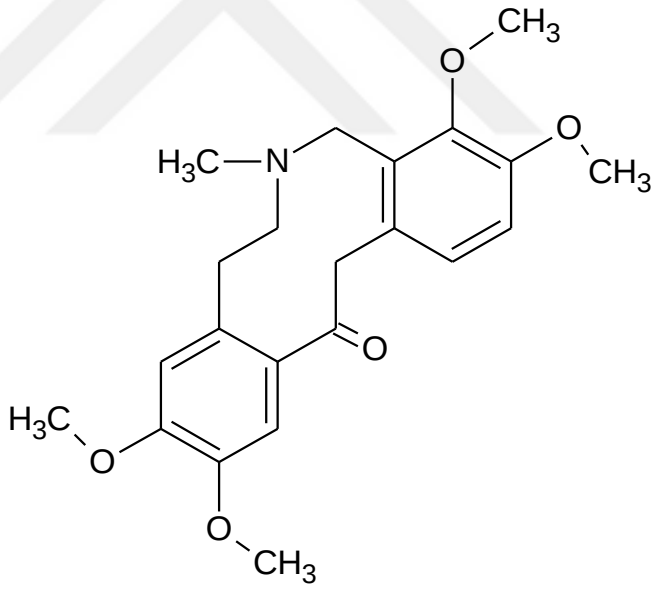
2.5.11. Protopine 11



	R_1	R_2	R_3
11a	H	O-CH2-O	
11b	H	OCH3	OCH3
11c	OCH3	OCH3	OCH3



11d



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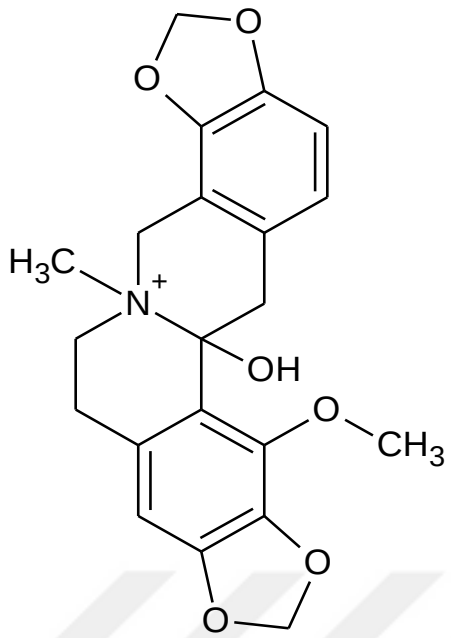

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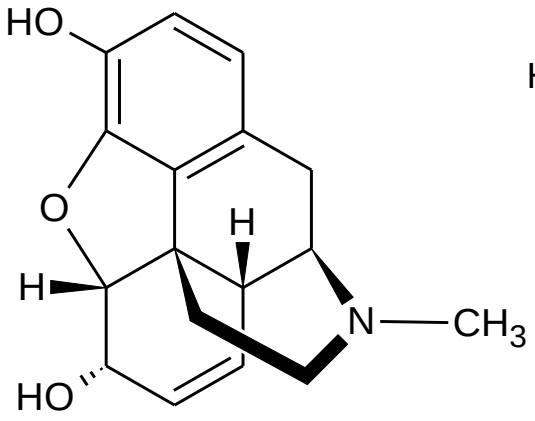

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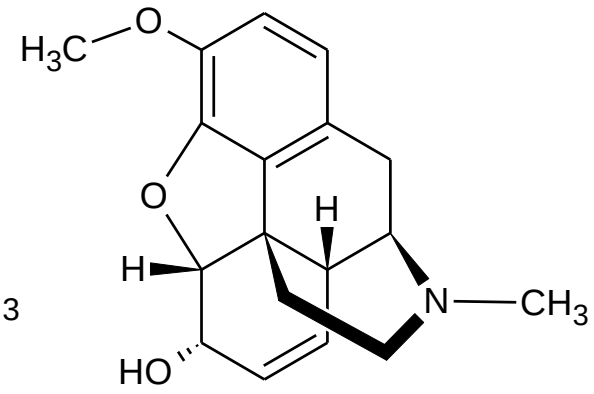
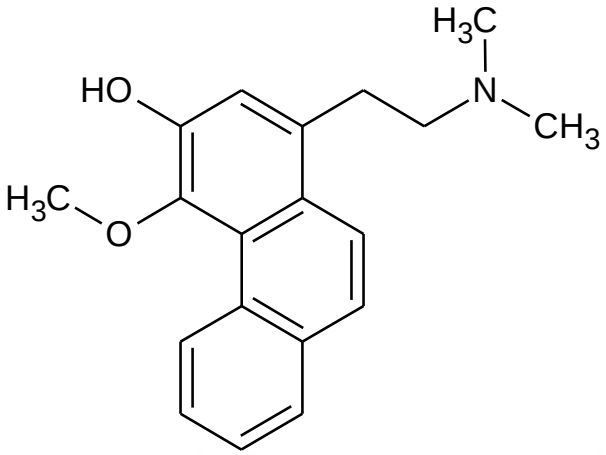


12b

2.5.13. Phenanthrene 13



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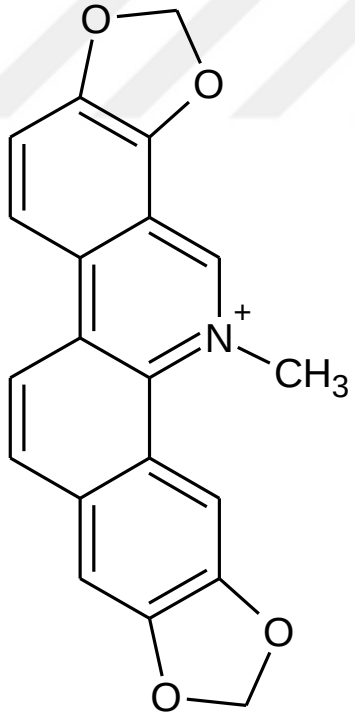
3.5.14. Benzophenanthridine 14



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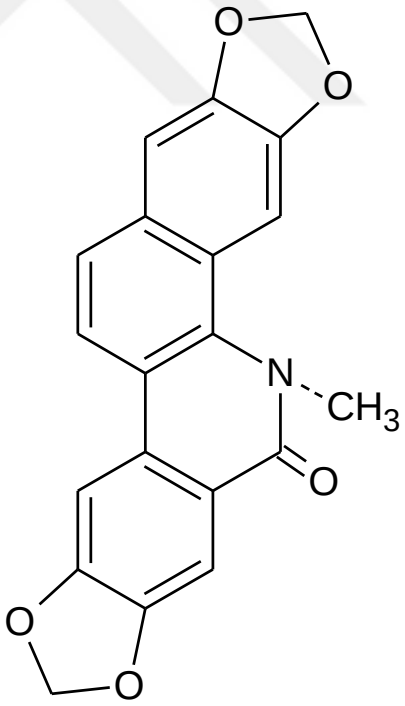

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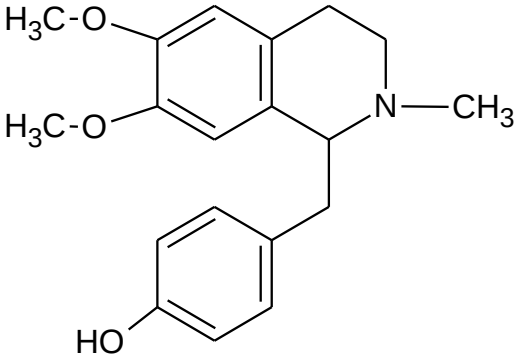
2.5.15. Benzylisoquinoline 15



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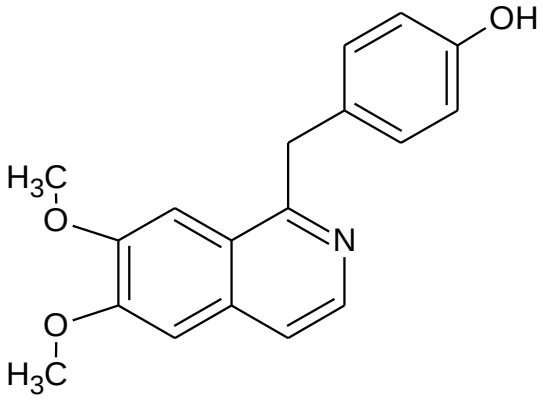

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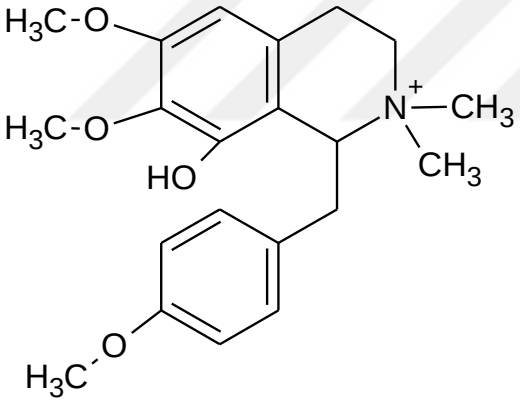

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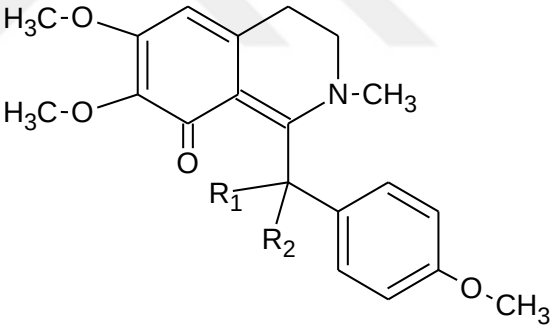
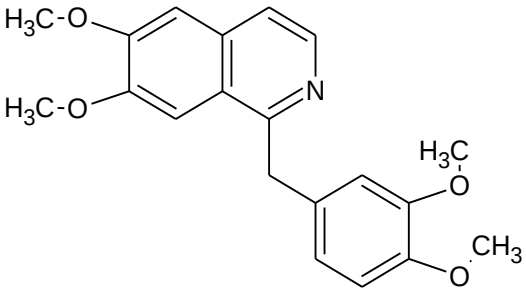


	R_1	R_2
15d	H	H
15e	-O-	



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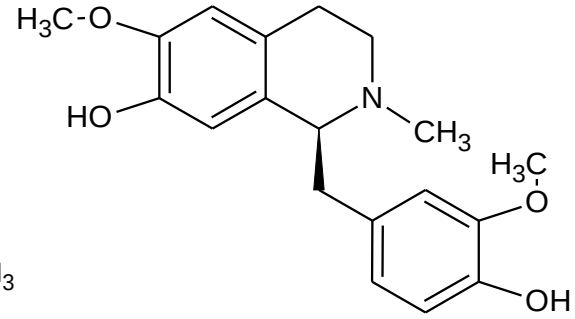

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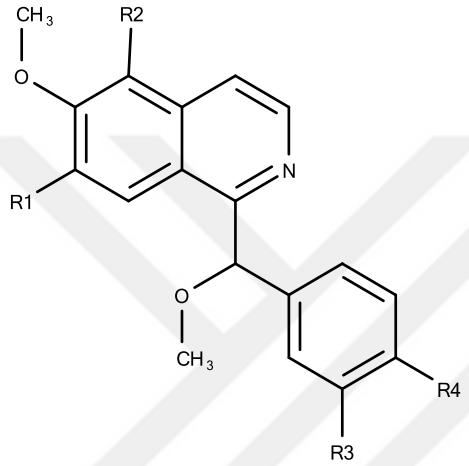
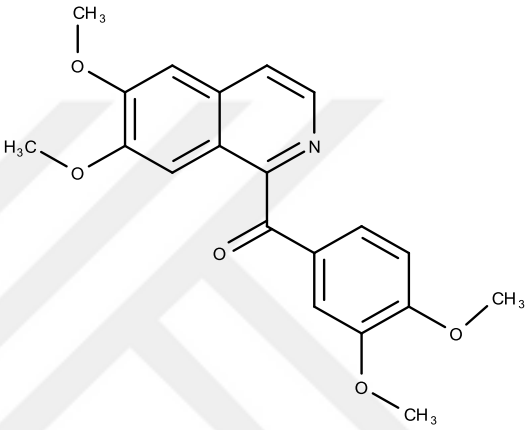


	R_1	R_2	R_3	R_4
15h	OCH_3	H	OCH_3	OCH_3
15i	OCH_3	OCH_3	$-OCH_2O-$	



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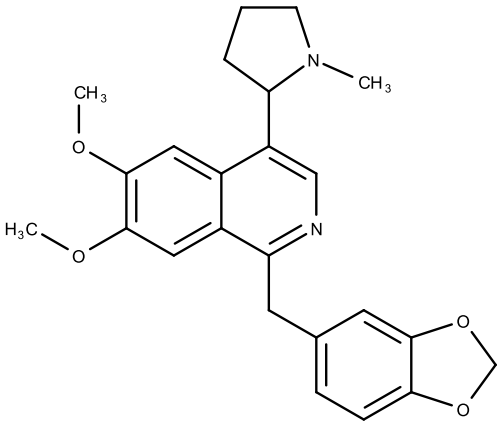

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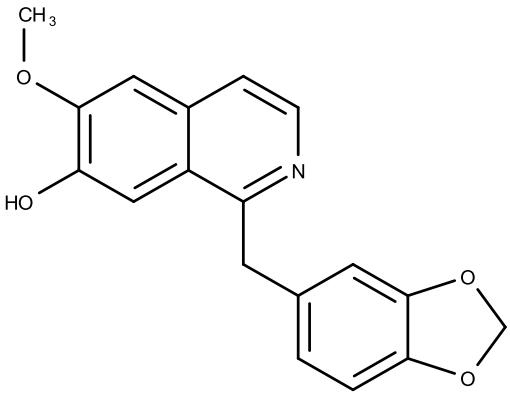
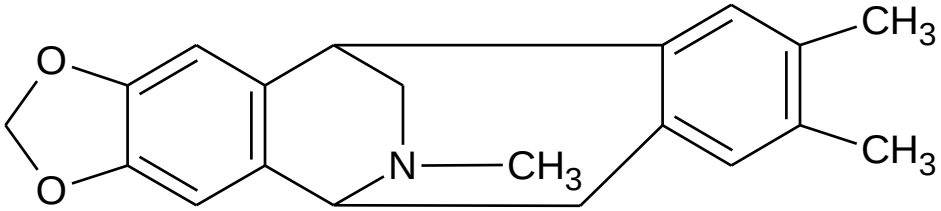
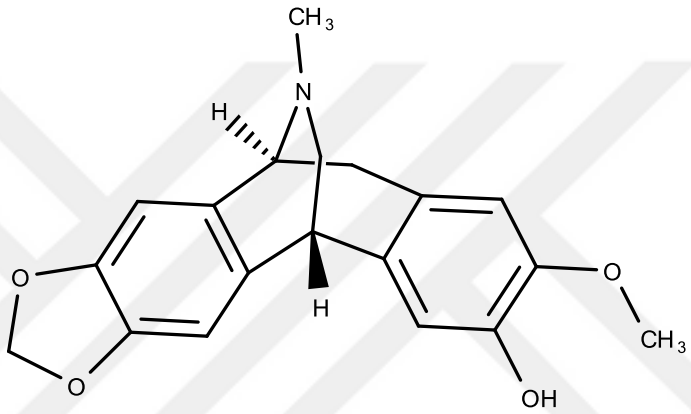


15l

2.5.16. Isopavine 16

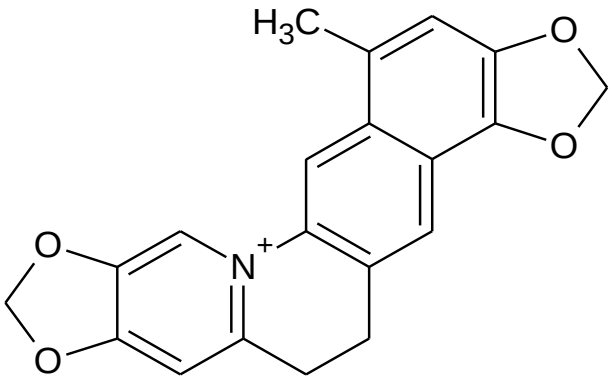


16a



16b

2.5.17. Phenanthridine 17



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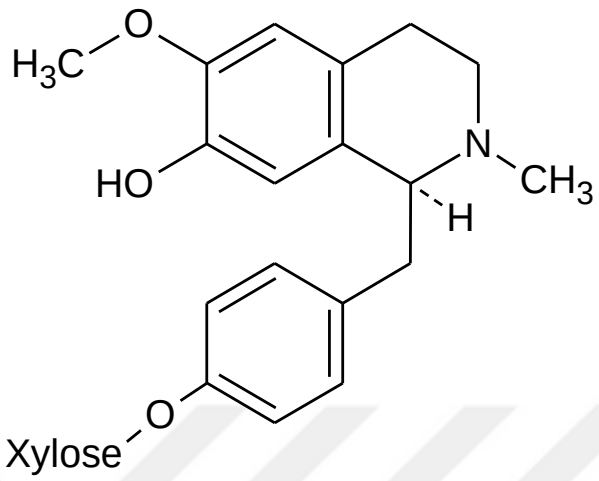
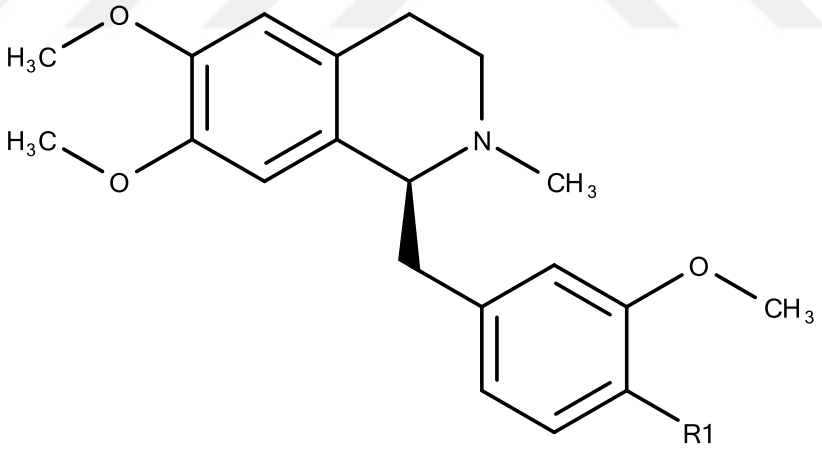
2.5.18. Tetrahydrobenzylisoquinoline 18

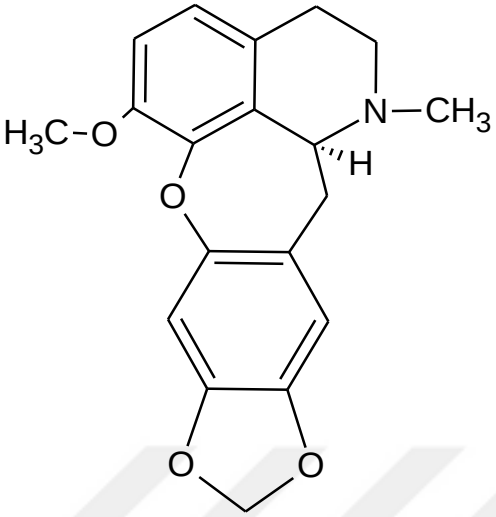


18a



	R_1
18b	OCH_3
18c	OH

2.5.19. Isoquinoline 19



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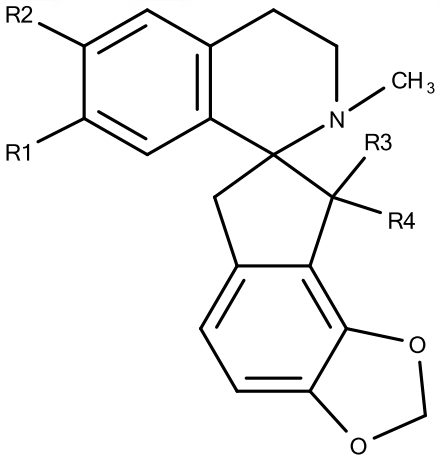
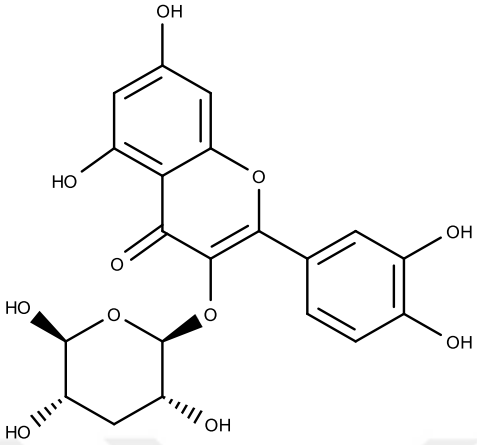


	R_1	R_2	R_3	R_4
20a	O-CH_2-O		-O-	
20b	OH	OCH_3	H	$OCOCH_3$

2.5.21. Phenolics



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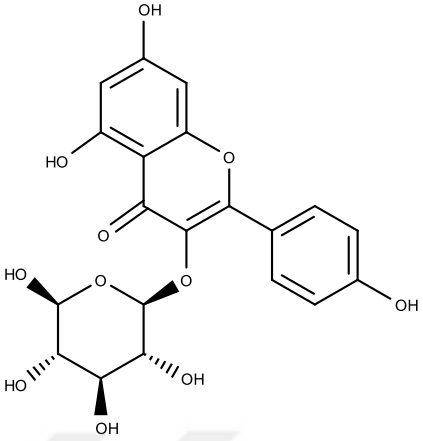

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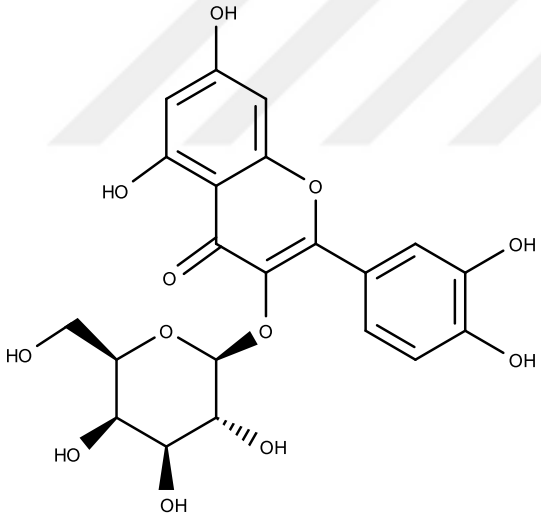

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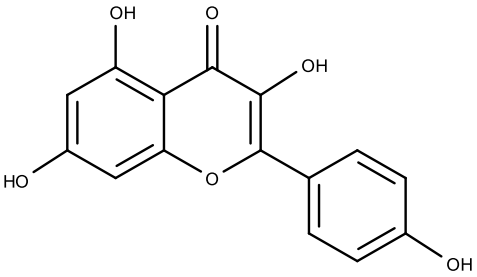

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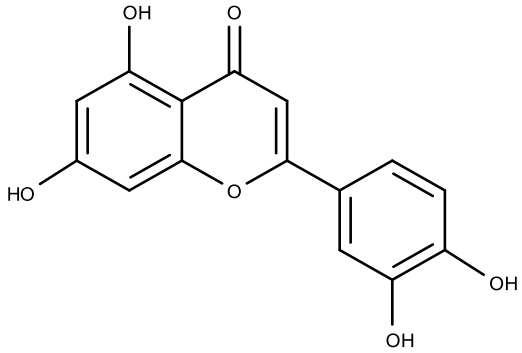

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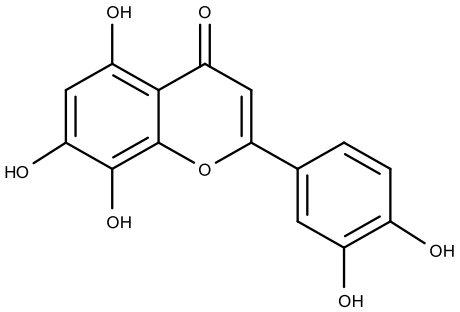

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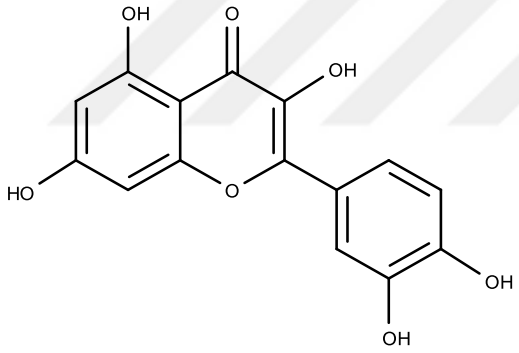

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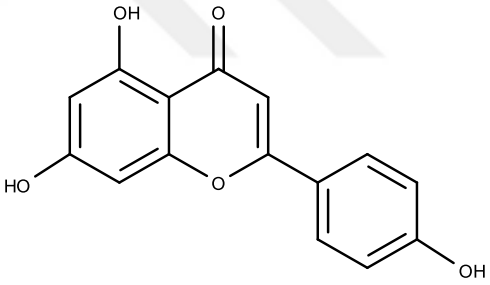

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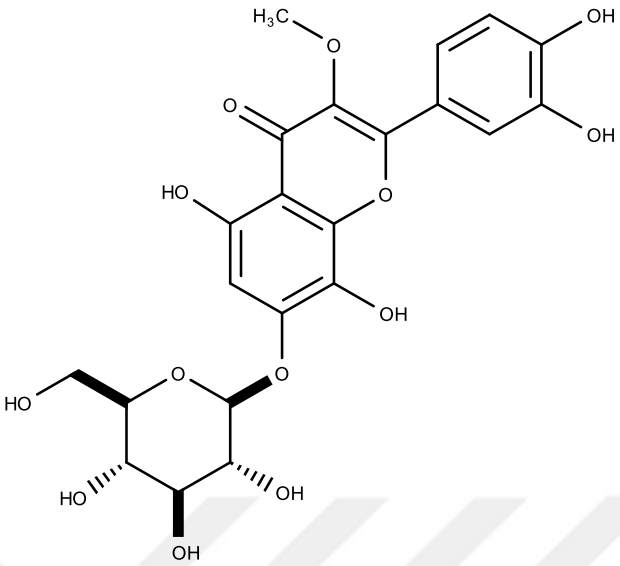

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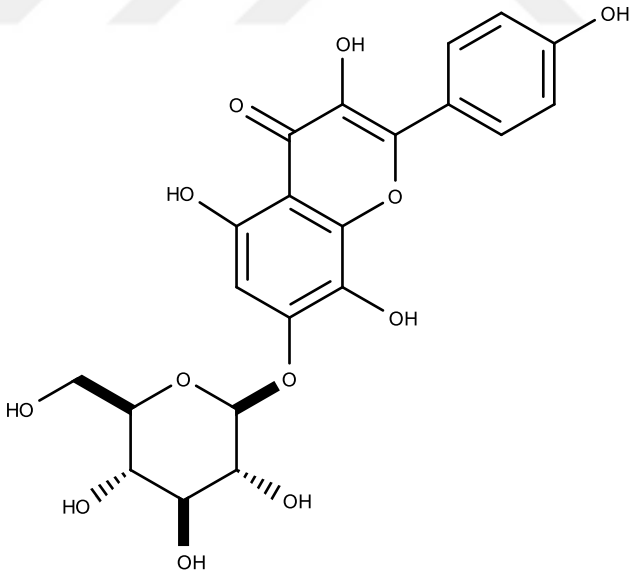

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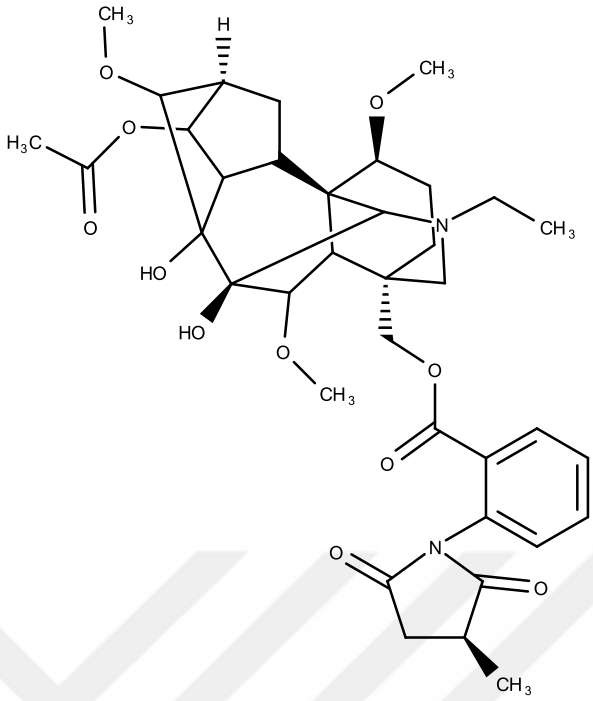
CH3
H
O
CH3
O
H3C
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O
N
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HO
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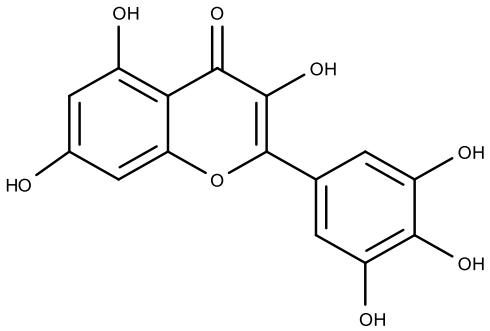
O
CH3

11


OH
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HO
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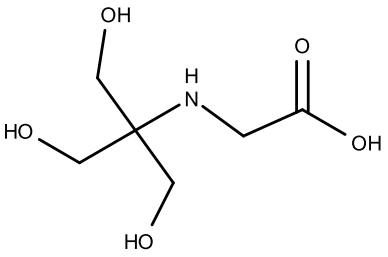
OH
OH
OH

12


OH
O
H
N
HO
OH
HO

13

2.6 Depression and BDNF

The main reason that causes depression is the imbalance in chemicals or hormones in the brain. Serotonin (5-HT) is the core hormone associated with depression. The other hormones which related to depression are dopamine (DA), epinephrine, norepinephrine (NE), histamine, acetylcholine, orexin A and orexin B. These hormones are important for the brain to function normally. Any changing in these hormones' level may affect the chemical balance in the brain leading to depression (120, 121).

Inflammatory cytokines can be released with over-activity in the sympathetic part of the brain during Major Depressive Disorder (MDD), these cytokines overlap with neurotrophic and monoaminergic signals. Cytokines also might destroy the sensitivity of central corticosteroid receptor, causing feedback disruption (Fig. 6) (122).

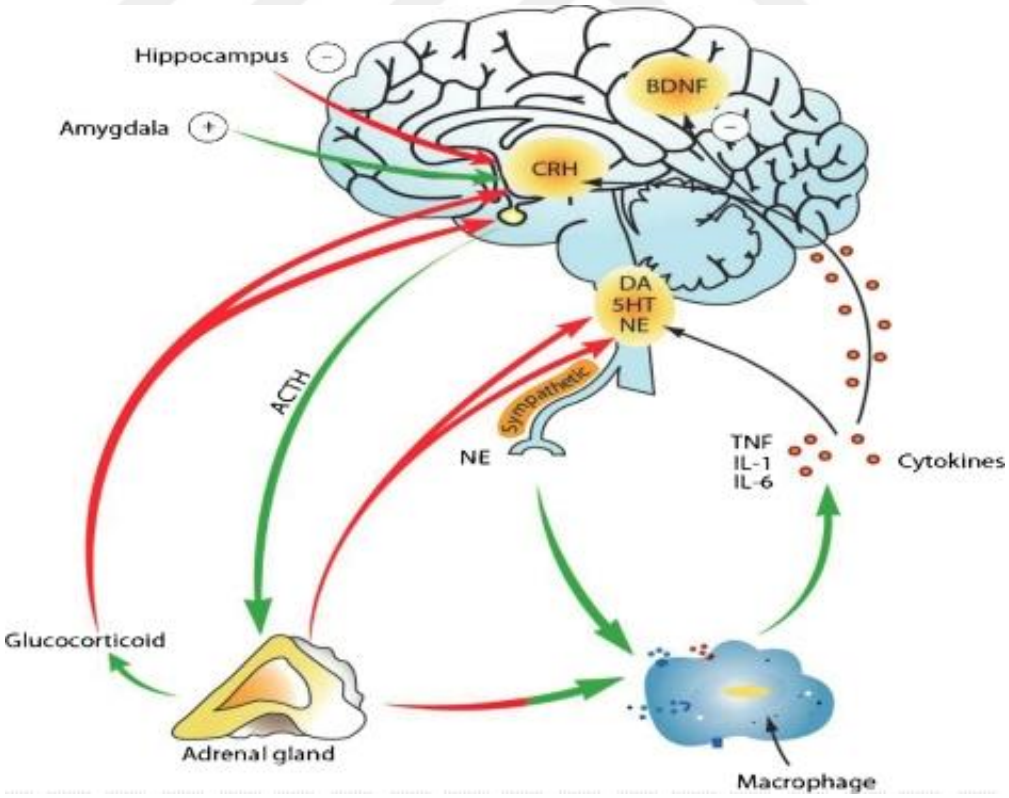


Figure 6. Molecular biology of depression (122).

2.6.1. Neurotrophins and Depression

Depression correlates with pathological atrophy of the neurons characterized by synaptic dysfunction in some part of the brain such as limbic and cortical regions. Neurotrophic factors involved in regulation of growth, development, differentiation, and survival of cell populations as well as their adaptation to environmental influences (4).

Neurotrophins family contains Nerve growth factor (NGF), BDNF, Neurotrophin-3 (NT-3) and Neurotrophin-4/5 (NT-4/5) (123). Many neurotrophic factors have a direct relation with depression and antidepressant action (124).

Patients with MDD are associated with a low level of the brain-derived neurotrophic factor. Most of Aantidepressants increase the level of BDNF in the brain and improve BDNF signaling in the prefrontal cortex and in the hippocampus (2).

2.6.2. BDNF

BDNF is a member of the Neurotrophins family that present in small amounts in the central nervous system (CNS); which support the survival of neurons. Gene expression of BDNF occurs in many regions of the CNS, including striate nucleus. BDNF is a trophic factor for dopaminergic neurons of the ventral midbrain, regulate voluntary motion, reward and emotion (125).

Structure of BDNF and it is receptor TrkB are shown in Figure 7 (126, 127). BDNF has two precursors, proBDNF and mature BDNF (mBDNF). proBDNF considered into passive and active pathways and mBDNF is known for it is biological activity. Lately, proBDNF is been recognized for it is biological activity by facilitating long-term depression through inducing apoptosis in the hippocampus. (128).

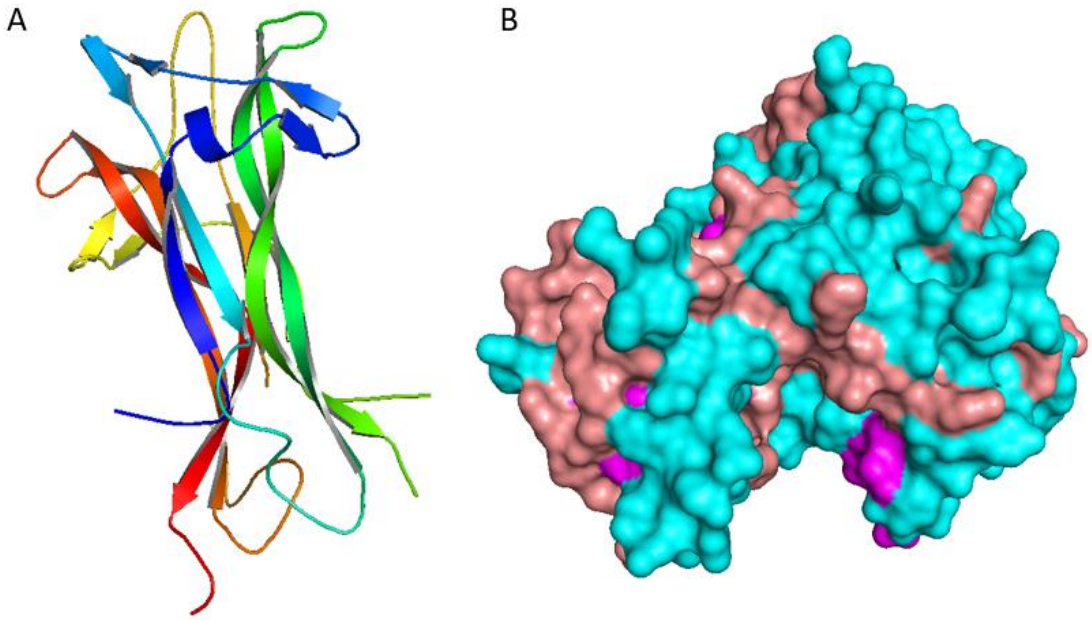


Figure 7. Structure of (A) BDNF, (B) TrkB receptor (126, 127).

2.6.3. BDNF Signaling

Abnormalities in cell signaling play an important role in pathophysiology and treatment of MDD. Studies show that cyclic adenosine monophosphate (cAMP) pathway changes in numerous sites in MDD. Antidepressants treatment regularly upregulate various part of cAMP pathway in mouse brain, including phosphorylated cAMP response element binding (CREB) and CREB levels. Studies revealed that antidepressants increase BDNF level in the hippocampus and cerebral cortex through downstream target genes of the cAMP pathway. It has been reported that people with MDD are associated with a decrease in the transcription factor CREB in the cerebral cortex (129).

Tropomyosin receptor kinase B (TrKB) is known as BDNF growth factor receptor. BDNF and TrKB are highly expressed in adult brain, stimulating cell signaling inside the neurons that are crucial for neuronal survival and behavior related plasticity (Figure 4) (130). BDNF binds to Tropomyosin receptor kinase B (TrkB) receptors to initiate numerous intracellular cascades, including phospholipase Cg (PLCg), mitogen-activated protein kinase/extracellular signal-regulated protein kinase (MAPK/ERK) and phosphoinositide 3-kinase (PI3K) pathways. The

biological effects of BDNF derive from the activation of similar related mechanisms (131). Additionally, BDNF also signals through common low-affinity neurotrophin receptor p75, which also implicated in neuronal plasticity and survival (132). The effect of BDNF on neurons weather is positive or negative via several intracellular cascades that are been initiated through activation of TrKB or p75 (Figure 8) (131).

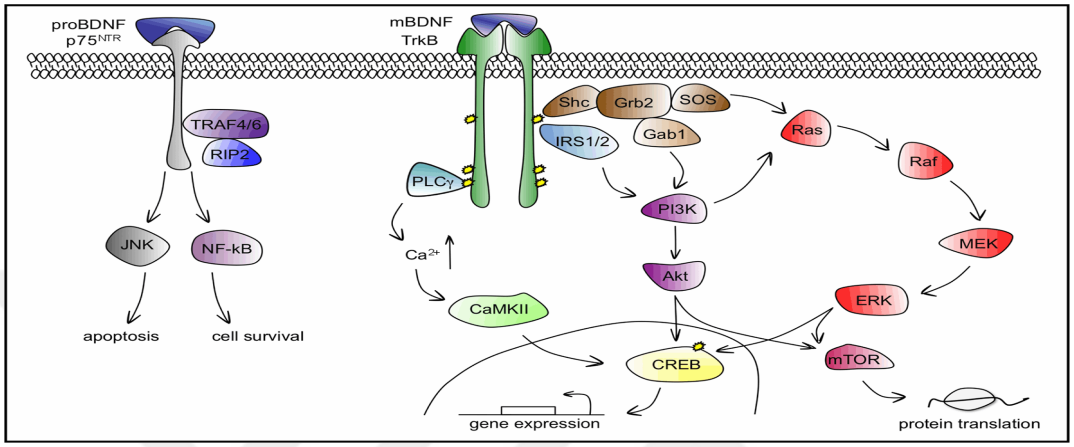


Figure 8. BDNF signaling; TrkB and p75NTR signaling pathways (131).

Role of BDNF in Depression

Antidepressants have been available for decades to treat MDD. Several antidepressants intensely increase monoamines level in the brain, but the necessity for frequent and repetitive administration of drugs, which led to the idea of long-term adaptation is important for the therapeutic effect of antidepressant. Since BDNF promotes neuronal development and survival, studies indicate that the long-term target of antidepressants treatment could possibly regulate BDNF. The supported hypothesis behind the role of BDNF in MDD is that antidepressants action is mediated by BDNF. The most common known presupposition assumes that antidepressants restore the level of neurotransmitters and any compromise neurotransmission, mostly serotoninergic and noradrenergic system has controlled our ideas about the mechanism of MDD (133). Several studies demonstrate that injection of BDNF in the mesencephalon has behavioral antidepressant effect in learned helplessness paradigms and forced-swim test. This suggests that increased BDNF expression has an antidepressant effect (134). All kind of antidepressants

treatment, including; lithium, and electroconvulsive shock treatment increase BDNF expression in the cortex and hippocampus (124).

Moreover, it has been shown that antidepressant treatment does not decrease immobility in forced swimming test in TrkB-T1 transgenic mice and BDNF knockout mice. There is no any reduction in immobility during forced swimming test with the administration of antidepressant, which indicates that the positive behavioral effect of antidepressant is completed by BDNF- signaling through TrkB receptor. Acute administration of antidepressant increases the immobility on forced swimming test, while chronic administration (21 days) is needed to prompt BDNF and Ntrk2 mRNAs expression in mice.

Therefore the effect of antidepressants on forced swimming test is more likely to be through an acute increase in signaling in the TrkB receptor or BDNF level or both instead of improving BDNF gene expression (135). MDD may not be due to an impairment in BDNF signal alone; instead, it may be triggered by multiple pathways deficits. On the other hand, BDNF-TrkB downregulation could be a result of compensation to upregulate other neurotrophic growth factors. Furthermore, development of MDD can be possibly due to genetic factors and stress (135).

BDNF and Neurodegenerative Diseases

BDNF has a crucial role in learning, memory and hippocampus-based synaptic plasticity. The interference of BDNF level would lead to decrease hippocampal function, memory and learning. BDNF also produces quick postsynaptic effects on glutamate receptors, N-Methyl-D-aspartic acid (NMDA) and ion channels; dysfunction of these receptors will cause problems with memory, behavior and thinking like in Alzheimer’s disease. (136).

2.6.4. Antidepressants Mechanisms

MDD is associated with a reduction in the hippocampus and prefrontal cortex volume. However, antidepressants promote numerous forms of neuronal plasticity

including neuronal maturation, synaptogenesis, neurogenesis in the hippocampus (124).

Most of the antidepressant drugs have many side effects including; sedation and metabolic syndrome (137). Several types of antidepressant drugs are available in the pharmaceutical market, only 60% of the patients are responsive to the medication (138). Thus, new more effective drugs with less adverse effects are still needed.

GABAergic System

GABAergic system is a very important to understand depression and anxiety. GABA neurotransmitter acts as an inhibitory in CNS on GABA-A, B and C receptors. Emrich et al. suggested that any dysfunction in GABAergic system could lead to mood disorders (139). Furthermore, numerous neuroimaging studies proposed that the reduction in GABA transmission is involved in MDD. Different drugs used to treat depression like anxiolytics and benzodiazepine, which directly improve GABA function by acting on α2 and α5 GABA subunit receptor. Moreover, selective GABA reuptake inhibitor Tiagabine® that targets GAT-1 (GABA transporter) has been used to treat anxiety and behavior disorders. It has been reported that there is a reduction in GABAergic neurons number in the orbitofrontal cortex of postmortem in many cases of MDD. The related mechanisms are unknown, some studies suggest it could be due to a decrease in BDNF level or decrease in neurogenesis of GABAergic neurons (139).

Monoaminergic System

Tricyclic antidepressants and monoamine oxidase inhibitors are one of the first effective drugs used to treat MDD by increasing the level of noradrenaline and 5-HT in the synapse. The presumption of monoamines for the pathophysiology of MDD, which led to the hypothesis of insufficient amount of 5-HT and noradrenaline in patients affected with the disease. Nevertheless, this hypothesis does not clarify the effectiveness of antidepressants in treating anxiety (139). In the following sections, we discuss different type of antidepressants.

2.6.5. Types of Antidepressants

Tricyclic Antidepressants (TCA)

TCA inhibit 5-HT and NE reuptake, which leads to increase 5-HT and NE concentration in the synaptic cleft. Clomipramine® and Imipramine® provide their action through upregulating the postsynaptic receptor. TCA adverse effects are orthostatic hypotension, sedation, dry mouth, weight gain, urinary retention, blurred vision and memory problems, which is due to their agonist effect on α-1 adrenergic, H_1-histaminic and anticholinergic receptors. (140).

Selective Serotonin Reuptake Inhibitors (SSRIs)

5-HT is associated with depression, mood and sleep disorders. 5-HT also can delay or induce fatigue in prolonged exercise (141). The core postulate of the pathology of MDD involves an insufficient amount of 5-HT, which decrease serotonergic function in the brain. SSRIs block any transportation of 5-HT, which increase 5-HT in the synaptic cleft, therefore enhancing serotonergic activity. Conversely, it has been reported that activation of nerve terminal $5\text{-}HT_{1B}$ autoreceptors and somatodendritic $5\text{-}HT_{1A}$ limits the therapeutic effectiveness of SRRI antidepressants (142, 143).

Monoamine Oxidase Inhibitors (MAOIs)

MAOIs are one of the first effective antidepressants. Having the ability to increase noradrenaline and 5-HT levels at the synapse (144). MAOIs inhibit monoamine oxidase enzyme (MAO), leading to an increase in the monoamine level followed by enhancing serotonin syndrome (145). MAOIs are the third line agents to treat resistant depression despite all the known high side effects and food interaction.

Noradrenergic Antidepressant

Noradrenaline is well-known neurotransmitter that involved in many psychological and physiological progression. Disruption of this neurotransmitter linked with series of psychological conditions. Various drugs target noradrenaline system, which shown to act as antidepressant. Currently, there is a number of antidepressants available with different degrees of selectivity on targeting noradrenergic system (146)..

3. MATERIALS AND METHODS

3.1. Plant Materials

Aerial parts of *Papaver* species were collected in May 2014 during the flowering period from eastern and middle regions of Anatolia. The species were identified by Prof. Dr. Galip Akaydın, Department of Plant Biology, Hacettepe University (Ankara, Turkey) where the herbarium samples are deposited (16019 to 16023 HEF).

- *Papaver glaucum*: It was collected in Şar Mahallesi, next to Mardin Museum, Artuklu/Mardin, Turkey (16019 HEF).
- *Papaver macrostomum*: It was collected in Eryaman, 550 meter away from Misbaşak İvedik-Fabrika, Etimesgut/Ankara, Turkey (16022 HEF).
- *Papaver rhoeas*: It was collected next to Ikea (200 meter) in Ankara/Turkey (16023 HEF).
- *Papaver lacerum*: It was collected next to the main road from Kilis (10 km away) toward Gaziantep direction (16020 HEF).
- *Papaver syriacum*: It was collected in Ceylanpınar close to the Syrian border (16021 HEF).

3.2. Chemicals and Reagents

Chlorpromazine, imipramine, tetrabenazine, alkest TW 80, 17β-estradiol (sigma, China), Dulbecco's modified eagle's medium (DMEM) (Biochrom, Germany), penicillin/streptomycin (Wisent, Canada), L- Glutamine (Lonza, Belgium), fetal bovine serum (FBS) (Biowest, USA), trypsin EDTA (Lonza, Belgium), PBS tablet (Biomatik, Canada), RIPA Lysis Buffer System (sc-24948t, Santa Cruz Biotechnology), human BDNF ELISA kit (Boster Biological Technology Co., LTD.), *n*-hexan (Sigma, Merck), DMSO (Sigma, USA), methanol (MeOH) (Sigma, Merck), chloroform ($CHCl_3$) (Sigma, Merck), ethyl acetate (EtOAc) (Sigma, Merck), dichloromethane (CH2Cl2) (Sigma, Merck), *n*-butanol (Merck), *t*-butanol, ethanol, acetic acid (Sigma, Merck).

3.3. Animals

Male and female albino Swiss mice (24–28 g) were purchased from the Kobay D.H.L. A.Ş (Ankara, Turkey).

3.4. Devices and Machines

o HPLC	Dionex-0631
o Distilled water machine	GFL-2004
o Autoclave	Hanshin Medical-HS 9041
o Water bath	Major Science-SWB 10L-1
o Centrifuge	KuBoTA-3500
o Laminar flow	Teknomar-Chemocell LRCX-UV
o HR-MS	Agilent 6530 Q-TOF LC-MS
o LC/ESI-MS/MS	Shimadzu LCMS-8030 triple quadrupole
o Lyophilizer	Virtis Freezemobile 6
o Microplate reader	BIO-TEK-µQuant
o Microscope	Leica- 090-131.001
o CO_2 incubator	SYNO-MCO-18 AIC-uv
o Centrifuge	Herolab-unicen 15D
o Spectrophotometer	BioTek-uquant 193379
o Deep freezer	nuve-DF290
o Elmasonic	Elma-TS70
o UV Cabin	CAMAG-022.9120
o Heater Table	CAMAG-022.3306
o Analytical balance	AnD-Ek-600H
o Sensitive scales	Denver instrument-SI-234
o Rotary evaporator	BÜCHI-R210
o Plant miller and sieves	AEG-AMEB80FYZ
o Oven	Electro mag-M5040

3.5. Extraction

The aerial parts of the *Papaver* species were dried in shade and powdered. The MeOH extracts were obtained by maceration of plant material with 1000 mL X 3 of MeOH for 3 days at a room temperature. The extract was filtered and dried under reduced pressure at 40 °C. The same extraction procedure was used for the biological activity tests. The dried extract was dissolved in 250 mL of water and extracted with 250 mL X 3 of EtOAc (Figure 9).

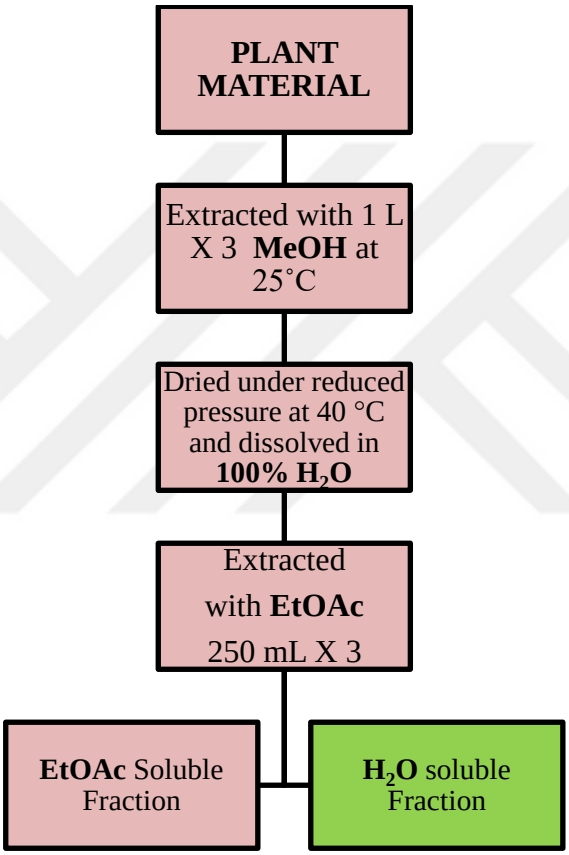


Figure 9. Extraction procedure.

3.6. Isolation

145 g of *P. lacerum* was extracted with the procedure that explained above to give 2.6 g EtOAc and 8.7 g of H_2O extracts. Due to the low amount of EtOAc extract and high contents of the chlorophyll, it was not chosen for further phytochemical investigations.

The H_2O soluble extract was separated by C-18 VLC (5x40 cm), eluting with 100% water (H_2O) with decrease in polarity (10% MeOH ⇨ 100% MeOH), assembling 300 mL per fraction. Four fractions (PL-17-1 – PL-17-4) were obtained. PL-17-2 was directly subjected to LH-20 Sephadex column (LH-20) (2.5x30 cm) eluting with CH_2Cl_2-MeOH (50:50) and two fractions (PL-18-1 – PL-18-2) were collected.

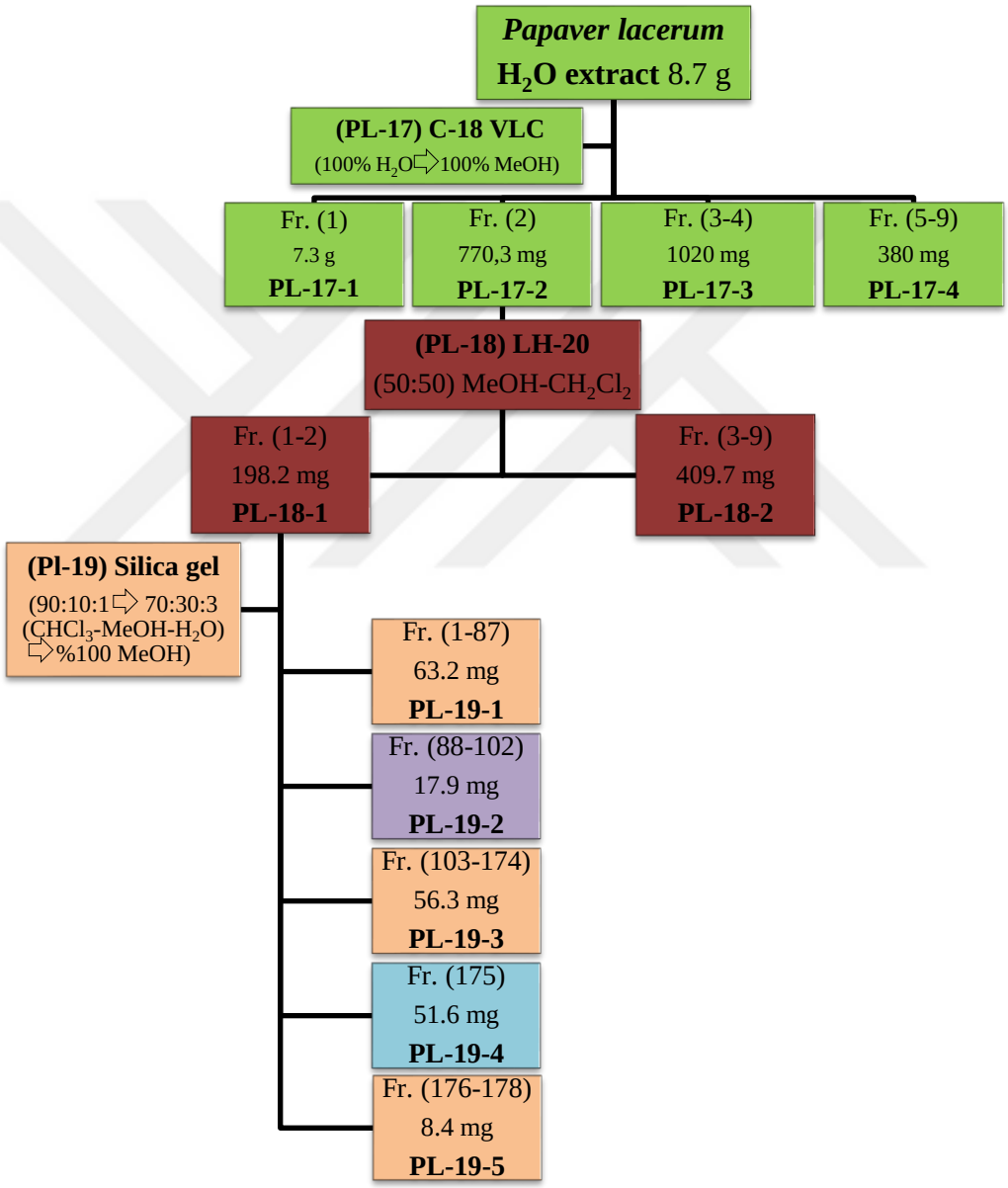


Figure 10. General isolation map.

PL-18-1 was applied to a silica gel column chromatography (3x40 cm), eluting with $CHCl_3$-MeOH-H_2O (90:10:1 ⇨ 70:30:3 and 100% MeOH) and five fractions (PL-19-1 – PL-19-5) were obtained; 5 mL per fraction (Figure 10).

Fraction PL-19-2 was separated by C-18 VLC (2x10cm), eluting with 100% H_2O with decrease in polarity (10% MeOH ⇨100% MeOH) and five fractions were collected (PL-27-1 – PL-27-5). Fraction Pl-27-5 was collected with 10% MeOH. PL-27-5 was subjected to LH-20 Sephadex column (LH-20) (2.5x30 cm) eluting with CH_2Cl_2-MeOH (50:50) and two fractions (PL-27-a-1 – PL-27-a-2) were obtained (Figure 11). Fraction PL-27a-1 was pure compound.

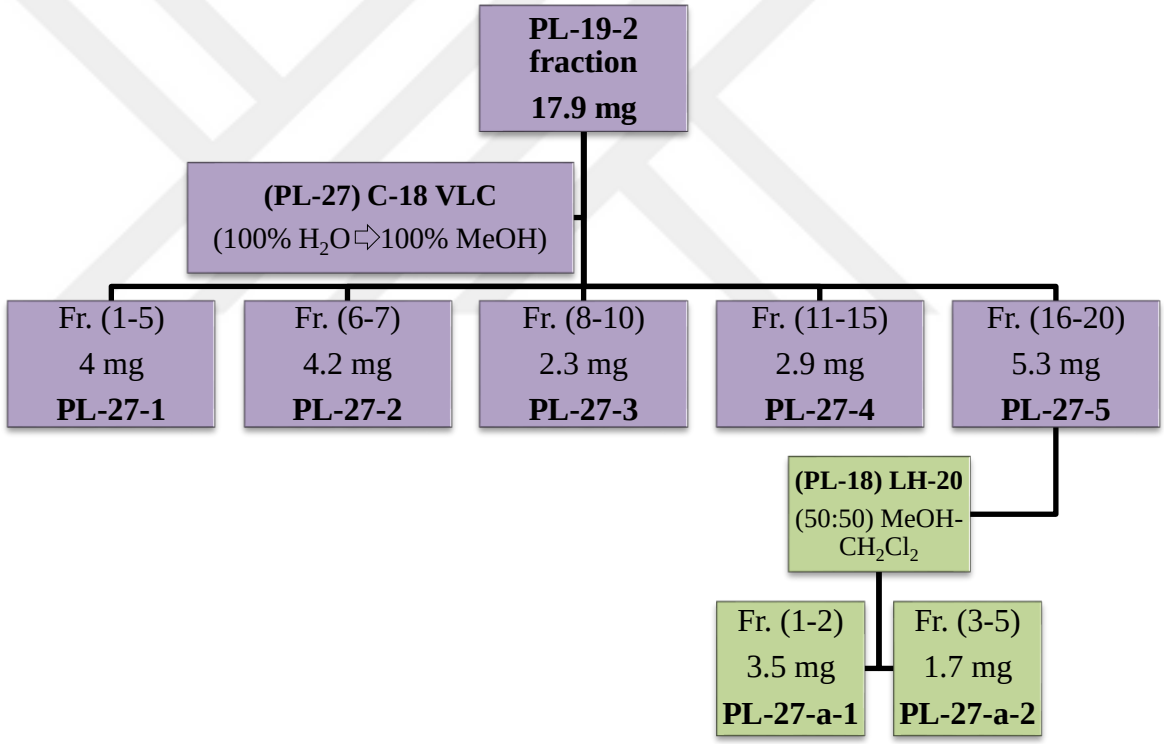


Figure 11. Isolation map for compound I.

Fraction PL-19-4 was subjected to a C-18 VLC (2x10 cm), eluting with (100% H_2O ⇨100% MeOH). Three fractions were collected, 5 mL each (PL-23-1 – PL-23-3) (Figure 12). Fraction PL-23-2 was pure compound.

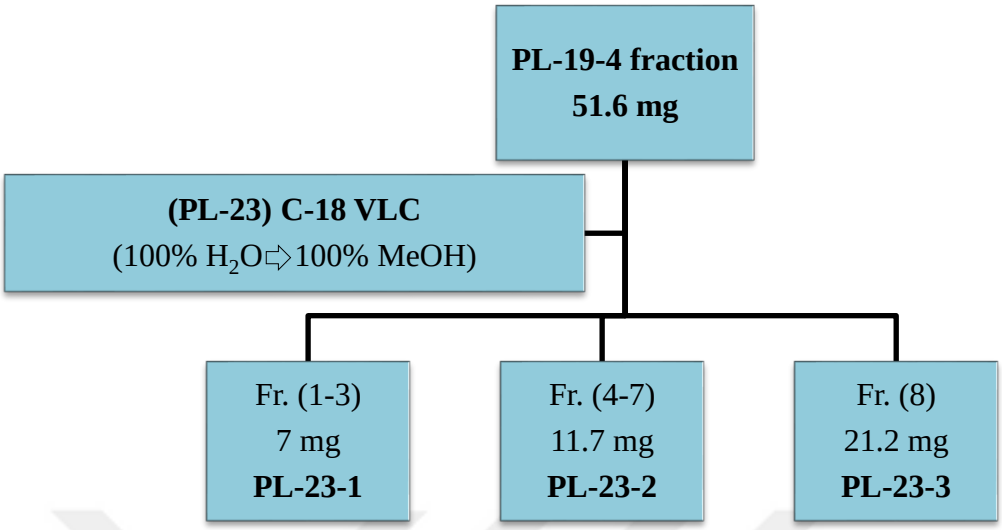


Figure 12. Isolation map for compound II.

3.7. LC-MS/MS Studies

LC/ESI-MS/MS was performed using a Shimadzu LCMS-8030 triple quadrupole mass spectrometer connected to a Shimadzu LC-20AXR chromatograph. The mass spectrometric detection was operated in positive electrospray ionization and multiple reaction monitoring mode. Hardware control and data acquisition and treatment were carried out Postrun Analysis Software.

Separations were carried out using a C18 column (2.1 x 50 mm, 3 µm) at the flow rate of 0.3 mL/min and at 40 ˚C. A gradient elution program with mobile phase A [0.1% (v/v) formic acid in water] and B [0.1% (v/v) formic acid in acetonitrile] was performed; B = 5% (0 min), 50% linearly increased (0–7 min) and 5% linearly decreasing (7-10 min). The ESI-MS/MS conditions were as follows: interface voltage, 4.5 kV; Q1 pre-rod bias voltage, -16V ((+)-pronuciferin) and -14 V (romerine); Q3 prerod bias voltage, -26 V or -19 V ((+)-pronuciferine) and -19 V or -17 V (romerine); collision energy, -22 eV; nebulizer gas flow rate, 3 L/min; drying gas flow rate, 15 L/min; desolvation line temperature, 250 ˚C; heat block temperature,

400 ˚C. The following transitions (precursor or product ion) 312.10 > 238.10, 280 > 191 m/z were used to monitor (+)-pronuciferin and romerine, respectively.

Romerine and (+)-pronuciferine stock solutions (1000 ppm) were prepared in MeOH and acetonitrile, respectively. The intermediate standard solutions for romerine and (+)-pronuciferine (10 ppm) were made from the stock solutions and were diluted with MeOH and acetonitrile. The stock solutions were stored in the refrigerator. Calibration standards were prepared by intermediate standard solutions to obtain concentrations of 10, 25, 50, 100, 250, 500, 750, 1000 ng/mL for romerine and (+)-pronuciferine.

3.8. Biological Activities

3.8.1. *In vivo* Studies

All the *in vivo* tests were realized in Dep. of Pharmacognosy, Gazi University. All animals were fasted for 12 hours before the administration of any doses. Extracts and drugs were mixed with Alkest TW 80 directly before using it and administrated orally before 1 hour of the experiments. Dosage was 0.25 mL/10 g in mice. With control animal only given 2% Alkest TW 80.

Effect on Spontaneous Motor Activity in Mice

After *p.o.* administration of drugs (30-50 mg/kg), *Papaver* extracts 100mg/kg, photocell activity meter was used to record locomotor activity beginning with 15 minutes then 60 and 120 minutes (147).

Effect on Normal Body Temperature

Thermistor thermometer (*Panlab 0331*) was used to measure rectal temperature of each mouse before the experiment and 1, 2, 4, 6, and 24 hour after *p.o.* administration of drugs (30-50 mg/kg), *Papaver* extracts 100mg/kg (147).

Forced Swimming Test in Mice

The test protocol was used as it was illustrated by (*Porsolt et al*) with slight modifications (1977) (148). After 1 hour of *p.o.* administration drugs (30-50 mg/kg), *Papaver* extracts 100mg/kg, mice were individually put in a glass container (diameter 10 cm, height 25 cm) filled up with 10 cm of 21–24 ˚C water. The total immobility time recorded in 6 minutes period. The time of immobility determined when the mouse stopped struggling and floated without any noticeable move in the water with slight movement to keep its head above water. Any decrease in immobility time indicates for an antidepressant-like effect (148).

3.8.2. *In silico* Study Design

To reveal the biological activities of the isolated compounds, a study has been designed to identify the molecular targets for each compound. Firstly a concensus scoring for the predicted targets was accomplished by using several software mentioned below, the targets with the highest score were selected.

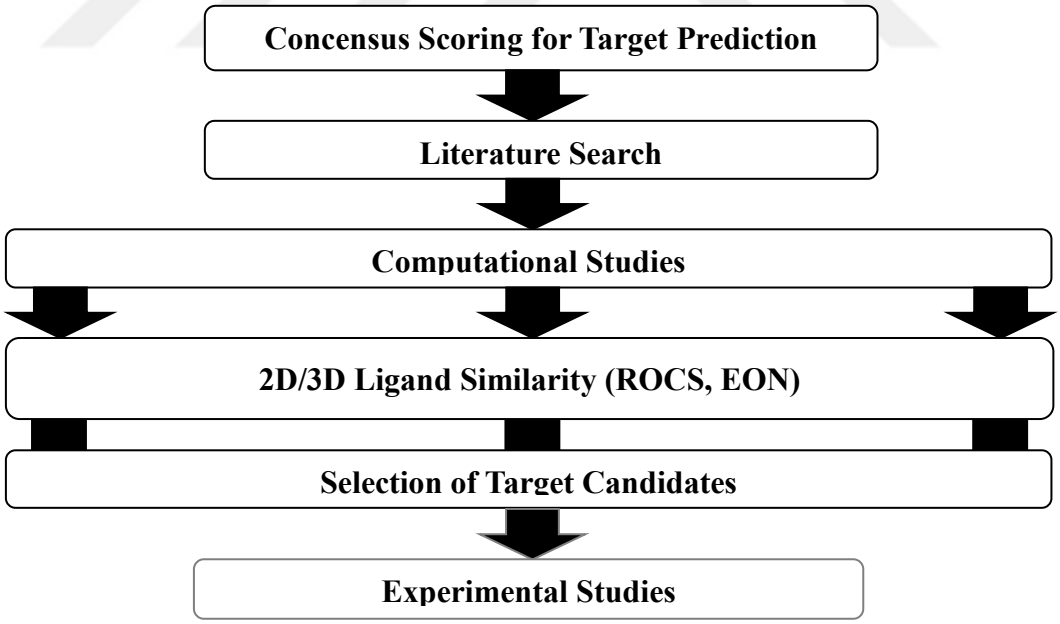


Figure 13. Systematic case study to identify molecular targets for the isolated compounds.

Secondly, a literature search was done to see if any of these obtained targets were tested experimentally. In this case the isolated Tyrosol-1-*O*-*β*-xylopyranosyl-(1→6)-*O*-*β*-glucopyranoside (TXG) and 5-*O*-(6-*O*-*α*-rhamnopyranosyl-*β*-glucopyranosyl) mevalonic acid (MRG) both have not been studied, especially compound II which is a new compound. Afterward structure virtual screening methods were used to identify 2D/3D Ligand Similarity. Lastly, the results were assessed to select the target candidates to be tested experimentally (Figure 13).

Target Prediction of Compounds I and II

Several target prediction software have been used to predict the molecular targets, CP, ChemProt (149); DP, DRAR-CPI (150); PM, PharmMapper (151); SEA, Similarity Ensemble Approach (152); SW, SwissTarget (153) and Spider (154). The top twenty one concensus molecular targets that were predicted across the six prediction software were used to complete the study. Structure of the compound I and II were drawn using MarvinSketch program and saved as (mol2). Smiles of compound I and II were generated using Online SMILES Translator and Structure File Generator website. Smiles were entered on the above mentioned software to identify the molecular target of compound I and II (Table 13).

Table 13. Compounds I and II smiles.

	Smile
Compound I	OCCC1=CC=C(OC2OC(COC3OCC(O)C(O)C3O)C(O)C(O)C2O)C=C1
Compound II	CC1OC(OCC2OC(OCCC(C)(O)CC(O)=O)C(O)C(O)C2O)C(O)C(O)C1O

Structure Virtual Screening

Virtual screening is a computational technique used in drug discovery research in recent years and it has become an important step in the drug discovery process. The screening involves the identification and compilation of relevant chemical structures from large chemical libraries (155). The chemicals identified are those most likely to bind to a protein target, typically a protein receptor selected by

using Openeye (OpenEye Scientific Software, Inc., Santa Fe, NM, USA, www.eyesopen.com) shape-similarity screening.

The theory of shape-similarity screening is derived from the idea that molecules possessing similar shapes (Using ROCS) and electrostatic (Using EON) capabilities might exhibit analogous biological activity. The method involves consideration of the atomistic and spatial characteristics of the target molecule. The pharmacophore and physical features of the molecule are quantitatively compared with a library of compounds. Molecules were drawn by MarvinSketch and the 3D structure was generated by Molecular Modeling and Simulations program (MOE) (156) (Figures 14, 15).

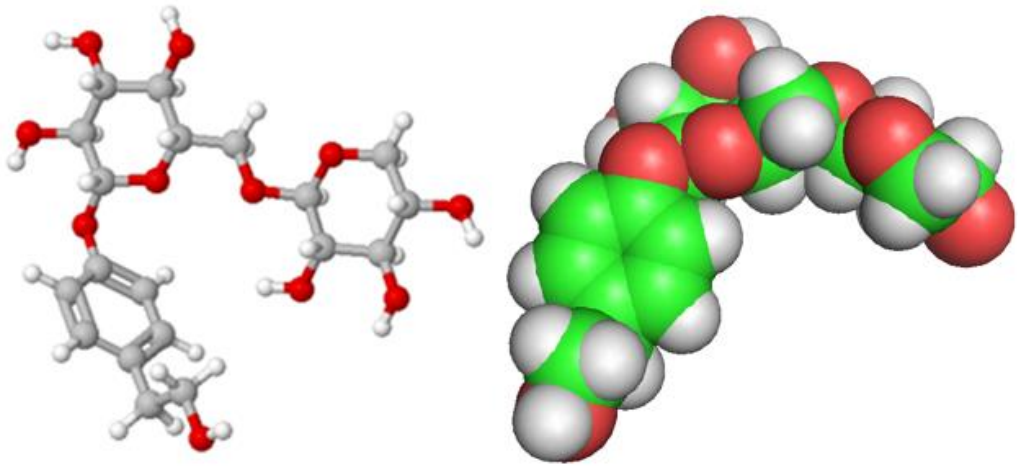


Figure 14. 3D Structure of compound I

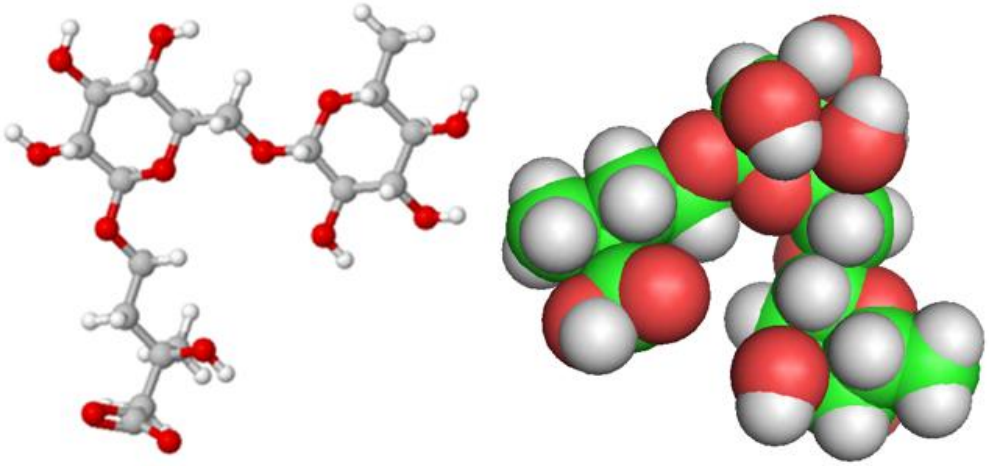


Figure 15. 3D Structure of compound II.

The molecules were subjected to ROCS (Rapid Overlay of Chemical Structures). ROCS generated a list of best 500 hits of similar shape (ShapeTanimoto score) and with similar color (ColorTanimoto score), TanimotoCombo is the sum of the ShapeTanimoto and ColorTanimoto score (157).

ROCS TanimotoCombo = Shape Tanimoto + ColorTanimoto

EON calculates the electrostatic potential of the molecules generated by ROCS and it arranges the hits from ROCS according to their electrostatic similarity for the input compound, with Poisson–Boltzman Electrostatic Tanimoto score and Tanimoto and Poisson–Boltzman score. ET combo is the sum of the two scores.

Electrostatic Taniomoto Combo = Shape Tanimoto + Poisson Boltzman Tanimoto

3.8.3. *In vitro* Studies

BDNF Expression Activity

SH-SY5Y cells were cultured in T-25 flask in DMEM 10% FBS, 1% penicillin- streptomycin and 1% L-glutamine and incubated in 3 mL media in 37 ˚C with 5% CO_2. Cells were passaged when the confluence reach 80% into T-75 flask in 8 mL media (158). After confluence reached 80%, cells were counted by hemocytometer. 3×10^5 cells were seeded in each well in 6 well-plates. In this experiment three different batches of cells were used (n= 3).

After the cells incubated in 6 well-plates for 24 hours, cells were treated with extracts/compounds and 17β-estradiol for 72 hours in a free serum medium containing 2 mL/ well (159). The final concentrations of the extracts were 100 µg/mL; the final concentrations of the pure compounds were 25 µg/mL; the final concentration of the positive control 17β-Estradiol was 10 µM. Three wells were only treated only with the carrier solvent as a Control.

After 48 hours, supernatant was removed and the cells were washed with PBS, then PBS was removed and chemical cell lysis was done by adding 100 µL of cell lysis solution (RIPA Lysis Buffer System) to each well and scraped by cell scraper. Then the mixture was transferred into labeled tube and mixed with vortex for few seconds and incubated for 30 minutes at 4˚C. After incubation, tubes were vortexed again and placed centrifuged at 14000G for 30 minutes at 0˚C.

After tubes were centrifuged, supernatants were transferred into new labeled tubes. Cell lysates were stored at -20˚C until time of use. To assess BDNF expression, BDNF human Elisa Kit was used from Boster's which is a standard sandwich enzyme-linked immune-sorbent technique (Figure 16). The effectiveness of the Elisa Kit was validated by using a control provided by the company. 578 pg of BDNF protein was reconstituted with 1 mL of sample diluent and mixed thoroughly. 100 µL of the mixture was added into the precoated 96-well plate. Instructions on the manual was followed to measure BDNF levels in the cell lysate.

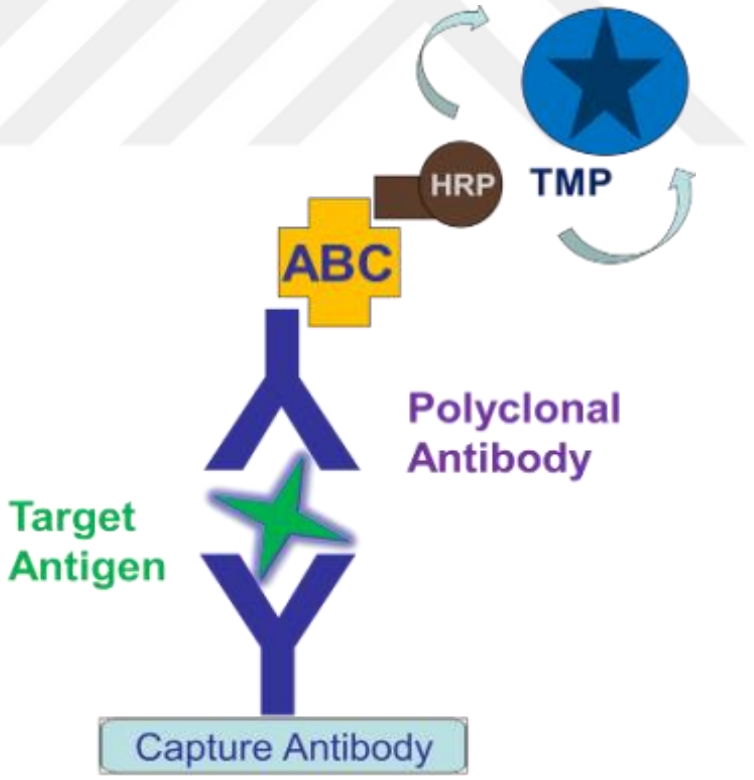


Figure 16. BDNF Elisa Kit Sandwich.

MTT Cytotoxicity Test

To evaluate cytotoxicity of *Papaver* extracts and the pure compounds, MTT Cell Cytotoxicity Assay was performed on HeLa cell line. All the compounds and active extracts were also tested against normal cell line L929 mouse fibroblasts to determine their cytotoxicity. HeLa/L929 cells were cultured in T-25 flask in DMEM 10% FBS, 1% penicillin- streptomycin and 1% L-glutamine and incubated in 3 mL media in 37 ˚C with 5% CO_2. Cells were passaged when the confluence reach 80% into T-75 flask in 8 mL media. After confluence reached 80%, cells were counted by hemocytometer. Cells were seeded in 96 well plates for 24 hours at 37 ◦C at a density of 1×10^4 cells per well. After 24 hours the cells were treated with extracts/compounds/drugs in a free serum medium containing 100 µL/well and incubated for 72 hours. Cisplatin was used as a positive control. After 72 hours 10 µL of (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (MTT) solution (25 mg/mL) was added to each well and incubated for 4 hours at 37 ˚C. Supernatants were removed and 100 µL of DMSO was added into each well and incubated again for 10 minutes. Color was measured with a 96-well plate reader at 450 nm. In this experiment three different batches of cells were used (n= 3). Cell viability percentages and IC_{50} values were calculated (160).

Cell viability (%) = OD treated/ OD control ×100

Colony Formation Assay

Colony formation assay is a cell survival assay established to assess the ability of a single cell to grow and form a colony. Colony is considered when it contains at least 50 cells (161). After culturing the cells, 1000 cells were seeded into each well in 6-well plates, with each sample as triplicate. After 9 days when cells formed colonies, cells were fixed with (70:30 MeOH: Acetic acid) for half an hour on bench. After fixation, MeOH:acetic acid solution was removed from each well and 1 mL of 0.2% crystal violet (v/v in water) was added into each well.

Plates were left to stain for half an hour on the bench. Stain was poured off and wells were washed with milliQ water to get rid of the non-specific staining. Wells were left to dry on bench and later the colonies were counted.

α-Glucosidase Inhibitory Assay

SPiDER Target Prediction Software predicted that compound I might targets glucosidase enzyme. To evaluate compound I activity, *α*-glucosidase inhibitory assay was used following *Watanabe et al*. (162), with slight modifications (163). *α*-glucosidase from yeast (0.7 unit) was liquefied in 100 mM (pH 7.0) phosphate buffer with sodium azide (0.2 g/L) and bovine serum albumin (2 g/L). *p*-nitrophenyl-*α*-D-glucopyranoside 5 mM was prepared as a substrate solution in (pH 7.0) phosphate buffer. Sample (20 µL) and enzyme solution (100 µL) were mixed and incubated for 5 minutes at 37˚C, afterward (100 µL) substrate solution was added to the mixture and incubated for 5 minutes at 37˚C, and the absorbance at 405 nm was read (10 minutes).

The concentrations of the *Papaver lacerum* extract were 5, 2.5, 1, 0.5, 0.25 and 0.1 µg/mL, the concentrations of compound I were 2.5, 1, 0.5, 0.25, 0.1and 0.05 µg/mL dissolved in DMSO, compared to the control (100 µL of DMSO). The inhibitory activity was calculated in percentage of inhibition.

$$\% \text{ inhibition} = \frac{Abs_{405nm}\text{ (control) - Abs405nm (extract/compound I)}}{\text{Abs405nm (control)}} \times 100$$

Statistic Analysis

Results were presented as mean ± SEM (n=3). Differences among groups were compared using one way analysis of variance (One-way ANOVA and non-parametric), followed by Tukey testing for multiple comparisons using GraphPad Prism program. *p* values of 0.05 or less were regarded as significant.

4. RESULTS

4.1. Structure Elucidation of the Compounds Isolated from *P. lacerum*

Phytochemical investigation on the *Papaver lacerum* resulted in isolation of two compounds.

4.1.1. Compound I

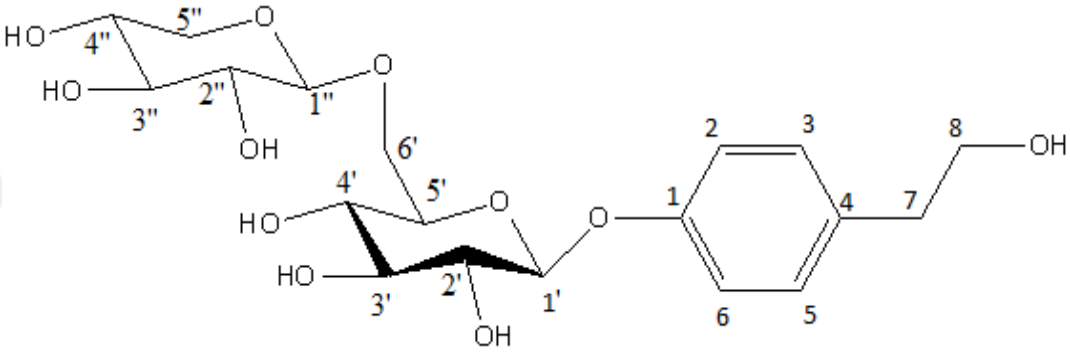


Figure 17. Structure of compound I.

Tyrosol-1-*O*-*β*-xylopyranosyl-(1→6)-*O*-*β*-glucopyranoside (TXG) (164)

$C_{19}H_{28}O_{11}$

(Molecular weight: 432.671 g/mol)

UV absorptions	223 and 273 nm
ESI-MS (*m/z*)	$[M+Na]^+$ 455.671
^{1}H NMR	Figures 18, 19. Table 14.
^{13}C NMR	Figures 20, 21. Table 14.
COSY	Figures 22, 23, 24.
HSQC	Figures 25, 26.
HMBC	Figures 27, 28, 29

Table 14. Compound I (TXG) in ^{1}H-NMR and ^{13}C- NMR spectral values (DMSO-d6, ^{1}H: 400 MHz, ^{13}C: 100 MHz) (δ in ppm, J in Hz).

No.	Carbon type	δ_C	δ_H / J
Aglycon			
1	C	156.17	-
2	CH	116.56	6.94 (1H, d, J = 8.5)
3	CH	130.12	7.09 (1H, d, J = 8.5)
4	C	133.13	-
5	CH	130.12	7.09 (1H, d, J = 8.5)
6	CH	116.56	6.94 (1H, d, J = 8.5)
7	CH_2	38.67	2.64 (2H, t, J = 7.02)
8	CH_2	62.8	3.52 (2H, t, J = 6.71)
Glucose			
1'	CH	101.12	4.71 (1H, d, J = 7.2)
2'	CH	73.66	3.19 (1H, m)
3'	CH	76.96	3.22 (1H, m)
4'	CH	70.03	3.14 (1H, dd, J = 8.73, 9.68)
5'	CH	76.88	3.46 (1H)*
$6'_a$	CH_2	68.60	3.91 (1H, dd, J = 1.19, 0.94)
$6'_b$			3.52 (1H, t, J = 0.45)
Xylose			
1"	CH	104.27	4.16 (1H, d, J = 7.4)
2"	CH	73.94	2.94 (1H, dd, J = 8.21, 1.096)
3"	CH	76.96	3.06 (1H, t, J = 8.63)
4"	CH	70.12	3.22 (1H, m)
$5''_a$	CH_2	66.07	3.64 (1H, dd, J = 5.3, 5.3)
$5''_b$			2.90 (1H)*

* Signals were overlapping

Compound I was isolated as a colorless powder. ESI-Mass exhibited an M+23 ion at m/z 455.671 suggesting the molecular formula $C_{19}H_{28}O_{11}$. The ^{1}HNMR signals revealed that compound 1 contains a para-substituted aromatic ring [δ_H 7.09 (2H, d, *J* = 8.5 Hz, H-3 and H-5) and 6.94 (2H, d, J = 8.5 Hz, H-2 and H-6)], and aliphatic protons [δ_H 2.64 (2H, t, *J* = 7.02 Hz, H-7) and 3.52 (2H, t, *J* = 6.71 Hz, H-8)] in the aglycon part (Table 14) and it was supported by the ^{13}C-NMR signals at δ 156.17 (C-1), 133.13 (C-4), 130.12 (C-3 and C-5), and 116.56 (C-2 and C-6), 38.67 (C-7) and 62.8 (C-8) (164) (Figure 20, 21). On the other hand ^{1}H spectrum illustrates the presence of two anomeric protons attributed to *β*-glucopyranoside and *β*-xylopyranosyl respectively at δ_H 4.71 (1H, d, *J* = 7.2 Hz, H-1′) and 4.16 (1H, d, *J* = 7.4 Hz, H-1″) (Figure 18, 19). The two anomeric protons correlated to the carbons at δ 101.12 (C-1′) and 104.27 (C-1″) respectively. In the HSQC spectrum (Figure 26). The inner sugar was *β*-glucopyranose [δ_H 4.71 (1H, d, *J* = 7.4 Hz, H-1′), 3.19, 3.22 (2H, m, H-2′ and H-3′), 3.14 (1H, dd, *J* = 8.73, 9.68 Hz, H-4′), 3.46 (1H, H-5′), 3.91 (1H, dd, 1.19, 0.94 Hz, H-6′a) and 3.52 (1H, t, *J* = 0.45 Hz, H-6′b) (Figure 19); δ_C δ 101.12 (C-1′), 73.66 (C-2′), 79.96 (C-3′), 70.03 (C-4′), 76.88 (C-5′) and 68.60 (C-6′)] (Figure 21). The outer sugar unit was *β*-xylopyranose [δ_H 4.16 (1H, d, *J* = 7.4 Hz, H-1″), 2.94 (1H, dd, *J* = 8.21, 1.09 Hz, H-2″), 3.06 (1H, t, *J* = 8.63 Hz, H-3″), 3.22 (1H, m, H-4″), 3.91 and 3.64 (1H, dd, *J*= 5.3, 5.3 Hz, H-5″a); δ_C 104.27 (C-1″), 73.94 (C-2″), 76.96 (C-3″), 70.12 (C-4″), 66.07 (C-5″)]. The correlation between H-1″ and C 6′ (δ 68.81) confirmed that *β*-xylopyranose, is connected to the *β*-glucopyranose from C 6′. In the HMBC spectrum (Figure 28) the correlation between H-1′ and C-1 (δ 156.17) suggested that *β*-glucopyranose is connected to the A2B2 ring. Also H-2 correlated to C 6 (δ 116.56) and H-4 correlated to C 5 (δ 130.12). Thus, the structure of compound I was confirmed to be tyrosol-1-*O*-*β*-xylopyranosyl-(1→6)-*O*-*β*-glucopyranoside with the molecular formula of $C_{19}H_{28}O_{11}$ (164).

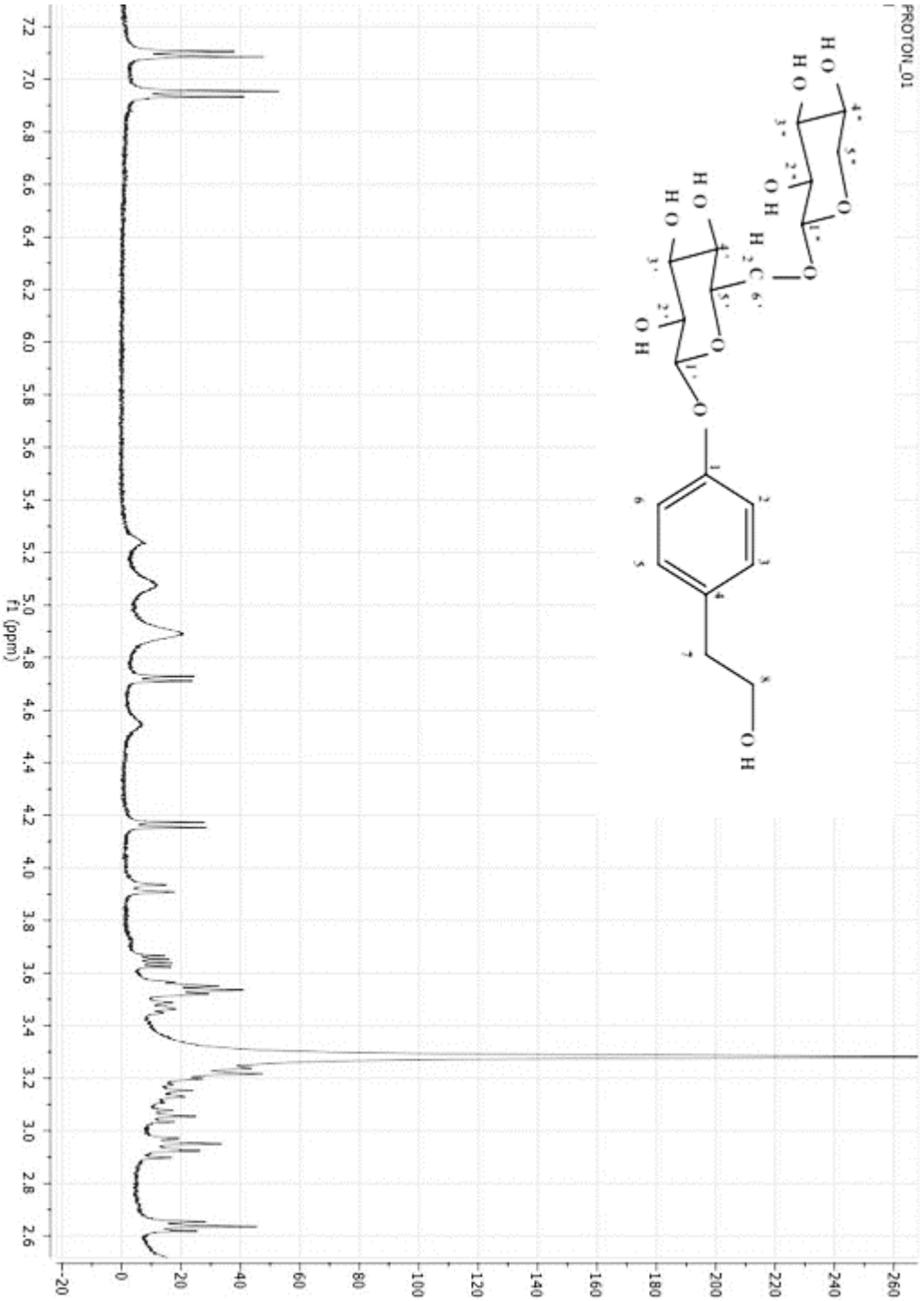


Figure 18. Compound I (TXG) ^{1}H-NMR spectrum (DMSO-d6, ^{1}H: 400 MHz) (Part I).

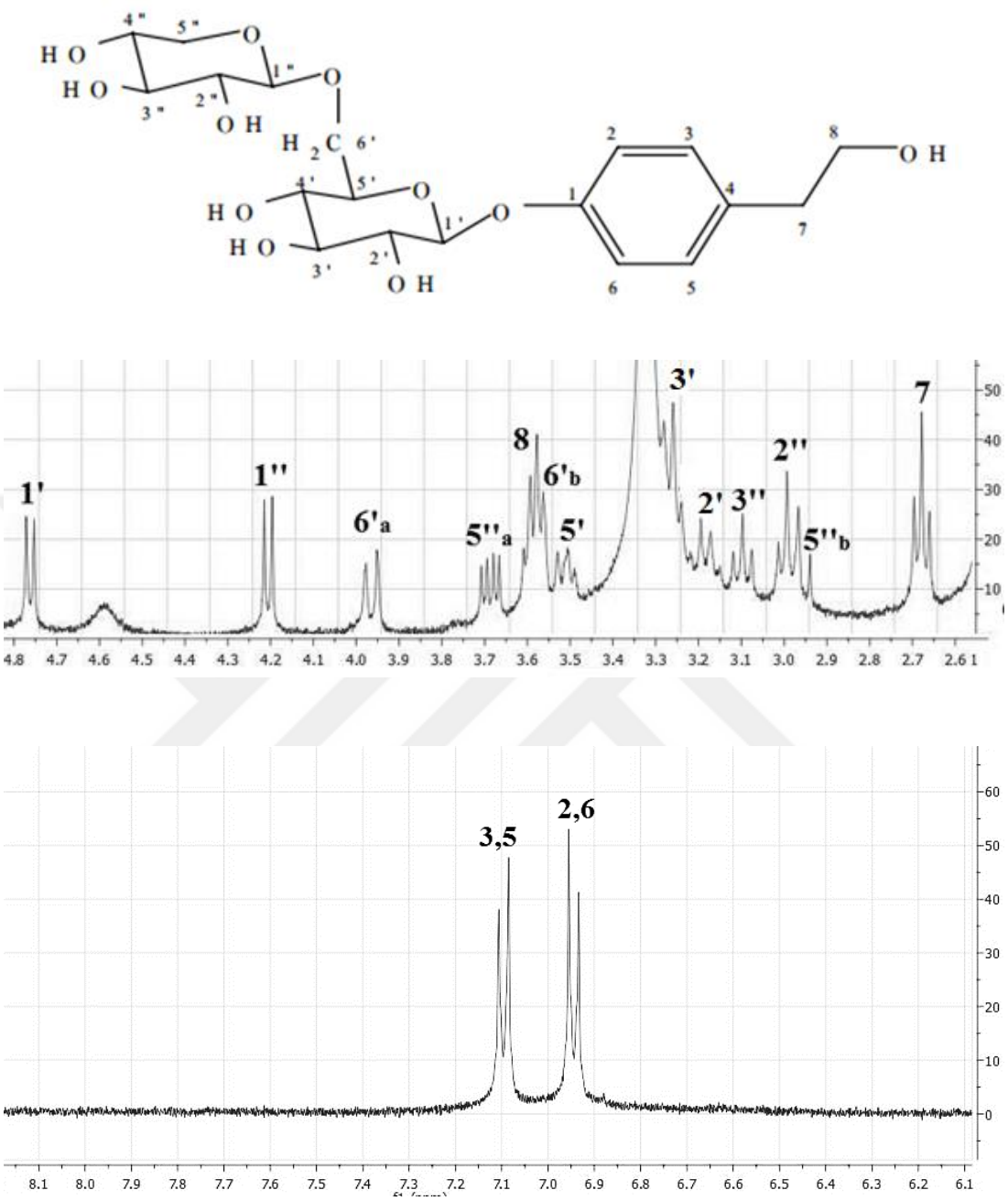


Figure 19. Compound I (TXG) ^{1}H-NMR spectrum (Part II)

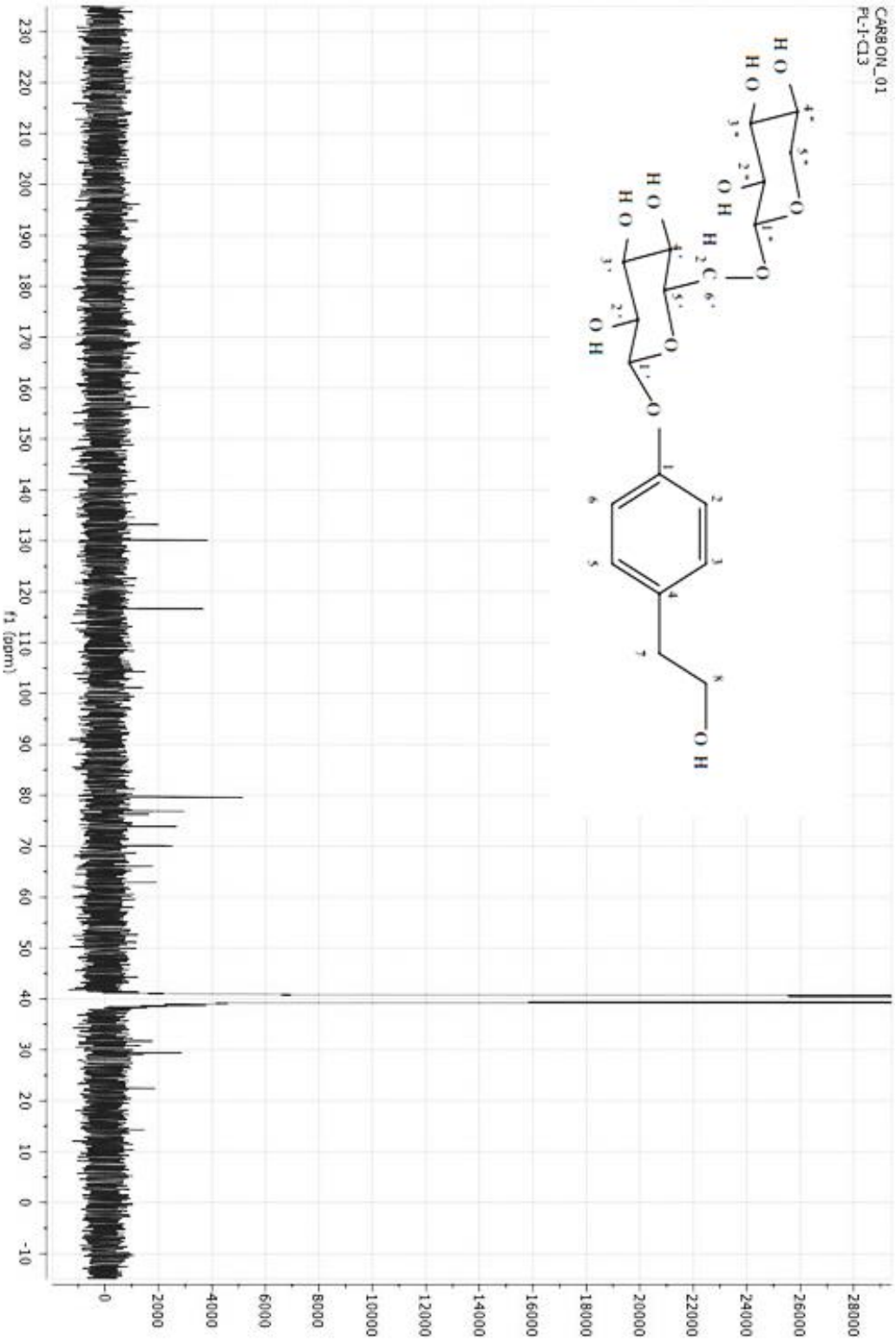


Figure 20. Compound I (TXG) ^{13}C NMR spectrum (DMSO-d6, ^{13}C: 100 MHz) (Part I).

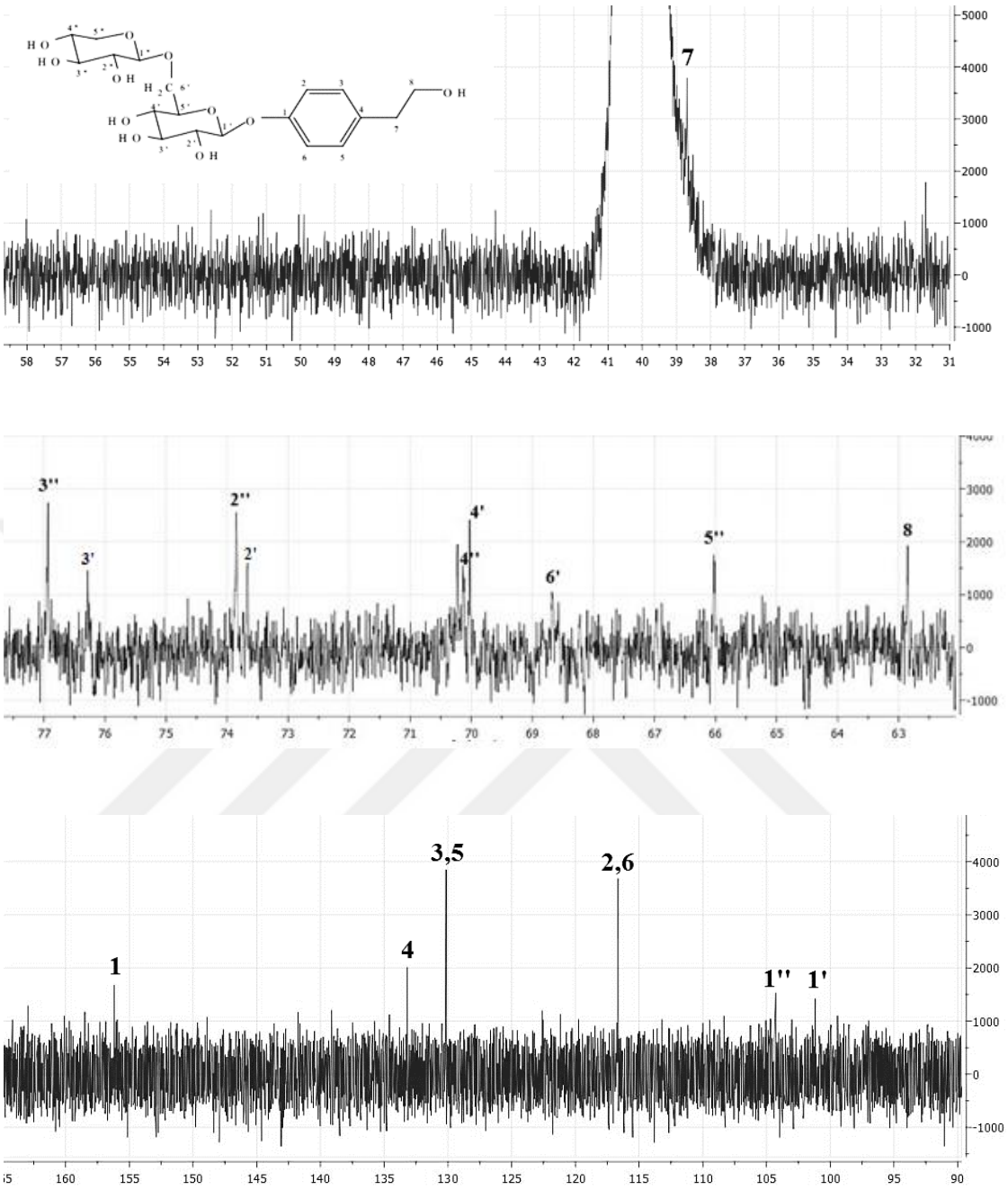


Figure 21. Compound I (TXG) ^{13}C NMR spectrum (Part II).

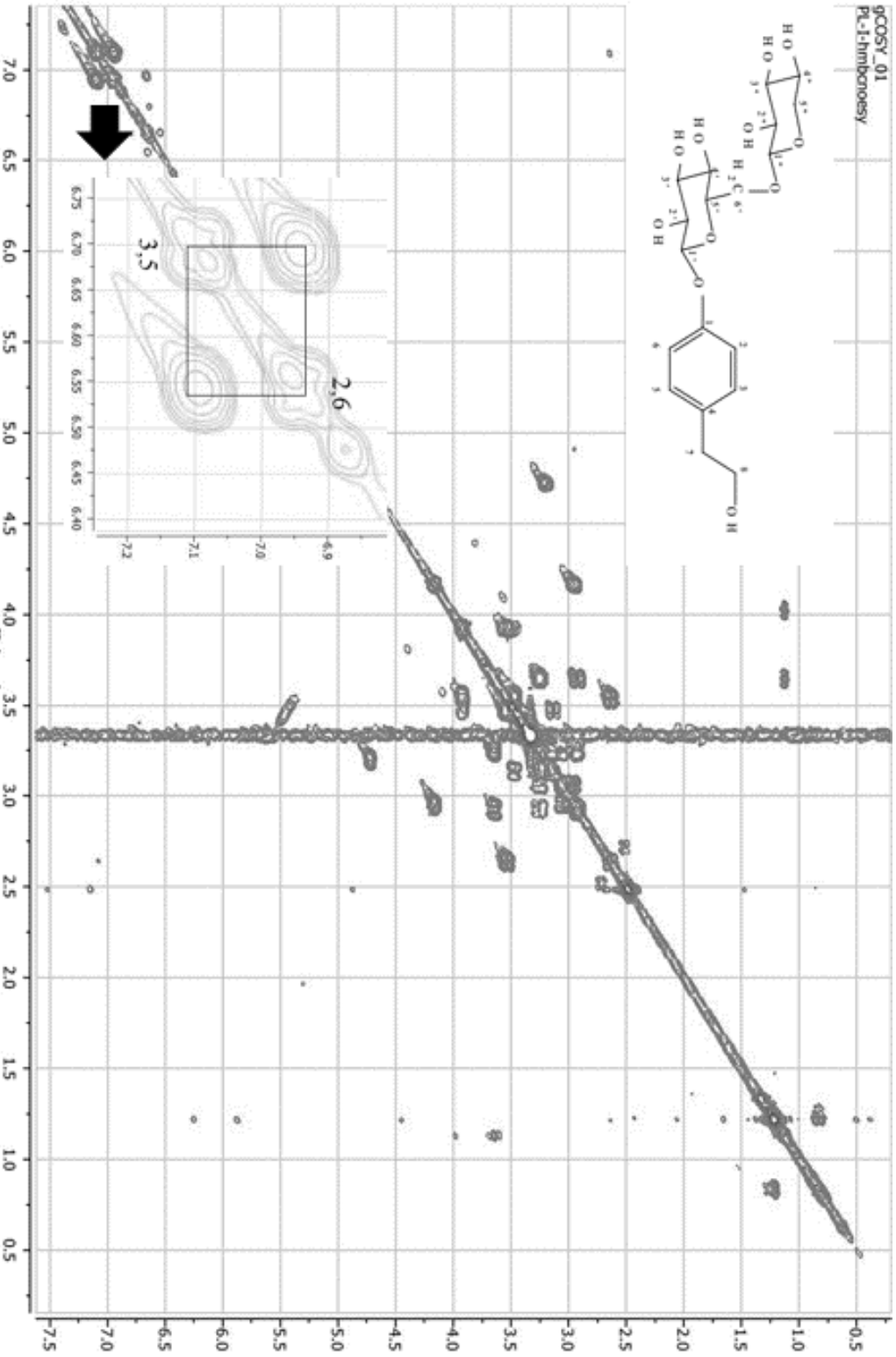


Figure 22. Compound I (TXG) 2D ^{1}H, ^{1}H homonuclear correlation spectroscopy (COSY) (Part I).

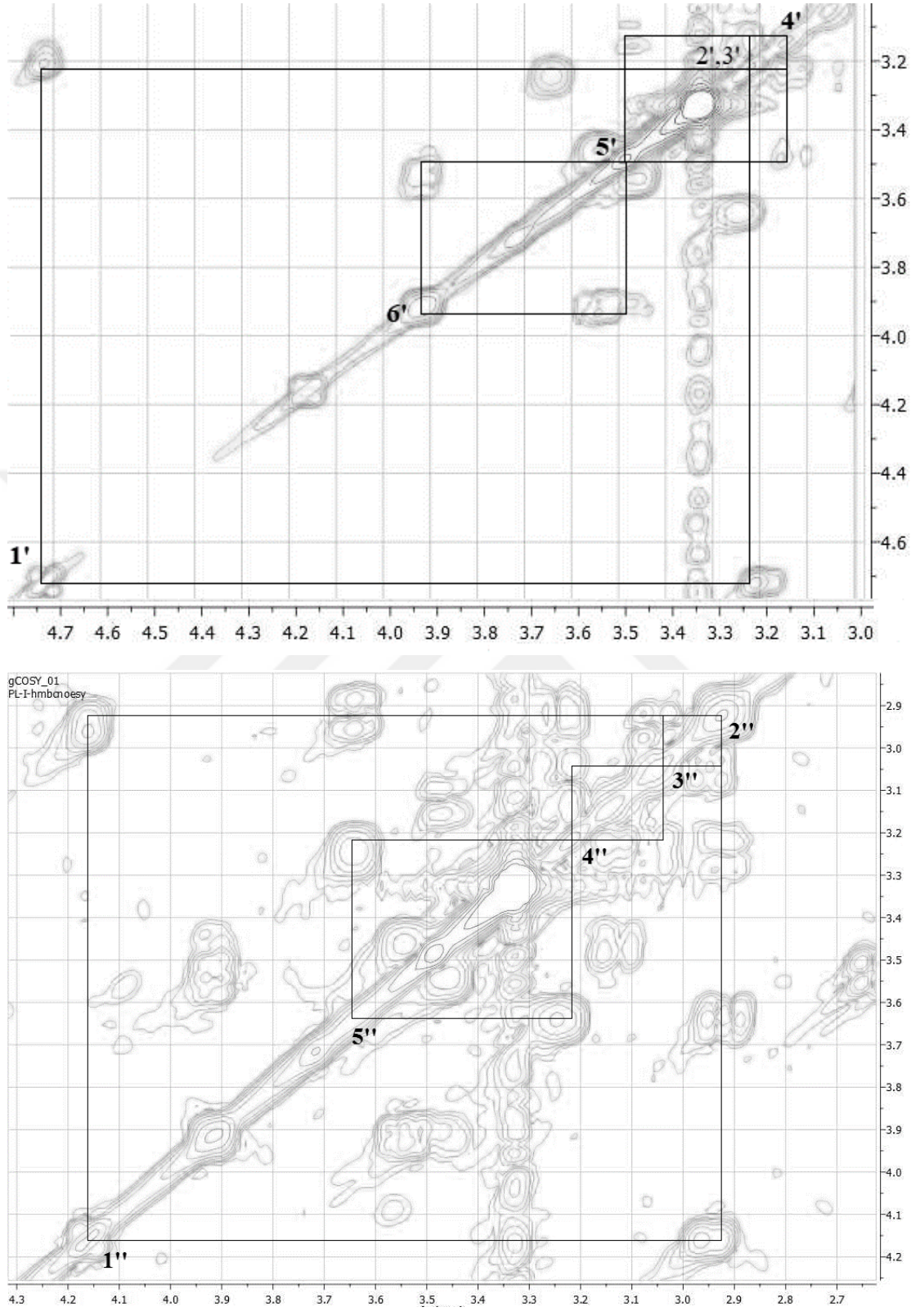


Figure 23. Compound I (TXG) 2D COSY (Part II).

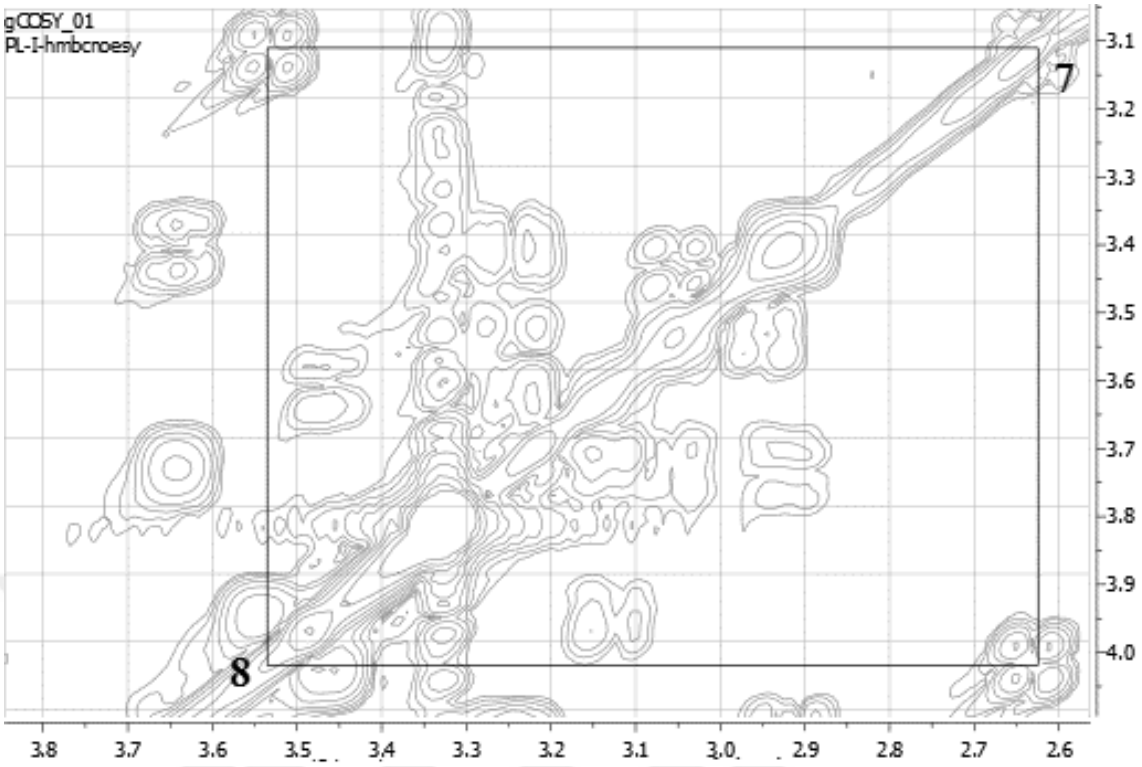


Figure 24. Compound I (TXG) 2D (COSY) (Part III).

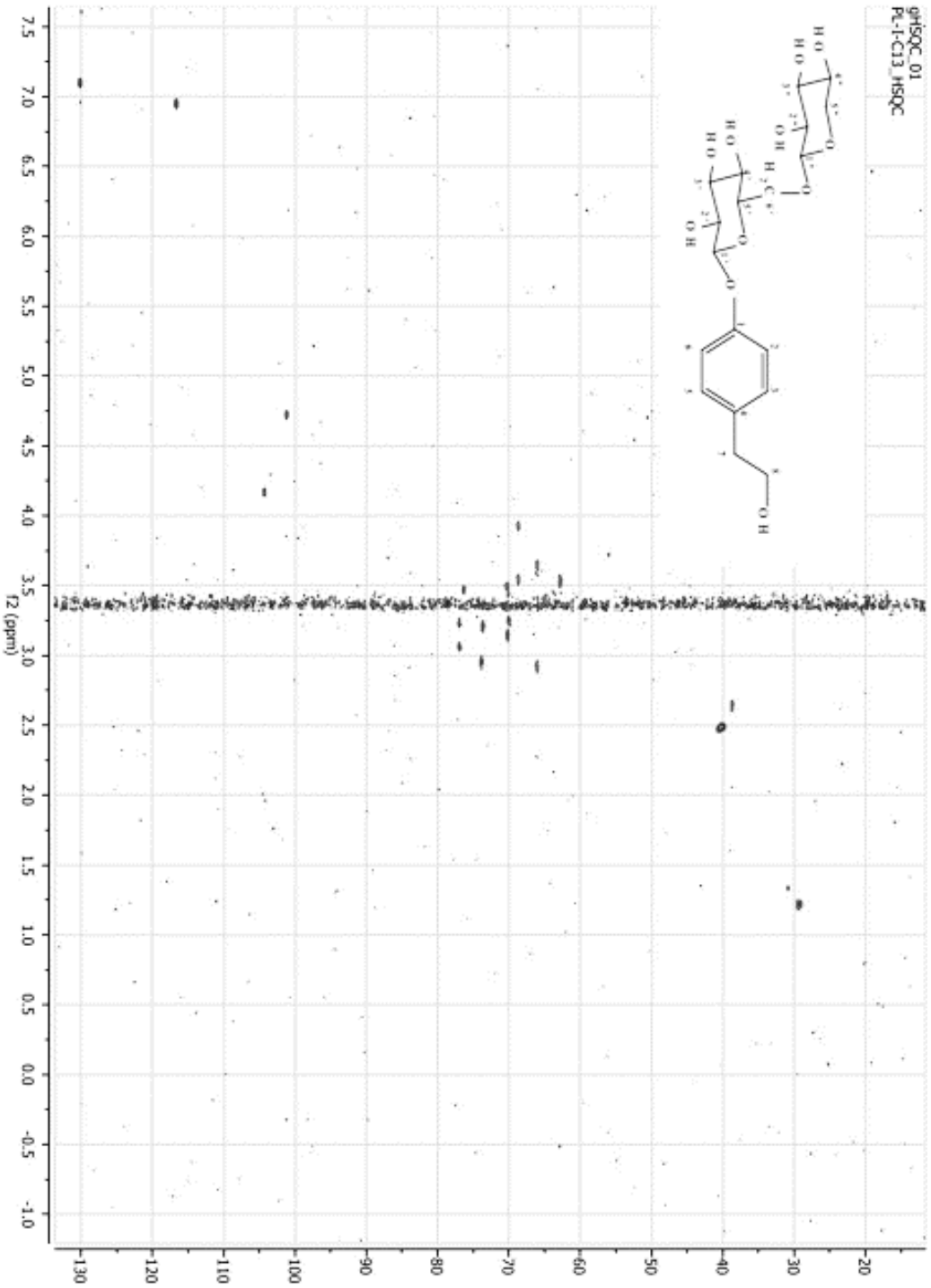


Figure 25. Compound I (TXG) 2D ^{1}H, ^{13}C heteronuclear single quantum coherence (HSQC) (Part I).

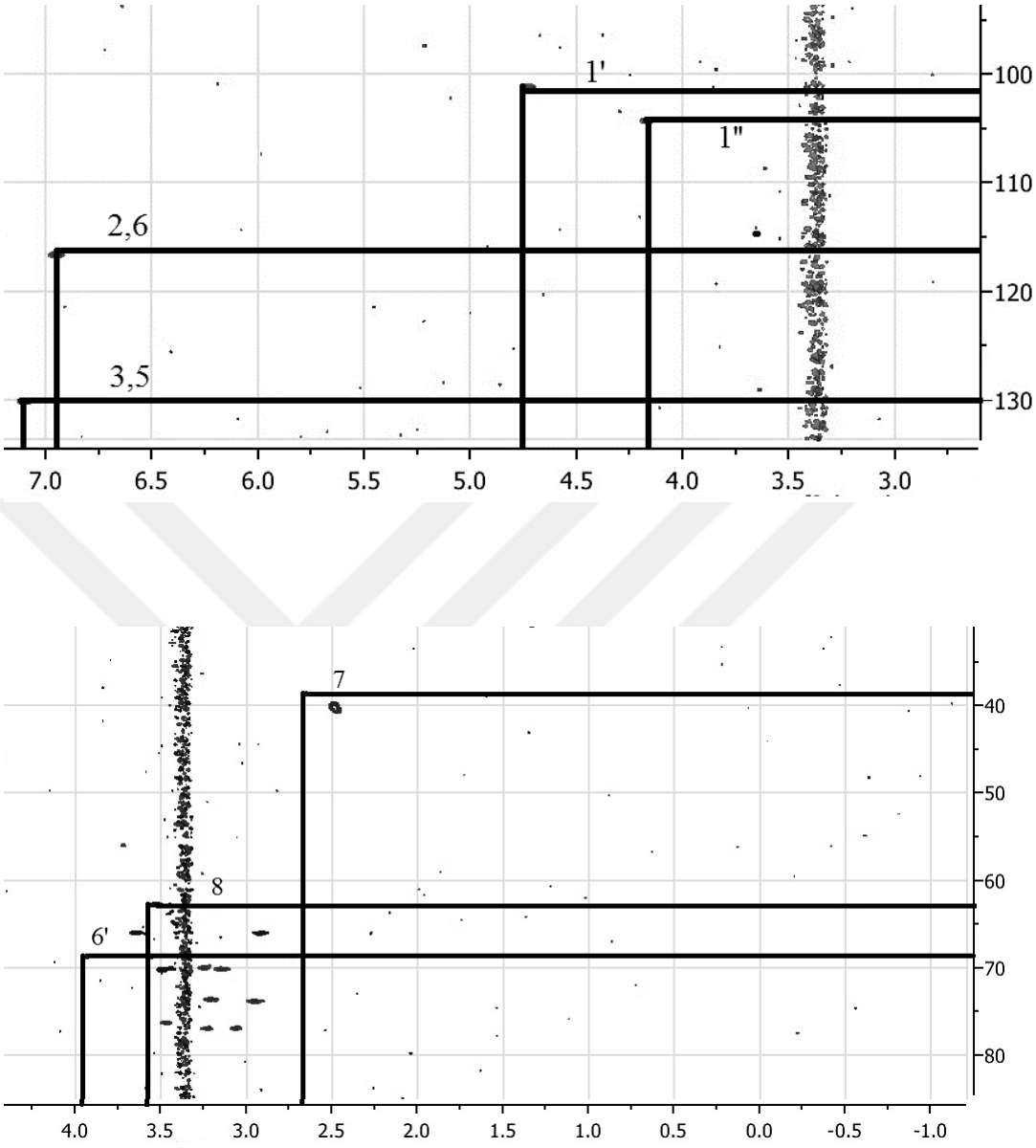


Figure 26. Compound I (TXG) HSQC (Part II).

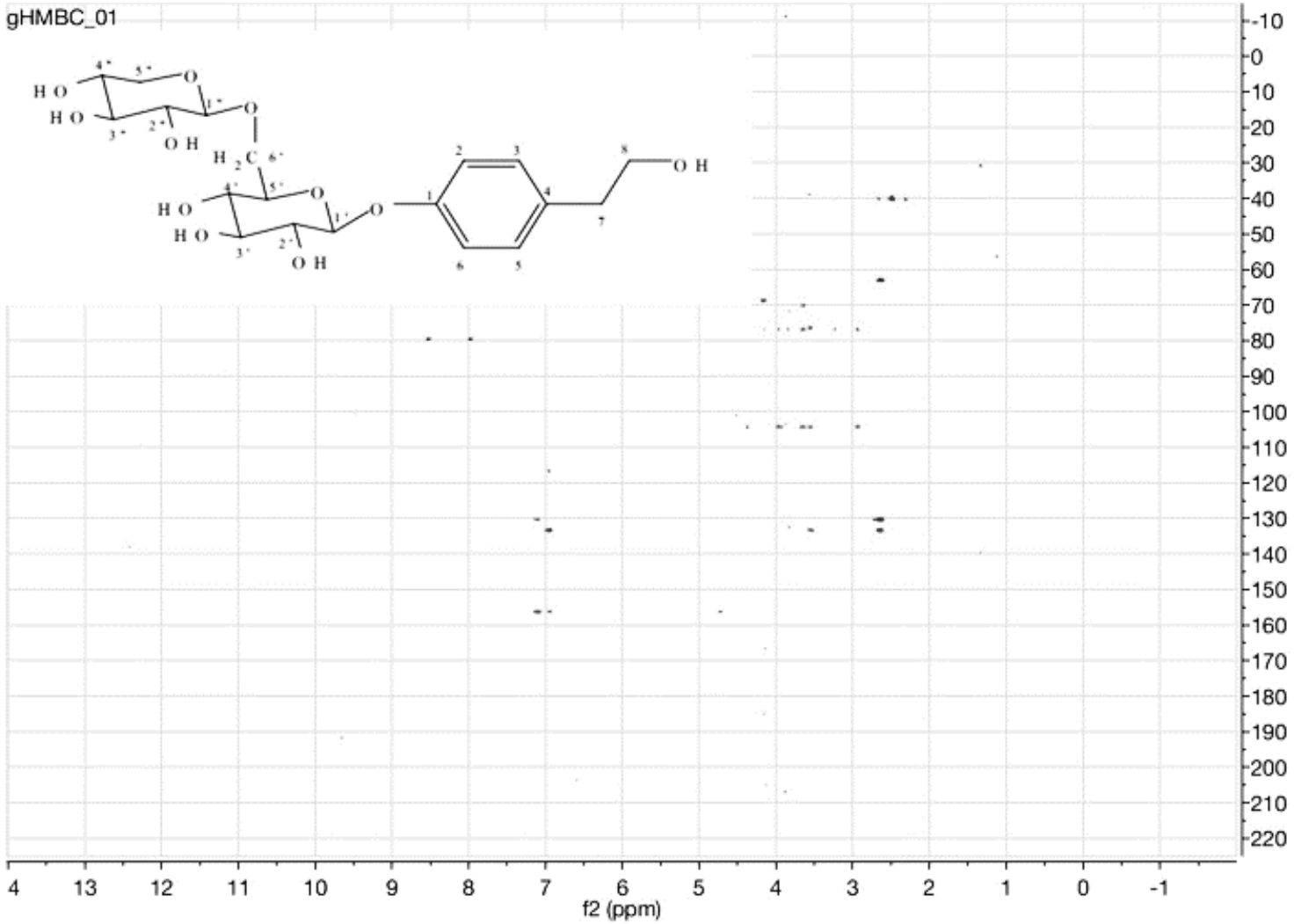


Figure 27. Compound I (TXG) 2D ^{1}H, ^{13}C Heteronuclear multiple-bond correlation spectroscopy (HMBC) (Part I).

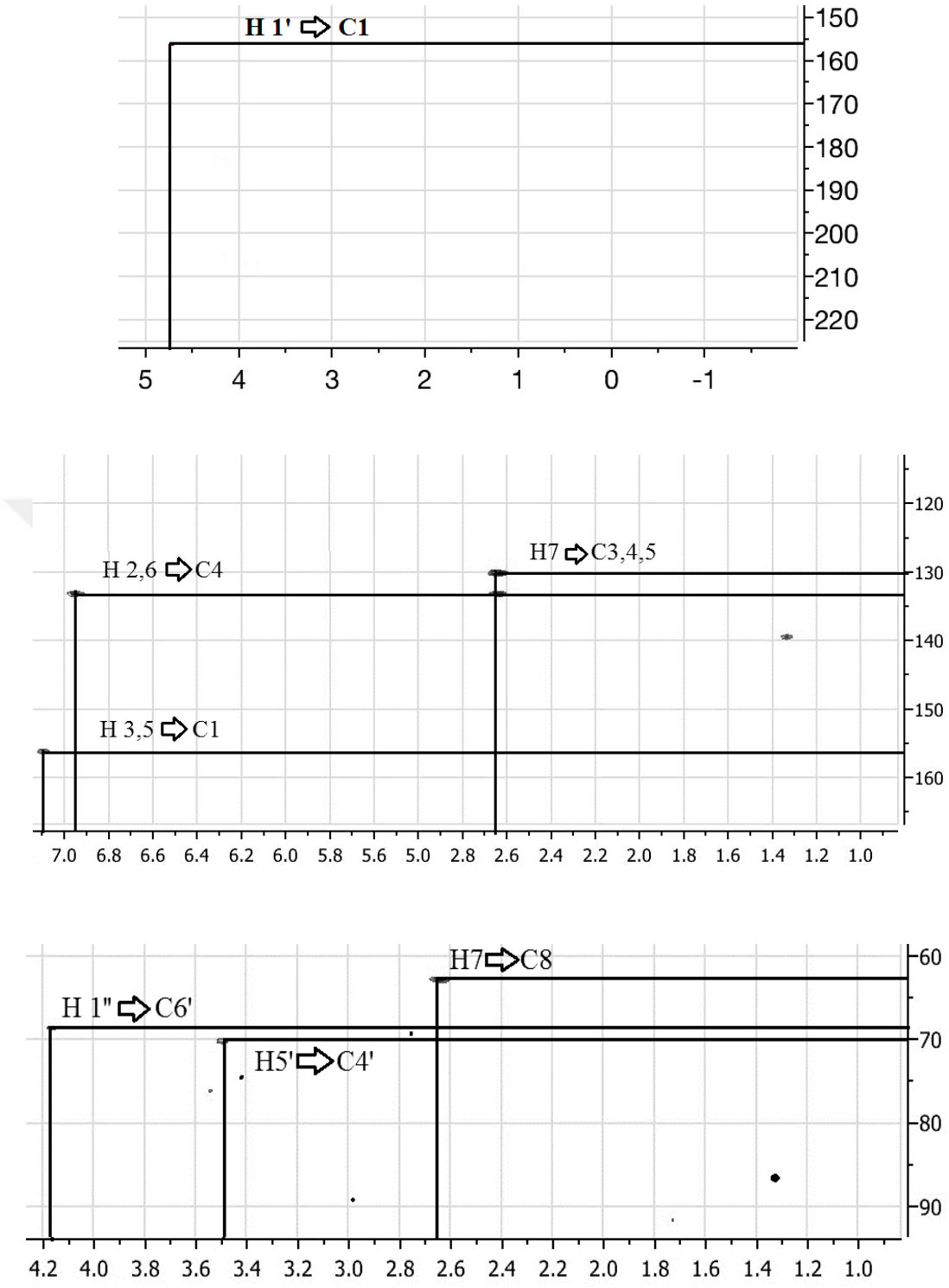


Figure 28. Compound I (TXG) HMBC (Part II).

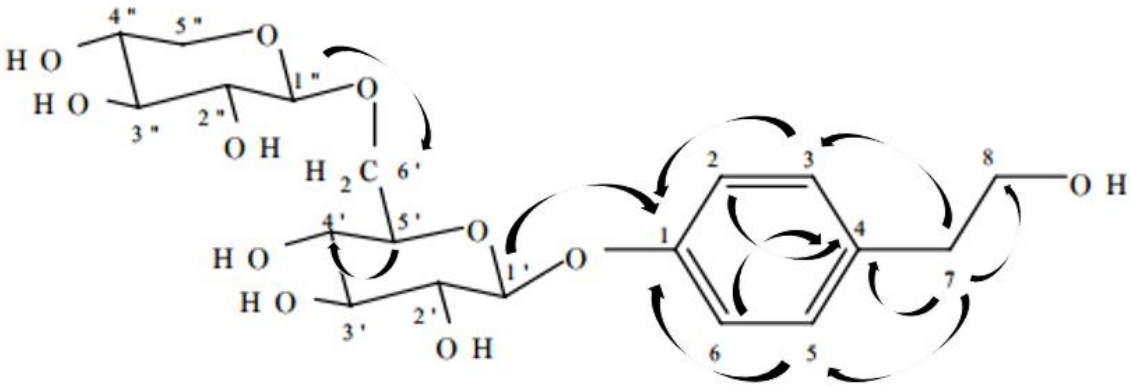


Figure 29. Compound I (TXG) HMBC correlation map.

4.1.2. Compound II

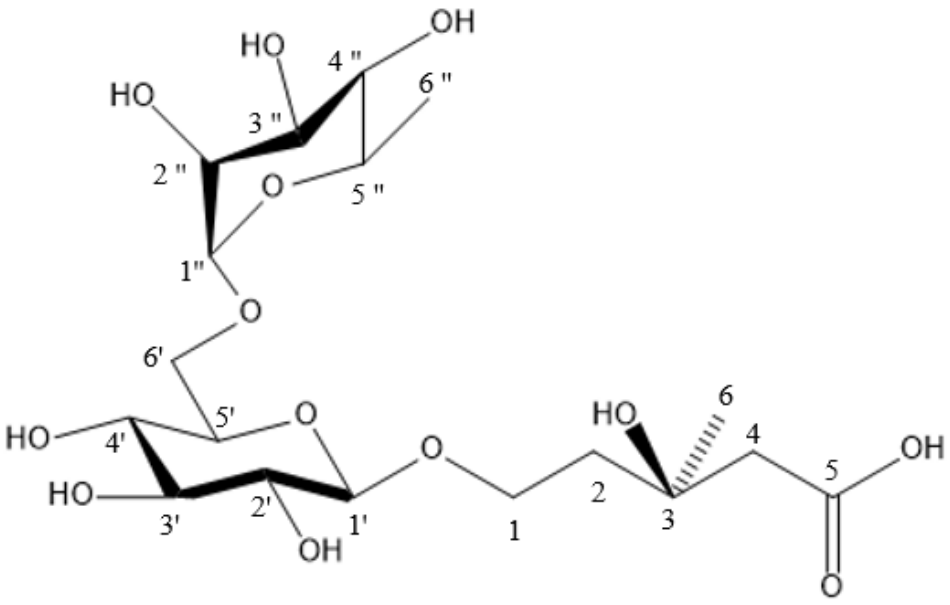


Figure 30. Structure of compound II.

5-*O*-(6-*O*-*α*-rhamnopyranosyl-*β*-glucopyranosyl) mevalonic acid (RGM)

$C_{18}H_{33}O_{13}$

(Molecular weight: 455.18)

UV absorptions	223 and 273 nm
HR-MS (*m/z*)	$[M+Na]^+$ 478.1872
^{1}H NMR	Figures 31, 32. Table 15.
^{13}C NMR	Figures 33, 34. Table 15.
COSY	Figures 35, 36, 37.
HSQC	Figures 38, 39.
HMBC	Figures 40, 41, 42.

Table 15. Compound II (MRG) in ^{1}H-NMR and ^{13}C- NMR spectral values (DMSO-d6, ^{1}H: 400 MHz, ^{13}C: 100 MHz) (δ in ppm, J in Hz).

No.	Carbon type	δ_C	δ_H / J
Aglycon			
1_a	CH_2	66.5	3.79 (1H, d, J = 9.94)
1_b			3.40 (1H, m)
2_a	CH_2	42.2	1.63 (1H, m)
2_b			1.53 (1H, m)
3	C	69.46	-
4_a	CH_2	47.3	2.07 (1H, d, J = 14.78)
4_b			1.90 (1H, d, J = 14.79)
5	C	177.9	-
6	CH_3	29.1	1.01 (3H, s)
Glucose			
1'	CH	103.8	4.02 (1H, d, J = 7.64)
2'	CH	73.8	2.89 (1H, m)
3'	CH	76.7	3.13 (1H, m)
4'	CH	70.78	3.42 (1H, m)
5'	CH	75.8	3.21 (1H, m)
$6'_a$	CH_2	67.8	3.79 (1H, d, J = 9.94)
$6'_b$			3.38 (1H, m)
Rhamnose			
1"	CH	101.8	4.56 (1H, d J = 1.01)
2"	CH	69.6	3.62 (1H, dd, J = 1.44/3.11)
3"	CH	68.9	3.42 (1H, m)
4"	CH	72.5	3.13 (1H, m)
5"	CH	70.96	2.92 (1H, m)
6"	CH_3	17.9	1.11 (3H, d, J = 6.29)

The compound II was isolated as a yellowish amorphous powder which has a molecular formula of $C_{18}H_{33}O_{13}$, pointed out by the [M+Na]+ ion peak at m/z 478.1872 in the HR-MS. When applied to the TLC plate, it appeared yellow in daylight and showed slight whitish fluorescence at UV-366 nm. When vanilin / H_2SO_4 was sprayed, it became yellow. In the ^{1}H-NMR spectrum (Figure 31, 32) there are two anomeric carbons at δ_H 4.02 (d, J = 7.64, H1') and δ_H 4.56 (d J = 1.01, H1"), which later attributed to β-glucose and α-rhamnose. Other protons belonging to sugar moieties appeared between δ_H 2.89 H2'and δ_H 3.79, H6'a for glucose and between δ_H 1.11, H6" and 3.62, H2" for rhamnose. Besides the glycose signals ^{13}C-NMR spectrum revealed that there is one $-CH_3$, three $-CH_2$ and two quaternary carbons, one of which is a carboxylic acid carbonyl at δ_C 177.9 and the other one is an oxygenated carbon at δ_C 69.46 (Figure 33, 34). Furthermore, down shielded CH_3 δ_C 29.1 in the ^{13}C-NMR spectrum and strong HMBC correlations from CH_3 to oxygenated carbon at δ_C 69.46 suggested that CH_3 was substituted at C-3. One of the methylene carbons was shifted quite downfield (C-1 δ_C 66.5) and HMBC correlations between anomeric proton of glucose to C-1 implied the glycosidation point of the structure (Figure 41). HMBC and COSY correlations lead the mevalonic acid structure as the aglycone part (Figure 35, 36). Therefore the structure of compound II was determined as a new compound 5-*O*-(6-*O*-α-rhamnopyranosyl-β-glucopyranosyl) mevalonic acid.

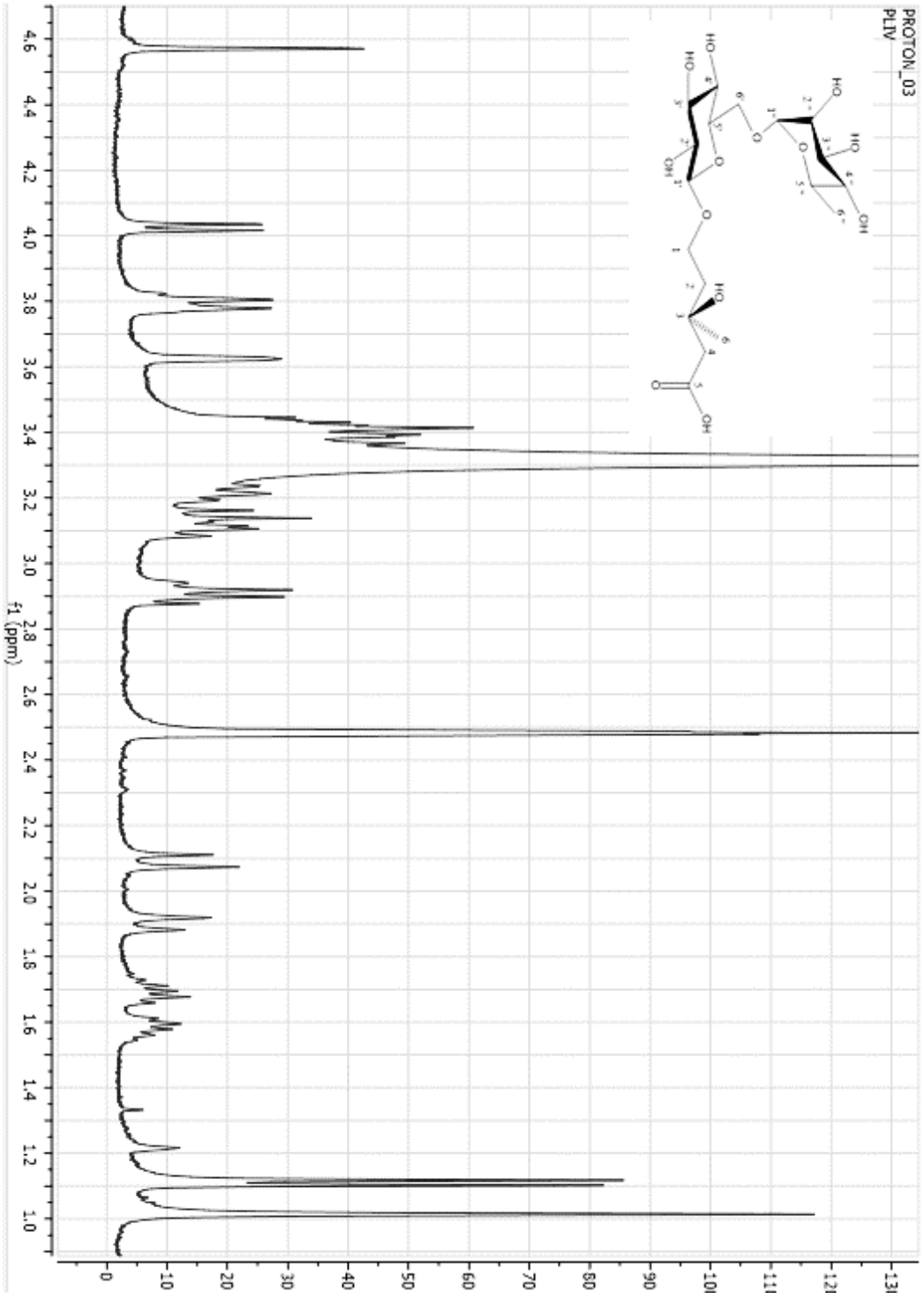


Figure 31. Compound II (MRG) ^{1}H-NMR spectrum (DMSO-d6, ^{1}H: 400 MHz) (Part I).

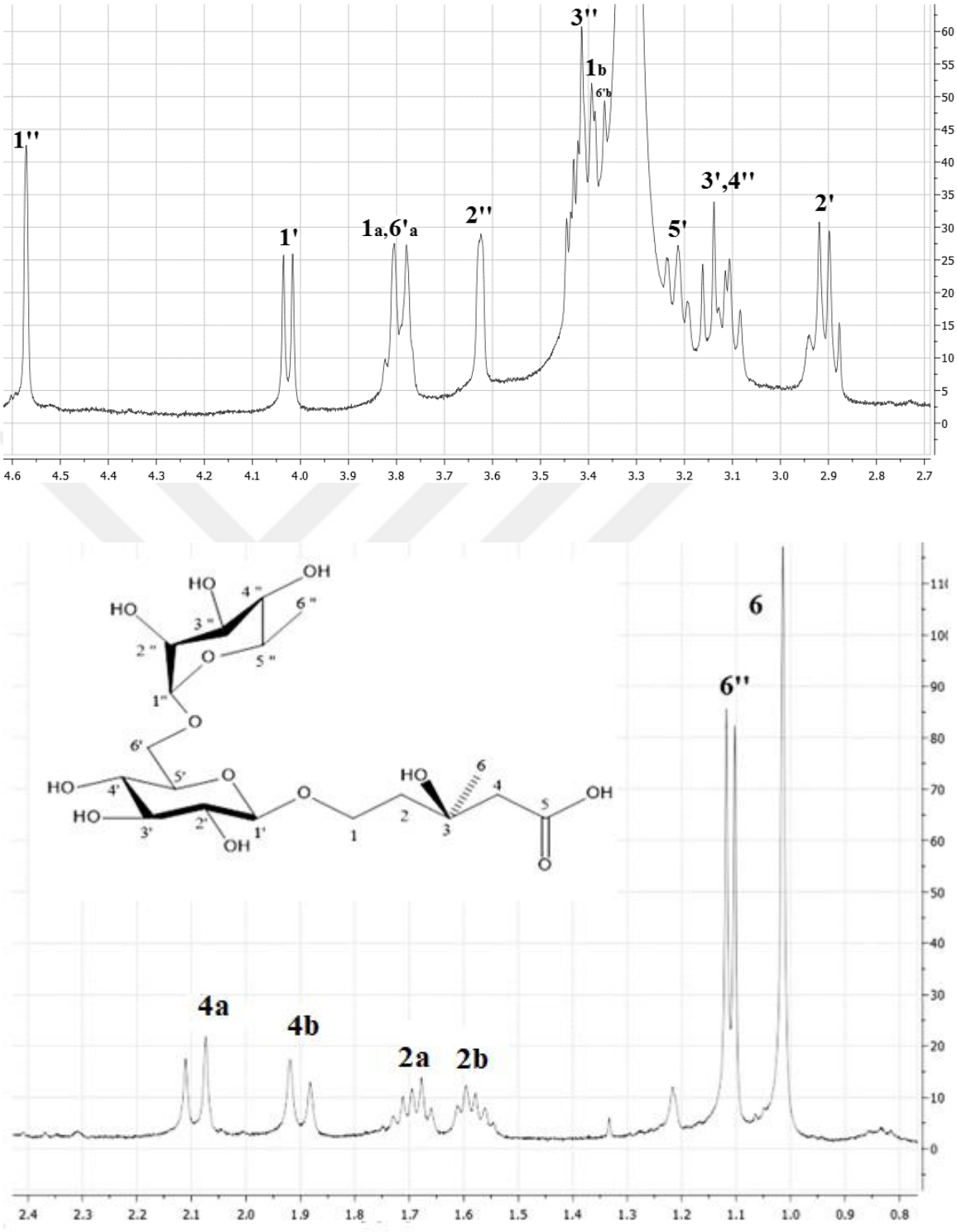


Figure 32. Compound II (MRG) ^{1}H-NMR spectrum (Part II)

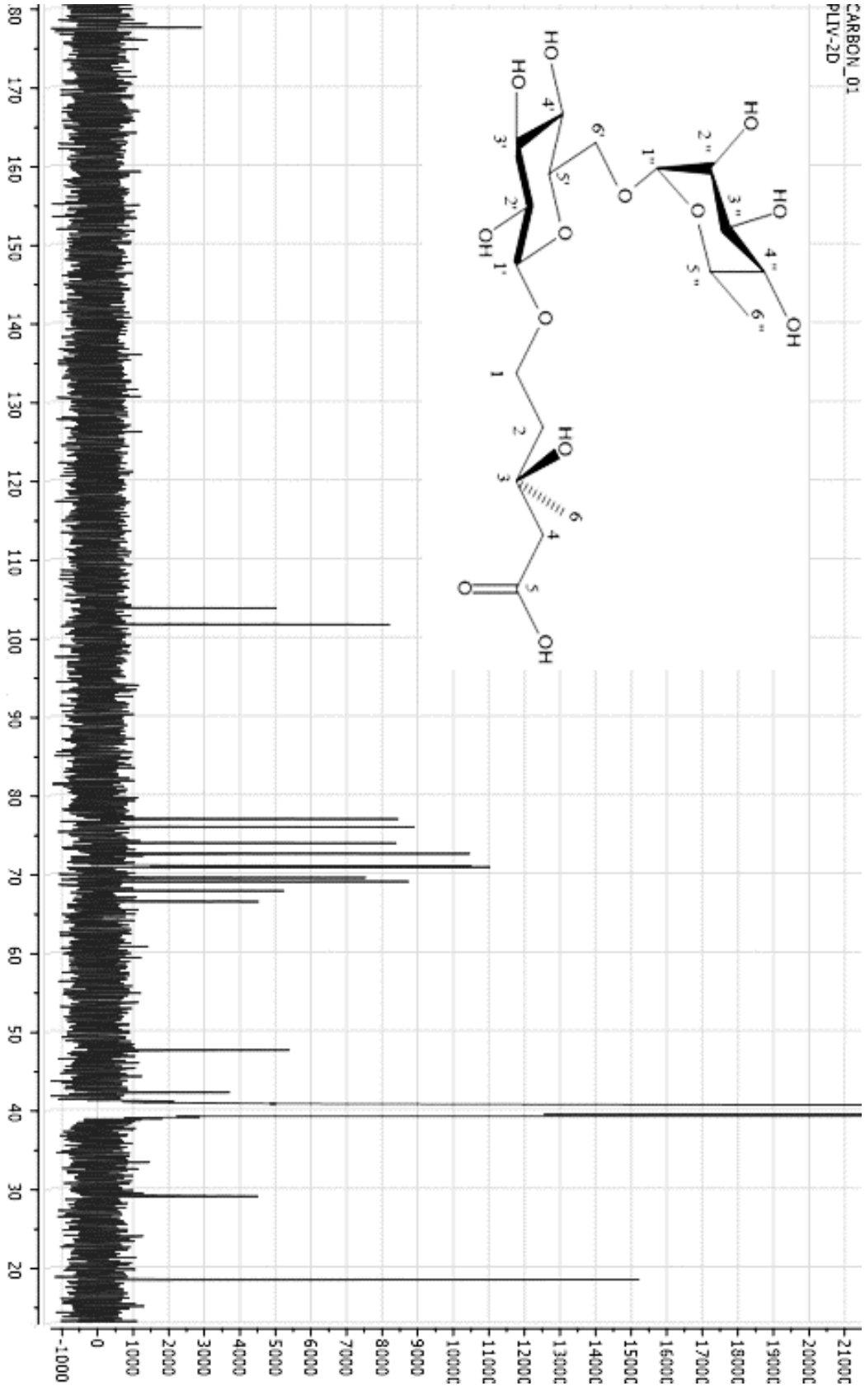


Figure 33. Compound II (MRG) ^{13}C NMR spectrum (DMSO-d6, ^{13}C: 100 MHz) (Part I).

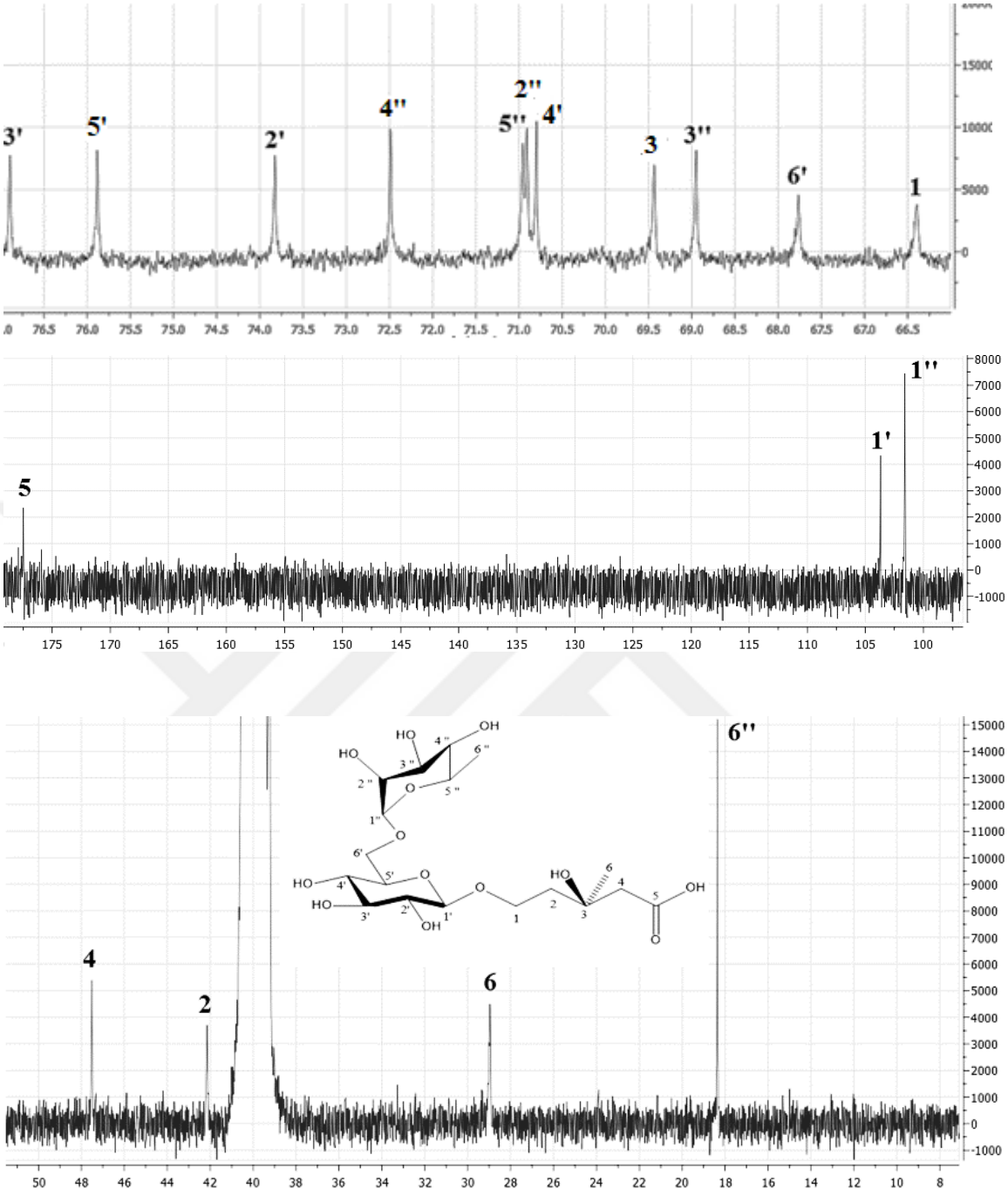


Figure 34. Compound II (MRG) ^{13}C NMR spectrum (Part II)

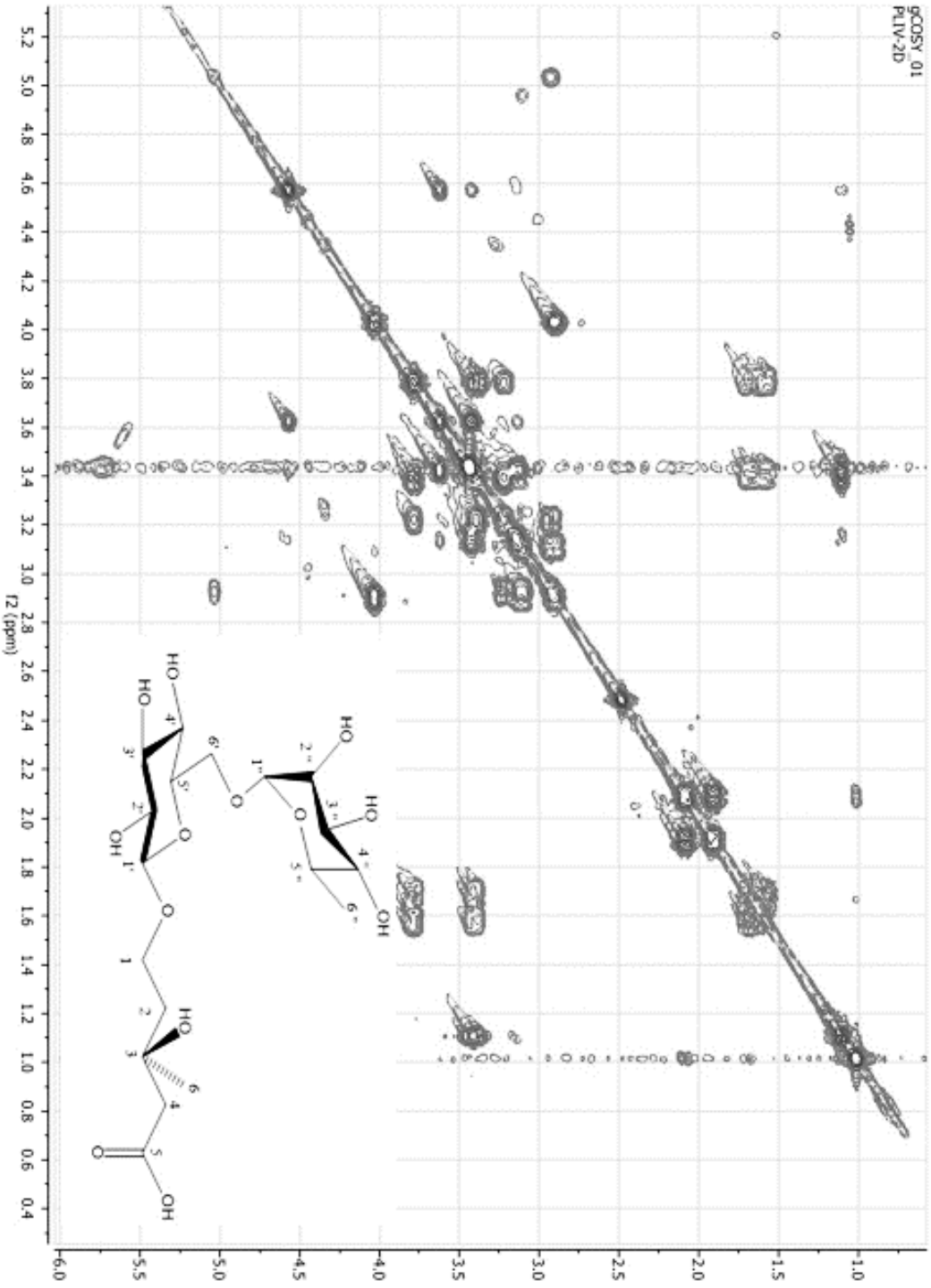


Figure 35. Compound II (MRG) 2D ^{1}H, ^{1}H homonuclear correlation spectroscopy (COSY) (Part I).

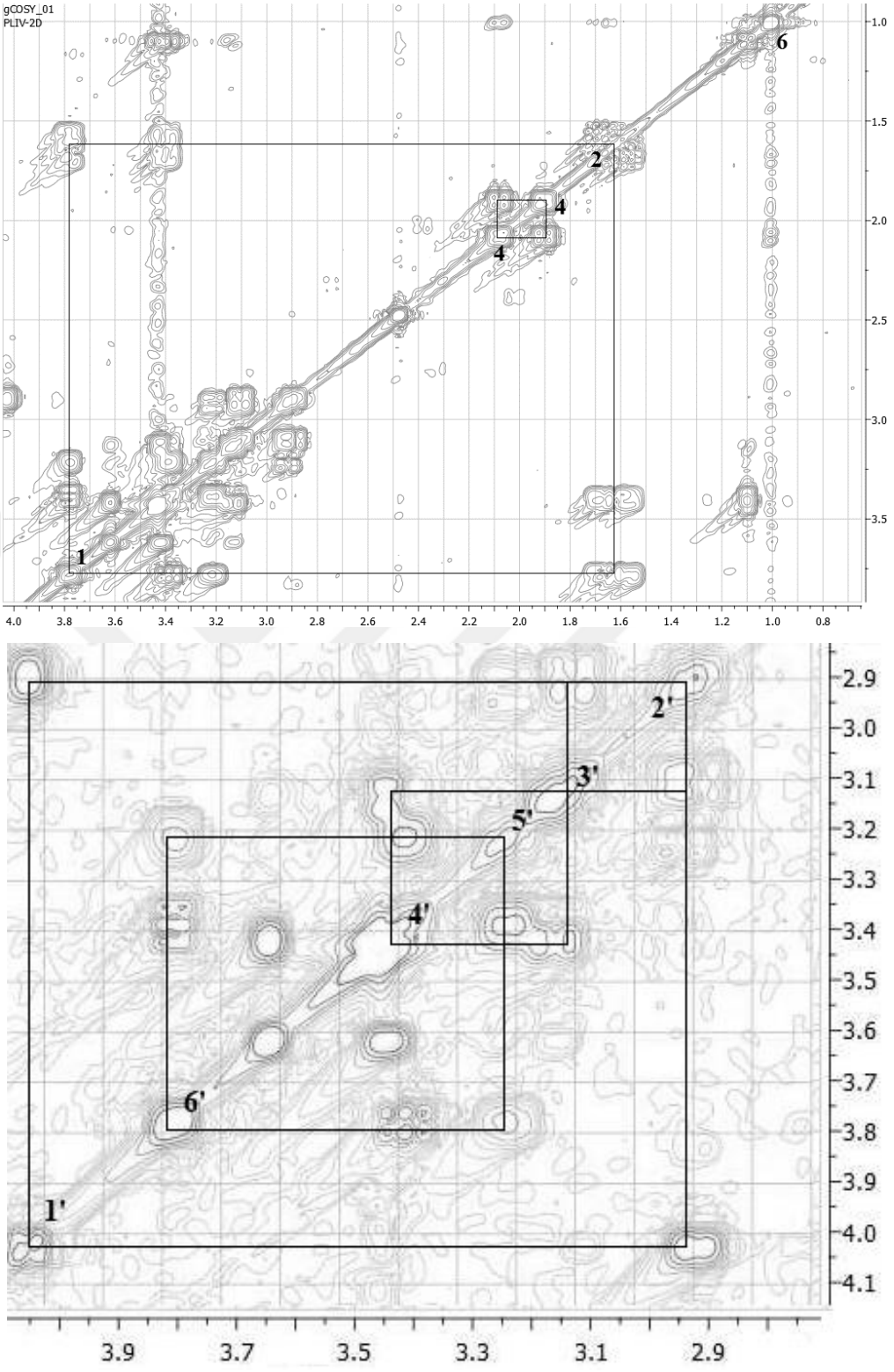


Figure 36. Compound II (MRG) COSY (Part II).

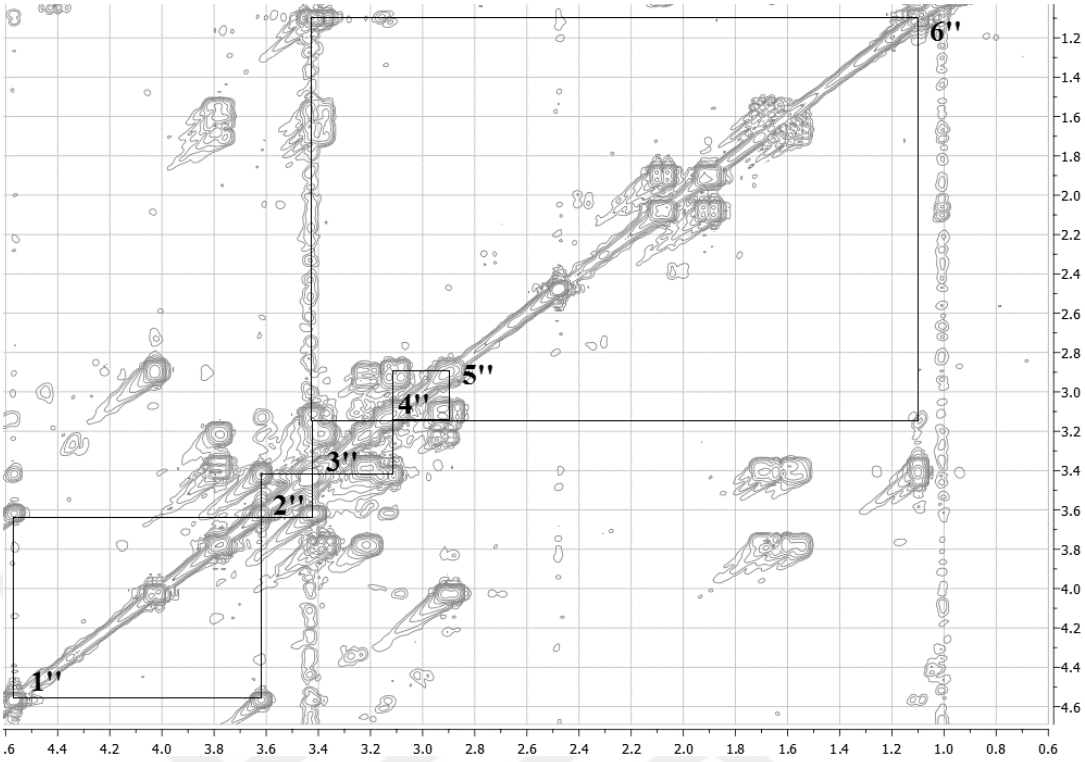


Figure 37. Compound II (MRG) COSY (Part III).

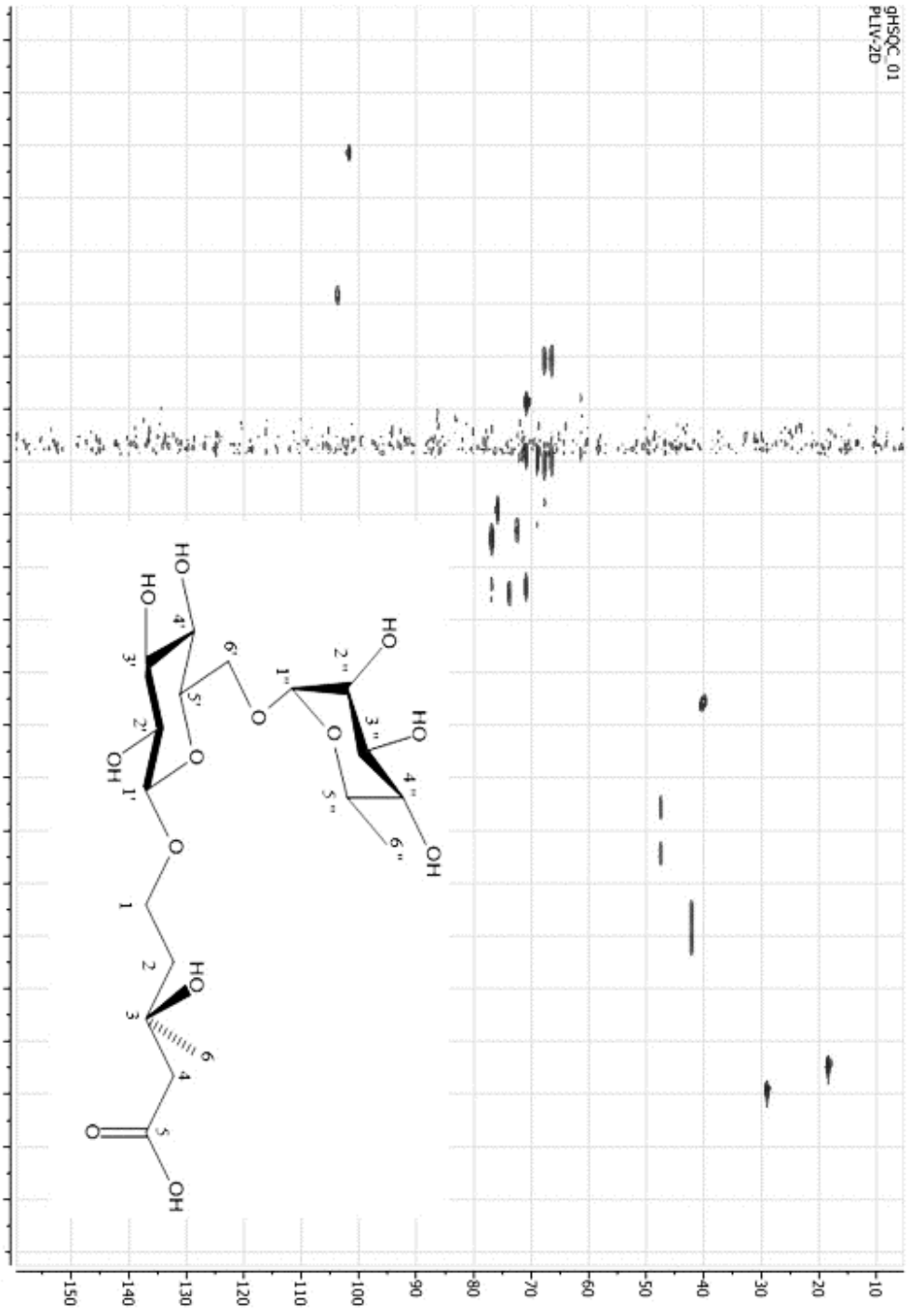


Figure 38. Compound II (MRG) 2D ^{1}H, ^{13}C heteronuclear single quantum coherence (HSQC) (Part I).

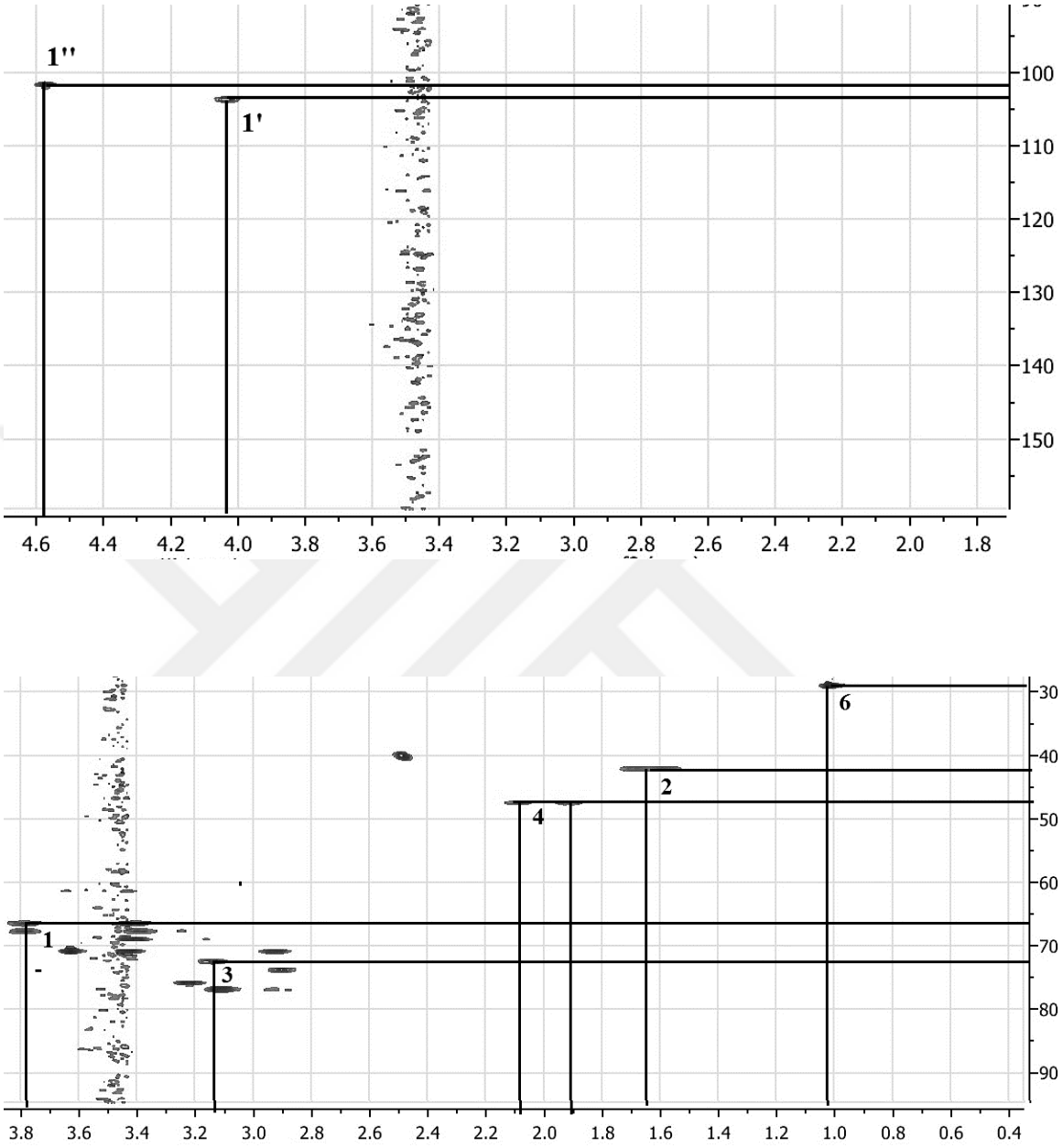


Figure 39. . Compound II (MRG) HSQC (Part II).

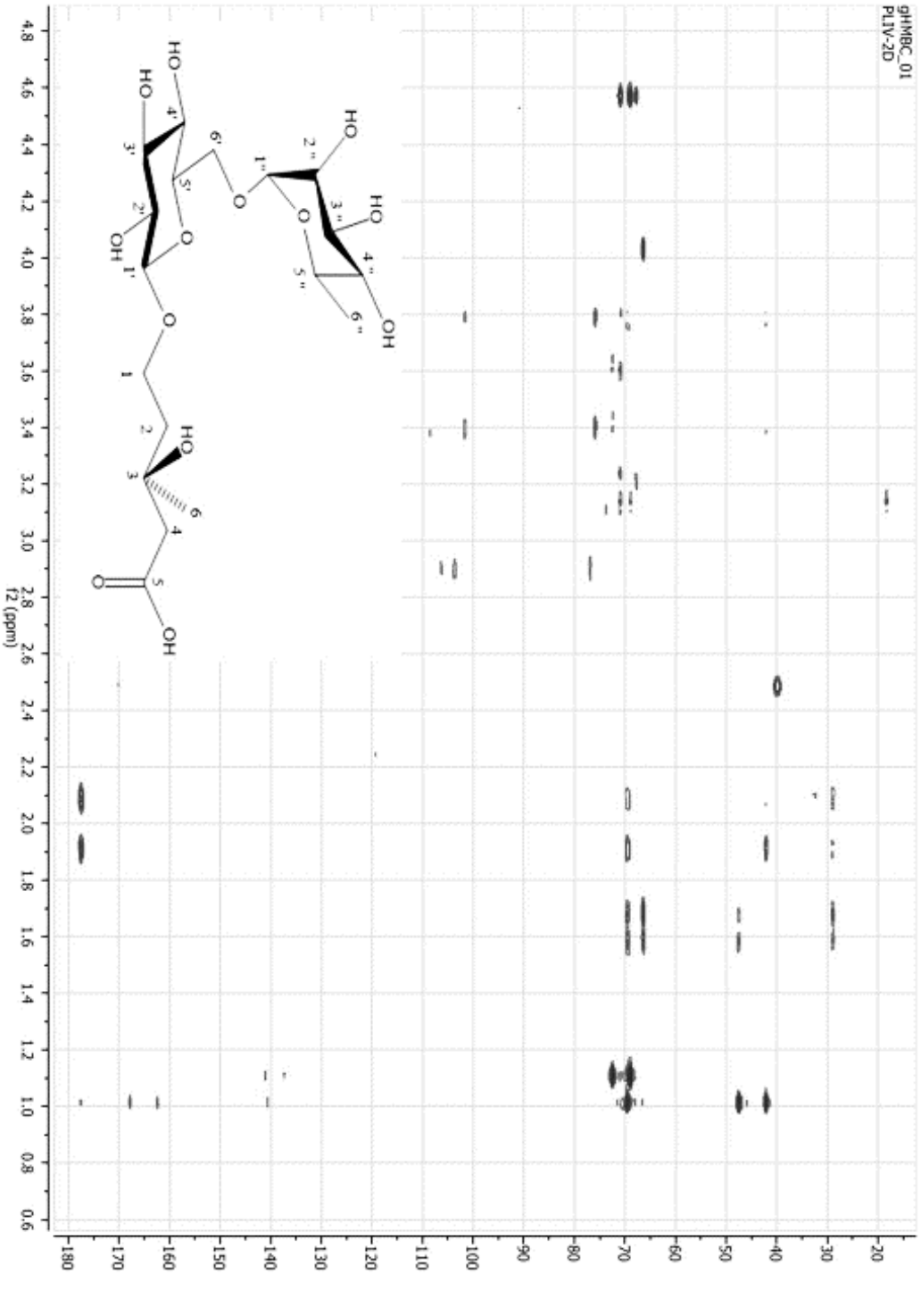


Figure 40. Compound II (MRG) 2D ^{1}H, ^{13}C Heteronuclear multiple-bond correlation spectroscopy (HMBC) (Part I).

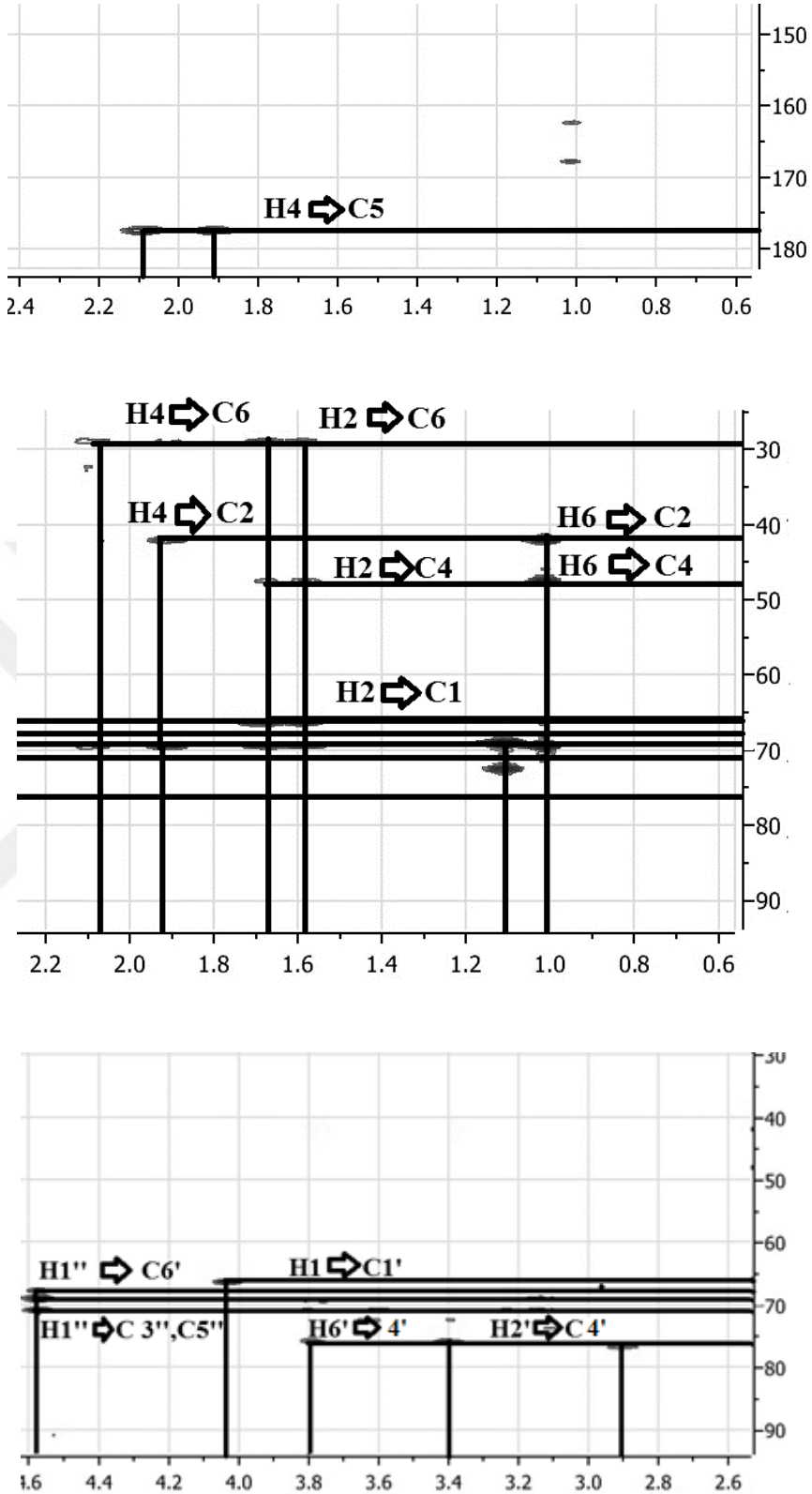


Figure 41. Compound II (MRG) HMBC (Part II).

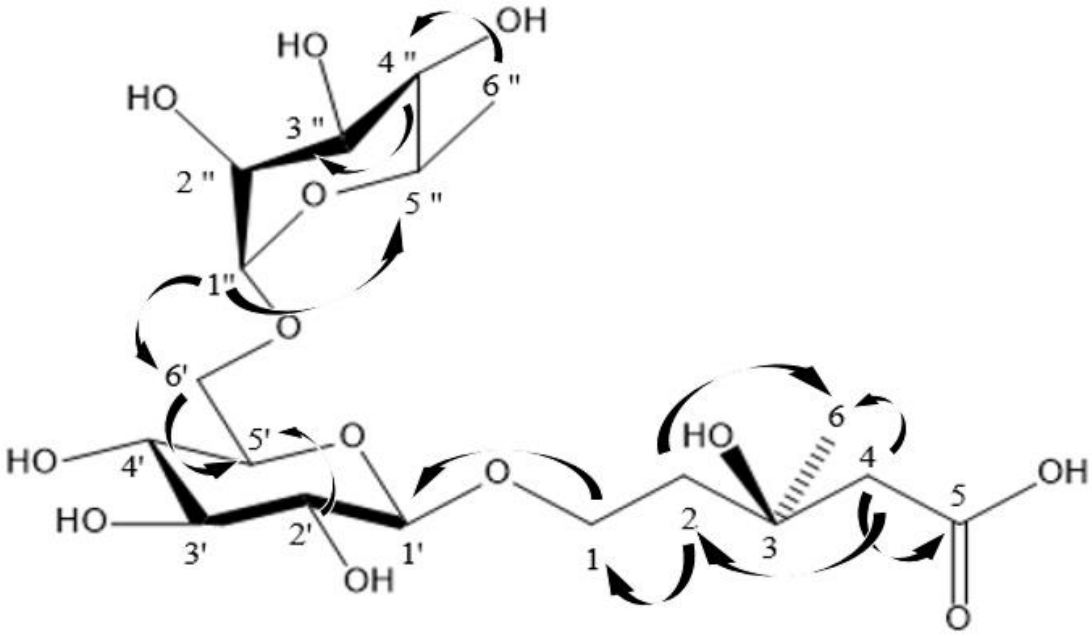


Figure 42. Compound II (MRG) HMBC correlation map.

4.2. LC-MS/MS Results

LC-MS/MS analysis clearly proved that roemerine and (+)-pronuciferine exist in *P. lacerum* MeOH extract (Figures 43-44).

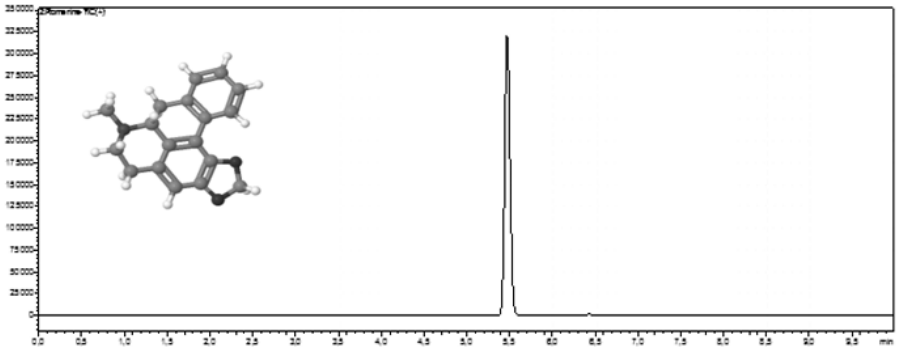


Figure 43. LC-MS/MS of roemerıne in *P. lacerum* MeOH extract.

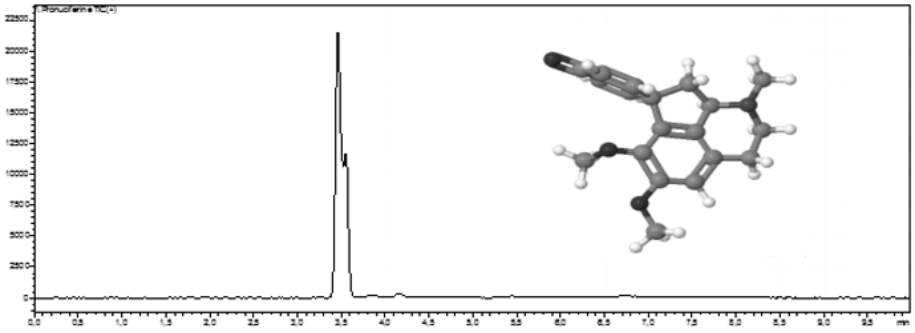


Figure 44. LC-MS/MS of (+)-pronuciferine in *P. lacerum* MeOH extract.

4.3. Biological Activity Results

4.3.1. *In vivo* Results

Forced Swimming Test in Mice

Immobility appeared in rodents during swimming depicts the desperation behavior as it seen in human depression. Immobility time decreases significantly with administration of antidepressants in mice (148). In the forced swimming test (Table 16) there was a significant inhibition in immobility ($p< 0.001$) by *P. lacerum* and ($p< 0.01$) by *P. syriacum* MeOH extracts. Both extracts caused decreasing in immobility time by more than 35% compare to imipramine 50 mg/kg 40%.

Table 16. Effect of *Papaver* extracts on the forced swimming test.

Treatment	Dose (mg/kg *p.o.*)	Duration of immobility (s) (mean±S.E.M.)	Variation (%)
Control	-	191.02 ± 6.23	-
P. glaucum	100	95.14 ± 5.08	2.15
P. lacerum	100	119.06 ± 4.12***	37.67
P. macrostomum	100	177.13 ± 5.22	7.27
P. syriacum	100	153.41 ± 4.14**	19.68
P. rhoeas	100	203.11 ± 7.04	6.32
Imipramin	30	142.25 ± 4.97**	25.53
	50	114.05 ± 6.23***	40.29

*** ($p< 0.001$), ** ($p< 0.01$).

Effect on Spontaneous Motor Activity in Mice

P. lacerum and *P. syriacum* MeOH extracts significantly antagonized *ptosis* ($p< 0.01$) and ($p< 0.5$) respectively. Both extracts also slightly antagonized motor depression that induced by tetrabenazine in mice (Table 17). This result indicates that both extracts have an antidepressant-like effect and may have an effect on monoamine neurotransmitters like noradrenaline, DA or 5-HT (165).

Table 17. Effect of *Papaver* MeOH extract on tetrabenazine (32 mg/kg *i.p.*) induced ptosis, akinesia.

Treatment	Dose (mg/kg *p.o.*)	*Ptosis* mean score (30 min)	Locomotor activity (%) (30 min)
Control	-	3.72 ± 0.25	0.00
P. glaucum	100	3.04 ± 0.32	10.00
P. lacerum	100	2.26 ± 0.13**	30.00
P. macrostomum	100	3.98 ± 0.26	00.00
P. rhoeas	100	3.17 ± 0.41	00.00
P. syriacum	100	2.39 ± 0.07*	30.00
Imipramin	25	0.00 ± 0.00***	100.00**

*** ($p< 0.001$), ** ($p< 0.01$), * ($p< 0.05$)

Effect on Normal Body Temperature

Previous study reports that patients with depression have shown an increase in body temperature through 5-HT or cytokine mechanism (166). Another study indicates that treating depressed patient with antidepressant can decrease 5-HT_{1A} receptor sensitivity which causes decrease in body temperature (167). The data in Table 18 shows the effect of *Papaver* extracts on body temperature in mice. Both *P. lacerum* and *P. syriacum* MeOH extracts showed significant ($p< 0.01$) decrease in the body temperature up to the fourth hour of administration compared to body temperature of control mice. Chlorpromazine caused significant ($p< 0.001$) hypothermia in body temperatures. Therefore, the results below indicate that both *P.*

lacerum and *P. syriacum* extracts with the disparity to typical antidepressants, were effective to decrease 5-HT_{1A} receptor sensitivity to induced hypothermia.

Table 18. Effect of *P. lacerum* extracts on the body temperature (mean ± s.e.m.).

Treatment	**Dose mg/Kg *p.o.***	**Mean decrease in rectal temperature (˚C)**				
		1 (h)	2 (h)	4 (h)	6 (h)	24 (h)
Control	100	1.34 ± 0.29	1.49 ± 0.31	2.03 ± 0.28	1.78 ± 0.30	1.65 ± 0.22
P. galucum	100	2.69 ± 0.26	3.15 ± 0.30	2.28 ± 0.19	1.06 ± 0.62	0.94 ± 0.56
P. lacerum	100	4.22 ± 0.34**	5.18 ± 0.45**	5.92 ± 0.23*	1.12 ± 0.18	0.74 ± 0.36
P. macrostomum	100	1.12 ± 0.69	1.33 ± 0.75	1.09 ± 0.41	1.42 ± 0.58	0.34 ± 0.16
P. rhoeas	100	2.13 ± 0.38	2.41 ± 0.48	2.94 ± 0.33	1.18 ± 0.25	0.39 ± 0.17
P. syriacum	100	3.98 ± 0.49**	4.02 ± 0.35**	3.74 ± 0.56*	1.03 ± 0.19	0.53 ± 0.15
Clorpromazin	50	5.93 ± 0.45***	7.15 ± 0.61***	7.03 ± 0.92**	1.21 ± 0.74	0.93 ± 0.62

*** ($p< 0.001$), ** ($p< 0.01$), * ($p< 0.05$)

4.3.2. *In silico* Results

Molecular Target

Compound I

The top twenty one concensus molecular targets that were predicted across the six prediction software are listed in Figure 45.

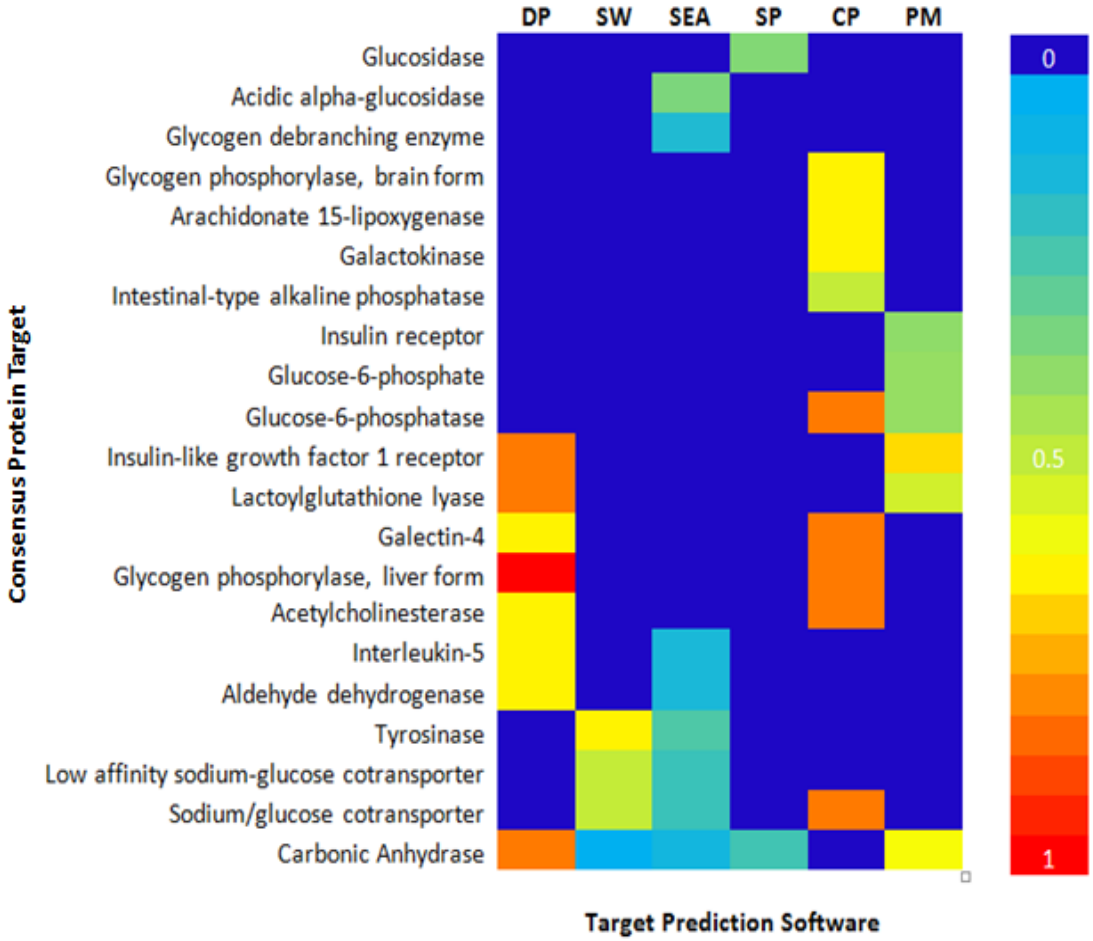


Figure 45. Concensus targets of six target prediction software. Normalized scores of different predicted targets by target prediction software for compound I.

Compound II

The top twenty three concensus molecular targets that were predicted across the six prediction software are listed in Figure 46.

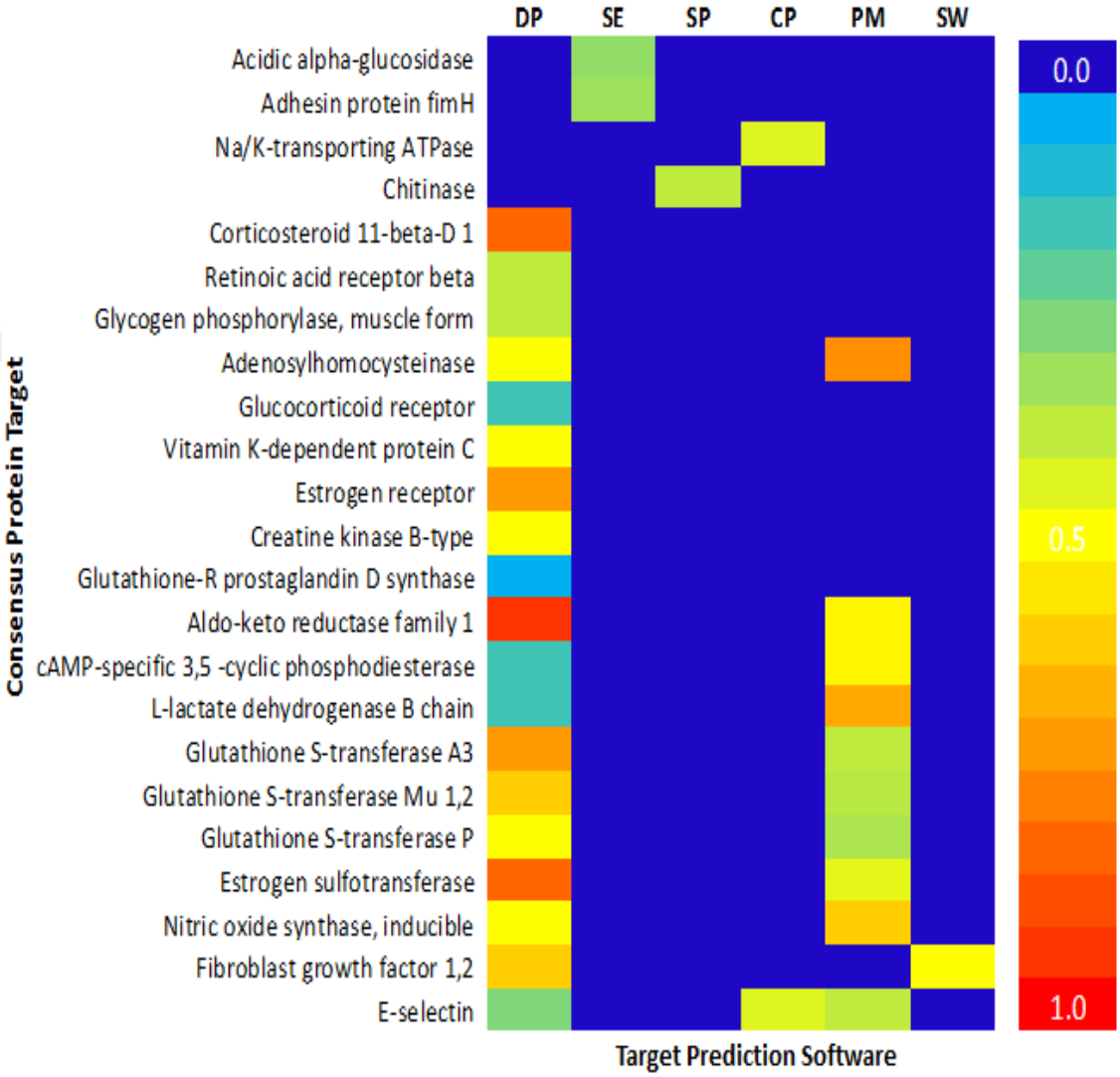


Figure 46. Concensus targets of six target prediction software. Normalized scores of different predicted targets by target prediction software for compound II.

Structure Virtual Screening

The 3D similarity of both compounds I, II were compared against library contains thousands of known ligands. After visualizing the result and compared the similarity of compounds I, II to each ligand in the top hits obtained by EON.

According to the fragment-hopping strategy used in previous studies, compounds with top-ranking scores 0.62 and higher for electrostatic Tanimoto (ET) and higher than 0.75 for TanimotoCombo are selected for similarity evaluation. Therefore, we examined the area with the highest values for 3D similarity to reference compounds I and II (168).

Compound I

Analyzing the 3D similarity result led to select three ligands, ET-Combo and TanimotoCombo scores for each compound is listed in Table 19. Similar ligands to the reference compound (compound II) are with drug bank ID; DB07115 targets carbonic anhydrase 13, DB07194 targets tyrosine-protein kinase SKY, while DB08907 targets sodium-glucose cotransporter-2 (169).

Table 19. Top ROCS and EON scores of active ligands compared to compound I.

Target		EON				ROCS	
	Drug Bank ID	ET-Pb	Shape Tanimoto	ET-Combo	Tanimoto Combo	Shape	Color
Carbonic anhydrase 13	DB07115	0.264	0.68	0.951	0.799	0.692	0.107
Tyrosine-protein kinase SKY	DB07194	0.321	0.61	0.937	0.80	0.617	0.183
Sodium-glucose co-transporter 2	DB08907	0.234	0.59	0.831	0.943	0.598	0.345

Compound 1 overlays with the most similar active ligands, DB07115 (Figure 47), DB07194 (Figure 48), DB08907 (Figure 49). The molecule with the gray color is compound I and the similar molecule is in cream color.

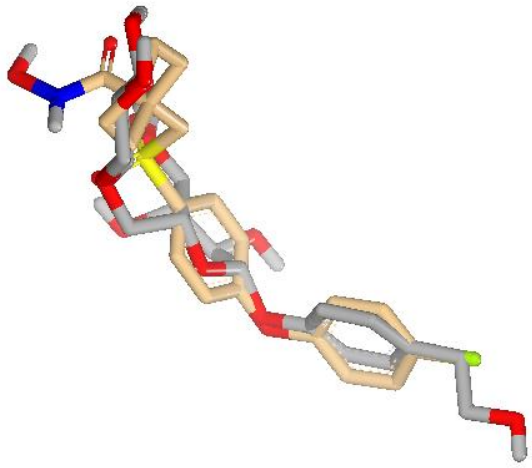


Figure 47. Overlay of compound I with DB07115.

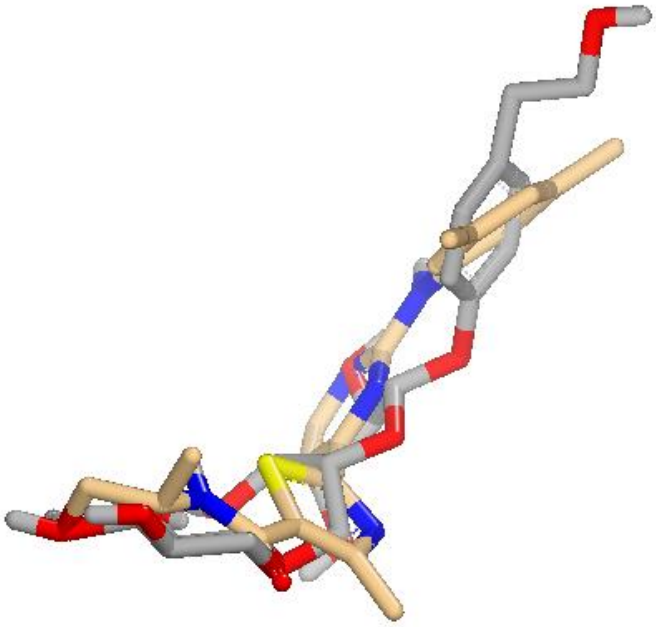


Figure 48. Overlay of compound I with DB07194.

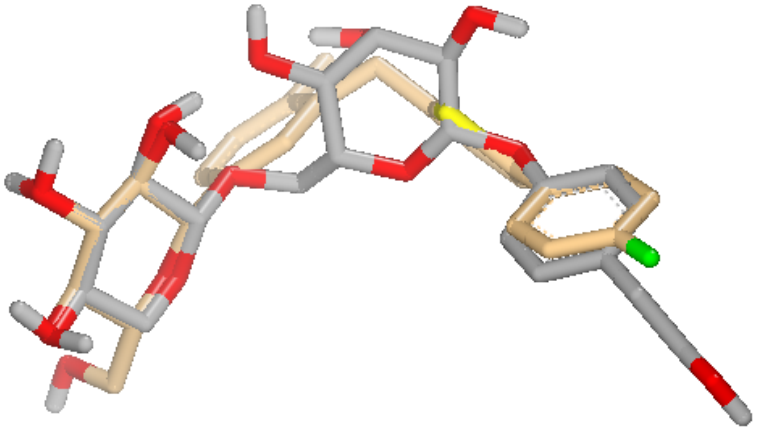


Figure 49. Overlay of compound I with DB08907.

Figures below show the electrostatics potential map of compound I with the most similar active ligands, DB07115 (Figure 50), DB07194 (Figure 51), DB08907 (Figure 52). Electrostatic grids are generated in two colors, red color (negative contour) and blue color (positive contour) (168).

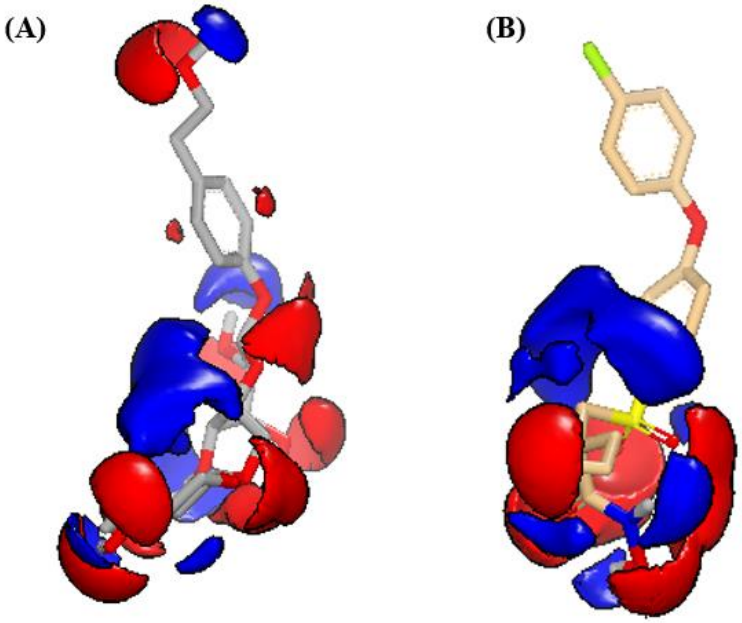


Figure 50. Electrostatics potential map of (A) Compound I, (B) DB07115.

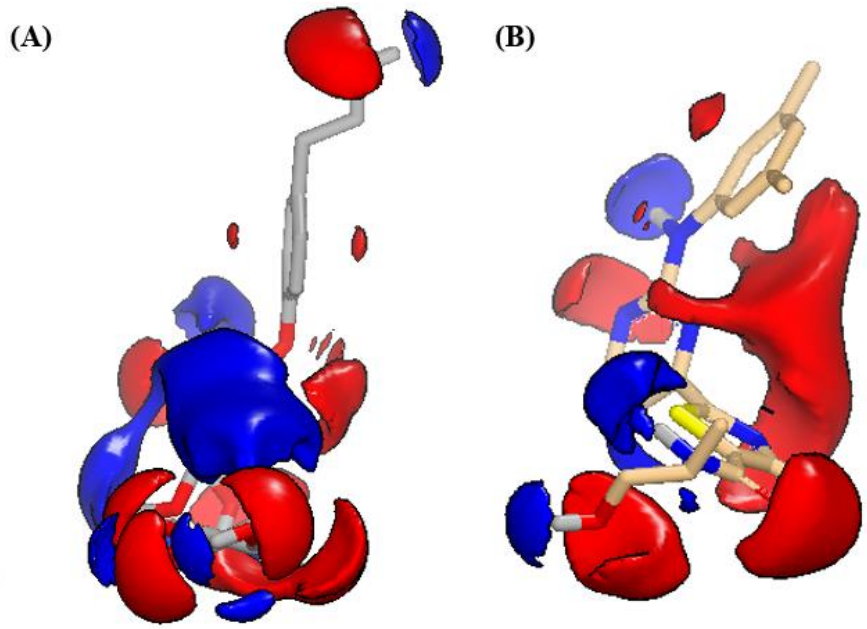


Figure 51. Electrostatics potential map of (A) Compound 1, (B) DB07194.

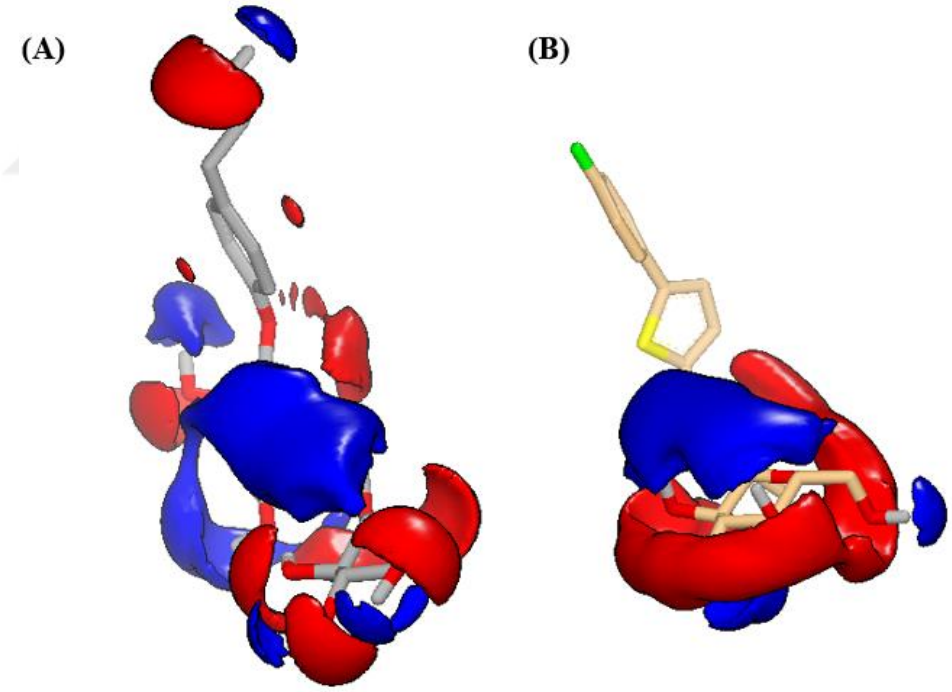


Figure 52 . Electrostatics potential map of (A) Compound 1, (B) DB08907.

The active three ligands were selected after analyzing the data from both target prediction software and 2D/3D ligand similarity. Four molecular targets were selected from molecular target identification:

a) Carbonic anhydrase.
b) Tyrosinase.
c) Sodium/glucose cotranspoter-2.
d) Interleukin-5.

Three similar ligands were selected from structural virtual screening:

a) Carbonic anhydrase.
b) Protein-tyrosine kinase Syk.
c) Sodium/glucose cotranspoter-2.

According to the literature, carbonic anhydrase isozymes have a critical role in cancer development by controlling the tumor's pH homeostasis that controls cancer cell behaviors, also carbonic anhydrase expression decreases in colorectal cancers (170).

Anti-tyrosinase antibodies in metastatic melanoma patients suggest that the Anti-tyrosinase antibodies responsible for some of the destruction of normal melanocytes (171).

On the other hand, protein-tyrosine kinase Syk is found in breast cancer cells with invasive growth and metastasis. Protein-tyrosine kinase Syk expression support information of cell cutlers and inhibits cell motility by improving cell-cell contacts. The hypothesis is that protein-tyrosine kinase Syk act as a tumor suppressor and effects through cell adhesion and motility (172).

Studies showed that interleukin-5 facilitates lung metastasis through controlling the scaffold immune microenvironment (173).

Lastly, sodium/glucose cotransporter-2 inhibitors are the new antidiabetic class that inhibits sodium/glucose cotransporter-2 in the kidney. Decreasing the glucose reabsorption in the proximal tubule, which leads to increase glucose in the urine excretion, improving glycemic control in diabetes patients. DB08907 is Canagliflozin®, which is a drug approved to treat diabetes by inhibiting

sodium/glucose cotransporter-2 (174). After selecting the target candidates, it was concluded that two biological activities should be considered for testing compound I:

Part I: **Anticancer activity** by targeting interleukins-5, tyrosinase, tyrosine-protein kinase SKY and carbonic anhydrase.

Part II: **Antidiabetic activity** by targeting sodium/glucose cotransporter-2.

Compound II

Analyzing the 3D similarity result led to select three ligands, ET-Combo and TanimotoCombo scores for each compound are listed in Table 20. The ligand with drug bank ID (169) (DB00429) targets Prostaglandin E_2 receptor EP_1 subtype, whereas DB02056 targets Aldo-keto reductase family 1 member C, while DB04754 targets Purine nucleoside phosphorylase.

Table 20. Top ROCS and EON scores of active ligands compared to compound II.

Target		EON			ROCS		
	Drug Bank ID	**ET-Pb**	**Shape Tanimoto**	**ET-Combo**	**Tanimoto Combo**	**Shape**	**Color**
Prostaglandin E_2 receptor EP_1 subtype	DB00429	0.51	0.96	1.47	0.929	0.723	0.723
Aldo-keto reductase family 1	DB02056	0.43	0.95	1.38	0.832	0.642	0.19
Purine nucleoside phosphorylase	DB04754	0.52	0.67	1.20	0.774	0.59	0.183

Compound II overlays with the most similar active ligands, DB00429 (Figure 53), DB02056 (Figure 54), DB04754 (Figure 55). The molecule with the gray color is compound II and the similar molecule is in cream color.

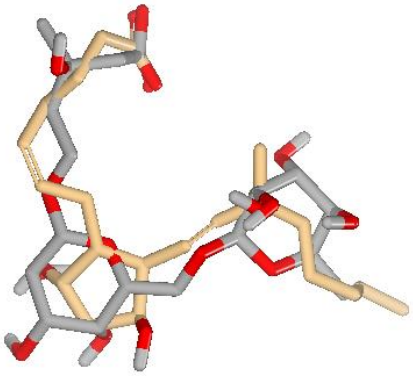


Figure 53. Overlay of compound II with DB00429.

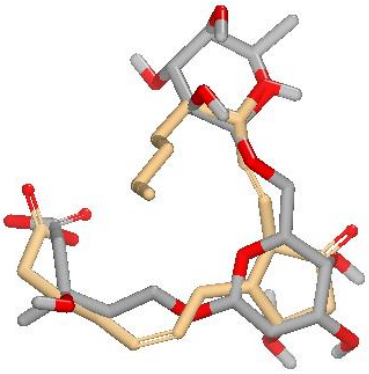


Figure 54. Overlay of compound II with DB02056.

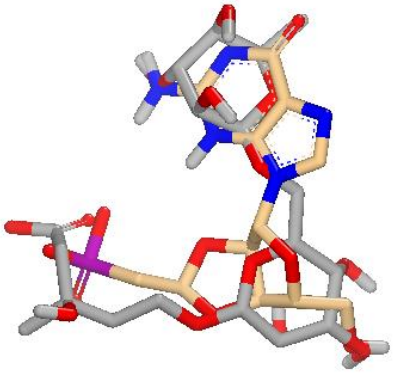


Figure 55. Overlay of compound II with DB04754.

Figures below show the electrostatics potential map of compound II with the most similar active ligands, DB00429 (Figure 56), DB02056 (Figure 57), DB04754 (Figure 58). Electrostatic grids are generated in two colors, red color (negative contour) and blue color (positive contour) (168).

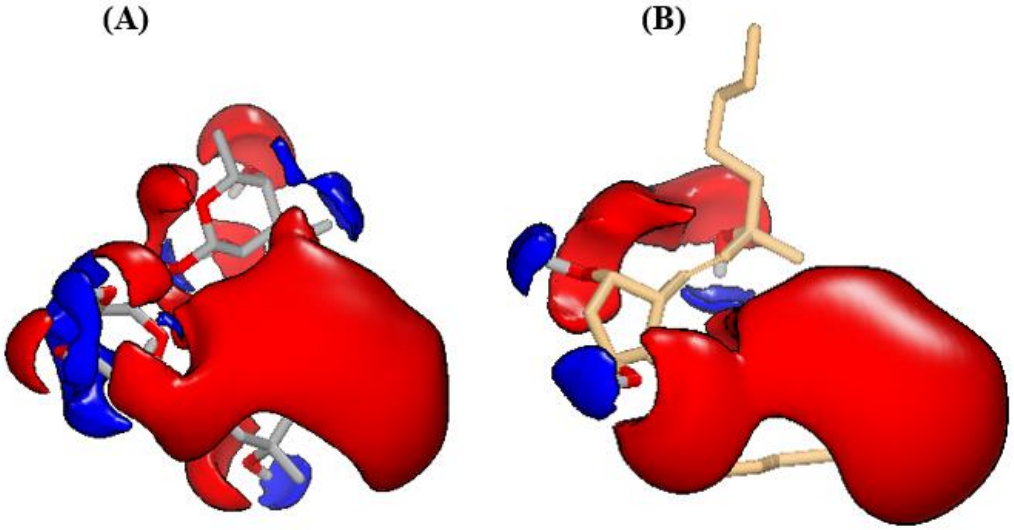


Figure 56. Electrostatics potential map of (A) Compound II, (B) DB00429.

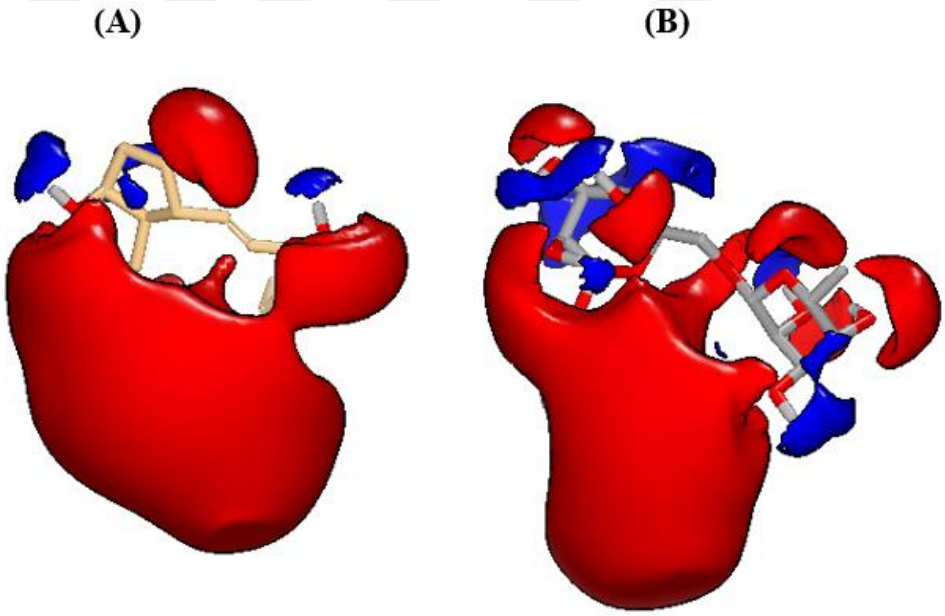


Figure 57. Electrostatics potential map of (A) Compound II, (B) DB02056.

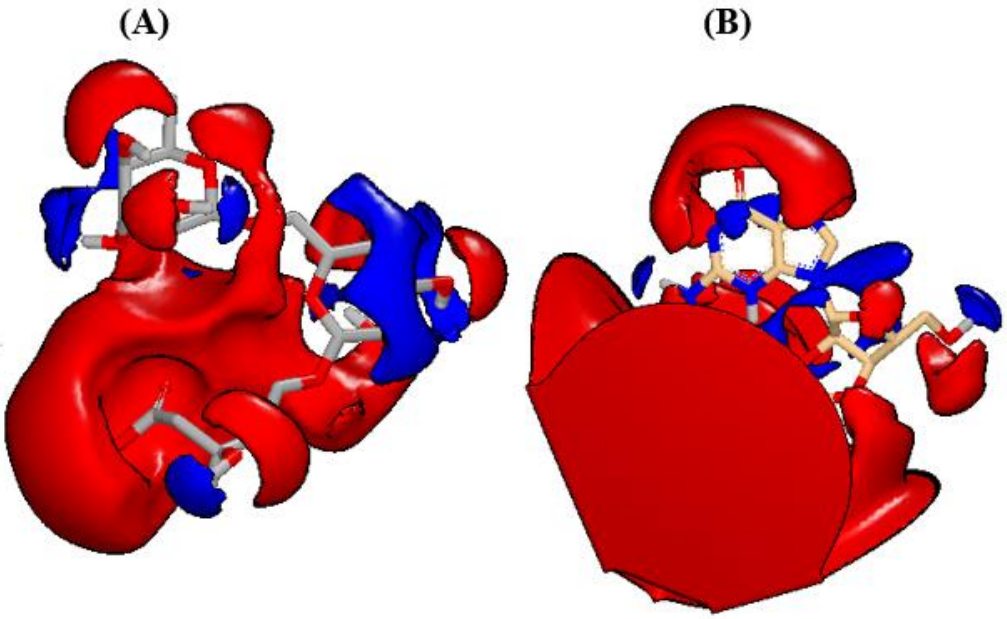


Figure 58 . Electrostatics potential map of (A) Compound II, (B) DB04754.

The active three ligands were selected after analyzing the data from both target prediction software and 2D/3D ligand similarity. Four molecular targets were selected from molecular target identification:

a) Glutathione-*R* prostaglandin *D* synthase.
b) Aldo-keto reductase family 1.
c) Fibroblast growth factor 1, 2.
d) E-selectin.

Three similar ligands were selected from structural virtual screening:

a) Prostaglandin E_2 receptor EP_1 subtype.
b) Aldo-keto reductase family 1.
c) Purine nucleoside phosphorylase.

According to the literature, glutathione-*R* prostaglandin *D* synthase which is predicted by molecular target software, is an enzyme responsible for the production of the prostaglandin D_2, which mediates inflammatory actions through prostaglandin DP_1 and DP_2 leading to an increase in vascular permeability, chemotaxis, peripheral vasodilatation (176), inhibition of platelet aggregation and bronchoconstriction (177). Structure virtual screening predicted that compound II is similar to a Prostaglandin

E_2 receptor EP_1 and EP_2 subtype agonist. Prostaglandin E_2 is synthesized by cytosolic and microsomal prostaglandin E synthases (178). Prostaglandin E_2 exerts its effect through four G-protein-coupled receptors, Prostaglandin E_2 subtypes EP_1, EP_2, EP_3, and EP_4 (175). Prostaglandin E2 has been linked to enhancing osteoclastic differentiation, which is involved in many inflammatory diseases such as osteomyelitis and rheumatoid arthritis (179).

Previous study shows that aldo-keto reductase family 1 member C gene may be associated with drug resistance in cancers, like cisplatin. This enzyme catalyzes steroids, lipid aldehydes and prostaglandins. It has been reported that there is a change in the expression of aldo-keto reductase family 1 member C in some malignant tumors. Also, studies reported that overexpression of aldo-keto reductase family 1 member C was narrowly associated with anti-cancer medications resistance. Mefenamic acid, which is aldo-keto reductase family 1 member C enzyme inhibitor increase the sensitivity of anti-cancer drugs (180).

Purine nucleoside phosphorylase is an enzyme that catalyzes the phosphorolysis of purine nucleosides. Birth defects in Purine nucleoside phosphorylase result in different immunodeficiency syndromes due to T cells depletion, with no notable decrease in the B cell count. Depletion of T cell caused by Purine nucleoside phosphorylase inhibition is after the accumulation of intracellular plasma 2'deoxyguanosine triphosphate and plasma 2'deoxyguanosine. Intracellular plasma 2'deoxyguanosine triphosphate is toxic to lymphocytes since it inhibits ribonucleotide reductase, leading to change in the intracellular deoxynucleotide pools, resulting in cellular apoptosis (181).

Fibroblast growth factors have very important roles in malignancies, wound healing, angiogenesis, endocrine signaling pathways and embryonic development, it also has a role in a tumor suppression. Several studies have reported that cervical cancers are associated with an increase in fibroblast growth factors levels. A recent study reported that the proliferation of HeLa cell increases when it is treated with fibroblast growth factors (182).

Tumor necrosis factor-α (TNF-α) is a cytokine produced by macrophages as well as neutrophils and lymphocytes. TNF-α precursor is a membrane TNF. Studies show that membrane TNF-α regulates inflammation on T cells. Soluble TNF- α form exerts biological effects by attributing to cytokine production, cell proliferation and apoptosis, whereas membrane TNF-α function is still not understood well. Membrane TNF-α activation in human T cells increases E-selectin expression, which is an adhesion molecule belonging to the selectin family that is known to be expressed in activated non-endothelial and endothelial cell types (183).

After selecting the target candidates, it was concluded that the two biological activities should be considered for experiment testing for compound II:

Part I: **Anticancer activity** by targeting Aldo-keto reductase family 1, Purine nucleoside phosphorylase, Fibroblast growth factor 1, 2 and E-selectin

Part II: **Anti-inflammatory** by targeting Glutathione-*R* prostaglandin *D* synthase and Prostaglandin E_2 receptor EP_1 subtype.

4.3.3. *In vitro* Results

BDNF Expression

Papaver lacerum MeOH extract, the most active species *in vivo*, appears to increase BDNF expression in SH-SY5Y cell line *in vitro*. The extract at 100 µg/mL and the pure compounds at 25 µg/mL were applied to SH-SY5Y cells for 48 hours. 17β-estradiol was used as a positive control with negative control (no treatment).

P. lacerum MeOH extract at 100 µg/mL significantly increased intracellular level of BDNF by 78.6% ($p< 0.0001$), where the MeOH extracts of *P. macrostomum, P. syriacum* increased BDNF level by 64.2% ($p< 0.0001$) and 47.1% ($p< 0.0001$) respectively. However *P. rhoeas* and *P. glacum* did increased BDNF by 45.2% and 50.8% respectively, but not as signifcant as the other extracts. (Figure 59).

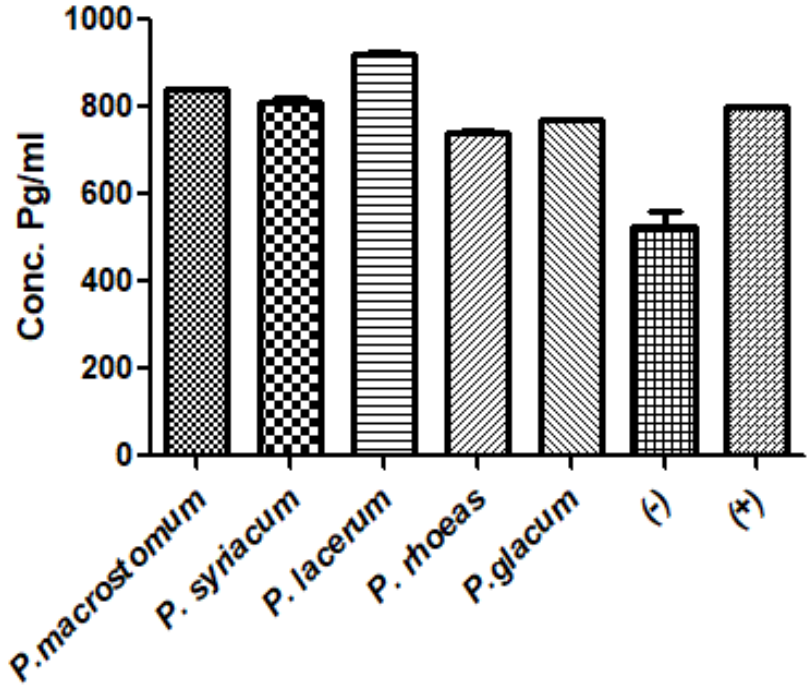


Figure 59. Effect of *Papaver* extracts on BDNF Level. (–) Control, (+) 17*β*-estradiol.

SH-SY5Y cells were treated with compounds I and II to evaluate their activity on BDNF expression. Result shows that there is no significant increase in BDNF levels for both compounds I and II.

To determine the activity related compounds in the active extract, the main alkaloid roemerine and the minor alkaloid (+)-pronuciferine were analyzed with the same method. Result shows that there is a significant increase compare to the control for more than 73, 36 % ($p< 0.0001$) in BDNF levels in SH-SY5Y cells for both concentration 20 and 10 μM respectively (Figure 60).

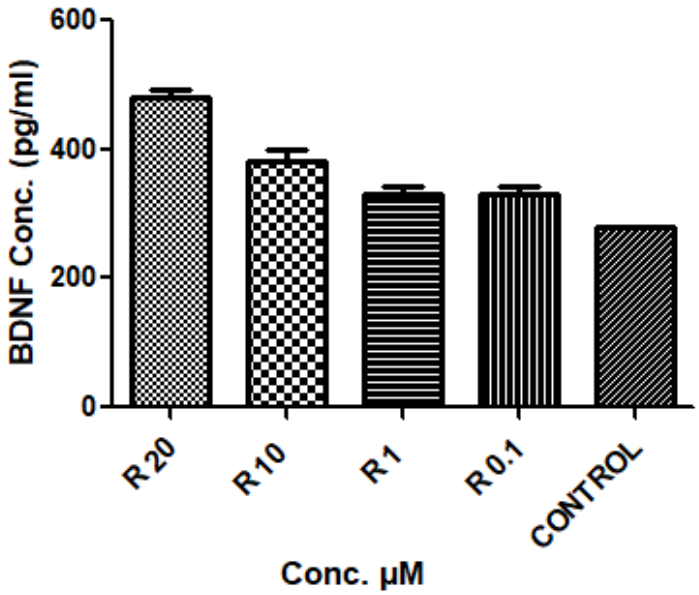


Figure 60. Effect of roemerine (R) on BDNF level.

Additionally, result shows that there is also a significant increase in BDNF levels when SH-SY5Y cells were treated with (+)-pronuciferine. There is an increase of 20.7, 20.6, 17 and 17 % ($p< 0.001$) at 20, 10, 1 and 0.1 µM respectively (Figure 61).

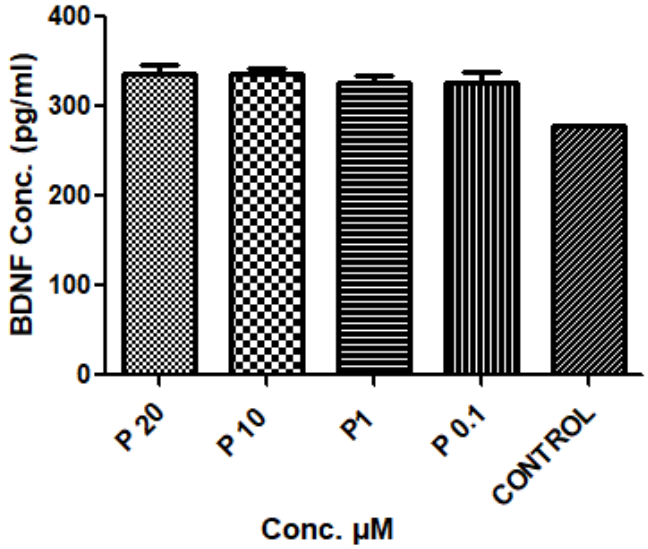


Figure 61. Effect of (+)-pronuciferine on BDNF level.

MTT Cytotoxicity Test

MTT Cytotoxicity assay was carried out to assess cytotoxicity of *Papaver* MeOH extracts and compounds I, II. The effect of *Papaver* MeOH extracts are shown in (Figure 62). *P. lacerum* and *P. rhoeas* were the most cytotoxic extracts with 87% ($p< 0.0001$), (80% $p< 0.001$) respectively, while there was no significant activity with *P. syriacum, P. glaucum* and *P. macrostomum.*

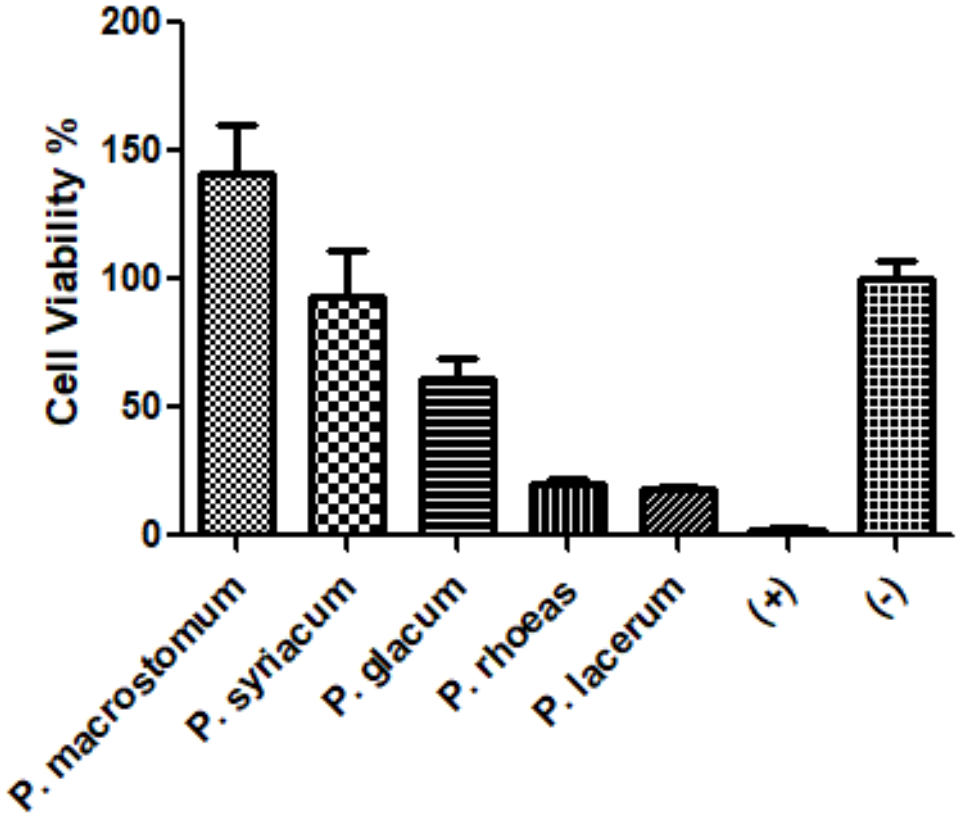


Figure 62. Cytotoxicity of *Papaver* extracts on HeLa cells. (+) cisplatine 30 µM, (-) Control.

Compounds I and II were also evaluated for their cytotoxic activity against HeLa cell line. Compound I was cytotoxic to HeLa cells at 100 µM by more than 40% ($p< 0.0001$) with IC_{50} equal to 66.4 µM (Figure 63).

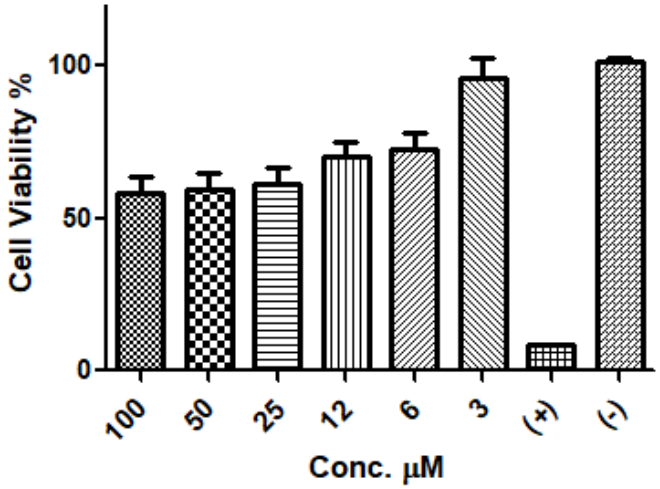


Figure 63. Cytotoxicity of compound I. (+) cisplatine 30 µM, (-) Control.

Compound II was cytotoxic in dose-dependent manner to HeLa cells, at 100 µM it was cytotoxic by more than 65% ($p< 0.0001$) with IC_{50} equal to 54 µM (Figure 64). Cisplatin 30 µM was used as a positive control. n=3, each with quadruplicate samples.

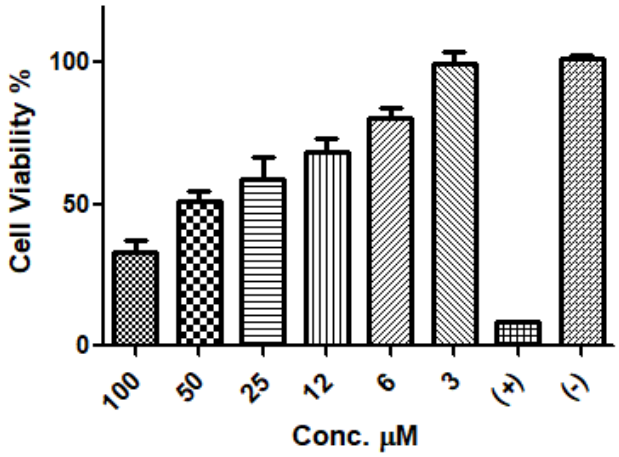


Figure 64. Cytotoxicity of compound II. (+) cisplatine 30 µM, (-) Control.

Roemerin was cytotoxic to HeLa cells at 100 µM by more than 97.7% ($p< 0.0001$) with $IC_{50}= 28$ (Figure 68), while (+)-pronuciferine did not show any cytotoxic effect. Cisplatin 30 µM was used as a positive control (Figure 65).

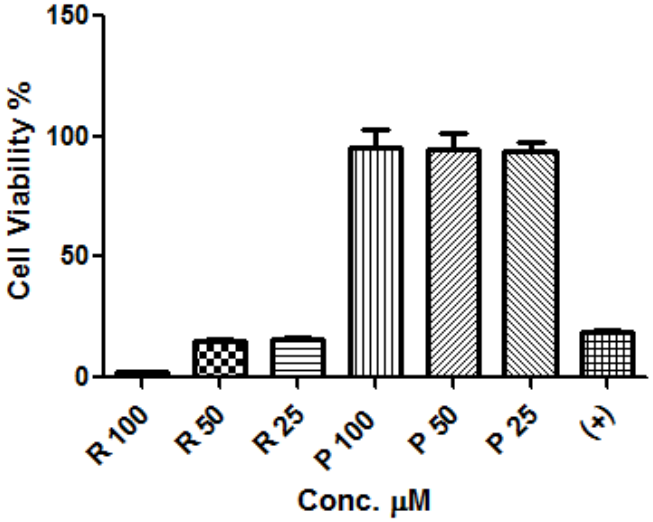


Figure 65. Cytotoxicity of roermerine (R) and. (+)-pronuciferine (P). (+) cisplatine 30 µM.

Normal Cell Line L929 Cell Viability Tests

All the compounds at 100 µM and the extracts at 100 µg/mL were not cytotoxic against normal cell line L929. In addition compound II and (+)-pronuciferine increased cell proliferation which may indicate to their protective effect (Figure 66).

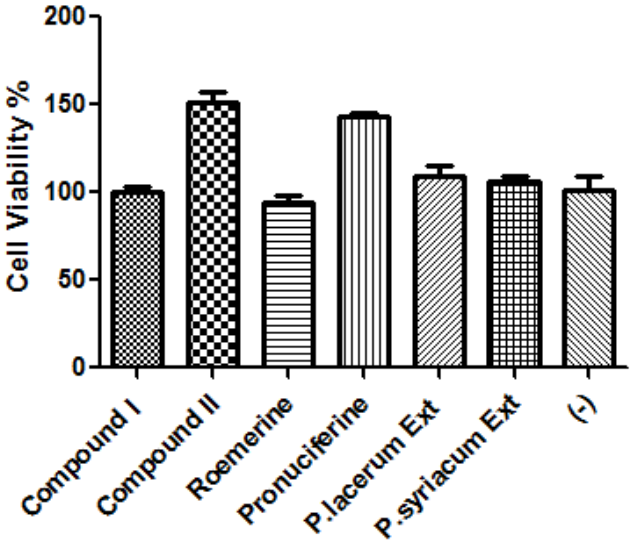


Figure 66. Cytotoxicity of all compounds and active extract. (-) Control.

Colony Formation Assay

Colony formation assay performed in 6-well plates, with clone's produced by HeLa cells. It was shown that after 9 days of treatment compound II and *P. lacerum* MeOH extract inhibit colony forming of HeLa cells. (A) Treated with compound II 100 μM, (B) Treated with *Papaver lacerum* extract 100 μg/mL, (C) Treated with compound I 100 μM, (D) Untreated controls (Figure 67).

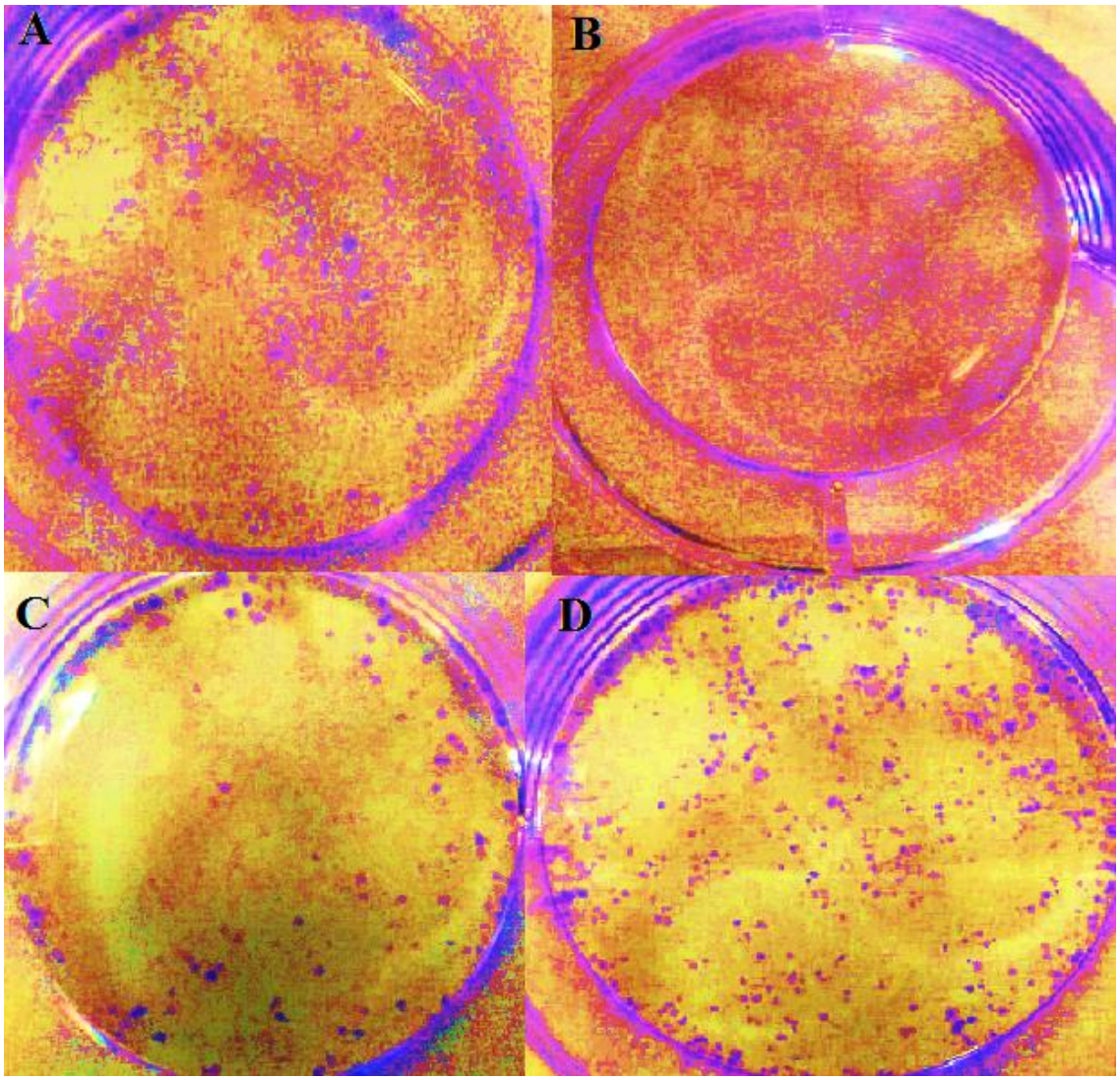


Figure 67. Colony formation assay A. Compound II. B. *Papaver lacerum* extract. C. Compound I. D. Control. Colonies are shown in purple dots.

α-Glucosidase Inhibition Assay

P. lacerum MeOH extract exhibited moderate activity regarding *α*-glucosidase with (IC_{50} 4.3 mg/mL) (figure 68). However, compound I did not show any efficacy as *α*-glucosidase inhibitors (figure 69) even though there was a possibility for activity according to *in silico* results.

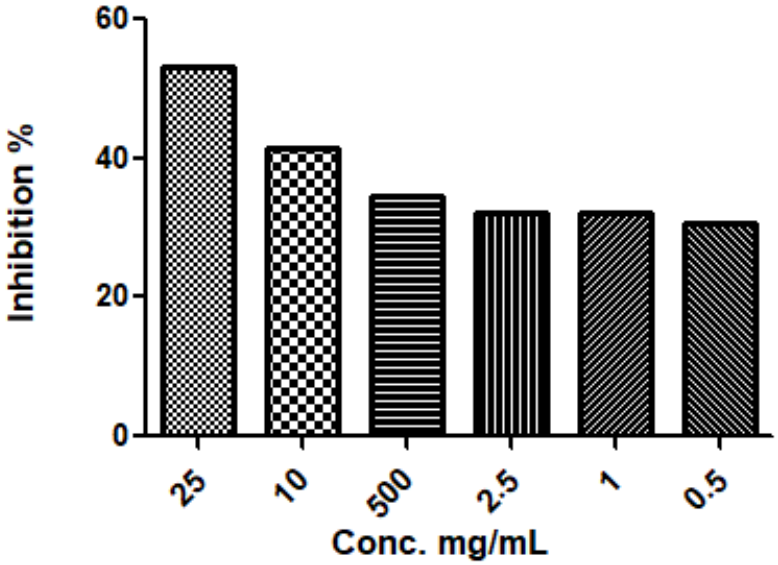


Figure 68. The effect of *Papaver lacerum* extract on *α*-glucosidase.

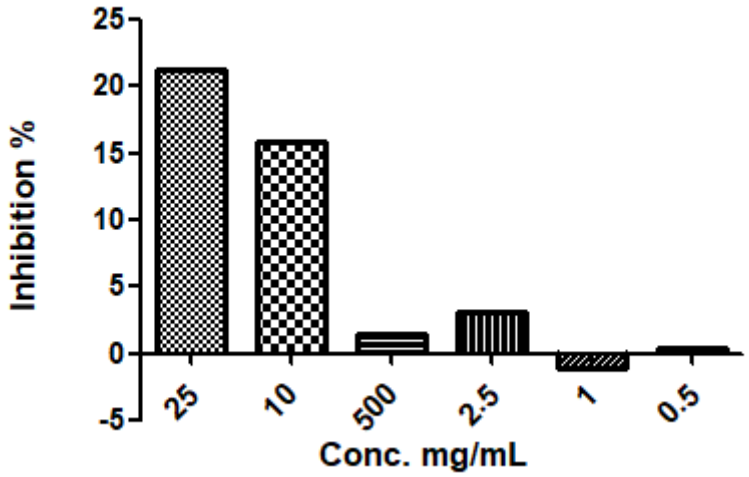


Figure 69. The effect of compound I on α-glucosidase.

5. DISCUSSION

15% of the patients with MDD ultimately present resistance of the treatment or refractory depression (184). Almost one-third of the patients with MDD do not response to the medication, and up to half of the patients with MDD partially response to their initial medication. After two years of treatment with antidepressants almost one-fifth of the patients poorly response to the medication and remain unwell. Some patients even after five years fail to recover (1 in 10). Any episode last more than two years are considered to be 'chronic depression' and it is either because delayed diagnosis or unsuitable treatment (185).

There are many reasons behind the pathophysiology of MDD. In the last decades, the principle regarding MDD pathophysiology has dictated to monoamine dysfunction, decreasing in the production of the monoamine or cell signaling malfunction such as cAMP. Recently more attention has been drawn on the abnormalities of neuroendocrinological, neurogenesis via a decrease in the level of BDNF, along with dysfunction in endogenous opioid, glutamatergic function and neurotransmission (186). Different brain part such as the hippocampus experience structural modifications in MDD and the hypothesis behind the hippocampal atrophy could explain the mood changing and memory instability in depressed people. Nevertheless the molecular biology source of these changes still unclear. While a reduction in neurogenesis, neuronal death, and alteration in the level of BDNF are directly related to loss of volume in hippocampal resign in MDD patients (187).

The starting point of this study was to find some natural products with possible antidepressant activity. The starting point to this study was to find some natural products with possible antidepressant activity. *Papaver* species are well known for their diverse biological activities such as anti-influenza, antidepressant, decrease morphine withdrawal symptoms, anti-inflammatory, antibacterial, cytotoxic, analgesics, sedative and antiviral effects. In addition to these facts, traditional usage of *P. rhoeas* on the treatment of some sort of mental disorders in Anatolia, led us to collect and study some *Papaver* spec. which have not been excessively studied chemically and biologically (46, 52).

Five *Papaver* species (*P. glaucum, P. macrostomum, P. rhoeas, P. lacerum and P. syriacum*) have been collected from eastern and middle regions of Turkey and their crude extracts were prepared with MeOH to screen their activity. Antidepressant-like activity of the five *Papaver* species were investigated using; forced swimming test, effect on spontaneous motor activity and effect on normal body temperature in mice. The dosage of extracts was 100 mg/kg, while the positive control imipramine dosage was 50 mg/kg. Extracts and imipramine were orally administrated, duration of immobility is expressed in seconds. *P. lacerum* and *P. syriacum* extracts decreased immobility time 119, 153 seconds respectively compare to the positive control imipramine at dosage 50 mg/kg decreased immobility time 114 seconds. Studies reported that brain stimulant such as psychotonics which are ineffective clinically as antidepressants, can also decrease immobility time in the forced swimming test. With the aim of evaluating the false positive possibility of anti-immobility effects in the forced swimming test result, a locomotor activity and ptosis test were performed to the mice. *P. lacerum* and *P. syriacum* extracts significantly antagonized ptosis ($p< 0.01$) and ($p< 0.5$) respectively, and slightly antagonized motor depression that induced by tetrabenazine in mice. Thus both extracts were only active in antagonized ptosis suggesting that the antidepressant-like effects of *P. lacerum* and *P. syriacum* extracts in the forced swimming test were unlikely to be false positives. A previous study indicated that treating depressed patient with antidepressants such as Ipsapirone® could decrease 5-HT_{1A} receptor sensitivity which causes decrease in body temperature (167). Measuring the rectal temperature of mice given *P. lacerum* and *P. syriacum* extracts were significantly lower than control mice ($p< 0.01$). Therefore, the results indicated that both *P. lacerum* and *P. syriacum* MeOH extracts with the disparity to typical antidepressants, were effective to decrease 5-HT_{1A} receptor sensitivity to induced hypothermia.

Several studies demonstrate that injection of BDNF in the mesencephalon possess behavioral antidepressant effects in learned helplessness paradigms and forced swimming test (20). Since BDNF promotes neuronal development and survival, studies indicate that the long-term target of antidepressants treatment could be possibly regulating BDNF. BDNF level acts also as a potential biomarker of cognitive performance (188). In addition, BDNF has the ability to initiate numerous

intracellular cascades that can attribute to promote neuronal differentiation and thus enhancing mood and memory (189).

Evaluation of the BDNF expression of the *P. lacerum* and *P. syriacum* extracts were completed to give an idea to on the mechanism of these species' antidepressant-like activities. BDNF expressions in SH-SY5Y cell line were evaluated for all *Papaver* MeOH extracts. BDNF was measured using the human BDNF ELISA Kit. Results are expressed as percentage in BDNF increase at 100 µg/mL of the extracts. After a continuous 48 hours exposure of the cells to the extracts, cells were lysed and BDNF levels were measured. In a consistency with the *in vivo* results *P. lacerum* and *P. syriacum* exhibited highest activity in the samples. BDNF protein expression levels were significantly high in cells treated with *P. lacerum* MeOH extract by 78.6% ($p< 0.0001$), where the MeOH extract of *P. macrostomum, P. syriacum* increased BDNF level by 64.2% ($p< 0.0001$), and 47.1% ($p< 0.0001$) respectively. Although both *P. rhoeas* and *P. glacum* increased BDNF level, however this increases were not as significant as the other species' results. The results therefore demonstrated that *Papaver lacerum* and *P. syriacum* extracts have antidepressant-like effects, which possibly through a neurotrophic action in the brain. According to the biological activity studies both *in vivo* and *in vitro P. lacerum* as the most active species was chosen for the phytochemical analysis. Since the alkaloid contents of the *P. lacerum* was studied earlier, and roemerine was isolated as major alkaloid while N-methyl asimilobine and the proaporphines; mecmibrine and pronuciferine were minor compounds. For this reason the isolation of alkaloids was not in the goals in this study and therefore alkaloid extraction was not performed (89).

One new and one known glycosides were isolated from the active extract. Compound I was identified as Tyrosol-1-*O*-*β*-xylopyranosyl-(1→6)-*O*-*β*-glucopyranoside and compound II as 5-*O*-(6-*O*-*α*-rhamnopyranosyl-*β*-glucopyranosyl mevalonic acid. Due to insufficient amount of compounds I, II to test them *in vivo,* it was decided to test their effect on BDNF expression in SH-SY5Y cells. Both compounds had moderate effects on BDNF which were not significant.

To identify the possible activity of TXG and RGM, *in silico* methods were approached; target identification and structure virtual screening were performed for

the molecules. *In silico* results implied that both compounds might exhibit cytotoxic activity against some cancer cells due to targeting carbonic anhydrase, protein-tyrosine kinase Syk for TXG and by targeting aldo-keto reductase family 1, purine nucleoside phosphorylase, fibroblast growth factor 1, 2 and E-selectin for RGM. To evaluate cytotoxicity of TXG and RGM, they were tested *in vitro* against HeLa cell line which carbonic anhydrase and fibroblast growth factor 1, 2 proteins play important roles in cell surviving. As a result, TXG was cytotoxic to HeLa cell line by more than 40% with IC_{50}= 66.4 whereas RGM was cytotoxic more than 65%. With IC_{50}= 54. To support this result, we also tested *P. lacerum* extract, it was significantly cytotoxic by more than 87% at 100 µg/mL. Neither the compounds nor the extract were cytotoxic against to healthy cells (L929) at same concentrations used in HeLa cells, 100 µM and 100 µg/mL, respectively. Furthermore, the results showed that TXG might have antidiabetic activity by inhibiting sodium/glucose cotransporter-2 and RGM has anti-inflammatory effect as prostaglandin E_2 receptor EP_1 subtype agonist. Since the only available method in our laboratory was α-glucosidase inhibition assay related to these targets. α-glucosidase inhibition assay was performed for the compound I as well as the extract. Neither of them exhibited significant activity.

Searching for the secondary metabolites that causing antidepressant-like effect, it was thought that the alkaloids might be related. Hence, it has been decided to check the commercially available alkaloids for their effects on BDNF expression. Roemerine as the major alkaloid in the extract and pronuciferine one of the minors were chosen for the tests. Moreover, preliminary structure virtual screening on roemerine resulted with similar ligands, the top ligands which share similar 3D structure and electrostatic are apomorphine which is DA agonist, Mirtazapine®, Orphenadrine® and Bromo-diphenydramine®, which have been used to treat atypical depression and psychosis. A recent study reported that roemerine has a strong selective affinity to bind to 5-HT_{2A} receptor with 20 – 400-fold higher affinity than binding to D_1, D_2 and 5-HT_{1A} receptors (190). While (+)-pronuciferine top similar ligands are Norfluoxetine® which is selective 5-HT reuptake inhibitor, Venlafaxine® which is 5-HT - NE reuptake inhibitor and Desvenlafaxine® which is selective serotonin and NE reuptake inhibitors. However, there is no any study reported in

literature regarding CNS activity of both roemerine and (+)-pronuciferine. In our *in vitro* tests roemerine increase in BDNF 73, 36 % ($p< 0.0001$) in BDNF levels for both concentration 20 and 10 μM respectively. Pronuciferine increase BDNF level 20.7, 20.6, 17 and 17 % ($p< 0.001$) at 20, 10, 1 and 0.1 μM respectively.

Since *P. lacerum* extract was cytotoxic to HeLa cells, we have tested these two alkaloids too. As a result roemerin was 97.7% cytotoxic at 100 μM with IC_{50}= 28, while pronuciferine did not show any activities. We also run cytotoxicity test on healthy cell line (L929) for both alkaloids and none of them possessed any cytotoxicity against L929 cell line at 100 μM concentration.

6. CONCLUSIONS AND FUTURE ASPECTS

The aim of this study was to figure out whether *Papaver* spec. are antidepressant *in vivo* and expressing BDNF *in vitro* or not, as well as determining secondary metabolites which might be related with the activities. At the end of the study, we have achieved our aim by finding natural compounds that have CNS activity, through increasing BDNF expression in SH-SY5Y cell line.

We conclude the following:

- *Papaver lacerum* extract within the 5 species possess strong antidepressant like activity *in vivo* and *in vitro*.
- We isolated one known; Tyrosol-1-*O*-*β*-xylopyranosyl-(1→6)-*O*-*β*-glucopyranoside (TXG) and one new glycoside; 5-*O*-(6-*O*-*α*-rhamnopyranosyl-*β*-glucopyranosyl mevalonic acid (RGM) from *Papaver lacerum.*
- *In silico* studies revealed the possible activities of the isolated compounds; anticancer and antidiabetic activities for TXG, anticancer and anti-inflammatory activities for RGM.
- Considering *in silico* results TXG and RGM were tested against HeLa cancer cell line and they both were significantly cytotoxic.
- The present of roemerine and (+)-pronuciferine alkaloids in the *P. lacerum* was confirmed by LC-MS/MS, which significantly increased BDNF expression in SH-SY5Y cells.
- Roemerine was significantly cytotoxic against HeLa cancer cell line.
- All extracts/compounds in this study are not toxic to L929 cell line at 100 μg/mL or 100 μM.

For the future studies we recommend that TXG should be tested for antidiabetic activity and RGM should be tested for anti-inflammatory activity. For the alkaloids roemerine and (+)-pronuciferine, their mechanism should be studied in detail on how they increase BDNF by determining their effect on TrKB and/or p75 receptors.

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APPENDIX 1. ETHICAL COMMITTEE APPROVAL

Evrak Tarih ve Sayısı: 16/02/2016-E.5516

T.C.
GAZİ ÜNİVERSİTESİ
Hayvan Deneyleri Yerel Etik Kurulu Başkanlığı

Sayı : 66332047-604.01.02-
Konu : Değerlendirme ve Onay

Sayın Prof.Dr. Funda Nuray YALÇIN
Hacettepe Üniversitesi
Eczacılık Fakültesi
Öğretim Üyesi

Araştırmacı grubu Funda Nuray YALÇIN, Esra AKKOL, Ömer BAYAZEİD ve Mert İLHAN'dan oluşan, G.Ü.ET-16.014 kod numaralı ve ***"Bazı Papaver L. Türleri Üzerinde Farmakognozik Araştırmalar"*** adlı başlıklı araştırma öneriniz incelenmiş ve Gazi Üniversitesi Hayvan Deneyleri Yerel Etik Kurul Yönergesindeki ilkelere uygun olduğu saptanarak onaylanmasına oy birliği ile karar verilmiştir.

Bilgilerinizi rica ederim.

It is unanimously approved that the research project numbered G.Ü.ET-16.014 and entitled ***"Pharmacognostical Studies on Some Papaver Species"*** is in compliance with Gazi University Animal Experiments Local Ethics Commitee regulations.

With my best regards.

e-imzalıdır
Prof. Dr. Leyla AÇIK
Kurul Başkanı

EK :
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GAZİ ÜNİVERSİTESİ
HAYVAN DENEYLERİ YEREL ETİK KURULU TOPLANTI KATILIM LİSTESİ

TOPLANTI TARİHİ :08.01.2016	TOPLANTI SAYISI : 01
ADI-SOYADI	**İMZA**
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Uzman Dr.Şeyda DİKER (Başkan Yrd.)	(GÖRÜŞ BİLDİRMEDİ)
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Curriculum Vitae

1. Identification:

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2. Degrees and Qualifications:

1. Master Degree in Clinical Pharmacology-University of Medical Sciences and Technology 2011.
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3. Research activities and conferences:

1. International Conference on Science and Society: Biopiracy and Phytomedicine, Mainz- Germany 24-27 July 2017-Short Lecture.

2. 9th Joint Natural Products Conference - Copenhagen, Denmark, 23-27 July 2016-Poster.

3. Trends in Natural Product Research: A Young Scientists Meeting of PSE and IUNG-PIB- Pulawy, Poland, 30 May -2 June 2016- Short Lecture.

4. International Congresses Annual Meeting of the Society for Medicinal Plant and Natural Product Research in Budapest, Hungary, 23 - 27 August 2015-Poster.

5. International Symposium: Natural Products and Drug Discovery – Future Perspectives in Vienna University of Technology/ Vienna, Austria, 13 -14 November 2014- Poster.

4. Projects:

1. TÜBİTAK Ph.D. fellowship (2215).
2. Hacettepe University Scientific Researches Coordination Unit (Project Number: 014 D11 301 003-734).