Indian Journal of Chemistry
Vol. 63, March 2024, pp. 253-262
DOI: 10.56042/ijc.v63i3.5887

Synthesis of some benzocoumarin and benzochromone: Antielastase, antihyaluronidase, anticollagenase and antidiabetic activities and *in silico* ADME and molecular docking studies

Onur Ertik*[a], Hakan Kolancılar[b] & Refiye Yanardag[a]

[a] Division of Biochemistry, Department of Chemistry, Faculty of Engineering, Istanbul University-Cerrahpaşa, 34320, Istanbul, Turkey

[b] Division of Pharmaceutical Chemistry, Department of Pharmaceutical Professional Sciences, Trakya University, Faculty of Pharmacy, 22030 Edirne, Turkey

E-mail: onur.ertik@btu.edu.tr

Received 28 September 2023; accepted(revised) 22 February 2024

Coumarins are compounds commonly found in nature and have many biological effects. So it is a molecule that continues to be popular. Benzocoumarin and benzochromone, which are coumarin derivatives, are a group of molecules obtained from plants and it is important to understand the biological activity of the derivatives of these molecules. In our study, the inhibitory effects of newly synthesized 9-hydroxy-1-methyl-3H-naphto[2,1-b]piran-3-on (benzocoumarin)(**1**) and 9-hydroxy-2-methyl-4H-naphto[2,1-b]piran-4-on (benzochromone)(**2**) on elastase, hyaluronidase, collagenase, alpha-amylase, and alpha-glucosidase were investigated. As a result of *in vitro* studies, the inhibition effect of compounds **1** (132.64±16.12 µM) and **2** (400.25±64.77 µM)), was observed relative to the dose, only the inhibition effect on collagenase was found to have a lower IC_{50} value than the positive control (479.03±38.40 µM). In line with the data obtained as a result of *in vitro* studies, molecular docking studies were also carried out for compounds **1** and **2** with collagenase. As a result, due to the inhibitory effect of compounds **1** and **2** on the collagenase activity, a way has been opened for further studies to prove it.

Keywords: Benzocoumarin, Benzochromone, Enzyme inhibition, Molecular docking, ADME

Benzocoumarin and benzochromone are both plant-derived compounds with known biological activities and benzocoumarin is a naturally occurring compound that has been isolated from various plant species, including *Ageratum conyzoides*, *Bassia cyanean*, *Cichorium pumilum*, and *Tridax procumbens*[1]. It has been shown to exhibit anti-inflammatory and antioxidant activities in various *in vitro* and *in vivo* studies. Benzochromone, on the other hand, is a compound that is found in the resin of the *Lignum vitae* plant, which is native to the Caribbean and South America. It has been shown to have anti-inflammatory and immunomodulatory properties, as well as being potentially useful for the treatment of cancer and diabetes[2].

The fact that they have a wide biological activity causes the popularity of studies on these two substances to continue. Determining the biological activities of these newly synthesized and/or isolated substances may enable new drug discoveries. Elastase catalyzes elastin, a protein found in connective tissues such as ligaments and blood vessels, whereas hyaluronidase degrades hyaluronic acid, a major component of synovial fluid found in joints. Collagenase is also an enzyme that degrades collagen, a structural protein found in connective tissues such as cartilage. Elastase, hyaluronidase, and collagenase are enzymes that are involved in a variety of physiological processes such as tissue remodelling, inflammation, extracellular matrix disintegration, and osteoarthritis (OA)[3-5]. OA is the most prevalent type of arthritis and a chronic joint illness. It is also known as degenerative joint disease or wear-and-tear arthritis. It typically affects cartilage, the protective, cushioning tissue that surrounds the ends of bones in a joint. The activities of elastase, hyaluronidase and collagenase enzymes are increased in OA and studies on the use and development of inhibitors for these enzymes in the treatment of this disease are continuing. Collagenase in particular primarily affects joint structures containing collagen type 1, causing joint instability, with minor direct effects on articular cartilage[6].

The incidence of diabetes is increasing day by day depending on genetic predisposition, nutritional habits, and lifestyle, and it is estimated that the

number of individuals with the disease will increase in the coming years. Therefore, the discovery of substances with antidiabetic properties is important both in terms of treatment and prevention of the disease. Inhibition of enzymes can be a suitable response to this approach, and inhibition of alpha-amylase (AA) and alpha-glucosidase (AG) enzymes, which are especially active in carbohydrate metabolism, is considered a biomarker in terms of maintaining blood sugar levels[7]. The discovery of compounds with inhibitory effects on both enzymes is important in terms of lowering and controlling blood glucose levels. In addition, the antioxidant/oxidant balance is disrupted in diabetic individuals and oxidative stress occurs. Oxidative stress is characterized by an increase in the amount of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and it has been reported that the increase in these reagents is associated with skin aging[8].

In skin aging, it is known that free radicals and ROS in the connective tissue of the skin, lipid peroxidation, cross-links in DNA proteins, inactivation of some antioxidant enzymes, polymerization of polysaccharides, protease, collagenase, the release of elastases and this effect causes skin aging[9]. Since the increase in the activity of these enzymes causes skin disinformation, their inhibition creates a protective approach in terms of skin protection. In addition, collagenase types play a key role in osteoarthritis (OA), especially Matrix metalloproteinases (MMPs) 1, 8 and 13[10].

In our previous study, we synthesized two benzocoumarins and a benzochromone using Pechmann condensation with 2,7-dihydroxy-naphthalene (2,7-DHN) and ethyl acetoacetate in the presence of sulphuric acid solution[11]. In this study, it was aimed to investigate newly synthesized 9-hydroxy-1-methyl-3H-naphto[2,1-b]pyran-3-one (angular benzocoumarin) (**1**) and 9-hydroxy-2-methyl-4H-naphto[2,1-b]pyran-4-one (angular benzochromone) (**2**) compounds on the inhibition effects on AA and AG activities to determine their antidiabetic properties as well as elastase, collagenase and hyaluronidase, which are effective in skin aging and OA.

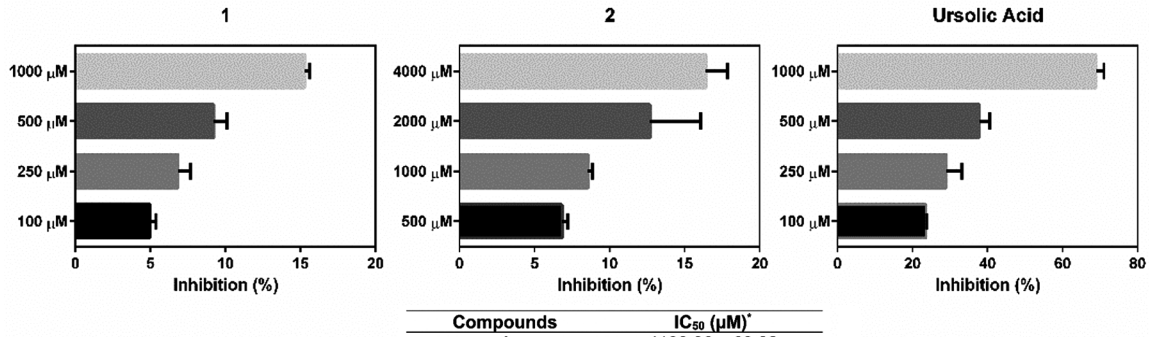
Results and Discussion

***In vitro* Enzyme Inhibition**

In Fig. 1, the elastase inhibition (%) and IC_{50} values of compounds **1**(4198.06±63.82 μM) and **2** (19952.44±4496.80 μM) and the positive control, ursolic acid (669.49±44.12 μM), are presented. Both synthesized compounds showed dose-dependent inhibition of elastase, but IC_{50} values were higher than ursolic acid. Elastase inhibitory activity of compounds **1** and **2**, and standard decreases in the order of ursolic acid >**1**>**2**.

The inhibition (%) and IC_{50} values of compounds **1** (1512.35±193.20 μM) and **2**, the standard epigallocatechin gallate (900.52±49.08 μM) on the hyaluronidase activity are given in Fig. 2. Compound **2** showed no inhibitory effect, while compound **1** showed hyaluronidase inhibitory activity due to dose increase. Hyaluronidase inhibitory activity of compounds **1** and **2**, and standard decreases in the order of epigallocatechin gallate >**1**.

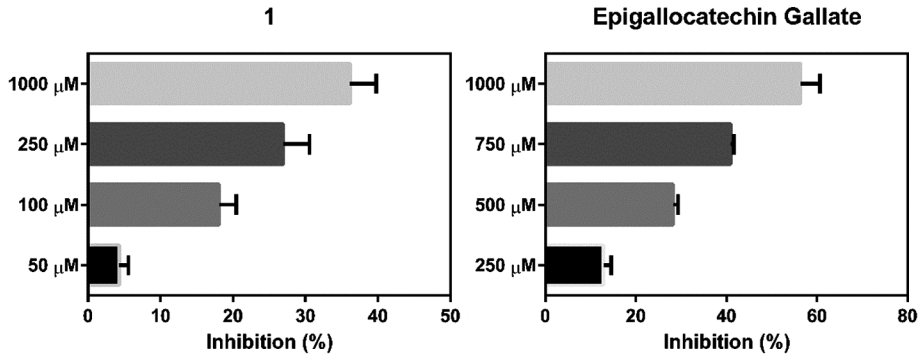
Fig. 3 gives the inhibition (%) and IC_{50} values of compounds **1** and **2** and the positive control epigallocatechin gallate on collagenase activity. It was determined that the inhibitory effect increased depending on the dose increase of compounds **1** (132.64±16.12 μM) and **2** (400.25±64.77 μM), and the IC_{50} value of both substances was lower than



Compounds	IC_{50} (μM)*
1	4198.06 ± 63.82
2	19952.44 ± 4496.80
Ursolic Acid	669.49 ± 44.12

*Mean±SD

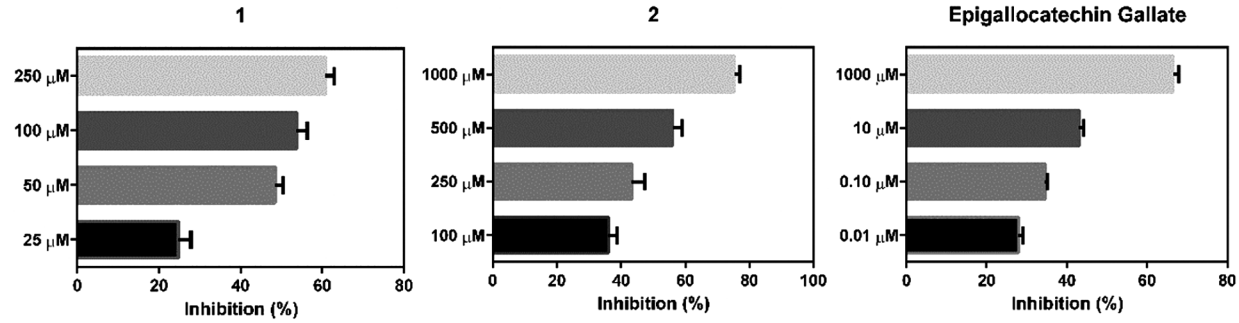
Fig. 1 — The inhibition (%) values and IC_{50} values of compounds 1 and 2, and standard on elastase activity



Compounds	IC_{50} (µM)*
1	1512.35 ± 193.20
2	N.D.
Epigallocatechin Gallate	900.52 ± 49.08

*Mean±SD

Fig. 2 — The inhibition (%) values and IC_{50} values of compounds **1** and **2**, and standard on hyaluronidase activity. N.D.: Not detected



Compounds	IC_{50} (µM)*
1	132.64 ± 16.12
2	400.25 ± 64.77
Epigallocatechin Gallate	479.03 ± 38.40

*Mean±SD

Fig. 3 — The inhibition (%) values and IC_{50} values of compounds 1 and 2, and standard on collagenase activity

epigallocatechin gallate (479.03±38.40 µM). Collagenase inhibitory activity of compounds **1** and **2**, and standard decreases in the order of **1**>**2**> epigallocatechin gallate.

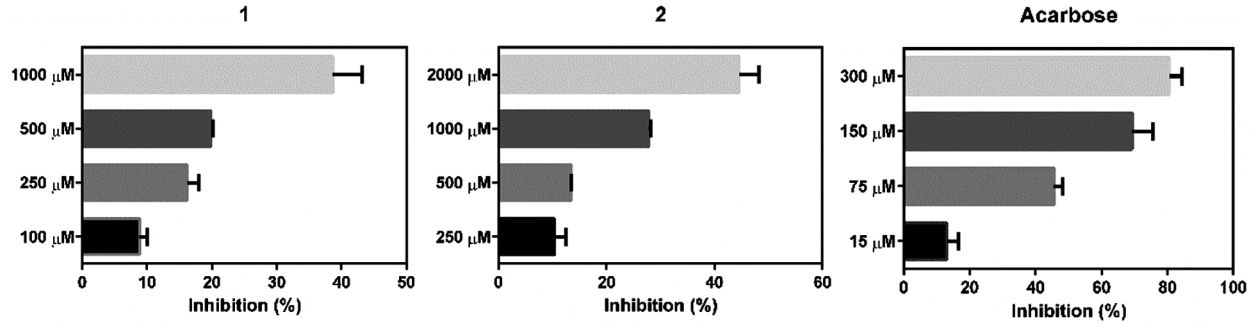
Alpha-amylase inhibition (%) and IC_{50} values of compounds **1** (1400.20±163.66 µM) and **2** (2232.77±135.79 µM), and acarbose (125.67±4.59 µM) are presented in Fig. 4. Both synthesized substances were found to have a dose-dependent inhibitory effect, but their IC_{50} values were calculated to be higher than acarbose. Alpha-amylase inhibitory activity of compounds **1** and **2**, and standard decreases in the order of acarbose >**1**>**2**.

Fig. 5 gives the alpha-glucosidase inhibition (%) and IC_{50} values for compounds **1** (3445.28±16.71 µM) and **2** (2722.66±16.43 µM) and the positive control acarbose (21.65±2.35). Compounds **1** and **2** were found to cause alpha-glucosidase inhibition depending on the dose increase. Alpha-glucosidase inhibitory activity of compounds **1** and **2**, and standard decreases in the order of acarbose >**2**>**1**.

Compounds **1** and **2** synthesized in our study inhibited the highest collagenase enzyme compared to the standards among elastase, hyaluronidase, collagenase, AA and AG enzymes. For this reason, the molecular docking examination in our study was performed on the collagenase enzyme.

In silico molecular docking, ADME and toxicity prediction

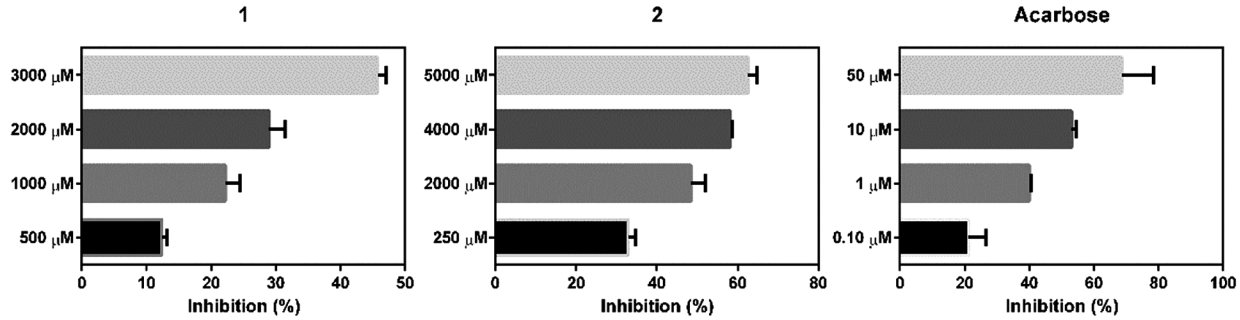
Table 1 shows the binding affinities of compounds **1**, **2**, and standards obtained with iGemdock (kcal/mol).



Compounds	IC_{50} (μM)*
1	1400.20 ± 163.66
2	2232.77 ± 135.79
Acarbose	125.67 ± 4.59

*Mean±SD

Fig. 4 — The inhibition (%) values and IC_{50} values of compounds 1 and 2, and standard on alpha-amylase activity



Compounds	IC_{50} (μM)*
1	3445.28 ± 16.71
2	2722.66 ± 16.43
Acarbose	21.65 ± 2.35

*Mean±SD

Fig. 5 — The inhibition (%) values and IC_{50} values of compounds **1** and **2**, and standard on alpha-glucosidase activity

Table 1 — Gemdock score of compounds 1 and 2, and standards (kcal/mol)

Compounds and Standards	*MMP-1*	*MMP-8*	*MMP-13*
1	–73.65	–89.81	–94.49
2	–76.62	–93.34	–95.12
EGCG	–96.70	–120.51	–112.14
Batimastat	–91.96	–73.23	–96.70
Marimastat	–87.83	–92.67	–91.07
Tanomistat	–87.14	–93.57	–120.45

When the results were examined, it was found that the binding affinity of compound **1** (–89.81 kcal/mol) to MMP-3 was higher than batimastat (–85.14 kcal/mol), while the binding affinity of compound **2** (–93.34 and –95.12 kcal/mol, respectively) was better than marimastat for both MMP-8 and MMP-13.

The CB-Dock results were given for compounds **1**, **2**, and standards in Table 2. Compound **1** (–7.8 and –8.5 kcal/mol) showed a high binding affinity to MMP-8 and MMP-13 when compared to standard marimastat (–7.4 and –7.0 kcal/mol). Also, the results highlighted that compound **2** (–7.9 and –8.5 kcal/mol, respectively) has a high binding affinity to MMP-8, and MMP-13 when compared to marimastat (–7.4 and –7.0 kcal/mol, respectively).

DockThor results are given in the Table 3 and the results showed that compound **1** (–7.835, and –8.083 kcal/mol, respectively) has higher binding affinity than marimastat (–7.359 and –7.871 kcal/mol) for MMP-1 and MMP-13. It was found that compound **2** (–7.463, –8.334 and –8.145 kcal/mol) has a higher binding affinity to MPP-1 and MMP-8 than the standard marimastat (–7.359 and –7.871 kcal/mol).

When we evaluated the results of iGemdock, DockThor, and CB-Dock, it was observed that compound **2** had a higher binding affinity for MMP-8 than marimastat with determination of molecular docking applications results. For that reason, compounds **1** and **2** was evaluated with marimastat and 2D and 3D plot of binding sites were determined with the help of DassaultSystèmes Discovery Studio, 2021.

The 3D interactions of protein-ligands obtained from CB-Dock and DockThor were given in

Table 2 — Autodock vina score obtained from CB-Dock 2 of compounds **1** and **2**, and standards (kcal/mol)

Compounds and Standards	*MMP-1*	*CurPocket ID*	*MMP-8*	*CurPocketID*	*MMP-13*	*CurPocket ID*
1	–6.5	*C1*	–7.8	*C1*	–8.5	*C1*
2	–7.8	*C1*	–7.9	*C1*	–8.5	*C1*
EGCG	–7.6	*C1*	–9.4	*C1*	–10.0	*C1*
Batimastat	–7.7	*C1*	–8.1	*C1*	–8.9	*C1*
Marimastat	–7.2	*C1*	–7.4	*C1*	–7.0	*C1*
Tanomistat	–8.1	*C1*	–9.0	*C1*	–10.6	*C1*

Table 3 — DockThor score of compounds 1 and 2, and standards (kcal/mol)

Compounds and Standards	*MMP-1*	*MMP-8*	*MMP-13*
1	–7.835	–7.593	–8.083
2	–7.463	–8.334	–8.145
EGCG	–8.399	–8.261	–8.788
Batimastat	–8.139	–8.596	–8.961
Marimastat	–7.359	–7.871	–8.318
Tanomistat	–8.339	–9.833	–9.084

Fig. 6 and 7. The green area represents the H-bond acceptors and the purple area represents the H-Bonds donors.

When the 2D diagrams of the results obtained from CB-Dock and DockThor are examined, it is seen that there are different interactions with different amino acids for compound **2** (Fig. 6 and Fig. 7). It can be argued that the reason for this difference is due to the use of different algorithms in both applications. However, both substances showed high collagenase inhibition *in vitro* and *in silico* studies. Also, EGCG has a higher binding affinity than compound **2** for all receptors.

The prediction of ADME, GI absorption, and BBB penetration of compounds **1** and **2** are given in Table 4. It was determined that both synthesized substances were suitable for Lipinski's Rule of Five, and both substances had high GI absorption and BBB penetration properties (Fig. 8).

Enzyme inhibition is one of the frequently preferred approaches in drug development studies. It is desirable that new drugs to be developed should be more effective than existing drugs and have fewer side effects. Therefore, studies continue for the discovery of new drugs for many diseases. In particular, the fact that a molecule exhibits more than one biological activity provides many advantages. Benzocoumarin and benzochromone are chemical structures frequently encountered in plants. Scientific studies are continuing to understand the significance of the inclusion of different substituents in the main structure and biological activity. The fact that benzocoumarin and benzochromone and their derivatives have many different biological activities maintains the popularity of these molecules and their derivatives[2].

OA is a degenerative joint disease that usually occurs with age. OA is caused by a combination of pathological conditions including progressive degeneration of the clamp cartilage, remodelling of the subchondral bone, and synovitis[12]. The formation of OA causes hardening of the cartilage in the joints and can affect the quality of life of patients with this condition. MMPs are a class of enzymes characterized as zinc-dependent endopeptidases and are involved in physiological processes such as during embryonic development[13] and wound healing **31**, tumour metastasis, osteoarthritis, rheumatoid arthritis, and atherosclerosis[14,15]. The MMPs are grouped under five different classes that include a) collagenases (MMP-1, 8, 13) that degrade mainly type II collagen besides type I and III collagen; b) the gelatinases (MMP-2, -9), which degrade mainly type IV collagen; c) the stromelysins (MMP-3, -10, -11), which degrade non-collagen matrix proteins; d) the matrilysins (MMP-14, -15, -16, -17, -24, -25) which degrade extracellular matrix; and e) a diverse subgroup enzymes including (MMP-7, -11, -12, -20 and MMP-23) which also degrade extracellular matrix[10]. In addition, collagen fibres provide the strength to the skin. In case of increased collagenase activity, a decrease in the resilience of the skin is observed due to the breakdown of collagen fibres. Therefore, inhibition of collagenase can be used to prevent skin irregularities that occur with aging[16]. Therefore, inhibition of MMPs is an ongoing area of drug development for the treatment of many diseases. In our study, it was determined that the *in vitro* collagenase inhibition activity of compounds **1** and **2** had a lower IC_{50} value than the positive control, EGCG. As a result of *in silico* studies, it was determined that the binding affinity of compound **2** was higher for MMP-8 than marimastat in iGemdock, DockThor, and CB-Dock results but not compound **1**. For compound 1, the same results were not obtained in all three docking programs. However, iGemdock and CB-Dock results for MMP-13 found that compound **1** showed higher binding affinity than marimastat.

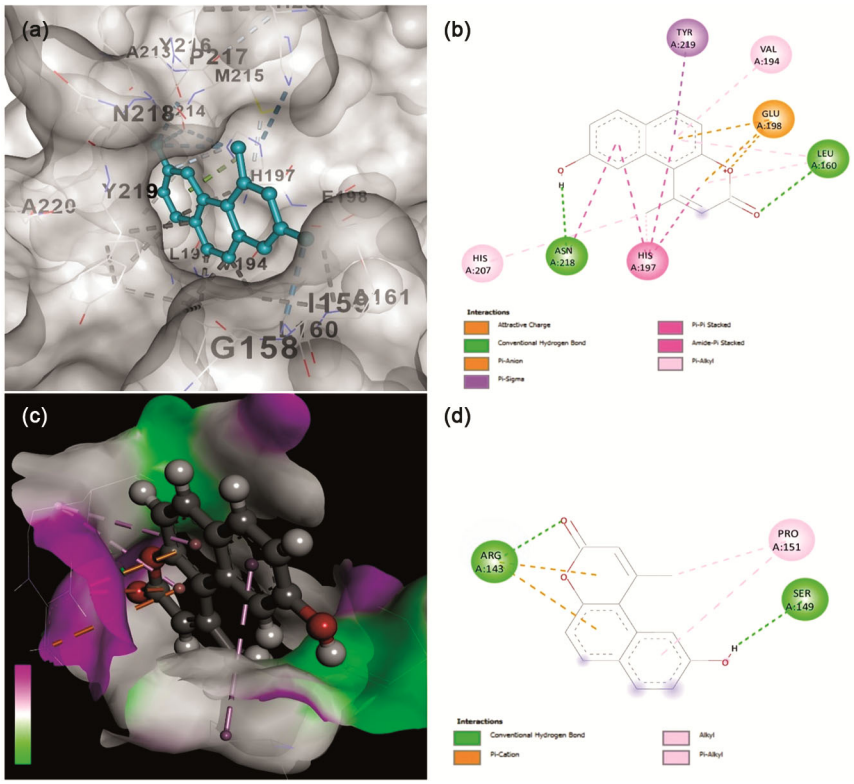


Fig. 6 — The protein-ligand interactions of compound 1. (A), (B) shows the 3D and 2D interactions of compound 1 in CB-Dock; (C), (D) shows the 3D and 2D interactions of compound 1 in DockThor

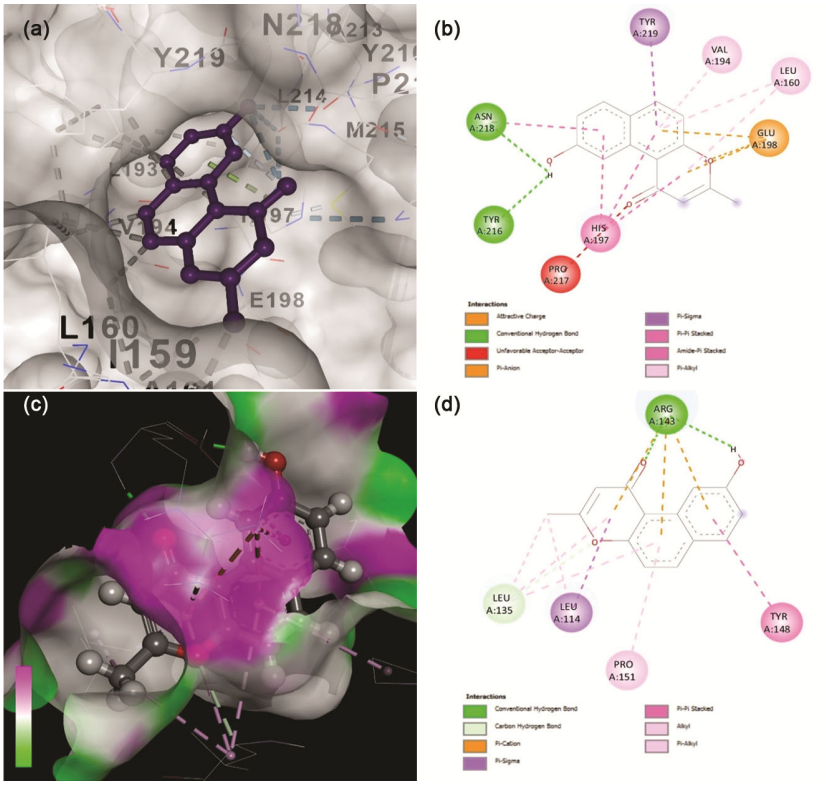


Fig. 7 — The protein-ligand interactions of compound 2. (A), (B) shows the 3D and 2D interactions of compound 2 in CB-Dock; (C), (D) shows the 3D and 2D interactions of compound 2 in DockThor

Table 4 — Lipinski's Rule of Five for absorption, distribution, metabolism, and excretion (ADME) analysis of compounds 1 and 2

Lipinski's Rule of Five						
Compounds	*Molecular Weight (g/mol)*	*Lipophilicity(MLogP)*	*H-bondDonors*	*H-bondAcceptors*	*RuleViolations*	*Drug-Likeness*
	<500	*<5*	*<5*	*<10*	*<2*	
1	226.23	2.26	1	3	0	Yes
2	226.23	1.44	1	3	0	Yes

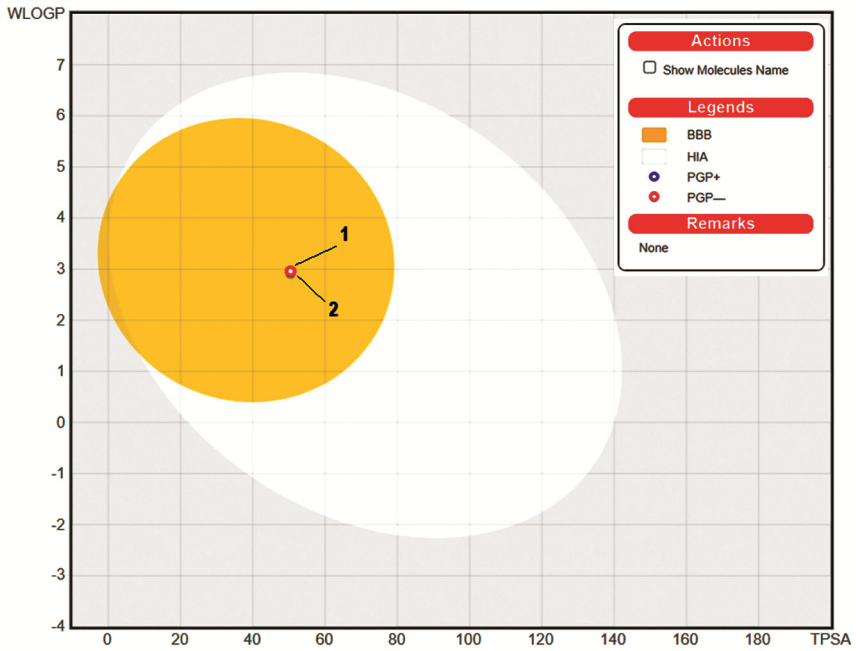


Fig. 8 — The BOILED-Egg plot of the compounds **1** and **2**. BBB; blood-brain barrier, HIA; human intestinal absorption, PGA+; is for molecules predicted to be evaluated from the central nervous system by the P-glycoprotein, PGA-; is for molecules predicted not to be evaluated from the central nervous system by the P-glycoprotein

MMP-8 is neutrophil collagenase and shows activity on collagen types 1, 2, and 3. In addition to neutrophils, MMP-8 can also be synthesized by various cells such as cytokine-stimulated mesenchymal cells, gingival fibroblasts, endothelial cells, odontoblasts, epithelial cells, macrophages, cancerous cells, plasma cells[17,18]. MMP-8 inhibition is very important because it is not involved in so many biological events. In our study, it was seen that compounds **1** and **2**, which showed collagenase inhibition, showed strong collagenase inhibition as a result of *in vitro* and *in silico* studies.

Studies in which coumarins show inhibition of MMPs are available in the literature. Yamazaki *et al.* reported that coumarin inhibited MMP-7 expression in human hepatoma cells[19]. It is known that osthol, which is also a natural coumarin, reduces MMP-9 activity[20]. The finding of the inhibitory effects of coumarins on MMPs causes continued interest in these studies. In the present study, it was found that compound **1** and **2** has a higher inhibitory effect than EGCG to collagenase according to *in vitro* studies. *In vitro* experiments, it was determined that compound **1** had a lower IC_{50} value than compound **2**, and it was determined that the binding affinity was higher than the standards in the molecular insertion methods used. According to molecular docking studies, compounds 1 and 2 demonstrated binding affinities for MMP-1, 8, and 13, with a strong attraction for MMP-8. When molecular docking experiments were compared to marimastat, an MMPs inhibitor, it was discovered that compounds 1 and 2 had better binding affinities. It can be argued that the differences in the chemical structures of compounds **1** and **2** alter the interactions with the collagenase.

In people with OA disease, there is an increase in the activity of elastase and hyaluronidase, and the increase in the activity of both enzymes helps in the degradation and wear of cartilage. Since it is important to protect cartilage in the joints, especially in individuals with OA, keeping the activity of these two enzymes under control may be helpful in the treatment

of the disease[5,21]. It has been determined that compounds 1 and 2 have inhibitory effects on elastase and hyaluronidase enzyme activities. However, IC_{50} values were above the standards for both enzymes. These inhibition effects can be increased by adding substituent groups to the structures of compounds 1 and 2.

AA and AG enzymes have antidiabetic effects, especially due to their blood glucose level-lowering effects. Therefore, inhibition of AA and AG is important in diabetes studies. Coumarin derivatives are known to cause AA and AG inhibition[22]. Similarly, benzocoumarins and benzochromone have been reported to have an inhibitory effect[2,23]. In our study, compounds 1 and 2 were found to cause dose-dependent inhibition of AA and AG, but their IC_{50} values were above positive controls. It can be suggested that compounds 1 and 2 can be the basic structure for inhibitor development studies for AA and AG, and their inhibition activities can be increased by adding different substituents to the side groups.

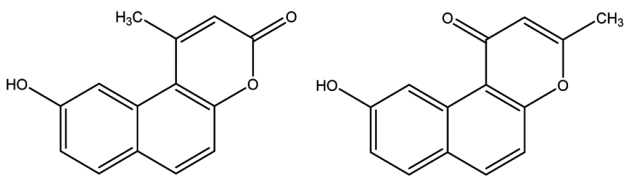
Experimental Details

Synthesis of compounds 1 and 2

In the previous study, the synthesis steps of compounds 1 and 2, ^{1}H NMR and ^{13}C NMR results are given[24]. IR spectra were recorded on a PerkinElmer Frontier FT-IR spectrophotometer. ^{1}H NMR (300 MHz) spectrum is obtained on Varian Mercury Plus model 300 MHz spectrometer. The chemicals such as 2,7-dihydroxynaphthalene, ethyl acetoacetate and H_2SO_4 were analytical grade and purchased from Merck, Aldrich and Fluka Chemical Companies and used without further purification. The purity determination of the substrates and reaction monitoring were accompanied by TLC using silica gel plates and molecular formulas of compounds 1 and 2 are given in Fig. 9.

In vitro Enzyme Inhibition Assays

In vitro elastase, hyaluronidase, collagenase, alpha-amylase and alpha-glucosidase inhibition were determined according to Moon *et al.*[25], Lee *et al.*[26], Thring *et al.*[27], Miller,[28] and Tao *et al.*[29], respectively.



Angular benzocoumarin (**1**) Angular benzochromone (**2**)

Fig. 9 — Benzocoumarin (1) and benzochromone (2)

Inhibitory activity was expressed as the percentage inhibition of all enzymes by using the following way:

$$\text{Inhibition (\%)} = [(A–B)/A]\times 100$$

where, A is the absorbance of without sample and B is the activity of the presence of the sample. The half maximal inhibitory concentrations (IC_{50} values) for all enzymes calculated regression equations prepared from the concentrations of samples and higher enzyme inhibitor activities were associated with lower IC_{50} values.

In silico molecular docking, ADME and toxicity prediction

Chemical structures of compounds **1** and **2** were written in the Avogadro program and then energy minimization of ligands with UFF was performed[30]. After this process, compounds **1** and **2** were saved as .pdb for use in molecular docking. EFGC, batimastat, marimastat and tanomastat, which are used as standard during docking operations, were downloaded from PubChem in .sdf format[31]. The 3D structures EGCG (PubChem CID: 65064), batimastat (PubChem CID: 5362422), marimastat (PubChem CID: 119031), and tanomastat (PubChem CID: 6918336) were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and converted as.pdb file with OpenBabel. The 3D structures of the collagenases were downloaded RCSB Protein Data Bank (http://www.rcsb.org). The receptors which are 2TCL (MMP-1), 3DPE (MMP-8) and 5BPA (MMP-13) were saved as .pdb format. After the preparation of the ligands and receptors, the molecular docking process was started.

Molecular docking for collagenase of compounds **1**, **2**, and standards was done using iGemdock[32], DockThor[33-35], and CB-Dock 2[36,37]. The active centers of the proteins were determined by DeepSite (https://playmolecule.com/deepsite/) and the active center scores of each enzyme and the coordinates are given in Table 5[38]. The centers with the highest scores

Table 5 — DeepSite scores of the MMP-1, MMP-3 and MMP-13

Enzymes	*ID*	*Centers*	*Score*
MMP-1	1	[71.7, 11.4, 10.6]	0.97
	2	[61.7, 17.4, 32.6]	0.85
	3	[65.7, –8.6, 10.6]	0.78
MMP-3	1	[4.9, –8.2, 7.8]	0.99
	2	[10.9, –0.2, 3.8]	0.99
	3	[18.9, –12.2, 11.8]	0.93
MMP-13	1	[9.5, 15.6, 58.0]	1.00
	2	[–14.5, 21.6, 44.0]	0.99
	3	[29.5, –4.4, 60.0]	0.99

Table 6 — The related cavity size is based on the results of CB-Dock for the receptors of MMP-1, MMP-3 and MMP-13

	MMP-1			*MMP-3*			*MMP-13*		
CurPocket ID\	*C1*	*C2*	*C3*	*C1*	*C3*	*C5*	*C1*	*C2*	*C4*
Cavity Volume (Å3)	215	174	135	1316	246	119	2380	1440	312
Center(x, y, z)	71, 8, 12	76, –2, 17	65, –6, 12	8, –5, 5	3, –12, 16	–4, –3, 12	–10, 21, 49	26, -3, 64	13, 6, 61
Dockin Size (x, y, z)	19, 19, 19	19, 19, 19	19, 19, 19	19, 19, 19	19, 19, 19	19, 19, 19	32, 19, 19	26, 19, 19	19, 19, 19

were accepted as the active center of the enzymes and these data were used in DockThor.

The virtual screening procedure of iGemdock consisted of four main steps which were: setting population size = 200, generations = 70, number of solutions = 2, and default setting = standard docking. Standard procedure was followed in DockThor (https://dockthor.lncc.br/v2/) and the number of evaluations: 1000000, population size: 750, initial seed: -1985, and a number of runs: 24 were set. The grid box was set to 20x20x20 and grid size was chosen ID1s for each enzyme for DockThor. CB-Dock2 (https://cadd.labshare.cn/cb-dock2/php/index.php) is a molecular docking server that is improved for protein-ligand blind docking, integrating cavity detection and docking. In the results of CB-Dock 2, information about the active center for each enzyme is given (Table 6).

The 3D and 2D interaction of protein-ligand was monitored by using DassaultSystèmes Discovery studio, 2021.

ADME estimation of compounds **1** and **2** was performed using the Swiss ADME (http://www.swissadme.ch/) online server (Molecular Modeling Group, Swiss Institute of Bioinformatics ©2019, online version)[39]. BOILED-Egg (brain or intestinal estimated permeation predictive model) method was used for the blood-brain barrier (BBB) and gastrointestinal (GI) absorption estimation of compounds **1** and **2**[40]**.** Also, predictions of compounds **1** and **2** in toxicity were made with ProTox-II (https://tox-new.charite.de/protox_II/)[41].

Conclusion

Inhibition of enzymes is used in the treatment of many diseases and is one of the important ways of scientific studies and drug development. MMPs are an important group of enzymes that play a role in many biological events, and therefore inhibition of MMPs can be seen as an approach in the treatment of diseases. OA is inflammation of the joints and is characterized by deterioration, wear and tear in the joint cartilage. As a result, damage begins in the joint and swelling and pain occur in the joint. The disease is most common in the feet, knees, waist, hips and fingers. It has been reported in the literature that the activity of elastase, hyaluronidase and collagenase (MMP-3, MMP-8, and MMP-13) increased in OA. The development of new inhibitors that can be used in the treatment of OA provides a treatment approach with multi-target. This study aimed to the inhibitory activity of newly synthesized compounds **1** and **2** on elastase, hyaluronidase and collagenase activity as well as alpha-amylase and alpha-glucosidase which are anti-diabetic targets. The data obtained in our study suggest that the newly synthesized compounds **1** and **2** have a high potential for OA-related enzymes, especially collagenase, and can be used in further studies in drug development studies with further research.

References

1 Jasim S F &Mustafa Y F, *J Med Chem Sci*, 5 (2022) 676.
2 Lv H N, Tu P F &Jiang Y, *Mini-Rev Med Chem,*14 (2014) 603.
3 Nagaya H, Ymagata T, Ymagata S, Iyoda K, Ito H, Hasegawa Y &Iwata H, *Ann Rheum Dis*, 58 (1999) 186.
4 Efstathiou M &Settas L, *J Rheumatol*, 28 (2017) 133.
5 Kaneva M K, *FEBS J*, 289 (2021) 113.
6 Boneva B, Ralchev N, Ganova P, Tchorbanov A &Mihaylova N, *Life*, 12 (2022) 1938.
7 Tundis R, Loizzo M R &Menichini F, *Mini Rev Med Chem*, *10* (2010) 315.
8 Verhaegen D, Smits K, Osório N &Caseiro A, *Encyclopedia*, 2 (2022) 1545.
9 Rinnerthaler M, Bischof J, Streubel M, Trost A &Richter K, *Biomolecules*, 5 (2015)545.
10 Roy H S, Dubey G, Sharma V K, Bharatam P V &Ghosh D, *J Biomol Struc Dyn*, 40 (2020) 2339.
11 Kolancilar H, *Trakya Univ J Sci*, 3 (2002) 7.
12 Li G, Yin J, Gao J, Cheng T S, Pavlos N J, Zhang C &Zheng M H, *Arthritis Res Ther*, 15 (2013) 223.
13 Shi J, Son M Y, Yamada S, Szabova L, Kahan S, Chrysovergis K, Wolf L, Surmak A &Holmbeck K, *Dev Biol*, 313 (2008) 196.
14 Hattori N, Mochizuki S, Kishi K, Nakajima T, Takaishi H, D'Armiento J &Okada Y, *Am J Pathol*, 175 (2009) 533.
15 Rose B J &Kooyman D L, *Dis, Markers,* 2016 (2016) 1.
16 Yang C C, Lin C Y, Wang H S &Lyu S R, *PLoS ONE*, 8 (2013) e79662.

17 Buffoli B, Rinaldi F, Labanca M, Sorbellini E, Trink A, Guanziroli E, Rezzani R &Rodella L F, *Int J Dermatol,* 53 (2013) 331.
18 Romanelli R, Mancini S, Laschinger C, Overall C M, Sodek J &McCulloch C A G, *Infect Immun,* 67 (1999) 2319.
19 Palosaari H, Wahlgren J, Larmas M, Rönk, H, Sorsa T, Salo T &Tjäderhane L, *J Dent Res,* 79 (2000) 77.
20 Yamazaki T &Tokiwa T, *Biol Pharm Bull,* 33 (2010) 1716.
21 Mao X, Yin W, Liu M, Ye M, Liu P, Liu J, Lian Q, Xu S &Pi R, *Brain Res,* 1385 (2011) 275.
22 Migliore A, *Clin Cases Miner Bone Metab,* 12 (2015) 31.
23 Zhao D G, Zhou A Y, Du Z, Zhang Y, Zhang K &Ma Y Y, *Fitoterapia*, 107 (2015) 122.
24 Liu Z &Ma S, *ChemMedChem*, 12 (2017) 819.
25 Moon J Y, Yim E Y, Song G, Lee N H &Hyun C G, *EurAsian J Biosci,* 4 (2010) 41.
26 Li K, Tay F R &Yiu C K Y, *Pharmacol Ther,* 207 (2020) 107465.
27 Thring T S, Hili P &Naughton D P, *BMC Comp Alter Med,* 9 (2009)27.
28 Miller G L, *J Anal Chem,* 31 (1959) 426.
29 Tao Y, Zhang Y, Cheng Y &Wang Y, *Biomed Chromatogr,* 27 (2012) 148.
30 Rappe A K, Casewit C J, Colwell K S, Goddard W A &Skiff W M, *J Am Chem Soc,* 114 (1999) 10024.
31 Kim S, Thiessen P A, Bolton E E, Chen J, Fu G, Gindulyte A, Han L, He J, He S, Shoemaker B A, Wang J, Yu B, Zhang J &Bryant S H, *Nucleic Acids Res,* 44 (2015) D1202.
32 Hsu K C, Chen Y F, Lin S R &Yang J M, *BMC Bioinform,* 12 (2011) 33.
33 de Magalhães C S, Almeida D M, Barbosa H J C &Dardenne L E, *Inform Sci,* 289 (2014) 206.
34 Santos K B, Guedes I A, Karl A L M &Dardenne L E, *J Chem Inform Model,* 60 (2020) 667.
35 Guedes I A, Barreto A M S, Marinho D, Krempser E, Kuenemann M A, Sperandio O, Dardenne L E &Miteva M A, *Sci Rep,* 11 (2021)3198.
36 Liu Y, Yang X, Gan J, Chen S, Xiao Z X &Cao Y, *Nucleic Acids Res,* 50 (2022) W159.
37 Yang J M & Chen C C, *Proteins*, 55 (2004) 288.
38 Jiménez J, Doerr S, Martínez-Rosell G, Rose A S &De Fabritiis G, *Bioinform,* 33 (2017)3036.
39 Daina A, Michielin O &Zoete V, *Sci Rep,* 7 (2017) 42717.
40 Daina A &Zoete V, *Chem Med Chem,* 11 (2016) 1117.
41 Banerjee, P, Eckert A O, Schrey A K &Preissner R, *Nucleic Acids Res,* 46 (2018) W257.