Journal of Enzyme Inhibition and Medicinal Chemistry

http://informahealthcare.com/enz
ISSN: 1475-6366 (print), 1475-6374 (electronic)

J Enzyme Inhib Med Chem, Early Online: 1–13
 DOI: 10.3109/14756366.2015.1063624

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RESEARCH ARTICLE

Coumarin or benzoxazinone based novel carbonic anhydrase inhibitors: synthesis, molecular docking and anticonvulsant studies

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Abstract

Among many others, coumarin derivatives are known to show human carbonic anhydrase (hCA) inhibitory activity. Since hCA inhibition is one of the underlying mechanisms that account for the activities of some antiepileptic drugs (AEDs), hCA inhibitors are expected to have anti-seizure properties. There are also several studies reporting compounds with an imidazole and/or benzimidazole moiety which exert these pharmacological properties. In this study, we prepared fifteen novel coumarin-bearing imidazolium and benzimidazolium chloride, nine novel benzoxazinone-bearing imidazolium and benzimidazolium chloride derivatives and evaluated their hCA inhibitory activities and along with fourteen previously synthesized derivatives we scanned their anticonvulsant effects. As all compounds inhibited purified hCA isoforms I and II, some of them also proved protective against Maximal electroshock seizure (MES) and ScMet induced seizures in mice. Molecular docking studies with selected coumarin derivatives have revealed that these compounds bind to the active pocket of the enzyme in a similar fashion to that previously described for coumarin derivatives.

Keywords

Anticonvulsant, benzoxazinone, carbonic anhydrase, coumarin, epilepsy, inhibition

History

Received 24 February 2015
Revised 12 June 2015
Accepted 13 June 2015
Published online 24 July 2015

Introduction

Epilepsy is a common neurological disorder that manifests itself in spontaneous, recurring attacks. Affecting more than 50 million people worldwide, epilepsy usually continues for a lifetime and requires permanent AED treatment, which is mainly symptomatic for the exact reason of this brain pathology remains unclear. Demand for new AEDs is high since the currently available ones fail to control seizures for approximately 30% of the patients and several serious side effects are reported[1–9].

Carbonic anhydrases (CAs; EC 4.2.1.1) catalyze the physiological hydration of CO_2 to yield bicarbonate and a proton. This reaction is involved in many physiological and pathological processes, including respiration and transport of CO_2 and bicarbonate between metabolizing tissues and lungs; pH and CO_2 homeostasis; electrolyte secretion in various tissues and organs; biosynthetic reactions such as gluconeogenesis, lipogenesis and ureagenesis; bone resorption; calcification; and tumorigenicity[10–18]. Many CA isoenzymes involved in these processes are important therapeutic targets to treat a range of disorders including edema, glaucoma, obesity, cancer, epilepsy and osteoporosis[19–24]. The active site of most CAs contains a zinc ion, hence the name metalloenzyme. Zn(II)forms three coordination bonds with histidine side chains and one with a water molecule, the former polarizing the oxygen of the water molecule to make it a stronger nucleophile for CO_2. CAs fall into four major classes, namely, cytosolic, mitochondrial, secreted and membrane-bound CAs, which in turn involve several isoforms. Among these, cytosolic and membrane-bound CAs are more widely distributed and more important as drug targets than other classes, especially isoform II is almost ubiquitous[25].

The CA inhibitors (CAIs) can be divided into four main classes[26]: (i) sulfonamides (and their isosteres, such as sulfamates, sulfamides and similar derivatives), (ii) phenols, (iii) polyamines, such as spermine, spermidine and (iv) coumarins and thiocoumarins. The primary sulfonamides (RSO_2NH_2) are classical CAIs. They are in clinical use for more than 50 years as antiglaucoma drugs. Furthermore, it has recently been reported that they have potential anticonvulsant properties. Sulthiame, topiramate, zonisamide[27–29] and lacosamide[30] are clinically used antiepileptics also showing potent inhibition of many hCA isozymes present in the brain. Most CAs inhibitors exert a Zn (II) dependent inhibition in that they either substitute Zn(II) or add to the coordination network. Unlike the other three groups, coumarins and thiocoumarins have an inhibition mechanism not dependent to Zn(II). The natural product, coumarin 6-(1*S*-hydroxy-3-methylbutyl)-7-methoxy-2*H*-chromen-2-one was shown to be hydrolyzed within the CA active site forming 2-hydroxy-cinnamic acid derivative, which possesses significant CA inhibitory properties. The latter, in turn, bound to the enzyme's active site in a completely unprecedented manner, not interacting with the Zn(II) ion but blocking the active site for substrate entrance[31,32]. Most recently,

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it was reported that some carboxylic acid derivatives inhibited hCA I, II, IX, XII with different inhibition mechanisms and this report makes carboxylic acids an interesting class of compound as inhibitors of metalloenzymes such as CAs[33].

Some of the CA inhibitors are clinically used to treat epilepsy. CA inhibition is a suggested mechanism among a few others for seizure control. Therefore, the elevation of CO_2 levels in the brain may play a crucial role in anti-seizure activity[34]. hCA isoforms II, VII and XIV are expressed in the choroid plexus, glial cells and oligodendrocytes and thought to play a role in signaling processes[35,36]. Targeting only carbonic anhydrase inhibition for the design of new anticonvulsant agents will not completely resolve seizures because several other molecular factors are involved in epilepsy etiology[7].

Coumarins are a member of a class of compounds called benzopyrones. Coumarin derivatives show various biological and pharmacological activities[37,38]. In addition to numerous activities of coumarin derivatives, they were reported to inhibit carbonic anhydrase activity[31,32] and control seizures induced with ScMet[39]. Benzimidazole consists of the fusion of benzene and imidazole. Benzimidazole bearing bioactive compounds was reported as having antihypertensive, anti-inflammatory, antimicrobial, antiviral, antioxidant, antitumor, lipid modulator and anticoagulant properties[40]. 1,4-Oxazinone derivatives have been used extensively for building bioactive compounds. Anticancer[41], antiulcer[42], antihypertensive[43], anti-inflammatory[44] and other biological activities of 1,4-oxazinones and benzoxazines were reported[45–48]. Beside various biological activities of 1,4-oxazinone and benzoxazine derivatives, anticonvulsant activities of benzoxazinones were reported in the literature[48].

In this article, with the prospect of potent CA inhibitory and anticonvulsant activities, we prepared fifteen novel coumarin and nine novel benzoxazinone-bearing imidazolium and benzimidazolium chloride derivatives (Schemes 1–4) and evaluated their inhibitory activities against purified hCA I and II isoforms. Together with fourteen derivatives available from our previous studies (Table 1), we investigated their inhibitor activities against seizures induced in mice by maximal electroshock (MES) and subcutaneous metrazole (ScMet). For selected coumarin derivatives, we performed molecular docking studies to obtain insights into binding mode of these ligands with key residues of the active site of hCA II.

Experimental

All reactions for the preparation of imidazolium and benzimidazolium salts were carried out in standard Schlenk type flasks. Chemicals were purchased from Sigma Aldrich (Istanbul, Turkey), Merck (Istanbul, Turkey), Alfa Aesar (Istanbul, Turkey). 6-(Chloroacetyl)-2*H*-1,4-benzoxazine-3(4*H*)-one, 1-methylimidazole, 1-butylimidazole and 1,2-dimethylimidazole were supplied commercially, controlled by ^{1}H-NMR and used without further purification. Melting points were determined by Electrothermal-9200 melting point apparatus. FT-IR spectra were recorded on ATR unit in the range of 400–4000 cm^{-1} with Perkin Elmer Spectrum 100 Spectrophotometer (Istanbul, Turkey). ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a Bruker AC300P FT spectrometer (Istanbul, Turkey) operating at 300.13 MHz (^{1}H), 75,47 MHz (^{13}C). Chemical shifts are given in ppm relative to TMS, coupling constants (*J*) in Hz. Elemental analyses were performed by IBTAM (Inonu University Scientific and Technological Research Central).

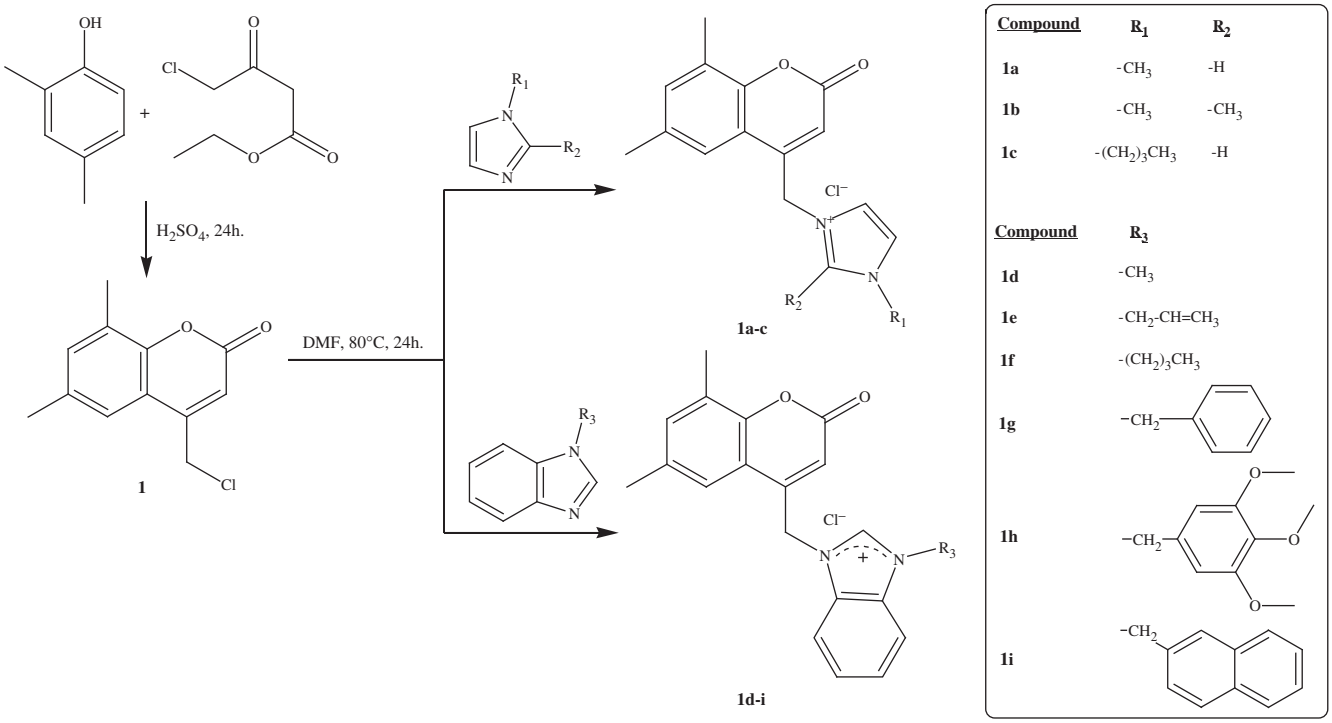
Synthesis and characterization of the compounds

Synthesis of 1-alkylbenzimidazoles and 1,1′-benzimidazoles

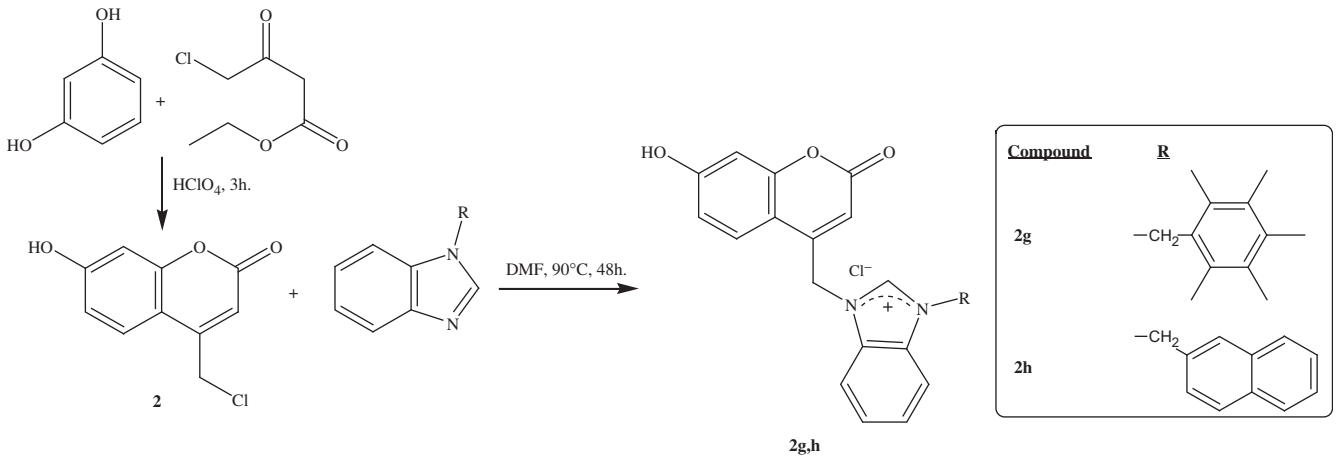
1-Alkylbenzimidazole derivatives were synthesized by the procedure described by Ozdemir et al.[49].

*Synthesis of 4-chloromethyl-6,8-dimethylcoumarin and 6,8-dimethylcoumarin bearing imidazolium and benzimidazolium salts **(1a–i)***

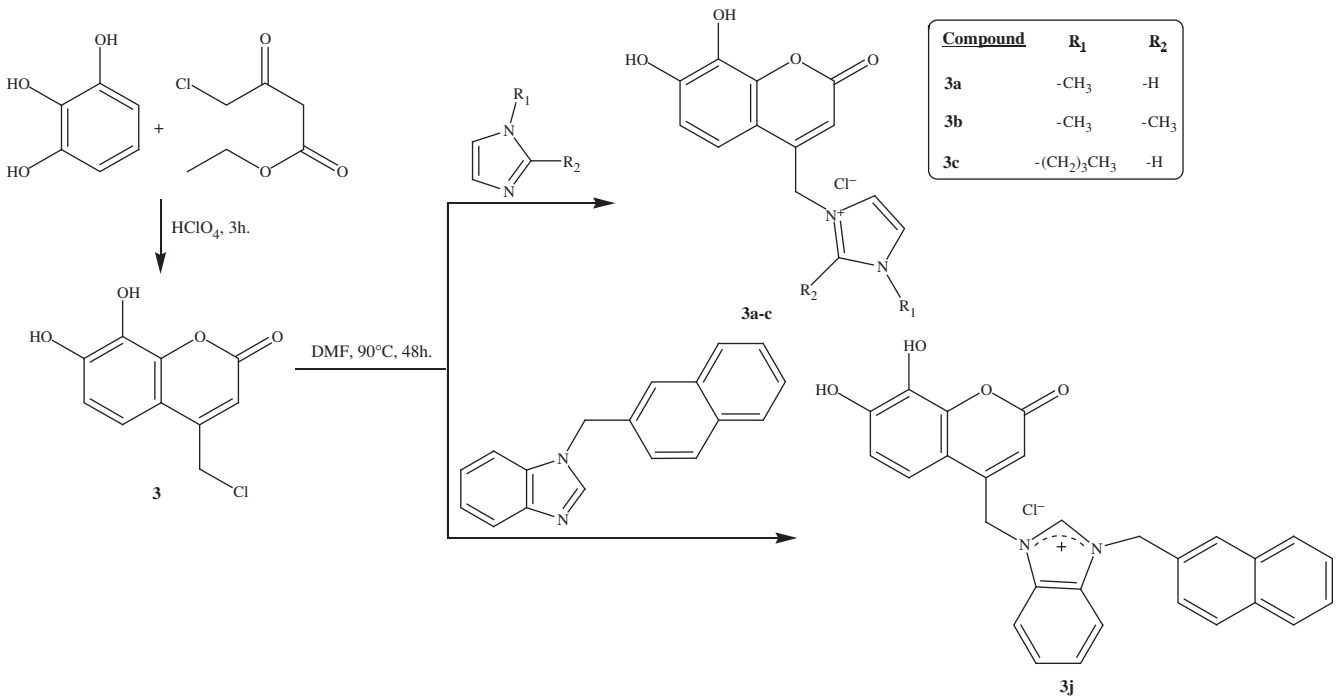
4-Chloromethyl-6,8-dimethylcoumarin (**1**) was synthesized according to the procedure described by Frasinyuk[50] (Scheme 1). Later, imidazolium and benzimidazolium salts (**1a–i**) were synthesized. About 10 mmol 1-alkylbenzimidazole or 1-alkylimidazole was dissolved in 5 mL DMF and 10 mmol compound **1** was added to this solution and the resulting mixture



Scheme 1. Synthesis of 6,8-dimethyl coumarin bearing imidazolium and benzimidazolium salts (**1a–i**).



Scheme 2. Synthesis of 7-hydroxy coumarin bearing benzimidazolium salts (**2g**, **h**).



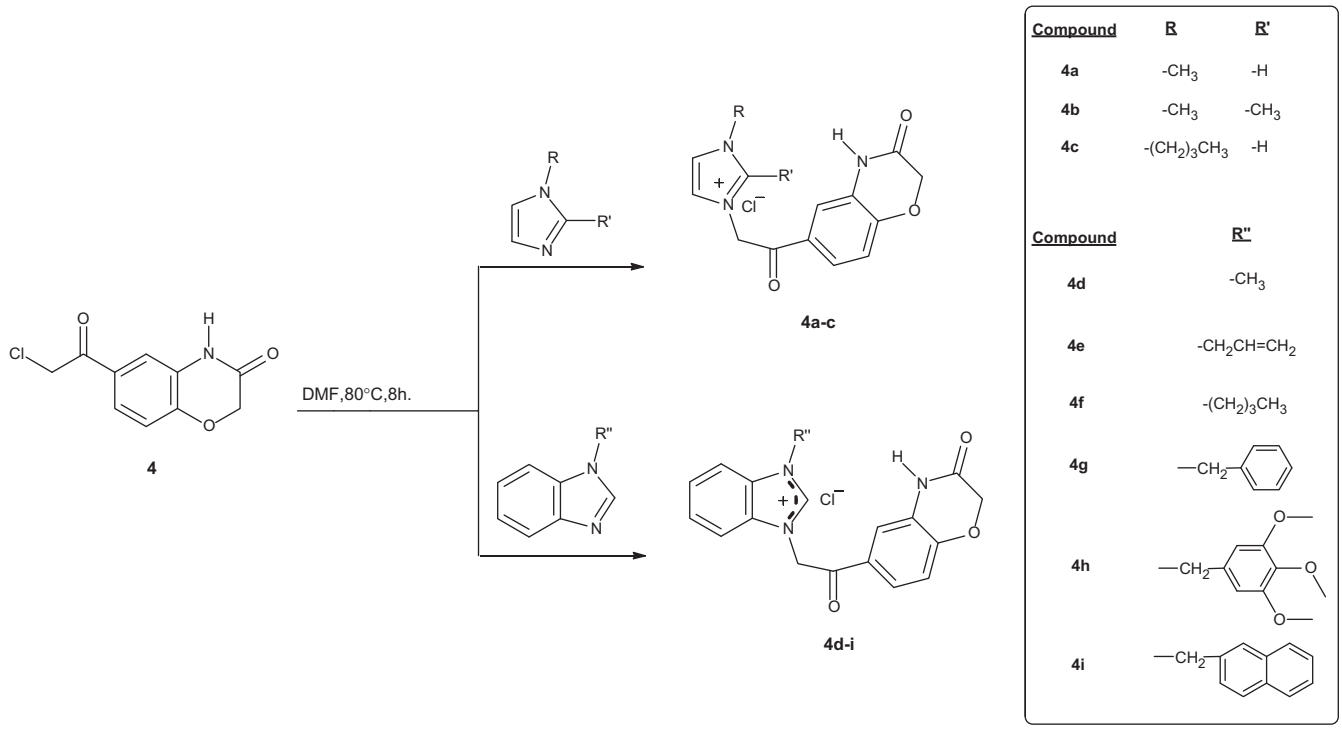
Scheme 3. Synthesis of 7,8-dihydroxy coumarin bearing imidazolium and benzimidazolium salts (**3a–c**, **j**).

was heated for 24 h at 80 °C. Then, the mixture was cooled to ambient temperature and 20 mL diethyl ether was added and the resulting precipitate was collected by filtration. The crude product was washed with hexane (2 × 10 mL) and diethyl ether (10 mL) then dried under reduced pressure.

*1-Methyl-3-(4-methylene-6,8-dimethyl-2H-chromen-2-one)imidazolium chloride (**1a**).* White solid, yield; 89%, m.p.: 271–273 °C; Anal Calcd for $C_{16}H_{17}ClO_2N_2$; C: 63.06, H: 5.62, N: 9.19, found: C: 62.88, H: 5.88, N: 9.30; IR (cm^{-1}): 1707 (–C=O), 1604 (–C=C–); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 9.41(s, 1H, –NC<u>H</u>N–), 7.42–7.91(4H, Ar-H, –NC<u>H</u> = C<u>H</u>N–), 6.06 (s, 1H, –C=C–<u>H</u>), 5.86 (s, 2H, coumarin–C<u>H</u>$_2$–N–), 3.91 (s, 3H, –N–C<u>H</u>$_3$), 2.35 (s, 3H, Ar-C<u>H</u>$_3$), 2.34 (s, 3H, Ar-C<u>H</u>$_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 160.0, 150.0, 149.9, 138.7, 135.2, 134.0, 125.9, 124.7, 123.4, 122.3, 116.8, 113.7, 48.8, 36.6, 20.8, 15.6.

*1-Methyl-2-methyl-3-(4-methylene-6,8-dimethyl-2H-chromen-2-one)imidazolium chloride (**1b**).* White solid, yield; 92%, m.p.: 288–289 °C; Anal Calcd for $C_{17}H_{19}ClO_2N_2$; C: 64.05, H: 6.01, N: 8.79, found: C: 64.23, H: 6.23, N: 8.62; IR (cm^{-1}): 1714 (–C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 7.45–7.82 (m, 4H, Ar-<u>H</u>, NC<u>H</u> = C<u>H</u>N), 5.89 (s, 2H, coumarin–C<u>H</u>$_2$–N–), 5.78 (s, 1H, –C=C–<u>H</u>), 3.84 (s, 3H, –N–C<u>H</u>$_3$), 2.60 (s, 3H, –NC(C<u>H</u>$_3$)N–), 2.40 (s, 3H, Ar-C<u>H</u>$_3$), 2.37 (s, 3H, Ar-C<u>H</u>$_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm:160.0, 150.1, 149.8, 146.4, 135.1, 133.7, 125.7, 123.7, 122.5, 122.1, 116.8, 111.7, 48.0, 35.6, 20.9, 15.6, 9.8.

*1-Butyl-3-(4-methylene-6,8-dimethyl-2H-chromen-2-one)imidazolium chloride (**1c**).* White solid, yield; 87%, m.p.: 262–264 °C; Anal Calcd for $C_{19}H_{23}ClO_2N_2$; C: 65.79, H: 6.68, N: 8.08, found: C: 65.65, H: 6.99, N: 7.98; IR (cm^{-1}): 3114 (–C=C–H), 1716 (–C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 9.60 (s, 1H,



Scheme 4. Synthesis of benzoxazinone bearing imidazolium and benzimidazolium salts (**4a**–**i**).

NCHN), 7.96 (s, 2H, NCH=CHN), 7.53 (s, 1H, Ar-H), 7.41 (s, 1H, Ar-H) 6.13 (s, 1H, C=C–H), 5.86 (s, 2H, coumarin–CH_2–N–), 4.24 (t, 2H, $J = 7.1$ Hz, $NCH_2CH_2CH_2CH_3$), 2.36 (s, 3H, Ar-CH3), 2.35 (s, 3H, Ar-CH_3), 1.82 (five, 2H, $J = 7.2$ Hz, $NCH_2CH_2CH_2CH_3$), 1.26 (six, 2H, $J = 7.5$ Hz, $NCH_2CH_2CH_2CH_3$), 0.91 (t, 3H, $J = 7.4$ Hz, $NCH_2CH_2CH_2CH_3$) ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 159.9, 150.0, 149.5, 137.7, 135.2, 133.9, 125.9, 123.6, 123.5, 122.3, 116.8, 114.5, 49.4, 48.9, 31.6, 19.3, 15.6, 13.4.

*1-Methyl-3-(4-methylene-6,8-dimethyl-2H-chromen-2-one)benzimidazolium chloride (**1d**).* White solid, yield; 88%, m.p.: 164–166 °C; Anal Calcd for $C_{20}H_{19}ClO_2N_2$; C: 67.70, H: 5.40, N: 7.89, found: C: 67.55, H: 5.68, N: 7.80; IR (cm^{-1}): 1714 (–C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 10.0 (s, 1H, NCHN), 7.46–8.14 (m, 6H, Ar-H), 6.23 (s, 2H, coum–CH_2–N–), 5.94 (s, 1H, –C=C–H), 4.16 (s, 3H, N–CH_3), 2.42 (s, 3H, Ar-CH_3), 2.38 (s, 3H, Ar-CH_3); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 160.0, 149.9, 149.5, 144.3, 135.2, 133.8, 132.6, 132.1, 131.5, 127.4, 127.0, 125.8, 122.6, 116.8, 114.4, 114.0, 112.6, 46.8, 34.1, 20.9, 15.6.

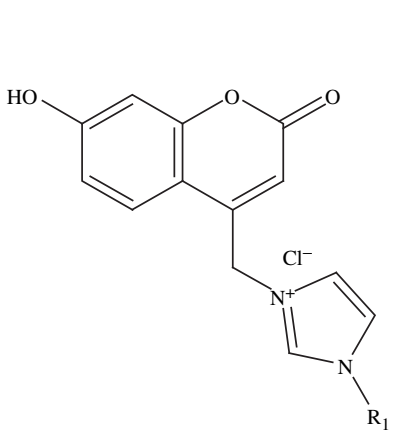
*1-Allyl-3-(4-methylene-6,8-dimethyl-2H-chromen-2-one)benzimidazolium chloride (**1e**).* White solid, yield; 78%, m.p.: 243–246 °C; Anal Calcd for $C_{22}H_{21}ClO_2N_2$; C: 69.38, H: 5.56, N: 7.34, found: C: 69.22, H: 5.79, N: 7.29; IR (cm^{-1}): 1706 (–C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 10.06 (s, 1H, –NCHN–), 7.47–8.14 (m, 6H, Ar-H), 6.17 (s, 2H, coumarin–CH_2–N–), 6.16 (ddt, 1H, –CH2–CH = CH′H″, $J_{H–CH2} = 5.9$ Hz, $J_{H–H'} = 10.3$ Hz, $J_{H–H''} = 16.3$ Hz), 5.99 (s, 1H, –C=C–H), 5.55–5.44 (dd, 2H, –CH_2–CH=CH′H″, $J_{H'–H''} = 1.1$ Hz, $J_{H–H''} = 15.99$ Hz), 5.25 (d, 2H, –CH_2–CH=CH′H″, $J_{CH2–H} = 5.9$ Hz), 2.42 (s, 3H, Ar-CH_3), 2.38 (s, 3H, Ar-CH_3); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 159.9, 149.9, 149.3, 144.1, 135.2, 133.8, 131.8, 131.4, 127.5, 127.3, 127.2, 125.9, 122.5, 121.7, 116.9, 114.7, 114.3, 112.9, 49.7, 47.1, 20.9, 15.6.

*1-Butyl-3-(4-methylene-6,8-dimethyl-2H-chromen-2-one)benzimidazolium chloride (**1f**).* White solid, yield; 85%, m.p.: 253–255 °C; Anal Calcd for $C_{23}H_{25}ClO_2N_2$; C: 69.60, H: 6.35, N: 7.06, found: C: 69.41, H: 6.59, N: 7.18; IR (cm^{-1}): 1722 (–C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 9.95 (s, 1H, NCHN), 7.16–8.19 (m, 6H, Ar-H), 6.10 (s, 2H, coumarin–CH_2–N–), 5.89 (s, 1H, –C=C–H), 4.84 (t, 2H, $J = 7.3$ Hz, –$NCH_2CH_2CH_2CH_3$), 2.36 (s, 3H, Ar-CH_3), 2.33 (s, 3H, Ar-CH_3), 1.89 (five, 2H, $J = 7.3$ Hz, $NCH_2CH_2CH_2CH_3$), 1.28 (six, 2H, $J = 7.3$ Hz, $NCH_2CH_2CH_2CH_3$), 0.88 (t, 3H, $J = 7.3$ Hz, $NCH_2CH_2CH_2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 160.1, 149.9, 149.1, 143.5, 135.3, 134.1, 131.8, 131.6, 127.6, 127.5, 126.0, 122.3, 116.7, 114.5, 114.1, 113.0, 47.4, 47.0, 30.7, 20.8, 19.5, 15.5, 13.8.

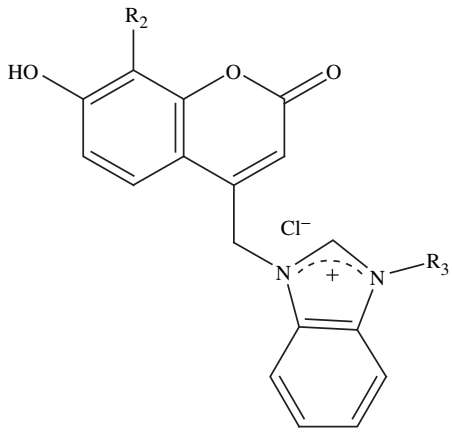
*1-Benzyl-3-(4-methylene-6,8-dimethyl-2H-chromen-2-one)benzimidazolium chloride (**1g**).* White solid, yield; 77%, m.p.: 219–221 °C; Anal Calcd for $C_{26}H_{23}ClO_2N_2$; C: 72.47, H: 5.38, N: 6.50, found: C: 72.34, H: 5.58, N: 6.40; IR (cm^{-1}): 1721 (–C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 10.24 (s, 1H, NCHN), 7.36–8.13 (m, 11H, Ar-H), 6.23 (s, 2H, –CH_2–N–), 6.06 (s, 1H, –C=C–H), 5.85 (s, 2H, –PhCH_2–N–), 2.40 (s, 3H, Ar-CH_3), 2.38 (s, 3H, Ar-CH_3); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 159.9, 150.0, 149.1, 144.1, 135.2, 134.2, 133.8, 131.9, 131.6, 129.5, 129.4, 129.3, 127.6, 127.5, 125.1, 122.5, 116.9, 114.7, 114.5, 113.3, 50.7, 47.2, 20.9, 15.6.

*1-(3,4,5-Trimethoxybenzyl)-3-(4-methylene-6,8-dimethyl-2H-chromen-2-one)benzimidazolium chloride (**1h**).* White solid, yield; 90%, m.p.: 158–160 °C; Anal Calcd for $C_{29}H_{29}ClO_5N_2$; C: 66.86, H: 5.61, N: 5.38, found: C: 66.67, H: 5.79, N: 5.28; IR (cm^{-1}): 1719 (–C=O), 1650 (–C=C–); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 10.13 (s, 1H, NCHN), 7.01–8.23 (m, 8H, Ar-H), 6.21 (s, 2H, coumarin–CH_2–N–), 5.97 (s, 1H, –C=C–H), 5.70 (s, 2H, –N–CH_2Ph), 3.78 (s, 6H, Ar-OCH_3), 3.74 (s, 3H, Ar-OCH_3), 2.39 (s, 3H, Ar-CH_3), 2.37 (s, 3H, Ar-CH_3); ^{13}C-NMR

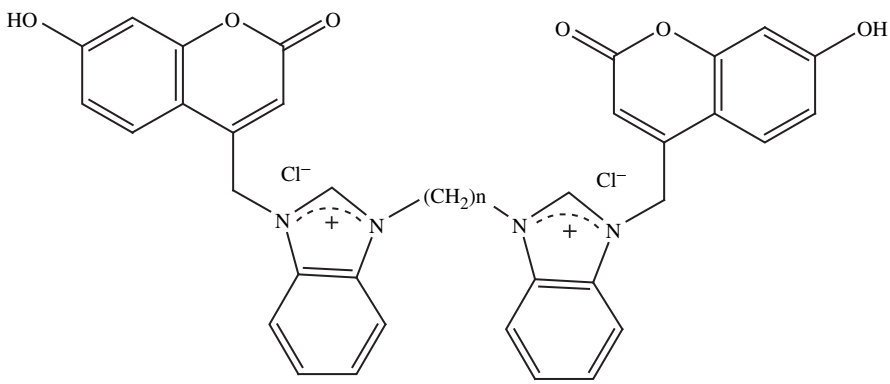
Table 1. Structures of **2a–j** and **3d–i** (these compounds were available from previous studies).



2a,b



2c-f, 3d-i



2i,j

Compounds	R_1	R_2	R_3	*n*
2a	$-CH_3$	–	–	–
2b	$-(CH_2)_3CH_3$	–	–	–
2c	–	–H	$-CH_3$	–
2d	–	–H	$-(CH_2)_3CH_3$	–
2e	–	–H	Benzyl	–
2f	–	–H	3,4,5-Trimethoxybenzyl	–
2i	–	–	–	4
2j	–	–	–	5
3d	–	–OH	$-CH_3$	–
3e	–	–OH	$-CH_3-CH=CH_2$	–
3f	–	–OH	$-(CH_2)_3CH_3$	–
3g	–	–OH	Benzyl	–
3h	–	–OH	3-Methylbenzyl	–
3i	–	–OH	3,4,5-Trimethoxybenzyl	–

(DMSO-d_6, 75 MHz) *δ*/ppm: 163.8, 159.9, 153.7, 149.9, 149.4, 143.8, 138.1, 135.2, 133.8, 131.9, 131.7, 129.3, 127.6, 127.4, 122.5, 116.8, 114.7, 112.9, 107.1, 60.5, 56.5, 36.3, 31.2,20.9, 15.6.

*1-(Naphthalen-2-ylmethyl)-3-(4-methylene-6,8-dimethyl-2H-chromen-2-one)benzimidazolium chloride (**1i**).* White solid, yield; 85%, m.p.: 283–286 °C; Anal Calcd for $C_{30}H_{25}ClO_2N_2$; C: 74.91, H: 5.24, N: 5.82, found: C: 74.75, H: 5.49, N: 5.78; IR (cm^{-1}): 1717 (–C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) *δ*/ppm; 10.12 (s, 1H, NC<u>H</u>N), 7.46–8.18 (m, 13H, Ar-<u>H</u>), 6.23 (s, 2H, coumarin–C<u>H</u>$_2$–N–), 6.07 (s, 1H, –C=C–<u>H</u>), 6.01 (s, 2H, –N–C<u>H</u>$_2$Ph), 2.39 (s, 3H, Ar-C<u>H</u>$_3$), 2.38 (s, 3H, Ar-C<u>H</u>$_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz) *δ*/ppm: 159.9, 150.0, 149.2, 144.2, 135.2, 133.8, 133.3, 133.2, 132.0, 131.8, 131.6, 129.3, 128.4, 128.2, 127.6, 127.5, 127.3, 127.2, 126.3, 125.9, 122.5, 116.9, 114.7, 114.5, 114.4, 113.2, 51.0, 47.2, 20.9, 15.6.

*Synthesis of 4-chloromethyl-7-hydroxycoumarin and 7-hydroxycoumarin bearing imidazolium and benzimidazolium salts (**2g**, **2h**)*

4-Chloromethyl-7-hydroxycoumarin (**2**) and compounds **2a–2f, 2i** and **2j** were previously synthesized by our group[51] (Scheme 2). Following the synthesis of compound **2**, benzimidazolium salts (**2g**, **2h**) were synthesized. About 10 mmol 1-alkylbenzimidazole was dissolved in 5 mL DMF and 10 mmol compound **2** was added to this solution and the resulting mixture was heated for 48 h at 90 °C. Later, the mixture was cooled to room temperature.

A 20 mL diethyl ether was added and the resulting precipitates were collected by filtration. The crude product was washed with hexane (2 × 10 mL) and diethyl ether (10 mL) then dried under reduced pressure.

*1-(2,3,4,5,6-Pentamethylbenzyl)-3-(4-methylene-7-hydroxy-2H-chromen-2-one)benzimidazolium chloride (**2g**).* White solid, yield; 52%, m.p.: 203–206 °C; Anal Calcd for $C_{29}H_{29}ClO_3N_2$; C: 71.23, H: 5.98, N: 5.73, found: C: 71.37, H: 6.30, N: 5.85; IR (cm^{-1}): 3260 (Ar-OH), 1710 (–C=O), 1610 (–C=C–); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 11.02 (s, 1H, Ar-OH), 9.23 (s, 1H, –NCHN–), 6.87–8.15 (m, 7H, Ar-H), 6.05 (s, 2H, coumarin–CH_2–N–), 5.78 (s, 2H, –N–CH_2Ph), 5.58 (s, 1H, –C=C–H), 2.25 (s, 3H, Ar-CH_3), 2.23 (s, 6H, Ar-CH_3), 2.22 (s, 6H, Ar-CH_3); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 162.8, 162.4, 160.4, 155.3, 150.1, 142.7, 134.3, 133.5, 132.3, 132.0, 127.7, 127.4, 126.2, 126.1, 114.7, 114.4, 113.8, 109.5, 107.8, 103.0, 47.0, 17.5, 17.2, 16.9.

*1-(Naphthalen-2-ylmethyl)-3-(4-methylene-7-hydroxy-2H-chromen-2-one)benzimidazolium chloride (**2h**).* White solid, yield; 60%, m.p.: 270–274 °C; Anal Calcd for $C_{28}H_{21}ClO_3N_2$; C: 71.72, H: 4.51, N: 5.97, found: C: 70.89, H: 4.55, N: 5.65; IR (cm^{-1}): 3320 (Ar-OH), 1713 (–C=O), 1615(–C=C–); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 10.99 (s, 1H, Ar-OH), 10.07 (s, 1H, NHCN), 6.96–8.14 (m, 14H, Ar-H), 6.16 (s, 2H, –CH_2–N–), 5.99 (s, 2H, –CH_2–N–), 5.80 (s, 1H, –C=C–H); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 162.4, 160.3, 150.6, 149.6, 144.2, 143.9, 133.2, 133.2, 133.1, 132.0, 131.7, 131.6, 129.3, 128.4, 128.2, 127.6, 127.4, 127.3, 126.3, 115.2, 114.6, 114.4, 113.1, 110.3, 109.1, 56.5, 50.9.

*Synthesis of 4-chloromethyl-7,8-dihydroxycoumarin and 7,8-dihydroxycoumarin bearing imidazolium and benzimidazolium salts (**3a–c, 3j**)*

Compounds **3d–i** were available from our previous study[52] (Scheme 3). 4-chloromethyl-7,8-dihydroxycoumarin (**3**) was synthesized according to procedure described by Gumus[44]. Subsequently, imidazolium and benzimidazolium salts (**3a–c**, **3j**) were synthesized. About 10 mmol 1-alkylbenzimidazole or 1-alkylimidazole was dissolved in 5 mL DMF and 10 mmol compound **3** was added to this solution and the resulting mixture was heated for 48 h at 90 °C. Later, the mixture was cooled to room temperature. A 20 mL diethyl ether was added and precipitate was collected by filtration. Crude product was washed with hexane (2 × 10 mL) and diethyl ether (10 mL) then dried under reduced pressure.

*1-Methyl-3-(4-methylene-7,8-dihydroxy-2H-chromen-2-one)imidazolium chloride (**3a**).* Yellow solid, yield; 47%, m.p.: 245–248 °C; Anal Calcd for $C_{14}H_{13}ClO_4N_2$; C: 54.47, H: 4.24, N: 9.07, found: C: 54.32, H: 4.58, N: 8.93; IR (cm^{-1}): 3150 (Ar-OH), 1724 (–C=O), 1615 (–C=C–); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 9.32 (s, 1H, –NCHN–), 7.88 (s, 1H, NCH = CHN), 7.82 (s, 1H, NCH = CHN), 7.18 (d, 1H, J = 8.5 Hz, Ar-H), 6.95 (d, 1H, J = 8.5 Hz, Ar-H), 5.81 (s, 1H, –C=C–H), 5.76 (s, 2H, coumarin–CH_2–N–), 3.90 (s, 3H, –N–CH_3); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 160.4, 150.6, 150.4, 143.8, 138.2, 133.1, 124.7, 123.5 115.1, 113.1, 109.5, 48.9, 36.6.

*1-Methyl-2-methyl-3-(4-methylene-7,8-dihydroxy-2H-chromen-2-one)imidazolium chloride (**3b**).* Yellow solid, yield; 48%, m.p.: 324–326 °C; Anal Calcd for $C_{15}H_{15}ClO_4N_2$; C: 55.82, H: 4.68, N: 8.68, found: C: 55.99, H: 4.86, N: 8.83; IR (cm^{-1}): 3205 (Ar-OH), 1731 (–C=O), 1660 (–C=C–); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 7.69 (d, 1H, J = 1.9 Hz, NCH = CHN), 7.65 (d, 1H, J = 1.9 Hz, NCH = CHN), 7.14 (d, 1H, J = 8.2 Hz, Ar-H), 6.91 (d, 1H, J = 8.2 Hz, Ar-H), 5.69 (s, 2H, coumarin–CH_2–N–), 5.51 (s, 1H, –C=C–H), 2.88 (s, 3H, –N–CH_3), 2.72 (s, 3H, –NC(CH_3)N–); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 163.1, 160.5, 150.4, 150.3, 146.2, 143.6, 123.5, 122.1, 115.2, 112.9, 110.3, 107.8, 47.9, 36.4, 9.7.

*1-Butyl-3-(4-methylene-7,8-dihydroxy-2H-chromen-2-one)imidazolium chloride (**3c**).* Yellow solid, yield; 43%, m.p.: 234–236 °C; Anal Calcd for $C_{17}H_{19}ClO_4N_2$; C: 58.21, H: 5.46, N: 7.99, found: C: 58.05, H: 5.79, N: 7.78; IR (cm^{-1}): 3280, 1715 (–C=O), 1616 (–C=C–); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 9.46 (s, 1H, –NCHN–), 7.93 (s, 2H, NCH = CHN), 7.16 (d, 1H, J = 8.2, Ar-H), 6.94 (d, 1H, J = 8.2 Hz, Ar-H), 5.81 (s, 2H, coumarin–CH_2–N–), 5.76 (s, 1H, –C=C–H), 4.07 (t, 2H, J = 7.2 Hz, $NCH_2CH_2CH_2CH_3$), 1.81 (five, 2H, J = 7.2 Hz, $NCH_2CH_2CH_2CH_3$), 1.26 (six, 2H, J = 7.2 Hz, $NCH_2CH_2CH_2CH_3$), 0.91 (t, 3H, J = 7.2 Hz, $NCH_2CH_2CH_2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 160.4, 150.8, 150.1, 143.8, 137.7, 133.2, 123.7, 123.4, 115.0, 113.2, 110.4, 109.8, 49.4, 49.0, 31.6, 19.3, 13.8.

*1-(Naphthalen-2ylmethyl)-3-(4-methylene-7,8-dihydroxy-2H-chromen-2-one)benzimidazolium chloride (**3j**).* White solid, yield; 60%, m.p.: 270–274 °C; Anal Calcd for $C_{28}H_{21}ClO_4N_2$; C: 69.35, H: 4.37, N: 5.78, found: C: 69.19, H: 4.55, N: 5.65; IR (cm^{-1}): 3320 (Ar-OH), 1713 (–C=O), 1615 (–C=C–); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 10.07 (s, 1H, NHCN), 6.96–8.14 (m, 13H, Ar-H), 6.13 (s, 2H, –CH_2–N–), 5.99 (s, 2H, –CH_2–N–), 5.82 (s, 1H, –C=C–H); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 160.3, 150.6, 149.6, 144.2, 143.9, 133.2, 133.2, 133.1, 132.0, 131.7, 131.6, 129.3, 128.4, 128.2, 127.6, 127.4, 127.3, 126.3, 115.2, 114.6, 114.4, 113.1, 110.3, 109.1, 56.5, 50.9.

*Synthesis of benzoxazinone bearing imidazolium and benzimidazolium salts (**4a–i**)*

About 10 mmol 1-alkylbenzimidazole or 1-alkylimidazole was dissolved in 5 mL DMF and 10 mmol 6-chloroacetyl-2*H*-1,4-benzoxazine-3(4*H*)-one was added to this solution and the resulting mixture was heated for 8 h at 80 °C (Scheme 4). Later, the mixture was cooled to room temperature. A 20 mL diethyl ether was added and precipitate was collected by filtration. The crude product was washed with hexane (2 × 10 mL) and diethyl ether (10 mL) then dried under reduced pressure.

*1-Methyl-3-(6-acetyl-2H-1,4-benzoxazine-3(4H)-one)imidazolium chloride (**4a**).* White solid, yield; 89%, m.p.: 291–294 °C; Anal Calcd for $C_{14}H_{14}ClO_3N_3$; C: 54.64, H: 4.59, N: 13.65, found: C: 54.79, H: 4.73, N: 13.60; IR (cm^{-1}): 1672 (–C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 11.16 (s, 1H, –N–H), 9.20 (s, 1H, –NCHN–), 7.14–7.80 (m, Ar-H, NCH = CHN), 6.06 (s, 2H, benzoxazinone–CH_2–N–), 4.74 (s, 2H, –C(O)–CH_2–O–), 3.96 (s, 3H, –N–CH_3); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 190.2, 164.4, 148.6, 138.3, 128.4, 128.1, 124.9, 124.4, 123.7, 116.8, 115.7, 67.2, 55.4, 36.4.

*1-Methyl-2-methyl-3-(6-acetyl-2H-1,4-benzoxazine-3(4H)-one)imidazolium chloride (**4b**).* White solid, yield; 91%, m.p.: 288–291 °C; Anal Calcd for $C_{15}H_{16}ClO_3N_3$; C: 55.99, H: 5.01, N: 13.06, found: C: 56.16, H: 4.73, N: 13.19; IR (cm^{-1}): 1688 (–C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 11.17 (s, 1H, –N–H), 7.15–7.75 (m, 5H, Ar-H, NCH = CHN), 6.05 (s, 2H, benzoxazinone–CH_2–N–), 4.73 (s, 2H, –C(O)–CH_2–O–), 3.86 (s, 3H, N–CH_3), 2.52 (s, 3H, –NC(CH_3)N–); ^{13}C-NMR (DMSO-d_6,

75 MHz) δ/ppm: 190.0, 164.4, 148.6, 146.3, 128.5, 128.1, 125.2, 122.8, 116.7, 115.9, 67.2, 54.5, 35.4, 9.7.

*1-Butyl-3-(6-acetyl-2H-1,4-benzoxazine-3(4H)-one)imidazolium chloride (**4c**).* White solid, yield; 88%, m.p.: 213–215 °C; Anal Calcd for $C_{17}H_{20}ClO_3N_3$; C: 58.37, H: 5.76, N: 12.01, found: C: 58.50, H: 5.87, N: 11.93; IR (cm^{-1}): 1697 (–C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 11.18 (s, 1H, –N–H), 9.29 (s, 1H, –NCHN–), 7.14–7.9 (m, 5H, Ar-H, NCH = CHN), 6.05 (s, 2H, benzoxazinone–CH_2–N–), 4.74 (s, 2H, –C(O)–CH_2–O–), 4.30 (t, 2H, $J = 7.0$ Hz, $NCH_2CH_2CH_2CH_3$), 1.81 (five, 2H, $J = 7.4$ Hz, $NCH_2CH_2CH_2CH_3$), 1.29 (six, 2H, $J = 7.5$ Hz, $NCH_2CH_2CH_2CH_3$), 0.93 (t, 3H, $J = 7.4$ Hz, $NCH_2CH_2CH_2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 190.2, 164.4, 148.6, 137.9, 128.4, 128.1, 124.9, 124.6, 122.4, 116.8, 115.7, 67.2, 55.5, 49.1, 31.9, 19.2, 13.8.

*1-Methyl-3-(6-acetyl-2H-1,4-benzoxazine-3(4H)-one)benzimidazolium chloride (**4d**).* White solid, yield; 84%, m.p.: 263–264 °C; Anal Calcd for $C_{18}H_{16}ClO_3N_3$; C: 60.43, H: 4.51, N: 11.74, found: C: 60.55, H: 4.43, N: 11.70; IR (cm^{-1}): 1681 (–C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 11.15 (s, 1H, –N–H), 9.83 (s, 1H, NCHN), 7.16–8.10 (m, 7H, Ar-H), 6.51 (s, 2H, benzoxazinone–CH_2–N–), 4.75 (s, 2H, –C(O)CH_2–O–), 4.21 (s, 3H, –N–CH_3); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 190.1, 164.5, 148.7, 144.2, 132.3, 131.9, 128.5, 128.1, 127.2, 126.9, 125.4, 116.8, 115.9, 114.3, 114.1, 67.3, 53.3, 33.9.

*1-Allyl-3-(6-acetyl-2H-1,4-benzoxazine-3(4H)-one)benzimidazolium chloride (**4e**).* White solid, yield; 81%, m.p.: 263–265 °C; Anal Calcd for $C_{20}H_{18}ClO_3N_3$; C: 62.58, H: 4.73, N: 10.95, found: C: 62.78, H: 4.60, N: 10.81; IR (cm^{-1}): 1694–1676 (–C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 11.14 (s, 1H, –N–H), 9.84 (s, 1H, NCHN), 7.19–8.08 (m, 7H, Ar-H), 6.40 (s, 2H, beznoxazinone–CH_2–N–), 6.18 (m, 1H, –CH_2–CH = CH_2), 5.32–5.45 (m, 4H, –CH_2–CH=CH_2), 4.76 (s, 2H, –C(O)–CH_2–O_); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 190.0, 164.5, 148.7, 144.0, 132.5, 131.5, 131.1, 128.9, 128.5, 128.1, 127.3, 127.1, 125.3, 120.9, 116.8, 115.9, 114.5, 114.3, 67.3, 53.4,49.4.

*1-Butyl-3-(6-acetyl-2H-1,4-benzoxazine-3(4H)-one)benzimidazolium chloride (**4f**).* White solid, yield; 88%, m.p.: 236–238 °C; Anal Calcd for $C_{21}H_{22}ClO_3N_3$; C: 63.08, H: 5.55, N: 10.51, found: C: 63.35, H: 5.40, N: 10.44; IR (cm^{-1}): 1686–1673 (–C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 11.13 (s, 1H, –N–H), 9.88 (s, 1H, NCHN), 7.19–8.18 (m, 7H, Ar-H), 6.38 (s, 2H, benzoxazinone–CH_2–N–), 4.76 (s, 2H, –C(O)–CH_2–O–), 4.63 (t, 2H, $J = 7.2$ Hz, $NCH_2CH_2CH_2CH_3$), 1.92 (five, 2H, $J = 7.5$ Hz, $NCH_2CH_2CH_2CH_3$), 1.36 (six, 2H, $J = 7.6$ Hz, $NCH_2CH_2CH_2CH_3$), 0.94 (t, 3H, $J = 7.4$ Hz, $NCH_2CH_2CH_2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 190.0, 164.5, 148.7, 143.9, 132.5, 131.1, 128.5, 128.1, 127.2, 127.1, 125.4, 116.8, 115.9, 114.5, 114.2, 67.3, 53.3, 47.0, 31.0, 19.5, 13.8.

*1-Benzyl-3-(6-acetyl-2H-1,4-benzoxazine-3(4H)-one)benzimidazolium chloride (**4g**).* White solid, yield; 94%, m.p.: 222–226 °C; Anal Calcd for $C_{24}H_{20}ClO_3N_3$; C: 66.44, H: 4.65, N: 9.68, found: C: 66.67, H: 4.48, N: 9.60; IR (cm^{-1}): 3385 (–N–H), 1689 (–C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 11.15 (s, 1H, –N–H), 10.02 (s, 1H, –NCHN–), 7.19–8.11 (sm, 12H, Ar-H), 6.41 (s, 2H, benzoxazinone–CH_2–N–), 5.93 (s, 2H, –N–CH_2Ph), 4.76 (s, 2H, –C(O)–CH_2–O–); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 189.5, 163.9, 148.2, 143.7, 133.9, 132.1, 130.5, 129.0, 129.3, 128.8, 128.3, 128.1, 127.6, 126.8, 126.7,124.8 116.3, 115.4, 114.1, 113.9, 66.8, 53.0, 49.9.

*1-(3,4,5-Trimethoxybenzyl)-3-(6-acetyl-2H-1,4-benzoxazine-3(4H)-one)benzimidazolium chloride (**4h**).* White solid, yield; 91%, m.p.: 174–177 °C; Anal Calcd for $C_{27}H_{26}ClO_6N_3$; C: 61.89, H: 5.00, N: 8.02, found: C: 61.62, H: 4.78, N: 8.38; IR (cm^{-1}): 1706 (–C=O), 1680 (–C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 11.10 (s, 1H, –N–H), 9.89 (s, 2H, NCHN), 6.96–8.18 (m, 9H, Ar-H), 6.37 (s, 2H, benzoxazinone–CH_2–N–), 5.78 (s, 2H, –N–CH_2Ph), 4.76 (s, 2H, –C(O)–CH_2–O–), 3.79 (s, 6H, Ar-OCH_3), 3.65 (s, 3H, Ar-OCH_3); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 190.0, 164.5, 162.9, 153.7, 148.8, 144.0, 138.1, 132.6, 131.0, 129.5, 128.5, 128.1, 127.4, 127.2, 125.3, 116.8, 115.8, 114.4, 106.7, 67.2, 60.5,50.7 36.3, 31.2.

*1-(Naphthalen-2-ylmethyl)-3-(6-acetyl-2H-1,4-benzoxazine-3(4H)-one)benzimidazolium chloride (**4i**).* White solid, yield; 86%, m.p.: 243–246 °C; Anal Calcd for $C_{28}H_{22}ClO_3N_3$; C: 69.49, H: 4.58, N: 8.68, found: C: 69.35, H: 4.63, N: 8.61; IR (cm^{-1}): 3410 (–N–H), 1687 (–C=O); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ/ppm; 11.14 (s, 1H, –N–H), 10.00 (s, 1H, NCHN), 7.19–8.15 (m, 14H, Ar-H), 6.40 (s, 2H, benzoxazinone–CH_2–N–), 6.09 (s, 2H, –N–CH_2Ph), 4.76 (s, 2H, –C(O)–CH_2–O–); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ/ppm: 189.5, 163.9, 148.2, 143.7, 132.7, 132.2, 131.3, 130.6, 128.9, 128.0, 127.9, 127.7, 127.6, 126.8, 126.7, 125.6, 124.8, 116.3, 115.4, 114.1, 113.9, 66.8, 53.0, 50.2.

Carbonic anhydrase inhibition

Preparation of hemolysate and purification from blood red cells

Blood samples (25 mL) were taken from healthy volunteers. They were anticoagulated with acid-citrate-dextrose, centrifuged at $1000 \times g$ for 20 min at 4 °C and the supernatant was removed. The packed erythrocytes were washed three times with 0.9% NaCl and then haemolysed in cold water. The ghosts and any intact cells were removed by centrifugation at $3100 \times g$ for 25 min at 4 °C, and the pH of the hemolysate was adjusted to pH 8.5 with solid Tris-base. The 25 mL hemolysate was applied to an affinity column containing L-tyrosine-sulfonamide-Sepharose-4B[53] equilibrated with 25 mM Tris–HCl/0.1 M Na_2SO_4 (pH 8.5). The affinity gel was washed with 50 mL of 25 mM Tris–HCl/22 mM Na_2SO_4 (pH 8.5). The human CA (hCA) isozymes were then eluted with 0.1 M NaCl/25 mM Na_2HPO_4 (pH 6.3) and 0.1 M CH_3COONa/0.5 M $NaClO_4$ (pH 5.6), which recovered hCA-I and hCA-II, respectively. Fractions of 3 mL were collected and their absorbance measured at 280 nm.

CA enzyme assay

Carbonic anhydrase activity was measured by the Maren method which is based on determination of the time required for the pH to decrease from 10.0 to 7.4 due to CO_2 hydration[54]. The assay solution was 0.5 M Na_2CO_3/0.1 M $NaHCO_3$ (pH 10.0) and Phenol Red was added as the pH indicator. CO_2-hydratase activity [enzyme units (EU)] was calculated using the equation $t_0 - t_c/t_c$ where t_0 and t_c are the times for pH change of the non-enzymatic and the enzymatic reactions, respectively.

In vitro *inhibition studies*

For the inhibition studies of coumarin different concentrations of these compounds were added to the enzyme. Activity percentage values of CA for different concentrations of each coumarin were determined by regression analysis using Microsoft Office 2000 Excel. CA enzyme activity without a coumarin solution was accepted as 100% activity.

Anticonvulsant activity

This study was approved by the Ethics Committee of Inonu University (Date: 13/01/2011, Number: 2011/A-09).

The compounds were tested for anticonvulsant activity according to MES and ScMet tests[23,24]. Phase I evaluation was designed to identify anticonvulsant activity and neurotoxicity with MES, ScMet and rotorod tests. Dual Impedance Stimulator (Harvard Apparatus 6020), Linear Isolated Stimulator (Biopac) and corneal electrodes were used for the evaluation of anticonvulsant activity. Suspensions of the compounds in 30% aqueous of PEG 400 were administered intraperitoneally at two dose levels (30 and 100 mg/kg) 30 min and 4 h after the administration. Four Balb-C mice (20–24 g) were used for each compound. The mice were obtained from the *Laboratory Animals Research Center of Inonu University*. Pentylenetetrazole was supplied by Sigma Chemical Co. and administered subcutaneously over the back of the neck. The rotorod toxicity test was performed for neurological deficits.

MES test

MES seizures were elicited with a 60 Hz alternating current of 50 mA intensity delivered for 0.2 s via corneal electrodes. A drop of 0.9% saline was instilled in the eyes prior to the application of the electrodes in order to prevent the animal death. Abolition of the hind limb tonic extension component of the seizure was defined as protection.

Subcutaneous pentylenetetrazole (metrazol) (ScM) test

Pentylenetetrazole (85 mg/kg) which produces seizures in greater than 95% of mice was administered as a 0.5% solution subcutaneously. The animals were observed for 30 min, failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5 s duration) was defined as protection.

Neurotoxicity test

The rotorod test was used to evaluate neurotoxicity. The animals were placed on a 1-inch-diameter knurled wooden rod rotating at 6 rpm. Normal mice remain on a rod rotating at this speed indefinitely. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials. In rats, neurological deficit is indicated by ataxia and loss of placing response and muscle tone.

In silico docking studies

Enzyme setup

Determination of the consistent receptor was based on previous studies[32]. Macromolecule file (PDB code: 3EFT) was modified using the ADT package version 1.5.6rc3 (Ankara, Turkey). All water molecules were deleted and polar hydrogens were added. Subsequently, Gasteiger charges were calculated and the generated pdbqt files were saved.

Ligands

Energy minimization of compounds **1i**, **2a**, **3g** and **3j** were carried out using GAMESS module for ChemOffice version Ultra 8.0.3 (Ankara, Turkey). All data were saved as pdb with the aid of Molegro Molecular Viewer version 2.5 (Ankara, Turkey). Further modification of these partial charges of pdb files was carried out through the ADT package so that the charges of the non-polar hydrogen atoms allocated to the atom to which the hydrogen is attached. These modified pdb files saved as pdbqt files. Appropriate grid box points were determined by centering on ligand separately for each compound.

Results and discussion

Synthesis and characterization of compounds

Twenty four compounds were synthesized according to the procedures in the literature and characterized by ^{1}H-NMR, ^{13}C-NMR, FT-IR spectroscopy and elemental analysis (Schemes 1–4). Among these, two 7-hydroxycoumarin (**2g**, **2h**) and four 7,8-dihydroxycoumarin derivatives (**3a–c**, **3j**) were synthesized by the reaction of 1-alkyl imidazoles/1-alkyl benzimidazoles with 7-hydroxy/7,8-dihydroxy coumarin derivatives. 6,8-Dimethylcoumarin derivatives (**1a–i**) were obtained through the reaction of 4-chloromethyl-6,8-dimethylcoumarin with 1-alkylimidazole/1-alkylbenzimidazole derivatives in DMF at 80 °C for 24 h.

Fourteen compounds (**2a–f**, **2i**, **2j**, **3d–i**) which bear 7-hydroxycoumarin or 7,8-dihydroxycoumarin were available from the previous studies. In addition to these compounds, two novel 7-hydroxycoumarin bearing compounds (**2g** and **2h**) and four novel 7,8-dihydroxycoumarin bearing compounds (**3a–c**, **3j**) were synthesized. These six novel compounds were synthesized according to the previously described procedures[51,52]. Target compounds were synthesized by the reaction of 1-alkyl imidazoles or 1-alkyl benzimidazoles with 7-hydroxy or 7,8-dihydroxy coumarin derivatives. 1-Alkylbenzimidazoles were synthesized by the procedure described by Ozdemir[49] and 4-chloromethyl-7,8-dihidroxycoumarin was synthesized procedure described by Gumus[55]. In ^{1}H-NMR spectra of compounds **2g** and **2h**, signals for –NCHN– protons were located at 9.23 and 10.07 ppm, while signals of hydroxide protons were located at 11.02 and 10.99 ppm, respectively. Signal of olefinic proton is a characteristic one for coumarin derivatives. For compounds **2g** and **2h**, these signals were located at 5.58 and 5.80 ppm, respectively. ^{13}C-NMR, IR spectra and elemental analysis results also supported the structures the compounds. In ^{1}H-NMR spectra of compounds **3a**, **3c** and **3j**, signals of –NCHN– protons were located at 9.32, 9.46 and 10.07 ppm, respectively. Signals of free hydroxide protons for compound **3j** were located at 10.53 and 9.51 ppm but they were not observed for compounds **3a–c**. Signals of olefinic protons for four compounds were located in the range of 5.51–5.82 ppm. ^{13}C-NMR, IR spectra and elemental analyses were supportive of the structures of these compounds too. Along with these compounds, nine novel 6,8-dimethylcoumarin bearing **(1a–i)** imidazolium and benzimidazolium salts were synthesized. For synthesis of compounds **1a–i**, first, 4-chloromethyl-6,8-dimethylcoumarin (compound **1**) was synthesized according to procedure described by Frasinyuk[50]. Compounds **1a–i** were than synthesized by the reaction of compound **1** with three different 1-alkylimidazole and six different 1-alkylbenzimidazole derivatives in DMF, at 80 °C for 24 h. In ^{1}H-NMR spectra of these compounds (except **1b**), signals of –NCHN– protons are located in the range of 9.41–10.24 ppm. Signals of olefinic protons are located in the range of 5.78–6.13 ppm. In ^{13}C-NMR, signals of carbonyl carbons belong to coumarin were located in the range of 159.9–162.4 ppm. IR spectrum and elemental analyses results were supportive of the structures of compounds **1a–i**. For all synthesized compounds, ^{1}H-NMR and ^{13}C-NMR were consistent with the literature[51,52,56]. Compounds **4a–i** were synthesized by the reaction of 6-(chloroacetyl)-2*H*-1,4-benzoxazine-3(4*H*)-one (compound **1**) with three different 1-alkylimidazole and six different 1-alkylbenzimidazole derivatives in DMF, at 80 °C for 8 h. In ^{1}H-NMR spectras of compounds **4a–i** (except **4b**), signals of –NCHN– protons were located in the range of 9.20–9.97 ppm. Free –N–H protons were located in the range of 11.10–11.86 ppm. ^{13}C-NMR, IR spectrum and elemental analysis were also matching with the structures of compounds **4a–i**. For all synthesized compounds, ^{1}H-NMR and ^{13}C-NMR were consistent with the literature[56].

Table 2. IC_{50} values of synthesized and previously reported compounds against hCA I and II inhibition.

Compounds	hCA I (μM)	hCA II (μM)
1a	11.76	17.17
1b	16.41	23.00
1c	10.11	14.52
1d	12.80	15.52
1e	7.79	9.91
1f	5.34	6.01
1g	15.62	19.67
1h	12.57	16.90
1i	9.46	8.92
2a[51]	264.00	327.00
2b[51]	218.00	188.00
2c[51]	138.00	137.00
2d[51]	147.00	221.00
2e[51]	153.00	154.00
2f[51]	79.00	88.00
2g	11.76	17.17
2h	16.41	23.00
2i[51]	81.00	94.00
2j[51]	99.00	89.00
3a	10.98	56.82
3b	11.72	26.81
3c	9.48	36.98
3d[52]	28.55	49.40
3e[52]	49.32	51.45
3f[52]	48.06	29.70
3g[52]	25.63	40.01
3h[52]	22.09	28.90
3i[52]	33.10	20.33
3j	4.99	6.01
4a	8.99	14.34
4b	18.09	20.16
4c	9.06	11.20
4d	15.49	22.92
4e	6.86	10.73
4f	14.71	24.28
4g	9.90	18.42
4h	7.61	9.88
4i	6.49	8.15
Acetazolamide[57]	3.30	2.40

CA inhibition, anticonvulsant activity and structure–activity relationship

For evaluating the CA inhibitory activity, all synthesized compounds were subjected to CA inhibition assay with CO_2 as substrate. The results showed that all synthesized compounds (**1–3j**) inhibited hCA I and II enzyme activity. The inhibition values of **1a–4i** against CAs are summarized in Table 2. We determined the IC_{50} values ranging between 4.99 and 18.09 μM for hCA I and 6.01–56.82 μM for hCA II. Among them, **3j** was found to be the most active (IC_{50}: 4.99 μM for hCA I and 6.01 μM for hCA II). The synthesized compounds have a lower affinity to CAI and II compared to acetazolamide (IC_{50}: 3.30 μM for hCA I 2.40 μM for hCA II), which is used in the treatment of glaucoma[57].

The results showed that both coumarin and benzoxazinone derivatives inhibited the hCA I and II enzyme activity.

We investigated anticonvulsant activities of the synthesized compounds considering the potential anticonvulsant activities of CAIs. Anticonvulsant activity and neurotoxicity screening tests (ScMet and rotorod) results of the synthesized compounds are summarized in Table 3. The most active compounds of **1**, **2**, **3** and **4** series were also scanned with MES test (Table 4). Compounds were tested at the dose of 30 mg/kg and 100 mg/kg at 0.5 and 4 h. Seventeen compounds **(1g**, **1i**, **2c–f**, **2h**, **3a**, **3e**, **3g–i**, **4c**, **4d**, **4f**, **4g** and **4i)** were active at 100 mg/kg at 0.5 h. Twelve compounds

Table 3. ScMet and neurotoxicity screening data in mice dosed ip with the compounds.

	ScMet				Toxicity			
	½ h		4 h		½ h		4 h	
	mg/kg		mg/kg		mg/kg		mg/kg	
Compounds	30	100	30	100	30	100	30	100
1a	*	*	*	*	*	*	*	*
1b	0/1	0/1	0/1	*	0/1	0/1	0/1	*
1c	0/1	*	0/1	0/1	0/1	*	0/1	0/1
1d	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
1e	0/1	*	*	*	0/1	*	*	*
1f	0/1	0/1	*	0/1	0/1	0/1	*	0/1
1g	**1/1**	**1/1**	0/1	**1/1**	0/1	0/1	**1/1**	**1/1**
1h	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
1i	**1/1**	**1/1**	**1/1**	*	0/1	0/1	0/1	*
2a	*	*	0/1	*	*	*	0/1	*
2b	0/1	*	*	*	0/1	*	*	*
2c	0/1	**1/1**	0/1	*	0/1	0/1	0/1	0/1
2d	0/1	**1/1**	0/1	0/1	0/1	0/1	0/1	**1/1**
2e	**1/1**	**1/1**	*	*	0/1	0/1	*	*
2f	**1/1**	**1/1**	0/1	**1/1**	0/1	0/1	0/1	0/1
2g	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
2h	0/1	**1/1**	0/1	0/1	0/1	**1/1**	0/1	0/1
2i	0/1	**1/1**	**1/1**	*	0/1	**1/1**	0/1	*
2j	0/1	*	0/1	**1/1**	0/1	0/1	0/1	0/1
3a	0/1	**1/1**	0/1	**1/1**	0/1	0/1	0/1	0/1
3b	0/1	*	0/1	0/1	0/1	*	0/1	0/1
3c	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
3d	**1/1**	*	0/1	0/1	0/1	*	0/1	0/1
3e	0/1	**1/1**	0/1	**1/1**	0/1	0/1	0/1	0/1
3f	0/1	*	0/1	**1/1**	0/1	*	**1/1**	**1/1**
3g	**1/1**	**1/1**	**1/1**	**1/1**	0/1	0/1	0/1	0/1
3h	**1/1**	*	0/1	0/1	0/1	*	0/1	0/1
3i	**1/1**	**1/1**	**1/1**	**1/1**	0/1	0/1	0/1	0/1
3j	0/1	0/1	0/1	**1/1**	0/1	0/1	0/1	0/1
4a	0/1	*	0/1	0/1	0/1	*	0/1	0/1
4b	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
4c	0/1	**1/1**	0/1	0/1	0/1	0/1	0/1	0/1
4d	**1/1**	**1/1**	**1/1**	*	0/1	0/1	0/1	0/1
4e	*	*	**1/1**	*	*	*	0/1	*
4f	**1/1**	**1/1**	0/1	0/1	0/1	0/1	0/1	0/1
4g	**1/1**	**1/1**	**1/1**	0/1	0/1	0/1	0/1	0/1
4h	0/1	0/1	0/1	**1/1**	0/1	0/1	0/1	0/1
4i	**1/1**	**1/1**	**1/1**	**1/1**	0/1	0/1	0/1	0/1

Compounds were administered to mice intraperitoneally at 30 and 100 mg/kg; seizure protection and neurotoxicity were measured 0.5 and 4 h after administration. ScMet: Subcutaneous metrazole test (number of animals protected/ number of animals tested). Toxicity: Rotarod test (number of animals exhibiting toxicity/number of animals tested). 0/1: no activity or toxicity at dose level. **1/1**: noticeable activity or toxicity at dose level (activity given in bold). *Animal death.

Table 4. MES screening data of **1i**, **2f**, **3g** and **3j** in mice dosed ip.

	MES			
	½ h		4 h	
Compounds	30 mg/kg	100 mg/kg	30 mg/kg	100 mg/kg
1i	**1/1**	**1/1**	**1/1**	**1/1**
2f	0/1	**1/1**	0/1	**1/1**
3g	0/1	**1/1**	0/1	**1/1**
3j	**1/1**	**1/1**	**1/1**	**1/1**
4i	**1/1**	**1/1**	**1/1**	**1/1**

Compounds were administered to mice intraperitoneally at 30 and 100 mg/kg; seizure protection was measured 0.5 and 4 h after administration. MES: Maximal electroshock test (number of animals protected/number of animals tested). 0/1: no activity at dose level. 1/1: noticeable activity at dose level (given in bold).

(**1g**, **1i**, **2e**, **2f**, **3d**, **3g–i**, **4d**, **4f**, **4g**, **4i**) showed protection at 30 mg/kg at 0.5 h. Animal deaths were observed with compounds **1a**, **1c**, **1e**, **2a**, **2b**, **2j**, **3b**, **3d**, **3f**, **4a** and **4e** at 100 mg/kg at 0.5 h. Although compound **3d** was active at 30 mg/kg at 0.5 h, animal death was observed at 100 mg/kg at 0.5 h. At 4 h at 100 mg/kg, only eleven compounds (**1g**, **1i**, **2f**, **3a**, **3e**, **3g**, **3h**, **3i**, **3j**, **4h** and **4i**) continued protection. When we decreased the dose to 30 mg/kg at 4 h, eight compounds (**1i**, **3g–i**, **4d**, **4e**, **4g** and **4i**) showed activity. Compounds **1i** and **4d** were active at 30 mg/kg at 4 h although they caused animal death at 100 mg/kg at 4 h.

From anticonvulsant activities of the compounds, some opinions about structure–activity relationship have emerged. We can classify the compounds in two different ways: (i) imidazole or benzimidazole bearing compounds, (ii) 6,8-dimethylcoumarin (**1a–i**) or 7-hydroxycoumarin (**2a–j**) or 7,8-dihydroxycoumarin (**3a–j**) or benzoxazinone (**4a–i**) bearing compounds. When compared, benzimidazolium derivatives are much more active than imidazolium derivatives. With the imidazolium derivatives no activity was observed, except compound **3a** (active at 100 mg/kg at 0.5 and 4 h). According to these results,

Table 5. Molecular docking binding scores and binding interactions of compounds **1i**, **2a**, **3g** and **3j** within the hCA II active site.

Compounds	Docking score (kcal/mol)	H-bonds	Close van der Waals contacts
3g	−5.9	–	Lys170, Phe231, Glu239
2a	−4.2	–	Lys170, Gly171
3j	−9.7	–	Gln92, Leu198, Val121, Phe 131, Pro202, Thr199, Zn262
1i	−9.5	–	Gln92, Leu198, Val121, Phe131, Asn67, Leu60, Asn62, Gly171

Residues participating in hydrogen bonds and close van der Waals contacts (<4 Å) with the inhibitors are shown.

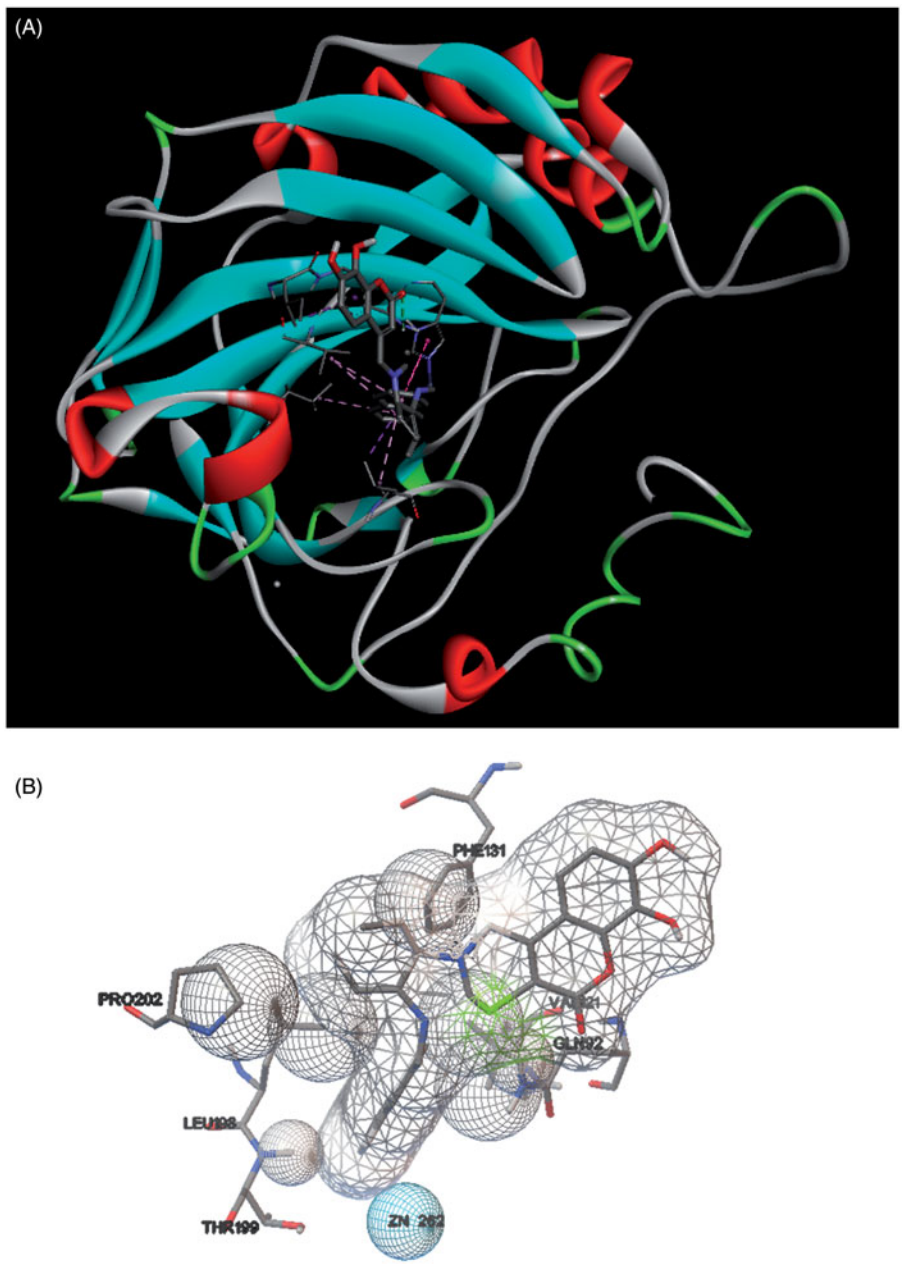


Figure 1. Docking of compound **3j** within the hCA II active site. (A) Discovery Studio 4.0 Client images and (B) ADT images.

addition of a benzene ring to the imidazolium derivatives increased lipophilicity of the compounds, making them more active than those with lower lipophilicity. In addition to these results, many imidazolium derivatives caused animal deaths. When we compare the activities of benzimidazolium derivatives, naphthalene bearing compounds (especially compounds **1i** and **3j**) showed better activity than other benzimidazolium derivatives. These results support the suggestion that lipophilicity increases anticonvulsant activity. Another factor that affect anticonvulsant activity is coumarin or benzoxazinone scaffold. 7-Hydroxycoumarin bearing imidazolium and benzimidazolium derivatives except **2f** (active at 100 mg/kg) were inactive, whereas 7,8-dihydroxycoumarin bearing derivatives were active. Compounds **3g–i** were active at any dose level at 0.5 and 4 h.

Some of the compounds were also scanned with MES test, all of which showed anti-MES activity at either dose level. **1i**, **3j** and **4i** exhibited anti MES activity at 30 and 100 mg/kg at both time intervals. Compounds **2f**, **3j** and **4i** showed anti MES activity at 100 mg/kg (Table 4). In the synthesized compounds, only **4i** showed anti ScMet and MES at any dose and time levels and toxicity was not observed for this compound.

Molecular docking studies

In order to obtain more insights into the binding mode, molecular docking studies were also performed for the coumarin derivative compounds (**1i**, **2a**, **3g** and **3j**) experimentally tested on animals and hCA activity, then all data were compared. The rationale of docking studies of coumarin derivatives is due to the fact that simple coumarin and 6-(1*S*-hydroxy-3-methylbutyl)-7-methoxy-2*H*-chromen-2-one were shown to be competitive inhibitor with CO_2 as substrate for the main isoforms for CA, i.e. human CA II[26,31,32]. Docking scores were obtained using Lamarckian Genetic Algorithm and scoring function of AutoDock 4. Afterwards, interactions were checked with the aid of ADT and Discovery Studio 4.0 Client (Ankara, Turkey). In general, the results achieved computationally were in good agreement with the experimental values. Docking scores and binding interactions of

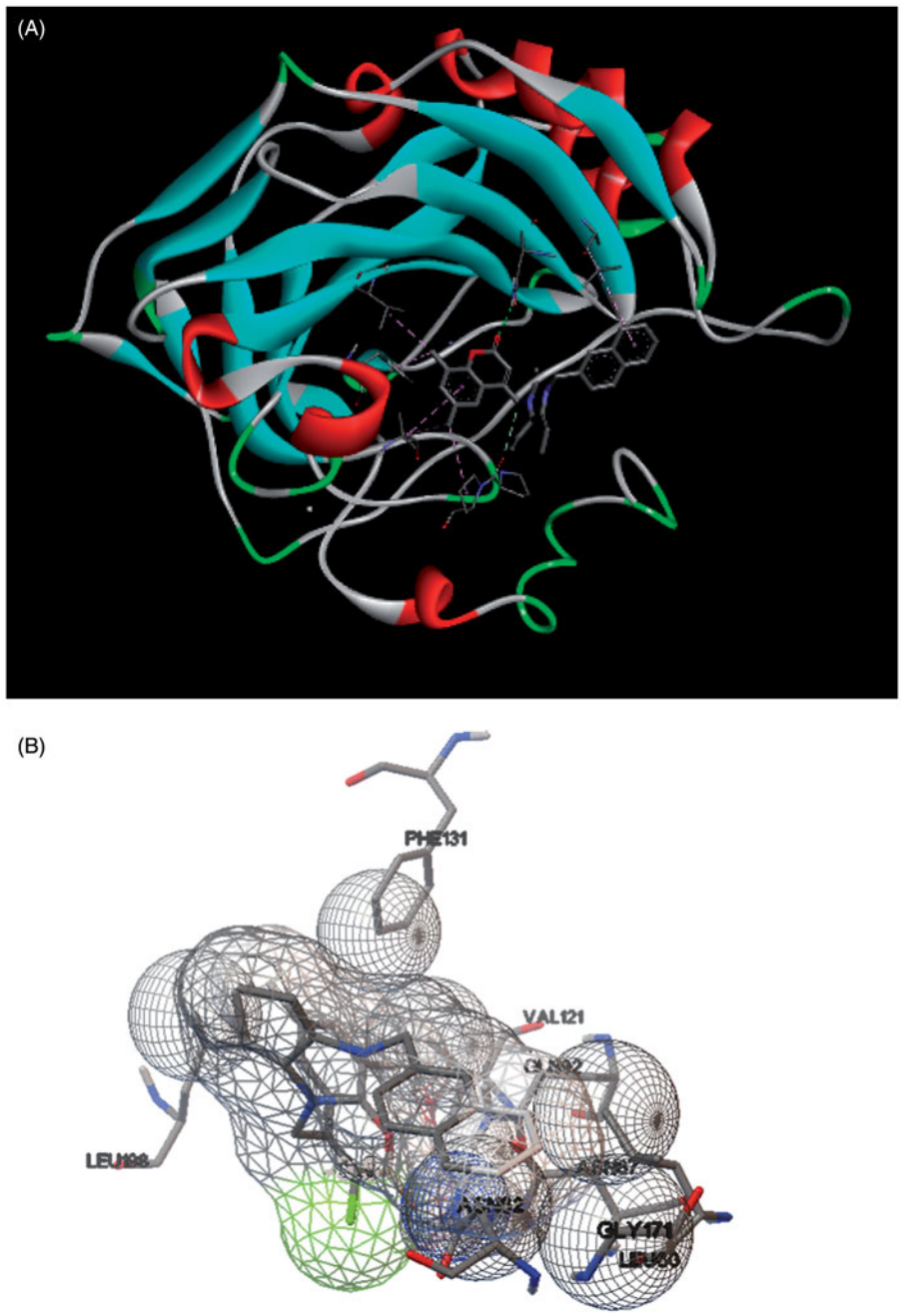


Figure 2. Docking of compound **1i** within the hCA II active site. (A) Discovery Studio 4.0 Client images and (B) ADT images.

compounds **1i**, **2a**, **3g** and **3j** with hCA II (PDB code: 3EFT) are presented in Table 5. Final images of compounds **1i** and **3j** for binding interactions are shown in Figures 1 and 2.

As seen from the data presented in Table 5, there are many amino acid residues participating in hydrogen bonds or close van der Waals (<4 Å) contacts with the inhibitors **1i**, **2a**, **3g** and **3j** when bound to the active site of hCA II. Most of these amino acids include Thr199 (one of the gate keeper residues of this enzyme) as well as the residues lining the CO_2 binding pocket, namely, Val121, Val143, Leu198, Val207 and Trp209[26,31,32]. Only compound **3j** was found coordinated to Zn(II) ion in the hCA II active site. Compound **3j** has two hydroxy groups on the coumarin scaffold. As mentioned in the ''Introduction'' section, phenols are inhibitors of coumarin derivatives and compounds **3j** may act in the active site of hCA II like phenols for binding Zn(II) ion besides non-Zinc binding interactions.

Conclusion

Coumarins are known as a novel type of hCA I. In this study, we have synthesized novel coumarin and benzoxazinone derivatives. All of the synthesized compounds inhibited hCA I and hCA II. Docking studies suggest that the mechanism of action of the compounds synthesized in this study is similar to that previously described for coumarin derivatives. CAI are known to exhibit anticonvulsant properties although their mechanisms of action have not fully been deciphered. With the prospect of potential anticonvulsant properties of CAIs, anticonvulsant activities of synthesized and previously described compounds were tested. Among the tested compounds, **4i** exhibited anti-SCM and anti-MES at either given dose level; neither animal death nor toxicity was observed. Our results suggest that these coumarin and benzoxazinone derivatives are likely to be adopted as candidates to treat glaucoma and epilepsy.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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