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### **RESEARCH ARTICLE**

### Synthesis and carbonic anhydrase inhibitory properties of novel coumarin derivatives

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#### Abstract

A newly series of water-soluble 1-alkyl-3-(4-methyl-7, 8-dihydroxy-2H-chromen-2-one) benzimidazolium chloride salts (3a-j) were synthesized and their inhibitory effects on the activity of purified human carbonic anhydrase (hCA) I and II were evaluated. hCA I and II from human erythrocytes were purified by a simple one step procedure by using Sepharose 4B-L-tyrosine-sulphanilamide affinity column. The result showed that all the synthesized compounds were inhibited the CA isoenzymes activity. Among them, **3g** and **3j** were found to be most active ( $IC_{so} = 22.09 \,\mu$ M and 20.33 µM) for hCA I and hCA II, respectively.

Keywords: Carbonic anhydrase, coumarin, benzimidazolium, inhibition

Coumarin derivatives were reported as a novel class of which is a which is a series of inhibitor of metalloenzyme carbonic anhydrase (CAM2) Benzimidazole consists of the fusion of imidazole. The most loss in nature in nature is N-ribosyl-dimethylbenzimidazole3, which serves as an axial ligand for cobalt in vitamin B12. Benzimidazole has been used widely as carbon skeleton for preparation N-heterocyclic carbenes (NHC). Benzimidazoles and their NHC's are usually used as ligand for transition metal complexes<sup>4</sup>. Besides using on synthesis of NHC's, antimicrobial activities of benzimidazolium salts were reported<sup>5</sup>.

The metalloenzyme CA (EC 4.2.1.1) catalyzes a very simple but critically important physiological reaction: the involvement of the CA enzyme family, which catalyzes the physiological hydration of CO<sub>2</sub> to yield bicarbonate and a proton, in many physiological/pathological processes open up widespread opportunities for the development of diverse, specific inhibitors for clinical application<sup>6-9</sup>.

The active site of most CAs contains a zinc ion  $(Zn^{2+})$ , which is essential for catalysis. The CA reaction is involved in many physiological and pathological processes, including respiration and transport of CO<sub>2</sub> and bicarbonate between metabolizing tissues and lungs; pH and CO<sub>2</sub> homeostasis; electrolyte secretion in various tissues and organs; biosynthetic reactions such as gluconeogenesis, lipogenesis and ureagenesis; bone resorption; calcification; and tumorigenicity<sup>10-18</sup>. Many of the CA isoenzymes involved in these processes are important therapeutic targets with the potential to be inhibited to treat a range of disorders including edema, glaucoma, obesity, cancer, epilepsy and osteoporosis<sup>19-24</sup>. Given the physiological importance of the CA, the metabolic impact of chemicals for crop production should receive greater study.

In this study, series of 10 new water-soluble 1-alkyl-3-(4-methyl-7, 8-dihydroxy-2H-chromen-2-one) benzimidazolium chloride salts (3a-j) derivatives were synthesized and their inhibitory effects on the activity of purified human carbonic anhydrase (hCA) I and II were evaluated.

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### **Materials and methods**

All reactions for the preparation of benzimidazolium salts were carried out under argon, flame-dried glassware using Standard Schlenk techniques. Chemicals were obtained by Sigma Aldrich. DMF used as solvent on synthesis of benzimidazolium salts was dried by  $P_2O_5$ .

Melting points were determined by Electrothermal-9200 melting point apparatus. FT-IR spectra were recorded on ATR unit in the range 400–4000 cm<sup>-1</sup> with Perkin Elmer Spectrum 100 Spectrophotometer. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR were recorded using a Bruker AC300P FT spectrometer operating at 300.13 MHz (1H), 75.47 MHz (<sup>13</sup>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 of 4-chloromethyl-7, 8-dihydroxy-2*H*-chromen-2-one (1)

4-chloromethyl-7, 8-dihydroxy-2*H*-chromen-2-one **(1)** was synthesized by the procedure of Gumus et al.<sup>25</sup>. Crude product purified by column chromatography (acetone/hexane 3/2 solvent system). Yield (yellow): 58%, melting point: 216–217°C<sup>26</sup>. FT-IR (cm<sup>-1)</sup>: 3542 and 3434 (Ar–OH), 3088, 1668 (C=O), 1611 (C=C). <sup>1</sup>H-NMR ( $\delta_{\rm H}$ , DMSO- $d_{\rm 6}$ ) :10.27 (s, 1H, -OH), 9.40 (s, 1H, -OH), 7.20-7.17 (d, *J* = 9 Hz, 1H, Ar-H), 6.87-6.84 (d, *J* = 9 Hz, 1H, Ar-H), 6.42 (s, 1H, -C=C-H), 4.93 (s, 2H, -CH<sub>2</sub>). <sup>13</sup>C-NMR ( $\delta_{\rm C}$ , DMSO- $d_{\rm 6}$ ): 160.6, 151.9, 150.2, 144.1, 132.9, 115.9, 112.8, 111.4, 110.6, 41.9.

### General procedure for the preparation of the 1-alkylbenzimidazole compounds (2a-2j)

1-alkylbenzimidazole compounds were synthesized by procedure of Ozdemir et al.<sup>27</sup>

### General procedure for synthesis of benzimidazolium salts (**3a-3j**)

10 mmol from 1-alkylbenzimidazole (**2a-j**) was solved in dried DMF (5 mL) and 10 mmol 4-chloromethyl-7, 8-dihydroxy-2*H*-chromen-2-one was added into the solution and the mixture heated 48 h at 90°C. After 48 h, diethyl ether was added to mixture and precipitates were collected by filtration. Crude product was washed with acetone and ethanol so that dried under reduced pressure.

### 1-methyl-3-(4-methyl-7, 8-dihydroxy-2*H*-chromen-2one) benzimidazolium chloride (**3a**)

Yield: 80%, mp: 306–308°C. Anal. cal. for.  $C_{18}H_{15}ClO_4N_2$ C:60.26, H:4.21, N:7.81; found C:60.31, H:4.24, N: 7.87. FT-IR (cm<sup>-1</sup>): 1561 (C-N), 1683 (C=O), 3250 (OH): <sup>1</sup>H-NMR ( $\delta_{\rm H}$ , DMSO- $d_{6'}$ ): 10.61 (s, 1H, -OH), 9.93 (s, 1H, NCHN), 9.5 (s, 1H, -OH), 7.00-8.12 (m, 6H, ArH), 6.14 (s, 2H, -CH<sub>2</sub>), 5.73 (s, 1H, -C=C-H), 4.14 (s, 3H, -CH<sub>3</sub>). <sup>13</sup>C-NMR ( $\delta_{c'}$ , DMSO- $d_{6}$ ): 160.3, 150.6, 149.8, 144.4, 143.8, 133.1, 132.5, 131.5, 127.4, 127.2, 115.3, 114.4, 114.0, 113.1, 110.3, 108.8, 46.8, 34.1.

### 1-(n-butyl)-3-(4-methyl-7,8-dihydroxy-2*H*-chromen-2one)benzimidazolium chloride (**3b**)

Yield: 68%. mp: 269.6°C. Anal. cal. for.  $C_{21}H_{21}ClO_4N_2$ C:62.92 H:5.28, N:6.99; found: C:62.93, H:5.28, N: 6.96. FT-IR (cm<sup>-1</sup>): 1566 (C-N), 1710 (C=O), 3320 (OH), <sup>1</sup>H-NMR ( $\delta_H$ , DMSO- $d_6$ ): 10.58 (s, 1H, -OH), 10.00 (s, 1H, NCHN), 9.5 (s, 1H, -OH), 6.96-8.19 (m, 6H, ArH), 6.10 (s, 2H, -CH<sub>2</sub>), 5.75 (s, 1H, -C=C-H), 4.52-4.57 (t, 2H, J = 7 Hz, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.89-1.99 (quint, 2H, J = 7 Hz, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.33-1.41 (six, 2H, J = 7 Hz, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 0.91-0.96 (t, 3H, J = 7 Hz, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>). <sup>13</sup>C-NMR ( $\delta$ C, DMSO- $d_6$ ): 160.3, 150.6, 149.6, 143.8, 143.7, 133.1, 131.8, 131.7, 127.5, 127.3, 115.3, 114.5, 114.2, 113.1, 110.3, 109.1, 56.5, 47.3, 30.8, 19.6, 13.9.

### 1-allyl-3-(4-methyl-7,8-dihydroxy-2*H*-chromen-2-one) benzimidazolium chloride (**3c**)

Yield: 82%. mp: 296–298°C. Anal. cal. for.  $C_{20}H_{17}ClO_4N_2$ C:62.42, H:4.45, N: 7.28; found: C: 62.45, H: 4.46, N: 7.25. FT-IR (cm<sup>-1</sup>): 1566 (C-N), 1712 (C=O), 3210 (OH). <sup>1</sup>H-NMR ( $\delta_{H'}$ , DMSO- $d_6$ ): 10.53 (s, 1H, -OH), 9.91 (s, 1H, NCHN), 9.51 (s, 1H, -OH), 6.96-8.06 (m, 6H, ArH), 6.18 (ddt, 1H, -CH<sub>2</sub>-CH=CH'H",  $J_{CH2-CH} = 5.88$  Hz,  $J_{H-H'} = 10.31$  Hz,  $J_{H-H'} = 17.17$  Hz ), 6.12 (s, 2H, -CH<sub>2</sub>), 5.74 (s, 1H, -C=C-H), 5.52-5.43 (dd, 2H, -CH<sub>2</sub>-CH=CH'H",  $J_{H'-H'} = 1.20$  Hz,  $J_{H-H'} = 15.60$  Hz), 5.23 (d, 2H, -CH<sub>2</sub>-CH=CH'H",  $J_{CH2-CH} = 5.91$  Hz). <sup>13</sup>C-NMR ( $\delta$ C, DMSO- $d_6$ ):160.3, 150.6, 149.6, 143.9, 143.8, 133.1, 131.8, 131.7, 131.4, 127.5, 127.4, 121.3, 115.3, 114.6, 114.3, 113.1, 110.4, 108.9, 56.5, 49.7.

### 1-(2-methoxyethyl)-3-(4-methyl-7,8-dihydroxy-2*H*chromen-2-one)benzimidazolium chloride (**3d**)

Yield: 25%. mp: 251–254°C. Anal. cal. for.  $C_{20}H_{19}ClO_5N_2$  C:59.63 H:4.75 N:6.95; found: C: 59.68, H: 4.80, N: 6.88. FT-IR (cm<sup>-1</sup>): 1560 (C-N), 1708 (C=O), 3380 (OH). <sup>1</sup>H-NMR ( $\delta_H$ , DMSO- $d_6$ ): 10.58 (s, 1H, -OH), 9.96 (s, 1H, NCHN), 9.51 (s, 1H, -OH), 6.98-8.20 (m, 6H, ArH), 6.16 (s, 2H, -CH<sub>2</sub>), 5.64 (s, 1H, -C=C-H), 4.75-4.78 (t, 2H, J = 4 Hz, -CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.82-3.85 (t, 2H, J = 4 Hz, -CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>), 3.28 (s, 3H, -CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>). <sup>13</sup>C-NMR ( $\delta_C$ , DMSO- $d_6$ ): 160.3, 150.6, 149.8, 144.2, 143.8, 133.1, 131.9, 131.5, 127.5, 127.3, 115.3, 114.7, 114.2, 113.1, 110.3, 108.6, 69.3, 58.7, 47.4, 46.98.

## 1-(2-ethoxyethyl)-3-(4-methyl-7,8-dihydroxy-2*H*-chromen-2-one)benzimidazolium chloride (**3e**)

Yield: 17%. mp: 219–223°C. Anal. cal. for.  $C_{21}H_{21}ClO_5N_2$ C:60.51 H: 5.08 N:6.72; found: C: 60.61, H: 5.15, N:6.63. FT-IR (cm<sup>-1</sup>): 1564 (C-N), 1709 (C=O), 3270 (OH). <sup>1</sup>H-NMR ( $\delta_H$ , DMSO- $d_6$ ): 10.6(s, 1H, OH), 9.94 (s, 1H, NCHN), 9.51 (s, 1H, OH), 6.98-8.21 (m, 6H, ArH), 6.17 (s, 2H, -CH<sub>2</sub>), 5.61 (s, 1H, C=C-H), 4.75-4.78 (t, 2H, J = 5 Hz,  $-CH_2CH_2CH_2CH_2CH_3$ ), 3.85-3.88 (t, 2H, J = 5 Hz,  $-CH_2CH_2OCH_2CH_3$ ), 3.45-3.50 (q, 2H, J = 7 Hz,  $-CH_2CH_2OCH_2CH_3$ ), 1.01-1.06 (t, 3H, J = 7 Hz,  $-CH_2CH_2OCH_2CH_3$ ), 1.01-1.06 (t, 3H, J = 7 Hz,  $-CH_2CH_2OCH_2CH_3$ ). <sup>13</sup>C-NMR ( $\delta_c$ , DMSO- $d_6$ , 300 MHz, ppm): 160.2, 150.7, 149.9, 144.1, 143.8, 133.1, 131.8, 131.6, 127.5, 127.3, 115.3, 114.7, 114.2, 113.1, 110.3, 108.6, 67.1, 66.1, 47.6, 47.0, 15.3.

#### 1-benzyl-3-(4-methyl-7,8-dihydroxy-2*H*-chromen-2one)benzimidazolium chloride (**3f**)

Yield: 70%, 288–289°C. Anal. cal. for.  $C_{24}H_{19}ClO_4N_2C:66.29$ H:4.40 N:6.44; found: C:66.33, H:4.42, N: 6.41, FT-IR (cm<sup>-1</sup>): 1564 (C-N), 1703 (C=O), 3220 (OH). <sup>1</sup>H-NMR ( $\delta_{H'}$ DMSO- $d_6$ ): 10.60 (s, 1H, -OH), 10.12 (s, 1H, NCHN), 9.51 (s, 1H, -OH), 6.99-8.07 (m, 11H, ArH), 6.13 (s, 2H, -CH<sub>2</sub>), 5.83 (s, 2H,-CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 5.81 (s, 1H, -C=C-H). <sup>13</sup>C-NMR ( $\delta_{C'}$ DMSO- $d_6$ ): 160.3, 150.6, 149.5, 144.1, 143.9, 134.2, 133.1, 131.9, 131.6, 129.5, 129.3, 129.0, 127.6, 127.4, 115.2, 114.6, 114.5, 113.2, 110.3, 109.2, 50.7, 47.2.

### 1-(3-methylbenzyl)-3-(4-methyl-7,8-dihydroxy-2*H*chromen-2-one)benzimidazolium chloride (**3g**)

Yield: 71%. mp: 241–245°C. Anal. cal. for  $C_{25}H_{21}ClO_4N_2$  C: 66.89, H:4.72, N:6.24; found: C:66.95, H:4.74, N:6.20. FT-IR (cm<sup>-1</sup>): 1567 (C- N), 1700 (C=O), 3450 (OH). <sup>1</sup>H-NMR ( $\delta_{H'}$ , DMSO- $d_6$ ): 10.58 (s, 1H, -OH), 10.06 (s, 1H, NCHN), 9.53 (s, 1H, -OH), 7.08-8.03 (m, 10H, ArH), 6.24 (s, 2H, -CH<sub>2</sub>), 5.79 (s, 1H, -C=C-H), 5.77 (s, 2H, -CH<sub>2</sub> $C_6H_4$ (CH<sub>3</sub>)), 2.3 (s, 3H, Ar-CH<sub>3</sub>). <sup>13</sup>C-NMR ( $\delta_{C}$ , DMSO- $d_6$ ): 160.3,150.6, 149.5, 144.0, 143.9, 138.9, 134.1, 133.1, 131.9, 131.5, 129.9, 129.4, 129.4, 127.6, 127.47, 126.01, 115.0, 114.6, 114.4, 113.1, 110.3, 109.3, 50.7, 47.2, 21.4.

### 1-(2,3,5,6-tetramethylbenzyl)-3-(4-methyl-7,8dihydroxy-2*H*-chromen-2-one) benzimidazolium chloride (**3h**)

Yield: 31%. mp: 288–289°C. Anal. cal. for.  $C_{28}H_{27}ClO_4N_2$ C:68.50 H:5.54 N:5.71; found: C:69.56 H:5.55, N:5.77. FT-IR (cm<sup>-1</sup>): 1567 (C-N), 1731 (C=O), 3440 (OH). <sup>1</sup>H-NMR ( $\delta_{H}$ , DMSO- $d_6$ ): 10.54 (s, 1H, -OH), 9.49 (s, 1H, NCHN), 9.26 (s, 1H, -OH), 6.94-8.31 (m, 7H, ArH), 6.02 (s, 2H, -C $H_2$ ), 5.78 (s, 2H, -C $H_2C_6H(CH_3)_4$ ), 5.60 (s, 1H, -C=C-H), 2.48 (s, 6H, 2xAr-C $H_3$ ), 2.18 (s, 6H, 2xAr-C $H_3$ ). <sup>13</sup>C-NMR ( $\delta_C$ , DMSO- $d_6$ ): 160.3, 150.6, 150.2, 143.7, 142.8, 134.9, 134.7, 133.0, 133.0, 132.3, 132.2, 128.8, 127.7, 127.4, 115.0, 114.6, 114.4, 113.1, 110.1, 107.9, 56.5, 47.23, 46.49, 20.6, 15.9.

### 1-(2,3,4,5,6-pentamethylbenzyl)-3-(4-methyl-7,8dihydroxy-2*H*-chromen-2-one) benzimidazolium chloride (**3i**)

Yield: 15%, mp: 272°C. Anal. cal. for.  $C_{29}H_{29}ClO_4N_2C:68.97$ , H:5.79, N: 5.55; found: C:69.05, H:5.85, N:5.47. FT-IR (cm<sup>-1</sup>): 1570 (C-N), 1705 (C=O), 3350 (OH). <sup>1</sup>H-NMR ( $\delta_{H'}$ , DMSO- $d_6$ ): 10.52 (s, 1H, -OH), 9.49 (s, 1H, NCHN), 9.23 (s, 1H, -OH), 6.94-8.34 (m, 6H, ArH), 6.01 (s, 2H, -CH<sub>2</sub>), 5.77 (s, 2H, -CH<sub>2</sub>C<sub>6</sub>(CH<sub>3</sub>)<sub>5</sub>), 5.59 (s, 1H, -C=C-H), 2.25 (s, 3H, Ar-CH<sub>3</sub>), 2.23 (s, 12 H, Ar-CH<sub>3</sub>). <sup>13</sup>C-NMR ( $\delta_{C'}$ , DMSO- $d_6$ ): 160.3, 150.6, 150.3, 143.7, 142.7, 136.8, 134.3, 133.5, 133.0, 132.25, 132.2, 127.7, 127.4, 126.1, 115.0, 114.7, 114.4, 113.1, 110.3, 107.9, 65.4, 47.00, 17.47,, 17.2, 16.9.

### 1-(3,4,5-trimethoxybenzyl)-3-(4-methyl-7,8dihydroxy-2*H*-chromen-2-one)benzimidazolium chloride (**3**j)

Yield: 86%. mp: 284–286°C. Anal cal. for.  $C_{27}H_{25}ClO_7N_2$  C: 61.78, H: 4.80, N:5.34; found: C:61.82, H:4.81, N:5.30. FT-IR (cm<sup>-1</sup>): 1563 (C-N), 1730 (C=O), 3231 (OH). <sup>1</sup>H-NMR ( $\delta_{H}$ , DMSO- $d_6$ ): 10.60 (s, 1H,-OH), 10.13 (s, 1H, NCHN), 9.51 (s, 1H, -OH), 6.98-8.20 (m, 8H, ArH), 6.14 (s, 2H, -CH<sub>2</sub>), 5.77 (s, 1H, -C=C-H), 5.70 (s, 2H, CH<sub>2</sub>C<sub>6</sub>H<sub>2</sub>(OCH<sub>3</sub>)<sub>3</sub>), 3.77 (s, 6H, Ar-OCH<sub>3</sub>), 3.65 (s, 3H, Ar-OCH<sub>3</sub>). <sup>13</sup>C-NMR ( $\delta_{C'}$ , DMSO- $d_6$ ): 162.9,160.3, 153.7, 150.6, 149.7, 143.9, 138.1, 133.1, 131.9, 131.6, 129.4, 127.6, 127.4, 115.2, 114.7, 114.4, 113.2, 110.4, 109.1, 107.0, 60.5, 56.5, 51.0, 47.09.

## Preparation of haemolysate and purification from blood red cells

Blood samples (25 mL) were taken from healthy human volunteers. They were anticoagulated with acid-citrate-dextrose (ACD), centrifuged at 2000g for 20 min at 4°C and the supernatant was removed. The packed erythrocytes were washed three times with 0.9% NaCI and then haemolysed in cold water. The ghosts and any intact cells were removed by centrifugation at 2000g for 25 min at 4°C, and the pH of the haemolysate was adjusted to pH 8.5 with solid Tris-base. The 25 mL haemolysate was applied to an affinity column containing L-tyrosine-sulfonamide-Sepharose-4B<sup>28</sup> equilibrated with 25 mM Tris-HCl/0.1 M Na<sub>2</sub>SO<sub>4</sub> (pH 8.5). The affinity gel was washed with 50 mL of 25 mM Tris-HCl/22 mM Na<sub>2</sub>SO<sub>4</sub> (pH8.5). The human CA (hCA) isozymes were then eluted with 0.1 M NaCl/25 mM Na<sub>2</sub>HPO<sub>4</sub> (pH 6.3) and 0.1 M CH<sub>2</sub>COONa/0.5 M NaClO<sub>4</sub> (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

CA 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<sup>29</sup>. The assay solution was 0.5 M Na<sub>2</sub>CO<sub>3</sub>/0.1 M NaHCO<sub>3</sub> (pH 10.0) and Phenol Red was added as the pH indicator.  $CO_2$ hydratase activity (enzyme units (EU)) was calculated by using the equation  $t_0$ - $t_c/t_c$  where  $t_0$  and  $t_c$  are the times for pH change of the nonenzymatic 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.

#### **Results and discussion**

In this study, series of 10 new water-soluble 1-alkyl-3-(4-methyl-7,8-dihydroxy-2*H*-chromen-2-one) benzimidazolium chloride salts (**3a-j**) derivatives were synthesized (Scheme 1) and their inhibitory effects on the activity of purified hCA I and II were evaluated. For the coumarin having an inhibition affect, the inhibitor concentration causing up to 50% inhibition (IC<sub>50</sub> values) was determined from the graphs (Figure 1 at Supplementary material). The result showed that these compounds (**3a-j**) inhibited the CA enzyme activity. The IC<sub>50</sub> values of **3a-j** analogues against hCA I and II were summarized in Table 1. Among the compounds synthesized, **3g** and **3j** was found to be most active one (IC<sub>50</sub> = 22.09  $\mu$ M and 20.33  $\mu$ M) for hCA I and hCA II, respectively.

Coumarins/thiocoumarins may possess various tautomeric forms, such as the zwitterionic benzo (thio) pyrylium phenoxides, which may bind within the CA active site similarly to phenols (Chart 2, step A)<sup>30</sup>, i.e. by anchoring to the zinc-bound water molecule/hydroxide ion<sup>31</sup>. It was reported that the coumarin-binding site in CAs may interact with diverse compounds, such as the antiepileptic drug lacosamide, which inhibits mammalian CAs I-XV, with inhibition constants in range of 331 nM-4.56  $\mu$ M<sup>32</sup>.

The natural product<sup>11,33</sup> coumarin 6-(1S-hydroxy-3methylbutyl)-7-methoxy-2*H*-chromen-2-one, as well as structurally related, simple coumarin derivatives possessing various substitution patterns at the heterocyclic ring<sup>11</sup>, were shown to be hydrolyzed within the CA active site with formation of 2-hydroxy-cinnamic acids, which possess significant CA inhibitory properties and bind in a completely unprecedented manner to the enzyme, not



Scheme 1. Synthesized Compounds

Table 1. The IC<sub>50</sub> values of coumarin compounds.

50		
Inhibitors	hCA I (µM)	hCA II (µM)
3a	28.55	49.4
3b	49.32	51.45
3c	48.06	29.7
3d	32.64	41.35
3e	32.86	33.16
3f	25.63	40.01
3g	22.09	28.9
3h	31.22	34.12
3i	29.5	28.96
3j	33.1	20.33
1		

hCA, human carbonic anhydrase.

interacting with the Zn(II) ion as the sulphonamide/sulfamate/sulfamide type of inhibitors<sup>33</sup>.

It was reported that the coumarin lacks classical zinc binding functional groups and exhibits an unusual binding mode that may be exploited to design isoform selective CA inhibitors. The inhibitor exhibits an extended two-arm conformation that effectively plugs the entrance to the active site<sup>33</sup>. The present investigation reported that coumarin derivatives (**3a-j**) had potent inhibitory effects on the hCAI and hCA II.

It has been reported that an original sulphonamide derivative of coumarin, namely 2-(8-methoxycoumarine-3-carbamido)-1,3,4-thiadia-zole-5-sulfonamide was synthesized. This compound was showed to inhibition effect against the hydratase activities of hCA I, hCA II (IC<sub>50</sub> values of 4.7, 9.6 and 25.3  $\mu$ M) and dog CA, respectively<sup>15,34</sup>. Our inhibition values were closes theirs.

Gupta and Kumaran (2005) reported that uncharged compounds cannot be made selective for cytosolic or membrane-bound isozyme since in both the cases the compounds appear to follow the same mechanism of inhibition. However, for the charged compounds the polarizability of the molecule seems to greatly favour the inhibition of the membrane-bound enzyme, and hence, they can be made selective for this enzyme by enhancing their polarizability, which is found to play no role in the inhibition of cytosolic enzymes<sup>35</sup>.

Barros et al. reported that reaction of 20 aromatic/ heterocyclic sulphonamides containing a free amino, imino, hydrazino or hydroxyl group, with 8-quinolinesulfonyl chloride afforded a series of water-soluble (as hydrochloride or triflate salts) compounds. The new derivatives were assayed as inhibitors of the zinc enzyme CA, and more precisely of three of its isozymes, CA I, II (cytosolic forms) and IV (membrane-bound form), involved in important physiological processes. Some of the best inhibitors synthesized were topically applied as 2% water solutions onto the eye of normotensive and glaucomatous albino rabbits, when strong and longlasting intraocular pressure (IOP) lowering was observed with many of them. This new compound is quite watersoluble as hydrochloride salt, behaves as a strong CA II inhibitor, and fared better than the parent molecule in lowering IOP in experimental animals<sup>26</sup>. Therefore, our

new water-soluble coumarins can effective inhibitor for CA isozymes.

In summary, enzyme inhibition is more important issue for drug design and biochemical applications<sup>36-41</sup>. The results showed that new coumarin derivatives inhibited the hCA I and II enzyme activity. Therefore, our results suggested that new coumarin derivatives are likely to be adopted as candidates to treat glaucoma and may be taken for further evaluation in *in vivo* studies.

### **Declaration of interest**

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