Full Paper

Synthesis and Carbonic Anhydrase Inhibitory Effects of Novel Sulfamides Derived from 1-Aminoindanes and Anilines

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Three 1-aminoindanes, four anilines and BnOH or *t*-BuOH were reacted with chlorosulfonyl isocyanate to give sulfamoyl carbamates. Pd-C catalysed hydrogenolysis reactions of carbamates or deprotection of the Boc group of the carbamates with CF_3CO_2H afforded seven novel sulfamides. Human carbonic anhydrase (hCA) isoenzymes I and II (hCA I and hCA II) were purified from fresh human blood erythrocytes with one-step affinity chromatography on Sepharose 4B-tyrosine-sulfanilamide. The inhibitory properties of the novel sulfamides on both isoenzymes were determined using the esterase activity with 4-nitrophenyl acetate (NPA) as substrate. The tested novel sulfamides derived from 1-aminoindanes and anilines effectively inhibited hCA I and II competitively in the nanomolar range. Among these compounds, the novel sulfamide derivative **17** showed the most potent inhibitory effect against hCA I (K_i: 153.88 nM), while sulfamide derivative **26** showed the highest inhibitory potential against hCA II (K_i: 117.80 nM).

Keywords: Aminoindane / Aniline / Carbonic anhydrase / Enzyme inhibition / Sulfamide / Sulfamoyl carbamate

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Introduction

Sulfamides are interesting compounds in the field of synthetic organic chemistry and medicinal chemistry [1, 2]. Some drugs, such as an injectable antibiotic doripenem (**1**) [3], dopaminergic and anti-hyperprolactinemic agent quinagolide (**2**) [4], and anticonvulsant **3** (JNJ-26990990) [5] contain sulfamide functional group in their structures. For this reason, the synthesis and the biological evaluation of novel sulfamides attract more attention on the scientific arena. For example, HIV-I protease inhibitory properties of **4** [6], β-secretase 1 inhibition of **5** [7] and carbonic anhydrase inhibitory activity of **6**–**7** [8, 9] have been reported (Fig. 1). In addition, there are numerous reports suggesting significant biological activities of the similar kind of compounds in the literature [10].

Carbon dioxide (CO_2) is a naturally occurring chemical compound. It is a fundamental chemical in various biological systems of organisms all over the phylogenetic tree. Its reaction with water is a slow process at the physiological pH (7.4). It needs the presence of a catalyst to become effective. These catalysts are the carbonic anhydrase (CA, EC 4.2.1.1) enzymes, a superfamily of metal containing enzymes [11–13]. The active site of majority of carbonic anhydrases contains a zinc ion. Therefore they are called as metalloenzymes. CA catalyses the hydration of carbon dioxide (CO_2) and the dehydration of carbonic acid (H_2CO_3), rapidly [14–16].

$$CO_2 + H_2O \overset{CA}{\Leftrightarrow} H_2CO_3 \Leftrightarrow HCO_3^- + H^+$$

CA is one of the fastest enzymes. Typical catalytic rates of the different isoforms of CA are in the range of 10^4 and 10^6 reactions per second [17]. CA isoenzymes are found in Archaea, prokaryotic and eukaryotic cells, and codified five distinct unrelated gene families. These distinct CA families are α-, β-, γ-, and δ-CAs. There is no significant amino acid sequence similarity in these families. Mammals, including humans,

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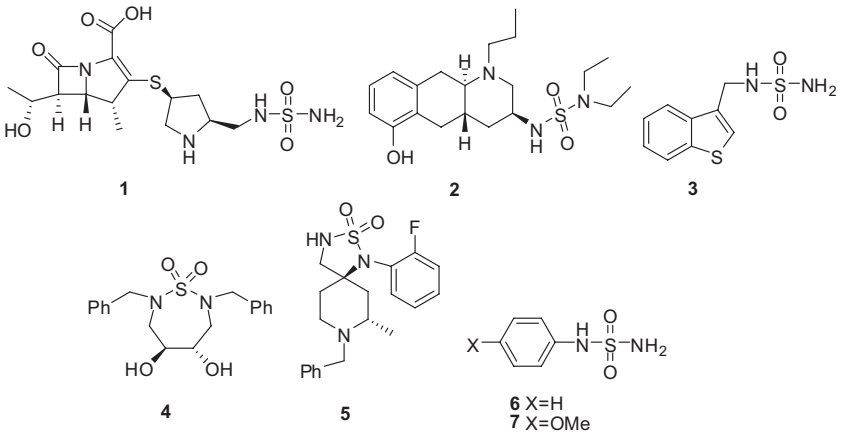


Figure 1. Some selected drugs **1–3** and biologically active sulfamides **4–7**.

possess only α-CAs, which is the first family of the CAs. Up to now sixteen different isozymes have been characterized. Of these, CA I, II, III, VII and XIII are cytosolic isozymes. Besides that, CA IV, IX, XII and XIV are membrane bound ones. Both of CA VA and VB are mitochondrial isoforms. CA VI is solely secreted isoenzyme. In addition, there are three acatalytic forms, which are called as CA-related proteins (CARPs). The CARPs and their functions remain unclear [18–20]. This CA family has different subcellular locality and tissue dispersion [21]. The namely CARP VIII, X and XI are the only known CARPs [11]. Nevertheless, CARP VIII can be turned into a functional CA by site-specific mutagenesis [22]. On the other hand, it has been reported that CA inhibitors (CAIs) have been extensively used for the management of altitude sickness [11], treatment of glaucoma, epilepsy, and obesity [23].

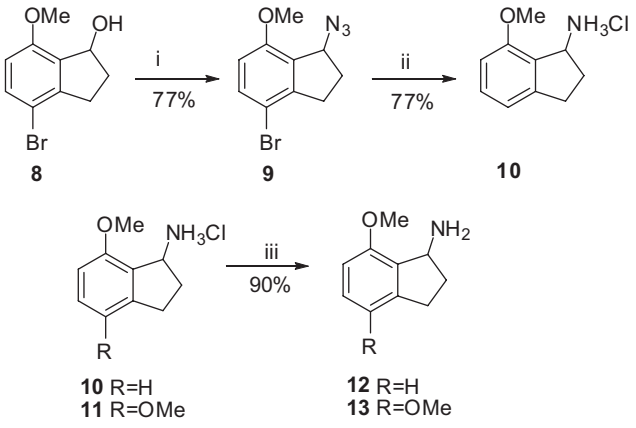
In our on-going researches on the synthesis and biological evaluation of novel sulfamide compounds, we have already addressed CA I and II inhibition properties of some the sulfamides synthesized from amino indanes [2], aminotetralins [24], and dopamine related compounds [12]. The results concluded from these investigations encouraged us on the synthesis of novel compounds and evaluate them for their CA I and II inhibitory properties. In this context, in the present work, we have synthesized and evaluated the CA I and CA II inhibitory actions of some novel sulfamide carbamates and sulfamides derived from methoxylated 1-aminoindanes and anilines. From the biological results, our second goal was to make a comparison about the structure activity relationships between two different series of compounds, *viz*. sulfamoyl carbamates and sulfamides.

Results and discussion

Chemistry

The synthesis of alcohol **8** was performed as described by Ling et al. [25]. The Mitsunobu reaction is one of the most convenient methods for the conversion of secondary alcohols to their azide derivatives [26, 27]. Specifically, the reactions of benzylic secondary alcohols with diphenylphosphoryl azide (DPPA) in the presence of DBU, also known as Mitsunobu reaction, are widely used for the synthesis of benzyl azides [28]. Applying the same procedure as discussed before to **8** afforded azide **9** which was converted to amine hydrochloride salt **10** via Pd-C catalysed hydrogenation in methanol (MeOH)–chloroform ($CHCl_3$). Generally, $CHCl_3$ is used for *in situ* production of HCl and converting amines to amine hydrochloride salts for easy crystallization, purification, and convenient storage [29]. Hydrochloride **11** was synthesized as described by Coutts and Malicky [30]. The amine hydrochlorides **10** and **11** were reacted with 10% NaOH to give amines **12** and **13** (Scheme 1).

On the other hand, amine **14** was synthesized according to the procedure described in the literature [31]. The reactions of 1 equivalent amines **12**–**14** and 1.2 equivalent benzyl alcohol



Scheme 1. The synthesis of amines **12** and **13**. Reagents and conditions: (i) DPPA, DBU/THF, 0–25°C. (ii) H_2/Pd-C, MeOH-$CHCl_3$, 25°C. (iii) 10% NaOH, MeOH, 0–25°C.

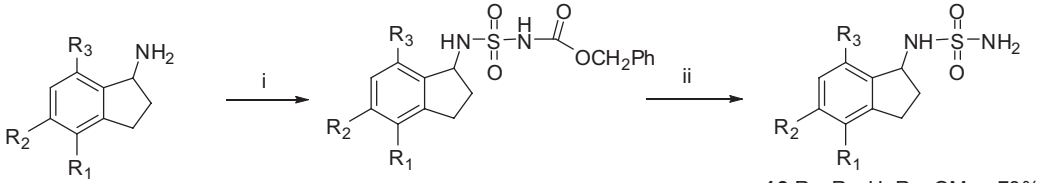
(BnOH) with chlorosulfonyl isocyanate (CSI) yielded new sulfamoyl carbamates **15**–**17**. Pd-C catalysed hydrogenolysis of carbamates yield amine related compounds [32]. Therefore, Pd-C catalysed hydrogenolysis of compounds **15**–**17** in MeOH furnished the title compounds novel sulfamides **18**–**20** with 72–78% yields (Scheme 2).

In addition, sulfamide carbamates can be easily prepared from amines, *tert*-BuOH and CSI [12]. Hence, commercially available methoxylated anilines **21**–**24** and 1.2 equiv. *tert*-BuOH were reacted with CSI to give sulfamide carbamates **25**–**28** with the yields ranging from 72–81%. Deprotection of Boc group with CF_3CO_2H is a useful method in the synthesis of target compounds [33]. Therefore, the reactions of carbamates **25**–**28** with CF_3CO_2H produced the title compounds **29**–**32** in high yields (Scheme 3). The chemical structures of all newly synthesized compounds were characterized by spectroscopic techniques, such as ^1H- and ^{13}C NMR, IR, and elemental analysis.

Carbonic anhydrase inhibitory properties

CA isozymes play vital physiological functions, such as respiration and acid-base balance, bone resorption, electrolyte secretion, calcification, and biosynthetic reactions, which require HCO_3^- as a substrate, in all organisms [34, 35]. The majority of CA isozymes constitute targets for the design and development of CA inhibitors for clinical practices [36]. It is well known that CA isozymes are effectively inhibited by aromatic, heterocyclic and inorganic sulfonamides as well as by metal complexing sulfonamides. The CAIs are regularly used for the treatment of elevated intracranial pressure in *Pseudotumor cerebri* and increased intraocular pressure in glaucoma while reducing the production of cerebrospinal fluid and aqueous humour, respectively [37]. The aromatic sulfonamides are classical CA inhibitors. Also, several potent sulfonamides have been reported as potential antitumor [38]. CAIs have a large number of applications in therapy, such as diagnostic tools and antiepileptics [39]. They are also used in the treatment of other neurological disorders [40].

In our previous studies, the synthesis of (3,4-dihydroxyphenyl)(2,3,4-trihydroxyphenyl)methanone and its derivatives [41], novel sulfamides [2], novel sulfonamide derivatives of aminotetralins and aminoindanes [28], novel benzylamine derivatives [42], sulfamide and sulfonamide analogues of dopamine related compounds [12, 43], sulfamides and sulfonamides incorporating tetralin scaffold [24] and bromophenols derivatives [44, 45] have been described as novel therapeutics. These newly synthesised compounds exhibited hCA I and II inhibition in the range between micromolar and nanomolar affinities. To continue our research on hCA inhibitors, sulfamide carbamates **15**–**17**, **25**–**28**, and sulfamides **18**–**20**, **29**–**32** were synthesized and investigated for their effects against hCA I and II isoforms. Therefore, at first, the inhibition effects of investigated compounds on hCA I and II were defined by drawing Lineweaver-Burk charts.

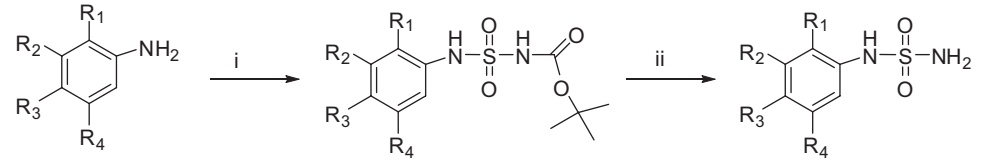


12 R_1=R_2=H, R_3=OMe
13 R_2=H, R_1=R_3=OMe
14 R_1=R_3=H, R_2=OMe

15 R_1=R_2=H, R_3=OMe, 62%
16 R_2=H, R_1=R_3=OMe, 62%
17 R_1=R_3=H, R_2=OMe, 60%

18 R_1=R_2=H, R_3=OMe, 78%
19 R_2=H, R_1=R_3=OMe, 72%
20 R_1=R_3=H, R_2=OMe, 78%

Scheme 2. The synthesis of sulfamides **18**–**20**. Reagents and conditions: (i) CSI, $PhCH_2OH/NEt_3$, CH_2Cl_2, 0–25°C. (ii) H_2/Pd-C, MeOH, 25°C.



21 R_2=R_4=H, R_1=R_3=OMe
22 R_1=R_3=H, R_2=R_4=OMe
23 R_2=R_3=H, R_1=R_4=OMe
24 R_1=H, R_2=R_3=R_4=OMe

25 R_2=R_4=H, R_1=R_3=OMe, 81%
26 R_1=R_3=H, R_2=R_4=OMe, 78%
27 R_2=R_3=H, R_1=R_4=OMe, 72%
28 R_1=H, R_2=R_3=R_4=OMe, 75%

29 R_2=R_4=H, R_1=R_3=OMe, 85%
30 R_1=R_3=H, R_2=R_4=OMe, 82%
31 R_2=R_3=H, R_1=R_4=OMe, 72%
32 R_1=H, R_2=R_3=R_4=OMe, 70%

Scheme 3. The synthesis of sulfamides **29**–**32**. Reagents and conditions: (i) CSI, *t*-BuOH/NEt_3, CH_2Cl_2, 0–25°C. (ii) TFA, CH_2Cl_2, 0–25°C.

Then, half maximal inhibitory concentrations (IC_{50}) and the mean inhibitory constant (K_i) values were calculated for synthesized compounds based on the drawn charts (Table 1). IC_{50} was used for measurement of the effectiveness of novel sulfamide carbamates and sulfamides in inhibiting both hCA isoenzymes.

In the first stage, we determined the inhibition effects of the carbamates **15–17**, **25–28**, and sulfamides **18–20**, **29–32** on the esterase activity of the cytosolic isoform hCA I and the physiologically dominant isoform CA II. The inhibition results are summarized in Table 1. It is well known that the active site of both CA isoenzyme inhibitors coordinate to the zinc ion. Among the investigated synthesized compounds, the compound **17** demonstrated to be an effective inhibitor of cytosolic isoenzyme hCA I (Table 1) with a K_i of 153.88 ± 04.01 nM. On the other hand, compound **26** showed the highest inhibitory activity on physiological dominate hCA II isoenzyme with K_i of 117.80 ± 17.87 nM. Also, the rest of sulfamides displayed moderate activity against cytosolic isoenzyme hCA I with K_i, which ranged from 169.58 ± 06.40 to 440.97 ± 16.28 nM, and on hCA II with K_i in the range of 158.91 ± 03.77–1070.25 ± 43.15 nM, respectively. The chemical structure of sulfamide **26**, the most powerful HCA II isoenzyme inhibitor, is shown in Fig. 2, as well as an estimated binding model of this compound to the active site of CA. Two hydrogen bonds have been modelled between the amide groups of sulfamide **26** and hydroxyl groups of Thr-199 and Thr-200 amino acid residues that are universally conserved in CAs.

Many inhibitors have been designed, developed, and synthesized for CA isoenzymes including CA I and II in the literature. Extensive researches on this issue continue to find novel applications for the widespread CAs inhibitors.

Table 1. Human carbonic anhydrase isoenzymes (hCA I and hCA II) inhibition values of some novel sulfamides (**15–31**) by an esterase bioassay with NPA as substrate.

	IC_{50} (nM)		K_i (nM)	
Compounds	**hCA I**	**hCA II**	**hCA I**	**hCA II**
15	234.20	183.86	321.88 ± 10.52	297.83 ± 14.22
16	228.26	184.26	271.05 ± 11.62	352.96 ± 16.81
17	262.50	196.04	153.88 ± 04.01	306.83 ± 13.51
18	388.89	270.07	440.97 ± 16.28	219.41 ± 03.61
19	523.52	266.74	337.81 ± 11.99	191.00 ± 10.48
20	410.54	410.54	360.94 ± 17.42	246.15 ± 05.03
25	337.55	207.42	424.83 ± 14.69	158.91 ± 03.77
26	224.56	159.20	262.22 ± 12.39	117.80 ± 17.87
27	294.14	151.00	233.97 ± 06.42	211.52 ± 04.16
28	359.44	220.28	335.14 ± 11.85	173.32 ± 03.17
29	497.13	259.64	380.15 ± 09.54	496.31 ± 13.91
30	146.94	256.76	174.53 ± 08.04	1070.25 ± 43.15
31	226.39	200.63	169.58 ± 06.40	331.31 ± 21.75

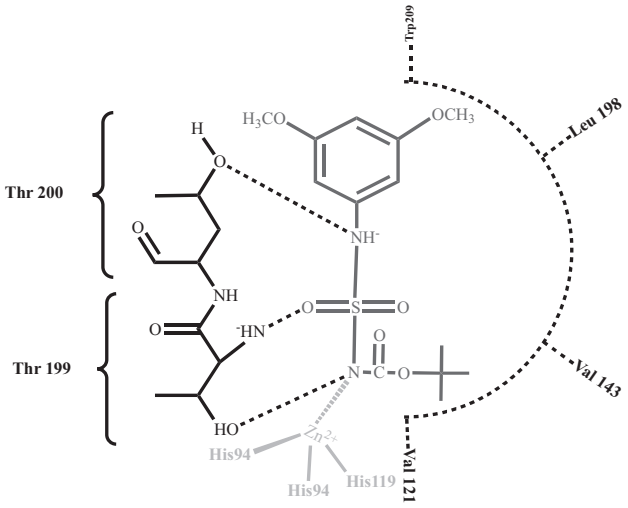


Figure 2. The proposed binding model of sulfamide **26** to CA by anchoring to the Zn^{2+} coordinated water/hydroxide ion (–OH).

Conclusion

In conclusion, we report the first synthesis of sulfamides, **18–20** and **29–32** from 1-aminoindanes **12–14** and anilines **21–24**. As carbamates and sulfamides are biologically active compounds and some of them are used in the treatment of some diseases. With this regard, our synthesized sulfamide carbamates **15–17** and **25–28** and sulfamides **18–20** and **29–32** hold a promise to be further developed as novel drugs. Further, our synthesized compounds showed inhibitory potential against the hCA I and II isoforms in the nanomolar range. These results and findings point out that synthesized novel sulfamide carbamates and sulfamides may be used as potent hCA isoenzyme inhibitors and can be potential candidates for treatment of glaucoma, epilepsy, and obesity.

Experimental

All chemicals and solvents are commercially available and they were used without purification or they were used after distillation and treatment with drying agents. Melting points were determined on a capillary melting apparatus (BUCHI 530) and are uncorrected. IR spectra were obtained from solutions in 0.1 mm cells with a Perkin–Elmer spectrophotometer. The ^{1}H- and ^{13}C NMR spectra were recorded on Varian and Bruker spectrometers at 400 and 100 MHz, respectively, and NMR shifts are presented as δ in ppm. The tetramethylsilane was used as an internal standard. Elemental analyses were performed on a Leco CHNS-932 apparatus. All column chromatography was performed on silica gel (60-mesh, Merck). PLC is preparative thin-layer chromatography: 1 mm of silica gel 60 PF (Merck) on glass plates.

Chemistry

The syntheses of alcohol **8** [25], hydrochloride **11** [30], and amine **14** [31] were performed as described earlier.

*Synthesis of 1-azido-4-bromo-7-methoxyindane (**9**) and afterwards*

Alcohol **8** (2.00 g, 8.23 mmol) was dissolved in dry THF (100 mL). Diphenylphosphoryl azide (DPPA; 2.71 g, 9.84 mmol) and DBU (1.49 g, 27.68 mmol) were added to this solution at 0°C under N_2 (g). The mixture was stirred for 2 h at the same temperature, then for 12 h at 25°C. The solvent was evaporated followed by the addition of 80 mL of dichloromethane (CH_2Cl_2) and 30 mL of 10% hydrochloric acid (HCl) to the residue. The organic phase was separated and washed with 10% HCl (2 × 20 mL). Organic layer was dried over Na_2SO_4 and the solvent was evaporated. The column chromatography of the residue on silica gel (30 g) with 10% EtOAc/hexane gave colourless oily azide **9** (1.70 g, 77% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39 (d, 1H, J = 8.6 Hz, Ar-H), 6.66 (d, 1H, J = 8.6 Hz, Ar-H), 5.17 (dd, 1H, Ar-H, J = 2.0, 7.5 Hz), 3.85 (s, 3H, OCH_3), 3.10–3.01 (m, 1H, CH), 2.90–2.82 (m, 1H, CH), 2.39–2.30 (m, 1H, CH), 2.14–2.05 (m, 1H, CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.3 (C), 146.3 (C), 133.4 (CH), 130.2 (C), 110.9 (CH), 110.7 (C), 65.0 (CH), 55.8 (OCH_3), 32.8 (CH_2), 31.8 (CH_2). IR (CH_2CI_2, cm^{-1}): 3393, 3047, 2935, 1734, 1600, 1435, 1319, 1266, 1074, 1054. Anal. calcd. for ($C_{10}H_{10}BrN_3O$): C, 44.80; H, 3.76; N, 15.67. Found: C, 44.80; H, 3.75; N, 15.70.

*1-Amino-7-methoxyindane hydrochloride (**10**) [46]*

Azide **9** (2.00 g, 7.46 mmol) was dissolved in MeOH (50 mL) and $CHCl_3$ (4 mL) in a 100 mL flask. After addition of Pd-C (50 mg) to this solution, a balloon filled with H_2 gas (3 L) was fitted to the flask. The mixture was deoxygenated by flushing with H_2. After hydrogenation of the mixture at RT for 20 h, the catalyst was removed by filtration. Crystallization of the residue with MeOH/Et_2O furnished **10** (1.40 g, 77% yield). White solid. Mp: 221–223°C. ^{1}H NMR (400 MHz, D_2O): δ 7.26 (t, 1H, Ar-H, J = 7.9 Hz), 6.85 (d, 1H, Ar-H, J = 7.9 Hz), 6.70 (d, 1H, Ar-H, J = 7.9 Hz), 4.8 (m, 1H, NCH), 3.75 (s, 3H, OCH_3), 3.03–3.94 (m 1H, CH), 2.87–2.79 (m 1H, CH), 2.53–2.49 (m 1H, CH), 1.95 (m 1H, CH). ^{13}C NMR (100 MHz, D_2O): δ 156.4 (C), 146.6 (C), 131.9 (CH), 125.1 (C), 117.6 (CH), 108.9 (CH), 55.4 (CN), 54.2 (OCH_3), 30.2 (CH_2), 30.0 (CH_2).

*1-Amino-4,7-dimethoxyindane hydrochloride (**11**)*

White solid. Mp: 244–246°C (lit; [24] Mp: 225–226°C). ^{1}H NMR (400 MHz, D_2O): δ 6.85 (A part of AB system, d, 1H, Ar-H, J = 8.8 Hz), 6.74 (B part of AB system, d, 1H, Ar-H, J = 8.8 Hz), 4.75 (dd, 1H, NCH, J = 5.3, J = 8.1 Hz), 4,65 (bs, NH_3 and H_2O), 3.70 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 3.93–2.86 (m, 1H, CH), 2.74–2.66 (m, 1H, CH), 2.52–2.43 (m, 1H, CH), 2.05–1.91 (m, 1H, CH). ^{13}C NMR (100 MHz, D_2O): δ 150.6 (C), 149.5 (C), 133.9 (C), 126.8 (C), 113.6 (CH), 110.0 (CH), 56.2 (CH–N) 55.7 (OCH_3), 54.5 (OCH_3), 29.8 (CH_2), 27.3 (CH_2).

*1-Amino-7-methoxyindane (**12**)*

Amine hydrochloride salt **10** (2.00 g, 10.02 mmol) was dissolved in MeOH (40 mL) and cooled to 0°C. A solution of 10% NaOH (20 mL) was added to this solution, the reaction mixture was stirred at RT for 3 h. After the most of MeOH was evaporated, CH_2Cl_2 (50 mL) and H_2O (20 mL) were added to the residue. Organic layer was separated and H_2O layer was extracted with CH_2Cl_2 (2 × 30 mL). Combined organic layers were dried over Na_2SO_4 and CH_2Cl_2 was evaporated. Oily aminoindane **12** (1.47 g, 90% yield) was synthesized and used without further purification and characterization, in the next step.

Compound **13** was also synthesized by the same procedure.

*Standard procedure for the synthesis of benzyl sulfamoylcarbamates: Synthesis of benzyl N-(7-methoxy-2,3-dihydro-1H-inden-1-yl)sulfamoyl carbamate (**15**)*

Benzylalcohol (0.93 g, 8.58 mmol) was added to a solution of CSI (0.87 g, 6.13 mmol) in CH_2Cl_2 (10 mL) at 0°C. A solution of amine **12** (1.00 g, 6.13 mmol) in CH_2Cl_2 (30 mL) and NEt_3 (0.68 g, 6.74 mmol) were added to the solution of CSI drop wise and stirred at 0°C for 1 h, then at RT for 3 h. The reaction mixture was cooled to 0°C and a solution of 0.1 N HCl (50 mL) was added to this mixture. Organic phase was separated and H_2O phase was extracted with H_2Cl_2 (2 × 25 mL). Combined organic layers were dried over Na_2SO_4. The solvent was evaporated. Column chromatography of the residue on silica gel (30 g) with 30% EtOAc/hexane yielded carbamate **15** (1.44 g, 62% yield). White solid. Mp: 133–135°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.7 (bs, 1H, NH), 7.34 (bs, 5H, Ar-H), 7.14 (t, 1H, Ar-H, J = 7.9 Hz), 6.86 (d, 1H, Ar-H, J = 7.9 Hz), 6.70 (d, 1H, Ar-H, J = 7.9 Hz), 5.72 (d, 1H, NH, J = 4.1 Hz), 5.18 (s, 2H, CH_2), 4.99–4.94 (m, 1H, NCH), 3.74 (s, 3H, OCH_3), 3.08–3.02 (m, 1H, CH), 2.84–2.76 (m, 1H, C–H), 2.45–2.36 (m, 1H, C–H), 2.30–2.20 (m, 1H, C–H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.2 (CO), 151.3 (C), 146.6 (C), 135.0 (C), 130.9 (CH), 128.9 (3CH), 128.5 (2CH), 127.7 (C), 117.7 (CH), 108.6 (CH), 68.5 (OCH_2), 58.0 (C–N), 55.4 (OCH_3), 33.9 (CH_2), 30.8 (CH_2). IR (CH_2CI_2, cm^{-1}): 3275, 2936, 2852, 1732, 1606, 1454, 1267, 1192, 1163, 1057. Anal. calcd. for ($C_{18}H_{20}N_2O_5S$): C, 57.43; H, 5.36; N, 7.44; S, 8.52. Found: C, 57.40; H, 5.35; N, 7.41; S, 8.49.

Carbamates **16** and **17** were also synthesized by the same method.

*Benzyl N-(4,7-dimethoxy-2,3-dihydro-1H-inden-1-yl)-sulfamoyl carbamate (**16**)*

62% yield. White solid. Mp: 137–139°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (bs, 1H, NH), 7.34 (bs, 5H, Ar-H), 6.72 (d, 1H, A part of AB system, Ar-H, J = 8.7 Hz), 6.62 (d, 1H, B part of AB system, Ar-H, J = 8.7Hz,), 5.73 (d, 1H, NH, J = 4.3 Hz), 5.18 (s, 2H, CH_2), 4.98–4.94 (m, 1H, NCH), 3.78 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 3.00–2.92 (m 1H, C–H), 2.80–2.73 (m 1H, C–H), 2.46–2.37 (m 1H, C–H), 2.29–2.21 (m 1H, C–H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 151.3 (CO), 150.6 (C), 150.1 (C), 135.0 (C), 134.3 (C), 129.4 (C), 128.92 (2CH), 128.90 (2CH), 128.5 (CH), 111.5 (CH), 109.4 (CH), 68.5 (OCH_2), 58.5 (CN), 55.9 (OCH_3), 55.7 (OCH_3), 33.8 (CH_2), 27.7 (CH_2). IR (CH_2CI_2, cm^{-1}): 3275, 2936, 1724, 1607, 1494, 1455, 1193, 1162, 1090, 1056, 1026. Anal. calcd. for ($C_{19}H_{22}N_2O_6S$): C, 56.15; H, 5.46; N, 6.89; S, 7.87. Found: C, 56.18; H, 5.43; N, 6.90; S, 7.86.

*Benzyl N-(5-methoxy-2,3-dihydro-1H-inden-1-yl)sulfamoyl carbamate (**17**)*

60% yield. White solid. Mp: 153–155°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.7 (bs, 1H, NH), 7.36 (bs, 5H, Ar-H), 7.14 (d, 1H, Ar-H, J = 8.3 Hz), 6.73 (s, 1H, Ar-H), 6.70 (dd, 1H, Ar-H, J = 2.0, 8.3 Hz), 5.38 (d, 1H, NH, J = 7.8 Hz), 5.17 (d, 2H, CH_2, J = 1.6 Hz), 4.81 (dd, 1H, NCH, J = 7.1, 13.9 Hz), 3.78 (s, 3H, OCH_3), 2.13–2.05 (m 1H, CH), 2.75–2.67 (m 1H, CH), 2.46–2.37 (m 1H, CH), 1.96–1.87 (m 1H, CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.6 (CO), 151.6 (C), 145.1 (C), 134.9 (C), 133.2 (C), 129.1 (CH), 129.0 (2CH), 128.9 (2CH), 125.3 (CH), 113.4 (CH),

110.1 (CH), 68.8 (OCH_2), 59.3 (CH-N), 55.7 (OCH_3), 34.1 (CH_2), 30.4 (CH_2). IR (CH_2CI_2, cm^{-1}): 3280, 3020, 2930, 1732, 1628, 1426, 1437, 1404, 1346, 1160, 1056, 1093. Anal. calcd. for ($C_{18}H_{20}N_2O_5S$): C, 57.43; H, 5.36; N, 7.44; S, 8.52. Found: C, 57.44; H, 5.38; N, 7.46; S, 8.50.

*Hydrogenolysis of benzyl sulfamoyl carbamates; synthesis of N-(7-methoxy-2,3-dihydro-1H-inden-1-yl)sulfamide (**18**)*

Pd-C (50 mg) was added to a solution of sulfamoylcarbamate **15** (1.40 g, 3.72 mmol) in MeOH (50 mL) into a 100 mL flask. A balloon filled with H_2 gas (3 L) was fitted to the flask. The mixture was deoxygenated by flushing with H_2 and then hydrogenated at RT for 4 h. The catalyst was removed by filtration. The residue was crystallized from EtOAc/hexane to give compound **18** (0.70 g, 78% yield). White solid. Mp: 122–124°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.24 (t, 1H, Ar-H, $J = 7.8$ Hz), 6.87 (d, 1H, Ar-H, $J = 7.8$ Hz), 6.70 (d, 1H, Ar-H, $J = 7.8$ Hz), 5.02 (dd, 1H, NCH, $J = 3.9$, 7.3 Hz), 4.77 (bs, 2H, NH_2), 3.86 (s, 3H, OCH_3), 3.09–3.01 (m 1H, CH), 2.85–2.78 (m 1H, CH), 2.49–2.40 (m 1H, CH), 2.30–2.22 (m 1H, CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.2 (C), 146.6 (C), 130.7 (CH), 128.7 (C), 117.9 (CH), 108.8 (CH), 57.7 (CN), 55.7 (OCH_3), 33.9 (CH_2), 30.9 (CH_2). IR (CH_2CI_2, cm^{-1}): 3279, 2942, 2846, 1605, 1406, 1341, 1307, 1266, 1163, 1073, 1057. Anal. calcd. for ($C_{10}H_{14}N_2O_3S$): C, 49.57; H, 5.82; N, 11.56; S, 13.23. Found: C, 49.60; H, 5.84; N, 11.54; S, 13.25.

*N-(4,7-Dimethoxy-2,3-dihydro-1H-inden-1-yl)sulfamide (**19**)*

72% yield. White solid. Mp: 133–135°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.72 (d, 1H, A part of AB system, Ar-H, $J = 8.7$ Hz), 6.67 (d, 1H, B part of AB system, Ar-H, $J = 8.7$ Hz), 5.05–5.00 (m, 2H, NCH and NH), 4.85 (bs, 2H, NH_2), 3.82 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.01–2.97 (m, 1H, C–H), 2.82–2.74 (m, 1H, C–H), 2.52–2.42 (m, 1H, C–H), 2.29–2.21 (m 1H, C–H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 150.8 (C), 150.1 (C), 134.3 (C), 130.5 (C), 111.2 (CH), 109.7 (CH), 58.2 (NC), 56.1 (OCH_3), 55.9 (OCH_3), 33.9 (CH_2), 27.8 (CH_2). IR (CH_2CI_2, cm^{-1}): 3356, 3276, 2939, 1549, 1496, 1463, 1439, 1303, 1258, 1163, 1088, 1058. Anal. calcd. for ($C_{11}H_{16}N_2O_4S$): C, 48.52; H, 5.92; N, 10.29; S, 11.77. Found: C, 48.55; H, 5.89; N, 10.26; S, 11.75.

*N-(5-Methoxy-2,3-dihydro-1H-inden-1-yl)sulfamide (**20**)*

78% yield. White solid. Mp: 112–114°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33 (d, 1H, Ar-H, $J = 8.3$ Hz), 6.79–6.74 (m, 2H, Ar-H), 4.91 (dd, 1H, NCH, $J = 7.3$, 14.7 Hz), 4.71 (bs, 2H, NH_2), 4.6 (d, 1H, NH, $J = 8.4$ Hz), 3.78 (s, 3H, OCH_3), 3.02–2.93 (m 1H, CH), 2.84–2.76 (m 1H, CH), 2.65–2.56 (m 1H, CH), 2.06–1.97 (m 1H, CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.5 (C), 145.2 (C), 134.2 (C), 125.4 (CH), 113.3 (CH), 110.2 (CH), 58.9 (CN), 55.7 (OCH_3), 35.1 (CH_2), 30.4 (CH_2). IR (CH_2CI_2, cm^{-1}): 3274, 3109, 3019, 2929, 1607, 1434, 1343, 1326, 1264, 1105, 1056. Anal. calcd. for ($C_{10}H_{14}N_2O_3S$): C, 49.57; H, 5.82; N, 11.56; S, 13.23. Found: C, 49.55; H, 5.80; N, 11.55; S, 13.20.

*Standard procedure for the synthesis of t-butyl sulfamoyl carbamates: t-Butyl N-(2,4-dimethoxyphenyl)sulfamoyl carbamate (**25**)*

t-BuOH (0.58 g, 7.83 mmol) and CSI (1.02 g, 7.18 mmol) were dissolved in CH_2Cl_2 (20 mL) at 0°C. A solution of **21** (1.00 g, 6.53 mmol) in CH_2Cl_2 (40 mL) and NEt_3 (0.80 g, 7.83 mmol) were added to the solution of CSI-*t*-BuOH drop wise and it was stirred at 0°C for 1 h then for 3 h at RT under N_2 atmosphere. The reaction mixture was cooled to 0°C and to this mixture was added a solution of 0.1 N HCl (70 mL). Organic phase was separated and H_2O phase was extracted with CH_2Cl_2 (2×30 mL). Combined organic layers were dried over Na_2SO_4 and the solvent was evaporated. The residue was crystallized from EtOAc/hexane to give **25** (1.23 g, 81% yield). Yellow solid (Mp: 138–140°C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39 (dm, 1H, Ar-H, $J = 8.3$ Hz), 7.24 (bs, 1H, NH), 7.08 (bs, 1H, NH), 6.47 (m, 1H, Ar-H), 6.45 (dm, 1H, Ar-H, $J = 8.3$ Hz), 3.82 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 1.45 (s, 9H, $3CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.7 (C), 151.9 (C=O), 149.1 (C), 123.9 (C), 118.5 (CH), 104.5 (CH), 99.2 (CH), 83.9 (C), 56.0 (OCH_3), 55.8 (OCH_3), 28.9 ($3CH_3$). IR (CH_2Cl_2, cm^{-1}) 3260, 2979, 2935, 1731, 1600, 1145, 929, 732. Anal. calcd. for ($C_{13}H_{20}N_2O_6S$): C, 46.98; H, 6.07; N, 8.43; S, 9.65. Found: C, 46.97; H, 6.10; N, 8.42; S, 9.63.

Compounds **26**–**28** were also synthesized by the same procedure.

*t-Butyl-N-(3,5-dimethoxyphenyl)sulfamoyl carbamate (**26**)*

White solid (78% yield, Mp: 40–42°C). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.27 (bs, 1H, NH), 7.22 (bs, 1H, NH), 6.41 (d, 2H, Ar-H, $J = 2.2$ Hz), 6.33 (t, 1H, Ar-H, $J = 2.2$ Hz), 3.78 (s, 6H, OCH_3), 1.41 (s, 9H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.5 (C), 150.1 (C=O), 137.8 (C), 100.9 (2CH), 98.8 (CH), 84.3 (C), 55.7 ($2OCH_3$), 28.1 ($3CH_3$). IR (CH_2Cl_2, cm^{-1}) 3260, 2979, 2935, 1731, 1600, 1144, 929, 732. Anal. calcd. for ($C_{13}H_{20}N_2O_6S$): C, 46.98; H, 6.07; N, 8.43; S, 9.65. Found: C, 46.99; H, 6.09; N, 8.40; S, 9.63.

*t-Butyl-N-(2,5-dimethoxyphenyl)sulfamoyl carbamate (**27**)*

White solid (72% yield, Mp: 148–150°C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 (bs, 1H, NH), 7.40 (bs, 1H, NH), 7.13 (d, 1H, Ar-H, $J = 2.9$ Hz), 6.47 (d, 1H, Ar-H, $J = 9.1$ Hz), 6.63 (dd, 1H, Ar-H, $J = 2.9$, 9.1 Hz), 3.77 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 1.44 (s, 9H, $3CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.9 (C=O), 149.1 (C), 143.3 (C), 126.2 (C), 111.6 (CH), 109.6 (CH), 106.6 (CH), 83.9 (C), 56.3 (OCH_3), 55.8 (OCH_3), 27.7 ($3CH_3$). IR (CH_2Cl_2, cm^{-1}) 3260, 2979, 2935, 1731, 1600, 1145, 929, 732. Anal. calcd. for ($C_{13}H_{20}N_2O_6S$): C, 46.98; H, 6.07; N, 8.43; S, 9.65. Found: C, 46.96; H, 6.10; N, 8.45; S, 9.62.

*t-Butyl-N-(3,4,5-trimethoxyphenyl)sulfamoyl carbamate (**28**)*

Yellow solid (75% yield, Mp, 158–160°C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (bs, 1H, NH), 6.65 (bs, 2H, Ar-H), 6.31 (bs, 1H, NH), 3.77 (s, 6H, $2OCH_3$), 3.66 (s, 3H, OCH_3), 1.41 (s, 9H, $3CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.5 (2C), 150.1 (C=O), 137.8 (C), 100.9 (2CH), 98.8 (C), 84.3 (C), 55.7 (OCH_3), 28.1 (CH_3). IR (CH_2Cl_2, cm^{-1}) 3254, 2974, 2935, 1717, 1606, 1134, 937, 742. Anal. calcd. for ($C_{14}H_{22}N_2O_7S$): C, 46.40; H, 6.12; N, 7.73; S, 8.85. Found: C, 46.43; H, 6.15; N, 7.74; S, 8.84.

*Standard procedure for the synthesis of sulfamides derived from anilines: Synthesis of N-(2,4-dimethoxyphenyl)-sulfamide (**29**)*

To a solution of **25** (1.00 g, 3.01 mmol) in CH_2Cl_2 (20 mL) was added trifluoro acetic acid (CF_3CO_2H) (3.43 g, 30.10 mmol) drop wise and it was stirred at 0°C for 2 h then at RT for 24 h under N_2 atmosphere. The reaction mixture was cooled to 0°C and to this mixture was added a solution of 1 N $NaHCO_3$ (100 mL). Organic phase was separated and H_2O phase was extracted with CH_2Cl_2 (2×40 mL). Combined organic layers were dried over Na_2SO_4 and the solvent was evaporated. The residue was crystallized from EtOAc/hexane to give sulfamide **29** (0.59 g, 85% yield). White solid.

Mp: 118–120°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.34 (d, 1H, Ar-H, $J = 8.7$ Hz), 7.09 (bs, 1H, NH), 6.61 (d, 1H, Ar-H, $J = 2.7$ Hz), 6.45 (dd, 1H, Ar-H, $J = 2.7$, 8.7 Hz), 6.13 (bs, 2H, NH_2), 3.86 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, DMSO-D_6): δ 154.3 (CH), 143.5 (CH), 128.9 (C), 111.9 (C), 107.4 (CH), 106.3 (C), 56.2 (OCH_3), 55.2 (OCH_3). IR (CH_2Cl_2, cm^{-1}) 3272, 3092, 2935, 1597, 1155, 922, 711. Anal. calcd. for ($C_8H_{12}N_2O_4S$): C, 41.37; H, 5.21; N, 12.06; S, 13.80. Found: C, 41.35; H, 5.20; N, 12.05; S, 13.84.

Compounds **30**–**32** were also prepared by the same procedure.

*N-(3,5-Dimethoxyphenyl)sulfamide (**30**)*

White solid (82% yield, Mp: 93–95°C). ^{1}H NMR (400 MHz, acetone-d_6): δ 6.51 (s, 2H, Ar-H), 6.20 (s, 1H, Ar-H), 3.72 (s, 6H, OCH_3). ^{13}C NMR (100 MHz, acetone-d_6): δ 161.6 (2C), 141.2 (C), 97.6 (2CH), 94.9 (CH), 54.9 (OCH_3). IR (CH_2Cl_2, cm^{-1}) 3266, 3109, 2914, 1603, 1148, 934, 724. Anal. calcd. for ($C_8H_{12}N_2O_4S$): C, 41.37; H, 5.21; N, 12.06; S, 13.81. Found: C, 41.49; H, 5.52; N, 11.97; S, 13.50.

*N-(2,5-Dimethoxyphenyl)sulfamide (**31**)*

White solid (72% yield, Mp: 108–110°C). ^{1}H-NMR (400 MHz, acetone-d_6): δ 7.11 (d, 1H, Ar-H, $J = 3.0$ Hz), 6.91 (d, 1H, Ar-H, $J = 8.9$Hz), 6.58 (dd, 1H, Ar-H, $J = 3.0$, 8.9 Hz), 3.81 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, acetone-d_6): δ 154.2 (C), 143.5 (C), 128.9 (C), 111.9 (CH), 107.4 (CH), 106.3 (CH), 55.7 (OCH_3), 55.1 (OCH_3). IR (CH_2Cl_2, cm^{-1}) 3272, 3092, 2935, 1597, 1155, 922, 711. Anal. calcd. for ($C_8H_{12}N_2O_4S$): C, 41.37; H, 5.21; N, 12.06; S, 13.81. Found: C, 41.39; H, 5.25; N, 12.04; S, 13.79.

*N-(3,4,5-Trimethoxyphenyl)sulfamide (**32**)*

70% yield. Yellow solid. Mp: 168–170°C. ^{1}H NMR (400 MHz, acetone-d_6): δ 6.64 (s, 2H, Ar-H), 3.77 (s, 6H, $2OCH_3$), 3.65 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, acetone-d_6): δ 153.8 (2C), 135.4 (2C), 134.9 (C), 98.3 (2CH), 59.9 (OCH_3), 55.9 ($2OCH_3$). IR (CH_2Cl_2, cm^{-1}) 3361, 3260, 2913, 1602, 1126, 1004. Anal. calcd. for ($C_9H_{14}N_2O_5S$): C, 41.21; H, 5.38; N, 10.68; S, 12.23. Found: C, 41.24; H, 5.40; N, 10.68; S, 12.20.

Biochemical methods

Purification of CA isoenzymes by affinity chromatography

Both CA isoenzymes (hCA I and II) were purified from fresh human erythrocytes by Sepharose 4B-L-tyrosine sulfanilamide affinity chromatography. This chromatographic method was described previously [47–49]. Briefly, erythrocytes were taken from human blood, following low-speed centrifugation at $2000 \times g$ for 20 min (Hermle, Z323 K, Grand rotor) by removal of plasma and buffy coat. The pH of haemolysate was adjusted to 8.7 with solid Tris. The homogenate was applied to the formerly prepared Sepharose 4B-L-tyrosine-sulfanylamide affinity column. Then column was equilibrated with Tris-HCl/Na_2SO_4 (pH 8.7; 25 mM/0.1 M). The affinity gel was washed with Tris-HCl/Na_2SO_4 (pH 8.7; 25 mM/22 mM). Finally, hCA I and II were eluted with NaCl/sodium phosphate (pH 6.3; 1.0 M/25 mM) and CH_3COONa/$NaClO_4$ (pH 5.6; 0.1 M/0.5 M), respectively [50–52].

Activity assays

Hydratase activity was assayed according to the Wilbur and Anderson's method [53]. CO-hydratase activity as an enzyme unit (EU) was calculated by using the following equation $(t_c - t_o)/t_c$, where t_o and t_c are the spending times for pH change of the nonenzymatic and the enzymatic reactions, respectively. On the other hand, esterase activity was assayed by following the change in absorbance at 348 nm of NPA to 4-nitrophenoxide ion over a period of 3 min at 25°C using a spectrophotometric method [53]. The enzymatic reaction, in a total volume of 3.0 mL, contained 1.4 mL Tris-SO_4 buffer (0.05 M, pH 7.4), 1 mL NPA (3 mM), distilled water (0.5 mL) and enzyme solution (0.1 mL).

Protein determination

During the purification studies, the quantity of protein was determined spectrophotometrically at 595 nm according to the Bradford's method [54]. Bovine serum albumin was used as standard protein [55].

SDS-polyacrylamide gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used in this study for separation of both isoenzymes. It was carried out according to Laemmli's procedure [56]. Briefly, it was applied in 10% and 3% acrylamide for the running and the stacking gel, respectively, containing SDS (0.1%). This method has been described in detail previously [57].

Inhibition assay

The inhibitory effects of some novel sulfamide carbamates and sulfamides **15**–**31** were examined *in vitro*. All compounds (**15**–**31**) were tested in triplicate at each concentration used. Both isoenzyme activities were measured in the presence of different concentration of each novel compound [58]. Control activity in the absence of inhibitor was taken as 100%. For each sulfamide, an Activity (%)-[Sulfamides] graph was obtained. To determine K_i values, several different concentrations were used. In these experiments, as stated previously NPA was used as a CA substrate at five different concentrations. The Lineweaver–Burk curves were used to determine kinetic parameters and inhibition constants [59, 60].

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