Full Paper

Novel Sulphamides and Sulphonamides Incorporating the Tetralin Scaffold as Carbonic Anhydrase and Acetylcholine Esterase Inhibitors

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Reactions of amino, aminomethyl tetralins and benzyl alcohol with chlorosulphonyl isocyanate (CSI) afforded sulphamoyl carbamates. The sulphamoyl carbamates were converted to sulphamides by palladium-catalysed hydrogenolysis. Sulphonamides were synthesized from the reactions of amines with $MeSO_2Cl$. Inhibition of human (h) carbonic anhydrase (CA) isoenzymes (hCA I, hCA II) and acetylcholine esterase (AChE) was investigated with the synthesized compounds. hCA I and hCA II were inhibited in the low micromolar or sub-micromolar range. The K_i values were in the range of 0.91–9.56 μM against hCA I and of 3.70–27.88 μM against hCA II. Sulphamides **11–13** and sulphonamides **14–16** had moderate inhibition capacity toward AChE. These findings suggest the novel sulphamides **11–13** and sulphonamides **14–16** as AChE and CA isoenzyme inhibitory agents.

Keywords: Acetylcholine esterase / Aminotetralins / Carbonic anhydrase / Sulphamides / Sulphamoyl carbamates / Sulphonamides

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Introduction

Sulphamides [1] and sulphonamides [2] are important biologically active compounds. In the last decades a lot of sulphamide and sulphonamide drugs have appeared in the markets. Quinagolide (**1**), a dopaminergic drug, is an antihyperprolactinemia agent [3]. Compound **2** (JNJ-26990990) has potential use in the treatment of depression, neuropathic and inflammatory pains [4]. The sulphonamide drug sultiame (**3**) is an anticonvulsant used in the treatment of epilepsy and West syndrome [5]. Acetazolamide (AZA, **4**), a drug also known as carbonic anhydrase (CA) inhibitor, is used in the treatment of glaucoma [6] and idiopathic intracranial hypertension [7] (Figure 1).

CA (E.C.: 4.2.1.1) is a protein that is well suited to serve as a model in many types of biophysical, bioanalytical and physical-organic chemical studies as well as for inhibitor drug design studies. This enzyme catalyses the hydration of carbon dioxide (CO_2) and the dehydration of bicarbonate ion (HCO_3^-) [8, 9].

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

The CA enzyme family is particularly attractive for biophysical studies of protein-ligand binding and inhibition studies for many reasons, among which (i) CA is a monomeric enzyme, single-chain protein of medium molecular weight (30 kDa). The most widespread isoforms (CA I and CA II) have no disulphide bonds, pendant sugar or phosphate groups; all the other isoforms have disulphide bonds, pendant sugar or phosphate groups. (ii) CA II is an inexpensive enzyme widely available. (iii) CA II is relatively easy to handle and purify, due in large part to its excellent stability under standard laboratory conditions. (iv) The amino acid sequences are available for most isoenzymes. (v) The structure and active site geometries have been defined in detail by X-ray diffraction analysis for most of the 13 catalytically active mammalian isoforms [8]. Its catalytic mechanism is well understood. (vi) It not only behaves as a CO_2 hydrase/dehydratese activity with a high turnover number but also possesses esterase activity with carboxylic acid esters, phosphoric acid esters and even

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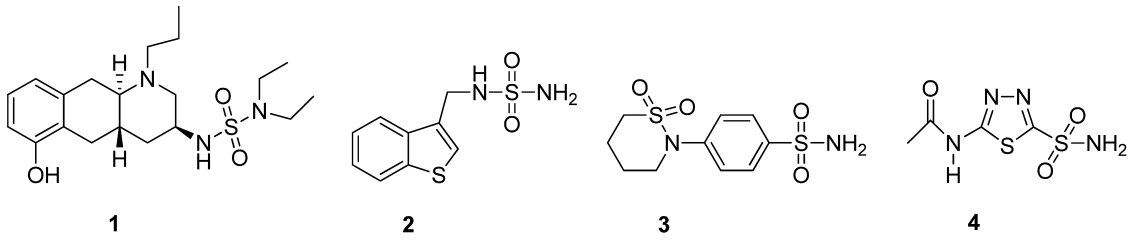


Figure 1. Some selected sulphamide and sulphonamide drugs (**1**–**4**).

sulphate esters [10–12]. (vii) Its inhibition mechanism by inhibitors that bind to the Zn^{2+} ion is fairly simple and well characterized [8, 13].

Alzheimer's disease is the most common form of dementia and neurodegenerative diseases. It is characterized by memory dysfunction. Reduction of acetylcholine levels in the brain is the most notable biochemical change in Alzheimer's disease [14]. Also, Alzheimer's disease can be treated by use of pharmaceuticals, which restore the level of acetylcholine through inhibition of acetylcholine esterase (AChE) [15]. Alzheimer's is predicted to affect 1 in 85 people globally by 2050 [16]. For treatment of Alzheimer's disease, some drugs such as galanthamine, tacrine and rivastigmine are used as AChE inhibitors. However, it has been reported that these drugs have adverse effects like hepatotoxicity and gastrointestinal disturbances [17–20]. Therefore, the safe and active AChE inhibitors have recently been gaining more attention.

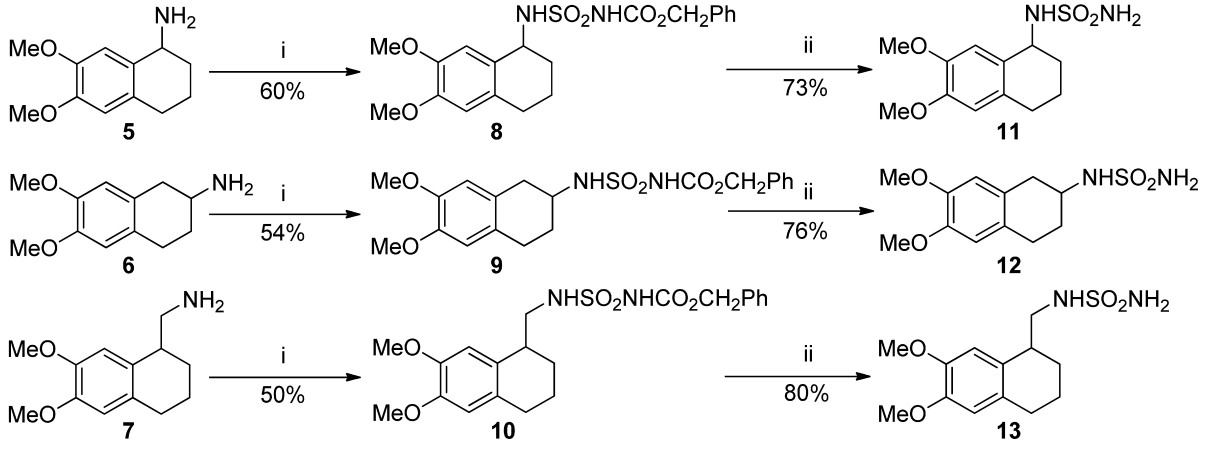
It is well known that aminotetralin derivatives show beneficial biological activities in CNS such as dopaminergic [21, 22], adrenergic and seratonergic activities [23]. The synthesis and biological evaluation of some novel sulphamides and sulphonamides derived from these biologically active tetralins will be useful for further synthetic and biological purposes. In this context, we report the synthesis of novel sulphamides **11**–**13** and sulphonamides **14**–**16**. We also evaluate CA isoenzymes (hCA I and hCA II) and AChE inhibitory effects of the synthesized novel sulphamides **11**–**13** and sulphonamides **14**–**16**.

Results and discussion

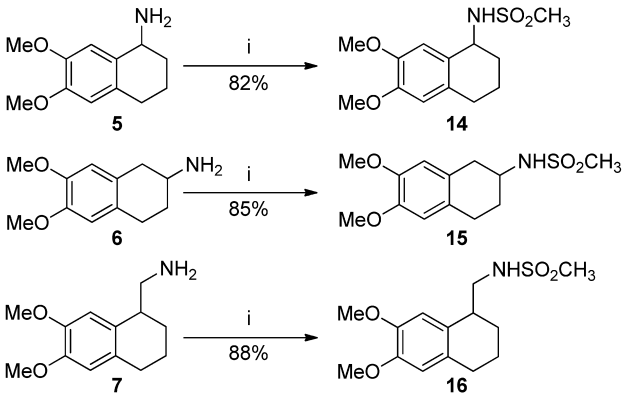
Chemistry

The synthesis of 1-aminotetralin **5** [24], and dopaminergic compounds **6** [25] and **7** [26] was carried out as described previously. The reactions of free amines and benzyl alcohol/*t*-BuOH with chlorosulphonyl isocyanate (CSI) in the presence of Et_3N to give sulphamoyl carbamates have been reported by us [9, 27]. By the similar approach, the reactions of amines **5**–**7** and 1.2 equiv. benzyl alcohol with CSI in the presence of Et_3N afforded novel sulphamide carbamates **8**–**10** in moderate yields. New sulphamides **11**–**13** were synthesized by Pd–C-catalysed hydrogenolysis of **8**–**10** with yields of 73, 76 and 80%, respectively (Scheme 1).

On the other hand, the treatment of amines with $MeSO_2Cl$ in the presence of Et_3N at 0–25°C yielded novel sulphonamides **14**–**16** in high yields (Scheme 2). The structures of all novel



Scheme 1. The synthesis of sulphamides **11**–**13**. (i) CSI/NEt_3/BnOH, CH_2Cl_2, 0–25°C, 4 h; (ii) H_2/Pd-C, MeOH, 25°C, 4 h.



Scheme 2. The synthesis of novel sulphonamides **14–16**. (i) $MeSO_2Cl/NEt_3$, CH_2Cl_2, 0–25°C, 15 h.

synthesized compounds were determined by ^1H and ^{13}C NMR techniques. The functional groups were characterized by IR.

CA isoenzymes purification and activity assay

Methods for isolation and purification of CA are well established and widely accessible. Commercial sources usually isolate isoenzymes hCA I and hCA II from fresh human red blood cells, in which CA is the second most abundant protein after haemoglobin. Research laboratories typically produce these isoenzymes by recombinant technology in *Escherichia coli* [28, 29]. This method provides the structural flexibility and specificity of site-directed mutagenesis, which is useful for biophysical and biochemical studies [18]. The blood protein and the wild-type recombinant construct are identical except for the N-terminus, which is a post-translationally acetylated Ser in the native CA and exists as Met-Ala or Ala in the recombinant version [9, 30]. The research laboratory procedure for the purification of hCA from red blood cells involves lysis of the cells and removal of the cellular remnants by centrifugation, followed by separation of haemoglobin from hCA on a sulphonamide-modified agarose affinity chromatography column [31, 32]. Divalent anions like sulphate do not bind appreciably in the active site of hCA. Therefore, it was used to remove nonspecifically bound protein by screening ionic interactions. Then, hCA I and hCA II can be eluted consecutively from the column. hCA I, which has a higher dissociation constant for sulphonamides than hCA II, is eluted with Tris-SO_4 buffer containing 0.2 M KI; hCA II elutes with Tris-SO_4 buffer containing 0.4 M NaN_3. Also, it was shown that CA isoenzymes from other species could be purified in a similar way, with only minor modifications. Commercial suppliers do not use the chromatographic methods, although their exact procedures are proprietary [31, 33].

In the present study, hCA I and hCA II isoenzymes were purified from fresh human erythrocytes. The purification of both CA isoenzymes was performed using a simple one-step method with Sepharose-4B-L-tyrosine-sulphanilamide affinity column chromatography [34, 35]. Human erythrocyte CA I isoenzyme was purified, 154.9-fold with a specific activity of 2088.6 EU mg^{-1} and overall yield of 22.8%; hCA II isoenzyme was purified, 245.6-fold with a specific activity of 9335.2 EU mg^{-1} and an overall yield of 38.3%.

CA isoenzymes inhibition effects

We report here the first study on the inhibitory effects of novel sulphamides **11–13** and sulphonamides **14–16** on the esterase activity of hCA I and hCA II. The inhibitor concentration that caused 50% inhibition (IC_{50}) was determined from activity versus (%)-[sulphamides] or [sulphonamides] plots and the average inhibition constant (K_i) values were calculated from Lineweaver–Burk plots (Table 1). The data in Table 1 show the following inhibition of hCA I and hCA II with the novel sulphamides **11–13** and sulphonamides **14–16** by an esterase assay, with 4-NPA as substrate.

Inhibition of the CA isoenzymes has pharmacologic applications in the field of anti-glaucoma, anti-convulsant, and anti-cancer agents. It is well known that sulphamides and sulphonamides are the main classes of CA inhibitors, which bind to the metal ion from the enzyme active site [27]. CA inhibitors block the function of CA enzyme. The myriad inhibitors of CA can be divided into four main groups:

i. Sulphamides and sulphonamides
ii. Sulphonic acid derivatives
iii. Small monoanions such as halides, azides and thiocyanate
iv. Aromatic rings or phenolic compounds.

The sulphonamides, sulphamides and sulphonic acid derivatives have K_i values in the picomolar to micromolar

Table 1. Human CA isoenzymes (hCA I and hCA II) and AChE inhibition data with the novel sulphamides and sulphonamides **11–16**.

Compounds	K_i (μM)		
	hCA-I	hCA-II	AChE
11	0.71 ± 0.11	0.69 ± 0.17	140.2 ± 26.8
12	1.24 ± 0.16	0.72 ± 0.02	56.7 ± 0.02
13	0.89 ± 0.18	1.45 ± 0.35	63.2 ± 22.5
14	1.26 ± 0.04	0.67 ± 0.09	365.6 ± 61.7
15	1.03 ± 0.27	0.87 ± 0.26	84.5 ± 18.1
16	1.56 ± 0.05	0.39 ± 0.13	537.3 ± 49.1
AZA[a)]	36.2 ± 0.00[b)]	3.70 ± 0.00[b)]	–
GAL[c)]	–	–	3.70 ± 0.00[c)]

[a)] AZA was used as positive standard inhibitor for both CA isoenzymes (CA I and CA II).
[b)] From ref. [37].
[c)] Galantamine was used as a reference compound for AChE inhibitory properties.

range, whereas the inorganic monoanions bind CA with K_i values in the micromolar to millimolar range [33]. Recently, it was observed by us that sulphamides show greater inhibitory effects than that of sulphonamides [27]. Similarly, it has been reported that phenolic inhibitors with aromatic rings have K_i values in the micromolar to millimolar range [10, 36–40].

The most obvious bioisosteres of sulphonamides are sulphamides, in which an additional electron-withdrawing atom/group is directly attached to the sulphamoyl function, generating compounds with general formula R–NH–SO_2NH_2. These groups were considered as interesting candidates for obtaining CAIs. In first, Supuran's group showed that the simple sulphamide binds to the metal ion in the Co^{2+}-substituted CA II [41], whereas the X-ray structures of the compound in adduct with CA II have been reported several years later [42]. Nowadays, there are a large number of aromatic, heterocyclic, aliphatic and sugar-based sulphamides, which were shown to possess highly effective inhibitory properties against all known mammalian isoforms [13]. Therefore, sulphamides constitute a highly important class of CAIs, with some derivatives clinically used for the treatment of epilepsy and obesity.

There have been several attempts to illuminate the inhibition mechanism of CA and to synthesize new compounds with higher inhibition potential. Novel CA inhibitors have been designed for pharmacological and medicinal approaches, and many inhibitors have been synthesized recently [43]. There are many studies in the literature on the interactions of different compounds and CA isoenzymes. Recently, the inhibitory effects of dantrolene [44], melatonin [45], vitamin E [46], morphine [47], ethanol [48], antioxidant phenols [49], phenolic acids [36], distinguished natural polyphenols products, prominent phenolic compounds [38] and bromophenols [50] with hCA I and hCA II have been investigated by our groups. Indeed, the inhibition profiles of hCA I and hCA II with novel sulphamides **11–13** and sulphonamides **14–16** are very variable, with inhibition constants ranging from the nanomolar (390 nM) to the submicromolar (1.56 μM) level. For this purpose, hCA I and hCA II inhibitory effects of the novel sulphamides **11–13** and sulphonamides **14–16** were tested under *in vitro* conditions and theirs K_i values are calculated and presented in Table 1.

We report here the initial study on the inhibitory effects of synthesized novel sulphamides **11–13** and sulphonamides **14–16** on the hydratase activity of hCA I and hCA II. The data in Table 1 show the following regarding the inhibition of hCA I and hCA II activity by novel sulphamides **11–13** and sulphonamides **14–16**. The novel sulphamides **11–13** and sulphonamides **14–16** investigated here showed effective inhibitory properties against the cytosolic isoforms hCA I and hCA II; however, the strongest inhibitory effect has been observed with novel sulphamide **11**, investigated here for the inhibition of the rapid cytosolic isoenzymes hCA I (K_i: 0.71 ± 0.11 μM). On the other hand, the strongest inhibitory effect has been observed with novel sulphonamide **16**, for hCA II (K_i: 0.39 ± 0.13 μM).

It had been reported that sulphamide and sulphamic acid act as moderate hCA II inhibitors, with inhibition constants of 1130 μM for sulphamide and 390 μM for sulphamic acid at the physiological pH (7.4), respectively [51]. In contrast to these literatures, in the current study, we observed strong inhibitory effect of new synthesized sulphamides **11–13** and sulphonamides **14–16** on hCA I and hCA II. K_i values of these sulphamide and sulphonamide compounds (**11–16**) are in the range of 0.89–1.56 μM for hCA I, and 0.39–1.45 μM for hCA II (Table 1). The arithmetic average K_i values of novel synthesized sulphamides and sulphonamides (**11–16**) are 1.12 μM for hCA I and 1.29 μM for hCA II. The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of novel sulphamides or sulphonamides **11–16** in inhibiting CA isoenzyme function. These values are commonly used as a measure of inhibition potency in biological and pharmacological research. K_i values were calculated by making use of IC_{50} values. A lower IC_{50} and K_i value reflects strong CA isoenzyme inhibition effect. The results obtained from Table 1 clearly showed that both the cytosolic isoenzymes hCA I and hCA II were effectively inhibited by sulphamide and sulphonamide compounds, with K_is within the range of 0.39–1.56 μM with a comparable potency as the reference compound AZA with K_i value of 36.2 μM. All novel synthesized sulphamides (**11–13)** and sulphonamides (**14–16)** acted as strong hCA I and hCA II inhibitors. The structure–activity relationship and inhibition properties of hCA II isoenzyme are particularly comparable to that what is outlined above for hCA I. Due to the two isoenzymes having a high sequence homology of amino acid present within the active site, all new synthesized sulphamide and sulphonamide compounds (**11–16)** had much stronger CA I and CA II inhibition effects than the clinically used AZA included in the assays as a standard inhibitor (Table 1).

All of the synthesized novel sulphamides and sulphonamides (**11–16**) have one aromatic ring with two methoxy groups. The aromatic ring in a chemical compound is known to be in favour of CA inhibition. These new synthesized sulphamides compounds have been investigated as CA isoenzyme inhibitors in this study. The rationale of investigating these compounds as CA inhibitors exists in the fact that the compounds with aromatic rings have been shown to be the only competitive inhibitor with CO_2 as the substrate for the main isoform of CA, i.e., human cytosolic isoenzymes (CA I and CA II).

CA inhibitors are a class of pharmaceuticals that suppress the activity of CA. Their clinical use has been established as anti-glaucoma agents, diuretics, antiepileptics, and in the

management of mountain sickness, gastric and duodenal ulcers, neurological disorders, or osteoporosis [52]. Sulphamides are indicated for the reduction of elevated intraocular pressure in patients with open-angle glaucoma or ocular hypertension and those who are insufficiently responsive to beta-blockers. The metal-complexing anions and the sulphamides with a terminal $-SO_2NH_2$ group coordinate to the Zn^{2+} ion. Simple anions such as HS−, CN−, NCO−, N_3−, HSO_3^-, I^- and $HCOO^-$ may bind with tetrahedral geometry or form trigonal-bipyramidal coordination [53] whereas the sulphamides replace the water coordinated to zinc and the 'deep-water' hydrogen-bonded to Thr199-NH. Both waters are present in the uncomplexed state [54]. Although sulphonamides were considered the moiety par excellence to coordinate the catalytic zinc and for designing potent CAIs, in recent years related functional groups such as sulphamide, sulphonamide and others have proven to be successful in the design of selective CAIs [55].

Inhibition effects of novel sulphamides **11–13** and sulphonamides **14–16** on AChE were determined by commercially available purified AChE (E.C. 3.1.1.7, Product no: C0663-50 UN, Sigma–Aldrich) from human erythrocytes based on the spectrophotometric method of Ellman et al. [56]. It was reported that the inhibition of AChE has been used for the determination of neurotoxic properties of chemicals capable of interfering with normal neurotransmission of the parasympathetic and sympathetic nervous system. It was well known that some chemical compounds like organophosphate and carbamate pesticides [57–60], enantiomeric inhibitors [61], metals and non-pesticide contaminants have also been reported to inhibit AChE enzymatic activity [62–65]. Also, it was demonstrated that the main AChE inhibitory effects were primarily associated with aromatic compounds and, to lesser degree, with aliphatic compounds [62].

All of these studies clearly showed that an investigation into the mechanism of action of AChE might lead to the design of the mechanism-based inhibitors, which could be of future therapeutic use. Different types of AChE inhibitors have been studied for the treatment of Alzheimer's disease. Some of the AChE inhibitors differ in their mechanism of action, metabolism and brain selectivity.

AChE was very effectively inhibited by novel sulphamides **11–13** and sulphonamides **14–16**, with K_is in the range of 56.04 ± 0.02 to 537.3 ± 49.1 μM. The K_i values of novel sulphamides **11–13** and sulphonamides **14–16** were similar to each other. The differences between the highest and the lowest K_i values of novel sulphamides **11–13** and sulphonamides **14–16** were only tenfold. The most active sulphamide was novel sulphamides **12**, and sulphonamides **15** with same functional groups demonstrated K_i values with 56.7 ± 0.02 and 84.5 ± 18.1 μM, respectively. On the other hand, galantamine (GAL), which is used for the treatment of mild-to-moderate Alzheimer's disease and various other memory impairments, has been shown to lower AChE inhibition activity (EC_{50}: 3.70 μM) [66].

Conclusions

In summary, three sulphamides **11–13** and three sulphonamides **14–16** were synthesized starting from known biologically active amines **5–7** for the first time. The synthesized sulphamoyl carbamates **8–10**, sulphamides **11–13** and sulphonamides **14–16** may be important synthons for further biological and synthetic purposes. In addition, several sulphamides **11–13** and sulphonamides **14–16** have been assayed for the inhibition of the physiologically relevant human CA isoenzymes (hCA I and hCA II). The compounds investigated here showed CA isoenzyme inhibitory effects, in the nanomolar to low micromolar range, by the esterase method. Toward hCA I sulphamide and sulphonamide compounds (**11–16**) the inhibition constant was in the range of 0.71–1.56 μM, and against hCA II it was in the range of 0.39–1.45 μM. In general, the compounds had comparable inhibitory activity with the clinically used AZA. Also, these findings clearly showed that novel synthesized sulphamides and sulphonamides (**11–16**) might be used as leads for generating potent hCA I and hCA II inhibitors, eventually targeting other isoforms that have not been assayed as yet for their interactions with such agents. Additionally, from this study it can be concluded that tested novel sulphamides **11–13** and sulphonamides **14–16** exhibited moderate inhibitory activity on AChE.

Experimental

All chemicals and solvents are commercially available and were used after distillation or 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 ^1H and ^{13}C NMR spectra were recorded on a 400 (100)-MHz Varian and 400 (100)-MHz Bruker spectrometer (δ in ppm, Me_4Si as the internal standard). Elemental analyses were performed on a Leco CHNS-932 apparatus. Column chromatography was performed on silica gel (60-mesh, Merck). PLC is preparative thick-layer chromatography: 1 mm of silica gel 60 PF (Merck) on glass plates.

Chemistry

1-Aminotetralin **5** [24], 2-aminotetralin **6** [22], and 1-aminomethyl tetralin **7** [26] were synthesized as described in the literature.

*Standard procedure for the synthesis of sulphamoylcarbamates: Benzyl N-(6,7-dimethoxy-1,2,3,4-tetrahydronaphthalen-1-yl)sulphamoylcarbamate (**8**)*

Benzylalcohol (0.50 g, 4.63 mmol) was added to a solution of CSI (0.66 g, 4.60 mmol) in CH_2Cl_2 (10 mL) at 0°C. A solution of amine **5**

(0.80 g, 3.86 mmol) in CH_2Cl_2 (30 mL) and NEt_3 (0.55 g, 5.40 mmol) was added to the solution of CSI dropwise and stirred at 0°C for 1 h, then at room temperature for 3 h. The reaction mixture was cooled to 0°C and to this mixture a solution of 0.1 N HCl (50 mL) was added. 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. Column chromatography of the residue on silica gel (30 g) with 30% EtOAc–hexane yielded carbamate **8** (0.98 g, 60%) as white solid. The same experimental procedure was also used for the synthesis of compounds **9** (%54) and **10** (50%).

*Benzyl N-(6,7-dimethoxy-1,2,3,4-tetrahydronaphthalen-1-yl)sulphamoylcarbamate (**8**)*

White solid. Mp 88–90°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.36–7.31 (m, 6H, 5Ph-H and NH), 6.85 (s, 1H, Ar–H), 6.52 (s, 1H, Ar–H), 5.37 (d, 1H, NH, J = 7.6 Hz), 5.20 (d, A part of AB; 1H, Ha of OCH_2, J = 12.1 Hz), 5.14 (d, B part of AB; 1H, Hb of OCH_2, J = 12.1 Hz), 4.53–4.51 (m, 1H, CH–N), 3.83 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 2.68–2.60 (m, 2H, CH_2), 1.91–1.87 (m, 2H, CH_2), 1.78–1.72 (m, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 151.6 (CO), 149.1 (C), 147.9 (C), 134.8 (C of Ph), 130.1 (C), 129.1 (CH of Ph), 129.0 (2CH of Ph), 128.6 (2CH of Ph), 126.5 (C), 111.7 (2CH), 68.7 (OCH_2), 56.12 (OCH_3), 56.06 (OCH_3), 53.2 (CH–N), 30.0 (CH_2), 28.7 (CH_2), 19.4 (CH_2). IR (CH_2Cl_2, cm^{-1}): 3417, 3332, 3272, 3193, 3056, 2928, 2855, 1688,1608, 1445, 1402, 1343, 1265, 1163, 1120, 1084, 1071. Anal. calcd. for ($C_{20}H_{24}N_2O_6S$): C, 57.13; H, 5.75; N, 6.66; S, 7.63. Found: C, 57.08; H, 5.82; N, 6.58; S, 7.65.

*Benzyl N-(6,7-dimethoxy-1,2,3,4-tetrahydronaphthalen-2-yl)sulphamoylcarbamate (**9**)*

White solid. Mp: 140–142°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.49 (bs, 1H, NH), 7.41–7.34 (m, 5H, Ph-H), 6.54 (s, 1H, Ar–H), 6.45 (s, 1H, Ar–H), 5.23 (d, 1H, NH, J = 6.9 Hz), 5.20 (s, 2H, OCH_2), 3.87 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.77–3.69 (m, 1H, CH–N), 2.97 (dd, A part of AB; 1H, Ha of CH_2, J = 5.0 and 16.0 Hz), 2.78–2.74 (m, 2H, CH_2), 2.63 (dd, B part of AB; 1H, Hb of CH_2, J = 8.0 and 16.0 Hz), 2.04–1.96 (m, 1H, H of CH_2), 1.81–1.72 (m, 1H, H of CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 151.2 (CO), 147.8 (C), 147.5 (C), 134.7 (C of Ph), 128.9 (CH of Ph), 128.8 (2CH of Ph), 128.5 (2CH of Ph), 126.8 (C), 124.7 (C), 112.0 (CH), 111.5 (CH), 68.6 (OCH_2), 56.0 (OCH_3), 55.9 (OCH_3), 50.7 (CH-N), 35.5 (CH_2), 29.1 (CH_2), 26.4 (CH_2). IR (CH_2Cl_2, cm^{-1}): 3257, 3064, 3030, 3002, 2935, 2840, 1727, 1610, 1517, 1452, 1352, 1295, 1241, 1226, 1159, 1114, 1071, 1017. Anal. calcd. for ($C_{20}H_{24}N_2O_6S$): C, 57.13; H, 5.75; N, 6.66; S, 7.63. Found: C, 57.11; H, 5.78; N, 6.64; S, 7.60.

*Benzyl-N-((6,7-dimethoxy-1,2,3,4-tetrahydronaphthalen-1-yl)methyl)sulphamoylcarbamate (**10**)*

White solid. Mp: 62–64°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.51 (bs, 1H, NH), 7.36–7.31 (m, 5H, Ph–H), 6.62 (s, 1H, Ar–H), 6.55 (s, 1H, Ar–H), 5.25 (t, 1H, NH, J = 6.3 Hz), 5.16 (s, 2H, OCH_2), 3.83 (s, 6H, 2OCH_3), 3.28–3.23 (m, 2H, CH and 1H of CH_2NH), 2.96–2.93 (m, 1H, CH_2NH), 2.67–2.64 (m, 2H, CH_2), 1.83–1.73 (m, 4H, 2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 151.5 (CO), 148.0 (C), 147.5 (C), 134.7 (C of Ph), 130.2 (C), 129.1 (CH of Ph), 129.0 (2CH of Ph), 128.6 (2CH of Ph), 127.8 (C), 112.3 (CH), 111.5 (CH), 68.8 (OCH_2), 56.3 (OCH_3), 56.0 (OCH_3), 48.9 (CH_2–N), 37.2 (CH), 29.3 (CH_2), 25.9 (CH_2), 19.7 (CH_2). IR (CH_2Cl_2, cm^{-1}): 3270, 3064, 3034, 2932, 2856, 2094, 1735, 1610, 1514, 1464, 1454, 1353, 1256, 1219, 1159, 1119, 1089, 1068, 1028. Anal. calcd. for ($C_{21}H_{26}N_2O_6S$): C, 58.05; H, 6.03; N, 6.45; S, 7.38. Found: C, 58.10; H, 5.99; N, 6.48; S, 7.32.

*Standard procedure for the synthesis of sulphamides (hydrogenolysis of sulphamoylcarbamates): N-(6,7-Dimethoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-sulphamide (**11**)*

Into a 100-mL flask Pd-C (50 mg) and sulphamoylcarbamate **8** (0.7 g, 1.66 mmol) in MeOH (50 mL) were added. 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 room temperature for 4 h. The catalyst was removed by filtration. Recrystallization of the residue from EtOAc–hexane gave **11** (0.35 g, 73% yield).

Sulphamides **12**–**13** were also synthesized by this procedure with yields of 76 and 80%, respectively.

*N-(6,7-Dimethoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-sulphamide (**11**)*

White solid. Mp: 152–155°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 6.95 (s, 1H, Ar–H), 6.52 (s, 1H, Ar–H), 4.78 (bs, 2H, NH_2), 4.62–4.54 (m, 2H, NH and CH–N), 3.84 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 2.69–2.63 (m, 2H, CH_2), 2.03–1.98 (m, 2H, CH_2), 1.82–1.78 (m, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 148.8 (C), 147.8 (C), 130.1 (C), 127.5 (C), 111.8 (CH), 111.5 (CH), 56.3 (OCH_3), 56.1 (OCH_3), 52.3 (CH–N), 30.7 (CH_2), 28.8 (CH_2), 19.4 (CH_2). IR (CH_2Cl_2, cm^{-1}): 3495, 3417, 3325, 2969, 2952, 2926, 2845, 1706, 1647, 1609, 1512, 1453, 1420, 1353, 1317, 1286, 1255, 1216, 1155, 1116, 1088, 1074, 1051, 1037, 1017. Anal. calcd. for ($C_{12}H_{18}N_2O_4S$): C, 50.33; H, 6.34; N, 9.78; S, 11.20. Found: C, 50.38; H, 6.40; N, 9.72; S, 11.18.

*N-(6,7-Dimethoxy-1,2,3,4-tetrahydronaphthalen-2-yl)-sulphamide (**12**)*

White solid. Mp: 158–160°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 6.56 (s, 1H, Ar–H), 6.54 (s, 1H, Ar–H), 4.76 (bs, 3H, NH and NH_2), 3.81–3.76 (m, 7H, 2OCH_3 and CH–N), 3.09 (dd, A part of AB; 1H, Ha of CH_2, J = 4.6 and 15.7 Hz), 2.82 (t, 2H, CH_2, J = 6.3 Hz), 2.71 (dd, B part of AB; 1H, Hb of CH_2, J = 8.0 and 15.7 Hz), 2.11–2.04 (m, 1H, H of CH_2), 1.87–1.78 (m, 1H, H of CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 147.6 (C), 147.4 (C), 126.9 (C), 125.2 (C), 111.9 (CH), 111.4 (CH), 55.9 (OCH_3), 55.88 (OCH_3), 50.1 (CH–N), 36.0 (CH_2), 29.4 (CH_2), 26.5 (CH_2). IR (CH_2Cl_2, cm^{-1}): 3501, 3338, 3252, 3098, 2927, 2851, 1735, 1610, 1547, 1517, 1460, 1449, 1345, 1328, 1265, 1247, 1222, 1208, 1158, 1112, 1071, 1015. Anal. calcd. for ($C_{12}H_{18}N_2O_4S$): C, 50.33; H, 6.34; N, 9.78; S, 11.20. Found: C, 50.27; H, 6.29; N, 9.83; S, 11.26.

*N-((6,7-Dimethoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-methyl)sulphamide (**13**)*

White solid. Mp: 105–107°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 6.71 (s, 1H, Ar–H), 6.55 (s, 1H, Ar–H), 4.89 (bs, 2H, NH_2), 4.80 (t, 1H, NH, J = 6.4 Hz), 3.84 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.34–3.25 (m, 2H, CH and 1H of CH_2–N), 2.96–2.94 (m, 1H, 1H of CH_2–N), 2.67–2.65 (m, 2H, CH_2), 1.83–1.73 (m, 4H, 2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 147.5 (C), 147.1 (C), 130.1 (C), 128.3 (C), 111.9 (CH), 111.6 (CH), 56.2 (OCH_3), 55.8 (OCH_3), 48.4 (CH_2–N), 37.1 (CH), 29.1 (CH_2), 25.7 (CH_2), 19.6 (CH_2). IR (CH_2Cl_2,

cm^{-1}): 3495, 3338, 3271, 2935, 2861, 2829, 1644, 1611, 1560, 1513, 1465, 1406, 1330, 1255, 1218, 1188, 1157, 1118, 1090, 1064, 1026. Anal. calcd. for ($C_{13}H_{20}N_2O_4S$): C, 51.98; H, 6.71; N, 9.33; S, 10.68. Found: C, 51.93; H, 6.75; N, 9.38; S, 10.63.

*Standard procedure for the synthesis of sulphonamides: N-(6,7-Dimethoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-methanesulphonamide (**14**)*

Amine **5** (0.5 g, 2.41 mmol) was dissolved in CH_2Cl_2 (30 mL) and this solution was cooled to 0°C. To this solution Et_3N (0.29 g, 2.89 mmol) and $MeSO_2Cl$ (0.28 g, 2.41 mmol) were added. The reaction mixture was stirred at room temperature. for 15 h. After the solvent was evaporated, the residue was chromatographed on silica gel (30 g) column with 20% EtOAc–hexane. Sulphonamide **14** was synthesized as colourless crystal (0.57 g, 82% yield).

*N-(6,7-Dimethoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-methanesulphonamide (**14**)*

Mp: 147–149°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 6.86 (s, 1H, Ar–H), 6.48 (s, 1H, Ar–H), 4,86 (d, 1H, NH, J = 8.4 Hz), 4.52–4.47 (m, 1H, CH–N), 3.78 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 2.96 (s, 3H, CH_3), 2.69–2.59 (m, 2H, CH_2), 1.99–1.94 (m, 1H, H of CH_2), 1.87–1.73 (m, 3H, CH_2 and H of CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 148.7 (C), 147.7 (C), 129.9 (C), 127.7 (C), 111.7 (CH), 111.6 (CH), 56.2 (OCH_3), 56.0 (OCH_3), 52.0 (CH–N), 42.3 (CH_3), 31.4 (CH_2), 28.7 (CH_2), 19.7 (CH_2). IR (CH_2Cl_2, cm^{-1}): 3512, 3287, 3053, 3002, 2935, 2857, 2834, 1728, 1610, 1514, 1462, 1450, 1321, 1256, 1219, 1188, 1148, 1118, 1090, 1079, 1054, 1022. Anal. calcd. for ($C_{13}H_{19}NO_4S$): C, 54.72; H, 6.71; N, 4.91; S, 11.24. Found: C, 54.68; H, 6.78; N, 4.96; S, 11.30.

Compounds **15** and **16** were also synthesized by the same procedure with the yields of 85 and 88%, respectively.

*N-(6,7-Dimethoxy-1,2,3,4-tetrahydronaphthalen-2-yl)-methanesulphonamide (**15**)*

White solid. Mp: 137–139°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 6.57 (s, 1H, Ar–H), 6.53 (s, 1H, Ar–H), 4.65 (d, 1H, NH, J = 7.6 Hz), 3.82–3.78 (m, 7H, 2OCH_3 and CH–N), 3.08 (dd, A part of AB; 1H, Ha of CH_2, J = 5.2 and 15.8 Hz), 3.01 (s, 3H, CH_3), 2.84 (t, 2H, CH_2, J = 6.3 Hz), 2.70 (dd, B part of AB; 1H, Hb of CH_2, J = 8.1 and 15.8 Hz), 2.13–2.07 (m, 1H, H of CH_2), 1.87–1.82 (m, 1H, H of CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 147.7 (C), 147.5 (C), 126.8 (C), 125.0 (C), 112.0 (CH), 111.5 (CH), 55.93 (OCH_3), 55.92 (OCH_3), 49.8 (CH–N), 41.9 (CH_3), 36.6 (CH_2), 30.0 (CH_2), 26.6 (CH_2). IR (CH_2Cl_2, cm^{-1}): 3512, 3275, 3064, 3002, 2932, 2852, 1736, 1610, 1547, 1518, 1462, 1448, 1411, 1316, 1265, 1250, 1226, 1150, 1114, 1073, 1017. Anal. calcd. for ($C_{13}H_{19}NO_4S$): C, 54.72; H, 6.71; N, 4.91; S, 11.24. Found: C, 54.71; H, 6.71; N, 4.90 S, 11.26.

*N-((6,7-Dimethoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-methyl)methanesulphonamide (**16**)*

Mp: 142–144°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 6.68 (s, 1H, Ar–H), 6.58 (s, 1H, Ar–H), 4.31 (t, 1H, NH, J = 6.2 Hz), 3.86 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.35 (t, 2H, CH_2–N, J = 6.2 Hz), 2.97–2.94 (m, 1H, CH), 2.92 (s, 3H, CH_3), 2.70–2.68 (m, 2H, CH_2), 1.85–1.74 (m, 4H, 2CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 148.0 (C), 147.5 (C), 130.3 (C), 128.0 (C), 112.3 (CH), 111.6 (CH), 56.3 (OCH_3), 56.1 (OCH_3), 48.5 (CH_2–N), 40.7 (CH_3), 37.8 (CH), 29.3 (CH_2), 26.0 (CH_2), 19.9 (CH_2). IR (CH_2Cl_2, cm^{-1}): 3434, 3274, 3008, 2928, 2851, 1735, 1609, 1514, 1464, 1448, 1404, 1317, 1255, 1217, 1195, 1150, 1118, 1088, 1062, 1026. Anal. calcd. for ($C_{14}H_{21}NO_4S$): C, 56.16; H, 7.07; N, 4.68; S, 10.71. Found: C, 56.10; H, 7.08; N, 4.72; S, 10.73.

Haemolysate preparation

Erythrocytes were isolated from fresh human blood, which was obtained from Ataturk University Hospital Blood Centre following low-speed centrifugation at 3000×*g* for 15 min (HERMLE Z 383 K) by removal of plasma and buffy coat. The red cells were washed twice with NaCl (0.9%) and haemolysed with 1.5 volumes of ice-cold water. Ghost and intact cells were then removed by high-speed centrifugation at 15,000×*g* for 30 min at 4°C and the pH of the haemolysate was adjusted to 8.7 with solid Tris [67].

Purification of hCA I and hCA II by affinity chromatography

Sepharose-4B-L-tyrosine affinity chromatography column was prepared according to our previous studies [35]. The pH-adjusted haemolysate was then subjected to affinity chromatography using a column packed with Sepharose 4B-L-tyrosine sulphanilamide resin [47]. For this purpose, pH value of affinity column was adjusted to 8.7 with solid Tris. Sepharose-4B-L-tyrosine-sulphanylamide affinity column was equilibrated with Tris-HCl (25 mM)/Na_2SO_4 (0.1 M, pH 8.7). The affinity gel was washed with Tris-HCl (25 mM)/Na_2SO_4 (22 mM, pH 8.7). hCA I and hCA II isoenzymes were eluted with NaCl (1.0 M)/sodium phosphate (0.25 M; pH 6.3) and sodium acetate (0.1 M)/$NaClO_4$ (0.5 M, pH 5.6), respectively. Column flow rate was 20 mL/h and 4-mL fractions were collected. All procedures were performed at 4°C.

Hydratase activity assay

CA hydratase activity was assayed by following the hydration of CO_2 according to the method described by Wilbur and Anderson [68]. The activity of CO_2-hydratase in enzyme units (EU) was calculated by using the equation $(t_o - t_c/t_c)$ where t_o and t_c are the times for pH change of the non enzymatic and the enzymatic reactions, respectively [69].

Esterase activity assay

Esterase activity of hCA I and hCA II was assayed by following the change in absorbance at 348 nm of 4-nitrophenyl acetate to 4-nitrophenolate ion over a period of 3 min at 25°C using a spectrophotometer according to the method described by Verpoorte et al. [70]. A reference measurement was obtained by preparing the mixture without the enzyme solution. All measurements were recorded in triplicate.

Quantitative protein determination

Quantitative protein determination was done by measuring the absorbance at 595 nm according to Bradford [71], explained previously [72] using bovine serum albumin as a standard.

CA inhibition

The inhibitory effects of the novel sulphamides **11–13** and sulphonamides **14–16** on CA enzyme activity purified from human erythrocytes were tested in triplicate at each concentration by using esterase activity assay. CA activities were measured in the presence of different substrate concentrations as previously described. The K_i values were determined from a series of

experiments using the novel sulphamides **11–13** and sulphonamides **14–16** and 4-nitrophenylacetate as the substrate at five different concentrations to construct Lineweaver–Burk [73] curves described previously [74].

Acetyl cholinesterase inhibition

The inhibitory effects of the novel sulphamides **11–13** and sulphonamides **14–16** on AChE activities were measured by slightly modifying the spectrophotometric method of Ellman et al. [56]. Acetylthiocholine iodide (AChI) was used as substrate of the reaction. 5,5′-Dithio-bis(2-nitrobenzoic)acid (DTNB, Product no: D8130-1G, Sigma–Aldrich) was used for the measurement of the AChE activity. Briefly, 100 μL of Tris/HCl buffer (1 M, pH 8.0), 10 μL of sample solution dissolved in deionized water at different concentrations, and 50 μL of AChE (5.32×10^{-3} U) solution were mixed and incubated for 10 min at 25°C. Then 50 μL of DTNB (0.5 mM) was added. The reaction was then initiated by the addition of 50 μL of AChI (10 mM, Product no: 01480-1G, Sigma–Aldrich). The hydrolysis of these substrates was monitored spectrophotometrically by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of AChI, at a wavelength of 412 nm [17]. Absorbance was measured at 405 nm every 45 s, three times consecutively.

In order to determine the effect of the novel sulphamides **11–13** and sulphonamides **14–16** on AChE, different concentrations of these novel compounds were added into the reaction medium. The enzyme activity was measured, and an experiment in the absence of drug was used as control. The IC_{50} values were obtained from activity (%) versus the novel sulphamides **11–13** and sulphonamides **14–16** concentration plots.

To determine the K_i constants in the media with the novel sulphamides **11–13** and sulphonamides **14–16** as inhibitor, different ACh concentrations were used. Inhibitor solution was added into the reaction medium, resulting in three different fixed concentrations of inhibitors. Lineweaver–Burk graphs [72] were drawn using $1/V$ versus and $1/[S]$ values. K_i constants were calculated from these graphs. Galantamine was generally used as a reference compound.

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