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RESEARCH ARTICLE

Synthesis of 4-(2-substituted hydrazinyl)benzenesulfonamides and their carbonic anhydrase inhibitory effects

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Abstract

In this study, 4-(2-substituted hydrazinyl)benzenesulfonamides were synthesized by microwave irradiation and their chemical structures were confirmed by ^{1}H NMR, ^{13}CNMR, and HRMS. Ketones used were: Acetophenone (**S1**), 4-methylacetophenone (**S2**), 4-chloroacetophenone (**S3**), 4-fluoroacetophenone (**S4**), 4-bromoacetophenone (**S5**), 4-methoxyacetophenone (**S6**), 4-nitroacetophenone (**S7**), 2-acetylthiophene (**S8**), 2-acetylfuran (**S9**), 1-indanone (**S10**), 2-indanone (**S11**). The compounds **S9**, **S10** and **S11** were reported for the first time, while **S1**–**S8** was synthesized by different method than literature reported using microwave irradiation method instead of conventional heating in this study. The inhibitory effects of 4-(2-substituted hydrazinyl)benzenesulfonamide derivatives (**S1**–**S11**) against hCA I and II were studied. Cytosolic hCA I and II isoenzymes were potently inhibited by new synthesized sulphonamide derivatives with K_{is} in the range of 1.79 ± 0.22–2.73 ± 0.08 nM against hCA I and in the range of 1.72 ± 0.58–11.64 ± 5.21 nM against hCA II, respectively.

Keywords

2-Acethylfuran, 2-acethylthiophene, acetophenones, carbonic anhydrase, enzyme inhibition, indanone, sulfonamide

History

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Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are zinc (Zn^{2+})-containing metalloenzymes that catalyze the reversible hydration of carbon dioxide (CO_2) to bicarbonate (HCO_3^-) and a proton (H^+)[1–3]:

$$CO_2 + H_2O \Leftrightarrow HCO_3^- + H^+$$

An understanding of the impact of this crucial equilibrium to human health continues to develop; e.g. the CA-mediated regulation of pH in the hypoxic tumor microenvironment is proposed for using as a therapeutic target[4–7]. There are six main classes of these enzymes: α-, β-, γ-, δ-, ζ- and η-CAs[8,9]. The α-, β-, δ- and η-CAs contain a Zn^{2+} ion at the active site, the γ-CAs are probably Fe^{2+} enzymes, while the metal ion is usually replaced by Cd^{2+} in the ζ-CAs[10]. Humans encode 12 catalytically active α-CA isozymes, which differ by molecular features, oligomeric arrangement, cellular localization, distribution in organs and tissues, expression levels, and kinetic properties[11–13]. These CAs comprise CA I, II, III, IV, VA, VB, VI, VII, IX, XII, XIII and XIV, all of which contain a zinc ion (Zn^{2+}) coordinated to the imidazole groups of three histidine residues and to the substrate H_2O/–OH that reacts with CO_2[14–17]. There are five cytosolic forms (CA I, II, III, VII and XIII), five membrane associated isozymes (CA IV, IX, XII, XIV and XV), two mitochondrial forms (CA VA and VB), and a secreted CA isoenzyme (CA VI). There are three additional non-catalytic CA isoforms (CA VIII, X and XI) whose functions remain unclear[18–20].

It was reported that the sulfonamide compounds ($R–SO_2NH_2$) coordinate to the active site Zn^{2+} and to block the reaction catalysis. The CAs inhibition has been of therapeutic interest for several decades. The clinical usage of carbonic anhydrase inhibitor (CAIs) has been established as antiglaucoma agents, diuretics and antiepileptic. CAIs were also used in the treatment of mountain sickness, osteoporosis, gastric and duodenal ulcers, and neurological disorders[21–23].

The aim of the study was to investigate the CA I and II inhibiting properties of the compounds to be synthesized. For this aim, ketones were changed as acetophenones derivatives, which carry a substituent at 4-position of phenyl ring that has electron donating or attracting property, 2-acethylthiophene, 2-acethylfuran, 1-indanone and 2-indanone.

Materials and methods

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained using a Varian Mercury Plus spectrometer (Varian Inc., Palo Alto, CA). Chemical shifts (δ) are reported in ppm. Mass spectra were undertaken on an HPLC-TOF Waters Micromass LCT Premier XE (Waters Corporation, Milford, MA) mass

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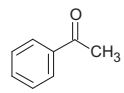
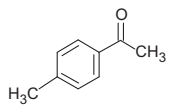
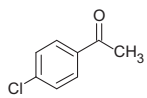
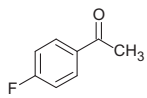
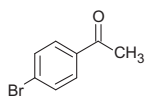
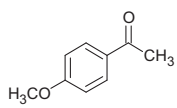
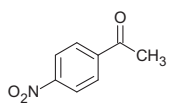
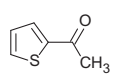
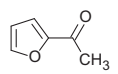
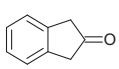
spectrometer using an electrospray ion source (ESI). Melting points were determined using an Electrothermal 9100 (IA9100, Bibby Scientific Limited, Staffordshire, UK) instrument and are uncorrected. Reactions were carried out in a CEM Discover Microwave Synthesis System, 908010 (Matthews, NC).

General procedure for the synthesis of compounds S1–S11

An appropriate ketone (9 mmol) [Acetophenone (**S1**), 4-methylacetophenone (**S2**), 4-chloroacetophenone (**S3**), 4-fluoroacetophenone (**S4**), 4-bromoacetophenone (**S5**), 4-methoxyacetophenone (**S6**), 4-nitroacetophenone (**S7**), 2-acetylthiophene (**S8**), 2-acetylfuran (**S9**), 1-indanone (**S10**), 2-indanone (**S11**)], 4-hydrazinobenzenesulfonamide hydrochloride (9 mmol) and sodium acetate (9 mmol) were dissolved in ethanol (20 mL). Reaction mixture was heated for 60 min at 78 °C, 150 W in a microwave oven. Reactions were followed by thin layer chromatography (TLC) using methanol:chloroform (4.5:0.5) solvent system. Ethanol was removed under vacuum. A solid separated out was filtered, dried and crystallized from ethanol or H_2O:*N*,*N*-dimethylformamide (1:4) to afford **S1–S11**. The yield (%) of compounds were as follows: **S1** (61), **S2** (63), **S3** (40), **S4** (86), **S5** (40), **S6** (70), **S7** (87), **S8** (50), **S9** (47), **S10** (38), **S11** (92). Chemical structures of known compounds were confirmed by ^{1}H NMR (data were not presented), and HRMS (Table 1). Structures of new compounds, **S9–S11**, were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS.

Table 1. The physical data of the compounds **S1–S11**.

Compounds	Ketone	Melting point (°C)	Crystallization solvent	Melting point (°C)	Crystallization solvent	HRMS $[MH]^+$ Calc. Mass	HRMS $[MH]^+$ Found Mass
S1		257–259	Ethanol	247–248†	DMF:H_2O†	290.0963	290.0958
S2		234–235	Ethanol	224–226†	Ethanol†	304.1120	304.1111
S3		237–238	DMF:H_2O	221–222†	DMF:H_2O†	324.0574	324.0562
S4		210–212	Ethanol	210–212‡	Ethanol‡	308.0869	308.0866
S5		241–243	Ethanol	210–212†	Ethanol†	368.0068	368.0058
S6		215–217	Ethanol	230–232‡	Ethanol‡	320.1069	320.1056
S7		266–268	Ethanol	254–255†	DMF:H_2O†	335.0814	335.0813
S8		217–218	Ethanol	230–232‡	Ethanol‡	296.0527	296.0518
S9		249–251	Ethanol	–	–	280.0756	280.0753
S10		247–249	DMF:H_2O	–	–	302.0963	302.0945
S11		214–216	DMF:H_2O	–	–	302.0970	302.0962

†Synthesis of some pyrazolyl benzenesulfonamide derivatives as dual anti-inflammatory antimicrobial agents[23].
‡Synthesis and biological evaluation of some 4-functionalized-pyrazoles as antimicrobial agents[46].

4-{2-[1-(Furan-2-yl)ethylidene]hydrazino} benzenesulfonamide (S9)

Melting point 249–251 °C. ^{1}H NMR (DMSO-d_6): δ 9.68 (s, 1H, NH), 7.64 (d, 1H, $J = 0.7$ Hz, furyl), 7.62 (d, 2H, $J = 8.8$ Hz, phenyl), 7.22 (d, 2H, $J = 8.8$ Hz, phenyl), 7.07 (s, 2H, SO_2NH_2), 6.76 (d, 1H, $J = 3.3$ Hz, furyl), 6.55 (d, 1H, $J = 3.7$ Hz, furyl), 2.17 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 153.2, 148.9, 144.1, 136.8, 134.1, 127.9, 112.6, 112.5, 109.5, 13.6; HRMS (ESI+) Calc. for $C_{12}H_{14}N_3O_3S$ $[MH]^+$ 280.0756; found 280.0753.

4-{2-(2,3-Dihydro-1*H*-inden-1-ylidene)hydrazino} benzenesulfonamide (S10)

Melting point 247–249 °C. ^{1}H NMR (DMSO-d_6): δ 9.58 (s, 1H, NH), 7.66–7.63 (m, 1H, phenyl of inden-1-on), 7.62 (d, 2H, $J = 8.8$ Hz, phenyl), 7.31–7.27 (m, 3H), 7.25 (d, 2H, $J = 8.8$ Hz, phenyl), 7.06 (s, 2H, SO_2NH_2), 3.05 (d, 2H, $J = 6.6$ Hz), 2.80 (d, 2H, $J = 6.6$ Hz); ^{13}C NMR (DMSO-d_6): δ 154.0, 149.3, 147.9, 139. 2, 133.5, 129.9, 127.9, 127.6, 126.3, 121.2, 112.2, 28.8, 27.5; HRMS (ESI+) Calc. for $C_{15}H_{16}N_3O_2S$ $[MH]^+$ 302.0963; found 302.094.

4-{2-(1,3-Dihydro-2*H*-inden-2-ylidene)hydrazino} benzenesulfonamide (S11)

Melting point 214–216 °C. ^{1}H NMR (DMSO-d_6/CD_3OD): δ 9.4 (s, 1H, NH), 7.59 (d, 2H, $J = 8.8$ Hz, phenyl), 7.21 (bs, 2H), 7.19 (d, 2H, d, 2H, $J = 5.5$ Hz), 7.13 (d, 2H, $J = 8.8$ Hz, phenyl), 7.04 (s, 2H, SO_2NH_2), 3.84 (s, 2H), 3.77 (s, 2H); ^{13}C NMR (CD_3OD): δ 153.9, 149.7, 139.7, 139.1, 132.3, 127.6, 126.9, 126.8, 124.9, 124.5, 111.6, 38.3, 34.3; HRMS (ESI+) Calc. for $C_{15}H_{16}N_3O_2S$ $[MH]^+$ 302.0970; found 302.0962.

Biochemistry

For determination of the inhibition effects of sulfonamides, both CA isoenzyme (hCA I and II) were purified by Sepharose-4B-L-tyrosine-sulfanilamide affinity chromatography in a single purification step[24–26]. The column material including Sepharose-4B-L-tyrosine-sulfanilamide was prepared according to a previous method[27–29]. Thus, homogenate solution acidity was adjusted to 8.7 with a pH-meter using solid Tris. Subsequently, the supernatant was transferred to the previously prepared Sepharose-4B-L-tyrosine-sulphanilamide affinity column[30–32]. The proteins flow in the column eluates was spectrophotometrically determined at 280 nm. For determination of both isoenzymes purity, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) for both isoenzymes was performed after a purification step. The presence and purity of both isoenzymes were visualized by SDS-PAGE (BIO-RAD Mini-PROTEAN® system casting stand, Shanghai, China). After this process, a single band was observed for each isoenzyme[33]. This protein imaging method was previously described[34–36]. In this application, the imaging method was performed out in 10 and 3% acrylamide for the running and the stacking gel, respectively, with 0.1% SDS[37,38].

Both CA isoenzymes activities were determined according to the method given by Verpoorte et al.[39] and described previously[40]. Briefly, the absorbance changing at 348 nm for *p*-nitrophenylacetate (NPA) to *p*-nitrophenolate (NP) was recorded over a 3-min period at the room temperature (25 °C) using a spectrophotometer (Shimadzu, UV-VIS Spectrophotometer, UVmini-1240, Kyoto, Japan)[41]. The protein quantity was spectrophotometrically measured at 595 nm during the purification steps according to the Bradford method[42]. As used in previous studies, bovine serum albumin (BSA) was used as the standard protein[43]. For determining the inhibition effect of each sulfonamide derivative, an activity (%)-[Sulfonamide] graph was drawn. To determine K_i values, three different sulfonamide derivatives concentrations were tested. In these experiments, NPA was used as the substrate at five different concentrations and the Lineweaver–Burk curves were drawn[44] as previously described[45].

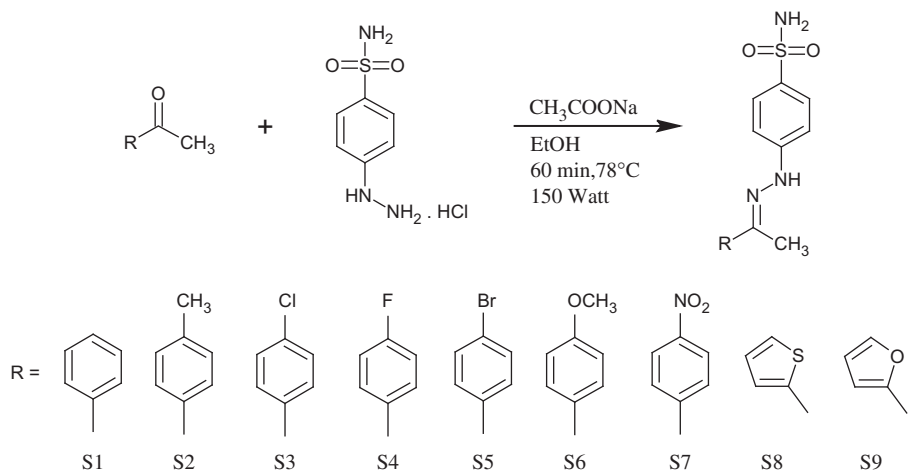
Results and discussion

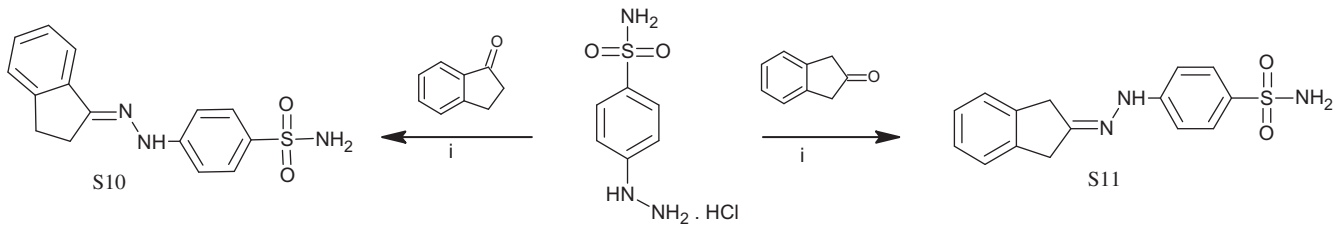
The compounds designed were synthesized successfully (Schemes 1 and 2) and their chemical structures were confirmed by spectral data. The physical data of the compounds were shown in Table 1. The compounds **S9–S11** are reported for the first time by a microwave irradiation. The synthetic procedure for the known compounds **S1–S8** was different than literature procedure[46,47]. Compounds **S1–S8** were synthesized by the microwave irradiation method in this study, while they were synthesized by a conventional heating method in literature[46,47]. Microwave irradiation method decreased the reaction time 2 or 3 times comparing to conventional heating. The yields of the reactions were 40–87% for **S1–S8** in microwave irradiation, while they were synthesized with the yield of 82–89% in conventional heating in literature[46,47].

Up to now, the sulfonamide group ($R\text{-}SO_2NH_2$) is the most important and largely used Zn^{2+}-binding function for the design of CAIs; accordingly, the majority of the clinically used CAIs are sulfonamides[48]. Since the first evidence of their CA inhibitory properties, these molecules were largely investigated by means of kinetic, pharmacological and physiological studies[14,48–50].

The first evidence that sulfonamides could act as potent CAIs came from a study, reported by Mann and Keilin[51]. Subsequently, many aromatic sulfonamides were synthesized and investigated for their CA inhibitory action[52]. Among these, benzenesulfonamides constitute the most common and best-characterized class.

Scheme 1. Synthesis of the compounds **S1–S9**.





i= Sodium acetate, ethanol, 60 min., 78° C, 150 Watt

Scheme 2. Synthesis of the compounds **S10** and **S11**.

Table 2. Human carbonic anhydrase isoenzymes (hCA I and II) inhibition values with 4-(2-substituted hydrazinyl)benzenesulfonamide derivatives (**S1**–**S11**) using esterase assay.

	K_i (nM)	
Compounds	hCA I	hCA II
S1	2.23 ± 0.14	8.02 ± 3.04
S2	2.42 ± 0.51	6.93 ± 2.51
S3	2.15 ± 0.42	7.91 ± 3.30
S4	2.67 ± 0.30	9.12 ± 4.42
S5	1.97 ± 0.23	11.64 ± 5.21
S6	2.73 ± 0.48	6.34 ± 2.64
S7	1.90 ± 0.43	1.97 ± 0.40
S8	2.73 ± 0.08	10.69 ± 4.73
S9	2.52 ± 0.38	9.43 ± 1.94
S10	2.06 ± 0.66	6.73 ± 2.18
S11	1.79 ± 0.22	1.72 ± 0.58
AZA*	5.41 ± 1.28	6.82 ± 1.59

*Acetazolamide (AZA) was used as a standard inhibitor for all hCA.

Up to now, a lot of studies have been reported on the interaction of benzenesulfonamides with CA isoenzymes. Especially, benzenesulfonamides include halogens, acetamido and alkoxycarbonyl moieties had good inhibitory properties and interesting physicochemical features were observed for compounds possessing carboxy-, ureido-, hydrazido-, thioureido- and methylamino-moieties[48]. It was reported that the interaction of the benzenesulfonamide moiety with the CA active site is rather similar, with the sulfonamide moiety involved in the canonical coordination of the Zn^{2+} catalytic ion and the phenyl ring. Also, the substituents of the phenyl ring can establish different types of interactions, which involve the hydrophobic, or the hydrophilic region of the active site[48].

Benzenesulfonamide derivatives have been largely investigated both in the search of more active CAIs and to develop compounds with a different biological activity[53]. So far, two different types of such derivatives have been characterized: those where the two sulfonamide moieties are present on the same phenyl ring[10] and those where the two sulfonamide moieties are present on two different phenyl rings, separated by urea, carboxyamido, guanidine moieties, etc., as spacers[53–55]. These sulfonamide derivatives have been largely clinically used for the treatment of glaucoma[56] and several neurological disorders[53].

Two physiologically relevant CA isoforms (hCA I and II) were included in our study. 4-(2-Substituted hydrazinyl)benzenesulfonamide derivatives (**S1**–**S11**) were tested for their inhibition properties against hCA I and II isoenzymes, showing generally an efficient inhibition. CA I and II inhibiting effects of the 4-(2-substituted hydrazinyl)benzenesulfonamide derivatives (**S1**–**S11**) are presented in Table 2. It was well known that developing isoenzyme-specific CAIs should be highly beneficial in obtaining novel classes of drugs devoid of various undesired side-effects[28]. We report here the first study on the inhibitory effects of 4-(2-substituted hydrazinyl)benzenesulfonamide derivatives (**S1**–**S11**) against hCA I and II using esterase activity.

Low cytosolic isoenzyme hCA I is ubiquitously expressed in the body, and can be found in high concentrations in the blood and gastrointestinal tract[57]. Specifically, hCA I is found in many tissues, however, but it was demonstrated that this isozyme is involved in retinal and cerebral edema, and its inhibition may be a valuable tool for fighting these conditions. Also, it was reported that if K_i value of a tested compound was less than 50 μM ($K_i > 50$ μM), it was considered low inhibition and this inhibitor was accepted to be inactive against hCA I[40,58]. The results obtained from this study clearly indicate that 4-(2-substituted hydrazinyl)benzenesulfonamide derivatives (**S1**–**S11**) have effective inhibition profile against slow cytosolic isoform hCA I, and cytosolic dominant rapid isozymes hCA II with low nanomolar range. These compounds bind to hCA I in the nanomolar range. K_i values are in the range of 1.79 ± 0.22–2.73 ± 0.08 nM for hCA I isoenzyme. On the other hand, acetazolamide (**AZA**) being a broad-specificity CA inhibitor owing to its widespread inhibition of CAs, showed K_i value of 5.41 ± 1.28 nM against hCA I. 4-{2-(1,3-dihydro-2H-inden-2-ylidene)hydrazino}benzenesulfonamide (**S11**), possessing 1,3-dihydro-2*H*-inden-2-one was the best hCA I inhibitor (K_i: 1.79 ± 0.22 nM). The inhibition effects of all 4-(2-substituted hydrazinyl)benzenesulfonamide derivatives (**S1**–**S11**) are higher than that of acetazolamide (AZA; K_i: 5.41 ± 1.28 nM). **AZA**, 5-Acetamido-1,3,4-thiadiazole-2-sulfonamide) is considered to be a good CA inhibitor and is approved for the treatment of a range of conditions including glaucoma, epilepsy and altitude sickness[5].

CA II is involved in several diseases including glaucoma, epilepsy, edema and probably altitude sickness. Against the physiologically dominant isoform hCA II, 4-(2-substituted hydrazinyl)benzenesulfonamide derivatives (**S1**–**S11**) demonstrated K_is of 1.72 ± 0.58–11.64 ± 5.21 nM (Table 2). As with CA I, the compound of **S11** (4-{2-(1,3-dihydro-2H-inden-2-ylidene)hydrazino}benzenesulfonamide) was the best hCA II inhibitor (K_i: 1.72 ± 0.58 nM). However, the average K_i value of 4-(2-substituted hydrazinyl)benzenesulfonamide derivatives (**S1**–**S11**) was found to be 2.28 nM for hCA I. Conversely, the average K_i value of these compounds for hCA II was found to be 7.32 nM. These results showed that 4-(2-substituted hydrazinyl)-benzenesulfonamide derivatives (**S1**–**S11**) have higher inhibition affinity toward hCA I than that of hCA II isoenzyme. Also, AZA, which may interact with the distinct hydrophobic and hydrophilic halves of the CA II active site, showed K_i value of 6.82 ± 1.59 nM.

Conclusion

The 4-(2-substituted hydrazinyl)benzenesulfonamide derivatives (**S1**–**S11**) were evaluated for their hCA I and II isoenzymes

inhibition properties. These compounds were found to be sufficiently active. hCA I and II isoenzymes were potently inhibited by new synthesized sulphonamide derivatives with K_is in the range of 1.79 ± 0.22–2.73 ± 0.08 nM against hCA I and in the range of 1.72 ± 0.58–11.64 ± 5.21 nM against hCA II, respectively.

Declaration of interest

The authors report no conflict of interest and are responsible for the contents and writing of the paper.

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