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Design, synthesis and biological evaluation of N-(5-methyl-isoxazol-3-yl/1,3,4-thiadiazol-2-yl)-4-(3-substitutedphenylureido) benzenesulfonamides as human carbonic anhydrase isoenzymes I, II, VII and XII inhibitors

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RESEARCH ARTICLE

Design, synthesis and biological evaluation of *N*-(5-methyl-isoxazol-3-yl/1,3,4-thiadiazol-2-yl)-4-(3-substitutedphenylureido) benzenesulfonamides as human carbonic anhydrase isoenzymes I, II, VII and XII inhibitors

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Abstract

A series of *N*-(5-methyl-isoxazol-3-yl/1,3,4-thiadiazol-2-yl)-4-(3-substitutedphenylureido) benzenesulfonamide derivatives has been designed, synthesized and screened for their *in vitro* human carbonic anhydrase (hCA; EC 4.2.1.1) inhibition potential. These newly synthesized sulfonamide compounds were assessed against isoforms hCA I, II, VII and XII, with acetazolamide (AAZ) as a reference compound. The majority of these compounds were found quite weak inhibitor against all tested isoforms. Compound **15** showed a modest inhibition potency against hCA I ($K_i = 73.7\ \mu M$) and hCA VII ($K_i = 85.8\ \mu M$). Compounds **19** and **25** exhibited hCA II inhibition with K_i values of 96.0 μM and 87.8 μM, respectively. The results of the present study suggest that, although the synthesized derivatives have weak inhibitory potential towards all investigated isoforms, some of them may serve as lead molecules for the further development of selective inhibitors incorporating secondary sulfonamide functionalities, a class of inhibitors for which the inhibition mechanism is poorly understood.

Keywords

Carbonic anhydrase, inhibitor, sulfonamide, synthesis

History

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Introduction

The carbonic anhydrases (CAs; EC 4.2.1.1) are a large group of ubiquitous zinc containing enzymes, present in all mammals, including human[1]. CAs are encoded by six distinct gene families known as alpha (α), beta (β), gamma (γ), delta (δ) zeta (ζ) and eta (η) and all human CAs belongs to class alpha[2–4]. These enzymes are well described by their different molecular features, oligomeric arrangement as well as kinetic properties[5]. Till date, a total number of 15 different human carbonic anhydrases (hCA) isoforms have been discovered, among which CA I-III, CA VII and CA XIII are present in cytosol; CA IV, CA IX, CA XII and CA XIV are membrane bound; CA VA and CA VB are restricted to the mitochondrion and CA VI is secreted in milk and saliva[6]. Functionally, CAs are primarily involved in catalyzing the rapid interconversion of CO_2 and H_2O to bicarbonate (HCO_3^-) and protons (H^+) using a metal hydroxide nucleophilic mechanism[7]. The hCAs play a pivotal role in a variety of physiological processes such as CO_2/bicarbonate transportation, respiration, electrolyte secretion, gluoconeogensis, lipogenesis, ureagenesis, bone resorption, neuronal excitability and tumorigenicity[8]. CAs have profound involvement in many pathological conditions like obesity, glaucoma, kidney dysfunction, osteoporosis, gastric ulcers, migraine, epilepsy and cancer[9]. For example CA II deficiency is the major defect in disease conditions such as osteopetrosis, cerebral calcification and renal tubular acidosis[10]. Another isoform, CA VII is implicated in generating neuronal excitation leading to the development of seizures as well as in the neuropathic pain control[11]. hCA II, IV and XII are druggable targets for anti-glaucoma agents while, hCA VII and hCA XIV are known drug targets for antiepileptic drugs. hCA IX and XII are anti-tumor drug targets.At present, CAs are attractive therapeutic targets to control these aforementioned diseases. The CAs are also essential for other living organism including pathogens.

Acetazolamide (AAZ) is considered as an excellent CA inhibitor (CAI) and is used for the treatment of glaucoma, epilepsy and altitude sickness[12]. Several other CAIs such as ethoxolamide (EZA), brinzolamide (BRZ), dorzolamide (DZA), methazolamide (MZA), and dichlorophenamide (DCP) are useful clinically as anti-glaucoma agents but, they are also used to treat various neurological as well as neuromuscular disorders such as epilepsy, genetic hemiplegic migraine and ataxia, tardive diskinesia, hypokalemic periodic paralysis, essential tremor and Parkinsons disease[13]. Unfortunately, the presently used CAIs are non-selective to a

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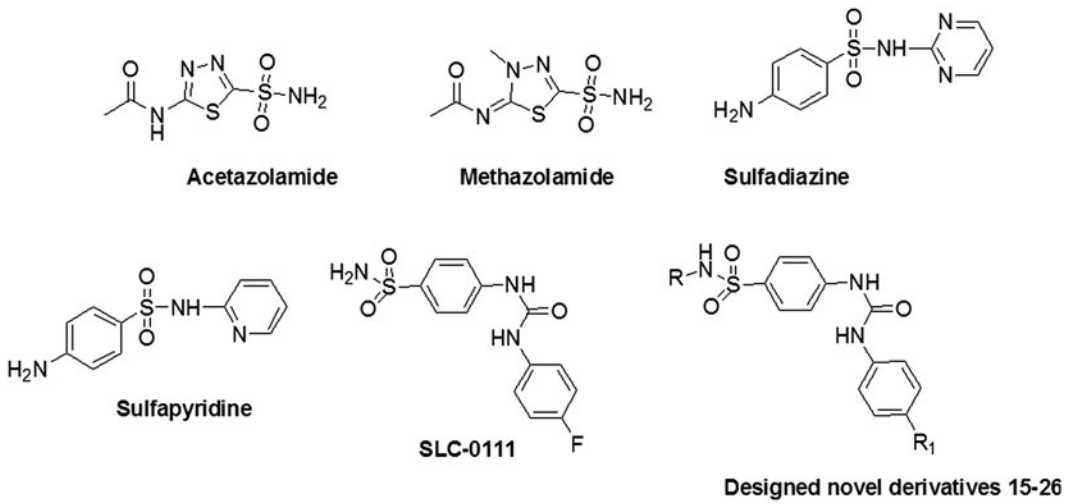


Figure 1. Sulfonamides CA inhibitors (acetazolamide, methazolamide and SLC-0111), sulfadrugs (sulfadiazine and sulfapyridine) and the designed novel compounds **15**–**26**.

particular CA isoform, which leads to the occurrence of peripheral side effects. Therefore, the development of novel CAIs having high potency and selectivity against specific isoform still represents a needful and challenging task to obtain newer compounds which could replace the ''old ones'', in consequence avoiding side effects as well as improving therapeutic safety.

Sulfonamides containing molecules have presented a remarkable history in CA inhibition. The primary sulfonamide group ($-SO_2NH_2$) is present in these molecules which bind as anions to the Zn^{2+} ion in the enzyme active site with high affinity, and block catalysis[14]. In the past, several research reports showed that the urea functional group along with sulfonamide also seemed to be beneficial to generate effective CA inhibitory activity[15]. In the present study, we have used sulfamethoxazole/sulfamethiazole to develop potent CAIs and also phenyl urea moiety was also installed to this pharmacophore to develop new CAIs. Figure 1 showed some known CAIs and the molecular framework of our synthesized compound. The synthesized derivatives have been well characterized by using 1H NMR, 13C NMR and mass spectroscopy. The purity was analyzed by HPLC. The esterase activity was performed to study the potential of the newly synthesized compounds (**15**–**26**) towards hCA I, hCA II, hCA VII and hCA XII inhibition.

Materials and methods

Chemistry

All the chemicals and reagents were obtained from Sigma Aldrich (St. Louis, MO), Alfa Aesar (Massachusetts), S.D Fine Chemicals (India) and Merck (Darmstadt, Germany) and solvents for reaction medium were dried by standard methods. Analytical thin layer chromatography (TLC) was performed on commercially available silica gel (Kieselgel 60, F_{254}) coated aluminum plates (Merck). Column chromatography purification was performed using silica gel Merck 100–200 mesh. Melting points were taken in open capillaries using model KSPII, KRUSS, (Germany). 1H NMR and 13C NMR were recorded on Jeol-400 MHz High Resolution NMR Spectrophotometer (JEOL USA, Inc., Peabody, MA). Chemical shifts in ^{1}H NMR are reported in parts per million and coupling constant (*J*) in Hertz (Hz) using DMSO-d_6 as solvent. The splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet. Mass spectra were recorded on an Agilent 6310 Ion trap LC/MS and the purity of final compounds was analyzed by using reverse phase HPLC (Shimadzu, Kyoto, Japan) with C-18 column.

General procedure for the synthesis of novel *N*-(5-methyl-isoxazol-3-yl/1,3,4-thiadiazol-2-yl)-4–(3-substitutedphenylureido) benzenesulfonamides (15–26)

Sulfamethoxazole/sulfamethiazole (10 mmol) was fully dissolved in dried DMF and then subsequent isocyanate (10 mmol) was added dropwise to the reaction mixture and heated at 90–100 °C for 5–6 h. The appeared precipitate was filtered and washed with hot petroleum ether as well as water. The obtained crude products were purified by column chromatography using chloroform/methanol (98:02) as an eluent to furnish target compounds (15–26).The purity of the synthesized compounds was analyzed by HPLC using acetonitrile/methanol (98:02) as mobile phase (Scheme 1).

N-(5-Methyl-isoxazol-3-yl)-4–(3-phenyl-ureido)-benzenesulfonamide (15)

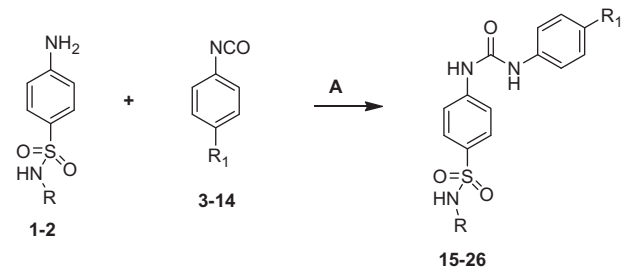
White solid; yield 1.74 g; mp 240–242 °C; ^{1}H NMR (DMSO-d_6,400 MHz): δ 2.28 (s, 3H, CH_3), 6.13 (s, 1H, CH), 6.98 (t, 1H, Ar-H, $J = 7.2$ Hz), 7.27 (t, 2H, Ar-H, $J = 7.6$ Hz), 7.44 (d, 2H, Ar-H, $J = 7.6$ Hz), 7.63 (d, 2H, Ar-H, $J = 9.1$ Hz), 7.75 (d, 2H, Ar-H, $J = 7.7$ Hz), 8.82 (s, 1H, NH), 9.15 (s, 1H, NH), 11.29 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 12.08, 95.3, 117.7, 118.4, 122.3, 128.1, 128.8, 131.4, 139.1, 144.3, 152.1, 157.6, 170.2. LC–MS: m/e; 372 (M^+). HPLC purity: 97.6%.

4-[3–(4-Fluoro-phenyl)-ureido]-*N*-(5-methyl-isoxazol-3-yl)-benzenesulfonamide (16)

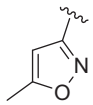
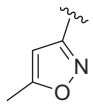
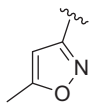
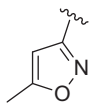
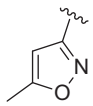
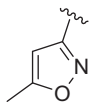
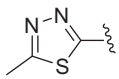
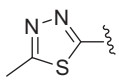
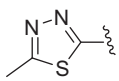
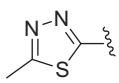
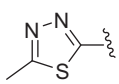
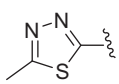
White solid; yield 1.92 g; mp 228–230 °C; ^{1}H NMR (DMSO-d_6,400 MHz): δ 2.28 (s, 3H, CH_3), 6.12 (s, 1H, CH), 7.12 (t, 2H, Ar-H, $J = 8.7$ Hz), 7.43–7.47 (m, 2H, Ar-H), 7.61 (d, 2H, Ar-H, $J = 9.1$ Hz), 7.76 (d, 2H, Ar-H, $J = 8.4$ Hz), 8.85 (s, 1H, NH), 9.10 (s, 1H, NH), 11.28 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 12.07, 95.3, 115.2, 115.5, 117.7, 120.2, 120.3, 128.1, 131.5, 135.4, 144.3, 152.2, 156.4, 157.6, 158.7, 170.2. LC–MS: m/e; 391 (M^{+1}). HPLC purity: 97.1%.

4-[3–(4-Chloro-phenyl)-ureido]-*N*-(5-methyl-isoxazol-3-yl)-benzenesulfonamide (17)

White solid; yield 2.35 g; mp 238–240 °C; ^{1}H NMR (DMSO-d_6,400 MHz): δ 2.28 (s, 3H, CH_3), 6.12 (s, 1H, CH), 7.33 (d, 2H, Ar-H, $J = 8.4$ Hz), 7.47 (d, 2H, Ar-H, J= 8.4 Hz), 7.62 (d, 2H, Ar-H, $J = 9.1$ Hz), 7.75 (d, 2H, Ar-H, $J = 8.4$ Hz), 8.97 (s, 1H, NH),



Scheme 1. Synthesis of *N*-(5-methyl-isoxazol-3-yl/1,3,4-thiadiazol-2-yl)-4-(3-substituted phenylureido)benzenesulfonamide derivatives. Reagent and conditions: A. Dried DMF reflux, 5–6 h.

Compound number	R-Group	R_1-Group
15		H
16		4-Fluoro
17		4-Chloro
18		4-Methyl
19		4-Methoxy
20		Benzyl
21		H
22		4-Fluoro
23		4-Chloro
24		4-Methyl
25		4-Methoxy
26		Benzyl

9.20 (s, 1H, NH), 11.28 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 12.09, 95.4, 117.8, 120.0, 125.9, 128.1, 131.6, 138.2, 144.1, 152.1, 157.6, 170.2. LC–MS: m/e; 406 (M^+). HPLC purity: 97.9%.

N-(5-Methyl-isoxazol-3-yl)-4-(3-*p*-tolyl-ureido)-benzenesulfonamide (18)

White solid; yield 1.49 g; mp 233–235 °C; ^{1}H NMR (DMSO-d_6,400 MHz): δ 2.23 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 6.12 (s, 1H, CH), 7.08 (d, 2H, Ar-H, $J = 7.6$ Hz), 7.32 (d, 2H, Ar-H, $J = 8.4$ Hz), 7.61 (d, 2H, Ar-H, $J = 9.1$ Hz), 7.74 (d, 2H, Ar-H, $J = 8.4$ Hz), 8.71 (s, 1H, NH), 9.11 (s, 1H, NH), 11.20 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 12.0, 20.3, 95.3, 117.3, 118.5, 128.1, 129.2, 131.2, 131.3, 136.6, 144.4, 152.1, 157.6, 170.2. LC–MS: m/e; 386 (M^+). HPLC purity: 99.4%.

4-[3–(4-Methoxy-phenyl)-ureido]-*N*-(5-methyl-isoxazol-3-yl)-benzenesulfonamide (19)

White solid; yield 1.84 g; mp 212–214 °C; ^{1}H NMR (DMSO-d_6,400 MHz): δ 2.28 (s, 3H, CH_3), 3.70 (s, 3H, CH_3), 6.12 (s, 1H, CH), 6.86 (d, 2H, Ar-H, $J = 8.4$ Hz), 7.34 (d, 2H, Ar-H, $J = 8.4$ Hz), 7.61 (d, 2H, Ar-H, $J = 8.4$ Hz), 7.73 (d, 2H, Ar-H, $J = 9.1$ Hz), 8.63 (s, 1H, NH), 9.08 (s, 1H, NH), 11.21 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 12.0, 55.1, 95.3, 114.0, 117.5, 120.3, 128.1, 131.2, 132.1, 144.5, 152.3, 154.8, 157.6, 170.2. LC–MS: m/e; 402 (M^+). HPLC purity: 99.7%.

4-(3-Benzyl-ureido)-*N*-(5-methyl-isoxazol-3-yl)-benzenesulfonamide (20)

White solid; yield 2.12 g; mp 218–220 °C; ^{1}H NMR (DMSO-d_6,400 MHz): δ 2.27 (s, 3H, CH_3), 4.29 (d, 2H, CH_2, $J = 6.1$ Hz), 6.10 (s, 1H, CH), 6.82 (t, 1H, NH, $J = 5.7$ Hz), 7.21–7.33 (m, 5H, Ar-H), 7.57 (d, 2H, Ar-H, $J = 9.1$ Hz), 7.69 (d, 2H, Ar-H, $J = 8.4$ Hz), 9.08 (s, 1H, NH), 11.22 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 12.0, 42.7, 95.3, 117.1, 126.8, 127.1, 128.1, 128.3, 130.7, 139.9, 145.0, 154.7, 157.7, 170.2. LC–MS: m/e; 387 (M^{+1}). HPLC purity: 99.4%.

N-(5-methyl-[1,3,4]thiadiazol-2-yl)-4-(3-phenyl-ureido)-benzenesulfonamide (21)

White solid; yield 2.16 g; mp 226–228 °C; ^{1}H NMR (DMSO-d_6,400 MHz): δ 2.45 (s, 3H, CH_3), 6.98 (t, 1H, Ar-H, $J = 7.6$ Hz), 7.28 (t, 2H, Ar-H, $J = 7.9$ Hz), 7.44 (d, 2H, Ar-H, $J = 8.4$ Hz), 7.58 (d, 2H, Ar-H, $J = 9.1$ Hz), 7.68 (d, 2H, Ar-H, $J = 9.1$ Hz), 8.77 (s, 1H, NH), 9.09 (s, 1H, NH), 13.81 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 16.1, 117.6, 118.4, 122.2, 127.1, 128.8, 134.3, 139.2, 143.5, 152.2, 154.3, 167.7. LC–MS: m/e; 390 (M^{+1}). HPLC purity: 96.8%.

4-[3-(4-Fluoro-phenyl)-ureido]-*N*-(5-methyl-[1,3,4]thiadiazol-2-yl)-benzene sulfonamide (22)

White solid; yield 1.64 g; mp 252–254 °C; ^{1}H NMR (DMSO-d_6,400 MHz): δ 2.45 (s, 3H, CH_3), 7.10–7.14 (m, 2H, Ar-H), 7.43–7.47 (m, 2H, Ar-H), 7.58 (d, 2H, Ar-H, $J = 8.3$ Hz), 7.68 (d, 2H, Ar-H, $J = 8.4$ Hz), 8.81 (s, 1H, NH), 9.10 (s, 1H, NH), 13.82 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): 16.1, 115.2, 115.5, 117.6, 120.2, 120.3, 127.0, 134.4, 135.6, 143.5, 152.3, 154.4, 156.4, 158.8, 167.7. LC–MS: m/e; 408 (M^{+1}). HPLC purity: 99.5%.

4-[3–(4-Chloro-phenyl)-ureido]-*N*-(5-methyl-[1,3,4]thiadiazol-2-yl)-benzene sulfonamide (23)

White solid; yield 2.0 g; mp 275–277 °C; ^{1}H NMR (DMSO-d_6,400 MHz): δ 2.44 (s, 3H, CH_3), 7.32 (d, 2H, Ar-H, $J = 9.1$ Hz),

7.48 (d, 2H, Ar-H, $J = 9.1$ Hz), 7.59 (d, 2H, Ar-H, $J = 8.4$ Hz), 7.69 (d, 2H, Ar-H, $J = 9.1$ Hz), 8.93 (s, 1H, NH), 9.14 (s, 1H, NH), 13.8 (s, 1H, NH).^{13}C NMR (DMSO-d_6): 16.1, 117.7, 120.0, 125.8, 127.1, 128.7, 134.5, 138.3, 143.3, 152.1, 154.4, 167.7. LC–MS: m/e; 423 (M^+). HPLC purity: 98.6%.

N-(5-methyl-[1,3,4]thiadiazol-2-yl)-4-(3-*p*-tolyl-ureido)-benzenesulfonamide (24)

White solid; yield 1.75 g; mp 258–260 °C; ^{1}H NMR (DMSO-d_6,400 MHz): δ 2.23 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 7.08 (d, 2H, Ar-H, $J = 8.4$ Hz), 7.32 (d, 2H, Ar-H, $J = 8.4$ Hz), 7.57 (d, 2H, Ar-H, $J = 9.9$ Hz), 7.67 (d, 2H, Ar-H, $J = 9.9$ Hz), 8.66 (s, 1H, NH), 9.05 (s, 1H, NH), 13.8 (s, 1H, NH).^{13}C NMR (DMSO-d_6): 16.09, 20.3, 117.5, 118.5, 127.0, 129.2, 131.1, 134.1, 136.6, 143.5, 152.2, 154.3, 167.7. LC–MS: m/e; 403 (M^+). HPLC purity: 96.7%.

4-[3-(4-Methoxy-phenyl)-ureido]-*N*-(5-methyl-[1,3,4]thiadiazol-2-yl)-benzenesulfonamide (25)

White solid; yield 2.0 g; mp 238–240 °C; ^{1}H NMR (DMSO-d_6,400 MHz): δ 2.48 (s, 3H, CH_3), 3.70 (s, 3H, CH_3), 6.86 (d, 2H, Ar-H, $J = 9.1$ Hz), 7.34 (d, 2H, Ar-H, $J = 9.1$ Hz), 7.57 (d, 2H, Ar-H, $J = 8.4$ Hz), 7.65 (d, 2H, Ar-H, $J = 8.4$ Hz), 8.58 (s, 1H, NH), 9.02 (s, 1H, NH), 13.8 (s, 1H, NH).^{13}C NMR (DMSO-d_6): 16.0, 55.1, 114.0, 117.4, 120.2, 127.0, 132.2, 134.0, 143.6, 152.3, 154.7, 167.7. LC–MS: m/e; 419 (M^+). HPLC purity: 99.3%.

4-(3-Benzyl-ureido)-*N*-(5-methyl-[1,3,4]thiadiazol-2-yl)-benzenesulfonamide (26)

White solid; yield 2.2 g; mp 270–272 °C; ^{1}H NMR (DMSO-d_6,400 MHz): δ 2.44 (s, 3H, CH_3), 4.28 (q, 2H, CH_2, $J = 6.0$ Hz), 6.76 (t, 1H, NH, $J = 6.1$ Hz), 7.20–7.33 (m, 5H, Ar-H), 7.53 (d, 2H, Ar-H, $J = 9.1$ Hz), 7.62 (d, 2H, Ar-H, $J = 8.4$ Hz), 9.02 (s, 1H, NH), 13.8 (s, 1H, NH).^{13}C NMR (DMSO-d_6): 16.0, 42.7, 117.0, 126.8, 127.0, 127.1, 128.3, 133.5, 140.0, 144.2, 154.2, 154.8, 167.6. LC–MS: m/e; 404 (M^{+1}). HPLC purity: 99.3%.

Carbonic anhydrase inhibition assay

An applied photophysics stopped-flow instrument has been used for assaying the CA catalyzed CO_2 hydration activity[16]. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.4) as buffer, and 20 mM Na_2SO_4 for maintaining constant the ionic strength (this anion is not inhibitory and has a $K_I > 200$ mM against these enzymes), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each measurement at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity, working with 10-fold decreasing inhibitor concentrations ranging between 0.1 nM and 10–100 μM (depending on the inhibitor potency, but at least five points at different inhibitor concentrations were employed for determining the inhibition constants). The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionized water and dilutions up to 0.1 nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-squares methods using the Cheng–Prusoff equation, and represent the mean from at least three different determinations. The human isoforms hCA I, II, VII and XII were recombinant enzymes produced as described earlier in our laboratory[17–32].

Result and discussion

Chemistry

The synthetic route utilized for the preparation of *N*-(5-methyl-isoxazol-3-yl/1,3,4-thiadiazol-2-yl)-4–(3-substitutedphenylureido) benzenesulfonamide derivativesis depicted in Scheme 1.

An equimolar ratio of sulfamethoxazole/sulfamethiazole and substituted phenyl isocyantes were reacted in dry DMF to afford *N*-(5-methyl-isoxazol-3-yl/1,3,4-thiadiazol-2-yl)-4–(3-substituted phenylureido)benzenesulfonamide derivatives. All compounds were purified by using column chromatography using chloroform/methanol as eluent. The purity of the final compounds was analyzed by HPLC analysis using acetonitrile/methanol (98:02) as mobile phase. All compounds presented percent purity more than 95% and were fully characterized by NMR and mass spectroscopy.

We have used SLC-0111 as lead molecule for designing these compounds[33,34], as this derivative recently completed successfully the Phase I clinical trials as an anti-tumor agent. In addition, a range of secondary and tertiary sulfonamides has been investigated as CAIs recently, some of them showing effective and selective inhibition of several important isoforms[35–49]. However, the inhibition mechanism with secondary and tertiary sulfonamides is unknown, as no X-ray crystal structures of such derivatives bound to the CA are available to date. Thus, the sulfonamides reported here incorporate in their molecule the urea fragment found in SLC-0111 and the secondary sulfonamide moiety present in sulfa drugs and several recently investigated CAIs[35–49].

In vitro carbonic anhydrase activity

All the synthesized compounds (**15**–**26**) were studied for the inhibition of four CA isozymes of human origin, i.e. hCA I, hCA II, hCA VII and hCA XII (Table 1). The following structure activity relationship (SAR) was obtained by analyzing CA inhibition data of Table 1:

(1) In the isoxazole subseries (**15**–**20**) compound substituted phenyl ring (compound **15**) at the terminal end has mild hCA inhibitory activity for both hCA I ($K_i = 73.7$ μM)and hCA VII ($K_i = 85.8$ μM) isoforms.
(2) In this subseries, compound substituted with para-methoxy phenyl (compound **19**) also exhibited low inhibition against hCA II ($K_i = 96.0$ μM). Moreover, this compound displayed a

Table 1. hCA I, II, VII and XII inhibition with compounds **15**–**26**, with **AAZ** as standard.

	K_I (μM)			
Compound	hCA I	hCA II	hCA VII	hCA XII
15	73.7	>100	85.8	>100
16	>100	>100	>100	>100
17	>100	>100	>100	>100
18	>100	>100	>100	>100
19	>100	96.0	>100	>100
20	>100	>100	>100	>100
21	>100	>100	>100	>100
22	>100	>100	>100	>100
23	>100	>100	>100	>100
24	>100	>100	>100	>100
25	>100	87.8	>100	>100
26	>100	>100	>100	>100
AAZ	0.25	0.012	0.005	0.006

Ki value of >100 μM towards hCA I, VII and XII, thus seems to inactive for these isoforms.

The remaining compounds in this subseries did not produce remarkable inhibitory potential against hCA I, II, VII and XII isoforms.

(3) In thiadiazole subseries (**21**–**26**), compound **25** comprising *para*-methoxy phenyl ring at its terminal end has shown a modest inhibitory potential against hCA II with Ki value of 87.8 μM. This compound was inactive against hCA I, VII and XII (Ki= >100 μM).

(4) Overall, the SAR study indicates that substitutions on the phenyl ring did not produce remarkable inhibitory potential against tested hCA isoforms except para-methoxy substitution. Therefore, further structural modifications are necessarily required to achieve more potent CAIs.

Conclusions

A set of *N*-(5-methyl-isoxazol-3-yl/1,3,4-thiadiazol-2-yl)-4–(3-substitutedphenylureido) benzenesulfonamide derivatives were synthesized in a good yield and were characterized by using NMR and mass spectroscopy techniques. All compounds were subjected for their *in-vitro* CA inhibitory activity and only compound **15** showed CA inhibition against hCA I and hCA VII isoform. The selectivity ratio of compounds **19** and **25** for hCA II isoform makes them interesting leads for the development of potent and selective hCA II inhibitor.

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Declaration of interest

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