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RESEARCH ARTICLE

Inhibition of carbonic anhydrase isoforms I, II, IX and XII with Schiff's bases incorporating iminoureido moieties

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Abstract

A series of new Schiff's bases was obtained from the sulfanilamide semicarbazone (4-aminosulfonylphenyl semicarbazide) and aromatic/heterocyclic aldehydes. The new compounds were designed to incorporate moieties known to induce effective inhibitory activity against carbonic anhydrase (CA, EC 4.2.1.1) isoforms involved in crucial physiologic or pathologic processes such as the cytosolic CA I and II or the transmembrane, tumor-associated CA IX and XII: the compounds were medium potency – weak CA I inhibitors, highly effective, low nanomolar CA II inhibitors, but few of them inhibited effectively CA IX and XII. This may probably due to the long spacer between the sulfamoylphenyl and imine fragments of the molecules, which probably induces a highly flexible conformation of the inhibitor bound to the active site of the enzyme, with destabilizing effects for the adduct. The detailed structure activity relationship for this class of inhibitors is discussed.

Keywords

Carbonic anhydrase, cytosolic/tumor-associated enzymes, isoform-selective inhibitor, Schiff's base, sulfonamide

History

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Introduction

Schiff's bases incorporating sulfonamide functionalities attached to aromatic/heterocyclic moieties of the general formula Ar–CH = N-linker-A-SO_2NH_2, have been investigated extensively as carbonic anhydrase (CA, EC 4.2.1.1)[1–15] inhibitors (CAIs starting with the 1990s when the first such compounds were reported by one of these groups[16–27]. Some of these derivatives were among the first reported sulfonamides showing good selectivity ratios for the inhibition of some human (h) CA isoforms, such as hCA I, II or IV[16,17].

The CA superfamily of enzymes, which act as catalysts for CO_2 hydration to bicarbonate and protons, comprises a large number of genetic families (six, i.e. the α-, β-, γ-, δ-, ζ- and η-CAs)[28–33]. Furthermore, numerous isoforms were discovered so far in most investigated organisms[1–8,34–38]. For example, 16 isozymes were described in mammals. Some of them are cytosolic (CA I, CA II, CA III, CA VII and CA XIII), others are membrane bound (CA IV, CA IX, CA XII, CA XIV and CA XV), two are mitochondrial (CA VA and CA VB) and one is secreted in saliva and milk (CA VI). Three of these proteins are acatalytic (CA VIII, X and XI)[1–3]. CA I and CA II are the two major CA isozymes present at high concentrations in the cytosol of red blood cells of most vertebrates, and CA II is also one of the most active of all CAs, together with the tumor-associated, transmembrane isoforms CA IX[1–3]. CA XII, another transmembrane isoform, as hCA IX, is also present in some tumors, but more diffuse in normal tissues compared to hCA IX[39–47].

The human (h) such enzyme, hCAs are therapeutic targets with the potential to be inhibited or activated, and these phenomena elicit a number of pharmacologic effects[28–47]. For example, inhibitors targeting hCA II/IV/XII are used for the treatment of glaucoma, those targeting hCA VII/XIV for epilepsy management, whereas some hCA II/IV inhibitors are used as diuretics[28–33]. hCA IX/XII inhibitors show applications as diagnostic tools for the imaging of hypoxic tumors, and several such agents are in early phase clinical development for the treatment of hypoxic tumors non-responsive to the classical chemo- or radiotherapy[1,39–47]. Inhibition of hCA IX/XII leads to the impairment of the growth of the primary tumor and metastases, and also to the depletion of the cancer stem cell population, all these phenomena being useful in the management of hypoxic tumors, for which few therapeutic options are available nowadays[1–3]. For such reasons, the design of new, potent and isoform-selective inhibitors of various CAs, may lead to clinical applications for treating a multitude of diseases[1–3]. The Schiff's base containing sulfonamide inhibitors are in fact just one of the inhibitor classes investigated for such purposes[16–27]. In addition to this, a number

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of Schiff's base derivatives have been reported to exert notable biological activity (antibacterial, antitubercular, antitumor, antileishmanial, DNA-binding activities, etc.)[27].

In this study, we report the synthesis of series of 12 N^4-(4-aminosulfonylphenyl)-N^1 (aryl/substitutedaryl/heteroaryl) semicarbazones derivatives, and investigation their CA inhibitory activity against four physiologically relevant human isoforms, hCA I, II, IX and XII.

Materials and methods

Chemistry

All the reagents and solvents were obtained from commercial suppliers and were used as received unless otherwise indicated. Solvents were dried, wherever necessary, according to standard procedures. All reactions were performed under N_2 atmosphere, unless otherwise indicated. Analytical silica gel 60 F_{254}-coated TLC plates were purchased from Sigma-Aldrich, and were visualized with UV light. Infrared (IR) spectra were recorded on Shimadzu – FTIR 8400S instrument (Shimadzu Corp. Tokyo, Japan) using KBr pellet technique. ^{1}H NMR spectra were routinely obtained with a Varian Mercury Plus 300 MHz NMR (Agilent Technologies, Santa Clara, CA) and Me_4Si was used as an internal standard. ^{13}C NMR spectra were obtained with Bruker Avance II 400 NMR spectrometer SAIF Punjab University Chandigarh. LC-MS spectra were recorded on 6110 AA Series Quadrupole LC/MS system (Agilent Technologies, Santa Clara, CA). Analytical HPLC analyses ware carried out with a Kromasil® 100-5C18 column (150 mm × 4.6 mm) on PerkinElmer Series 200 HPLC system with autosampler and PDA detector (PerkinElmer, Inc., Waltham, MA) using ACN:Water (50:50) mobile phase (isocratic elution). Melting points were recorded using a Veego® (VMP)-D capillary melting point apparatus (Veego Instruments Corp. Mumbai, India) and are uncorrected.

*Procedure for synthesis of 4-ureidobenzenesulfonamide (**1**)*

In a 250-mL beaker, sulfanilamide (0.03 mol) was dissolved in a mixture of glacial HOAc (13 mL) and hot water (30 mL)[48]. A solution of sodium cyanate (0.05 mol) in hot water (25 mL) was added to the above mixture with continuous stirring. It was allowed to stand for 30 min, then cooled in ice-bath and vacuum filtered, dried and recrystallized from EtOH. Yield: 70%; mp: 175 °C(178–179 °C)[49]; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.20 (s, 2H, CO–NH_2), 7.15 (s, 2H, SO_2–NH_2), 7.66–7.64 (d,1H, C–H aromatic), 7.62–7.59 (d, 1H, C–H aromatic), 7.55–7.51 (d, 1H, C–H aromatic), 7.52–7.40 (d, 1H, C–H aromatic), 6.01 (s, 1H, Ar–NH); IR (KBr) cm^{-1}: 3462 (N–H *str* of NH_2), 3365 (N–H *str*), 3227 (aromatic C–H *str*), 1693 (C = O *str*), 1533 (aromatic C = C *str*), 1409, 1321, 1155 (S = O *str*).

*Procedure for synthesis of 4-aminosulfonylphenyl semicarbazide (**2**)*

In a 250-mL RBF, equimolar quantities of **1** (0.05 mol) and hydrazine hydrate (0.05 mol) in EtOH (2.5 ml) were refluxed for 27 h[48]. The progress of the reaction was monitored by TLC using $CHCl_3$:MeOH (8:2) as the mobile phase. The 2/3rd volume of EtOH was removed under reduced pressure and then the reaction mixture was poured onto crushed ice. The resultant precipitate was filtered, washed with H_2O and dried. The crude product was recrystallized from EtOH. Yield: 56%; mp: 207 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 8.98 (s, 1H, CO–NH), 7.15 (s, 2H, SO_2–NH_2), 4.40 (s, 2H, NH_2), 7.60–7.68 (m, 5H, Ar–NH and C–H aromatic); IR (KBr) cm^{-1}: 3365 (N–H *str* of NH_2), 3281 (N–H *str*), 3074 (aromatic C–H *str*), 1676 (C = O *str*), 1521 (aromatic C = C *str*), 1408, 1301, 1153 (S = O *str*).

*General procedure for synthesis of N^4-(4-aminosulfonylphenyl)-N^1-(aryl/substituted aryl/heteroaryl) semicarbazones (**3a–l**)*

In a 250-mL flask, equimolar quantities of **2** (0.05 mol) and aromatic aldehyde (0.05 mol) were dissolved in EtOH (50 mL)[50]. Glacial HOAc (2–3 mL) was added to adjust the pH to 5–6. The reaction mixture was further refluxed for 10–25 h. After completion of reaction (as seen from TLC), the mixture was concentrated under reduced pressure, poured onto the crushed ice and the precipitated product was filtered, washed twice with ice-cold water and recrystallized from EtOH.

Synthetic and spectral characterization details for compounds **3a–l** can be found in Supplementary Material.

*2-(4-Fluorobenzylidene)-N-(4-sulfamoylphenyl) hydrazinecarboxamide (**3a**)*

Purity (HPLC): 98%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 10.93 (s, 1H, CO–NH), 9.24 (s, 1H, Ar–NH), 7.96 (s, 1H, N = CH), 7.91–7.94 (m, 1H, C–H aromatic), 7.87–7.84 (d, 2H, C–H aromatic), 7.75–7.72 (d, 2H, C–H aromatic), 7.30–7.24 (m, 3H, C–H aromatic), 7.22 (s, 2H, SO_2NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 152.4, 141.7, 137.2, 134.8 (J_{C-F} = 243 Hz), 136.7, 133.5, 130.2, 128.9, 128, 127.0, 126.5, 118.5. LC-MS (ESI) $(M + H)^+$: 337.12; IR (KBr) cm^{-1}: 3375 (NH *str* of NH_2), 3333 (N–H *str*), 3099 (aromatic C–H *str*), 1687 (C = O *str*), 1593 (C = N *str*), 1533 (aromatic C = C *str*), 1411, 1321, 1153 (S = O *str*), 1095 (C–F *str*).

*2-(4-Bromobenzylidene)-N-(4-sulfamoylphenyl) hydrazinecarboxamide (**3b**)*

Purity (HPLC): 97 %; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.0 (s, 1H, CO–NH), 9.27 (s, 1H, Ar–NH), 7.94 (s, 1H, N = CH), 7.87–7.85 (d, 2H, C–H aromatic, J = 7.2 Hz), 7.84–7.82 (d, 2H, C–H aromatic, J = 6.6 Hz), 7.76–7.73 (d, 2H, C–H aromatic, J = 8.7 Hz), 7.64–7.61 (d, 2H, C–H aromatic, J = 8.4 Hz), 7.22 (s, 2H, C–H SO_2NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 152.7, 141.9, 139.1, 136.9, 128.7, 126.7, 123.9, 122.3, 120.8, 117.8.; LC-MS (ESI; M)$^+$: 397.13 $(M + 2)^+$: 399.09; IR (KBr) cm^{-1}: 3371, 3338 (N–H *str* of NH_2), 3254 (N–H *str*), 3097 (aromatic C–H *str*),1695 (C = O *str*) 1587 (C = N *str*), 1531 (aromatic C = C *str*), 1410, 1340, 1157 (S = O *str*), 702 (C-Br *str*).

*2-(4-Nitrobenzylidene)-N-(4-sulfamoylphenyl) hydrazinecarboxamide (**3c**)*

Purity (HPLC): 97%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.0 (s, 1H, CO–NH), 9.27 (s, 1H, Ar–NH), 7.94 (s, 1H, N = CH), 8.28–8.25 (d, 2H, C–H aromatic, J = 9 Hz), 8.16–8.14 (d, 2H, C–H aromatic, J = 8.7 Hz), 7.88–7.85 (d, 2H, C–H aromatic, J = 8.7 Hz), 7.77–7.47 (d, 2H, C–H aromatic, J = 8.7 Hz), 7.22 (s, 2H, SO_2NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 152.7, 142.0, 141.5, 139.1, 137.2, 131.2, 128.9, 126.7, 126.4, 118.4. LC-MS (ESI; $M + H)^+$: 364.08; IR (KBr) cm^{-1}: 3350 (N–H *str* of NH_2), 3246 (N–H *str*), 3086 (aromatic C–H *str*), 1687 (C = O *str*), 1589 (C = N *str*), 1537 (aromatic C = C *str*), 1410, 1338, 1147 (S = O *str*), 1323 (C–NO_2 *str*).

*2-(2,4-Dichlorobenzylidene)-N-(4-sulfamoylphenyl) hydrazinecarboxamide (**3d**)*

Purity (HPLC): 97%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.17 (s, 1H, CO–NH), 9.32 (s, 1H, Ar–NH), 8.32 (s, 1H, N = CH), 8.86–8.83 (d, 2H, C–H Aromatic), 8.44–8.41 (d, 1H, C–H Aromatic), 7.76–7.33 (d, 2H, C–H Aromatic), 7.52–7.50 (dd, 1H, C–H Aromatic), 7.53 (s, 1H, C–H Aromatic), 7.21 (s, 2H, SO_2NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 152.6, 148.2,

142.0, 139.0, 137.5, 136.1, 132.8, 129.6, 126.3, 123.4, 121.3, 118.9; LC-MS (ESI; M)$^+$: 387.10, (M + 2)$^+$: 389.05; IR (KBr) cm^{-1}: 3390, 3346 (N–H *str* of NH_2), 3265 (N–H *str*), 2966 (aromatic C–H *str*), 1697 (C = O *str*), 1587 (C = N *str*), 1537 (aromatic C = C *str*), 1410, 1327, 1157 (S = O *str*), 678 (C–Cl *str*).

*2-(4-Hydroxybenzylidene)-N-(4-sulfamoylphenyl) hydrazinecarboxamide (**3e**)*

Purity (HPLC): 99%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 10.71 (s, 1H, CO–NH), 9.84 (s, 1H, Ar–NH), 9.13 (s, 1H, OH), 7.88 (s, 1H, N = CH), 7.87–7.85 (d, 2H, C–H Aromatic), 7.74–7.71 (d, 2H, C–H Aromatic), 7.69–7.66 (d, 2H, C–H Aromatic), 6.86–6.82 (d, 2H, C–H Aromatic), 7.21 (s, 2H, SO_2NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 152.8, 142.1, 140.1, 137.3, 130.6, 130.42, 129.0, 126.3, 118.6, 115.8; LC-MS (ESI; M + H)$^+$: 335.16; IR (KBr) cm^{-1}: 3446 (O–H *str*), 3369, 3315 (NH *str* of NH_2), 3227 (N–H *str*), 3095 (aromatic C–H *str*), 1693 (C = O *str*), 1587 (C = N *str*), 1529 (aromatic C = C *str*), 1415, 1327, 1159 (S = O *str*).

*2-(4-Methylbenzylidene)-N-(4-sulfamoylphenyl) hydrazinecarboxamide (**3f**)*

Purity (HPLC): 99%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 10.85 (s, 1H, CO–NH), 9.19 (s, 1H, Ar–NH), 7.93 (s, 1H, N = CH), 7.88–7.85 (d, 2H, C–H aromatic), 7.78–7.74 (d, 2H, C–H aromatic), 7.76–7.71 (d, 2H, C–H aromatic), 7.26–7.23 (d, 2H, C–H aromatic), 7.22 (s, 2H, SO_2NH_2), 2.4 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 152.6, 148.2, 147.3, 140.5, 138.9, 137.5, 136.1, 132.7, 126.3, 123.4, 118.9, 39.6; LC-MS (ESI; M + H)$^+$: 333.18; IR (KBr) cm^{-1}: 3363 (N–H *str*), 3257 (N–H *str*), 3105 (aromatic C–H *str*), 1689 (C = O *str*), 1589 (C = N *str*), 1533 (aromatic C = C *str*), 1411, 1325, 1161 (S = O *str*).

*2-((1H-Indol-3-yl)methylene)-N-(4-sulfamoylphenyl) hydrazinecarboxamide (**3g**)*

Purity (HPLC): 99%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.56 (s, 1H, CO–NH), 10.54 (s, 1H, Ar–NH), 8.91 (s,1H, NH of indole), 8.23–7.17 (m, 1H of CH = N, 2H, SO_2NH_2 and 9H of C–H aromatic); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 158·2, 152.6, 142.1, 137.5, 134.8, 132.8, 131.4, 130.5, 127.4, 127.3, 127.0, 126.7, 126.3, 118.8; LC-MS (ESI; M + H)$^+$: 358.14; IR (KBr) cm^{-1}: 3344 (N–H *str*), 3277 (N–H *str*), 3115 (aromatic C–H *str*), 1695 (C = O *str*), 1585 (C = N *str*), 1531 (aromatic C = C *str*), 1410, 1317, 1155 (S = O *str*).

*2-(2-Chlorobenzylidene)-N-(4-sulfamoylphenyl) hydrazinecarboxamide (**3h**)*

Purity (HPLC): 97%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.14 (s, 1H, CO–NH), 9.29 (s, 1H, Ar–NH), 8.38 (s, 1H, N = CH), 7.22 (s, 2H, SO_2NH_2), 7.87–7.84 (d, 2H, C–H aromatic), 7.76–7.73 (d, 2H, C–H aromatic), 7.63–7.40 (m, 4H, C–H aromatic); LC-MS (ESI; M + H)$^+$: 353.04; IR (KBr) cm^{-1}: 3375 (N–H *str*), 3230 (N–H *str*), 3095 (aromatic C–H *str*), 1695 (C = O *str*), 1591 (C = N *str*), 1537 (aromatic C = C *str*), 1411, 1334, 1174 (S = O *str*).

*2-(2-Thienylidene)-N-(4-sulfamoylphenyl) hydrazinecarboxamide (**3i**)*

Purity (HPLC): 98%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 10.82 (s, 1H, CO–NH), 8.99 (s, 1H, Ar–NH), 8.18 (s, 1H, N = CH), 7.80–7.78 (d, 2H, C–H aromatic), 7.74–7.72 (d, 2H, C–H aromatic), 7.65–7.64 (d, 1H, C–H thiophene), 7.46–7.45 (d, 1H, C–H thiophene), 7.20 (s, 2H, SO_2NH_2), 7.13–7.10 (t, 1H, C–H thiophene); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 158·9, 152.8, 142.2, 141.7, 137.1, 129.8, 128.5, 126.4, 125.1, 118.4; LC-MS (ESI) (M + H)$^+$: 325.10; IR (KBr) cm^{-1}: 3367 (N–H of NH_2 *str*), 3261 (N–H *str*), 3084 (aromatic C–H *str*), 1687 (C = O *str*), 1589 (C = N *str*), 1531 (aromatic C = C *str*), 1411, 1327, 1149 (S = O *str*), 761 (C–S *str*).

*2-(Anthracen-9-ylmethylene)-N-(4-sulfamoylphenyl) hydrazinecarboxamide (**3j**)*

Purity (HPLC): 98%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.12 (s, 1H, CO–NH), 9.21 (s, 1H, Ar–NH), 9.14 (s, 1H, N = CH), 8.7 (s, 1H, C–H aromatic), 8.52–8.50 (d, 2H, C–H aromatic), 8.17–8.15 (d, 2H, C–H aromatic), 7.80–7.58 (m, 8H, C–H aromatic), 7.19 (s, 2H, SO_2NH_2); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 152.7, 142.1, 140.0, 139.1, 137.4, 136.9, 133.3, 131.3, 129.1, 128.7, 126.5, 126.3, 122.7, 118.7; LC-MS (ESI; M + H)$^+$: 419.15; IR (KBr) cm^{-1}: 3414 (NH *str* of NH_2), 3282 (NH *str*), 2916 (aromatic C–H *str*), 1647 (C = O *str*) 1595 (imine C = N *str*), 1545 (aromatic C = C *str*), 1471, 1311, 1155 (S = O *str*).

*2-(3-Nitrobenzylidene)-N-(4-sulfamoylphenyl) hydrazinecarboxamide (**3k**)*

Purity (HPLC): 98%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.13 (s, 1H, CO–NH), 9.40 (s, 1H, Ar–NH), 8.66 (s, 1H, N = CH), 7.22 (s, 2H, SO_2NH_2), 8.33 (d, 1H, C–H aromatic), 8.24–8.23 (d, 1H, C–H aromatic), 8.10 (s, 1H, C–H aromatic), 7.77–7.74 (d, 2H, C–H aromatic), 7.75–7.73 (d, 2H, C–H aromatic), 7.70 (s, 1H, C–H aromatic); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 159.3, 152.6, 142.1, 137.4, 133.9, 131.1, 126.9, 126.3, 124.3, 121.8, 121.7, 118.8; LC-MS (ESI; M + H)$^+$: 364.0718; IR (KBr) cm^{-1}: 3362 (N–H *str* of NH_2), 3211 (N–H *str*), 3082 (aromatic C–H *str*), 1703 (C = O *str*), 1589 (C = N *str*), 1535 (aromatic C = C *str*), 1410, 1350, 1155 (S = O *str*), 1327 (C–NO_2 *str*).

*2-(2-Fluorobenzylidene)-N-(4-sulfamoylphenyl) hydrazinecarboxamide (**3l**)*

Purity (HPLC): 99%; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.08 (s, 1H, CO–NH), 9.27 (s, 1H, Ar–NH), 8.20 (s, 1H, N = CH), 7.23 (s, 2H, SO_2NH_2), 8.36–8.30 (t, 1H, C–H aromatic), 7.87–7.85 (d, 2H, C–H aromatic), 7.76–7.73 (d, 2H, C–H aromatic), 7.50–7.29 (m, 3H, C–H aromatic); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 142.1, 138.9, 137.1, 130.9, 129.5, 128.8, 128.3, 126.8, 125.5, 125.1, 124.5, 118.5; LC-MS (ESI; M + H)$^+$: 337.0773; IR (KBr) cm^{-1}: 3373 (N–H *str*), 3269 (N–H *str*), 3099 (aromatic C–H *str*), 1687 (C = O *str*), 1591 (C = N *str*), 1537 (aromatic C = C *str*), 1413, 1301, 1159 (S = O *str*), 1103 (C–F *str*).

CA activity measurements and inhibition studies

An SLX Applied Photophysics stopped-flow instrument has been used for measuring the catalytic activity and inhibition with a CO_2 hydration assay method[51]. 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) or 20 mM Tris (pH 8.3) as buffers and 20 mM Na_2SO_4 or $NaClO_4$ (for maintaining constant ionic strength). The initial rates of the CA-catalyzed CO_2 hydration reaction were followed for a period of 10–100 s. The concentrations of substrate (CO_2) ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants, with at least six traces of the initial 5–10% of

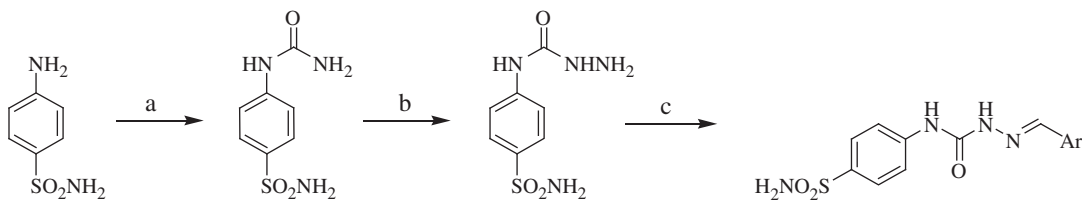
the reaction being used for determining the initial velocity, for each inhibitor. The uncatalyzed rates determined were subtracted from the total observed rates. Stock solutions of inhibitors (10 mM) were prepared in distilled-deionized water and dilutions up to 0.01 nM were done with the assay buffer. Enzyme and inhibitor solutions were pre-incubated prior to assay for 15 min (at room temperature), in order to allow for the formation of the E–I complex. The inhibition constants were obtained by non-linear least-squares methods using PRISM 3 and the Cheng–Prusoff equation as reported earlier by our groups[52–55]. The CAs were recombinant proteins obtained in-house as reported earlier[50–52]. The concentrations of enzyme used in the assay were: 16.0 nM for hCAI, 9.1 nM for hCA II; 9.5 nM for hCA IX and 12.6 nM for hCA XII, respectively.

Results and discussion

Chemistry

The synthesis of target compounds was carried out as outlined in Scheme 1. Sulfanilamide was reacted with sodium cyanate in the presence of glacial acetic acid, leading to 4-ureidobenzenesulfonamide (**1**). 4-Ureidobenzenesulfonamide **1** was further used to synthesize 4-aminosulfonylphenyl semicarbazide (**2**) through condensation with hydrazine hydrate in ethanol. Compound **2** was then used to synthesize N^4-(4-aminosulfonylphenyl)-N^1-(aryl/substituted–aryl/heteroaryl) semicarbazone derivatives **3a–l** by reaction with the appropriate heterocyclic/aromatic aldehydes in the presence of glacial acetic acid and ethanol, which yielded the Schiff's bases **3a–l**. The products obtained in this way were purified by recrystallization from ethanol. Table 1 lists their relevant physicochemical data.

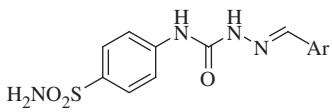
Structures of the synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR and LC-MS. In the IR spectra, formation of the Schiff's bases was confirmed by disappearance of the $-NH_2$ group and appearance of the imine N = CH band, characterized by an absorption at 1589–1591 cm^{-1}. The semicarbazone derivatives presented N–H stretching vibration at 3344–3390 cm^{-1}, CO–NH stretching vibrations at 1687–1703 and 3211–3277 cm^{-1}, respectively, in their IR spectra. Aromatic C = C stretching vibrations were observed in the 1529–1537 cm^{-1} region, whereas aromatic C–H stretching vibration in the 2966–3115 cm^{-1} region. In addition, the strong SO_2 stretching vibrations were observed in the region 1410–1415, 1301–1350 and 1147–1159 cm^{-1}. The ^{1}H-NMR spectra of compounds **3a–l** displayed singlets at $\delta = 7.94$–9.14 ppm (for N = CH), proton which confirms the formation of Schiff's bases. The singlet in the range of $\delta = 10.5$–11.56 ppm was observed for CO–NH protons; the singlet at $\delta = 9.19$–10.54 ppm corresponding to Ar–NH protons; whereas the broad singlet at $\delta = 7.19$–8.20 ppm corresponding to the SO_2NH_2 protons. In all compounds, a multiplet was observed within the range of $\delta = 8.44$–6.82 ppm for aromatic protons. In compound **3e**, the singlet at $\delta = 9.13$ ppm was assigned to the OH proton and the singlet at $\delta = 2.4$ ppm for the CH_3 protons of **3f**. A singlet at



Sulfanilamide **1** **2** **3a-3l**

Scheme 1. Synthesis of compounds **3a–l**. Reagents and conditions were: (a) NaCNO, glacial AcOH; (b) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 27 h; (c). ArCHO, glacial AcOH, EtOH, reflux, 10–25 h.

Table 1. Structures and physicochemical properties of compounds **1–3** reported here.



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Compound code	Ar	Molecular formula	Mol. Wt.	Melting Point (°C)	R_f*	Yield (%)
1	–	$C_7H_9N_3O_3S$	215.0	175	0.48	75
2	–	$C_7H_{10}N_4O_3S$	230.0	207	0.33	56
3a	4-F–C_6H_4	$C_{14}H_{13}FN_4O_3S$	336.11	243	0.71	69
3b	4-Br–C_6H_4	$C_{14}H_{13}BrN_4O_3S$	397.25	256	0.76	65
3c	4-NO_2–C_6H_4	$C_{14}H_{13}N_5O_5S$	363.09	275	0.64	72
3d	2,4-Cl_2–C_6H_3	$C_{14}H_{12}Cl_2N_4O_3S$	387.25	245	0.79	77
3e	4-OH–C_6H_4	$C_{14}H_{14}N_4O_4S$	334.36	230	0.82	62
3f	4-CH_3–C_6H_4	$C_{15}H_{16}N_4O_3S$	332.39	231	0.75	79
3g	3-Indolyl	$C_{16}H_{15}N_5O_3S$	357.4	224	0.70	68
3h	2-Cl–C_6H_4	$C_{14}H_{13}ClN_4O_3S$	352.8	228	0.79	71
3i	2-Thienyl	$C_{12}H_{12}N_4O_3S_2$	324.39	242	0.77	65
3j	9-Anthranyl	$C_{22}H_{18}N_4O_3S$	418.48	249	0.85	82
3k	3-NO_2–C_6H_4	$C_{14}H_{13}N_5O_5S$	363.09	223	0.67	74
3l	2-F–C_6H_4	$C_{14}H_{13}FN_4O_3S$	336.1	241	0.80	66

*TLC mobile phase – $CHCl_3$:MeOH (8:2).

$\delta = 8.91$ ppm corresponding to NH of indole was observed for compound **3g.**

CA inhibition

The CA inhibitory properties of sulfonamides **3a–l** reported here were investigated against four physiologically relevant isoforms, hCA I, II (cytosolic) as well as hCA IX and XII (transmembrane, tumor-associated enzymes). The following structure activity relationship can be observed from the data of Table 2:

(i) The slow cytosolic isoform hCA I was inhibited with medium efficacy by four sulfonamides investigated here, i.e. **3b, g, h** and **i**, which showed inhibition constants in the range of 71.2–95.2 nM, being thus more effective than the standard drug acetazolamide **AAZ** (Table 1). These derivatives incorporate 4- and 2-Cl-phenyl- as well as heterocyclic (indolyl and thienyl) moieties. It may be observed that changing the substitution pattern at the Ar moiety has a detrimental effect on the hCA I inhibitory activity, as compounds incorporating other moieties (4-nitro, 3-nitro, 4-Me, 3,4-dichloro-, etc) are much weaker inhibitors of this isoform, with K_Is in the range of 250–6530 nM (Table 2).

(ii) The physiologically dominant hCA II (drug target for antiglaucoma agents) was efficiently inhibited by many of the sulfonamides investigated here, such as **3a–c, 3e–j** and **3l**, which showed K_Is in the range of 4.0–19.5 nM, being more potent or equipotent the standard drug AAZ. Thus, unlike hCA I discussed above, many substitution patterns of the Ar moiety in the sulfonamides **3** lead to effective inhibitors of this isoform. Only two compounds, **3d** (2,4-dichlorophenyl derivative) and **3k** (3-nitrophenyl derivative) were medium potency hCA II inhibitors, with K_Is in the range of 51.6–75.0 nM. Thus, small differences in the nature or substitution pattern of the Ar moiety (e.g. the regiomers **3c** and **3k**, which only differ by the position of the NO_2 moiety) of these derivatives leads to drastic changes in the inhibition pattern of the corresponding compounds (Table 2).

(iii) The hCA IX inhibition profile with the compounds investigated here was less interesting, however. Only compound **3 a** (4-fluorophenyl derivative) showed an activity similar to AAZ, with a K_I of 27.3 nM, whereas all other compounds were much less effective as inhibitors of this transmembrane isoform, with inhibition constants in the range of 90.4–424 nM (Table 2).

(iv) A rather similar behavior to that mentioned above for hCA IX; was also observed for the inhibition of the second transmembrane isoform, hCA XII. Two compounds, **3i** and **3j** (incorporating 2-thienyl and 9-anthranyl moieties, respectively) were effective hCA XII inhibitors (K_Is in the range of 21.5–23.8 nM), whereas the remaining derivatives were medium potency hCA XII inhibitors (K_Is in the range of 42.3–95.8 nM). The structure activity relationship is thus rather flat, with a reduced variation in the inhibition constants over the entire series of derivatives. This may be due to the quite long linker between the sulfamoylphenyl and arylimine fragments of the molecules investigated here, which probably induces a too flexible conformation of the inhibitor molecule when bound to the enzyme active site. This leads to a destabilization of the enzyme–inhibitor adduct and as a consequence the weaker inhibitory power of these Schiff's bases compared to similar derivatives[17–25] possessing a shorter linker between the two fragments mentioned above.

Table 2. hCA I, II, IX and XII Inhibition data of compounds **3a–l** investigated in the paper, by a stopped-flow CO_2 hydrase assay[51].

	K_i (nM)*			
Compound	hCA I	hCA II	hCA IX	hCA XII
3a	675	5.6	27.3	95.8
3b	95.2	6.2	90.4	92.3
3c	1580	7.4	102	94.1
3d	6530	75.0	98.1	90.7
3e	250	6.1	176	60.3
3f	802	6.7	105	87.4
3g	71.2	4.0	103	63.1
3h	77.6	4.2	213	42.3
3i	79.3	4.6	395	23.8
3j	1640	19.5	381	21.5
3k	4930	51.6	415	83.9
3l	507	4.7	424	79.1
AAZ	250	12	25	5.8

AAZ was used as standard drug.
*Mean from three different assays. The errors were in the range of 5–10% of the reported values.

Conclusion

We report here a new series of new Schiff's bases, which was obtained from the sulfanilamide semicarbazone (4-aminosulfonylphenyl semicarbazide), by reaction with aromatic/heterocyclic aldehydes. The compounds were designed to incorporate moieties known to induce effective inhibitory for CA isoforms involved in crucial physiologic or pathologic processes such as the cytosolic CA I and II or the transmembrane, tumor-associated CA IX and XII. The investigated compounds were medium potency – weak CA I inhibitors, highly effective, low nanomolar CA II inhibitors, but few of them inhibited effectively CA IX and XII. This may probably due to the long spacer between the sulfamoylphenyl and imine fragments of the molecules, which probably induces a highly flexible conformation of the inhibitor bound to the active site of the enzyme, with destabilizing effects for the adduct. The detailed structure activity relationship for this class of inhibitors is discussed.

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Declaration of interest

Most authors declare no conflict of interest. CTS declares conflict of interest, being author on many patents claiming CA inhibitors with various pharmacologic applications.

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Supplementary material available online
Supplementary Figures S1–S36.