Synthesis of a new series of 3-functionalised-1-phenyl-1,2,3-triazole sulfamoylbenzamides as carbonic anhydrase I, II, IV and IX inhibitors

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ABSTRACT
The synthesis of a novel series of 3-functionalised benzenesulfonamides incorporating phenyl-1,2,3-triazole with an amide linker was achieved by using the “click-tail” approach. The new compounds, including the intermediates, were assayed as inhibitors of human carbonic anhydrase (CA, EC 4.2.1.1) isozymes hCA I and II (cysotolic isozymes) and also for hCA IV and IX (transmembrane isozymes) taking acetazolamide as standard drug. Most of these compounds exhibited excellent activity against all these isozymes. hCA I was inhibited with Ki in the range of 50.8–966.8 nM, while the glaucoma associated hCA II was inhibited with Ki in the range of 65.5–760.0 nM. Isozyme hCA IV was inhibited with Ki in the range of 65.3–957.5 nM, whereas the tumor associated hypoxia induced hCA IX was inhibited with Ki in the range of 30.8–815.9 nM. The structure activity relationship study for the 3-functionalised-1-phenyl-1,2,3-triazole sulfamoylbenzamides against these isozymes was also inferred from the results.

1. Introduction
Many approaches for the development of sulfonamide carbonic anhydrase (CA, EC 4.2.1.1) inhibitors have been explored with the aim to achieve better selectivity profiles towards the different human (h) isozymes of the enzyme. CAs are ubiquitous Zn containing metalloenzymes present in all life phyla catalyzing the reversible hydration of carbon dioxide to bicarbonate anion and a proton by using a metal hydroxide nucleophilic mechanism and is being crucial for a variety of physiological and patho- logical processes such as maintenance pH and CO2 homeostasis, electrolyte secretion, bone resorption/calcification, gluconeogenesis, cell differentiation and proliferation, neurotransmission (in mammals), and virulence and tissue colonization (in pathogens)1. Up until now it has already been reported that sulfonamides, sulfamates, sulfamides, hydroxamates incorporate efficient zinc binding groups (ZBGs) and directly bind to the metal ion within the enzyme cavity2. Thus, from the drug design viewpoint the classical sulfonamide group (–SO2NH2) is the interesting target where the classical sulfonamide group is the recognition motif for small molecules. In deprotonated form it binds to the zinc (II) ion in the active site thereby inhibiting the binding of the endogenous substrates (CO2 and H2O) and hence reducing the catalytic ability of the enzyme, similar to the transition state of the endogenous reaction constituting two additional H-bonds with Thr199 residue (Figure 1)3,4. The binding pattern for the sulfonamide moiety exhibits a common feature among the active site super structure of all the 15 human isozymes belonging to class. The active site consists of a tetrahedral Zn2+ coordinated to the imidazole side chains of the three histidine residues present at the base of the funnel shaped active site cavity. Till now seven genetically distinct families have been identified the α, β, γ, δ, ε, η, θ-CAs, which are very much different from the α-CAs mentioned above5–7.

The hCA isozymes differ in their subcellular location, tissue distribution, and molecular and kinetic properties. Basically, four isozymes are cytosolic (I, II, III, and VII), five membrane-bound (IV, IX, XII, XIV, and XV), two mitochondrial (VA and VB), and CAVI is secreted in saliva and milk. Among these CA IV and XV are having GPI (glycosylphosphatidylinositol) tails anchored to the membrane while CAs IX, XII, XIV are transmembrane proteins possessing just one membrane domain5. Despite that, all these five membrane-bound isozymes are commonly termed as extracellular CAs due to having their active sites outside the cell. Many sulfonamide-based drugs (Figure 2) such as acetazolamide (AAZ), methazolamide, ethoxzolamide, dorzolamide, brinzolamide, dichlorophenamide, and celecoxib are used clinically for many years as diuretics, anti-epileptics, anti-glaucoma, or as anti-tumor agents9,10.

Recently many researchers in this field are focusing on isoenzyme selective sulfonamide inhibitors by using two strategies such as the ring and the tail approach (first described by Supuran’s group)11. The first one consists in modulating the ring directly linked to the sulfonamide moiety and the later one entails attaching different tails to the aromatic/heterocyclic ring bearing the ZBG (Figure 1). The respective tail moieties of the ligand have the ability to specifically interact with amino acid residues (most variable among various isozymes) present at the rim of the active site pocket. Hence, the tail approach is followed mostly. It was also possible to harmonise the physicochemical properties (most
crucial for activity) of the CAIs by selecting tails with a diverse chemical nature. Although diverse types of sulfonamide derivatives have already been reported for CA inhibition, it is necessary to explore this class further for better CA inhibitory profiles.

Nowadays, click chemistry is widely used to obtain metallo-enzyme CA inhibitors belonging to sulfonamide and coumarin classes. To synthesise 1,4-disubstituted 1,2,3-triazoles, the copper-catalyzed azide-alkyne cycloadditions also well known as "click chemistry" has played a pivotal role in medicinal chemistry. The 1,2,3-triazole ring is a bioisostere of the amide bond and maintains high stability under basic as well as acid hydrolysis, reductive and oxidative conditions. It also has high dipole moment and capability of H-bonding in the in-vivo environment. Due to its aromatic character, it may undergo π-stacking interactions with relevant amino acid residues within the enzyme cavity. In recent years, CAIs belonging to sulfonamide and coumarin classes have been obtained by recurrent use of click chemistry. Thus, owing to the versatility of click chemistry in medicinal chemistry and drug discovery, it was combined with the tail approach, together termed as "click tailing" for the first time in 2006, for the development of cell membrane impermeable CA inhibitors. Authors have explored the reversal of CA isoenzyme selectivity from hCA II to hCA IX by tethering a sugar triazole tail on to the CA anchor pharmacophore (Figure 3(a,b))

Thus in the present study we decided to synthesise a novel series of 1,2,3-triazole derivatives linked to a 3-sulfamoyl moiety with an amide linker via the “click tailing” approach and to test them as CA inhibitors.

2. Materials and methods

2.1. General

All the commercially available reagents were used without further purification. Solvents were dried and distilled wherever necessary prior to use using standard methods. All the air and moisture sensitive reactions were performed under inert conditions using clean and dried glassware and syringe technique to transfer solutions. Reactions were monitored by TLC using Merck silica gel 60 F-254 plates. Purification was performed by column chromatography on silica gel (60–120 mesh) using a mixture of DCM and methanol as eluent. Melting points were acquired on Stuart digital melting point apparatus/SMP 30 in open capillary tubes and uncorrected. Nuclear Magnetic Resonance (1H NMR and 13C NMR) spectra were recorded by using an Avance bruker 500 and 125 MHz spectrometer in DMSO-d6 as solvent and tetramethylsilane as internal standards.

Figure 1. Zinc coordination within CA active site showing: (a) hydration of CO2 to HCO3− and (b) a sulfonamide inhibitor bound to the zinc ion and the gate keeping residues Thr199-Glu106, conserved in all α-CAs [4].

Figure 2. Clinically used classical sulfonamide CA inhibitors.

Figure 3. (a) AAZ, MZA, EZA, DCP, BRZ, Celecoxib, DZA. (b) Structure of (a) 3-(4-tert-butylphenyl)-1,2,3-triazole (PTB) and (b) 1,5-diaryl-1,2,3-triazole (DAA) and (c) 1,3,4-thiadiazole sulfonamide exhibiting exclusively inhibitory action against hCA II (Figure 3(h)) [18]. Apart from this report there are no reports on the 3-sulfamoyl substituted derivatives as CAIs.
standard. Chemical shifts are reported as δ values in parts per million (ppm) and coupling constants (J) are expressed in Hz. Multiplicities are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets). HRMS were determined with Agilent QTOF mass spectrometer 6540 series instrument and were performed in the ESI techniques at 70 eV.

2.2. Chemistry

2.2.1. Synthesis of 3-(chlorosulfonyl)benzoic acid derivatives (2a–d)
To the stirred chlorosulfonic acid (13.6 ml, 204.5 mmol) at 0 °C, 4-Substituted benzoic acid derivatives (1a–d) (5 g, 40.9 mmol) were added portion wise and then stirred at 110 °C for 5 to 8 h. After completion of the reaction (monitored by TLC) it was cooled to RT and then the reaction mixture is poured into crushed ice (200 g) with vigorous stirring. The solid obtained was filtered off and the residue collected and washed with 50 ml water and dried in vacuo to obtain desired intermediate (2a–d) as white solid with 70–85% yield.

2.2.2. Synthesis of 3-(sulfamoyl)benzoic acids (3a–d)
To the ice-cold solution of ammonium hydroxide (25% in water) (5 ml, 204.5 mmol) at 0 °C, 3-(chlorosulfonyl)benzoic acid derivatives (2a–d) (5 g, 40.9 mmol) were added portion wise and then stirred at 110 °C for 5 to 8 h. After completion of the reaction (monitored by TLC) it was cooled to RT and then the reaction mixture is poured into crushed ice (200 g) with vigorous stirring. The solid obtained was filtered off and the residue collected and washed with 50 ml water and dried in vacuo to obtain desired intermediate (2a–d) as white solid with 70–85% yield.

2.2.3. Synthesis of N-(prop-2-yn-1-yl)-3-sulfamoylbenzamide (4a–d)
To the stirred solution of 3-(sulfamoyl)benzoic acid derivatives 3a–d (0.5 g, 2.5 mmol) in dry DMF (5 ml), EDCI (2.75 mmol), and HOBT (2.75 mmol) were added under inert conditions and the resultant solution stirred for 30 min at room temperature. This was followed by addition of propargyl amine (2.75 mmol) and the resultant solution was stirred at room temperature until the reaction was completed (monitored by TLC). After completion of the reaction as indicated by TLC, the reaction mixture was quenched with ice and the precipitate obtained is filtered and washed with ice cold water. The crude product was purified by column chromatography using alumina as the stationary phase and DCM: Methanol (97:3) as eluent to afford the products as white solid in 70–80% yield.

2.2.4. Synthesis of N-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)-3-sulfamoylbenzamides (6a–z) via click chemistry
N-(prop-2-yn-1-yl)-3-sulfamoylbenzamides 4a–d (0.08 g, 0.34 mmol) and phenyl azides (5a–m) (0.37 mmol) were dissolved in tBuOH:H2O (1:1, 5 ml) followed by the addition of CuSO4.5H2O (0.07 mmol) and sodium ascorbate (0.14 mmol). The resultant solution was kept for stirring till completion of the reaction (TLC monitoring). Solvents were removed under vacuum and the residue was purified by column chromatography using silica gel (60–120 mesh) as the stationary phase and methanol in DCM (0–5%) as the mobile phase. The pure products (6a–z) were collected in 52–98% yield.

2.2.4.1. 3-Sulfamoylbenzamido acid (3a): White solid, Yield 95%; 1H NMR (500 MHz, DMSO) δ 13.42 (s, 1H), 8.40 (t, J = 1.7 Hz, 1H), 8.15 (dd, J = 7.7, 1.1 Hz, 1H), 8.06 (dd, J = 7.9, 1.3 Hz, 1H), 7.72 (dd, 8.26, 7.9 Hz, 1H) ppm.
White solid; yield: 98%; m.p.: 212–213 °C; 1H NMR (500 MHz, DMSO) δ 9.36 (t, J = 5.6 Hz, 1H), 8.97 (s, 1H), 8.74 (t, J = 2.1 Hz, 1H), 8.44–8.40 (m, 1H), 8.37 (t, J = 1.6 Hz, 1H), 8.34–8.29 (m, 1H), 8.14–8.10 (m, 1H), 8.00–7.96 (m, 1H), 7.89 (t, J = 8.2 Hz, 1H), 7.70 (t, J = 7.8 Hz, 1H), 7.44 (s, 2H), 4.66 (d, J = 5.6 Hz, 2H), 13C NMR (125 MHz, DMSO) δ 165.57, 149.07, 147.01, 144.95, 137.73, 135.26, 132.00, 130.77, 129.60, 128.76, 126.46, 125.39, 123.47, 122.05, 115.13, 35.42. HRMS (ESI) m/z: [M + H]+ calculated for C19H22N5O6S 415.1441, found 415.1442.

2.2.5.3. N-((1-(3-cyanophenyl)-1H-1,2,3-triazol-4-yl)methyl)-3-sulfamoylbenzamide (6d). White solid; yield: 96%; m.p.: 209–210 °C; 1H NMR (500 MHz, DMSO) δ 9.21 (t, J = 5.4 Hz, 1H), 8.33 (t, J = 1.7 Hz, 1H), 8.10–8.03 (m, 1H), 8.01–7.96 (m, 1H), 7.69 (dd, J = 14.2, 6.4 Hz, 1H), 7.45 (s, 2H), 4.09 (dd, J = 5.5, 2.5 Hz, 2H), 3.15 (t, J = 2.5 Hz, 1H). 13C NMR (125 MHz, DMSO) δ 165.31, 144.96, 135.00, 130.68, 129.71, 128.85, 125.32, 81.50, 73.49, 29.14. HRMS (ESI) m/z: [M + Na]+ calculated for C16H15N6O5S 298.0248, found 298.0250.

2.2.5.4. N-((1-(3-fluorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-3-sulfamoylbenzamide (6f). White solid; yield: 87%; m.p.: 230–231 °C; 1H NMR (500 MHz, DMSO) δ 9.39 (t, J = 5.6 Hz, 1H), 8.73 (s, 1H), 8.37 (t, J = 7.8 Hz, 1H), 7.98 (d, J = 7.8 Hz, 1H), 7.69 (t, J = 7.8 Hz, 1H), 7.44 (s, 2H), 4.66 (d, J = 5.5 Hz, 2H), 13C NMR (125 MHz, DMSO) δ 165.56, 146.40, 144.92, 137.17, 135.32, 130.78, 130.34, 129.58, 120.93, 127.81, 125.38, 121.71, 120.46, 35.45. HRMS (ESI) m/z: [M + H]+ calculated for C19H22N5O6S 417.1325, found 417.1326.

2.2.5.5. N-((1-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)benzamide (6b). White solid; yield: 98%; m.p.: 230–231 °C; 1H NMR (500 MHz, DMSO) δ 9.34 (t, J = 5.6 Hz, 1H), 8.73 (s, 1H), 8.37 (t, J = 7.8 Hz, 1H), 7.98 (d, J = 7.8 Hz, 1H), 7.69 (t, J = 7.8 Hz, 1H), 7.44 (s, 2H), 4.64 (d, J = 5.5 Hz, 2H), 13C NMR (125 MHz, DMSO) δ 165.56, 146.40, 144.92, 137.17, 135.32, 130.78, 130.34, 129.58, 120.93, 127.81, 125.38, 121.71, 120.46, 35.45. HRMS (ESI) m/z: [M + H]+ calculated for C19H22N5O6S 448.1285, found 448.1293.

2.2.5.6. 3-Sulfamoyl-N-((1-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-4-yl)methyl)benzamide (6b). White solid; yield: 98%; m.p.: 230–231 °C; 1H NMR (500 MHz, DMSO) δ 9.34 (t, J = 5.6 Hz, 1H), 8.73 (s, 1H), 8.37 (t, J = 7.8 Hz, 1H), 7.98 (d, J = 7.8 Hz, 1H), 7.69 (t, J = 7.8 Hz, 1H), 7.44 (s, 2H), 4.64 (d, J = 5.5 Hz, 2H), 13C NMR (125 MHz, DMSO) δ 165.56, 146.40, 144.92, 137.17, 135.32, 130.78, 130.34, 129.58, 120.93, 127.81, 125.38, 121.71, 120.46, 35.45. HRMS (ESI) m/z: [M + H]+ calculated for C19H22N5O6S 448.1285, found 448.1293.
2.2.5.5. **N**-((1-(2,3-dimethylphenyl)-1H-1,2,3-triazol-4-ylmethyl)-3-sulfamoylbenzamide (6g).** White solid; yield: 86%, m.p: 205–206 °C; ¹H NMR (500 MHz, DMSO) δ 9.30 (t, J = 5.5 Hz, 1H), 8.36 (s, 1H), 8.27 (s, 1H), 8.09 (t, J = 9.4 Hz, 1H), 7.97 (d, J = 7.8 Hz, 1H), 7.68 (t, J = 7.8 Hz, 1H), 7.44 (s, 2H), 7.39 (d, J = 7.6 Hz, 1H), 7.28 (t, J = 7.7 Hz, 1H), 7.20 (d, J = 7.8 Hz, 1H), 4.65 (d, J = 5.5 Hz, 2H), 2.34 (s, 3H), 1.96 (s, 3H). 13C NMR (125 MHz, DMSO) δ 165.59, 145.15, 144.91, 139.81, 136.95, 135.40, 132.64, 131.48, 130.78, 129.57, 126.89, 126.62, 125.37, 124.36, 35.42, 20.34, 14.40. HRMS (ESI) m/z: [M + H]⁺ calculated for C₁₁H₁₈N₂O₄S 388.1074, found 388.1082.

2.2.5.6. **N**-((1-(2,4-dimethylphenyl)-1H-1,2,3-triazol-4-ylmethyl)-3-sulfamoylbenzamide (6h).** White solid; yield: 89%, m.p: 189–191 °C; ¹H NMR (500 MHz, DMSO) δ 9.29 (t, J = 5.4 Hz, 1H), 8.35 (d, J = 1.5 Hz, 1H), 8.29 (s, 1H), 8.10 (d, J = 7.8 Hz, 1H), 7.97 (d, J = 8.3 Hz, 1H), 7.68 (t, J = 7.8 Hz, 1H), 7.43 (s, 2H), 7.27 (d, J = 7.7 Hz, 2H), 7.19 (d, J = 8.0 Hz, 1H), 4.64 (d, J = 5.5 Hz, 2H), 2.36 (s, 3H), 2.11 (s, 3H). 13C NMR (125 MHz, DMSO) δ 165.56, 145.17, 144.91, 139.78, 135.39, 134.46, 133.12, 132.23, 130.78, 129.57, 126.89, 126.83, 126.18, 125.37, 125.06, 35.41, 21.07, 17.82. HRMS (ESI) m/z: [M + H]⁺ calculated for C₁₈H₂₀N₅O₃S 386.1281, found 386.1287.

2.2.5.7. **4-Chloro-N**-((1-phenyl-1H,1,2,3-triazol-4-ylmethyl)-3-sulfamoylbenzamide (6i).** White solid; yield: 83%, m.p: 199–201 °C; ¹H NMR (500 MHz, DMSO) δ 9.38 (t, J = 5.5 Hz, 1H), 8.70 (s, 1H), 8.51 (d, J = 2.1 Hz, 1H), 8.10 (d, J = 8.3, 2.2 Hz, 1H), 7.90 (d, J = 8.5, 0.9 Hz, 2H), 7.76 (t, J = 10.0 Hz, 1H), 6.79 (s, 2H), 7.59 (dd, J = 10.8, 5.0 Hz, 2H), 7.47 (dd, J = 17.5, 10.1 Hz, 1H), 4.63 (d, J = 5.5 Hz, 2H). 13C NMR (125 MHz, DMSO) δ 164.76, 142.86, 141.63, 137.16, 136.38, 136.15, 132.87, 130.45, 130.14, 127.93, 125.57, 133.06, 132.09 (d, J = 4.4 Hz), 128.79, 122.05, 98.59, 60.68, 56.56, 35.45. HRMS (ESI) m/z: [M + H]⁺ calculated for C₁₈H₁₄ClFN₅O₃S 376.0874, found 376.0881.

2.2.5.8. **4-Chloro-3-sulfamoyl-N**-((1-(3,4,5-trimethoxyphenyl)-1H,1,2,3-triazol-4-ylmethyl)-benzamide (6j).** White solid; yield: 95%, m.p: 249–251 °C; ¹H NMR (500 MHz, DMSO) δ 9.41 (t, J = 5.5 Hz, 1H), 8.73 (s, 1H), 8.51 (d, J = 2.0 Hz, 1H), 8.10 (d, J = 8.3, 2.1 Hz, 1H), 7.77 (d, J = 8.3 Hz, 1H), 7.69 (s, 2H), 7.20 (s, 2H), 4.63 (d, J = 5.5 Hz, 2H), 3.87 (s, 6H), 3.71 (s, 3H). 13C NMR (125 MHz, DMSO) δ 164.72, 154.01, 146.06, 141.66, 137.89, 133.80, 133.57, 133.06, 132.09 (d, J = 4.4 Hz), 128.79, 122.05, 98.59, 60.68, 56.56, 35.45. HRMS (ESI) m/z: [M + H]⁺ calculated for C₁₆H₁₆ClFN₅O₃S 428.0896, found 428.0899.

2.2.5.9. **4-Chloro-N**-((1-(4-methoxyphenyl)-1H,1,2,3-triazol-4-ylmethyl)-3-sulfamoylbenzamide (6k).** White solid; yield: 90%, m.p: 172–174 °C; ¹H NMR (500 MHz, DMSO) δ 9.37 (t, J = 5.5 Hz, 1H), 8.59 (s, 1H), 8.51 (d, J = 2.1 Hz, 1H), 8.09 (dd, J = 8.3, 2.2 Hz, 1H), 7.82–7.75 (m, 3H), 7.69 (s, 2H), 7.15–7.08 (m, 2H), 4.61 (d, J = 5.5 Hz, 2H), 3.82 (s, 3H). 13C NMR (125 MHz, DMSO) δ 164.76, 156.69, 146.01, 141.64, 133.69 (d, J = 14.6 Hz), 132.07, 130.62, 128.79, 122.11, 121.71, 115.35, 56.04, 35.50. HRMS (ESI) m/z: [M + H]⁺ calculated for C₁₇H₁₃F₃N₅O₃S 444.0748, found 444.0757.

2.2.6. **4-Chloro-N**-((1-(4-fluorophenyl)-1H,1,2,3-triazol-4-ylmethyl)-3-sulfamoylbenzamide (6l).** White solid; yield: 83%, m.p: 189–190 °C; ¹H NMR (500 MHz, DMSO) δ 9.39 (t, J = 5.6 Hz, 1H), 8.69 (s, 1H), 8.51 (d, J = 2.2 Hz, 1H), 8.10 (dd, J = 8.3, 2.2 Hz, 1H), 7.98–7.91 (m, 2H), 7.78 (d, J = 8.3 Hz, 1H), 7.70 (s, 2H), 7.47–7.39 (m, 2H), 4.62 (d, J = 5.6 Hz, 2H). 13C NMR (125 MHz, DMSO) δ 164.76, 163.03, 146.35, 141.64, 133.76, 133.58, 130.57, 128.78, 122.79 (d, J = 8.8 Hz, 1H), 121.98, 117.25, 117.07, 35.46. HRMS (ESI) m/z: [M + H]⁺ calculated for C₁₇H₁₄F₃CN,O₃S 410.0484, found 410.0492.
7.30 (d, J = 146.70, 133.73 (d, J = 153.90) oro-3-sulfamoylbenzamide (6i). White solid, yield: 62%, m.p: 270°C (125 MHz, DMSO) δ 165.24, 158.65, 146.63, 137.18, 133.22, 131.61, 130.33, 129.00, 127.88, 126.09, 121.66, 120.44, 112.76, 56.97, 35.37. HRMS (ESI) m/z: [M + H]+ calculated for C18H18N5O4S 390.1031, found 390.1037.

2.2.6.7. 4-Fluoro-3-sulfamoyl-N-((1-(p-tolyl)-1H,1,2,3-triazol-4-yl)methyl)benzamide (6r). White solid, yield: 69%, m.p: 249–251°C; 1H NMR (500 MHz, DMSO) δ 9.40 (t, J = 5.6 Hz, 1H), 8.82 (s, 1H), 8.48–8.43 (m, 1H), 8.38 (dd, J = 7.0, 2.2 Hz, 1H), 8.30 (ddd, J = 8.3, 2.0, 0.9 Hz, 1H), 8.19 (ddd, J = 8.5, 4.5, 2.3 Hz, 1H), 7.97–7.92 (m, 1H), 7.79 (ddd, J = 13.4, 5.3 Hz, 1H), 7.77 (s, 2H), 7.57 (dd, J = 18.6, 8.9 Hz, 1H), 4.63 (d, J = 5.6 Hz, 1H); 13C NMR (125 MHz, DMSO) δ 164.67, 146.91, 137.55, 133.79 (d, J = 9.4 Hz, C); 1H NMR (500 MHz, DMSO) δ 164.65, 146.16, 133.80 (d, J = 9.3 Hz, C); 13C NMR (125 MHz, DMSO) δ 165.24, 158.65, 146.63, 137.18, 133.22, 131.61, 130.33, 129.00, 127.88, 126.09, 121.66, 120.44, 112.76, 56.97, 35.37. HRMS (ESI) m/z: [M + H]+ calculated for C17H17FN5O3S 390.1031, found 390.1037.

2.2.6.8. 4-Fluoro-3-sulfamoyl-N-((1-(p-tolyl)-1H,1,2,3-triazol-4-yl)methyl)benzamide (6t). White solid, yield: 62%, m.p: 153–155°C, 1H NMR (500 MHz, DMSO) δ 9.36 (t, J = 5.4 Hz, 1H), 8.74 (s, 1H), 8.37 (dd, J = 6.9, 1.9 Hz, 1H), 8.22 – 8.15 (m, 1H), 7.89 (d, J = 8.8 Hz, 2H), 7.79 (d, J = 8.8 Hz, 2H), 7.76 (s, 2H), 7.56 (t, J = 9.2 Hz, 1H), 4.59 (t, J = 19.7 Hz, 2H); 13C NMR (125 MHz, DMSO) δ 164.65, 146.16, 133.83 (d, J = 9.3 Hz, C); 13C NMR (125 MHz, DMSO) δ 165.24, 158.65, 146.63, 137.18, 133.22, 131.61, 130.33, 129.00, 127.88, 126.09, 121.66, 120.44, 112.76, 56.97, 35.37. HRMS (ESI) m/z: [M + H]+ calculated for C17H17FN5O3S 390.1031, found 390.1037.

2.2.6.9. 4-Fluoro-3-sulfamoyl-N-((1-phenyl-1H,1,2,3-triazol-4-yl)methyl)-4-sulfonylbenzamide (6u). White solid, yield: 58%, m.p: 210–212°C; 1H NMR (500 MHz, DMSO) δ 9.18 (t, J = 5.6 Hz, 1H), 8.68 (s, 1H), 8.34 (d, J = 2.3 Hz, 1H), 8.14 (dd, J = 8.7, 2.3 Hz, 1H), 7.90 (dd, J = 8.5, 1.0 Hz, 2H), 7.58 (dd, J = 7.9 Hz, 2H), 7.48 (t, J = 7.4 Hz, 1H), 7.29 (d, J = 8.8 Hz, 1H), 7.16 (s, 2H), 4.61 (d, J = 5.5 Hz, 2H), 3.98 (s, 3H); 13C NMR (125 MHz, DMSO) δ 165.24, 158.65, 146.63, 137.18, 133.22, 131.61, 130.33, 129.00, 127.88, 126.09, 121.66, 120.44, 112.76, 56.97, 35.37. HRMS (ESI) m/z: [M + H]+ calculated for C17H18N5O4S 390.1031, found 388.1079.

2.2.7. 4-Fluoro-3-sulfamoylbenzamide (6v). White solid, yield: 52%, m.p: 222–224°C; 1H NMR (500 MHz, DMSO) δ 9.18 (t, J = 5.5 Hz, 1H), 8.66 (s, 1H), 8.33 (d, J = 2.1 Hz, 1H), 8.13 (dd, J = 8.7, 2.1 Hz, 1H), 8.01–7.85 (m, 2H), 7.48–7.37 (m, 2H), 7.29 (d, J = 8.7 Hz, 1H), 7.16 (s, 2H), 4.60 (d, J = 5.5 Hz, 2H), 3.96 (s, 3H); 13C NMR (125 MHz, DMSO) δ 165.24, 158.68, 146.70, 133.73 (d, J = 2.8 Hz), 132.22, 131.61, 127.87, 126.07, 122.79 (d, J = 8.7 Hz), 121.92, 117.23, 110.05, 112.77, 56.97, 35.34. HRMS (ESI) m/z: [M + H]+ calculated for C17H18N5O4S 406.0980, found 406.0987.
for the formation of the E-I complex. Triplicate experiments were done for each inhibitor concentration, and the values reported throughout the paper are the mean of such results. The inhibition constants were obtained by non-linear least-squares methods using the Cheng-Prusoff equation, as reported earlier, and represent the mean from at least three different determinations. All CA isoforms used here were recombinant proteins obtained as reported earlier by our group.

3. Result and discussion

3.1. Chemistry

The present work was aimed at designing molecules, which target specifically CA I, II, IX, XII based on previously reported data. The synthesis of the designed 3-functionalised benzenesulfonamide linked triazoles (6a–z) was performed according to the general synthetic route as illustrated in Scheme 1. Commercially available 4-substituted benzoic acid (1a–d) were treated with chlorosulfonic acid at 110°C to afford the 3-(chlorosulfonyl)benzoic acids (2a–d), which were treated with ammonium hydroxide solution at 0°C to get the corresponding 3-sulfamoylbenzoic acids (3a–d). In the next step, the 3-sulfamoyl benzoic acids (3a–d) were coupled with propargyl amine in presence of EDCI and HOBt in the presence of dry DMF to afford the corresponding sulfamoylbenzamide alkyne intermediates (4a–d). In the final step the sulfamoylbenzamide alkyne intermediates (4a–d) were subjected to click chemistry reaction with substituted azide intermediates in the presence of CuSO4, sodium ascorbate in t-BuOH and H2O (1:1) solvent system to afford the desired target derivatives (6a–z) in good to excellent yields. The phenyl azide intermediates were previously prepared from the corresponding anilines through diazotization, using concentrated HCl, NaNO2, and NaN3.

3.2. CA inhibition

The newly synthesised compounds 3-sulfamoylbenzamide linked 1,2,3-triazoles (Figure 4) (6a–z) as well as the intermediates (3a–d) and (4a–d) (listed in Table 1) were screened for their CA inhibitory activities against four physiologically significant isoforms, the cytosolic hCA I (associated with edema) and II (associated with glaucoma) as well as the membrane bound hCA IV (associated with glaucoma and retinitis pigmentosa) and transmembrane hCA IX (associated with tumors) by means of the stopped flow carbon dioxide assays in comparison to AAZ as standard CAI (Table 2). The results of the CA inhibitory assay are discussed below:

- The cytosolic isoform hCA I was strongly inhibited by all the synthesised compounds with Ki ranging between 50.8 nM and 9.755 μM range. Among all the synthesised compounds only three compounds 6k, 6l, and 6o were found to be more potent hCA I inhibitors with Ki 50.8–86.7 nM compared to the standard AAZ (Ki = 250 nM), in-fact the compound 6k is almost four fold more active than that of AAZ. The remaining compounds including the intermediates 3a–d and 4a–d were showing the weakest inhibition (Ki ranging between 312.1 and 9755 nM). It was also observed that the 4-chloro and 4-fluoro substituted 3-benzenesulfonamide derivatives were exhibiting the better inhibition as compared to the methoxy derivatives.
- The cytosolic isoform hCA II was strongly inhibited by some of the synthesised compounds with Ki ranging between 6.5 nM and 0.760 μM. Compounds 6l and 6m showed excellent inhibition with Ki 6.5 and 7.8 nM, respectively, compared to the standard AAZ (Ki = 12.1 nM). The compounds 6a–6f, 6u–6z, 3a, 3b, 4d were found to be the weakest inhibitors with Ki ranging between 36.6 and 150 μM.

Figure 4. General structure of the synthesised molecules.

Scheme 1. Synthesis of 1,2,3-triazole 3-sulfamoylbenzamide hybrids (6a–z). Reagent and reaction conditions: (i) HSO3Cl, 110°C, 6–8 h, 70–80%; (ii) NH4OH sol., 0°C, 2 h; (iii) Propargyl amine, EDCI, HOBt, anhydrous DMF, rt, 16–24 h, 74–85%; (iv) substituted phenyl azides, CuSO4, Sodium ascorbate, t-BuOH: H2O (1:1), 40°C, 4–6 h, 52–98%.
1.382–9.647 μM and the remaining were found weak inhibitors with $K_i < 800$ nM. The results show that the 4-chloro substituted 3-benzenesulfonamide derivatives were more potent inhibitors of hCA II (Table 2).

The membrane bound isoform hCA IV was weakly inhibited by all of the synthesised molecules with $K_i$ ranging between 78.8 nM and 2.470 μM except one compound 6l, which was found to be most potent with $K_i$ 65.3 nM compared to the

Table 1. List of synthesised compounds 3a-d, 4a-d and 6a-z.

| No. | Structure | No. | Structure | No. | Structure |
|-----|-----------|-----|-----------|-----|-----------|
| 3a  | ![Structure](image_url) | 6e  | ![Structure](image_url) | 6p  | ![Structure](image_url) |
| 3b  | ![Structure](image_url) | 6f  | ![Structure](image_url) | 6q  | ![Structure](image_url) |
| 3c  | ![Structure](image_url) | 6g  | ![Structure](image_url) | 6r  | ![Structure](image_url) |
| 3d  | ![Structure](image_url) | 6h  | ![Structure](image_url) | 6s  | ![Structure](image_url) |
| 4a  | ![Structure](image_url) | 6i  | ![Structure](image_url) | 6t  | ![Structure](image_url) |
| 4b  | ![Structure](image_url) | 6j  | ![Structure](image_url) | 6u  | ![Structure](image_url) |
| 4c  | ![Structure](image_url) | 6k  | ![Structure](image_url) | 6v  | ![Structure](image_url) |
| 4d  | ![Structure](image_url) | 6l  | ![Structure](image_url) | 6w  | ![Structure](image_url) |
| 6a  | ![Structure](image_url) | 6m  | ![Structure](image_url) | 6x  | ![Structure](image_url) |
| 6b  | ![Structure](image_url) | 6n  | ![Structure](image_url) | 6y  | ![Structure](image_url) |
| 6c  | ![Structure](image_url) | 6o  | ![Structure](image_url) | 6z  | ![Structure](image_url) |
| 6d  | ![Structure](image_url) | 6p  | ![Structure](image_url) | 6q  | ![Structure](image_url) |
the phenyl ring of 1,2,3-triazole moiety led to a dramatic change in their inhibition against hCA II and IV with 4b, poor inhibition, except 3a showing strong inhibition against all four isoforms hCA I, II, IV, and IX, respectively. The newly synthesised compounds showed weak inhibitory potency against hCA IX whereas compound 6i showed excellent inhibition against hCA I, II, and IV with $K_I$ values of 50.8, 6.5, and 65.3 nM with compared to AAZ as standard drug with $K_I = 250, 12.1, 74$ nM, respectively. Furthermore, the results of hCA inhibition clearly indicate that few of the compounds containing electron withdrawing substitution on both the phenyl rings (6k, 6i, 6m, and 6o) showed strong inhibitory activity against three isoforms hCA I, II, IV. Hence, it may be concluded that the newly synthesised 1,2,3-triazole 3-sulfamoylbenzamide derivatives exhibit potent hCA inhibitory properties.

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### Disclosure statement

No potential conflict of interest was reported by the authors.

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### Table 2. Inhibition of hCA isos I, II, IV and IX with compounds 6a–z, 3a–d, 4a–d, and AAZ as standard inhibitor

| Cmp | hCA I | hCA II | hCA IV | hCA IX |
|-----|-------|--------|--------|--------|
| 6a  | 752.7 | 4491   | 9421.0 | 7976   |
| 6b  | 854.7 | 8967   | 9142   | 4904   |
| 6c  | 951.9 | 9271   | 9272   | 6924   |
| 6d  | 866.9 | 2973   | 8835   | 7423   |
| 6e  | 430.8 | 2357   | 9298   | 3188   |
| 6f  | 887.9 | 9012   | 6673   | 6746   |
| 6g  | 447.5 | 366.4  | 8584   | 6128   |
| 6h  | 693.0 | 515.6  | 1758   | 225.8  |
| 6i  | 312.1 | 71.2   | 170.4  | 84.4   |
| 6j  | 478.6 | 51.4   | 2282   | 211.9  |
| 6k  | 86.7  | 56.5   | 80.0   | 41.6   |
| 6l  | 50.8  | 6.5    | 65.3   | 64.3   |
| 6m  | 811.2 | 7.8    | 89.9   | 34.6   |
| 6n  | 890.2 | 49.7   | 240.0  | 84.5   |
| 6o  | 69.2  | 73.5   | 174.8  | 63.1   |
| 6p  | 368.9 | 91.1   | 91.8   | 234.6  |
| 6q  | 425.9 | 328.7  | 5314   | 30.8   |
| 6r  | 836.2 | 260.3  | 215.3  | 67.5   |
| 6s  | 696.0 | 402.2  | 3397   | 67.9   |
| 6t  | 696.0 | 372.1  | 514.6  | 71.5   |
| 6u  | 680.8 | 2898   | 9349   | 702.9  |
| 6v  | 571.6 | 9647   | 5352   | 6821   |
| 6w  | 966.8 | 4158   | 7621   | 525.6  |
| 6x  | 7562 | 6390   | 9337   | 93.3   |
| 6y  | 555.1 | 2329   | 9082   | 187.3  |
| 6z  | 952.0 | 5367   | 8935   | 768.1  |
| 3a  | 8769 | 8656   | 8914   | 9415   |
| 3b  | 8847 | 1382   | 2470   | 8976   |
| 3c  | 5750 | 760.0  | 957.5  | 9570   |
| 3d  | 9755 | 9183   | 8777   | 9674   |
| 4a  | 9280 | 513.9  | 8456   | 9362   |
| 4b  | 4931 | 73.5   | 78.8   | 446.9  |
| 4c  | 747.2 | 164.5  | 429.1  | 815.9  |
| 4d  | 9501 | 5531   | 6843   | 9221   |
| AAZ | 250  | 12.1   | 74     | 25.8   |

$^*$Mean from three different assays, by a stopped flow technique (errors were in the range of 5–10% of the reported values).

Supprisingly, the structure activity relationship (SAR) studies revealed that the derivatives containing –Cl, –F substitution on the C4 position of 3-sulfamoylbenzamide moiety (6i–6t) were exhibiting strong inhibition against all four isoforms hCA I, II, IV, and IX, whereas all the intermediates i.e. the benzoic acid derivatives 3a–3d and N-proaprgyl benzamide 4a–4d derivatives showed very poor inhibition, except 4b, which showed moderate inhibition against hCA II and IV with $K_I$ 0.073 and 0.078 μM, respectively. The compound 6l showed excellent inhibition against hCA I, II, IV as compared to AAZ the standard drug used in these assays. It was also observed from SAR studies that the substitution on the phenyl ring of 1,2,3-triazole moiety led to a dramatic change of inhibition potency. The 4-substituted –F, –CF3, –OMe (6k, 6l, 6m, and 6o) showed strong inhibition against hCA I, II, IV. Hence,
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