Synthesis and biological evaluation of benzenesulphonamide-bearing 1,4,5-trisubstituted-1,2,3-triazoles possessing human carbonic anhydrase I, II, IV, and IX inhibitory activity

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Abstract

A library of benzenesulphonamides incorporating 1,2,3-triazole rings functionalised with ester, carboxylic acid, carboxamidine, carboxyxydrazide, and hydroxymethyl moieties were synthesised. The carbonic anhydrase (CAs, EC 4.2.1.1) inhibitory activity of the new compounds was assessed against four human (h) isoforms, hCA I, hCA II, hCA IV, and hCA IX. Among them, hCA II and IV are anti-glaucoma drug targets, being involved in aqueous humour secretion within the eye. hCA I was inhibited with Ki's ranging between 8.3 nM and 0.8737 μM. hCA II, the physiologically dominant cytosolic isoform, was excellently inhibited by these compounds, with Ki's in the range of 1.6–9.4 nM, whereas hCA IV was effectively inhibited by most of them, with Ki's in the range of 1.4–55.3 nM. Thirteen of the twenty sulphonamides were found to be excellent inhibitors of tumour associated hCA IX with Ki's < 9.5 nM. Many of the new compounds reported here showed low nM inhibitory action against hCA II, IV, and IX, isoforms involved in glaucoma and some tumours, making them interesting candidates for further medicinal chemistry/pharmacologic studies.

Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) belong to family of zinc metalloenzymes found in variety of organisms, including higher vertebrates and humans. As all the seven families of CAs known to date (α, β, γ, δ, ζ, η, and ζ-class) are involved in the reversible hydration/dehydration of carbon dioxide and bicarbonate ions, therefore, play vital roles in various important physiological processes, such as respiration, electrolyte secretion in variety of tissues/organs, biosynthetic reactions (i.e. lipogenesis, glucogenesis, and ureagenesis), bone resorption, calcification, etc. However, several studies demonstrated that abnormal levels or activities of these enzymes have been associated with various human diseases. Out of the sixteen isoforms of human associated α-class of CAs, some isoforms are involved in pertinent pathologies, such as glaucoma, epilepsy, obesity, altitude sickness, retinitis pigmentosis, cancer, etc. Therefore, the carbonic anhydrase inhibitors (CAs) have applications as therapeutic agents, such as antiglaucoma, antiobesity, antiadrenergic, antiinflammatory, anti-angiogenic, antitumor, and anti-infective agents. However, designing and synthesising isoform-selective inhibitors are a challenging task for obtaining a drug with minimum side effect.

Primary sulphonamide bearing heterocyclic compounds form a part of potent CAs in which binding group binds to the Zn(II) ion as anion in a tetrahedral geometry. A large number of drugs belonging to this class, like acetazolamide (AZA), methazolamide (MZA), ethoxzolamide (EZA), dorzolamide (DZA), etc., are in clinical use from past many years targeting different therapeutic areas.

Recently our research group has reported some fused 1,2,4-triazoles and 4-functionalised pyrazoles bearing benzenesulphonamide as selective inhibitors of CA IX and XII. Further, 1,2,3-triazole ring containing compounds are gaining interest in diverse therapeutic areas like antiproliferative, antimicrobial, antitubercular, anticancer, antifungal, antibacterial, anti-inflammatory, antiobesity, antiviral, etc., as well as in several DNA-alkylating, crosslinking agents, and β-lactamase inhibitors. Also some 1,2,3-triazole ring containing selective CAs have been reported. Motivated by these findings and continuing our interest in the design of various classes of heterocyclic based compounds of potential medicinal interest, we turned our attention towards the synthesis of a small library of novel 4-functionalised 1-aryl-5-alkyl/aryl-1,2,3-triazoles bearing a primary sulphonamide group on the phenyl ring at N-1 position of 1,2,3-triazole scaffold with different functionalities at C-4, such as ester, carboxylic acid, carboxamidine, hydrazinocarbonyl, and hydroxymethyl (Figure 1) in order to investigate their carbonic anhydrases inhibition against isoforms hCA I, II, IV, and IX.

Materials and methods

General

All the commercially available chemicals were used without further purification. All the solvents were dried and/or purified according to standard procedures prior to use. All the reactions were...
DMSO were heated at 70 °C, N–(DMSO-d₆) as solvent, and tetramethylsilane (TMS) as internal standard at room temperature. Chemical shifts are reported as δ values in parts per million (ppm) downfield from TMS. High resolution mass spectra were obtained from a MicroMass ESI-TOF MS spectrometer. Multiplicities are described as singlet (s), doublet (d), doublet of doublet (dd), doublet of a doublet of a doublet (ddd), doublet of triplet (dt), triplet (t), quartet (q), multiplet (m), exchangeable proton (ex) for NMR assignments and strong (s), medium (m), broad (br) for IR assignments. The coupling constants are expressed in hertz (Hz).

**Synthesis of ethyl 1-[4-(aminosulfonyl)phenyl]-5-(alkyl/aryl)-1H-1,2,3-triazole-4-carboxylate (4a–4d)**

General procedure: A mixture of appropriate β-diketoester **11a–11d** (2.00 mmol) and piperidine (5 mol%) dissolved in 3 ml DMSO were heated at 70 °C in silicon oil bath for 5 min followed by addition of 4-azidobenzenesulphonamide (2.02 mmol). After addition, reaction mixture was allowed to stir at 70 °C for 4–6 h. Reaction was monitored through thin-layer chromatography. After completion, reaction mixture was poured into water after cooling to afford required product **4a–4d**. The crude product thus obtained was recrystallised with ethanol.

**Ethyl 1-[4-(aminosulfonyl)phenyl]-5-methyl-1H-1,2,3-triazole-4-carboxylate (4a)**

Yield 91%; white solid; mp: 212 °C; IR(KBr) (ν, cm⁻¹): 3302, 3070 (m, N–H stretch), 2924 (m, –CH₃ stretch), 1728 (s, C=O stretch), 1335, 1165 (s, SO₂stretch); ¹H NMR (400 MHz, DMSO-d₆) δ (ppm): 8.08 (dd, J = 8.8 Hz, J = 2.0 Hz, 2H, Ar), 7.88 (dd, J = 8.8 Hz, J = 2.0 Hz, 2H, Ar), 7.61 (s, 2H, SO₂NH₂), 4.37 (q, J = 7.2 Hz, 2H, CH₂), 2.58 (s, 3H, –CH₃), 1.35 (t, J = 7.2 Hz, 3H, CH₃); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 160.92, 145.31, 139.48, 137.47, 136.08, 136.14, 127.15, 125.98, 60.50, 14.12, 9.73; HRMS (ESI-MS) m/z 333.0640 (M + Na)⁺, C₁₂H₁₄N₂O₃SNa⁺, calcd 333.0634.

**Ethyl 1-[4-(aminosulfonyl)phenyl]-5-phenyl-1H-1,2,3-triazole-4-carboxylate (4b)**

Yield 74%; pale yellow solid; mp: 176 °C; IR(KBr) (ν, cm⁻¹): 3371, 3263, 3061 (m, N–H stretch), 1704 (s, C=O stretch), 1342, 1335, 1159 (s, SO₂ stretch); ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.96 (dd, J = 7.2 Hz, J = 1.2 Hz, 2H, Ar), 7.56–7.48 (m, 5H, Ar), 7.32 (dd, J = 8.4 Hz, J = 1.2 Hz, 2H, Ar), 7.06 (s, 2H, SO₂NH₂), 4.33 (q, J = 7.2 Hz, 2H, CH₂), 1.28 (t, J = 7.2 Hz, 3H, CH₃); ¹³C NMR (100 MHz, CDCl₃) δ (ppm): 165.33, 149.56, 146.45, 142.84, 141.97, 135.02, 134.95, 133.38, 132.17, 130.08, 130.05, 65.94, 18.81; HRMS (ESI-MS) m/z 395.0785 (M + Na)⁺, C₁₇H₁₄N₄O₃SNa⁺, calcd 395.0789.

**Ethyl 1-[4-(aminosulfonyl)phenyl]-5-(4-methoxyphenyl)-1H-1,2,3-triazole-4-carboxylate (4c)**

Yield 74%; pale yellow solid; mp: 184 °C; IR(KBr) (ν, cm⁻¹): 3302, 3070 (m, N–H stretch), 1728 (s, C=O stretch), 1342, 1157 (s, SO₂ stretch); ¹H NMR (400 MHz, DMSO-d₆) δ (ppm): 7.93 (dd, J = 6.8 Hz, J = 2.0 Hz, 2H, Ar), 7.59 (d, J = 8.8 Hz, 2H, Ar), 7.54 (s, 2H, SO₂NH₂), 7.32 (d, J = 8.8 Hz, 2H, Ar), 6.97 (d, J = 6.8 Hz, J = 2.0 Hz, 2H, Ar), 4.23 (q, J = 7.2 Hz, 2H, CH₂), 3.77 (s, 3H, –CH₃), 1.18 (t, J = 7.2 Hz, 3H, CH₃); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 160.31, 149.56, 146.45, 142.84, 141.97, 135.02, 134.95, 133.38, 132.17, 130.08, 130.05, 65.94, 18.81; HRMS (ESI-MS) m/z 395.0785 (M + Na)⁺, C₁₇H₁₄N₄O₃SNa⁺, calcd 395.0789.

**Ethyl 1-[4-(aminosulfonyl)phenyl]-5-(2-naphthyl)-1H-1,2,3-triazole-4-carboxylate (4d)**

Yield 92%; dirty white solid; mp: 180 °C; IR(KBr) (ν, cm⁻¹): 3325, 3240 (m, N–H stretch), 1728 (s, C=O stretch), 1335, 1165 (s, SO₂ stretch), 7.96 (dd, J = 8.8 Hz, J = 2.0 Hz, 2H, Ar), 7.61 (s, 2H, SO₂NH₂), 4.37 (q, J = 7.2 Hz, 2H, CH₂), 2.58 (s, 3H, –CH₃), 1.35 (t, J = 7.2 Hz, 3H, CH₃); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 160.92, 145.31, 139.48, 137.47, 136.08, 127.15, 125.98, 60.50, 14.12, 9.73; HRMS (ESI-MS) m/z 333.0640 (M + Na)⁺, C₁₂H₁₄N₂O₃SNa⁺, calcd 333.0634.
Synthesis of 1-[4-(aminosulfonyl)phenyl]-5-alkyl/aryl-1,2,3-triazole-4-carboxylic acid (5a–5d)

General procedure: An appropriate 1,2,3-triazolic ester 4a–4d was dissolved in 20%aq NaOH solution (5 ml) and refluxed for 4 h. Then cooled the solution to room temperature, added ice to it and neutralised with concd HCl which resulted into the precipitation of a white solid. The solid was filtered off, washed with water, dried and recrystallised from appropriate solvent which afforded the pure products 5a–5d.

1-[4-(Aminosulfonyl)phenyl]-5-methyl-1,2,3-triazole-4-carboxylic acid (5a)

Yield 94%; white solid; mp: 198 °C; IR(KBr) (ν, cm⁻¹): 3348, 3225 (m, N–H stretch), 3078 (br, O–H stretch), 2905 (m, –CH₃ stretch), 1667 (s, C=O stretch), 1334, 1150 (s, SO₂ stretch); ¹H NMR (400 MHz, DMSO-d₆) δ (ppm): 10.40 (s, 1H, C=O), 7.59–7.60 (m, 5H, Ar), 7.49 (m, ex, 3H, OH/NH, Ar), 7.37 (m, 5H, Ar), 7.29 (d, J = 7.3 Hz, 2H Ar), 7.13 (d, J = 2.0 Hz, 2H Ar), 7.09 (d, J = 8.4 Hz, 2H Ar), 7.05 (d, J = 8.4 Hz, 2H Ar), 6.95 (d, J = 8.8 Hz, 2H Ar), 3.77 (s, 3H, –CH₃); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 161.69, 160.16, 144.85, 140.75, 137.98, 137.01, 131.85, 128.67, 126.32, 117.31, 113.72, 55.18; HRMS (ESI-MS) m/z 397.0577 (M + Na)⁺, C₁₁H₁₂N₂O₄SNa⁺, calcd 397.0582.

1-[4-(Aminosulfonyl)phenyl]-5-phenyl-1,2,3-triazole-4-carboxylic acid (5b)

Yield 85%; white solid; mp: 156 °C; IR(KBr) (ν, cm⁻¹): 3379, 3263 (m, N–H stretch), 3063 (br, O–H stretch), 1713 (s, C=O stretch), 1342, 1165 (s, SO₂ stretch); ¹H NMR (400 MHz, DMSO-d₆) δ (ppm): 12.66 (s, br, 1H, –COOH), 7.90 (dd, J = 6.8 Hz, J = 1.6 Hz, 2H, Ar), 7.59–7.57 (m, 4H, Ar, SO₂NH₂), 7.45–7.40 (m, 5H, Ar); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 162.12, 145.24, 139.22, 136.83, 127.16, 125.94, 9.74; HRMS (ESI-MS) m/z 305.0325 (M + Na)⁺, C₁₀H₁₀N₄O₄SNa⁺, calcd 305.0321.

1-[4-(Aminosulfonyl)phenyl]-5-(4-methoxyphenyl)-1,2,3-triazole-4-carboxylic acid (5c)

Yield 98%; dirty white solid; mp: 148–150 °C; IR(KBr) (ν, cm⁻¹): 3333, 3242 (m, N–H stretch), 3055 (br, O–H stretch), 2905 (m, –CH₃ stretch), 1705 (s, C=O stretch), 1335, 1165 (s, SO₂ stretch); ¹H NMR (400 MHz, DMSO-d₆) δ (ppm): 13.02 (s, br, 1H, –COOH), 7.90 (dd, J = 6.8 Hz, J = 1.6 Hz, 2H, Ar), 7.56 (d, J = 8.8 Hz, 2H Ar), 7.52 (s, 2H, SO₂NH₂), 7.30 (d, J = 8.8 Hz, 2H Ar), 6.95 (d, J = 8.8 Hz, 2H Ar), 3.77 (s, 3H, –CH₃); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 161.69, 160.16, 144.85, 140.75, 137.98, 137.01, 131.85, 128.67, 126.32, 117.31, 113.72, 55.18; HRMS (ESI-MS) m/z 397.0577 (M + Na)⁺, C₁₁H₁₂N₂O₄SNa⁺, calcd 397.0582.

1-[4-(Aminosulfonyl)phenyl]-5-(naphthyl)-1,2,3-triazole-4-carboxylic acid (5d)

Yield 93%; pale yellow solid; mp: 198–200 °C; IR(KBr) (ν, cm⁻¹): 3356, 3256 (m, N–H stretch), 3078 (br, O–H stretch), 1690 (s, C=O stretch), 1335, 1165 (s, SO₂ stretch); ¹H NMR (400 MHz, DMSO-d₆) δ (ppm): 12.98 (s, br, 1H, COOH), 8.04 (d, J = 8.4 Hz, 1H, Ar), 7.97 (d, J = 8.0 Hz, 1H, Ar), 7.76 (dd, J = 8.8 Hz, J = 2.0 Hz, 2H Ar), 7.66 (dd, J = 7.6 Hz, J = 1.2 Hz, 1H, Ar), 7.58–7.39 (m, 8H, Ar, SO₂NH₂); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 161.28, 144.94, 139.32, 138.88, 137.83, 132.67, 133.06, 130.29, 129.48, 128.42, 127.30, 126.66, 126.40, 125.61, 125.11, 124.32, 123.39; HRMS (ESI-MS) m/z 395.0800 (M + H)⁺, C₁₁H₁₂N₂O₄SH⁺, calcd 395.0814.
Yield 92%; white solid; mp: 216–218 °C; IR(KBr) (ν, cm⁻¹): 3325, 3178, 3078, 3016 (m, N–H stretch), 1670 (s, C=O stretch), 1342, 1165 (s, SO₂ stretch); ¹H NMR (400 MHz, DMSO-d₆) δ (ppm): 9.79 (s, ex, 1H, NH), 8.06 (dd, J = 6.8 Hz, J = 2.0 Hz, 2H, Ar), 7.88 (dd, J = 6.8 Hz, J = 2.0 Hz, 2H, Ar), 7.57 (s, 2H, SO₂NH₂), 4.50 (s, ex, 2H, NH₂), 2.58 (s, 3H, –CH₃); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 161.10, 144.86, 140.91, 137.94, 137.25, 132.71, 131.17, 130.07, 129.54, 128.36, 127.09, 126.64, 126.30, 125.55, 125.09, 124.45, 123.58; HRMS (ESI-MS) m/z 416.0805 (M + Na)⁺, calc 416.0794.

Synthesis of 4-[4-(hydrazinocarbonyl)-5-alkyl/aryl-1H-1,2,3-triazol-1-yl]benzenesulphonamide (7a)

General procedure: A solution of appropriate 1,2,3-triazolic ester 4a–4d (1.93 mmol) and hydrazine hydrate (5.81 mmol) in ethanol (15 ml) was refluxed for 10–12 h. The reaction was monitored through thin-layer chromatography. After completion, reaction mixture was concentrated and allowed to cool to room temperature. Solid thus obtained was filtered and crystallised from EtOH/THF (1:1) to afford the desired compounds 7a–7d in good yields.

Yield 92%; white solid; mp: 216–218 °C; IR(KBr) (ν, cm⁻¹): 3325, 3178, 3078, 3016 (m, N–H stretch), 1670 (s, C=O stretch), 1342, 1165 (s, SO₂ stretch); ¹H NMR (400 MHz, DMSO-d₆) δ (ppm): 9.88 (s, ex, 1H, NH), 7.89 (dd, J = 6.8 Hz, J = 2.0 Hz, 2H, Ar), 7.58 (dd, J = 6.8 Hz, J = 2.0 Hz, 2H, Ar), 7.54 (s, 2H, SO₂NH₂), 7.42–7.38 (m, 5H, Ar), 4.49 (s, ex, 2H, NH₂); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 161.67, 145.35, 139.22, 138.69, 138.45, 130.93, 130.10, 128.64, 127.31, 126.82, 125.99; HRMS (ESI-MS) m/z 381.0640 (M + Na)⁺, C₁₉H₁₇NO₄SNa⁺, calc 381.0745.

Yield 70%; white solid; mp: 206–210 °C; IR(KBr) (ν, cm⁻¹): 3402, 3317, 3186, 3094 (m, N–H stretch), 1668 (s, C=O stretch), 1335, 1157 (s, SO₂ stretch); ¹H NMR (400 MHz, DMSO-d₆) δ (ppm): 9.88 (s, ex, 1H, NH), 7.89 (dd, J = 6.8 Hz, J = 2.0 Hz, 2H, Ar), 7.58 (dd, J = 6.8 Hz, J = 2.0 Hz, 2H, Ar), 7.74 (m, 2H, CH₂), 5.24 (t, ex, 1H, OH), 4.65 (d, J = 5.6 Hz, 2H, Ar), 3.85 (s, 3H, CH₃); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 159.67, 144.86, 140.91, 137.94, 137.25, 132.71, 131.17, 130.07, 129.54, 128.36, 127.09, 126.64, 126.30, 125.55, 125.09, 124.45, 123.58; HRMS (ESI-MS) m/z 431.0894 (M + Na)⁺, C₁₉H₁₇NO₄SNa⁺, calc 431.0903.

Yield 55%; pale yellow solid; mp: 174–176 °C; IR(KBr) (ν, cm⁻¹): 3225 (br, O–H stretch), 3225, 3171, 3070 (m, N–H stretch), 1342, 1157 (s, SO₂ stretch); ¹H NMR (400 MHz, DMSO-d₆) δ (ppm): 7.97 (d, J = 8.8 Hz, 2H, Ar), 7.48–7.44 (m, 5H, Ar), 4.65 (d, J = 5.6 Hz, 2H, Ar), 2.82 (s, 3H, CH₃); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 165.14, 150.34, 149.16, 143.87, 140.36, 135.92, 132.09, 129.70, 122.94, 119.31, 60.14, 59.41.

Yield 72%; white solid; mp: 156–160 °C; IR(KBr) (ν, cm⁻¹): 3279, 3063 (m, N–H stretch), 1659 (s, C=O stretch), 1327, 1165 (s, SO₂ stretch); ¹H NMR (400 MHz, DMSO-d₆) δ (ppm): 9.53 (s, ex, 1H, NH), 8.04 (d, J = 8.0 Hz, 1H, Ar), 7.98 (d, J = 8.0 Hz, 1H, Ar), 7.76 (d, J = 8.8 Hz, 2H, Ar), 7.63 (d, J = 7.2 Hz, 1H, Ar), 7.58–7.44 (m, 7H, Ar), SO₂NH₂); 7.35 (d, J = 8.4 Hz, 1H, Ar), 4.45 (s, ex, 2H, NH₂); ¹³C NMR (100 MHz, DMSO-d₆) δ (ppm): 159.26, 145.30, 140.74, 137.82, 133.31, 133.22, 131.73, 130.67, 130.19, 128.90, 127.66, 127.20, 126.84, 126.01, 125.61, 124.94, 123.88; HRMS (ESI-MS) m/z 431.0894 (M + Na)⁺, C₁₉H₁₇NO₄SNa⁺, calc 431.0903.
Table 1. Inhibitory potency data for compounds 4a–4d, 5a–5d, 6a–6d, 7a–7d, and 8a–8d against isozymes hCA I, hCA II, hCA IV, and hCA IX.

| Compound | R          | hCA I | hCA II | hCA IV | hCA IX |
|----------|------------|-------|--------|--------|--------|
| 4a       | −CH₃       | 9.1   | 3.2    | 3.5    | 6.6    |
| 4b       | −C₆H₅      | 245.5 | 6      | 5.1    | 9.5    |
| 4c       | 4-C₆H₄-OCH₃| 63    | 6.1    | 4.5    | 40     |
| 4d       | 2-Naphthyl | 68.2  | 5.8    | 5      | 7.1    |
| 5a       | −CH₃       | 9.6   | 1.6    | 269.4  | 8.5    |
| 5b       | −C₆H₅      | 645.7 | 97.9   | 55.3   | 45.9   |
| 5c       | 4-C₆H₄-OCH₃| 489.1 | 9      | 2.4    | 42.3   |
| 5d       | 2-Naphthyl | 56.2  | 8.9    | 4.1    | 4.2    |
| 6a       | −CH₃       | 8.3   | 1.9    | 211.9  | 9.5    |
| 6b       | −C₆H₅      | 69.1  | 9.1    | 1.4    | 28.3   |
| 6c       | 4-C₆H₄-OCH₃| 873.7 | 6.2    | 1.7    | 6.8    |
| 6d       | 2-Naphthyl | 771.7 | 6      | 4      | 6.7    |
| 7a       | −CH₃       | 15.1  | 7.6    | 227.8  | 9.3    |
| 7b       | −C₆H₅      | 73.7  | 38     | 22.7   | 26.1   |
| 7c       | 4-C₆H₄-OCH₃| 91.2  | 5.6    | 4      | 8.1    |
| 7d       | 2-Naphthyl | 82.5  | 1.6    | 2      | 7.2    |
| 8a       | −CH₃       | 354.2 | 58.9   | 359.6  | 30.6   |
| 8b       | −C₆H₅      | 78.9  | 9.4    | 8.4    | 58.7   |
| 8c       | 4-C₆H₄-OCH₃| 811.8 | 4      | 1.9    | 5.8    |
| 8d       | 2-Naphthyl | 213.5 | 8.1    | 1.9    | 7.8    |
| AZA      |            | 250   | 12.1   | 74     | 25.8   |

AZA: acetazolamide (reference compound).

Table 1. Inhibitory potency data for compounds 4a–4d, 5a–5d, 6a–6d, 7a–7d, and 8a–8d against isozymes hCA I, hCA II, hCA IV, and hCA IX.

| Compound | R          | hCA I | hCA II | hCA IV | hCA IX |
|----------|------------|-------|--------|--------|--------|
| 4a       | −CH₃       | 9.1   | 3.2    | 3.5    | 6.6    |
| 4b       | −C₆H₅      | 245.5 | 6      | 5.1    | 9.5    |
| 4c       | 4-C₆H₄-OCH₃| 63    | 6.1    | 4.5    | 40     |
| 4d       | 2-Naphthyl | 68.2  | 5.8    | 5      | 7.1    |
| 5a       | −CH₃       | 9.6   | 1.6    | 269.4  | 8.5    |
| 5b       | −C₆H₅      | 645.7 | 97.9   | 55.3   | 45.9   |
| 5c       | 4-C₆H₄-OCH₃| 489.1 | 9      | 2.4    | 42.3   |
| 5d       | 2-Naphthyl | 56.2  | 8.9    | 4.1    | 4.2    |
| 6a       | −CH₃       | 8.3   | 1.9    | 211.9  | 9.5    |
| 6b       | −C₆H₅      | 69.1  | 9.1    | 1.4    | 28.3   |
| 6c       | 4-C₆H₄-OCH₃| 873.7 | 6.2    | 1.7    | 6.8    |
| 6d       | 2-Naphthyl | 771.7 | 6      | 4      | 6.7    |
| 7a       | −CH₃       | 15.1  | 7.6    | 227.8  | 9.3    |
| 7b       | −C₆H₅      | 73.7  | 38     | 22.7   | 26.1   |
| 7c       | 4-C₆H₄-OCH₃| 91.2  | 5.6    | 4      | 8.1    |
| 7d       | 2-Naphthyl | 82.5  | 1.6    | 2      | 7.2    |
| 8a       | −CH₃       | 354.2 | 58.9   | 359.6  | 30.6   |
| 8b       | −C₆H₅      | 78.9  | 9.4    | 8.4    | 58.7   |
| 8c       | 4-C₆H₄-OCH₃| 811.8 | 4      | 1.9    | 5.8    |
| 8d       | 2-Naphthyl | 213.5 | 8.1    | 1.9    | 7.8    |
| AZA      |            | 250   | 12.1   | 74     | 25.8   |

AZA: acetazolamide (reference compound).

Results and discussion

Chemistry

The synthetic route adopted for the synthesis of 4-functionalised 1,2,3-triazole compounds (4a–4d, 5a–5d, 6a–6d, 7a–7d, and 8a–8d) is outlined in Scheme 1. 1,2,3-Triazole-4-carboxylates 4a–4d, the supreme compounds to carry out the complete conversion, were synthesised by reactions of 4-azidobenzenesulphonamide (10) with differently substituted β-ketoesters (11a–11d) in the presence of organic base. 4-Azidobenzenesulphonamide (10) in turn was prepared from sulphanilamide (9) via diazotisation followed by treatment with sodium azide. After the synthesis, 1,2,3-triazole-4-carboxylates 4a–4d were converted to corresponding carboxylic acids 5a–5d by hydrolysis with a strong base and

Scheme 1. Synthetic pathway to the sulphonamides 4a–4d, 5a–5d, 6a–6d, 7a–7d, and 8a–8d. Reagents and conditions: (i) HCl, NaNO₂, H₂O, 0 °C, 15 min; (ii) NaN₃, 0 °C, 30 min; (iii) Piperidine, DMSO, 70 °C, 4 h; (iv) NaOH, reflux, 3 h then H₂O; (v) NH₃ solution, stir, 22 h; (vi) NH₂NH₂.H₂O, EtOH, reflux, 10–12 h; (vii) LiAlH₄, dry THF, reflux, 2 h then H₂O.
corresponding carboxamide derivatives 6a–6d by treatment with ammonia solution. 1,2,3-Triazole-4-hydrazinocarbonyl derivatives 7a–7d were obtained by treating their corresponding esters 4a–4d with hydrazine hydrate ethanol while 1,2,3-triazole-4-hydroxymethyl derivatives 8a–8d were prepared by treating esters 4a–4d with lithium aluminium hydride (Scheme 1).

The structures of all the newly synthesised compounds (4a–4d, 5a–5d, 6a–6d, 7a–7d, and 8a–8d) were characterised by a rigorous analysis of their IR, 1H NMR and 13C NMR spectral data. Structures were further confirmed by their HRMS data. In FT-IR, a strong characteristic absorption band for C=O stretch was observed in the range 1704–1728 cm⁻¹ for 1,2,3-triazole-4-carboxylates 4a–4d, 1690–1713 cm⁻¹ for 1,2,3-triazole-4-carboxylic acids 5a–5d, 1674–1690 cm⁻¹ for 1,2,3-triazole-4-carboxamides 6a–6d and 1659–1670 cm⁻¹ for 1,2,3-triazole-4-hydrazinocarbonyl derivatives 7a–7d while no such absorption band was observed in 1,2,3-triazole-4-hydroxymethyl compounds 8a–8d showing the complete reduction of ester group to hydroxymethyl group. The NMR spectra of ethyl esters of 1,2,3-triazole-4-carboxylic acids 4a–4d displayed a quartet in the range 8 4.37–4.40 ppm of two protons and a triplet in the range δ 0.81–1.35 ppm of three protons for ethyl group. Conversion of ester compounds 4a–4d to the corresponding carboxylic acids 5a–5d was confirmed by a downfield exchangeable singlet around δ 13.00 ppm due to COOH with disappearance of signals due to ethyl group protons. Similarly, 1,2,3-triazole-4-carboxamides 6a–6d were characterised by two exchangeable singlets in the range δ 7.94–8.04 ppm and 7.52–7.54 ppm corresponding to NH and OH protons, target 1,2,3-triazole-4-hydrazinocarbenyls 7a–7d showed two exchangeable singlets in the range δ 9.53–9.88 ppm due to NH proton and δ 4.45–4.50 ppm due to NH₂ protons whereas 1,2,3-triazole-4-hydroxymethyl compounds 8a–8d displayed a triplet around δ 5.11–5.25 ppm of OH and a doublet around δ 4.48–4.65 ppm of CH₂ protons. The presence of sulphonamide group in all the target 4-functionalised 1,2,3-triazole compounds (4–8) was evident from a broad singlet, exchangeable in D₂O, appearing in the range δ 7.44–7.56 ppm.

**CA inhibition**

All the newly synthesised 4-functionalised 1,2,3-triazole compounds (4a–4d, 5a–5d, 6a–6d, 7a–7d, and 8a–8d) were evaluated against cytosolic isoenzymes hCA I & hCA II and membrane bound isoenzymes hCA IV & hCA IX for their CA inhibition potential by using stopped-flow CO₂ hydrase assay method and acetazolamide (AZA) was chosen as reference drug for the assay. In general, all the assayed compounds have shown significant inhibitory action against the reported isozymes, with low nanomolar inhibition constant (Ki). Inhibition data of the compounds as given in Table 1 let the following insights regarding CAs inhibitory properties.

i. The cytosolic isofrom hCA I was in general significantly inhibited by all the newly synthesised compounds (4a–4d, 5a–5d, 6a–6d, 7a–7d, and 8a–8d) with Ki in the range 8.3 nM–0.8737 μM (Table 1). It is pertinent to mention that 5-CH₃ substituted derivatives of newly synthesised compounds except 8a were most effective inhibitors of hCA I with Ki ≤ 15.1nM as compared to corresponding 5-aryl derivatives. At the same time some compounds namely 5b, 5c, 6c, 6d, 8a, and 8c showed weaker inhibition potential as compared to reference drug AZA (Ki = 250 nM) against hCA I that is off-target while inhibiting hCA II and IV in glaucoma and hCA IX in tumours.

ii. Nearly all the newly synthesised compounds (4a–4d, 5a–5d, 6a–6d, 7a–7d, and 8a–8d) showed better inhibitory potential in low nanomolar range with Ki ≤ 9.4 nM except three compounds namely 5b, 7b, and 8a against the most abundant isofrom hCA II as compared to standard drug AZA (Ki = 12.1 nM). Some of the tested compounds mainly 5-CH₃ derivatives (4a, 5a, and 6a) and two other compounds (7d and 8c) exhibited inhibitory potency (Ki ≤ 4 nM) several times better than AZA (Table 1).

iii. All the tested compounds except some 5-CH₃ derivatives namely 5a, 6a, 7a, and 8a showed excellent inhibitory potential with Ki in the range of 1.4–55.3 nM against membrane bound isozyme hCA IV as compared to standard drug AZA. Most of the compounds (4a–4d, 5c–5d, 6b–6d, 7c–7d, and 8b–8d) have their inhibitory potency (Ki ≤ 8.4 nM) several folds superior than AZA (Ki ≤ 74 nM) against hCA IV which is one of the drug target for designing antiglaucoma drugs (Table 1).

iv. In general, all the tested compounds except few (4c, 5b, 5c, 6b, 7b, 8a, and 8b) have shown better activity profile (Ki ≤ 9.5 nM) against tumour associated membrane bound isozyme hCA IX as compared to reference drug AZA (Ki = 25.8 nM). It is significant to mention here that, in the broader sense, derivatives with 5-CH₃ and 5-(naphtha-2-yl) substitution have shown better activity as compared to other derivatives (Table 1).

v. Interestingly compounds possessing rather bulky scaffolds were milder inhibitors of cytosolic isofrom hCA I, over other isozymes (hCA II, IV, and IX) and is mainly due to the fact that the active site cavity of hCA I is smaller than other isozymes hCA II, IV and IX, because of the presence of two His residues (i.e. His 200 and His 67)⁴⁹. Overall comparison of activity, in terms of SAR, reveals that all the compounds except derivatives with 5-CH₃ group were better selective for hCA II and IV over hCA I in the broader sense. Therefore, these compounds can be important candidates for designing antiglaucoma drugs. However, their good activity against both hCA II and hCA IX suggests that structure of compounds needs further modification for getting better selectivity for tumour associated hCA IX over hCA II.

**Conclusions**

In this paper, we report a series of twenty novel compounds of 4-functionalised 1-aryl-5-alkyl/aryl-1,2,3-triazole compounds (4a–4d, 5a–5d, 6a–6d, 7a–7d, and 8a–8d) bearing a primary sulphonamide group on the phenyl ring at N-1 position of 1,2,3-triazole scaffold with different functionalities at C-4, such as ester, carboxylic acid, carboxamide, hydrazinocarbonyl, and hydroxymethyl which were evaluated against four isozymes, hCA I, II, IV, and IX. Most of the compounds performed better against aforementioned isozymes showing low nanomolar potency as compared to reference drug acetazolamide. Out of twenty newly synthesised compounds, seventeen compounds (except 5b, 7b, and 8a) showed low nanomolar affinity (Ki ≤ 9.4 nM) for hCA II, sixteen compounds except the derivatives with 5-CH₃ substitution have displayed excellent activity (Ki ≤ 55.3 nM) for hCA IV and thirteen compounds (except 4c, 5b, 5c, 6b, 7b, 8a, and 8b) have shown better activity (Ki ≤ 9.5) for hCA IX while most of compounds with bulkier substitution at C-5 were medium to weaker inhibitors of hCA I with Ki values in the range of 56.2–873.7 nM. In short, reported compounds have shown remarkable activity against hCA I, II, IV, and IX isozymes from which it can be concluded that 1,2,3-triazoles
scaffold deserve to be investigated further as a novel scaffold for CAIs.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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