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RESEARCH PAPER



Evaluation of sulphonamide derivatives acting as inhibitors of human carbonic anhydrase isoforms I, II and *Mycobacterium tuberculosis* β -class enzyme Rv3273

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ABSTRACT

A series of novel sulphonamide derivatives was obtained from sulphanilamide which was N4-alkylated with ethyl bromoacetate followed by reaction with hydrazine hydrate. The hydrazide obtained was further reacted with various aromatic aldehydes. The novel sulphonamides were characterised by infrared, mass spectrometry, ¹H- and ¹³C-NMR and purity was determined by high-performance liquid chromatography (HPLC). Human (h) carbonic anhydrase (CA, EC 4.2.1.1) isoforms hCA I and II and *Mycobacterium tuberculosis* β -CA encoded by the gene Rv3273 (mtCA 3) inhibition activity was investigated with the synthesised compounds which showed promising inhibition. The K_s were in the range of 54.6 nM–1.8 μ M against hCA I, in the range of 32.1 nM–5.5 μ M against hCA II and of 127 nM–2.12 μ M against mtCA 3.

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1. Introduction

Sulphonamides are interesting biologically active compounds. There are numerous sulphonamide drugs available on the markets for the treatment of various diseases¹. Sulphonamide derivatives such as acetazolamide, methazolamide, ethoxzolamide, dichlorophenamide, dorzolamide and brinzolamide have been clinically used for decades as inhibitors of the zinc enzyme carbonic anhydrase (CA, EC 4.2.1.1). A diverse research trend in the past years has led to the obtaining of diuretic, anti-glaucoma, anti-cancer, anti-convulsant, anti-diabetic, and anti-obesity agents based on CA inhibitors (CAIs) of the sulphonamide type^{2–6}. Sulphonamides act as strong CAIs by binding as anions to the zinc metal ion within the enzyme active site⁷.

CA has various roles in physiological events such as carbon dioxide and bicarbonate transport processes, respiration, pH balancing, CO₂ homeostasis, electrolyte secretion, biosynthetic reactions¹. Distinct, evolutionarily non-related gene families of CAs are present in various organisms, out of which the α -class is present in humans, as 15 different isoforms (hCA I–XIV). hCA I is present in red blood cells and in many tissues but its physiological function is still unknown; however, it is known that hCA I is associated with retinal and cerebral edema, and the inhibition of CA I may be helpful in curing such conditions^{1–5}. hCA II, the physiologically dominant isoform, is another enzyme which is associated with several disease conditions such as epilepsy, edema, glaucoma and altitude sickness^{1–5}. Furthermore, it has also emerged in the past few years that these enzymes can be used as potential target for designing anti-infective drugs with a novel mechanism of action⁶.

In this article, we report a synthetic strategy for the generation and characterisation of some sulphonamide derivatives. The novel sulphonamides were purified and characterised using IR, Mass spectrometry, ¹H and ¹³C-NMR for confirmation of their structure,

and purity of the compounds was determined by using HPLC techniques. The newly synthesised compounds were analysed as inhibitors of human hCA I and II, and the bacterial, β -class enzyme from *Mycobacterium tuberculosis* (mtCA 3) encoded by the gene Rv3273⁸.

2. Material and method

2.1. 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 N₂ atmosphere, unless otherwise indicated. Analytical silica gel 60 F₂₅₄-coated TLC plates were purchased from Sigma-Aldrich (Milan, Italy), and were visualised with UV light. IR spectra (ATR) were recorded on a Quest ATR Diamond Accessory (Black) P31482 & Shimadzu 8100 infrared spectrophotometer. ¹H-NMR was recorded at 300 MHz in DMSO-d₆ as solvent using TMS as an internal reference standard at Sophisticated Analytical Instrument Facility (SAIF). Molecular ion peaks of some of the synthesised compounds were recorded using LCMS at Laxai- Avanti Life Sciences Pvt. Ltd Hyderabad, India. Melting points were recorded using a Veego[®] (VMP)-D capillary melting point apparatus (Veego Instruments Corp., Mumbai, India) and are uncorrected. Percent Purity of synthesised compounds was determined by performing RP-HPLC.

General procedure for synthesis of ethyl 2-((4-sulphamoylphenyl) amino) acetate (2)⁹

In a solution of sulphanilamide (0.01 mol) in absolute ethanol (20 ml), ethyl bromoacetate (0.01 mol) and anhydrous potassium

carbonate (0.6 g) were added and the reaction mixture was heated under reflux for 12 h. The potassium salt was filtered off and excess of ethanol was removed under reduced pressure. The residue solidifies on cooling to give compound (**2**). Yield: 70%, R_f : 0.72 (chloroform:methanol 9:1); M.P.: 141–144 °C; IR (ATR) cm^{-1} : 3356 (N–H str of NH_2), 3271 (N–H str), 2993 (Ar C–H str), 2904 (aliphatic C–H str), 1728 (C=O str), 1597 (C=C str), and 1138 (S=O str).

General procedure for synthesis of 4-((2-hydrazinyl-2-oxoethyl)amino) benzenesulphonamide (**3**)¹⁰

In a solution of compound (**2**) (0.01 mol) in absolute ethanol (15 ml), hydrazine hydrate (0.02 mol) was added and the reaction mixture was heated under reflux for 4 h. The excess of ethanol was removed under vacuum and the reaction mixture was allowed to cool. The reaction mixture was then diluted with ice-cold water. The precipitate obtained was filtered, washed with cold water, dried and recrystallised from ethanol. Yield: 50%; R_f : 0.46 (chloroform:methanol 9:1); M.P.: 174–177 °C, $^1\text{H-NMR}$ (300 MHz, DMSO-d_6) δ ppm: 9.15 (s, 1H, –CONH), 7.51–7.47 (dd, 2H, Ar–H), 6.93 (s, 2H, – $\text{SO}_2\text{-NH}_2$), 6.62–6.61 (dd, 2H, Ar–H), 6.58 (t, 1H, Ar–NH), 4.25 (d, 2H, – $\text{CH}_2\text{-}$), 3.69–3.67 (d, 2H, – NH_2); IR (ATR, cm^{-1}): 3344 (N–H str of NH_2), 3294 (N–H str), 3034 (Ar C–H str), 2943 (aliphatic C–H str), 1654 (C=O str), 1589 (Ar C=C), and 1139 (S=O str).

General procedure for synthesis of (E)-1-(4-chlorophenyl)-3-phenylprop-2-en-1-one derivatives (4a–4k)^{11–13}

4-Chloro-acetophenone (0.01 mol) and various substituted aromatic aldehydes (0.01 mol) were mixed in ethanol (40 ml) in a conical flask placed in an ice bath. To this, 60% NaOH solution (10 ml) was added dropwise with continuous stirring for 30 min. The mixing was continued for another 2–3 h maintaining the ice bath. The mixture was kept in a refrigerator overnight. Reaction completion was confirmed by TLC (hexane:ethyl acetate = 2:1). Then, it was diluted with ice-cold water, filtered, washed well with cold water, dried in air and recrystallised from rectified methanol.

General procedure for synthesis of 4-aryl/heteroaryl but-3-en-2-one derivatives (4l–4u)¹⁴

A mixture of aldehyde (1 equiv) and acetone (13.6 equiv) was added in aqueous 60% NaOH solution. The mixture was stirred at 40 °C. Reaction completion was confirmed by TLC (hexane:ethyl acetate = 2:1). On completion of reaction, 25 cm^3 of water were added to reaction mixture to afford the crude as an oil. The product was extracted with AcOEt ($3 \times 25 \text{ cm}^3$) and dried in air. Reaction with 4-((2-hydrazinyl-2-oxoethyl)amino) benzenesulphonamide (**3**) led to the final products.

General procedure for synthesis of 4-((2-(3-(4-chlorophenyl)-5-aryl/heteroaryl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)amino)benzenesulphonamide derivatives (5a–5k)¹⁵

A mixture of chalcone (E)-1-(4-chlorophenyl)-3-aryl/heteroaryl-prop-2-en-1-one derivatives (4a–4k) (0.01 mol) and 4-((2-hydrazinyl-2-oxoethyl) amino) benzenesulphonamide (**3**) (0.02 mol) in 20 ml ethanol was refluxed for 2 h. To this, alcoholic KOH solution (10 ml, 60%) was added dropwise with continuous stirring for 30 min. The reaction mixture was refluxed further for 2 h and stirred overnight. Reaction was monitored by TLC using chloroform:methanol (0.8:0.2). The resulting solution was poured on ice-cold water. Precipitate obtained was filtered and recrystallised from ethanol.

General procedure for synthesis of 4-((2-(3-methyl-5-aryl/heteroaryl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)amino)benzenesulphonamidederivatives (5l–5u)¹⁶

4-Aryl/heteroaryl-but-3-en-2-one derivatives (**4l–4u**) (0.01 mol) were dissolved in hot glacial acetic acid. To this solution 4-((2-hydrazinyl-2-oxoethyl)amino)benzenesulphonamide (**3**) (0.015 mol) was added and refluxed. Reaction was monitored by TLC using chloroform:methanol (9:1). The resulting solution was poured on ice-cold water. Precipitate obtained was filtered and recrystallised from 90% ethanol.

General synthesis of 4-((2-(arylmethylidene)hydrazinyl)-2-oxoethyl)amino)benzene sulphonamide derivatives (6a–6j)^{17–19}

In a 250-ml flask, equimolar quantity of hydrazide (**3**) (0.05 mol) and aromatic aldehyde (0.05 mol) were dissolved in EtOH (50 ml). Glacial acetic acid (2–3 ml) was added to adjust the pH to 5–6. The reaction mixture was refluxed for 3 h. The progress of reactions was monitored using TLC using hexane:ethyl acetate (8:2) as the mobile phase. After completion of reaction (as seen from TLC), the mixture was poured onto the crushed ice and the precipitated product was filtered, washed twice with ice-cold water, dried and recrystallised from water:ethanol.

Using the above general procedure, the following compounds were prepared and characterised.

4-((2-(3-(4-Chlorophenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)amino) benzenesulphonamide (5a)

Yield: 70%; R_f : 0.53 (chloroform:methanol 9:1); M.P.: 140–144 °C; purity (HPLC): 88.87%; $^1\text{H-NMR}$ (300 MHz, DMSO-d_6) δ ppm: 7.98–7.86 (dd, 2H, Ar–H), 7.57–7.50 (m, 4H, Ar–H), 7.47–7.20 (m, 5H, Ar–H), 6.93 (s, 2H, – SO_2NH_2), 6.67–6.64 (dd, 2H, Ar–H), 6.59–6.55 (t, 1H, Ar–NH), 5.61–5.56 (dd, 1H, Hx), 4.49–4.30 (dd, 2H, – $\text{CH}_2\text{-}$), 3.93–3.83 (dd, 1H, Ha), 3.21–3.13 (dd, 1H, Hb); $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6): δ = 167.07, 154.42, 151.38, 142.22, 135.8, 131.15, 130.11, 129.17, 129.00, 128.85, 127.67, 125.91, 111.62, 60.61, 45.15, 42.2; IR (ATR, cm^{-1}): 3390, 3306 (N–H str of NH_2), 3234 (N–H str), 3074 (Ar C–H str), 2939 (aliphatic C–H str), 1683 (C=O str), 1155 (S=O str), 738 (C–Cl str); LC-MS (ESI; M)⁺: 468.5.

4-((2-(3-(4-Chlorophenyl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)amino) benzenesulphonamide (5b)

Yield: 68%; R_f : 0.46 (chloroform:methanol 9:1); M.P.: 179–181 °C; purity (HPLC): 98.02%; $^1\text{H-NMR}$ (300 MHz, DMSO-d_6) δ ppm: 7.86–7.84 (dd, 2H, Ar–H), 7.54–7.49 (m, 4H, Ar–H), 7.28–7.24 (dd, 2H, Ar–H), 7.10–7.06 (t, 2H, Ar–H), 6.89 (s, 2H, – $\text{SO}_2\text{NH}_2\text{-}$), 6.66–6.64 (dd, 2H, Ar–H), 6.48–6.45 (t, 1H, Ar–NH), 5.62–5.58 (dd, 1H, Hx), 4.46–4.30 (dd, 2H, – $\text{CH}_2\text{-}$), 3.91–3.84 (dd, 1H, Ha), 3.20–3.15 (dd, 1H, Hb); $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6): δ = 167.19, 160.64, 154.47, 151.43, 138.36, 135.78, 131.17, 130.12, 129.22, 128.94, 128.14, 127.67, 115.85, 111.65, 59.98, 45.15, 42.12; IR (ATR, cm^{-1}): 3390, 3308 (N–H of NH_2), 3236 (N–H str), 3074 (Ar C–H str), 2924 (aliphatic C–H str), 1683 (C=O str), 1157 (S=O str), 738 (C–Cl str); LC-MS (ESI; M)⁺: 486.8.

4-((2-(3-(4-Chlorophenyl)-5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)amino) benzenesulphonamide (5c)

Yield: 75%; R_f : 0.44 (chloroform:methanol 9:1); M.P.: 182–185 °C; purity (HPLC): 99.11%; $^1\text{H-NMR}$ (300 MHz, DMSO-d_6) δ ppm: 7.88–7.86 (dd, 2H, Ar–H), 7.57–7.47 (m, 4H, Ar–H), 7.29–7.20 (dd,

2H, Ar-H), 7.16–7.10 (t, 2H, Ar-H), 6.93 (s, 2H, $-\text{SO}_2\text{NH}_2-$), 6.66–6.63 (dd, 2H, Ar-H), 6.58–6.55 (t, 1H, Ar-NH), 5.62–5.56 (dd, 1H, Hx), 4.42–4.35 (dd, 2H, $-\text{CH}_2-$), 3.92–3.82 (dd, 1H, Ha), 3.22–3.15 (dd, 1H, Ha); $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6): $\delta = 166.81, 151.5, 142.96, 137.27, 133.84, 131.56, 131.09, 129.27, 128.74, 128.19, 127.7, 123.78, 111.62, 44.00$; IR (ATR, cm^{-1}): 3362, 3300 (N-H of NH_2), 3230 (N-H str), 3080 (Ar C-H str), 2846 (aliphatic C-H str), 1683 (C=O str), 1155 (S=O str), 736 (C-Cl str), 580 (C-Br str).

4-((2-(3-(4-Chlorophenyl)-5-(4-methylphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl) amino)benzenesulphonamide (5d)

Yield: 70%; R_f : 0.57 (chloroform:methanol 9:1); M.P.: 151–154 °C; purity (HPLC): 100%; $^1\text{H-NMR}$ (300 MHz, DMSO-d_6) δ ppm: 7.85–7.80 (dd, 2H, Ar-H), 7.58–7.50 (m, 4H, Ar-H), 7.10 (s, 4H, Ar-H), 6.89 (s, 2H, SO_2NH_2), 6.70–6.64 (dd, 2H, Ar-H), 6.45–6.42 (t, 1H, Ar-NH), 5.57–5.53 (dd, 1H, Hx), 4.46–4.30 (dd, 2H, CH_2), 3.98–3.81 (dd, 1H, Ha), 3.18–3.12 (dd, 1H, Hb), 2.27 (s, 3H, Ar- CH_3); $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6): $\delta = 167.02, 154.44, 151.4, 139.28, 137.23, 135.76, 131.16, 130.32, 129.19, 128.84, 128.14, 127.69, 125.89, 111.64, 60.41, 45.16, 42.2, 21.12$; IR (ATR, cm^{-1}): 3362, 3294 (N-H of NH_2), 3232 (N-H str), 3082 (Ar C-H str), 2853 (aliphatic C-H str), 1680 (C=O str), 1151 (S=O str), 740 (C-Cl str).

4-((2-(3-(4-Chlorophenyl)-5-(pyridin-4-yl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)amino) benzenesulphonamide (5e)

Yield: 71%; R_f : 0.41 (chloroform:methanol 9:1); M.P.: 161–164 °C; $^1\text{H-NMR}$ (300 MHz, DMSO-d_6) δ ppm: 8.5 (s, 2H, Pyr-H), 7.88–7.85 (dd, 2H, Ar-H), 7.57–7.51 (m, 4H, Ar-H), 7.26–7.24 (dd, 2H, Ar-H), 6.93 (s, 2H, SO_2NH_2), 6.68–6.58 (dd, 2H, Ar-H), 6.61–6.58 (t, 1H, Ar-NH), 5.63–5.57 (dd, 1H, Hx), 4.54–4.32 (dd, 2H, CH_2), 3.95–3.85 (dd, 1H, Ha), 3.25–3.17 (dd, 1H, Hb); $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6): $\delta = 168.78, 154.19, 151.09, 149.92, 149.12, 135.22, 130.73, 129.75, 128.86, 127.18, 120.81, 111.24, 59.23, 44.56, 41.12$; IR (ATR, cm^{-1}): 3390, 3309 (N-H of NH_2), 3238 (N-H str), 3074 (Ar C-H str), 2926 (aliphatic C-H str), 1685 (C=O str), 1521 (C=N str), 1153 (S=O str), 736 (C-Cl str); LC-MS (ESI; M^+): 469.10.

4-((2-(3-(4-Chlorophenyl)-5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl) amino)benzenesulphonamide (5f)

Yield: 67%; R_f : 0.47 (chloroform:methanol 9:1); M.P.: 159–162 °C; purity (HPLC): 100%; $^1\text{H-NMR}$ (300 MHz, DMSO-d_6) δ ppm: 7.88–7.85 (dd, 2H, Ar-H), 7.57–7.51 (m, 5H, Ar-H), 7.29–7.25 (dd, 2H, Ar-H), 7.16 (s, 1H, Ar-H), 6.93 (s, 2H, SO_2NH_2), 6.69–6.66 (dd, 2H, Ar-H), 6.63–6.59 (t, 1H, Ar-NH), 5.82–5.77 (dd, 1H, Hx), 4.57–4.38 (dd, 2H, CH_2), 4.02–3.92 (dd, 1H, Ha), 3.29–3.23 (dd, 1H, Hb); $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6): $\delta = 167.29, 154.58, 151.42, 138.82, 135.87, 131.41, 131.24, 130.06, 129.98, 129.4, 129.19, 128.9, 127.89, 127.69, 126.9, 111.69, 58.37, 45.15, 41.1$; IR (ATR, cm^{-1}): 3267, 3207 (N-H of NH_2), 3146 (N-H str), 3097 (Ar C-H str), 2947 (aliphatic C-H str), 1670 (C=O str), 1195 (S=O str), 759 (C-Cl str); LC-MS (ESI; M^+): 503.40.

4-((2-(3-(4-Chlorophenyl)-5-(2-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl) amino)benzenesulphonamide (5g)

Yield: 72%; R_f : 0.41 (chloroform:methanol 9:1); M.P.: 161–163 °C; purity (HPLC): 98.58%; $^1\text{H-NMR}$ (300 MHz, DMSO-d_6) δ ppm: 10.85 (s, 1H, Ar-OH), 7.85–7.78 (m, 3H, Ar-H), 7.55–7.50 (m, 3H, Ar-H), 7.48–7.41 (dd, 3H, Ar-H), 6.92 (s, 2H, SO_2NH_2), 6.69–6.66 (dd, 3H, Ar-H), 6.48–6.45 (t, 1H, Ar-NH), 4.35–4.32 (dd, 2H, CH_2), 4.01–3.99

(dd, 1H, Ha), 3.35 (dd, 1H, Hb); $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6): $\delta = 171.95, 151.54, 147.39, 137.25, 134.4, 131.04, 129.16, 128.73, 128.4, 128.17, 127.72, 111.63, 44.44$; IR (ATR, cm^{-1}): 3566 (OH str), 3356, 3300 (N-H of NH_2), 3259 (N-H str), 3072 (Ar C-H str), 2924 (aliphatic C-H str), 1670 (C=O str), 1147 (S=O str), 729 (C-Cl str).

4-((2-(3-(4-Chlorophenyl)-5-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)amino)benzenesulphonamide (5h)

Yield: 70%; R_f : 0.61 (chloroform:methanol 9:1); M.P.: 170–173 °C; $^1\text{H-NMR}$ (300 MHz, DMSO-d_6) δ ppm: 7.88–7.82 (dd, 2H, Ar-H), 7.57–7.47 (m, 4H, Ar-H), 6.92 (s, 2H, SO_2NH_2), 6.87–6.80 (dd, 2H, Ar-H), 6.71–6.65 (dd, 2H, Ar-H), 6.63–6.59 (t, 1H, Ar-NH), 5.82–5.77 (dd, 1H, Hx), 4.57–4.38 (dd, 2H, CH_2), 4.02–3.92 (dd, 1H), 3.29–3.23 (dd, 1H, Hb); $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6): $\delta = 167.18, 154.48, 149.29, 148.54, 135.67, 134.81, 131.16, 130.29, 129.22, 128.92, 127.66, 117.88, 112.31, 111.62, 110.01, 60.43, 56.02, 55.94$; IR (ATR, cm^{-1}): 3329 (N-H of NH_2), 3209 (N-H str), 3087 (Ar C-H str), 2899 (aliphatic C-H str), 1689 (C=O str), 1153 (S=O str), 732 (C-Cl str).

4-((2-(3-(4-Chlorophenyl)-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl) amino)benzenesulphonamide (5i)

Yield: 68%; R_f : 0.45 (chloroform:methanol 9:1); M.P.: 148–150 °C; purity (HPLC): 96.06%; $^1\text{H-NMR}$ (300 MHz, DMSO-d_6) δ ppm: 7.89–7.87 (dd, 2H, Ar-H), 7.54–7.50 (m, 4H, Ar-H), 7.37–7.35 (dd, 1H, Ar-H), 7.05–7.04 (dd, 1H, Ar-H), 6.94–6.92 (dd, 1H, Ar-H), 6.91 (s, 2H, SO_2NH_2), 6.66–6.64 (dd, 2H, Ar-H), 6.57–6.54 (t, 1H, Ar-NH), 5.93–5.89 (dd, 1H, Hx), 4.41–4.28 (dd, 2H, CH_2), 3.90–3.83 (dd, 1H, Ha), 3.41–3.40 (dd, 1H, Hb); $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6): $\delta = 167.3, 154.67, 151.49, 135.8, 131.16, 130.1, 129.31, 129.03, 127.66, 127.14, 125.48, 125.2, 111.65, 56.14, 45.13, 41.89$; IR (ATR, cm^{-1}): 3390, 3308 (N-H of NH_2), 3236 (N-H str), 3074 (Ar C-H str), 2924 (aliphatic C-H str), 1683 (C=O str), 1157 (S=O str), 738 (C-Cl str); LC-MS (ESI; M^+): 475.0.

4-((2-(5-(Anthracen-9-yl)-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl) amino)benzenesulphonamide (5j)

Yield: 71%; R_f : 0.62 (chloroform:methanol 9:1); M.P.: 190–192 °C; purity (HPLC): 100%; $^1\text{H-NMR}$ (300 MHz, DMSO-d_6) δ ppm: 8.75–7.61 (m, 4H, Ar-H), 8.22–8.02 (m, 4H, Ar-H), 7.59–7.49 (m, 12H, Ar-H), 6.95–6.92 (dd, 3H, Hx, SO_2NH_2), 6.75–6.68 (dd, 2H, Ar-H), 6.58–6.55 (t, 1H, Ar-NH), 4.38–4.35 (dd, 2H, CH_2), 4.01–4.00 (dd, 1H, Ha), 3.35 (dd, 1H, Hb); $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6): $\delta = 166.86, 151.57, 146.71, 137.28, 134.37, 131.66, 131.38, 131.15, 131.05, 130.07, 129.93, 129.81, 129.4, 128.74, 128.42, 128.21, 127.82, 127.74, 127.52, 125.89, 125.84, 125.55, 125.45, 111.63, 44.43, 44.17$; IR (ATR, cm^{-1}): 3342 (N-H of NH_2), 3277 (N-H str), 3051 (Ar C-H str), 2862 (aliphatic C-H str), 1672 (C=O str), 1161 (S=O str), 761 (C-Cl str).

4-((2-(3-(4-Chlorophenyl)-5-(1H-indol-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl) amino)benzenesulphonamide (5k)

Yield: 73%; R_f : 0.55 (chloroform:methanol 9:1); M.P.: 174–177 °C; purity (HPLC): 100%; $^1\text{H-NMR}$ (300 MHz, DMSO-d_6) δ ppm: 11.23 (s, 1H, Indole-NH), 8.23–8.15 (dd, 2H, Ar-H), 7.73–7.71 (dd, 1H, Ar-H), 7.57–7.55 (dd, 2H, Ar-H), 7.44 (dd, 1H, Ar-H), 7.22–7.18 (t, 2H, Ar-H), 6.90 (s, 2H, SO_2NH_2), 6.71–6.65 (t, 3H, Ar-H, Hx), 6.52–6.49 (t, 1H, Ar-NH), 4.37–4.31 (dd, 2H, CH_2), 3.88–3.86 (dd, 1H, Ha), 3.50–3.43 (dd, 1H, Hb); $^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6): $\delta = 165.68, 151.6, 141.54, 137.53, 131.47, 131.02, 130.3, 127.77, 124.76, 124.58, 122.93, 121.94, 120.98, 120.71, 112.26, 111.89, 111.75, 111.56$

56.67, 44.06; IR (ATR, cm^{-1}): 3174 (N-H of NH_2), 3113 (N-H *str*), 3041 (Ar C-H *str*), 2820 (aliphatic C-H *str*), 1672 (C=O *str*), 1122 (S=O *str*), 740 (C-Cl *str*).

4-((2-(3-Methyl-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl) amino) benzene sulphonamide (5l)

Yield: 79%; R_f : 0.63 (chloroform:methanol 9:1); M.P.: 211–213 °C; purity (HPLC): 99.16%; $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ ppm: 7.68–7.61 (dd, 2H, Ar-H), 7.53–7.51 (m, 3H, Ar-H), 7.47–7.45 (dd, 2H, Ar-H), 7.34–7.32 (dd, 1H, H_x), 7.19–7.08 (dd, 1H, H_b), 6.95 (s, 2H, $\text{SO}_2\text{-NH}_2$), 6.67–6.64 (dd, 2H, Ar-H), 6.51–6.48 (t, 1H, Ar-NH), 4.27–4.22 (dd, 2H, CH_2), 3.99–3.95 (dd, 1H, CHH_a), 2.08 (s, 3H, CH_3); IR (ATR) cm^{-1} : 3404 (N-H *str* of NH_2), 3302 (N-H *str*), 3078 (aromatic C-H *str*), 2885 (aliphatic C-H *str*), 1693 (C=O *str*), 1610 (C=N), 1143 (S=O *str*); LC-MS (ESI; $\text{M} + 1$) $^+$: 373.1.

4-((2-(5-(4-Methoxyphenyl)-3-methyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl) amino)benzenesulphonamide (5m)

Yield: 85%; R_f : 0.65 (chloroform:methanol 9:1); M.P.: 235–237 °C; purity (HPLC): 98.89%; $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ ppm: 7.58–7.56 (dd, 2H, Ar-H), 7.50–7.48 (dd, 2H, Ar-H), 7.46–7.44 (dd, 1H, H_x), 6.95–6.93 (dd, 1H, H_b), 6.92–6.90 (dd, 2H, Ar-H), 6.8 (s, 2H, $\text{SO}_2\text{-NH}_2$), 6.69–6.67 (dd, 2H, Ar-H), 6.32–6.30 (t, 1H, Ar-NH), 4.26–4.25 (dd, 2H, CH_2), 4.0–3.99 (dd, 1H, CHH_a), 3.79 (s, 3H, OCH_3), 2.08 (s, 3H, CH_3); IR (ATR) cm^{-1} : 3404 (N-H *str* of NH_2), 3333 (N-H *str*), 3248 (O-C *str*) 3030 (Ar C-H *str*), 2839 (aliphatic C-H *str*), 1660 (C=O *str*), 1604 (C=N), 1153 (S=O *str*); LC-MS (ESI; $\text{M} + 1$) $^+$: 403.1.

4-((2-(5-(4-Chlorophenyl)-3-methyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl) amino)benzenesulphonamide (5n)

Yield: 85%; R_f : 0.61 (chloroform:methanol 9:1); M.P.: 221–225 °C; purity (HPLC): 98.89%; $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ ppm: 7.61–7.54 (dd, 2H, Ar-H), 7.54–7.51 (dd, 2H, Ar-H), 7.43–7.33 (dd, 2H, Ar-H), 7.01–6.99 (dd, 1H, H_x), 6.97–6.93 (dd, 1H, H_b), 6.87 (s, 2H, $\text{SO}_2\text{-NH}_2$), 6.69–6.67 (dd, 2H, Ar-H), 6.32–6.30 (t, 1H, Ar-NH), 4.26–4.25 (dd, 2H, CH_2), 3.99–3.40 (dd, 1H, CHH_a), 2.1 (s, 3H, CH_3); IR (ATR) cm^{-1} : 3408 (N-H *str* of NH_2), 3288 (N-H *str*), 2885 (aliphatic C-H *str*), 1697 (C=O *str*), 1608 (C=N), 1145 (S=O *str*), 810 (C-Cl *str*); LC-MS (ESI; $\text{M} + 1$) $^+$: 407.1.

4-((2-(3-Methyl-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl) amino)benzene sulphonamide (5o)

Yield: 78%; R_f : 0.7 (chloroform:methanol 9:1); M.P.: 233–235 °C; purity (HPLC): 99.46%; $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ ppm: 7.76–7.75 (dd, 2H, Ar-H), 7.57–7.55 (dd, 2H, Ar-H), 7.35–7.34 (dd, 1H, H_x), 7.11–7.09 (dd, 2H, Ar-H), 6.87 (s, 2H, $\text{SO}_2\text{-NH}_2$), 6.84–6.81 (dd, 1H, H_b), 6.64–6.60 (t, 1H, Ar-NH), 6.62–6.60 (dd, 2H, Ar-H), 4.24–4.23 (dd, 2H, CH_2), 3.97–3.96 (dd, 1H, CHH_a), 2.27 (s, 3H, Ar- CH_3), 2.04 (s, 3H, CH_3); IR (ATR) cm^{-1} : 3319 (NH *str* of NH_2), 3232 (NH *str*), 3113 (Ar C-H *str*), 2916 (aliphatic C-H *str*), 1668 (C=O *str*), 1600 (C=N), 1151 (S=O *str*); LC-MS (ESI; $\text{M} + 1$) $^+$: 387.1.

4-((2-(5-(4-Fluorophenyl)-3-methyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl) amino)benzenesulphonamide (5p)

Yield: 76%; R_f : 0.81 (chloroform:methanol 9:1); M.P.: 217–219 °C; purity (HPLC): 99.67%; $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ ppm: 7.68–7.64 (dd, 2H, Ar-H), 7.53–7.51 (dd, 2H, Ar-H), 7.28–7.22 (dd, 2H, Ar-H), 7.12–7.08 (dd, 1H, H_x), 6.94 (s, 2H, $\text{SO}_2\text{-NH}_2$), 6.68–6.66

(dd, 1H, H_b), 6.68–6.66 (dd, 2H, Ar-H), 6.52–6.49 (t, 1H, Ar-NH), 4.27–4.25 (dd, 2H, CH_2), 3.99–3.97 (dd, 1H, CHH_a), 2.1 (s, 3H, CH_3); IR (ATR) cm^{-1} : 3406 (NH *str* of NH_2), 3294 (NH *str*), 3051 (Ar C-H *str*), 2901 (aliphatic C-H *str*), 1693 (C=O *str*), 1600 (C=N), 1307 (C-F *str*), 1145 (S=O *str*); LC-MS (ESI; $\text{M} + 1$) $^+$: 391.23.

4-((2-(5-(2-Chlorophenyl)-3-methyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl) amino)benzenesulphonamide (5q)

Yield: 76%; R_f : 0.64 (chloroform:methanol 9:1); M.P.: 202–205 °C; purity (HPLC): 99.40%; $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ ppm: 7.54–7.52 (dd, 2H, Ar-H), 7.47–7.41 (dd, 1H, H_x), 7.37–7.28 (m, 4H, Ar-H), 7.00–6.94 (dd, 1H, H_b), 6.90 (s, 2H, $\text{SO}_2\text{-NH}_2$), 6.68–6.66 (dd, 2H, Ar-H), 6.46–6.43 (t, 1H, Ar-NH), 4.27–4.25 (dd, 2H, CH_2), 4.0–3.9 (dd, 1H, CHH_a), 2.1 (s, 3H, CH_3); IR (ATR) cm^{-1} : 3406 (NH *str* of NH_2), 3279 (NH *str*), 3064 (Ar C-H *str*), 2885 (aliphatic C-H *str*), 1693 (C=O *str*), 1610 (C=N), 1141 (S=O *str*), 746 (C-Cl *str*); LC-MS (ESI; $\text{M} + 1$) $^+$: 407.1.

4-((2-(5-(2-Hydroxyphenyl)-3-methyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl) amino)benzenesulphonamide (5r)

Yield: 86%; R_f : 0.59 (chloroform:methanol 9:1); M.P.: 211–213 °C; purity (HPLC): 99.35%; $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ ppm: 9.69 (s, 1H, Ar-OH), 7.55–7.53 (dd, 2H, Ar-H), 7.50–7.48 (dd, 1H, Ar-H), 7.25–7.2 (dd, 1H, Ar-H), 7.09–7.05 (dd, 1H, Ar-H), 6.97–6.92 (dd, 1H, H_x), 6.87–6.856 (dd, 1H, H_b), 6.85 (s, 2H, $\text{SO}_2\text{-NH}_2$), 6.80–6.76 (dd, 1H, Ar-H), 6.67–6.65 (dd, 2H, Ar-H), 6.34–6.31 (t, 1H, Ar-NH), 4.24–4.23 (dd, 2H, CH_2), 3.97–3.96 (dd, 1H, CHH_a), 2.1 (s, 3H, CH_3); IR (ATR) cm^{-1} : 3336 (NH *str*), 3252 (OH *str*), 2893 (aliphatic C-H *str*), 1658 (C=O *str*), 1600 (C=N), 1153 (S=O *str*); LC-MS (ESI; $\text{M} + 1$) $^+$: 389.1.

4-((2(5-(4-Hydroxyphenyl)-3-methyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl) amino)benzenesulphonamide (5s)

Yield: 77%; R_f : 0.50 (chloroform:methanol 9:1); M.P.: 243–246 °C; purity (HPLC): 99.57%; $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ ppm: 9.52 (s, 1H, Ar-OH), 7.55–7.53 (dd, 2H, Ar-H), 7.36–7.3 (dd, 2H, Ar-H), 6.92–6.86 (dd, 1H, H_b), 6.84 (s, 2H, $\text{SO}_2\text{-NH}_2$), 6.76–6.74 (dd, 2H, Ar-H), 6.67–6.64 (dd, 2H, Ar-H), 6.54–6.53 (dd, 1H, H_x), 6.52–6.49 (t, 1H, Ar-NH), 4.23–4.21 (dd, 2H, CH_2), 3.96–3.95 (dd, 1H, CHH_a), 2.06 (s, 3H, CH_3); IR (ATR) cm^{-1} : 3389 (NH *str* of NH_2), 3315 (NH *str*), 3263 (OH *str*), 3020 (Ar C-H *str*), 2902 (aliphatic C-H *str*), 1680 (C=O *str*), 1602 (C=N), 1143 (S=O *str*); LC-MS (ESI; $\text{M} + 1$) $^+$: 389.1.

4-((2-(5-(4-(Dimethylamino)phenyl)-3-methyl-4,5-dihydro-1Hpyrazol-1-yl)-2-oxoethyl) amino)benzenesulphonamide (5t)

Yield: 86%; R_f : 0.7 (chloroform:methanol 9:1); M.P.: 239–240 °C; purity (HPLC): 99.26%; $^1\text{H-NMR}$ (400 MHz, DMSO-d_6) δ ppm: 7.53–7.51 (dd, 2H, Ar-H), 7.43–7.41 (dd, 2H, Ar-H), 6.99–6.95 (dd, 1H, H_b), 6.93 (s, 2H, $\text{SO}_2\text{-NH}_2$), 6.72–6.70 (dd, 2H, Ar-H), 6.72–6.70 (dd, 1H, H_x), 6.68–6.66 (dd, 2H, Ar-H), 6.50–6.47 (t, 1H, Ar-NH), 4.25–4.24 (dd, 2H, CH_2), 3.97–3.95 (dd, 1H, CHH_a), 2.07 (s, 3H, CH_3); IR (ATR) cm^{-1} : 3325 (N-H *str* of NH_2), 3236 (N-H *str*), 3107 (Ar C-H *str*), 2899 (aliphatic C-H *str*), 1660 (C=O *str*), 1602 (C=N *str*), 1311 (C-N *ter. amine str*), 1238 (C-N *aromatic str*) 1153 (S=O *str*); LC-MS (ESI; $\text{M} + 1$) $^+$: 416.1.

4-((2-(3-Methyl-5-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl) amino)benzenesulphonamide (5u)

Yield: 82%; R_f: 0.63 (chloroform:methanol 9:1); M.P.: 197–199 °C; purity (HPLC): 99.099%; ¹H-NMR (400 MHz, DMSO-d₆) δ ppm: 7.55–7.53 (dd, 2H, Ar-H), 7.38–7.37 (dd, 1H, Ar-H), 7.13–7.08 (dd, 1H, Ar-H), 7.08–7.06 (dd, 1H, H_b), 7.01–7.00 (dd, 1H, Ar-H), 6.83 (s, 2H, SO₂-NH₂), 6.66–6.64 (dd, 2H, Ar-H), 6.46–6.45 (dd, 1H, H_x), 6.26 (t, 1H, Ar-NH), 4.23–4.22 (dd, 2H, CH₂), 3.97–3.96 (dd, 1H, CHH₃), 2.05 (s, 3H, CH₃); IR (ATR) cm⁻¹: 3402 (NH str of NH₂), 3296 (NH str), 3074 (Ar C-H str), 2881 (aliphatic C-H str), 1691 (C=O str), 1600 (C=N), 1143 (S=O str); LC-MS (ESI; M + 1)⁺: 379.1.

4-((2-(2-Benzylidenehydrazinyl)-2-oxoethyl) amino) benzenesulphonamide (6a)

Yield: 80%; R_f: 0.69; M.P.: 223–226 °C; ¹H-NMR (400 MHz, DMSO-d₆) δ ppm: 11.51 (s, 1H, NH), 8.02 (s, 1H, CH), 7.72–7.67 (m, 4H), 7.45–7.38 (m, 5H), 6.87 (s, 2H, NH₂), 6.32 (t, 1H, NH), 4.33 (d, 2H, CH₂); ¹³C-NMR (400 MHz, DMSO-d₆) δ ppm: 43.49 (CH₂), 170.60 (C=O), 143.67 (N=CH); IR (ATR) cm⁻¹: 3313 (N-H str of NH₂), 3255 (N-H str), 3173 (Ar C-H str), 3070 (aliphatic C-H str), 1681 (C=N str), 1145 (S=O str); LC-MS (ESI; M + 1)⁺: 333.2.

4-((2-(2-(4-Methylbenzylidene) hydrazinyl)-2-oxoethyl)amino)benzene sulphonamide (6b)

Yield: 85%; R_f: 0.75; M.P.: 220–225 °C. ¹H-NMR (400 MHz, DMSO-d₆) δ ppm: 11.44 (s, 1H, NH), 7.98 (s, 1H, CH), 7.59–7.57 (m, 4H), 7.23–7.19 (dd, 2H), 6.86 (s, 2H, NH₂), 6.70–6.65 (dd, 2H), 6.30 (t, 1H, NH), 4.29 (d, 2H, CH₂), 2.39 (s, 3H, CH₃); ¹³C-NMR (400 MHz, DMSO-d₆) δ ppm: 43.47 (CH₂), 170.44 (C=O), 143.78 (N=CH), 21.03 (CH₃); IR (ATR) cm⁻¹: 3319 (N-H str of NH₂), 3257 (N-H str), 3194 (Ar C-H str), 3078 (aliphatic C-H str), 1683 (C=N str), 1145 (S=O str); LC-MS (ESI; M)⁺: 346.9.

4-((2-(2-(2-Hydroxybenzylidene)hydrazinyl)-2-oxoethyl) amino) benzenesulphonamide (6c)

Yield: 85%; R_f: 0.82; M.P.: 213–216 °C. ¹H-NMR (400 MHz, DMSO-d₆) δ ppm: 11.50 (s, 1H, NH), 10.01 (s, 1H, OH), 8.31 (s, 1H, CH), 7.58–7.54 (dd, 2H), 7.45–7.43 (d, 1H), 7.27–7.21 (d, 1H), 6.86 (s, 2H, NH₂), 6.69–6.65 (m, 4H), 6.41 (t, 1H, NH), 4.29 (d, 2H, CH₂); ¹³C-NMR (400 MHz, DMSO-d₆) δ ppm: 43.48 (CH₂), 170.10 (C=O), 144.10 (N=CH); IR (ATR) cm⁻¹: 3443 (O-H str), 3369 (N-H str of NH₂), 3265 (N-H str), 3113 (Ar C-H str), 3093 (aliphatic C-H str), 1681 (C=N str), 1120 (S=O str); LC-MS (ESI; M)⁺: 348.3.

4-((2-Oxo-2-(2-(pyridin-2-ylmethylene) hydrazinyl) ethyl) amino) benzenesulphonamide (6d)

Yield 80%; R_f: 0.78; M.P.: 245–250 °C. ¹H-NMR (400 MHz, DMSO-d₆) δ ppm: 11.74 (s, 1H, NH), 8.02 (s, 1H, CH), 7.95–7.93 (m, 4H), 7.86–7.79 (d, 1H), 7.39–7.36 (d, 1H), 6.91 (s, 2H, NH₂), 6.46 (t, 1H, NH), 4.36 (d, 2H, CH₂); ¹³C-NMR (400 MHz, DMSO-d₆) δ ppm: 43.50 (CH₂), 170.82 (C=O), 144.19 (N=CH); IR (ATR) cm⁻¹: 3354 (N-H str of NH₂), 3236 (N-H str), 3097 (Ar C-H str), 3072 (aliphatic C-H str), 1685 (C=N str), 1145 (S=O str); LC-MS (ESI; M + 1)⁺: 334.3.

4-((2-(2-(3,4-Dimethoxybenzylidene)hydrazinyl)-2-oxoethyl)amino) benzenesulphonamide (6e)

Yield: 86%; R_f: 0.84; M.P.: 230–233 °C. ¹H-NMR (400 MHz, DMSO-d₆) δ ppm: 11.40 (s, 1H, NH), 7.94 (s, 1H, CH), 7.57–7.54 (m, 4H), 7.20–6.94 (m, 3H), 6.88 (s, 2H, NH₂), 6.69 (t, 1H, NH), 4.31 (d, 2H,

CH₂), 3.85 (s, 6H, 2-OCH₃); ¹³C-NMR (400 MHz, DMSO-d₆) δ ppm: 43.48 (CH₂), 170.28 (C=O), 143.46 (N=CH), 55.11 (2O-CH₃); IR (ATR) cm⁻¹: 3306 (N-H str of NH₂), 3248 (N-H str), 3171 (Ar C-H str), 3086 (aliphatic C-H str), 1683 (C=N str), 1143 (S=O str), 1255 (C-O str); LC-MS (ESI; M + 1)⁺: 393.29.

4-((2-(2-(4-Methoxybenzylidene) hydrazinyl)-2-oxoethyl) amino) benzenesulphonamide (6f)

Yield: 85%; R_f: 0.79; M.P.: 240–245 °C. ¹H-NMR (400 MHz, DMSO-d₆) δ ppm: 11.40 (s, 1H, NH), 7.96 (s, 1H, CH), 7.66–7.61 (m, 4H), 6.99–6.95 (dd, 2H), 6.90 (s, 2H, NH₂), 6.70–6.65 (dd, 2H), 6.43–6.41 (t, 1H, NH), 4.29 (d, 2H, CH₂), 3.81 (s, 3H, OCH₃); ¹³C-NMR (400 MHz, DMSO-d₆) δ ppm: 43.52 (CH₂), 170.37 (C=O), 143.71 (N=CH), 55.49 (O-CH₃); IR (ATR) cm⁻¹: 3294 (N-H str of NH₂), 3275 (N-H str), 3198 (Ar C-H str), 3099 (aliphatic C-H str), 1680 (C=N str), 1143 (S=O str); LC-MS (ESI; M)⁺: 362.9.

4-((2-(2-(4-Hydroxybenzylidene)hydrazinyl)-2-oxoethyl) amino) benzenesulphonamide (6g)

Yield 80%; R_f: 0.81; M.P.: 214–218 °C. ¹H-NMR (400 MHz, DMSO-d₆) δ ppm: 11.25 (s, 1H, NH), 9.73 (s, 1H, OH), 7.85 (s, 1H, CH), 7.50–7.43 (m, 4H), 6.77–6.72 (dd, 2H), 6.63–6.60 (dd, 2H), 6.82 (s, 2H, NH₂), 6.58 (t, 1H, NH), 4.20 (d, 2H, NH); ¹³C-NMR (400 MHz, DMSO-d₆) δ ppm: 43.48 (CH₂), 170.10 (C=O), 144.10 (N=CH); IR (ATR) cm⁻¹: 3392 (O-H str), 3313 (N-H str of NH₂), 3292 (N-H str), 3211 (Ar C-H str), 3022 (aliphatic C-H str), 1685 (C=N str), 1136 (S=O str); LC-MS (ESI; M + 1)⁺: 349.3.

4-((2-(2-(4-Hydroxy-3,5-dimethoxybenzylidene)hydrazinyl)-2-oxoethyl)amino) benzenesulphonamide (6h)

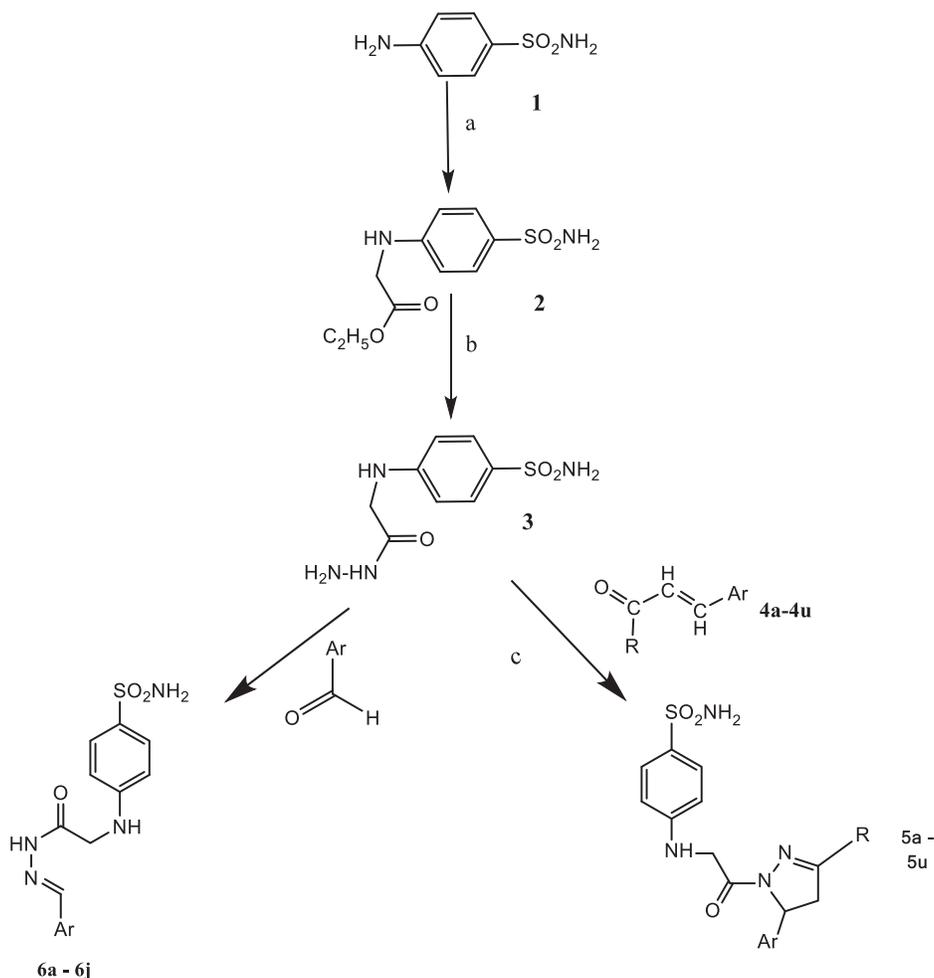
Yield: 85%; R_f: 0.85; M.P.: 255–260 °C. ¹H-NMR (400 MHz, DMSO-d₆) δ ppm: 11.33 (s, 1H, NH), 9.83 (s, 1H, OH), 7.82 (s, 1H, CH), 7.51–7.47 (dd, 2H), 6.83–6.81 (dd, 2H), 6.88 (s, 2H, NH₂), 6.63–6.56 (s, 2H), 6.35 (t, 1H, NH), 4.26 (d, 2H, CH₂), 3.78 (s, 6H, 2OCH₃); ¹³C-NMR (400 MHz, DMSO-d₆) δ ppm: 43.48 (CH₂), 170.29 (C=O), 147.98 (N=CH), 55.95 (O-CH₃); IR (ATR) cm⁻¹: 3408 (O-H str), 3304 (N-H str of NH₂), 3279 (N-H str), 3196 (Ar C-H str), 3106 (aliphatic C-H str), 1685 (C=N str), 1143 (S=O str), 1219 (C-O str), LC-MS (ESI; M + 1)⁺: 409.3.

4-((2-Oxo-2-(2-(3,4,5-trimethoxybenzylidene) hydrazinyl)ethyl) amino) benzenesulphonamide (6i)

Yield: 80%; R_f: 0.80; M.P.: 250–253 °C. ¹H-NMR (400 MHz, DMSO-d₆) δ ppm: 11.33 (s, 1H, NH), 7.82 (s, 1H, CH), 7.51–7.47 (dd, 2H), 6.83–6.81 (dd, 2H), 6.88 (s, 2H, NH₂), 6.63–6.56 (dd, 2H), 6.35 (t, 1H, NH), 4.26 (d, 2H, CH₂), 3.78 (s, 6H, 2OCH₃), 3.82 (s, 3H, OCH₃); ¹³C-NMR (400 MHz, DMSO-d₆) δ ppm: 40.21 (CH₂), 170.47 (C=O), 143.62 (N=CH), 55.11 (O-CH₃), 60.03 (2O-CH₃); IR (ATR) cm⁻¹: 3304 (N-H str of NH₂), 3271 (N-H str), 3201 (Ar C-H str), 3107 (aliphatic C-H str), 1683 (C=N str), 1144 (S=O str), 1236 (C-O str); LC-MS (ESI; M + 1)⁺: 423.3.

4-((2-(2-(2,3-Dimethoxybenzylidene)hydrazinyl)-2-oxoethyl)amino)-benzenesulphonamide (6j)

Yield: 80%; R_f: 0.77; M.P.: 240–244 °C. ¹H-NMR (400 MHz, DMSO-d₆) δ ppm: 11.40 (s, 1H, NH), 7.94 (s, 1H, CH), 7.57–7.54 (dd, 2H), 7.32–7.31 (dd, 2H), 7.20–6.94 (m, 3H), 6.88 (s, 2H, NH₂), 6.69–6.65 (t, 1H, NH), 4.31 (d, 2H, CH₂), 3.85 (s, 6H, OCH₃); ¹³C-NMR (400 MHz, DMSO-d₆) δ ppm: 43.48 (CH₂), 170.28 (C=O), 143.46



Scheme 1. Synthesis of compounds **5a–5u** and **6a–6j**. Reagents and conditions were: (a) Br-CH₂COOC₂H₅, K₂CO₃, EtOH, reflux, 12 h; (b) NH₂NH₂·H₂O, EtOH, reflux, 4–6 h. (c) For compounds **5a–5k** EtOH, KOH, reflux, 4 h, overnight stirring and for **5l–5u** glacial acetic acid, reflux.

(N=CH), 55.11 (2O-CH₃); IR (ATR) cm⁻¹: 3387 (N-H *str* of NH₂), 3255 (N-H *str*), 3186 (Ar C-H *str*), 3070 (aliphatic C-H *str*), 1687 (C=N*str*), 1172 (S=O *str*), 1230 (C-O *str*); LC-MS (ESI; M)⁺: 392.9.

and the Cheng-Prusoff algorithm by using non-linear least squares fitting as reported earlier^{21–24}.

2.2. CA activity/inhibition studies

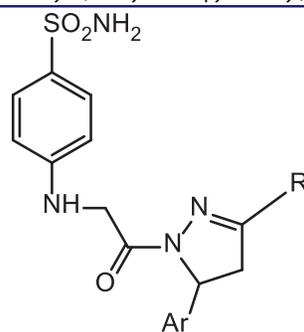
An Sx.18Mv-R Applied Photophysics (Oxford, UK) stopped-flow instrument has been used to assay the catalytic activity of various CA isozymes for CO₂ hydration reaction²⁰. Phenol red (at a concentration of 0.2 mM) was used as indicator, working at the absorbance maximum of 557 nm, with 10 mM Hepes (pH 7.5) or 10 mM Tris (pH 8.5) as buffers, and 0.1 M Na₂SO₄ (for maintaining constant ionic strength, which is not inhibitory against these enzymes), following the CA-catalysed CO₂ hydration reaction for a period of 10 s at 25 °C. The CO₂ concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and activation constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial rate. The uncatalysed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitors (10 mM) were prepared in distilled-deionised diluted to 1 nM using the assay buffer. Inhibitor and enzyme solutions were pre-incubated together for 15 min (standard assay at room temperature) prior to assay, in order to allow for the formation of the enzyme-inhibitor complex. The inhibition constant (K_i), was obtained by considering the classical Michaelis-Menten equation

3. Results and discussion

3.1. Chemistry

The key intermediate **1** (ethyl 2-((4-sulphamoylphenyl) amino) acetate) was obtained in good yields by the reaction of sulphanilamide with ethyl bromoacetate in the presence of potassium carbonate (no sulphonamide N-alkylation occurred), **Scheme 1**. During optimisation, the same reaction was performed with sodium carbonate instead of potassium carbonate. The results were not satisfactory, and when the equimolar ratio of both the reactants in the presence of K₂CO₃ and ethanol as a solvent was used to obtain compound **1**. 4-((2-Hydrazinyl-2-oxoethyl)amino)benzene sulphonamide (**2**) was obtained by the reaction of hydrazine hydrate with **1** in equimolar ratios. The reaction was refluxed for 3 h at 70 °C producing the compound with yields of 70–72%. Reaction of **2** with various substituted aromatic aldehydes afforded derivatives **6**. When using unsaturated aldehydes, a cyclisation reaction occurred after the Schiff base formation, leading to compounds **5** (**Scheme 1**).

The structures of 4-((2-(3-alkyl/aryl-5-ary/heteroaryl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)amino)benzenesulphonamides **5a–5u** were confirmed by mass spectrometry (MS), Fourier-transform infrared spectroscopy (FTIR) and ¹H and ¹³C-nuclear magnetic

Table 1. Physicochemical properties of 4-((2-(3-alkyl/aryl-5-ary/heteroaryl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)amino)benzenesulphonamide derivatives (**5a–5u**).**5a–5u**

Compound code	R	Ar	Molecular formula	Mol. Wt.	Yield (%)
5a	p-Cl-C ₆ H ₄	Phenyl	C ₂₃ H ₂₁ N ₄ O ₃ SCl	468.10	70
5b		4-Fluorophenyl	C ₂₃ H ₂₀ N ₄ O ₃ SClF	486.09	68
5c		4-Bromophenyl	C ₂₃ H ₂₀ N ₄ O ₃ SClBr	547.85	75
5d		4-Methylphenyl	C ₂₄ H ₂₃ N ₄ O ₃ SCl	482.12	70
5e		Pyridin-4-yl	C ₂₂ H ₂₀ N ₅ O ₃ SCl	469.94	71
5f		2-Chlorophenyl	C ₂₃ H ₂₀ N ₄ O ₃ SCl ₂	503.40	67
5g		2-Hydroxyphenyl	C ₂₃ H ₂₁ N ₄ O ₄ SCl	484.96	72
5h		3,4-Dimethoxy phenyl	C ₂₅ H ₂₅ N ₄ O ₅ SCl	529.01	70
5i		Thiophen-2-yl	C ₂₁ H ₁₉ N ₄ O ₃ S ₂ Cl	474.98	68
5j		Anthran-9-yl	C ₃₁ H ₂₅ N ₄ O ₃ SCl	569.13	71
5k		Indol-3-yl	C ₂₅ H ₂₂ N ₅ O ₃ SCl	507.99	73
5l	Me	Phenyl	C ₁₈ H ₂₀ N ₄ O ₃ S	372.44	79
5m		4-Methoxyphenyl	C ₁₉ H ₂₂ N ₄ O ₄ S	402.46	85
5n		4-Chlorophenyl	C ₁₈ H ₁₉ N ₄ O ₃ SCl	406.88	85
5o		4-Methylphenyl	C ₁₉ H ₂₂ N ₄ O ₃ S	386.46	78
5p		4-Fluorophenyl	C ₁₈ H ₁₉ N ₄ O ₃ SF	390.43	76
5q		2-Chlorophenyl	C ₁₈ H ₁₉ N ₄ O ₃ SCl	406.88	76
5r		2-Hydroxyphenyl	C ₁₈ H ₂₀ N ₄ O ₄ S	388.44	86
5s		4-Hydroxyphenyl	C ₁₈ H ₂₀ N ₄ O ₄ S	388.44	77
5t		4-(Dimethyl)amine phenyl	C ₂₀ H ₂₅ N ₅ O ₃ S	415.5	86
5u		2-Thienyl	C ₁₆ H ₁₈ N ₄ O ₃ S ₂	378.46	82

resonance (NMR). The structures of compounds were analysed by FTIR spectra which revealed that N–H stretches of amines in the region 3500–3000 cm⁻¹. Spectra revealed presence of C=O stretching vibrations of amide in the region 1695–1630 cm⁻¹, aliphatic C–H stretching vibration was observed in 2920–2800 cm⁻¹ and C=N stretching vibration was observed at 1615–1564 cm⁻¹. Further evidence for formation of target compound was obtained from ¹H-NMR spectra which provided diagnostic tool for the positional elucidation of the protons. The Ar–NH proton was appeared at δ = 6.58 ppm as triplet. The formed pyrazoline was confirmed with doublet of doublet for CH₂ giving signal δ = 4.0–3.8 ppm (H_a), H_b at δ 7.1–6.6 ppm (compounds **5l–5u**) and at δ = 3.26–3.13 ppm (compounds **5a–5k**), CH giving signal at δ = 7.4–7.1 ppm (H_x of series 5l–5u) and δ = 5.61–5.56 ppm (H_x of series **5a–5k**). The characteristic doublet signal of aromatic protons was observed between δ = 7.9 and 6.6 ppm. The NH protons of SO₂–NH₂ as singlet between δ = 6.94 and 6.80 ppm was observed. Singlet in the range of δ = 6.59–6.2 ppm for Ar–NH protons was observed. The characteristic singlet signal of CH₃ protons in series 5l–5u was observed between δ = 2.1 and 2.04 ppm. Mass spectroscopy was done for newly synthesised compounds. The base peak *m/z* for the compounds were found as (M + 1)⁺ with respective to their molecular weight except for 5i it is was found to be (M)⁺.

High-performance liquid chromatography (HPLC) was done for newly synthesised compounds. Using area normalisation method, the percent purity for the compounds was found to be above 88%. The structures of novel Schiff bases **6a–6j** were confirmed by MS, FTIR and ¹H and ¹³C-NMR. The IR spectra displayed an intense

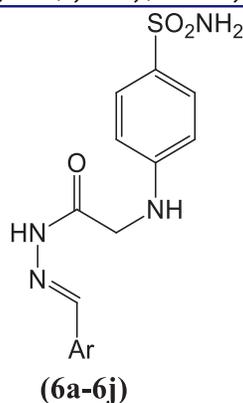
absorption band in the range of 1615–1630 cm⁻¹, characteristic of the carbonyl groups. Additionally, intense bands, originating from the stretching vibration of the C=N group of the azomethine were observed at 1685 and 3313 cm⁻¹ for N–H *str* of NH₂ following the aliphatic C–H *str* displayed vibration at 3022 cm⁻¹. Further, we observed ¹H and ¹³C-NMR interpretation for singlet peak at chemical shift δ range in 7.95–8.02 ppm and 143.71–144.10 ppm, confirming the presence of azomethine group in the compound, respectively (Tables 1 and 2).

3.2. CA inhibition

The compounds **5** and **6** reported here were investigated as inhibitors of three CAs involved in crucial physiologic processes and known to act as drug targets, i.e. the human (h) isoforms hCA I and II (belonging to the α -CA class) and the bacterial enzyme mtCA3 from *Mycobacterium tuberculosis* (a β -class CA) (Table 3). Acetazolamide (AAZ), a clinically used sulphonamide has been employed as standard inhibitor in the assay.

As seen from data of Table 3, all investigated compounds inhibited the three enzymes, but generally with a medium potency. Thus, the inhibition constants (K_is) were in the range of 54.6 nM–1.8 μ M against hCA I, in the range of 32.1 nM–5.5 μ M against hCA II and of 127 nM–2.12 μ M against mtCA 3, showing a quite flat structure–activity relationship, except for some particular cases which will be discussed in detail.

Thus, for hCA I, the best inhibitors were **5d**, **5j** and **6c**, with K_is ranging between 54.6 and 93.4 nM, being thus much better

Table 2. Physicochemical properties of 4-((2-(aryl(methylidene)hydrazinyl)-2-oxoethyl)amino) benzene sulphonamide derivatives (**6a–6j**).

Compound code	Ar	Molecular Formula	Molecular weight	Yield (%)
6a	Phenyl	C ₁₅ H ₁₆ N ₄ O ₃ S	332.38	80
6b	4-Methylphenyl	C ₁₆ H ₁₈ N ₄ O ₃ S	346.40	85
6c	2-Hydroxyphenyl	C ₁₅ H ₁₆ N ₄ O ₄ S	348.38	85
6d	Pyridin-2-yl	C ₁₄ H ₁₅ N ₅ O ₃ S	333.37	80
6e	3,4-Dimethoxyphenyl	C ₁₇ H ₂₀ N ₄ O ₅ S	392.43	86
6f	4-Methoxyphenyl	C ₁₆ H ₁₈ N ₄ O ₄ S	362.40	85
6g	4-Hydroxyphenyl	C ₁₅ H ₁₆ N ₄ O ₄ S	348.38	80
6h	4-Hydroxy-3,5-dimethoxy	C ₁₇ H ₂₀ N ₄ O ₆ S	408.43	85
6i	3,4,5-Trimethoxy phenyl	C ₁₈ H ₂₂ N ₄ O ₆ S	422.46	80
6j	2,3-Dimethoxyphenyl	C ₁₇ H ₂₀ N ₄ O ₅ S	392.43	80

Table 3. hCA I, II and mtCA 3 inhibition data of compounds **5** and **6** reported in the article, by a stopped-flow CO₂ hydrase assay²⁰.

Compound	K _i (nM) ^a		
	hCA I	hCA II	mtCA 3
5a	306	282	1800
5b	441	500	486
5c	634	2850	732
5d	93.4	426	175
5e	175	42.3	138
5f	445	433	215
5g	174	578	147
5h	280	424	186
5i	276	373	233
5j	91.8	2020	157
5k	440	392	127
5l	760	810	1583
5m	2374	5545	>10,000
5n	835	944	1288
5o	898	3461	275
5p	665	903	250
5q	742	648	623
5r	721	83.1	1592
5s	738	73.6	265
5t	842	5154	1735
5u	798	126	1452
6a	220	549	>10,000
6b	266	914	>10,000
6c	54.6	32.1	2145
6d	316	418	>10,000
6e	1802	758	736
6f	648	703	2128
6g	327	255	254
6h	292	483	2003
6i	890	446	216
6j	785	240	232
AAZ	250	12.1	104

AAZ was used as standard drug.

^aMean from three different assays. The errors were in the range of ±10% of the reported values.

inhibitors compared to the standard acetazolamide (K_i of 250 nM, Table 3). These compounds incorporate *p*-chlorophenyl and *p*-tolyl moieties (**5d**), *p*-chlorophenyl and 9-anthranil (**5j**) moieties, and

the 2-hydroxyphenyl fragment in the case of **6c**, which are in fact not very different from those found in compounds showing a much worse inhibitory pattern (e.g. **5c**, **5k**, **6e**, etc.). Thus, the explanation that we propose is that the quite long and flexible linker between the benzenesulphonamide fragment and the imine or heterocyclic parts of the molecule, affords for a multitude of diverse orientation of the tail present in these compounds, which is probably detrimental to a tight binding, except for the few cases mentioned above, i.e. **5d**, **5j** and **6c**, for which probably some of these conformations assure good interactions with the enzyme active site. However, for the majority of these derivatives, these various conformations/orientations may be not favourable, which explain why most of them have inhibition constants in the high nanomolar–micromolar range (Table 3).

More or less the same situation was observed for the inhibition of hCA II, but for this isoform the most effective inhibitors were **5e**, **5r**, **5s** and **6c**, with K_s ranging between 32.1 and 83.1 nM (Table 3). Compound **5e** is the only derivative incorporating a 4-pyridyl moiety, which seems to be effective in inducing strong hCA II inhibitory effects, whereas the remaining ones incorporate 2- or 4-hydroxyphenyl groups. However, the change of these groups to halogens or to methoxy leads to a strong loss of inhibitory effects. The explanation we propose is the same as above for the discussion of hCA I inhibition data. mtCA3 was also effectively inhibited by several of the new compounds, such as **5e**, **5g**, **5j** and **5k**, which showed K_s ranging between 127 and 157 nM (acetazolamide has an inhibition constant of 104 nM, being only slightly more effective compared to these sulphonamides). However, the largest majority of these derivatives showed K_s in the range of 250 nM–2.12 μM, being thus much less effective inhibitors.

4. Conclusion

We report here a new series of sulphonamide derivatives, which was obtained by reaction of a hydrazide derivative with aromatic/heterocyclic aldehydes, followed by an eventual cyclisation to a

five-membered heterocyclic system. 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 hCA I and hCA II and the bacterial enzyme mtCA3 from *Mycobacterium tuberculosis*. The compounds acted as effective-medium potency inhibitors, with K_s in the range of 54.6 nM–1.8 μ M against hCA I, in the range of 32.1 nM–5.5 μ M against hCA II and of 127 nM–2.12 μ M against mtCA 3.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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