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RESEARCH PAPER

4-(3-Alkyl/benzyl-guanidino)benzenesulfonamides as selective carbonic anhydrase VII inhibitors

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ABSTRACT

The treatment of chronic neuropathic pain remains one of the most challenging of all neurological diseases and very much an art. There exists no consensus for the optimal management of this condition at the moment. Gaining inspiration from recent studies which pointed out the involvement of brain-associated carbonic anhydrase (CA, EC 4.2.1.1) isoform VII in the pathology of various neurodegenerative diseases, which highlighted the relationship between selective inhibition of this isozyme and relieve of neuropathic pain, herein we report the synthesis and CA VII inhibitory activity of novel 4-(3-alkyl/benzyl-guanidino)benzenesulfonamides. Ten benzyl-substituted and five alkyl-substituted 4-guanidinobenzenesulfonamide derivatives were obtained, some of which (**7c**, **7h**, **7m** and **7o**) exhibited satisfactory selectivity towards CA VII over CA I and II, with K_I-s in the subnanomolar range and good selectivity indexes for inhibiting the target versus the off-target isoforms.

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1. Introduction

As its name suggests, carbonic anhydrases (CAs, EC 4.2.1.1) are enzymes which catalyse the reversible hydration/dehydration of carbon dioxide (CO_2) to bicarbonate (HCO_3^-) and protons (H^+)[1]. These enzymes are constitutively produced in all tissues, organs and cells and comprise 15 different isoforms (CA I, II, III, VA, VB, VI, VII, VIII, IX, X, XI, XII, XIII, XIV and XV) in humans, according to their subcellular localisation[2]. There is increasing evidence that CAs play a key role in a variety of diseases, including edoema, epilepsy, cancer, glaucoma, haemolytic anaemia, obesity, sterility and other disorders[3]. Hence, isoform-selective targeting hCAs is an important approach for discovery and development of selective, effective and safe novel drugs[4].

Primary sulfonamides were discovered as CA inhibitors (CAIs) in the '40s of the last century, and majority of the drugs launched in the next decades (diuretics, antiepileptics, or antiglaucoma agents) belonged to this class of compounds or to their isosteres such as the sulfamates and sulfamides[5–9]. A major pitfall of the first generation of CAIs was their lack of isoform selectivity, keeping in mind that in humans are present at least 12 catalytically active and three acatalytic isoforms[5–13]. In last decade a discovery was made and the new generation of CAIs belonging to coumarins and sulfocoumarins and their bioisosteres showed significant isoform-selective inhibition profiles, as demonstrated in a number of studies[14–32]. This is principally due to the fact that these compounds possess a distinct inhibition mechanism compared to the sulfonamides, which coordinate to the zinc ion from the CA active site as anions[5–13]. Recently so-called tail approach also has proven to give considerable inhibition selectivity among CA isoforms in case of primary sulfonamides[31,32]. This approach was chosen also for this study.

Neuropathic pain is a neurological disorder caused by a lesion or disease affecting the peripheral or central nervous system[33]. Often, patients with chronic neuropathic pain experience severe and unrelenting pain; thus sometimes opioid analgesics are prescribed to relieve pain[34]. Anticonvulsant drugs acting at calcium channels (e.g. pregabalin and gabapentin) and antidepressant agents (e.g. duloxetine) are the first-line options for management of this pain[35,36]. However, their efficacies are not high, and also associated with several side effects. Therefore, undoubtedly there is an unmet medical need to discover a new pharmacological class for the treatment of neuropathic pain.

Although the mechanisms of neuropathic pain for big extend remain unclear, recent studies have highlighted the involvement of the brain-associated CA VII isoform in the pathology of this syndrome[37]. Thereby, isoform-selective CAVII inhibitors are recognised as promising agents for management of neuropathic pain. Needless to say that primary sulfonamides ($R\text{-}SO_2NH_2$) are the main class of CA inhibitors and logically utmost studies on the inhibition of CA VII are relying on the use of sulfonamide-based compounds[38]. Intriguingly, recent works by one of our groups indicated that the incorporation of guanidine moiety into sulfonamide-containing compounds resulting in CA inhibitors with enhanced efficiency and selectivity (Figure 1)[39–41]. Keeping these interesting facts in mind and in connection with our works on the field of CA inhibitors[16,19,27,42], we decided to synthesis a series of novel 4–(3-alkyl/benzyl-guanidino)benzenesulfonamides and investigate their inhibitory activity against CA VII (Figure 2).

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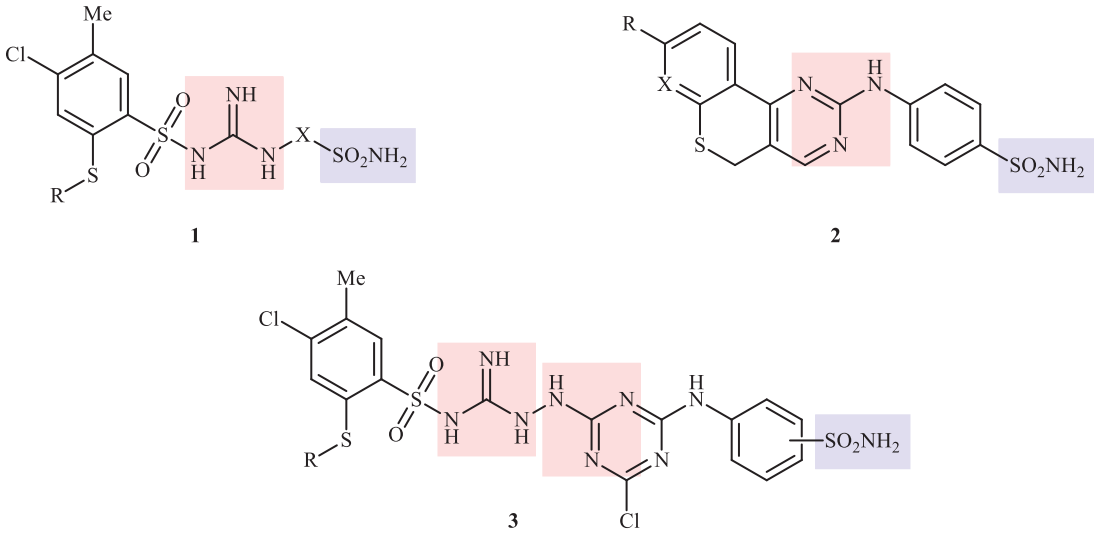


Figure 1. Selected examples of the guanidine-containing sulfonamide CAIs.

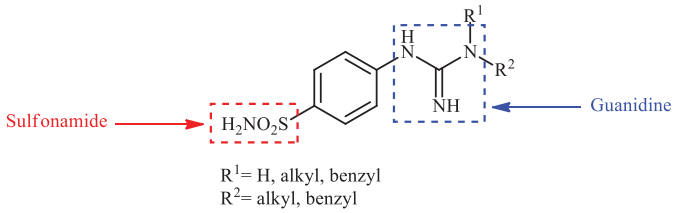


Figure 2. General structure of 4-(3-alkyl/benzyl-guanidino)benzenesulfonamides discussed in the paper.

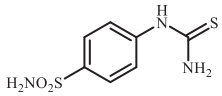
2. Experimental section

2.1. Chemistry

Reagents, starting materials and solvents were obtained from commercial sources and used as received. Thin-layer chromatography was performed on silica gel, spots were visualised with UV light (254 and 365 nm). NMR spectra were recorded on Bruker 300 spectrometer with chemical shifts values (δ) in ppm relative to TMS using the residual DMSO-d_6 signal (^{1}H 2.50; ^{13}C 39.52). High-resolution mass spectra (HRMS) were recorded on a mass spectrometer with a Q-TOF micro mass analyser using the ESI technique.

2.2. Synthesis

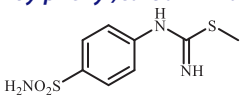
2.2.1. 4-Thioureidobenzenesulfonamide (5)



To a solution of 4-aminobenzensulfonamide (30 g, 174.3 mmol) in 3.5 M HCl (180 ml), which was heated at 70 °C and cooled to room temperature, KSCN (16.94 g, 174.3 mmol) was added and the mixture was refluxed for 3 h. After cooling to room temperature, the reaction mixture was diluted with ice-cold water. Solids were collected by filtration, washed with water and air dried to afford **5** (12.1 g, 31%) as white powder.

^{1}H NMR (300 MHz, DMSO-d_6) $\delta = 7.32$ (s, 2H), 7.69 (d, 2H, $J = 8.6$ Hz), 7.77 (d, 2H, $J = 8.6$ Hz), 10.02 (s, 1H) ppm ^{13}C NMR (75 MHz, DMSO-d_6) $\delta = 122.8$, 127.3, 139.8, 143.9, 182.8 ppm MS (ESI) $[M + H]^+$: *m/z* 232.0.

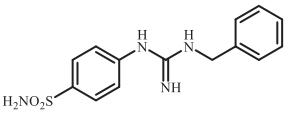
2.2.2. Methyl (4-sulfamoylphenyl)carbamimidothioate (6)



To a solution of 4-thioureidobenzenesulfonamide (**5**) (10.0 g, 43.28 mmol) in DMF (100 ml), MeI (2.69 ml, 43.28 mmol) at room temperature was added and the mixture was heated at 40 °C for 2.5 h. After cooling to room temperature water (150 ml) was added and the mixture was extracted with EtOAc (3 × 50 ml). Organic layer was washed with aq. sat. $NaHCO_3$ (2 × 50 ml) and aq. sat. NH_4Cl (50 ml), and dried over Na_2SO_4. Solvent evaporation in vacuum afforded **6** (7.43 g, 70%) as white powder.

^{1}H NMR (300 MHz, DMSO-d_6) $\delta = 2.37$ (s, 3H), 6.63 (s, 2H), 6.94 (s, 2H), 7.22 (s, 2H), 7.71 (d, 2H, $J = 8.4$ Hz) ppm ^{13}C NMR (75 MHz, DMSO-d_6) $\delta = 14.2$, 122.8, 127.7, 138.0, 153.9, 157.0 ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_8H_{12}N_3O_2S_2$) 246.0371. Found 246.0372.

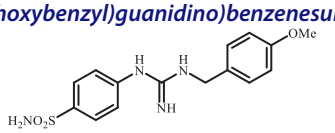
2.2.3. 4-(3-Benzyl-guanidino)benzenesulfonamide (7a)



To a solution of **6** (300 mg, 1.22 mmol) in DMSO (8 ml), benzylamine (1.066 ml, 9.76 mmol) was added and the reaction mixture was stirred in sealed tube at 130 °C for 2 h. After cooling to room temperature water (40 ml) was added and the mixture was extracted with EtOAc (3 × 20 ml). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was dissolved in *i*PrOH (5 ml) under gentle heating and product was precipitated by addition of hexanes (40 ml). Precipitate was collected by filtration and dried under vacuum to afford the **7a** as white solids (187 mg, 50%).

^{1}H NMR (300 MHz, DMSO-d_6 +D_2O) $\delta = 4.39$ (2H, s), 6.93 (d, 2H, $J = 7.8$ Hz), 7.29 (s, 1H), 7.37 (s, 4H), 7.65 (d, 2H, $J = 7.8$ Hz) ppm ^{13}C NMR (75 MHz, DMSO-d_6 +D_2O) $\delta = 44.4$, 123.1, 127.2, 127.3, 127.7, 128.7, 135.1, 140.7, 152.6, 154.9 ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_{14}H_{17}N_4O_2S$) 305.1072. Found 305.1078.

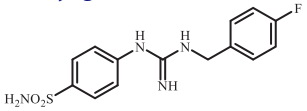
2.2.4. 4-(3-(4-Methoxybenzyl)guanidino)benzenesulfonamide (7b)



To a solution of **6** (300 mg, 1.22 mmol) in DMSO (8 ml), (4-methoxyphenyl)methanamine (1.275 ml, 9.76 mmol) was added and the reaction mixture was stirred in sealed tube at 130 °C for 2 h. After cooling to room temperature water (40 ml) was added and mixture was extracted with EtOAc (3 × 20 ml). The organic phase was dried over Na_2SO_4. Solvent evaporation under reduced pressure afforded **7b** (305 mg, 74%) as white powder.

^{1}H NMR (300 MHz, DMSO-d_6 + D_2O) $\delta = 3.74$ (s, 3H), 4.30 (s, 2H), 6.90 (s, 2H), 6.93 (s, 2H), 7.29 (d, 2H, $J = 8.4$ Hz), 7.64 (d, 2H, $J = 8.4$ Hz) ppm ^{13}C NMR (75 MHz, DMSO-d_6 + D_2O) $\delta = 44.7$, 56.3, 114.9, 123.8, 128.1, 129.9, 133.5, 135.7, 153.5, 155.9, 159.4 ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_{15}H_{19}N_4O_3S$) 335.1178. Found 335.1180.

2.2.5. 4-(3-(4-Fluorobenzyl)guanidino)benzenesulfonamide (7c)



To a solution of **6** (300 mg, 1.22 mmol) in DMSO (8 ml), (4-fluorophenyl)methanamine (1.110 ml, 9.76 mmol) was added and the reaction mixture was stirred in sealed tube at 130 °C for 2 h. After cooling to room temperature water (40 ml) was added and mixture was extracted with EtOAc (3 × 20 ml). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was dissolved in *i*PrOH (5 ml) under gentle warming and product was precipitated by addition of hexanes (40 ml). Precipitate was collected by filtration and dried in vacuum to afford **7c** (354 mg, 90%) as white powder.

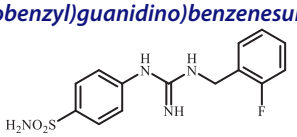
^{1}H NMR (300 MHz, DMSO-d_6 + D_2O) $\delta = 4.36$ (s, 2H), 6.92 (d, 2H, $J = 8.1$ Hz), 7.15–7.43 (m, 4H), 7.64 (d, 2H, $J = 8.1$ Hz) ppm ^{13}C NMR (75 MHz, DMSO-d_6 + D_2O) $\delta = 44.5$, 116.2 (d, $J = 20.9$ Hz), 123.8, 128.1, 130.5 (d, $J = 7.6$ Hz), 136.1, 137.7, 153.3, 155.2, 162.4 (d, $J = 240.6$ Hz) ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_{14}H_{16}N_4O_2FS$) 323.0978. Found 323.0990.

2.2.6. 4-(3-(3-Fluorobenzyl)guanidino)benzenesulfonamide (7d)

To a solution of **6** (300 mg, 1.22 mmol) in DMSO (8 ml), (3-fluorophenyl)methanamine (1.113 ml, 9.76 mmol) was added and the reaction mixture was stirred in sealed tube at 130 °C for 2 h. After cooling to room temperature water (40 ml) was added and mixture was extracted with EtOAc (3 × 20 ml). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was dissolved in *i*PrOH (5 ml) under gentle warming and product was precipitated by addition of hexanes (40 ml). Precipitate was collected by filtration and dried in vacuum to afford **7d** (286 mg, 72%) as white powder.

^{1}H NMR (300 MHz, DMSO-d_6 + D_2O) $\delta = 4.42$ (s, 2H), 6.93 (d, 2H, $J = 6.9$ Hz), 7.07–7.41 (m, 4H), 7.65 (d, 2H, $J = 6.9$ Hz) ppm ^{13}C NMR (75 MHz, DMSO-d_6 + D_2O) $\delta = 40.6$, 114.6 (d, $J = 30.7$ Hz), 114.9 (d, $J = 30.7$ Hz), 123.8, 124.4, 128.1, 131.4 (d, $J = 8.1$ Hz), 136.2, 144.8 (d, $J = 6.9$ Hz), 153.3, 155.0, 163.5 (d, $J = 241.5$ Hz) ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_{14}H_{16}N_4O_2SF$) 323.0978. Found 323.0986.

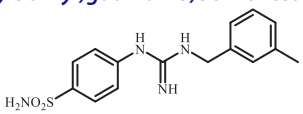
2.2.7. 4-(3-(2-Fluorobenzyl)guanidino)benzenesulfonamide (7e)



To a solution of **6** (300 mg, 1.22 mmol) in DMSO (8 ml), (2-fluorophenyl)methanamine (1.110 ml, 9.76 mmol) was added and the reaction mixture was stirred in sealed tube at 130 °C for 2 h. After cooling to room temperature water (40 ml) was added and mixture was extracted with EtOAc (3 × 20 ml). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was dissolved in *i*PrOH (5 ml) under gentle warming and product was precipitated by addition of hexanes (40 ml). Precipitate was collected by filtration and dried in vacuum to afford **7e** (193 mg, 49%) as white powder.

^{1}H NMR (300 MHz, DMS-O-d_6 + D_2O) $\delta = 4.43$ (s, 2H), 6.92 (d, 2H, $J = 8.3$ Hz), 7.127.50 (m, 4H), 7.64 (d, 2H, $J = 8.3$ Hz) ppm ^{13}C NMR (75 MHz, DMSO-d_6 + D_2O) $\delta = 39.1$, 116.2 (d, $J = 21.0$ Hz), 123.7, 125.5 (d, $J = 3.2$ Hz), 128.0 (d, $J = 6.6$ Hz), 128.2, 129.9 (d, $J = 8.19$ Hz), 130.7 (d, $J = 4.6$ Hz), 136.0, 153.1, 155.2, 160.3 (d, $J = 242.4$ Hz) ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_{14}H_{16}N_4O_2FS$) 323.0978. Found 323.0992.

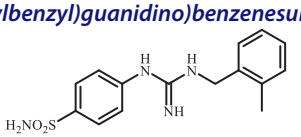
2.2.8. 4-(3-(3-Methylbenzyl)guanidino)benzenesulfonamide (7f)



To a solution of **6** (300 mg, 1.22 mmol) in DMSO (8 ml), *m*-tolylmethanamine (1.224 ml, 9.76 mmol) was added and the reaction mixture was stirred in sealed tube at 130 °C for 2 h. After cooling to room temperature water (40 ml) was added and mixture was extracted with EtOAc (3 × 20 ml). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was dissolved in *i*PrOH (5 ml) under gentle warming and product was precipitated by addition of hexanes (40 ml). Precipitate was collected by filtration and dried under vacuum to afford **7f** (252 mg, 65%) as white powder.

^{1}H NMR (300 MHz, DMSO-d_6 + D_2O) $\delta = 2.33$ (s, 3H), 4.36 (s, 2H), 6.94 (d, 2H, $J = 7.6$ Hz), 7.10–7.26 (m, 4H), 7.65 (d, 2H, $J = 7.6$ Hz) ppm ^{13}C NMR (75 MHz, DMSO-d_6 + D_2O) $\delta = 22.3$, 45.2, 123.8, 125.6, 128.0, 128.6, 129.2, 129.4, 136.0, 138.6, 141.3, 153.4, 155.4 ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_{15}H_{19}N_4O_2S$) 319.1229. Found 319.1241.

2.2.9. 4-(3-(2-Methylbenzyl)guanidino)benzenesulfonamide (7g)

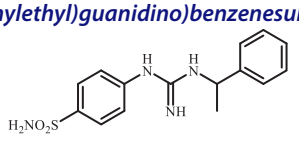


To a solution of **6** (300 mg, 1.22 mmol) in DMSO (8 ml), *o*-tolylmethanamine (1.210 ml, 9.76 mmol) was added and the reaction mixture was stirred in sealed tube at 130 °C for 2 h. After cooling to room temperature water (40 ml) was added and mixture was extracted with EtOAc (3 × 20 ml). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced

pressure. The residue was dissolved in *i*PrOH (5 ml) under gentle warming and product was precipitated by addition of hexanes (40 ml). Precipitate was collected by filtration and dried in vacuum to afford **7g** (315 mg, 81%) as white powder.

^{1}H NMR (300 MHz, DMSO-d_6 +D_2O) $\delta = 2.32$ (s, 3H), 4.36 (s, 2H), 6.94 (d, 2H, $J = 8.3$ Hz), 7.16–7.33 (m, 4 H), 7.65 (d, 2H, $J = 8.3$ Hz) ppm ^{13}C NMR (75 MHz, DMSO-d_6 +D_2O) $\delta = 19.8$, 43.5, 123.9, 127.1, 128.1, 128.1, 128.8, 131.3, 136.1, 137.0, 139.0, 153.5, 155.3 ppm HRMS (ESI) $[M+H]^+$: *m/z* calcd for ($C_{15}H_{19}N_4O_2S$) 319.1229. Found 319.1237.

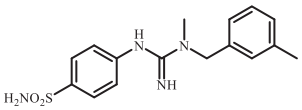
2.2.10. 4-(3-(1-Phenylethyl)guanidino)benzenesulfonamide (7h)



To a solution of **6** (300 mg, 1.22 mmol) in DMSO (8 ml), 1-phenylethanamine (1.258 ml, 9.76 mmol) was added and the reaction mixture was stirred in sealed tube at 130 °C for 2 h. After cooling to room temperature water (40 ml) was added and mixture was extracted with EtOAc (3 × 20 ml). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was dissolved in *i*PrOH (5 ml) under gentle warming and product was precipitated by addition of hexanes (40 ml). The precipitate was collected by filtration and dried in vacuum to afford **7h** (232 mg, 59%) as white powder.

^{1}H NMR (300 MHz, DMSO-d_6 +D_2O) $\delta = 1.41$ (d, 3H, $J = 5.9$ Hz), 4.97 (q, 1H, $J = 5.9$ Hz), 6.85 (d, 2H, $J = 7.6$ Hz), 7.25 (s, 1H), 7.38 (app s, 4H), 7.62 (d, 2H, $J = 7.6$ Hz) ppm ^{13}C NMR (75 MHz, DMSO-d_6 +D_2O) $\delta = 24.5$, 50.6, 123.8, 127.2, 127.8, 128.1, 129.6, 135.9, 146.8, 152.7, 155.5 ppm HRMS (ESI) $[M+H]^+$: *m/z* calcd for ($C_{15}H_{19}N_4O_2S$) 319.1229. Found 319.1225.

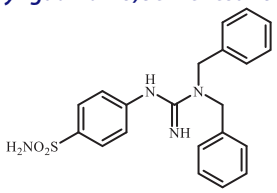
2.2.11. 4-(3-Methyl-3-(3-methylbenzyl)guanidino)benzenesulfonamide (7i)



To a solution of **6** (300 mg, 1.22 mmol) in DMSO (8 ml), *N*-methyl-1-(*m*-tolyl)methanamine (1.466 ml, 9.76 mmol) was added and the reaction mixture was stirred in sealed tube at 130 °C for 2 h. After cooling to room temperature water (40 ml) was added and mixture was extracted with EtOAc (3 × 20 ml). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residual solid was washed with *i*PrOH (20 ml) and dried in vacuum to afford **7i** (292 mg, 72%) as white powder.

^{1}H NMR (300 MHz, DMSO-d_6 +D_2O) $\delta = 2.33$ (s, 3H), 2.88 (s, 3H), 4.59 (s, 2H), 6.90 (d, 2H, $J = 8.4$ Hz), 7.09-7.29 (m, 4H), 7.67 (d, 2H, $J = 8.4$ Hz) ppm ^{13}C NMR (75 MHz, DMSO-d_6 +D_2O) $\delta = 22.4$, 36.2, 53.2, 123.7, 125.6, 128.2, 128.8, 129.1, 129.6, 135.5, 138.8, 139.9, 154.5, 156.4 ppm HRMS (ESI) $[M+H]^+$: *m/z* calcd for ($C_{16}H_{21}N_4O_2S$) 333.1385. Found 333.1395.

2.2.12. 4-(3,3-Dibenzyl-guanidino)benzenesulfonamide (7j)



To a solution of **6** (300 mg, 1.22 mmol) in DMSO (8 ml), dibenzylamine (1.876 ml, 9.76 mmol) was added and the reaction mixture was stirred in sealed tube at 130 °C for 2 h. After cooling to room temperature water (40 ml) was added and mixture was extracted with EtOAc (3 × 20 ml). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was dissolved in *i*PrOH (5 ml) under gentle warming and product was precipitated by addition of hexanes (40 ml). The precipitate was collected by filtration and dried in vacuum to afford **7j** (279 mg, 58%) as white powder.

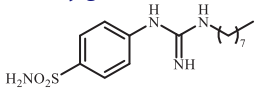
^{1}H NMR (300 MHz, DMSO-d_6 +D_2O) $\delta = 4.58$ (s, 4H), 6.91 (dd, 2H, $J = 6.8$, 1.6 Hz), 7.29–7.40 (m, 10H), 7.66 (dd, 2H, $J = 6.8$, 1.6 Hz) ppm ^{13}C NMR (75 MHz, DMSO-d_6 +D_2O) $\delta = 50.7$, 123.7, 128.2, 128.4, 129.7, 135.7, 139.7, 154.17, 156.06 ppm HRMS (ESI) $[M+H]^+$: *m/z* calcd for ($C_{21}H_{23}N_4O_2S$) 395.1542. Found 395.1553.

2.2.13. 4-(3-(2-Phenoxyethyl)guanidino)benzenesulfonamide (7k)

To a solution of **6** (300 mg, 1.22 mmol) in DMSO (8 ml), 2-phenoxyethanamine (1.277 ml, 9.76 mmol) was added and the reaction mixture was stirred in sealed tube at 130 °C for 6 h. After cooling to room temperature water (40 ml) was added and mixture was extracted with EtOAc (3 × 20 ml). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was dissolved in *i*PrOH (5 ml) under gentle warming and product was precipitated by addition of hexanes (40 ml). The precipitate was collected by filtration and dried under vacuum to afford **7k** (118 mg, 29%) as orange powder.

^{1}H NMR (300 MHz, DMSO-d_6+D_2O) $\delta = 3.48$–3.56 (m, 2H), 4.04-4.11 (m, 2H), 6.85–7.01 (m, 5H), 7.31 (q, 2H, $J = 7.4$ Hz), 7.65 (d, 2H, $J = 8.4$ Hz) ppm ^{13}C NMR (75 MHz, DMSO-d_6+D_2O) $\delta = 41.2$, 67.7, 115.6, 115.7, 121.9, 123.9, 128.1, 130.8, 136.2, 153.5, 159.7 ppm HRMS (ESI) $[M+H]^+$: *m/z* calcd for ($C_{15}H_{19}N_4O_3S$) 335.1178. Found 335.1193.

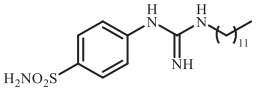
2.2.14. Synthesis of 4-(3-octylguanidino)benzenesulfonamide (7l)



To a solution of **6** (300 mg, 1.22 mmol) in DMSO (8 ml), octan-1-amine (1.613 ml, 9.76 mmol) was added and the reaction mixture was stirred in sealed tube at 130 °C for 2 h. After cooling to room temperature water (40 ml) was added and mixture was extracted with EtOAc (3 × 20 ml). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was dissolved in *i*PrOH (5 ml) under gentle warming and product was precipitated by addition of hexanes (40 ml). The precipitate was collected by filtration and dried in vacuum to afford **7l** (338 mg, 85%) as white powder.

^{1}H NMR (300 MHz, DMSO-d_6+D_2O) $\delta = 0.82–0.90$ (m, 3H), 1.28 (br. s 10H), 1.42–1.50 (m, 2H), 3.11 (t, 2H, $J = 6.7$ Hz), 6.88 (d, 2H, $J = 8.4$ Hz), 7.61 (d, 2H, $J = 8.4$ Hz) ppm ^{13}C NMR (75 MHz, DMSO-d_6 +D_2O) $\delta = 15.3$, 23.5, 27.8, 30.0, 30.1, 30.5, 32.6, 41.7, 124.0, 128.1, 135.7, 153.8, 156.1 ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_{15}H_{27}N_4O_2S$) 327.1855. Found 327.1867.

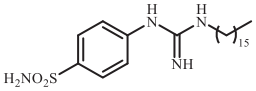
2.2.15. 4–(3-Dodecylguanidino)benzenesulfonamide (7m)



To a solution of **6** (300 mg, 1.22 mmol) in DMSO (8 ml), dodecan-1-amine (1.809 g, 9.76 mmol) was added and the reaction mixture was stirred in sealed tube at 130 °C for 2 h. After cooling to room temperature water (40 ml) was added and mixture was extracted with EtOAc (3 × 20 ml). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was dissolved in *i*PrOH (5 ml) under gentle warming and product was precipitated by addition of hexanes (40 ml). The precipitate formed was collected by filtration and dried in vacuum to afford **7m** (196 mg, 42%) as white powder.

^{1}H NMR (300 MHz, DMSO-d_6 +D_2O) $\delta = 0.88$ (t, 3H, $J = 6.3$ Hz), 1.15–127 (m, 18H), 1.47 (s, 2H), 3.14 (t, 2H, $J = 6.3$ Hz), 6.88 (d, 2H, $J = 8.1$ Hz), 7.61 (d, 2H, $J = 8.1$ Hz) ppm ^{13}C NMR (75 MHz, DMSO-d_6 +D_2O) $\delta = 15.1$, 23.3, 27.7, 29.9, 30.0, 30.2 (br), 30.4, 32.5, 41.5, 123.7, 127.9, 135.5, 153.4, 156.1 ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_{19}H_{35}N_4O_2S$) 383.2481. Found 383.2490.

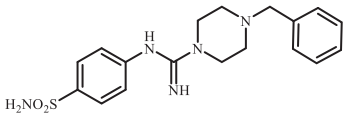
2.2.16. Synthesis of 4-(3-hexadecylguanidino)benzenesulfonamide (7n)



To a solution of **6** (300 mg, 1.22 mmol) in DMSO (8 ml), hexadecan-1-amine (2.356 g, 9.76 mmol) was added and the reaction mixture was stirred in sealed tube at 130 °C for 2 h. After cooling to room temperature, it was filtered through the sintered glass crucible. Water (40 ml) was added to the filtrate and the mixture was extracted with EtOAc (3 × 20 ml). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was washed with $CHCl_3$ (50 ml) and dried under vacuum to afford **7n** (221 mg, 41%) as white powder.

^{1}H NMR (300 MHz, DMSO-d_6 +D_2O) $\delta = 0.84$ (br. s, 3H), 1.23 (br. s, 28H), 3.09 (br. s, 2H), 6.88 (br. s, 2H), 7.61 (br. s, 2H) ppm ^{13}C NMR (75 MHz, DMSO-d_6+ D_2O) $\delta = 15.2$, 23.6, 28.0, 30.6 (br), 32.8, 41.9, 124.0, 128.2, 135.9, 153.8, 155.9 ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_{23}H_{43}N_4O_2S$) 439.3107. Found 439.3114.

2.2.17. 4-Benzyl-N-(4-sulfamoylphenyl)piperazine-1-carboximidamide (7o)

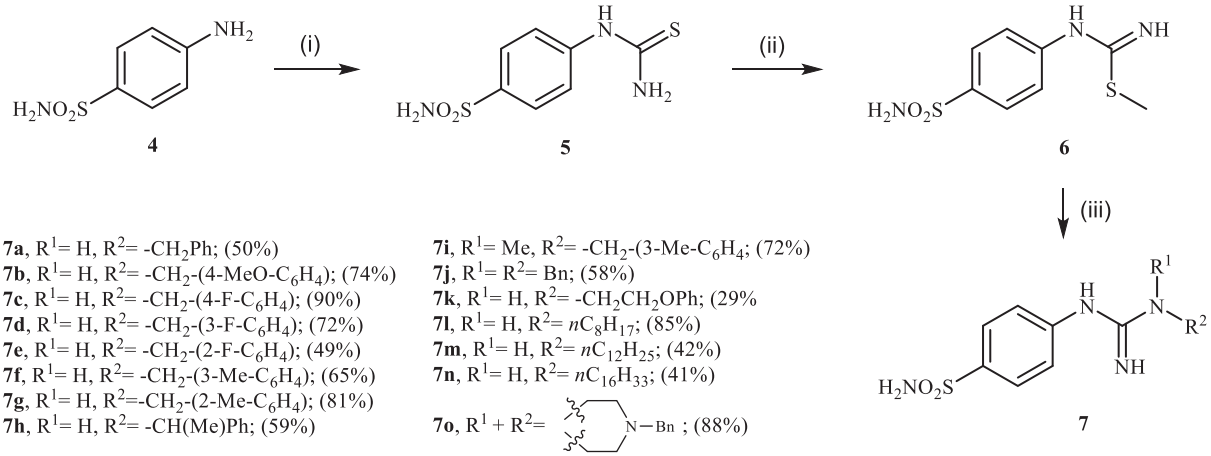


To a solution of **6** (300 mg, 1.22 mmol) in DMSO (8 ml), 1-benzylpiperazine (1.696 ml, 9.76 mmol) was added and the reaction mixture was stirred in sealed tube at 130 °C for 6 h. After cooling to room temperature water (40 ml) was added and the mixture was extracted with EtOAc (3 × 20 ml). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residual oily solid was washed with Et_2O (20 ml) and dried under vacuum to afford **7o** (402 mg, 88%) as white powder.

^{1}H NMR (300 MHz, DMSO-d_6 +D_2O) $\delta = 2.41$ (s, 4H), 3.38 (s, 4H), 3.52 (s, 2H), 6.86 (d, 2H, $J = 8.3$ Hz), 7.28-7.39 (m, 5H), 7.65 (d, 2H, $J = 8.3$ Hz) ppm ^{13}C NMR (75 MHz, DMSO-d_6 +D_2O) $\delta = 45.9$, 53.5, 63.3, 123.7, 128.1, 128.2, 129.4, 130.2, 135.9, 139.0, 154.2, 156.1 ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_{18}H_{24}N_5O_2S$) 374.1651. Found 374.1649.

2.3. CA inhibitory assay

An applied photophysics stopped-flow instrument has been used for assaying the CA-catalysed CO_2 hydration activity[43]. Phenol red (at a concentration of 0.2 mM) was used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalysed CO_2 hydration reaction for a period of 10 – 100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor, at least six traces of the initial 5 – 10% of the reaction have been used for determining the initial velocity. The uncatalysed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled-deionised water, and dilutions up to 0.01 nM



Scheme 1. Reagents and conditions: (i) KSCN, aq. 3.5 M HCl, reflux, 3 h, 31%; (ii) MeI, DMF, 40 °C, 2.5 h, 70%; (iii) HNR^1R^2 (8 equiv.), DMSO, 130 °C, 2–6 h.

were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 6 h at room temperature prior to assay in order to allow for the formation of the E-I complex. The inhibition constants were obtained by nonlinear least-squares methods using PRISM 3 and the Cheng-Prusoff equation, as reported earlier[44–53], and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in-house as reported earlier[54–57].

3. Results and discussion

3.1. Chemistry

Desired 4–(3-alkyl/benzyl-guanidino)benzenesulfonamides **7a–o** were obtained in three step synthesis (Scheme 1). In the first step 4-aminobenzenesulfonamide (**4**) was reacted with KSCN under acidic conditions, thus obtaining intermediate-4-thioureidobenzenesulfonamide (**5**). In the intermediate **5** the reactive thiourea functionality was selectively converted into methyl carbamimidothioate **6** through the treatment with methyl iodide in DMF in the absence of base or catalyst. In the subsequent reaction methyl carbamimidothioate **6** was reacted various primary and secondary aliphatic and benzylic amines in DMF at elevated temperature, affording the desired 4-(3-alkyl/benzyl-guanidino)benzenesulfonamides **7** in satisfying to high yields ranging from 29% to 90%.

3.2. Carbonic anhydrase inhibition

The obtained series of 4–(3-alkyl/benzyl-guanidino)benzenesulfonamides **7a–o** were investigated for their CA inhibitory properties by using a stopped-flow CO_2 hydrase assay[43] and three human CA isoforms (hCA I, II and VII) known to be drug targets for neurological conditions[37,58–62] (Table 1).

As seen from data of Table 1, benzenesulfonamides **7a–o** did not significantly inhibit the cytosolic isoforms hCA I, which is considered as being an off-target isoform in our study. The ubiquitous hCA II was significantly inhibited by many benzenesulfonamides **7** studied here. Compounds **7a–e**, **7h** and **7k** had K_Is ranging from 1.6 to 59.1 nM, in most cased being lower or comparable to those of the non-selective CA inhibitor acetazolamide (**AAZ**), which has a K_I of 12 nM. The rest of the derivatives **7** strongly inhibited CA II, with K_I values in the low nanomolar or subnanomolar range. Neuropathic pain associated CA VII was also effectively inhibited by most of the sulfonamides **7**, even in subnanomolar range for compounds **7f**, **7g**, **7i** and **7m–o**. However, compounds **7c**, **7h**, **7m** and **7o** (nanomolar and subnanomolar inhibitors) also exhibited remarkable selectivity towards CA VII compared to the off-target isoforms CA I and CA II.

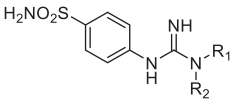
The selectivity indexes for the inhibition of hCA VII over hCA I and II for the new compounds reported here are shown in Table 2.

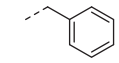
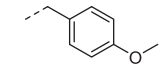
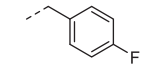
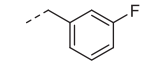
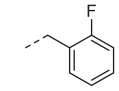
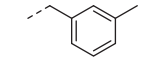
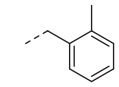
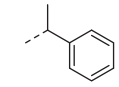
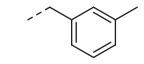
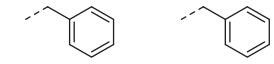
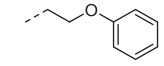
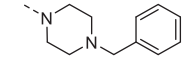
It may be seen that all new compounds **7a–7o** were highly selective for the inhibition of CA VII over CA I, with selectivity indexes in the range of 12.8 – 61300. On the other hand, only compounds **7c, 7h, 7m** and **7o** showed selectivity for inhibiting CA VII over CA II, with selectivity indexes of 1.66 – 8.72. Many of these new sulfonamides (e.g. **7d, 7e, 7g, 7i** and **7j**) were in fact highly hCA II selective inhibitors.

4. Conclusion

A series of novel 4-(3-alkyl/benzyl-guanidino)benzenesulfonamide derivatives with various long alkyl chains and functional groups on benzyl moieties through the direct catalyst-free desulfidative amination of easily accessible methyl (4-sulfamoylphenyl)carbamimidothioate with respective primary and secondary amines were obtained. The new derivatives were assayed as inhibitors of the zinc metalloenzyme CA. Three pharmacologically relevant human (h) isoforms (CA I, CA II and CA VII) were investigated. No significant inhibition of hCA I was observed, whereas some of the new derivatives were effective, low nanomolar or even subnanomolar

Table 1. Inhibition data of human CA isoforms I, II and VII using **AAZ** as standard drug.



Compound	R_1	R_2	K_i (nM)[a] CA I	CA II	CA VII
7a		H	746.5	7.7	10.1
7b		H	698.7	17.7	25.1
7c		H	989.1	59.1	15.0
7d		H	781.7	1.6	60.7
7e		H	2988.8	3.2	44.9
7f		H	2932.2	0.3	0.9
7g		H	3163.0	0.07	0.4
7h		H	1319.0	9.6	1.1
7i		CH_3	1137.7	0.08	0.7
7j			5845.5	0.6	3.2
7k		H	4183.3	6.9	8.7
7l		H	729.8	0.9	2.2
7m		H	930.3	0.4	0.2
7n		H	2154.0	0.06	0.08
7o			3676.8	0.1	0.06
AAZ	–	–	250.0	12.0	2.5

[a]Mean from three different assays, by a stopped-flow technique (errors were in the range of ±5–10% of the reported values).

Table 2. Selectivity indexes for hCA VII over hCA I and II inhibition with compounds **7a–7o** and acetazolamide as standard inhibitor.

	Selectivity index[a]	
Compound	hCA VII/hCA I	hCA VII/hCA II
7°	73.9	0.76
7b	27.8	0.70
7c	65.9	3.94
7d	12.8	0.02
7e	66.5	0.07
7f	3257	0.33
7g	7907	0.17
7h	1199	8.72
7i	1625	0.11
7j	1826	0.18
7k	480	0.79
7l	331	0.41
7m	4651	2.00
7n	26,925	0.75
7o	61,300	1.66
AAZ	100	4.80

[a]Calculated as the ratio K_I(CA I or II)/K_I(CA VII).

hCA II and CA VII inhibitors. Four novel sulfonamide derivatives **7c**, **7h**, **7m** and **7o** having low nanomolar or subnanomolar K_I values and significant selectivity towards neuropathic pain related CA VII have a potential for further investigation as potential neuropathic pain attenuation agents.

Disclosure statement

No potential conflict of interest was reported by all author(s) except CTS. CT Supuran is Editor-in-Chief of the Journal of Enzyme Inhibition and Medicinal Chemistry. He was not involved in the assessment, peer review, or decision-making process of this paper. The authors have no relevant affiliations of financial involvement with any organisation or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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