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2-((1H-Benzo[d]imidazol-2-yl)amino)benzo[d]thiazole-6-sulphonamides: a class of carbonic anhydrase II and VII-selective inhibitors

Morteza Abdoli[a], Claudiu T. Supuran[b] and Raivis Žalubovskis[a,c]

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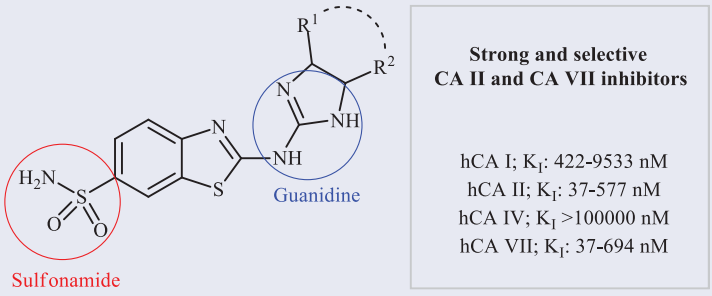
ABSTRACT

A small library of substituted cyclic guanidine incorporated benzothiazole-6-sulphonamides was synthesized. All obtained compounds were investigated for their inhibitory activity against the key brain-associated human carbonic anhydrase isoform hCA VII (a promising target for the treatment of neuropathic pain) and three isoforms expressed in brain and other tissues, hCA I, II, and IV. Sulphaguanidine derivatives **9a–d** were inactive on the all investigated isoforms while the primary sulphonamide containing guanidines **6a–c** and **7a–c** were inactive towards hCA IV but displayed inhibiting properties on hCA I, II, and VII with K_{Is} values in the low nanomolar to micromolar ranges. The results indicated that isoforms hCA II and VII were potently and selectively inhibited by these compounds, whereas the cytosolic hCA I was less sensitive to inhibition. The derivatives reported in this study might be useful for design of more potent and selective inhibitors of hCA II and VII.

GRAPHICAL ABSTRACT



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Carbonic anhydrase isozyme VII; neuropathic pain; sulphonamides; guanidines; inhibitors

Introduction

Human carbonic anhydrases (hCA, EC 4.2.1.1), are a large family of zinc-metalloenzymes with twelve catalytically active isoforms (hCA I, hCA II, hCA III, hCA IV, hCA VA, hCA VB, hCA VI, hCA VII, hCA IX, hCA XII, CA XIII, hCA XIV), which are vital for respiration, pH regulation, ion transport, metabolism, biosynthetic reactions and other physiological processes[1]. However, the abnormal activity of each isoform stimulates the initiation of various pathological processes in which a disturbed cellular pH buffering or metabolism plays a significant role[2]. Therefore, hCAs are regarded as promising therapeutic targets for many diseases, and the design of isoform-selective inhibitors has attracted the attention of researchers over the last 40 years[3]. In this context, brain-associated isoform CA VII, has been recently validated as an important target in neuropathic pain[4], a disease which results from damage or dysfunction of the nervous system and whose patients suffer from the lack of effective treatment options[5,6].

Sulphonamides are the largest group of sulphur-containing drugs and the chief class of zinc-binding carbonic anhydrases inhibitors (CAIs)[7,8]. Acetazolamide, methazolamide, ethoxzolamide, brinzolamide, and dorzolamide were the earliest drugs approved as carbonic anhydrase inhibitors and still currently used in the treatment of various diseases such as glaucoma, duodenal ulcers, epilepsy and as diuretics[8]. As shown in Figure 1, the structural similarity of all five drugs is that they share the sulphonamide moiety bound to a heterocycle. High inhibitory potency against hCA VII is another similarity of these clinically used drugs[9]. However, they are not isoform selective inhibitors and are active against the majority of isoforms, with inhibition constants in low nanomolar range. Due to their poor selectivity against a single isoform, another thing they have in common is the multitude of side effects. Ethoxzolamide is the most potent hCA VII inhibitor in this series (K_I of 0.8 nM). However, it also inhibits hCA I, II, IV–VII, IX, and XII–XIV in moderate to low nanomolar ranges. Therefore,

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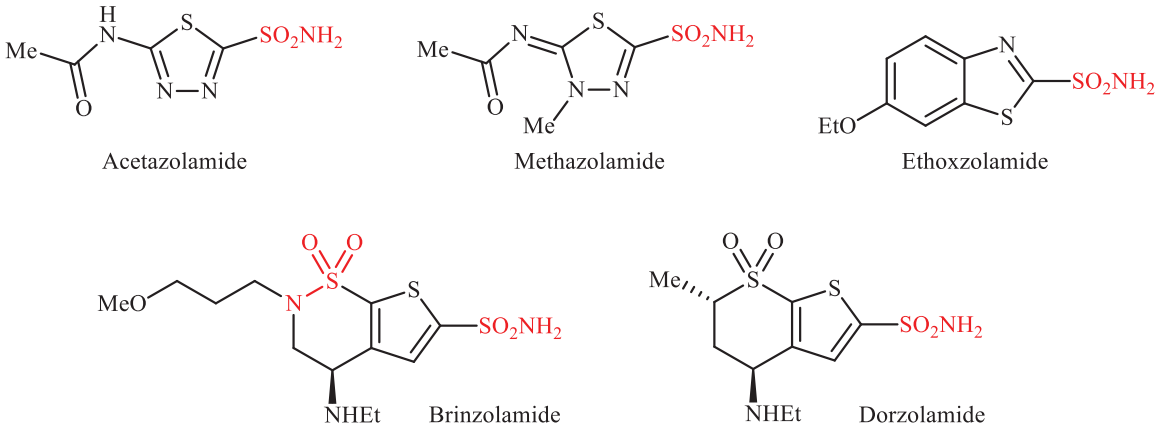


Figure 1. Chemical structures of acetazolamide, methazolamide, ethoxzolamide, brinzolamide, and dorzolamide.

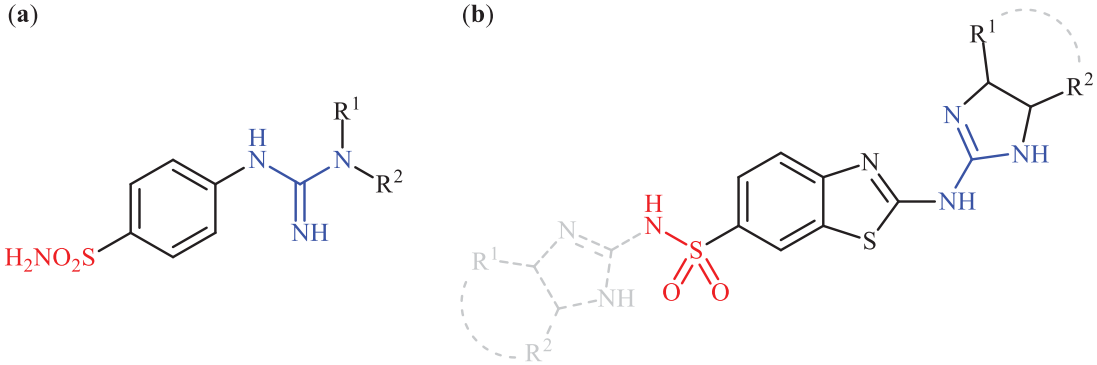


Figure 2. (a) General structure of 4-(3-alkyl/benzyl-guanidino)benzenesulphonamides developed by our group as hCA VII selective inhibitors; (b) general structure of cyclic guanidine incorporated benzothiazole-6-sulphonamides discussed in the paper.

the search for structural improvement of this drug to produce less-toxic, more efficient, and isoform selective agents is an ongoing struggle[10]. Very recently, we disclosed that incorporation of the guanidine moieties as tails to classical benzenesulphonamide scaffolds improved selectivity against hCA VII versus ubiquitous hCA I and CA II (Figure 2(a))[11]. Considering all these facts and in connection with our works in the development of selective CAIs[12], with the objectives of development of hCA VII selective inhibitors, in this study, we present the synthesis of three different sets of novel cyclic guanidine incorporated benzothiazole-6-sulphonamides (Figure 2(b)) and evaluate their capability to inhibit three cytosolic isozymes (hCA I, II, and VII) and one membrane-bound isozyme (hCA IV).

Results and discussion

Chemistry

The rationale for obtaining the novel compounds reported here consisted in replacing phenyl ring in the 4-guanidinobenzenesulphonamide scaffold, a structure recently reported by our group as selective inhibitor of hCA VII[11], by a heteroaryl ring, leading thus to guanidine-heteroarene sulphonamide which presumably show enhanced hCAs inhibitory activity.

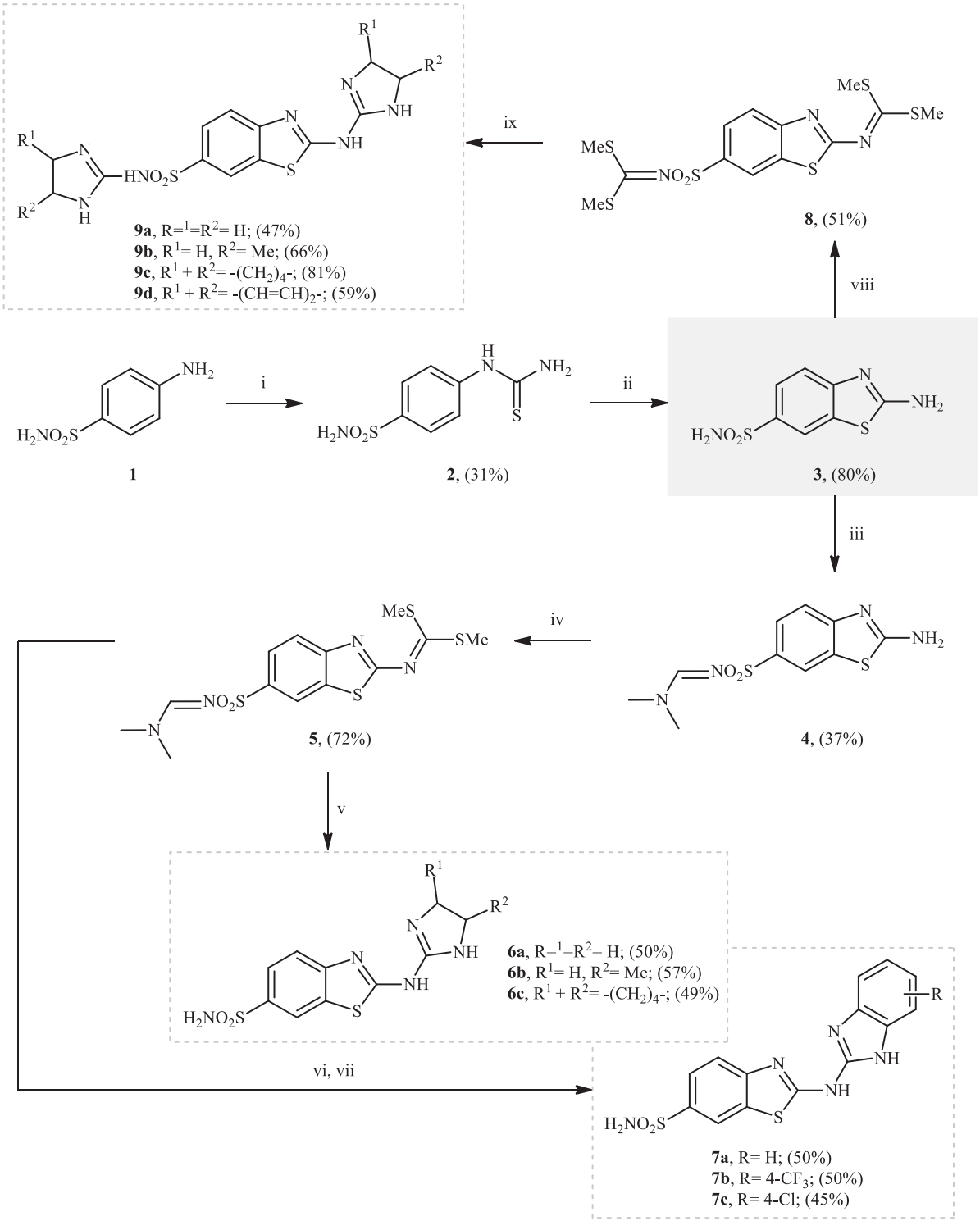
The synthesis of three different sets of 2-((imidazolyl)amino)-benzothiazole-6-sulphonamides **6**, **7**, and **8** was performed as illustrated in Scheme 1. 4-Thioureidobenzenesulphonamide **2** was prepared by the reaction of 4-aminobenzenesulphonamide **1** with KSCN under acidic conditions. Consequently, the intermediate **2** was treated with Br_2 using $CHCl_3$ as a solvent to afford the key intermediate **3** which upon treatment with *N,N*-dimethylformamide *N,N*-dimethylacetamide (DMF-DMA) in DMF provided the *N*-protected sulphonamide intermediate **4**. Subsequently, the intermediate **4** was reacted with CS_2 then MeI to yield dimethyl carbonimidodithioate **5**. The reaction between intermediate **5** and over-stoichiometric amounts of simple aliphatic diamines at elevated temperature was then performed to afford heteroayl sulphonamide-substituted cyclic guanidines **6** in moderate yields. Similarly, the intermediate **5** was reacted with various 1,2-phenylenediamines to give fluorescent 2-((benzoimidazol-2-yl)amino)benzothiazole-6-sulphonamides **7**. However, in these cases, an additional deprotection step was required. Finally, double *N*-dithiocarbonatation of 2-aminobenzothiazole-6-sulphonamide **3** with CS_2/MeI was carried out to form intermediate **8** which was subsequently reacted with 1,2-diamines to afford the desired *N*-(imidazol-2-yl)-2-((imidazol-2-yl)amino)benzothiazole-6-sulphonamides **9**.

Carbonic anhydrase inhibition

The inhibition data against four human CA isoforms expressed in brain, hCA I, II, IV (off-target) and VII (target for neuropathic pain drug discovery) with the newly synthesised sulphonamides **6a–c**, **7a–c**, and **9a–d** along with the reference drug acetazolamide (AAZ), are shown in Table 1.

The following structure activity relationship (SAR) can be figured out from the inhibition data of Table 1:

i. Among the entire series, only compounds bearing primary sulphonamide moiety **6a–c** and **7a–c** were moderately inhibited the cytosolic isoform hCA I, whereas sulphaguanidine

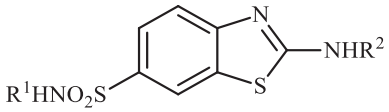


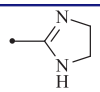
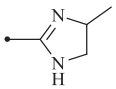
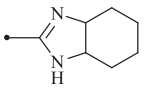
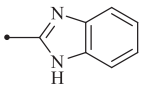
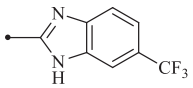
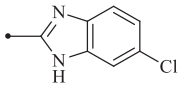
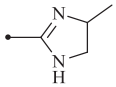
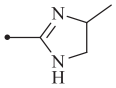
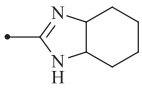
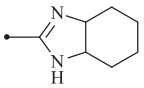
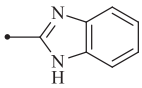
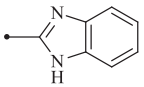
Scheme 1. Reagents and conditions: (i) KSCN (1 equiv.), 3.5 M HCl, 90 °C, 3 h; (ii) Br_2 (1.5 equiv.), $CHCl_3$, reflux, 4.5 h; (iii) DMF-DMA (1 equiv.), DMF, 0 °C, 2.5 h; (iv) CS_2 (1.7 equiv.), KOH (2.5 equiv.), MeI (2.5 equiv.), DMF/H_2O (3:1); (v) aliphatic diamine (10 equiv.), DMSO, 120 °C, 15 h; (vi) 1,2-phenylenediamine (8 equiv.), DMSO, 120 °C, 20 h; (vii) $N_2H_4.H_2O$, r.t., 1 h; (viii) CS_2 (4 equiv.), KOH (5 equiv.), MeI (5 equiv.), DMF/H_2O (3:1); (ix) aliphatic diamine (15 equiv.), DMSO, 120 °C, 15 h.

derivatives **9a–d** were entirely inactive against this isoform (K_I > 100 μM). The SAR showed that compounds containing imidazoline core **6a–c** displayed better inhibitory capability against this isoform, with K_{Is} ranging between 442.4 and 7590 nM, as compared to the benzimidazoline-substituted ones **7a–c** (K_{Is} of 4927–9533 nM). Notably, the inhibitory potency was reduced significantly when any functionality was presented on the periphery of(benz)imidazoline rings. As shown in Table 1, 2-((4,5-dihydro-imidazol-2-yl)amino) benzothiazole-6-sulphonamide **6a** exhibited the best activity of all the evaluated compounds, albeit approximately two-fold less effective than standard drug, AAZ.

ii. The physiologically most relevant isoform hCA II was inhibited by compounds **6a–c** and **7a–c** more than hCA I (K_{Is} in the range 37.6–577.6 nM). The results showed that sulphaguanidines **9a–d** were also inactive against this isoform.

Table 1. Inhibition data of human CA isoforms CAI, II, IV and VII with benzothiazole-6-sulphonamide substituted five-membered (bi)cyclic guanidines **6**, **7**, **9** using acetazolamide (AAZ) as standard drug.



			K_I (nM)[a]			
Compound	R^1	R^2	hCA I	hCA II	hCA IV	hCA VII
6a	–H		422.4	37.6	>100 000	56.3
6b	–H		568.5	48.1	>100 000	37.4
6c	–H		7590	65.6	>100 000	82.5
7a	–H		4927	84.0	>100 000	66.9
7b	–H		8102	375.0	>100 000	451.9
7c	–H		9533	577.6	>100 000	694.4
9a			>100 000	>100 000	>100 000	>100 000
9b			>100 000	>100 000	>100 000	>100 000
9c			>100 000	>100 000	>100 000	>100 000
9d			>100 000	>100 000	>100 000	>100 000
AAZ	–	–	250	12.5	74	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).

Again imidazoline-incorporated sulphonamides **6a–c** demonstrated superior inhibitory activities (K_{Is} in the range 37.6–65.6 nM) compared to benzimidazoline-substituted derivatives **7a–c** (K_{Is} of 84.0–577.6 nM). Once more, the optimal substitution seems to be an unsubstituted imidazoline ring, in **6a**, which is the most effective hCA II inhibitor in the series investigated here.

iii. As seen from data of Table 1, none of the tested compounds showed inhibitory action against the transmembrane isoform hCA IV ($K_I > 100$ μM), which is considered as being an off-target isoform in our study. Notably, AAZ shows low nanomolar inhibitory activity against this isoform with a K_I of 74nM.

iv. Similar to the inhibitory pattern of investigated compounds against ubiquitous CA I and CA II, sulphaguanidine derivatives **9a–d** failed to inhibit the neuropathic pain associated hCA VII. However, the rest of the derivatives **6a–c** and **7a–c** effectively inhibited this isoform, with K_{Is} values ranging from 37.4to 694.4 nM. Although again analogues **6a–c** exhibited better inhibitory activity when compared to compounds **7a–c**, in this case, the SAR was not very flat. Indeed, compound **6a** has the highest inhibitory effect for the off-target isoforms hCA I and hCA II while methyl-substituted compound **6b** showed superior inhibitory potency towards hCA VII. Although this compound displayed 15-fold higher selectivity for hCA VII over hCA I, it did not show significant selectivity towards hCA VII versus hCA II (hCA VII/hCA II ≈ 1.3).

Conclusion

We have synthesised three small sets of novel cyclic guanidine incorporated benzothiazole-6-sulphonamides (**6a–c**, **7a–c**, and

9a–d) and screened them against four human CA isoforms expressed in brain, hCA I, II, IV and VII. Among them, compounds bearing primary sulphonamide moiety **6a–c** and **7a–c** showed potent inhibitory activity against three cytosolic isoforms hCA I, II and VII whereas they did not display any inhibitory activity towards membrane-bound isoform hCA IV. The SAR indicated that generally compounds of series **6a–c** displayed better hCA inhibitory activity compared to the derivatives **7a–c**. It was also found that hCA II and VII were the considerably most sensitive to these inhibitors than hCA I. However, the newly developed compounds did not display remarkable selectivity towards hCA VII versus hCA II. Therefore, further optimisation and exploration of this kind of novel scaffolds required to development of new isoform-selective CAIs with better inhibition potency and fewer side effects.

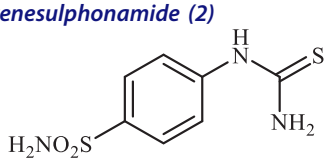
Experimental section

Chemistry methods

Starting materials, reagents and solvents were purchased from commercial sources and used as received without any type of further purification. Thin-layer chromatography (TLC) was performed on silica gel, spots were visualised with UV light (254 and 365 nm). NMR spectra were recorded on Bruker 300 and 500 spectrometers in DMSO or DMF with chemical shifts values (δ) in ppm relative to tetramethylsilane (TMS). High-resolution mass spectra (HRMS) were recorded on a mass spectrometer with a Q-TOF micro mass analyser using the ESI technique.

Synthesis

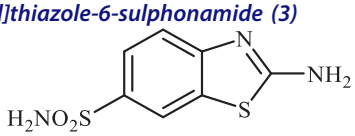
4-Thioureidobenzenesulphonamide (2)



4-Aminobenzensulphonamide (**1**) (30 g, 174.3 mmol) was dissolved in 3.5 M HCl (180 ml) under gentle warming. The solution was cooled down to r.t. and then KSCN (16.94 g, 174.3 mmol) was added to the reaction mixture and the mixture was refluxed for 3 h. After cooling to room temperature, the reaction mixture was diluted with ice-cold water (approximately 200 ml). The formed solids were collected by filtration, washed with water and air dried to afford **2** (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

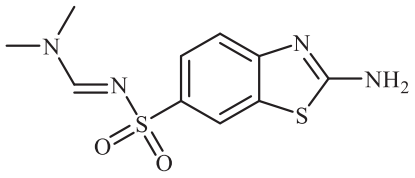
2-Aminobenzo[d]thiazole-6-sulphonamide (3)



To a suspension of 4-thioureidobenzenesulphonamide (**2**) (10 g, 43.3 mmol) in $CHCl_3$ (120 ml) was added dropwise a solution of Br_2 (3.27 ml, 64.9 mmol) in $CHCl_3$ (15 ml) and stirred at 70 °C for 4.5 h. After cooling to rt volatiles were removed under reduced pressure to give a lumpy solid that was dissolved in H_2O (120 ml). The aqueous solution was treated with ammonium hydroxide until the pH adjusted to greater than 9–10 and then stirred at 90 °C for 1 h. The formed precipitate was collected by filtration, washed with H_2O and cold ethanol and dried under vacuum to afford compound **3** (7.9 g, 80%) as off-white solid.

^{1}H NMR (300 MHz, DMSO-d$_6$) $\delta = 7.25$ (s, 2H), 7.47 (d, 1H, $J = 8.4$ Hz), 7.72 (d, 1H, $J = 8.4$ Hz), 7.90 (s, 2H), 8.17 (s, 1H) ppm ^{13}C NMR (75 MHz, DMSO-d$_6$) $\delta = 118.2$, 120.1, 124.6, 131.9, 137.1, 156.4, 170.3 ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_7H_8N_3O_2S_2$) 230.0058. Found 230.0062.

N′-((2-aminobenzo[d]thiazol-6-yl)sulphonyl)-N,N-dimethylformimidamide (4)

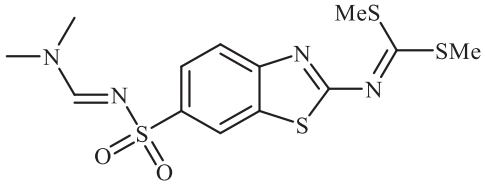


To an ice-cooled stirred solution of 2-aminobenzo[d]thiazole-6-sulphonamide (**3**) (20 g, 87.3 mmol) in DMF (160 ml), DMF-DMA (11.6 ml, 87.3 mmol) was added dropwise and stirring continued at 0 °C for 2 h. After completion of the reaction, the mixture was extracted with DCM (3 × 100 ml). The combined organic layers were washed with water (1 × 100 ml) and concentrated under reduced pressure to 30 ml and then treated with $CHCl_3$ (250 ml). The precipitated solid was collected by filtration, washed with $CHCl_3$ (3 × 25 ml) and dried to afford the target product **4** (9.3 g, 37%) as yellowish powder.

Note, due to the precipitation of considerable amounts of the desired product by adding Na_2SO_4 to the organic extracts, extracts collected were not dried over Na_2SO_4 and directly concentrated under vacuum.

^{1}H NMR (500 MHz, DMSO-d$_6$) $\delta = 2.92$ (s, 3H), 3.16 (s, 3H), 7.41 (d, 1H, $J = 8.4$ Hz), 7.62 (d, 1H, $J = 8.4$ Hz), 7.88 (s, 2H), 8.14 (s, 1H), 8.22 (s, 1H) ppm ^{13}C NMR (125 MHz, DMSO-d$_6$) $\delta = 35.9$, 41.8, 118.0, 120.4, 124.8, 132.0, 135.8, 156.3, 160.4, 170.3 ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_{10}H_{13}N_4O_2S_2$) 285.0480. Found 285.0490.

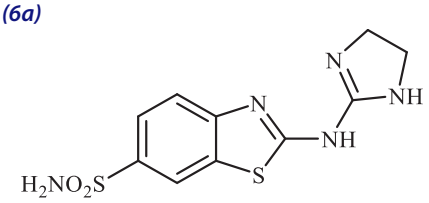
Dimethyl (6-(N-((dimethylamino)methylene)sulphamoyl)benzo[d]-thiazol-2-yl)carbonimidodithioate (5)



To an ice cooled solution of *N*′-((2-aminobenzo[*d*]thiazol-6-yl)sulphonyl)-*N*,*N*-dimethylformimidamide (**4**) (10 g, 35.2 mmol) and CS_2 (3.6 ml, 59.8 mmol) in DMF (75 ml) was dropwise added a solution of KOH (4.75 g, 88.0 mmol) in H_2O (25 ml) at a such rate that the temperature kept below 10 °C. After 1 h stirring, MeI (5.47 ml, 88.0 mmol) was dropwise added and the reaction mixture was stirred at the indicated temperature for another 1 h. Then water (60 ml) and hexanes (12 ml) were added to the mixture and the solution was vigorously stirred for 20 min. Subsequently, the solids formed in the organic phase were collected by filtration and washed with water and cold ethanol to afford compound **5** (9.85 g, 72%) as yellow solids.

^{1}H NMR (500 MHz, DMSO-d$_6$) $\delta = 2.67$ (s, 6H), 2.95 (s, 3H), 3.19 (s, 3H), 7.84 (d, 1H, $J = 8.4$ Hz), 7.94 (d, 1H, $J = 8.4$ Hz), 8.28 (s, 1H), 8.50 (s, 1H) ppm ^{13}C NMR (125 MHz, DMSO-d$_6$) $\delta = 16.6$, 36.0, 41.9, 121.5, 122.8, 125.0, 135.0, 139.6, 153.9, 160.7, 170.8, 178.4 ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_{13}H_{17}N_4O_2S_4$) 389.0234. Found 389.0247.

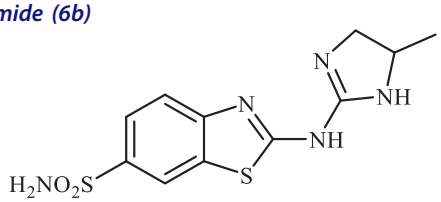
2-((4,5-Dihydro-1H-imidazol-2-yl)amino)benzo[d]thiazole-6-sulphonamide (6a)



A mixture of dimethyl (6-(*N*-((dimethylamino)methylene)sulphamoyl)benzo[*d*]thiazol-2-yl)carbonimidodithioate (**5**) (0.4 g, 1.03 mmol) and ethane-1,2-diamine (0.69 ml, 10.3 mmol) in DMSO (5.0 ml) was stirred at 120 °C for 15 h. After completion of the reaction, the mixture was cooled to room temperature and treated with water (25 ml) and then extracted with EtOAc (3 × 25 ml). The combined organic extracts were washed with water (3 × 25 ml) and concentrated to obtain a residue that was washed with Et$_2$O to afford the compound **6a** (0.153 g, 50%) as white powder.

^{1}H NMR (500 MHz, DMSO-d$_6$) $\delta = 3.63$ (s, 4H), 7.27 (s, 2H), 7.60 (d, 1H, $J = 8.4$ Hz), 7.74 (d, 1H, $J = 8.4$ Hz), 8.07 (s, 2H), 8.17 (s, 1H) ppm ^{13}C NMR (125 MHz, DMSO-d$_6$) $\delta = 42.7$, 119.0, 119.9, 124.2, 132.0, 137.9, 155.5, 162.3, 177.1 ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_{10}H_{12}N_5O_2S_2$) 298.0432. Found 298.0439.

2-((5-Methyl-4,5-dihydro-1H-imidazol-2-yl)amino)benzo[d]thiazole-6-sulphonamide (6b)

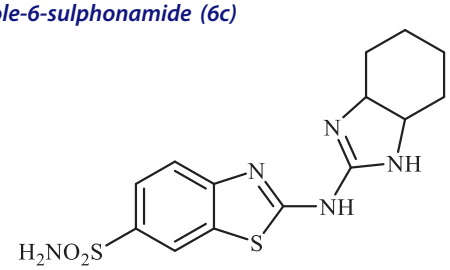


A mixture of dimethyl (6-(*N*-((dimethylamino)methylene)sulphamoyl)benzo[*d*]thiazol-2-yl)carbonimidodithioate (**5**) (0.4 g, 1.03 mmol) and propane-1,2-diamine (0.88 ml, 10.3 mmol) in DMSO (5.0 ml) was stirred at 120 °C for 15 h. After completion of the reaction, the mixture was cooled to room temperature and treated with water (25 ml) and then extracted with EtOAc (3 × 25 ml). The combined organic extracts were washed with water (3 × 25 ml) and concentrated to obtain a residue that was washed with Et$_2$O to afford compound **6b** (0.184 g, 57%) as white powder.

Note, due to the precipitation of considerable amounts of the desired product by adding Na_2SO_4 to the organic extracts, extracts collected were not dried over Na_2SO_4 and directly concentrated under vacuum.

^{1}H NMR (500 MHz, DMSO-d$_6$) $\delta = 1.27$ (d, 3H, $J = 5.6$ Hz), 3.18–3.22 (m, 1H), 3.76 (d, 1H, $J = 9.2$ Hz), 4.03–4.08 (m, 1H), 7.27 (s, 2H), 7.60 (d, 1H, $J = 8.4$ Hz), 7.47 (d, 1H, $J = 8.4$ Hz), 8.07 (s, 1H), 8.14 (s, 1H), 8.17 (s, 1H) ppm ^{13}C NMR (125 MHz, DMSO-d$_6$) $\delta = 21.8$, 49.8, 50.5, 118.9, 119.9, 124.2, 131.9, 137.9, 155.5, 161.2, 177.1 ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_{11}H_{14}N_5O_2S_2$) 312.0589. Found 312.0604.

2-((3a,4,5,6,7,7a-hexahydro-1H-benzo[d]imidazol-2-yl)amino)benzo[d]thiazole-6-sulphonamide (6c)

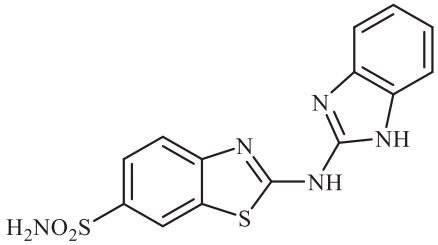


A mixture of dimethyl (6-(*N*-((dimethylamino)methylene)sulphamoyl)benzo[*d*]thiazol-2-yl)carbonimidodithioate (**5**) (0.4 g, 1.03 mmol) and cyclohexane-1,2-diamine (1.24 ml, 10.3 mmol) in DMSO (5.0 ml) was stirred at 120 °C for 15 h. After completion of the reaction, the mixture was cooled to room temperature and treated with water (25 ml) and then extracted with EtOAc (3 × 25 ml). The combined organic extracts were washed with water (3 × 25 ml) and concentrated to obtain an orange solid that was dispersed in *t*-BuOMe (30 ml) and then filtered-off. The collected solution was treated with hexanes (40 ml) and the precipitated solids were collected by filtration and washed with hexanes and dried to afford compound **6c** (0.177 g, 49%) as white powder.

Note, due to the precipitation of considerable amounts of the desired product by adding Na_2SO_4 to the organic extracts, extracts collected were not dried over Na_2SO_4 and directly concentrated under vacuum.

^{1}H NMR (500 MHz, DMSO-d$_6$) $\delta = 1.28$–1.52 (m, 4H), 1.73–1.84 (m, 2H), 2.07–2.16 (m, 2H), 3.12–3.21 (m, 2H), 7.28 (s, 2H), 7.66 (d, 1H, $J = 7.7$ Hz), 7.76 (d, 1H, $J = 7.7$ Hz), 8.20 (s, 1H), 8.22 (s, 2H) ppm ^{13}C NMR (125 MHz, DMSO-d$_6$) $\delta = 24.5$, 29.8, 62.5, 119.3, 120.0, 124.3, 132.1, 138.2, 155.4, 163.3, 176.7 ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_{14}H_{18}N_5O_2S_2$) 352.0902. Found 352.0917.

2-((1H-benzo[d]imidazol-2-yl)amino)benzo[d]thiazole-6-sulphonamide (7a)

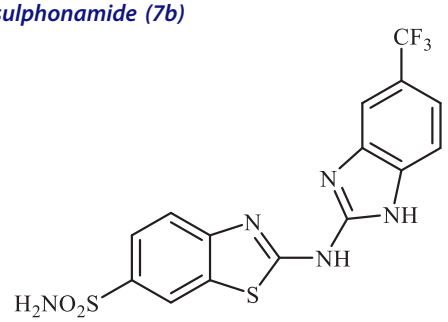


A mixture of dimethyl (6-(*N*-((dimethylamino)methylene)sulphamoyl)benzo[*d*]thiazol-2-yl)carbonimidodithioate (**5**) (0.4 g, 1.03 mmol) and *o*-phenylenediamine (0.89 g, 8.24 mmol) in DMSO (5.0 ml) was stirred at 120 °C for 15 h. After completion of the reaction, the mixture was cooled to room temperature, hydrazine hydrate (2 ml) was dropwise added and the reaction mixture was stirred for 1 h. Then water (30 ml) and ethyl acetate (30 ml) were added to the mixture and the solution was stirred for 10 min and then remained still for a few minutes. Subsequently, the solids formed in the organic phase were collected by filtration and washed with EtOAc to afford compound **7a** (0.179 g, 50%) as white powder.

^{1}H NMR (500 MHz, DMSO-d$_6$) $\delta = 7.19$–7.24 (m, 2H), 7.33 (s, 2H), 7.44–7.49 (m, 2H), 7.75 (d, 1H, $J = 7.9$ Hz), 7.83 (d, 1H, $J = 7.9$ Hz), 8.26 (s, 1H), 12.27 (s, 2H) ppm ^{13}C NMR (125 MHz, DMF-d$_7$) $\delta = 111.5$, 118.2, 119.6, 122.6, 124.0, 131.0, 131.9, 137.8, 151.2,

154.8, 174.1 ppm HRMS (ESI) $[M+H]^+$: *m/z* calcd for ($C_{14}H_{12}N_5O_2S_2$) 346.0432. Found 346.0434.

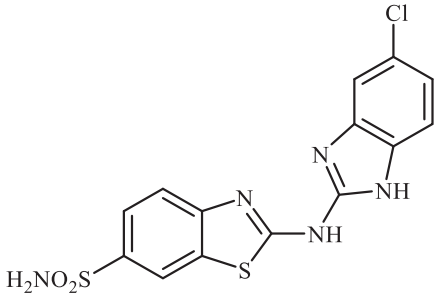
2-((6-(Trifluoromethyl)-1H-benzo[d]imidazol-2-yl)amino)benzo[d]-thiazole-6-sulphonamide (7b)



A mixture of dimethyl (6-(*N*-((dimethylamino)methylene)sulphamoyl)benzo[*d*]thiazol-2-yl)carbonimidodithioate (**5**) (0.4 g, 1.03 mmol) and 4-(trifluoromethyl)benzene-1,2-diamine (1.45 g, 8.24 mmol) in DMSO (5.0 ml) was stirred at 120 °C for 15 h. After completion of the reaction, the mixture was cooled to room temperature, hydrazine hydrate (2 ml) was dropwise added and the reaction mixture was stirred for 1 h. Subsequently, the mixture was treated with water (50 ml) and the formed solids were collected by filtration, washed with *t*-BuOMe (50 ml) and dried under vacuum to afford compound **7b** (0.213 g, 50%) as pink powder.

^{1}H NMR (500 MHz, DMSO-d_6) $\delta = 7.37$ (s, 2H), 7.51–7.87 (m, 5H), 8.34 (s, 1H), 12.48 (s, 2H) ppm ^{13}C NMR (125 MHz, DMF-d_7) $\delta = 109.4$, 112.5, 117.8, 119.3, 120.0, 123.2 (q, $J = 31.80$ Hz), 124.3, 125.5 (q, $J = 271.4$ Hz), 131.4, 133.3, 135.9, 138.4, 152.6, 171.2 ppm HRMS (ESI) $[M+H]^+$: *m/z* calcd for ($C_{15}H_{11}N_5O_2F_3S_2$) 414.0306. Found 414.0313.

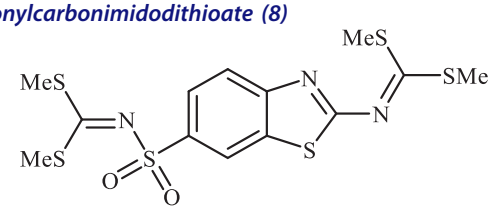
2-((6-Chloro-1H-benzo[d]imidazol-2-yl)amino)benzo[d]thiazole-6-sulphonamide (7c)



A mixture of dimethyl (6-(*N*-((dimethylamino)methylene)sulphamoyl)benzo[*d*]thiazol-2-yl)carbonimidodithioate (**5**) (0.4 g, 1.03 mmol) and 4-chlorobenzene-1,2-diamine (1.17 g, 8.24 mmol) in DMSO (5.0 ml) was stirred at 120 °C for 15 h. After completion of the reaction, the mixture was cooled to room temperature, hydrazine hydrate (2 ml) was dropwise added and the reaction mixture was stirred for 1 h. Then water (30 ml) and ethyl acetate (30 ml) were added to the mixture and the solution was stirred for 10 min and then remained still for a few minutes. Subsequently, the solids formed in the organic phase were collected by filtration and washed with EtOAc to afford compound **7c** (0.177 g, 45%) as dark grey powder.

^{1}H NMR (500 MHz, DMSO-d_6) $\delta = 7.20$ (d, 1H, $J = 8.2$ Hz), 7.44 (d, 1H, $J = 8.2$ Hz), 7.35 (s, 2H), 7.49 (s, 1H), 7.70 (d, 1H, $J = 8.2$ Hz), 7.85 (d, 1H, $J = 8.2$ Hz), 8.31 (s, 1H), 12.32 (s, 2H) ppm ^{13}C NMR (125 MHz, DMF-d_7) $\delta = 111.9$, 113.0, 117.9, 119.8, 122.4, 124.2, 127.0, 131.6, 133.4, 138.2, 151.8, 153.3, 170.7, 172.2 ppm HRMS (ESI) $[M+H]^+$: *m/z* calcd for ($C_{14}H_{11}N_5O_2S_2Cl$) 380.0043. Found 380.0049.

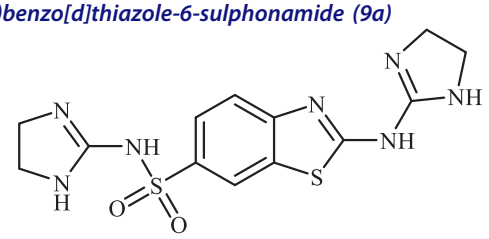
Dimethyl (2-((bis(methylthio)methylene)amino)benzo[d]thiazol-6-yl)sulphonylcarbonimidodithioate (8)



To an ice cooled solution of 2-aminobenzo[*d*]thiazole-6-sulphonamide(**3**) (3 g, 1.31 mmol) and CS_2 (3.16 ml, 5.24 mmol) in DMF (20 ml) was dropwise added a solution of KOH (3.67 g, 6.55 mmol) in H_2O (10 ml). After 1 h stirring at 0 °C, MeI (4.08 ml, 6.55 mmol) was dropwise added and the reaction mixture was stirred at the indicated temperature for additional 1 h. Then the mixture was treated with water (100 ml) and the precipitated solid was collected by filtration under vacuum, washed with water, EtOH and then acetone to afford compound **8** (2.9 g, 37%) as yellowish powder.

^{1}H NMR (300 MHz, 80 °C, DMSO-d_6) $\delta = 2.61$ (s, 3H), 2.62 (s, 3H), 2.68 (s, 3H), 2.69 (s, 3H), 7.95–8.0 (m, 2H), 8.61 (s, 1H) ppm ^{13}C NMR (75 MHz, 80 °C, DMSO-d_6) $\delta = 16.1$, 16.6, 122.0, 122.4, 134.8, 136.7, 154.5, 170.9, 177.9, 186.0 ppm HRMS (ESI) $[M+H]^+$: *m/z* calcd for ($C_{13}H_{16}N_3O_2S_6$) 437.9567. Found 437.9574.

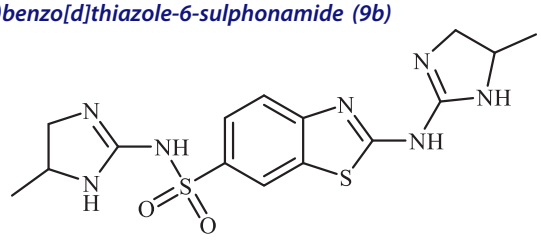
N-(4,5-Dihydro-1H-imidazol-2-yl)-2-((4,5-dihydro-1H-imidazol-2-yl)amino)benzo[d]thiazole-6-sulphonamide (9a)



A mixture of dimethyl (2-((bis(methylthio)methylene)amino)benzo[*d*]thiazol-6-yl)sulphonylcarbonimidodithioate (**8**) (0.4 g, 1.03 mmol) and ethane-1,2-diamine (0.916 ml, 15.45 mmol) in DMSO (5.0 ml) was stirred at 120 °C for 15 h. After completion of the reaction, the mixture was cooled to room temperature and treated with water (70 ml) and EtOAc (30 ml) and was vigorously stirred for 30 min. Subsequently, the solids formed in the organic phase were collected by filtration, washed with Et_2O (30 ml) and dried under vacuum to compound **9a** (0.159 g, 47%) as pink powder.

^{1}H NMR (500 MHz, DMSO-d_6) $\delta = 3.46$ (s, 4H), 3.62 (s, 4H), 7.48 (s, 2H), 7.54 (d, 1H, $J = 7.8$ Hz), 7.68 (d, 1H, $J = 7.8$ Hz), 8.04 (s, 2H), 8.13 (s, 1H) ppm ^{13}C NMR (125 MHz, DMSO-d_6) $\delta = 42.5$, 42.7, 118.8, 119.9, 124.3, 131.7, 138.2, 155.1, 161.3, 162.2, 176.7 ppm HRMS (ESI) $[M+H]^+$: *m/z* calcd for ($C_{13}H_{16}N_7O_2S_2$) 366.0807. Found 366.0816.

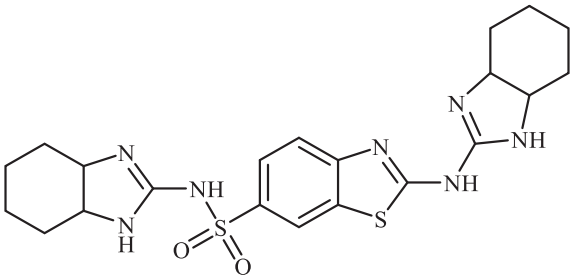
N-(4,5-Dihydro-1H-imidazol-2-yl)-2-((4,5-dihydro-1H-imidazol-2-yl) amino)benzo[d]thiazole-6-sulphonamide (9b)



A mixture of dimethyl (2-((bis(methylthio)methylene)amino)benzo[*d*]thiazol-6-yl)sulphonylcarbonimidodithioate (**8**) (0.4 g, 1.03 mmol) and propane-1,2-diamine (1.17 ml, 15.45 mmol) in DMSO (5.0 ml) was stirred at 120 °C for 15 h. After completion of the reaction, the mixture was cooled to room temperature and treated with water (70 ml) and EtOAc (30 ml) and was vigorously stirred for 30 min. Subsequently, the solids formed in the organic phase were collected by filtration, washed with Et_2O (30 ml) and dried under vacuum to compound **9b** (0.240 g, 66%) as pink powder.

^{1}H NMR (500 MHz, DMSO-d_6) $\delta = 1.15$ (d, 3H, $J = 6.0$ Hz), 1.27 (d, 3H, $J = 6.0$ Hz), 3.00–3.04 (m, 1H), 3.18–3.21 (m, 1H), 3.59 (t, 1H, $J = 9.2$ Hz), 3.76 (t, 1H, $J = 9.2$ Hz), 3.84–3.91 (m, 1H), 4.01–4.08 (m, 1H), 7.43 (s, 1H), 7.54 (d, 1H, $J = 8.4$ Hz), 7.63 (s, 1H), 7.68 (d, 1H, $J = 8.4$ Hz), 8.05 (s, 1H), 8.11 (s, 1H), 8.13 (s, 1H) ppm ^{13}C NMR (125 MHz, DMSO-d_6) $\delta = 21.6$, 21.8, 49.5, 49.8, 50.4, 50.4, 118.8, 119.8, 124.2, 131.7, 138.3, 155.1, 160.2, 161.2, 176.7 ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_{15}H_{20}N_7O_2S_2$) 394.1120. Found 394.1133.

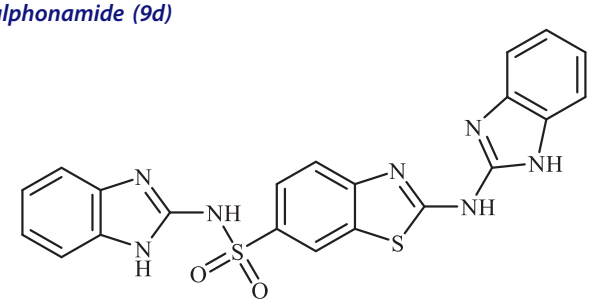
N-(3a,4,5,6,7,7a-hexahydro-1H-benzo[d]imidazol-2-yl)-2-((3a,4,5,6, 7,7a-hexahydro-1H-benzo[d]imidazol-2-yl)amino)benzo[d]thiazole-6-sulphonamide (9c)



A mixture of dimethyl (2-((bis(methylthio)methylene)amino)benzo[*d*]-thiazol-6-yl)sulphonylcarbonimidodithioate (**8**) (0.4 g, 1.03 mmol) and cyclohexane-1,2-diamine (1.65 ml, 15.45 mmol) in DMSO (5.0 ml) was stirred at 120 °C for 15 h. After completion of the reaction, the mixture was cooled to room temperature and treated with water (100 ml) and the formed precipitate was collected by filtration, washed with H_2O and *t*-BuOMe and dried under vacuum to afford compound **9c** (0.352 g, 81%) as white solid.

^{1}H NMR (500 MHz, DMSO-d_6) $\delta = 1.20$–1.52 (m, 8H), 1.66–1.82 (m, 4H), 1.94–2.17 (m, 4H), 2.94–3.05 (m, 2H), 3.17–3.21 (m, 2H), 7.61 (s, 1H), 7.65–7.83 (m, 3H), 8.06–8.31 (m, 3H) ppm ^{13}C NMR (125 MHz, DMSO-d_6) $\delta = 24.4$, 24.5, 29.6, 29.8, 62.3, 62.5, 119.2, 119.9, 124.3, 131.9, 138.3, 155.1, 162.6, 163.2, 176.4 ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_{21}H_{28}N_7O_2S_2$) 474.1746. Found 474.1750.

N-(3a,4,5,6,7,7a-hexahydro-1H-benzo[d]imidazol-2-yl)-2-((3a,4,5,6, 7,7a-hexahydro-1H-benzo[d]imidazol-2-yl)amino)benzo[d]thiazole-6-sulphonamide (9d)



A mixture of dimethyl (2-((bis(methylthio)methylene)amino)benzo[*d*]thiazol-6-yl)sulphonylcarbonimidodithioate (**8**) (0.4 g, 1.03 mmol) and *o*-phenylenediamine (1.48 g, 15.45 mmol) in DMSO (5.0 ml) was stirred at 120 °C for 15 h. After completion of the reaction, the mixture was cooled to room temperature, hydrazine hydrate (2 ml) was dropwise added and the reaction mixture was stirred for 1 h. Subsequently, the mixture was treated with water (100 ml) and the formed precipitate was collected by filtration, washed with H_2O and *t*-BuOMe and dried under vacuum to afford compound **9d** (0.249 g, 51%) as grey solid.

^{1}H NMR (500 MHz, DMSO-d_6) $\delta = 6.94$–7.60 (m, 8H), 7.63–7.98 (m, 2H), 8.36 (s, 1H), 11.95 (s, 2H), 12.23 (s, 2H) ppm ^{13}C NMR (125 MHz, DMSO-d_6) $\delta = 111.2$, 111.6, 118.2, 119.4, 119.6, 122.8, 124.0, 130.4, 131.5, 131.9, 138.7, 151.3, 151.6, 154.3, 173.7 ppm HRMS (ESI) $[M + H]^+$: *m/z* calcd for ($C_{21}H_{16}N_7O_2S_2$) 462.0807. Found 462.0813.

CA inhibition assays

An applied photophysics stopped-flow instrument has been used for assaying the CA-catalysed CO_2 hydration activity[13]. 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 were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min 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[14] and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in-house as reported earlier[15].

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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