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Sulfonamides incorporating boroxazolidone moieties are potent inhibitors of the transmembrane, tumor-associated carbonic anhydrase isoforms IX and XII

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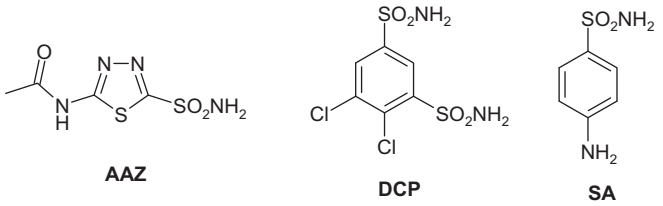
ABSTRACT

A new series of sulfonamides was synthesized by the reaction of the boroxazolidone complex of L-lysine with isothiocyanates incorporating sulfamoyl moieties and diverse organic scaffolds. The obtained thioureas have been investigated as inhibitors of four physiologically relevant human carbonic anhydrase (hCA, EC 4.2.1.1) isoforms, hCA I, II, IX and XII. Inhibition between the low nanomolar to the micromolar range has been observed against them, with several low nanomolar and tumor-CA selective inhibitors detected. These boron-containing compounds might be useful for the management of hypoxic tumors overexpressing hCA IX/XII by means of boron neutron capture therapy, a technique not investigated so far with inhibitors of this enzyme.

Carbonic anhydrase (CA, EC 4.2.1.1) isoforms IX (CA IX) and XII (CA XII) have recently been shown to be druggable targets for imaging and treatment of hypoxic tumors.[1–7] CA IX is one of the most strongly overexpressed genes in response to hypoxia in a high number of human cancer cells.[2–4] This enzyme is a multidomain protein[1,6] with the CA subdomain situated outside the cell and possessing a very high CO_2 hydrase catalytic activity.[1,6,7] This makes CA IX a key player in the regulation of the tumor pH.[1–7] CA IX expression is strongly increased in many types of solid tumors, such as gliomas/ependymomas, mesotheliomas, papillary/follicular carcinomas, as well as carcinomas of the bladder, uterine cervix, kidneys, esophagus, lungs, head and neck, breast, brain, vulva, and squamous/basal cell carcinomas, among others.[8,9] Furthermore, such hypoxic tumors do not generally respond to the classic chemo- and radiotherapy, and the strong acidification produced by CA IX overexpression also triggers the development of metastases.[8,9] Another isoform which is sometimes associated with cancers is CA XII, which similar to CA IX is a transmembrane enzyme with an extracellular active site and good activity for the hydration of CO_2 to bicarbonate and protons.[10] Recently, it has been shown by several groups[1–3] that the genetic silencing[2b] or pharmacologic inhibition[1,2a,c,d,11] of CA IX and XII have a strong anticancer effect, with growth delay of both the primary tumor and the metastases.[11]



Sulfonamides represent one of the classical chemotypes associated with potent CA inhibition.[1,12–15] They bind in deprotonated form to the Zn(II) ion from the enzyme active site, and also make a wide range of polar, hydrophobic and/or stacking interactions with amino acid residues from the enzyme cavity, leading to highly stable enzyme-inhibitor adducts.[1,12–15] Indeed, many aromatic, heterocyclic, aliphatic and sugar sulfonamides show activities in the range of the low micromolar to the low nanomolar for

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inhibiting various CA isoforms, among which also CA IX and XII.[1,12–15] However, the mammalian CA family comprises 16 isoforms, 13 of which are catalytically active and most of which are indiscriminately inhibited by classical sulfonamide CA inhibitors (CAIs) such as acetazolamide **AAZ**, dichlorophenamide **DCP** or sulfanilamide **SA**.[1,12–15] These and other sulfonamides are in clinical use as diuretics, antiglaucoma, anticonvulsant or antiinfective drugs for more than 50 years.[1] The strong correlations between CA IX/XII and tumors, and the possibility to inhibit these enzymes by sulfonamides and other CAIs, envisages the use of specific CA IX/XII inhibitors as antitumor drugs and diagnostic agents for hypoxic tumors, a rather new and unexpected application for CAIs.[1–4] Thus, there is a stringent need to design specific CAIs for the tumor-associated isoforms CA IX and XII, which should possess decreased affinity for the main offtarget CA isoforms (CA I and II, which are rather abundant in many tissues and participate in important physiological processes).[1,12–15]

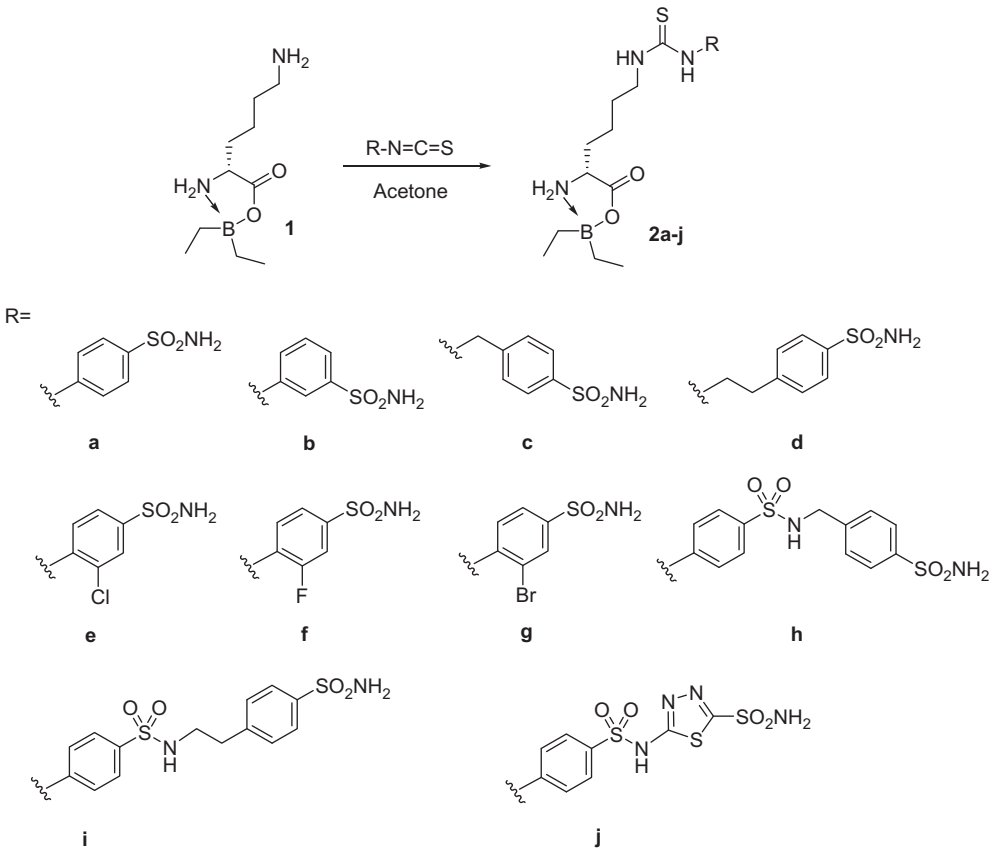
Continuing our interest in the design of various classes of CAIs, we report here the synthesis and evaluation as enzyme inhibitors of a new class of boron-containing compounds,[16] obtained starting from the boroxazolidone complex of L-lysine **1** (Scheme 1).[17]

The key intermediate **1** has only the ε-amino group enough reactive to be derivatized with isothiocyanates incorporating sulfamoyl moieties of the type RNCS, leading to thioureas of types **2a–2j** which have been investigated earlier as CAIs. Indeed, many compounds incorporating a thiourea linker between the aromatic benzenesulfonamide moiety and the tail have been reported by our groups to possess potent CA inhibitory effects against several physiologically relevant isoforms, such as CA II, IV, IX and XII among others.[18] Furthermore, the X-ray crystal structure of one such thioureido sulfonamide in complex with CA II has been reported,[19] giving interesting hints for the design of CAIs incorporating this functionality. We were interested to design CAIs incorporating boron-containing moieties, such as derivatives **2a–2j** reported here, due to their potential use as agents for boron neutron capture therapy of hypoxic tumors, a field not explored up until now in detail.[20] The new derivatives incorporate various aromatic (3-amino- and 4-aminobenzenesulfonamide derivatives) and heterocyclic (1,3,4-thiadizole-2-sulfonamide) sulfonamide heads and linkers of diverse nature/length, in order to generate chemical diversity and obtain a complete structure–activity relationship (SAR) for the inhibition of CAs. Compounds **2a–2j** have been characterized in detail by physico-chemical methods which confirmed their structures.[16]

Compounds **2a–2j** reported here and standard CAIs of the sulfonamide type were screened for the inhibition of the tumor-associated isoforms hCA IX and XII (h = human isoform) as well as the offtarget, cytosolic hCA I and II (Table 1).[21]

The following SAR has been observed for the inhibition of these four CA isozymes with sulfonamides **2a–2j**:

(i) Activities from the low nanomolar to the micromolar have been observed for the inhibition of the slow cytosolic isoform hCA I with derivatives **2a–2j**. The inhibition constants of these compounds were in the range of 3.2–6390 nM (Table 1). Derivatives **2h–2**i were very potent, low nanomolar hCA I inhibitors (K_Is of 3.2–6.8 nM). They incorporate a longer scaffold compared to other sulfonamides investigated here, which was shown[18] to be associated with very potent CA inhibitory activity against many isoforms, this fact being confirmed also for the boroxazolidone-containing derivatives investigated in the present paper. The sulfanilamide,



Scheme 1. Synthesis of the sulfonamides incorporating boroxazolidone moieties of type **2a–2j**.

Table 1
hCA I, II, IX and XII inhibition data with sulfonamides **2a–2j** and **AAZ–SA** as standard inhibitors, by a stopped-flow CO_2 hydration assay method.[21] The selectivity ratios for the inhibition of the tumor-associated over the cytosolic isoforms are also provided

Compd	K_i^* (nM)				Selectivity ratios			
	hCA I[a]	hCA II	hCA IX[b]	hCA XII[b]	I/IX	I/XII	II/IX	II/XII
2a	91	9.6	9.3	6.4	9.8	14.2	1.03	1.5
2b	3870	88	9.5	7.1	407.4	545.0	9.3	12.4
2c	96	9.6	9.5	7.0	10.1	13.7	1.0	1.4
2d	94	9.7	9.6	6.3	9.8	14.9	1.0	1.5
2e	6390	351	91	78	70.2	81.9	3.8	4.5
2f	3650	295	89	79	41.0	46.2	3.3	3.7
2g	6340	486	92	81	68.9	78.3	5.3	6.0
2h	5.1	13.9	7.7	6.9	0.66	0.74	1.8	2.0
2i	6.8	22.1	9.0	7.8	0.75	0.87	2.4	2.8
2j	3.2	15.0	8.6	7.0	0.37	0.45	1.7	2.1
AAZ	250	12	25	5.7	10.0	43.8	0.48	2.1
DCP	1200	38	50	50	24.0	24.0	0.76	0.76
SA	25,000	240	294	37	85.0	675	0.81	6.5

[a] Full length, cytosolic isoform.
[b] Catalytic domain, recombinant enzyme.
* Errors in the range of ±5–10% of the reported value, from three different determinations.

homosulfanilamide and 4-aminoethylbenzenesulfonamide derivatives **2a, 2c** and **2d** were on the other hand medium potency hCA I inhibitors, with K_Is in the range of 91–94 nM. The metanilamide (**2b**) and halogenosulfanilamide (**2e–2g**) derivatives were much weaker CAIs compared to the previously discussed compounds, with K_Is in the range of 3.65–6.39 μM (Table 1).

(ii) The new derivatives **2a–2j** showed inhibition constants in the range of 9.6–486 nM against the physiologically dominant, offtarget isoforms hCA II (Table 1). Again the SAR was rather well defined, with the simple derivatives **2a, 2c** and **2d** (all incorporating a 4-sulfamoylphenyl moieties) being quite effective inhibitors (K_Is of 9.6–9.7 nM), whereas the sulfonylated-sulfonamides **2i–2j** were slightly less effective (but still significant) hCA II inhibitors (K_Is of 13.9–22.1 nM), in the same range as the clinically used sulfonamides acetazolamide **AAZ** and dichlorophenamide **DCP**. The metanilamide derivative **2b** was a medium potency inhibitor (K_I of 88 nM) whereas the halogenated sulfanilamides incorporating chlorine, fluorine or bromine (**2e–2g**) showed a significant loss of activity compared to the parent sulfanilamide derivative **2a**, with inhibition constants of 295–486 nM (the loss of activity increased with the increase of the atomic weight of the halogen present in these molecules).

(iii) The tumor-associated isoform hCA IX was strongly inhibited by the benzenesulfonamides **2a–2d** (both *para*- and *meta*-sulfamoyl groups were equally effective for this isoform inhibition) and by the sulfonylated-sulfonamides with a longer molecule such as **2h–2j**, which showed K_Is in the range of 7.7–9.6 nM. The halogenated sulfanilamides **2e–2g** were around one order of magnitude less effective as hCA IX inhibitors (compared to **2a**), with K_Is in the range of 81–92 nM. It should be observed that many of the new sulfonamides **2** reported here are much more effective hCA IX inhibitors compared to the clinically used derivatives **AAZ** and **DCP**, or than **SA**. Thus, the introduction of the boroxazolidone complex of L-lysine moiety is highly beneficial for obtaining highly potent hCA IX inhibitors based on the relatively weak lead compound **SA**.

(iv) The SAR described above for hCA IX inhibition was very similar for the inhibition of the second tumor-associated isoform, hCA XII (which shares a rather high degree of homology with CA IX).[22] Indeed, compounds **2a–2d** and **2h–2j** showed highly effective hCA XII inhibitory properties, with K_Is of 6.3–7.8 nM, whereas the halogenated sulfanilamides **2e–2g** were less effective hCA XII inhibitors (K_Is of 78–81 nM).

(v) The selectivity ratios for inhibiting the target isoforms (in this case hCA IX and XII) over the offtarget ones (in this case hCA I and II) represent significant challenges when designing CAIs.[1] Usually the offtarget isoform hCA I is less important as many classes of sulfonamides show a diminished activity against it, compared to the inhibition of hCA II, which is usually sulfonamide-avid.[1,12] For this specific class of CAIs reported here, it may be observed however that some derivatives (**2h–2j**) are highly effective, low nanomolar hCA I inhibitors. Thus, the selectivity ratios against this isoform will be also analyzed. As seen from data of Table 1, the selectivity ratios for the inhibition of hCA IX over hCA I were in the range of 0.37–407.4, having thus both isoform IX over I selective compounds as well as the reverse among the newly prepared sulfonamides reported here. Indeed, **2b** is an hCA IX over hCA I selective inhibitor, whereas **2j** is hCA I over hCA IX selective (but with not a high selectivity ratio). The selectivity ratios for inhibiting hCA XII over hCA I were in the range of 0.45–545, and again the same two compounds were selective both for hCA XII over hCA I (**2b**) and for hCA I over hCA XII (**2j**).

The selectivity ratio for the inhibition of hCA IX and XII over hCA II is usually a problematic issue, since all classical CAIs (e.g., **AAZ, DCP**)[1] have higher affinity for CA II than for CA IX. As a consequence such compounds should not be used for the selective inhibition of the tumor-associated isozyme as they will preponderantly inhibit CA II (offtarget) instead of CA IX (target enzyme). For the derivatives **2** reported here, we observed selectivity ratios for inhibiting hCA IX over hCA II in the range of 1.0–9.3, and for inhibiting hCA XII over hCA II in the range of 1.4–12.4. The most tumor-associated isoform selective inhibitor (over hCA II) was **2b**. Indeed, this compound also showed selectivity for the inhibition of the tumor-associated isoforms over hCA I, as discussed above, and it can be concluded that we have discovered an interesting, potent and selective CA IX/XII inhibitor incorporating boron in its molecule.

In conclusion, we report here a new series of sulfonamides prepared by the reaction of the boroxazolidone complex of L-lysine with isothiocyanates incorporating sulfamoyl moieties and diverse organic scaffolds. The obtained thioureas have been investigated as

inhibitors of four physiologically relevant CA isoforms, hCA I, II, IX and XII. Inhibition between the low nanomolar to the micromolar range has been observed against them, with several low nanomolar and tumor-CA selective inhibitors detected. These boron-containing compounds might be useful for the management of hypoxic tumors overexpressing CA IX/XII by means of boron neutron capture therapy.

Acknowledgements

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16. The boroxazolidone complex of L-lysine **1** was prepared as previously described.[17] ^{1}H NMR (400 MHz), DMSO-d_6) δ = −0.02–0.20 (m, 4H), 0.45–0.48 (m, 6H), 1.16–1.20 (m, 2H), 1.28–1.31 (m, 4H), 2.10 (br s, 1H), 3.19–3.21 (m, 2H), 4.00 (br s, 2H, NH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 8.85, 8.94, 12.10,12.70, 23.16, 28.59, 30.19, 50.44, 54.31, 173.99. MS (ESI$^+$/ESI$^-$) *m*/*z*: 215.27 [M+H]$^+$, 237.21 [M+Na]$^+$, 429.42 [2M+H]$^+$, 213.35 [M−H]$^-$, 259.28 [M+Cl]$^-$, 427.29 [2M−H]$^-$.
*General procedure for the synthesis of the thiourea **2a–2j**:*
One equivalent of **1** was mixed with 1 equiv of the corresponding substituted isothiocyanatophenyl-sulfonamide in acetone. The mixture was refluxed overnight, then concentrated under vacuum. The crude product was purified by silica gel column chromatography using methylene chloride/methanol (95:5) as eluent.
N^{ε}-[(4-Sulfamoylphenyl)thioureido]-L-lysinatodiethylboron (**2a**): Yield 68%; mp 177–179 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.18–0.26 (m, 4H), 0.67–0.73 (m, 6H), 1.42–1.47 (m, 2H), 1.54–1.59 (m, 4H), 1.81–1.84 (m, 1H), 3.45–3.47 (m, 2H), 5.55 (t, 1H, ^{6}NH, *J* = 5.62 Hz), 7.27 (s, 2H), 7.64 (d, 2H), 7.71 (d, 2H, *J* = 8.46 Hz), 9.78 (s, 1H, NH); ^{13}C NMR (101 MHz, DMSO-d_6) δ 8.89, 8.91, 12.13, 12.83, 23.08, 27.86, 30.22, 43.59, 54.32, 121.40, 126.20, 138.23, 142.27, 174.02, 180.10. MS (ESI$^+$/ESI$^-$) *m*/*z*: 429.21 [M+H]$^+$, 451.15 [M+Na]$^+$, 427.21 [M−H]$^-$, 463.22 [M+Cl]$^-$.
N^{ε}-[(3-Sulfamoylphenyl)thioureido]-L-lysinatodiethylboron (**2b**): Yield 75%; mp 98–100 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.19–0.25 (m, 4H), 0.67–0.73 (m, 6H), 1.40–1.47 (m, 2H), 1.54–1.62 (m, 4H), 1.81–1.84 (m, 1H), 3.45–3.47 (m, 2H), 5.55 (t, 1H, ^{6}NH, *J* = 5.65 Hz), 7.37 (s, 2H), 7.48–7.51 (m, 2H), 7.66–7.68 (m, 1H), 7.93 (s, 1H), 9.77 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 8.89, 8.90, 12.13, 12.78, 23.08, 27.94, 30.22, 43.55, 54.32, 119.40, 120.73, 125.67, 128.99, 140.03, 144.18, 174.00, 180.43; MS (ESI$^+$/ESI$^-$) *m*/*z*: 429.01 [M+H]$^+$, 451.16 [M+Na]$^+$, 427.14 [M−H]$^-$, 463.17[M+Cl]$^-$.
N^{ε}-[(4-Sulfamoylphénylmethyl) thioureido]-L-lysinatodiethylboron (**2c**): Yield 72%; mp 75–87 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.17–0.27 (m, 4H), 0.67–0.73 (m, 6H), 1.40–1.47 (m, 2H), 1.54–1.57 (m, 4H), 1.78–1.82 (m, 1H), 2.86 (t, 2H, *J* = 6.80 Hz), 3.60–3.61 (m, 2H), 5.52 (t, 1H, ^{6}NH, *J* = 6.30 Hz), 7.31 (s, 2H), 7.44 (d, 2H, *J* = 8.34 Hz), 7.74 (d, 2H, *J* = 8.34 Hz), ^{13}C NMR (101 MHz, DMSO-d_6) δ 8.89, 8.91, 12.10, 12.75, 23.00, 28.32, 30.22, 34.55, 47.02, 54.32, 125.66, 129.07, 141.99, 143.58, 174.01, 183.42; MS (ESI$^+$/ESI$^-$) *m*/*z*: 443.15 [M+H]$^+$, 465.23 [M+Na]$^+$, 441.15 [M−H]$^-$, 477.17 [M+Cl]$^-$.
N^{ε}-[(4-Sulfamoylphenylethyl)thioureido]-L-lysinatodiethylboron (**2d**): Yield 81%; mp 79–81 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.17–0.25 (m, 4H), 0.66–0.72 (m, 6H), 1.40–1.47 (m, 2H), 1.54–1.57 (m, 4H), 1.78–1.82 (m, 1H), 2.87 (t, 2H, *J* = 6.94 Hz), 3.42–3.44 (m, 2H), 3.60–3.61 (m, 2H), 5.52 (t, 1H, *J* = 5.94 Hz), 7.30 (s, 2H), 7.39 (d, 2H, *J* = 8.20 Hz), 7.73 (d, 2H, *J* = 8.20 Hz); ^{13}C NMR (101 MHz, DMSO-d_6) δ 8.88, 8.91, 12.06, 12.79, 23.00, 28.31, 30.20, 34.58, 43.4, 53.50, 54.32, 125.65, 129.06, 141.99, 143.57, 174.01, 183.21; MS (ESI$^+$/ESI$^-$) *m*/*z*: 457.22 [M+H]$^+$, 479.23 [M+Na]$^+$, 455.22 [M−H]$^-$, 491.11 [M+Cl]$^-$.
N^{ε}-[(2-Chloro-4-sulfamoylphenyl)thioureido]-L-lysinatodiethylboron (**2e**): Yield 74%; mp 62–64 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.18–0.27 (m, 4H), 0.67–0.73 (m, 6H), 1.46–1.48 (m, 2H), 1.56–1.59 (m, 4H), 1.81–1.83 (m, 1H), 3.45–3.48 (m, 2H), 5.54 (t, 1H, *J* = 6.06 Hz), 7.47 (s, 2H), 7.69–7.71 (dd, 1H, *J* = 8.45 Hz, *J* = 2.02 Hz), 7.85 (d, 1H, *J* = 2.02 Hz), 8.05–8.07 (d, 1H, *J* = 8.46 Hz), 8.37 (t, 1H, *J* = 5.2 Hz), 9.35 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 8.85, 8.90, 12.12, 12.77, 23.07, 27.83, 30.19, 43.87, 54.12, 64.86, 124.17, 126.55, 127.78, 139.26, 140.99, 174.00, 180.66; MS (ESI$^+$/ESI$^-$) *m*/*z*: 463.16 [M+H]$^+$, 485.23 [M+Na]$^+$, 461.02 [M−H]$^-$, 497.18 [M+Cl]$^-$, 463.16 [M+H]$^+$, 485.23 [M+Na]$^+$.
N^{ε}-[(2-Fluoro-4-sulfamoylphenyl)thioureido]-L-lysinatodiethylboron (**2f**): Yield 83%; mp 93–95 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.18–0.27 (m, 4H), 0.66–0.73 (m, 6H), 1.43–1.48 (m, 2H), 1.55–1.60 (m, 4H), 1.81–1.84 (m, 1H), 3.45–3.48 (m, 2H), 5.54 (t, 1H, ^{6}NH, *J* = 6.00 Hz), 7.45 (s, 2H), 7.57 (d, 1H, *J* = 2.02 Hz), 7.59 (s, 1H), 7.62 (d, 1H, *J* = 2.02 Hz), 8.35 (t, 1H, *J* = 5.2 Hz), 9.58 (s, 1H,); ^{13}C NMR (101 MHz, DMSO-d_6) δ 8.88, 8.90, 12.08, 12.73, 23.06, 27.85, 30.20, 43.83, 54.30, 64.86, 112.98, 113.21, 121.41, 126.35, 139.00, 173.99, 180.64; MS (ESI$^+$/ESI$^-$) *m*/*z*: 447.08 [M+H]$^+$, 469.16 [M+Na]$^+$, 915.33 [2M+Na]$^+$, 445.15 [M−H]$^-$, 481.13 [M+Cl]$^-$, 291.18 [2M−H]$^-$, 447.08 [M+H]$^+$, 469.16 [M+Na]$^+$, 915.33 [2M+Na]$^+$;
N^{ε}-[(2-Bromo-4-sulfamoylphenyl)thioureido]-L-lysinatodiethylboron (**2g**): Yield 63%; mp 68–70 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.18–0.26 (m, 4H), 0.67–0.73 (m, 6H), 1.46–1.48 (m, 2H), 1.56–1.59 (m, 4H), 1.81–1.85 (m, 1H), 3.45–3.47 (m, 2H), 5.54 (t, 1H, ^{6}NH, *J* = 6.06 Hz), 7.48 (s, 2H), 7.74 (dd, 1H, *J* = 8.46 Hz, *J* = 1.64 Hz), 7.92 (d, 1H, *J* = 8.46 Hz), 8 (d, 1H, *J* = 2.02 Hz), 8.36 (m, 1H), 8.29 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 8.89, 8.90, 12.07, 12.79, 23.08, 27.89, 30.20, 43.90, 54.29, 64.86, 124.71, 128.56, 129.65, 140.69, 141.39, 173.99, 180.77; MS (ESI$^+$/ESI$^-$) *m*/*z*: 507.00 [M+H]$^+$, 529.05 [M+Na]$^+$, 504.96 [M−H]$^-$, 541.18 [M+Cl]$^-$, 1047.08 [2M+Cl]$^-$.

N^{ε}-[(4-(4-Sulfamoylbenzyl)sulfamoylphenyl)thioureido]-L-lysinatodiethylboron (**2h**): Yield 57%; mp 85–87 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.15–0.23 (m, 4H), 0.64–0.70 (m, 6H), 1.43–1.45 (m, 2H), 1.52–1.58 (m, 4H), 1.78–1.80 (m, 1H), 3.44–3.47 (m, 2H, H_5), 4.00 (d, 2H), 5.52 (t, 1H, ^{6}NH, J = 5.93 Hz), 7.30 (s, 2H), 7.41 (d, 2H, J = 7.20 Hz), 7.71 (d, 2H, J = 7.20 Hz), 8.13 (t, 1H, CH_2, J = 4.80 Hz), 9.98 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 8.88, 8.90, 12.04, 12.74, 23.07, 27.82, 30.21, 43.58, 45.48, 54.31, 125.78, 127.78, 141.98, 142.86, 173.99, 180.51.; MS (ESI$^+$/ESI$^-$) m/z: 598.31 [M+H]$^+$, 620.17 [M+Na]$^+$, 596.09 [M−H]$^-$, 632.11 [M+Cl]$^-$.

N^{ε}-[(4-(4-Sulfamoylphenylethyl)sulfamoylphenyl)thioureido]-L-lysinatodiethylboron (**2i**): Yield 61%; mp 73–75 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.21–0.25 (m, 4H), 0.67–0.73 (m, 6H), 1.45–1.47 (m, 2H), 1.56–1.58 (m, 4H), 1.81–1.82 (m, 1H), 2.76 (t, 2H, J = 7.20H), 2.95–3.0 (m, 2H), 3.47 (m, 2H), 5.55 (t, 1H, ^{6}NH, J = 6.06 Hz), 7.30 (s, 2H), 7.35 (d, 2H, J = 7.80 Hz), 7.71 (d, 2H, J = 7.80 Hz), 8.26 (t, 1H, J = 4.80 Hz), 10.0 (s, 1H), ^{13}C NMR (101 MHz, DMSO-d_6) δ 8.88, 8.90, 12.09, 12.79, 23.06, 27.82, 30.20, 34.87, 43.55, 43.58, 54.31, 125.60, 129.16, 142.08, 142.92, 173.99, 180.02; MS (ESI$^+$/ESI$^-$) m/z: 612.16 [M+H]$^+$, 634.16 [M+Na]$^+$, 610.20 [M−H]$^-$, 646.36 [M+Cl]$^-$.

N^{ε}-[(4-(5-Sulfamoyl-[1,3,4]thiadiazol-2-ylsulfamoyl)-phenyl)thioureido]-L-lysinatodiethylboron (**2j**): Yield 52%; mp 135–137 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.17–0.25 (m, 4H), 0.67–0.72 (m, 6H), 1.43–1.47 (m, 2H), 1.54–1.57 (m, 4H), 1.80–1.82 (m, 1H), 3.44 (m, 2H), 7.50 (s, 1H), 7.49 (d, 2H, J = 8.70 Hz), 7.62 (d, 2H, J = 8.70 Hz), 5.54 (t, 1H, J = 5.87 Hz), 7.77 (s, 2H), 8.26 (s, 1H), 9.82 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 8.88, 8.91, 12.56, 12.76, 23.11, 27.86, 30.22, 43.62, 54.32, 121.25, 126.28, 135.17, 136.20, 141.63, 157.40, 174.02, 179.94; MS (ESI$^+$/ESI$^-$) m/z: 592.13 [M+H]$^+$, 614.03 [M+Na]$^+$, 590.09 [M−H]$^-$, 626.11 [M+Cl]$^-$.

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