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Structure–activity relationship for aryl and heteroaryl boronic acid inhibitors of hormone-sensitive lipase

Søren Ebdrup,* Poul Jacobsen, Anupma Dhanda Farrington and Per Vedsø

Novo Nordisk A/S, Novo Nordisk Park, 2760 Måløv, Denmark

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Abstract—A range of aryl and heteroaryl boronic acids were tested for their in vitro hormone-sensitive lipase inhibitory properties. (2-Benzyloxy-5-fluorophenyl)boronic acid, (2-benzyloxy-5-chlorophenyl)boronic acid and 5-bromothiophene-2-boronic acid were found to be the most potent HSL inhibitors with IC_{50} values of 140, 17 and 350 nm, respectively.

1. Introduction

Type 2 diabetes including the metabolic syndrome is a chronic multifactorial metabolic disease. One of the characteristics of the disease is elevated levels of fatty acids (FAs) in the circulation, which is widely considered a major pathogenetic factor. It has been shown that elevated FA levels are associated with peripheral insulin resistance, decreased glucose uptake by muscle tissue and increased hepatic triglyceride (TG) release to the circulation.[1–3]

Hormone-sensitive lipase (HSL) is a multifunctional tissue lipase that plays a central role in the overall energy homeostasis as it is the rate limiting enzyme in the release of FA from adipocytes.[4] HSL catalyzes the first and second step in the breakdown of triglycerides, releasing two molecules of FA and monoacylglycerol, which is further hydrolyzed by monoacylglycerol lipase.[5]

HSL has a broad substrate specificity as it catalyzes the hydrolysis of both cholesteryl esters and acylglycerides.[4] The activity of HSL is regulated via phosphorylation/dephosphorylation primarily controlled by catecholamines and insulin.[6]

The enzyme belongs to a class of hydrolases that adapt the α/β hydrolase fold and contains a catalytic triad of serine, histidine and aspartic acid and an oxyanion hole.[7]

Due to the dysregulated metabolism of type 2 diabetic patients and the elevated level of FA the interest in targeting HSL with the aim of reducing insulin resistance and dyslipidemia in obese, prediabetic and diabetic individuals has recently gained considerable interest.

Thus carbamoyl triazoles[8] (**1**), carbamoyl isoxazole-5-ones[9] (**2**), oxadiazoles[10,11] (**3**), cyclipostines[12] (**4**), carbazates[13] (**5**) and pyrrolopyrazinediones[14] (**6**) have been identified as HSL inhibitors. Most of the HSL inhibitors described are expected to be pseudosubstrate inhibitors[8] (inhibition mechanism for pyrrolopyrazinediones unknown). A few carbamoyltriazoles (**1**, R1 = Cl and R2 = CF_3CH_2 or **1**, R1 = OCF_3 and R2 = CH_3)[8] and a carbamoyl isoxazole-5-one (**2**, R1 and R2 forms a *S*-3-methyl substituted piperidine ring and R3 = CH-$(CH_3)_2$)[8] are the only selective HSL inhibitors described (Fig. 1).

Reversible hydrolase inhibitors such as boronic acids have been extensively used in the development of enzyme inhibitors of peptidases/proteases and lipases and employed as pharmaceutical agents.[15] In this paper the HSL structure–activity relationship for a range of aryl and heteroaryl boronic acids will be presented together with counter screening data for selected hydrolases.

2. Results and discussion

Boronic acids are strong Lewis acids due to the incomplete boron electron shell. Depending on the phenyl substitution, most phenylboronic acids have a pK_a in the

* Corresponding author. Tel.: +45 4443 4830; fax: +45 4466 3939; e-mail: sebd@novonordisk.com

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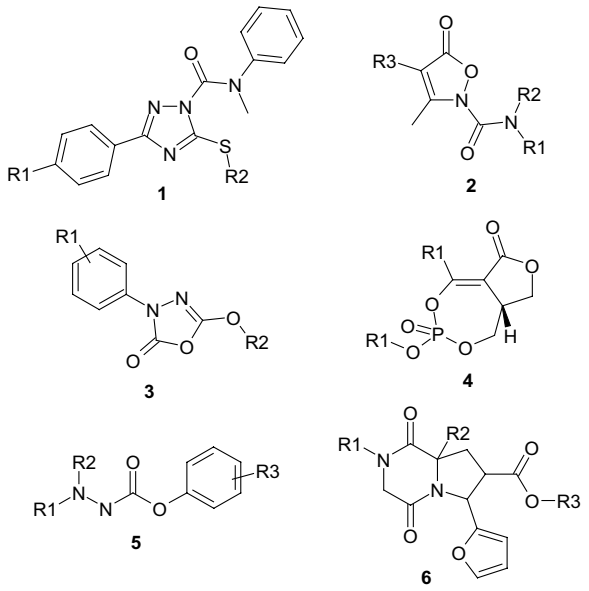


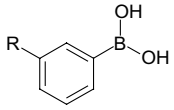
Figure 1. Structure of HSL inhibitors.

range 4.5–9.0 and suitable substitution of the aryl ring can provide boronic acids with the correct properties for ready conversion from a neutral and trigonal planar sp^2 boron to an anionic tetrahedral sp^3 boron under physiological conditions.[15] This sp^2–sp^3 transition state conversion is similar to that seen for the amide and ester carbon atom during cleavage of natural substrates.

Due to the described characteristics of aryl and heteroaryl boronic acids as reversible hydrolase inhibitors we decided to explore if it was possible to find aryl or heteroaryl boronic acids, which were capable of inhibiting HSL. Different substituted phenyl and thiopheneboronic acids were tested and selected compounds were also counter screened for butyrylcholin esterase (BChE) and acetylcholine esterase (AChE) inhibitory activity.

As can be seen from Table 1 phenylboronic acids bearing an electron withdrawing group in the meta position (**7a**–**e**) are capable of inhibiting HSL with IC_{50} values between 0.85 and 5.9 μM. The calculated pK_a values (Table 1) of the most active *meta* substituted phenylboronic acids are about 7.5 whereas the inactive analogues (**7h**,**7f**–**j**) all have higher pK_a values (7.8–8.6) suggesting that they are not capable of forming a strong interaction with the active site serine as compared to **7a**–**e**. Despite the high pK_a value of 8.0, **7g** is capable of inhibiting HSL with an IC_{50} value of 4.6 μM. As a reference, the experimental determined pK_a value of phenylboronic acid is 8.8.[16] and the HSL IC_{50} value of phenylboronic acid is above 10 μM.

Table 1. Activity and selectivity of *meta* substituted phenylboronic acids



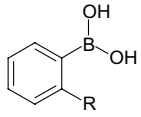
No.	R	HSL IC_{50}, μM	BChE IC_{50}, μM	Calculated pK_a
7a[a]	Br	5.9 ± 0.55	>10	7.6
7b[a]	Cl	4.4	>50	7.6
7c[a]	F	5.9	>50	7.5
7d[a]	OF_3C	0.85 ± 0.45	>50	7.4
7e[a]	CF_3	1.0 ± 0.20	>50	7.6
7f	CO_2Et	>50	na	7.8
7g[a]	SMe	4.6	>50	8.0
7h	OEt	>50	na	8.1
7i[a]	OBu[b]	21	>50	8.1
7j	Me	>50	na	8.6

[a] AChE IC_{50} > 50 μM.
[b] OBu: Butyl ether.

ortho Substituted phenylboronic acids were also tested (Table 2) but only **8f** and **8g** were active with IC_{50} values of 3.4 and 1.1 μM, respectively. Due to the relatively high calculated pK_a value of 8.6 it is expected that lipophilic interactions of the butyl and benzyl ether groups are responsible for the surprisingly low IC_{50} values. Compound **8a**–**e**, which have lower pK_a values than **8f** and **8g** were not active.

Only one *para* substituted phenylboronic acid (**9c**, Table 3) has an IC_{50} value in the low micromole range (1.3 μM) in contrast to its relatively high pK_a value of 8.4. Surprisingly **9d**,**9e** and **9g** were not active despite

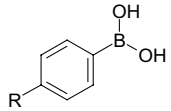
Table 2. Activity and selectivity of *ortho* substituted phenylboronic acids



No.	R	HSL IC_{50}, μM	BChE IC_{50}, μM	Calculated pK_a
8a	Br	>50	na	8.3
8b	Cl	>50	na	8.2
8c[a]	F	>50	>10	8.3
8d[a]	CF_3	>50	11	8.1
8e[a]	NO_2	>50	>10	7.8
8f[a]	OBu[b]	3.4	>50	8.6
8g[a]	OBn[c]	1.1	21	8.6

[a] AChE IC_{50} > 50 μM.
[b] OBu: Butyl ether.
[c] OBn: Benzylether.

Table 3. Activity and selectivity of *para* substituted phenylboronic acids



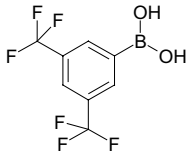
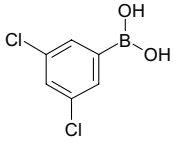
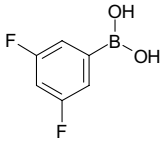
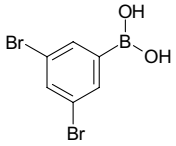
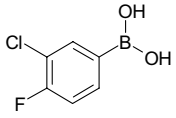
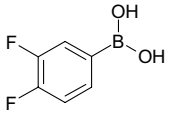
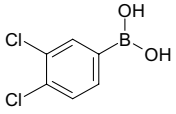
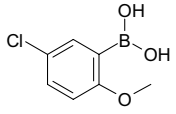
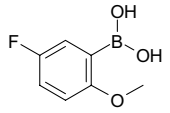
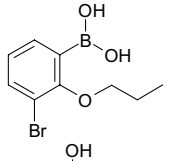
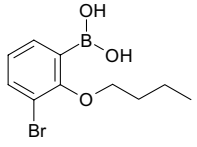
No.	R	HSL IC_{50}, μM	AChE/BChE IC_{50}, μM	Calculated pK_a
9a	OCH_2Ph	>50	na	8.7
9b	F	>50	na	8.7
9c	Cl	1.3	>50	8.4
9d	CF_3	>10	>50	7.8
9e	OCF_3	>50	>50	8.3
9f	OMe	>50	>50	9.0
9g	SO_2Me	>50	na	7.2

lower calculated pK_a values. Steric hindrance might be responsible for this lack of inhibitory activity.

As most of the potent phenylboronic acids have electron withdrawing groups in the *meta* and *para* positions we tested different disubstituted phenylboronic acids possessing two electron withdrawing groups **10a**–**g** (Table 4). Three dihalo-phenylboronic acids were active in the low micromole range. **10c** (3,5-difluoro), **10e** (3-chloro-4-fluoro) and **10f** (3,4-difluoro) had IC_{50} values of 2.2, 2.6 and 6 μM, respectively. Surprisingly **10g** (3,4-dichloro) was not active even though both mono substituted analogues **7b** and **9c** were active.

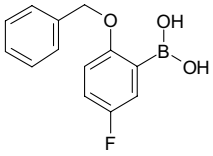
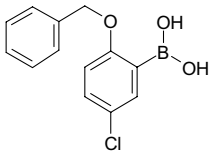
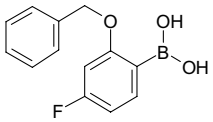
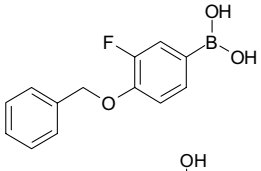
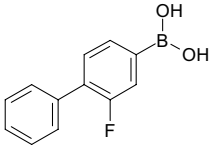
The *ortho* ether structure element of **8f** and **8g** and the *meta* and *para* electron withdrawing structure element

Table 4. Activity and selectivity of disubstituted phenylboronic acids

No.	Structure	HSL IC_{50}, μM	AChE/BChE IC_{50}, μM	Calculated pK_a
10a		>50	na	6.6
10b		>10	na	6.6
10c		2.2	>50	6.5
10d		>50	na	6.6
10e		2.6	>50	7.6
10f		6	>50	7.6
10g		>50	na	7.4
10h		28	>50	7.6
10i		>10	na	7.5
10j		>50	>50	7.6
10k		>50	>50	7.6

(*continued on next page*)

Table 4 (*continued*)

No.	Structure	HSL IC_{50}, μM	AChE/BChE IC_{50}, μM	Calculated pK_a
10l[a]		0.14 ± 0.028	>50	7.5
10m		0.017 ± 0.070	>10	7.6
10n		0.76	>10	8.7
10o		>50	na	7.6
10p		>50	na	7.5

[a] Hepatic lipase (HL) IC_{50} > 10 μM; lipoprotein lipase (LPL) IC_{50} > 10 μM; and pancreatic lipase (PL) IC_{50}: 7μM.

of **7a**–**e** and **9c** were combined in the search for more potent inhibitors. The 2-methoxy-5-chloro analogue **10h** was a weak inhibitor with an IC_{50} value of 28 μM. The *ortho* propoxy and butoxy ethers **10j** and **10k** were inactive but surprisingly **10l** and **10m** bearing a benzyloxy group in the 2-position and a fluoro or a chloro group in the 5-position were potent HSL inhibitors with IC_{50} values of 140 nm and 17 nm, respectively. The 4-fluoro regioisomer **10n** was also active with an IC_{50} value of 0.76 μM. The pK_a values of **10l** and **10n** were in the same range as the pK_a values of **7b**,**7c** and **9b**. Compound **10o**, the 4-benzyloxy isomer of **10l**, was not capable of inhibiting HSL which is in agreement with data for **9a**.

Table 5. Activity and selectivity of thiopheneboronic acids

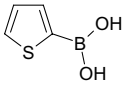
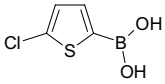
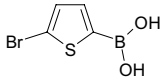
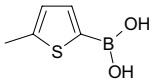
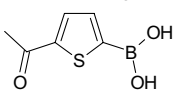
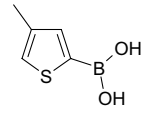
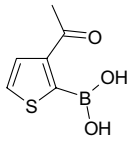
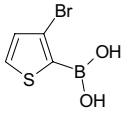
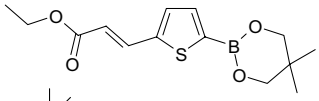
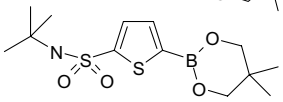
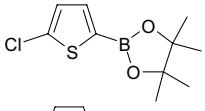
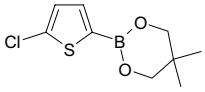
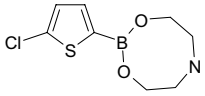
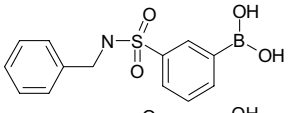
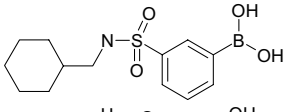
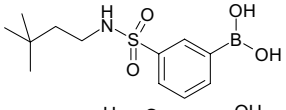
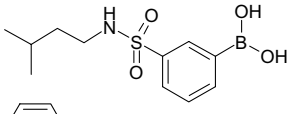
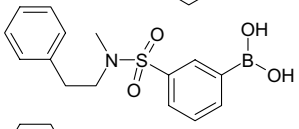
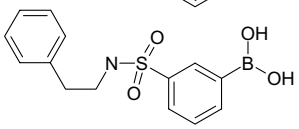
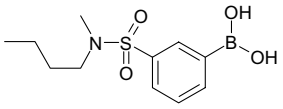
No.	Structure	HSL IC_{50}, μM	BChE IC_{50}, μM	Calculated pK_a
11a		>50	na	8.4
11b[b]		0.71 ± 0.38	>50	8.3
11c[a]		0.35 ± 0.053	>50	8.2
11d[b]		>50	>50	8.7
11e		>50	na	7.5
11f[b]		>10	>50	8.5

Table 5 (*continued*)

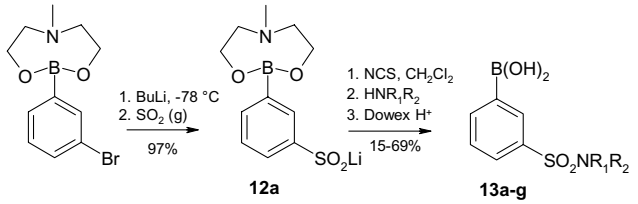
No.	Structure	HSL IC_{50}, μM	BChE IC_{50}, μM	Calculated pK_a
11g		>50	na	8.1
11h		>50	na	8.1
11i		>50	na	7.9[c]
11j		>50	na	7.9[c]
11k[b]		1.28	>10	8.3[c]
11l[b]		1.09	>10	8.3[c]
11m[b]		1.1	>10	8.3[c]

[a] Hepatic lipase (HL) IC_{50} > 10 μM; lipoprotein lipase (LPL) IC_{50} > 10 μM; and pancreatic (PL) IC_{50}: >10 μM.
[b] AChE IC_{50} > 50 μM.
[c] Calculated pK_a value of corresponding boronic acid.

Table 6. Activity and selectivity of 3-sulfonamide substituted phenylboronic acids

No.	Structure	HSL IC_{50}, μM	BChE IC_{50}, μM	Calculated pK_a
13a[a]		>50	>10	7.4
13b[a]		>50	>10	7.4
13c[a]		>50	>10	7.4
13d[a]		>50	>50	7.4
13e[a]		>50	>50	7.5
13f[a]		7.5	>10	7.4
13g[a]		>50	>50	7.5

[a] AChE IC_{50} > 50 μM.



Scheme 1. Synthesis of 3-sulfonamide substituted phenylboronic acids.

A range of thiopheneboronic acids were also tested (Table 5) and surprisingly the chloro and bromo substituted analogues **11b** and **11c** were potent HSL inhibitors with IC_{50} values of 710 nm and 350 nm, respectively. The two compounds both have calculated pK_a values of about 8.3, which is considerably higher than the corresponding *meta* substituted phenylboronic acids ($pK_a \approx 7.6$). Surprisingly, the acetyl analogue **11e** was inactive despite a lower pK_a value (7.5). The extended analogues **11i** and **11j** were also inactive indicating that only small substituents are accepted in the 5-position of the thiophene ring. The boronic ester analogues **11k**–**m** of **11b** were all equipotent with the boronic acid **11b** suggesting that the boronic esters are hydrolyzed to **11b** in the aqueous assay media.

Electron withdrawing groups in the *meta* position of the phenylboronic acid seem in general to be the most favourable pattern. Thus a series of 3-sulfonamide substituted phenylboronic acids **13a**–**g** were synthesized employing our recently described protocol[17] in which the 3-bromo substituted *N*-methyldiethanolamine protected phenylboronic acid undergoes selective bromine–lithium exchange at −78 °C upon treatment with *n*-BuLi (Scheme 1). The lithio intermediate was trapped with sulfur dioxide providing the corresponding lithium sulfinate **12a** in 97% yield. Oxidation with *N*-chlorosuccinimide gives the sulfonyl chloride, which upon reaction with a range of alkyl amines produce the corresponding 3-sulfonamide substituted arylboronic acids **13a** and **13g** (Table 6). However, all compounds except **13f** (IC_{50} of 7.5 μM) were inactive.

3. Conclusion

In conclusion, a range of substituted phenyl and thiopheneboronic acids have been identified as HSL inhibitors with IC_{50} values in the low to high nm range. The phenylboronic acids **10l** and **10m** and the thiopheneboronic acid **11c** were the most potent HSL inhibitors with IC_{50} values of 140, 17 and 350 nm, respectively.

It seems as if large substituents are only accepted in the *ortho* and *meta* position of phenylboronic acids. In general, it seems like steric hindrance and lipophilic interactions are of greater importance for the inhibitory activity than the actual pK_a value of the boronic acid. As **8g** and **11l**–**n** are more potent than what would have been expected from their calculated pK_a values it is expected that lipophilic interaction between the *ortho* benzyloxy group of the inhibitors and HSL is important for the inhibitory properties.

The two HSL inhibitors **10l** and **11c** were also tested for hepatic lipase (HL), lipoprotein lipase (LPL) and pancreatic lipase (PL) activity. None of the compounds inhibited HL and LPL but unfortunately **10l** inhibited PL with an IC_{50} value of 7 μM. Further investigations will be conducted with the aim of identifying selective HSL inhibitors and to evaluate the biological importance of targeting HSL. Inhibitors of HSL might have a future for the treatment of type 2 diabetes, the metabolic syndrome and impaired glucose tolerance.

4. Experimental

4.1. General methods and materials

All reactions involving air-sensitive reagents were performed under nitrogen using syringe-septum cap techniques. The glassware was flame dried prior to use. $MgSO_4$ were used to dry solutions. Solvents were removed in vacuo by rotary evaporation. Melting points were recorded on a Büchi 535 and are uncorrected. NMR spectra were recorded on a Bruker AMX 400 or Bruker DRX 300 instrument with tetramethylsilane (TMS) as internal standard. Liquid chromatography-mass spectrometry (LC–MS) analysis was performed on HP1100 MSD equipped with a Waters Xterra® MS C-18 5 μm 3.0 × 50 mm column. All solvents and reagents were obtained from commercial sources and used without further purification. Butyl lithium was titrated prior to use.

4.2. 3-[5-(5,5-Dimethyl-[1,3,2]dioxaborinan-2-yl)-thiophen-2-yl]-acrylic acid ethyl ester (11i)

A solution of 2,2,6,6-tetramethylpiperidine (1.53 mL, 9 mmol) in anhydrous THF (50 mL) was stirred under nitrogen. The mixture was cooled to −5 °C (internal temperature). *n*-Butyllithium (1.6 M in hexanes, 5.5 mL, 8.8 mmol) was added over 6 min. The mixture was stirred for an additional 30 min at −5 °C before being cooled to −105 °C N_2(l)/Et_2O. 3-Thiophen-2-yl-acrylic acid ethyl ester (1.46 g, 8 mmol) dissolved in THF (30 mL) was added over 5 min and the solution was stirred for another 30 min. Then triisopropylborate (2.3 mL, 10 mmol) was added and the reaction mixture was allowed to warm up to room temperature over 1 h. Glacial acetic acid (0.52 mL, 9 mmol) and neopentylglycol (1.04 g, 10 mmol) was added followed by stirring for 30 min. Addition of aqueous NH_4Cl followed by extraction with CH_2Cl_2, drying of the combined organic phases ($MgSO_4$), filtration and evaporation produced a crude product, which was crystallized from heptane–EtOAc to give 0.96 g (41%) of the title compound as yellow crystals. ^1H NMR ($CDCl_3$): δ 7.77 (d, 1H), 7.48 (d, 1H), 7.27 (d, 1H), 6.27 (d, 1H), 4.26 (q, 2H), 3.77 (s, 4H), 1.35 (t, 3H), 1.04 (s, 6H). LC–MS: *m*/*z* 227 (M-pinacol+H), 249 (M-pinacol+Na).

4.3. 5-(5,5-Dimethyl-[1,3,2]dioxaborinan-2-yl)-thiophene-2-sulfonic acid *tert*-butylamide (11j)

A solution of diisopropylamine (420 mg, 4.16 mmol) in anhydrous THF (4 mL) was stirred under nitrogen.

The mixture was cooled to −5 °C (internal temperature). *n*-Butyllithium (1.6 M in hexanes, 2.5 mL, 4 mmol) was added over 3 min. The mixture was stirred an additional 30 min at −5 °C before being cooled to −78 °C. Thiophene-2-sulfonic acid *tert*-butylamide (438 mg, 2 mmol) dissolved in THF (4 mL) was added over 2 min and the suspension was stirred for another 45 min. Triisopropylborate (760 mg, 4 mmol) was added and the reaction mixture was allowed to warm up to room temperature over 30 min. Addition of glacial acetic acid (0.24 mL, 4.2 mmol) and neopentylglycol (235 mg, 2.26 mmol) followed by stirring for 2 h. Addition of aqueous NH_4Cl followed by extraction with CH_2Cl_2, drying of the combined organic phases ($MgSO_4$), filtration and evaporation produced 684 mg slightly impure product. This was recrystallized from heptane–EtOAc (5:1) to give 106 mg (16%) of the title compound as crystals. ^{1}H NMR ($CDCl_3$): δ 7.60 (d, 1H), 7.41 (d, 1H), 4.52 (br s, 1H), 3.77 (s, 4H), 1.30 (s, 9H), 1.04 (s, 6H). LC–MS: *m*/*z* 286 (M-pinacol+Na).

4.4. 2-(5-Chlorothiophen-2-yl)-4,4,5,5-tetramethyl-[1,3,2]dioxaborolane (11k)

5-Chloro-2-thiopheneboronic acid (1 mmol) and pinacol (1 mmol) in toluene was stirred at room temperature for 2 h. The solution was washed with water and evaporated to dryness to give the title compound (20%) as a volatile oil. ^{1}H NMR ($CDCl_3$): δ 7.41 (d, 1H), 6.98 (d, 1H), 1.34 (s, 12H). LC–MS: *m*/*z* 162 (M-pinacol+H).

4.5. 2-(5-Chlorothiophen-2-yl)-5,5-dimethyl-[1,3,2]dioxaborinane (11l)

5-Chloro-2-thiopheneboronic acid (1 mmol) and neopentylglycol (1 mmol) in toluene was stirred at room temperature for 2 h. The solution was washed with water and evaporated to dryness to give the title compound (54%) as crystals. Mp 42–48 °C; ^{1}H NMR ($CDCl_3$): δ 7.33 (d, 1H), 6.95 (d, 1H), 3.74 (s, 4H), 1.03 (s, 6H). LC–MS: *m*/*z* 162 (M-neopentylglycol+H).

4.6. 2-(5-Chlorothiophen-2-yl)-[1,3,6,2]dioxazaborocane (11m)

5-Chloro-2-thiopheneboronic acid (1 mmol) and diethanolamine (1 mmol) in toluene was stirred at room temperature for 2 h. The solution was evaporated to dryness and recrystallized from heptane–*iso*-propanol (4:1) to give the title compound (28%) as crystals. Mp 188–190 °C; ^{1}H NMR (DMSO-d_6): δ 7.10 (br s, 1H), 6.94 (d, 1H), 6.82 (d, 1H), 3.88–3.73 (m, 4H), 3.15–3.03 (m, 2H), 2.90–2.81 (m, 2H). LC–MS: *m*/*z* 162 (M-diethanolamine+H).

4.7. Lithium; 3-(6-methyl-[1,3,6,2]dioxazaborocan-2-yl)-benzenesulfinate (12a)

To a stirred solution of 3-bromobenzeneboronic acid *N*-methyldiethanolamine cyclic ester (3.31 g, 11.7 mmol) in dry THF (100 mL) was added dropwise 1.43 M solution in hexanes *n*-BuLi (7.4 mL, 10.5 mmol) over a 3 min period at −78 °C. The mixture was stirred at −78°C for 15 min. Then gaseous sulfur dioxide (ca. 5 g) was added causing an immediate precipitation and a 40 °C increase in the internal temperature. The mixture was allowed to warm to room temperature and stirred for 1 h. The precipitated lithium sulfinate was isolated by filtration under N_2(g), washed with THF (50 mL) and dried in vacuo providing 2.81 g (97%) of the title compound as a solid: ^{1}H NMR (DMSO-d_6): δ 7.66 (s, 1H), 7.39–7.32 (m, 2H), 7.17 (t, 1H), 3.97-3.84 (m, 4H), 3.27–3.21 (m, 2H), 2.97–2.89 (m, 2H), 2.18 (s, 3H).

4.8. Representative procedure for preparation of sulfonamide substituted phenylboronic acids: 3-benzylsulfamoylbenzeneboronic acid (13a)

Lithium; 3-(6-methyl-[1,3,6,2]dioxazaborocan-2-yl)-benzenesulfinate (275 mg, 1.0 mmol) was suspended in CH_2Cl_2 (2 mL). *N*-Chlorsuccinimide (147 mg, 1.10 mmol) was added and the mixture was stirred at room temperature for 1 h. Benzylamine (0.23 mL, 2.1 mmol) was added and the reaction mixture was stirred for 1 h at room temperature and then Dowex 50WX2-400 cation exchange resin (ca. 1 g) was added and the mixture stirred for a further 1 h. The resin was removed by filtration and extracted with CH_2Cl_2–MeOH (9:1). 1 N NaOH was added to the combined organic filtrates and the aqueous phase was washed with CH_2Cl_2. The aqueous phase was acidified with 1 N HCl and the resulting crystals were isolated by filtration to give 107 mg (37%) of the title compound: ^{1}H NMR (DMSO-d_6 + DCl): δ 8.24 (s, 1H), 8.03 (d, 1H), 7.85 (dt, 1H), 7.56 (t, 1H), 7.31–7.21 (m, 5H), 3.96 (s, 2H). LC–MS: *m*/*z* 314 (M+Na).

4.9. 3-Cyclohexylmethylsulfamoylbenzeneboronic acid (13b)

3-Cyclohexyl-methylamine was used instead of benzylamine. Yield 21%. ^{1}H NMR (DMSO-d_6 + DCl): δ 8.21 (s, 1H), 8.03 (d, 1H), 7.83 (dt, 1H), 7.58 (t, 1H), 2.55 (d, 2H), 1.68–1.65 (m, 4H), 1.40–1.25 (m, 1H), 1.20–1.04 (m, 3H), 0.87–0.72 (m, 2H). LC–MS: *m*/*z* 298 (M+H), 320 (M+Na).

4.10. 3-(3,3-Dimethyl-butylsulfamoyl)benzene-boronic acid (13c)

3,3-Dimethyl-butylamine was used instead of benzylamine. Yield 24%. ^{1}H NMR (DMSO-d_6 + DCl): δ 8.21 (s, 1H), 8.04 (d, 1H), 7.84 (dt, 1H), 7.58 (t, 1H), 2.77–2.70 (m, 2H), 1.31–1.23 (m, 2H), 0.80 (s, 9H). LC–MS: *m*/*z* 286 (M+H), 308 (M+Na).

4.11. 3-(3-Methyl-butylsulfamoyl)benzeneboronic acid (13d)

3-Methyl-butylamine was used instead of benzylamine. Yield 15%. ^{1}H NMR (DMSO-d_6 + DCl): δ 8.23 (s, 1H), 8.04 (d, 1H), 7.84 (dt, 1H), 7.58 (t, 1H), 2.74 (t, 2H), 1.54 (apparent sep, 1H), 0.78 (d, 6H). LC–MS: *m*/*z* 272 (M+H), 294 (M+Na).

4.12. 3-(Methyl-phenethylsulfamoyl)benzeneboronic acid (13e)

N-Methyl-phenylethylamine was used instead of benzylamine. Yield 69%. ^{1}H NMR (DMSO-d_6 + DCl): δ 8.17 (s, 1H), 8.08 (d, 1H), 7.78 (d, 1H), 7.60 (t, 1H), 7.33–7.19 (m, 5H), 3.18 (t, 2H), 2.77 (t, 2H), 2.69 (s, 3H). LC–MS: *m*/*z* 320 (M+H), 342 (M+Na).

4.13. 3-(Phenethylsulfamoyl)benzeneboronic acid (13f)

Phenethylamine was used instead of benzylamine. Yield 25%. ^{1}H NMR (DMSO-d_6 + DCl): δ 8.24 (s, 1H), 8.05 (d, 1H), 7.84 (dt, 1H), 7.58 (t, 1H), 7.40–7.14 (m, 5H), 2.96 (t, 2H), 2.68 (t, 2H). LC–MS: *m*/*z* 306 (M+H), 328 (M+Na).

4.14. 3-(Butylmethylsulfamoyl)benzeneboronic acid (13g)

N-Methyl-butylamine was used instead of benzylamine. Yield 68%. ^{1}H NMR (DMSO-d_6 + DCl): δ 8.16 (s, 1H), 8.08 (d, 1H), 7.79 (d, 1H), 7.61 (t, 1H), 2.92 (t, 2H), 2.63 (3, 3H), 1.43 (p, 2H), 1.27 (sextet, 2H), 0,87 (t, 3H). LC–MS: *m*/*z* 272 (M+H), 294 (M+Na).

4.15. Enzyme assay

The inhibition of the different lipases (HSL, HL, LPL, PL) was determined in phosphorlipid stabilized emulsion assays.[8] The assays were based on a fluorochrome-labelled triacylglyceride octadec-9-enoic acid 2-[12-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-dodecanoyloxy]-1-octadec-9-enoyloxymethyl-ethyl ester. The AChE and BChE assays were based on the substrates acetylcholine and butyrylthiocholine, respectively.[8]

Maximum concentration of inhibitors for determination of IC_{50} values was 100 μM with 5-fold dilution steps in six concentrations. Each data point was determined as the average of two measurements. IC_{50} values were calculated using Prism ver. 4.0 by fitting the data to a sigmoidal dose–response (variable slope) nonlinear regression analyses.

4.16. Calculation of pK_a values

The computed values were calculated using the pK_a software from ACDLabs (ACDLabs, Toronto, Canada, version 6.0).

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