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Design and synthesis of boron-containing PDE4 inhibitors using soft-drug strategy for potential dermatologic anti-inflammatory application

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ABSTRACT

PDE4 inhibitors are a validated approach as anti-inflammatory agents but are limited by systemic side effects including emesis. We report a soft-drug strategy incorporating a carboxylic ester group into boron-containing PDE4 inhibitors leading to the discovery of a series of benzoxaborole compounds with good potency (for example IC_{50} = 47 nM of compound **2**) and low emetic activity. These compounds are intended for dermatological use further limiting possible systemic side effects.

Cyclic nucleotide phosphodiesterase (PDE) enzymes have been drug targets in many diseases and several non-specific PDE inhibitors, such as theophylline and doxofylline (see Fig. 1), have been approved to treat patients.[1]

The phosphodiesterase 4 (PDE4) is one of the eleven families of PDEs and, during the last 30 years, PDE4 inhibitors have been a significant focus of research and development by numerous organizations in the quest to discover therapeutic agents for the treatment of inflammation-associated diseases such as asthma and chronic obstructive pulmonary disease (COPD).[2] More than 30 new chemical entities (NCEs) of PDE4 inhibitors have progressed to various clinical stages, but only a small portion of these NCEs are currently in active development at advanced clinical phases.[2a–c] The challenges for developing PDE4 inhibitors are multi-factorial including lack of efficacy in many cases and, more often, safety-related issues. Vomiting, nausea, arteritis and immunosuppression are among the serious adverse events of PDE4 inhibitors.[2d–f] The reported side effects take place in the brain, gastrointestinal tract, arteries or whole body due to systemic exposure of PDE4 inhibitors.

Topical therapeutic application of boron-containing NCEs has been one of our focuses[3] and recently two boron-containing PDE4 inhibitors (AN2728 & AN2898; see Fig. 2)[3d–f] have been identified as anti-inflammatory agents undergoing clinical development for potential topical treatment of psoriasis and atopic dermatitis. For a topically used drug, the possible systemic side effects are expected to be relatively low as compared to for the systemic usage. In an ideal case, a topically applied drug exerts its therapeutic action in the target area of the skin and then converts into inactive and non-toxic metabolites if any drug penetrates the skin and reaches systemic circulation. This so-called soft-drug strategy[4] may further improve the therapeutic index of boron-con-

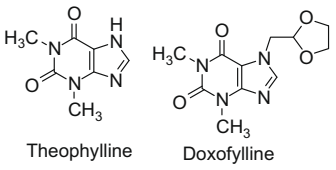

Figure 1. Theophylline has been launched as oral sustained release formulation for treating asthma and respiratory disease (once daily or twice daily). Doxofylline has been launched in Italy for treating asthma in expectation of having fewer side effects than its close analog, theophylline.

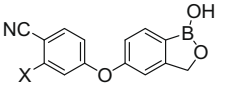

Figure 2. Chemical structures of AN2728 and AN2898 that are in clinical development.

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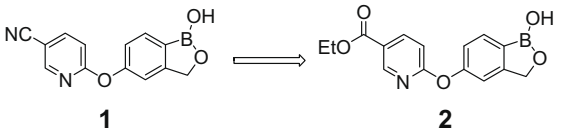
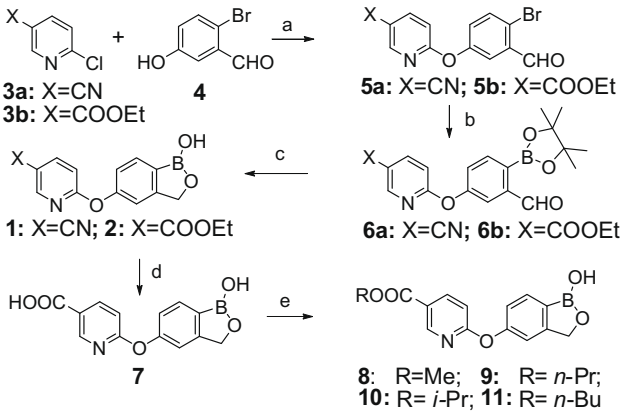


Figure 3. A boron-containing PDE4 inhibitor **1** was used as a starting point of a soft-drug approach for the discovery of more potent carboxylic ester compound **2** with fewer side effects.

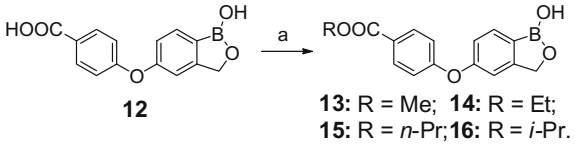


Scheme 1. Reagents and conditions: (a) K_2CO_3, DMF, 65–80 °C overnight; (b) bis(pinacolato)diboron, $PdCl_2(dppf)_2$, 1,4-dioxane, N_2, 80 °C 14 h; (c) $NaBH_4$, EtOH, and then 6 N HCl, H_2O, EtOH (overall yield 60% for **2** from 1st step; (d) NaOH, H_2O, MeOH, rt overnight, and then HCl, H_2O, yield 95%; (e) for **8** (R = Me), H_2SO_4, MeOH, reflux overnight, N_2, yield 15%; for **9–11** (R = *n*-Pr, *i*-Pr and *n*-Bu), CDI, DCM/THF/DMF (1:1:1, v/v/v), N_2, rt overnight, and then ROH and catalytic amount NaH, reflux, 2–4 h, yield 61%, 72% and 69%, respectively.

taining PDE4 inhibitors. This article describes our soft-drug approach starting from compound **1** (see Fig. 3) to the discovery of a series of benzoxaborole carboxylic ester compounds, such as compound **2**, with good potency against PDE4.

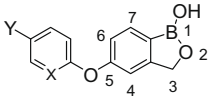
The synthetic methodologies for the preparation of cyanopyridyloxybenzoxaborole (**1**), the ester compounds (**2**, **8**–**11**) and their corresponding carboxylic acid (**7**) are shown in Scheme 1.[5] Nucleophilic substitution of the chloro atom in **3a** and **3b** by the phenolic group in 2-bromo-5-hydroxybenzaldehyde **4** in the presence of a base provided the coupling products **5a** and **5b** which were catalytically boronylated to replace the bromo with bis(pinacolato)diboron generating intermediates **6a** and **6b**. Reduction of compounds **6a** and **6b** with sodium borohydride converted the aldehyde moiety into hydroxymethyl group that was simultaneously cyclized to the adjacent borate and subsequently hydrolyzed to benzoxaborole **1** and **2** upon addition of aqueous hydrochloride. Hydrolysis of compound **2** afforded acid compound **7** that was used for the preparation of other ester analogues **8–11** under normal esterification conditions.[5]

For the SAR study, non-pyridyl phenoxybenzoxaborole acid (**12**) and esters (**13–16**) were also synthesized as shown in Scheme 2.



Scheme 2. Reagents and conditions: (a) for **13** and **14** (R = Me and Et), H_2SO_4, MeOH or EtOH, reflux overnight, N_2; for **15** and **16** (R = *n*-Pr and *i*-Pr), H_2SO_4, *n*-PrOH or *i*-PrOH, 100 °C two days, N_2.

Table 1
PDE4 IC_{50} results for benzoxaborole compounds **1**, **2**, **7–16** and **AN2728**



Compds	X	Y	PDE4 IC_{50} (μM)
1	N	CN	0.18
2	N	COOEt	0.047
7	N	COOH	5% inhib@10uM
8	N	COOMe	0.084
9	N	COOPr-*n*	0.13
10	N	COOPr-*i*	0.113
11	N	COOBu-*n*	0.0945
AN2728	CH	CN	0.49
12	CH	COOH	6.42
13	CH	COOMe	0.287
14	CH	COOEt	0.219
15	CH	COOPr-*n*	0.763
16	CH	COOPr-*i*	0.13

The acid compound **12** was conveniently converted to the corresponding esters under normal esterification condition.[5]

Since the goal of the soft-drug approach is to identify a potent boron-containing PDE4 inhibitor with an ester group that may convert to the corresponding carboxylic acid with much less activity against PDE4, screening against PDE4 of compounds **1**, **2**, **7–16** and **AN2728** have been performed using the previously described procedure[3f] and the results are summarized in Table 1.

As a starting point, 5-(5-cyano-2-pyridyloxy) benzoxaborole (**1**) has a reasonable potency of IC_{50} = 0.18 μM against PDE4. After the cyano group is replaced with carboxylic esters, improvement of potencies was observed with IC_{50} in the range of 47–130 nM. The ester alkyl group has an impact on the potency and the sequence of the potency is Et (**2**) > Me (**8**) ≈ *n*-Bu (**11**) > *i*-Pr (**10**) ≈ *n*-Pr (**9**), which is not correlated to the lipophilicity. Further modification of the functional group to COOH (**7**) resulted in the loss of activity (5% inhibition at 10 μM) against PDE4. For the 5-phenoxybenzoxaborole series, very similar activity pattern was observed, and the ester compounds (**13–16**) are generally more potent than the cyano (**AN2728**) and the acid (**12**). Again, the acid (**12**) is the least potent among this series. For cross comparison between these two pyridyloxy and phenoxy series, the pyridyloxybenzoxaboroles are more potent except the carboxylic compound (**7**). Since the most potent ester (**2**) and least potent acid (**7**) fit the soft-drug strategy well, more anti-inflammatory screenings for selected cytokine release inhibitions from THP-1 cells[3f] were conducted and the data are shown in Table 2.

IC_{50}s of the acid **7** against release of TNF-α, IL-2, IFN-γ, IL-5 and IL-10 are greater than 10 μM indicating this acid compound is inactive of inhibiting cytokine release whereas the ester **2** has good potencies of IC_{50}s in the range between 0.10 and 0.46 μM. These activity differences, in addition to the two compound's PDE4 potency difference, further support the soft-drug approach of using active ester compound that may hydrolyze in the systemic circulation into the acid. Therefore, plasma stabilities of ester compounds (**2**, **8–11** and **13–16**) and their conversion into the corresponding

Table 2
Cytokine inhibition results for compounds **2** (ester) and **7** (acid)

Compds	TNF-αIC_{50}[a] (μM)	IL-2 IC_{50}[a] (μM)	IFN-γ IC_{50}[a] (μM)	IL-5 IC_{50}[a] (μM)	IL-10 IC_{50}[a] (μM)
Ester **2**	0.20	0.10	0.10	0.20	0.46
Acid **7**	>10	>10	>10	>10	>10

[a] Values are means of triplicate.[3f]

Table 3
Stability and conversion results of compounds **2**, **8–11** and **13–16** in mouse plasma and PBS buffer[a]

Compds	Ester group	% Change of ester in PBS	% Formed of acid in PBS	% Change of ester in plasma	% Formed of acid in plasma
2	COOEt	−2.4	ND (**7**)	−72.5	ND (**7**)
8	COOMe	−9.8	0.4 (**7**)	−42.4	31.6 (**7**)
9	$COOPr^n$	−13.6	0.5 (**7**)	−92.5	79.8 (**7**)
10	$COOPr^i$	2.4	1.4 (**7**)	−60.5	36.4 (**7**)
11	$COOBu^n$	−8.9	0.4 (**7**)	−98.0	76.6 (**7**)
13	COOMe	−8.9	ND (**12**)	−43.6	29.4 (**12**)
14	COOEt	−3.2	ND (**12**)	−76.2	53.6 (**12**)
15	$COOPr^n$	−8.8	ND (**12**)	−94.9	58.8 (**12**)
16	$COOPr^i$	−10.6	ND (**12**)	−58.9	27.6 (**12**)

[a] Values are means of triplicate after 1 h incubation in mouse plasma (CD-1, female, K2EDTA) or PBS buffer (pH 7.4) at 37 °C. ND = not determined.

acid compounds (**7** and **12**) are very critical. LC–MS analysis of ester samples in plasma and PBS buffer gave the results[6] as shown in Table 3.

In general, the percentage loss of the esters and their conversions into the corresponding acids in PBS buffer are very minor as indicated by the data in Table 3. In plasma, high percentages of the esters (42–98%) disappeared were mainly converted to their corresponding acids. Consistently in both of pyridyl and phenyl series, the stability order in mouse plasma is COOMe > $COOPr^i$ > COOEt > $COOPr^n \approx COOBu^n$, which might be the combining effects of various esterase activities and susceptibility for hydrolysis, and steric hindrance of ester compounds. It can be concluded that the esters studied were hydrolyzed to the corresponding acid as a major product in mouse plasma at the physiological condition.

Furthermore, pharmacokinetic properties of ester compound **2** were investigated and the acid compound **7** was monitored simultaneously.[7] The results are summarized in Figs. 4–6 and Table 4. Following subcutaneous dose of ester compound **2** in mice at 100 mg/kg single dosage, analysis of the plasma samples indicates that the ester rapidly converts to the corresponding acid which increases to its maximum concentration at 2 h (Fig. 4).

After oral administration, the ester converts rapidly to the acid with maximum concentration at 15 min (Fig. 5) indicating an instantaneous hydrolysis during the absorption and distribution of the ester to the blood. A very similar result was observed when the ester compound was dosed intravenously as shown in Figure 6. The similar declined rates of ester and acid indicates the conversion rate of ester to acid is the rate limiting step. As presented in Table 4, the ester has a very short $t_{1/2}$'s and t_{max}'s (0.25–1.03 h) that fit the criteria for soft-drug profile. The subcutaneous bioavailability is high ($F\%$ = 97.8%) in comparison to the oral bioavailability ($F\%$ = 10.8%).

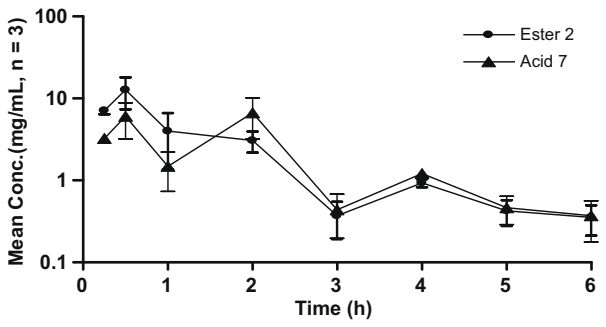


Figure 4. Ester **2** and acid **7** plasma concentrations after subcutaneous (sc) dose of ester **2** in mice (CD-1, n = 3) at 100 mg/kg dose (MO-1).

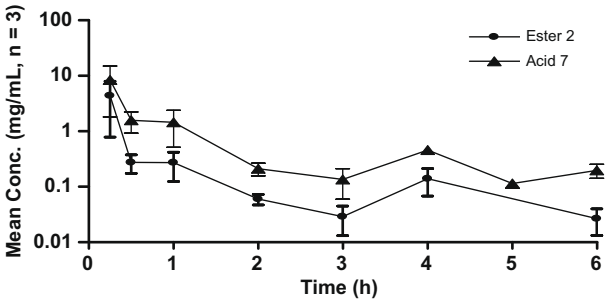


Figure 5. Ester **2** and acid **7** plasma concentrations after oral dose of ester **2** in mice (CD-1, n = 3) at 100 mg/kg single dosage (1%CMC).

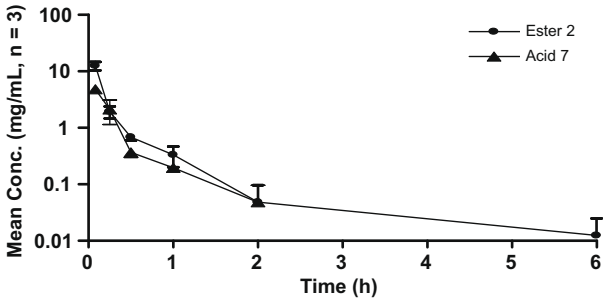


Figure 6. Ester **2** and acid **7** plasma concentrations after intravenous (iv) dose of ester **2** in mice (CD-1, n = 3) at 25 mg/kg single dosage (MO-1).

Table 4
Pharmacokinetic parameters of ester **2** in mice plasma

Dosing method [a]	$t_{1/2}$ (h)	t_{max} (h)	C_{max} (μg/mL)	$F\%$ [b]	AUC_{last} (h μg/mL)	AUC_{inf} pred (h μg/mL)
sc	NA	0.50	12.7	97.8	14.5	15.2
po	NA	0.25	4.39	10.8	1.66	1.68
iv	1.03	NA	12.5	100	NA	3.88

[a] Mice (CD-1, not fasted) were dosed at 100 mg/kg single dosage for sc and po, and 25 mg/kg single dosage for iv (n = 3 per group).
[b] Calculated with non-compartment model and iv bioavailability ($F\%$) is considered to be 100%. NA = not available.

Since esterase is present in the skin, the ester compound **2** was also tested in phorbol ester-induced mouse ear edema model[3f] to investigate its in vivo efficacy by skin penetration under dermatologic treatment. As summarized in Table 5, compound **2** showed significant inhibition against the ear edema caused by phorbol ester after dosing at 1 mg/ear × 2 suggesting that this compound has good skin bioavailability, skin stability and in vivo anti-inflammatory activity. Rolipram exhibited similar activity only at a higher dosage in this model.

The ester compound **2** in vitro plasma stability and in vivo pharmacokinetic results above indicate its rapid conversion to the corresponding PDE4-inactive acid, and presumably this might lead to less systemic toxicity of the compound. Because there is a

Table 5
Inhibitory activity of **2** against phorbol ester-induced mouse ear edema

Compound	Dose (mg/ear)	Inhibition (%)
2	1	59
Dexamethasone	1	68
Rolipram	1	6
	3	53

Table 6
Emesis test results of ester **2** in *Suncus murinus*[8]

Compd	po dose (mg/kg)	N [a]	Number of vomiting episodes	Onset of vomiting at (min)[b]	Last of vomiting[b] (min)	Vomiting[b] (±)
Rolipram	10	1	8	15	11	+
		2	11	3	39	+
		3	19	2	7	+
2	10 or 30	1	0	NV	NV	NV
		2	0	NV	NV	NV
		3	0	NV	NV	NV
		4	0	NV	NV	NV
2	100	1	1	37	NV	+
		2	1	4	NV	+
		3	0	NV	NV	NV
		4	0	NV	NV	NV

[a] Body weights of *Suncus murinus* are between 50 and 70 g. Experimental procedure is given in the reference section.[8]

[b] The symbol of '+' stands for positive result with vomiting and 'NV' for no vomiting.

possibility that variable amount of a topically applied PDE4 inhibitor, depending on the dosage, could enter the systemic circulation, the ester compound **2** was tested in an emesis model and compared to rolipram[8] to verify that this soft-drug approach could result in a potent PDE4 compound with less systemic side effect. As shown in Table 6, all of the animals in the group dosed with rolipram at 10 mg/kg (po, 3 animals) had vomiting episodes (38-time episodes with 57 min in total). In comparison, no vomiting was observed for the two groups dosed with ester compound **2** at 10 mg/kg (po, 4 animals) and at 30 mg/kg (po, 4 animals). Half of the animals (2/4 animals) in the group with 100 mg/kg oral dosage had a single vomiting episode less than a minute during the 90 min observation time. The results demonstrated that ester compound **2** has less emetic activity than rolipram, and the low emesis is possibly due to ester's conversion to PDE4-inactive acid, which is consistent with the soft-drug approach.

In summary, the soft-drug strategy has been used for designing boron-containing chemical entities for topical anti-inflammatory application. Two structural series, 5-(substituted-pyridyloxy)benzoxaborole and 5-(substituted-phenoxy)benzoxaborole, have been synthesized. In general, esters show good potency against PDE4 while the acid is inactive. One of the ester compounds, 5-(5-ethoxycarbonyl-2-pyridyloxy) benzoxaborole (**2**), has broad anti-inflammatory activities and converts rapidly to the corresponding inactive acid (**7**) in vitro in plasma and in vivo in mice. This ester compound has demonstrated in vivo anti-inflammatory activity and improved safety properties with less emetic activity as compared to rolipram. More studies on the benzoxaborole compounds are in progress for their potential topical application.

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4. (a) Bodor, N. *Trends Pharmacol. Sci.* **1982**, *3*, 53; (b) Bodor, N. *Adv. Drug Res.* **1984**, *13*, 255.
5. *Synthesis of 6-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-5-yloxy)nicotinonitrile (**1**)*: This compound was prepared with **3a** as starting material using the same procedures described below for compound **2**.
*Synthesis of ethyl 6-(1-hydroxy-1,3-dihydrobenzo[c][1,2] oxaborol-5-yloxy)nicotinate (**2**)*: To a mixture of ethyl 6-chloronicotinate (**3b**, 18.6 g, 0.1 mol) and 2-bromo-5-hydroxy benzaldehyde (**4**, 20.1 g, 0.1 mol) in dry DMF (200 mL) was added K_2CO_3 (20.8 g, 1.5 equiv) under nitrogen atmosphere and the mixture was stirred at 65–80 °C for 30.5 h. After being cooled to room temperature, the mixture was filtered, evaporated and pumped overnight to give brown oil (38.26 g) with 81.5% coupling conversion to ethyl 6-(4-bromo-3-formyl-phenoxy)nicotinate (**5b**) as indicated by ^{1}H NMR. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.17 (s, 1H), 8.66–8.65 (m, 1H), 8.33 (dd, J = 8.7&2.4 Hz, 1H), 7.86 (d, J = 8.7 Hz, 1H), 7.60 (d, J = 2.7 Hz, 1H), 7.50 (dd, J = 8.4&2.7 Hz, 1H), 7.23 (dd, J = 8.7& 0.6 Hz, 1H), 4.30 (q, J = 7.2 Hz, 2H) and 1.29 (t, J = 7.2 Hz, 3H) ppm. To the solution of the resulting oil intermediate (**5b**) in 1,4-dioxane (450 mL) was added bis(pinacolato)diboron (30.5 g, 0.12 mol), KOAc (29.5 g, 0.3 mol) and $PdCl_2(dppf)_2$ (1.95 g, 2.5 mol %). The mixture was degassed with N_2 and heated at 80 °C for 14 h with stirring. The resulting dark mixture was filtered and evaporated. The residue was dissolved in minimum volume of EtOAc, passed through a short silica gel column eluted with hexane/EtOAc (2:1) to remove the dark color giving brown oil (46.4 g) mainly containing ethyl 6-(3-formyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)nicotinate (**6b**). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.39 (s, 1H), 8.67–8.65 (m, 1H), 8.35–8.31 (m, 1H), 7.83 (d, J = 8.1 Hz, 1H), 7.64 (d, J = 2.7 Hz, 1H), 7.50 (dd, J = 7.8 & 2.7 Hz, 1H), 7.24 (d, J = 8.1 Hz, 1H), 4.30 (q, J = 7.2 Hz, 2H), 1.34 (s, 12H) and 1.29 (t, J = 7.2 Hz, 3H) ppm. To the solution of the pinacolboron aldehyde (**6b**, 46.4 g) in EtOH (450 mL, 200 proof) at 0 °C was added $NaBH_4$ (5 g) in portions and the mixture was stirred overnight with slow increasing to room temperature. The mixture was cooled with ice bath again and water (50 mL) was added and followed with slow addition of 6 N HCl (50 mL). After being stirred for 30 min, the mixture was evaporated to remove EtOH and then water (200 mL) was added, neutralized with $NaHCO_3$-saturated water. The mixture was extracted with EtOAc, concentrated and loaded to a short and big silica gel column eluted with hexane/EtOAc (2:1, v/v) to remove dark impurity. The oil obtained contained pinacol impurity that also complicates ^{1}H NMR spectrum. The oil was dissolved in minimum acetone, and then water was added slowly with sonication at same time to participate the solid product. The solid was collected by filtration and washed with pentane and hexane, dried overnight under high vacuum to give ethyl 6-(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-5-yloxy)nicotinate (**2**) as a cream solid (17.84 g) in 60% overall yield (three steps). Mp 110–113 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.21 (s, 1H), 8.68 (d, J = 2.4 Hz, 1H), 8.30 (dd, J = 8.4 & 2.1 Hz, 1H), 7.76 (d, J = 8.1 Hz, 1H), 7.21 (d, J = 1.5 Hz, 1H), 7.15 (d, J = 8.7 Hz, 1H), 7.12 (dd, J = 7.8 & 2.1 Hz, 1H), 4.97 (s, 2H), 4.30 (q, J = 7.5 Hz, 2H) and 1.29 (t, J = 7.5 Hz, 3H) ppm. Purity (HPLC): 95.3% at 220 nm and 95.4% at 254 nm. MS: m/z = 300 (M+1, ESI+) and m/z = 298 (M−1, ESI−).
*Synthesis of 6-(1-Hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-5-yloxy)nicotinic acid (**7**)*: Ethyl 6-(1-hydroxy-1,3-dihydrobenzo[*c*][1,2]oxaborol-5-yloxy)nicotinate (**2**, 2.99 g, 10 mmol) was dissolved in freshly opened THF (100 mL), and 1 N NaOH (38 mL) was added. The mixture was stirred at room temperature under N_2 overnight. Then 6 N HCl (6.5 mL) was added, rotary evaporated to remove THF, filtered and washed with water and hexane. The solid was dried overnight under high vacuum to afford the bis-acid compound **7** (2.57 g, 9.48 mmol, yield 94.8%) as a slightly brown solid. Mp >200 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.21 (s, 1H), 9.21 (s, 1H), 8.65 (d, J = 2.1 Hz, 1H), 8.28 (dd, J = 8.4 & 2.4 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.21 (d, J = 1.5 Hz, 1H), 7.14–7.11 (m, 2H) and 4.97 (s, 2H) ppm. Purity (HPLC): 97.2% at 220 nm and 97.8% at 254 nm. MS: m/z = 272 (M+1, ESI+) and m/z = 270 (M−1, ESI−).
*Synthesis of methyl 6-(1-hydroxy-1,3-dihydrobenzo[c][1,2] oxaborol-5-yloxy)nicotinate (**8**)*: The mixture of acid **7** (0.8 g, 2.95 mmol) and 96%H_2SO_4 (1 g) in MeOH (130 mL) was refluxed overnight under N_2. Normal work-up and flash column chromatography over silica gel eluted with hexane/EtOAc (1:1, v/v) provided the title methyl ester compound **8** as a white solid (0.127 g, yield 15%). Mp 156–158 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.22 (s, 1H), 8.68 (dd, J = 2.4 & 0.6 Hz, 1H), 8.31 (dd, J = 8.7 & 2.4 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.22 (d, J = 1.2 Hz, 1H), 7.17–7.11 (m, 2H), 4.97 (s, 2H) and 3.84 (s, 3H) ppm. Purity (HPLC): 98.0% at 220 nm and 100% at 254 nm. MS: m/z = 286 (M+1, ESI+) and m/z = 284 (M−1, ESI−).
*Synthesis of n-propyl 6-(1-hydroxy-1,3-dihydrobenzo[c][1,2] oxaborol-5-yloxy)nicotinate (**9**)*: The mixture of acid **7** (0.5 g, 1.84 mmol) and a coupling agent CDI (0.66 g, 4.06 mmol, 2.2 equiv) in a mixed solvent of CH_2Cl_2 (50 mL), THF (30 mL) and DMF (40 mL) was stirred at rt overnight under N_2. Then anhydrous *n*-PrOH (30 mL) was injected into the mixture, and catalytic amount of NaH (60%, 10 mg) was added. The mixture was refluxed under N_2 for 2 h and then evaporated. The residue was dissolved in EtOAc, washed with 0.5 N HCl, then with $NaHCO_3$ solution (pH 8), dried and

evaporated. The sticky solid was dissolved in minimum acetone followed by addition of hexane with sonication and cooling to generate the title *n*-propyl ester product **9** as an off-white solid (0.354 g, 1.13 mmol, yield 61.3%). Mp 89–94 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.22 (s, 1H), 8.69 (d, J = 2.4 Hz, 1H), 8.31 (dd, J = 8.7 & 2.7 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.22 (s, 1H), 7.17–7.12 (m, 2H), 4.97 (s, 2H), 4.22 (t, J = 6.3 Hz, 2H), 1.70 (sextet, J = 6.9 Hz, 2H) and 0.94 (t, J = 7.2 Hz, 3H) ppm. Purity (HPLC): 98.3% at 220 nm and 98.3% at 254 nm. MS: m/z = 314 (M+1, ESI+) and m/z = 312 (M−1, ESI−).

*Synthesis of isopropyl 6-(1-hydroxy-1,3-dihydrobenzo[c][1,2] oxaborol-5-yloxy)nicotinate (**10**)*: This compound was prepared by adapting the procedure described above for **9** with increase of refluxing time to 4 h. Yield 71.8%. Mp 95–101 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.21 (s, 1H), 8.66 (d, J = 2.4 Hz, 1H), 8.29 (dd, J = 8.7 & 2.1 Hz, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.21 (d, J = 1.8 Hz, 1H), 7.16–7.11 (m, 2H), 5.12 (septet, J = 6.0 Hz, 1H), 4.97 (s, 2H) and 1.30 (d, J = 6.3 Hz, 6H) ppm. Purity (HPLC): 98.2% at 220 nm and 96.6% at 254 nm. MS: m/z = 314 (M+1, ESI+) and m/z = 312 (M−1, ESI−).

*Synthesis of n-butyl 6-(1-hydroxy-1,3-dihydrobenzo[c][1,2] oxaborol-5-yloxy)nicotinate (**11**)*: This compound was prepared by using the procedure described above for **10**. Yield 68.5%. Mp 75–80 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.22 (s, 1H), 8.68–8.67 (m, 1H), 8.32–8.29 (dm, J_d = 8.7 Hz, 1H), 7.76 (d, J = 7.5 Hz, 1H), 7.22 (s, 1H), 7.17–7.12 (m, 2H), 4.97 (s, 2H), 4.26 (t, J = 6.3 Hz, 2H), 1.66 (pentatet, J = 7.5 Hz, 2H), 1.39 (sextet, J = 7.5 Hz, 2H) and 0.90 (t, J = 7.5 Hz, 3H) ppm. Purity (HPLC): 100% at 220 nm and 100% at 254 nm. MS: m/z = 328 (M+1, ESI+).

*Synthesis of 4-(1-hydroxy-1,3-dihydrobenzo[c][1,2]oxaborol-5-yloxy)benzoic acid (**12**)*: This compound was prepared by hydrolysis from AN2728 and the procedure was reported previously.[3f]

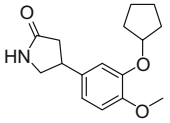
*Synthesis of methyl 4-(1-hydroxy-1,3-dihydro-benzo[c][1,2]oxaborol-5-yloxy)benzoate (**13**)*: To a solution of acid **12** (800 mg, 2.96 mmol) in methanol (50 mL) was added 4 drops of conc. H_2SO_4. The resulting solution was refluxed overnight under N_2. Solvent was evaporated under vacuum. The residue was dissolved in EtOAc (30 mL) and washed with saturated $NaHCO_3$ (30 mL). Then the mixture was acidified to pH 3 with 1 N HCl. The organic layer was dried over $MgSO_4$, filtered, and evaporated. The crude product was purified by silica gel column chromatography eluted with 10% EtOAc/hexane providing 630 mg of the methyl ester compound **13** in 75% yield. ^{1}H NMR 400 MHz (400 MHz, DMSO-d_6): δ 9.22 (s, 1H), 7.98 (d, J = 9.0 Hz, 2H), 7.78 (d, J = 7.8 Hz, 1H), 7.30–7.10 (m, 4H), 4.97 (s, 2H) and 3.83 (s, 3H) ppm. Purity (HPLC): 96.2% at 220 nm and 96.2 % at 254 nm. MS: m/z = 285 (M+1, ESI+).

*Synthesis of ethyl 4-(1-hydroxy-1,3-dihydro-benzo[c][1,2]oxaborol-5-yloxy)benzoate (**14**)*: This compound was prepared in 39% yield by using the procedure above for **13** with EtOH as the solvent. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.21 (s, 1H), 7.98 (d, J = 9.0 Hz, 2H), 7.78 (d, J = 8.2 Hz, 1H), 7.11–7.03 (m, 4H), 4.96 (s, 2H), 4.30 (q, J = 7.4 Hz, 2H) and 1.31 (t, J = 7.0 Hz, 3H) ppm. Purity (HPLC): 97.7% at 220 nm and 99.5% at 254 nm. MS: m/z = 299 (M+1, ESI+).

*Synthesis of n-propyl 4-(1-hydroxy-1,3-dihydro-benzo[c][1,2]oxaborol-5-yloxy)benzoate (**15**)*: This compound was prepared using the procedure above for **13** with *n*-PrOH as the solvent. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.21 (s, 1H), 7.99 (d, J = 9.0 Hz, 2H), 7.78 (d, J = 7.8 Hz, 1H), 7.16–7.05 (m, 4H), 4.96 (s, 2H), 4.22 (t, J = 6.7 Hz, 2H), 1.76–1.65 (m, 2H) and 0.96 (t, J = 7.41 Hz, 3H) ppm. Purity (HPLC): 96.5% at 220 nm and 97.6% at 254 nm. MS: m/z = 313 (M+1, ESI+).

*Synthesis of isopropyl 4-(1-hydroxy-1,3-dihydro-benzo[c][1,2]oxaborol-5-yloxy)benzoate (**16**)*: This compound was prepared using the procedure described above for **13** with *i*-PrOH as the solvent in 60% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.22 (s, 1H), 7.98 (d, 2H), 7.78 (d, 1H), 7.13–7.05 (m, 4H), 5.16–5.08 (m, 1H), 4.96 (s, 2H) and 1.33 (d, 6H). HPLC purity: 97.4% at 220 nm and 98.2% at 254 nm. MS: m/z = 313 (M+1, ESI+).

6. Benzoxaborole stabilities in mouse plasma and buffer were tested by using the following procedure. Four 96-well plates were divided into two groups for handling compound solutions in mouse plasma (CD-1, Female, K_2EDTA, purchased from Bioreclamation) and in phosphate buffer saline (PBS, pH 7.4, purchased from Sigma). To two plates (one for time 0 and the other for time 60 min), 90 μL of plasma was added to each well. To other two plates, 90 μL of PBS was added. Ten microliters of each compound PBS solution (50 μM) was added to the well resulting in 5 μM final sample concentration. Time 60 min plate was placed in water bath set at 37 °C with mild shaking and time 0 min plate was left on ice and was quenched with 300 μL of internal standard working solutions (ISWS, 0.1 μg/mL in acetonitrile or methanol depending on the structures of compounds tested) on ice after the preparation. After incubation for 1 h, the time 60 min plate was placed on ice and then 300 μL of ISWS was added to each well. Both plasma plates were vortexed and then centrifuged at approximate 1800 rpm for 10 min. To a 96-well autosampler plate, 200 μL of supernatant of all samples were transferred and proceed for LC/MS/MS analysis (MS instrument API3000 assembled with Shimadzu LC-10AD system). Each compound was incubated and processed in triplicate.
7. Pharmacokinetic parameters were calculated using a non-compartment model for iv, po and sc in WinNonlin Pro, ver. 5.2. Following single iv dosing at 25 mg/kg of the ester dissolved in MO-1 (1-methyl-2-pyrrolidone/propylene glycol/PEG-400, 10:40:50, v/v/v), ester reached maximum plasma concentration (C_{max}) of 12.5 μg/mL at 5 min. The mean half-time of ester was 1.03 h and mean clearance was 6.44 L/h/mL. After po and sc dosing of the ester suspended in 1% carboxymethylcellulose (CMC) at 100 mg/kg/route, the mean C_{max} of 4.39 μg/mL and 12.7 μg/mL were achieved at 0.25 h for po and 0.25 h for sc, respectively. The mean bioavailability was approximately 10.8% for po dosing and 97.8% for sc dosing. The measurable acid was observed and its profiles were similar to its corresponding ester in all dosing groups. The acid was not quantified and no pharmacokinetics parameters were generated.
8. (a) Emetic activities of rolipram and ester **2** were tested with the procedure as described below. Male animals (*Suncus murinus*, body weights 50–70 g, purchased from CLEA Japan Inc.) were administered orally with rolipram at 10 mg/kg or ester compound **2** at 10, 30 and 100 mg/kg in 1% carboxymethylcellulose (1% CMC) at a dosing volume of 10 mL/kg. Then animals were observed for retching and emetic responses for 90 min post-dosing. A positive emetic episode was defined as disgorgement of the stomach contents generally preceded by retching and its occurrence in one or more of 3 or 4 test animals was considered significant. Additionally, the number, onset and duration of emetic episodes were recorded; (b) Structure of rolipram [4-(3-(cyclopentyloxy)-4-methoxyphenyl)pyrrolidin-2-one, *C* Log *P* = 1.715, molecular weight = 275.34]:



Rolipram