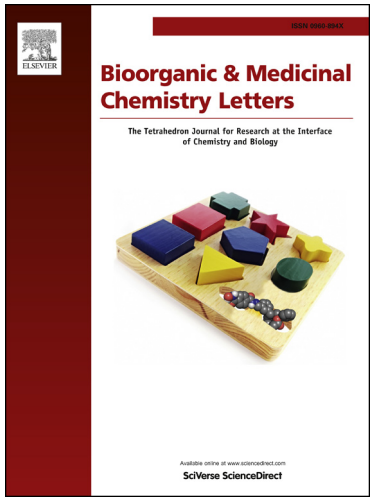

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Arylboronic acids as dual-action FAAH and TRPV1 ligands

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ABSTRACT

A series of 31 arylboronic acids designed on the basis of the pharmacophore model for a variety of TRPV1 antagonists was prepared and tested on FAAH and TRPV1 channel. Four of them, that is, compounds **3c**, **4a**, **5a**,**b** acted as dual FAAH/TRPV1 blockers with IC_{50} values between 0.56 and 8.11 μM whereas ten others (compounds **1c**,**f-i**, **2c-f**, **4b**) inhibited FAAH and activated/desensitized TRPV1.

In recent years, there has been an increasing interest in the therapeutic potential of boron-containing compounds as enzyme inhibitors, boron neutron capture therapy (BNCT) agents, and drug delivery devices.[1] For example, bortezomib, a dipeptidyl boronic acid approved in 2003 by the FDA for the treatment of multiple myeloma (MM) and mantle cell lymphoma, stands out as one of the most potent proteasome inhibitors so far available.[2] Boronic acids inhibit hydrolytic enzymes such as serine proteases[1e,3] through the formation of a tetracoordinate-boronate complex by coordination of the catalytic serine residue, thus acting as transition-state analogues.[1e,4] In 2008, Minkkilä et al. reported that a series of commercially available phenyl-, heteroaryl-, alkyl-, and alkenylboronic acids behaved as potent and selective fatty acid amide hydrolase (FAAH) inhibitors[5] and, at the same time, some boronic acid derivatives were patented as reversible FAAH inhibitors by Infinity Pharmaceuticals.[6]

FAAH is a member of the amidase signature family of serine hydrolases[7] that cleaves and regulates a broad range of endogenous signalling lipid amides, such as the prototypical endocannabinoid anandamide (AEA),[8] the anti-inflammatory substance palmitoylethanolamide (PEA),[9] the sleep-inducing agent oleamide (OA),[10] and the analgesic and satiety regulating mediator oleoylethanolamide (OEA).[11] Thus, blockade of FAAH represents a promising approach for the treatment of various disease states such as pain,[12] inflammatory,[13] and neuropsychiatric disorders,[14] and, toward this ends, many classes of FAAH inhibitors, including α-ketoheterocycles, carbamates, and ureas, have been reported.[15] Although in animal models FAAH inhibitors have proved to be effective analgesic agents, devoid of the CNS side effects that arise when a cannabinoid receptors agonist is used,[16] data on their clinical efficacy are less encouraging. For example, the potent and selective FAAH inhibitor PF-04457845, evaluated as an analgesic in randomized, placebo-controlled phase II clinical trials, was apparently ineffective.[17] Among the reasons for this failure, the fact that FAAH inhibition might indirectly lead to activation of other, non-cannabinoid receptors involved in nociception, such as transient receptor potential vanilloid 1 (TRPV1) channel, should be taken into account.[18] TRPV1, a non-selective cation channel belonging to the large family of transient receptor potential (TRP) ion channels,[19] is activated by a variety of stimuli, including noxious heat, protons, endogenous substances such as AEA, OEA, and lipoxygenase products, and exogenous compounds such as capsaicin (Figure 1), and has emerged as a promising therapeutic target for pain management.

Recent findings of us suggest that compounds that inhibit both FAAH and TRPV1 may be more efficacious in pain relief than those targeting only one such protein.[20] As continuation of our efforts to identify new molecules able to target simultaneously both FAAH and TRPV1 receptors, we hypothesized that the incorporation of a boronic acid group (as the FAAH inhibiting moiety) into the pharmacophore model for a variety of TRPV1 antagonists (Figures 1 and 2)[19h,l] could represent a simple strategy for the development of combined FAAH/TRPV1 blockers. The model can be generalized as a central hydrogen-bond acceptor/

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E-mail addresses: enrico.morera@uniroma1.it (E. Morera), luciano.depetrocellis@icb.cnr.it (L. De Petrocellis).

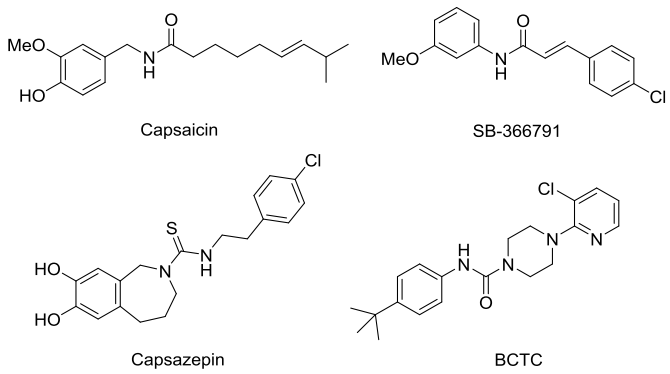


Figure 1. Structure of capsaicin and of selected TRPV1 antagonists.

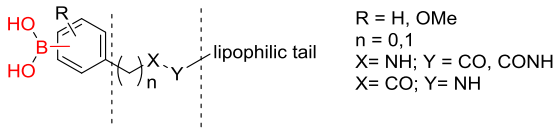


Figure 2. Design of arylboronic acid derivatives as FAAH/TRPV1 blockers.

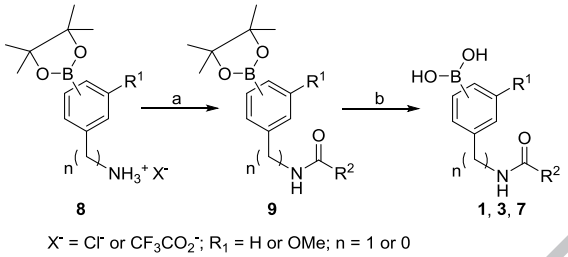
donor motif flanked by a lipophilic tail on one side and an aromatic group that incorporates a hydrogen-bond acceptor on the other side.

Accordingly, 31 arylboronic acids inspired by this model were prepared and tested on FAAH and TRPV1 channel (compounds **1**-**7**, Table 1).

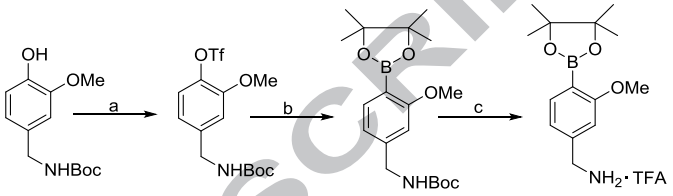
All the boronic acids were prepared as pinacol boronate esters and subsequent deprotection with sodium metaperiodate.[21] In detail, amides **1**, **3**, and **7** were synthesized by acylation of the corresponding aminobenzyl or anilino boronates **8** with the appropriate carboxylic acids R^2CO_2H, using 1-hydroxybenzotriazole (HOBt)/*N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) as the carboxylate activator (Scheme 1), followed by cleavage of the boronates **9**.[22] The non-commercially available 4-(aminomethyl)-2-methoxyphenylboronic acid pinacol ester was prepared by a palladium-catalyzed cross-coupling reaction of diboron pinacol ester (B_2pin_2) with the appropriate aryl triflate (Scheme 2).[23] Amides **2** and **4** were obtained by reaction of the activated carboxylic acids **10** with the appropriate amines RNH_2, followed by cleavage of the boronates **11** (Scheme 3).[22] Ureas **5** and **6** were prepared by reaction of the corresponding 4-aminobenzyl or anilino boronates **8** with 4-*tert*-butylphenylisocyanate followed by cleavage of the boronates **12**.[22]

The effects of arylboronic acids **1**-**7** on [^{14}C]anandamide hydrolysis by rat membranes (which express FAAH as the only AEA hydrolyzing enzyme) and on intracellular Ca^{2+} elevation in HEK293 cells stably transfected with the human TRPV1 cDNA are shown in Table 1.[24] The TRPV1 antagonist or desensitizing activity was assessed by adding the test compounds 5 min before stimulation of cells with the reference agonist capsaicin. Data obtained for the two most potent boronic acids reported by Minkkilä et al.[5] (compounds **13** and **14**), compounds **15**, **16**,[25] the selective TRPV1 antagonist SB-366791,[26] and two 'dual-target' agents previously identified by us [*N*-arachidonoylserotonin (AA-5-HT) and the piperazinyl carbamate OMDM-198][20] are also included in the Table.

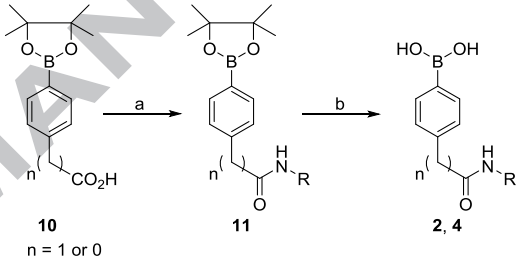
The majority of arylboronic acids **1**-**7** showed, as expected, fairly good FAAH inhibitory activities (compounds **1c**,**e**-**i**, **2c**-**f**, **3c**-**e**, **4a**-**c**,**e**-**g**, **5a**,**b**), irrespective of the nature of the functional group X-Y connecting arylboronic acid and lipophilic moieties and of the presence or absence of a methylene bridge. The boronic acid functionality was essential for FAAH inhibitory activity, as



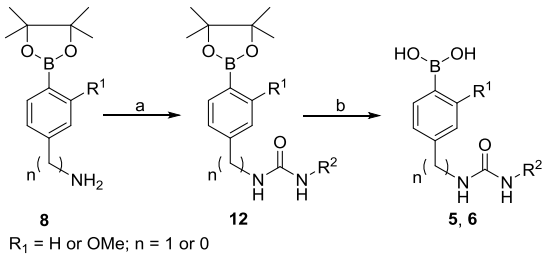
Scheme 1. Reagents and conditions: (a) R^2CO_2H, HOBt/EDC, DMF, rt, 1 h, then appropriate amine, Et_3N, DMF, rt, 16 h. (b) $NaIO_4$, 2 N HCl, THF/H_2O (3:1), rt, 3-5 h.



Scheme 2. Reagents and conditions: (a) $PhNTf_2$, Et_3N, CH_2Cl_2, rt, 16 h; (b) B_2pin_2, $PdCl_2$, DPPF, AcOK, dioxane, 100 °C, 4h; (c) TFA/CH_2Cl_2 (3:1), rt., 40 min.



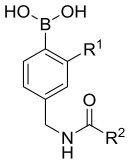
Scheme 3. Reagents and conditions: (a) HOBt/EDC, DMF, rt, 1 h, then RNH_2, DMF, rt, 16 h; (b) $NaIO_4$, 2 N HCl, THF/H_2O (3:1), rt, 3-5 h.



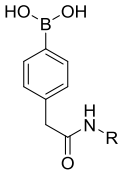
Scheme 4. Reagents and conditions: (a) *t*Bu-4-PhNCO, Et_3N, DMF, rt, 16 h; (b) $NaIO_4$, 2 N HCl, THF/H_2O (3:1), rt, 3-5 h.

demonstrated by the lack of activity of the analogues of **2f**, **15** and **16**. The choice of **15** and **16** as reference compounds was driven by the high, submicromolar modulating potency of the boronic acid **2f** on both FAAH and TRPV1. Notably, however, the movement of the $B(OH)_2$ group away from the para-position resulted in a loss of activity (compare compounds **3c** and **7**). In their study, Minkkilä et al.[5] found that para-substituted compounds were indeed more potent FAAH inhibitors than the meta-substituted ones, a result which was ascribed to the more steric tolerance near the enzyme's catalytic site for the para-substitution. A similar explanation may be invoked for the decrease in activity of the 2-methoxy-substituted compounds **1h**,**i**, **5b** as compared to their unsubstituted counterparts **1f**,**g**, **5a**. The choice of an ω-arylaliphatic chain as the lipophilic tail appeared to be beneficial for optimal activity on FAAH with a positive relationship with the chain length (compare compounds **1d**,**f**, and **g**; **2b**,**d**,**e**, and **f**; **3d** and **e**; **4c**,**f**, and **g**). Conversely, the

Table 1. Results of TRPV1 and FAAH assays of arylboronic acids **1-7**, **13**, and **14**[a]



1



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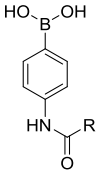

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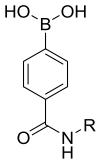

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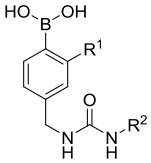
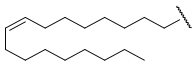
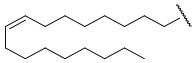
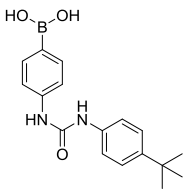
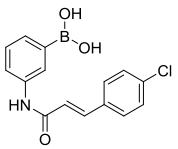
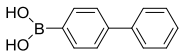
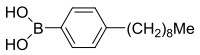
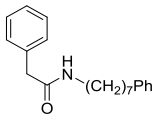
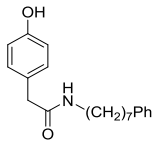
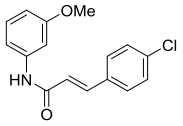
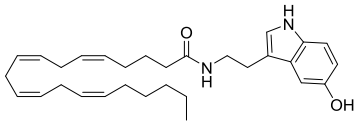
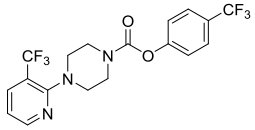


5

Compound	R^1	R or R^2	TRPV1 (efficacy %)[b]	TRPV1 (EC_{50} μM)	TRPV1 (IC_{50} μM)[c]	FAAH (IC_{50} μM)
1a	H		42.2	0.022	0.047	> 10
1b	OMe		49.2	0.020	0.032	> 10
1c	H	Ph-4-Ph	22.0	4.23	7.11	0.33
1d	H	$(CH_2)_2Ph$	60.2	0.32	0.23	> 10
1e	H	CH=CHPh-4-Cl	< 10	ND	> 10	1.51
1f	H	$(CH_2)_5Ph$	41.1	1.53	2.89	1.08
1g	H	$(CH_2)_7Ph$	54.7	1.00	0.50	0.47
1h	OMe	$(CH_2)_5Ph$	59.3	1.46	1.53	1.32
1i	OMe	$(CH_2)_7Ph$	62.5	0.051	0.034	1.77
2a	H	Ph-4-*t*-Bu	< 10	ND	> 10	> 10
2b	H	CH_2Ph	< 10	ND	> 10	> 10
2c	H	CH_2Ph-4-Ph	27.4	0.31	5.53	0.28
2d	H	$(CH_2)_4Ph$	44.9	1.99	2.19	1.17
2e	H	$(CH_2)_5Ph$	62.8	2.25	2.53	0.63
2f	H	$(CH_2)_7Ph$	52.5	0.37	0.37	0.25
3a	H	Ph-4-*t*-Bu	39.3	0.52	0.78	> 10
3b	H	CH_2Ph-4-Ph	< 10	ND	> 10	> 10
3c	H	CH=CHPh-4-Cl	< 10	ND	1.39	0.56
3d	H	$(CH_2)_5Ph$	< 10	ND	> 10	0.57
3e	H	$(CH_2)_7Ph$	< 10	ND	> 10	0.39
4a	H	Ph-4-*t*-Bu	< 10	ND	0.75	5.50
4b	H	Ph-4-Ph	12.5	3.97	> 10	0.15
4c	H	CH_2Ph	< 10	ND	> 10	2.87
4d	H	$(CH_2)_2$Ph-4-OH	< 10	ND	> 10	> 10
4e	H	CH_2Ph-4-Ph	< 10	ND	> 10	1.86
4f	H	$(CH_2)_4Ph$	< 10	ND	> 10	0.58
4g	H	$(CH_2)_5Ph$	< 10	ND	> 10	0.18
5a	H	Ph-4-*t*-Bu	< 10	ND	4.13	1.99
5b	OMe	Ph-4-*t*-Bu	< 10	ND	8.11	4.28
6			< 10	ND	1.95	> 10
7			< 10	ND	0.041	> 10

13		<10	ND	>10	0.79 (0.021)[d]
14		<10	ND	9.10	2.92 (0.0091)[d]
15		54.6	0.66	1.21	> 10
16		64.7	0.17	0.26	> 10
SB-366791		< 10	ND	0.19 (0.27)[e]	> 10
AA-5-HT		< 10	ND	0.27[f]	8.0[f]
OMDM-198		< 10	ND	1.0[f]	3.36[f]

[a] Data are means of n = 4 separate determinations. Standard errors are not shown for the sake of clarity and were never higher than 10% of the means.
[b] As percent of ionomycin (4 μM). ND, not determined when efficacy is lower than 10%.
[c] Determined against the effect of capsaicin (0.1 μM).
[d] Data from ref. 5.
[e] Data from ref. 19h.
[f] Data from ref. 20b.

presence of an aliphatic chain (compounds **1a**,**b**) was detrimental for this activity. Boronic acids **13** and **14** were reported by Minkkilä et al.[5] to inhibit FAAH in the low nanomolar range (IC_{50} = 21 and 9.1 nM, respectively), while the corresponding values recorded by us were one and three orders of magnitude higher (0.79 and 2.92 μM, respectively). FAAH assays were however performed at different pH values (7.4 by Minkkilä et al.,[5] and FAAH optimal pH = 9 by us) and it is well known that pharmacological properties of FAAH are highly pH-dependent.[27] In particular, rat brain hydrolysis of [^{3}H]AEA shows an optimum around pH 8-9.

As concerns TRPV1 assays, benzylic amides **1** and benzylic reverse amides **2** exhibited an overall superiority over their aryl counterparts **3** and **4**, and ureas **5** and **6**. A reversed trend was however observed with the 4-chlorostyryl and 4-*t*-butylphenyl moieties (compare compounds **1e** and **3c**; **2a**, **3a**, and **4a**; **5a** and **6**). The EC_{50} and IC_{50} values were, with the exception of **2c**, **3c**, **4a**,**b**, **5a**,**b**, **6**, and **7**, comparable and thus, only **3c**, **4a**, **5a**,**b**, **6**, and **7** behaved as 'true' antagonists, while **2c** and **4b** acted as weakly desensitizing agonists. The boronic functional group was fully compatible with a TRPV1 modulating action but not essential for it, as demonstrated by the comparison between compounds **2f** and **15**,**16**, and **7** and SB-366791, and the inactivity of compounds **1e**, **3d**,**e**, **4c**,**e**,**f**,**g**, **13**, and **14**.

Thirteen of the compounds examined, that is, **1c**,**f-i**, **2c-f**, **3c**, **4a**,**b**, **5a**,**b** targeted both FAAH and TRPV1 channel. In particular, four of them, that is, **3c**, **4a**, **5a**,**b** acted as dual FAAH/TRPV1 blockers, the first three compounds comparing favorably in terms of potency with the other chemotypes so far presented by us.[20] The ability of these compounds to inhibit FAAH and activate/desensitize or directly antagonize TRPV1, i.e. to target simultaneously two distinct targets involved in nociception, qualify them as promising antinociceptive, antihyperalgesic, and antioedemic agents in chronic and inflammatory pain preclinical studies.

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22. *General procedure for the preparation of arylboronic acids **1**, **3**, **7***. To a stirred solution of the appropriate carboxylic acid (1.0 mmol) in DMF (1 mL) were added at 0 °C HOBt (1.0 mmol) and EDC (1.0 mmol). The mixture was stirred for 15 min at 0 °C and for 1 h at room temperature. Then, the pinacol boronate ester amine hydrochloride or trifluoroacetate **8** (1.0 mmol) and Et_3N (1.0 mmol) were added, and the mixture was stirred overnight at room temperature. The mixture was diluted with brine and extracted with AcOEt. The organic phase was washed with 2 N HCl solution, saturated $NaHCO_3$, and brine, dried (Na_2SO_4), and evaporated under vacuum. The residue was purified by column chromatography (silica gel, hexane/AcOEt mixtures). Sodium periodate (3 mmol) was added at room-temperature to a solution of the purified pinacol boronate ester **9** (1.0 mmol) in THF/H_2O (15/5 mL) and then 2 N HCl (0.40 mL) was added. The solution was stirred at room temperature for 3-5 h, concentrated under vacuum, diluted with water, and extracted with AcOEt. The organic phase was separated, washed twice with water, dried (Na_2SO_4), and evaporated under vacuum. The residue was crystallized from THF/H_2O to afford pure title compound. *General procedure for the synthesis of arylboronic acids **2** and **4***. To a stirred solution of the carboxylic acid **10** (1.0 mmol) in DMF (1 mL) were added at 0 °C HOBt (1.0 mmol) and EDC (1.0 mmol). The mixture was stirred for 15 min at 0 °C and for 1 h at room temperature. Then the appropriate amine (1.0 mmol) was added and the mixture was stirred overnight at room temperature. The mixture was diluted with brine and extracted with AcOEt. The organic phase was washed with 2 N HCl solution, saturated $NaHCO_3$, and brine, dried (Na_2SO_4), and evaporated under vacuum. The residue was purified by column chromatography (silica gel, hexane/AcOEt mixtures). Sodium periodate (3 mmol) was added at room-temperature to a solution of the purified pinacol boronate ester **11** (1.0 mmol) in THF/H_2O (15/5 mL) and then 2 N HCl (0.40 mL) was added. The solution was stirred at room temperature for 3-5 h, concentrated under vacuum, diluted with water, and extracted with AcOEt. The organic phase was separated, washed twice with water, dried (Na_2SO_4), and evaporated under vacuum. The residue was crystallized from THF/H_2O to afford pure title compound. *General procedure for the synthesis of arylboronic acids **5** and **6***. A solution of 4-*tert*-butylphenyl isocyanate (1.0 mmol), pinacol boronate esters amine hydrochloride or trifluoroacetate **8** (1.0 mmol) and Et_3N (1.2 mmol) in dry DMF (5 mL) was stirred overnight at room temperature. The mixture was diluted with brine and extracted with AcOEt. The organic phase was washed twice with brine, dried (Na_2SO_4), and evaporated under vacuum. The residue was purified by column chromatography (silica gel, hexane/AcOEt mixtures). Sodium periodate (3 mmol) was added at room-temperature to a solution of the purified pinacol boronate ester urea **12** (1.0 mmol) in THF/H_2O (15/5 mL) and then 2 N HCl (0.40 mL) was added. The solution was stirred at room temperature for 3-5 h, concentrated under vacuum, diluted with water, and extracted with AcOEt. The organic phase was washed twice with water, dried (Na_2SO_4), and evaporated under vacuum. The residue was crystallized from THF/H_2O to afford pure title compound **5**. *Data for selected compounds*: compound **3c**: mp > 230°C; IR 3302, 1661, 1626, 1589, 1524, 1340, 1242, 1124, 1013, 972, 817 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 6.86 (1H, d, J = 16.0 Hz), 7.52 (2H, d, J = 8.4 Hz), 7.59 (1H, d, J = 16.0 Hz), 7.66 (4H, m), 7.77 (2H, d, J = 8.4 Hz), 7.94 (2H, s), 10.25 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 117.95, 123.03, 128.98, 129.33, 133.60, 134.13, 134.84, 138.77, 140.71, 163.29. Compound **4a**: mp > 230 °C; IR 3349, 2959, 1656, 1638, 1517, 1407, 1320, 1251, 1100, 1008, 835, 729 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.28 (9H, s), 7.36 (2H, d, J = 8.8 Hz); 7.70 (2H, d, J = 8.8 Hz), 7.93 (4H, s), 8.26 (2H, s), 10.19 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 31.18, 34.00, 120.15, 125.15, 126.44, 133.95, 136.19, 136.52, 145.99, 165.43. Compound **5a**: mp > 230 °C. IR 3319, 2959, 1639, 1598, 1550, 1408, 1359, 1231, 1114, 1016, 816 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.24 (9H, s), 4.30 (2H, d, J = 6.0 Hz), 6.54 (1H, t, J = 6.0 Hz), 722-7.33 (6H, m), 7.52 (2H, d, J = 7.8 Hz), 8.00 (2H, s), 8.46 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 31.17, 33.69, 42.66, 117.45, 125.10, 125.95, 134.07, 137.70, 142.14, 143.24, 155.19.
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24. *Fatty amide hydrolase (FAAH) assays*. The effect of compounds on the enzymatic hydrolysis of anandamide was obtained using membranes prepared from rat brain, incubated with the test compounds and [^{14}C]AEA (2.4 µM) in 50 mM Tris-HCl, pH 9, for 30 min at 37 °C. [^{14}C]Ethanolamine produced from [^{14}C]AEA hydrolysis was measured by scintillation counting of the aqueous phase after extraction of the incubation mixture with 2 volumes of $CHCl_3/CH_3OH$ = 2/1 (by volume). Data are expressed as the concentration exerting 50% inhibition of AEA hydrolysis (IC_{50}), calculated by GraphPad. *TRPV1 channel assays*. HEK293 (human embryonic kidney) cells stably over-expressing recombinant human TRPV1 were grown on 100 mm diameter Petri dishes as mono-layers in minimum essential medium (MEM) supplemented with non-essential amino acids, 10% fetal bovine serum, and 2

mM glutamine, and maintained at 5% CO_2 at 37 °C. The effect of the substances on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was determined by using the selective intracellular fluorescent probe Fluo-4. On the day of the experiment, cells were loaded for 1 h at room temperature with the methyl ester Fluo-4-AM (4 µM in dimethyl sulfoxide containing 0.02% Pluronic F-127, Invitrogen) in MEM without fetal bovine serum, then were washed twice in Tyrode's buffer (145 mM NaCl, 2.5 mM KCl, 1.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10mM D-glucose, and 10 mM HEPES, pH 7.4), resuspended in the same buffer, and transferred (about 100,000 cells) to the quartz cuvette of the spectrofluorimeter (Perkin-Elmer LS50B equipped with PTP-1 Fluorescence Peltier System; PerkinElmer Life and Analytical Sciences, Waltham, MA, USA) under continuous stirring. The changes in $[Ca^{2+}]_i$ were determined before and after the addition of various concentrations of test compounds by measuring cell fluorescence (λ_{EX} = 488 nm, λ_{EM} = 516 nm) at 25 °C. Curve fitting (sigmoidal dose–response variable slope) and parameter estimation were performed with GraphPad Prism® (GraphPad Software Inc., San Diego, CA). Potency was expressed as the concentration of test substances exerting a half-maximal agonist effect (i.e., half-maximal increases in $[Ca^{2+}]_i$) (EC_{50}). The efficacy of the agonists was determined by normalizing their effect to the maximum Ca^{2+} influx effect on $[Ca^{2+}]_i$ observed with application of 4 µM ionomycin (Cayman). When significant, the values of the effect on $[Ca^{2+}]_i$ in wild-type (i.e., not transfected with any construct) HEK293 cells were taken as baseline and subtracted from the values obtained from transfected cells. Antagonist/desensitizing behaviour was evaluated against capsaicin (0.1 µM) , by adding the test compounds in the quartz cuvette 5 min before stimulation of cells with agonists. Data are expressed as the concentration exerting a half-maximal inhibition of agonist-induced $[Ca^{2+}]_i$ elevation (IC_{50}), which was calculated again using GraphPad Prism® software. The effect on $[Ca^{2+}]_i$ exerted by agonist alone was taken as 100%. Dose response curves were fitted by a sigmoidal regression with variable slope. All determinations were performed at least in triplicate. Statistical analysis of the data was performed by analysis of variance at each point using ANOVA followed by the Bonferroni's test.

25. Prepared by reaction of HOBt/EDC-activated 2-phenylacetic acid or 2-(4-hydroxyphenyl)acetic acid with 7-phenylheptan-1-amine.
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Graphical Abstract

Arylboronic acids as dual-action FAAH and TRPV1 ligands

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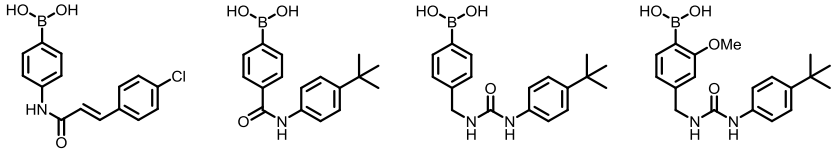

	3c	**4a**	**5a**	**5b**
FAAH IC_{50}	0.56 μM	5.50 μM	1.99 μM	4.28 μM
TRPV1 IC_{50}	1.39 μM	0.75 μM	4.13 μM	8.11 μM