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Aminoquinazolines as TRPV1 antagonists: Modulation of drug-like properties through the exploration of 2-position substitution

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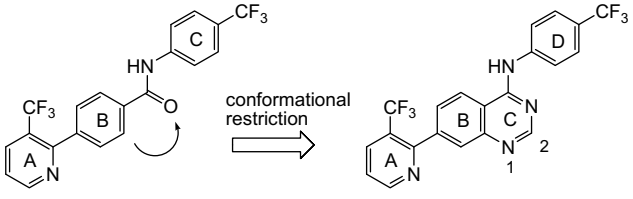
ABSTRACT

A focused SAR exploration of the lead 4-aminoquinazoline TRPV1 antagonist **2** led to the discovery of compound **18**. In rats, compound **18** is readily absorbed following oral dosing and demonstrates excellent in vivo potency and efficacy in an acute inflammatory pain model.

TRPV1 (VR1) is a member of the transient receptor potential (TRP) family of ion channels and is highly expressed on sensory neurons that innervate many organs, including the skin, bladder, and gut. Activation of TRPV1 on sensory neurons by chemical stimulants, including capsaicin and resiniferatoxin, as well as low pH and heat, leads to an influx of calcium and sodium ions through the channel, causing depolarization of the cell and transmission of painful stimuli. Since the cloning of TRPV1 more than ten years ago,[1] a great deal of research has been carried out to better understand this ion channel and its pharmacology.[2] It is hypothesized that blockade of the channel should provide a clinically effective therapeutic method to treat pain. In addition, evidence suggests that a number of other disorders may be treatable through modulation of TRPV1 signaling.[2] Therefore, the identification of selective and potent antagonists of TRPV1 has become a focus of attention within the pharmaceutical industry.[3] Indeed, several TRPV1 antagonists have reached human testing and early clinical results for some of these trials have been reported.[4,2]

In a previous communication we outlined the discovery of a novel class of TRPV1 antagonists, the aminoquinazolines, which were derived from the conformational restriction of a biarylamide series (Fig. 1),[5] which in turn was derived from the initial arylurea series.[6] The prototypical compound **2** was superior to its forerunners in a number of respects, most notably in vitro potency. In addition, the quinazoline **2** demonstrated good in vivo efficacy, fully reversing carrageenan-induced thermal hyperalgesia in rats following oral dosing (MED 0.1 mg/kg).[5b] Although excellent oral exposure was achieved with **2** using a vitamin E TPGS[7] dosing vehicle, the compound plasma levels were significantly diminished upon dosing as a standard methylcellulose suspension (Table 5). Hence, a primary goal was to improve the oral exposure of this series through modulation of the drug-like properties of **2**. Because the quinazoline skeleton has had a history of use in pharmaceutical research, a secondary goal was to enhance the structural novelty of this class through appropriate substitutions. An important new aspect of the quinazoline series is that it allowed access to a hitherto unexplored region of space within the urea/biarylamide TRPV1 pharmacophore, the area accessible by substitution of carbon-2



1
hTRPV1 = 14 nM

2
hTRPV1 = 1 nM

Figure 1. Genesis of quinazolines (**2**) from biarylamides (**1**).

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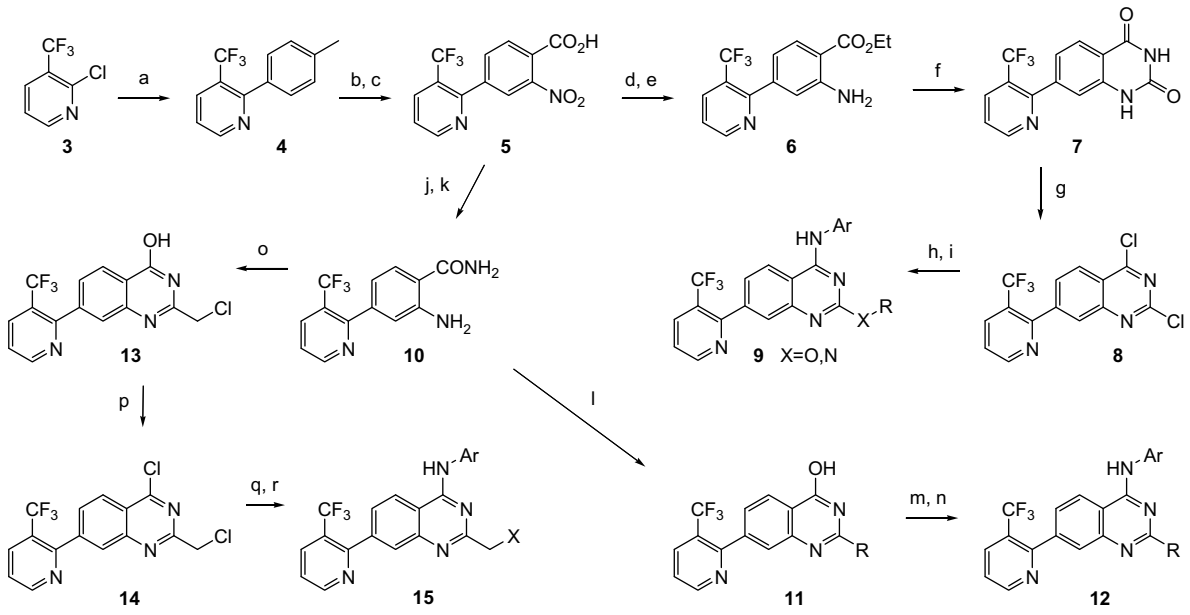
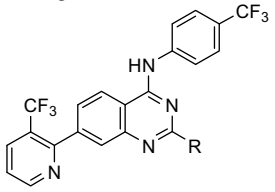


Scheme 1. Reagents and conditions: (a) 4-methylphenylboronic acid, 2 M Na_2CO_3, $Pd(PPh_3)_4$, DME, 80 °C, 83%; (b) HNO_3, 0 °C, 2 h, 100%; (c) $KMnO_4$, pyridine, H_2O, reflux, 16 h, 63%; (d) EtOH, 30 psi H_2, Pd/C, 92%; (e) EtOH, HCl (gas), reflux, 6 h, 85%; (f) KOCN, HOAc, 100 °C, 16 h, then NaOH; (g) $POCl_3$, reflux, 16 h; (h) 1.2 equiv Ar-NH_2, IPA, diisopropylethylamine, 80 °C, 6 h; (i) excess NR_1R_2, sealed tube, 100 °C or ROH, NaH, THF, reflux, 16 h; (j) $SOCl_2$, reflux, 2 h, then NH_3 (gas), CH_2Cl_2, 0 °C, 30 min, 77%; (k) Raney Ni, 40 psi H_2, 4 h, EtOH, 95%; (l) RCOCl, THF, 25 °C, 1 h, then 15% NaOH, 50 °C, 30 min; (m) $POCl_3$, reflux, 16 h; (n) Ar-NH_2, IPA, diisopropylethylamine, 80 °C, 6 h; (o) $ClCH_2C(OMe)_3$, reflux, 3 h, 70%; (p) $POCl_3$, 2,6-lutidine, $CHCl_3$, reflux, 16 h, 82%; (q) Ar-NH_2, acetonitrile, 80 °C, 4 h; (r) ROH, NaH, THF or NR_1R_2, DMA.

(C-2) (Fig. 1). This letter highlights our efforts to enhance the oral exposure and structural novelty of **2** through structural exploration of the quinazoline C-2 position and D-ring. Our goal was to do this while maintaining low single-digit nM potency at TRPV1. The general synthetic approach used to access these C-2 substituted compounds is shown in Scheme 1. At the outset of our investigation, several oxygen- and nitrogen-linked analogs at C-2 were found to lack the desired hTRPV1 potency (<5 nM). For example, insertion of a methoxy group at the 2-position of **2** yielded a compound which was 154 nM at hTRPV1. More importantly, the O- and N-linked analogs were not as novel structurally and thus were less appealing from an intellectual property perspective. Therefore, our efforts were quickly directed toward the exploration of a variety of carbon-linked analogs, both ether-containing (Table 1) and amine-containing (Table 2). Within this first group, several alkoxy-alkyl analogs met our in vitro potency criterion of <5 nM (**18**, **19**, **20**, **21**, and **24**, Table 1). Not surprisingly, these analogs did little to improve the aqueous solubility of **2**, although they were found to be sufficiently stable in rat liver microsomes to warrant further evaluation. C-2 substituents containing acidic functionality were moderately (**22**, **23**) or weakly active (**27**) and had significantly improved solubility. It is important to note that in principle, the phosphate analog **22** could hydrolyze to the highly potent alcohol **17** and thus we cannot rule out the possibility that small amounts of unmasked **17** contribute to the activity observed for **22**.[9] However, the experimental protocol used in the FLIPR assay and the observed potency of other acidic analogs suggest that **22** has intrinsic potency at TRPV1.

It was anticipated that the introduction of basic functional groups at C-2 would significantly alter the physicochemical properties of **2**. Indeed, this approach has been used successfully by others as a means to improve the aqueous solubility of TRPV1 antagonists.[3e] As shown in Table 2, it was possible to retain our targeted TRPV1 potency (<5 nM) with several basic amino-methyl derivatives (**33**, **35**, and **36**), as well as in two of the extended chain amine analogs (**42** and **43**). The trends in Table 2 suggested that the potency of these amines increases with enhanced lipophilicity. In comparing **29** to **31**, **35** to **36**, and **38** to **37**, for example, the *c*log*P*[10] values increase by 1.24, 1.04, and 1.26 log units, respectively. Although the pK_a values for some are sufficiently high to ensure a partially ionized species at physiological pH (**36** pK_a 7.51, **42** pK_a 7.86),[11] the higher lipophilicities and molecular weights of these more potent compounds led to minimal improvements in aqueous solubility (compare **38** with **37**, e.g.). In addition, side-chains featuring a basic amine tended to introduce a higher cardiovascular risk as measured by their enhanced inhibitory activity at the hERG channel (see e.g., **33**, **35**, **36**, **42**, and **43**, vs **2**, Table 4).

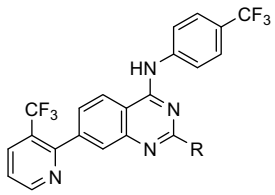
Table 1
2-Alkoxyalkyl quinazoline analogs

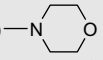
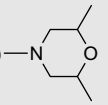
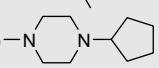
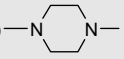
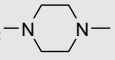
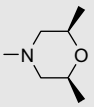


Compound	R	hTRPV1-cap[a] (nM)	HTSol[b] (μg/mL)
2	H	0.8 ± 0.07	0 [pK_a 4.54][c]
16	Me	0.5 ± 0.13	0
17	CH_2OH	4 ± 1.20	0.6
18	CH_2OMe	1.5 ± 0.20	0.2 [pK_a 3.7]
19	CH_2OEt	0.9 ± 0.16	0.6
20	CH_2O^iPr	0.3 ± 0.08	0.04
21	CH_2OCH_2Ph	0.5 ± 0.11	0.1
22	CH_2OPO_3H	46 ± 14	>138
23	$CH_2OCH_2CO_2H$	81 ± 22	125
24	$(CH_2)_2OMe$	2 ± 0.54	0
25	$(CH_2)_2OH$	22 ± 5.0	0.02
26	$(CH_2)_3OH$	25 ± 7.4	0.06
27	$(CH_2)_2CO_2H$	1627 ± 578	125
28	$(CH_2)_2SO_2Me$	36 ± 9.5	0.2

[a] Values are IC_{50}s ± SEM at human TRPV1 receptor activated by capsaicin ($n \geqslant 4$).
[b] See Ref. 8.
[c] See Ref. 11.

Table 2
2-Aminoalkyl quinazoline analogs

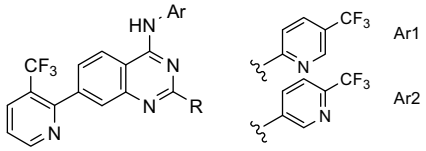


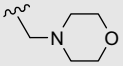
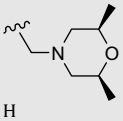
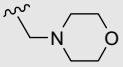
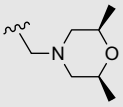
Compound	R	hTRPV1cap[a] (nM)	HTSol[b] (μg/mL)
29	$-CH_2NHMe$	26 ± 8.6	10
30	$-CH_2NH^iPr$	35 ± 10	0
31	$-CH_2NH^tBu$	8 ± 2.1	8
32	$-CH_2NH$-cyclohexyl	6 ± 1.8	—
33	$-CH_2NEt_2$	1.2 ± 0.32	0
34		25 ± 5.4	25
35		2.1 ± 0.21	2
36		0.4 ± 0.13	0.04 [pK_a 7.51][c]
37		8 ± 2.1	0
38		62 ± 13	57 [pK_a 7.68]
39		50 ± 11	36
40		69 ± 15	28
41		184 ± 35	86
42		3 ± 0.32	2 [pK_a 7.86]
43		2 ± 0.42	0.2

[a,b,c] See Table 1.

Having established some of the 2-position groups which impart the greatest TRPV1 potency, we explored the consequences of utilizing 2- and 3-aminopyridines as replacements for the 4-trifluoromethylaniline (TFMA) D-ring. These D-rings offered another avenue to enhance structural novelty. These modifications were well tolerated in the biarylamide series provided that a CF_3 substituent was present at the '*para*' position.[12] Excellent TRPV1 potency could also be achieved in the biarylamide series with 4-*tert*-butyl substitution on the D-ring, but these analogs were metabolically unstable. Indeed, adequate TRPV1 potency was demonstrated with either aminopyridine D-ring when used in conjunction with morpholino-methyl (**47**, **55**), small alkoxy-methyl groups (**45**, **50**, **51**), or H (**44**, **48**) at C-2 (Table 3). Compared to the TFMA-containing analogs, the 2-aminopyridines (Ar1, Table 3) were approximately 4-fold less potent, and the 3-aminopyridines (Ar2, Table 3) approximately 6- to 12-fold less potent at human TRPV1, respectively. However, the hERG data suggested that the pyridine D-rings were beneficial with respect to hERG channel inhibition. For example, compare **46** (30% inhibition at 3 μM) vs **35** (53%) and **51** (5%) vs **20** (61%) (Table 4). No significant change was observed with respect to aqueous solubility upon switching to the aminopyridine D-rings. Like their trifluoromethylaniline counterparts, the potent aminopyridine D-ring compounds (**44**, **46**, **48**, **50**, and **51**) were found to be stable in both rat and human liver microsome preparations.[13] Based on their overall profiles (including in vitro potency, metabolic stability, and hERG blockade), **44**, **48**, **55**, and **18** were advanced into rat pharmacokinetic experiments (Table 5). Both **44** and **18** demonstrated better oral exposure than our benchmark compound (**2**) when dosed in their freebase forms in methyl cellulose vehicle (AUCs of 7617 and 3251 ng h/mL, respectively). The low clearance (4.2 mL/min/kg) and large volume of distribution (12 L/kg) exhibited by **44** translated into a 38 h half-life in rat. Unfortunately, subsequent investigation of the chemical stability of **44** showed that this compound and other quinazolines incorporating 2-aminopyridines at the 4-position (D-ring) were too hydrolytically labile to warrant further evaluation. Indeed, stability studies in 0.1 N HCl at 40 °C over 6 h (6 time points) indicated that

Table 3
Quinazoline analogs with 2- and 3-aminopyridine D-rings



Compound	Ar	R	hTRPV1[a] (nM)	HTSol[b] (μg/mL)
44	Ar1	H	2.5 ± 0.45	ND [pK_a 3.26][c]
45	Ar1	CH_2OMe	12 ± 4.2	ND
46	Ar1		9 ± 1.5	2
47	Ar1		1 ± 0.10	0
48	Ar2	H	7 ± 0.71	0 [pK_a 3.48]
49	Ar2	CH_2OMe	25 ± 5.8	4
50	Ar2	CH_2OEt	4.5 ± 0.42	0
51	Ar2	CH_2O^iPr	1 ± 0.17	0
52	Ar2	$(CH_2)_3OH$	380 ± 100	5
53	Ar2	$(CH_2)_2CO_2H$	>3000	125
54	Ar2		24 ± 4.4	31
55	Ar2		2 ± 0.23	7

[a,b,c] See Table 1.

Table 4
hERG data for selected aminoquinazolines

Compound	hERG @ 3 μM[a]	SD[b]
2	8	6.9
16	25	3.3
17	68	10.7
18	44	10.7
19	35	11.4
20	61	12.5
21	11	8.6
24	35	4.1
33	86	6.3
35	53	6.5
36	40	3.5
42	90	6
43	79	8.3
44	13	3.5
46	30	5.1
48	0.2	5.3
51	5	(n = 2)
55	23	2.6

[a] Data are average percentage block of hERG potassium channels using an in vitro electrophysiological whole cell (Cos7) assay.
[b] Standard deviation ($n \geqslant 3$).

Table 5
Rat pharmacokinetics for selected aminoquinazolines[a]

Compound	iv dose (mg/kg)	Cl (mL/min/kg)	V_{ss} (L/kg)	$T_{1/2}$ (h)	po dose (mg/kg)	AUC_{0-in} (ng h/mL)	T_{max} (h)	C_{max} (ng/mL)	f (%)
2	2.7	23	13	8	2.0	121	3.0	13	8
					2.0[b]	1426	0.67	101	99
44	1.5	4.2	12	38	2.0	7617	5.7	137	89
48	1.5	22	5.5	4.2	2.0[b]	1449	1.7	211	94
					2.1[c]	982	1.8	137	61
55	—	—	—	—	2.0	218	0.9	93	—
18	1.0	5.3	3.5	6.9	2.0	3251	3.7	215	50

[a] Dosed in 50% PEG-400/water (iv) and 0.5% methylcellulose, 0.1% triacetin (po) unless otherwise indicated.
[b] Vehicle was 2% vitamin E TPGS/water.
[c] Dosed as the HCl salt.

44 degrades by ~70% after 6 h and ~57% after 4 h (approximate stomach transit time in humans). The hydrolysis products were identified as the parent quinazolin-4-one and the 2-amino-5-trifluromethyl-pyridine. By comparison, **48** degraded by only 3% and **18** by only 1%, both over 6 h. Adequate exposure was achieved with **48** when dosed in vitamin E-TPGS (Table 5) or in MC as the HCl salt (AUC 982 ng h/mL @ 2.1 mg/kg). The potent amino-methyl analog **55** was stable in rat microsomal preparations[13] but was poorly absorbed following oral dosing in methylcellulose (Table 5). The 2-methoxymethyl analog (**18**) exhibited the best overall pharmacokinetic profile in rat with low clearance (5.3 mL/min/kg), moderate volume of distribution (3.5 L/kg), and a half-life of about 7 h. In addition, **18** is 4- to 5-fold more potent than **48** in vitro (1.5 nM vs 7.0 nM). Quinazoline **18** fully inhibited carrageenan-induced thermal hyperalgesia (CITH) in rats following a 3 mg/kg oral dose and led to a significant reversal of thermal hyperalgesia at doses as low as 0.3 mg/kg (Fig. 2). Although it proved to be particularly difficult to increase aqueous solubility starting from our lead aminoquinazoline (**2**), the excellent cellular permeability of **18** may be one of the factors contributing to the excellent in vivo performance of this compound. Experiments in MDCK-MDR1 cells suggest that p-glycoprotein-mediated efflux mechanisms are not an issue with this compound (Table 6). These data are important in light of evidence that central exposure may be an important parameter for achieving broad spectrum analgesic effects with TRPV1 antagonists in preclinical pain models.[15] Consistent with the robust effect observed in CITH, **18** demonstrated significant brain penetration with a brain-to-plasma ratio of ~1.8 (Table 6).

In summary, initial SAR exploration of lead aminoquinazoline (**2**) at C-2 has led to a number of potent TRPV1 antagonists with improved pharmacokinetic properties. In addition, we have further demonstrated the utility of this series with respect to efficacy in an acute inflammatory pain model in rats. The 2-methoxymethyl analog **18** exhibits good in vitro and in vivo potency (CITH) and is well absorbed following oral dosing. Unfortunately, hERG data suggest that cardiac QT interval prolongation may be a potential risk with this compound (Table 5). To address this potential liability, further optimization of this series will be the topic of future communications.

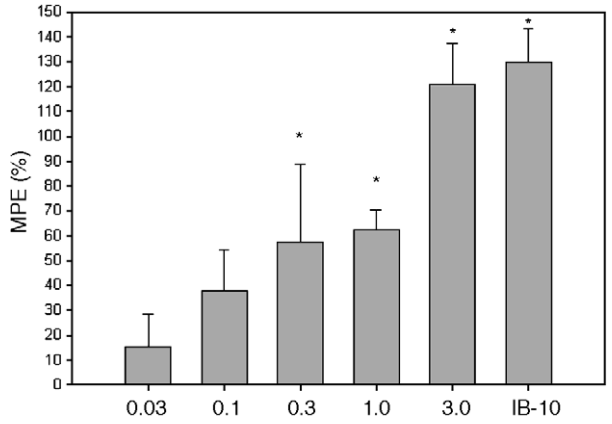


Figure 2. Effect of **18** (0.03, 0.1, 0.3, 1.0, and 3.0 mg/kg) and ibuprofen (10 mg/kg) on carrageenan-induced thermal hyperalgesia following oral dosing in rats. $^{*}p < 0.05$ (see Ref. 14).

Table 6
Key in vitro and in vivo data for **18**

hTRPV1-cap (nM)[a]	rTRPV1-pH (nM)[b]	MDCK P_{App} ($\times 10^{-6}$ cm)[c]	hERG (%In @ 3 μM)[d]	CITH MED (mg/kg)[e]	b/p[f]
1.5	0.5	A → B 14 (±5) B → A 17 (±2)	44	0.3	1.8

[a] IC_{50} at human TRPV1 receptor activated by capsaicin.
[b] IC_{50} at rat TRPV1 receptor activated by low pH.
[c] Permeability in MDCK cells over-expressing Pgp.
[d] See Table 4.
[e] Minimum effective oral dose to reverse carrageenan-induced thermal hyperalgesia (CITH).
[f] Brain-to-plasma ratio at 4 h following a 3 mg/kg oral dose in rats (vit E-TPGS vehicle).

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7. Vitamin E TPGS is D-alpha-tocopheryl polyethylene glycol 1000 succinate.
8. Aqueous solubility was assessed using a high-throughput format assay (HTSol) by diluting DMSO stock solutions of the test article into pH 7.4 buffer and shaking for 4 h at room temperature. Solutions were then filtered and analyzed (HPLC/MS).
9. There is ample precedent for successfully using phosphate pro-drugs for the parenteral delivery of poorly water-soluble drugs. Preliminary in vitro experiments in our laboratory suggested that in fact the phosphate group on **22** could be readily cleaved in the presence of alkaline phosphatase. Phosphatase cleavage experiments: 550 μmol of substrate **22**, 20 μL of calf intestine alkaline phosphatase (equiv to 20 U), and 150 μL of buffer (pH 7.6), at 40 °C for 2 h. Disappearance of **22** and appearance of **17** were monitored by HPLC.
10. Octanol–water partition coefficients (*c*log*P*) were calculated using the BioByte ClogP 4.0 estimator, as implemented in the Daylight Chemical Information System software, v. 4.71. Daylight Chemical Information Systems, Mission Viejo, CA.
11. pK_a values were determined potentiometrically.
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13. Compounds were incubated with pooled human or rat liver microsomes. The rates of oxidative metabolism were measured under the following conditions: compound, 1 μM final concentration; final microsomal protein concentration approximately 1 mg/mL; NADPH, 0.5 mM; pH 7.4 sodium phosphate buffer. Incubations were performed at 39 °C and were initiated by the addition of NADPH. Samples (50 μL) were taken from each incubation well at about 0.25, 1, 5, 10, and 30 min and added to 75 μL of ice-cold acetonitrile. The aliquot mixtures were mixed and then centrifuged at 3000 rpm for 15 min. The supernatant was analyzed for parent compound using LC/MS and the percent metabolized was calculated based on disappearance of parent compound.
14. Sprague–Dawley rats (n = 11–12/group) were administered vehicle, Ibuprofen, or **18** via oral gavage (2% vitamin E-TPGS vehicle) 4 h pre-test. Carrageenan (1%) was injected subcutaneously into the plantar surface of the paw 3 h pre-test. Results are expressed as % of maximum potential effect (MPE, y-axis) wherein 0% MPE is equivalent to a vehicle treatment group response and 100% MPE is equivalent to complete reversal of thermal hyperalgesia. Statistical analysis was performed by one way analysis of variance with post hoc analysis using Fisher's least significant difference test. Statistical significance was accepted as $P < 0.05$.
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