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Discovery of small molecule antagonists of TRPV1

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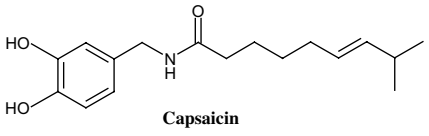
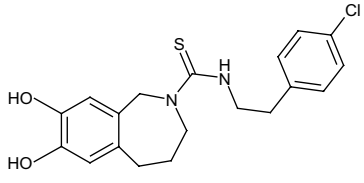
Abstract—Small molecule antagonists of the vanilloid receptor 1 (TRPV1, also known as VR1) are disclosed. Ureas such as **5** (SB-452533) were used to explore the structure activity relationship with several potent analogues identified. Pharmacological studies using electrophysiological and FLIPR Ca^{2+} based assays showed compound **5** was an antagonist versus capsaicin, noxious heat and acid mediated activation of TRPV1. Study of a quaternary salt of **5** supports a mode of action in which compounds from this series cause inhibition via an extracellularly accessible binding site on the TRPV1 receptor.

The pungent component of hot chili pepper, capsaicin and its analogues, are activators of the ion channel originally called vanilloid receptor 1 (VR1) but now referred to as TRPV1 according to an updated nomenclature.[1] Activation of TRPV1 on sensory neurons by capsaicin, heat or low pH is thought to lead to an influx of calcium and sodium ions through the integral channel causing a depolarisation of the cell and eventually leading to the sensation of pain. In humans, activation of TRPV1 by topical application of capsaicin initially induces pain that is followed by densensitisation leading to an analgesic effect. Several potent TRPV1 agonists have been identified[2] but the side effects associated with the development of these agents have hindered rapid progress. A TRPV1 antagonist, however, offers a rapid onset of analgesic action by simply blocking TRPV1 mediated signalling potentially avoiding the side effects associated with an agonist induced desensitisation strategy. Furthermore, because of its different mode of action, a TRPV1 antagonist may offer an improved safety profile in comparison with established analgesics such as NSAIDs[3] and hence may have therapeutic potential for the treatment of chronic pain arising from both tissue or nerve injury.

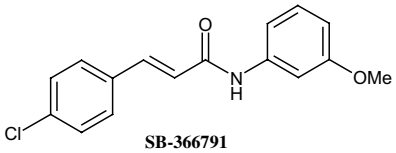
In 1992, capsazepine[4] was reported as the first competitive TRPV1 antagonist. This compound, however, has nonspecific actions, at both voltage-gated calcium channels[5] and nicotinic acetylcholine receptors,[6] at concentrations commonly used to block TRPV1 mediated responses. We recently reported[7] that SB-366791



Capsaicin



Capsazepine



SB-366791

Keywords: TRPV1; Vanilloid antagonist; VR1; SB-452533; SB-366791.
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was a novel, potent and selective competitive inhibitor of human and rat TRPV1 that represented an improved tool compound for the study of TRPV1 function.

More recently, other workers have sought to exploit this exciting area and several different structural classes of TRPV1 antagonists have been published.[8]

As part of our continuing effort to identify novel TRPV1 templates, a high throughput screen (HTS) was performed using a FLIPR (fluorescence imaging plate reader)-based Ca^{2+} assay versus our in-house compound collection. This Fluo-3 based assay allowed the identification of compounds, which were capable of antagonising the capsaicin-mediated activation of human TRPV1 stably expressed in 1321N1 cells, as previously described.[7] Compound **5** was identified as the most potent urea TRPV1 antagonist (pK^b 7.8) from the HTS campaign. We therefore initiated an investigation of the SAR of this urea template using array chemistry and a detailed characterisation of the antagonist properties of this compound versus the acid- and heat-mediated activation of TRPV1 using FLIPR and electrophysiological techniques (see Figs. 2 and 3).

Synthesis of the urea analogues (**1**–**20**) was readily accomplished from commercially available isocyanates and *N*-(2-aminoethyl)-*N*-ethyl-*m*-toluidine. Other required substituted amines were conveniently prepared from the appropriate substituted *N*-ethyl anilines and 2-aminoethyl bromide hydrochloride in toluene at reflux (2:1 stoichiometry) (Fig. 1).[9]

Compounds were screened in the FLIPR[7,10] assay using capsaicin as the agonist and the data are presented in Table 1.

Scrutiny of the substitution pattern on the LHS Ar group revealed that good antagonist activity was retained with 2-Cl **4** but surprisingly the corresponding fluoro **3** was 10-fold less potent. The unsubstituted compound **1** also showed an acceptable level of potency. Interestingly, for bromo substitution the order of potency was 2-Br > 3-Br > 4-Br. The dichloro analogues (**9**, **10**) showed comparable potency to **5** but the 1-naphthyl compound **8** was less active. Further SAR work established *N*-ethyl group (R1) as being optimum as replacement by NH (**11**), *N*-methyl (**12**), *N*-benzyl (**13**) or *N*-acetyl (**14**) did not enhance activity.

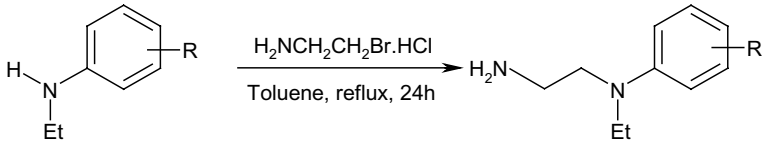


Figure 1. Preparation of *N*-(2-aminoethyl)-*N*-ethyl-phenyl derivatives.

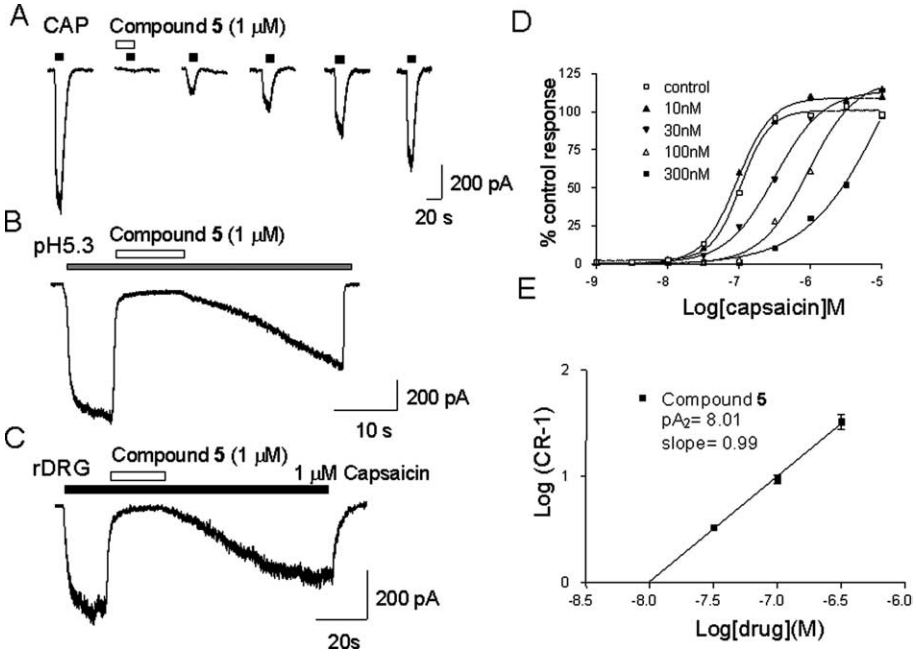


Figure 2. Inhibition of human TRPV1 receptors by **5**. (A and B) Whole cell currents were recorded from HEK293. TRPV1 cells using the patch clamp technique. TRPV1-mediated responses to 1 μM capsaicin (CAP) or acid (pH 5.3) were reversibly inhibited by co-application of 1 μM **5** ($n = 3$). Similarly, in (C), 1 μM **5** was demonstrated to inhibit the native capsaicin receptor present in rat dorsal root ganglia neurons ($n = 4$). (D) Concentration–response curves for capsaicin-evoked Ca^{2+}-entry in HEK293. TRPV1 cells recorded using a fluorescence imaging plate reader (FLIPR)[10] under control conditions, or in the presence of increasing concentrations of **5** (10–300 nM), as indicated. (E) Schild analysis of the data shown in (D) exemplified a competitive mechanism of antagonism of TRPV1 by **5**, with a pA2 = 8.01.

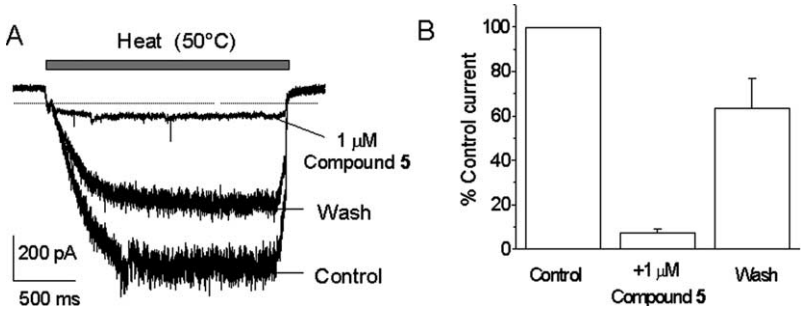
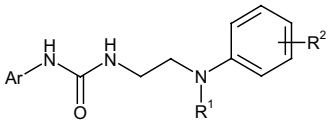


Figure 3. Inhibition of the heat-mediated activation of human TRPV1 receptors by **5**. Whole cell currents evoked by noxious heat (50 °C, applied for the duration indicated by the bar) were recorded from HEK293. TRPV1 cells using the patch clamp technique.[11] Heat-activated currents were reversibly inhibited by 1 μM **5** (93 ± 2% inhibition relative to control, $n = 3$).

Table 1. Antagonist activity at TRPV1

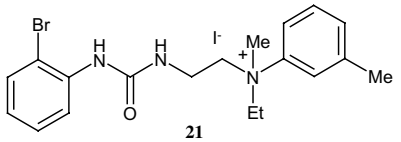


Compound	Ar	R^1	R^2	pK_b
1	Ph	Et	3-Me	7.2
2	2-Me–Ph	Et	3-Me	6.9
3	2-F–Ph	Et	3-Me	6.8
4	2-Cl–Ph	Et	3-Me	7.7
5	2-Br–Ph	Et	3-Me	7.8
6	3-Br–Ph	Et	3-Me	7.4
7	4-Br–Ph	Et	3-Me	7.2
8	1-Naphthyl	Et	3-Me	7.1
9	2,3-diCl–Ph	Et	3-Me	7.7
10	2,5-diCl–Ph	Et	3-Me	7.6
11	2-Br–Ph	H	3-Me	<6.8
12	2-Br–Ph	Me	3-Me	7.4
13	2-Br–Ph	CH_2Ph	3-Me	<6.7
14	2-Br–Ph	COMe	3-Me	<6.6
15	2-Br–Ph	Et	H	7.2
16	2-Br–Ph	Et	2-Me	6.9
17	2-Br–Ph	Et	4-Me	8.1
18	2-Br–Ph	Et	3-F	7.6
19	2-Br–Ph	Et	3,4-diF	7.8
20	2-Br–Ph	Et	3-F-4-Me	8.2
SB-366791				7.7
Capsazepine				7.5

Substitution (R^2) on RHS phenyl ring was investigated next and unsubstituted phenyl (**15**) and 2-Me (**16**) showed lower activity but in contrast the 4-Me analogue (**17**) was more potent. Replacement of the 3-Me by 3-F substituent (**18**) maintained activity, as did the 3,4-diF pattern (**19**). Encouragingly, a combination of 3-F-4-Me substitution (**20**) gave the most potent compound.

Compound **5** was investigated in electrophysiology experiments using the whole cell patch clamp method and its mode of action was studied in detail in further FLIPR experiments. The data show that **5** is a potent and reversible inhibitor of capsaicin, low pH and heat-mediated activation of human TRPV1 (see Figs. 2 and 3) and acts as a competitive inhibitor at the capsaicin binding site on the receptor (Fig. 2D and E).

Having established key SAR we then turned our attention towards investigation of the binding site of ureas such as **5**. Interestingly, the capsaicin binding site on TRPV1 has been reported to be accessible from the intracellular side of the receptor.[11] However, recent data now suggests that intracellular application of vanilloids is insufficient for activation of TRPV1.[12] The true location of the capsaicin binding site on TRPV1 therefore remains unclear. As **5** is a competitive inhibitor of the capsaicin binding site we thought it would be interesting to determine its site of action. Quaternary salts are unlikely to permeate through cell membranes and therefore the methiodide salt of **5** was prepared by stirring in acetone with methyl iodide at ambient temperature for 7 days.



21

This quaternary salt **21** showed moderate antagonist activity in the FLIPR assay (pK_b 7.0) in comparison with **5**. Electrophysiological assays suggested that its binding site and, therefore, the binding site for this template is likely to be accessible only from the extracellular surface as **21** was inactive when applied intracellularly at up to 100 μM concentration and yet was clearly active when applied extracellularly (Fig. 4).

Compound **5** was also potent as an antagonist at the cloned recombinant rat TRPV1 receptor in HEK293 cells with a pK_b of 7.7 versus capsaicin and pIC_{50} of 7.0 versus pH. This level of activity was confirmed in native receptor: **5** showed a pK_b of 7.4 versus capsaicin-evoked responses in rat dorsal root ganglian neurones. Furthermore, good potency was also observed for **5** in HEK293 cells at the cloned guinea pig TRPV1 receptor (pK_b of 7.5) and the human TRPV1 receptor (pK_b 7.7) versus capsaicin in the same cell line.

In conclusion, we have identified a potent series of TRPV1 antagonists, as exemplified by **5**, which show

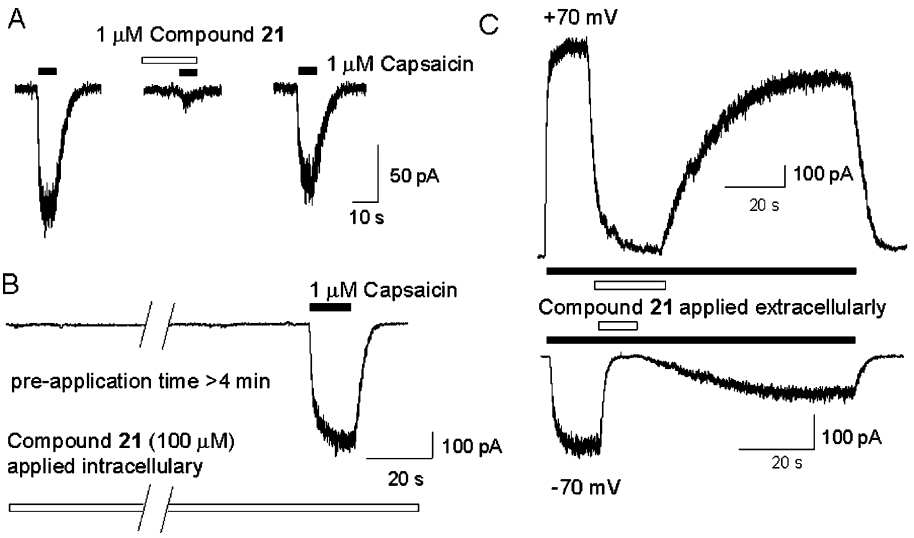


Figure 4. Inhibition of human TRPV1 receptors by the quaternary salt **21**. (A) The antagonist activity of the quaternary salt **21** was confirmed by demonstrating inhibition of TRPV1-mediated whole-cell responses to 1 μM capsaicin ($n = 3$). (B) Application of a high concentration (100 μM) of **21** to the intracellular side of the receptor did not result in inhibition of capsaicin-mediated responses ($n = 5$). In these experiments **21** was included in the intracellular solution and a pre-application period of >4 min was employed after whole cell mode was achieved to ensure that the compound had sufficient time to diffuse into the cell. (C) In the same test cells as those studied in (B) subsequent extracellular addition of compound **21** to responses recorded at both negative (−70 mV) and positive (+70 mV) membrane potentials resulted in a full blockade of TRPV1 ($n = 5$).

activity against capsaicin, noxious heat and low pH mediated activation of TRPV1. In addition **5** is equipotent across species (human, rat and guinea pig) with a good level of antagonistic activity. High intrinsic clearance determined in vitro in both rat and human liver microsomes[13] means that **5** is unsuitable for in vivo studies. However, it does provide an excellent tool compound for the study of the pharmacology of TRPV1 in vitro or ex vivo. Further work that addresses the pharmacokinetics properties of **5** (SB-452533)[14] is ongoing and will be reported in a future publication.

References and notes

1. Caterina, M. J.; Schumacher, M. A.; Tominaga, M.; Rosen, T. A.; Levine, J. D.; Julius, D. *Nature* **1997**, *389*, 816–824; Benham, C. D.; Davis, J. B.; Randall, A. D. *Neuropharamcology* **2002**, *42*, 873–888; Gunthorpe, M. J.; Benham, C. D.; Randall, A.; Davis, J. B. *Trends Pharmacol. Sci.* **2002**, *23*, 183–191.
2. Urban, L.; Campbell, E. A.; Panesar, M.; Patel, S.; Chaudhry, N.; Kane, S.; Buchheit, K.; Sandells, B.; James, I. F. *Pain* **2000**, *89*, 65–74.
3. Forrest, J. B.; Camu, F.; Greer, I. A.; Kehlet, H.; Abdalla, M.; Bonnet, F.; Ebrahim, S.; Escolar, G.; Jage, J.; Pocock, S.; Velo, S.; Langman, M. J. S.; Bianchi Porro, G.; Samama, M. M.; Heitlinger, E. *Br. J. Anaesth.* **2002**, *88*, 227–233.
4. Bevan, S.; Hothi, S.; Hughes, G.; James, I. F.; Rang, H. P.; Shah, K.; Walpole, C. S.; Yeats, J. C. *Br. J. Pharmacol.* **1992**, *107*, 544–552.
5. Docherty, R. J.; Yeats, J. C.; Piper, A. S. *Br. J. Pharmacol.* **1997**, *121*, 1461–1467.
6. Liu, L.; Simon, S. A. *Neurosci. Lett.* **1997**, *228*, 29–32.
7. Gunthorpe, M. J.; Rami, H. K.; Jerman, J. C.; Smart, D.; Gill, C. H.; Soffin, E. M.; Luis Hannan, S.; Lappin, S. C.; Egerton, J.; Smith, G. D.; Worby, A.; Howett, L.; Owen, D.; Nasir, S.; Davies, C. H.; Thompson, M.; Wyman, P. A.; Randall, A. D.; Davis, J. B. *Neuropharmacology* **2004**, *46*, 133–149.
8. Bakthavatchatam, R.; Blum, C. A.; Brielmann, H. L.; Caldwell, T. M.; De Lombaert, S. WO Patent 02/08221; Suh, H.; Oh, U. T.; Kim, H. D.; Lee, J. W.; Park, H. G.; Park, Y. H. WO Patent 02/16317, WO Patent 02/16318, WO Patent 02/16319; McIntyre, P.; James, I. F. U.S. Patent 6,406,908; Bo,Y. Y.; Chakrabarti, P. P.; Chen, N.; Doherty, E. M.; Fotsch, C. H. WO Patent 03/049702.
9. Rami, H. K.; Thompson, M.; Wyman, P. WO Patent 02/072536.
10. Smart, D.; Jerman, J. C.; Gunthorpe, M. J.; Brough, S. J.; Ranson, J.; Cairns, W.; Hayes, P. D.; Randall, A. D.; Davis, J. B. *Eur. J. Pharmacol.* **2001**, *417*, 51–58; Jerman, J. C.; Gray, J.; Brough, S. J.; Ooi, L.; Owen, D.; Davis, J. B.; Smart, D. *Br. J. Anaesth.* **2002**, *89*, 882–887.
11. Jung, J.; Hwang, S. W.; Kwak, J.; Lee, S.; Kang, C.; Kim, W. B.; Kim, D.; Oh, U. *J. Neurosci.* **1999**, *19*, 529–538.
12. Vyklicky, L.; Lyfenko, A.; Kuffler, D. P.; Vlachova, V. *Neuroreport* **2003**, *14*, 1061–1065.
13. Stevens, A. J. unpublished results. Intrinsic clearance rates in liver microsomes were rat >50 and human 41 mL/min/g liver.
14. Characterisation data for **5** (SB-452533): ^{1}H NMR (250 MHz, $CDCl_3$) δ (ppm): 8.00 (d, 1H), 7.50 (d, 1H), 7.26 (m, 1H), 7.10 (m, 1H), 6.92 (m, 1H), 6.55 (m, 4H), 4.95 (br, 1H), 3.47 (m, 4H), 3.37 (q, 2H), 2.30 (s, 3H), and 1.14 (t, 3H). $MH^+ = 376, 378$.