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Discovery of SB-705498: A potent, selective and orally bioavailable TRPV1 antagonist suitable for clinical development

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Abstract—Small molecule antagonists of the vanilloid receptor TRPV1 (also known as VR1) are disclosed. Pyrrolidinyl ureas such as **8** and **15** (SB-705498) emerged as lead compounds following optimisation of the previously described urea SB-452533. Pharmacological studies using electrophysiological and FLIPR-Ca^{2+}-based assays showed that compounds such as **8** and **15** were potent antagonists versus the multiple chemical and physical modes of TRPV1 activation (namely capsaicin, acid and noxious heat). Furthermore, **15** possessed suitable developability properties to enable progression of this compound into in vivo studies and subsequently clinical development.

The vanilloid receptor[1] (TRPV1, previously referred to as VR1) is a non-selective cation channel, predominantly expressed by neurons of the peripheral nervous system, which plays a key role in the detection of noxious painful stimuli such as acid (low pH), noxious heat and vanilloids such as capsaicin (the pungent component of hot chili peppers).

Based on knowledge gleaned from the biological effects of capsaicin,[2] the study of TRPV1 null mice and recent understanding of the role of TRPV1 receptors in nociceptive pathways and models of disease, it is increasingly clear that TRPV1 represents an exciting novel target for the treatment of chronic pain and a range of other disorders.[3]

Early efforts at manipulation of the TRPV1 receptor for the treatment of pain centred on the use of agonists such as capsaicin and resiniferatoxin. Both of these agents are thought to induce analgesia, via the desensitisation or elimination of TRPV1-bearing nerve terminals. The initial TRPV1-receptor activation, however, often leads to pain and extreme discomfort. In addition, such therapy may also be associated with systemic side-effects. The delayed onset of action coupled with an appreciation of these side-effects has hindered the development of systemically bioavailable TRPV1 agonists.

Indeed, more recent efforts have focused on small molecule TRPV1 antagonists since these agents may be expected to have a faster onset of action and should avoid the side-effects associated with TRPV1 agonist-based therapies. Furthermore, because of its different mode of action, a TRPV1 antagonist may offer an improved safety profile in comparison with current established therapy. The appearance of many new TRPV1 antagonists in the literature means that these predictions may soon be evaluated in the clinic.[3]

Keywords: SB-705498; TRPV1; TRPV1 antagonist; VR1; Pain; SB-452533; SB-366791; Electrophysiology; Capsaicin; Noxious heat; FCA.
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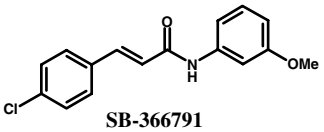

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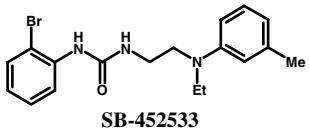

SB-452533

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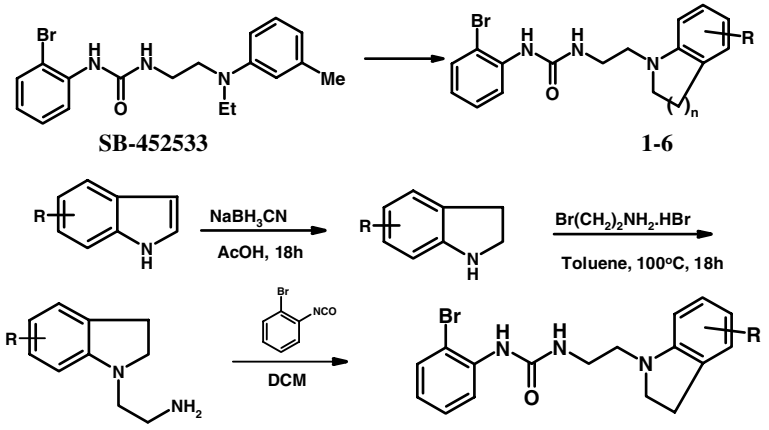

Figure 1. Cyclisation of *N*-ethyl group and generic synthetic scheme.

We recently reported[4] that SB-366791, a novel, potent and selective competitive antagonist of human and rat TRPV1 represented a useful tool compound for the study of TRPV1 function. Unfortunately, the poor pharmacokinetic properties of this compound, like the prototypical TRPV1 antagonist capsazepine before it, made it unsuitable for further progression. More recently we described[5] the synthesis and SAR around the urea SB-452533, a compound identified by high throughput screening (HTS). SB-452533 showed good potency against capsaicin, noxious heat and low pH mediated activation of TRPV1 but it too was deemed unsuitable for progression due to its high intrinsic clearance determined in vitro in both rat and human liver microsomes (intrinsic clearance rates in liver microsomes: rat > 50 and human 41 mL/min/g liver).

In this communication, we report our continuing efforts to identify selective TRPV1 antagonists with improved pharmacokinetic properties in order to: (i) enable the assessment of the safety and efficacy of such molecules in vivo and (ii) identify a molecule suitable for clinical development.

Since the high clearance associated with SB-452533 series could be due to potential dealkylation of the *N*-ethyl group, our initial attempt to improve the pharmacokinetic properties focused on cyclisation of the *N*-ethyl group onto the right-hand side (RHS) phenyl ring affording 2,3-dihydroindoline derivatives (Fig. 1).

The synthesis of the dihydroindole urea derivatives (Table 1) was readily achieved by reduction of appropriately substituted indole using sodium cyanoborohydride, alkylation with 2-bromoethylamine followed by addition of 2-bromophenyl isocyanate to afford compounds **1**–**5** as previously published[6] (see Fig. 1). The parent dihydroindoline compound **1** was 10-fold less potent in comparison to SB-452533. Encouragingly, introduction of either a methyl or fluoro substituent increased potency with the 4-position showing optimum potency but still lower when compared with SB-452533. The tetrahydroquinoline homologue derivative[6] **6** also showed good potency, suggesting minimal overall conformational change and therefore tolerated. However, despite the encouraging potency achieved with both the dihydroindole derivatives **1**–**5** and the homologue tetrahydroquinoline **6**, there was no improvement in their overall in vitro PK profile (e.g., for **5** and **6** rat CLi > 50 mL/min/g liver and human 19 and > 45 mL/min/g liver, respectively).

Table 1. Antagonist activity (FLIPR) versus capsaicin at TRPV1 for compounds **1**–**6**

Compound	*n*	R	pK_b
1	1	H	6.8
2	1	4-Me	7.4
3	1	5-Me	7.3
4	1	4-F	7.4
5	1	5-F	7.1
6	2	6-Me	7.4
SB-452533	—	—	7.8

Our attention next turned to alternative cyclisation of the *N*-ethyl group onto the ethylene linker, leading to the pyrrolidinyl urea derivatives. Preparation of analogues **7**–**11** was readily achieved as outlined in generic Figure 2. Palladium-assisted coupling of an appropriately substituted bromobenzene with the 3-amino Boc-protected pyrrolidine derivative afforded the 3-amino Boc-protected *N*-arylpyrrolidine. Deprotection under acidic conditions followed by treatment with 2-bromophenyl isocyanate afforded analogues **7**–**11** in good overall yield.[7] Preparation of the analogues **12**–**20** was readily achieved as outlined in Figure 3 for synthesis of **15**.

In this pyrrolidine series, the stereochemical requirement(s) for activity was initially investigated (Table 2). With the 3-Me phenyl RHS both the *R* and *S* enantiomers were prepared. The *R* enantiomer (**8**) clearly showed at least 10-fold greater activity in comparison with the *S* enantiomer (**7**) and demonstrated chiral discrimination at the receptor site. Encouragingly, the SAR determined from the SB-452533 series could be applied to the pyrrolidinyl ureas. Thus, the 3-methyl (**8**) and 3-fluoro (**9**) showed equivalent potency to SB-452533 whilst the disubstituted analogues **10** and **11** exhibited slightly higher potency. Results from rat and human liver microsomes for analogues **8**–**11** showed

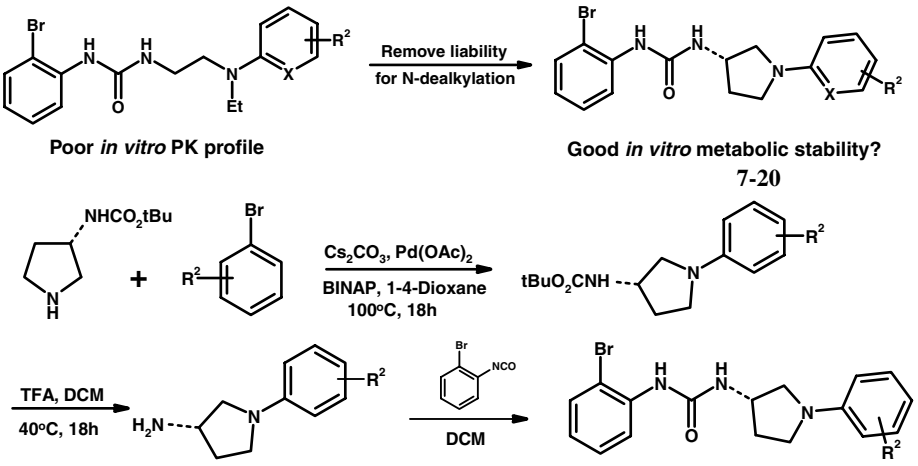

Figure 2. Design of pyrrolidine analogues and generic synthetic scheme for analogues **7**–**11.**

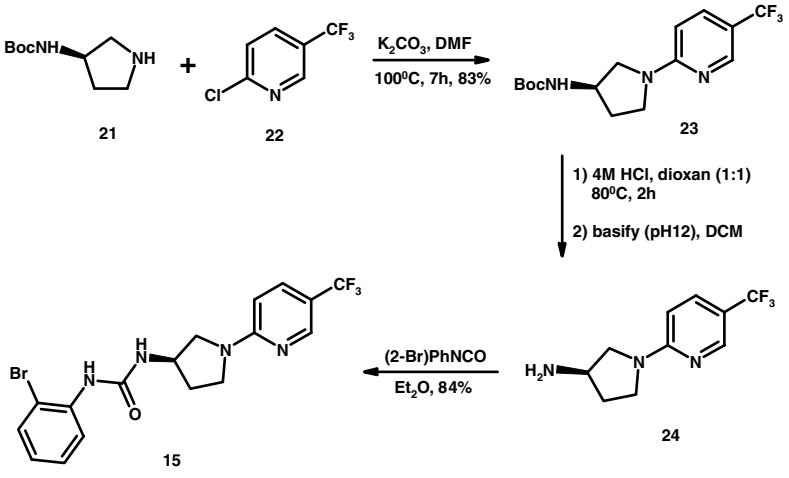

Figure 3. Synthesis of compound **15**.

Table 2. Antagonist activity (FLIPR) versus capsaicin at TRPV1 for pyrrolidine urea analogues **7**–**20**

Compound	Stereochemistry	X	R^2	pK_b
7	*S*	C	3-Me	<6.8
8	*R*	C	3-Me	7.7
9	*R*	C	3-F	7.8
10	*R*	C	3,4-DiF	8.1
11	*R*	C	3-F-4-Me	8.3
12	*R*	N	H	<6.6
13	*R*	N	3-CF_3	7.1
14	*R*	N	4-CF_3	6.9
15	*R*	N	5-CF_3	7.5
16	*R*	N	6-CF_3	7.2
17	*R*	N	5-Br	7.2
18	*R*	N	5-Cl	7.3
19	*R*	N	5-CN	6.7
20	*R*	N	5-Me	<6.7

that the intrinsic clearance rates were now much lower than for previous compounds ($\leqslant$11 and $\leqslant$4 mL/min/g liver in rat and human, respectively) and, hence, the potential for assessing key exemplars of the series in vivo could be usefully explored. However, all the analogues **8**–**11** possess lipophilic left-hand side (LHS) and RHS and, not surprisingly showed poor aqueous solubility (<0.1 mg/mL); an improvement in the solubility profile was desired. The LHS SAR demonstrated by the SB-452533 series precluded introduction of polar substituents such as amino groups since this was found to lower activity and so modification of the properties of the RHS aryl group was investigated.

Evaluation of the parent 2-pyridyl compound **12** (Table 2) indicated poor activity at TRPV1 but introduction of a trifluoromethyl group in the 6-position (equivalent to 3-Me position when X = CH, e.g., as in **8**) showed an encouraging level of activity (analogue **16**). Further investigation of the isomeric trifluoromethyl analogues showed that the 5-pyridyl position was optimum (compound **15**) as the alternative isomers (**13** and **14**) showed lower potency. Having established the 5-pyridyl position as optimum for substitution, alternative substituents were investigated. Neither the 5-bromo (**17**) nor the 5-chloro (**18**) showed an increase in potency compared to **15** and replacement by 5-cyano (**19**) and 5-methyl (**20**) attenuated activity.

Table 3. Antagonist activity (FLIPR) versus capsaicin at rat and guinea pig TRPV1 receptor and pharmacokinetic profile for **15**

Parameter	Rat	Guinea pig	Dog
pK_b	7.5	7.3	nd
CLi (mL/min/g liver)	<0.5	4.2	0.9
CLb (mL/min/kg)	4	34	11
V_{ss} (L/kg)	0.9	3.6	1.9
$t_{1/2}$ (h)	3.1	2.6	3.4
F_{po} (%)	86[a]	39[b]	42[a]

[a] At 3 mg/kg dose.
[b] At 1 mg/kg dose.
nd, not determined.

The selectivity of **15** was defined by broad receptor profiling (CEREP) and other in vitro assays where it showed little or no activity versus a wide range of ion channels, receptors and enzymes.[8] Furthermore, **15** showed improved solubility (0.4 mg/mL in simulated gastric fluid) and therefore, emerged as the optimized analogue from the pyrrolidine urea series. Compound **15** (pK_b 7.5 ± 0.2), SB-705498, was further investigated and a full in vitro and in vivo package generated (Table 3).

The synthesis of compound **15** as detailed above (Fig. 3) exemplifies a readily accessible route to pyrrolidinyl ureas and was applied to preparation of analogues[7] highlighted in Table 2. From commercially available starting materials (**21** and **22**) the pyridyl intermediate **23** was prepared in high yields (K_2CO_3, DMF, 100 °C, 7 h, 83%). Deprotection of **23** under acidic conditions followed by basification afforded the primary amine **24** which was used without purification in the next step. Addition of **24** to an ethereal solution of 2-bromophenyl isocyanate furnished **15** in an overall yield of 62% without the need for chromatography.

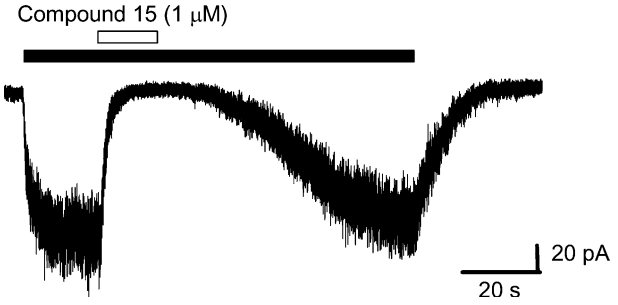

Figure 4. Inhibition of the capsaicin-mediated activation of human TRPV1 by **15**. Whole cell currents were recorded from HEK293 · TRPV1 cells using the patch clamp technique. TRPV1-mediated responses to 1 μM capsaicin (black bar) were completely and reversibly inhibited by co-application of 1 μM **15** (open bar; 102 ± 1% inhibition, $n = 5$).

Compound **15** showed good antagonist potency in FLIPR assays against both the rat and guinea pig TRPV1. Furthermore, the low intrinsic clearance translated to low in vivo clearance in the rat, dog and moderate in the guinea pig with good bioavailability and an acceptable volume of distribution and half-life across species (Table 3).

Compound **15** was also investigated in electrophysiology experiments using the whole cell patch clamp method, as described previously.[4,5] The data show that **15** is a rapid, potent and reversible inhibitor of capsaicin-mediated activation of human TRPV1 (Fig. 4).

Further aspects of the pharmacological properties namely effects of pyrrolidinyl ureas on the alternative modes of activation of TRPV1 were also investigated. Compound **8** showed full blockade of heat activation of TRPV1 and this was fully reversible following drug washout (Fig. 5). Compound **15** also produced full blockade of heat (97 ± 3% at 1 μM) as well as pH activation (104 ± 3% at 1 μM, $n = 5$) of hTRPV1.

Overall, these results show that these pyrrolidinyl ureas such as **8** and **15** exhibit potent and reversible blockade against the multiple modes of TRPV1 activation, namely the vanilloid (capsaicin), heat- and acid-mediated activation of the receptor (full data to be reported elsewhere).

Having initially demonstrated that **15** showed good overall in vitro efficacy and oral bioavailability in rat, the in vivo activity of **15** was investigated in the capsaicin-induced secondary hyperalgesia model[9] in the rat (Fig. 6). This model demonstrates the compound's antagonist activity in vivo against the specific TRPV1 agonist capsaicin.

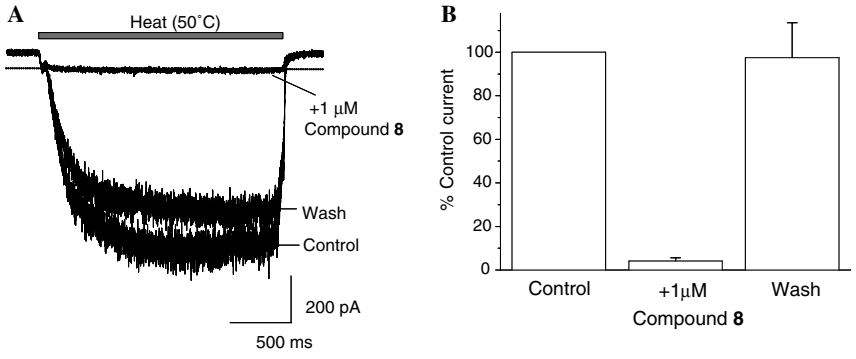

Figure 5. Inhibition of human TRPV1 receptors by **8**. Whole cell currents were recorded from HEK293 · TRPV1 cells using the patch clamp technique. TRPV1-mediated responses to heat were completely and reversibly inhibited by co-application of 1 μM **8**.

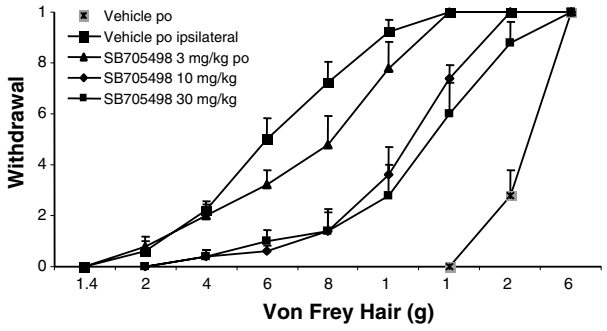

Figure 6. Activity of compound **15** (SB-705498) in the capsaicin induced secondary hyperalgesia model in the rat (3–30 mg/kg po at 2 h pre-treatment time).

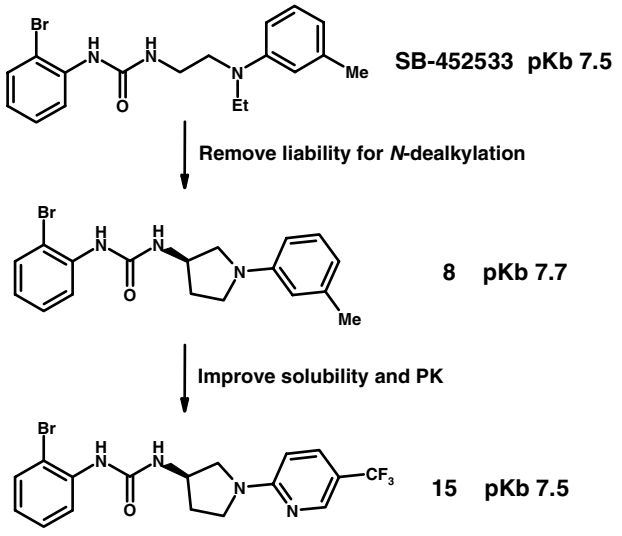

Figure 7. Design of pyrrolidine analogues.

As an early indicator of potential pharmacodynamic activity (consistent with activity at the target receptor), **15** showed excellent activity at 10 and 30 mg/kg po with good reversal of allodynia. Compound **8** also showed a similar level of activity at the equivalent dose (data not presented). Furthermore, compound **15** was also shown to give 80% reversal of allodynia in the guinea pig FCA model at 10 mg/kg po (full data to be reported elsewhere). This latter result suggests the potential for a TRPV1 antagonist in the treatment of inflammatory pain.

In conclusion, we have shown that modification of the HTS hit SB-452533 led to **8** that showed improved in vitro metabolic stability. Several cycles of lead optimisation chemistry, as summarized in Figure 7, detail the process of identification of our cyclised pyrrolidine ureas. Further SAR work identified **15** that exhibited an excellent in vitro and in vivo PK profile together with highly encouraging activity in preclinical models of pain (Fig. 6) and activities across different modalities (capsaicin, heat and low pH). Compound **15**[10] (SB-705498) is currently undergoing clinical trials and the outcome, as well as full in vivo biology details, will be reported elsewhere.

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10. Characterisation data for **15** (SB-705498): ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.41 (1H, s), 8.12 (1H. dd, *J* 8.3, 1.4 Hz), 7.76–7.79 (2H, m), 7.55 (1H, dd, *J* 8.0, 1.4 Hz), 7.47 (1H, d, *J* 6.7 Hz), 7.28 (1H, t, *J* 8.5 Hz), 6.89 (1H, t, *J* 7.8 Hz), 6.62 (1H, d, *J* 9.0 Hz), 4.34–4.36 (1H, m), 3.67–3.71 (1H, m), 3.56 (2H, br s), 3.31–3.41 (1H, m), 2.19–2.28 (1H, m) and 1.94–1.98 (1H, m). MH^+ = 429, 431.