

Identification of novel, small molecule Bradykinin B1 antagonists with oral activity in rodent models of pain and inflammation



David Hallett, Alastair Parkes, James Madden, Xiaolu Wang, Bart Ellenbroek, Frederic Machet, Tom Schwagarus, Christina Platt, Nicole Höschen, Mark Gemkow, Rachel Grigg, Andrea Cesura and John Kemp

Abstract

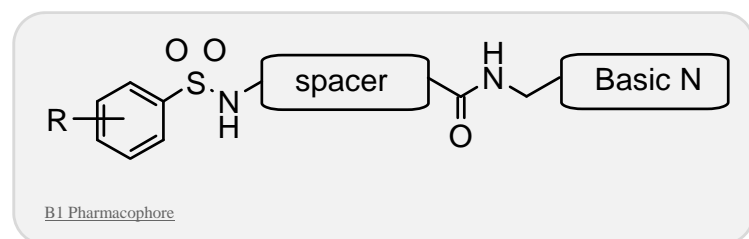
The Bradykinin B1 and B2 receptors are GPCRs that play a role in pain and inflammation. The B2 receptor is constitutively expressed under normal physiological conditions and its activation in response to inflammatory or noxious insult is believed to account for acute pain responses. In contrast, the B1 receptor is not expressed in healthy tissue but is upregulated following tissue injury. Activation of B1 receptors is believed to play a significant role in the chronic phase of inflammatory pain responses. Therefore, an orally active, small molecule B1 antagonist has been the goal of a number of drug discovery companies.

Our approach to finding small molecule B1 antagonists is to identify leads which, in contrast to the majority of known B1 antagonists, are active at both rodent and human B1 receptors thus enabling them to be profiled in rodent models of pain and inflammation.

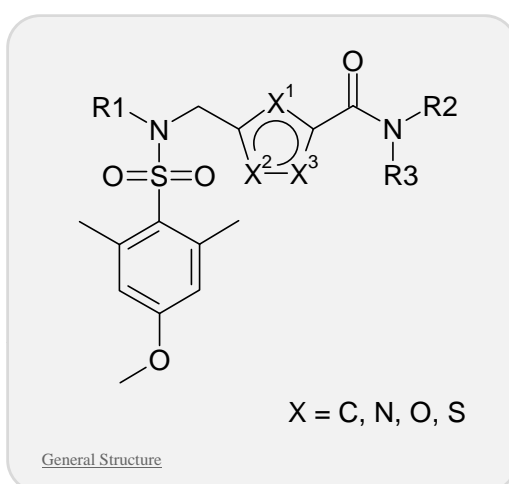
This poster describes how we identified and improved a novel series of small molecule B1 antagonists which resulted in the discovery of potent, selective compounds that were found to be orally active in rodent models of pain and inflammation.

Lead Identification

In 2005 Marceau [1] described pharmacophoric elements that are common to many published non-peptide B1 antagonists. Interestingly, most of the examples cited are active at both human and rodent B1 receptors. The structural elements typically consisted of an aryl sulfonamide, a spacer unit and an amide containing a basic centre as shown below:



This concept was used by our medicinal chemists to generate a number of novel "spacer" variants which were broadly consistent with this scheme. These "virtual" structures were filtered by overlaying them with the simple pharmacophore shown and subsequently ranked by docking into an in-house homology model of the human B1 receptor [2]. Preferred examples were then synthesised and active scaffolds were functionalised with a range of sulfonyl chlorides and basic amines. This approach led to the identification of compounds with the general structure shown below:



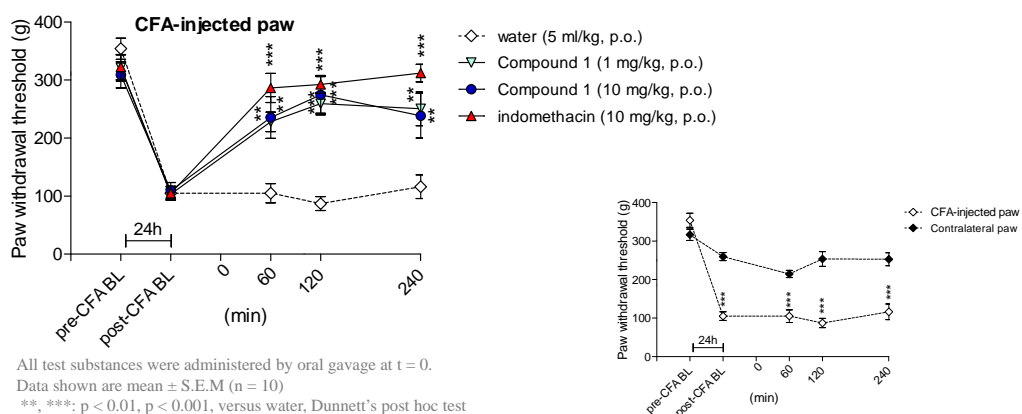
Exploratory SAR scoping afforded **compound 1**, which contained a furan as the central heterocyclic ring. This had acceptable human and rat B1 potency and a generally good *in vitro* profile for an early lead. The compound was not a CYP inhibitor (5 isoforms), it had no major off-target activities in a Cerep panel nor did it cause reversion mutations in an exploratory Ames assay (\pm S9). It also showed acceptable solubility, permeability (weak P-gp signal) and good free fraction in plasma. Parameters to optimise included human B1 activity, human metabolic stability and hERG. Rat PK data demonstrated that **compound 1** had good oral bioavailability and so it was used as a tool for *in vivo* pharmacology studies.

Parameter	Cmpd 1
hB1 IC ₅₀ (nM)	40
rB1 IC ₅₀ (nM)	0.6
Solubility @ pH 7.4 (mg/mL)	>1.0
In vitro stability, t_{1/2} (minutes)	
Liver microsomes	
Human	5
Rat	29
PPB (% unbound)	11
Caco-2 (A-B x10 ⁻⁶ cm/s)	4.3
CaCo-2 (B-A/A-B)	2.5
CYP (5 isoforms) IC ₅₀ (μM)	>45
hERG Ephys IC ₅₀ (μM)	3.9
Cerep Panel (53 assays)	Clean

Route (dose)	Parameter	Cmpd 1
Rat i.v. (1 mg/Kg)	CL _p (mL/min/kg)	46.5
	V _{ss} (L/Kg)	11.7
	t _{1/2} (h)	3.3
Rat p.o. (normalised to 1 mg/Kg)	C _{max} (nM)	37
	T _{max} (h)	2
	t _{1/2} (h)	4.7
	F _{po} (%)	40
	AUC _{0-∞} (mM.h)	0.26

Compound 1 was profiled in an IL-1B/DABK pharmacodynamic (PD) model which measures paw edema (data not shown), using a Sanofi B1 antagonist as a positive control [2].

Compound 1 was found to be active after oral administration with a minimum efficacious dose of 0.3 mg/kg. The compound also attenuated both thermal and mechanical hyperalgesia in a rat CFA model although the effect in the latter endpoint was more robust:

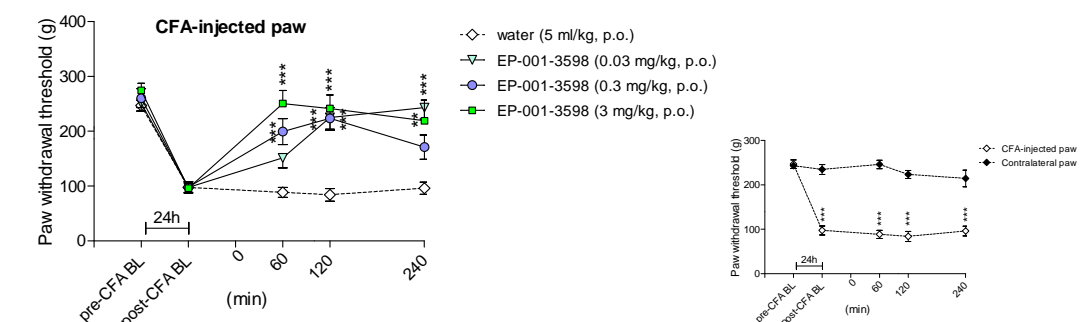


After demonstrating oral efficacy with an exemplar of this class of compounds we turned our attention to addressing the shortcomings of **compound 1**. Changes to the heterocyclic spacer were found to attenuate hERG and small modifications to R1, R2 and R3 afforded **compound 2** (an oxadiazole) with improved hB1 potency, enhanced selectivity over hERG and with improved, but still sub-optimal microsomal stability in primates. Free fraction in plasma (measured by equilibrium dialysis) was also increased.

Parameter	Cmpd 2
hB1 IC ₅₀ (nM)	0.7
rB1 IC ₅₀ (nM)	0.5
Solubility @ pH 7.4 (mg/mL)	>1.0
In vitro stability, t_{1/2} (minutes)	
Liver microsomes	
Mouse	372
Rat	74
Dog	71
Cyno	12
Human	10
PPB (% unbound)	72
Caco-2 (A-B x10 ⁻⁶ cm/s)	3.8
CaCo-2 (B-A/A-B)	3.1
CYP (5 isoforms) IC ₅₀ (μM)	>50
hERG Ephys IC ₅₀ (μM)	27

Route (dose)	Parameter	Cmpd 2
Rat i.v. (1 mg/Kg)	CL _p (mL/min/Kg)	53
	V _{ss} (L/Kg)	8.3
	t _{1/2} (h)	2.5
Rat p.o. (normalised to 1 mg/Kg)	C _{max} (nM)	38
	T _{max} (h)	2
	t _{1/2} (h)	2.0
	F _{po} (%)	26
	AUC _{0-∞} (mM.h)	0.16

Data from rat PK studies showed the compound was orally bioavailable, but there was no improvement in clearance or half life - the increased free fraction likely off-setting the improved *in vitro* stability. **Compound 2** was orally active at doses as low as 0.03 mg/Kg in both the rat PD model (data not shown) and in rat CFA (mechanical hyperalgesia):



In order to assess the predictivity of *in vitro* stability data multi-species PK studies were undertaken, and these showed a good correlation between *in vitro* stability and oral bioavailability across species. Oral bioavailability in mice, rats, dogs and cynomolgus monkey was 100%, 26%, 30% and 2%, respectively.

Conclusion

We have developed a series of novel B1 antagonists that are orally bioavailable and orally active in rat models. Poor *in vitro* stability in primates remains an area for optimisation and this remains an ongoing objective. Metabolite ID studies are currently guiding rational medicinal chemistry efforts.

References

- [1] F. Marceau, Trends in Pharmacological Sciences 2005 (26), 116-118
- [2] J. Gougat, J Pharmacol Exp Ther 2004 (309), 661-669