Letters

Potent and Orally Bioavailable Non-Peptide Antagonists at the Human Bradykinin B_1 Receptor Based on a 2-Alkylamino-5-sulfamoylbenzamide Core

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Abstract: The bradykinin B_1 receptor is rapidly induced after inflammation or tissue trauma and appears to play an important role in the maintenance of hyperalgesia in inflammatory conditions. Here, we describe the optimization process to identify novel, potent non-peptide human B_1 receptor antagonists based on a 2-alkylamino-5-sulfamoylbenzamide core. Optimized derivatives are selective, functional B_1 antagonists with low nanomolar affinity and exhibit oral bioavailability in animals.

Chronic pain affects a significant proportion of people worldwide and causes unnecessary suffering, disability, and a poor quality of life. There is a great medical need for new medicines to treat chronic nociceptive pain primarily because of the side effects that are frequently observed with existing treatments such as nonsteroidal antiinflammatory drugs (NSAIDs) and opioids.

The kinins are potent 9−10 amino acid peptide hormones involved in inflammatory, vascular, and pain processes and are released following inflammation, tissue damage, or other noxious stimulation.[1] In humans, the two endogenous kinins bradykinin (BK, Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) and kallidin (KD, Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) are formed by proteolytic cleavage of high- and low-molecular-weight kininogen precursor proteins by plasma or tissue kallikrein (serine protease) enzymes, respectively.

The kinin peptides exert their biological effects by activating two distinct BK receptors (termed B_1 and B_2), which belong to the rhodopsin superfamily of G-protein-coupled receptors (GPCRs) but have low (36%) sequence homology to one another. Across species, there are also considerable differences in the amino acid sequences of the BK B_1 receptors.

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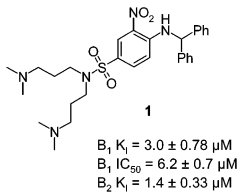
Chart 1. Bradykinin B_1 Antagonist Lead **1**



While BK and KD have high affinity for constitutive BK B_2 receptors, these kinins have low affinity for BK B_1 receptors, which are generally present at low levels under normal conditions. However, B_1 receptors are rapidly induced and expressed in peripheral tissues and cells within 2−5 h after tissue injury or inflammation and are activated by the metabolic fragments des-Arg^9-BK and des-Arg^{10}-KD.[2]

Compelling evidence suggests that the BK B_1 receptor plays an important role in chronic nociceptive pain associated with inflammatory conditions.[3] In animal models of persistent nociceptive pain, it has also been demonstrated that peptide B_1 receptor antagonists are effective in reversing inflammatory hyperalgesia.[4] Thus, a potent, selective, orally bioavailable B_1 antagonist should have significant potential as a novel therapy for inflammatory pain.

Several classes of non-peptide antagonists for BK B_2 receptors have been disclosed in the scientific literature,[5,6] and more recently the discovery of non-peptide antagonists for the B_1 receptor have been described.[7,8] In this Letter, we describe our own efforts in the optimization of a micromolar lead compound to afford potent, selective, and orally bioavailable non-peptide BK B_1 receptor antagonists based on the 2-alkylamino-5-sulfamoylbenzamide core.

An in-house collection of non-peptide bradykinin B_2 antagonists, generated in our previous research that afforded bradyzide and related analogues,[9−11] was assayed for binding affinity at the cloned human bradykinin B_1 receptor expressed in HEK293 cells. This screening process identified the 4-alkylamino-3-nitrobenzene sulfonamide **1** (Chart 1) as an interesting lead, having single-digit micromolar binding affinity (expressed as the inhibition constant $K_I \pm$ SEM) at both human B_1 and B_2 receptors, expressed in HEK293 and Cos-7 cells, respectively. **1** also behaved as a functional antagonist at human B_1 receptors expressed in Cos-7 cells, inhibiting the Ca^{2+} efflux induced by des-Arg^{10}-KD with $IC_{50} = 6.2\ \mu$M.

To rapidly investigate the importance of the bis-amine and the benzhydryl moieties in **1**, analogues were prepared. Thus, in separate reactions, eight different polar amines (see Chart 2) were coupled with commercially available 4-chloro-3-nitrobenzenesulfonyl chloride in THF in the presence of Et_3N to form the corresponding sulfonamide intermediates, which were each used crude for the subsequent nucleophilic aryl chloride displacement (DMSO at 120 °C) with nine

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Chart 2. Amines Used for Initial Array of Analogues of **1**

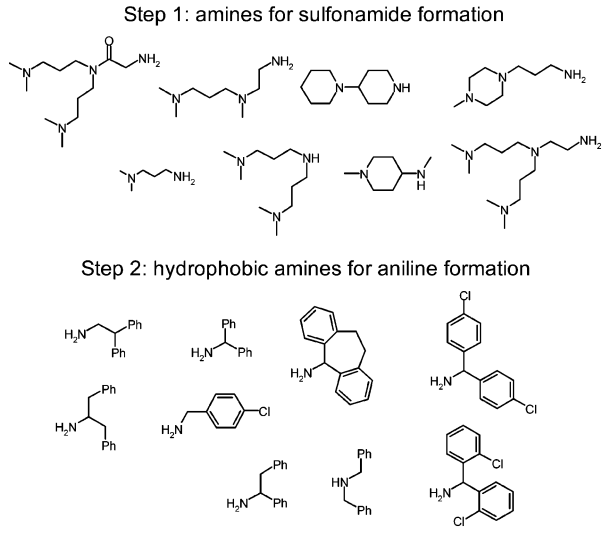
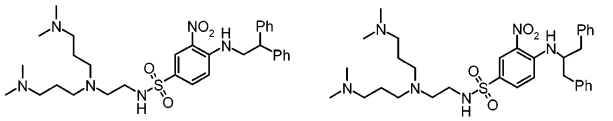
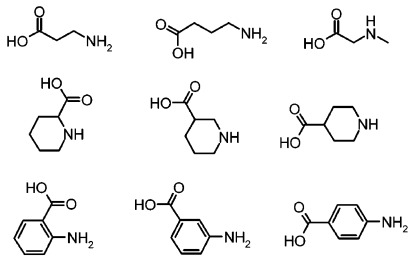


Chart 3. More Potent Analogues of **1** from First Array



2 B_1 K_i = 0.239 ± 0.030 μM **3** B_1 K_i = 0.255 ± 0.048 μM

Chart 4. Amino Acid Linkers Used in Second Array



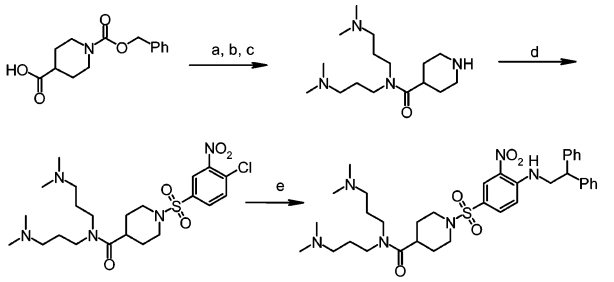
separate hydrophobic amines (Chart 2). The 71 new derivatives obtained by this process were screened for binding affinity at the human B_1 receptor and compared with that of **1**.

The results indicated that while the majority of the members of the array were equiactive with **1** or less potent (particularly when the bis-amine motif was absent), a 10-fold increase in binding affinity was observed in two analogues where (i) an ethylene spacer had been introduced between the sulfonamide and the bis-amine and (ii) the benzhydryl group had been replaced by the 2,2-diphenylethyl or the dibenzylmethyl group (**2** and **3**, Chart 3). It is interesting that neither of these modifications, in the absence of the other, increased B_1 receptor binding affinity over that observed with **1**.

A subsequent array of analogues was prepared where the ethylene spacer in **2** and **3** was replaced with a variety of acyclic and cyclic amino acids (shown in Chart 4). For this array, 2,2-diphenylethylamine, dibenzylmethylamine, and 3,3-diphenylpropylamine were used as the hydrophobic amine partners. A representative synthesis for this array is shown in Scheme 1.

Results from the B_1 binding assay indicated that although the inclusion of methylaminoacetic acid, piperidine-2-carboxylic acid, and the aromatic amino acids as spacers was not useful, incorporation of the 3-aminopropionic acid, piperidine-3-carboxylic acid, and piperidine-4-carboxylic acid spacers increased receptor binding affinity relative to the simple ethylene spacer. In particular, the piperidine-4-carboxylic acid derivative of **2** (**4**, Scheme 1) exhibited $K_I = 0.018$ μM.

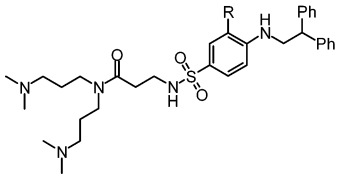
Scheme 1. Synthesis of Piperidine-4-carboxylic Acid Analogue **4**[a]



4 K_i = 0.018 ± 0.003 μM

[a] Conditions: (a) $(COCl)_2$, PhMe, DMF, room temp; (b) 3,3′-iminobis(*N*,*N*′-dimethylaminopropylamine), CH_2Cl_2; (c) H_2, 10% Pd/C, MeOH; (d) 4-chloro-3-nitrobenzenesulfonyl chloride, NEt_3, THF; (e) 2,2′-diphenylethylamine, DMSO, 150 °C.

Table 1. Binding Affinity of Nitro Group Replacements



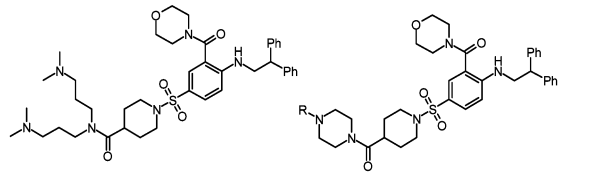
compd	R	B_1 K_I (μM)
5	NO_2	0.144 ± 0.022
6	Cl	>1
7	$C(O)NMe_2$	0.136 ± 0.022
8	C(O)morpholine	0.012 ± 0.003

In parallel to the optimization of the sulfonamide-bis-amine spacer group, analogues were prepared in which the undesirable aromatic nitro group was replaced with alternative substituents. In this investigation, the 3-aminopropionic acid spacer was used with the 2,2-diphenylethyl group (Table 1). While the chloro derivative **6** had low affinity for B_1 receptors, the tertiary amide **7** exhibited a binding affinity similar to that of the parent nitro analogue **5**. Although primary and secondary amide derivatives were found to be inactive (as was the parent carboxylic acid), the utility of a tertiary amide as a nitro replacement was confirmed when morpholine was used in place of dimethylamine. This change resulted in **8**, which had an affinity similar to that of **4**.

Subsequently a hybrid molecule was prepared, which combined the optimal amino acid spacer (piperidine-4-carboxylic acid used in **4**) and nitro replacement (morpholine amide used in **8**). This analogue (**9**, Chart 5) was found to be a highly potent B_1 ligand, with a binding K_I of 0.001 μM. **9** also behaved as a functional antagonist in Cos-7 cells, with an IC_{50} of 0.012 μM.

As discussed above, analogues prepared during the initial optimization of **1** that lacked the bis-amine motif tended to lose binding affinity at the B_1 receptor. With the discovery of **9**, we decided to reexplore the bis-amine

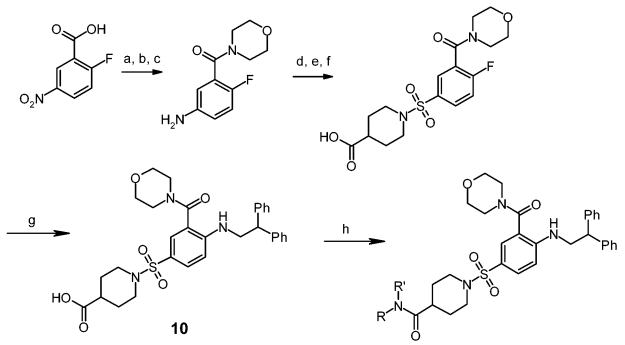
Chart 5



9
B_1 K_i = 0.001 ± 0.0001 μM

11 R = Me B_1 K_i = 0.014 ± 0.002 μM
12 R = Pr^i B_1 K_i = 0.011 ± 0.002 μM

Scheme 2[a]



[a] Conditions: (a) $(COCl)_2$, PhMe, DMF, room temp; (b) morpholine, NEt_3, THF; (c) Zn, AcOH; (d) $NaNO_2$, HCl, AcOH; (e) SO_2, $CuCl_2$, AcOH, H_2O; (f) isonipecotic acid, Na_2CO_3, dioxane−water; (g) 2,2-diphenylethylamine, DMSO, 120 °C; (h) isopropyl chloroformate, NMM, THF, −40 °C, 40 min, followed by amine.

region to find a smaller monobasic amine to replace the iminobis(*N*,*N*′-dimethylpropylamine).

To expedite this, the advanced intermediate **10** was prepared according to the synthetic route shown in Scheme 2. The carboxyl group of **10** was then activated via the corresponding mixed anhydride and coupled with a range of cyclic and acyclic amines.

It soon became apparent that it was possible to replace the bis-amine with a number of cyclic and acyclic monoamines to afford analogues that retained a respectable level of B_1 binding affinity. As anticipated, the oral absorption profiles of some of the monoamines were superior to those of **9**. For example, while **9** could not be detected in plasma after oral administration to rats, the two piperazine derivatives **11** and **12** (Chart 5) were found to exhibit reasonable oral absorption. This improvement was thought to be due to the reduction in overall molecular weight and the lower basicity of the piperazine nitrogens (pK_a of 6.5 and 7.6 for **11** and **12**, respectively). Thus, after an oral dose of 7 mg/kg, administered as a suspension in 0.5% methyl cellulose, the hydrochloride salts of **11** and **12** exhibited C_{max} of 913 and 196 nM and oral bioavailabilities of 42% and 15%, respectively. After intravenous administration to rats (0.7 mg/kg), the half-lives for **11** and **12** were estimated at 161 and 37 min, respectively. In dogs, a 20 mg/kg dose of **11** exhibited an oral bioavailability of 35% (C_{max} = 10.3 μM) when administered as an aqueous hydroxypropyl β-cyclodextrine inclusion complex.

11 and **12** also exhibited at least 500-fold selectivity for human bradykinin B_1 receptors over a panel of 30 G-protein-coupled receptors (including human BK B_2 receptors), 7 ion channels (including sodium, potassium, and calcium channels), and cyclooxygenase enzymes.

In conclusion, we have described the optimization of a micromolar lead **1** to afford a novel series of nonpeptide antagonists for the human bradykinin B_1 receptor. The optimized derivatives are potent and selective antagonists with reasonable oral bioavailability in animals and as such have potential as a novel therapy for pain associated with inflammatory conditions.

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Supporting Information Available: ^{1}H NMR spectra (400 MHz), HPLC and HRMS characterization data for all compounds, elemental analyses for **1**, **11**, and **12**, and details of human B_1 receptor binding and functional bioassays. This material is available free of charge via the Internet at http://pubs.acs.org.

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