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Discovery of a Potent, Non-peptide Bradykinin B_1 Receptor Antagonist

Dai-Shi Su,* M. Kristine Markowitz, Robert M. DiPardo, Kathy L. Murphy,‡ C. Meacham Harrell,‡ Stacy S. O'Malley,‡ Richard W. Ransom,‡ Raymond S. L. Chang,‡ Sookhee Ha, Fred J. Hess,‡ Douglas J. Pettibone,‡ Glenn S. Mason,§ Susan Boyce,§ Roger M. Freidinger, and Mark G. Bock

Departments of Medicinal Chemistry and Neuroscience, Merck Research Laboratories, West Point, Pennsylvania 19486, and Neuroscience Research Centre, Merck Research Laboratories, Terlings Park, Harlow, Essex, CM20 2QR, England

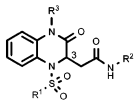
Received March 27, 2003; E-mail: daishi_su@merck.com

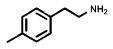
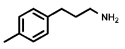
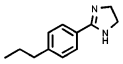
Bradykinin (BK) is an autacoid peptide produced by the catalytic action of kallikrein enzymes on plasma and tissue precursors termed kininogens. It plays an important role in the pathophysiological processes accompanying pain and inflammation. Its biological actions are mediated by two known G-protein-coupled receptors named B_1 and B_2. The BK B_2 receptor is constitutively expressed in most cell types, whereas the BK B_1 receptor is induced during inflammatory insults.[1] In animal models, BK B_1 receptor agonists produce hyperalgesia, an effect blocked by peptide BK B_1 receptor antagonists.[2] Recent data from transgenic BK B_1 receptor knockout mice has implicated a role for the BK B_1 receptor in inflammation and algesia.[3] Accordingly, selective and effective BK B_1 receptor antagonists hold promise as novel therapeutic agents for the treatment of pain and inflammation. In this communication we report the development of the first non-peptide BK B_1 antagonist with subnanomolar affinity for the BK B_1 receptor and functional potency in an animal pain model.

The discovery process was initiated with a high-throughput screen (HTS) of the Merck sample collection which uncovered the dihydroquinoxalinone lead compound, **1**. In racemic form, **1** exhibits a K_i value of 1.4 μM for binding to the human BK B_1 receptor with selectivity vs the human BK B_2 receptor (K_i > 10 μM) (Table 1). To improve the potency and pharmacological parameters of this screening lead we followed classical structure activity relationship (SAR) trends. This optimization process was also facilitated by a modeling study wherein we docked **1** into a homology model of the BK B_1 receptor constructed on the crystal structure of bovine rhodopsin (Figure 1).[4,5] The latter exercise suggested that **1** interacts mainly with transmembrane spanning domains 3 (TM3) and TM7 of the receptor. The aromatic ring of the sulfonamide chain and the dihydroquinoxalinone core are both accommodated by a hydrophobic pocket formed by residues Ile97, Trp98, Trp103, Ile113, and Phe302. Residue Gln 295 interacts with the sulfonamide oxygen of **1** via a hydrogen bond. A weak interaction is also observed between residue Asn114 and the N1 nitrogen of the dihydroquinoxalinone ring in **1**. On the basis of this analysis, it was determined that the phenyl sulfonamide moiety plays a key role in the substrate−receptor binding event and was therefore targeted for initial modification.

Among the first analogues prepared which displayed measurable advances over the lead compound **1** was derivative **2** (Table 1).[6] The potency of racemic **1** was improved by increasing the lipophilicity of the phenyl sulfonamide group and introducing a 2-methoxyl group in the phenyl amide ring. Resolution of **2** provided compound **3**, the *S*-enantiomer, and **4**, the corresponding *R*-enantiomer. As anticipated, receptor binding affinity and the configuration of the asymmetric center are linked. In this case, the *R*-isomer is approximately 2.5-fold more potent than the *S*-isomer.

Table 1. Receptor Binding Affinities of Dihydroquinoxalinone Analogues



Compound	R^1	R^2	R^3	3-Stereo	Ki (nM)[a,b]
1	4-methyl phenyl	phenyl	H	R, S	1400
2	2,4,6-trimethyl phenyl	2-methoxy phenyl	H	R, S	335
3	2,4,6-trimethyl phenyl	2-methoxy phenyl	H	S	840
4	2,4,6-trimethyl phenyl	2-methoxy phenyl	H	R	302
5	3,4-dichloro phenyl	2-methoxy phenyl	H	R	294
6	3,4-dichloro phenyl	2-methoxy phenyl	Me	R	>10000
7	3,4-dichloro phenyl	2,3,4-trimethoxy phenyl	H	R, S	51
8	3,4-dichloro phenyl		H	R	2.59
9	3,4-dichloro phenyl		H	R	0.98
10	3,4-dichloro phenyl		H	R, S	3.7
11	3,4-dichloro phenyl		H	R	0.034

[a] All K_i values are calculated for competition binding assays utilizing cloned human BK B_1 receptor and are the average of at least two experiments. [b] The human BK B_2 K_i values for all compounds are greater than 10,000 nM.

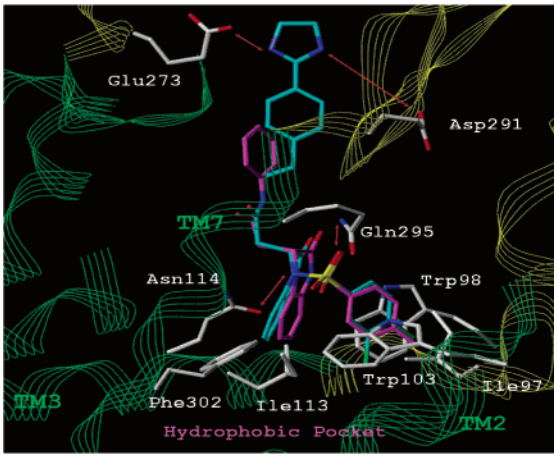


Figure 1. Homology model of BK B_1 receptor with **1** and **11** docked in the putative binding site. Transmembrane domains and extracellular loops are represented in green and yellow ribbons, respectively. Analogues **1** and **11** are colored in magenta and cyan, respectively.

‡ Department of Neuroscience, Merck Research Laboratories.
§ Neuroscience Research Centre, Merck Research Laboratories.

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Replacement of trimethyl phenyl in **4** with the 3,4-dichloro phenyl ring yielded a compound of equivalent potency (compound **5**). Capping the N1 nitrogen atom with a methyl group, which interrupts the interaction between the N1 nitrogen and Asn114 in the TM3 region as suggested by our model, caused a loss of potency (compound **6**). The increase in electron density of the phenyl amide ring in **5**, by the addition of two methoxyl groups, resulted in a further potency increase (compound **7**). Other neutral functional groups at various positions of the phenyl amide ring were examined (data not shown), but the 2,3,4-trimethoxyl groups were preferred. Replacement of the sulfonamide group with other functionalities resulted in a significant decrease in potency (data not shown) which supports the modeling observation of a hydrogen-bond interaction between residue Gln 295 in TM7 and one of the oxygen atoms in the sulfonamide group.

In close proximity to the hypothesized receptor binding site is the extracellular domain 4 (EC4) containing two acidic residues, Glu273 and Asp291. We speculated that the receptor binding affinity of **7** could be further strengthened by exploiting a potential binding interaction between the ligand and EC4. Therefore, a basic amino group was introduced to give **8** and subsequently **9**, which displayed a further 50-fold boost in receptor binding potency. Since there are two accessible acidic residues associated with EC4, a logical extension was to incorporate a bidentate basic group. Accordingly, the 4-ethylamine group in **8** was replaced with an imidazoline ring, yielding compound **10**. The imidazoline ring in **10** appears to be suitably positioned to simultaneously interact with Glu273 and Asp291 by insertion of an ethyl linker between the side chain amide nitrogen and the phenyl imidazoline ring system. This modification afforded **11**, the optimal compound in this study which displays subnanomolar affinity for the human BK B_1 receptor.

Receptor mutagenesis was utilized in an attempt to further delineate regions of the BK B_1 receptor that are involved with binding **11**. Mutation of amino acid residue Asn114 in TM3 of the human BK B_1 receptor to Ala results in an approximately 15-fold loss in affinity for **11**, with no loss in affinity for kinin peptides. This amino acid residue is on the same helical face as Lys118, a residue that has been implicated in binding the C-terminal carboxyl group of the peptide agonist des-Arg[10]-kallidin.[7] Significantly, the binding affinity of **11** is not affected by the Lys118Ala mutation. Therefore, **11** appears to be interacting with a region in TM3 that is in close proximity, but is not identical, to that involved with binding peptide agonists.

Previous mutagenesis studies have also implicated TM7 of the BK B_1 kinin receptor in binding peptide agonists.[8] We found that mutation of Gln295, in TM7, to Ala results in an approximately 26-fold loss in affinity for **11**. In contrast, the affinity of Gln295Ala for kinin peptides is unaltered. This result further supports the contention that the amino acid residues that bind **11** are distinct from those involved with binding peptide agonists.

Analogue **11** exhibits excellent binding affinities across BK B_1 receptors in different species (K_i: 0.034 nM (human), 0.05 nM (rabbit), 1.28 nM (dog), and 62.0 nM (rat)). Relative to the human B_1 receptor, the affinity of the dog and rat BK B_1 receptors for **11** is reduced 38- and 1800-fold, respectively. The EC4 domain of the human BK B_1 receptor has been implicated in determining the species selectivity for kinin peptides.[9] We tested the affinity of **11** for mutant chimeric receptors, in which this region of the human BK B_1 receptor was replaced with that of either the dog or rat BK B_1 kinin receptor. The affinity of the dog EC4 chimera is reduced 6-fold for **11** and the rat EC4 chimeric receptor affinity is reduced 30-fold. It can reasonably be concluded that EC4 confers a portion of the species selectivity observed for **11**.

Analogue **11** is selective for the BK B_1 versus the BK B_2 receptor (human BK B_2, $K_i > 10$ μM), and it demonstrates excellent functional antagonist potencies in a FLIPR assay[10] that are in concert with the receptor binding affinities. In addition, **11** is selective versus a number of human opioid receptor subtypes (IC_{50}: 7.6 μM (μ), 3.2 μM (δ), and 7.3 μM (κ)), and in a panel of assays[11] representing 170 enzymes, receptors, and transporters, **11** exhibited over 5000-fold selectivity for the human BK B_1 receptor.

To assess the in vivo antinociceptive efficacy of **11**, it was examined in a rabbit assay of inflammatory hyperalgesia.[12] In this model, **11** inhibits the spinal nociceptive reflex response to noxious pinch of an inflamed paw in a dose-dependent manner. It is efficacious at inhibiting the nociceptive reflex response to both low- and high-intensity stimuli. Analogue **11** is more effective than morphine at inhibiting low-intensity stimuli, indicating that it possesses potent antinociceptive activity.

The compounds disclosed in this work represent the first generation of dihydroquinoxalinones which are a useful base for the design of BK B_1 receptor antagonists. In particular, the pharmacokinetic and physicochemical properties of these compounds are suboptimal. Analogue **11** is the most prominent member of the series to emerge from this study, and its further evaluation, as well as those of its congeners, is in progress. The results from these studies will be disclosed in due course.

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Supporting Information Available: Assay protocols, all experimental details for **11**, and characterization of **3**−**11** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Fluorescence imaging plate reader, IC_{50}: 0.18 nM (human), 0.26 nM (rabbit), 16.30 nM (dog), and 163.5 nM (rat). See Supporting Information for assay protocol.

(11) Panlabs (MDS Pharma Services, Bothell, WA).

(12) Intravenous administration, ID_{50}: 3.5 μg/kg (low-intensity stimuli) and 16.4 μg/kg (high-intensity stimuli). For morphine, ID_{50}: 299 μg/kg (low-intensity stimuli). See Supporting Information for results and the assay protocol.

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