Design of Potent Non-Peptide Competitive Antagonists of the Human Bradykinin B_2 Receptor

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Bradykinin (1, Figure 1) is a linear nonapeptide plasma and tissue hormone[1] with the structure $Arg^1Pro^2Pro^3Gly^4$-$Phe^5Ser^6Pro^7Phe^8Arg^9$. Bradykinin is released upon tissue injury or trauma via the rapid enzymatic cleavage of kininogens by the proteolytic processing of plasma kallikreins.[2] This peptide hormone has been implicated in numerous pathophysiological processes[3,4] and is a potent pain-producing agent.[5,6] An antagonist of the bradykinin B_2 receptor has been suggested as a potential therapeutic agent to treat inflammation and pain.[4,7] Intensive research efforts have identified peptide antagonists of the bradykinin receptor.[8,9] However to date, there have been no reports of potent non-peptide antagonists.[10] We report herein the design of the first non-peptide agents which bind to the human bradykinin B_2 receptor and display competitive antagonism in several *in vitro* functional assays.

Initial screening efforts directed toward the discovery of a small molecule possessing bradykinin B_2 receptor activity afforded charged molecules as leads exemplified by the bis-phosphonium cation, **2** [IC_{50} = 3.9 μM ([^{3}H]-bradykinin binding to guinea pig ilieum homogenates)].[11] Replacement of the alkyl chain spacer in **2** with a more rigid biphenyl spacer gave rise to a class of bis-phosphonium salts represented by **3**. Compound **3** demonstrated modest potency (K_i = 3.4 ± 0.3 μM) against the human IMR 90 fetal lung fibroblast bradykinin B_2 receptor[12] and also displayed competitive antagonism against bradykinin-stimulated contractility in the guinea pig ileum with a pA_2 = 7.1 ± 0.1.

The distance separating the positively charged phosphonium groups in **3** was determined to be *ca.* 10 Å by molecular modeling. This 10-Å separation is in agreement with the distance separating the positively charged terminal arginine residues (Arg^1 and Arg^9) in **1**, assuming a β-turn conformation at the carboxy termini of bradykinin.[13] It has been demonstrated from previously disclosed peptide SAR data that an aromatic residue in position 8 or a D-aromatic residue in position 7 is an absolute requirement for high-affinity binding.[2,14] Implicit in these data is the existence of a distinct hydrophobic binding site in the B_2 receptor. We believed that to significantly enhance the potency in our series, it would be necessary to engage this critical hydrophobic binding site. Therefore, we sought to further modify the scaffold in **3** in such a way as to permit the incorporation of an additional hydrophobic functionality.

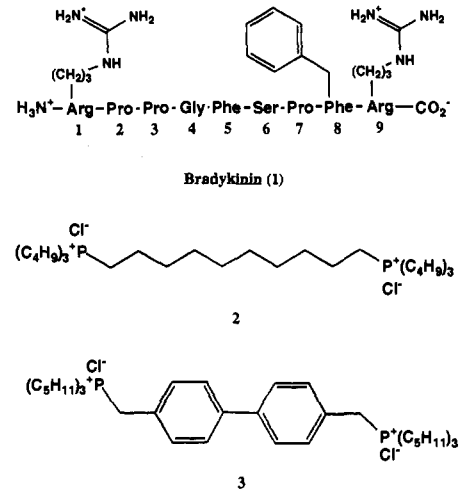

Figure 1. Structures of bradykinin (1) and bis-phosphonium salts **2** and **3**.

From a design aspect, we chose to use an α-amino acid as a core from which to construct a bis-charged backbone having a charge separation comparable to that of the biphenyl spacer. This strategy allowed us to probe the receptor for the putative hydrophobic binding site. At this juncture, we also chose to substitute one of the phosphonium groups in **3** with a guanidinium moiety. This gave us the option of modulating the pK_a and lipophilicity of the guanidyl moiety to further augment the overall activity of our series.

A wide variety of analogs with hydrophobic side chains were incorporated into the amino acid-based scaffold[15] and were evaluated for their B_2 receptor affinity. A small, representative group of these analogs is shown in Table I. Compounds which lack an aromatic side chain (**4**, R = H; **5**, R = $CH_2CH(CH_3)_2$) have no appreciable binding affinity (K_i > 100 μM). In contrast, incorporation of a lipophilic aromatic side chain (**6**, R = CH_2Ph) which was designed to mimic the Phe^8 residue in the natural ligand **1**, afforded antagonists with moderate potency. The activity of **6** could be modulated, depending on the nature of the substituents attached to the guanidyl nitrogens. For example, when the isopropyl group of **6** (K_i = 25 μM) was exchanged for a cyclohexyl group as in **7** (K_i = 3.3 μM), a 6-fold increase in binding affinity was noted, giving rise to an antagonist with a potency comparable to that of **3**. As the size of the aromatic side chain was increased from phenyl to naphthyl, guanidine **8** emerged as the most potent antagonist. Guanidine **8** exhibited a K_i = 60 nM and demonstrated competitive antagonism in radioligand binding and bradykinin-mediated functional assays (pA_2 = 7.1 ± 0.5). Compound **8** demonstrated submicromolar activity at the rat muscarinic receptor (K_i = 350 nM) and is 25–100-fold more selective for the bradykinin receptor when compared to all other receptor assays in which it has been tested.[16] Most significantly, **8** (R = CH_2(2-naphthyl)) is about 20 times more potent then **9** (R = CH_2(1-naphthyl); K_i = 1.0 μM), where the only change in the two molecules is the attachment point of the aromatic naphthylene ring. This well-defined SAR suggests that the putative hydrophobic binding site in the receptor prefers large aromatic groups oriented in a specific fashion in three-dimensional space.

Using **8** as a lead structure, analogs were synthesized where the remaining charged phosphonium group was

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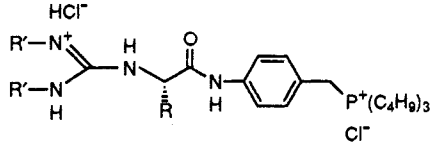
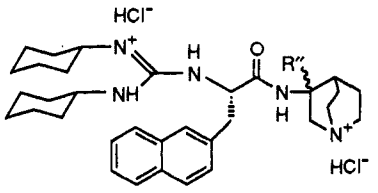
Table I. Bradykinin B_2 Receptor Affinities (K_i) for the α-Amino Acid-Based Antagonists 4–10b[18]



compd	R	R′	K_i (μM)
4	H	isopropyl	>100
5	$CH_2CH(CH_3)_2$	cyclohexyl	>100
6	CH_2Ph	isopropyl	25 ± 6.4
7	CH_2Ph	cyclohexyl	3.3 ± 0.2
8	CH_2(2-naphthyl)	cyclohexyl	0.06 ± 0.01
9	CH_2(1-naphthyl)	cyclohexyl	1.0 ± 0.15



compd	R″		K_i
10	H	diastereomeric mixture	0.41 ± 0.1
10a	H	diastereomer 1	0.21 ± 0.08
10b	H	diastereomer 2	0.55 ± 0.08

replaced with other functional groups bearing a positive charge at physiological pH. The protonated quinuclidine ion has been shown to have a disperged charge, rather than a point charge like an ammonium ion.[17] This charge dispersion is similar to the diffuse charge on the phosphonium group and for this reason the quinuclidine moiety was selected as an isostere of the trialkyl phosphonium group. (*R,S*)-3-Aminoquinuclidine was coupled to the L-2-naphthylalanine scaffold. This resulted in the formation of a 1:1 diastereomeric mixture, **10**, possessing molecular dimensions very similar to the lead structure **8**. Mixture **10** bound to the human bradykinin B_2 receptor with a K_i = 410 nM and displayed competitive antagonism in the guinea pig ileum contractility assay (pA_2 = 6.9 ± 0.6).[11] Separation of **10** into its respective diastereomers **10a** and **10b**[18] and evaluation for B_2 binding activity revealed that diastereomer **10a** bound to the human bradykinin B_2 receptor with a K_i = 210 nM, while diastereomer **10b** possessed a K_i = 550 nM.

In summary, we have successfully designed a series of potent non-peptide competitive antagonists of the human bradykinin B_2 receptor using an α-amino acid scaffold. The series of analogs displays a well-defined SAR, strongly suggesting binding in a specific fashion to the B_2 receptor. In equilibrium radioligand binding studies, guanidines **8** and **10a** display reversible, competitive binding affinities of 60 and 210 nM, respectively. The presence of the two positively charged residues and the hydrophobic naphthylene core in **8** and **10a** bear structural resemblance to the positively charged terminal arginine residues (Arg^1 and Arg^9) and the salient hydrophobic phenylalanine residue (Phe^8) in the native peptide ligand. This archetypical class of non-peptide bradykinin B_2 receptor antagonists may hold promise for the design of other agents having enhanced receptor affinity and optimal *in vivo* bioactivity.

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Supplementary Material Available: The experimental procedure for the synthesis of guanidine **8** is provided (4 pages). Ordering information is given on any current masthead page.

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