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STRUCTURE ACTIVITY RELATIONSHIPS OF NON-PEPTIDE BRADYKININ B_2 RECEPTOR ANTAGONISTS

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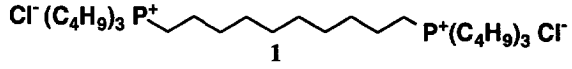
Abstract: A series of non-peptide competitive antagonists of the human IMR 90 fetal lung fibroblast bradykinin B_2 receptor have been designed and synthesized. Compound **15** binds with an affinity constant K_i = 60 nM. The SAR leading to the design of these non-peptide antagonists is described.

The nonapeptide bradykinin, ArgProProGlyPheSerProPheArg, is a potent pain producing agent and is involved in numerous pathophysiological processes.[1] Intensive efforts have identified peptide antagonists of the bradykinin B_2 receptor as potential therapeutic agents to treat pain and inflammation.[2] In an earlier communication we reported the first non-peptide agents which bound to the human bradykinin B_2 receptor and displayed competitive antagonism in several *in vitro* functional assays.[3] In this paper we describe our rationale for the design and structure activity relationships for several classes of non-peptide antagonists discovered in this study.

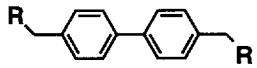
Screening of our chemical library for small molecules displaying bradykinin B_2 receptor affinity afforded charged molecules as leads. The bis phosphonium cation **1** [IC_{50} = 3.9 μM (^{3}H-bradykinin binding to guinea pig ileum homogenates)] bound with modest potency to the receptor,[4] however it did not display competitive antagonism. With the goal of achieving competitive antagonism and optimizing binding affinity the flexible alkyl chain spacer in **1** was replaced with a rigid biphenyl spacer. The lipophilicity around the charged phosphonium groups was varied, and the phosphonium moiety was replaced by other functional groups. Table I contains a set of molecules **2-6** [5] which suggest an important role of lipophilicity around the charged group for activity.



1

Most important was the discovery of compound **4** which was equipotent compared to **1**, but also displayed competitive antagonism against bradykinin stimulated contractility in the guinea pig ileum with a pA_2 = 7.1 ± 0.1.[4]

TABLE I. Human IMR 90 fetal fibroblast bradykinin receptor affinities (K_i) for compound **2-6**.

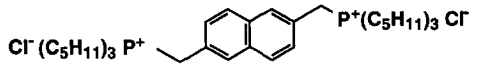
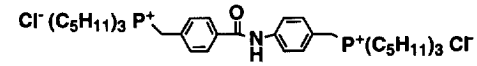
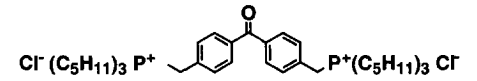
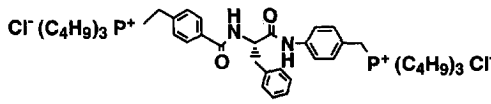


Compd	R	K_i (μM)
2	$-P^+(C_3H_7)_3\ Cl^-$	>25
3	$-P^+(C_4H_9)_3\ Cl^-$	20.7
4	$-P^+(C_5H_{11})_3\ Cl^-$	3.4
5	$-NH_2$	>25
6	$-N^+(C_4H_9)_3\ Cl^-$	>25

Using **4** as a lead structure, compounds **7**, **8** and **9** (Table II) were prepared possessing a charge separation comparable to **4**. The similar activity of these molecules suggests that their scaffolds are acting as a spacer separating the two charges by approximately 10 Å, and that the aromatic portion of these scaffolds are not contributing to binding to the receptor. This 10 Å separation is in agreement with the distance separating the positively charged terminal arginine residues in bradykinin, assuming a β turn conformation at the carboxy termini.[6] Peptide SAR data suggest the existence of a distinct hydrophobic binding site in the B_2 receptor occupied by the phenylalanine residue in position 8 of bradykinin or a D-aromatic residue in position 7 for peptide antagonists.[7] We sought to modify the non-peptide scaffold in such a way as to engage this critical hydrophobic binding pocket. Initially we chose to insert an α-amino acid between the amide linkage of compound **8**, resulting in **10**. In this way we would be able to readily substitute a large variety of side chain functionality into a series of analogs, in an attempt to fill the putative Phe[8] hydrophobic pocket. The bis-phosphonium **10** was synthesized and evaluated for receptor binding. This compound however, displayed weak bradykinin B_2 receptor affinity (> 25 μM). Molecular modeling indicated that the charge separation in **10** is closer to 13 Å than to the desired 10 Å. This may account for the poor activity observed for **10**. We therefore sought to replace the N-benzoyl(benzyl phosphonium) moiety in **10** with a guanidinum moiety attached directly to the amino acid core. This effectively reduced the separation of charges closer to 10 Å and permitted modulation of the guanidinyl group to further enhance the activity of the series.

Table III lists the key analogs **11-26** from which a well defined structure activity relationship emerged. The glycine analog **11** was inactive as predicted due to lack of a hydrophobic side chain. The introduction of an aromatic side chain in **12** resulted in weak binding activity, albeit more potent than **10**. Notably a threefold increase in binding affinity was achieved by changing the phenyl group of **12** to a naphthyl group as in **13**. Increasing the lipophilicity around the charged guanidinium moiety by replacement of the N-isopropyl group for an N-cyclohexyl group, gave a dramatic increase in receptor binding for this series of analogs. The phenylalanine analog **14** is 6-fold more potent than **12**, and the naphthylalanine analog **15** is ca. 2-orders of magnitude more potent than the analog **13**.

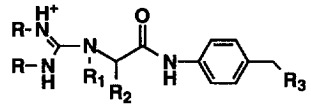
TABLE II. Human IMR 90 fetal fibroblast bradykinin receptor affinities (K_i) for compound **7-10**.

Compd	Structure	K_i (μM)
7		3.3
8		1.1
9		0.77
10		>25

Compound **15** bound to the human IMR 90 fetal lung fibroblast bradykinin B_2 receptor with a K_i = 60 nM and demonstrated competitive antagonism in radioligand binding and bradykinin mediated functional assays with a $pA_2 = 7.1 \pm 0.5$. Although compound **15** bound to the rat muscarinic receptor with a K_i = 350 nM, it was 25-100 fold more selective for the bradykinin receptor when compared to a number of other receptor assays.[4] An increase in lipophilicity around the phosphonium in **15** had little or no effect on increase of potency, therefore lipophilicity must be near optimum for **15**. In all examples it was found that the corresponding D-enantiomers of the amino acid cores produced compounds that were generally 2-fold weaker in binding versus the L-enantiomer. The side chains of the α-amino acid core were also varied (analogs **18-24**, Table III). This was done in order to ascertain the importance of the side chain towards binding affinity. The attachment point of the naphthalene moiety was found to be critical for tight binding, as shown by the 20-fold decrease in activity when 1-naphthylalanine was used as the core scaffold in **18**. Changing the naphthylalanine core to a leucine core in **19** was detrimental for binding activity. These data are indicative of a hydrophobic pocket which prefers large aromatic groups oriented in a specific fashion. Other changes to the side chain's length or aromatic character were better tolerated, however the 2-naphthylalanine core was clearly preferred. The seemingly isosteric change of 2-naphthylalanine to 2-benzothienylalanine showed a 7-fold decrease in potency, however a typtophan replacement reduced the potency by at least 40-fold.

The next point of variation was around the guanidinyl functionality. Interestingly the receptor tolerated N-methylation of the α-nitrogen in compound **25**. Aryl substitution on the guanidyl nitrogens in **26** was accepted equally as well as the cyclohexyl groups. This was an interesting discovery because now we had a means of modulating the pK_a of the substituted guanidine functionality. Lowering the pK_a of the guanidine closer to physiological pH would increase the partition between charged and uncharged species, thus potentially aiding in absorption of the molecule and transport across membranes.[8] Removal of the guanidyl function and replacement

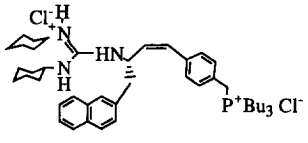
TABLE III. Human IMR 90 fetal lung fibroblast bradykinin B_2 receptor affinities (K_i) for compounds **11-26**.



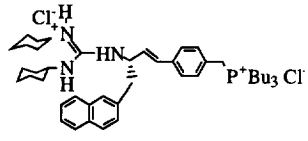
Compd	R	R_1	R_2	R_3	K_i (μM)
11	isopropyl	H	H[a]	$P^+(C_4H_9)_3$ Cl^-	>100
12	isopropyl	H	CH_2Ph[a]	$P^+(C_4H_9)_3$ Cl^-	24.9
13	isopropyl	H	CH_2(2-naphthyl)[a]	$P^+(C_4H_9)_3$ Cl^-	7.05
14	cyclohexyl	H	CH_2Ph[a]	$P^+(C_4H_9)_3$ Cl^-	3.25
15	cyclohexyl	H	CH_2(2-naphthyl)[a]	$P^+(C_4H_9)_3$ Cl^-	0.06
16	cyclohexyl	H	CH_2(2-naphthyl)[a]	$P^+(C_5H_{11})_3$ Cl^-	0.06
17	cyclohexyl	H	CH_2(2-naphthyl)[a]	$P^+(Ph)_3$ Cl^-	0.11
18	cyclohexyl	H	CH_2(1-naphthyl)[a]	$P^+(C_4H_9)_3$ Cl^-	1.03
19	cyclohexyl	H	$CH_2CH(CH_3)_2$[a]	$P^+(C_4H_9)_3$ Cl^-	>100
20	cyclohexyl	H	2-naphthyl[a]	$P^+(C_4H_9)_3$ Cl^-	3.44
21	cyclohexyl	H	CH_2(1-thienyl)[a]	$P^+(C_4H_9)_3$ Cl^-	2.57
22	cyclohexyl	H	CH_2CH_2Ph[a]	$P^+(C_4H_9)_3$ Cl^-	4.65
23	cyclohexyl	H	CH_2(2-benzothienyl)[a]	$P^+(C_4H_9)_3$ Cl^-	0.43
24	cyclohexyl	H	CH_2(3-indole)[a]	$P^+(C_4H_9)_3$ Cl^-	2.70
25	cyclohexyl	CH_3	CH_2(2-naphthyl)[a]	$P^+(C_4H_9)_3$ Cl^-	0.07
26	$CH_3C_6H_5$	H	CH_2(2-naphthyl)[b]	$P^+(C_4H_9)_3$ Cl^-	0.14

a: L-enantiomer
b: D-enantiomer

with a primary amine resulted in an inactive compound (data not shown) suggesting that the substituted guanidyl is a critical functional group for receptor binding. In order to evaluate whether a cis or trans amide bond was required for binding, the cis and trans olefin isosteres of **15** were synthesized. The cis olefin was 3-fold less potent then the trans olefin. This suggests a trans amide bond in **15** as the bioactive conformation.



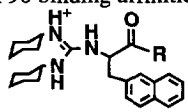
$K_{i\ cis}$ = 0.76 μM

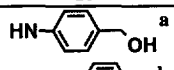
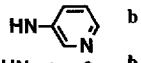
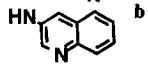
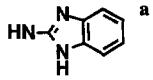
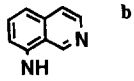
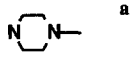
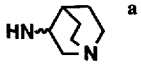
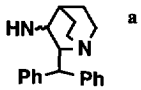
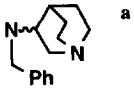


$K_{i\ trans}$ = 0.25 μM

Finally a series of analogs **27-35** were synthesized to determine the importance of the charged phosphonium group towards receptor affinity. The benzyl phosphonium moiety was replaced by a series of polar functional groups as listed in Table IV. Ultimately, we desired to replace the charged phosponium moiety because of suspected toxicology [9] and oral bioavailablity problems.[8] The decrease in potency observed for the set of monocharged compounds (**27 - 31**) indicated a critical role of a charged functionality in this position.

TABLE IV. Human IMR 90 binding affinities (K_i) for compounds **27-35**.



Compd	R	K_i (μM)
27	 [a]	4.95
28	 [b]	3.15
29	 [b]	1.30
30	 [a]	5.30
31	 [b]	2.54
32	 [a]	>25
33	 [a]	0.41
33a	diastereomer a [c]	0.21
33b	diastereomer b [c]	0.55
34	 [a]	17.9
35	 [a]	0.82

a: L-enantiomer
b: D-enantiomer
c: absolute stereochemistry was not determined.

Therefore, the charged benzyl phosphonium moiety was replaced by a functional group which would be charged at physiological pH. Compounds **32** and **33** were synthesized, each containing an amine which would be expected to be fully protonated. Compound **32** bound quite weakly to the receptor in contrast to the 1:1 diasteromeric mixture **33** which bound only 7-fold weaker to the receptor then **15**. Mixture **33** 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 with a $pA_2 = 6.9 \pm 0.6$. The quinuclidine and the tributyl phosphonium moieties both are expected to have a dispersed charge, rather than a point charge like an ammonium ion.[10] This may be the explanation as to why **33** binds to the receptor so much better than the amine **32**. The mixture **33** was separated by HPLC into its respective diastereomers **33a** and **33b**. Evaluation for receptor binding activity revealed that

33a bound about 2-fold better to the receptor than its diastereomer **33b** ($K_{i\ 33a}$ = 210 nM; $K_{i\ 33b}$ = 550 nM). Increasing the steric bulk around the charged quinuclidine nitrogen by substitution in the 2-position to give compound **34**, eliminated activity. However N-benzylation of the amine nitrogen producing **35** only resulted in a 2-fold loss of activity compared to **33**.

In conclusion we have successfully designed a series of potent non-peptide competitive antagonists of the human bradykinin B_2 receptor. The structure activity relationships which have emerged from this study point at the necessity of two charges separated by about 10 Å and surrounded by a critical amount of lipophilicity. A large aromatic group oriented in a specific way is required for high affinity binding. The essential charged residues separated by 10 Å and the hydrophobic aromatic group may mimic the terminal arginine residues and the hydrophobic phenylalanine (Phe^8) of the native ligand.

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