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1-Benzylbenzimidazoles: The discovery of a novel series of bradykinin B_1 receptor antagonists

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ABSTRACT

The design, synthesis, and structure–activity studies of a novel series of BK B_1 receptor antagonists based on a 1-benzylbenzimidazole chemotype are described. A number of compounds, for example, **38g**, with excellent affinity for the cynomolgus macaque and rat bradykinin B_1 receptor were discovered.

The kinins, bradykinin (BK) and kallidin (KD) and their metabolites [des-Arg^9]bradykinin (DABK) and [des-Arg^{10}]kallidin (DAKD) provoke a number of acute and chronic inflammatory pathways resulting in pain, edema and vasodilation.[1] These effects are mediated by the G-protein coupled receptors BK B_1 and B_2.[2] The BK B_2 receptor is constitutively expressed, whereas the BK B_1 receptor expression in peripheral tissues is induced following injury.[3] Recent studies in mice however, suggest that the BK B_1 receptor is constitutively expressed in the central nervous system, suggesting a potential central role for this receptor as well.[4] BK B_1 receptor null mice exhibit decreased inflammatory response and hyperalgesia supporting the hypothesis that BK B_1 receptor antagonists will be effective anti-inflammatory analgesic drugs. A number of selective, non-peptide, potent BK B_1 receptor antagonists have been described.[5] Early disclosures were dominated by relatively high molecular weight aryl sulfonamides including the extensively studied SSR240612[6] and NVP-SAA164.[7] Recently, a number of non-sulfonamide templates have emerged including the pyrazole **1**,[8] cyclopropane-carboxamide **2**[9] and the benzodiazepine **3**[10a] (Fig. 1). The biaryl **2** exhibited a large difference between its reported human and rat affinity whereas the benzodiazepine **3** displayed similar potency at both human and rat receptors. Planning to study the in vivo efficacy of the compounds prepared in the present study in rodent models of pain, the benzodiazepine **3** appeared to be an excellent starting point in this regard, although, the molecular weight of **3** was high (M_W = 601) and the oral bioavailability[10a] in rodent was low (F < 4%).[10b] We considered that replacement of the phenethylbenzodiazepine moiety of **3** by a lower molecular weight surrogate could lead to compounds potent at both human and rat BK B_1 receptors and with improved oral bioavailability.

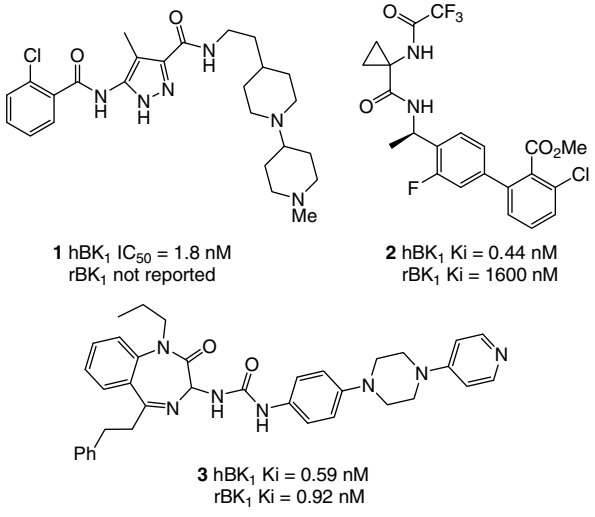

Figure 1. Structures of non-sulfonamide BK B_1 receptor antagonists.

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The benzimidazole core has been considered to be a privileged structure, being found in compounds that modulate a number of GPCRs and ligand-gated ion channels and in marketed drugs such as omeprazole.[11] We considered that the benzodiazepine core **4** could be replaced by a suitably substituted benzimidazole-2-carboxamide, for example, **5** (Fig. 2) and that exploration of the central linker and the basic tail (R) could lead to a novel series of BK B_1 receptor antagonists. Our endeavors in this regard are reported herein.

Potential alignments of low-energy conformers of the BK B_1 receptor antagonist **4** and the proposed new template **5** were first examined to better understand the relative dispositions of the pharmacophores of the two templates. The analyses were performed using the GASP algorithm.[12] The results were encouraging and revealed that there was significant, although by no means perfect, overlap between **4** and **5** with respect to several of the presumed key pharmacophore features over the entire surface of the two templates (Fig. 3). In particular, the pyridylpiperazine moiety at one end overlapped well. At the opposite end, the aromatic ring of the benzyl substituent of **5** matched with the aryl ring of the benzodiazepine, both in the location of the ring centroids and relative orientation of the π-planes. In the middle portion of the molecule, the imine nitrogen of benzimidazole overlapped with that of the benzodiazepine, while the oxygen of the carboxamide had good correspondence with that of the urea. Although the two sets of hydrogen bond acceptors are slightly displaced from each other, the lone pairs point in similar directions. Consequently, template **5** appeared to be a promising mimic of **4** in its ability to interact with the BK B_1 receptor.

The compounds described in the study were synthesized from the readily available 1-benzyl-benzimidazol-2-carboxylic acid (**6**)[13] as exemplified by the synthesis of the final compounds **7** and **9** (Scheme 1). 2-Chloro-1,3-dimethylimidazolinium chloride (DMC)[14] coupling of the acid **6** with commercially available 4-pyridylpiperazine directly gave the amide **7**. Alternatively, DMC or benzotriazolyloxytris(dimethylamino) phosphonium hexafluorophosphate (BOP) mediated coupling of the acid **6** with glycine ethyl ester followed by saponification with aqueous sodium hydroxide gave the acid **8**. A second amide coupling reaction with 4-pyridylpiperazine gave the target amide **9** in good overall yield. Following

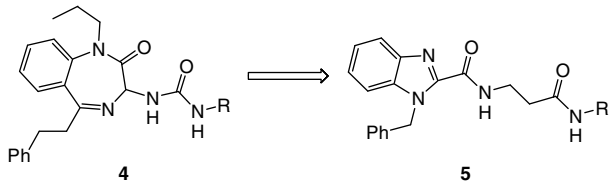


Figure 2. 1-Benzylbenzimidazoles as potential BK B_1 receptor antagonists.

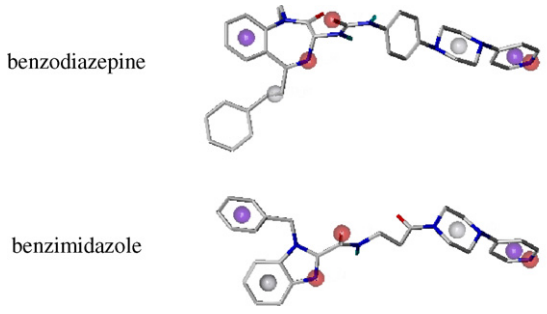
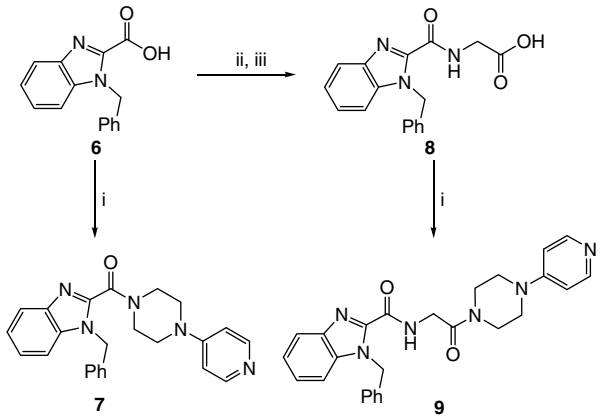


Figure 3. Common pharmacophore features in aligned low energy conformations of benzodiazepine and benzimidazole cores. Shared hydrogen bond acceptors are shown in red, centroids of aromatic rings in purple, and hydrophobic units in gray.

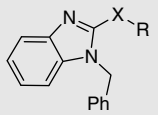
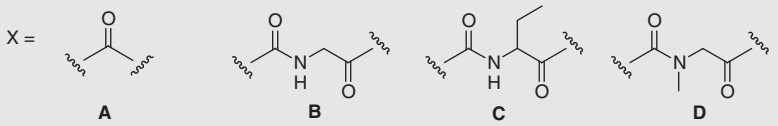
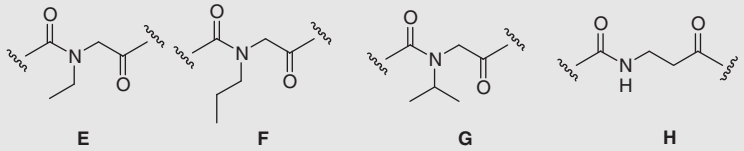
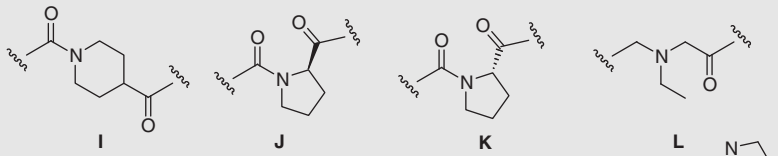
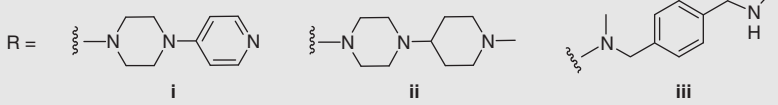


Scheme 1. Reagents and conditions: (i) DMC, DIEA, 4-pyridylpiperazine, MeCN (96%); (ii) DMC, DIEA, $NH_2CH_2CO_2Et$, MeCN (87%); (iii) NaOH, MeOH (95%).

analogous sequences to those outlined in Scheme 1, but using alternate amino esters as the linkers and the appropriate terminal amine moiety, the compounds described in Tables 1–3 were prepared. BK B_1 receptor antagonist potency was determined using cynomolgus macaque BK B_1 receptor-expressing CHO cells and a fluorescent imaging plate reader (FLIPR) to measure intracellular Ca^{2+} transients. Inhibition of 0.1 nM (approximate EC_{50} concentration) DAKD-induced fluorescence was measured at multiple compound concentrations to determine a functional IC_{50} at the BK B_1 receptor. Compound potency at the rat BK B_1 receptor was determined in an analogous manner. A number of analogues made during the study were also evaluated as potential substrates for the xenobiotic efflux pump P-glycoprotein (P-gp), this was important because a Pgp substrate may limit access to central nervous system BK B1 receptors which may be critical for analgesic activity.[4] The P-gp efflux ratio was assessed by calculating the ratio of the apparent permeability (P_{app}) in the basolateral to apical direction versus P_{app} in the apical to basolateral direction across a monolayer of MDR1 transfected MDCK cells.[15]

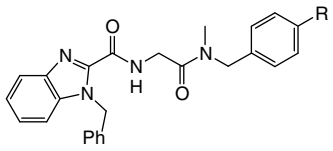
Given the basic character of the endogenous B_1 receptor ligands DABK and DAKD, and that of a number of reported small molecule BK B_1 ligands, we initially chose to keep the terminal amine region of the targets as 4-pyridylpiperazine, 1-(1-methyl-4-piperidin-4-yl)piperazine or *N*-methylbenzylimidazoline and varied the nature of the linker between this group and the benzimidazole. Direct attachment of 4-pyridylpiperazine or 1-(1-methyl-4-piperidin-4-yl)piperazine to the 2-carboxylbenzimidazole gave analogues **7** and **10**, which were inactive but the imidazoline analogue **11** was the first active compound in the series, albeit in the low micromolar range. Attachment of the amines to the benzimidazole via a glycine linker, however, gave compounds with sub micromolar activity (**9**, IC_{50} = 310 nM and **12**, IC_{50} = 686 nM) and in the case of the imidazoline **13** potency under 10 nM. To capitalize upon this promising activity, the effects of substitutions to the glycine linker were examined (compounds **14–24**). C-alkylation brought about a loss in activity, for example, **14** and **15** and N-methylation resulted in a loss in activity for the 4-pyridyl analogue **16** but a boost in activity for the 4-methylpiperidine analogue **17** relative to their parent analogues **9** and **12**. The *N*-ethyl and *N*-propyl analogues, however, were considerably more active, in particular the 4-methylpiperidine analogues **19** and **22** (IC_{50} = 16 and 18 nM, respectively), while the imidazoline **20** was as active as its un-alkylated parent (IC_{50} = 6 nM). Branching of the alkyl chain, for example, **23** and **24** resulted in a significant reduction in potency. Increasing the length of the linker (e.g., β-alanine analogues **25** and **26**) rendered compounds of similar activity to their glycine counterparts, whereas imidazoline **27** was an order of magnitude weaker. Con-

Table 1
Effects of the linker (X) and the amine tail (R) on *c*BK B_1 receptor potency

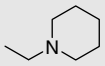
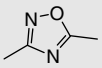
Compound	X	R	*c*BK B_1 IC_{50}[a] (nM)	*r*BK B_1 IC_{50}[a] (nM)	P-gp[b]	P_{app}[c] (10^{-6} cm/s)	Compound key
7	A	i	>5000				
10	A	ii	>5000				
11	A	iii	1016				
9	B	i	310	417			
12	B	ii	686				
13	B	iii	5	155	23	1.0	
14	C	i	>5000				
15	C	ii	1303				
16	D	i	1176				
17	D	ii	268				
18	E	i	278				
19	E	ii	16	15	55	0.6	
20	E	iii	6.0	345			
21	F	i	355	396			
22	F	ii	18	23			
23	G	i	>5000				
24	G	ii	465				
25	H	i	962				
26	H	ii	601				
27	H	iii	54				
28	I	i	2311				
29	I	ii	4021				
30	I	iii	188				
31	J	i	4074				
32	J	ii	3219				
33	K	i	>5000				
34	K	ii	884				
35	L	i	>5000				
36	L	ii	>5000				

[a] IC_{50} values are means of at least two determinations.
[b] MDR1 directional transport ratio (B to A)/(A to B).
[c] Passive permeability (A to B).

Table 2
Imidazoline bioisosteres



37a-l

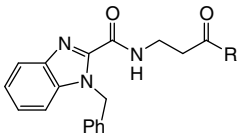
Compound	R	*c*BK B_1 IC_{50}, nM[a]	Compound	R	*c*BK B_1 IC_{50}, nM[a]
37a		>5000	**37g**		407
37b		>5000	**37h**		330
37c	CN	>5000	**37i**		196
37d		14	**37j**		1167
37e		718	**37k**		2130
37f		626	**37l**		>5000

[a] IC_{50} values are means of at least two determinations.

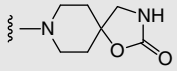
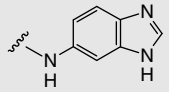
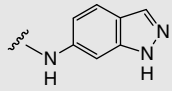
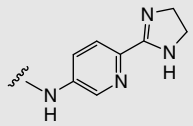
straint of the linker into a cyclic system (compounds **28**–**34**) gave a large decrease in activity and the importance of the 2-carbonyl was underscored by the lack of activity observed for the 2-methylene analogues **35** and **36**. A number of the compounds were further characterized. The pyridine **9** was evaluated at the rat receptor and encouragingly was found to be of similar potency (rBK_1 IC_{50} = 417 nM) as at the cynomolgus macaque receptor. The 4-methylpiperidine **19** was also of similar potency at the rat receptor (rBK_1 IC_{50} = 15 nM) as at the cynomolgus macaque receptor. Despite a relatively high molecular weight (M_W = 502), compound **19** had excellent aqueous solubility (120 μg/mL at pH 7.4) and was also found to be stable in rat liver microsomes.[16] The pharmacokinetic properties of **19** were examined in rodents (10 mpk PO, 0.5% MC/0.1% TA) but poor oral exposure (F < 5%) was observed. In addition, compound **19** demonstrated both poor permeability [P_{app} = 0.6 (10^{-6} cm/s)] and a potential P-gp liability (BA/AB = 55). The imidazolines **13** and **20** were also evaluated at the rat receptor, and in contrast to the 4-pyridine and 4-methylpiperidine analogues (**9**, **19**, **21**, and **22**) were found to be considerably weaker (rBK_1 IC_{50} = 155 and 345 nM, respectively) than at the cynomolgus receptor. Compound **13** also had a potential P-gp liability (BA/AB = 23). To confirm that **13** was a P-gp substrate, the compound was also tested in the presence of 25 μM cyclosporine, a potent P-gp inhibitor.[17] An improvement in both apparent permeability [P_{app} = 11 (10^{-6} cm/s)] and efflux ratio (BA/AB = 1.5) was the result, confirming **13** to be a P-gp substrate.

To capitalize on the excellent cynomolgus macaque receptor potency observed for the imidazoline analogues, and in an attempt to increase rat receptor potency and reduce P-gp liability, a number of bioisosteres for the imidazoline were prepared (Table 2, compounds **37a–l**). Examples included basic, acidic and neutral heterocycles but a significant reduction in potency was the result of all modifications of this type. Examination of the 4-cyano analogue **37c** (which lacks a basic amine moiety) in MDR1 transfected MDCK cells revealed reduced P-gp susceptibility (BA/AB = 3) and reasonable permeability [P_{app} = 7 (10^{-6} cm/s)] indicating, that at least in part, the P-gp liability of the series was related to the presence of a basic moiety in the molecule.

Table 3
β-Alanine linked analogues



38a-g

Compound	R	*c*BK B_1 IC_{50}[a]
38a		>5000
38b		1990
38c		2912
38d		>5000
38e		744
38f		524
38g		2

[a] IC_{50} values are means of at least two determinations.

The β-alanine linked compounds **25**–**27** had shown encouraging *in vitro* activity and a number of additional analogues with this linker were also prepared (Table 3). The spirocyclic and bicyclic analogues **38a–f** had weak activity but the 2-imidazoline-5-aminopyridine analogue **38g** had excellent cynomolgus potency (IC_{50} = 2 nM) and in contrast to the benzyl linked imidazolines (e.g., **13** and **20**) a similar activity in the rat assay (rBK_1 IC_{50} = 0.8 nM). As with all previous imidazolines, compound **38g** had a high P-gp susceptibility (BA/AB = 42).

In summary, the hypothesis of replacing the benzodiazepine core of a known BK B_1 receptor antagonist was investigated and a novel series of benzimidazoles were discovered. The 2-carboxyl group was important for activity and a number of different linkers and amine groups were studied. Pyridine and piperidine moieties displayed little difference between cynomolgus and rat activity (e.g., **9**, **19**, **21**, and **22**) but the analogues with phenyl-imidazoline moieties were generally an order of magnitude weaker at the rat receptor (e.g., **13** and **20**). The combination of β-alanine linker and 2-imidazoline-5-aminopyridine group, however, led to the

identification of a compound **38g** of excellent potency at both cynomolgus and rat receptors. A number of compounds were assessed in MDR1 transfected MDCK cells and found to be P-gp substrates. The hydrogen bond acceptor located in the tail of these compounds appeared to facilitate the efflux. Further optimization of the benzimidazole series with respect to improving pharmacokinetic properties will be reported in due course.

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16. One hundred percent parent compound remaining after 10 min; compounds at 1 μM concentration were incubated at 37 °C in the presence of 0.5 mg/ml male pooled rat liver microsomes and NADPH was added to commence the reaction. After 0 and 10 min aliquots were sampled and reactions terminated by dispensing into ice cold acetonitrile with an internal standard. Samples were centrifuged for 15 min at 3000 rpm and analyzed by LC–MS using electrospray ionization detection. The disappearance of parent compound was determined by monitoring the M+1 ion in selective ion mode. The percent parent remaining was calculated by the ratio of the signal response at 10 min with that of the 0 min and corrected by using the internal standard recovery.
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