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5-Piperazinyl pyridine carboxamide bradykinin B_1 antagonists

Scott D. Kuduk,[a,*] Christina Ng Di Marco,[a] Ronald K. Chang,[a] Michael R. Wood,[a] June J. Kim,[a] Kathy M. Schirripa,[a] Kathy L. Murphy,[b] Richard W. Ransom,[b] Cuyue Tang,[c] Maricel Torrent,[d] Sookhee Ha,[e] Thomayant Prueksaritanont,[c] Douglas J. Pettibone[b] and Mark G. Bock[a]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, Sumneytown Pike, PO Box 4, West Point, PA 19486, USA*
[b]*Department of Neuroscience Drug Discovery, Merck Research Laboratories, Sumneytown Pike, PO Box 4, West Point, PA 19486, USA*
[c]*Department of Drug Metabolism, Merck Research Laboratories, Sumneytown Pike, PO Box 4, West Point, PA 19486, USA*
[d]*Molecular Systems, Merck Research Laboratories, Sumneytown Pike, PO Box 4, West Point, PA 19486, USA*
[e]*Basic Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*

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Abstract—A series of 2,3-diaminopyridine bradykinin B_1 antagonists was modified to mitigate the potential for bioactivation. Removal of the 3-amino group and incorporation of basic 5-piperazinyl carboxamides at the pyridine 5-position provided compounds with high affinity for the human B_1 receptor.

The production of bradykinin (BK) peptides transpires subsequent to tissue injury and noxious stimuli resulting in a variety of physiological effects, including pain and inflammation.[1] There are two distinct G-protein-coupled bradykinin receptors, designated as B_1 and B_2, that regulate these effects.[2] The constitutively expressed B_2 receptor mediates the acute pain response following injury and is activated by the peptides bradykinin (BK = Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) and kallidin (Lys-BK). Their corresponding metabolites, [des-Arg^9]BK and [des-Arg^{10}]kallidin, are substrates for the inducible B_1 receptor.[3] Bradykinin B_1 receptor antagonists have been shown to ameliorate pain responses in animal models[4] indicating the potential for treating inflammatory pain such as osteoarthritis via a novel mechanism.[5] Additional evidence for the function of B_1 antagonists has been obtained from studies in both B_1 receptor knockout mice and, more recently, in transgenic mice expressing the human B_1 receptor.[6]

We have previously described the preparation and biological evaluation of a series of non-peptide, 2,3-diaminopyridine BK B_1 antagonists.[7] These compounds exhibited excellent affinity for the human B_1 receptor (hBK_1), suitable pharmacokinetic properties, and good in vivo efficacy in rabbit models of hyperalgesia and inflammation. However, evidence that 2,3-diaminopyridines such as **1** are subjected to bioactivation as depicted in Figure 1 impaired their development.[8] Herein, we report our efforts to modify the 2,3-diaminopyridine nucleus to yield analogs which retain the beneficial properties of their progenitors.

The design rationale was based on the premise that removal or transposition of the 3-amino group on the diaminopyridine nucleus would lead to analogs incapable of forming reactive intermediates via the bioactivation route shown in Figure 1. Thus, we were pleased to discover that removal of the 3-cyanoacetamide side chain yielded a truncated analog which still retained modest affinity for the human B_1 receptor (Fig. 2). This result held out the prospect that the A-ring in **2** is a promising scaffold for further exploration.

Scheme 1 shows the route employed to prepare the 4-substituted pyridine derivatives of Table 1. Negishi

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* Corresponding author. E-mail: scott_d_kuduk@merck.com

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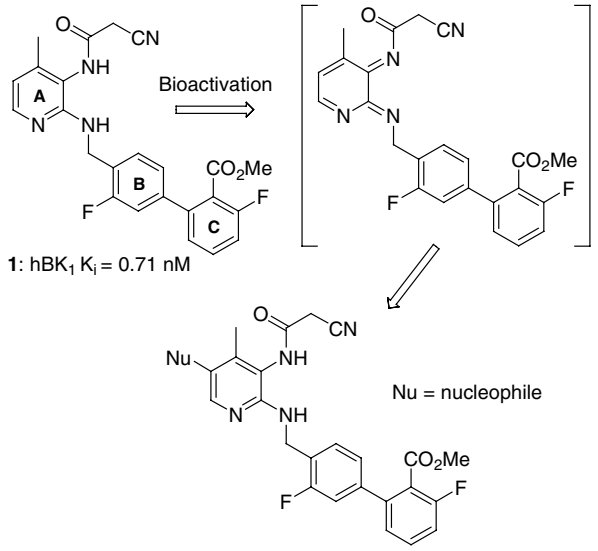


Figure 1. Bioactivation route of 2,3-diaminopyridines.

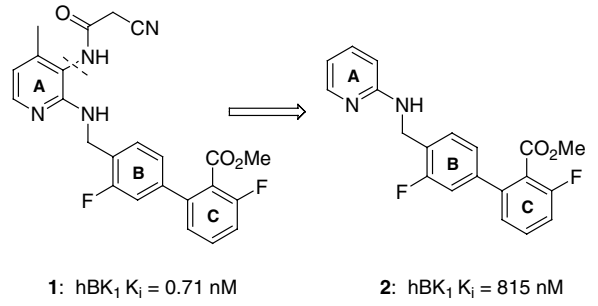
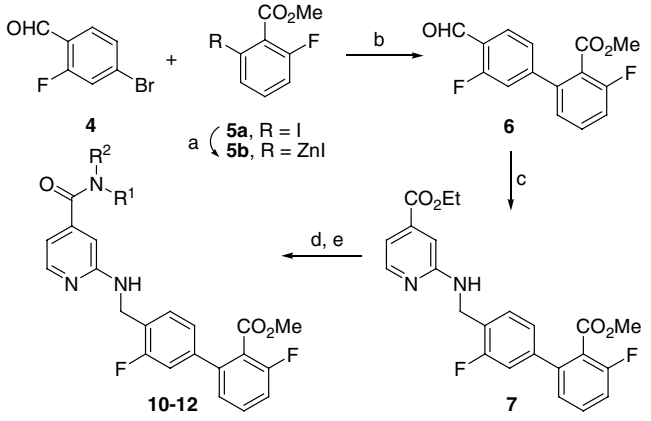
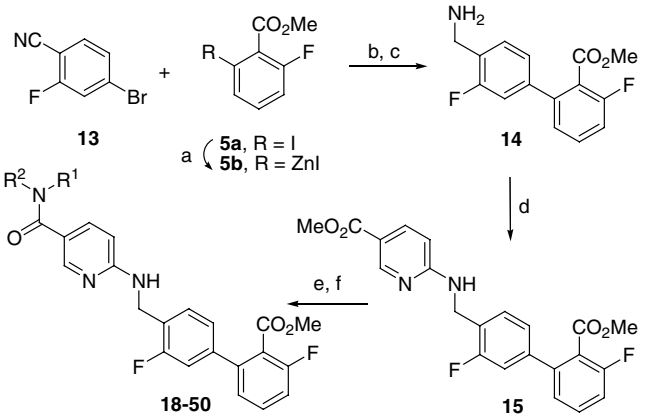


Figure 2. Modification of lead B_1 antagonist **1**.



Scheme 1. Reagents and conditions: (a) Rieke Zn, THF, 60 °C; (b) $Pd(Ph_3P)_4$, 60 °C, THF; (c) 2-amino-4-carboxyethylpyridine, $NaBH(OAc)_3$, AcOH, DCE; (d) NaOH, THF, H_2O; (e) EDCI, TEA, R_2R_1NH, HOBt, CH_2Cl_2.

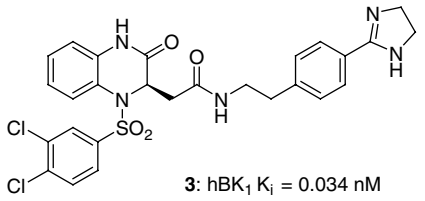
cross-coupling between bromide **4** and zinc reagent **5b** provided the requisite biphenyl aldehyde **6**. Reductive amination of **6** with 2-amino-4-carboxyethylpyridine led to ester **7**. Selective hydrolysis of the ethyl ester and subsequent EDCI mediated coupling with the appropriate amines provided compounds **10**–**12**.[9]



Scheme 2. Reagents and conditions: (a) Rieke Zn, THF, 60 °C; (b) $Pd(Ph_3P)_4$, 60 °C, THF; (c) Raney Ni, H_2, NH_3–MeOH; (d) methyl 6-chloronicotinate, TEA, MeOH, 110 °C; (e) NaOH, THF, 60 °C; (f) EDCI, TEA, amine, HOBt, CH_2Cl_2.

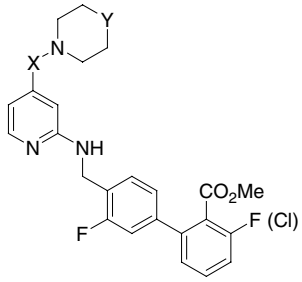
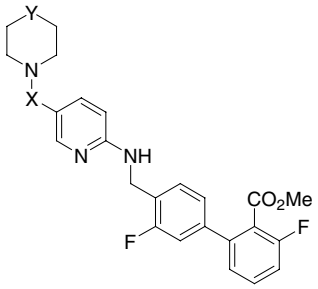
The preparation of 5-substituted pyridine derivatives of Tables 1 and 2 is illustrated in Scheme 2. Negishi cross-coupling of bromide **13** with zincate **5b** proceeded smoothly and was followed by nitrile reduction to afford amine **14**. Displacement of the chlorine of methyl 6-chloronicotinate with amine **14** provided ester **15**. Subsequent hydrolysis and EDCI coupling produced the desired 5-substituted analogs **18**–**50**.

In an earlier report, we disclosed a series of dihydroquinoxalinones, exemplified by **3**, whose optimization was facilitated by a theoretical study using a BK B_1-rhodopsin homology model.[10] A key finding in the latter study was that incorporation of a basic moiety led to enhanced affinity for the human B_1 receptor. Analogous studies with aminopyridine **2** (vide infra) indicated that a basic pharmacophore extending from the 4- or 5-position on the pyridine ring could potentially lead to similarly favorable interactions.



Accordingly, the pyridine A-ring of **2** was substituted with a variety of piperidine and piperazine derivatives. The compounds in Table 1 show that human B_1 receptor binding affinities are influenced by the position, as well as the nature of the linking unit. For example, a piperidine ring linked to the 4-position of the pyridine core via a methylene unit (**8**) showed a modest increase in receptor affinity relative to **2**, but attachment at the 5-position afforded the more potent compound **16**. A 20-fold decrease in affinity was observed when a piperidine ring was replaced with a morpholine ring at the 5-position. 5-Position substitution was also favored when the linking unit was changed to an amide (cf. **10** and **18**). Overall, substitution at the 4-position for this series of

Table 1. Effect of linker at the 4- or 5-position on receptor affinities

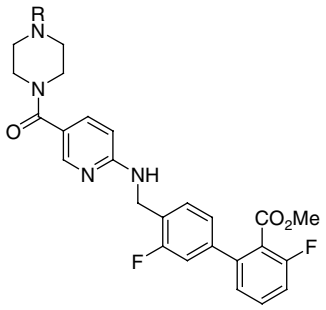
Compound	X	Y	hK_i[b] (nM)
 **8-12**			
8[a]	CH_2	CH_2	611
9[a]	CH_2	O	>3000
10	CO	CH_2	>3000
11	CO	O	2100[c]
12	CO	NCH_3	893
 **16-20**			
16	CH_2	CH_2	9.1
17	CH_2	O	184[c]
18	CO	CH_2	70[c]
19	CO	O	80[c]
20	CO	NCH_3	4.4

[a] Compound has a chlorine ortho to the ester in place of fluorine.
[b] Values represent the average of two experiments; standard deviation is ±25%.
[c] Denotes $n = 1$.

compounds either had minimal effect or led to loss in binding affinity, whereas the addition of substituents to the 5-position yielded analogs with improved potency. The *N*-methyl piperazinyl amide **20** emerged as a key lead compound from this first phase of optimization.

Extended SAR work on compound **20** was centered on evaluating piperazine nitrogen substituents (Table 2). Acylation or sulfonylation (**21**–**24**) led to significant decreases in potency indicating a preference for the basic piperazine nitrogen. With regard to unsubstituted alkyl groups (**25**–**32**), a trend favoring larger groups became evident. However, it appears that the *i*-butyl group in **30** is optimum since the mere insertion of a methylene unit to give the homologous isopentyl group (**31**) resulted in a 6-fold reduction in affinity. While fluoroethyl piperazine **37** was equipotent with ethyl piperazine **26**, the difluoro-(**38**), and trifluoroethyl analogs (**39**) were less potent, which was consistent with the decreased basicity of the corresponding piperazine nitrogen.

Table 2. Effect of piperazine *N*-substituent on receptor affinity



Compound	R	hK_i[a] (nM)
21	*t*-BuCO	213[b]
22	Ac	17[b]
23	Ms	23[b]
24	CO_2Me	34[b]
25	H	5.5
26	Et	3.4
27	Pr	1.7
28	Bu	1.4
29	*i*-Pr	2.2
30	*i*-Bu	1.5
31	*i*-Pent	9.5
32	2-Bu	1.5
33	Crotyl	3.3
34	Allyl	8.0[b]
35	Homoallyl	4.0
36	Propargyl	25[b]
37	CH_2CH_2F	5.2
38	CH_2CHF_2	28[b]
39	CH_2CF_3	52[b]
40	*c*Bu	2.9
41	*c*Pent	1.5
42	*c*Hex	2.8
43	CH_2*c*Pr	0.85
44	CH_2*c*Bu	3.4
45	CH_2*c*Pent	1.7
46	Benzyl	26
47	Phenyl	62[b]
48	2-Pyridyl	65[b]
49	3-Pyridyl	3.5
50	4-Pyridyl	0.045

[a] Values represent the average of two experiments.
[b] Denotes $n = 1$.

Further receptor binding potency improvements of **28** were probed with cycloalkane (**40**–**42**), cycloalkylmethyl (**43**–**45**), *N*-benzyl (**46**), and *N*-aryl (**47**–**50**) substituents. In all instances, the receptor potency ceiling established by **28** could not be significantly superseded. However, the judicious placement of a nitrogen atom into **47** yielded the 4-pyridyl analog **50**, the most potent analog to be identified among the pyridinecarboxamide BK B_1 antagonists.

To address the potential for bioactivation of the pyridine carboxamides, diaminopyridine **1** and compound **50** were incubated in rat and human liver microsomes. As predicted, diaminopyridine **1** underwent extensive metabolism after incubation with human liver microsomes (HLM) and rat liver microsomes (RLM) (supplemented with NADPH and glutathione (GSH)), and produced a number of GSH-adducts as major metabo-

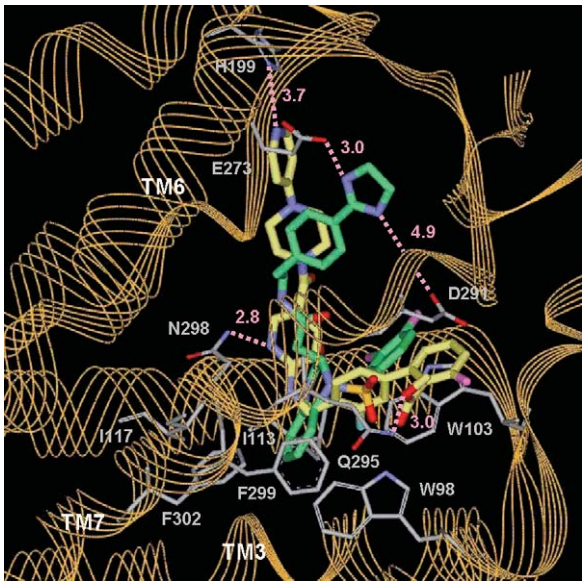


Figure 3. Compounds **3** (green) and **50** (yellow) bound to the homology model of the human B_1 receptor.

lites. Based upon mass spectral analysis, the formation of these conjugates appeared to involve modification of the diaminopyridine ring. However, compound **50** was more resistant to metabolism and formation of GSH-adducts was not detected, indicating that the metabolic pathway for **50** is unlikely to include a reactive pyridine intermediate (cf. Fig. 1).

Since the optimization of compound **3** was facilitated by modeling studies,[10,11] the activity of **50** may be rationalized in similar terms. As seen in Figure 3, the southern hydrophobic portion of the receptor binding site accommodates both the biphenyl group of **50** and the dihydroquinoxalinone moiety of **3**. Whereas the phenylimidazoline moiety of **3** reaches residues Glu273

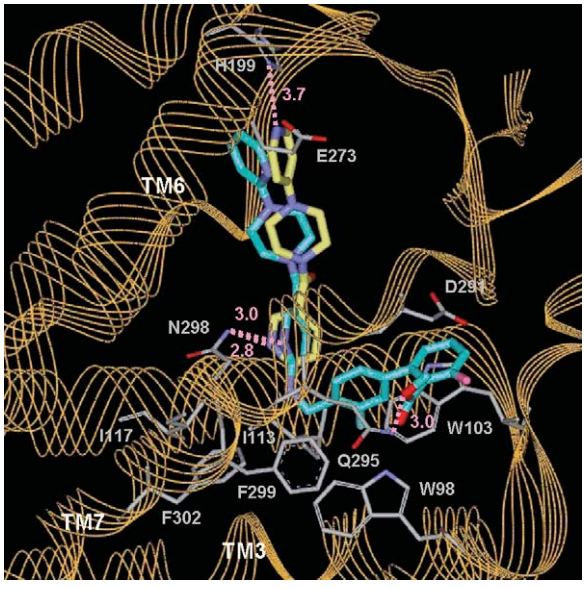


Figure 4. Compounds **48** (magenta) and **50** (yellow) bound to the homology model of the human B_1 receptor.

Table 3. Bradykinin mutant binding affinities

Mutant[a]	**48**	**50**	**3**
WT	79	0.041	0.034
E273	286	0.05	0.0078
D291	85	0.08	0.049
Q295	10,000	5.12	0.89
N298	726	0.83	0.038

[a] Human K_i values (nM) represent the average of three experiments.

(3.0 Å) and Asp291, compound **50** is too 'long'. Accordingly, the 4-pyridyl tail is positioned toward His199.

Docking of the 2-pyridyl analog, **48**, in the homology model (Fig. 4) further illustrates the binding advantage of compound **50** over other analogs in Table 2. Compound **48** lacks the ability to bind to His199 and no other residue can compensate for the polar interaction seen in **50**.

In support of the modeling studies, mutagenic binding experiments were conducted. Residues Glu273, Asp291, Gln295, and Asn298 were selected to confirm that the piperazine series would use the northern pocket differently than compound **3** (Table 3).

Mutations at positions 273 and 291 would not be expected to significantly alter the potency of compounds **48** and **50**. Consistent with the model, the potency of **50** in the wildtype (wt) remains within the same order of magnitude for these mutants. Mutation of residue Gln295, located at the bottom of the binding pocket, renders both **3** and **50** incapable of making good interactions (as in the wt) leading to a dramatic reduction in potency. The proposed models show that compound **3** does not make any direct interaction with Asn298, consistent with the mutant data. On the other hand, both **48** and **50** pair their pyridine N with residue 298. Such a polar interaction becomes weaker when a shorter Ser replaces the original Asn leading to the slight decrease in potency for both **48** and **50.**

To address the potential for bioactivation of the 2,3-diaminopyridine ring, the attendant 3-cyanoacetamide group in compound **1** was deleted to afford a scaffold for further exploration. Incorporation of a 5-piperazinylpyridine pharmacophore dramatically increased affinity for the human B_1 receptor and gave compound **50** which did not form detectable GSH-adducts when incubated with NADPH–fortified HLM and RLM.

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