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Bradykinin B_1 antagonists: SAR studies in the 2,3-diaminopyridine series

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Dedicated to Professor Iwao Ojima on the occasion of his 60th birthday.

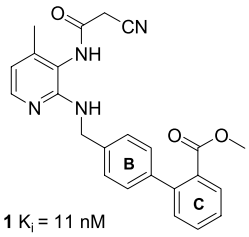
Abstract—SAR study of the biphenyl region of 2,3-diaminopyridine bradykinin B_1 antagonists was investigated with non-aromatic carbo- and heterocyclic rings. A piperidine ring was found to be a good replacement for the proximal phenyl ring while replacement of the distal phenyl was optimal with a cyclohexyl group leading to a dramatic improvement in affinity for the B_1 receptor.

1. Introduction

Bradykinin (BK) peptides are rapidly formed in plasma after tissue injury and exert a variety of physiological effects, such as pain and inflammation.[1] Two known G-protein-coupled receptors, designated as B_1 and B_2, regulate these effects.[2] The constitutively expressed B_2 receptor is believed responsible for the immediate acute pain response following tissue injury and is mediated by the peptides, bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) and kallidin (Lys-BK). Their corresponding metabolites, [des-Arg9]bradykinin and [des-Arg10]kallidin, are agonists for the B_1 receptor, which is induced in the hours following the injury.[3]

Bradykinin B_1 receptor agonists have been shown in animal models to produce hyperalgesia, which is blocked by peptide-derived B_1 antagonists.[4,5] Also, transgenic B_1 receptor knockout mice exhibit reduced sensitivity to painful stimuli, while appearing normal in all other respects. In addition to peripheral B_1 receptors, a central role for the B_1 receptor has been implied based on evidence that it is constitutively expressed in the central nervous systems of rats and mice.[6–8] Thus, bradykinin B_1 receptor antagonists are considered good prospects as novel therapeutic agents for the treatment of chronic pain and inflammation.[9]

We recently disclosed the preparation and biological evaluation of a series of non-peptide, 2,3-diaminopyridine bradykinin B_1 antagonists (Fig. 1).[10] These compounds displayed excellent selectivity and exhibited good human bradykinin B_1 antagonist activity. The purpose of this communication is to report the results of an expanded SAR study to identify alternative biphenyl 'isosteres' that exhibit enhanced receptor affinities and improved physical properties relative to lead compound **1**.



1 K_i = 11 nM

Figure 1. Lead diaminopyridine bradykinin B_1 antagonist **1**.

Keywords: Bradykinin; Diaminopyridine.
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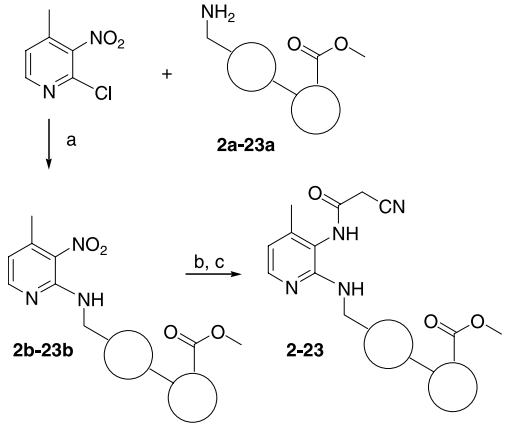
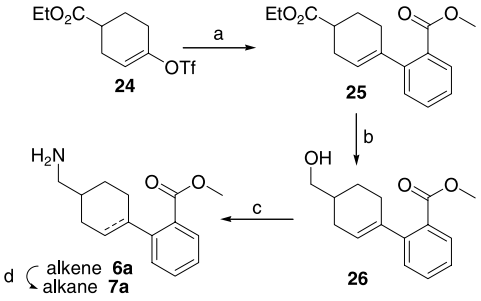
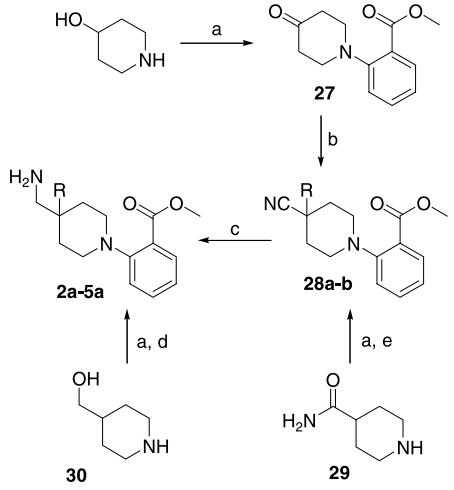


Scheme 1. Reagents and conditions: (a) TEA, THF, 70 °C; (b) $SnCl_2$, MeOH, 60 °C; (c) cyanoacetic acid, EDC, HOBt, DMF, TEA.

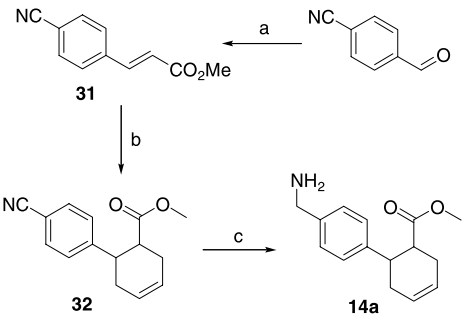
Scheme 1 outlines the general route employed for preparing the 2,3-diaminopyridine derivatives highlighted in this report. Displacements of the chlorine in 2-chloro-3-nitro-4-methylpyridine with the appropriate amines **2a**–**23a** (Schemes 2–7), were carried out in THF in the presence of TEA. Reduction of the nitro group was accomplished with $SnCl_2$ in MeOH followed by an EDC-mediated coupling of the resultant amine with cyanoacetic acid to afford test compounds **2**–**23**.



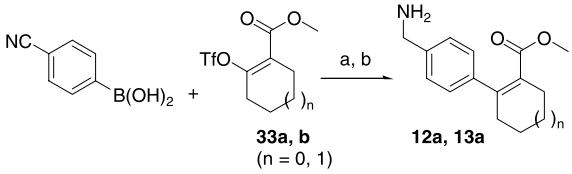
Scheme 2. Reagents and conditions: (a) $Pd(Ph_3P)_4$, 2-IZn-methylbenzoate, THF, 70 °C; (b) (i)—NaOH, THF; (ii)—BH_3; THF, 0 °C; (c) (i)—MsCl, TEA, CH_2Cl_2; (ii)—NaN_3, DMF; (iii)—Ph_3P, THF-H_2O; (d) Pd/C, EtOAc, H^2.



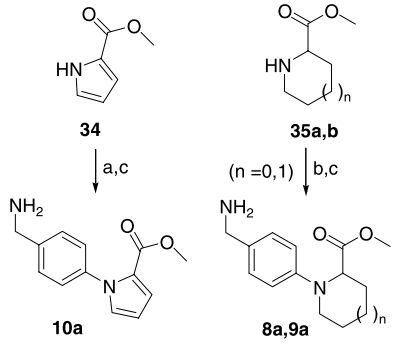
Scheme 3. Reagents and conditions: (a) (i)—2-Fluoromethyl benzoate, K_2CO_3, DMSO, 120 °C; (ii)—TPAP–NMO, CH_2Cl_2; (b) TMSCN, ZnI_2, DMF; (c) RaNi, NH_3–MeOH, H_2; (d) (i)—MsCl, TEA, CH_2Cl_2; (ii)—NaN_3, DMF; (iii)—Ph_3P, THF-H_2O; (e) (i)—TFAA, TEA, CH_2Cl_2; (ii)—LiHMDS, MeI, THF, 0 °C.



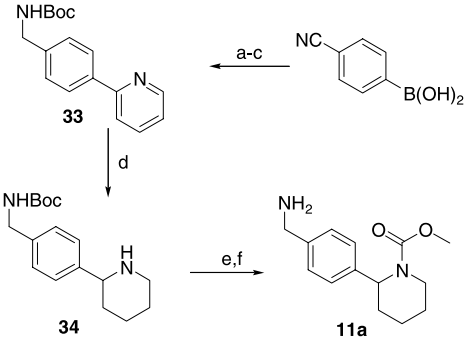
Scheme 4. Reagents and conditions: (a) Ph_3PCHCO_2Me, toluene, 80 °C; (b) butadiene, toluene, 180 °C, 3–7 days; (c) RaNi, H_2, NH_3–MeOH.



Scheme 5. Reagents: (a) $Pd(OAc)_2$, Ph_3P, K_2CO_3, THF; (b) RaNi, NH_3–MeOH, H_2.



Scheme 6. Reagents and conditions: (a) 4-Fluorobenzonitrile, NaH, DMSO, 60 °C; (b) 4-fluorobenzonitrile, K_2CO_3, DMSO, 120 °C; (c) RaNi, NH_3–MeOH, H_2.



Scheme 7. Reagents: (a) $Pd(OAc)_2$, 2-bromopyridine, Ph_3P, K_2CO_3, THF; (b) RaNi, NH_3–MeOH, H_2; (c) Boc_2O, CH_2Cl_2; (d) EtOH, $Pd(OH)_2$, H_2 (60 psi); (e) $ClCO_2Me$, TEA, CH_2Cl_2; (f) HCl (g) EtOAc.

Scheme 2 shows the preparation of amines in which the B-ring phenyl is replaced with cycloalkane rings. Negishi coupling of triflate **24** with the corresponding zinc reagent afforded **25**. Selective hydrolysis of the ethyl ester was followed by reduction to the alcohol with borane. Subsequent conversion of **26** to the amine and reduction of the olefin produced the requisite amines.

Compounds bearing a B-ring piperidine in place of phenyl were prepared as shown in Scheme 3. Heating 2-fluoromethyl benzoate with 4-piperidin-ol followed by oxidation afforded **27**. Conversion to the cyanohydrin **28a** ($R = OH$) and reduction with Raney Nickel afforded **4a**. In a similar fashion, piperidines **29** and **30** could be transformed to the desired amines.

Two routes were employed for preparation of compounds in which the C-ring phenyl is replaced with cycloalkyl derivatives. The first general example is shown in Scheme 4. A thermal Diels–Alder reaction of cinnamate **31** with butadiene afforded the necessary *trans*-4,5-cyclohexene **32**. Reduction of the nitrile with Raney Nickel in NH_3–MeOH produced the desired amine **14a** without reduction of the olefin.[11] Employing the *cis*-cinnamate afforded the *cis*-cyclohexene, while cyclopentadiene or cyclohexadiene were used as the other dienes to afford the bicyclic analogs (not shown for amines **15a**, **19a**–**22a**).

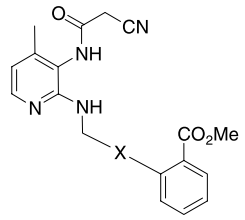
The second route employed a Suzuki coupling of the appropriate triflates **33a** and **b** with 4-cyanophenylboronic acid followed by reduction to produce **12a** and **13a** (Scheme 5).

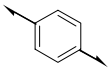
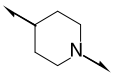
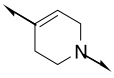
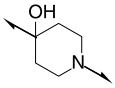
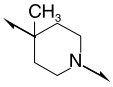
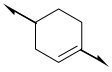
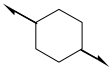
In Scheme 6 is outlined the synthesis of the amines used to prepare compounds **8**–**10** in which the C-ring phenyl is replaced with an amine heterocycle. Nucleophilic displacement of 4-fluorobenzonitrile with the appropriate nitrogen nucleophile was followed by reduction to afford the desired amines **8a**–**10a**.

Scheme 7 illustrates the preparation of the amine used for the synthesis of **11**. Suzuki coupling of 2-bromopyridine with 4-cyanophenyl boronic acid was followed by reduction of the nitrile and Boc protection to yield biaryl **33**. The HCl salt of the pyridine was then reduced with Pearlman's catalyst to provide piperidine **34**. The piperidine nitrogen was acylated with methyl chloroformate and Boc removal afforded **11a**.

The central phenyl B-ring in **1** was replaced with several alternative ring systems as shown in Table 1. Replacement of the phenyl with a 4-piperidine ring afforded **2**, which exhibited equivalent bradykinin B_1 receptor affinity and lower log *P* and human plasma protein binding compared to **1**. The piperidine proved very sensitive to further modification as all other derivatives which were tested had reduced potency. For example, introduction of an olefin (**3**) led to a 10-fold loss in affinity while addition of a hydroxy or methyl group on the piperidine further decreased receptor binding affinity (entries **4**–**5**).

Table 1. Human bradykinin B_1 receptor affinities and physical properties for B-ring phenyl replacements



Compounds	X	*h* K_i (nM)[a]	log *P*	PB[b] (%)
1		11	2.5	99
2		13	2.1	93
3		130	nd	nd
4		>5000	2.1	65
5		3500	2.6	90
6		25	2.5	99
7[c]		385	2.7	nd

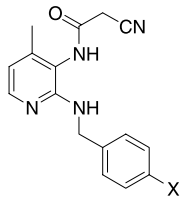
[a] Values represent the average of three experiments; standard deviation is ±25%.
[b] Protein binding measured using 10% human serum.
[c] Approximately 2:1 mixture of *cis*:*trans* isomers.

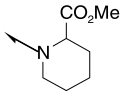
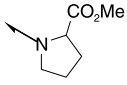
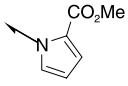
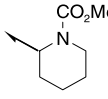
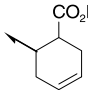
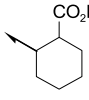
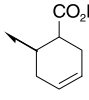
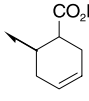
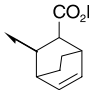
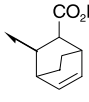
The piperidine plays a key role as replacement of **2** with a cyclohexene (**6**) led to ~2-fold reduction in human B_1 receptor affinity, while the cyclohexane **7** led to a >30-fold reduction. Overall, piperidine **2** was the best replacement for phenyl as it provided an equipotent compound with more desirable physical properties. The reduced protein binding is of particular interest as significant protein shifts have complicated in vivo studies.[10b]

The exchange of the C-ring with alternative ring systems was examined (Table 2). The aryl C-ring proved to be less amenable to replacement. For example, although a piperidine ring (**2**) proved to be an effective replacement for the phenyl, compound **8** bearing a C-ring piperidine in place of the phenyl was devoid of B_1 receptor binding affinity. The related pyrrolidine derivative **9** was also devoid of potency.

However, pyrrole **10** retained substantial potency indicating that a planar or aromatic ring is preferred. Moving the piperidine to the 2-position as in **11** also afforded an inactive compound. It appears that conformational requirements of the C-ring are more stringent than the B-ring.

Table 2. Human bradykinin B_1 receptor affinities and physical properties for C-ring phenyl replacements



Compounds	X	*h* K_i (nM)[a]	log *P*	PB[b] (%)
1		11	2.5	99
8		>5000	2.7	78
9		>5000	nd	nd
10		250	2.4	81
11		>5000	nd	nd
12		35	3.0	96
13		126	nd	nd
14 (± *trans*)		0.83	2.7	96
15 (± *cis*)		7	3.3	96
16 (± *trans*)		2.2	2.7	95
17 (+)		1.3	2.3	nd
18 (−)		1.0	2.4	nd
19 (± *trans* exo)		38	nd	nd
20 (± *trans* endo)		142	3.1	nd
21 (± *trans*)		54	3.4	nd
22 (± *trans*)		730	nd	nd

[a] Values represent the average of three experiments, standard deviation is ±25%.
[b] Protein binding measured using 10% human serum.

The most promising results were obtained with the C-ring carbocyclic derivatives. The 1,2-cyclohexene derivative **12** showed ~3-fold drop in potency compared to **1**, and the related 1,2-cyclopentene was even less potent indicating the preference for the six-membered ring. Relocation of the olefin to provide the *trans*-4,5-cyclohexene analog **14** led to a dramatic increase in potency. Racemic compound **14** exhibited a K_i of 0.83 nM, approximately 10-fold greater than **1**. The closely related racemic *cis*-cyclohexene was less potent with a K_i of 7 nM. Reduction of *trans*-olefin **14** produced saturated *trans*-cyclohexane **16** with a K_i of 2.2 nM. Both *trans*-cyclohexane **16** and *trans*-cyclohexene **14** exhibit higher affinity for the B_1 receptor than the original biphenyl lead compound **1**. Nonetheless, the physical properties of this series of carbocyclic C-ring replacements were not substantially different from **1**.

The resolution of racemic cyclohexene **14** afforded **17** and **18**. Both compounds have similar receptor affinity indicating that this segment of the scaffold appears not to have any stereochemical influence on the K_i. In an effort to rigidify the carbocyclic C-rings, bicyclic cyclohexane derivatives were prepared (**19**–**22**), but all proved significantly less potent than the parent compounds **14** and **16**. Overall, carbocyclic replacements for the C-ring proved to be the optimum. In particular, the *trans*-isomers were preferred with cyclohexene **14** and cyclohexane **16** as the best substitution for the C-ring phenyl in terms of providing a significant improvement in potency, with a modest reduction of protein binding.

2. Pharmacokinetic studies

To evaluate the pharmacokinetic attributes of the 2,3-diaminopyridines bearing alternative B- and C-ring systems, the pharmacokinetic properties for selected compounds were determined in the rat as summarized in Table 3. Compound **2** in which the piperidine replaced the central B-ring, led to an increase in $t_{1/2}$ with a reduced clearance. Coupled with the good human bradykinin B_1 affinity of **2**, this improvement in PK provides support for further inspection of the piperidine as a phenyl replacement in the 2,3-diaminopyridine series. Both *trans*-cyclohexene **14** and cyclohexane **16** showed poor oral bioavailability and were rapidly cleared in the rat compared to **1**. We speculate, given

Table 3. Rat pharmacokinetics for selected compounds

Compound	F (%)[a]	$t_{1/2}$[b]	Cl[b]	Vd_{ss}[b]
1	18	1.9	4.3	0.36
4	22	3	2.2	0.24
14	5	1.0	29	0.55
16	4	0.5	30	0.38
23	18	1.2	17	0.47

[a] Sprague–Dawley rats. Dose: 2 mg/kg i.v.; 10 mg/kg p.o.
[b] Cl in ml/min/kg, $t_{1/2}$ in h, Vd_{ss} is in L/kg.

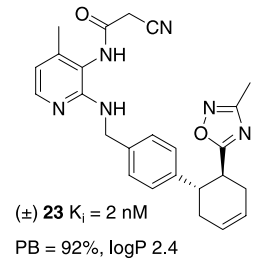


Figure 2. Cyclohexene analog **23** containing an oxadiazole.

that the absence of conjugation with an aromatic ring, that the methyl ester of the cyclohexene ring is more prone to hydrolysis than the ester in **1**. Accordingly, an ester isostere of **14** was prepared in the form of 3-methyloxadiazole **23**. Indeed, the PK of **23** was improved over **14** and gave similar values in terms of rat $t_{1/2}$ and clearance compared to **1**. Consequently, the highly potent C-ring *trans*-cyclohexenes also appear to have suitable pharmacokinetic properties and qualify as new lead structures in the 2,3-diaminopyridine series (Fig. 2).

In summary, we have successfully identified alternative ring systems as suitable replacements for the biphenyl motif in a series of 2,3-diaminopyridine-based bradykinin B_1 receptor antagonists. A 4-piperidine ring replacement for the B-ring phenyl provided compound **2**, which proved to be equipotent with lead compound **1** and exhibited improved pharmacokinetic and physical properties. Concerning the C-ring, a *trans*-cyclohexene ring led to a 10-fold increase in receptor binding affinity and provided a compound with equivalent PK properties relative to **1** through employment of an ester isostere. Additional studies of bradykinin B_1 antagonists employing these new ring systems will be reported in due course.

Acknowledgments

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References and notes

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11. The olefin in the C-ring of **14a** was later reduced to the cyclohexane derivative (Raney Nickel) after the pyridine was attached to give **14b** as shown in Scheme 1.