2,3-Diaminopyridine Bradykinin B_1 Receptor Antagonists

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Abstract: Bradykinin B_1 receptor antagonists embody a potentially novel approach for the treatment of chronic pain and inflammation. A series of 2,3-diaminopyridine B_1 antagonists was optimized to have sub-nanomolar affinity and good pharmacokinetic properties. Lead compounds were shown to exhibit good efficacy in rabbit in vivo models of pain and inflammation.

Bradykinin (BK) peptides are rapidly produced after tissue injury and produce a variety of physiological effects, most notably, pain and inflammation.[1] These effects are mediated by two G-protein coupled receptors designated as B_1 and B_2.[2] The constitutively expressed B_2 receptor evokes the acute pain response following tissue injury and is activated by the peptides bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) and kallidin (Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg). The corresponding metabolites, [des-Arg9]BK (DABK) and [des-Arg10]-kallidin, act as agonists for the B_1 receptor, which is induced following proinflammatory and painful stimuli.[3] Animal models have shown that agonists produce hyperalgesia which can be successfully blocked by peptide-derived B_1 antagonists.[4,5] In addition, B_1 receptor knockout mice have shown reduced sensitivity to painful stimuli, while appearing normal in all other respects. Along with a role in the periphery, a B_1 mechanism in the central nervous system (CNS) has been implied on the basis of evidence that the B_1 receptor is constitutively expressed in the CNS of rats and mice and that B_1 antagonists are active when administered centrally.[6−12] Taken together, it appears that antagonists for the bradykinin B_1 receptor may have potential as novel therapeutic agents for the treatment of pain and inflammation.[13]

We have previously reported non-peptide bradykinin B_1 antagonists based on benzodiazepine[14] and dihydroquinoxaline[15] scaffolds. Although both series gave rise to compounds with high affinities for the human B_1 receptor and exhibited activity in rodent pain models, we explored a third structural series with an improved pharmacokinetic and pharmacodynamic profile.[16]

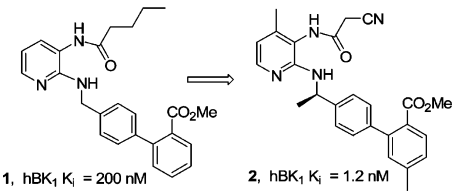

Figure 1.

Screening of the Merck sample collection identified the promising lead compound **1**, which contains a 2,3-diaminopyridine ring and the biphenyl "privileged structure motif"[17] found in a number of marketed drugs (Figure 1). Initial SAR work on **1** rapidly led to the identification of the more potent compound **2**.[18] Despite its high affinity and selectivity for the human B_1 receptor (hB_1) over the hB_2 receptor (K_i > 10 μM), compound **2** exhibited poor PK properties, hindering further pharmacological evaluation. Metabolic profiling suggested that ester hydrolysis and oxidation of the aryl methyl groups were major liabilities. Accordingly, an objective was to block these primary sites of metabolism to obtain more efficacious compounds. Herein we report a series of potent 2,3-diaminopyridine-based bradykinin B_1 antagonists with pharmacokinetic properties suitable for further development.

The compounds in this study were prepared as shown in Scheme 1. Displacement of the chloride in **3** with (*R*)-4-bromo-α-methylbenzylamine in refluxing *n*-BuOH, followed by conversion of the 4-hydroxyl with $POCl_3$, afforded **4**. Reduction of the nitro group was accomplished with $SnCl_2$ in MeOH, and subsequent conversion[19] of the bromide yielded boronate **5**. Suzuki[20] coupling of **5** with the appropriate aryl bromides and acylation produced the final products **6a**−**i**.

K_i values (nM) were determined radiometrically using the appropriate radioligand and Chinese hamster ovary (CHO) cells stably expressing the hB_1 or hB_2 receptors. In vitro functional activity was assessed in standard FLIPR experiments (IC_{50}, nM) employing functionally active hB_1 or rabbit B_1 receptors. Full details for the above experiments are described in the Supporting Information.

Data for several key compounds are shown in Table 1. All compounds were B_1 selective (IC_{50} > 10 μM vs the B_2 receptor). Replacement of the aryl methyl groups in compound **2** at the 4-position of the pyridine ring and 5-position of the distal aryl ring with chlorine trended toward an increase in potency. When the chlorine on the biphenyl in **6a** was relocated from the 5′- to the 3′-position to afford **6b**, a similar trend was observed. Compounds bearing a fluorine adjacent to the ester (e.g. **6c**) were also potent throughout the series. A net result of these substitutions was increased in vitro metabolic stability and improved pharmacokinetic properties (cf. **6a** vs **6b**, **6c**).

An approach to further improve the metabolic stability involved the preparation of a number of replacements for the metabolically labile ester. Exchange of the methyl ester in **6c** with CF_3, CN, and CONHMe yielded

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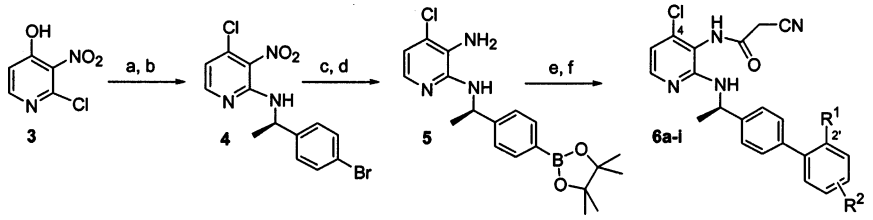
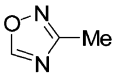
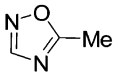
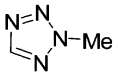
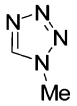
Scheme 1. Synthesis of Biphenyl Compounds **6a**−**i**[a]



[a] (a) (*R*)-4-Br-α-methylbenzylamine, *n*-BuOH, TEA, 120 °C; (b) $POCl_3$, CH_3CN; (c) $SnCl_2$, MeOH; (d) Pd(dppf)Cl_2, KOAc, pinacoldiboron ester, DMSO; (e) arylbromide, Pd(OAc)$_2$, K_2CO_3, THF−H_2O, 80 °C; (f) cyanoacetic acid, EDCI, HOBt.

Table 1. Bradykinin B_1 Receptor Binding Affinities and Pharmacokinetic Parameters for Test Compounds

Compound[a]	R^1	R^2	hBK_1[b]	h FLIPR	Rat PK[c] F%, $t_{1/2}$, CL	Dog PK[d] F%, $t_{1/2}$, CL
2	CO_2Me	5'-Me	1.2	1.4	21, 0.6, 42	nd
6a	CO_2Me	5'-Cl	0.8	1.5	6, 0.5, 25	2, 0.9, 5.5
6b	CO_2Me	3'-Cl	0.7	0.85	43, 1.3, 24	23, 6.3, 8.7
6c	CO_2Me	3'-F	0.4	0.25	50, 2.1, 11	34, 2.7, 9
6d		3'-F	0.5	0.94	62, 8.8, 9.5	14, 2.1, 7.3
6e		3'-F	0.7	0.78	50, 12.9, 4.9	25, 2.0, 5.9
6f		3'-F	0.5	1.17	73, 7.8, 9	69, 3.8, 2.4
6g		3'-F	6.0	nd	nd	nd
6h	CF_3	3'-F	1.1	nd	52, 1.8, 36	nd
6i	CN	3'-F	2.0	nd	80, 0.9, 8.3	nd

[a] All compounds were >95% pure by HPLC and characterized by ^{1}H NMR and HRMS. [b] Values represent the numerical average of at least two experiments. Interassay variability was ±25% for the binding assays (K_i, nM) and ±25% for the FLIPR experiments (IC_{50}, nM). [c] *F*% oral bioavailability, half-life is represented in hours, CL in mL/min/kg. Sprague−Dawley rats ($n = 3$). Oral dose = 10 mg/kg, iv dose = 2 mg/kg. Interanimal variability was less than 20%. [d] Mongrel dogs ($n = 2$). Oral dose 3 mg/kg, iv dose = 1 mg/kg. Interanimal variability was less than 20% for all values.

potent analogues that did not show marked improvement in terms of pharmacokinetic properties. Since oxadiazoles have been used extensively as ester isosteres,[21] both the 3-methyl (**6d**) and 5-methyl (**6e**) oxadiazoles were prepared and found to have affinity for the hB_1 receptor comparable to that of the ester analogues. While tetrazoles are recognized to be isosteric with carboxylic acids, N-substituted tetrazoles have been less utilized as ester surrogates. The N-2 substituted methyl tetrazole (**6f**) was determined to be equipotent with the oxadiazoles, while the N-1 methyl isomer (**6g**) exhibited 12-fold lower affinity.

The structural modifications cited above resulted in increased potency for compounds in this series, as well as significant improvements in pharmacokinetic (PK) properties. Compound **6a**, in which both methyl groups were replaced with chlorine, showed a 2-fold decrease in clearance in the rat. Metabolite identification indicated that the corresponding carboxylic acid of **6a** was the predominant metabolism product. The chlorine in **6a** was moved from the 5′- to the 3′-position on the grounds that its steric bulk might attenuate hydrolysis and lead to improved PK properties. In fact, this proved to be the case. Compound **6b** exhibited improved PK properties over **6a**, particularly in the dog. Surprisingly, **6c** bearing the smaller fluorine at the 3′-position had a PK profile similar to that of the chloro substituted derivative, indicating that metabolic stability is dependent on a subtle interplay between electronic and steric parameters.

The half-lives of both oxadiazoles **6d** and **6e** was markedly increased over the ester, particularly in the rat, where **6e** showed a half-life of almost 13 h. The most impressive advance was obtained with the tetrazole **6f**. This compound exhibited the best balance of bioavailability in rat and dog with half-lives of 7.8 and 3.8 h, respectively.

Table 2. Species Differences for Selected Compounds

compd	K_i (nM)[a] rat	K_i (nM) rabbit	IC_{50}(nM) rabbit (FLIPR)
6b	35.7	1.1	7.2
6c	35.3	0.69	2.4
6d	81	2.7	12.3
6f	119	3.0	3.9
6g	12	41.5	*b*

[a] Values represent the numerical average of at least two experiments. Interassay variability was ± 25% for the binding assays (K_i, nM) and ± 30% for the FLIPR experiments (IC_{50}, nM). [b] Not determined.

Table 3. In Vivo Experiments for Compounds **6b**, **6c**, and **6f**

compd[a]	rabbit LPS AD_{50} (mg/kg)[a]	rabbit CFA ID_{50} (mg/kg)	protein shift
6b	0.80 ± 0.06	0.9 ± 0.6	41
6c	0.57 ± 0.04	2.1 ± 0.9	58
6f	0.28 ± 0.04	3.1 ± 0.2	19

[a] Three rabbits were used in each experiment with iv dosing. See Supporting Information for full experimental protocols.

In general, the compounds in this 2,3-diaminopyridine series are B_1 receptor selective for the human and rabbit over the rat (Table 2). For this reason, the characterization of lead compounds using classic rodent models of pain and inflammation proved problematic. To accommodate this species difference, the in vivo efficacy of key compounds was examined in two rabbit models.

In the first pharmacodynamic readout, functional B_1 receptors in the vasculature were induced by iv administration of lipopolysaccharide (LPS), as evidenced by a depressor blood pressure response to the B_1 agonist DABK (1 μg/Kg iv).[22] Antagonist dose−responses to the depressor effects were generated by pretreatment with rising doses of test compounds before repeated doses of DABK. Results from selected compounds are shown in Table 3. All compounds studied (**6b**, **6c**, and **6f**) were potent with AD_{50} values of less than 1 mg/kg. With the exception of **6f**, the B_1 antagonist activity is consistent with the rabbit B_1 receptor binding affinity. While **6f** displays lower binding affinity relative to **6b** and **6c**, its relatively greater antagonist activity can be attributed to lower nonspecific plasma protein binding. In support of this result, the addition of rabbit plasma (50%) to the binding assay showed that **6f** (19× shift) indeed exhibited the lowest protein shift among these compounds (**6b** and **6c**, 41× and 58× shift, respectively).

To assess the antinociceptive activity of **6b**, **6c**, and **6f**, mechanical hyperalgesia was induced in the rabbit using complete Freund's adjuvant (CFA).[4] In this model, all three compounds were potent in a dose dependent manner to inhibit the spinal nociceptive reflex response to a noxious pinch of the inflamed rabbit hind paw (Table 3).

By way of comparison, morphine gave an ID_{50} of ~0.5 mg/kg, indicating the potential of these bradykinin antagonists as potent antinociceptive agents. It is interesting to note that the rank order of potency among **6b**, **6c**, and **6f** in the mechanical hyperalgesia model is reversed relative to that of the blood pressure model. For example, **6f** is 2−3× more potent in the rabbit LPS model compared to **6b**, while the opposite is true for the rabbit CFA model. This may be attributable to a possible CNS site of action for the B_1 antagonists in the hyperalgesia model. As a result, a compound's ability to cross the blood−brain barrier may affect the degree of efficacy. Further experiments are underway to evaluate the CNS role of the B_1 receptor in nociception.

In summary, a novel class of 2,3-diaminopyridine-based bradykinin B_1 receptor antagonists has been elaborated in terms of potency, selectivity, and pharmacokinetic properties. Several of these compounds exhibit good in vivo efficacy in rabbit models of hyperalgesia and inflammation. Moreover, they retain suitable pharmacokinetic and physicochemical properties for additional study to determine their potential for clinical evaluation.

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Supporting Information Available: ^{1}H NMR and HRMS data for new compounds. Experimental details for the bradykinin B_1/B_2 binding and FLIPR assays, the rabbit CFA and blood pressure models, and rat/dog pharmacokinetic protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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