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2,3-Diaminopyridine as a platform for designing structurally unique nonpeptide bradykinin B_1 receptor antagonists

Dong-Mei Feng,[a,*] Jenny M. Wai,[a] Scott D. Kuduk,[a] Christina Ng,[a] Kathy L. Murphy,[b] Richard W. Ransom,[b] Duane Reiss,[b] Raymond S. L. Chang,[b] Charles M. Harrell,[b] Tanya MacNeil,[c] Cuyue Tang,[d] Thomayant Prueksaritanont,[d] Roger M. Freidinger,[a] Douglas J. Pettibone[b] and Mark G. Bock[a]

[a]*Departments of Medicinal Chemistry, Merck Research Laboratories, West Point, PA 19486, USA*
[b]*Neuroscience Drug Discovery, Merck Research Laboratories, West Point, PA 19486, USA*
[c]*Department of Metabolic Disorders, Merck Research Laboratories, Rahway, NJ 07065, USA*
[d]*Drug Metabolism, Merck Research Laboratories, West Point, PA 19486, USA*

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Abstract—A novel class of 2,3-diaminopyridine bradykinin B_1 receptor antagonists is disclosed. Structure–activity relationship studies (SARs) that led to compounds with significantly improved potency and pharmacokinetic properties relative to the lead compound are described.

1. Introduction

Kinins are a group of peptides that include the nonapeptide, bradykinin (BK) (Arg[1]-Pro[2]-Pro[3]-Gly[4]-Phe[5]-Ser[6]-Pro[7]-Phe[8]-Arg[9]) and the decapeptide, kallidin (Lys[1]-Arg[2]-Pro[3]-Pro[4]-Gly[5]-Phe[6]-Ser[7]-Pro[8]-Phe[9]-Arg[10]). The kinins are formed in plasma and various tissues in response to inflammatory insults, infection, or tissue trauma.[1] Once released, kinins exert most of their biological effects by activating at least two subtypes of specific G-protein coupled cell surface receptors, designated as B_1 and B_2.[2] The B_2 receptors appear to be constitutively expressed in most peripheral and central tissues under normal physiological conditions.[3] On the other hand, the B_1 receptors are typically expressed only at low levels, but can be functionally upregulated in the periphery and CNS by pro-inflammatory and noxious stimuli.[4] The role of BK B_1 receptors to mediate responses to pain in animals has been established using selective B_1 receptor antagonists and B_1 knockout mice.[5,6] These findings imply that B_1 receptor antagonists have therapeutic potential in treating inflammatory pain such as osteoarthritis, as well as in ameliorating neuropathic pain conditions.[2,7,8] The identification of potent and selective, small molecule BK B_1 receptor antagonists is currently an area of intense research. In this context, we have previously disclosed the discovery of nonpeptide BK B_1 receptor antagonists that avidly bind the human B_1 receptor and exhibit in vivo efficacy in animal models of pain.[5e,f] In our continuing search for diverse chemical structures that exhibit affinity for the BK B_1 receptor and which also have the potential for improved pharmacokinetic properties, we uncovered a novel 2,3-diaminopyridine, compound **1** (Fig. 1), by means of a receptor binding screen. It is the chemical elaboration of this lead compound **1**, which afforded

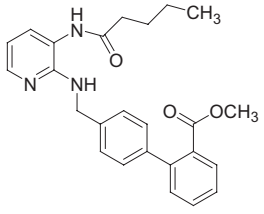

Figure 1. B_1 receptor binding screening lead, **1**.

Keywords: Bradykinin B_1; Receptor; Antagonists; 2,3-Diaminopyridine.
* Corresponding author. Tel.: +1 2156527838; fax: +1 2156523971; e-mail: dongmei_feng@merck.com

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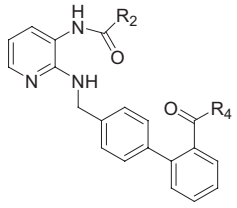
analogs with improved human BK B_1 receptor binding potencies and pharmacokinetic properties in rodents, that forms the basis of this communication.

2. Chemistry

The compounds described in this study are tabulated in Tables 1 and 2. The general preparative method used to access the compounds in Table 1 is outlined in Scheme 1.

Table 1. Effects of 3-amidopyridine modifications on BK B_1 receptor binding affinities and PK properties



Compound[a]	R_2	R_4	hBK$_1$ K_i (nM)[b]	Rat PK[c]		
				F%	$t_{1/2}$	CL
1	n-C_4H_9	OCH_3	200	2	0.2	30
2	n-C_3H_7	OCH_3	88	15	0.4	23
3	i-C_3H_7	OCH_3	230		ND	
4	n-C_2H_5	OCH_3	119		ND	
5	$CH_2N(CH_3)_2$	OCH_3	1280		ND	
6	CH_2OCH_3	OCH_3	390		ND	
7	$CH_2SO_2CH_3$	OCH_3	40		ND	
8	CH_2CF_3	OCH_3	12	9	0.2	35
9	CH_2CN	OCH_3	11	28	0.8	18
10	CH_2CF_3	$NHCH_3$	33	95	0.3	15

[a] All compounds were >98% pure by HPLC and characterized by ^{1}H NMR and HRMS.
[b] Values represent the numerical average of at least two experiments. Inter-assay variability was ±20% for the binding assays.
[c] F% oral bioavailability, half-life is represented in hours, and CL in mL/min/kg.

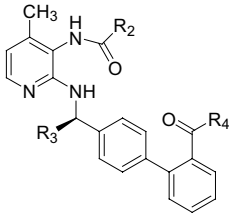
Accordingly, the commercially available 4′-methylbiphenyl-2-carboxylic acid methyl ester **A** was transformed via the four-step sequence of bromination, 2-amino-3-nitropyridine alkylation, reduction, and acylation to afford analogs **2**–**9**, **11**, and **13**. Alternatively, intermediate **C** could be diverted to give the methyl amides **10** and **15** following standard procedures. The compounds listed in Table 2 were derived according to the synthetic routes outlined in Scheme 2. In this way, 2-chloro-3-nitro-4-methylpyridine was reacted with (*R*)-4-bromo-α-methylbenzylamine to give the 2-aminopyridine adduct, which yielded intermediate **E** after reduction with stannous chloride. Subsequent boronate ester formation of **E** and Suzuki-coupling with methyl 2-bromobenzoate yielded intermediate **F**. The latter was acylated in the standard fashion to give **12** and **14** or saponified and converted to the methyl amide **16** in the usual manner.

3. Biological results and discussion

K_i values (nM) were determined radiometrically using the appropriate radioligand and Chinese hamster ovary (CHO) cells stably expressing the human BK B_1 receptor.[9] The protocol for determining pharmacokinetic properties using Sprague–Dawley rats ($n = 3$) wherein the oral dose is 10 mg/kg and the IV dose is 2 mg/kg, was identical to that previously described. Interanimal variability was less than 20%.[5e]

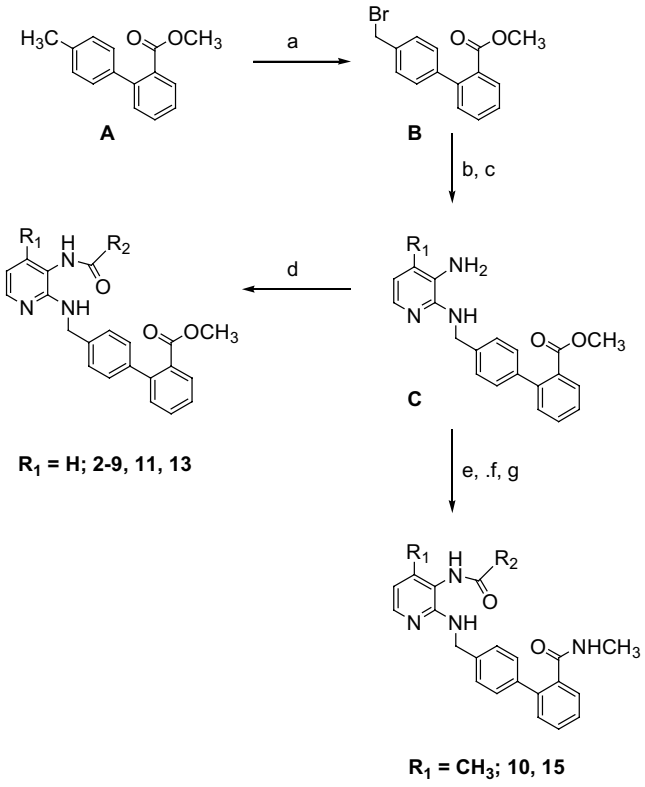
The initial objective in optimizing the pharmacological profile of the screening lead compound **1** was to increase its human BK B_1 receptor binding affinity. Therefore, our attention was immediately drawn to the 3-amide function following the observation that shortening the chain length of the *n*-pentanoyl amide in **1**, by one carbon atom, resulted in a 2-fold boost in potency (cf. **1** and **2**). An extensive analoging effort ensued, which

Table 2. 4-Methyl pyridine analogs



Compound[a]	R_2	R_3	R_4	hBK$_1$ K_i (nM)[b]	Rat PK[c]		
					F%	$t_{1/2}$	CL
11	CH_2CF_3	H	OCH_3	7.1	40	0.4	14
12	CH_2CF_3	(*R*)CH_3	OCH_3	1.8	17	0.9	8.0
13	CH_2CN	H	OCH_3	11.5	19	2.0	4.3
14	CH_2CN	(*R*)CH_3	OCH_3	2.6	27	0.2	18.5
15	CH_2CF_3	H	$NHCH_3$	11	79	1.0	30
16	CH_2CF_3	(*R*)CH_3	$NHCH_3$	6.0	25	0.5	31

[a] All compounds were >98% pure by HPLC and characterized by ^{1}H NMR and HRMS.
[b] Values represent the numerical average of at least two experiments. Inter-assay variability was ±20% for the binding assays.
[c] F% oral bioavailability, half-life is represented in hours, and CL in mL/min/kg.

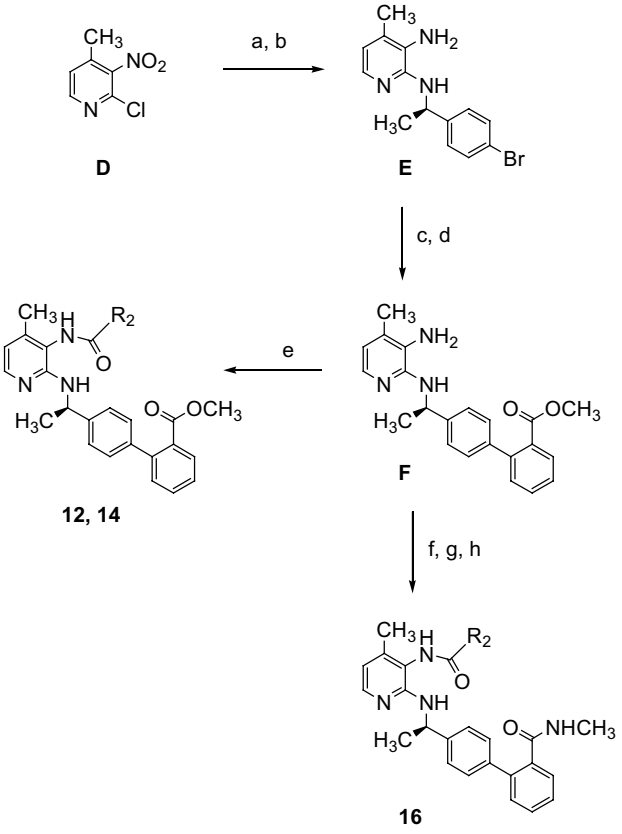


Scheme 1. Reagents and conditions: (a) NBS, AIBN, CCl_4, 85%; (b) 2-amino-3-nitropyridine, NaH, 0 °C, DMF; (c) $SnCl_2$, MeOH, 70 °C, 72% (for b, c); (d) carboxylic acid, EDCI, HOBt, 95%; (e) 4 N NaOH, MeOH, H_2O, 50 °C; (f) carboxylic acid, EDCI, HOBt; (g) NH_2CH_3, EDCI, HOBt, 77% (for e, f, g).

was designed to flesh out the SAR related specifically to the 3-amide function. As the BK B_1 receptor binding data for selected compounds in Table 1 indicate, binding affinity is clearly influenced by steric and electronic factors. Thus, increased steric bulk (**1**, **3**), as well as the incorporation of electron donating groups (**5**, **6**) at the 3-amide group have a detrimental effect on potency. Conversely, the introduction of electron withdrawing groups on the 3-amide side chain resulted in improved binding affinities. Among the functional groups, which were surveyed, the 3,3,3-trifluoropropionamide (**8**) and cyanoacetamide (**9**) were optimum. While these minor structural changes to **1** had a significant effect on BK B_1 receptor binding affinity, these modifications alone had no marked influence on pharmacokinetic (PK) properties. We surmised that the methyl ester on the distal aromatic ring of compounds **1**–**9** was, in part, responsible for their poor PK profile. The latter assumption was born out by the improved bioavailability and reduced clearance rate of the corresponding *N*-methyl amide **10**.

To gain further insight toward improving the PK properties of lead compounds exemplified by structures **8**–**10**, we examined their in vitro stabilities in rat liver microsomal preparations (data not shown). Metabolite profiling indicated that the 4-position of the pyridine ring was oxidized in all compounds, which were examined. Consequently, we installed a methyl group at the 4-pyridine position in an attempt to mitigate this metabolic pathway (Table 2). The latter modification yielded the intended result. The 3,3,3-trifluoropropionamido methyl ester **11** and cyanoacetamido methyl ester **13** were essentially equipotent with their respective des-methyl analogs, **8** and **9**. Moreover, improvements in oral bioavailability, half life, and clearance were also realized. A similar trend was observed with the 3,3,3-trifluoropropionamido methyl amides **10** and **15**, effectively making the 2,3-diamino-4-methylpyridine ring the new lead template.

During the course of optimizing the newly identified lead compounds, **11** and **13**, additional points of metabolism were identified. Not surprisingly, among these, the benzylic position of the central aromatic ring appeared especially vulnerable. In response, a methyl group was installed to yield **12** and **14**, respectively. Both compounds display improved human B_1 receptor binding affinity relative to their progenitors and in the case of the 3,3,3-trifluoromethylpropionamide **12**, a modest improvement in clearance and half life was observed compared to **11**. The (*S*)-configured enantiomers of closely related analogs were also examined but in each instance, showed substantially reduced human B_1



Scheme 2. Reagents and conditions: (a) (*R*)-4-bromo-α-methylbenzylamine, TEA, *n*-BuOH, 110 °C; (b) $SnCl_2$, MeOH, 70 °C, 75% (for a, b); (c) Pd(dppf)Cl_2, KOAc, pinacoldiboron ester, DMSO, 80 °C; (d) methyl 2-bromobenzoate, Pd(dppf)Cl_2, K_2CO_3, DMSO, 80 °C, 75% (for c, d); (e) carboxylic acid, EDCI, HOBt, 95%; (f) 4 N NaOH, MeOH, H_2O, 50 °C; (g) carboxylic acid, EDCI, HOBt; (h) NH_2Me, EDCI, HOBt, 77% (for f, g, h).

receptor binding affinity (data not shown). Similarly, replacement of the (*R*)-benzylic methyl group in **14** with ethyl or methoxymethyl groups resulted in a significant diminution of human B_1 receptor binding affinity. It is apparent that the installation of a benzylic methyl group benefits human B_1 receptor binding potency in this series of compounds but its effects on their PK properties is not as definitive. This observation also obtains for the methyl amides **15** and **16**.

In summary, we have disclosed the novel 2,3-diaminopyridine screening lead **1**, which displays modest receptor binding affinity for the human bradykinin B_1 receptor and described its structural elaboration to give compounds with up to 100-fold enhanced binding potency and significantly improved PK properties. These SAR studies, guided by in vitro metabolism experiments using rat liver microsomes, demonstrate that the 2,3-diamino-4-methylpyridine ring system is a viable platform for the design of a new generation of low molecular weight, selective BK B_1 receptor antagonists with desirable PK properties.[10] These studies are continuing and their outcomes will be reported in due course.

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