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Potent bradykinin B_1 receptor antagonists: 4-Substituted phenyl cyclohexanes

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Abstract—Selective bradykinin (BK) B_1 receptor antagonists have been shown to be antinociceptive in animal models and could be novel therapeutic agents for the treatment of pain and inflammation. Elucidation of the structure–activity relationships of the biphenyl moiety of the lead compound **1** provided a potent new structural class of BK B_1 receptor antagonists.
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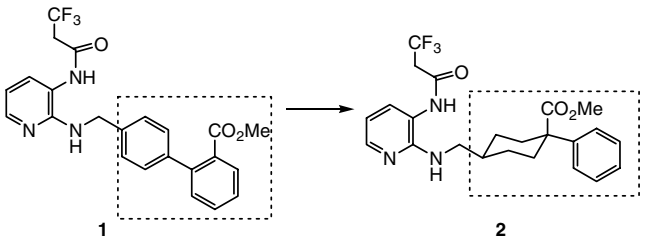
Bradykinin (BK) is an autacoid peptide produced by the catalytic action of kallikrein enzymes on plasma and tissue precursors termed kininogens. It plays an important role in the pathophysiological processes accompanying pain and inflammation. Its biological actions are mediated by two known G-protein coupled receptors named B_1 and B_2. The BK B_2 receptor is constitutively expressed in most cell types and evokes acute pain responses following tissue injury, whereas the BK B_1 receptor is induced during inflammatory insults or painful stimuli.[1] In animal models, BK B_1 receptor agonists, such as des-Arg^9-bradykinin (DABK) and des-Arg^{10}-kallidin (DAK), produce hyperalgesia, an effect that can be blocked by peptide BK B_1 receptor antagonists, such as des-Arg^9-Leu^8-bradykinin (DALBK) and des-Arg^{10}-Leu^9-kallidin (DALK).[2] A study result from the BK B_1 receptor knockout mouse has implicated a role for the BK B_1 receptor in inflammation, algesia, and neuropathic pain.[3] In addition to the accepted peripheral mode of action of the BK B_1 receptor, the BK B_1 receptor has also been accorded a central role on the basis of recent results which demonstrate that the BK B_1 receptor is constitutively expressed in the central nervous system (CNS) of mice and rats.[4] Accordingly, selective and effective BK B_1 receptor antagonists hold promise as novel therapeutic agents for the treatment of pain and inflammation.[5]

The current study was initiated with the previously reported biphenyl diaminopyridine lead compound **1** (Scheme 1).[6] This paper reports the revelation and SAR study results of a structural alternative, 4-substituted phenyl cyclohexane (**2**), that exhibits excellent binding affinity and good receptor occupancy in an ex vivo receptor occupancy assay which has been designed to determine the extent of CNS penetration of compound.



Scheme 1. Lead compound and replacement of biaryl phenyl core.

Keywords: Bradykinin; B_1 receptor; B_1 receptor antagonists; 4-Substituted phenyl cyclohexanes.
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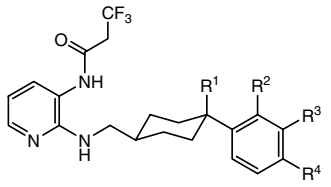
Table 1. Binding affinities of select BK B_1 antagonists



Compound	R^1	R^2	R^3	R^4	K_i[a] (nM)
2	CO_2Me	H	H	H	1645
3	OAc	H	H	H	1451
4	OH	H	H	H	478
5	CN	H	H	H	14
6	CN	F	H	H	18
7	CN	H	F	H	3.7
8	CN	H	H	F	60
9	CN	H	CF_3	H	24.5
10	CN	H	CO_2Me	H	49
11	CN	F	F	H	2
12	CN	H	Cl	Cl	30
13	CN	Cl	Cl	H	4.9
14	CN	Cl	H	H	3.7
15	CN	OCF_3	H	H	3.6
16	CN	CF_3	H	H	0.4

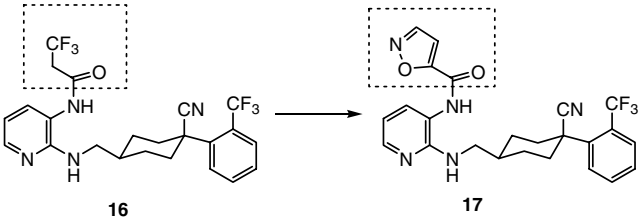
[a] Values represent the numerical average of at least two experiments. Interassay variability was ±25%.

The central ring of the lead compound **1** can be replaced with a number of alternative heterocycles and cycloalkyls.[7] We found that the bis-substitution at the 4-position of cyclohexane ring is well tolerated (analogue **2**, Table 1).[8] The methyl ester of compound **2** was replaced with several other functional groups as shown in Table 1. Replacement of the methyl ester with an amide, hydroxymethyl, or 3-methyl-1,2,4-oxidiazole proved to be ineffective (data not shown). However, replacement of the ester with acetoxy yielded an equipotent compound (**3**). Hydrolysis of the acetate derivative gave the alcohol, **4**, with a threefold binding affinity enhancement. Replacement of hydroxyl to a cyano group improved the binding affinity about 30-fold (compound **5**). The importance of stereochemistry of the 4-substituted phenyl cyclohexane was also investigated with the *cis* isomer (**5**) imparting greater potency (about 100-fold) than the *trans* isomer (data not shown).

With the potency enhancing *cis* cyano group at 4-position, we then examined substitution on the phenyl ring. The fluorine atom was used as a probe. Among the three positions on the substituted phenyl ring, *meta*-fluoro compound **7** showed fourfold improvements over the lead compound **5**. Other substituents were introduced at the *meta* position, but with significant loss of potency (compounds **9** and **10**). Difluoro analogue **11** was slightly more potent, but multiple chlorine substitution on the phenyl ring did not increase the binding affinity (compounds **12** and **13**). Although the *ortho*-fluoro analogue (**6**) was not as potent as *meta*-fluoro compound (**7**), the *ortho* chlorine analogue, **14**, was about fourfold more potent than parent analogue **5** and equally potent as compound **7**. Further modification of the *ortho* substituents led to the trifluoromethyl compound **16** with subnanomolar binding affinity. Compound **16** shows good functional antagonist potency in a Fluorescence Imaging Plate Reader assay (FLIPR) with an IC_{50} of 1.15 nM. Compound **16** was examined in a rat pharmacokinetic experiment.[9] However, it exhibits high plasma clearance (37.7 mL/min/kg), a short half-life (0.8 h), and poor bioavailability.

Incorporation of alternatives to the trifluoroethyl amide side chains from the SAR study of the original series into compound **16** led to a potent isoxazole analogue, **17**, with a K_i of 0.6 nM (Scheme 2). Compound **17** demonstrates excellent functional antagonist potency in a FLIPR assay with an IC_{50} of 0.45 nM which is in accord with the receptor binding affinity. Although compound **17** shows improvement versus **16** in terms of pharmacokinetic parameters, it exhibits modest clearance (16.1 and 21.3 mL/min/kg) and short half-life (1 and 1.3 h) in rat and dog, respectively.[10]

To determine the extent to which the compounds in this series occupy the human BK B_1 receptor, **17** was examined in an ex vivo receptor occupancy study in transgenic rats in which the human BK B_1 receptor is constitutively over-expressed.[11] In this study, **17** showed dose-dependent occupancy following iv infusion over 30 min (Table 2). At a dose of 6 mg/kg, compound **17** shows 77% and 84% of receptor occupancy in both brain and spinal cord with concentrations of 666 and 468 nM, respectively. These data suggest that this compound penetrates the blood–brain barrier and occupies the receptor in the CNS which is in accordance with the finding that compound **17** is not a substrate for P-glyco-

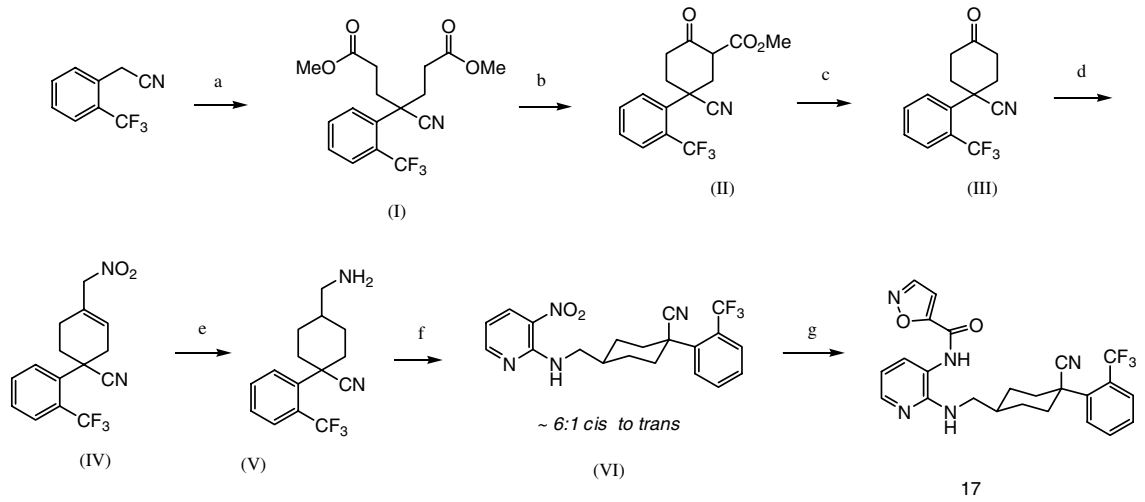


16 **17**

Scheme 2.

Table 2. Ex vivo receptor occupancy study results of compound **17**

	Occupancy (%)	Concn (nM)
0.06 mpk		
Plasma	—	24
Brain	19	<26
Spinal cord	3	<26
0.6 mpk		
Plasma	—	274
Brain	58	62
Spinal cord	50	35
6 mpk		
Plasma	—	3216
Brain	77	666
Spinal cord	84	468



Scheme 3. Reagents and conditions: (a) methyl acrylate, Triton B, AcCN, reflux, 83%; (b) NaH, DME, reflux, 88%; (c) DMSO, NaCl, 150 °C, 61%; (d) CH_3NO_2, ethylene diamine (cat.), reflux, 86%; (e) Raney Ni, H_2, MeOH; (f) 2-chloro-3-nitro pyridine, TEA, THF, silica gel chromatography, 50% for two steps; (g) Raney Ni, H_2, MeOH, then isoxazole-5-carbonyl chloride, 50% for two steps.

protein (P-gp) mediated efflux (MDR1, (B/A)/(A/B): 2.5, Passive permeability (Papp): 36×10^{-6}cm/s).[12,13]

Compound **17** was prepared according to the route depicted in Scheme 3. All other compounds were synthesized in an analogous fashion. Double Michael reactions of commercially available benzoacetonitrile with methyl acrylate afforded adduct I. Dieckmann cyclization followed by decarboxylation of II yielded cyclohexanone, III. Henry reaction and reduction of the resulting vinyl nitro intermediate IV provided the amine derivative (V) as a mixture of *cis* and *trans* isomers (ratio: 6:1). Nucleophilic aromatic substitution of 2-chloro-3-nitro pyridine by the amine (V) delivered the desired aminopyridine compound. At this stage the mixture of *cis* and *trans* isomers can be easily separated by flash chromatography. Reduction of nitro by hydrogenation and coupling with the isoxazole-5-carbonyl chloride provided the target compound **17**.

In summary, we have successfully identified alternative isosteres for the biphenyl moiety of the lead compound **1**. The compounds disclosed in this paper represent a new structural class of BK B_1 receptor antagonists. Compound **17** is an optimal member of the series to emerge from this study and exhibits promising ex vivo receptor occupancy in transgenic rats. Further evaluation and modification of these compounds to improve their pharmacokinetic properties are in progress. The results from these studies will be disclosed in due course.

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