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Bradykinin B_1 antagonists: Biphenyl SAR studies in the cyclopropanecarboxamide series

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Abstract—SAR study of the biphenyl region of cyclopropanecarboxamide derived bradykinin B_1 antagonists was examined. Incorporation of a pyridine in place of the proximal phenyl ring and chlorination of the distal phenyl ring proved to be well tolerated and provided compounds with improved pharmacokinetic profiles, CNS penetration, and enhanced receptor occupancy.

Bradykinin (BK) peptides are rapidly produced in plasma after tissue insult and exert a variety of physiological effects, including 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 initial acute pain response following tissue injury and is mediated by the peptides bradykinin (BK = Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) and kallidin (Lys-BK). Their corresponding metabolites, [des-Arg9]bradykinin and [des-Arg10]-kallidin, serve as agonists for the B_1 receptor, which is induced in the hours following the injury.[3]

In support of their therapeutic potential,[4] bradykinin B_1 receptor antagonists have been shown to ameliorate pain responses in animal models[5,6] and 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 system of rats and mice.[7–9] Thus, CNS penetrant bradykinin B_1 receptor antagonists are of considerable interest as they may have superior efficacy relative to peripheral B_1 antagonists, and might also find additional application in the treatment of neuropathic pain.[10]

We recently reported the synthesis and biological characterization of a series of biphenylcyclopropane carboxamide based bradykinin B_1 receptor antagonists as exemplified by compound **1**, which was selected for further development.[11] While **1** exhibited high affinity for the human B_1 receptor and was reasonably CNS penetrant in animals, it lacked ideal pharmacokinetic properties and exhibited only moderate CNS receptor occupancy in a transgenic mouse model. In this communication we describe our efforts to improve these properties via modulation of the biphenyl region, with the goal of finding a backup candidate to compound **1** (Fig. 1).

The general synthesis of target compounds **2a**–**p** is illustrated in Scheme 1. In situ conversion of the appropriate

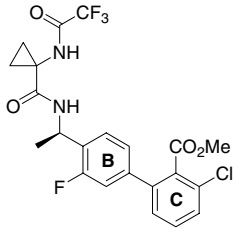

Figure 1. Bradykinin B_1 antagonist **1**.

Keywords: Bradykinin; B_1; Antagonists; Biphenyl.
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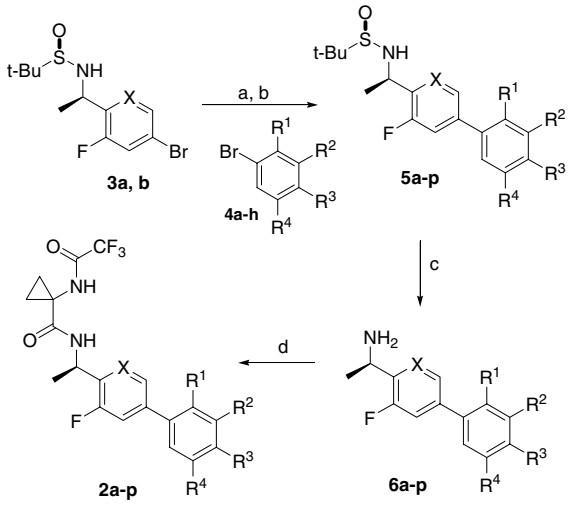
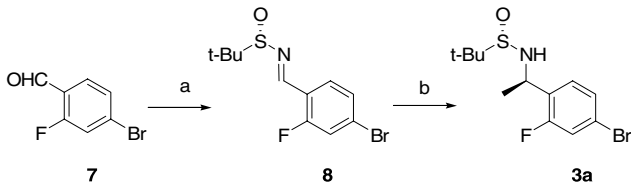


Scheme 1. Reagents and conditions: (a) $Pd(dppf)Cl_2$, KOAc, pinacol-diboron ester, DMSO, 80 °C; (b) Arylbromide **4a**–**h**, $Pd(dppf)Cl_2$, K_2CO_3, DMSO–H_2O, 80 °C; (c) 4 N HCl/dioxane, MeOH, 0 °C; (d) EDCI, HOBt, TEA, 1-(trifluoroacetyl)aminocyclopropanecarboxylic acid.

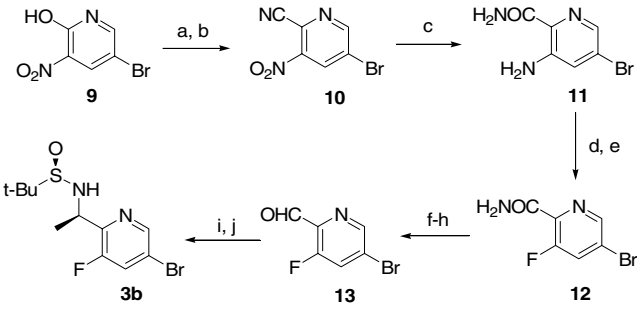
bromide **3a**, **b** to the pinacol boronate ester is followed by a Suzuki coupling with the requisite C-ring bromide **4a**–**h** to afford the biaryls **5a**–**p**. Removal of the sulfinamide group to provide amines **6a**–**p** was accomplished using HCl in MeOH followed by subsequent EDCI-mediated acylation with 1-(trifluoroacetyl)aminocyclopropanecarboxylic acid to provide **2a**–**p**.

The synthesis of phenyl bromide **3a** is shown in Scheme 2. The chiral center was constructed employing the Ellman *tert*-butanesulfinimine methodology.[12] Aldehyde **7** was condensed with (*S*)-*tert*-butane sulfinamide in the presence of magnesium sulfate to provide imine **8**. Addition of methylmagnesium chloride in DCM provided **3a** in good yield with 9:1 diastereoselection.

Scheme 3 details the synthesis of pyridyl bromide **3b**. The hydroxy group of pyridine **9** was converted to a bromide which underwent selective cyanation to afford **10**. Reduction of the nitro group with stannous chloride was accompanied by contaminant hydrolysis of the nitrile to amide **11**. Diazotization of **11** was followed by thermal decomposition of the resultant diazonium tetrafluoroborate salt in toluene to afford fluoride **12**. The amide was converted to aldehyde **13** in three steps via the intermediacy of a Weinreb amide. Subsequent imine formation and addition of methylmagnesium chloride as described for **3a** provided **3b** in 6:1 diastereoselection. It should be noted that the (*R*)-*tert*-butane



Scheme 2. Reagents and condition: (a) (*S*)-*tert*-butane sulfinamide, CH_2Cl_2, $MgSO_4$; (b) MeMgCl, CH_2Cl_2, −48 °C.



Scheme 3. Reagents and conditions: (a) $POBr_3$, toluene, 80 °C; (b) CuCN, DMF, 120 °C; (c) $SnCl_2$, MeOH, 50 °C; (d) $NOBF_4$, CH_2Cl_2; (e) toluene, 100 °C; (f) 12 N HCl, 100 °C; (g) *N*-methoxymethylamine, EDCI, TEA, DMF; (h) $LiAlH_4$, ether, −78 °C; (i) (*R*)-*tert*-butane sulfinamide, PPTS, CH_2Cl_2, $MgSO_4$; (j) MeMgCl, CH_2Cl_2, −48 °C.

sulfinamide was required as there was a reversal of diastereoselectivity observed due to the presence of the pyridine nitrogen at the 2-position.[13]

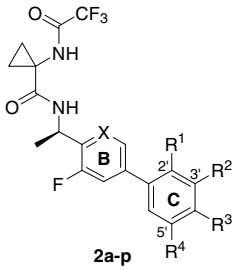
SAR work has shown that the trifluoroacetamide was essential to provide compounds that are not substrates for human P-glycoprotein (P-gp) mediated efflux which otherwise would limit human CNS exposure.[11] The cyclopropane 'A-ring' carboxamide sector of **1** has been optimized in order to avoid prohibitively high levels of bioactivation while maintaining high B_1 receptor affinity.[14,15] Accordingly, our focus for improving this series was directed toward the biphenyl region of **1** (designated as the B- and C-rings). Results for chosen compounds **2a**–**p** are shown in Table 1.

Moving the 3′-chloro group to the 4′-position on the distal C-ring (**2a**, R^3 = Cl) led to a >300-fold loss in potency while a chloro at the 5′-position provided an analog with good potency (**2b**, hK_i = 2.05 nM) for further SAR evaluation. Since we have previously observed that the 3′-chloro group was essential to mitigate hydrolysis of the methyl ester,[11] a number of heterocyclic isosteres were incorporated into **2a** to evaluate their effects on potency and pharmacokinetic properties. The 5-methyloxadiazole (**2d**) and 2-methyltetrazole (**2e**) showed equivalent B_1 receptor affinity compared to **2b**, while 3-methyloxadiazole (**2c**) was less potent. Moreover, both **2d** and **2e** had improved rat pharmacokinetic profiles in terms of longer half-lives and decreased clearance relative to **1**, while maintaining low P-gp transport susceptibility.

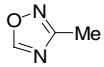
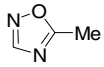
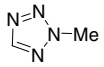
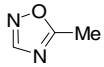
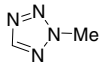
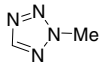
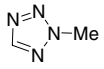
The most exciting results were obtained when the 5′-chloro group was incorporated onto the C-ring in combination with the 3′-chloro group. The resulting dichloro compound (**2f**) exhibited a dramatically improved rat half-life of 4.6 h and a low clearance of 1.9 mL/min/kg while maintaining equivalent B_1 receptor affinity relative to **1**. The 3′-fluoro analog (**2g**) was equipotent, but showed a poorer rat pharmacokinetic profile further validating the importance of the 3′-chloro group at mitigating the ester hydrolysis.

To further improve upon **2f**, replacement of the methyl ester with heterocyclic isosteres and other esters was

Table 1. Bradykinin B_1 receptor binding affinities and pharmacokinetics



2a-p

Compound	R^1	R^2	R^3	R^4	X	hBK_1[a]	P-gp[b]	P_{app}[c]	Rat F (%)[d]	Rat $t_{1/2}$	Rat Cl
1	CO_2Me	Cl	H	H	CH	0.44	1.9	34	34	0.4	40
2a	CO_2Me	H	Cl	H	CH	134	nd	nd	—	—	—
2b	CO_2Me	H	H	Cl	CH	2.05	nd	nd	—	—	—
2c		H	H	Cl	CH	7.60	nd	nd	—	—	—
2d		H	H	Cl	CH	1.39	2.4	31	38	1.4	5.3
2e		H	H	Cl	CH	1.45	2.7	28	47	3.8	2.7
2f	CO_2Me	Cl	H	Cl	CH	0.46	1.8	18	38	4.6	1.9
2g	CO_2Me	F	H	Cl	CH	0.68	2.1	27	21	0.8	13
2h	CO_2Et	Cl	H	Cl	CH	1.85	1.7	25	52	2.2	12.1
2i		Cl	H	Cl	CH	2.4	2.7	23	39	5.6	0.8
2j		Cl	H	Cl	CH	1.58	2.6	28	55	8.7	0.8
2k		F	H	Cl	CH	1.1	3.5	23	64	3.5	2.3
2l	CO_2Me	Cl	H	H	N	0.77	1.9	25	21	1.2	19.6
2m	CO_2Me	Cl	H	Cl	N	2.6	2.2	29	19	1.3	16.6
2n	CO_2Et	Cl	H	H	N	1.2	2.6	28	52	0.8	6.8
2o	CO_2Et	Cl	H	Cl	N	1.8	2.2	25	57	2.0	12.6
2p		Cl	H	Cl	N	3.8	9.1	27	65	6.4	1.2

[a] Values represent the numerical average of at least two experiments. Interassay variability was ±25% (K_i, nM).
[b] MDR1 directional transport ratio (*B* to *A*)/(*A* to *B*). Values represent the average of three experiments and interassay variability was ±20%.
[c] Passive permeability (10^{-6} cm/s).
[d] *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%.

examined. Ethyl ester **2h** led to a decrease in both B_1 receptor potency and rat pharmacokinetic properties, while heterocycles **2i**–**k** were tolerant of the replacement with respect to receptor affinity. While the heterocyclic isosteres (**2i**–**k**) proved to have excellent rat pharmacokinetic profiles, their P-gp transport ratios began to rise with the increasing number of heteroatoms they presented. For example, tetrazole **2k** (P-gp = 3.5) was above the acceptable level for defining compounds as P-gp substrates (P-gp transport ratio >2.5), while **2i**–**j** were borderline.[16] The apparent permeabilities (P_{app}) of all the compounds examined were well above the generally accepted value for CNS penetrant compounds ($P_{app} > 15 \times 10^{-6}$ cm/s). Hence the esters remained as the most promising candidates for further evaluation.

Compound **1** was highly lipophilic and showed low aqueous solubility. In an effort to improve the physical properties, a nitrogen was inserted into the B-ring phenyl of selected compounds (**2l**–**p**, Table 1). The pyridine analog of **1** (**2l**) showed similar potency with an improved half-life and reduced clearance in the rat. Moreover, the inclusion of an additional heteroatom

in the form of the B-ring pyridine did not lead to an increase in human P-gp susceptibility. Addition of the 5′-chloro on the C-ring provided compound **2m**, but did not greatly improve pharmacokinetics in the rat as was noted for the B-ring phenyl (compounds **1** → **2f**). The related ethyl ester variants (**2n–o**) also showed good B_1 receptor affinity and superior oral bioavailability (>50%) in the rat. As exemplified by tetrazole **2p**, heterocyclic replacements for the ester were not tolerated in combination with the B-ring pyridine, due to the unacceptably high human P-gp efflux ratios.

To further evaluate modifications made to the biphenyl region of **1**, select compounds were evaluated in the ex vivo transgenic receptor mouse occupancy model.[17] In addition, CNS penetration studies in African green monkey (AGM) were implemented.[18] Results are shown in Table 2.

Addition of the 5′-chloro group to the C-ring (**2f**) exhibited improvement in both receptor occupancy efficiency (Occ_{90} = 450 nM) and increased CNS brain to plasma ratio in the AGM compared to **1**. The closely related tetrazole **2j** did not result in any improvement in occupancy or CNS levels over the ester. The most important result obtained was the boost in receptor occupancy efficiency afforded by the compounds containing the B-ring pyridine. For example, pyridine **2l** had an Occ_{90} of 350 nM (vs 520 nM for **1**) without lessening the CNS levels in the AGM. Gratifyingly, the inclusion of the 5′-chloro group on the C-ring of **2l** provided **2m** which exhibited the best occupancy (Occ_{90} = 210 nM) in the series, as well as the highest brain to plasma ratio in the AGM for compounds bearing the B-ring pyridine (B/P = 0.8). It is interesting to note that many of the compounds showing improved Occ_{90}'s had somewhat weaker B_1 affinity in the binding assay. We believe the improved receptor occupancy of compounds bearing the B-ring pyridine is likely attributed to subtle improvement in physical properties rendered by the presence of the nitrogen heteroatom (lower log P and protein binding).

Table 2. Receptor occupancy and monkey CNS penetration for selected compounds

Compound	Occ_{90}[a] (nM)	AGM brain/plasma[b]	PB[c]	log P
1	520	0.4	98.6	>3.6
2f	450	1.1	97.4	>3.8
2j	540	0.3	99.1	>3.8
2l	350	0.5	97.2	3.2
2m	210	0.8	97.5	3.3
2n	225	0.7	98.4	3.6

[a] Values are means of at least eight experiments.
[b] Values are a mean of two experiments.
[c] Protein binding measured using 10% rat serum.

Table 3. Dog and Rhesus Pharmacokinetics for select compounds

Compound	Dog PK[a]			Rhesus PK[b]		
	F (%)	$t_{1/2}$ (h)	Cl	F (%)	$t_{1/2}$ (h)	Cl
1	33	1.8	9.0	31	1.7	13
2f	19	1.6	20	20	3.9	11.2
2j	87	13	1.2	75	4.2	3.4
2m	66	6.6	6.2	12	2.5	16.9
2n	20	2.3	10	12	1.1	11.5

[a] Mongrel dogs (n = 2). Oral dose 3 mg/kg, iv dose = 1 mg/kg. Interanimal variability was less than 20% for all values.
[b] Rhesus monkeys (n = 2). Oral dose 3 mg/kg, iv dose = 1 mg/kg. Interanimal variability was less than 20% for all values.

Preferred compounds were examined for additional pharmacokinetic evaluation in dog and rhesus (Table 3). While addition of the 5′-Cl in compound **2f** led to an improvement in rat PK relative to **1** (Table 1), little benefit was seen in dog and monkey. Replacing the ester with a tetrazole provided the compound (**2j**) which had the best profile with half-lives in the dog and rhesus of 13 and 4.2 hours, respectively. While compound **2m**, the B-ring pyridine version of compound **2f**, showed minimal improvement in rhesus, a significant increase in bioavailability (66%) and half-life (6.6 h) was observed in dog. The closely related analog **2n**, which lacks the 5′-Cl group and has an ethyl ester in place of methyl ester, had very similar PK properties in these species compared to **1**.

In conclusion, a series of cyclopropanecarboxamide containing compounds, bearing modifications to the biphenyl sector, was prepared and evaluated as bradykinin B_1 receptor antagonists. Incorporation of a pyridine in the proximal phenyl ring led to enhanced receptor occupancy, while addition of a chlorine at the 5′-position of the distal phenyl ring led to improvements in terms of pharmacokinetics and greater CNS penetration. These enhancements are best exemplified by compounds **2l–n** which represent a significant advance over lead compound **1**.

Acknowledgment

We are grateful to the analytical department for log P and protein binding measurements.

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18. Compound was administered iv as a bolus injection at 2 mg/kg to conscious African green monkeys ($n = 2$). At 30 min post-dose plasma samples were taken and the animal was euthanized with pentobarbital and the brain and spinal cord removed.