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A new class of bradykinin B_1 receptor antagonists with high oral bioavailability and minimal PXR activity

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Abstract—The design and synthesis of a novel class of human bradykinin B_1 antagonists featuring difluoroethyl ether and isoxazole carboxamide moieties are disclosed. Compound **7g** displayed excellent pharmacokinetic properties, efficient ex vivo receptor occupancy, and low potential for P450 induction via PXR activation.

The kinin-related peptides are potent endogenous algesic and pro-inflammatory substances. The kinins mediate a number of acute and chronic inflammatory responses including vasodilation, edema, cellular infiltration, and pain.[1–3] These effects are mediated by two G-protein coupled receptors, B_1 and B_2.[4–8] B_2 receptors are normally present in the periphery in many cell types involved in pain responses. Bradykinin B_1 receptors are not normally highly expressed, but instead are rapidly induced in response to inflammatory and painful stimuli. The inducible nature of the B_1 receptor has led to the belief that B_1 receptors are most relevant during longer term inflammatory pain conditions, while B_2 receptors are important mediators of acute pain and the early inflammatory response. This hypothesis was initially supported by studies with peripheral administration of peptidyl kinin agonists and antagonists in non-inflamed, inflamed, and neuropathic pain models.[9–12] Recently, however, studies with B_1 knockout mice have generated a broader view of the potential role of B_1 mechanisms in both non-inflammatory and inflammatory pain, acutely and chronically.[13,14]

We previously reported non-peptide human bradykinin B_1 antagonists[15] based on the benzodiazepine,[16] dihydroquinoxaline,[17] diaminopyridine,[18] and cyclopropanecarboxamide scaffolds.[19] SAR studies in the latter series indicated that incorporation of a trifluoroacetamide group led to analogs that were not substrates for the xenobiotic efflux pump P-glycoprotein (P-gp), and had acceptable CNS penetration.[20] As a follow up to this work, we investigated a related series that had excellent binding affinity, good brain penetration, and reasonable pharmacokinetic properties as exemplified by compound **1** (Fig. 1).[21] However, compound **1** still possessed certain metabolic liabilities related to the methyl ester

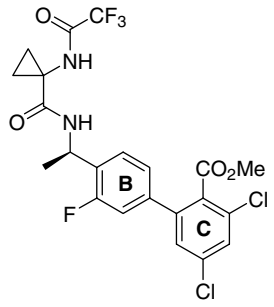

Figure 1. Lead compound **1**.

Keywords: Bradykinin; GPCR; Pain; PXR; P-glycoprotein.
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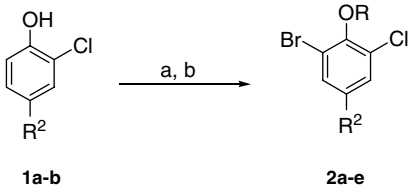
moiety, and it was shown to activate the pregnane X receptor (PXR).[22] Activation of PXR leads to induction of CYP3A4, a cytochrome P450 enzyme that is responsible for the metabolism of many drugs.[23] It is possible, therefore, that PXR activation could cause potential drug–drug interactions. In this paper, we describe our synthetic efforts to modify compound **1** in order to improve its pharmacokinetic profile and eliminate PXR activity.

The compounds described in this study are listed in Tables 1–4 and the general preparative method used to access the compounds is outlined in Schemes 1 and 2. Commercially available phenols **1a**–**b** were brominated with NBS followed by alkylation with the appropriate alkyl triflate or halide to provide **2a**–**e**.

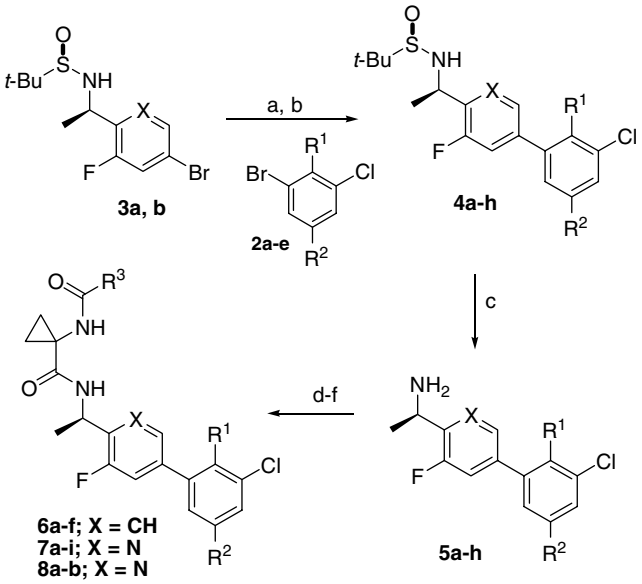
The synthesis of compounds **3a** and **3b** has been previously described,[21] and these were readily converted to the pinacol boronate derivatives which underwent smooth Suzuki coupling with **2a**–**e** to produce the protected biphenyl amines **4a**–**h** (Scheme 2). Deprotection of the sulfinamide in the standard fashion afforded the biphenylamines **5a**–**h**. Subsequent EDCI mediated acylation with 1-(*tert*-butoxycarbonyl)aminocyclopropanecarboxylic acid followed by Boc removal and acylation with the appropriate carboxylic acid afforded target compounds **6a**–**f** for evaluation.

A number of ether replacements were examined for the metabolically labile C-ring methyl ester as shown in Table 1. All compounds displayed nanomolar affinity for the hBK B_1 receptor and had good pharmacokinetics in the rat. For example, ethyl ethers **6a**–**b** both showed good affinity, but the addition of the chlorine at the 5′ position (R^2 = Cl) of the phenyl C-ring had notable improvement in terms of half-life in rat. The difluoroethyl ether (**6c**) maintained similar rat pharmacokinetics to **6b**, but markedly improved binding potency (hK_i = 0.2 nM) whilst the more hindered isopropyl ether **6d** lost about 10-fold in terms of receptor binding. Adding a fluorine to each methyl group (**6e**) returned significant binding affinity, but proved to also confer unacceptable P-gp susceptibility (BA/AB ratio = 5.2). Interestingly, the trifluoroethyl ether **6f** lacked sufficient permeability (<15) for further evaluation.

It has been previously observed that compounds containing a pyridine B-ring had markedly improved physical properties and enhanced CNS penetration than compounds containing a phenyl B-ring.[21] On this basis we chose to incorporate the pyridine B-ring into compound **6c** as a lead structure for further efforts to modify the PXR activation potential by replacement of the trifluoroacetamide moiety.



Scheme 1. Reagents and condition: (a) NBS, CH_3CN, 30 min; (b) i—NaH, HMPA; ii—ROTf or RI or RBr.

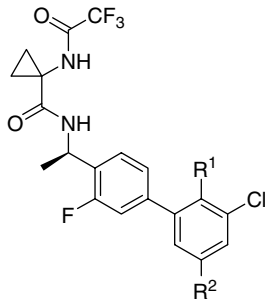


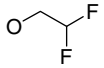
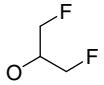
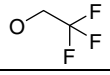
Scheme 2. Reagents and conditions: (a) pinacoldiboron ester, KOAc, DMSO, Pd(dppf)$C1_2$, 80 °C; (b) Pd(dppf)$C1_2$, 80 °C; K_2CO_3, DMSO; (c) 4 *N* HCl dioxane, 0 °C; (d) *tert*-butoxycarbonyl-aminocyclopropane carboxylic acid, EDCI, HOAt, DMF, TEA; (e) HCl, ethyl acetate, 0 °C; (f) R_3CO_2H, EDCI, HOAt, DMF, TEA.

After surveying a variety of heterocyclic replacements (data not shown) for the trifluoroacetamide, a series of promising isoxazoles was settled on for further examination. The compounds in Table 2 summarize the results of this endeavor. First, it should be noted that incorporation of the B-ring pyridine into compound **6c** to provide **7a** was reasonably well tolerated in terms of hBK B_1 binding characteristics, maintained good P-gp and dog pharmacokinetic properties, but did not reduce the PXR activation liability for the compound. Replacement of the trifluoroacetamide with a 3-isoxazole carboxamide (**7b**) maintained excellent receptor affinity and dog pharmacokinetics relative to compound **1**, but more importantly had the desired effect of significantly reducing the PXR activation to 23% relative to the effect of rifampicin at 10 μM. Alkyl group incorporation and modification to the 5-position of the isoxazole (compound **7c**–**e**) eroded receptor binding to a modest extent and began to confer undesirable susceptibility to P-gp mediated efflux with larger alkyls (**7e**).

The corresponding 5-isoxazole carboxamide (**7f**) was also examined and found to have enhanced binding affinity (hK_i = 0.4 nM) and maintained low PXR activation potential relative to **1**, but was a modest P-gp substrate (BA/AB = 3.9). Incorporation of an electron donating methoxy group (**7g**) afforded a desirable P-gp profile, improved the dog half-life, and exhibited the lowest PXR activation potential amongst the series (8% at 10 μM relative to rifampicin). The related ethoxy isoxazole **7h** maintained a similar profile to **7g**, while the 3-chloro isoxazole **7i** exhibited very good receptor affin-

Table 1. Effects of alkyl ether modifications on BK B_1 receptor binding affinities and PK properties



Compound	R^1	R^2	hBK_1[a]	P-gp[b]	P_{app}[c]	Rat F%[d]	Rat $t_{1/2}$	Rat Cl	PXR (% act at 10 μM)[e]
1	CO_2Me	Cl	0.4	1.8	18	38	4.6	19	77
6a		H	0.9	1.9	29	48	1.9	16	nd
6b		Cl	1.3	1.9	18	38	7.5	11.5	62
6c		Cl	0.2	2.1	26	nd	7.6	5.8	95
6d		Cl	2.3	nd	nd	nd	nd	nd	nd
6e		Cl	0.9	5.2	28	56	5.0	1.6	nd
6f		Cl	1.8	2.0	6.3	35	27	1.1	45

[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%.
[e] Relative to rifampicin.

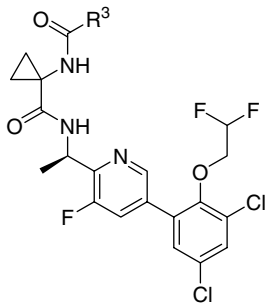
ity with a long dog half-life, but the PXR activation increased considerably.

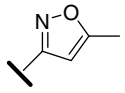
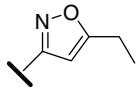
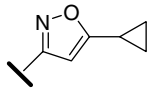
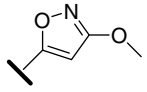
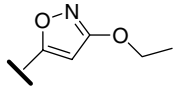
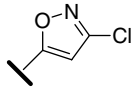
To further characterize compounds in Table 2 and related analogs, ex vivo receptor binding experiments were carried out. The transgenic rat construct employed in these studies expresses the hBK B_1 receptor broadly throughout the CNS, including the brain and spinal cord, under the mouse neuronal specific enolase promoter.[24] This rat model has been successfully used to measure the efficiency with which a compound binds to the hBK B_1 receptor expressed in the CNS. In addition, studies of pharmacokinetics in rats and brain penetration in African Green Monkey (AGM) were carried out for selected compounds.

As shown in Table 3, the ethyl ether variant (**8a**) of compound **1** showed a modest decrease in B_1 receptor affinity that was reflected in the Occ_{90} of this analog. Additionally, **8a** has significant PXR activation, and replacement with the 5-isoxazole carboxamide (**8b**) dramatically reduced this activity showing the trifluoroacetamide is a major contributor to the PXR problem. Moreover, the receptor occupancy of **8b** was modestly improved (Occ_{90} = 380 nM) over **1** (Occ_{90} = 450 nM) and has similar brain to plasma ratio in the monkey. Derivatives **7c** and **7g** both had Occ_{90}s commensurate with the level of binding affinity, exhibit CNS penetration in monkey, and have low PXR activation. In order to make a distinction among compounds **7c**, **7g**, and **8b**, the pharmacokinetics for these compounds of interest were compared across species (Dog and Rhesus Monkey) and results of this comparison are presented in Table 4.

It is clear from the dog and rhesus data shown in Table 4 (as well as the rat data shown in Table 3) that the ether replacements for the ester led to a dramatic improvement in pharmacokinetic properties across species. The properties of 5-methyl isoxazole **7c** were significantly better in the dog, but did not show any improvement over **1** in rhesus. The related 3-methoxy isoxazole **7g** showed superior bioavailability (>90%) and the longest half-lives in dog (21 h) and rhesus (6.2 h) for any analogs examined in this study. Ethyl ether variant **8b** exhibited low bioavailability in rat (7%) and rhesus

Table 2. Effects of isoxazole modifications on BK B_1 receptor binding affinity, P-gp transport, Dog pharmacokinetic properties, and PXR activity



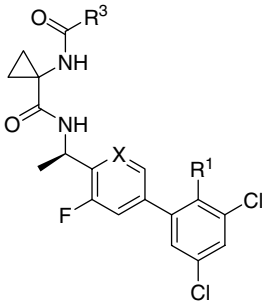
Compound	R^3	hBK_1[a]	P-gp[b]	P_{app}[c]	Dog $t_{1/2}$[d]	Dog Cl	PXR (% act at 10 μM)[e]
7a	CF_3	1.5	2.2	23	21	2.3	99
7b		1.2	1.8	35	18	2.0	23
7c		1.4	1.5	23	9.4	4.5	28
7d		1.9	2.0	25	4.5	14	37
7e		2.6	3.8	18	6.9	13	58
7f		0.4	3.9	29	4.0	5.9	21
7g		0.5	2.0	28	21	1.2	8
7h		1.1	2.1	25	13.5	3.0	37
7i		0.2	1.6	26	34	2.6	68

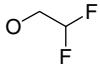
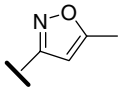
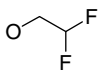
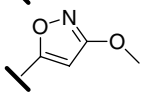
[a] Values represent the numerical average of at least two experiments. Interassay variability was ±25% for the binding assays (K_i, nM).
[b] MDR1 Directional transport ratio (B to A)/(A to B). Values represent the average of three experiments and interassay variability was ±20%.
[c] Passive permeability (10^{-6} cm/s).
[d] Mongrel dog cassette ($n = 2$). IV dose = 1 mg/kg. Interanimal variability was less than 20% for all values. Half-life is represented in hours, and Cl in mL/min/kg.
[e] Relative to rifampicin.

(3%) with shorter half-lives across species relative to **7g** exemplifying the clear advantage of the difluoroethyl ether moiety in terms of pharmacokinetic properties.

To further establish the reduction in PXR activity was driven largely by the incorporation of the isoxazole heterocycle in lieu of the trifluoroacetamide, the 3-methoxyisoxazole version of compound **1** was prepared in the form of compound **9**. This analog exhibited similar receptor binding and gratifyingly also showed a marked decrease in PXR activation relative to **1**. However, the P-gp profile was not satisfactory with a directional transport ratio of 5.1 due to the presence of the isoxazole in combination with the methyl ester. It is likely that the extra heteroatoms present due to the ester of **9** confer this P-gp susceptibility, while the difluoroethyl ether in **7g** balances out this risk and is devoid of the P-gp liability. Thus, while the replacement of the trifluoroacetamide with the isoxazole carboxamide was essential to address the PXR issue, the ether replacement for the ester was required in order to make up for the commensurate rise in P-gp liability

Table 3. Ex vivo occupancy and CNS penetration in AGM for selected compounds



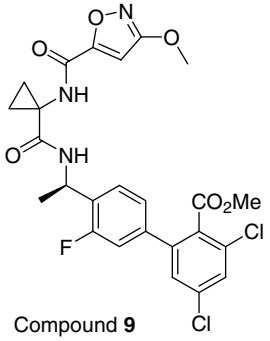
Compound	R^1	R^3	X	hBK_1[a]	Rat F%[b]	Rat $t_{1/2}$	Rat Cl	Occ_{90} (nM)[a]	AGM CNS/Plasma[c]	PXR (% act at 10 μM)[d]
1	CO_2Me	CF_3	CH	0.4	38	4.6	19	450	1.1	77
8a		CF_3	N	1.5	8	5	11.3	1450	2.2	89
8b			N	0.6	7	1.9	10	380	1.4	15
7c			N	1.4	30	3	5.1	940	0.9	28
7g			N	0.5	44	4.5	2.4	540	0.7	8

[a] Values represent the numerical average of at least two experiments. Interassay variability was ±25% (K_i, nM).
[b] 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%.
[c] Values are means of two experiments.
[d] Relative to rifampicin.

Table 4. 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	19	1.6	20	20	3.9	11.2
7c	21	9.4	4.5	25	1.6	9.1
7g	94	21	1.2	93	6.2	1.6
8b	55	4.5	7.0	3	1.2	15

[a] Mongrel dogs ($n = 2$). Oral dose 3 mg/kg, IV dose = 1 mg/kg. Interanimal variability was less than 20% for all values and Cl is in mL/min/kg.
[b] Rhesus Monkeys ($n = 2$). Oral dose 3 mg/kg, IV dose = 1 mg/kg. Interanimal variability was less than 20% for all values and Cl in mL/min/kg.



Compound **9**
hBK1 = 0.4 nM
PXR = 40% @ 10 μM
P-gp = 5.1

In conclusion, systematic structural modifications to compound **1** led to analogs displaying reduced potential for P450 induction via activation of the PXR pathway. Exchange of the methyl ester in compound **1** with a difluoroethyl ether moiety yielded compounds with good hBK B_1 receptor affinity and marked improvements in pharmacokinetic profiles. Replacement of the trifluoromethyl functional group in **1** with 3-methoxyisoxazole gave compounds with reduced susceptibility to P-gp mediated efflux, attenuated PXR activation, and excellent pharmacokinetic properties. The combination of all of the above modifications in one hybrid structure resulted in the discovery of compound **7g** which displayed high hBK B_1 receptor binding affinity and efficiently occupied the hBK B_1 receptor in the CNS of the transgenic mouse. Accordingly, **7g** was selected for further evaluation as a potential clinical candidate.

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