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Bradykinin B_1 receptor antagonists: An α-hydroxy amide with an improved metabolism profile

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ABSTRACT

A series of carbo- and heterocyclic α-hydroxy amide-derived bradykinin B_1 antagonists were prepared and evaluated. A 4,4-difluorocyclohexyl α-hydroxy amide was incorporated along with a 2-methyl tetrazole in lieu of an oxadiazole to afford a suitable compound with good pharmacokinetic properties, CNS penetration, and clearance by multiple metabolic pathways.

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One component of the response of humans and animals to tissue injury is mediated by bradykinin (BK) peptides which are produced in plasma soon after injury occurs. The physiological effects of these peptides, including pain and inflammation,[1,2] are regulated by the G-protein-coupled receptors, B_1 and B_2.[3–5] The B_2 receptor is constitutively expressed, and the current thinking is that it is responsible for the incipient acute pain response following injury. The native ligands for the B_2 receptor are the peptides bradykinin (BK = Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) and kallidin (Lys-BK). The metabolites of these peptides, [des-Arg9] bradykinin and [des-Arg10] kallidin, are agonists for the B_1 receptor, which is induced subsequent to the occurrence of the injury.

Animal models have demonstrated that bradykinin B_1 receptor antagonists attenuate the response to painful stimuli.[6–9] It has been further shown that transgenic B_1 knockout mice exhibit reduced sensitivity to painful stimuli while appearing normal in all other respects.[10] In addition, experimental evidence has been gathered that indicates that the B_1 receptor is constitutively expressed in the central nervous system of rats and mice.[11] This would imply a role for the B_1 receptor not only in the periphery but also in the CNS. Therefore, CNS penetrant bradykinin B_1 receptor antagonists are highly desirable because they may be much more efficacious than peripheral B_1 antagonists.[12]

Recently, we reported a series of α-hydroxy amide-based bradykinin BK_1 receptor antagonists.[13] For example, compound **1** bearing an oxadiazole ester surrogate had sub-nanomolar affinity for the B_1 receptor, good pharmacokinetics across three species, and exhibited efficient receptor occupancy in an ex vivo transgenic mouse model. However, **1** was metabolized exclusively by a single P450 in the form of CYP2B6 raising the possibility for drug–drug interactions (Fig. 1). In addition, this oxidation by CYP2B6 led to a major circulating metabolite in vivo (**2**), which also showed excellent hBK_1 binding affinity and good pharmacokinetic properties. Accordingly, we sought to prepare compounds that would be oxidized by multiple CYP enzymes and/or have alternative clear-

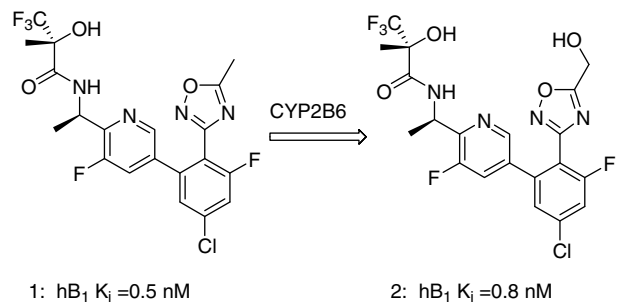

Figure 1. Metabolism of compound **1**.

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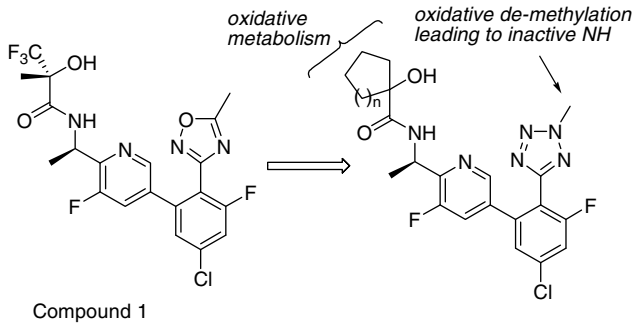


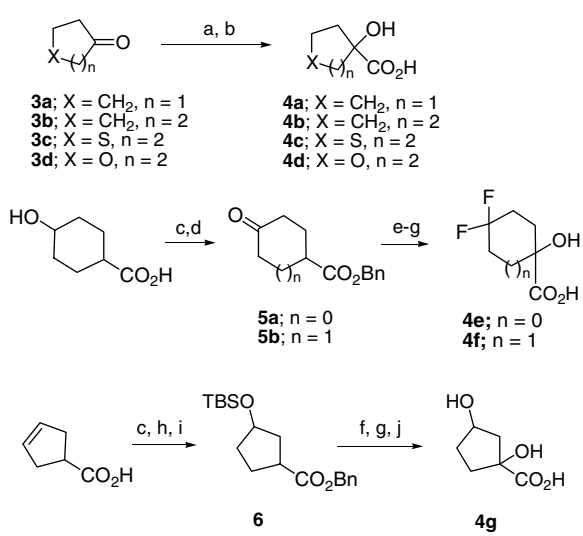
Figure 2. Strategy to address metabolism.

ance pathways, while avoiding the production of a major circulating active metabolite.

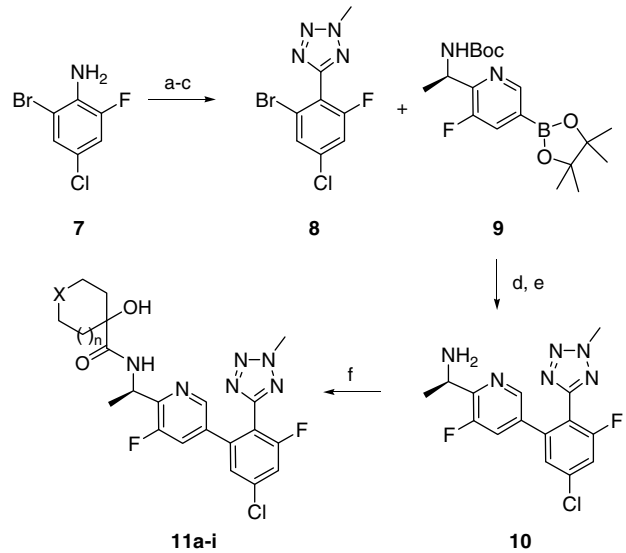
The strategy to overcome these issues is shown in Fig. 2. Previous work on a series of related biaryl bradykinin B_1 antagonists has shown that an *N*-2-methyl tetrazole was an effective isostere for a methyl ester.[14] Oxidative dealkylation led to the acidic N–H-tetrazole which had no significant affinity for the B_1 receptor. Therefore, we wanted to incorporate a tetrazole in lieu of the oxadiazole to address the circulating metabolite issue. To confer additional oxidation potential to the molecule, hopefully by P450's other than CYP2B6, we planned to remove the metabolism resistant trifluoromethyl group and incorporate an aliphatic cyclopentane ring.

The methods used to prepare the α-hydroxy carboxylic acids **4a–g** are shown in Scheme 1. When the ketone starting materials were available (**3a–d**), the compounds were prepared via hydrolysis of the intermediate cyanohydrins. Alternatively, the compounds could be prepared from the corresponding esters, via hydroxylation using the method described by Davis.[15]

The requisite tetrazole **8** was prepared in a three-step sequence (diazotization–cyanation, tetrazole formation, and methylation) from commercially available aniline **7** (Scheme 2). The *N*-2-methyl tetrazole **8** was formed as a 1:1 mixture with the *N*-1 isomer (not shown), but was readily separable. Suzuki coupling of **8** with boronate **9**,[16] followed by removal of the Boc group with HCl, provided amine **10**. Final acylation with the appropriate acid **4a–g**, afforded target molecules **11a–i**. Compound **11d** was prepared via TPAP oxidation of **11b**.



Scheme 1. Reagents and conditions: (a) NaCN, H_2O, $Na_2S_2O_3$. (b) HCl, reflux. (c) BnBr, K_2CO_3, DMF. (d) PCC, DCM. (e) Deoxo-fluor, CH_2Cl_2, rt. (f) KHMDS, THF, −78 °C, Davis Oxaziridine. (g) Pd/C, H_2, EtOAc. (h) i—BH_3, THF. ii—$NaBO_3$, H_2O. (i) TBSCl, imidazole, DMF. (j) 1 *N* HCl, THF.



Scheme 2. Reagents and conditions: (a) $NOBF_4$, KCN. (b) $TMSnN_3$, toluene, 120 °C. (c) MeI, K_2CO_3, DMF. (d) $PdCl_2$·dppf, DMSO, K_2CO_3, H_2O. (e) HCl, EtOAc. (f) **4a–g**, EDC, TEA, HOBt, DMF.

Compounds **11a–i** were evaluated for human bradykinin B_1 binding affinity and susceptibility to efflux by the transporter P-glycoprotein (P-gp). In addition, pharmacokinetic properties in dog were screened to further triage analogs for additional testing.[17] Results are shown in Table 1.

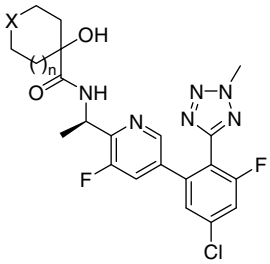
Cyclopentane derivative **11a** proved to have high affinity for the B_1 receptor, similar to that for **1**, and was not a substrate for human P-gp (BA/AB ratio = 2.1). This compound also exhibited low dog clearance, but a shorter half-life relative to **1**. Incorporation of a sulfide (**11b**) was similarly well tolerated with only a slight increase in P-gp susceptibility, but sulfoxide and sulfone analogs **11c-d** proved to be impressive substrates for P-gp. This is likely due to incorporation of the additional heteroatom(s), as the related ketone **11e**, which despite having impressive binding and dog pharmacokinetic properties, also proved to be subject to P-gp-mediated efflux.

In order to avoid the undesired effects on P-gp and to improve the pharmacokinetic profile of **11a**, a difluoromethylene unit was incorporated into the cyclopentane ring. This led to a loss of symmetry to produce diastereomers **11f** and **11g** after chromatographic resolution. Both diastereomers maintained good B_1 binding affinity, but it was fortuitous that the modestly more potent **11g** also exhibited a markedly better half-life in the dog ($t_{1/2}$ = 19 h) with a more preferable P-gp profile. Homologation to the symmetrical six-membered ring analogs was also investigated. Tetrahydropyran **11h** exhibited good affinity, but continued the trend of increasing P-gp efflux (BA/AB = 8.2) with additional heteroatom incorporation. However, difluorocyclohexane **11i** exhibited excellent binding affinity (hK_i = 0.41 nM), P-gp transport, and dog pharmacokinetic profiles (F = 91%) comparable to difluorocyclopentane **11g**.

Analogs **11a**, **11g**, and **11i** were selected for further triage via determination of rat pharmacokinetics and brain penetration in African green monkey. In addition, ex vivo receptor binding experiments were investigated using a transgenic rat construct, which expresses the hBK B_1 receptor broadly throughout the CNS, including the brain and spinal cord.[18] The binding efficiency of a compound to the hBK B_1 receptor expressed in the CNS can therefore be determined with this rat model. Results are shown in Table 2.

With respect to rat pharmacokinetics, difluoro derivatives **11g** and **11i** exhibited better bioavailability (>60%) and lower clearance relative to cyclopentane **11a** (F = 26%, Cl = 10 mL/min/kg). The pharmacokinetic advantage of the difluoro analogs is across spe-

Table 1
Bradykinin B_1 receptor binding affinities, P-gp transport properties, and dog pharmacokinetics



Compound	X	*n*	hBK_1 [a] (nM)	P-gp[b]	P_{app} (10^{-6} cm/s)[c]	Dog *F*% [d]	Dog $t_{1/2}$ [d]	Dog CL [d]
1	–	–	0.66	1.9	27	84	11	0.5
11a	CH_2	0	0.48	2.1	29	nd	4.7	0.6
11b	S	0	0.39	2.9	31	nd	5.4	3.2
11c	S(O)	0	1.6	14.3	24	nd	3.1	2.3
11d	$S(O)_2$	0	0.69	29	22	nd	9.9	0.5
11e	C=O	0	0.67	16	31	nd	9.8	0.3
11f	CF_2	0	1.0	2.6	29	nd	3.0	0.3
11g	CF_2	0	0.67	1.9	32	85	19	0.2
11h	O	1	0.96	8.2	24	nd	nd	nd
11i	CF_2	1	0.41	2.0	31	91	20.3	1.0

[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[a] Mongrel dogs ($n = 2$). Oral dose 3 mg/kg in methocel, IV dose = 1 mg/kg in DMSO. Interanimal variability was less than 20% for all values.

Table 2
Rat pharmacokinetics and transgenic rat receptor occupancy for select compounds

Compound	Rat *F*%[a]	Rat $t_{1/2}$ (h)[a]	Rat CL[a]	Occ_{90} [b]	B/P[c]
11a	26	5.5	10	214	nd
11g	96	4.1	5.2	120	0.55
11i	62	5.6	2.2	140	0.63

[a] *F*% oral bioavailability, half-life is represented in hours, CL in mL/min/kg. Sprague–Dawley rats ($n = 3$). Oral dose = 10 mg/kg in methocel, IV dose = 2 mg/kg in DMSO. Interanimal variability was less than 20%.
[b] Values represent the numerical average of at least two experiments. Interassay variability was ±25% (K_i, nM).
[c] African Green Monkeys ($n = 2$). IV dose = 2 mg/kg. Interanimal variability was less than 20% for all values.

cies and consistent with high microsomal and hepatocyte stability for these three compounds (data not shown).

Both difluoro analogs proved to be very efficient in the ex vivo receptor occupancy assay compared to the cyclopentane derivative. In addition, **11g** and **11i** were found to be brain penetrant in African green monkey with brain to plasma ratios of 0.55 and 0.63, respectively. However, the symmetrical difluorocyclohexane **11i** showed better dose proportionality in rat and dog, and was selected for more in depth metabolic studies.

Metabolic profiling revealed that compound **11i** was metabolized by multiple pathways in human microsomes and hepatocytes (Fig. 3). *N*-Demethylation was mediated by CYP2B6 similar to the hydroxylation of **1** into metabolite **2**. However, unlike the case for **2**, the *N*-desmethyl metabolite had 30 fold weaker affinity (hK_i = 21 nM) for the hB_1 receptor. In addition, CYP3A4 mediated hydroxylation of the difluorocyclohexane ring was seen as a second oxidative pathway. Direct conversion of the tertiary hydroxyl of **11i** into the acyl glucuronide was also observed, although this pathway is much more significant in rat hepatocytes than other species. Hydrolysis of the amide bond was also noted as a fourth biotransformation pathway.

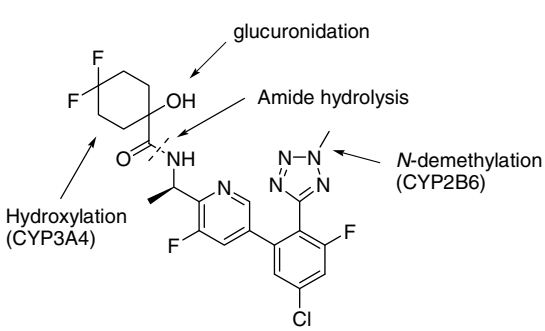


Figure 3. Metabolic profile of **11i**.

In conclusion, a series of 5- and 6-membered carbocyclic and heterocyclic α-hydroxy amide-derived bradykinin B_1 antagonists bearing an *N*-2 methyl tetrazole as an oxadiazole replacement was prepared and evaluated. A number of compounds with excellent B_1 binding affinity, good pharmacokinetic properties, and desirable P-gp transport properties was elucidated. In particular, 4,4-difluorocyclohexane **11i** possessed similar properties to oxadiazole **1**, but was found to undergo biotransformation by multiple pathways lowering the risk of any potential drug–drug interactions. In addition, no major active circulating metabolites were observed. Accordingly, **11i** was selected for additional pre-clinical development as a backup to compound **1**.

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