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Identification of novel, orally bioavailable spirohydantoin CGRP receptor antagonists

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Abstract—A rapid analogue approach to identification of spirohydantoin-based CGRP antagonists provided novel, low molecular weight leads. Modification of these leads afforded a series of nanomolar benzimidazolinone-based CGRP receptor antagonists. The oral bioavailability of these antagonists was inversely correlated with polar surface area, suggesting that membrane permeability was a key limitation to absorption. Optimization provided compound **12**, a potent CGRP receptor antagonist (K_i = 21 nM) with good oral bioavailability in three species.

Migraine is a chronic, highly disabling disorder that afflicts more than 10% of adults and is often characterized by severe, unilateral headache.[1] The pathophysiology is thought to involve dilation of cranial blood vessels and activation of the trigeminovascular system.[2] The preferred treatment for acute migraine is the triptan class of 5-$HT_{1B/1D}$ receptor agonists, although these compounds are contraindicated for patients with cardiovascular disease because they are potent vasoconstrictors.[1] A number of lines of evidence have implicated the neuropeptide calcitonin gene-related peptide (CGRP) as a key player in migraine pathology.[3] Moreover, the potent, intravenously administered CGRP receptor antagonist BIBN4096BS (olcegepant) demonstrated clinical proof of concept for the acute treatment of migraine headache.[4] BIBN4096BS achieved comparable efficacy to that seen historically for triptans and no serious adverse effects or changes in hemodynamics were observed.[5] Therefore, a CGRP receptor antagonist suitable for oral administration may represent a significant advance in acute migraine therapy.

Our program to develop orally bioavailable, small molecule CGRP receptor antagonists began with the identification of benzodiazepinone **1** (Fig. 1, CGRP K_i = 4800 nM) via high throughput screening.[6] Initial work focused on replacement of the tetralin spirohydantoin fragment with a variety of piperidinyl privileged structures and led to nanomolar antagonists, including some with modest oral bioavailability.[6,7]

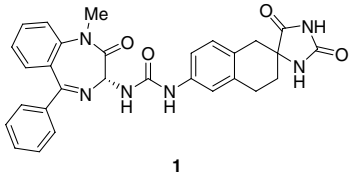

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Figure 1. High throughput screening lead.

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In a complementary approach, we sought to replace the benzodiazepinone moiety in **1** using a rapid analogue strategy, as illustrated in Figure 2. Briefly, three spirohydantoin templates (two tetralins and one indane) were chosen to display potential benzodiazepinone replacements from different vectors with respect to the hydantoin pharmacophore. The templates were derivatized using several linkers (e.g., X = NH(CO)NH, CONH, SO_2NH, NHCO) to attach a wide variety of fragments (generally with $M_w < 200$).

Screening of such analogues yielded a number of structurally diverse antagonists with $K_i < 100$ μM, including representatives of the three templates shown in Figure 2 and a variety of linkers. Compounds **2** and **3** (Table 1), in which a simple benzoxazolinone was substituted for the benzodiazepinone in **1**, were among the most potent leads and were selected for further evaluation. The tetralin **2** ($K_i = 607$ nM) exhibited improved intrinsic potency relative to **1** combined with reduced molecular weight, while indane **3** offered a further boost in potency ($K_i = 309$ nM). A small potency advantage for indanes versus tetralins was consistently observed in this series of compounds so the remaining discussion will focus on indanyl analogues.

Methylation of the spirohydantoin moiety provided **4** (Table 1), which exhibited improved potency compared with **3** in the radioligand binding assay and as a functional antagonist in the cell-based assay ($IC_{50} = 1400$ nM).[9] Resolution of the racemic **4** demonstrated that the potency essentially resided in the (*R*)-enantiomer (**5**), while the (*S*)-enantiomer (**6**) was significantly less active. The preference for (*R*)-indanylspirohydantoin was consistent in this series of antagonists, therefore only the (*R*)-enantiomers of the analogues in Table 2 will be discussed (vide infra). Replacement of the benzoxazolinone in lead **3** with benzimidazolinone gave compound **7**, which was equipotent with **3** in the binding assay but about 4-fold more potent in the cell-based assay. Substitution at the benzimidazolinone nitrogen led to the methyl analogue **8** and phenyl analogue **9**, both of which exhibited enhanced intrinsic potency (Table 1). Combining the modifications in compounds **5** and **7** afforded benzimidazolinone **10** (Table 2), which possessed similar affinity to **5** for the CGRP receptor ($K_i = 43$ nM).

The unsubstituted benzimidazolinone nitrogen in compound **10** could be derivatized with a wide variety of substituents with no loss in potency. A subset of these modifications, in which this position is substituted with a series of cyclic groups, is shown in Table 2. The most potent of these analogues were the thiazole **13** and isothiazole **15**, but a range of aryl, heteroaryl, and saturated rings were well tolerated. In general, there was a good correlation between the K_i values obtained in the radioligand binding assay and the IC_{50} values for functional antagonism, with the latter value being 3- to 5-fold less potent than the former. Because a range of substituents on the benzimidazolinone were consistent with good potency, we reasoned that this may be an ideal site to

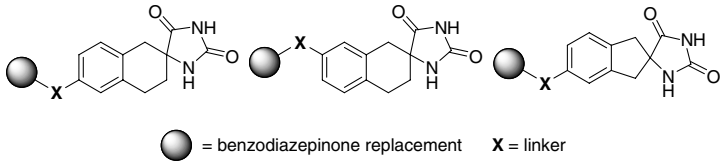
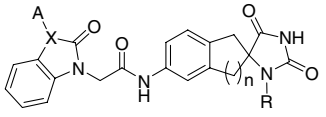


Figure 2. Rapid analogue strategy.

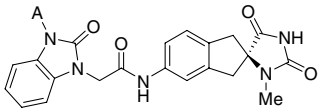
Table 1. CGRP antagonist activity of benzoxazolinone and benzimidazolinone compounds

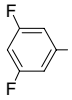
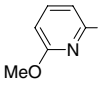


Compound	A	X	*n*	R	Stereo[a]	CGRP K_i[b,c] (nM)	cAMP IC_{50}[b,d] (nM)
2	—	O	2	H	*RS*	607 ± 35 (3)	3300 (2)
3	—	O	1	H	*RS*	309 ± 61 (6)	2900 (2)
4	—	O	1	Me	*RS*	103 ± 23 (4)	1400 (2)
5	—	O	1	Me	*R*	48 ± 5 (3)	350 (2)
6	—	O	1	Me	*S*	1050 ± 230 (3)	—
7	H	N	1	H	*RS*	280 ± 100 (4)	690 (1)
8	Me	N	1	H	*RS*	78 ± 11 (4)	530 ± 180 (3)
9	Ph	N	1	H	*RS*	87 ± 35 (6)	430 (2)

[a] Stereochemistry of spirohydantoin.
[b] Mean value ± standard deviation, where appropriate; number of replicates in parentheses.
[c] K_i values for competition with ^{125}I-hCGRP determined using membranes from HEK293 cells stably expressing cloned human CLR/RAMP1 (line E10).[8]
[d] Inhibition of CGRP-induced cAMP production in the E10 cell line.[8]

Table 2. CGRP antagonist activity of benzimidazolinone compounds



Compound	A	CGRP K_i (nM)[a]	cAMP IC_{50} (nM)[a]
10	H	43 ± 25 (7)	120 (2)
11		29 ± 11 (5)	180 (2)
12		21 ± 8 (7)	78 ± 23 (3)
13		8.7 ± 2.5 (5)	24 (2)
14		20 ± 4 (5)	61 ± 3 (3)
15		6.8 ± 1.7 (7)	39 (2)
16		15 ± 6 (4)	54 (2)
17		10 ± 3 (9)	50 (2)
18		25 ± 4 (5)	110 (2)
19		12 ± 1 (5)	64 (2)
20		9.5 ± 5.8 (8)	50 ± 23 (3)
21		17 ± 2 (4)	64 (2)

[a] See footnote to Table 1 for details.

modify physical properties in an attempt to modulate the pharmacokinetic profile of such compounds.

Table 3 summarizes pharmacokinetic data determined in rats for compounds **11**–**21**. The compounds are presented in order of increasing calculated polar surface area[10] (PSA) and it is apparent that there is an inverse correlation between this parameter and oral bioavailability (F). For example, analogues **11**–**13**, which had the lowest PSA values, had good oral bioavailability (F = 20–36%). On the other hand, analogues **19**–**21**, which had the highest PSA values, exhibited essentially no bioavailability (F = 0–1%). This behavior is consistent with oral absorption being limited by membrane permeability.[10]

It should be noted that oral bioavailability is also dependent on other variables, including solubility, rate of dissolution, pK_a, and metabolism.[12] Perhaps because the compounds in Table 3 were closely related in structure, some of the variability in these other parameters was minimized and a trend related to PSA was observed more clearly. For example, pK_a was probably not a major factor since none of the compounds were very basic. In terms of metabolism, the bioavailable compounds in Table 3 had low clearance but other compounds with similar clearance were not orally bioavailable (e.g., **17** and **19**). Thus, low clearance may be necessary but not sufficient for oral bioavailability in these analogues.

It is clear that compounds must possess sufficient solubility in order to be orally bioavailable.[13] However, most of these analogues had poor aqueous solubility and there was no clear relationship with bioavailability. Compounds **12** and **13** (F = 20–36%) had extremely low solubility (<1 μg/mL at pH 7.4), while compounds **20**

Table 3. Pharmacokinetic data for benzimidazolinone CGRP antagonists

Compound	PSA[a] (Å^2)	F[b] (%)	iv $t_{1/2}$[c] (h)	Cl[c] (mL/min/kg)	Vd_{ss}[c] (L/kg)	P_{app}[d] ($\times 10^{-6}$ cm/s)
11	117	24	6.5	3.1	1.4	15
12	125	36	1.6	3.4	0.47	11
13	127	20	2.0	3.7	0.54	13
14	128	5	0.9	9.0	0.43	4
15	131	1	1.0	60	2.0	—
16	134	13	1.2	11	0.84	—
17	137	0	2.8	7.5	1.8	—
18	138	2	1.1	23	0.50	—
19	140	1	1.1	11	0.64	8
20	146	0	0.5	46	0.95	4
21	158	0	1.1	31	0.80	0.3

[a] Polar surface area calculated by method of Clark[10] and was an average of the values for 8–10 energy-minimized conformers.
[b] Oral bioavailability in rats following dosing (10 mpk) in 1% methylcellulose.
[c] Pharmacokinetic parameters in rats following iv dosing (2 mpk) in DMSO.
[d] Passive permeability through CACO-2 monolayer determined using 20 μM test compound in the presence of 5 μM cyclosporin.[11]

and **21** ($F = 0\%$) were more soluble by orders of magnitude (>100 μg/mL at pH 7.4). It appeared that these analogues possessed minimal solubility but that it could be sufficient for reasonable oral absorption. Passive permeability determined on a limited number of analogues using CACO-2 monolayers was generally consistent with the inverse correlation between F and PSA (see Table 3). Thus, those compounds with good permeability ($P_{app} > 10 \times 10^{-6}$ cm/s) were orally bioavailable ($F \geqslant 20\%$) while those with lower permeability ($P_{app} < 10 \times 10^{-6}$ cm/s) were poor ($F \leqslant 5\%$). Interestingly, compounds **14**, **19**, and **20** exhibited poor oral bioavailability but had CACO-2 P_{app} values that are often consistent with good absorption.[12] Apparently, the low solubility of analogues in this series led to a more stringent requirement for good intestinal permeability.

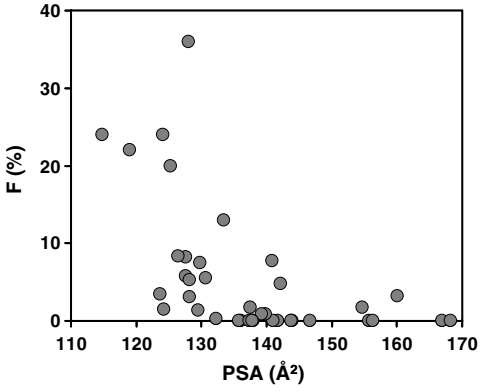


Figure 3. Oral bioavailability in rats (%) versus calculated polar surface area (Å^2) for spirohydantoin CGRP antagonists. Oral bioavailability and polar surface area determined as described in Table 3. The graph represents the results for 39 benzoxazolinone and benzimidazolinone spirohydantoin analogues.

The relationship between oral bioavailability and polar surface area seen in Table 3 was observed generally for related spirohydantoins. Figure 3 shows a plot of oral bioavailability in rats against PSA for compounds in this structural class. For such analogues, it appears that the probability of good oral bioavailability is much better for compounds with PSA < 130 Å^2 than for those compounds with PSA > 140 Å^2, consistent with published data.[10] The recognition that membrane permeability was a key limitation to oral absorption in this structural class, combined with the use of PSA as a readily calculated surrogate for permeability, facilitated the design of orally bioavailable spirohydantoin CGRP antagonists.

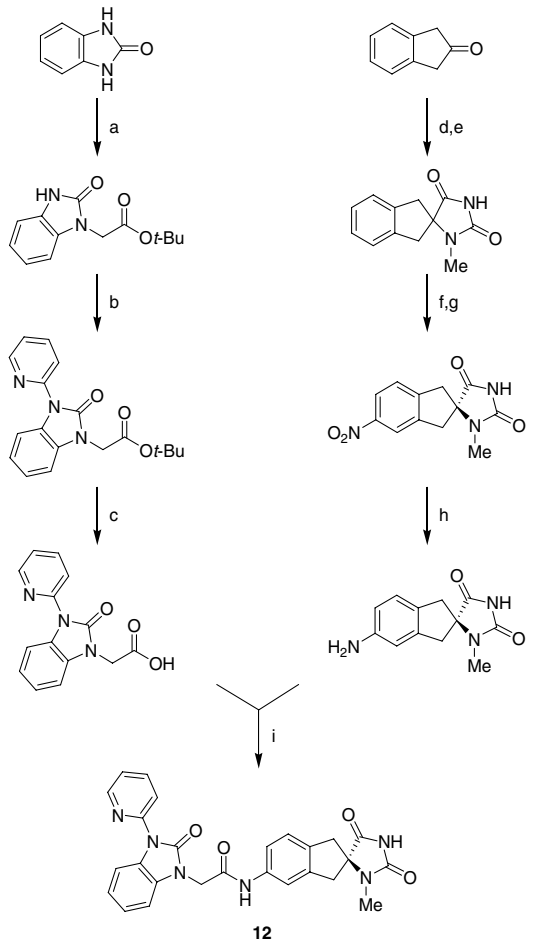
Compound **12**, which possessed the best combination of oral bioavailability in rats and functional potency in this series, was selected for more detailed pharmacokinetic characterization. A summary of these data is shown in Table 4. In analogy with the observations in rats, **12** was orally bioavailable in dogs ($F = 83\%$) and rhesus ($F = 29\%$), and exhibited low plasma clearance in all three species. Importantly, compound **12** was not an inhibitor of CYP3A4, CYP2C9, or CYP2D6 ($IC_{50} > 10$ μM for these enzymes).

The synthesis of compound **12**, which is representative of the methodology used to prepare the compounds described herein, is shown in Scheme 1.[14]

Table 4. Pharmacokinetic data for compound **12**

Species	po dose[a] (mpk)	iv dose[b] (mpk)	F (%)	iv $t_{1/2}$ (h)	Cl (mL/min/kg)	Vd_{ss} (L/kg)
Rat	10	2	36	1.6	3.4	0.47
Dog	2	0.5	83	4.8	1.7	0.69
Rhesus	2	0.5	29	9.6	0.85	0.31

[a] Compound dosed in 1% methylcellulose.
[b] Compound dosed in DMSO.



Scheme 1. Synthesis of compound **12**. Reagents and conditions: (a) NaH, *tert*-butyl bromoacetate, DMF, 0 °C, 27%; (b) 2-bromopyridine, Cu, CuCl, KOAc, py, 100 °C, 84%; (c) HCl, EtOAc, 0 °C, 100%; (d) $MeNH_3Cl$, KCN, MeOH, H_2O, 0 °C to rt, then HCl, Et_2O, 75%; (e) KOCN, AcOH, H_2O, then HCl, H_2O, 79%; (f) 90% HNO_3, 60%; (g) ChiralPak AD, 90:10 CH_3CN/MeOH, second major peak is (*R*)-enantiomer; (h) H_2, Pd/C, EtOAc, MeOH, 100%; (i) EDC, HOBT, DIEA, DMF, 89%.

Most of the carboxylic acid intermediates containing benzimidazolinone or benzoxazolinone moieties were synthesized using standard alkylation or arylation chemistry, such as the sequential derivatization of both benzimidazolinone nitrogens illustrated in Scheme 1. The spirohydantoins were derived from 2-indanone via Bucherer–Bergs methodology, followed by nitration with fuming nitric acid. Separation of the enantiomers of the 5-nitroindane intermediate was achieved using a ChiralPak AD column, to provide the active (*R*)-enantiomer.[15] Following reduction to the corresponding aniline, EDC-mediated coupling with the carboxylic acid of interest provided the final compound.

In conclusion, a novel series of spirohydantoin-based CGRP receptor antagonists was identified using a rapid analogue strategy. In this series of compounds, membrane permeability was apparently a key limitation to oral absorption and consideration of calculated polar surface area values facilitated the design of orally bioavailable compounds. These studies led to compound **12**, which combined good potency with excellent pharmacokinetics in three species.

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