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Identification of potent, highly constrained CGRP receptor antagonists

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ABSTRACT

A novel series of potent CGRP receptor antagonists containing a central quinoline ring constraint was identified. The combination of the quinoline constraint with a tricyclic benzimidazolinone left hand fragment produced an analog with picomolar potency (**14**, CGRP K_i = 23 pM). Further optimization of the tricycle produced a CGRP receptor antagonist that exhibited subnanomolar potency (**19**, CGRP K_i = 0.52 nM) and displayed a good pharmacokinetic profile in three preclinical species.

Migraine is a disabling neurovascular disorder that affects approximately 13% of the general population.[1] A migraine attack is characterized by unilateral head pain and is often accompanied by phonophobia, photophobia, and nausea. Calcitonin gene-related peptide (CGRP), a 37 amino acid neuropeptide, has been implicated in the pathogenesis of migraine, and therefore, antagonism of the CGRP receptor has been proposed as a novel approach for migraine therapy.[2] Clinical studies have demonstrated the effectiveness of CGRP receptor antagonists for the acute treatment of migraine with both the intravenously-administered olcegepant[3] and the orally-administered telcagepant.[4] Furthermore, recently published Phase III clinical results have shown telcagepant 300 mg to have similar efficacy to a positive control (zolmitriptan 5 mg), with fewer reported adverse events.[5] Our research program continued to focus on developing non-peptidic, orally bioavailable CGRP receptor antagonists as novel therapeutics.

A previous communication from these laboratories described the discovery of novel CGRP receptor antagonist **1** (Fig. 1).[6] Subsequent communications have described the evolution of both the left and right hand fragments of **1** to produce more potent antagonists. Optimization of the benzimidazolinone fragment provided the more potent tricyclic analog **2**;[7] whereas replacement of the spirohydantoin fragment with a spiroazaoxindole further improved potency to provide **3**.[8] While compounds **2** and **3** are very potent CGRP receptor antagonists, they exhibit poor overall pharmacokinetic profiles. Calculated polar surface area (PSA) has been shown to correlate with intestinal permeability.[9] In a related series of spirohydantoin compounds, an inverse relationship between PSA and bioavailability has been observed.[6] In an effort to reduce the PSA of **2** (158 Å^2) and **3** (149 Å^2), replacement of the central amide linker, one area of the molecules that had thus far eluded modification, was investigated.

A torsion plot with respect to rotation around the central amide of **1** indicated a low energy conformation that was planar with respect to the amide carbonyl and aryl group of the spiroindane (i.e., $\Phi = 0°$ or 180°). Substitution on the aryl group at the positions *ortho* to the amide linker was predicted to bias one of two possible planar conformations. To that end, the *o*-fluoro analogs **4** and **5** were synthesized, with each predicted to favor only one of the low energy, planar conformations by approximately 3 kcal/mol (Fig. 2). The similar potency of compounds **1** (K_i = 20 nM) and **4** (K_i = 55 nM), and the loss of potency with compound **5** (K_i = 720 nM), suggested that the bioactive conformation with respect to the central amide was the one depicted for structure **4** in Figure 2 (i.e., $\Phi = 0°$).

In order to further elucidate the preferred binding mode of compounds like **1**, we sought to incorporate a constraint that would

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mimic the proposed bioactive conformation of **1**. Evaluation of the two benzimidazole analogs **6** (K_i = 320 nM) and **7** (K_i = 6600 nM) provided further evidence for the proposed bioactive conformation, albeit with the loss of activity observed with **6** suggesting the presentation of the left hand fragment was suboptimal (Fig. 3). The benzoxazole **8** suffered an additional loss of potency (K_i = 6000 nM) compared to the corresponding benzimidazole **6** (K_i = 320 nM). It was anticipated that expansion of the constraint to a six membered ring might better approximate the geometry of the central amide of **1**. Indeed, when the racemic quinoxaline **9** was evaluated, a modest increase in potency was observed (K_i = 185 nM). The quinoline analog **10** exhibited an even greater improvement in potency, with a K_i = 17 nM making it equipotent to amide **1** and providing further support for the proposed bioactive conformation.

The quinoline constraint was evaluated with a number of left hand fragments, both with the spiroazaoxindole and spirohydantoin moieties (Table 1). In the related amide series of compounds, the spiroazaoxindole imparted a significant improvement in potency over the corresponding hydantoins.[8] However, we anticipated that the more polar spirohydantoin moiety might help improve the physical properties (e.g., solubility, protein binding) of the lipophilic quinoline analogs, as their calculated log $D_{7.4}$ (ACD/Labs 11.0) was typically at least one log unit lower than for the corresponding spiroazaoxindoles. The simple 3,4-dihydro-2-quinolinone **11** exhibited similar potency to the lead compound **10** (K_i = 8.9 nM). As anticipated, replacement of the spirohydantoin with the spiroazaoxindole (**12**) provided an order of magnitude increase in potency in both the binding and cell-based assays. Combination of the quinoline with an optimized tricyclic benzimidazolinone[7] produced subnanomolar CGRP receptor antagonist **13** (K_i = 0.30 nM). Once again, incorporation of the spiroazaoxindole produced a significant increase in potency, with the corresponding compound **14** exhibiting picomolar potency (K_i = 23 pM) in the binding assay. Unfortunately, compound **14** showed no bioavailability when dosed in rats (Table 2). In an effort to further reduce the PSA of **14** (130 Å^2), modification or deletion of one of the remaining polar amides was investigated. Modification of either the spirohydantoin or azaoxindole has been shown to drastically reduce the potency of related compounds.[8] Therefore, effort was focused on modification of the amides of the tricyclic benzimidazolinone rings. Simple methylation of the secondary amide in the tricycle caused a marked loss of potency (**15** and **16** vs **13** and **14**), as well as a more pronounced serum shift in the functional assay. Replacement of the benzimidazolinone with an indoline served to reduce PSA and introduce a weakly basic amine into the structure, which we hoped would improve solubility in acidic environments.[10] Indolines **17** (PSA = 116 Å^2) and **18** (PSA = 108 Å^2, data for most potent epimers shown) showed similar potency in the binding assay to **15** and **16**, however, the serum shift observed in the functional assay was attenuated. Gratifyingly, these indoline analogs demonstrated improved bioavailability and reduced intrinsic clearance (Table 2) in the rat, especially in the spirohydantoin series (rat F = 41%, Cl = 11 mL/min/kg). Addition of a 3-methyl substituent to the indoline produced analogs **19** and **20**, which exhibited similar potency to the benzimidazolone tricycles **13** and **14**. Fortunately, the improved pharmacokinetic profile was maintained with addition of the methyl group. Homologation of the 3-indoline substituent to an ethyl group provided no potency advantage (**21** and **22**), and in the case of spirohydantoin **21** increased the serum shift in the functional assay (IC_{50} = 44 nM).

The combination of the indoline tricycle with the spirohydantoin quinoline provided compounds with superior bioavailability in rats (F = 34–65%) compared to the corresponding spiroazaoxindole quinolines (F = 6–9%). Interestingly, it was also noted that the difference in potency between the spirohydantoins **17** and **19** and spiroazaoxindoles **18** and **20** was somewhat less than the order of magnitude we had typically observed. Based on the combination of potency and rat pharmacokinetics, compound **19** was selected for more in-depth evaluation.

The sodium salt of compound **19** displayed a good overall pharmacokinetic profile in rats, dogs, and rhesus monkeys (Table 3), with low clearance in all three species, ranging from 1.6 mL/min/

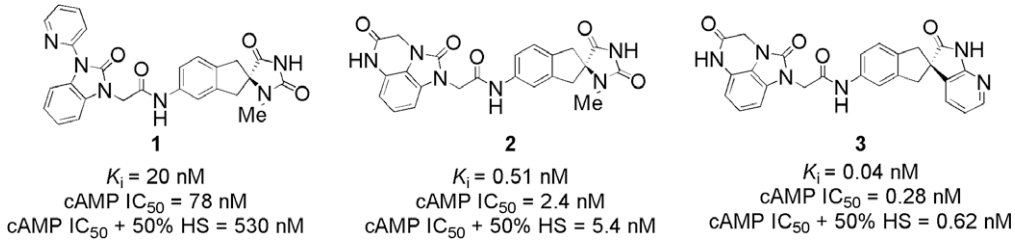


Figure 1. Previously disclosed, potent CGRP receptor antagonists.

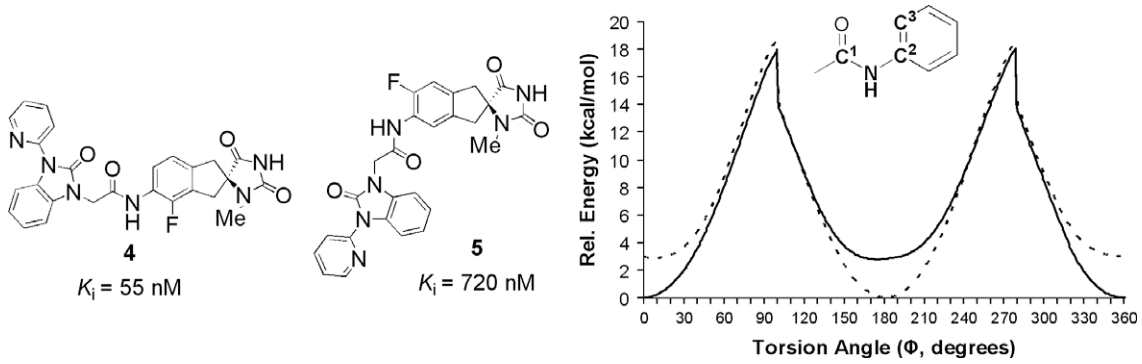


Figure 2. Plot of torsional energy strain for rotation about the central amide (C^1–N–C^2–C^3) of **4** (solid line) and **5** (dashed line).

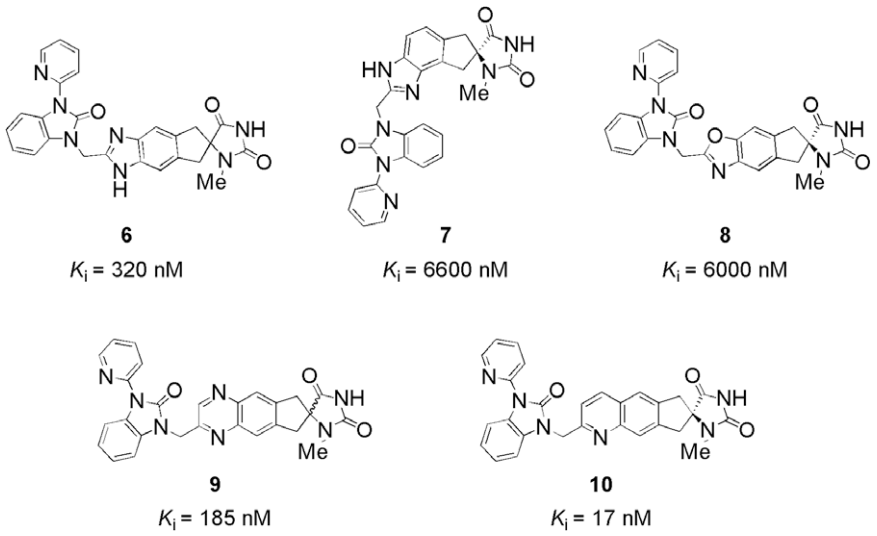
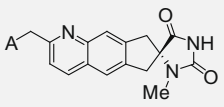


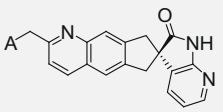
Figure 3. CGRP receptor antagonists with central constraints.

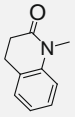
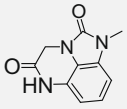
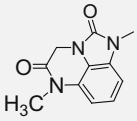
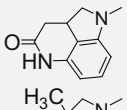
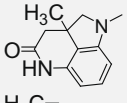
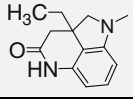
kg (dog) to 14 mL/min/kg (rat). After an intravenous dose, the plasma half-life ranged from 1.6 h (rat) to 6.1 h (dog). The oral bioavailability was good (34–78%) in all three species in a number of different dosing vehicles. The good bioavailability observed when **19** was dosed as a suspension in methylcellulose (F = 38–59%) indicates that poor aqueous solubility is not greatly limiting its absorption. In fact, only marginal effects were observed when **19** was dosed in more solubilizing formulations, such as Imwitor:Tween (rat F = 34%) or PEG400 (dog F = 78%, rhesus F = 44%).

Compound **19** was further tested in a primate model to evaluate its ability to act in vivo as a CGRP receptor antagonist. When dosed in rhesus monkeys in the capsaicin-induced dermal vasodilation phar-

Table 1
Comparison of CGRP receptor antagonist activity of spriroazaoxindole and spirohydantoin





A	Compound	CGRP K_i[a,b] (nM)	cAMP IC_{50}[a,c] (nM)	cAMP IC_{50} + HS[a,d] (nM)	Compound	CGRP K_i[a,b] (nM)	cAMP IC_{50}[a,c] (nM)	cAMP IC_{50} + HS[a,d] (nM)
	**11**	8.9 (2)	50 (1)	850 (1)	**12**	1.4 ± 0.33 (13)	4.7 ± 1.6 (3)	81 ± 61 (3)
	**13**	0.30 (1)	1.7 ± 0.55 (3)	14 ± 11 (3)	**14**	0.023 ± 0.0032 (4)	0.077 ± 0.024 (3)	2.5 ± 0.27 (3)
	**15**	3.0 ± 0.25 (4)	33 ± 20 (3)	1200 ± 380 (3)	**16**	0.41 (2)	1.2 (1)	95 (1)
	**17**	1.5 ± 0.21 (7)	7.3 ± 4.6 (5)	43 ± 20 (5)	**18**	0.55 ± 0.35 (12)	0.86 ± 0.38 (9)	4.1 ± 1.9 (9)
	**19**	0.52 ± 0.068 (4)	2.2 ± 0.42 (4)	6.5 ± 1.3 (4)	**20**	0.12 ± 0.030 (4)	0.52 ± 0.18 (3)	1.3 ± 0.27 (3)
	**21**	1.3 (1)	7.2 (2)	44 (2)	**22**	0.072 ± 0.015 (4)	0.68 ± 0.077 (3)	1.5 ± 0.35 (3)

[a] Mean value ± standard deviation, where appropriate; number of replicates in parentheses.
[b] K_i values for inhibition of ^{125}I-hCGRP binding determined using membranes from HEK293 cells stably expressing human CLR/RAMP1.[5]
[c] Inhibition of CGRP-induced cAMP production in HEK293 cells stably expressing human CLR/RAMP1.[5]
[d] Inhibition of CGRP-induced cAMP production in HEK293 cells stably expressing human CLR/RAMP1in the presence of 50% human serum.[5]

Table 2
Pharmacokinetics of compounds in rats

Compound	po dose (mpk)	iv dose[a] (mpk)	F[b] (%)	iv $t_{1/2}$ (h)	Cl (mL/min/kg)
11	10	2	10[I]	0.70	38
12	10	2	3[I]	0.35	56
14	5	1	0[II]	0.45	40
17	10	2	41[III]	1.1	11
18	5	1	8[III]	0.32	19
19	10	2	34[III]	1.6	14
20	10	2	6[III]	0.62	28
21	10	2	65[I]	1.1	44
22	10	2	9[III]	0.61	11

[a] Compound dosed in DMSO.
[b] Compound dosed orally in (I) 1% methylcellulose, or (II) 90% PEG 400:10% water, or (III) 50% Imwitor:50% Tween.

Table 3
Pharmacokinetics of compound **19** (sodium salt)

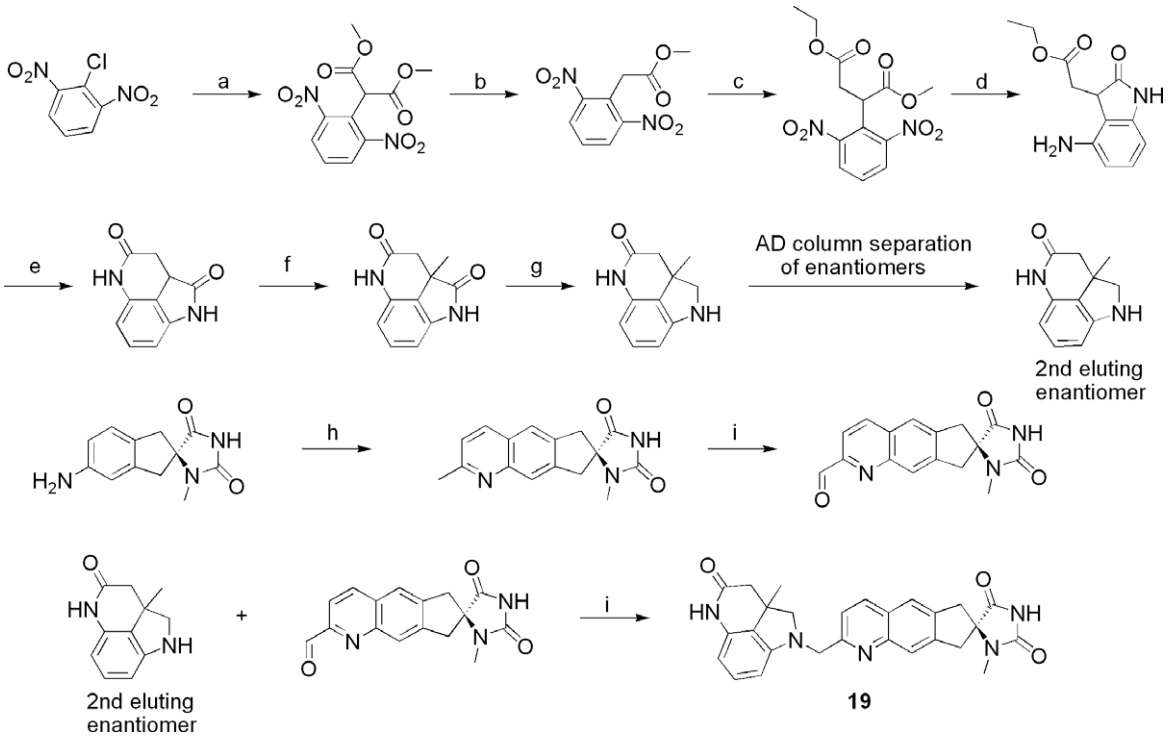
Species	po dose (mpk)	iv dose[a] (mpk)	F[b] (%)	iv $t_{1/2}$ (h)	Cl (mL/min/kg)
Rat	10	2	45	1.6	14
Dog	1	0.5	59	6.1	1.6
Rhesus	2	0.5	38	1.7	5.6

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

macodynamic assay,[4c] plasma levels of 5.5 μM of **19** were required to inhibit ninety percent of the capsaicin-induced vasodilation response (EC_{90} = 5.5 μM). This value, although slightly less potent than the number obtained for the clinical compound telcagepant, demonstrated in vivo potency for this class of CGRP receptor antagonists.

The synthesis of compound **19** is shown in Scheme 1.[11] Alkylation of dimethylmalonate with 2-chloro-1,3-dinitrobenzene, followed by decarboxylation produced methyl (2,6-dinitrophenyl) acetate. Further alkylation with ethyl bromoacetate provided the 2,6-dinitrophenyl substituted succinic diester, which underwent reduction of the nitro groups and monocyclization upon treatment with iron and ammonium chloride. The tricyclic core was formed upon heating in xylenes and acetic acid. The 3-methyl substituent was installed by alkylation with methyl iodide and sodium hydride. Selective reduction of the oxindole carbonyl was achieved in good yield upon treatment with DIBAH. Chiral resolution on a ChiralPak AD column produced the tricyclic indoline intermediate in eight total steps and overall 10% yield. The quinoline aldehyde was produced in a two step sequence from the known spirohydantoin aniline.[6] A Skraup-type reaction between the aniline and crotonaldehyde, using *p*-chloranil as the oxidant, produced the 2-methylquinoline in good yield.[12] Oxidation of the methyl group with selenium dioxide produced the desired aldehyde. The final reductive alkylation between the tricyclic indoline and quinoline aldehyde was carried out using sodium cyanoborohydride in methanol to produce **19**.

In conclusion, good evidence for the bioactive conformation of CGRP receptor antagonists linked by a central amide was provided by the use of conformational constraints. In particular, replacement of the central amide with a quinoline ring produced a novel series of potent CGRP receptor antagonists. Furthermore, the combination of the quinoline constraint with a tricyclic benzimidazolinone left hand fragment produced analogs with picomolar potency in both the radioligand binding assay and the cell-based functional assay. Further optimization of the tricycle produced a subnanomolar CGRP receptor antagonist (**19**) that also exhibited good in vivo potency in a pharmacodynamic assay in rhesus monkeys. In addition, **19** displayed a good pharmacokinetic profile in three preclinical species.



Scheme 1. Synthesis of compound **19**. Reagents and conditions: (a) $CH_2(CO_2Me)_2$, KO*t*-Bu, DMA, 90 °C, 90%; (b) LiCl, H_2O, DMSO, 100 °C, 99%; (c) $BrCH_2CO_2Et$, Cs_2CO_3, DMF, 75%; (d) Fe, $NH_4Cl_{(aq)}$, EtOH, 90 °C, 95%; (e) AcOH, xylenes, 140 °C, 75%; (f) NaH, CH_3I, DMF, 0 °C, 65%; (g) DIBAH, THF, 0 °C, 70%; (h) crotonaldehyde, *p*-chloranil, 1-BuOH, HCl, 120 °C, 79%; (i) SeO_2, dioxane, 100 °C, 87%; (j) $NaBH_3CN$, AcOH, MeOH, 86%.

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