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Design and synthesis of potent antagonists containing rigid spirocyclic privileged structures for the CGRP receptor

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ABSTRACT

We report the synthesis of rigid spirocyclic systems as conformationally constrained variants of the Ala-Phe-NH_2 dipeptide amide C-terminus of the calcitonin gene-related peptide (CGRP). CGRP receptor antagonists containing these moieties displayed potent affinity, functional antagonism and excellent oxidative stability. Structure–activity relationship studies demonstrated the relative importance of hydrogen bond donor/acceptor functionalities and the preferred orientation of an aromatic ring. Antagonists showed potent and full reversal of CGRP-induced dilation of ex vivo human intracranial arteries.

Migraine is a neurovascular disorder that afflicts more than 12% of adults and is often characterized by severe headache, nausea, and sensitivity to external stimuli lasting more than 4 h.[1] The current standard of therapy involves treatment with 5-$HT_{1B/1D}$ agonists (triptans) that cause direct vasoconstriction of blood vessels through activation of 5-HT_{1B} receptors.[2] Because of their vasoconstrictive properties, triptans are contraindicated in patients with cardiovascular disease. Thus, a migraine drug that is devoid of cardiovascular liabilities is expected to have a significant advantage over existing agents.[3]

Calcitonin gene-related peptide (CGRP) is a 37-amino acid neuropeptide widely distributed in both the peripheral and central nervous system (CNS), principally localized in afferent and central neurons and displaying a number of biological effects, including potent vasodilation.[4] Activation of trigeminal sensory neurons and increases in serum levels of CGRP are strongly implicated in the pathophysiology of migraine.[5] Clinical proof of concept for CGRP receptor antagonists as an effective migraine therapy has been demonstrated with intravenous administration of olcegepant (BIBN-4096) **1** and through oral administration of a structurally distinct antagonists, telcagepant (MK-0974), MK-3207 and BI 44370 TA.[6]

In this context, we initiated a research program aimed at identifying a CGRP receptor antagonist suitable for rapid onset of migraine relief. At the outset of our program, there existed a limited number of small molecule CGRP antagonists (e.g., **1–2**, Fig. 1).[7] Their key structural features were: an aromatic central amino acid core; a GPCR (G-protein coupled receptor) privileged structure containing an aromatic moiety attached to the central amino acid at the N-terminus; and a basic or hydrophobic moiety attached to the central core unit at the C-terminus. Based on the simplicity and good potency of these antagonists we prepared compounds **3–5** (Fig. 1).

Encouraged by the initial potencies of **3**–**5**, we set out to identify: (i) additional novel GPCR privileged substructures that preferably contained fewer amide bonds and degrees of freedom for improved affinity and a more favorable pharmacokinetic profile; (ii) novel amino acids that provided improved binding affinity and selectivity; and (iii) a key recognition element that defined the bio-active conformation of CGRP antagonists. This strategy led us to design and synthesize constrained CGRP receptor antagonists with reduced peptide character that should have improved oral exposure. In this Letter we would like to disclose our initial results of this research program. We and others[8] hypothesized that the piperidinyldihydroquinazolinone in **4** might mimic the Ala-Phe-NH_2 at the C-terminus of the CGRP peptide (Fig. 2). A recent crystal structure of the ectodomain of the CGRP receptor in complex with two known CGRP antagonists confirmed the importance of this key pharmacophore containing a hydrogen bond donor–acceptor pair and an aromatic moiety.[9] In light of the known liability of facile benzylic oxidation associated with the piperidinyldihydroquinazolinone heterocycle in **4**,[10] our investigation began with the design of alternate GPCR privileged scaffolds that were less prone or free from benzylic oxidation.

Spiropiperidine systems containing hydrogen bond donor and acceptor moieties constrained in a five- or six-membered ring with a fused or pendant aromatic moiety were initially considered as

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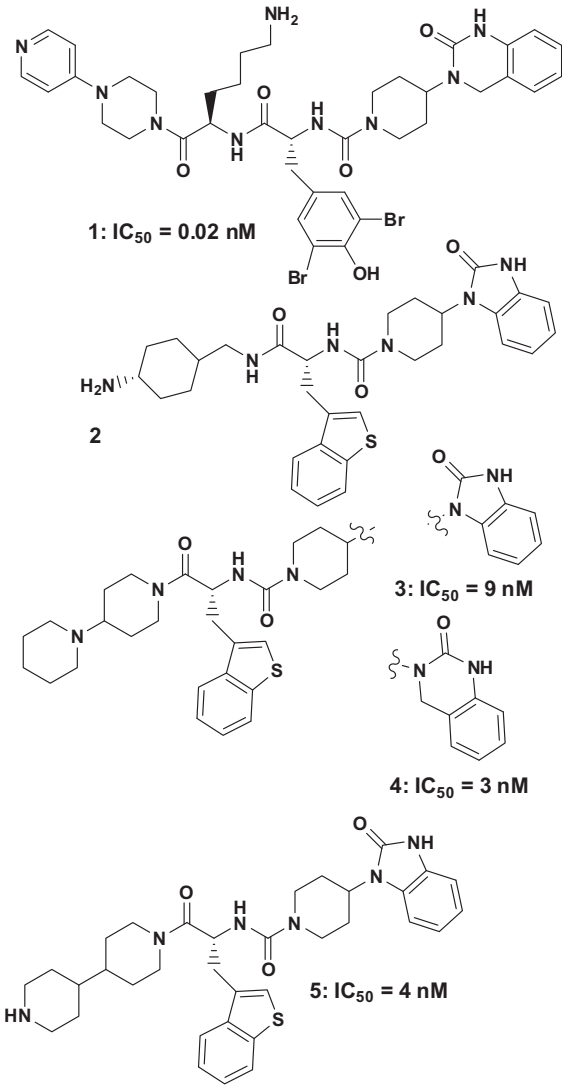


Figure 1. Known CGRP antagonists.

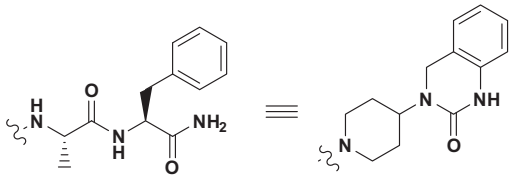
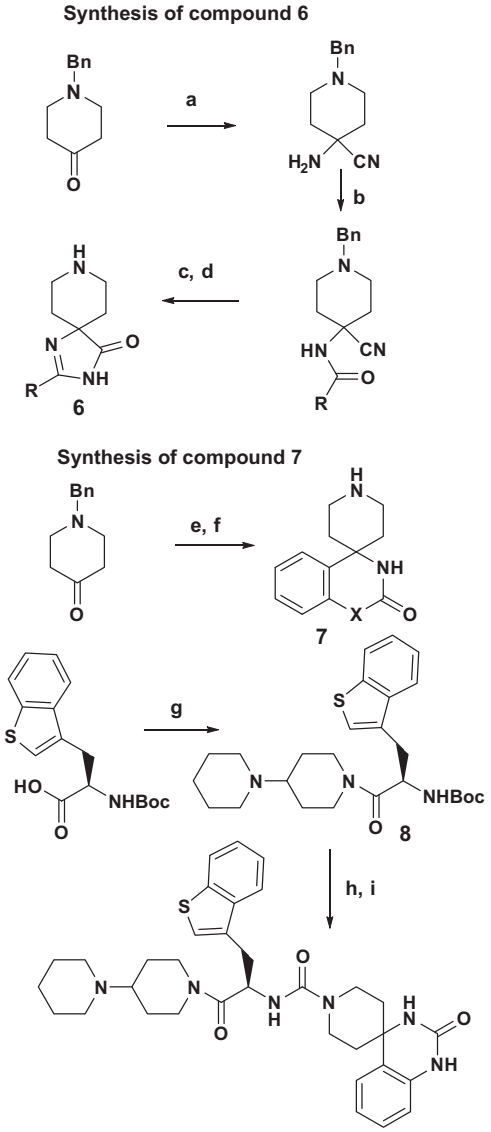


Figure 2. Key pharmacophore common in -Ala-Phe-NH_2 of CGRP(8–37) peptide and dihydroquinazolinone.



Scheme 1. Reagents and conditions: (a) NaCN, NH_4Cl, MeOH, 25 °C, 12 h, 91%; (b) RCOCl, Et_3N, DMAP, CH_2Cl_2, 25 °C, 12 h, 72–91%; (c) 30% H_2O_2, 6 M NaOH, reflux, 3 h, 54–68%; (d) HCl in dioxane, 25 °C, 3 h, 95%; (e) *N*-phenylurea, 100 °C, polyphosphoric acid, 3 h, 45%; (f) 10% Pd on carbon, MeOH, 6.0 M HCl (cat), H_2,(1 atm), 12 h, 96%; (g) 4-piperidinopiperidine, PyBop, Et_3N, CH_2Cl_2, 91%; (h) dioxane–HCl, 25 °C, 4 h, 95%; (f) *N*,*N*′-disuccinimidyl carbonate, diisopropylethylamine, CH_2Cl_2, 25 °C, 3 h.

mimetics of the Ala-Phe-NH_2 dipeptide amide and piperidinyldihydroquinazolinone replacements.

Convergent syntheses of the desired targets are shown in Scheme 1. The spiropiperidine intermediates used in the synthesis of CGRP receptors antagonists were prepared using known literature methods.[11] Novel spiropiperidines were prepared according to the synthetic scheme shown in Scheme 1. 1-Benzyl-4-piperidone was converted to α-amino nitrile under standard Strecker conditions in 85% yield. The amine was reacted with various acid chlorides in the presence of triethylamine to give amides in 80–95% yields. Hydrolysis of the nitrile with 30% hydrogen peroxide/6 M sodium hydroxide followed by debenzylation provided the spiropiperidines (R = Ph, cyclohexyl, 3-pyridyl, 4-pyridyl) **6** in 52–75%yields. Spiropiperidine **7** was prepared by reacting 1-benzyl-4-piperidone with *N*-phenylurea and polyphosphoric acid at 100 °C followed by debenzylation in 45% overall yield. Treatment of Boc-(D)-3-benzothienylalanine and 4-piperidinopiperidine with PyBOP provided **8** in excellent yield. The amino protecting group was removed under acidic conditions (TFA). The amine was then coupled to various spiropiperidines mediated by disuccinimidyl carbonate to give the desired compounds **9**–**20** (Fig. 3) in 70–90% yields.

All target compounds were tested in human neuroblastoma SK-N-MC cells that endogenously expressed the CGRP receptor with an identical sequence to the cloned human CGRP receptor. The ability of compounds to compete for the radioligand [^{125}I]CGRP endogenous peptide human alpha CGRP was measured using a radioligand competition assay to determine IC_{50}'s. Functional antagonism for selected compounds was determined by measuring

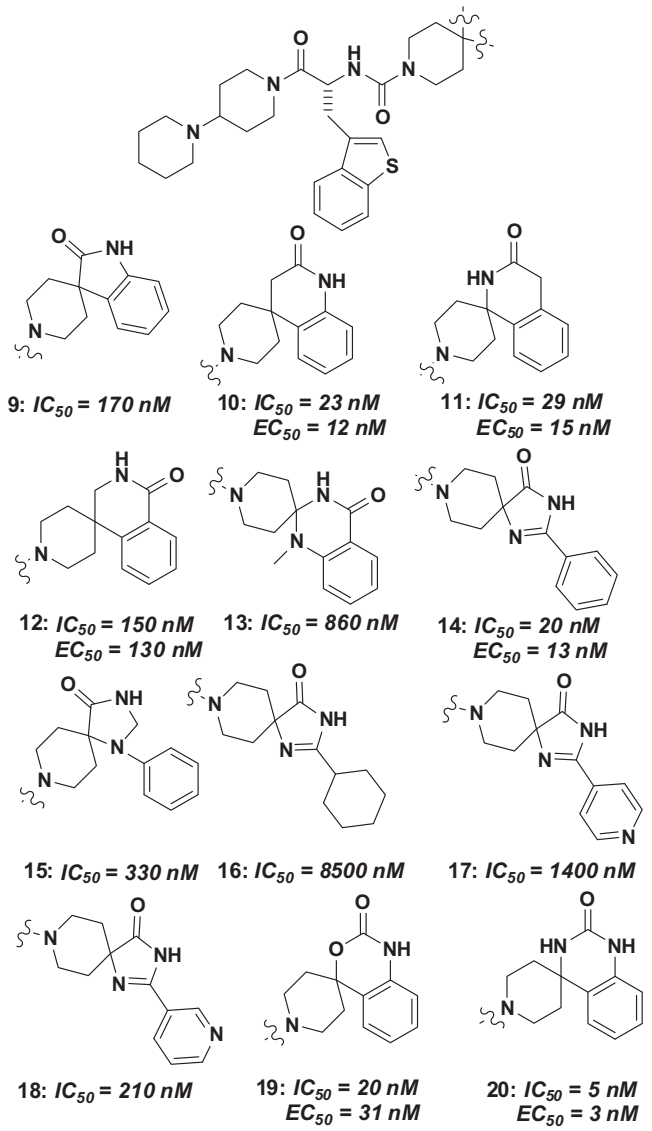


Figure 3. Structure–activity relationships of CGRP antagonists containing spirocyclic moieties.

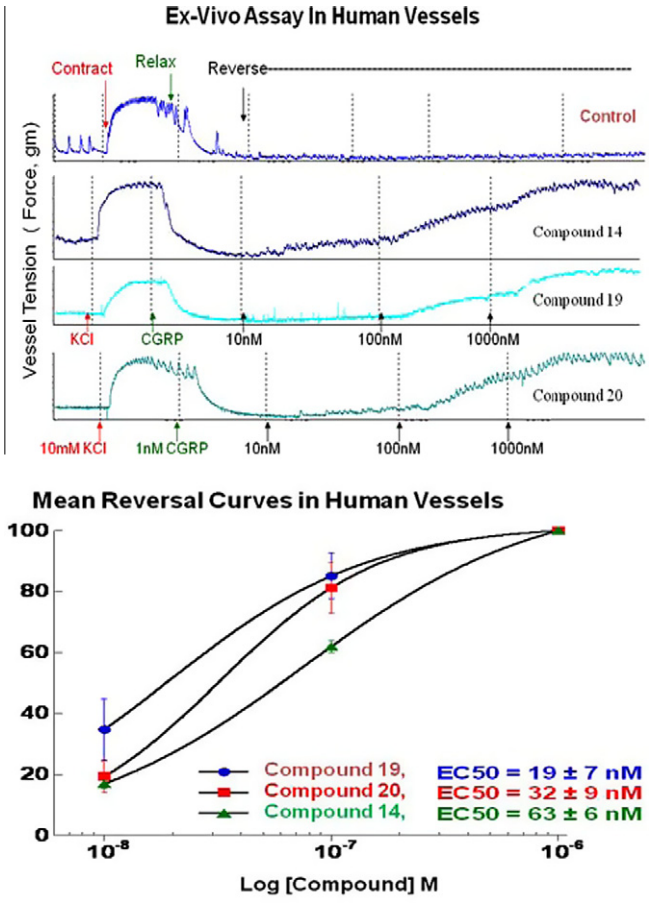


Figure 4. Reversal of CGRP-induced artery dilation.

their ability to inhibit CGRP-stimulated formation of cyclic AMP in attached whole SK-N-MC cells (EC_{50}).[12]

CGRP-receptor binding data obtained for the compounds **9**–**20** is presented in Fig. 3. Homologation of **9** to give **10** enhanced CGRP receptor binding potency. The reversal of donor (NH) and acceptor (C=O) moieties in **12** versus **10** led to a decrease in affinity that was restored by homologation between the carbonyl and phenyl ring in **11**. This preference for a single atom separation between the carbonyl and phenyl group was further demonstrated by the poor affinity of quinazolinone **13**. The spirocyclic imidazolinone **14** with a pendant phenyl group was eightfold more potent compared with its fused congener **9**. Substituting the aromatic moiety with cyclohexyl (**16**), 4-pyridinyl (**17**) or 3-pyridyl (**18**) led to a significant loss of binding potency suggesting the preference for a neutral aromatic group at C2 of the imidazolinone. In an effort to maximize the putative hydrogen bond donor/acceptor ability of spiropiperidines with the CGRP receptor, compounds **19** and **20** were synthesized. Compound **20** was found to be the most potent in the series in line with the expected highest electron density on the urea carbonyl oxygen (best H-bond acceptor in the group).

Reversal of CGRP-induced dilation of intracranial arteries is believed to be an ex vivo measure which models the clinical treatment conditions where migraine-related CGRP release precedes initiation of acute therapy. Accordingly, Compounds **14, 19** and **20** were tested for their ability to reverse CGRP-induced dilation in human intracranial artery preparations.[12] Pre-contracted vessels were dilated using CGRP and then the dilation was reversed with a CGRP receptor antagonist. In this protocol,[12] antagonist treatment reverses CGRP-induced artery dilation in a dose dependent manner. In the tracings shown in Figure 4, compounds **14, 19** and **20** were tested at 10, 100 and 1000 nM. Compounds **14, 19** and **20** showed potent and full reversal of CGRP-induced dilation of ex vivo human intracranial arteries with EC_{50} values in the range 19–65 nM. In general the rank order of activity tracked well across radioligand binding, cell-based functional and ex vivo human vessel assays (e.g., 14 and 19), although there were a few exceptions (e.g., 20).

Selected CGRP receptor antagonists containing the benzothiophene moiety and spirocyclic substructures were profiled in various assays for metabolic stability, oxidative stability, cytochrome P450 inhibition and Caco-2 permeability. In general, these compounds showed excellent metabolic stability, oxidative stability, and favorable CYP inhibition profiles (IC_{50}'s >40 μM) with the exception of CYP 3A4 (IC_{50}'s <1 μM). However, Caco-2 cell permeability was poor for these compounds, most likely because of a high degree of amide bond polarity and/or P-glycoprotein efflux. Specific profiling data for compound **20** is shown here. The metabolism of compound **20** was evaluated in pooled human and rat liver microsomes. The rate of metabolism at 3 μM was low in both species after 10 min (human: 82% compound remaining; rat:

100% compound remaining). Compound **20** inhibited recombinant CYP1A2, 2C9, 2C19 with IC_{50}'s >40 μM, but was a potent inhibitor of CYP3A4 with an IC_{50} of 0.1 μM, most likely hydrophobic benzothiophene moiety contributing to CYP3A4 recognition.

In summary, we have identified novel CGRP receptor antagonists by incorporating rigid spirocyclic moieties that mimic the Ala-Phe-NH_2 C-terminus of CGRP into a known benzothiophene-containing core structure. SAR investigations helped to define the optimal spatial relationship between the –(C=O)NH– hydrogen bond acceptor–donor pair and the phenyl ring. Selected CGRP antagonists displayed potent receptor binding, functional potency, and oxidative stability. These compounds also caused reversal of CGRP-induced dilation of isolated human intracranial arteries, an ex vivo migraine model, suggesting potential efficacy as anti-migraine agents.

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