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Non-peptide calcitonin gene-related peptide receptor antagonists from a benzodiazepinone lead

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Abstract—High-throughput screening of the Merck sample collection identified benzodiazepinone tetralin-spirohydantoin **1** as a CGRP receptor antagonist with micromolar activity. Comparing the structure of **1** with those of earlier peptide-based antagonists such as BIBN 4096 BS, a key hydrogen bond donor–acceptor pharmacophore was hypothesized. Subsequent structure activity studies supported this hypothesis and led to benzodiazepinone piperidinyldihydroquinazolinone **7**, CGRP receptor $K_i = 44$ nM and $IC_{50} = 38$ nM. Compound **7** was orally bioavailabile in rats and is a lead in the development of orally bioavailable CGRP antagonists for the treatment of migraine.

Migraine is a neurovascular disorder in which neuropeptide release and intracranial blood vessel dilation play an important role.[1] Characteristic symptoms of migraine include severe headache, nausea, and sensitivity to light and sound lasting 4–72 h.[2] Approximately 12% of the general population experience migraine headaches, but since migraine is underdiagnosed and undertreated, this may be an underestimate.[3]

The current standard for the treatment of migraine is 5-$HT_{1B/1D}$ receptor agonists that form the triptan class of migraine drugs.[4] Part of the triptan mechanism of action involves direct vasoconstriction of blood vessels through activation of 5-HT_{1B} receptors.[5] Although selective for intracranial over coronary vessel constriction, triptans are contraindicated in patients with cardiovascular disease. A migraine drug that does not cause direct vasoconstriction would have significant therapeutic value.

Calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide present in perivascular sensory nerve fibers and one of the most potent vasodilators known.[6] The CGRP receptor is especially abundant in the trigeminal vasculature, a network of intracranial vessels associated with sensory neurons of the trigeminal ganglion.[7] Activation of trigeminal sensory neurons and CGRP release are strongly implicated in the pathophysiology of migraine headache.[8] Therefore, a CGRP receptor antagonist is an attractive therapeutic target for the treatment of migraine.[9,10]

In placebo-controlled clinical trials, the potent Boehringer–Ingelheim CGRP receptor antagonist BIBN 4096 BS was efficacious in alleviating migraine headache pain.[11] Two hours following a 2.5 mg iv dose, 69% of patients reported significant pain relief. The therapeutic response was similar to that observed for triptans in previous clinical trials. No evidence of direct vasoconstriction or effects on hemodynamics was observed with BIBN 4096 BS.[11,12] The Boehringer–Ingelheim clinical trial is proof-of-concept for treating acute migraine headache with a CGRP receptor antagonist. Efficacy

Keywords: CGRP receptor antagonist; Benzodiazepinone; CGRP; CLR/RAMP1; Migraine.
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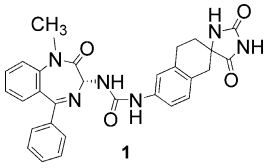
was achieved by blocking the pathological intracranial response to CGRP and was not a consequence of direct vasoconstriction, since the latter was not observed.

The CGRP antagonist BIBN 4096 BS was administered by intravenous infusion in clinical trials. We sought to identify a suitable lead compound for a program aimed at developing an orally active CGRP antagonist to treat acute migraine headache. High-throughput screening identified benzodiazapinone **1** as a modest inhibitor of CGRP binding to native human CGRP receptors (K_i = 4.8 μM, SK-N-MC cell membranes). Follow-up studies demonstrated that benzodiazapinone **1** was a functional antagonist of CGRP-stimulated cAMP production in SK-N-MC cells (IC_{50} = 6 μM). Binding and functional assays using a recombinant form of the human CGRP receptor obtained similar results. The structure of **1** was unique compared to previously published CGRP antagonists based on D-dibromotyrosine ('Compound **1**')[13] or the dipeptide D-dibromotyrosine-L-lysine (BIBN 4096 BS)[14] (Fig. 1). In contrast to amino acid-based inhibitors, HTS lead **1** linked two heterocycles through a urea group: the GPCR 'privileged structure'[15] *N*-methyl 3-aminobenzodiazepinone and the spirohydantoin derived from β-tetralone (spiro ring fusion as a mixture of isomers).



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Simple substituent additions to **1** did not increase potency, but more substantial structural changes were successful. It was noted that potent CGRP antagonists incorporated another GPCR privileged structure, either piperidinylbenzimidazolone in the case of 'Compound 1',[13] or the closely related piperidinyldihydro-quinazolinone in BIBN 4096 BS.[14] In these compounds, the benzimidazolone and dihydroquinazolinone have a C(O)NH hydrogen bond donor–acceptor pair embedded in the heterocycle, similar to the hydantoin in **1**. This led us to speculate that 'Compound **1**', BIBN 4096 BS, and **1** shared a common hydrogen bond donor–acceptor pharmacophore Eq. 1. An overlay of energy minimized

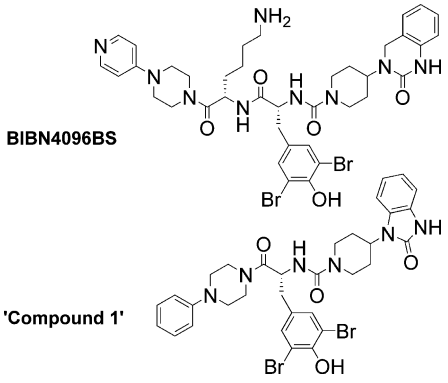
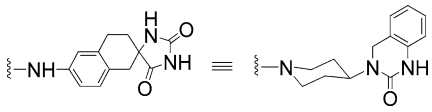


Figure 1. Previously reported CGRP receptor antagonists.

Figure 2. Overlay of energy minimized piperidinyldihydroquinazolinone (blue) and tetralin spirohydantoin (yellow) from Eq. 1.

structures of piperidinyldihydroquinazolinone and one enantiomer of the tetralin spirohydantoin showed good alignment between carbonyl amide oxygen–NH pairs (Fig. 2).

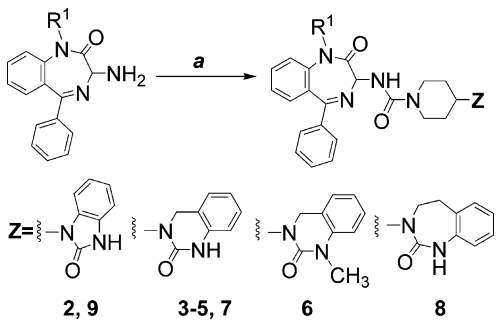


(1)

To test the hypothesis of a common pharmacophore, the 2-aminotetralin spirohydantoin substructure in **1** was replaced with piperidinylbenzimidazolone or piperidinyldihydroquinazolinone, to form hybrid structures **2** and **3**.

The required 3-aminobenzodiazepine[15–18] was treated with *p*-nitrophenylchloroformate and triethylamine, followed by the appropriate 4-substituted piperidine,[19] to give compounds **2**–**5** and **7**–**9** (Scheme 1). Compound **6** was obtained by treating **5** with 1 equiv of NaH and CH_3I in DMF.[20]

The CGRP receptor is heterodimeric and is comprised of the family B GPCR calcitonin-like receptor (CLR) and the accessory protein, receptor activity modifying protein 1 (RAMP1).[21,22] Co-expression of CLR and RAMP1 is required to form a functional CGRP receptor. A human neuroblastoma cell line (SK-N-MC) expresses the native human CGRP receptor and was used to determine the binding affinity.[23] A cell line expressing recombinant human CLR/RAMPl exhibited pharmacology identical to that of the native receptor. Binding studies were conducted with [I^{125}]CGRP using E10 (human cloned receptor)[24] or SK-N-MC (native human receptor) cell membranes and results reported as a K_i. The IC_{50} for antagonism of CGRP-stimulated increases in intracellular cAMP was determined in E10 cells expressing the human cloned receptor.



Scheme 1. Synthesis of substituted piperidine benzodiazepinone analogs. Reagents and conditions: (a) *p*-nitrophenylchloroformate, DMSO/THF, TEA, 0 °C; 4-Z-piperidine, TEA.

Hybrid structures were synthesized to link the *N*-methyl 3-aminobenzodiazepinone component of screening lead **1** to either piperidinylbenzimidazolone (forming **2**) or to piperidinyldihydroquinazolinone (forming **3**). Although benzimidazolone **2** was inactive at the concentrations tested, benzodiazepine dihydroquinzolinone enantiomers **3a** (3*S*-enantiomer) and **3b** (3*R*-enantiomer) had low micromolar activity.

Overall, the *R*-enantiomer **3b** was approximately 5-fold more potent than the original lead **1** (Table 1). SAR studies showed that small lipophilic N-1 benzodiazepinone substituents were well tolerated. Slight increases in potency were noted for *N*-ethyl (**4**) and *N*-isopropyl (**5**) substituents. In partial support of the hydrogen bond donor–acceptor pharmacophore model, N-alkylation of the dihydroquinazoline nitrogen was highly detrimental to activity (compare **5** with *N*-methyl analog **6**), although steric effects between the ligand and receptor cannot be ruled out as the cause of poor activity.

Changing the N-1 methyl substituent in **3b** to trifluoroethyl increased potency 50-fold. *N*-Trifluoroethyl benzodiazepinone **7** was one of the most potent analogs prepared, with $K_i = 44$ nM. In cell culture, **7** blocked CGRP-stimulated cAMP production with an $IC_{50} = 38$ nM. We found that compounds containing the dihydroquinazoline substructure required protection from light and air to prevent dihydroquinazolinone ring oxidation. The ring expanded 1,3-benzodiazepin-2-one **8** was stable in air and was found to be equipotent to **7**. When the optimized N-1 trifluoroethyl benzodiazepine substituent in the dihydroquinazolinone series was re-introduced to benzimidazolone **2** ($K_i > 20{,}000$ nM), potency increased more than 10-fold (**9**, $K_i = 2370$ nM), consistent with dihydroquinazolinone structure–activity relationships.

Receptor kinetic studies were carried out on compound **7**. Competitive binding studies with [^{125}I]CGRP and **7** (E10 membranes, human recombinant CGRP receptor) showed $K_i = 48$ nM. In the same cell line, compound **7** demonstrated competitive antagonism of CGRP-stimulated cAMP production. Increasing concentrations of

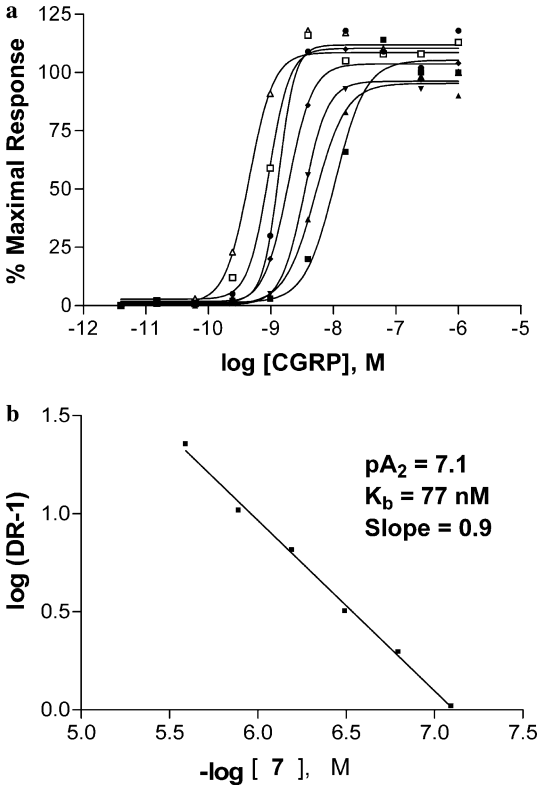


Figure 3. Concentration–response curves and Schild plot analysis of compound **7**. (a) Concentration–response curves of CGRP-induced cAMP production in E10 cells expressing human recombinant CLR/RAMP1 in the presence and absence of compound **7**. ■ = 2560 nM; ▲ = 1280 nM; ▽ = 640 nM; ♦ = 320 nM; ● = 160 nM; □ = 80 nM; △ = 0 nM. (b) Schild plot showing the effect of compound **7** on CGRP-induced cAMP production in E10 cells expressing recombinant CLR/RAMP1. DR is the ratio of CGRP EC_{50} values in the presence and absence of compound **7**. The *X*-intercept is equal to the pA_2 and the K_b calculated using the formula $pA_2 = -\log K_b$.

Table 1. Binding affinity and antagonist activity for compounds **1**–**9**, compound **1** and BIBN 4096 BS on the human CGRP receptor (CLR/RAMP1)

Compound	R^1	BZD C-3	CLR/RAMP1 K_i[a] (nM)	*n*	CLR/RAMP1 IC_{50}[b] (nM)	*n*
1	—	*R*	4250 ± 275	4	57% inh (17 μM)	2
2	CH_3	*R,S*	>13,000	5	—	
3a	CH_3	*S*	8400 ± 800	3	4450	1
3b	CH_3	*R*	2200 ± 87	4	3640	1
4	CH_3 CH_2	*R*	897 ± 118	4	990	1
5	*i*-Pr	*R,S*	1060 ± 658	5	1810	1
6	*i*-Pr	*R,S*	33% inh (100 μM)[c]	1	—	
7	CF_3 CH_2	*R*	44 ± 17	7	38 ± 24	5
8	CF_3 CH_2	*R*	61 ± 12	6	43	1
9	CF_3 CH_2	*R*	2370 ± 321	3	1,580	1
Compound **1**	—	—	83	2	75	2
BIBN	—	—	0.005 ± .001	5	0.100 ± .058	7

[a] K_i values were determined using E10 cell membranes expressing a human cloned CLR/RAMP1 receptor and are expressed as arithmetic means of n determinations, along with the standard deveriation.[24]

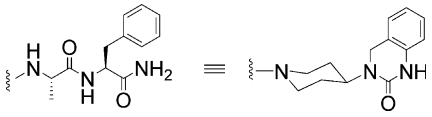
[b] IC_{50} values were determined using E10 cells expressing a human cloned CLR/RAMP1 receptor and measured the inhibition of CGRP-mediated cAMP production.[24]

[c] K_i value was determined using SK-N-MC cell membranes as previously described.[23]

CGRP produced a rightward shift in the dose–response curve for **7** with no change in maximal response (Fig. 3). In good agreement with binding experiments, a Schild plot analysis yielded K_b = 77 nM (pA_2 = 7.1). The slope of the line was 0.9, indicating a 1:1 association of antagonist and receptor. In contrast to the human CGRP receptor, **7** was much less potent on the rat CGRP receptor, inhibiting only 35% of [^{125}I]CGRP binding at 100 μM. Differences in human and rat RAMP1 sequence, most notably at position 74 (Trp in human and Lys in rat), were responsible for the species difference in affinity, an effect also observed for BIBN 4096 BS.[23] In other studies, **7** was inactive on the human adrenomedullin receptor CLR/RAMP2.[21]

A key objective for the program was to identify a CGRP antagonist lead that could be optimized for oral bioavailability. To this end, the pharmacokinetic profile of **7** was determined in rats and dogs. In rats, **7** had an iv $t_{1/2}$ = 1.7 h, Cl = 20 mL/min/kg, and had modest but consistent oral bioavailability when dosed as a suspension in 1% aqueous methocel (F = 10%, C_{max} = 240 nM, 10 mpk) (n = 3). In dogs, **7** was not detected in plasma after an oral dose of 2 mpk (1% aqueous methocel, n = 2).

A nonpeptide screening lead **1** was identified that had micromolar affinity for the human CGRP receptor and was a functional CGRP antagonist in cells. SAR studies of benzodiazepinone **1** led to a 100-fold increase in potency and identified **7** as a promising new lead. Benzodiazepinone **7** was shown to be orally bioavailable in rats, thus offering an excellent lead for the pursuit of potent, bioavailable CGRP antagonists for the treatment of migraine. A hypothesis of a key hydrogen bond donor–acceptor antagonist pharmacophore was proposed and guided antagonist design. A recent publication discussed the importance of the dihydroquinazolinone NH of BIBN 4096 BS for CGRP receptor antagonist activity. It is interesting to note that a similar hydrogen bond donor–acceptor pharmacophore is also present in CGRP(8–37) and related peptide antagonists, in the form of the critical C-terminal peptide amide.[25] Thus, it is possible that the dihydroquinazolinone substructure in **7** and BIBN 4096 BS functions as a conformationally constrained Ala-Phe-NH_2 dipeptide amide Eq. 2.



(2)

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