Bioorganic & Medicinal Chemistry Letters 21 (2011) 6705–6708

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Identification of a novel RAMP-independent CGRP receptor antagonist

C. Blair Zartman [a,*], Ian M. Bell [a], Steven N. Gallicchio [a], Samuel L. Graham [a], Stefanie A. Kane [b], John J. Mallee [b], Ruth Z. Rutledge [b], Christopher A. Salvatore [b], Joseph P. Vacca [a], Theresa M. Williams [a]

[a] Department of Medicinal Chemistry, Merck Research Laboratories, West Point, PA 19486, USA
[b] Department of Pain Research, Merck Research Laboratories, West Point, PA 19486, USA

ARTICLE INFO

Article history:
Received 16 August 2011
Revised 14 September 2011
Accepted 15 September 2011
Available online 21 September 2011

Keywords:
CGRP
Hydroxypyridine
RAMP-independent
HIV integrase

ABSTRACT

Identification of an HIV integrase inhibitor with micromolar affinity for the CGRP receptor led to the discovery of a series of structurally novel CGRP receptor antagonists. Optimization of this series produced compound **16**, a low-molecular weight CGRP receptor antagonist with good pharmacokinetic properties in both rat and dog. In contrast to other nonpeptide antagonists, the activity of **16** was affected by the presence of divalent cations and showed evidence of an alternative, RAMP-independent CGRP receptor binding site.

Calcitonin gene-related peptide (CGRP), a 37-amino acid neuropeptide first discovered in the early 1980's, is produced by alternative splicing of the calcitonin gene and is widely distributed in the central and peripheral nervous systems.[1,2] The CGRP receptor is a heterodimer comprised of the G-protein-coupled receptor (GPCR), calcitonin receptor-like receptor (CLR) plus the receptor activity-modifying protein 1 (RAMP1) (Fig. 1).[3] The same CLR protein is also known to associate with other receptor activity-modifying proteins (RAMP2 or RAMP3) to produce high affinity receptors for the peptide adrenomedullin. Similarly, the related calcitonin receptor (CTR) can dimerize with RAMP1 to form a high affinity amylin receptor.[4]

In many tissues, CGRP-containing nerves are closely associated with blood vessels and CGRP is known to be a potent vasodilator.[2] Additionally, a number of lines of evidence have pointed to a key role for CGRP in migraine pathophysiology and this has led to interest in the development of small molecule antagonists of the CGRP receptor as a possible treatment for migraine and pain.[5] Boehringer Ingelheim reported the first selective small molecule CGRP receptor antagonist olcegepant **1** (Fig. 2), which exhibited impressive affinity for human CGRP receptors (K_i = 14 pM).[6] Intravenous administration of olcegepant provided clinical proof of concept for CGRP receptor antagonists in the treatment of migraine[7] and these findings have been subsequently corroborated with the orally bioavailable compounds telcagepant (MK-0974) **2**,[8,9] MK-3207 **3**[10,11] (Fig. 2) and BI 44370 TA.[12]

Profiling of the HIV integrase inhibitor L-870,810,[13] compound **5** (Fig. 2), revealed that it possessed micromolar affinity for the CGRP receptor. Since this compound represented a distinct chemotype from other known CGRP receptor antagonists, further investigation seemed warranted. Screening of other HIV integrase inhibitors identified a number of other active compounds and suggested that the cyclic sultam was important for antagonism of the CGRP receptor (data not shown). With these preliminary data in

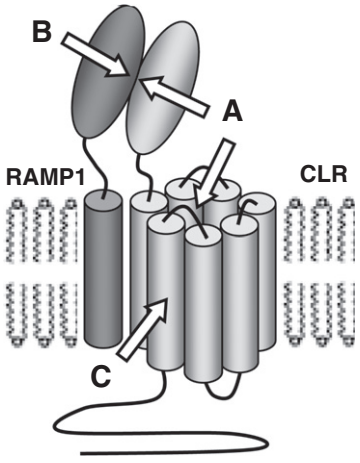

Figure 1. Schematic representation of CGRP receptor. CLR is shown in light gray and RAMP1 in dark gray. Putative binding sites for various ligands are labeled as **A** (CGRP), **B** (most small molecule antagonists), and **C** (compound **16**).

* Corresponding author.
E-mail address: blair_zartman@merck.com (C.B. Zartman).

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doi:10.1016/j.bmcl.2011.09.056

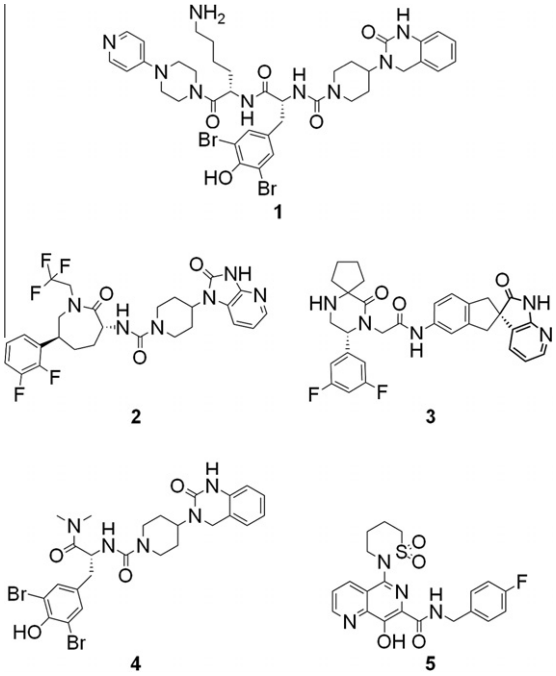


Figure 2. CGRP receptor antagonists olcegepant (**1**), telcagepant (**2**), MK-3207 (**3**), compound **4** and L-870,810 (**5**).

hand, we initiated an effort to characterize and optimize this novel lead series.

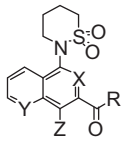
As noted previously, the lead compound **5** had micromolar affinity for the CGRP receptor (K_i = 7300 nM). Evaluation of a number of alternatives to the 4-fluorobenzylamide moiety of **5** revealed a preference for fluoro substitution of the aromatic ring (compare compounds **5**, **6** and **9** in Table 1). In particular, the 3,5-difluorobenzylamide (compound **10**) offered improved potency compared with benzylamide **6**. In addition to lipophilic, fluoroaromatic substituents, moderately polar groups were also tolerated, as exemplified by the pyridine **7** and the *tert*-butyl ester **8**.

Exploration of the naphthyridine core (Table 1) revealed some of the key pharmacophoric elements for compounds of this class. The inactivity of compound **11** highlights the importance of the appended hydroxyl group. Replacement of the naphthyridine core with an isoquinoline (**12**) or quinoline (**13**) also produced compounds with diminished activity. The ester analog **14** lost all activity, implying that the more basic amide carbonyl of **10** may be required for activity.

In the context of the 3,5-difluorobenzyl amide moiety, SAR around a truncated pyridine core was also explored (Table 2). Simple deletion of the western pyridine ring from naphthyridine **10** led to a 20-fold loss in receptor affinity (compound **15**), but incorporation of a bromine atom provided compound **16**, which possessed similar potency to the naphthyridine analog. As in the naphthyridine series, the hydroxyl substituent appeared to be critical for activity and methylation (**17**) caused a significant loss in potency. Also in analogy with the naphthyridines, the glycine *tert*-butyl ester derivative **18** possessed similar receptor affinity to the benzyl amides. With compound **18** in hand, an array of glycine amides were explored leading to the most active compound in this series, compound **21** (K_i = 710 nM).

The lack of activity observed with compounds **11**, **13**, **14** (Table 1) and **17** (Table 2) was of significant interest. One interpretation of these data was that the presence of the hydroxyl moiety and the coplanarity of the basic amide carbonyl and the naphthyridine ring were crucial for activity. Taken together with the fact that the original lead **5** evolved from the known metal-chelating diketo acid

Table 1
Selected CGRP receptor antagonists and naphthyridine analogs



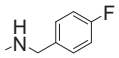
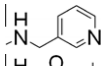
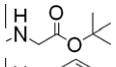
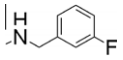
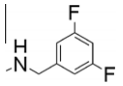
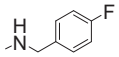
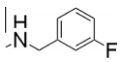
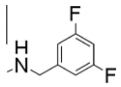
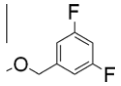
Compd	R	X	Y	Z	CGRP binding K_i, nM[a,b]	CGRP cAMP IC_{50}, nM[a,c]
2	–	–	–	–	0.78 ± 0.05 (10)[e]	2.2 ± 0.29 (8)[d,e]
3	–	–	–	–	0.024 ± 0.001 (3)[f]	0.12 ± 0.02 (6)[d,f]
4	–	–	–	–	9.3 ± 3.1 (7)	41 ± 12 (11)
5		N	N	OH	7300 ± 1700 (3)	28,000 (1)
6		N	N	OH	16,000 (2)	NA
7		N	N	OH	14,000 (2)	NA
8		N	N	OH	8000 (1)	>10,000 (1)
9		N	N	OH	5200 ± 1400 (4)	>10,000 (1)
10		N	N	OH	1900 (1)	2600 (1)
11		N	N	H	>100,000 (1)	NA
12		N	CH	OH	26,000 (1)	NA

Table 1 (*continued*)

Compd	R	X	Y	Z	CGRP binding K_i, nM[a,b]	CGRP cAMP IC_{50}, nM[a,c]
13		CH	N	OH	>100,000 (1)	NA
14		N	N	OH	>100,000 (1)	NA

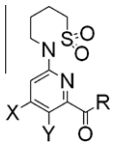
[a] Number of replicates given in parentheses (NA, not tested).
[b] K_i values for inhibition of ^{125}I-hCGRP binding determined using native human receptor in SK-*N*-MC cell membranes.[14]
[c] Inhibition of CGRP-stimulated cAMP production in SK-*N*-MC cells.[14]
[d] Inhibition of CGRP-stimulated cAMP production in HEK293 cells stably expressing CLR/RAMP1.[14]
[e] Data from Ref. 14.
[f] Data from Ref. 15.

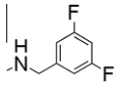
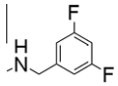
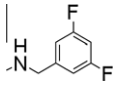
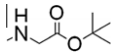
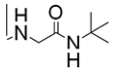
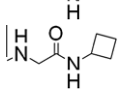
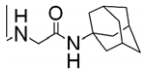
pharmacophore found in many HIV integrase inhibitors[13] this suggested that metal binding was critical for the interaction of these compounds with the CGRP receptor. The HIV integrase inhibitors related to lead compound **5** are thought to bind to divalent cations in the active site of the enzyme. We noted that our standard CGRP receptor binding assay contained 5 mM $MgCl_2$ and hypothesized that an interaction with this metal ion may be important for the activity of compounds like **5**.

In order to investigate this possibility, CGRP and a number of representative antagonists were analyzed in the binding assay both in the presence and absence of 5 mM $MgCl_2$ (Table 3). The small molecule CGRP receptor antagonist **4**, a truncated analog of olcegepant, was essentially unaffected by removal of magnesium chloride from the assay. In contrast, it was clearly evident that the hydroxypyridine **16** required Mg^{2+} for binding to the CGRP receptor and its K_i value shifted >35-fold in the absence of added magnesium. Related analogs, such as **21**, also lost significant receptor affinity without added $MgCl_2$, suggesting that the binding of this novel class of antagonists to the CGRP receptor requires magnesium ions, in contrast to other known small molecule CGRP receptor antagonists. The native agonist CGRP and the peptide antagonist CGRP (8–37) were unaffected by the presence or absence of magnesium ions.

Radioligand binding studies utilizing chimeric receptors generated by exchanging regions of the CLR with corresponding regions of the calcitonin receptor (CTR), a related class II G-protein-coupled receptor, followed by coexpression with RAMP1 afforded further insight into the binding of these hydroxypyridine-based CGRP receptor antagonists.[16] Compound **16** (designated "compound 4" in Ref. 16) was shown to bind to CGRP by interaction with transmembrane region 7 (TM7) of CLR independent of RAMP1, while small molecule antagonists related to olcegepant were shown to bind RAMP1-dependently to the amino terminus of CLR (Fig. 1).[16] Because **16** interacted with the CGRP receptor in a RAMP-independent manner, it exhibited a different selectivity profile to that observed for many CGRP receptor antagonists, such as telcagepant (**2**). For example, while **2** possessed good selectivity against the human adrenomedullin receptor CLR/RAMP2 (K_i >100 μM), **16** did not

Table 2
Selected hydroxypyridine compounds



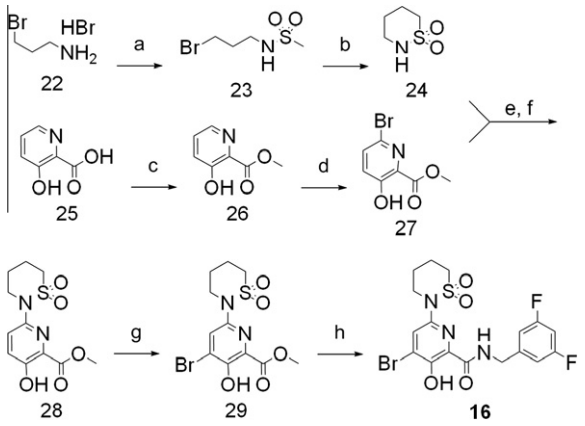
Compd	R	X	Y	CGRP binding K_i, nM[a,b]	CGRP cAMP IC_{50}, nM[a,c]
15		H	OH	45,000 (1)	NA
16		Br	OH	3300 ± 1100 (11)	2700 (2)
17		Br	OCH_3	>100,000 (1)	NA
18		Br	OH	1700 ± 500 (6)	9800 (2)
19		Br	OH	11,000 (2)	>10,000 (1)
20		Br	OH	1800 (2)	2400 (1)
21		Br	OH	710 (2)	2100 (1)

[a] Number of replicates given in parentheses (NA, not tested).
[b] K_i values for inhibition of ^{125}I-hCGRP binding determined using native human receptor in SK-*N*-MC cell membranes.[14]
[c] Inhibition of CGRP-stimulated cAMP production in SK-*N*-MC cells.[14]

Table 3
Effect of magnesium on CGRP and selected CGRP receptor antagonists

Compd	K_i, nM[a] 5 mM $MgCl_2$	K_i, nM[a] 0 mM $MgCl_2$	Shift
4	9 (2)	8 (2)	0.9
16	2700 (3)	>100,000 (3)	>35
21	840 (1)	5600 (1)	6.7
CGRP (**8–37**)	5.5 (1)	4.5 (1)	0.8
CGRP	0.01 (1)	0.01 (1)	1

[a] Number of replicates given in parentheses. K_i values determined in the presence and absence of 5 mM $MgCl_2$ for the inhibition of ^{125}I-CGRP binding to SK-N-MC cell membranes.



Scheme 1. Synthesis of compound **16**. Reagents and conditions: (a) TEA, methanesulfonyl chloride, THF, 5 °C, 95%; (b) *N*,*N*-diisopropylamine, 1,10-phenanthroline, *n*-BuLi in hexanes, THF, −20 °C to rt, 77%; (c) H_2SO_4, CH_3OH, 79%; (d) Br_2, H_2O, 79%; (e) Cu(I)O, pyridine, 130 °C; then EDTA, CH_2Cl_2, H_2O; (f) H_2SO_4, CH_3OH, 34% over two steps; (g) NBS, CH_3Cl, 80 °C, 47%; and (h) 3,5-difluorobenzylamine, toluene, reflux, 42%.

Table 4
Pharmacokinetic data for compound **16**

Species	F (%)	iv $t_{1/2}$ (h)	Cl (mL/min/kg)	Vd_{ss} (L/kg)
Rat	26[a]	5.4[c]	1.1	0.4
Dog	13[b]	7.1[d]	5.1	3.0

[a] Dosed at 10 mpk in 1% methylcellulose.
[b] Dosed at 1 mpk in 1% methylcellulose.
[c] Dosed at 2 mpk in DMSO.
[d] Dosed at 0.5 mpk in DMSO.

(K_i = 7500 nM).[14] Additionally, **2** had reduced affinity for non-primate CGRP receptors such as the rat receptor (K_i = 1200 nM), and much of this species selectivity was dictated by sequence differences in RAMP1, notably by residue 74.[17,18] Unsurprisingly, since **16** does not appear to interact with RAMP1, it did not exhibit significantly lower affinity for the rat CGRP receptor (K_i = 6800 nM).[16]

The synthesis of compound **16**, which is representative of the methodology used to synthesize the related analogs in Table 2, is shown in Scheme 1.[19] The cyclic sultam was synthesized in two steps[20] from commercially available 3-bromopropan-1-amine hydrobromide and the methyl 6-bromo-3-hydroxypyridine-2-carboxylate was obtained in two steps from commercially available 3-hydroxypyridine-2-carboxylic acid. An Ullmann coupling of the two followed by bromination with NBS and direct amidation of the ester with amines yielded compound **16** and closely related analogs. Amidation with *tert*-butyl glycinate followed by hydrolysis and standard amide couplings allowed for the synthesis of related amides **19**–**21**.

The pharmacokinetic properties of compound **16** were evaluated in rat and dog. As detailed in Table 4, **16** exhibited moderate oral bioavailability in rat and dog, with low plasma clearance and good plasma half-lives in both species.

In conclusion, a novel series of CGRP receptor antagonists has been characterized. Optimization of naphthyridine **5** led to the orally bioavailable hydroxypyridine **16**, which possessed a 10-fold improvement in functional potency. Compound **16** interacted with the CGRP receptor in a magnesium-dependent fashion at a binding site that involved TM7 and was independent of RAMP1. As such, this CGRP receptor antagonist is both structurally and functionally distinct from previously reported small molecule antagonists of this receptor.

Acknowledgments

The authors thank Yvonne M. Leonard, Cynthia Miller-Stein and Audrey Wallace for animal dosing and pharmacokinetic analysis; the MRL West Point analytical, mass spectrometry and NMR spectroscopy groups for analytical and spectroscopy data; and Neville Anthony, Scott Ceglia, Robert Gomez and John Wai for providing compounds **5**, **6** and **9** and for helpful discussions.

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