Bioorganic & Medicinal Chemistry Letters 23 (2013) 1870–1873

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

The synthesis and SAR of calcitonin gene-related peptide (CGRP) receptor antagonists derived from tyrosine surrogates. Part 2 ☆

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ARTICLE INFO

Article history:
Received 2 November 2012
Revised 28 December 2012
Accepted 2 January 2013
Available online 24 January 2013

Keywords:
CGRP receptor antagonist
Calcitonin gene-related peptide (CGRP)
Migraine headache
Tyrosine surrogates
CYP 3A4 inhibition

ABSTRACT

Various substituted indazole and benzoxazolone amino acids were investigated as D-tyrosine surrogates in highly potent CGRP receptor antagonists. Compound **3**, derived from the 7-methylindazole core, afforded a 30-fold increase in CGRP binding potency compared with its unsubstituted indazole analog **1**. When dosed at 0.03 mg/kg SC, compound **2** (a racemic mixture of **3** and its (*S*)-enantiomer) demonstrated robust inhibition of CGRP-induced increases in mamoset facial blood flow up to 105 min. The compound possesses a favorable predictive in vitro toxicology profile, and good aqueous solubility. When dosed as a nasal spray in rabbits, **3** was rapidly absorbed and showed good intranasal bioavailability (42%).

Triptans, the current standard of care for migraine, are 5-$HT_{1B/1D}$ receptor agonists. Their efficacy depends on a vasoconstrictive effect on blood vessels. Although selective for intracranial over coronary vessel constriction, triptans are contraindicated in patients with cardiovascular disease and hypertention.[2] Thus, there is a clear need for acute migraine therapies without cardiovascular liabilities. Calcitonin gene-related peptide (CGRP) is a 37-amino acid neuropeptide that is expressed in trigeminal ganglia nerves, and has been implicated in the pathogenesis of migraine.[3] Increased levels of CGRP are observed during a migraine attack, and intravenous (iv) administration of CGRP can induce migraine in migraineurs.[4] The potent CGRP receptor antagonist, BIBN4096BS (olcegepant), when doses iv demonstrated antimigraine efficacy that was minimally equivalent to the triptans, without cardiovascular or other serious adverse effects.[5] Orally administered CGRP receptor antagonists have also recently demonstrated clinical utility in acute migraine.[6] In this context, we undertook a medicinal chemistry effort to identify a potent CGRP antagonist that could be delivered by another convenient route of administration.[7]

Although oral formulations are usually the most convenient route for delivering medicines, intranasal delivery would seem ideally suited for the treatment of migraine. A nasal spray can be expected to achieve a more rapid onset of action in comparison with traditional oral formulations.[8,9] Moreover, while nausea can limit the usefulness of oral medicines in migraneurs, intranasal dosing offers the possibility of treating even those patients suffering from migraine-related emesis.[10] However, an effective intranasal formulation imposes a number of stringent compound requirements. First, the molecule must have excellent plasma protein-adjusted binding potency because of the need to deliver a fairly low dose into the nasal cavity.[11] Also, the compound should have very high aqueous solubility as the entire dose must be delivered in a small volume of water or aqueous buffer (⩽100 μL/nostril).[12]

Previously, we have reported the identification and SAR of novel CGRP receptor antagonists derived from heterocyclic tyrosine surrogates.[1] Compound **1** (Fig. 1) was found to be a potent antagonist with a reasonable cytochrome P450 (CYP) profile, and it showed activity in a primate model of CGRP-induced facial blood flow when dosed subcutaneously. In this Letter, we report the SAR efforts that led to compound **3**, a highly potent CGRP receptor antagonist that was the direct precursor to BMS-694153, our first potential intranasal clinical candidate.[13] We also describe the activity of **3** in ex vivo and in vivo models relevant to migraine.[13]

☆ See Ref. 1.

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0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.01.011

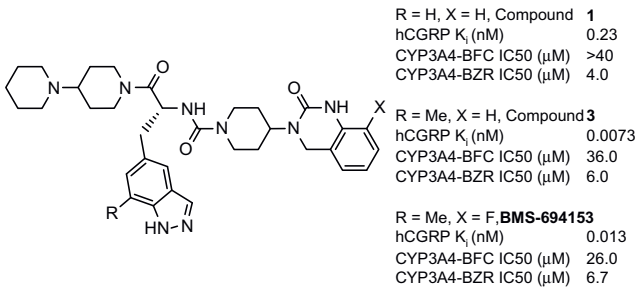
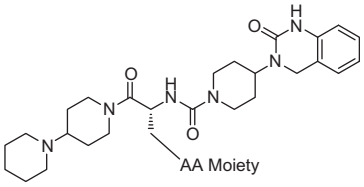
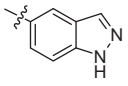
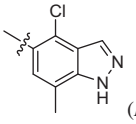
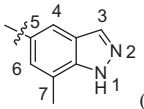
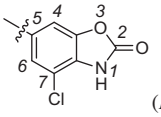
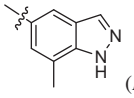
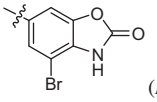
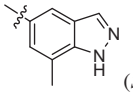
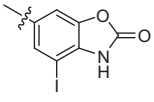
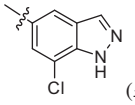
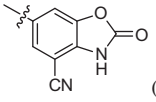
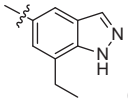
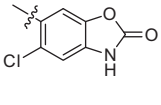
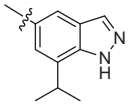
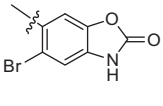
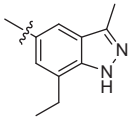
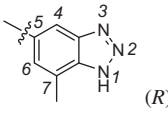


Figure 1.

As a surrogate of tyrosine, the indazole-containing amino acid in compound **1** gave us the best balance of CGRP receptor antagonist potency and acceptable CYP inhibition.[1] Therefore, we first decided to study the effects of substitution on the indazole core, and then study the effects of substitution on related heterocycles. The results of this study are summarized in Table 1. We were pleased to see that the racemic amino acid with a 7-Me substitution on the indazole (**2**) showed excellent binding potency (K_i = 0.012 nM). As expected from our previous studies, activity resided primarily with the *R*-enantiomer (**3**, K_i = 0.0073 nM), which was 30-fold more potent than compound **1**. It was also encouraging to see that inhibition of CYP3A4 metabolism of BZR, our strongest isoform interaction in this series, was unchanged in comparison with compound **1** (CYP 3A4 BZR IC_{50}, 6.0 vs 4.0 μM, respectively, Table 1). This effectively improved our safety margin versus CYP 3A4 by greater then 30-fold. Replacement of the 7-Me in **2** with -Cl (**5**) or -Et (**6**) resulted in comparable CGRP receptor binding activity but increased CYP inhibition (3A4 BZR IC_{50}'s 0.18 and 0.45 μM for **5** and **6**, respectively, Table 1). The corresponding 7-*i*-Pr analog (**7**) was significantly less potent (K_i = 0.22 nM), and the disubstituted compounds **8** and **9** were modestly less potent (K_i = 0.048 and 0.032 nM, respectively, Table 1). These results suggested that the 7-Me indazole was the best fit for the D-amino acid binding pocket in the CGRP receptor.

Table 1
SAR of central amino acid moieties



Compd	AA moiety (stereochemistry)	K_i (nM)	CYP3A4 (IC_{50}, μM) BZR (BFC)[a]	Compd	AA moiety (stereochemistry)	K_i (nM)	CYP3A4 (IC_{50}, μM) BZR (BFC)
1	 (*R*)	0.23	4.0 (>40)	**9**	 (*R*)	0.032	1.6 (3.5)
2	 (±)	0.012	1.1 (24)	**10**	 (*R*)	0.039	17 (>4 0)
3	 (*R*)	0.0073	6.0 (36)	**11**	 (*R*)	0.023	7.2 (>40)
4	 (*S*)	27	NA	**12**	 (*R*)	0.055	5.5 (32)
5	 (±)	0.011	0.18 (13)	**13**	 (*R*)	11	NA
6	 (±)	0.0050	0.45 (25)	**14**	 (*R*)	0.28	5.0 (>40)
7	 (±)	0.22	0.37 (NA)	**15**	 (*R*)	0.14	2.3 (18)
8	 (±)	0.048	6.9 (>40)	**16**	 (*R*)	0.015	7.3 (>40)

[a] BZR = 7-benzyloxy-resorufin and BFC = 7-benzyloxy-4-trifluoromethylcoumarin.

Previous results from our laboratories indicated that benzooxazolone and benzotriazole moieties showed CGRP receptor antagonist potencies and CYP inhibition profiles that were comparable to indazole **1**.[1] Thus we prepared a small series of halogen-substituted benzoxazolones. The 7-Cl (**10**, K_i = 0.039 nM), 7-Br (**11**, K_i = 0.023 nM), and 7-I (**12**, K_i = 0.055 nM) analogs all demonstrated very good CGRP receptor binding, albeit somewhat inferior to **3** (K_i = 0.0073 nM), with CYP3A4 inhibition that became undesirably more potent with increasing size of the halogen substituent. Substitution at the 6-position of the benzoxazolones with Cl (**14**) and Br (**15**) both resulted in a significant loss of potency (K_i = 0.28 and 0.14 nM, respectively, Table 1) compared with the corresponding 7-substituted analogues (**10** and **11**), but similar to the unsubstituted benzoxazolone (compound **25** in Ref. 1, K_i = 0.27 nM). Interestingly, the 7-CN analog (**13**) was almost 300-fold less potent than the 7-Cl compound (**10**), suggesting a very strict requirement for hydrophobicity in that small binding pocket in the CGRP receptor. Compound **16**, the 7-Me substituted benzotriazole, was comparable to **3** in terms of CGRP receptor binding potency and CYP inhibition.

Functional activity of new compounds was determined by measuring concentration-dependent inhibition of CGRP-stimulated cAMP production in SK-N-MC cells.[13] All compounds shown in Table 1 demonstrated full antagonism of the human CGRP receptor (for **3**, EC_{50} = 0.017 nM). Compound **3** was chosen for further study. First, we examined the ability of **3** to antagonize CGRP-induced blood vessel dilation in an assay utilizing ex vivo human intracranial arteries.[13] In this experiment, each vessel ring was mounted between two wire hooks and attached to a force transducer that measured arterial tone. Vessels were first contracted with 10 mM KCl, then fully dilated with 1 nM hαCGRP. Dilation was then reversed by the cumulative addition of increasing concentrations of **3** in half-log amounts. In this study, compound **3** potently reversed CGRP-induced dilation on human intracranial arteries with an EC_{50} = 0.050 nM. Commensurate with its improved binding affinity, compound **3** was 24-fold more potent than compound **1** (EC_{50} = 1.2 nM) in this assay.

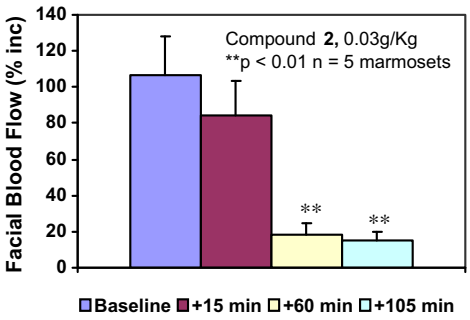
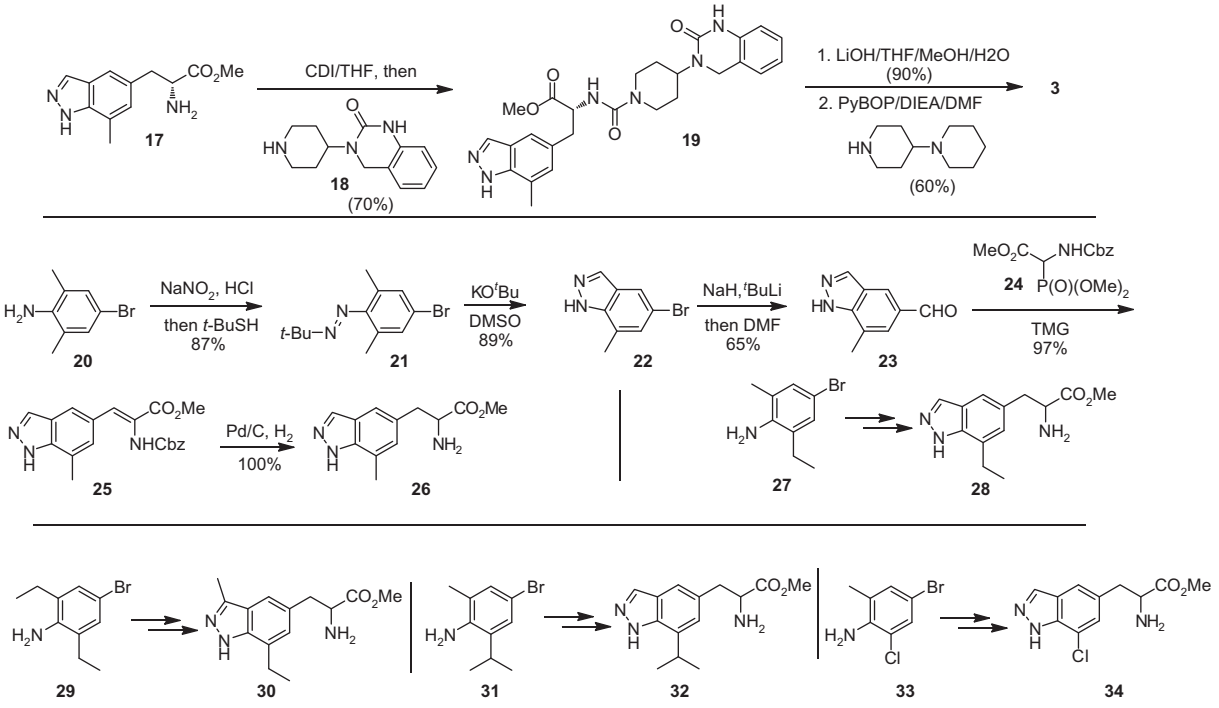


Figure 2. Marmoset facial blood flow.

Our marmoset facial blood flow model was used to assess in vivo efficacy of our CGRP receptor antagonists.[13] In this model, marmosets were anesthetized and facial blood flow was measured and increased by iv administration of hαCGRP (10 μg/kg) at 45 min intervals (−30, 15, 60, and 105 min). The effect of antagonist **2** (the racemate of **3**), delivered subcutaneously (SC) at 0 min, on CGRP-induced changes in facial blood flow was measured by laser Doppler flowmetry. In this study, compound **2** (0.03 g/kg, SC) demonstrated strong inhibition at 60 and 105 min (Fig. 2). Comparing efficacy versus exposure of **2** at 15 min (4.7 nM), 60 min (13 nM) and 105 min (11 nM), showed that plasma levels of **2** above 10 nM were associated with robust in vivo activity, consistent with what we had previously seen for BMS-694153.[13] CGRP-induced increases in marmoset facial blood flow were similar across all time points in vehicle-treated animals.

Intranasal administration of **3** was explored in rabbits. The aqueous solubility of amorphous **3**·HCl in the pH range 4.0–6.2 was between 23 and 13 mg/mL. When intranasally dosed as a spray at a concentration of 10 mg/mL, the HCl salt of **3** was rapidly and efficiently absorbed from the nasal cavity of rabbits. Measurable plasma levels were seen as early as 2 min and T_{max} occurred within 10 min. The intranasal bioavailability of a 0.6 mg/kg dose of a 10 mg/mL solution dose of **3** was 42%.



Scheme 1. Synthesis of compound **3** and amino esters **26**, **28**, **30**, **32** and **34**.

Compound **3** had a favorable CYP profile with IC_{50}'s >40 μM for CYP isoforms 1A2, 2C19, 2C9 and 2D6. Its IC_{50}'s for CYP3A4 were 36 μM (BFC) and 6.0 μM (BZR). Compound **3** was negative in the Ames test, and it demonstrated only low levels of hERG channel inhibition and PXR activation.

The representative syntheses of compound **3** and the amino acids required for compounds **2**–**16** is outlined in Scheme 1. The coupling of amino ester **17**[14] and quinazolinone **18**[15] afforded urea **19**. Saponification of the methyl ester of **19** with LiOH gave the carboxylic acid, which was coupled with 1,4′-bipiperidine to afford compound **3** (Scheme 1).

The synthesis of amino ester **26** began with the treatment of aniline **20** with $NaNO_2$, followed by quenching of the resulting diazonium ion with *t*-BuSH to afford **21**. Reaction of **21** with KO^{t}Bu in DMSO gave indazole bromide **22**,[16] which was converted to aldehyde **23** by reaction with NaH/tBuLi and quenching with DMF. The olefination[17] of aldehyde **23** using phosphonate **24** provided enamide **25**. Hydrogenation of **25** afforded amino ester **26**. In a similar manner, amino esters **28**, **30**, **32** and **34** were prepared from the corresponding anilines **27**, **29**, **31** and **33** (Scheme 1). The synthesis of other amino esters used in the compounds shown in Table 1 have been reported previously.

In summary, we explored substituted indazole and benzoxazolone amino acids as D-tyrosine surrogates in CGRP receptor antagonists. We found that compound **3**, derived from the 7-methylindazole core, afforded a 30-fold increase in CGRP binding potency compared with the corresponding unsubstituted indazole **1**. When dosed at 0.03 mg/kg SC, **2** (racemate of **3**) demonstrated robust inhibition of CGRP-induced increases in mamoset facial blood flow out to 105 min. Compound **3** also possessed a favorable predictive in vitro toxicology profile. However, despite showing good IN bioavailability in rabbits, its aqueous solubility of **3** was not deemed sufficient for continued development. Further efforts resulted in the discovery of BMS-694153, a compound with superior aqueous solubility.[13]

Acknowledgment

We thank the members of Discovery Analytic Sciences for their help in the chiral purification and characterization of compounds contained herein.

References and notes

1. For Part I, see: Han, X.; Civiello, R. L.; Conway, C. M.; Cook, D. A.; Davis, C. D.; Macci, R.; Pin, S. S.; Ren, S. X.; Schartman, R.; Signor, L. J.; Thalody, G.; Widmann, K. A.; Xu, C.; Chaturvedula, P. V.; Macor, J. E.; Dubowchik, G. M. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4723.
2. Silberstein, S. D. *Lancet* **2004**, *363*, 381.
3. (a) Goadsby, P. J. *Drugs* **2005**, *65*, 2557; (b) Edvinsson, L. *Cephalalgia* **2004**, *24*, 611; (c) Williamson, D. J.; Hargreaves, R. J. *Microsc. Res. Tech.* **2001**, *53*, 167.
4. Lassen, L. H.; Haderslev, P. A.; Jacobson, V. B.; Iversen, H. K.; Sperling, B.; Olesen, J. *Cephalalgia* **2002**, *22*, 54.
5. Olesen, J.; Diener, H. C.; Husstedt, I. W.; Goadsby, P. J.; Hall, D.; Meier, U.; Pollentier, S.; Lesko, L. M. N. *Engl. J. Med.* **2004**, *350*, 1104.
6. Oral CGRP receptor antagonist medicinal chemistry efforts have been reported in: (A) For MK-0974 Paone, D. V.; Shaw, A. W.; Nguyen, D. N.; Burgey, C. S.; Deng, J. Z.; Kane, S. A.; Koblan, K. S.; Salvatore, C. A.; Mosser, S. D.; Johnston, V. K.; Wong, B. K.; Miller-Stein, C. M.; Hershey, J. C.; Graham, S. L.; Vacca, J. P.; Williams, T. M. *J. Med. Chem.* **2007**, *50*, 5564; For MK-3207 (b) Bell, I. M.; Gallicchio, S. N.; Wood, M. R.; Quigley, A. G.; Stump, C. A.; Zartman, C. B.; Fay, J. F.; Li, C.; Lynch, J. J.; Moore, E. L.; Mosser, S. D.; Prueksaritanont, T.; Regan, C. P.; Roller, S.; Salvatore, C. A.; Kane, S. A.; Vacca, J. P.; Selnick, H. G. *ACS Med. Chem. Lett.* **2010**, *1*, 24.
7. (a) IDDB, Thomson Scientific reports that the development of BIBN4096BS (IV delivery) has been discontinued.; (b) Merck & Co., Inc. announced discontinuation of the clinical development program for MK-3207 (oral delivery) on Sept. 10, 2009.
8. Behl, C. R.; Pimplaskar, H. K.; Sileno, A. P.; DeMeireles, J.; Romeo, V. D. *Adv. Drug Deliv. Rev.* **1998**, *29*, 89.
9. Rapoport, A. *Cephalalgia* **2001**, *21*, 13.
10. Rapoport, A. M.; Bigal, M. E.; Tepper, S. J.; Sheftell, F. D. *CNS Drugs* **2004**, *18*, 671.
11. Wermeling, D. P.; Miller, J. L.; Rudy, A. C. *Drug Deliv. Technol.* **2002**, *2*, 56.
12. Merkus, P.; Guchelaar, H.-J.; Bosch, D. A.; Merkus, F. W. H. M. *Neurology* **2003**, *60*, 1669.
13. Degnan, A. P.; Chaturvedula, P. V.; Conway, C. M.; Cook, D. A.; Davis, C. D.; Denton, R.; Han, X.; Macci, R.; Mathias, N. R.; Moench, P.; Pin, S. S.; Ren, S. X.; Schartman, R.; Signor, L. J.; Thalody, G.; Widmann, K. A.; Xu, C.; Macor, J. E.; Dubowchik, G. M. *J. Med. Chem.* **2008**, *51*, 4858. For biology experimental procedures to measure [^{125}I]CGRP binding, CYP inhibition, reversal of CGRP-induced dilation in ex vivo human intracranial artery, Schild analysis, in vivo efficacy in marmoset facial blood flow, and pharmacokinetics properties, see the Supplementary data in this Letter.
14. For the synthesis of the amino acids not shown in Scheme 1, see: (a) Han, X.; Civiello, R. L.; Fan, H.; Wu, D.; Gao, Q.; Chaturvedula, P. V.; Macor, J. E.; Dubowchik, G. M. *J. Org. Chem.* **2008**, *73*, 8502; (b) Han, X.; Jiang, X.-J.; Civiello, R. L.; Degnan, A. P.; Chaturvedula, P. V.; Macor, J. E.; Dubowchik, G. M. *J. Org. Chem.* **2009**, *74*, 3993.
15. Rudolf, K.; Eberlein, W.; Engel, W.; Pieper, H.; Entzeroth, M.; Hallermayer, G.; Doods, H. *J. Med. Chem.* **2005**, *48*, 5921.
16. Dell'Erba, C.; Novi, M.; Petrillo, G.; Tavani, C. *Tetrahedron* **1994**, *50*, 3529.
17. Schmidt, U.; Griesser, H.; Leitenberger, V.; Lieberknecht, A.; Mangold, R.; Myer, R.; Riedl, B. *Synthesis* **1992**, 487.