Letters

Discovery of (*R*)-4-(8-Fluoro-2-oxo-1,2-dihydroquinazolin-3(4*H*)-yl)-*N*-(3-(7-methyl-1*H*-indazol-5-yl)-1-oxo-1-(4-(piperidin-1-yl)piperidin-1-yl)propan-2-yl)piperidine-1-carboxamide (BMS-694153): A Potent Antagonist of the Human Calcitonin Gene-Related Peptide Receptor for Migraine with Rapid and Efficient Intranasal Exposure

Andrew P. Degnan,* Prasad V. Chaturvedula,* Charles M. Conway, Deborah A. Cook, Carl D. Davis, Rex Denton, Xiaojun Han, Robert Macci, Neil R. Mathias, Paul Moench, Sokhom S. Pin, Shelly X. Ren, Richard Schartman, Laura J. Signor, George Thalody, Kimberly A. Widmann, Cen Xu, John E. Macor, and Gene M. Dubowchik

Departments of Neuroscience Chemistry, Neuroscience Biology, Metabolism and Phamacokinetics, Bioanalytical Research, Discovery Toxicology, Pharmaceutics, and Pharmaceutics Development, Bristol-Myers Squibb Research & Development, 5 Research Parkway, Wallingford, Connecticut 06492

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Abstract: Calcitonin gene-related peptide (CGRP) has been implicated in the pathogenesis of migraine. Early chemistry leads suffered from modest potency, significant CYP3A4 inhibition, and poor aqueous solubility. Herein, we describe the optimization of these leads to give **4** (BMS-694153), a molecule with outstanding potency, a favorable predictive toxicology profile, and remarkable aqueous solubility. Compound **4** has good intranasal bioavailablity in rabbits and shows dose-dependent activity in validated in vivo and ex vivo migraine models.

Introduction

It has been hypothesized that migraine headache is associated with the dilation of cranial blood vessels.[1] The current standard of care, the triptans, are 5-$HT_{1B/1D}$ agonists and are believed to act, in part, by the active, nonselective vasoconstriction of intracranial arteries.[2] However, because triptans are nonselective vasoconstrictors, they are associated with a number of unpleasant cardiovascular side effects and are contraindicated in patients with hypertension or ischemic heart disease.[3] Calcitonin gene-related peptide (CGRP), an extremely potent vasodilator, has been implicated in the pathogenesis of migraine.[4] Studies have shown that plasma levels of CGRP, a 37 amino acid peptide, are elevated during migraine attacks.[5] In addition, it has recently been demonstrated that intravenous administration of the potent CGRP receptor antagonist BIBN4096BS is accompanied by the alleviation of pain in migraneurs.[6] Importantly, preliminary reports indicate that efficacy was achieved in the absence of the cardiovascular side effects associated with the use of triptans.

* To whom correspondence should be addressed. For A.P.D.: Phone, 203-677-5750; Fax, 203-677-7702; E-mail, andrew.degnan@bms.com. For P.V.C.: Phone, 203-677-6699; Fax, 203-677-7702; E-mail, prasad.chaturvedula@bms.com.

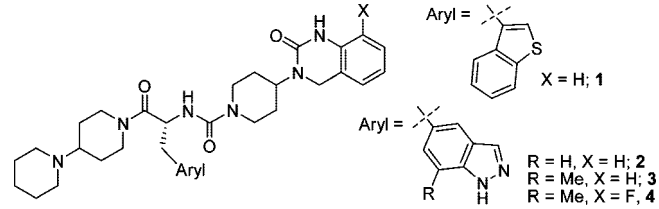

Figure 1. Compounds **1**−**4**.

Table 1. Screening Data for Compounds **1**−**4**

compound	1	2	3	4
hCGRP K_i (nM)	0.55	0.26	0.010	0.013
CYP3A4-BFC[a] IC_{50} (μM)	0.87	>40	36	26
CYP3A4-BZR[b] IC_{50} (μM)	0.084	4.0	6.0	6.7
solubility (mg/mL)	NA	NA	15[c]	>500[d]

[a] BFC=7-benzyloxy-4-trifluoromethylcoumarin. [b] BZR=7-Benzyloxyresorufin. [c] Amorphous material (pH 5). [d] Crystalline (pH 6.8).

Although this compound effectively demonstrated the first clinical proof-of-concept, its acceptance in the market may be limited in its current injectible formulation. In this context, we undertook a medicinal chemistry effort to identify a potent CGRP antagonist that could be administered by a more convenient route.[7,8]

Intranasal (IN) delivery would seem ideally suited for the treatment of migraine. Nasal sprays can be expected to deliver a faster onset of action over traditional oral formulations.[9] Indeed, studies with marketed IN triptans show that plasma concentrations rise more rapidly and offer faster pain relief than their oral forms.[10] Additionally, nausea sometimes limits the usefulness of oral compounds in migraneurs.[11] A nasal spray offers the possibility of treating even those patients suffering from migraine-related emesis. The decision to pursue an IN formulation imposed a number of compound requirements. First, the molecule must have very high receptor potency and a large free fraction in plasma because of the need to deliver a low dose to the nasal cavity.[12] Also, the compound should have very high aqueous solubility as the entire dose must be delivered in a small volume of water ($\leq$100 μL/nostril).[13] Herein we describe the identification of the potent CGRP antagonist **4** (BMS-694153) for IN delivery.

A careful survey of journal and patent literature,[14] revealed a number of structural motifs common to known CGRP antagonists. As such, the synthesis of compound **1** was undertaken which incorporated several of these elements (Figure 1). Compound **1** was found to be a potent inhibitor of CGRP in the primary binding assay (Table 1). Unfortunately, further evaluation of **1** revealed it to be a potent inhibitor of cytochrome P450 3A4 (CYP3A4). To better understand the role that the benzthiophene side chain played in the inhibition of CYP3A4, a number of analogues with varied amino acid side chains were prepared and tested for their ability to inhibit CYP3A4. One heterocycle that proved very promising in this regard was the indazole of compound **2**, which conferred excellent potency in the CGRP binding assay while greatly reducing CYP3A4 inhibition. Further work in the indazole chemotype led to compound **3**, containing a 7-methylindazole. This substitution resulted in a dramatic increase in receptor potency while

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preserving acceptable CYP3A4 inhibition. The profile of **3** in a series of in vitro assays designed to predict pharmacokinetic properties indicated low clearance based on the stability of the compound in liver microsomes (human, rat, and mouse). Of the major human metabolizing enzymes, only CYP3A4 was moderately inhibited by **3** (other major isoforms >30 μM). Although **3** emerged as an attractive lead, it did not have sufficient aqueous solubility (Table 1) to support IN dosing. A survey of closely related analogues revealed that compound **4**, differing only in the inclusion of a fluorine at C-8 of the quinazolinone, had dramatically improved solubility (>500 mg/mL) while retaining the positive attributes of **3** (vide infra) (Figure 1). It is possible that the fluorine serves to polarize the NH bond of the adjacent urea, rendering it a more potent hydrogen bond donor and thereby enhancing solvation by water.

The binding affinity of **4** for the human CGRP receptor was determined by inhibition of ^{125}I-CGRP binding in SK-N-MC cell membranes. Compound **4** displayed concentration-dependent competitive inhibition with a $K_i = 0.0128 \pm 0.0005$ nM ($n = 10$). Functional receptor antagonism for **4** was determined by measuring concentration-dependent inhibition of CGRP-stimulated cAMP production in SK-N-MC cells. The compound was shown to be a full, competitive antagonist with $EC_{50} = 0.0345 \pm 0.010$ nM ($n = 4$). A measured K_b of 0.0161 nM was in agreement with the binding K_i.

The selectivity of **4** for the CGRP receptor over other closely related receptors[15] in the calcitonin receptor family was assessed using radioligand binding assays. Compound **4** displayed greater than 10000-fold selectivity for CGRP over adrenomedullin receptors 1 and 2, calcitonin, and amylin receptors 1 and 3. Species-dependent CGRP receptor binding affinity of **4** was determined for two additional species. In the marmoset, compound **4** displayed affinity similar to that to that of the human receptor. No inhibition of CGRP binding to the rat receptor was observed at concentrations up to 1000 nM. This result is consistent with earlier reports that small molecule hCGRP-receptor antagonists show species-specific differences with relatively poor activity against nonprimate receptors.[16] This is thought to be due to differences in receptor activity-modifying protein 1 (RAMP1), one of the component proteins of the CGRP receptor (vide infra).

Ex Vivo and in Vivo Studies

To examine the ability of **4** to antagonize CGRP-induced blood vessel dilation, the compound was tested in a number of assays utilizing ex vivo human intracranial arteries. In these studies, each vessel ring was mounted between two wire hooks and attached to a force transducer that measured arterial tone. To test the antidilatory effect of **4**, vessels were first contracted with 10 mM potassium chloride (KCl), then fully dilated with 1 nM hαCGRP, and finally dilation was reversed by the cumulative addition of increasing concentrations of **4** in half-log units. Data analysis was performed for each vessel individually, fitting the concentration−response data to a four-parameter logistic function by nonlinear regression analysis to estimate the EC_{50} values. This study showed that compound **4** potently reverses CGRP-induced dilation (Figure 2, $EC_{50} = 0.06$ nM).

In a second experiment with ex vivo human intracranial arteries, the shift of the CGRP dose−response curve was measured at various concentrations of **4** (Schild Analysis).[15] Each wire-mounted artery ring was preincubated for 30 min with a single concentration (0.1−30 nM) of antagonist **4**, then contracted with 10 mM K^+, followed by increasing concentrations of hαCGRP to achieve full relaxation. This assay again

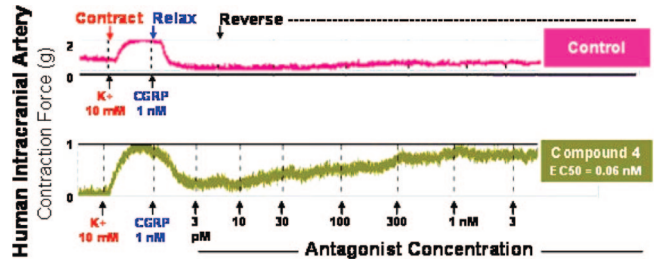


Figure 2. Reversal protocol in human intracranial arteries.

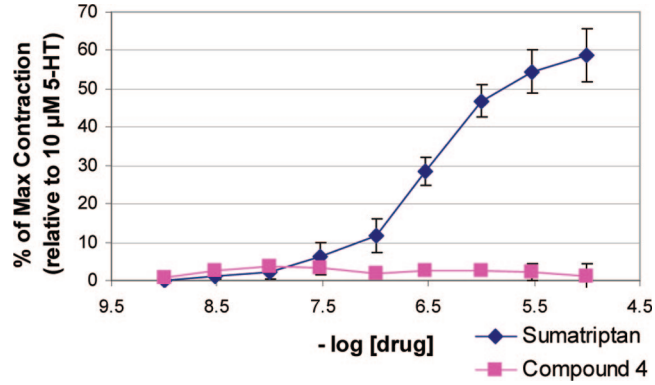


Figure 3. Effect of sumatriptan and compound **4** on ex vivo human coronary arteries.

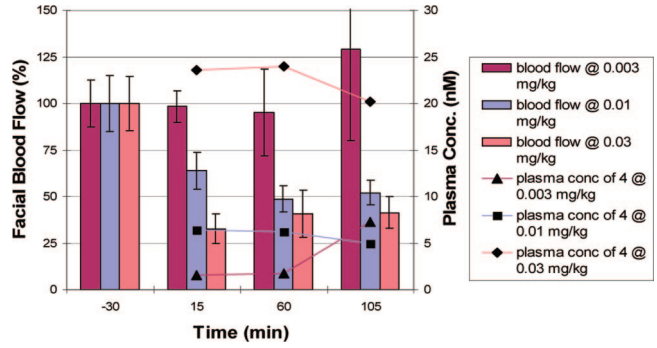
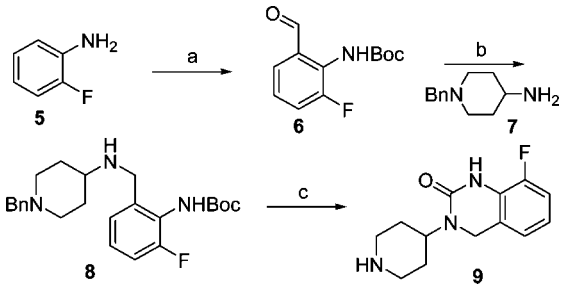


Figure 4. Marmoset facial blood flow.

showed **4** to be a potent antagonist of CGRP-induced dilation ($K_b = 0.028$ nM).

Active vasoconstriction by 5-$HT_{1B/1D}$ agonists reflects a key liability of the triptans, which constrict human coronary artery and are contraindicated in patients with coronary artery disease. To study the role of **4** in active vasoconstriction, basal artery tension was measured upon cumulative addition of the antagonist in ex vivo human coronary arteries. As a positive control, serotonin (10 μM) was added to each tissue bath at the end of a test session to provide a measure of maximal contractility in each vessel. Compound **4** did not produce measurable contraction in ex vivo human arteries up to 10 μM (Figure 3). In contrast, sumatriptan produced concentration-dependent vessel contraction up to ca. 59% of maximum ($EC_{50} = 311$ nM). The absence of active constriction exhibited by **4** is a reflection of the different mechanism of action for CGRP antagonists, which is an antidilatory effect (returning dilated vessels to normal). This suggests that this class of agents may be free from the mechanism-based cardiovascular liabilities associated with triptans. To summarize, our studies on ex vivo human intracranial arteries show that **4** was effective at (i) reversing and (ii) inhibiting CGRP-induced dilation of human intracranial arteries but does not by itself constrict human coronary arteries.

Scheme 1. Synthesis of 8-Fluoroquinazolinone[a]



[a] (a) (i) Boc_2O/THF/Δ (100%); (ii) 2 equiv *t*-BuLi/THF, then DMF (96%). (b) $NaBH_4$/THF (77%). (c) (i) Pyr/(56%); (ii) Pd/H_2/HOAc (100%).

Current animal models of migraine primarily use rats.[17] However, because small molecule hCGRP-receptor antagonists (including **4**) show poor activity against rodent CGRP receptors,[16] higher species were required to examine in vivo efficacy against this target. Marmosets are the only animal reported to have human-like CGRP receptor pharmacology. This is thought to be due to the presence of a specific amino acid residue (Trp74) in the RAMP1 sequence that is responsible for the phenotype of the human receptor. Although primate migraine models have been developed, they are generally invasive, terminal procedures.[18,19] As such, a novel noninvasive marmoset recovery model for in vivo efficacy assessment of CGRP-receptor antagonists was developed that utilizes facial blood flow as a surrogate for intracranial artery diameter. In brief, marmosets were anesthetized and facial blood flow was increased by intravenous (IV) administration of hαCGRP (10 μg/kg) at 45 min intervals (−30, 15, 60, and 105 min). The effect of antagonist **4**, delivered at 0 min, on the hαCGRP-induced changes in facial blood flow was measured by laser Doppler flowmetry. In this model (Figure 4), compound **4** (0.003−0.03 mg/kg) showed dose-dependent inhibition of CGRP-induced increases in marmoset facial blood flow upon subcutaneous (SC) dosing. Robust inhibition was observed at 0.03 mg/kg at 15, 60, and 105 min postdose. At the next lower dose, 0.01 mg/kg, strong inhibition was seen at 60 and 105 min, but not 15 min. No effect was seen at 0.003 mg/kg. CGRP-induced increases in marmoset facial blood flow were similar across all time points in vehicle-treated animals. Comparing efficacy vs exposure at the 15 min test time, plasma levels of **4** above 10 nM were associated with robust in vivo efficacy.

Compound **4** did not exhibit significant oral bioavailability in either the cynomolgus monkey or rat ($F_{po} \leq 0.3\%$), presumably because of poor intrinsic permeability (Caco-2 P*c* < 15 nm/s). Because of the high aqueous solubility of **4** in the pH range of 1−6.8 (>500 mg/mL), the IN route of administration was explored in rabbits.[15] The IN pharmacokinetic profile indicates that **4** is rapidly and efficiently absorbed from the nasal cavity of rabbits when sprayed as a solution. T_{max} occurred within 10 min for all doses. The IN bioavailability at doses of 1.0 and 0.3 mg/kg was 59% and 55%, respectively. Maximal plasma concentrations were 1200 and 360 nM, respectively. For the iv leg (0.5 mg/kg), mean clearance (Cl) was 13 ± 5.7 mL/min/kg, volume of distribution (V_{ss}) was 2.7 ± 1.0 L/kg, and $T_{1/2}$ was 11 h. Compound **4** was systemically present for 24 h (>10 nM) after IN administration, suggesting the potential for rapid onset and sustained effect. IN bioavailability in rabbit was comparable to that observed in rats dosed intratracheally (73%) and subcutaneously (43% with 25 mM PBS vehicle; 81−157% with PG:D5W vehicle). In the cynomolgus monkey, the SC bioavailability was 76% using a PG:D5W vehicle.

Binding of **4** (10 μM) to serum proteins in vitro was low over a range of species, including human (f_u = 35−77%). Compound **4** inhibited recombinant CYP3A4 (IC_{50} = 25 μM, BFC; 7.8 μM, BZR), recombinant CYP2D6 (IC_{50} = 25 μM, AMMC assay), and CYP2D6-mediated metabolism of dextromethorphan in human liver microsomes (IC_{50} = 1.6 μM). Inhibition of recombinant CYP1A2, CYP2C9, and CYP2C19 and also the CYP3A4-mediated metabolism of testosterone was characterized by an IC_{50} of greater than 40 μM. Compound **4** at 30 μM produced ≈19% inhibition of hERG channel activity, suggesting a low potential for QT related electrocardiographic changes. The combined results from sodium patch-clamp and Purkinje fiber action potential V_{max} suggest that **4** has minimal impact on the sodium channel. Compound **4** at 10 μM showed no significant potential for off-target liabilities in a broad panel of receptor and ion channel binding and enzyme activity assays.

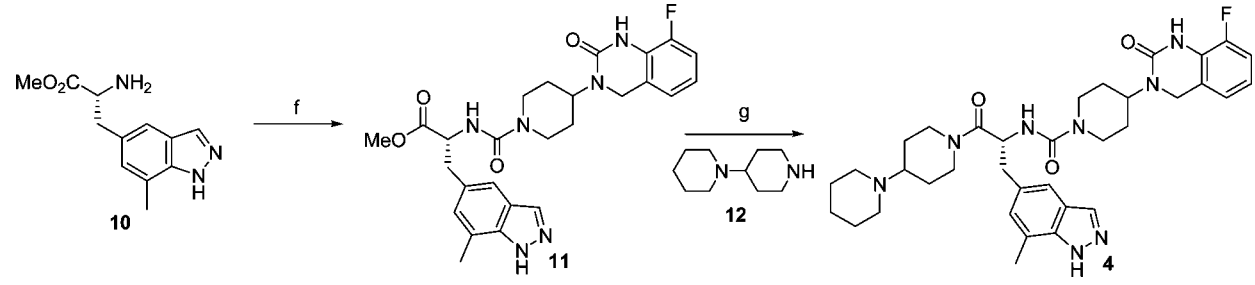
Chemistry

The synthesis of quinazolinone **9** began with the protection of 2-fluoroaniline as its *tert*-butyl carbamate (Scheme 1). The incorporation of the carbamate facilitated an *ortho*-lithiation by *tert*-butyllithium to afford aldehyde **6** upon trapping with dimethylformamide.[20] Reductive amination with 4-amino-1-benzylpiperidine completed the backbone of this fragment. Upon heating, the secondary amine underwent an intramolecular cyclization with the extrusion of *tert*-butanol to afford the cyclic urea. Catalytic hydrogenation cleaved the benzyl protecting group to afford **9**, ready for coupling.

Fragment **9** and amino ester **10**[21] were efficiently united with carbonyldiimidazole to install the urea in 78% yield. Saponification of the methyl ester with lithium hydroxide gave a nearly quantitative yield of the carboxylic acid. Finally, PyBOP mediated coupling with the commercially available 4-piperidinyl-piperidine completed the synthesis of **4** (Scheme 2).

In summary, we have discovered compound **4**, a high-affinity (K_i = 0.0128 nM) CGRP receptor antagonist that incorporated a novel 7-methylindazole amino acid as a key central subunit. Introduction of a fluorine to the 3,4-dihydro-2(1*H*)-quinazoli-

Scheme 2. Synthesis of Compound **4**[a]



[a] (f) CDI/THF, then **9** (78%). (g) (i) LiOH/THF/MeOH/H_2O (96%); (ii) PyBOP/DIEA/DMF/DCM (63%).

none substituent led to dramatic increase in aqueous solubility (>500 mg/mL, pH 1.0−6.8). The compound reverses CGRP-induced dilation of ex vivo human intracranial arteries. At low doses (0.03 mg/kg, SC), **4** shows robust inhibition of CGRP-induced increases in marmoset facial blood flow, with rapid onset of action (15 min) in vivo. Compound **4** exhibited a favorable IN pharmacokinetic profile in rabbits with $F_{IN} = 59\% \pm 22\%$ and high plasma levels within 10 min, suggesting the potential for rapid relief of migraine suffering.

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Supporting Information Available: Experimental details and analytical data for the preparation of compounds **1**−**4**, **6**, and **8**−**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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