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QUININE ANALOGS AS NON-PEPTIDE CALCITONIN GENE-RELATED PEPTIDE (CGRP) RECEPTOR ANTAGONISTS

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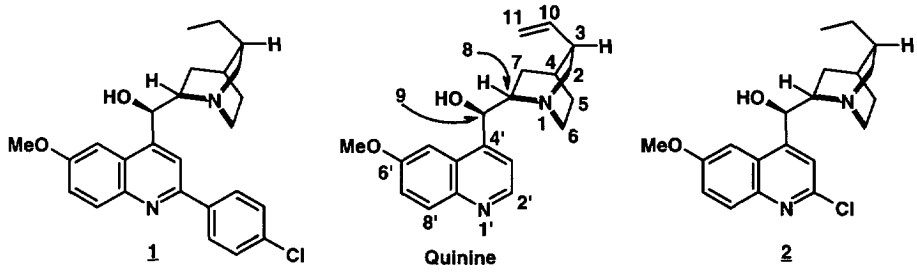
Abstract: A high-throughput screen identified quinine analog **1** as an antagonist of the human calcitonin gene-related peptide (hCGRP) receptor. Thus, compound **1** displaces [^{125}I]-CGRP from the hCGRP receptor and inhibits CGRP-mediated cAMP production with IC_{50} values of 5.9 ± 1.2 μM and 26 ± 5 μM, respectively. A limited structure–activity study of **1** is described. © 1997 Elsevier Science Ltd.

Calcitonin gene-related peptide (CGRP), a 37 amino acid peptide, exists in two homologous forms, α-CGRP and β-CGRP, that have been reported to display similar biological activities.[1] CGRP is a potent vasoactive substance, which is synthesized and released from sensory nerves, and produces a wide range of pharmacological responses including potent peripheral and cerebral vasodilation, cardiac acceleration, regulation of calcium metabolism, reduction of intestinal motility, regulation of glucose metabolism (reduction of insulin secretion and insulin sensitivity), and reduction of growth hormone release. High affinity CGRP binding sites have been identified and characterized in central and peripheral tissues. Multiple CGRP receptors have been observed and are classified according to their pharmacological properties as being of type 1, 2, or 3.[2,3] Recently, the human $CGRP_1$ receptor has been cloned and characterized.[4] Stable expression of the human CGRP receptor cDNA in embryonic kidney 293 (HEK-293) cells produced specific, high affinity binding sites for CGRP that displayed pharmacological and functional properties similar to native $CGRP_1$ receptors. The $CGRP_1$ receptor, part of the VIP/calcitonin family of receptors, contains seven transmembrane domains and is coupled, via a G-protein, to the activation of adenylyl cyclase. CGRP has been implicated as a mediator of cerebral vasodilation leading to migraine and may play a role in the onset of non-insulin dependent diabetes (type 2 diabetes) via promotion of insulin resistance. Therefore, a potent and selective CGRP receptor antagonist has the potential of being a novel and effective treatment for migraine, type 2 diabetes, and neurogenic inflammation.[5]

As part of an effort to identify non-peptide antagonists of the CGRP receptor, a high-throughput screen was established using porcine lung membranes. Screening of the SmithKline Beecham compound collection identified quinine analog **1**[6] as being active with an IC_{50} of 6.0 μM. Subsequent confirmation using human

SK-N-MC membranes, which possess $CGRP_1$ receptors, showed that **1** displaced [^{125}I]-CGRP with an IC_{50} of 5.9 ± 1.2 μM and inhibited CGRP-mediated cAMP production (SK-N-MC cells) with an IC_{50} of 26 ± 5 μM. Thus, compound **1** is a nonpeptide antagonist of the $hCGRP_1$ receptor, albeit, with modest potency. As quinine and 10,11-dihydroquinine were inactive in the hCGRP assay, it was clear that the 4-chlorophenyl substituent at the 2′-position was critical for activity. In order to rapidly explore variations at this position, we employed a general synthetic scheme utilizing a Pd^0-mediated cross-coupling reaction between an aryl boronic acid and the π-deficient 2′-chlorodihydroquinine (**2**) according to the procedure reported by Mitchell et al.[7] The results of this study are shown in Table 1.

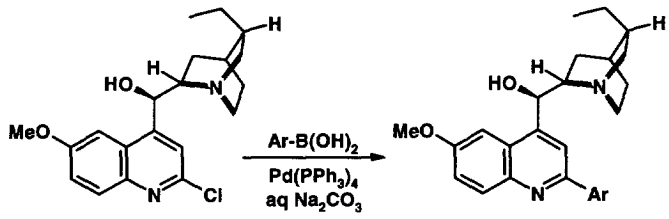


2′-Chlorodihydroquinine (**2**) was prepared by treating the aryl N-oxide of dihydroquinine with $POCl_3$ as described by Ochiai et al.[8] Reaction of **2** with an aryl boronic acid in the presence of a catalytic amount of tetrakis(triphenylphosphine)palladium(0), prepared in situ, provided the desired biaryl compounds with yields of 60–85% (Table 1).[9] The radioligand binding assay utilized [^{125}I]-hCGRP binding to membranes of the human neuroblastoma cell line SK-N-MC while the cAMP functional assay was performed using SK-N-MC whole cells according to previously published procedures.[4]

As illustrated in Table 1, with respect to the radioligand binding assay, the 2′-aryl moiety shows a preference for lipophilic groups such as chlorine (**1**), trifluoromethyl (**3**, **4**), and phenoxyphenyl (**5**). An unsubstituted phenyl group at the 2′-position resulted in poor activity (**6**); however, a degree of activity was restored in the 1-naphthyl analog (**7**). The presence of alkyl ethers on the aryl ring resulted in a marked decrease in activity (**8**, **9**). Replacing the phenyl group with the heterocycle thiophene (**10**) resulted in complete loss of activity. Multiple halogen groups on the phenyl ring (**11, 12**) resulted in reduced activity compared to the monochloro analog (**1**). The SAR also tends to support a preference for electron poor aryl systems as opposed to electron rich systems. This is further illustrated by the observation that the 3-amino analog **13** was inactive whereas the 3-nitro analog **14**, while less active than **1**, did possess activity. Although not exhaustive, the study indicated that within the series the 4-chlorophenyl and 4-trifluoromethylphenyl groups (i.e., **1** and **3**) were optimal. Analysis of selected compounds in the cAMP functional assay revealed that compounds active in the radioligand binding assay were also able to antagonize the functional response of CGRP. In the absence of

CGRP, these compounds (100 μM) produced no change in the basal levels of cAMP indicating the lack of both agonist activity and non-specific inhibition of adenylyl cyclase.

Table 1: In vitro activity of 2′-aryl analogs.



No.[a]	Ar	binding[b] IC_{50}, μM	cAMP[b] IC_{50}, μM
1	(4-Cl)Ph	5.9 ± 1.2	26 ± 5
3	(4-CF_3)Ph	5.2 ± 0.8	24 ± 6
4	(3-CF_3)Ph	9.9 ± 2.5	20 ± 6
5	(4-PhO)Ph	12 ± 3	22
6	Ph	96 ± 6	65
7	1-naphthyl	33 ± 8	23
8	(3, 4-OCH_2O-)Ph	74 ± 18	60
9	(4-MeO)Ph	>100	ND
10		>100	ND
11	(2, 4-diCl)Ph	37 ± 5	28
12	(3-Cl, 4-F)Ph	17 ± 2	14
13	(3-NH_2)Ph	>100	>100
14	(3-NO_2)Ph	41 ± 14	ND

ND, not determined.

[a] All compounds were tested as dihydrochloride salts.

[b] The IC_{50} values are stated as the mean of at least three determinations ± standard error. All other values are the mean IC_{50} of two concentration-response curves.

Compound **1** was also assayed using the recently reported recombinant human $CGRP_1$ receptor ($rhCGRP_1$) expressed in HEK-293 cells.[4] Evaluation of **1** in the radioligand binding assay using membrane preparations of the rhCGRP receptor produced an IC_{50} of 5.5 ± 1.8 μM. This value is in close agreement with the results obtained using SK-N-MC cell membranes. This is consistent with the endogenous SK-N-MC human receptor and the rhCGRP receptor both being type 1 receptors. The selectivity of **1** was also assessed at a number of receptors using radioligand binding assays (including A-II, VIP, GLP-1, ADM, ET_A and ET_B).[10] At concentrations up to 30 μM, compound **1** did not significantly effect (<20%) ligand binding to these receptors.

The quinine analog **1** was identified via a high-throughput screen as an antagonist of the $hCGRP_1$ receptor. To our knowledge, compounds such as **1** and **3** represent the first reported nonpeptide CGRP receptor antagonists. In addition, these lead structures define a scaffold from which new compounds may be designed. Although **1** and **3** exhibit low micromolar activity in the radioligand binding assay, these compounds may, nevertheless, prove to be useful tools with which to study the physiological role of CGRP. Additional SAR studies based upon this quinine scaffold will be reported in due course.

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9. All compounds exhibited satisfactory infrared, ^{1}HNMR (400 MHz), mass spectroscopic and/or elemental anaysis data.
10. Abbreviations: A-II, angiotensin-II; VIP, vasoactive intestinal peptide; GLP-1, glucagon-like peptide 1; ADM, adrenomedullin; ET_A and ET_B, endothelin A and B.

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