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Design, synthesis, and structure–activity relationships of a series of 2-Ar-8-methyl-5-alkylaminoquinolines as novel CRF_1 receptor antagonists

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ABSTRACT

We designed and synthesized a series of 2-Ar-8-methyl-5-alkylaminolquinolines as potent corticotropin-releasing factor 1 (CRF_1) receptor antagonists. The structure–activity relationships of substituents at each position (R^3, R^5, $R^{5'}$, and R^8) was investigated. By derivatization, three compounds (**6**, **14b**, and **14c)** were identified as orally active CRF_1 receptor antagonists.

Corticotropin-releasing factor (CRF), isolated by Vale et al. in 1981 as a 41-amino acid-long peptide, is the primary regulator of the hypothalamic-pituitary-adrenal (HPA) stress response.[1] Elevated CRF concentration in patients with depression[2] and a blunted corticotropin response to CRF in patients with depression and anxiety[3] have been observed in clinical studies, which suggests that CRF receptor antagonists may be useful in the treatment of depression and anxiety. CRF exerts its biological functions by binding to 2 GPCR-subfamily receptors, the CRF_1 and CRF_2 receptors.[4–7] The CRF_1 receptor is abundantly found in the pituitary and is involved in the regulation of adrenocorticotropic hormone (ACTH), a key mediator of the stress response.[8–10] Therefore, several pharmaceutical research groups have focused on the discovery of CRF_1 receptor antagonists for the treatment of depression or other stress-related disorders, while the benefits of blocking the CRF_2 receptor remain uncertain. To date, several CRF_1 receptor antagonists have been reported; prototypical antagonists are illustrated in Figure 1. CRF_1 receptor antagonists, **1** (R121919),[11] **2** (CP-154526),[12,13] **3** (DMP696),[14] and **4** (CP-316311),[15,16] exhibited high in vitro affinity to the CRF_1 receptor and demonstrated significant activity in animal models. However, the results of using these antagonists in clinical studies remain ambiguous. Antagonist **1** exhibited efficacy in a small open-label clinical study on major depression,[17] but antagonist **4** failed in a double-blind study on

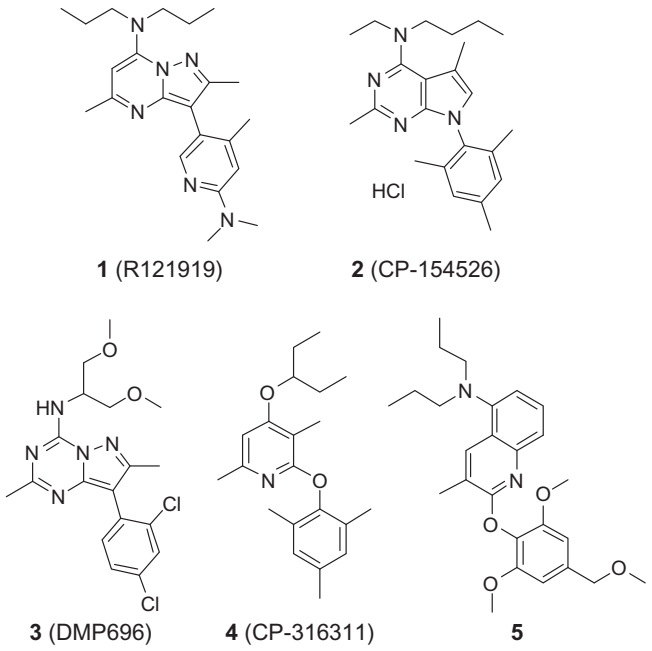

Figure 1. Known CRF_1 receptor antagonists.

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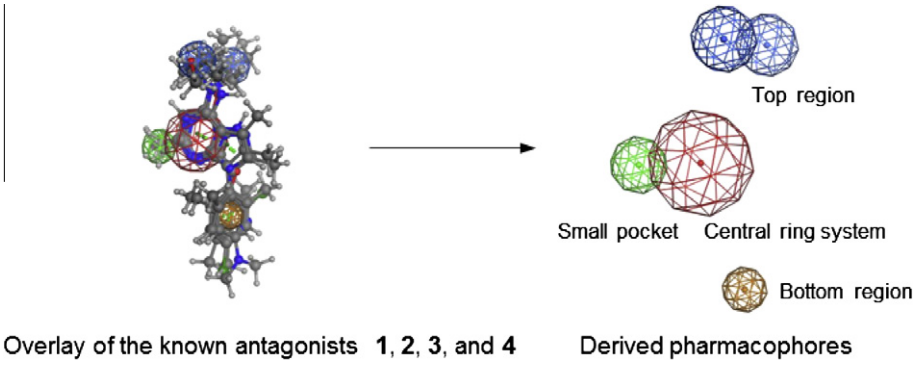


Figure 2. Overlay of known CRF_1 receptor antagonists and derived pharmacophores.

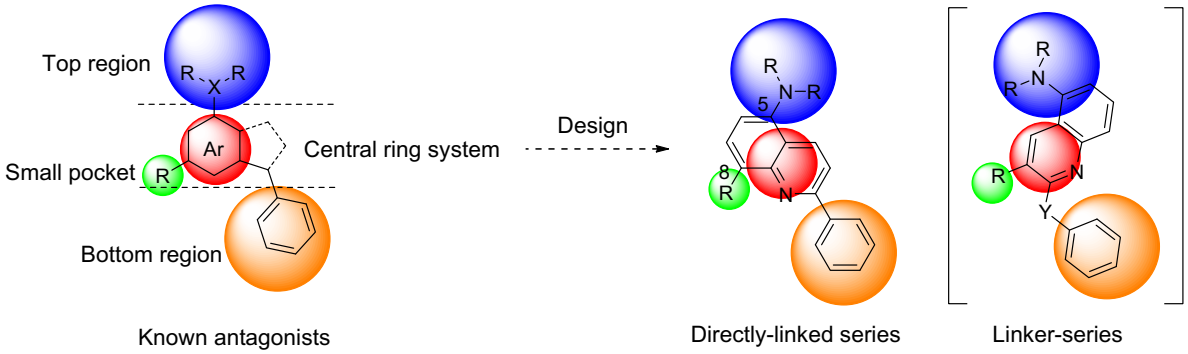


Figure 3. Design of a novel CRF_1 receptor antagonist.

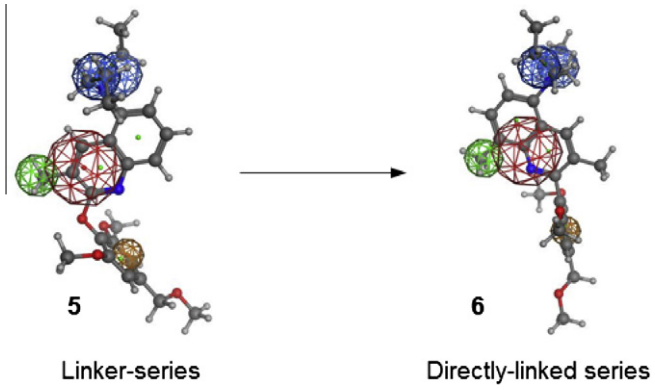
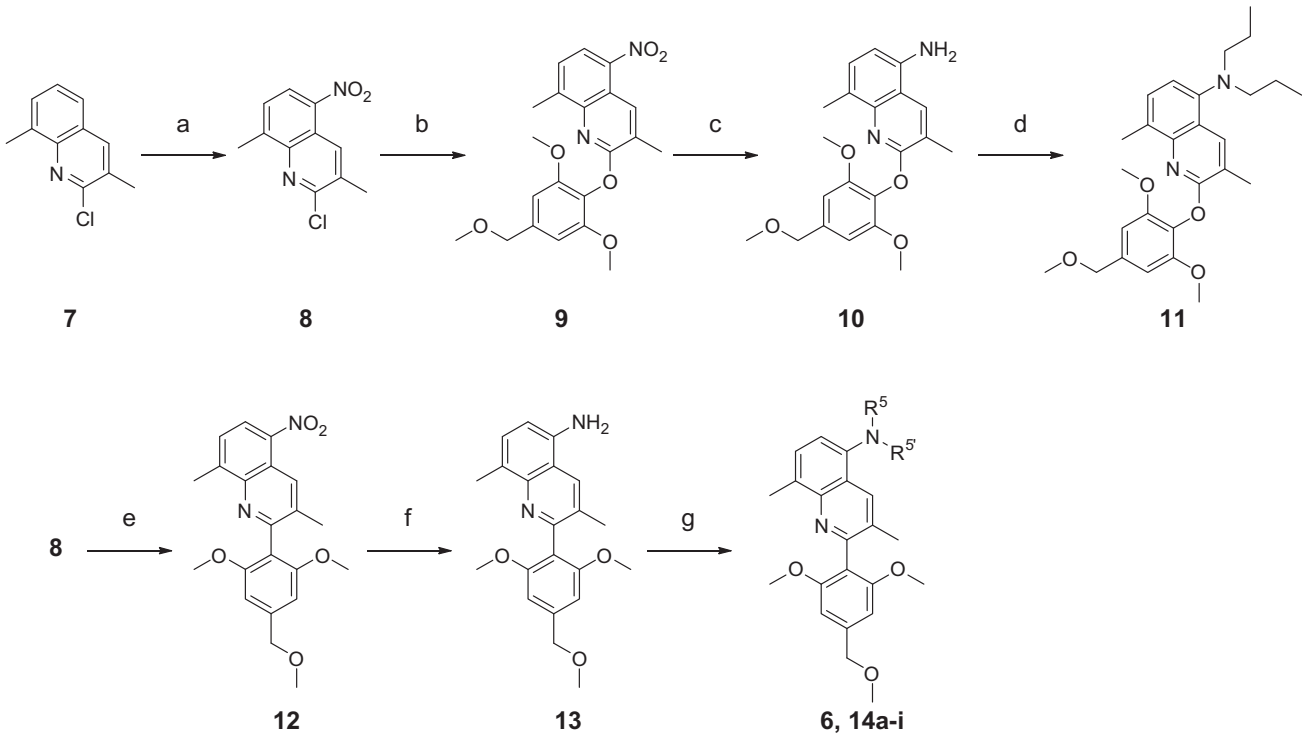


Figure 4. Molecular simulation of compounds **5** and **6**.

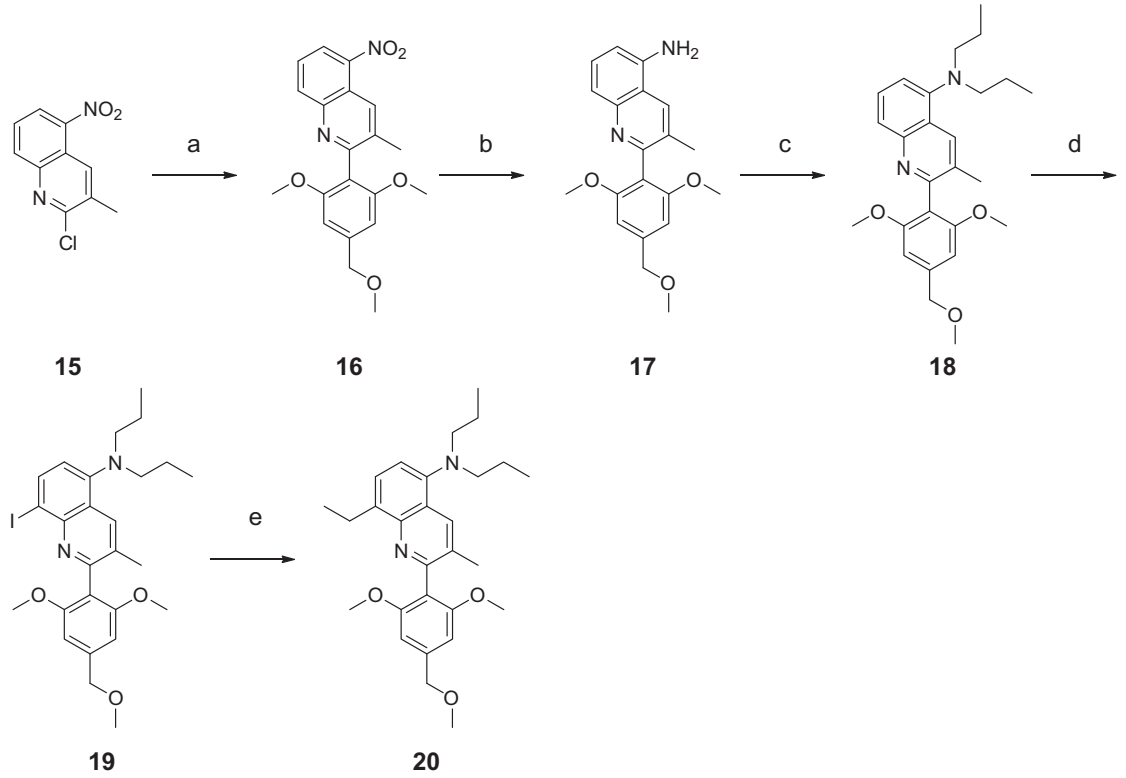
depression.[18] Thus, it is essential to discover structurally diverse CRF_1 receptor antagonists, and to amass the clinical data conveyed by the results of their usage, in order to clarify the role of CRF in humans.

We have previously reported a 5-alkylaminoquinoline derivative **5** as a novel CRF_1 receptor antagonist, which is structurally distinct from other antagonists because this compound possesses 6–6-membered ring with a characteristic substitution pattern,[19] and have been exploring compound designs to obtain further structurally diverse CRF_1 receptor antagonists. This report describes a novel series of such compounds. Using the same method previously reported by us, we extracted four pharmacophores from known antagonists (Fig. 2) and performed molecular design to find structures that could dock to these pharmacophores. The analyses were performed using molecular operating environment (MOE) from Ryoka System Inc. As a result, we designed a novel structure in a directly linked series; various ways of docking to the pharmacophores were obtained by changing only the substitution patterns, while quinoline was retained as the core template (Fig. 3). Compared to the compound **5** series (linker-series), the quinoline template was inverted, and the Y linker atom has been removed. In addition, the substituent at the C_8 position may occupy the small pocket of a pharmacophore. Consequently, the structure for compound **6** was designed, and in silico simulation used to assess whether this structure could dock to the pharmacophores, which it appeared to be capable of doing (Fig. 4). We then set out to synthesize compound **6** and its derivatives.

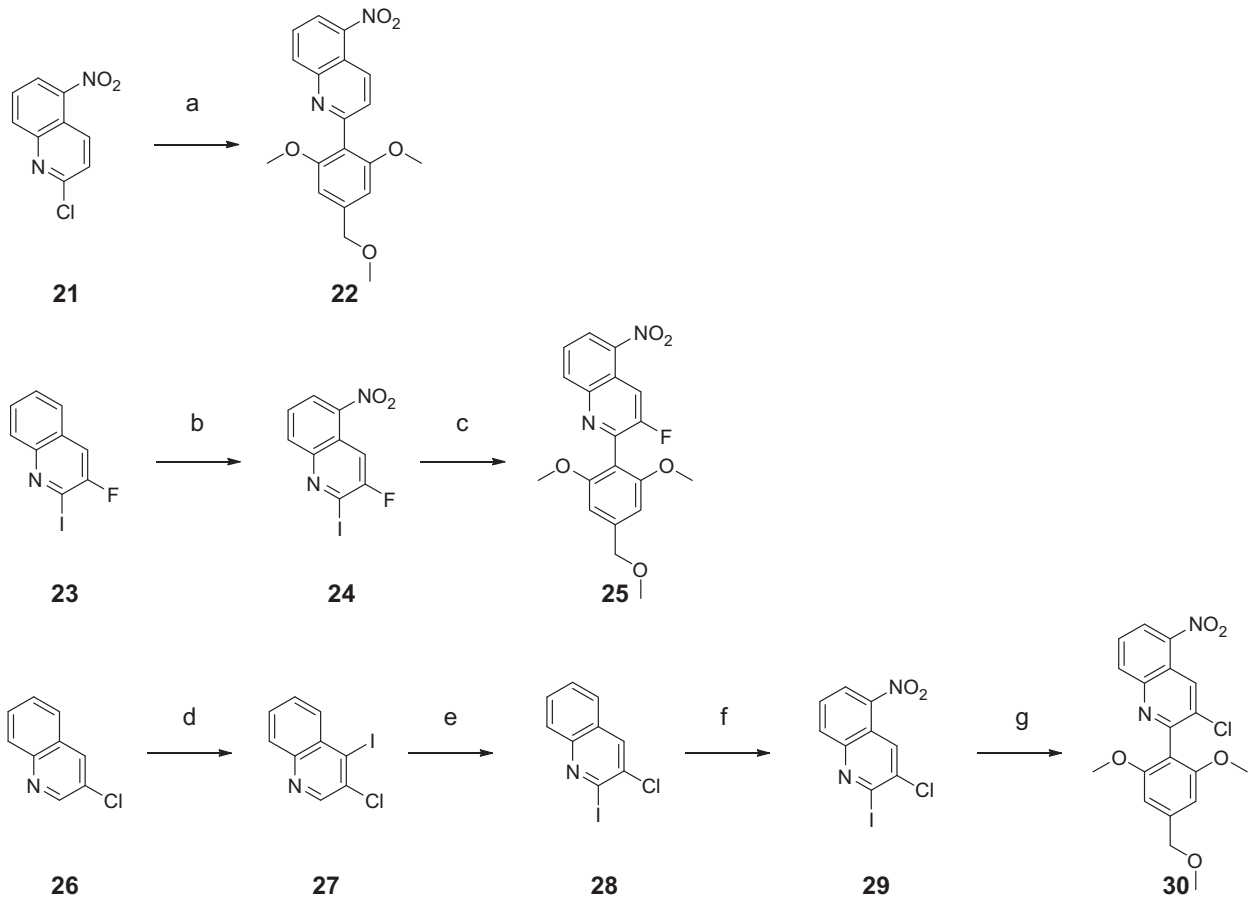
The synthesis of compound **11** is described in Scheme 1. Nitration of commercially available **7** was conducted in the presence of HNO_3/H_2SO_4 to obtain intermediate **8**. The position of the nitro-group was determined by NMR analysis.[20] In the next reaction, this intermediate was coupled to a phenol moiety in the presence of a palladium catalyst, to yield intermediate **9**. Reduction of the nitro-group of intermediate **9**, using Fe, followed by reductive amination in the presence of $NaBH(OAc)_3$ as a reducing reagent, yielded target compound **11**. A Suzuki-Miyaura cross-coupling reaction of intermediate **8** with boronic acid produced **12**.[21] Reduction of the nitro-group of **12**, using Fe, followed by reductive amination with aldehydes or ketones, or a substitution reaction with alkyl halides, generated target compounds **6** and **14a–i**. Compound **20** was synthesized as described in Scheme 2. Compound **18** was synthesized in a manner similar to that described for compounds **6** and **14a–i**, starting with compound **15**, which was prepared using a method analogous to that previously reported.[19] Iodation of **18** afforded 8-iodoquinoline **19**. Target compound **20** was prepared by conversion of the iodine group to an ethyl group. Intermediates **22**, **25**, and **30** were prepared in a manner similar to that described for compound **12**, starting with **21**, **23**,[22] and **28**, respectively (Scheme 3). Intermediate **28** was prepared using a method analogous to that reported by



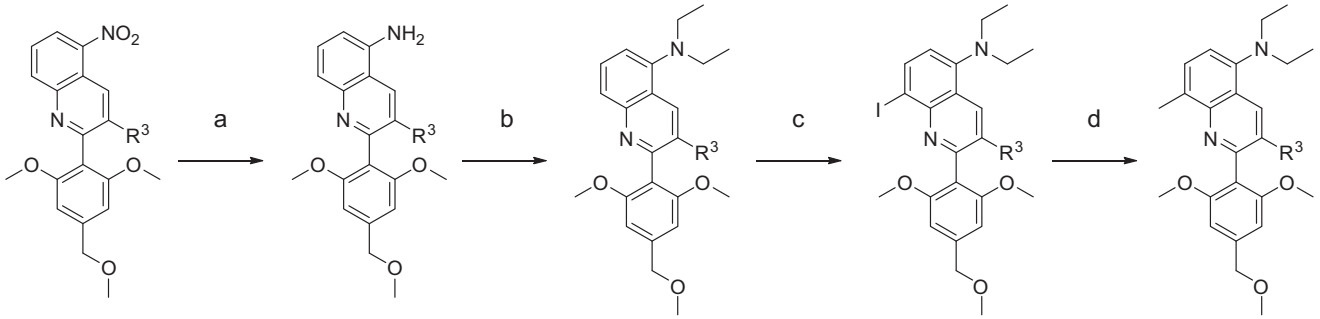
Scheme 1. Reagents and conditions: (a) HNO_3, H_2SO_4, −20 °C, (27%); (b) 2,6-dimethoxy-4-(methoxymethyl)phenol, $Pd(OAc)_2$, 2-di-*tert*-butylphosphino-2′,4′,6′-triisopropylbiphenyl, K_3PO_4, PhMe, reflux, (34%); (c) Fe, saturated aqueous NH_4Cl, EtOH reflux, (83%); (d) propionaldehyde, $NaBH(OAc)_3$, AcOH, THF, RT, (69%); (e) 2,6-dimethoxy-4-(methoxymethyl)phenyl boronic acid, $Pd(OAc)_2$, PPh_3, K_2CO_3, H_2O, DME, reflux, (95%); (f) Fe, saturated aqueous NH_4Cl, EtOH reflux, (99%); (g) aldehyde or ketone, $NaBH(OAc)_3$, AcOH, THF, RT, (for **6**, 19%, for **14a**, 26%, for **14b**, 35%, for **14c**, 72%, for **14f**, 25%, for **14g**, 76%, for **14h**, 21%), or 2-bromoethyl methyl ether, K_2CO_3, DMF, 100 °C, (**14i**, 65%), then aldehyde, $NaBH(OAc)_3$, AcOH, THF, RT, (for **14d**, 83%, for **14e**, 75%).



Scheme 2. Reagents and conditions: (a) 2,6-dimethoxy-4-(methoxymethyl)phenyl boronic acid, $Pd(OAc)_2$, PPh_3, K_2CO_3, H_2O, DME, reflux, (87%); (b) H_2, Pd/C, EtOAc, RT; (c) propionaldehyde, α-picoline-borane, AcOH, MeOH, RT, (75%, 2 steps); (d) NIS, DMF, RT, (79%); (e) Et_2Zn, bis(tri-*tert*-butylphosphine)palladium, 1,4-dioxane, 70 °C, (21%).



Scheme 3. Reagents and conditions: (a) 2,6-dimethoxy-4-(methoxymethyl)phenyl boronic acid, $Pd(PPh_3)_4$, 1 M Na_2CO_3 aq, EtOH, PhMe, reflux, (quant.); (b) fuming HNO_3, H_2SO_4, 0 °C; (c) 2,6-dimethoxy-4-(methoxymethyl)phenyl boronic acid, $Pd(PPh_3)_4$, 1 M Na_2CO_3 aq, EtOH, PhMe, reflux, (30%, 2 steps); (d) I_2, LDA, THF, −78 °C, (49%); (e) LDA, THF, −78 °C, (56%); (f) fuming HNO_3, H_2SO_4, 0 °C; (g) 2,6-dimethoxy-4-(methoxymethyl)phenyl boronic acid, $Pd(PPh_3)_4$, 1 M Na_2CO_3 aq, EtOH, PhMe, reflux, (37%, 2 steps).

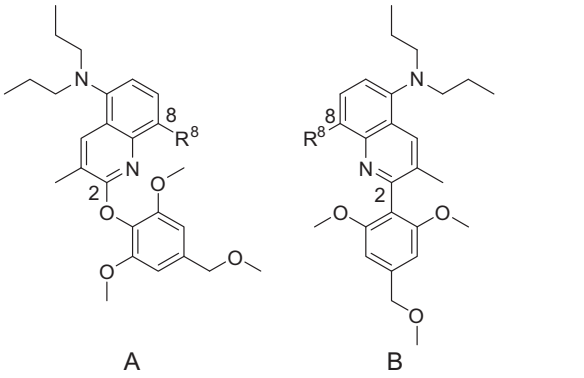


Scheme 4. Reagents and conditions: (a) Fe, saturated aqueous NH_4Cl, EtOH reflux, (for **31a**, 99%, for **31b**, quant., for **31c**, 97%); (b) acetaldehyde, $NaBH(OAc)_3$, AcOH, THF, RT, (for **32a**, 72%, for **32b**, 72%, for **32c**, 69%); (c) NIS, DMF, RT, (for **33a**, 79%, for **33b**, 75%, for **33c**, 77%); (d) Me_2Zn, bis(tri-*tert*-butylphosphine)palladium, 1,4-dioxane, 70 °C, (for **34a**, 95%, for **34b**, 85%, for **34c**, 98%).

Patrick Rocca et al.,[22] starting with 3-chloro-4-iodoquinoline **27**. The position of the nitro-groups of compounds **24** and **29** were determined by NMR analysis.[23] The synthesis of compounds **34a**, **34b**, and **34c** is described in Scheme 4. By using a procedure similar to that described for **20**, compounds **22**, **25**, and **30** were transformed to yield target compounds **34a**, **34b**, and **34c**, respectively.

The compounds in this study were first screened for their ability to inhibit [^{125}I] CRF binding to the membranes of cells expressing the human CRF_1 receptor.[24] Compounds with high-binding affinities were then evaluated in further functional studies. The functional assay evaluated the CRF antagonistic function of the compounds, that is, inhibition of CRF-induced cyclic adenosine

Table 1
Effects of R^8



No.	Type	R^8	Binding IC_{50} (nM)	Functional IC_{50} (nM)
5	A	H	138	163
11	A	Me	>1000	NT
18	B	H	276	97
6	B	Me	88	70
20	B	Et	115	375

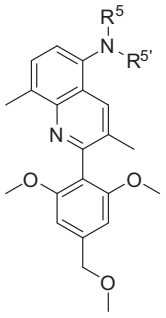
NT = not tested.

monophosphate (cAMP) production in human CRF_1 receptor-expressing HEK293 cells.[25]

Our initial investigation aimed to assess the effects of substituents at the C_2 and C_8 positions. To this end, several derivatives, shown in Table 1, were prepared. As seen for compounds **5** and **18**, binding affinity was reduced by removing the linker atom. It was noteworthy that introduction of a methyl group to the C_8 position resulted in quite opposite effects between the compounds in the linker-series (Type A) and those in the directly linked series (Type B). When compared with compound **5**, compound **11** exhibited a markedly reduced binding affinity. However, compound **6** exhibited approximately 3-fold higher binding affinity than did compound **18**. This opposite effect of the methyl group supports the results of the pharmacophore analysis shown in Figure 4. The methyl group at the C_8 position in type B compounds could occupy the small pocket of a pharmacophore, leading to improved binding affinity. On the other hand, the methyl group in type A compounds was not related to the structure of any pharmacophores and may have a negative impact on the conformation of Ar–O group in the bottom region, leading to a reduction in binding affinity. In brief, we hypothesized that a series of compounds with different modes of binding to the CRF_1 receptor could be obtained by simply removing the linker atom of a previously reported series of compounds. By removing the linker atom, the conformation of compound **6** becomes more rigid than that of compound **5**, which might be a reason for higher binding affinity and more potent antagonistic activity observed in compound **6**. Replacement of methyl at the C_8 position with an ethyl group (**20**) resulted in a retained binding affinity, but reduction in antagonistic activity. Thus far, methyl was the most suitable substituent at the C_8 position.

To further comprehend the SAR of the directly linked series, the effects of R^5 and $R^{5'}$ were examined with the methyl substituent retained at the C_8 position (Table 2). Replacement of di-*n*-propyl with di-methyl resulted in a lowered binding affinity as well as antagonistic activity (**14a**). Di-ethyl (**14b**) and di-cyclopropylmethyl (**14c**) derivatives again improved the binding affinity as well as the antagonistic activity. From the results of compound **14d** and **14e**, it appears that a methoxyethyl substituent is also tolerated. These results indicated that substituents larger than a dimethyl amine group are requisite at this position for high-binding affinity and potent antagonistic activity. Moreover, introduction of a tetrahydropyran moiety reduced the binding affinity (**14f**, **14g**), which indicated that large size of a cyclic group was intolerable at this position. Secondary amines were also examined, but resulted in markedly lowered activity (**14h** and **14i**). Among these derivatives, compound **14b**, a di-ethyl amine derivative, exhibited the highest binding affinity and the most potent antagonistic activity.

Table 2
Effects of R^5 and $R^{5'}$

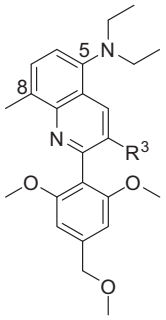


No.	R^5	$R^{5'}$	Binding IC_{50} (nM)	Functional IC_{50} (nM)
6	*n*-Propyl	*n*-Propyl	88	70
14a	Me	Me	212	>1000
14b	Ethyl	Ethyl	79	24
14c	Cyclopropylmethyl	Cyclopropylmethyl	88	46
14d	Me	Methoxyethyl	89	174
14e	Ethyl	Methoxyethyl	109	23
14f	Cyclopropylmethyl	CH_2-4-THP	286	NT
14g	Cyclopropylmethyl	4-THP	246	NT
14h	H	3-Pentyl	>1000	NT
14i	H	Methoxyethyl	>1000	NT

NT = not tested.

The effects of R^3 were also examined (Table 3). To allow comparison to compound **14b**, methyl and di-ethyl amine were retained at the C_8 and C_5 positions, respectively. Removing the methyl or replacement of the methyl group with fluorine led to a decreased binding affinity and antagonistic activity (**34a**, **34b**). In addition, a chloro derivative, **34c**, retained the binding affinity and antagonistic activity compared to **14b**. These results indicated that substituents at this position influenced activity, although this position was not related to any pharmacophores according to our pharmacophore analysis (Fig. 4). Furthermore, the dihedral angle between the quinoline and Ar group in the bottom region may

Table 3
Effects of R^3



No.	R^3	Binding IC_{50} (nM)	Functional IC_{50} (nM)
14b	Me	79	24
34a	H	184	381
34b	F	144	131
34c	Cl	81	45

Table 4
Effects of orally administered compounds on CRF-induced fecal pellet output in rats

No.	Fecal weight (g)		% Inhibition	Statistical significance
	Vehicle + CRF	Vehicle + CRF + compound		
6	1.323 ± 0.283	0.481 ± 0.137	64	*
14b	1.323 ± 0.283	0.492 ± 0.146	63	*
14c	1.216 ± 0.152	0.567 ± 0.218	53	*

Each value represents the mean ± S.E.M., 6–7 rats/group (*P <0.05, unpaired t test).
Compounds (10 mg/kg) were orally administered 1 h before intravenous injection of CRF (10 μg/kg). Fecal weight was measured 4 h after CRF injection.

be important for antagonistic activity, because the substituents at this position would have a considerable impact on the angle. Moreover, a twisted conformation for the Ar moiety in the bottom region may be favored, judging from the finding that substituted derivatives (**14b**, **34b**, and **34c**) exhibited better binding affinities and antagonistic activities than an unsubstituted derivative **34a**.

The in vivo functional antagonism of compounds with potent in vitro affinity was determined using a CRF-induced defecation model. It is well known that exogenously injected CRF increases fecal weight in rats and that CRF_1 receptor antagonists can block this CRF-induced defecation.[26] Three compounds (**6**, **14b**, and **14c**) were evaluated in this model. Compounds (10 mg/kg) were orally administered 1 h before intravenous injection of CRF (10 μg/kg) and the effect on CRF-induced fecal pellet output was evaluated; the results are shown in Table 4. All compounds caused a statistically significant decrease in fecal weight compared to a group who received only vehicle + CRF (6–7 rats/group; P <0.05, unpaired t-test). These results confirmed the antagonistic activity of these compounds against the CRF_1 receptor in vivo.

In summary, 2-Ar-8-methyl-5-alkylaminoquinoline derivatives were designed as novel CRF_1 receptor antagonists in this study. When compared to a previously reported 2-aryloxy-5-alkylaminoquinoline series, higher binding affinity and more potent antagonistic activity were obtained in in vitro assays by removing the linker atom and by introducing substituents at the C_8 position. The core template quinoline remained unchanged, but we expect that its binding mode to the receptor may have been changed, according to the results shown in Figure 4 and Table 1. We obtained compounds with a high in vitro potency (**6**, **14b**, **14c**, **14e**, and **34c**) through the preparation and evaluation of the derivatives at each position (R^3, R^8, R^5, and $R^{5'}$). Among these, compounds **6**, **14b**, and **14c** exhibited in vivo functional antagonism when orally administered in a CRF-induced defecation model. Further optimization will be reported in due course.

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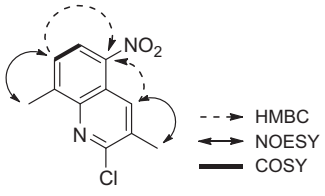
20. 

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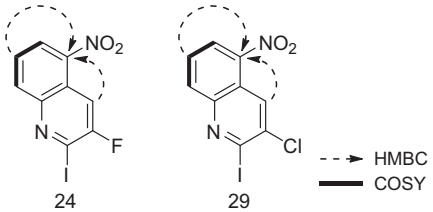
23. 

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24. For the binding assay, HEK293 cells expressing the human CRF_1 receptor were cloned.[4] Screening of CRF_1 receptor binding was performed using the scintillation proximity assay (SPA™, Amersham Pharmacia, UK) using 96-well plates. Cell membrane (5 μg/well), wheat germ agglutinin coated SPA beads (1 mg/well), [^{125}I] human/rat CRF (0.1 nM), and diluted test compound solution were suspended in 150 μL of assay buffer (137 mM NaCl, 8.1 mM Na_2HPO_4, 2.7 mM KCl, 1.5 mM KH_2PO_4, 10 mM $MgCl_2$, 2 mM EGTA, 1.5% bovine serum albumin (BSA), Protease inhibitor cocktail (Roche, Diagnostics GmbH), pH 7.0). Total binding and nonspecific binding were measured in the absence and presence of 0.4 μM unlabeled Sauvagine, respectively. Plates were shaken gently and incubated for over 2 h at room temperature. The plates were centrifuged (260 × g, 5 min, room temperature), and the radioactivity was detected using a TopCount (Perkin Elmer, MA, USA) 1 min counting time per well. Each count was corrected by subtracting the non-specific binding, and was represented as a percentage of total binding. The IC_{50} value of each compound was calculated using a concentration-response curve.
25. To determine the activities of the antagonists, their effects on CRF-stimulated intracellular cyclic AMP (cAMP) accumulation were examined on HEK293 cells expressing the human CRF_1 receptor, as described previously.[4] cAMP was

measured using an enzyme immunoassay (EIA) kit (Amersham Pharmacia, UK). HEK293 cells expressing the human CRF_1 receptor were seeded into 96-well plates (5×10^4 cells/well) in DMEM containing 0.1% fetal bovine serum and 1 mM 3-isobutyl-1-methylxanthine, which is a phosphodiesterase inhibitor. After 30 min of pre-incubation, the diluted test compounds were added to the wells and incubated for a further 30 min at 37 °C. The cells were then stimulated with 1 nM human/rat CRF for 30 min at 37 °C and collected by centrifugation (630 × g, 5 min, 4 °C). After aspiration of the medium, the cells were lysed with the EIA kit lysis buffer. The amount of intracellular cAMP was measured according to the manufacturer's instructions. Basal levels of cAMP (i.e., in the absence of CRF) were subtracted from the measured values and these were then expressed as a percentage of total production. The IC_{50} value of each compound was calculated using a concentration–response curve.

26. Saunders, P. R.; Maillot, C.; Million, M.; Tache, Y. *Eur. J. Pharmacol.* **2002**, *435*, 231.