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Design, synthesis and structure–activity relationships of 5-alkylaminolquinolines as a novel series of CRF_1 receptor antagonists

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ABSTRACT

A series of 5-alkylaminolquinolines was designed and synthesized as potential novel CRF_1 receptor antagonists. The structure–activity relationships (SARs) of the substituents on each position (R^2, R^3, R^5 and $R^{5'}$) were investigated.

Corticotropin-releasing factor (CRF) is a 41-amino acid peptide that acts as the prime regulator of the hypothalamic-pituitary-adrenal (HPA) axis.[1] CRF is a major modulator of the body's overall response to stressors[2–6] and there is evidence supporting the hypothesis that over production of CRF may underlie the pathology of stress-related disorders, such as depression and anxiety.[7–10] CRF acts through a class-B G protein-coupled receptor (GPCR), which is divided to two subtypes, CRF_1 receptor and CRF_2 receptor.[11–14] CRF_1 receptor is the most abundant subtype found in the pituitary and is involved in the regulation of adrenocorticotropic hormone (ACTH), a key mediator of the body's response to stress.[15–17] Therefore a CRF_1 receptor antagonist is hypothesized to be a valuable target for the treatment of stress-related disorders. Prototypical CRF_1 receptor antagonists are illustrated in Figure 1. Preclinical studies on CRF_1 antagonists **1** (R121919),[18] **2** (CP-154526),[19,20] **3** (DMP696),[21] and **4** (CP-316311)[22,23] support the hypothesis that CRF_1 receptor antagonists have the potential to be used for the treatment of stress-related disorders. However, clinical studies looking at the role of CRF in the pathophysiology of depression have been equivocal. CRF_1 receptor antagonist **1** (R121919) showed some efficacy in a small open label clinical study for major depression,[24] but **4** (CP-316311) was found to be ineffective in a double-blind, placebo-controlled study for depression using sertraline as a positive control.[25] Further clinical studies would be necessary to elucidate the reasons for the differences in the success of these trials. The accumulation of clinical data using a number of structurally diverse CRF_1 receptor antagonists is an efficient way to investigate the role of CRF in humans and to discover new drugs for treating depression and anxiety. Here we report the generation of novel CRF_1 receptor antagonists that have distinctive structural features compared to the currently known antagonists.

First, we aligned low-energy conformers of the known antagonists shown in Figure 1 to extract pharmacophores using MOE (Molecular Operating Environment) from Ryoka System Inc. The analysis indicated the following structural features (Fig. 2): (1) the top region is occupied by a lipophilic group such as a di-alkyl-amine, (2) the bottom region consists of substituted phenyl rings, (3) there is a central ring system that fixes a certain conformation between the top and bottom regions, (4) there is a small pocket in which a methyl group fits. Potential variations in the structures of the top and bottom regions are thought to be limited. However, the central core appears to be relatively adaptable, with the presence of a number of different hetreoaromatic systems observed. Therefore, we examined the possibility of modifying the central core structure, while leaving the positions of the top and bottom regions intact. Molecular design was performed to find a motif that fitted each pharmacophore and this suggested that a 6-6-membered ring was appropriate as the central ring system, with side chains placed at the appropriate positions (Fig. 3). Quinoxaline derivative **10a** was selected as a preliminary structure, then in silico simulation was performed to clarify whether this molecule fitted the pharmacophore model prior to synthesizing the other derivatives. The results indicated that the compound adequately occupied all of the pharmacophores (Fig. 4).

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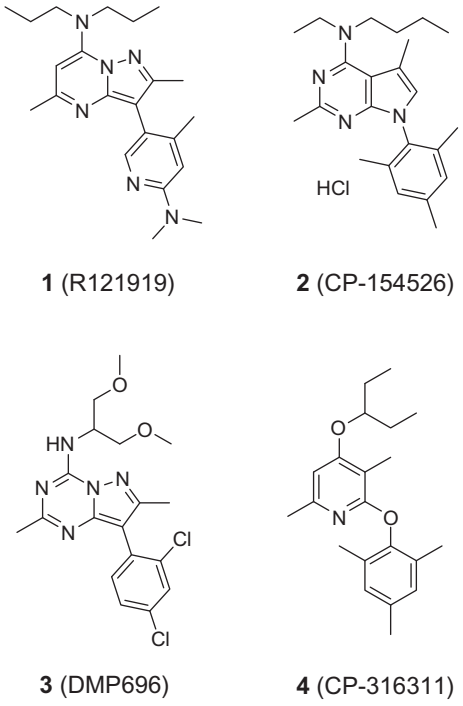


Figure 1. Known CRF_1 receptor antagonists.

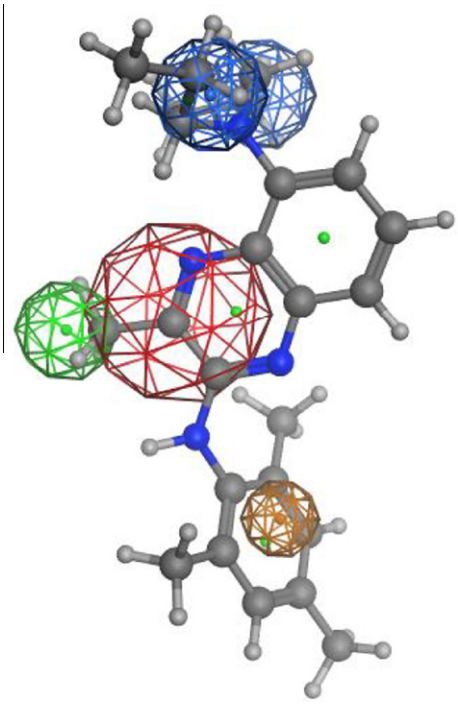


Figure 4. Molecular simulation of compound **10a**.

The synthesis of compounds **10a–b** is described in Scheme 1. Intermediate **6** was prepared by condensation of commercially available 3-nitro-o-phenylenediamine (**5**) with pyruvic acid. Chlorination using $POCl_3$ followed by a substitution reaction with $ArNH_2$ afforded intermediate **8**. Hydrogenation of the nitro group in **8** gave the amine **9** and the following reductive amination in the presence of α-picoline-borane as a reducing agent, gave the target derivatives **10a–b**. Compounds **16–18** were prepared as depicted in Scheme 2. Chlorination using $POCl_3$ of commercially available 5-nitro-1,4-dihydro-quinoxaline-2,3-dione (**11**) afforded di-chloro **12**, which was transformed into intermediate **13** using sodium methoxide. A substitution reaction of **13** with $ArNH_2$

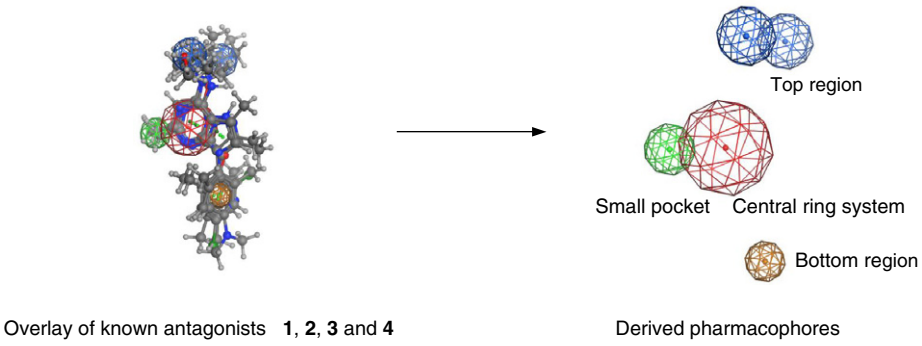


Figure 2. Overlay of known CRF_1 receptor antagonists and derived pharmacophores.

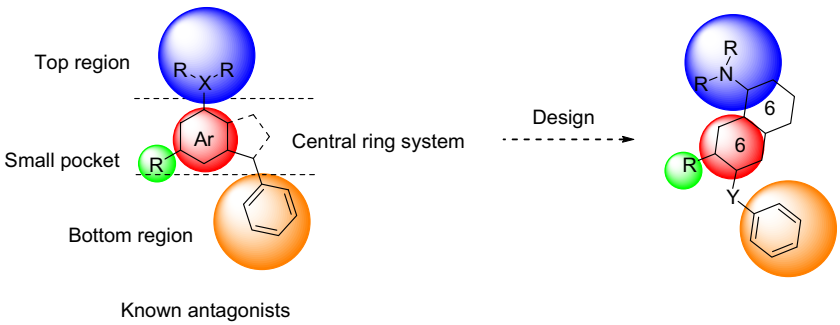
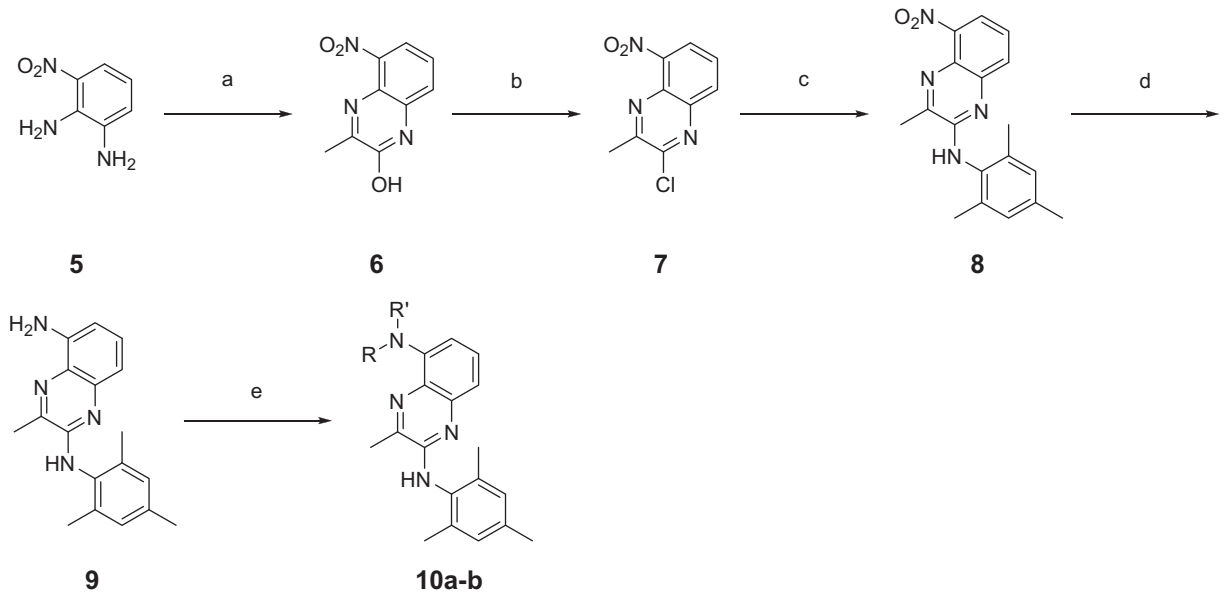
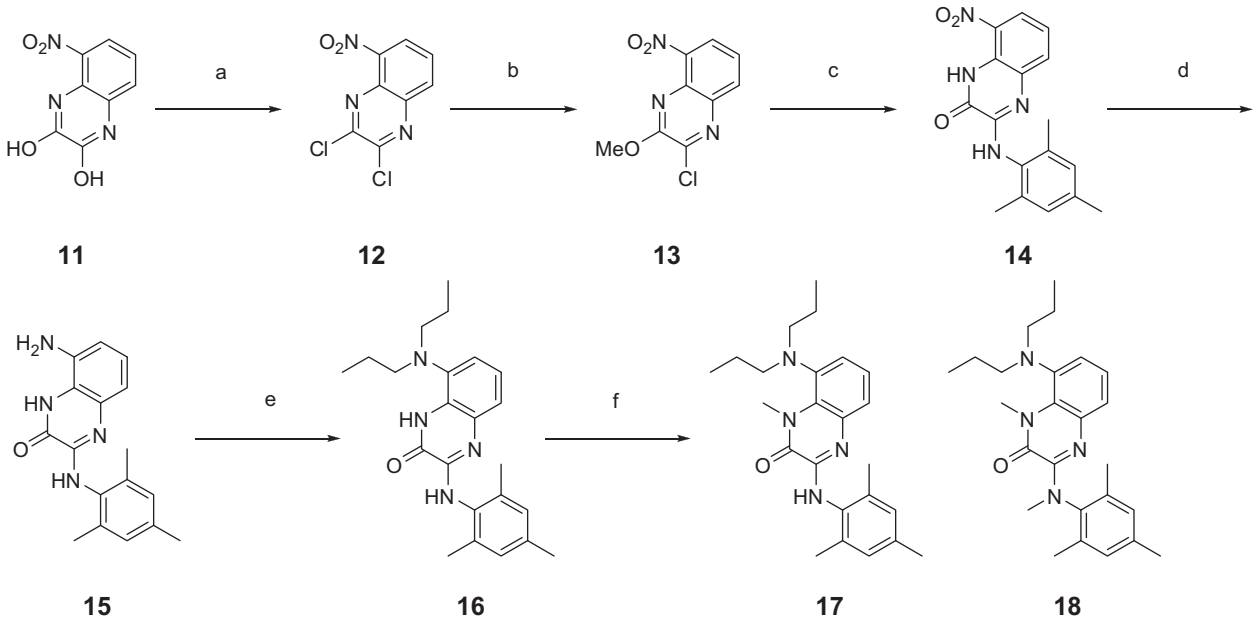


Figure 3. Design of a novel CRF_1 receptor antagonist.



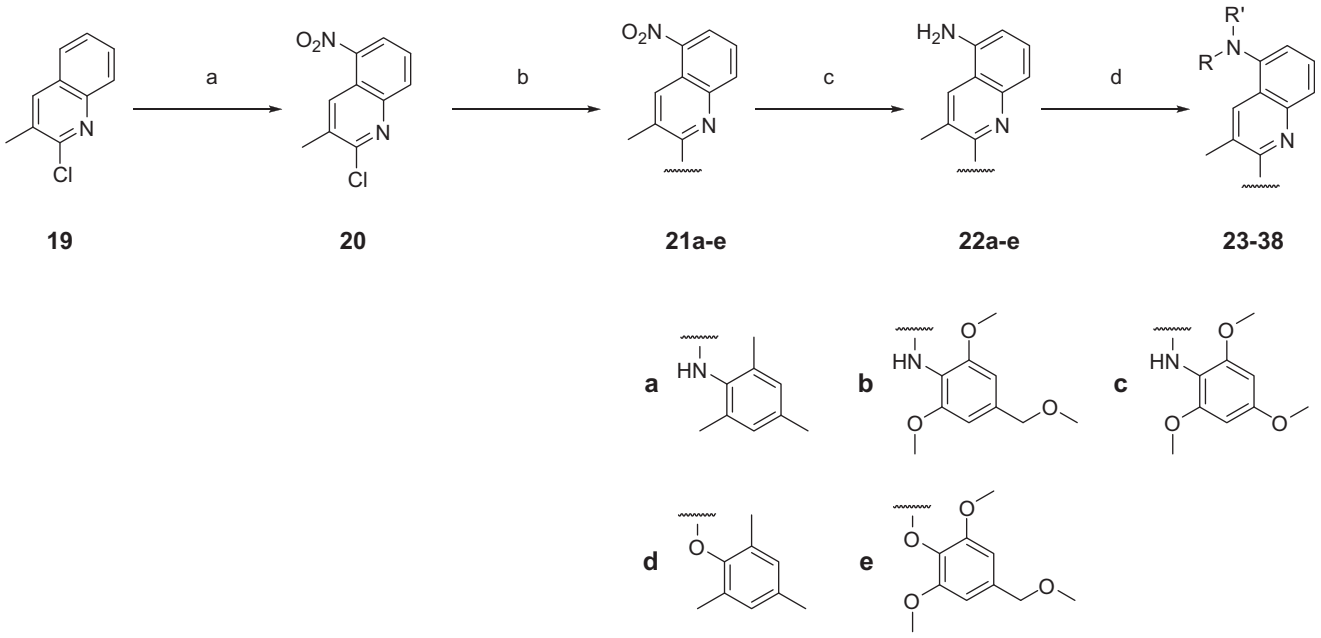
Scheme 1. Reagents and conditions: (a) Pyruvic acid, oxalyl chloride, DMF, DCM, rt, (46%); (b) $POCl_3$, *N*,*N*-diethylaniline, reflux, (59%); (c) 2,4,6-trimethylaniline, NMP, 150 °C; (d) H_2, Pd/C, EtOH, THF, rt, (22%, two steps); (e) aldehyde or ketone, α-picoline-borane, AcOH, MeOH, rt.



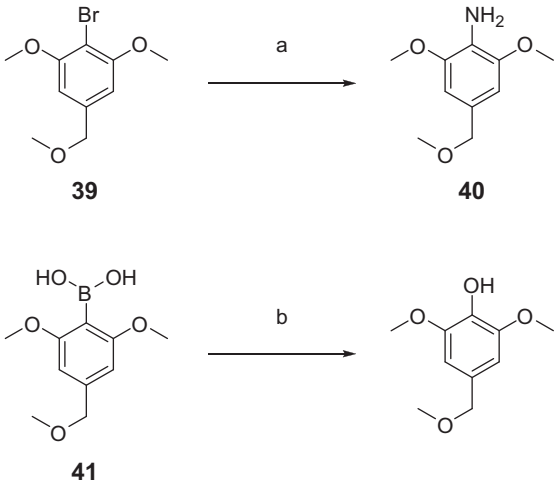
Scheme 2. Reagents and conditions: (a) $POCl_3$, *N*,*N*-diethylaniline, reflux; (b) NaOMe, MeOH, DMF, rt, (18%, two steps); (c) 2,4,6-trimethylaniline, NMP, 150 °C; (d) H_2, Pd/C, EtOH, THF, rt, (61%, two steps); (e) propionaldehyde, α-picoline-borane, AcOH, MeOH, rt, (75%); (f) NaH, MeI, DMF, rt, (**17**:26%, **18**:36%).

produced intermediate **14** with the accompanying demethylation of the methoxy-quinoxaline moiety. Hydrogenation of **14** followed by reductive amination in the presence of α-picoline-borane as a reducing agent, afforded target compound **16**. The results of NOESY analysis of compound **15** confirmed the position of 2,4,6-trimethylaniline moiety.[26] Methylation of **16** in the presence of MeI/NaH produced a mixture of **17** and **18**, which was separable by silica gel column chromatography. The synthesis of the quinoline derivatives is illustrated in Scheme 3. Nitration of commercially available starting material **19** was performed with HNO_3/H_2SO_4 to afford intermediate **20**. The position of the nitro group was determined by NMR analysis.[27] A coupling reaction with $ArNH_2$ or ArOH afforded intermediate **21a**–**e**. Hydrogenation of **21a**–**e** followed by reductive amination in the presence of α-picoline-borane as a reducing agent, afforded compounds **23**–**38**. The synthesis of 2,6-dimethoxy-4-methoxymethylaniline (**40**) and 2,6-dimethoxy-4-methoxymethylphenol (**42**) is described in Scheme 4. Compound **40** was synthesized from compound **39** via azidation. Hydrolysis of **41** using H_2O_2 afforded compound **42**.

The compounds in this study were screened for their ability to inhibit [^{125}I] CRF binding to cell membranes expressing the human CRF_1 receptor.[28] Table 1 summarizes the results obtained for the synthesized quinoxaline derivatives. Compound **10a** exhibited a high binding affinity. This result not only showed the validity of our pharmacophore model but also encouraged further derivatization of the quinoxaline template to advance current understanding of the structure–activity relationship (SAR) involved. Both secondary and tertiary amines were found to be well tolerated in the top



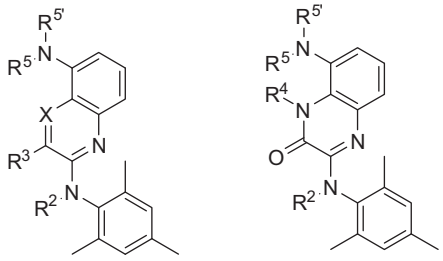
Scheme 3. Reagents and conditions: (a) HNO_3, H_2SO_4, −20 °C, (46%); (b) For **21a**, 2,4,6-trimethylaniline, NMP, 220 °C (Microwave irradiation), (39%), for **21b**, 2,6-dimethoxy-4-methoxymethylaniline, $Pd_2(dba)_3$, 2-(di-^{t}butylphosphino)-biphenyl, ^{t}BuONa, PhMe, reflux, (33%), for **21c**, 2,4,6-trimethoxyaniline, $Pd_2(dba)_3$, 2-(di-^{t}butylphosphino)-biphenyl, ^{t}BuONa, PhMe, reflux, (34%), for **21d**, 2,4,6-trimethylphenol, K_3PO_4, $Pd(OAc)_2$, 2-di- ^{t}butylphosphino-2′,4′,6′-triisopropylbiphenyl, PhMe, reflux, (19%), for **21e**, 2,6-dimethoxy-4-methoxymethylphenol, K_3PO_4, $Pd(OAc)_2$, 2-di- ^{t}butylphosphino-2′,4′,6′-triisopropylbiphenyl, PhMe, reflux, (27%); (c) H_2, Pd/C, EtOAc, rt; (d) aldehyde or ketone, α-picoline-borane, AcOH, MeOH, rt.



Scheme 4. Reagents and conditions: (a) *n*-BuLi, diphenylphosphoryl azide, THF, −78 °C to −15 °C then Sodium bis(2-methoxyethoxy)aluminum hydride, −78 °C to rt, (60%); (b) 30% H_2O_2, H_2O, rt, (96%).

region (**10a** and **10b**, respectively), with little change in activity observed. Changing the core template from quinoxaline to quinoxalinone was also tolerated (**16**). However, the introduction of a methyl group to R^4 (**17**) resulted in a decrease in activity. Moreover, introduction of a methyl group to R^2 caused complete loss of activity (**18**). This loss of activity is possibly caused by the conformational change of the $(n\text{-Pr})_2$N- or Ar–N– moiety on the central ring system. Further modification of the central core was carried out in order to fine-tune the conformation for maximum activity. Alteration of the conformation of the $(n\text{-Pr})_2$N- moiety was attempted by replacing the nitrogen atom next to the C_3 position in the quinoxaline core with a carbon atom (quinoline **23**). This quinoline exhibited a higher binding affinity than the original quinoxaline (**10a** vs **23**), suggesting that the conformation of

Table 1
Effects of R^2, R^3, R^4, R^5 and $R^{5'}$ on the quinoxaline and the quinoline

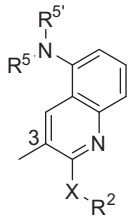


No.	X	R^5	$R^{5'}$	R^3	R^4	R^2	Binding IC_{50} (nM)
10a	N	*n*-Propyl	*n*-Propyl	Me	—	H	155
10b	N	4-Heptyl	H	Me	—	H	197
16	—	*n*-Propyl	*n*-Propyl	—	H	H	100
17	—	*n*-Propyl	*n*-Propyl	—	Me	H	672
18	—	*n*-Propyl	*n*-Propyl	—	Me	Me	>1000
23	C	*n*-Propyl	*n*-Propyl	Me	—	H	88

$(n\text{-Pr})_2$N- moiety in the quinoline template could give higher levels of activity than that in the quinoxaline template. Thus, further derivatization was conducted using the quinoline template.

The effects of the substituents R^5, $R^{5'}$ and R^2 were next explored (Table 2). The comparison between compounds **23** and **24** demonstrates that a small alkyl group was well tolerated, with very little difference in activity apparent. The large disparity in binding affinity between compounds **25** and **26** indicates that the length or size of the substituents at R^5 and $R^{5'}$ is highly restricted. It is hypothesized that the lipophilic nature of the compounds may be one of the issues hampering the clinical development of nonpeptide CRF_1 receptor antagonists.[29,30] The compounds developed here also exhibit high lipophilicity (ClogP 9.17 for **23** calculated by Daylight version 4.94). Thus, we attempted to convert the 2,4,6-trimethylphenyl, a highly lipophilic substituent, to a more hydrophilic Ar group such as 2,6-dimethoxy-4-methoxymethylphenyl (**27**) or 2,4,6-trimethoxyphenyl (**28**). From our previous experience, we

Table 2
Effects of R^2, R^5 and $R^{5'}$ on the nitrogen-linked quinoline and oxygen-linked quinoline



No.	X	R^5	$R^{5'}$	R^2	Binding IC_{50} (nM)
23	NH	*n*-Propyl	*n*-Propyl	2,4,6-Trimethylphenyl	88
24	NH	Cyclopropylmethyl	Cyclopropylmethyl	2,4,6-Trimethylphenyl	75
25	NH	Cyclopropylmethyl	CH_2-4-THP	2,4,6-Trimethylphenyl	742
26	NH	Cyclopropylmethyl	4-THP	2,4,6-Trimethylphenyl	90
27	NH	*n*-Propyl	*n*-Propyl	2,6-Dimethoxy-4-methoxymethylphenyl	472
28	NH	*n*-Propyl	*n*-Propyl	2,4,6-Trimethoxyphenyl	>1000
29	O	*n*-Propyl	*n*-Propyl	2,4,6-Trimethylphenyl	79
30	O	*n*-Propyl	*n*-Propyl	2,6-Dimethoxy-4-methoxymethylphenyl	138
31	O	Cyclopropylmethyl	CH_2-4-THP	2,6-Dimethoxy-4-methoxymethylphenyl	762
32	O	Ethyl	CH_2-4-THP	2,6-Dimethoxy-4-methoxymethylphenyl	646
33	O	Cyclopropylmethyl	4-THP	2,6-Dimethoxy-4-methoxymethylphenyl	157
34	O	Ethyl	4-THP	2,6-Dimethoxy-4-methoxymethylphenyl	323
35	O	3-Pentyl	H	2,6-Dimethoxy-4-methoxymethylphenyl	>1000
36	O	Cyclohexyl	H	2,6-Dimethoxy-4-methoxymethylphenyl	>1000
37	O	Ethyl	Pyridin-4-ylmethyl	2,6-Dimethoxy-4-methoxymethylphenyl	>1000
38	O	Ethyl	Ethyl	2,6-Dimethoxy-4-methoxymethylphenyl	70

found that these substituents could successfully reduce lipophilicity without loss of activity. In the results obtained here, although the ClogP values of **27** and **28** were lower (7.45 and 7.60, respectively) than that of **23** (9.17), both compounds exhibited less activity. We speculated that this loss of activity might be caused by the planarity of the Ar-NH- moiety, as the methoxy substituent on the *ortho*-position of the phenyl ring forms an intramolecular hydrogen bond with the NH group. This interpretation is supported by our pharmacophore model (Fig. 4). To confirm the effect of a linker atom, oxygen-linked derivatives were prepared (**29** and **30**). Compound **29** retained a level of binding affinity comparable to **23**. On the other hand, compound **30**, which had 2,6-dimethoxy-4-methoxymethylphenyl on R^2, exhibited higher binding affinity than **27**. This result supports the hypothesis that the flat angle caused by the intramolecular hydrogen bond between NH and the *ortho*-substituent of the phenyl ring, is an unfavorable conformation. Changing the linker atom to oxygen would keep the twisted conformation of Ar-O- moiety, even in compounds with a methoxy substituent at the *ortho*-position, by avoiding the formation of an intramolecular hydrogen bond. To further understand the SAR of this oxygen-linked series, derivatization of the dialkylamine moiety of compound **30** was carried out. From the results of **31**, **32**, **33** and **34**, a 4-tetrahydropyranyl (THP) substituent was more favored than a CH_2-4-tetrahydropyranyl substituent, which was similar to the nitrogen-linked series (**25** and **26**). Introduction of secondary amines or replacement of a di-alkyl-amine with a pyridine moiety resulted in a remarkable reduction in activity (**35**, **36** and **37**). Among these compounds, **38**, which has diethyl amine moiety, exhibited both a better binding affinity and a lower ClogP value (5.37), indicating that the oxygen-linked series has the potential to overcome the trade-off relationship between lipophilicity and activity.

Among those derivatives with high binding affinities, compounds **10a**, **16**, **23**, **26**, **30**, and **38** were subjected to a functional assay to evaluate their antagonism to the CRF_1 receptor. The inhibition of CRF-induced cAMP (cyclic adenosine monophosphate) production was measured for human CRF_1 receptor-expressing HEK293 cells.[31] The results are summarized in Table 3. Compounds **30** and **38** exhibited potent activity, while compounds **10a**, **16**, **23**, and **26** were slightly less active.

In summary, the pharmacophore was extracted by analysis of the common structural features of the currently known CRF_1 receptor antagonists. Molecular design based on the pharmacophore subsequently identified 5-dialkyaminoquinolines and 5-dialkylaminoquinoxalines as potential novel CRF antagonists. Through preparation and evaluation of these identified derivatives, the SAR of substituents on each position (R^2, R^3, R^4, R^5, $R^{5'}$) was grasped. As a result, a number of compounds with high binding affinities and potent antagonistic activities were found (**10a**, **16**, **23**, **26**, **30**, and **38**). Future work will aim to optimize these compounds further in order to achieve the development of a novel CRF_1 receptor antagonist for clinical application.

Table 3
Results of the functional assay

No.	Functional IC_{50} (nM)
10a	563
16	469
23	372
26	255
30	163
38	167

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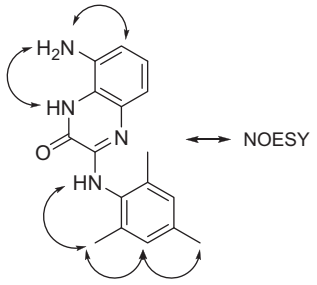

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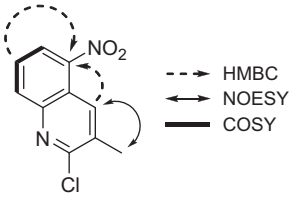

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28. For the binding assay and functional assay, HEK293 cells expressing the human CRF_1 receptor were cloned.[11] Screening of CRF_1 receptor binding was performed using the scintillation proximity assay (SPA™, Amersham Pharmacia, UK) using 96-well 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.

29. Hsin, L. W.; Tian, X.; Webster, E. L.; Coop, A.; Caldwell, T. M.; Jacobson, A. E.; Chrousos, G. P.; Gold, P. W.; Habib, K. E.; Ayala, A.; Eckelman, W. C.; Contoreggi, C.; Rice, K. C. *Bioorg. Med. Chem.* **2002**, *10*, 175.
30. Zorrilla, E. P.; Koob, G. F. *Drug Discovery Today* **2010**, *15*, 371.

31. 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.[11] 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.