# Design and Synthesis of a Series of Non-Peptide High-Affinity Human Corticotropin-Releasing Factor$_1$ Receptor Antagonists

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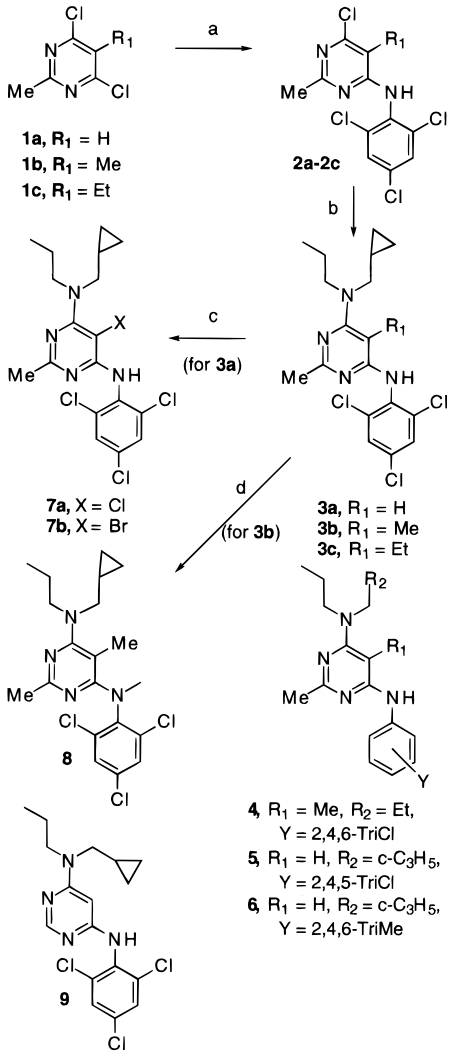
Corticotropin-releasing factor (CRF), a 41 amino acid peptide of hypothalamic origin, plays a major role in coordinating the endocrine, behavioral, and autonomic responses to stressful stimuli.[1−3] The actions of CRF are mediated through high-affinity receptors which are part of the superfamily of G-protein-coupled seven transmembrane proteins.[4] Recently two members of the CRF receptor family have been cloned expressed and characterized. These receptors demonstrate a different sequence, pharmacology, and regional distribution within the central nervous system and the periphery. The $CRF_1$ receptor demonstrates high affinity for CRF and its related mammalian analogs,[4−7] as well as for non-mammalian related peptides including sauvagine (frog) and urotensin I (sucker fish).[8] The $CRF_2$ family of receptors, while sharing sequence homology with the $CRF_1$ receptor subtype, demonstrates low affinity for CRF itself but retains its high affinity for the nonmammalian forms of the peptide.[8,9]

While there is very little known about the physiology and function of the $CRF_2$ subfamily of receptors, a substantial amount of preclinical and clinical data exists that suggests that CRF and its high-affinity receptor play a major role in mediating various neurological and psychological disorders, including major depression, anxiety, and a variety of stress-related disorders (for reviews, see refs 2, 3, and 10). Treatments for these disorders have been hampered by the fact that ligands for these receptors have thus far been large peptides.[11] We report the design and synthesis of high affinity and selective non-peptide $CRF_1$ receptor antagonists, 4-anilino-6-aminopyrimidines (illustrated by **3b)**, targeted for the treatment of CRF-mediated disorders.

The reaction sequence for the synthesis of **3a**−**c, 4, 5, 6**, and **9** is illustrated with **3a**−**c** in Scheme 1. N-Methylation of the anilino nitrogen on **3b** with excess methyl iodide and 1 equiv of sodium hydride in THF gave **8**. Pyrimidines **11** and **12** (see Chart 1 and Supporting Information for Scheme 2 with synthesis) were prepared by a similar sequence as that used for the synthesis of **3a**−**c** starting with commercially available 2,6-dichloro-6-methylpyrimidine (**10)** and separating the anilinopyrimidines **11** and **12** by flash chromatography.

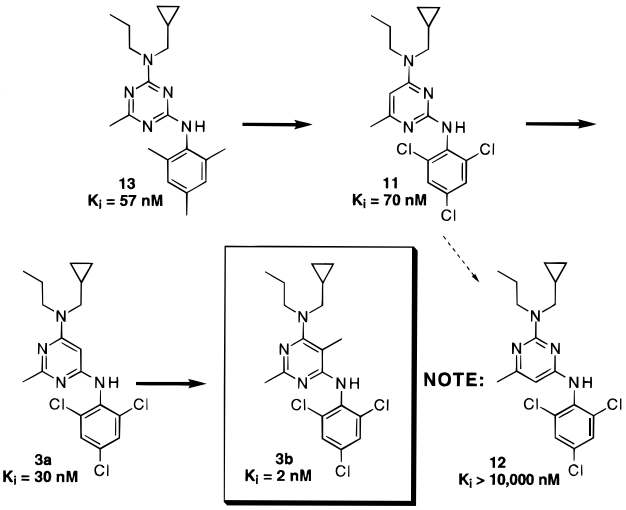
The inhibition of [$^{125}$I]CRF binding to cells expressing the human $CRF_1$ receptor was used to identify specific receptor antagonists in a radioligand binding assay.[12] The CRF receptors expressed in these cell lines demonstrated reversible, saturable, high-affinity binding to CRF with the pharmacological and functional characteristics comparable to those found in a variety of animal or human tissues.[12] CRF receptor binding assays were carried out essentially as previously described.[13]

**Scheme 1**[a]




[a] (a) 2,4,6-$Cl_3C_6H_2NH_2$, NaH, THF, RT or reflux; (b) c-$C_3H_5CH_2NHCH_2CH_2CH_3$, 100−190 °C; (c) NBS or NCS, $CHCl_3$, reflux; (d) NaH, MeI, THF, room temperature.

**Chart 1.** Sequence Followed in the Design of $CRF_1$ Receptor Antagonists

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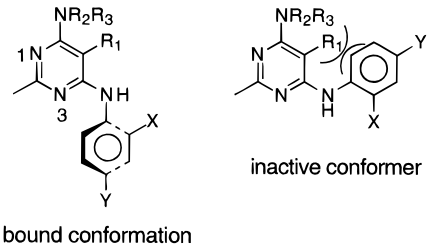


**Figure 1.** Proposed conformation for anilinopyrimidines to bind to the $CRF_1$ receptor and comparison with an inactive conformer.

**Table 1.** Inhibition ($K_i$) Values for Receptor Antgonists in Cells Stably Transfected with Human $CRF_1$ Receptors[a]

| compd | $K_i$ (nM) | compd | $K_i$ (nM) |
|---|---|---|---|
| **3a** | 30 | **7a** | 1.7 |
| **3b** | 2.3 | **7b** | 2.0 |
| **3c** | 3.8 | **8** | 150 |
| **4** | 2.5 | **9** | >10000 |
| **5** | 253 | D-PheCRF(12−41) | 20 |
| **6** | 1390 | | |

[a] Compounds were tested at 6−12 doses for their ability to inhibit [$^{125}$I]CRF binding as described in text. Data are representative of duplicate determinations with the experiments repeated two or three times.

The design of **3b** starting from triazine **13**[14] and the structure−activity relationship of related pyrimidines **11**, **3a**, and **12** with regard to $CRF_1$ receptor binding activity is outlined in Chart 1. Removal of the 1- or 5-nitrogen from triazine **13** ($K_i$ = 57 nM), optimized by rapid microscale synthesis (RMS),[14] gave pyrimidine **11** ($K_i$ = 70 nM) or **3a** ($K_i$ = 30 nM), respectively. However, changing the position of the 3-nitrogen (i.e. **12**) resulted in complete loss of activity. This $CRF_1$ receptor binding data led to a proposed pharmacophore model for binding to the $CRF_1$ receptor illustrated in Figure 1. In this model the bound conformation of the molecule requires the anilino group to be orthogonal to and below the pyrimidine (or triazine) ring. In addition, the nitrogen at the 3-position of **3a**, **3b**, **11**, and **13,** but absent in **12**, provides a critical hydrogen-bonding site. Consistent with the model, addition of a substituent at the 5-position of the pyrimidine ring resulted in a 30−50-fold increase in activity. Thus, addition of a methyl group (**3b**) or halogen (chloro, **7a**, or bromo, **7b**) provided compounds with $K_i$ values in the 2 nM range (see Table 1). 2,4,6-Trichloroanilinopyrimidines were synthesized (**3a**−**c**, **4**, **6**, **8**, and **9**) on the basis of the 2,4,6-trisubstituted aryl group required for optimal activity for triazine **13** (Chart 1), the preference for trichloro over other substituents in related series (see Chart 1 and **3a** vs **6**), and the preference for an orthogonal relationship between the phenyl and pyrimidine rings with this substitution pattern. 2,4,5-Trisubstituted aryl compound **5** was over 10-fold less active than the corresponding 2,4,6-trisubstituted aryl compound **3a**. Interestingly, addition of a methyl group on the anilino nitrogen (**8**) resulted in over 100-fold loss in activity. As in the triazine **13**,[14] *N*-propyl-*N*-cyclopropylmethyl was optimal for the N6 amino group, but unlike the triazine series *N*,*N*-dipropyl was equally active (**4**).

While direct inhibition of binding activity gives a valid measure of the potency of a compound at a specific receptor, functional tests must be employed in order to determine whether the compounds can act as agonists or antagonists at these receptors. Using membrane preparations of the stable cell lines transfected with the $CRF_1$ receptor, the $CRF_1$ antagonist activity of the series was demonstrated by inhibition of CRF-stimulated cAMP production with **3a**, **4**, and **7a** using D-PheCRF-(12−41) as the standard ($IC_{50}$ = 3700, 250, 100, and 200 nM, respectively). Inhibition of CRF-stimulated adenylate cyclase activity was performed as previously described.[15] None of the compounds demonstrated any effects on basal cAMP production (that is in the absence of CRF or other stimulator), indicating that these compounds are devoid of agonist activity at this receptor subtype. In addition, these compounds could inhibit CRF-stimulated ACTH release from primary rat anterior pituitary cell cultures (data not shown), further demonstrating antagonist activity at $CRF_1$ receptors. In order to test for CRF receptor selectivity, the compounds synthesized in this series (Table 1) were assessed for inhibition of cAMP production in cells transfected with the human $CRF_{2\alpha}$ receptor and were completely devoid of activity (data not shown).

In conclusion, we have demonstrated the design and synthesis of selective, high-affinity non-peptide $CRF_1$ receptor antagonists **3b**, **3c**, **4**, **7a**, and **7b** with $K_i$ values in the low nanomolar range. In addition, these compounds demonstrate *in vitro* inhibition of CRF-stimulated cAMP production in stable lines transfected with the human $CRF_1$ subtype. These compounds, and further modifications, will be of particular significance in establishing the utility and potential of $CRF_1$ receptor antagonists in the treatment of depression and anxiety-related disorders.

**Supporting Information Available:** A brief description of the biological assays, synthetic procedures for new compounds, and analytical data (8 pages). Ordering information is given on any current masthead page.

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