Design and Synthesis of Tricyclic Imidazo[4,5-*b*]pyridin-2-ones as Corticotropin-Releasing Factor-1 Antagonists

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Abstract: The synthesis and SAR studies of tricyclic imidazo-[4,5-*b*]pyridin-2-ones as human corticotropin-releasing factor receptor (CRF_1) antagonists are discussed herein. Compound **16g** was identified as a functional antagonist that inhibited CRF-stimulated cyclic adenosine monophosphate production and CRF-induced adrenocorticotrophic hormone release. Pharmacokinetics studies in rats showed that **16g** was orally bioavailable, had good brain penetration, and had a moderate half-life. In our effort to identify CRF_1 antagonists with improved pharmacokinetics properties, **16g** exhibited a favorably lower volume of distribution.

Corticotropin-releasing factor (CRF, also known as corticotropin-releasing hormone), a 41 amino acid neuropeptide isolated from mammalian brain, is the prime regulator of the hypothalamic-pituitary-adrenal (HPA) stress response.[1] CRF mediates its actions through high-affinity binding to its receptors, CRF_1 and CRF_2, both of which are members of the class B subfamily of G-protein-coupled receptors. Prolonged activation of the central CRF system may play a fundamental role in the etiology of major depression.[2] Hypersecretion of hypothalamic CRF manifests itself in a down-regulation of CRF receptors in the anterior pituitary as demonstrated by the blunted adrenocorticotrophic hormone (ACTH) response to peripherally administered CRF in severely depressed patients. Because of the key role that CRF plays in stress response, centrally acting CRF_1 antagonists are expected to have utility in treatment of stress-related psychiatric disorders such as anxiety and depression.[3]

Many non-peptide CRF_1 antagonists from different chemical classes have been reported in recent years, and several representative examples are illustrated in Figure 1. Potent CRF_1 antagonists such as **1** (CP-154,526),[4] **2** (antalarmin),[5] **3** (NBI-27914),[6] and **4** (DMP696)[7] have been widely studied in many different animal models of CRF-related behavior. Although these compounds possess excellent in vitro activities as CRF_1 antagonists,

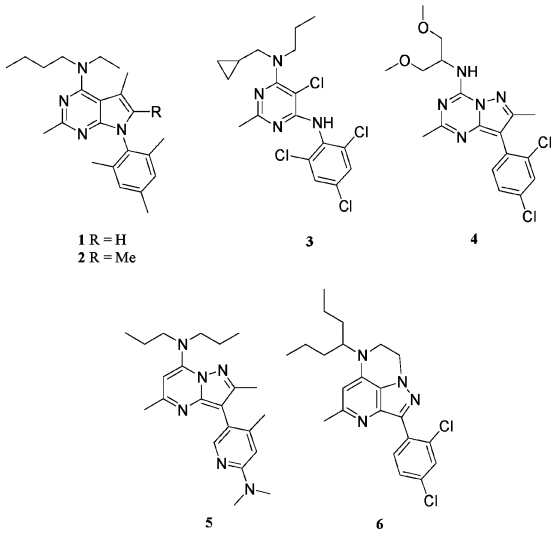

Figure 1. Some small-molecule CRF_1 receptor antagonists.

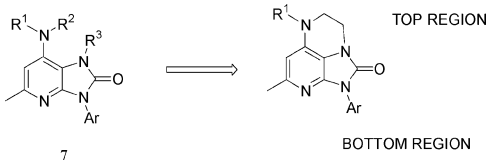

Figure 2. Design of tricyclic CRF_1 receptor antagonists.

and several of them exhibited in vivo efficacy in the anxiety models when dosed orally, most of these compounds reported earlier suffer from high lipophilicity and poor water solubility.[8] For example, **1** has a very long half-life (51 h) and high volume of distribution (105 L/kg) in rats.[9] More recent efforts on the discovery of more hydrophilic CRF_1 antagonists generated **5** (NBI-30775/R121919),[10] which has antidepressant efficacy in an open label phase IIA clinical trial in major depressive disorder patients.

Compounds **1**−**5** are based on monocyclic or bicyclic cores. We envisioned that synthesizing compounds with a third ring on the core structure would restrict the conformational freedom of their flexible "top region" (Figure 2) and thus would result in compounds that would populate a smaller subset of the biologically relevant conformations required for optimal biological activity. We recently reported on a series of conformationally constrained tricyclic pyrazolo[4,3-*b*]pyridines such as **6**, which was found to be a potent functional antagonist of CRF_1.[11] In our effort to prepare potent CRF_1 antagonists with reduced lipophilicity, we have made tricyclic analogues of the polar 1,3-dihydroimidazo[4,5-*b*]pyridin-2-one series[12] (Figure 2), and the initial SAR and pharmacokinetics profiles of representative compounds from this series of CRF_1 antagonists are reported herein.

For the synthesis of tricyclic analogues we employed two general methods. In the first method (Scheme 1), a substituted pyridine ring was prepared (**13**), followed by construction of the imidazolone ring (**15**), and finally closure of the tetrahydropyrazine ring. The starting material 2,4-dichloro-6-methyl-3-nitropyridine (**9**) was readily prepared from commercially available 4-hy-

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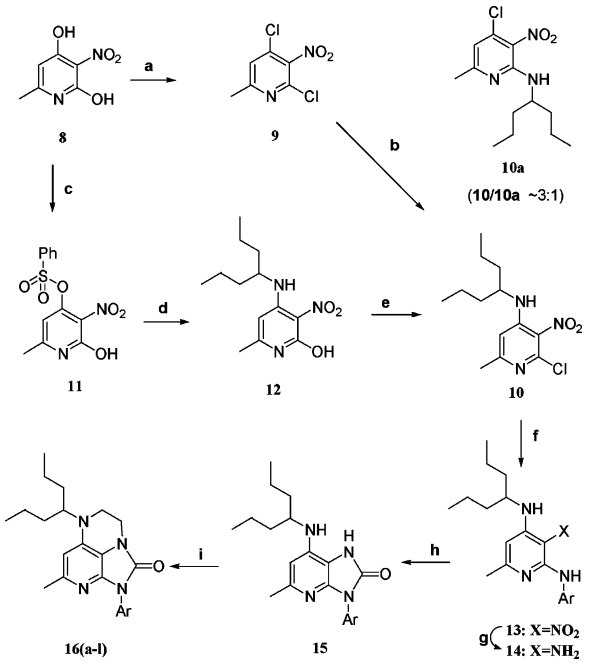
Scheme 1[a]



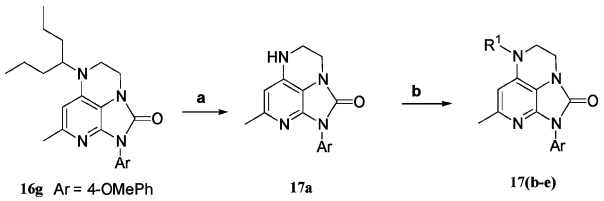
[a] Reagents and conditions: (a) $POCl_3$, ACN, 82 °C, 85%; (b) 4-aminoheptane, Et_3N, ACN, −10 °C, 40%; (c) $PhSO_2Cl$, Et_3N, DMAP, 60 °C, 91%; (d) 4-aminoheptane, TsOH, acetonitrile, 68 °C, 80%; (e) $POCl_3$, acetonitrile, DIPEA, 90 °C, 87%; (f) $ArNH_2$, ACN, 65 °C, 95%; (g) $Na_2S_2O_4$, THF/water, room temp, 91%; (h) triphosgene, NEt_3, 0 °C, 80%; or carbonyldiimidazole, NEt_3, DMF, 76%; (i) 1,2-dibromoethane, DMF, NaH, room temp, 30−80%.

droxy-6-methyl-3-nitro-2(1*H*)-pyridone (**8**) and $POCl_3$.[12] **9** was then treated with 4-aminoheptane in acetonitrile at −10 °C to give a 3:1 mixture of **10** and its regioisomer (**10a**). In an improved version of this method, **8** was selectively monosulfonylated with benzenesulfonyl chloride, and the resulting sulfonate intermediate **11** was directly treated with 4-aminoheptane. This one-pot procedure yielded **12** in 73% yield with no purification issues. Chlorination of **12**, followed by reaction with substituted anilines at elevated temperatures (~140 °C), afforded the condensation products **13**. The 5-nitro group of **13** was reduced with sodium hydrosulfite to provide the corresponding diaminopyridines **14**, which were cyclized with triphosgene to form the imidazolone ring. The third ring was installed by treatment of **15** with 1,2-dibromoethane using sodium hydride as base in DMF or sodium hydroxide as base under phase-transfer conditions.

With the above synthetic routes, the upper amino substituent R^1 was fixed at the beginning of the synthesis. We developed an alternative approach (Scheme 2) to explore the SAR at this position while keeping the bottom Ar substituent constant. Dealkylation of **16g**[13] was carried out in neat concentrated sulfuric acid to yield the secondary amine **17a**. Top region R^1 groups were then installed via base-mediated alkylation of intermediate **17a** to yield the final compounds **17b**−**e**. Compounds **18a**−**c** and **19a**−**c** were also prepared according to the synthetic route in Scheme 1 with the 3-pentyl or 1-methoxy-2-butyl group as the top amino substituent.

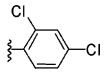
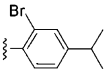
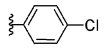
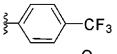
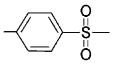
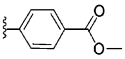
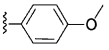
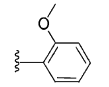
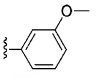
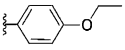
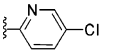
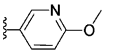
The compounds thus synthesized were tested for binding affinity at the cloned human CRF_1 receptor expressed in leukocyte tyrosine kinase cells with sauvagine as the radiolabeled ligand, and the K_i values were determined from dose−response curves using concentrations from 1 nM to 10 μM as described.[14]

Scheme 2[a]



[a] Reagents and conditions: (a) concentrated H_2SO_4, 65 °C, 50%; (b) R^1Br or R^1I, NaH or ^{t}BuOK, DMF, 20−50 °C, 15−70%.

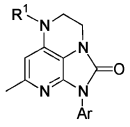
Table 1. Binding Affinities of **16a**−**l** to the Human CRF_1 Receptor

Compds	Ar	K_i(nM)[a]
2	---	3.1
6	---	4.5
16a		5.1
16b		1.1
16c		12
16d		17
16e		460
16f		920
16g		2
16h		44
16i		130
16j		140
16k		23
16l		60

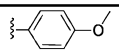
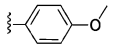
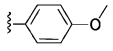
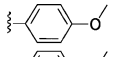
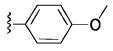
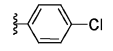
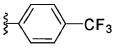
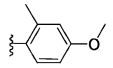
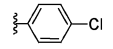
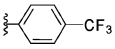
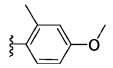
[a] Receptor binding was conducted as described previously. Data are the average of three or more independent determinations. Typical standard errors were less than 30%.

The binding activity data of substitutions at the bottom aryl region of the tricyclic imidazolone series are summarized in Table 1. The top amino substituent (R^1) was initially kept constant as 4-heptyl, since this substituent was potent in our other tricyclic series (i.e., **6**).[11] The SAR pattern that emerged was in part similar to that seen for other CRF_1 antagonist scaffolds in that 2,4-disubstituted aryl groups gave good potency, particularly with lipophilic and relatively small substituents (**16a**,**b**). Analogue **16a** with a 2,4-dichlorophenyl group demonstrated a potent K_i of 5.1 nM, while the slightly bigger 2-bromo-4-isopropyl analogue **16b** was even more potent (K_i = 1.1 nM). Our goal, however, was to identify polar substituents to help offset the contribution of the amino substituent (R^1) to the overall lipo-

Table 2. Binding Affinities of **17b**−**e**, **18a**−**c**, and **19a**−**c** to the Human CRF_1 Receptor



17, **18**, **19**

Compds	R^1	Ar	K_i(nM)[a]
16g	4-heptyl		2
17b	isopropyl		> 10,000
17c	n-butyl		8,800
17d	3-pentyl		8
17e	1-MeO-2-butyl		150
18a	3-pentyl		58
18b	3-pentyl		170
18c	3-pentyl		20
19a	1-MeO-2-butyl		200
19b	1-MeO-2-butyl		190
19c	1-MeO-2-butyl		1,000

[a] Receptor binding was conducted as described previously. Data are the average of three or more independent determinations. Typical standard errors were less than 30%.

philicity of the compounds. Even though removal of the 2-chloro substitution in **16a** resulted in a 2-fold loss of potency (**16c**), it demonstrated that the 2-aryl substituent is not required for good activity in this series. Accordingly, a series of 4-monosubstituted aryl analogues (**16d**−**g**) were prepared to look for simple and less lipophilic groups as the bottom. Although a strong electron-withdrawing trifluoromethyl group at the 4-position was tolerated (**16d**, 17 nM), the polar, electron-withdrawing methylsulfone and carboethoxy substituents dramatically reduced the binding affinity as seen in **16e** and **16f** (K_i = 465 and 920 nM, respectively). Incorporation of a less lipophilic, electron-donating methoxy group at the different positions on the bottom phenyl ring suggested that the 4-position (**16g**, 2 nM) was preferred over the 2- or 3-position analogues in potency. Moreover, extending the 4-methoxy to 4-ethoxy led to a decrease in potency by 2 orders of magnitude. Incorporation of a substituted pyridine ring on the bottom (**16k**,**l**) lowered the lipophilicity further but provided less active analogues than the corresponding phenyl compounds.

Binding results from variation of the upper region amino substituent R^1 are depicted in Table 2. In our attempt to reduce overall lipophilicity, we investigated smaller alkyl replacements for the 4-heptyl group of **16g**. From this study, it is clear that the top region nitrogen substituents of tricyclic **17** participitate in a key binding interaction with the CRF receptor. Alkyl substituents smaller than the isopropyl group resulted in a total loss of binding activity, while *n*-butyl analogue **17c** exhibited only very weak binding. The 3-pentyl analogue had very good affinity (**17d**, 8 nM), even though it was still about 4-fold less potent than **16g**. Insertion of an oxygen atom into the five-carbon chain of **17d** (as shown in **17e**) decreased affinity further.

Further studies were carried out to determine whether the SAR of the bottom aryl region would be different if the heptyl substituent of **16** was replaced by a smaller, less lipophilic group. Binding data for representative 3-pentyl compounds **18a**,**b** and methoxybutyl compounds **19a**,**b** are also presented in Table 2. The results in general paralleled those seen in the 4-heptyl series, and the uniformally lower affinity of these compounds served to confirm the requirement for a lipophilic upper amino substituent for optimal potency. Adding a 2-aryl substitution to reimburse the loss of affinity from the top region (as shown in **18c** and **19c**) failed to improve the binding affinity.

Compound **16g** was very weakly basic with a pK_a of 4.9, and it possessed moderate lipophilicity with a log *P* value of 4.5, which was comparable to the reported value[10] for **5** (log *P* = 4.9). Even though the free base had limited solubility in water (0.34 mg/mL), its L-malic acid salt was readily soluble in 1 N HCl (38 mg/mL). This compound had essentially no affinity for the CRF_2 receptor expressed in Chinese hamster ovary (CHO) cells at 10 μM.[15] **16g** showed no significant binding at 10 μM to over 60 different receptors, ion channels, and transporters. As a consequence, **16g** was further evaluated for functional CRF_1 antagonism in vitro and in vivo. Compound **16g** dose-dependently inhibited CRF-stimulated cyclic adenosine monophosphate (cAMP) production in CHO cells expressing the CRF_1 receptor[16] with an IC_{50} of 71 nM, which was comparable to that of **5** (IC_{50} = 50 nM). In a second functional assay, **16g** was also found to functionally antagonize CRF-stimulated ACTH release from rat primary anterior pituitary cell cultures.[14] The result showed that **16g** was a potent antagonist in this assay with an IC_{50} of 150 nM.

We further determined the pharmacokinetics profile of **16g** in male Sprague-Dawley rats, following intravenous and oral administration at 10 mg/kg. After an iv injection, **16g** had a high plasma clearance (CL = 53.4 mL min^{-1} kg^{-1}) and a small volume of distribution (V_d = 7.2 L/kg) in rats, which resulted in a terminal half-life of 1.6 h. After oral administration of **16g**, the C_{max} in plasma and brain occurred simultaneously at 1 h after dosing. The mean maximal concentrations (C_{max}) in plasma and brain tissue via oral administration were 356 ng/mL and 1180 ng/g, respectively. Oral bioavailability was estimated to be 30%, and on the basis of the AUC (0−6 h) ratio, the brain tissue was exposed to approximately 280% of the plasma concentration of **16g** at this dosage. **16g** had a notably smaller volume of distribution and shorter terminal half-life in rats than the previous leading CRF antagonists such as **1** (V_d = 105 L/kg, $t_{1/2}$ = 51 h)[9] and **4** (DMP696, V_d = 22 L/kg, $t_{1/2}$ = 16 h).[7] In light of these results, **16g** was further evaluated in vivo.

The in vivo functional antagonism of **16g** was determined using the CRF-induced ACTH release assay in normal rats. As shown in Figure 3, intravenous CRF (0.3 nmol/kg) administration in rats produced a robust increase in plasma levels of ACTH (p < 0.0007).

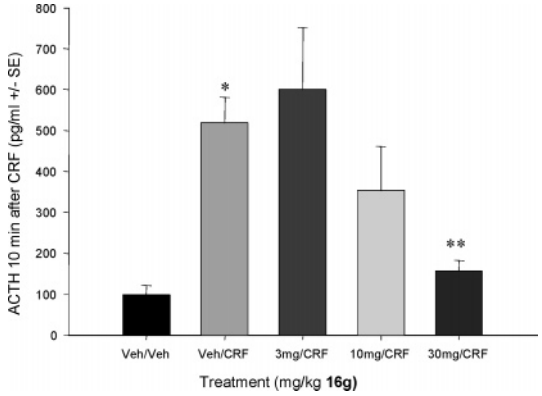


Figure 3. Dose-dependent effects of **16g** on CRF-induced ACTH release 10 min following CRF administration: (*) $p < 0.0007$ vs 10 mL/kg 5% D-mannitol (w/v) in water (Veh); (**) $p < 0.002$ vs CRF.

Compound **16g** significantly attenuated this effect in a dose-dependent manner with statistically significant reductions observed at the 10 and 30 mg/kg doses ($p < 0.002$ vs CRF). The observed attenuation of ACTH was 86% at the 30 mg/kg dose.

In conclusion, we have shown that tricyclic imidazo-[4,5-*b*]pyridin-2-one compounds are potent CRF_1 antagonists. The antagonistic activity of these compounds was demonstrated by their high affinities in binding to the CRF_1 receptor and inhibition of CRF-stimulated cAMP production. A representative from this series, **16g**, possessed acceptable physicochemical properties for use as a central nervous system agent, showed a good overall in vitro profile, and had appropriate pharmacokinetics (especially favorably small volume of distribution) in rats. **16g** not only inhibited CRF-stimulated ACTH release in vitro but also dose-dependently attenuated the elevation in plasma ACTH levels induced by CRF in rats in vivo. These results confirmed that **16g** is a functional CRF_1 antagonist with improved physicochemical properties and a good pharmacokinetics profile. Further evaluation of **16g** will be reported in due course.

Supporting Information Available: Experimental procedures for the synthesis and characterization of representative compounds, experimental details of the binding and functional assays, and X-ray structure for **16g**. This material is available free of charge via the Internet at http://pubs.acs.org.

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