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3-Aryl Pyrazolo[4,3-*d*]pyrimidine Derivatives: Nonpeptide CRF-1 Antagonists

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Abstract—The synthesis of a series of 3-aryl pyrazolo[4,3-*d*]pyrimidines as potential corticotropin-releasing factor (CRF-1) antagonists is described. The effects of substitution on the aromatic ring, the amino group and the pyrazolo ring on CRF-1 receptor binding were investigated.

Corticotropin releasing factor (CRF) is a 41-amino acid peptide that acts as a modulator of the body's responses to stress.[1] Compelling clinical evidence exists for the hypothesis that over-stimulation of CRF may underlie the pathology of several diverse neuropsychiatric diseases including depression, anxiety, and stress-related disorders.[2] Until now, two G-protein coupled receptors for CRF (CRF-1 and CRF-2) have been identified in mammals. They are encoded by two separate genes and display distinct pharmacology and distribution within the CNS and periphery.[3,4] Although the therapeutic benefits of blocking the CRF-2 receptor remain to be established, evidence exists that antagonism of the CRF-1 receptor produces anxiolytic and antidepressant effects in animals. In an attempt to produce clinically useful therapeutic agents, several series of small molecule CRF-1 receptor antagonists have been reported, as exemplified by pyrrolopyrimidine **1** (CP154526),[5] triazolopyrimidine **2**[6] and pyrazolo[1,5-*a*]pyrimidines **3** (NBI 30775/R121919) (Fig. 1).[7–9]

A large body of evidence gathered with the antagonist **1** ($K_i = 2.7$ nM), and others,[10] supports the CRF concept preclinically.[11] Indeed, recent clinical results with R121919 **3** ($K_i = 5$ nM) have turned out to be very encouraging, despite an open label design.[12] Further development of R121919 was eventually discontinued due to clinical signs of hepatotoxicity and the search for new chemical matter is still ongoing. In this paper, we describe the synthesis and structure–activity based optimization of a new series of CRF-1 antagonists, the 3-aryl pyrazolo[4,3-*d*]pyrimidines **4**.

The compounds described in this study are listed in Tables 1–3, and the methods used for their synthesis are outlined in Schemes 1 and 2. Reaction of the appropriately aryl substituted acetophenone **6** with sodium hydride and diethyl oxalate gave the pyruvate **7** in good yield (CAUTION: Careful heating of this reaction is necessary as an exotherm at 75 °C was observed). Treatment of **7** with dinitrogen trioxide (generated from sodium nitrite and HCl) in ethanol gave the oxime **8**.[13] Cyclization of **8** with methyl hydrazine gave a mixture of the nitroso pyrazoles **9** and **10**, which were separated by flash chromatography. Reduction of the nitroso group with sodium dithionite in aqueous THF gave the respective amino-pyrazoles **11** and **12**.

Treatment of the pyrazole **11** with benzyl thioacetimidate hydrobromide[14] in refluxing pyridine afforded the pyrazolopyrimidinone **13** in moderate yield. Heating **13** with phosphorus oxychloride in the presence of *N,N*-diethyl aniline gave the corresponding chloride **14** in high yield. Displacement of the chloride of **14** with the appropriate amine afforded the target 7-amino substituted pyrazolo[4,3-*d*]pyrimidines **15**.

Pyrazole **12** was subjected to the same sequence of reactions outlined in Scheme 1 and gave the corresponding 1-methyl analogues. The unsubstituted, *N*-ethyl and *N*-propyl substituted pyrazolo[4,3-*d*]pyrimidines (Table 1) were prepared employing analogous chemistry to that outlined in Schemes 1 and 2 but using hydrazine, ethyl

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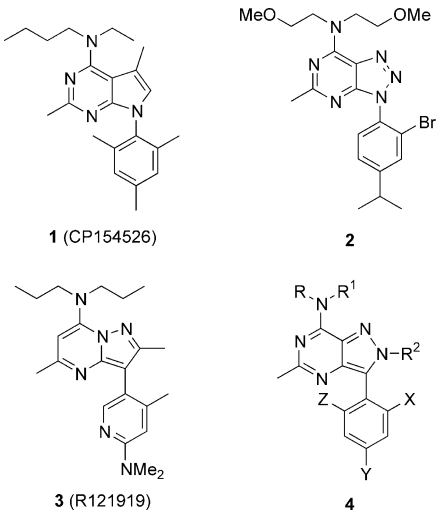
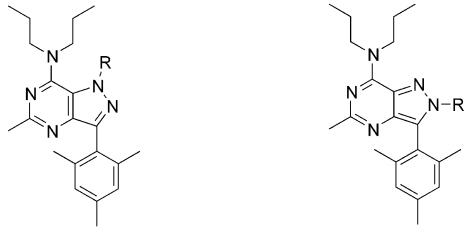


Figure 1. Examples of small molecule CRF-1 receptor antagonists.

Table 1. Effects of 1- and 2-pyrazolo[4,3-*d*]pyrimidine substituents on CRF-1 binding



Compd	R	K_i, nM
16a	H	16±2
16b	Me	1±0.4
16c	Et	15±3
16d	Pr	62±15
17a	Me	3±1
17b	Et	3±1
17c	Pr	11±3
CP154526 (**1**)	—	2.7

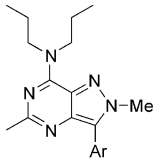
K_i values are the mean of three independent experiments.

hydrazine and propyl hydrazine respectively in the cyclization step.

The affinity of the compounds (Tables 1–3) for the CRF-1 receptor were determined by using a modified version of the assay described by Grigoriadis and De Souza[15] by examining their displacement of ^{125}I-sauvagine from CRF-1 receptors endogenously expressed in IMR-32 human neuroblastoma cells.

The effects of alkyl substituents at the 1- and 2-position of the pyrazolo[4,3-*d*]pyrimidine on CRF-1 receptor binding were examined first (Table 1). The 3-mesityl and 7-dipropylamine substituents were kept constant throughout. The unsubstituted analogue **16a** was found to have good affinity for the CRF-1 receptor (K_i = 16 nM). Introduction of a methyl group to the 1-position gave compound **16b** and a large increase in binding affinity (K_i = 1 nM). Increasing the chain length (ethyl **16c** and propyl **16d**) resulted in a significant loss in affinity. A similar, but less pronounced trend was observed for the 2-alkyl substituted analogues **17a**–**17c**. The 2-methyl **17a** and 2-ethyl **17b** analogues were of similar affinity to **16b** and while the affinity of the corresponding 2-propyl **17c** analogue was reduced the magnitude of the loss was greatly reduced. Compounds **16b**, **17a**, and **17b** all displayed binding affinity comparable to that reported for CP154526 **1**.[5b]

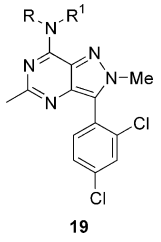
Table 2. Effects of the 3-aryl substituent on CRF-1 binding



Compd	Ar	K_i, nM
17a	2,4,6-Trimethyl	3±1
18a	Ph	>5000
18b	2-Chloro	765±93
18c	3-Chloro	>5000
18d	4-Chloro	>5000
18e	2,6-Dichloro	20±4
18f	2,4-Dichloro	2±1
18g	2,4-Methoxy	10±3
18h	2-Methyl-4-chloro	4±2
CP154526 (**1**)	—	2.7

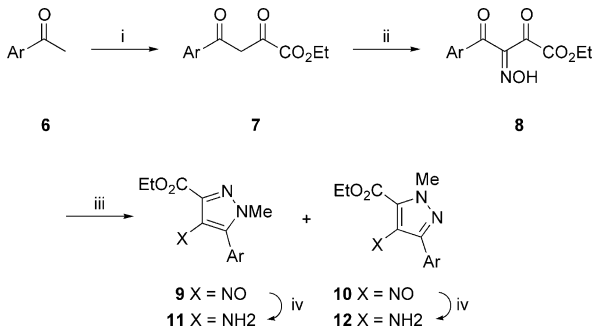
K_i values are the mean of three independent experiments.

Table 3. Effect of the 7-amino substituent on CRF-1 binding

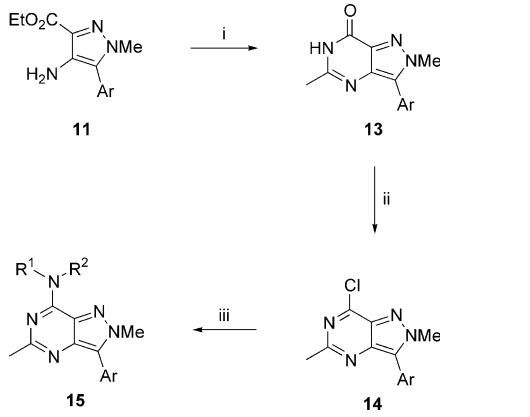


Compd	R	R^1	K_i, nM
19a	Methyl	H	>5000
19b	Ethyl	H	192±82
19c	*n*Propyl	H	24±10
19d	*n*Butyl	H	25±9
19e	*n*Pentyl	H	38±17
19f	*n*Hexyl	H	112±31
19g	Cyclopentyl	H	17±4
19h	Cyclohexyl	H	174±39
19i	$CH(Et)_2$	H	16±8
18f	*n*Propyl	*n*Propyl	2±1
19j	$(CH_2)_2OMe$	$(CH_2)_2OMe$	3±2
19k	Ethyl	*n*Butyl	2±1
19l	Ethyl	*n*Propyl	3±2
19m	Ethyl	*n*Hexyl	29±15
19n	*n*Propyl	$(CH_2)_2NMe_2$	145±71
19o	*n*Propyl	$(CH_2)_2N(CH_2)_4$	115±33
19p	*n*Propyl	$(CH_2)_2N(CH_2)_5$	561±212
CP154526 (**1**)	—	—	2.7

K_i values are the mean of three independent experiments.



Scheme 1. Reagents and conditions: (i) NaH, diethyl oxalate, PhMe, reflux, (71–89%); (ii) N_2O_3, EtOH, (85–94%); (iii) $MeNHNH_2$·HCl, MeOH–H_2O (69–78%); (iv) $Na_2S_2O_4$, THF–H_2O, (59–68%).



Scheme 2. Reagents and conditions: (i) benzylthioacetimidate·HBr, pyridine, reflux, (47–55%); (ii) $POCl_3$, *N,N*-diethylaniline, reflux, (89–93%); (iii) NHR^1R^2, ethanol, reflux (68–89%).

The effects of the substituents on the 3-aryl group were also investigated (Table 2). The 7-dipropyl amine and the 2-methyl substituent were kept constant in the pyrazolopyrimidine. For optimal CRF-1 binding, previous studies have suggested that the aryl ring lies out of the plane of the core heterocycle and our results are in accord with that model.[16] The unsubstituted phenyl analogue **18a** and the 3- and 4-chloro analogues (**18c** and **18d**) were all inactive. An *ortho* substituent, however, on the aromatic ring enforces an active conformation and the 2-chloro analogue **18b** showed moderate affinity ($K_i = 765$ nM). A large increase in affinity was observed by addition of a second *ortho* substiutent (**18e**, $K_i = 20$ nM). The 2,4,6-trimethylphenyl analogue **17a** described earlier gave a further increase in affinity. A *para* substituent in combination with one *ortho* substituent also gave compounds of a similar affinity (**18f**, $K_i = 2$ nM, **18g**, $K_i = 10$ nM, and **18h**, $K_i = 4$ nM).

The final region of the pyrazolopyrimidine that was investigated was the 7-amino substituent. The 2-methyl substituent was fixed in the pyrazolopyrimidine and the 2,4-dichlorophenyl was retained at the 3-position. Secondary amino-pyrazolopyrimidines ($R^1 = H$) **19a–i** were examined first (Table 3). A dramatic increase in binding was observed as the alkyl chain increased, with the propyl and butyl analogues **19c** and **19d** proving the most active. The activity decreased as the alkyl chain was increased to pentyl and hexyl (**19e** and **19f**). The cyclopentyl analogue **19g** had good affinity ($K_i = 17$ nM), but the cyclohexyl analogue **19h** was less well tolerated. The open chain analogue of **19g**, the 3-pentyl amino derivative **19i**, was of similar affinity. The tertiary amines **18f** and **19j–19l** provided active compounds (K_i's < 5 nM) so long as the steric requirement was not exceeded (**19m**, $K_i = 29$ nM). In an attempt to increase the solubility of the series, several basic amines were incorporated into the side chain (**19n–19p**), however, a substantial loss in affinity was the result of this modification.

It next became important to determine the functional activity of these compounds at the CRF-1 receptor. Compounds **16b** and **17a** were shown to inhibit sauvagine stimulated cAMP accumulation in AtT20 cells expressing the CRF-1 receptor with K_i's of 5 and 22 nM, respectively.[17] This data confirms that **16b** and **17a** are functional antagonists at the CRF-1 receptor.

In summary, a series of 3-aryl pyrazolo[4,3-*d*]pyrimidines has been described having affinity for the CRF-1 receptor. Small alkyl substituents were well tolerated at both the 1- and 2-position of the pyrazolopyrimidine. An ortho substituent on the 3-aryl group was essential for binding and a second substituent at the 4-position gave a further increase in binding affinity. Tertiary amines at the 7-position were optimal as long as the steric requirement was not exceeded. Seven of the compounds described had affinities comparable ($K_i < 3$ nM) to that reported for CP154526 **1**.[5b]

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