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Synthesis of 3-Phenylpyrazolo[4,3-*b*]pyridines Via a Convenient Synthesis of 4-Amino-3-arylpyrazoles and SAR of Corticotropin-Releasing Factor Receptor Type-1 Antagonists

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Abstract—3-Phenylpyrazolo[4,3-*b*]pyridines were synthesized via a cyclization of 4-amino-3-phenylpyrazoles **11**–**13** with ethyl acetoacetate. These compounds were found to be potent CRF_1 antagonists. The 2-alkylpyrazolo[4,3-*b*]pyridines were more polar but less active than the corresponding 1-alkyl-isomers.

Corticotropin releasing factor (CRF), a 41-amino acid peptide produced in hypothalamus and extracts its function by acting on the CRF receptors in the pituitary gland, is a major modulator of the body's responses to stress. Clinical evidences suggest that over-stimulation of CRF may result in several neuropsychiatric diseases including depression, anxiety and stress related disorders. Ever since the discovery of the first nonpeptide CRF_1 antagonist CP-154,526 (**1**) in 1996,[1] many different classes of small molecules were reported as potent CRF_1 antagonists.[2] In the efforts to search for small-molecule CRF_1 receptor antagonists for treatment of anxiety/depression and other related diseases, we[3] and others[4] have synthesized a series of pyrazolo[1,5-*a*]pyrimidines. Although these derivatives possess very potent receptor binding affinity and functional antagonism, like many other classes of CRF_1 antagonists, this series of compounds such as **2a** are very lipophilic and have poor water-solubility. CP-154,526 has extremely high volume of distribution in rats, resulting in a very long half-life,[5] that may imply potential accumulation in the body and thus may cause unexpected toxicity. We have been able to optimize the pyrazolo[1,5-*a*]pyrimidine series by incorporating a basic aminopyridine group represented by compound **2b** (NBI 30775).[6] We also reported recently[7] a series of 4-aminoquinolines such as **3**, which was designed and optimized based on the high basicity of this core (4-aminoquinoline has a pK_a value of 9.1).[8] Recently, a series of pyrazolo[4,3-*d*]pyrimidines **4** and **5** was reported as potent CRF_1 antagonists with low nanomolar binding affinity.[9] Again this series of derivatives may suffer from high lipophilicity and poor water solubility. Here, we report the synthesis and initial SAR study of a series of 3-phenylpyrazolo[4,3-*b*]pyridines as CRF_1 antagonists (Fig. 1).

The rationale for the design of this series of compounds is as follow. Since the benzene-fused 4-aminopyridine ($pK_a = 9.17$),[10] that is 4-aminoquinoline ($pK_a = 9.1$),[8] is much more basic than 4-aminoquinazoline ($pK_a = 5.73$),[11] the pyrazolo-fused 4-aminopyridine structure (pyrazolo[4,3-*b*]pyridine) would be more basic (estimated $pK_a \sim 7.8$)[12] than the corresponding ring-fused pyrimidines such as pyrrolo[2,3-*b*]pyrimidine (**1**), pyrazolo[1,5-*a*]pyrimidine (**2**) and pyrazolo[4,3-*d*]pyrimidine (**4**). However, the chemistry of this bicyclic heterocycle remains unexplored due to a lack of efficient synthetic routes to the key intermediate 4-amino-3-arylpyrazoles **11**–**13** (Schemes 1 and 2). There are few reports on the synthesis of 4-amino-3-arylpyrazoles in the literature.[13–15] For example, a five-step synthesis is reported via the cyclization of ethyl 3-benzoyl-3-nitroso-2-oxopropionate with hydrazine or methylhydrazine, followed by saponification and decarboxylation, but the

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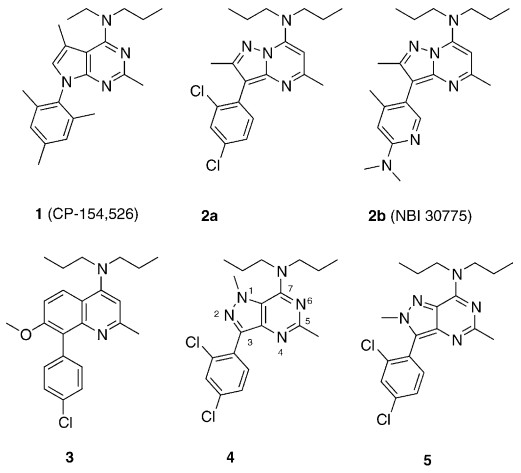
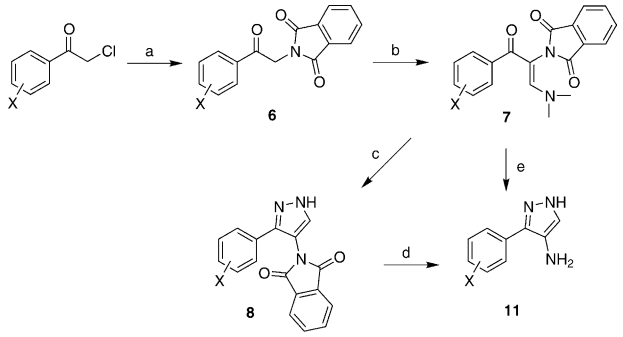
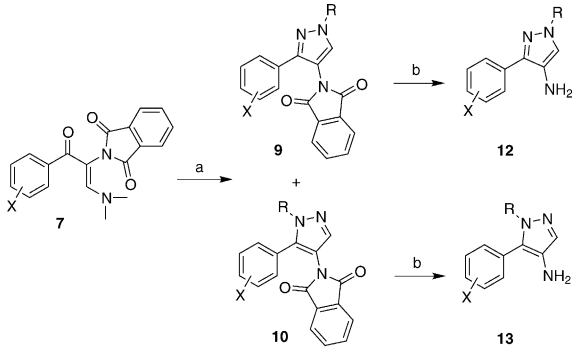


Figure 1. Some bicyclic small-molecule CRF_1 antagonists.



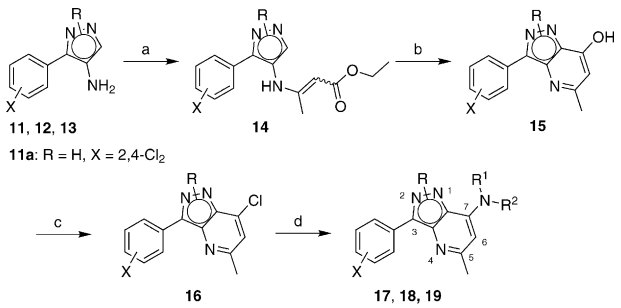
Scheme 1. Reagents and conditions: (a) sodium phthalimide, DMF; (b) DMFDMA, reflux; (c) $NH_2NH_2{\cdot}HCl$, aq EtOH, reflux; (d) NH_2NH_2, EtOH, reflux; (e) NH_2NH_2, EtOH, rt, 1 h, then reflux.



Scheme 2. Reagents and conditions: (a) $RNHNH_2{\cdot}H_2SO_4$, aq EtOH, reflux, then, separation; (b) NH_2NH_2, EtOH, reflux.

overall yield is low (less than 1%).[9] Here, we report the synthesis of a series of pyrazolo[4,3-*b*]pyridines via an efficient synthesis of 4-aminopyrazoles. The initial SAR of the 1-alkyl- and 2-alkyl-pyrazolo[4,3-*b*]pyridines as CRF_1 receptor antagonists will also be discussed.

Reaction of α-phthaloylaminoacetophenone derivatives **6**, obtained from α-bromo- or α-chloro-acetophenones and potassium phthalimide in DMF or ethanol,[16] with *N*,*N*-dimethylformamide dimethyl acetal (DMFDMA) proceeded smoothly at reflux (with or without DMF as solvent) to give the crude enamines **7** in quantitative yields after concentration in vacuo. The enamines **7** could be easily purified by ether trituration to precipitate the products (Scheme 1). Reaction of **7** with 2.5 equiv of hydrazine in ethanol at room temperature for 1 h followed by refluxing the mixture for 2 h gave 4-amino-3-phenylpyrazoles **11** in 70–85% yield (two steps from **6**). Similarly, cyclization of **7** with alkylhydrazine afforded 4-amino-3-arylpyrazoles **12** and **13**, in good yields, as a mixture of regio-isomers.



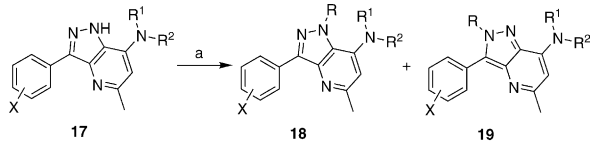
Scheme 3. Reagents and conditions: (a) ethyl acetoacetate, benzene; (b) Ph_2O, heat; (c) $POCl_3$, reflux; (d) R^1R^2NH, heat.

It is worth noting that 4-aminopyrazoles **11–13** are labile compounds and they turn to dark color when exposed to air. We discovered that the use of alkylhydrazine hydrochloride or sulfate salts avoided the formation of the des-phthaloyl compounds even with a large excess of hydrazine reagent or longer reaction time. Thus, cyclization of hydrazine hydrochloride with **7** in refluxing aqueous ethanol (EtOH–H_2O 10:1) for 2–16 h gave the 3-aryl-4-phthaloylaminopyrazoles **8** in about 85% yield, which was deprotected with hydrazine in refluxing ethanol to afford the 4-amino-3-arylpyrazoles **11** (Scheme 1). A similar method was used to prepared compounds **12** and **13**. Thus, cyclization of **7** with methylhydrazine sulfate gave a mixture of **9** and **10** in a ratio of about 2:1. These two regio-isomers were easily separated by chromatography on silica gel. Deprotection of the phthaloyl group of compound **9** or **10** proceeded in high yields (>90%) with two equivalents of hydrazine free base in refluxing ethanol for an hour (Scheme 2). The assignment of the two isomeric structures of **9/10**, and **12/13** are based on proton NMR analyses and confirmed by NOE NMR experiments.[17]

We applied the aminopyrazoles **11**, **12**, and **13** to synthesize pyrazolo[3,4-*b*]pyridines **17**, **18**, and **19**, which is outlined in Scheme 3 and illustrated as follows. 4-Amino-3-(2,4-dichlorophenyl)-1*H*-pyrazole (**11a**) was obtained from readily available α,2′,4′-trichloroacetophenone in 65% yield. Compound **11a** was heated for two h with ethyl acetoacetate (2 equiv), in the presence of catalytic amount of toluenesulfonic acid, in benzene with a Dean–Stark water trap to remove water. The crude enamine **14a** was used directly, after concentration in vacuo, for cyclization. Thus, **14a** was added into a hot phenyl ether solution at 240 °C for 10 min to give the pyrazolo[3,4-*b*]pyridone **15a** as a brown solid. Conversion of the pyridone **15a** to the corresponding chloropyrazolo[3,4-*b*]pyridine **16a** was accomplished by refluxing compound **15a** in $POCl_3$ for 2 h. The amine replacement reaction proceeded in an excess amount of

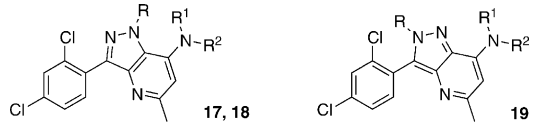
primary or secondary amine, promoted by toluenesulfonic acid at 160 °C in a sealed reacti-vial for 6 h, to give the desired pyrazolo[3,4-*b*]pyridine **17a**. Finally, alkylation of **17** with alkyl halide in the presence of sodium hydride in DMF gave two isomers **18** and **19**, which were separated by chromatography on silica gel (Scheme 4). Their isomeric structures were assigned based on proton NMR analyses as described before and confirmed by NOE NMR experiments. This synthetic route was not optimized but is very efficient for the rapid generation of **18** and **19** analogues from readily available α-chloro- or α-bromoacetophenones. Alternatively, compounds **18** and **19** were also prepared from the corresponding 1-alkyl-4-aminopyrazoles **12** and **13**, respectively, according to a similar procedure described above.

The desired compounds **17**, **18**, and **19** were assayed on the binding affinities towards the human CRF_1 receptor stably express on HEK273 cells,[18] and the results are summarized in Table 1 and Table 2. The 1*H*-pyrazolo[4,3-*b*]pyridines **17a–c** showed only moderate binding affinity (K_i 26–60 nM), and they were much less active than the corresponding pyrazolo[1,5-*a*]pyrimidine analogues such as **2a**. One explanation for the decrease in binding affinity could be that the polar nature of the NH functionality of **17a** may be harmful to receptor



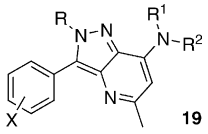
Scheme 4. Reagents and conditions: (a) RX, NaH, DMF, rt.

Table 1. Comparison of binding affinity between 1-alkyl and 2-alkylpyrazolo[4,3-*b*]pyridines



Compd	R	R^1NR^2	K_i (nM)
17a	H	Bu_2N	40
17b		*c*-$PrCH_2NPr$	26
17c		BuNEt	60
18a	Me	Bu_2N	0.62±0.35
18b		*c*-$PrCH_2NPr$	1.7±0.7
18c		BuNEt	2.0±0.2
18d		Pr_2N	3.3±1.5
18e	Et	Bu_2N	2.1±0.2
18f		*c*-$PrCH_2NPr$	1.7±0.7
18g		BuNEt	4.2±0.7
18h	Pr	Bu_2N	180
18i	*i*-Pr	Bu_2N	16.1±1.0
19a	Me	Bu_2N	32.5±5.7
19b		*c*-$PrCH_2NPr$	11.0±4.8
19c		BuNEt	35.0±5.0
19d		Pr_2N	4.4±2.0
19e	Et	Bu_2N	32.8±4.0
19f		*c*-$PrCH_2NPr$	46
19g		BuNEt	32
19h	Pr	Bu_2N	64
19i	*i*-Pr	Bu_2N	37

Table 2. SAR of the substituents on the 3-phenyl group



Compd	R	X	R^1NR^2	K_i (nM)
19d	Me	2,4-Cl_2	Pr_2N	4.4±2.0
19j		2,4-Cl_2	$BuNCH_2CH_2PhOMe$-4	80
19k		4-Cl	Pr_2N	81.5±25.5
19l		H	Pr_2N	230
19m		2,4,6-Me_3	Bu_2N	27.5±7.5
19n		2-Cl-4-MeO	Bu_2N	70
19o	Et	2-Cl-4-Me	*c*-$PrCH_2NPr$	32.0±4.0
19p		2-Cl-4-Me	Bu_2N	59

binding. The missing methyl group corresponding to the 2-position of pyrazolo[1,5-*a*]pyrimidine **2a** (K_i = 1 nM) could also account for the 10-fold loss in activity. The 2-alkyl-pyrazolo[4,3-*b*]pyridines **19**, especially the 2-methyl analogue **19d** which had the same geometry as **2a**, were much more polar than the corresponding 1-alkyl-isomers **18** and pyrazolo[1,5-*a*]pyrimidine **2** on silica gel. Thus, methylation (**19b**) at the 2-position of **17b** (K_i = 26 nM) only slightly increased binding affinity, whereas ethylation (**19f**) hardly changed the binding (**19f** vs **17b**). Alkylation of **17c** with propyl or isopropyl group (**19h** and **19i**) also had minimal impact on the binding affinity. The best compound from this subseries, the dipropylamino derivative **19d,** had a K_i value of 4.4 nM, which is comparable to the corresponding pyrazolo[4,3-*d*]pyrimidine analogue **5** (K_i = 3 nM), but less active than the pyrazolo[1,5-*a*]pyrimidine **2a** (K_i = 1.3 nM).

The 1-alkyl pyrrazolo[4,3-*b*]pyridine derivatives **18**, although they were less polar than their corresponding isomers **19**, were still much more polar on silica gel than the pyrazolo[1,5-*a*]pyrimidines **2** and exhibited increased binding affinity. Thus, **18b** and **18c** were 15- and 30-fold, respectively, more active than **17b** and **17c**; and **18a** with a K_i value of 0.62 nM was 65-fold better than **17a**. The dipropylamino derivative **18d** (K_i = 1.8 nM) had comparable activity with the corresponding 1-methylpyrazolo[4,3-*d*]pyrimidine **4** (K_i = 1 nM) and pyrazolo[1,5-*a*]pyrimidine **2a** (K_i = 1.3 nM). The ethyl derivatives **18e**, **18f** and **18g** were slightly less active (< 3-fold) than the corresponding methyl analogues **18a–c**. Interestingly, the bulky isopropyl compound **18i** (K_i = 16 nM) was only 25-fold less active than the optimal methyl analogue **18a**, but the longer propyl derivative **18h** (K_i = 180 nM) lost almost 300-fold activity (Table 1).

The effects of the substitution on the 3-phenyl group of the 2-methylpyrazolo[4,3-*b*]pyridines **19** were also investigated and the results are summarized in Table 2. Similar to the pyrazolo[1,5-*a*]pyrimidine series, the 2,4-dichlorophenyl is the most favored group at this position. In the current series, loss of the 4-chlorine of the 2,4-dichlorophenyl group of **19d** resulted in almost 20-fold decrease in binding affinity (**19k**, K_i = 81 nM).

The un-substituted phenyl analogue **19l** had a K_i value of 230 nM, much less active than **19d**. The 2,4,6-trimethylphenyl derivative **19m** ($K_i = 28$ nM) was similarly active as **19a**, but the 2-chloro-4-methoxyphenyl was not able to mimic the 2,4-chlorophenyl group and the resulting compound **19n** ($K_i = 70$ nM) was 3-fold less active than **19a**. Other than 2,4,6-trimethylphenyl ring, the 2-chloro-4-methylphenyl group was a reasonably good replacement of the 2,4-dichlorophenyl ring (**19o** and **19b**, $K_i = 22$ and 11 nM, respectively).

In general, the 1-alkyl-pyrazolo[4,3-*b*]pyridines **18** were much more active than the corresponding 2-alkyl-isomers **19**, and the best compound from isomers **19** had a K_i value of 4.4 nM (**19d**) on binding to the CRF_1 receptor. The high polarity, which may contribute to the lower binding affinity, is certainly an advantage for designing CRF_1 antagonists with desirable pharmacokinetic profile and eventually in vivo efficacy.

Selective compounds from this series were further tested for functional antagonism on the CRF_1 receptor. Thus, in a CRF-stimulated c-AMP production assay, compounds **18a**, **18b**, and **18c** inhibited c-AMP accumulation with low nanomolar IC_{50} values (15.8, 5.0, and 16 nM, respectively), while compounds **19d** was less active ($IC_{50} = 365$ nM). All compounds were examined for activity in a CRF_2-receptor binding assay as previously described[19] and none of the listed compounds showed a does-dependent inhibition of binding and none had a greater than 40% inhibition at a concentration of 10 μM. These data demonstrate these compounds are selective CRF_1 antagonists.

In conclusion, condensation of a phthaloylaminoacetophenone derivatives **6** with *N*,*N*-dimethylformamide dimethyl acetal gave the enamines **7**, which was cyclized with hydrazine to give the pivotal intermediate 4-amino-3-arylpyrazoles **10**, **11**, and **12**. Application of this key intermediate towards the synthesis of pyrazolo[4,3-*b*]pyridines **17**–**19** resulted in the discovery of a class of very potent and relatively more polar (most likely more basic) CRF_1 receptor antagonists. Isomers **19**, which were more polar on silica gel chromatography, were less active than the corresponding **18** subseries. Isomers **18** possessed a basic core structure (calculated pK_a value for **18d** was 10.4, ACD) and displayed excellent potency. The optimization of **18** will be reported in the following paper.

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