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Synthesis of 1-Methyl-3-phenylpyrazolo[4,3-*b*]pyridines Via a Methylation of 4-Phthalimino-3-phenylpyrazoles and Optimization toward Highly Potent Corticotropin-Releasing Factor Type-1 Antagonists

Charles Q. Huang, Keith Wilcoxen, James R. McCarthy, Mustaph Haddach, Dimitri Grigoriadis and Chen Chen*

Department of Medicinal Chemistry and Department of Pharmacology, Neurocrine Biosciences, Inc., 10555 Science Center Drive, San Diego, CA 92121, USA

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Abstract—1-Methyl-3-phenylpyrazolo[4,3-*b*]pyridines were synthesized via a cyclization reaction of 1-methyl-4-amino-3-phenylpyrazoles **8** with ethyl acetoacetate. Optimization of this series of compounds resulted in CRF_1 antagonists with subnanomolar binding affinity. Compounds bearing a polar group such as methoxy or hydroxy were also found to be very active.

In the preceding paper[1] we described the synthesis and initial SAR studies of a series of 3-phenylpyrazolo[4,3-*b*]pyridines (**1**, **2**, and **3**, Fig. 1) as corticotrophin-releasing factor type-1 antagonists, on the assumption that this series of compounds may be more polar than 3-phenylpyrazolo[1,5-*a*]pyrimidines which we developed earlier and suffered, like many other known CRF_1 antagonists, high lipophilicity. Because the pyrazolo-fused 4-aminopyridine is similar to 4-aminoquinoline ($pK_a = 9.08$),[2] it may offer a core with high basicity (estimated $pK_a > 7.8$),[3] which is desirable for compounds with better pharmacokinetic profile. Although isomers **3** are more polar than **2**, compounds from this subseries were somewhat less active than **2**. Because of the lack of highly active compounds **3** from our initial SAR, we turn our attention to the 1-alkylpyrazolo[4,3-*b*]pyridines **2** in our efforts to discover higher hydrophilic CRF_1 antagonists. Here we report the detailed SAR and optimization of physicochemical properties on this subseries of compounds.

The synthesis of 1-alkyl-3-phenylpyrazolo[4,3-*b*]pyridines described in Scheme 1 is based on the new synthetic method for 4-phthalimido-3-phenylpyrazoles **5**.[4] Thus, cyclization of enamines **4** with methylhydrazine sulfate in a mixture of solvents ($EtOH/H_2O = 10:1$) at reflux gave 1-methyl-3-phenyl-4-phthalimidopyrazoles **7** and 1-methyl-5-phenyl-4-phthalimidopyrazoles **6** as mixtures with a ratio ranging from 3:1 to 3:2. The two isomers **6** and **7** were easily separated by chromatography on silica gel. Alternatively, 4-phthalimidopyrazolo[4,3-*b*]pyrimidines **5**, which were obtained from the cyclization of enamines **4** with hydrazine hydrochloride in refluxing aqueous ethanol ($EtOH–H_2O$, 10:1) in 85–95% yields, were alkylated with alkyl halide in dry DMF promoted by sodium hydride to give the corresponding alkyl analogues **7**. This regio-selective procedure avoided the formation of **6** as a by-product from the alkylhydrazine cyclization. Compounds **7** were then deprotected with two equivalents of hydrazine in refluxing ethanol to give the 4-aminopyrazoles **8** which were converted to the desired 1-alkyl-3-phenyl-7-chloropyrazolo[4,3-*b*]pyridines **9** as described in Scheme 2. Compounds **9** were subjected to a nucleophilic replacement with various alkyl amines to give the desired products **11**. By using the above reaction, we also introduced amines bearing a hetero-atoms. Thus reactions of **9** with primary amines such as 3-methoxypropylamine under the similar conditions gave compounds **10**, which were alkylated with alkyl halide in DMF to give the tertiary amine products **12**.

*Corresponding author. Tel.: +1-858-658-7600; fax: +1-858-658-7601; e-mail: cchen@neurocrine.com

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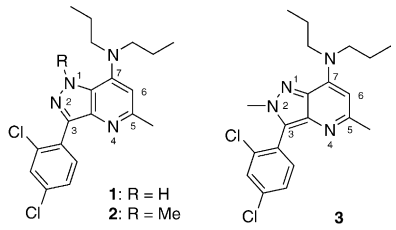
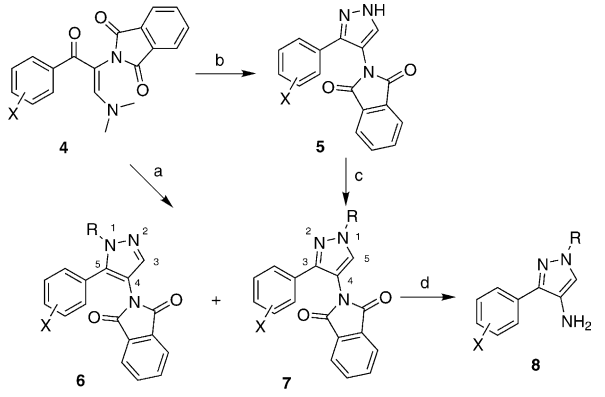
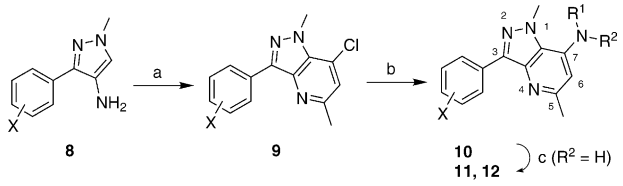


Figure 1. 1-Methyl- and 2-methyl-3-phenyl-7-aminopyrazolo[4,3-*b*]-pyridines **2** and **3**.



Scheme 1. Reagents and conditions: (a) $RNHNH_2{\cdot}H_2SO_4$, EtOH–H_2O, reflux; (b) $NH_2NH_2{\cdot}HCl$, EtOH–H_2O, reflux; (c) RX, NaH, DMF, rt; (d) NH_2NH_2, EtOH, reflux.



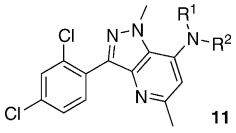
Scheme 2. Reagents and conditions: (a) (i) ethyl acetoacetate, benzene, reflux; (ii) Ph_2O, 260 °C; (iii) $POCl_3$, reflux; (b) R^1R^2NH, TsOH, 140 °C; (c) R^2X, NaH, DMF, rt.

The binding affinity of the compounds prepared were determined as CRF_1 antagonists by using a binding assay reported by Grigoriadis, and using [^{125}I]-sauvagine from CRF_1 receptor expressed in HEK293 cells.[5] The effects of alkyl substituents at the 7-position of the 1-methyl-3-(2,4-dichlorophenyl)-5-methyl-7-aminopyrazolo[4,3-b]pyridines on the binding to the CRF_1 receptor were summarized in Table 1 and 2.

The mono-butylamino derivative **11a** ($K_i = 170$ nM) was found to be only moderately active, but *N*-methyl-*N*-propylamino analogue (**11b**, $K_i = 24$ nM) was much improved. Increasing the chain length from ethyl to hexyl resulted in a gain in binding affinity (0.62–4.4 nM), and the dibutylamino (**11l**) was optimal with a K_i value of 0.62 nM. Interestingly, this result was slightly different from the pyrazolo[1,5-*a*]pyrimidines, in which dipropylamine or *N*-butyl-*N*-ethylamine is the optimal side chain.[6] The requirement of a more lipophilic group for optimal binding indicates that the more basic pyrazolo[1,5-*a*]pyrimidine core needs to be off-set with a more hydrophobic group. This hypothesis was further supported by compounds **11m–o** (K_i's = 0.9-1.3 nM) that bear a highly lipophilic group such as *N*-butyl-*N*-pentylamine. Only the branched 2-pentyl derivative **11p** was less active ($K_i = 7$ nM).

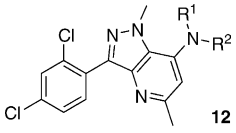
Although compounds from this series had high binding affinity, they were still very lipophilic (calculated logD

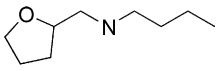
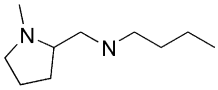
Table 1. Effects of some small lipophilic alkyl group



Compd	R^1NR^2	K_i (nM)
11a	*n*-BuNH	170
11b	*n*-PrNMe	24±9
11c	*n*-PrNEt	4.1±2.0
11d	*c*-$PrCH_2NEt$	4.4±0.8
11e	$(n\text{-Pr})_2N$	3.3±1.5
11f	*c*-$PrCH_2NPr$-*n*	1.7±0.7
11g	*c*-$PrCH_2NBu$-*n*	1.3±0.6
11h	*c*-$PrCH_2NBu$-*i*	3.7±1.2
11i	*c*-$PrCH_2N(C_5H_{11})$-*n*	1.5±0.5
11j	*c*-$PrCH_2N(C_6H_{13})$-*n*	1.0±0.1
11k	*n*-BuNEt	2.0±0.2
11l	*n*-Bu_2N	**0.62±0.35**
11m	*n*-(C_5H_{11})NEt	1.3±0.7
11n	*n*-(C_5H_{11})NPr-*n*	1.2±1.2
11o	*n*-(C_5H_{11})NBu-*n*	0.9±0.1
11p	$(Et)_2CH$NBu	7.0±3

Table 2. Effects of seven-side chain with a polar group

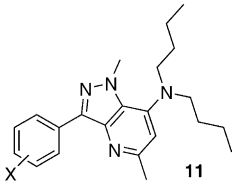


Compd	R^1NR^2	K_i (nM)
12a	$MeOCH_2CH_2NMe$	32±11
12b	$MeOCH_2CH_2NEt$	**3.2±0.8**
12c	$MeOCH_2CH_2NPr$-*n*	**5.1±0.8**
12d	$MeOCH_2CH_2NPr$-*i*	9.7±3.0
12e	$MeOCH_2CH_2NBu$-*n*	10.4±0.5
12f	$MeOCH_2CH_2NBu$-*i*	10.5±0.5
12g	$MeOCH_2CH_2N(C_5H_{11})$-*n*	6.7±0.6
12h	$MeOCH_2CH_2N(C_6H_{13})$-*n*	4.0±1.2
12i	$(MeOCH_2CH_2)_2N$	8.2±0.7
12j	$MeOCH_2CH_2CH_2NEt$	12.5±1.1
12k	$MeOCH_2CH_2CH_2NPr$-*n*	9.8±3.2
12l	$MeOCH_2CH_2CH_2NBu$-*n*	18±4
12m	$MeSCH_2CH_2NBu$-*n*	1.4±0.2
12n		11.2±2.8
12o		380
12p	4-$PyCH_2NBu$-n	68±11
12q	4-$PyCH_2CH_2NBu$-*n*	34±13
12r	2-$PyCH_2CH_2NBu$-*n*	13±2
12s	4-$HOPhCH_2NH$	>10,000
12t	4-$HOPhCH_2NPr$-*n*	13±8
12u	$HOCH_2CH_2NPr$-*n*	**4.4±0.9**
12v	$HOCH_2CH(n\text{-Pr})NH$	62

value for **11e** was 4.0, ACD software). In order to reduce the lipophilicity of this series of compounds, a reduction of logP/logD at least one log unit is desirable. This means an introduction of a hydroxyl or at least a methoxy group. The results from compounds bearing a hetero-atom are summarized in Table 2. The *N*-methyl-*N*-methoxyethylamino compound (**12a**, $K_i = 32$ nM) had comparable affinity to the *N*-methyl-*N*-propyamine **11b** ($K_i = 24$ nM); similarly the *N*-ethyl-*N*-methoxyethylamine **12b** had a K_i value of 3.2 nM, close to the *N*-ethyl-*N*-propyl analogue (**11c**, $K_i = 4.1$ nM). Unfortunately, increasing the length of this alkyl chain did not further increase activity. Instead, a decrease in binding affinity for the butyl or the isobutyl group (**12e** and **12f** $K_i = 10.4$ and 10.5 nM, respectively) was observed. Substitution with the polar bis(methoxyethyl)amino group resulted in compound **12i** ($K_i = 8.2$ nM) with less activity. *N*-Methoxypropyl-*N*-alkylamines (**12j–l**) were 2- to 4-fold less active (10–18 nM) than the 2-methoxyethylamino analogues, and *N*-(tetrahydrofuranmethyl)-*N*-butylamine (**12n**, $K_i = 11$ nM) was also less active. As we expected, **12o**, which bears a basic pyrrolidinemethylamino side chain, was much less active in binding affinity ($K_i = 380$ nM). Attempt to incorporate a less basic pyridine moiety into the side chain also was not very successful, and compounds **12p–r** all had K_i values of >10 nM. Although the *N*-methylthioethyl-*N*-butylamine **12m** was very potent ($K_i = 1.4$ nM), this compound might not be polar enough. However, the *N*-(hydroxyethyl)-*N*-propylamine **12u** had a K_i value of 4.4 nM, while the 1-hydroxy-2-pentylamine analogue (**12v**, $K_i = 62$ nM) was not very active. Another interesting compound is **12t** that had an acidic phenol moiety and still had a K_i value of 13 nM, much better than its despropyl analogue **12s** (inactive).

We also examined the possible alternatives for the 3-(2,4-dichlorophenyl) group (Table 3). While the 2-chloro-4-methoxy- and 2,4,6-trimethylphenyl replacements (**11q** and **11t**) resulted in compounds with about 8-fold less active; 2-chloro-4-methyl, especially 2-chloro-4,6-dimethylphenyl analogues (**11r** and **11s**) had comparable binding affinity. On the other hand, the 4-chlorophenyl compound was significantly less active (**11u**, $K_i = 160$ nM) in binding affinity.

Table 3. Substitution effects on the 3-phenyl group



11

Compd	X	K_i (nM)
11l	2,4-Cl_2	0.62±0.35
11q	2-Cl-4-MeO	4.8±0.9
11r	2-Cl-4-Me	2.8±0.9
11s	2-Cl-4,6-Me_2	2.1±0.7
11t	2,4,6-Me_3	5.1±0.2
11u	4-Cl	340

Table 4. CRF functional antagonistic activity[a] of selected compounds

Compd	K_i (nM)	IC_{50} (nM)
11f	1.7±0.7	16
11g	1.3±0.6	3.0
11k	2.0±0.2	16
11l	**0.62±0.35**	5.0
12b	**3.2±0.8**	15
12c	**5.1±0.8**	36
12i	8.2±0.7	38
12u	**4.4±0.9**	18

[a]Inhibition of CRF-stimulated cAMP production.

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 **11f**, **11g**, **11k**, **11l**, **12b**, and **12u** displayed low nanomolar IC_{50} values while compounds **12c** and **12i** were slightly less active (Table 4). These data seems to be in agreement with the binding affinity which measures the interaction between the small molecule ligands and the CRF_1 receptor. All compounds were examined for activity in a CRF_2-receptor binding assay as previously described[7] 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-selective antagonists.

In conclusion, we developed a regio-selective synthesis of 1-alkyl-3-phenyl-7-aminopyrazolo[4,3-b]pyridines. These compounds were tested as CRF_1 antagonists and many of them were highly potent. This core structure, comparing with 4-aminoquinoline ($pK_a = 9.1$), should be more basic than the previous pyrazolo[1,5-*a*]pyrimidine, pyrazolo[4,3-*d*]pyrimidine and pyrrolo[2,3-*d*]pyrimidine, therefore, more hydrophilic. Compound **2l** with a dibutylamino side chain had a K_i value of 0.62 nM, but was still quite lipophilic (calculated values for pK_a 10.4, logP > 6 and logD > 4, ACD software). However, the hydroxy and methoxy analogues **12b** and **12u**, despite of slightly decreased binding affinity (K_i ~4 nM) and functional antagonistic activity (cAMP IC_{50} ~15 nM), possess desirable physicochemical properties (calculated values for **12b**: logP 4.3, logD 2.7; for **12u**: logP 4.2, logD 1.8, ACD software), which are required for good PK profiles and in vivo efficacy. Results for further studies of these compounds will be reported elsewhere in due course.

References and Notes

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