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Design and synthesis of 3-(2-pyridyl)pyrazolo[1,5-*a*]pyrimidines as potent CRF_1 receptor antagonists

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Abstract—A series of 3-(2-pyridyl)pyrazolo[1,5-*a*]pyrimidines was designed and synthesized as antagonists for the corticotrophin-releasing factor-1 (CRF_1) receptor. Several compounds such as **20c** ($K_i = 10$ nM) exhibited good binding affinities at the CRF_1 receptor. In addition, **20c** had adequate solubility in water.

Corticotropin-releasing factor (CRF), a neuropeptide isolated from mammalian brain,[1] has been implicated as the mediator for the integrated physiological response to stress.[2] CRF acts via two receptor subtypes, CRF_1, and CRF_2, which belong to the Class B G-protein-coupled receptor superfamily.[3] The binding of CRF to CRF_1 receptor in the hypothalamus is responsible for the increased release of ACTH and other peptides.[4] Inhibition of CRF_1 receptors has been shown to reduce ACTH levels in plasma of stressed animals.[5] Many small molecules from different chemical classes have been identified as potent CRF_1 receptor antagonists (**1**–**9**, Fig. 1).[6] Although several of them have exhibited brain penetration and oral activity in anxiety and depression animal models, many earlier reported CRF_1 antagonist compounds, such as **1**–**6**,[7] suffer from high lipophilicity and poor

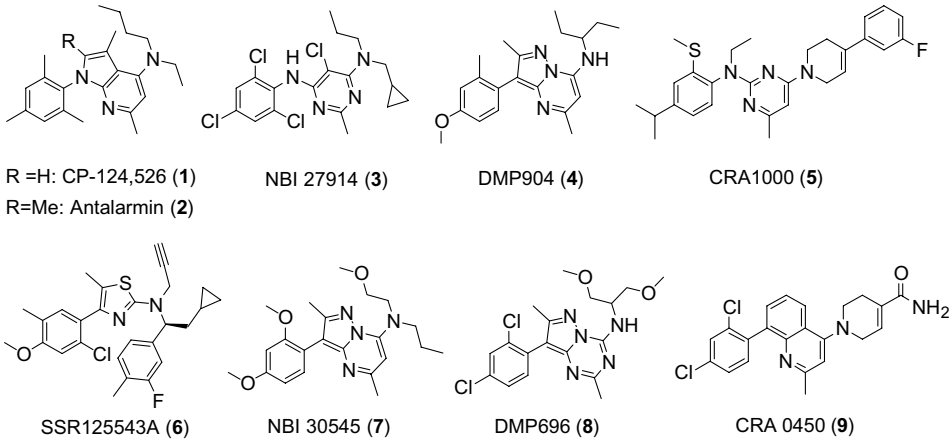

Figure 1. Some small molecule CRF_1 receptor antagonists.

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Table 1. Calculated log *P*

Compound	*C* log *P*[a]
1	8.43
2	8.89
3	9.71
4	4.80
5	5.98
6	7.75
7	2.65
8	3.32
9	3.89

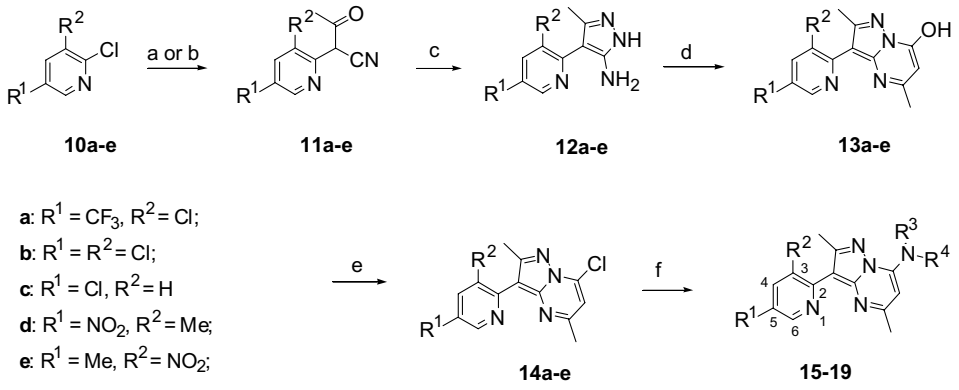
[a] ACD/LogP software.

water solubility.[8] These undesirable properties make it more difficult to develop these compounds into pharmaceutical agents. For example, CP-124,526 exhibits a very long half-life associated with high volume distribution in rats.[9] Recent efforts on discovery of more hydrophilic CRF_1 antagonists have generated compounds such as **7**–**9** with more suitable physicochemical properties.[10] By using ACD/LogP software,[11] we calculated *C* log *P* values of **1**–**9** (Table 1) to assess the relative lipophilicities of these compounds. *C* log *P* values of compounds **1**–**3**, **5**, and **6** are $\geqslant 6$, much higher than that of compounds **7**–**9**, which have *C* log *P* values of <4. In our efforts to identify potent CRF_1 antagonists with desirable physicochemical properties, we synthesized NBI 30545 (**7**)[10a] by incorporation of polar alkoxy groups into the 3-phenylpyrazolo[1,5-*a*]pyrimidine molecules.[12] The calculated log *P* of **7** was 3.18, a very desirable value for a CNS agent.[13] As an alternative, replacement of the lipophilic 3-phenyl group of the 3-phenylpyrazolo[1,5-*a*]pyrimidine with a weakly basic and hydrophilic pyridine moiety should also decrease the lipophilicity of this class of compounds. In addition, alternation of substituents on the pyridine ring will change the basicity and hydrophilicity. Here we report the synthesis and structure–activity relationships of the 3-(2-pyridyl)pyrazolo[1,5-*a*]pyrimidines as CRF_1 receptor antagonists.

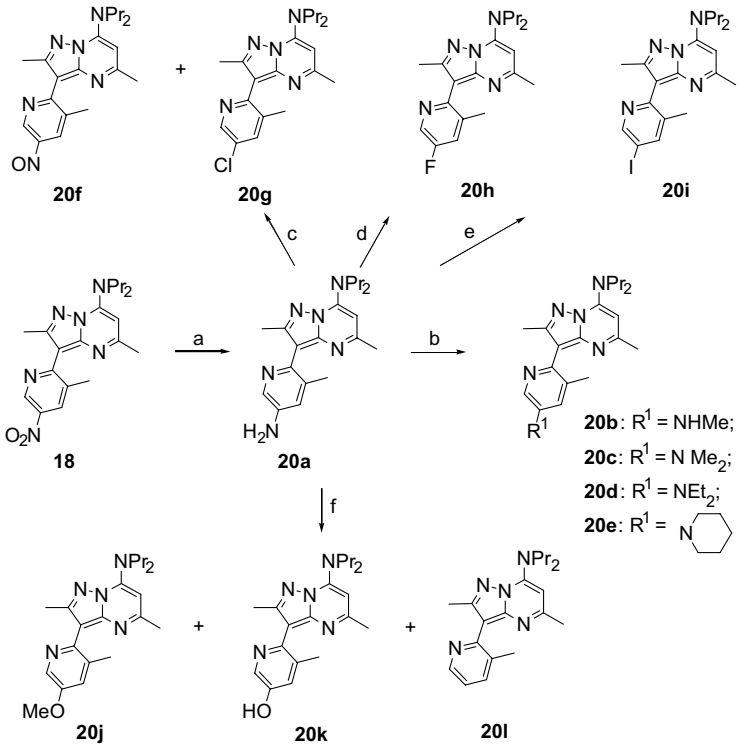
A general synthetic route[14] for the preparation of 3-(2-pyridyl)-7-alkylaminopyrazolo[1,5-*a*]pyrimidines **15**–**19** was developed as outlined in Scheme 1. Reaction of 2,3-dichloro-5-trifluoromethylpyridine **10a** with *tert*-butyl cyanoacetate in the presence of sodium hydride, followed by decarboxylation under acidic conditions, afforded the substituted 3-chloro-5-trifluoromethylpyridin-2-ylacetonitrile (55% yield for two steps), which was then acetylated with ethyl acetate in the presence of sodium hydride to afford the ketone **11a** in quantitative yield. Compounds **11b** and **11c** were synthesized in a similar manner (13% and 40%, respectively, from **10b** and **10c**).[15] Alternatively, reaction of 2-chloropyridine **10d** or **10e** with acetoacetonitrile sodium salt, obtained from 2-methylisoxazole and sodium ethoxide,[16] afforded the 2-(2-pyridyl)acetoacetonitrile **11d** in 61% yield, or **11e** in 19% yield. Cyclizations of **11a**–**e** with hydrazine hydrobromide in a mixture of ethanol–water (10:1) at reflux gave the corresponding 3-amino-4-(2-pyridyl)-5-methylpyrazoles **12a**–**e** in good yields (40–65%), which were subjected to a second cyclization with ethyl acetoacetate in refluxing dioxane to give the pyrazolo[1,5-*a*]pyrimidinones **13a**–**e** as white solids (18–52% yield). Conversion of **13** to the corresponding 7-chloropyrazolo[1,5-*a*]pyrimidines **14a**–**e** was accomplished in 80–91% yields with $POCl_3$ in refluxing acetonitrile. Finally, the target compounds **15**–**19** were obtained by the reaction of chloropyrimidines **14** with an alkylamine in refluxing acetonitrile in excellent yields (80–90%).

Compounds **18** and **19** were further modified as shown in Schemes 2 and 3, respectively. Palladium-catalyzed hydrogenation of **18** gave the corresponding amino analog **20a**, which was further elaborated to various derivatives. Alkylation of **20a** with a standard reductive amination protocol gave 5-methylamino (**20b**), dimethylamino (**20c**), diethylamino (**20d**) and piperidin-1-yl compound (**20e**), respectively, with the corresponding aldehyde. **20a** was also subjected to a Sandmeyer chlorination protocol to afford the chlorinated compound **20g**, a nitroso by-product **20f** was isolated under these conditions. 5-Fluoro- (**20h**) and 5-iodopyridine (**20i**) were synthesized in a similar manner. Diazotization of compound **20a**, followed by methanol treatment, afforded the desired 5-methoxy analog (**20j**), along with a 5-hydroxypyridyl compound **20k** and a reduced by-product **20l** from this reaction.

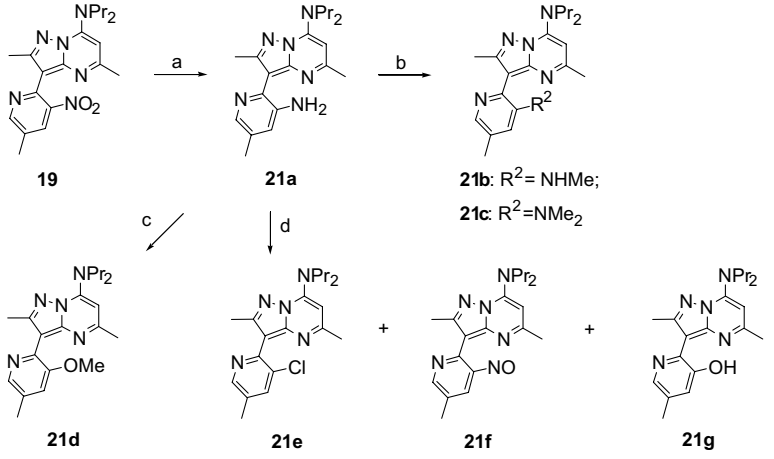
Similarly, 3-nitropyridine **19** was reduced to give the corresponding 3-amino analog **21a**, which was further



Scheme 1. Synthesis of the 3-(2-pyridyl)pyrazolo[1,5-*a*]pyridimidines: (a) MeC(ONa) = CHCN/DMSO; (b) (i) $NCCH_2COOBu$-*t*/*t*-BuOK/THF, (ii) TFA, (iii) NaH/EtOAc/THF; (c) NH_2NH_2HBr/H_2O/EtOH; (d) ethyl acetoacetate/dioxane/reflux; (e) $POCl_3$/reflux; (f) R^3R^4NH/acetonitrile/reflux.



Scheme 2. Chemical modification of the 5-position of the pyridine: (a) H_2/Pd–C/EtOH; (b) RCHO/$NaCNBH_3$/AcOH; (c) $NaNO_2$/HCl/CuCl; (d) $NaNO_2$/HBF_4; (e) $NaNO_2$/HCl/CuI; (f) $NaNO_2$/HCl/MeOH.



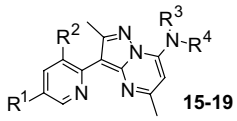
Scheme 3. Chemical modification of the 3-position of the pyridine: (a) H_2/Pd–C/EtOH; (b) aq CH_2O/$NaCNBH_3$/AcOH; (c) $NaNO_2$/HCl/MeOH; (d) $NaNO_2$/HCl/CuCl.

modified as showed in Scheme 3. Methylation of **21a** with a standard reductive amination protocol gave the 3-methylamino (**21b**) and 3-dimethylamino analog (**21c**). Compound **21a** was converted to the corresponding 3-methoxy analog **21d** by diazotization, followed by methanol treatment. Sandmeyer chlorination of **21a** provided the 3-chloro analog **21e**, 3-nitroso (**21f**) and 3-hydroxy compound (**21g**) were obtained as two by-products.

These compounds were tested for their binding affinities at the cloned human CRF_1 receptor in a competition binding assay as described,[17] and the K_i values were determined from concentration–response curves using concentrations ranging from 1 nM to 10 μM. The structure–activity relationships of these compounds are summarized in Tables 2 and 3. Selected compounds were also measured for their abilities to inhibit CRF-stimulated c-AMP production in cells expressing the human CRF_1 receptor to assess their functional antagonism in a manner similar to that previously reported.[18] Antalarmin (**2**), which was utilized as a positive control, demonstrated potent binding and function activities ($K_i = 2.6$ nM and c-AMP $IC_{50} = 16$ nM).

Results from a quick survey at the 7-position of the 3-(2-pyridyl)pyrazolo[1,5-*a*]pyrimidine on **15** and **16** using the preferred dialkylamine side-chains of the

Table 2. SAR of the 7-amino group of 3-(2-pyridyl)pyrazolopyrimidines (**15**–**19**)

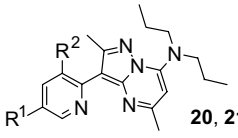


15-19

Compound	R^1	R^2	R^3NR^4	K_i (nM)[a]	$C\log P$[b]
15a	CF_3	Cl	NPr_2	1.2	5.91
15b	CF_3	Cl	$PrNCH_2Pr$-c	0.8	5.75
15c	CF_3	Cl	PrNBu-n	1.2	6.44
15d	CF_3	Cl	$PrNCH_2Ph$	1.7	6.74
15e	CF_3	Cl	$N(Bu\text{-}n)_2$	1.1	6.98
15f	CF_3	Cl	$N(CH_2CH_2OMe)_2$	12	3.62
16a	Cl	Cl	NPr_2	3.2	5.18
16b	Cl	Cl	$PrNCH_2Pr$-c	3.9	5.01
16c	Cl	Cl	$PrNCH_2CH_2OMe$	3.2	4.03
16d	Cl	Cl	$N(CH_2CH_2OMe)_2$	78	2.88
17	Cl	H	NPr_2	88	4.44
18	NO_2	Me	NPr_2	9.1	3.81
19	Me	NO_2	NPr_2	14	3.74

[a] K_i values were average of at least two independent measurements.
[b] ACD/LogP software.

Table 3. SAR of modified pyridine analogs (**20** and **21**)



20, 21

Compound	R^1	R^2	K_i (nM)[a]	$C\log P$[b]
20a	NH_2	Me	85	3.41
20b	NHMe	Me	40	3.29
20c	NMe_2	Me	10	4.76
20d	NEt_2	Me	13	5.82
20e	$N(CH_2)_5$-c	Me	17	5.27
20f	NO	Me	100	4.90
20g	Cl	Me	6.7	3.97
20h	F	Me	19	4.00
20i	I	Me	6.6	5.13
20j	OMe	Me	10	4.33
20k	OH	Me	530	3.95
20l	H	Me	110	4.08
21a	Me	NH_2	40	3.32
21b	Me	NHMe	13	2.98
21c	Me	NMe_2	23	4.76
21d	Me	OMe	25	4.81
21e	Me	Cl	6.2	5.08
21f	Me	NO	>10,000	4.18
21g	Me	OH	>10,000	3.69

[a] K_i values were average of at least two independent measurements.
[b] ACD/LogP software.

corresponding 3-phenylpyrazolo[1,5-*a*]pyrimidines suggested the dipropylamino moiety was one of the best groups at this position for high CRF_1 receptor binding affinity (Table 2). The *N*-propyl-*N*-2-methoxyethylamine derivative **16c** ($K_i = 3.2$ nM) possessed similar binding affinity as the corresponding dipropylamine **16a** ($K_i = 3.2$ nM). However, the calculated $\log P$ value of **16c** was a log unit lower than that of **16a**. These results certainly offers a possible alternative to design analogs with lower lipophilicity from this series.

The structure–activity relationship study of the pyridine ring was performed on the 7-dipropylaminopyrazolo[1,5-*a*]pyrimidine. The 3,5-dichloropyridin-2-yl compound **16a** ($K_i = 3.2$ nM) was 27-fold better in binding affinity than the 5-chloropyridin-2-yl analog **17** ($K_i = 88$ nM), and this again implies the importance of the orthogonal relationship between the pyridyl ring and the bicyclic core for high CRF_1 receptor binding.[7b,19] Since the nitrogen atom of a pyridine is smaller than a CH moiety of a phenyl group, an '*ortho*'-substituted pyridine is expected to be required for it to achieve this orthogonal conformation. The binding affinity was further improved when a more lipophilic trifluoromethyl group was incorporated at the 5-position of the pyridine (**15a**, $K_i = 1.2$ nM).

Further SAR studies on the 3- and 5-substituted-pyridyl compounds are summarized in Table 3. The 3-methyl-5-nitropyridin-2-yl compound **18** exhibited good binding to the receptor ($K_i = 9.1$ nM), while the more hydrophilic amino-analog **20a** ($K_i = 85$ nM) was almost 10-times lower in binding affinity. Compound **20a** could be rescued by methylation (**20c**, $K_i = 10$ nM) or ethylation (**20d**, $K_i = 13$ nM) of the NH_2 moiety. A group as large as a piperidine was tolerated at this position (**20e**, $K_i = 17$ nM), although **20c** was the preferred compound since its lipophilicity was lower than the other two (the $C\log P$ value was 4.76 for **20c**). Among the halogenated pyridine analogs (**20g**–**i**), the two more lipophilic 5-chloro and 5-iodo-derivatives **20g** and **20i** exhibited better binding than the 5-fluoro analog **20h**. The 5-methoxypyridin-2-yl analog **20j** also possessed good binding affinity ($K_i = 10$ nM) and desirable hydrophilicity ($C\log P = 4.33$). However, the 5-hydroxy pyridine (**20k**, $K_i = 530$ nM) had lower binding affinity than the unsubstituted pyridine **20l** ($K_i = 110$ nM).

Substitution at the 3-position of the pyridine ring was studied on derivatives from the 3-nitropyridine **19**, which possessed a K_i of 14 nM. Interestingly, among the

3-aminopyridin-2-yl analogs (**21a**–**c**), the *N*,*N*-dimethylamino derivative (**21c**, $K_i = 23$ nM) was not superior in binding to the corresponding mono-methyl analog (**21b**, $K_i = 13$ nM). On the other hand, a chloro-substitution at this 3-position gave a compound (**21e**) with high binding affinity ($K_i = 6.2$ nM). In comparison, a methoxy group at this position was less favored and **21d** ($K_i = 25$ nM) was 4-fold less active than **21e**. A hydroxy group at this position completely abolished the binding of **21g** to the receptor ($K_i > 10\,\mu$M).

Several compounds from this 3-(2-pyridyl)pyrazolo[1,5-*a*]pyrimidine series were identified to possess low nanomolar binding affinities (**20c**, **20g**, **20j**, **21b**, and **21e**). One advantage of using a pyridine ring to replace the 3-phenyl group is to increase hydrophilicity of this series of compounds. The calculated log*P* values for many of these pyridine derivatives were around 4 (Tables 2 and 3), which is about one log unit lower than the corresponding phenyl analogs. For example, **20c** ($C\log P = 4.76$), **20j** ($C\log P = 4.33$), **21b** ($C\log P = 2.98$) and **21e** ($C\log P = 4.81$) could be considered as possessing suitable lipophilicity.

Selected compounds from this series demonstrated their functional antagonism by dose-dependent inhibition of CRF-stimulated c-AMP release from cells expressing the CRF_1 receptor.[18] For example, **16a**, **18** and **20c** exhibited IC_{50} values of 49, 180, and 150 nM, respectively, in this assay. Importantly, the hydrochloride salt of **20c** had >2 mg/mL of solubility in water, which should be adequate for further pharmaceutical development.

In conclusion, we designed and synthesized a series of 3-(2-pyridyl)pyrazolo[1,5-*a*]pyrimidines to address the high lipophilicity and poor water solubility of some analogs from this series reported earlier.[12] Calculation of lipophilicity using ACD/LogP software suggests that many of the pyridine compounds possess adequate lipophilicity/hydrophilicity. Several compounds bearing a substituted pyridine ring exhibited good binding affinities at the CRF_1 receptor. For example, compound **20c** had a $C\log P$ value of 4.76, and as a CRF_1 receptor antagonist, it had a K_i value of 10 nM in a competition binding assay and an IC_{50} of 150 nM in inhibition of CRF-stimulated c-AMP production. In addition, **20c** also had relatively good solubility in water.

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