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Synthesis and structure–activity relationships of N^3-pyridylpyrazinones as corticotropin-releasing factor-1 (CRF_1) receptor antagonists

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ABSTRACT

A series of N^3-pyridylpyrazinones was investigated as corticotropin-releasing factor-1 receptor antagonists. It was observed that the binding affinity of analogues containing a pyridyl group was influenced not only by the substitution pattern on the pyridyl group, but also by the pK_a of the pyridyl nitrogen. Analogues containing a novel 6-(difluoromethoxy)-2,5-dimethylpyridin-3-amine group were among the most potent $\boldsymbol{N^3}$-pyridylpyrazinones synthesized. The synthesis and SAR of N^3-pyridylpyrazinones is described herein.

Corticotropin-releasing factor (CRF), a 41 amino acid neuropeptide[1] that is secreted from the paraventricular nucleus of the hypothalamus, functions as the primary physiological regulator of the hypothalamic–pituitary–adrenal (HPA) axis, coordinating the body's endocrine response to stress by regulating the release of adrenocorticotropin hormone (ACTH) from the anterior pituitary gland.[2,3] Two well-characterized receptor subtypes, CRF_1 and CRF_2, have been identified. These G-protein coupled receptors are widely distributed throughout the central and peripheral nervous systems.[4] A body of evidence exists supporting the hypothesis that excessive levels of CRF contribute to stress-related disorders such as depression and anxiety, and that antagonists of CRF_1 receptors may be able to successfully treat these conditions.[2,4,5] The potential that CRF_1 receptor antagonists offer to provide a novel mechanism for the treatment of depression and anxiety has captured the attention of numerous research groups.[6]

We identified **1** (Fig. 1) as a CRF_1 receptor antagonist with good pharmacokinetic properties and efficacy in rats in the Defensive Withdrawal model of anxiety.[7] However, further advancement of this compound was precluded due to the formation of significant levels of glutathione (GSH) adducts upon incubation with liver microsomes.[8] The formation of excessive levels of GSH adducts, an indication of reactive metabolite formation, was of concern due to the potential for reactive metabolites to be involved in idiosyncratic drug toxicity.[9,10] An optimization strategy was subsequently developed to reduce the level of reactive metabolites.[11] One aspect involved replacement of the phenyl group in **1** with a pyridyl group. In a study comparing a small number of pyridyl and phenyl groups, it was found that pyridyl groups showed reduced levels of metabolic activation relative to phenyl groups.[9] On the basis of these results, a broader investigation of the structure–activity relationships (SAR) of pyridyl-containing analogues was undertaken, ultimately leading to the discovery of **2** (BMS-764459),[11] in which we observed a substantial reduction in the formation of GSH adduct metabolites relative to the N^3-phenylpyrazinone analogue (**1**). The initial SAR studies described in this

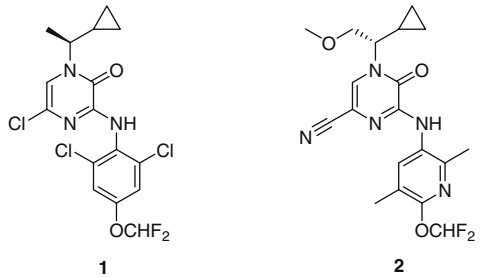

Figure 1. Structures of **1** and **2**.

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article, were carried out using the 5-chloropyrazinone core for ease of synthesis. A limited number of 5-cyanopyrazinone derivatives of some of the more potent N^3-pyridylpyrazinone analogues were subsequently synthesized, and the results have been previously disclosed.[11] The synthesis and SAR of 5-chloro-N^3-pyridylpyrazinone analogues is described herein.

The previously described synthetic route[12] to prepare 1-alkyl-3-*N*-aryl-5-chloropyrazinones is summarized in Scheme 1. Briefly, treatment of alkylamine hydrochlorides **3** with chloroacetonitrile in the presence of potassium iodide and potassium carbonate furnished aminoacetonitrile intermediate **4** in high yield. Intermediate **4** was then condensed with oxalyl chloride to form dichloropyrazinones **5**.[13] Coupling of **5** with a variety of aryl amines in the presence of NaHMDS then furnished the desired pyrazinone products (**6**).

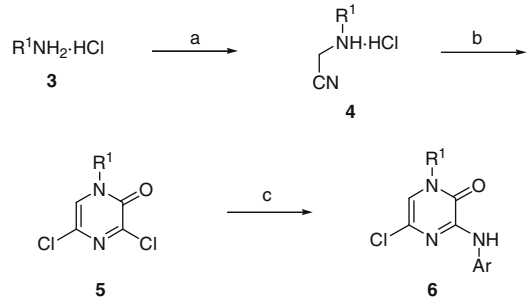
A variety of aminopyridines were available from commercial sources; others were prepared as shown in Schemes 2–6 or as cited. These substituted pyridines were chosen for direct comparison with previously described phenyl substituents. The synthesis of pyridine **9** was completed in three steps from **7** (Scheme 2). After treatment of **7** with dimethylamine to form **8**, installation of the second methyl group was achieved by way of a vicarious nucleophilic substitution reaction using trimethylsulfoxonium iodide in the presence of sodium hydride. Reduction of the nitro group with tin chloride furnished **9**.

The synthesis of pyridine **12** was completed in five steps from **10**[14] (Scheme 3). Demethylation of **10** followed by bromination with pyridinium tribromide afforded compound **11**. Alkylation of **11** with dimethylsulfate proceeded in good yield. Palladium catalyzed coupling with methylboronic acid followed by reduction of the nitro group afforded compound **12**.

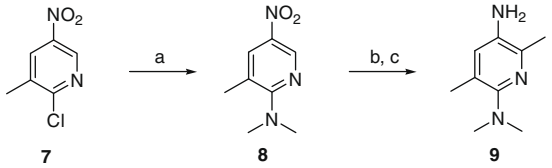
The synthesis of pyridine **15** is illustrated in Scheme 4. A methyl group was installed at the 6-position of **13**[15] by the vicarious nucleophilic substitution reaction described above. Subsequent reduction of the nitro group with tin chloride furnished compound **15**.

The synthesis of pyridine **18** began with demethylation of **16**[16] with concd HCl to furnish **17** in high yield (Scheme 5). Installation of the difluoromethyl group was achieved by either stirring **17** in acetonitrile in the presence of NaH, CsF, and trimethylsilyl 2,2-difluoro-2-(fluorosulfonyl)acetate or by stirring **17** in acetonitrile in the presence of NaH and 2,2-difluoro-2-(fluorosulfonyl)acetic acid.[11] Both conditions afforded the difluoromethyl ether product in high yield. Reduction of the nitro group by hydrogenation in the presence of palladium on carbon provided **18** in high yield.

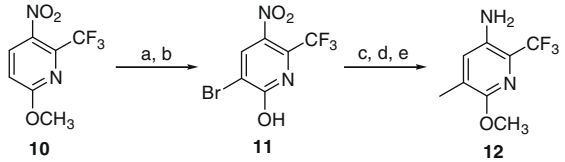
Synthesis of pyridine **21** was accomplished in four steps (Scheme 6). Compound **20** was prepared using a two step, one pot reaction sequence. The crude material was subsequently treated with the sodium salt of chlorodifluoroacetic acid in the presence of K_2CO_3 resulting in the installation of the difluoromethoxy group in 31% yield over three steps.[17] Reduction of the nitro group completed the synthesis of **21**.



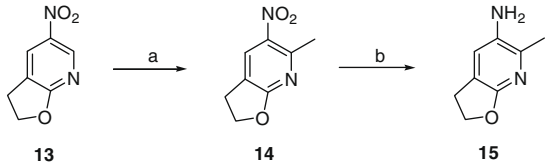
Scheme 1. Reagents and conditions: (a) chloroacetonitrile, KI, K_2CO_3, CH_3CN, 50 °C (84–96%); (b) $(COCl)_2$, toluene, 55 °C (43–71%) or $(COCl)_2$, dioxane/CH_2Cl_2, 55 °C (69–74%); (c) NaHMDS, $ArNH_2$, THF.



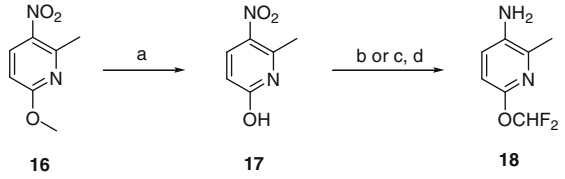
Scheme 2. Reagents and conditions: (a) $HNMe_2$ in THF, reflux (86%); (b) NaH, $Me_3SO^+I^-$, DMSO (93%); (c) $SnCl_2{\cdot}H_2O$, EtOH (49%).



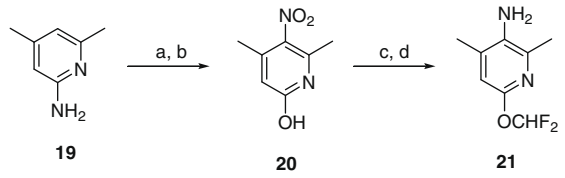
Scheme 3. Reagents and conditions: (a) LiI, 2,4,6-collidine (79%); (b) pyridinium tribromide, DMF (70%); (c) K_2CO_3, Me_2SO_4, DMF (59%); (d) $MeB(OH)_2$, $PdCl_2(dppf)_2$, K_3PO_4, DMF (53%); (e) H_2, Pd/C, MeOH (87%).



Scheme 4. Reagents and conditions: (a) NaH, $Me_3SO^+I^-$, DMSO (25%); (b) $SnCl_2{\cdot}2H_2O$, EtOH (62%).



Scheme 5. Reagents and conditions: (a) concd HCl, reflux (97%); (b) NaH, $FSO_2CF_2CO_2SiMe_3$, CsF, CH_3CN (91%); (c) NaH, $FSO_2CF_2CO_2H$, CH_3CN (83%); (d) H_2, Pd/C, EtOH (99%).

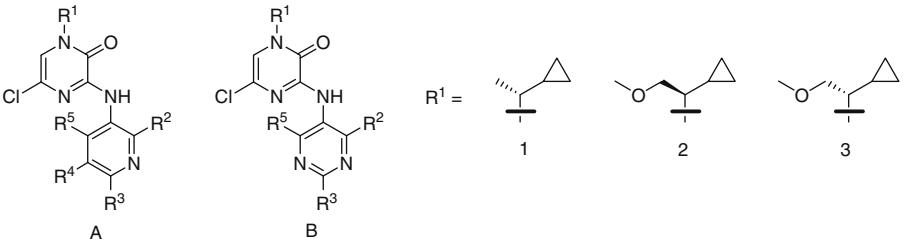


Scheme 6. Reagents and conditions: (a) HNO_3, H_2SO_4; (b) HNO_3, H_2O; (c) ClF_2CCO_2Na, K_2CO_3, DMF (31%, three steps); (d) H_2, Pd/C, EtOH (88%).

Compounds were tested in a CRF_1 receptor binding titration assay using rat frontal cortex homogenate, in which inhibition of specific binding of [^{125}I]Tyr-ovine-CRF by our test compounds was measured to determine their receptor binding affinity.[12] As part of the optimization process, test compounds were subsequently incubated with rat and human liver microsomes to evaluate their metabolic stability.

The phenyl group SAR previously developed demonstrated that inclusion of both an *ortho*- and *para*-substituent relative to the nitrogen linker proved to be important for potency.[12] In addition,

Table 1
CRF_1 receptor binding affinity of analogues containing either pyridyl or pyrimidyl groups



Compd	Structure	R^1	R^2	R^3	R^4	R^5	IC_{50}[a] (nM)	Met stab[b,c] (HLM, % remaining)	Met stab[c,d] (RLM, % remaining)
6a	A	1	Me	OMe	H	H	11 ± 2	89	60
6b	A	1	OMe	OMe	H	H	17 ± 6	43	25
6c	A	1	CF_3	OMe	H	H	1.6 ± 0.4	96	65
6d	A	1	Me	OMe	H	Me	12 ± 0.3	79	81
6e	A	1	Me	Me	H	Me	65 ± 7	87	76
6f	A	1	Me	OMe	Me	H	1.5 ± 0.5	77	55
6g	A	1	Me	OCH_2–	–CH_2	H	740 ± 110	ND[e]	ND
6h	A	1	Me	OEt	Me	H	2.7 ± 1.0	93	81
6i	A	1	Me	NMe_2	Me	H	0.68 ± 0.16	54	29
6j	A	1	Me	$N(CH_2)_4$	Me	H	6.8 ± 0.4	57	26
6k	A	1	Me	CF_3	H	H	720 ± 210	ND	ND
6l	A	1	CF_3	OMe	Me	H	1.2 ± 0.4	67	77
6m	A	1	Me	$OCHF_2$	H	H	1.2 ± 0.3	97	74
6n	A	1	Me	$OCHF_2$	H	Me	2.1 ± 0.3	89	84
6o	A	1	Me	$OCHF_2$	Me	H	0.30 ± 0.03	100	86
6p	A	2	Me	$OCHF_2$	H	H	0.80 ± 0.02	98	29
6q	A	3	Me	$OCHF_2$	H	H	1.2 ± 0.1	89	52
6r	A	3	Me	$OCHF_2$	H	Me	4.7 ± 0.5	74	5
6s	A	3	Me	$OCHF_2$	Me	H	0.24 ± 0.10	93	89
6t	B	1	Me	OMe	—	OMe	240 ± 40[f]	86	81
6u	B	1	OMe	Me	—	CN	140 ± 1[f]	ND	ND
6v	B	2	Me	OMe	—	OMe	31 ± 14[f]	71	25
6w	B	3	Me	OMe	—	OMe	90 ± 2[f]	45	18

[a] All values are the average of at least $n = 3 \pm$ standard deviation unless indicated otherwise. The IC_{50} of *o*-CRF = 2.9 ± 1.0 nM in this assay and the IC_{50} of DMP-696 = 1.2 ± 0.2 nM in this assay.
[b] HLM = human liver microsomes.
[c] % Remaining after 10 min.
[d] RLM = rat liver microsomes.
[e] ND = not determined.
[f] Value determined by two measurements.

incorporation of a third substituent at the positions of either R^4 or R^5 (as shown in structure A, Table 1) further improved the potency of the phenyl-based compounds. As a result, our investigation of the pyridyl group SAR began with compounds that contained both an *ortho*- and a *para*-substituent. The CRF_1 receptor binding affinities for N^3-pyridylpyrazinone analogues and a small number of N^3-pyrimidylpyrazinone analogues (prepared similar to the method in Scheme 1 using commercially available or synthesized pyrimidines) are shown in Table 1.

The SAR of three substituents at R^2 was determined (Table 1, **6a**–**6c**). An analogue with a methyl group at R^2 was similar in potency to the corresponding methoxy analogue (compare **6a** vs **6b**). Compound **6c**[18] ($R^2 = CF_3$), however, proved to be nearly 10-fold more potent than **6a**.

Compounds with a variety of groups at R^3 were synthesized and the results are shown in Table 1 (entries **6d**–**6m**). In addition, an additional substituent at either R^4 or R^5 was included in most of these compounds. It was found that unlike the phenyl analogues,[12] including an additional methyl group at R^5 did not result in an improvement in binding affinity (compare **6d**[19] vs **6a**). However, compound **6f** with a methyl group at R^4, was nearly 10-fold more potent than **6a**, possibly due to an additional hydrophobic interaction. The bicyclic analogue (**6g**) where R^3 and R^4 are tied together to form a five-membered ring was 500-fold less potent than compound **6f** where R^3 = OMe and R^4 = Me.

Compounds with additional groups at R^3 were also quite potent (**6h**–**6j**).[20,21] Remarkably, **6k**[22] (IC_{50} = 720 nM) was much less potent than most of the other analogues in this series.[23] Unlike compounds with a methyl group at R^2, incorporation of a methyl group at R^4 when $R^2 = CF_3$ did not result in improved potency (compare **6c** vs **6l** and **6a** vs **6f**).

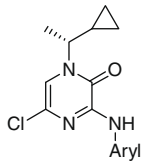
Subsequent to the synthesis of the above analogues, a compound containing a difluoromethoxy group at R^3 was prepared. Compound **6m** (IC_{50} = 1.2 nM) was 10-fold more potent than the corresponding analogue with a methoxy group at R^3 (compare with **6a**). A variety of additional difluoromethoxy analogues were synthesized. Incorporation of a methyl group at R^5 resulted in a modest decrease in potency (compare **6n** vs **6m** or **6r** vs **6q**). However, a further improvement in potency versus compound **6m** was observed when a methyl group was incorporated at R^4, resulting in a compound with an IC_{50} of 0.30 nM (**6o**). Analogues with the novel 6-(difluoromethoxy)-2,5-dimethylpyridin-3-amine group[24] (e.g., **6o**) proved to be among the most potent in this series of compounds.

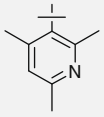
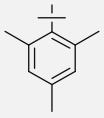
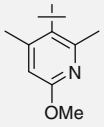
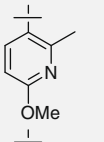
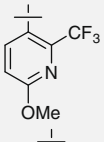
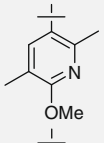
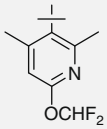
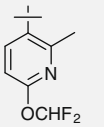
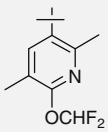
Analogues containing a pyrimidyl group in place of the pyridyl group were also synthesized[25,26] (**6t**–**6w**). These compounds were generally less potent than the pyridyl-based analogues.

In addition to the placement of substituents on the pyridyl group, the basicity of the pyridyl nitrogen also played a role in influencing binding affinity to the CRF_1 receptor. The pK_a of the

pyridyl nitrogen in a subset of the above compounds was measured (Table 2). Compound **6e**[27] with a mildly basic pyridyl nitrogen (pK_a = 7.9) had an IC_{50} of 65 nM. The corresponding phenyl analogue (**6x**) had an IC_{50} of 1.7 nM indicating that the CRF_1 receptor has little tolerance for a basic nitrogen in the aryl substituent. Replacement of a methyl group in **6e** with a methoxy group (**6d**) lowered the pK_a of the protonated pyridyl nitrogen to 3.8. The IC_{50} of this compound was 12.0 nM. Removal of one of the methyl groups (**6a**) had little effect on the pK_a and no significant effect on the IC_{50} compared to **6d**. Replacement of the remaining methyl group in **6a** with a trifluoromethyl group (**6c**) resulted in a further improvement of the IC_{50} to 1.6 nM and also further lowering of the pK_a to <2. Compound **6f** was similar in potency to **6c**. Replacement of the methoxy group in compounds **6d**, **6a**, and **6f** with a difluoromethoxy group led to compounds **6n**, **6m**, and **6o**, respectively, each with an improved binding affinity relative to the corresponding methoxy analogues. Analogues with the difluoromethoxy group had superior potency compared to other pyridyl-containing analogues. To summarize, the results shown in Table 2 indicate that the CRF_1 receptor shows little tolerance for basic groups in the aryl region.

Table 2
Relationship between CRF_1 receptor binding affinity and pyridyl nitrogen pK_a



Compd	Aryl	IC_{50} (nM)	pK_a[a]
6e		65 ± 7	7.9
6x		1.7 ± 0.2	NA[b]
6d		12 ± 0.3	3.8
6a		11 ± 2	3.2
6c		1.6 ± 0.4	<2
6f		1.5 ± 0.5	3.6
6n		2.1 ± 0.3	<2
6m		1.2 ± 0.3	<2
6o		0.30 ± 0.03	<2

[a] pK_a measurements were determined using a spectrophotometric titration method.[28]
[b] NA = not applicable.

A comparison of compounds **6a**, **6d** and **6f** versus **6m**, **6n** and **6o**, respectively, indicated that the difluoromethoxypyridyl analogues showed improved metabolic stability in rat and human liver microsomal incubations (Table 1) compared to the methoxypyridyl analogues. Additional SAR studies with analogues containing the difluoromethoxypyridyl group led to the discovery of **2**, a compound which was selected for further development.[11]

In conclusion, efforts to identify suitable phenyl group replacements resulted in the discovery of highly potent pyridyl-containing analogues. It was found that the CRF_1 receptor binding affinity of analogues containing a pyridyl group was influenced not only by the substitution pattern on the pyridyl group, but also by the pK_a of the pyridyl nitrogen. In general, analogues with pyridyl groups wherein the pK_a of the pyridyl nitrogen was low showed improved potency, indicating that the CRF_1 receptor appears to have limited tolerance for basic groups in the pocket where the pyridyl group binds. Analogues containing the novel 6-(difluoromethoxy)-2,5-dimethylpyridin-3-amine group proved to be among the most potent in this series of compounds.

Acknowledgment

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18. Reduction of the nitro group in **10** by hydrogenation afforded 6-methoxy-2-(trifluoromethyl)pyridin-3-amine in 97% yield.
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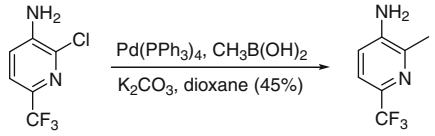
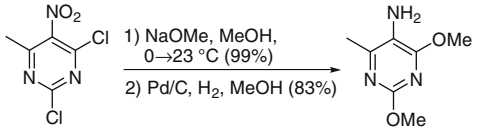
20. 6-Ethoxy-2,5-dimethylpyridin-3-amine was prepared in a manner analogous to 6-methoxy-2,5-dimethylpyridin-3-amine as described in Ref. 11.
21. 2,5-Dimethyl-6-(pyrrolidin-1-yl)pyridin-3-amine was prepared in a manner analogous to N^2,N^2,3,6-tetramethylpyridine-2,5-diamine (**9**).
22. 2-Methyl-6-(trifluoromethyl)pyridin-3-amine (**23**) was prepared by palladium catalyzed coupling of **22** with methylboronic acid.



22 **23**

23. By comparison, the IC_{50} for a closely related phenyl-based analogue (2,6-dichloro-4-CF_3) was 4.1 nM as described in Ref. 7.
24. 6-(Difluoromethoxy)-2,5-dimethylpyridin-3-amine was synthesized as described in Ref. 11.
25. 2,4-Dimethoxy-6-methylpyrimidin-5-amine (**25**) was synthesized from commercially available 2,4-dichloro-6-methyl-5-nitropyrimidine **24** in two steps.



24 **25**

26. 5-Amino-6-methoxy-2-methylpyrimidine-4-carbonitrile was synthesized using a procedure analogous to that described in Al-Azmi, A.; Booth, B. L.; Pritchard, R. G.; Proenca, F. J. R. P. *J. Chem. Soc., Perkin Trans.* **2001**, *1*, 485.
27. 2,4,6-Trimethylpyridin-3-amine was prepared according to the procedure in Beck, J. P.; Arvanitis, A. G.; Curry, M. A.; Rescinito, J. T.; Fitzgerald, L. W.; Gilligan, P. J.; Zaczek, R.; Trainor, G. L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 967.
28. The pK_a values were determined in the pH range between 2 and 11, and the data were obtained using a Sirius GLpK_a instrument. The pK_a was measured using the spectrophotometric titration method and the compound was dissolved in varying concentrations of acetonitrile/water with extrapolation to 0% cosolvent.