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Optimization of 3-phenylpyrazolo[1,5-*a*]pyrimidines as potent corticotropin-releasing factor-1 antagonists with adequate lipophilicity and water solubility

Chen Chen,[a,*] Keith M. Wilcoxen,[a] Charles Q. Huang,[a] James R. McCarthy,[a] Takung Chen[c] and Dimitri E. Grigoriadis[b]

[a]*Department of Medicinal Chemistry, Neurocrine Biosciences, Inc., 10555 Science Center Drive, San Diego, CA 92121, USA*
[b]*Department of Pharmacology, Neurocrine Biosciences, Inc., 10555 Science Center Drive, San Diego, CA 92121, USA*
[c]*Department of Preclinical Development, Neurocrine Biosciences, Inc., 10555 Science Center Drive, San Diego, CA 92121, USA*

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Abstract—In our efforts to identify potent CRF_1 antagonists with proper physicochemical properties, a series of 3-phenylpyrazolo[1,5-*a*]pyrimidines bearing polar groups, such as amino, hydroxyl, methoxy, sulfoxide, were designed and synthesized. Several positions of the core structure were identified, where a polar group was tolerated with slight reduction in receptor binding. NBI 30545 (**18n**) was found to have good binding affinity and potent antagonistic activity at the human CRF_1 receptor. Moreover, this compound had proper lipophilicity ($\log D = 2.78$) and good solubility in water (>10 mg/mL), and exhibited good plasma and brain exposure when given orally.

1. Introduction

Corticotropin-releasing factor (CRF), a 41-amino acid peptide produced in hypothalamus, is a major modulator of the body's responses to stress. CRF exerts its function by binding to and activating the CRF_1 and CRF_2 receptors, both belonging to the class B G-protein-coupled receptor superfamily.[1] Clinical evidence suggests that over-stimulation of the CRF system may result in several diverse neuropsychiatric diseases including depression, anxiety and stress related disorders, and that CRF antagonists have the potential to treat these diseases. Since the first nonpeptide CRF antagonist CP-154,526 (**1**) disclosed in 1996,[2] many potent small molecule CRF antagonists from different chemical classes have been reported.[3] Among them a series of 3-phenylpyrazolo[1,5-*a*]pyrimidines, exemplified by PD171729 (**2**)[4b] and DMP904 (**3**),[4c] have been synthesized by several groups.[4] Like many other small molecule CRF_1 antagonists reported previously (**4**–**7**),[5] this series of compounds such as **2** suffer from high lipophilicity and poor water solubility.[6] We calculated the Clog *P* values of **1**–**7** using the ACD/LogP software[7] to assess the relative lipophilicities of these molecules. All of the compounds, except DMP904 (**3**), have Clog *P* values close to or greater than 6,[8] much too high as ideal CNS agents since a preferred log *P* value should be between 2 and 3.5.[9] Recently, CRF_1 receptor antagonists with lower lipophilicity have been identified.[10] For example, DMP696 (**8**) is a potent CRF_1 antagonist with good in vitro activity, good pharmacokinetic property and in vivo efficacy in anxiety animal models. The Clog *P* value of **8** is 3.32.[11] Here we report the synthesis and structure–activity relationships of 3-phenylpyrazolo[1,5-*a*]pyrimidines such as NBI 30545 (**18n**)[12] in our efforts to identify potent CRF_1 antagonists with proper physicochemical properties (Fig. 1).

2. Chemistry

The synthesis of the desired 3-phenylpyrazolo[1,5-*a*]-pyrimidines **18**–**20** was started from the substituted phenylacetonitriles **9** as described in Schemes 1 and 2. β-Ketonitriles **10** and **11** were prepared by reaction of **9** with sodium hydride in THF, followed by a carboxylic

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* Corresponding author. Tel.: +1-858-658-7600; fax: +1-858-658-7619; e-mail: cchen@neurocrine.com

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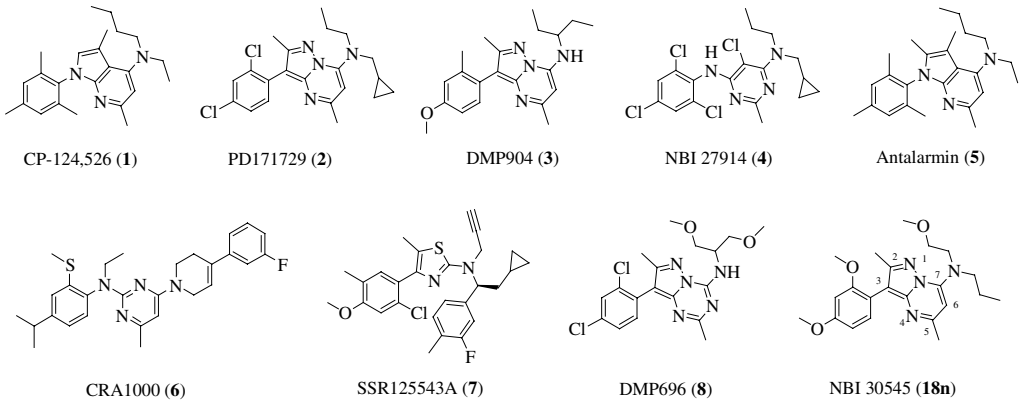
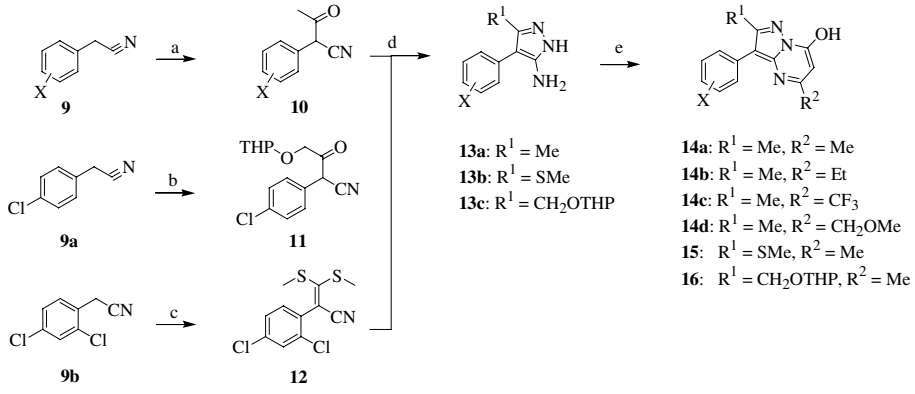
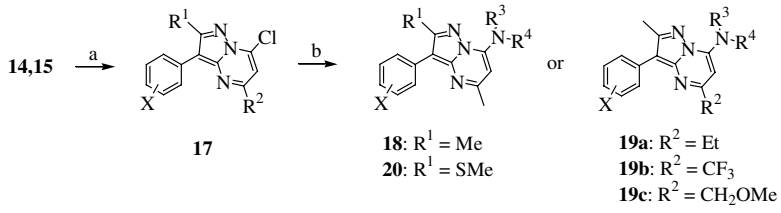


Figure 1. Some small molecule CRF_1 receptor antagonists.



Scheme 1. Reagents and conditions: (a) NaH/THF, then AcOEt; (b) NaH/THF, then $THPOCH_2COOEt$; (c) i. NaH/THF/rt, ii. CS_2, iii. MeI; (d) $NH_2NH_2{\cdot}HCl$/aq EtOH/reflux; (e) R^2COCH_2COOEt/dioxane/reflux.

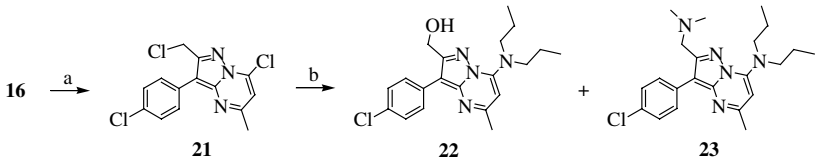


Scheme 2. Reagents and conditions: (a) $POCl_3$/reflux; (b) R^3R^4NH/reflux.

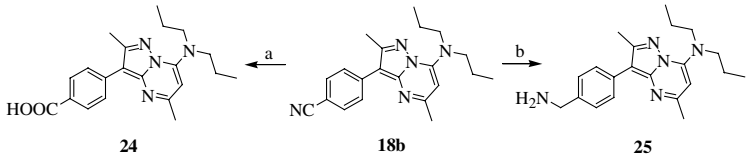
ester.[4c] Compound **12** was prepared as a yellowish solid by reaction of **9b** with 2.5 equiv of sodium hydride in THF, followed by excess amount of carbon disulfide and then methyl iodide. The 3-phenylpyrazolo[1,5-*a*]-pyrimidinones **14**–**16** were synthesized using a procedure similar to that reported.[13] In brief, **10**–**12** were cyclized with hydrazine hydrochloride in refluxing aqueous ethanol to give the aminopyrazoles **13**, which were subjected to a second cyclization with a β-ketoester in refluxing dioxane to give the pyrazolo[1,5-*a*]pyrimidinones **14**–**16** as white solids. Compounds **14** and **15** were converted to the corresponding chloropyrimidines **17** with $POCl_3$ at reflux, which were subsequently reacted with an alkylamine to afford the desired products **18**–**20**.

Reaction of **16** with $POCl_3$ at reflux gave the intermediate **21**, which, after aqueous workup and without further purification, was treated with dimethylamine in acetonitrile first at room temperature, followed by excess dipropylamine at reflux to provide a mixture of the 2-hydroxylmethyl **22** and the 2-(dimethylamine)methyl **23** that were separated by chromatography (Scheme 3). Hydrolysis of the 4-cyanophenyl analog **18b** under acidic conditions (6 N HCl/EtOH, reflux) gave the corresponding carboxylic acid **24**. On the other hand, reduction of **18b** with boran/THF gave the 4-aminomethylphenyl analog **25** (Scheme 4).

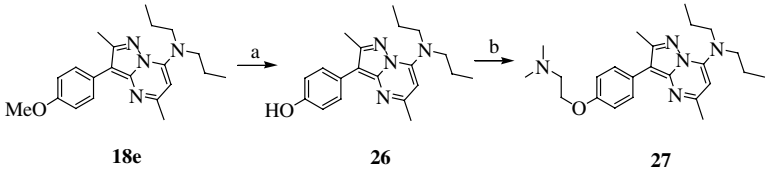
Demethylation of **18e** with BCl_3 in dichloromethane at 0 °C to rt gave the phenol **26**, which was alkylated with 2-dimethylaminoethyl chloride under basic conditions (K_2CO_3/EtOH) to give the amine **27** (Scheme 5). Oxidation of the methylsulfide **20** with 1.5 equiv of *m*-CPBA in dichloromethane at 0 °C afforded the corresponding



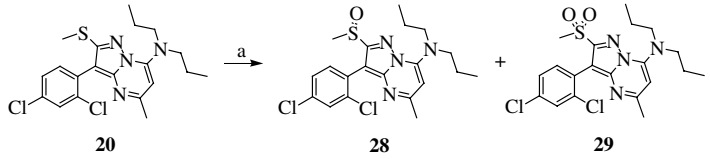
Scheme 3. Reagents and conditions: (a) $POCl_3$/reflux; (b) Me_2NH/ACN/rt, then Pr_2NH/reflux.



Scheme 4. Reagents and conditions: (a) 6 N HCl/EtOH/reflux; (b) BH_3/THF.



Scheme 5. Reagents and conditions: (a) BCl_3/CH_2Cl_2, 0 °c to rt; (b) $Me_2NCH_2CH_2Cl{\cdot}HCl/K_2CO_3$/EtOH/△.



Scheme 6. Reagents and conditions: (a) *m*-CPBA/CH_2Cl_2/rt.

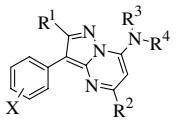
sulfoxide **28** and sulfone **29**, which were separated by chromatography on silica gel (Scheme 6).

3. Results and discussion

The synthesized compounds were tested for their binding affinities at the cloned human CRF_1 receptor in a competition binding assay as described,[14] and the K_i values were determined from concentration–response curves using concentrations ranging from 100 pM to 10 μM. The structure–activity relationships of these compounds are summarized in Table 1. Selected compounds were also evaluated for functional antagonism in the same cell line as that used in the binding studies above however utilizing a live whole cell preparation to measure intracellular cAMP accumulation, and further determined in an assay based on their ability to inhibit CRF-stimulated ACTH release in cultured rat anterior pituitary cells.[15] These results are summarized in Table 2. Antalarmin (**5**), which is utilized as a positive control, displayed a K_i of 2.6 nM in the binding assay and an IC_{50} of 16 nM in the cAMP inhibitory assay.

Although compound **2** exhibits very good binding affinity ($K_i = 1.3$ nM in our assay, 3.2 nM, reported)[4b] at the human CRF_1 receptor, it is highly lipophilic with a Clog*P* value of 5.86. Moreover, its mesylate salt is largely insoluble in water. In order to reduce its lipophilicity, we introduced polar groups such as a hydroxyl, methoxy, sulfoxide and weakly basic amino group into a different position of this core structure. The 2-, 3- and 4-methoxyphenyl compounds **18d**, **18e** and **18f** had Clog*P* values of about 4.7, and among them the 4-methoxy analog **18f** possessed the best binding affinity ($K_i = 3.6$ nM). Incorporation of an additional methoxy group into **18f** further lowered the lipophilicity and slightly increased binding affinity. Thus, the 3,4-dimethoxyphenyl compound **18h** had a K_i of 1.3 nM and Clog*P* of 4.45. Similarly, the 2,4-dimethoxyphenyl analog **18i** possessed a K_i value of 1.1 nM and Clog*P* of 4.33. Optimization at the 7-position of the pyrazolopyrimidine with different alkylamines resulted in several compounds with good binding affinity as well as proper lipophilicity. For example, **18l** and **18n** possessed K_i values of 2.4 and 3.4 nM, and Clog*P* values of 4.16 and 3.18, respectively. The binding affinities of these

Table 1. SAR and Clog *P* of substituted 3-phenylpyrazolo[1,5-*a*]pyrimidines[17]



Compound	X	R^1	R^2	R^3NR^4	K_i (nM)[a]	Clog *P*[b]
5	—	—	—	—	2.6	8.89
2	2,4-Cl	Me	Me	$PrNCH_2Pr$-*c*	1.3	5.86
18a	4-Cl	Me	Me	Pr_2N	1.6	5.54
18b	4-CN	Me	Me	Pr_2N	3.5	4.28
18c	H	Me	Me	Pr_2N	69	4.97
18d	2-MeO	Me	Me	Pr_2N	15	4.63
18e	3-MeO	Me	Me	Pr_2N	24	4.73
18f	4-MeO	Me	Me	Pr_2N	3.6	4.78
18g	4-MeO	Me	Me	$PrNCH_2Pr$-*c*	2.4	4.49
18h	3,4-MeO	Me	Me	Pr_2N	1.3	4.45
18i	2,4-MeO	Me	Me	Pr_2N	1.1	4.33
18j	2,4-MeO	Me	Me	Et_2CHNH	3.9	3.89
18k	2,4-MeO	Me	Me	EtNBu-*n*	1.9	4.33
18l	2,4-MeO	Me	Me	$PrNHCH_2Pr$-*c*	2.4	4.16
18m	2,4-MeO	Me	Me	$PrNCH_2CH_2OH$	29	2.51
18n	2,4-MeO	Me	Me	$PrNCH_2CH_2OMe$	3.4	3.18
18o	2,4-MeO	Me	Me	$(MeOCH_2CH_2)_2N$	43	2.03
19a	4-Cl	Me	Et	Pr_2N	100	6.07
19b	4-Cl	Me	CF_3	Pr_2N	>10,000	6.80
19c	4-Cl	Me	CH_2OMe	Pr_2N	>10,000	4.82
20	2,4-Cl	Me	SMe	$PrNCH_2Pr$-*c*	0.73	6.09
22	4-Cl	CH_2OH	Me	Pr_2N	11	3.93
23	4-Cl	CH_2NMe_2	Me	Pr_2N	>10,000	4.84
24	4-COOH	Me	Me	Pr_2N	>10,000	4.74
25	$4\text{-}CH_2NH_2$	Me	Me	Pr_2N	>10,000	3.84
27	$4\text{-}OCH_2CH_2NMe_2$	Me	Me	Pr_2N	>10,000	4.63
28	2,4-Cl	Me	SOMe	$PrNCH_2Pr$-*c*	8.6	3.73
29	2,4-Cl	Me	SO_2Me	$PrNCH_2Pr$-*c*	150	3.68

[a] Values are average of two or more independent determinations.
[b] ACD/LogP software.

Table 2. Inhibition of CRF-stimulated cAMP production and ACTH release

Compound	K_i (nM)	cAMP IC_{50} (nM)	ACTH IC_{50} (nM)
5	2.6	16.3	—
2	1.3	33	—
18a	1.6	38	118
18i	1.1	34	106
18j	3.9	38	—
18n	3.4	76	37
22	11	470	—
28	8.6	210	—

compounds are comparable to **2**, however, their lipophilicities are much lower than **2** (Clog $P = 5.86$).

Attempts to incorporate a methoxy group at the 5-position of the core were unsuccessful. Thus, **19c** was inactive ($K_i > 10\,\mu M$). This could simply be due to a stereo-effect since the 5-ethyl analog **19a** ($K_i = 100\,nM$) had a much higher binding K_i than the corresponding 5-methyl compound **18a** ($K_i = 1.6\,nM$). The CF_3-analog **19b** also exhibited no binding ($K_i > 10\,\mu M$).

The 2-position of the core structure seemed to be less sensitive to a polar substituent. Thus, the 2-hydroxymethylpyrazolo[1,5-*a*]pyrimidine **22** exhibited a K_i value of 11 nM, about sevenfold reduction in binding affinity from **18a**. The sulfoxide **28** had a K_i value of 8.6 nM, about 12-fold lower than the methylthio analog **20** ($K_i = 0.73\,nM$). The calculated log *P* values of **22** (3.93) and **28** (3.73) were, however, much lower than **18a** and **20**. The bulky sulfone group at the 2-position of pyrazolo[1,5-*a*]pyrimidine caused a large reduction in binding affinity (**29**, $K_i = 150\,nM$). The weakly basic dimethylamino moiety at the 2-methyl group of **18a** completely abolished its binding (**23**, $K_i > 10\,\mu M$). These data may suggest this position is more sensitive to bulkiness than a polar group.

Because of the success in incorporation of the hydrophilic methoxyl moiety at the 3-phenyl group, we further explored several other alternatives. Both the acidic carboxylate (**24**) and basic amine (**25**) substitutions at the *para*-position of the 3-phenyl group gave inactive analogs. Attempts to distance the basic moiety from the core structure were also unsuccessful (**27**, $K_i > 10\,\mu M$).

These results suggest that, for this series of compounds, introduction of a polar methoxy group at 7-position side chain had little change in receptor binding. For example, **18i** and its oxygen-inserted analog **18n** had K_i values of 1.1 and 3.4 nM, respectively. However, the additional oxygen of **18n** decreased the lipophilicity (log *P*) about one log unit. The 5-position did not tolerate the meth-

oxy moiety and a group larger than the methyl. The 3-phenyl group was open to multiple methoxy substituents, but did not tolerate acidic and basic functionalities at the *para*-position. Introducing a polar moiety at the 2-position was somewhat successful. The 2-hydroxymethyl (**22**) and 2-sulfoxide (**28**) analogs possessed acceptable binding affinities and proper lipophilicities.

Compounds of interest were selected for measurement of their antagonistic activities in functional assays including inhibition of CRF-stimulated cAMP production in cells expressing the human CRF_1 receptor, and CRF-stimulated ACTH release in rat anterior pituitary primary cell cultures (Table 2). All compounds tested exhibited functional antagonism in both assays. For example, **28** exhibited an IC_{50} value of 210 nM in the cAMP assay, and **18n** had IC_{50} values of 76 nM in the cAMP assay and 37 nM in the ACTH assay.

The experimentally measured log *P* value of **18n** was 2.78 (octanol–PBS buffer, pH 7.4, HPLC detection), which approximately matches the calculated value (3.18). In contrast with **2**, which is quite insoluble in water, the mesylate salt of **18n** had very good water solubility (>10 mg/mL). Compound **18n** also had a good pharmacokinetic profile in rats. After intravenous injection to Sprague–Dawley rats (10 mg/kg), the volume of distribution was calculated to be 4.7 L/kg, indicating extensive tissue distribution. The clearance of 43 mL/min kg for this compound was high in this species, and this resulted in a relatively short half-life of 1.1 h. At the dose of 10 mg/kg po, the plasma and brain AUC values were 1182 ng/ml h and 1472 ng/g h, respectively. This resulted in an oral bioavailability of 30.5% and a brain/plasma ratio of 1.2. These data indicate **18n** had good plasma and brain exposure when administrated orally. In comparison, the high lipophilic CP-124,526 (**1**, Clog $P = 8.43$) has a long half-life (51 h) associated with high volume of distribution (105 L/kg) in rats.[16]

4. Conclusion

SAR studies at the 2-, 3-, 5- and 7-positions of the 3-phenylpyrazolo[1,5-*a*]pyrimidine towards more hydrophilic derivatives revealed that the 5-position was very sensitive to any replacement of the optimal methyl group. However, the 2-, 3- and 7-positions were somewhat tolerated to a small hydrophilic group, with a slight reduction in binding affinity. Several compounds such as **18n** were identified with good binding affinity, potent functional antagonistic activity and suitable lipophilicity for a CNS agent. Compound **18n** also possessed good plasma and brain exposure after oral administration.

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17. For most compounds assayed 2–5 times, the K_i values were highly reproducible with an average standard deviation of <50%.