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Discovery of pyrrolo[2,3-*d*]pyrimidin-4-ones as corticotropin-releasing factor 1 receptor antagonists with a carbonyl-based hydrogen bonding acceptor

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ABSTRACT

A new class of pyrrolo[2,3-*d*]pyrimidin-4-one corticotropin-releasing factor 1 (CRF_1) receptor antagonists has been designed and synthesized. In general, reported CRF_1 receptor antagonists possess a sp^2-nitrogen atom as hydrogen bonding acceptor (HBA) on their core scaffolds. We proposed to use a carbonyl group of pyrrolo[2,3-*d*]pyrimidin-4-one derivatives as a replacement for the sp^2-nitrogen atom as HBA in classical CRF_1 receptor antagonists. As a result, several pyrrolo[2,3-*d*]pyrimidin-4-one derivatives showed CRF_1 receptor binding affinity with IC_{50} values in the submicromolar range. Ex vivo ^{125}I-sauvagine binding studies showed that 2-(dipropylamino)-3,7-dimethyl-5-(2,4,6-trimethylphenyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (**16b**) (30 mg/kg, po) was able to penetrate into the brain and inhibit radioligand binding to CRF_1 receptors (frontal cortex, olfactory bulb, and pituitary) in mice. We identified pyrrolo[2,3-*d*]pyrimidin-4-one derivatives as the first CRF_1 antagonists with a carbonyl-based HBA.

Corticotropin-releasing factor (CRF), a 41-amino acid neuropeptide secreted in the hypothalamus, was isolated from ovine brain and characterized in 1981.[1] Over the past 30 years, CRF has been thought to function as the prime regulator of the hypothalamic-pituitary-adrenocortical (HPA) axis and to play an important role as a neurotransmitter in the mediation of stress-related behaviors.[2–5] The CRF receptor, a class B G-protein-coupled receptor, has two subtypes (CRF_1 and CRF_2).[6,7] CRF_1 receptor-knockout mice showed anxiolytic-like behaviors in various tests and blunted HPA axis responses to stress.[8] These findings indicated that CRF_1 receptor antagonists would be an important target for developing novel drugs for stress-related disorders such as depression and anxiety.

The clinical efficacy of CRF_1 receptor antagonists against depression and anxiety is still unclear, but data from few clinical studies show that these antagonists tend to be efficacious. In a small open-label phase IIa clinical trial, R121919 (**1**) effectively reduced the Hamilton depression and anxiety scale (HAM-D and HAM-A) scores in patients with major depression.[9] Subchronic treatment with NBI-34041 (**2**) attenuated the neuroendocrine response to psychosocial stress in a placebo-controlled clinical study.[10] These data suggested that non-peptide CRF_1 receptor antagonists could be useful therapeutic agents in the treatment of depression and anxiety. On the other hand, in a double-blind, placebo-controlled clinical study of CP-316,311 (**3**), the change in the HAM-D score from baseline to the final visit was not significantly different between the CP-316,311 and placebo groups.[11] Pexacerfont (**4**)[12,13] and emicerfont (**5**)[14] also failed in clinical trials for major depression, general anxiety disorder, and irritable bowel syndrome. However, GSK561679 (**6**)[15] and SSR125543 (**7**)[16] are still being tested for the potential treatment of post-traumatic stress disorder (PTSD) in phase II by the Emory university group[17] and for depression and PTSD in phase II by Sanofi-Aventis,[18] respectively. In addition, CRF_1 receptor antagonists are still regarded as a relevant target for diseases resulting from elevated levels of CRF, for example, alcohol abuse, pain, and stress-related disorders.[19,20] Identification of structurally diverse CRF_1 receptor antagonists has been an active area of drug discovery research.

A variety of small molecule CRF_1 receptor antagonists have been reported in the literature and patents[21] since the disclosure of CP-154,526 (**8**) in 1996.[22] Most of the reported compounds such as R121919 (**1**), pexacerfont (**4**), emicerfont (**5**), GSK-561679 (**6**), and CP-154,526 (**8**) have a bicyclic heterocyclic core. Moreover, potent CRF_1 receptor antagonists with a monocyclic core, for example, CP-316,311 (**3**) and SSR125543 (**7**), and those with a tricyclic core, for example, NBI-34041 (**2**), have been reported. All of them possess a sp^2-nitrogen atom as a hydrogen bonding acceptor (HBA) on the core scaffold (Fig. 1).

In our investigation of a new class of CRF_1 receptor antagonists, we proposed to use a carbonyl group as a replacement for a sp^2-nitrogen atom as HBA on the reported CRF_1 receptor antagonists. We anticipated that the unique compounds with a carbonyl-based HBA would not only exhibit potent affinity to CRF_1

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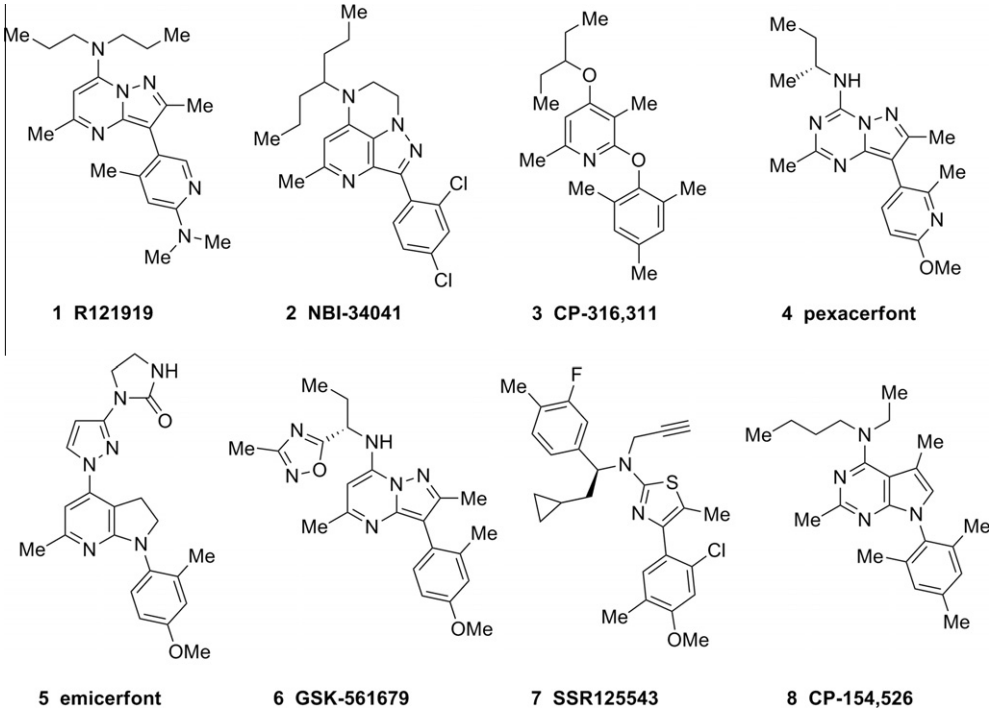


Figure 1. Reported CRF_1 antagonists.

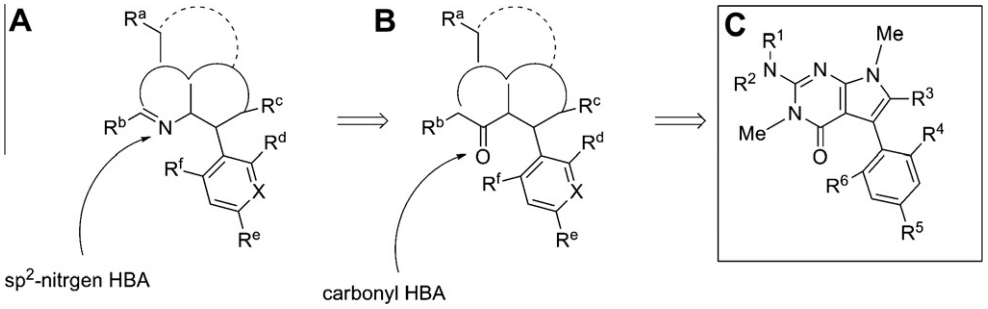


Figure 2. (a) Pharmacophore of the reported CRF_1 antagonists, (b) design of the carbonyl HBA antagonists and (c) pyrrolo[2,3-*d*]pyrimidin-4-one with the carbonyl HBA.

receptors due to their electrostatic interaction but also show properties different from those of the reported antagonists. In this article, we describe our successful efforts in discovering a new class of pyrrolo[2,3-*d*]pyrimidin-4-one CRF_1 receptor antagonists containing a carbonyl-based HBA, their structure–activity relationships (SAR), and biological profile.

In an effort to design a new class of CRF_1 antagonists, analysis of a general pharmacophore model constructed from the reported antagonists was very useful (Fig. 2(a)). The pharmacophore contains common structural features, which include (1) a monocyclic, a bicyclic, or a tricyclic core with a sp^2-nitrogen as HBA; (2) a large lipophilic group (R^a) attached to the core at the opposite position to the sp^2-nitrogen; (3) a pendant aryl ring minimally substituted in the *ortho* and the *para* positions orthogonal to the core ring; and (4) a small alkyl group (R^b) at the left side of the sp^2-nitrogen. Whereas SARs have been explored with various core structures, the sp^2-nitrogen as the HBA has never been given attention. We were interested in the replacement of the sp^2-nitrogen atom with a carbonyl group with negative charge as HBA (Fig. 2(b)). Carbonyl oxygen with two lone-pairs can provide wider angle for hydrogen-bonding than aromatic sp^2-nitrogen with one-lone pair. Considering the HBA in this case is affected by the intramolecular steric

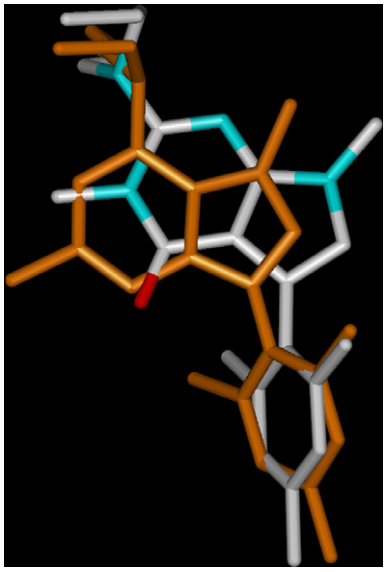
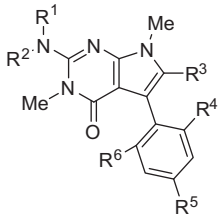


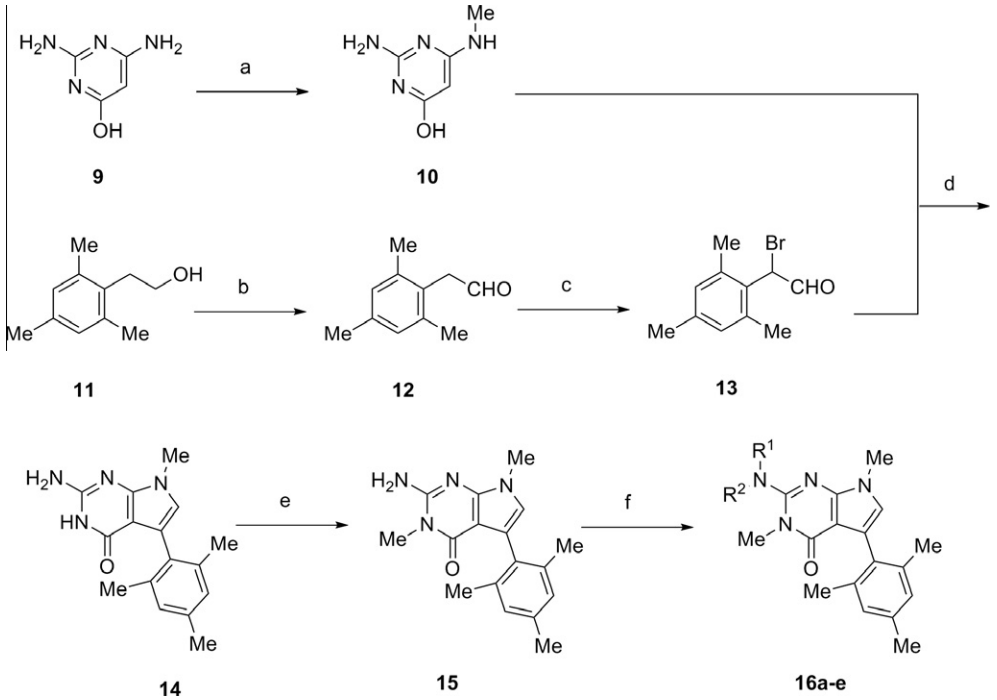
Figure 3. Superimposition of CP-154,526 **8** (orange) and **16b** (white).

Table 1
hCRF$_1$ binding affinities of pyrrolo[2,3-*d*]pyrimidin-4-one derivatives



Compound	R^1	R^2	R^3	R^4	R^5	R^6	hCRF$_1$ IC_{50}[a] (μM)	Log *P*[b]
16a	Et	Et	H	Me	Me	Me	7.7 (3.7–16.5)	3.45
16b	*n*Pr	*n*Pr	H	Me	Me	Me	0.12 (0.061–0.23)	4.52
16c	*n*Bu	*n*Bu	H	Me	Me	Me	0.97 (0.49–1.9)	5.58
16d			H	Me	Me	Me	0.14 (0.091–0.21)	4.19
16e			H	Me	Me	Me	3.8 (1.8–7.8)	3.16
21a	*n*Pr	*n*Pr	Me	Me	Me	Me	1.1 (0.67–1.9)	4.98
21b	*n*Pr	*n*Pr	Me	H	Me	Me	3.3 (0.97–11)	4.52
29a	*n*Pr	*n*Pr	H	H	Me	Me	1.6 (1.1–2.2)	4.05
29b	*n*Pr	*n*Pr	H	OMe	OMe	OMe	3.1 (2.5 -3.8)	1.92
29c	*n*Pr	*n*Pr	H	OMe	OMe	H	3.0 (0.97–9.0)	2.50
29d	*n*Pr	*n*Pr	H	CF_3	CF_3	H	3.0 (1.8- 4.9)	5.36
CP-316,311 (**3**)							0.092 (0.079–0.11)	6.20
CP-154,526 (**8**)							0.011 (0.0059–0.020)	6.63

[a] IC_{50} values represent 95% confidence intervals.
[b] Calculated by ACD Physchem Batch (Ver.10).



Scheme 1. Reagents: (a) $MeNH_2$, AcOH, 80%; (b) $PySO_3$, Et_3N, DMSO, 99%; (c) TMS-Br, DMSO/MeCN; (d) K_2CO_3, DMSO, 68% in two steps; (e) MeI, NaH, DMF, 62%; (f) alkylhalide, NaH, DMF, 8–87%.

hindrance from the vicinity aromatic ring, the wide angle of HBA capability of the carbonyl-based HBA can be the advantage to form a hydrogen bond feasibly with a hydrogen bond donor of the receptor. On the basis of these considerations, we thought that a pyrrolo[2,3-*d*]pyrimidin-4-one scaffold[23,24] could be an appropriate core structure with a carbonyl-based HBA. In addition, each key feature could be flexibly installed in this scaffold by using the pharmacophore model (Fig. 2(c)).

Alignment of the carbonyl group in the designed pyrrolo[2,3-*d*]pyrimidin-4-one derivative **16b** and the sp^2-nitrogen atom in CP-154,526 (**8**) resulted in good overlap of other moieties (Fig. 3). In the designed pyrrolo[2,3-*d*]pyrimidin-4-one derivatives,

alkyl substituents (R^1 and R^2) on the 2-amino group would be important to occupy the large lipophilic pocket on the top region. The steric repulsion between the *ortho* substituent on the pendant aromatic ring and the core ring would also play a significant role to generate the orthogonal conformation. On the other hand, the substituent at the 6-position (R^3) of the core ring might inhibit binding to the receptor due to its exterior location. From a different point of view, several of the reported antagonists, such as **2**, **3**, **7**, and **8**, show high lipophilicity (Log *P* calculated by ACD Physchem Batch (Ver.10): **2**, 5.75; **3**, 6.20; **7**, 7.75; **8**, 6.63). However, the pyrrolo[2,3-*d*]pyrimidin-4-one scaffold offers the potential to provide derivatives with Log *P* values closer to the range of 2–4 which is preferred for access to the central nervous system (Table 1).[25,26] Therefore, we prepared a series of pyrrolo[2,3-*d*]pyrimidin-4-ones, which were investigated for their ability to act as CRF_1 receptor antagonists with a carbonyl-based HBA.

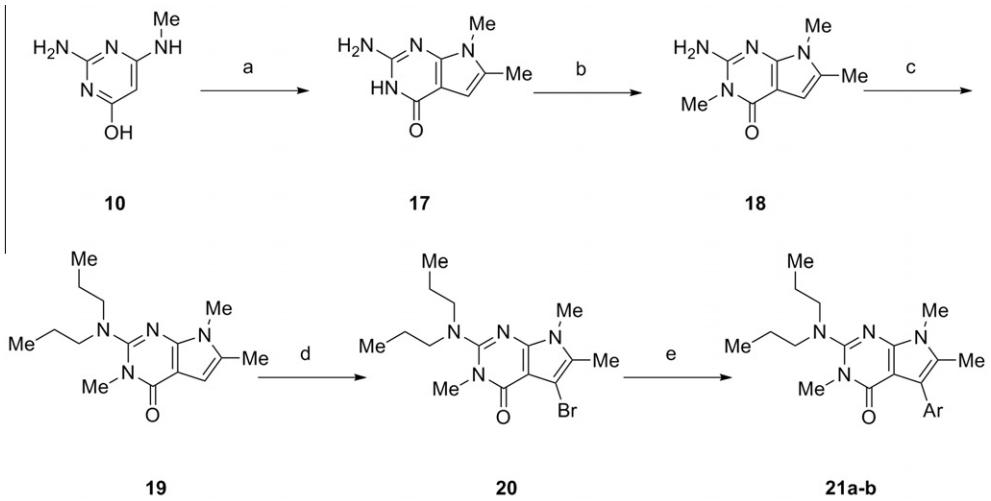
Our initial efforts focused on the substituent variation at the 2-position (R^1 and R^2) of pyrrolo[2,3-*d*]pyrimidin-4-one. The desired pyrrolo[2,3-*d*]pyrimidin-4-one derivatives **16a–e** were synthesized as illustrated in Scheme 1. 2-Amino-4-hydroxy-6-methylaminopyrimidine **10** was prepared from commercially available 2,4-diamino-6-hydroxypyrimidine **9** and methylamine. 2-(2,4,6-Trimethylphenyl)ethanol **11** was converted to 2,4,6-trimethylphenylacetaldehyde **12** under Swern oxidation conditions. Bromination of compound **12** with bromotrimethylsilane yielded α-bromoaldehyde **13**, which was cyclized with compound **10** under basic conditions to afford pyrrolo[2,3-*d*]pyrimidin-4-one **14**. Treatment of compound **14** with sodium hydride and iodomethane chemoselectively yielded 3-*N*-methylpyrrolo[2,3-*d*]pyrimidin-4-one **15**. Alkylation of the 2-amino group of compound **15** by various alkyl halides afforded the desired pyrrolo[2,3-*d*]pyrimidin-4-ones **16a–e**.[27]

We established a different synthetic route to prepare variations of the 5-aromatic group. This route permitted the installation of the aromatic group at the final step (Scheme 2). The reaction of 2-amino-4-hydroxy-6-methylaminopyromidine **10** and chloroacetone in the presence of sodium acetate yielded the 6-methylpyrrolo[2,3-*d*]pyrimidin-4-one derivative **17**, which was chemoselectively methylated at the 3-position to yield the 3-methyl derivative **18**. Alkylation of the 2-amino group of compound **18**, followed by bromination with *N*-bromosuccinimide (NBS) provided 5-bromo-2-(dipropylamino)pyrrolo[2,3-*d*]pyrimidin-4-one derivative **20** as a key intermediate to introduce an aromatic ring at the 5-positon. The palladium-catalyzed coupling reaction of compound **20** with an arylboronic acid afforded the desired pyrrolo[2,3-*d*]pyrimidin-4-ones **21a** and **21b**.

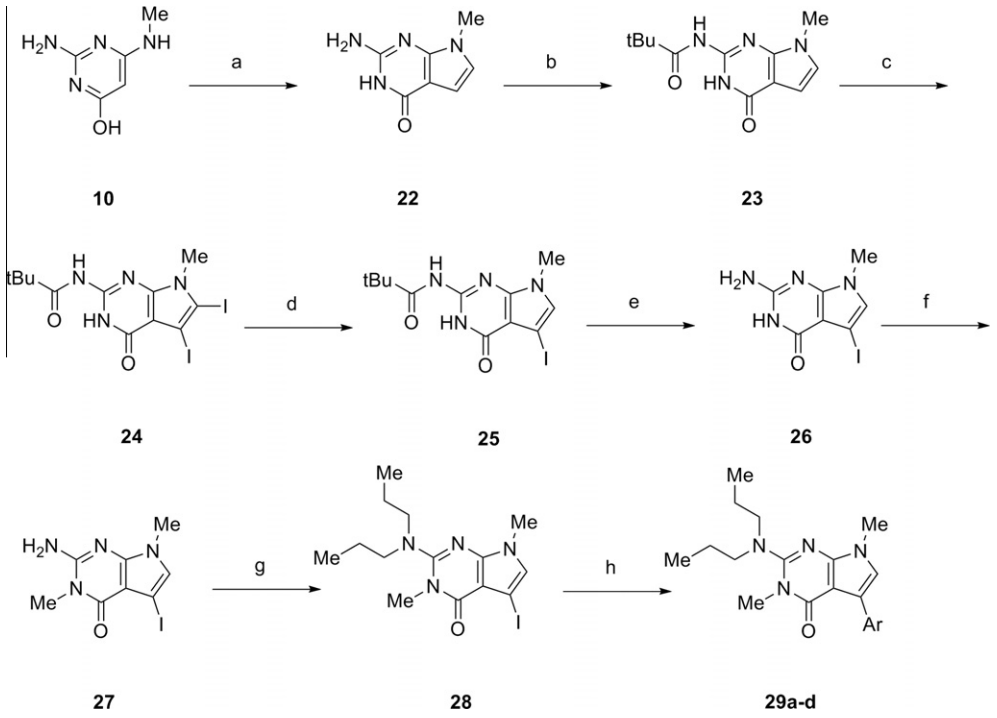
Preparation of the unsubstituted derivatives **29a–d** at the 6-position also required 5-halogenated pyrrolo[2,3-*d*]pyrimidin-4-one derivative **28** as a key intermediate (Scheme 3). We prepared the 5-iodopyrrolo[2,3-*d*]pyrimidin-4-one derivative **26** by using a modified method of Taylor et al.[28] After protection of the 2-amino group of pyrrolo[2,3-*d*]pyrimidin-4-one derivative **22**, treatment of compound **23** with excess amounts of *N*-iodosuccinimide (NIS) yielded the 5,6-diiodo derivative **24**. Regioselective deiodination of compound **24** was successfully conducted by treatment with zinc in acetic acid to afford the 5-monoiodo derivative **25**. After removing the protecting group at the 2-amino group, the target compounds **29a–d** were prepared by the method described in Scheme 2.

In order to confirm the function of the carbonyl group as the HBA, we prepared 4-chloro (**30**), 4-hydroxy (**31**), and 4-methoxy (**35**) derivatives. Chlorination of pyrrolo[2,3-*d*]pyrimidin-4-one derivative **14** with phosphorus oxychloride, followed by alkylation of 2-amino group provided the 4-chloropyrrolo[2,3-*d*]pyrimidine derivative **30**. Compound **30** was converted into 4-hydroxy derivative **31** by treatment with an alkaline solution. We determined that compound **31** was in the hydroxyl pyrimidine and not in the pyrimidone form based on the chemical shift of 6.11 ppm assigned to the 4-hydroxy proton.[29] The chemical shift of the amide NH proton in the pyrimidone form **32** should be found at 8–9 ppm (Scheme 4). Methylation of compound **14** by dimethyl sulfate yielded a mixture of 3-*O*-methyl **33** and 4-*N*-methyl **34** derivatives (11% and 32%, respectively). The 4-methoxy derivative **33** was alkylated by *n*-propyl iodide to afford the desired 4-methoxypyrrolo[2,3-*d*]pyrimidine **35** (Scheme 5).

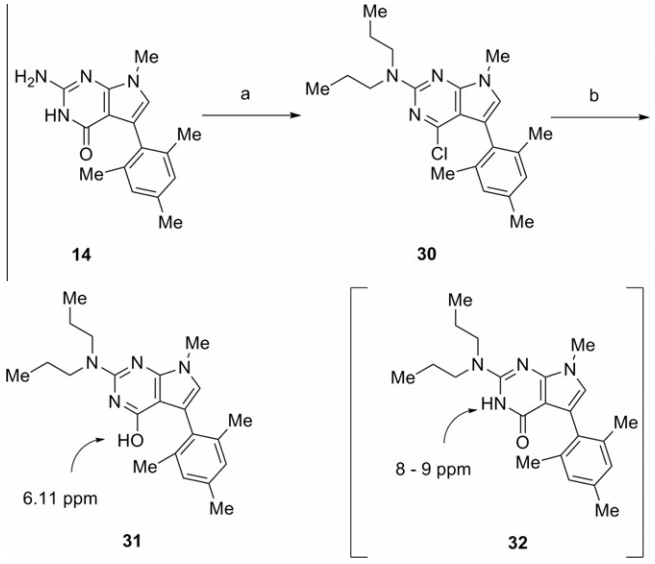
The synthesized compounds **16a–e**, **21a**, **21b**, **29a–d**, **30**, **31**, and **35** were screened for their inhibitory activities of ovine [^{125}I]CRF binding to human CRF_1 receptors expressed on Chinese hamster ovary (CHO) cellular membranes (Tables 1 and 2). We found that the pyrrolo[2,3-*d*]pyrimidin-4-ones **16a–e**, **21a**, **21b**, and **29a–d** with the carbonyl group exhibited potent CRF_1 receptor binding affinity (IC_{50} = 0.12–7.7 μM). The most active compound **16b** (IC_{50} = 0.12 μM) in this series was found to be as potent as CP-316,311 (**3**) (IC_{50} = 0.092 μM), evaluated in phase II. Unexpectedly, the binding affinity of **16b** was less than that of the superimposed CP-154,526 (**8**). Lone-pairs on the carbonyl oxygen atom of **16b** would be pointed slightly different direction from that of the sp^2-nitrogen of **8**. In addition, compound **16b** was inactive for CRF2α



Scheme 2. Reagents: (a) chloroacetone, NaOAc, EtOH/H_2O, 44%; (b) MeI, NaH, DNF, 74%; (c) *n*PrI, NaH, DMF, 42%; (d) NBS, DMF, 54%; (e) $ArB(OH)_2$, $Pd(Ph_3P)_4$, Na_2CO_3, DME, 10–14%.



Scheme 3. Reagents: (a) chloroacetaldehyde, K_2CO_3, MeOH, 22%; (b) (i) *t*BuCOCl, pyridine; (ii) aq NH_3, 75%; (c) NIS, DMF, 70%; (d) Zn, AcOH, H_2O, 78%; (e) NaOH, THF/H_2O, 91%; (f) MeI, NaH, DNF, 55%; (g) *n*PrI, NaH, DMF, 73%; (h) $ArB(OH)_2$, $Pd(Ph_3P)_4$, Na_2CO_3, DME, 24–44%.



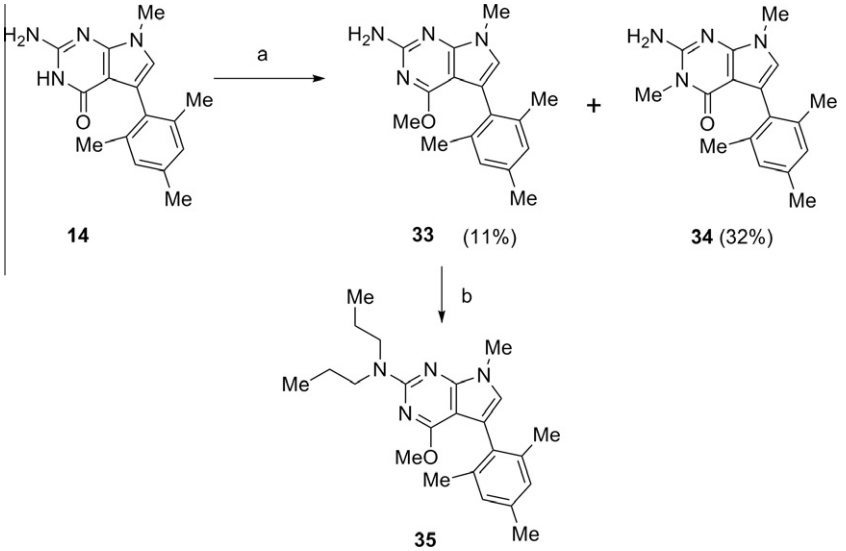
Scheme 4. Reagents: (a) (i) $POCl_3$, $PhNEt_2$; (ii) *n*PrI, NaH, DMF, 31%; (b) 1 N NaOH in H_2O, 81%.

and $CRF_{2\beta}$ receptors (IC_{50}, >10 μM). Compound **16b** also inhibited human CRF-stimulated cAMP accumulation with IC_{50} value of 1.2 μM (0.83–1.7 μM; 95% confidential interval) in CHO cells expressing human CRF_1 receptor, indicating that this compound functioned as CRF_1 receptor antagonist. The non-carbonyl derivatives, 4-chloro (**30**), 4-hydroxy (**31**), and 4-methoxy (**35**) analogues, were significantly less active (IC_{50}, >10 μM) (Table 2). These data indicated that the carbonyl group of the pyrrolo[2,3-*d*]pyrimidine derivatives functioned as HBA to bind selectively to the CRF_1 receptor. Our hypothesis of the carbonyl-based HBA could be supported by these results.

We attempted to investigate the primary SAR for these unique CRF_1 receptor antagonists with the carbonyl-based HBA. We initially determined the effect of the 2-amino group on CRF_1 receptor binding affinity (Table 1). The *N*-di-*n*-propylamino **16b** and *N*-di-(cyclopropylmethyl)amino **16d** derivatives displayed high binding affinity (IC_{50} = 0.12 and 0.14 μM, respectively). Replacement of the di-*n*-propylamino group with the *N*-diethylamino moiety resulted in a 70-fold lower binding affinity (**16a**, IC_{50} = 7.7 μM). *N*-Di-*n*-butylamino derivative **16c** (IC_{50} = 0.97 μM) with longer alkyl chains was also ninefold less active than the *N*-di-*n*-propylamino derivative **16b**. The cyclic tertiary amine **16e** displayed diminished affinity (IC_{50} = 3.8 μM). These data suggested that the 2 alkyls of C3 chain length (i.e., *n*-propyl and cyclopropyl) as 2-amino substituents adequately occupied the large lipophilic pocket on the top region in Figure 2(b).

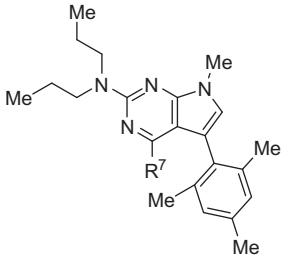
Substituent effects at the 6-position orthogonal to the 5-aryl group to the pyrrolo[2,3-*d*]pyrimidine core ring were also studied. Introduction of a methyl group into compound **16b** at the 6-position resulted in a 10-fold lower binding affinity (**21a**, IC_{50} = 1.1 μM). Comparison of **21b** versus **29a** of the 5-(2,4-dimethylphenyl) derivatives also showed that the 6-methyl analogue **21b** was less active than the 6-unsubstituted analogue **29a**. These results suggested that the substituent at the 6-position of the core ring inhibited binding to the receptor due to its exterior location as expected from the superimposed forms of **16b** and **8**.

We also briefly investigated the variation in substituents of the 5-phenyl group on binding affinity. The 2,4,6-trimethylphenyl derivative **16b** exhibited more potent binding affinity than the 2,4-dimethylphenyl derivative **21b** (**16b**, IC_{50} = 0.12 μM and **21b**, IC_{50} = 3.3 μM). The orthogonal conformation of the aromatic ring could be supported by the two ortho substituents. Introduction of electron donating groups, that is, methoxy groups, led to diminished binding affinity (**29b**, IC_{50} = 3.1 μM and **29c**, IC_{50} = 3.0 μM). Compounds with electron withdrawing groups, that is, trifluoromethyl groups, also did not show improved binding affinity (compare **29c** vs **29d**). These results suggested that the electron effects on the 5-phenyl group were independent of the binding activity.



Scheme 5. Reagents: (a) Me_2SO_4, K_2CO_3, acetone; (b) *n*PrI, NaH, DMF, 4%.

Table 2
$hCRF_1$ binding affinities of pyrrolo[2,3-*d*]pyrimidines



Compound	R^7	$hCRF_1$ IC_{50} (μM)
30	Cl	>10
31	OH	>10
35	OMe	>10

The penetration into the brain and the CRF_1 receptor binding of the most active compound **16b** in the unique carbonyl-based HBA series was evaluated by ex vivo ^{125}I-sauvagine (CRF_1 receptor ligand) binding in mice (Table 3).[30] Compound **16b** showed displacement of ^{125}I-sauvagine in the frontal cortex, olfactory bulb, and pituitary gland in mice at 30 mg/kg (po). The inhibitory rate of ^{125}I-sauvagine binding to the pituitary gland (53%) with regard to peripheral CRF_1 receptor binding was higher than that to the cortex (35%) and olfactory bulb (22%) in the brain. The relative brain/plasma ratio (0.42–0.66) estimated by ex vivo binding studies was sufficiently acceptable for a CNS drug. These data indicated that compound **16b** penetrated into the brain after oral absorption and bound to CRF_1 receptors in the CNS.

In conclusion, we identified a new class of pyrrolo[2,3-*d*]pyrimidin-4-one derivatives as CRF_1 receptor antagonists with a carbonyl-based HBA. Eleven pyrrolo[2,3-*d*]pyrimidin-4-one derivatives, prepared by efficient synthetic routes, showed CRF_1 receptor binding affinity, and their carbonyl group on the pyrrolo[2,3-*d*]pyrimidine core would function as HBA to bind CRF_1 receptors. Meanwhile, the non-carbonyl derivatives, 4-chloro (**30**), 4-hydroxy (**31**), and 4-methoxy (**35**) analogues, were significantly less active (IC_{50}, >10 μM). These findings would provide support for the hypothesis that the carbonyl group acted as a replacement for the sp^2-nitrogen atom as HBA in classical CRF_1 receptor antagonists.

Table 3
Ex vivo studies of **16b** at 30 mg/kg (po) in mice

^{125}I-sauvagine binding inhibition (%)		
Frontal cortex	Olfactory bulb	Pituitary
35	22	53

The representative compound **16b** in this series showed potent and selective CRF_1 receptor binding affinity in in vitro and ex vivo tests. Compound **16b** was also well absorbed orally and penetrated into the brain in mice. Further optimization studies of the pyrrolo[2,3-*d*]pyrimidin-4-one derivatives with a carbonyl-based HBA will be reported in due course.

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References and notes

1. Vale, W.; Spiess, J.; River, C.; River, J. *Science* **1981**, *213*, 1394.
2. Dzierba, C. D.; Hartz, R. A.; Bronson, J. J. *Annu. Rep. Med. Chem.* **2008**, *43*, 3.
3. Owens, M. J.; Nemeroff, C. B. *Pharmacol. Rev.* **1991**, *43*, 425.
4. Holsboer, F. *J. Psychiatry Res.* **1999**, *33*, 181.
5. Grigoriadis, D. E.; Haddach, M.; Ling, N.; Saunders, J. *Curr. Med. Chem.* **2001**, *1*, 63.
6. Perrin, M. H.; Grace, C. R.; Riek, R.; Vale, W. W. *Ann. N.Y. Acad. Sci.* **2006**, *1070*, 105.
7. Spiess, J.; Dautzenberg, F. M.; Sydow, S.; Hauger, R. L.; Ruhmann, A.; Blank, T.; Radulovic, J. *Trends Endocrinol. Metab.* **1998**, *9*, 140.
8. Timpl, P.; Spanagel, R.; Sillabar, I.; Kresse, A.; Reul, J. M.; Stalla, G. K.; Blanquet, V.; Steckler, T.; Holsboer, F.; Wurst, W. *Nat. Genet.* **1998**, *19*, 162.
9. Zobel, A. W.; Nickel, T.; Kunzel, H. E.; Ackl, N.; Sonntag, A.; Ising, M.; Holsboer, F. *J. Psychiatry Res.* **2000**, *309*, 171.
10. Ising, M.; Zimmermann, U. S.; Kunzel, H. E.; Uhr, M.; Foster, A. C.; Learned-Coughlin, S. M.; Holsboer, F.; Grigoriadis, D. E. *Neuropsychopharmacology* **2007**, *32*, 1941.
11. Binneman, B.; Feltner, D.; Kolluri, S.; Shi, Y.; Qiu, R.; Stiger, T. *Am. J. Psychiatry* **2008**, *165*, 617.

12. Coric, V.; Feldman, H. H.; Oren, D. A.; Shekhar, A.; Pultz, J.; Dockens, R. C.; Wu, X.; Gentile, K. A.; Huang, S.-P.; Emison, E.; Delmonte, T.; D'Souza, B. B.; Zimbroff, D. L.; Grebb, J. A.; Goddard, A. W.; Stock, E. G. *Depress. Anxiety* **2010**, *27*, 417.
13. Sweetser, S. R.; Linker Nord, S. J.; Burton, D. D.; Grudell, A.; Eckert, D. J.; Manini, M. L.; Busciglio, I.; Tong, G.; Dockens, R. C.; Zinsmeister, A. R.; Camilleri, M. *Gastroenterology* **2008**, *134*, Abstr T1405.
14. Thoua, N. M.; Hobson, A. R.; Dukes, G. E.; Kelleher, D. L.; Keller, D. L.; Hicks, K. J.; Boardley, R. L.; Raeburn, A. J.; Emmanuel, A. V. *Gastro* **2009**, London, UK, November 2009, OP097.
15. Tellew, J. E.; Lanier, M.; Moorjani, M.; Lin, E.; Luo, Z.; Slee, D. H.; Zhang, X.; Hoare, S. R. J.; Grigoriadis, D. E.; Denis, Y. S.; Romano Di Fabio, R. D.; Modugno, E. D.; Saunders, J.; Williams, J. P. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7259.
16. Gully, D.; Geslin, M.; Serva, L.; Fontaine, E.; Roger, P.; Lair, C.; Darre, V.; Marcy, C.; Pierre-Eric Rouby, P.-E.; Simiand, J.; Guitard, J.; Gout, G.; Steinberg, R.; Rodier, D.; Griebel, G.; Philippe Soubrie, P.; Pascal, M.; Pruss, R.; Scatton, B.; Maffrand, J.-P.; Fur, G. L. *J. Pharmacol. Expert Ther.* **2002**, *301*, 322.
17. ClinicalTraials.gov (A service of the U.S. National Institutes of Health; http://clinicaltrial.gov), October 23, 2010.
18. *Sanofi-Aventis company report* (http://en.sanofi-aventis.com), R&D Portfolio as of February 9, 2011.
19. Gehlert, D. R.; Cippitelli, A.; Thorsell, A.; Lê, A. D.; Hipskind, P. A.; Hamdouchi, C.; Lu, J.; Hembre, E. J.; Cramer, J.; Song, M.; McKinzie, D.; Morin, M.; Ciccocioppo, R.; Heilig, M. *J. Neurosci.* **2007**, *27*, 2718.
20. Hummel, M.; Cummons, T.; Lu, P.; Mark, L.; Harrison, J. E.; Kennedy, J. D.; Whiteside, G. T. *Neuropharmacology* **2010**, *59*, 160.
21. Tellew, J. E.; Luo, Z. *Curr. Top. Med. Chem.* **2008**, *8*, 506.
22. Schulz, D. W.; Mansbach, R. S.; Sprouse, J.; Braselton, J. P.; Collins, J.; Corman, M.; Dunaiskis, A.; Fraci, S.; Schmidt, A. W.; Seeger, T.; Seymour, P.; Tingley, F. D., III; Winston, E. N.; Chen, Y. L.; Heym, J. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, 10477.
23. Aso, K.; Hitaka, T.; Yukishige, K.; Ootsu, K.; Akimoto, H. *Chem. Pharm. Bull.* **1995**, *43*, 256.
24. Aso, K.; Imai, Y.; Yukishige, K.; Ootsu, K.; Akimoto, H. *Chem. Pharm. Bull.* **2001**, *49*, 1280.
25. Hitchcock, S. A.; Pennington, L. D. *J. Med. Chem.* **2006**, *49*, 7559.
26. Wager, T. T.; Chandrasekaran, R. Y.; Hou, X.; Troutman, M. W.; Verhoest, P. R.; Villalobos, A.; Will, Y. A. C. S. *Chem. Neurosci.* **2010**, *1*, 420.
27. All new compounds gave satisfactory analytical data. For **16b**: ^{1}H NMR ($CDCl_3$) δ 0.90 (6H, t, J = 7.5 Hz), 1.61 (4H, m), 2.11 (6H, s), 2.29 (3H, s), 3.11 (4H, t, J = 7.5 Hz), 3.47 (3H, s), 3.70 (3H, s), 6.43 (1H, s), 6.90 (2H, s). MS calculated: 380. Found: 381 (M+H). Mp: 100–102 °C. Anal. Calcd for $C_{23}H_{32}N_4O$: C, 72.60; H, 8.48; N, 14.72. Found: C, 72.64; H, 8.66; N, 14.36. For **16d**: ^{1}H NMR ($CDCl_3$) δ 0.12–0.17 (4H, m), 0.45–0.52 (4H, m), 1.01–1.10 (2H, m), 2.11 (6H, s), 2.29 (3H, s), 3.10 (4H, d, J = 6.6 Hz), 3.52 (3H, s), 3.72 (3H, s), 6.45 (1H, s), 6.90 (2H, s). MS calcd: 404; found: 405 (M+H). Mp: 218–221 °C. Anal. Calcd for $C_{25}H_{32}N_4O$: C, 74.22; H, 7.97; N, 13.85. Found: C, 74.20; H, 7.89; N, 13.83.
28. Taylor, E. C.; Kuhnt, D.; Shih, C.; Rinzel, S. M.; Grindey, G. B.; Barredo, J.; Jannatipour, M.; Moran, R. G. *J. Med. Chem.* **1992**, *35*, 4450.
29. For **31**: ^{1}H NMR ($CDCl_3$) δ 0.94 (6H, t, J = 7.5 Hz), 1.67 (4H, m), 2.06 (6H, s), 2.30 (3H, s), 3.57 (4H, t, J = 7.5 Hz), 3.69 (3H, s), 6.11 (1H, s), 6.48 (1H, s), 6.90 (2H, s). MS calcd: 366; found: 367 (M+H).
30. *Ex vivo binding assay in mice*: The test compound or the corresponding vehicle was administered po to mice (10 per group) at 30 mg/kg, 60 min before mouse decapitation and organ removal (frontal cortex, olfactory bulb, and pituitary). Tissues were homogenized in lysis buffer (50 mM Tris–HCl, 10 mM $MgCl_2$, 2 mM ethylenediaminetetraacetic acid (EDTA), 100 KU/mL aprotinin) and diluted (final protein concentration: 5 mg/mL). [^{125}I]-sauvagine binding was performed using membrane homogenates in the presence of 100 pM of [^{125}I]-sauvagine in lysis buffer containing 0.1% bovine serum albumin (BSA), 0.5% dimethyl sulfoxide (DMSO), and 0.05% 3-[(3-cholamido-propy1)-dimethylammonio]-l-propanesulfonate (CHAPS) in a final volume of 200 μL. After incubation at room temperature for 2 h, the incubation mixture was filtered through Whatman GF/C filter presoaked in 0.3% polyethyleneimine. The filters were washed six times with ice-cold wash buffer (phosphate buffered saline (PBS) containing 0.05% CHAPS and 0.01% Triton X-100) and dried. The radioactivity was determined using a gamma scintillation counter. Nonspecific binding was determined using 1 μM unlabeled selective CRF_1 antagonist R121919. The mean value of the 2 independent experiments was expressed as inhibitory rate of [^{125}I]-sauvagine binding.