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2-Aryl-3,6-dialkyl-5-dialkylaminopyrimidin-4-ones as Novel CRF-1 Receptor Antagonists

Kevin J. Hodgetts, Taeyoung Yoon, Jianhua Huang, Michael Gulianello, Andrzej Kieltyka, Renee Primus, Robbin Brodbeck, Stéphane De Lombaert and Darío Doller*

Neurogen Corporation, 35 NE Industrial Road, Branford, CT 06405, USA

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Dedicated to the memory of Prof. Eduardo G. Gros and Prof. Alicia M. Seldes

Abstract—The discovery, synthesis and structure–activity studies of a novel series of 2-arylpyrimidin-4-ones as CRF-1 receptor antagonists is described. These compounds are structurally simple and display appropriate physical properties for CNS agents

In spite of numerous marketed treatments, anxiety and depression are psychiatric disorders which constitute a major health concern. Agents with increased efficacy or a reduced side-effects profile are actively sought by pharmaceutical companies. Corticotropin-releasing factor (CRF) is a 41-aminoacid peptide characterized as the principal mediator of the effects of stress via the hypothalamic-pituitary-adrenocortical (HPA) axis. CRF receptors are 7-transmembrane Gαs-protein coupled receptors. They are classified in two subtypes, CRFR-1 and CRFR-2; these two share ca. 70% homology. Extensive biological research led to the hypothesis that CRF-1 receptor antagonists might be useful in treating anxiety and depression.[1] The conclusions from an open-label clinical study involving R121919, a small-molecule CRF-1 receptor antagonist, were reported recently. The outcome was considered encouraging as R121919 produced a decrease in symptoms of both depression and anxiety.[2] Eventually, its clinical development was discontinued, seemingly due to elevation of liver enzymes. Other CRF-1 antagonists (structures not disclosed) are reportedly undergoing clinical trials at the present time.

A number of CRF-1 receptor antagonists have been reported. They can be classified into two topologies based on distinct structural features.[1] The diversity of chemical matter among leading series is rather narrow, particularly considering the compounds which reached advanced preclinical development or clinical stage (e.g., topology 1: CP-154,526, R121919, DMP 904, NGD 98-1; topology 2: SSR125543A; Fig. 1). In our studies we set out to explore the possibility of identifying novel small-molecule CRF-1 receptor antagonists. Specifically, our strategy was to seek an appropriate linker to replace the bicyclic or tricyclic cores found in most reported antagonists. In addition, we envisioned that such a linker would have the potential for decreasing the molecular weight relative to existing antagonists. Herein we report our work leading to the discovery of 2-aryl-3,6-dialkyl-5-dialkylaminopyrimidin-4-ones (e.g., **1**) as CRF-1 receptor antagonists.

Planning for an appropriate central scaffold we considered the following facts. Prior structure–activity relationship studies for bicyclic and tricyclic CRF-1 receptor antagonists had demonstrated that certain structural features are needed for small-molecule high-affinity binding to the receptor.[1] First, an out-of-plane relationship between the bottom aromatic ring and the heterocyclic core is key. Second, at least one nitrogen atom is required on the central ring, presumably playing the role of hydrogen bond acceptor. The position of this nitrogen atom is critical for activity. Two topologies for CRFR-1 antagonists have been proposed. These differ in the number of atoms linking the bottom aromatic group and this essential nitrogen atom on the central heterocycle.[1] Findings from our preliminary studies were in agreement with this model (Fig. 2). We decided to keep the top portion as a di-*n*-propylamino group for our

*Corresponding author. Tel.: +1-203-488-8201x3114; fax: +1-203-481-5290; e-mail: ddoller@nrgn.com

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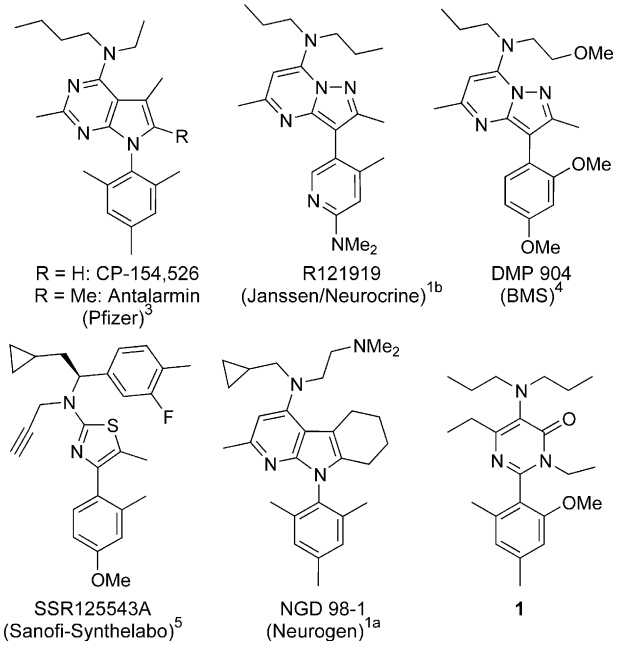


Figure 1. Structures of selected CRF-1 receptor antagonists.

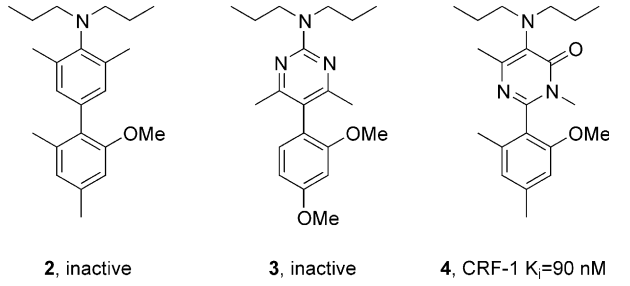


Figure 2. Early analogues defining the need for a nitrogen atom on a specific position of the central ring.

exploratory work, as it produced analogues with acceptable affinity levels. While no significant binding was observed for aniline **2** or pyrimidine **3**, pyrimidone **4** did show affinity for CRFR-1. This suggested that a nitrogen atom on a specific location of the central ring is also needed for CRFR-1 binding in this new pyrimidone-based template, and that the optimal position was that in **4** and not that in **3**.

After defining a novel CRF-1 receptor binding template, the next step became trying to improve its binding affinity towards the receptor. Results from our structure–activity relationship studies are summarized in Table 1. Affinities were determined by examining the displacement of [^{125}I]-sauvagine from CRF-1 receptors endogenously expressed in IMR-32 human neuroblastoma cells.[6,7] Functional antagonism of this series was established in a secondary assay, measuring inhibition of sauvagine-stimulated cAMP accumulation in AtT_{20} cells which endogenously express CRFR-1.[6,8] Compounds screened in this functional assay had K_i values between 10 and 100 nM (Table 1). These values tracked reasonably well with the binding affinity.

Conclusions from our SAR studies are as follows. In terms of affinity for the CRF-1 receptor, *N*-unsubstituted pyrimidones were typically inactive or much less active than their *N*-alkyl analogues. *N*-ethyl substitution on the pyrimidone ring provided a number of potent analogues (e.g., **1**, **6**, **16**, **25**, **29**). Further increasing the size of the alkyl group or introducing solubilizing groups (**14**, **26**)[9] resulted in significant loss of affinity. Similarly, ethyl substitution on position 6 of the pyrimidone ring led to analogues with high affinity (**1** and **13**). While 2,4,6-trisubstitution on the aryl ring provided some of the more potent analogues, it was not essential for potency, as appropriate 2,4-disubstitution also resulted in compounds of comparably high affinity (e.g., **29**). The presence of a methoxy group in one of the *ortho* positions resulted in an increase in affinity with respect to a methyl substituent (e.g., **4** vs **15**, **6** vs **16**). The poor affinity of analogues bearing a hydrogen on the *para* position underscores the importance of such substitution (**19**, **20** and **22**). These substituent effects on affinity parallel those observed for a number of CRF-1 receptor antagonists.[1]

By appropriate tuning of the bottom phenyl ring and the substitution on the pyrimidone core, potent CRF-1 antagonists were obtained. As expected, the required introduction of alkyl groups on the pyrimidone core resulted in an overall increase in lipophilic character and a decrease in aqueous solubility at pH 7.4. Calculated Mlog P and polar surface area (PSA) values were generally within the range which predicts reasonably good membrane permeability and brain penetration.[10] Disappointingly, human microsomal half-lives for these compounds were too short to predict appropriate plasma concentrations over a useful period of time. To explore the potential for oral plasma and brain exposure of this series a rat pharmacokinetic study was carried out on a representative. Compound **6** showed negligible plasma exposure following oral administration in rats ($F = 2.6 \pm 0.5\%$ at a 20 mg/kg dose, $n = 3$; $C_{max} = 234 \pm 52$ ng/mL, $T_{max} = 0.6 \pm 0.4$ h, $T_{1/2} = 3.3 \pm 0.3$ h, $Cl = 16.6 \pm 9.5$ mL/min/kg, $Vd = 11.5 \pm 0.2$ L/Kg). The brain to plasma ratio was $B/P = 0.9 \pm 0.4$ mL/g.

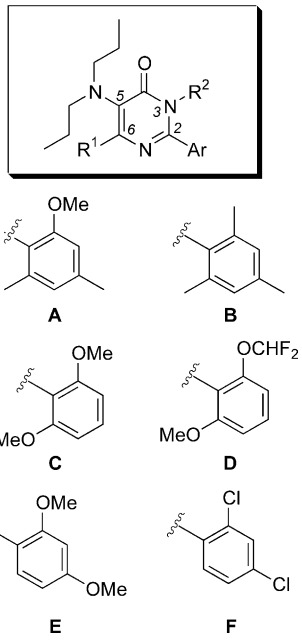
Noticing that some *N*-unsubstituted pyrimidin-4-ones (**5**, **12**, **17**, **18**, **21**, **23**) had more-acceptable microsomal stabilities than their *N*-Me or *N*-Et counterparts, we speculated that CYP-450-mediated *N*-dealkylation might be the major metabolic event leading to short half-lives. Unfortunatelly, attempts at increasing microsomal half-lives by blocking this dealkylation process through fluorination or introduction of heteroatoms on the *N*-alkyl substituent group did not prove successful. These compounds were found to be much weaker (**9**, **14**, **26**) or reasonably potent but still metabolically unstable (**10**). This can be explained by the existence of several metabolic routes for these compounds.

Analogue Syntheses

Pyrimidin-4-ones reported in Table 1 were synthesized starting from compound **30** as exemplified in Scheme 1 for analogue **1**. Nitration of commercially available 2,4-dihydroxy-6-methylpyrimidine **30** yielded nitropyrimidine **31**, which was stepwise converted to chloropyr-

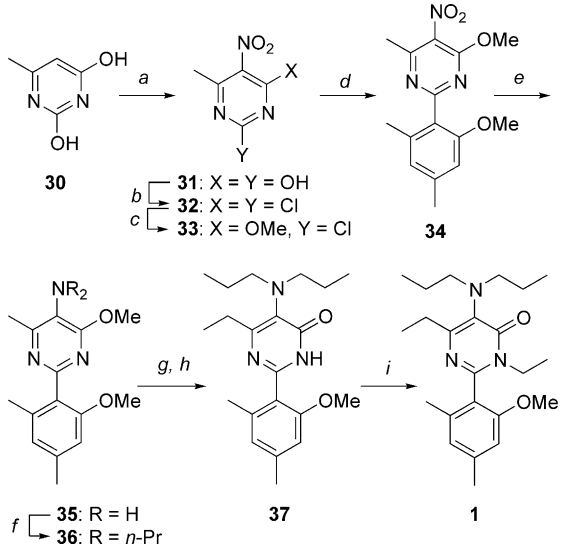
Table 1. CRF-1 receptor affinity, microsomal stability and physical properties for compounds **1** and **4–29**

Compd	R^1	R^2	Ar[a]	CRF-1 K_i, nM[b]	Microsomal $T_{1/2}$, min[c]	Solubility (μg/mL)[d]	PSA, Å^2[e]	MlogP[e]	AtT_{20} K_i, nM[f]	Definitions
4	Me	Me	**A**	90	2.5	3.3	41.2	3.30		
5	Me	H	**A**	930	15.5	10.3	53.2	3.08		
6	Me	Et	**A**	21	3.6	1.3	40.4	3.52	26	
7	Me	*n*-Pr	**A**	190	3.0	<0.1	36.3	3.74		
8	Me	*i*Pr	**A**	47	2.1	<0.1	28.9	3.74		
9	Me	CH_2CF_3	**A**	1630	3.4	<0.1	35.5	3.84		
10	Me	CH_2CH_2F	**A**	39	3.0	<0.1	36.3	3.63	90	
11	Me	Bn	**A**	IA	2.2	0.4	34.9	4.33		
12	Et	H	**A**	560	8.1	3.8	52.4	3.30		
13	Et	Me	**A**	7	3.3	2.5	39.9	3.52		
1	Et	Et	**A**	4	2.3	<0.1	35.3	3.74	7	
14	Me	$(CH_2)_2N(CH_2)_4$	**B**	IA	4.2	25.2	31.8	4.09		
15	Me	Me	**B**	135	4.6	2.2	29.6	3.82		
16	Me	Et	**B**	72	3.6	0.9	26.2	4.04		
17	Me	H	**B**	IA	7.5	0.4	40.6	3.82		
18	Me	H	**C**	IA	13.3	23.6	65.0	2.35		
19	Me	Me	**C**	530	1.8	6.5	52.6	2.57		
20	Me	Et	**C**	310	1.0	20.5	48.2	2.80		
21	Me	H	**D**	IA	21.6	ND	64.5	2.69		
22	Me	Et	**D**	620	1.8	<0.1	47.8	3.12		
23	Me	H	**E**	IA	9.4	7.9	60.2	2.35		
24	Me	Me	**E**	470	6.0	41.5	53.7	2.57		
25	Me	Et	**E**	38	3.0	17.5	52.3	2.80	68	
26	Me	$(CH_2)_2OMe$	**E**	4000	1.3	n/a	64.5	2.26		
27	Me	*n*Pr	**E**	720	2.3	4.9	52.3	3.02		
28	Me	$CH_2(CH_2)_3$	**E**	260	1.5	20.1	50.2	3.23		
29	Me	Et	**F**	15	1.9	0.9	29.4	4.36	36	



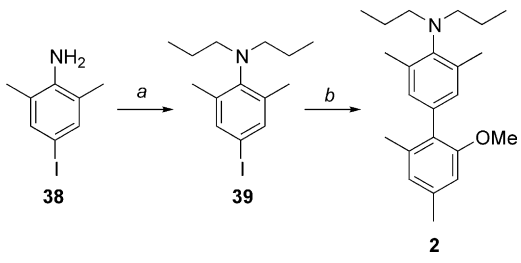
[a]For Ar group definitions see Table 1.
[b]According to ref 7. K_i values are means of at least two determinations; standard errors lower than 25%. IA = inactive. ND = not determined.
[c]Human microsomes.
[d]At pH 7.4.
[e]Values calculated using AIDD software.[11]
[f]Functional assay. See refs 6 and 8.

imidine **33**. Suzuki coupling of **33** under standard conditions afforded nitropyrimidine **34**. Reduction to aminopyrimidine **35** by catalytic hydrogenation at atmospheric pressure (balloon), followed by reductive amination yielded quantitatively dialkylaminopyrimidine **36**. Alkylation of the acidic methyl group on the pyrimidine ring was accomplished by treatment with a slight excess of lithium diisopropylamide followed by quenching with methyl iodide. Hydrolysis of the methoxypyrimidine to pyrimidone **37** under acidic conditions, followed by base-promoted alkylation produced *N*-ethyl pyrimidone **1**. Small amounts of the isomeric 4-methoxypyrimidines were reaction byproducts.

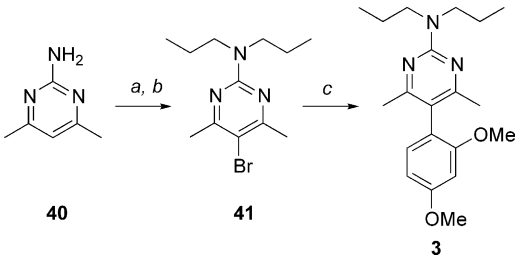


Scheme 1. Reagents and conditions: (a) KNO_3, H_2SO_4 (75%); (b) $OPCl_3$ $PhNEt_2$, reflux (60%); (c) MeONa, MeOH (35%); (d) $Pd[(PPh_3)]_4$, 2,4-Me_2-6-$(MeO)C_6H_2B(OH)_2$, $NaHCO_3$, toluene, 70 °C (65%); (e) H_2, Pd/C (100%); (f) Propionaldehyde, AcOH, $NaBH(OAc)_3$, 1,2-dichloroethane, rt (100%); (g) (1) LDA, THF, −78 °C. (2) MeI, −78 °C to rt (75%); (h) HCl 1M (100%); (i) EtI, NaH, THF, rt (50%).

Synthesis of probe compounds **2** and **3** was carried out as outlined in Schemes 2 and 3. For the case of **2**, starting out with commercially available 4-iodo-2,6-dimethylaniline **38**, reductive amination to dialkylamine **39,** followed by Suzuki coupling yielded biphenylamine **2**. For pyrimidine **3** (Scheme 3) the starting material was compound **40**. Reductive amination followed by bromination produced bromide **41**.



Scheme 2. Reagents and conditions: (a) Propionaldehyde, AcOH, $NaBH(OAc)_3$, 1,2-dichloroethane (100%); (b) $Pd[(PPh_3)]_4$, 2,4-Me_2-6-$(MeO)C_6H_2B(OH)_2$, $NaHCO_3$, toluene, 70 °C (35%).



Scheme 3. Reagents and conditions: (a) Propionaldehyde, AcOH, $NaBH(OAc)_3$, 1,2-dichloroethane (70%). (b) Br_2, $CHCl_3$, rt, 95%. (c) $Pd[(PPh)_3]_4$, 2,4-$(MeO)_2C_6H_3B(OH)_2$, $NaHCO_3$, toluene, 70 °C (15%).

Suzuki coupling yielded final pyrimidine **3**, albeit in low overall yield.

In summary, we have described the discovery, synthesis and structure–affinity relationship study of a new series of CRF-1 receptor antagonists. These are structurally simple and have physical properties consistent with CNS-active drugs. At this stage, their unacceptable pharmacokinetic profile, presumably due to low microsomal stability or poor absorption, rendered this series of compounds unsuitable for further preclinical development. However, it can be expected that related series could be found with similar pharmacophoric elements to those reported herein and possessing improved permeability characteristics and/or metabolic stability. The results of our continuing efforts to solve these issues will be reported in due course.

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References and Notes

1. For recent reviews, see: (a) Holsboer, F. *Curr. Opinion Invest. Drugs* **2003**, *4*, 46. (b) Kehne, J.; De Lombaert, S. *Curr. Drug Targets* **2002**, *1*, 467. (c) Lanier, M.; Williams, J. P. *Expert Opin. Ther. Patents* **2002**, *12*, 1619. (d) Grigoriadis, D. E.; Haddach, M.; Ling, N.; Saunders, J. *Curr. Med. Chem.* **2001**, *1*, 63. (e) Gilligan, P. J.; Robertson, D. W.; Zaczek, R. *J. Med. Chem.* **2000**, *43*, 1641.
2. Zobel, A. W.; Nickel, T.; Kunzel, H. F.; Ackl, N.; Sonntag, A.; Ising, M.; Holsboer, F. *J. Psych. Res.* **2000**, *34*, 171.
3. Webster, E. L.; Lewis, D. B.; Torpy, D. J.; Zachman, E. K.; Rice, K. C.; Chrousos, G. P. *Endocrinology* **1996**, *137*, 5747.
4. He, L.; Gilligan, P. J.; Zaczek, R.; Fitzgerald, L. W.; Mc Elroy, J.; Shen, H. S. L.; Saye, J. A.; Kalin, N. H.; Shelton, S.; Christ, D.; Traimor, G.; Hartig, P. *J. Med. Chem.* **2000**, *43*, 449.
5. Gully, D.; Geslin, M.; Serva, L.; Fontaine, E.; Roger, P.; Lair, C.; Dane, V.; Marcy, C.; Rouby, P. E.; Simiand, J.; Guitard, J.; Gout, G.; Steinberg, R.; Rodier, D.; Griebel, G.; Soubrie, P.; Pascal, M.; Pruss, R.; Scatton, B.; Maffrand, J. P.; Le Fur, G. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 322. Griebel, G.; Simiand, J.; Steinberg, R.; Jung, M.; Gully, D.; Roger, P.; Geslin, M.; Scatton, B.; Maffrand, J. P.; Soubrie, P. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 333.
6. Yuan, J.; Gulianello, M.; De Lombaert, S.; Brodbeck, R.; Kieltyka, A.; Hodgetts, K. J. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2133.
7. Grigoriadis, D. E.; De Souza, E. B. *Methods Neurosci.* **1991**, *5*, 510.
8. Chaki, S.; Okuyama, S.; Nakazato, A.; Kumagai, T.; Okubo, T.; Ikeda, Y.; Oshida, Y.; Hamajima, Y.; Tomisawa, K. *Eur. J. Pharmacol.* **1999**, *371*, 205.
9. Wermuth E. G. In *The Practice of Medicinal Chemistry*. Wermuth, E. G., Ed. Academic Press: New York, 1996; chapter 35, pp 755–776.
10. Atkinson, F.; Cole, S.; Green, C.; van de Waterbeemd, H. *Curr. Med. Chem-CNS Agents* **2002**, *2*, 229.
11. http://www.neurogen.com/technology.htm