Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2067–2070

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

PYRAZOLO[1,5-a]PYRIMIDINE CRF-1 RECEPTOR ANTAGONISTS

David J. Wustrow,* Thomas Capiris, Ronald Rubin, James A. Knobelsdorf, Hyacinth Akunne, M. Duff Davis, Robert MacKenzie, Thomas A. Pugsley, Kim T. Zoski, Thomas G. Heffner, and Lawrence D. Wise

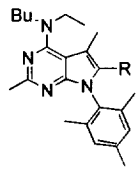
Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor MI, 48105 U.S.A.

Received 8 April 1998; accepted 22 May 1998

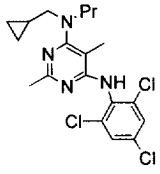
Abstract: A series of 3-phenylpyrazolo[1,5-**a**]pyrimidines was prepared and found to have affinity for the human CRF-1 receptor. The 3-dimensional structure of one of the most potent analogs in this series, **10d**, was determined by X-ray crystallography and suggests the spatial requirements for potent CRF-1 receptor binding affinity in this series.

Corticotrophin releasing factor (CRF) is a primary endocrine factor necessary for the activation of the hypothalamic-pituitary-adrenal (HPA) axis as a physiological response to stressful stimuli. The binding of CRF to CRF-1 receptors in the hypothalamus is responsible for the increased release of ACTH and other peptides.[1] Prolonged activation of brain CRF receptors is thought to be related to the psychological effects of stress leading to anxiety and depression and blockade of CRF-1 receptor activation has been proposed as a novel approach for the treatment of these psychiatric disorders.[2]

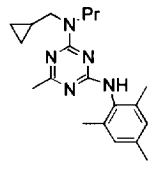
Small molecule CRF-1 receptor antagonists exemplified by compounds (**1-5**) have been reported in the literature.[3-7] All are comprised of a core heterocyclic ring supporting amino, methyl and substituted aryl functionalities.[8] In a search for novel heterocyclic templates carried out on the Parke–Davis compound library, pyrazolo[1,5-**a**]pyrimidines of the general structure **6** were discovered having a topographical similarity to known CRF-1 receptor antagonists. In this paper, we disclose structure–activity relationship studies around this nucleus leading to the discovery of a potent class of CRF-1 receptor antagonists.



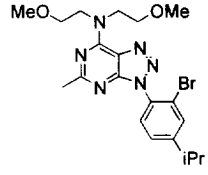
1 R = H
2 R = Me



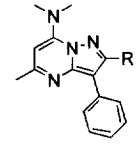
3



4



5

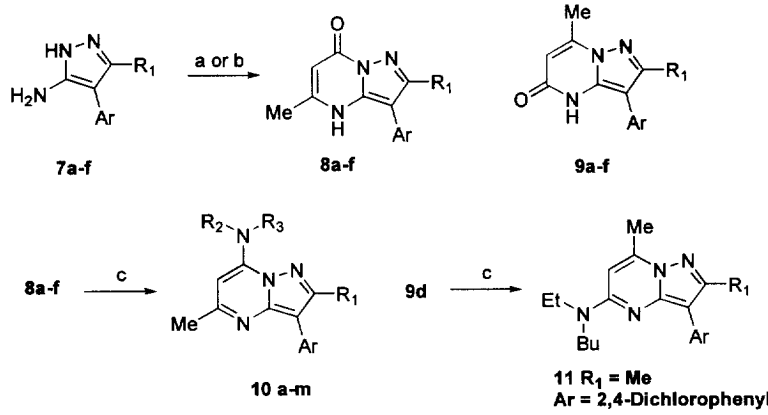


6

A general scheme for the synthesis of the pyrazolo[1,5-**a**]pyrimidine targets is outlined in Scheme 1. Condensation of a series of 3-phenyl-2-aminopyrazoles **7**[9] with ethyl acetoacetate in the presence of tosic acid in refluxing toluene resulted in the production of the regioisomeric pyrazolo[1,5-**a**]pyrimidines **8** and **9** with the predominant regiosomers **8** being isolated by crystallization. In one case it was found that when the condensation was carried out using ammonium chloride in refluxing toluene regioisomer **9d** (R_1 = Me, Ar = 2,4-

0960-894X/98/$ - see front matter
PII: S0960-894X(98)00372-2

dichlorophenyl) predominated and could be isolated by column chromatography. Pyrazolopyrimidinones **8a–f** and **9d** were converted to their corresponding chloro derivatives and reacted with various amines giving the 7-aminopyrazolo[1,5-**a**]-pyrimidines **10a–m** and **11** as the final products. The regiochemistry of products **10** and **11** (and by extension **8** and **9**) was unambiguously determined by an X-ray diffraction study of **10d** (R_1 = Me, Ar = 2,4-dichlorophenyl, R_2 = Et, R_3 = nBu). (Figure 1).



Scheme 1. Synthesis of substituted pyrazolopyrimidines. (a) Ethylacetoacetate, *p*-TSA, toluene reflux; (b) NH_4Cl, toluene reflux; (c) i. $POCl_3$, ii. NHR_2R_3.

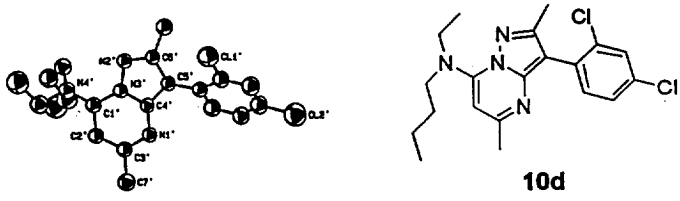
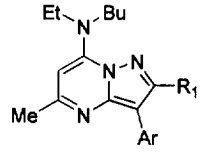


Figure 1. X-ray structure of **10d**.

The CRF-1 receptor binding assay was performed with cloned human CRF-1 receptors expressed in CHO-cells using [^{125}I]o-CRF as the ligand in a manner similar to that previously reported.[10] The prototypical compound in this series was the unsubstituted phenyl analog **10a**, which was found to have affinity for the CRF-1 receptor (K_i = 511 nM). Introduction of a 2-chloro substituent on the phenyl ring (compound **10b**) resulted in an over 30-fold increase in binding affinity for the CRF-1 receptor. The 4-chlorophenyl analog **10c** also had improved receptor affinity (K_i = 77 nM). Introduction of a 2,4-dichlorophenyl substituent resulted in compound **10d** having a K_i of 5 nM and was the most potent phenyl substituent pattern in the series. Interestingly the 2,4,6-trimethylphenyl analog **10e**, having the phenyl substitution pattern found in CP 154526 (**1**), had approximately

17-fold lower potency than **10d**. In the pyrazolopyrimidine series the 2-chloro substituent appears to contribute more to binding affinity than does the 4-chloro substituent. This is due at least in part to the fact that the 2-methyl substituent helps to induce a perpendicular orientation between the phenyl and pyrazolopyrimidine rings. Indeed this is the conformation adopted by the 2,4-dichlorophenyl ring as determined in the X-ray crystallographic study of **10d**. Replacement of the 2-methyl substituent with hydrogen (compound **10f**) resulted in an 8-fold decrease in binding affinity. A methyl group in the 2 position may help keep the phenyl ring in an orientation perpendicular to pyrazole ring.

Table 1. Effects of 2 and 3-pyrazolo[1,5-**a**]pyrimidine substituents



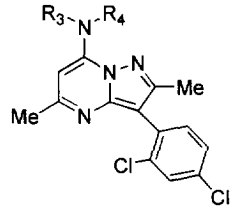
Compound	Ar	R_1	CRF-1 Binding (K_i, nM)
10a	phenyl	Me	511
10b	2-chlorophenyl	Me	15
10c	4-chlorophenyl	Me	77
10d	2,4-dichlorophenyl	Me	5
10e	2,4,6-trimethylphenyl	Me	93
10f	2,4-dichlorophenyl	H	40
1 (CP 154526)	---	---	56

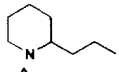
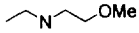
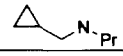
Next the effect of substitution of the 7-amino group was examined (Table 2). The X-ray structure of compound **10d** revealed that the alkyl groups on the 7-amino group extended in a plane perpendicular to the pyrazolopyrimidine ring. To determine if this was indeed important for activity, the ethyl butyl amino group of **10d** was replaced with a series of cyclic amines. The morpholino analog **10g** was over 60-fold less potent at binding to the CRF-1 receptor. The piperidine analog **10h** was nearly 8-fold less potent than **10d**. However, compound **10i** having a propyl group appended to the piperdine ring was nearly equipotent to compound **10d**. The propyl group of **10i** would be able to occupy space similar to that of butyl chain of **10d**. The cyclopropyl methyl analog **10j** showed good affinity for the CRF receptor; however, the monoalkyl analog **10k** was approximately 13-fold less potent at the CRF receptor. Compound **10l** having a 7-methoxylamino functionality was about 40 fold less potent at the CRF-1 receptor, but when the methoxy group was present on the alkyl chain as in compound **10m**, only a 2-fold drop in binding affinity was observed. The 5-amino analog **11** was inactive in the CRF-1 receptor binding assay.

In summary, a series of pyrazolopyrimidines exemplified by **10a** was discovered as having affinity for the CRF-1 receptor. An X-ray structure of one of the most potent compounds, **10d**, and the results of the SAR

study suggests that extension of the 3-phenyl ring and the alkyl groups on the 7-amino functionality out of the plane of the core heterocycle are required for optimum CRF receptor binding affinity. From these studies a number of pyrazolo[1,5-**a**]pyrimidines having high affinity for the CRF-1 receptor have been characterized.[11] Further studies detailing the consequences of their antagonist effects towards the CRF-1 receptor in vitro and in vivo will be reported elsewhere.

Table 2. Effects of 7-amino substituents



No.	NR_3R_4	CRF-1 Binding (K_i, nM)	No.	NR_3R_4	CRF-1 Binding (K_i, nM)
10g		310	**10k**		69
10h		41	**10l**		216
10i		6.4	**10m**		11
10j		3.2			

References

1 Dieterich, K. D.; Lehnert, H.; Desouza, E. B. *Endocrinol. Diabetes* **1998**, *105*, 65.

2. Heit, S. O.; Michael, J.; Plotsky, P.; Nemeroff, C. B. *Neuroscientist* **1997**, *3*,186.

3. Chen, Y. L.; Mansbach, R. S.; Winter, S. M.; Brooks, E; Collins, J.; Corman, M. L; Dunaiskis, A. R.; Faraci, W. S.; Gallaschun, R. J.; Schmidt, A; Schulz, D. W. *J. Med. Chem.* **1997**, *40*, 1749.

4. Webster, E. L.; Lewis D. B.; Torpy, D. J.; Zachman, E. K.; Rice, K. C.; Chrousos, G. P. *Endocrinology* **1996**, *137*, 5747.

5. Chen, C.; Dagnino, R. Jr; De Souza, E. B.; Grigoriadis, D. E.; Huang, C. Q.; Kim, K. I.; Liu, Z.; Moran, T.; Webb, T. R.; Whitten, J. P.; Xie, Y. F.; McCarthy, J. R. *J. Med. Chem.* **1996**, *39*, 4358.

6. Whitten, J. P.; Xie, Y. F.; Erickson, P. E.; Webb, T. R.; DeSouza, E. B.; Grigoriadis, D. E.; McCarthy, J. R. *J. Med. Chem.* **1996**, *39*, 4354.

7. Gilligan, P. J.; Hartig, P. R.; Robertson D. W.; Zaczek, R. *Annu. Rep. Med. Chem.* **1997**, *32*, 41.

8. For a review see: Christos, T. E.; Arvanitis, A. *Expert Opin. Ther. Pat.* **1998**, *8*,143.

9. Julian, P. L.; Oliver, J. J.; Kimball, R. H.; Pike, A .B.; Jefferson, G. D. *Org Synth* **1943**, *Coll. Vol. 2*, 487; Senga, K.; Nivinson, T.; Wilson, H. R.; Robins R. K. *J. Med. Chem.* **1981**, *24*, 610; Elnagdi, M. H.; Elmoghayar, M. R.; Elgemeie, G. E. *Adv. Het. Chem.* **1987**, *41*, 319.

10. Olianas, M. C.; Lampis G.; Onali, P. J. *NeuroChem.* **1995**, *64*, 402.

11. During the course of these investigations similar compounds appeared in the patent literature: Chen, C.; Webb, T. R.; McCarthy, J. R.; Moran, T. J.; Wilcoxen, K. M.; PCT Int. Appl. WO 97/29109; Arvanitis, A.J.; Charvat, R.J. PCT Int. Appl. WO 98/03510