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Bioorganic & Medicinal Chemistry Letters 14 (2004) 2083–2086
Bioorganic & Medicinal Chemistry Letters

Design, synthesis, and SAR of 2-dialkylamino-4-arylpyrimidines as potent and selective corticotropin-releasing factor$_1$ (CRF$_1$) receptor antagonists

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Received 16 December 2003; accepted 11 February 2004

Abstract—A series of 2-dialkylamino-4-phenylpyrimidines (**7**) was designed and synthesized as CRF$_1$ antagonists. SAR studies of this series resulted in the discovery of potent and selective antagonists **7b** and **7n** bearing a 4-(2,4,6-trisubstituted-phenyl) ring and a bulky 2-(*N*-bis(cyclopropane)methyl-*N*-propyl)amino group.

Corticotrophin-releasing factor (CRF), a 41 amino acid peptide, was first isolated, sequenced, and characterized by Vale and co-workers.[1] It is the key regulator of an organism's response to stress and as such, mediates the endocrine, autonomic, behavioral, and immune response to stressful stimuli. In addition, this hormone has been demonstrated to exhibit all of the characteristics of a bona fide neurotransmitter. The binding of CRF to CRF$_1$ receptors in the pituitary gland is responsible for the increased release of ACTH and other peptides.[2] On the other hand, 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.[3]

Recent discovery of non-peptide antagonists for CRF receptors has begun a new era of study for this neurotransmitter. Several small molecules have been reported to have good CRF$_1$ receptor antagonistic activity.[4] For example, pyrrolo[2,3-*d*]pyrimidine (**1**, CP-154,526),[5] anilinopyrimidine (**2**, NBI 27914),[6] and pyrazolo[1,5-*a*]pyrimidine (**3**, DMP904)[7] show good profiles in vitro, as well as oral activity in vivo in several relevant animal model studies. In a recent human clinical trial, NBI 30775 (**4**) demonstrated encouraging, albeit anecdotal, results in a group of severely depressed patients.[8] While all these compounds comprise of a core heterocyclic ring bearing amino, methyl, and substituted aryl functionalities, the first reported small molecule **5** has a relatively simple structure.[9] To search for novel heterocyclic templates, 2-dialkylamino-4-phenylpyrimidines of the general structure **7** were designed based on a topographical similarity to previously reported 1-alkyl-3-amino-5-aryl-1*H*-[1,2,4]triazole (**6**)[10] and 4-aryl-5-methyl-2-dialkylaminothiazole (**15**, SSR125543A).[11] In this paper, we discuss the structure–activity relationship studies around this nucleus **7** that leads to the discovery of a potent class of CRF$_1$ receptor antagonists.

The synthesis of the 2-dialkylaminopyrimidines (**7**) is outlined in Scheme 1. Condensation of substituted acetophenones (**8**) with *N*,*N*-dimethylformamide dimethyl acetal or diethyl acetal at reflux gave the corresponding enamines (**9**).[12] Cyclization of the enamines (**9**) with a guanidine hydrochloride in the presence of sodium methoxide in ethanol at reflux resulted in the production of the 2-amino-4-arylpyrimidines (**10**).[13] Reductive amination of the aminopyrimidine (**10**) with a ketone or aldehyde offered the 2-alkylaminopyrimidine (**11**), which could be further alkylated with an alkyl halide in the presence of sodium hydride in DMF to give 2-dialkylamino-4-arylpyrimidine (**7**). The target compounds (**7**) could also be synthesized from direct cyclization of the enamine (**9**) with an *N*,*N*-dialkylguanidine, which is readily available from bis(benzyloxycarbonyl)-*S*-methylthiourea and dialkylamine according to published

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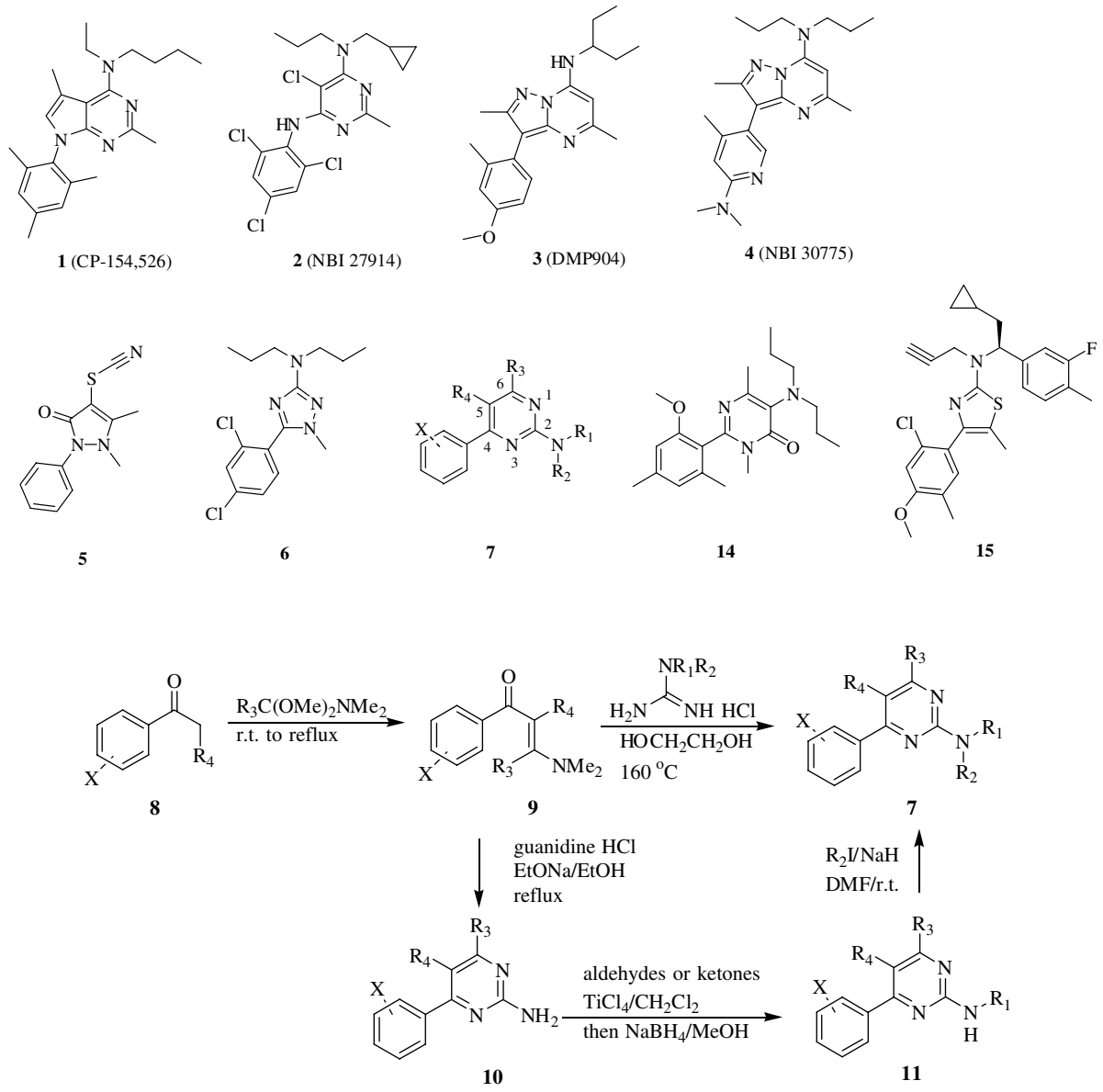
doi:10.1016/j.bmcl.2004.02.053

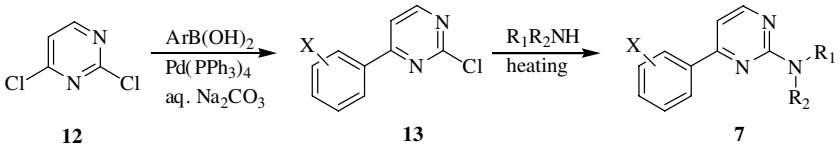


Scheme 1.

procedures.[14] A third method to generate the desired targets (**7**) is described in Scheme 2. Thus 2,4-dichloropyrimidine (**12**) was subjected to a coupling reaction with phenylboronic acid catalyzed by tetrakis(triphenylphosphine)palladium(0) under Suzuki conditions to give the corresponding 2-chloro-4-phenylpyrimidine (**13**).[15] Replacement of the chlorine on compound **13** with an amine gave the desired pyrimidine (**7**).

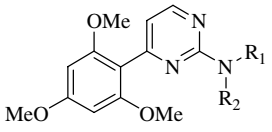
The CRF_1 receptor binding assay was performed on cloned human CRF_1 receptors expressed in CHO cells using ovine [^{125}I]-CRF as the radiolabeled ligand in a manner similar to that previously reported[6] and the results are listed in Table 1. The prototypical compound in this series was the 2-{*N*-[α-(cyclopropane)benzyl]-*N*-propylamino}-4-(2,4,6-trimethoxyphenyl)-pyrimidine **7c**, which closely mimics the known thiazole CRF_1 antagonist SSR125543A (**15**),[11] and it was found to have moderate binding affinity for the CRF_1 receptor ($K_i = 240$ nM). Replacement of the benzyl with the 2-thiophenylmethyl group (**7d**, $K_i = 250$ nM) did not affect the affinity, while replacement with 3-thiophenylmethyl or 3-furanylmethyl group resulted in reduced activity (**7e** and **7f**, $K_i = 2$ and 0.7 μM, respectively). However, introduction of a second cyclopropyl group resulted in an over 15-fold increase in binding affinity (**7b**, $K_i = 15$ nM). On the other hand, replacement of the *N*-propyl of **7b** with an allyl group decreased the activity almost sevenfold (**7a**, $K_i = 100$ nM), while deletion of the *N*-propyl group resulted in an inactive compound (**11**). These results indicate that the substitution pattern on the 2-amino group is very important for high CRF_1 receptor binding (Table 1).

The effect of the substitution on the 4-phenyl ring as well as the 5- and 6-position of the core pyrimidine was also



Scheme 2.

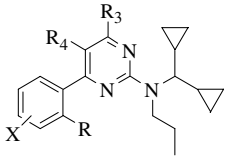
Table 1. Effects of 2-amino substituents on binding to CRF_1 receptor



Compound	R^1	R^2	K_i (nM)[a]
10	H	H	Inactive
11	Bis(cyclopropane)methyl	H	Inactive
7a	Bis(cyclopropane)methyl	Allyl	100
7b	Bis(cyclopropane)methyl	Pr	15
7c	α-(Cyclopropane)benzyl	Pr	240
7d	1-(2-Thiophenyl)cyclopropyl	Pr	250
7e	1-(3-Thiophenyl)cyclopropyl	Pr	2,000
7f	1-(3-Furyl)cyclopropyl	Pr	700
7g	1-Naphthyl	Pr	Inactive
7h	Cyclopentyl	Pr	2,200

[a] K_i values are means of two or more determinations.

Table 2. Effects of 4-phenyl substituents on binding to CRF_1 receptor



Compound	X	R	R^4	R^3	K_i (nM)[a]
7i	2,4-MeO	H	H	H	460
7j	2,4-MeO	H	Me	H	19
7k	2,4-MeO	H	Et	H	860
7l	2,4-MeO	H	COOMe	H	Inactive
7m	2,4-MeO	F	H	H	63
7n	2,4-MeO	Cl	H	H	11
7b	2,4-MeO	MeO	H	H	15
7o	2,4-MeO	MeO	Me	H	20
7p	4-Br	H	Me	H	49
7q	4-Br	Me	Me	H	63
7r	4-Br	H	Me	Me	Inactive

[a] K_i values are means of two or more determinations.

examined (Table 2). The 2,4-dimethoxy analogue **7i** was found to have a K_i value of 460 nM, which is much less active than the 2,4,6-trimethoxy analogue **7b**. However, introduction of a methyl substituent on the 5-position of pyrimidine re-gained the binding affinity (**7j**, $K_i = 19$ nM). Replacement of the methyl with a larger ethyl group resulted in over 40-fold loss of binding (**7k**, $K_i = 860$ nM), while the bulky and polar methoxycarbonyl group at this position abolished the activity completely (**7l**). These results suggest the dihedral angle between the 4-phenyl and the core pyrimidine is critical for high affinity binding.[6,16]

Because of the importance of the combination of 4-*ortho*-substituted phenyl ring and 5-substituted pyrimidine, we further screened different *ortho*-substituted 2,4-dimethoxyphenyl groups. Introduction of a fluorine at the 6-position of the 4-phenyl group increased almost eightfold the binding affinity (**7m**, $K_i = 63$ nM), while a chlorine gave much a better analogue (**7n**, $K_i = 11$ nM). Introduction of a methyl group at the 5-position of **7b** actually did not affect the binding affinity (**7o**, $K_i = 20$ nM). Similarly, incorporation of a methyl group at the *ortho*-position of the 4-(4-bromophenyl) ring of **7p** had hardly any effect in binding (**7p**, $K_i = 49$ nM, vs **7q**, $K_i = 63$ nM). These results again emphasize the orthogonal relation between the 4-phenyl ring and the pyrimidine core. Finally, the fact that introduction of a methyl group at the 6-position of the pyrimidine core of **7p** abolished binding affinity (**7r**) implies this region is very sensitive to stereo effect.

Selected compounds were tested to determine their functional activity at the CRF_1 receptor. Functional antagonism was assessed 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. All compounds were tested in the presence or absence of peptide agonist in order to determine if any of the non-peptides exhibited any intrinsic agonist activity. Compounds **7b** and **7n** dose-dependently inhibited CRF-stimulated cAMP production in CHO cells expressing the CRF_1 receptor with EC_{50} values of 990 nM and 1.6 μM, respectively.[17] In addition to the functional cAMP assay, compounds **7b** and **7n** were also tested and found to functionally antagonize CRF-stimulated ACTH release from primary rat anterior pituitary cell cultures.[18] Neither compounds **7b** and **7n** nor any other non-peptide molecules examined had any functional intrinsic activity for cAMP or ACTH release suggesting that these molecules are indeed functional CRF_1 receptor antagonists. Table 3 summarizes all these results.

This series of compounds was also tested on the $CRF_{2\alpha}$ receptor binding assay using cloned human $CRF_{2\alpha}$

Table 3. In vitro activity profiles of compounds **7b** and **7n**

Compound	K_i CRF_1 (nM)	K_i $CRF_{2\alpha}$ (nM)	EC_{50} cAMP CRF_1 (nM)[a]	EC_{50} ACTH CRF_1 (nM)[a]
7b	15	Inactive	990	970
7n	11	Inactive	1600	1100

[a] EC_{50} values are means of two or more determinations.

receptors as previously described[19] to access the selectivity of this series of compounds. Compounds **7b** and **7n**, which exhibited K_i values of 16 and 11 nM, respectively, at the CRF_1 receptor, showed no activity (less than 40% inhibition at a concentration of 10 μM) at the $CRF_{2\alpha}$ receptor. Thus, compound **7b** exhibited good selectivity profile. In a NovaScreen™ performed on 66 neurotransmitter receptors (such as D_1, $GABA_a$, H_1, and 5HT), brain/gut peptides (such as bradykinin, CGRP, NPY, and somatostatin), and growth factors and ion channels (Ca^{2+} and Na^+), **7b** showed no significant affinity at 5 μM.

The basic pharmacophore for this series of compounds is a six-membered heterocyclic ring supporting a lipophilic dialkylamino group and a substituted aryl ring, which needs to be orthogonal to the pyrimidine core. This pharmacophore seems to be different from other more popular CRF_1 antagonists such as **1**–**4**, in which a nitrogen served as hydrogen-bond acceptor and a methyl group are critical for high affinity binding to the CRF_1 receptor, and the lipophilic side chain is relative small.[16] However, for **7**, a bulky bis(cyclopropane)methyl group on the 2-amino was very important for high binding affinity, and the 2-nitrogen may serve as a hydrogen-bonding acceptor. A recent publication disclosed a series of arylpyrimidinones such as **14**, which also showed SAR different from traditional bicyclic cores.[20] This difference in pharmacophore requirements may offer the advantage to design potent CRF_1 antagonists with different structural features.

In summary, a series of 2-dialkyl-4-arylpyrimidines exemplified by **7b** and **7n** was discovered as potent and selective CRF_1 receptor antagonists. SAR studies suggest that the bis(cyclopropane)methyl group on the 2-amino functionality and the 2,4,6-trisubstituted aryl or 2,4-disubstituted aryl with a small alkyl group at the 5-position of the pyrimidine core are required for optimum CRF_1 receptor binding affinity. From this study a number of 2-dialkyl-4-arylpyrimidines with high binding affinity for the CRF_1 receptor have been characterized. In addition, these compounds demonstrated in vitro inhibition of CRF-stimulated cAMP production in stable lines transfected with human CRF_1 receptor subtype and ACTH release from primary rat anterior pituitary cell cultures. The different SAR from the previously reported series should provide us with a different venue to search for novel CRF_1 receptor antagonists.

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