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4-ARYL-2-ANILINOPYRIMIDINES AS CORTICOTROPIN-RELEASING HORMONE (CRH) ANTAGONISTS

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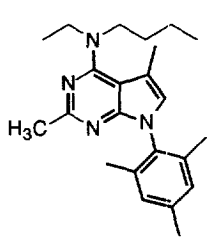
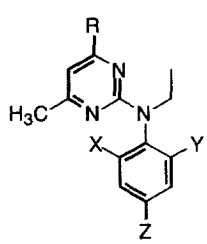
Abstract: A series of 4-aryl-2-(N-ethylanilino)pyrimidines has been synthesized as corticotropin-releasing hormone (CRH) inhibitors. The effect of substitution on each aromatic ring on receptor binding was investigated.

Corticotropin releasing hormone or factor (CRH or CRF) is a 41 amino acid peptide, which is the prime regulator of the hypothalmus–pituitary–adrenocortical (HPA) axis and as such coordinates the endocrine, behavioral, and autonomic responses to stress.[1–3] Hypersecretion of CRH in the central nervous system is associated with both major depression and a spectrum of anxiety-related disorders. Two 7-transmembrane receptors for CRH (CRH_1 and CRH_2) have been identified[4,5] and while no nonpeptide antagonists to CRH_2 have been reported, several series of small molecule antagonists for the CRH_1 receptor have appeared in the literature.[6–13] The pharmacology of one of these molecules (CP154526-1 K_i = 2.7 nM), including its activity in animal models of anxiety and depression, has been described in great detail..[6] These studies offer considerable hope that CRH_1 antagonists may eventually play a role in the treatment of depression or anxiety-related disorders.

In our work, we found compounds with high affinity to CRH_1 among a series of 2-(N-ethylanilino)-6-methylpyrimidines (**1**) incorporating a wide variety of substituents in the 4-position of the pyrimidine ring.[12] Since many of the compounds we examined were 4-(dialkylamino)pyrimidines, of which a representative example is **2** (K_i = 9 nM), we decided to expand our SAR investigation by synthesizing a series of 4-aryl-2-anilinopyrimidines **3**. While the lowest energy conformation of 4-aminopyrimidines such as **2** would likely place



CP154526-1



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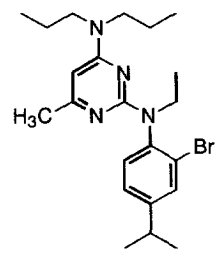

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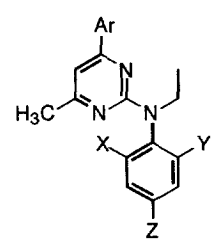

3

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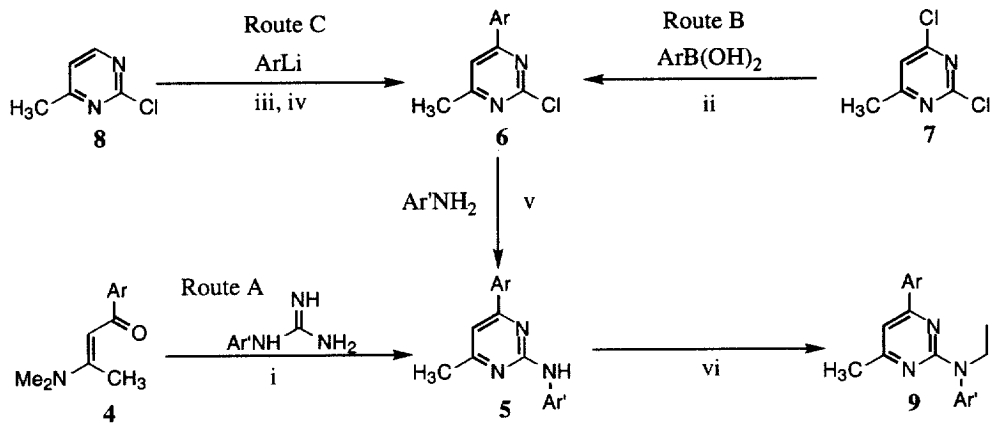
the carbons adjacent to the 4-nitrogen roughly in the plane of the pyrimidine ring, the 4-aryl-pyrimidines **3** are expected to adopt conformations where the aryl and pyrimidine rings are orthogonal. These 4-arylpyrimidines would have a 3-dimensional structure quite different from our earlier compounds, and as a class, their own perhaps distinctive SAR.

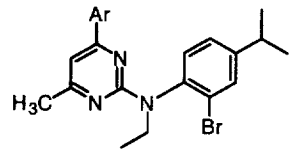


Reagents and conditions: *(i) 2-methoxyethanol, reflux; (ii) $(Ph_3P)_4Pd$, benzene, 1 M Na_2CO_3, reflux; (iii) ether, -30–0 °C; (iv) DDQ, ether - THF, 0 °C; (v) ethylene glycol, reflux; or NaH, toluene, reflux; (vi) ethyl iodide, NaH, DMSO.*

The 4-aryl-2-anilinopyrimidines were prepared by one of the three synthetic routes depicted above. In the condensation route (Route A), ß-dimethylaminobutenones **4** were condensed with an arylguanidine to afford 2-anilino-4-arylpyrimidines. The ß-dimethylaminobutenones **4** were prepared by heating acetophenones with neat dimethylformamide dimethylacetal. Bulky ortho-substituents on the acetophenone or the arylguanidine did not interfere significantly with either of these steps, but the preparation of ortho-disubstituted arylguanidines[14] required more drastic reaction conditions than preparation of the mono-substituted guanidines.[15] In route B, 4-aryl-2-chloropyrimidines **6** were synthesized by displacement of the 4-chloride of 2,4-dichloropyrimidine **7** with arylboronates. Under classical Suzuki conditions, reaction occurred exclusively at the 4-position with no displacement of the 2-chloride observed.[16] In route C, addition of aryllithium reagents (generated either by orthometalation or by metal-halogen exchange using alkyllithium reagents) to 2-chloro-4-methylpyrimidine **8** followed by DDQ oxidation[17] also afforded 2-chloropyrimidines **6**. The chloride of **6** was displaced with 2,4-disubstituted anilines under neutral conditions in refluxing ethylene glycol and with 2,4,6-trisubstituted anilines in the presence of NaH in refluxing toluene. In all three routes, N-alkylation of anilinopyrimidines **5** (using NaH in DMSO) afforded the target compounds **9**.

Affinity of these compounds for CRH_1 was determined by examining their displacement of ^{125}I-tyr-ovine-CRH from cloned human $hCRH_1$ receptors expressed in 293EBNA cells.[18] We chose to carry out exploratory studies (Table 1) on anilinopyrimidines with the 2-bromo and 4-isopropyl groups on the aniline ring, a substitution pattern which had afforded potent ligands in our earlier series of 4-(dialkylamino)pyrimidines.[12] Anilinopyrimidine **10a**, with an unsubstituted phenyl group in the 4-position, had only modest affinity (109 nM)

Table 1. Binding affinities of 2-(2-bromo-4-isopropylanilino)pyrimidines



Compd	Ar	K_i (nM)	Synth. Rte.
10a	Phenyl	109	A
10b	2-Ph-phenyl	8.4	C
10c	3-Ph-phenyl	84.6	C
10d	4-Ph-phenyl	inact.	C
10e	1-Napthyl	7.8	B
10f	2-Me-phenyl	18.4	B
10g	2-F-phenyl	23.4	B
10h	2-Cl-phenyl	13.5	A
10i	2-Br-phenyl	24.0	A
10j	2-CF_3-phenyl	3.1	B
10k	2-NO_2-phenyl	2.4	A
10m	2-CN-phenyl	5.8	A
10n	2-COOH-phenyl	3536	a
10p	2-(5-Tetrazolyl)phenyl	662	b
10q	2-NH_2-phenyl	2359	c
10r	2-NHMe-phenyl	369	d
10s	2-NMe_2-phenyl	78.2	e
10t	2-NMe_3+-phenyl	inact.	f
10u	3-CF_3-phenyl	17.4	B
10v	4-CF_3-phenyl	inact.	B
10w	2,6-Dichlorophenyl	10.1	B
10x	2,4,6-Trimethylphenyl	11.2	C

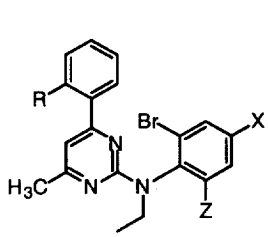
Reagents and conditions: *(a)* ***10m****: 12 N HCl, reflux. (b)* ***10m****: NaN_3, toluene, reflux. (c)* ***10k****: Fe, HOAc, MeOH, reflux. (d)* ***10q****: Me_2SO_4, $NaHCO_3$, CH_3CN, reflux. (e)* ***10r****: Me_2SO_4, $NaHCO_3$, CH_3CN, reflux. (f)* ***10q****: $MeSO_3CF_3$, di-t-butyl-pyridine, CH_2Cl_2.*

for the CRH_1 receptor. In order to probe the overall size and shape of the receptor pocket occupied by the aryl group, a bulky phenyl substituent was introduced at each position around the ring. Surprisingly, a meta-phenyl group was tolerated and an ortho-phenyl group increased the affinity to 8.4 nM. Substitution with a 1-naphthyl group (7.8 nM) confirmed the presence of open space adjacent to the ortho- and meta-postions. The para derivative **10d** was completely inactive.

The improved affinity afforded by an ortho-phenyl substituent prompted preparation of numerous analogs with other ortho substituents. Substitution with a variety of ortho groups increased affinity for the $hCRH_1$ receptor, with the highest affinity compounds being those substituted with strongly electron withdrawing groups such as cyano (5.8 nM), nitro (2.4 nM), and trifluoromethyl (3.1 nM). Despite the large amount of space that is available to ortho-substituents, negatively-charged groups (**10n** and **10p**), a strongly electron-withdrawing positively-charged group (**10t**), and groups with hydrogen bond donors (**10q** and **10r**) at this position all diminished affinity. Finally, bis-ortho substitution was also found to be tolerated. Both the 2,6-dichloro- and

2,4,6-trimethylphenyl compounds suffered no loss in activity when compared to their monosubstituted analogs. This finding complicated interpretation of the SAR for compounds with a single ortho substituent. Ortho groups that increased affinity could do so by interacting productively with the receptor at sites adjacent to either the 2- or 6-positions. Groups which diminished affinity must have produced unfavorable interactions at both sites.

Table 2



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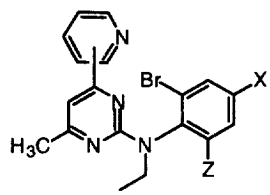
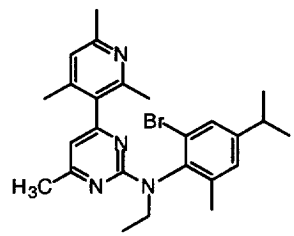


12a 2-pyridyl
12b 3-pyridyl



12c

Compd	R	X	Z	K_i (nM)	Synth. Rte.
10j	CF_3	i-Pr	H	3.1	B
11a	CF_3	Br	H	5.8	A
11b	CF_3	SMe	H	2.5	a
11c	CF_3	SO_2Me	H	20.6	b
11d	CF_3	$COCH_3$	H	4.6	c
11e	CF_3	NMe_2	OMe	9.4	B
11f	CF_3	OMe	OMe	8.6	B
11g	H	OMe	OMe	13.9	A
11h	2-Cl	OMe	OMe	9.1	A
11i	2-OMe	OMe	OMe	54.8	C
12a		OMe	OMe	222	C
12b		OMe	OMe	229	C
12c		i-Pr	H	24.9	A

Reagents and conditions: *(a)* ***11a****: NaSMe, $(Ph_3P)_4Pd$, DMSO, reflux.*[12] *(b)* ***11b****: MCPBA, CH_2Cl_2. (c)* ***11a****: $(Ph_3P)_4Pd$, $(Ph_3P)_2PdCl_2$, (1-ethoxyvinyl)tributyltin, toluene, reflux; then KF, ether, RT.*[12]

We next turned our attention to the variation of substitution on the aniline ring. In our earlier work with 4-methyl and 4-(dimethylamino)-2-anilinopyrimidines, a number of favorable substitution patterns on the aniline ring were identified.[12] While keeping the 4-(2-trifluoromethylphenyl) group constant, several 4-aryl-2-anilinopyrimidines with these aniline substituents were prepared. Table 2 reveals that replacing the 2-bromo-4-isopropylaniline ring with this group of selected anilines results in most cases in little or no loss in binding affinity. In some cases, changes to the aniline ring increased potency (**10a** vs **11g**, **10h** vs **11h**). The potency of unsubstituted **11g** (13.9 nM) demonstrates that an ortho substituent is not required for high affinity.

Since our most potent compounds had an electron deficient aryl group in the pyrimidine 4-position, three 4-pyridyl-2-anilinopyrimidines (**12a–12c**) were also prepared. Despite the fact that pyridine is an electron-

deficient aromatic ring, compounds substituted with simple pyridines had lower affinity for the receptor than the best electron-deficient phenyl rings. However, the similar affinity observed with 2,4,6-trimethylphenyl **10x** and 2,4,6-trimethylpyridyl **12c**, suggests that a requirement for desolvation of the pyridine nitrogen prior to binding may contribute to the low affinity of **12a** and **12b**.

In conclusion, we have found that 4-aryl-groups are potential replacements for the 4-(dialkylamino) substituents of earlier 2-anilinopyrimidine CRH_1 antagonists. In particular, 4-aryl-2-anilinopyrimidines when ortho substituted with electron withdrawing groups are high-affinity antagonists of the CRH_1 receptor.

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14. For example, 2-bromo-4,6-dimethoxyguanidine was prepared by refluxing the corresponding aniline hydrochloride in 50% aq ethanol with 3 equival of cyanamide for 1.5 h, followed after addition of 1.5 equival of HCl and 3 more equival of cyanamide, with an additional 2 h of reflux. Preparation of arylguanidines with a single ortho substituent did not require additional cyanamide.
15. These compounds were prepared as described in ref 12.
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18. For a detailed description of the isolation of cell membranes containing cloned human CRH_1 receptors for use in the binding assay as well as a description of the binding assay itself see ref 12. K_i values are the average of at least two determinations for $K_i < 50$ nm, otherwise they are single determinations. Selected compounds were shown to be silent antagonists in that they completely inhibited CRH-stimulated adenylyl cyclase activity in membranes from rat cortex without any effect by themselves (data not shown).