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Synthesis of benzoylpyrimidines as antagonists of the corticotropin-releasing factor-1 receptor

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Abstract—A series of benzoylpyrimidines derived from the anilinepyrimidine CRF_1 antagonists were synthesized. Several synthetic routes were developed to explore the SAR of this series of compounds. Compounds such as **8d** ($K_i = 15$ nM) exhibited high binding affinities at the human CRF_1 receptor.

1. Introduction

Corticotropin-releasing factor (CRF) is a 41 amino acid peptide C-terminal amide, which stimulates adrenocorticotropic hormone (ACTH) release from the pituitary[1] and is a neuromodulator or neurotransmitter, which is involved in neural stress induced responses in the brain.[2] CRF exerts its action by binding to and activating the CRF_1 and CRF_2 receptors, both belong to the class B G-protein-coupled receptor superfamily, which have recently been cloned and expressed.[3] The pharmacology, physiology and role for CRF in CNS disorders have been extensively reviewed.[4]

Clinical evidence suggests that over-stimulation of CRF may result in several diverse neuropsychiatric diseases including depression, anxiety and stress related disorders, and therefore CRF antagonists have the potential to treat these diseases. Since the first nonpeptide CRF antagonist CP-154,526 (**1**) disclosed in 1996,[5] many potent small molecule CRF antagonists from different chemical classes have been reported.[6] A series of analinopyrimidines exemplified by NBI 27914 (**2**) has been reported as potent CRF_1 antagonists (Fig. 1).[7] We have proposed that the dihedral angle between the phenyl group and the pyrimidine ring of **2** is very important for receptor binding.[7] To further explore and understand the structure–activity relationships of these CRF_1 antagonists, we replaced the nitrogen linker in **2** with a carbon moiety, which may alter the relation of the two aromatic rings. In addition, this change eliminates the potential liability of the aniline functionality. Here we report the synthesis and SAR of this class of compounds as CRF_1 receptor antagonists.

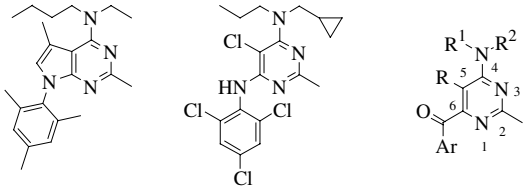

Figure 1. Small molecule CRF_1 antagonists.

2. Chemistry

Several different synthetic routes were investigated in order to obtain the benzoylpyrimidine derivatives with various substitutions at the 4-, 5- and 6-positions of the pyrimidine ring. The initial synthesis of **7** is showed in Scheme 1. 4-Chloro-2,6-dimethylpyrimidine was

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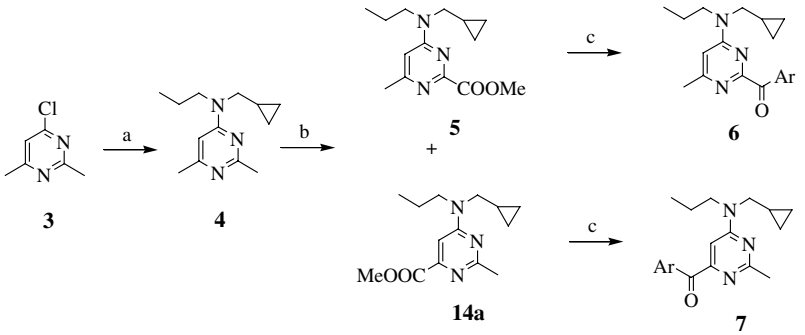


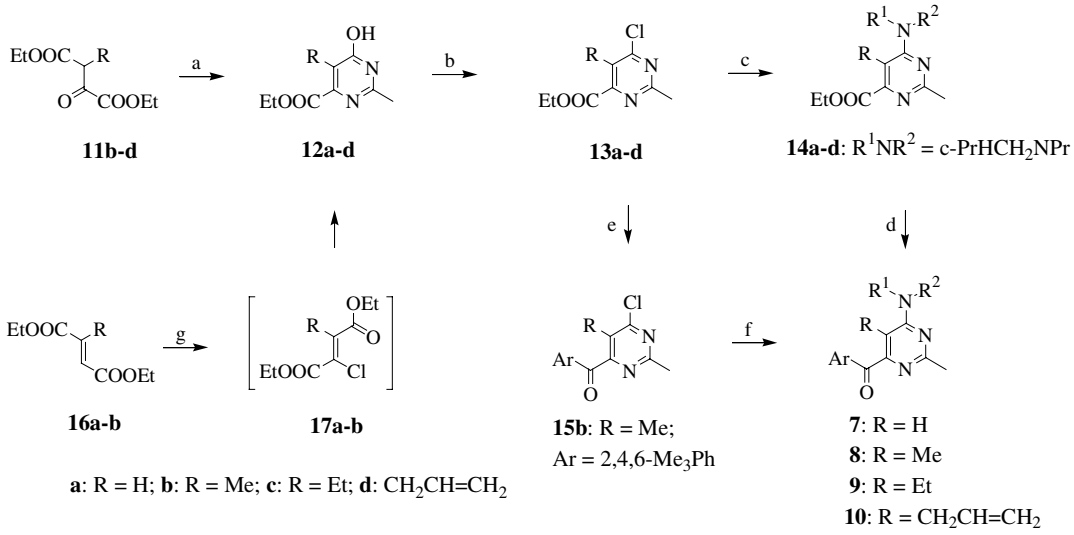
Scheme 1. Reagents and conditions: (a) *c*-PrCH$_2$NHPr/heat, 93%; (b) i. SeO_2, ii. $SOCl_2$/MeOH, then separation, 20%; (c) ArMgX/THF, 40–60%.

allowed to react with N-propyl-N-cyclopropanemethylamine to give the aminopyrimidine **4** in 93% yield. Although Sakasai et al.[8] have shown that it is possible to regioselectively oxidize 2,4-dimethylpyrimidine to give pyrimidine-4-carboxylic acid, applying these conditions (SeO_2/pyridine, reflux, followed by esterification) to **4** gave a mixture of isomers **5** and **14a**, along with a diester by-product. Compounds **5** and **14a** were readily separated by flash column chromatography in about 20% yield each. Reaction of **5** or **14a** with an aryl Grignard reagent afforded the ketones **6** and **7**, respectively, in moderate yields (40–60%). The regio-isomeric structures of **6a** and **7a** were assigned based on observed NOE between the 5-proton and the 6-methyl group of **6a**.

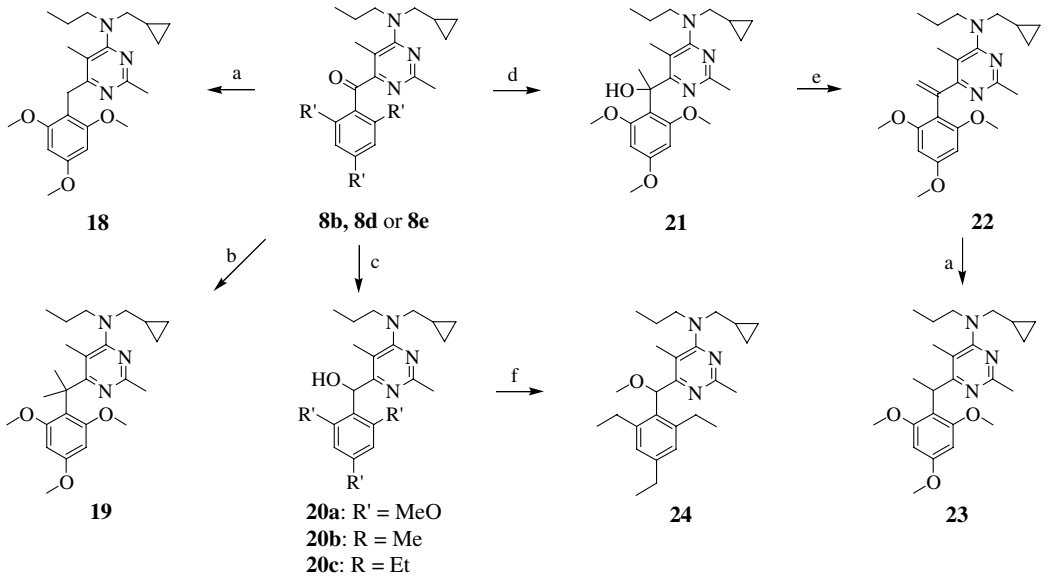
The 5-alkyl pyrimidinones **12b–d** were obtained in moderate yields (10–40%) by the reaction of 3-alkyl-2-oxosuccinates **11b–d** with acetamidine hydrochloride in the presence of NaOEt, similar to the reported procedure.[9] Treatment of **12** with $POCl_3$ gave the corresponding 4-chlorpyrimidines **13**, in almost quantitative yields, which were converted to the 4-aminopyridines **14** with N-propyl-N-cyclopropanemethylamine in about 90% after purification. Finally, **14a–d** were transformed into the desired 6-benzoylpyrimidines **7–10** in good yields (40–80%) with various aryl Grignard reagents (Scheme 2). Alternately, the 4-chloropyrimidine **13b** was treated with 2,4,6-trimethylphenylmagnesium bromide to give the 4-chloro-6-(2,4,6-trimethylbenzoyl)pyrimidine **15b**, in 90% yield, which was subjected to a nucleophilic replacement with various alkylamines to afford the final products **8g–p**.

Since the cyclization of 2-oxosuccinate with acetamidine provided the pyrimidines in relative poor yield (10% for **12a**),[9] we explored other alternatives to synthesize the pyrimidine-esters **12a–d**. Thus, reaction of diethyl fumarate (**16a**, containing a trace of DMF) with chlorine gas gave a chlorinated intermediate,[10] which up on treatment with sodium ethoxide in ethanol gave presumably the chlorofumarate **17a**. Cyclization of **17a** with acetamidine hydrochloride in the presence of a base such as sodium ethoxide afforded the desired pyrimidine **12a** in 56% yield. Similarly, **12b** was synthesized from **16b**.

The benzoylpyrimidines **8b–e** were further modified as showed in Scheme 3. Palladium-catalyzed hydrogena-



Scheme 2. Reagents and conditions: (a) acetamidine·HCl/NaOEt/EtOH, 10–40%; (b) $POCl_3$/reflux, ~100%; (c) *c*-PrCH$_2$NHPr/heat, 90%; (d) ArMgX/THF, 40–80%; (e) 2,4,6-Me_3PhMgBr/THF, 93%; (f) R^1R^2NH/heat, 30–90%; (g) i. Cl_2/CH_2Cl_2, ii. NaOEt/EtOH, iii. acetamidine·HCl, ~60%.



Scheme 3. Reagents and conditions: (a) H_2/Pd/EtOH; (b) $Me_3Al/Me_3SiOTf/CH_2Cl_2$; (c) $NaBH_4$/MeOH; (d) MeMgBr/THF; (e) $MsCl/Et_3N$; (f) $MsCl/Et_3N$, then NaOMe/MeOH.

tion of **8b** afforded the methylene **18** in 45% yield. The *gem*-dimethyl compound **19** was isolated from the treatment of **8b** with trimethylaluminium–trimethylsilyltriflate in dichloromethane.[11] Reactions of **8b** with methyl Grignard reagent provided the tertiary alcohol **21**, which was further modified. Treatment of **21** with methanesulfonyl chloride in the presence of triethylamine afforded the olefin **22**, which was reduced under hydrogenation conditions to give the ethylidene **23**. Reduction of ketone **8b**, **8d** and **8e** gave the corresponding alcohols **20a–c** in good yields. Conversion of **20c** to the corresponding methyl ether **24** was accomplished with methanesulfonyl chloride and triethylamine, followed by sodium methoxide in methanol.

3. Results and discussion

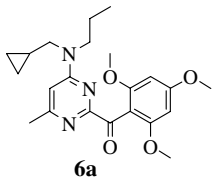
The synthesized compounds were tested for their binding affinities at the cloned human CRF_1 receptor in a competition binding assay as previously described, and the K_i values were determined from concentration–response curves using concentrations ranging from 1 nM to 10 μM.[7,12a] The structure–activity relationships of these compounds are summarized in Table 1. Selected compounds were also measured for their abilities to inhibit CRF-stimulated cAMP production in cells expressing CRF_1 receptor to assess their functional antagonism.[12]

Previous studies have led to a proposed pharmacophore for potent CRF receptor antagonists.[7,13] A major feature of this pharmacophore is the orthogonal relation between an aromatic group and a nitrogen-containing heterocyclic ring. In the anilinopyrimidine series (i.e., **2**), a 2,6-substituted analine at the 6-position and a small group such as methyl and chloro moiety at the 5-position of the pyrimidine are crucial for high binding affinity to the CRF_1 receptor. Since we speculated a replacement of the nitrogen linker of **2** with a sp^2-carbon of a carbonyl group may alter the geometry of the two aromatic rings, this change may provide compounds with different side-chain decorations. In addition, this modification should also change the physicochemical property of this series, and remove the potentially undesirable aniline moiety.

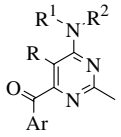
The initial 5-unsubstituted pyridines **7** had modest binding affinities at the CRF_1 receptor. For example, **7b** with a 2,4,6-trimethylphenyl group had a K_i value of 200 nM. Introduction of a methyl group at the 5-position of the pyrimidine increased the binding affinity over 13-fold (**8d**, $K_i = 15$ nM). In comparison to **8d**, the 2-methylphenyl analog **8c** exhibited a K_i value of 810 nM, a 54-fold reduction in binding affinity, the 2,4,6-trimethoxyphenyl analog (**8b**, $K_i = 38$ nM), however, was only slightly less active, and the 2,4,6-triethylphenyl compound displayed a similar K_i value (**8e**, $K_i = 13$ nM). Both the 5-position of the pyrimidine and the *ortho*-position of the 6-benzoyl group have direct impact on the relative conformation of these two aromatic rings. Interestingly, 5-ethyl and the bigger 5-allyl group had minimal effect on the receptor binding ($K_i = 55$ and 31 for **9** and **10**, respectively).

The SAR study from the anilinopyrimidine series has previously established for the alkylamine at the 4-position of the pyrimidine, and a small aliphatic side-chain such as dipropylamine and N-propyl-N-cyclopropanemethylamine is optimal for the receptor binding.[7] For this benzoylpyrimidine series, it also seems to be true. Thus, N-propyl-N-cyclopropylmethylamine proved to be the most effective side-chain at the 4-position of the pyrimidine. Other small hydrophobic amine substituents were tolerated (**8g** and **8h**). Cyclic amines yielded derivatives that were much less active (**8m** and **8o**). These results seem to indicate a preference for the size

Table 1. Structure–activity relationships of benzoylpyrimidines **6–10**



6a



7-10

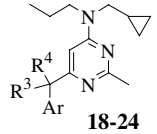
Compound	R	R^1NR^2	Ar	K_i (nM)
2				2.7
6a				630
7a	H	*n*-PrNCH$_2$Pr-*c*	2,4,6-MeOPh	1100
7b	H	*n*-PrNCH$_2$Pr-*c*	2,4,6-MePh	200
7c	H	*n*-PrNCH$_2$Pr-*c*	2,3,4,5,6-MePh	>10,000
8a	Me	*n*-PrNCH$_2$ Pr-*c*	2,4-MeOPh	810
8b	Me	*n*-PrNCH$_2$Pr-*c*	2,4,6-MeOPh	38
8c	Me	*n*-PrNCH$_2$Pr-*c*	2-MePh	810
8d	Me	*n*-PrNCH$_2$Pr-*c*	2,4,6-MePh	15
8e	Me	*n*-PrNCH$_2$Pr-*c*	2,4,6-EtPh	13
8f	Me	*n*-PrNCH$_2$Pr-*c*	2,3,4,5,6-MePh	5100
8g	Me	*n*-Pr$_2$N	2,4,6-MePh	58
8h	Me	(CH$_2$=CHCH$_2$)$_2$N	2,4,6-MePh	38
8i	Me	EtNHBn-*n*	2,4,6-MePh	470
8j	Me	PrNBn-*n*	2,4,6-MePh	180
8k	Me	*n*-PrNCH$_2$CH$_2$OH	2,4,6-MePh	540
8l	Me	(MeOCH$_2$CH$_2$)$_2$N	2,4,6-MePh	1200
8m	Me	Pyrrolidin-1-yl	2,4,6-MePh	>10,000
8n	Me	(2*S*)-MeOCH$_2$-pyrrolidin-1-yl	2,4,6-MePh	380
8o	Me	Morpholin-4-yl	2,4,6-MePh	>10,000
8p	Me	Et$_2$CHNH	2,4,6-MePh	220
9	Et	*n*-PrNCH$_2$Pr-*c*	2,4,6-MePh	55
10	CH$_2$=CHCH$_2$	*n*-PrNCH$_2$Pr-*c*	2,4,6-MePh	31

and/or orientation of the hydrophobic substituents at this position.

We intended to break the possible conjugation between the carbonyl group and the aromatic rings by modifying the C=O moiety. The methylene analog (**18**, $K_i = 26$ nM) of **8b** exhibited the similar binding affinity as the parent, however, the ethylidene (**23**, $K_i = 480$ nM) and the hydroxymethylene (**20a**, $K_i = 1.9$ μM) were much less active. This decrease in binding affinity was most likely caused by conformational change since both the CHMe and CHOH groups are not much larger in size than the C=O moiety. The bulkier *gem*-dimethyl group abolished the binding affinity of **19**. The low activity of the alcohols **20** caused at least partially by the polarity of the hydroxyl group, since the corresponding methyl ether (**24**, $K_i = 90$ nM) recovered some activity from its hydroxyl analog (**20c**, $K_i = 360$ nM). An attempt to mimic the C=O group with a C=CH$_2$ moiety was also unsuccessful and the resultant compound exhibited almost 20-fold reduction in binding (**22**, $K_i = 560$ nM). These results imply the importance of the correct dihedral angle between the phenyl group and the pyrimidine ring for high binding affinity to the CRF$_1$ receptor (Table 2).

Selected compounds were tested for functional antagonism, and these compounds were able to inhibit CRF-stimulated release in CHO cells expressing the CRF$_1$ receptor. For example, compound **8b** dose-dependently inhibited cAMP production with an IC_{50} value of

Table 2. Structure–activity relationships of benzylpyrimidines **18–24**



18-24

Compound	Ar	CR^3R^4	K_i (nM)
18	2,4,6-MeOPh	CH$_2$	26
23	2,4,6-MeOPh	CHMe	480
20a	2,4,6-MeOPh	CHOH	1900
20b	2,4,6-MePh	CHOH	79
20c	2,4,6-EtPh	CHOH	360
24	2,4,6-EtPh	CHOMe	90
19	2,4,6-MeOPh	C(Me)$_2$	>10,000
21	2,4,6-MeOPh	C(OH)Me	1900
22	2,4,6-MeOPh	C=CH$_2$	560

1.0 μM (average of four independent measurements), and **8d** had an IC_{50} of 1.2 μM (average of three measurements). In addition, **8d** was also tested and found to inhibit CRF-stimulated ACTH release from primary rat anterior pituitary cell cultures with an IC_{50} value of 0.98 μM.[14] Neither compounds **8b** and **8d** nor any other analogs examined had any functional intrinsic activity for cAMP release suggesting that these molecules are indeed functional CRF$_1$ receptor antagonists.

Conformational analyses of compounds **2**, **8b**, **18** and **19** using MedChem Explorer[15] revealed that there was a

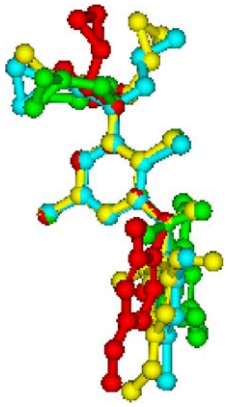


Figure 2. Overlay of low-energy conformers of compounds **2** (light blue), **8b** (yellow), **18** (green) and **19** (red).

good match between the low-energy conformers of **2** and **8b**. The phenyl ring of **18** or **19** was not aligned well with that of **2** (Fig. 2). Since the methylene moiety of **18** is much more flexible than the *gem*-dimethyl substituted carbon of **19**, the correct conformer of **18** for receptor binding will cost much less energy than that of **19**. That may explain why both **8b** and **18** had similar binding affinity while **19** was inactive.

4. Conclusion

The structure–activity relationships of the novel benzoylpyrimidines as antagonists of the CRF_1 receptor were explored. Synthetic approaches were developed that allowed for the facile divergent modification of the 6-aryl or the 4-amino substituents. The carbonyl functionality of the benzoyl group was found to be the most desirable among the carbon linkers explored. This is probably related to a proper dihedral angle between the two aromatic rings. Several potent compounds were discovered during this study (e.g., **8d** and **8e**).

Acknowledgements

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