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PURIN-8-ONES AS CORTICOTROPIN-RELEASING HORMONE (CRH-R1) RECEPTOR ANTAGONISTS

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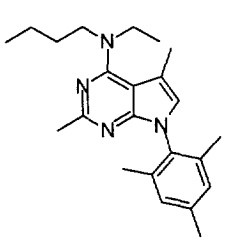
Abstract: A series of purin-8-ones was prepared and discovered to have excellent binding affintity to the CRH-R1 receptor. Structure-activity studies focused on amine side-chain optimization, urea substitution and pyridyl isostere incorporation. Thus, the highly potent purin-8-ones show promise as a new class of potential anxiolytics and/or antidepressants.

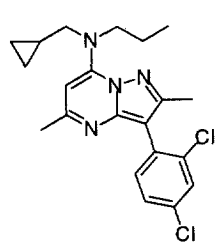
Corticotropin releasing hormone (CRH) is a 41-amino acid neurotransmitter that modulates mammalian stress response via hypothalamic-pituitary-adrenal (HPA) axis regulation.[1–3] The observation that depressed patients have increased CSF concentrations of CRH correlates with the hypothesis that CRH hyperactivity may contribute to the symptomatology observed in anxiety related disorders. A growing body of preclinical pharmacology continues to support the theory that small molecule antagonists of the CRH-R1 receptor may prove useful as novel anxiolytics and/or antidepressants.[1,4]

Several accounts of *in vitro* CRH-R1 heterocyclic antagonists have appreared in the literature,[5] including CP154526-1[6] and more recently pyrazolopyrimidine **1**[7] (Figure 1). We have recently reported the discovery and structure–activity relationships for the structurally related triazolopyrimidines **2**.[8] These bicyclic cores share a common pharmacophore, with bulky amine side chains, 2,4-di and 2,4,6-trisubstituted aryl groups and methyl-substituted pyrimidines being necessary for optimal activity. In this report, we disclose the synthesis and structure–activity studies of purin-8-ones and related pyridine isosteres (Figure 1) that led to the discovery of this potent new series of compounds having excellent binding affinity for the CRH-R1 receptor.[9]

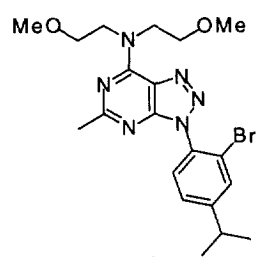
Figure 1



CP154526-1



1



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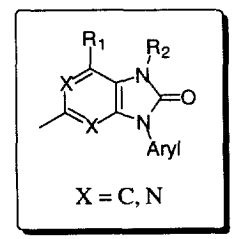

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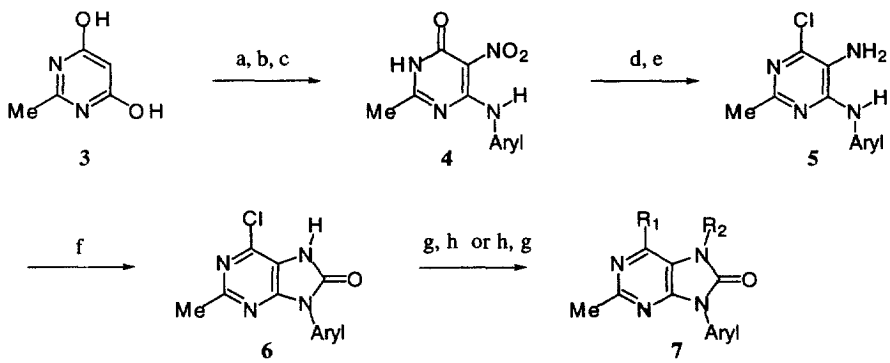
General procedures for the synthesis of purine-8-ones are described in Scheme 1. Briefly, commercially available **3** was nitrated and treated with $POCl_3$ to afford the intermediate 4,6-dichloro-2-methyl-5-nitropyrimidine. Due to the reactivity of the dichloro pyrimidine, however, attempted addition of anilines consistently afforded a complicated mixture of mono- and bis-addition products. A solution was devised, whereby aniline addition was conducted in DMSO as solvent. Upon workup, this afforded the mono-adducts **4** as the pyrimidones – in effect resulting in an in-situ protection of the second reactive center. Chlorination with $POCl_3$ and iron reduction of the nitro group provided diamines **5** which were readily cyclized to the purin-8-one cores **6**. Finally, intermediates **6** were treated with primary and secondary amines and alkylated to provide target compounds **7** or the order could be reversed, depending on the desired analoging objectives. However, when amination preceeded urea alkylation, the selectivity for N vs. O alkylation decreased from >20/1 to ca. 10/1 when R_2 = Me due to increased steric bulk near the site of alkylation.

Scheme 1

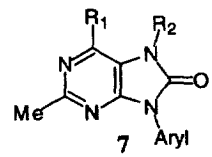


Reagents and conditions: (a) HNO_3, 0 °C to rt, 1.5 h, 85%; (b) $POCl_3$, diethylaniline, reflux, 2 h, 80–90%; (c) H_2N-Aryl, DMSO; 18 h, 75-85%; (d) $POCl_3$, reflux, 0.5 h, 80–95%; (e) Fe, AcOH, MeOH, reflux, 4 h, 70–80%; (f) $COCl_2$ (10 eq.), toluene, reflux, 3 h, 90–95%; (g) amine (excess, neat, sealed tube), 110 °C, 36 h, 85-95%, (h) KOH, R_2-LG, acetone, rt, 2 h, 60–95%.

Table 1 details our initial structure–activity studies for the purin-8-ones. New compounds were screened against transfected human CRH-R1 receptors expressed in HEK 293E cells using ^{125}I-tyr-oCRH as the displaced radioligand.[10] Based on optimized aryl SAR from previous work,[11] 2-bromo-4-isopropylphenyl was selected and fixed for preliminary studies. The aminated product **7a** (R_1 = N(Et)nBu) was found to have poor affinity at the CRH-R1 receptor; a dramatic improvement was realized by N-methylation to afford **7b** (Ki = 5.0 nM). When the steric bulk of R_2 was increased further (**7c**, R_2 = CH_2cPr), activity decreased > 20-fold. With R_2 binding affinity optimized for smaller alkyl groups, attention shifted to R_1 amine optimization.

It was rapidly discovered that symmetric and non-symmetric dialkyl amine substitution was equipotent (**7b**, **7d** and **7e** with K_i = 5.0, 7.6, and 5.7 nM, respectively). Small dialkyl amines (i.e., NMe_2) were not examined due to previous SAR understanding.[8] Primary amines were also examined, and generally these side chains were less potent (**7f**, K_i = 15.1 nM), especially when a polar alkoxy group was introduced (**7g**, K_i = 42.8

Table 1 Structure–Activity Relationships for Purin-8-one CRH-R1 Antagonists



7

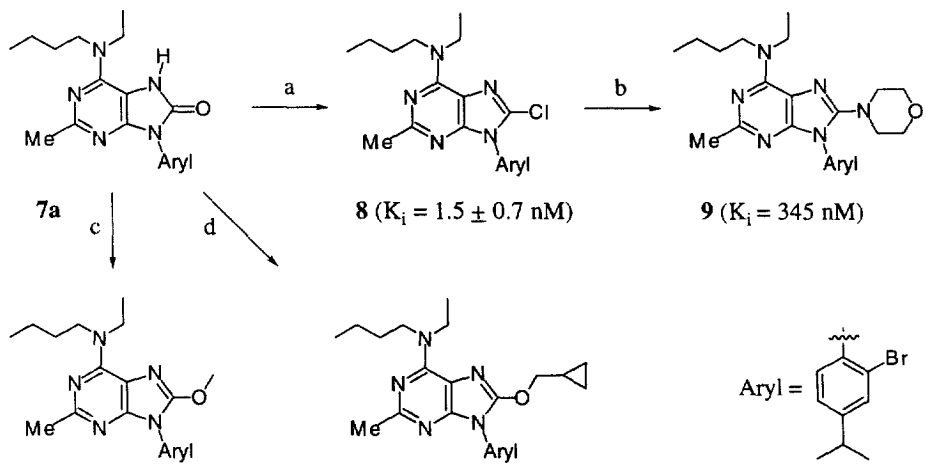
Compd	R_1	Aryl	R_2	K_i (nM, hCRH R1)[a]
7a	N(Et)n-Bu	2-bromo-4-isopropyl	H	890
7b	N(Et)n-Bu	2-bromo-4-isopropyl	Me	5.0 ± 2.4
7c	N(Et)n-Bu	2-bromo-4-isopropyl	CH_2cPr	117
7d	N(n-Pr)$_2$	2-bromo-4-isopropyl	Me	7.6 ± 1.2
7e	N(n-Pr)CH_2cyclo-Pr	2-bromo-4-isopropyl	Me	5.7 ± 2.7
7f	N(H)CH(Et)n-Bu	2-bromo-4-isopropyl	Me	15.1 ± 7.6
7g	N(H)CH(Et)CH_2OMe	2-bromo-4-isopropyl	Me	42.8 ± 17.8
7h	N(H)CH_2CH_2OMe	2-bromo-4-isopropyl	Me	1179
7i	N(CH_2CH_2OMe)$_2$	2-bromo-4-isopropyl	Me	130
7j	N(Et)CH_2CH_2OMe	2-bromo-4-isopropyl	Me	47.9 ± 11.9
7k	N(CH_2c-Pr)CH_2CH_2OMe	2-bromo-4-isopropyl	Me	12.2 ± 2.1
7l	N(benyzl)CH_2CH_2OMe	2-bromo-4-isopropyl	Me	26.2 ± 7.8
7m	N(Et)n-Bu	2,4,6-trimethyl	Me	4.4 ± 1.0
7n	N(n-Pr)CH_2cyclo-Pr	2,4,6-trimethyl	Me	6.1 + 1.2
7o	N(H)CH(Et)n-Bu	2,4,6-trimethyl	Me	17.4 + 1.2
7p	N(CH_2CH_2OMe)$_2$	2,4,6-trimethyl	Me	698
7q	N(Et)n-Bu	2,4,6-trimethyl-3-pyridyl	Me	124
7r	N(n-Pr)CH_2cyclo-Pr	2,4,6-trimethyl-3-pyridyl	Me	113
7s	N(n-Pr)$_2$	2,4,6-trimethyl-3-pyridyl	Me	259

[a]Binding affinity was measured at transfected human CRH R1 receptors expressed in HEK 293E cells using ^{125}I-tyr-oCRH as the displaced radioligand.[10] Values are the average of n ≥ 2 when K_i < 50 nM.

nM). This latter observation became more pronounced when the alkyl branch α to the nitrogen was removed to provide **7h** (K_i = 1179 nM). Although addition of a second alkoxy side chain improved binding affinity (**7i**, K_i = 130 nM), good activity was not observed until the second alkoxy side chain was replaced with a cyclopropyl methyl group (**7k**, K_i = 12.2 nM). In the course of this work we also examined the congeners where Aryl = 2,4,6-trimethylphenyl. Here, the SAR mirrored the 2,4-disubstituted analogs with the dialkylamine **7m** = **7n** > **7o** >> **7p** with respect to CRH-R1 receptor binding affinity. The concluding SAR efforts in this area examined Aryl = 2,4,6-trimethyl-3-pyridyl[12] with the goal of maintaining potency and lowering lipophilicity. Unfortunately, this effort to improve hydrophilicity was not tolerated as all the analogs prepared (**7q–s**) were K_i > 100 nM in the CRH-R1 receptor assay.

We next focused attention on modification of the 8-oxo functionality in order to explore new SAR. Synthetic details and CRH-R1 binding affinities are provided in Scheme 2. From **7a**, treatment with $POCl_3$ afforded the 8-chloro target **8**. This compound was found to be highly potent and was subsequently treated with morpholine under refluxing conditions to provide **9**. Consistent with earlier SAR observations of 8-phenyl substituted purine derivatives,[8] purine **9** displayed greater than two orders of magnitude decrease in CRH-R1 affinity. We believe this marked decrease in activity is likely due to unfavorable receptor-ligand interaction as a result of steric considerations over electronic. Additional polar functionality was incorporated at C-8 via the oxophilic alkylation of intermediate **7a** to afford **10**. This compound, despite bearing a polar C-8 methoxy, displayed excellent CRH-R1 receptor affinity. Once again, however, when the steric bulk was increased to provide **11**, a decrease in receptor affinity was noted.

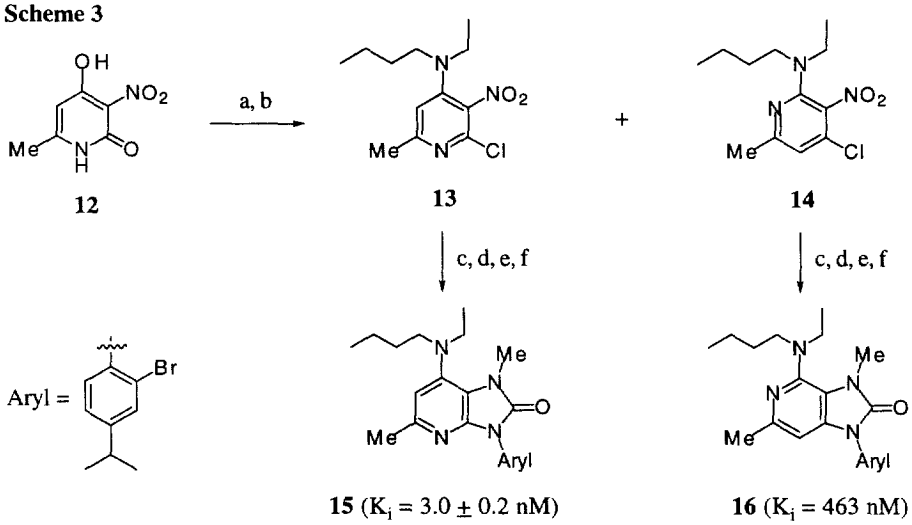
Scheme 2



Reagents and conditions: (a) $POCl_3$, reflux, 72 h, 30%; (b) morpholine (excess, neat), reflux, 72 h, 75%; (c) Me_3OBF_4, CH_2Cl_2, rt, 36 h, 40%; (d) cyclopropylmethyl bromide, KOH, acetone, rt, 12 h, 45%.

Finally, we examined pyridine isosteres of the purin-8-ones with the goal of increasing basicity and solubility. Starting with commercially available **12**, chlorination under the standard conditions and treatment with N-butyl-N-ethylamine afforded a regiomeric mixture of the addition products **13** and **14** (Scheme 3) which were separated by column chromatography and characterized by nOe experiments.[8] Intermediates **13** and **14** were then carried on in parallel to afford the target compounds **15** and **16**, respectively. Although **16** was weakly active, the pyridyl regiomer **15** possessed excellent binding affinity to the CRH-R1 receptor (K_i = 3.0 nM) which was generally equipotent with pyrimidine **7b** (K_i = 5.0 nM). Additionally, **15** had a measured solubility of 0.102 mg/ml in 0.1 N HCl, which represented a 3.0-fold improvement over the measured solubility of **7b**.

Scheme 3



Reagents and conditions: (a) $POCl_3$, diethylaniline, reflux, 3 h, 95%; (b) N-ethyl-N-butylamine (excess, neat), i-Pr_2NEt, reflux, 5 h, 85% (**13**:**14**, 3.0:1.0); (c) H_2N-Aryl, neat, 140 °C, 5–24h; (d) $Na_2S_2O_4$, NH_4OH, dioxane, rt, 2 h; (e) $COCl_2$ (10 eq.), toluene, reflux, 3 h; (f) KOH, MeI, acetone, rt, 1–2 h, (yields 20–50% over 4 steps, from **13**/**14**).

In conclusion, we have discovered a series of purin-8-ones **7** and the related pyridyl isostere **15** that display excellent CRH-R1 receptor affinity *in vitro*. Structure–activity relationships were developed that support small alkyl substitution at R_2 (Figure 1). Methyl was found to be optimal. At R_1, dialkyl amines were preferred; alkoxy substitution was only tolerated on one side chain, and only when balanced with an appropriate alkyl group. In the limited aryl substitution studies we undertook, 2-bromo-4-isopropylphenyl analogs were equipotent with 2,4,6-trimethylphenyl analogs and incorporation of nitrogen in the latter series proved detrimental to receptor affinity. Further detailed studies of this class of compounds as potential antagonists of the CRH-R1 receptor will be reported in due course.

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12. Analogs **7q–s** were prepared by a slight modification of Scheme 1. Treatment of 4,6-dichloro-2-methyl-5-nitropyrimidine (generated from **3** by Steps a, b) with 2,4,6-trimethyl-3-aminopyridine[13] afforded the desired mono-addition adduct. The remaining reaction sequence proceeded as described in Scheme 1 with Step h proceeding Step g.
13. Obtained from the nitration (85%) of 2,4,6-collidine (Plazek, E. *Ber. Dtsch. Chem. Ges.* **1939**, *72*, 577) followed by reduction (H_2, Pd/C, 50 psi, 95%).