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5-Arylamino-1,2,4-triazin-6(1*H*)-one CRF_1 receptor antagonists

William D. Schmitz [a,*], Allison B. Brenner [a], Joanne J. Bronson [a], Jonathan L. Ditta [a], Corrine R. Griffin [b], Yu-Wen Li [c], Nicholas J. Lodge [b], Thaddeus F. Molski [b], Richard E. Olson [a], Xiaoliang Zhuo [c], John E. Macor [a]

[a] *Neuroscience Discovery Chemistry, Bristol-Myers Squibb Research and Development, 5 Research Parkway, Wallingford, CT 06492, USA*
[b] *Neuroscience Discovery Biology, Bristol-Myers Squibb Research and Development, 5 Research Parkway, Wallingford, CT 06492, USA*
[c] *Preclinical Candidate Optimization, Bristol-Myers Squibb Research and Development, 5 Research Parkway, Wallingford, CT 06492, USA*

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ABSTRACT

A series of 5-arylamino-1,2,4-triazin-6(1*H*)-ones was synthesized and evaluated as antagonists at the corticotropin releasing factor receptor. Formation of CYP-mediated oxidative reactive metabolites previously observed in a related N^3-phenylpyrazinone structure was minimized by incorporation of the additional ring nitrogen found in the triazinones.

Major depressive disorder treatment remains an area of unmet medical need. Worldwide, the incidence of depression is estimated at approximately 121 million affected people.[1] Current standard of care includes treatment with serotonin and/or norepinephrine transporter reuptake inhibitors. However, there are several limitations associated with these treatment regimens including slow onset of action, a significant number of nonresponders and/or recurrence of depressive events. A number of alternative treatment paradigms have been investigated such as coupled use of neurotransmitter receptor antagonists/reuptake inhibitors, augmentation strategies, and neuropeptide modulators.[2,3] This latter category includes corticotropin releasing factor receptor antagonists. Corticotropin releasing factor (CRF) is a 41 amino acid peptide released by the hypothalamus. CRF functions as the primary activator of the hypothalamic–pituitary–adrenal (HPA) axis, which in turn regulates the body's response to stress.[4] The potential for treatment of a wide variety of disorders including depression, anxiety, ischemia, irritable bowel syndrome, Alzheimer's disease, immune system disorders and substance abuse via modulation of CRF receptors has been hypothesized due to the broad applicability of the HPA axis in stress related conditions that may play a role in the etiology of these diseases.[5] Some examples of non-peptidic small molecule CRF_1 antagonists that have been investigated in clinical trials include R121919,[6] CP-376395,[7] BMS-561388[8] and BMS-562086[9] (Fig. 1). Many of the compounds reported to be in preclinical development consist of similar bicyclic heterocyclic core structures that generally exhibit potent activity at the CRF_1 receptor.[10] A number of monocyclic heterocyclic based CRF_1 receptor antagonists have also been described[5a,9,11] including pyrazolones,[12] thiazoles, pyrimidines, pyrazines and triazines.

We have recently disclosed the N^3-phenylpyrazinone structure as a competent class of potent and orally active CRF_1 receptor antagonists.[13] One of the liabilities associated with certain N^3-phenylpyrazinones was the potential for oxidation at the pyrazinone core and subsequent formation of reactive intermediates leading to the formation of glutathione (GSH) adducts.[14] Previous in vitro metabolite studies in liver microsomes suggested that pyrazinone oxidation was a primary pathway for metabolism. Detection of glutathione adducts was consistent with formation of a reactive species on the pyrazinone core. Additionally, in vivo studies conducted in bile duct cannulated rats showed that for pyrazinones

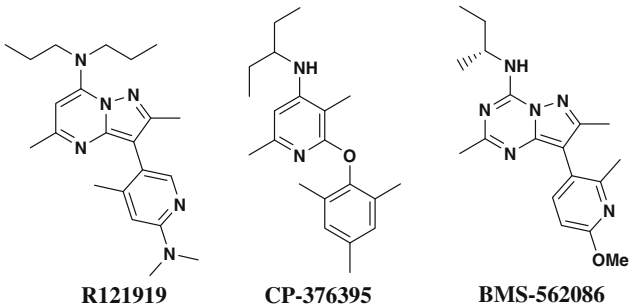

Figure 1. Small molecule CRF_1 antagonists.

* Corresponding author. Tel.: +1 203 677 6562; fax: +1 203 677 7702.
E-mail address: william.schmitz@bms.com (W.D. Schmitz).

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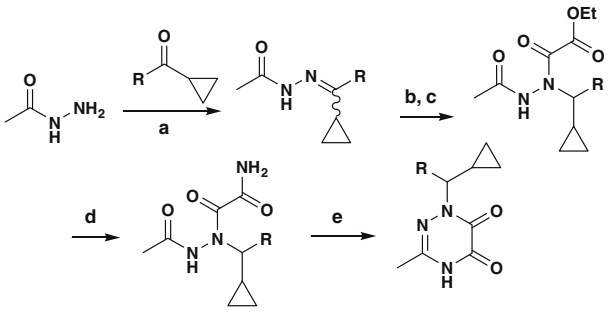
such as BMS-665053, there was extensive metabolism with pyrazinone oxidation products comprising the majority of drug-related materials in plasma.[15] The pathway leading to these adducts was proposed to occur through the intermediacy of an epoxide formed at the C5–C6 double bond of BMS-665053 (Fig. 2). This type of metabolic pathway has been previously reported.[16] The potential for such reactive intermediates to participate in idiosyncratic drug reactions[17] prompted us to consider modifications to the core structure that could potentially reduce such oxidative metabolism. We previously reported attenuation of pyrazinone core metabolism via introduction of electron-withdrawing groups such as cyano at C-5.[14] An alternative approach in our strategy involved modification of the electronic properties of the heterocyclic core structure via replacement of the pyrazinone with a 1,2,4-triazine-6-one.[18]

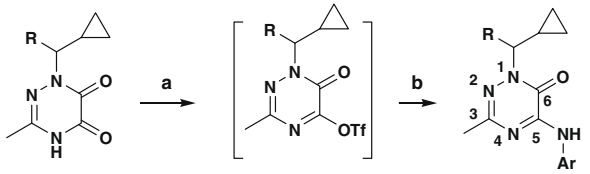
The synthesis of the 1,2,4-triazine-5,6-dione core structure was completed as shown in Scheme 1 starting with acetylhydrazide. Condensation with a cyclopropyl ketone gave an imine of undetermined geometry which was directly reduced and treated with chloroethyloxalate. Conversion of the ester to the primary oxamide provided the penultimate intermediate for cyclization to the core structure. Initial methods used to effect this cyclization included treatment with trimethylsilyl iodide or triflate in dichloromethane at room temperature. While these conditions did provide the desired material, the yields were highly variable and these reactions were not readily amenable to large scale preparation. An improved procedure was realized with base induced cyclization in 1 M NaOH using microwave heating and very short reaction times to give modest yet reliable yields of the triazine dione suitable for use without further purification.

Preparation of the final compounds was accomplished as shown in Scheme 2. Activation of the dione core as the imino triflate and displacement with a variety of substituted anilines or aminopyridines under basic conditions gave 5-arylamino-1,2,4-triazin-6(1*H*)-ones. Alternatively, this coupling could be carried out under neutral conditions using palladium catalysis at elevated temperatures for cases in which the prior method failed.

Previous investigation in the pyrazinone series had shown that preferred structural elements in the hydrophobic top region were cyclopropyl accompanied by either a small alkyl or alkoxyalkyl substituent; therefore the target compounds in this series were



Scheme 1. Reagents and conditions: (a) EtOH, reflux (85%); (b) Pt_2O, AcOH, 50 psi H_2; (c) $ClCOCO_2Et$, Et_3N, CH_2Cl_2; (d) NH_4OH, THF/EtOH (89% c b through d); (e) 1 M NaOH, 100 °C, 20–30 min (20–30%).



Scheme 2. Reagents and condition: (a) Tf_2O, 2,4,6-collidine, CH_2Cl_2; (b) NaHMDS, THF or $Pd(OAc)_2$, BINAP, K_2CO_3, THF, 70 °C.

all designed utilizing these top structural motifs (Table 1). Additionally, since we had shown previously with the pyrazinones bearing either 2,4,6- or 2,4,5-anilino substitutions at the 5-position were preferred, we focused on these analogs in the triazinone series. Compounds were tested in a CRF_1 receptor binding titration assay using rat frontal cortex homogenate in which inhibition of specific binding of [^{125}I]Tyr-ovine-CRF by our test compounds was measured to determine their receptor binding affinity.

Diverse substituents on the lower aryl ring were tolerated with respect to steric and electronic considerations (Tables 1–3). In the 2,4,6-substituted aniline series, substitution of chlorine for methyl in the 2- and 4-positions resulted in reduced potency (**1A** vs **2A**). However, compounds having chlorine in the 2- and 6-position were more potent than their respective methyl analogs (**4A** and **4C** vs **3A** and **3C**). Replacing the methyl ether in the 4-position of

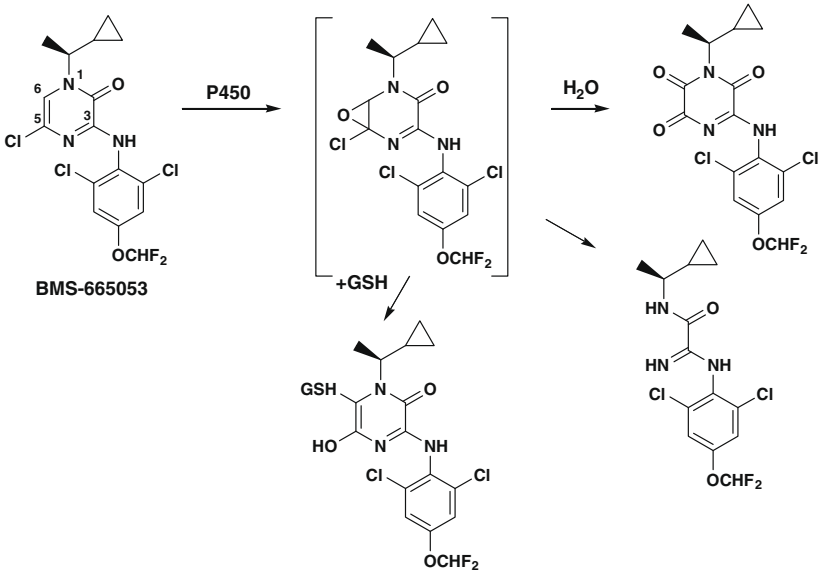
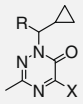
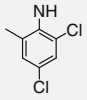
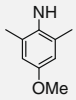
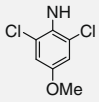
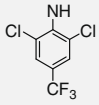
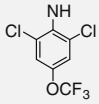
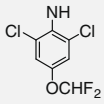
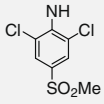


Figure 2. Proposed core oxidative metabolic pathway of N^3-phenylpyrazinones.

Table 1
rCRF$_1$ binding affinities of 5-(2,4,6-substituted)arylamino-1,2,4-triazin-6(1*H*)-ones[a]

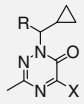


R = (A) Me; (B) Et; (C) CH_2OMe

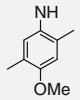
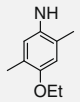
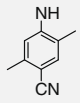
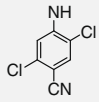
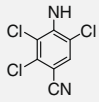
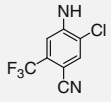
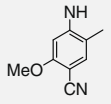
X	Compound	rCRF$_1$ IC_{50}[b]
	**1A**	19 (4)
	**2A**	33 (5)
	**3A** **3C**	68 (19) 51 (2)
	**4A** **4C**	15 (1) 6.3 (1.6)
	**5A** **5B** **5C**	14 (2) 6.3 (1.6) 11 (2)
	**6A** **6C**	39 (5) 11 (2)
	**7A** **7B** **7C**	9.9 (2.5) 2.1 (0.8) 6.3 (1.2)
	**8A**	20 (1)

[a] Values are means of three experiments, standard deviation is given in parentheses.
[b] IC_{50} of o-CRF = 2.9 ± 1.0 nM in this assay.

Table 2
rCRF$_1$ binding affinities of 5-(2,4,5-substituted)arylamino-1,2,4-triazin-6(1*H*)-ones[a]



R = (A) Me; (B) Et; (C) CH_2OMe

X	Compound	rCRF$_1$ IC_{50}
	**9A** **9B**	5.0 (2.5) 0.89 (0.10)
	**10A**	28 (8)
	**11A** **11B**	60 (13) 9.3 (3.7)
	**12A**	270 (50)
	**13A**	10 (3)
	**14A**	3300 (200)
	**15A** **15B**	94 (25) 13 (6)

[a] Values are means of three experiments, standard deviation is given in parentheses.

compound **4C** with an electron-withdrawing substituent resulted in similar potency for the $-CF_3$ analogs **5A** and **5C**. Only a modest decrease in potency was observed when the larger, more polar methylsulfonyl group was introduced in compound **8A**. No improvement in potency was realized by incorporating the $-CF_3$ phenol ether in compounds **6A** and **6C**. However, when a slight change was made from a $-CF_3$ ether to $-CHF_2$ ether, improved potencies were observed (compounds **7A–C**). Consistent with previous observations in the pyrazinone series,[13] a general rank ordering of compound IC_{50}'s with respect to alkyl substitution on the triazinone N1 position was: 1-cyclopropylethyl > 1-cyclopropyl-2-methoxyethyl ⩾ 1-cyclopropylpropyl-1-amine. The most potent compound in the 2,4,6-series was **7B**.

For select compounds in the 2,4,5-substituted aniline series, a significant improvement in potency was observed relative to the 2,4,6-substituted series (Table 2). For example, migration of the 6-methyl in **3A** to the 5-position as in **9A** gave a >10-fold increase in potency. Interestingly, even though a diverse array of substituents were tolerated in compounds with potent rCRF$_1$ binding affinities, the relative positions and combinations of these groups showed significant effects on binding. Compound **12A**, with chlorine at the 2- and 5-position, was only modestly active at 270 nM. Incorporation of an additional chlorine at the 6-position as in compound **13A** improved the potency by 27-fold to 10 nM. While electron-withdrawing substituents such as $-CF_3$, $-SO_2Me$ and –CN were tolerated (compounds **5A–C** and **8B**, Table 1; compounds **11–15**, Table 2), aryl groups bearing multiple electron-withdrawing groups were less active (**12A** vs **14A**, Table 2). One notable example from the 2,4,5-trisubstituted series was compound **9B**, which was the most potent compound tested at 0.89 nM.

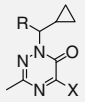
Structures possessing a substituted 3-amino pyridyl substituent at the 5-position of the triazinone were found to have potencies equal to or better than the aniline-derived compounds (Table 3). Earlier work in the pyrazinone series had shown either a 2,6- or 2,5,6-substitution pattern on the pyridine was optimal.[19] Initial results for the triazinones suggested that this trend was conserved. By comparison, the 2,4,6-substitution pattern on the pyridyl group was disfavored (compare **5A**, Table 1 to **16A**, Table 3), thus we focused on pyridyl analogs with either the 2,6- or 2,5,6-substitution patterns. Alkyl ethers were the preferred groups at the 6-position, and a significant improvement in potency was observed upon replacing the methyl ether with a difluoromethyl ether (compare **20C** vs **21C**, Table 3). Introduction of an additional nitrogen as in pyrimidine **22A** resulted in a substantial loss of activity which was also consistent with observed results in the pyrazinone series.[20]

The effect of stereogenicity on the CRF$_1$ binding profile of individual enantiomers was also assessed. It was found that there was

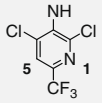
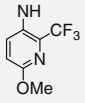
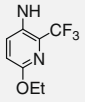
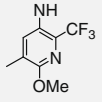
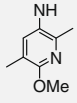
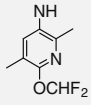
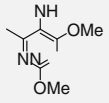
generally a preference for the (*S*-) enantiomer within the anilino series and for the (*R*-) enantiomer in the 3-aminopyridine series (Table 4) although the difference between any enantiomer pair was no greater than 10-fold. Of particular note was that compounds possessing a 1-cyclopropyl-2-methoxyethyl fragment exhibited the greatest potency differences (5- to 11-fold; Table 4, entries 5–9). This trend was in contrast to that observed previously for pyrazinones, wherein compounds containing this functionality showed little to no differentiation in activity across enantiomer pairs.[13b] Absolute configuration was assigned based on single crystal X-ray analyses for compounds **4A** and **4C** and subsequent correlation of optically active triazinone core intermediates used for syntheses of final products.[21]

Compound **17A** was chosen for further evaluation to assess its in vitro metabolic profile, particularly with respect to core oxidation. Previous experience with pyrazinone compounds bearing the identical trifluormethyl methoxy pyridine residue found in **17A** suggested that this was one of the more metabolically stable pendant aryl groups under our assay conditions which allowed for a more straightforward analysis of metabolism specifically at the triazinone core.[19] Accordingly, results of incubation with human and rat liver microsomes for triazinone **17A** showed a significant decrease in the total percentage of metabolized compound resulting from core oxidation (Table 5). The levels of core oxidation products resulting from the proposed CYP-mediated epoxidation were below the limits of detection. The levels of GSH adducts attributed to reactive oxidative metabolites at the core structure were estimated at <1% (detectable only by mass spectral analysis).

Table 3
$rCRF_1$ binding affinities of 5-(di- and tri-substituted-pyridin-3-ylamino)-1,2,4-triazin-6(1*H*)-ones[a]



R = (A) Me; (B) Et; (C) CH_2OMe

X	Compound	$rCRF_1$ IC_{50}
	**16A**	260 (10)
	**17A**	6.7 (0.6)
	17B	3.7 (0.4)
	17C	3.7 (0.3)
	**18A**	12 (2)
	18B	3.6 (0.8)
	18C	3.0 (0.3)
	**19B**	2.7 (1.0)
	**20A**	26 (7)
	20B	6.5 (1.6)
	20C	20 (2)
	**21C**	1.1 (0.0)[b]
	**22A**	1100 (100)

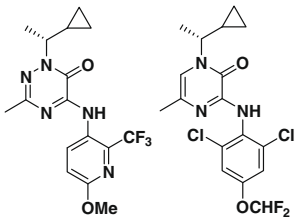
[a] Values are means of three experiments, standard deviation is given in parentheses.
[b] Data from most potent single enantiomer.

Table 4
$rCRF_1$ binding affinities of individual enantiomers for select triazinones.[a]

Entry	Compound	(*R*-)	(*S*-)
1	**4A**	8.3 (3.7)	7.5 (0.4)
2	**7A**	13 (2)	7.2 (2.0)
3	**17A**	3.5 (1.0)	12 (1)
4	**18A**	8.6 (0.6)	23 (1)
5	**4C**	69 (10)	7.2 (2.0)
6	**7C**	23 (9)	4.4 (1.8)
7	**17C**	2.3 (1.7)	15 (4)
8	**18C**	2.4 (0.8)	27 (2)
9	**21C**	1.1 (0.0)	6.5 (1.3)

[a] Values are means of three experiments, standard deviation is given in parentheses.

Table 5
Liver microsome incubation comparison of select pyrazinones and triazinones[a]



Compound	Species	Compound remaining[b] (%)	Core oxidation (%)	GSH core adducts (%)
17A	Human	85	ND[c]	<1
17A	Rat	37	ND[c]	<1
23	Human	71	8	20
23	Rat	12	7	56

[a] Data from (*R*)-enantiomers.
[b] Thirty minute incubation with liver microsomes at 37 °C. For experimental details see Ref. 15.
[c] ND, not detected.

The major metabolic pathway for triazinone **17A** was found to be O-demethylation (~70% of total), along with smaller amounts of oxidation products on the pyridyl ring (~2%). Only trace amounts (⩽0.1%) of GSH adducts at the pyridyl ring were detected.[15] Included for reference in Table 5 is the data for pyrazinone **23**[15] which possesses an analogous core structure.

Finally, to further assess the viability of the triazinone chemotype, central target engagement was measured using an ex vivo binding assay. Compounds **9B** and **18C** were shown to occupy rat CRF_1 receptors in the parietal cortex at 64% and 46%, respectively.[22] The antagonist activity of the triazinones at CRF_1 was confirmed by evaluating both enantiomers of compound **21C** in a cAMP assay, using Y79 cells in which potent antagonist activity (7.3 nM, *R*-enantiomer; 29 nM, *S*-enantiomer) against 0.5 nM CRF was observed.

In summary, a series of 5-arylamino-1,2,4-triazin-6(1*H*)-ones was found to be potent CRF_1 receptor antagonists. Additionally, oxidative metabolism of the heterocyclic core was not detected, thereby minimizing the potential for formation of reactive metabolites, improving the overall metabolic profile of triazinone-based compounds relative to N^3-phenylpyrazinones.

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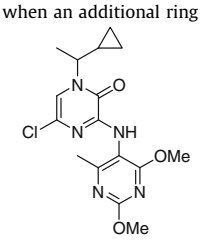
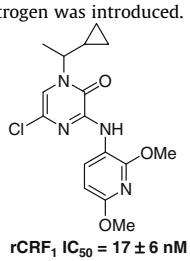
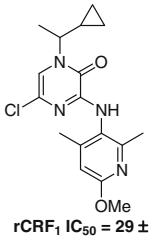
20. Pyrimidine substituted pyrazinones were generally less potent than structurally related pyridyl analogs.[19] Data shown below for closest structurally related pyrazinones also suggested a decrease in binding at CRF_1 when an additional ring nitrogen was introduced.



$rCRF_1$ IC_{50} = 240 ± 40 nM



$rCRF_1$ IC_{50} = 17 ± 6 nM



$rCRF_1$ IC_{50} = 29 ± 12 nM

21. Racemic 1,2,4-triazine-5,6,(1*H*, 4*H*)-dione cores were separated by chiral chromatography. Representative conditions: Chiralcel OD column, 4.6 × 250 mm, 10 μm; solvents: A = EtOH, B = heptane; 95% B for 35 min; flow rate: 0.8 mL/min, 281 nm. Alternatively, in some cases final racemic triazinone compounds were separated by chiral chromatography. Representative conditions: Chiralpak AD-H column, 4.6 × 250 mm, 5 m; solvents: 91% CO_2–9% ethanol; temperature: 35 °C; pressure: 100 bar; flow rate: 2 mL/min; UV at 210 nm.
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