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Letters

Arylamidrazones as Novel Corticotropin Releasing Factor Receptor Antagonists

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Figure 1. Some advanced small-molecule CRF receptor antagonists.[13,16,17]

Abstract: The arylamidrazones have been found to be potent corticotropin releasing factor (CRF) receptor antagonists structurally distinct from previously reported CRF_1 antagonists. Attempts to modify the arylamidrazone core suggested an important role for the anilino NH moiety. The right-hand-side 2-nitro feature in lead **1** could be replaced with substituents methyl, chloro, cyano, or trifluoromethyl with a 4- to 10-fold reduction in receptor binding. With appropriate left-hand-side modifications, this potency loss could be recovered.

Introduction. The role of corticotropin releasing factor (CRF) in the physiology of stress has been an area of active research since the isolation of the 41 amino acid protein in 1981.[1] It has since been established that CRF (also known as corticotropin releasing hormone, CRH) modulates the activity of the hypothalamus—pituitary—adrenal axis (HPA), regulating "fight or flight" responses to stressors. Two subtypes of CRF receptors have been identified, CRF_1 and CRF_2, with the latter existing as splice variants α, β, and γ.[2] While most drug discovery efforts have focused on CRF_1 receptors, CRF_2 receptor subtypes have begun to attract attention,[3] especially since the discovery of subtype-specific ligands.[4] The overall correlation between clinical

outcome and subtype specificity has yet to be established and remains an active area of research.

Direct intracerebroventricular (icv) injection of CRF itself elicits a behavioral and physiological cascade very similar to that resulting from environmental sources of stress.[5] In addition to these normal functions, CRF has been invoked in a wide variety of pathological conditions ranging from anxiety and depression,[6] substance abuse,[7] eating disorders,[8] premature parturition,[9,10] immune disease,[11] and gastrointestinal maladies.[12]

Potent small-molecule CRF receptor antagonists have been discovered, several of which are under early clinical evaluation. Only recently have data become available detailing in vivo profiles of development compounds, since most preclinical and clinical investigations remain active areas within the pharmaceutical industry. Three recent examples are illustrated in Figure 1.

Pfizer has described CP-154,526,[13] and the effects of chronic adminstration in rat were shown to be anxiolytic.[14] Adrenal insufficiency, a plausible adverse effect, was not observed, consistent with the clinical utility for small-molecule CRF receptor antagonists. The Neurocrine compound NBI 30775 (R121919) has advanced to

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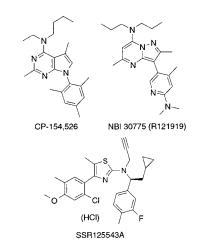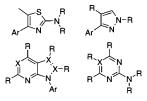**Figure 2.** Representative small-molecule CRF receptor antagonist chemotypes.[18]
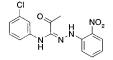

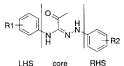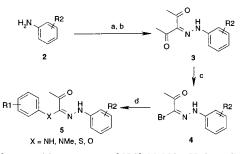**Figure 3.** Initial lead arylamidrazone **1**.

Figure 4. Arylamidrazone strategy: optimization of LHS, RHS, and core.

Scheme 1[a]

[a] Conditions: (a) concentrated HCl, $NaNO_2$, H_2O, 0 ºC, 30 min; (b) 2,4-pentanedione, NaOAc, 3:1 H_2O/EtOH, 0 °C to room temperature, 3 h; (c) 2.5:1 $AcOH$/Ac_2O, NaOAc, Br_2, 0 °C to room temperature, 30 min; (d) $ArNH_2$, ArNHMe, ArSH, or ArOH, DMF, I-Pr_2NEt, room temperature, o/n.

Table 1. K_i (nM) Data for Derivatives of **1**[25]

compd	X	R2	K_i (nM)
1	NH	2-NO_2	51
8	NMe	2-NO_2	8600
9	O	2-NO_2	>10000
10	NH	H	2720
11	NMe	H	>10000
12	O	H	>10000
13	NH	2-CF_3	538
14	NMe	2-CF_3	>10000
15	O	2-CF_3	>10000
16	NH	2-Cl	417
17	NMe	2-Cl	>10000
18	S	2-Cl	>10000
19	NH	2-CN	415
20	NMe	2-CN	>10000
21	NH	2-Me	216
22	NMe	2-Me	>10000
23	O	2-Me	>10000
24	S	2-Me	>10000
25	NH	2-SO_2Me	>10000
26	NH	2-SO_2NH_2	>10000
27	NH	4-Me	2285
28	NH	4-Br	1459

reported. After the compound was tested against the human CRF_1 receptor, its K_i value was determined to be 51 nM.[22]

Efforts were directed toward several potential issues. First, the role of individual structural features in receptor binding was unknown, information best acquired by exploring the structure–activity relationships (SAR) for the series. Second, several chemical features, including the nitro group, the aniline moieties, and the arylamidrazone core, were seen as potential liabilities in a drug candidate. These areas were therefore targeted for investigation.

Chemistry

To systematically explore the SAR of the arylamidrazone series, the structure was divided into three parts (Figure 4) to elucidate the effect on receptor binding (vide infra).

Analogues were prepared in a three-step sequence starting with commercially available anilines **2**. In situ diazotization followed by condensation with 2,4-pentanedione gave the diketohydrazones **3**, which were converted to the hydrazonyl bromides **4** by bromination in acetic acid/acetic anhydride.[23] Reaction of the hydrazonyl bromides with the appropriate aniline, phenol, or thiophenol gave the aza, oxo, and thia derivatives **5** (Scheme 1).[24]

Optimization and SAR. Holding the 3-chlorophenyl moiety constant (LHS), arylamidrazone derivatives were synthesized modifying both the phenylhydrazone substitution (RHS) and the core connectivity "X". As shown in Table 1, the 2-nitrophenyl substituent in **1** (RHS) could be replaced with small substituents such as methyl, chloro, cyano, or trifluoromethyl without complete elimination of receptor binding. Although potency was reduced 4- to 10-fold, (**13**, **16**, **19**, **21**), the possibility of compensatory optimizations elsewhere in the mol-

an open label phase IIa trial,[15] which, although preliminary, suggested reductions in clinical scoring of anxiety and depression. Rodent in vivo data for the compound were also recently disclosed.[16] Sanofi-Synthelabo has also described the rodent in vivo profile of SSR125543A.[17]

Although multiple general chemical classes of CRF receptor antagonists have been identified (Figure 2),[18] there is still considerable interest in novel, unprecedented chemotypes, especially given the substantial prior art.[19] Furthermore, potential chemotype-specific pharmacokinetic, selectivity, or clinical liabilities provide ample incentive for unique antagonists. It is within this context that we sought to develop structurally unprecedented antagonists of CRF.

To generate novel leads, our proprietary screening library known as the universal informer library (UIL) was evaluated. This chemically diverse screening library (13 600 compounds) was designed to generate an optimal amount of information from the screening data (both actives and inactives), as described previously.[20] When the UIL was screened against the rat CRF_1 receptor,[21] lead compound **1** was identified (Figure 3).

Compound **1** was an arylamidrazone, a novel structural class for which no CRF antagonism had been

Table 2. K_i (nM) Data for LHS and RHS Derivatives of **1**[25,a]

compd	R1	R2	K_i (nM)
29	H	Me	526
30	2-Cl	Me	>10000
31	3-Cl	Me	216
32	4-Cl	Me	1832
33	3-Me	Me	526
34	**3-F**	**Me**	**43**
35	3-OMe	Me	1403
36	3-OEt	Me	>10000
37	3-SMe	Me	>10000
38	3-CN	Me	>10000
39	3-ethynyl	Me	62
40	3-CF₃	Me	291
41	H	Cl	299
42	2-Cl	Cl	>10000
43	3-Cl	Cl	417
44	4-Cl	Cl	3867
45	3-Me	Cl	115
46	**3-F**	**Cl**	**43**
47	3-OMe	Cl	652
48	3-OEt	Cl	>10000
49	3-SMe	Cl	>10000
50	3-CN	Cl	nd
51	**3-ethynyl**	**Cl**	**35**
52	3-CF₃	Cl	nd
53	H	CF₃	2261
54	2-Cl	CF₃	>10000
55	3-Cl	CF₃	538
56	4-Cl	CF₃	>10000
57	3-Me	CF₃	1422
58	3-F	CF₃	197
59	3-OMe	CF₃	6953
60	3-OEt	CF₃	>10000
61	3-SMe	CF₃	>10000
62	3-CN	CF₃	>10000
63	3-ethynyl	CF₃	66
64	3-CF₃	CF₃	>10000
65	H	H	6230
66	2-Cl	H	>10000
67	3-Cl	H	2720
68	4-Cl	H	>10000
69	3-Me	H	4708
70	3-OMe	H	>10000
71	H	CN	1079
72	2-Cl	CN	>10000
73	3-Cl	CN	415
74	4-Cl	CN	2207
75	3-Me	CN	650
76	3-OMe	CN	7771

a Compounds with K_i < 51 nM are labeled in bold.

ecule suggested plausible non-nitro-containing analogues. Potential formal charge bioisosteres for the nitro substituent gave inactive compounds (**25**, **26**). Substitution at the 2 position was also favored over 4-substituted analogues (**21**, **27**).

Variations of the LHS core anilino linker "X" indicated a strong preference for X = NH over NMe, thio, or oxo analogues. In all cases, such core replacements gave K_i's greater than 1 μM. The importance of the anilino NH moiety itself was confirmed by substitution of N-methylated aniline nucleophiles (X = NMe), which gave rise to compounds with dramatically attenuated activity (**8**, **11**, **14**, **17**, **20**, **22**). Similarly, analogues with oxygen and sulfur connectivity were uniformly inactive (**9**, **12**, **15**, **18**, **23**, **24**). The obligate H-bond feature (X = NH) was therefore retained, and efforts were focused on derivatives retaining the arylamidrazone core.

Next, the RHS 2-nitro replacements identified were held constant and the LHS aromatic substituents were varied. Fortunately, it was discovered that through appropriate LHS modifications, the 4- to 10-fold potency loss observed upon nitro replacement could be recovered. For example, 3-fluoro derivatives (**34**, **46**) and 3-ethynyl-substituted **51** led to compounds more potent than the original lead **1**, sans nitro, thus achieving an important optimization objective. Similar 3-ethynyl derivatives **39** and **63** were essentially equipotent. These results are summarized in Table 2.

Conclusion. We have discovered a novel series of CRF₁ receptor antagonists that are structurally novel and distinct from other known CRF antagonists. Preliminary attempts to modify the arylamidrazone core suggested an important role for the anilino NH moiety, in contrast to the customary obligate hydrogen bond acceptor.[3] Retention of this feature and optimization of substituents on the LHS and RHS aromatic rings identified several des-nitro compounds more potent than **1**. The polarity (calculated log P = 3−5) of the compounds contrasts with those of most known CRF antagonists, which are typically more lipophilic,[18] suggesting potential physicochemical and pharmacokinetc benefits. Further in vitro and in vivo studies on the arylamidrazone series shall be disclosed in due course.

Supporting Information Available: Experimental procedures, ¹H NMR, LC/MS, and HRMS data for compounds shown in tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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(25) Mean K_i values from at least two determinations were reported, obtained according to ref 22. If deviation between initial duplicate K_i values exceeded 15%, additional duplicate studies were performed until this criterion was met and determinations were averaged. Retests were also performed on compounds essential to SAR analysis.