Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3997–4000

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

2-Arylaminothiazoles as High-Affinity Corticotropin-Releasing Factor 1 Receptor (CRF_1R) Antagonists: Synthesis, Binding Studies and Behavioral Efficacy

Gene M. Dubowchik,[a,*] Jodi A. Michne,[a] Dmitry Zuev,[a] Wendy Schwartz,[a] Paul M. Scola,[a] Clint A. James,[b] Edward H. Ruediger,[b] Sokhom S. Pin,[a] Kevin D. Burris,[a,†] Lynn A. Balanda,[a] Qi Gao,[a] Dedong Wu,[a] Lawrence Fung,[a,‡] Tracey Fiedler,[a] Kaitlin E. Browman,[a,§] Matthew T. Taber[a] and Jie Zhang[a,¶]

[a]*Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 5100, Wallingford, CT 06492-7660, USA*
[b]*Bristol-Myers Squibb Pharmaceutical Research Institute, Candiac, Quebec, Canada J5R 1J1*

Received 5 June 2003; revised 13 August 2003; accepted 27 August 2003

Abstract—2-Arylamino-4-trifluoromethyl-5-aminomethylthiazoles represent a novel series of high-affinity corticotropin releasing factor-1 receptor (CRF_1R) antagonists that are prepared in three steps in good overall yields. Herein, we report binding SAR as well as anxiolytic activity of an exemplary compound (**7a**, $K_i = 8.6$ nM) in a mouse canopy model.

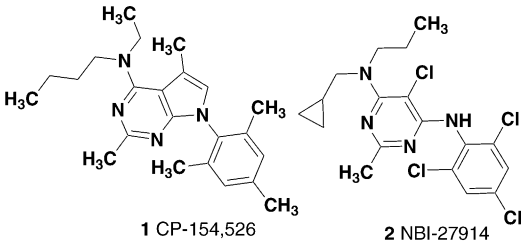
Corticotropin-releasing factor (CRF), a 41-residue neuropeptide, first isolated from ovine hypothalamus, coordinates the neuroendocrine, autonomic and behavioral responses to stress by stimulating the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland.[1,2] Stress-induced secretion of ACTH by CRF initiates the synthesis and release of adrenal glucocorticoids, which subsequently suppress the synthesis of CRF and ACTH, thereby restoring homeostasis of the hypothalamic–pituitary–adrenal (HPA) axis.[3] Hypersecretion of CRF in the central nervous system may lead to a variety of psychiatric and stress-related illnesses, such as anxiety, depression, obsessive-compulsive and post-traumatic stress disorders.[4] Support for this hypothesis is given by the detection of marked elevations of CRF in the cerebrospinal fluid in a large portion of individuals diagnosed with major depression and anxiety disorders. Furthermore, the CRF levels were shown to correlate with severity of illness.[5] Following antidepressant treatment, the increased CRF levels observed in depressed patients were reduced.[6] Commensurate with its role as the principal regulator of mammalian physiological and behavioral responses to stress, CRF has also been shown to mediate several immune system functions through its effect on glucocorticoid plasma levels.[7] The neuropeptide carries out its diverse set of roles through binding to CRF_1 and CRF_2 receptors,[8] which belong to the family of transmembrane G-protein-coupled receptors.

Various animal studies have suggested that antagonism of the effects of CRF binding to CRF_1 receptors (CRF_1R) present in the CNS represents a novel target for the treatment of depression and anxiety. As a result, numerous classes of non-peptide small molecules have been reported as selective CRF_1R antagonists.[9,10] Extensive analysis of structure–activity relationships (SARs) has led to the identification of structural features common to most CRF_1R antagonists.[9] These are exemplified by **1**[11] and **2**:[12] a heteroaromatic core with an sp^2-hybridized nitrogen, a small alkyl group on the atom next to that nitrogen, and an aryl ring attached to

*Corresponding author. Fax: +1-203-677-7702; e-mail: gene.dubowchik@bms.com
†Present address: Palatin Technologies Inc., 175 May St., Edison, NJ 08837, USA.
‡Present address: Neurogen Corp., 35 N.E. Industrial Rd., Branford, CT 06405, USA.
§Present address: Abbott Laboratories, Dept. R4N5, Bldg. AP9A, 100 Abbott Park Rd., Abbott Park, IL 60064-6125, USA.
¶Present address: Aventis Pharmaceuticals, PO Box 6800, Bridgewater, NJ 08807-0800, USA.

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.08.055

it containing one or two *ortho*-substituents to enforce what has been proposed to be the active, mutually orthogonal conformation. In addition, most potent CRF_1R antagonists possess a *para*-substituent on the pendant aryl ring as well as a branched side chain attached to the core heterocycle.



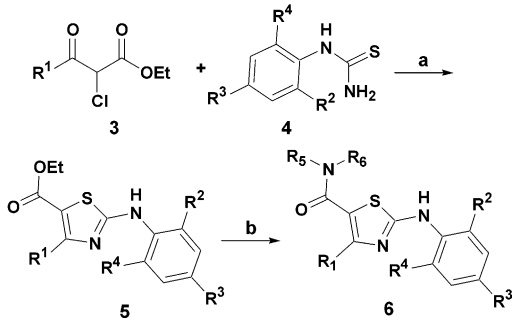
1 CP-154,526
K_i = 3.7 nM
(lit. K_i = 2.7 nM)[11]

2 NBI-27914
K_i = 20 nM
(lit. K_i = 1.7 nM)[12]

In search of novel antidepressive/anxiolytic agents, we have synthesized 2-arylamino-4-trifluoromethyl-5-aminomethylthiazoles that are high-affinity CRF_1R antagonists. These compounds were easily prepared in three steps from commercially available starting materials. As shown in Scheme 1, the aminothiazole was assembled by condensation of ethyl 2-chloro-3-oxobutyrates **3** with aryl thioureas **4**.[13] Hydrolysis of the esters **5** was attempted (LiOH, H_2O, THF, rt, 12 h), but the resulting carboxylates were easily decarboxylated. Instead, direct amidation under Weinreb conditions[14] provided amides **6** cleanly and in good yields.

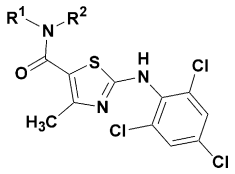
CRF_1R binding affinities were determined by displacement of [^{125}I]Tyr-*o*-CRF from $hCRF_1R$ endogenously expressed on IMR-32 human neuroblastoma cells.[15] Initial work (Table 1) showed that the (*N*-cyclopropylmethyl-*N*-*n*-propyl)amide **6a** was superior to some other small branched structural motifs commonly used by others.[9] In addition, the very low affinity demonstrated by MeO-containing compounds **6e** and **6f** suggested that polar atoms in this region might not be well tolerated by the receptor.

We decided to explore structural changes in other parts of the chemotype while retaining the *N*-cyclopropylmethyl-*N*-*n*-propyl side chain (Table 2). The moderate activity of **6a** was reduced by replacement of all the chlorines with CH_3 (**6g**). The Dupont group had reported that an ethyl group often confers best affinity for a



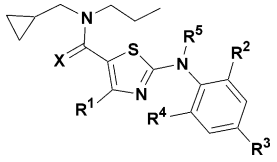
Scheme 1. Reagents and conditions: (a) EtOH, reflux, 16 h, 85–97%; (b) Me_3Al, R^5-NH-R^6, $PhCH_3$, reflux, 14 h, 68–92%.

Table 1. $hCRF_1R$ binding affinities of thiazole amides **6**



Compd	R^1	R^2	K_i (nM)
6a	*c*PrCH$_2$	*n*Pr	360
6b	*n*Pr	*n*Pr	820
6c	*n*Bu	Et	2800
6d	Et	Et	5000
6e	MeOCH$_2$CH$_2$	Et	16,000
6f	MeOCH$_2$CH$_2$	MeOCH$_2$CH$_2$	51,000

Table 2. $hCRF_1R$ binding affinities of thiazoles **6–8**

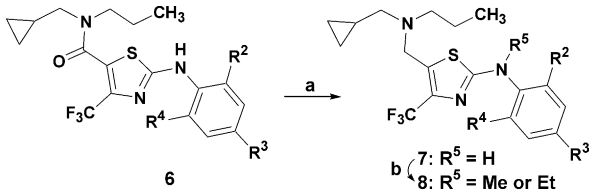


Compd	X	R^1	R^2	R^3	R^4	R^5	K_i (nM)
6g	O	Me	Me	Me	Me	H	2600
6h	O	Et	Cl	Cl	Cl	H	11,000
6i	O	CF_3	Cl	Cl	Cl	H	31,000
7a	H_2	CF_3	Cl	Cl	Cl	H	8.6
7b	H_2	Me	Cl	Cl	Cl	H	—[a]
7c	H_2	CF_3	Cl	Cl	Me	H	16
7d	H_2	CF_3	Br	Me	Me	H	21
7e	H_2	CF_3	Cl	Me	Me	H	23
8a	H_2	CF_3	Cl	Me	Me	Me	100
8b	H_2	CF_3	Cl	Me	Me	Et	67
7f	H_2	CF_3	Cl	CF_3	Cl	H	28
7g	H_2	CF_3	Br	Me	Br	H	28
7h	H_2	CF_3	Me	Me	Me	H	60
7i	H_2	CF_3	Me	Cl	Me	H	73
7j	H_2	CF_3	Br	Br	Br	H	7.8
7k	H_2	CF_3	Br	iPr	H	H	2900
8c	H_2	CF_3	Br	iPr	H	Et	56
7l	H_2	CF_3	Cl	Cl	H	H	4600
7m	H_2	CF_3	MeO	MeO	H	H	5100
7n	H_2	CF_3	Et	H	Et	H	>40,000

[a]Compound was unstable to purification.

CRF_1R antagonist containing a five-membered A-ring, while methyl substitution is optimal when the A-ring is a six-membered aromatic heterocycle.[18] Surprisingly, the 4-ethylthiazole **6h** showed a 30-fold loss of activity in comparison with **6a**. Replacement with CF_3 (**6i**) further reduced affinity.

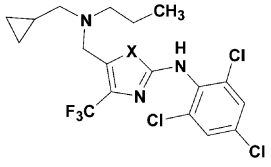
Concern that the amide group in **6** might be imposing unfavorable conformational rigidity, or that the polarity of the carbonyl oxygen might be disfavored in the binding pocket, led us to reduce it to give the corresponding 5-aminomethylthiazoles **7**. For the 4-trifluoromethylthiazoles, 1 M BH_3–THF followed by methanolysis (Scheme 2) gave high yields of stable products (**7a** and **7c**–**p**).[19] However, in the case of **6a**, these



Scheme 2. Reagents and conditions: (a) (i) BH_3–THF (3 equiv), reflux, 16 h; (ii) MeOH, reflux, 1 h (56–88%); (b) NaH, MeI or EtI, THF, rt, 14 h, 95%-quant.

conditions led to decomposition, presumably because the resulting 4-methyl-2-aminothiazole **7b** is so electron-rich that loss of the side-chain amine is facile. Using a non-Lewis acidic reducing agent (Red-Al, PhMe, rt, 8 h), **7b** could be generated and even purified by preparative LC. However, decomposition occurred upon concentration of the product-containing fractions. Clearly, the 5-aminomethyl-2-aminothiazole core requires at least one electron-withdrawing group to be viable under ambient conditions.

As shown in Table 2, amide reduction of **6i** resulted in a 3500-fold gain in CRF_1R affinity for the product, **7a**. An X-ray crystal structure of the HCl salt of **7a** (Fig. 1) shows an orthogonal relationship between the two rings in the crystal form. The oxazole analogue (**9**) of **7a** was prepared using a different synthetic sequence.[20] An almost 400-fold loss of binding affinity for **9** ($K_i = 3.5$ μM) suggests that the large sulfur atom is lipophilic enough to be tolerated by the CRF_1R and may serve to maintain mutual orthogonality between the rings.



7a: X = S, K_i = 8.6 nM
9: X = O, K_i = 3500 nM

Sequential replacement of aryl chlorines with CH_3 led to a systematic diminution of activity for **7c**, **7e**, **7i**, and **7h**. No advantage was seen with the larger *o*-Br substituents in **7d** or **7j**. Removal of one *o*-Cl from **7a** resulted in a 500-fold reduction in activity for **7l**, while two other 2,4-disubstitution patterns (**7k** and **7m**) gave similarly low activity. A single example of a 2,6-disubstitution pattern (**7n**) was inactive.

Alkylation of the linking nitrogen atom between the core heterocycle and aryl ring with methyl or ethyl groups has been found to be advantageous for some CRF_1R antagonist chemotypes. This was easily effected by treatment of **7** with excess NaH in THF followed by methyl- or ethyl iodide. The remarkably low nucleophilicity of the aminomethyl nitrogen was attested to by the fact that 6 equiv of alkyl iodide could be used without formation of detectable quaternized product. Using **7f** as a test case for trisubstituted phenyl compounds, we found that *N*-alkylation with methyl (**8a**) or ethyl (**8b**) had a somewhat deleterious effect on CRF_1R binding. In contrast, the affinity of the 2-bromo-4-isopropylphenyl compound **7k** was increased 50-fold by *N*-ethyl substitution (**8c**).

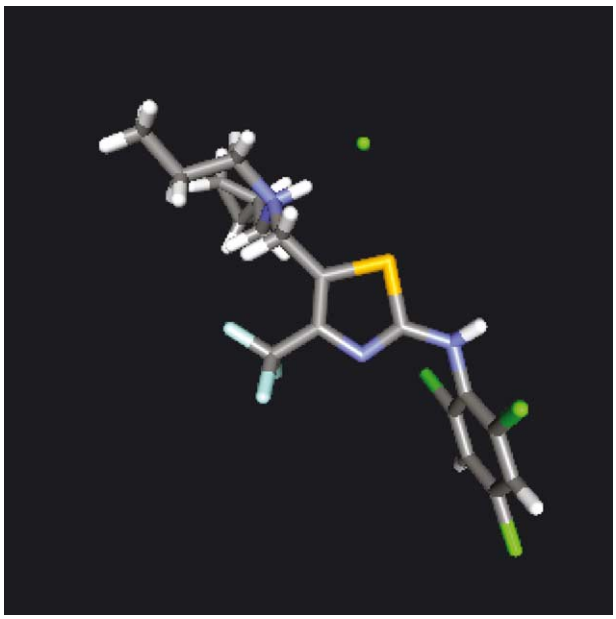


Figure 1. X-ray structure of **7a** (HCl salt).

Table 3. Rat PK Parameters for **7a** (10 mg/kg, po; 5 mg/kg, iv)

$T_{1/2}$	7.6 h
Cl	16 mL/min/kg
F_{po}	9% (±3%)
V_d	1.1 L/kg
B/P (2 h)	2.0
AUC (plasma), 0-4 h	1730 ng/mL*h

Compound **7a** was chosen for further study. Table 3 shows the results of a pharmacokinetic study in rats. The HCl salt of **7a** showed moderate clearance and volume of distribution with good brain uptake and an acceptable plasma half-life. However, oral bioavailability was low.

In order to determine its potential as an anxiolytic agent, **7a** (HCl salt) was tested in a mouse canopy stretched attend posture (SAP) model.[21] In this paradigm, mice are placed on a well-lit black platform, a portion of which is covered by a clear red canopy attached by a central pillar. SAPs are characterized by forward elongations of the body, exhibited when the mouse is standing still or moving slowly forward. This behavior is investigative in nature, and is considered an important behavioral indicator of anxiety in mice. Active compounds reduce the number of SAPs, indicating anxiolytic potential. When given ip at 32 and 64 mg/kg, **7a** significantly reduced SAPs in a dose-dependent manner in comparison with vehicle-treated mice (Fig. 2). CP-154,526 **1** showed comparable activity at 32 mg/kg, as did buspirone at 2 mg/kg.

In summary, we have discovered a new class of high-affinity CRF_1R antagonists, 2-arylamino-4-trifluoromethyl-5-aminomethylthiazoles, that can be prepared in three

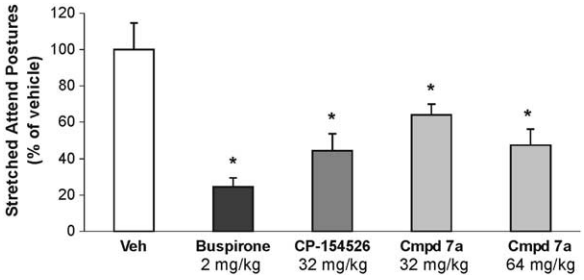
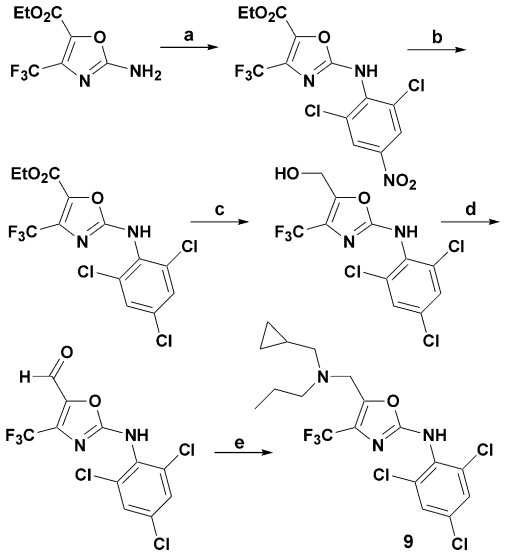


Figure 2. Canopy test results in which a reduction in stretched attend postures corresponds to putative anxiolytic activity.[20] Data represents the mean ± SEM of 10 mice (BALBc) per group. Asterices indicate significant difference from vehicle, $p < 0.05$ (Dunnett's test).

steps in good overall yields. An exemplary compound, **7a**, demonstrated anxiolytic activity in a mouse behavioral model, suggesting potential use of these compounds as anxiolytic agents.

References and Notes

1. Owens, M. J.; Nemeroff, C. B. *Pharmacol. Rev.* **1991**, *43*, 425.
2. Grigoriadis, D. E.; Haddach, M.; Ling, N.; Saunders, J. *Curr. Med. Chem. CNS Agents* **2001**, *1*, 63.
3. Plotsky, P. M. *J. Neuroendocrinol.* **1991**, *3*, 1.
4. Kasckow, J. W.; Baker, D.; Geracioti, T. D. *Peptides* **2001**, *22*, 845.
5. Holsboer, F. J. *Psychiatric Res.* **1999**, *33*, 181.
6. Banki, C. M.; Karmasci, L.; Bissette, G.; Nemeroff, C. B. *Eur. Neuropsychopharmacol.* **1992**, *2*, 107.
7. Webster, E. L.; Torpy, D. J.; Elenkov, I. J.; Chrousos, G. P. *Ann. N.Y. Acad. Sci.* **1998**, *840*, 21.
8. Takahashi, L. K. *Neurosci. Behav. Rev.* **2001**, *25*, 627.
9. Gilligan, P. J.; Robertson, D. W.; Zaczek, R. *J. Med. Chem.* **2000**, *43*, 1641.
10. McCarthy, J. R.; Heinrichs, C.; Grigoriadis, D. E. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic: San Diego, CA, 1999; Vol. 34, p 11.
11. Schulz, D. W.; Mansbach, R. S.; Sprouse, J.; Braselton, J. P.; Collins, J.; Corman, M.; Dunaiskis, A.; Faraci, S.; Schmidt, A. W.; Seeger, T.; Seymour, P.; Tingley, F. D., 3rd; Winston, E. N.; Chen, Y. L.; Heym, J. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10477.
12. Chen, C.; Dagnino, R.; De Souza, E. B.; Grigoriadis, D. E.; Huang, C. Q.; Kim, K. I.; Liu, Z.; Moran, T.; Webb, T. R.; Whitten, J. P.; Xie, Y. F.; McCarthy, J. R. *J. Med. Chem.* **1996**, *39*, 4358.
13. Dodson, R. M.; King, L. C. *J. Am. Chem. Soc.* **1945**, *67*, 2242.
14. Levin, J. I.; Turos, E.; Weinreb, S. M. *Synth. Commun.* **1982**, *12*, 989.
15. Membranes were prepared from IMR-32 cells as previously described[16] and incubated with [^{125}I]Tyr-*o*-CRF (100 pM) and increasing concentrations of test compound for 100 min at 25 °C (assay buffer: 50 mM Tris (pH 7.2), 10 mM $MgCl_2$, 0.5% BSA, 0.005% Triton X-100, 10 μg/mL aprotinin and 10 μg/mL leupeptin). Assays were stopped by addition of ice-cold buffer. Non-specific binding was defined with 10 μM *o*-CRF. These compounds are full antagonists of the CRF_1R, as determined by their ability to inhibit CRF stimulated cAMP production in IMR-32 cells.[16] For **7a**, functional $EC_{50} = 42$ nM (90% inhibition). Compound **7a** was also tested against the CRF_2R and found to have $IC_{50} > 10$ μM.[17]
16. Dieterich, K. D.; DeSouza, E. B. *Brain Res.* **1996**, *733*, 113.
17. Suman-Chauhan, N.; Carnell, P.; Franks, R.; Webdale, L.; Gee, N. S.; McNulty, S.; Rossant, C. J.; Van Leeuwen, D.; MacKenzie, R.; Hall, M. D. *Eur. J. Pharmacol.* **1999**, *379*, 219.
18. Wilde, R. G.; Klaczkiewicz, J. D.; Carter, K. L.; Gilligan, P. J.; Olson, R. E.; Frietze, W. E.; Buckner, W. H.; Curry, M. A.; Robertson, D. W.; Sun, J. H.; Arneric, S. P.; Hartig, P.; Fitzgerald, L. W.; Marshal, W. J. *219th ACS National Meeting*, San Francisco, CA, 2000; MEDI 317.
19. After overnight reflux in 1:1 concd HCl/MeOH, **7a** was found to be unchanged. All new compounds gave satisfactory analytical data. For **7a**: ^{1}H NMR ($CDCl_3$) δ 0.05 (2H, q), 0.45 (2H, m), 0.84 (4H, t and m), 1.42 (2H, m), 2.34 (2H, d), 2.48 (2H, ABq), 3.75 (2H, d), 7.45 (2H, s), 8.42 (1H, brs). Mass spec.: 474.12 $(MH)^+$. Anal. calcd for $C_{18}H_{19}N_3SF_3Cl_3$: C 42.45, H 3.96, N 8.25. Found: C 42.56, H 3.94, N 8.07.
20. Synthesis of **9** (yields are unoptimized):



Reagents and conditions: (a) 3,4,5-trichloronitrobenzene, K_2CO_3, DMF, 80 °C, 48 h (80%); (b) (i) $SnCl_2$, EtOH, reflux, 1 h (79–96%); (ii) $CuCl_2$, *t*BuONO, MeCN, 65 °C, 1 h (35%); (c) $LiAlH_4$, THF, 0 °C to rt (18%); (d) Dess–Martin, CH_2Cl_2, rt (59%); (e) (i) *c*-$PrCH_2NHnPr$, $(MeO)_3CH$, DMF, AcOH; (ii) $NaBH(OAc)_3$, (10%).

21. Grewal, S. S.; Shepherd, J. K.; Bill, D. J.; Fletcher, A.; Dourish, C. T. *Psychopharmacology* **1997**, *133*, 29.