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Bioorganic & Medicinal Chemistry Letters 15 (2005) 3870–3873
Bioorganic & Medicinal Chemistry Letters

Synthesis, structure–activity relationships, and anxiolytic activity of 7-aryl-6,7-dihydroimidazoimidazole corticotropin-releasing factor 1 receptor antagonists

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Received 31 March 2005; revised 24 May 2005; accepted 26 May 2005
Available online 28 June 2005

Abstract—7-Aryl-6,7-dihydroimidazoimidazoles represent a novel series of high-affinity corticotropin-releasing factor 1 receptor antagonists. Here, we report their synthesis and SAR as well as behavioral activity of two exemplary compounds, **7b** and **7k**, in a mouse canopy model of anxiety.

Corticotropin-releasing factor (CRF), a 41-residue neuropeptide, secreted in the hypothalamus, mediates stress responses by stimulating the release of adrenocorticotropic hormone (ACTH) from the pituitary. The resulting secretion of ACTH initiates the release of adrenal glucocorticoids, which impose their pathophysiological effects through the hypothalamic–pituitary–adrenal axis (HPA).[1–3] The clinical relevance of the HPA/stress/depression hypothesis has been supported by the fact that high CRF levels have been detected in the cerebrospinal fluid in more than half of depressed patients, and that treatment with antidepressants normalize these levels.[4] These findings, along with the desire to target a new antidepressant mechanism that might avoid problems associated with current therapies, have inspired a number of groups to develop highly selective, small molecule CRF1 receptor (CRF1R) antagonists. Initial clinical work has shown promise for this target,[5] but an optimized compound, free of liabilities, has yet to appear. The CRF receptor, a G-protein-coupled receptor (class B), has two well-characterized subtypes, CRF1R and CRF2R. The receptor is mainly expressed in the central nervous system, primarily in the cortex, cerebellum, hippocampus, amygdala, olefactory bulb, and pituitary.[6]

Nearly all known nonpeptidic CRF1R antagonists (e.g., **1** and **2**) share the following structural features: a heteroaromatic core with an sp^2-hybridized nitrogen, a small alkyl group on the atom next to that nitrogen, a branched tertiary amine side-chain attached to this core, and an aryl (or heteroaryl) ring also attached to it containing at least one ortho substitution to reinforce the active, mutually orthogonal conformation (Fig. 1).[7] In this letter, we report our efforts in the design, synthesis, binding studies and behavioral efficacy of a novel series of 7-aryl-6,7-dihydroimidazoimidazole CRF1R antagonists (**7** and **8**).

The synthesis of imidazoles **7** and **8** is outlined in Schemes 1 and 2. *N*-arylethylenediamines **3** were treat-

Keywords: Corticotropin-releasing factor 1 receptor antagonists; SAR; Anxiolytic activity; Mouse canopy model.
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doi:10.1016/j.bmcl.2005.05.117

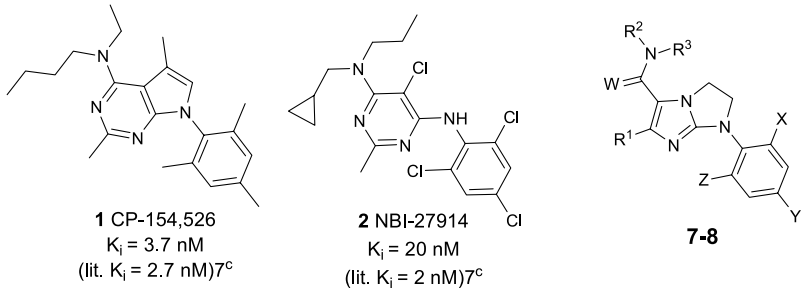
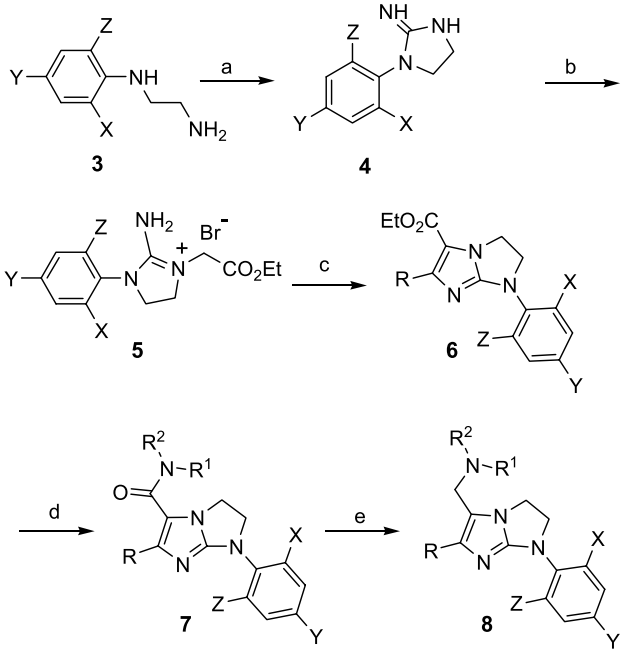


Figure 1. Small-molecule CRF1R antagonists.

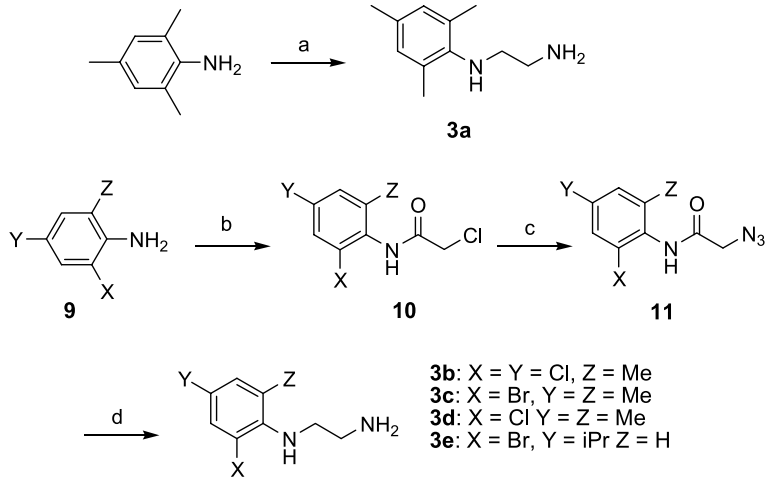
ed with cyanogen bromide in ethanol at 150 °C with the condenser open to the air, to give cyclic guanidines **4**.[8] These were alkylated with ethyl bromoacetate in acetone at reflux. After acetone was removed, the residues were refluxed in either acetic (for R = Me) or propionic (for R = Et) anhydride, along with their respective sodium salts to afford esters **6**.[9] Amidation under Weinreb conditions[10] with secondary amines afforded amides **7**.[11] Finally, employment of the non-Lewis acidic-reducing agent, Red-Al, to reduce amides **7** produced amines **8**.[11]



Scheme 1. Reagents and conditions: (a) BrCN, EtOH, 150 °C, 40 min. (b) Bromoethyl acetate, acetone, reflux, 12 h. (c) $(RCO)_2O$, RCO_2Na, 170 °C, 8 h 15–30% for three steps. (d) R^1NHR^2, $AlMe_3$, PhMe, 80 °C, 14 h, 70–90%. (e) Red-Al, PhMe, rt, 24 h, 40–70%.

N-arylethylenediamines **3** were prepared as shown in Scheme 2. Reaction of 2,4,6-trimethylaniline with 2-bromoethylamine-HBr in toluene at reflux afforded diamine **3a**.[12] This methodology did not extend to anilines containing electron-withdrawing groups. For a wider study of aryl substitution, a new synthesis of *N*-arylethylenediamines **3b–e** was developed. Di- or tri-substituted anilines **9** were acylated with chloroacetic anhydride to afford chlorides **10**. Treatment of **10** with NaN_3 gave azides **11** in high yields. Both the amide and azide groups were smoothly reduced by BH_3-THF to afford diamines **3b–e** following methanolysis.

CRF1R-binding affinities were determined by displacement of [^{125}I]Tyr-*o*-CRF from *h*CRF1R endogenously expressed on IMR-32 human neuroblastoma cells.[13] We first looked at the requirements for alkyl substitution at the 2-position of the imidazole as well as the tolerance for amide or amine substitution at position 3. The results for a series of *N*-cyclopropylmethyl-*N*-*n*-pro-



Scheme 2. Reagents and conditions: (a) 2-bromoethylamine-HBr salt, PhMe, reflux, 14 h, 45%. (b) $(ClCH_2CO)_2O$, $(ClCH_2)_2$, rt, 1 h, 80–90%. (c) NaN_3, KI, DMF, 50 °C, 4 h, 60–90%. (d) BH_3-THF, THF, reflux, 14 h, 70–88%.

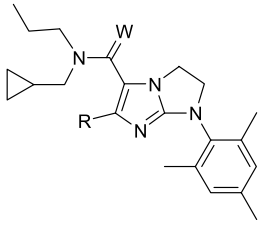
pyl amides and amines are summarized in Table 1. In contrast to the related series of aminothiazoles,[15] tertiary amides in this series were significantly more potent than amines (**7a**, **b**, vs **8a**, **b**). As reported for other series of 5-membered bicyclic CRF antagonists,[16] the ethyl group in **7b** conferred best activity.

Next, a series of 2-ethylimidazole-3-amides containing a pendent 2,4,6-trimethylphenyl ring were prepared to explore SAR preferences for the amide side-chain. The best activity was seen with compounds containing the cyclopropylmethyl side-chain (**7b** and **7j–n**) along with a small alkyl (**7b**, **7j**, and **7n**) or fluorinated alkyl (**7k–m**) chain. When cyclopropylmethyl was replaced by *n*-propyl or *n*-butyl (**7c**, **d**), there was a modest loss of affinity, while extension by one methylene group to give cyclopropylethyl (**7o–q**) resulted in a >10-fold reduction in potency. Benzyl, or substituted benzyl, derivatives (**7r–w**) showed poor activity, but a single phenylethyl example (**7x** vs **7r**) was ca. 20-fold more potent, perhaps because of greater conformational mobility. An attempt to introduce polarity into the side-chain (**7e**) was unsuccessful as were efforts to tie up the side-chains into 6-membered rings (**7z–cc**). These results argue for a relatively small, hydrophobic-binding pocket in which a 'pseudo-aromatic' group such as cyclopropylmethyl binds well, either because of size or the restricted conformation of the side-chain amide linkage, larger groups such as benzyl do not (Table 2).

Finally, a small selection of 2-ethylimidazole-3-cyclopropylamides containing four different pendent halo-aromatic rings were prepared with either *n*-propyl or trifluoroethyl side-chain (Table 3). While mono-bromination (**7hh–ii**) and mono- and di-chlorination (**7dd–gg**) gave little-or-no potency advantage over corresponding 2,4,6-trimethyl analogues (**7b** and **7k**), the loss of activity seen with the 2-bromo-4-isopropylphenyl compounds (**7jj–kk**) suggests the need for two ortho substituents in this chemotype.

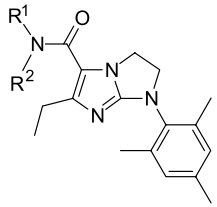
Compounds **7b** and **7k** were chosen for further in vivo study. Table 4 shows the results of a pharmacokinetic study of **7b** in rats. The compound showed a high-to-moderate clearance with good oral bioavailability, but a fairly low brain-to-plasma ratio.

Table 1. *h*CRF_1R-binding affinities of amides **7a**, **b** and amines **8a**, **b**



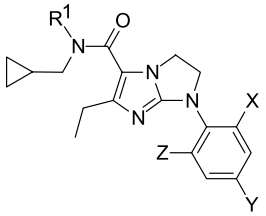
Compound	R	W	K_i (nM)
7a	Me	O	220
7b	Et	O	42
8a	Me	H_2	6600
8b	Et	H_2	19,000

Table 2. *h*CRF_1R-binding affinities of amides **7b–cc**



Compound	R^1	R^2	K_i (nM)
7c	nPr	nPr	100
7d	nBu	Et	290
7e	$Me_2NCH_2CH_2$	Et	>10,000
7f	Allyl	nPr	740
7g	Allyl	Allyl	3000
7h	CF_3CH_2	nPr	61
7i	$CF_3CH_2CH_2$	nPr	220
7b	$cPrCH_2$	nPr	42
7j	$cPrCH_2$	Et	94
7k	$cPrCH_2$	CF_3CH_2	41
7l	$cPrCH_2$	$CF_3CF_2CH_2$	63
7m	$cPrCH_2$	$CF_3CH_2CH_2$	73
7n	$cPrCH_2$	$cPrCH_2$	68
7o	$cPrCH_2CH_2$	nPr	770
7p	$cPrCH_2CH_2$	Et	610
7q	$cPrCH_2CH_2$	$CF_3CH_2CH_2$	650
7r	$PhCH_2$	nPr	4100
7s	*m*-F-$PhCH_2$	nPr	4200
7t	*p*-Cl-$PhCH_2$	nPr	3000
7u	$PhCH_2$	$CF_3CH_2CH_2$	660
7v	*p*-Cl-$PhCH_2$	$CF_3CH_2CH_2$	270
7w	$PhCH_2$	$PhCH_2$	>10,000
7x	$PhCH_2CH_2$	nPr	220
7y	Ph	H	>10,000
7z	Morpholine		>10,000
7aa	Piperazine		>10,000
7bb	4-Acetylpiperazine		>10,000
7cc	4-(2-F-phenyl)piperazine		>10,000

Table 3. *h*CRF_1R-binding affinities of amides **7dd–kk**



Compound	X	Y	Z	R^1	K_i (nM)
7dd	Cl	Me	Me	CF_3CH_2	41
7ee	Cl	Me	Me	nPr	94
7ff	Cl	Cl	Me	CF_3CH_2	24
7gg	Cl	Cl	Me	nPr	75
7hh	Br	Me	Me	CF_3CH_2	26
7ii	Br	Me	Me	nPr	126
7jj	Br	iPr	H	CF_3CH_2	270
7kk	Br	iPr	H	nPr	1559

We used the mouse canopy stretched attend posture (SAP) model to determine the anxiolytic potential of compounds **7b** and **7k** (Fig. 2).[17] Behavioral efficacy is

Table 4. Rat PK parameters for **7b** (10 mg/kg, po; 2 mg/kg, iv)[a]

Cl	35 mL/min/kg
V_d	5.7 L/kg
$AUC_{0-24\ h}$ (plasma, po)	1524 ng h/mL
B/P (2 h)	0.21
F_{po}	32%
C_{max} (po)	250 ng/mL
T_{max} (po)	4 h

[a] Dosing vehicle was 10/10/80 Cremphor/DMSO/water. Dosing volumes were 1 and 3 mL/kg for iv and po, respectively. Brain to plasma concentration ratio (B/P) was determined after IV administration.

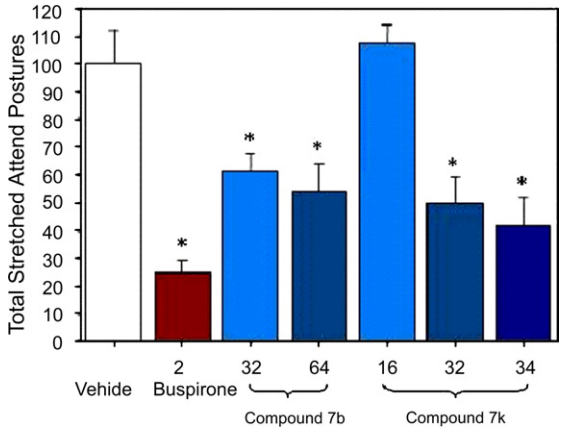


Figure 2. Canopy test results in which a reduction in stretched attend posture corresponds to putative anxiolytic activity. Data represent means ± SEM of 10 mice (BALBc) per group. Asterisk indicate significant difference from vehicle, $p < 0.05$ (Dunnett's test).

indicated by a reduction in SAPs. Both compounds, given ip, significantly reduced SAPs in a dose-dependent manner at 32 and 64 mg/kg, while compound **7k** was inactive at 16 mg/kg. Buspirone (2 mg/kg) was included in the study as a positive control.

In summary, 7-aryl-6,7-dihydroimidazoimidazoles represent a novel series of high-affinity CRF1R antagonists. Representative compounds show anxiolytic activity in a mouse canopy model.

Acknowledgments

We thank Dr. John Macor and Dr. Joanne Bronson for critical reading of the manuscript.

References and notes

1. Vale, W.; Spiess, J.; Rivier, C.; Rivier, J. *Science* **1981**, *213*, 1394.
2. Owens, M. J.; Nemeroff, C. B. *Pharmacol. Rev.* **1991**, *43*, 425.
3. Chrousos, G. P. *New Engl. J. Med.* **1995**, *332*, 1351.
4. Holsboer, F. *J. Psychiat. Res.* **1999**, *33*, 181.
5. Zobel, A.; Nickel, T.; Kunzel, H. F.; Ackl, N.; Sonntag, A.; Ising, M.; Holsboer, F. *J. Psychiat. Res.* **2000**, *34*, 171.
6. Hauger, R. L.; Grigoriadis, D. E.; Dallman, M. F.; Plotsky, P. M.; Vale, W. W.; Dautzenberg, F. M. *Pharmacol. Rev.* **2003**, *55*, 21.
7. (a) Kehne, J. H.; Maynard, G. D.; De Lombaert, S.; Krause, J. E. *Ann. Rep. Med. Chem.* **2003**, *38*, 11; (b) Grigoriadis, D. E.; Haddach, M.; Ling, N.; Saunders, J. *Curr. Med. Chem.—Central Nervous System Agents* **2001**, *1*, 63; (c) Gilligan, P. J.; Robertson, D. W.; Zaczek, R. *J. Med. Chem.* **2000**, *43*, 1641; (d) Keller, P. A.; Elfick, L.; Garner, J.; Morgan, J.; McCluskey, A. *Bioorg. Med. Chem.* **2000**, *8*, 1213.
8. Scherz, M. W.; Fialeix, M.; Fischer, J. B.; Reddy, N. L.; Server, A. C.; Sonders, M. S.; Tester, B. C.; Webber, E.; Wong, S. T.; Keana, J. F. W. *J. Med. Chem.* **1990**, *33*, 2421.
9. (a) Spasov, A. A.; Larionov, N. P.; Sibiryakova, T. B.; Verovskii, V. E.; Anisimova, V. A.; Dudchenko, G. P.; Baldenkov, G. N.; Men'shikov, M. Y. *Khim.-Farm. Zh.* **1998**, *32*, 17; (b) Anisimova, V. A.; Kuz'menko, T. A.; Spasov, A. A.; Bocharova, I. A.; Orobinskaya, T. A. *Pharm. J. Chem.* **1999**, *33*, 361.
10. Levin, J. I.; Turos, E.; Weinreb, S. M. *Synth. Commun.* **1982**, *12*, 989.
11. All new compounds gave satisfactory analytical data. For **7b**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.83 (s, 2H), 4.12–4.01 (m, 4H), 3.56 (t, J = 7.1 Hz, 2H), 3.35 (d, J = 6.8 Hz, 2H), 2.42 (q, J = 7.5 Hz, 2H), 2.21 (s, 3H), 2.17 (s, 6H), 1.59 (quint, J = 7.2 Hz, 2H), 1.11 (t, J = 7.5 Hz, 3H), 0.95–0.90 (m, 1H), 0.86 (t, J = 7.3 Hz, 3H), 0.51–0.49 (m, 2H), 0.17–0.14 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.1, 156.9, 147.6, 137.3, 136.9, 133.9, 129.6, 115.6, 53.6, 51.2, 48.1, 42.9, 22.6, 21.0, 20.9, 18.2, 13.7, 11.2, 10.0, 3.8; MS: 395.30 $(MH)^+$. For **8b**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.86 (s, 2H), 4.13–3.97 (m, 4H), 3.47 (s, 2H), 2.45 (t, J = 7.2 Hz, 2H), 2.39 (q, J = 7.6 Hz, 2H), 2.31 (d, J = 6.5 Hz, 2H), 2.24 (s, 3H), 2.19 (s, 6H), 1.48–1.42 (m 2H), 1.32–26 (m, 1H), 1.06 (t, J = 7.4 Hz, 3H), 0.87 (t, J = 7.2 Hz, 3H), 0.50–0.46 (m, 2H), 0.09–0.07 (m, 2H); MS: 381.27 $(MH)^+$.
12. Heidempergher, F.; Pillan, A.; Pinciroli, V.; Vaghi, F.; Arrigoni, C.; Bolis, G.; Caccia, C.; Dho, L.; McArthur, R.; Varasi, M. *J. Med. Chem.* **1997**, *40*, 3369.
13. Membranes were prepared from IMR-32 cells as previously described[14] 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, 0.01 mg/mL aprotinin, and 0.01 mg/mL leupeptin]. Assays were stopped by addition of ice-cold buffer. Nonspecific binding was defined with 0.01 mM *o*-CRF. These compounds are full antagonists of the CRF1R, as determined by their ability to inhibit CRF-stimulated *c*AMP production in IMR-32 cells.[14] Compounds **7b** and **7k** were also tested against the CRF2R and found to have IC_{50}'s > 10 μM.[18]
14. Dieterich, K. D.; DeSouza, E. B. *Brain Res.* **1996**, *733*, 113.
15. Dubowchik, G. M.; Michne, J. A.; Zuev, D.; Schwartz, W.; Scola, P. M.; James, C. A.; Ruediger, E. H.; Pin, S. S.; Burris, K.; Balanda, L. A.; Gao, Q.; Wu, D.; Fung, L.; Fiedler, T.; Browman, K. E.; Taber, M. T.; Zhang, J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3997.
16. Gilligan, P. J.; Olson, R. E.; Frietze, W. E.; Buckner, W. H.; Currry, M. A.; Robertson, D. W.; Sun, J. H.; Arneric, S. P.; Hartig, P.; Fitzgerald, L. W.; Marchal, W. J. 219th ACS National Meeting: San Francisco, CA, **2000**; Medi 317.
17. Grewal, S. S.; Shepherd, J. K.; Bill, D. J.; Fletcher, A.; Dourish, C. T. *Psychopharmacology* **1997**, *133*, 29.
18. 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.