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# An orally active corticotropin releasing factor 1 receptor antagonist from 8-aryl-1,3a,7,8-tetraaza-cyclopenta[*a*]indenes

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**Abstract**—8-Aryl-1,3a,7,8-tetraaza-cyclopenta[*a*]indenes represent a novel series of high-affinity corticotropin-releasing factor-1 receptor (CRF1R) antagonists. Herein we report the synthesis and SAR around the tricyclic core and the anxiolytic activity of an orally dosed exemplary compound **9d** ($K_i$ = 8.0 nM) in a mouse canopy model.


Depression is a common and serious illness. In any given 1-year period, 9.5% of the population, or about 18.8 million American adults, suffer from a depressive illness.[1] The treatment of depression has evolved from tricyclic antidepressants (TCA) in the 1970s to Selective Serotonin Reuptake Inhibitors (SSRI) in the 1990s propelled by the deeper mechanistic understanding of the disease.[2] Although the SSRIs are better tolerated than their predecessors, agents with increased efficacy, better side-effect profiles, and faster onset of action are still needed.

The CRF is a 41-amino acid neuropeptide, secreted in the hippocampus. It imposes its physiological effects on depression and other neuropsychiatric disorders via the hypothalamic–pituitary–adrenal (HPA) axis.[3] The CRF receptor, a G-protein coupled receptor, has two well-characterized subtypes (CRF1 and CRF2).[4] Compelling clinical evidence[3] supports the hypothesis that over-production of CRF may underlie the pathology of depression, anxiety, and stress-related disorders, and that antagonists of CRF1R could be useful for the treatment of depression.[5]

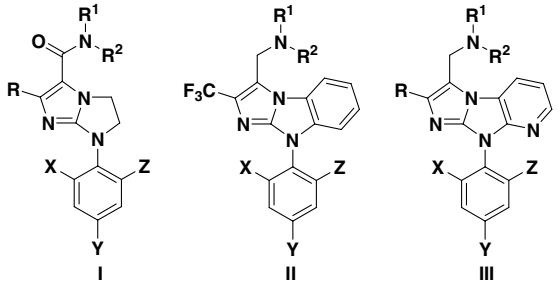


**Figure 1.** The rational progression of CRF1R antagonist chemotypes.

As a continuation of our work on 1-aryl-2,3-dihydroimidazoimidazoles (**I**),[6] we have reported the synthesis of 8-aryl-1,3a,8-triaza-cyclopenta[*a*]indenes (**II**).[7] An exemplified compound (X, Y, Z = Me, $R^1$ = *n*-Pr, $R^2$ = *c*-Pr$CH_2$) had good microsomal stability, binding affinity ($K_i$ = 24 nM), and reasonable PK properties. However, this compound is quite lipophilic (c log *P* = 8.2)[8] and poorly soluble in water (<0.01 μg/mL).[9] These unwanted properties were probably in part due to the presence of the highly lipophilic $CF_3$ group. We reasoned that if we could incorporate less hydrophobic electron withdrawing groups or elements into the core to replace the $CF_3$ group, we could lower c log *P* and increase aqueous solubility. Therefore, 8-aryl-1,3a,7,8-tetraaza-cyclopenta[*a*]indenes (**III**) (Fig. 1) were proposed. We report here the SAR of this chemotype along with detailed studies of compound **9d**.

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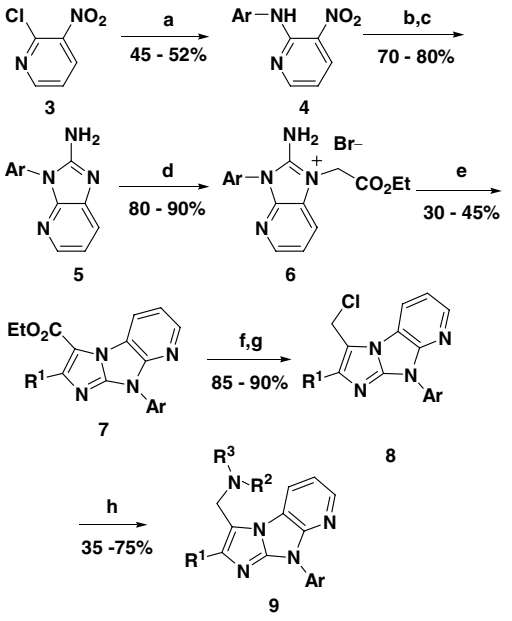


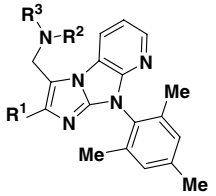

**Scheme 1.** Reagents and conditions: Ar = 2,4,6-trimethylphenyl or 2-chloro-4,6-dimethylphenyl (a) 4.0 equiv aniline, 1.5 equiv KF, 130 °C, 16 h; (b) 1 atm $H_2$, Pd/C, EtOAc, rt, 4 h, 80%; or $Na_2S_2O_4$, THF/$H_2O$/concd $NH_4OH$ (1:1:1), rt, 70%; (c) 1.8 equiv BrCN, 1.8 equiv $NaHCO_3$, EtOH, 0 °C, 8 h, then 80 °C, 8 h; (d) 1.2 equiv $BrCH_2CO_2Et$, acetone, 65 °C, 16 h; (e) 2.5 equiv $R^1CO_2Na$, $(R^1CO)_2O$, 160 °C, 4 h; (f) 5 equiv *i*-$Bu_2AlH$, PhMe, 0 °C, 1 h; (g) 2 equiv $SOCl_2$, $CH_2Cl_2$, 0 °C, 1 h; (h) 5 equiv $R^2NHR^3$–HCl, 7 equiv *i*-$Pr_2NEt$ (or 5 equiv $R^2NHR^3$, 2 equiv *i*-$Pr_2NEt$), MeCN, rt, 1 h, then **8**, rt, 24 h.

The synthesis of these compounds is outlined in Scheme 1. The reaction of 2-chloro-3-nitro-pyridine **3** with 2,4,6-trisubstituted anilines afforded compound **4**,[10] which was reduced to the corresponding diamines either by hydrogenation ($H_2$, Pd/C) or $Na_2S_2O_4$[11] reduction. After trying out various combinations of solvents, temperatures, and bases, it was found that these diamines could be reacted with BrCN in EtOH in the presence of $NaHCO_3$ at 80 °C to afford guanidines **5** in good yield. Treatment of guanidines **5** with $BrCH_2CO_2Et$ in refluxing acetone afforded bromides **6**, which were heated at reflux with acid salts $RCO_2Na$ in the corresponding anhydrides, $(RCO)_2O$, to produce esters **7**.[12] DIBAL-H reduction of esters **7** and treatment of the resulting alcohols with $SOCl_2$ yielded chlorides **8**. In a parallel fashion, chlorides **8** were reacted with a series of mainly secondary amines[13] to afford amines **9**.[14]

CRF1R binding affinities were determined as described previously.[15] We first studied the effect of different core alkyl groups ($R^1$) on binding affinity and aqueous solubility. Since solubility assessments were performed on amorphous TFA salts, the data are primarily useful in gauging the effect of structural changes within a chemotype. As shown in Table 1, representative compounds containing $CF_3$, Et, and Me (**9a**–**d**) were nearly equipotent. However, while $CF_3$ substitution led to extremely low aqueous solubility (<0.1 μg/mL), Me and Et substituted compounds showed excellent aqueous solubilities (>50 μg/mL).[9]

Next, a series of analogues where $R^1$ = Me were prepared to explore substitution of the amino side chains (Table 2). As in our previous studies,[6,7,17] 2,4,6-trisubstituted pendant aryl groups were required for effective binding to the CRF1R in our chemotypes. In order to minimize molecular weight, only 2,4,6-trimethyl phenyl and 2-chloro-4,6-dimethyl phenyl were chosen, and resulting differences in receptor binding affinity were small (<4-fold for all comparable examples). Tertiary amines are clearly much more favored by the receptor than secondary amines, such that both lipophilic (1-ethyl-propylamine, **9an**) and polar (2-methoxy-1-methoxymethyl-ethylamine, **9ao**) secondary amines were relatively inactive. Polar groups on the amino side chains are not tolerated in this chemotype; the tertiary amines derived from morpholine (**9af**) and di-methoxyethyl amine (**9am**) showed very low affinities. For pyridylmethyl and pyridylethyl containing compounds, the different pyridyl substitution pattern (2-, 3- or 4-position) caused several fold changes in potency (>8-fold for **9ai** vs **9ag**; >5-fold for **9al** vs **9aj**). In general, tertiary amines containing one *n*-Pr group provided the most potent examples (i.e., **9c**, **9g**, and **9r**), with a variety of hydrophobic (especially aromatic, vinylic or small cyclo-alkyl) groups favored on the opposing side. Exceptions were ethylamines (**9d** and **9w**) where the large phenethyl moiety presumably can compensate for the removal of one methylene group. Historic SAR[5] of a

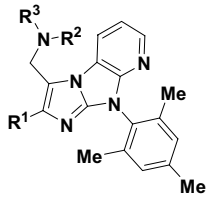
**Table 1.** The effect of $R^1$ substitution on aqueous solubility of CRF1R antagonists **9a**–**d**



| Compound | $R^1$ | $R^2$ | $R^3$ | Aqueous solubility[a] (μg/mL) | $K_i$ (nM) |
|---|---|---|---|---|---|
| **9a** | $CF_3$ | *c*-$PrCH_2$ | *n*-Pr | <0.1 | 16 |
| **9b** | Et | *c*-$BuCH_2$ | *n*-Pr | 52 | 17 |
| **9c** | Me | *c*-$BuCH_2$ | *n*-Pr | 61 | 8.2 |
| **9d** | Me | $PhCH_2CH_2$ | Et | 54 | 7.8 |

[a] Amorphous TFA salts. Solubilities were determined using a medium throughout assay at pH 6.5; see Ref. 10 for details. TFA, trifluoroacetic acid.

**Table 2.** *h*CRFR1 binding affinities of CRF1R antagonists **9e–ao**




| Compound | X | $R^2$ | $R^3$ | $K_i$ (nM) |
|---|---|---|---|---|
| **9e** | Cl | *c*-$PrCH_2$ | *n*-Pr | 11 |
| **9f** | Me | *c*-$PrCH_2$ | *n*-Pr | 15 |
| **9g** | Cl | *c*-$BuCH_2$ | *n*-Pr | 6.0 |
| **9h** | Cl | $PhCH_2$ | *n*-Pr | 28 |
| **9i** | Cl | Allyl | *n*-Pr | 11 |
| **9j** | Me | Allyl | *n*-Pr | 41 |
| **9k** | Cl | *n*-Pr | *n*-Pr | 15 |
| **9l** | Cl | *n*-Bu | *n*-Bu | 18 |
| **9m** | Cl | Allyl | Allyl | 28 |
| **9n** | Me | Allyl | Allyl | 26 |
| **9o** | Me | *c*-$PrCH_2$ | *c*-$PrCH_2$ | 23 |
| **9p** | Cl | *c*-$PrCH_2CH_2$ | *n*-Pr | 15 |
| **9q** | Me | *c*-$PrCH_2CH_2$ | *n*-Pr | 43 |
| **9r** | Cl | $PhCH_2CH_2$ | *n*-Pr | 5.9 |
| **9s** | Cl | *c*-$PrCH_2$ | Et | 36 |
| **9t** | Me | *c*-$PrCH_2$ | Et | 93 |
| **9u** | Cl | *c*-$BuCH_2$ | Et | 20 |
| **9v** | Cl | *c*-$PrCH_2CH_2$ | Et | 50 |
| **9w** | Cl | $PhCH_2CH_2$ | Et | 6.4 |
| **9x** | Cl | *n*-Bu | Et | 36 |
| **9y** | Cl | Et | Et | 360 |
| **9z** | Cl | 3-Phenyl-pyrrolidinyl | | 8.4 |
| **9aa** | Cl | 2-Benzyl-pyrrolidinyl | | 48 |
| **9ab** | Cl | 2-Phenyl-pyrrolidinyl | | 580 |
| **9ac** | Cl | 3-Benzyl-pyrrolidinyl | | 700 |
| **9ad** | Cl | 3-Phenethyl-pyrrolidinyl | | 700 |
| **9ae** | Cl | 2-Phenethyl-pyrrolidinyl | | 1300 |
| **9af** | Cl | Morpholinyl | | 1400 |
| **9ag** | Cl | 4-$PyCH_2$ | Me | 1200 |
| **9ah** | Cl | 3-$PyCH_2$ | Me | 2700 |
| **9ai** | Cl | 2-$PyCH_2$ | Me | >10,000 |
| **9aj** | Cl | 3-$PyCH_2CH_2$ | Me | 570 |
| **9ak** | Cl | 2-$PyCH_2CH_2$ | Me | 2800 |
| **9al** | Cl | 4-$PyCH_2CH_2$ | Me | 3200 |
| **9am** | Cl | $MeOCH_2CH_2$ | $MeOCH_2CH_2$ | 4100 |
| **9an** | Cl | 2-Pentyl | H | 2900 |
| **9ao** | Cl | $(MeOCH_2)CH$ | H | 5600 |

large variety of CRF1R antagonists shows that the area of the receptor in which these side chains bind is intolerant of more than one polar atom, usually an ether oxygen. It is tempting to see the need for highly lipophilic side chains in our aminomethylene-containing antagonists as a compensation for the presence of the more polar basic amino group. A number of racemic, phenyl-containing cyclic amines were prepared. It is interesting to note that the compound derived from 3-phenyl-pyrrolidine (**9z**) was much more potent than the isomeric 2-phenyl-pyrrolidine (**9ab**), mirroring, to some extent, the advantage of the acyclic phenethyl **9r** over the benzyl **9h**. Limited flexibility in these phenyl-substituted cyclic amines presumably makes substitution position even more critical for potency.

To characterize the pharmacokinetics of compound **9d**, it was administered iv (tail vein) and by oral gavage to groups of mice. The data are summarized in Table 3. After iv dosing, the compound showed low whole body clearance and a moderate elimination half-life. The low (4%) bioavailability after po dosing may be reflective of limited absorption and/or high first-pass metabolism by the liver. That first-pass metabolism may be more significant than poor absorption is suggested by the high rate of metabolism of the compound in mouse liver microsomes (0.30 nmol/min/kg). Despite the low bioavailability, the serum concentrations are sufficient for activity, as the dose used was in the efficacious range (Fig. 2).

Compound **9d** was assessed in a mouse canopy stretched attend posture (SAP) model of anxiety, as previously described.[18] When dosed po at 30 and 60 mg/kg, **9d** significantly reduced SAPs in a dose-dependent manner in comparison with vehicle-treated mice (Fig. 3).

**Table 3.** Pharmacokinetic parameters of compound **9d** in Balb/c mice

| Parameters[a] | iv[b] 5.8 mg/kg | po 23 mg/kg |
|---|---|---|
| CL (mL/min/kg) | 5.2 | — |
| $V_{ss}$ (L/kg) | 0.48 | — |
| $t_{1/2}$ (h) | 3.0 | — |
| AUC $_{0-t}$ (nM–h) | 17794 | 2738 |
| $C_{max}$ (nM) | — | 1189 |
| $T_{max}$ (h) | — | 0.5 |
| $F$ (%) | — | 4 |

[a] Values are the averages calculated from three animals at six time points through 8 h following dosing.
[b] Dosing vehicle: PEG400/ethanol (9:1, v/v).

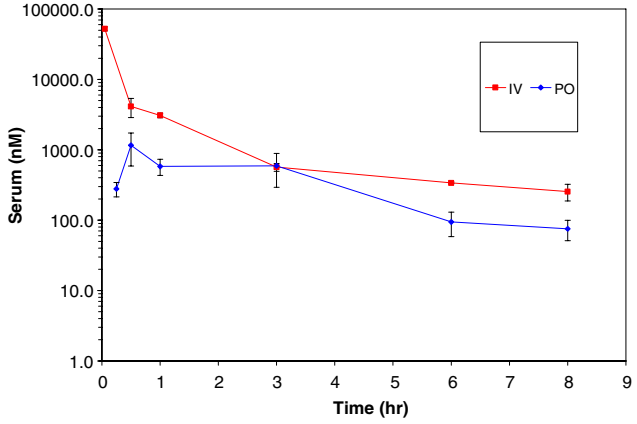



**Figure 2.** Serum levels after iv (5.8 mg/kg) and po (23 mg/kg) administration of compd **9d** to mice (Means ± SD of 3 mice/time point).

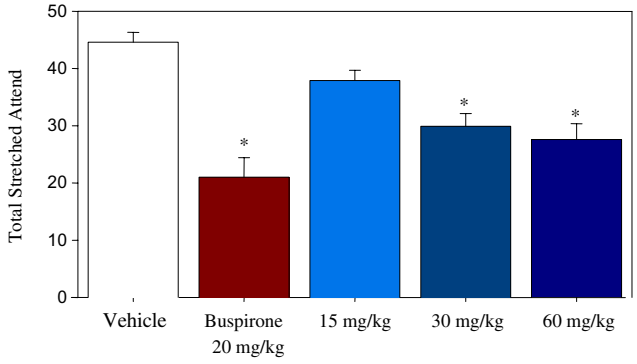



**Figure 3.** Canopy test results of compound **9d** (po, 30% PEG400/70% water, 15 min pretreatment). Data represent means ± SEM of 10 mice (Balb/c) per group. Asterisks indicate significant difference from vehicle-treated mice, $p < 0.05$ (Dunnett's test).

In conclusion, we have discovered a novel class of potent CRF1R antagonists, 8-aryl-1,3a,7,8-tetraaza-cyclopenta[*a*]indenes. A representative compound, **9d**, when dosed orally in mice, showed significant, dose-dependent activity in a mouse canopy stretched attend posture model, indicative of its potential use as an oral anxiolytic agent.

## Acknowledgment

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8. clog*P* was calculated using a licensed program from Tripos.
9. Aqueous solubility was determined as follows: 10 mg/mL stock solutions of each compound were prepared in neat DMSO. The stock solutions were diluted 100-fold into a 96-well plate with 25 mM phosphate buffer, pH 6.5. The plate was sealed and sonicated for 3 min. The plate was shaken for 4 h at room temperature to allow the compounds to equilibrate. Standards were prepared at 0.05 mg/mL from the DMSO stock by diluting 200-fold into ACN:$H_2O$ (1:1). The plate was centrifuged for 30 min to separate compound from solution. The supernatant and standards were analyzed by HPLC to determine the solubility.
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14. All new compounds gave satisfactory analytical data. See Scheme 1, in which Ar = 2,4,6-trimethylphenyl and R = Me. For **4a**: $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 9.38 (s, 1H), 8.51 (d, $J$ = 5.2 Hz, 1H), 8.38 (1H), 7.00 (s, 2H), 6.73 (s, 1H), 2.34 (s, 3H), 2.18 (s, 6H); $^{13}$C NMR ($CDCl_3$,125 MHz) $\delta$ 156.2, 152.1, 137.2, 135.7, 135.4, 132.3, 129.1 (vs), 128.3, 112.8, 21.1, 18.5. For **5a**: $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 7.95 (dd, $J$ = 5.0, 1.5 Hz, 1H), 7.53 (dd, $J$ = 8.0, 1.5 Hz, 1H), 7.03 (s, 2H), 7.00 (dd, $J$ = 8.0, 5.0 Hz, 1H), 5.82 (br s, 2H), 2.32 (s, 3H), 1.99 (s, 6H); $^{13}$C NMR ($CDCl_3$,125 MHz) $\delta$ 154.3, 147.7, 140.1, 140.0, 137.3, 135.2, 129.9 (vs), 129.4, 128.0,

122.0, 117.8, 21.2, 17.8; mass spec.: 253.18 $(MH)^+$. For **7a**: $^1H$ NMR ($CDCl_3$, 500 MHz) $\delta$ 8.77 (dd, $J = 8.1$, 1.2 Hz, 1H), 8.29 (dd, $J = 4.9$, 1.2 Hz, 1H), 7.23 (dd, $J = 8.1$, 5.0 Hz, 1H), 7.04 (s, 2H), 4.48 (q, $J = 7.1$ Hz, 2H), 2.65 (s, 3H), 2.34 (s, 3H), 1.98 (s, 6H), 1.49 (t, $J = 7.1$ Hz, 3H); $^{13}C$ NMR ($CDCl_3$,125 MHz) $\delta$ 161.1, 152.1, 147.4, 147.0, 144.0, 139.8, 136.7, 129.7, 127.9, 123.9, 122.4, 120.0, 117.0, 60.4, 21.1, 17.9, 16.4, 14.7; mass spec.: 363.30 $(MH)^+$. For **9d**: HCl:$^1H$ NMR (DMSO-$d_6$, 500 MHz) $\delta$ 8.81 (d, $J = 8.2$ Hz, 1H), 8.37 (d, $J = 4.9$ Hz, 1H), 7.50 (dd, $J = 8.2$, 4.9 Hz, 1H), 7.37–7.32 (m, 4H), 7.28–7.25 (m, 1H), 7.16 (s, 2H), 5.09 (dd, $J = 15.9$, 3.3 Hz, 1H), 5.01 (dd, $J = 15.9$, 6.0 Hz, 1H), 3.54–3.46 (m, 2H), 3.42–3.36 (m, 2H), 3.27–3.22 (m, 2H), 2.49 (s, 3H), 2.37 (s, 3H), 1.98 (s, 3H), 1.92 (s, 3H), 1.39 (t, $J = 7.3$ Hz, 3H); $^{13}C$ NMR (DMSO-$d_6$, 125 MHz) $\delta$ 145.5, 144.4, 142.8, 139.7, 137.1, 136.7, 136.5, 129.2, 128.61 (vs), 128.58 (vs), 127.0, 126.7, 122.1, 118.6, 117.9, 110.4, 66.3, 51.5, 46.2, 45.2, 28.7, 20.6, 17.4, 12.2, 8.6; Mass spec.: 452.32 $(MH)^+$.

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 the addition of ice-cold buffer. Non-specific binding was defined with 10 μM *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.
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