Supporting Information

Design and Synthesis of Tricyclic Imidazo[4,5-b]pyridin-2-ones as Corticotropin-Releasing Factor-1 (CRF_1) Antagonists

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Experimental Section

Chemistry

Elemental analysis results are indicated by atom symbols and are within 0.4% of theoretical values except where indicated. ^{1}H NMR spectra were recorded on a Varian Spectrometer (Mercury 300Hz) using TMS as the internal standard and $CDCl_3$ as solvent except where indicated. Final products were purified by Gilson preparatory HPLC system, which was connected to a mass spectrometer and fractional collector. The fractional collector was triggered by the desired mass. All final compounds after purification were re-analyzed by reverse phase HPLC-MS system (HP-4500 series with APCI mode for mass detection) and determined to be at least 98% pure based on two UV absorbance wavelengths (220 nM, 254 nM) and total ion current (TIC) monitoring from the mass spectrometer.

6-Methyl-3-nitro-4-(1-propyl-butylamino)-pyridin-2-ol (12). To a solution of 4-hydroxy-6-methyl-3-nitro-2-pyridone (**8**, 931 g, 5.47 mol) in anhydrous tetrahydrofuran (THF, 8.0 L) was added 4-dimethylaminopyridine (33 g, 0.27 mol). Triethylamine (725 mL, 5.20 mol) was added carefully while keeping the temperature below 35ºC. The resulting bright yellow slurry was refluxed for 2 hours, benzenesulfonyl chloride (700 mL, 5.47 mol) in anhydrous THF (500 mL) was added dropwise over 1 hour, and the mixture was refluxed for 1 hour. To the mixture was carefully charged 4-heptylamine (630 g, 5.47 mol) in THF (500 mL) over 30 minutes. Triethylamine (762 mL, 5.47 mol) was charged over 30 minutes, the mixture was heated to reflux over 3 hours and then held at 40 ºC for 12 hours. The mixture was cooled to ambient temperature and the slurry was filtered over celite. The celite was washed with THF (200 mL). The solvent was removed *in vacuo* and the crude product diluted with EtOAc (4 L). The resulting precipitate (organic salts) was filtered and the filter cake washed with 1 L of EtOAc. The filtrate was washed with 2 L of D.I. water, and twice with 2 L of brine. The solvent was removed *in vacuo* affording a precipitate. The precipitate was slurried with EtOAc (1 L), chilled, and the precipitate collected by filtration affording the desired product (**12,** 949 g). The mother liquor was filtered through a plug of silica gel, eluting with 1:1 EtOAc/hexanes until the eluant became clear. The silica gel was then rinsed with pure EtOAc, which when concentrated, afforded an additional 120 g of product. All product was combined affording **12** (1069 g, 73%). ^{1}H NMR ($CDCl_3$) δ 0.93 (t, J = 7.3 Hz, 6H), 1.32-1.43 (m, 4H), 1.53 -1.64 (m, 4H), 2.31 (s, 3H), 3.54-3.61 (m, 1H), 5.65 (s, 1H), 9.28 (d, J = 8.5 Hz, 1H), 11.52 (br s, 1H); MS (CI) *m/z* 268.1 (MH^+).

4-(4-Heptyl-amino)-2-amino-(4-methoxyphenyl)-6-methyl-3-nitropyridine (13g). To a solution of **12** (619 g, 2.32 mol) in anhydrous acetonitrile (3.0 L) was added phosphorus oxychloride (277 mL, 2.96 mol), followed by addition of DMF (36 mL, 0.46 mol). The mixture was heated to 50 ºC for 2 hours. The mixture was chilled to 0 ºC and the

precipitate filtered. The precipitate was washed with chilled acetonitrile (500 mL) until the filtrate wash was devoid of color. The crude precipitate **10** was dissolved in acetonitrile (1.5 L). To the slurry was charged dropwise a solution of *p*-anisidine (286 g, 2.32 mol) in anhydrous acetonitrile (500 mL). The mixture was heated to 65 ºC for 8 h. The mixture was cooled to ambient temperature and carefully quenched with 2 N NaOH (~ 2 L) while keeping the temperature below 30ºC. The organic phase was separated and washed with brine. The combined aqueous phases were extracted with EtOAc (2 L) and the organic phase was washed with brine. All the organic phases were combined and the solvent removed *in vacuo*. The residue was dried using a heptane azeotrope and the crude diluted with 1 L of warm heptane and seeded. The solution was chilled in a freezer for 16 hours. The solid was collected by filtration and washed with 500 mL of heptane affording 620 g of title compound. The mother liquor was filtered through a plug of silica gel eluting with heptane then 9:1 heptane/EtOAc affording an additional 130 g of title compound. The combined products afforded **13g** (750 g, 87%) as a bright red solid: ^{1}H NMR ($CDCl_3$) δ 0.94 (t, *J* = 7.2 Hz, 6H), 1.35-1.46 (m, 4H), 1.55 -1.63 (m, 4H), 2.28 (s, 3H), 3.55-3.61 (m, 1H), 3.81 (s, 3H), 5.93 (s, 1H), 6.88 (d, *J* = 9.1 Hz, 2H), 7.62 (d, *J* = 9.1 Hz, 2H), 9.25 (d, *J* = 8.1 Hz, 1H), 11.12 (br s, 1H); MS (CI) *m/z* 373.1 (MH^+). Anal. Calcd for $C_{20}H_{28}N_4O_3$: C, 64.49; H, 7.58; N, 15.04. Found: C, 64.29; H, 7.44; N, 14.58.

3-(4-Methoxy-phenyl)-5-methyl-7-(1-propyl-butylamino)-1,3-dihydro-imidazo[4,5-b]pyridin-2-one (15g). To the solution of sodium hydrosulfite (1360 g, 6.64 mol) in water (7.0 L) was slowly added 10 N NaOH (664 mL). A solution of **13g** (620 g, 1.66 mol) in THF (4 L) was added in portions. A slight exotherm occurs during the addition, bringing the reaction temperature to 35-40ºC. The reaction was stirred at ambient temperature for 2 hours. The organic phase was separated and the solvent removed *in vacuo* affording a dark tan gum. The aqueous phase was extracted with DCM (2 L) and the aqueous phase was removed. The crude gum isolated from above was diluted

with DCM (2 L) and combined with the DCM extracts. The mixture was chilled to 10 ºC, 5 N NaOH (2.6 L, 13.28 mol) was added, and the mixture was vigorously agitated. A solution of triphosgene (163 g, 0.55 mol) in dichloromethane (400 mL) was carefully added drop wise over 1 hour, while maintaining a reaction temperature below 25ºC. Upon completion of the addition, the reaction was stirred for 14 h, gradually warming to ambient temperature. The mixture was diluted with EtOAc (6 L) and brine (4 L), and the pH value was adjusted to ~ 6 with 6 N HCl (500 mL). The aqueous phase was removed and the organic phase washed with brine. The solvent was removed *in vacuo* and the crude oil diluted with 1 L of EtOAc. The solution was filtered over celite and approximately half of the solvent was removed. Hexane (1.5 L) was added, and the mixture was allowed to sit at ambient temperature for 14 hours. The precipitate was collected by filtration, washed with 1:4 EtOAc/hexane (500 mL) and dried *in vacuo* at 50 ºC to afford 295 g of the title compound. The filtrate was chromatographed with 1:4 EtOAc hexanes affording an additional 75g of title product. The combined **15g** (370 g, 60%) was an off-white solid: ^{1}H NMR ($CDCl_3$) δ 0.79-0.89 (m, 6H), 1.19 -1.33 (m, 8H), 2.37 (s, 3H), 3.37-3.43 (m, 1H), 3.76 (s, 3H), 4.90 (d, *J* = 8.8 Hz, 1H), 6.13 (s, 1H), 6.89 (d, *J* = 9.0 Hz, 2H), 7.42 (d, *J* = 9.0 Hz, 2H), 11.05 (br s, 1H),; MS (CI) *m/z* 369.1 (MH^+). Anal. Calcd for $C_{21}H_{28}N_4O_2$: C, 68.45; H, 7.66; N,15.20. Found: C, 68.18; H, 7.30; N,15.05.

1-(4-Methoxy-phenyl)-7-methyl-5-(1-propyl-butyl)-4,5-dihydro-1*H*,3*H*-1,2a,5,8-tetraaza-acenaphthylen-2-one (16g). To a solution of **15g (**370 g, 1.00 mol) and tetrabutylammonium bromide (64 g, 0.20 mol) in dichloromethane (1.6 L) was added 50% aqueous NaOH (1600 g, 20 mol). 1,2-dibromoethane (431 mL, 5 mol) was added portion wise with vigorous stirrring. The mixture was heated to reflux for 2 hr and then cooled to ambient temperature. The mixture was diluted with ice water (1200 mL), and the aqueous phase was removed. The organic phase was washed with water (2X), brine, dried ($MgSO_4$), filtered, and the solvent removed *in vacuo.* The crude product was

chromatographed on silica gel by eluting with 1:6 to 1:2 EtOAc/toluene to yield **16g** (276 g, 70%) as an off-white powder: ^{1}H NMR ($CDCl_3$) δ 0.93 (t, *J* = 7.3 Hz, 6H), 1.25 -1.38 (m, 4H), 1.51 -1.62 (m, 4H), 2.37 (s, 3H), 3.38 (t, *J* = 4.9 Hz, 2H), 3.71-3.78 (m, 1H), 3.83 (s, 3H), 4.01 (t, *J* = 4.9 Hz, 2H), 6.25 (s, 1H), 7.01 (d, *J* = 8.8 Hz, 2H), 7.60 (d, *J* = 8.8 Hz, 2H); MS (CI) *m/z* 395.1 (MH^+). HRMS (FAB) *m/z* calcd for $C_{23}H_{30}N_4O_2Na$ (MNa^+) 417.2266, found 417.2277. Anal. Calcd for $C_{23}H_{30}N_4O_2$: C, 70.02; H, 7.66; N, 14.20. Found: C, 70.33; H, 7.83; N, 14.27.

1-(4-Methoxy-phenyl)-7-methyl-5-(1-propyl-butyl)-4,5-dihydro-1*H*,3*H*-1,2a,5,8-tetraaza-acenaphthylen-2-one hydrochloride. To a solution of **16g** free base (43 g, 110 mmol) in EtOAc (100 mL) and MTBE (100 mL), HCl/diethyl ether (2.0 M, 60 mL, 120 mmol) was added carefully, and a thick precipitate appeared. Diethyl ether was removed *in vacuo*, and the slurry was then heated to reflux for 1 hour. The mixture was cooled to ambient temperature and filtered. The filter cake was washed with EtOAc and dried to afford the title compound (40 g, 85%) as an off-white, fine-granular solid: mp 176.4-177.6 ºC; ^{1}H NMR ($CDCl_3$) δ 0.91 (t, *J* = 7.3 Hz, 6H), 1.23 -1.31 (m, 4H), 1.57 -1.63 (m, 4H), 2.74 (s, 3H), 3.54 (t, *J* = 5.0 Hz, 2H), 3.79 (s, 3H), 3.82-3.90 (m, 1H), 4.01 (t, *J* = 5.0 Hz, 2H), 6.33 (s, 1H), 7.02 (d, *J* = 8.9 Hz, 2H), 7.39 (d, *J* = 8.9 Hz, 2H); MS (CI) *m/z* 395.1 (MH^+). Anal. Calcd for ($C_{23}H_{30}N_4O_2 \bullet HCl \bullet H_2O$): C, 61.53; H, 7.41; N, 12.48. Found: C, 61.16; H, 7.47; N, 12.19.

1-(2,4-Dichloro-phenyl)-7-methyl-5-(1-propyl-butyl)-4,5-dihydro-1*H*,3*H*-1,2a,5,8-tetraaza-acenaphthylen-2-one (16a). ^{1}H NMR ($CDCl_3$) δ 0.93 (t, *J* = 7.5 Hz, 6H), 1.25-1.38 (m, 4H), 1.52 -1.62 (m, 4H), 2.40 (s, 3H), 3.40 (t, *J* = 4.7 Hz, 2H), 3.72-3.78 (m, 1H), 3.90-4.11 (m, 2H), 6.26 (s, 1H), 7.26-7.57 (m, 3H); MS (CI) *m/z* 433.00 (MH^+); HRMS (FAB) *m/z* calcd for $C_{22}H_{27}{}^{35}Cl{}^{35}Cl\ N_4O/C_{22}H_{27}{}^{35}Cl\ {}^{37}ClN_4O$ (MH^+) 433.1562/435.1532, found 433.1558/435.1528. Anal. Calcd for $C_{22}H_{26}Cl_2N_4O$: C, 60.97; H, 6.05; N, 12.93. Found: C, 61.18; H, 5.94; N, 12.68.

1-(2-Bromo-4-isopropyl-phenyl)-7-methyl-5-(1-propyl-butyl)-4,5-dihydro-1*H*,3*H*-1,2a,5,8-

tetraaza-acenaphthylen-2-one (16b). ^{1}H NMR ($CDCl_3$) δ 0.93 (t, *J* = 7.5 Hz, 6H), 1.26 (d, *J* = 6.9 Hz, 6H), 1.27-1.35 (m, 4H), 1.52 -1.61 (m, 4H), 2.41 (s, 3H), 2.94 (hept, *J* = 6.9 Hz, 1H), 3.40 (t, *J* = 4.7Hz, 2H), 3.73-3.82 (m, 1H), 3.89-4.11 (m, 2H), 6.26 (s, 1H), 7.27-7.58 (m, 3H); MS (CI) *m/z* 485.10 (MH^+); HRMS (FAB) *m/z* calcd for $C_{25}H_{34}{}^{79}BrN_4O/C_{25}H_{34}{}^{81}BrN_4O$ (MH^+) 485.1916/487.1896, found 485.1922/487.1902. Anal. Calcd for $C_{25}H_{33}BrN_4O$: C, 61.85; H, 6.85; N, 11.54. Found: C, 61.74; H, 6.88; N, 11.69.

1-(4-Chloro-phenyl)-7-methyl-5-(1-propyl-butyl)-4,5-dihydro-1*H*,3*H*-1,2a,5,8-tetraaza-acenaphthylen-2-one (16c). ^{1}H NMR ($CDCl_3$) δ 0.86 (t, *J* = 7.3 Hz, 6H), 1.17 -1.32 (m, 4H), 1.43 -1.57 (m, 4H), 2.38 (s, 3H), 3.32 (t, *J* = 5.0 Hz, 2H), 3.63-3.73 (m, 1H), 3.92 (t, *J* = 5.0 Hz, 2H), 6.20 (s, 1H), 7.36 (d, *J* = 8.4 Hz, 2H), 7.72 (d, *J* = 8.4 Hz, 2H); MS (CI) *m/z* 399.0 (MH^+). Anal. Calcd for ($C_{22}H_{27}ClN_4O \bullet H_2O$): C, 63.38; H, 7.01; N, 13.44. Found: C, 63.49; H, 6.94; N, 13.08.

1-(4-Methanesulfonyl-phenyl)-7-methyl-5-(1-propyl-butyl)-4,5-dihydro-1*H*,3*H*-1,2a,5,8-tetraaza-acenaphthylen-2-one (16e). ^{1}H NMR ($CDCl_3$) δ 0.92 (t, *J* = 7.3 Hz, 6H), 1.24 -1.38 (m, 4H), 1.49 -1.60 (m, 4H), 2.47 (s, 3H), 2.99 (s, 3H), 3.40 (t, *J* = 4.8 Hz, 2H), 3.72-3.79 (m, 1H), 4.00 (t, *J* = 4.8 Hz, 2H), 6.30 (s, 1H), 7.69 (d, *J* = 8.8 Hz, 2H), 8.23 (d, *J* = 8.8 Hz, 2H); HRMS (ESI) *m/z* calcd for $C_{23}H_{30}N_4O_3S$ (MH^+) 443.2111, found 443.2115.

1-(4-Methoxycarbonyl-phenyl)-7-methyl-5-(1-propyl-butyl)-4,5-dihydro-1*H*,3*H*-1,2a,5,8-tetraaza-acenaphthylen-2-one (16f). ^{1}H NMR ($CDCl_3$) δ 0.93 (t, *J* = 7.3 Hz, 6H), 1.23 -1.36 (m, 2H), 1.52 -1.65 (m, 6H), 2.46 (s, 3H), 3.39 (t, *J* = 5.1 Hz, 2H), 3.71-3.79 (m, 1H), 3.92 (s, 3H), 4.00 (t, *J* = 5.1 Hz, 2H), 6.28 (s, 1H), 8.05 (d, *J* = 8.7 Hz, 2H), 8.16 (d, *J* = 8.7 Hz, 2H); MS (CI) *m/z* 423.0 (MH^+). Anal. Calcd for ($C_{24}H_{30}N_4O_3 \bullet$ EtOAc): C, 65.86; H, 7.50; N, 10.97. Found: C, 66.11; H, 7.23; N, 11.06.

1-(5-Chloro-pyridin-2-yl)-7-methyl-5-(1-propyl-butyl)-4,5-dihydro-1*H*,3*H*-1,2a,5,8-tetraaza-acenaphthylen-2-one (16k). ^{1}H NMR ($CDCl_3$) δ 0.92 (t, *J* = 7.2 Hz, 6H), 1.25 -1.38 (m, 4H), 1.50 -1.62 (m, 4H), 2.47 (s, 3H), 3.39 (t, *J* = 5.0 Hz, 2H), 3.72-3.78 (m, 1H), 3.99 (t, *J* = 5.0 Hz, 2H), 6.29 (s, 1H), 7.76 (d, *J* = 8.7 Hz,

1H), 7.82 (dd, *J* = 8.7, 2.1 Hz, 1H), 8.61 (d, *J* = 2.1 Hz, 1H); MS (CI) *m/z* 400.0 (MH^+). Anal. Calcd for $C_{21}H_{26}ClN_5O$: C, 63.07; H, 6.55; N, 17.51. Found: C, 63.23; H, 6.53; N, 17.21.

1-(6-Methoxy-pyridin-3-yl)-7-methyl-5-(1-propyl-butyl)-4,5-dihydro-1*H*,3*H*-1,2a,5,8-tetraaza-acenaphthylen-2-one (16l). ^{1}H NMR ($CDCl_3$) δ 0.92 (t, *J* = 7.3 Hz, 6H), 1.25 -1.38 (m, 4H), 1.51 -1.66 (m, 4H), 2.43 (s, 3H), 3.39 (t, *J* = 5.0 Hz, 2H), 3.70-3.77 (m, 1H), 3.96 (s, 3H), 4.01 (t, *J* = 5.0 Hz, 2H), 6.26 (s, 1H), 6.86 (d, *J* = 8.9 Hz, 1H), 7.95 (dd, *J* = 8.9, 2.7 Hz, 1H), 8.57 (d, *J* = 2.7 Hz, 1H); MS (CI) *m/z* 396.1 (MH^+). Anal. Calcd for $C_{22}H_{29}N_5O_2$: C, 66.81; H, 7.39; N, 17.71. Found: C, 66.67; H, 7.11; N, 17.45.

1-(4-Methoxy-phenyl)-7-methyl-4,5-dihydro-1*H*,3*H*-1,2a,5,8-tetraaza-acenaphthylen-2-one (17a). A solution of **16g** (4.72 g, 12 mmol) in conc. sulfuric acid (15 mL) was heated at 65 ^{o}C for 10 h, the reaction mixture was cooled down to ambient temperature and was poured on ice. Solid KOH was added until the solution became neutral, and then $NaHCO_3$ was added until the pH value reached 9. EtOAc was added, and the solids were removed by filtration. The biphasic mixture was separated in a separation funnel, and the aqueous phase was extracted three more times with EtOAc. Combined organic extracts were dried over Na_2SO_4, evaporated, and purified by column chromatography with silica gel (1: 1 hexanes/acetone) to obtain the desired product (**17a**, 1.76 g, 50%): ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3H), 3.58-3.64 (m, 2H), 3.82 (s, 3H), 4.03-4.09 (m, 2H), 4.22 (br s, 1H), 6.25 (s, 1H), 7.05 (d, *J* = 8.7 Hz, 2H), 7.83 (d, *J* = 8.7 Hz, 2H); MS (CI) *m/z* 297.10 (MH^+). Anal. Calcd for $C_{16}H_{16}N_4O_2$: C, 64.85; H, 5.44; N, 18.91. Found: C, 64.66; H, 5.71; N, 18.63.

5-(1-Ethyl-propyl)-1-(4-methoxy-phenyl)-7-methyl-4,5-dihydro-1*H*,3*H*-1,2a,5,8-tetraaza-acenaphthylen-2-one (17d). To a solution of **17a** (1.22 g, 4.1 mmol) in anhydrous DMF (10 mL) was added t-BuOK (1.23 g, 11 mmol), and the reaction mixture was stirred at ambient temperature for 5 min. 3-Bromopentane (1.24 g, 8.2 mmol) was added slowly in several portions, and the reaction mixture was stirred at rt for 5 h. The reaction was quenched with water, and EtOAc was added. The organic phase was washed with brine, dried (Na_2SO_4), evaporated,

and purified by column chromatography with silica gel (2: 1 hexanes/EtOAc) to obtain the desired product (**17d**, 795 mg, 53%): ^{1}H NMR ($CDCl_3$) δ 0.91 (t, *J* = 7.7 Hz, 6H), 1.53 -1.65 (m, 4H), 2.43 (s, 3H), 3.37 (t, *J* = 5.2 Hz, 2H), 3.49-3.58 (m, 1H), 3.83 (s, 3H), 3.99 (t, *J* = 5.2 Hz, 2H), 6.26 (s, 1H), 7.01 (d, *J* = 9.0 Hz, 2H), 7.60 (d, *J* = 9.0 Hz, 2H); MS (CI) *m/z* 367.10 (MH^+). Anal. Calcd for $C_{21}H_{26}N_4O_2$: C, 68.83; H, 7.15; N, 15.29. Found: C, 68.59; H, 7.33; N, 15.25.

5-Isopropyl-1-(4-methoxy-phenyl)-7-methyl-4,5-dihydro-1*H*,3*H*-1,2a,5,8-tetraaza-acenaphthylen-2-one (17b). ^{1}H NMR ($CDCl_3$) δ 1.27 (t, *J* = 6.6 Hz, 6H), 2.45 (s, 3H), 3.42 (t, *J* = 5.2 Hz, 2H), 3.83 (s, 3H), 4.02 (t, *J* = 5.2 Hz, 2H), 4.04-4.11 (m, 1H), 6.28 (s, 1H), 7.01 (d, *J* = 9.1 Hz, 2H), 7.61 (d, *J* = 9.1 Hz, 2H); HRMS (ESI) *m/z* calcd for $C_{19}H_{22}N_4O_2$ (MH^+) 339.1815, found 339.1818.

5-Butyl-1-(4-methoxy-phenyl)-7-methyl-4,5-dihydro-1*H*,3*H*-1,2a,5,8-tetraaza-acenaphthylen-2-one (17c). ^{1}H NMR ($CDCl_3$) δ 0.98 (t, *J* = 7.1 Hz, 3H), 1.36-1.44 (m, 2H), 1.61-1.73 (m, 2H), 2.45 (s, 3H), 3.32 (t, *J* = 7.5 Hz, 2H), 3.49 (t, *J* = 5.0 Hz, 2H), 3.83 (s, 3H), 4.05 (t, *J* = 5.0 Hz, 2H), 6.22 (s, 1H), 7.00 (d, *J* = 8.8 Hz, 2H), 7.61 (d, *J* = 8.8 Hz, 2H); HRMS (ESI) *m/z* calcd for $C_{20}H_{24}N_4O_2$ (MH^+) 353.1972, found 353.1981.

5-(1-Methoxymethyl-propyl)-1-(4-methoxy-phenyl)-7-methyl-4,5-dihydro-1*H*,3*H*-1,2a,5,8-tetraaza-acenaphthylen-2-one (**17e**)**.** ^{1}H NMR ($CDCl_3$) δ 0.96 (t, *J* = 7.3 Hz, 3H), 1.64 -1.75 (m, 2H), 2.45 (s, 3H), 3.33 (s, 3H), 3.47-3.55 (m, 5H), 3.83 (s, 3H), 3.88-3.96 (m, 1H), 4.03-4.09 (m, 1H), 6.28 (s, 1H), 7.00 (d, *J* = 9.2 Hz, 2H), 7.59 (d, *J* = 9.2 Hz, 2H); HRMS (ESI) *m/z* calcd for $C_{21}H_{26}N_4O_3$ (MH^+) 383.2078, found 383.2084.

5-(1-Ethyl-propyl)-7-methyl-1-(4-trifluoromethyl-phenyl)-4,5-dihydro-1*H*,3*H*-1,2a,5,8-tetraaza-acenaphthylen-2-one (18b). ^{1}H NMR ($CDCl_3$) δ 0.92 (t, *J* = 7.5 Hz, 6H), 1.57 -1.69 (m, 4H), 2.46 (s, 3H), 3.40 (t, *J* = 5.1 Hz, 2H), 3.51-3.60 (m, 1H), 4.03 (t, *J* = 5.1 Hz, 2H), 6.31 (s, 1H), 7.75 (d, *J* = 8.8 Hz, 2H), 8.07 (d, *J* = 8.8 Hz, 2H); HRMS (ESI) *m/z* calcd for $C_{21}H_{23}F_3N_4O$ (MH^+) 405.1897, found 405.1897.

5-(1-Ethyl-propyl)-1-(4-methoxy-2-methyl-phenyl)-7-methyl-4,5-dihydro-1*H*,3*H*-1,2a,5,8-tetraaza-acenaphthylen-2-one (18c). ^{1}H NMR ($CDCl_3$) δ 0.93 (t, *J* = 7.5 Hz, 6H), 1.59 -1.68 (m, 4H), 2.21 (s, 3H), 2.40 (s, 3H), 3.41 (t, *J* = 5.1 Hz, 2H), 3.50-3.59 (m, 1H), 3.81 (s, 3H), 3.95-4.07 (m, 2H), 6.25 (s, 1H), 6.81-6.88 (m, 2H), 7.22 (d, *J* = 8.6 Hz, 1H); HRMS (ESI) *m/z* calcd for $C_{22}H_{28}N_4O_2$ (MH^+) 381.2285, found 381.2285.

1-(4-Chloro-phenyl)-5-(1-methoxymethyl-propyl)-7-methyl-4,5-dihydro-1*H*,3*H*-1,2a,5,8-tetraaza-acenaphthylen-2-one trifluoroacetate (19a). ^{1}H NMR ($CDCl_3$) δ 0.97 (t, *J* = 7.4 Hz, 3H), 1.64 -1.77 (m, 2H), 2.53 (s, 3H), 3.33 (s, 3H), 3.55 (d, *J* = 5.7 Hz, 2H), 3.63 (t, *J* = 4.8 Hz, 2H), 3.92-3.99 (m, 2H), 4.09-4.16 (m, 1H), 6.37 (s, 1H), 7.35-7.46 (m, 4H); MS (CI) *m/z* 387.1 (MH^+). Anal. Calcd for ($C_{20}H_{23}Cl\ N_4O_2 \bullet$ TFA): C, 52.75; H, 4.83; N, 11.19. Found: C, 52.63; H, 4.68; N, 10.96.

Biology

In vitro **Binding and Functional Studies**. Radioligand binding assays and functional inhibition of CRF-induced cAMP production were performed in L-CRF_1 cell membranes. Equilibrium binding of unlabeled ligands was measured in duplicate by inhibition of radioligand binding ([^{125}I]sauvagine) to LtK^- cells expressing the human CRF_1 receptor. Assay buffer (30 µl DPBS, 1.5mM KH_2PO_4, 8.1mM Na_2HPO_4, 2.7mM KCl, 138mM NaCl) supplemented with 10mM $MgCl_2$, 2mM ethylene glycol-bis[β-aminoethyl]-N,N,N',N'-tetraacetic acid, pH 7.4), 20µl unlabeled ligand, 50µL radioligand and 100µL L-CRF_1 cell membranes were sequentially added to low protein-binding 96 well plates (Corning #3605). The final concentration of radioligand was approximately 90 pM for [^{125}I]sauvagine with a total of 5µg of membrane . Unlabeled compounds were serially diluted for final concentrations of 10 pM to 1 µM. Following a two-hour incubation at room temperature, bound and free radioligand were separated by rapid vacuum filtration. In all assays total radioligand bound to the filter (total binding) was less than 20% of the total amount of radioligand added. Non-specific binding was determined in the presence of an excess of the

unlabeled analogue of the radioligand. Bound and non-specific radioactivity was monitored using a Packard Cobra II gamma counter (78% efficiency) and analyzed using the non-linear curve-fitting algorithm software Prism™ (GraphPad Inc., CA).

CRF-Stimulated cAMP Production in Cells Expressing Human CRF_1 Receptors. One day prior to assay L-hCRF_1 cells were transferred to 96-well tissue culture plates (100,000 cells / well in 200 µL medium). On the day of assay, the medium was removed and the cells washed with 200 µL DPBS. Following aspiration of DPBS, 75 µL cAMP assay buffer was added to each well (DMEM without phenol red, supplemented with 2 mM glutamine, 1 mM sodium pyruvate, 10 mM HEPES, 50 IU / mL penicillin, 50µg/mL streptomycin and 1 mM IBMX). Corticotropin-releasing factor and non-peptides were then added in a volume of 25µL cAMP assay buffer at various concentrations for inhibition, and the cells incubated for 30 minutes at 37°C in 5% CO_2. Total cAMP produced was measured by chemiluminescent immunoassay (Tropix, Bedford, MA) and measured on an Analyst™ (LJL Biosystems Inc., CA). All IC_{50} values were calculated using the non-linear curve-fitting algorithm software Prism™ as above.

CRF-Stimulated ACTH Release from Cultured Rat Anterior Pituitary Cells. For the inhibition of ACTH release from primary rat pituitary cell cultures, five whole pituitaries are collected from 7 week-old female SD rats. Pituitaries are washed six times with HEPES buffer (2.5 g/L BSA; 10 mg/L Deoxyribonuclease I; 8.0 g/L NaCl; 0.37 g/L KCl; 100 mg/L Sodium Phosphate dibasic; 6.0 g/L HEPES; 2.0 g/L Glucose) and minced. The tissue is then digested with 10 mL collagenase for 1.5 hours at 37ºC, with trituration every 30 minutes. The digest is then transferred to a 50 mL conical centrifuge tube and centrifuge at 1000 rpm for 4 minutes. The supernatant is discarded and the pellet resuspended in 10 mL neuraminidase solution and incubated for 9 minutes at 37ºC. The suspension is centrifuged at 1000 rpm for 5 minutes and the pellet washed once with 10 mL BBM-T medium (11.49 g/L Custom Media Mixture, Irvine Scientific, CA; 1.83 g $NaCO_3$/L; 2.4 g HEPES/L; 2.0 g/L BSA;

10.0 mg/L Transferrin; 50,000 I.U./L Penicillin and Streptomycin; 1 μg/L Insulin; 0.1 μg/L EGF; 0.4 μg/L T3; 0.7 μg/L PTH; 10 μg/L Glucagon). The resulting pellet is finally resuspended in 3% FCS/BBM-T medium and cultured in 96-well tissue culture plates for 2-3 days at a density of 40,000 cells/well in a final volume of 200 μL medium. For the assay of antagonists, cells are washed once with BBM-T, test samples are added in various concentrations (1 μM to 1 pM) with 0.5 nM r/hCRF in 200 mL BBM-T and incubated for 4 hours incubation at 37ºC. The medium is then aspirated and assessed for ACTH release using a standard RIA kit (MP Biomedicals, NY). Again, all data was analyzed using the non-linear curve-fitting algorithm software Prism™ as above.

***In vivo* CRF-Induced ACTH Release in Rats.** Three days prior to testing with compound, rats were anesthetized (n = 6 per group) with isoflurane and implanted with a femoral vein catheter (IITC #26A; PE 10 silastic) in the right groin area. The catheter was secured in place with 4-0 suture. A gastric catheter was placed in the stomach and sutured with a purse string suture (4-0 suture) to secure the cannula in place. The cannulae were fed subcutaneously to the dorsal section of the rat (behind the ears), where they exited and were sutured in place. All external incisions were closed using standard wound clips. On the day of testing, fed rats were weighed, and then connected to PE50 tubing via manosil tubing. They were then placed in opaque collection containers, with the PE50 tubing drawn through the top of the container, and habituated to the containers for 1 hour. Following a baseline blood draw, Compound **16g** (3, 10 or 30 mg/kg) or vehicle (2 mL/kg) was infused via the intragastric tube. Sixty minutes later, CRF (0.3nmol/kg) or vehicle (0.5mL/kg) was injected i.v. The CRF vehicle was a 0.1% BSA, 10mM acetic acid solution. Blood was drawn at 2, 10 and 30 minutes following the CRF injection and collected in EDTA-coated tubes and centrifuged at 2500 rpm (4ºC) for 20 minutes. Plasma was frozen (-80ºC) until the time of assay. The ACTH levels were determined in these samples using a standard ACTH RIA kit (MP Biomedicals, NY), with

sample ACTH values calculated from a log-transformed standard curve. ACTH values over time were analyzed using repeated measures, mixed design ANOVA. Peak (10 minute time point) ACTH values were analyzed using one-way ANOVA, with Fischer's PLSD as the post-hoc method of testing dose group differences.

Rat Pharmacokinetics. Oral bioavailability studies were conducted in male Sprague-Dawley rats and test articles were administered to the rats by oral gavage and intravenous injection (10 mg/kg; N = 3/time point) in water solution. The dosing solution was prepared in purified water and filtered through 0.2µm Nylon filter prior to administration (2 mL / Kg). Blood samples from each dosing route were taken at pre-determined time points for pharmacokinetic analysis. All plasma samples were flash frozen in liquid nitrogen within 10 minutes of sampling and stored in -76 °C or below until analysis. The bioanalytical method applied for the measurement of test articles in plasma along with added internal standard consisted of precipitation with 200 µL of acetonitrile from 50 µL of plasma, centrifugation and recovery of the supernatant, which was dried down in vacuum and then reconstituted in acetonitrile-water solutions before introduction into an LC-MS/MS system for analysis. The lower limit of quantification (LLOQ) for the analytical methods was 5 ng/mL of test article in plasma. All pharmacokinetic parameters were calculated from a non-compartmental model using WinNonlin program version 3.2.

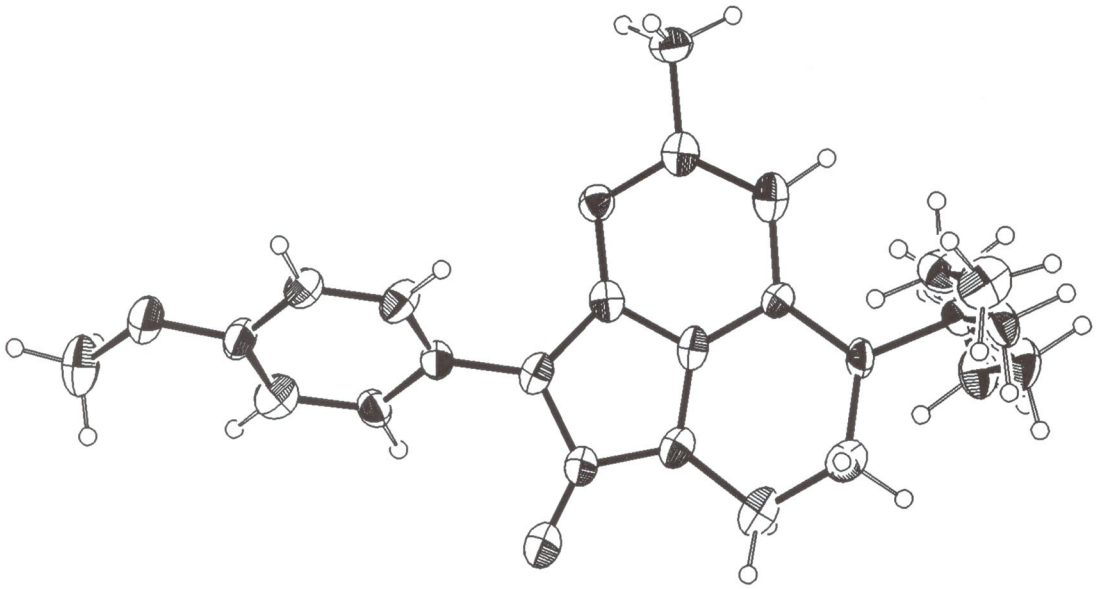

Figure 4. X-Ray structure for **16g**.