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The Discovery of 4-(3-Pentylamino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)-pyrazolo-[1,5-*a*]-pyrimidine: A Corticotropin-Releasing Factor ($hCRF_1$) Antagonist

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Abstract—Structure–activity relationship studies led to the discovery of 4-(3-pentylamino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)-pyrazolo-[1,5-*a*]-pyrimidine **11-31** (DMP904), whose pharmacological profile strongly supports the hypothesis that $hCRF_1$ antagonists may be potent anxiolytic drugs. Compound **11-31** ($hCRF_1$ $K_i = 1.0 \pm 0.2$ nM ($n = 8$)) was a potent antagonist of $hCRF_1$-coupled adenylate cyclase activity in HEK293 cells ($IC_{50} = 10.0 \pm 0.01$ nM versus 10 nM r/hCRF, $n = 8$); α-helical CRF(9-41) had weaker potency ($IC_{50} = 286 \pm 63$ nM, $n = 3$). Analogue **11-31** had good oral activity in the rat situational anxiety test; the minimum effective dose for **11-31** was 0.3 mg/kg (po). Maximal efficacy (approximately 57% reduction in latency time in the dark compartment) was observed at this dose. Chlordiazepoxide caused a 72% reduction in latency at 20 mg/kg (po). The literature compound **1** (CP154526-1, 30 mg/kg (po)) was inactive in this test. Compound **11-31** did not inhibit open-field locomotor activity at 10, 30, and 100 mg/kg (po) in rats. In beagle dogs, this compound (5 mg/kg, iv, po) afforded good plasma levels. The key iv pharmacokinetic parameters were $t_{1/2}$, CL and $V_{d,ss}$ values equal to 46.4 ± 7.6 h, 0.49 ± 0.08 L/kg/h and 23.0 ± 4.2 L/kg, respectively. After oral dosing, the mean C_{max}, T_{max}, $t_{1/2}$ and bioavailability values were equal to 1260 ± 290 nM, 0.75 ± 0.25 h, 45.1 ± 10.2 h and 33.1%, respectively. The overall rat behavioral profile of this compound suggests that it may be an anxiolytic drug with a low motor side effect liability.

Introduction

Corticotropin releasing factor (CRF) is a 41 amino acid peptide, a pituitary secretagogue of adrenocorticotropin hormone (ACTH) and a neurotransmitter.[1–4] Since its isolation and characterization, clinical and preclinical studies have documented the pivotal role of CRF in the regulation of endocrine, autonomic and behavioral responses to stress. Rapid release of CRF mediates many of the body's responses to stressors and intracerebroventricular (icv) administration of CRF replicates many of the behavioral and physiological effects of stress.[5–8] Intracerebroventricular administration of peptidic CRF antagonists, such as α-helical CRF(9-41) and astressin, block not only the effects of exogenously administered CRF but also the effects of natural stressors.[3] Transgenic mice, which overexpress CRF, demonstrate heightened stress-related behaviors and have elevated function of the hypothalamus–adrenal–pituitary (HPA) axis. Mice, which have null mutations for CRF, exhibit a blunted endocrine response to stress.[3] The above data, taken together with neuroanatomical

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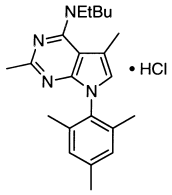
studies on the localization of CRF receptors and the innervation of key brain centers by CRF-containing fibers,[9] support the proposal that CRF is the primary component of the central response to stress.

Clinical studies have implicated CRF in the etiology of depression and affective disorders.[10–17] Maladaptive response to stress and the cognate chronic elevation of corticosteroid levels may be a common basis for the production of major depression. This hypercortisolemia is most likely the result of chronic hypersecretion of hypothalamic CRF.[18] CRF levels in the cerebrospinal fluid (CSF) are elevated in depressed patients as is the number of CRF neurons in the hypothalami of such individuals.[14] Successful treatment of depressive symptoms is accompanied by a lowering of CSF CRF levels and plasma cortisol levels to normal ranges. CSF CRF levels are also elevated relative to controls during alcohol withdrawal, anorexia nervosa, obsessive–compulsive disorder and post-traumatic stress syndrome.[3]

The functions of CRF are mediated through two CRF receptor subtypes and one binding protein.[3,8,19] The heptahelical G-protein-coupled receptor subtypes, CRF_1 and CRF_2, are distinguished by the relative affinities and efficacies of peptide agonists as well as anatomical distribution. The latter type has three splice variants: α, β, and γ[3,19] Signal transduction through each receptor subtype involves stimulation of cyclic adenosine monophosphate (c-AMP) production; agonists stimulate c-AMP production for both subtypes. CRF_1 sites are found primarily in the rat pituitary, cerebral cortex, hippocampus and amygdala. In rats, the CRF_2 receptor is primarily a rat peripheral receptor with lower distribution in discrete CNS regions: lateral septum, choroid plexus, hypothalamus and sympathetic nuclei. The CRF binding protein is widely distributed in the CNS, including hippocampus and amygdala and in human, but not rodent, plasma. The central functions of the CRF receptor subtypes and the central binding protein have been the subject of intense investigation with peptidic antagonists and antisense reagents.[8] Modulation of CRF function therefore offers the tantalizing prospect of developing new therapeutic agents for anxiety, depression, Alzheimer's disease and obesity but clinical studies with non-peptidic selective agents are needed to truly test these hypotheses.[3,8]

Pyrrolopyrimidine **1** (CP154526-1)[20–23] is a non-peptidic CRF_1-selective antagonist, which has been studied at length. This compound has high affinity for $hCRF_1$ receptors (human $K_i = 2.4$ nM (IMR32 neuroblastoma cells)) and blocks the behavioral effects of CRF in rats. Compound **1** reduced the r/hCRF-enhanced acoustic startle responses of rats. Compound **1** is also effective in the fear-potentiated acoustic startle test in rats (maximal efficacy at 17.8 mg/kg (ip)). In the rat learned helplessness model for depression, administration of **1** (32 mg/kg, ip, 60 min pretest, single dose) completely counters the effects of inescapable foot shocks for three consecutive days. In the same test, there was no significant effect with the imipramine (17.8 and 32 mg/kg, sc). These data suggest potential anxiolytic or antidepressant utilities for CRF_1 antagonists.



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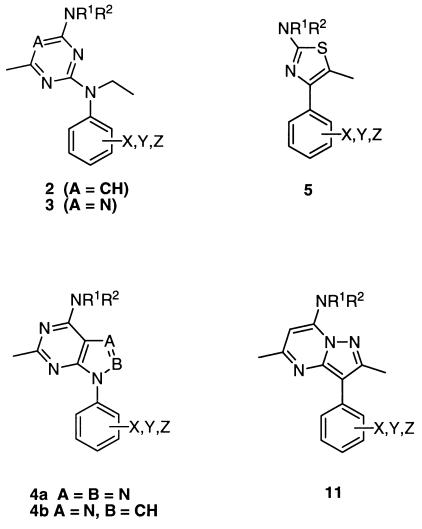
Our interest in pyrazolo-[1,5-*a*]-pyrimidines **11** as potential CRF antagonists arose from an analysis of the structure–activity relationships published for other CRF antagonist series (Scheme 1).[24–27] We have previously reported that anilino-pyrimidines/-triazines **2** and **3** as well as triazolo-pyrimidines **5** are potent $hCRF_1$ antagonists, some of which have excellent pharmacokinetic profiles in multiple species (Scheme 1). Researchers at Sanofi have identified aryl thiazoles **4** to be high affinity CRF antagonists. Overlay of these structures via molecular modeling techniques suggested to us and others that alternate scaffolds, such as the pyrazolo-[1,5-*a*]-pyrimidine core, are possible, providing that the peripheral functional groups are held in approximately the same spatial orientation to maximize binding affinity to the CRF_1 receptor.[28–30] We report herein the structure–activity relationships leading to the discovery of 4-(3-pentylamino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)pyrazolo-[1,5-*a*]-pyrimidine **11-31** (DMP904), whose pharmacological profile strongly supports the hypothesis that $hCRF_1$ antagonists may be potent anxiolytic drugs.[31]

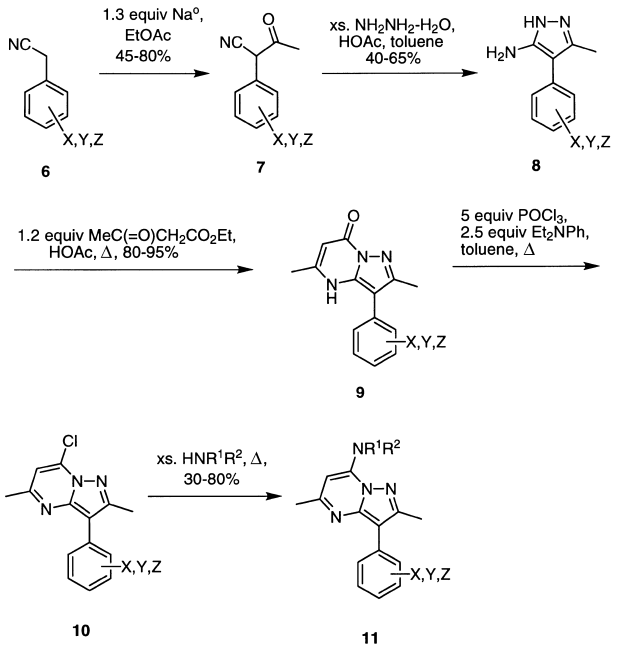
Results and Discussion

Chemistry

Pyrazolo-[1,5-*a*]-pyrimidines **11** were synthesized according to the procedures outlined in Scheme 2. Substituted phenylacetonitriles **6** were condensed with ethyl acetate to generate keto-nitriles **7** in 45–80% yields. Reaction of intermediates **7** with hydrazine-hydrate in



Scheme 1.



Scheme 2.

the presence of acetic acid and toluene produced aminopyrazoles **8** in 40–85% yields. Subsequent condensation of pyrazoles **8** with ethyl acetoacetate in refluxing acetic acid provided pyrazolopyrimidinones **9** in 80–95% yields. Reaction of **9** with phosphorus oxychloride in the presence of *N*,*N*′-diethylaniline afforded chloro intermediates **10**, which were purified by flash chromatography and stored at reduced temperature to minimize hydrolysis back to the precursors **9**. Reaction with neat alkyl or dialkyl amines gave the desired target compounds **11** in 30 to 80% overall yields.

Pharmacology. A multivariate approach to screening was utilized to efficiently identify the optimal analogues. A cloned human $hCRF_1$ receptor binding assay, in which inhibition of specific binding of ^{125}I-tyr-o-CRF by our test compounds was measured to determine their receptor binding affinity, began our testing sequence for the pyrazolopyrimidines. High affinity analogues were next evaluated in a rat ex vivo binding assay to estimate plasma levels as a function of time. However, this bioassay was not able to discriminate parent compounds from active metabolites. Therefore, rat or dog pharmacokinetic studies were conducted on the leading compounds. High affinity ligands ($K_i \leq 10$ nM) were also evaluated in a $hCRF_1$-mediated c-AMP production assay to measure antagonist potency. Behavioral effects of the test compounds were studied in rat models for anxiety: the situational anxiety test and the elevated plus maze model, while motor deficits were monitored in the rat locomotor activity or rotarod tests.

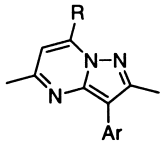
Structure–activity studies commenced in the 2,4-dimethylphenyl series (Table 1). A variety of substituted alkylamino or dialkylamino analogues were prepared and evaluated in the $hCRF_1$ receptor binding assay, based on the published precedent in other bicyclic CRF antagonist series. The diethylamino and 3-pentylamino analogues **11-2** and **11-1** had the best receptor binding affinity, but acylamino analogue **11-10** was a poorer ligand.

The focus of our studies then shifted to the identification of the optimal phenyl substitution pattern (Table 1). Replacement of a methyl group with a chlorine at either the 2- or 4-positions had minimal effect on receptor binding affinity. Comparable affinities were found for the corresponding analogues in the 2,4-dimethylphenyl, 4-chloro-2-methylphenyl, 2-chloro-4-methylphenyl and 2,4-dichlorophenyl series (e.g. compare **11-2** (mean $hCRF_1$ $K_i = 1.1$ nM) with **11-11** (mean $hCRF_1$ $K_i = 1.5$ nM) or **11-17** (mean $hCRF_1$ $K_i = 1.3$ nM)). 2,4-Disubstituted analogues generally had high receptor binding affinity than those compounds with 2,4,6-trisubstitution.[31] In the series where the amino side chain is fixed either as a diethylamino or a 3-pentylamino group, various 2,4-disubstituted phenyl patterns enhanced receptor binding affinity in similar fashion (e.g. analogues **11-1**, **11-12**, **11-18**, **11-22**, **11-29** and **11-31** had mean $hCRF_1$ K_i values equal to 0.8, 1.5, 1.7, 1.3, 1.5, and 1.0 nM, respectively). Introduction of alkoxy substituents at the 5-position reduced receptor binding affinity when the 2-substituent was bromine (e.g. compare **11-36** (mean $hCRF_1$ $K_i = 4.1$ nM) with **11-39** (mean $hCRF_1$ $K_i = 34.6$ nM)). The effect was less dramatic when chlorine was the 2-substituent (e.g. compare **11-28** (mean $hCRF_1$ $K_i = 1.2$ nM) with **11-41** (mean $hCRF_1$ $K_i = 2.6$ nM)).

Compounds were then evaluated in a rat ex vivo binding assay to estimate plasma exposure after oral administration. In this test, compounds were administered orally at 10 mg/kg and plasma samples were drawn at 1 and 3 h post-dose. The plasma samples were diluted 10-fold with assay medium and submitted to the $hCRF_1$ receptor binding assay. Table 2 delineates the percent inhibition values as a function of time. All the analogues, which were tested, had superior exposure in the rat relative to that of the reference compound **1** (CP154526-1), which had plasma exposures below the limit of detection for this bioassay. While the exposures of these compounds appeared to be high, the bioassay can not discriminate parent compound from active metabolite(s).

Pyrazolopyrimidine **11-31** (DMP904) ($hCRF_1$ $K_i = 1.0 \pm 0.2$ nM ($n = 8$)) was advanced to secondary in vitro studies based on its high affinity for $hCRF_1$ receptors and its projected duration of exposure in the rat ex vivo binding assay.[31] This compound was a potent antagonist of $hCRF_1$-coupled adenylate cyclase activity in HEK293 cells ($IC_{50} = 10.0 \pm 0.01$ nM versus 10 nM r/hCRF, $n = 8$); α-hCRF(9-41) had weaker potency ($IC_{50} = 286 \pm 63$ nM, $n = 3$). The antagonist activity profile of **11-31** with $hCRF_1$ receptors has been processed through a Schild analysis; it has been shown to be a non-competitive antagonist.[31–33] This compound had no affinity for $hCRF_{2-}$ receptors expressed in HEK293 cells and no affinity for the CRF-binding protein.[31,33] Analogue **11-31** was also submitted to several receptor binding assays in the NovaScreen program (Hanover,

Table 1. $hCRF_1$ Receptor binding and physical data[a–c]



Example	R	Ar	Mean $hCRF_1$ K_i (nM)	Formula	mp (°C)
11-1	NH-3-pentyl	2,4-Me_2Ph	0.8 ± 0.2 (5)	$C_{21}H_{28}N_4$	Oil
11-2	NEt_2	2,4-Me_2Ph	1.1 ± 0.3 (4)	$C_{20}H_{26}N_4$	92–94
11-3	NHCH(Et)CH_2OMe	2,4-Me_2Ph	1.7 ± 0.5 (4)	$C_{21}H_{28}N_4O$	Oil
11-4	NPrCH_2CH_2CN	2,4-Me_2Ph	1.8 ± 0.5 (3)	$C_{22}H_{27}N_5$	143
11-5	NBuCH_2CH_2CN	2,4-Me_2Ph	2.0 ± 0.7 (3)	$C_{23}H_{29}N_5$	115–117
11-6	N(c-Pr)CH_2CH_2CN	2,4Me_2Ph	3.4 ± 0.8 (3)	$C_{22}H_{25}N_5$	133 (dec)
11-7	$N(CH_2CH_2OMe)_2$	2,4-Me_2Ph	5.7 ± 2.9 (4)	$C_{22}H_{30}N_4O_2$	87–89
11-8	$NHCH(CH_2OMe)_2$	2,4-Me_2Ph	6.4 ± 2.1 (4)	$C_{21}H_{28}N_4O_2$	103–105
11-9	NHEt	2,4-Me_2Ph	10.6 ± 4.2 (10)	$C_{18}H_{22}N_4$	Oil
11-10	NAcEt	2,4-Me_2Ph	28.9 ± 0.1 (3)	$C_{20}H_{24}N_4O$	Oil
11-11	NEt_2	2-Cl-4-MePh	1.5 ± 1.1 (4)	$C_{19}H_{23}ClN_4$	129–130
11-12	NH-3-pentyl	2-Cl-4-MePh	1.5 ± 1.0 (4)	$C_{20}H_{25}ClN_4$	139–141
11-13	N(c-Pr)CH_2CH_2CN	2-Cl-4-MePh	3.0 ± 1.1 (3)	$C_{21}H_{22}ClN_5$	162
11-14	NHCH(Et)CH_2OMe	2-Cl-4-MePh	3.2 ± 1.2 (3)	$C_{20}H_{25}ClN_4O$	112–113
11-15	$N(CH_2CH_2OMe)_2$	2-Cl-4-MePh	4.5 ± 2.2 (3)	$C_{21}H_{27}ClN_4O_2$	77–78
11-16	$NHCH(CH_2OMe)_2$	2-Cl-4-MePh	4.6 ± 1.0 (4)	$C_{20}H_{25}ClN_4O_2$	131–133
11-17	NEt_2	2-Me-4-ClPh	1.3 ± 0.4 (3)	$C_{19}H_{23}ClN_4$	113–114
11-18	NH-3-pentyl	2-Me-4-ClPh	1.7 ± 0.9 (5)	$C_{20}H_{25}ClN_4$	94–96
11-19	$N(CH_2CH_2OMe)_2$	2-Me-4-ClPh	2.2 ± 0.9 (4)	$C_{21}H_{27}ClN_4O_2$	Oil
11-20	$NHCH(CH_2OMe)_2$	2-Me-4-ClPh	2.3 ± 0.7 (3)	$C_{20}H_{25}ClN_4O_2$	113–114
11-21	NE*t*Bu	2,4-Cl_2Ph	0.9 ± 0.1 (3)	$C_{20}H_{24}Cl_2N_4$	86–87
11-22	NH-3-pentyl	2,4-Cl_2Ph	1.3 ± 0.4 (3)	$C_{19}H_{22}Cl_2N_4$	175–176
11-23	$NHCH(CH_2OMe)_2$	2,4-Cl_2Ph	2.1 ± 0.5 (3)	$C_{19}H_{22}Cl_2N_4O_2$	107
11-24	NHCH(Et)CH_2OMe	2,4-Cl_2Ph	2.3 ± 1.3 (4)	$C_{19}H_{22}Cl_2N_4O$	110–111
11-25	$N(CH_2CH_2OMe)_2$	2,4-Cl_2Ph	3.0 ± 1.3 (4)	$C_{20}H_{24}Cl_2N_4O_2$	72–74
11-26	NPrCH_2CH_2CN	2,4-Cl_2Ph	3.1 ± 1.4 (3)	$C_{20}H_{21}Cl_2N_5$	83–85
11-27	$N(CH_2CH_2OMe)_2$	4-ClPh	6.8 ± 0.4 (3)	$C_{20}H_{25}ClN_4O_2$	69
11-28	NEt_2	2-Cl-4-MeOPh	1.2 ± 0.7 (4)	$C_{19}H_{23}ClN_4O$	78–80
11-29	NH-3-pentyl	2-Cl-4-MeOPh	1.5 ± 0.6 (3)	$C_{20}H_{25}ClN_4O$	145–147
11-30	NEt_2	2-Me-4-MeOPh	0.8 ± 0.5 (4)	$C_{20}H_{26}N_4O$	97–99
11-31	NH-3-pentyl	2-Me-4-MeOPh	1.0 ± 0.2 (8)	$C_{21}H_{28}N_4O$	104–106
11-32	NPrCH_2CH_2CN	2-Me-4-MeOPh	1.8 ± 0.5 (3)	$C_{22}H_{27}N_5O$	118–119
11-33	N(c-Pr)CH_2CH_2CN	2-Me-4-MeOPh	3.0 ± 0.9 (3)	$C_{22}H_{25}N_5O$	177
11-34	NHCH(Et)CH_2OMe	2-Me-4-MeOPh	3.5 ± 0.3 (4)	$C_{21}H_{28}N_4O_2$	Oil
11-35	$NHCH(CH_2OMe)_2$	2-Me-4-MeOPh	9.9 ± 0.9 (3)	$C_{21}H_{28}N_4O_3$	115–116
11-36	$NHCH(CH_2OMe)_2$	2-Br-4-MeOPh	4.1 ± 0.8 (3)	$C_{20}H_{25}BrN_4O_3$	128–131
11-37	$N(CH_2CH_2OMe)_2$	2-Br-4-MeOPh	5.7 ± 2.0 (3)	$C_{21}H_{27}BrN_4O_3$	74–76
11-38	NH-3-pentyl	2-Br-4,5-$(MeO)_2$Ph	4.9 ± 0.9 (3)	$C_{21}H_{27}BrN_4O_2$	159–161
11-39	$NHCH(CH_2OMe)_2$	2-Br-4,5-$(MeO)_2$Ph	34.6 ± 5.4 (3)	$C_{21}H_{27}BrN_4O_4$	90–93
11-40	$N(CH_2CH_2OMe)_2$	2-Br-4,5-$(MeO)_2$Ph	41.0 ± 12.9 (3)	$C_{22}H_{29}BrN_4O_4$	110
11-41	NEt_2	2-Br-4,5-$(MeO)_2$ph	2.6 ± 0.4 (4)	$C_{20}H_{25}ClN_4O_2$	100–101
11-42	NH-3-pentyl	2-Br-4,5-$(MeO)_2$Ph	4.5 ± 1.3 (8)	$C_{21}H_{27}ClN_4O_2$	169–170
11-43	$N(CH_2CH_2OMe)_2$	2-Br-4,5-$(MeO)_2$Ph	19.7 ± 1.0 (3)	$C_{22}H_{29}ClN_4O_4$	98–100
α-hel-CRF(9-41)	—	—	7.5 ± 1.0 (20)	—	—
1	—	—	1.6 ± 0.9 (11)	—	—

[a]Standard deviations are reported.
[b]Parenthetical values are the number of determinations.
[c]c-Pr = cyclopropyl.

MD). No affinity at 10 μM was detected for adenosine, monoamine (adrenergic (α1, α2, α3, β), dopaminergic, or serotonergic receptors), histaminergic H_1 or H_2, muscarinic, $GABA_a$, $GABA_b$, oxytocin, vasopressin V_1, glycine, CGRP, prostaglandin (LTB_4, LTD4 and thromboxane A_2) or opioid receptors. Similarly, the compound had no affinity at 10 μM for ion channels (glutamate (NMDA or Cl sites), calcium (types L or N) or calcium activated potassium sites) and no monoamine oxidase A or B inhibition was detected at the same concentration. There was weak inhibition at dopamine uptake sites (51% @ 10 μM).

Compound **11-31** had good oral activity in the rat situational anxiety test and the rat elevated plus maze model.[31,34,35] The minimum effective dose for **11-31** was 0.3 mg/kg (po) in the rat situational anxiety test.[34] Maximal efficacy (approximately 57% reduction in latency time in the dark compartment) was observed at this dose and after 10 and 30 mg/kg po. Chlordiazepoxide

Table 2. Rat ex vivo bnding data[a,b]

Example	Mean hCRF$_1$ K_i (nM)	N[c]	1 h	3 h
11-2	1.1	4	57.0 ± 9.2	32.2 ± 6.1
11-3	1.7	5	42.3 ± 23.0	27.3 ± 4.0
11-5	2.0	5	29.1 ± 3.4	18.7 ± 11.5
11-7	5.4	4	16.3 ± 10.9	23.0 ± 5.0
11-18	1.7	9	88.9 ± 7.4	89.9 ± 13.5
11-19	2.2	5	48.3 ± 10.5	49.3 ± 14.7
11-25	3.0	5	57.9 ± 11.4	54.5 ± 12.3
11-28	1.2	5	86.7 ± 7.8	91.3 ± 3.1
11-30	0.8	4	64.2 ± 11.5	52.8 ± 17.9
11-31	1.0	11	82.2 ± 7.9	87.4 ± 4.6
11-42	4.5	5	57.9 ± 12.6	62.5 ± 15.7
1	1.6	10	< 10	< 10

[a]Standard deviations are reported.
[b]See Table 1 for the statistics associated with the mean K_i values.
[c]N = the number of rats employed in the studies.

caused a 72% reduction in latency at 20 mg/kg (po). The literature compound **1** (CP154526-1, 30 mg/kg (po)) was inactive in this test. Compound **11-31** was also effective in the rat elevated plus maze model, the ED50 was 5 mg/kg (po).[31,35] However, **11-31** did not inhibit open-field locomotor activity at 10, 30 and 100 mg/kg (po), while chlordiazepoxide did at 30 mg/kg (po, 50% reduction relative to controls). This compound did not cause any significant motor deficits at 30 mg/kg (po) in the rat rotarod model.[34] These results indicate that **11-31** may be a potent anxiolytic drug, for which there is a good separation between the doses which cause motor side effects and those which lead to behavioral efficacy.

Some peripheral effects of **11-31** were examined in the rat since CRF receptors populate peripheral as well as central tissues. CRF sites are present in rat testes, ovaries and components of the immune system, the GI tract, heart and skeletal muscle tissues.[3] Single dosing of **11-31** (30 mg/kg, po) had no statistically significant effect on gastrointestinal (GI) motility; carbachol (0.3 mg/kg, ip) significantly increased GI motility, while atropine (3.0 mg/kg, po) decreased GI motility in the same experimental protocol. Compound **11-31** (30 mg/kg, po) had no statistically significant effect on acute renal function up to 24 h post-dose in rats. In contrast, the positive control furosemide (30 mg/kg, po) elevated renal output and reduced osmolality and glucose, urea, creatinine, sodium, potassium and chloride levels.[36]

Compound **11-31** did not cause statistically significant cardiovascular or pulmonary effects in anesthetized dogs (n = 4) after intravenous administration of **11-31**. Escalating doses of **11-31** (0.3, 1.0, 3.0 and 5.0 mg/kg) in a mixed solvent vehicle (*N*,*N*-dimethylacetamide: polyethylene glycol:water: 1:7:2) were administered at 30 min intervals. Peak plasma concentrations were 1.55 ± 0.24, 2.60 ± 0.16, 10.85 ± 2.82 and 13.82 ± 4.39 nM for the 0.3, 1.0, 3.0, and 5.0 mg/kg doses, respectively. Mean arterial blood pressure, contractility, heart rate and the lead II ECG wave form, including QT interval were within the ranges for control values. Respiration rate, tidal volume, minute volume, peak expiratory flow as well as arterial pO_2, pCO_2 and pH were unchanged for the duration of the experiment.[36]

Compound **11-31** had a promising dog pharmacokinetic profile (Table 3). In beagle dogs, this compound (5 mg/kg, iv, po) afforded good plasma levels. Intravenous administration using a mixed solvent vehicle (*N*,*N*-dimethylacetamide:ethanol:propylene glycol:water: 1:2:6:1) generated mean $t_{1/2}$, CL and $V_{d,ss}$ values equal to 46.4 ± 7.6 h, 0.49 ± 0.08 L/kg/h and 23.0 ± 4.2 L/kg, respectively, after iv administration. After oral dosing, the mean C_{max}, T_{max}, $t_{1/2}$ and bioavailability values were equal to 1260 ± 290 nM, 0.75 ± 0.25 h, 45.1 ± 10.2 h and 33.1%, respectively.[37]

Conclusion

Analogue **11-31** (DMP904) is a potent hCRF$_1$ antagonist, which has good efficacy in two rat models for anxiety. In the rat situational anxiety test, this compound appears to be a more potent anxiolytic agent after oral administration than chlordiazepoxide and compound **1** (CP154526-1). Based on the currently available data, the peripheral effects in rats and dogs for this compound appear to be minimal. Compound **11-31** has an excellent oral pharmacokinetic profile in dogs. The overall profile of this compound suggests that it may be an anxiolytic drug with a low motor side effect liability.

Experimental

Chemistry

Analytical data were recorded for the compounds described below using the following general procedures (Table 4). Proton NMR spectra were recorded on Varian VXR or Unity 300 FT-NMR instruments (300 MHz); chemical shifts were recorded in ppm (δ) from an internal tetramethysilane standard in deuterochloroform or deuterodimethylsulfoxide as specified below. Coupling constants (J) were recorded in Hertz (Hz). Mass spectra (MS) were recorded on a Finnegan MAT 8230 spectrometer or a Hewlett-Packard 5988A model spectrometer (both using chemi-ionization (CI) with NH_3 as the carrier gas). Gas chromatography–mass spectroscopy was run occasionally using the former instrument. Chemi-ionization high resolution mass

Table 3. Single dose dog pharmacokinetic data for compound **11-31** (5 mg/kg)[a]

Parameter	iv	po
N[b]	4	4
$t_{1/2}$ (h)	46.4 ± 7.6	45.1 ± 10.2
C_{max} (nM)	—	1260 ± 290
T_{max}	—	0.75 (0.5–1.0)[c]
AUCT (nM h)	24,420 ± 3260	8120 ± 270
AUC (nM h)	29,520 ± 4790	9780 ± 310
$V_{d,ss}$ (L/kg)	23.0 ± 4.2	—
CL (L/h/kg)	0.49 ± 0.08	—
% Bioavailability	—	33.1

[a]The mean values are reported. Standard deviations are reported.
[b]N is the number of animals used in the study.
[c]The parenthetical value is the range for the value.

Table 4. Analytical data

Example no.	MS $(M+H)^+$ calcd (found)	Quantitative analysis theory (found)	mp (°C)	Example no.	MS $(M+H)^+$ calcd (found)	Quantitative analysis theory (found)	mp (°C)
11-1	337.2388 (337.2392)		Oil	**11-22**		C 60.48 H 5.89 N 14.85 (C 60.62 H 5.88 N 14.82)	175–176
11-2	323.2233 (323.2236)	C 74.50 H 8.137 N 17.38 (C 74.43 H 7.90 N 17.16)	92–94	**11-23**	(409,411)		107
11-3	353.2333 (353.2341)		Oil	**11-24**		C 58.02 H 5.65 N 14.24 (C 58.11 H 5.52 N 14.26)	110–111
11-4	362.2337 (362.2345)	C 73.10 H 7.538 N 19.37 (C 73.18 H 7.59 N 18.81)	143	**11-25**	423.1355 (423.1337)		72–74
11-5	376.2486 (376.2501)	C 73.57 H 7.78 N 18.65 (C 73.55 H 7.79 N 18.64)	115–117	**11-26**		C 59.71 H 5.26 N 14.85 (C 59.94 H 5.09 N 17.23)	83–85
11-6	360.2182 (360.2188)	C 73.51 H 7.01 N 19.48 (C 73.57 H 7.15 N 19.12)	133 (dec)	**11-27**	389.1720 (389.1744)		69
11-7	383.2427 (383.2447)	C 69.08 H 7.915 N 14.65 (C 68.85 H 7.83 N 14.54)	87–89	**11-28**	358.1547 (358.1560)	C 63.59 H 6.46 N 15.61 (C 63.22 H 6.24 N 15.27)	78–80
11-8	369.2291 (369.2291)	C 68.45 H 7.669 N 15.21 (C 68.35 H 7.49 N 14.91)	103–105	**11-29**	372.1701 (372.1717)	C 64.42 H 6.77 N 15.02 (C 64.08 H 6.63 N 14.96)	145–147
11-9	295.1919 (295.1923)		Oil	**11-30**	339.2182 (339.2185)	C 70.98 H 7.74 N 16.55 (C 71.15 H 7.46 N 16.56)	97–99
11-10	337.2017 (337.2028)		Oil	**11-31**	353.2334 (353.2341)	C 71.56 H 8.017 N 15.90 (C 71.48 H 7.99 N 15.88)	108
11-11	343.1685 (343.1690)	C 66.59 H 6.76 N 16.34 (C 66.69 H 6.82 N 16.20)	129–130	**11-32**	378.2274 (378.2294)	C 70.00 H 7.219 N 18.55 (C 70.05 H 7.22 N 18.36)	118–119
11-12	357.1846 (357.1846)	C 67.31 H 7.06 N 15.70 Cl 9.93 (C 67.32 H 6.95 N 15.50 Cl 9.93)	139–141	**11-33**	376.2125 (376.2137)	C 70.38 H 6.71 N 18.65 (C 70.35 H 6.82 N 18.83)	177
11-13	380.1634 (380.1642)	C 66.39 H 5.847 N 18.44 Cl 9.33 (C 66.29 H 5.51 N 18.36 Cl 9.31)	162	**11-34**	369.2278 (369.2291)		Oil
11-14	373.1800 (373.1795)	C 64.42 H 6.767 N 15.02 (C 64.59 H 6.51 N 14.81)	112–113	**11-35**	385.2243 (385.2240)	C 65.60 H 7.34 N 1457 (C 65.42 H 7.24 N 14.37)	115–116
11-15	403.1899 (403.1901)		77–78	**11-36**	448.1092 (448.1110)	C 53.46 H 5.62 N 12.47 Br 17.78 (C 53.91 H 5.81 N 12.12 Br 18.00)	128–131
11-16	389.1742 (389.1744)	C 61.77 H 6.49 N 14.41 Cl 9.13 (C 61.90 H 6.66 N 13.62 Cl 9.25)	131–133	**11-37**	462.1238 (462.1267)	C 54.43 H 5.87 N 12.09 (C 54.52 H 5.86 N 11.91)	74–76
11-17	342.1597 (342.1611)	C 66.56 H 6.76 N 16.34 (C 66.70 H 6.73 N 16.13)	113–114	**11-38**	447.1396 (447.1396)	C 56.38 H 6.08 N 12.52 Br 17.86 (C 56.42 H 5.77 N 12.46 Br 18.15)	159–161
11-18	357.1848 (357.1846)	C 67.31 H 7.06 N 15.70 (C 67.12 H 6.86 N 15.53)	94–96	**11-39**	479.1289 (479.1294)		90–93
11-19	403.1887 (403.1901)		Oil	**11-40**	493.1441 (493.1450)	C 53.55 H 5.92 N 11.36 Br 16.20 (C 53.64 H 5.92 N 11.29 Br 16.33)	110
11-20	389.1733 (389.1744)	C 61.77 H 6.489 N 14.41 Cl 9.126 (C 62.06 H 6.37 N 14.25 Cl 9.12)	113–114	**11-41**	388.1649 (388.1666)	C 61.77 H 6.49 N 14.41 (C 62.13 H 6.42 N 14.11)	100–101
11-21		C 61.38 H 6.18 N 14.32 (C 61.54 H 6.12 N 14.37)	86–87	**11-42**	403.1834 (403.1902)	C 62.30 H 6.75 N 13.91 (C 62.30 H 6.66 N 13.61)	169–170
				11-43	449.1938 (449.1956)		98–100

spectra (CI-HRMS) were obtained on a VG 7-VSE instrument with NH_3 as the carrier gas. Combustion analyses were performed by Quantitative Technologies Inc., Whitehouse, NJ. Melting points were measured on a Buchi Model 510 melting point apparatus or a Thomas Hoover capillary apparatus and are uncorrected. Reagents were purchased from commercial sources and, when necessary, purified prior to use according to the general procedures outlined by D. Perrin and W. L. F. Armarego.[38] Chromatography was performed on silica gel using the solvent systems indicated below. For mixed solvent systems, the volume ratios are given. Otherwise, parts and percentages are by weight. All reactions were performed under a nitrogen atmosphere using magnetic stirring. Reactions requiring anhydrous conditions were performed in glassware, which had been flame-dried or oven-dried with purging under a nitrogen atmosphere. Reactions using aqueous media were run under the ambient atmosphere. Anhydrous magnesium sulfate ($MgSO_4$) was used routinely to dry the combined organic layers from extractions. Solvent was routinely removed in vacuo, using a rotary evaporator, followed by evacuation with vacuum pump.

Commonly used abbreviations are: EtOAc (ethyl acetate), MeOH (methanol), EtOH (ethanol), DMF (*N*,*N*-dimethylformamide), HOAc (acetic acid), THF (tetrahydrofuran), and TLC (thin-layer chromatography).

2-Methyl-4-methoxyphenylacetonitrile. A mixture of 1-bromomethyl-2-methyl-4-methoxybenzene[39] (35 g, 163 mmol) and sodium cyanide (39.9 g, 813 mmol) in DMF (800 mL) was stirred at reflux temperature for 23 h. Solvent was removed in vacuo and the residue was treated with a 1 N NaOH solution (500 mL). The aqueous mix was extracted three times with CH_2Cl_2. The combined organic layers were washed twice with brine, dried and filtered. Solvent was removed in vacuo. The residue was purified by column chromatography (EtOAc:hexanes: 1:9) to give a solid, which was used without further purification: ^1H NMR ($CDCl_3$) δ 7.25 (br d, 1H, $J=8$, C_6-H), 6.80–6.70 (m, 2H, C_3-H and C_5-H), 3.80 (s, 3H, OCH_3), 3.60 (s, 2H, CH_2CN); MS (GC) m/z 162 $(M+H)^+$.

1-Cyano-1-(2-methyl-4-methoxyphenyl)propane-2-one. To a solution of 2-methyl-4-methoxyphenylacetonitrile

(7.0 g, 43.4 mmol) in ethyl acetate (150 mL) were added sodium pellets (1.2 g, 52.1 mmol) portionwise at room temperature. The reaction mixture was heated to reflux for 16 h. Upon cooling to room temperature, the resulting suspension was filtered and washed with copious amounts of ether. The collected white solid was dissolved in water. The water solution was acidified by adding acetic acid until pH 5–6. The mixture was extracted with ethyl acetate; the combined organic layers were dried, filtered and concentrated to dryness to provide a white solid (5.3 g, 60% yield): ^{1}H NMR ($CDCl_3$) δ 7.30 (dd, 1H, J=8,1, C_6-H), 6.85–6.75 (m, 2H, C_3-H and C_5-H), 4.75 (s, 1H, CHCN), 3.80 (s, 3H, OCH_3), 2.35, 2.30, 2.25, 2.20 (4s, 6H, CH_3 and $COCH_3$); MS (CI) m/z 204 $(M+H)^+$.

5-Amino-4-(2-methyl-4-methoxyphenyl)-3-methylpyrazole. A mixture of 1-cyano-1-(2-methyl-4-methoxyphenyl)-propane-2-one (5.3 g, 26.1 mmol), hydrazine monohydrate (2.6 mL, 52.2 mmol), acetic acid (5.1 mL, 88.7 mmol) and toluene (125 mL) was heated at reflux using a Dean–Stark trap for 5 h. The reaction mixture was cooled to room temperature and concentrated. The residue was dissolved in 6 N HCl and the resulting solution was extracted with ether:hexanes (1:1) three times (25 mL). The aqueous layer was basified by addition of concentrated ammonium hydroxide solution until pH 11. The resulting mixture was extracted with ethyl acetate three times. The combined organic layers were dried and concentrated in vacuo to give a pale brown viscous oil (3.6 g, 66% yield). ^{1}H NMR ($CDCl_3$) δ 7.08 (d, 1H, J=8, C_6-H), 6.75–6.85 (m, 2H C_3-H and C_5-H), 3.83 (s, 3H), 2.19 (s, 3H); MS(CI) 218 $(M+H)^+$.

2,5-Dimethyl-3-(2-methyl-4-methoxyphenyl)-pyrazolo[1,5-α]pyrimidin-7-one. 5-Amino-4-(2-methyl-4-methoxyphenyl)-3-methylpyrazole (5.3 g, 24.4 mmol) was dissolved in glacial acetic acid (30 mL) with stirring. Ethyl acetoacetate (3.4 mL, 26.8 mmol) was then added dropwise to the resulting solution. The reaction mixture was then heated to reflux temperature and stirred for 16 h, then cooled to room temperature. Ether (100 mL) was added and the resulting precipitate was collected by filtration. Drying in vacuo afforded a white solid (4.7 g, 68% yield): mp >200 °C; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 9.69 (s, 1H, NH), 7.08 (d, 1H, J=8, phenyl C_6-H), 6.72–6.67 (m, 2H, phenyl C_3-H and C_5-H), 5.49 (s, 1H, C_6-H), 3.77 (s, 3H, OCH_3), 2.37 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 2.10 (s, 3H, CH_3); CI-MS: 284 $(M+H)^+$.

7-Chloro-2,5-dimethyl-3-(2-methyl-4-methoxyphenyl)-pyrazolo[1,5-α]pyrimidine. A mixture of 2,5-dimethyl-3-(2-methyl-4-methoxyphenyl)-pyrazolo[1,5-*a*]pyrimidin-7-one (3.0 g, 10.6 mmol), phosphorus oxychloride (8.2 g, 5.0 mL, 52.9 mmol), *N*,*N*-diethylaniline (1.9 g, 2.0 mL, 12.7 mmol) and toluene (20 mL) was stirred at reflux temperature for 3 h, then it was cooled to ambient temperature. The volatiles were removed in vacuo. Flash chromatography (EtOAc:hexane: 1:2) on the residue gave 7-chloro-5-methyl-3-(2-methyl-4-methoxyphenyl)-pyrazolo[1,5-*a*]pyrimidine (2.87 g, 90% yield) as a yellow oil: ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.17 (d, 1H, J=8, phenyl C_6-H), 6.88 (d, 1H, J=3, phenyl C_3-H), 6.81 (dd, 1H, J=8,3, phenyl C_5-H), 6.78 (s, 1H, C_6-H), 3.84 (s, 3H, OCH_3), 2.54 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 2.17 (s, 3H, CH_3); CI-MS: 302 $(M+H)^+$.

7-(Pentyl-3-amino)-2,5-dimethyl-3-(2-methyl-4-methoxyphenyl)pyrazolo[1,5-*a*]pyrimidine (11-31). A solution of 3-pentylamine (12.7 g, 146 mmol) and 7-chloro-5-methyl-3-(2-methyl-4-methoxyphenyl)pyrazolo[1,5-*a*]pyrimidine (8.8 g, 29.2 mmol) was stirred at reflux temperature for 16 h; then it was cooled to ambient temperature. The reaction mixture was then poured onto water (100 mL) and mixed. Three extractions with EtOAc, washing the combined organic layers with brine, drying, filtration and removal of solvent in vacuo produced a yellow solid. Recrystallization from hexane afforded a white solid (8.8 g, 45% yield): mp 108 °C; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 6.86 (d, 1H, J=3 Hz, phenyl C_3-H), 6.77–6.81 (dd, 1H, J=9, 3 Hz, phenyl C_5-H), 5.99 (bd, 1H, J=9 Hz, phenyl C_6-H), 5.77 (s, 1H, C_6-H), 3.83 (s, 3H, OCH_3), 3.40–3.48 (m, 1H, NHC*H*), 2.44 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.21 (s, 3H, CH_3), 1.58–1.82 (m, 4H, CH_2CH_3), 1.02 (t, 6H, J=7, CH_2CH_3). Anal. calcd for $C_{21}H_{28}N_4O$: C 71.56, H, 8.02, N 15.90, Found: C, 71.48, H, 7.99, N 15.88.

Biology

The cloned human receptor binding assay was performed as described previously.[24] The rat and human CRF-coupled adenylate cyclase assays have been reported elsewhere.[24,40]

The rat ex vivo assay is a variation of the cloned human receptor binding assay. Test compounds (10 mg/kg) were orally administered in a 0.5% methocel suspension. Plasma samples were drawn from Sprague–Dawley rats at 1 and 3 h post-dose. Plasma samples were then diluted 10-fold with assay medium and aliquots of these diluted samples were analyzed in the standard binding assay.

The rat situational anxiety, elevated plus maze and rotarod tests were performed as described previously.[41–43] Pharmacokinetic parameters were determined in dogs after intravenous (iv) or oral (po) doses of 5 mg/kg each. Dogs (n=4) were given the compound intravenously in a cosolvent vehicle (*N*,*N*-dimethylacetamide:ethanol: propylene glycol:water: 1:2:6:1), or orally in 0.25% methylcellulose suspension (with 0.1% Tween 80 in some cases). Blood samples were collected from jugular veins at pre-dose, 5, 15, 30 min and 1, 2, 4, 8, 10, 12, 16, 24, 32, 48, 56, and 72 h after dosing.

Plasma samples were analyzed after extraction of test compounds by simple liquid–liquid extraction. LC/MS/MS analysis was performed on a Sciex (Thornhill, Ontario) Model APIII triple quadrapole mass spectrometer interfaced with a turbo Ion Spray ionization source. The liquid chromatography consisted of a Perkin–Elmer series 200 solvent delivery system (Norwalk, CT), a Perkin–Elmer ISS 200 autoinjector and a Waters Symmetry octyl mini-bore column (2.1×50 mm).

Cardiovascular pharmacodynamic testing was performed in anesthetized dogs. Mongrel dogs ($n=8$) of both sexes (weights = 7.0 to 12.3 kg, HRP Inc., Cumberland, VA) were anesthetized with sodium pentobarbital (30 mg/kg, iv) and mechanically ventilated (15 breaths/min) using a Harvard respirator (Harvard Apparatus, South Natick, MA). The cephalic veins were cannulated for compound administration and infusion of sodium pentobarbital at 6 mg/kg/h. A micromanometer tip catheter (Millar Instruments, Houston, TX) was placed in the left ventricle to measure left ventricular pressure. The first derivative of the left ventricular pressure signal, $LV+dP/dt$, was obtained by the modular Instruments software. Arterial blood pressure was monitored from a cannulated brachial artery with a Statham pressure transducer (Spectamed-Viggio, Oxnard, CA). Blood pressure, left ventricular pressure, left ventricular dP/dt, and a lead II ECG were recorded on a Modular Instruments polygraph. Compound **11-31** was administered iv at 0.3, 1.0, 3.0 and 5.0 mg/kg at intervals of 30 min. The vehicle was composed of *N*,*N*-dimethylacetamide, propylene glycol, ethanol and water (1:6:1:2) and the infusion rate was 0.033 mL/kg/min. over 6 min for the 0.3 and 1.0 mg/kg doses, 0.06 mL/kg/min over 10 min for the 3 mg/kg dose and 0.0625 mL/kg/min for 16 min for the 5.0 mg/kg dose.

Respiratory function was monitored in anesthetized dogs. Mongrel dogs were treated with sodium pentobarbital as described above, but were allowed to breathe spontaneously. Test compound was administered as described above for the cardiovascular test. The femoral artery and vein were cannulated to collect blood samples for determination of blood gases, pH and compound plasma levels. Arterial blood pressure was measured as described previously. A balloon was positioned in the lower third of the esophagus to record pleural pressure. The endotracheal tube was attached to a Hans Rudolph pneumotachograph (3500 Series, Kansas City, MO) connected to a Validyne differential pressure transducer (DP45-14) for the measurement of air flow. The esophageal balloon was similarly attached to one side of a second differential pressure transducer (Validyne, DP45-24), the other side of which was connected to a side arm inserted in the tracheal cannula adjacent to the pneumotachograph to measure intrapleural pressure. The vehicle was composed of *N*,*N*-dimethylacetamide, polyethylene glycol 400 (PEG400), ethanol and water (1:6:1:2) and the infusion rate was 0.033 mL/kg/min over 6 min for 0.3 and 1.0 mg/kg dose. Arterial blood samples were taken at −10, 0, 6, 15, 30, 60 and 90 min to measure arterial pH, pO_2 and pCO_2 using a Ciba–Corning 278 pH/blood gas analyzer (Ciba–Corning Diagnostics Corp., Medfield, MA).

Renal function was assessed in conscious male Sprague–Dawley rats (Caesarian-derived, 199–224 g). The animals were volume-loaded with isotonic saline containing compound **11-31** (10 mg/kg, po) or furosemide (30 mg/kg, po) in methylcellulose at a total volume of 25 mL/kg. The initial urine samples were collected during the first 5 h period postdose, then between 5 and 24 h. Total volume and osmolality (Fiske 2400 Osmometer, Fiske Associates, Norwood, MA) were measured and glucose, urea, creatinine, sodium, potassium and chloride concentrations were determined (Dimension Clinical analyzer, DuPont, Wilmington, DE).

Gastrointestinal motility was evaluated by gross observation and transit of an orally administered charcoal suspension (10% w/v charcoal in 0.25% methylcellulose) in fasted male Sprague–Dawley rats (178–222 g, Caesarian-derived). Compound **11-31** (30 mg/kg, po), atropine (3.0 mg/kg, po) and carbachol (0.3 mg/kg, ip) were administered in the standard vehicles. The charcoal suspension (5 mL/kg) was administered orally 60 min after the dose of **11-31** and 30 min after the doses of carbachol or atropine. Five minutes after the charcoal meal, the rats were euthanized with CO_2 and the gastrointestinal tract (stomach to sigmoid colon) was removed. The distance from the pyloric sphincter to the charcoal at its most distal location in the small intestine was measured and compared to the pyloric sphincter to cecum measurement. The results were expressed as a percentage relative to methocel controls.

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References

1. Rivier, J.; Spiess, J.; Vale, W. *Proc. Natl. Acad. Sci. USA* **1983**, *80*, 4851.
2. Vale, W.; Spiess, J.; Rivier, C.; Rivier, J. *Science* **1981**, *213*, 1394.
3. Gilligan, P. J., Hartig, P. R., Robertson, D. W., Zaczek, R. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic: San Diego, 1997; Vol. 32, pp 41–50.
4. De Souza, E. B.; Insel, T. R.; Perrin, M. H.; Rivier, J.; Vale, W. W.; Kuhar, M. J. *J. Neurosci.* **1985**, *5*, 3189.
5. Koob, G. F., Heinrichs, S. C., Pich, E. M., Menzaghi, F., Baldwin, H., Miczek, K., Britton, K. T. In *Corticotropin Releasing-factor, Ciba Foundation Symposium 172*; Chadwick, D. J.; Marsh, J.; Ackrill, K., Eds.; J. Wiley and Sons: Chicester, 1993: pp 277–291.
6. Webster, E. L.; Torpy, D. J.; Elenkov, I. J.; Chrousos, G. P. *Ann. N.Y. Acad. Sci.* **1998**, *840*, 21.
7. Chrousos, G. P. *New Engl. J. Med.* **1995**, *332*, 1351.
8. DeSouza, E. B., Grigoriadis, D. E. In *Psychopharmacology; The Fourth Generation of Progress*; Bloom, F. E.; Kupfer, D. J., Eds.; Raven: New York, 1995; pp 505–517.
9. Sawchenko, P. E., Swanson, L. W. In *Corticotropin-releasing Factor: Basic and Clinical Studies of a Neuropeptide*; Desouza, E. B.; Nemeroff, C. B., Eds.; CRC, Boca Raton, 1990; pp 29–51.
10. Owens, M. J., Nemeroff, C. B. In *Corticotropin-releasing Factor, Ciba Foundation Symposium 172*; Chadwick, D. J, Marsh, J.; Ackrill, K., Eds.; J. Wiley and Sons: Chichester, 1993; pp 296–316.
11. Banki, C. M.; Bisette, G.; Arato, M.; O'Connor, L.; Nemeroff, C. B. *Am. J. Psychiatry* **1987**, *144*, 873.

12. France, R. D.; Urban, B.; Krishnan, R. R.; Bissette, G.; Banki, C. M.; Nemeroff, C. B.; Spielman, F. J. *Biol. Psychiatry* **1988**, *23*, 86.
13. Arato, M.; Banki, C. M.; Bissette, G.; Nemeroff, C. B. *Biol. Psychiatry* **1989**, *25*, 355.
14. Raadsheer, F. C.; Hoogendijk, W. J. G.; Stam, F. C.; Tilders, F. J. H.; Swaab, D. F. *Neuroendocrinology* **1994**, *60*, 436.
15. Gold, P. W.; Chrousos, G.; Kellner, C.; Post, R.; Roy, A.; Augerinos, P.; Schultz, H.; Oldfield, E.; Loriaux, D. L. *Am J. Psychiatry* **1984**, *141*, 619.
16. Holsboer, F.; Muller, O. A.; Doerr, H. G. *Psychoneuroendocrinology* **1984**, *9*, 147.
17. Gold, P. W.; Loriaux, D. L.; Roy, A.; Kling, M. A.; Calkabrese, J. R.; Kellner, C. H.; Nieman, L. K.; Post, R. M.; Pickar, D.; Gallucci, W.; Augerinos, P.; Paul, S.; Oldfield, E. H.; Cutler, G. B.; Chrousos, G. P. *New Eng. J. Med* **1986**, *314*, 1329.
18. Sapolsky, R. M. *Arch. Gen. Psychiatry* **1989**, *46*, 1047.
19. Kostich, W. A.; Chen, A.; Sperle, K.; Largent, B. L. *Mol. Endocrinology* **1998**, *12*, 1077.
20. Chen, Y. L.; Mansbach, R. S.; Winter, S. M.; Brooks, E.; Collins, J.; Corman, M. L.; Dunaiskis, A. R.; Faraci, W. S.; Gallaschun, R. J.; Schmidt, A.; Schulz, D. W. *J. Med. Chem* **1997**, *40*, 1749.
21. 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.; Winston, E. N.; Chen, Y. L.; Heym, J. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 10477.
22. Mansbach, R. S.; Brooks, E. N.; Chen, Y. L. *Eur. J. Pharmacol* **1997**, *322*, 21.
23. Griebel, G.; Perrault, G.; Sanger, D. J. *Psychopharmacology* **1998**, *138*, 55.
24. Arvanitis, A. G.; Gilligan, P. J.; Chorvat, R. J.; Cheeseman, R. S.; Christos, T. E.; Bakthavatchalam, R.; Beck, J. P.; Cocuzza, A. J.; Hobbs, F. W.; Wilde, R. G.; Arnold, C.; Chidester, D.; Curry, M.; He, L.; Hollis, A.; Klaczkiewicz, J.; Krenitsky, P. J.; Rescinito, J. P.; Scholfield, E.; Culp, S.; De Souza, E. B.; Fitzgerald, L.; Grigoriadis, D.; Tam, S. W.; Wong, Y. N.; Huang, S.-M.; Shen, H. *J. Med. Chem.* **1999**, *42*, 808.
25. Hodge, C. N.; Aldrich, P. E.; Wasserman, Z. R.; Fernandez, C. H.; Arvanitis, A.; Chorvat, R. J.; Cheeseman, G.; Christos, T. E.; Schofield, P.; Krenitsky, P.; Gilligan, P. J.; Ciganek, E.; Strucely, P. *J. Med. Chem.* **1999**, *42*, 819.
26. Chorvat, R. J.; Bakthavatchalam, R.; Beck, J. P.; Gilligan, P. J.; Wilde, R. G.; Cocuzza, A.; Hobbs, F. W.; Cheeseman, R. S.; Curry, M.; Rescinito, J. P.; Krenitsky, P.; Chidester, D.; Yarem, J.; Klackewicz, J. D.; Hodge, C. N.; Aldrich, P. E.; Wasserman, Z. R.; Fernandez, C. H.; Zaczek, R.; Fitzgerald, L.; Huang, S.-M.; Shen, H. L.; Wong, Y. N.; Chien, B. M.; Quon, C. Y.; Arvanitis, A. *J. Med. Chem.* **1999**, *42*, 833.
27. Courtmanche, G.; Gautier, C.; Gully, D.; Roger, P.; Valette, G.; Wermuth, C.-G. US Patent 5464847, 1995.
28. Eyermann, J., personal communication.
29. Chen, C.; Webb, T. R.; McCarthy, J. R.; Moran, T. J.; Wilcoxen, K. M. International Publication Number WO9729109, 1997.
30. Wustrow, D. J.; Capiris, T.; Rubin, R.; Knobelsdorf, J. A.; Akunne, H.; Davis, M. D.; Mackenzie, R.; Pugsley, T. A.; Zoski, K. T.; Heffner, T. G.; Wise, L. D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2067.
31. This work was previously presented in part: Gilligan, P. J.; Baldauf, C. A.; Cocuzza, A. J.; Chidester, D. R.; Fitzgerald, L. W.; Zaczek, R.; Shen, H.-S. L. *American Chemical Society National Meeting*, Boston, MA, 1998 (Abstract MEDI 135).
32. Berridge, C. W.; Dunn, A. J. *Brain Res. Rev* **1990**, *15*, 71.
33. Fitzgerald, L. W.; Smith, M. A.; Baldauf, C.; He, L.; Gilligan, P.; Hartig, P.; Zaczek, R., personal communication.
34. McElroy, J., personal communication.
35. Smith, M. A., personal communication.
36. Saye, J. A., personal communication.
37. Shen, H. L., personal communication.
38. Perrin, D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*; 3rd ed.; Pergamon: New York, 1988.
39. Ghera, E.; Plemenitas, A.; Ben-David, Y. *Synthesis* **1984**, 504.
40. Battaglia, G.; Webster, E. L.; De Souza, E. B. *Synapse* **1987**, *1*, 572.
41. Takahashi, L. K.; Kalin, N. H.; Vanden Burgt, J. A.; Sherman, J. E. *Behav. Neurosci.* **1989**, *103*, 648.
42. File, S. E.; Johnston, A. L.; Baldwin, H. A. *Stress Med.* **1988**, *4*, 221.
43. File, S. E. *Neuropharmacology* **1987**, *26*, 877.