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Review Article

Corticotropin-releasing factor$_1$ receptor as a target for therapeutic intervention

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CONTENTS

Introduction

Corticotropin-releasing factor (CRF), a 41 amino acid peptide which regulates the release of adrenocorticotropin (ACTH) from the anterior pituitary (1), has been shown to mediate stress-induced changes in the autonomic nervous system, neuroendocrine function and behavior (1-3). Receptors for CRF are distributed throughout the central and peripheral nervous systems (3). Intracerebroventricular (i.c.v.) administration of CRF to laboratory animals has behavioral effects similar to those observed in both anxiety and depression, such as altered locomotor activity (4, 5), increased anxiety in an elevated plus-maze (6), cocaine withdrawal-induced anxiety (7), social defeat-induced anxiety (8), diminished food intake (9), decreased sexual behavior (10) and sleep disruption (11).

Clinical findings have indicated that patients with depression or posttraumatic stress disorder have significantly elevated concentrations of CRF in cerebrospinal fluid as compared with normal controls (12, 13). In addition, patients with depression, anxiety, anorexia nervosa or posttraumatic stress disorder exhibited blunted ACTH responses to i.v. CRF (14, 15), suggesting that their CRF receptor level may be downregulated, possibly due to chronic hypersecretion of CRF (16).

In situ hybridization studies indicate that at least two CRF receptors subtypes, CRF$_1$ and CRF$_{2\alpha}$, are expressed in the mammalian brain (17-19). The heterogeneous anatomical distribution patterns of CRF$_1$ and CRF$_{2\alpha}$ mRNA expression suggest distinct functional roles for each receptor in CRF-related central nervous system circuits (17, 20). While CRF$_1$ receptor expression is abundant in the neocortical, cerebellar and sensory-related structures, CRF$_{2\alpha}$ receptor expression is generally localized to specific subcortical structures, including the lateral septum and various hypothalamic nuclei (17). In addition, CRF$_{2\beta}$ has been reported to be absent in the rat brain but abundant in rat heart and skeletal muscle (21).

The level of CRF$_1$ mRNA in the hypothalamic paraventricular nucleus (PVN) is increased under various kinds of stress such as i.p. hypersonic saline injection (22), immune challenge (23) and immobilization (24), and is decreased with glucocorticoid treatment or adrenalectomy (24). The amount of stress-induced increase in CRF$_1$ mRNA level in the PVN corresponded to the increase in CRF binding (22). CRF$_{2\alpha}$ mRNA was also relatively highly expressed in the PVN even under non-stressful conditions (17). However, CRF$_{2\alpha}$ mRNA levels in the PVN were not altered by corticosterone administration, adrenalectomy or lipopolysaccharide injection (25). CRF$_1$ and CRF$_2$ receptor knockdown was achieved within brain regions related to behavioral reactivity to stressors by chronic, central administration of antisense oligonucleotides and confirmed autoradiographically (26). CRF$_1$, but not CRF$_2$, knockdown produced a significant anxiolytic-like effect in defensive withdrawal compared with that in a vehicle-treated and two missense oligonucleotide-negative control groups. In contrast, antisense treatment neither altered endocrine nor behavioral reactivity to a swim stressor (26). These findings provide evidence that CRF$_1$ may be involved in autoregulation of CRF secretion, especially in stressful situations.

α-Helical CRF$_{(9\text{-}41)}$, a peptide CRF receptor antagonist which was initially reported (16), has been extensively studied *in vivo* in exploration of the physiological roles of endogenous CRF systems in mediating various stress-induced hormonal and behavioral effects (16, 27-29). Furthermore, several peptide CRF receptor antagonists or agonists have been designed from CRF and/or α-helical CRF$_{(9\text{-}41)}$ (30-38). Intravenous administration of astressin (cyclo(30-33)[D-Phe^{12},Nle^{21,38},Glu^{30}]-r/hCRF$_{(12\text{-}41)}$), a peptide CRF$_1$ receptor antagonist,

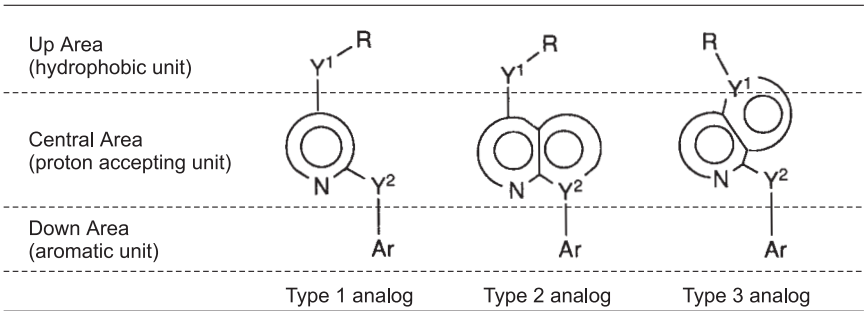


Fig. 1.

reduced secretion of ACTH both in adrenalectomized rats and that induced by electroshock (34). Notably, however, use of peptide antagonists in clinical treatment might be limited, since good enteral absorption and/or penetration into brain of such agents is not expected.

On the other hand, since thiazole derivative **1** and pyrazole derivative **2** were reported as nonpeptide CRF antagonists in patent applications (39, 40), various nonpeptide CRF$_1$ receptor antagonists (**3-28**) have been discovered and reported with biological characterizations and/or structure-activity relationships (41-82). These reports suggest that CRF$_1$ receptor antagonists may be useful for the treatment of depression, anxiety and/or stress-related diseases. The CRF$_1$ receptor antagonist might thus be an interesting target for therapeutic intervention.

Nonpeptide CRF$_1$ antagonists

Since the thiazole (39) and pyrrole (40) derivatives represented by compounds **1** and **2** were previously reported in a patent application as nonpeptide CRF antagonists with no suitable biological data, various nonpeptide antagonists have been studied and reported (41-82). These CRF$_1$ receptor antagonists consist of 3 units: a hydrophobic unit (Up Area), a proton accepting unit (Central Area) and an aromatic unit (Down Area) (Fig. 1). Furthermore, nonpeptide CRF$_1$ antagonists can be classified into 3 types: aromatic hetero-monocyclic analogs (type 1); aromatic hetero-bicyclic analogs containing Y^2 in the ring (type 2) and aromatic hetero-bicyclic analogs containing Y^1 in the ring (type 3) (Fig. 1). Typical compounds of these 3 types are shown in Schemes 1-3. Binding data for these typical antagonists are shown in Table I.

Structure-activity relationships

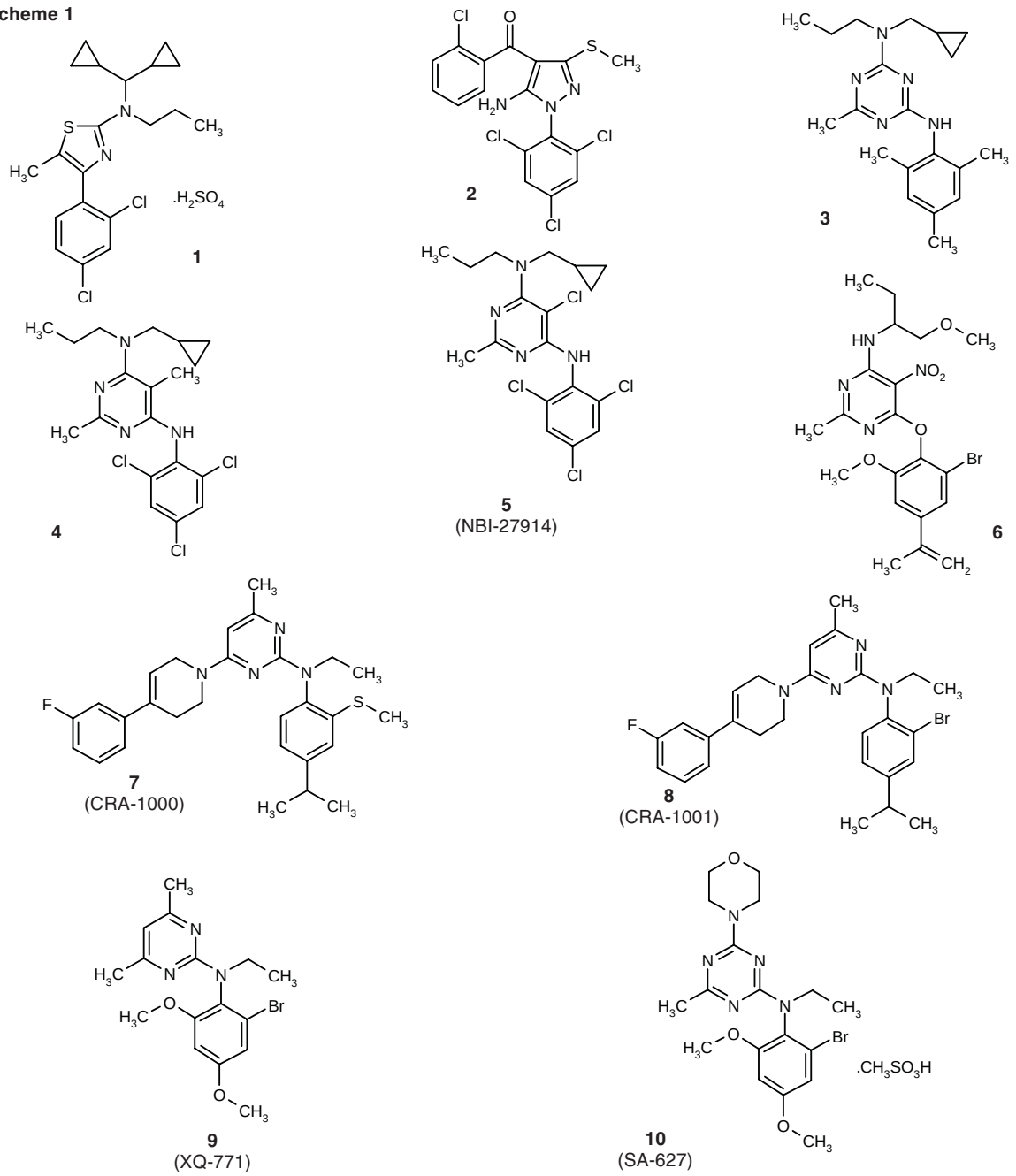
In the Down Area, aryl moieties attached with 2- and/or 6- and 4-positional substituents are preferred for high affinity for CRF$_1$ receptors. The 2- and/or 6-positional substituents yield an orthogonal or orthogonal-like conformation between moieties in the Central and Down

Table I: Nonpeptide CRF$_1$ receptor antagonists.

Compound	Binding affinity for CFR$_1$ receptors K_i (nM)	Cells or tissue[b]	Refs.
3	57	Human	41
4	2.3	Human	
5 (NBI-27914)			42-44
6	0.9	h (HEK293)	45
7 (CRA-1000)	20.6[a]	r (frontal cortex)	46-50
	15.7[a]	r (pituitary)	47, 48
8 (CRA-1001)	22.3[a]	r (frontal cortex)	46-48
	18.6[a]	r (pituitary)	47, 48
9 (XQ-771)	12	h (HEK293)	51
	5	r (frontal cortex)	64
10 (SA-627)	32	h (HEK293)	51
11 (CP-154,526)	2.7	h (IMR32)	52-62
	5.7	r (frontal cortex)	52, 59
	1.4	r (pituitary)	59
12 (antalarmin)	1.4	r (frontal cortex)	63
	1.9	r (pituitary)	63
13	1	R (frontal cortex)	64
14 (SC-241)	3.7	h (HEK293)	66
15	5.7	h (HEK293)	67
16	4.1	h (HEK293)	68
17 (PD-171729)	5	h (CHO)	69-71
18 (NBI-30545)	2.8	Human	72
19 (SP-904)	1.0	h (HEK293)	73
	0.6	r (HEK293)	73
20 (DMP-696	1.6	h (HEK293)	74, 75
	2.1	r (HEK293)	74
21 (NBI-31200)	0.5	h (LtK)	76
22	4	h (HEK293)	77
23	6	h (HEK293)	78
24 (SJ-948)	14.2	h (HEK293)	79
25	0.5	h (HEK293)	80
26	0.6	h (HEK293)	81
27	4.1	h (HEK293)	81
28	2.8	h (HEK293)	82

[a]IC_{50} value. [b]h (HEK293): human CRF$_1$ receptors expressed in HEK293 cells; r (frontal cortex): rat frontal cortex homogenate; r (pituitary): rat pituitary homogenate; h (IMR32): human CRF$_1$ receptors expressed in IMR32 neuroblastoma cell membranes; h(CHO): human CRF$_1$ receptors expressed in CHO cells; h(LtK): human CRF$_1$ receptors expressed in LtK-mouse fibroblast cells; r (HEK293): rat CRF$_1$ receptors expressed in HEK293 cells.

Scheme 1



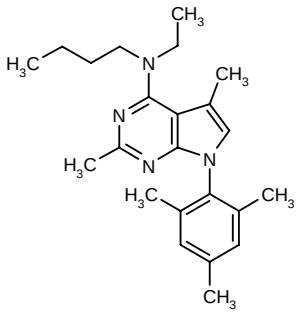
Areas (42, 51, 64). R and NO_2 groups in the Central Area of compounds **4** and **6** and an ethyl group on the nitrogen atom of compounds **7-10** are important for positioning aryl moieties of the Down Area. Aromatic and basic nitrogen in the Central Area is required for hydrogen bonding between CRF_1 receptor and ligand and/or to maintain the orthogonal or orthogonal-like conformation between moieties in the Central and Down Areas.

Theoretical and spectroscopic studies of the conformational relationships between moieties in the Down and Central Areas have yielded type 2 analog **13** and **14** as typical compounds (64, 66). The hetero-bicyclic structures of the Central Area of type 2 and 3 analog are useful for positioning the aryl moieties of the Down Area to obtain high affinity for CRF_1 receptors.

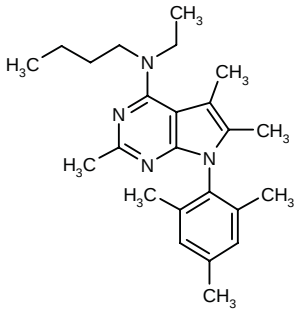
In any type of analog, the 4-positional substituents of the phenyl group in the Down Area, a middle size alkyl group or halogen atom (Br or Cl) is required for interaction with the hydrophobic pocket of CRF_1 receptors.

N,N-Dialkylaminobutylamino groups containing alkoxyalkyl groups are typical moieties in the Up Area.

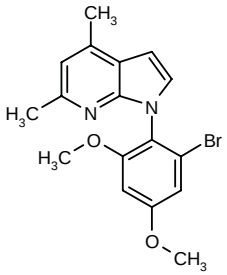
Scheme 2



11 (CP-154,526)



12 (antalarmin)



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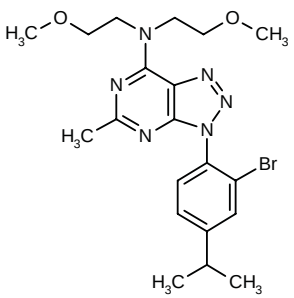
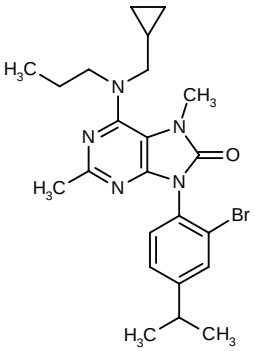


14 (SC-241)



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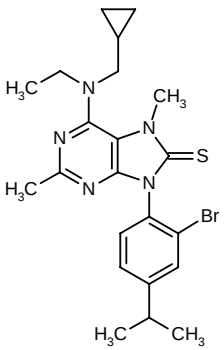

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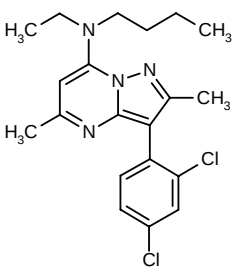
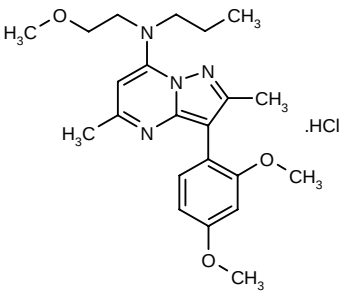
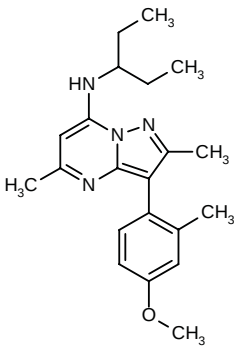


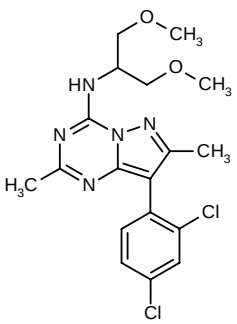
17 (PD-171729)



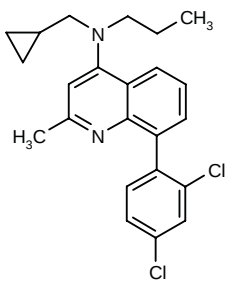
18 (NBI-30545)



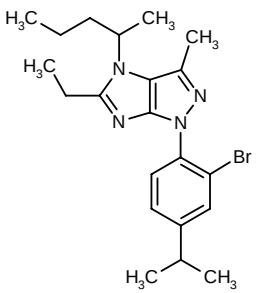
19 (SP-904)



20 (DMP-696)



21 (NBI-31200)



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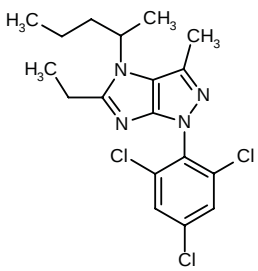

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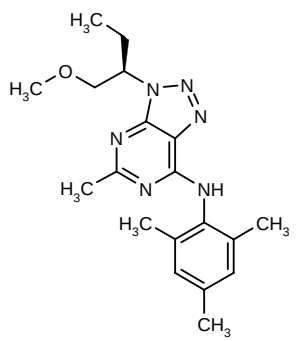
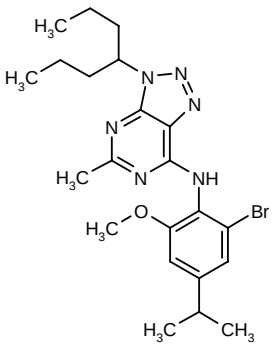
Scheme 3



24 (SJ-948)



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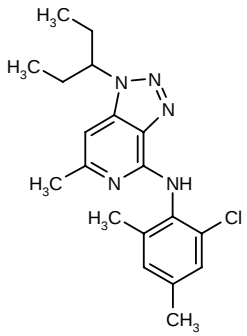

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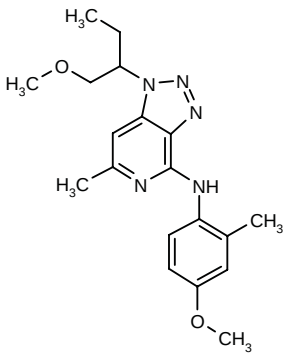

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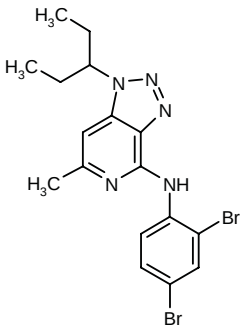

28

Notably, however, compounds **5** and **13** containing a methyl group and compounds **7** and **8** containing a 4-aryl-1,2,3,6-tetrahydropyridino group in the Up Area have high affinities for CRF_1 receptors. The Up Area has wider restriction than the Central and Down Areas for the substituent of CRF_1 receptor antagonist.

Compounds **6**, **19** and **20** have a unique structure: a mono-secondary-alkylamino group in the Up Area as compared to compounds **3-5**, **11**, **12**, **14-18** and **21**, which have secondary alkylamino groups in the Up Area. Methoxymethyl and/or ethyl groups of this mono-secondary-alkylamino group play the role of two alkyl groups of secondary alkylamino groups in the Up Area of compounds **3-5**, **11**, **12**, **14-18** and **21** in interacting with CRF_1 receptors. This successful displacement of the secondary alkylamino group by the mono-secondary-alkylamino group yielded type 2 analog **22** and **23** and type 3 analog **24-28**. Furthermore, moieties of the Central and Down Areas of compounds **6** and **17-21** are connected with an oxygen and carbon atom, respectively, instead of the nitrogen atom of compounds **3-5**, **7-16** and **22-28**. This suggests that Y^2 does not directly influence CRF_1 receptor affinity but might control relative distance and/or conformation of moieties in the Central and Down Areas to produce high CRF_1 receptor affinity.

Preclinical studies

NBI-27914, NBI-30545 and NBI-31200

NBI-27914 (5 mg/kg s.c.) increased the time spent in the open arms in the elevated plus-maze test in rats (43). In a test of restraint-induced defensive behavior, the compound (5 mg/kg s.c.) attenuated defensive withdrawal behavior (43). NBI-27914 (5 mg/kg s.c.) reversed the effects of i.c.v. infusion of CRF_1 but not that of urocortin ($CRF_{2\alpha}$ receptor agonist)-induced defensive behavior (43). Furthermore, NBI-27914 (10-20 mg/kg i.p. and 20 mg/kg p.o.) increased the latency and decreased the duration of i.c.v. infused CRF (0.15 nmol/l μl)-induced seizure in infant rats (44).

NBI-30545 inhibited [^{125}I]-sauvagine binding to human CRF_1 receptor expressed in LtK mouse fibroblast cells with K_i values of 2.8 nM (72). NBI-30545 displayed an oral bioavailability of 80% in mice and 10% in rats. In a model of CRF-induced stress, NBI-30545 (20 mg/kg p.o.) inhibited CRF-induced locomotor activity in rats. The compound also showed a dose-dependent decrease in stress-induced anxiogenic behavior in the elevated plus-maze test in rats at oral doses up to 20 mg/kg (72).

NBI-31200 inhibited [^{125}I]-sauvagine binding to human CRF_1 receptors expressed in LtK mouse fibroblast

cells with K_i values of 0.5 nM (76). CRF-induced ACTH secretion was inhibited by NBI-31200 (IC_{50} = 16 nM) (76).

CRA-1000 and CRA-1001

CRA-1000 and CRA-1001 dose-dependently inhibited [^{125}I]-ovine CRF binding to membranes of rat frontal cortex, with IC_{50} values of 20.6 and 22.3 nM, respectively (46, 48). Likewise, CRA-1000 and CRA-1001 dose-dependently inhibited [^{125}I]-ovine CRF binding to membranes of rat pituitary, with IC_{50} values of 15.7 and 18.6 nM, respectively (48). In contrast, neither compound had affinity for the $CRF_{2\beta}$ receptors when examined using rat heart (46-48). CRF-induced cAMP accumulation was dose-dependently inhibited by both compounds in AtT-20 cells (IC_{50}s = 377 and 187 nM, respectively) and COS-7 cells expressing CRF_1 receptors (IC_{50}s = 79 and 68 nM, respectively), while they did not attenuate the CRF response in COS-7 cells expressing $CRF_{2\alpha}$ receptors (48). CRF-induced ACTH secretion was dose-dependently inhibited by both compounds in AtT-20 cells (IC_{50}s = 142 and 363 nM, respectively) (47).

CRA-1000 (1-10 mg/kg p.o.) and CRA-1001 (1-10 mg/kg p.o.) dose-dependently reversed swim stress-induced reduction of the time spent in the light area in a light/dark exploration task in mice (48). In nonstressful conditions, neither compound at doses of 3-10 mg/kg p.o. affected the time mice spent in the light area in the same task (48). CRA-1000 (0.1-1 mg/kg p.o.) and CRA-1001 (0.3-10 mg/kg p.o.) dose-dependently reversed the effects of i.c.v. infusion of CRF (1 μg/10 μl) on the time spent in the open arms in the elevated plus-maze in rats (48).

Lesioning of the olfactory bulb induced hyperemotionality, and this effect was inhibited by acute and chronic administration of CRA-1000 and CRA-1001 at doses of 3-10 mg/kg p.o. in rats. The firing rate of locus ceruleus (LC) neurons was increased by i.c.v. infused CRF (1 μg/10 μl). This excitation of LC neurons was significantly blocked by pretreatment with CRA-1000 (0.5-5 mg/kg i.v.) and CRA-1001 (1-5 mg/kg i.v.) (48).

Neither of the compounds at doses up to 100 mg/kg p.o. had effects on hexobarbital-induced anesthesia in mice, rotarod test in mice, spontaneous locomotor activity in mice and passive avoidance task in rats (48).

A 60-min period of restraint significantly shortened pentobarbital/Na-induced sleeping time, which was completely reversed by CRA-1000 (1-10 mg/kg i.p.) (49). The inhibition of food intake and increase in locomotor activity induced by emotional stress using a communication box were reversed by CRA-1000 (1-10 mg/kg i.p.) (50).

Both compounds exhibited 12-18% oral bioavailability at 10 mg/kg in rats (48).

*XQ-771, SA-627, SC-241, SP-904, DMP-696, SJ-948, **6** and **27***

XQ-771 (51), SA-627 (51), SC-241 (66), SP-904 (73), DMP-696 (74) and SJ-948 (79) inhibited [^{125}I]-ovine CRF binding to membranes containing cloned human CRF_1 receptors, with K_i values of 12, 32, 3.7, 1.0, 1.6 and 14.2 nM, respectively.

In rats, XQ-771 (51), SA-627 (51), SA-241 (66) and DMP-696 (74) had oral bioavailabilities of 4% (30 mg/kg), 19% (30 mg/kg), 17% (1 mg/kg) and 37% (5 mg/kg), respectively. In dogs, SA-627 (51), SA-241 (66), SP-904 (73), SJ-948 (79) and DMP-696 (74) had respective oral bioavailabilities of 20% (5 mg/kg), 30% (1 mg/kg), 33% (10 mg/kg), 25% (10 mg/kg) and 50% (1 mg/kg).

SP-904 increased the time rats spent in the open arms in the elevated plus-maze, with an ED_{50} of 5 mg/kg p.o. (73).

DMP-696 showed good oral activity in a rat situational anxiety test (minimum effective dose: 10 mg/kg), plus-maze test (equally as effective as chlordiazepoxide at 10 mg/kg), ACTH stress model in Brattelboro rats, human intruder test in rhesus monkeys (21 mg/kg reduced lip smacking by 60%) and the air-blast model in rhesus monkeys (21 mg/kg reduced struggle time by 50%) (75). Furthermore, in rats, unlike chlordiazepoxide, DMP-696 (100 mg/kg) caused no ataxia or sedation (75).

DMP-696 had no affinity for 30 different receptors and ion channels, including serotonin, dopamine, histamine and vasopressin receptors, and α and β adrenoceptors (74).

Compound **26** exhibited the highest affinity for CRF_1 receptors (K_i = 0.6 nM) among the N-aryl aminotriazolopyridines but yielded poor pharmacokinetic results in dogs (AUC = 39.1 nM·h/ml; C_{max} = 30.0 nM; $t_{1/2}$ = 1.2 h). Compound **27**, though less potent *in vitro* (K_i = 4.1 nM), had a better pharmacokinetic profile (AUC = 281.1 nM.h/ml; C_{max} = 124.0 nM and $t_{1/2}$ = 2.8 h) (81).

CP-154,526

CP-154,526 dose-dependently inhibited [^{125}I]-ovine CRF binding to membranes of rat frontal cortex (52), hippocampus (60) and pituitary (60) with IC_{50} values of 5.5, 0.5 and 0.04 nM, respectively. CP-154,526 dose-dependently blocked CRF-stimulated adenylate cyclase activity in membranes prepared from rat cortex (K_i = 3.7 nM) (59). The agent (1-30 mg/kg s.c.) also dose-dependently antagonized the stimulatory effects of exogenous CRF (4 μg/kg i.v.) on plasma ACTH level (59).

Preweaned rat pups were separated from their litter causing them to emit a series of ultrasonic vocalizations which are known to be suppressed by benzodiazepines and CP-154,526 (ED_{50}s = 5-10 mg/kg i.p.) (53). The potential anxiolytic activity of CP-154,526 (3.2-17.8 mg/kg i.p.) was dose-dependent in a fear-potentiated startle paradigm (54, 59). CP-154,526 (5-80 mg/kg i.p.) dose-dependently increased the number of licks in a Vogel conflict test (58). I.c.v. administered CRF (3 μg/rat)-induced excitation of LC neurons was significantly blocked by pretreatment with CP-154,526 (1-5.6 mg/kg i.v.) (59), and the compound (0.16 mg/kg i.p.) also blocked recombinant human IL-1β (5 μg/kg i.p.)-induced fever in rats (60). At a

dose of 1 mg/kg (i.p.), the agent increased the time rats spent in the open arms in the elevated plus-maze (60). However, these effects were not dose-dependent, since higher doses (3.2 and 10 mg/kg i.p.) did not have significant effects in this paradigm (60). CP-154,526 (15 and 30 mg/kg s.c.) attenuated stress-induced relapse to drug seeking in cocaine- and heroin-trained rats (62).

CP-154,526 (10-32 mg/kg i.p.) reversed escape deficit when administered 60 min prior to the test session, but had no effects on the performance of control rats pre-exposed to inescapable stress (61). The compound decreased immobility after acute and subchronic dosing schedules in the Porsolt forced swim test as compared to standard antidepressants which tended to be ineffective in this model when acutely administered (55). CP-154,526 exhibited no muscle relaxant side effects at doses up to 40 mg/kg i.p. (53) and showed a 37% oral bioavailability at 10 mg/kg in rats (52).

Antalarmin

Antalarmin inhibited [^{125}I]-ovine CRF binding to rat pituitary, cerebellum and frontal cortex homogenates with K_i values of 1.9, 1.3 and 1.4 nM, respectively (63). The compound (20 mg/kg i.p.) also antagonized the stimulatory effects of exogenous CRF (4.74 ng/0.5 ml i.p.) on plasma ACTH levels (64) and at 20 mg/kg (i.p.), inhibited carrageenin-induced s.c. inflammation (63).

PD-171729

PD-171729 bound to human CRF_1 expressed in CHO cells with a K_i value of 5 nM (69). PD-171729 blocked the activation of adenyl cyclase in CHO cells expressing human CRF_1 receptors with an IC_{50} of 357 nM (71) and antagonized the stimulatory effects of exogenous CRF on plasma ACTH levels (ED_{50} = 4 mg/kg i.v.) without affecting basal levels of ACTH (71). PD-171729 dose-dependently reduced CRF-stimulated locomotion in acclimated rats, providing further evidence of its antagonism of CRF *in vivo* (71). PD-171729 (1-20 mg/kg p.o.) dose-dependently increased catecholamine synthesis in the hypothalamus and hippocampus, though not in the striatum and cerebellum in rats (71). The agent had no effects on serotonin synthesis (71). CRF (10 μg/rat i.c.v.) caused an 80% increase in brain norepinephrine (NE) release in cortex with a corresponding increase in the level of its metabolite, MHPG (70); pretreatment with PD-171729 (5-20 mg/kg p.o.) dose-dependently reduced this CRF-induced NE response (70).

Conclusions

Various nonpeptide CRF_1 receptor antagonists have been reported with structure-activity relationships and/or biological characterizations. Pharmacokinetic data have been reported for some nonpeptide CRF antagonists although data are not complete since the concentration of these compounds in brain, a target tissue of CRF receptor antagonist, has not been reported. However, the structure-activity relationships and pharmacokinetic data could be useful for directing future design of CRF receptor antagonists.

The results of biological characterization of several CRF_1 receptor antagonists suggest that CRF_1 receptor antagonists may be useful for the treatment of depression, anxiety and/or stress-related diseases, and that the CRF_1 receptor could be an interesting target for therapeutic intervention. It should be pointed out that the diversity of the pharmacological effects of CRF_1 receptor antagonists may result in side effects. However, it is clear that the CRF_1 receptor is an exciting target in the development of central nervous system drugs and studies on CRF_1 receptor antagonists may soon reach clinical trials.

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