Drugs of the Future 2007, 32(9): 809-822

CCC: 0377-8282/2007
DOI: 10.1358/dof.2007.032.09.1131450

Review Article

Neuropeptide receptors: novel therapeutic targets for depression and anxiety disorders

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Abstract

Recently, numerous neuropeptides (short-chain amino acids) have been suggested to play pivotal roles in stress responses. Neuropeptides are synthesized in restricted brain regions and act as neurotransmitters or neuromodulators in the brain. Moreover, their expression and secretion are altered upon exposure to stress, resulting in several stress responses, including depressive and anxiety-like behaviors. Neuropeptides exert their effects through specific receptors, most of which belong to the G-protein-coupled receptor (GPCR) superfamily. A series of neurochemical and behavioral studies utilizing pharmacological tools and genetically engineered animals have delineated the role of each receptor subtype in stress-related responses. In particular, agents that act on receptors for vasopressin, melanin-concentrating hormone (MCH), neuropeptide Y (NPY), melanocortin, galanin or nociceptin/orphanin FQ could be attractive targets for drug discovery for the treatment of stress-related disorders such as depression and anxiety disorders.

Introduction

Current medications for both mood and anxiety disorders are dominated by drugs that act through monoaminergic or GABAergic transmission. Monoamine reuptake inhibitors, such as selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs), are now widely prescribed not only for depression but also for anxiety disorders, and have become the first-line treatment for these disorders. These drugs, however, require several weeks for their effects to become noticeable, and only about 50% of patients with major depressive disorder exhibit remission, although approximately 70% show some improvement. Moreover, these drugs have side effects such as nausea and sexual dysfunction, which can result in medication being discontinued in many patients. Although many patients with anxiety disorders have been successfully treated with short-term benzodiazepine use, long-term use increases the risk of drug dependence and tolerance. Therefore, current strategies for drug discovery for the treatment of mood and anxiety disorders have shifted to focus on molecules targeting systems other than the monoaminergic and GABAergic systems.

Stress is well recognized as the primary cause of depression and anxiety disorders. In the brain, neuropeptides acting as neurotransmitters or neuromodulators have been suggested to play a pivotal role in stress responses. Generally, these peptides are locally synthesized in restricted brain regions, and their expression and secretion are changed upon stress exposure. Moreover, these neuropeptides are deeply involved in the regulation of hypothalamus-pituitary-adrenal (HPA) axis activity, and they also modulate the activity of monoaminergic systems.

The important role of neuropeptide receptors in depression and anxiety can be well exemplified by antagonists for tachykinin receptors (NK_1 and NK_2) and the corticotropin-releasing factor (CRF) receptor CRF_1. Indeed, both an NK_1 receptor antagonist (1) and an NK_2 receptor antagonist have proven effective for the treatment of major depressive disorder in phase II studies, and saredutant, an NK_2 receptor antagonist, is presently being tested in a phase III clinical trial for depression. Although Merck announced the discontinuation of the phase III clinical development of aprepitant because of a lack of demonstrable efficacy for depression (2), several NK_1 receptor antagonists are still being pursued in clinical trials for depression. Moreover, CRF_1 receptor antagonists are being tested in clinical trials, and R-121919, which was discontinued because of hepatotoxicity, was proven

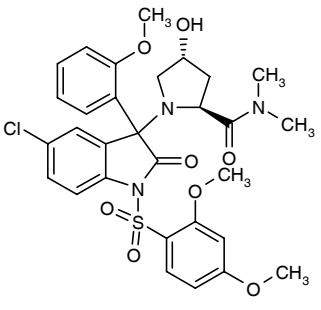
to be effective for major depressive disorder in a small open-label trial (3).

Recently, in addition to these well-studied neuropeptides, numerous neuropeptides and their receptors have been identified and their roles in stress-related responses have been clarified using pharmacological tools as well as genetically engineered animals. In this review, we will focus on neuropeptide systems which have recently emerged as attractive and promising targets for the treatment of depression and anxiety disorders.

Vasopressin receptors

Arginine-vasopressin (AVP) is a cyclic nonapeptide that is thought to be the principal factor in the regulation of adrenocorticotropic hormone (ACTH) release from the pituitary (4). In addition to its involvement in the regulation of HPA axis activity, AVP is believed to play a role in mood regulation. Upon stress stimulation, AVP is released from the median eminence into the pituitary portal circulation, where it strongly potentiates the effects of CRF on the release of ACTH. Clinically, both the plasma AVP levels and the intensity of AVP immunoreactivity in the paraventricular nucleus (PVN) are elevated in patients with major depression compared to healthy controls (5, 6), and AVP release is significantly correlated with anxiety symptoms in healthy subjects challenged with an anxiogenic CCK_B agonist (7), implicating a role for AVP in these stress-related disorders.

AVP exerts its effects via three receptor subtypes, V_{1a}, V_{1b} and V_2 receptors, all of which are G-protein-coupled receptors (GPCRs) coupled to G_q/G_{11} protein (8). Of these, the V_{1b} receptor has been reported to be involved in emotional processes based on a series of studies using SSR-149415 (**1**; Fig. 1), a nonpeptide V_{1b} receptor antagonist with high affinity, selectivity and potent antagonist activity (9). SSR-149415 has been reported to exhibit antidepressant- and anxiolytic-like activities in a variety of rodent models of depression and anxiety, with more pronounced effects in models involving stressful situations (9-11), and displays a different profile compared to a CRF_1 receptor antagonist (12). The extrahypothalamic nuclei (the lateral septum, central, basolateral and medial nucleus of the amygdala) are also reportedly involved



SSR-149415 (**1**)

Fig. 1. V_{1b} receptor antagonist.

in the antidepressant effect of SSR-149415; among these nuclei, however, only the basolateral nucleus is involved in the anxiolytic effect (13, 14). In contrast, the pituitary V_{1b} receptor (which is responsible for regulating the HPA axis) has been reported to play a role in the anxiolytic effect in the social interaction test (15), but not in the antidepressant effect in the forced swimming test (9). Therefore, the neuroendocrine mechanisms underlying the antidepressant and anxiolytic effects of V_{1b} receptor antagonists may differ. Moreover, the repeated administration of a V_{1b} receptor antagonist, like CRF_1 receptor antagonists and fluoxetine, reversed stress-induced reductions in adult hippocampal neurogenesis, thought to be a cause of depression, in a mouse chronic mild stress model, while neither a V_{1b} receptor antagonist nor a CRF_1 receptor antagonist had significant effects on hippocampal neurogenesis in nonstressed animals (16). SSR-149415 is currently being tested in a phase II clinical trial for depression.

Melanin-concentrating hormone receptors

Melanin-concentrating hormone (MCH) is a cyclic 19-amino-acid peptide that was originally isolated from the Chum salmon pituitary as a 17-amino-acid peptide (17). In mammals, MCH is produced predominantly by neurons in the lateral hypothalamus and zona incerta with extensive projections throughout the brain (18); it exerts numerous physiological effects, including increasing food intake and the induction of depressive and anxiety-like behaviors, as shown by the observation that MCH-null mice became lean (19) and displayed an antidepressive phenotype (20).

Two receptor subtypes have been reported, the MCH1 and the MCH2 receptor, both of which belong to the GPCR superfamily and were originally cloned as orphan GPCR receptors (SLC-1/GPR24 and SLT, respectively) (21). Emerging lines of evidence have suggested that the MCH1 receptor mediates not only the regulation of feeding behavior but also emotional states. The MCH1 receptor is densely expressed in the nucleus accumbens shell, a brain region involved in motivation and reward, and it has been reported that local injection of MCH into the nucleus accumbens shell induces a depressive phenotype in the forced swimming test, while the local injection of an MCH1 receptor antagonist produces antidepressant effects (20). MCH1-null mice have been reported to display anxiolytic-like behavior in several animal models of anxiety (open field, elevated plus-maze, social interaction, stress-induced hyperthermia) (22-24). Moreover, female, but not male, mice carrying a null mutation of the MCH1 receptor gene reportedly exhibited antidepressant phenotypes in the forced swimming test and the tail suspension test (23). Smith *et al.* (25) reported that knockout mice lacking the MCH1 receptor exhibited enhanced mesolimbic dopaminergic activity, which may explain in part the mechanism of antidepressant-like behavior seen in MCH1 receptor-null mice. In addition, the MCH1 receptor has been reported

to be densely distributed in the PVN of the hypothalamus, where it has a stimulatory effect on HPA axis activity through CRF secretion (26); this mechanism may be involved in the depressive and anxiety-related effects of MCH. Moreover, the expression of MCH1 receptor mRNA in the hippocampus was significantly upregulated following chronic mild stress for 5 weeks in mice, which concomitantly exhibited depressive behavior in the tail suspension test, and the repeated administration of fluoxetine reversed both the increase in MCH1 receptor expression and the depressive behavior (23).

The systemic administration of nonpeptide MCH1 receptor antagonists (Fig. 2) such as ATC-0175 (**2**), ATC-0065 (**3**) and SNAP-7941 (**4**) has been reported to exert antidepressant and anxiolytic effects in a variety of rodent models of depression and anxiety (27, 28), although one report concluded that MCH1 receptor antagonists, including SNAP-7941, did not have any effect in the forced swimming, tail suspension and Vogel conflict tests (29). Recently, another MCH1 receptor antagonist, GW-3430 (**5**), has been reported to reverse MCH-induced anxiety-like behavior and increase plasma corticosterone levels, as well as to exhibit anxiolytic effects in wild-type but not MCH1 receptor-null mice (24). Moreover, a novel non-peptide MCH1 receptor antagonist, SNAP-94847 (**6**), reportedly exerted an anxiolytic effect in the light/dark test and an anxiolytic/antidepressant effect in novelty-suppressed feeding after both acute and chronic (28 days) dosing, while it had no effect in the forced swimming test in mice (30). Interestingly, chronic administration of SNAP-94847 increased progenitor cell proliferation in the dentate gyrus, but the suppression of hippocampal neurogenesis by X-irradiation did not alter the antidepressant effect of SNAP-94847 on novelty-suppressed feeding; thus, the mechanism responsible for the effects of SNAP-94847 may be distinct from that of SSRIs (30).

Since the prototype MCH1R antagonist T-226296, the (–)-enantiomer of **7**, was first discovered by Takeda (31),

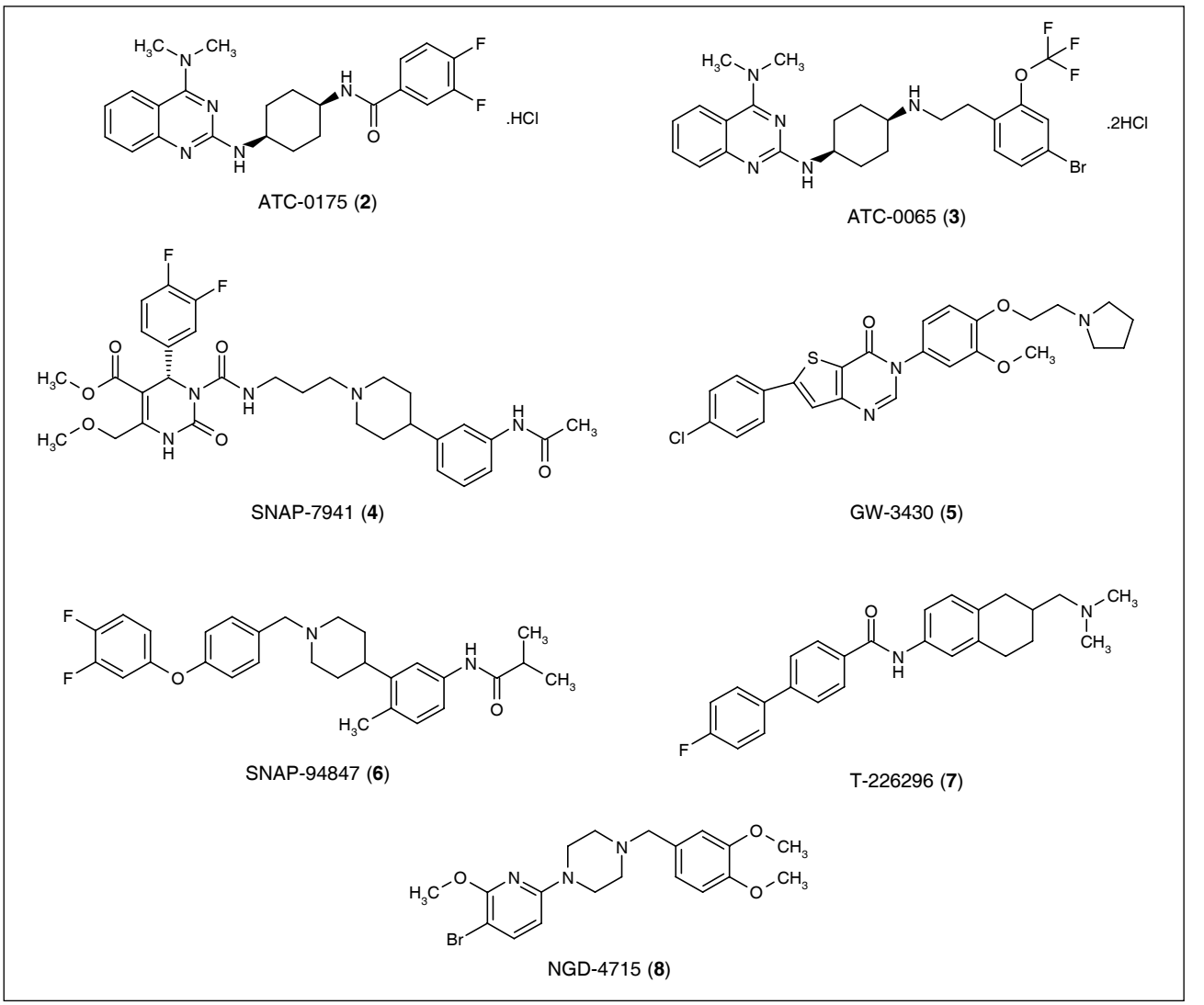


Fig. 2. MCH1 receptor antagonists.

a plethora of MCH1 receptor-selective antagonists have been discovered. Nevertheless, the development of almost all of these antagonists has been stopped because of an association with hERG blockade, leading to drug-induced Q-T_c prolongation, and poor pharmacokinetic profiles.

Taisho and Arena discovered a series of 4-(dimethylamino)quinazolines that acted as MCH1 receptor antagonists using a high-throughput screening (HTS) of an in-house GPCR-directed library, exemplified by ATC-0175 (IC_{50} = 3.4 nM for the MCH1 receptor; IC_{50} = 260 nM for the α_{2A}-adrenoceptor; IC_{50} = 2700 nM for the neuropeptide Y [NPY] Y_5 receptor; $t_{1/2}$ in human liver microsomes = 25 min) and ATC-0065 (IC_{50} = 16 nM for the MCH1 receptor; IC_{50} = 340 nM for the α_{2A}-adrenoceptor; IC_{50} = 420 nM for the NPY Y_5 receptor; $t_{1/2}$ in human liver microsomes = 73 min) (32-34).

Lundbeck reported that SNAP-7941, featuring a 4-arylpiperidine structure, was a competitive antagonist of the MCH1 receptor in a phosphoinositide accumulation assay (pA_2 = 9.24) (27). SNAP-94847 was also identified as an MCH1 receptor antagonist (pA_2 = 7.81) (30).

To date, only three compounds, GW-3430 (GlaxoSmithKline), AMG-076 (undisclosed structure; Amgen) and NGD-4715 (**8**; Neurogen), have progressed to clinical trials for obesity.

Neuropeptide Y receptors

Neuropeptide Y (NPY) is a highly conserved 36-amino-acid peptide that is abundantly expressed in the central and peripheral nervous system (35). NPY is the most abundant peptide in the central nervous system and is expressed in numerous brain areas, including the ventral and dorsal striatum, limbic structures and brainstem (the locus coeruleus); it is often co-localized with norepinephrine and GABA, as well as other neuropeptides, such as somatostatin and agouti-related protein (36, 37). NPY plays an important role in numerous physiological processes, including food intake, cognition and pain perception.

Human studies have suggested a link between low levels of NPY and depressive and anxiety disorders. Lower plasma NPY levels were observed in depressed patients compared to control subjects (38, 39), and robust reductions in NPY levels were observed in the cerebrospinal fluid (CSF) of medication-free subjects with treatment-refractory depression (40), while plasma NPY levels did not differ among subjects with panic disorders, subjects with social phobia or control subjects (41). In a *post mortem* study, NPY immunoreactivity was significantly lower in the frontal cortex and caudate nucleus from suicide victims compared to age-matched controls, with an even more robust reduction seen in a subgroup of suicide victims with a history of depression (42). In contrast, antidepressant treatment (citalopram) increased NPY levels in the CSF, and this change was associated with a change in the Hamilton Depression Scale (HAM-D) score (43). Likewise, an increase in NPY levels in the CSF was observed following electroconvulsive therapy (ECT), which paralleled the clinical recovery (44).

Alterations in NPY levels have also been linked to depression and anxiety in animal studies. Genetic animal models of depression, such as the Flinders Sensitive Line (FLS) and fawn-hooded rats showed lower NPY levels in the hippocampus compared to those in the control group (45, 46). NPY levels (mRNA or immunoreactivity) in the hippocampus were decreased in a chronic mild stress model (47), a learned helplessness model (48) and after maternal deprivation (49). In contrast, antidepressant treatments (antidepressants, mood stabilizer, wheel running, ECT) increased (50) or attenuated the changes in the NPY levels in the hippocampus (45, 48, 49, 51). Thus, the NPY expression level may be related to depressive behavior and antidepressant effects.

Several lines of evidence have shown that NPY modulates anxiety and depressive behavior in a variety of behavioral tests. The injection of NPY into the cerebroventricle or specific brain nuclei has been reported to exert anxiolytic and antidepressant effects in several animal models, such as the Geller-Seifter conflict test, the learned helplessness test and the forced swimming test (52-54).

Studies of mutant rodents further support the role of NPY in depression and anxiety. NPY-null mice have been reported to display anxiety-like behavior (open field, acoustic startle) (55), while rats overexpressing NPY in the hippocampus showed attenuated anxiety-like behavior induced by stress (elevated plus-maze, punished drinking) (56). Moreover, increased NPY levels in the amygdala caused by the injection of viral vectors encoding NPY reduced anxiety-like behavior in the elevated plus-maze task (57).

The actions of NPY are mediated through five receptor subtypes (Y_1, Y_2, Y_4, Y_5, Y_6), all of which belong to GPCRs that inhibit the production of cAMP (58). Of these, Y_1 and Y_2 receptors are of interest in terms of depression and anxiety. Of note, the Y_2 receptor acts as a presynaptic NPY autoreceptor; thus, blockade increases the release and synthesis of NPY. Y_1 receptor agonists mimic the anxiolytic action of NPY (52), while Y_1 receptor antagonists increase anxiety (57, 59) or block NPY-induced antidepressant effects (54). Consistent with these findings, knockout mice lacking the Y_1 receptor increased their voluntary ethanol consumption (60), and a Y_1 receptor antisense oligonucleotide produced anxiogenic effects (61). On the other hand, Y_2 receptor antagonists have been reported to exert antidepressant effects in the learned helplessness test (54) and anxiolytic effects in the elevated plus-maze task (62).

Contradictory results were obtained for Y_2 receptor agonists, the effects of which may depend on the site of injection in the brain and the types of behavior tested. Moreover, mice lacking the Y_2 receptor exhibited an antidepressant-like phenotype in the forced swimming test and an anxiolytic-like phenotype in the elevated plus-maze task, the light/dark exploration test and the open field test (63). These results indicate that blockade of

presynaptic Y_2 receptors enhances the release of NPY, resulting in the stimulation of a postsynaptic NPY receptor, presumably the Y_1 receptor, and the induction of anxiolytic effects. Therefore, both Y_1 receptor agonism and Y_2 receptor antagonism may be interesting approaches for the treatment of depression and anxiety, as well as alcohol dependence, which is frequently a co-morbid condition.

Boehringer Ingelheim identified the first potent, selective, nonpeptide NPY Y_2 receptor antagonist, BIIE-0246 (**9**; Fig. 3), which binds to the human Y_2 receptor with high affinity (IC_{50} = 3.3 nM) (64), while having virtually no affinity for Y_1, Y_4 and Y_5 receptors.

Bristol-Myers Squibb disclosed a new class of benzothiophenes, exemplified by compound **10** (Fig. 3), synthesized using a solid split pool methodology, with moderate Y_2 affinity, but did not comment on the functional activity (IC_{50} = 450 nM) (65).

Johnson & Johnson reported that a series of small-molecule Y_2 ligands (Fig. 3), exemplified by the piperidinylindoline cinnamide JNJ-2765074 (**11**; IC_{50} = 4000 nM), were found using HTS (66). Through a structure-activity relationship (SAR) study of the series, JNJ-5207787 (**12**) was identified as a moderately active nonpeptide Y_2 receptor antagonist (IC_{50} = 100 nM). This compound was also found to be more than 100-fold selective *versus* human Y_1, Y_4 and Y_5 receptors, as evaluated using radioligand binding.

To date, a nonpeptide Y_1 receptor agonist has not been reported, although several potent nonpeptide Y_1 receptor antagonists have been discovered.

Melanocortin receptors

Melanocortins (ACTH and α-, β- and γ-melanocyte-stimulating hormone [α-, β- and γ-MSH]) are derived from pro-opiomelanocortin (POMC) through enzymatic processing, and participate in a wide range of physiological functions. Five subtypes of melanocortin receptors have been identified (MC_1-MC_5), all of which are coupled to G_s (67). In the brain, MC_3 and MC_4 receptors are the main melanocortin receptors expressed, with small amounts of the MC_5 receptor also detected. Of these receptors, the MC_4 receptor has been shown to play a role in the regulation of emotion and stress responses. MC_4 receptor agonists reportedly induce stress-related behaviors in rodents, while MC_4 receptor antagonists block stress-related behaviors (68, 69). Moreover, the MC_4 receptor regulates HPA axis activity, presumably through the regulation of CRF secretion in the PVN of the hypothalamus (70). α-MSH and MC_4 receptor agonists have also been reported to produce anxiety-like behaviors in rodents (69, 71, 72).

Nonpeptide MC_4 receptor antagonists (Fig. 4), such as MCL-0129 (**13**) and MCL-0042 (**14**), have been reported to exhibit antidepressant and anxiolytic effects in several animal models of depression and anxiety, without causing sedation or motor dysfunction (73, 74). Anxiolytic and antistress effects were observed using another selective peptidomimetic MC_4 receptor antagonist, MCL-0020 (**15**) (69). Interestingly, MCL-0129 exerted antidepressant effects after only a single dose, as evaluated using a learned helplessness paradigm (73), while fluvoxamine

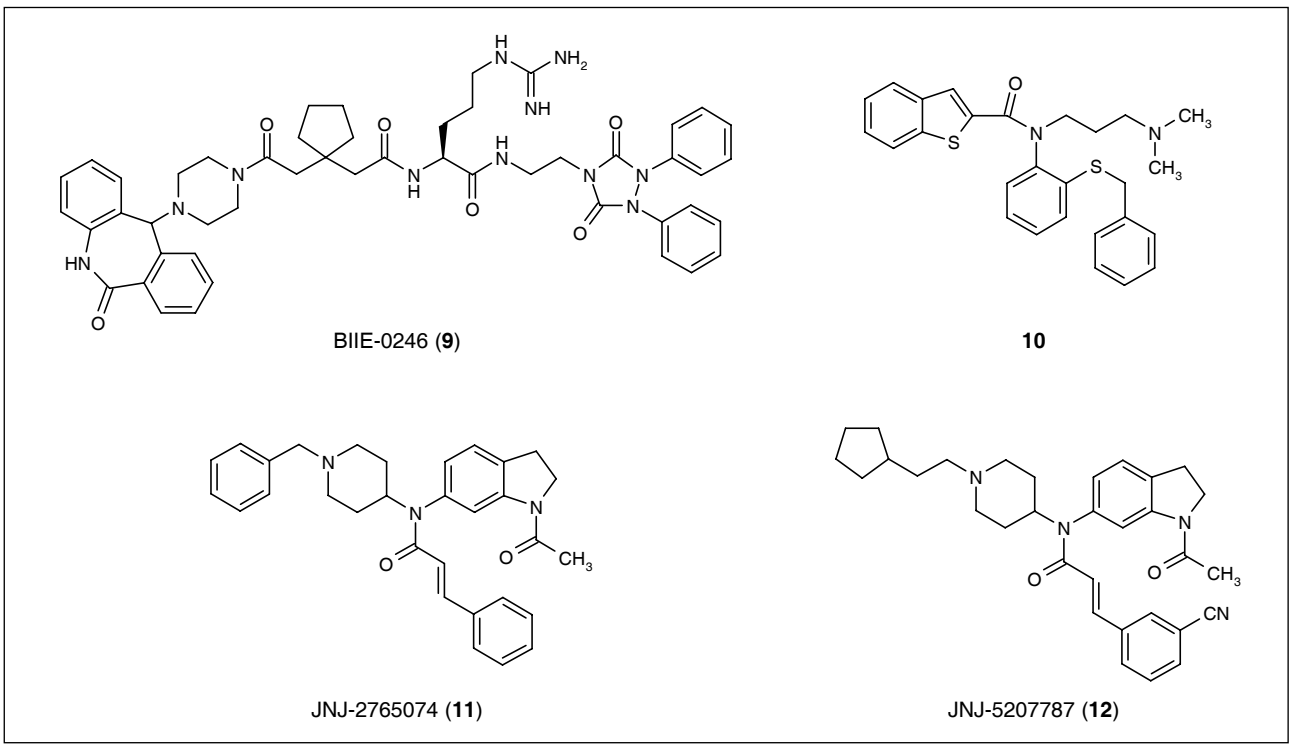


Fig. 3. Y_2 receptor antagonists.

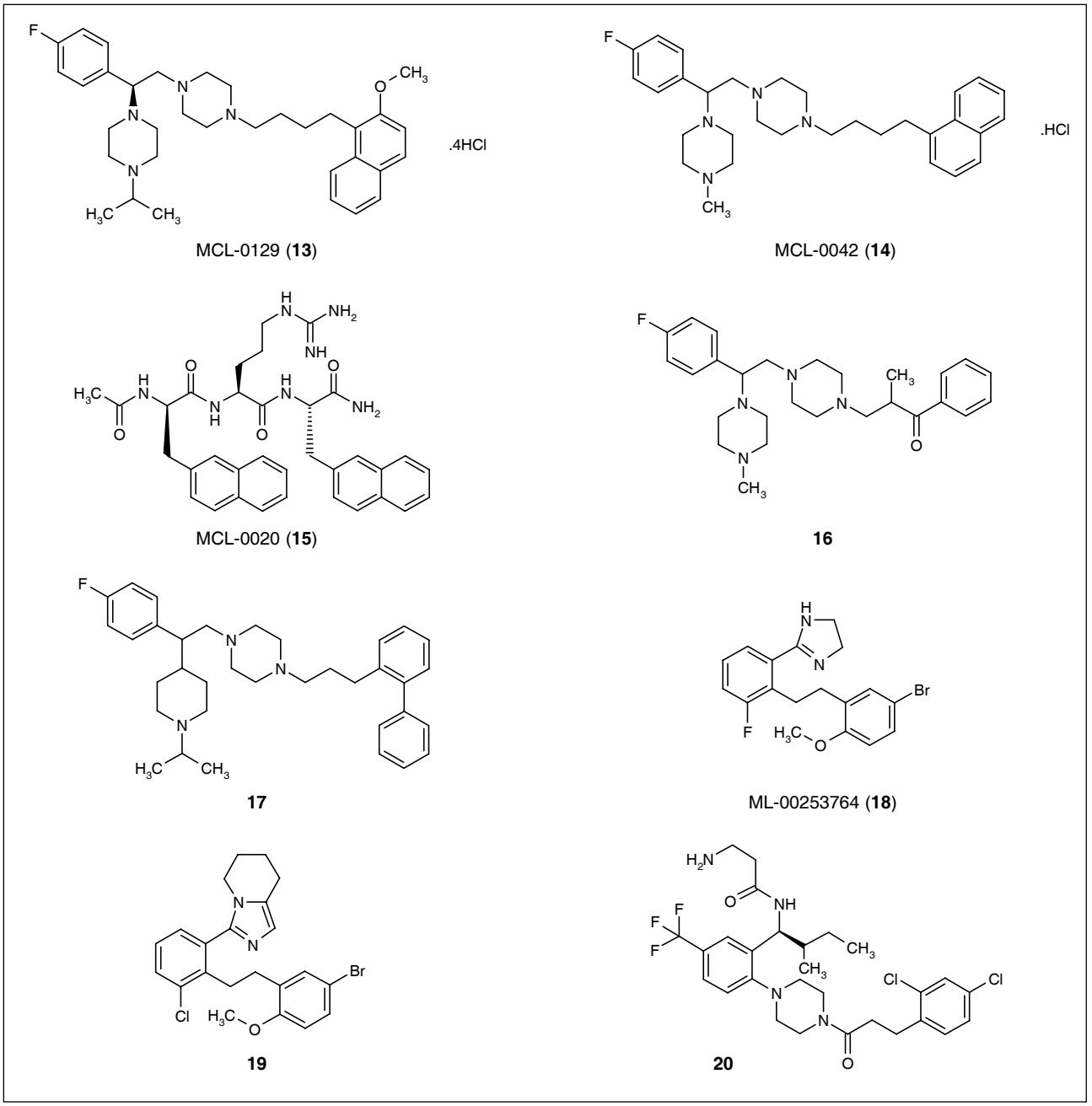


Fig. 4. MC_4 receptor antagonists.

exhibited this effect only after subchronic administration, indicating that MCL-0129 has a rapid onset of action.

Compound **16**, first reported by Amgen to be an inhibitor of agouti-related protein (AGRP) binding to the MC_4 receptor (IC_{50} = 52 nM), exhibits moderate inhibition (IC_{50} = 217 nM) of NDP-MSH binding and reduces α-MSH-stimulated cAMP production at high concentrations (75). At the same time, Taisho independently identified compound **16** as an MC_4 receptor antagonist using HTS of an in-house library (76). Through an elaborate investigation of the SAR around the bis-piperazine derivatives, MCL-0129, a (–)-enantiomer of compound **13**, was identified as one of the most potent known antagonists of the MC_4 receptor. Furthermore, to ameliorate the strong basicity among the bis-piperazine derivatives, the mono-piperazine derivatives, exemplified by compound **17**, were discovered as a new class of MC_4 receptor antagonists (77). Compound **17** significantly reversed anxiety-like behavior caused by swim stress in the elevated plus-maze task upon oral dosing in rats (1 mg/kg).

Millennium identified the lead compound ML-00253764 (**18**) as an MC_4 receptor antagonist using HTS (K_i = 160 nM; IC_{50} = 103 nM) (78). ML-00253764 efficiently penetrates the blood-brain barrier after s.c. admin-

istration in mice, leading to the effective attenuation of tumor-induced weight loss. However, ML-00253764 exhibited low plasma exposure when dosed p.o. or i.v. in rats and displayed rapid plasma clearance, which may stem from the metabolic instability and basicity of the imidazoline ring system. To overcome these limitations, the replacement of the imidazoline ring with an imidazole ring was investigated; this led to the discovery of compound **19**, which exhibits increased plasma levels following p.o. or i.v. administration and a markedly reduced plasma clearance (79).

Neurocrine Biosciences disclosed a series of piperazinebenzylamine derivatives as MC_4 receptor-selective ligands, which interestingly exert both agonist and antagonist activities with only subtle alterations to their chemical structures (80-84). Among these, compound **20** was identified as one of the most potent known antagonists for the MC_4 receptor (K_i = 13 nM) (84). While compound **20** gave high plasma concentrations following oral administration, it exhibited a very low brain/plasma ratio.

Galanin receptors

Galanin is a 29-amino-acid (in humans) or a 30-amino-acid neuropeptide found in central and peripheral tissues (85). In the central nervous system, galanin is co-localized with serotonin and norepinephrine, as shown by the detection of galanin immunoreactivity in the serotonin-producing neurons of the dorsal raphe nucleus (86) and in brainstem norepinephrine-producing neurons of the locus coeruleus (86). Galanin produces effects on cognition, feeding, seizures, sexual behavior and pain threshold (86).

Several lines of evidence indicate that galanin plays an important role in emotion and stress-related behaviors, although its effects are complex –possibly stemming from the differential actions of each receptor subtype; its actions are also task- and brain region-dependent. The intracerebroventricular injection of galanin produces anxiolytic effects in the Vogel conflict test (87), while injection into the amygdala led to an anxiogenic effect in the same paradigm (88). The injection of galanin into the ventral tegmental area, but not into the lateral ventricles, midbrain reticular formation or hypothalamus, significantly increased immobility in the forced swimming test, while galantide (M15), a galanin receptor antagonist, reduced immobility (an antidepressant effect) (89). Consistent with these results, in the FSL, a putative animal model of depression, increased galanin binding sites were observed in the dorsal raphe nucleus (90). On the other hand, galanin reportedly failed to show an antidepressant effect in the tail suspension test in C57BL/6J mice (91), indicating that the depressive behavior induced by galanin may also be task- or strain-dependent.

To date, three galanin receptor subtypes (GAL1, GAL2, GAL3) have been identified, all of which are GPCRs that are coupled to different effector systems. Both GAL1 and GAL3 are coupled to inhibitory G-proteins G_i/G_o, while GAL2 is additionally coupled to the G_q/G_{11} type of G-proteins for the transmission of stimulatory effects. Among the galanin receptor subtypes, the involvement of GAL3 in depression and anxiety has been demonstrated using selective nonpeptide antagonists that can be systemically administered. Swanson *et al.* (92) reported that SNAP-37889 (**21**; Fig. 5), a potent and selective GAL3 antagonist, exhibited anxiolytic effects in

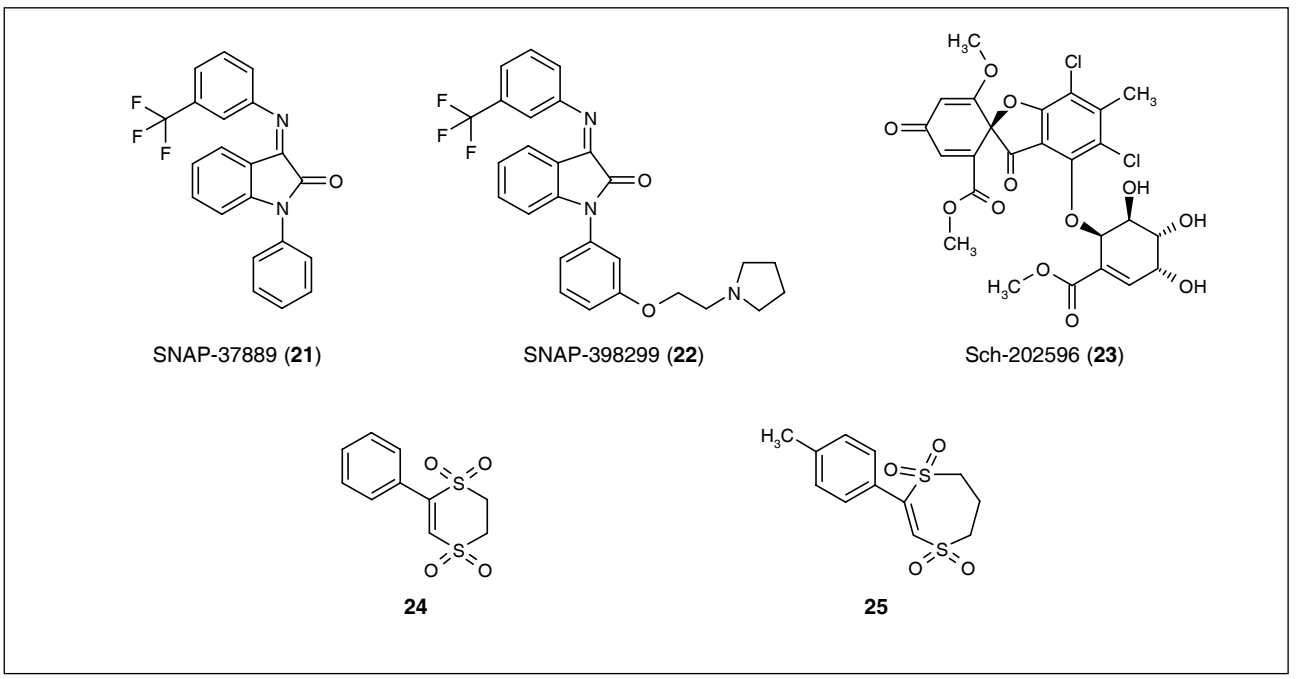


Fig. 5. GAL1 and GAL3 receptor antagonists.

several animal models, including the social interaction test, the Vogel conflict test, separation-induced vocalization in guinea pig pups and the stress-induced hyperthermia test, while antidepressant effects were seen in the forced swimming test, with increased swimming behavior. Of note, the anxiolytic and antidepressant effects of SNAP-37889 were observed following subchronic dosing, with no signs of tolerance. Moreover, SNAP-37889 antagonized the galanin-induced reduction of serotonin release in the hippocampus, and SNAP-398299 (**22**; Fig. 5), another selective GAL3 antagonist, attenuated the galanin-inhibited firing of dorsal raphe serotonin neurons (92). Therefore, GAL3 antagonists may exert antidepressant and anxiolytic effects by attenuating the tonic inhibition of galanin on serotonergic transmission. Moreover, another group has reported the antidepressant effects of a GALR3 antagonist patented by Lundbeck, but which differs from the above-mentioned SNAP compounds in the forced swimming test and the tail suspension test, while no effects were seen in the elevated plus-maze (93).

Galmic, a low-affinity GAL1 agonist, exhibited antidepressant effects in the forced swimming test (94). Likewise, galanon, a nonpeptide agonist at both GAL1 and GAL2 receptors, produced a dose-dependent antidepressant effect in the forced swimming test (95). Moreover, GAL1-null mice displayed increased anxiety-like behaviors in the elevated plus-maze test, although anxiety-like behaviors were not observed in the light/dark exploration and open field tests (96), and a depressive-like phenotype was not observed in the tail suspension test (91). Of note, higher levels of corticosterone and ACTH were seen after the elevated plus-maze task, compared with the levels after the light/dark and open field tests (96), indicating that highly stressful conditions may be necessary before an anxiety-like phenotype can be detected.

The role of GAL2 in depression and anxiety is not fully understood. Chronic administration (14 days) of fluoxetine reportedly increased the number of GAL2 sites in the dorsal raphe nucleus, albeit not significantly in the locus coeruleus, with no change in the number of GAL1 sites (95), suggesting that increased GAL2 levels in these monoaminergic nuclei may be involved in the action of antidepressants. Because GAL2 is the only galanin receptor subtype that exerts an excitatory input through the G_q/G_{11} protein, the authors speculated that fluoxetine treatment induced a relative shift in the effects of galanin on the neurons in the dorsal raphe nucleus toward a greater influence exerted though GAL2, which would result in increased firing rates in the serotonin neurons of the dorsal raphe nucleus. In contrast, mice lacking GAL2 have been reported to exhibit normal behavior in the tail suspension test (97). Recently, GAL2-null mice have been reported to display anxiety-like behavior specifically in the elevated plus-maze task but not in other anxiety tests, including the light/dark exploration, stress-induced hyperthermia and open field tests (98). The absence of an anxiety-like phenotype in GAL2 knockout mice in the stress-induced hyperthermia and open field tests has also been reported (97). Therefore, although GAL2 may play some role in emotional states, the significance of its role remains to be determined using selective GAL2 agonists/antagonists.

A novel spirocoumaranone, Sch-202596 (**23**; Fig. 5), isolated from a fungal fermentation broth of *Aspergillus* spp. by Schering-Plough in 1997, is a weak GAL1 antagonist (IC_{50} = 1700 nM) (99).

The R.W. Johnson Pharmaceutical Research Institute discovered that dithiin-1,1,4,4-tetroxide (**24**; Fig. 5) acted as a GAL1 antagonist using HTS (IC_{50} = 2700 nM) (100). SAR studies based on **24** ultimately led to the discovery of dithiipin-1,1,4,4-tetroxide (**25**; Fig. 5), the first reported nonpeptide submicromolar GAL1 antagonist (IC_{50} = 190 nM).

Lundbeck characterized 3-arylimino-2-indolones, exemplified by SNAP-37889, as GAL3 antagonists (101). SNAP-37889 displayed potent antagonist activity (K_b = 29 nM) and high affinity for human GAL3 (K_i = 17 nM), but no affinity for human GAL1 or GAL2 (K_i > 10 μM). However, because compound SNAP-37889 has low water solubility (1 μg/ml, pH 7.4 buffer), a basic amino functionality was incorporated to improve its solubility (102). Amine-containing indolones have significantly improved solubility compared with non-amine-containing indolones. A potent antagonist, SNAP-398299 (K_i = 5 nM), with an improved solubility of 48 μg/ml in pH 7.4 buffer has also been discovered.

Nociceptin receptors

Nociceptin/orphanin FQ (N/OFQ) is a 17-amino-acid peptide that was independently isolated in 1995 by two groups and was characterized as an endogenous ligand for the N/OFQ receptor (NOP receptor) (103, 104).

In 1997, Jenck *et al.* (105) first reported the anxiolytic effects of N/OFQ in a battery of behavioral models of anxiety (light/dark exploration test, elevated plus-maze, urocortin-induced anxiety-like behavior in the open field test, operant conflict test), that were later confirmed in different paradigms (106, 107). In contrast, knockout mice lacking the prepro-N/OFQ gene displayed increased anxiety-like behaviors in three anxiety models (open field, elevated plus-maze and light/dark tests) (108). Anxiolytic effects induced by stimulation of the NOP receptor were demonstrated by Jenck *et al.* (109), who showed that a selective nonpeptide NOP receptor agonist, Ro-64-6198 (**26**; Fig. 6), produced anxiolytic effects in several animal models of anxiety. Ro-64-6198 exerted anxiolytic effects in the elevated plus-maze test, the modified Geller-Seifter conflict test and fear-potentiated startle that were comparable to those produced by benzodiazepines. The anxiolytic effects of Ro-64-6198 were observed across multiple species, including rats, mice and guinea pigs (110). Of note, Ro-64-6198 dosing often produced abnormal body posture characterized by a flattened posture with splayed hind limbs in rats, albeit at doses higher than the anxiolytic doses (110). No differences in anxiety-like behavior were observed between $NOP^{-/-}$ and wild-type mice (110), and an NOP receptor antagonist did not alter the anxiety-

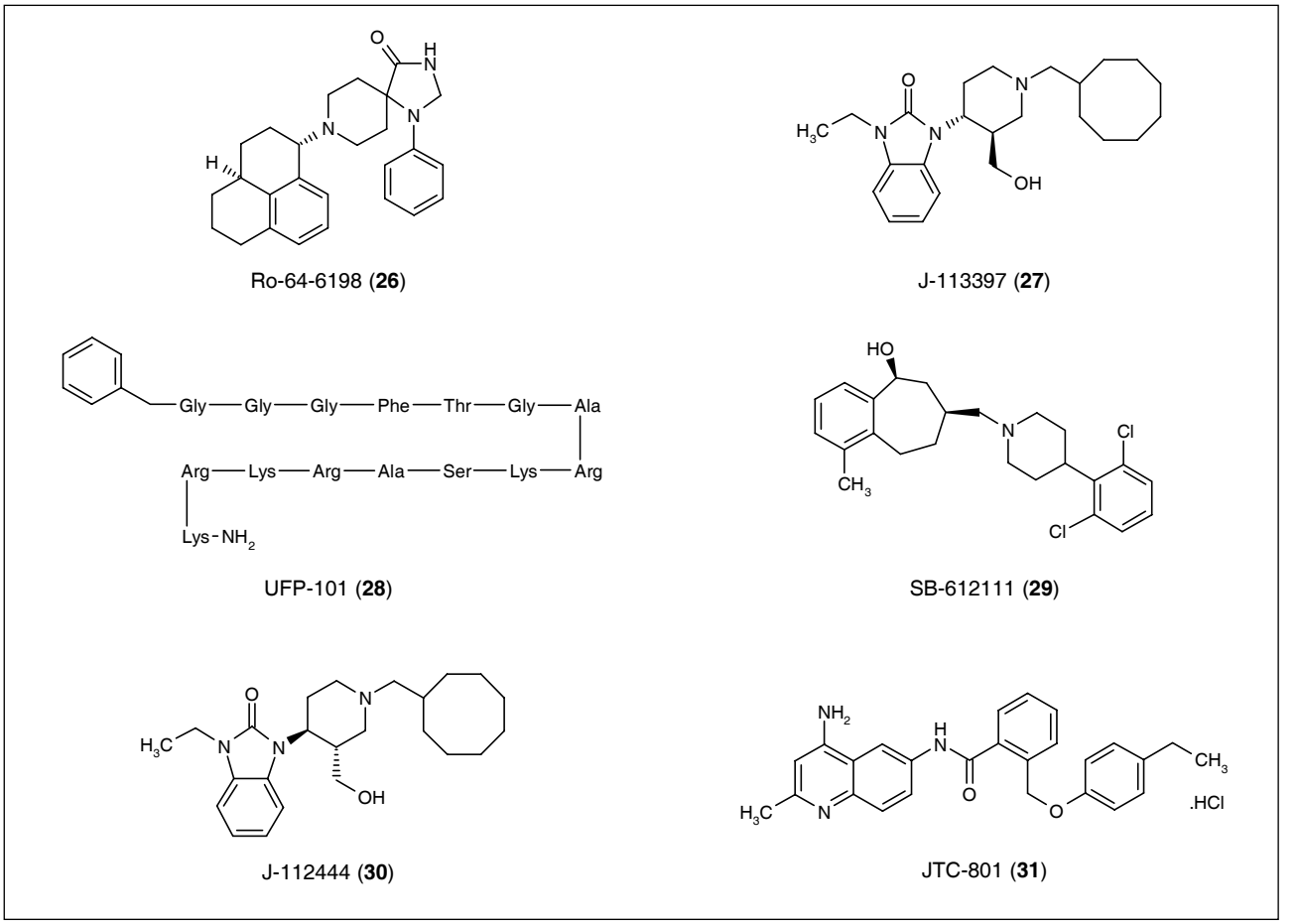


Fig. 6. NOP receptor agonist and antagonists.

like behavior, although it attenuated the anxiolytic effects of Ro-64-6198 (110), raising the question of whether N/OFQ-NOP receptor signaling may exert tonic regulation on basal anxiety levels. Recently, NOP receptor-null mice have been reported to display anxiety-related behaviors in a complex manner, with anxiogenic effects observed in the elevated plus-maze and light/dark tests, anxiolytic effects observed in the novelty-suppressed feeding and the elevated T-maze test, and no changes observed in the open field, hole-board, marble-burying and stress-induced hyperthermia tests (111).

Redrobe *et al*. (112) reported that both the peptide NOP receptor antagonist [NPhe^1]N/OFQ(1-13)NH_2 and the nonpeptide antagonist J-113397 (**27**; Fig. 6) reduced the immobility of mice subjected to the forced swimming test, without affecting locomotor activity. The antidepressant-like effects of NOP receptor antagonists were confirmed using another peptide NOP receptor antagonist, UFP-101 (**28**; Fig. 6) (113, 114), and a nonpeptide antagonist, SB-612111 (**29**; Fig. 6) (115), in the forced swimming and tail suspension tests; these effects were reversed by N/OFQ. Moreover, knockout mice lacking the NOP receptor displayed a significant reduction in immobility time in both the forced swimming test and the tail suspension test compared to in wild-type mice (113-115). These findings suggest that NOP receptor blockade induces antidepressant effects in rodents. Of note, all of the data on the antidepressant effects of NOP receptor antagonists were obtained after acute administration and no data are available for subchronic administration.

Although the detailed neural mechanisms by which NOP receptor antagonists exert their antidepressant effects have not been fully investigated, interactions between the N/OFQ-NOP receptor and monoaminergic systems have been indicated. Indeed, N/OFQ inhibits both serotonin and norepinephrine release and neuronal activity in both the locus coeruleus and dorsal raphe neurons (114, 116). The antidepressant effect of UFP-101 was prevented by pretreatment with *p*-chlorophenylalanine methyl ester, a serotonin synthesis inhibitor, whereas *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine, a neurotoxin for noradrenergic neurons, did not affect the antidepressant effect of UFP-101 (114). Therefore, serotonergic transmission may be involved in the antidepressant effects of NOP receptor antagonists, although the involvement of the noradrenergic system cannot be ruled out because the NOP receptor antagonist increased climbing behavior without changing swimming behavior in the forced swimming test (114), similar to the results obtained using norepinephrine reuptake inhibitors.

Banyu discovered the first small nonpeptide NOP receptor antagonist, J-113397, which has potent antagonist activity (IC_{50} = 5.6 nM) and high selectivity over opioid receptors, including mu, kappa and delta receptors (> 600-fold) (117). The resolved enantiomer of J-113397, J-112444 (**30**; Fig. 6), differed considerably in its affinity for the NOP receptor, with J-113397 having approximately 360-fold higher affinity than that measured for J-112444.

Japan Tobacco discovered novel 4-aminoquinoline-based NOP receptor antagonists (118). One of the optimum compounds, JTC-801 (**31**; Fig. 6), was confirmed to be a potent NOP receptor antagonist (K_i = 8.2 nM). JTC-801 displayed approximately 12.5-, 129- and 1,055-fold selectivity for the NOP receptor over mu, kappa and delta opioid receptors, respectively.

GlaxoSmithKline disclosed that SB-612111, which contains a common central piperidine core for NOP receptor ligands, was a potent antagonist at the NOP receptor (K_b = 5 nM), with significant selectivity relative to mu (174-fold), delta (6,391-fold) and kappa (486-fold) opioid receptors (119).

Conclusions and future directions

To date, several stress-related neuropeptides have been identified, and the relationship between some neuropeptide systems and depression/anxiety has been well demonstrated in animal models. Moreover, the efficacies of antagonists at tachykinin receptors, the CRF_1 receptor and the V_{1b} receptor have been evaluated in clinical trials. Given that stress may have a pivotal causal role in depression and anxiety and that neuropeptides are synthesized in rather restricted brain regions, with their levels changing upon stress exposure, the efficacy of most agonists/antagonists for neuropeptide receptors in animal models is a common and promising characteristic, suggesting their possible use as both antidepressants and anxiolytics (with the exception of the N/OFQ system) devoid of sedation or motor dysfunction, in marked contrast to the effects of benzodiazepines and monoamine reuptake inhibitors.

In addition to the above-mentioned neuropeptide receptors, numerous neuropeptide systems have been implicated in stress responses and have been proposed as potential therapeutic targets for depression and anxiety. Recently, several novel neuropeptides have been identified using a reverse pharmacology technique as endogenous ligands for formally cloned orphan GPCRs. Based on the distribution of peptide precursors and their receptors, as well as behavioral pharmacological studies using genetically engineered animals, some of the peptide systems have been shown to play a role in anxiety and depression. Among them, neuropeptides related to the sleep-wake cycle and feeding might be of particularly great interest, because depressive symptoms include abnormalities in appetite, sleep and circadian rhythms, and decreased REM latency is highly replicable in depression (120, 121). These are well exemplified by orexin, ghrelin, neuromedin U and, more recently, neuropeptide S, as all of these peptide systems have been reported to be related to stress and anxiety/depressive behaviors (122-125).

The list of neuropeptide systems related to depression and anxiety is growing. The validation of these neuropeptide systems in animal models using pharmacological and molecular tools, as well as mutant rodents, would greatly contribute to our understanding of the implications of each neuropeptide system. Eventually, clinical evaluation using small molecules (agonists/antagonists) for neuropeptide receptors that have excellent pharmacokinetic profiles could delineate whether each neuropeptide system could be an effective target for the treatment of depressive and anxiety disorders.

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