Drugs of the Future 2011, 36(6): 473-484

CCC: 0377-8282/2011
DOI: 10.1358/dof.2011.36.6.1653891





# THERAPEUTIC TARGETS FOR ANXIETY DISORDERS

*L.A. Sorbera, C. Dulsat and E. Rosa*

Thomson Reuters, Barcelona, Spain

## CONTENTS



## SUMMARY

*Anxiety disorders are a group of psychiatric illnesses characterized by extreme or pathological anxiety, resulting in alterations in mood or emotional tone. They are the most frequent of all mental disorders encountered in clinical practice and include generalized anxiety disorder, panic disorder, agoraphobia, obsessive–compulsive disorder, post-traumatic stress disorder, social anxiety disorder and specific phobias. Treatments for anxiety disorders are varied and depend on each individual case. In general, standard therapy includes benzodiazepines or tricyclic antidepressants targeting 5-hydroxytryptamine, dopamine, GABA, β-adrenoceptors, metabotropic glutamate, cholecystokinin, NMDA and opioid receptors, among others. However, anxiolytic therapy continues to be associated with serious and debilitating adverse effects. Researchers actively continue to search for effective treatments for anxiety disorders, with special attention given to the identification of novel targets for therapeutic intervention. This article presents those drug targets that are currently under active investigation for the treatment of anxiety disorders.*

## INTRODUCTION

Anxiety is defined as the pathological counterpart of normal fear and can be manifested by alterations in mood, thinking, behavior and physiological activity. In contrast to fear, which is an acute response to a known, external and well-defined threat, anxiety is a chronic response to an unknown, internal and vague threat. Anxiety disorders are the most frequent of all mental disorders encountered in clinical practice, and they are associated with significant comorbidity and often overlap with other mood, eating or substance abuse disorders. For example, up to 88% of men and 79% of women with post-traumatic stress disorder (PTSD) suffer from at least one other mood disorder and approximately 50% have three or more comorbid psychiatric disorders (1-3).

**Correspondence:** L.A. Sorbera, PhD. Thomson Reuters, Provença 398, 08025 Barcelona, Spain. E-mail: lisa.sorbera@thomsonreuters.com.

Several anxiety disorders have been identified, although they are all characterized by extreme or pathological anxiety as the principal cause of alterations in mood or emotional tone. They include generalized anxiety disorder (GAD), panic disorder, agoraphobia, obsessive–compulsive disorder (OCD), PTSD, social anxiety disorder and specific phobias. GAD is characterized by excessive, unfocused anxiety or worry lasting for more than 6 months. Symptoms may include feelings of threat, restlessness, irritability and tension, as well as sleep disturbances, palpitations, dry mouth, trembling and perspiration. Panic disorder is defined by recurrent attacks of anxiety that rapidly peak and result in a change in behavior; it is often poorly recognized in primary healthcare settings and left undertreated. Agoraphobia is a severe and pervasive anxiety triggered by being in situations from which escape would be difficult (e.g., airplanes), being alone outside one's home or being in crowded places. It is frequently detected as a comorbidity of panic disorder, and tends to be both underdiagnosed and undertreated. OCD is an anxiety disorder characterized by recurrent, intrusive images, impulses or thoughts that lead to significant anxiety or distress (obsessions), alone or associated with repetitive, ritualistic behaviors or mental acts performed to relieve anxiety (compulsions). Comorbidities are common and include depression, GAD, agoraphobia, panic disorder, social phobia and alcohol and/or drug dependence. PTSD is characterized by the following triad of symptoms: re-experiencing, avoidance and hyperarousal. The highest rates of PTSD for females and males are seen in crime victims (rape in particular) and combat exposure, respectively. In both genders, the sudden death of a loved one, a history of chronic and extreme abuse, trauma and exposure to acts of terrorism are also significant causes of PTSD. Social anxiety disorder, also known as social phobia, is a persistent and debilitating condition that is frequently associated with comorbidities. Subjects with social phobia have a marked and persistent fear of manifesting symptoms of anxiety (e.g., panic attacks) when in unfamiliar situations or when exposed to scrutiny. These lead to avoidance of situations that may cause embarrassment or humiliation and can potentially result in extreme isolation. Specific phobias are a group of persistent, excessive fears directed toward a specific object or situation (e.g., spiders, dogs, heights, needles, etc.). They affect as many as 12.5% of the population and can lead to a potentially maladaptive response characterized by avoidance of the object or situation and potentially severe anxiety reactions when confronted with said object or situation (1, 3-9).

The pathophysiology of anxiety appears to involve the amygdala, a major component of the limbic system located in the temporal lobe, which is responsible for the acquisition and expression of fear conditioning. The amygdala processes specific sensory input and assigns it an emotional significance. Abnormal amygdalic function may be responsible for anxiety, stress or phobias. Various factors are known to contribute to the likelihood of developing an anxiety disorder, including life experiences, psychological traits and genetic factors. Individuals vary significantly in the level of individual tolerance to anxiety and stress, such that an experience that is exhilarating for one person is anxiety-provoking for another. Gonadal hormones may also contribute to the pathogenesis of anxiety, which could account for the higher incidence of anxiety disorders among women. Other suggested risk factors include stress or trauma early in life, streptococcal bacterial infection and structural abnormalities in the brain. Some pharmaceuticals (e.g., CNS stimulants, antidepressants), medical disorders (i.e., hypertension, arrhythmia, anemia, hyper- or hypothyroidism, asthma, epilepsy, etc.) and psychiatric conditions (i.e., dementia, schizophrenia, substance abuse, etc.) may also cause symptoms of anxiety (1, 4, 10, 11).

Treatments for anxiety disorders are extremely varied and depend on each individual case. Typically, patients are treated with benzodiazepines or tricyclic antidepressants accompanied by psychotherapy. Benzodiazepines have a faster onset of effect, while antidepressants tend to have improved safety and tolerability. The efficacy of a given drug depends on the nature of the particular anxiety disorder being treated. Other therapeutic options include targeting 5-hydroxytryptamine (5-HT), dopamine, GABA, β-adrenoceptors, metabotropic glutamate, cholecystokinin (CCK), NMDA and opioid receptors, among others. However, anxiolytic therapy continues to be associated with serious and debilitating adverse effects, such as addiction, withdrawal problems and/or impaired cognitive and motor skills (1, 5, 8, 9, 12-14).

The search for effective treatment strategies for anxiety continues, with research focusing on the identification of novel targets for drug development. Those targets which are currently under active investigation for anxiety disorders are discussed below (Fig. 1). Table I provides a selection of products under active development for each target and Table II includes selected patents.

## TARGETS

### $\alpha_1/\alpha_2$-Adrenoceptors

$\alpha_1$-Adrenoceptors are a subtype of α-adrenoceptor that signal via $G_{\alpha q/11}$ proteins. Signaling involves phospholipase C (PLC)-mediated cleavage of phosphatidylinositol 4,5-bisphosphate ($PIP_2$), resulting in an increase in inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). DAG interacts with calcium channels of the endoplasmic and sarcoplasmic reticulum, increasing intracellular $Ca^{2+}$ release. Activation of this receptor type results in excitatory signals in the CNS and therapeutic antagonism of the receptor may be effective in treating anxiety and other psychiatric or neurological disorders. On the other hand, $\alpha_2$-adrenoceptors, another subtype of α-adrenoceptor, signal via $G_i/G_o$ proteins and exert inhibitory effects within the CNS. $\alpha_2$-Adrenoceptor agonists may therefore be effective in the treatment of anxiety (8, 15).

### β-Adrenoceptors

β-Adrenoceptors are G protein-coupled receptors (GPCRs) present in effector tissues that bind endogenous catecholamines, such as norepinephrine and epinephrine. Three isoforms have been described: $\beta_1$, $\beta_2$ and $\beta_3$. While $\beta_1$- and $\beta_2$-adrenoceptors are widely distributed, the distribution of $\beta_3$-adrenoceptors is predominantly in adipocytes. All three isoforms are coupled to $G_s$ proteins ($\beta_2$-adrenoceptors also couple to $G_i$). Studies have shown that psychosocial stress resulting in altered immune function and the development of psychological disorders, such as anxiety and depression, is mediated via β-adrenoceptors. Moreover, β-adrenoceptors may be upregulated in patients suffering from anxiety disorders. Antagonism of these receptors may therefore be effective in the treatment of anxiety disorders (16-18).

### AMPA receptor

The AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor is a non-NMDA-type ionotropic transmembrane receptor for the excitatory neurotransmitter glutamate that is involved in learning and memory; AMPA is the synthetic ligand for these receptors. Studies have shown that changes in the number of synaptic AMPA receptors may be responsible for synaptic plasticity (i.e., the neuronal mechanism required for learning and memory). AMPA receptor antagonists are being studied for the treatment of several respiratory, neurological and psychiatric disorders. Non-competitive AMPA receptor antagonists have been shown to potently suppress anxiety-like behavior (19-22).

### Carbonic anhydrases

Carbonic anhydrases (EC 4.2.1.1) are a family of zinc-containing enzymes that catalyze the conversion of $CO_2$ and $H_2O$ to $HCO_3^-$ and $H^+$, a reaction essential for many physiological processes, such as facilitating transport and elimination of $CO_2$ from tissues. Ten human isozymes have been identified: three cytosolic isozymes (CA-I, -II, -III), five membrane-bound isozymes (CA-IV, -VII, -IX, -XII, -XIV), one mitochondrial isozyme (CA-V) and one secreted salivary isozyme (CA-VII); there are also several related proteins which lack catalytic activity. The isozymes facilitate the intracellular diffusion of $CO_2$ and protons ($H^+$). Inhibition of carbonic anhydrases, particularly the cytosolic CA-II and membrane-bound CA-VII isoforms, has been shown to produce anxiolytic effects and may therefore be a therapeutic option in the treatment of anxiety disorders (5, 23).

### Cholecystokinin $CCK_2$ (CCK-B/gastrin) receptor

Cholecystokinin (CCK) is a linear peptide that is synthesized as a preprohormone and then proteolytically cleaved to generate a family of peptides having the same carboxy termini. Full biological activity is retained in CCK8 (8 amino acids), but peptides of 33, 38 and 59 amino acids are also produced. CCK peptides are secreted from mucosal epithelial cells in the first segment of the small intestine (duodenum) and are involved in facilitating digestion within the small intestine. CCK is also produced by neurons in the enteric nervous system and is widely distributed throughout the brain. CCK exerts its actions via two seven-transmembrane-spanning GPCRs: the $CCK_1$ receptor found abundantly on pancreatic acinar cells and the $CCK_2$ receptor found in brain and stomach. Activation of the $CCK_2$ receptor stimulates the phosphatidylinositol pathway, thus

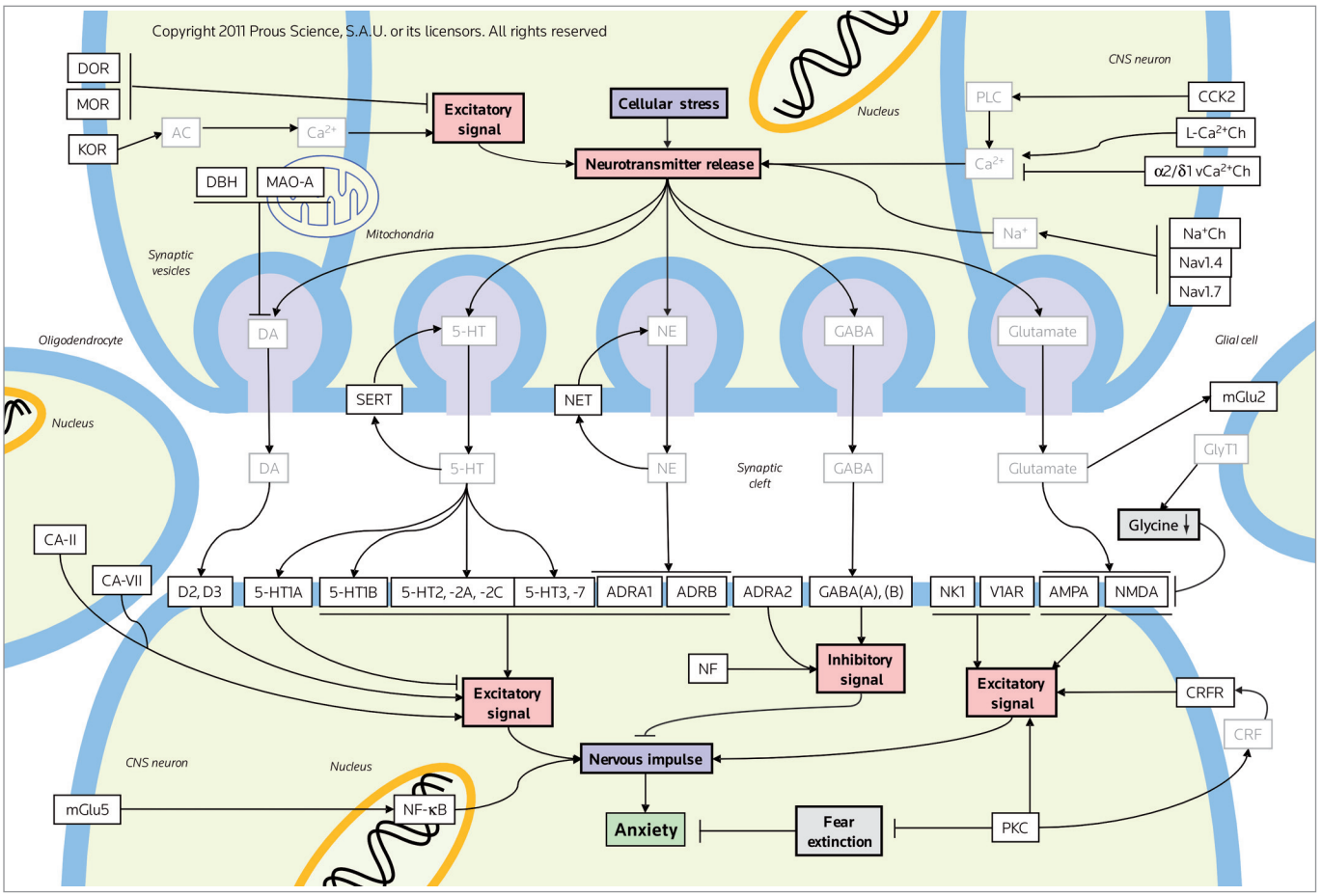


**Figure 1.** Anxiety targetscape. A diagram showing an overall cellular and molecular landscape or comprehensive network of connections among the current therapeutic targets for the treatment of anxiety and their biological actions. Gray or lighter symbols are targets that are not validated (i.e., targets not associated with a product that is currently under active development for anxiety). Abbreviations: $\alpha 2/\sigma$ $vCa^{2+}$ Ch: voltage-gated calcium channel $\alpha 2/\sigma$ subunit; AC: adenylate cyclase; ADRA: $\alpha$-adrenoceptor; ADRB: $\beta$-adrenoceptor; AMPA: AMPA receptor; CA: carbonic anhydrase; CCK2: cholecystokinin $CCK_2$ receptor; CRF: corticotropin-releasing factor; CRFR: corticotropin-releasing factor receptor; D2: dopamine $D_2$ receptor; D3: dopamine $D_3$ receptor; DA: dopamine; DBH: dopamine $\beta$-hydroxylase; DOR: $\sigma$ opioid receptor; GABA: $\gamma$-aminobutyric acid; GABA(A): $GABA_A$ receptor; GABA(B): $GABA_B$ receptor; GlyT1: sodium- and chloride-dependent glycine transporter 2; 5-HT: serotonin; 5-HT1A/1B/2/2A/2C/3/7: $5\text{-}HT_{1A/1B/2/2A72C/3/7}$ receptors; KOR: $\kappa$ opioid receptor; L-$Ca^{2+}$ Ch: L-type calcium channel; MAO-A: monoamine oxidase type A; mGlu2/5: metabotropic glutamate $mGlu_{2/5}$ receptors; MOR: $\mu$ opioid receptor; $Na^+$ Ch: sodium channel; Nav1.4/1.7: voltage-gated sodium channels $Na_v1.4/1.7$; NE: norepinephrine; NET: norepinephrine transporter; NF: neurotrophic factor; NF-$\kappa$B: nuclear factor NF-$\kappa$B; NK1: tachykinin $NK_1$ receptor; NMDA: NMDA receptor; PKC: protein kinase C; PLC: phospholipase C; SERT: sodium-dependent serotonin transporter; V1AR: vasopressin $V_{1A}$ receptor.

increasing intracellular $Ca^{2+}$ mobilization and promoting cell growth. The CCKergic system has been implicated in anxiety-, panic- and stress-related behavior. Studies have shown that selective $CCK_2$ receptor agonists produce anxiogenic-like effects, while $CCK_2$ receptor antagonists exert anxiolytic-like responses in several models of anxiety (13, 24-28).

### Corticotropin-releasing factor (CRF) receptors

Corticotropin-releasing factor (CRF), also known as corticotropin-releasing hormone (CRH), comprises a family of hormones (CRF, urocortin I, II and III), receptors ($CRF_1$ and $CRF_2$) and a CRF-binding protein. CRF is released from the hypothalamus to stimulate pituitary release of ACTH and its release is inhibited by glucocorticoids. CRF is suspected to play a major role in mediating stress responses or stress-induced disorders. It is overproduced in clinically depressed patients and may be dysregulated in individuals with anxiety disorders. Thus, antagonism of CRF receptors may be effective in the treatment of anxiety and depression (9, 13, 24, 29).

### Dopamine $\beta$-hydroxylase (DBH)

DBH (EC 1.14.17.1), also known as dopamine $\beta$-monooxygenase, is an enzyme that converts dopamine to norepinephrine in the synaptic vesicles of postganglionic sympathetic neurons. Norepinephrine has been implicated as playing a crucial role in several psychiatric disor-

***Table I.*** *Selected targets and products launched or being actively investigated for anxiety (from Thomson Reuters Integrity [SM]).*

| Target name | Product | Source | Phase |
|---|---|---|---|
| $\alpha_1$-Adrenoceptor | Prazosin hydrochloride | Yale University | III |
| $\alpha_2$-Adrenoceptor | Ecstasy | Multidisciplinary Association for Psychedelic Studies | II |
| β-Adrenoceptor | Propranolol hydrochloride | AstraZeneca | Launched |
| | Oxprenolol hydrochloride | Novartis | Launched |
| AMPA receptor | Topiramate | University of Florida | III |
| Carbonic anhydrase 2 (CA-II) | Topiramate | University of Florida | III |
| Carbonic anhydrase 7 (CA-VII) | Sulpiride | Sanofi | L-1968 |
| Cholecystokinin $CCK_2$ (CCKB/gastrin) receptor | Itriglumide | Rottapharm | II |
| Corticotropin-releasing factor $CRF_1$ receptor | Verucerfont | GlaxoSmithKline | II |
| | Pivagabine | CeNeRx BioPharma | I/II |
| | GSK-586529 | GlaxoSmithKline | I |
| | SSR-125543 | Sanofi | I |
| Corticotropin-releasing factor $CRF_2$ receptor | Pivagabine | CeNeRx BioPharma | I/II |
| Dopamine β-hydroxylase | Nepicastat hydrochloride | Synosia Therapeutics | II |
| Dopamine $D_2$ receptor | Trifluoperazine hydrochloride | GlaxoSmithKline | L-1959 |
| | Sulpiride | Sanofi | L-1968 |
| | Fluphenazine hydrochloride | Bristol-Myers Squibb/Sanofi | Launched |
| Dopamine $D_3$ receptor (isoform 1) | Pramipexole hydrochloride | GlaxoSmithKline | I |
| $GABA_A$ receptor | Chlordiazepoxide hydrochloride | Roche | L-1960 |
| $GABA_B$ receptor | Midazolam maleate | NovaDel Pharma | Preclinical |
| 5-$HT_{1A}$ receptor | Buspirone hydrochloride | Bristol-Myers Squibb | L-1985 |
| | Tandospirone citrate | Dainippon Sumitomo Pharma | L-1996 |
| | Lu-AA-21004 | Lundbeck/Takeda | III |
| | Osemozotan hydrochloride | MediciNova | II/III |
| | TGWOOAD/AA | Fabre-Kramer | II |
| | GSK-163090 | GlaxoSmithKline | I |
| 5-$HT_{1B}$ receptor | GSK-163090 | GlaxoSmithKline | I |
| 5-$HT_2$ receptor | TGWOOAD/AA | Fabre-Kramer | II |
| | Ecstasy | Multidisciplinary Association for Psychedelic Studies | II |
| 5-$HT_{2A}$ receptor | Carpipramine | Pierre Fabre | L-1997 |
| | Quetiapine fumarate | AstraZeneca | Pre-reg |
| 5-$HT_{2C}$ receptor | Cyamemazine | Sanofi | L-1972 |
| 5-$HT_3$ receptor | Cyamemazine | Sanofi | L-1972 |
| | Lu-AA-21004 | Lundbeck/Takeda | III |
| 5-$HT_7$ receptor | Lu-AA-21004 | Lundbeck/Takeda | III |
| Monoamine oxidase type A (MAO-A) | CX-157 | CeNeRx BioPharma | I |
| NMDA receptor | Cycloserine | Emory University | I |
| | Memantine hydrochloride | SUNY | III |
| | D-Serine | Hebrew University | II |
| Nuclear factor NF-κB | *N*-Acetylcysteine | Yale University | II |
| σ Opioid (DOR) receptor | Sedatin | Immunotech Developments | Preclinical |
| | AZD-2327 | AstraZeneca | II |
| μ Opioid (MOR) receptor | Sedatin | Immunotech Developments | Preclinical |

*Continued*

***Table I.*** *Cont. Selected targets and products launched or being actively investigated for anxiety (from Thomson Reuters Integrity SM).*

| Target name | Product | Source | Phase |
|---|---|---|---|
| Sodium-dependent serotonin transporter (SERT) | Clomipramine hydrochloride | Novartis | L-1967 |
| | Fluoxetine hydrochloride | Lilly | L-1987 |
| | Duloxetine hydrochloride | Lilly | L-2007 |
| | Diazepam | Roche | L-1961 |
| | Oxacepam | Wyeth Pharmaceuticals | L-1966 |
| | Clorazepate dipotassium | Sanofi | L-1968 |
| | Lorazepam | Pfizer | L-1972 |
| | Bromazepam | Roche | L-1975 |
| | Clobazam | Sanofi | L-1975 |
| | Clonazepam | Roche | L-1975 |
| | Clotiazepam | Esteve | L-1979 |
| | Prazepam | Pfizer | L-1980 |
| | Ethyl loflazepate | Sanofi | L-1982 |
| | Halazepam | Merck & Co. | L-1982 |
| | Alprazolam | Pfizer | L-1983 |
| | Nordazepam | Bouchara-Recordati | L-1985 |
| | Tofizopam | Mochida | L-1986 |
| | Ketazolam | Novartis | L-1987 |
| | Etizolam | Fournier Pierrel Farma | L-1989 |
| | Bentazepam | Abbot | Launched |
| | Brotizolam | Boehringer Ingelheim | Launched |
| | Sufentanil citrate/triazolam | AcelRx | II |
| | NSD-721 | NeuroSearch | I |
| | TGCS01AA | Fabre-Kramer | Preclinical |
| Tachykinin $NK_1$ receptor | Orvepitant | GlaxoSmithKline | II |
| | Vofopitant hydrochloride | National Institute of Mental Health | II |
| Vasopressin $V_{1A}$ receptor | SRX-246 | Azevan | I |
| Voltage-gated calcium channel $\alpha 2/\sigma$ subunit | Pregabalin | Pfizer | L-2006 |
| Voltage-gated sodium channel | Trifluoperazine hydrochloride | GlaxoSmithKline | L-1959 |
| | Rufinamide | Synosia Therapeutics | II |
| Voltage-gated sodium channel $Na_v1.4$ | Trifluoperazine hydrochloride | GlaxoSmithKline | L-1959 |
| Voltage-gated sodium channel $Na_v1.7$ | Trifluoperazine hydrochloride | GlaxoSmithKline | L-1959 |

ders, including anxiety (PTSD in particular) and substance abuse, and selective antagonism of DBH could be effective in the treatment of these disorders (30, 31).

### Dopamine receptors

The dopamine $D_2$ receptor is a seven-transmembrane-spanning GPCR protein ($G_i/G_o$) that, like the $D_1$ receptor, binds dopamine present in the CNS in basal ganglia. The $D_3$ receptor is the longest isoform of the $D_2$-like receptor subfamily. Both $D_2$ and $D_3$ receptors inhibit cAMP synthesis by coupling to $G_{\alpha i/o}$ and also regulate $Ca^{2+}$ and $K^+$ ion channels via PLC when it forms hetero-oligomers, particularly with the $D_1$ receptor. This $D_1$–$D_2$ receptor hetero-oligomer has been proposed to facilitate a distinctive dopamine-mediated $Ca^{2+}$ signal, with important effects on synaptic plasticity. In disorders such as anxiety, bipolar disorder and schizophrenia, transmission in discrete dopamine pathways may involve hypoactivation–hyperactivation of dopamine receptors, particularly the $D_2$ and $D_3$ subtypes. However, full agonists or pure $D_2$ receptor antagonists may not be optimal therapeutic approaches due to their inability to restore the aberrant dopamine pathways to a normal level of basal tone. $D_2$ receptor partial agonists, on the other hand, may stabilize activity in dopamine pathways by dampening excessive and/or by restoring deficient $D_2$ receptor stimulation and achieving a desired level of basal activity. The $D_2$ receptor is currently one of the most widely studied targets for antipsychotic drugs and $D_2$ receptor antagonists and partial agonists have been validated for the treatment of psychiatric diseases, such as schizophrenia, anxiety, depression and bipolar disorder. Studies have shown a reduction in the expression of dopamine $D_3$ receptor mRNA in individuals suffering from anxiety, schizophrenia and bipolar disorder, thus suggesting a distorted homeostasis of dopamine receptor subtypes in these illnesses. $D_3$ receptor modulators have been shown to exert antipsychotic properties and are under development for the treatment of diseases such as schizophrenia, depression, anxiety and bipolar disorder (31-37).

***Table II.*** *Selected patents for targets being validated for anxiety (from Thomson Reuters Integrity SM).*

| Target | Patent | Source | Phase |
|---|---|---|---|
| AMPA receptor | WO 2010038167 | Pfizer | Biological testing |
| | WO 2010041162 | Pfizer | Biological testing |
| N-type calcium channel $Ca_v2.2$ | WO 2010001179 | Lectus Therapeutics | Biological testing |
| | WO 2010035032 | Lectus Therapeutics | Biological testing |
| Voltage-gated calcium channel $\alpha2/\sigma$ subunit | WO 2010084797 | Daiichi Sankyo | Biological testing |
| | WO 2010084798 | Daiichi Sankyo | Biological testing |
| Corticotopin-releasing factor $CRF_1$ receptor | WO 2010014687 | Bristol-Myers Squibb | Biological testing |
| | WO 2010015628 | Novartis | Biological testing |
| | WO 2010015655 | Novartis | Biological testing |
| | WO 2010096426 | Emory University | Biological testing |
| Dopamine $D_3$ receptor | WO 2010031735 | F. Hoffmann-La Roche | Biological testing |
| $GABA_A$ receptor | WO 2010123441 | AstraZeneca | Biological testing |
| | WO 2010025407 | Concert Pharmaceuticals | Biological testing |
| | WO 2010055124 | NeuroSearch | Biological testing |
| | WO 2010055125 | NeuroSearch | Biological testing |
| | WO 2010055126 | NeuroSearch | Biological testing |
| | WO 2010055127 | NeuroSearch | Biological testing |
| | WO 2010055128 | NeuroSearch | Biological testing |
| | WO 2010055129 | NeuroSearch | Biological testing |
| | WO 2010055130 | NeuroSearch | Biological testing |
| | WO 2010055131 | NeuroSearch | Biological testing |
| | WO 2010055132 | NeuroSearch | Biological testing |
| | WO 2010055133 | NeuroSearch | Biological testing |
| | WO 2010084182 | Universitaet Wien | Biological testing |
| 5-$HT_2$ receptor | JP 2010155802 | RaQualia Pharma | Biological testing |
| | WO 2010031735 | F. Hoffmann-La Roche | Biological testing |
| | WO 2010059393 | Janssen Pharmaceutica | Biological testing |
| | WO 2010080357 | Boehringer Ingelheim Pharma | Biological testing |
| 5-$HT_3$ receptor | WO 2010060037 | Auspex Pharmaceuticals | Biological testing |
| 5-$HT_7$ receptor | WO 2010059390 | Janssen Pharmaceutica | Biological testing |
| | WO 2010059393 | Janssen Pharmaceutica | Biological testing |
| Metabotropic glutamate $mGlu_2$ receptor | WO 2010025890 | Addex Pharma/Ortho-McNeil-Janssen Pharmaceuticals | Biological testing |
| | WO 2010060589 | Addex Pharma/Ortho-McNeil-Janssen Pharmaceuticals | Biological testing |
| Metabotropic glutamate $mGlu_5$ receptor | WO 2010011570 | H. Lundbeck | Biological testing |
| | WO 2010049366 | GlaxoSmithKline | Biological testing |
| | WO 2010063487 | Merz Pharma | Biological testing |
| | WO 2010123451 | AstraZenec | Biological testing |
| | WO 2010130423 | Addex Pharma/Ortho-McNeil-Janssen Pharmaceuticals | Biological testing |
| NMDA receptor | WO 2010032123 | Biotechnology Research Corp. (BRC) | Biological testing |
| | WO 2010088408 | Emory University | Biological testing |
| | WO 2010088414 | Emory University | Biological testing |
| Sodium-dependent serotonin transporter (SERT) | WO 2010006945 | F. Hoffmann-La Roche | Biological testing |
| Tachykinin $NK_1$ receptor | US 2010137332 | Auspex Pharmaceuticas | Biological testing |
| | WO 2010007032 | Glaxo Wellcome | Biological testing |
| | WO 2010015626 | GlaxoSmithKline | Biological testing |
| | WO 2010032856 | Takeda Pharmaceutical | Biological testing |
| Vasopressin $V_{1A}$ receptor | WO 2010060836 | F. Hoffmann-La Roche | Biological testing |

### $GABA_A$ and $GABA_B$ receptors

GABA (γ-aminobutyric acid) is the major inhibitory neurotransmitter in the brain and spinal cord that acts via $GABA_A$, $GABA_B$ and $GABA_C$ receptors. $GABA_A$ receptors are widely distributed throughout the CNS. They are ionotropic and can be activated by several different compounds. They are suggested to be involved in the modulation of vigilance, anxiety, muscle tension, epileptogenic activity and memory functions. $GABA_B$ receptors are also widely distributed throughout the CNS and in peripheral autonomic terminals and are metabotropic, distinguishing them from ionotropic $GABA_A$ receptors. $GABA_B$ receptors are coupled to G proteins and activation causes a decrease in $Ca^{2+}$ and an increase in $K^+$ influx through the membrane. $GABA_B$-induced changes in $Ca^{2+}$ conductance are thought to be associated with the P/Q-type and N-type, and possibly L-type, $Ca^{2+}$ channels and with several different types of $K^+$ channels. GABA transmission has been shown to be disrupted in patients suffering from depression, bipolar disorder and anxiety, and $GABA_A$ and $GABA_B$ receptor modulators may be effective in improving these conditions (19, 25, 38-44).

### 5-HT receptors

Serotonin (5-hydroxytryptamine, 5-HT) is a biogenic amine neurotransmitter synthesized in neurons of the raphe nucleus in the brain stem and present in high concentrations in the hypothalamus and basal ganglia. The serotonergic system innervates almost all areas of the brain and spinal cord. 5-HT is involved in a wide variety of behaviors, including affective state, sleep–wakefulness, feeding behavior, sexual behavior, temperature regulation, circadian rhythmicity, locomotion, neuroendocrine secretion, pain, hallucinogenesis and memory. $5\text{-}HT_{1A}$ and $5\text{-}HT_{1B}$ receptors are GPCRs ($G_i/G_o$) that signal via a decrease in intracellular levels of cAMP. In contrast, the $5\text{-}HT_7$ receptor subtype is coupled to $G_s$, which activates adenylate cyclase and consequently increases intracellular levels of cAMP. The $5\text{-}HT_{2A}$ and $5\text{-}HT_{2C}$ receptors are also GPCRs that couple to the $G_{\alpha q}$ signal transduction pathway. $G_{\alpha q}$ stimulates PLC activity, which subsequently causes the release of DAG and IP3; IP3 then activates protein kinase C (PKC) and induces $Ca^{2+}$ release. The $5\text{-}HT_3$ receptor is a ligand-gated ion channel for serotonin involved in synaptic transmission. Activation of the $5\text{-}HT_3$ receptor can regulate the release of other neurotransmitters, such as dopamine, acetylcholine, GABA and norepinephrine, modulating the anxiolytic, antipsychotic and procognitive effects of these compounds. 5-HT receptor subtypes have been implicated in the pathogenesis of anxiety, as well as several other psychiatric and neurological disorders, including Alzheimer's disease, schizophrenia, depression, anxiety and bipolar disorder (9, 12, 45-64).

### Kainate receptors

Kainate receptors, also known as ionotropic kainate glutamate receptors (GluRs), are $K^+$-type excitatory ion channels that are gated by the neurotransmitter kainate. These receptors overlap considerably with AMPA receptors and are often grouped together and referred to as AMPA/kainate receptors. Several of the kainate receptor subtypes are involved in synaptic transmission and modulate the presynaptic release of glutamate and GABA. Kainate receptor sensitivity to glutamate may mediate the pathophysiological expression of anxiety states. Moreover, studies have shown that antagonism of kainate receptors results in anxiolytic-like effects. Thus, the selective targeting of kainate receptors may be effective in the treatment of anxiety disorders (19, 43, 65).

### Metabotropic glutamate $mGlu_2$ and $mGlu_5$ receptors

The $mGlu_2$ and $mGlu_5$ receptors are GPCRs ($G_i/G_o$ and $G_q$, respectively) for glutamate (type II and I, respectively), the main, ubiquitously distributed excitatory neurotransmitter in the CNS. The $mGlu_2$ receptor signals via inhibition of the cAMP cascade and serves to modulate the function of other receptors (such as the NMDA receptor), changing the synapse's excitability. Activation of this receptor decreases glutamate release from presynaptic neurons and thus modulates NMDA neurotransmission. Deregulation of the $mGlu_2$ receptor is implicated in schizophrenia and anxiety. The $mGlu_5$ receptor activates PLC and is also associated with $Na^+$ and $K^+$ channels and serves to modulate the function of other receptors (e.g., NMDA receptors), also changing the excitability of the synapse. The $mGlu_5$ receptor can increase both excitatory presynaptic potentials and inhibitory postsynaptic potentials. Activation of $mGlu_5$ receptors by selective agonists potentiates NMDA-induced responses and may be effective as a therapy for diseases involving NMDA hypofunction, such as schizophrenia, depression and anxiety (19, 66-70).

### Monoamine oxidase type A (MAO-A)

Monoamine oxidases are involved in the catabolism of the neurotransmitters serotonin, norepinephrine and dopamine (MAO-A) and dietary amines such as phenylethylamine (MAO-B), which are important for maintaining normal balanced levels of dopamine. Both MAO-A and MAO-B in the brain have been implicated in the etiology of several neurological and psychiatric disorders. Selective inhibition of brain MAO-A has been shown to significantly increase levels of dopamine, norepinephrine and serotonin in the striatum and hippocampus, and improve cognition, as well as attenuating depression and anxiety (8, 12, 23).

### Neurotrophic factors (NFs)

Neurotrophic factors (NFs) are a family of proteins that mediate the maintenance and survival of neurons. Members include brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), glial cell line-derived neurotrophic factor (GDNF) and nerve growth factor (NGF). NFs regulate neuronal growth via modulation of associated metabolic functions (i.e., protein synthesis) and neurotransmitter production. They also play a pivotal role in brain development and synaptic plasticity. NFs may be effective therapeutic targets for several neurodegenerative diseases, as well as psychiatric disorders, including stress and anxiety (19, 71-73).

### Norepinephrine transporter (NET)

The norepinephrine transporter (NET; sodium-dependent noradrenaline transporter, solute carrier family 6 member 2) is an $Na^+$-coupled transporter for norepinephrine. Monoamine transporters, in general, act in neurons by sequestering monoamines from nerve terminals. The NET regulates norepinephrine signaling in the central and peripheral nervous system by mediating its clearance and mod-

ulating its presence in the synaptic cleft. Selective NET inhibitors increase norepinephrine in the brain and cause secondary increases in prefrontal dopamine. NET inhibitors are being investigated for the treatment of several neurological and psychiatric disorders related to norepinephrine or dopamine deregulation, including schizophrenia, depression and anxiety (8, 12, 30, 31, 74, 75).

### NMDA receptor

The NMDA (*N*-methyl-D-aspartate) receptor is a subtype of the glutamate receptors that binds the excitotoxic amino acid NMDA in neurons. Activation of the NMDA receptor results in the opening of an associated ion channel pore, allowing influx of $Na^+$, $K^+$ and $Ca^{2+}$, of which the latter is thought to play a critical role in synaptic plasticity. The receptor mediates long-term potentiation of the signaling involved in learning, memory and cognition. Synaptic overactivity results in excessive glutamate release, thus overstimulating postsynaptic cell membrane receptors (i.e., NMDA receptor), which, upon activation, open associated ion channel pores and increase ion influx. The consequence is neuronal cell injury and death. The glutamate system appears to play a crucial role in the pathophysiology and treatment of anxiety disorders and antagonism of the NMDA receptor may be an effective treatment option (19, 29, 40).

### Nuclear factor NF-κB

Nuclear factor NF-κB (NF-κB) is a protein transcription factor and intracellular mediator of the inflammatory cascade involved in the generation of adhesion molecules (ICAM-1, VCAM-1), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), cytokines (e.g., IL-1β, IL-2, TNF-α, IL-6, IFN-γ) and chemokines (e.g., IL-8). Other genes that are regulated by NF-κB include those encoding the IL-2 receptor, the IL-12 p40 subunit and proto-oncogene c-Myc. NF-κB has been implicated in various aspects of neuroplasticity, including long-term potentiation, and cellular apoptosis and differentiation. It is active in neurons and studies have shown that altered NF-κB transcriptional activity modifies neuronal circuitry responsible for emotional behavior. Thus, targeting of NF-κB may be effective in the treatment of mood disorders and anxiety (76, 77).

### Opioid receptors

Opioid receptors are found in regions of the brain that bind morphine, as well as in areas that are unrelated (e.g., striatum) and related (e.g., along the aqueduct of Sylvius) to pain. Subtypes include μ (MOR), κ (KOR), δ (DOR) and NOP (ORL1) receptors, all of which have been shown to have antinociceptive effects. Natural ligands for these receptors are the opiate peptide neurotransmitters (derived from proopiomelanocortin [POMC], proenkephalin, prodynorphin or pronociceptin/orphanin FQ), although opiates (e.g., morphine) are potent agonists that mimic the action of the natural transmitters. They are widely distributed in other areas of the body, including the gut. κ Opioid receptors have been implicated in several opioid-mediated neurological and psychiatric functions, and antagonism of these receptors may be beneficial in treating such psychiatric conditions as anxiety and depression. The μ opioid receptor is expressed both pre- and postsynaptically and can restimulate or disinhibit the release of presynaptic GABA. μ Opioid receptors inhibit neurotransmitter release by reducing $Ca^{2+}$ influx and enhancing $K^+$ conductance. They are specific receptors for β-endorphin, and their activation has been shown to be involved in the regulation of behavioral responses in psychiatric conditions such as anxiety. Agonism of this receptor could be effective in treating such conditions. δ Opioid receptors are also implicated in regulating anxiety- and depression-related behaviors and δ receptor agonists have been shown to induce anxiolytic-like effects (78-85).

### Protein kinase C (PKC)

PKC is a family of enzymes (EC 2.7.11) that phosphorylates proteins on serine or threonine residues, usually in the presence of calcium. PKCs are activated by receptor-mediated hydrolysis of membrane phospholipids, which are involved in intracellular signaling for cell growth. The classical PKCs (α, β1, β2 and γ) are $Ca^{2+}$-dependent and can be activated endogenously by diacylglycerol (DAG) or non-physiologically by phorbol esters, although the specific physiological substrates for these enzymes are not yet known; several $Ca^2$-independent isoforms have also been identified. PKCγ is exclusively expressed in neurons and is involved in various neuronal functions. Among other actions, PKC regulates the intracellular trafficking of the dopamine $D_3$ receptor in the CNS through sequestration and further desensitization by phosphorylation. The dopamine $D_3$ receptor is expressed mostly in parts of the brain that control emotional behaviors and its deregulation has been involved in the pathogenesis of psychiatric diseases, such as anxiety, schizophrenia and bipolar disorder (86-89).

### Sodium-dependent serotonin transporter

The sodium-dependent serotonin transporter (solute carrier family 6 member 4, 5HTT, SERT) is a 5-HT transporter that terminates serotonergic transmission. It is a membrane uptake carrier dependent on $Na^+$ and $Cl^-$ that transports 5-HT from the extracellular synaptic space back to the inside of 5-HT nerve terminals. SERT inhibitors increase extracellular concentrations of serotonin and amplify signals sent by 5-HT neurons. Polymorphic regions in the SERT promoter and variations in its gene *5-HTTLPR* have been implicated in neuropsychiatric and mood disorders. Agents that block SERT as selective 5-HT reuptake inhibitors may be effective in the treatment of diseases affected by altered 5-HT neurotransmission, such as anxiety, bipolar disorder and schizophrenia (8, 12, 74, 75, 90-92).

### Tachykinin $NK_1$ receptor

Tachykinins are a group of polypeptides, or neurokinins, including substance P, neurokinin A (NKA) and neurokinin B (NKB), which share four of five carboxyl terminal amino acids (Phe-Xaa-Gly-Leu-Met-$NH_2$). They are localized in both the central and peripheral nervous systems and cause hypotension, contraction of the smooth muscle of the gut and bladder, and secretion of saliva. The effects of the tachykinins are mediated via three tachykinin receptor subtypes, $NK_1$, $NK_2$ and $NK_3$, which are members of the superfamily of seven-transmembrane-spanning GPCRs. Tachykinin transmission has been shown to be upregulated in patients suffering from anxiety and stress-related disorders, and $NK_1$ receptor antagonists may be effective in the treatment of these disorders (9, 13, 93-96).

### Vasopressin $V_{1A}$ receptor

The vasopressin $V_{1A}$ receptor is a GPCR for arginine vasopressin (AVP), which, upon ligand binding, activates the phosphatidylinositol-calcium second messenger system. $V_{1A}$ receptors are widely distributed in the CNS, where vasopressin regulates social behavior, anxiety and mood changes. Antagonism of the vasopressin $V_{1A}$ receptor may be a therapeutic strategy for treating psychiatric conditions, such as anxiety and depression (97-99).

### Voltage-gated calcium channels

The L-type calcium channel is a large transmembrane ion channel (voltage-gated calcium channel) with selective permeability for calcium ions. These channels are essential for neuronal signal transmission and intracellular signal transduction and are structurally related to T-type calcium channels, exhibit sustained conductance, are slow inactivating and are regulated by cAMP-dependent protein kinase (e.g., phosphorylation enhances the probability of channel opening). They are found on skeletal, cardiac and smooth muscle cells and within the nervous system, where they are expressed on neurons and neuroendocrine cells. It has been suggested that modulation of intracellular calcium levels by voltage-gated calcium channels may be involved in neuronal death. In several psychiatric disorders, including anxiety, administration of antidepressant medications, behavioral sensitization processes and neuronal calcium dysregulation can all lead to apoptosis of critical brain circuitry that regulates emotion. Thus, targeting dysregulation in calcium levels in the CNS by administering L-type calcium channel blockers may potentially alter the progression of anxiety, depression and bipolar disorder. The $\alpha 2/\delta$ subunit of voltage-gated calcium channels regulates calcium current density and activation/inactivation kinetics of the channel. $\alpha 2/\delta$ is the regulatory subunit for the P/Q-type (*CACNA1A*), N-type (*CACNA1B*), L-type (*CACNA1C* or *CACNA1D*) and T-type calcium channels (*CACNA1G*). Overexpression of the $\alpha 2/\delta$ subunit may induce apoptosis. Activation is thus considered a therapeutic option for the treatment of various psychiatric and neurological disorders, including anxiety and depression (8, 12, 100-102).

### Voltage-gated sodium channels

The sodium channel is a plasma membrane-bound ion channel permeable to $Na^+$. The channels are ubiquitous and are classified as either voltage-gated (expressed on central and peripheral neurons, skeletal muscle, cardiac myocytes) or ligand-gated, as are nicotinic receptors in neuromuscular junctions that bind acetylcholine. The fast voltage-gated sodium channel is composed of an $\alpha$ and $\beta$ subunit. The $\alpha$ subunit (including $Na_v1.4$ and 1.7) contains four repeat domains (I-IV), each containing six membrane-spanning regions (S1-S6); the S4 segment is the voltage sensor. The channel's voltage sensitivity is mediated by positive amino acids located at every third position. When stimulated by an alteration in transmembrane voltage, this region moves toward the extracellular side of the cell membrane and the channel becomes more permeable to ions. The ions are conducted through a pore, which can be broken into two regions comprising a largely extracellular portion of "P-loops", which is responsible for ion selectivity, and the more cytoplasmic portion, formed by the combined S5 and S6 regions of the four domains. The region that links domains III and IV serves to physically plug or block the channel after extended activation, thereby inactivating it. Inhibition of voltage-gated sodium channels results in stabilization of neuronal membranes and the subsequent modulation of presynaptic transmitter release of excitatory amino acids (e.g., glutamate). Thus, sodium channel regulation is important in diseases associated with neurotransmitter deregulation and voltage-gated sodium channel blockers may have potential for use in the treatment of various neurological and psychiatric disorders, including anxiety (103-106).

## DISCLOSURES

The authors state no conflicts of interest.

## REFERENCES


1. Thomson Reuters Integrity Disease Briefings: Anxiety (online publication). Updated 2011.
2. Hidalgo, R.B., Davidson, J.R. *Selective serotonin reuptake inhibitors in post-traumatic stress disorder.* J Psychopharmacol 2000, 14(1): 70-6.
3. Stanley, M.A., Beck, J.G. *Anxiety disorders*. Clin Psychol Rev 2000, 20(6): 731-54.
4. Aupperle, R.L., Paulus, M.P. *Neural systems underlying approach and avoidance in anxiety disorders.* Dialogues Clin Neurosci 2010, 12(4): 517-31.
5. Durant, C., Christmas, D., Nutt, D. *The pharmacology of anxiety*. Curr Top Behav Neurosci 2010, 2: 303-30.
6. Pêgo, J.M., Sousa, J.C., Almeida, O.F., Sousa, N. *Stress and the neuroendocrinology of anxiety disorders*. Curr Top Behav Neurosci 2010, 2: 97-117.
7. Allgulander, C. *Novel approaches to treatment of generalized anxiety disorder*. Curr Opin Psychiatry 2010, 23(1): 37-42.
8. Ravindran, L.N., Stein, M.B. *The pharmacologic treatment of anxiety disorders: A review of progress.* J Clin Psychiatry 2010, 71(7): 839-54.
9. Sheehan, D.V., Sheehan, K.H. *Current approaches to the pharmacologic treatment of anxiety disorders.* Psychopharmacol Bull 2007, 40(1): 98-109.
10. Garakani, A., Matthew, S.J., Charney, D.S. *Neurobiology of anxiety disorders and implications for treatment.* Mt Sinai J Med 2006, 73(7): 941-9.
11. Wand, G. *The anxious amygdala: CREB signaling and predisposition to anxiety and alcoholism*. J Clin Invest 2005, 115(10): 2697-9.
12. Schneier, F.R. *Pharmacotherapy of social anxiety disorder*. Expert Opin Pharmacother 2011, 12(4): 615-25.
13. Steckler, T. *Developing small molecule nonpeptidergic drugs for the treatment of anxiety disorders: Is the challenge still ahead?* Curr Top Behav Neurosci 2010, 2: 415-28.
14. Cloos, J.M., Ferreira, V. *Current use of benzodiazepines in anxiety disorders*. Curr Opin Psychiatry 2009, 22(1): 90-5.
15. Mansur, S.S., Terenzi, M.G., Neto, J.M., Faria, M.S., Paschoalini, M.A. *Changes in food intake and anxiety-like behaviors after clonidine injected into the median raphe nucleus*. Behav Brain Res 2010, 212(1): 71-7.
16. Fu, A., Li, X., Zhao, B. *Role of beta1-adrenoceptor in the basolateral amygdala of rats with anxiety-like behavior*. Brain Res 2008, 1211: 85-92.
17. Lee, I.S., Kim, K.J., Kang, E.H., Yu, B.H. *Beta-adrenoceptor affinity as a biological predictor of treatment response to paroxetine in patients with acute panic disorder*. J Affect Disord 2008, 110(1-2): 156-60.
18. Schank, J.R., Liles, L.C., Weinshenker, D. *Norepinephrine signaling through beta-adrenergic receptors is critical for expression of cocaine-induced anxiety*. Biol Psychiatry 2008, 63(11): 1007-12.

19. Kaplan, G.B., Moore, K.A. *The use of cognitive enhancers in animal models of fear extinction*. Pharmacol Biochem Behav 2011, Epub ahead of print.
20. Yamada, D., Wada, K., Sekiguchi, M. *Facilitating actions of an AMPA receptor potentiator upon extinction of contextually conditioned fear response in stressed mice.* Neurosci Lett 2011, 488(3): 242-6.
21. Kapus, G.L., Gacsαlyi, I., Vegh, M. et al. *Antagonism of AMPA receptors produces anxiolytic-like behavior in rodents: Effects of GYKI 52466 and its novel analogues*. Psychopharmacology (Berl) 2008, 198(2): 231-41.
22. da Cunha, I.C., Lopes, A.P., Steffens, S.M. et al. *The microinjection of AMPA receptor antagonist into the accumbens shell, but not into the accumbens core, induces anxiolysis in an animal model of anxiety.* Behav Brain Res 2008, 188(1): 91-9.
23. Farooq, M.U., Moore, P.W., Bhatt, A., Aburashed, R., Kassab, M.Y. *Therapeutic role of zonisamide in neuropsychiatric disorders*. Mini Rev Med Chem 2008, 8(10): 968-75.
24. Wang, H., Spiess, J., Wong, P.T., Zhu, Y.Z. *Blockade of CRF1 and CCK2 receptors attenuated the elevated anxiety-like behavior induced by immobilization stress*. Pharmacol Biochem Behav 2011, 98(3): 362-8.
25. Antonelli, T., Tomasini, M.C., Mazza, R., Fuxe, K., Gaetani, S., Cuomo, V., Tanganelli, S., Ferraro, L. *Cannabinoid CB1 and cholecystokinin CCK2 receptors modulate, in an opposing way, electrically evoked [3H]GABA efflux from rat cerebral cortex cell cultures: Possible relevance for cortical GABA transmission and anxiety*. J Pharmacol Exp Ther 2009, 329(2): 708-17.
26. Chung, L., Moore, S.D. *Cholecystokinin enhances GABAergic inhibitory transmission in basolateral amygdala.* Neuropeptides 2007, 41(6): 453-63.
27. Herdade, K.C., Strauss, C.V., Zangrossi Júnior, H., Viana, M.B. *Effects of medial amygdala inactivation on a panic-related behavior*. Behav Brain Res 2006, 172(2): 316-23.
28. Mantovani, M., Van Velthoven, V., Fuellgraf, H., Feuerstein, T.J., Moser, A. *Neuronal electrical high frequency stimulation enhances GABA outflow from human neocortical slices.* Neurochem Int 2006, 49(4): 347-50.
29. Delawary, M., Tezuka, T., Kiyama, Y. et al. *NMDAR2B tyrosine phosphorylation regulates anxiety-like behavior and CRF expression in the amygdala*. Mol Brain 2010, 3: 37.
30. Itoi, K., Sugimoto, N. *The brainstem noradrenergic systems in stress, anxiety and depression*. J Neuroendocrinol 2010, 22(5): 355-61.
31. LeBlanc, J., Ducharme, M.B. *Plasma dopamine and noradrenaline variations in response to stress.* Physiol Behav 2007, 91(2-3): 208-11.
32. Zarrindast, M.R., Mahboobi, S., Sadat-Shirazi, M.S., Ahmadi, S. *Anxiolytic-like effect induced by the cannabinoid CB1 receptor agonist, arachydonilcyclopropylamide (ACPA), in the rat amygdala is mediated through the D1 and D2 dopaminergic systems.* J Psychopharmacol 2011, 25(1): 131-40.
33. Leggio, G.M., Micale, V., Le Foll, B., Mazzola, C., Nobrega, J.N., Drago, F. *Dopamine D3 receptor knock-out mice exhibit increased behavioral sensitivity to the anxiolytic drug diazepam*. Eur Neuropsychopharmacol 2011, 21(4): 325-32.
34. Hayden, E.P., Klein, D.N., Dougherty, L.R., *The dopamine D2 receptor gene and depressive and anxious symptoms in childhood: Associations and evidence for gene-environment correlation and gene-environment interaction.* Psychiatr Genet 2010, 20(6): 304-10.
35. Furmark, T. *Neurobiological aspects of social anxiety disorder*. Isr J Psychiatry Relat Sci 2009, 46(1): 5-12.
36. Schneier, F.R., Abi-Dargham, A., Martinez, D., Slifstein, M., Hwang, D.R., Liebowitz, M.R., Laruelle, M. *Dopamine transporters, D2 receptors, and dopamine release in generalized social anxiety disorder*. Depress Anxiety 2009, 26(5): 411-8.
37. Micale, V., Cristino, L., Tamburella, A., Petrosino, S., Leggio, G.M., Drago, F., Di Marzo, V. *Altered responses of dopamine D3 receptor null mice to excitotoxic or anxiogenic stimuli: Possible involvement of the endocannabinoid and endovanilloid systems*. Neurobiol Dis 2009, 36(1): 70-80.
38. Appel, K., Rose, T., Fiebich, B., Kammler, T., Hoffmann, C., Weiss, G. *Modulation of the ã-aminobutyric acid (GABA) system by Passiflora incarnata L.* Phytother Res 2011, 25(6): 838-43.
39. Tasan, R.O., Bukovac, A., Peterschmitt, Y.N., Sartori, S.B., Landgraf, R., Singewald, N., Sperk, G. *Altered GABA transmission in a mouse model of increased trait anxiety*. Neuroscience 2011, 183: 71-80.
40. Papadopoulos, A., Chandramohan, Y., Collins, A., Droste, S.K., Nutt, D.J., Reul, J.M. *GABAergic control of novelty stress-responsive epigenetic and gene expression mechanisms in the rat dentate gyrus*. Eur Neuropsychopharmacol 2011, 21(4): 316-24.
41. Uusi-Oukari, M., Korpi, E.R. *Regulation of GABA(A) receptor subunit expression by pharmacological agents.* Pharmacol Rev 2010, 62(1): 97-135.
42. Koek, W., France, C.P., Cheng, K., Rice, K.C. *GABAB receptor-positive modulators: Enhancement of GABAB receptor agonist effects in vivo*. J Pharmacol Exp Ther 2010, 335(1): 163-71.
43. Wu, L.J., Kim, S.S., Zhuo, M. *Molecular targets of anxiety: From membrane to nucleus*. Neurochem Res 2008, 33(10): 1925-32.
44. Jacobson, L.H., Cryan, J.F. *Evaluation of the anxiolytic-like profile of the GABAB receptor positive modulator CGP7930 in rodents.* Neuropharmacology 2008, 54(5): 854-62.
45. Sörman, E., Wang, D., Hajos, M., Kocsis, B. *Control of hippocampal theta rhythm by serotonin: Role of 5-HT2c receptors*. Neuropharmacology 2011, 61(3): 489-94.
46. Rogel-Salazar, G., López-Rubalcava, C. *Evaluation of the anxiolytic-like effects of clomipramine in two rat strains with different anxiety vulnerability (Wistar and Wistar-Kyoto rats): Participation of 5-HT1A receptors.* Behav Pharmacol 2011, 22(2): 136-46.
47. Freeman-Daniels, E., Beck, S.G., Kirby, L.G. *Cellular correlates of anxiety in CA1 hippocampal pyramidal cells of 5-HT1A receptor knockout mice*. Psychopharmacology (Berl) 2011, 213(2-3): 453-63.
48. da Silva, E.S., Poltronieri, S.C., Nascimento, J.O., Zangrossi, H. Jr., Viana, M.B. *Facilitation of 5-HT(2A/2C)-mediated neurotransmission in the ventromedial hypothalamic nucleus decreases anxiety in the elevated T-maze*. Behav Brain Res 2011, 216(2): 692-8.
49. Matthys, A., Haegeman, G., Van Craenenbroeck, K., Vanhoenacker, P. *Role of the 5-HT7 receptor in the central nervous system: From current status to future perspectives.* Mol Neurobiol 2011, 43(3): 228-53.
50. Nikolaus, S., Antke, C., Beu, M., Müller, H.W. *Cortical GABA, striatal dopamine and midbrain serotonin as the key players in compulsive and anxiety disorders—Results from in vivo imaging studies*. Rev Neurosci 2010, 21(2): 119-39.
51. Graeff, F.G., Zangrossi, H. Jr. *The dual role of serotonin in defense and the mode of action of antidepressants on generalized anxiety and panic disorders*. Cent Nerv Syst Agents Med Chem 2010, 10(3): 207-17.
52. Mongeau, R., Martin, C.B., Chevarin, C., Maldonado, R., Hamon, M., Robledo, P., Lanfumey, L. *5-HT2C receptor activation prevents stress-induced enhancement of brain 5-HT turnover and extracellular levels in the mouse brain: Modulation by chronic paroxetine treatment*. J Neurochem 2010, 115(2): 438-49.
53. Mombereau, C., Kawahara, Y., Gundersen, B.B., Nishikura, K., Blendy, J.A. *Functional relevance of serotonin 2C receptor mRNA editing in antidepressant- and anxiety-like behaviors.* Neuropharmacology 2010, 59(6): 468-73.

54. Smit-Rigter, L.A., Wadman, W.J., van Hooft, J.A. *Impaired social behavior in 5-HT(3A) receptor knockout mice.* Front Behav Neurosci 2010, 4: 169.
55. Walstab, J., Rappold, G., Niesler, B. *5-HT(3) receptors: Role in disease and target of drugs.* Pharmacol Ther 2010, 128(1): 146-69.
56. Leopoldo, M., Lacivita, E., Berardi, F., Perrone, R., Hedlund, P.B. *Serotonin 5-HT7 receptor agents: Structure-activity relationships and potential therapeutic applications in central nervous system disorders.* Pharmacol Ther 2011, 129(2): 120-48.
57. Kakui, N., Yokoyama, F., Yamauchi, M., Kitamura, K., Imanishi, T., Inoue, T., Koyama, T. *Anxiolytic-like profile of mirtazapine in rat conditioned fear stress model: Functional significance of 5-hydroxytryptamine 1A receptor and alpha1-adrenergic receptor.* Pharmacol Biochem Behav 2009, 92(3): 393-8.
58. Hiroi, R., Neumaier, J.F. *Estrogen decreases 5-HT1B autoreceptor mRNA in selective subregion of rat dorsal raphe nucleus: Inverse association between gene expression and anxiety behavior in the open field.* Neuroscience 2009, 158(2): 456-64.
59. Mnie-Filali, O., Lambas-Señas, L., Scarna, H., Haddjeri, N. *Therapeutic potential of 5-HT7 receptors in mood disorders.* Curr Drug Targets 2009, 10(11): 1109-17.
60. Hedlund, PB. *The 5-HT7 receptor and disorders of the nervous system: An overview.* Psychopharmacology (Berl) 2009, 206(3): 345-54.
61. Holmes, A. *Genetic variation in cortico-amygdala serotonin function and risk for stress-related disease.* Neurosci Biobehav Rev 2008, 32(7): 1293-314.
62. Muraki, I., Inoue, T., Koyama, T. *Effect of co-administration of the selective 5-HT1A receptor antagonist WAY 100,635 and selective 5-HT1B/1D receptor antagonist GR 127,935 on anxiolytic effect of citalopram in conditioned fear stress in the rat.* Eur J Pharmacol 2008, 586(1-3): 171-8.
63. Watson, J.M., Dawson, L.A. *Characterization of the potent 5-HT(1A/B) receptor antagonist and serotonin reuptake inhibitor SB-649915: Preclinical evidence for hastened onset of antidepressant/anxiolytic efficacy.* CNS Drug Rev 2007, 13(2): 206-23.
64. Massé, F., Hascoët, M., Dailly, E., Bourin, M. *Effect of noradrenergic system on the anxiolytic-like effect of DOI (5-HT2A/2C agonists) in the four-plate test.* Psychopharmacology (Berl) 2006, 183(4): 471-81.
65. Läck, A.K., Ariwodola, O.J., Chappell, A.M., Weiner, J.L., McCool, B.A. *Ethanol inhibition of kainate receptor-mediated excitatory neurotransmission in the rat basolateral nucleus of the amygdala.* Neuropharmacology 2008, 55(5): 661-8.
66. Trabanco, A.A., Cid, J.M., Lavreysen, H., Macdonald, G.J., Tresadern, G. *Progress in the development of positive allosteric modulators of the metabotropic glutamate receptor 2.* Curr Med Chem 2011, 18(1): 47-68.
67. Fell, M.J., Witkin, J.M., Falcone, J.F. et al. *N-(4-((2-(Trifluoromethyl)-3-hydroxy-4-(isobutyryl)phenoxy)methyl)benzyl)-1-methyl-1H-imidazole-4-carboxamide (THIIC), a novel metabotropic glutamate 2 potentiator with potential anxiolytic/antidepressant properties: In vivo profiling suggests a link between behavioral and central nervous system neurochemical changes.* J Pharmacol Exp Ther 2011, 336(1): 165-77.
68. Paterson, N.E., Iwunze, M., Davis, S.F., Malekiani, S.A., Hanania, T. *Comparison of the predictive validity of the mirror chamber and elevated plus maze tests in mice.* J Neurosci Methods 2010, 188(1): 62-70.
69. Spanka, C., Glatthar, R., Desrayaud, S., Fendt, M., Orain, D., Troxler, T., Vranesic, I. *Piperidyl amides as novel, potent and orally active mGlu5 receptor antagonists with anxiolytic-like activity.* Bioorg Med Chem Lett 2010, 20(1): 184-8.
70. Kotlinska, J., Bochenski, M. *The influence of various glutamate receptors antagonists on anxiety-like effect of ethanol withdrawal in a plus-maze test in rats.* Eur J Pharmacol 2008, 598(1-3): 57-63.
71. Moonat, S., Sakharkar, A.J., Zhang, H., Pandey, S.C. *The role of amygdaloid brain-derived neurotrophic factor, activity-regulated cytoskeleton-associated protein and dendritic spines in anxiety and alcoholism.* Addict Biol 2011, 16(2): 238-50.
72. Frielingsdorf, H., Bath, K.G., Soliman, F., Difede, J., Casey, B.J., Lee, F.S. *Variant brain-derived neurotrophic factor Val66Met endophenotypes: Implications for posttraumatic stress disorder.* Ann NY Acad Sci 2010, 1208: 150-7.
73. Kaplan, G.B., Vasterling, J.J., Vedak, P.C. *Brain-derived neurotrophic factor in traumatic brain injury, post-traumatic stress disorder, and their comorbid conditions: Role in pathogenesis and treatment.* Behav Pharmacol 2010, 21(5-6): 427-37.
74. Zhang, P., Cyriac, G., Kopajtic, T., Zhao, Y., Javitch, J.A., Katz, J.L., Newman, A.H. *Structure-activity relationships for a novel series of citalopram (1-(3-(dimethylamino)propyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile) analogues at monoamine transporters.* J Med Chem 2010, 53(16): 6112-21.
75. Bandelow, B. *The medical treatment of obsessive-compulsive disorder and anxiety.* CNS Spectr 2008, 13(9, Suppl. 14): 37-46.
76. Lehmann, M.L., Brachman, R.A., Listwak, S.J., Herkenham, M. *NF-kappaB activity affects learning in aversive tasks: Possible actions via modulation of the stress axis.* Brain Behav Immun 2010, 24(6): 1008-17.
77. Kassed, C.A., Herkenham, M. *NF-kappaB p50-deficient mice show reduced anxiety-like behaviors in tests of exploratory drive and anxiety.* Behav Brain Res 2004, 154(2): 577-84.
78. Hudzik, T.J., Maciag, C., Smith, M.A. et al. *Preclinical pharmacology of AZD2327 - A highly selective agonist of the {delta} opioid receptor.* J Pharmacol Exp Ther 2011, Epub ahead of print.
79. Solati, J., Zarrindast, M.R., Salari, A.A. *Dorsal hippocampal opioidergic system modulates anxiety-like behaviors in adult male Wistar rats.* Psychiatry Clin Neurosci 2010, 64(6): 634-41.
80. van Rijn, R.M., Brissett, D.I., Whistler, J.L. *Dual efficacy of delta opioid receptor-selective ligands for ethanol drinking and anxiety.* J Pharmacol Exp Ther 2010, 335(1): 133-9.
81. Bruchas, M.R., Chavkin, C. *Kinase cascades and ligand-directed signaling at the kappa opioid receptor.* Psychopharmacology (Berl) 2010, 210(2): 137-47.
82. Carr, G.V., Lucki, I. *Comparison of the kappa-opioid receptor antagonist DIPPA in tests of anxiety-like behavior between Wistar Kyoto and Sprague Dawley rats.* Psychopharmacology (Berl) 2010, 210(2): 295-302.
83. Sudakov, S.K., Bashkatova, V.G., Kolpakov, A.A., Trigub, M.M. *Peripheral administration of loperamide and methylnaloxone decreases the degree of anxiety in rats.* Bull Exp Biol Med. 2010, 149(3): 273-5.
84. Ide, S., Sora, I., Ikeda, K., Minami, M., Uhl, G.R., Ishihara, K. *Reduced emotional and corticosterone responses to stress in mu-opioid receptor knockout mice.* Neuropharmacology 2010, 58(1): 241-7.
85. Kiosterakis, G., Stamatakis, A., Diamantopoulou, A., Fameli, M., Stylianopoulou, F. *Long-term effects of neonatal handling on mu-opioid receptor levels in the brain of the offspring.* Dev Psychobiol 2009, 51(5): 439-49.
86. Varadarajulu, J., Lebar, M., Krishnamoorthy, G. et al. *Increased anxiety-related behaviour in Hint1 knockout mice.* Behav Brain Res 2011, 220(2): 305-11.
87. Wrobel, L.J., Dupré, A., Raggenbass, M. *Excitatory action of vasopressin in the brain of the rat: Role of cAMP signaling.* Neuroscience 2011, 172: 177-86.
88. Barbier, E., Wang, J.B. *Anti-depressant and anxiolytic like behaviors in PKCI/HINT1 knockout mice associated with elevated plasma corticosterone level.* BMC Neurosci 2009, 10: 132.

89. Lesscher, H.M., McMahon, T., Lasek, A.W., Chou, W.H., Connolly, J., Kharazia, V., Messing, R.O. *Amygdala protein kinase C epsilon regulates corticotropin-releasing factor and anxiety-like behavior.* Genes Brain Behav 2008, 7(3): 323-33.

90. Moya, P.R., Fox, M.A., Jensen, C.L., Laporte, J.L., French, H.T., Wendland, J.R., Murphy, D.L. *Altered 5-HT2C receptor agonist-induced responses and 5-HT2C receptor RNA editing in the amygdala of serotonin transporter knockout mice.* BMC Pharmacol 2011, 11: 3.

91. Calapoðlu, M., Sahin-Calapoðlu, N., Karaçöp, A., Soyöz, M., Elyýldýrým, U.Y., Avþaroðlu, S. *Serotonin transporter bi- and triallelic genotypes and their relationship with anxiety and academic performance: a preliminary stu*dy. Neuropsychobiology 2011, 63(2): 103-11.

92. Charoenphandhu, J., Teerapornpuntakit, J., Nuntapornsak, A., Krishnamra, N., Charoenphandhu, N. *Anxiety-like behaviors and expression of SERT and TPH in the dorsal raphé of estrogen- and fluoxetine-treated ovariectomized rats*.Pharmacol Biochem Behav 2011, 98(4): 503-10.

93. Ebner, K., Sartori, S.B., Singewald, N. *Tachykinin receptors as therapeutic targets in stress-related disorders.* Curr Pharm Des 2009, 15(14): 1647-74.

94. Katsouni, E., Sakkas, P., Zarros, A., Skandali, N., Liapi, C. *The involvement of substance P in the induction of aggressive behavior*. Peptides 2009, 30(8): 1586-91.

95. Singewald, N., Chicchi, G.G., Thurner, C.C. et al. *Modulation of basal and stress-induced amygdaloid substance P release by the potent and selective NK1 receptor antagonist L-822429*. J Neurochem 2008, 106(6): 2476-88.

96. Carvalho, M.C., Masson, S., Brandão, M.L., de Souza Silva, M.A. *Anxiolytic-like effects of substance P administration into the dorsal, but not ventral, hippocampus and its influence on serotonin.* Peptides 2008, 29(7): 1191-200.

97. Caffrey, M.K., Nephew, B.C., Febo, M. *Central vasopressin V1a receptors modulate neural processing in mothers facing intruder threat to pups*. Neuropharmacology 2010, 58(1): 107-16.

98. Simon, N.G., Guillon, C., Fabio, K. et al. *Vasopressin antagonists as anxiolytics and antidepressants: Recent developments.* Recent Pat CNS Drug Discov 2008, 3(2): 77-93.

99. Egashira, N., Tanoue, A., Matsuda, T. et al. *Impaired social interaction and reduced anxiety-related behavior in vasopressin V1a receptor knockout mice.* Behav Brain Res 2007, 178(1): 123-7.

100. Aupperle, R.L., Ravindran, L., Tankersley, D. et al. *Pregabalin influences insula and amygdala activation during anticipation of emotional images.* Neuropsychopharmacology 2011, 36(7): 1466-77.

101. Cui, X.Y., Zhao, X., Chu, Q.P., Chen, B.Q., Zhang, Y.H. *Influence of diltiazem on the behavior of zolpidem-treated mice in the elevated-plus maze test*. J Neural Transm 2007, 114(2): 155-60.

102. Shinnick-Gallagher, P., McKernan, M.G., Xie, J., Zinebi, F. *L-type voltage-gated calcium channels are involved in the in vivo and in vitro expression of fear conditioning.* Ann NY Acad Sci 2003, 985: 135-49

103. Sarris, J., LaPorte, E., Schweitzer, I. *Kava: A comprehensive review of efficacy, safety, and psychopharmacology.* Aust NZ J Psychiatry 2011, 45(1): 27-35.

104. Papale, L.A., Paul, K.N., Sawyer, N.T., Manns, J.R., Tufik, S., Escayg, A. *Dysfunction of the Scn8a voltage-gated sodium channel alters sleep architecture, reduces diurnal corticosterone levels, and enhances spatial memory.* J Biol Chem 2010, 285(22): 16553-61.

105. Mahoney, K., Moore, S.J., Buckley, D. et al. *Variable neurologic phenotype in a GEFS+ family with a novel mutation in SCN1A*. Seizure 2009, 18(7): 492-7.

106. Clare, J.J., Tate, S.N., Nobbs, M., Romanos, M.A. *Voltage-gated sodium channels as therapeutic targets.* Drug Discov Today 2000, 5(11): 506-520.