# REVIEW ARTICLE

# Anxiolytic properties of compounds that counteract oxidative stress, neuroinflammation, and glutamatergic dysfunction: a review

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**Objective:** Anxiety disorders are highly prevalent and the efficacy of the available anxiolytic drugs is less than desired. Adverse effects also compromise patient quality of life and adherence to treatment. Accumulating evidence shows that the pathophysiology of anxiety and related disorders is multifactorial, involving oxidative stress, neuroinflammation, and glutamatergic dysfunction. The aim of this review was to evaluate data from animal studies and clinical trials showing the anxiolytic effects of agents whose mechanisms of action target these multiple domains.
**Methods:** The PubMed database was searched for multitarget agents that had been evaluated in animal models of anxiety, as well as randomized double-blind placebo-controlled clinical trials of anxiety and/or anxiety related disorders.
**Results:** The main multitarget agents that have shown consistent anxiolytic effects in various animal models of anxiety, as well in clinical trials, are agomelatine, N-acetylcysteine (NAC), and omega-3 fatty acids. Data from clinical trials are preliminary at best, but reveal good safety profiles and tolerance to adverse effects.
**Conclusion:** Agomelatine, NAC and omega-3 fatty acids show beneficial effects in clinical conditions where mainstream treatments are ineffective. These three multitarget agents are considered promising candidates for innovative, effective, and better-tolerated anxiolytics.

**Keywords:** Anxiety; agomelatine; N-acetylcysteine; omega-3 fatty acids

## Introduction

Anxiety has been defined as a state of high arousal and enhanced vigilance in the absence of immediate threat.[1] It is characterized by subjective experiences (such as persistent worry and tension) in addition to physiological changes (such as sweating and increased heart rate). Though healthy individuals may present sporadic anxiety, it becomes pathological if persistent, disruptive, and disproportionate.[2] Anxiety disorders have global lifetime prevalence rates as high as 28%,[3] and include social phobia, panic disorder, agoraphobia, and generalized anxiety disorder (GAD).[4] Though obsessive-compulsive disorders (OCD) and posttraumatic stress disorder (PTSD) present marked anxiety symptoms, the DSM-5 categorizes these conditions as obsessive-compulsive and related disorders and trauma and stressor-related disorders, respectively.

In addition to drug therapy, the current treatment of anxiety disorders involves lifestyle interventions, such as physical exercise and mindfulness-based stress reduction, as well as psychological interventions, such as cognitive behavioral therapy, which are difficult to implement. The main drug classes used to treat anxiety disorders are GABAergic or serotonergic agents, including benzodiazepines (BZD), 5-HT$_{1A}$ serotonin receptor agonists, and selective serotonin reuptake inhibitors (SSRIs).[5] Unfortunately, however, not all patients respond to the available medications.[6] Moreover, BZDs and SSRIs are associated with unwanted adverse effects, including sedation, memory deficits, dependence, withdrawal syndrome, sexual dysfunction, and weight gain.[5] While these adverse effects decrease adherence to treatment, the better-tolerated 5-HT$_{1A}$ agonist buspirone has the slowest onset of action and its efficacy is limited to GAD.[7,8]

Despite its high prevalence, few effective therapeutic targets have been identified for anxiety disorders. The expectation that highly selective agents acting on specific molecular targets would yield better and safer psychiatric drugs has not yet been met.[9] A newer approach involving multi-targeted agents [10,11] recognizes the complex pathophysiology underlying psychiatric disorders. In anxiety disorders, oxidative stress,[12-14] neuroinflammation,[15] and glutamatergic hyperactivity[16-18] are now recognized as key contributing factors.

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*Anxiety and neurochemical damage*

Glutamatergic hyperactivity, a key feature in brain injuries, triggers a complex chain of events, including oxidative stress, mitochondrial dysfunction, and cellular signaling that result in inflammatory response and/or cell death.[19,20] Since glutamatergic hyperactivity is characteristic of anxiety,[17,18] oxidative stress and neuroinflammation are relevant.

Abnormalities in glutamate neurotransmission are among the biological mechanisms underlying stress response and anxiety disorders.[17] Anxiety disorders seem to result from a hyperactive glutamatergic system deregulating inhibitory/excitatory balance in the brain.[16,18] Metabotropic glutamatergic 2/3 ($mGlu_{2/3}$) receptors stand out as a potential target for anxiety-modulating drugs (Pitsikas).[16] Presynaptically located, $mGlu_{2/3}$ receptors are present in several brain areas where glutamate hyperactivity is associated with anxiety, including the cortex, thalamus, striatum, amygdala, and hippocampus.[21,22] The activation of $mGlu_{2/3}$ receptors limits neuronal glutamate release,[23] and agonists of such receptors show anxiolytic activity in diverse animal models of anxiety.[16]

An association between anxiety and oxidative stress has been documented in rodents and humans. Hovatta et al.[24] found a positive correlation between glyoxalase I and glutathione reductase I gene expression and anxiety phenotypes on stress-related behaviors in isogenic mice. Overexpression of the glyoxalase I gene has also been reported for naturally anxious mice.[25] Bouayed et al.[26] reported a positive correlation between markers of peripheral oxidative stress and anxious behavior in mice. Increased anxiety-like behavior accompanied by oxidative stress has been documented in rodents exposed to psychological stress,[27] chronic restraint stress,[28] and oxidative stress inducers.[29-31] Changes in antioxidant defenses and elevated lipid peroxidation products have been reported in GAD,[32-34] OCD,[35-39] panic disorder,[40] and social phobia.[41,42] Anxious women were found to have a lower total antioxidant capacity in the blood than controls.[43]

Associations between deregulation of the hypothalamic pituitary adrenal axis (HPA) and anxiety disorders are widely recognized, resulting in changes in the levels of pro- and anti-inflammatory cytokines and cortisol.[15,44] Inflammatory cytokines and immune cells can access the brain and alter behavior, including the synthesis, release, and reuptake of neurotransmitters such as glutamate, serotonin, and dopamine, which are affected by cytokines and their signaling pathways.[45] The kynurenine pathway is also activated by cytokines, generating neuroactive metabolites that influence dopamine and glutamate transmission and, by depleting tryptophan, regulate the synthesis of serotonin.[45]

Increased peripheral cytokine expression is associated with increased anxiety in mice.[46,47] Mice overexpressing interleukin (IL)-6 or tumor necrosis factor (TNF) exhibit an anxiogenic phenotype.[48,49] Several human studies have also shown a correlation between anxiety, neuroinflammation, and the immune system.[15,44] Injection of the immune activator lipopolysaccharide (LPS) induced anxiety symptoms in normal volunteers,[50] and a positive correlation between anxiety and increased levels of inflammatory markers (such as TNF-$\alpha$ and IL-6) has been repeatedly documented in anxiety disorders.[15,43,51,52]

Strategies to minimize and/or counteract the damage resulting from these accompanying neurochemical processes may lead to innovation in the field of anxiolytic drug research. As a key step in translational research is target validation, the aim of this study is to review drug candidates known to counteract oxidative stress, neuroinflammation, and glutamatergic hyperfunction that have undergone preclinical and clinical analyses relevant to anxiety disorders.

## Methods

The PubMed database was searched through March 2017. The search strategy used successive combinations of the following terms (compounds whose multi-target mechanisms of action have been well-established in the literature, including modulation of oxidative stress and/or neuroinflammation and/or glutamate hyperactivity): ascorbic acid, vitamin C, vitamin A, vitamin E, tocopherol, vitamin D, polyphenols, flavonoids, $mGlu_{2/3}$ modulator, melatonin, agomelatine, N-acetylcysteine, omega-3 fatty acids, omega-3 polyunsaturated fatty acids (PUFA) AND anxiety. The results were initially limited to clinical trials. The criterion for a compound's inclusion in this review was evidence of anxiolytic effects in both randomized double-blind placebo-controlled clinical trials and animal models. When no such studies were found for a given compound, it was excluded from further analysis. For compounds that had been tested in clinical trials, we also carried out searches for the compound AND each of these conditions (which are classified as anxiety disorders or have a strong relation with anxiety-related symptoms): generalized anxiety disorder, social phobia, specific phobia, panic disorder, obsessive-compulsive disorder, post-traumatic stress disorder, trichotillomania, nail biting, and excoriation (skin-picking) disorder. The publications were assessed for relevance to the selected topics. The search was limited to texts in English. To select articles for inclusion, all the abstracts found using the search criteria were read.

## Results and discussion

We found that agomelatine, N-acetylcysteine (NAC), and omega-3 PUFA are the main agents fitting the inclusion criteria that have demonstrated antioxidant, anti-inflammatory, and glutamatergic effects.

*Agomelatine*

Agomelatine, a synthetic analog of melatonin, is a high-affinity agonist of $MT_1$ and $MT_2$ melatonin receptors.[53,54] Agomelatine antagonizes 5-$HT_{2C}$ serotonin receptors, an effect thought to be involved in its anxiolytic effects.[55] Agomelatine also modulates glutamate neurotransmission in regions associated with mood and cognition, such as the prefrontal and frontal cortex,[55] the hippocampus,

and the amygdala.[56] In rats submitted to prenatal restraint stress, agomelatine blocked the stress-induced glutamate release in the prefrontal cortex[57] and regularized glutamate release and the expression of mGlu$_{2/3}$ receptor mRNA in the hippocampus.[58]

Agomelatine decreased lipid peroxidation levels and nitrite contents in the brains of mice submitted to chemically induced seizures[59] and protected cultured PC-12 neuronal cells from cytosolic reactive oxygen species production, as well as increased glutathione.[60]

Agomelatine was able to reduce LPS-induced upregulation of proinflammatory cytokines IL-6 and IL1-β both within and outside rat brains. These effects were accompanied by inhibition of nuclear factor kappa B (NF-κB) translocation and microglia activation.[61] Microglia are resident macrophages normally present in the healthy brain that perform active tissue scanning and can respond quickly to any microenvironment change.[62] Agomelatine also modified the expression of enzymes associated with the kynurenine pathway, possibly protecting the brain from the neurotoxic consequences of the conversion of kynurenine to quinolinic acid, an N-methyl-D-aspartate (NMDA) receptor agonist.[61]

Though the antidepressant properties of agomelatine have been better characterized,[63] its anxiolytic effects have been reported in different animal models[58,64-66] (Table 1). In most animal studies, agomelatine's anxiolytic effects were documented after acute administration. However, Morley-Fletcher et al. reported that agomelatine administered for 3 or 6 weeks prevented prenatal restraint stress (in the elevated plus-maze) as well as reversed the reduced hippocampal levels of mGlu$_{2/3}$ and mGlu$_5$ receptors in rats.[58] These effects were restricted to rats submitted to restraint stress, which suggests that agomelatine modulation of mGlu$_{2/3}$ receptors may be especially relevant in stressed subjects.

Most of the available clinical data on agomelatine as an anxiolytic refer to GAD patients and were published by the same group. The first clinical trial was published in 2008 (Table 2), in which GAD patients (comorbidity free) were randomized to receive agomelatine or placebo for 12 weeks.[65] This randomized double-blind placebo controlled trial (RDBCT) revealed that agomelatine (25-50 mg/day) was superior to placebo in the primary outcome (Hamilton Anxiety Rating Scale), as well as secondary outcome measures (clinical response, insomnia, and associated disability). In this study, agomelatine was well tolerated and discontinuation symptoms were lower in agomelatine than placebo patients.[80] An open-label study with agomelatine 25-50 mg/day for 16 weeks followed by a multicenter RDBCT (with the same doses of agomelatine) for 26 weeks was conducted to evaluate long-term tolerability to agomelatine and its efficacy in preventing relapse. The results showed that agomelatine was well tolerated and superior to placebo in preventing relapse.[81] A third trial compared agomelatine with escitalopram and placebo. The multicenter RDBCT showed that agomelatine and escitalopram were comparable regarding improved symptomatology, but escitalopram had a higher incidence of adverse events than placebo.[82] A recent trial evaluated the minimal effective optimal dose of agomelatine in GAD

patients: the 12-week multicenter RDBCT showed that 10 and 25 mg/kg are better than placebo, and the best response was obtained with 25 mg.[83]

Data on other anxiety disorders are very limited and present too many confounding factors to allow meaningful conclusions.[99] Stein et al. reviewed data from three placebo-controlled short-term trials[100-102] and three comparative studies of agomelatine vs. the SSRI antidepressants venlafaxine,[103] fluoxetine,[104] and sertraline[105] in major depression patients with anxiety symptoms, finding that agomelatine had a greater effect on anxiety symptoms than placebo or antidepressants.[106]

Adverse events reported with agomelatine are mostly perceived as mild to moderate and include headache, dizziness, somnolence, fatigue, and gastrointestinal symptoms.[107] Elevation of liver transaminase levels and rare cases of hepatic failure were seen only with 50 mg/day.[107] The use of agomelatine was not associated with discontinuation symptoms,[108,109] a relevant aspect considering its beneficial effects on sleep disturbances observed in patients with depression and/or anxiety.[80,82]

## NAC

NAC is a precursor of cysteine (required for the production of the primary endogenous antioxidant glutathione) and can directly sequester oxidants.[110] NAC supplementation results in additional cysteine, which activates the cystine/glutamate antiporter (also called x[c]-system), predominantly expressed by astrocytes in the brain. The cysteine dimer, cystine, is taken up by astrocytes and exchanged for glutamate, which activates mGluR$_{2/3}$ receptors on presynaptic neurons and reduces the synaptic release of glutamate.[110]

NAC has anti-inflammatory properties as result of multiple mechanisms. Through its direct antioxidant effect and as a glutathione (GSH) precursor, NAC inhibits the activation of the proinflammatory transcription factor NF-κB, which downregulates the expression of several proinflammatory genes.[111-113] Microglia inhibition also seems to be important in NAC's ability to reduce neuroinflammation.[114,115] Therefore, by stimulating GSH synthesis and regulating the cystine/glutamate antiporter, glutamate excitotoxicity, and oxidative stress, NAC inhibits microglia, macrophage activation, and the production of cytokines and oxidative species.[114,116]

The anti-inflammatory properties of NAC have been documented in animal models of ischemic and traumatic brain injury,[117-119] LPS-induced pulmonary edema,[120] and lethal endotoxemia.[121] In humans, NAC has reduced lung inflammation (Blackwell et al.[122]) decreased proinflammatory cytokines in burn[123] and dialysis patients,[124] and caused a reduction of proinflammatory cytokines, as well as shown antioxidant effects in cardiac injury after aortic aneurysm repair.[125]

Egashira et al. found that acute NAC (but not α-tocopherol) inhibited marble-burying behavior in mice (Table 1), suggesting that this anxiolytic-like effect is related to glutamate modulation rather than antioxidant effects.[67] Chen et al. showed that NAC reversed valproate-induced social interaction deficit and anxiety-like behavior in rats

**Table 1** Anxiolytic-like effects of mult-itarget compounds: preclinical studies

| Compound/dose | Treatment duration | Species | Behavioral tests | Effects | Reference |
|---|---|---|---|---|---|
| **Agomelatine** | | | | | |
| 2.5-80 mg/kg, i.p. | Acute | Rats | EPM, SI, UV, VCT | Anxiolytic | Millan et al.[65] |
| 10-75 mg/kg, i.p. | Acute | Rats | Conditioned footshock-induced UV, EPM, VCT, | Anxiolytic | Papp et al.[66] |
| 20-40 mg/kg, i.p. | Acute | Rats | EPM, NIH, PD, SSWS | Anxiolytic in the EPM | Loiseau et al.[64] |
| 40-50 mg/kg, i.p. | Chronic | Rats | EPM, FST | Prevented prenatal restraint-induced anxiety-like behavior in the EPM | Morley-Fletcher et al.[58] |
| **NAC** | | | | | |
| 50 mg/kg, i.p., | Acute | Mice | MBB | Inhibited marble-burying behavior | Egashira et al.[67] |
| 150 mg/kg, i.p. | 10 days | Rats | EPM, OF, SI | Reversed valproate-induced anxiety-like behavior and social interaction deficit | Chen et al.[68] |
| 30 or 60 mg/kg, i.p. | 11 days | Mice | HB, SP | Prevented rhythm disruption-induced anxiety in the HB | Pilz et al.[69] |
| 0.1, 1.0 and 10 mg/L of tank water | Acute | Zebrafish | L/D, NT | Anxiolytic in the L/D, prevented acute stressor-induced anxiety-like behavior in NT | Mocelin et al.[70] |
| 60-150 mg/kg, i.p. | Acute and subacute (4 days) | Mice | ETM, HB, L/D, OF, SI, SIH | Anxiolytic (except at the elevated T-maze). | Santos et al.[71] |
| **Omega-3** | | | | | |
| Diet supplemented with DHA | Chronic | Mice | OF, L/D, MWM | Anxiolytic in the L/D | Carrié et al.[72] |
| Diet supplemented with different combinations of omega-3 PUFA | Chronic | Rats | EPM, OF | Attenuated i.c.v. IL-1 beta-induced anxiety. | Song et al.[73] |
| Diet supplemented with different proportions of ethyl-EPA | Chronic | Rats | EPM, OF | Attenuated the i.c.v. IL-1 beta-induced anxiety | Song et al.[74] |
| Diet supplemented with EPA + DHA | Chronic | Rats | EPM, modified FST, MWM | Counteracted restraint-induced anxiety | Ferraz et al.[75] |
| Diet supplemented with long-chain omega-3 PUFA | Chronic | Grey mouse lemur (Microcebus murinus) | OF | Anxiolytic | Vinot et al.[76] |
| Diet supplemented with EPA + DHA | Chronic | Rats | Avoidance conditioning, EPM | Prevented restraint stress-induced anxiety | Pérez et al.[77] |
| 10:1 omega-6/omega-3 diet supplemented with DHA for three generations | Chronic | Mice | EPM, OF | Anxiolytic in third generation male offspring | Jašarević et al.[78] |
| Diet supplemented with long-chain omega-3 PUFA | Chronic | Grey mouse lemur (Microcebus murinus) | OF, Barnes maze | Anxiolytic | Pifferi et al.[79] |

DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; EPM = elevated plus maze; ETM = elevated T-maze; FST = forced swim test; HB = hole-board; i.c.v = intracerebroventricular; IL = interleukin; i.p. = intraperitoneal; L/D = light/dark; MBB = marble-burying behavior; MWM = Morris water maze; NAC = N-acetylcysteine; NIH = novelty-induced hypophagia; NOR = novel object recognition test; NT = novel tank; OF = open field; PD = punished drinking test; PUFA = polyunsaturated fatty acid; SI = social interaction; SIH = stress-induced hyperthermia; SP = social preference; SSWS = safety signal withdrawal schedule (operant conflict procedure); UV = ultrasonic vocalization test; VCT = vogel conflict test.

**Table 2** Anxiolytic effects of multitarget compounds: clinical trials

| Compound/disorder | Study design | Study size | Daily dose and treatment duration | Main measures/ instruments | Results | Reference |
|---|---|---|---|---|---|---|
| **Agomelatine** | | | | | | |
| GAD | RDBCT | 121 | 25-50 mg, 12 weeks | CGI, HARS, LSEQ, SDS | Anxiolytic | Stein et al.[80] |
| GAD | Open-label treatment followed by a multicenter RDBCT | 477 | 25-50 mg, 16 weeks (open-label) followed by 26 weeks (RDBCT) | CGI, DESS, HAD, HARS, LSEQ, SDS | Anxiolytic and well-tolerated in long-term treatment. Superior to placebo in preventing relapse. | Stein et al.[81] |
| GAD | Multicenter, RDBCT | 412 | 25-50 mg, 12 weeks | CGI, HADS, LSEQ, SDS | Anxiolytic effect similar to escitalopram, with lower adverse events incidence. | Stein et al.[82] |
| GAD | RDBCT | 412 | 10-25 mg, 12 weeks | HARS | Anxiolytic, placebo-agomelatine difference greater with the higher dose. | Stein et al.[83] |
| **NAC** | | | | | | |
| TTM | RDBCT | 50 | 1,200-2,400 mg, 12 weeks | CGI, HARS MGH-HPS, PITS | Reduced hair-pulling | Grant et al.[84] |
| OCD (refractory to SRI) | RDBCT | 39 | Initially 600 mg, doubling weekly to a maximum dose of 2,400 mg (add-on treatment to SRI), 12 weeks | CGI-S, Y-BOCS | Improved mean CGI-S and Y-BOCS scale scores | Afshar et al.[85] |
| Chronic nail biting | RDBCT | 25 | 800 mg, 2 months | Nail length | Decreased nail biting over the short term | Ghanizadeh et al.[86] |
| OCD | RDBCT | 44 | 3,000 mg (add-on treatment), 16 weeks | Y-BOCS | Decreased Y-BOCS score | Sarris et al.[87] |
| PTSD and SUD | RDBCT | 35 | 2,400 mg, 8 weeks | CAPS, PCL-M, VAS | Improved PTSD and craving | Back et al.[88] |
| Skin-picking disorder | RDBCT | 53 | 1,200-3,000 mg, 12 weeks | Measures of skin-picking severity: CGI-S and modified Y-BOCS | Decreased skin-picking | Grant et al.[89] |
| OCD | RDBCT | 44 | 2,000 mg (add-on treatment to fluvoxamine), 10 weeks | Y-BOCS | Decreased scores in Y-BOCS | Paydary et al.[90] |
| **Omega-3** | | | | | | |
| Test anxiety | Placebo controlled trial | 126 | 90 mg of α-linolenic acid (omega-3) and 360 mg of linoleic acid (omega-6 fatty acid), 3 weeks | Standardized rating scale | Improved variables associated with test anxiety | Yehuda et al.[91] |
| SUD | RDBCT | 24 | 3 g, 3 months | Modified version of the POMS (baseline and monthly) | Decreased anxiety scores progressively | Buydens-Branchey & Branchey[92] |
| SUD | RDBCT | 22 | 3 g, 3 months | Modified version of POMS | Decreased anxiety scores | Buydens-Branchey et al.[93] |
| Healthy young adults | RDBCT | 68 | 2.5 g, 12 weeks | BAI, CES-D | Decreased anxiety | Kiecolt-Glaser et al.[94] |
| Alcoholic patients | RDBCT | 31 | 60 mg EPA + 252 mg DHA, 3 weeks | PSS | Decreased anxiety/stress | Barbadoro et al.[95] |

**Table 2** (continued)

| Compound/disorder | Study design | Study size | Daily dose and treatment duration | Main measures/ instruments | Results | Reference |
|---|---|---|---|---|---|---|
| Early postmyocardial infarction | RDBCT | 52 | 1 g + standard pharmacotherapy, 1 month | BDI, ESQ, STAI-S, STAI-T, used at the baseline (3rd day of acute myocardial infarction) and after one month | Decreased anxiety (STAI-S) | Haberka et al.[96] |
| PMS | RDBCT | 124 | 2 g, 3 months | VAS | Decreased anxiety severity and duration | Sohrabi et al.[97] |
| Japanese accident survivors (at risk for developing PTSD) | RDBCT | 83 | 1,470 mg DHA + 147 mg EPA, 12 weeks | Monitoring of heart rate and skin conductance, script-driven imagery of their traumatic event | Decreased heart rate | Matsumura et al.[98] |

BAI = Beck Anxiety Inventory; BDI = Beck Depression Inventory; CAPS = Clinician Administered PTSD Scale; CES-D = Center for Epidemiological Studies Depression Scale; CGI = Clinical Global Impression Scale; CGI-S = Clinical Global Impression - Severity of Illness; DESS = Discontinuation Emergent Signs and Symptoms checklist; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; ESQ = Emotional State Questionnaire; GAD = generalized anxiety disorder; HAD = Hospital Anxiety and Depression Scale; HARS = Hamilton Anxiety Rating Scale; LSEQ = Leeds Sleep Evaluation Questionnaire; MGH-HPS = Massachusetts General Hospital Hair Pulling Scale; NAC = N-acetylcysteine; OCD = obsessive-compulsive disorder; PCL-M = PTSD Checklist-Military; PITS = Psychiatric Institute Trichotillomania Scale; PMS = premenstrual syndrome; POMS = Profiles of Mood States; PSS = Perceived Stress Scale; PTSD = posttraumatic stress disorder; PUFA = polyunsaturated fatty acid; RDBCT = randomized double-blind placebo-controlled trial; SDS = Sheehan Disability Scale; SRI = serotonin reuptake inhibitor; STAI-S = State-Trait Anxiety Inventory in a Specific Situation; STAI-T = State-Trait Anxiety Inventory as a General Trait; SUD = substance use disorder; TTM = trichotillomania; VAS = Visual Analog Scale; Y-BOCS = Yale-Brown Obsessive-Compulsive Scale.

(considered an experimental model of autism). The effects were that mGlu$_{2/3}$ receptor antagonist LY341495 blocked dependent mGLU$_{2/3}$ receptors. Accordingly, NAC also reduced enhanced presynaptic excitatory neurotransmission to normal levels in the amygdala of valproate-exposed rats.[68] NAC prevented rhythm disruption-induced anxiety in mice.[69] The anxiolytic effects of NAC have also been documented in the light/dark model and in stress-induced anxiety behavior in zebrafish,[70] as well as in the hole-board test, the light/dark model, the open field test, social interaction, and stress-induced hyperthermia in mice.[71] One report suggested that NAC may have anxiogenic properties, but this conclusion is questionable since it was based only on decreased time spent in the center of an open field, which was also accompanied by decreased locomotion.[84]

Very few studies have been designed to evaluate the specific effects of NAC in patients diagnosed with an anxiety disorder. Back et al. conducted a study with NAC in veterans diagnosed with comorbid PTSD and substance use disorder (SUD) in which the patients were treated with NAC or placebo, along with group cognitive-behavioral therapy. NAC decreased self-reported and clinician-rated PTSD symptoms, and the symptoms remained significantly lower after the drug was discontinued at one-month of follow-up. Patients receiving NAC also reported decreased cravings.[88]

NAC has been evaluated in obsessive-compulsive and related disorders where anxiety is a key component. An RDBCT conducted by Grant et al. revealed significant improvement in trichotillomania patients after 12 weeks of treatment[126]; this result was substantiated by a number of case reports.[127-129] In pediatric trichotillomania patients, an RDBCT found no significant reduction in hair pulling compared to placebo[130]; in this trial, the authors suggested that the improvement was associated with psycho-education about trichotillomania rather than drug treatment, since significant improvement in several measures of hair pulling was observed regardless of treatment time. A series of case reports showed that NAC is effective against excoriation disorder,[127,131-133] and an RDBCT concluded that NAC significantly reduced skin-picking symptoms.[89] Berk et al. found a reduced nail biting frequency in three patients enrolled in a bipolar disorder treatment protocol with NAC.[134] Ghanizadeh et al.'s finding that NAC decreases nail biting in the short, but not the long term is somewhat questionable considering the lower dose and shorter treatment time used in their RDBCT compared to the case reports.[86]

It has been reported that NAC has beneficial effects in children, adolescents, and adults with OCD.[135,136] Afshar et al. conducted an RDBCT with NAC as an add-on treatment in OCD patients refractory to SSRIs, finding that NAC improved Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) and Clinical Global Impression-Severity of Illness (CGI-S) scores, but not those on the Clinical Global Impression–Improvement Scale (CGI-I); full responders at the end of the study were significantly higher than placebo.[85] Sarris et al. conducted an RDBCT using NAC as add-on treatment (mainly to SSRIs) in OCD patients; the primary outcome measure was the Y-BOCS,

conducted every 4 weeks. At week 12 there was a significant reduction in Y-BOCS score, but the difference dissipated at week 16.[87] A third RDBCT was performed with moderate-to-severe OCD patients, randomized to receive fluvoxamine plus placebo or fluvoxamine plus NAC. NAC showed a significant effect on Y-BOCS score.[90]

### Omega-3

Adequate dietary levels of PUFA, including omega-3 fatty acids, are essential for health since they are important components of cholesterol esters and phospholipids in the neuronal cell membrane. Changes in the composition of these membrane phospholipids can affect the regulation of neurotransmitter release, receptors, ion channels, and enzyme activity.[137,138] Omega-3 and omega-6 PUFAs are cleaved from membrane phospholipids and converted via different pathways to mediators that have opposing effects: arachidonic acid mediators are derived from omega-6 fatty acids and are proinflammatory, while mediators derived from omega-3 fatty acids have anti-inflammatory effects. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the two main types of omega-3 PUFA, and fish oil is their main dietary source. It has been suggested that EPA may play a role in brain function by counteracting arachidonic acid-mediated signaling, decreasing immune-inflammatory responses mediated by omega-6 derived eicosanoids, which have been linked to the pathophysiology of anxiety and other mental disorders.[92,139] Moreover, by inhibiting proinflammatory cytokine secretion, omega-3 may also decrease corticosteroid release from the adrenal gland, reducing the mood-altering effects associated with increased cortisol,[140] and hence reducing the impact of cortisol on anxiety.

Several studies have investigated the effects of omega-3 fatty acids in animal models of anxiety (Table 1). Most of the rodent studies involved long-term diet supplements with DHA or a combination of EPA and DHA. Carrié et al. used a DHA-supplemented diet in mice previously fed with a semisynthetic balanced diet or a diet deficient in alpha-linolenic acid (ALA) (another type of omega-3 fatty acid) until the age of 8 months. The supplemented diet showed anxiolytic effects, regardless of the previous diet condition, and restored water maze performance, which had been impaired in the ALA deficient diet group.[72] Jašarević et al. treated female mice for three generations with an omega-6/omega-3 supplemented diet and found that the male offspring of the third generation showed decreased anxiety-like behavior.[78] Rat diets supplemented with different combinations of PUFAs counteracted the anxiogenic effects of intracerebroventricular administered IL-1 beta[73,74] and restraint stress.[75,77] The anxiolytic effect of omega-3 supplementation has also been demonstrated in adult male grey mouse lemurs (*Microcebus murinus*), a nocturnal Malagasy prosimian primate.[76,79]

Low omega-3 levels in erythrocyte membranes have been observed in patients with anxiety disorders.[141-143] Nevertheless, most trials investigating omega-3 in anxiety focused on anxiety symptoms in different conditions rather than anxiety disorders themselves. In an RDBCT with healthy young adults, Kiecolt-Glaser et al. showed

that EPA and DHA supplementation decreased anxiety symptoms and LPS-stimulated production of IL-6.[94] Yehuda et al. showed that a mixture of ALA and linolenic acid, given to university students experiencing significant anxiety associated with upcoming exams (test anxiety), improved variables associated with test anxiety (e.g., appetite, mood, concentration, fatigue, academic organization, sleep) and lowered cortisol levels.[91] The anxiolytic effects of omega-3 supplementation were found in patients with acute myocardial infarction[96] and women diagnosed with premenstrual syndrome (PMS).[97] In an RDBCT, Buydens-Branchey & Branchey investigated the effects of a mixture of EPA + DHA supplementation in patients with a history of substance abuse, finding that the supplementation progressively decreased anxiety scores, which remained decreased three months after treatment was discontinued.[138] In a subsequent similarly designed RDBCT, the same group showed that increases in circulating omega-3 levels paralleled decreases in anxiety scores.[93] Similar results were found with male alcoholic patients in a residential rehabilitation program: this small-sample RDBCT showed that fish oil (a source of omega-3 fatty acids) decreased stress/anxiety ratings and reduced basal levels of cortisol.[95] In a placebo-controlled crossover trial, Fux et al. showed that EPA is ineffective as an add-on treatment to SSRI in OCD patients, though the reliability of their results is questionable due to the small sample size and the high placebo response.[144] Matsuoka et al. reported that omega-3 supplementation was not superior to placebo for PTSD symptom prevention three months after accidental injury.[145] In a cohort of Japanese accident survivors at risk of developing PTSD, the same group reported that short-term supplementation with DHA and EPA lowered heart rates during script-driven imagery and/or resting, whereas the baseline heart rate did not differ from the placebo group.[98]

In addition to the compounds discussed above (agomelatine, NAC, and omega-3 fatty acids), we also found some evidence of anxiolytic effects in clinical trials and animal studies for ascorbic acid (vitamin C) and the mGlu$_{2/3}$ receptor agonist LY354740. Although ascorbic acid has presented anxiolytic effects in different animal models in rats,[146] mice,[147] and zebrafish,[148] evidence of its anxiolytic effects in humans is limited. Only one small randomized double-blind placeb-controlled clinical trial (n=42) with ascorbic acid conducted with normal volunteers was found: its results were that ascorbic acid decreased anxiety levels.[149] Although studies with LY354740 showed robust anxiolytic activity in several animal models, as well as in a few clinical trials, larger clinical trials were interrupted due to reports of seizures in animal studies.[16]

One limitation of our study is the likely existence of publication bias in this field. Despite the possibility that many negative results concerning this topic may have been deterred from publication, our main goal was to present the available data for compounds with a robust body of evidence.

## Conclusion

We reviewed three compounds that may counteract key biochemical correlates of anxiety states. Despite a

reasonable body of evidence showing anxiolytic properties, the results show that the clinical data is deficient. Data from clinical trials are more indicative than conclusive, and larger trials specifically designed for anxiety disorders are needed. Nevertheless, the beneficial effect observed in clinical conditions where mainstream treatments are ineffective should not be overlooked.

Regarding safety and tolerability, clinical trials and toxicity studies have shown that agomelatine,[106,150] NAC,[111] and omega-3[151] were generally well tolerated and free from serious adverse effects. The most common side effects reported were headache, dizziness, somnolence, fatigue, and gastrointestinal symptoms for agomelatine,[150] gastrointestinal symptoms, with headache for NAC[111] and a fish aftertaste and nausea with omega-3.[140,151]

In conclusion, due to the prevalence and morbidity of anxiety disorders, the potential translational value of the biochemical basis of anxiety, and the safety profile of these compounds, investment in larger clinical trials seems justified.

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## Disclosure

The authors report no conflict of interest.

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