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Recent Developments in Novel Antidepressants Targeting $\alpha 4\beta 2$ -Nicotinic Acetylcholine Receptors

Miniperspective

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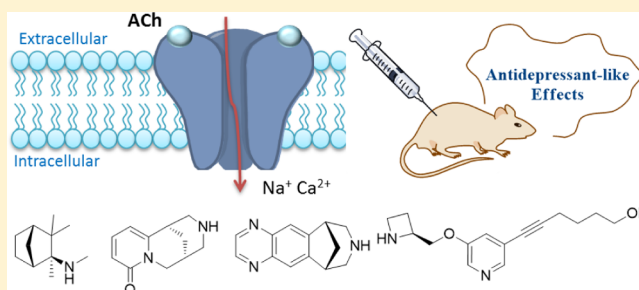
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ABSTRACT: Nicotinic acetylcholine receptors (nAChRs) have been investigated for developing drugs that can potentially treat various central nervous system disorders. Considerable evidence supports the hypothesis that modulation of the cholinergic system through activation and/or desensitization/inactivation of nAChR holds promise for the development of new antidepressants. The introductory portion of this Miniperspective discusses the basic pharmacology that underpins the involvement of $\alpha 4\beta 2$ -nAChRs in depression, along with the structural features that are essential to ligand recognition by the $\alpha 4\beta 2$ -nAChRs. The remainder of this Miniperspective analyzes reported nicotinic ligands in terms of drug design considerations and their potency and selectivity, with a particular focus on compounds exhibiting antidepressant-like effects in preclinical or clinical studies. This Miniperspective aims to provide an in-depth analysis of the potential for using nicotinic ligands in the treatment of depression, which may hold some promise in addressing an unmet clinical need by providing relief from depressive symptoms in refractory patients.



INTRODUCTION

Depression is a common and frequently severe psychological condition with a distinct change of mood, characterized by sadness, loss of interest and pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, and feelings of tiredness and poor concentration, affecting approximately 120 million people worldwide.¹ Numerous therapeutic agents exist for the treatment of depression that target monoamine transporters regulating the uptake of the neurotransmitters dopamine, serotonin, and norepinephrine.² However, a considerable proportion of patients respond poorly to these drugs,³ as demonstrated by the NIMH-funded sequenced treatment alternatives to relieve depression (STAR*D) study conducted between 2001 and 2006, which highlighted the inadequacy of current medications for major depressive disorder (MDD).⁴ Therefore, there is still an urgent need for potent pharmacotherapies associated with novel biological mechanisms of action. In this Miniperspective, we review the association between depression and nicotinic acetylcholine receptors (nAChRs), especially the $\alpha 4\beta 2$ -nAChR

subtype, from the perspective of clinical and preclinical findings. We highlight the most recently developed $\alpha 4\beta 2$ -nAChR agonists and antagonists that exhibit antidepressant-like effects in vivo.

The cholinergic hypothesis of depression proposes that hyperactivity of the cholinergic system over that of the adrenergic system leads to depression (Figure 1).⁵ Several lines of evidence from rodent and human studies support this hypothesis. Flinders sensitive rats, a line selectively bred for increased cholinergic sensitivity, were found to exhibit several depression-like behaviors,^{6,7} and increased acetylcholine (ACh) signaling in the hippocampus was found to promote behaviors in mice related to anxiety and depression.⁸ In humans, physostigmine, which potentiates cholinergic transmission by inhibiting acetylcholinesterase (AChE), the enzyme that breaks down ACh, produces depressive-like symptoms in individuals with and without a history of depression.⁵ Administration of the

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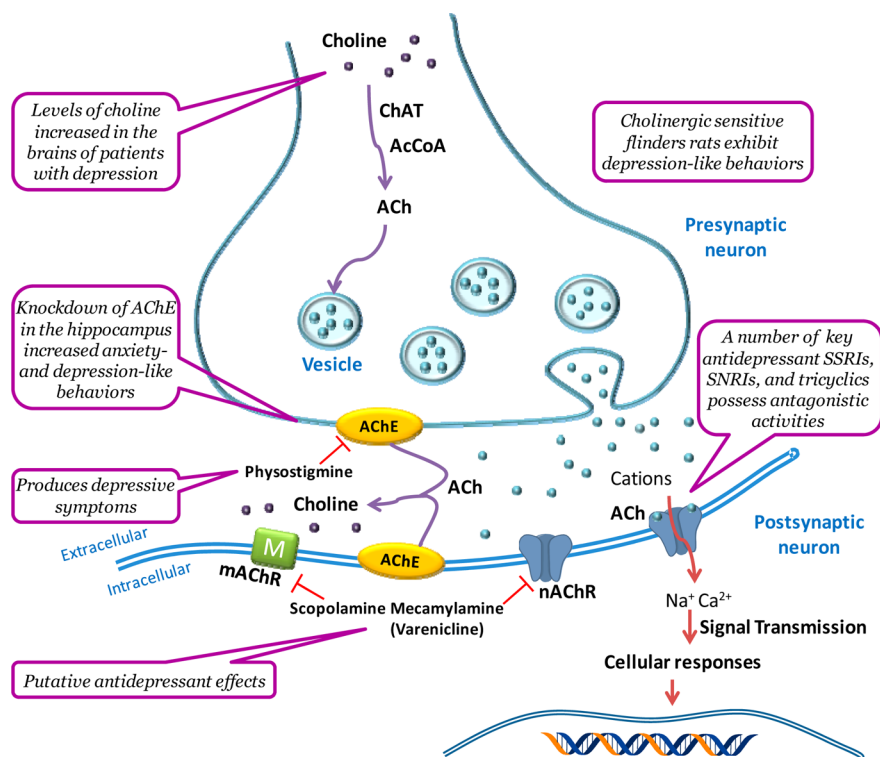


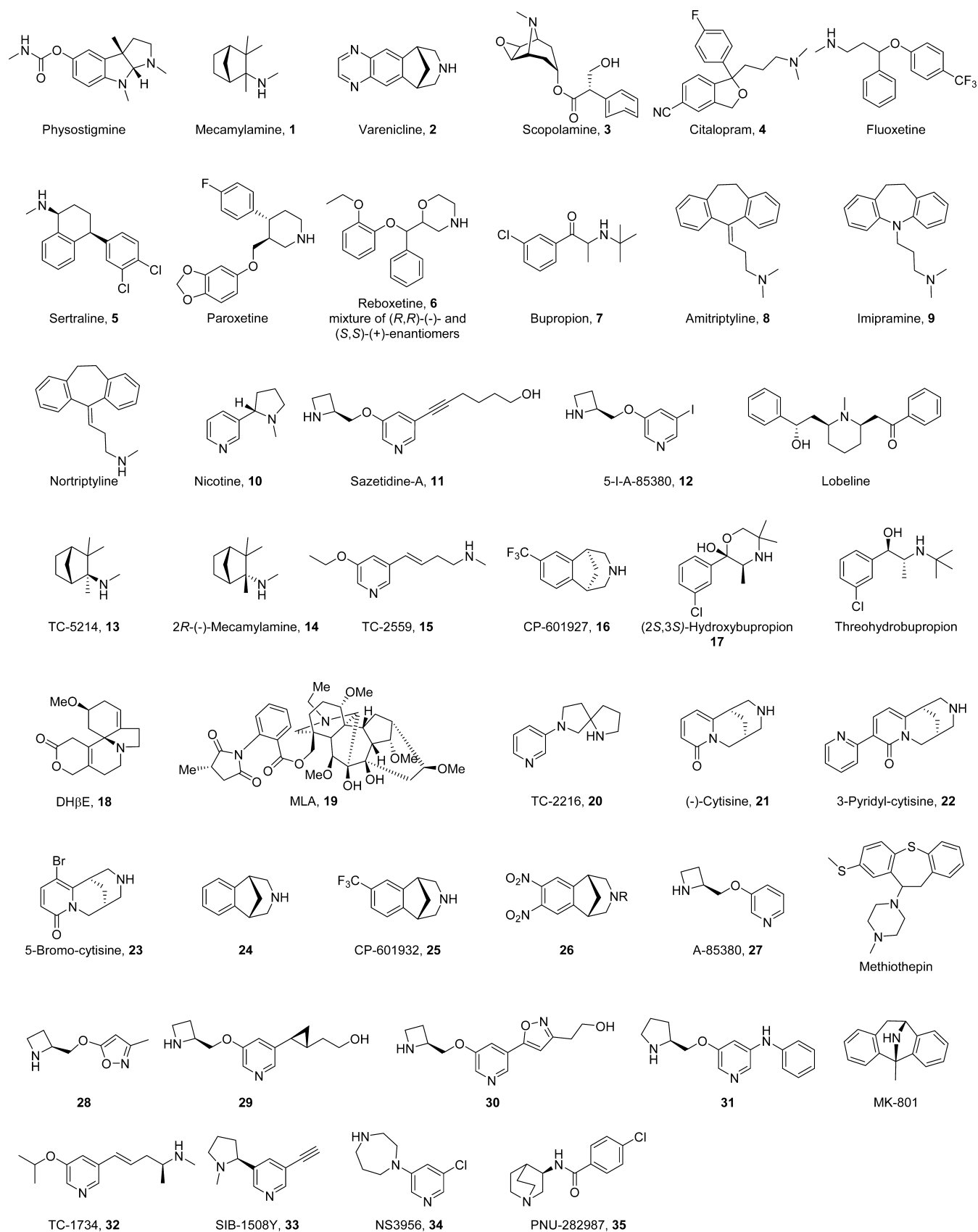
Figure 1. Role of the cholinergic system in depression. The cholinergic hypothesis of depression postulates a hyperactivity of the cholinergic system over that of the adrenergic system in the brain. Choline (the rate-limiting precursor to endogenous ACh) crosses the blood–brain barrier to enter the brain and is actively transported into the cholinergic presynaptic terminals by an active uptake mechanism. The neurotransmitter ACh is synthesized from choline and acetyl coenzyme A, catalyzed by the enzyme choline acetyl transferase. ACh is sequestered into secretory vesicles by vesicular ACh transporters. Once released from the presynaptic terminals, ACh can interact with a variety of presynaptic and postsynaptic receptors. Two classes of the cholinergic ACh receptors are muscarinic (G protein-coupled) and nicotinic (ionotropic). Once activated, nAChRs form transient open cationic channels that allow the ions Na⁺, K⁺, and Ca²⁺ to flow across the plasma membrane and induce cellular responses. Prolonged exposure to ACh or nicotinic agonist causes a gradual decrease in the rate of this ionic response, leading to a high affinity, longer-lasting functionally inactive state, referred to as desensitization. ACh has its signal terminated primarily by the enzyme AChE, unlike many other monoaminergic neurotransmitters where reuptake mechanisms predominate.

nonselective nicotinic antagonist mecamylamine (**1**) (Scheme 1),^{9–11} the partial agonist varenicline (**2**),¹² or the muscarinic antagonist scopolamine (**3**)^{13,14} demonstrated putative antidepressant-like effects, especially in treatment-resistant patients receiving their regular psychotropic medications such as the selective serotonin reuptake inhibitor (SSRI) citalopram (**4**).⁹ Magnetic resonance imaging studies have shown that the levels of choline, the rate-limiting precursor to endogenous ACh, were elevated in the brains of patients with depression as well as in the frontal cortex of adolescents with depression.^{15,16} Additionally, a number of key antidepressants such as SSRIs (fluoxetine, sertraline (**5**), paroxetine, and citalopram **4**), the norepinephrine reuptake inhibitor reboxetine (**6**), the norepinephrine dopamine reuptake inhibitor bupropion (**7**), and tricyclics (amitriptyline (**8**), imipramine (**9**), and nortriptyline)¹⁷ have all been shown to possess antagonistic activities at nAChRs,^{18–20} although, in most cases, drug concentrations achieved in the human brain would not be adequate to affect nAChR functions.²¹ Nicotine (**10**) itself, and some nicotinic agonists or antagonists, can potentiate the antidepressant-like effects of the SSRIs and SNRIs in rodent models,^{22,23} likely due to the common end point of reduced function due to receptor desensitization by agonists or antagonism. The development of nAChR ligands to attenuate cholinergic activity to treat depression could conceivably help to treat depressive symptoms in refractory patients.

■ NICOTINIC ACETYLCHOLINE RECEPTORS AND DEPRESSION

The two major types of cholinergic receptors are muscarinic ACh receptors (mAChRs) and nAChRs, both of which are widely distributed in the central and peripheral nervous systems.²⁴ G protein-coupled mAChRs are believed to be involved in mood regulation and AChE-induced depressive behavior.^{5,13,14,25} Neuronal nAChRs belong to the ligand-gated ion channel superfamily of neurotransmitter receptors. Varying combinations of nAChR subunits ($\alpha 1$ – $\alpha 10$, $\beta 1$ – $\beta 4$, γ , δ , and ϵ ; $\alpha 2$ – $\alpha 7$ and $\beta 2$ – $\beta 4$ are expressed in the brain) assemble into pentameric ion channels, allowing for diverse pharmacological properties.²⁶ Each nAChR subunit consists of a large amino-terminal extracellular domain (ECD), a transmembrane domain comprising four α -helices (M1–M4), and a variable cytoplasmic domain between M3 and M4. ACh binding sites are thought to form between the subunit interfaces of the ECD bound by the C-loop containing the face of an α -type subunit and the face of an adjacent subunit. When acutely activated by endogenous ACh or exogenous nicotinic ligands, nAChRs form transient open cationic channels that allow the ions Na⁺, K⁺, and Ca²⁺ to flow across the plasma membrane and induce cellular responses.²⁷ Prolonged exposure to ACh or nicotinic agonists causes a gradual decrease in the rate of this ionic response, leading to a longer-lasting functionally inactive state through a process referred to as desensitization. nAChRs have been found to contribute to mood

Scheme 1. Compound Structures



control by regulating behavioral reinforcement in the mesolimbic dopamine system, corticotropin releasing factor and function in the hypothalamic–pituitary–adrenal (HPA) axis, circadian

rhythms in the suprachiasmatic nucleus, and cytoplasmicity in the hippocampus.¹¹ Given nAChR subtype diversity and their involvement in the modulation of various key neurotransmitter

systems, including dopamine, serotonin, norepinephrine, glutamate, and γ -aminobutyric acid (GABA), nicotinic ligands have the potential to treat a multitude of neurological and psychiatric disorders, including depression.^{9,28}

The $\alpha 4\beta 2$ heteropentameric and $\alpha 7$ homopentameric subtypes are the two major nAChR subtypes expressed in the brain (Figure 2).²⁹ Both subtypes are implicated in the mediation

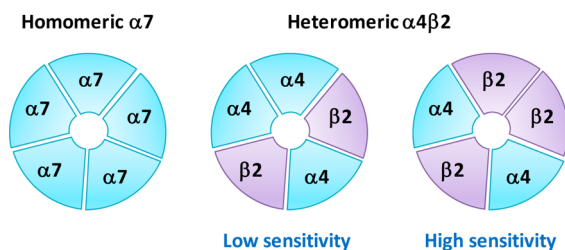


Figure 2. Selected nAChR subtypes. The high sensitivity (HS) $\alpha 4\beta 2$ -nAChR has a presumed $\alpha 4/\beta 2$ subunit ratio of 2:3 and exhibits comparatively high sensitivity to nicotinic agonists, whereas the low sensitivity (LS) $\alpha 4\beta 2$ -nAChR, at which nicotinic agonists have lower observed potency, is composed presumably of $\alpha 4$ and $\beta 2$ subunits in a 3:2 ratio.

of the pharmacological and behavioral effects of compound **10** and in nAChR-mediated modulation of monoamine release^{30,31} and are likely involved in the antidepressant effects of nicotinic ligands. Studies investigating the role of nAChRs in depression have focused primarily on the $\alpha 4\beta 2^*$ (asterisk indicates possible assembly with other subunits) and $\alpha 7$ -nAChR subtypes. $\alpha 4\beta 2^*$ -nAChRs are widely distributed in the neuroanatomic regions implicated in depression, including the thalamus, basal ganglia, striatum, hypothalamus, amygdala, ventral tegmental area (VTA), locus coeruleus, and dorsal raphe nucleus,⁹ and $\alpha 7$ -nAChRs are highly expressed in the hypothalamus, hippocampus, and cortex.^{32,33} $\alpha 4\beta 2^*$ -nAChRs are thought to regulate the release of monoamine neurotransmitters through action in these areas.^{34,35} $\beta 2$ -knockout mice showed decreased immobility in the forced swim test (FST) compared to that of wild-type mice, indicating that the absence of $\beta 2$ -nAChRs-mediated signaling could manifest in an antidepressant-like phenotype in vivo.³⁶ The antidepressant-like effect of the nAChR antagonist compound **1** was diminished when the $\beta 2$ - or $\alpha 7$ -subunits were knocked out.³⁷ Similarly, the antidepressant-like effects of the nAChR agonist sazetidine-A (**11**)³⁸ and the tricyclic antidepressant compound **8**³⁶ were absent in mice lacking the $\beta 2$ -subunit. Additionally, the efficacies of compounds **4** and **6** in the mouse FST were enhanced by agonists at either $\alpha 4\beta 2^*$ - or $\alpha 7$ -nAChRs.²³ These findings suggest the involvement of $\beta 2$ - and

$\alpha 7$ -receptor subtypes in mediating the antidepressant-like effects of nicotinic ligands.

Clinical studies provide additional evidence for a relationship between $\alpha 4\beta 2^*$ -nAChRs and depression. Single photon emission computed tomography (SPECT) using the $\alpha 4\beta 2^*$ -nAChR specific radioligand [¹²³I]5-I-A-85380 ([¹²³I]**12**) revealed that the $\beta 2^*$ -nAChR availability across all brain regions in depressed patients was lower than that in healthy subjects.³⁹ Additionally, positron emission tomography using 2-[¹⁸F]fluoro-3-(2-[S]-2-azetidylmethoxy)-pyridine showed reduced levels of ligand binding to $\alpha 4\beta 2^*$ -nAChR in Parkinson's patients with depressive symptoms.⁴⁰ It was recently reported that the clinical action of compound **6** may be at least partially due to its inhibitory action on $\alpha 4\beta 2$ -nAChR.⁴¹

Designing a nicotinic ligand to provide maximal therapeutic efficacy and minimal side effects depends on a ligand's ability to specifically target the desired combination of nAChR subtypes. Observations that nAChR $\alpha 6$ subunits are not widely distributed in the brain but are most prevalent in midbrain dopaminergic regions in the mammalian CNS suggest their potential involvement in mood control. As such, targeting $\alpha 6^*$ -nAChR may be indicated.^{11,42–44} In the basal ganglia, the VTA and substantia nigra, in particular, $\alpha 6$ - and possibly nAChR $\beta 3$ subunits, are included in $\alpha 4\beta 2^*$ -nAChRs that appear to have high affinity for nicotinic agonists.³⁴ $\alpha 3\beta 4^*$ -nAChR subtypes are expressed at relatively low levels in the brain⁴⁵ except for the interpeduncular nucleus, fasciculus retroflexus, and median habenula.⁴⁶ However, activation or blockade of $\alpha 3\beta 4^*$ -nAChR, which are also expressed in the peripheral nervous system, may result in side effects in vivo, including dysregulation of the autonomic nervous system.^{47,48} Selective and potent partial agonists of $\alpha 4\beta 2^*$ -nAChRs, especially those with low affinity for $\alpha 3\beta 4^*$ -nAChRs, are considered to have higher efficacy and likely fewer side effects in rodent behavioral models, although the involvement of $\alpha 3\beta 4^*$ -nAChRs in mood control cannot be completely ruled out.^{49–51} We also point out that because of their roles as “accessory” subunits that are incapable of forming functional receptors alone or even in combination with nAChR α or β subunits (except, perhaps, as $\alpha 7\alpha 5$ -nAChR), we have not delved deeply into the potential roles played by the $\alpha 5$ subunits. However, $\alpha 5$ subunits can integrate into $\alpha 3\beta 4^*$ - and $\alpha 4\beta 2^*$ -nAChRs and perhaps into $\alpha 6^*$ -nAChRs to further extend the diversity in receptor subtypes and isoforms, which may affect pharmacological profiles.^{52,53} Continuing studies will extend our knowledge of the role of $\alpha 5$ subunits in nAChRs.

The essential pharmacophore presented by nicotinic ligands consists of a cationic center (e.g., a quaternized or protonated nitrogen) and a hydrogen-bond acceptor (e.g., the pyridine nitrogen atom in the case of compound **10**). The cationic nitrogen binds to a tryptophan residue of the principal α -subunit,

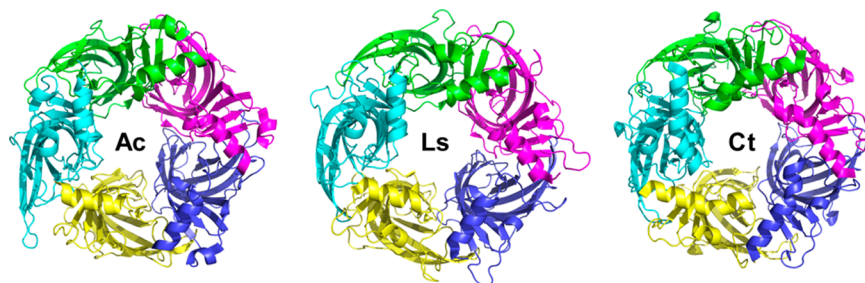


Figure 3. Top view of X-ray crystal structures of Ac-, Ls-, and Ct-AChBPs. The figure was generated using PDB files 2BR7, 1I9B, and 4B5D by PyMOL.

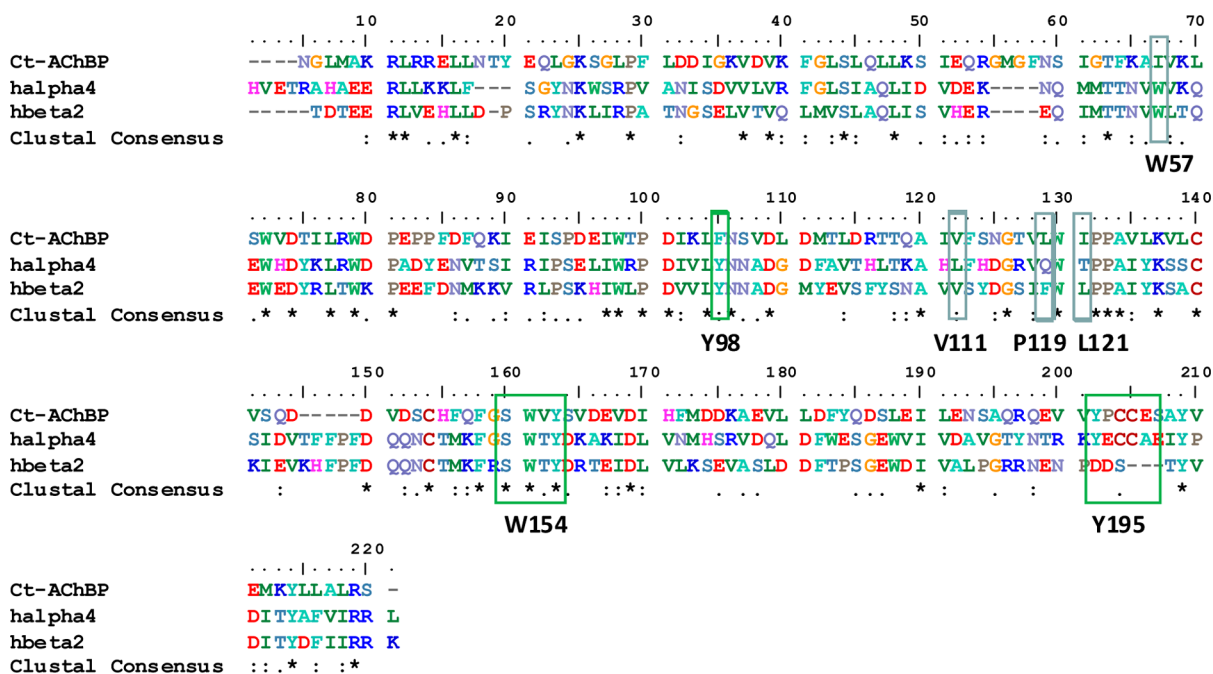


Figure 4. Amino acid sequence alignment of the *Ct*-AChBP with extracellular domains of nAChR $\alpha 4$ or $\beta 2$ subunits. Green boxes highlight positions of key residues of the $\alpha 4$ subunit, and blue boxes outline key residues of the $\beta 2$ subunit. Residue numeration refers to that for the human $\alpha 4$ subunit. (This is for the mature $\alpha 4$ subunit, not including cleavage of the leaders sequence including the translational start methionine residue.)

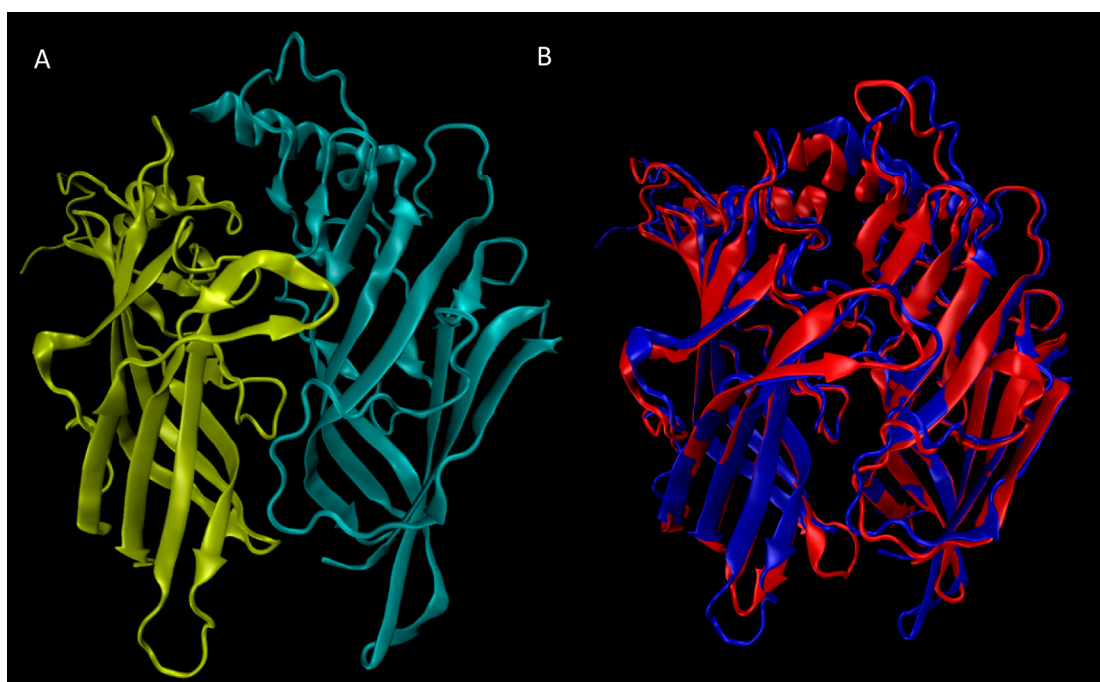


Figure 5. Homology model of the human $\alpha 4\beta 2$ -nAChR ECD including the ligand binding interface: (A) ribbon structure representation colored by subunit (yellow, $\alpha 4$; azure, $\beta 2$) for the human $\alpha 4\beta 2$ -nAChR ECD and (B) superimposition of the modeled structure (in blue) with the experimental template from *Ct*-AChBP (in red).

forming a cation- π interaction.^{54–56} The hydrogen-bond acceptor has been shown to bind to the complementary β -subunit, likely mediated by a water molecule to the backbone NH of a Leu residue in the $\alpha 4\beta 2$ receptor.^{57,58} Additionally, various studies support the idea that agonist interactions with the principal α -subunit are important for binding affinity, while interactions with key amino acids present in the complementary β -subunit affect agonist efficacy.^{59,60}

High-resolution crystal structures of integral membrane protein mammalian nAChRs are not yet available. Invertebrate pentameric acetylcholine binding protein (AChBP) subunits share 20–24% sequence identity with that of the homologous ECDs of nAChR subunits. However, the putative binding site and some aromatic residues found in nAChR subunits are reported to be conserved. Therefore, AChBPs have been characterized and used to provide insights into ligand recognition

sites and other elements of nAChR subunit ECDs and ligand binding site interfaces. The majority of ligand-bound structures have been obtained with AChBP from *Aplysia californica* (*Ac*) or *Lymnaea stagnalis* (*Ls*) (Figure 3). The *Ls*-AChBP was postulated to be a more suitable surrogate of $\alpha 4\beta 2$ -nAChR for a series of 1-(pyridin-3-yl)-1,4-diazepane analogues because the Trp 53 in *Ls*-AChBP subunits is Tyr in *Ac*-AChBP subunits, which interacts less favorably with the quaternized or protonated nitrogen of nicotinic ligands. Recently, the nonmolluscan acetylcholine binding protein from the marine annelid *Capitella teleta* (*Ct*), namely, *Ct*-AChBP, was identified⁶¹ and reported to more closely mimic $\alpha 4\beta 2$ -nAChR than does the *Ac*-AChBP based on comparisons of the ligand binding affinities.⁶² The X-ray crystal structure of *Ct*-AChBP with lobeline or compound **2** bound along with mutagenic studies highlight the importance of key interactions that are responsible for receptor activation or desensitization and for location of key residues in loops D (W57) and E (V111, F119, and L121) in the complementary subunit opposed to the α subunit at the ligand binding subunit interface.

Because of the higher homology between *Ct*-AChBP and the human $\alpha 4$ and $\beta 2$ subunits (as computed by ClustalX: $\alpha 4$ /*Ct*-AChBP = 64.6% and $\beta 2$ /*Ct*-AChBP = 62.1%), especially for key residues involved in ligand recognition (Figure 4), a homology model was generated and refined based on a *Ct*-AChBP for the ligand binding domain of the human $\alpha 4\beta 2$ -nAChR. A ribbon structure for the ECD modeled $\alpha 4\beta 2$ -nAChR shows a 10-stranded β -sandwich capped by an N-terminal α -helix for each subunit (Figure 5A), for which the modeled β -sandwich can be nicely superimposed on that of *Ct*-AChBP (Figure 5B). This homology model may provide useful information for designing other selective $\alpha 4\beta 2$ -nAChR ligands.⁶³

■ nAChR AGONISTS AND ANTAGONISTS EXHIBITING ANTIDEPRESSANT-LIKE EFFECTS

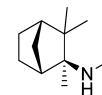
The diversity of nAChR subtypes provides an opportunity to generate subtype-specific ligands that could treat a variety of conditions, although the high sequence homology across individual subtypes of brain nAChRs poses a substantial challenge for the development of subtype-selective nicotinic drugs. Both academia and industry have contributed to a growing body of literature concerning the rational design of potential antidepressant ligands that more selectively target $\beta 2^*$ -nAChR than does compound **10**.

Rodent behavioral models have been used to assess antidepressant-like effects of these new chemical entities. The most widely used behavioral assay for antidepressant-like efficacy is the FST, in which a mouse or rat is placed in a beaker of water and the amount of time the animal spends passively floating is measured. Clinically therapeutic antidepressants typically reduce the time an animal will spend immobile. Similarly, the tail suspension test (TST) measures immobility time when the rodent is suspended by its tail. This test is relatively short in duration, and in the case where an antidepressant agent is given, the animal will struggle for longer periods of time compared to vehicle treated animals.⁶⁴ The novelty-suppressed feeding (NSF) test is a behavioral paradigm originally utilized to measure anxiolytic-like effects of drugs, but more recently the NSF assay has been proposed as a behavioral platform sensitive to the chronic but not acute administration of antidepressants. In NSF, food-deprived mice experience a conflict between feeding and the fear of exploring the novel environment of a brightly lit open area or an unfamiliar cage containing food. Chronic treatment with antidepressants reduces latency to eat in the novel

environment. Additionally, an alternative version of the NSF has been advanced that is called the novelty-induced hypophagia (NIH) test, but it utilizes a palatable food to eliminate the need for food restriction. In general, these models predict the onset of action of antidepressants consistent with the therapeutic time course found in humans and therefore validate the NSF/NIH tests as useful behavioral paradigms to gauge the antidepressant efficacy of compounds.⁶⁵

■ nAChR ANTAGONISTS

(1*R*,2*S*,4*S*)-*N*,2,3,3-Tetramethylbicyclo[2.2.1]heptan-2-amine, **13**.



TC-5214, **13**

IC_{50, $\alpha 4\beta 2$} : 0.5–3.2 μ M
 IC_{50, $\alpha 3\beta 4$} : 0.2–0.6 μ M
 IC_{50, $\alpha 7$} : 1.2–4.6 μ M
 IC_{50, $\alpha 1\beta 1\gamma\delta$} : 0.6–2.2 μ M

The nicotinic ligand that has received the most attention as a potential antidepressant is compound **1**, a racemic non-competitive and nonselective antagonist of nAChRs (IC_{50, $\alpha 3\beta 4$} = 91–610 nM, IC_{50, $\alpha 4\beta 2$} = 0.6–2.5 μ M, and IC_{50, $\alpha 7$} = 1.6–6.9 μ M). Originally developed as an antihypertensive agent, anecdotal reports of mood modulation and a hypothesis that traditional antidepressants might be acting in part through noncompetitive antagonism of nAChRs¹⁹ led to a preliminary controlled study that demonstrated therapeutic effects of compound **1** for mood disorders, including MDD, that were comorbid with Tourette's disorder.^{10,50} However, the subgroup sizes were very small, and the comorbidity with Tourette's disorder precluded any conclusions from being drawn about the antidepressant efficacy of compound **1** monotherapy in patients who do not have Tourette's disorder.

In studies using receptor expression in *Xenopus* oocytes, the 2*S*(+)-enantiomer of compound **1**, TC-5214 (**13**),⁵¹ was found to dissociate more slowly than 2*R*(-)-mecamylamine (**14**) from $\alpha 4\beta 2$ - and $\alpha 3\beta 4$ -nAChRs, as well as more slowly from $\alpha 4\beta 2$ - than $\alpha 3\beta 4$ -nAChRs. IC₅₀ values for compound **13** at $\alpha 3\beta 4$ -, $\alpha 4\beta 2$ -, $\alpha 7$ -, or $\alpha 1\beta 1\gamma\delta$ -nAChRs are similar to those of compound **14** (IC_{50, $\alpha 3\beta 4$} = 0.2–0.6 and 0.05–0.4 μ M, IC_{50, $\alpha 4\beta 2$} = 0.5–3.2 and 0.5–1.7 μ M, IC_{50, $\alpha 7$} = 1.2–4.6 and 2.2–5.8 μ M, and IC_{50, $\alpha 1\beta 1\gamma\delta$} = 0.6–2.2 and 0.3–1.1 μ M).⁵¹ Further pharmacological studies suggested that compound **13** is a more efficacious antagonist of LS $\alpha 4\beta 2$ -nAChRs than compound **1** and can act as a positive allosteric modulator at HS $\alpha 4\beta 2$ -nAChRs.⁶⁶ This was hypothesized to be mechanistically important; however, the positive allosteric modulation at $\alpha 4\beta 2$ -nAChRs was inferred from a slight potentiation of the HS $\alpha 4\beta 2$ -selective agonist TC-2559 (**15**) in a mixed population of HS and LS $\alpha 4\beta 2$ -nAChRs. Potentiation of ACh was not demonstrated, and because the effect was not reproduced in a pure population of HS $\alpha 4\beta 2$ -nAChRs, activation of LS $\alpha 4\beta 2$ -nAChRs by the coapplication of compounds **15** and **13** was not excluded.

Compound **13** exhibited higher anxiolytic- and antidepressant-like effects in several animal models than racemic mecamylamine (forced swim and social interaction tests, light/dark assay). These behavioral activities were attributed to antagonist effects at $\alpha 4\beta 2^*$ -nAChRs.⁶⁷ Moreover, compound **13** showed a superior preclinical safety profile compared to that of either the racemic compound or the 2*R*(-)-enantiomer. Compound **13**

was found to be well-tolerated in acute and chronic toxicity studies in different animal models (mice, rats, and dogs) and showed acceptable pharmacologic, pharmacokinetic, and metabolic profiles for therapeutic development.

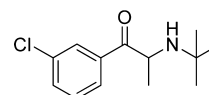
Targacept advanced compound 13 to a phase 2 study as an augmentation in patients who were inadequate responders to the SSRI compound 4. Initial results were very promising, with patients showing an average 6 point improvement on the primary end point, the Hamilton depression rating scale (HAM-D), as well as improvement on other secondary measures.⁶⁸ A collaboration with AstraZeneca (Targacept and AstraZeneca's RENAISSANCE program) followed, advancing compound 13 to phase 3 development as an adjunctive therapy in MDD patients who were inadequate responders to SSRI or SNRI monotherapy. Unfortunately, four phase 3 trials (two fixed and two flexible dose trials) failed to meet the primary end point of achieving a greater change in the Montgomery-Asberg depression rating scale total score for the experimental group receiving an adjunctive therapy of compound 13 combined with an SSRI or SNRI than for the placebo group receiving monotherapy with an SSRI or SNRI alone.^{69–72}

There are several possible explanations for the failure of compound 13 in the phase 3 trials. One explanation is that the studies that utilized compound 13 as an adjunct therapy had an unusually high placebo response. Every dose group (compound 13 and placebo) showed at least a 40% improvement in the MADRS total score after 8 weeks of adjunct treatment. A contemporaneous phase 2b monotherapy study of compound 13, which had a lower placebo response, provided some indications of a dose-related antidepressant response, but the study was terminated early.⁷³ It has been suggested that pursuing compound 13 as a monotherapy may have led to a better outcome than as a combination therapy with an SSRI or SNRI.^{73,74} Subjects in placebo groups in the combination study received SSRI or SNRI treatment, and manipulation of the serotonergic or noradrenergic system may have interfered with the antidepressant response of compound 13.⁷³ Additionally, other authors have suggested that a combination study with a tricyclic antidepressant, which would have antimuscarinic effects, rather than an SSRI or SNRI devoid of antimuscarinic activity, may have been more effective. An antidepressant with antimuscarinic effects could have pro-nicotinic effects through a neuro-adaptive upregulation of cholinergic tone, which could be normalized by compound 1. Alternatively, combining an antinicotinic with an antimuscarinic may have produced a complementary effect in reducing a hypercholinergic state than either treatment alone. Papke and Picciotto⁷⁴ also suggest that compound 1, in particular, the 2*S*-(+)-enantiomer 13, may not have been the best choice for the clinical trials. The rationale for using compound 13 was based on a study that suggested that compound 13 had both activating and inhibitory effects at $\alpha 4\beta 2$ -nAChRs, a better in vivo profile in animal studies, and a better safety profile than the 2*R*-(-)-enantiomer.^{67,75} However, more recent studies found little in vitro evidence to differentiate the pharmacological properties of compound 1 and the 2*R*- and 2*S*-enantiomers and only a modest difference in potency in vivo in the tail flick test between the stereoisomers.⁷⁶ Furthermore, compound 1 is an antagonist that is more potent at inhibiting ganglionic $\alpha 3\beta 4$ -nAChRs than $\alpha 4\beta 2$ -nAChRs in the brain that are thought to be the primary target of hypercholinergic activity associated with depression.^{25,77} A recent estimate of the free compound 1 concentration in the brain indicates that it is likely insufficient to be pharmacologically relevant, blocking approx-

imately 20% of $\alpha 4\beta 2$ -nAChR function.²¹ Further evidence that compound 13 did not provide sufficient inhibition on nAChRs comes from the failure of CP-601927 (16),⁷⁸ an $\alpha 4\beta 2$ partial agonist that was tested in a phase 2 clinical trial as an augmentation in treatment resistant subjects with major depression.^{79,80} Weber et al.²¹ propose that compound 16, which was predicted to inhibit only 23% of $\alpha 4\beta 2$ -nAChRs,⁸¹ would not translate into improved antidepressant efficacy.

Although it is possible that compound 13 could be an effective monotherapy, given its poor nAChR subtype selectivity and the failure in the phase 3 clinical trial, it seems unlikely that this ligand will have a future in the treatment of depressive disorders. The disappointing failure of compound 13 illustrates the need for a better understanding of the optimal pharmacological properties to maximize clinical antidepressant efficacy. More potent nAChR inhibitors that block >25% $\alpha 4\beta 2$ nAChR would likely be more effective than compound 1 or compound 13. Inhibition may be achieved with more potent antagonists, partial agonists with very low intrinsic activity, or desensitizing agents.

2-(*tert*-Butylamino)-1-(3-chlorophenyl)propan-1-one, 7.



Bupropion, 7

IC₅₀, $\alpha 4\beta 2$: 12 μ M

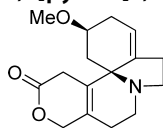
IC₅₀, $\alpha 3\beta 4$: 1.8 μ M

IC₅₀, $\alpha 1$: 7.9 μ M

Compound 7⁸² is an α -aminoketone believed to elicit antidepressant effects by acting as a dopamine and norepinephrine reuptake inhibitor. It was first reported to show efficacy as a smoking-cessation aid in nondepressed patients in 1994 and has subsequently proven to be a noncompetitive antagonist at a variety of nAChR subtypes.^{83,84} Compound 7 is most potent as a dopamine reuptake inhibitor, with an IC₅₀ of 550 nM, while potencies at norepinephrine transporters and a number of nAChR subtypes fall in the low micromolar range. However, it has long been appreciated that clinical outcomes correlate poorly with plasma concentrations of compound 7⁸⁵ and that active metabolites reaching higher and longer-lasting concentrations in plasma likely play the leading role. The principal metabolite, 2*S*,3*S*-hydroxybupropion (17), has reduced potency at dopamine transporters and most nAChR subtypes, whereas its potency at norepinephrine transporters and $\alpha 4\beta 2$ -nAChRs is enhanced.⁷⁶ It appears that a determining factor of compound 7's efficacy is its metabolism to sufficiently high concentrations of compound 17, as differences in its plasma concentrations between responders and nonresponders was the most statistically significant difference in a 2006 study.⁸⁶ Plasma concentrations reported for compound 17 were in the low micromolar range. Although compound 7 and another active metabolite, threohydrobupropion, were present at lower concentrations, they may have additive effects in combination with the principal metabolite that provide a significant contribution to a complex pharmacology mediated by norepinephrine transporters, dopamine transporters, and $\alpha 4\beta 2$ -nAChRs. Evaluation of compound 7 and its hydroxyl metabolites in the mouse FST showed that compound 17 was equally potent to that of compound 7, whereas the 2*S*,3*R*-isomer showed no effect on immobility.⁸³ The 2*S*,3*S*-isomer was also a more potent inhibitor of [³H]-norepinephrine uptake and [³H]dopamine uptake as well as a more potent antagonist of $\alpha 4\beta 2$ -nAChR function than that of the

2*S*,3*R*-isomer, suggesting that the 2*S*,3*S*-isomer may be a better candidate than compound **7** for antidepressant treatment.

(2*S*,13*bS*)-2-Methoxy-2,3,5,6,8,9,10,13-octahydro-1*H*,12*H*-pyrano[4',3':3,4]pyrido[2,1-*i*]indol-12-one, **18.**



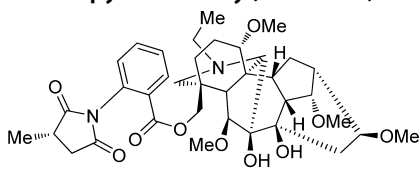
DHβE, **18**

IC₅₀, α4β2: 30 nM

IC₅₀, α3β4: 23 μM

Dihydro-β-erythroidine (DHβE, **18**)⁸⁷ is an alkaloid that is isolated from the seeds of *Erythrina L.*, a genus of trees and shrubs that is found in the tropics and subtropics around the globe. In Central America, *Erythrina* had an important role in folk medicine during precolonial times. Compound **18** acts as a competitive antagonist at α4β2-nAChRs (IC₅₀,α4β2 = 30 nM and IC₅₀,α3β4 = 23 μM)⁸⁷ that blocks compound **10**-induced dopamine release from rat striatal slices (IC₅₀ = 30 nM).⁸⁸ Compound **18** shows antidepressant-like effects in the mouse FST (3.0 mg/kg) and TST (1.0 and 3.0 mg/kg) without affecting locomotor activity.⁸⁹ Additionally, compound **18** is able to potentiate the antidepressant-like effects of compound **9** (4 and 20 mg/kg) in the TST.²²

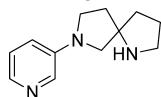
(3*S*,6*S*,6*aS*,7*R*,7*aR*,8*S*,9*R*,10*S*,11*aR*,12*S*,12*aS*,13*S*)-1-Ethyl-11*a*,12-dihydroxy-6,8,10,13-tetramethoxydodecahydro-2*H*-3,6*a*,12-(epiethane[1,1,2]triyil)-7,9-methanonaphtho[2,3-*b*]azocin-3(4*H*)-yl)methyl 2-((*S*)-3-methyl-2,5-dioxypyrrolidin-1-yl)benzoate, **19.**



MLA, **19**

Methyllycaconitine (MLA, **19**),⁹⁰ a complex diterpenoid alkaloid isolated from the seeds of *Delphinium brownii*, is a competitive and selective α7-nAChR antagonist with an IC₅₀ value in the subnanomolar range, as tested in *Xenopus* oocytes and α7-transfected SH-SY5Y cells.^{90,91} Compound **19** was found to partially inhibit anatoxin-evoked dopamine release from rat striatal slices⁹² and decrease the time spent immobile in NMRI female mice in the mouse FST (10 mg/kg) and TST (10 mg/kg) without affecting locomotor activity.⁸⁹ Another study, however, found no effect of compound **19** (10 mg/kg) in the FST in BALB/c male mice.³⁸ The discrepancies between these studies, which may be related to mouse strain, gender, or procedural differences, warrant further investigation into the role of α7 nAChR inhibition and antidepressant function.

7-(Pyridin-3-yl)-1,7-diazaspiro[4.4]nonane, **20.**



TC-2216, **20**

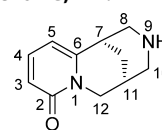
Another α4β2-nAChR antagonist is the Targacept compound TC-2216 (**20**).⁹³ This compound was reported to show beneficial effects in preclinical studies, thereby promoting its further development for the treatment of depression and anxiety disorders.⁹³ A phase 1 clinical trial of racemic compound **20** was initiated in 2008, but no further trials were conducted.

■ nAChR AGONISTS

Modulation of nAChRs with partial agonists is pharmacologically complex, as it involves potentially simultaneous and interacting effects. Under acute conditions, partial agonists elevate baseline cholinergic tone while simultaneously lowering the ceiling of nAChR-mediated signaling. Depending on potency, dose, and exposure, partial agonists could have an additive effect with endogenous ACh, thereby lowering the threshold for ACh signaling while simultaneously lowering the efficacy of that signal. Under conditions of chronic administration, partial agonists desensitize nAChR, and it is believed that the resulting attenuation of cholinergic signaling is the principal pharmacotherapeutic end point.

■ CYTISINE AND DERIVATIVES

(1*R*,5*S*)-1,2,3,4,5,6-Hexahydro-8*H*-1,5-methanopyrido[1,2-*a*][1,5]diazocin-8-one, **21.**



(-)-Cytisine, **21**

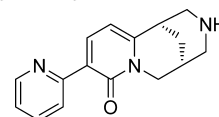
K_i, α4β2: 2 nM

K_i, α3β4: 480 nM

K_i, α7: 5890 nM

(-)-Cytisine (**21**),⁹⁴ a tricyclic quinolizidine alkaloid isolated from the seeds of *Cytisus laburnum L.*, has been used to treat tobacco dependence in Eastern Europe since the 1960s. Compound **21** is an α4β2-nAChR partial agonist (K_i,α4β2 = 2 nM) and an α3β4 (K_i,α3β4 = 480 nM) and α7 (K_i,α7 = 5890 nM) full agonist that shows antidepressant-like activities in several rodent models,⁹⁵ presumably mediated by a reduction of neuronal activity in the basolateral amygdala.⁹⁶ Compound **21** (10 μM) was reported to exhibit 56% of the response relative to that of compound **10** and to inhibit 30% of the current evoked by compound **10** (10 μM) in *Xenopus* oocytes expressing human α4β2-nAChRs.⁹⁷ However, it was reported to have unfavorable side effects, which include nausea, vertigo, abdominal pain, respiratory stimulation, and muscle weakness.⁹⁸ Mineur et al. observed that compound **21** was not tolerated by mice at a dose of >1.5 mg/kg.⁹⁵ Additionally, the poor absorption and brain penetration^{94,99–101} of compound **21** may also limit its application as a clinical antidepressant.

(1*R*,5*S*)-9-(Pyridin-2-yl)-1,2,3,4,5,6-hexahydro-8*H*-1,5-methanopyrido[1,2-*a*][1,5]diazocin-8-one, **22.**



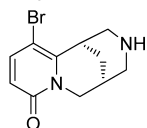
3-pyridyl-cytisine, **22**

K_i, α4β2: 0.91 nM

K_i, α3β4: 119 nM

K_i, α7: 1100 nM

(1*R*,5*S*)-11-Bromo-1,2,3,4,5,6-hexahydro-8*H*-1,5-methanopyrido[1,2-*a*][1,5]diazocin-8-one, **23**.



5-bromo-cytisine, **23**

$K_{i, \alpha 4 \beta 2^*}$: 0.3 nM

$K_{i, \alpha 3 \beta 4^*}$: 3.8 nM

$K_{i, \alpha 7^*}$: 28 nM

SAR studies of cytosine analogues revealed that phenyl ring replacements of the pyridone ring of compound **21** reduced the binding affinities and functional efficacies.⁹⁷ Substitution of **21** at the C-3 ($-\text{CH}=\text{CH}_2$,¹⁰² $-\text{NO}_2$,¹⁰³ or pyridinyl⁹⁵), C-4 ($-\text{CH}_3$,¹⁰² or C-5 ($-\text{Br}$)⁷⁸ position could lead to the same or improved $\alpha 4 \beta 2$ -nAChR binding affinity, whereas substitution at the piperidine nitrogen,^{97,103,104} the C-3 position (aromatic rings),¹⁰² or at the C-12 position ($-\text{CO}_2\text{CH}_3$ or $-\text{COCH}_2\text{CH}_3$)¹⁰² reduced affinity. Introduction of substituents on the piperidine ring nitrogen,⁹⁷ C-4 ($-\text{CH}_3$ or $-\text{CH}_2\text{OH}$),¹⁰² or the C-5 ($-\text{NO}_2$)¹⁰³ position increases the selectivity for $\alpha 4 \beta 2$ receptor. Among these substituted cytosine analogues, 3-(2-pyridyl)cytosine (**22**)⁹⁵ and 5-bromocytisine (**23**)⁹⁵ were further tested in mouse antidepressant efficacy models. Both compounds displayed high affinity at $\alpha 4 \beta 2^*$ -nAChRs ($K_{i, \alpha 4 \beta 2^*}$ = 0.9 for **22** and 0.3 nM for **23**), whereas compound **22** was found to show lower affinities at $\alpha 3 \beta 4^*$ -nAChRs and $\alpha 7^*$ -nAChRs ($K_{i, \alpha 3 \beta 4^*}$ = 119 nM and $K_{i, \alpha 7^*}$ = 1100 nM for **22** vs $K_{i, \alpha 3 \beta 4^*}$ = 3.8 nM and $K_{i, \alpha 7^*}$ = 28 nM for **23**). In electrophysiology assays, compound **22** activates both HS and LS $\alpha 4 \beta 2^*$ -nAChRs ($\text{EC}_{50, \text{HS}}$ = 12 nM and $\text{EC}_{50, \text{LS}}$ = 31 nM) expressed in oocytes with low efficacies (less than 10% relative to the efficacy of ACh at both HS and LS receptors) while exhibiting little agonism at $\alpha 3 \beta 4^*$.⁹⁵ In the same assays, compound **23** activated both HS and LS $\alpha 4 \beta 2^*$ -nAChRs with similar potencies ($\text{EC}_{50, \text{HS}}$ = 13 nM and $\text{EC}_{50, \text{LS}}$ = 15 nM) and efficacies (17% relative to ACh). A comparison of binding and functional data for compound **21** and its selected analogues are presented in Table 1. The

Table 1. Binding Affinities and Maximal Responses and Potencies of Compounds 2 and 21–23 with Respect to Activation of nAChRs Expressed in Oocytes^{95,101}

ID	ref	$\alpha 4 \beta 2$ activation				$\alpha 4 \beta 2$ inactivation	
		LS efficacy	HS efficacy	LS EC_{50} (nM)	HS EC_{50} (nM)	LS IC_{50} (nM)	HS IC_{50} (nM)
ACh	95	100%	100%	73	1.7		
2	101		22%		1400	50 000	70
21	101		6.5%		2000	28 000	50
22	95	3%	8%	31	12		
23	95	17%	17%	15	13		

antidepressant-like effects of compound **22** were demonstrated in the TST (0.6 mg/kg, but not at 0.3 or 0.9 mg/kg), FST (0.3–0.9 mg/kg), and chronic NSF tests (15 days at 0.3 mg/kg, but not at 0.6 mg/kg) in C57BL/6 mice. On the other hand, compound **23** (0.3–1.2 mg/kg) failed to show any significant effects in the same tests 30 min postintra-peritoneal injection.⁹⁵ It has been suggested that the lack of efficacy of compound **23** was likely due to the low brain penetration, as 50 ng of the compound in 1 μL of artificial cerebrospinal fluid showed a significant antidepressant-like effect in the TST when administered centrally.

(1*R*,5*S*)-7-(Trifluoromethyl)-2,3,4,5-tetrahydro-1*H*-1,5-methanobenzo[d]azepine, **16**. (1*S*,5*R*)-7-(Trifluoromethyl)-2,3,4,5-tetrahydro-1*H*-1,5-methanobenzo[d]azepine, **25**. (6*R*,10*S*)-7,8,9,10-Tetrahydro-6*H*-6,10-methanoazepino[4,5-*g*]quinoxaline, **2**. Inspired by morphine and its simplified analogues [3.3.1]- and [3.2.1]-bicyclic benzomorphans, Coe et al. identified the simplified cytosine analogue, benzazapine **24**,¹⁰⁵ as a nicotinic antagonist with a K_i value of 20 nM. Attachment of a trifluoromethyl group to the C-4 position of benzazapine **24** gives **16** and CP-601932 (**25**)⁷⁸ (Figure 6). Compound **16** is a selective $\alpha 4 \beta 2$ -nAChR partial agonist with weaker activity at the $\alpha 3 \beta 4$ -nAChRs ($K_{i, \alpha 4 \beta 2}$ = 1.2 nM vs $K_{i, \alpha 3 \beta 4}$ = 102 nM),⁷⁸ whereas its enantiomer, **25**, shows similar affinities at both receptor subtypes, with K_i values of 21 nM and lower affinities for the $\alpha 6$ - and $\alpha 7$ -nAChR subtypes (K_i > 300 nM).^{106,107} Compound **16** decreased the time spent immobile in the FST at 0.75, 1, and 1.5 mg/kg, whereas no significant effect was found in the TST at the same doses.⁷⁸ Moreover, compound **16** was found to be relatively safe and well-tolerated in phase 1 single- and multiple-dose studies, but it failed to show a statistically significant change on the primary efficacy scale in favor of the drug when used in the augmentation of antidepressant therapy in major depression in a phase 2 clinical study.¹⁰⁸

Nitration of benzazapine **24** in the presence of 2 equiv of nitronium triflate ($\text{CF}_3\text{SO}_2\text{O}^-\text{NO}_2^+$) in dichloromethane afforded the 4,5-dinitrated benzazapine **26**¹⁰⁵ with an unexpected regioselectivity (the meta-isomer was found to be less than 10% of the mixture). Reduction of **26** to the diamine, condensation with glyoxal, and deprotection followed by salt formation provided compound **2** (Figure 6).¹⁰⁵ This achiral quinoxaline **2** is the most publicized cytosine analogue, which is known as varenicline. Its tartrate salt has been launched and marketed by Pfizer for smoking cessation in the U.S. (Chantix), Canada, Europe, and other countries (Champix). Compound **2** is a potent partial agonist at $\alpha 4 \beta 2^*$ -nAChRs, with a K_i value of 0.4 nM and an agonist efficacy of 40–60% of that of compound **10**.^{109,110} In *Xenopus laevis* oocytes expressing $\alpha 4 \beta 2$ -nAChRs, compound **2** was found to have an EC_{50} of 2.3 μM and an efficacy of 13.4% relative to that of ACh.¹⁰⁹ It potently blocks compound **10** binding to $\alpha 4 \beta 2^*$ -nAChRs and produces a sustained increase in dopamine release to 60% of the maximal effect of compound **10** (188% at 0.32 mg/kg sc).¹¹⁰ In addition, 1 mg/kg po compound **2** reduced the dopamine-enhancing effects of a subsequent dose of 0.32 mg/kg sc compound **10** to the level of the effect of compound **2** alone.¹¹⁰ In a SPECT imaging study, compound **2** (0.5 mg) was found to completely saturate $\alpha 4 \beta 2^*$ -nAChRs in human brain.¹¹¹ The dissociation half-life of compound **2** is about 5.4 h in a mouse ex vivo receptor occupancy study.³⁸ Compound **2** alone has antidepressant-like effects in the FST and was able to augment the effects of compound **5** when administered in combination.¹¹² It enhanced the mood and cognitive function associated with compound **10** withdrawal in patients in clinical trials for smoking cessation.¹¹³ In an 8 week open-label study, compound **2** augmented the effects of traditional antidepressants in depressed smokers who derived minimal benefit from standard antidepressant treatment.¹² Recently, studies on a larger scale of community volunteers who wanted to quit smoking revealed that 12 week administration of compound **2** (0.5 mg/day for days 1–3 followed by 0.5 mg twice a day for days 4–7 and 1 mg twice a day thereafter) was coupled with a generalized suppression of

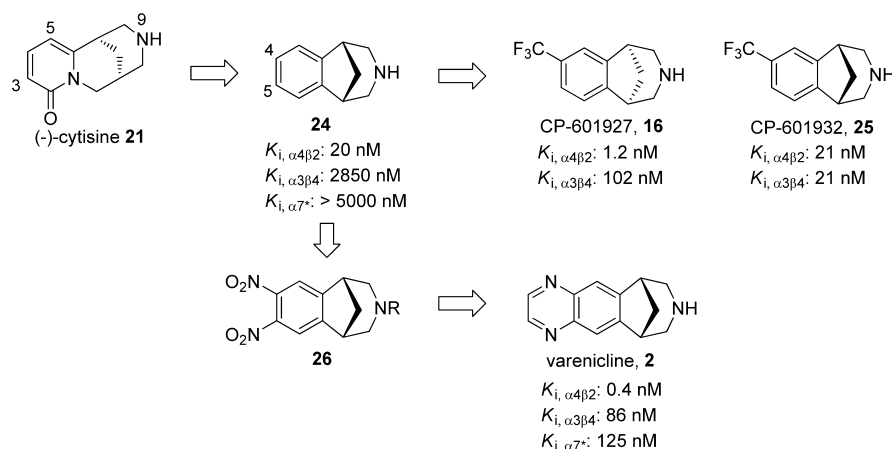
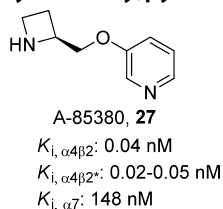


Figure 6. Selected nicotinic benzazapine analogues.

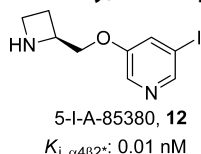
depression compared with that of the placebo-controlled treatment.¹¹⁴

■ A-85380 AND ANALOGUES

(S)-3-(Azetidin-2-ylmethoxy)pyridine, 27.



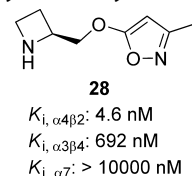
(S)-3-(Azetidin-2-ylmethoxy)-5-iodopyridine, 12.



The pyridine ether-based ligands, in which a CH_2O linker is inserted between the pyrrolidine ring (or in case of its analogues, an azetidine ring) of compound 10 and its pyridine ring have attracted considerable interest as $\alpha 4 \beta 2$ -nAChR agonists due to their high potency.^{115–118} A-85380 (27)¹¹⁹ is a 3-pyridyl ether that exhibits both high potency and selectivity for $\beta 2$ -containing nAChRs, primarily $\alpha 4 \beta 2^*$ in brain ($K_{i, \alpha 4 \beta 2^*}$ = 0.02–0.05 nM for rat brain receptors and 0.04 nM for human brain receptors) relative to that of human $\alpha 7$ -nAChRs ($K_{i, \alpha 7}$ = 148 nM) or muscle-type $\alpha 1 \beta 1 \gamma \delta$ -nAChRs ($K_{i, \alpha 1 \beta 1 \gamma \delta}$ = 314 nM).^{119,120} Functionally, compound 27 acts as a potent activator of both human $\alpha 4 \beta 2$ -nAChRs (EC_{50} = 0.7 μM) and ganglionic $\alpha 3 \beta 4^*$ -nAChRs (EC_{50} = 0.8 μM), the effects of which are blocked by pretreatment with compound 1. In the FST, compound 27 was found to have antidepressant-like effects that could be blocked by pretreatment with the nAChR antagonists compounds 1 and 18, as well as by the nonselective serotonin receptor antagonist methiothepin, suggesting that compound 27 exerts its effects via neuronal nicotinic receptor activation of serotonergic pathways.^{119,121} As exemplified by compound 12¹²⁰ ($K_{i, \alpha 4 \beta 2^*}$ = 0.01 nM), introduction of a halogen substituent at the C-2 (fluoro only), C-5, or C-6 position of compound 27 leads to ligands that retain subnanomolar affinity for the $\alpha 4 \beta 2^*$ -nAChRs, as measured by radioligand binding assays utilizing rat brain membrane preparations.¹²⁰ Similar to compound 27, 12 was also found to decrease immobility in the mouse FST.³⁸ Recently,

$\beta 2^*$ -nAChR availability was found to be decreased across brain regions in depressed patients compared to that of healthy subjects using SPECT with the $\beta 2^*$ -selective radioligand tracer [¹²³I]12.³⁹ A unifying explanation of these effects stems from the idea that desensitization and antagonism provide similar end points for agonists and antagonists. However, because agonists have an acute activation phase, behavioral assays may measure effects due to activation and/or inactivation of nAChRs. In this context, one would expect coadministration of an antagonist to reduce the behavioral signature of an agonist administered alone. More complicated explanations involving multiple nAChR subtypes also can be made, but they are highly speculative.

(S)-5-(Azetidin-2-ylmethoxy)-3-methylisoxazole, 28.



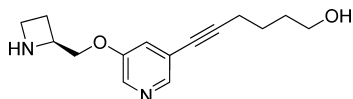
Replacement of the pyridine core of these ether-based ligands with a methylisoxazole group led to compound 28, which binds to $\alpha 4 \beta 2$ -nAChRs with higher affinity than to the $\alpha 3 \beta 4$ -nAChRs ($K_{i, \alpha 4 \beta 2}$ = 4.6 nM vs $K_{i, \alpha 3 \beta 4}$ = 692 nM).¹²² The functional potency of compound 28 (1.2 μM) at the $\alpha 4 \beta 2$ -nAChR is similar to that of compound 2 (1.4 μM), whereas the efficacy of compound 28 is higher (110% vs 53%) in cells heterologously expressing a mixture of HS and LS $\alpha 4 \beta 2$ -nAChRs (Table 2). Broad screening showed that this compound was highly selective for nAChRs and did not have significant binding affinity to the other 45 neurotransmitter receptors and transporters tested. In the mouse FST, compound 28 was found to decrease immobility at 1–5 mg/kg i.p. and 5 mg/kg po. This compound showed no significant hERG or CYP (1A2, 2B6, 2C9, 2C19, 2D6, and 3A4) inhibition at 10 μM . Between 53.4 and 73.7% of compound 28 was found to remain unchanged after 60 min incubation with mouse or human liver microsomes (1 and 10 μM).¹²²

Table 2. Functional Potencies and Efficacies of Ligands 2, 10, 11, and 28–31: Agonism and Inactivation at Human $\alpha 4\beta 2$ -nAChRs^{122–125}

compound	agonism			inactivation	
	EC ₅₀ (nM)	efficacy (%) ^a	efficacy HS (%) ^b	IC ₅₀ (nM)	efficacy (%)
2	1400	53		110	85
11	5.8	55	100	4.8	37
28	1200	110	92	169	78
29	10	21	92	9.4	63
30	42	22	61	31	68
31	8.4, 2300 ^c	21	45	58	85
10	290	88	110	430	93

^aThe efficacies were measured in a mixture of HS and LS $\alpha 4\beta 2$ -nAChRs. ^bThe efficacy values were extrapolated using compound 11 defined as a full agonist at the HS $\alpha 4\beta 2$ -nAChR. ^cCompound 31 activates both HS and LS $\alpha 4\beta 2$ -nAChRs with sufficient selectivity to distinguish activity at each subtype. Efficacy of 31 at LS $\alpha 4\beta 2$ -nAChRs is approximately 17%.

(S)-6-(5-(Azetidin-2-ylmethoxy)pyridin-3-yl)hex-5-yn-1-ol, 11.



Sazetidine-A, 11
 $K_i, \alpha 4\beta 2$: 0.4 nM
 $K_i, \alpha 3\beta 4$: > 10⁴ nM
 $K_i, \alpha 7$: > 10⁴ nM

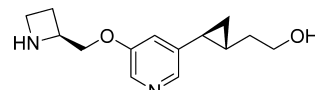
Compound 11, an analogue of compound 27 having an alkynyl substituent attached to the 5-position of the pyridine ring, was identified as a highly potent full agonist at HS $\alpha 4\beta 2$ -nAChR and a low efficacy agonist at LS nAChR (Table 2).¹²⁶ Compared with compound 27, the selectivity of compound 11 at $\alpha 4\beta 2$ - relative to ganglionic $\alpha 3\beta 4$ *- and homomeric $\alpha 7$ -nAChRs is greatly improved.^{127,128} Compound 11 potently binds to $\alpha 4\beta 2$ -nAChRs ($K_i = 0.4$ nM) but not to $\alpha 3\beta 4$ *- ($K_i > 10^4$ nM) or $\alpha 7$ -nAChRs ($K_i > 10^4$ nM). Ex vivo receptor occupancy in the thalamus showed prolonged receptor occupancy. The dissociation half-life of compound 11 (8–24 h) was found to be significantly longer compared with that of compound 12 (3–5 h),³⁸ which is likely due to the presence of the side chain in compound 11.

Robust antidepressant-like effects of compound 11 were observed in the FST, TST, and NIH assays, with no effect on baseline locomotor activity when given chronically.^{35,38,129} Compound 11 (1 mg/kg) significantly reduced immobility 2, 3, and 4 h but not 5 h after administration in the mouse FST. Repeated administration of compound 11 in the FST at 1 and 3

mg/kg doses for 2 weeks did not result in tolerance. Moreover, its activity in the FST was completely blocked by either compound 18 or compound 1,³⁸ suggesting that activation of nAChRs may be responsible for the activity of compound 11 in this behavioral model of antidepressant efficacy. In addition, $\beta 2$ subunit knockout mice did not show any antidepressant response to compound 11 in the FST, suggesting that interaction with $\beta 2$ *-nAChRs plays a key role in the behavioral activity of compound 11.³⁸ Stimulation of dopamine and noradrenaline release in rat striatal slices was observed with compound 11, an effect that was blocked by nicotinic antagonists 18 and compound 1.¹²⁶ In addition, the $\alpha 6$ *-selective antagonist α -conotoxin MII (100 nM) reduced the maximum effect of compound 11-induced dopamine release in rat by 48%, suggesting that $\alpha 6$ *-nAChRs may be involved in the antidepressant-like effects of compound 11.¹²⁶

Compound 11 has been reported to have other behavioral effects including increased hypothermia.^{130,131} It is unlikely that the hypothermic effects of compound 11 are related to its antidepressant-like effects observed in the FST. Although reduced, $\beta 2$ knockout mice still showed a hypothermic response to compound 11,¹³¹ whereas the antidepressant-like effects of compound 11 was completely abolished in $\beta 2$ knockout mice.³⁸ The hypothermic effects of compound 11 (1 mg/kg) returned to baseline approximately 2 h postinjection,¹³² whereas the antidepressant-like effect persisted up to 4 h.³⁸ Compound 11 has shown additional beneficial behavioral effects in animal models including analgesia,¹³³ improving attentional function after disruption with compound 3 or MK-801,^{132,134} reducing anxiety following withdrawal from chronic compound 10,¹³⁵ decreasing body weight gain following chronic treatment,¹³⁶ and decreasing compound 10 self-administration and alcohol consumption.^{137–139} Interestingly, unlike compound 10 or compound 2, compound 11 did not upregulate nAChR in the brain after chronic administration¹⁴⁰ and did not maintain receptor upregulation following chronic compound 10 administration,¹³⁶ providing further evidence that compound 11 may exert its behavioral effects through a unique mechanism.

2-((1R,2S)-2-(5-(((S)-Azetidin-2-yl)methoxy)pyridin-3-yl)cyclopropyl)ethan-1-ol, 29.



29
 $K_i, \alpha 4\beta 2$: 0.1 nM
 $K_i, \alpha 3\beta 4$: 6520 nM
 $K_i, \alpha 7$: > 10000 nM

Table 3. Binding Affinities of Compounds 10, 11, and 27–31 at Seven nAChR Subtypes^{122–125}

compound	K_i (nM) ^a							selectivity $\alpha 4\beta 2/\alpha 3\beta 4$
	$\alpha 2\beta 2$	$\alpha 2\beta 4$	$\alpha 3\beta 2$	$\alpha 3\beta 4$	$\alpha 4\beta 2$	$\alpha 4\beta 4$	$\alpha 4\beta 2$ * ^b	
11				>10 000	0.4		0.9	24 000
27					0.05		0.05	
28	4.3	311	8.7	692	4.6	86.0	12.0	150
29	0.1	249	3.0	6520	0.1	82.6	0.5	65 200
30	1.0	935	15.4	>10 000	0.6	1790	3.0	>16 300
31	1.7	559	40.6	5640	1.2	16.9	1.4	4700
10	5.5	70	29	260	4.9	23	9.8	53

^a K_i values were determined by competition for [³H]epibatidine binding sites using radioligand binding. ^b $\alpha 4\beta 2$ *, prepared from rat forebrain.

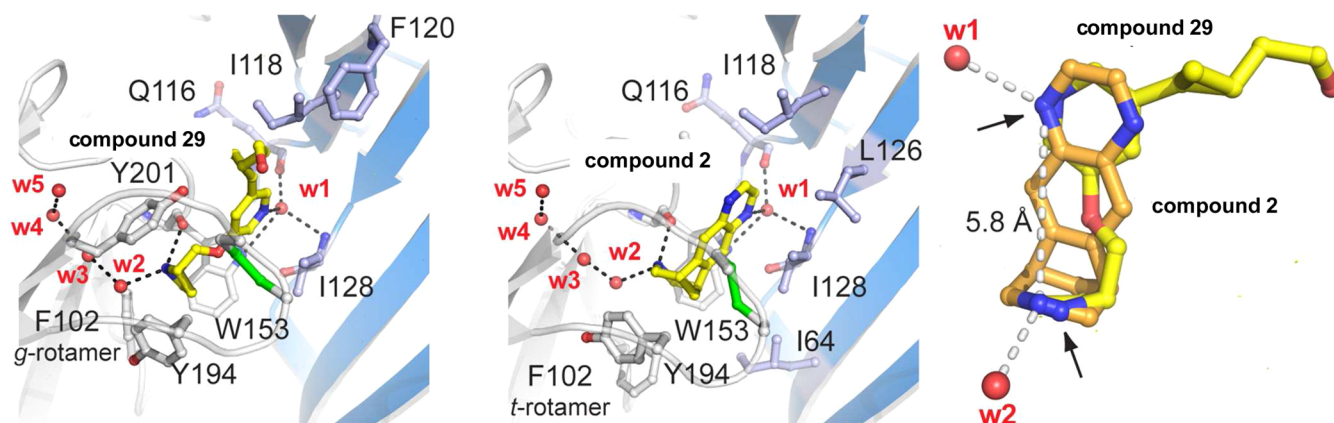
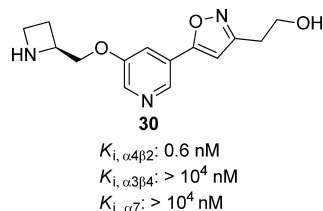
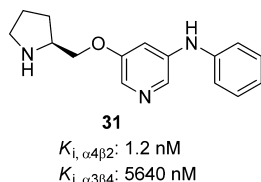


Figure 7. X-ray crystal structure of the *Ct*-AChBP in complex with compound 2 or 29.⁶³

(S)-2-(5-(5-(Azetidin-2-ylmethoxy)pyridin-3-yl)isoxazol-3-yl)ethan-1-ol, 30.



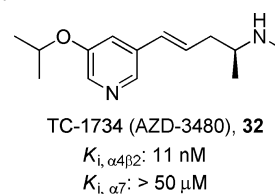
(S)-N-Phenyl-5-(pyrrolidin-2-ylmethoxy)pyridin-3-amine, 31.



Replacing the acetylene bond of compound 11 with a substituted cyclopropane or an isoxazole ring lead to compounds 29 and 30, respectively.^{63,124,125,141} Introduction of different amino groups at the 5-position of the pyridine ring yielded a series of 3-alkoxy-5-aminopyridine derivatives exemplified by compound 31.¹²³ These compound 11 analogues (29–31) act as $\alpha4\beta2$ -nAChRs partial agonists (Table 2) with low nanomolar binding affinities ($K_i = 0.1$ – 1.2 nM) and excellent subtype selectivity at $\beta2^*$ - over $\beta4^*$ -nAChRs (Table 3). Compound 29 has been cocrystallized with *Ct*-AChBP, and its binding mode was found to be similar to that reported for compound 2; both compounds rely on the presence of two cationic centers spaced ~ 5.8 Å from each other for their binding interactions, including cation– π interactions with W153 (loop B) and Y201 (loop C) as well as a set of H-bonds with the backbone atoms of W153 (loop B), Q116 (loop E), and I128 (loop E). (Figure 7.) The hydroxyethyl group of 29 appears to be flexible and is able to adopt different orientations, thus highlighting the ability to carry out structural modifications of this appendage without causing significant alterations in activity. The antidepressant-like properties of compounds 29–31 were demonstrated in the classical mouse FST (1–30 mg/kg). The lack of significant interactions with other neurotransmitter receptors and transporters widely distributed throughout the CNS as well as the favorable preliminary absorption, distribution, metabolism, excretion, and toxicity (ADME-Tox) profiles place these specific $\alpha4\beta2$ -nAChR ligands in a favorable position to be further studied as new antidepressant drug candidates.

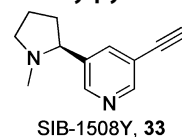
■ OTHER LIGANDS

(S,E)-5-(5-Isopropoxy)pyridin-3-yl)-N-methylpent-4-en-2-amine, 32.



TC-1734 (32) is a potent $\alpha4\beta2$ -nAChR partial agonist ($K_i = 11$ nM) and is selective over $\alpha7$ -nAChRs ($K_i > 50\,000$ nM).¹⁴² Compound 32 (10 μ M) showed no significant inhibition when tested at 135 other receptor and enzyme systems. In functional studies, the agonist activity of compound 32 was measured using a $^{86}\text{Rb}^+$ efflux assay in rat thalamic synaptosomes ($\alpha4\beta2$ subtype, $\text{EC}_{50} = 220$ nM, efficacy = 57%) and the dopamine-release assay in striatal synaptosomes ($\alpha4\beta2/\alpha6/\alpha3\beta2$, $\text{EC}_{50} = 106$ nM, efficacy = 55%). Compound 32 (up to 100 μ M) did not show any detectable effects at either muscle-type ($\alpha1\beta1\gamma\delta$) or ganglionic ($\alpha3\beta4$) nAChRs. Compound 32 had favorable pharmacokinetic and metabolic profiles and was well-tolerated in acute and chronic oral toxicity studies in various animal species (mice, rats, and dogs), as well as in a human clinical studies using either single- or repeated-dose oral administration.¹⁴³ Compound 32 exhibited antidepressant-like effects in the mouse FST when administered intraperitoneally, with a significant reduction in immobility at the minimal dose of 1 μ mol/kg, and locomotor activity was not affected by repeated administration.¹⁴² In clinical trials, compound 32 has been studied in a variety of indications characterized by varying types and degrees of cognitive impairment, such as attention deficit/hyperactivity disorder, mild cognitive impairment, and age-associated memory impairment.^{144,145} Currently, Targacept is recruiting patients for a phase 2b study with this compound in order to assess efficacy, safety, and tolerability in patients with mild to moderate dementia due to Alzheimer's disease.^{146,147}

(S)-3-Ethynyl-5-(1-methylpyrrolidin-2-yl)pyridine, 33.



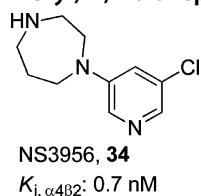
SIB-1508Y (33)¹⁴⁸ is a selective $\alpha4\beta2$ -nAChR partial agonist ($\text{EC}_{50} = 1.8$ μ M; efficacy = 49% relative to that of compound 10), having no activity at homomeric $\alpha7$ - or muscle-type $\alpha1\beta1\gamma\delta$ -

Table 4. Clinical and Preclinical Evidence for the Viability of Targeting nAChRs in Depression

ID	receptor subtype selectivity	antidepressant/anxiolytic results	ref
2	$\alpha 4\beta 2$ partial agonist, less potent $\alpha 3\beta 4$ and $\alpha 7$ full agonist	Improved FST; augmented the antidepressant effects in depressed smokers	12, 38, and 109–114
11	$\alpha 4\beta 2$ partial agonist	Improved FST, TST, and NIH	35, 38, and 126–129
12	$\alpha 4\beta 2$ full agonist	Improved FST	38, 39, and 120
13	Nonselective noncompetitive antagonist	Improved FST, social interaction test, light/dark assay; failed phase 3 clinical study as add-on in treating resistant patientsdark assay	19, 21, 50, 51, and 66–72
16	$\alpha 4\beta 2$ partial agonist	Improved FST Failed as augmentation of antidepressant therapy for major depression in phase 2 clinical study	78 and 108
18	$\alpha 4\beta 2$ competitive antagonist	Improved FST and TST	22 and 87–89
19	$\alpha 7$ antagonist	Improved FST and TST	89–92
20	$\alpha 4\beta 2$ antagonist	Antidepressant and anxiolytic like response in preclinical studies	93
21	$\alpha 4\beta 2$ partial agonist, $\alpha 3\beta 4$ and $\alpha 7$ full agonist	Antidepressant-like activities in several rodent models; safety issues, poor absorption and limited brain penetration	94–101
22	$\alpha 4\beta 2$ partial agonist	Improved FST, TST, and chronic NSF	95 and 97
27	$\alpha 4\beta 2$ partial agonist, $\alpha 3\beta 4$ agonist	Improved FST	119–121
28	$\alpha 4\beta 2$ partial agonist, less potent $\alpha 3\beta 4$ agonist	Improved FST	122
29	$\alpha 4\beta 2$ partial agonist	Improved FST	63 and 125
30	$\alpha 4\beta 2$ partial agonist	Improved FST	124 and 141
31	$\alpha 4\beta 2$ partial agonist	Improved FST	123
32	$\alpha 4\beta 2$ partial agonist	Improved FST	142–147
33	$\alpha 4\beta 2$ partial agonist	Improved learned helplessness	148–152
34	$\alpha 4\beta 2$ partial agonist	No effect alone in FST; enhanced the antidepressant-like effect of SSRI (compound 4) and SNRI (compound 6)	23 and 58
35	$\alpha 7$ agonist	No effect alone at FST; enhanced the antidepressant-like effect of SSRI (compound 4) and SNRI (compound 6)	23 and 153

nAChRs and weak activity at ganglionic $\alpha 3\beta 4$ -nAChRs ($EC_{50} = 23 \mu M$; efficacy = 52% relative to that of compound 10).¹⁴⁸ In vitro, compound 33 stimulated dopamine release from slices of rat striatum prepared from various brain regions, and the effect was blocked by nAChR antagonists compound 1 or compound 18.¹⁴⁹ On the other hand, compound 33 was found to show relatively weak effects on NE or 5-HT release. Subchronic or chronic administration of compound 33 demonstrated robust antidepressant-like effects in the learned helplessness model in rat, which were significantly blocked by nAChR antagonist compound 1 in the subchronic study.¹⁵⁰ In addition, compound 33 was also found to improve cognitive and motor function in monkey models of Parkinson's disease.^{151,152}

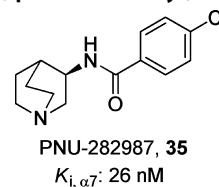
1-(5-Chloropyridin-3-yl)-1,4-diazepane, 34.



NS3956 (**34**)²³ was found to be a partial agonist at human $\alpha 4\beta 2$ -nAChRs ($K_i = 0.36$ nM) with good selectivity ($K_{i, \alpha 7} = 399$ nM and $K_{i, \alpha 1} = 944$ nM). In the oocyte expression system, this compound was very potent at HS $\alpha 4\beta 2$ -nAChRs ($EC_{50} = 11$ nM), with an efficacy of 47% of the response to a maximally efficacious concentration of ACh.²³ At LS $\alpha 4\beta 2$ -nAChRs, potency and efficacy were both reduced, with an EC_{50} of 604 nM and an efficacy of 38% relative to that of ACh. In reference to the subtype selectivity and off-target activity, actions at $\alpha 7$ - or $\alpha 3\beta 4$ -nAChRs of compound 34 were weak or absent, and it did not show any significant interactions with the monoamine transporters, including 5-HT, DA, and NE. Moreover, compound 34 exhibited an ED_{50} value of 0.033 mg/kg in the

[³H]epibatidine binding assay when tested in vivo, indicating that it was able to cross the BBB. Interestingly, compound 34 (0.3–3.0 mg/kg) was shown to be inactive in the mouse FST when tested alone, whereas when tested as a combination therapy, it significantly enhanced the antidepressant-like effects of the SSRI compound 4 and the SNRI compound 6 at 1.0 mg/kg.²³ These results suggest the possibility of a synergistic interaction between nicotinic agonism and the action of antidepressant medications. In vitro binding studies along with the detailed interactions with *Ls*-AChBP revealed that a bromine substitution at the 6-position increased the efficacy at HS $\alpha 4\beta 2$ -nAChR, and a relatively small substitution, such as chloro, bromo, or ethoxy, at the 5-position of the pyridine ring in a 1-(pyridin-3-yl)-1,4-diazepane scaffold improved selectivity for $\alpha 4\beta 2$ - over $\alpha 7$ -nAChRs.⁵⁷

(R)-4-Chloro-N-(quinuclidin-3-yl)benzamide, 35.



PNU-282987 (**35**)¹⁵³ is a potent and selective $\alpha 7$ -nAChR agonist ($K_i = 26$ nM) with good selectivity over $\alpha 3\beta 4$ - or $\alpha 1\beta 1\gamma\delta$ -nAChRs ($\geq 60 \mu M$). Compound 35 did not show significant interactions with monoamine, muscarine, glutamate, or GABA receptors when tested at concentrations of 1 μM , with the exception of the 5-HT₃ receptor, where it showed a K_i value of 930 nM. Furthermore, compound 35 was able to evoke whole-cell currents from cultured rat hippocampal neurons and enhance GABAergic synaptic activity in hippocampal slices.¹⁵³ Compound 35 (10–20 mg/kg) show no antidepressant-like activity when tested alone, but it significantly enhanced the

antidepressant-like effect of the SSRI compound **4** or the SNRI compound **6**.²³

CONCLUSIONS

Considerable evidence supports the hypothesis that hyperactivity of the cholinergic system over that of the adrenergic system leads to depression. Clinical and preclinical studies provide evidence that modulating nAChRs may lead to antidepressant effects when given alone and in combination with traditional antidepressants (summarized in Table 4). Compound **10** has a clear and long-recognized activity as an antidepressant, and a childhood history of depression elevates the risk for those individuals becoming users of tobacco products, reflecting self-medication with compound **10** as a “street” antidepressant.¹⁵⁴ It is difficult to determine the critical drug features that are responsible for compound **10**'s antidepressant activity, given the pharmacodynamic and pharmacokinetic variables and the various effects of compound **10** on nAChRs in vitro. Our understanding is incomplete about where and which nAChR subtypes are involved in excitatory neurotransmission and/or in modulation of neurotransmission mediated by monoamines or other chemical messengers implicated in mood regulation. Pharmacokinetics bring additional uncertainties with regard to drug availability and efficacy in vivo and translation of ligand activities in vitro to those in vivo. For example, effects of several agonists in animal models are attenuated by coadministered antagonists, suggesting that nAChR activation contributes to some antidepressant-like drug effects. Antagonists alone also elicit antidepressant-like effects, suggesting that inhibition of nAChR function through processes such as desensitization also contribute to antidepressant efficacy, consistent with the cholinergic/adrenergic hypothesis. Moreover, compound **10** and many nicotinic ligands have limited selectivity across nAChR subtypes or subtype isoforms, meaning that they could have a matrix of effects across subtypes as well as on the activation–inactivation axis. There still is a need for reliable assays for several nAChR subtypes, in vitro, complicating the interpretation of results and strategies in identification of promising compounds. It is also important to be mindful that behavioral tests in animals measure changes in behavior that correlate with clinical efficacies of currently available antidepressants and do not necessarily predict effective antidepressant activity in humans, particularly in treatment-resistant patients.

Recent phase 3 findings indicated that the nAChR antagonist compound **13**, the 2S-(+)-enantiomer of the broad-spectrum, noncompetitive nAChR antagonist, compound **1**, did not show efficacy as an adjunct therapy for patients that were nonresponders to traditional antidepressant treatment. This suggests that simple antagonism of what is likely to be a number of nAChR subtypes has an insufficient antidepressant effect. However, the lack of nAChR subtype specificity and the marginal free concentration in brain are also significant issues for compound **13** that may have contributed to the negative outcomes in the phase 3 trials. Those outcomes are not likely to be accurate predictors of effects of nicotinic full or partial agonists with greater nAChR subtype selectivity.

Designing a compound that has actions more like those of compound **10** (short-lived nAChR agonism mediated by binding to the orthosteric sites followed by receptor desensitization) may produce a more efficacious antidepressant. Factors outlined above need to be addressed toward optimization of ligand action of the desired type and at the appropriate nAChR subtypes.

These challenges aside, preclinical evidence shows potent antidepressant-like efficacy of compound **11** and some of its analogues in the mouse FST. These ligands have high selectivity at $\alpha 4\beta 2^*$ -nAChR over other nAChR subtypes and act as partial agonists at the $(\alpha 4)_2(\beta 2)_3$ -nAChR isoform. These findings suggest and support the hypothesis that ligands selectively targeting the $(\alpha 4)_2(\beta 2)_3$ -nAChR isoform may hold significant promise as efficacious antidepressants. These ligands would offer advantages over nicotinic agents that are relatively nonselective and produce adverse side effects through actions at peripheral nAChRs or at central non- $\alpha 4\beta 2^*$ -nAChRs.

Aside from their selectivity for $\alpha 4\beta 2^*$ -nAChR, compound **11** and its analogues have favorable pharmacokinetic and toxicological profiles. Advancement of the best of these ligands to and through clinical trials still requires substantial investment of effort and capital. Our work funded under a National Cooperative Drug Discovery and Development program has provided important impetus to such advancement, but private sector engagement and commitment to treatment of depression or other psychiatric disorders will be necessary. This poses an additional challenge given the perhaps more proximal benefit of attention on new drugs to treat diseases of aging and metabolism and acute/chronic pain than to treat psychiatric disorders that are poorly understood, perhaps because of their etiopathogenic heterogeneity. If nAChR ligands are to be prescribed as antidepressants, then it may happen through the inverse route that compound **7** took to be prescribed as a smoking-cessation aid. $\alpha 4\beta 2^*$ -selective agonists and partial agonists that are attractive as potential antidepressants may also be efficacious as smoking-cessation aids. It is entirely possible that this class of compounds will first reach the clinic as an improvement over compound **2** and that antidepressant efficacy is ascertained thereafter. Regardless, studies with $\alpha 4\beta 2^*$ -nAChR-selective ligands have already contributed to an improved understanding about nAChR subtypes and isoforms, and elucidation of roles played by the nicotinic system in the effects of these ligands certainly have illuminated the neurobiology underlying depression.

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Notes

The authors declare no competing financial interest.

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Ronald J. Lukas received undergraduate and early graduate training in Physics at the State University of New York (SUNY) and Columbia University before earning a Ph.D. in Biophysics from the SUNY Health Sciences Center in Brooklyn and serving as a postdoctoral fellow in Chemical Biodynamics at the University of California, Berkeley, and in Neurobiology at Stanford University. He since has directed the Laboratory of Neurochemistry at the Barrow Neurological Institute, conducting multidisciplinary work concerning nicotinic acetylcholine receptor structure, function, and roles in neuropsychiatric disorders.

Alan P. Kozikowski is a Professor of Medicinal Chemistry and Pharmacognosy at the University of Illinois at Chicago. He has previously held positions at the University of Pittsburgh, the Mayo Clinic, and the Georgetown University Medical Center. He has made numerous contributions to the CNS and cancer fields and published over 500 research articles. His interest in epigenetics led to the identification of highly selective HDAC6 inhibitors for application to orphan indications such as Rett syndrome and Charcot-Marie-Tooth disease. Some of his designed PSMA inhibitors have been advanced to phase 2 clinical trials for prostate cancer imaging. His efforts in the GSK-3 area have recently led to a new startup company to advance novel therapeutics for pancreatic and brain cancers.

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■ ABBREVIATIONS USED

STAR*D, sequenced treatment alternatives to relieve depression; MDD, major depressive disorder; nAChR(s), nicotinic acetylcholine receptor(s); SSRI(s), selective serotonin reuptake inhibitor(s); SNRI(s), serotonin norepinephrine reuptake inhibitor(s); ACh, acetylcholine; AChE, acetylcholinesterase;

mAChRs, muscarinic ACh receptors; ECD, extracellular domain; HPA, hypothalamic–pituitary–adrenal; GABA, γ -aminobutyric acid; LS, low sensitivity; HS, high sensitivity; VTA, ventral tegmental area; SPECT, single photon emission computed tomography; CNS, central nervous system; AChBP(s), acetylcholine binding protein(s); *Ac*, *Aplysia californica*; *Ls*, *Lymnaea stagnalis*; *Ct*, *Capitella teleta*; FST, forced swim test; TST, tail suspension test; NSF, novelty-suppressed feeding; NIH, novelty-induced hypophagia; ADME-Tox, absorption, distribution, metabolism, excretion, and toxicity

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