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# Recent Developments in Novel Antidepressants Targeting α4β2-Nicotinic Acetylcholine Receptors

## Miniperspective

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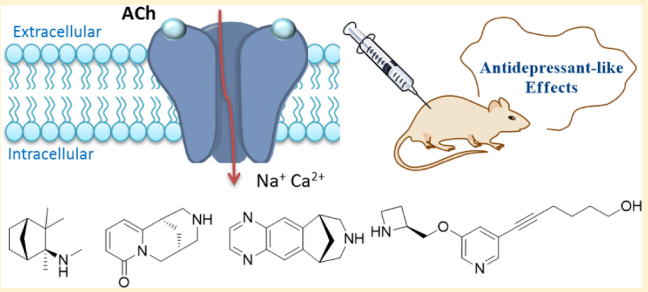
**ABSTRACT:** Nicotinic acetylcholine receptors (nAChRs) have been investigated for developing drugs that can potentially treat various central nervous system disorders. Considerable evidence supports the hypothesis that modulation of the cholinergic system through activation and/or desensitization/inactivation 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 α4β2-nAChRs in depression, along with the structural features that are essential to ligand recognition by the α4β2-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 α4β2-nAChR subtype, from the perspective of clinical and preclinical findings. We highlight the most recently developed α4β2-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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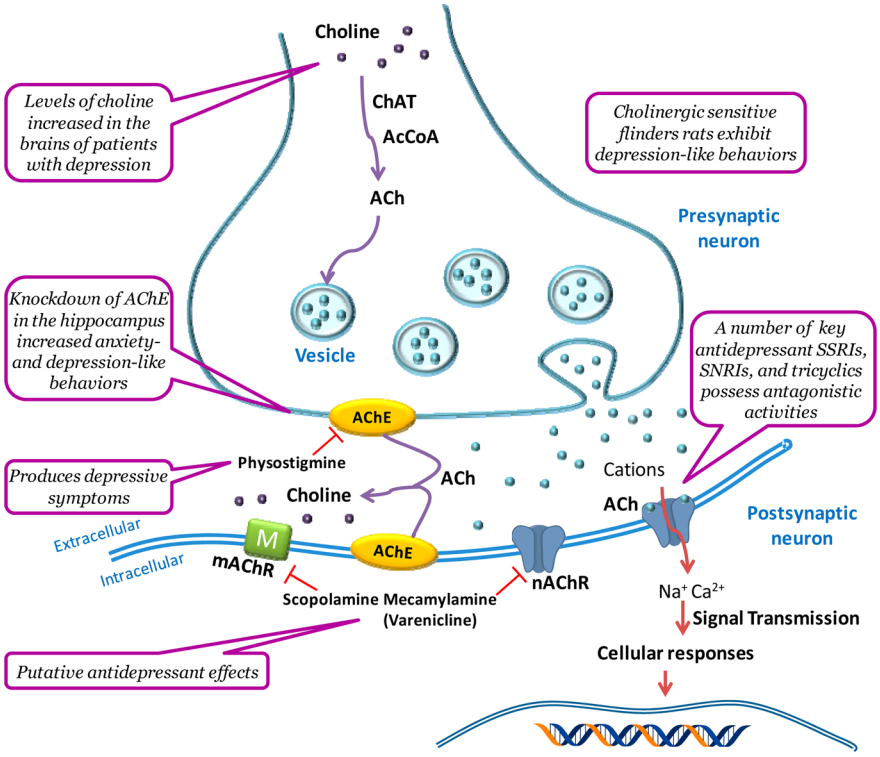


**Figure 1.** Role of the cholinergic system in depression. The cholinergic hypothesis of depression postulates a hyperactivity of the cholinergic system over that of the adrenergic system in the brain. Choline (the rate-limiting precursor to endogeneous ACh) crosses the blood–brain barrier to enter 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 $Ca^{2+}$ 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 ($\alpha1-\alpha10$, $\beta1-\beta4$, $\gamma$, $\delta$, and $\varepsilon$; $\alpha2-\alpha7$ and $\beta2-\beta4$ 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 $\alpha$-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 $\alpha$-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 $Ca^{2+}$ 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


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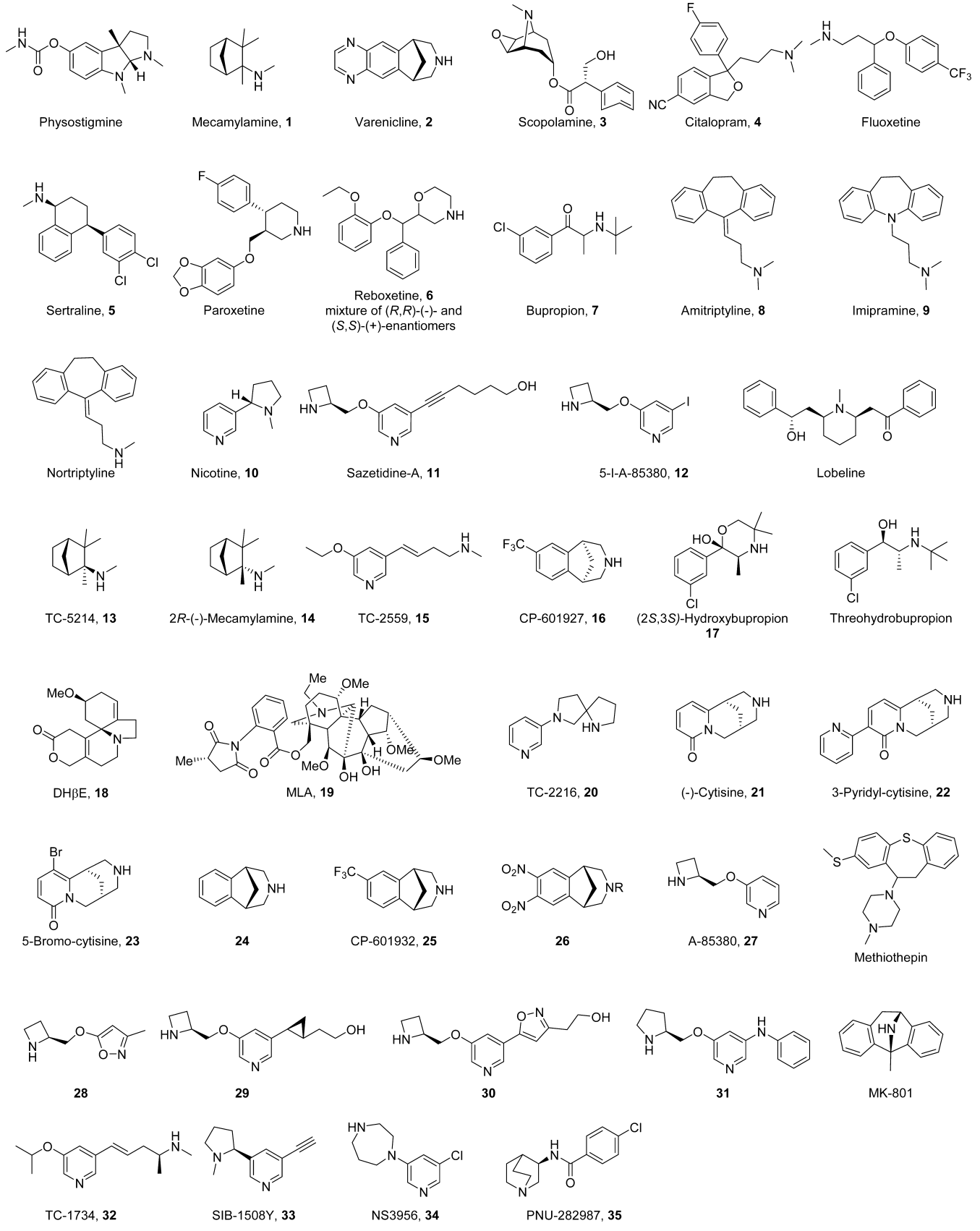
**Scheme 1. Compound Structures**




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 cytoplasticity in the hippocampus.[11] Given nAChR subtype diversity and their involvement in the modulation of various key neurotransmitter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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 α4β2 heteropentameric and α7 homopentameric subtypes are the two major nAChR subtypes expressed in the brain (Figure 2).[29] Both subtypes are implicated in the mediation

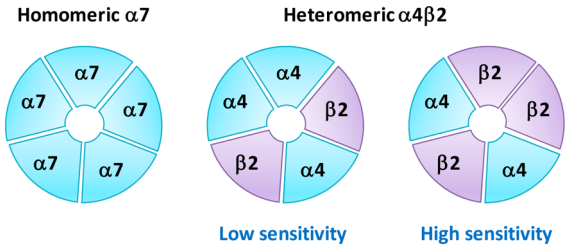


**Figure 2.** Selected nAChR subtypes. The high sensitivity (HS) α4β2-nAChR has a presumed α4/β2 subunit ratio of 2:3 and exhibits comparatively high sensitivity to nicotinic agonists, whereas the low sensitivity (LS) α4β2-nAChR, at which nicotinic agonists have lower observed potency, is composed presumably of α4 and β2 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 α4β2* (asterisk indicates possible assembly with other subunits) and α7-nAChR subtypes. α4β2*-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 α7-nAChRs are highly expressed in the hypothalamus, hippocampus, and cortex.[32,33] α4β2*-nAChRs are thought to regulate the release of monoamine neurotransmitters through action in these areas.[34,35] β2-knockout mice showed decreased immobility in the forced swim test (FST) compared to that of wild-type mice, indicating that the absence of β2-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 β2- or α7-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 β2-subunit. Additionally, the efficacies of compounds **4** and **6** in the mouse FST were enhanced by agonists at either α4β2*- or α7-nAChRs.[23] These findings suggest the involvement of β2- and α7-receptor subtypes in mediating the antidepressant-like effects of nicotinic ligands.

Clinical studies provide additional evidence for a relationship between α4β2*-nAChRs and depression. Single photon emission computed tomography (SPECT) using the α4β2*-nAChR specific radioligand [$^{123}$I]5-I-A-85380 ([$^{123}$I]**12**) revealed that the β2*-nAChR availability across all brain regions in depressed patients was lower than that in healthy subjects.[39] Additionally, positron emission tomography using 2-[$^{18}$F]fluoro-3-(2[*S*]-2-azetidinylmethoxy)-pyridine showed reduced levels of ligand binding to α4β2*-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 α4β2-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 α6 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 α6*-nAChR may be indicated.[11,42−44] In the basal ganglia, the VTA and substantia nigra, in particular, α6- and possibly nAChR β3 subunits, are included in α4β2*-nAChRs that appear to have high affinity for nicotinic agonists.[34] α3β4*-nAChR subtypes are expressed at relatively low levels in the brain[45] except for the interpeduncular nucleus, fasciculus retroflexus, and median habenula.[46] However, activation or blockade of α3β4*-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 α4β2*-nAChRs, especially those with low affinity for α3β4*-nAChRs, are considered to have higher efficacy and likely fewer side effects in rodent behavioral models, although the involvement of α3β4*-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 α7α5-nAChR), we have not delved deeply into the potential roles played by the α5 subunits. However, α5 subunits can integrate into α3β4*- and α4β2*-nAChRs and perhaps into α6*-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 α5 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,

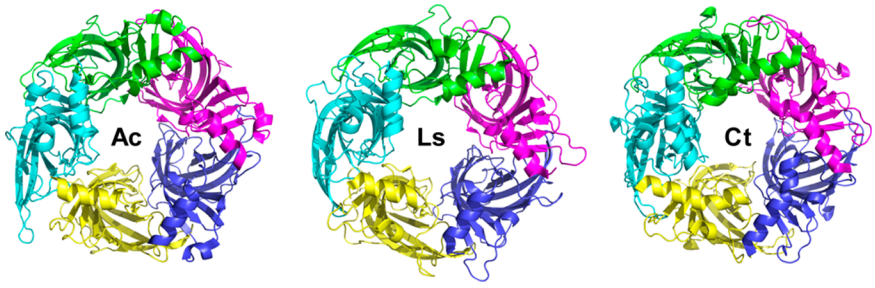


**Figure 3.** Top view of X-ray crystal structures of *Ac*-, *Ls*-, and *Ct*-AChBPs. The figure was generated using PDB files 2BR7, 1I9B, and 4B5D by PyMOL.


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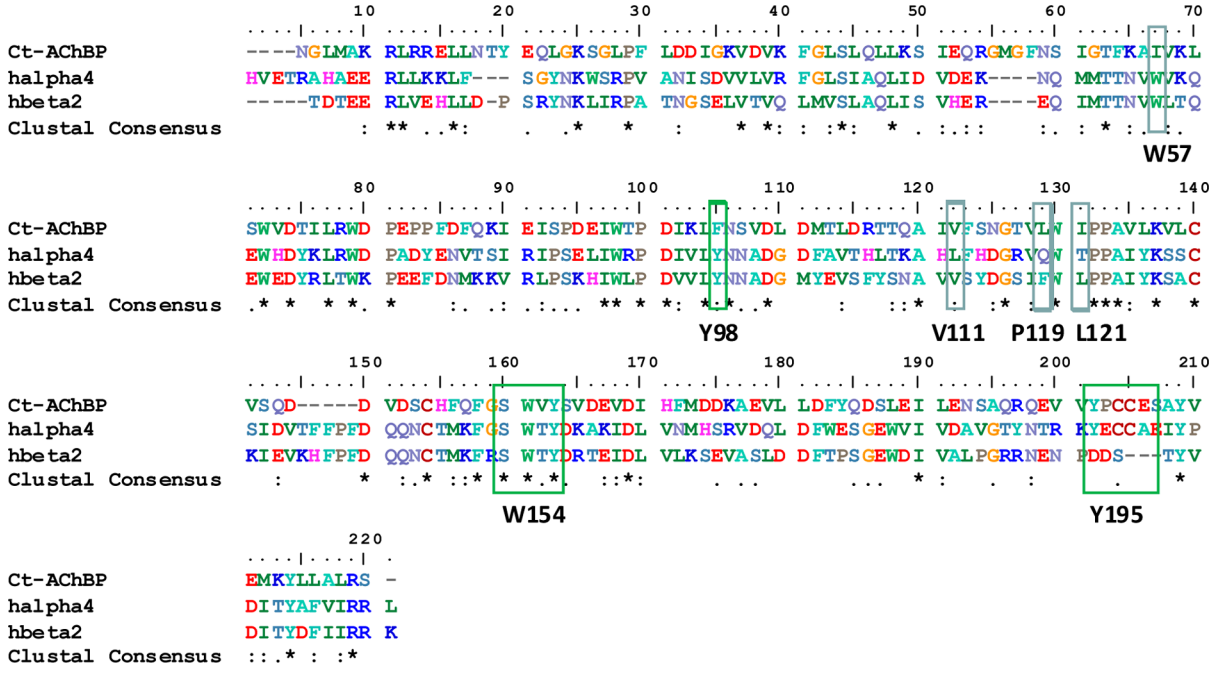


**Figure 4.** Amino acid sequence alignment of the *Ct*-AChBP with extracellular domains of nAChR $\alpha$4 or $\beta$2 subunits. Green boxes highlight positions of key residues of the $\alpha$4 subunit, and blue boxes outline key residues of the $\beta$2 subunit. Residue numeration refers to that for 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.).

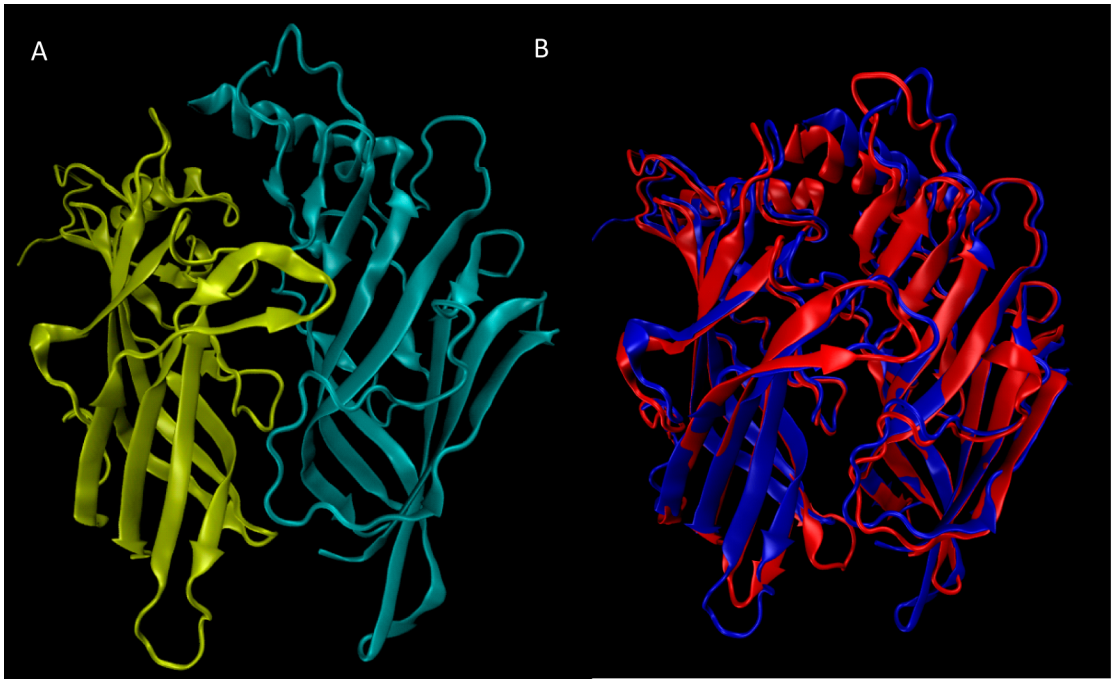


**Figure 5.** Homology model of the human $\alpha4\beta2$-nAChR ECD including the ligand binding interface: (A) ribbon structure representation colored by subunit (yellow, $\alpha$4; azure, $\beta$2) for the human $\alpha4\beta2$-nAChR ECD and (B) superimposition of the modeled structure (in blue) with the experimental template from *Ct*-AChBP (in red).

forming a cation−$\pi$ interaction.[54−56] The hydrogen-bond acceptor has been shown to bind to the complementary $\beta$-subunit, likely mediated by a water molecule to the backbone NH of a Leu residue in the $\alpha4\beta2$ receptor.[57,58] Additionally, various studies support the idea that agonist interactions with the principal $\alpha$-subunit are important for binding affinity, while interactions with key amino acids present in the complementary $\beta$-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


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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 $\alpha4\beta2$-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 identified[61] and reported to more closely mimic $\alpha4\beta2$-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 $\alpha$ subunit at the ligand binding subunit interface.

Because of the higher homology between *Ct*-AChBP and the human $\alpha4$ and $\beta2$ subunits (as computed by ClustalX: $\alpha4$/*Ct*-AChBP = 64.6% and $\beta2$/*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 $\alpha4\beta2$-nAChR. A ribbon structure for the ECD modeled $\alpha4\beta2$-nAChR shows a 10-stranded $\beta$-sandwich capped by an N-terminal $\alpha$-helix for each subunit (Figure 5A), for which the modeled $\beta$-sandwich can be nicely superimposed on that of *Ct*-AChBP (Figure 5B). This homology model may provide useful information for designing other selective $\alpha4\beta2$-nAChR ligands.[63]

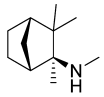
## nAChR AGONISTS AND ANTAGONISTS EXHIBITING ANTIDEPRESSANT-LIKE EFFECTS

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 $\beta2$*-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**

$IC_{50,\ \alpha4\beta2}$: 0.5-3.2 μM
$IC_{50,\ \alpha3\beta4}$: 0.2-0.6 μM
$IC_{50,\ \alpha7}$: 1.2-4.6 μM
$IC_{50,\ \alpha1\beta1\gamma\delta}$: 0.6-2.2 μM

The nicotinic ligand that has received the most attention as a potential antidepressant is compound **1**, a racemic noncompetitive and nonselective antagonist of nAChRs ($IC_{50,\alpha3\beta4}$ = 91−610 nM, $IC_{50,\alpha4\beta2}$ = 0.6−2.5 $\mu$M, and $IC_{50,\alpha7}$ = 1.6−6.9 $\mu$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 nAChRs[19] 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 $\alpha4\beta2$- and $\alpha3\beta4$-nAChRs, as well as more slowly from $\alpha4\beta2$- than $\alpha3\beta4$-nAChRs. $IC_{50}$ values for compound **13** at $\alpha3\beta4$-, $\alpha4\beta2$-, $\alpha7$-, or $\alpha1\beta1\gamma\delta$-nAChRs are similar to those of compound **14** ($IC_{50,\alpha3\beta4}$ = 0.2−0.6 and 0.05−0.4 $\mu$M, $IC_{50,\alpha4\beta2}$ = 0.5−3.2 and 0.5−1.7 $\mu$M, $IC_{50,\alpha7}$ = 1.2−4.6 and 2.2−5.8 $\mu$M, and $IC_{50,\alpha1\beta1\gamma\delta}$ = 0.6−2.2 and 0.3−1.1 $\mu$M).[51] Further pharmacological studies suggested that compound **13** is a more efficacious antagonist of LS $\alpha4\beta2$-nAChRs than compound **1** and can act as a positive allosteric modulator at HS $\alpha4\beta2$-nAChRs.[66] This was hypothesized to be mechanistically important; however, the positive allosteric modulation at $\alpha4\beta2$-nAChRs was inferred from a slight potentiation of the HS $\alpha4\beta2$-selective agonist TC-2559 (**15**) in a mixed population of HS and LS $\alpha4\beta2$-nAChRs. Potentiation of ACh was not demonstrated, and because the effect was not reproduced in a pure population of HS $\alpha4\beta2$-nAChRs, activation of LS $\alpha4\beta2$-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 $\alpha4\beta2$*-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**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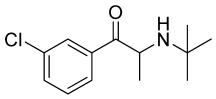
was found to be well-tolerated in acute and chronic toxicity studies in different animal models (mice, rats, and dogs) and showed acceptable pharmacologic, pharmacokinetic, and metabolic profiles for therapeutic development.

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 Picciotto[74] 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 $\alpha4\beta2$-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 $\alpha3\beta4$-nAChRs than $\alpha4\beta2$-nAChRs in the brain that are thought 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 approximately 20% of $\alpha4\beta2$*-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 $\alpha4\beta2$ 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 $\alpha4\beta2$-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% $\alpha4\beta2$ 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**

$IC_{50,\ \alpha4\beta2}$: 12 μM
$IC_{50,\ \alpha3\beta4}$: 1.8 μM
$IC_{50,\ \alpha1}$: 7.9 μM

Compound **7**[82] is an $\alpha$-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 $IC_{50}$ 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 **7**[85] 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 $\alpha4\beta2$-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 $\alpha4\beta2$-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 [$^3$H]-norepinephrine uptake and [$^3$H]dopamine update as well as a more potent antagonist of $\alpha4\beta2$-nAChR function than that of the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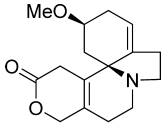
2*S*,3*R*-isomer, suggesting that the 2*S*,3*S*-isomer may be a better candidate than compound 7 for antidepressant treatment.

**(2*S*,13b*S*)-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.**

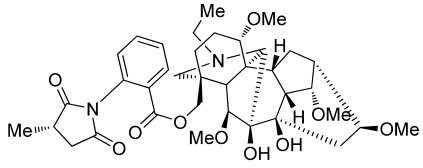


DHβE, **18**

$IC_{50}$, $\alpha4\beta2$: 30 nM
$IC_{50}$, $\alpha3\beta4$: 23 μM

Dihydro-*β*-erythroidine (DH*β*E, **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 $\alpha4\beta2$-nAChRs ($IC_{50,\alpha4\beta2}$ = 30 nM and $IC_{50,\alpha3\beta4}$ = 23 μM)[87] that blocks compound **10**-induced dopamine release from rat striatal slices ($IC_{50}$ = 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]

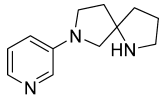
**((3*S*,6*S*,6a*S*,7*R*,7a*R*,8*S*,9*R*,10*S*,11a*R*,12*S*,12a*S*,13*S*)-1-Ethyl-11a,12-dihydroxy-6,8,10,13-tetramethoxydodecahydro-2*H*-3,6a,12-(epiethane[1,1,2]triyl)-7,9-methanonaphtho[2,3-*b*]azocin-3(4*H*)-yl)methyl 2-((*S*)-3-methyl-2,5-dioxopyrrolidin-1-yl)benzoate, 19.**



MLA, **19**

Methyllycaconitine (MLA, **19**),[90] a complex diterpenoid alkaloid isolated from the seeds of *Delphinium brownii*, is a competitive and selective $\alpha7$-nAChR antagonist with an $IC_{50}$ value in the subnanomolar range, as tested in *Xenopus* oocytes and $\alpha7$-transfected SH-SY5Y cells.[90,91] Compound **19** was found to partially inhibit anatoxin-evoked dopamine release from rat striatal slices[92] 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 $\alpha7$ nAChR inhibition and antidepressant function.

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



TC-2216, **20**

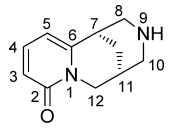
Another $\alpha4\beta2$-nAChR antagonist is the Targacept compound TC-2216 (**20**).[93] This compound was reported to show beneficial effects in preclinical studies, thereby promoting its further development for the treatment of depression and anxiety disorders.[93] A phase 1 clinical trial of racemic 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.**

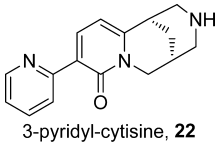


(-)-cytisine, **21**

$K_{i,\alpha4\beta2}$: 2 nM
$K_{i,\alpha3\beta4}$: 480 nM
$K_{i,\alpha7*}$: 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 $\alpha4\beta2$-nAChR partial agonist ($K_{i,\alpha4\beta2}$ = 2 nM) and an $\alpha3\beta4$ ($K_{i,\alpha3\beta4}$ = 480 nM) and $\alpha7$ ($K_{i,\alpha7}$ = 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 $\alpha4\beta2$-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**

$K_{i,\alpha4\beta2*}$: 0.91 nM
$K_{i,\alpha3\beta4*}$: 119 nM
$K_{i,\alpha7*}$: 1100 nM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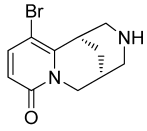
**(1*R*,5*S*)-11-Bromo-1,2,3,4,5,6-hexahydro-8*H*-1,5-methanopyrido[1,2-*a*][1,5]diazocin-8-one, 23.**




5-bromo-cytisine, **23**

$K_{i,\alpha4\beta2^*}$: 0.3 nM
$K_{i,\alpha3\beta4^*}$: 3.8 nM
$K_{i,\alpha7^*}$: 28 nM

SAR studies of cytisine 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 ($-CH{=}CH_2$,[102] $-NO_2$,[103] or pyridinyl[95]), C-4 ($-CH_3$),[102] or C-5 (-Br)[78] position could lead to the same or improved $\alpha4\beta2$-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 ($-CO_2CH_3$ or $-COCH_2CH_3$)[102] reduced affinity. Introduction of substituents on the piperidine ring nitrogen,[97] C-4 ($-CH_3$ or $-CH_2OH$),[102] or the C-5 ($-NO_2$)[103] position increases the selectivity for $\alpha4\beta2$ receptor. Among these substituted cytisine analogues, 3-(2-pyridyl)cytisine (**22**)[95] and 5-bromocytisine (**23**)[95] were further tested in mouse antidepressant efficacy models. Both compounds displayed high affinity at $\alpha4\beta2$*-nAChRs ($K_{i,\alpha4\beta2^*}$ = 0.9 for **22** and 0.3 nM for **23**), whereas compound **22** was found to show lower affinities at $\alpha3\beta4$*-nAChRs and $\alpha7$*-nAChRs ($K_{i,\alpha3\beta4^*}$ = 119 nM and $K_{i,\alpha7}$ = 1100 nM for **22** vs $K_{i,\alpha3\beta4^*}$ = 3.8 nM and $K_{i,\alpha7}$ = 28 nM for **23**). In electrophysiology assays, compound **22** activates both HS and LS $\alpha4\beta2$*-nAChRs ($EC_{50,HS}$ = 12 nM and $EC_{50,LS}$ = 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 $\alpha3\beta4$*.[95] In the same assays, compound **23** activated both HS and LS $\alpha4\beta2$*-nAChRs with similar potencies ($EC_{50,HS}$ = 13 nM and $EC_{50,LS}$ = 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]**

| | | $\alpha4\beta2$ activation | | | | $\alpha4\beta2$ inactivation | |
|---|---|---|---|---|---|---|---|
| ID | ref | 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 postintraperitoneal 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 $\mu$L 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 cytisine 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 $\alpha4\beta2$-nAChR partial agonist with weaker activity at the $\alpha3\beta4$-nAChRs ($K_{i,\alpha4\beta2}$ = 1.2 nM vs $K_{i,\alpha3\beta4}$ = 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 $\alpha6$- and $\alpha7$-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 ($CF_3SO_2O^-NO_2^+$) 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 cytisine 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 $\alpha4\beta2$*-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 $\alpha4\beta2$-nAChRs, compound **2** was found to have an $EC_{50}$ of 2.3 $\mu$M and an efficacy of 13.4% relative to that of ACh.[109] It potently blocks compound **10** binding to $\alpha4\beta2$*-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 $\alpha4\beta2$*-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


dx.doi.org/10.1021/jm401937a | J. Med. Chem. 2014, 57, 8204−8223

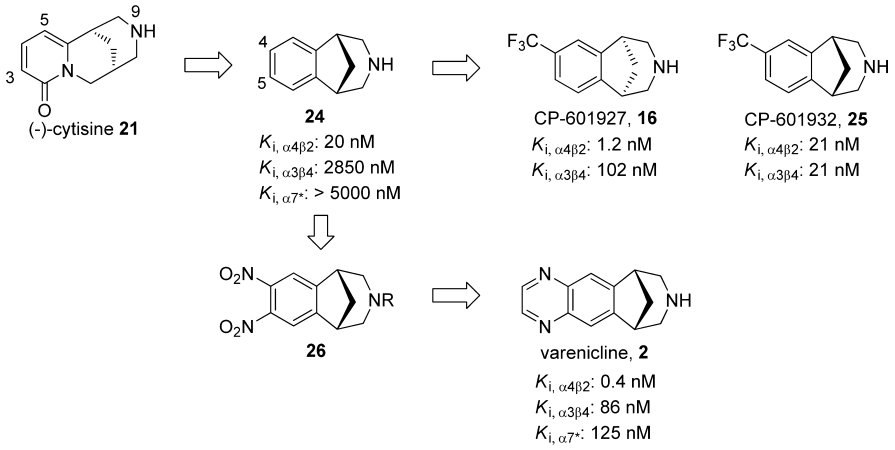
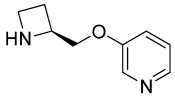



**Figure 6.** Selected nicotinic benzazapine analogues.

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

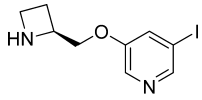
## A-85380 AND ANALOGUES

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



A-85380, **27**
$K_{i,\alpha4\beta2}$: 0.04 nM
$K_{i,\alpha4\beta2^*}$: 0.02-0.05 nM
$K_{i,\alpha7}$: 148 nM

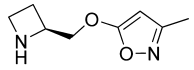
### (*S*)-3-(Azetidin-2-ylmethoxy)-5-iodopyridine, 12.



5-I-A-85380, **12**
$K_{i,\alpha4\beta2^*}$: 0.01 nM

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 $\alpha4\beta2$-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 $\beta2$-containing nAChRs, primarily $\alpha4\beta2^*$ in brain ($K_{i,\alpha4\beta2^*}$ = 0.02−0.05 nM for rat brain receptors and 0.04 nM for human brain receptors) relative to that of human $\alpha7$-nAChRs ($K_{i,\alpha7}$ = 148 nM) or muscle-type $\alpha1\beta1\gamma\delta$-nAChRs ($K_{i,\alpha1\beta1\gamma\delta}$ = 314 nM).[119,120] Functionally, compound **27** acts as a potent activator of both human $\alpha4\beta2$-nAChRs ($EC_{50}$ = 0.7 $\mu$M) and ganglionic $\alpha3\beta4^*$-nAChRs ($EC_{50}$ = 0.8 $\mu$M), 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 **12**[120] ($K_{i,\alpha4\beta2^*}$ = 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 $\alpha4\beta2^*$-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, $\beta2^*$-nAChR availability was found to be decreased across brain regions in depressed patients compared to that of healthy subjects using SPECT with the $\beta2^*$-selective radioligand tracer [$^{123}$I]**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.



**28**
$K_{i,\alpha4\beta2}$: 4.6 nM
$K_{i,\alpha3\beta4}$: 692 nM
$K_{i,\alpha7}$: > 10000 nM

Replacement of the pyridine core of these ether-based ligands with a methylisoxazole group led to compound **28**, which binds to $\alpha4\beta2$-nAChRs with higher affinity than to the $\alpha3\beta4$-nAChRs ($K_{i,\alpha4\beta2}$ = 4.6 nM vs $K_{i,\alpha3\beta4}$ = 692 nM).[122] The functional potency of compound **28** (1.2 $\mu$M) at the $\alpha4\beta2$-nAChR is similar to that of compound **2** (1.4 $\mu$M), whereas the efficacy of compound **28** is higher (110% vs 53%) in cells heterologously expressing a mixture of HS and LS $\alpha4\beta2$-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 $\mu$M. 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 $\mu$M).[122]


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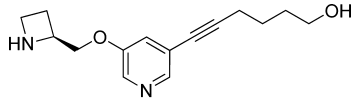
**Table 2. Functional Potencies and Efficacies of Ligands 2, 10, 11, and 28−31: Agonism and Inactivation at Human $\alpha4\beta2$-nAChRs[122−125]**

| | agonism | | | inactivation | |
|---|---|---|---|---|---|
| compound | $EC_{50}$ (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 |

[a]The efficacies were measured in a mixture of HS and LS $\alpha4\beta2$-nAChRs. [b]The efficacy values were extrapolated using compound **11** defined as a full agonist at the HS $\alpha4\beta2$-nAChR. [c]Compound **31** activates both HS and LS $\alpha4\beta2$-nAChRs with sufficient selectivity to distinguish activity at each subtype. Efficacy of **31** at LS $\alpha4\beta2$-nAChRs is approximately 17%.

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



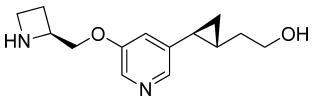

Sazetidine-A, **11**
$K_{i,\ \alpha4\beta2}$: 0.4 nM
$K_{i,\ \alpha3\beta4}$: > $10^4$ nM
$K_{i,\ \alpha7}$: > $10^4$ nM

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 $\alpha4\beta2$-nAChR and a low efficacy agonist at LS nAChR (Table 2).[126] Compared with compound **27**, the selectivity of compound **11** at $\alpha4\beta2$- relative to ganglionic $\alpha3\beta4$*- and homomeric $\alpha7$-nAChRs is greatly improved.[127,128] Compound **11** potently binds to $\alpha4\beta2$-nAChRs ($K_i$ = 0.4 nM) but not to $\alpha3\beta4$*- ($K_i$ > $10^4$ nM) or $\alpha7$-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 responsible for the activity of compound **11** in this behavioral model of antidepressant efficacy. In addition, $\beta2$ subunit knockout mice did not show any antidepressant response to compound **11** in the FST, suggesting that interaction with $\beta2$*-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 $\alpha6$*-selective antagonist $\alpha$-conotoxin MII (100 nM) reduced the maximum effect of compound **11**-induced dopamine release in rat by 48%, suggesting that $\alpha6$*-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, $\beta2$ knockout mice still showed a hypothermic response to compound **11**,[131] whereas the antidepressant-like effects of compound **11** was completely abolished in $\beta2$ 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 administration[140] 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-((1*R*,2*S*)-2-(5-(((*S*)-Azetidin-2-yl)methoxy)pyridin-3-yl)cyclopropyl)ethan-1-ol, 29.**




**29**
$K_{i,\ \alpha4\beta2}$: 0.1 nM
$K_{i,\ \alpha3\beta4}$: 6520 nM
$K_{i,\ \alpha7}$: > 10000 nM

**Table 3. Binding Affinities of Compounds 10, 11, and 27−31 at Seven nAChR Subtypes[122−125]**

| | $K_i$ (nM)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| compound | $\alpha2\beta2$ | $\alpha2\beta4$ | $\alpha3\beta2$ | $\alpha3\beta4$ | $\alpha4\beta2$ | $\alpha4\beta4$ | $\alpha4\beta2$*[b] | selectivity $\alpha4\beta2/\alpha3\beta4$ |
| **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 [$^3$H]epibatidine binding sites using radioligand binding. [b]$\alpha4\beta2$*, prepared from rat forebrain.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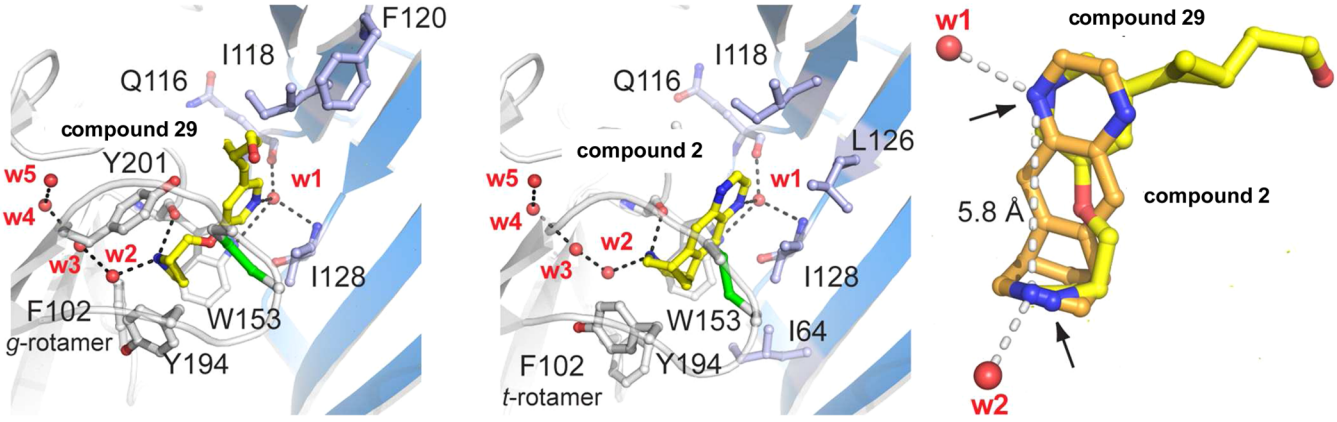
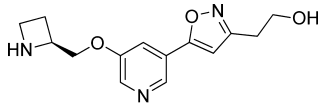



**Figure 7.** X-ray crystal structure of the *Ct*-AChBP in complex with compound **2** or **29**.[63]

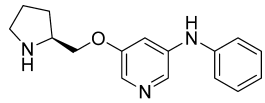
**(*S*)-2-(5-(5-(Azetidin-2-ylmethoxy)pyridin-3-yl)isoxazol-3-yl)ethan-1-ol, 30.**



**30**

$K_{i,\alpha4\beta2}$: 0.6 nM
$K_{i,\alpha3\beta4}$: > $10^4$ nM
$K_{i,\alpha7}$: > $10^4$ nM

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



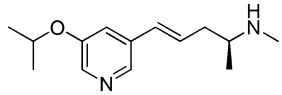
**31**

$K_{i,\alpha4\beta2}$: 1.2 nM
$K_{i,\alpha3\beta4}$: 5640 nM

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–$\pi$ 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-Isopropoxypyridin-3-yl)-*N*-methylpent-4-en-2-amine, 32.**



TC-1734 (AZD-3480), **32**

$K_{i,\alpha4\beta2}$: 11 nM
$K_{i,\alpha7}$: > 50 μM

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 $\mu$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 $^{86}Rb^+$ efflux assay in rat thalamic synaptosomes ($\alpha4\beta2$ subtype, $EC_{50}$ = 220 nM, efficacy = 57%) and the dopamine-release assay in striatal synaptosomes ($\alpha4\beta2/\alpha6/\alpha3\beta2$, $EC_{50}$ = 106 nM, efficacy = 55%). Compound **32** (up to 100 $\mu$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 $\mu$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**

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


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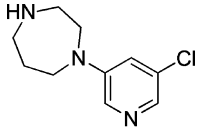
**Table 4. Clinical and Preclinical Evidence for the Viability of Targeting nAChRs in Depression**

| ID | receptor subtype selectivity | antidepressant/anxiolytic results | ref |
|---|---|---|---|
| **2** | $\alpha4\beta2$ partial agonist, less potent $\alpha3\beta4$ and $\alpha7$ full agonist | Improved FST; augmented the antidepressant effects in depressed smokers | 12, 38, and 109−114 |
| **11** | $\alpha4\beta2$ partial agonist | Improved FST, TST, and NIH | 35, 38, and 126−129 |
| **12** | $\alpha4\beta2$ 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** | $\alpha4\beta2$ partial agonist | Improved FST Failed as augmentation of antidepressant therapy for major depression in phase 2 clinical study | 78 and 108 |
| **18** | $\alpha4\beta2$ competitive antagonist | Improved FST and TST | 22 and 87−89 |
| **19** | $\alpha7$ antagonist | Improved FST and TST | 89−92 |
| **20** | $\alpha4\beta2$ antagonist | Antidepressant and anxiolytic like response in preclinical studies | 93 |
| **21** | $\alpha4\beta2$ partial agonist, $\alpha3\beta4$ and $\alpha7$ full agonist | Antidepressant-like activities in several rodent models; safety issues, poor absorption and limited brain penetration | 94−101 |
| **22** | $\alpha4\beta2$ partial agonist | Improved FST, TST, and chronic NSF | 95 and 97 |
| **27** | $\alpha4\beta2$ partial agonist, $\alpha3\beta4$ agonist | Improved FST | 119−121 |
| **28** | $\alpha4\beta2$ partial agonist, less potent $\alpha3\beta4$ agonist | Improved FST | 122 |
| **29** | $\alpha4\beta2$ partial agonist | Improved FST | 63 and 125 |
| **30** | $\alpha4\beta2$ partial agonist | Improved FST | 124 and 141 |
| **31** | $\alpha4\beta2$ partial agonist | Improved FST | 123 |
| **32** | $\alpha4\beta2$ partial agonist | Improved FST | 142−147 |
| **33** | $\alpha4\beta2$ partial agonist | Improved learned helplessness | 148−152 |
| **34** | $\alpha4\beta2$ partial agonist | No effect alone in FST; enhanced the antidepressant-like effect of SSRI (compound **4**) and SNRI (compound **6**) | 23 and 58 |
| **35** | $\alpha7$ 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 $\alpha3\beta4$-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.**

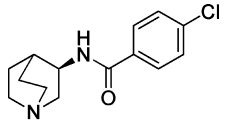



NS3956, **34**

$K_{i,\alpha4\beta2}$: 0.7 nM

NS3956 (**34**)[23] was found to be a partial agonist at human $\alpha4\beta2$-nAChRs ($K_i$ = 0.36 nM) with good selectivity ($K_{i,\alpha7}$ = 399 nM and $K_{i,\alpha1}$ = 944 nM). In the oocyte expression system, this compound was very potent at HS $\alpha4\beta2$-nAChRs ($EC_{50}$ = 11 nM), with an efficacy of 47% of the response to a maximally efficacious concentration of ACh.[23] At LS $\alpha4\beta2$-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 $\alpha7$- or $\alpha3\beta4$-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 [$^3$H]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 $\alpha4\beta2$-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 $\alpha4\beta2$- over $\alpha7$-nAChRs.[57]

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




PNU-282987, **35**

$K_{i,\alpha7}$: 26 nM

PNU-282987 (**35**)[153] is a potent and selective $\alpha7$-nAChR agonist ($K_i$ = 26 nM) with good selectivity over $\alpha3\beta4$- or $\alpha1\beta1\gamma\delta$-nAChRs (≥60 $\mu$M). Compound **35** did not show significant interactions with monoamine, muscarine, glutamate, or GABA receptors when tested at concentrations of 1 $\mu$M, with the exception of the 5-$HT_3$ 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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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, particularity in treatment-resistant patients.

Recent phase 3 findings indicated that the nAChR antagonist compound **13**, the 2*S*-(+)-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 $\alpha4\beta2$*-nAChR over other nAChR subtypes and act as partial agonists at the $(\alpha4)_2(\beta2)_3$-nAChR isoform. These findings suggest and support the hypothesis that ligands selectively targeting the $(\alpha4)_2(\beta2)_3$-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-$\alpha4\beta2$*-nAChRs.

Aside from their selectivity for $\alpha4\beta2$*-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. $\alpha4\beta2$*-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 $\alpha4\beta2$*-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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**Han-Kun Zhang** completed his Ph.D. in Medicinal Chemistry in 2009 at Shanghai Institute of Materia Medica, Chinese Academy of Sciences, under the mentorship of Dr. Li-Hong Hu. His dissertation research centered on the discovery of novel antidiabetic and antitumor agents derived from natural products. He began his postdoctoral research career at the University of Illinois at Chicago, under the direction of Dr.

Alan P. Kozikowski, where his research focus was on the development of novel nAChR ligands and HDAC inhibitors. In 2013, he joined the School of Life Sciences, East China Normal University, as an Associate Professor, and his current research interests are focused on GPCR-based drug discovery.

**Barbara J. Caldarone** is the Director of the NeuroBehavior Laboratory at the Harvard NeuroDiscovery Center and an Instructor at Brigham and Women's Hospital in Boston, Massachusetts. She completed her postdoctoral training at Yale University School of Medicine, where she began studying the relationship between nicotinic acetylcholine receptors and depression; she continued this work in industry at PsychoGenics, where she worked on characterizing novel nicotinic ligands for the treatment of depression. She currently works with investigators from the Harvard research community in utilizing mouse behavioral models to help understand and develop treatments for neurodegenerative and psychiatric disorders.

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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, $\gamma$-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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