

Nicotinic ACh Receptors

Susan Wonnacott

Department of Biology & Biochemistry, University of Bath, Bath, BA2 7AY, UK, Email: S.Wonnacott@bath.ac.uk

Susan Wonnacott is a Professor of Neuroscience in the Department of Biology and Biochemistry at the University of Bath. Her research focuses on understanding the roles of nicotinic acetylcholine receptors in the mammalian brain and the molecular and cellular events initiated by acute and chronic nicotinic receptor stimulation.

Introduction

The nicotinic acetylcholine receptor (nAChR) is the prototype of the cys-loop family of ligand-gated ion channels that also includes GABA_{A-p} {formerly GABA_C}, glycine and 5-HT₃ receptors, as well as invertebrate glutamate-, histamine-, ACh-, and 5-HT-gated chloride channels.¹⁻³ More recently, homologous ligand-gated ion channels (albeit lacking the hitherto definitive 'cys-loop') have been identified in prokaryotes and are likely to represent the ancestral form from which current day 'cys-loop' receptors descended.^{4,5} In vertebrates, nAChRs constitute a family of proteins serving many physiological functions:

- at the neuromuscular junction postsynaptic nAChRs mediate skeletal muscle contraction;
- in the autonomic nervous system, ganglionic nAChRs are responsible for fast excitatory transmission; they are also found in presynaptic nerve endings of sympathetic, parasympathetic and sensory neurons;
- 'neuronal' nAChRs are present throughout the brain and spinal cord, where they exert a largely modulatory influence;
- 'neuronal' nAChRs also occur on non-neuronal cells, including glial, immune and endothelial cells, where they are presumed to respond to paracrine ACh.

The structural and functional diversity within this receptor family⁶ has kindled interest in nAChRs as potential therapeutic targets for a wide variety of medical conditions, and has spurred drug discovery programs. This has resulted in the development of subtype-selective ligands that complement the generous armamentarium of natural products directed at nAChRs, to provide an increasing portfolio of tools for nAChR research.^{7,8}

nAChR Structure

nAChRs in vertebrate skeletal muscle, and their counterparts in the electrogenic organs of *Torpedo* and *Electrophorus*, were the first receptors to be studied and have been characterized in exquisite functional and structural detail. This was possible because the neuromuscular junction enabled detailed electrophysiological measurements of nAChR function to be made, in early studies by Langley and Dale, followed by the pioneering work of Katz and Miledi, and development of single channel recording by Neher and Sakmann.^{1,8} *Torpedo* and *Electrophorus* electric organs provided a high density of nAChRs that facilitated high resolution structural studies^{9,10} and biochemical characterization.¹

High affinity snake α -toxins, notably α -bungarotoxin (α -Bgt), enabled the nAChR protein to be purified from *Torpedo* electroplax and resolved into 4 different subunits, designated α , β , γ and δ .¹¹ An additional subunit, ϵ , was subsequently identified in nAChRs

Contents

Introduction.....	1
nAChR Structure.....	1
Sites on the nAChR for Ligand Interactions.....	2
Diversity of nAChR Subtypes.....	3
nAChR Stoichiometry.....	3
Invertebrate nAChRs.....	4
Distribution and Physiological Significance of nAChR Subtypes.....	4
Nicotinic Ligands for Neuronal nAChR.....	6
Agonists.....	6
Antagonists.....	13
Positive Allosteric Modulators.....	21
Future Perspectives for nAChR Research.....	24
References.....	25
Nicotinic Compounds.....	29

purified from adult skeletal muscle. In the early 1980s, these subunits were cloned and the era of the molecular analysis of nAChR commenced.¹ The muscle endplate nAChR has the subunit combination and stoichiometry ($\alpha 1$)₂ $\beta 1\epsilon\delta$, whereas extrajunctional ($\alpha 1$)₂ $\beta 1\gamma\delta$ nAChRs predominate in fetal or denervated muscle, and electric organs.¹² Each of the five subunits comprising the nAChR spans the lipid bilayer to create a water-filled pore (Figure 1). Each subunit consists of 4 transmembrane segments; the second transmembrane segment (M2) lines the ion channel. The extracellular N-terminal domain of every subunit contains a 'cys-loop' that is the signature sequence of this ligand-gated ion channel family: two cysteine residues (Cys 128, 142, *Torpedo* $\alpha 1$ subunit numbering), separated by 13 amino acids, form a disulfide bond to create a loop that has been implicated in the transduction of agonist binding into channel opening.^{13,14}

The principal agonist binding site resides in the N-terminal domain of α subunits, close to the pair of adjacent ('vicinal') cysteine residues (Cys 192, 193, *Torpedo* numbering) that define an α subunit. Mutagenesis and photoaffinity labeling experiments have highlighted the importance of 4 aromatic residues (Tyr 93, Trp 149, Tyr 190, Tyr 198, *Torpedo* numbering), consistent with 3 polypeptide loops of the α subunit (loops A–C) contributing to the principal agonist binding site.^{14,15} These aromatic residues stabilize bound ligands through π -cation interactions.

The muscle nAChR subunits are arranged in clockwise order – $\alpha 1$, $\beta 1$, δ , $\alpha 1$, γ/ϵ – with the two agonist binding sites occurring at the α - δ and α - γ/ϵ interfaces¹² (see Figure 3). The adjacent subunit (δ or γ/ϵ) also contributes to the agonist binding site (complementary site: polypeptide loops D–F). One consequence of this is that the α - δ and α - γ/ϵ binding sites are not identical, and this is reflected in differences in ligand affinity.^{12,22} However, occupancy of both binding sites is required for effective opening of the channel.

Knowledge of ligand binding to nAChRs has been greatly augmented by the crystal structure of a soluble ACh binding protein (AChBP), first identified in the snail *Lymnaea stagnalis* and subsequently also cloned from *Aplysia californica* and *Bulinus truncatus*.^{16–18} Each subunit of this pentameric secreted protein is homologous to the N-terminal domain of a nAChR subunit (20–24% sequence identity), with conservation of all the residues implicated in ACh binding to the muscle nAChR. The ability to crystallize these proteins for X-ray diffraction studies has provided a high resolution view of the extracellular portion of the receptor, notably of the binding site at the interface between adjacent subunits, and the interaction of ligands with these sites.^{14,17,19} Co-crystallization of the AChBP with different ligands, and molecular modeling approaches in which the crystal structure of the AChBP is modified to accommodate sequence differences found in the extracellular domains of various nAChR subunits, can be exploited to identify subtype-selective nAChR ligands.^{20,21}

Whereas the function of the AChBP is to sequester ACh, binding of ACh to nAChRs increases the probability of channel opening, thus promoting cation flux. Upon agonist binding, nAChRs undergo an allosteric transition from the predominantly closed, resting conformation to an open state that allows an influx of Na⁺, and, to some extent (depending on nAChR subtype, see Figure 3), Ca²⁺,

accompanied by an efflux of K⁺ under normal physiological conditions. In the closed state the ion channel is occluded by a 'hydrophobic girdle' that constitutes a barrier to ion permeation.⁹ Agonist binding in the extracellular domain promotes a conformational change that results in a rotational movement of the M2 helices lining the pore, twisting the girdle to widen the pore by ~3 Å, sufficient for ion permeation.^{9,23} At the muscle endplate, the ensuing depolarization elicits muscle contraction.

Despite the presence of agonist, the nAChR channel closes within seconds to minutes, in order to enter a desensitized state (Figure 2). In this condition, the nAChR is refractory to activation. Multiple desensitized states have been proposed to exist.²⁴ In the active (open) conformation, the nAChR binds agonists with low affinity (e.g. K_d of muscle nAChR for ACh ~50 μ M). The desensitized states display higher affinity for agonist binding (K_d of muscle nAChR for ACh ~1–5 μ M); thus the desensitized nAChR retains bound agonist despite its non-conducting state. The transitions between resting, open and desensitized states can be influenced by allosteric modulators (see below).

Sites on the nAChR for Ligand Interactions (Figure 1)

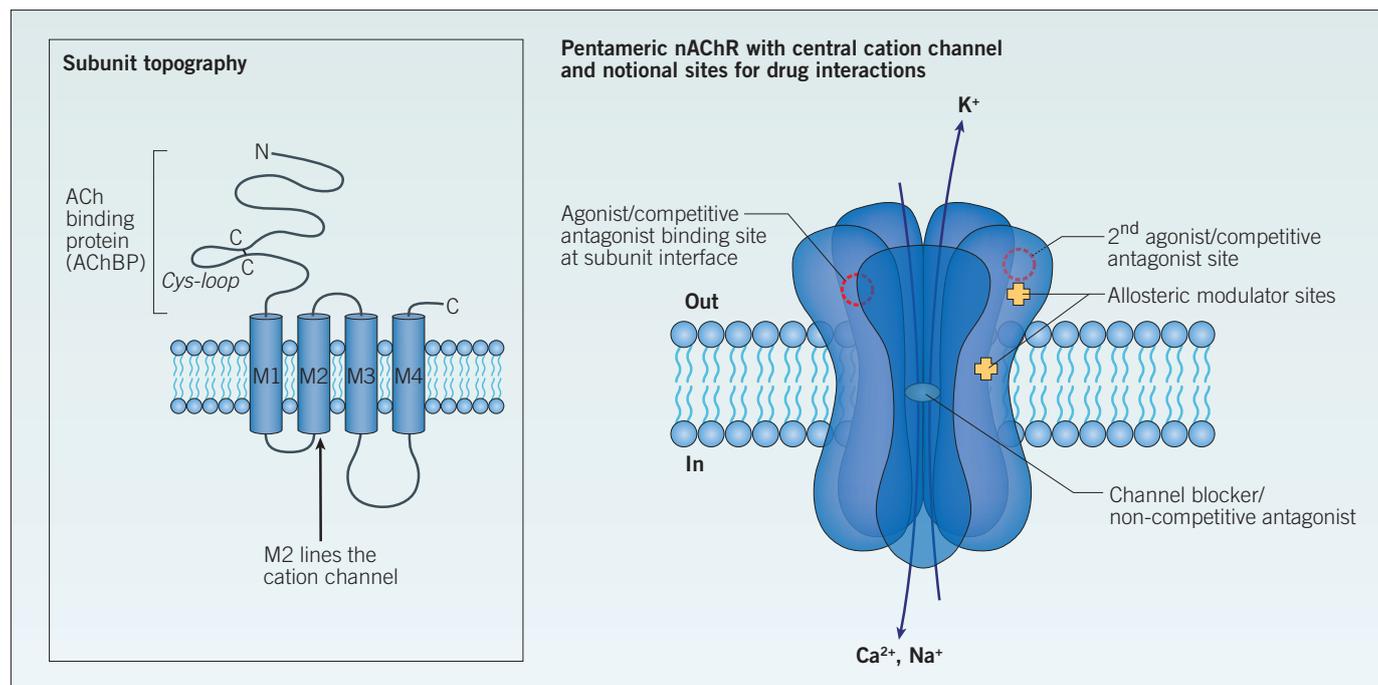
Agonists bind at subunit interfaces in the extracellular domain, as described above.¹⁴ Agonists stabilize the mobile loop-C of the principal binding subunit in close apposition to the adjacent (complementary) subunit. Competitive antagonists also bind at or close to the agonist binding sites, preventing access to agonists. In contrast to agonists, competitive antagonists stabilize loop-C in an extended conformation that precludes channel opening.^{17,19,25} Thus the channel remains closed and access to the agonist binding site is prevented.

In contrast to full agonists, exemplified by the endogenous ligand ACh, partial agonists activate the nAChR with low efficacy, despite interacting with the same agonist binding site.²⁶ Such ligands also exhibit competitive inhibition through competition with other, more efficacious agonists for occupancy of the binding site. Thus they combine weak agonism with a degree of antagonism.⁸ The efficacy displayed by a partial agonist is not an intrinsic property of the ligand but a consequence of the interaction with a particular nAChR subtype.¹³⁹ For example, the efficacies of synthetic agonists TC 2559 and sazetidine A at $\alpha 4\beta 2$ nAChRs are determined by the subunit stoichiometry.²⁷ The complementary subunit has been implicated as the major determinant of efficacy, based on a comparison of cytisine and a novel agonist NS 3861.³⁷

Non-competitive antagonists bind at sites distinct from agonist binding sites to prevent receptor activation or block channel function. Channel blocking compounds may interact specifically with residues in the mouth or lumen of the pore to occlude the ion channel. In addition, any small, positively charged species may be predicted to channel block, and many agonists, including ACh, do this at high concentrations.²⁸ The efficiency of channel blockade is 'state dependent' as access to the channel requires the channel to be open. Hence the speed of block will be influenced by the state of the receptor: resting, open or desensitized (Figure 2).

Allosteric modulators are non-competitive ligands that act at a variety of distinct sites to positively or negatively influence agonist

Figure 1 | General structure of a nAChR



Left: The topography of a single nAChR subunit. The two cysteine residues forming the signature cys-loop are indicated. The extracellular N-terminal domain corresponds to the AChBP.

Right: Schematic of a generic nAChR showing 5 subunits spanning the membrane to form a central ion channel. Notional sites of action of interacting drugs are shown: two agonist binding sites are indicated at extracellular subunit interfaces, consistent with a heteromeric nAChR. Competitive antagonist sites overlap the agonist binding sites. Extracellular and intramembrane sites have been proposed for different allosteric modulators. Non-competitive antagonists may occlude or block the ion channel, as indicated, or may act at other sites. See text for further details.

interactions and/or nAChR function.²⁹ This diverse class includes inorganic cations, steroids, anesthetics and amines. Subtype-selective positive allosteric modulators (PAMs), in particular, are attracting attention as potential therapeutic drugs.⁸ Their sites of interaction on the nAChR are diverse, ranging from non-canonical binding sites in the extracellular N-terminus to locations within the membrane spanning domains (Figure 1; see section on PAMs, p21).³⁰⁻³²

Diversity of nAChR Subtypes (Figure 3)

The classical studies of Paton and Zaimis³³ demonstrated that nicotinic responses in autonomic neurons are pharmacologically distinct from their counterparts in skeletal muscle, providing the first indication of nAChR heterogeneity. The quest for nAChRs in the brain, necessary to explain the psychoactive actions of nicotine, identified binding sites for ¹²⁵I- α -Bgt and [³H]-nicotine.^{34,35} Their distinct pharmacological profiles and anatomical distributions in rodent brain raised the (then) novel and controversial prospect of nAChR heterogeneity. Following the first publication³⁶ of a cloned neuronal nAChR subunit (α 3) in 1986, eleven novel 'neuronal' nAChR subunits have been identified in mammals (α 2- α 7, α 9, α 10, β 2- β 4), with an additional subunit, α 8, cloned from avian species.^{6,8}

α 2- α 6 subunits form heteromeric nAChRs with β subunits. α subunits are defined by the presence of a pair of vicinal cysteines equivalent to those that characterize the muscle α 1 subunit. This led to the supposition that all α subunits could

constitute a principal agonist binding site in neuronal nAChRs. However, the α 5 subunit is not capable of fulfilling this role as it lacks the critical tyrosine from loop C (Tyr 190, *Torpedo* α 1 labeling).³⁸ β subunits lack the N-terminal vicinal cysteines but β 2 and β 4 subunits contain the tryptophan residue characteristic of loop D; hence these subunits can act like γ and δ muscle nAChR subunits to provide the complementary agonist binding site at an $\alpha\beta$ interface. The absence of this key tryptophan residue in the β 3 subunit makes it the true homolog of the muscle β 1 subunit that does not contribute to an agonist binding site. Indeed, the sequence similarity between α 5 and β 3 subunits^{46,8} (see Figure 3) is consistent with both having this role, although other subunits can also occupy the position equivalent to β 1.^{39,40} Issues of nAChR subunit nomenclature are reviewed by Hurst *et al.*⁸

nAChR Stoichiometry

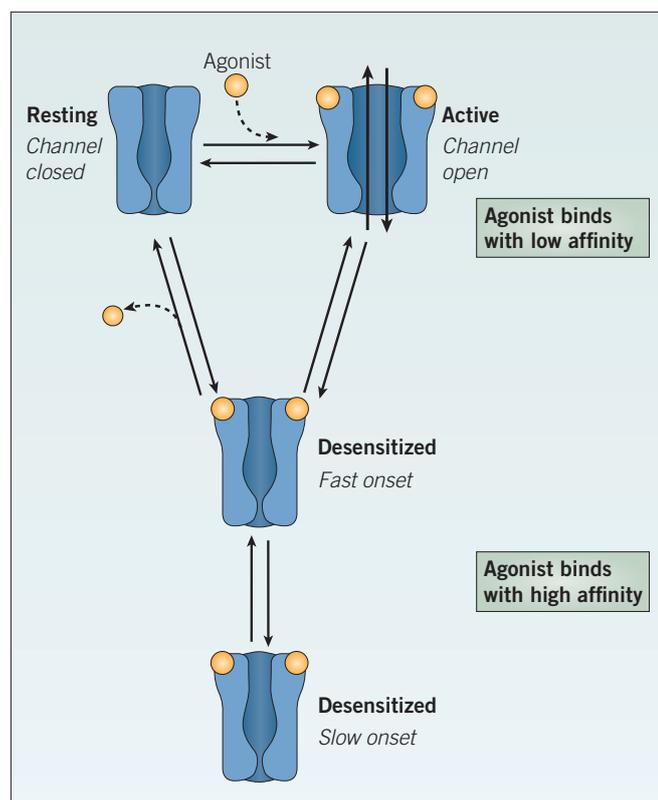
Heteromeric neuronal nAChRs comprised of α and β subunits include two pairwise combinations of α 2, α 3, α 4 or α 6 with β 2 or β 4 subunits, to provide two agonist binding sites in an arrangement analogous to that of the muscle nAChR (Figure 3). The fifth position, corresponding to that occupied by the β 1 subunit in muscle nAChR, can accommodate any subunit (although rules of assembly constrain the subunit combinations in a given nAChR, Figure 3). The subunit occupying this position (sometimes referred to as the 'accessory' subunit)⁴⁰ can influence assembly, trafficking and functional properties of the resultant nAChR subtype, including agonist potency, conductance, Ca^{2+}

permeability and desensitization kinetics.^{27,40} For example, agonists and competitive antagonists typically have higher potency at $(\alpha 4)_2(\beta 2)_3$ nAChRs, compared with $(\alpha 4)_3(\beta 2)_2$ nAChRs.²⁷ Nicotine upregulates $\alpha 4\beta 2$ nAChRs by acting as a chaperone to stabilize the $(\alpha 4)_2(\beta 2)_3$ stoichiometry.^{41,42} $\alpha 3\beta 4$ nAChRs also exhibit stoichiometry-dependent differences in agonist and antagonist sensitivities and nicotine-induced upregulation, in heterologous expression systems.^{37,43,44}

The fifth subunit contributes to a putative allosteric site analogous to the benzodiazepine binding site in GABA_A receptors.¹⁴ There is evidence that some allosteric modulators bind to neuronal nAChRs at this location, depending on which subunit occupies the fifth position. For example, the anthelmintic morantel acts at this site in mammalian $\alpha 3\beta 2$ nAChRs to enhance channel gating.³⁰ Site-directed mutagenesis, combined with cysteine-substitution methods, indicated that $\beta 2$ occupies the fifth position to create a non-canonical binding site ($\beta 2$ – principal face; $\alpha 3$ – complementary face).³⁰ Conversely, the PAM NS 9283 is proposed to interact selectively with an $\alpha 4\beta 2$ heteromer with stoichiometry $(\alpha 4)_3(\beta 2)_2$, which places $\alpha 4$ in the fifth position.⁴⁶ However, the precise subunit stoichiometry of native nAChRs remains poorly established.

In contrast, the $\alpha 7$, $\alpha 8$ and $\alpha 9$ subunits are distinguished by their ability to form robust homomeric receptors, sensitive to α -Bgt, when expressed in *Xenopus* oocytes. Evidence indicates that native α -Bgt-sensitive nAChRs in mammalian brain are predominantly homomeric, being formed of $\alpha 7$ subunits.⁶ However, compared with heteromeric nAChRs, heterologous expression of $\alpha 7$ nAChRs is much less efficient in non-neuronal mammalian cultured cells. This paradox was resolved by the

Figure 2 | Relationship between the major conformational states of a nAChR



discovery that the endoplasmic reticulum-resident chaperone RIC-3 can promote the formation of functional $\alpha 7$ nAChRs in non-permissive cells.⁴⁷ This highlights the importance of ancillary proteins in regulating the assembly, trafficking, stabilization and turnover of nAChR subtypes.⁴⁸

In a homopentamer, the same type of subunit provides both principal and complementary faces of the agonist binding site,⁶⁶ resulting in five putative binding sites per receptor homomer (Figure 3). An interesting question concerns the number of sites that must be occupied by an agonist for efficient channel activation. Elegant studies to determine the single channel activity of receptors composed of different proportions of wildtype and mutated (low conductance) subunits indicated that occupancy of 3 non-consecutive sites within a pentamer is necessary for full channel activity.⁴⁹

Despite its ability to form homomeric nAChRs, in physiological systems the $\alpha 9$ subunit co-assembles with $\alpha 10$ to form heteromeric nAChRs, with the stoichiometry $(\alpha 9)_2(\alpha 10)_3$.^{50,51} On its own, $\alpha 10$ appears to be incapable of forming a homomeric receptor.⁵² In avian tissues $\alpha 7\alpha 8$ heteromers exist and nAChRs comprised of $\alpha 7$ and $\beta 2$ subunits have been proposed to occur in heterologous expression systems and in mammalian brain.^{53–55}

Invertebrate nAChRs

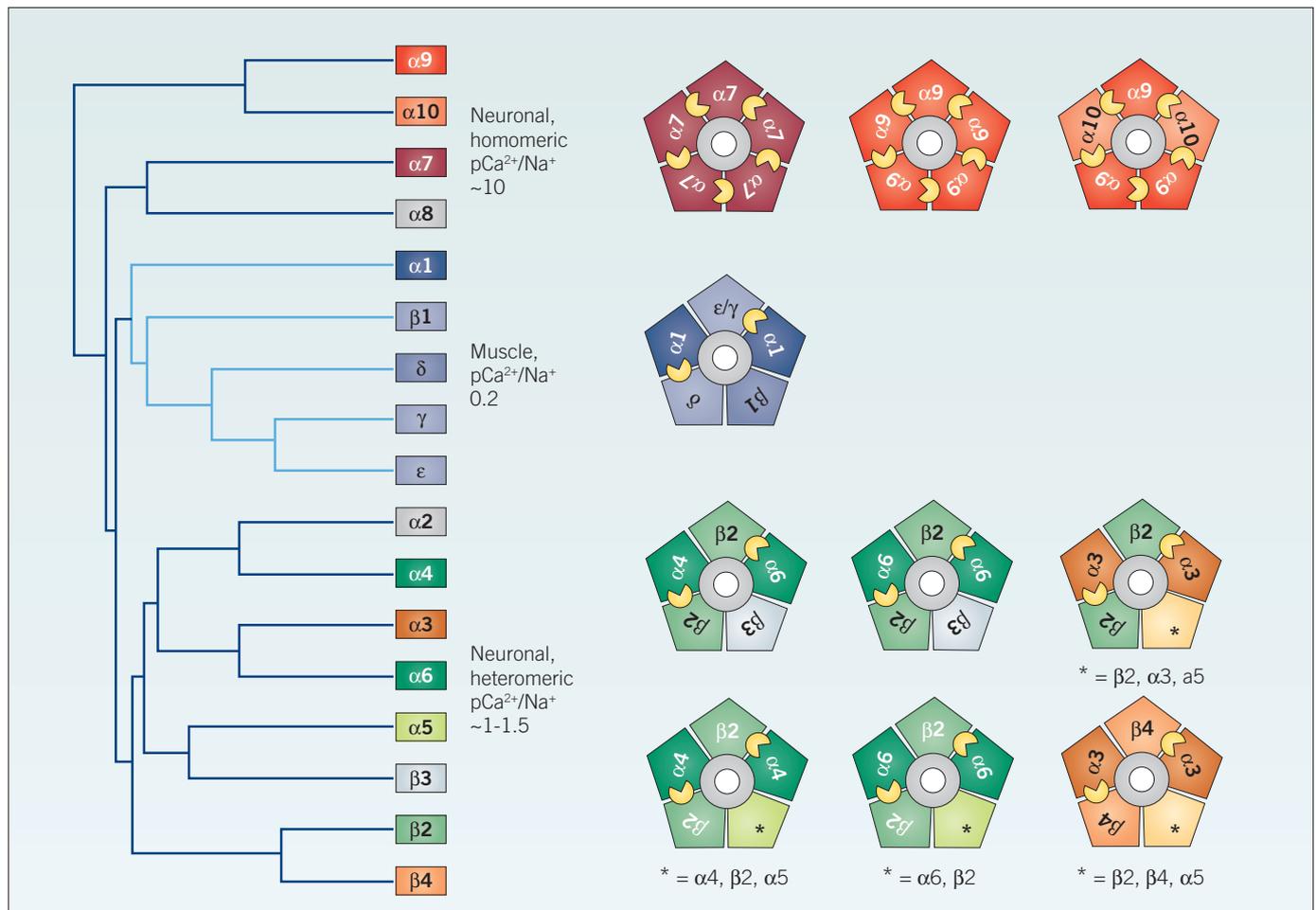
A distinct but related gene family of α and β nAChR subunits has been uncovered in invertebrates. The *C. elegans* genome sequence incorporates the largest number of such subunits, with at least 32 reported to date, 22 of which have been classified as α subunits.⁵⁶ This contrasts with a lower number of subunits in parasitic nematodes, where they represent clinical and veterinary targets.⁵⁶ Insects, including the fruit fly (*Drosophila melanogaster*), malaria mosquito (*Anopheles gambiae*), and honey bee (*Apis mellifera*) have 10–12 nAChR subunits that comprise the major excitatory receptor class in the insect central nervous system.^{57,58} This distinct gene family of nAChR subunits has allowed the development of selective agonists – the neonicotinoids that target insect or helminth nAChRs, for pesticide or veterinary applications.⁵⁹ Mutagenesis, electrophysiology and computer modeling suggest that the subunit providing the complementary face of the binding site confers the selectivity for neonicotinoids of invertebrate over mammalian nAChRs. Concerns about the impact of neonicotinoids on ‘friendly’ insects, such as bees, highlights the challenge to generate more discriminating ligands that can distinguish between insect species.⁶⁰

Distribution and Physiological Significance of nAChR Subtypes

Autonomic neurons (including sympathetic ganglia, parasympathetic innervation, sensory ganglia, chromaffin, neuroblastoma and PC12 cells) typically express $\alpha 3$, $\alpha 5$, $\alpha 7$, $\beta 2$ and $\beta 4$ subunits,^{61,62} with the likely assembly of $\alpha 3\beta 4$, $\alpha 3\beta 4\alpha 5$, $\alpha 3\beta 4\beta 2$ and $\alpha 7$ nAChRs. Additional subunits (including $\alpha 4$, $\alpha 6$, $\alpha 9$ and $\alpha 10$) have been reported in dorsal root ganglia;^{63–65} nAChRs in these sensory neurons are of interest as therapeutic targets for modulating nociceptive signals.

In the mammalian brain there is a heterogeneous distribution of $\alpha 2$ - $\alpha 7$ and $\beta 2$ - $\beta 4$ subunits, with distinct and often extensive and

Figure 3 | Heterogeneity of vertebrate nAChRs



Left: Cladogram of all vertebrate nAChR subunits cloned to date, adapted from Novère *et al*⁴⁶⁸

Right: Cartoons illustrate viable pentameric mammalian subunit combinations, with putative agonist binding sites indicated by small yellow crescents between adjacent subunits. See text for further details.

*Indicates the possible inclusion of additional unspecified subunits

overlapping expression patterns.^{8,66} $\alpha 4$, $\beta 2$ and $\alpha 7$ are the most wide-spread subunits, with $\alpha 4\beta 2^*$ (where * indicates the possible inclusion of additional unspecified subunits),⁶⁷ and $\alpha 7$ nAChRs having a somewhat complementary distribution. In contrast to the well-established role of nAChRs in synaptic signaling at the neuromuscular junction and in sympathetic ganglia, there are rather few reports of neuronal nAChRs mediating cholinergic synaptic transmission in the CNS. However, there is abundant evidence in the brain for presynaptic nAChRs that modulate the release of many different neurotransmitters⁶⁸ and this has led to the supposition that the majority of nAChRs are located presynaptically. In addition, nAChRs also exist on somatodendritic regions,⁶⁹ in perisynaptic and extrasynaptic locations.^{70,355} The current perspective is that presynaptic and extrasynaptic nAChRs modulate short and longer term neuronal activity in response to non-synaptic ('paracrine') levels of ACh (or choline, in the case of $\alpha 7$ nAChRs).^{71,72} Nevertheless, a few examples of synaptic nAChRs mediating synaptic transmission at cholinergic synapses in the brain have been documented.^{73,74}

The $\alpha 7$ nAChR is particularly prominent in hippocampus and cortex (including the prefrontal cortex (PFC)),⁷⁵ where it is commonly associated with GABAergic interneurons and

glutamatergic synapses.^{72,77} There are numerous accounts of the highly Ca²⁺ permeable $\alpha 7$ nAChRs contributing to synaptic plasticity and long term potentiation, notably in the hippocampus,^{71,72,76,78} and working memory in the PFC.³⁵⁵ Genetic linkage studies and pharmacological evidence have implicated $\alpha 7$ nAChRs in schizophrenia.⁷⁹ A substantial body of evidence has encouraged the aim of targeting $\alpha 7$ nAChRs to treat cognitive and attentional deficits in a variety of conditions including schizophrenia, ADHD and Alzheimer's disease.⁸ $\alpha 7$ and $\alpha 4\beta 2$ nAChRs also collaborate in modulating synaptic activity.^{69,80}

$\alpha 4\beta 2$ nAChRs with stoichiometry $(\alpha 4)_2(\beta 2)_3$ have high affinity for nicotine (and account for >90% of [³H]-nicotine binding to brain tissues⁸¹). Transgenic knockout of either of these subunits eliminates nicotine self-administration, whereas virally targeted re-expression of the $\beta 2$ subunit in mesolimbic areas of $\beta 2$ knockout mice recovers this behavior, implicating a role for $\alpha 4\beta 2$ nAChRs in nicotine addiction.^{82,83} A similar strategy has uncovered a role for $\beta 2^*$ nAChRs in the prelimbic area of the PFC in attention.⁸⁴ $\alpha 4\beta 2$ nAChRs are highly expressed in the thalamus.⁸⁵ Their putative role in thalamo-cortical circuitry provides a rationale for the ability of gain of function mutations in the channel-forming M2 domain, or adjacent M3 domain, of either

the $\alpha 4$ or $\beta 2$ subunit to give rise to autosomal dominant nocturnal frontal lobe epilepsy.⁸⁶

The $\alpha 3$, $\alpha 5$ and $\beta 4$ nAChR subunit genes form an evolutionarily conserved gene cluster, found on human chromosome 15, and they are often, but not always, co-expressed.⁸ $\alpha 3$ and $\beta 4$ nAChR subunits have a more restricted distribution in the CNS, compared with $\alpha 4$ and $\beta 2$, whereas the $\alpha 5$ subunit is expressed more widely, at low to moderate levels.⁸⁷⁻⁸⁹ Polymorphisms in these 3 genes, notably D398N within the coding region of the $\alpha 5$ subunit gene, are associated with increased vulnerability to tobacco addiction and/or risk of lung cancer.⁹⁰ This association inspired studies in mice that implicate $\alpha 5$ -containing nAChRs in the medial habenular – interpeduncular nucleus (IPN) pathway in the negative regulation of nicotine consumption, making such receptors a potential target for smoking cessation.^{91,92}

In cortex, thalamus, hippocampus and striatum, $\alpha 5$ is associated with $\alpha 4$ and $\beta 2$ subunits in 15-40% of $\alpha 4\beta 2^*$ nAChRs.⁸⁹ Somatodendritic or terminal $\alpha 4\beta 2\alpha 5$ nAChRs have been identified in dopamine neurons in the ventral tegmental area (VTA) and substantia nigra and in corticothalamic projection neurons.⁹³⁻⁹⁵ $\alpha 5$ confers increased Ca^{2+} permeability and enhanced rates of desensitization to heteromeric nAChRs,⁴⁰ can alter sensitivity to ACh, and renders $\alpha 4\beta 2$ nAChRs resistant to nicotine-induced upregulation.^{89,96-98}

$\alpha 6$ and $\beta 3$ subunits are commonly expressed together and exhibit a limited distribution, largely restricted to catecholaminergic neurons and neurons of the visual pathway.^{6,99,100} These subunits contribute to nAChRs of complex subunit composition on dopaminergic terminals, including $\alpha 6\beta 2\beta 3$ and $\alpha 4\alpha 6\beta 2\beta 3$ nAChRs⁹³ (Figure 3). The latter subtype is inferred to have the highest sensitivity to nicotine ($\text{EC}_{50} = 0.23 \mu\text{M}$) and sazetidine A.^{101,102} The $\beta 3$ subunit is suggested to influence the assembly, stability and/or targeting of $\alpha 6$ -containing nAChRs¹⁰³⁻¹⁰⁵ and a dominant negative role has been inferred from studies of recombinant nAChR subunits.^{106,107}

The $\alpha 2$ nAChR subunit has a limited expression pattern in the rodent CNS, being largely restricted to the IPN.¹⁰⁸ Its distribution in the primate brain appears to be more extensive.¹⁰⁹ Transgenic mice with a targeted deletion of the $\alpha 2$ subunit gene exhibit little change in phenotype, although some nicotine-modulated behaviors, including nicotine self-administration and withdrawal, are potentiated.¹¹⁰ This is consistent with the role of the IPN in restraining addictive behavior, alluded to above.^{91,92} $\alpha 2\beta 4$ nAChRs are also inferred to participate in the motoneuron-Renshaw cell synapse of the mouse spinal cord, a rare model of nicotinic synaptic transmission in the CNS.¹¹¹

Mechanosensory hair cells express $\alpha 9$ and $\alpha 10$ nAChR subunits that co-assemble to generate predominantly heteromeric nAChRs. $\alpha 9\alpha 10$ nAChRs exhibit an unusual mixed nicotinic/muscarinic pharmacology: although they are activated by ACh, nicotine and other agonists act as competitive antagonists at $\alpha 9\alpha 10$ nAChRs.^{52,124} $\alpha 9\alpha 10$ nAChRs are highly Ca^{2+} permeable; they mediate the effects of the efferent olivocochlear system on auditory processing.^{51,112} These subunits are not expressed in the brain but have been reported, together or separately, in a variety of non-neuronal cells and tissues, as well as in sensory neurons where they are putative antinociceptive targets.¹¹³

Expression of numerous ‘neuronal’ nAChR subunits has also been detected in diverse non-neuronal cells. These include astrocytes, macrophages, keratinocytes, endothelial cells of the vascular system, muscle cells, lymphocytes, intestinal epithelial cells and various cell-types of the lungs.¹¹⁴ mRNAs encoding most nAChR subunits have been detected in such cells but the identity and functional significance of assembled nAChRs in non-neuronal cells is less clear. $\alpha 7$ nAChRs in non-neuronal cells have excited interest because of their high Ca^{2+} permeability and ability to engage Ca^{2+} -dependent signaling cascades, but there are arguments for both adverse and beneficial influences.^{115,116}

Nicotinic Ligands for Neuronal nAChR

Due to the critical roles of muscle and ganglionic nAChRs in normal vertebrate physiology, Nature has elaborated a diverse array of plant and animal toxins that target these receptors, and their counterparts in the CNS. The nicotinic pharmacopoeia continues to be augmented by the generation of synthetic ligands in response to the perceived validity of neuronal nAChRs as therapeutic targets. As a consequence, the listing that follows is primarily directed at neuronal nAChRs. The $\alpha 7$ nAChR, in particular, has attracted a substantial number of selective agonists, antagonists and allosteric modulators (see Tables 2, 3 and 7, respectively). There remains a scarcity of subtype-selective tools for heteromeric nAChRs, especially ligands that can discriminate between nAChR subtypes with subtle differences in subunit composition and stoichiometry. Most notable is the dearth of synthetic subtype-selective antagonists (see Table 3), perhaps reflecting the lack of therapeutic potential associated with nAChR blockade. Some compensation for this deficit is afforded by the α -conotoxins (Table 4). A striking development has been the generation of subtype-selective PAMs (Table 7). Representatives of these various classes of ligand that are currently available from Tocris are briefly discussed below. For more comprehensive accounts of some of the families of synthetic nicotinic ligands see References 7, 8 and 117.

Agonists

nAChR agonists are structurally diverse, comprising natural products from a variety of plant and animal species and synthetic molecules derived from structure activity relationship programs or screening of drug libraries.^{8,117} In equilibrium binding studies, non-selective agonists like nicotine typically bind with highest affinity (K_i) to the $(\alpha 4)_2(\beta 2)_3$ nAChR, with 2–3 orders of magnitude lower affinity at $\alpha 7$ nAChRs and with intermediate affinity at $\alpha 3^*$ nAChRs. Binding reflects the high affinity desensitized state of the nAChR (Figure 2) that is stabilized during the prolonged incubation period of such assays. Agonists are ~10-fold more potent at $\alpha 9\alpha 10$ nAChRs compared with their affinities for $\alpha 7$ nAChRs.¹¹⁸ With respect to functional potency, EC_{50} values are typically 1–3 orders of magnitude higher than K_i values. This difference is greatest for $\alpha 4\beta 2$ nAChRs, so that EC_{50} values between subtypes may be more similar than suggested by their binding affinities. This has spurred the quest for subtype-selective nAChR agonists to exclusively activate a particular subtype in a mixed population.

Functional potency of novel ligands is typically assessed by electrophysiological recording or Ca^{2+} flux assays of recombinant

nAChR subunits expressed in *Xenopus* oocytes or mammalian cells.^{119,120,121,272} Evaluation in native systems is complicated by the likely presence of a multiplicity of nAChRs of complex and often unknown subunit composition. A practical aspect arises from the method of analysis of rapidly desensitizing currents, notably those recorded from cells expressing $\alpha 7$ nAChRs. Measurements of the peak height (amplitude) of electrophysiological responses to construct concentration response relationships, as is conventionally done, underestimate the potency of agonists activating $\alpha 7$ nAChRs; concentration response relationships based on net change analysis (area under the curve) provide more accurate (and more potent) EC_{50} values.¹²² For more slowly desensitizing nAChRs, like $\alpha 4\beta 2$ and $\alpha 3\beta 4$, these two methods are in good agreement with respect to EC_{50} values and Hill coefficients.¹²³

Classical agonists (Table 1)

These well-established agonists are non-selective and can activate all nAChR subtypes (with the possible exception of $\alpha 9^*$ nAChRs),¹²⁴ with varying degrees of potency and efficacy. The general rank order of potency is:

Epibatidine > Anatoxin A > Nicotine = Cytisine \geq
ACh > Carbamoylcholine

Acetylcholine chloride (ACh)

The endogenous agonist that activates all nAChRs

ACh is the endogenous agonist for all nAChR subtypes. It is a full agonist and a popular choice for activating nAChRs in electrophysiological experiments (commonly used at 100 μ M). Its utility is compromised by (i) its lack of selectivity for nAChRs versus muscarinic receptors and (ii) its susceptibility to hydrolysis. A muscarinic antagonist (typically atropine, ~ 1 μ M) and an acetylcholinesterase inhibitor may be necessary adjuncts; some of these agents may also interact directly with nAChRs. Choline, a substrate and hydrolysis product of ACh, is a selective, full agonist of $\alpha 7$ nAChRs ($EC_{50} = 1.6$ mM; about an order of magnitude less potent than ACh).^{125,126} Choline has weak partial agonist activity at $\alpha 3\beta 4^*$ nAChRs,¹²⁶ and also antagonizes nAChRs.¹²⁷

(\pm)-Anatoxin A fumarate

A potent non-selective agonist with properties similar to those of ACh

Anatoxin A is a potent, semi-rigid, stereoselective agonist originally isolated from freshwater blue green algae, *Anabaena flos aqua*.¹²⁸ Activity resides in the natural (+)-enantiomer. At muscle nAChRs, anatoxin A is about 8 times more potent than ACh¹²⁹ but it is 20–100 times more potent than ACh in activating neuronal nAChR subtypes (EC_{50} values in the high nM – low μ M range).^{130,131} As a secondary amine, anatoxin A crosses the blood brain barrier readily, and the (+)-enantiomer is behaviorally effective when administered systemically at doses of 100–200 μ g/kg in rats.¹³² Despite similarities, its responses were qualitatively different from those of nicotine, a conclusion supported by subsequent studies.^{133,134}

Carbamoylcholine chloride (carbachol)

A weak, non-selective agonist that is more potent at muscarinic receptors

Carbamoylcholine is the carbamate analog of ACh; it is hydrolysis-

resistant but retains potency at muscarinic receptors and is commonly used as a non-selective muscarinic agonist. It has been frequently employed to study muscle nAChRs¹³⁵ but has lower affinity at $\alpha 4\beta 2$ and $\alpha 7$ nAChRs.¹³⁶ N-Methylation of the carbamate nitrogen to yield N-methylcarbamoylcholine recovers high (nanomolar) binding affinity at $\alpha 4\beta 2$ nAChRs, comparable to ACh.¹³⁶ N-Methylation also confers substantial selectivity for nAChRs over muscarinic receptors.

(-)-Cytisine

A relatively potent agonist with variable efficacy dependent on nAChR subtype and subunit stoichiometry

(-)-Cytisine is a rigid tricyclic quinolizidine alkaloid found in plants of the *Leguminosae* family. It is comparable to nicotine with respect to its high affinity binding to $\alpha 4\beta 2$ nAChRs ($K_i \sim 1$ nM). However, its differential interactions with other nAChR subtypes has enabled subpopulations of nicotinic binding sites labeled by [³H]-epibatidine to be distinguished by their high or low affinity for cytisine.¹³⁷ Its efficacy varies with subunit composition;³⁷ it has very low or negligible efficacy at $(\alpha 4)_2(\beta 2)_3$ nAChRs, but displays $\sim 50\%$ efficacy at $(\alpha 4)_3(\beta 2)_2$ nAChRs.^{138,139} These properties have made cytisine a lead compound for drug discovery programs,¹⁴⁰ most notably for smoking cessation^{141,142} and depression.¹⁴³ Cytisine has been shown to be effective in behavioral experiments at doses of 1–3 mg/kg in rats^{144,145} or 1–5.6 mg/kg in mice.^{146,147} It is less potent than nicotine *in vivo*, a fact ascribed to its lower lipophilicity.¹⁴⁴ Cytisine shows only partial generalization to nicotine in a drug discrimination test, attributed to its partial agonist profile at $\alpha 4\beta 2$ nAChRs.^{148,149} Like nicotine, it offers some protection against 6-hydroxydopamine lesions *in vivo*.¹⁴⁵

(\pm)-Epibatidine

A very potent and efficacious non-selective agonist

(+)-Epibatidine was originally obtained from skin extracts of the Amazonian frog, *Epidobates tricolor*.¹⁵⁰ This bicyclic alkaloid (comprising an azabicycloheptane structure coupled to a chloropyridyl moiety) is one of the most potent nicotinic agonists, with both enantiomers showing similar activity. It binds to multiple heteromeric nAChRs with sub-nanomolar affinities.¹⁵¹ The functional potency of epibatidine is also exceptionally high with sub-micromolar EC_{50} values for heteromeric and $\alpha 8$ neuronal nAChR subtypes, whereas $\alpha 7$ and muscle nAChRs exhibited EC_{50} values in the low micromolar range.^{117,152,153} *In vivo*, epibatidine displays potent non-opioid analgesic activity¹⁵⁰ but its therapeutic window is very narrow, attributed to side effects arising from its lack of nAChR subtype selectivity. Both spinal and central loci for epibatidine's antinociceptive actions have been proposed.^{154–156} Effective doses reported for *in vivo* administration are: 0.25–10 μ g/kg¹⁵⁶ or 0.1–1.0 μ g¹⁵⁵ intrathecal (rat), 1–10 μ g/kg s.c.,^{154,157} 0.01–0.3 μ g local infusion.¹⁵⁶

(-)-Nicotine ditartrate

The eponymous nicotinic agonist activates all nAChR subtypes except $\alpha 9^*$

The tobacco alkaloid (-)-nicotine is the prototypic nAChR agonist that has been used historically to classify nAChRs. All nAChR subtypes are activated by nicotine (with the exception of $\alpha 9$ and $\alpha 9\alpha 10$ nAChRs, which are blocked by nicotine).¹²⁴ It binds preferentially and with high affinity to $\alpha 4\beta 2$ nAChRs ($K_i \sim 1$ nM),¹⁵⁸

and activates neuronal nAChRs with EC_{50} values in the micromolar range.⁸ Nicotine crosses the blood brain barrier readily and its pharmacokinetics and metabolism are well documented.¹⁵⁹ Recommended doses for *in vivo* research have been compiled.¹⁶⁰ Behavioral responses often show a bell-shaped dose-response profile with maximum responses in rats elicited by doses of 0.4 mg/kg s.c. or less. Doses are normally reported as the free base concentration of nicotine, and correspond to 3-times higher concentrations of the tartrate salt. Since the latter forms acidic solutions, it should be pH-neutralized for *in vivo* administration.

The primary metabolite of nicotine is (-)-cotinine.¹⁵⁹ It is credited with having weak agonist activity at certain nAChR subtypes^{161,162} (EC_{50} = 340 μ M for rat striatal dopamine release).¹⁶³ *In vivo*, its high concentration, relative to nicotine, and long half-life may promote nAChR desensitization, in both human smokers and animal subjects.^{159,163}

Other synthetic agonists (Table 2)

A 582941

An $\alpha 7$ nAChR-selective partial agonist

A 582941 (Octahydro-2-methyl-5-(6-phenyl-3-pyridazinyl)-pyrrolo-[3,4-c]pyrrole) is an $\alpha 7$ nAChR-selective agonist that binds with high affinity (K_i = 10 nM, rat) to these receptors.¹⁶⁴ It behaves as a partial agonist at $\alpha 7$ nAChRs (EC_{50} = 2.4 μ M; 60% efficacy) but

is devoid of appreciable activity at other nAChR subtypes tested. It was effective in a number of behavioral tests when given *in vivo* at doses of 0.01–1 μ mol/kg (administered i.p. in rodents or i.m. in monkeys),¹⁶⁴ 1–10 mg/kg in rats^{165,166} or 0.04–4 mg/kg in mice.¹⁶⁷

A 844606

An $\alpha 7$ nAChR-selective partial agonist

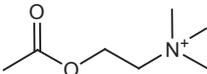
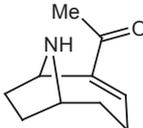
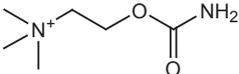
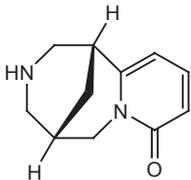
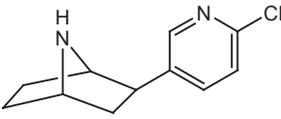
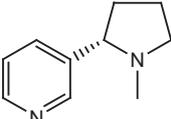
A 844606 (2-(Hexahydro-5-methylpyrrolo[3,4-c]pyrrol-2(1*H*)-yl)-9*H*-xanthen-9-one) is an $\alpha 7$ nAChR-selective partial agonist derived from a well-known interferon inducer tilerone.¹⁶⁸ Tilerone itself possesses potent affinity for $\alpha 7$ nAChRs and has inspired structure-activity relationship studies.¹⁶⁹ A 844606 was found to bind with high affinity (IC_{50} = 11 nM, rat) and potently activated $\alpha 7$ nAChRs in *Xenopus* oocytes (EC_{50} ~ 2 μ M).¹⁶⁸ A carbon-11 labeled version has been investigated as a potential PET ligand.¹⁷⁰

1-Acetyl-4-methylpiperazine hydrochloride (AMP HCl)

A weak non-selective agonist

The hydrochloride (HCl) salt of 1-acetyl-4-methylpiperazine (AMP) is a brain accessible version of the nAChR agonist AMP methiodide.¹⁷¹ It exhibits lower potency than the methiodide and differs from nicotine in its pharmacodynamic actions. AMP has been employed along with other agonists to demonstrate a

Table 1 | Classical nAChR agonists

Agonista	Structure	Comment/Selectivity ^a
Acetylcholine (2809)		Endogenous nAChR agonist; also activates muscarinic receptors. Readily hydrolyzed
(±)-Anatoxin A (0789)		Potent ACh-like, nAChR-specific, hydrolysis-resistant agonist
Carbamoylcholine (carbachol) (2810)		Non-hydrolyzable analog of ACh; also activates muscarinic receptors. $\alpha 1\beta 1\gamma/\epsilon\delta$ > neuronal nAChRs
(-)-Cytisine (1390)		Potent partial agonist at $\alpha 4\beta 2$ nAChRs; greater efficacy at other subtypes
(±)-Epibatidine (0684)		Very potent agonist. Heteromeric neuronal nAChRs > $\alpha 7$, $\alpha 1\beta 1\gamma/\epsilon\delta$ nAChRs
(-)-Nicotine (3546)		Potent agonist. Heteromeric neuronal nAChRs > $\alpha 7$, $\alpha 1\beta 1\gamma/\epsilon\delta$ nAChRs

(**Bold** text denotes compounds available from Tocris at time of publication)

^a See text for details and references

nicotinic component within the visual responses of superior colliculus neurons in the rat.¹⁷²

4-Acetyl-1,1-dimethylpiperazinium iodide

A weak non-selective agonist

4-Acetyl-1,1-dimethylpiperazinium iodide is a less potent analog of isoarecolone.¹⁷³ This class of compound is of interest because of its structural rigidity and similarity to the classical ganglionic agonist DMPP.¹⁷⁴

(+)-Anabasine hydrochloride

A weak non-selective agonist

(+)-Anabasine is a tobacco alkaloid.¹⁷⁵ Unusually, anabasine shows similar binding affinity at $\alpha 4\beta 2$ and $\alpha 7$ nAChRs ($K_i \sim 0.5 \mu\text{M}$).¹⁷⁶ Functional selectivity for $\alpha 7$ nAChRs is enhanced in the naturally occurring analog anabaseine (see below). Anabasine is effective behaviorally at 1–10 mg/kg s.c.^{177,178} Intravenous LD_{50} in mice is 1.3 mg/kg, and high doses produced muscle co-ordination deficits (presumably by interacting with muscle nAChRs).¹⁷⁹

AR-R 17779 hydrochloride

An $\alpha 7$ nAChR-selective agonist

AR-R 17779 ((3S)-Spiro[1-azabicyclo[2.2.2]octane-3,5'-oxazolidine]-2'-one hydrochloride) was one of the first $\alpha 7$ -selective agonists to be reported.¹⁸⁰ It is a structurally rigid spirooxazolidone with 100-fold greater affinity for binding to $\alpha 7$ nAChRs than $\alpha 4\beta 2$ nAChRs. It activated $\alpha 7$ nAChR currents in *Xenopus* oocytes ($\text{EC}_{50} = 10 \mu\text{M}$) whereas no activation of $\alpha 4\beta 2$, $\alpha 3\beta 4$, $\alpha 3\beta 2$ or 5-HT₃ receptors was observed, at concentrations up to 1 mM.¹⁸¹ Central effects were observed with doses of AR-R 17779 of 1–2 mg/kg s.c.^{182,183} Its lack of effect at 20 mg/kg in attentional tasks has been interpreted as evidence that $\alpha 7$ nAChRs are not involved.¹⁸⁴

3-Bromocytisine

A potent non-selective agonist with high affinity for $\alpha 4\beta 2$ nAChRs

3-Bromocytisine is the most potent of a series of cytosine derivatives halogenated at different positions of the pyridine ring.^{185,186} It is an order of magnitude more potent than cytosine but exhibits the same agonist profile as the parent molecule.^{186,187} It binds to $\alpha 4\beta 2$ nAChRs with picomolar affinity ($K_i \sim 80 \text{ pM}$) and displays a biphasic activation curve with $\alpha 4\beta 2$ nAChRs expressed in *Xenopus* oocytes ($\text{EC}_{50} = 5 \text{ pM}$ and 9 nM), consistent with a differential interaction with the two stoichiometries of this recombinant receptor.¹⁸⁷ It is effective *in vivo* at 0.1–0.2 mg/kg (rat).¹⁸⁸

DMAB-anabaseine dihydrochloride

A weak agonist with some preference for $\alpha 7$ nAChRs

DMAB-anabaseine dihydrochloride (4-[(5,6-Dihydro[2,3'-bipyridin]-3(4H)-ylidene)methyl]-N,N-dimethylbenzamine dihydrochloride) is a derivative of anabaseine (a naturally occurring toxin with a distinctive nicotinic profile favoring $\alpha 7$ nAChRs)¹⁸⁹ and has a nicotinic profile that has been superceded by the more efficacious derivative DMXB (GTS 21).¹⁹⁰ DMAB-anabaseine (2 mg/kg) produced some benefits in combating learning and auditory deficits.^{190,191}

GTS 21

A partial agonist at $\alpha 7$ nAChRs and an antagonist at $\alpha 4\beta 2$ nAChRs

GTS 21 (3-[(2,4-Dimethoxyphenyl)methylene]-3,4,5,6-tetrahydro-2,3'-bipyridine dihydrochloride; also known as DMXB), initially reported to be a nicotinic ligand with cognitive-enhancing properties,^{190,484} was subsequently shown to be a partial agonist at $\alpha 7$ nAChRs and a weak antagonist at $\alpha 4\beta 2$ and other heteromeric nAChRs.⁴⁸⁵ Co-crystallization of the AChBP with GTS 21 indicates that the benzylidene substituent prevents full closure of binding site loop-C, which may explain its partial agonist / antagonist profile.⁴⁸⁶ There are species differences in potency and efficacy of GTS 21 at rat ($\text{EC}_{50} = 5 \mu\text{M}$; 32% efficacy) and human ($\text{EC}_{50} = 11 \mu\text{M}$; 9% efficacy) $\alpha 7$ nAChRs, attributed to amino acid differences in the binding site loops.⁴⁸⁷ Pharmacokinetic studies in rats indicate that it rapidly crosses the blood brain barrier and its elimination half-life from plasma is 1.7 h.⁴⁸⁸ GTS 21 (0.1–10 mg/kg i.p.) is effective in rodent models of sensory gating and cognitive deficits^{485,489} and GTS 21 improved attention and working memory in patients with schizophrenia.⁷⁹ Its principal hydroxy metabolites exhibit similar activity profiles to the parent compound.^{8,490}

5-Iodo-A-85380 dihydrochloride

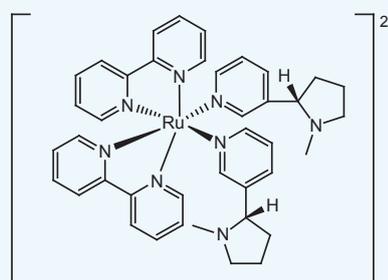
A potent $\beta 2$ -selective agonist

Iodination of A-85380 to generate an iodinated radioligand produced improved functional selectivity for $\beta 2$ -containing nAChRs over other nAChR subtypes.^{192,193} This is useful for discriminating $\beta 2$ - from $\beta 4$ -containing nAChRs.¹⁹⁴ 5-Iodo-A-85380 is a potent agonist at $\beta 2^*$ nAChRs ($\text{EC}_{50} = 13 \text{ nM}$),¹⁹³ and is effective *in vivo*; for example, it improved auditory gating in mice when given at 0.1–1 mg/kg i.p.¹⁹⁵ and partially reduced L-DOPA induced motor dysfunction when given twice daily at $\sim 0.2 \mu\text{mol/kg}$.¹⁹⁶ 5-Iodo-A-85380 incorporating the short-lived radioisotope ¹²³I is in use in humans as a SPECT neuroimaging ligand for evaluating $\alpha 4\beta 2$ nAChRs.¹⁹⁷

Caged Nicotine

RuBi-Nicotine

Cat. No. 3855

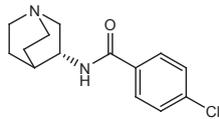
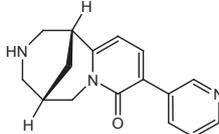
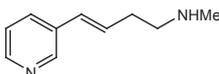
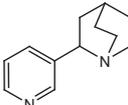
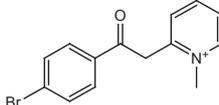
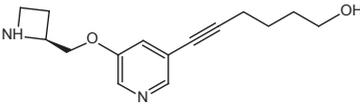
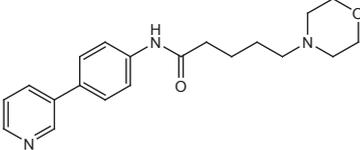
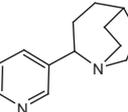
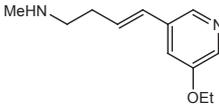
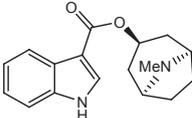
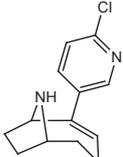
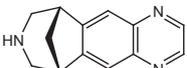


RuBi-Nicotine is a ruthenium-bisbipyridine-caged nicotine $[\text{Ru}(\text{bpy})_2(\text{Nic})_2]^{2+}$ that can be excited by irradiation with light in the visible spectrum.⁴⁶⁹ The compound undergoes rapid photolysis to release nicotine (time constant 17.3 ns), and exhibits a high quantum yield. RuBi-Nicotine induces action potential propagation in Retzius neurons of leech ganglia, with no detectable toxicity at a concentration of 1 mM.⁴⁶⁹

Table 2 | Other nAChR agonists, including novel synthetic structures

Agonist ^a	Structure	Comment/Selectivity ^a	Effective Concentration Range ^b	
			<i>In vitro</i>	<i>In vivo</i>
A 582941 (4341)		An $\alpha 7$ nAChR-selective partial agonist	5–10 μM	0.1–10 mg/kg
A 844606 (4477)		An $\alpha 7$ nAChR-selective partial agonist	5–10 μM	
1-Acetyl-4-methylpiperazine (0351)		A weak non-selective nAChR agonist, structurally related to isoarecolone and DMPP		
4-Acetyl-1,1-dimethylpiperazinium (0352)		A weak non-selective nAChR agonist, structurally related to isoarecolone and DMPP		
(+)-Anabasine (1971)		A weak non-selective nAChR agonist, with relatively high affinity for $\alpha 7$ nAChRs		1–10 mg/kg
AR-R17779 (3964)		An $\alpha 7$ nAChR-selective agonist	10–100 μM	1–2 mg/kg
3-Bromocytisine (3549)		A potent non-selective nAChR agonist	1–100 nM	0.1–0.2 mg/kg
DMAB-anabaseine (2241)		A weak nAChR agonist with some preference (but lower efficacy) for $\alpha 7$ nAChRs		2 mg/kg
GTS 21 (4557)		A partial agonist at $\alpha 7$ nAChRs and a weak antagonist at $\alpha 4\beta 2$ and other heteromeric nAChRs	5–100 μM	0.1–10 mg/kg
5-Iodo-A-85380 (1518)		A potent $\beta 2^*$ -selective nAChR agonist	10–100 nM	0.1–1 mg/kg
(-)-Lobeline (1077)		An atypical nicotinic partial agonist that also interacts with several non-nicotinic targets	0.1–10 μM	0.3–4 mg/kg
PHA 543613 (3092)		An $\alpha 7$ nAChR-selective agonist, weak activity at 5-HT ₃ receptors	0.3–30 μM	0.3–1.0 mg/kg
PHA 568487 (3134)		An $\alpha 7$ nAChR-selective agonist		

Table 2 | (continued)

Agonist ^a	Structure	Comment/Selectivity ^a	Effective Concentration Range ^b	
			<i>In vitro</i>	<i>In vivo</i>
PNU 282987 (2303)		An $\alpha 7$ nAChR-selective agonist	1–10 μM	1–30 mg/kg
3-pyr-Cytisine (4125)		A selective partial agonist at $\alpha 4\beta 2$ nAChRs	10–100 μM	0.3–0.9 mg/kg
RJR 2403 (1053)		An $\alpha 4\beta 2$ nAChR-selective agonist	0.1–10 μM	10–200 mg/kg
RJR 2429 (1271)		A broad spectrum agonist: $\alpha 4\beta 2 > \alpha 1\beta 1\gamma\delta > \alpha 3\beta 4^*$ nAChRs		
S 24795 (3518)		A weak $\alpha 7$ nAChR-selective agonist	10–100 μM	0.3–1.0 mg/kg
Sazetidine A (2736)		A potent stoichiometry-dependent agonist at $\alpha 4\beta 2$ nAChRs	10–100 nM	0.01–3 mg/kg
SEN 12333 (4441)		An $\alpha 7$ nAChR-selective full agonist	10–100 μM	1–10 mg/kg
TC 1698 (2518)		An $\alpha 7$ nAChR-selective full agonist; partial agonist at $\alpha 1\beta 1\epsilon\delta$ nAChRs; competitive antagonist at $\alpha 4\beta 2$ nAChRs	10 μM	
TC 2559 (2737)		An $\alpha 4\beta 2$ nAChR-selective agonist	0.1–10 μM	0.3–10 mg/kg
Tropisetron (2459)		A 5-HT ₃ receptor antagonist and $\alpha 7$ nAChR partial agonist; inhibits $\alpha 3\beta 4$ nAChRs	1–10 μM	1–3 mg/kg
UB 165 (1348)		A potent nAChR agonist, especially at $\alpha 4\beta 2$ nAChRs	0.1–1 μM	
Varenicline (3754)		A partial agonist at $\alpha 6\beta 2\beta 3^* > \alpha 4\beta 2 > \alpha 3\beta 4$ nAChRs; full agonist at $\alpha 7$ nAChRs	0.1–10 μM	0.1–3 mg/kg

(**Bold** text denotes compounds available from Tocris at time of publication)

^a See text for details and references

^b Range of concentrations typically used to achieve substantial activation of main target nAChR subtype in rodents (unless otherwise stated)

* Indicates the possible inclusion of additional unspecified subunits

(-)-Lobeline hydrochloride

A nicotinic partial agonist with multiple non-nicotinic targets

(-)-Lobeline, an alkaloid from the Indian tobacco *Lobelia inflata*, has been known as a ganglionic drug for over half a century but is an atypical nicotinic ligand. Lobeline binds with high affinity to $\alpha 4\beta 2^*$ nAChRs ($K_i \sim 10$ nM), and displays agonist, antagonist (desensitizer) and potentiator actions.^{198–200} Its partial agonist profile has prompted investigation of its utility in combating drug dependence.^{201,202} However, lobeline is promiscuous and inhibits vesicular monoamine transporters ($IC_{50} \sim 1$ μ M) and plasma membrane dopamine transporters ($IC_{50} = 40–100$ μ M),²⁰³ μ opioid receptors ($IC_{50} = 1$ μ M)²⁰⁴ and potassium channels ($IC_{50} = 15$ μ M, $K_v1.5$ channel).²⁰⁵ Lobeline (0.3–4 mg/kg) shows some nicotine-like effects *in vivo*,²⁰⁶ although blockade of other targets may also contribute, for example, to its antidepressant-like effects.²⁰⁷

PHA 543613 dihydrochloride

An $\alpha 7$ nAChR-selective agonist with activity at 5-HT₃ receptors

PHA 543613 (N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]furo[2,3-c]pyridine-5-carboxamide) is a novel $\alpha 7$ -selective agonist ($K_i = 8.8$ nM; $EC_{50} = 65$ nM) with negligible activity at other nAChRs and weaker activity at 5-HT₃ receptors ($K_i = 500$ nM).²⁰⁸ PHA 543613 displayed rapid brain penetration and was effective in behavioral tasks (0.3–1.0 mg/kg, rat)²⁰⁸ and provoked neurochemical changes *in vivo* (0.3–1.0 mg/kg, mice).²⁰⁹

PHA 568487

An $\alpha 7$ nAChR-selective agonist

PHA 568487 is a quinuclidine $\alpha 7$ -selective agonist, designed to overcome limitations in *in vivo* tolerability of previous structures.²¹⁰

PNU 282987

A potent $\alpha 7$ nAChR-selective agonist

PNU 282987 (N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide) is a potent and selective $\alpha 7$ nAChR agonist ($K_i = 27$ nM; $EC_{50} = 150$ nM), with weak activity at 5-HT₃ receptors ($K_i = 0.9$ μ M).^{211,212} It has been widely used to target $\alpha 7$ nAChRs. It is reported to be effective *in vivo* at 1–30 mg/kg i.p.,^{213,214} 1 mg/kg i.v.²¹² or 10–40 nM injected locally into the brain.²¹⁵

3-pyr-Cytisine

A weak $\alpha 4\beta 2$ nAChR-selective partial agonist

3-pyr-Cytisine (1R,5S)-1,2,3,4,5,6-Hexahydro-9-(3-pyridinyl)-1,5-methano-8H-pyrido[1,2-a][1,5]diazocin-8-one) is the pyridine homolog of 3-bromocytisine (see above).¹⁴³ It is a relatively weak partial agonist at $\alpha 4\beta 2$ nAChRs ($EC_{50} = 30$ μ M) with little effect at $\alpha 3\beta 4$ or $\alpha 7$ nAChRs. 3-pyr-Cytisine produced antidepressant-like effects in mice, when administered at 0.3–0.9 mg/kg i.p.¹⁴³ 3-pyr-Cytisine (0.3 mg/kg) did not affect intracranial self-stimulation thresholds in rats, in contrast to nicotine and varenicline.²¹⁶ However, it was as potent as nicotine as a secretagogue when applied to PC12 cells (1–100 μ M).²¹⁷

RJR 2403 oxalate

An $\alpha 4\beta 2$ nAChR-selective agonist

RJR 2403 (also known as *trans*-metan nicotine or TC-2403) was first generated by opening the pyrrolidine ring of nicotine; it also occurs naturally as a minor tobacco alkaloid. It shows some functional selectivity for $\alpha 4\beta 2$ nAChRs ($K_i = 26$ nM; $EC_{50} =$

730 nM) compared with other nAChR subtypes^{218,219} and has been used to support the presence of presynaptic $\alpha 4\beta 2$ nAChRs on projections to the dorsal raphe nucleus.²²⁰ RJR 2403 is effective *in vivo*, with a profile that recapitulates some, but not all, of nicotine's effects, consistent with its greater nAChR subtype selectivity.²²¹ RJR 2403 also produced antinociception in mice when administered at 10–30 mg/kg²²² or 10–200 mg/kg;²²³ it was at least 10-fold less potent than nicotine in both studies.

RJR 2429 dihydrochloride

An $\alpha 4\beta 2$ nAChR-selective agonist

RJR 2429 ((\pm)-2-(3-pyridinyl)-1-azabicyclo[2.2.2]octane) has an unusual profile.²²⁴ It conforms to the pattern expected of a partial agonist at $\alpha 4\beta 2$ nAChRs, binding with high affinity ($K_i = 1$ nM) while acting as a more effective antagonist than agonist. However, it was more efficacious in eliciting striatal dopamine release ($EC_{50} = 2$ nM), suggesting an enhanced agonist action at more complex $\beta 2^*$ nAChRs. It was also an exceptionally potent agonist at $\alpha 1\beta 1\gamma\delta$ nAChRs ($EC_{50} = 60$ nM), but was relatively weak at activating ganglionic ($\alpha 3\beta 4^*$) nAChRs.

S 24795

A weak $\alpha 7$ nAChR-selective partial agonist

S 24795 (2-[2-(4-bromophenyl)-2-oxoethyl]-1-methylpyridinium iodide) is a weak partial agonist at $\alpha 7$ nAChRs ($EC_{50} = 34$ μ M; efficacy = 14% of ACh responses).²²⁵ It showed a memory-enhancing effect in mice at 0.3–1.0 mg/kg,²²⁶ and facilitated release of A β peptide from $\alpha 7$ nAChRs *in vitro* (10–100 μ M) and *in vivo* (0.3–1.0 mg/kg).^{227,228}

Sazetidine A dihydrochloride

A potent $\alpha 4\beta 2$ nAChR-selective agonist with stoichiometry-dependent efficacy

Sazetidine A, an analog of A-85380 with an acetylene substituent in the 5 position (analogous to 5-iodo-A-85380), was first reported to be a potent desensitizing agent at $\alpha 4\beta 2$ nAChRs.²²⁹ Subsequent studies indicated an agonist action that is highly dependent on subunit stoichiometry, with very limited activation of ($\alpha 4$)₃($\beta 2$)₂ nAChRs (6%) but full agonist activity at ($\alpha 4$)₂($\beta 2$)₃ nAChRs ($EC_{50} = 6$ nM).²³⁰ Sazetidine A also potently activated $\alpha 4\alpha 6\beta 2\beta 3$ nAChRs ($EC_{50} = 19–44$ nM), and was 5–10 times less potent, and less efficacious, at activating $\alpha 6\beta 2\beta 3$ nAChRs ($EC_{50} = 0.15$ μ M).¹⁰² It has much weaker affinity for $\alpha 3\beta 4$ and $\alpha 7$ nAChRs.²³¹ Sazetidine A (3 mg/kg s.c.) reduced ethanol and nicotine self-administration in rats,²³² and ameliorated nicotine withdrawal-induced behavior when given systemically (0.01–0.1 mg/kg i.p.) or by local infusion into the hippocampus (10 pM–1 nM),²³³ raising interest in it as a lead compound for combating drug addiction.

SEN 12333

An $\alpha 7$ nAChR-selective agonist

SEN 12333 (N-[4-(3-pyridinyl)phenyl]-4-morpholinopentanamide), also known as WAY 317538, is an $\alpha 7$ nAChR-selective full agonist ($K_i = 260$ nM; $EC_{50} = 1.6$ μ M).²³⁴ While devoid of functional potency at other targets, inhibition of heteromeric nAChRs and other receptors at low micromolar concentrations was reported. SEN 12333 (1–10 mg/kg) was effective *in vivo* in a number of tests within a narrow therapeutic window, interpreted as reflecting an inverted 'U' dose-response relationship.^{234,235}

TC 1698 dihydrochloride

An $\alpha 7$ nAChR-selective agonist

TC 1698 (2-(3-Pyridinyl)-1-azabicyclo[3.2.2]nonane dihydrochloride) is a potent and efficacious agonist at $\alpha 7$ nAChRs ($EC_{50} = 440$ nM), a partial agonist at muscle nAChRs ($EC_{50} = 20$ μ M), and a competitive antagonist at $\alpha 4\beta 2$ nAChRs ($IC_{50} = 0.3$ μ M versus 30 μ M ACh).²³⁶ TC 1698 (10 μ M) produced α -Bgt-sensitive changes in the JAK2 survival pathway *in vitro*.

TC 2559 difumarate

An $\alpha 4\beta 2$ nAChR-selective agonist

TC 2559 ((E)-N-Methyl-4-[3-(5-ethoxy-pyridinyl)]-3-buten-1-amine), the 5-ethoxy derivative of RJR 2403, is comparable with nicotine in eliciting striatal dopamine release ($EC_{50} \sim 0.2$ μ M) but lacks activity at muscle or ganglionic nAChRs,²³⁷ suggesting a selectivity for $\alpha 4\beta 2^*$ nAChRs. This preference was confirmed using recombinant nAChRs expressed in cell lines.²³⁸ In contrast to most other agonists, TC 2559 displayed high efficacy at low sensitivity ($\alpha 4$)₃($\beta 2$)₂ nAChRs.¹³⁸ TC 2559 (0.3–10 mg/kg) generalized to the nicotine discriminative stimulus¹⁴⁸ and when administered at 0.6–10 μ mol/kg it showed similar efficacy to nicotine in amelioration of scopolamine-impaired memory, but differed from nicotine in other *in vivo* measures.²³⁷

Tropisetron

A potent $\alpha 7$ nAChR-selective agonist and 5-HT₃ receptor antagonist

Tropisetron, a 5-HT₃ receptor antagonist,²³⁹ is a potent partial agonist at $\alpha 7$ nAChRs ($EC_{50} = 1$ μ M).¹⁸¹ It is a potent antagonist of $\alpha 9\alpha 10$ nAChRs ($IC_{50} = 70$ nM),²⁴⁰ with higher concentrations inhibiting $\alpha 3\beta 4$ nAChRs.¹⁸¹ MLA-sensitive central or behavioral effects indicate that tropisetron (1–3 mg/kg) can activate $\alpha 7$ nAChRs *in vivo*.^{241,242}

UB 165 difumarate

A potent partial agonist at $\alpha 4\beta 2$ nAChRs

UB 165 comprises the azabicyclononene bicycle of anatoxin A and the chloropyridyl moiety of epibatidine and exhibits intermediate potency, with stereoselectivity comparable to that of anatoxin A. Therefore UB 165 is a potent agonist at $\alpha 4\beta 2^*$ nAChRs ($K_i \sim 0.3$ nM; $EC_{50} = 50$ nM) but in contrast to the parent molecules, it is a partial agonist at this subtype.^{243,244}

Varenicline tartrate

A potent $\beta 2^*$ nAChR-selective partial agonist

Varenicline (Chantix™ (USA); Champix™ (EU)) is a cytosine congener developed to exploit the $\alpha 4\beta 2^*$ nAChR selectivity and partial agonism of the parent compound, as an aid to smoking cessation.¹⁴¹ It is a partial agonist at $\alpha 4\beta 2$ nAChRs ($EC_{50} \sim 2$ μ M; 13% efficacy relative to ACh)^{245,246} and $\alpha 6\beta 2\beta 3^*$ nAChRs ($EC_{50} = 0.1$ – 0.2 μ M; 6–27%).^{40,247} It also activates $\alpha 3\beta 4$ nAChRs with lower potency and greater efficacy ($EC_{50} \sim 50$ μ M; 75%) and is a relatively potent (possibly dependent on species), full agonist at $\alpha 7$ nAChRs ($EC_{50} = 0.8$ – 18 μ M).^{245,246} Genetic mouse models have been exploited to show that $\alpha 4^*$ nAChRs are necessary and sufficient for varenicline-induced reduction of alcohol consumption.²⁴⁸ Varenicline is effective *in vivo* at doses of 0.01–6.0 mg/kg (given s.c. or p.o.)^{249,250} and has a half-life of 4 and 17 hours in rats and humans respectively, with little metabolism.²⁵¹

Antagonists

As already mentioned, there are rather few nAChR antagonists. In addition to their use as tools for defining nAChR responses and mechanisms, *in vitro* and *in vivo*, antagonists have also been employed to generate animal models of impaired nicotinic function, reviewed by Roegge *et al.*²⁵² Most have a long pedigree, many emanating from natural products, while some newer synthetic molecules are starting to arise from SAR (structure-activity relationship) programs. Increasing awareness of the subunit complexity of native nAChRs emphasizes the need for nAChR subtype-selective antagonists. However, that very complexity, including differences in stoichiometry and subtle species differences, makes the identification and rigorous characterization of subtype-selective ligands challenging.

Competitive antagonists (Table 3)

By interacting with the nAChR at, or close to, the agonist binding site, the inhibition achieved by competitive antagonists can, by definition, be overcome by increasing the agonist concentration. Hence competitive antagonism is referred to as 'surmountable', shifting the concentration response relationship for the agonist to the right (e.g. (+)-tubocurarine block of ACh-evoked currents in *Xenopus oocytes*;²⁵³ dihydro- β -erythroidine (DH β E) versus nicotine-evoked [³H]-dopamine release).¹⁸⁶ Consequently the degree of functional blockade achieved by a given concentration of competitive antagonist will be influenced by the experimental conditions, notably the agonist concentration.

Benzoquinonium dibromide

A classical neuromuscular blocking agent

Benzoquinonium was developed as a muscle relaxant for surgical anesthesia in the 1950s.²⁵⁴ It was employed in early studies of muscle nAChRs.²⁵⁵ Benzoquinonium is not selective, inhibiting muscle and ganglionic nAChRs with comparable potency ($IC_{50} = 6$ μ M)²⁵⁶ and it is also used as an effective nicotinic antagonist in invertebrate preparations.^{257,258} Benzoquinonium is also a weak allosteric potentiator of muscle and neuronal nAChR subtypes at 0.1–10 μ M, via a site distinct from the agonist binding site that is shared by galanthamine and other novel ligands.²⁵⁹

bPiDDB

Centrally active compound with a profile consistent with selective and potent blockade of $\alpha 6\beta 2^*$ nAChRs; weaker action at putative $\alpha 3\beta 4^*$ nAChRs

bPiDDB (1,1'-(1,12-Dodecanediyl)bis[3-methylpyridinium] dibromide) is a synthetic *N-n*-alkylnicotinium compound that potently blocked around 60% of nicotine-induced dopamine release from striatal slices ($IC_{50} = 2$ nM) in a competitive manner, without competing for radioligand binding to $\alpha 4\beta 2$ or $\alpha 7$ nAChRs, which was interpreted as evidence of specificity for $\alpha 6\beta 2^*$ nAChRs.^{260,261} bPiDDB was effective in attenuating nicotine-evoked dopamine release *in vivo* in the rat following systemic (1–3 mg/kg s.c) or local administration (0.1–10 μ M by reverse dialysis).²⁶² It also blocked nicotine-evoked noradrenaline release from rat hippocampal slices (putative $\alpha 3\beta 4^*$ nAChRs)¹⁹⁴ in a non-competitive manner ($IC_{50} = 430$ nM).⁴⁴⁶ This may reflect the sensitivity of $\beta 4^*$ nAChRs to bPiDDB, reported in a study of recombinant nAChR subtypes expressed in *Xenopus oocytes*, which revealed a non-competitive mode of action.²⁶³ bPiDDB did

not block nicotine-evoked conditioned responding.²⁷⁸ Originally envisaged as a smoking cessation agent, toxicity of bPiDDB has led to the development of new analogs.²⁶⁴

α-Bungarotoxin (*α*-Bgt)

A very potent, pseudo-irreversible antagonist of $\alpha 1\beta 1\gamma/\epsilon\delta$, $\alpha 7$ - $\alpha 9^*$ and some invertebrate nAChRs

A polypeptide snake toxin isolated from the venom of the Taiwanese banded krait, *Bungarus multicinctus*, α -Bgt was instrumental in the characterization and purification of muscle nAChRs.¹ This 8 kDa 'three finger' peptide is the most potent of the 'long' α -neurotoxins at muscle and *Torpedo* nAChRs. It binds to $\alpha 1\beta 1\gamma/\epsilon\delta$, $\alpha 7$ - $\alpha 9^*$ and some invertebrate nAChRs with high affinity ($K_i \sim 1$ nM).^{124,176,280} Nuclear magnetic resonance spectroscopy (NMR), X-ray crystallography and modeling studies identified loop-C of the agonist binding site as the major locus of interaction, with fingers I and II of α -Bgt enveloping loop-C, which adopts an open or extended conformation. α -Bgt forms cation- π interactions with essential Tyr 190 within loop-C of muscle/*Torpedo* $\alpha 1$ subunit^{265,266} and the equivalent Tyr 184 of the $\alpha 7$ nAChR.²⁶⁷

The high affinity binding shown by α -Bgt is a consequence of very slow association and dissociation kinetics, with implications for its practical application. Typically a pre-incubation of up to one hour with a low nanomolar concentration (10 nM) of toxin is necessary to achieve a complete blockade. This is commonly circumvented by increasing the concentration 10 or 100-fold and decreasing the preincubation time. This strategy is possible because, even at micromolar concentrations, α -Bgt does not appear to interact with other nAChR subtypes (α/β heteromeric neuronal nAChRs). The very slow dissociation kinetics (especially at muscle nAChRs) mean that functional blockade is not readily reversed by washout,²⁶⁸ which may be advantageous or problematic. It does not access the brain if given peripherally *in vivo* but has been effective if administered by the intracerebroventricular route (i.c.v.; 1.25 nM).²⁶⁹

Dihydro- β -erythroidine (DH β E)

A non- $\alpha 7$ nAChR antagonist with a preference for $\beta 2$ -containing subtypes

An alkaloid originating from *Erythrina* seeds, DH β E is a purely competitive antagonist of neuronal nAChRs. The crystal structure of DH β E bound to the AChBP suggests a novel mode of interaction with loop-C of the agonist binding site.²⁷⁰ Sub-micromolar concentrations of DH β E block recombinant $\alpha 4\beta 2$ nAChRs but it is 10–50 fold less potent at other subtypes, including $\alpha 3\beta 4$, $\alpha 7$ and $\alpha 9$ nAChRs.^{124,263,271} A similar difference in sensitivity is shown by native $\beta 2^*$ and $\beta 4^*$ nAChRs.¹⁸⁶ $\alpha 6$ -containing nAChRs have been difficult to express in heterologous systems but using chimeric constructs, $\alpha 6\beta 2^*$ nAChRs appear to be about 10-fold less sensitive to DH β E than $\alpha 4\beta 2$ nAChRs,^{263,272} a result compatible with inferences from native systems.²⁷³ DH β E is typically employed at a concentration of 1–10 μ M to selectively block $\beta 2^*$ nAChRs *in vitro*. DH β E is also effective *in vivo*, typically administered at concentrations of 2–4 mg/kg s.c. in both rats^{274,275} and mice.^{276,277} Both higher and lower doses have also been reported, e.g. doses up to 10 mg/kg were employed to

block nicotine's conditioned stimulus effects in rats,²⁷⁸ while DH β E was used at 1 mg/kg to explore the roles of $\beta 2^*$ nAChRs in motor function, in mice bearing hypersensitive Leu9'Ala mutations in the $\alpha 4$ subunit.²⁷³

Methyllycaconitine citrate (MLA)

A reversible $\alpha 7$ nAChR antagonist

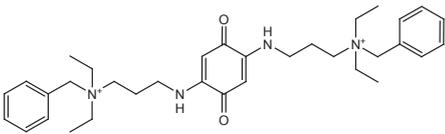
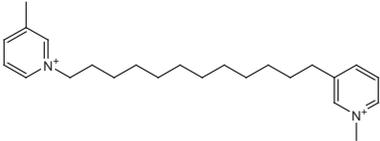
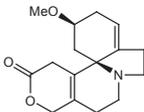
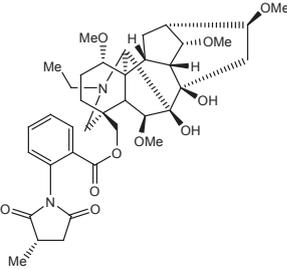
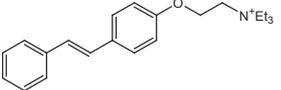
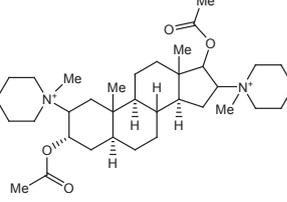
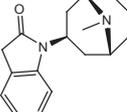
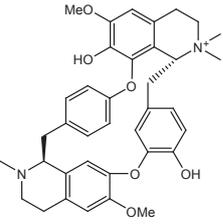
MLA is a norditerpenoid alkaloid produced by *Delphinium* sp. It is a potent competitive antagonist, selective for $\alpha 7$ nAChRs.^{279,470} MLA binds to $\alpha 7$ nAChRs with a K_i of approximately 1 nM, and inhibition of $\alpha 7$ nAChRs by MLA is rapid and reversible, making it a useful alternative to α -Bgt. Like α -Bgt, MLA also potently blocks $\alpha 9$ and $\alpha 9\alpha 10$ nAChRs and some invertebrate nAChRs with low nanomolar affinity,^{124,280} and potently binds to the AChBP.¹⁷ Crystallization of MLA bound to the AChBP shows that it binds at the subunit interface forming the agonist binding site without closing loop-C.¹⁷ Unlike α -Bgt, MLA discriminates between $\alpha 7$ and muscle nAChRs, and is 3 orders of magnitude less potent at blocking the latter.²⁷⁹ It is a weak antagonist of $\alpha 4\beta 2$ nAChRs ($IC_{50} \sim 0.2$ μ M), and may also bind non-competitively at the non-canonical $\alpha 4$ - $\alpha 4$ interface of $(\alpha 4)_3(\beta 2)_2$ nAChRs.⁴⁷⁰ Of more practical concern for brain studies is MLA's ability to inhibit $\alpha 6\beta 2^*$ nAChRs with only ~30-fold lower affinity ($K_i \sim 30$ nM) than at $\alpha 7$ nAChRs.^{272,281} Therefore, this antagonist is selective, rather than specific, for $\alpha 7$ over other nAChR subtypes. This can be problematic for *in vivo* studies where the local concentration of MLA is not known, especially in areas of catecholaminergic cell bodies or innervation which exhibit high levels of $\alpha 6$ expression. MLA accesses the brain following systemic injection (4–10 mg/kg s.c. in rodents)^{277,282} but brain uptake may be diminished after chronic nicotine treatment.²⁸³ MLA is effective by i.c.v. administration (10 μ g, rat).²⁸⁴

MG 624

A putative $\alpha 7$ nAChR antagonist but has other actions, especially in mammalian systems

The 4-oxystilbene derivative MG 624 (*N,N,N*-triethyl-2-[4-(2-phenylethenyl)phenoxy]ethanaminium iodide) is a selective and potent antagonist of recombinant chicken $\alpha 7$ nAChRs ($IC_{50} = 100$ nM). It is ~30 fold less potent at chicken muscle-type or $\alpha 4\beta 2$ nAChRs.^{285,286} MG 624 (100 nM) effectively blocked nicotine-induced airway contractions in murine trachea²⁸⁷ and when applied at 20 μ M, inhibited nicotine-induced angiogenesis in various *in vitro* preparations from different species.²⁸⁸ The latter effect has been ascribed to specific blockade of $\alpha 7$ nAChRs. However, the nAChR subtype specificity of MG 624 in mammalian species is less well established. MG 624 potently attenuated nicotine-evoked [³H]-dopamine and [³H]-noradrenaline release in rat hippocampal slices ($IC_{50} \sim 0.5$ μ M), in contrast to the $\alpha 7$ nAChR-selective antagonists α -conotoxin lml, α -Bgt and MLA.²⁸⁹ Moreover, although MG 624 rather weakly inhibited the binding of a putative $\alpha 7$ nAChR-selective radioligand, [³H]-CHIBA-1001, to rat brain membranes ($K_i = 1.6$ μ M), nicotine, α -Bgt and MLA failed to displace [³H]-CHIBA-1001 binding.²⁹⁰ The specificity of [³H]-CHIBA-1001 is highly questionable and the ability of MG 624 to interact with this site confounds, rather than confirms, the $\alpha 7$ nAChR-credentials of this antagonist.

Table 3 | Competitive nAChR antagonists

Agonist ^a	Structure	Comment/Selectivity ^a	Effective Concentration Range ^b	
			<i>In vitro</i>	<i>In vivo</i>
Benzoquinonium (0424)		A classical neuromuscular blocking agent; broad specificity	1–5 µg/ml (10–50 µM)	15–75 µM (limpet)
bPiDDB (3221)		$\alpha 6\beta 2^* > \alpha 3\beta 4^*$?	10–100 nM	1–3 mg/kg s.c.
α-Bungarotoxin (2133)		Pseudo-irreversible antagonist of muscle, $\alpha 7$, $\alpha 8$, $\alpha 9^*$ and some invertebrate nAChRs; requires lengthy preincubation	1–100 nM	
Dihydro-β-erythroidine (2349)		$\beta 2^* \gg \beta 4^*$, $\alpha 7$, $\alpha 8$, $\alpha 9^*$	1–10 µM	2–4 mg/kg s.c.
Methyllycaconitine (1029)		A potent competitive antagonist with selectivity for homomeric nAChRs. $\alpha 7$, $\alpha 8$, $\alpha 9^* > \alpha 6^* > \alpha 3^* > \alpha 4^*$, muscle	10–100 nM	4–10 mg/kg s.c.
MG 624 (1356)		Putative $\alpha 7$ -selectivity for chicken recombinant nAChRs; specificity for mammalian nAChRs uncertain	0.1–20 µM	n.d.
Pancuronium (0693)		Muscle $\gg \gg$ neuronal nAChRs	1–10 nM	
SR 16584 (4424)		$\alpha 3\beta 4 \gg \alpha 4\beta 2$, $\alpha 7$ (recombinant nAChRs)	0.5–10 µM	
(+)-Tubocurarine (2820)		Potent blocker of muscle nAChRs at nM concentrations; antagonist of neuronal nAChRs at µM concentrations; also blocks 5-HT ₃ > GABA _A receptors	10–30 µM (neuronal nAChRs)	

(**Bold** text denotes compounds available from Tocris at time of publication)

^a See text for details and references

^b Range of concentrations typically used to achieve substantial functional blockade of main target nAChR subtype in rodents (unless otherwise stated); *in vivo* data not given for neuromuscular blocking agents due to peripheral toxicity

* indicates the possible inclusion of additional unspecified subunits

n.d. = Not determined s.c. = Subcutaneous delivery

Pancuronium bromide

A potent, selective, non-depolarizing antagonist of muscle nAChRs

Pancuronium is a steroidal neuromuscular blocking agent²⁹¹ used clinically for reversible neuromuscular blockade in anesthesia and intensive care. Pancuronium is about 10-fold more potent than (+)-tubocurarine at muscle nAChRs.²⁹² IC₅₀ values for inhibition of recombinant immature/extrajunctional muscle (α1)₂β1γδ nAChRs and adult postsynaptic muscle (α1)₂β1εδ nAChRs are in the low nanomolar range,^{293,294} with (α1)₂β1γδ nAChRs showing slightly greater sensitivity to pancuronium than their mature counterparts.²⁹⁵ In contrast to (+)-tubocurarine, pancuronium has a preference for binding to the αδ interface over the αε site.²⁹⁶ Sensitivity of neuronal nAChRs to pancuronium is 3 orders of magnitude lower than that of muscle nAChRs, with IC₅₀ values in the micromolar range.¹²³

SR 16584

A novel ligand showing selective antagonism at recombinant α3β4 nAChRs

SR 16584 (1,3-Dihydro-1-(3-exo)-9-methyl-9-azabicyclo[3.3.1]non-3-yl]-2H-indol-2-one) was identified through a SAR screen.²⁹⁷ In ligand binding assays, SR 16584 displaces [³H]-epibatidine binding to rat α3β4 nAChRs expressed in HEK cells with a K_i of 500 nM, but does not displace radioligand binding to α4β2 or α7 nAChRs, indicative of at least 200-fold selectivity for α3β4 nAChRs. This selectivity for α3β4 over α4β2 nAChRs is attributed to the larger size of the bicyclic azabicyclononane ring, compared with that of epibatidine, together with the introduction of the hydroindolinone ring. These features also confer an antagonist profile, demonstrated by the inhibition of epibatidine-induced Ca²⁺ influx in HEK cells transfected with α3β4 nAChRs (IC₅₀ =

10 μM), with no evidence of α3β4 nAChR activation by SR 16584 alone.²⁹⁷ Although the inhibition of [³H]-epibatidine binding implies a competitive interaction with the agonist binding site, saturation binding experiments suggested that SR 16584 provokes a change in K_d as well as B_{max}, interpreted as evidence of a degree of non-competitive binding.²⁹⁷ It is not known if the inhibition of Ca²⁺ influx was surmountable at increasing epibatidine concentrations. This promising compound merits more studies (including its activity at α3β2 nAChRs) to provide a better defined pharmacological profile.

(+)-Tubocurarine chloride (TC)

Classical non-selective nAChR antagonist

A product of the South American shrub *Chondodendron tomentosum*, TC has an intriguing history since its early use as an arrow poison by South American natives.²⁹⁸ It contributed to classical studies of muscle nAChRs, including the realization that the two non-identical agonist binding sites can be distinguished pharmacologically: TC binds with higher affinity to the α-ε interface than to the α-δ interface of adult mouse nAChRs.^{12,299} Low nanomolar concentrations of TC block muscle nAChRs (IC₅₀ = 18 nM) whereas it inhibits neuronal nAChRs at micromolar concentrations, with IC₅₀ values between 1 and 20 μM.¹²³ While blockade of α4β2 nAChRs is clearly competitive,^{123,253} a non-competitive mode of action has been inferred at α7 or α3β4 nAChRs.^{123,300} TC also binds to the AChBP with high affinity.³⁰¹ Despite its rigid structure, TC adopted at least 3 different binding orientations at the inter-subunit binding site. This versatility may account for the ability of TC to interact with other cys-loop receptors. TC is a potent antagonist of 5-HT₃ receptors, binding with a K_i of approximately 0.1 μM, with a concentration of

Table 4 | α-Conotoxins

α-Conotoxin ^a	Structure	Selectivity ^a
ACV 1 (Vc1.1)	Gly-Cys-Cys-Ser-Asp-Pro-Arg-Cys-Asn-Tyr- Asp-His-Pro-Glu-Ile-Cys-NH ₂	α9α10 nAChRs, plus actions at other nAChRs and other targets
AulB (3120)	Gly-Cys-Cys-Ser-Tyr-Pro-Pro-Cys-Phe-Ala- Thr-Asn-Pro-Asp-Cys-NH ₂	α3β4 > α7 nAChRs
EI (3124)	Arg-Asp-Hyp-Cys-Cys-Tyr-His-Pro-Thr-Cys- Asn-Met-Ser-Asn-Pro-Gln-Ile-Cys-NH ₂	α1β1δγ, α3β4 > α4β2 nAChRs
lml	Gly-Cys-Cys-Ser-Asp-Pro-Arg-Cys-Ala-Trp- Arg-Cys-NH ₂	Moderate potency at α7, α9 nAChRs (rat); species differences
MII (1340)	Gly-Cys-Cys-Ser-Asn-Pro-Val-Cys-His-Leu- Glu-His-Ser-Asn-Leu-Cys-NH ₂	α3β2, α6-containing nAChRs
PIA (3121)	Arg-Asp-Pro-Cys-Cys-Ser-Asn-Pro-Val-Cys- Thr-Val-His-Asn-Pro-Gln-Ile-Cys-NH ₂	α6β2* > α6β4 > α3β2 > α3β4 nAChRs
PnIA (3123)	Gly-Cys-Cys-Ser-Leu-Pro-Pro-Cys-Ala-Ala- Asn-Asn-Pro-Asp-Tyr-Cys-NH ₂	α3β2 > α7 nAChRs

(**Bold** text denotes compounds available from Tocris at time of publication)

^a See text for details and references

* Indicates the possible inclusion of additional unspecified subunits

1–10 μM inhibiting 5-HT₃ receptor-mediated currents in hippocampal interneurons and transfected HEK-293 cells.^{302–304} Higher concentrations of TC also block GABA_A receptor function.^{305,306}

α -Conotoxins (Table 4)

The α -conotoxins are highly selective, often very potent, typically competitive, peptide antagonists of nAChRs that are produced by marine cone snails.³⁰⁷ The venom from such organisms contains a cocktail of toxins to immobilize and kill their prey and predators. Careful analysis of the individual peptides has identified some discriminating research tools. The α -conotoxins are 12–19 amino acids in length and all share a conserved disulfide bonding pattern imposed by 4 conserved cysteine residues. Some α -conotoxins display a degree of selectivity for particular nAChR subtypes that has not been realized by other antagonists, making them therapeutic drug leads.³⁰⁸ In addition to their deployment as selective antagonists they are also useful molecular probes for interrogating nAChR structure and function.

There are a few caveats associated with their experimental use as antagonists. Their exquisite selectivity can make extrapolation between species problematic, α -conotoxin Iml being a prime example (see below). Synthetic peptides can differ from naturally produced peptides that may undergo post-translational modification, which could modify their characteristics (e.g. α -conotoxin PnIA is sulfated).³⁰⁷ As peptides, conotoxins are prone to stick to plastic surfaces etc., and BSA (0.1–1 mg/ml) is commonly added to counter this problem.^{263,309} Their peptidic nature precludes the usual routes of systemic administration *in vivo*.

ACV 1 (α -conotoxin Vc1.1) (Table 5)

A potent antagonist of $\alpha 9\alpha 10$ nAChRs, with actions at other targets

ACV 1 was identified in venom duct mRNA from *Conus victoriae*.³¹⁰ It was initially shown to be a competitive inhibitor of nicotine-stimulated catecholamine release and nicotine-evoked currents in chromaffin cells ($\text{IC}_{50} = 1\text{--}3 \mu\text{M}$), indicative of an interaction with $\alpha 3\beta 4^*$ nAChRs.^{310,311} It also blocked $\alpha 6$ -containing nAChRs at sub- μM concentrations. It was effective in models of neuropathic pain,^{309,312} attributed to its potent antagonism of $\alpha 9\alpha 10$ nAChRs³¹³ (Table 5a). It is regarded as a useful lead compound for this clinical condition.³¹³ However $\alpha 9\alpha 10$ nAChRs have been challenged as the therapeutic target; instead, an agonist action at GABA_B receptors modulating Ca²⁺ channels has been proposed^{314–316} and questioned.¹¹³ The jury is still out.³¹³ ACV 1 has been administered *in vivo* by intrathecal (0.2 – 2 nM, rat)³¹⁷ and intramuscular routes (0.36 – 36 μg , rat).^{313,318}

α -Conotoxin AulB

A modest antagonist of $\alpha 3\beta 4$ nAChRs, with weaker activity at $\alpha 7$ nAChRs

α -Conotoxin AulB was initially purified from the venom of *Conus aulicus*.³⁴⁶ It was found to be a selective antagonist of $\alpha 3\beta 4$ nAChRs ($\text{IC}_{50} = 0.75 \mu\text{M}$) with little effect on other heteromeric nAChRs tested, as it does not block $\alpha 9\alpha 10$ nAChRs³¹⁸ but does display some activity at $\alpha 7$ nAChRs (34% block at 3 μM).³⁴⁶ Its specificity for $\alpha 3\beta 4^*$ nAChRs has been exploited in the characterization of nAChR subtypes modulating transmitter release and ionic responses *in vitro* (typically used at

5–10 μM).^{347–349} α -conotoxin AulB has also been administered into the brains of rats *in vivo*, by local microinjection (2.5–25 pM);^{350,351} the i.c.v. route of administration was employed in mice (3.5–14 pM).²⁷⁷ Intramuscular (0.36–36 μg) or intrathecal injections (0.02–2 nM) were reported to be effective in reversing signs of neuropathic pain,^{317,318} although a partial inhibition of voltage operated Ca²⁺ channels (as reported for ACV 1) was also observed.³¹⁸

α -Conotoxin EI

A structurally interesting α -conotoxin that preferentially blocks $\alpha 1\beta 1\delta\gamma$ and $\alpha 3\beta 4$ nAChRs

α -Conotoxin EI was purified from the venom of *Conus ermineus*, an Atlantic fish-hunting *Conus*.³¹⁹ α -conotoxin EI competitively antagonizes muscle-type nAChRs. It binds preferentially to the $\alpha 1/\delta$ subunit interface of *Torpedo* nAChRs but is much less discriminating between the two agonist binding sites of mammalian muscle nAChRs. Structurally, α -conotoxin EI resembles α -conotoxins that target neuronal, rather than muscle, nAChRs.³²⁰ Subsequently α -conotoxin EI has been found to block $\alpha 3\beta 4$ nAChRs more effectively than $\alpha 4\beta 2$ nAChRs when applied at 10 μM .³²¹ Potentiating effects following brief application of nanomolar concentrations of α -conotoxin EI were also noted.

α -Conotoxin Iml (Table 5)

A selective antagonist at rodent $\alpha 7$ and $\alpha 9$ nAChRs but potently blocks human $\alpha 3\beta 2$ nAChRs

α -Conotoxin Iml is a 12 amino acid peptide originally isolated from *Conus imperialis*.³²² It was first described as a selective antagonist of rat $\alpha 7$ and $\alpha 9$ nAChRs, with muscle nAChRs requiring more than 2 orders of magnitude higher concentrations for blockade.³²³ However, α -conotoxin Iml appears to show considerable species differences in its selectivity. It is more efficacious at producing neuromuscular blockade in frog preparations,³²² and its propensity to block nicotine-evoked catecholamine secretion in bovine chromaffin cells ($\text{IC}_{50} = 2.5 \mu\text{M}$) has been attributed to inhibition of $\alpha 3\beta 4^*$ nAChRs in this species.³²⁴ When tested on recombinant human nAChRs it proved to be most potent at blocking $\alpha 3\beta 2$ nAChRs³²⁵ (Table 5b).

α -Conotoxin Iml has been exploited to probe binding interactions with $\alpha 7$ nAChRs,^{307,326} and with an AChBP,¹⁷ as well as serving as a template for developing novel $\alpha 7$ nAChR-selective antagonists.³⁰⁸ It has been used to define rodent $\alpha 7$ nAChR responses *in vitro*.^{327,328} *In vivo*, i.c.v. injections (5–10 nM) in rodents produced complex seizures (likely due to blockade of $\alpha 7$ nAChRs in the hippocampus) with a high incidence of death, while i.p. injections were without effect.³²²

α -Conotoxin MII

A potent and selective antagonist of $\alpha 3\beta 2$ and $\alpha 6$ -containing nAChRs

α -conotoxin MII, from *Conus magus*, was initially hailed as being a selective antagonist of $\alpha 3\beta 2$ nAChRs ($\text{IC}_{50} = 0.5 \text{ nM}$)³²⁹ but was subsequently revealed to recognize $\alpha 6$ -containing nAChRs,³³⁰ which are at least as sensitive to inhibition by α -conotoxin MII ($\text{IC}_{50} = 0.4 \text{ nM}$).³³¹ This specificity appears to extend to primate nAChRs.³³² α -conotoxin MII has been widely used *in vitro* to investigate the roles of $\alpha 6\beta 2^*$ nAChRs in modulating nigrostriatal

Table 5 | Relative selectivity and potencies of select conotoxins

a) Selectivity of ACV 1 ^{309,311}								
nAChR Subtype	$\alpha 9\alpha 10$	$\alpha 6/\alpha 3\beta 2\beta 2^a$	$\alpha 6/\alpha 3\beta 4\alpha$	$\alpha 3\beta 4$	$\alpha 3\alpha 5\beta 2$	$\alpha 3\beta 2$	$\alpha 3\alpha 5\beta 4, \alpha 4\beta 2, \alpha 7, \alpha 1\beta 1\delta\gamma$	
IC ₅₀ (nM)	19	140	980	4200	7200	7300	>30,000	
b) Potency of α -Conotoxin Iml at rat ³²³ and human ³²⁵ nAChRs								
Species	Rat				Human			
nAChR Subtype	$\alpha 7$	$\alpha 9$	$\alpha 1\beta 1\delta\gamma$	$\alpha 3\beta 4, \alpha 4\beta 2$	$\alpha 3\beta 2$	$\alpha 7$	$\alpha 3\beta 4$	$\alpha 4\beta 2, \alpha 1\beta 1\delta\epsilon$
IC ₅₀ (μ M)	0.22	1.8	51	No effect	0.04	0.6	3.4	>10
c) Selectivity of α -Conotoxin PIA ³³⁹								
nAChR Subtype	$\alpha 6/\alpha 3\beta 2\alpha$	$\alpha 6/\alpha 3\beta 2\beta 3\alpha$	$\alpha 3\beta 2$	$\alpha 6/\alpha 3\beta 4\alpha$	$\alpha 3\beta 4$	$\alpha 4\beta 2, \alpha 1\beta 1\delta\epsilon$		
IC ₅₀ (nM)	0.69	0.95	74	30	518	>10,000		

^aChimeric $\alpha 3/\alpha 6$ subunits comprised of the N-terminal extracellular domain of $\alpha 6$ and transmembrane domains of $\alpha 3$ subunits, to facilitate heterologous nAChR formation and expression⁴³³

dopamine release in normal and Parkinsonian models (typical concentrations for selective blockade are 10–100 nM).^{101,333,334} Intrathecal administration of α -conotoxin MII (0.02 – 2 nM) was effective in a neuropathic pain model.³¹⁷ It has also been administered locally by injection (0.25–25 μ M),^{335,336} reverse dialysis (1–10 μ M),³³⁷ or delivered i.c.v. (30 nM)³³⁸ to achieve central blockade.

α -Conotoxin PIA (Table 5)

A potent and selective antagonist of $\alpha 6$ -containing nAChRs

α -Conotoxin PIA was cloned from *Conus purpurascens*.³³⁹ It blocked $\alpha 6\beta 2^*$ nAChRs at low nanomolar concentrations and was two orders of magnitude less potent at $\alpha 3\beta 2$ nAChRs. This was the first antagonist reported to distinguish between $\alpha 6$ - and $\alpha 3$ -containing nAChRs. It also preferentially blocks $\beta 2$ - over $\beta 4$ -containing nAChRs; the order of potency is $\alpha 6\beta 2^* > \alpha 6\beta 4 > \alpha 3\beta 2 > \alpha 3\beta 4$ (Table 5c). Recovery from blockade by α -conotoxin PIA was quicker at $\alpha 3$ -containing nAChRs. Recombinant rat and human subtypes were similarly sensitive to α -conotoxin PIA.³³⁹ It is also effective on native nAChRs, partially blocking nicotine-stimulated dopamine release from striatal synaptosomes (IC₅₀ = 1.5 nM),³⁴⁰ consistent with the involvement of $\alpha 6\beta 2^*$ nAChRs. α -Conotoxin PIA (1 nM) was used to define the presence of $\alpha 6$ -containing nAChRs on GABAergic terminals.³⁴¹ *In vivo* perfusion of α -conotoxin PIA into the rat ventral tegmental area by reverse dialysis (10 μ M) attenuated nicotine-evoked increases in dopamine overflow and locomotion.³³⁷ In this study, α -conotoxin PIA was an order of magnitude less potent than α -conotoxin MII, but its greater selectivity for native $\alpha 6\beta 2^*$ nAChRs over $\alpha 3\beta 2^*$ nAChRs was affirmed.

α -Conotoxin PnIA

A potent antagonist of $\alpha 3\beta 2$ nAChRs, with weaker activity at $\alpha 7$ nAChRs

α -Conotoxin PnIA was purified from the venom of *Conus pennaceus*.³⁴² It preferentially inhibits $\alpha 3\beta 2$ nAChRs (IC₅₀ = 9.5 nM) while displaying weaker antagonism of $\alpha 7$ nAChRs (IC₅₀ = 252 nM).³⁴³ It has attracted most interest because the related α -conotoxin PnIB shows the reverse selectivity, prompting structure activity studies.^{307,344,345} α -Conotoxin PnIA was less effective at $\alpha 3\beta 4, \alpha 4\beta 2$ and $\alpha 1\beta 1\delta\gamma$ nAChRs.³⁴³ Its effect on $\alpha 6$ -containing nAChRs does not appear to have been reported.

Non-competitive antagonists (Table 6)

Non-competitive antagonists, by definition, do not compete for binding to the agonist binding sites. Hence they do not displace the binding of conventional agonist or competitive antagonist radioligands, and their inhibition is not surmountable with increasing agonist concentration (e.g. comparison of blockade of ACh-evoked currents by catestatin (non-competitive) and DH β E (competitive) at $\alpha 3\beta 4$ nAChRs).³⁵² Insurmountable blockade has practical advantages for reliable nAChR inhibition. Non-competitive antagonists interact with distinct sites on the nAChR to inhibit receptor function; typically they block the nAChR channel, but a variety of sites and modes of antagonism are encountered.³⁵³

Catestatin

A peptide open channel blocker of $\alpha 3\beta 4$ and other neuronal nAChRs

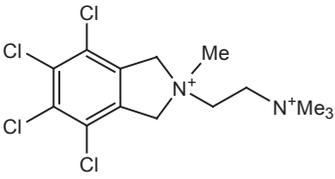
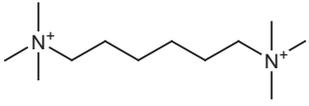
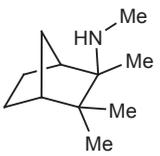
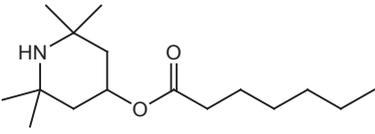
Catestatin, a naturally occurring, 21 amino acid fragment of chromogranin A that is secreted from chromaffin cells, is a potent inhibitor of nicotinic-cholinergic-stimulated catecholamine secretion (IC₅₀ ~0.8 μ M).^{352,354} The peptide confers an antihypertensive protection, while naturally occurring polymorphisms and knockout of catestatin result in varying degrees of hypertension. Catestatin inhibits recombinant rat $\alpha 3\beta 4$ nAChRs expressed in *Xenopus* oocytes in a reversible, non-competitive, voltage-, and use-dependent manner.³⁵² This profile is consistent with open-channel blockade. Docking of the catestatin structure onto a homology model of the $\alpha 3\beta 4$ nAChR indicated major interactions with the extracellular domain of the $\alpha 3$ subunit, at sites distinct from the agonist binding site, such that the peptide occludes the ion channel from the receptor vestibule, consistent with its non-competitive mode of action.³⁵⁶ $\alpha 7, \alpha 3\beta 2$ and $\alpha 4\beta 2$ nAChRs were similarly sensitive to 0.1–10 μ M catestatin.³⁵²

Chlorisondamine diiodide

A ganglionic blocker which produces long-lasting inhibition of central nAChRs

This bisquaternary nicotinic antagonist was originally used as a ganglionic blocker, inhibiting nAChRs that mediate synaptic transmission in sympathetic and parasympathetic ganglia.³⁵⁷ When administered *in vivo* it is unique in producing a persistent blockade of nAChRs within the CNS. Inhibition lasts for weeks or even months, in contrast to a transient ganglionic blockade. For

Table 6 | Non-competitive nAChR antagonists

Antagonist ^a	Structure	Comment/Selectivity ^a	Effective Concentration Range ^b	
			<i>In vitro</i>	<i>In vivo</i>
Catestatin (2722)	Arg-Ser-Met-Arg-Leu-Ser-Phe-Arg-Ala-Arg-Gly-Tyr-Gly-Phe-Arg-Gly-Pro-Gly-Leu-Gln-Leu	A peptide with specificity for $\alpha 3\beta 4$ and other neuronal nAChRs	0.1–10 μM	
Chlorisondamine (1001)		Long-lasting inhibition of CNS nAChRs		10 mg/kg 5 μg , i.c.v.
Hexamethonium (4111)		Ganglionic blocker that does not cross blood brain barrier	1–100 μM	2–10 mg/kg; 12–18 ng, i.c.v.
Mecamylamine (2843)		Centrally active ganglionic blocker/heteromeric nAChRs > $\alpha 7$	1–10 μM	1–3 mg/kg
TMPH (2438)		Slowly reversible inhibitor of heteromeric neuronal nAChRs	1–10 μM	

(**Bold** text denotes compounds available from Tocris at time of publication)

^a See text for details and references

^b Range of concentrations typically used to achieve substantial functional blockade of main target nAChR subtype in rodents (unless otherwise stated)

example, nicotine-evoked [³H]-noradrenaline release was abolished from rat hippocampal synaptosomes prepared 3 weeks after administration of chlorisondamine *in vivo* (10 mg/kg s.c.).³⁵⁸ It is hypothesized that this long lasting inhibition may arise from an intracellular accumulation of the drug, based on the retention of tritium following administration of radiolabeled chlorisondamine.³⁵⁸ Its persistent action is particularly useful for antagonizing brain nAChRs during lengthy behavioral protocols or chronic studies involving repeated or continuous nicotine administration.³⁵⁹ However, it should be noted that systemic administration of centrally effective doses of chlorisondamine (10 mg/kg s.c.) will elicit some transient ganglionic effects due to peripheral blockade. The drug can also be delivered locally into the brain (5 μg , i.c.v.) to interrogate the roles of discrete brain regions in nicotinic responses.¹⁸⁸ The long lasting inhibition allows for recovery from stereotaxic injection (3 days¹⁸⁸) before behavioral testing. The nAChR selectivity of chlorisondamine is unclear.

Hexamethonium bromide

A ganglionic blocker that does not access the brain

Hexamethonium was also first recognized as a ganglionic nAChR blocking agent, and used to treat hypertension, among other conditions.³⁶⁰ It is a voltage-dependent, open channel blocker of ganglionic nAChRs.³⁶¹ The hydrophilic nature of this polymethylene bistrimethylammonium compound limits its ability to cross the blood-brain barrier and access the brain. Hence it is used in comparative *in vivo* studies with a centrally acting antagonist

(typically mecamylamine) to establish if a particular behavior is centrally or peripherally mediated.^{362,363} Typical doses for systemic administration are in the range 2–10 mg/kg.^{364,365} Hexamethonium is relatively non-selective, blocking muscle and diverse neuronal nAChR subunit combinations with an IC_{50} value of ~10–20 μM ,²⁴⁶ and is used at 1–100 μM to block neuronal nAChRs *in vitro*.^{366,367} It is an effective antagonist of central nicotinic effects if delivered directly into the brain.³⁶⁵

Mecamylamine hydrochloride

Widely used antagonist for *in vitro* and *in vivo* studies

Mecamylamine was also developed as a ganglionic blocker for the treatment of hypertension.³⁶⁸ Unlike hexamethonium, this secondary amine readily crosses the blood brain barrier to exert both central and peripheral effects. More recently mecamylamine has been considered as a lead compound for the treatment of neuropsychiatric conditions.^{8,369} It has become the archetypal non-competitive antagonist for neuronal nAChRs. Molecular docking studies suggest that as a result of its predominantly protonated form, mecamylamine is attracted to the channel mouth and interacts with a luminal site, consistent with it being an open channel blocker.³⁷⁰ Mecamylamine inhibits most neuronal nAChRs with IC_{50} values typically in the range 0.1–5 μM , with $\alpha 3\beta 4$ appearing slightly more sensitive than other nAChRs.^{246,271} Ten μM mecamylamine is typically used to achieve a complete block *in vitro*. Mecamylamine's antagonism of $\alpha 7$ nAChRs is readily reversible,³⁷¹ with higher concentrations

needed for effective blockade of this subtype. Mecamylamine (1–10 μM) also antagonizes muscle nAChRs in a non-competitive manner and high concentrations of mecamylamine (100 μM) can transiently inhibit NMDA receptors.^{135,371} Mecamylamine crosses the blood brain barrier freely and has been widely used as a general nicotinic antagonist in behavioral experiments. It is typically administered at 1–3 mg/kg in rodents to block CNS nAChRs *in vivo*, although doses as high as 10 mg/kg have been reported.²⁵² It has also been effective after i.c.v. administration.³⁷²

2,2,6,6-Tetramethylpiperidin-4-yl heptanoate hydrochloride (TMPH)

A potent and slowly reversible inhibitor of heteromeric neuronal nAChRs

TMPH is a synthetic derivative of the parent bis-tetramethylpiperidine compound BTMPS. The latter produces a nearly irreversible non-competitive block of neuronal nAChRs (IC_{50} ~200 nM) by interacting with the channel-forming domain of β subunits, whereas its inhibition of muscle nAChRs is readily reversible.³⁷³ Similarly, low micromolar concentrations of TMPH produced a long-lasting inhibition of heteromeric nAChRs comprised of $\alpha 3$ or $\alpha 4$ with $\beta 2$ or $\beta 4$ subunits (IC_{50} ~100–400 nM), whereas blockade of $\alpha 7$ and muscle nAChRs was readily reversible, allowing the different classes of nAChRs to be distinguished.³⁷⁴ However, incorporation of an additional subunit (either $\alpha 5$, $\alpha 6$ or $\beta 3$) into heteromeric nAChRs resulted in less potent inhibition by TMPH. It was also effective *in vivo* (1–5 mg/kg),^{375,376} inhibiting nicotine-induced analgesia, nicotine discrimination and levamisole-induced seizures. A selective action was implied by the insensitivity of nicotine-induced hypothermia and locomotor effects to TMPH (up to 20 mg/kg).³⁷⁵

Other compounds producing non-competitive inhibition of neuronal nAChRs

Many molecules that have other primary targets also act as non-competitive antagonists of nAChRs. These agents cannot be considered to be specific for nAChRs, but the interactions can be of pharmacological or physiological relevance or they may raise practical concerns. The examples mentioned here are representative of some of the more well-known or topical classes. Compounds in bold typeface are currently available from Tocris.

Antidepressants and antipsychotics

Bupropion was originally developed as an antidepressant but is now marketed as an aid to smoking cessation (Zyban™). Its principal pharmacological target is inhibition of dopamine and noradrenaline transporters, but at low micromolar concentrations it also acts as a non-competitive inhibitor of various neuronal nAChR subtypes.^{377–379} The possibility that this interaction contributes to the efficacy of bupropion as a smoking cessation agent is debated.³⁸⁰ The arguments are complicated by the efficacy of bupropion metabolites and species differences.³⁸¹ A photoreactive derivative of bupropion has been developed to interrogate its sites of interaction, reported to be within the M2 pore-forming domain of the nAChR.³⁸² Low micromolar concentrations of other antidepressants including **fluoxetine**, **sertraline** and **paroxetine** also inhibit nAChRs (IC_{50} = 1–12 μM),^{377,383,384} although it is argued that this interaction is unlikely to contribute to their antidepressant actions.³⁸⁵ Both typical (chlorpromazine,

haloperidol) and atypical antipsychotics (**clozapine**, **quetiapine**)³⁸⁶ and the anti-epileptic drug **lamotrigine**³⁸⁷ also interact with nAChR channels.

Ca²⁺ channel blockers

Inhibitors of L-type voltage-operated Ca²⁺ channels inhibit nAChRs in chromaffin cells or neuroblastoma cell lines, with IC_{50} values in the low micromolar range. These drugs include **verapamil** and **diltiazem**, and the dihydropyridines **nimodipine**, **nifedipine** and **nitrendipine**.^{388–390} The N/P/Q-type calcium channel blocker ω -conotoxin **MV1C** and the N-type blocker ω -conotoxin **GVIA** have also been reported to block nAChRs expressed in *Xenopus* oocytes, with rat $\alpha 3\beta 4$ nAChRs being more susceptible than $\alpha 7$ nAChRs.³⁸⁹ The block of $\alpha 3\beta 4$ nAChRs by ω -conotoxins was shown to be reversible, whereas these inhibitors exert a longer lasting inhibition of voltage-operated calcium channels. This difference has been exploited to discriminate between these two targets.³⁴⁷

NMDA receptor antagonists

(+)-**MK 801 maleate** ((+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine) is an open channel blocker of the NMDA receptor (IC_{50} = 0.4 μM), with no effect on AMPA or kainate receptors.³⁹¹ It is also an open channel blocker of $\alpha 4\beta 2$ (IC_{50} = 15 μM) and $\alpha 7$ nAChRs (IC_{50} = 15 μM),^{392,393} being about 40 times less potent at nAChRs compared with NMDA receptors.³⁹⁴ Other NMDA receptor blockers that also interact with nAChRs, typically in the low micromolar range, include the dissociative anesthetics **phencyclidine** and **ketamine**,^{377,395} and the Alzheimer's drug **memantine**.³⁹⁶ The endogenous tryptophan metabolite **kynurenic acid**, a competitive antagonist of the glycine site of NMDA receptors, has been reported to be a non-competitive antagonist of $\alpha 7$ nAChRs (IC_{50} = 7 μM),³⁹⁸ although this is controversial.^{399,400} In a recent report, kynurenic acid was advocated as a negative modulator of $\alpha 7$ nAChR-mediated enhancement of marijuana's rewarding effects.⁴⁸²

Steroids

Steroids, including corticosterone, progesterone, estradiol and hydrocortisone, inhibit native and recombinant neuronal nAChRs with IC_{50} values ranging from 0.1–10 μM .^{401–403} Fluorescence spectroscopy has identified the membrane lipid – nAChR interface as the site of action of steroids (and also of free fatty acids).⁴⁰⁴ Progesterone modulates $\alpha 5$ nAChR subunit expression, although this probably represents an indirect mechanism.⁴⁰⁵

Strychnine hydrochloride

Strychnine, from the plant *Strychnos nux-vomica*, is a potent competitive antagonist of glycine receptors. It also interacts with other members of the cys-loop family. Strychnine non-competitively blocked muscle $\alpha 1\beta 1\gamma\delta$ nAChRs (IC_{50} = 7 μM)⁴⁰⁶ and heteromeric neuronal nAChRs ('type II' putative $\alpha 4\beta 2^*$ nAChRs; IC_{50} = 38 μM).⁴⁰⁷ In contrast it was a more potent, competitive antagonist of $\alpha 9\alpha 10$ nAChRs (IC_{50} ~20 nM)^{118,124} and $\alpha 7$ nAChRs (IC_{50} = 1 μM).⁴⁰⁷ Like (+)-tubocurarine, it has been shown to interact with multiple sites on the AChBP.³⁰¹

Amyloid β -peptide 1–42 ($\text{A}\beta_{1-42}$)

There are numerous reports that $\text{A}\beta_{1-42}$, the endogenous agent that accumulates in Alzheimer's disease, interacts with nAChRs,

principally the $\alpha 7$ subtype. However, the reported effects of $A\beta_{1-42}$ on nAChR function are very varied and not easily reconciled.⁴⁰⁸ Non-competitive, voltage-independent block of $\alpha 7$ nAChRs in rat hippocampal cultures or *Xenopus* oocytes has been described ($IC_{50} = 7.5 - 90$ nM);^{409,410} heteromeric $\alpha 7\beta 2$ nAChRs in basal forebrain cholinergic neurons are purported to show high sensitivity to blockade by oligomeric $A\beta_{1-42}$.⁵⁴ However, others have failed to observe any antagonism or have reported activation of $\alpha 7$ nAChRs by very low (picomolar) concentrations of $A\beta_{1-42}$.⁴¹¹ Inconsistencies in the literature may be due to variables such as the membrane environment of the nAChR and the physical state of $A\beta_{1-42}$.⁴⁰⁸ There is an emerging view that $\alpha 7$ nAChRs (having a presynaptic, postsynaptic or glial location) may mediate some physiological and/or pathological effects of $A\beta_{1-42}$ at glutamatergic synapses.⁴¹²⁻⁴¹⁵

Positive Allosteric Modulators (Table 7)

Positive allosteric modulators (PAMs)²⁹ interact with sites separate from the agonist binding site, and alter the energy barrier between resting, open and/or desensitized states (Figure 2). They lower the energy barrier from resting to activated (open) nAChR, or raise the barrier from open to desensitized nAChR, resulting in an increased probability of receptor activation and hence a potentiation of agonist action.⁸ PAMs have no discernible agonist activity⁴¹⁶ (although some potentiators, notably galanthamine and physostigmine, can themselves increase the frequency of single channel events).⁴¹⁷ They embrace a wide variety of structures, including the endogenous molecule SLURP-1 that is a PAM at peripheral $\alpha 7$ nAChRs.^{418,419} The modulators are therapeutically attractive as they offer a means of enhancing the nicotinic effects of endogenous agonist (ACh), preserving the spatial and temporal specificity imposed by cholinergic innervation. The emergence of subtype-selective PAMs, notably $\alpha 7$ -selective PAMs, has prompted extensive searches for new molecules with PAM activity, using conventional drug discovery approaches or novel *in silico* methods.⁴²⁰⁻⁴²²

As a result of comparing the properties of several PAMs, two classes of $\alpha 7$ -selective PAMs have been distinguished, termed type I and type II.^{29,416} Type I PAMs lower the energy barrier between resting and open states to enhance agonist responses (sensitivity / efficacy) without altering the response time-course (dictated by the open to desensitized transition of the receptor). NS 1738 is a commonly used representative.^{423,424} Type II PAMs raise the barrier for desensitization, enhancing the agonist response and its duration. PNU 120596 was the first compound in this class to be described.⁸ Type II PAMs are useful research tools for unveiling functional $\alpha 7$ nAChRs that are otherwise undetectable with methods that lack sufficient temporal and functional sensitivity to capture the normally very brief responses.^{425,426}

Interrogation of the sites with which PAMs interact on the nAChR has identified a number of candidates. Type I and II PAMs have been proposed to bind at a common, mutually exclusive site located in an intrasubunit transmembrane cavity.^{31,421,423,427} This interaction is analogous to that proposed for neurosteroids and volatile anesthetics binding to the GABA_A receptor, and might correspond to a conserved modulatory site.⁴²⁸ However, alternative, extracellular sites have been proposed for type I PAMs, notably the M2-M3 loop for genistein and NS 1738.^{416,429}

Homology modeling and mutagenesis has defined an extracellular site on $\alpha 4\beta 2$ and $\alpha 7$ nAChRs for non-selective PAMs represented by galanthamine.^{430,449} while photoaffinity labeling has identified multiple sites.⁴³¹ Moreover, at least 3 small molecule PAM binding sites were described in the extracellular domain of an $\alpha 7$ nAChR model, using an automatic pocket finding program.⁴²² Some modulators (e.g. morantel and zinc) are purported to bind at a non-canonical subunit interface^{30,432} and galanthamine's greater potentiation of $\alpha 5$ -containing nAChRs has invoked comparison with the benzodiazepine site of GABA_A receptors.⁴⁰

Both type I and type II PAMs have been reported to show *in vivo* efficacy.⁴⁵⁷ This has raised concern that chronic administration of a type II PAM (modeling a long term therapeutic application) might have neurotoxic consequences due to prolonged Ca^{2+} influx²⁶⁹ but this has not been supported by subsequent experimental observations.⁴²⁶ However, despite enthusiasm for the PAM concept as a therapeutic approach, no positive allosteric modulators have progressed to clinical development thus far.⁸

A 867744

An $\alpha 7$ -selective type II PAM with inhibitory actions at heteromeric nAChRs

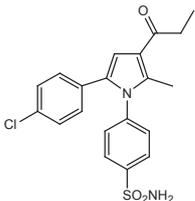
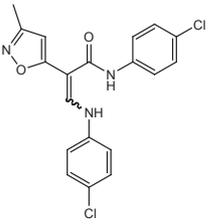
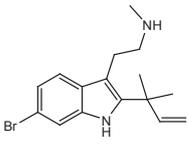
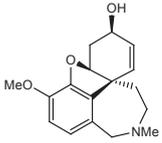
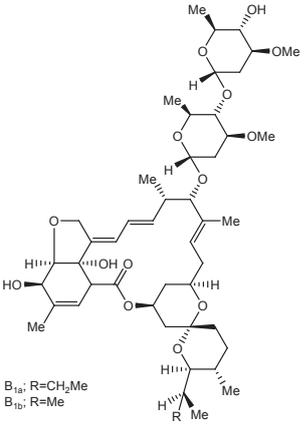
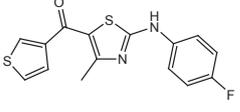
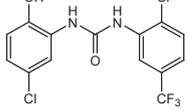
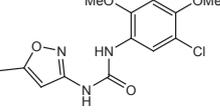
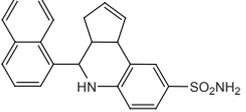
A 867744 (4-[5-(4-chlorophenyl)-2-methyl-3-propionyl-1H-pyrrol-1-yl]benzenesulfonamide), an optimized pyrrole-sulfonamide,⁴³⁴ potentiated ACh-evoked responses in *Xenopus* oocytes expressing $\alpha 7$ nAChRs up to 7.3 fold ($EC_{50} \sim 1$ μ M), without displaying any intrinsic agonist activity itself.⁴³⁵ A 867744 shifted the concentration-response curve for ACh to the left, prevented desensitization in the continued presence of ACh and reactivated desensitized $\alpha 7$ nAChRs. Similar effects were observed on native $\alpha 7$ nAChRs in hippocampal slice preparations. Although A 867744 did not compete for [³H]-MLA binding sites it did differ from other type II PAMs in partially displacing the binding of [³H]-A 585539,⁴³⁵ a novel $\alpha 7$ -selective radioligand.⁴³⁶ It is not clear if the locus of this interaction is responsible for the PAM activity of A 867744. A 867744 does not potentiate responses from $\alpha 4\beta 2$ or $\alpha 3\beta 4$ nAChRs or 5-HT₃ receptors, but low micromolar concentrations inhibited $\alpha 4\beta 2$ and $\alpha 3\beta 4$ nAChRs.⁴³⁵ No cytotoxicity was observed following exposure of cultured cells to A 867744 for up to 3 days.⁴²⁶

CCMI

An $\alpha 7$ -selective type I PAM

First described as Compound 6, CCMI ([N-(4-chlorophenyl)]-alpha-[(4-chlorophenyl)-aminomethylene]-3-methyl-5-isoxazoleacetamide) was identified as an $\alpha 7$ -selective PAM in a screen of GABA_A receptor PAMs.²⁶⁹ It potentiated agonist-evoked currents (from 5% to $\sim 50\%$ of I_{max}) in *Xenopus* oocytes expressing $\alpha 7$ nAChRs ($EC_{50} \sim 0.5$ μ M). Potentiation of GABA_A receptors was weaker and there were no effects on $\alpha 4\beta 2$, $\alpha 3\beta 4$ or $\alpha 1\beta 1\gamma\delta$ nAChRs or 5-HT₃ receptors. The concentration-response curve for ACh was shifted to the left but the rapid desensitization kinetics were unaffected. These characteristics are consistent with a type I PAM. In contrast to type II PAMs, CCMI (1 nM - 3 μ M) produced only modest increases in intracellular Ca^{2+} and no change in ERK phosphorylation, in cells also exposed to an $\alpha 7$ nAChR agonist.⁴²⁶ CCMI was behaviorally effective in rodents at a dose (0.3 mg/kg) that was confirmed to penetrate the brain following i.v. or oral administration in mice.²⁶⁹

Table 7 | Allosteric potentiators

Positive Allosteric Modulator (PAM)	Structure	Comment/Selectivity ^a	Effective Concentration Range ^b	
			<i>In vitro</i>	<i>In vivo</i>
A 867744 (4571)		α 7-selective type II PAM; may inhibit heteromeric nAChRs	5–10 μ M	n.d.
CCMI (3837)		α 7-selective type I PAM; weak GABA _A receptor potentiator	1–3 μ M	0.3 mg/kg
Desformylflustrabromine (3328)		α 4 β 2- and α 2 β 2-selective PAM with type II characteristics; inhibitory at higher concentrations (\geq 10 μ M)	1–5 μ M	3–6 mg/kg
Galanthamine (0686)		Non-selective weak potentiator; inhibitory at higher concentrations (\geq 10 μ M)	1–3 μ M	1–3 mg/kg
Ivermectin (1260)		α 7-selective type I PAM; activates several other Cys-loop receptors	30 μ M	
LY 2087101 (4141)		Weak type I PAM at α 7 nAChRs; more potent potentiator of α 4 β 2 nAChRs	1–10 μ M (α 4 β 2) 10–30 μ M (α 7)	
NS 1738 (2995)		α 7-selective type I PAM; inhibits α 4 β 2 and α 3 β 4 nAChRs at \geq 10 μ M	10 μ M	10–30 mg/kg
PNU 120596 (2498)		α 7-selective type II PAM	2–10 μ M	1–30 mg/kg
TQS (4233)		α 7-selective type II PAM; inhibits other nAChRs at \geq 10 μ M	10 μ M	

(**Bold** text denotes compounds available from Tocris at time of publication)

^a See text for details and references

^b Range of concentrations typically used to achieve substantial functional potentiation of main target nAChR subtype in rodents

Desformylflustrabromine hydrochloride

A putative $\alpha 4\beta 2$ -selective PAM that also potentiates $\alpha 2\beta 2$ nAChRs; inhibitory at higher concentrations

First identified as a natural product in bryozoan *Flustra foliacea* (a marine organism that is a common bryozoan in the North Sea),⁴³⁷ desformylflustrabromine was found to potentiate $\alpha 4\beta 2$ nAChRs by increasing the open probability of the receptor channel.⁴³⁸ The synthetic, water soluble HCl salt has been studied in more detail and displays a complex profile. It potentiates ACh-evoked responses from recombinant $\alpha 4\beta 2$ nAChRs up to 3-fold ($EC_{50} = 120$ nM), shifting the concentration response curve for ACh to the left,⁴³⁹ and has a greater effect on responses to partial agonists.⁴⁴⁰ There is some evidence that it can re-activate desensitized $\alpha 4\beta 2$ nAChRs in the continued presence of agonist,⁴⁴⁰ and desformylflustrabromine HCl (1 μ M) can prevent the inhibition of $\alpha 4\beta 2$ nAChRs by β -amyloid (1 μ M).²¹³ However, at higher concentrations (>10 μ M) desformylflustrabromine HCl inhibits $\alpha 4\beta 2$ nAChRs, and this is likely due to channel blockade. Thus the concentration response curve for desformylflustrabromine HCl is bi-phasic.^{439,441}

The specificity profile of desformylflustrabromine relies on a single study of recombinant nAChRs in *Xenopus* oocytes. The natural compound was reported to be devoid of potentiating activity at $\alpha 3\beta 2$, $\alpha 3\beta 4$, $\alpha 4\beta 4$ and $\alpha 7$ nAChRs when tested at 10 μ M with 100 μ M ACh.⁴³⁸ More recently desformylflustrabromine HCl was shown to potentiate and inhibit $\alpha 2\beta 2$ nAChRs in a similar manner to its interactions with $\alpha 4\beta 2$ nAChRs ($EC_{50} = 446$ nM; $IC_{50} = 11$ μ M).²¹³ The lack of potentiation of $\alpha 7$ nAChRs has been confirmed, with the finding that low micromolar concentrations of desformylflustrabromine HCl inhibit $\alpha 7$ nAChRs ($IC_{50} = 2-44$ μ M).^{439,441}

In vivo, desformylflustrabromine HCl (3, 6 mg/kg s.c.) decreased nicotine self-administration (an $\alpha 4\beta 2^*$ nAChR-mediated response)⁸² without substituting for nicotine itself.⁴⁴² It is not clear if the potentiating or inhibitory activity (or both) contributed to this action. Brain penetration of desformylflustrabromine HCl was suggested by the presence of the compound in CSF for at least 90 minutes after systemic administration; the half-life for desformylflustrabromine HCl in blood was estimated to be 8.6 hours.⁴⁴²

Galanthamine

A weak non-selective potentiator, with greater efficacy at $\alpha 5$ -containing nAChRs

Galanthamine is an acetylcholinesterase inhibitor that was among the first nAChR potentiators to be reported.²⁵⁹ It did not discriminate between major nAChR subtypes and weakly potentiated (by ~30%) nAChR responses evoked by sub-maximal concentrations of nicotinic agonists, shifting the concentration-response curve to the left.^{443,444} Alone, galanthamine can activate single-channel currents in muscle and neuronal cells but the probability of channel opening is too low to generate macroscopic (whole cell) currents.⁴¹⁷ Its locus of action was established as distinct from the agonist binding site.⁴⁴⁵ nAChRs incorporating the $\alpha 5$ subunit show enhanced potentiation (200%) by galanthamine⁴⁰ and this has been exploited to distinguish native heteromeric nAChRs.⁷⁷ Higher concentrations of galanthamine (>1 μ M) inhibit nAChR responses by acting as an open channel blocker.^{40,443}

A number of other compounds also act as weak non-selective potentiators, in a similar manner to galanthamine, including the acetylcholinesterase inhibitor **physostigmine**, the opiate codeine, the neuromuscular blocking agent **benzoquinonium** (Table 3) and the neurotransmitter 5-HT.^{259,417,447,448} Site-directed mutagenesis, electrophysiology and molecular docking has identified Thr 197 as important for the interaction of these diverse ligands with neuronal nAChRs: this residue lies close to both the agonist binding site and the cys-loop, leading to a rationale for its potentiating effect.^{430,449} However, using the intrinsic photoreactivities of galanthamine and physostigmine to label sites of interaction within muscle-type nAChRs, multiple binding sites were identified.⁴³¹ The sensitivity to galanthamine conferred by the $\alpha 5$ subunit⁴⁰ raises further questions about its site and mechanism of potentiation.

Ivermectin

An $\alpha 7$ -selective type I PAM with actions at other cys-loop receptors

Ivermectin is a macrocyclic lactone and an anthelmintic drug. At nanomolar concentrations it activates glutamate-gated chloride channels (cys-loop receptors exclusive to invertebrate species³). At micromolar concentrations ivermectin activates GABA_A and glycine receptors, potentiates $\alpha 7$ nAChRs^{32,450} and has no effect on 5-HT₃ receptors. Ivermectin conforms to the criteria of a type I PAM at $\alpha 7$ nAChRs and potentiates ACh-evoked currents with an EC_{50} value of 6.8 μ M.⁴⁵¹ Its site of action has been localized to an intrasubunit transmembrane cavity.⁴⁵¹ Another anthelmintic, levamisole, targets nAChRs in parasitic nematodes and is a common contaminant of cocaine.⁴⁵² It reputedly shows dual potentiation and inhibition of ACh-evoked responses recorded from *Xenopus* oocytes expressing human $\alpha 3^*$ nAChRs.⁴⁵³ This dual behavior is reminiscent of the modulatory effects of galanthamine and physostigmine described above.

LY 2087101

Relatively non-selective PAM that potentiates $\alpha 4\beta 2$ nAChRs and is a weak type I PAM at $\alpha 7$ nAChRs

LY 2087101, a (2-amino-5-keto)thiazole compound, has the characteristics of a weak type I PAM in its potentiation of peak currents evoked from $\alpha 7$ nAChRs, at concentrations of 3–30 μ M.⁴⁵⁴ It is a more potent potentiator of $\alpha 4\beta 2$ nAChRs ($EC_{50} = 1$ μ M) but without effect on $\alpha 1\beta 1\delta\gamma$, $\alpha 3\beta 2$ or $\alpha 3\beta 4$ heterologously expressed nAChRs. Mutagenesis and computer docking simulations predict that LY 2087101 may bind to the $\alpha 7$ nAChR within a transmembrane intrasubunit cavity that is also a putative site for type II PAMs.³¹

NS 1738

A type I PAM selective for $\alpha 7$ nAChRs; inhibits $\alpha 4\beta 2$ and $\alpha 3\beta 4$ nAChRs

NS 1738 (*N*-(5-Chloro-2-hydroxyphenyl)-*N'*-[2-chloro-5-(trifluoromethyl)phenyl]urea) is a type I PAM at rat $\alpha 7$ nAChRs expressed in *Xenopus* oocytes ($EC_{50} = 3.9$ μ M) or human $\alpha 7$ nAChRs expressed in GH4C1 cells ($EC_{50} = 1.6$ μ M).⁴⁵⁵ Maximal responses to ACh were increased 2–6 fold, with little effect on the potency of ACh or the desensitization kinetics of $\alpha 7$ nAChRs,⁴⁵⁵ and it does not reactivate the desensitized receptor.⁴²³ NS 1738 is a selective type I PAM for $\alpha 7$ nAChRs, giving no potentiation of $\alpha 4\beta 2$, $\alpha 3\beta 4$ or $\alpha 1\beta 1\gamma\delta$ nAChRs, or 5-HT₃ receptors; inhibition of

the heteromeric neuronal nAChRs was observed at concentrations of 10 μ M and above.⁴⁵⁵ In rats, NS 1738 shows modest brain penetration and it improved cognitive function *in vivo* in scopolamine-impaired rats (Morris water maze task), when administered at 10 and 30 mg/kg i.p.⁴⁵⁵ NS 1738 was inferred to interact with the extracellular domain of α 7 nAChRs, including the M2–M3 segment,⁴²⁹ although other studies have proposed that it competes for the same (or overlapping) intrasubunit transmembrane site that binds PNU 120596.⁴²³ Perhaps consistent with their binding to a common site, NS 1738 (30 mg/kg i.p.) partially blocked the antinociceptive effects of PNU 120596 in mice, without having any antinociceptive action itself.⁴²⁴

PNU 120596

Prototypical type II PAM for α 7 nAChRs

First published in 2005,⁴⁵⁶ PNU 120596 (*N*-(5-Chloro-2,4-dimethoxyphenyl)-*N'*-(5-methyl-3-isoxazolyl)-urea) has become the most widely used type II PAM, with over 60 citations to date. PNU 120596 selectively potentiates α 7 nAChRs (EC_{50} = 200 nM).⁴⁵⁶ It increases the magnitude of agonist-evoked responses several fold and extends the timecourse of responses by preventing desensitization; these properties led to the classification and definition of type II PAMs.⁴¹⁶ It has been widely used in a variety of *in vitro* studies to magnify α 7 nAChR responses^{425,458–460} and is predicted to bind at an intrasubunit transmembrane site on the α 7 nAChR.^{31,427} Five PNU binding sites per α 7 homomer are predicted and potentiation is highly co-operative, with occupancy of all 5 sites required for maximum potentiation.⁴⁶¹

PNU 120596 accesses the brain after i.p. administration.⁴²⁴ It is effective *in vivo* in rats against auditory gating deficits (1 mg/kg i.v.),⁴⁵⁶ cognitive dysfunction (2 mg/kg i.p.),⁴⁶² cerebral ischemia (30 mg/kg s.c., 1 mg/kg i.v.)⁴⁶³ and inflammatory hyperalgesia (30 mg/kg i.p.),⁴⁶⁴ and provoked the overflow of dopamine (1 mg/kg i.p.).⁴⁶⁵ In mice PNU 120596 is reported to be antinociceptive (4 and 8 mg/kg i.p.).⁴²⁴ Its sub-chronic administration⁴²⁴ was without adverse effects, and no cytotoxicity was observed *in vitro*.⁴²⁶ Daily injections (30 mg/kg; 7 days) did not alter brain ¹²⁵I- α Bgt binding sites in rats, in contrast to the upregulation produced by repeated agonist administration.⁴⁶⁶ PNU 120596 has some solubility issues. For *in vivo* administration it is typically prepared as a stock solution in 5% DMSO + 5% solutol dissolved in 0.9% sterile saline.²¹³

TQS

An α 7 nAChR-selective type II PAM

TQS (3a,4,5,9b-Tetrahydro-4-(1-naphthalenyl)-3H-cyclopentan[c]quinoline-8-sulfonamide) selectively potentiated α 7 nAChR responses (up to 4-fold increase in peak current; EC_{50} = 3 μ M) and decreased current decay times, consistent with the characteristics of a type II PAM.⁴¹⁶ It is devoid of agonist activity but, in contrast to PNU 120596, TQS inhibits α 4 β 2, α 3 β 4 and α 1 β 1 γ δ nAChRs.^{416,467} Mutagenesis and computer-docking simulations identify a transmembrane binding site close to that proposed for PNU 120596, using the same methodology.⁴²⁸ A related compound, 4-BP-TQS, in which the naphthyl group is replaced by a 4-bromophenyl entity, is a potent, atypical, non-

desensitizing agonist at α 7 nAChRs, in addition to displaying type II PAM activity.^{428,467} 4-BP-TQS does not compete for the orthosteric agonist binding site but interacts with the transmembrane site shared by TQS and PNU 120596.

Future Perspectives for nAChR Research

In the seven years since the previous version of this review there have been substantial developments in our understanding of the molecular structure of nAChRs, including insights into their subunit stoichiometry, assembly and trafficking, as well as an increasing knowledge of their cellular functions and roles in physiological and pathological processes. However, like the proverbial onion, each layer of knowledge reveals further sets of questions. To help tackle these questions, this period has also seen a growth in the number of new nicotinic ligands published, with a welcome increase in the number of nAChR subtype-selective agonists and PAMs.⁸

These synthetic successes are likely to pave the way for more novel nicotinic probes. RuBi-Nicotine⁴⁶⁹ (page 9) is an example: this caged nicotine will have applications for the rapid, localized activation of nAChRs. There is also the prospect of monitoring nAChR behavior via detection mechanisms coupled to subunit proteins, such as Förster resonance energy transfer (FRET)⁴⁷¹ or optochemical technology.⁴⁷² While the use of radioligands for quantifying nAChRs has declined in recent years, there has been a growth in the development of novel radioligands, based on subtype-selective nicotinic ligands, for *in vivo* analysis of nAChRs by positron emission tomography (PET) studies.^{473,474} Antibodies remain problematic for identifying nAChRs, in brain tissue at least,⁴⁷⁵ but peptide toxins offer alternative approaches. Fluorescently-labeled α -Bgt has been a valuable marker for labeling muscle and α 7 nAChRs in cells and tissues,^{476,477} and fluorescent labeling is being extended to the α -conotoxins with some success.⁴⁷⁸ Biotinylated α -Bgt offers additional approaches, for example detection by fluorophore-labeled anti-biotin antibodies (for signal amplification)⁴⁷⁹ or single particle tracking of nAChRs in live cells using streptavidin quantum dots.^{70,479,480} Indeed, α -Bgt binding motifs have been incorporated into α 3 subunits⁴⁸³ and other membrane proteins⁴⁸¹ in order to take advantage of α -Bgt-linked detection agents.

With such technological advances and new chemical entities we can look forward to resolving some of the mysteries that still surround nAChRs and their roles in health and disease.

References

1. Changeux (2012) *J.Biol.Chem.* **287** 40207.
2. Jones and Sattelle (2006) *Invert.Neurosci.* **6** 123.
3. Wolstenholme (2012) *J.Biol.Chem.* **287** 40232.
4. Tasneem et al. (2005) *Genome Biol.* **6** R4.
5. Duret et al. (2011) *Proc.Natl.Acad.Sci.U.S.A.* **108** 12143.
6. Millar and Gotti (2009) *Neuropharmacology* **56** 237.
7. Gündisch and Eibl (2011) *Expert Opin.Ther.Pat.* **21** 1867.
8. Hurst et al. (2013) *Pharmacol.Ther.* **137** 22.
9. Unwin (2013) *Q.Rev.Biophys.* **20** 1.
10. Zuber and Unwin (2013) *Proc.Natl.Acad.Sci.U.S.A.* **110** 10622.
11. Rafferty et al. (1980) *Science* **208** 1454.
12. Karlin (2002) *Nat.Rev.Neurosci.* **3** 102.
13. Sine and Engel (2006) *Nature* **440** 448.
14. Bouzat (2012) *J.Physiol.Paris* **106** 23.
15. Grutter et al. (2004) *Curr.Top.Med.Chem.* **4** 645.
16. Smit et al. (2003) *Ann.N.Y.Acad.Sci.* **998** 81.
17. Hansen et al. (2005) *EMBO J.* **24** 3635.
18. Celie et al. (2005) *J.Biol.Chem.* **280** 26457.
19. Celie et al. (2004) *Neuron* **41** 907.
20. Rucktooa et al. (2009) *Biochem.Pharmacol.* **78** 777.
21. Li et al. (2011) *Nat.Neurosci.* **14** 1253.
22. Nayack and Auerbach (2013) *Proc.Natl.Acad.Sci.U.S.A.* **110** 13654.
23. Taly et al. (2009) *Nat.Rev.Drug Discov.* **8** 733.
24. Changeux et al. (2005) *Science* **308** 1424.
25. Taly et al. (2011) *J.Mol.Graph.Model.* **30** 100.
26. Lape et al. (2008) *Nature* **454** 722.
27. Mantione et al. (2012) *Future Med.Chem.* **4** 2217.
28. Ogden and Colquhoun (1985) *Proc.R.Soc.Lond.B.Biol.Sci.* **225** 329.
29. Bertrand and Gopalakrishnan (2007) *Biochem.Pharmacol.* **74** 1155.
30. Seo et al. (2009) *J.Neurosci.* **29** 8734.
31. Young et al. (2008) *Proc.Natl.Acad.Sci.U.S.A.* **105** 14686.
32. Lynagh and Lynch (2012) *Trends Pharmacol.Sci.* **33** 432.
33. Paton and Zaimis (1997) *Br.J.Pharmacol.* **120** 60.
34. Romano and Goldstein (1980) *Science* **210** 647.
35. Clarke et al. (1992) *Trends Pharmacol.Sci.* **13** 407.
36. Boulter et al. (1986) *Nature* **319** 368.
37. Harpsøe et al. (2013) *J.Biol.Chem.* **288** 2559.
38. Wang et al. (1996) *J.Biol.Chem.* **271** 17656.
39. Corringer et al. (2000) *Annu.Rev.Pharmacol.Toxicol.* **40** 431.
40. Kuryatov et al. (2008) *Mol.Pharmacol.* **74** 132.
41. Nelson et al. (2003) *Mol.Pharmacol.* **63** 332.
42. Srinivasan et al. (2011) *J.Gen.Physiol.* **137** 59.
43. Grishin et al. (2010) *J.Biol.Chem.* **285** 22254.
44. Krashia et al. (2010) *PLoS One* **5** e13611.
45. Mazzo et al. (2013) *J.Neurosci.* **33** 12316.
46. Grupe et al. (2013) *Br.J.Pharmacol.* **168** 2000.
47. Millar (2008) *Br.J.Pharmacol.* **153** S177.
48. Vallés et al. (2012) *Biochim.Biophys.Acta.* **1818** 718.
49. Rayes et al. (2009) *J.Neurosci.* **29** 6022.
50. Vetter et al. (2007) *Proc.Natl.Acad.Sci.U.S.A.* **104** 20594.
51. Elgoyhen et al. (2012) *J.Physiol.Paris* **106** 47.
52. Elgoyhen et al. (2001) *Proc.Natl.Acad.Sci.U.S.A.* **98** 3501.
53. Khiroug et al. (2002) *J.Physiol.* **540** 425.
54. Liu et al. (2009) *J.Neurosci.* **29** 918.
55. Murray et al. (2012) *Mol.Pharmacol.* **81** 175.
56. Holden-Dye et al. (2013) *Parasitol.Int.* **62** 606.
57. Millar and Denholm (2007) *Invert.Neurosci.* **7** 53.
58. Jones and Sattelle (2010) *Adv.Exp.Med.Biol.* **683** 25.
59. Matsuda et al. (2009) *Mol.Pharmacol.* **76** 1.
60. Blacquièrre et al. (2012) *Ecotoxicology* **21** 973.
61. Mao et al. (2006) *Mol.Pharmacol.* **70** 1693.
62. David et al. (2010) *Eur.J.Neurosci.* **31** 978.
63. Genzen et al. (2001) *J.Neurophysiol.* **86** 1773.
64. Lips et al. (2002) *Neuroscience* **115** 1.
65. Hone et al. (2012) *FASEB J.* **26** 917.
66. Gotti et al. (2006) *Trends Pharmacol.Sci.* **27** 482.
67. Lukas et al. (1999) *Pharmacol.Rev.* **51** 397.
68. Wonnacott (1997) *Trends Neurosci.* **20** 92.
69. Mameli-Engvall et al. (2006) *Neuron* **50** 911.
70. Bürli et al. (2010) *PLoS One* **5** e11507.
71. Dajas-Bailador and Wonnacott (2004) *Trends Pharmacol.Sci.* **25** 317.
72. Gu (2012) *J.Neurosci.* **32** 12337.
73. Jones et al. (1999) *Trends Neurosci.* **22** 555.
74. Klein and Yakel (2005) *J.Physiol.* **568** 881.
75. Wallace and Bertrand (2013) *Biochem.Pharmacol.* **85** 1713.
76. Broide and Leslie (1999) *Mol.Neurobiol.* **20** 1.
77. Poorhuis et al. (2013) *J.Neurosci.* **43** 4843.
78. Griguoli et al. (2013) *J.Neurosci.* **33** 1044.
79. Olincy and Freedman (2012) *Handb.Exp.Pharmacol.* **213** 211.
80. Mao et al. (2011) *J.Neurosci.* **31** 6710.
81. Zoli (1998) *J.Neurosci.* **18** 4461.
82. Stoker and Markou (2013) *Curr.Opin.Neurobiol.* **23** 493.
83. Orejarena et al. (2012) *Neuropharmacology* **63** 235.
84. Guillem et al. (2011) *Science* **333** 888.
85. Picard et al. (2013) *Neuroimage* **79** 42.
86. Hoda et al. (2008) *Mol.Pharmacol.* **74** 379.
87. Wada et al. (1990) *Brain Res.* **526** 45.
88. Flora et al. (2000) *J.Neurochem.* **75** 18.
89. Mao et al. (2008) *J.Neurochem.* **104** 446.
90. Berrettini and Doyle (2012) *Mol.Psychiatry* **17** 856.
91. Fowler et al. (2011) *Nature* **471** 597.
92. Frahm et al. (2011) *Neuron* **70** 522.
93. Livingstone and Wonnacott (2009) *Biochem.Pharmacol.* **78** 744.
94. Heath et al. (2010) *Neuropsychopharmacology* **35** 2324.
95. Exley et al. (2012) *J.Neurosci.* **32** 2352.
96. Gerzanich et al. (1998) *J.Pharmacol.Exp.Ther.* **286** 311.
97. Tapia et al. (2007) *Mol.Pharmacol.* **71** 769.
98. Chatterjee et al. (2013) *PLoS One* **8** e68300.
99. Le Novère et al. (1996) *Eur.J.Neurosci.* **8** 2428.
100. Yang et al. (2009) *Acta Pharmacol.Sin.* **30** 740.
101. Salminen et al. (2007) *Mol.Pharmacol.* **71** 1563.
102. Kuryatov and Lindstrom (2011) *Mol.Pharmacol.* **79** 126.
103. Gotti et al. (2005) *Mol.Pharmacol.* **67** 2007.
104. Drenan et al. (2008) *Mol.Pharmacol.* **73** 27.
105. Dash and Lukas (2012) *J.Biol.Chem.* **287** 14259.
106. Broadbent et al. (2006) *Mol.Pharmacol.* **70** 1350.
107. Dash et al. (2011) *J.Biol.Chem.* **286** 37976.
108. Wada et al. (1989) *J.Comp.Neurol.* **284** 314.
109. Han et al. (2000) *Eur.J.Neurosci.* **12** 3664.
110. Lotfipour et al. (2013) *J.Neurosci.* **33** 7728.
111. Lamotte d'Incamps and Ascher (2013) *Biochem.Pharmacol.* **86** 1114.
112. Lipovsek et al. (2012) *Proc.Natl.Acad.Sci.U.S.A.* **109** 4308.
113. McIntosh et al. (2009) *Biochem.Pharmacol.* **78** 693.
114. Wessler and Kirkpatrick (2008) *Br.J.Pharmacol.* **154** 1558.
115. Schuller (2009) *Nat.Rev.Cancer* **9** 195.
116. Filippini et al. (2012) *Curr.Drug Targets* **13** 644.
117. Jensen et al. (2005) *J.Med.Chem.* **48** 4705.
118. Baker et al. (2004) *Mol.Pharmacol.* **65** 453.
119. Dunlop et al. (2007) *Biochem.Pharmacol.* **74** 1172.
120. Sidach et al. (2009) *J.Neurosci.Methods* **182** 17.
121. Malysz et al. (2009) *Assay Drug Dev.Technol.* **7** 374.
122. Papke et al. (2002) *Br.J.Pharmacol.* **137** 49.

123. Jonsson et al. (2006) *Anesthesiology* **105** 521.
124. Verbitsky et al. (2000) *Neuropharmacology* **39** 2515.
125. Papke et al. (1996) *Neurosci.Lett.* **213** 201.
126. Alkondon et al. (1997) *Eur.J.Neurosci.* **9** 2734.
127. Alkondon and Albuquerque (2006) *J.Pharmacol.Exp.Ther.* **318** 268.
128. Wonnacott and Gallagher (2006) *Marine Drugs* **4** 228.
129. Swanson et al. (1986) *Mol.Pharmacol.* **29** 250.
130. Thomas et al. (1993) *J.Neurochem.* **60** 2308.
131. Feuerbach et al. (2005) *Neuropharmacology* **48** 215.
132. Stolerman et al. (1992) *Neuropharmacology* **31** 311.
133. MacPhail et al. (2007) *Toxicology* **234** 83.
134. Jarema et al. (2008) *Neurotoxicol.Teratol.* **30** 220.
135. Varanda et al. (1985) *Mol.Pharmacol.* **28** 128.
136. Jensen et al. (2003) *Mol.Pharmacol.* **64** 865.
137. Marks (2007) *Neuropharmacology* **53** 390.
138. Moroni et al. (2006) *Mol.Pharmacol.* **70** 755.
139. Tavares Xda (2012) *J.Am.Chem.Soc.* **134** 11474.
140. Pérez et al. (2012) *Nat.Prod.Rep.* **29** 555.
141. Coe et al. (2005) *Bioorg.Med.Chem.Lett.* **15** 2974.
142. Etter et al. (2008) *Drug Alcohol Depend.* **92** 3.
143. Mineur et al. (2007) *Neuropharmacology* **52** 1256.
144. Reavill et al. (1990) *Neuropharmacology* **29** 619.
145. Abin-Carriquiry et al. (2008) *Eur.J.Pharmacol.* **589** 80.
146. Ferger et al. (1998) *Eur.J.Pharmacol.* **360** 155.
147. Cunningham and McMahon (2011) *Eur.J.Pharmacol.* **654** 47.
148. Smith et al. (2007) *Psychopharmacology (Berl)*. **190** 157.
149. Jutkiewicz et al. (2011) *J.Pharmacol.Exp.Ther.* **339** 194.
150. Badio and Daly (1994) *Mol.Pharmacol.* **45** 563.
151. Xiao and Kellar (2004) *J.Pharmacol.Exp.Ther.* **310** 98.
152. Gerzanich et al. (1995) *Mol.Pharmacol.* **48** 774.
153. Alkondon and Albuquerque (1995) *J.Pharmacol.Exp.Ther.* **274** 771.
154. Damaj et al. (1998) *J.Pharmacol.Exp.Ther.* **284** 1058.
155. Khan et al. (1998) *Neuropharmacology* **37** 1515.
156. Cucchiario et al. (2006) *Neuropharmacology* **50** 769.
157. Ganesh et al. (2008) *Eur.J.Pharmacol.* **584** 93.
158. Marubio et al. (1999) *Nature* **398** 805.
159. Benowitz et al. (2009) *Handb.Exp.Pharmacol.* **192** 29.
160. Matta et al. (2007) *Psychopharmacology (Berl)*. **190** 269.
161. Buccafusco and Terry Jr (2003) *Life Sci.* **72** 2931.
162. O'Leary et al. (2008) *J.Pharmacol.Exp.Ther.* **325** 646.
163. Oliver et al. (2007) *Vaccine* **25** 7354.
164. Bitner et al. (2007) *J.Neurosci.* **27** 10578.
165. Thomsen et al. (2008) *Neuroscience* **154** 741.
166. Thomsen et al. (2011) *PLoS One* **6** e27014.
167. Kohlhaas et al. (2012) *Psychopharmacology (Berl)*. **220** 823.
168. Briggs et al. (2008) *Br.J.Pharmacol.* **153** 1054.
169. Schrimpf et al. (2012) *Bioorg.Med.Chem.Lett.* **22** 1633.
170. Toyohara et al. (2010) *PLoS One* **5** e8961.
171. Garcha et al. (1993) *Psychopharmacology (Berl)*. **110** 347.
172. Binns and Salt (2000) *Vis.Neurosci.* **17** 283.
173. Spivak et al. (1986) *Eur.J.Pharmacol.* **120** 127.
174. Manetti et al. (1999) *Bioorg.Med.Chem.Lett.* **7** 457.
175. Dewey and Xie (2013) *Phytochemistry* **94** 10.
176. Marks et al. (1986) *Mol.Pharmacol.* **30** 427.
177. Stolerman et al. (1995) *Psychopharmacology (Berl)*. **117** 430.
178. Mastropaolo et al. (2004) *Behav.Brain Res.* **153** 419.
179. Welch et al. (2013) *Toxicol.Appl.Pharmacol.* **266** 366.
180. Mullen et al. (2000) *J.Med.Chem.* **43** 4045.
181. Papke et al. (2004) *Bioorg.Med.Chem.Lett.* **14** 1849.
182. Levin et al. (1999) *Behav.Pharmacol.* **10** 675.
183. Van Kampen et al. (2004) *Psychopharmacology (Berl)*. **172** 375.
184. Grottick et al. (2003) *Neuropsychopharmacology* **28** 880.
185. Imming (2001) *Eur.J.Med.Chem.* **36** 375.
186. Abin-Carriquiry et al. (2006) *Eur.J.Pharmacol.* **536** 1.
187. Houlihan et al. (2001) *J.Neurochem.* **78** 1029.
188. Abin-Carriquiry et al. (2010) *Eur.J.Pharmacol.* **634** 89.
189. Kem et al. (1997) *J.Pharmacol.Exp.Ther.* **283** 979.
190. Arendash et al. (1995) *Brain Res.* **674** 252.
191. Stevens et al. (1998) *Psychopharmacology (Berl)*. **136** 320.
192. Mukhin et al. (2000) *Mol.Pharmacol.* **57** 642.
193. Mogg et al. (2004) *Neuropharmacology* **47** 848.
194. Kennett et al. (2012) *Nicotine Tob.Res.* **14** 1339.
195. Wildeboer and Stevens (2008) *Brain Res.* **1224** 29.
196. Huang et al. (2011) *Neuropharmacology* **60** 861.
197. Cavanagh et al. (2010) *Synapse* **64** 111.
198. Miller et al. (2000) *Neuropharmacology* **39** 2654.
199. Dwoskin and Crooks (2001) *J.Pharmacol.Exp.Ther.* **298** 395.
200. Kaniaková et al. (2011) *Eur.J.Pharmacol.* **658** 108.
201. Farook et al. (2009) *Physiol.Behav.* **97** 503.
202. Stead and Hughes (2012) *Cochrane Database Syst.Rev.* **2** CD000124.
203. Crooks et al. (2011) *Curr.Top.Med.Chem.* **11** 1103.
204. Miller et al. (2007) *Dug Alcohol Depend.* **89** 282.
205. Jeong et al. (2010) *Pflugers Arch.* **460** 851.
206. Levin and Christopher (2003) *Pharmacol.Biochem.Behav.* **76** 133.
207. Roni and Rahman (2013) *Prog.Neuropsychopharmacol.Biol.Psychiatry* **41** 44.
208. Wishka et al. (2006) *J.Med.Chem.* **49** 4425.
209. Kita et al. (2013) *Psychopharmacology (Berl)*. **225** 543.
210. Shilliday et al. (2010) *Drug Metab.Lett.* **4** 162.
211. Bodnar et al. (2005) *J.Med.Chem.* **48** 905.
212. Hajós et al. (2005) *J.Pharmacol.Exp.Ther.* **312** 1213.
213. Pandya and Yakel (2013) *Neuropharmacology* **70** 35.
214. Young et al. (2013) *Behav.Brain Res.* **240** 119.
215. Brunzell and McIntosh (2012) *Neuropsychopharmacology* **37** 1134.
216. Igari et al. (2014) *Neuropsychopharmacology* **39** 445.
217. Turcanu et al. (2012) *Neurosci.Lett.* **516** 212.
218. Bencherif et al. (1996) *J.Pharmacol.Exp.Ther.* **279** 1413.
219. Papke (2000) *J.Neurochem.* **75** 204.
220. Garduño et al. (2012) *J.Neurosci.* **32** 15148.
221. Lippiello et al. (1996) *J.Pharmacol.Exp.Ther.* **279** 1422.
222. Damaj et al. (1999) *J.Pharmacol.Exp.Ther.* **291** 390.
223. Rowley et al. (2008) *Anesth.Analg.* **107** 1052.
224. Bencherif et al. (1998) *J.Pharmacol.Exp.Ther.* **284** 886.
225. Lopez-Hernandez et al. (2007) *Neuropharmacology* **53** 134.
226. Beracochea et al. (2008) *Psychopharmacology (Berl)*. **196** 555.
227. Wang et al. (2009) *J.Neurosci.* **29** 10961.
228. Wang et al. (2010) *Biol.Psychiatry* **67** 522.
229. Xiao et al. (2006) *Mol.Pharmacol.* **70** 1454.
230. Zwart et al. (2008) *Mol.Pharmacol.* **73** 1838.
231. Liu et al. (2013) *J.Med.Chem.* **56** 3000.
232. Rezvani et al. (2010) *Psychopharmacology (Berl)*. **211** 161.
233. Turner et al. (2013) *Neuropsychopharmacology* **38** 2035.
234. Roncarati et al. (2009) *J.Pharmacol.Exp.Ther.* **329** 459.
235. Haydar et al. (2009) *Bioorg.Med.Chem.* **17** 5247.
236. Marrero et al. (2004) *J.Pharmacol.Exp.Ther.* **309** 16.
237. Bencherif et al. (2000) *Eur.J.Pharmacol.* **409** 45.
238. Chen et al. (2003) *Neuropharmacology* **45** 334.
239. Richardson et al. (1985) *Nature* **316** 126.
240. Rothlin (2003) *Mol.Pharmacol.* **63** 1067.
241. Cui et al. (2009) *Eur.J.Pharmacol.* **609** 74.
242. Kohnomi et al. (2010) *Brain Res.* **1353** 152.
243. Sharples et al. (2000) *J.Neurosci.* **20** 2783.
244. Cao et al. (2005) *Neuropharmacology* **48** 72.
245. Mihalak et al. (2006) *Mol.Pharmacol.* **70** 801.
246. Papke et al. (2010) *J.Pharmacol.Exp.Ther.* **333** 501.
247. Bordia et al. (2012) *J.Pharmacol.Exp.Ther.* **342** 327.
248. Hendrickson et al. (2010) *J.Neurosci.* **30** 10169.
249. Rollema et al. (2007) *Neuropharmacology* **52** 985.
250. Wildeboer-Andrud and Stevens (2011) *Pharmacol.Biochem.Behav.* **100** 17.

251. Obach et al. (2006) *Drug Metab.Dispos.* **34** 121.
252. Roegge et al. (2006) Nicotinic receptor antagonists in rats. In: Levin and Buccafusco ed. *Animal Models of Cognitive Impairment*. CRC Press, Boca Raton, FL.
253. Bertrand et al. (1990) *Proc.Natl.Acad.Sci.U.S.A.* **87** 1993.
254. Bowman (1958) *Br.J.Pharmacol.Chemother.* **13** 521.
255. Christ and Blaber (1968) *J.Pharmacol.Exp.Ther.* **160** 159.
256. Lukas (1989) *J.Pharmacol.Exp.Ther.* **251** 175.
257. Walker et al. (1993) *Comp.Biochem.Physiol.C.* **106** 49.
258. Bini et al. (2006) *J.Exp.Zool.A.Comp.Exp.Biol.* **305** 472.
259. Pereira et al. (1993) *J.Pharmacol.Exp.Ther.* **265** 1474.
260. Dwoskin et al. (2004) *Bioorg.Med.Chem.Lett.* **14** 1863.
261. Dwoskin et al. (2008) *J.Pharmacol.Exp.Ther.* **326** 563.
262. Rahman et al. (2008) *Br.J.Pharmacol.* **153** 792.
263. Papke et al. (2008) *Neuropharmacology* **54** 1189.
264. Wooters et al. (2011) *Br.J.Pharmacol.* **163** 346.
265. Fuchs et al. (2003) *Ann.N.Y.Acad.Sci.* **998** 93.
266. Dellisanti et al. (2007) *Nat.Neurosci.* **10** 1222.
267. Huang et al. (2013) *Biochem.J.* **454** 303.
268. Wu et al. (2004) *J.Pharmacol.Exp.Ther.* **311** 80.
269. Ng et al. (2007) *Proc.Natl.Acad.Sci.U.S.A.* **104** 8059.
270. Shahsavari et al. (2012) *PLoS One* **7** e40757.
271. Harvey et al. (1996) *J.Neurochem.* **67** 1953.
272. Capelli et al. (2011) *Br.J.Pharmacol.* **163** 313.
273. Soll et al. (2013) *Neuropharmacology* **73** 19.
274. Jutkiewicz et al. (2011) *J.Pharmacol.Exp.Ther.* **339** 194.
275. Guy and Fletcher (2013) *Psychopharmacology (Berl)*. **225** 429.
276. André et al. (2011) *Neuropharmacology* **60** 617.
277. Jackson et al. (2013) *Neuropharmacology* **70** 228.
278. Struthers et al. (2009) *Pharmacol.Biochem.Behav.* **94** 319.
279. Ward et al. (1990) *FEBS Lett.* **270** 45.
280. Dupuis et al. (2011) *J.Neurophysiol.* **106** 1604.
281. Mogg et al. (2002) *J.Pharmacol.Exp.Ther.* **302** 197.
282. Feng et al. (2011) *Behav.Brain Res.* **220** 100.
283. Lockman et al. (2005) *J.Neurochem.* **94** 37.
284. van Kampen et al. (2004) *Psychopharmacology (Berl)*. **172** 375.
285. Gotti et al. (1998) *Br.J.Pharmacol.* **124** 1197.
286. Maggi et al. (1999) *Br.J.Pharmacol.* **126** 285.
287. Xu et al. (2010) *Respir.Res.* **11** 13.
288. Brown et al. (2012) *Angiogenesis* **15** 99.
289. Cao et al. (2005) *J.Pharmacol.Exp.Ther.* **312** 1298.
290. Tanibuchi (2010) *Brain Res.* **1348** 200.
291. Buckett (1968) *Ir.J.Med.Sci.* **7** 565.
292. Wenningmann and Dilger (2001) *Mol.Pharmacol.* **60** 790.
293. Paul et al. (2002) *Anesth.Analg.* **94** 597.
294. Jonsson Fagerlund et al. (2009) *Anesthesiology* **110** 1244.
295. Yost and Winegar (1997) *Cell Mol.Neurobiol.* **17** 35.
296. Liu and Dilger (2009) *Mol.Pharmacol.* **75** 166.
297. Zaveri et al. (2010) *J.Med.Chem.* **53** 8187.
298. Lee (2005) *J.R.Coll.Physicians Edinb.* **35** 83.
299. Wang et al. (2003) *J.Biol.Chem.* **278** 32284.
300. Bertrand et al. (1992) *Neurosci.Lett.* **146** 87.
301. Brams et al. (2011) *PLoS Biol.* **9** e1001034.
302. McMahon and Kauer (1997) *J.Neurophysiol.* **78** 2493.
303. Brown et al. (1998) *J.Physiol.* **507** 653.
304. Yan et al. (2006) *Mol.Pharmacol.* **70** 571.
305. Schwartz and Mindlin (1988) *J.Pharmacol.Exp.Ther.* **244** 963.
306. Wotring and Yoon (1995) *Neuroscience* **67** 293.
307. Azam and McIntosh (2009) *Acta Pharmacol.Sin.* **30** 771.
308. Armishaw (2010) *Toxins (Basel)* **2** 1471.
309. Vincler et al. (2006) *Proc.Natl.Acad.Sci.U.S.A.* **103** 17880.
310. Sandall et al. (2003) *Biochemistry* **42** 6904.
311. Clark et al. (2005) *Proc.Natl.Acad.Sci.U.S.A.* **102** 13767.
312. Satkunathan et al. (2005) *Brain Res.* **1059** 149.
313. van Lierop et al. (2013) *ACS Chem.Biol.* **8** 1815.
314. Nevin et al. (2007) *Mol.Pharmacol.* **72** 1406.
315. Callaghan et al. (2008) *J.Neurosci.* **28** 10943.
316. Adams et al. (2012) *Br.J.Pharmacol.* **166** 486.
317. Napier et al. (2012) *Neuropharmacology* **62** 2202.
318. Klimis et al. (2011) *Pain* **152** 259.
319. Martinez et al. (1995) *Biochemistry* **34** 14519.
320. Park et al. (2001) *J.Biol.Chem.* **276** 49028.
321. López-Vera et al. (2007) *FEBS J.* **274** 3972.
322. McIntosh et al. (1994) *J.Biol.Chem.* **269** 16733.
323. Johnson et al. (1995) *Mol.Pharmacol.* **48** 194.
324. Broxton et al. (1999) *J.Neurochem.* **72** 1656.
325. Ellison et al. (2004) *Biochemistry* **43** 16019.
326. Yu et al. (2011) *PLoS Comput.Biol.* **7** e1002011.
327. Pereira et al. (1996) *J.Pharmacol.Exp.Ther.* **278** 1472.
328. Barik and Wonnacott (2006) *Mol.Pharmacol.* **69** 618.
329. Cartier et al. (1996) *J.Biol.Chem.* **271** 7522.
330. Champiaux et al. (2002) *J.Neurosci.* **15** 1208.
331. McIntosh et al. (2004) *Mol.Pharmacol.* **65** 944.
332. Kulak et al. (2002) *Mol.Pharmacol.* **61** 230.
333. Exley et al. (2013) *Eur.J.Neurosci.* [Epub ahead of print].
334. McCallum et al. (2005) *Mol.Pharmacol.* **68** 737.
335. Löf et al. (2007) *Psychopharmacology (Berl)*. **195** 333.
336. Brunzell et al. (2010) *Neuropsychopharmacology* **35** 665.
337. Gotti et al. (2010) *J.Neurosci.* **30** 5311.
338. Okada et al. (2008) *Eur.J.Pharmacol.* **587** 322.
339. Dowell et al. (2003) *J.Neurosci.* **23** 8445.
340. Azam and McIntosh (2005) *J.Pharmacol.Exp.Ther.* **312** 231.
341. Yang et al. (2011) *J.Neurosci.* **31** 2537.
342. Fainzilber et al. (1994) *Biochemistry* **33** 9523.
343. Luo et al. (1999) *Biochemistry* **38** 14542.
344. Everhart et al. (2003) *J.Pharmacol.Exp.Ther.* **306** 664.
345. Hogg et al. (2003) *J.Biol.Chem.* **278** 26908.
346. Luo et al. (1998) *J.Neurosci.* **18** 8571.
347. Kulak et al. (2001) *J.Neurochem.* **77** 1581.
348. Grady et al. (2001) *J.Neurochem.* **76** 258.
349. Park et al. (2006) *Pflugers Arch.* **452** 775.
350. Glick et al. (2011) *Eur.J.Pharmacol.* **669** 71.
351. McCallum et al. (2012) *Neuropharmacology* **63** 434.
352. Herrero et al. (2002) *J.Neurosci.* **22** 377.
353. Peng et al. (2013) *Mol.Pharmacol.* **84** 459.
354. Mahata et al. (2010) *Regul.Pept.* **162** 33.
355. Yang et al. (2013) *Proc.Natl.Acad.Sci.U.S.A.* **110** 12078.
356. Sahu et al. (2012) *J.Cell.Sci.* **125** 2323.
357. Plummer et al. (1955) *J.Pharmacol.Exp.Ther.* **115** 172.
358. Reuben et al. (1998) *Br.J.Pharmacol.* **125** 1218.
359. Costa et al. (2001) *Brain Res.* **888** 336.
360. Hunter (1950) *Lancet* **1** 251.
361. Ascher et al. (1979) *J.Physiol.* **295** 139.
362. DeNoble and Mele (2006) *Psychopharmacology (Berl)*. **184** 266.
363. Ortiz et al. (2012) *Nicotine Tob.Res.* **14** 711.
364. Benwell et al. (1995) *Br.J.Pharmacol.* **114** 454.
365. Malin et al. (1997) *Pharmacol.Biochem.Behav.* **58** 695.
366. Marks et al. (1999) *J.Pharmacol.Exp.Ther.* **289** 1090.
367. Eaton et al. (2003) *Mol.Pharmacol.* **64** 1283.
368. Ford et al. (1956) *Am.J.Med.Sci.* **232** 129.
369. Bacher et al. (2009) *Expert Opin.Pharmacother.* **10** 2709.
370. Arias et al. (2010) *Int.J.Biochem.Cell Biol.* **42** 1007.
371. Papke et al. (2001) *J.Pharmacol.Exp.Ther.* **297** 646.
372. Yalcin et al. (2009) *J.Physiol.Pharmacol.* **60** 133.
373. Francis et al. (1998) *Biophys.J.* **74** 2306.
374. Papke et al. (2005) *Mol.Pharmacol.* **67** 1977.
375. Damaj et al. (2005) *Eur.J.Pharmacol.* **521** 43.

376. Rehni and Singh (2010) *Naunyn Schmiedebergs Arch.Pharmacol.* **382** 279.
377. Fryer and Lukas (1999) *J.Pharmacol.Exp.Ther.* **288** 88.
378. Slemmer et al. (2000) *J.Pharmacol.Exp.Ther.* **295** 321.
379. Sidhpura (2007) *Eur.J.Pharmacol.* **567** 102.
380. Arias (2009) *Int.J.Biochem.Cell Biol.* **41** 2098.
381. Damaj et al. (2004) *Mol.Pharmacol.* **66** 675.
382. Pandhare et al. (2012) *Biochemistry* **51** 2425.
383. García-Colunga et al. (1997) *Proc.Natl.Acad.Sci.U.S.A.* **94** 2041.
384. Feuerbach et al. (2005) *Neuropharmacology* **48** 215.
385. Weber et al. (2013) *Neuropharmacology* **72** 88.
386. Grinevich et al. (2009) *Neuropharmacology* **57** 183.
387. Zheng et al. (2010) *J.Pharmacol.Exp.Ther.* **335** 401.
388. Villarroya et al. (1997) *Eur.J.Pharmacol.* **320** 249.
389. Herrero et al. (1999) *Br.J.Pharmacol.* **127** 1375.
390. Ween et al. (2010) *Neurochem.Int.* **57** 269.
391. Huettner et al. (1988) *Proc.Natl.Acad.Sci.U.S.A.* **85** 1307.
392. Briggs and McKenna (1996) *Neuropharmacology* **35** 407.
393. Buisson and Bertrand (1998) *Mol.Pharmacol.* **53** 555.
394. Amador and Dani (1991) *Synapse* **7** 207.
395. Ho and Flood (2004) *Anesthesiology* **100** 657.
396. Aracava et al. (2005) *J.Pharmacol.Exp.Ther.* **312** 1195.
397. Lee et al. (2012) *PLoS One* **7** e40326.
398. Hilmas et al. (2001) *J.Neurosci.* **21** 7463.
399. Albuquerque and Schwarcz (2013) *Biochem.Pharmacol.* **85** 1027.
400. Stone and Darlington (2013) *Br.J.Pharmacol.* **169** 1211.
401. Valera et al. (1992) *Proc.Natl.Acad.Sci.U.S.A.* **89** 9949.
402. Ke and Lukas (1996) *J.Neurochem.* **67** 1100.
403. Garbus et al. (2001) *Neuroreport* **12** 227.
404. Fernandez-Ballester et al. (1994) *Biochem.Soc.Trans.* **22** 776.
405. Gangitano et al. (2009) *Genes Brain Behav.* **8** 398.
406. García-Colunga and Miledi (1999) *Proc.Natl.Acad.Sci.U.S.A.* **96** 4113.
407. Matsubayashi et al. (1998) *J.Pharmacol.Exp.Ther.* **284** 904.
408. Parri et al. (2011) *Biochem.Pharmacol.* **82** 931.
409. Liu et al. (2001) *Proc.Natl.Acad.Sci.U.S.A.* **98** 4734.
410. Grassi et al. (2003) *J.Physiol.* **547** 147.
411. Mehta et al. (2009) *J.Neurochem.* **109** 1452.
412. Snyder et al. (2005) *Nat.Neurosci.* **8** 1051.
413. Abbott et al. (2008) *Neurobiol.Aging* **29** 992.
414. Puzo and Arancio (2013) *J.Alzheimers Dis.* **33** S111.
415. Talantova et al. (2013) *Proc.Natl.Acad.Sci.U.S.A.* **110** E2518.
416. Grönlén et al. (2007) *Mol.Pharmacol.* **72** 715.
417. Schratzenholz et al. (1996) *Mol.Pharmacol.* **49** 1.
418. Chimienti et al. (2003) *Hum.Mol.Genet.* **12** 3017.
419. Kawashima et al. (2012) *Ann.N.Y.Acad.Sci.* **1261** 7.
420. Faghiih et al. (2007) *Recent Pat.CNS Drug Discov.* **2** 99.
421. Sattelle et al. (2009) *Biochem.Pharmacol.* **78** 836.
422. Dey and Chen (2011) *J.Biomol.Struct.Dyn.* **28** 695.
423. Collins et al. (2011) *Neuropharmacology* **61** 1306.
424. Freitas et al. (2013) *J.Pharmacol.Exp.Ther.* **344** 264.
425. Dickinson et al. (2007) *J.Neurochem.* **100** 1089.
426. Hu et al. (2009) *Br.J.Pharmacol.* **158** 1857.
427. daCosta et al. (2011) *J.Neurosci.* **31** 13870.
428. Gill et al. (2011) *Proc.Natl.Acad.Sci.U.S.A.* **108** 5867.
429. Bertrand et al. (2008) *Mol.Pharmacol.* **74** 1407.
430. Luttmann et al. (2009) *ChemMedChem* **4** 1874.
431. Hamouda et al. (2013) *J.Neurosci.* **33** 485.
432. Moroni et al. (2008) *J.Neurosci.* **28** 6884.
433. Kuryatov et al. (2000) *Neuropharmacology* **39** 2570.
434. Faghiih et al. (2009) *J.Med.Chem.* **52** 3377.
435. Malysz et al. (2009) *J.Pharmacol.Exp.Ther.* **330** 257.
436. Anderson et al. (2008) *J.Pharmacol.Exp.Ther.* **324** 179.
437. Peters et al. (2004) *Planta Med.* **70** 883.
438. Sala et al. (2005) *Neurosci.Lett.* **373** 144.
439. Kim et al. (2007) *Bioorg.Med.Chem.Lett.* **17** 4855.
440. Weltzin and Schulte (2010) *J.Pharmacol.Exp.Ther.* **334** 917.
441. German et al. (2011) *J.Med.Chem.* **54** 7259.
442. Liu (2013) *Psychopharmacology (Berl)*. **230** 203.
443. Samochocki et al. (2003) *J.Pharmacol.Exp.Ther.* **305** 1024.
444. Dajas-Bailador et al. (2003) *Mol.Pharmacol.* **64** 1217.
445. Akk and Steinbach (2005) *J.Neurosci.* **25** 1992.
446. Smith et al. (2009) *Biochem.Pharmacol.* **78** 889.
447. Storch et al. (1995) *Eur.J.Pharmacol.* **290** 207.
448. Militante et al. (2008) *Mol.Pharmacol.* **74** 764.
449. Ludwig et al. (2010) *J.Recept.Signal Transduct.Res.* **30** 469.
450. Krause et al. (1998) *Mol.Pharmacol.* **53** 283.
451. Collins and Millar (2010) *Mol.Pharmacol.* **78** 198.
452. Larocque and Hoffman (2012) *Clin.Toxicol.(Phila)*. **50** 231
453. Levandoski et al. (2003) *Eur.J.Pharmacol.* **471** 9.
454. Broad et al. (2006) *J.Pharmacol.Exp.Ther.* **318** 1108.
455. Timmermann et al. (2007) *J.Pharmacol.Exp.Ther.* **323** 294.
456. Hurst et al. (2005) *J.Neurosci.* **25** 4396.
457. Pandya and Yakel (2013) *Biochem.Pharmacol.* **86** 1054.
458. El Kouhen et al. (2009) *Br.J.Pharmacol.* **156** 638.
459. Grybko et al. (2011) *Eur.J.Neurosci.* **33** 1786.
460. Shen and Yakel (2012) *J.Mol.Neurosci.* **48** 14.
461. daCosta and Sine (2013) *Proc.Natl.Acad.Sci.U.S.A.* **110** 6595.
462. McLean et al. (2012) *J.Psychopharmacol.* **26** 1265.
463. Kalappa et al. (2013) *Br.J.Pharmacol.* **169** 1862.
464. Munro et al. (2012) *Br.J.Pharmacol.* **167** 421.
465. Livingstone et al. (2009) *Eur.J.Neurosci.* **29** 539.
466. Christensen et al. (2010) *J.Neurochem.* **114** 1205.
467. Gill et al. (2013) *PLoS One* **8** e55047.
468. Le Novère et al. (2002) *J.Neurobiol.* **53** 447.
469. Filevich et al. (2010) *J.Inorg.Biochem.* **104** 1248.
470. Absalom et al. (2013) *J.Biol.Chem.* **288** 26521.
471. Srinivasan et al. (2012) *Int.J.Mol.Sci.* **13** 10022.
472. Tochitsky et al. (2012) *Nature Chem.* **10** 105.
473. Brust et al. (2012) *Curr.Drug Targets* **13** 594.
474. Wong et al. (2013) *J.Nucl.Med.* **54** 1308.
475. Moser et al. (2007) *J.Neurochem.* **102** 479.
476. Chang and Fischbach (2006) *J.Neurosci.* **26** 11295.
477. Shelukhina et al. (2009) *J.Neurochem.* **109** 1087.
478. Hone et al. (2013) *J.Neurochem.* **114** 994.
479. Gomez-Varela and Berg (2013) *J.Neurosci.* **33** 17062.
480. Sekine-Aizawa and Haganir (2004) *Proc.Natl.Acad.Sci.U.S.A.* **101** 17114.
481. Hannan et al. (2013) *Methods Enzymol.* **521** 109.
482. Justinova et al. (2013) *Nature Neurosci.* **16** 1652.
483. Caffery et al. (2009) *Eur.J.Neurosci.* **30** 2064.
484. Woodruff-Pak et al. (1994) *Brain Res.* **645** 309.
485. Kem (2000) *Behav. Brain Res.* **113** 169.
486. Hibbs et al. (2009) *EMBO J.* **28** 3040.
487. Stokes et al. (2004) *Mol.Pharmacol.* **66** 14.
488. Mahnir et al. (1998) *Biopharm.Drug Dispos.* **19** 147.
489. Callahan et al. (2014) *Psychopharmacology (Berl)*. [Epub ahead of print].
490. Kem et al. (2004) *Mol.Pharmacol.* **65** 56.

Nicotinic Compounds Available from Tocris

Catalog No.	Product Name	Primary Action
α4β2 Nicotinic Receptors		
Agonists		
5017	A 85380	High affinity and selective α 4 β 2 nAChR agonist
5079	ABT 089	High affinity and selective α 4 β 2 nAChR partial agonist
6576	ABT 594	Selective α 4 β 2 nAChR agonist
5557	Dianicline	Selective α 4 β 2 nAChR partial agonist
1053	RJR 2403	Selective α 4 β 2 nAChR agonist
2737	TC 2559	Selective α 4 β 2 nAChR partial agonist
3754	Varenicline	Selective α 4 β 2 nAChR partial agonist; orally active
Ligands		
2736	Sazetidine A	α 4 β 2 nAChR ligand; may act as an agonist or desensitizer
Modulators		
5112	NS 9283	Positive allosteric modulator of α 4 β 2 nAChRs
α7 Nicotinic Receptors		
Agonists		
4234	4BP-TQS	α 7 nAChR agonist; acts at an allosteric site
4341	A 582941	α 7 nAChR partial agonist
4477	A 844606	Selective α 7 nAChR partial agonist
3964	AR-R 17779	Selective α 7 nAChR agonist
4557	GTS 21	α 7 nAChR partial agonist
6139	NS 6740	High affinity α 7 nAChR partial agonist; anti-inflammatory
3092	PHA 543613	Potent and selective α 7 nAChR agonist
3134	PHA 568487	Selective α 7 nAChR agonist
2303	PNU 282987	Selective α 7 nAChR agonist
3518	S 24795	α 7 nAChR partial agonist
4441	SEN 12333	α 7 nAChR agonist; also histamine H ₃ antagonist
5559	SSR 180711	Selective α 7 nAChR partial agonist
2518	TC 1698	α 7-selective nAChR agonist
2459	Tropisetron	α 7 nAChR partial agonist; also potent 5-HT ₃ antagonist
Antagonists		
2133	α-Bungarotoxin	Selective α 7 nAChR antagonist
3405	COG 133	α 7 nAChR antagonist; ApoE peptide fragment
2813	D-Amphetamine	α 7 nAChR antagonist; also induces dopamine, 5-HT and noradrenalin release
1029	Methyllycaconitine	Selective α 7 neuronal nAChR antagonist
1356	MG 624	α 7 neuronal nAChR antagonist
Modulators		
4571	A 867744	Positive allosteric modulator of α 7 nAChRs
3837	CCMI	Positive allosteric modulator of α 7 nAChRs
3091	FR 236924	Enhances α 7 nAChR activity; PKC ϵ inhibitor
1260	Ivermectin	Positive allosteric modulator of α 7 nAChRs; also positive allosteric modulator of P2X ₄ receptors
2995	NS 1738	Positive allosteric modulator of α 7 nAChRs; active <i>in vivo</i>
2498	PNU 120596	Positive allosteric modulator of α 7 nAChRs; active <i>in vivo</i>
4233	TQS	Positive allosteric modulator of α 7 nAChRs
Other Nicotinic Receptor Subtypes		
Agonists		
4831	NS 3861	α 3 β 2 full agonist; also α 3 β 4 partial agonist
4764	SIB 1553A	nAChR agonist; displays selectivity for β 4 subunit-containing receptors
Antagonists		
3120	α-Conotoxin AulB	Selective α 3 β 4 nAChR antagonist

Catalog No.	Product Name	Primary Action
3124	α-Conotoxin EI	α1β1δγ selective nAChR antagonist
1340	α-Conotoxin MII	Potent and selective α3β2 and β3 nAChR antagonist
3121	α-Conotoxin PIA	Selective antagonist of α6-containing nAChRs
3123	α-Conotoxin PnIA	Selective α3β2 nAChR antagonist
4424	SR 16584	Selective α3β4 nAChR antagonist
Non-Selective Nicotinic Receptors		
Agonists		
0351	1-Acetyl-4-methylpiperazine	nAChR agonist
0352	4-Acetyl-1,1-dimethylpiperazinium	nAChR agonist
2809	Acetylcholine	Endogenous neurotransmitter; agonist for all nAChR subtypes
1971	(+)-Anabasine	High affinity nAChR partial agonist
0789	(±)-Anatoxin A	nAChR agonist
3549	3-Bromocytisine	Potent α4β4, α4β2 and α7 nAChR agonist
2810	Carbamoylcholine	Non-selective cholinergic agonist
5236	CC4	High affinity and subtype-selective α6β2 and α4β2 nAChR partial agonist
3110	(-)-Cotinine	Primary metabolite of nicotine
1390	(-)-Cytisine	Potent and selective neuronal nAChR agonist
0684	(±)-Epibatidine	High affinity nAChR agonist
1518	5-Iodo-A-85380	High affinity α4β2 and α6β2 subtype-selective nAChR agonist
1527	5-Iodo-A-85380, 5-trimethylstannyl N-BOC derivative	Precursor to 5-Iodo-A-85380 (Cat. No. 1518)
1077	(-)-Lobeline	nAChR partial agonist
3546	(-)-Nicotine	Prototypical nAChR agonist
1271	RJR 2429	nAChR agonist; displays selectivity for α4β2, α1βγδ and α3β4 subtypes
4766	SIB 1508Y	Potent agonist at α4β2, α2β4, α4β4 and α3β4 nACh receptors
1348	UB 165	Subunit selective nAChR agonist; agonist at α3β2 and partial agonist at α4β2
Antagonists		
0424	Benzoquinonium	nAChR antagonist
4128	Bisindolylmaleimide II	Potent nAChR antagonist; also potent PKC inhibitor
3221	bPiDDB	Orthosteric neuronal nAChR antagonist
2722	Catestatin	Non-competitive nAChR antagonist
1001	Chlorisondamine	nAChR antagonist; long-lasting
2349	Dihydro-β-erythroidine	α4β2, muscle type and <i>Torpedo</i> nAChR antagonist
4111	Hexamethonium	Non-competitive nAChR antagonist
2843	Mecamylamine	Non-competitive nAChR antagonist
6552	PAMP-20 (human)	Non-competitive nAChR antagonist; also MRGPRX2 agonist
0693	Pancuronium	Neuromuscular nAChR antagonist
2438	TMPH	Neuronal nAChR antagonist
2820	(+)-Tubocurarine	nAChR antagonist
Modulators		
5963	CMP1	Potent positive allosteric modulator of α4β2 nAChRs; also inhibitor of (α4) ₂ (β2) ₃ , muscle-type and <i>Torpedo</i> nAChRs
3328	Desformylflustrabromine	Positive allosteric modulator of α4β2 nAChRs; also muscle-type nAChR inhibitor
2241	DMAB-anabaseine	nAChR agonist
0686	Galanthamine	Allosteric potentiator of nAChR potentiator; cholinesterase inhibitor
4141	LY 2087101	Allosteric potentiator of α4β2, α7 and α4β4 nAChRs
Caged Nicotinic Compounds		
6706	DPNB-ABT 594	Caged ABT 594 (Cat. No. 6576); suitable for one- or two-photon photolysis
6700	PA Nic	Caged nicotine; photoactivated by one-photon and two-photon excitation
3855	RuBi-Nicotine	Caged nicotine; rapidly excitable by visible light
Other Nicotinic Compounds		

Catalog No.	Product Name	Primary Action
Modulators		
0750	PG-9	Presynaptic cholinergic modulator
0751	SM-21	Presynaptic cholinergic modulator; also selective σ_2 antagonist
Others		
1695	Cisapride	Stimulates ACh release; increases intestinal motility
2330	DMP 543	Potent ACh release enhancer; orally active
0537	MR 16728	Stimulates ACh release
2851	Nefiracetam	Cognitive enhancer
Acetylcholine Transporters		
Inhibitors		
5725	ML 352	High affinity and selective presynaptic choline transporter (CHT) inhibitor
0653	(+)-Vesamicol	Inhibits ACh transport
Cholinesterases		
Inhibitors		
3069	Acephate	Anticholinesterase insecticide
0388	Ambenonium	Cholinesterase inhibitor
3713	Cryptotanshinone	Acetylcholinesterase inhibitor; also displays multiple other activities
4385	Donepezil	Potent AChE inhibitor
0686	Galanthamine	Cholinesterase inhibitor
4086	PE 154	Fluorescent, potent AChE and BChE inhibitor
2967	Phenserine	Cholinesterase inhibitor
0622	Physostigmine	Cholinesterase inhibitor
4440	Rivastigmine	Dual AChE and BChE inhibitor
0965	Tacrine	Cholinesterase inhibitor

For a complete and up-to-date product listing please visit | [tocris.com](https://www.tocris.com)

R&D SYSTEMS

NOVUS
BIOLOGICALS

TOCRIS

protein simple

ACD™

bio-**techne**®

Global info@bio-techne.com bio-techne.com/find-us/distributors TEL +1 612 379 2956
North America TEL 800 343 7475 Europe | Middle East | Africa TEL +44 (0)1235 529449
China info.cn@bio-techne.com TEL +86 (21) 52380373

bio-techne.com



For research use or manufacturing purposes only. Trademarks and registered trademarks are the property of their respective owners.

RV_NicotinicACh_29044