Review

For reprint orders, please contact: reprints@future-science.com

Future Medicinal Chemistry

# Designing selective modulators for the nicotinic receptor subtypes: challenges and opportunities

Dina Manetti[1], Cristina Bellucci[1], Niccolò Chiaramonte[1], Silvia Dei[1], Elisabetta Teodori[1] & Maria Novella Romanelli*,[1]

[1]University of Florence, Department of Neurosciences, Psychology, Drug Research & Child Health (NEUROFARBA) – Section of Pharmaceutical & Nutraceutical Sciences, via Ugo Schiff 6, 50019 Sesto Fiorentino, Italy

* Author for correspondence: novella.romanelli@unifi.it

Nicotinic receptors are membrane proteins involved in several physiological processes. They are considered suitable drug targets for various CNS disorders or conditions, as shown by the large number of compounds which have entered clinical trials. In recent years, nonconventional agonists have been discovered: positive allosteric modulators, allosteric agonists, site-specific agonists and silent desensitizers are compounds able to modulate the receptor interacting at sites different from the orthodox one, or to desensitize the receptor without prior opening. While these new findings can further complicate the pharmacology of these proteins and the design and optimization of ligands, they undoubtedly offer new opportunities to find drugs for the many therapeutic indications involving nicotinic receptors.

First draft submitted: 20 July 2017; Accepted for publication: 22 September 2017; Published online: 13 February 2018

**Keywords:** allosteric modulators • drug design • nicotinic receptors

Nicotinic acetylcholine receptors (nAChR) are ligand-gated ion channels (LGIC), members of a family called Cys-loop receptors owing to the presence, near the N-terminal extracellular domain, of a conserved disulfide bridge between two Cys residues separated by 13 amino acids. These proteins have a pentameric structure, formed of five subunits assembled around a central pore. nAChRs have been known for more than a century, but their fundamental role in the pathophysiology of the CNS emerged only in the last part of the 20th century, after cloning and expression of the neuronal subtypes [1]. The importance of these proteins as drug targets is proven by the high number of compounds that have entered clinical trials for several different CNS applications, some of which are listed in Table 1. However, despite all the effort, success has only been achieved so far for smoking cessation; in many instances, for other diseases or conditions, drug candidates were found endowed with insufficient efficacy and adverse effects.

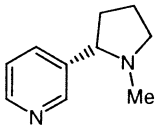
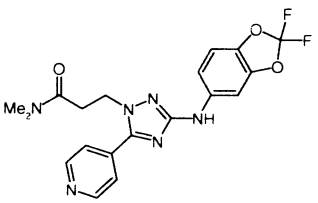
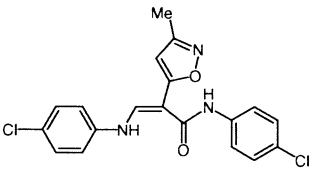
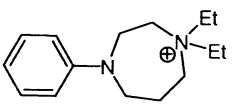
The design of nAChR drugs has been hampered by the complexity of these proteins, and by a lack of structural information on the whole channel, information which has only recently been obtained [50]. On the one hand, complexity refers to the high number of possible subtypes and many physiological processes in which these proteins are involved. Several excellent reviews have been published on this topic, which will only be partly addressed in this paper, and readers are advised to refer to them for in-depth information. On the other hand, some intriguing properties of the protein itself have emerged in recent times, such as the increased numbers of binding sites and their different ability to modulate receptor activity, which will be mentioned in this review. While this complexity may create difficulties for drug design, it also offers opportunities which are yet to be exploited.

## Subunit composition

nAChR subtypes consist of five subunits assembled to form a channel. Twelve subunits have been cloned from neuronal tissues, classified as nine alpha (α2–α10) and three beta (β2–β4); they can be joined into homomeric nAChRs (α7–α9; the α8 subunit has been found in avian but not in mammalian tissue) or heteromeric nAChRs,

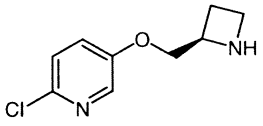
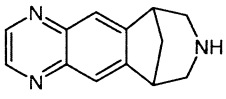
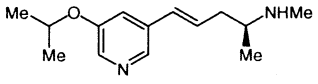
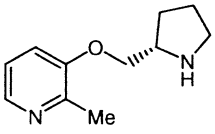
newlands press

**Table 1. Nicotinic agents that are or have been studied in clinical trials by the US NIH†.**

| Drug | Action on nAChR | Pathology; Phase (status) | Results‡ | Ref. |
|---|---|---|---|---|
|  **1** Nicotine | Agonist | Down syndrome; I,II (R) | | |
| | | Mild cognitive impairment; I,II (R) | | |
| | | Parkinson's disease; I,II (C) | | |
| | | Major depressive disorder; IV (R) | | |
| | | ASD; I (R) | | |
| | | Chemo brain; II (R) | | |
| | | Schizophrenia§ | Low or medium doses of nicotine i.v. did not alter symptoms of schizophrenia or attention Modest improvement of nicotine on attention, not in other cognitive functions, in smoking but not in nonsmoking patients | [2,3] |
|  **3** JNJ39393406 | α7 PAM | Schizophrenia, smoking cessation in schizophrenia; II | Failed to reverse basic deficits of information processing | [4] |
|  **4** AVL-3288 | α7 PAM | Schizophrenia; I | Positive although nonsignificant effects on cognition | [5] |
|  **9** ASM024 | α7 silent agonist | Asthma; II | No significant inhibition of allergen-induced asthmatic response and related airway inflammation. Side effect: cough | [6] |
| | | COPD; II (C) | | |

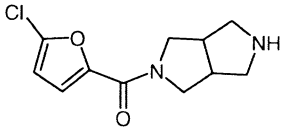
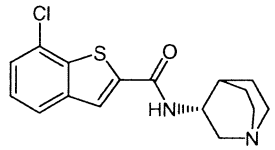
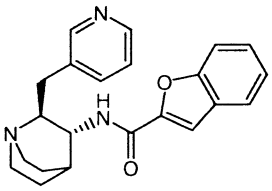
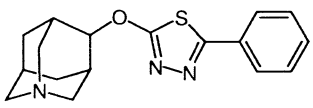
†[42].
‡Information was taken from peer-reviewed papers or from Web news.
§Phase nonreported.
¶From [7].
#Healthy volunteers.
††[43]
‡‡[44]
§§[45].
¶¶Formula and activity retrieved from [46].
##Definition reported on www.clinicaltrial.gov (NCT01293669).
†††[47,48].
‡‡‡[49].
§§§Low-dose nicotine gel, NCT00316537.
A: Active; AChEI: Acetylcholinesterase inhibitors; AD: Alzheimer's disease; ADHD: Attention-deficit hyperactivity disorder; ASD: Autism spectrum disorder; b.i.d.: Twice daily; C: Completed; COPD: Chronic obstructive pulmonary disease; fNMRI: Functional Nuclear Magnetic Resonance Imaging; METH: Methamphetamine; nAChR: Nicotinic acetylcholine receptor; PAM: Positive allosteric modulator; R: Recruiting; T: Terminated; U: Unknown.

**Table 1. Nicotinic agents that are or have been studied in clinical trials by the US NIH[†] (cont.).**

| Drug | Action on nAChR | Pathology; Phase (status) | Results[‡] | Ref. |
|---|---|---|---|---|
|  **17** ABT-594 | α4β2 agonist | Diabetic neuropathic pain; II | Significant efficacy in the treatment of diabetic peripheral neuropathic pain. Side effects: nausea, dizziness, vomiting, abnormal dreams and asthenia | [7] |
|  **21** Varenicline | α4β2 partial agonist; α7 agonist | Nicotine dependence in depression; IV | Increase in smoking cessation in smokers with current or past depression without exacerbation of depression or anxiety | [8] |
| | | Schizophrenia; III | Cognitive improvements as adjunctive treatment with antipsychotics. Side effects: nausea and headache | [9] |
| | | Alzheimer's disease; II | No improvement of cognition in Korean patients with mild-to-moderate Alzheimer's disease. Side effects: gastrointestinal | [10] |
| | | Parkinson's disease; II,IV (R, A) | | |
| | | Obesity; II,III (R) | | |
| | | Alcohol use disorder[§] | Inhibition of alcohol consumption in a population of heavy drinkers | [11] |
| | | Methamphetamine dependence; I | Significant improvement of reaction time on the n-back for visual stimuli in METH-dependent volunteers | [12] |
| | | Cocaine dependence; II (C) | | |
| | | Smoking cessation in schizophrenia; III | Significant efficacy in smoking reduction in people with schizophrenia | [13] |
|  **23** AZD3480, TC1734, ispronicline | α4β2 partial agonist | ADHD; II | Significant improvement of cognitive and clinical ADHD symptoms. Dose: 50 mg | [14] |
| | | Schizophrenia; II | No efficacy. Psychiatric side effects recorded | [15] |
| | | Alzheimer's disease; II | The study was inconclusive | [16] |
|  **24** ABT089 | α4β2 agonist | Nicotine addiction; II (T) | | |
| | | Alzheimer's disease; II (T) | | |
| | | ADHD; II | Effective in a pilot study but non in a larger trial | [17] |

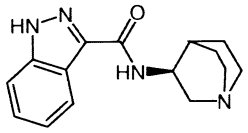
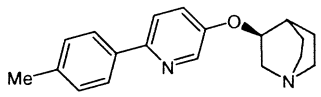
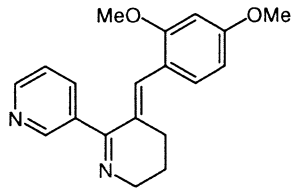
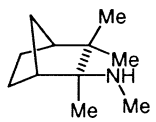
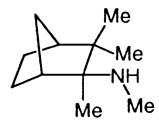
[†][42].
[‡]Information was taken from peer-reviewed papers or from Web news.
[§]Phase nonreported.
[¶]From [7].
[#]Healthy volunteers.
[††][43]
[‡‡][44]
[§§][45].
[¶¶]Formula and activity retrieved from [46].
[##]Definition reported on www.clinicaltrial.gov (NCT01293669).
[†††][47,48].
[‡‡‡][49].
[§§§]Low-dose nicotine gel, NCT00316537.
A: Active; AChEI: Acetylcholinesterase inhibitors; AD: Alzheimer's disease; ADHD: Attention-deficit hyperactivity disorder; ASD: Autism spectrum disorder; b.i.d.: Twice daily; C: Completed; COPD: Chronic obstructive pulmonary disease; fNMRI: Functional Nuclear Magnetic Resonance Imaging; METH: Methamphetamine; nAChR: Nicotinic acetylcholine receptor; PAM: Positive allosteric modulator; R: Recruiting; T: Terminated; U: Unknown.

**Table 1. Nicotinic agents that are or have been studied in clinical trials by the US NIH† (cont.).**

| Drug | Action on nAChR | Pathology; Phase (status) | Results‡ | Ref. |
|---|---|---|---|---|
|  **25** AZD1446, TC6683 | α4β2 agonist | *; I | Side effects: nausea, headache and dizziness | [18] |
| | | ADHD; II | No significant improvements of ADHD symptoms after 2 weeks of treatment | [19] |
| | | Alzheimer's disease; II (C) | | |
|  **26** EVP6124, MT4666, encenicline | α7 partial agonist | Schizophrenia; I | Positive effect on cognitive functions in patients treated with antipsychotic drugs | [20] |
| | | Alzheimer's disease; IIb | After 23 weeks 26 showed improved cognitive performance with respect to placebo. Dose: 2 mg/die | [21] |
| | | Smoking cessation; II (T) | | |
|  **27** TC5619 | α7 agonist | Schizophrenia; II | The positive results of exploratory study were not confirmed in larger trials | [22] |
| | | Alzheimer's disease; I | Discontinued | ‡‡ |
| | | ADHD; II | Discontinued | ‡‡ |
|  **28** ABT126 | α7 agonist | Schizophrenia; II | Lack of a consistent effect on cognition in nonsmoking subjects with schizophrenia; positive effect on negative symptoms | [23,24] |
| | | Alzheimer's disease; IIb | No significant improvement in subjects with mild-to-moderate AD, alone or with AChEI. Side effects: agitation, constipation, diarrhea, fall, headache | [25,26] |

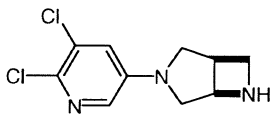
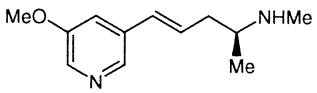
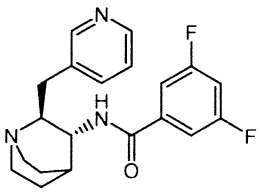
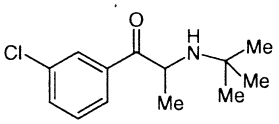
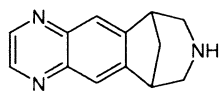
†[42].
‡Information was taken from peer-reviewed papers or from Web news.
§Phase nonreported.
¶From [7].
#Healthy volunteers.
††[43]
‡‡[44]
§§[45].
¶¶Formula and activity retrieved from [46].
##Definition reported on www.clinicaltrial.gov (NCT01293669).
†††[47,48].
‡‡‡[49].
§§§Low-dose nicotine gel, NCT00316537.
A: Active; AChEI: Acetylcholinesterase inhibitors; AD: Alzheimer's disease; ADHD: Attention-deficit hyperactivity disorder; ASD: Autism spectrum disorder; b.i.d.: Twice daily; C: Completed; COPD: Chronic obstructive pulmonary disease; fNMRI: Functional Nuclear Magnetic Resonance Imaging; METH: Methamphetamine; nAChR: Nicotinic acetylcholine receptor; PAM: Positive allosteric modulator; R: Recruiting; T: Terminated; U: Unknown.

**Table 1. Nicotinic agents that are or have been studied in clinical trials by the US NIH† (cont.).**

| Drug | Action on nAChR | Pathology; Phase (status) | Results‡ | Ref. |
|---|---|---|---|---|
|  **29** MEM3454, RG3487 | α7 agonist | Alzheimer's disease; II (C) | | |
| | | Schizophrenia; II | No improvement of cognitive deficits but improvement of negative symptoms. Side effect: constipation | [27] |
|  **30** AQW051 | α7 partial agonist | Schizophrenia; II | Following treatment with **32**, brain activation, measured by means of fNMRI, was not associated with changes in cognitive performance | [28] |
| | | Levodopa-induced dyskinesia in Parkinson's Disease; II | No significant reduction of dyskinesia or Parkinsonian severity | [29] |
| | | Alzheimer's disease; II (T) | | |
|  **31** GTS21, DMXB-A | α7 partial agonist and α4β2 antagonist | ADHD; II (C) | | |
| | | Alzheimer's disease; II (C) | | |
| | | Schizophrenia; II | Significant effects on attention/vigilance and working memory; improvement of negative symptoms | [30] |
| | | Tobacco use disorder; II (A) | | |
| | | Obesity; I (R) | | |
|  S(+)-**32** TC5214 | Antagonist | Major depressive disorder; III | No efficacy was shown in patients with inadequate response to prior antidepressant therapy. Side effects: constipation, dizziness, dry mouth | [31,32] |
|  **32** mecamylamine | Antagonist | Cognition in schizophrenia§ | Worsening of performance relative to placebo and varenicline, to a greater extent in participants with schizophrenia than in healthy controls | [33] |

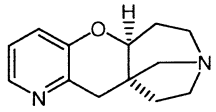
†[42].
‡Information was taken from peer-reviewed papers or from Web news.
§Phase nonreported.
¶From [7].
#Healthy volunteers.
††[43]
‡‡[44]
§§[45].
¶¶Formula and activity retrieved from [46].
##Definition reported on www.clinicaltrial.gov (NCT01293669).
†††[47,48].
‡‡‡[49].
§§§Low-dose nicotine gel, NCT00316537.
A: Active; AChEI: Acetylcholinesterase inhibitors; AD: Alzheimer's disease; ADHD: Attention-deficit hyperactivity disorder, ASD: Autism spectrum disorder; b.i.d.: Twice daily; C: Completed; COPD: Chronic obstructive pulmonary disease; fNMRI: Functional Nuclear Magnetic Resonance Imaging; METH: Methamphetamine; nAChR: Nicotinic acetylcholine receptor; PAM: Positive allosteric modulator; R: Recruiting; T: Terminated; U: Unknown.

**Table 1. Nicotinic agents that are or have been studied in clinical trials by the US NIH[†] (cont.).**

| Drug | Action on nAChR | Pathology; Phase (status) | Results[‡] | Ref. |
|---|---|---|---|---|
| | | Alcohol dependence; II | No significant effect on alcohol consumption in patients with alcohol use disorders | [34] |
| | | Autism; I | No significant benefit was suggested | [35] |
| | | Smoking cessation; II | No evidence of efficacy as adjuvant with nicotine-replacement therapy | [36] |
|  | α4β2 agonist | Diabetic neuropathic pain; II | No efficacy at the dose of 1–4 mg b.i.d. | [37] |
| **33** ABT894, sofinicline | | | | |
| | | ADHD; II | Significant reduction of ADHD symptoms in adults. Dose: 4 mg b.i.d. Side effects: nausea, dizziness, headache and fatigue | [38] |
|  | α4β2 agonist | Pain; II | In postoperative dental pain, the primary end point was not met | [§§] |
| **34** TC2696 | | | | |
|  | α7 agonist;[¶¶] open channel stabilizer[##] | Asthma; II (C) | Some end points were met | [---] |
| **35** TC6987 [¶¶] | | | | |
| | | Type 2 diabetes mellitus; II (C) | Discontinued | [‡‡‡] |
|  | | Smoking cessation; III | Combined use of **21** and **35**, compared with **21** alone, increased prolonged abstinence at 12 and 26 weeks but not at 52 weeks. Side effects: anxiety and depressive symptoms Higher efficacy, after 12-week treatment, in patients not responding to prequit nicotine patch treatment | [39,40] |
|  | | | | |
| **36**, bupropion and **21**, varenicline | | | | |
| | | Alcohol use disorder; II (A) | | |

[†][42].
[‡]Information was taken from peer-reviewed papers or from Web news.
[§]Phase nonreported.
[¶]From [7].
[#]Healthy volunteers.
[---][43]
[‡‡][44]
[§§][45].
[¶¶]Formula and activity retrieved from [46].
[##]Definition reported on www.clinicaltrial.gov (NCT01293669).
[---][47,48].
[‡‡‡][49].
[§§§]Low-dose nicotine gel, NCT00316537.
A: Active; AChEI: Acetylcholinesterase inhibitors; AD: Alzheimer's disease; ADHD: Attention-deficit hyperactivity disorder; ASD: Autism spectrum disorder; b.i.d.: Twice daily; C: Completed; COPD: Chronic obstructive pulmonary disease; fNMRI: Functional Nuclear Magnetic Resonance Imaging; METH: Methamphetamine; nAChR: Nicotinic acetylcholine receptor, PAM: Positive allosteric modulator; R: Recruiting, T: Terminated; U: Unknown.

**Table 1. Nicotinic agents that are or have been studied in clinical trials by the US NIH† (cont.).**

| Drug | Action on nAChR | Pathology; Phase (status) | Results‡ | Ref. |
|---|---|---|---|---|
|  | α4β2 Partial agonist | Smoking cessation; III | No efficacy for smoking cessation beyond the initial treatment phase. Side effects: diarrhea, nausea | [41] |
| **37** SSR591813, Dianicline | | | | |
| ATG002§§§ | Agonist | Diabetic foot ulcers; I,II (T) | | |

†[42].
‡Information was taken from peer-reviewed papers or from Web news.
§Phase nonreported.
¶From [7].
#Healthy volunteers.
††[43]
‡‡[44]
§§[45].
¶¶Formula and activity retrieved from [46].
##Definition reported on www.clinicaltrial.gov (NCT01293669).
†††[47,48].
‡‡‡[49].
§§§Low-dose nicotine gel, NCT00316537.
A: Active; AChEI: Acetylcholinesterase inhibitors; AD: Alzheimer's disease; ADHD: Attention-deficit hyperactivity disorder; ASD: Autism spectrum disorder; b.i.d.: Twice daily; C: Completed; COPD: Chronic obstructive pulmonary disease; fNMRI: Functional Nuclear Magnetic Resonance Imaging; METH: Methamphetamine, nAChR: Nicotinic acetylcholine receptor; PAM: Positive allosteric modulator; R: Recruiting; T: Terminated; U: Unknown.

the latter being composed by α(α2–α7) and β(β2–β4) subunits or by α9 and α10 [51]. At the neuromuscular junction, the muscle-type nicotinic receptor is formed by α1, β1, δ and γ (fetal) or ε (adult) [1].

Based on binding studies, the nicotinic receptors can also be classified as sensitive and insensitive to α-Bungarotoxin (αBgtx). αBgtx-sensitive receptors contain α7–α10 subunits, but also the muscle-type receptor is αBgtx-sensitive. The αBgtx-insensitive receptors are made up of combinations of neuronal α and β subunits [52]. More than 90% of these receptor subtypes in rodent brain are α4β2* nAChRs (* indicates the possible presence of other subunits): autoradiographic studies of [$^3$H]-nicotine binding sites in brain showed correspondence with an expression pattern for α4 and β2 subunits ([53] and references therein).

The 12 neuronal subunits can theoretically associate into many combinations, raising the number of possible receptor subtypes; however, it is not known if all possible combinations give functional receptors. There is evidence that some particular combinations are more abundant and physiologically relevant: for instance, in neurons the α7 subtype is mainly homomeric; the α4β2 subtype is the most abundant heteromeric subtype in brain of several species; the α3β4 is the most expressed at ganglions (see [1,54] and references therein). In many instances, the initial screening of new nicotinic ligands, including some of the compounds reported in Table 1, has been performed on heterologously expressed homomeric α7 and heteromeric α4β2 subtypes.

However, the definition of selectivity may change over time due to new experimental findings on subunit assembly, among which stoichiometry, which will be discussed later. For instance, it has recently been shown that the α7 subunit also forms heteromeric channels: α7 and β2 subunits can coassemble in both heterologously expressed systems and in native brain neurons, showing, with respect to homomeric α7 channels, different functional properties and sensitivity to agonists (reviewed in [55]). Tissue and subcellular localization, or sensitivity to oligomeric amyloid-$\beta_{1-42}$ protein ($A\beta_{1-42}$) are some intriguing properties of this subtype, suggesting a different role with respect to homomeric α7 receptors [55,56].

Heteromeric receptors may be formed of two, three or four different subunits, giving functional combinations differing in pharmacological and biophysical properties [52]. It is difficult to study these receptors in native tissues because different combinations can colocalize in the same area [57]. Expression in recombinant systems may speed up receptor characterization and may help in finding new ligands to be used as pharmacological tools to study the receptors in native tissues. For instance, the $(\alpha3\beta4)_2\alpha5$ subtype has been expressed in HEK293 and CHO cells and a library of compounds was tested by means of high-throughput screening (HTS) to discover compounds able to discriminate between the $(\alpha3\beta4)_2\alpha5$ and $(\alpha3)_2(\beta4)_3$ subtypes [58,59]. Also the α6β2β3 has been expressed in HEK293 cells and studied by means of HTS. In this case, in order to overcome problems of functional expression, a chimeric α subunit was used that was formed of the extracellular domain of α6 (containing the acetylcholine

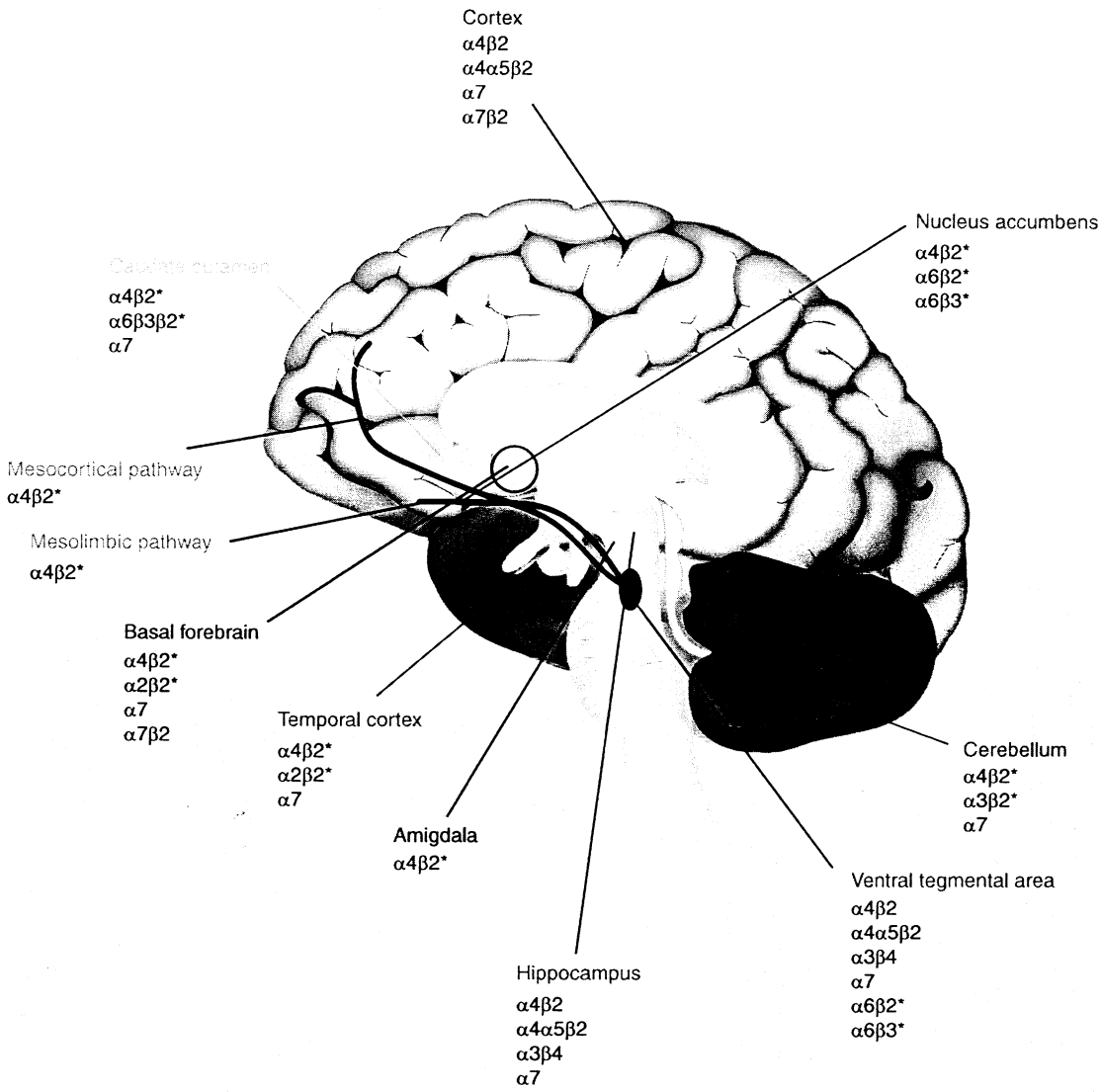
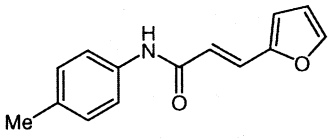



**Figure 1. Nicotinic acetylcholine receptor distribution in human brain.**
Reproduced with permission from [57], and implemented.

[ACh]-binding site) and the transmembrane domain of the α3 subunit, and a mutation was introduced into the β3 pore-lining region [60].

## nAChR distribution & function

The α or β subunits are differently expressed in various neuroanatomical regions (Figure 1). The α4β2* receptors are widely distributed throughout the brain; the α7* subtypes are also highly expressed, particularly in the hippocampus, while other subunits have a more restricted localization [1,52,54,57]. nAChRs may have a pre-, post- or nonsynaptic location, which accounts for different modulating activities on transmission and neuronal excitation. Most nAChRs have a presynaptic localization and modulate the release of both excitatory and inhibitory neurotrasmitters. Therefore, the activation of nAChRs can have opposite modulatory effects on the same circuits depending on whether they induce the release of excitatory or inhibitory neurotrasmitters [1]. In particular, dopamine release is modulated by α4β2*, α3β2* and α6* nAChRs in nigrostriatal terminals [61], while presynaptic α7 nAChRs regulate




2 (PAM2)

**Figure 2. Structure of the positive allosteric modulator, PAM-2 (2).**

glutamate and GABA release [62]. Presynaptic nAChRs are also involved in regulating the release of ACh, serotonin, and noradrenaline [63].

Nicotinic receptors play a role in synaptic plasticity and development, and participate in cognitive processes. Several diseases or conditions have been associated with changes in function or expression of nicotinic receptors (see [64] and references therein, and section on 'Therapeutic potential' in this paper), some of which such as epilepsy or schizophrenia, may have a genetic cause. In many instances, the involvement of nAChRs has been evidenced by using knock-out mice [65,66].

## nAChR modulators

Nicotinic ligands are usually classified as agonists, antagonists or allosteric modulators; the prototypical agonist is nicotine (**1**, structure in Table 1). Agonists bind to the orthosteric site, fully or partially activating the receptor. Competitive antagonists bind at or near the ACh-binding site and their interaction is mutually exclusive with that of agonists, while pore blockers behave as noncompetitive antagonists, binding within the channel and thus hindering the ion flow. The majority of the compounds that have entered clinical trials are full or partial agonists interacting at the orthosteric site. The difficulty in designing subtype-selective ligands lies in the high degree of homology within the orthosteric sites: several compounds are known which are able to activate or block more than one subtype, or that behave as agonist on one subtype and as antagonist on another.

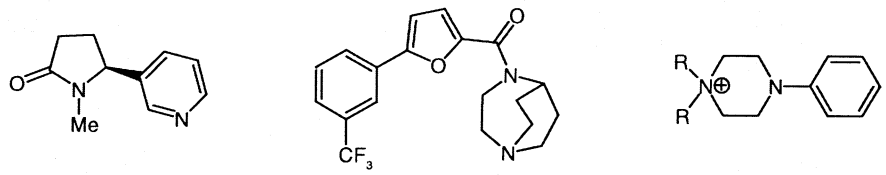
Allosteric modulators interact with sites different from those of the neurotransmitter, and affect the transition between functional states [64]. Positive allosteric modulators (PAM) increase the apparent affinity of the receptor for the agonist and reduce the concentration required to achieve channel opening, while negative allosteric modulators behave in the opposite way. The α7 nAChR PAMs have been classified as either type I or type II on the basis of a difference in their effect on receptor desensitization. Type I PAMs increase the apparent peak amplitude of agonist-evoked responses with little effect on desensitization kinetics, whereas type II PAMs reduce desensitization and reactivate, to a certain extent, desensitized α7 nAChRs [67].

Receptor modulation with allosteric ligands is gaining interest because these molecules have the potential to be more subtype selective than orthosteric modulators since the neurotransmitter-binding site is highly conserved, while the allosteric-binding sites are not. In addition, PAMs may have more than one binding site [68]; interaction with those sites may modulate differently activation/desensitization mechanisms, which can also affect selectivity. PAMs are also attractive molecules because they are able to stimulate the receptor up to a physiological level, their action depending upon the concentration of the endogenous agonist. At the same time, this property may be a limitation in conditions where availability of the endogenous agonist is impaired, for instance, due to neuronal loss. Moreover, the ability of type II α7 PAM to reactivate desensitized α7 receptors may cause some trouble due to the high calcium permeability of these proteins, which in turn may cause calcium overload and toxicity [69].

In animal models, several α7 PAMs have demonstrated efficacy in different experimental conditions. As an example, compound **2** (PAM-2, Figure 2), a recently discovered type II PAM at α7 receptors, showed procognitive and antidepressant activity in rodents, reduced neuropathic and inflammatory pain, and ameliorated ketamine-induced cognitive impairments [70–73]. However, two PAMs (**3**, JNJ-39393406 and **4**, AVL-3288, Table 1) have entered clinical trials, giving contrasting results (see later in this review). Therefore, more work is needed to understand the real therapeutic potential of PAMs.

## Silent agonists (silent desensitizers)

nAChRs can be desensitized by continuous or repeated exposure to an agonist (e.g., **1**), resulting in a progressive decrease in response to a subsequent stimulus. α4* receptors desensitize slowly after exposure to a low amount of




**5**, cotinine **6**, NS6740 **7**, DMPP (R = Me)
**8**, DEPP (R = Et)

**Figure 3. Chemical structure of silent agonists.**

agonist, but desensitization is long lasting; α7* receptors desensitize after exposure to high agonist concentration, but desensitization is readily reversible [74].

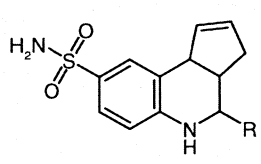
Receptor desensitization is a conformational transition to a nonconducting agonist-bound state: after an initial receptor activation producing an ion current, the system is actually blocked, with the channel being in a conformation that has high affinity for agonist [74]. Recently, two different desensitized states have been recognized for the α7 receptors, obtained under different conditions of agonist application, one sensitive and the other insensitive to type II PAM [75]. Desensitization has been suggested as playing an important role in shaping the effects of nicotinic receptor activation, and altered desensitization mechanisms have been associated with some disease conditions such as autosomal dominant nocturnal frontal lobe epilepsy, congenital myasthenic syndrome and cancer (see [76] and references therein).

Since the consequence of desensitization is a nonconducting state of the receptor, it is not clear if agonists produce a particular pharmacological effect and actually function as inhibitors. Indeed, in several instances, some nAChR antagonists have been shown to evoke agonist-like nicotinic effect [77]. In this context, the activity of ligands affecting desensitization, in a way that is different with respect to classical agonists, could cast some light. For instance, type II α7-PAMs, as mentioned above, when coadministered with an agonist (i.e., ACh) reactivate one of the desensitized states of the receptor; allosteric agonists, which do not induce desensitization, will be discussed in the next section.

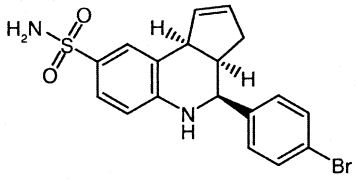
Compounds able to directly desensitize the receptor without prior activation are called 'silent agonists' or 'silent desensitizers' [77,78]; such behavior has been reported for nicotine (**1**) at very low concentration (10 nM) on α4β2 receptors expressed in HEK293 cells [79], or for **5** (cotinine, Figure 3), a nicotine metabolite, on ganglionic nicotinic receptors in rats [80].

Recently, silent agonists have mainly been studied on α7 receptors, and an example of such compounds is **6** (NS6740; Figure 3): *in vitro* characterization revealed a very weak activation (<2%) of the α7 receptor and antagonistic properties toward ACh; agonist-like effects were revealed when coadministered with a type II PAM, which reactivates the conducting state, or when tested on a slowly desensitizing mutated receptor [81]. *In vivo*, this molecule did not improve cognitive performance in the mouse passive avoidance test [81], but it blocked the procognitive activity of the nicotinic partial agonist BMS-902483 in the mouse novel object recognition test [82]. These findings suggest that activation of α7 receptors is an important step in α7-mediated cognitive improvement. On the other hand, **6** is active in different models of chronic (formalin test and chronic constrictive nerve injury-induced neuropathy) but not acute pain (tail-flick and hot-plate tests) [83]. As well as the α7 antagonist methyllycaconitine, **6** is able to reduce lipopolysaccharide-induced TNF-α release from microglia [84]. Indeed, the α7 nAChR-mediated modulation of TNF-α release has been associated with a desensitized, nonconductive state of this receptor [85].

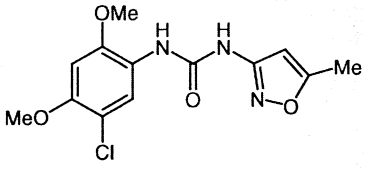
Silent agonists are ligands binding to the orthosteric site, whose pharmacophore should, at least partially overlap with that of agonists. Some structure–activity relationships have been inferred by Papke *et al.*: while the simplest structure-activating nicotinic receptor is tetramethylammonium, replacing methyl with ethyl groups gave tetraethylammonium, which is the minimal pharmacophore for α7 silent agonism [86]. In a similar way, by replacing the two methyl groups with ethyl moieties, the ganglion stimulating and α7 full agonist dimethylphenylpiperazinium iodide (**7**) was transformed into the α7 silent agonist diethylphenylpiperazinium (**8**, Figure 3) whose structure was then further manipulated to obtain more potent molecules [87].



**10**, 4BP-QTS (R = 4-Br-phenyl)
**11**, TQS (R = 1-nafthyl)



(+)-**10** GAT107



**12**, PNU120596

**Figure 4. Structure of positive allosteric modulators and allosteric activators.**

Silent agonists can be either permanently charged compounds or tertiary amines. While amines are required to study receptors in the CNS, ammonium ions can be used for modulating peripheral α7 receptors, for instance, in neuropathy and inflammation. Indeed, a permanently charged compound, the homopiperazinium derivative **9** (ASM024, Table 1), has entered clinical trials for stable moderate asthma (see section on 'Pain and inflammation').

## Allosteric agonists

Recently, allosteric agonists have also been reported, in other words, molecules able to directly activate the receptor acting at a site different from that of the neurotransmitter. Compound **10** (4BP-TQS; Figure 4), a compound structurally related to the type II PAM TQS (**11**) [88], potentiated the activity of ACh after coapplication, exhibiting a type II PAM behavior. However, when administered alone, it was able to directly activate the α7 receptor. Methyllycaconitine antagonized the effect of **10** in a noncompetitive way, suggesting an interaction on a different binding site, which was confirmed by the activity on mutated receptors [89]. Several other analogs have been tested and some structure–activity relationships have been established, showing that small changes in structure can produce a different receptor modulation, going from direct activation to positive, negative or silent allosteric modulation [90–92]. Interestingly, contrary to ACh and other α7 orthosteric agonists, **10** activated the receptor relatively slowly and it did not produce a rapid desensitization but rather a very slow desensitization that occurred over a period of minutes [90]. Subtype selectivity was checked on recombinant receptors in *Xenopus* oocytes: 4BP-TQS was able to activate only α7 receptors, but it behaved as antagonist on α3β4, α4β2 and muscle-type receptors. In primary rat hippocampal neurons, **10** increased calcium influx with micromolar potency, therefore showing activity also on native α7 receptors [93].

The binding site of (+)-**10** (GAT107, the active enantiomer [94]) has been studied by site-directed mutagenesis. The two kinds of activity, direct activation and positive allosteric modulation, seem to be produced by interaction at two distinct sites: one should be the same transmembrane site where PAMs like **12** (PNU-120596; Figure 4) bind, while the other site, required for direct activation, seems to be at the extracellular domain vestibule, in a solvent accessible position (Figure 5A) [92]. Up to now, (+)-**10** has been tested in models of acute and chronic pain, being active only on the latter, like **6** and also like other α7 agonists and PAMs [83,95–96]. Interestingly, in the mouse formaline test, the antinociceptive activity of the selective α7 agonist PNU-282987 was found dependent on the activation of peroxisome proliferator-activated receptors-α (PPARα), since it was blocked by the PPARα antagonist GW6471. However, GW6471 was not effective on the antinociceptive activity of **12**, **6** or (+)-**10**, suggesting that PPARα activity is involved only when the α7 receptor is activated from the orthosteric site [97].

## Site-specific agonists

A feature greatly affecting receptor properties is subunit stoichiometry. When expressed in recombinant systems, α4 and β2 subunits can coassemble in two different complexes, composed of 3α4 and 2β2 or 2α4 and 3β2 subunits and characterized, respectively, by low and high sensitivity to agonists [99,100]. This feature is not restricted to this subunit combination and it has also been found for α3β4, α2β2 and α6β2 [101–103]. Significantly, there is evidence that combinations of α4 and β2 subunits with different stoichiometries exist in the brain [104,105], possibly associated with different modes of transmission or different physiological functions [106].

The difference in properties between $(\alpha4\beta2)_2\beta2$ and $(\alpha4\beta2)_2\alpha4$ is due to the presence of an additional binding site: the α4/α4 interface, evidenced by means of subunit concatamers with fixed arrangement order, which produces

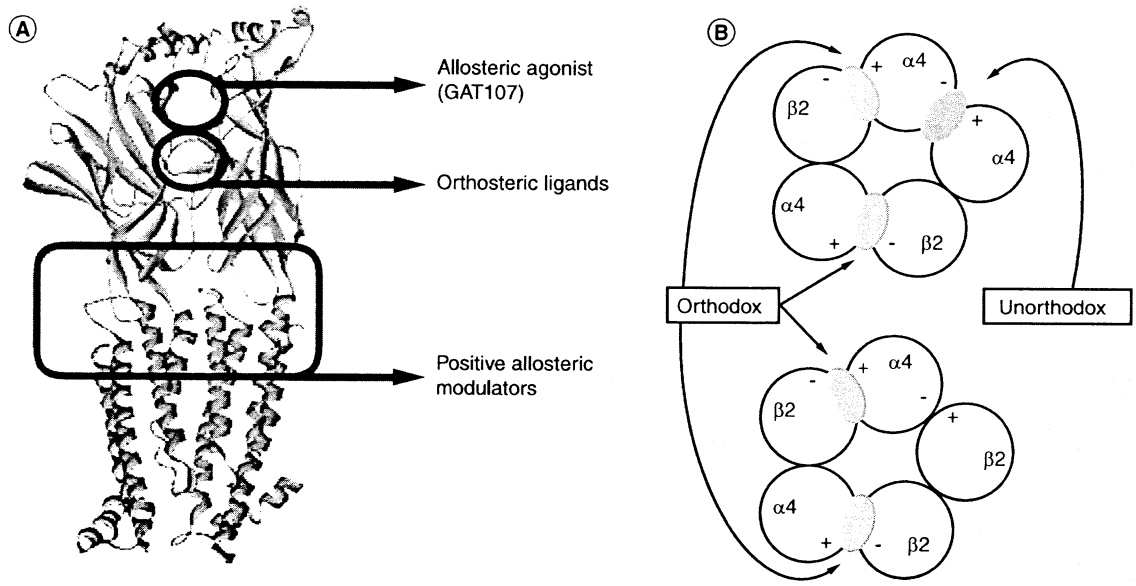



**Figure 5. Binding sites on the nicotinic receptor. (A)** Location of the binding site for nicotinic activators. The orthosteric-binding site is in the extracellular domain, at the subunit interface; orthosteric, site-specific and silent agonists bind in this region. The interaction site for the allosteric agonist GAT107 seems located nearby, in the vestibule [92]. Positive allosteric modulators bind in a region that comprises the gating interface and the transmembrane domain [98]. **(B)** The two different stoichiometries of α4β2 receptors, with indication of the orthodox- and unorthodox-binding sites.

a functional agonist binding site, called unorthodox as a way to differentiate it from those (i.e., the orthodox ones) at the α4/β2 interfaces (Figure 5B) [107]. The list of subunits that can form unorthodox-binding sites comprises α2–6 and β3 (reviewed by Wang and Lindstrom [98]), thus it includes α5 and β3 subunits which were in the past thought unable of involvement in the formation of functional binding sites.

The number of ACh-binding sites has always been related to the number of α subunits, which provide the principal component (positive face). For instance, homomeric α7 receptors contain five equivalent binding sites: the occupancy of only one is required for maximal activation while higher occupancy increases agonist sensitivity [108]. The unorthodox site represents a third, nonequivalent agonist binding site on heteromeric receptor, affecting channel properties such as conductance, mean open lifetime and desensitization [109–112]. Pharmacological properties also change: at $(\alpha4\beta2)_2\alpha4$ (the low-sensitive subtype), agonists usually show lower potency (higher $EC_{50}$ values) with respect to $(\alpha4\beta2)_2\beta2$ (the high-sensitive subtype). On the other hand, efficacy does not change in a parallel way. For instance, the efficacy of epibatidine (**13**) is greater on $(\alpha4\beta2)_2\alpha4$ than on $(\alpha4\beta2)_2\beta2$, while the reverse is true for **14** and **15** (TC2559 and sazetidine-A, respectively, Figure 6) [107]. This can be explained by the ability of epibatidine to bind to both orthodox and unorthodox sites, the latter being responsible for the more efficacious activation. On the contrary, agonists such as **14** and **15** are selective toward the α4/β2 site: the low or absent affinity for the unorthodox α4/α4 site limits or precludes activation of the $(\alpha4\beta2)_2\alpha4$ subtype.

Agonists which behave as α4/α4 site-specific modulators are also known [111]. One such compound is **16** (NS9283; Figure 6), which was initially classified as α4β2 PAM for its potentiating activity toward nicotinic agonists *in vitro* and, *in vivo*, in different models for pain [113–115]; subsequent studies showed that it interacts with the α4/α4-binding site [116,117]. **16** is selective for the unorthodox site, with respect to the canonical α4/β2 one, and therefore is able to discriminate between the $(\alpha4\beta2)_2\alpha4$ and $(\alpha4\beta2)_2\beta2$ receptors. Unfortunately, it is not selective for the α4/α4 interface. In fact, it was able to also activate receptors with unorthodox sites formed of α2/α4, α4/α2, α4/α3, α4/α4, α4/α6 subunits, being however inactive on α3/α3, α4/β2, α4/β4 and α7/α7

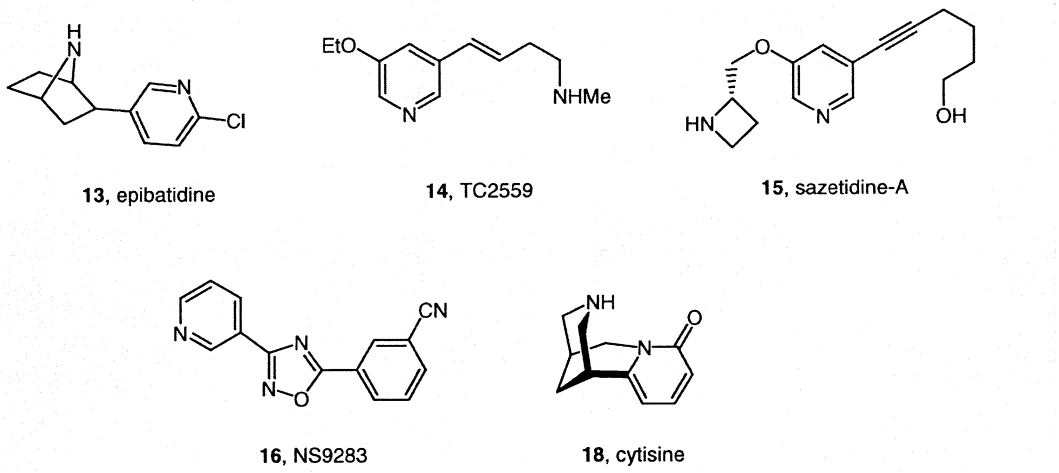



**Figure 6. Structure of epibatidine and of site-selective agonists.**

sites [111,116]. From a structural point of view, **16** is not an allosteric ligand since it binds to one of the ACh-binding sites. However, binding only at the accessory site is not enough to activate the receptor [111], so the *in vivo* activity of this molecule depends on the endogenous ACh or on the addition of a nicotinic agonist. Administered alone, **16** showed procognitive activity in different models of cognition [116], and in medial prefrontal cortex it enhanced glutamate release mediated by the cholinergic neurons or evoked by low concentration of nicotine [118]. Moreover, in several animal models coadministration with **17** (ABT-594; Table 1) potentiates the analgesic activity but not adverse effects [115].

On the contrary, compound **14** (TC2559; Figure 6) is a selective modulator for the α4/β2-binding site, activating the $(\alpha4\beta2)_2\beta2$ receptor but showing very low efficacy at the $(\alpha4\beta2)_2\alpha4$ subtype [107]. In addition, it shows *in vitro* selectivity for α4β2 compared with other subtypes [119,120] and displays interesting pharmacological properties: it increased dopamine release in rat ventral tegmental area slices [120] and exhibited analgesic activity in different rodent models of pain [121,122]. **14** significantly improved performances of rats in two different cognitive tasks [119] but, unexpectedly, suppressed stimulation-induced long-term potentiation in rat dentate gyrus, an effect that was prevented by the nicotinic antagonist dihydro-β-erythroidine [123].

The binding ability of the agonist is dependent on the complementary face of the adjacent subunit, as demonstrated for **15** (Figure 6), **16**, and cytisine (**18**) by site-directed mutagenesis [124]. Three residues on the complementary component are hydrophobic (V109, F117 and L119) on β2 and can interact with the hexynyl group of **15** with higher affinity with respect to the analogous residues of the α4 subunit (H114, Q122 and T124), which are hydrophilic. The triple mutation of the residues in α4 to resemble β2 gave a receptor with stoichiometry $(\alpha4\beta2)_2\alpha4$, which can be activated by **15**, while the wild type is not, but cannot be activated by **16,** while the wild type can be. In a similar way, mutation of F117Q in the β2 subunit gave a $(\alpha4\beta2)_2\beta2$ receptor that was activated by **18**, which is ineffective on the wild type.

These findings may be useful to design site-specific agonists, although more work is still needed to understand if these molecules are only pharmacological tools to study receptor stoichiometry or whether they have therapeutic potential.

## Structure of the binding site & mode of interaction of nicotinic agonists

The structure of the nicotinic receptor has been extensively studied by means of electron microscopy in the final decades of the last century, thanks to the possibility of purifying this protein from Torpedo ray, which contains the muscle-type receptor in large quantities. These studies led to the creation of a model of the whole channel at 4 Å resolution [125]. This was the only structure of the whole channel available up to 2016 when the crystal structure of the high affinity $(\alpha4\beta2)_2\beta2$ receptor was resolved in complex with nicotine, in the desensitized form, as the first example of crystal structure of a heteromeric pentameric LGIC [50]. This structure revealed important

features in the binding site and in the overall architecture which can be compared with other Cys-loop receptors and LGICs. Moreover, it also allowed exploration of the differences between the binding site and the β2/α4 and β2/β2 interfaces and led to a possible explanation of why in these locations the binding of nicotine is precluded. The structural features emerging from recent crystal structures of $(\alpha4\beta2)_2\beta2$, the extracellular domain of $(\alpha2)_5$ [126] and of monomeric α9 [127] have recently been reviewed [128].

Until resolution of the $(\alpha4\beta2)_2\beta2$ receptor, the architecture of the orthosteric-binding site was known from the crystal structure of *Lymnaea stagnalis* acetylcholine-binding protein (AChBP) [129] and several other analogs (reviewed in [130]). AChBP is a structural homolog of the extracellular domain of nAChR, the region harboring the ACh-binding sites. Several nicotinic ligands have been shown to bind to AChBPs with high affinity [131]. The discovery of these proteins was important in the design of new nicotinic ligands, which was carried out not only by means of homology models of the binding site but also by exploiting the possibility of cocrystallization and by applying fragment-based techniques. As a matter of fact, in the Protein Data Bank (PDB), up to July 2017, the number of structures of AChBP, alone or in complex with ligands, was greater than 110. Some examples of successful strategies can be found in [132–136].

The structure of AChBP in complex with nicotinic ligands provided the first picture of the possible arrangement of the binding site, partly suggested from previous studies [137] and later confirmed by the structure of the whole channel. The ACh-binding site is made of six (A–F) loops: A–C are on the principal component (positive side, provided by the α subunit), while D–F are on the complementary component (negative side, provided by the β subunit, by another α subunit in a homomeric receptor, or by the γ or δ subunits in the muscle-type receptor). This description is valid for the orthodox site of heteromeric receptors, while in the unorthodox site both components can be given by α and β subunits, depending on the arrangement. Several aromatic residues (the aromatic cage, among which are a Trp residue in loop B, and three Tyr residues: one in loop A and two in loop C) are present in the active site and can establish the pivotal π–cation interaction with the ammonium group of ACh. Usually this interaction is made by the TrpB residue. When the cationic group is a protonated amine, there is in the AChBP-**1** complex an additional interaction between the agonist and TrpB, consisting of a H-bond between the $NH^+$ and the backbone CO. The other important interaction, as predicted by nicotinic pharmacophoric models (see [138] and references therein), is the H-bond formed by the acceptor group of the ligand (the pyridine nitrogen of nicotine or the carbonyl oxygen atom of ACh). In the complex AChBP-**1**, this H-bond is possible thanks to a water molecule, connected to the NH group of a metionine and to the CO moiety of a leucine in loopE. These two residues become Asn and Leu, respectively, in the nicotinic receptors, and are conserved in all subtypes.

Although in the recently resolved crystal structure of the **1**-$(\alpha4\beta2)_2\beta2$ receptor complex only the π–cation interaction is visible, all three interactions are important in agonist activation, as demonstrated by the studies performed by the group of Dougherty and Lester using nonsense suppression methodology [139]. This technique consists of replacing the key residues with unnatural amino acids showing lower abilities to be involved in H-bond or π–cation interactions: the potency of agonists to activate the mutated receptors, measured by means of electrophysiology and compared with wild-type receptor, indicates if that particular interaction is significant for the function of the protein. The results strongly support the idea that contribution of the three interactions varies depending on both agonist and receptor subtype (reviewed in [140]).

Limiting the analysis to the π–cation interaction (Figure 7), at the muscle-type receptor, ACh and **13** interact only with TrpB, and not with TyrA and TyrC2. On the contrary, and surprisingly, for **1** the π–cation interaction with TrpB is already weak, so that its reduction does not substantially affect nicotine potency. At the α4β2 receptor, both ACh and **1** interact strongly with TrpB, and not with the other residues of the aromatic cage. However, secondary amines such as **19** (metanicotine), **20** (nornicotine), **21** (varenicline) and **22** (TC299423, a α6-preferring agonist [141]) establish a functional π–cation interaction with both TrpB and TyrC2 [142]. On the α7 subtype, π–cation interaction is formed between ACh and TyrA, and between **13** and both TyrA and TyrC2, but not with TrpB. On the α6β2 subtype, π–cation interaction with TrpB is strong for ACh but not for **1**, as is the case on the muscle-type receptor, nor for **22** [103]. These findings may give useful hints for designing selective nicotinic ligands, thus providing a structural basis for molecules with particular geometric or conformational features.

## Therapeutic potential

Below we discuss the role of nAChRs in the etiology and treatment of pathologies for which nicotinic ligands have reached clinical trials (compound structures are shown in Table 1).

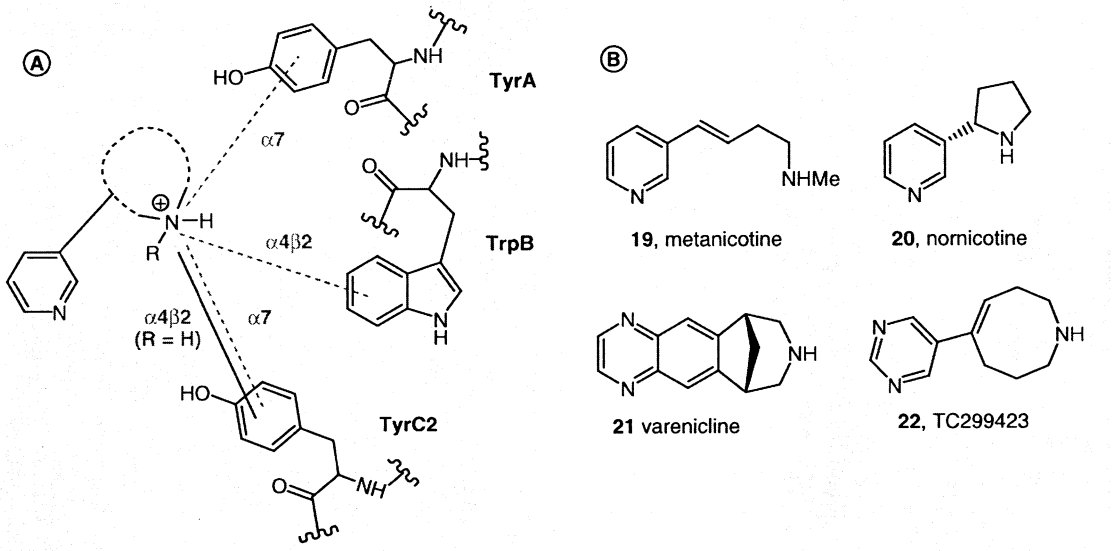



**Figure 7. Cation–π interactions of protonated amines within the nAChR binding site, studied by means of nonsense suppression methodology. (A)** The cationic nitrogen makes a cation–π interaction (blue) with TrpB in the α4β2 receptors; when the nitrogen atom is in a secondary amine, a similar interaction is made also with TyrC2. On the α7 receptor, the cation–π interaction is made with TyrC2 and/or with a TyrA (red). See also text for explanation. **(B)** Structure of some of the ligands used in these studies.

## Neurological disorders

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by loss of memory and other cognitive functions; histopathological markers include the presence of neuritic plaques, formed of β-amyloid protein ($A\beta_{1-42}$), and of neurofibrillary tangles, due to hyperphosphorylated tau protein. Postmortem analysis of brains from AD patients showed an important decrease of nAChRs, in particular of the α4β2* subtype in the cerebral cortex and α7* subtype in the hippocampus; the latter seems to correlate with the severity of cognitive impairment [143]. $A\beta_{1-42}$ interacts with nicotinic receptors [144]. Affinity of Aβ for α7 nAChRs is in the picomolar range and α7 subunits are colocalized with $A\beta_{1-42}$ in senile plaques [145]; heteromeric α7β2 nAChRs were much more sensitive to $A\beta_{1-42}$ inhibition than homomeric α7 nAChRs [146]. There are some findings implicating α4β2 nAChRs in AD: Aβ has inhibitory effects on these and other β2* receptors [147], while no colocalization was found between Aβ with α4 subunit [148]. The therapeutic benefits of employing α7 nAChR agonists for AD therapy are due to the procognitive effects of α7 nAChR agonists and to the protective role of α7 nAChRs in relation to $A\beta_{1-42}$ neurotoxicity.

However, also α7 antagonists can interfere with nAChR-Aβ interaction [148]. In fact, the interruption of α7 nAChR function could be beneficial in the treatment of AD, as demonstrated on a transgenic mouse model of AD overexpressing amyloid precursor protein but lacking the α7 *nAChR* gene [149]. At the moment, this approach is not being pursued. As a matter of fact, the compounds that have been tested in clinical trials act as full or partial agonists at α4β2 (**23–25**) and α7 (**26–30**) receptors, or show a dual α7 and α4β2 activity (**21** and **31**; Table 1). The compounds tested so far have limited success: only EVP6124 (**26**) seems effective in improving cognitive performance in patients with mild-to-moderate AD [21].

An AD-like pathology also occurs in adults with Down's syndrome, in the third decade and beyond. The production and deposition of amyloidogenic Aβ peptides are faster due to the extra copy of the *APP* gene on chromosome 21 [150]. α7 nAChR modulation has been proposed as treatment [151] but presently only the nonselective agonist nicotine (**1**) is being tested in a Phase I–II clinical trial.

Parkinson's disease (PD), characterized by a prominent decline in the nigrostriatal dopaminergic pathway, is characterized also by a pronounced decline in nAChR expression in the cerebral cortex of patients [152]. The decline is particularly evident in α6β2* nAChRs and less in the α4β2* receptor subtype, while there is no change in α7 receptor expression. An explanation may be the diverse localization of these subtypes in this tissue: α6β2* nAChRs are primarily located on incoming dopaminergic afferents, while α7 receptors are positioned on nondopaminergic

terminals, and α4β2* are on dopaminergic, GABAergic and serotoninergic neurons [153]. The cholinergic and dopaminergic systems are closely connected and this finding supports the hypothesis that nAChR drugs may be useful for PD therapy, also because nicotine facilitates dopamine release on nigrostriatal neurons. Moreover, epidemiological studies have shown that smoking is inversely associated with PD [154] and the lower risk of contracting the disease depends on smoking duration, intensity and how recent is cessation. Nicotinic ligands that have entered clinical trials are **21** (varenicline), **30** (AQW051) and nicotine, tested for treatment of excessive daytime sleepiness, of gait and balance impairment and of levodopa-induced dyskinesia.

## Psychiatric diseases & neurodevelopmental disorders

Schizophrenia is a neurodevelopmental disorder that shows symptoms that can be classified as positive, negative or cognitive. The high percentage of smokers among schizophrenic patients, the positive effect of nicotine on cognitive processes and other evidence (reviewed in [30]) have suggested the modulation of α7 receptor as possible therapeutic intervention. As seen in AD, also in brains from schizophrenic patients, α7 nAChR protein expression is decreased to different degrees according to the cortex region (frontal, parietal and dorsolateral prefrontal) [155,156]. Although there is less biological data linking the α4β2 nAChR to schizophrenia, this receptor has also been targeted for the treatment of cognitive impairments associated with this disease [64]. The systemic administration of type II α7 and α4β2 nAChR PAMs has recently been suggested for the treatment of CNS disorders and in particular for schizophrenia [106,157]. Indeed α7* nAChR agonists and PAMs currently appear to be the most effective hope for the treatment of schizophrenia. The amelioration of cognitive deficits is important to improve negative symptoms and the quality of life, also because antipsychotic medication has a slight effect on cognitive symptoms in schizophrenic patients.

Several α7 agonists (**26–31**) and PAM (**3**, **4**) entered clinical trials. Some interesting results were found for **28**, **29** and **31**, especially on negative symptoms. Clinical trials on PAMs gave contrasting results: compound **3** (JNJ-39393406), a type II α7-PAM developed at Janssen Pharmaceutical, was tested on regularly smoking male patients with schizophrenia, but failed to reverse basic deficits of information processing [5]. A Phase I trial on healthy subjects for **4** (AVL-3288, a type I α7-PAM) indicated positive although nonsignificant effects on cognition, which need to be confirmed in Phase II trials [4]. Varenicline, partial agonist for the α4β2 nAChRs and full agonist for the α7 nAChRs, improves cognition in schizophrenic patients [9,158]. Also **23** (AZD-3480, α4β2 partial agonist) and nicotine have completed clinical trials, but with disappointing results (Table 1).

Depression is a severe psychological condition that is usually treated with antidepressant drugs that target monoamine transporters regulating the uptake of neurotrasmitters (dopamine, serotonin and norepinephrine). The hypothesis that there is a cholinergic hypertone in major depression is an old theory [159] supported by new studies on animals and humans. In fact, using MRI studies, levels of choline were shown to be increased in the brains of patients with depression, and choline levels have been related to alterations in cholinergic function [160]. Moreover, physostigmine, an acetylcholinesterase inhibitor that potentiates cholinergic transmission, produced depression-like symptoms [159], while the administration of α7 or α4β2 nicotinic antagonists, or compounds that induce desensitization, reduced depressive symptoms [143]. Single photon emission computed tomography (SPECT) and positron emission tomography (PET) studies using α4β2* nAChR-specific radioligands revealed that, across all brain regions, the β2* nAChR availability in depressed patients or in PD patients with depressive symptoms was lower than in healthy subjects [161,162]. In general, the inhibition of nAChR activity is beneficial in the treatment of depression and the effects are synergistic with antidepressants such as imipramine, citalopram and reboxetine. Antagonists alone have also shown antidepressant-like effects in several animal models, suggesting that inhibition of nAChR function through processes such as desensitization also contributes to antidepressant efficacy [163]. However, in two Phase III clinical trials, TC-5214 [(S)-**32**] was tested as adjunct therapy in patients with depression but did not show efficacy [31]. On the contrary, varenicline, tested as smoking cessation agent in patients suffering or having suffered from depression, did not exacerbate the disease [8,92]. Trials with nicotine are on-going.

Neurodevelopmental disorders such as autism spectrum disorder (ASD) and attention-deficit hyperactivity disorder (ADHD), although most commonly considered in childhood, can be lifelong conditions. ASD is characterized by deficits in social interaction, stereotypic behavior, difficulties in verbal and nonverbal communication, and cognitive impairment. nAChR alterations are correlated to autism. In particular, a reduced expression of both α4 and β2 subunits was observed in frontal, parietal and cerebellar cortex but not in thalamus [164]. α4β2 nAChR loss results from an impaired post-translational mechanism regulating its expression, since there is no reduction in mRNA expression [165]. In contrast to the loss of α4 and β2 subunits, there is a compensatory increase in the

expression of the α7 subunit. The alterations in AChRs in ASD may also serve as an early molecular biomarker: monitoring, by means of PET, of α4β2 nAChR levels in the frontal, parietal and cerebellar cortex might provide a clinical tool to assess the effectiveness of pharmacotherapies for autism [166]. The extensive loss of α4β2 nAChRs provides a rationale for drugs that can upregulate and activate α4β2 nAChRs: agonists and PAMs for this subtype can help restore its levels of expression and may be beneficial in correcting deficits of ASD. So far, however, only α4β2 antagonists like mecamylamine **32** have been tested in clinical trials, with negative results [35], while the trial on nicotine is ongoing (Table 1).

ADHD is the most common psychiatric disorder of childhood. ADHD was classically attributed to dysregulation of the catecholamine system but the positive effects of cholinergic agonists on attention and memory have led pharmaceutical companies to explore the utility of nicotinic agonists for ADHD treatment. In this context, the α4β2 subtype seems to be of particular importance [167], even if the main challenge to progress in ADHD treatment remains the lack of knowledge regarding causes of the disease. Several α4β2 (**23–25**, **33**) and α7 agonists (**27**, **31**) underwent clinical trials (Table 1). Promising results have been achieved for ispronicline (AZD3480, **23**) [14] and ABT894 (**33**) [38] but not for **24**, **25**, and **27** [17,19] (Table 1).

## Pain & inflammation

Many years ago, nicotinic receptors emerged as interesting drug targets for pain treatment [168]. More than 20 years ago, the analgesic properties of epibatidine (**13**) [169] were discovered. Since **13** is a potent agonist at α4β2* receptors, although not selective, research initially focused on this subtype and several potent α4β2 full agonists have been synthesized. These compounds, which include ABT-594 (**17**) and TC2696 (**34**), are endowed with strong analgesic properties but a narrow therapeutic window [95,170]. ABT594 was tested in a Phase II clinical trial, showing efficacy but limited tolerability due to side effects [7]. Even though research is very active in this field, only the α4β2 agonist ABT-894 (**33**) has completed a Phase II clinical trial for diabetic neuropathic pain, and did not confirm the results of **17** [37]. Interestingly, as mentioned above, it has been shown that the safety profile of **17** can be improved by using the α4/α4 site-selective ligand NS9283 (**16**), suggesting the combination of a α4β2 full agonist and a α4β2 selective potentiator as new therapeutic strategy [115]

It has also been known for a long time that α7 stimulation produces antinociceptive activity [171]. However, the development of α7 agonists may not be a priority given that long-term treatment can lead to receptor desensitization and/or inactivation, thus suggesting possible adaptations to their effects after chronic use [172]. More recently, α7 type II PAMs and silent agonists have been evaluated in preclinical models for pain [83,96] and have confirmed that α7 nAChR modulators can be an attractive and versatile alternative to α4β2 agonists.

In addition to α4β2* and α7* receptors, other nAChRs are known to be involved in pain. In particular, the α5 subunit plays a significant role in nociception and the α4β2α5* subtype may be a target for nicotinic analgesic agents [173]. Furthermore, dorsal root ganglion neurons coexpress the α9 and α10 nAChR subunits, suggesting a role for the α9/α10 nAChRs in pain processing [174]. This was supported by a recent study showing that RgIA4, a peptide with high selectivity and potent inhibition of the α9α10 nAChR subtype, prevented chemotherapy-induced neuropathic pain in rats [175].

Inflammatory states normally produce pain and the α7 receptor plays an important role also in the cholinergic control of inflammation, regulating the production of proinflammatory cytokine through neuronal and non-neuronal mechanisms [176,177]. It has recently been reported that the activation of α7 nAChRs on non-neuronal cells, such as macrophages, efficiently suppressed TNF-α synthesis [178]; high TNF-α levels are involved in delaying diabetic wound healing through an excessive fibroblast apoptosis and reduced fibroblast density [179]. The selective α7 nAChR agonist PNU282987 (Figure 8) significantly reduced the TNF-α level of wounds in a murine model, by inhibiting its production through activation of α7 nAChR on macrophages and accelerated the healing process. So far, only ATG002 (a nicotine-based gel) has been tested for diabetic foot ulcer in a clinical trial (Table 1). However, as said before, also modulation of α7 receptors by means of silent agonists reduced TNF-α release, supporting the hypothesis that this action is associated with a desensitized, nonconductive state of this receptor [85].

Also chronic inflammatory diseases of the airways, like allergic and nonallergic asthma, can be treated with α7 agonists. Administration of α7 nAChR agonists significantly attenuates the development and function of innate lymphoid cells (ILCs) and in particular group 2 ILCs (ILC2s), abolishing both airway hyper-reactivity and allergic inflammation. Therefore, the cholinergic pathways have a protective role in the pathogenesis of asthma and α7 nAChR agonists can be useful for the treatment of ILC2-mediated asthma [180]. The α7 modulator TC6987 (**34**)

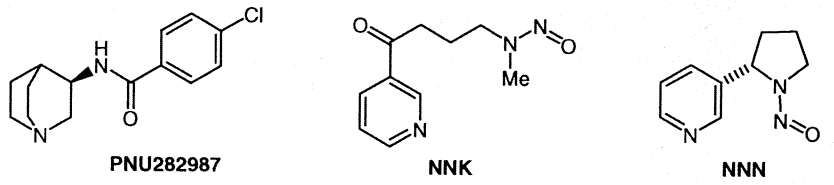



**Figure 8. Structure of PNU282987 and of carcinogenic nicotinic nitrosamines.**

and the α7 silent agonist **9** [181] have completed clinical trials in moderate and allergic asthma; the latter did not show efficacy [6].

## Food behavior

The involvement of nAChRs in food intake behaviors, regulating bodyweight, energy homeostasis and physical activity is due to central and peripheral sites of action. One mechanism proposed for the nicotine-induced reduction of appetite and bodyweight is an increased activity of α3β4*, α7 and α4β2 nicotinic receptors in pro-opiomelanocortin neurons of the hypothalamus, resulting in stimulation of the melanocortin system [182]. A primary potential pathway for α7nAChR mediation of eating bahaviors involves hypothalamic cholinergic input. Cholinergic innervations are abundant in the hypothalamus and there are high levels of α7 nAChR expression, leading to activation of pro-opiomelanocortin neurons. Another proposed mechanism is that a long-term sugar consumption causes an imbalance in expression of dopamine D1, D2 and D3 receptors and of α4β2* and α6β2* nAChRs in nucleus accumbens [183], a change similar to that observed in addiction to substances of abuse [184]. Therefore, targeting the α7 nAChRs may be a useful therapeutic strategy to treat and manage obesity: GTS-21 (**31**) is in Phase I clinical trial in this regard. The α4β2 partial agonist and α7 full agonist varenicline reduces sugar consumption, and it is in a Phase III clinical trial for control of weight gain after cessation of smoking. In mice, constant infusion of sazetidine-A (**15**) decreased food intake robustly as well as bodyweight; the proposed mechanism is desensitization of α4β2* subtype [182].

Recently, it has been reported that the regulation of glucose and energy homeostasis may be due to nAChRs in nonneuronal cells. Mice pancreatic islets present a predominant expression of α7 and β2 nAChR subunits but at lower levels than in the CNS, while α5 and β2 nAChR subunits are prevalent in human islets. Mice with double α7 and β2 deficiency show increased spontaneous physical activity and no change in bodyweight, although a modification of body composition was observed (increased lean and bone mass and decreased fat pads) [185]. Compound **35** (TC-6987), an α7 modulator, has been studied in Phase II clinical trial for the treatment of Type 2 diabetes mellitus.

## Substance abuse

nAChRs are important pharmacological targets for the development of medications to treat alcohol and drug dependence. The drugs approved for smoking cessation are the α4β2 partial agonists varenicline (**21**, approved by the US FDA) and cytisine (**18**, approved in Europe) and bupropion (**36**, approved by the FDA), which behaves as α3β2/α4β2 antagonist [186]. Nicotinic ligands such as the α4β2 agonists ABT-089 (**24**) and dianicline (**37**), and the association of **21** and **36** have been studied in Phase II/III clinical trials for smoking cessation. Dianicline did not show efficacy beyond the initial treatments [41]. With respect to **21** alone, the association between **21** and **36** was more effective in the initial treatment phase but not over longer periods [39,40]. Recently, the involvement of α7 and α6β2* have been investigated and there is preclinical evidence that inhibition of α6β2* and activation of α7 can be novel therapeutic strategies with significant decreases in motivation to self-administer nicotine [187]. In animal models, selective α6 antagonists were effective in reducing nicotine self-administration (see [186] and references therein). These substances should have fewer side effects compared with the nonselective antagonist mecamylamine since the localization of α6* receptors is more restricted. Mecamylamine has been extensively tested in clinical trials for smoking cessation and has yielded contrasting results [36,188]. GTS-21 (**31**), with a dual α7 agonist and α4β2 antagonist activity, is undergoing a Phase II clinical trial for tobacco use disorder, while the trial involving the α7 agonist EVP-6124 (**26**) has been terminated.

Regarding other drug dependences, nAChR antagonists were found to decrease cocaine self-administration [189] and to play a critical role in regulating the rewarding effects of cannabinoids [190]. Alcohol dependence can be treated with α4β2 partial agonists such as cytisine (**18**) and varenicline (**21**); the latter was found efficacious in reducing alcohol consumption in both animal models and humans. Also sazetidine-A (**15**) was able to reduce alcohol intake in alcohol-preferring rats [191]. A Phase III clinical trial on mecamylamine (**32**) did not show significant effects on alcohol consumption [34].

## Cancer

nAChRs are expressed also in cancer cells and contribute to the development and progression of cancers either directly, through the activity of nicotine and its derived carcinogenic nitrosamines, or indirectly through the deregulation of the nAChRs. Chronic exposure to nicotine or nicotine-derived carcinogenic nitrosamines upregulates α7 and α9 nAChRs and desensitizes α4β2 nAChRs, leading to activation of oncogenic pathways, promoting tumor angiogenesis and inhibition of apoptosis in multiple types of cancers [192]. The α7* and α4β2* receptors regulate differently the release of neurotransmitters that have opposite actions on cell proliferation. It has been proposed that an imbalance between the activity of these two receptors affects cancer growth in smokers [193]. The tobacco-specific carcinogenic nitrosamines, NNK and NNN (Figure 8), interact with nAChRs: NNK was found to bind with high affinity to α7 receptors and to stimulate proliferation of small-cells lung cancer cells, an effect which was antagonized by α-Bgtx, suggesting α7 activation [194]. On α4β2 receptors, NNK behaved as a partial agonist, while NNN was an antagonist [195]. Several pieces of evidence suggest that α7 antagonists may be useful in blocking cancer cell proliferation but their use *in vivo* could be problematic, considering the physiological processes in which α7 receptor activation is involved. On the contrary, α7 nAChR stimulation protects neurons from oxaliplatin toxicity through an astrocyte-mediated mechanism [196]. Both varenicline and nicotine have been tested in cancer patients but only for smoking cessation because smokers have a higher risk of treatment complications with respect to nonsmokers.

In cancer patients, during and following chemotherapy, cognitive deficits referred to as 'chemo-brain' may appear. The study of 'chemo-brain' is complicated by difficulties with animal models [197] and currently there is no approved treatment for this condition. At present, transdermal nicotine is being studied in a Phase II clinical trial for the treatment of chemobrain in patients who are one to 5 years postchemotherapy.

## Conclusion & future perspective

One of the main accomplishments in the last 10 years of research on nicotinic receptors is the discovery of compounds able to modulate nAChRs through different sites, either allosteric or orthosteric. This is significant for different reasons. First, modulation from allosteric sites should allow, in principle, achievement of subtype selectivity which has been difficult to obtain with orthosteric ligands, and which has been among the reasons for failure of early clinical trials. Second, PAMs may represent for the nicotinic receptors what benzodiazepines have been for the GABA-A receptor, in other words, the possibility to activate the receptor without exceeding the maximum physiological level of the tissue. Third, compounds affecting desensitization in different ways make it possible to study the contribution of this mechanism in the activity of the receptor. Fourth, the multiplicity of orthosteric-binding sites within heteromeric receptors offers the possibility to discriminate among receptors with similar subunits but in different stoichiometry. Medicinal chemists therefore can exploit these different druggable sites to design new ligands.

The challenges for the future are, however, numerous. Selectivity still may be the highest obstacle to overcome. In this regard, a great help may come from structural studies: it is likely that in the near future other crystal structures of nicotinic receptors will become available, providing an up-close look at different subunit interfaces. Optimization of PAMs is important but it may not be straightforward since they do not show a general pharmacophore: PAMs have been discovered by means of HTS techniques using different libraries of compounds and may bind to more than one site, thus giving multiple structure–activity relationships. Allosteric activators are a promising new class of nicotinic modulators but unfortunately at present only compounds showing dual agonistic-PAM activity are available. It will be necessary to discover in the future 'pure' allosteric agonists to understand the consequence of receptor activation by this mechanism. Last, but not least, optimization of potency and selectivity and careful preclinical and clinical investigations will clarify if silent desensitizers, site-selective agonists, PAMs and allosteric agonists could be useful only as pharmacological tools, or if they really possess a therapeutic potential that is exploitable within the many disorders or conditions in which nicotinic receptors are involved.

## Executive summary

- Nicotinic receptors are pentameric membrane proteins; 12 neuronal subunits have been cloned which can theoretically associate into many combinations, raising the number of possible receptor subtypes and making the design of selective ligands difficult.
- Nicotinic receptors are modulated by compounds interacting at orthosteric or allosteric sites, located in the extracellular domain or in the transmembrane region. Orthosteric ligands can act at orthodox and/or unorthodox sites; compounds acting only on the latter are able to discriminate among receptor subtypes made up of the same subunits but with different stoichiometry.
- Agonists activate and desensitize the receptors; silent agonists block the receptor in the desensitized state without prior activation. Positive allosteric modulators potentiate agonist activity and reactivate the desensitized receptor.
- Nicotinic receptors are involved in many different physiological and pathological processes and therefore are important drug targets for several disorders or conditions.
- Careful preclinical and clinical investigations are necessary to discover the therapeutic usefulness of nonconventional nicotinic receptor modulators.

### Acknowledgements

The support of the University of Florence is acknowledged by the authors. The authors thank Ms Lori Hetherington for the revision of the English language.

### Financial & competing interests disclosure

The financial support for this work comes from the University of Florence (intramural grants). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Writing assistance was utilized in the production of this manuscript.

## References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Albuquerque EX, Pereira EFR, Alkondon M, Rogers SW. Mammalian nicotinic acetylcholine receptors: from structure to function. *Physiol. Rev.* 89(1), 73–120 (2009).
2. Boggs DL, Ranganathan M, Sewell RA, Madonick S, D'Souza DC. Pilot study of intravenous nicotine effects on cognitive performance in schizophrenia. *Schizophr. Res.* 150(1), 323–324 (2013).
3. Quisenaerts C, Morrens M, Hulstijn W *et al.* The nicotinergic receptor as a target for cognitive enhancement in schizophrenia: barking up the wrong tree? *Psychopharmacology* 231(3), 543–550 (2014).
4. Gee KW, Olincy A, Kanner R *et al.* First in human trial of a type I positive allosteric modulator of alpha7-nicotinic acetylcholine receptors: pharmacokinetics, safety, and evidence for neurocognitive effect of AVL-3288. *J. Psychopharmacol.* 31(4), 434–441 (2017).
5. Winterer G, Gallinat J, Brinkmeyer J *et al.* Allosteric alpha-7 nicotinic receptor modulation and P50 sensory gating in schizophrenia: a proof-of-mechanism study. *Neuropharmacology* 64, 197–204 (2013).
6. Boulet L-P, Gauvreau GM, Cockcroft DW *et al.* Effects of ASM-024, a modulator of acetylcholine receptor function, on airway responsiveness and allergen-induced responses in patients with mild asthma. *Can. Respir. J.* 22(4), 230–234 (2015).
7. Rowbotham MC, Duan RW, Thomas J, Nothaft W, Backonja M-M. A randomized, double-blind, placebo-controlled trial evaluating the efficacy and safety of ABT-594 in patients with diabetic peripheral neuropathic pain. *Pain* 146(3), 245–252 (2009).
8. Anthenelli RM, Morris C, Ramey TS *et al.* Effects of varenicline on smoking cessation in adults with stably treated current or past major depression: a randomized trial. *Ann. Intern. Med.* 159(6), 390–400 (2013).
9. Shim J-C, Jung D-U, Jung S-S *et al.* Adjunctive varenicline treatment with antipsychotic medications for cognitive impairments in people with schizophrenia: a randomized double-blind placebo-controlled trial. *Neuropsychopharmacology* 37(3), 660–668 (2012).
10. Kim SY, Choi SH, Rollema H *et al.* Phase II crossover trial of varenicline in mild-to-moderate Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.* 37(3–4), 232–245 (2014).
11. Mitchell JM, Teague CH, Kayser AS, Bartlett SE, Fields HL. Varenicline decreases alcohol consumption in heavy-drinking smokers. *Psychopharmacology* 223, 299–306 (2012).
12. Kalechstein AD, Mahoney JJ, Verrico CD, De La Garza R. Short-term, low-dose varenicline administration enhances information processing speed in methamphetamine-dependent users. *Neuropharmacology* 85, 493–498 (2014).

13. Jeon D-W, Shim J-C, Kong B-G *et al.* Adjunctive varenicline treatment for smoking reduction in patients with schizophrenia: a randomized double-blind placebo-controlled trial. *Schizophr. Res.* 176(2), 206–211 (2016).
14. Potter AS, Dunbar G, Mazzulla E, Hosford D, Newhouse PA. AZD3480, a novel nicotinic receptor agonist, for the treatment of attention-deficit/hyperactivity disorder in adults. *Biol. Psychiatry* 75(3), 207–214 (2014).
15. Velligan D, Brenner R, Sicuro F *et al.* Assessment of the effects of AZD3480 on cognitive function in patients with schizophrenia. *Schizophr. Res.* 134(1), 59–64 (2012).
16. Frölich L, Ashwood T, Nilsson J, Eckerwall G. Effects of AZD3480 on cognition in patients with mild-to-moderate Alzheimer's fisease: a Phase IIb dose-finding study. *J. Alzheimer's Dis.* 24, 363–374 (2011).
17. Childress A, Sallee FR. Pozanicline for the treatment of attention-deficit/hyperactivity disorder. *Exp. Opin. Investigat. Drugs* 23(11), 1585–1593 (2014).
18. Boström E, Bohnstedt KC, Jostell K-G *et al.* Population pharmacokinetics and safety of AZD1446, a neuronal nicotinic receptor agonist, administered in healthy volunteers. *Clin. Pharmacol. Drug Dev.* 3(1), 63–71 (2014).
19. Jucaite A, Öhd J, Potter AS *et al.* A randomized, double-blind, placebo-controlled crossover study of $\alpha4\beta2^*$ nicotinic acetylcholine receptor agonist AZD1446 (TC-6683) in adults with attention-deficit/hyperactivity disorder. *Psychopharmacology* 231(6), 1251–1265 (2014).
20. Preskorn SH, Gawryl M, Dgetluck N, Palfreyman M, Bauer LO, Hilt DC. Normalizing effects of EVP-6124, an alpha-7 nicotinic partial agonist, on event-related potentials and cognition: a proof of concept, randomized trial in patients with schizophrenia. *J. Psychiatr. Pract.* 20, 12–24 (2014).
21. Deardorff WJ, Shobassy A, Grossberg GT. Safety and clinical effects of EVP-6124 in subjects with Alzheimer's disease currently or previously receiving an acetylcholinesterase inhibitor medication. *Exp. Rev. Neurother.* 15(1), 7–17 (2015).
22. Walling D, Marder SR, Kane J *et al.* Phase 2 trial of an alpha-7 nicotinic receptor agonist (TC-5619) in negative and cognitive symptoms of schizophrenia. *Schizophr. Bull.* 42(2), 335–343 (2016).
23. Haig G, Wang D, Othman AA, Zhao J. The α7 nicotinic agonist ABT-126 in the treatment of cognitive impairment associated with schizophrenia in nonsmokers: results from a randomized controlled Phase 2b study. *Neuropsychopharmacology* 41, 2893–2902 (2016).
24. Haig GM, Bain EE, Robieson WZ, Baker JD, Othman AA. A randomized trial to assess the efficacy and safety of ABT-126, a selective α7 nicotinic acetylcholine receptor agonist, in the treatment of cognitive impairment in schizophrenia. *Am. J. Psychiatry* 173(8), 827–835 (2016).
25. Florian H, Meier A, Gauthier S *et al.* Efficacy and Safety of ABT-126 in subjects with mild-to-moderate Alzheimer's disease on stable doses of acetylcholinesterase inhibitors: a randomized, double-blind, placebo-controlled study. *J. Alzheimer's Dis.* 51, 1237–1247 (2016).
26. Gault LM, Lenz RA, Ritchie CW *et al.* ABT-126 monotherapy in mild-to-moderate Alzheimer's dementia: randomized double-blind, placebo and active controlled adaptive trial and open-label extension. *Alzheimer's Res. Ther.* 8(1), 44 (2016).
27. Umbricht D, Keefe RSE, Murray S *et al.* A randomized, placebo-controlled study investigating the nicotinic α7 agonist, RG3487, for cognitive deficits in schizophrenia. *Neuropsychopharmacology* 39(7), 1568–1577 (2014).
28. Barch DM, Marder SR, Harms MP *et al.* Task-related fMRI responses to a nicotinic acetylcholine receptor partial agonist in schizophrenia: a randomized trial. *Progr. Neuropsychopharmacol. Biol. Psychiatry* 71, 66–75 (2016).
29. Trenkwalder C, Berg D, Rascol O *et al.* A placebo-controlled trial of AQW051 in patients with moderate to severe levodopa-induced dyskinesia. *Mov. Disord.* 31(7), 1049–1054 (2016).
30. Freedman R. α7-nicotinic acetylcholine receptor agonists for cognitive enhancement in schizophrenia. *Annu. Rev. Med.* 65(1), 245–261 (2014).
31. Vieta E, Thase ME, Naber D *et al.* Efficacy and tolerability of flexibly-dosed adjunct TC-5214 (dexmecamylamine) in patients with major depressive disorder and inadequate response to prior antidepressant. *Eur. Neuropsychopharmacol.* 24(4), 564–574 (2014).
32. Möller H-J, Demyttenaere K, Olausson B *et al.* Two Phase III randomised double-blind studies of fixed-dose TC-5214 (dexmecamylamine) adjunct to ongoing antidepressant therapy in patients with major depressive disorder and an inadequate response to prior antidepressant therapy. *World J. Biol. Psychiatry* 16(7), 483–501 (2015).
33. Roh S, Hoeppner SS, Schoenfeld D, Fullerton CA, Stoeckel LE, Evins AE. Acute effects of mecamylamine and varenicline on cognitive performance in non-smokers with and without schizophrenia. *Psychopharmacology* 231(4), 765–775 (2014).
34. Petrakis IL, Ralevski E, Gueorguieva R *et al.* Mecamylamine treatment for alcohol dependence: a randomized controlled trial. *Addiction* 133(1), 6–14 (2018).
35. Arnold LE, Aman MG, Hollway J *et al.* Placebo-controlled pilot trial of mecamylamine for treatment of autism spectrum disorders. *J. Child Adolesc. Psychopharmacol.* 22(3), 198–205 (2012).
36. Glover ED, Laflin MT, Schuh KJ *et al.* A randomized, controlled trial to assess the efficacy and safety of a transdermal delivery system of nicotine/mecamylamine in cigarette smokers. *Addiction* 102(5), 795–802 (2007).
37. Rowbotham MC, Arslanian A, Nothaft W *et al.* Efficacy and safety of the $\alpha4\beta2$ neuronal nicotinic receptor agonist ABT-894 in patients with diabetic peripheral neuropathic pain. *Pain* 153(4), 862–868 (2012).

38. Bain EE, Robieson W, Pritchett Y *et al.* A randomized, double-blind, placebo-controlled Phase 2 study of α4β2 agonist ABT-894 in adults with ADHD. *Neuropsychopharmacology* 38(3), 405–413 (2013).
39. Ebbert JO, Hatsukami DK, Croghan IT *et al.* Combination varenicline and bupropion sr for tobacco-dependence treatment in cigarette smokers: a randomized trial. *JAMA* 311(2), 155–163 (2014).
40. Rose JE, Behm FM. Combination treatment with varenicline and bupropion in an adaptive smoking cessation paradigm. *Am. J. Psychiatry* 171(11), 1199–1205 (2014).
41. Tonstad S, Holme I, Tønnesen P. Dianicline, a novel α4β2 nicotinic acetylcholine receptor partial agonist, for smoking cessation: a randomized placebo-controlled clinical trial. *Nicotine Tob. Res.* 13(1), 1–6 (2011).
42. NIH. ClinicalTrials.gov http://clinicaltrials.gov
43. Bradanicline. Adis Insight. http://adisinsight.springer.com/drugs/800026693
44. Marketwatch. Targacept ends development of ADHD drug. https://www.marketwatch.com/story/targacept-ends-development-of-adhd-drug-2012-09-17
45. US FDA. FDA News: targacept reports results of pain trial. https://www.fdanews.com/articles/101721-targacept-reports-results-of-pain-trial
46. Attenua, Inc.: WO2017023700 (2017).
47. Donohue J. Targacept's TC-6987 shows promise in asthma but not diabetes. *CPhI Online.* www.cphi-online.com/targacept-s-tc6987-shows-promise-in-asthma-but-news031272.html
48. Quintaro P. Targacept announces revised top-line results from exploratory Phase II study. *Benzinga.* www.benzinga.com/news/12/04/2483872/targacept-announces-revised-top-line-results-from-exploratory-phase-2-study
49. TC6987. Adis Insight. http://adisinsight.springer.com/drugs/800031656
50. Morales-Perez CL, Noviello CM, Hibbs RE. X-ray structure of the human α4β2 nicotinic receptor. *Nature* 538(7625), 411–415 (2016).
51. Gotti C, Clementi F. Neuronal nicotinic receptors: from structure to pathology. *Prog. Neurobiol.* 74(6), 363–396 (2004).
52. Gotti C, Clementi F, Fornari A, Gaimarri A, Guiducci S, Manfredi I. Structural and functional diversity of native brain nicotinic receptors. *Biochem. Pharmacol.* 78(7), 703–711 (2009).
53. Papke RL. Merging old and new perspectives on nicotinic acetylcholine receptors. *Biochem. Pharmacol.* 89(1), 1–11 (2014).
54. Gotti C, Zoli M, Clementi F. Brain nicotinic acetylcholine receptors: native subtypes and their relevance. *Trends Pharmacol. Sci.* 27(9), 482–491 (2006).
55. Wu J, Liu Q, Tang P *et al.* Heteromeric α7β2 nicotinic acetylcholine receptors in the brain. *Trends Pharmacol. Sci.* 37(7), 562–574 (2016).
56. Lykhmus O, Gergalova G, Koval L, Zhmak M, Komisarenko S, Skok M. Mitochondria express several nicotinic acetylcholine receptor subtypes to control various pathways of apoptosis induction. *Int. J. Biochem. Cell Biol.* 53, 246–252 (2014).
57. Zoli M, Pistillo F, Gotti C. Diversity of native nicotinic receptor subtypes in mammalian brain. *Neuropharmacology* 96(Pt B), 302–311 (2015).
58. Kirsch GE, Fedorov NB, Kuryshev YA, Liu Z, Armstrong LC, Orr MS. Electrophysiology-based assays to detect subtype-selective modulation of human nicotinic acetylcholine receptors. *ASSAY Drug Dev. Technol.* 14(6), 333–344 (2016).
59. Ray C, Soderblom EJ, Bai Y, Carroll FI, Caron MG, Barak LS. Probing the allosteric role of the α5 subunit of α3β4α5 nicotinic acetylcholine receptors by functionally selective modulators and ligands. *ACS Chem. Biol.* 12(3), 702–714 (2017).
60. Armstrong LC, Kirsch GE, Fedorov NB *et al.* High-throughput patch clamp screening in human α6-containing nicotinic acetylcholine receptors. *SLAS Discov.* 22(6), 686–695 (2017).
61. Salminen O, Murphy KL, Mcintosh JM *et al.* Subunit composition and pharmacology of two classes of striatal presynaptic nicotinic acetylcholine receptors mediating dopamine release in mice. *Mol. Pharmacol.* 65(6), 1526–1535 (2004).
62. Bertrand D, Lee C-HL, Flood D, Marger F, Donnelly-Roberts D. Therapeutic potential of α7 nicotinic acetylcholine receptors. *Pharmacol. Revi.* 67, 1025–1073 (2015).

•• **A comprehensive review on the role and pharmacology of α7 receptors.**

63. Jensen AA, Frølund B, Liljefors T, Krogsgaard-Larsen P. Neuronal nicotinic acetylcholine receptors: structural revelations, target identifications, and therapeutic inspirations. *J. Med. Chem.* 48, 4705–4745 (2005).
64. Hurst R, Rollema H, Bertrand D. Nicotinic acetylcholine receptors: from basic science to therapeutics. *Pharmacol. Ther.* 137(1), 22–54 (2013).

• **A comprehensive review providing an overview of the compounds in clinical trials.**

65. Maskos U. Emerging concepts: novel integration of *in vivo* approaches to localize the function of nicotinic receptors. *J. Neurochem.* 100(3), 596–602 (2007).
66. Changeux J-P. Nicotine addiction and nicotinic receptors: lessons from genetically modified mice. *Nat. Rev. Neurosci.* 11(6), 389–401 (2010).

67. Mazurov AA, Speake JD, Yohannes D. Discovery and development of α7 nicotinic acetylcholine receptor modulators. *J. Med. Chem.* 54(23), 7943–7961 (2011).

68. Arias HR. Positive and negative modulation of nicotinic receptors. In: *Advances in Protein Chemistry and Structural Biology.* Rossen D (Ed.). Academic Press, Amsterdam, The Netherlands, 153–203 (2010).

69. Williams DK, Wang J, Papke RL. Positive allosteric modulators as an approach to nicotinic acetylcholine receptor-targeted therapeutics: advantages and limitations. *Biochem. Pharmacol.* 82(8), 915–930 (2011).

•• **A critical review on the pros and cons of positive allosteric modulators.**

70. Potasiewicz A, Kos T, Ravazzini F *et al.* Pro-cognitive activity in rats of 3-furan-2-yl-N-p-tolyl-acrylamide, a positive allosteric modulator of the α7 nicotinic acetylcholine receptor. *Br. J. Pharmacol.* 172(21), 5123–5135 (2015).

71. Targowska-Duda KM, Wnorowski A, Budzynska B, Jozwiak K, Biala G, Arias HR. The positive allosteric modulator of α7 nicotinic acetylcholine receptors, 3-furan-2-yl-N-p-tolyl-acrylamide, enhances memory processes and stimulates ERK1/2 phosphorylation in mice. *Behav. Brain Res.* 302, 142–151 (2016).

72. Potasiewicz A, Hołuj M, Kos T, Popik P, Arias HR, Nikiforuk A. 3-Furan-2-yl-N-p-tolyl-acrylamide, a positive allosteric modulator of the α7 nicotinic receptor, reverses schizophrenia-like cognitive and social deficits in rats. *Neuropharmacology* 113(Pt A), 188–197 (2017).

73. Targowska-Duda KM, Feuerbach D, Biala G, Jozwiak K, Arias HR. Antidepressant activity in mice elicited by 3-furan-2-yl-N-p-tolyl-acrylamide, a positive allosteric modulator of the α7 nicotinic acetylcholine receptor. *Neurosci. Lett.* 569, 126–130 (2014).

74. Giniatullin R, Nistri A, Yakel JL. Desensitization of nicotinic ACh receptors: shaping cholinergic signaling. *Trends Neurosci.* 28(7), 371–378 (2005).

75. Williams DK, Wang J, Papke RL. Investigation of the molecular mechanism of the α7 nicotinic acetylcholine receptor positive allosteric modulator PNU-120596 provides evidence for two distinct desensitized states. *Mol. Pharmacol.* 80(6), 1013–1032 (2011).

76. Cecchini M, Changeux J-P. The nicotinic acetylcholine receptor and its prokaryotic homologues: structure, conformational transitions & allosteric modulation. *Neuropharmacology* 96(Pt B), 137–149 (2015).

77. Buccafusco JJ, Beach JW, Terry AV. Desensitization of nicotinic acetylcholine receptors as a strategy for drug development. *J. Pharmacol. Exp. Ther.* 328(2), 364–370 (2009).

78. Chojnacka K, Papke RL, Horenstein NA. Synthesis and evaluation of a conditionally-silent agonist for the α7 nicotinic acetylcholine receptor. *Bioorg. Med. Chem. Lett.* 23(14), 4145–4149 (2013).

79. Paradiso KG, Steinbach JH. Nicotine is highly effective at producing desensitization of rat α4β2 neuronal nicotinic receptors. *J. Physiol.* 553(3), 857–871 (2003).

80. Buccafusco JJ, Shuster LC, Terry AV Jr. Disconnection between activation and desensitization of autonomic nicotinic receptors by nicotine and cotinine. *Neurosci. Lett.* 413(1), 68–71 (2007).

81. Briggs CA, Grønlien JH, Curzon P *et al.* Role of channel activation in cognitive enhancement mediated by α7 nicotinic acetylcholine receptors. *Br. J. Pharmacol.* 158(6), 1486–1494 (2009).

82. Pieschl RL, Miller R, Jones KM *et al.* Effects of BMS-902483, an α7 nicotinic acetylcholine receptor partial agonist, on cognition and sensory gating in relation to receptor occupancy in rodents. *Eur. J. Pharmacol.* 807, 1–11 (2017).

83. Papke RL, Bagdas D, Kulkarni AR *et al.* The analgesic-like properties of the alpha7 nAChR silent agonist NS6740 is associated with non-conducting conformations of the receptor. *Neuropharmacology* 91, 34–42 (2015).

84. Thomsen MS, Mikkelsen JD. The α7 nicotinic acetylcholine receptor ligands methyllycaconitine, NS6740 and GTS-21 reduce lipopolysaccharide-induced TNF-α release from microglia. *J. Neuroimmun.* 251(1–2), 65–72 (2012).

85. Suzuki T, Hide I, Matsubara A *et al.* Microglial α7 nicotinic acetylcholine receptors drive a phospholipase C/$IP_3$ pathway and modulate the cell activation toward a neuroprotective role. *J. Neurosci. Res.* 83(8), 1461–1470 (2006).

86. Papke RL, Chojnacka K, Horenstein NA. The minimal pharmacophore for silent agonism of the α7 nicotinic acetylcholine receptor. *J. Pharmacol. Exp. Ther.* 350(3), 665–680 (2014).

87. Quadri M, Papke RL, Horenstein NA. Dissection of N,N-diethyl-N′-phenylpiperazines as α7 nicotinic receptor silent agonists. *Bioorg. Med. Chem.* 24(2), 286–293 (2016).

88. Halvard Grønlien J, Håkerud M, Ween H *et al.* Distinct profiles of α7 nAChR positive allosteric modulation revealed by structurally diverse chemotypes. *Mol. Pharmacol.* 72(3), 715–724 (2007).

89. Gill JK, Savolainen M, Young GT, Zwart R, Sher E, Millar NS. Agonist activation of α7 nicotinic acetylcholine receptors via an allosteric transmembrane site. *Proc. Natl Acad. Sci. USA* 108(14), 5867–5872 (2011).

90. Gill JK, Dhankher P, Sheppard TD, Sher E, Millar NS. A series of α7 nicotinic acetylcholine receptor allosteric modulators with close chemical similarity but diverse pharmacological properties. *Mol. Pharmacol.* 81(5), 710–718 (2012).

91. Gill-Thind JK, Dhankher P, D'oyley JM, Sheppard TD, Millar NS. Structurally similar allosteric modulators of α7 nicotinic acetylcholine receptors exhibit five distinct pharmacological effects. *J. Biol. Chem.* 290(6), 3552–3562 (2015).

92. Horenstein NA, Papke RL, Kulkarni AR *et al.* Critical molecular determinants of α7 nicotinic acetylcholine receptor allosteric activation: separation of direct allosteric activation and positive allosteric modulation. *J. Biol. Chem.* 291(10), 5049–5067 (2016).

93. Gill JK, Chatzidaki A, Ursu D, Sher E, Millar NS. Contrasting properties of α7-selective orthosteric and allosteric agonists examined on native nicotinic acetylcholine receptors. *PLoS ONE* 8(1), e55047 (2013).

94. Thakur GA, Kulkarni AR, Deschamps JR, Papke RL. Expeditious synthesis, enantiomeric resolution, and enantiomer functional characterization of (4-(4-Bromophenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline-8-sulfonamide (4BP-TQS): an allosteric agonist-positive allosteric modulator of α7 nicotinic acetylcholine receptors. *J. Med. Chem.* 56(21), 8943–8947 (2013).

95. Gao B, Hierl M, Clarkin K *et al.* Pharmacological effects of nonselective and subtype selective nicotinic acetylcholine receptor agonists in animal models of persistent pain. *Pain* 149(1), 33–49 (2010).

96. Freitas K, Carroll FI, Damaj MI. The antinociceptive effects of nicotinic receptors α7-positive allosteric modulators in murine acute and tonic pain models. *J. Pharmacol. Exp. Ther.* 344(1), 264–275 (2013).

97. Donvito G, Bagdas D, Toma W *et al.* The interaction between alpha 7 nicotinic acetylcholine receptor and nuclear peroxisome proliferator-activated receptor-α represents a new antinociceptive signaling pathway in mice. *Exp. Neurol.* 295, 194–201 (2017).

98. Wang J, Lindstrom J. Orthosteric and allosteric potentiation of heteromeric neuronal nicotinic acetylcholine receptors. *Br. J. Pharmacol.* doi:10.1111/bph.13745 (2017) (Epub ahead of print).

•• **A recent review on the different binding sites of nicotinic receptor modulators.**

99. Nelson ME, Kuryatov A, Choi CH, Zhou Y, Lindstrom J. Alternate stoichiometries of α4β2 nicotinic acetylcholine receptors. *Mol. Pharmacol.* 63(2), 332–341 (2003).

• **The first experimental evidence on the existence of subtypes with different stoichiometry.**

100. Moroni M, Zwart R, Sher E, Cassels BK, Bermudez I. α4β2 Nicotinic receptors with high and low acetylcholine sensitivity: pharmacology, stoichiometry, and sensitivity to long-term exposure to nicotine. *Mol. Pharmacol.* 70(2), 755–768 (2006).

101. Krashia P, Moroni M, Broadbent S *et al.* Human α3β4 neuronal nicotinic receptors show different stoichiometry if they are expressed in xenopus oocytes or mammalian HEK293 cells. *PLoS ONE* 5(10), e13611 (2010).

102. Dash B, Lukas RJ, Li MD. A signal peptide missense mutation associated with nicotine dependence alters α2*-nicotinic acetylcholine receptor function. *Neuropharmacology* 79, 715–725 (2014).

103. Post MR, Limapichat W, Lester HA, Dougherty DA. Heterologous expression and nonsense suppression provide insights into agonist behavior at α6β2 nicotinic acetylcholine receptors. *Neuropharmacology* 97, 376–382 (2015).

104. Gotti C, Moretti M, Meinerz NM *et al.* Partial deletion of the nicotinic cholinergic receptor α4 or β2 subunit genes changes the acetylcholine sensitivity of receptor-mediated $^{86}Rb^{+}$ efflux in cortex and thalamus and alters relative expression of α4 and β2 subunits. *Mol. Pharmacol.* 73(6), 1796–1807 (2008).

105. Maurer JJ, Sandager-Nielsen K, Schmidt HD. Attenuation of nicotine taking and seeking in rats by the stoichiometry-selective alpha4beta2 nicotinic acetylcholine receptor positive allosteric modulator NS9283. *Psychopharmacology* 234(3), 475–484 (2017).

106. Grupe M, Grunnet M, Bastlund JF, Jensen AA. Targeting α4β2 nicotinic acetylcholine receptors in central nervous system disorders: perspectives on positive allosteric modulation as a therapeutic approach. *Basic Clin. Pharmacol. Toxicol.* 116(3), 187–200 (2015).

107. Carbone AL, Moroni M, Groot-Kormelink PJ, Bermudez I. Pentameric concatenated $(\alpha4)_2(\beta2)_3$ and $(\alpha4)_3(\beta2)_2$ nicotinic acetylcholine receptors: subunit arrangement determines functional expression. *Br. J. Pharmacol.* 156(6), 970–981 (2009).

108. Andersen N, Corradi J, Sine SM, Bouzat C. Stoichiometry for activation of neuronal α7 nicotinic receptors. *Proc. Natl Acad. Sci. USA* 110(51), 20819–20824 (2013).

109. Harpsøe K, Ahring PK, Christensen JK, Jensen ML, Peters D, Balle T. Unraveling the high- and low-sensitivity agonist responses of nicotinic acetylcholine receptors. *J. Neurosci.* 31(30), 10759–10766 (2011).

110. Mazzaferro S, Bermudez I, Sine SM. α4β2 Nicotinic acetylcholine receptors: relationships between subunit stoichiometry and function at the single channel level. *J. Biol. Chem.* 292(7), 2729–2740 (2017).

111. Wang J, Kuryatov A, Sriram A *et al.* An accessory agonist binding site promotes activation of α4β2* nicotinic acetylcholine receptors. *J. Biol.Chem.* 290(22), 13907–13918 (2015).

112. Benallegue N, Mazzaferro S, Alcaino C, Bermudez I. The additional ACh binding site at the α4(+)/α4(−) interface of the $(\alpha4\beta2)_2\alpha4$ nicotinic ACh receptor contributes to desensitization. *Br. J. Pharmacol.* 170(2), 304–316 (2013).

113. Malysz J, Dyhring T, Ahring P *et al. In vitro* pharmacological profile of a novel α4β2 positive allosteric modulator NS9283 (A-969933). *Biochem. Pharmacol.* 78, 919–920 (2009).

114. Lee C-H, Zhu C, Malysz J *et al.* α4β2 neuronal nicotinic receptor positive allosteric modulation: an approach for improving the therapeutic index of α4β2 nAChR agonists in pain. *Biochem. Pharmacol.* 82(8), 959–966 (2011).

115. Zhu CZ, Chin C-L, Rustay NR *et al.* Potentiation of analgesic efficacy but not side effects: co-administration of an α4β2 neuronal nicotinic acetylcholine receptor agonist and its positive allosteric modulator in experimental models of pain in rats. *Biochem. Pharmacol.* 82(8), 967–976 (2011).

116. Timmermann DB, Sandager-Nielsen K, Dyhring T *et al.* Augmentation of cognitive function by NS9283, a stoichiometry-dependent positive allosteric modulator of α2- and α4-containing nicotinic acetylcholine receptors. *Br. J. Pharmacol.* 167(1), 164–182 (2012).

117. Olsen JA, Kastrup JS, Peters D, Gajhede M, Balle T, Ahring PK. Two distinct allosteric binding sites at α4β2 nicotinic acetylcholine receptors revealed by NS206 and NS9283 give unique insights to binding activity-associated linkage at cys-loop receptors. *J. Biol. Chem.* 288(50), 35997–36006 (2013).

118. Grupe M, Paolone G, Jensen AA, Sandager-Nielsen K, Sarter M, Grunnet M. Selective potentiation of $(\alpha4)_3(\beta2)_2$ nicotinic acetylcholine receptors augments amplitudes of prefrontal acetylcholine- and nicotine-evoked glutamatergic transients in rats. *Biochem. Pharmacol.* 86(10), 1487–1496 (2013).

119. Bencherif M, Bane AJ, Miller CH, Dull GM, Gatto GJ. TC-2559: a novel orally active ligand selective at neuronal acetylcholine receptors. *Eur. J. Pharmacol.* 409(1), 45–55 (2000).

120. Chen Y, Sharples TJW, Phillips KG *et al.* The nicotinic α4β2 receptor selective agonist, TC-2559, increases dopamine neuronal activity in the ventral tegmental area of rat midbrain slices. *Neuropharmacology* 45(3), 334–344 (2003).

121. Cheng L-Z, Han L, Fan J, Huang L-T, Peng L-C, Wang Y. Enhanced inhibitory synaptic transmission in the spinal dorsal horn mediates antinociceptive effects of TC-2559. *Mol. Pain* 7, 56 (2011).

122. Saika F, Kiguchi N, Kobayashi Y, Kishioka S. Peripheral alpha4beta2 nicotinic acetylcholine receptor signalling attenuates tactile allodynia and thermal hyperalgesia after nerve injury in mice. *Acta Physiol.* 213(2), 462–471 (2015).

123. Wang Y, Sherwood JL, Lodge D. The α4β2 nicotinic acetylcholine receptor agonist TC-2559 impairs long-term potentiation in the dentate gyrus *in vivo*. *Neurosci. Lett.* 406(3), 183–188 (2006).

124. Marotta CB, Rreza I, Lester HA, Dougherty DA. Selective ligand behaviors provide new insights into agonist activation of nicotinic acetylcholine receptors. *ACS Chem. Biol.* 9(5), 1153–1159 (2014).

125. Unwin N. Refined structure of the nicotinic acetylcholine receptor at 4 Å resolution. *J. Mol. Biol.* 346, 967–989 (2005).

126. Kouvatsos N, Giastas P, Chroni-Tzartou D, Poulopoulou C, Tzartos SJ. Crystal structure of a human neuronal nAChR extracellular domain in pentameric assembly: ligand-bound α2 homopentamer. *Proc. Natl Acad. Sci. USA* 113(34), 9635–9640 (2016).

127. Zouridakis M, Giastas P, Zarkadas E, Chroni-Tzartou D, Bregestovski P, Tzartos SJ. Crystal structures of free and antagonist-bound states of human α9 nicotinic receptor extracellular domain. *Nat. Struct. Mol. Biol.* 21(11), 976–980 (2014).

128. Giastas P, Zouridakis M, Tzartos SJ. Understanding structure-function relationships of the human neuronal acetylcholine receptor: insights from the first crystal structures of neuronal subunits. *Br. J. Pharmacol.* doi:10.1111/bph.13838 (2017) (Epub ahead of print).

129. Smit AB, Syed NI, Schaap D *et al.* A glia-derived acetylcholine-binding protein that modulates synaptic transmission. *Nature* 411(6835), 261–268 (2001).

130. Shahsavar A, Gajhede M, Kastrup JS, Balle T. Structural studies of nicotinic acetylcholine receptors: using acetylcholine-binding protein as a structural surrogate. *Basic Clin. Pharmacol. Toxicol.* 118(6), 1742–7843 (2016).

131. Rucktooa P, Smit AB, Sixma TK. Insight in nAChR subtype selectivity from AChBP crystal structures. *Biochem. Pharmacol.* 78, 777–787 (2009).

132. Ulens C, Akdemir A, Jongejan A *et al.* Use of acetylcholine binding protein in the search for novel α7 nicotinic receptor ligands. *In silico* docking, pharmacological screening, and X-ray analysis. *J. Med. Chem.* 52(8), 2372–2383 (2009).

133. Ussing CA, Hansen CP, Petersen JG *et al.* Synthesis, pharmacology, and biostructural characterization of novel α4β2 nicotinic acetylcholine receptor agonists. *J. Med. Chem.* 56(3), 940–951 (2013).

134. De Kloe GE, Retra K, Geitmann M *et al.* Surface plasmon resonance biosensor based fragment screening using acetylcholine binding protein identifies ligand efficiency hot spots (LE hot spots) by deconstruction of nicotinic acetylcholine receptor α7 ligands. *J. Med. Chem.* 53(19), 7192–7201 (2010).

135. Zhang H-K, Eaton JB, Yu L-F *et al.* Insights into the structural determinants required for high-affinity binding of chiral cyclopropane-containing ligands to α4β2-nicotinic acetylcholine receptors: an integrated approach to behaviorally active nicotinic ligands. *J. Med. Chem.* 55(18), 8028–8037 (2012).

136. Mazurov AA, Kombo DC, Hauser TA *et al.* Discovery of (2S,3R)-N-[2-(Pyridin-3-ylmethyl)-1-azabicyclo[2.2.2]oct-3-yl]benzo[b]furan-2-carboxamide (TC-5619), a selective α7 nicotinic acetylcholine receptor agonist, for the treatment of cognitive disorders. *J. Med. Chem.* 55(22), 9793–9809 (2012).

137. Corringer P-J, Le Novère N, Changeux J-P. Nicotinic receptors at the aminoacid level. *Annu. Rev. Pharmacol. Toxicol.* 40, 431–458 (2000).

138. Glennon RA, Dukat M. α4β2 nACh receptor pharmacophore models. *Bioorg. Med. Chem. Lett.* 14, 1841–1844 (2004).

139. Dougherty DA. Cys-loop neuroreceptors: structure to the rescue? *Chem. Rev.* 108, 1642–1653 (2008).

140. Van Arnam EB, Dougherty DA. Functional probes of drug–receptor interactions implicated by structural studies: cys-loop receptors provide a fertile testing ground. *J. Med. Chem.* 57(15), 6289–6300 (2014).

- **A review summarizing the results of the missens mutation technique on various nicotinic receptor subtypes.**

141. Wall TR. *Effects of TI-299423 on neuronal nicotinic acetylcholine receptors [PhD thesis].* California Institute of Technology, CA, USA (2015).

142. Post MR, Tender GS, Lester HA, Dougherty DA. Secondary ammonium agonists make dual cation-π interactions in α4β2 nicotinic receptors. *eNeuro* 4(2), pii: ENEURO.0032-17.2017 (2017).

143. Taly A, Corringer P-J, Guedin D, Lestage P, Changeux J-P. Nicotinic receptors: allosteric transitions and therapeutic targets in the nervous system. *Nat. Rev. Drug Discov.* 8(9), 733–750 (2009).

144. Govoni S, Mura E, Preda S *et al.* Dangerous liaisons between beta-amyloid and cholinergic neurotransmission. *Curr. Pharm. Des.* 20(15), 2525–2538 (2014).

145. Wang H-Y, Lee DHS, Davis CB, Shank RP. Amyloid peptide $A\beta_{1-42}$ binds selectively and with picomolar affinity to α7 nicotinic acetylcholine receptors. *J. Neurochem.* 75(3), 1155–1161 (2000).

146. Liu Q, Huang Y, Shen J, Steffensen S, Wu J. Functional α7β2 nicotinic acetylcholine receptors expressed in hippocampal interneurons exhibit high sensitivity to pathological level of amyloid β peptides. *BMC Neurosci.* 13(1), 155 (2012).

147. Lamb PW, Melton MA, Yakel JL. Inhibition of neuronal nicotinic acetylcholine receptor channels expressed in *Xenopus* Oocytes by $\beta$-amyloid$_{1-42}$ peptide. *J. Mol. Neurosci.* 27(1), 13–21 (2005).

148. Lombardo S, Maskos U. Role of the nicotinic acetylcholine receptor in Alzheimer's disease pathology and treatment. *Neuropharmacology* 96, 255–262 (2015).

149. Dziewczapolski G, Glogowski CM, Masliah E, Heinemann SF. Deletion of the α7 nicotinic acetylcholine receptor gene improves cognitive deficits and synaptic pathology in a mouse model of Alzheimer's disease. *J. Neurosci.* 29(27), 8805–8815 (2009).

150. Wisniewski KE, Dalton AJ, Crapper Mclachlan DR, Wen GY, Wisniewski HM. Alzheimer's disease in Down's syndrome: clinicopathologic studies. *Neurology* 35, 957–996 (1985).

151. Deutsch SI, Burket JA, Benson AD. Targeting the α7 nicotinic acetylcholine receptor to prevent progressive dementia and improve cognition in adults with Down's syndrome. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 54(0), 131–139 (2014).

152. Burghaus L, Schütz U, Krempel U, Lindstrom J, Schröder H. Loss of nicotinic acetylcholine receptor subunits α4 and α7 in the cerebral cortex of Parkinson patients. *Parkinsonism Relat. Disord.* 9(5), 243–246 (2003).

153. Quik M, Wonnacott S. α6β2* and α4β2* nicotinic acetylcholine receptors as drug targets for Parkinson's disease. *Pharmacol. Rev.* 63(4), 938–966 (2011).

154. Ritz B, Rhodes SL. After half a century of research on smoking and PD, where do we go now? *Neurology* 74(11), 870–871 (2010).

155. Leonard S, Breese C, Adams C *et al.* Smoking and schizophrenia: abnormal nicotinic receptor expression. *Eur. J. Pharmacol.* 393(1), 237–242 (2000).

156. Thomsen MS, Hansen HH, Timmerman DB, Mikkelsen JD. Cognitive improvement by activation of alpha7 nicotinic acetylcholine receptors: from animal models to human pathophysiology. *Curr. Pharm. Des.* 16, 323–343 (2010).

157. Dineley KT, Pandya AA, Yakel JL. Nicotinic ACh receptors as therapeutic targets in CNS disorders. *Trends Pharmacol. Sci.* 36(2), 96–108 (2015).

•• **Updated review on the evidences of the involvement of nicotinic receptors in several CNS disorders.**

158. Wing VC, Wass CE, Bacher I, Rabin RA, George TP. Varenicline modulates spatial working memory deficits in smokers with schizophrenia. *Schizophrenia Res.* 149(1), 190–191 (2013).

159. Janowsky DS, El-Yousef MK, Davis JM, Sekerke HJ. A cholinergic-adrenergic hypothesis of mania and depression. *Lancet* 2, 632–635 (1972).

160. Charles HC, Lazeyras F, Krishnan KRR, Boyko O, Payne M, Moore D. Brain choline in depression: *in vivo* detection of potential pharmacodynamic effects of antidepressant therapy using hydrogen localized spectroscopy. *Prog. Neuropsychopharmacol. Biol. Psychiat.* 18, 1121–1127 (1994).

161. Saricicek A, Esterlis I, Maloney KH *et al.* Persistent β2*-nicotinic acetylcholinergic receptor dysfunction in major depressive disorder. *Am. J. Psychiatry* 169(8), 851–859 (2012).

162. Meyer PM, Strecker K, Kendziorra K *et al.* Reduced alpha4beta2*-nicotinic acetylcholine receptor binding and its relationship to mild cognitive and depressive symptoms in Parkinson disease. *Arch. Gen. Psychiatry* 66, 866–877 (2009).

163. Yu L-F, Zhang H-K, Caldarone BJ, Eaton JB, Lukas RJ, Kozikowski AP. Recent developments in novel antidepressants targeting α4β2-nicotinic acetylcholine receptors. *J. Med. Chem.* 57(20), 8204–8223 (2014).

164. Martin-Ruiz CM, Lee M, Perry RH, Baumann M, Court JA, Perry EK. Molecular analysis of nicotinic receptor expression in autism. *Mol. Brain Res.* 123(1), 81–90 (2004).

165. Lee M, Martin-Ruiz C, Graham A *et al.* Nicotinic receptor abnormalities in the cerebellar cortex in autism. *Brain* 125, 1483–1495 (2002).

166. Anand R, Amici SA, Ponath G, Robson JI, Nasir M, Mckay SB. Nicotinic acetylcholine receptor alterations in autism spectrum disorders – biomarkers and therapeutic targets. In: *Autism – A Neurodevelopmental Journey from Genes to Behaviour.* Eapen V (Ed.). Intech, Rijeka, Croatia (2011).

167. Fleisher C, Mcgough J. Sofinicline: a novel nicotinic acetylcholine receptor agonist in the treatment of attention-deficit/hyperactivity disorder. *Exp. Opin. Investigat. Drugs* 23(8), 1157–1163 (2014).

168. Umana IC, Daniele CA, Mcgehee DS. Neuronal nicotinic receptors as analgesic targets: it's a winding road. *Biochem. Pharmacol.* 86(8), 1208–1214 (2013).

169. Sullivan JP, Decker MW, Brioni JD *et al.* (±)-Epibatidine elicits a diversity of *in vitro* and *in vivo* effects mediated by nicotinic acetylcholine receptors. *J. Pharmacol. Exp. Ther.* 271(2), 624–631 (1994).

170. Donnelly-Roberts DL, Puttfarcken PS, Kuntzweiler TA *et al.* ABT-594 [(*R*)-5-(2-azetidinylmethoxy)-2-chloropyridine]: a novel, orally effective analgesic acting via neuronal nicotinic acetylcholine receptors: I. *In vitro* characterization. *J. Pharmacol. Exp. Ther.* 285(2), 777–786 (1998).

171. Damaj MI, Meyer EM, Martin RB. The antinociceptive effects of α7 nicotinic agonists in an acute pain model. *Neuropharmacology* 39(13), 2785–2791 (2000).

172. Papke RL, Kem WR, Soti F, López-Hernández GY, Horenstein NA. Activation and desensitization of nicotinic α7-type acetylcholine receptors by benzylidene anabaseines and nicotine. *J. Pharmacol. Exp. Ther.* 329(2), 791–807 (2009).

173. Jackson KJ, Marks MJ, Vann RE *et al.* Role of α5 nicotinic acetylcholine receptors in pharmacological and behavioral effects of nicotine in mice. *J. Pharmacol. Exp. Ther.* 334(1), 137–146 (2010).

174. Lips KS, Pfeil U, Kummer W. Coexpression of α9 and α10 nicotinic acetylcholine receptors in rat dorsal root ganglion neurons. *Neuroscience* 115(1), 1–5 (2002).

175. Romero HK, Christensen SB, Di Cesare Mannelli L *et al.* Inhibition of α9α10 nicotinic acetylcholine receptors prevents chemotherapy-induced neuropathic pain. *Proc. Natl Acad. Sci. USA* 114(10), e1825–e1832 (2017).

176. Rosas-Ballina M, Tracey KJ. Cholinergic control of inflammation. *J. Intern. Med.* 265(6), 663–679 (2009).

177. Zdanowski R, Krzyzowska M, Ujazdowska D, Lewicka A, Lewicki S. Role of α7 nicotinic receptor in the immune system and intracellular signaling pathways. *Cent. Eur. J. Immunol.* 40(3), 373–379 (2015).

178. Dong M-W, Li M, Chen J *et al.* Activation of α7nAChR promotes diabetic wound healing by suppressing AGE-induced TNF-α production. *Inflammation* 39(2), 687–699 (2016).

179. Siqueira MF, Li J, Chehab L *et al.* Impaired wound healing in mouse models of diabetes is mediated by TNF-α dysregulation and associated with enhanced activation of forkhead box O1 (FOXO1). *Diabetologia* 53(2), 378–388 (2010).

180. Galle-Treger L, Suzuki Y, Patel N *et al.* Nicotinic acetylcholine receptor agonist attenuates ILC2-dependent airway hyperreactivity. *Nat. Commun.* 7, 13202 (2016).

181. Assayag EI, Beaulieu M-J, Cormier Y. Bronchodilatory and anti-inflammatory effects of ASM-024, a nicotinic receptor ligand, developed for the treatment of asthma. *PLoS ONE* 9(1), e86091 (2014).

182. Dezfuli G, Kellar KJ, Dretchen KL, Tizabi Y, Sahibzada N, Gillis RA. Evidence for the role of β2* nAChR desensitization in regulating body weight in obese mice. *Neuropharmacology* 110(Pt A), 165–174 (2016).

183. Shariff M, Quik M, Holgate J *et al.* Neuronal nicotinic acetylcholine receptor modulators reduce sugar intake. *PLoS ONE* 11(3), e0150270 (2016).

184. Hoebel BG, Avena NM, Rada P. Accumbens dopamine-acetylcholine balance in approach and avoidance. *Curr. Opin. Pharmacol.* 7(6), 617–627 (2007).

185. Somm E, Guérardel A, Maouche K *et al.* Concomitant alpha7 and beta2 nicotinic AChR subunit deficiency leads to impaired energy homeostasis and increased physical activity in mice. *Mol. Genet. Metab.* 112(1), 64–72 (2014).

186. Crooks PA, Bardo MT, Dwoskin LP. Nicotinic receptor antagonists as treatments for nicotine abuse. In: *Advances in Pharmacology.* Linda PD (Ed.). Academic Press, Amsterdam, The Netherlands, 513–551 (2014).

187. Brunzell DH, Mcintosh JM, Papke RL. Diverse strategies targeting α7 homomeric and α6β2* heteromeric nicotinic acetylcholine receptors for smoking cessation. *Ann. NY Acad. Sci.* 1327(1), 27–45 (2014).

188. Rose JE. Disrupting nicotine reinforcement. *Ann. NY Acad. Sci.* 1141(1), 233–256 (2008).

189. Hansen ST, Mark GP. The nicotinic acetylcholine receptor antagonist mecamylamine prevents escalation of cocaine self-administration in rats with extended daily access. *Psychopharmacology* 194(1), 53–61 (2007).

190. Solinas M, Scherma M, Fattore L *et al.* Nicotinic α7 receptors as a new target for treatment of cannabis abuse. *J. Neurosci.* 27(21), 5615–5620 (2007).

191. Rahman S, Engleman EA, Bell RL. Nicotinic receptor modulation to treat alcohol and drug dependence. *Fronti. Neurosci.* 8, 426 (2015).

192. Zhao Y. The oncogenic functions of nicotinic acetylcholine receptors. *J. Oncol.* 2016 9650481 (2016).

193. Schuller HM. Is cancer triggered by altered signalling of nicotinic acetylcholine receptors? *Nat. Rev. Cancer* 9(3), 195–205 (2009).

194. Schuller HM, Orloff M. Tobacco-specific carcinogenic nitrosamines. *Biochem. Pharmacol.* 55(9), 1377–1384 (1998).

195. Brusco S, Ambrosi P, Meneghini S, Becchetti A. Agonist and antagonist effects of tobacco-related nitrosamines on human α4β2 nicotinic acetylcholine receptors. *Front. Pharmacol.* 6 201 (2015).

196. Di Cesare Mannelli L, Tenci B, Zanardelli M, Failli P, Ghelardini C. Alpha7 nicotinic receptor promotes the neuroprotective functions of astrocytes against oxaliplatin neurotoxicity. *Neural Plast.* 2015, 396908 (2015) .

197. Philpot RM. Potential use of nicotinic receptor agonists for the treatment of chemotherapy-induced cognitive deficits. *Neurochem. Res.* 40(10), 2018–2031 (2015).