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

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Abstract

Nicotinic receptors are membrane proteins involved in several physiological processes; they are considered suitable drug targets for several CNS disorders or conditions, as shown by the large number of compounds which entered clinical trials. In recent times non conventional 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. If, on one side, these new findings further complicate the pharmacology of these proteins and the design and optimization of ligands, they indeed offer new opportunities to find drugs for the many therapeutic indications involving nicotinic receptors.

Keywords

Drug design, Nicotinic receptors, Allosteric modulators

Introduction

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, being formed by five subunits assembled around a central pore. nAChRs are known from more than a century, but only in the last part of 1900 their fundamental role in the pathophysiology of the central nervous system has emerged, 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 which entered clinical trials for several different CNS indications; some are listed in Table 1. However, despite all the efforts, the only therapeutic application so far is smoking cessation; in many instances, for other diseases or conditions, drugs candidates were found endowed with insufficient efficacy and to adverse effects.

The design of nAChR drugs has been hampered by the complexity of these proteins, and by the lack of structural information on the whole channel, which have been obtained only recently [2]. Complexity refers, on one side, to the high number of possible subtypes and to the many physiological processes in which these proteins are involved; on this topic, which will only be partly addressed in this paper, several excellent reviews have been published, to which the reader is referred for in-depth information. On the other side, 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. If, on one side, this complexity may create difficulties in drug design, it also offers several opportunities, 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 in 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, the latter being composed by α(α2-α7) and β(β2-β4) subunits or by α9 and α10 [3]. 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 be also 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 by combinations of neuronal α and β subunits [4]. More than 90% of these receptor subtypes in rodent brain are α4β2* nAChRs[1]: autoradiographic studies of [^{3}H]-nicotine binding sites in brain showed a correspondence with expression pattern for α4 and β2 subunits ([5] and references therein).

The twelve neuronal subunits can theoretically associate into many combinations, raising the number of the 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 from several species; the α3β4 is the most expressed at ganglions (see [1], [6] and references therein). In many instances, the initial

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[1] :* indicates the possible presence of other subunits

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 subunits assembly, among which stoichiometry, which will be discussed later. For instance, it has been recently shown that the α7 subunit forms also heteromeric channels: α7 and β2 subunits can co-assemble 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 [7]). Tissue and subcellular localization, or sensitivity to oligomeric Amiloid-β_{1-42} protein ($A\beta_{1-42}$) are some intriguing properties of this subtype, suggesting a different role with respect to homomeric α7 receptors [7, 8].

Heteromeric receptors may be formed by 2, 3 or 4 different subunits, giving functional combinations differing in pharmacological and biophysical properties [4]. It is difficult to study these receptors in native tissue, because different combinations can co-localize in the same area [9]. Expression in recombinant systems may speed up receptor characterization, and it 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 or in CHO cells and a library of compounds was tested by means of High Throughput Screening (HTS), disclosing compounds able to discriminate between the $(\alpha3\beta4)_2\alpha5$ and $(\alpha3)_2(\beta4)_3$ subtypes [10, 11]. Also the α6β2β3 has been expressed in HEK293 cells and studied by means of HTS; in this case, to overcome problems of functional expression, a chimeric α subunit was used, formed by the extracellular domain of α6 (containing the ACh binding site) and the transmembrane domain of the α3 subunit, and a mutation was introduced into the β3 pore-lining region [12].

nAChR distribution and function

The α or β subunits are differently expressed in various neuroanatomical regions (Fig. 1). The α4β2* receptors are widely distributed throughout the brain; the α7* subtype are also highly expressed, particularly in the hippocampus; other subunits have a more restricted localization [1, 4, 6, 9]. nAChRs may have a pre-, post- and non-synaptic location, accounting for different modulating activities on transmission and neuronal excitation. Most of 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 [13], while presynaptic α7 nAChRs regulate glutamate and GABA release [14]. Presynaptic nAChRs are also involved in regulating the release of ACh, serotonin and noradrenaline [15].

Nicotinic receptors have roles in synaptic plasticity and development, and participate in cognitive processes. Several diseases or conditions have been associated to changes in function or expression of nicotinic receptors (see [16] and references therein, and the section on Therapeutic potential); some, 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 [17, 18].

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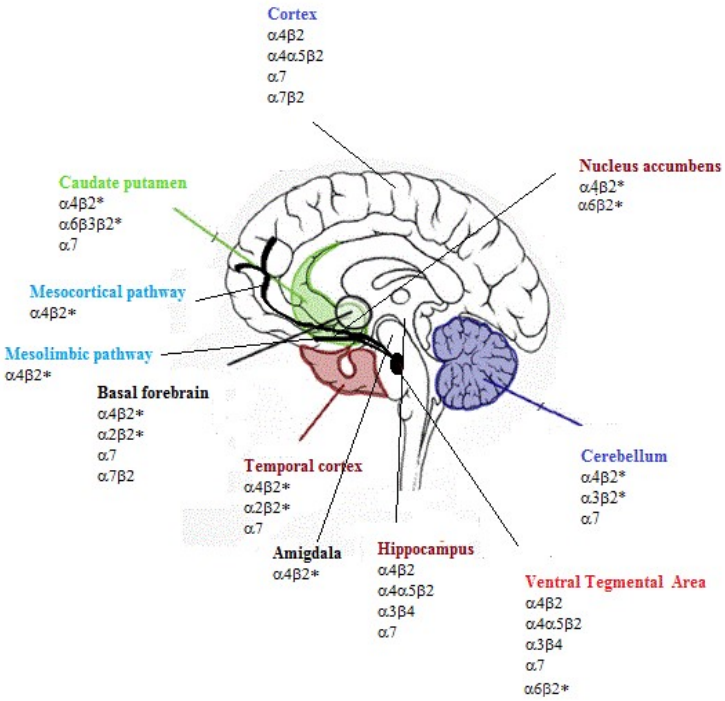

Figure 1. nAChR distribution in human brain (modified from ref.[9] with permission)

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 behaves as non competitive antagonists, binding within the channel, thus hindering the ion flow. The majority of the compounds which entered clinical trials are full or partial agonists interacting at the orthosteric site. The difficulty in designing subtype-selective ligands is due to the high degree of homology within the orthosteric sites; several compounds are known which are able to activate or to 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 [16]. Positive allosteric modulators (PAM) increase the receptor apparent affinity for the agonist and reduce the concentration required to achieve channel opening, while negative allosteric modulators (NAM) 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 [19].

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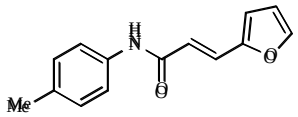
Receptor modulation with allosteric ligands is gaining interest because these molecules have the potentiality to be more subtype selective than orthosteric modulators, since the neurotransmitter binding site is highly conserved, while the allosteric binding sites are not. In addition, PAMs may

have more than one binding site [20]; interaction with those sites may differently modulate activation/desensitization mechanisms, which can also affect selectivity. PAMs are also attractive molecule 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 the 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 may cause calcium overload leading to toxicity [21].



2 (PAM2)

Figure 2. Structure of the Positive Allosteric Modulator PAM-2 (**2**).

On animal models, several α7 PAMs have demonstrated efficacy in different experimental conditions: as an example, compound **2** (PAM-2, Fig. 2), a recently disclosed type II PAM at α7 receptors, showed procognitive and antidepressant activity in rodents, reduced neuropathic and inflammatory pain, and ameliorated ketamine-induced cognitive impairments [22-25]. However, two PAMs (**3**, JNJ-39393406 and **4**, AVL-3288, Table I) have entered clinical trials, giving contrasting results (see later). 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 progressive decrease in response to a subsequent stimulus. α4* receptors desensitize slowly after exposure to low amount of agonist, but desensitization is long lasting; α7* desensitize after exposure to high agonist concentration, but desensitization is readily reversible [26].

Receptor desensitization is a conformational transition to a non-conducting 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 [26]. Recently, for the α7 receptors two different desensitized states have been recognized, obtained under different conditions of agonist application, one sensitive and the other insensitive to type II PAM [27]. Desensitization has been suggested to play 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 ref [28] and references therein).

Since the consequence of desensitization is a non-conducting state of the receptor, it is not clear if agonists produce a particular pharmacological effect actually functioning as inhibitors; indeed, in several instances some nAChR antagonists have been shown to evoke agonist-like nicotinic effect [29]. In this context, the activity of ligands affecting desensitization in a different way with respect to classical agonists, could cast some light. For instance, type II α7-PAMs, as said before, when co-administered with an agonist (i.e. ACh) reactivate one of the desensitized state of the receptor; allosteric agonists, which do not induce desensitization, will be discussed in the next section.

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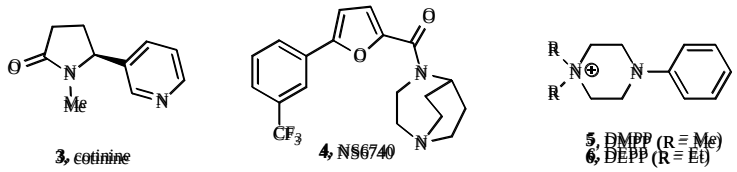

Figure 3. Chemical structure of silent agonists

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Compounds able to directly desensitize the receptor without prior activation are called "silent agonists" or "silent desensitizers"[29, 30]; such behavior has been reported for nicotine (**1**) at very low concentration (10 nM) on α4β2 receptors expressed in HEK293 cells [31], or for **5** (cotinine, Fig. 3), a nicotine metabolite, on ganglionic nicotinic receptors in rats [32].

Recently, silent agonists have been studied mainly on α7 receptors, and an example of such compounds is **6** (NS6740, Fig. 3): in vitro characterization revealed a very weak activation (<2%) of the α7 receptor, and antagonistic properties toward ACh; agonist-like effect were revealed when co-administered with a type II PAM, which reactivate the conducting state, or when tested on a slowly-desensitizing mutated receptor [33]. In vivo, this molecule did not improve cognitive performance in the mouse passive avoidance test [33], but it blocked the procognitive activity of the nicotinic partial agonist BMS-902483 in the mouse novel object recognition test [34]: these findings suggest that activation of α7 receptors is an important step in the 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) [35]; as well as the α7 antagonist methyllycaconitine (MLA), **6** is able to reduce lipopolysaccharide-induced tumor necrosis factor-α (TNF-α) release from microglia [36]. Indeed, the α7 nAChR-mediated modulation of TNF-α release has been associated to a desensitized, non-conductive state of this receptor [37].

Silent agonists are ligands binding to the orthosteric site; their pharmacophore should, at least partially overlap with that of agonist. Some structure-activity relationships have been derived by Papke and coworkers: while the simplest structure activating nicotinic receptor is tetramethylammonium, replacing methyl with ethyl groups gave tetraethylammonium, which is minimal pharmacophore for α7 silent agonism [38]. In a similar way, by replacing the two methyl groups with ethyl moieties, the ganglion stimulating and α7 full agonist dimethylphenylpiperazinium iodide (**7**, DMPP) was transformed into the α7 silent agonist diethylphenylpiperazinium (**8**, DEPP, Fig. 3), whose structure was then further manipulated to obtain more potent molecules [39].

Silent agonists can be either permanently charged compounds or tertiary amines: while amines are required to study receptors in the central nervous system, 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 asthma).

Allosteric agonists

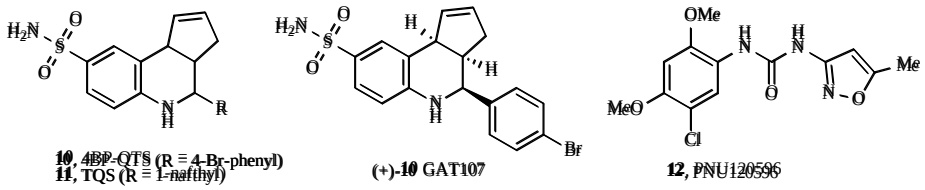

Figure 4. Structure of PAMs and allosteric activators.

Recently, allosteric agonists have also been disclosed, i.e. molecules able to directly activate the receptor acting at a site different from that of the neurotransmitter. Compound **10** (4BP-TQS, Fig. 4), a compound structurally related to the type II PAM TQS (**11**) [40], potentiated the activity of ACh when co-administered with **11**, showing a type II PAM behavior, but when administered alone, it was able to directly activate the α7 receptor. MLA antagonized the effect of **10** in a non competitive way, suggesting an interaction on a different binding site, which was confirmed by the activity on mutated receptors [41]. Several other analogues have been tested, and some structure-activity relationships has been derived, showing that small changes in structure can produce a different receptor modulation, going from direct activation to positive, negative or silent allosteric modulation [42-44]. Interestingly, contrary to ACh and other α7 orthosteric agonists, **10** activated the receptor relatively slowly and it did not produce a rapid desensitization, rather a very slow desensitization that occurred over a period of minutes [42]. 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 [45].

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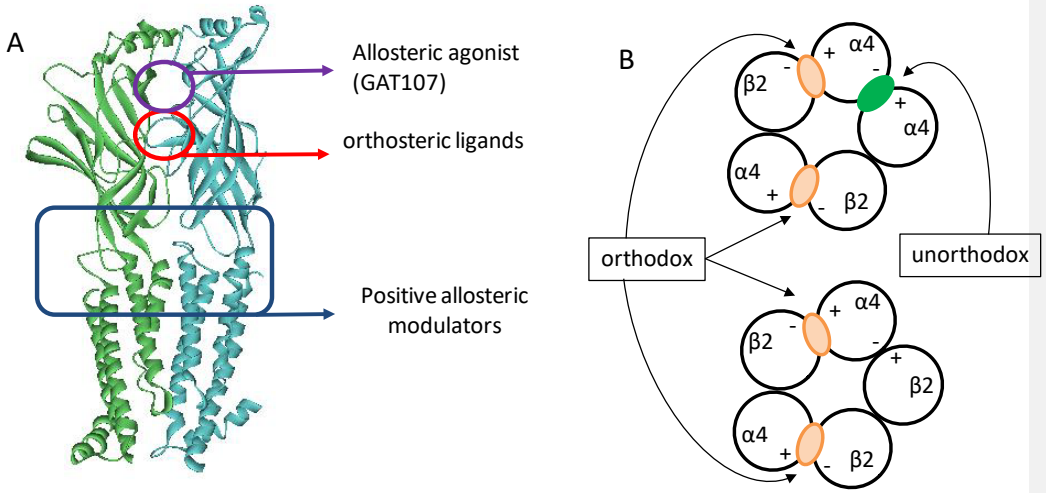

Figure 5. Binding sites on the nicotinic receptor. A) Location of the binding site for nicotinic activators. The orthosteric binding site is in the extracellular domain, at the subunit interface; orthosteric, site-specific and silent agonists bind in this region. The interaction site for the allosteric agonist GAT107 seems located nearby, in the vestibule [44]. Positive allosteric modulators bind in a region that comprises the gating interface and the transmembrane domain [46]. B) The two different stoichiometries of α4β2 receptors, with indication of the orthodox and unorthodox binding sites

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The binding site of **(+)-10** (GAT107, the active enantiomer [47]), 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, Fig. 4) bind, while the other site, required for direct activation, seems to be at the extracellular domain vestibule, in a solvent accessible position (Fig. 5A) [44]. Up to now, **(+)-10** has been tested in models of acute and chronic pain, being active only on the latters, like **6** and also like other α7 agonists and PAMs [35, 48, 49]. 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 [50].

Site specific agonists

A feature greatly affecting receptor properties is subunit stoichiometry. When expressed in recombinant systems, α4 and β2 subunits can co-assemble in two different complexes, formed by 3α4 and 2β2 or by 2α4 and 3β2 subunits, and characterized, respectively, by low and high sensitivity to agonists [51, 52]. This feature is not restricted to this subunit combination, but it has been found also for α3β4, α2β2 and α6β2 [53-55]; significantly, there is evidence that combinations of α4 and β2 subunits with different stoichiometries exist in the brain [56, 57], possibly associated with different modes of transmission or different physiological functions [58].

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's order, produces a functional agonist binding site, called unorthodox to differentiate it from those (the orthodox ones) at the α4/β2 interfaces (Fig. 5B) [59]. The list of subunits that can form unorthodox binding sites comprises α2-6 and β3 (reviewed by Wang [46]), thus including α5 and β3 subunits which were thought in the past unable to be involved in the formation of functional binding sites.

The number of ACh binding sites have 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 [60]. The unorthodox site represent a third, non equivalent agonist binding site on heteromeric receptor, affecting channel properties such conductance, mean open lifetime and desensitization [61-64]. Pharmacological properties are also changed: 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 similar way: for instance, the efficacy of epibatidine (**13**) is higher on $(\alpha4\beta2)_2\alpha4$ than on $(\alpha4\beta2)_2\beta2$, while the reverse is true for **14** and **15** (respectively TC2559 and sazetidine-A, Fig. 6) [59]. 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 with respect to the α4/α4 one: the low or absent affinity for the unorthodox site limits or precludes activation of the $(\alpha4\beta2)_2\alpha4$ subtype.

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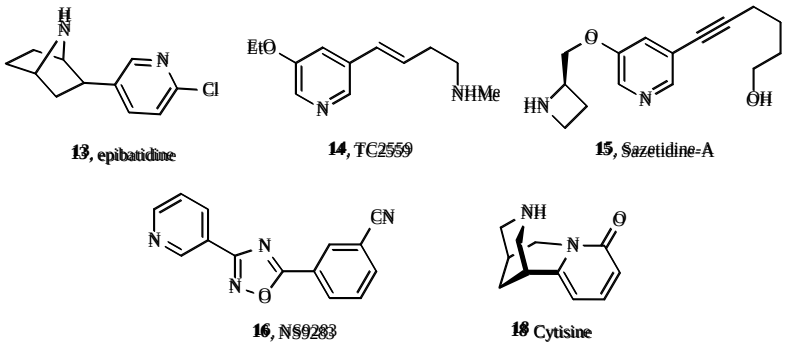

Figure 6. Structure of epibatidine and of site-selective agonists

Agonists are also known, which behave as α4/α4 site-specific modulators [63]. One of such compound is **16** (NS9283, Fig. 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 [65-67]; subsequent studies showed that it interacts with the α4/α4 binding site [68, 69]. **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, since it was able to activate also receptors showing unorthodox sites formed by α2/α4, α4/α2, α4/α3, α4/α4, α4/α6 subunits, being however inactive on α3/α3, α4/β2, α4/β4 and α7/α7 sites [63, 68]. 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 [63] so the in vivo activity of this molecule depends on the endogenous ACh or on the addition of a nicotinic agonist. **16**, administered alone, showed pro-cognitive activity in different models of cognition [68], and in medial prefrontal cortex it enhanced glutamate release mediated by the cholinergic neurons or evoked by low concentration of nicotine [70]. Moreover, co-administration with **17** (ABT-594, Table 1) in several animal models potentiate the analgesic activity but not adverse effects [67].

Compound **14** (TC2559, Fig. 6), on the contrary, is a selective modulators 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 [59]. In addition, **14** shows in vitro selectivity for α4β2 with respect to other subtypes [71] [72], and displays interesting pharmacological properties: it increased dopamine release in rat ventral tegmental area (VTA) slices [72] and it showed analgesic activity in different rodent models of pain [73, 74]. **14** significantly improved performances of rats in two different cognitive tasks [71] but unexpectedly, it suppressed stimulation-induced long-term potentiation (LTP) in rat dentate gyrus, an effect that was prevented by the nicotinic antagonist Dihydro-β-erythroidine (DHβE) [75].

The binding ability of the agonist is dependent on the complementary face of the adjacent subunit, as demonstrated for **15** (Fig. 6), **16** and cytisine (**18**) by site-directed mutagenesis [76]. 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

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be. In a similar way, mutation of F117Q in the β2 subunit gave a $(\alpha4\beta2)_2\beta2$ receptor which was activated by **18**, which is ineffective on the wild type.

These findings may be useful to design site-specific agonists; more work is needed to understand if these molecules can be only pharmacological tools to study receptor stoichiometry or may have a therapeutic potential.

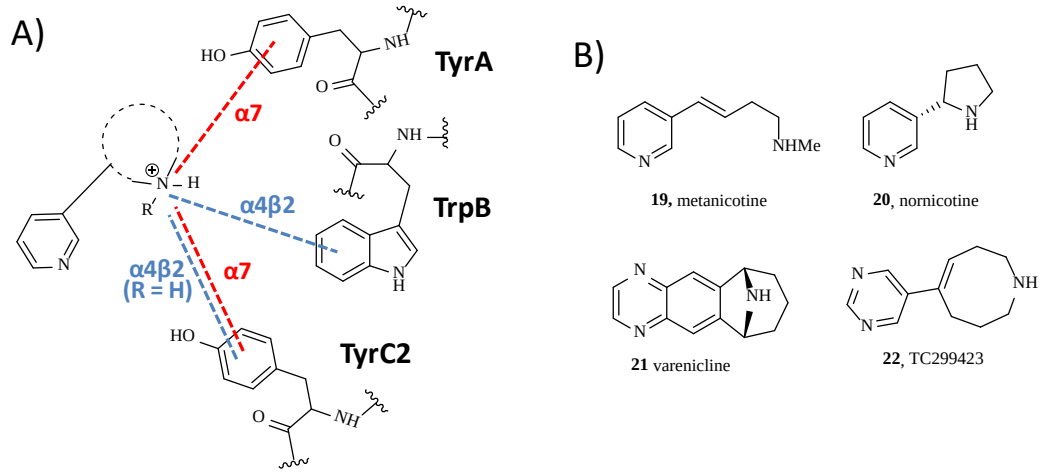

Figure 7. A) Cation-π interactions established at the agonist binding site, measured by means of the nonsense suppression methodology. The cationic nitrogen makes a cation-π interaction (blue) with TrpB in the α4β2 receptors; when the nitrogen atom is in a secondary amine, a similar interaction is made 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

Structure of the binding site and mode of interaction of nicotinic agonists

The structure of nicotinic receptor has been extensively studied by means of electron microscopy in the last decades of 1900, thanks to the possibility of purifying this protein from Torpedo ray, which contains the muscle-type receptor in high quantity, and ending up with a model of the whole channel at 4 Å resolution [77]. 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, being the first example of crystal structure of a heteromeric pentameric ligand-gated ion channel [2]. This structure revealed important features in the binding site and in the overall architecture, which can be compared with other Cys-loop receptors and ligand-gated ion channels. Moreover, it allowed also to explore the differences between the binding site and the β2/α4 and β2/β2 interfaces, suggesting explanation on 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 $(\alpha2)_5$ [78] and of monomeric α9 [79] have been recently reviewed [80].

Until the 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) [81] and of several other analogues (reviewed in [82]). AChBP is a structural homologue 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 [83]. The discovery of these proteins was important in the design of new nicotinic ligands, which was performed not only by means of

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homology models of the binding site, but also by exploiting the possibility of co-crystallization and by applying fragment-based techniques. As a matter of fact, up to July 2017, in the PDB the number of structures of AChBP, alone or in complex with ligands, was higher than 110. Some examples of successful strategies can be found in refs [84-88].

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 [89] and later confirmed by the structure of the whole channel. The ACh binding site is made by six (A-F) loops: A-C are on the principal component (positive side, provided by the α subunit) while D-F 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 a 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, in the AChBP-**1** complex there is 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 ref [90] and references therein) is the H-bond made 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 residue become Asn and Leu, respectively, in the nicotinic receptors, and are conserved in all subtypes.

Although in the recently solved crystal structure of the **1**-$(\alpha4\beta2)_2\beta2$ receptor complex only the π-cation interaction is visible, all the three interactions are important in agonist activation, as demonstrated by the studies performed by the group of Dougherty and Lester using the nonsense suppression methodology [91]. This technique consists in 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 for activating the mutated receptors, measured by means of electrophysiology and compared to wild-type receptor, then shows if that particular interaction is significant for the function of the protein. The results strongly support the idea that the contribution of the three interactions varies depending on both agonist and receptor subtype (reviewed in [92]).

Limiting the analysis to the π-cation interaction (Fig. 7), at the muscle-type receptor, ACh and **13** interact only with TrpB, 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 [93]) establish a functional π-cation interaction with both TrpB and TyrC2 [94]. On the α7 subtype, the 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, the π-cation interaction with TrpB is strong for ACh but not for **1**, as it happens on the muscle-type receptor, nor for **22** [55]. These findings may give useful hints for designing selective nicotinic ligands, providing a structural basis for molecules with particular geometric or conformational features.

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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.

Neurological disorders

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by loss of memory and other cognitive functions; the histopathological markers are the presence of neuritic plaques, formed by β-amyloid protein ($A\beta_{1-42}$), and of neurofibrillary tangles, due to hyperphosphorylated tau protein. Post-mortem analysis of brains from AD patients showed a large decrease of nAChRs, in particular the α4β2* subtype in the cerebral cortex and the α7* subtype in the hippocampus; the latter seems to correlate with the severity of cognitive impairment [95]. $A\beta_{1-42}$ interacts with nicotinic receptors [96]. Affinity of Aβ for α7 nAChRs is in the picomolar range and α7 subunits are co-localized with $A\beta_{1-42}$ in senile plaques [97]; heteromeric α7β2 nAChRs were much more sensitive to $A\beta_{1-42}$ inhibition than homomeric α7 nAChRs [98]. There are some findings implicating α4β2 nAChRs in AD: Aβ has inhibitory effects on these and other β2* receptors [99], while no co-localization was found between Aβ with α4 subunit [100]. The therapeutic benefits of employing α7 nAChR agonists for AD therapy is due to pro-cognitive 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 [100]. 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 (APP) but lacking the α7 nAChR gene [101]. At the moment this approach is not 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 had limited success: only EVP6124 (**26**) seems effective in improving cognitive performance in patients with mild-to-moderate AD [102].

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 [103]. α7 nAChR modulation has been proposed as treatment [104] but presently, only the non-selective agonist nicotine (**1**) is being tested in a phase 1-2 clinical trial.

Parkinson's disease (PD), characterized by a prominent decline in the nigrostriatal dopaminergic pathway, is characterized also by a pronounced decline in AChR expression in the cerebral cortex of PD patients [105]. The decline is particularly evident in α6β2* nAChRs, smaller in the α4β2* receptor subtype while there is no change in α7 receptor expression. The explanation may be due to the diverse localization of these subtypes in this tissue: α6β2* nAChRs are primarily located on incoming dopaminergic afferents, while α7 are positioned on non-dopaminergic terminals, and α4β2* are on dopaminergic, GABAergic and serotoninergic neurons [106]. 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 shown that smoking is inversely associated

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with PD [107] and the lower risk to contract the disease depends on smoking duration, intensity and how recent is cessation. Nicotinic ligands which 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 and neurodevelopmental disorders

Schizophrenia is a neurodevelopmental disorder which 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 evidences (reviewed in [108]) 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 in different percentage according to the cortex region (frontal, parietal and dorsolateral prefrontal) [109, 110]. Although there are 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 [16]. Recently the systemic administration of type II α7 and α4β2 nAChR PAMs has been suggested for the treatment of central nervous system disorders and in particular for schizophrenia [58, 111]. At present, indeed α7* nAChR agonists and PAMs appear to be the most effective hope for the treatment of schizophrenia: the amelioration of cognitive deficits is important for improving negative symptoms and the quality of life, also because the 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 result 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 [112]. A Phase 1 trial on healthy subject for **4** (AVL-3288, a type I α7-PAM), showed indications of positive although non-significant effects on cognition, which need to be confirmed in Phase 2 trials [113]. Varenicline, partial agonist for the α4β2 nAChRs and full agonist for the α7 nAChRs, improves cognition in schizophrenic patients [114, 115]. 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 antidepressants 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 [116], supported by new studies on animals and humans. In fact, using magnetic resonance imaging 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 [117]. Moreover, physostigmine, an acetylcholinesterase inhibitor that potentiates cholinergic transmission, produced depressive-like symptoms [116], while the administration of α7 or α4β2 nicotinic antagonists, or compounds that induce desensitization, reduced depressive symptoms [95]. SPECT (single photon emission computed tomography) and PET (positron emission tomography) 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 that in healthy subjects [118, 119]. In general, the inhibition of nAChR activity is beneficial in the treatment of depression and the effects are synergistic with antidepressants such as imipramine,

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citalopram and reboxetine. Antagonists alone 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 [120]. However, in two phase 3 clinical trials, TC-5214 [(S)-**32**] was tested as adjunct therapy in patients with depression but did not show efficacy [121]. On the contrary, Varenicline, tested as smoking cessation agent in patients suffering or having suffered from depression, did not exacerbated the disease [44][Anthenelli, 2013 #1185. 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 non-verbal communication and by 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 no in thalamus [Martin-Ruiz, 2004 #1139]. α4β2 nAChR loss results from an impaired post-translational mechanism regulating its expression, since there is no reduction in mRNA expression [122]. 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: the 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 [123]. 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 to restore its levels of expression and may be beneficial in correcting deficits of ASD. So far, however, only α4β2 antagonists like mecamylamine **32** has been tested in clinical trials, with negative results [124], while the trial on nicotine is ongoing (Table1).

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 [125] even if the main challenge to progress in ADHD treatment remains the lack of knowledge of the 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**) [126] and ABT894 (**33**) [127] but not for **24**, **25** and **27** [128, 129] (Table 1).

Pain and inflammation

Since many years the nicotinic receptors have emerged as interesting drug targets for pain treatment [130]. More than 20 years ago the analgesic properties of epibatidine (**13**) [131] were discovered; since **13** is a potent agonist at α4β2* receptors, even if not selective, research has been initially focused on this subtype, and several potent α4β2 full agonists have been synthesized. These compounds, among which are ABT-594 (**17**) and TC2629 (**36**), are endowed with strong analgesic properties, but a narrow therapeutic window [48, 132]. ABT594 was tested in a phase 2 clinical trial, showing efficacy but limited tolerability due to side effects [133]. Even though research is very active in this field, only the α4β2 agonist ABT-894 (**33**) has completed a phase 2 clinical trial for diabetic neuropathic pain, not confirming the results of **17** [134]. Interestingly, as mentioned before, it has been shown that the safety profile of **17** can be improved by using the

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$\alpha 4/\alpha 4$ site-selective ligand NS9283 (**16**), suggesting the combination of a $\alpha 4\beta 2$ full agonist and a $\alpha 4\beta 2$ selective potentiator as new therapeutic strategy [67]

It is known from long time also that $\alpha 7$ stimulation produces antinociceptive activity [135]. However, the development of $\alpha 7$ agonists may not be a priority given that a long-term treatment can lead to receptor desensitization and/or inactivation, suggesting possible adaptations to their effects after chronic use [136]. More recently, $\alpha 7$ type II PAMs and silent agonists have been evaluated in preclinical models for pain [35, 49], confirming that $\alpha 7$ nAChR modulators can be an attractive and versatile alternative to $\alpha 4\beta 2$ agonists.

Besides the $\alpha 4\beta 2^*$ and $\alpha 7^*$ receptors, other nAChRs are also known to be involved in pain. In particular, the $\alpha 5$ subunit plays a significant role in nociception and the $\alpha 4\beta 2\alpha 5^*$ subtype may be the target for nicotinic analgesic agents [137]. Furthermore, dorsal root ganglion neurons coexpress the $\alpha 9$ and $\alpha 10$ nAChR subunits, suggesting a role for the $\alpha 9/\alpha 10$ nAChRs in pain processing [138]. This was supported by a recent study showing that RgIA4, a peptide showing highly selectivity and potent inhibition of the $\alpha 9\alpha 10$ nAChR subtype, prevented chemotherapy-induced neuropathic pain in rats [139].

Inflammatory states normally produce pain, and the $\alpha 7$ receptor plays an important role also in the cholinergic control of inflammation, regulating the production of pro-inflammatory cytokine through neuronal and non-neuronal mechanisms [140, 141]. Recently it has been reported that the activation of $\alpha 7$ nAChRs on nonneuronal cells, like macrophages, efficiently suppressed TNF-α synthesis [142]; high TNF-α levels are involved in delaying diabetic wound healing through an excessive fibroblast apoptosis and reduced fibroblast density [143]. The selective $\alpha 7$ nAChR agonist PNU282987 (Fig. 8) significantly reduced the TNF-α level of wounds in a murine model, by inhibiting its production through activation of $\alpha 7$ 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 $\alpha 7$ receptors by means of silent agonists reduced TNF-α release, supporting the hypothesis that this action is associated to a desensitized, non-conductive state of this receptor [37].

Also chronic inflammatory diseases of the airways, like allergic and non-allergic asthma, can be treated with $\alpha 7$ agonists. Administration of $\alpha 7$ 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 (AHR) and allergic inflammation. Therefore, the cholinergic pathways have a protective role in the pathogenesis of asthma, and $\alpha 7$ nAChR agonists can be useful for the treatment of ILC2-mediated asthma [144]. The $\alpha 7$ modulator TC6987 (**34**) and the $\alpha 7$ silent agonist **9** [145] have completed clinical trials in moderate and allergic asthma; the latter did not show efficacy [146].

Food behavior

The involvement of nAChRs in food intake behaviors, in regulating body weight, in 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 body weight is an increased activity of $\alpha 3\beta 4^*$, $\alpha 7$ and $\alpha 4\beta 2$ nicotinic receptors in pro-opiomelanocortin (POMC) neurons of the hypothalamus, resulting in stimulation of the melanocortin system [147]. A primary potential pathway for $\alpha 7$nAChR mediation of eating bahaviors involves hypothalamic cholinergic input.

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Cholinergic innervations are abundant in the hypothalamus, and there are high levels of α7 nAChR expression, leading to activation of POMC neurons. Another mechanism proposed 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 (NAc) [148], a change similar to that observed for addiction to substances of abuse [149]. Therefore, targeting the α7 nAChRs may be a useful therapeutic strategy to treat and manage obesity: GTS-21 (**31**) is in phase 1 clinical trial for this indication. The α4β2 partial agonist and α7 full agonist varenicline reduces sugar consumption; it is in a phase 3 clinical trial for controlling weight gain after stopping smoking. In mice, constant infusion of sazetidine-A (**15**) decreased food intake robustly and body weight; the proposed mechanism is desensitization of α4β2* subtype [147].

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. Double α7 and β2 deficiency mice shows increased spontaneous physical activity, no change in body weight though associated with a modification of body composition with increased lean and bone mass and decreased fat pads [150]. Compound **34** (TC-6987), an α7 modulator, has been studied in phase 2 clinical trial for the treatment of type 2 diabetes mellitus.

Substance of 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 FDA) and cytisine (**18**, approved in Europe) and bupropion (**35**, approved by FDA), behaving as α3β2/α4β2 antagonist [151]. Nicotinic ligands like the α4β2 agonists ABT-089(**24**) and dianicline (**37**), and the association of **21** and **35** have been studied in phase 2/3 clinical trials for smoking cessation. Dianicline did not show efficacy beyond the initial treatments [152]; with respect to **21** alone, the association between **21** and **35** was more effective in the initial treatment phase but not after longer times [153, 154]. 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 a significant decreases in motivation to self-administer nicotine [155]. In animal models selective α6 antagonists were effective in reducing nicotine self-administration (see [151] and references therein); these substances should have less side effects compared to the non-selective antagonist mecamylamine, since the localization of α6* receptors is more restricted. Mecamylamine has been extensively tested in clinical trials for smoking cessation yielding contrasting results [156, 157]. GTS-21, with a dual α7 agonist and α4β2 antagonist activity, is undergoing a phase 2 clinical trial for tobacco use disorder, while the trial involving the α7 agonist EVP-6124 (**26**) has been terminated.

Regarding other drug dependence, nAChR antagonists were found to decrease cocaine self-administration [158] and to have a critical role in regulating the rewarding effects of cannabinoids [159]. Alcohol dependence can be treated with α4β2 partial agonists such as cytisine (**18**) and varenicline (**21**); the latter was found efficacious in reducing alcohol drinking in both animals models and humans. Also sazetidine-A (**15**) was able to reduce alcohol drinking in alcohol-preferring rats [160]. A phase 3 clinical trial on mecamylamine did not show significant effects on alcohol consumption [161].

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Cancer

Nicotinic acetylcholine receptors 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, to promotion of tumor angiogenesis and inhibition of apoptosis in multiple types of cancers [162]. The α7* and α4β2* receptors differently regulate the release of neurotransmitters which have opposite actions on cell proliferation; it has been proposed that unbalance between the activity of these two receptors affects cancer growth in smokers [163]. The tobacco-specific carcinogenic nitrosamines, NNK and NNN (Fig. 8) interact with nAChRs: NNK was found to bind with high affinity to α7 receptors and to stimulate Small Cells Lung Cancer (SCLC) cells proliferation, an effect which was antagonized by α-Bgtx suggesting α7 activation [164]. On α4β2 receptors, NNK behaved as a partial agonist, while NNN was an antagonist [165]. Several evidences suggest that α7 antagonists may be useful to block cancer cells 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 [166]. Both varenicline and nicotine have been tested in cancer patients, but only for smoking cessation, because smokers have higher risk of treatment complications with respect to non-smokers.

In cancer patients, during and following chemotherapy, cognitive deficits referred as "chemo-brain" may appear. The study of "chemo-brain" is complicated by the difficulties to have animal models [167], and currently there is no approved treatment for this indication. At present, transdermal nicotine is studied in a phase 2 clinical trial for the treatment of chemo-brain in patients who are 1-5 years post-chemotherapy.

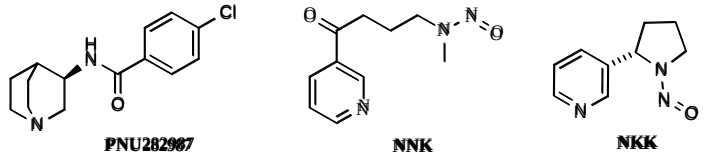

Figure 8. Structure of PNU282987 and of carcinogenic nicotinic nitrosamines

Conclusions and future directions

One of the main achievement in the last ten years of research on nicotinic receptors is the discovery of compounds able to modulate nAChRs through different sites, either allosteric or orthosteric; this is important for different reasons. First, modulation from allosteric sites should allow, in principle, to achieve the subtype selectivity which has been difficult to obtain with orthosteric ligands, and which has been among the reasons for failure in clinical trials. Second, PAMs may represent for the nicotinic receptors what benzodiazepine have been for GABA-A receptor, i.e. the possibility to activate the receptor without exceeding the maximal physiological level of the tissue. Third, compounds affecting desensitization in different way give the opportunity to study the contribution of this mechanisms in the activity of the receptor. Fourth, the multiplicity of orthosteric binding sites within heteromeric receptors offers the possibility to discriminate among

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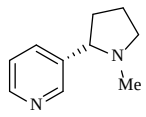
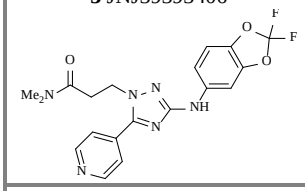
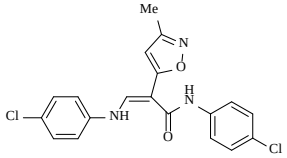
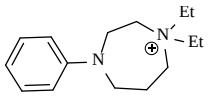
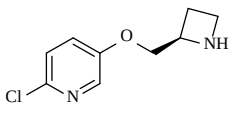
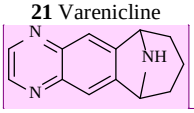
receptor with similar subunits but in different stoichiometry. The medicinal chemists therefore have the possibility to exploit these different druggable sites to design new ligands.

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, giving a close picture of 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, so 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 disclose 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 compounds useful only as pharmacological tools, or if they really possess a therapeutic potential, exploitable within the many disorders or conditions, in which nicotinic receptors are involved.

Table 1. Nicotinic agents that are or have been studied in clinical trials by U.S. National Institutes of Health[a].

Drug	Action on nAChR	Pathology; Phase (Status[b])	Results [c] [source]
1 Nicotine 	Agonist	Down syndrome; 1,2 (R)	
		Mild Cognitive Impairment; 1,2 (R)	
		Parkinson's disease; 1,2 (C)	
		Major depressive disorder; 4 (R)	
		ASD; 1 (R)	
		Chemo Brain; 2 (R)	
		Schizophrenia; [d]	Low or medium doses of nicotine i.v. did not alter symptoms of schizophrenia or attention [168] Modest improvement of nicotine on attention, not in other cognitive functions, in smoking but not in non-smoking patients [169]
3 JNJ39393406 	α7 PAM	Schizophrenia, Smoking cessation in schizophrenia; 2	Failed to reverse basic deficits of information processing [113]
4 AVL-3288 	α7 PAM	Schizophrenia; 1	Positive although non-significant effects on cognition [112]
9 ASM024 	α7 Silent agonist	Asthma; 2	No significant inhibition of allergen-induced asthmatic response and related airway inflammation. Side effect: cough. [146]
		COPD; 2 (C)	
17 ABT-594[e] 	α4β2 Agonist	Diabetic neuropathic pain; 2	Significant efficacy in the treatment of diabetic peripheral neuropathic pain. Side effects: nausea, dizziness, vomiting, abnormal dreams, and asthenia. [133]
21 Varenicline 	α4β2 Partial Agonist; α7 Agonist	Nicotine dependence in depression; 4	Increase in smoking cessation in smokers with current or past depression without exacerbation of depression or anxiety. [170]
		Schizophrenia; 3	Cognitive improvements as adjunctive treatment with antipsychotics. Side effects: nausea and headache. [114]

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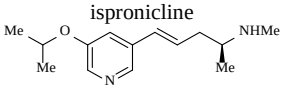
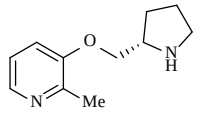
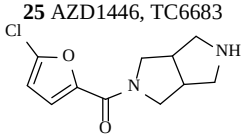
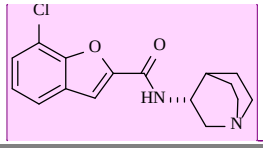
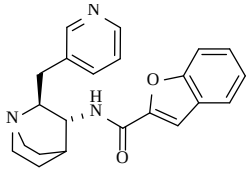
		Alzheimer's disease; 2	No improvement of cognition in korean patients with mild-to-moderate Alzheimer's disease. Side effects: gastrointestinal. [171]
		Parkinson's disease; 2,4 (R, A)	
		Obesity; 2,3 (R)	
		Alcohol use disorder; [d]	Inhibition of alcohol consumption in a population of heavy drinkers. [172]
		Methamphetamine Dependence; 1	Significant improvement of reaction time on the n-back for visual stimuli in METH-dependent volunteers. [173]
		Cocaine dependence; 2 (C)	
		Smoking cessation in schizophrenia; 3	Significant efficacy in smoking reduction in people with schizophrenia. [174]
23 AZD3480, TC1734, ispronicline 	α4β2 Partial Agonist	ADHD; 2	Significant improvement of cognitive and clinical ADHD symptoms. Dose: 50 mg. [126]
		Schizophrenia; 2	No efficacy. Psychiatric side effects recorded. [175]
		Alzheimer's disease; 2	The study was inconclusive. [176]
24 ABT089 	α4β2 Agonist	Nicotine addiction; 2 (T)	
		Alzheimer's disease; 2 (T)	
		ADHD; 2	Effective in a pilot study but non on a larger trial. [128]
25 AZD1446, TC6683 	α4β2 Agonist	-; 1	Side effects: nausea, headache and dizziness [177]
		ADHD; 2	No significant improvements of ADHD symptoms after 2 weeks of treatment . [129]
		Alzheimer's disease; 2 (C)	
26 EVP6124, MT4666, encenicline 	α7 partial agonist	Schizophrenia; 1	Positive effect on cognitive functions in patients treated with antipsychotic drugs. [178]
		Alzheimer's disease; 2b	(At 28 showed improved cognitive performance with respect to placebo. Dose: 2 mg/die. [102]
		Smoking cessation; 2 (T)	
27 TC5619 	α7 Agonist	Schizophrenia; 2	The positive results of exploratory study were not confirmed in larger trials. [179]
		Alzheimer's disease ; 1	Discontinued [http://adisinsight.springer.com/drugs/800026693]
		ADHD; 2	Discontinued [http://www.marketwatch.com/story/targacept-ends-development-of-adhd-drug-2012-09-17]

Commento [u13]: Forse meglio inserire Healthy invece che -

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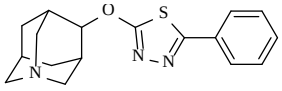
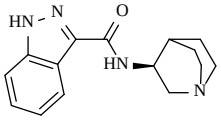
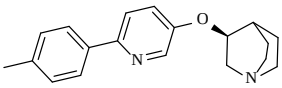
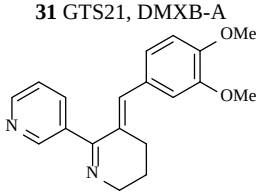
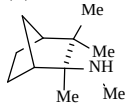
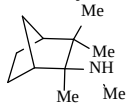
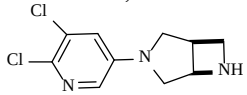
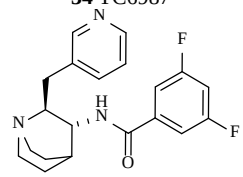
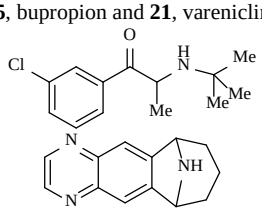
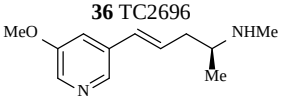
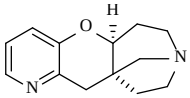
Commento [u14]: È un benzotiofene

28 ABT126 	α7 agonist	Schizophrenia; 2	Lack of a consistent effect on cognition in non-smoking subjects with schizophrenia; positive effect on negative symptoms. [180, 181]
		Alzheimer's disease; 2b	No significant improvement in subjects with mild-to-moderate ~~AD, alone or with AChEI.~~ Side effects: agitation, constipation, diarrhea, fall, headache. [182, 183]
29 MEM3454, RG3487 	α7 agonist	Alzheimer's disease; 2 (C)	
		Schizophrenia; 2	No improvement of cognitive deficits but improvement of negative symptoms. Side effect: constipation. [184]
30 AQW051 	α7 partial agonist	Schizophrenia; 2	Following treatment with **32**, brain activation, measured by means of fNMRI, was not associated with changes in cognitive performance. [185]
		Levodopa-induced Dyskinesia in Parkinson's Disease; 2	No significant reduction of dyskinesia or parkinsonian severity. [186]
		Alzheimer's disease; 2 (T)	
31 GTS21, DMXB-A 	α7 partial agonist and α4β2 antagonist	ADHD; 2 (C)	
		Alzheimer's disease; 2 (C)	
		Schizophrenia; 2	Significant effects on attention/vigilance and working memory; improvement of negative symptoms. [108]
		Tobacco use disorder; 2 (A)	
		Obesity; 1 (R)	
S(+)-**32** TC5214 	Antagonist	Major depressive disorder; 3	No efficacy was shown in patients with inadequate response to prior antidepressant therapy. Side effects: constipation, dizziness, dry mouth. [121] [187]
32 mecamylamine 	Antagonist	Cognition in schizophrenia; [d]	Worsening of performance relative to placebo and varenicline, to a greater extent in participants with schizophrenia than in healthy controls. [188]
		Alcohol dependence; 2	No significant effect on alcohol consumption in patients with alcohol use disorders. [161]
		Autism; 1	No significant benefit was suggested. [124]
		Smoking cessation; 2	No evidence of efficacy as adjuvant with nicotine-replacement therapy. [156]
33 ABT894, sofinicline 	α4β2 Agonist	Diabetic neuropathic pain; 2	No efficacy at the dose of 1-4 mg BID. [134]
		ADHD; 2	Significant reduction of ADHD symptoms in adults. Dose: 4 mg BID. Side effects: nausea, dizziness, headache, and fatigue. [127]

Commento [u15]: 2b ??

34 TC6987 [f] 	α7 agonist; [f] open channel stabilizer [g]	Asthma; 2 (C)	
		Type 2 diabetes mellitus; 2 (C)	
35, bupropion and **21**, varenicline 		Smoking cessation; 3	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. [154] Higher efficacy, after 12 weeks treatment, in patients not responding to prequit nicotine patch treatment. [153]
		Alcohol use disorder; 2 (A)	
36 TC2696 	α4β2 Agonist	Pain; 2	In Postoperative Dental Pain the primary end point was not met. *[http://www.nomura.com/resources/npv/pdfs/12-03-07-Targacept.pdf]*
37 SSR591813, Dianicline 	α4β2 Partial Agonist	Smoking cessation; 3	No efficacy for smoking cessation beyond the initial treatment phase. Side effects: diarrhea, nausea. [152]
ATG002[h]	Agonist	Diabetic foot ulcers; 1,2 (T)	

[a] http://clinicaltrials.gov, by U.S. National Institutes of health; [b] C: Completed; A: Active, R: Recruiting; T: Terminated; U: Unknown; [c] Information were taken from peer-reviewed papers or from Web news. [d] Phase non reported; [e] From ref [133]; [f] Retrieved from ref. [189189]. [g] Definition reported on www.clinicaltrial.gov (NCT01293669). [h] Low-dose Nicotine gel, NCT00316537.

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Executive summary

- Nicotinic receptors are pentameric membrane proteins; twelve neuronal subunits have been cloned, which can theoretically associate into many combinations, raising the number of the 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 by the same subunits but with different stoichiometry.

- Agonists activate and desensitize the receptors; silent agonists block the receptor in the desensitize state without prior activation. Positive allosteric modulators potentiate the 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 non-conventional nicotinic receptor modulators.

References

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

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

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

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

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

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

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

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

Commento [N16]: Da rivedere secondo la nuova numerazione

1. Albuquerque EX, Pereira EFR, Alkondon M, Rogers SW. Mammalian Nicotinic Acetylcholine Receptors: From Structure to Function. *Physiological Reviews* 89(1), 73-120 (2009).
2. Morales-Perez CL, Noviello CM, Hibbs RE. X-ray structure of the human α4β2 nicotinic receptor. *Nature* 538 411-415 (2016).
3. Gotti C, Clementi F. Neuronal nicotinic receptors: from structure to pathology. *Prog. Neurobiol.* 74 363-396 (2004).
4. Gotti C, Clementi F, Fornari A, Gaimarri A, Guiducci S, Manfredi I. Structural and functional diversity of native brain nicotinic receptors. *Biochemical Pharmacology* 78 703-711 (2009).
5. Papke RL. Merging old and new perspectives on nicotinic acetylcholine receptors. *Biochemical Pharmacology* 89 1-11 (2014).
6. Gotti C, Zoli M, Clementi F. Brain nicotinic acetylcholine receptors: native subtypes and their relevance. *Trends in Pharmacological Sciences* 27(9), 482-491 (2006).
7. Wu J, Liu Q, Tang P *et al*. Heteromeric α7β2 Nicotinic Acetylcholine Receptors in the Brain. *Trends in Pharmacological Sciences* 37(7), 562-574 (2016).
8. 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. *The International Journal of Biochemistry & Cell Biology* 53 246-252 (2014).
9. Zoli M, Pistillo F, Gotti C. Diversity of native nicotinic receptor subtypes in mammalian brain. *Neuropharmacology* 96, Part B 302-311 (2015).
10. 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 and Drug Development Technologies* 14(6), 333-344 (2016).
11. 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 Chemical Biology* 12(3), 702-714 (2017).

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Formattato: Inglese (Regno Unito)

Formattato: Inglese (Regno Unito)

Formattato: Inglese (Regno Unito)

Formattato: Inglese (Regno Unito)

12. Armstrong LC, Kirsch GE, Fedorov NB *et al*. High-Throughput Patch Clamp Screening in Human α6-Containing Nicotinic Acetylcholine Receptors. *SLAS DISCOVERY: Advancing Life Sciences R&D* 22(6), 686-695 (2017).
13. 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).
14. Bertrand D, Lee C-HL, Flood D, Marger F, Donnelly-Roberts D. Therapeutic Potential of a7 Nicotinic Acetylcholine Receptors. *Pharmacological Reviews* 67 1025–1073 (2015).
15. 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).
16. Hurst R, Rollema H, Bertrand D. Nicotinic acetylcholine receptors: From basic science to therapeutics. *Pharmacology & Therapeutics* 137(1), 22-54 (2013).
17. Maskos U. Emerging concepts: novel integration of in vivo approaches to localize the function of nicotinic receptors. *Journal of Neurochemistry* 100(3), 596-602 (2007).
18. Changeux J-P. Nicotine addiction and nicotinic receptors: lessons from genetically modified mice. *Nat Rev Neurosci* 11(6), 389-401 (2010).
19. Mazurov AA, Speake JD, Yohannes D. Discovery and Development of α7 Nicotinic Acetylcholine Receptor Modulators. *Journal of Medicinal Chemistry* 54(23), 7943-7961 (2011).
20. Arias HR. Chapter 5 - Positive and negative modulation of nicotinic receptors. In: *Advances in Protein Chemistry and Structural Biology*, Rossen D (Ed.^(Eds).Academic Press 153-203 (2010).
21. Williams DK, Wang J, Papke RL. Positive allosteric modulators as an approach to nicotinic acetylcholine receptor-targeted therapeutics: Advantages and limitations. *Biochemical Pharmacology* 82(8), 915-930 (2011).
22. 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. *British Journal of Pharmacology* 172(21), 5123-5135 (2015).
23. 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. *Behavioural Brain Research* 302 142-151 (2016).
24. 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, Part A 188-197 (2017).
25. 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. *Neuroscience Letters* 569 126-130 (2014).
26. Giniatullin R, Nistri A, Yakel JL. Desensitization of nicotinic ACh receptors: shaping cholinergic signaling. *Trends in Neurosciences* 28(7), 371-378 (2005).
27. 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. *Molecular Pharmacology* 80(6), 1013-1032 (2011).
28. Cecchini M, Changeux J-P. The nicotinic acetylcholine receptor and its prokaryotic homologues: Structure, conformational transitions & allosteric modulation. *Neuropharmacology* 96 137-149 (2015).
29. Buccafusco JJ, Beach JW, Terry AV. Desensitization of Nicotinic Acetylcholine Receptors as a Strategy for Drug Development. *Journal of Pharmacology and Experimental Therapeutics* 328(2), 364-370 (2009).
30. Chojnacka K, Papke RL, Horenstein NA. Synthesis and evaluation of a conditionally-silent agonist for the α7 nicotinic acetylcholine receptor. *Bioorganic & Medicinal Chemistry Letters* 23(14), 4145-4149 (2013).

Formattato: Inglese (Regno Unito)

31. Paradiso KG, Steinbach JH. Nicotine is Highly Effective at Producing Desensitization of Rat α4β2 Neuronal Nicotinic Receptors. *The Journal of Physiology* 553(3), 857-871 (2003).
32. Buccafusco JJ, Shuster LC, Terry Jr AV. Disconnection between activation and desensitization of autonomic nicotinic receptors by nicotine and cotinine. *Neuroscience Letters* 413(1), 68-71 (2007).
33. Briggs CA, Grønlien JH, Curzon P *et al*. Role of channel activation in cognitive enhancement mediated by α7 nicotinic acetylcholine receptors. *British Journal of Pharmacology* 158(6), 1486-1494 (2009).
34. 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. *European Journal of Pharmacology* 807 1-11 (2017).
35. 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).
36. Thomsen MS, Mikkelsen JD. The α7 nicotinic acetylcholine receptor ligands methyllycaconitine, NS6740 and GTS-21 reduce lipopolysaccharide-induced TNF-α release from microglia. *Journal of Neuroimmunology* 251(1–2), 65-72 (2012).
37. Suzuki T, Hide I, Matsubara A *et al*. Microglial α7 nicotinic acetylcholine receptors drive a phospholipase C/IP3 pathway and modulate the cell activation toward a neuroprotective role. *Journal of Neuroscience Research* 83(8), 1461-1470 (2006).
38. Papke RL, Chojnacka K, Horenstein NA. The Minimal Pharmacophore for Silent Agonism of the α7 Nicotinic Acetylcholine Receptor. *Journal of Pharmacology and Experimental Therapeutics* 350(3), 665-680 (2014).
39. Quadri M, Papke RL, Horenstein NA. Dissection of N,N-diethyl-N'-phenylpiperazines as α7 nicotinic receptor silent agonists. *Bioorganic & Medicinal Chemistry* 24(2), 286-293 (2016).
40. Halvard Grønlien J, Håkerud M, Ween H *et al*. Distinct Profiles of α7 nAChR Positive Allosteric Modulation Revealed by Structurally Diverse Chemotypes. *Molecular Pharmacology* 72(3), 715-724 (2007).
41. Gill JK, Savolainen M, Young GT, Zwart R, Sher E, Millar NS. Agonist activation of α7 nicotinic acetylcholine receptors via an allosteric transmembrane site. *Proceedings of the National Academy of Sciences* 108(14), 5867-5872 (2011).
42. 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. *Molecular Pharmacology* 81(5), 710-718 (2012).
43. 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. *Journal of Biological Chemistry* 290(6), 3552-3562 (2015).
44. 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. *Journal of Biological Chemistry* 291(10), 5049-5067 (2016).
45. 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).
46. Wang J, Lindstrom J. Orthosteric and allosteric potentiation of heteromeric neuronal nicotinic acetylcholine receptors. *British Journal of Pharmacology* doi:doi: 10.1111/bph.13745 (2017).
47. 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. *Journal of Medicinal Chemistry* 56(21), 8943-8947 (2013).
48. 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).

Formattato: Inglese (Regno Unito)

49. Freitas K, Carroll FI, Damaj MI. The Antinociceptive Effects of Nicotinic Receptors α7-Positive Allosteric Modulators in Murine Acute and Tonic Pain Models. *Journal of Pharmacology and Experimental Therapeutics* 344(1), 264-275 (2013).
50. 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. *Experimental Neurology* 295 194-201 (2017).
51. Nelson ME, Kuryatov A, Choi CH, Zhou Y, Lindstrom J. Alternate Stoichiometries of α4β2 Nicotinic Acetylcholine Receptors. *Molecular Pharmacology* 63(2), 332-341 (2003).
52. 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. *Molecular Pharmacology* 70(2), 755-768 (2006).
53. 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).
54. 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).
55. 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).
56. 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. *Molecular Pharmacology* 73(6), 1796-1807 (2008).
57. 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).
58. 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 & Clinical Pharmacology & Toxicology* 116(3), 187-200 (2015).
59. Carbone AL, Moroni M, Groot-Kormelink PJ, Bermudez I. Pentameric concatenated (α4)2(β2)3 and (α4)3(β2)2 nicotinic acetylcholine receptors: subunit arrangement determines functional expression. *British Journal of Pharmacology* 156(6), 970-981 (2009).
60. Andersen N, Corradi J, Sine SM, Bouzat C. Stoichiometry for activation of neuronal α7 nicotinic receptors. *Proceedings of the National Academy of Sciences* 110(51), 20819-20824 (2013).
61. Harpsøe K, Ahring PK, Christensen JK, Jensen ML, Peters D, Balle T. Unraveling the High- and Low-Sensitivity Agonist Responses of Nicotinic Acetylcholine Receptors. *The Journal of Neuroscience* 31(30), 10759-10766 (2011).
62. Mazzaferro S, Bermudez I, Sine SM. α4β2 Nicotinic Acetylcholine Receptors: relationships between subunit stoichiometry and function at the single channel level. *Journal of Biological Chemistry* 292(7), 2729-2740 (2017).
63. Wang J, Kuryatov A, Sriram A *et al*. An Accessory Agonist Binding Site Promotes Activation of α4β2* Nicotinic Acetylcholine Receptors. *Journal of Biological Chemistry* 290(22), 13907-13918 (2015).
64. Benallegue N, Mazzaferro S, Alcaino C, Bermudez I. The additional ACh binding site at the α4(+)/α4(−) interface of the (α4β2)2α4 nicotinic ACh receptor contributes to desensitization. *British Journal of Pharmacology* 170(2), 304-316 (2013).
65. Malysz J, Dyhring T, Ahring P *et al*. In vitro pharmacological profile of a novel α4β2 positive allosteric modulator NS9283 (A-969933). *Br. J. Pharmacol.* 78 919-920 (2009).
66. 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. *Biochemical Pharmacology* 82(8), 959-966 (2011).
67. 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

Formattato: Inglese (Regno Unito)

allosteric modulator in experimental models of pain in rats. *Biochemical Pharmacology* 82(8), 967-976 (2011).
68. 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. *British Journal of Pharmacology* 167(1), 164-182 (2012).
69. 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. *Journal of Biological Chemistry* 288(50), 35997-36006 (2013).
70. Grupe M, Paolone G, Jensen AA, Sandager-Nielsen K, Sarter M, Grunnet M. Selective potentiation of (α4)3(β2)2 nicotinic acetylcholine receptors augments amplitudes of prefrontal acetylcholine- and nicotine-evoked glutamatergic transients in rats. *Biochemical Pharmacology* 86(10), 1487-1496 (2013).
71. Bencherif M, Bane AJ, Miller CH, Dull GM, Gatto GJ. TC-2559: A novel orally active ligand selective at neuronal acetylcholine receptors. *European Journal of Pharmacology* 409(1), 45-55 (2000).
72. 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).
73. 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. *Molecular Pain* 7 1744-8069-1747-1756 (2011).
74. 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 Physiologica* 213(2), 462-471 (2015).
75. Wang Y, Sherwood JL, Lodge D. The α4β2 nicotinic acetylcholine receptor agonist TC-2559 impairs long-term potentiation in the dentate gyrus in vivo. *Neuroscience Letters* 406(3), 183-188 (2006).
76. Marotta CB, Rreza I, Lester HA, Dougherty DA. Selective Ligand Behaviors Provide New Insights into Agonist Activation of Nicotinic Acetylcholine Receptors. *ACS Chemical Biology* 9(5), 1153-1159 (2014).
77. Unwin N. Refined Structure of the Nicotinic Acetylcholine Receptor at 4 Å Resolution. *J. Mol. Biol.* 346 967-989 (2005).
78. 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. *Proceedings of the National Academy of Sciences* 113(34), 9635-9640 (2016).
79. 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).
80. 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. *British Journal of Pharmacology* doi:10.1111/bph.13838 n/a-n/a (2017).
81. Smit AB, Syed NI, Schaap D *et al*. A glia-derived acetylcholine-binding protein that modulates synaptic transmission. *Nature* 411 261-268 (2001).
82. Shahsavar A, Gajhede M, Kastrup JS, Balle T. Structural Studies of Nicotinic Acetylcholine Receptors: Using Acetylcholine-Binding Protein as a Structural Surrogate. *Basic & Clinical Pharmacology & Toxicology* 118(6), 1742-7843 (2016).
83. Rucktooa P, Smit AB, Sixma TK. Insight in nAChR subtype selectivity from AChBP crystal structures. *Biochemical Pharmacology* 78 777-787 (2009).
84. 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. *Journal of Medicinal Chemistry* 52(8), 2372-2383 (2009).

Formattato: Inglese (Regno Unito)
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Formattato: Inglese (Regno Unito)

85. Ussing CA, Hansen CP, Petersen JG *et al*. Synthesis, Pharmacology, and Biostructural Characterization of Novel α4β2 Nicotinic Acetylcholine Receptor Agonists. *Journal of Medicinal Chemistry* 56(3), 940-951 (2013).
86. 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. *Journal of Medicinal Chemistry* 53(19), 7192-7201 (2010).
87. 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. *Journal of Medicinal Chemistry* 55(18), 8028-8037 (2012).
88. 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. *Journal of Medicinal Chemistry* 55(22), 9793-9809 (2012).
89. Corringer P-J, Le Novère N, Changeux J-P. Nicotinic receptors at the aminoacid level. *Annu. Rev. Pharmacol. Toxicol.* 40 431-458 (2000).
90. Glennon RA, Dukat M. $\alpha 4\beta 2$ nACh Receptor pharmacophore models. *Bioorg. Med. Chem. Lett.* 14 1841-1844 (2004).
91. Dougherty DA. Cys-loop neuroreceptors: structure to the rescue? *Chemical Reviews* 108 1642-1653 (2008).
92. Van Arnam EB, Dougherty DA. Functional Probes of Drug–Receptor Interactions Implicated by Structural Studies: Cys-Loop Receptors Provide a Fertile Testing Ground. *Journal of Medicinal Chemistry* 57(15), 6289-6300 (2014).
93. Wall TR. Effects of TI-299423 on Neuronal Nicotinic Acetylcholine Receptors. *Pasadena, CA.* Ph.D. Thesis (2015).
94. Post MR, Tender GS, Lester HA, Dougherty DA. Secondary Ammonium Agonists Make Dual Cation-π Interactions in α4β2 Nicotinic Receptors. *eneuro* 4(2), (2017).
95. 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).
96. Govoni S, Mura E, Preda S *et al*. Dangerous Liaisons between Beta-Amyloid and Cholinergic Neurotransmission. *Current Pharmaceutical Design* 20(15), 2525-2538 (2014).
97. Wang H-Y, Lee DHS, Davis CB, Shank RP. Amyloid Peptide Aβ1-42 Binds Selectively and with Picomolar Affinity to α7 Nicotinic Acetylcholine Receptors. *Journal of Neurochemistry* 75(3), 1155-1161 (2000).
98. 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 Neuroscience* 13(1), 155 (2012).
99. Lamb PW, Melton MA, Yakel JL. Inhibition of Neuronal Nicotinic Acetylcholine Receptor Channels Expressed in *Xenopus* Oocytes by β-Amyloid$_{1-42}$ Peptide. *J. Mol. Neurosci.* 27(13-21), (2005).
100. Lombardo S, Maskos U. Role of the nicotinic acetylcholine receptor in Alzheimer's disease pathology and treatment. *Neuropharmacology* 96 255-262 (2015).
101. 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. *The Journal of Neuroscience* 29(27), 8805-8815 (2009).
102. 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. *Expert Review of Neurotherapeutics* 15(1), 7-17 (2015).
103. 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).

Formattato: Inglese (Regno Unito)

104. 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. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 54(0), 131-139 (2014).
105. 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 & Related Disorders* 9(5), 243-246 (2003).
106. Quik M, Wonnacott S. α6β2* and α4β2* Nicotinic Acetylcholine Receptors As Drug Targets for Parkinson's Disease. *Pharmacological Reviews* 63(4), 938-966 (2011).
107. 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).
108. Freedman R. α7-Nicotinic Acetylcholine Receptor Agonists for Cognitive Enhancement in Schizophrenia. *Annual Review of Medicine* 65(1), 245-261 (2014).
109. Leonard S, Breese C, Adams C *et al*. Smoking and schizophrenia: abnormal nicotinic receptor expression. *European Journal of Pharmacology* 393(1), 237-242 (2000).
110. Thomsen MS, Hansen HH, Timmerman DB, Mikkelsen JD. Cognitive improvement by activation of α7 nicotinic acetylcholine receptors: from animal models to human pathophysiology. *Curr. Pharm. Des.* 16 323-343 (2010).
111. Dineley KT, Pandya AA, Yakel JL. Nicotinic ACh receptors as therapeutic targets in CNS disorders. *Trends in Pharmacological Sciences* 36(2), 96-108 (2015).
112. 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).
113. 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. *Journal of Psychopharmacology* 31(4), 434-441 (2017).
114. 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).
115. Wing VC, Wass CE, Bacher I, Rabin RA, George TP. Varenicline modulates spatial working memory deficits in smokers with schizophrenia. *Schizophrenia Research* 149(1), 190-191 (2013).
116. Janowsky DS, El-Yousef MK, Davis JM, Sekerke HJ. A cholinergic-adrenergic hypothesis of mania and depression. *Lancet* 2 632-635 (1972).
117. 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. Neuro-psychopharmacol. Biol. Psychiat.* 18 1121-1127 (1994).
118. Saricicek A, Esterlis I, Maloney KH *et al*. Persistent β2*-Nicotinic Acetylcholinergic Receptor Dysfunction in Major Depressive Disorder. *American Journal of Psychiatry* 169(8), 851-859 (2012).
119. 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).
120. Yu L-F, Zhang H-K, Caldarone BJ, Eaton JB, Lukas RJ, Kozikowski AP. Recent Developments in Novel Antidepressants Targeting α4β2-Nicotinic Acetylcholine Receptors. *Journal of Medicinal Chemistry* 57(20), 8204-8223 (2014).
121. 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. *European Neuropsychopharmacology* 24(4), 564-574 (2014).
122. Lee M, Martin-Ruiz C, Graham A *et al*. Nicotinic receptor abnormalities in the cerebellar cortex in autism. *Brain* 125 1483-1495 (2002).
123. Anand R, Amici SA, Ponath G, Robson JI, Nasir M, Mckay SB. Nicotinic Acetylcholine Receptor Alterations in Autism Spectrum Disorders – Biomarkers and Therapeutic Targets. *Autism - A Neurodevelopmental Journey from Genes to Behaviour* doi:DOI: 10.5772/20752 (2011).

Formattato: Inglese (Regno Unito)

124. Arnold LE, Aman MG, Hollway J *et al*. Placebo-Controlled Pilot Trial of Mecamylamine for Treatment of Autism Spectrum Disorders. *Journal of Child and Adolescent Psychopharmacology* 22(3), 198-205 (2012).
125. Fleisher C, Mcgough J. Sofinicline: a novel nicotinic acetylcholine receptor agonist in the treatment of attention-deficit/hyperactivity disorder. *Expert Opinion on Investigational Drugs* 23(8), 1157-1163 (2014).
126. 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. *Biological Psychiatry* 75(3), 207-214 (2014).
127. Bain EE, Robieson W, Pritchett Y *et al*. A Randomized, Double-Blind, Placebo-Controlled Phase 2 Study of [alpha]4[beta]2 Agonist ABT-894 in Adults with ADHD. *Neuropsychopharmacology* 38(3), 405-413 (2013).
128. Childress A, Sallee FR. Pozanicline for the treatment of attention-deficit/hyperactivity disorder. *Expert Opinion on Investigational Drugs* 23(11), 1585-1593 (2014).
129. Jucaite A, Öhd J, Potter AS *et al*. A randomized, double-blind, placebo-controlled crossover study of α4β2* nicotinic acetylcholine receptor agonist AZD1446 (TC-6683) in adults with attention-deficit/hyperactivity disorder. *Psychopharmacology* 231(6), 1251-1265 (2014).
130. Umana IC, Daniele CA, Mcgehee DS. Neuronal nicotinic receptors as analgesic targets: It's a winding road. *Biochemical Pharmacology* 86(8), 1208-1214 (2013).
131. Sullivan JP, Decker MW, Brioni JD *et al*. (+/-)-Epibatidine elicits a diversity of in vitro and in vivo effects mediated by nicotinic acetylcholine receptors. *Journal of Pharmacology and Experimental Therapeutics* 271(2), 624-631 (1994).
132. 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. *Journal of Pharmacology and Experimental Therapeutics* 285(2), 777-786 (1998).
133. 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).
134. Rowbotham MC, Arslanian A, Nothaft W *et al*. Efficacy and safety of the α4β2 neuronal nicotinic receptor agonist ABT-894 in patients with diabetic peripheral neuropathic pain. *Pain* 153(4), 862-868 (2012).
135. Damaj MI, Meyer EM, R. Martin B. The antinociceptive effects of α7 nicotinic agonists in an acute pain model. *Neuropharmacology* 39(13), 2785-2791 (2000).
136. 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. *Journal of Pharmacology and Experimental Therapeutics* 329(2), 791-807 (2009).
137. Jackson KJ, Marks MJ, Vann RE *et al*. Role of α5 Nicotinic Acetylcholine Receptors in Pharmacological and Behavioral Effects of Nicotine in Mice. *Journal of Pharmacology and Experimental Therapeutics* 334(1), 137-146 (2010).
138. 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).
139. Romero HK, Christensen SB, Di Cesare Mannelli L *et al*. Inhibition of α9α10 nicotinic acetylcholine receptors prevents chemotherapy-induced neuropathic pain. *Proceedings of the National Academy of Sciences* 114(10), E1825-E1832 (2017).
140. Rosas-Ballina M, Tracey KJ. Cholinergic control of inflammation. *Journal of Internal Medicine* 265(6), 663-679 (2009).
141. Zdanowski R, Krzyżowska M, Ujazdowska D, Lewicka A, Lewicki S. Role of α7 nicotinic receptor in the immune system and intracellular signaling pathways. *Central-European Journal of Immunology* 40(3), 373-379 (2015).
142. 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).

Formattato: Inglese (Regno Unito)

143. 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).
144. Galle-Treger L, Suzuki Y, Patel N *et al*. Nicotinic acetylcholine receptor agonist attenuates ILC2-dependent airway hyperreactivity. 7 13202 (2016).
145. 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).
146. 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. *Canadian Respiratory Journal : Journal of the Canadian Thoracic Society* 22(4), 230-234 (2015).
147. 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, Part A 165-174 (2016).
148. Shariff M, Quik M, Holgate J *et al*. Neuronal Nicotinic Acetylcholine Receptor Modulators Reduce Sugar Intake. *PLOS ONE* 11(3), e0150270 (2016).
149. Hoebel BG, Avena NM, Rada P. Accumbens dopamine-acetylcholine balance in approach and avoidance. *Current Opinion in Pharmacology* 7(6), 617-627 (2007).
150. 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. *Molecular Genetics and Metabolism* 112(1), 64-72 (2014).
151. Crooks PA, Bardo MT, Dwoskin LP. Chapter Thirteen - Nicotinic Receptor Antagonists as Treatments for Nicotine Abuse. In: *Advances in Pharmacology*, Linda PD (Ed.^(Eds).Academic Press 513-551 (2014).
152. 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 & Tobacco Research* 13(1), 1-6 (2011).
153. Rose JE, Behm FM. Combination Treatment With Varenicline and Bupropion in an Adaptive Smoking Cessation Paradigm. *American Journal of Psychiatry* 171(11), 1199-1205 (2014).
154. 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).
155. Brunzell DH, Mcintosh JM, Papke RL. Diverse strategies targeting α7 homomeric and α6β2* heteromeric nicotinic acetylcholine receptors for smoking cessation. *Annals of the New York Academy of Sciences* 1327(1), 27-45 (2014).
156. 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).
157. Rose JE. Disrupting Nicotine Reinforcement. *Annals of the New York Academy of Sciences* 1141(1), 233-256 (2008).
158. 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).
159. Solinas M, Scherma M, Fattore L *et al*. Nicotinic α7 Receptors as a New Target for Treatment of Cannabis Abuse. *The Journal of Neuroscience* 27(21), 5615-5620 (2007).
160. Rahman S, Engleman EA, Bell RL. Nicotinic receptor modulation to treat alcohol and drug dependence. *Frontiers in Neuroscience* 8(426), (2015).
161. Petrakis IL, Ralevski E, Gueorguieva R *et al*. Mecamylamine Treatment for Alcohol Dependence: A Randomized Controlled Trial. *Addiction* doi:10.1111/add.13943 n/a-n/a (2017).
162. Zhao Y. The Oncogenic Functions of Nicotinic Acetylcholine Receptors. *Journal of Oncology* 2016 9 (2016).
163. Schuller HM. Is cancer triggered by altered signalling of nicotinic acetylcholine receptors? *Nat Rev Cancer* 9(3), 195-205 (2009).

Formattato: Inglese (Regno Unito)

164. Schuller HM, Orloff M. Tobacco-Specific Carcinogenic Nitrosamines. *Biochemical Pharmacology* 55(9), 1377-1384 (1998).
165. Brusco S, Ambrosi P, Meneghini S, Becchetti A. Agonist and antagonist effects of tobacco-related nitrosamines on human α4β2 nicotinic acetylcholine receptors. *Frontiers in Pharmacology* 6(201), (2015).
166. 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 Plasticity* 2015 10 (2015).
167. Philpot RM. Potential Use of Nicotinic Receptor Agonists for the Treatment of Chemotherapy-Induced Cognitive Deficits. *Neurochemical Research* 40(10), 2018-2031 (2015).
168. Boggs DL, Ranganathan M, Sewell RA, Madonick S, D'souza DC. Pilot study of Intravenous Nicotine Effects on Cognitive Performance in Schizophrenia. *Schizophrenia Research* 150(1), 323-324 (2013).
169. 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).
170. 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. *Annals of Internal Medicine* 159(6), 390-400 (2013).
171. Kim SY, Choi SH, Rollema H *et al*. Phase II Crossover Trial of Varenicline in Mild-to-Moderate Alzheimer's Disease. *Dementia and Geriatric Cognitive Disorders* 37(3-4), 232-245 (2014).
172. Mitchell JM, Teague CH, Kayser AS, Bartlett SE, Fields HL. Varenicline decreases alcohol consumption in heavy-drinking smokers. *Psychopharmacology* 223 299-306 (2012).
173. 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).
174. 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. *Schizophrenia Research* 176(2), 206-211 (2016).
175. Velligan D, Brenner R, Sicuro F *et al*. Assessment of the effects of AZD3480 on cognitive function in patients with schizophrenia. *Schizophrenia Research* 134(1), 59-64 (2012).
176. Frölich L, Ashwood T, Nilsson J, Eckerwall G. Effects of AZD3480 on Cognition in Patients with Mild-to-Moderate Alzheimer's Disease: A Phase IIb Dose-Finding Study *J Alzheimer's Dis* 24 363-374 (2011).
177. Boström E, Bohnstedt KC, Jostell K-G *et al*. Population pharmacokinetics and safety of AZD1446, a neuronal nicotinic receptor agonist, administered in healthy volunteers. *Clinical Pharmacology in Drug Development* 3(1), 63-71 (2014).
178. 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).
179. 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. *Schizophrenia Bulletin* 42(2), 335-343 (2016).
180. 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).
181. 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. *American Journal of Psychiatry* 173(8), 827-835 (2016).
182. 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).

Formattato: Inglese (Regno Unito)
Formattato: Inglese (Regno Unito)
Formattato: Inglese (Regno Unito)
Formattato: Inglese (Regno Unito)
Formattato: Inglese (Regno Unito)

183. 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 Research & Therapy* 8(1), 44 (2016).
184. Umbricht D, Keefe RSE, Murray S *et al*. A Randomized, Placebo-Controlled Study Investigating the Nicotinic [alpha]7 Agonist, RG3487, for Cognitive Deficits in Schizophrenia. *Neuropsychopharmacology* 39(7), 1568-1577 (2014).
185. Barch DM, Marder SR, Harms MP *et al*. Task-related fMRI responses to a nicotinic acetylcholine receptor partial agonist in schizophrenia: A randomized trial. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 71 66-75 (2016).
186. Trenkwalder C, Berg D, Rascol O *et al*. A Placebo-Controlled Trial of AQW051 in Patients With Moderate to Severe Levodopa-Induced Dyskinesia. *Movement Disorders* 31(7), 1049-1054 (2016).
187. 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. *The World Journal of Biological Psychiatry* 16(7), 483-501 (2015).
188. 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).
189. Liang J, Dicpinigaitis P, Cunning B, Devita R: (2017).