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Allosteric modulators of the $\alpha 4\beta 2$ subtype of Neuronal Nicotinic Acetylcholine Receptors

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Abstract

Nicotinic acetylcholine receptors are ligand-gated ion conducting transmembrane channels from the Cys-loop receptor super-family. The $\alpha 4\beta 2$ subtype is the predominant heteromeric subtype of nicotinic receptors found in the brain. Allosteric modulators for $\alpha 4\beta 2$ receptors interact at a site other than the orthosteric site where acetylcholine binds. Many compounds which act as allosteric modulators of the $\alpha 4\beta 2$ receptors have been identified, with both positive and negative effects. Such allosteric modulators either increase or decrease the response induced by agonist on the $\alpha 4\beta 2$ receptors. Here we discuss the concept of allosterism as it pertains to the $\alpha 4\beta 2$ receptors and summarize the important features of allosteric modulators for this nicotinic receptor subtype.

Keywords

Nicotinic acetylcholine receptor; Allosteric modulation; Positive allosteric modulators; Negative allosteric modulators

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) are cation-conducting pentameric transmembrane proteins that are activated by the endogenous neurotransmitter acetylcholine (ACh) [1]. These receptors are formed by the assembly of a variety of the 17 different subunits ($\alpha 1$ - $\alpha 10$, $\beta 1$ - $\beta 4$, γ , δ , ϵ) thus far known to exist, resulting in different subtypes of functional nAChRs [2]. The nAChRs at the neuromuscular junctions are composed of $\alpha 1$, $\beta 1$, γ , δ , ϵ subunits and referred to as the muscle type of nAChRs, while those present at the synapse or other tissues in the nervous system are composed of α (2–10) and β (2–4) subunits and referred to as the neuronal type of nAChRs [3, 4]. The main subtypes of nAChRs widely expressed in the brain are the homomeric $\alpha 7$ and the heteromeric $\alpha 4\beta 2$ receptors [5–10]. The $\alpha 4\beta 2$ receptors are expressed in two different stoichiometries, either the ($\alpha 4$)₂($\beta 2$)₃ stoichiometry which binds to ACh and nicotine with high affinity, or the ($\alpha 4$)₃($\beta 2$)₂ stoichiometry which binds to ACh and nicotine with low affinity [11, 12]. The $\alpha 4\beta 2$ receptors have been implicated in a number of neurological conditions including (but not limited to) nicotine addiction [13], Alzheimer's disease [14], depression [15], impaired

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cognitive functions [16] and autism [17]. Positive allosteric modulators (PAMs) are rapidly being developed for neuronal nAChRs and they represent a promising new approach for treating disorders involving the $\alpha 4\beta 2$ receptors [18–21].

1.1. Allosteric modulation of the $\alpha 4\beta 2$ receptors

According to the allosteric theory, the $\alpha 4\beta 2$ receptor protein has multiple conformations (states) [22]. The binding of ligand to the receptor reduces the free energy of the stabilized conformation and the energy from this ligand binding interaction provides the energy required to stabilize a certain conformation of the receptor [23]. Accordingly, an agonist for the $\alpha 4\beta 2$ receptor is a ligand which stabilizes the open ion conducting conformation of the receptor. The binding site of ACh occurs at the $\alpha 4(+)/\beta 2(-)$ interface between the subunits forming the $\alpha 4\beta 2$ receptor. For the purposes of this review, we have defined the ACh binding site on the $\alpha 4\beta 2$ receptor as the orthosteric site. In addition to the orthosteric site, multiple distinct binding sites on the $\alpha 4\beta 2$ receptor can be present where different ligands can bind. All such sites other than the orthosteric site are defined as allosteric sites. This is the situation in the related GABA_A receptor which has multiple allosteric sites [24–26]. If an allosteric ligand stabilizes the open ion conducting conformation of the receptor channel that is induced by the agonist, such ligands are termed positive allosteric modulators (PAMs) for the receptor [18, 27, 28].

On a macroscopic level for purposes of this review, we will define PAMs of the $\alpha 4\beta 2$ receptors as ligands that increase potency (i.e. an increase in the apparent affinity; Fig. 1A & B) by acting at a site different from the orthosteric site [28]. PAMs for the $\alpha 4\beta 2$ receptor can also increase efficacy (maximal response amplitude), however based on our working definition here, this is not a requirement for a PAM [28, 29]. The change in efficacy caused by PAMs is rather varied and is discussed in section 1.2. On a single channel level the effect of PAMs for $\alpha 4\beta 2$ receptors translates into an increase in the number of openings or an increase in mean open times of the channel, indicating the stabilization of the open state of the receptor [18].

Some PAMs, in addition to being allosteric modulators by acting at an allosteric binding site, also act as partial or full agonists for the $\alpha 4\beta 2$ receptor [20]. If such compounds are applied along with an agonist, then an increase in receptor response amplitude is observed over what would normally be achieved by the agonist alone. This is seen with some of the 2-amino-5-ketothiazole compounds when tested on the $\alpha 4\beta 2$ receptors [20]. Such ligands may be classified as allosteric agonists or allosteric partial agonists; however for the purposes of this review, we do not consider these to be pure PAMs. As we have defined here, a pure PAM is one which binds to an allosteric site on the receptor, but fails to activate the channel by itself.

Negative allosteric modulators (NAMs) are ligands which *decrease* the maximal current (efficacy) or affinity of the agonist, and many NAMs have been discovered for the $\alpha 4\beta 2$ receptor [30–35]. Similar to the PAMs, the NAMs bind to a site on the $\alpha 4\beta 2$ receptors other than the orthosteric site, and inhibit receptor function by preferentially favoring a non-conducting conformation of the receptor. NAMs for the $\alpha 4\beta 2$ receptor may bind in the lumen of the channel or at non-luminal sites on the receptor such as the extracellular-transmembrane interface, the intracellular loop or anywhere else on the protein [32, 33]. There are several compounds for $\alpha 4\beta 2$ receptors that are referred to as allosteric antagonists or noncompetitive antagonists [32, 33, 35–37], all of which would be considered to be NAMs as long as they bind to a site distinct from the orthosteric site and inhibit the function of the $\alpha 4\beta 2$ receptors.

There may also be compounds yet to be discovered for $\alpha 4\beta 2$ receptors which could potentially bind to the same allosteric site as a PAM on the receptor and competitively inhibit its binding and actions. For example for the GABA_A receptors, one such compound is Ro15-1788 (Flumazenil) which binds to the benzodiazepine site and acts as an allosteric antagonist [25, 38, 39]. Such ligands for the $\alpha 4\beta 2$ receptor, in particular or for other subtypes of nAChR, in general, may be consequential as they can inhibit the potentiation induced by PAMs by competing for the same allosteric binding site.

1.2. Efficacy changes in $\alpha 4\beta 2$ receptors by PAMs

The effect of PAMs on potentiating the maximal response amplitudes of $\alpha 4\beta 2$ receptors is varied. While a PAM increases the apparent affinity (potency) of the agonist for the $\alpha 4\beta 2$ receptors, it may or may not change the maximal response amplitude (efficacy) elicited. Galantamine and desformylflustrabromine (dFBr) are both PAMs for the $\alpha 4\beta 2$ receptors and display different effects on receptor efficacy. Galantamine when co-applied with ACh on human $\alpha 4\beta 2$ nAChRs stably expressed in cultured HEK-293 cells causes an increase in apparent affinity without any change in efficacy (Fig. 1A) [29]. In contrast, dFBr (when co-applied with ACh) increases both apparent affinity and efficacy (Fig. 1B) [28]. This later effect is similar to that is seen with PNU-120596 on $\alpha 7$ receptors [40]. In the related GABA_A receptor, chlordiazepoxide, a PAM acting via the benzodiazepine site, shifts the GABA dose-response curve to the left (i.e. increased potency), but does not increase the maximal GABA response amplitude [41]. However, another drug tracazolate, a pyrazolopyridine derivative which is structurally diverse from benzodiazepines, acts via the benzodiazepine site to increases both potency and efficacy [42]. A similar situation may exist in the $\alpha 4\beta 2$ nAChRs.

Based on their effect on the receptor response profiles, PAMs for the $\alpha 7$ nAChRs have been classified into two types [19]; type I PAMs predominantly affect the peak current of the response, while type II PAMs affect the peak response and the time course of the agonist-evoked response [19]. While this classification may adequately describe the different types of PAMs for $\alpha 7$ receptors, this may not be the case with $\alpha 4\beta 2$ receptor PAMs.

2. Positive allosteric modulators of $\alpha 4\beta 2$ subtype of nAChRs

Many ligands are known to act as allosteric modulators for the $\alpha 4\beta 2$ subtypes of nAChRs. Our knowledge of specific domains crucial to the binding of PAMs and the functional effects produced by them is currently incomplete. In the section that follows, the different allosteric ligands for the $\alpha 4\beta 2$ receptors are discussed.

2.1 Steroids

The primary mechanistic action of steroid hormones derived from cholesterol is to control gene transcription through activation of nuclear receptors. However they also directly influence the function of a variety of ligand-gated ion channels. It has been demonstrated that albumin-bound and unbound progesterone are equally capable of inhibiting $\alpha 4$ -subunit containing receptor activity in *Xenopus* oocytes [34]. Since albumin-bound progesterone cannot cross the cell membrane, an extracellular site of action for progesterone on the $\alpha 4$ subunit was suggested [34]. Progesterone was tested for its ability to potentiate $\alpha 4\beta 2$ receptors, but it failed to do so, indicating that it can be considered a NAM.

The estrogenic steroid 17- β -estradiol (17-BE) (Fig. 2A) has differential effects on $\alpha 4\beta 2$ receptors. It potentiated the human but inhibited the rat receptors without potentiation [43]. This difference in action of 17-BE in these two species of $\alpha 4\beta 2$ receptors was used to locate the binding site on the human receptor responsible for potentiation. Using site directed mutagenesis, it was determined that the short C-terminal end of the $\alpha 4$ subunit with the

amino acid sequence AGMI was the key determinant for 17-BE potentiation of human $\alpha 4\beta 2$ receptors (Fig. 3B) and this site was shown to be different than the steroid inhibition site [44]. Furthermore it seems as if the binding site for 17-BE is portable since it can be moved to the C-terminus of the $\beta 2$ subunit and to another location on a subunit that contributes to an ACh-binding site [45]. Therefore the complex modulation of $\alpha 4\beta 2$ receptors by steroids seems to be determined by both the chemical structure of the steroid and the amino acid sequence of the nAChR.

There is evidence suggesting a transmembrane location of an inhibitory binding site for different steroids in other subtypes of nAChRs [33], however such experiments are yet to be done for the $\alpha 4\beta 2$ receptors. Single channel studies have confirmed that mechanistically, 17-BE increases the open probability of $\alpha 4\beta 2$ receptors [46] and that it appears to be a pure PAM for this subtype of nAChR.

2.2 Zinc

Ionic zinc (Zn^{2+}) is a key modulator of neuronal excitability. It is present in neurons throughout the brain, especially in the cerebral cortex and the hippocampus [47]. It also modulates the function of many ligand-gated ion channel receptors including GABA_A [48] and glycine receptors [49]. For many of the different nAChR subtypes, Zn^{2+} can both potentiate and/or inhibit receptor function, depending on a variety of factors [50]. For the $\alpha 4\beta 2$ receptor, the half-maximal concentration of Zn^{2+} for potentiation of ACh responses (pEC_{50}) was about 16 μM and the half-maximal concentration for inhibition (IC_{50}) was about 440 μM [50]. The ACh response amplitudes on $\alpha 4\beta 2$ receptors were potentiated by ~260% with Zn^{2+} . Furthermore the potentiation and inhibition of these receptors by Zn^{2+} is pH dependent, but voltage independent [51].

Of the two different stoichiometries of the $\alpha 4\beta 2$ receptors found in the central nervous system, Zn^{2+} potentiates only the low ACh affinity stoichiometry receptors [i.e. $(\alpha 4)_3(\beta 2)_2$] while inhibiting both high [i.e. $(\alpha 4)_2(\beta 2)_3$] and low affinity stoichiometries of the receptor [51]. The inhibition of Zn^{2+} on the high affinity $(\alpha 4)_2(\beta 2)_3$ receptor is voltage-dependent, while it is voltage-independent on low affinity $(\alpha 4)_3(\beta 2)_2$ receptors [51]. Based on these observations and through site-directed mutagenesis studies, the $\beta 2(+)/\alpha 4(-)$ subunit interface was proposed as the Zn^{2+} inhibition site on the high and low affinity $\alpha 4\beta 2$ receptors and the $\alpha 4(+)/\alpha 4(-)$ subunit interface was proposed as the site for the potentiating action of Zn^{2+} on low affinity receptors (Fig. 3A) [51]. Single channel studies suggest that Zn^{2+} potentiates $\alpha 4\beta 4$ receptors by increasing the burst frequency of the receptor [52]. Since Zn^{2+} potentiates $\alpha 4\beta 4$ and $\alpha 4\beta 2$ receptors, the mechanism of action of Zn^{2+} on $\alpha 4\beta 2$ receptors may be similar to its mechanism of action on $\alpha 4\beta 4$ receptors. Finally, Zn^{2+} can be considered to be a pure PAM for $\alpha 4\beta 2$ receptors since it does not show any agonistic actions.

2.3 Thiazole (2-amino-5-keto) and Carbamate Analogues

Three novel (2-amino-5-keto) thiazole analogues (e.g. LY-2087101) (Fig. 2B) have been reported to be allosteric modulators of neuronal nAChRs [20]. These compounds potentiated some, but not all of the various subtypes of nAChRs, including $\alpha 4\beta 2$ receptors [20]. All three compounds enhanced the potency and maximal efficacy of different nAChR agonists on $\alpha 4\beta 2$ receptors, a profile typical of allosteric potentiators [20]. At concentrations required for potentiation, the compounds did not displace [^3H]-epibatidine from the agonist-binding site and potentiation was observed at all agonist concentrations, suggesting a non-competitive allosteric mechanism of action [20]. Interestingly at concentrations higher than that required for potentiation, these compounds also showed intrinsic agonist activity which

was blocked by competitive and non-competitive nAChR antagonists [20]. Thus these compounds are not pure PAMs for $\alpha 4\beta 2$ receptors.

Recently a series of carbamate analogues which were optimized for CNS penetration were reported to potentiate $\alpha 4\beta 2$ nAChRs [53]. These compounds increased the response of $\alpha 4\beta 2$ receptors to ACh [54] without competing for binding with the orthosteric ligand cytosine [53]. These carbamate analogues represent a new class of compounds with immense therapeutic potential.

2.4 Desformylflustrabromine (dFBr)

Desformylflustrabromine (dFBr) (Fig. 2C) is a metabolite of the marine bryozoan *Flustra foliacea* which is common in the North Sea [55]. Some of the tryptophan-derived metabolites of *Flustra* were first shown to have muscle relaxant properties [56]. Flustramine A, another *Flustra* extract has also been shown to block potassium channels [57]. When the *Flustra* derivatives were tested on the $\alpha 4\beta 2$ and $\alpha 7$ nAChR subtypes using radioligand binding assays, it was determined that dFBr had low μM affinities for these receptors [58]. dFBr extracted from its natural source was tested functionally on various subtypes of nAChRs, including $\alpha 4\beta 2$, expressed in *Xenopus* oocytes [18]. When co-applied with ACh, dFBr selectively potentiated but did not inhibit $\alpha 4\beta 2$ receptors. By using synthetic dFBr, it was later shown that the ACh-induced responses of $\alpha 4\beta 2$ receptor were potentiated in the nano-molar range ($\text{pEC}_{50} = 120 \text{ nM}$) and inhibited in the micro-molar range ($\text{IC}_{50} = 150 \mu\text{M}$) [28]. This potentiation induced by dFBr was reversible and concentration dependent. Furthermore in single channel recordings, dFBr acts by either increasing the opening rate constant or decreasing the closing rate constant of the $\alpha 4\beta 2$ receptors, without changing the conductance or reversal potential [18]. It has been proposed that dFBr potentiates $\alpha 4\beta 2$ receptors by an allosteric mechanism while its inhibition is via an open channel block [27]. Recently we have shown that in addition to enhancing the current amplitude of both $\alpha 4\beta 2$ and $\alpha 2\beta 2$ nAChRs, dFBr also prevents the inhibition of these channels by the β -amyloid peptide ($\text{A}\beta_{1-42}$), a result that suggests that similar compounds may be useful in combating the inhibition of these receptors by $\text{A}\beta_{1-42}$ in patients suffering with Alzheimer's Disease [59]. There is no evidence of dFBr acting as an agonist on any subtype of nAChRs.

2.5 Acetylcholinesterase inhibitors

Acetylcholinesterase (AChE) is the enzyme that rapidly hydrolyzes ACh. Some AChE inhibitors such as galantamine (Fig. 2D) and physostigmine (Fig. 2E) also act as noncompetitive allosteric agonists on nAChRs. Recent reports based on homology models for the ligand binding domain of human $\alpha 7$ and $\alpha 4\beta 2$ receptors have identified the key amino acid residues which are presumably, an important part of the binding site for these compounds [60]. This binding site region overlaps the ACh binding site on nAChR and is located on the outer surface of the ligand binding domain. This suggests that galantamine, physostigmine and ACh bind to different sites on nAChR and that allosteric potentiation may arise from a direct interplay between both these sites [60].

Physostigmine and galantamine have effects on $\alpha 4\beta 2$ receptors that are similar to their action on muscle nicotinic receptors [61]. Galantamine shows no effect on $\alpha 4\beta 2$ receptors expressed in *Xenopus* oocytes. In contrast however, in HEK293 cells expressing human $\alpha 4\beta 2$ receptors, galantamine reduces the apparent EC_{50} of ACh by half but has no effect on the efficacy [29]. Like galantamine, physostigmine also shows differential effects on receptor function depending on the expression system. For example physostigmine activates chicken $\alpha 4\beta 2$ receptors expressed in M10 cells through a binding sight that is insensitive to ACh [61], however it does not elicit currents from $\alpha 4\beta 2$ receptors when expressed in *Xenopus* oocytes. When physostigmine is co-applied with low concentration ($1 \mu\text{M}$) of ACh

on $\alpha 4\beta 2$ receptors expressed in *Xenopus* oocytes, it potentiates ionic currents, however it inhibits them when co-applied with higher ACh concentrations [62]. A two-site equilibrium receptor occupation model predicts that these dual effects of physostigmine are due to its binding to a second equivalent agonist recognition site combined with noncompetitive ion channel block [62, 63]. Based on this mechanism, it can be concluded that the binding site of physostigmine on $\alpha 4\beta 2$ receptors is also a low affinity ACh binding site distinct from the high affinity site that activates the receptor [62].

Both physostigmine and galantamine activate single channels in excised membrane patches from cultured M10 cells expressing the neuronal $\alpha 4\beta 2$ receptors [61] and both are thought to potentiate neuronal nAChRs by stabilizing the open state of the receptor channel [64]. Galantamine and physostigmine lack selectivity in their allosteric action because they potentiate both homomeric $\alpha 7$ and other heteromeric subtypes of nAChRs. The beneficial effects of these two drugs in restoring cholinergic tone in the synapse is expected to be enhanced by their dual actions as acetylcholinesterase inhibitors and allosteric modulators of nAChRs [65].

3. Negative allosteric modulators of $\alpha 4\beta 2$ subtype of nAChRs

The $\alpha 4\beta 2$ subtype of nAChRs is targeted by a number of exogenous and endogenous compounds that allosterically modulate its function [33]. Such compounds are structurally diverse and inhibit the functioning of the receptor and are referred to as negative allosteric modulators (NAMs). Compounds that are true NAMs have a distinct allosteric binding site on the $\alpha 4\beta 2$ receptor, whether it is on the extracellular domain or the intracellular loop, the transmembrane domain or in the pore of the ion channel itself.

Drugs like dizocilpine (+)-MK-801, amantadine and memantine act as open channel blockers for the $\alpha 4\beta 2$ receptor and have a specific binding site in the pore of the receptor channel [37], therefore they are classified as NAMs for this receptor. The antidepressant milnacipran [66] and steroids such as progesterone [34] are some of the other known NAMs for the $\alpha 4\beta 2$ receptors, as is UCI-30002 [N-(1,2,3,4-tetrahydro-1-naphthyl)-4-nitroaniline] which has been shown to significantly reduce nicotine self-administration in rats [30]. KAB-18 is another $\alpha 4\beta 2$ nAChR NAM that contains three phenyl rings, one piperidine ring and one ester bond linkage was developed with the aid of SAR computational designing and molecular biology approaches [31].

While NAMs for $\alpha 4\beta 2$ receptors indicate the presence of an allosteric binding site on the receptor, they may not offer any distinct advantage as compared to a competitive antagonist in terms of drug development. However, NAMs can be useful experimental tools for studying the functional aspects of the $\alpha 4\beta 2$ nAChR function.

4. Conclusions

Due to their importance in the regulation of neuronal signaling and their apparent role in neurological disorders, a significant effort has been made to develop nAChR ligands, particularly those that can act selectively on individual nAChR subtypes. For the $\alpha 4\beta 2$ receptors, two broad types of ligands are being actively pursued. These can be classified as orthosteric ligands (ligands that bind at the ACh binding site) and allosteric ligands (ligands binding outside of the orthosteric ACh binding site). Orthosteric ligands have received much attention. Partial agonists like varenicline, which targets the $\alpha 4\beta 2$ nAChR subtype, provides a treatment option for smoking cessation and nicotine addiction [67, 68]. However, most partial or full agonists cause desensitization of receptor responses [69, 70] and up regulation of receptors expression [71, 72]. Hence, the utility of either partial or full agonists can be limited. Subtype selective agonists have also proved difficult to develop, probably due to the

conserved nature of the orthosteric binding sites[54]. Given the variety of allosteric binding sites that apparently exist on nAChRs, it may be easier to develop subtype selective allosteric (rather than orthosteric) ligands since allosteric sites are likely to be less conserved across nAChR subtypes. Therefore, these ligands may display greater subtype selectivity.

PAMs bind to allosteric binding sites and produce an increase in the potency of the agonist and often in the amplitude of responses [19]. Allosteric ligands that enhance the currents elicited by ACh provide an alternate approach to treating disorders of decreased nAChR activity. Pure PAMs potentiate responses in the presence of endogenous agonists and do not, by themselves, activate the receptor. This can prevent receptor desensitization and preserve cholinergic modulation of synaptic transmission. This is in contrast to orthosteric ligands like nicotine which produce receptor up-regulation and/or desensitization [69–72]. In pathological conditions where nAChR-mediated signaling in the CNS is decreased, PAMs could be used to maintain or restore normal levels of nAChR-mediated synaptic transmission without directly altering other cholinergic signaling (e.g. that through the mAChRs).

Since $\alpha 4\beta 2$ receptors are implicated in the patho-physiology of many diseases and conditions, PAMs for these receptors could be important therapeutic agents in the treatment of a variety of neuro-pathological conditions. The therapeutic applicability of the benzodiazepine class of compounds, for example, is an amazing success story in the related GABA receptor [24, 26]. Allosteric modulatory ligands for nAChRs provide many advantages over conventional agonists. PAMs for the $\alpha 4\beta 2$ nAChR clearly have the potential to develop into clinically applicable drugs targeting these receptors. Such compounds will provide a novel means of treatment for various neurological disorders where these receptors play a critical role.

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Abbreviation

ACh	acetylcholine
nAChRs	neuronal nicotinic acetylcholine receptors
dFBr	desformylflustrabromine
PAM	positive allosteric modulator
NAM	negative allosteric modulator
17-BE	17- β -Estradiol

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Fig. 1. Effect of $\alpha 4\beta 2$ nAChRs PAMs, galantamine and dFBr on efficacy. A) Galantamine does not change the maximal response obtained by ACh in $\alpha 4\beta 2$ nAChR stably expressed in cultured HEK-293 cells [29]. B) dFBr increases the maximal response obtained by ACh in $\alpha 4\beta 2$ nAChR expressed in *Xenopus* oocytes [28].

Fig. 2.
Structure of PAMs for $\alpha 4\beta 2$ nAChRs. A) 17- β -estradiol, B) LY-2087101, C) Desformylflustrabromine, D) Galantamine, E) Physostigmine.

Fig. 3.

A. Location of Zn^{2+} sites in relation to the ACh binding sites on the $(\alpha 4)_3(\beta 2)_2$ stoichiometry of the $\alpha 4\beta 2$ nAChRs. The $\alpha 4(+)/\alpha 4(-)$ interface influences the potentiation of $(\alpha 4)_3(\beta 2)_2$ receptors by Zn^{2+} . The $\alpha 4(-)/\beta 2(+)$ interface present in both stoichiometry of $\alpha 4\beta 2$ receptors is responsible for the inhibitory action of Zn^{2+} [51]. B) Location of the 17-BE binding site on the C-terminal region of $\alpha 4$ -subunit of the $\alpha 4\beta 2$ nAChRs [44, 45].