Molecular Pathways

Nicotinic acetylcholine receptor-based blockade: Applications of molecular targets for cancer therapy

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Running Title: Nicotinic acetylcholine receptor-targeted cancer therapy
Abstract

The nicotinic acetylcholine receptor (nAChR) was first characterized in 1970 as a membrane receptor of a neurotransmitter and an ion channel. nAChRs have been demonstrated to be involved in smoking-induced cancer formation in multiple types of human cancer cells. *In vitro* and *in vivo* animal studies show that homo-pentameric nAChR inhibitors, such as methyllycaconitine and α-Bgtx, can attenuate nicotine-induced proliferative, angiogenic, and metastatic effects in lung, colon, and bladder cancer cells. Recent publications have demonstrated that α9-nAChR is important for breast cancer formation. Several α9-nAChR-specific antagonists (such as α-ImI, α-ImI, Vc1.1, RgIA, and It14a) produce an analgesic effect in many *in vivo* studies. Additionally, Vc1.1 has functioned in a variety of animal pain models and has currently entered phase II clinical trials. For cancer therapy, natural compounds such as garcinol and EGCG have been found to block nicotine- and estrogen-induced breast cancer cell proliferation through inhibition of the α9-nAChR signaling pathway. Therefore, a detailed investigation the carcinogenic effects of nAChRs and their specific antagonists will enhance our understanding of their value as targets for clinical translation.
Background

**Biological functions of nAChRs**

Based on the ligand-binding properties, nAChRs have been divided into two classes: (a) α-bungarotoxin (α-Bgtx)-binding nAChRs containing α7 or α9 subunits, which form homopentamers, and (b) α-Bgtx non-binding nAChRs, which contain α2-α6 and β2–β4 subunits, which form heteromeric receptors with high affinities for receptor agonists (such as acetylcholine and nicotine) (1, 2). Each of these receptor subtypes has distinct electrophysiological and pharmacological properties. For example, The α4β2-containing nAChRs have the highest nicotine-binding affinity in mammalian cells (3). Once α4β2 nAChRs are stimulated, dopamine is released in the brain reward pathway which results in smoking addiction. In another case, α7- and α9-nAChR receptors have unique properties pertaining to the regulation of signaling mechanisms found in sensory epithelia (α7-nAChR) and other non-neuronal (α9-nAChR) cell types. Homopentamers of α7-nAChR are the most well-investigated subtype and important channels for Ca^{2+}-dependent mechanisms, including activating second messengers such as PKA, PKC, PI3K/Akt, and MAPK (Figure 1) (4). These signaling pathways are closely correlated to the formation of several cancers. In particular, α7-nAChR is associated with lung (5), bladder (6), and colon cancers (7) while α9-nAChR is associated with breast cancer (8-10) (Figure 1). The functional diversity of the nAChR family offers abundant prospects for the design of novel therapies. Therefore, nAChRs have been investigated as drug targets for nervous system disorders (11).

Homopentamers of α7-nAChR are the most well-investigated subtype and are known to desensitize rapidly and have a Ca^{2+}:Na^{+} permeability ratio (12) that is around four-fold higher than those of other nAChRs. The opening of nAChR channels impacts several Ca^{2+}-dependent mechanisms, including activating second messengers (4) such as PKA, PKC, PI3K/Akt, and MAPK (Figure 1). These signaling pathways are closely correlated to the formation of several cancers. In particular, α7-nAChR is associated with lung (5), bladder (6), and colon cancers (7) and α9-nAChR is associated with breast cancer (8-10) (Figure 1).

nAChRs are ligand-gated cation channels, and different subtypes are known to be differentially permeable to calcium ions (Ca^{2+}) (13). In non-neuronal cells, the role of nAChR-mediated calcium entry is less understood. The calcium permeability of
homomeric receptors is significantly higher than that of heteromeric nAChRs. In particular, the α7-nAChR subtype has one of the highest calcium permeabilities; the activation of this receptor can raise cytoplasmic calcium levels and trigger a series of calcium-dependent intracellular processes (14). Several studies have observed the presence of nAChRs in several non-neuronal, non-excitable cells, such as cells from the bronchial epithelium, endothelial cells, keratinocytes, immune cells, vascular smooth muscle cells and cells from other tissue types. The presence of these receptors in non-neuronal cells seems to suggest that they have distinct functions beyond neurotransmission (15). Most recently, several studies have indicated that α7-nAChRs primarily mediate endothelial cell proliferation, invasion and angiogenesis (16-19). Additionally, the presence of α7-nAChR inhibitors, such as methyllycaconitine (MLA) and α-Bgtx, can reverse the pro-angiogenic effects of nicotine (16, 17). However, it is important to note that both α-Bgtx and MLA bind with high affinity to α9-nAChR. Therefore, α9-nAChR may be partially involved in nicotine-induced pro-angiogenic effects (20). In particular, α7-nAChRs have been found to activate the MAP kinase, PI3-kinase/Akt and NF-κB pathways, thereby mediating angiogenesis (16-18).

- Molecular structure of nAChRs

Acetylcholine receptors (AChRs) are integral membrane proteins that respond to the binding of acetylcholine (ACh), which is synthesized, stored and finally released by cholinergic neurons (21). Like many other ligand-activated neurotransmitter receptors, AChRs have been classified according to either their pharmacological properties or their relative affinities for various molecules. Therefore, they can be divided into two major subtypes: (a) the metabotropic muscarinic receptors (mAChRs) found in vertebrate skeletal muscles, which mediate neuromuscular transmission at the neuromuscular junction and are particularly responsive to muscarine (22), and (b) the ionotropic nicotinic receptors (nAChRs), which are particularly responsive to nicotine and found not only throughout the peripheral and central nervous systems but also in non-neuronal tissues (23). Both share the properties of being activated by the endogenous neurotransmitter ACh and of being expressed by both neuronal and non-neuronal cells throughout the body (24).

Previous papers have demonstrated that mAChRs are ligand-gated ion channel receptors primarily expressed in skeletal neuromuscular junctions, and they are
composed of five subunits, including two α1 subunits, and one each of β1, δ, and γ (or ε, depending on the stage of development) (25). Only two types of mACHRs are constructed from this complex subunit pool; one of the types is composed of α1, β1, δ, and γ subunits, and the other is composed of α1, β1, δ, and ε subunits, both at ratios of 2:1:1:1. In contrast, nAChRs were originally cloned from a neuronal-like cell line and brain cDNA libraries. They are expressed throughout the nervous system, and they functionally increase neuronal excitability and facilitate synaptic transmission (26). nAChRs can form either homopentamers or heteropentamers containing ten α-subunits (termed α1-α10), four non-α subunits (termed β1-β4), δ, γ, and ε. Notably, α8 has only been identified in avian libraries and has not been found in mammals (24). Expression of these nAChR subunits has been observed in many other cell types, including endothelial cells, glial cells, immune cells, and keratinocytes, as well as in gastrointestinal, lung, bladder, colon and breast tissues.

- nAChR-mediated signaling pathways

The first evidence that nAChRs regulate cancer growth was reported by Dr. H. M. Schuller in 1989 (27). In the following decades, studies have highlighted that nAChRs are key molecules acting as central regulators of a complex network that governs growth (5, 28), angiogenesis (29), metastasis (7) and apoptosis (30) during carcinogenesis in response to the tumor microenvironment. In addition, nAChRs stimulate intracellular signaling pathways in a cell type-specific manner. Figure 1 shows that nicotine-induced upregulation of growth factors (such as VEGF and βFGF) and their receptors is one of the major molecular mechanisms underlying the pro-angiogenic effects of nAChRs in several types of cancer cells (18, 29, 31). Most intriguingly, VEGF and βFGF-induced human microvascular endothelial cell (HMVEC) migration requires nAChR activation. These studies suggest that nAChR and VEGF mediate distinct but interdependent pathways of angiogenesis (32). Furthermore, nicotine has been shown to induce activation of NF-κB through the MAP kinase p38 and PI3K/AKT signaling pathways, promoting the survival, proliferation and angiogenesis of endothelial cells (32). Another study further demonstrated that AKT survival signals play an important role in the nicotine-mediated carcinogenic process in human breast cancer cells (10).

A recent study demonstrated that estrogen- and nicotine-induced α9-nAChR
expression is transcriptionally regulated by the estrogen receptor (ER) through the PI3K/Akt or MAPK signaling machinery, which mediates phosphorylation of the ER at multiple sites (Figure 1)(9). Phosphorylated ERs can also act indirectly by altering the activities of other transcription factors (e.g., Sp1, AP1, or NF-κB) at their cognate sites on DNA (33). Figure 1 shows the potential transcription factor response elements in the promoter regions of α9-nAChR, including two activating protein 1 (AP1) sites and one vitamin D receptor (VDR) site, both of which are responsive to ER binding (34). Thus, the signaling pathways induced by nicotine and estrogen explain the effects of hormones and smoking on human breast cancer formation.

Smoking-induced cancer formation

Cigarette smoking has a strong etiological association with the development and progression of several types of cancers. In cigarette smoking, the nicotine-derived metabolic derivatives 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N-nitrosonornicotine (NNN) have been proven to act as initiators of carcinogenesis especially in lung, breast, and bladder cancers (5, 35-38). In contrast, nicotine is a demonstrated co-carcinogenic factor that promotes carcinogenesis in tobacco replacement therapies (39). However, the mechanisms of nicotine-mediated cell transformation are still uncertain.

1. Cell transformation induced by nicotine and its metabolic derivatives

The average plasma nicotine concentration of active smokers is about 100 nM - 1 mM (40). The most potent cigarette smoke carcinogens are polycyclic aromatic hydrocarbons and nicotine-specific metabolites, such as NNK and NNN (41). Nicotine and NNK are considered to be carcinogens that react with DNA, and most reports have suggested that the chemical properties of the resulting DNA adducts can cause the diverse genetic changes known to exist in human cancers (35). Accordingly, nicotine and its derivatives have the properties of both promoting (nicotine itself) and initiating (NNN, and NNK) smoking-induced carcinogenesis. Our recent study demonstrated that nicotine rapidly (< 30 min) binds to α9-nAChR in normal human breast epithelial (MCF-10A) cells at a physiologically relevant concentration (8 nM) that is below the average plasma concentration of nicotine in active smokers. Similar results were also observed in human small cell lung cancer cells and pulmonary adenocarcinoma cells (42). Such results suggest that apart from active cigarette...
smoking, passive exposure to smoke is still a hazardous source of nicotine. NNK-triggered human breast epithelial cell transformation has been demonstrated in several previous papers upon long-term (>1 month) exposure to a low concentration of NNK (100 pM) (37). Additionally, our recent study demonstrated that nicotine (10 μM) can induce transformation in normal breast epithelial cells (MCF-10A) upon forced expression of α9-nAChR (43). Another important concern is that nAChRs can be induced in response to nicotine, NNN, and NNK exposure, which produces a detrimental feedback effect on receptor-ligand binding signaling. Such results indicate that receptor-mediated effects may play an important role in nicotine-induced carcinogenesis. Therefore, understanding nAChR-mediated mechanisms could offer useful and abundant prospects for the design of novel cancer therapies.

2. nAChR activation-mediated tumorigenesis

Cell type-specific oncogenesis occurs in response to different nAChR combinations. For example, high levels of α7-nAChR expression promote cancer cell proliferation and metastasis in lung, gastrointestinal and bladder tissues through ERK and Akt signal transduction (44). Apart from α7-nAChRs, α4β2-nAChRs are evolutionarily the oldest heteromeric nAChR receptor and are predominately expressed in mammalian brain and lung tissues (44). It has also been shown that the α4β2-nAChR subtype is crucial in mediating the effects of nicotine-induced dopamine release (45). These effects promote cigarette smoking behavior through uncontrolled nicotine addiction, and they thus play a major role in disease progression (46). The α9-nAChR homopentamer was originally identified in hair cells of the inner ear and is involved in synaptic transmission between efferent nerves and hair cells (47). Recently, α9-nAChR has been found to have diverse functions, such as keratinocyte adhesion (48), immune responses (49), neuropathic pain (50) and even breast cancer formation (43). The correlation between α9-nAChR mRNA expression level and disease outcome in breast tumor patients was evaluated in a recent study (43). In this study, 186 (67.3%) of the 276 paired samples expressed α9-nAChR mRNA at higher levels (mean 7.84-fold) in breast cancer than in surrounding normal tissue. The highest α9-nAChR mRNA expression levels were detected in smoking-related, advanced-stage breast cancer tissues. These observations have led to the conclusion that nicotine binding to nAChRs may play a crucial role in human
breast cancer formation.

3. nAChR activation-mediated angiogenesis

Angiogenesis is a complex combinatorial process that is regulated by a balance between pro- and anti-angiogenic molecules (16-18). An imbalance between pro-angiogenic and anti-angiogenic factors can result in pathological situations, either through deficit conditions (e.g., in inefficient healing, tissue ischemia) or through excess angiogenesis (e.g., atherosclerotic plaque development, diabetic retinopathy and cancer). Increasing evidence has demonstrated that nicotine enhances the angiogenic response to inflammation (the disc angiogenesis model), ischemia (the femoral artery ligation model), atherosclerosis (ApoE-deficient mice) and neoplasia (the Lewis lung cancer model). These processes are mediated by nAChRs and may involve the production of nitric oxide, prostacyclin, βFGF and/or VEGF (17, 51). These growth factors stimulate endothelial cells (ECs) in the existing vasculature to proliferate and to migrate through the tissue to form new endothelialized channels (52, 53). Accordingly, βFGF is known to be involved in nicotine-induced angiogenesis because antibodies against βFGF markedly prevent nicotine-induced angiogenesis (54). Inhibition of nicotinic receptors completely prevents the βFGF-mediated migration of endothelial cells. Furthermore, nAChR-mediated stimulation of angiogenesis was found to be completely dependent on activation of the PI3K/Akt, MAPK and NF-κB pathways, as shown by abrogation of network formation by the PI3K inhibitor LY294002 (54). A further investigation found that α7-nAChR knockout mice exhibited an attenuated angiogenic response to ischemia and inflammation, including a 27% abrogation of the angiogenic response. Notably, antagonists of nAChRs such as mecamylamine and α-Bgtx markedly attenuate VEGF-induced angiogenesis (17). Altogether, these findings indicate that an intricate interplay is present between the pro-angiogenic factors VEGF and βFGF and the nAChR cholinergic pathway. Modulation of the activity of this pathway may represent a new therapeutic avenue for disorders characterized by inadequate or pathological angiogenesis.

4. nAChR activation-mediated metastasis

Cell invasion and metastasis are crucial processes in tumor development.
Numerous factors play a role in the regulation of this process, including growth factors, kinases, phosphatases, and extracellular matrix components. Many growth factors and their downstream effectors are aberrantly activated or overexpressed, contributing to growth deregulation in cancer. It has been reported that the addition of nicotine increases EGF receptor (EGFR) expression in lung, breast, gastric, and colon cells (55-58). After recruiting downstream effectors, growth-related receptors often exert their functions by organizing their downstream effectors to initiate signaling cascades in such pathways as PKC, PI3K/Akt and MAPK, which affect various biological processes including cell migration and cancer cell invasion (59, 60). Nicotine has been shown to induce the phosphorylation of multiple subtypes of calpains, resulting in enhanced cell migration, and specifically lung cancer metastasis (61, 62). Emerging evidence shows that nicotine potently induces secretion of multiple types of calpain from lung cancer cells, which promotes the cleavage of various substrates in the extracellular matrix, facilitating metastasis and tumor progression (63). Accordingly, nicotine-treated mice have shown markedly higher tumor recurrence (59.7%) than vehicle-treated mice (19.5%). Nicotine was also found to increase the metastasis of dorsally implanted Line-1 tumors to the lungs 9-fold. Treatment with α-Bgtx significantly inhibited nicotine-induced proliferation of Line-1 cells, thereby suggesting that an nAChR subunit is mainly responsible for mediating the proliferative effect of nicotine. Another study has also confirmed that NNK enhances the migration of HT29 and DLD-1 colon cancer cells through α7-nAChR-mediated downregulation of the adhesion molecule E-cadherin and upregulation of its transcriptional repressors Snail and ZEB1 (7). By interfering with α7-nAChR protein expression or treating with an α7-nAChR-specific inhibitor (MLA), α7-nAChR mediated NNK-enhanced colon cancer cell migration is significantly attenuated.

Clinical-Translational Advances

- nAChRs are molecular targets for drug development

It is well documented that brain nAChRs participate in physiological functions such as attention, memory and cognition. Clinical data also suggest their involvement in the pathogenesis of several disorders, including Alzheimer’s disease, Parkinson’s disease, schizophrenia, and depression. These results have inspired hypotheses in a
variety of preclinical and (to a lesser extent) clinical models that nAChRs may be a therapeutic target for studying the effects of L-form nicotine (64). In 2001, the crystal structure of the nAChR protein allowed its characterization as an ACh-binding protein (65). To date, there are two rapidly developing fields of nicotinic receptor target drugs. One field is focused on aiding drug discovery by bridging the gap between the assembly, activity, and conformational transitions of nicotinic receptors. The other field relies on the development of therapeutically applicable nicotinic receptor ligands that are either competitive (including agonist and antagonist binding) or non-competitive (allosteric ligand binding). The positions of noncompetitive (allosteric) ligand-binding sites on heteropentameric nAChRs differ in that the non-α neuronal subunit takes the place of the γ or δ subunit. Competitive ligand-binding sites are formed at the interfaces between homopentameric neuronal α subunits (α7, α8, and α9 nAChRs) and between α (α2-4 or α6) and β subunits (β2 or β4) in heteropentameric nAChRs (66).

Over the last few years, the availability of high-resolution x-ray crystal structures of nAChRs in their ligand-free and ligand-bound forms has greatly increased our knowledge regarding nAChR structure and function (67). However, the main difficulty in drug design is that at least 12 genes encode the neuronal nAChR subunits, and their gene products (9α and 3β subunits) assemble in various combinations, forming a broad diversity of pentamers with distinct pharmacological properties. Accordingly, treating pathological conditions involving various neuronal subtypes requires the design of subtype-specific drugs. However, the majority of known nAChR ligands and designed drugs are not subtype-specific (11). Recent studies in preclinical models have demonstrated that nicotine can enhance wound healing via nAChR stimulation (18). Similar results have been obtained in several neurological diseases associated with aging and reduced angiogenesis. For example, decreased levels of nAChRs were detected in the cerebrovascular cells of Alzheimer's disease patients (68), suggesting that nicotine-based therapy could be appropriate for neurological disorders. However, a previous paper demonstrated that α7-nAChR agonists used for therapeutic revascularization after myocardial infarction may have pro-atherogenic activity (31). Additional studies should be performed to assess the optimum balance of nAChR activity regulation in clinical patients.

- nAChR antagonists as potential agents for molecular cancer therapy:
Neurotoxins are commonly used to distinguish between nAchR subunit combinations (69). The neurotoxins lophotoxin, neosurugatoxin, and Bgtx (70, 71) and the alkaloids DH βE (72) and erysodine (73) are competitive nAChR antagonists that display selectivity for β2-containing nAChRs, particularly the α4β2 subtype (74). Among these nAChRs, the α7-nAChRs are known to be overexpressed in small cell lung carcinomas associated with smoking (75). In this case, in vitro experiments have suggested that malignant growth can be halted using snake neurotoxins (α-neurotoxins) or snail conotoxins (α-conotoxins) (76, 77), as they are competitive antagonists of α7-nAChR (78). In vivo animal studies have further demonstrated that α7-nAChR inhibitors, such as MLA (79, 80) and α-Bgtx (81), can reverse the pro-angiogenic effects of nicotine and inhibit cancer cell growth (16, 17, 19, 82, 83). In a lung airway epithelial cell model (84), normal human bronchial epithelial (NHBE) cells were forced to transform by nicotinic activation of Akt, altering their growth characteristics. Dysregulated NHBE growth after nicotine administration is consistent with in vivo observations that active smokers have increased proliferative indices when compared with those of former smokers. Protection from prolonged serum deprivation-induced apoptosis conferred by nicotine was found to be attenuatable by LY294002 or DH βE, and protection conferred by NNK was attenuatable by LY294002 or α-BTX. These studies show that in addition to promoting cellular survival and/or transformation, nicotine-induced nAChR activation or NNK-induced Akt signaling is required to diminish contact inhibition and reduce cellular dependence on exogenous growth factors and the extracellular matrix. These studies have revealed that specific antagonists can decrease the high levels of α7-nAChR expression in human cancer cells. In addition to α7-nAChR-specific antagonists, α9-nAChR-specific antagonists reduce ascending afferent excitatory pain pathway signaling and have analgesic effects in in vivo studies (50, 85). As shown in Table 1, several nicotinic antagonists have either launched or entered phase II clinical trials (86, 87). Unfortunately, most of the antagonists have also caused a variety of side effects due to the lack of nAChR subtype specificity. Among these α9-nAChR-specific antagonists (50, 88-92), Vc1.1 has profound analgesic effects in a variety of animal pain models and has currently entered phase II clinical trials (93). Vc1.1 should have off-label use potential against α9-nAChR-mediated cancer formation in the future.

- Interference with α9-nAChR expression as a novel strategy for drug
development

Our recent study demonstrated that α9-nAChR was preferentially overexpressed in human breast tumor tissue in comparison to normal tissue (43). Normal breast epithelial cells (MCF-10A) were transformed by long-term nicotine treatment (10 μM, > 2 month) together with forced expression of α9-nAChR using the Tet-inducible adenovirus system. The established α9-nAChR over-expressing cells (Tet-Off group) were transplanted into nude mice and resulted in increased tumor growth volume (2.33-fold) when compared to the control (Tet-On) group. Moreover, specific inhibition of α9-nAChR expression using RNA-interference (siRNA) or compounds derived from plants was found to concomitantly inhibit cancer cell growth, soft-agar colony formation, and tumor growth in SCID mice (8, 10, 94). Nicotine-induced breast cancer cell proliferation can be inhibited by garcinol (1 μM) derived from the edible fruit *Garcinia indica* through down-regulation of α9-nAChR and cyclin D3 expression (95). A combination of luteolin and quercetin (0.5 μM each) synergistically down-regulates α9-nAChR expression in human breast cancer cells (10). Another study found that the tea polyphenol (-)-epigallocatechin-3-gallate (EGCG, 10 μM) attenuates estradiol (10 nM)- and nicotine (10 μM)-induced α9-nAChR protein expression in human breast cancer cells (94). They further demonstrated that combined treatment with EGCG profoundly inhibits [³H]-Nic/α9-nAChR binding activity, resulting in reduced soft-agar colony formation (> 50%) in MCF-7 breast cancer cells (94). Several previous papers also demonstrated that *in vivo* growth of lung and breast cancer cell lines with functional estrogen receptors is initiated by estradiol (1~10 nM) stimulation (9, 96-99). Such results imply that EGCG (or other natural compounds such as garcinol, luteolin and quercetin) could be used as to block smoking (nicotine)- or hormone (estradiol)-induced cancer cell proliferation by inhibiting the nAChR signaling pathway.

**Conclusion**

Over the past few years, the technique of high-resolution x-ray crystallography has revealed the structures and functions of nAChRs in both their ligand-free and ligand-bound forms. It has been shown that nAChRs are selectively overexpressed in a variety of cancers, such as α7-nAChR in lung cancer and α9-nAChR in breast cancer. Studies have shown that inhibited nAChR protein levels significantly attenuate nicotine and NNK-induced cell proliferation in tumor models. Although
there are several nAChR-specific agonists and antagonists that have been used in clinical trials for the treatment of various diseases (excluding cancer), a selective antagonist specifically targeting the over-expressed nAChR subtypes that appear in human cancer cells could represent a novel therapeutic strategy. The development of nAChR-specific antagonists for clinical translation is both timely and relevant, and it will enhance our understanding of the carcinogenic role of nAChRs.
Figure 1. nAChR signal transduction. The nicotinic agonists at the top of the signaling cascades are listed in the order of their affinity for nAChRs. NNK and nicotine bind with highest affinity to the homopentameric nAChRs such as the α7 and α9 subunits, whereas acetylcholine and NNN seem to preferentially bind to heteropentameric nAChRs (α4β2, α3β2, or α3β4). In lung cancer cells and neuronal cells, the signaling pathways downstream of α7- and heteropentameric-nAChRs promote proliferation and angiogenesis by mediating Ca\(^{2+}\)-dependent activation of PKA, PKC, PI3K/Akt, and MAPK through the induction of the transcription factors NF-kB, AP1, CREB, and mTOR. On the other hand, nicotine inhibits apoptosis by activating survival factors such as Myc, XIAP and survivin proteins. In this context, nicotine is also plays a pro-angiogenic role by releasing MMPs, VEGF, and βFGF from endothelial cells and in the tumor microenvironment. However, in breast tumors, the α9 subunit is the most abundant nAChR, and downstream signaling pathways such as MAPK and PI3K/Akt can be activated through nicotine and E2 stimulation. The downstream transcription factors AP1 and VDR can bind the promoter region of α9-nAChR by activating the ER at Ser167 and Ser118, or at Ser 104/106 sites through the PI3K/Akt and MAPK pathways. These signal transduction cascades eventually form a strong positive feedback loop and result in tumor progression. N: nicotine, E2: estrogen.
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Table: nAChR inhibitors with potential clinical applications for cancer therapy.

<table>
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<tr>
<th>Compound</th>
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<th>Receptor</th>
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<td>Dihydro-β-erythroidine (DHβE)</td>
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<td>α4β2</td>
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<td>(81)</td>
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