Drugs of the Future 2007, 32(2): 161-170

CCC: 0377-8282/2007
DOI: 10.1358/dof.2006.032.02.1064020

Review Article

Selective α7 nicotinic acetylcholine receptor ligands for the treatment of neuropsychiatric diseases

Lisa M. Broad, Emanuele Sher, Peter C. Astles, Ruud Zwart, Michael J. O'Neill*

*Eli Lilly and Co., Ltd., Lilly Research Centre, Erl Wood Manor, Windlesham, Surrey GU20 6PH, U.K.*Correspondence: e-mail: Oneill_Michael_J@Lilly.com*

CONTENTS

Abstract

Recent developments have yielded a number of selective α7 nicotinic acetylcholine receptor (nAChR) ligands allowing the critical evaluation of α7 nAChRs as targets for the treatment of neuropsychiatric and neurodegenerative diseases. We review the *in vitro* and *in vivo* profiles of these α7 nAChR-selective ligands and preliminary data on their use in man.

Introduction

Nicotinic acetycholine receptors (nAChRs) are pentameric ion channels that are widely expressed throughout the central and peripheral nervous systems and in the periphery, including skeletal muscle, epithelial and endothelial cells and immune cells. Neuronal nAChRs are composed of either heteromeric (α2-6/β2-4) or homomeric (α7-10) subunit combinations. The homomeric α7 receptor is one of the most abundant nicotinic receptors in the human brain, together with heteromeric α4β2* (* implying that further subunits are likely to be present in the pentamer). These nAChRs have an overlapping distribution, but α7 is more heavily expressed in the hippocampus, cortex, thalamic nuclei, ventral tegmental area and substantia nigra. In contrast to α4β2* receptors, α7 receptors are also expressed in the autonomic nervous system, where they have an auxiliary role to heteromeric α3β4 receptors in mediating synaptic transmission.

The presence of α7 nAChRs at high density in areas involved in learning and memory, and their physiological role in the modulation of neurotransmitter release, particularly glutamate and GABA, has focused attention on this subtype. The importance of α7 receptors in the modulation of both glutamatergic and GABAergic synapses can also be envisaged by the unexpectedly high concentration of α7 receptors within hippocampal synapses (1). Moreover, the ability of α7 receptors to potentiate glutamate release has been confirmed not only in rodents but also in human neocortical synaptosomes (2). *In vivo* studies with nonselective nAChR agonists have revealed activity in animal models of attention and memory performance, while nAChR antagonists cause deficits in these models (3, 4). However, the adverse side effect profile of these nonselective nAChR agonists limits their clinical utility. Selective α7 ligands are expected to retain efficacy while affording a broader therapeutic window.

In addition to a physiological role, α7 receptors are further implicated in pathological conditions, with a genetic linkage to deficits in auditory and sensory gating in schizophrenic patients (5) and decreased α7 nAChR protein expression reported in patients with diseases associated with memory impairment, including schizophrenia and Alzheimer's disease (6, 7). Distinctive features of α7 nAChRs include high permeability to calcium, rapid desensitization and sensitivity to block by methyllycaconitine and α-bungarotoxin. The relative lack of suitable antagonist tools for this receptor is worth noting. α-Bungarotoxin is a large peptide and does not cross the blood-brain barrier. Methyllycaconitine, while it does enter the brain, will also block other receptors at similar (α6*) or higher (most heteromeric nAChRs) concentrations. While antagonist tools would still be helpful for further *in vivo* validation of this target, the most relevant advances have been in the development of agonists with varying intrinsic efficacy, and more recently positive allosteric modulators. The rationale for these different approaches includes the hypothesis that partially efficacious agonists would be predicted to act as agonists in the absence or in the pres-

ence of low concentrations of the endogenous neurotransmitter, but as antagonists in the presence of high concentrations of the endogenous neurotransmitter. This profile has been speculated to be beneficial in terms of ceiling effects and safety margins (8). Importantly, both partial and fully efficacious agonists desensitize α7 nAChRs at lower concentrations than those required for activation, leading to some speculation that limited *in vivo* effects might be seen with chronically administered agonists. The use of selective positive allosteric modulators is argued to be a more physiological approach to target α7 nAChRs, as only endogenous cholinergic neurotransmission would be amplified without direct or tonic stimulation of the target.

In vitro pharmacology of nAChR ligands

Structure and basic pharmacology of various classes of ligands

A range of structurally diverse α7 nAChR ligands have now been developed, including agonists with varying intrinsic efficacy and allosteric modulators (Fig. 1). Examples of α7 agonists displaying high efficacy include AR-R17779 (9), PSAB-OFP (10, 11) and PNU-282987 (12, 13), while agonists displaying more partial efficacy include DMXB-A (GTS-21) (14), SSR-180711 (15) and Mitsubishi's "compound 23" (16) (see also Fig. 2).

DMXB-A, or GTS-21 (**1**), a natural alkaloid derivative, was the first selective α7 agonist described, and despite its rather weak micromolar potency at α7 nAChRs and reported cross-reactivity with α4β2 and serotonergic 5-HT_3 receptors (14, 17, 18), it has been used extensively for *in vivo* preclinical studies and evaluated in clinical trials. Metabolites have also been studied and the primary metabolite, 4-OH-GTS-21 (**2**), is itself a partial α7 nAChR agonist, reportedly more potent then DMXB-A itself. The 4-OH metabolite is also reported to cause less "irreversible" inactivation of α7 receptors, an effect prominent with the parent compound (19). Notably, 4-OH-GTS-21 and a 2-OH-MBA, another metabolite, are also partial agonists at 5-HT_3 receptors (20).

Improved ligands subsequently disclosed by AstraZeneca included the spiro-oxazolidinone AR-R17779 (**3**), which exhibits *in vitro* selectivity for α7 (K_i = 92 nM) over α4β2 nAChRs (K_i = 16 μM) and acts as a highly efficacious agonist, displaying 96% maximal efficacy (9). A structurally related compound, PSAB-OFP (**5**), retains its profile as an efficacious α7 agonist (86% efficacy) but also demonstrates agonist activity at 5-HT_3 receptors at similar concentrations (11). Further developments led to ligands with increased potency and selectivity over 5-HT_3 receptors and improved pharmacokinetic properties (10, 18). However, to date, AR-R17779 has been the most widely profiled of these compounds *in vivo*.

More recently, Mitsubishi Pharma reported several leads from a series of (*S*)-spiro[1-azabicyclo[2.2.2]-octane-3,5'-oxazolidin]-2'-ones (**6** and **7**) as potent α7 nAChR agonists (16, 22, 23). The most recent of these, compound **7**, was shown to be a partial agonist at α7 nAChRs in cultured hippocampal neurons and displayed clear selectivity in binding studies for α7 (K_i = 3 nM) over a panel of cell-surface receptors, including α4β2 nAChRs (K_i > 10,000 nM). However, it was also found to act as an antagonist at 5-HT_3 receptors (K_i = 10 nM) (16).

Pfizer has also described a series of quinuclidine benzamides that are α7 nAChR agonists (12). Medicinal chemistry initially led to PNU-282987 (**8**), which displayed selectivity for α7 nAChRs (K_i = 27 nM) over other nAChR subtypes in binding assays, but acted as an antagonist at 5-HT_3 receptors (K_i = 1662 nM). More recently, Pfizer reported on PHA-543613 (**9**), another potent α7 nAChR agonist (K_i = 9 nM), which was once again selective *versus* other nAChR subtypes but still displayed 5-HT_3 receptor cross-reactivity (K_i = 511 nM) (24).

Sanofi-Aventis recently disclosed SSR-180711 (**10**) as a potent and selective partial agonist at α7 AChRs (15). SSR-180711 displayed high affinity for recombinant human α7 AChRs (K_i = 22 nM) and > 250-fold selectivity over other nAChRs; at 10 μM it was devoid of activity in a CEREP screen of 100 standard receptors.

In November 2005, preclinical data from Targacept on TC-5619 (**11**) were presented at a meeting in Washington, D.C. (25). TC-5619 demonstrated high binding affinity for α7 receptors (K_i = 0.3 nM) and was shown to be a full α7 receptor agonist (EC_{50} = 33 nM).

In addition to these publications, there have been a number of recent patent filings claiming compounds with α7 receptor-agonist activity. Novartis (**12** and **13**) (26, 27) and Abbott (**14**) (28) have described 3-pyrazinoxyquinuclidines, while AstraZeneca has discovered a series of 4- and 5-substituted imidazoles (**15** and **16**) (29, 30). Structures disclosed in patents from AstraZeneca (**17**) (31) and sanofi-aventis (**18**) (32) have achieved replacement of the quinuclidine present in many α7 agonists. No precise *in vitro* data are published on these structures, but a U.S. patent (28) contains detailed *in vivo* evaluation of **14** in a model of sensory gating. The exact structures of Memory Pharmaceuticals' phase I candidate MEM-3454 and back-up compounds such as MEM-63908 are unknown at present. Memory has a patent filing (33) covering anabaseine analogues such as **19** related to GTS-21, and a number of recent patents (34-36) describing isoxazole, indazole and isothiazoles linked to quinuclidine or related azabicyclics (**20** and **21**). Bayer has patented related quinuclidine derivatives substituted with biarylamides **22** (37) or benzothiophenes (38).

In summary, a range of structurally diverse α7 agonists which are potent and selective for α7 over other nAChR subtypes are now available. Cross-reactivity at 5-HT_3 receptors is still a feature for some but not all of these agonists. These compounds display a range of efficacies as α7 agonists *in vitro* and several possess favorable pharmacokinetic properties, making them amenable to *in vivo* evaluation. It would therefore be interesting to compare their *in vivo* profile with this in mind (see later).

In contrast, relatively few selective nAChR positive allosteric modulators (or potentiators) have been reported

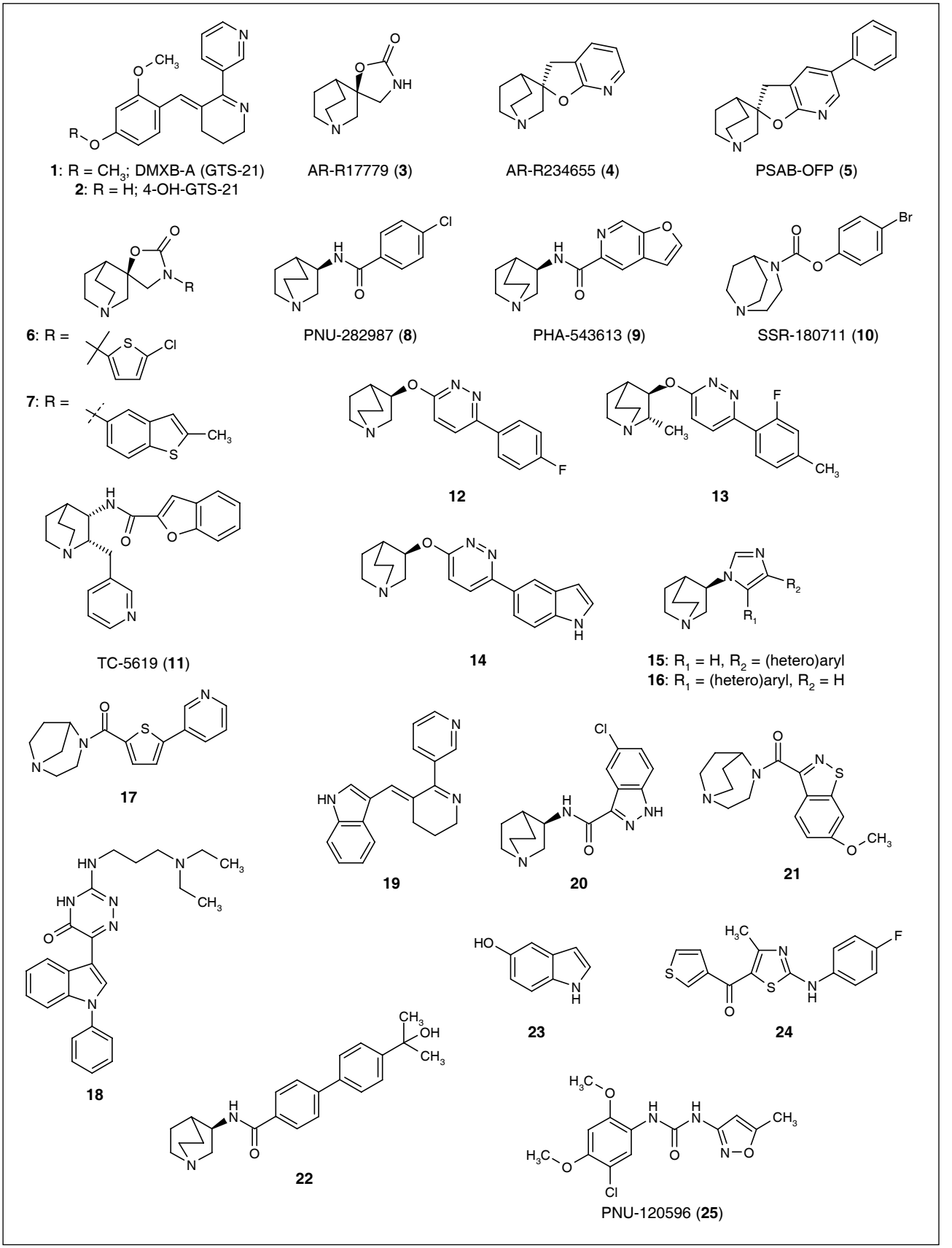

Fig. 1. Structures of α7 nAChR agonists and allosteric modulators.

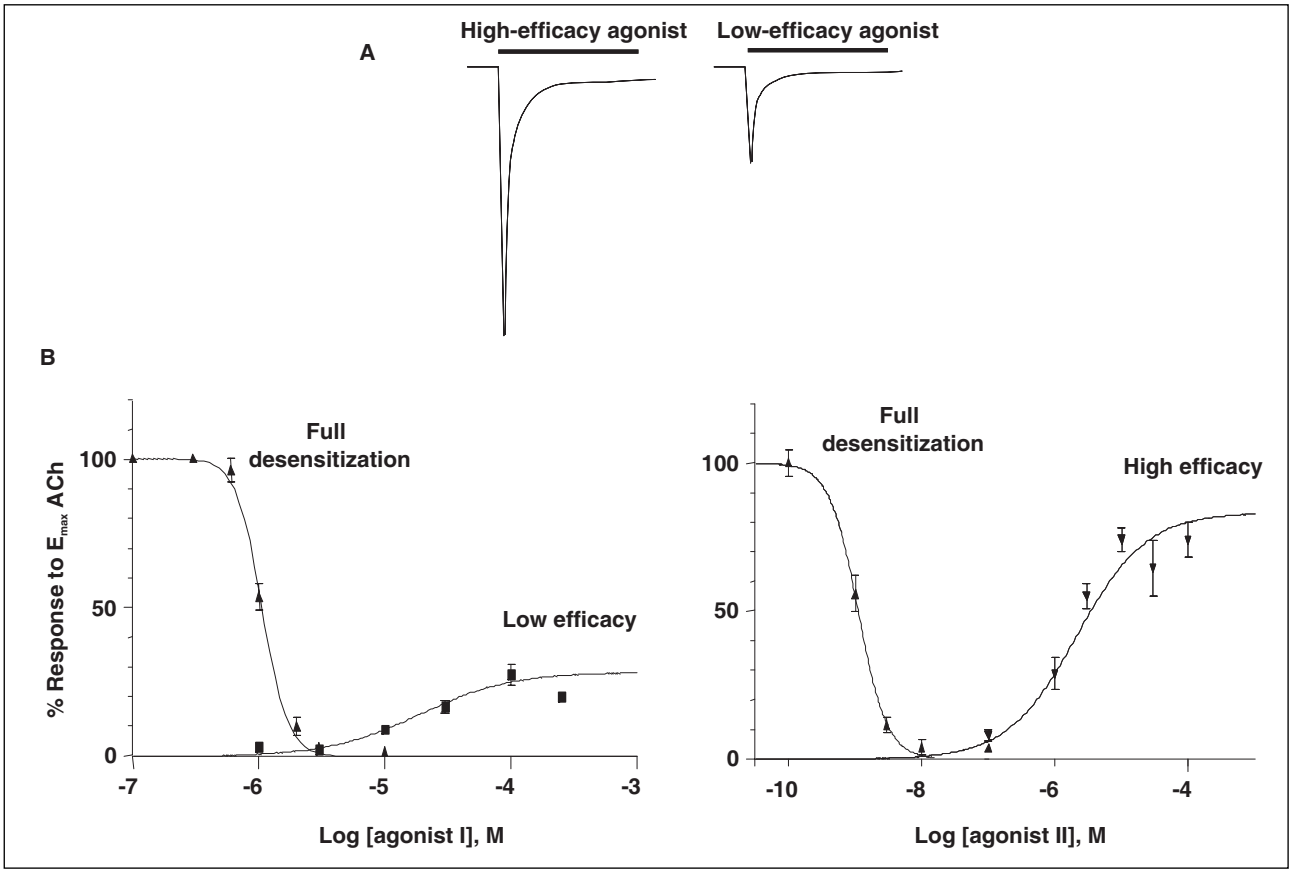

Fig. 2. Profile of full and partial agonists at human α7 nAChRs expressed in *Xenopus* oocytes. **A**. Maximal α7 nAChR current responses to a high- or low-efficacy agonist. **B**. Concentration-response curves for activation of α7 nAChRs by high- and low-efficacy agonists and for desensitization of responses to a subsequent application of 1 μM ACh, as indicated.

to date. The 5-hydroxyindole **23** (39) and ivermectin (40), for example, selectively potentiate α7 over other nAChRs, but display low potency and/or cross-reactivity with other ligand-gated ion channels. Galantamine, an acetylcholinesterase inhibitor used for the treatment of Alzheimer's disease, also acts as a positive allosteric modulator of nAChRs (41) but is very weak, not selective for the α7 subtype and also modulates NMDA receptors. More recently, a group of novel (2-amino-5-keto)thiazole compounds (*e.g.*, **24**) was reported to selectively potentiate α7 and α4β2 nAChRs, but not ganglionic or muscle-type nAChRs or other ligand-gated ion channels (42). PNU-120596 (**25**) was the first allosteric potentiator reported as α7-selective, with no effects on α4β2 or α3β4 nAChRs (43). New selective α7 potentiators have recently been described by Lilly as well (44). Positive allosteric modulators have been shown to: 1) increase agonist potency; 2) increase agonist efficacy; and 3) decrease the rate of desensitization, although not all modulators alter all parameters to the same degree. These agents also appear to increase the window in which agonists activate α7 nAChRs without causing desensitization (Fig. 3). At lower concentrations, they act to potentiate agonist-induced responses, but can act themselves as "activators" at high concentrations (42, 44).

Electrophysiological effects

The profile of several of the α7-selective agonists and allosteric modulators has been studied *in vitro* using various rodent neuronal preparations, including electrophysiological recordings from hippocampal neuronal cultures and slices. We have shown that in neonatal hippocampal slices, AR-R17779 stimulates GABA release by activating α7 nAChRs, which mediates the generation of giant depolarizing potentials, events thought to facilitate the consolidation and fine-tuning of hippocampal synapses (45). The activation of somatic α7 currents and the potentiation of spontaneous GABA release using *in vitro* hippocampal preparations have also been reported with the full agonist PNU-282987 (13). In cultured rat hippocampal neurons, the partial agonist SSR-180711 has been shown to induce inward currents attributable to somatodendritic α7 nAChRs, as well as GABA release through the activation of presynaptic α7 nAChRs. In mouse hippocampal slices, SSR-180711 increased the amplitude of glutamatergic excitatory postsynaptic currents (EPSCs) and GABAergic inhibitory postsynaptic currents (IPSCs), as well as long-term potentiation (LTP). This activity was absent in α7-null mice. LTP was also induced in rat hippocampal slices, an effect blocked by α-bungarotoxin (15).

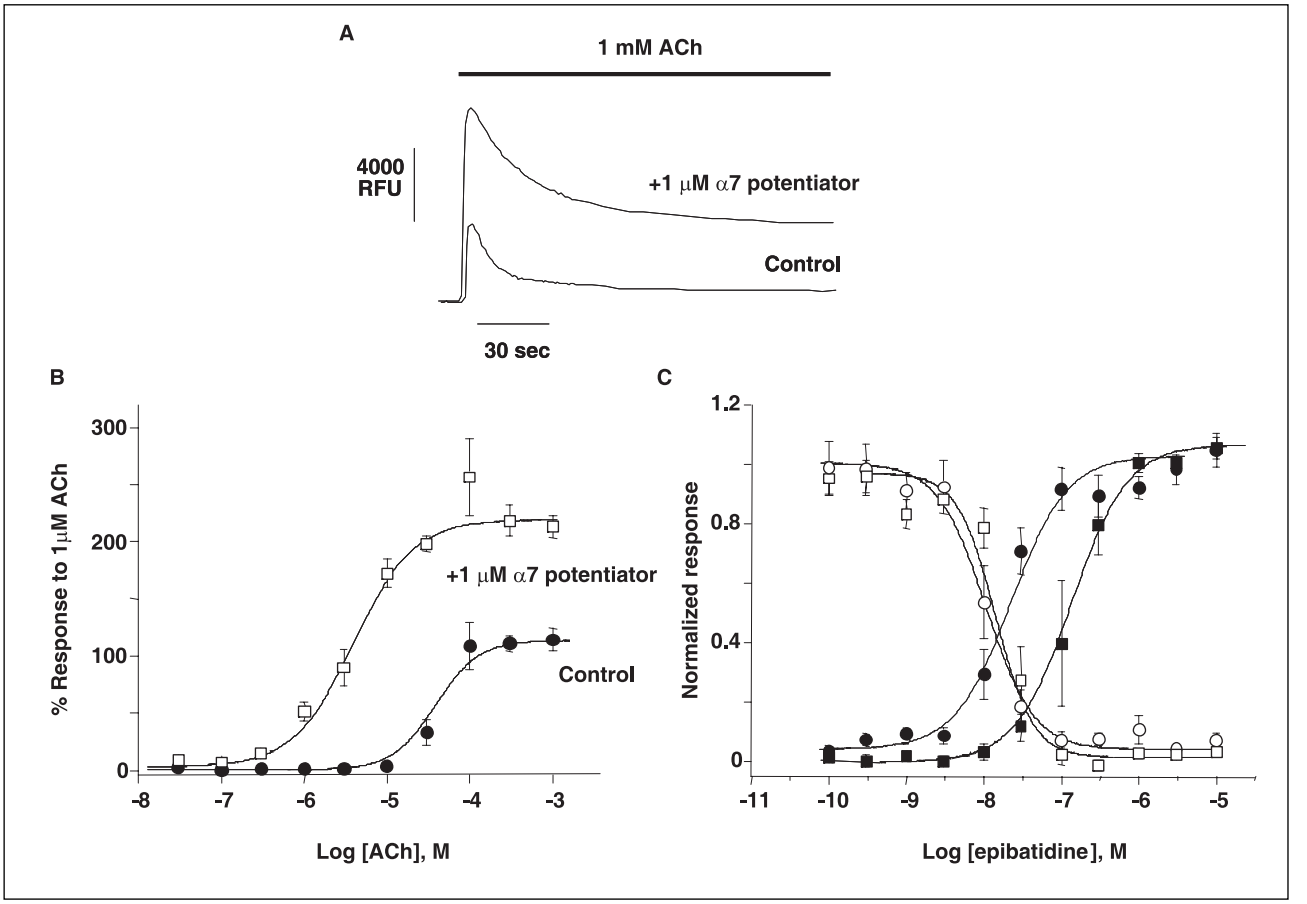

Fig. 3. Profile of an α7 nAChR allosteric modulator on recombinant human α7 nAChRs stably expressed in GH4 cells**. A**. Calcium responses to a maximal concentration of ACh in the absence or presence of an α7 potentiator, as indicated. **B.** Concentration-response curves for activation of α7 nAChRs by ACh in the absence or presence of 1 μM α7 potentiator. **C.** Concentration-response curves for activation of α7 nAChRs by epibatidine in the absence (filled squares) or presence (filled circles) of 1 mM 5-hydroxyindole and for desensitization of responses to a subsequent application of 1 μM epibatidine (open squares and open circles, respectively).

The above data are somewhat different from those reported by Fuji and Sumikawa in a similar LTP protocol in rat hippocampal slices (46). They proposed that nicotine potentiated LTP by both activating α4β2 receptors and inactivating α7 receptors. This was mainly based on findings that the α7 antagonist methyllycaconitine potentiated LTP. Biton *et al.* did not see any potentiation of LTP with α-bungarotoxin and their data with the selective α7 agonist are quite convincing (15). The various effects of α7 agonists on synaptic transmission in the hippocampus might well represent the cellular substrates for the *in vivo* effects reported with some of these compounds in cognitive tasks and auditory gating tests. Indeed, activation of nicotinic receptors has been reported to induce LTP in mouse brain *in vivo* (47).

The electrophysiological properties of the new α7 potentiators are quite striking. Both the compounds reported by Lilly (44) and by Pfizer (43) dramatically increase the amplitude of agonist-evoked currents of both recombinant and native α7 receptors. This is associated with marked inhibition of receptor desensitization. At the single channel level, the compounds increase the frequency of openings and the single channel open time, while reducing the closed time. At least in our experiments, changes in conductance and rectification of α7 receptors have also been observed. The α7 potentiators, as expected, significantly facilitate the agonist-induced effects on GABA and glutamate release.

In vivo actions of nAChR ligands

Cognition

In agreement with the localization and functional role of α7 nAChRs in areas involved in cognitive processing, α7 receptor knockout mice have been reported to have a performance deficit in delayed matching to place (48), suggesting a mild impairment of working/episodic memory. Further studies reported deficits in attentional tasks such as the 5-choice serial reaction test (49, 50) and in an odor span test (50). Otherwise, knockout mice have normal development and no obvious basal behavioral phenotype, although the effects of nicotine and selective nicotinic ligands still have to be investigated in detail in these animals.

Although the data with α7 transgenic mice are recent, numerous published studies have evaluated nicotine in rodent models of cognition (3, 4). Nicotine has been reported to improve performance in both wild-type and α7 knockout mice (51), although improvement appears to depend on how the task is carried out. In a more recent study, it was reported that nicotine (0.001-1.0 mg/kg) was unable to rescue deficits in the 5-choice serial reaction time observed in α7 knockout mice (49). Grottick and Higgins evaluated the effects of nicotine and subtype-selective compounds on a 5-choice serial reaction time test in rats (52). Nicotine (0.2 mg/kg s.c.) and the α4β2 agonist SIB-1765F (5 mg/kg s.c.) increased correct responding and decreased response latencies across the treatment week, whereas the α7 agonist AR-R17779 (20 mg/kg s.c.) had no effect. In nicotine-pretreated rats, the decrease in latency and increase in premature responses induced by nicotine (0.2 mg/kg s.c.) to a target stimulus of 150 ms were fully antagonized by dihydro-β-*erythro*-iodine (3 mg/kg s.c.), but not by methyllycaconitine (5 mg/kg i.p.). The authors suggested that α7 receptors do not play a role in any of the behavioral effects of nicotine observed in the 5-choice serial reaction time test, whereas a high-affinity site, perhaps α4β2, is more likely involved.

Many of the earlier pharmacological studies linking α7 receptor activation to cognition were carried out with GTS-21. Nanri and co-workers reported that GTS-21 improved ischemia-induced deficits in passive avoidance in gerbils (53) and ischemia-induced deficits in radial arm maze performance in rats (54). In both cases, the improvements were also associated with neuroprotection in the hippocampus. In addition, chronic treatment (20 weeks) with GTS-21 was reported to protect against neuronal cell loss caused by nucleus basalis magnocellularis (nBM) lesions in rats (55). GTS-21 was also reported to provide some cognitive improvement in mice, rats and monkeys (56, 57), and in some cases this appeared to be sensitive to mecamylamine (56), suggesting the involvement of nAChRs. Positive effects on some aspects of cognition were reported in a phase I clinical study in young healthy males (see 57 for review).

However, until recently, there were limited data on the effects of highly selective agonists on behavioral endpoints. Studies from Levine and co-workers reported that AR-R17779 improved learning in two radial-arm maze tasks and reversed working memory impairment caused by fimbria-fornix lesions (58). In a social recognition test, AR-R17779 improved performance in unimpaired animals (1, 3, 10 and 30 mg/kg s.c.) employing a 24-h retention interval and reversed the scopolamine-induced deficit (0.3 and 1 mg/kg s.c.) after a 15-min retention interval (59). The effects of AR-R17779 (1 mg/kg s.c.) in unimpaired animals were reversed by methyllycaconitine. However, there has been some debate as to whether the compound reaches sufficient brain levels to act as a selective α7 agonist.

Bayer has recently reported that other more selective compounds such as *N*-[(3*R*)-1-azabicyclo[2.2.2]oct-3-yl]-7-[2-(methoxy)phenyl]-1-benzofuran-2-carboxamide (ABBF) also show efficacy in this social recognition memory test and were also active in working memory tasks (60). This area of work is being pursued further by En Vivo Pharmaceuticals since Bayer closed its CNS activities, with the first of these compounds progressing to phase I (Gerhard Koenig, communication at GTCbio meeting, Washington, D.C., 2006).

Mitsubishi Pharma reported that its potent, selective α7 partial agonist (**7**) blocked scopolamine-induced deficits in the 8-arm radial maze at doses of 3 and 10 mg/kg p.o. (16).

Memory Pharmaceuticals (in collaboration with Roche) has reported a series of compounds, with the lead compounds being MEM-63908 (preclinical development for Alzheimer's disease) and MEM-3454 (prephase IIa). In a phase I study in healthy volunteers, MEM-3454 proved to be safe and generally well tolerated up to and including a dose of 450 mg as single ascending doses. It was also safe and generally well tolerated up to and including a dose of 150 mg as multiple ascending doses. In addition, cognition data generated in the MAD study using the Cognitive Drug Research (CDR) battery demonstrated that a dose of 15 mg of MEM 3454 administered once daily for 13 days showed a statistically significant effect on Quality of Episodic Secondary Memory (QESM) scores, one of the study's primary efficacy variables. QESM is a composite score derived from memory tests in the CDR battery that measure the efficiency with which study participants are able to remember words and pictures. The other doses administered in the study did not show a similarly statistically significant effect, although there was a trend toward efficacy at the 50-mg dose.

In addition, α7 receptors have been reported to mediate neuroprotection in several *in vitro* (glutamate toxicity, Aβ toxicity, serum deprivation) culture systems (see 61 for review). Of particular relevance to Alzheimer's disease are reports that β-amyloid peptide Aβ(1-42) binds selectively and with picomolar affinity to α7 nAChRs (62) and can alter the function of these receptors (63, 64). Further studies have reported accelerated plaque accumulation, associative learning deficits and upregulation of α7 nAChR protein in transgenic mice co-expressing mutant human presenilin 1 and amyloid precursor proteins (APPs) (65). Several molecular pathways (66-68) for an interaction to prevent amyloid formation and toxicity have been proposed. It has also been reported that nicotine can reduce amyloidosis in transgenic mice carrying the Swedish mutation of human APP, which develop brain Aβ deposits (69), and α7 receptors have been suggested as a target to rescue deficits in hippocampal LTP induction in Aβ-infused rats (70).

In summary, there is evidence that activating nAChRs both improves cognitive function (baseline or impaired) and provides protection against neurotoxicity (induced by amyloid accumulation or neurotoxin).

Schizophrenia

Genetic polymorphism in the α7 nAChR gene *CHRNA7* has been linked to cognitive and sensory

deficits in schizophrenic patients. Moreover, in general, positive effects have been reported with α7-selective ligands in auditory gating assays, with some of the most compelling data coming from studies with DMXB-A. Nicotine and DMXB-A (71, 72) have been extensively tested in rodent models and were found to correct deficits in auditory gating (see reviews 73 and 74). In a recent clinical proof-of-concept trial with DMXB-A, significant neurocognitive improvement was found on the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) scale total score, particularly for the lower DMXB-A dose compared with placebo (75). The effects were greater than those of nicotine in a similar study. A significant improvement in P50 inhibition also occurred. However, due to the mixed pharmacological profile of DMXB-A and its active metabolites at α4β2 nAChRs and 5-HT_3 receptors, confirmation with some of the more selective ligands would be required to further establish that the effects are mediated via α7 nAChRs.

Pfizer's selective α7 agonists PNU-282987 (1 or 3 mg/kg i.v.) and PHA-543613 corrected auditory gating deficits produced by amphetamine in rodent models (13). PHA-543613 also improved performance in the rat novel object recognition test (24). Likewise, Mitsubishi compounds **6** (23) and **7** (16) showed some effect in inhibiting MK-801-induced gating deficits in rats, and the latter compound also increased extracellular dopamine levels in the frontal cortex. Sanofi-Aventis recently reported that its selective partial α7 nAChR agonist SSR-180711 reversed MK-801-induced deficits in retention of episodic memory in rats (object recognition) and selective attention impaired by neonatal phencyclidine (PCP) treatment, and restored MK-801- or PCP-induced memory deficits in the Morris or linear maze, with a minimum effective dose (MED) of 1-3 mg/kg (76). SSR-180711 also increased extracellular levels of dopamine in the prefrontal cortex (MED = 1 mg/kg) and enhanced (3 mg/kg) spontaneous firing of retrosplenial cortex neurons in rats. The selectivity of SSR-180711 was confirmed, as these effects were abolished by methyllycaconitine (3 mg/kg i.p. and 1 mg/kg i.v., respectively). The compound also had antidepressant-like actions and it was concluded that SSR-180711 was a promising new agent for the treatment of the cognitive symptoms of schizophrenia.

Targacept also reported that its α7 agonist TC-5619 has the potential for managing both the cognitive dysfunction and the psychosis associated with schizophrenia (25). This was based on: 1) data from an object recognition paradigm, which demonstrated that TC-5619 administered orally had positive effects over a wide dose range (0.3-10 mg/kg); and 2) data indicating that the hyperactivity induced by dopamine overstimulation was attenuated by TC-5619 (0.3 and 1.0 mg/kg).

There has been only one report to date of *in vivo* studies with selective α7 positive allosteric modulators. Pfizer recently reported that PNU-120596 corrected the deficits in auditory gating produced by amphetamine (43). While there is evidence from *in vitro* studies that allosteric modulators can vary in terms of their overall potency and that there appears to be no separation between the effects of α7 agonists displaying partial and full efficacy and allosteric modulators in many of the *in vivo* assays, most compounds show a similar level of activity in tests such as auditory gating.

In summary, there appears to be consistent evidence that nicotine and α7 agonists and potentiators can correct deficits in auditory gating assays. The data from behavioral assays such as prepulse inhibition (77, 78) is more variable and much debated. However, the links between smoking and schizophrenia (6, 73) are compelling and have helped strengthen the case for a role of nicotinic receptors in schizophrenia (79, 80).

Summary and conclusions

In the last 5-10 years much effort has been focused on developing selective nAChR agonists. Nicotine is known to enhance attention and several studies have also reported other cognitive benefits. Until recently, the lack of selective ligands for α7 receptors has hampered progress. However, several companies have now reported potent subtype-selective α7 agonists and allosteric modulators. Despite some concerns that α7 receptors are rapidly desensitized by many of the agonists, promising preclinical effects have been observed in rodent models of cognition and there are also effects on auditory gating. It is not clear if the effects on auditory gating will translate into the clinic, but the emerging picture indicates that modulating α7 receptors could be a useful approach for treating cognitive impairment, gating deficits and cognitive impairment associated with schizophrenia. A recent proof-of-concept study with DMXB-A in 12 patients with schizophrenia reported improvement in both neurocognitive and P50 auditory-evoked potential inhibition endpoints. Several companies have now progressed (or are close to progressing) their lead candidates to the clinic. Data to date suggest that these molecules are well tolerated and further clinical data are awaited.

References

1. Fabian-Fine, R., Skehel, P., Errington, M.L. et al. *Ultrastructural distribution of the alpha7 nicotinic acetylcholine receptor subunit in rat hippocampus.* J Neurosci 2001, 21(20): 7993-8003.

2. Marchi, M., Risso, F., Viola, C. et al. *Direct evidence that release-stimulating alpha7 nicotinic cholinergic receptors are localized on human and rat brain glutamatergic axon terminals.* J Neurochem 2002, 80: 1071-8.

3. Levin, E.D. *Nicotinic receptor subtypes and cognitive function.* J Neurobiol 2002, 53(4): 633-40.

4. Levin, E.D., Bradley, A., Addy, N. et al. *Hippocampal alpha 7 and alpha 4 beta 2 nicotinic receptors and working memory.* Neuroscience 2002, 109(4): 757-65.

5. De Luca, V., Wong, A.H., Muller, D.J. et al. *Evidence of association between smoking and alpha7 nicotinic receptor subunit gene in schizophrenia patients.* Neuropsychopharmacology 2004, 29(8): 1522-6.

6. Leonard, S., Breese, C., Adams, C. et al. *Smoking and schizophrenia: Abnormal nicotinic receptor expression*. Eur J Pharmacol 2000, 393(1-3): 237-42.

7. Perry, E., Martin-Ruiz, C., Lee, M. et al. *Nicotinic receptor subtypes in human brain ageing, Alzheimer and Lewy body diseases*. Eur J Pharmacol 2000, 393(1-3): 215-22.

8. Hogg, R.C., Bertrand, D. *Partial agonists as therapeutic agents at neuronal nicotinic acetylcholine receptors*. Biochem Pharmacol 2006, Epub ahead of print.

9. Mullen, G., Napier, J, Balestra, M. et al. *(-)-Spiro[1-azabicyclo[2.2.2]octane-3,5'-oxazolidin-2'-one], a conformationally restricted analogue of acetylcholine, is a highly selective full agonist at the alpha 7 nicotinic acetylcholine receptor*. J Med Chem 2000, 43(22): 4045-50.

10. Phillips, E., Mack, R., Macor, J. et al. (Astra AB) *Novel spiroazabicyclic heterocyclic compounds*. WO 9903859.

11. Broad, L.M., Felthouse, C., Zwart, R. et al. *PSAB-OFP, a selective alpha 7 nicotinic receptor agonist, is also a potent agonist of the 5-HT3 receptor*. Eur J Pharmacol 2002, 452(2): 137-44.

12. Bodnar, A.L., Cortes-Burgos, L.A., Cook, K.K. et al. *Discovery and structure-activity relationship of quinuclidine benzamides as agonists of alpha7 nicotinic acetylcholine receptors.* J Med Chem 2005, 48(4): 905-8.

13. Hajos, M., Hurst, R.S., Hoffmann, W.E. et al. *The selective alpha7 nicotinic acetylcholine receptor agonist PNU-282987 [N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide hydrochloride] enhances GABAergic synaptic activity in brain slices and restores auditory gating deficits in anesthetized rats.* J Pharmacol Exp Ther 2005, 312(3): 1213-22.

14. De Fiebre, C.M., Meyer, E.M., Henry, J.C. et al. *Characterization of a series of anabaseine-derived compounds reveals that the 3-(4)-dimethylaminocinnamylidine derivative is a selective agonist at neuronal nicotinic α7/^{125}I-α-bungaratoxin receptor subtypes.* Mol Pharmacol 1995, 47: 164-71.

15. Biton, B., Bergis, O.E., Galli, F. et al. *SSR180711, a novel selective α7 nicotinic receptor partial agonist: (1) Binding and functional profile*. Neuropsychopharmacology 2007, 32(1):1-16.

16. Tatsumi, R., Fujio, M., Takanashi, S. et al. *(R)-3'-(3-Methylbenzo[b]thiophen-5-yl)spiro[1-azabicyclo[2,2,2]octane-3,5'-oxazolidin]-2'-one, a novel and potent alpha7 nicotinic acetylcholine receptor partial agonist displays cognitive enhancing properties*. J Med Chem 2006, 49(14): 4374-83.

17. Briggs, C.A., Anderson, D.J., Brioni, J.D. et al. *Functional characterization of the novel neuronal nicotinic acetylcholine receptor ligand GTS-21 in vitro and in vivo.* Pharmacol Biochem Behav 1997, 57(1-2): 231-41.

18. Astles, P.C., Baker, S.R., Boot, J.R. et al. *Recent progress in the development of subtype selective nicotinic acetylcholine receptor ligands*. Curr Drug Targets CNS Neurol Disord 2002, 1(4): 337-48.

19. Kem, V.R., Mahnir, V.M., Prokai, L. et al. *Hydroxy metabolites of the Alzheimer's drug candidate 3-[(2,4-dimethoxy)benzylidene]-anabaseine dihydrochloride (GTS-21): Their molecular properties, interactions with brain nicotinic receptors and brain penetration*. Mol Pharmacol 2004, 65: 56-67.

20. Machu, T.K., Hamilton, M.E., Frye, T.F. et al. *Benzylidene analogs of anabaseine display partial agonist and antagonist properties at the mouse 5-hydroxytryptamine3A receptor*. J Pharmacol Exp Ther 2001, 299: 1112-9.

21. Phillips, E., Schmiesing, R. (AstraZeneca AB). *Novel biarylcarboxamides*. WO 0160821.

22. Tatsumi, R., Seio, K., Fujio, M. et al. *(+)-3-[2-(Benzo[b]thiophen-2-yl)-2-oxoethyl]-1-azabicyclo[2.2.2]octane as potent agonists for the alpha7 nicotinic acetylcholine receptor*. Bioorg Med Chem Lett 2004, 14(14): 3781-4.

23. Tatsumi, R., Fujio, M., Satoh, H. et al. *Discovery of the alpha7 nicotinic acetylcholine receptor agonists. (R)-3'-(5-Chlorothiophen-2-yl)spiro-1-azabicyclo[2.2.2]octane-3,5'-[1',3']oxazolidin-2'-one as a novel, potent, selective, and orally bioavailable ligand*. J Med Chem 2005, 48(7): 2678-86.

24. Wishka, D.G., Walker, D.P., Yates, K.M. et al. *Discovery of N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]furo[2,3-c]pyridine-5-carboxamide, an agonist of the alpha7 nicotinic acetylcholine receptor, for the potential treatment of cognitive deficits in schizophrenia: Synthesis and structure-activity relationship*. J Med Chem 2006, 49(14): 4425-36.

25. Jordan, K.J., Gatto, G.J., Hauser, T.A. et al. *Assessment of a full α7 nicotinic acetylcholine receptor agonist, TC-5619, in animal models of schizophrenia*. 35th Annu Meet Soc Neurosci (Nov 12-15, Washington, D.C.). 2005, Abst 952.10.

26. Feuerbach, D., Hurth, K., Ritchie, T.J. (Novartis AG; Novartis Pharma GmbH). *Aza-bicycloalkyl ethers and their use as alpha7-nAChR agonist*. WO 2004022556.

27. Feuerbach, D., Roy, B.L., Troxler, T.J., Hurth, K. (Novartis AG; Novartis Pharma GmbH). *3-(Heteroaryl-oxy)-2-alkyl-1-azabicycloalkyl derivatives as alpha.7-nAChR ligands for the treatment of CNS diseases*. WO 2006005608.

28. Kohlhaas, K.L., Rueter, L.E., Bitner, R.S. (Abbott Lab.). *Alpha7 neuronal nicotinic receptor ligand and antipsychotic compositions*. US 2006311686.

29. Empfield, J., Phillips, E., Throner, S. (AstraZeneca AB). *4-Substituted imidazoles.* WO 2005049612.

30. Empfield, J., Phillips, E., Throner, S. (AstraZeneca AB). *5-Substituted imidazoles.* WO 2005049611.

31. Ernst, G., Frietze, W., Jacobs, R., Phillips, E. (AstraZeneca AB). *Nicotinic acetylcholine receptor ligands*. WO 2005061510.

32. Benedetti, Y., Bohme, A., Genevois-Borella, A., Touyer, G., Zhang, J. (Aventis Pharma SA). *Triazine derivatives, their preparation and therapeutic application thereof*. WO 2006111662.

33. Herbert, B., Nguyen, T.M., Tehim, A., Hopper, A.T., Xie, W. (Memory Pharmaceuticals Corp.). *Anabaseine derivatives useful in the treatment of neurodegenerative diseases.* WO 2004019943.

34. Xie, W., Herbert, B., Schumacher, R., Nguyen, T.M., Ma, J., Gauss, C.M., Tehim, A. (Memory Pharmaceuticals Corp.). *Indazoles, benzothiazoles, benzoisothiazoles, benzisoxazoles, and preparation and uses thereof*. WO 2005092890.

35. Xie, W., Herbert, B., Nguyen, T.M., Gauss, C.M., Tehim, A. (Memory Pharmaceuticals Corp.). *Indazoles, benzothiazoles, and benzoisothiazoles, and preparation and uses thereof.* WO 2004029050.

36. Xie, W., Herbert, B., Schumacher, R.A., Ma, J., Nguyen, T.M., Gauss, C.M., Tehim, A. (Memory Pharmaceuticals Corp.).

1H-Indazoles, benzothiazoles, 1,2-benzoisoxazoles, 1,2-benzoisothiazoles, and chromones and preparation and uses thereof. WO 2005111038.

37. Luithle, J., Boess, F.-G., Erb, C. et al. (Bayer AG). *Azabicyclic N-biarylamides with affinity for the alpha-7 nicotinic acetylcholine receptor*. WO 03078431.

38. Flessner, T., Boess, F.-G., Erb, C. et al. (Bayer Healthcare AG). *Benzothiophene urea, benzofurane urea, and indole urea, and use of the same as alpha-7 AChR agonists*. WO 2004013136.

39. Zwart, R., De Filippi, G., Broad, L.M. et al. *5-Hydroxyindole potentiates human alpha 7 nicotinic receptor-mediated responses and enhances acetylcholine-induced glutamate release in cerebellar slices*. Neuropharmacology 2002, 43(3): 374-84.

40. Krause, R.M., Buisson, B., Bertrand, S. et al. *Ivermectin: A positive allosteric effector of the α7 neuronal nicotinic acetylcholine receptor*. Mol Pharmacol 1998, 53: 283-94.

41. Samochocki, M., Hoffle, A., Fehrenbacher, A. et al. *Galantamine is an allosterically potentiating ligand of neuronal nicotinic but not of muscarinic acetylcholine receptors*. J Pharmacol Exp Ther 2003, 305(3): 1024-36.

42. Broad, L.M., Zwart, R., Pearson, K.H. et al. *Identification and pharmacological profile of a new class of selective nicotinic acetylcholine receptor potentiators*. J Pharmacol Exp Ther 2006, 318(3): 1108-17.

43. Hurst, R.S., Hajos, M., Raggenbass, M. et al. *A novel positive allosteric modulator of the alpha7 neuronal nicotinic acetylcholine receptor: In vitro and in vivo characterization*. J Neurosci 2005, 25(17): 4396-405.

44. Sher, E., Zwart, R., De Filippi, G. et al. *Pharmacological and electrophysiological characterisation of a new class of selective alpha-7 nicotinic receptor potentiators*. 35th Annu Meet Soc Neurosci (Nov 12-15, Washington, D.C.) 2005, Abst 951.10.

45. Maggi, L., Sher, E., Cherubini, E. *Regulation of GABA release by nicotinic acetylcholine receptors in the neonatal rat hippocampus*. J Physiol 2001, 536: 89-100.

46. Fujii, S., Sumikawa, K. *Acute and chronic nicotine exposure reverse age-related declines in the induction of long-term potentiation in the rat hippocampus*. Brain Res 2001, 894(2): 347-53.

47. Matsuyama, S., Matsumoto, A., Enomoto, T. et al. *Activation of nicotinic acetylcholine receptors induces long-term potentiation in vivo in the intact mouse dentate gyrus*. Eur J Neurosci 2000, 12(10): 3741-7.

48. Fernandes, C., Hoyle, E., Dempster, E. et al. *Performance deficit of alpha7 nicotinic receptor knockout mice in a delayed matching-to-place task suggests a mild impairment of working/episodic-like memory*. Genes Brain Behav 2006, 5(6): 433-40.

49. Hoyle, E., Genn, R.F., Fernandes, C. et al. *Impaired performance of alpha7 nicotinic receptor knockout mice in the five-choice serial reaction time task*. Psychopharmacology (Berl) 2006, 189(2): 211-23.

50. Young, J.W., Crawford, N., Kelly, J.S. et al. *Impaired attention is central to the cognitive deficits observed in alpha 7 deficient mice*. Eur Neuropsychopharmacol 2007, 17(2): 145-55.

51. Young, J.W., Finlayson, K., Spratt, C. et al. *Nicotine improves sustained attention in mice: Evidence for involvement of the alpha7 nicotinic acetylcholine receptor.* Neuropsychopharmacology 2004, 29(5): 891-900.

52. Grottick, A.J., Higgins, G.A. *Effect of subtype selective nicotinic compounds on attention as assessed by the five-choice serial reaction time task*. Behav Brain Res 2000, 117(1-2): 197-208.

53. Nanri, M., Yamamoto, J., Miyake, H. et al. *Protective effect of GTS-21, a novel nicotinic receptor agonist, on delayed neuronal death induced by ischemia in gerbils.* Jpn J Pharmacol 1998, 76(1): 23-9.

54. Nanri, M., Miyake, H., Murakami, Y. et al. *GTS-21, a nicotinic agonist, attenuates multiple infarctions and cognitive deficit caused by permanent occlusion of bilateral common carotid arteries in rats.* Jpn J Pharmacol 1998, 78(4): 463-9.

55. Nanri, M., Kasahara, N., Yamamoto, J. et al. *GTS-21, a nicotinic agonist, protects against neocortical neuronal cell loss induced by the nucleus basalis magnocellularis lesion in rats*. Jpn J Pharmacol 1997, 74(3): 285-9.

56. Meyer, E.M., Tay, E.T., Papke, R.L. et al. *3-[2,4-Dimethoxybenzylidene]anabaseine (DMXB) selectively activates rat alpha7 receptors and improves memory-related behaviors in a mecamylamine-sensitive manner*. Brain Res 1997, 768(1-2): 49-56.

57. Kem, W.R. *The brain alpha7 nicotinic receptor may be an important therapeutic target for the treatment of Alzheimer's disease: Studies with DMXBA (GTS-21).* Behav Brain Res 2000, 113(1-2): 169-81.

58. Levin, E.D., Bettegowda, C., Blosser, J. et al. *AR-R17779, an alpha7 nicotinic agonist, improves learning and memory in rats*. Behav Pharmacol 1999, 10(6-7): 675-80.

59. Van Kampen, M., Selbach, K., Schneider, R. et al. *AR-R 17779 improves social recognition in rats by activation of nicotinic alpha7 receptors.* Psychopharmacology (Berl) 2004, 172(4): 375-83.

60. Boess, F.G., De Vry, J., Erb, C. et al. *The novel α7 nicotinic acetylcholine receptor agonist N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-7-[2-(methoxy)phenyl]-1-benzofuran-2-carboxamide (ABBF) improves working and recognition memory in rodents*. J Pharmacol Exp Ther 2007, Epub ahead of print.

61. O'Neill, M.J., Murray, T.K., Lakics, V. et al. *The role of neuronal nicotinic acetylcholine receptors in acute and chronic neurodegeneration.* Curr Drug Targets CNS Neurol Disord 2002, (4): 399-411.

62. Wang, H.Y., Lee, D.H., Davis, C.B. et al. *Amyloid peptide Abeta(1-42) binds selectively and with picomolar affinity to alpha7 nicotinic acetylcholine receptors*. J Neurochem 2000, 75(3): 1155-61.

63. Liu, Q., Kawai, H., Berg, D.K. *Beta-amyloid peptide blocks the response of alpha 7-containing nicotinic receptors on hippocampal neurons*. Proc Natl Acad Sci USA 2001, 98(8): 4734-9.

64. Dineley, K.T., Bell, K.A., Bui, D. et al. *Beta-amyloid peptide activates alpha 7 nicotinic acetylcholine receptors expressed in Xenopus oocytes*. J Biol Chem 2002, 277(28): 25056-61.

65. Dineley, K.T., Xia, X., Bui, D. et al. *Accelerated plaque accumulation, associative learning deficits, and up-regulation of alpha 7 nicotinic receptor protein in transgenic mice co-expressing mutant human presenilin 1 and amyloid precursor proteins*. J Biol Chem 2002, 277(25): 22768-80.

66. Kihara, T., Shimohama, S., Sawada, H. et al. *Alpha 7 nicotinic receptor transduces signals to phosphatidylinositol 3-kinase to block A beta-amyloid-induced neurotoxicity*. J Biol Chem 2001, 276(17): 13541-6.

67. Shaw, S., Bencherif, M., Marrero, M.B. *Janus kinase 2, an early target of alpha 7 nicotinic acetylcholine receptor-mediated neuroprotection against Abeta-(1-42) amyloid*. J Biol Chem 2002, 277(47): 44920-4.

68. Wang, H.Y., Li, W., Benedetti, N.J. et al. *Alpha 7 nicotinic acetylcholine receptors mediate beta-amyloid peptide-induced tau protein phosphorylation*. J Biol Chem 2003, 278(34): 31547-53.

69. Nordberg, A., Hellstrom-Lindahl, E., Lee, M. et al. *Chronic nicotine treatment reduces beta-amyloidosis in the brain of a mouse model of Alzheimer's disease (APPsw)*. J Neurochem 2002, 81(3): 655-8.

70. Chen, L., Yamada, K., Nabeshima, T. et al. *Alpha7 nicotinic acetylcholine receptor as a target to rescue deficit in hippocampal LTP induction in beta-amyloid infused rats*. Neuropharmacology 2006, 50(2): 254-68.

71. Simosky, J.K., Stevens, K.E., Kem, W.R. et al. *Intragastric DMXB-A, an alpha7 nicotinic agonist, improves deficient sensory inhibition in DBA/2 mice*. Biol Psychiatry 2001, 50(7): 493-500.

72. O'Neill, H.C., Rieger, K., Kem, W.R. et al. *DMXB, an alpha7 nicotinic agonist, normalizes auditory gating in isolation-reared rats*. Psychopharmacology (Berl) 2003, 169(3-4): 332-9.

73. Leonard, S., Adler, L.E., Benhammou, K. et al. *Smoking and mental illness*. Pharmacol Biochem Behav 2001, 70(4): 561-70.

74. Martin, L.F., Kem, W.R., Freedman, R. *Alpha-7 nicotinic receptor agonists: Potential new candidates for the treatment of schizophrenia*. Psychopharmacology (Berl) 2004, 174(1): 54-64.

75. Olincy, A., Harris, J.G., Johnson, L.L. et al. *Proof-of-concept trial of an alpha7 nicotinic agonist in schizophrenia*. Arch Gen Psychiatry 2006, 63(6): 630-8.

76. Pichat, P., Bergis, O.E., Terranova, J.P. et al. *SSR180711, a novel selective alpha7 nicotinic receptor partial agonist: (II) Efficacy in experimental models predictive of activity against cognitive symptoms of schizophrenia.* Neuropsychopharmacology 2007, 32: 17-34.

77. Schreiber, R., Dalmus, M., De Vry, J. *Effects of alpha 4/beta 2- and alpha 7-nicotine acetylcholine receptor agonists on prepulse inhibition of the acoustic startle response in rats and mice*. Psychopharmacology (Berl) 2002, 159(3): 248-57.

78. Suemaru, K., Yasuda, K., Umeda, K. et al. *Nicotine blocks apomorphine-induced disruption of prepulse inhibition of the acoustic startle in rats: Possible involvement of central nicotinic alpha7 receptors*. Br J Pharmacol 2004, 142(5): 843-50.

79. Duncan, E., Madonick, S., Chakravorty, S. et al. *Effects of smoking on acoustic startle and prepulse inhibition in humans*. Psychopharmacology (Berl) 2001, 156(2-3): 266-72.

80. Ripoll, N., Bronnec, M., Bourin, M. *Nicotinic receptors and schizophrenia*. Curr Med Res Opin 2004, 20(7): 1057-74.