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ASUNTO	Radicación	10-131169
	Trámite	011
	Evento	378
	Actuación	519
	Oficio	- - - 186 34
	Folios	019

Patente de Invención		Patente de Modelo de Utilidad	☐
Vía Nacional		Cap. I	
Vía PCT (fase nacional)		Cap. II	☐

No. Solicitud Internacional PCT/US2009/041076
Fecha de Presentación Internacional 20 de abril de 2009.

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 178 a 373	22/octubre/2010
Reivindicaciones:	Folios 373 a 378	22/octubre/2010
	Folios 406 a 416	13/diciembre/2010
Dibujos:	Folios 422 a 432	13/diciembre/2010
Prioridades:	US 61047211	23/abril/2008
	US 12432299	14/abril/2009

2. Análisis de la Prioridad

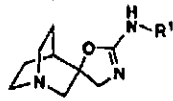
Se acepta la reivindicación de prioridades porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

Título de la solicitud: "Compuesto de quinuclidina como ligandos del receptor nicotínico de acetilcolina alfa-7"

El problema planteado en esta solicitud consiste en suministrar ligandos del receptor nicotínico α -7 útiles para el tratamiento de diversas transtornos del sistema nervioso central, en especial transtornos afectivos y neurodegenerativos.

Para resolver este problema técnico la solicitud en estudio proporciona un compuesto de la fórmula (I) y composiciones que lo contienen



Compuesto I

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07D 498/20; A61K 31/439 ; A61P 25/28.

4. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad más antigua: abril 23 de 2008 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

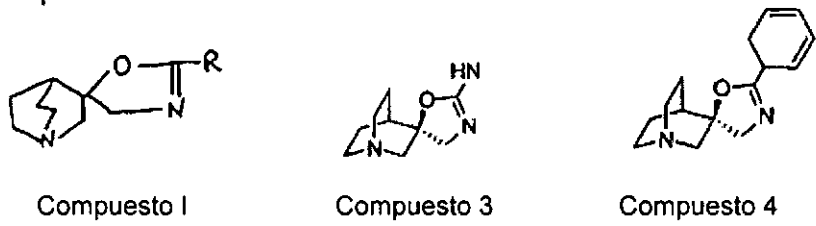
Ítem	Documento	Título	Fecha de Publicación*
D1	ES 2137926	Compuestos espiro heterocíclicos puenteados y no puenteados	16/octubre/1991
D2	Curr. Med. Chem. – Central Nervous System Agents, 2005, 5, 171-184	α7 Nicotinic Receptor Agonists as Potential Therapeutic Drugs for Schizophrenia	2005

D1: http://www.espatentes.com/pdf/2137926_t3.pdf divulga (Pág. 2, Ren. 5-10) Un grupo de compuestos 4- y 5-espiro(1,3-oxazolinas y 1,3-tiazolinas) en las que el anillo que está espiro-conectado a la oxazolina o tiazolina es un anillo saturado heterocíclico con o sin puentes, que contiene un átomo de nitrógeno, refiriéndose además a las composiciones farmacéuticas que contienen los espiro compuestos. Estos compuestos son potencialmente útiles para el tratamiento de enfermedades del sistema nervioso central y periférico.

De igual modo, refiere que los compuestos (Pág. 11, Ren. 55-60) son en general potencialmente útiles para el tratamiento de demencia presenil y senil, entre otras caracterizados porque la enfermedad son alteraciones en las que una hipofunción colinérgica central ha sido implicada como mínimo de cierta manera.

En otro aparte, indica (Pág. 12, Ren. 5-30) que los espiro-compuestos de la invención pueden ser utilizados en combinación con inhibidores de la acetilcolinesterasa tales como fisostigmina o tetrahidroaminoacridina; en combinación con precursores de acetilcolina tales como colina o lecitina; en adición a medicamentos "nootrópicos" tales como piracetam, aniracetam, oxiracetam o pramiracetam, entre otros principios activos.

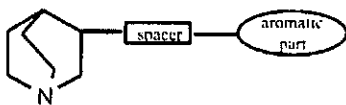
Adicionalmente, a través de las realización preferidas del compuesto I (Pág. 2, Ren. 35-45) da a conocer los ejemplos 3 y 4 correspondientes a los compuestos 2-Amino-espiro(1,3-oxazolin-5,3') quinuclidina y 2-Fenil-espiro(1,3-oxazolin-5,3')quinuclidina, los cuales se representan a continuación.



Finalmente, refiere para el radical R un grupo extenso de sustituyentes dentro del cual indica (Pág. 42, Ren. 27-45) la selección de R entre NH₂, NH-C₁₋₆-alquilo y N(C₁₋₆alquilo)₂ cuando R forma parte de la fórmula I.

D2: divulga el compuesto AR-R17779 (Pág.175, Col .1) correspondiente al (-) espiro[1-azabicyclo[2.2.2]octano-3,5'-oxazolidin-2'-ona], el cual es un potente agonista α7 nAChRs el cual es altamente selectivo para el subtipo α7 sobre α4β2. Indicando

que (Pág.175, Col .2) agonistas $\alpha 7$ nAChRs tiene un potencial terapéutico para alteraciones cognitivas in enfermedades neuropsiquiátricas incluyendo la esquizofrenia. A continuación refiere la hipótesis que los derivados 1-azabicyclo[2.2.2]octano, que tienen una parte aromática y un grupo espaciador en la posición 3, puede tener actividad agonista en $\alpha 7$ nAChR como se representa a continuación, como estrategia de síntesis para agonista $\alpha 7$ nAChR.

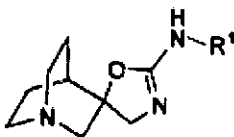


Indicando que el enfoque sintético de la relación estructura actividad de los derivados de 3-sustituido 1-azabicyclo[2.2.2]octano, muestra que la introducción de un grupo estructural α o β -naftil como parte aromática mejora la afinidad $\alpha 7$ nAChRs. Más sin embargo, el reemplazo de la parte naftil por un grupo benzo [b]tiofeno-2-il incrementa esta afinidad por $\alpha 7$ nAChRs.

5. Examen de Patentabilidad (Art 14 D486)

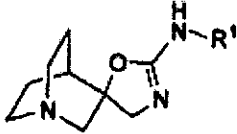
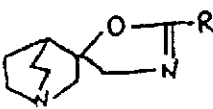
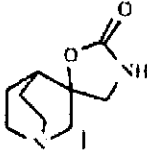
Nivel Inventivo (Art18 D486)

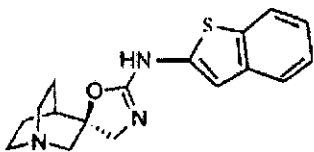
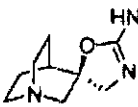
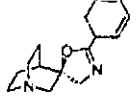
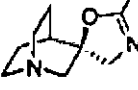
La reivindicación 1 consiste en compuesto de fórmula i, caracterizados por ser derivados de la 2-aminosustituidos-espiro(1,3-oxazolin-5,3')quinuclidina



Compuesto I

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Solicitud	D1	D2
Un compuesto de fórmula I  Compuesto I	El compuesto 	Divulga que 1.) el compuesto AR-  R17779
R ¹	R ¹	
se selecciona del grupo constituido por isoxazolilo, pirazolilo; oxazolilo, tiazolilo, imidazolilo, oxadiazolilo, tiadiazolilo, triazolilo, piridinilo, pirazinilo, piridazinilo, pirimidinilo, triazinilo, quinolinilo, isoquinolinilo, quinoxalinilo, quinazolinilo, naftiridinilo, indazolilo, indolilo, 2-indolonilo, bencisoxazolilo, benzoisotiazolilo, benzoxazolilo, benzotiazolilo, bencimidazolilo, furopiridinilo, tienopiridinilo, tienopirimidinilo, isotiazolopiridinilo, tiazolopiridinilo, tiazolopiridinonilo, tiazolopirazinilo, tiazolopirimidinilo, triazolopiridinilo, triazolopirazinilo, pirrolotriazinilo, 5,6-dihidrobenzo[h]quinazolinilo, 5H-cromeno[4,3-d]pirimidinilo, 6,7-dihidro-5H-clclopenta[d]pirimidinilo, 5,6,7,8-tetrahidroquinazolinilo, 7,8-dih;droquinazolin-5(6H)-onilo y tetrahidrobenzotiazolilo sustituidos	amino-C ₁₋₆ -alquilo, mono-(C ₁₋₆ -alquilo)amino-C ₁₋₆ -alquilo, di-(C ₁₋₆ -alquilo)amino-C ₁₋₆ -alquilo	correspondiente al (-) espiro[1] azabicyclo[2.2.2]octano-3,5'-oxazolidin-2'-sustituido], el cual es un potente agonista $\alpha 7$ nAChRs el cual es altamente selectivo para el subtipo $\alpha 7$ sobre $\alpha 4\beta 2$. 2.) da a conocer la hipótesis que los derivados 1-azabicyclo[2.2.2]octano, que tienen una parte aromática y un grupo espaciador en la posición 3, puede tener actividad agonista en $\alpha 7$ nAChR como se representa a continuación, como estrategia de síntesis para agonista $\alpha 7$ nAChR. 3.) y que derivados de 3-sustituido 1-azabicyclo[2.2.2]octano, muestran que la introducción de un grupo estructural α o β -naftil como parte aromática mejora la afinidad $\alpha 7$ nAChRs. Sin embargo, el reemplazo de la parte naftil por un grupo benzo [b]tiofeno-2-il incrementa esta afinidad por $\alpha 7$ nAChRs.

Realización Preferidas	Realización Preferidas	
 Ejemplo 1	 Compuesto 3	
	 Compuesto 4	
	 Compuesto 1	

El documento D1 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 1 porque divulga una serie de compuesto derivados de la 2-amino sustituidos-espiro(1,3-oxazolin-5,3')quinuclidina útiles como agonista colinérgico y para el tratamiento de enfermedades del sistema nervioso central y periférico.

La diferencia entre la invención, definida en la reivindicación 1 y el compuesto 3 del documento D1 es que la invención define que en la posición 2 de anillo espiro(1,3-oxazolin-5,3')quinuclidina un sustituyentes amino sustituido por un radical heteroarilo mono o biciclo y el documento D1 refiere como posibles sustituyentes del radical amino cadena alquílicas de 1 a 6 átomos.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo modificar el sustituyente en la posición 2 del anillo espiro(1,3-oxazolin-5,3')quinuclidina de los compuestos del documento D1 para así obtener compuestos ligandos del receptor nicotínico α -7 útiles para el tratamiento de diversas transtornos del sistema nervioso central, en especial transtornos afectivos y neurodegenerativos.

El efecto que logra al “modificar el sustituyente en la posición 2 del anillo espiro(1,3-oxazolin-5,3')quinuclidina de los compuestos del documento D1 es obtener compuestos alternativos ligandos del receptor nicotínico α -7 útiles”.

Sin embargo, dado que el documento D1 refiere (Pág. 22, Ren 49-54) que el compuesto de la invención (el amino análogo de AF125) fue también activo como agonista colinérgico y que el documento D2 refiere que los derivados 1-azabicyclo[2.2.2]octano, que tienen una parte aromática y un grupo espaciador en la posición 3, puede tener actividad agonista en α 7 nAChR y que los derivados de 3-sustituido 1-azabicyclo[2.2.2]octano, muestran que la introducción de un grupo estructural α o β -naftil como parte aromática mejora la afinidad α 7 nAChRs y que sin embargo, el reemplazo de la parte naftil por un grupo benzo [b]tiofeno-2-il incrementa esta afinidad por α 7 nAChRs.

En consecuencia, una persona del oficio normalmente versada en la materia, estaría motivada o tendría suficientemente expectativa de éxito modificar el sustituyente en la posición 2 del anillo espiro(1,3-oxazolin-5,3')quinuclidina de los compuestos del documento D1 para obtener nuevos agonista en α 7 nAChR alternativos empleando el grupo 2-amino-oxazolin-5,3' como grupo espaciador e introduciendo un anillo arilo o heteroarilo para así obtener agonista en α 7 nAChR como lo sugiere el documento D2, Para así llegar al objeto de la reivindicación 1. Por lo cual la reivindicación 1 para el primer grupo inventivo se considera obvia.

Puesto que las reivindicaciones dependientes 2-12 no mencionan características inventivas adicionales para el primer grupo inventivo tampoco se pueden considerar inventivas

6. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	ES 2137926	1-12	Nivel Inventivo
D2	Curr. Med. Chem. – Central Nervous System Agents. 2005, 5, 171-184	1-12	Nivel Inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante (60 días) y presentarla por escrito.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha 23 OCT. 2012

Elaborado por: Lenin Roberto Guerrero. 
Revisado Por: Consuelo Leguizamón Leguizamón
Aprobado por: José Luis Salazar López.

$\alpha 7$ Nicotinic Receptor Agonists as Potential Therapeutic Drugs for Schizophrenia

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Abstract: Deficient inhibitory processing of the P50 auditory evoked potential is a measurable marker observed in schizophrenia. Several lines of evidence suggest that $\alpha 7$ nicotinic receptors ($\alpha 7$ nAChRs) play a critical role in P50 auditory sensory gating in the human brain. Similar to schizophrenic patients, DBA/2 mice spontaneously exhibit a deficit in inhibitory processing of the P20-N40 auditory evoked potential, which is a rodent analogue of the human P50 auditory evoked potential. Agonists at $\alpha 7$ nAChRs improve deficient inhibitory processing of the P20-N40 auditory gating potential in DBA/2 mice. In this article, we review the role of $\alpha 7$ nAChRs in the pathophysiology of schizophrenia, and $\alpha 7$ nAChR agonists and indirect agonists (5-hydroxytryptamine-3 (5-HT₃) receptor antagonists, positive allosteric modulators (galantamine, 5-hydroxyindole, PNU-120596), FK960, FR236924) at $\alpha 7$ nAChRs as potential therapeutic drugs for the treatment of schizophrenia. In addition, we also discuss the role of kynurenic acid as an endogenous antagonist of $\alpha 7$ nAChRs in brain.

Keywords: $\alpha 7$ nicotinic receptors, auditory gating, sensory gating, cognition, schizophrenia.

SCHIZOPHRENIA

Schizophrenia is a chronic psychotic mental disorder that affects about one percent of the world's general population. This illness has varied and ominous symptoms that generally begin in late adolescence or early adulthood and usually continue throughout life. Schizophrenia is characterized by three broad types of symptoms: positive symptoms (e.g., hallucinations, delusion, or bizarre behaviors), negative symptoms (e.g., blunted affect, anhedonia, avolition or apathy, and alogia), and cognitive impairment (e.g., problems in attention and concentration, psychomotor speed, learning and memory, and executive function) [1,2]. Positive and negative symptoms vary in intensity over time; patients may have predominantly one type at any particular time. In contrast, cognitive impairment is a core feature of the illness, and predicts vocational and social disabilities for patients [1,2].

The cause of schizophrenia is unknown, but several lines of evidence suggest that genetic factors and environmental factors (e.g., prenatal and perinatal events - including maternal influenza, rubella, malnutrition, diabetes mellitus, and smoking during pregnancy - and obstetric complications) contribute to the pathophysiology of this illness [1-9]. Furthermore, schizophrenia may be a neurodevelopmental and progressive disorder with multiple biochemical abnormalities involving the dopamine, serotonin, acetylcholine (ACh), glutamate, and γ -aminobutyric acid (GABA) systems [1,2,10-23].

SCHIZOPHRENIA AND SMOKING

In several countries, the prevalence of cigarette smoking among schizophrenic patients is extraordinarily high (70% or greater) [24-29]. While the prevalence of smoking is declining in the general population, it is not declining in the patients with psychiatric disorders, particularly in schizophrenia. It has also been reported that the percentage of schizophrenic patients who quit smoking is much lower when compared with the normal US population [25,30]. The reason underlying the high frequency of smoking in schizophrenic patients is currently unclear. It has also been suggested that smoking may be a form of self-treatment for patients with schizophrenia, since nicotine has been shown to reduce negative symptoms [24-26,28]. Interestingly, it has also been reported that schizophrenic patients are usually smoking before diagnosis [31], suggesting that impaired neurotransmission by nicotine might be involved in the pathophysiology of schizophrenia.

$\alpha 7$ NICOTINIC RECEPTORS

(S)-(-)-Nicotine (Fig. 1, hereafter simply nicotine), the main addictive component of tobacco, activates and desensitizes nicotinic ACh receptors (nAChRs). In the central nervous system (CNS), nAChRs normally respond to ACh (Fig. 1) and modulate neuronal excitability and synaptic communication [32-35]. The health consequences of smoking and the mechanisms involved with nicotine dependence continue to be subjects of great interest [36-40]. In addition, recent attention has been directed to the potential role of nAChRs in diseases and therapeutic targets [13,14,41-51].

The nAChRs are ligand-gated ion channels that are distributed all over the human CNS and that each consists of

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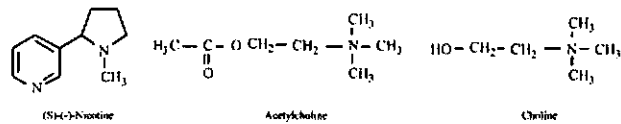


Fig. (1). Chemical structures of (S)-(-)-nicotine, acetylcholine (ACh) and choline.

five subunits (a combination of α and β subunits). At present, nine α ($\alpha 2$ - $\alpha 10$) and three β ($\beta 2$ - $\beta 4$) subunits have been identified and cloned in humans. The numerous combinations of α and β subunits or of α subunits alone can generate many subtypes of nAChRs with different physiologies, pharmacologies and anatomical distributions [35]. Two major subtypes exist in the brain, those comprised of $\alpha 4\beta 2$ and those comprised of $\alpha 7$ subunits (Fig. 2). The former contribute >90% of the high affinity binding sites for nicotine in the rat brain [52]. The low affinity binding sites ($\alpha 7$ subunits) for nicotine are recognized by their nanomolar affinity for α -bungarotoxin [53]. The $\alpha 7$ nAChRs have an unusually high permeability to Ca^{2+} compared to other subtypes and exhibit exceptionally rapid desensitization following exposure to agonists [39,54-58]. Choline (Fig. 1) is an essential physiological component of the cerebrospinal fluid (CSF) and is important for the structural integrity of cell membranes, ACh synthesis, and lipid and cholesterol transport and metabolism. Several lines of evidence suggest that choline is a full agonist of $\alpha 7$ nAChRs, but not other nAChR subtypes [59-62].

The $\alpha 7$ nAChRs are assumed to comprise five $\alpha 7$ subunits and differ from other subtypes of nAChRs. In particular, the $\alpha 7$ nAChRs may play a distinct role in regulating neuronal plasticity. By elevating intracellular Ca^{2+} levels in discrete neuronal locations, these ligand-gated ion channels may influence numerous physiological processes in the developing and adult CNS [35,54,55,63]. Several lines of evidence suggest that both pre- and postsynaptic $\alpha 7$ nAChRs

modulate transmitter release in the brain through Ca^{2+} -dependent mechanisms, and that the $\alpha 7$ nAChRs play a role in regulating neuronal growth and differentiation in the developing CNS [35,54,63,64]. Furthermore, it has been proposed that intracellular Ca^{2+} may be coregulated by N-methyl-D-aspartate (NMDA) receptors and $\alpha 7$ nAChRs in the brain [54]. Together with NMDA receptors, postsynaptic $\alpha 7$ nAChRs may serve to regulate intracellular Ca^{2+} levels in neurons, whereas presynaptic $\alpha 7$ nAChRs could serve as a feedback mechanism for modulating glutamatergic transmission (Fig. 3). Thus, it is possible that a close interaction between cholinergic and glutamatergic pathways, mediated by $\alpha 7$ nAChRs and NMDA receptors, may play a role in the pathophysiology of schizophrenia.

SCHIZOPHRENIA AND $\alpha 7$ nAChRs

The binding sites ($\alpha 7$ subtype) labeled by [^{125} I] α -bungarotoxin are different from the high affinity binding sites ($\alpha 4\beta 2$ subtype) for nicotine [65]. In the hippocampus, [^{125} I] α -bungarotoxin binds most intensely to inhibitory interneurons in the CA3 region of Ammon's horn and in the hilus of the dentate gyrus [66]. These authors subsequently suggested that inhibitory interneurons with $\alpha 7$ nAChRs are possible candidates for medication of the habituation of auditory responses in the hippocampus because activation of the interneurons via $\alpha 7$ nAChRs would increase the inhibitory synaptic input to pyramidal neurons and thereby diminish the responsiveness of these pyramidal neurons to sensory stimulation [67]. This parallels studies of postmortem human

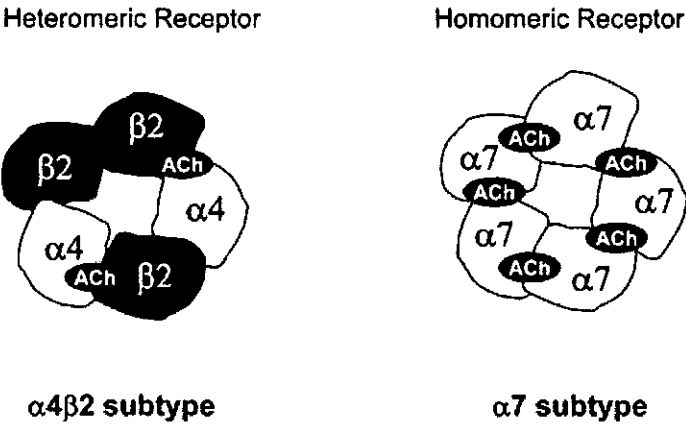


Fig. (2). Receptor assembly of $\alpha 4\beta 2$ subunits and $\alpha 7$ subunits of nicotinic receptors in brain.

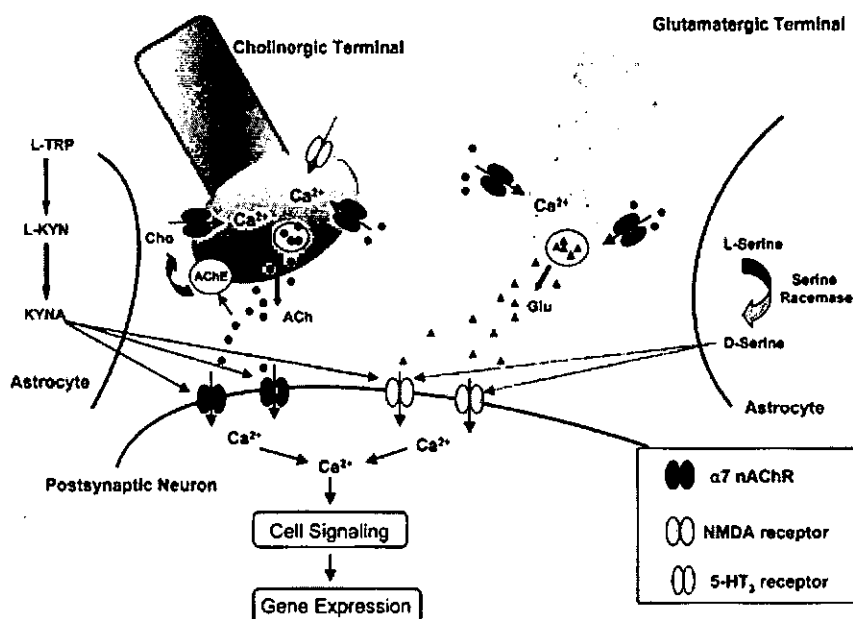


Fig. (3). Role of $\alpha 7$ nicotinic receptors ($\alpha 7$ nAChRs) and NMDA receptors in the neuron-glia communication in the brain. Acetylcholine (ACh) released from the nerve terminal of cholinergic neurons binds to $\alpha 7$ nAChRs on the postsynaptic neurons. By stimulation at $\alpha 7$ nAChRs on the presynaptic terminals, glutamate released from the nerve terminals of glutamate neurons binds to NMDA receptors on the postsynaptic neurons. Thus, the increase in intracellular Ca^{2+} that arises from activation of $\alpha 7$ nAChRs and NMDA receptors lead to cell signaling and gene expression. Kynurenic acid synthesized from L-tryptophan in astrocytes, and, as a non-competitive antagonist, kynurenic acid binds to $\alpha 7$ nAChRs and glycine sites on NMDA receptors. D-Serine is synthesized from L-serine via serine racemase in astrocytes. Glutamate binds to AMPA receptors on astrocyte that stimulate the release of D-serine. Released D-serine binds to glycine site on NMDA receptors. Thus, $\alpha 7$ nAChRs and NMDA receptors can exert a wide range of influences through Ca^{2+} signals, from changes in synaptic plasticity in the brain.

tissue that documented a decreased expression of hippocampal $\alpha 7$ nAChRs in schizophrenic patients [68]. Thus, it seems that schizophrenic patients have fewer $\alpha 7$ nAChRs in the hippocampus, a condition which may lead to failure of cholinergic activation of inhibitory interneurons, manifest clinically as decreased gating of the response to sensory stimulation [67,69,70].

It has been shown that [^{125} I] α -bungarotoxin binding is reduced in the thalamic reticular nucleus of schizophrenic subjects [71], and that $\alpha 7$ nAChR protein levels are reduced in the frontal cortex in patients with schizophrenia [72]. Furthermore, Marulle *et al.* [73] observed a reduction of $\alpha 7$ nAChRs but an increase of [^{3} H]cytisine binding to $\alpha 4\beta 2$ nAChRs in the cingulate cortex of schizophrenic patients.

In regard to the search for peripheral biological markers for schizophrenia, Perl *et al.* [74] investigated the mRNA levels of $\alpha 7$ nAChRs in peripheral blood lymphocytes of schizophrenic patients and healthy controls. They found a significant reduction of $\alpha 7$ nAChR mRNA levels on lymphocytes of schizophrenic patients [74]. This reduction was

not a result of medication, because the non-medicated patients displayed the same levels of reduction in $\alpha 7$ nAChR mRNA. In addition, the possibility that the observed decrease in $\alpha 7$ nAChR mRNA levels resulted from nicotine consumption in smoking was excluded, because healthy smokers exhibited the same levels of $\alpha 7$ nAChR mRNA as non-smokers [74]. These findings suggest that mRNA levels of $\alpha 7$ nAChRs in peripheral blood lymphocytes may serve a reliable peripheral biological marker for schizophrenia.

SENSORY GATING DEFICITS IN SCHIZOPHRENIA AND $\alpha 7$ nAChRs

Deficits of sensory gating in schizophrenia derive from the clinical observation that patients report failures of information processing characterized by poor sensory gating [67,75-77]. The underlying problem is evident in the inability of people with schizophrenia to adequately filter their response to incoming sensory stimulation, as measured by their inhibitory processing of the P50 auditory evoked potential. The P50 auditory evoked potential is a positive electroen-

cephalographic waveform that occurs 50 msec after presentation of an auditory stimulus. When pairs of auditory stimuli are presented, with a 500 msec interstimulus interval, schizophrenic patients fail to adequately inhibit the P50 response to the second stimulus. Normal subjects, however, have significantly reduced responses to the second stimulus [67,75,78,77]. The reduced response to the second stimulus reflects inhibitory processing of the information that may function to protect the individual from being overwhelmed by incoming, repetitive sensory information. It is known that nicotine transiently normalizes the P50 auditory evoked-potential deficits in schizophrenic patients [79,80].

Genetic linkage analysis of the P50 auditory-evoked potential deficit in families of patients with schizophrenia has revealed a peak LOD score at 15q13-q14, and the LOD score was 5.3 (theta=0.00) at the D15S1360 marker, which is located in intron 2 of the gene for the $\alpha 7$ nAChR subunit (CHRNA7) [81]. The CHRNA7 gene is located on chromosome 15q13-q14, a region linked with schizophrenia in several earlier studies [82,83]. The CHRNA7 has a partial duplication of exons 5-10, including the intervening introns (CHRFAM7) that map approximately 0.5 Mb proximal to the full-length CHRNA7 gene [84]. The D15S1360 microsatellite repeats, in intron 2 of the CHRNA7 gene, cosegregate with an auditory gating deficit in family linkage studies of schizophrenic patients [81]. Furthermore, in a mutation screening of the CHRNA7 gene from schizophrenics and controls, Leonard *et al.* [85] identified the promoter polymorphisms that decreased the subunit transcription and P50 inhibition in schizophrenia. Moreover, an association has been demonstrated between the homozygous 113 bp allele on

D15S1360 polymorphism of the CHRNA7 gene and smoking risk in schizophrenia [86]. Taken together, these results suggest that the CHRNA7 gene is likely susceptible to the deficits of sensory gating P50 in schizophrenia [12,13,29,67,69,70,76,81,85].

$\alpha 7$ NICOTINIC RECEPTOR AGONISTS

While there are a number of nicotinic receptor agonists known to be selective for the $\alpha 4\beta 2$ subtype, there are some agonists which bind the $\alpha 7$ nAChRs selectively over other subtypes [42,47,49]. Anabascine (2-(3-pyridyl)-3,4,5,6-tetrahydropyridine) (Fig. 4), a naturally occurring substance in nemertines, is an agonist at the neuromuscular junction [87] and is structurally related to nicotine. The more well known compound anabasine (neonicotinic; 3-(2-piperidinyl)pyridine) (Fig. 4) is a weak nicotinic alkaloid found in tobacco that lacks the imine double bond present in anabascine. Three analogues of anabasine, 3-(2,4)-dimethoxybenzylidene anabaseine (DMXB-A; also known as GTS-21), 3-(4)-dimethylaminobenzylidene anabaseine (DMAB), and 3-(4)-dimethylaminocinnamylidene (DMAC), have been reported to be functionally selective for the $\alpha 7$ nAChRs [88] (Fig. 4). Compared with anabaseine and the other derivatives, DMAC was the most potent at displacing [¹²⁵I] α -bungarotoxin binding (putative $\alpha 7$ subtype) and the least potent at displacing [³H]cytisine binding (putative $\alpha 4\beta 2$ subtype) to brain membranes. These anabaseine derivatives were partial agonists at $\alpha 7$ nAChRs [88]. Furthermore, DMXB-A bound to human $\alpha 4\beta 2$ nAChRs ($K_i=20$ nmol/L) 100-fold more potently than to human $\alpha 7$ nAChRs, and was 18- and 2-fold less potent than (-)-nicotine at human $\alpha 4\beta 2$

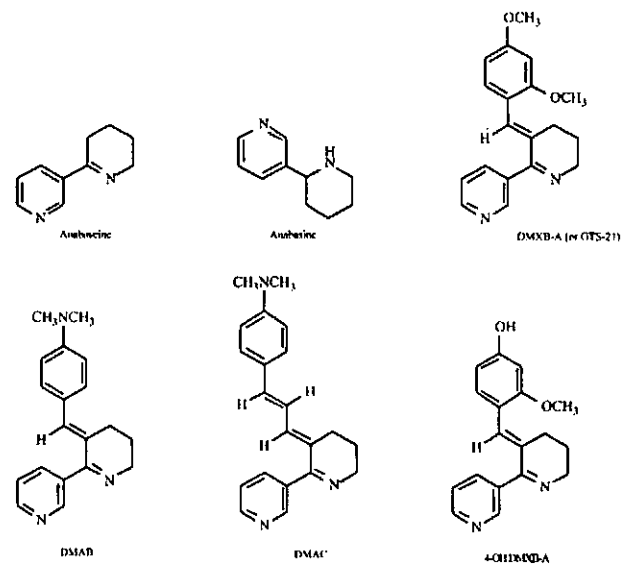


Fig. (4). Chemical structures of anabascine, anabaseine, DMXB-A, DMAB, DMAC and OH-DMXB-A.

and $\alpha 7$ nAChRs, respectively [89]. The primary human metabolite, 3-(4-hydroxy-2-methoxybenzylidene) anabaseine (4-OH DMXB-A; Fig. 4), of DMXB-A exhibited a similar level of efficacy for human $\alpha 7$ nAChRs [90,91]. Initial (phase I) clinical studies on DMXB-A have been reported [92]. DMXB-A was administered to 87 healthy volunteers. Initially, the effects of single doses (range, 1-250 mg) were assessed. The elimination half-life ranged between 0.5 and 1.0 h for DMXB-A and its major phase I metabolite, 4-OH DMXB-A. No serious adverse effects were reported with these doses. At twice daily doses of 75 and 150 mg for 5 days, DMXB-A improved the cognitive function of young adult volunteers. Furthermore, DMXB-A improved long-term memory as well as working memory and attention, as measured by the Cognitive Drug Research test battery [92]. Therefore, it is of great interest to examine the effects of DMXB-A in schizophrenic patients, who have been shown suggested to have low levels of deficits in the functioning of $\alpha 7$ nAChRs [68].

Researchers at Astra Zeneca reported the profile of AR-R17779, (-)-spiro[1-azabicyclo[2.2.2]octane-3,5'-oxazolidin-2'-one] (Fig. 5), a potent full agonist of the $\alpha 7$ nAChRs that is highly selective for the $\alpha 7$ subtype over the $\alpha 4\beta 2$ subtypes [93]. AR-R17779 was prepared as shown in Fig. 5. Reaction of methyl 3-hydroxy-1-azabicyclo[2.2.2]octane-3-acetate with hydrazine followed by Curtius rearrangement led to the desired carbamate. Resolution of the carbamate was accomplished via the dibenzoyl-D (or L)-tartarate salt. AR-R17779 has been widely used as a selective full agonist at the $\alpha 7$ nAChRs. For example, AR-R17779 failed to stimulate locomotor activity in both nicotine-nontolerant and -sensitized rats, whereas nicotine and the putative agonist SIB1765F, [$\pm$]-5-ethynyl-3-(1-methyl-2-pyrrolidinyl) pyridine fumarate (Fig. 6) [92], at $\alpha 4\beta 2$ nAChRs increased the activity under both experimental conditions, suggesting a negligible role of $\alpha 7$ nAChRs in nicotine-induced hyperlocomotion and reward in the rat [95]. Furthermore, it has been reported that chronic administration of both nicotine and SIB1765F, but not AR-R17779, resulted in an enhanced locomotor response to acute challenge with either nicotine or SIB1765F but not AR-R17779, suggesting that the $\alpha 4\beta 2$ subtype plays a role in both the initiation and expression of sensitization to the psychomotor stimulant effects of nicotine [96]. Moreover, ad-

ministration of AR-R17779 improved learning in two radial-arm maze tasks and reversed working memory impairment caused by fimbria-fornix section [97]. These findings suggest that $\alpha 7$ nAChRs play a role in learning and memory, and that agonists at $\alpha 7$ nAChRs might have therapeutic potential for cognitive impairments in neuropsychiatric diseases, including schizophrenia.

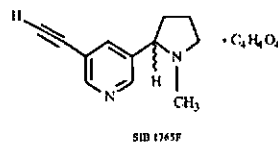


Fig. (6). Chemical structure of SIB1765F.

Macor and Wu reported some derivatives of 1-azabicyclo[2.2.2]oct-3-yl phenylcarbamate as agonists at $\alpha 7$ nAChRs [98] (Fig. 7). In order to develop novel agonists at $\alpha 7$ nAChRs, we hypothesized that 1-azabicyclo[2.2.2]octane

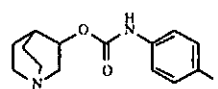


Fig. (7). 1-Azabicyclo[2.2.2]oct-3-yl phenylcarbamate derivatives.

derivatives, bearing an aromatic part and a spacer group at the 3-position, may have agonist activity at $\alpha 7$ nAChRs [99] (Fig. 8). We reported a synthetic approach and the structure activity relationship of 3-substituted 1-azabicyclo [2.2.2]

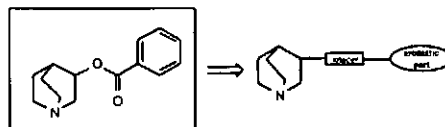
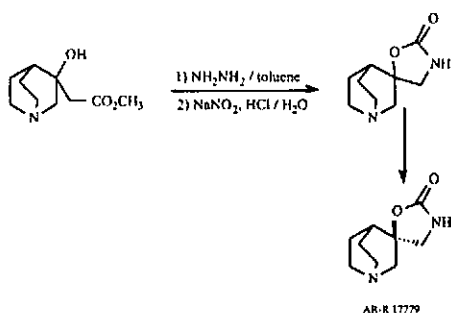
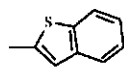
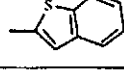
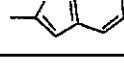
Fig. (8). Synthetic strategy for $\alpha 7$ nAChR agonists.

Fig. (5). Synthesis of AR-R17779.

octane derivatives. As shown in Table 1, the introduction of an α or β -naphthyl moiety as the aromatic part dramatically enhanced the affinity at $\alpha 7$ nAChRs (Table 1). Moreover, the replacement of the naphthyl part with the benzo[b]thiophen-2-yl increased the affinity for $\alpha 7$ nAChRs (Table 1). The (S)-stereoisomer of the benzo[b]thiophen derivative was more potent than that of the (R)-isomer; however, the compound showed poor bioavailability [99]. Table 2 shows the binding data of benzo[b]thiophen-2-yl derivatives. Alkylene analogues were more potent than their corresponding ketone analogues [99]. Among these derivatives, (+)-3-[2-(benzo[b]thiophen-2-yl)-2-oxoethyl]-1-azabicyclo[2.2.2]octane is a potent and partial agonist of $\alpha 7$ nAChRs [99] (Fig. 9). Furthermore, we reported the structure-activity relationships and pharmacokinetic profiles of the series of compounds leading

Table 1. Binding Affinities of 1-Azabicyclo[2.2.2]octane Derivatives



Spacer	Aromatic part	$\alpha 7$ affinity (IC ₅₀ : μ mol/L)
—OCO—	Ph	2.6
—OCH ₂ —	Ph	3.7
—OCH ₂ —	2-Naphthyl	0.15
—OCH ₂ —	1-Naphthyl	0.73
—OCH ₂ —		0.059
—OCH ₂ —		0.515
—OCH ₂ —		0.026

to the discovery of (R)-3'-(5-chlorothiophen-2-yl) spiro-1-azabicyclo[2.2.2]octane-3,5'-[1',3']oxazolidin-2'-one [100] (Fig. 10) This compound has potent binding affinity (K_i = 9 nmol/L for $\alpha 7$ nAChRs) and good selectivity toward the other nicotinic subtypes ($\alpha 4\beta 2$ and $\alpha 1\beta 2\gamma 6$). Also, this compound has good oral bioavailability and brain permeability.

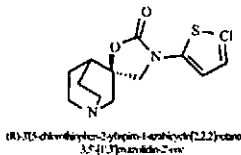
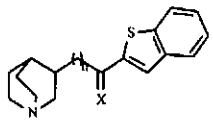


Fig. (10). Chemical structure of (R)-3'-(5-chlorothiophen-2-yl) spiro-1-azabicyclo[2.2.2]octane-3,5'-[1',3']oxazolidin-2'-one.

Interestingly, this compound (10 mg/kg, p.o.) significantly improved dizocilpine ((+)-MK-801) (3 mg/kg)-induced auditory gating deficits in rats, suggesting that this compound has the potential to improve sensory gating deficits in schizophrenic patients [100]. Moreover, we have developed a novel partial $\alpha 7$ nAChR agonist, W-56203, (R)-3'-(3-methylbenzo [b]thiophen-5-yl) spiro[1- azabicyclo[2.2.2] octane-3,5'-oxazolidin-2'-one, (Fig. 11) at $\alpha 7$ nAChRs (Katayama *et al.*, The 33rd SFN Annual Meeting, 2003). W-56203 bound to $\alpha 7$ nAChRs with a K_i value (3 nmol/L). No significant binding of W-56203 was detected at $\alpha 4\beta 2$

Table 2. Binding Affinities of Benzo[b]thiophen-2-yl Derivatives



N	X	$\alpha 7$ affinity (IC ₅₀ : μ mol/L)
0	O	0.95
0	H ₂	0.11
1	O	0.15
1	H ₂	0.023
2	O	1.7
2	H ₂	0.37

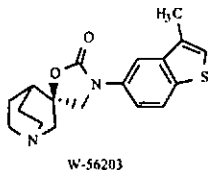


Fig. (11). Chemical structure of W-56203.

nAChRs or muscarinic receptors. Furthermore, W-56203 showed no binding to other known receptors (dopamine D₁ and D₂, 5-HT_{1A}, 5-HT₂, adrenergic $\alpha 1$, $\alpha 2$, histamine H₁ and H₂) or ion channels (NMDA and α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)), although W-56203 exhibited a moderate affinity to 5-HT₁ receptors. In cultured hippocampal neurons, W-56203 evoked a rapidly desensitizing inward current, which was blocked by the selective $\alpha 7$ nAChR antagonist methyllycaconitine (MLA, 1 nmol/L) (Fig. 12). Interestingly, W-56203 has been shown to significantly improve dizocilpine-induced auditory gating deficits in rats (Katayama *et al.*, The 33rd SFN Annual Meeting, 2003). These results suggest that W-56203 is an orally active and partial agonist at $\alpha 7$ nAChRs, and that W-56203 would be a useful drug for the treatment of schizophrenia.

Researchers at Targacept Inc. reported that 2-(3-pyridyl)-1-azabicyclo[3.2.2]nonane (TC-1698; Fig. 13) was a highly selective agonist of $\alpha 7$ nAChRs [101]. TC-1698 exhibited a

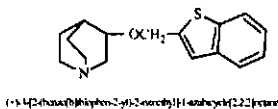


Fig. (9). Chemical structure of (+)-3-[2-(benzo[b]thiophen-2-yl)-2-oxoethyl]-1-azabicyclo[2.2.2]octane.

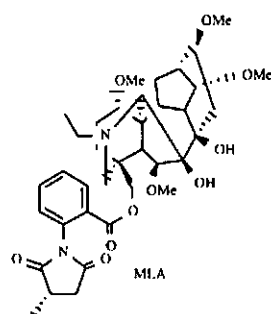


Fig. (12). Chemical structure of MLA.

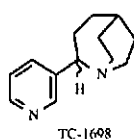


Fig. (13). Chemical structure of TC-1698.

Ki of 11 nmol/L in the binding assay of [^{3} H]MLA to rat hippocampal membranes, whereas TC-1698 (10 μ mol/L) had no or very low affinity for other receptors. TC-1698 exerts neuroprotective effects via activation of the JAK2/PI3K cascade, which can be neutralized through activation of the angiotensin II receptors [101]. These findings suggest that JAK2 plays a central role in the $\alpha 7$ nAChR activation of the JAK2-PI3K cascade in PC12 cells, which ultimately contribute to $\alpha 7$ nAChR mediated neuroprotection.

Researchers at Pfizer reported the selective $\alpha 7$ nAChR agonist PNU-282987, N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide hydrochloride (Fig. 14) [102, 103]. PNU-282987 binds to $\alpha 7$ nAChR with a Ki of 27 nmol/L, and showed evoked whole-cell currents from cultured rat hippocampal neurons that were sensitive to the selective $\alpha 7$ nAChR antagonist MLA [102,103]. Systemic administration of PNU-282987 (1 mg/kg, i.v.) significantly improved d-amphetamine-induced sensory gating deficits in chloral hydrate-anesthetized rats. These findings suggest that PNU-282987 may be useful for treating the cognitive and attentional deficits of schizophrenia [102,103].

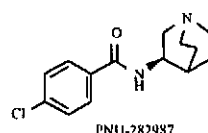


Fig. (14). Chemical structure of PNU-282987.

Very recently, researchers at Sanofi-Aventis demonstrated a novel $\alpha 7$ nAChR agonist SSR180711A, 4-bromophenyl 1,4-diazabicyclo[3.2.2]nonane-4-carboxylate hydrochloride (Fig. 15; Biton *et al.*, The 34th SFN Annual Meeting, 2004). Binding assays show that SSR180711A has a high and selective affinity for the human (Ki=78 nmol/L) and rat (Ki=50 nmol/L) $\alpha 7$ nAChRs. This ligand inhibits, in a dose-dependent manner, the *ex vivo* [^{3} H] α -bungarotoxin binding in mouse cortical homogenates after both i.p. and p.o. administration. At recombinant human $\alpha 7$ nAChRs, SSR180711A displays a partial agonist profile. Furthermore, it has been reported that SSR180711A improves cognitive deficits in a variety of rat models related to schizophrenia. This drug restores the selective attention deficit induced by PCP administration at the neonatal stage. This action is reversed by the selective $\alpha 7$ nAChR antagonist MLA, suggesting that $\alpha 7$ nAChRs play a role in the mechanism of action of SSR180711A (Pichat *et al.*, The 34th SFN Annual Meeting, 2004). This drug also restores a short-term episodic memory impairment and a spatial working memory deficit induced by PCP or dizocilpine. In addition, it has been shown to enhance the *in vivo* retrosplenial cortex firing in rats (Pichat *et al.*, The 34th SFN Annual Meeting, 2004). The retrosplenial cortex is known to be a corticolimbic structure involved in neuropathological changes related to NMDA receptor hypofunction [104-110]. This drug also enhances extracellular dopamine release in the prefrontal cortex (Pichat *et al.*, The 34th SFN Annual Meeting, 2004). Taken together, these findings suggest that SSR180711A has a potential to improve cognitive deficits associated with schizophrenia. Therefore, future clinical studies of SSR180711A on cognitive deficits in schizophrenic patients would be of great interest.

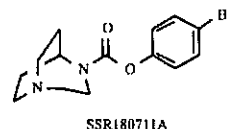


Fig. (15). Chemical structure of SSR180711A.

ANIMAL MODELS OF SENSORY GATING DEFICITS AND $\alpha 7$ NICOTINIC RECEPTOR AGONISTS

The hippocampal P20-N40 wave in DBA/2 mice has been used to model the neurobiology and pharmacology of the human P50 processing deficit [111-115]. Inhibition of the P20-N40 response and expression of $\alpha 7$ nAChRs in the hippocampus were found to be significantly correlated across nine inbred strains of mice [112]. This correlation showed that mouse strains with the fewest hippocampal $\alpha 7$ nAChRs had the least inhibition of the P20-N40 response to the second of paired stimuli [112]. In particular, the DBA/2 strain of inbred mice was shown to fail to attenuate their response to the second stimulus and to have significantly decreased expressions of the $\alpha 7$ nAChRs in their hippocampi [112]. This parallels studies of postmortem human tissue that documented the decreased expression of hippocampal $\alpha 7$ nAChRs in schizophrenic patients [68-70]. Interestingly, subcutaneous or intragastric injection of DMXB-A has been demonstrated to normalize deficient P20-N40 inhibition in

DBA/2 mice [113,114]. Therefore, agonists at $\alpha 7$ nAChRs are a drug candidate that may prove efficacious in normalizing deficient P50 processing in schizophrenic patients [12,13,29].

$\alpha 7$ nAChRs AND 5-HYDROXYTRYPTAMINE-3 (5-HT₃) RECEPTORS

Both $\alpha 7$ nAChRs and 5-hydroxytryptamine-3 (5-HT₃) receptors are members of the superfamily of ligand-gated ion channels [56,116-118] (Fig. 16). These two receptors share the greatest similarity within the family, displaying approximately 30% sequence homology [119]. Tropisetron and ondansetron (Fig. 17) are potent 5-HT₃ receptor antagonists that are widely used in the treatment of patients with chemotherapy-induced or postoperative nausea and vomiting [120,121]. It has been reported that tropisetron is a partial agonist of $\alpha 7$ nAChRs with a high affinity, whereas ondansetron is a weak agonist of $\alpha 7$ nAChRs [122,123]. Macor *et al.* [122] synthesized the replacement of the azabicyclo[3.2.1]heptane amine in tropisetron with the azabicyclo[2.2.2]hexamine amine, 1-Azabicyclo[2.2.2]oct-3-yl 1H indole-3-carboxylate and 1-Azabicyclo[2.2.2]oct-3-yl 1H indole-1-carboxylate (Fig. 18) were found to be partial agonists of $\alpha 7$ nAChRs with a high affinity, whereas they also bound to 5-HT₃ receptors with high affinities [122]. We found that tropisetron improves the deficient inhibitory processing of P20-N40 in DBA/2 mice, and that improvement of tropisetron could be antagonized by

coadministration of the selective $\alpha 7$ nAChR antagonist MLA [124]. These findings suggest that tropisetron improves abnormal auditory gating of P20-N40 in DBA/2 mice via $\alpha 7$ nAChRs. Recently, Adler *et al.* [125] demonstrated that ondansetron, a highly selective 5-HT₃ receptor antagonist, improves deficits of P50 suppression in medicated schizophrenic patients [125]. In addition, we have found that tropisetron improves deficits of P50 suppression in schizophrenic patients [126]. Taken together, these results indicate that both agonist activity at $\alpha 7$ nAChRs and antagonist activity at 5-HT₃ receptors for tropisetron might be implicated in the therapeutic action of normalization of P50 suppression by tropisetron. Furthermore, it would be of great interest to study the effects of tropisetron on cognitive dysfunction in schizophrenic patients, since P50 auditory gating deficits have been associated with attentional deficits in schizophrenia [77,127].

It has been reported that PSAB-OFP, (R)-(-)-5'-phenylspiro[1-azabicyclo[2.2.2] octano-3,2'-(3'-H) furo[2,3-b]pyridine (Fig. 19) , binds to human recombinant $\alpha 7$ nAChRs. However, PSAB-OFP also displayed high affinity binding to 5-HT₃ receptors. These results show that PSAB-OFP is an equipotent, partial agonist of both $\alpha 7$ nAChRs and 5-HT₃ receptors [128]. It has been found that agonist activity on one receptor translates to antagonist activity at the other receptor. For example, the endogenous ligands, ACh and 5-HT, act as antagonists on their counterparts [129,130]. The 5-

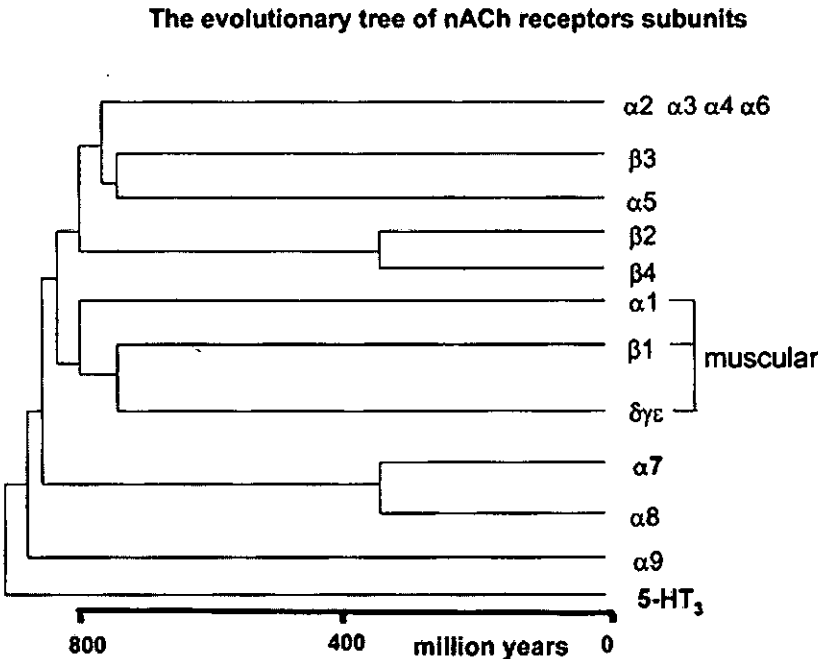


Fig. (16). The evolutionary tree of nACh receptors subunits and 5-HT₃ receptors.

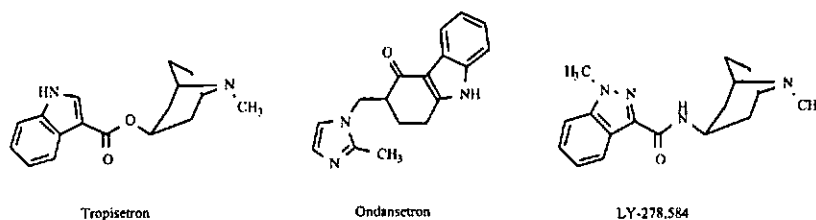


Fig. (17). Chemical structures of 5-HT₃ receptor antagonists (tropisetron, ondansetron, LY-278,584).

HT₃ receptor antagonist tropisetron, but not ondansetron or LY278,584, is a partial agonist of the $\alpha 7$ nAChRs [122]. The dramatic influence of small changes in ligand [131] or receptor structure [132] on the functional activity at $\alpha 7$ nAChRs and 5-HT₃ receptors might provide an understanding of the basis of the cross-reactivity and thereby allow development of selective ligands.

Furthermore, the combination of galantamine with risperidone has shown therapeutic benefits for two treatment-resistant patients with schizophrenia whose successful treatment with clozapine had been discontinued because of agranulocytosis [138]. Thus, it is likely that this dual action (cholinesterase inhibition and allosteric potentiation of nAChRs) may be implicated in the mechanisms of action of galantamine.

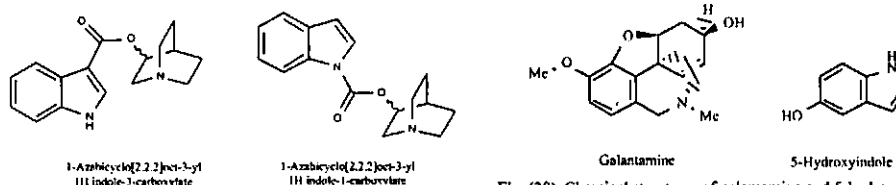


Fig. (20). Chemical structures of galantamine and 5-hydroxyindole.

Fig. (18). Chemical structures of 1-azabicyclo[2.2.2]oct-3-yl 1H indole-3-carboxylate and 1-azabicyclo[2.2.2]oct-3-yl 1H indole-1-carboxylate.

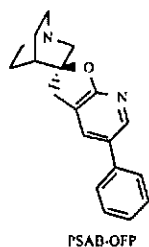


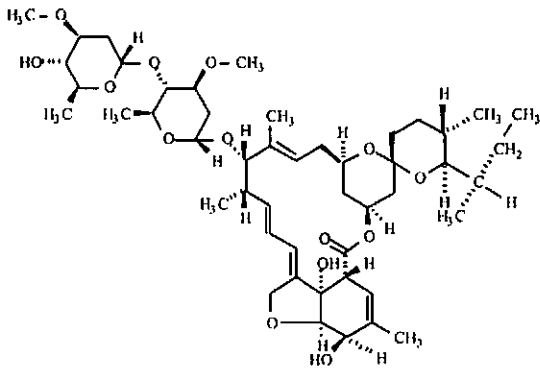
Fig. (19). Chemical structure of PSAB-OFF.

POSITIVE MODULATORS OF $\alpha 7$ nAChRs

Positive allosteric modulators potentiate the response to nicotinic receptor agonists by acting at a site other than the agonist binding site or channel area. Galantamine (acetylcholinesterase inhibitor) (Fig. 20), an approved drug for treatment of Alzheimer's disease, is also a potent allosteric potentiator of nicotinic receptors, including $\alpha 7$ nAChRs [133-136]. Galantamine has been reported to improve nega-

tive symptoms in patients with schizophrenia [137]. Furthermore, the combination of galantamine with risperidone has shown therapeutic benefits for two treatment-resistant patients with schizophrenia whose successful treatment with clozapine had been discontinued because of agranulocytosis [138]. Thus, it is likely that this dual action (cholinesterase inhibition and allosteric potentiation of nAChRs) may be implicated in the mechanisms of action of galantamine.

In has been reported that 5-hydroxyindole (Fig. 20) potentiates 5-HT₃ receptors [141-144]. It is, therefore, of interest to examine the effects of 5-hydroxyindole on $\alpha 7$ nAChRs, because $\alpha 7$ nAChRs belong to the superfamily of ligand-gated ion channels, including 5-HT₃ receptors. Gurley *et al.* (The 30th SFN Annual Meeting, 2000) reported that $\alpha 7$ nAChRs could be potentiated by 5-hydroxyindole. In *Xenopus* oocytes expressing $\alpha 7$ nAChRs, 5-hydroxyindole potentiated sub-maximal ACh (60 μ M/L)-induced ion currents in a concentration-dependent manner, although 5-hydroxyindole itself did not act as an agonist of $\alpha 7$ nAChRs [145]. Furthermore, it has been reported that 5-hydroxyindole potentiated $\alpha 7$ nAChR-mediated increases in intracellular free Ca²⁺ levels in cells expressing $\alpha 7$ nAChRs, and that the ACh-induced release as well as the 5-hydroxyindole-induced enhancement of release were blocked by the $\alpha 7$ nAChR antagonists, MLA or α -bungarotoxin, suggesting a role of $\alpha 7$ nAChRs [145].



Ivermectin (22,23-dihydroavermectin B_{1a})

Fig. (21). Chemical structure of ivermectin.

Researchers at Pfizer reported the novel positive allosteric modulator of the $\alpha 7$ nAChRs, PNU-120596, 1-(5-chloro-2,4-dimethoxy-phenyl)-3-(5-methyl-isoxazol-3-yl)-urea (Fig. 22) [146]. PNU-120596 increased agonist-evoked Ca^{2+} flux mediated by an engineered variant of the human $\alpha 7$ nAChRs. This compound increased the frequency of ACh-evoked GABAergic postsynaptic currents measured in pyramidal neurons, and greatly enhanced the ACh-evoked inward currents in hippocampal interneurons. Interestingly, systemic administration of PNU-120596 (0.1 - 3 mg/kg, i.v.) improved auditory gating deficits induced by administration of d-amphetamine (1 mg/kg). These findings suggest that PNU-120596 represents a new class of molecule that enhances $\alpha 7$ nAChR function and thus has the potential to treat psychiatric and neurological disorders [146].

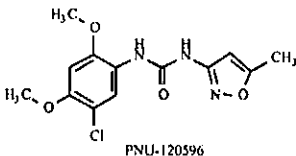


Fig. (22). Chemical structure of PNU-120596.

Taking these results together, it is likely that the identification of allosteric positive modulators for $\alpha 7$ nAChRs may lead to the design of novel classes of ligands that would be useful therapeutic drugs for neuropsychiatric diseases, including schizophrenia, although the precise mechanisms by which allosteric positive modulators potentiate the $\alpha 7$ nAChR-mediated responses are currently unknown.

FK960 AND FR236924

FK960 (N-(4-acetyl-1-piperazinyl)-p-fluorobenzamide monohydrate) (Fig. 23), which activates somatostatinergic neurotransmission, has considerable potential for the treat-

ment of cognitive impairment, including that associated with Alzheimer's disease [147]. In hippocampal CA1 neurons, FK960 significantly increased the amplitude of the excitatory postsynaptic potentials, and the modulatory action of FK960 was blocked by treatment with MLA or α -bungarotoxin [148]. These findings suggest that FK960 increases the quantal release of glutamate from Schaffer collateral-commissural nerve terminals in the hippocampal CA1 areas either by changing the ambient levels of ACh or by positively modulating the activity of $\alpha 7$ nAChRs on glutamatergic nerve terminals [148]. In considering the role of $\alpha 7$ nAChRs in the pathophysiology of schizophrenia, would be of great interest to study the effects of FK960 in schizophrenic patients, since FK960 has been tried as a therapeutic drug in Alzheimer's disease.

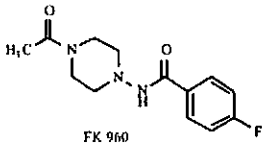


Fig. (23). Chemical structure of FK960.

Essential fatty acids, such as arachidonic acid (Fig. 24), linoleic acid (Fig. 24), and linolenic acid (Fig. 24) have been thought to play a role as raw materials for bioactive lipid mediators such as prostaglandins and/or leukotrienes. However, several recent findings suggest that these *cis*-unsaturated free acids induce a long-lasting facilitation of hippocampal synaptic transmission that resembles long-term potentiation, as a result of enhancing the activity of nAChRs via a protein kinase C (PKC) pathway [149-151]. Researchers at Fujisawa Pharmaceutical Co., Ltd. reported a newly synthesized linoleic acid derivative (FR236924; 8-(2-(2-pentylcyclopropan-1-yl) methyl) cyclopropyl) octanoic acid, Fig. 24) with a cyclopropane ring instead of *cis*-double

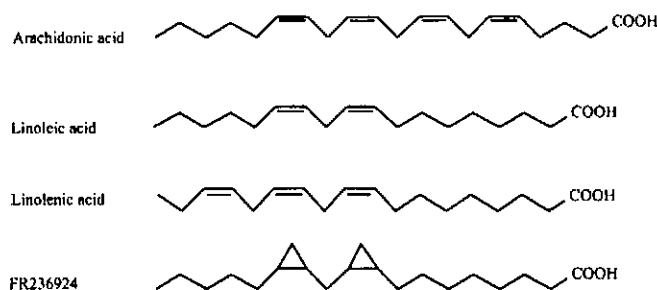


Fig. (24). Chemical structures of arachidonic acid, linoleic acid, linolenic acid and FR236924.

bonds. FR236924 (10 $\mu\text{mol/L}$) induced a gradually developing and persistent potentiation of $\alpha 7$ nAChR responses [152]. The effect of FR236924 was inhibited by the selective PKC inhibitor GF109203X or the $\alpha 7$ nAChR antagonist α -bungarotoxin [152]. Furthermore, it has been reported that FR236924 increased the rate of AMPA receptor-mediated miniature excitatory postsynaptic currents, without affecting the amplitude, triggered by nicotine in rat hippocampal CA1 pyramidal neurons, and that the effect could be inhibited by GF109203X or α -bungarotoxin [153]. FR236924 stimulated glutamate release from rat hippocampal slices and in the hippocampus of freely moving rats, and the effect was also inhibited by GF109203X or α -bungarotoxin [153]. These findings suggest that FR236924 stimulates glutamate release by functionally targeting presynaptic $\alpha 7$ nAChRs on the glutamatergic terminals under the influence of PKC, responsible for the facilitatory action on hippocampal synaptic transmission. It is thus possible that FR236924 would be a useful drug for studying the regulatory mechanisms underlying cognitive functions linked to *cis*-unsaturated free fatty acids and $\alpha 7$ nAChRs.

KYNURENIC ACID AS AN ENDOGENOUS ANTAGONIST OF $\alpha 7$ nAChR

Kynurenic acid is synthesized via kynurenine from the essential amino acid L-tryptophan, and kynurenic acid is produced and released by astrocytes in the brain [21,133,154,155] (Fig. 3). Interestingly, it has been reported that the levels of kynurenic acid are increased in the CSF and brain of schizophrenic patients [156,157]. In addition to its well-characterized action as a competitive antagonist of the glycine site on the NMDA receptors, kynurenic acid also acts as a noncompetitive antagonist of the $\alpha 7$ nAChRs [154,155] (Fig. 3). Administration of L-kynurenine, a precursor of kynurenic acid together with probenecid, an inhibitor of organic acid transport, increased levels (500-fold) of kynurenic acid in the hippocampus of rats, and also disrupted auditory sensory gating in rats. In contrast, administration of L-701,324, a centrally acting antagonist of the glycine site of NMDA receptors, failed to disrupt auditory gating in rats, suggesting that elevated levels of kynurenic acid produce the disruption in auditory processing through $\alpha 7$ nAChR [158].

D-Serine is synthesized from L-serine via serine racemase in astrocytes (Fig. 3). Glutamate binds to AMPA receptors on astrocytes that stimulate the release of D-serine. The released D-serine binds to glycine sites on NMDA receptors [20,21,159-161]. Several findings suggest that D-serine plays a role as an endogenous agonist on glycine sites of the NMDA receptors [20,21,159-161]. We reported that serum levels of D-serine are significantly reduced in patients with schizophrenia, supporting the NMDA receptor hypofunction in schizophrenia [19,162]. Furthermore, we found that the ratio of D-serine to total serine in the CSF of first episode and drug naive schizophrenic patients was significantly lower than that of age-matched normal controls [163]. These data suggest that the synthetic and/or metabolic pathway of D-serine may be impaired in the brains of schizophrenic patients [21,163]. It would thus seem that both kynurenic acid and D-serine may act as endogenous ligands at glycine sites of the NMDA receptor and thereby play a role in NMDA receptor hypofunction in schizophrenia [21] (Fig. 3).

Taken together, these results indicate that blockade of $\alpha 7$ nAChR in the hippocampus by elevated levels of kynurenic acid is likely to lead to auditory gating deficits in schizophrenic patients, and that disruption of reciprocal astrocyte-neuron signaling mechanisms involving kynurenic acid and D-serine may play a role in the pathophysiology of schizophrenia [21] (Fig. 3).

CONCLUDING REMARKS

As described above, $\alpha 7$ nAChRs play a role in the deficits of P50 auditory sensory gating in schizophrenia. Direct ($\alpha 7$ nAChR agonists) or indirect agonists (5-HT₂ receptor antagonists, positive allosteric modulators, FK960, FR236924, PNU-120596) of $\alpha 7$ nAChRs would be useful as potential therapeutic drugs for the treatment of schizophrenia, since deficits of P50 auditory sensory gating have been associated with the cognitive impairments in schizophrenia. Considering the role of kynurenic acid and D-serine as endogenous ligands at glycine sites of the NMDA receptors, it is possible that agents which could decrease the levels of kynurenic acid or increase the levels of D-serine in the brain would be possible therapeutic drugs for schizophrenia.

In addition, patients with Alzheimer's disease have also been reported to show deficits of P50 auditory sensory gating relative to controls [164]. It has also been suggested that the disturbed sensory gating in patients with Alzheimer's disease might result from cholinergic dysfunction or $\alpha 7$ nAChR loss. Furthermore, it has been shown that $\alpha 7$ nAChRs might be implicated in the disposition of β -amyloid as well as cognitive impairments in Alzheimer's disease [165,166]. Therefore, it is also likely that agonists of $\alpha 7$ nAChRs would be useful as potential therapeutic drugs for the treatment of Alzheimer's disease.

ABBREVIATIONS

ACh	=	Acetylcholine
GABA	=	γ -Aminobutyric acid
nAChR	=	Nicotinic acetylcholine receptor
CNS	=	Central nervous system
CSF	=	Cerebrospinal fluid
NMDA	=	N-methyl-D-aspartate
CHRNA7	=	$\alpha 7$ nAChR subunit gene
DMXB-A	=	3-(2,4)-Dimethoxybenzylidene anabaseine
(GTS-21)		
DMAB	=	3-(4)-Dimethylaminobenzylidene anabaseine
DMAC	=	3-(4)-Dimethylaminocinnamylidene
4-OH DMXB-A	=	3-(4-Hydroxy-2-methoxybenzylidene) anabaseine
AR-R17779	=	(-)-Spiro[1-azabicyclo[2.2.2]octane-3,5'-oxazolidin-2'-one]
SIB1765F	=	[$\pm$]-5-Ethynyl-3-(1-methyl-2-pyrrolidinyl)pyridine fumarate
W-56203	=	(R)-3'-(3-Methylbenzo[b]thiophen-5-yl)spiro[1-azabicyclo[2.2.2]octane-3,5'-oxazolidin]-2'-one
AMPA	=	α -Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
MLA	=	Methyllycaconitine
TC-1698	=	2-(3-Pyridyl)-1-azabicyclo[3.2.2]nonane
PNU-282987	=	N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide hydrochloride
SSR180711A	=	4-Bromophenyl 1,4-diazabicyclo[3.2.2]nonane-4-carboxylate hydrochloride
PCP	=	Phencyclidine
PSAB-OPF	=	(R)-(-)-5'-Phenylspiro[1-azabicyclo[2.2.2]octane-3,2'-(3'-H)furo[2,3-b]pyridine
PNU-120596	=	1-(5-Chloro-2,4-dimethoxy-phenyl)-3-(5-methyl-isoxazol-3-yl)-urea

FK 960	=	N-(4-Acetyl-1-piperazinyl)-p-fluorobenzamide monohydrate
PKC	=	Protein kinase C
FR 236924	=	8-(2-((2-Pentylcyclopropan-1-yl)methyl)cyclopropyl)octanoic acid

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