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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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Señores

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIVISIÓN DE NUEVAS CREACIONES

E. _____ S. _____ D. _____

Re: Expediente N° 07-002.330

Solicitud de Patente de Invención Titulada: "DERIVADOS DE 3-(HETEROAIL-OXI)-2-ALQUIL-1-AZA-BICICLOALQUILO COMO LIGANDOS ALFA-7-NACHR PARA EL TRATAMIENTO DE ENFERMEDADES DEL SISTEMA NERVIOSO CENTRAL (SNC)"

Solicitante: NOVARTIS A.G.

Nuestra Ref.: P2006/000269

RESPUESTA AL OFICIO No. 02239, NOTIFICADO POR FIJACIÓN EN LISTA No. 41

DE FECHA 11 DE FEBRERO DE 2011

(PRIMER EXAMEN DE FONDO)

CARLOS R. OLARTE, mayor de edad y vecino de Bogotá, identificado tal como aparece al pie de mi firma, en mi carácter de apoderado de la sociedad solicitante, me permito manifestar lo siguiente ante el auto emanado por ese Despacho, dentro del plazo legal de prórroga previsto en el artículo 45 de la Decisión 486, en los siguientes términos:

1. En primer lugar, con el ánimo de brindar mayor claridad a la materia reivindicada, me permito presentar un **Capítulo Reivindicatorio Modificado**, que reemplaza en su totalidad al Capítulo Reivindicatorio presentado al momento de radicar la solicitud.

Así entonces, el Capítulo Reivindicatorio Modificado adjunto contiene los siguientes cambios:

- Se han eliminado las Reivindicaciones 2, 4 a 6, y 8 a 15;
- En la Reivindicación 1 se ha limitado el significado de los radicales A, B, X y R, y se han eliminado los radicales R¹ a R⁵; y

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- Se han introducido las nuevas Reivindicación 3 a 7, que encuentran soporte en la página 11, líneas 5 y 6, Ejemplos 3h-3p, y 3dI, Ejemplos 3k, 3I y 3m, respectivamente.

Valga anotar que las modificaciones efectuadas al Capítulo Reivindicatorio cumplen a cabalidad con lo estipulado en el Artículo 34 de la Decisión 486, ya que éstas de ninguna manera implican una ampliación de la protección correspondiente a la divulgación contenida en la solicitud inicial, por el contrario, éstas corresponden a una franca limitación de la materia reivindicada.

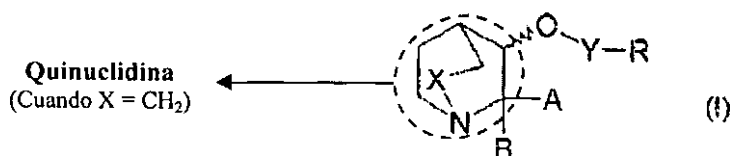
2. a- El Examinador señala que las Reivindicaciones 8 a 13 no son patentables porque reclaman métodos de tratamiento y usos.

b- Considero superada la anterior objeción, en tanto que se han eliminado las Reivindicaciones 8 a 13 del Capítulo Reivindicatorio Modificado adjunto.

3. a- El Examinador señala que la patente WO2004/022556 (D1), afecta el nivel inventivo de la presente invención.

El Examinador señala que D1 enseña compuestos azabicycloalquil éteres que se diferencian de la presente solicitud en la presencia de un alquilo C_{1-7} en A o B, o la formación de un cicloalquilo C_{3-7} a partir de A y B. Sin embargo, el Examinador concluye que dicha diferencia estructural no proporciona un efecto sorprendente, ya que presenta el mismo efecto modulador selectivo del receptor $\alpha 7$ nAChR con un valor pEC_{50} entre aproximadamente 5 y 9, y por lo tanto, para cualquier persona medianamente versada en la materia habría sido obvio realizar la modificación estructural propuesta.

b- i) En primer lugar, llamo respetuosamente la atención del Examinador sobre las limitaciones realizadas en el Capítulo Reivindicatorio Modificado adjunto, en donde específicamente se reivindican derivados 2-metil-3-(5-indolil-heteroariloxi)-1-azabicyclo[2.2.2]octano (vale la pena aclarar en este punto que la fracción 1-azabicyclo[2.2.2]octano es conocida como el grupo quinuclidina), de fórmula:



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útiles como agonistas del receptor nicotínico acetilcolina (nAChR), en donde A es hidrógeno; B es un grupo metilo que se encuentra en posición trans respecto al oxígeno de la molécula; X representa CH₂; Y representa un grupo piridazina, pirimidina, piridina o 1,3,4-tiadiazol; y R representa específicamente un grupo 5-indolilo.

ii) En cuanto a D1, este documento divulga compuestos derivados de 1-azabicycloalcanos, que se encuentran sustituidos en la posición 3 por un grupo heteroariloxi, que a su vez se encuentra sustituido por un grupo heteroarilo mono o policíclico, espaciado opcionalmente por un enlace amino/aminoalquil, y que son útiles como agonistas nAChR.

Ahora bien, dichos compuestos difieren de la presente invención no sólo en los elementos señalados por el Examinador. Los compuestos de D1 no se encuentran sustituidos en la posición 2 del núcleo azabicycloalcano, y menos por un metilo en posición trans. De hecho, no se encuentra en D1 sugerencia alguna que le permitiera a la persona medianamente versada en la materia desarrollar específicamente un núcleo quinuclidina sustituido en posición 2 por un metilo trans, y que a través de un puente de oxígeno se encontrara unido un grupo pirimidina, piridina, o 1,3,4-tiaziazol, sustituido específicamente por un grupo 5-indolilo.

Como evidencia de que el arte previo disponible en el momento en que se radicó la presente solicitud no sugería o enseñaba los compuestos aquí reivindicados, presento como anexos a este memorial dos artículos: i) Bunelle et al¹. (Anexo 1) y ii) Mazurov et al²., en los que se concluye que la introducción de un sustituyente en la posición dos del núcleo quinuclidina, disminuye significativamente la afinidad por el blanco, de los agonistas $\alpha 7$. Específicamente en el Anexo 1 se establece en la página 324, líneas 1 y 2, que:

“la introducción de un sustituyente 2-metilo en el núcleo quinuclidina causa una pérdida de 3 veces la afinidad sobre el $\alpha 7$ ” (mi traducción, mi subrayado)

De otra parte, en el Anexo 2 se comparan quinuclidin-amidas y carbamatos, y se establece en la página 1574, líneas 14 a 17, que:

¹ Bunelle et al. “Design of Ligands for the Nicotinic Acetylcholine Receptors: The Quest for Selectivity”. Current Topics in Medicinal Chemistry, Vol. 4, Pages 299-334. 2004.

² Mazurov et al. “Selective $\alpha 7$ Nicotinic Acetylcholine Receptor Ligands”. Current Medicinal Chemistry. Vol. 13. Pages 1567-1584. 2006.

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“la incorporación de grupos pequeños (metilo) o grandes (bencilo) en la posición 2 del anillo azabicclico, generalmente, causa un detrimento en la afinidad” (mi traducción, mi subrayado)

Teniendo en cuenta la anterior información, que particularmente cubre diferentes tipos de derivados de quinuclidina (amidas y carbamatos), la persona medianamente versada en la materia se habría inclinado por evitar la introducción de un grupo sustituyente en la posición 2 del anillo quinuclidina, ya que según las enseñanzas del estado de la técnica, dicha introducción llevaría a una reducción significativa en la afinidad. En consecuencia, el técnico medianamente versado en la materia no hubiera encontrado motivación para introducir cualquier sustituyente, y menos aún un metilo trans en la posición 2, con el fin de resolver el problema de proporcionar agonistas con una mayor selectividad por el receptor $\alpha 7$ nACh.

Por último, me permito presentar el Anexo 3, en donde se compara la actividad del compuesto 3-[6-(1h-indol-5-il)-piridazin-3-iloxi]-2-metil-1-aza-biciclo[2.2.2]octano (Ejemplo 3k), y el mismo compuesto sin el sustituyente metilo en posición 2. Como se enseña en el Anexo 3, el Ejemplo de la presente solicitud presenta un EC_{50} de **$13 \pm 0,1nM$** , y una eficacia del $89 \pm 5\%$, mientras que el compuesto más cercano presenta un EC_{50} de **$16 \pm 0,2nM$** y una eficacia del $93 \pm 14\%$ (la eficacia de los dos compuestos se obtuvo al realizar un ensayo comparativo con epibatidina). Es importante señalar que un aspecto muy importante de este tipo de compuestos agonistas del receptor $\alpha 7$ nACh es retener la potencia ya que están destinados para el uso crónico de pacientes de edad, (por ejemplo, enfermedades neurodegenerativas, como Alzheimer) entre otros. Teniendo en cuenta lo anterior, una pérdida de 3 veces la potencia (EC_{50}) puede traducirse en una dosis tres veces más alta, y por lo tanto, un fácil incremento en los efectos adversos que para un tratamiento crónico en dicha población de pacientes puede alcanzar un nivel inaceptable. En el presente caso, el compuesto de la presente invención presenta un EC_{50} menor, y una eficacia similar.

iii) Según todo lo anterior, se puede concluir fácilmente que los elementos estructurales específicos del compuesto de la presente invención no podían preverse o predecirse de las enseñanzas de D1. Ahora bien, según los lineamientos del Manual Andino de Patentes (MAP), las invenciones en el campo de la química tienen nivel inventivo cuando tienen una estructura inesperada (no derivable por la persona medianamente versada en la materia), o presente un efecto técnico inesperado o mejorado impredecible del arte previo³. En el presente

³ Manual Andino de Patentes. WIPO, EPO, Secretaría de la Comunidad Andina. 2004. ISBN9978-43-855-6. Sección 10.8.1

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caso, no solo la estructura de química es inesperada, sino también la actividad proporcionada por las mismas, lo que es una clara evidencia de nivel inventivo.

Ahora bien, es importante señalar que el análisis de nivel inventivo no puede estar basado en si es o no posible obtener la invención reclamada, pues claramente era posible, toda vez que la estructura química reclamada en realidad existe. Esta aproximación conllevaría a que ninguna invención sería patentable, volviendo así inoperante el sistema de patentes.

Determinado aquello que es diferente con respecto al arte previo, como se realizó anteriormente, es muy importante que quién examine la materia de la solicitud de patente la analice como un todo. No es dable a quien examina una patente simplemente desglosar sus elementos separadamente y luego empezar a buscar referencias en el estado del arte que los incluyan, como si de armar un rompecabezas con piezas aisladas, o una colcha de retazos, se tratara.

Para el Examinador resulta simple argumentar que cualquier persona medianamente versada en la materia hubiera podido llegar a los compuestos de la presente invención de manera obvia, sin considerar los aspectos descritos anteriormente. Por el contrario, nada en D1 enseña, o incluso sugiere, y aún menos divulga, los compuestos aquí reivindicados, es más, el arte previo establece que una de las sustituciones realizadas en los compuestos aquí reivindicados va en detrimento con la selectividad de las moléculas (sustitución en el anillo quinuclidina), al punto que la persona medianamente versada en la materia, no habría encontrado motivación alguna para fabricar los compuestos aquí reivindicados, y por el contrario, habría evitado siquiera pensar en sustituir la posición 2 del núcleo quinuclidina.

Sin embargo, contrario a las enseñanzas y sugerencias del estado de la técnica, los investigadores encontraron que los compuestos de la presente invención, que contienen un metilo en posición trans sustituyendo el carbono 2 del núcleo quinuclidina, no sólo no pierden la afinidad con respecto al receptor $\alpha 7$ nACh, sino que presentan mejores resultados (EC_{50}) frente a compuestos análogos que no contienen dicha sustitución, lo que a todas luces es una evidencia de nivel inventivo.

4. Finalmente, deseo poner bajo su conocimiento que para el concepto inventivo comprendido en la presente solicitud, la oficina de patentes en Europa ha otorgado el privilegio de patente.

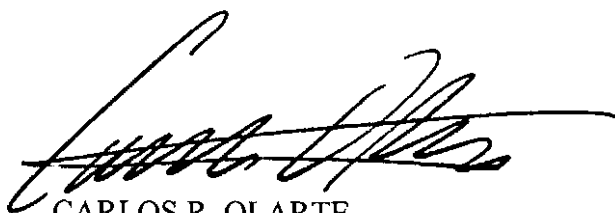
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Si bien es cierto que las concesiones de patentes en oficinas de otros países no son vinculantes en el sentido que obliguen a la SIC a tomar una decisión en el mismo sentido, considero que dichas concesiones sugieren el cumplimiento de los requisitos de patentabilidad de la materia

5. Por todo lo anterior, considero que las objeciones presentadas por el Examinador en su examen de fondo No. 141 han sido resueltas y por consiguiente solicito respetuosamente que el Despacho tenga por cumplidos los requisitos de patentabilidad y proceda con la concesión de la patente.

Atentamente,



CARLOS R. OLARTE

C.C. No. 79.782.747 de Bogotá

T.P. 74.295

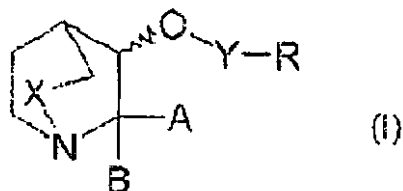
Anexo:

- Capítulo Reivindicatorio Modificado.
- Anexo 1: Bunnelle et al. "Design of Ligands for the Nicotinic Acetylcholine Receptors: The Quest for Selectivity". Current Topics in Medicinal Chemistry, Vol. 4, Pages 299-334. 2004.
- Anexo 2: Mazurov et al. "Selective $\alpha 7$ Nicotinic Acetylcholine Receptor Ligands". Current Medicinal Chemistry. Vol. 13. Pages 1567-1584. 2006.
- Anexo 3: Biological Data to support European Patent Application No. 05 0578 825.3.

CAPITULO REIVINDICATORIO MODIFICADO

(Primer Examen de Fondo)

1. Un compuesto de Fórmula I

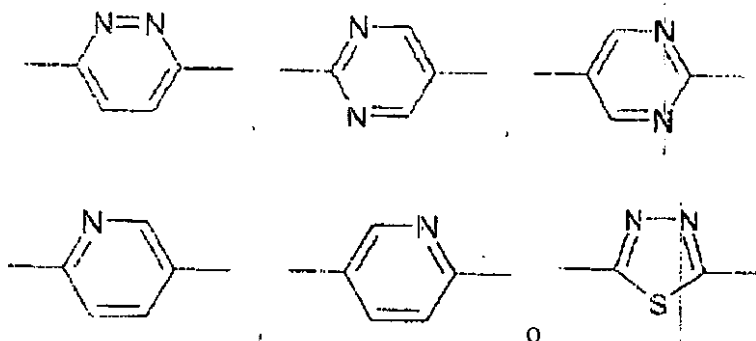


en donde

A representa hidrógeno, B representa metilo y B se encuentra en la posición trans respecto al oxígeno;

X representa CH₂;

Y representa un grupo de fórmula

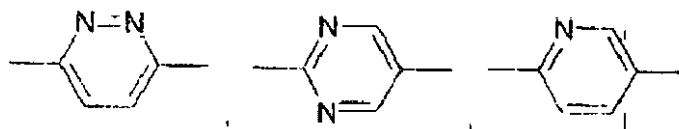


en donde el enlace izquierdo se encuentra unido al oxígeno, y el enlace derecho se encuentra unido al grupo R;

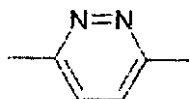
R representa 5-indolilo;

en forma de base libre o en forma de sal de adición de ácido.

2. Un compuesto de Fórmula 1 de acuerdo con la Reivindicación 1, en donde Y es un grupo de fórmula:



3. Un compuesto de Fórmula 1 de acuerdo con la Reivindicación 1, en donde Y es:



4. Un compuesto de Fórmula I de acuerdo con la Reivindicación 1 seleccionado entre:

(2SR,3RS)-3-[5-(1H-indol-5-il)-piridin-2-iloxi]-2-metil-1-aza-biciclo[2.2.2]octano;
(+)-3-[5-(1H-indol-5-il)-piridin-2-iloxi]-2-metil-1-aza-biciclo[2.2.2]octano;
(-)-3-[5-(1H-indol-5-il)-piridin-2-iloxi]-2-metil-1-aza-biciclo[2.2.2]octano;
(2SR,3RS)-3-[6-(1H-indol-5-il)-piridazin-3-iloxi]-2-metil-1-aza-biciclo[2.2.2]

octano;

(2S,3R)-3-[6-(1H-indol-5-il)-piridazin-3-iloxi]-2-metil-1-aza-biciclo[2.2.2]octano;
(2R,3S)-3-[6-(1H-indol-5-il)-piridazin-3-iloxi]-2-metil-1-aza-biciclo[2.2.2]octano;
(2SR,3RS)-3-[5-(1H-indol-5-il)-pirimidin-2-iloxi]-2-metil-1-aza-biciclo[2.2.2]

octano;

(2S,3R)-3-[5-(1H-indol-5-il)-pirimidin-2-iloxi]-2-metil-1-aza-biciclo[2.2.2]octano;
(2R,3S)-3-[5-(1H-indol-5-il)-pirimidin-2-iloxi]-2-metil-1-aza-biciclo[2.2.2]octano;

y

(2SR,3RS)-3-[6-(1H-indol-5-il)-piridin-3-iloxi]-2-metil-1-aza-biciclo[2.2.2]octano.

5. Un compuesto de Fórmula I de acuerdo con la Reivindicación 1, en donde dicho compuesto es (2SR,3RS)-3-[6-(1H-indol-5-il)-piridazin-3-iloxi]-2-metil-1-aza-biciclo[2.2.2]octano.

6. Un compuesto de Fórmula I de acuerdo con la Reivindicación 1, en donde dicho compuesto es (2S,3R)-3-[6-(1H-indol-5-il)-piridazin-3-iloxi]-2-metil-1-aza-biciclo[2.2.2]octano.

7. Un compuesto de Fórmula I de acuerdo con la Reivindicación 1, en donde dicho compuesto es (2R,3S)-3-[5-(1H-indol-5-il)-pirimidin-2-iloxi]-2-metil-1-aza-biciclo[2.2.2]octano.

8. Una composición farmacéutica que comprende un compuesto de Fórmula I como se ha definido en cualquiera de las Reivindicaciones 1 a 7 en base libre o en forma de una sal de adición de un ácido farmacéuticamente aceptable, en asociación con un vehículo o diluyente farmacéuticamente aceptable.

Design of Ligands for the Nicotinic Acetylcholine Receptors: The Quest for Selectivity

William H. Bunnelle*, Michael J. Dart, and Michael R. Schrimpf

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Abstract: In the last decade, nicotinic acetylcholine receptors (nAChRs) have emerged as important targets for drug discovery. The therapeutic potential of nicotinic agonists depends substantially on the ability to selectively activate certain receptor subtypes that mediate beneficial effects. The design of such compounds has proceeded in spite of a general shortage of data pertaining to subtype selectivity. Medicinal chemistry efforts have been guided principally by binding affinities to the $\alpha 4\beta 2$ and/or $\alpha 7$ subtypes, even though these are not predictive of agonist activity at either subtype. Nevertheless, a diverse family of nAChR ligands has been developed, and several analogs with promising therapeutic potential have now advanced to human clinical trials. This paper provides an overview of the structure-affinity relationships that continue to drive development of new nAChR ligands.

Key Words: Nicotine, epibatidine, acetylcholine, quinuclidine, nAChR, $\alpha 4\beta 2$, $\alpha 7$, SAR.

INTRODUCTION

The profound physiological effects of the tobacco alkaloid nicotine are mediated by a set of ion channels whose endogenous ligand is acetylcholine. Multiple subtypes of nicotinic acetylcholine receptors (nAChRs) are differentially located throughout the CNS and periphery, and the functional roles of these diverse receptor types are just beginning to be elucidated [1-3]. In the CNS, nAChRs modulate functions of many major neurotransmitter systems, and thereby influence a broad range of brain functions [4]. This is reflected in the beneficial effects of nicotine in treatment of cognitive disorders, neurodegenerative conditions such as Alzheimer's and Parkinson's diseases, schizophrenia, depression, and pain, among others [5-7]. Likewise, nAChRs are implicated in nicotine's well-known side effects, including cardiovascular and gastrointestinal effects, addiction liability, and a possible role in angiogenesis facilitating tumor growth [8-10]. The existence of multiple nAChR subtypes suggests that it should be possible to eliminate undesired side effects through development of nAChR ligands that are suitably selective for subtypes that mediate the therapeutically beneficial function [11,12].

Over the past fifteen years, drug discovery efforts have greatly expanded the number and structural diversity of nAChR ligands. These have been detailed in several excellent reviews [13-16]. This current paper is meant to provide an overview of the challenges of developing subtype-selective nAChR agonists and a summary of recent developments in the area.

nAChR RECEPTOR ORGANIZATION

nAChRs are composed of a pentagonal array of subunit proteins, forming an ion channel in the center. The prototype

and best-characterized receptor is found at the neuromuscular junction (NMJ), where it mediates chemical transmission across the nerve-muscle synapse [17]. This nAChR comprises four different subunit proteins: two $\alpha 1$ subunits along with $\beta 1$, δ and γ (the latter replaced by ϵ in later developmental stages) [18]. Neuronal nAChRs are built from a separate and distinct group of subunits ($\alpha 2$ -10 and $\beta 2$ -4 have been identified to date) [19,20]. The α subunits are associated with the agonist binding domain, characterized by a disulfide bridge between vicinal cysteine residues in the extracellular N-terminal region of the protein. Nevertheless, adjacent (generally non- α) subunits also contribute to the binding site, so activity at any nAChR depends on the entire subunit composition.

In the resting state (absent agonist), the ion channel is closed. Binding of agonist to the receptor causes transient opening of the channel, allowing influx of cations (K^+ , Na^+ , and/or Ca^{2+}) into the cell that triggers other events (e.g., membrane depolarization, initiation of action potential). Within a time frame of milliseconds to seconds, the channel closes again, a process known as desensitization. Indeed, multiple desensitized states are likely involved [21]. Ultimately, dissociation of the agonist returns the receptor to the resting state. The responsiveness to agonist, the ion conductance and selectivity, and rate of desensitization are all characteristics of individual receptor subtypes determinant for their particular function.

Structural information on the nAChR is based mainly on investigations of the NMJ subtype. There are two agonist-binding sites, at the $\alpha\gamma$ and $\alpha\delta$ interfaces, and receptor activation requires occupation of both. Extensive study of photoaffinity labels and site-directed mutagenesis confirm that key binding-site residues are contributed by both subunits [18]. Critical structural detail has been provided by recent publication of the x-ray structure of acetylcholine binding protein (AChBP), a pentameric protein that resembles the extracellular binding domain of nAChRs [22]. This structure confirms the main features of the receptor binding domain, in particular the convergence of three tyrosine and

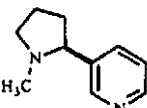
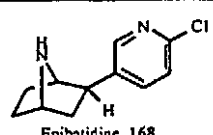
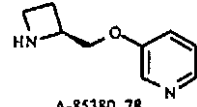
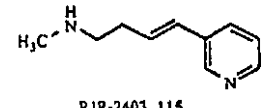
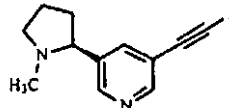
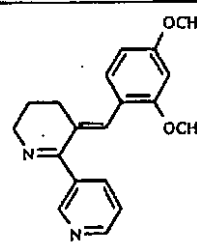
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two tryptophan side chains to frame a 'box' of electron-rich aromatic rings [23]. It is now well-established that the trimethylammonium moiety of acetylcholine binds in this hydrophobic box, determined largely by a strong cation- π interaction with Trp149 of the α subunit [24-27]. Homology models indicate that the 'cation box' is conserved across neuronal subtypes as well. Unwin's elegant time-resolved electron microscopic studies reveal the gross changes in NMJ receptor morphology from resting state through activation by agonist and ultimate desensitization [17,28,29]. The manner by which binding of a small agonist leverages

these conformational reorganizations is not well understood, but must involve some induced change in the geometry of the ligand-binding domain. A crucial point to be appreciated here is that the nAChR pharmacophore is most certainly different for the active state of the receptor compared to the desensitized state(s). Equilibrium binding experiments, including ligand photoaffinity labeling, will normally access the desensitized state of the receptor, and thereby reveal detail that is different from the active form [30].

The overall architecture of neuronal nAChRs is similar to

Table I. Pharmacological Data for Key nAChR Ligands.

Ligands	Binding (K_i , nM)			Receptor activation (EC_{50})			Refs
	Rat $\alpha 4\beta 2$	Rat $\alpha 7$	Torpedo $\alpha 3\beta \gamma \delta$	ha4 $\beta 2$	ha3 $\beta 4^*$	ha7	
 Nicotine, 1	1.0 nM	4000 nM	1000 nM	4.6 μ M (100%)	21 μ M (100%)	83 μ M (100%)	[308] [45] [309]
 Epibatidine, 168	0.04 nM	230 nM	2.7 nM	0.02 μ M (156%)	0.007 μ M (153%)	1.3 μ M (80%)	[110] [310] [309]
 A-85380, 78	0.05 nM	100 nM	310 nM	0.7 μ M (163%)	0.7 μ M (113%)	8.9 μ M (100%)	[110] [46]
 RJR-2403, 115	26 nM	36,000 nM	No data	4.3 μ M (83%)	Inactive	No data	[135] [137] [139] [138]
 SIB-1508Y, 22	4 nM	No data	52,000 nM	1.8 μ M (49%)	100 μ M (<20%)	inactive	[66] [65] [139]
 GTS-21, 322	19 nM	650 nM	1100 nM	No activation	No activation	12% @ 1 mM	[308] [309]

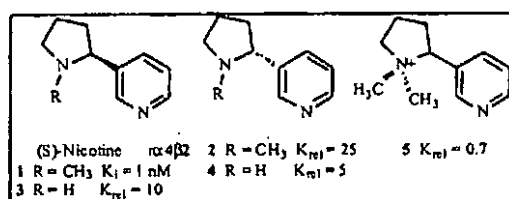
that of the NMJ receptor, but a greater diversity of subunits provides for many more receptor subtypes [1,18,19]. Combinations with the $(\alpha)_2(\beta)_3$ stoichiometry are common, but some alpha subunits (notably $\alpha 7$, but also $\alpha 8-9$) form functional, homopentameric receptors [19]. At least one receptor with overall the $(\alpha)_2(\beta)_3$ stoichiometry has been described [31]. In native tissue, radioligand binding differentiates two major classes of nAChRs. One group is defined by high-affinity (~ 1 nM) binding of radiolabeled agonists, e.g., [^{3} H]-nicotine, and has been identified as mainly the $\alpha 4\beta 2$ combination. The other group is characterized by lower (micromolar) affinity for nicotine, but high affinity for the snake toxin α -bungarotoxin (Btx), and is assigned as the $\alpha 7$ homopentamer [32]. Combinations of $\alpha 3$ with $\beta 4$, and possibly other subunits including $\alpha 5$ or $\beta 2-3$ [31,33,34] are expressed in the autonomic ganglia; the uncertain composition of receptor subtypes is reflected in designations like $\alpha 3\beta 4^*$, where the asterisk indicates the possible involvement of other subunits.

THE QUEST FOR SELECTIVITY

Expression systems with various cloned subunit combinations have been developed [32], and these have been useful as pharmacological standards to help characterize and identify receptors in native tissue, as well as to evaluate subtype selectivity for a limited number of nAChR ligands. While these tools have been useful in helping to elucidate the role(s) of various receptor subtypes, they have had limited impact on the development of subtype-selective nAChR ligands. Most obvious is that *in vitro* selectivities can reflect only the subtypes against which the ligand has been evaluated – an example is the assignment of the $\alpha 3\beta 2$ nAChR as the key subtype mediating striatal dopamine release, based largely on the putative selectivity of the antagonist α -conotoxin MII [35]. Subsequent study revealed that conotoxin MII is also a potent inhibitor of $\alpha 6$ -containing subtypes, which has caused revision of the $\alpha 3\beta 2$ hypothesis to include involvement of $\alpha 6$ [36]. Moreover, it must be emphasized that recombinant expression systems are artificial constructs, and evidence exists that the pharmacology is not always identical to native receptor subtypes [37-39]. The use of recombinant receptors is not widespread, and comparative data for only relatively few compounds have been published [3,40-42]. A comparison of binding and functional data for select compounds is presented in Table 1. Precise assay conditions differ between laboratories, leading to considerable variability in reported data, even for key standards. For example, reported values for the binding affinity of nicotine to the $\alpha 4\beta 2$ nAChR in rat brain membranes range from 0.8 – 8 nM [43,44], while those for binding at the rat $\alpha 7$ subtype (130 – 6000 nM) [45,46] exhibit an even larger spread. The values in Table 1 are chosen as somewhat representative, and perhaps reflect a bias for data from our own laboratory. Nevertheless, they provide a sense of the comparative potencies and subtype selectivities of the best-known compounds – these have served as templates for further SAR and are discussed in more detail below. On the other hand, available data for the rather large number of new nAChR ligands is limited mainly to radioligand binding at the $\alpha 4\beta 2$, and to a lesser degree, the $\alpha 7$ subtypes [13]. In order to facilitate comparison of compounds from different

laboratories, we have reported binding data as K_{rel} , the ratio of the binding constant for the test compound to that of nicotine from the same laboratory.

Consideration of the structures of acetylcholine (ACh) and (-)-nicotine suggests a minimal pharmacophore consisting of a quaternized or protonated (cationic) nitrogen, and a hydrogen-bond acceptor (the C=O of ACh and the pyridine of nicotine). These basic elements are retained in several more sophisticated pharmacophore models that are reviewed in [47]. So far, all of these models reflect binding affinity for the $\alpha 4\beta 2$ receptor, and the shortage of data for other subtypes precludes any analysis of selectivity. Likewise, the reliance on binding affinity necessarily leads to modeling of the desensitized state of the receptor, and available data indicate that binding affinities do not correlate well with potencies for receptor activation [40]. Finally, it is worth noting that, at the NMJ receptor, the cation- π interaction established for acetylcholine is substantially attenuated for nicotine [24], suggesting differing binding modes for these agonists. In any case, nicotine's interaction with the NMJ receptor is much weaker than with neuronal subtypes (see below), even though the putative cation-binding site is well conserved.

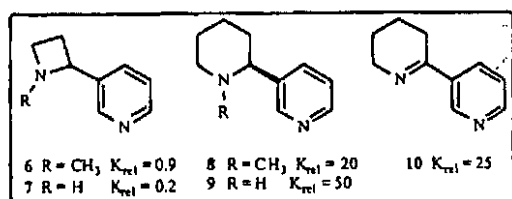


NICOTINE

As the prototype ligand for nAChRs, nicotine has been studied extensively for structure-activity trends. Indeed, (S)-nicotine (1) appears to be well-optimized for activity at these receptors, since most structural modifications result in diminished potency as measured by binding affinity to the high-affinity nAChR (mainly $\alpha 4\beta 2$) in rat brain. Nicotine exhibits significant stereoselectivity, as the (R)-enantiomer (2) binds with 10-30-fold lower affinity [48-50] than the natural (S)-isomer. The N-methyl group of (S)-nicotine contributes substantially to its receptor binding, as (S)-nicotine (3) suffers a 10-fold drop in potency compared to nicotine [49,50]. Interestingly, this effect is reversed for the (R)-enantiomers, as the demethylated analog (4) is 5 times more potent than (R)-nicotine (2) [50]. N-Alkyl groups other than methyl cause a sharp reduction in binding affinity – the N-ethyl homolog of nicotine suffers a 17-fold drop in potency, with even greater attenuation for larger alkyl groups [51]. On the other hand, the dimethyl quaternary salt (5) retains potency similar to nicotine [49].

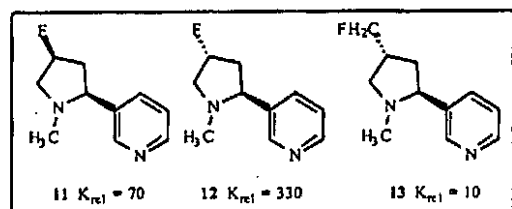
Variations in the azacycle ring size have been explored and the azetidine analog to nicotine, 1-methyl-2-(3-pyridyl)azetidine (MPA, 6), exhibits essentially equivalent affinity to rat brain receptors [49,52,53]. Unlike nicotine, demethylation of the azetidine, as in (7) appears to enhance binding potency [48], but since the available data pertains to

racemic material, it is not known whether there is a differential effect on enantiomers.



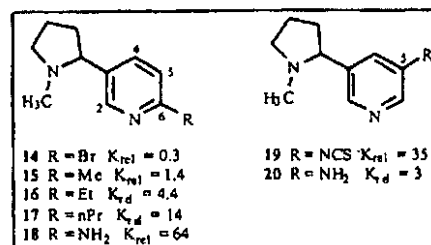
Ring expansion of nicotine leads to compounds of substantially lower affinity at the $\alpha 4\beta 2$ subtype for both *N*-methyl (8) and *N*-H (9) variants [48,54]. On the other hand, it is worth noting that (*S*)-anabasine (9), an isomer of nicotine also found in tobacco, is a more potent ligand at the $\alpha 7$ nAChR than it is at the $\alpha 4\beta 2$ subtype [55] and in fact is five-fold more potent than nicotine at the $\alpha 7$ receptor [55]. Both anabasine and its 1,2-dehydro analog anabaseine (10), a toxin originally isolated from marine worms, fully activate $\alpha 7$ receptors expressed in oocytes, but are weak partial agonists at the $\alpha 4\beta 2$ receptor [55,56]. Anabaseine, especially, has served as a template for $\alpha 7$ -selective compounds (see below).

This feature does not extend to the eight-membered ring analogs of 8-10, which have been disclosed [57]. While these compounds are moderately potent ligands for $\alpha 4\beta 2$ nAChRs, they are significantly less active at the $\alpha 7$ subtype.

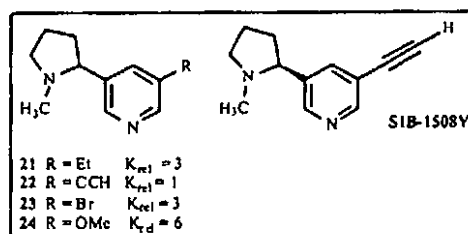


Substitution on the pyrrolidine ring of nicotine has been thoroughly studied – all of the modifications cause diminished potency as measured by affinity to $\alpha 4\beta 2$ nAChRs [58,59]. QSAR analysis of this data indicates that the steric effects are most important, and are amplified when the substituent is near the pyridine or pyrrolidine nitrogen [59], presumably reflecting the limited space in the cation-binding pocket that must accommodate the pyrrolidine ring. A feature not explained by the QSAR equations is the unexpectedly low potency for the *trans*-4-fluoronicotine (12), especially relative to that of 11, where the fluorine occupies the more sterically-sensitive *cis* orientation. Presumably, an unfavorable electronic interaction at the receptor interferes with effective binding of 12; the additional methylene link in 13 is sufficient to mitigate this effect.

While variations in the pyrrolidine ring of nicotine have generally caused a substantial loss of binding affinity, alterations of the pyridine ring have proved to be a more rewarding path to potent analogs. Substitutions at the 2- and 4-pyridine positions, *ortho* to the pyrrolidine attachment,



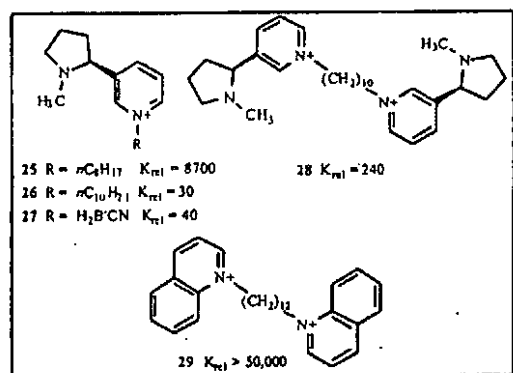
appear to be poorly tolerated [13,48], although very few examples have been reported. Much better success has been achieved with substitutions at the 5- and 6- positions of the pyridine. Halogens at the 6-position (e.g., 14) provide a modest enhancement of binding affinity at the $\alpha 4\beta 2$ receptor compared to nicotine, and small alkyl groups (15-17) are accommodated with only a modest decrease in potency [60]. Analysis of a range of substituents suggests that increased lipophilicity of the group enhances binding affinity, but this effect must be balanced against an opposing steric effect [60]. The 6-Cl, 6-Me, and 6-Et analogs of nicotine are all somewhat more potent than nicotine in the mouse tail-flick model of antinociception, considered to be mediated by $\alpha 4\beta 2$ receptors [61]. In contrast, the *n*-propyl homolog (17) blocks this effect, suggesting a change in functional activity from agonist to antagonist with increasing size of the pyridine 6-substituent [62]. 5-Substituted nicotines have been introduced as affinity labels for neuronal nAChRs [63,64]. The (racemic) isothiocyanate 19 is some 35-fold less potent than nicotine, but irreversibly blocks the high-affinity ($\alpha 4\beta 2$) brain receptor at doses that are accessible for *in vivo* use [63]. The interaction appears to involve only the alpha subunit, and is presumed to represent reaction at the vicinal cysteines near the binding site [64]. Likewise, tritiated versions of 5-amino and 5-iodo nicotine have been developed as photoaffinity labels. The racemic 5-amino analog 20 is only 3-fold less potent than nicotine, a difference more than compensated by the improved efficiency of its photoreactions [64].



The SIBIA group has found that agonist activity of nicotine derivatives is strongly influenced by the pyridine 5-substitution, even when binding affinities do not reflect the difference [65,66]. Thus, the 5-ethynyl analog 22 exhibits affinity for rat $\alpha 4\beta 2$ receptors that is virtually equivalent to nicotine, while the 5-ethyl analog 21 is only 2-3 times weaker. In functional assays at human recombinant nAChRs,

22 is more potent (although somewhat less efficacious) than nicotine toward activation of the $\alpha 4\beta 2$ nAChR (Table 1), while 21 has no detectable functional activity at this subtype. The activity of 22 resides in the (*S*)-enantiomer, designated SIB-1508Y, which is particularly efficacious toward stimulating release of dopamine, attributed to enhanced activity (compared to nicotine) at $\beta 2$ -containing subtypes. Moreover, SIB-1508Y does not activate $\alpha 7$ receptors and is less potent than nicotine at $\beta 4$ -containing receptors. On the basis of its improved pharmacological profile compared to nicotine, SIB-1508Y was selected for preclinical, and ultimately clinical evaluation for treatment of Parkinson's disease [66]. The Sibia group has also reported that replacement of ethynyl in 22 with phenyl causes a shift in subtype selectivity toward activation of $\beta 4$ receptors [67].

Another demonstration of differences in functional activity that are not evident from binding affinities comes from a recent study of 5-bromo (23) and 5-methoxynicotine (24) [68], ligands that bind with slightly lower affinity than nicotine. *In vitro* assays indicate that 23 is a weak partial agonist at $\alpha 4\beta 2$ receptors [66,68], but it is inactive in classical *in vivo* assays of nicotinic function [68]. The 5-methoxy derivative 24, on the other hand, blocks the antinociceptive effect of nicotine, but not its effects on locomotor activity, suggesting that it has antagonist properties toward at least some nAChRs.

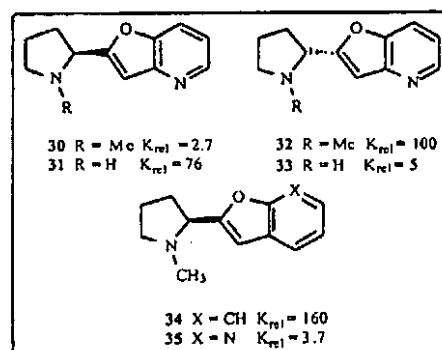


Finally, nicotine pyridinium salts, where the pyridine nitrogen has been alkylated, have been investigated as antagonists at nAChR subtypes [69]. Like its lower homologs, the *n*-octyl nicotininium (NONI, 25) exhibits very poor affinity for the $\alpha 4\beta 2$ receptor (20 μ M) [70]. Nevertheless, 25 is comparatively potent (IC₅₀ = 0.62 μ M) toward inhibition of nicotine-evoked release of dopamine from striatal tissue [71], once thought to be mediated by $\alpha 3\beta 2$ receptors, but likely involving $\alpha 6$ and other $\alpha 3$ -containing subtypes as well [36]. Not surprisingly, 25 is a much weaker antagonist (IC₅₀ = 10 μ M) of nicotine-induced ⁸⁶Rb⁺ efflux from rat thalamic synaptosomes, governed by $\alpha 4\beta 2$ nAChRs. In sharp contrast stands the *n*-decyl homolog (NDNI, 26), which exhibits much improved affinity for the $\alpha 4\beta 2$ receptor [72] and corresponding increase in potency (IC₅₀ = 14 nM) [71] in the thalamic synaptosome assay. Conversely, 26 does not inhibit (IC₅₀ > 100 μ M) dopamine

release, indicating a reversal of subtype selectivity for the two-carbon extension of the nicotininium alkyl chain. Recently, this series was extended to include alkylene-linked pyridinium dimers like 28, but these are relatively weak ligands for $\alpha 4\beta 2$ receptors [72]. The bis(quinolinium) analog 29 was found to be selective for the $\alpha 7$ subtype, with 1.6 μ M binding affinity (compared to 0.45 μ M for nicotine). Finally, a boron complex of nicotine (27), a neutral analog to the *N*-alkyl nicotininium compounds, retains respectable affinity for the $\alpha 4\beta 2$ receptor [73].

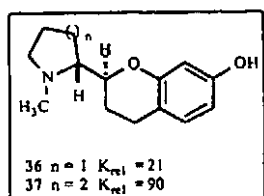
PYRIDINE REPLACEMENTS

In addition to placement of substituent groups on the pyridine of nicotine, SAR studies have led to modified structures where the pyridine ring is replaced altogether. Substituted phenyl derivatives have been evaluated for binding affinity to the rat $\alpha 4\beta 2$ nAChR [74]. Similar trends as for pyridine substitution were observed – *ortho* substituents are not tolerated, *para* halogens enhance potency – but even the best compounds are still 40-fold less potent than (*S*)-nicotine.

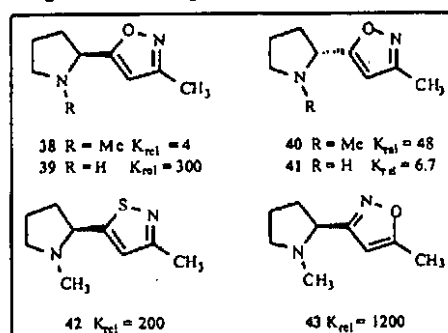


Elliot and coworkers described furo[2,3-*b*]pyridine analogs with comparable potency to nicotine, and improved agonist selectivity [75]. The furo[2,3-*b*]pyridine 30 is a potent ligand for rat brain $\alpha 4\beta 2$ receptors (K_i = 2.7 nM), but exhibits very low affinity for the $\alpha 7$ subtype. Moreover, 30 was slightly more potent than nicotine as an agonist in cells expressing recombinant human $\alpha 4\beta 2$ nAChRs, with much lower activity at ganglionic-type ($\alpha 3\beta 4^*$) receptors in IMR-32 cells. Structure-activity trends in this series are similar to those for nicotine. Removal of the *N*-methyl group (31) causes a 30-fold loss of binding affinity, but in the (*R*)-enantiomer series, the NH compound (33) is more potent than (32). Both the presence and the location of the pyridine nitrogen are critical to high-affinity binding. Thus, the benzofuran analog 34 has low affinity for the $\alpha 4\beta 2$ receptor, and a systematic walk of the nitrogen around the pyridine reveals that only the positions proximal to the ring fusion (i.e., 30 and 35) provide a good fit for receptor binding [76]. Dihydrofuro[2,3-*b*]pyridine analogs of 30 have also been disclosed, but no specific data were provided [77].

The structurally-related chroman derivatives 36 and 37 have been shown to bind with modest affinity to $\alpha 4\beta 2$ receptors [78]. The hydroxyl substituent is critical for

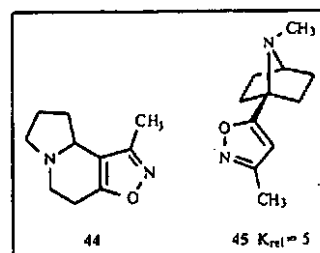


receptor binding, and it is tempting to speculate that this group fills the role of the ring nitrogen discussed above. Other placements of the hydroxyl group, however, are not effective. The compounds have been evaluated as racemates, but the alternative *syn* diastereomers are much less potent. Against recombinant human nAChRs expressed in oocytes, 36 and 37 were both found to be substantially more effective toward activation of the $\alpha 3\beta 2$ and $\alpha 7$ subtypes compared to the $\alpha 4\beta 2$ receptor, and both effect dopamine release from striatal synaptosomes with potency comparable to nicotine. Analog 36 fails to effect cortical ACh release, and is therefore thought to be an antagonist at the $\alpha 4\beta 2$ nAChR [78].



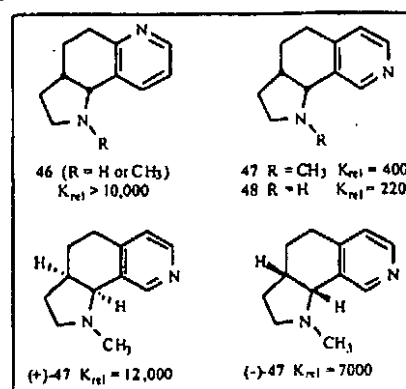
The Abbott group has explored isoxazole as a potential bioisostere for pyridine, leading to clinical evaluation of ABT-418 (38) as a cognition-enhancing drug with an improved therapeutic index relative to nicotine [79]. As for the furopyridines, the *N*-methyl analogs in the (*S*)-enantiomer series have substantially (at least 50-fold) higher affinity for the rat $\alpha 4\beta 2$ nAChR compared to the secondary amines (39), while the opposite trend (NH more potent than NMe) is observed among the (*R*)-enantiomers (40 vs 41) [80]. Groups larger than methyl on the isoxazole can be accommodated with little erosion of binding affinity, but polar groups (CO₂Et and CH₂OH) are not well tolerated. A variety of substitutions around the pyrrolidine were investigated [81,82]; as for nicotine, all of these significantly decreased binding affinity. Finally, modifications of the isoxazole nucleus, for example replacing oxygen by sulfur, as in the isothiazole 42, or interchanging nitrogen and oxygen, to give the regioisomeric isoxazole 43, result in drastic loss of potency [80]. An attempt to rigidify the ABT-418 structure led to 44, which has no detectable affinity for neuronal nAChRs [83]. Since this structure has the incorrect point of attachment to the isoxazole ring, the lack of activity is not surprising. A more interesting structure is the

bridgehead analog 45 [84], which has a binding affinity essentially equivalent to that of ABT-418.

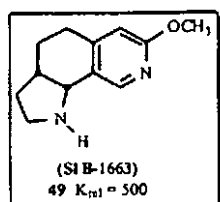


RIGID NICOTINES

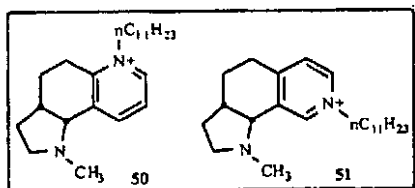
Nicotine possesses conformational flexibility chiefly around the pyrrolidine-pyridine link. Rigidified analogs might have improved potency or subtype selectivity compared to nicotine, and several approaches have been pursued. The *anti*-fused analog 46 was first described by Chavdarian *et al.* [85], but this compound has little activity at nAChRs (K_i at $\alpha 4\beta 2$ and $\alpha 7 > 10 \mu M$) [73]. The alternate *syn*-fused isomer 47 is somewhat more potent ($K_i = 400$ nM and 590 nM at rat $\alpha 4\beta 2$ and $\alpha 7$, respectively) [73], but still much less active than nicotine. In this case, demethylation (48) improves activity at the $\alpha 4\beta 2$ nAChR while sharply attenuating affinity for $\alpha 7$ ($K_i = 20 \mu M$) [73]. The individual enantiomers of 47 have been prepared [86]; curiously, (+)-47 with an absolute configuration corresponding to (*S*)-nicotine, is less potent than (-)-47 by *in vitro* assays. Nevertheless, (+)-47 is more active in a variety of *in vivo* models of nicotinic effects, but these could not be blocked by the nicotinic antagonist mecamylamine, suggesting that the observed effects are driven by non-nicotinic pharmacology [87]. In contrast, the methoxy analog SIB-1663 (49) has been reported to exhibit *in vivo* effects, including antinociception in the tail-flick assay, that are blocked with mecamylamine [88]. SIB-1663 has somewhat lower affinity for rat $\alpha 4\beta 2$ nAChRs compared to 48, but its functional profile at human recombinant subtypes reveals improved efficacy at $\alpha 2\beta 4$ and $\alpha 3\beta 4$ constructs. It was suggested that these subtypes might be responsible for the *in vivo* effects, but this has not been substantiated.



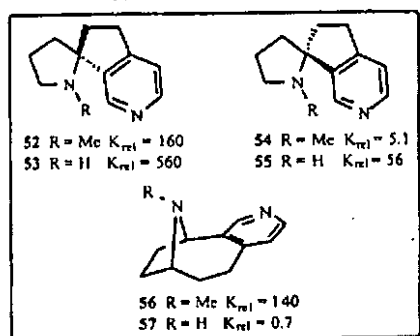
[†] Dan, M. J.; Anderson, D. J. Unpublished observation.



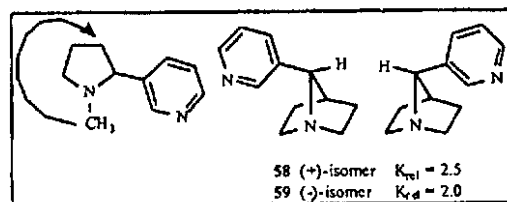
Like the nicotine analogs described above, alkylpyridinium analogs of the fused nicotine analogs have also been investigated [89,90]. The constraint of the ring systems abolishes activity at the $\alpha 4\beta 2$ and $\alpha 7$ subtypes, but the compounds retain reasonable potency for inhibition of nicotine-evoked dopamine release. Both *anti* and *syn* analogs (e.g., 50 and 51) were effective in this assay, with IC_{50} s in the 30-40 nM range [90].



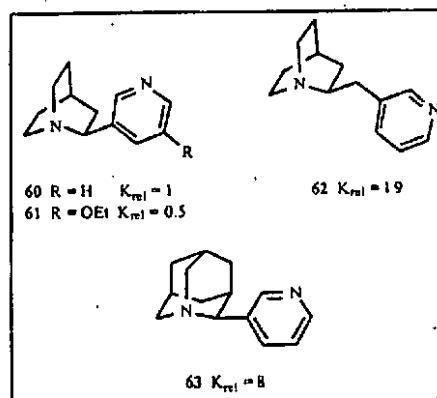
In the fused nicotine analogs, the pyrrolidine and pyridine rings are constrained to a roughly coplanar orientation, while the energetically-preferred conformation of nicotine (and protonated nicotine) places these rings in nearly perpendicular planes [91,92]. This conformation is achieved in the spiro-annulated analogs 52-55, reported by Ullrich and coworkers [93]. The rigid nicotine analog (54) is the first to mimic both the potency and the SAR of the natural alkaloid. As is the case for nicotine, the spirofused analogs exhibit substantial differences in affinity for the (*R*)- (52) vs the (*S*)- (54) configuration. Moreover, demethylation of 54 results in a 10-fold drop in potency, reminiscent of the similar effect for (*S*)-nicotine. These results are certainly consistent with the suggestion that 54 captures the preferred binding conformation for nicotine – its slightly lower binding affinity is reasonably attributed to the additional substitution on the pyridine ring.



Pyrido[3,4-*b*]homotropane (PHT, 57) also locks the pyridine in an orientation roughly perpendicular to the pyrrolidine ring. Although the individual enantiomers have now been prepared [94], biological data have been reported only for the racemate [95]. Here the SAR differs from nicotine – while the NH analog (57) is as potent as nicotine, *N*-methylation (56) causes pronounced attenuation of binding affinity.

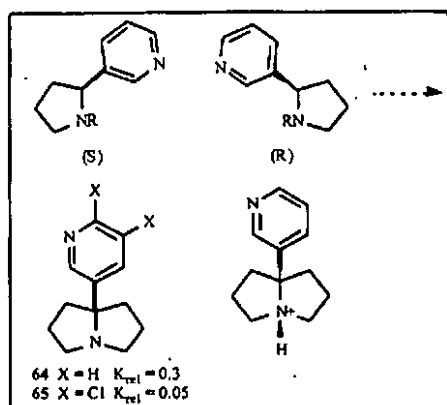


Another interesting modification is evident in the aza-norbornanes 58 and 59, where the *N*-methyl group of nicotine is extended and tethered back to the 3-position of the pyrrolidine. One way to consider this structure is that the basic nitrogen is no longer stereogenic, and the *N*-alkyl group is always pseudoaxial with respect to the pyrrolidine ring. Individual enantiomers for the pyridine attachment have been prepared [96]. Although the absolute configurations have not been established, both antipodes bind with high affinity [93], and nearly equipotent with (*S*)-nicotine.

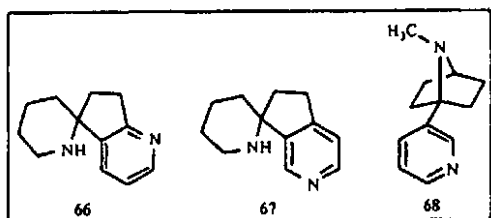


The Targacept group has explored homologous structures. Thus, RJR-2429 (60) binds with high affinity to rodent $\alpha 4\beta 2$ receptors, but appears to act as an antagonist at this subtype [97]. On the other hand, 60 is a potent agonist at the central ($\alpha 6$ and $\alpha 3$ -containing?) nAChRs involved in dopamine release, as well as peripheral receptors including the NMJ and ganglionic ($\alpha 3\beta 4^*$) subtypes [97]. As for other series discussed in this review, substitution on the pyridine 5-position modulates subtype selectivity. Thus, the 5-ethoxy derivative 61 is 2.5-fold less potent than 60 toward stimulation of dopamine release, but 10-fold less active at the NMJ and ganglionic nAChRs [98]. Insertion of a methylene spacer between the pyridine and the azabicyclic ring (62) results in decreased binding affinity, and even more profound loss of

agonist activity at the aforementioned subtypes [99]. On the other hand, analogs of 60 with ring-expansion of the azabicyclic system retain high affinity binding (data not shown) [100]. Replacement with the azaadamantane skeleton as in 63 confers antagonist activity toward dopamine release ($IC_{50} = 700$ nM), and this compound is a weak partial agonist at NMJ and ganglionic subtypes [101].



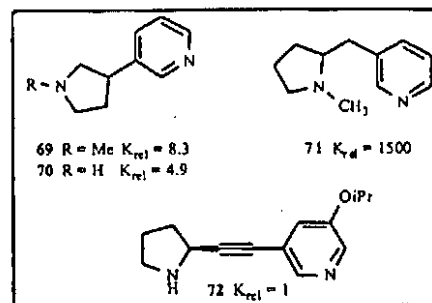
The pyrrolizidine 64 illustrates an interesting structural constraint - formally, this molecule arises by bridging the nicotine *N*-methyl back to the pyrrolidine 2-position. Alternatively, the pyrrolizidine can be viewed as a juxtaposition of (*S*) and (*R*) configurations of the pyrrolidine, and the plane of symmetry renders the molecule achiral [76,102,103]. A significant issue here is that protonation of the basic nitrogen will reasonably occur *cis* to the pyridine, in accord with the computed preference for nicotine itself [91]. The pyridine derivative 64 is in fact a more potent ligand than (*S*)-nicotine at the rat $\alpha 4\beta 2$ nAChR, and the dichloro analog 65 has affinity in the 50 pM range [103].



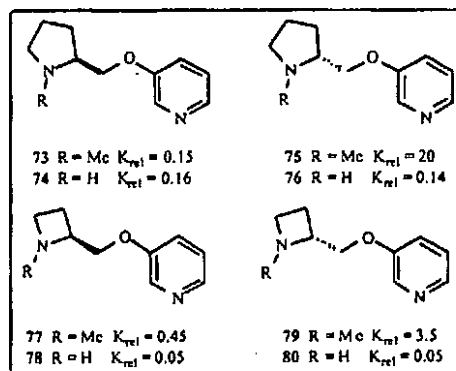
A number of other interesting constrained nicotine derivatives have been synthesized, but biological data has not yet been reported. These include a set of spiro-annulated anabasines 66 and 67 [104], and the bridgehead-substituted 7-azanorbomane 68 (conceptually the reverse of 58/59, where now it is the methylated nitrogen group that is stereogenic) [105].

LINKER MODIFICATIONS

While connection of the pyrrolidine to the 3-position of the pyridine seems to be required for good activity, the



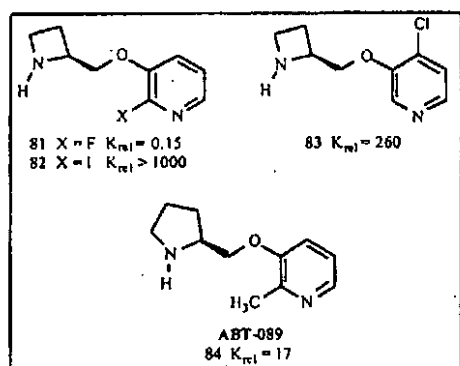
pyridine can be moved to the alternate position of the pyrrolidine with only a small loss of potency. Thus, (racemic) isonicotine 69 is eight-fold less potent than nicotine [106]. The demethylated analog 70 actually has slightly better affinity at the rat $\alpha 4\beta 2$ nAChR while, as for nicotine itself, *N*-alkyl substituents larger than methyl are poorly tolerated [107]. Displacement of the pyridine as in isonicotine amounts to a one-carbon extension of the link between the basic nitrogen and the heterocycle. Homonicotine (71), where the link is extended in the exocyclic fashion, is markedly less effective [108]. Rigidification with the alkyne link (72) affords relatively potent ligands [109], but the analogous alkenyl-linked compounds are comparatively ineffective.



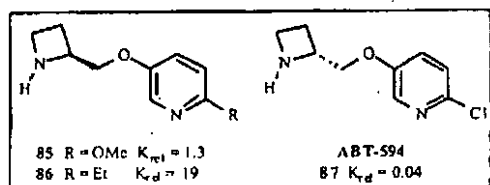
Incorporation of an ether link in the tether results in some very potent nAChR ligands [110]. The (*S*)-pyrrolidine ether 73 binds to rodent $\alpha 4\beta 2$ nAChRs with seven-fold higher affinity than does nicotine. 73 is a full agonist at recombinant human $\alpha 4\beta 2$ nAChRs, as well as the ganglionic ($\alpha 3\beta 4^*$) subtype expressed in IMR32 cells, but is substantially more potent at the $\alpha 4\beta 2$ receptor. High enantioselectivity is evident with this series, so that the (*R*)-enantiomer 75 exhibits much lower affinity for the rat $\alpha 4\beta 2$ receptor than does 73, and is not an agonist in either functional assay. In contrast, both demethylated enantiomers 74 and 76 bind with affinities essentially equal to that of 73, and are full agonists at $\alpha 4\beta 2$ and $\alpha 3\beta 4^*$ nAChRs. The functional potencies reveal an interesting trend in that the NH analogs 74 and 76 are more potent than 73 at the ganglionic ($\alpha 3\beta 4^*$) subtype, but

less potent at the $\alpha 4\beta 2$ nAChR, so that the subtype selectivity of 73 is essentially lost in the NH derivatives.

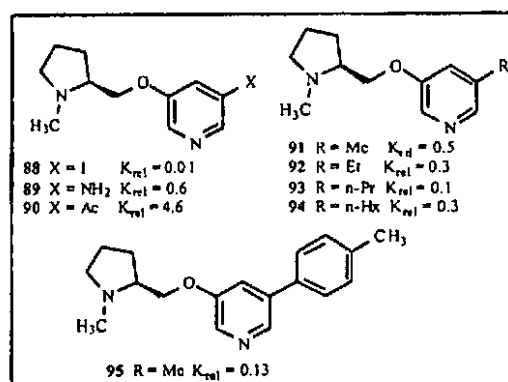
Ring contraction to the azetidines 77-80 results in some of the most potent nAChR ligands yet reported. As for the pyrrolidines, enantioselectivity in binding the *N*-methyl analogs favors the (*S*)-isomer 77 over 79, but the preference here is not as great. In contrast to the pyrrolidine series, both nor-methyl enantiomers 78 and 80 exhibit substantially improved binding affinities compared to 77 and 79. The NH compounds 78 and 80 potently activate both $\alpha 4\beta 2$ and $\alpha 3\beta 4^*$ nAChRs, but with virtually no subtype selectivity. *N*-Methylation attenuates (10- to 100-fold) the potencies at both subtypes, and also reduces maximal efficacies. The effect is somewhat more pronounced on the $\alpha 3\beta 4^*$ subtype, so that 77 exhibits modest selectivity (about 8-fold) for activation of $\alpha 4\beta 2$ vs $\alpha 3\beta 4^*$ subtypes.



Pyridine substituents have been thoroughly evaluated: as for nicotine, tolerance for substitution at the 2- and 4-positions is limited compared to the 6-, and especially the 5-position. Thus, 2-halo derivatives of 78 exhibit a sharp loss of binding affinity with increasing substituent size (see 81 and 82) [111], while the 4-chloro analog (83) is 5000 times less potent than 78 [112]. It has been suggested that larger 2-pyridine substituents disfavor the ligand conformer that is preferred for receptor binding. Nevertheless, the exceptional intrinsic potency of the pyridyl ether system makes some loss of activity tolerable. For example, ABT-089 (84), with a 2-methyl substituent, has 100-fold weaker binding affinity than its parent (74) [113], but this compound is as potent and efficacious as nicotine toward stimulation of ACh release in hippocampal synaptosomes, as well as an *in vivo* model of cognitive function [114]. As ABT-089 does not activate human $\alpha 4\beta 2$ or $\alpha 3\beta 4^*$ receptors *in vitro*, it is likely that these effects result from activation of other nAChR subtypes. ABT-089 also causes weak activation of the $\alpha 7$ receptor, but is considerably less potent than nicotine.

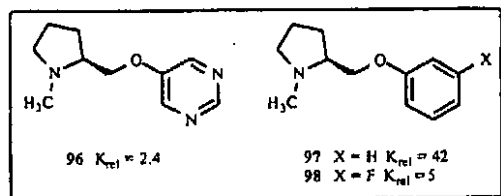


The effects of substitution at the pyridine 6-position are similar to those already described for nicotine. Small groups such as halogens and methyl are readily accommodated. The 6-methoxy derivative (85) has binding affinity comparable to nicotine (albeit 25-fold weaker than 78), but the ethyl analog (86) is 15-fold weaker than 85 [112]. As for nicotine, the 6-halogen substitution improves *in vivo* activity [115]. ABT-594 (87) was identified as a potent analgesic agent whose effects are mediated by central nAChRs [116], and has since advanced to phase 2 clinical trials for treatment of chronic and neuropathic pain [117].

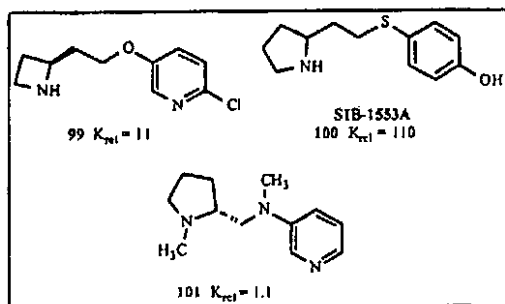


The pyridine 5-position accommodates a very broad range of substituents while maintaining good binding affinity at the $\alpha 4\beta 2$ subtype [112,118,119]. The 5-iodo derivative (88) has been developed as a radioligand with exceptional affinity for the $\alpha 4\beta 2$ nAChR and good selectivity (>5000 fold) for binding at this subtype compared to $\alpha 3\beta 4^*$, $\alpha 7$, and the NMJ receptor [45]. Strongly electron-donating (89) and electron-withdrawing (90) groups can be incorporated with retention of good binding affinity. In contrast to the other pyridine positions, steric effects of 5-substituents have little impact on binding to the $\alpha 4\beta 2$ receptor, and alkyl groups larger than methyl (compare 91-94) actually result in enhanced binding potency. On the other hand, these substituents have a large impact on the ability of the ligand to activate the ion channels. For instance, the ethyl analog (92) is a partial agonist at both $\alpha 4\beta 2$ and $\alpha 3\beta 4$ nAChRs, but the *n*-propyl homolog (93) appears to function as an antagonist at both receptor types [118]. Likewise, a series of aryl and heteroaryl substituents at the pyridine 5-position of 73 have been evaluated. These compounds (e.g., 95) retain subnanomolar binding affinity at $\alpha 4\beta 2$ nAChRs, but function as potent antagonists of the $\alpha 3\beta 4^*$ subtype (for 95, $IC_{50} = 800$ nM) [119]. Thus, it appears that this region of the ligand is important for modulating functional activity, and substitutions here may serve to distinguish different receptor subtypes, as well as to differentiate features of the inactive (desensitized, binding) state of the receptor from the activated state.

As for nicotine, the 3-pyridine attachment for 73 is optimal. Connection at the 2- or 4-pyridine positions results in a dramatic loss of affinity [120]. Among other bis(aza)-aromatics, only 5-pyrimidinyl (96) approaches the activity of

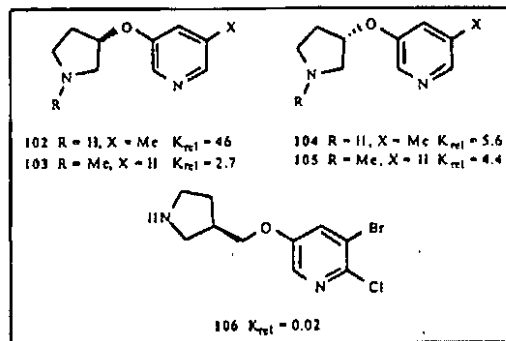


the 3-pyridinyl ethers. In the same vein, a series of substituted phenyl ethers have been studied. The *meta* fluoro phenyl ether (98) is comparatively potent [121], and it has been proposed that the fluorine atom might fulfill the hydrogen bond-acceptor role ascribed to the pyridine nitrogen, albeit with diminished effectiveness.

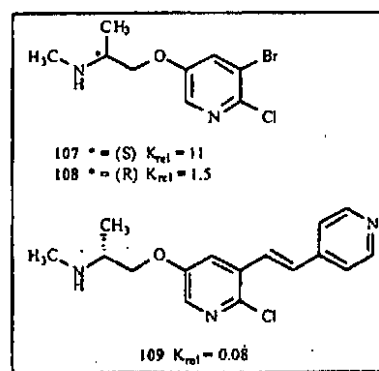


An extensive list of binding data for these pyridinyl ethers has now been published [122]. Similar to nicotine, substitutions on the pyrrolidine ring of 73 are generally not well-tolerated. *N*-Alkyl groups larger than methyl lead to a sharp attenuation of binding affinity, as does extension of the ether link as in 99. A similar effect is seen in the thioether 100 (STB-1553A) reported by SIBIA [67], which binds with relatively low affinity to $\alpha 4\beta 2$ nAChRs. On the other hand, STB-1553A displays functional selectivity for $\beta 4$ -containing subtypes, and has been shown to improve some attentional deficits in rodents and primates, although these effects have not been definitively linked to nAChR activity [123]. Nitrogen-linked pyridines analogous to 73 are generally much less potent, and only the compound 101 with methyl substitution at both the pyrrolidine and linking nitrogens approaches the binding affinity of nicotine [124]. In distinct contrast to the pyridyl ethers, it is the (*R*)-enantiomer 101 that is more potent in the amine-linked analogs. Moreover, incorporation of 6-chloro substitution on the pyridine, and/or ring contraction to the corresponding azetidine, both modifications that enhance activity of the ethers, is highly detrimental to activity of the amine-linked analogs. These SAR differences suggest that, despite their structural similarities, 73 and 101 may bind via significantly different orientations at the nAChR.

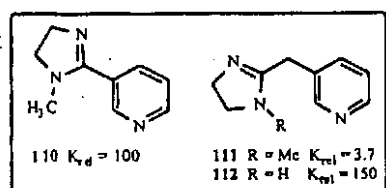
Alternative arrangements of the pyridyl ether scaffold have been developed. The 3-pyrrolidinyl-3'-pyridyl ethers [125-128] maintain the three-atom (CCO) tether between the basic nitrogen and the pyridine ring, but allow less conformational flexibility (fewer rotatable bonds) than



pyridyl ethers like 73. Moderately potent ligands have been developed, and some SAR is evident. For NH-pyrrolidines, the (*R*)-stereochemistry at the ether junction appears to be preferred (102 vs 104) [127], but the difference disappears with *N*-methylation (103 vs 105) [120]. While larger *N*-alkyl groups are not tolerated, the pyridine 5-position can accommodate sterically bulky groups [125-127]. Ring contraction to the azetidine abolishes nAChR activity, while the corresponding piperidines are roughly equipotent to the pyrrolidines [125]. Extension of the linker by insertion of a methylene into the pyrrolidine-oxygen bond provides a substantial boost in binding affinity [127]. Again, the (*R*)-configuration is more potent, and better activity is found for the non-methylated pyrrolidine analogs (106).

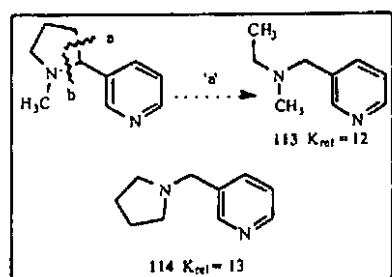


Even the simplest version of the pyridyl ether motif can provide moderately potent compounds [129,130]. A methyl branch *alpha* to the amino substituent improves binding affinity, but larger groups are not tolerated. Nitrogen substitution strongly influences potency in the order $NH_2 > NHMe > NMe_2$; for the NHMe compounds, appreciable enantioselectivity in favor of the (*R*)-isomer is evident (107 vs 108) [129]. Substituent effects on the pyridine ring are consistent with those described above for the azacycle pyridyl ethers, with high-affinity binding achieved with large 5-substituents (109). Functional data for this series have not been reported.



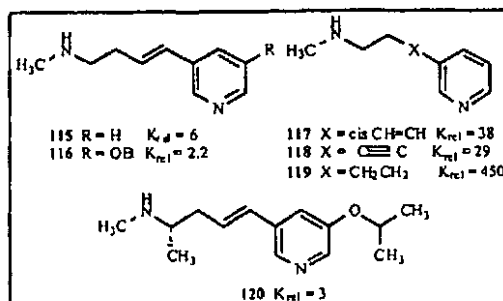
AZA-RING MODIFICATIONS

Azanicotine (110) [108] substitutes the basic amine of nicotine with an amidine function, with resulting loss of the stereogenic center. Azanicotine is much less potent than nicotine, perhaps a consequence of conjugation that will tend to favor a coplanar orientation of the rings. Insertion of a methylene linker (111) disrupts the conjugation, and restores nanomolar binding affinity. The change from 110 to 111 is particularly dramatic when compared to the profound attenuation of potency that accompanies the equivalent modification of nicotine (cf. 71). As for nicotine, demethylation of 111 (112) results in loss of potency [131]. A number of other linker modifications for azanicotine have been evaluated [131], including ethers, amines, alkyl and vinyl, but all of these are inferior to 111.

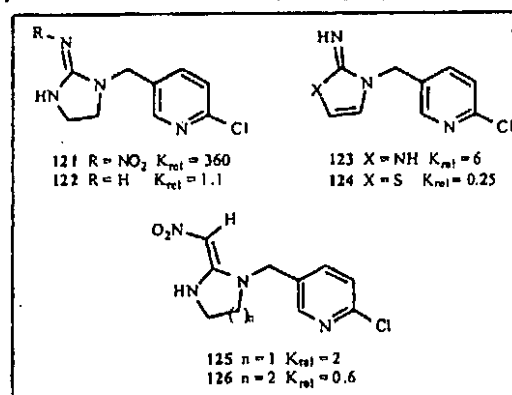


Opening the ring of nicotine can be done in two distinct ways, reflecting different connecting paths from pyridine to the pyrrolidine nitrogen. Cleavage to maintain the short connecting path (as in 'a') leads to aminomethylpyridines like 113 [54,132], with a significant reduction in binding affinity relative to nicotine. The related pyrrolidine 114 is nearly equipotent to 113, but other alkylation motifs are decidedly less effective. A recent evaluation of the effects of pyridine substitution on activity of 113 compared to nicotine (as well as the corresponding ether analogs like 107) reveals differences between the series that suggest non-equivalent binding modes for each [133].

The alternative ring-opening mode ('b') of nicotine results in a four-atom tether between amine and pyridine – the chemical manifestation [134] of this formal cleavage of nicotine leads to *trans*-metanicotine (115, TC-2403), a highly selective nAChR ligand [135]. Only slightly less potent than nicotine at central $\alpha 4\beta 2$ nAChRs, 115 has very low affinity for the $\alpha 7$ receptor, and is virtually devoid of activity at peripheral (ganglionic ($\alpha 3\beta 4^*$) and muscle) subtypes. Metanicotine (115) exhibits cognitive improvement in rodent models of working and reference memory [136]



and is effective in various models of pain [137], with improved threshold for side effects typical of nicotine. Alkoxy substitution on the pyridine 5-position (116) enhances potency [138] and retains the subtype selectivity [139], while modifications of the *E*-olefin as in 117–119 result in diminished binding affinity [140]. A shorter tether [131] or other *N*-substitutions appear to be detrimental. A methyl branch *alpha* to the amine can be tolerated – in the case of 120, the (*S*)-configuration (shown) is 5-fold more potent than the enantiomeric (*R*)-form [141].



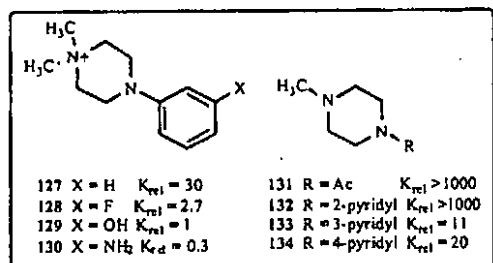
NEONICOTINOIDS

Owing to its toxicity toward a variety of agricultural pests, nicotine has been used historically as a crop protectant [142]. Nicotine, however, is substantially more potent at mammalian receptors than it is at the family of unique nAChR subtypes that populate insect nervous systems. Efforts to identify insect-selective agents that are safer and more effective have led to development of so-called neonicotinoids, exemplified by imidacloprid (121) [143], which has low affinity at mammalian nAChRs, but is nearly 1000-fold more potent than nicotine at insect receptors [144]. Other neonicotinoid analogs involve replacement of the nitroimine moiety of 121 by cyanoimine or nitromethylene. A key feature conferring selectivity for insect nAChRs is thought to be interaction of the 'negatively-charged tip' (e.g., nitro or cyano group) with positively-charged amino acids (arginine or lysine) in the insect nAChR [145] that are absent in mammalian receptors.

While detailed discussion of insect receptors is beyond the scope of this review (for leading references, see [146]), some neonicotinoid analogs exhibit activity at mammalian receptors. For example, 122, a minor metabolite of 121 lacking the nitro group [147], is as potent as nicotine at the rat $\alpha 4\beta 2$ receptor, with several hundred-fold lower affinity to typical insect receptors [144]. The effects of pyridine substitution are similar to those for nicotine. Removal of chlorine results in a small decrease in affinity [147], while azide substitution is accommodated at the 5- (but not 4-) position (data not shown) [144]. Interestingly, a 5-azido substitution on imidacloprid actually enhances binding affinity to the rat $\alpha 4\beta 2$ receptor [148]. Unsaturation in the diaza-ring (123) is detrimental, but the sulfur analog 124 is even more potent than nicotine [147]. Curiously, while the nitro group of 121 causes a dramatic attenuation of $\alpha 4\beta 2$ activity, the nitromethylene analogs 125 and 126 are nearly as potent as nicotine toward the high-affinity ($\alpha 4\beta 2$) site in the mammalian CNS [149,150], while retaining potent activity at insect receptors.

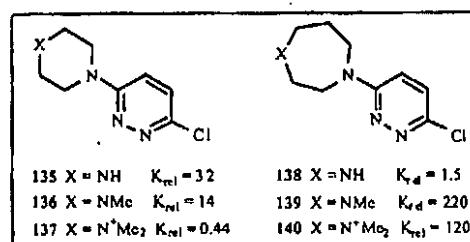
DIAMINES

1, 1 - Dimethyl - 4 - phenylpiperazinium (127, DMPP) represents an unusual pharmacophore for nAChR binding. Although the quaternary ammonium functionality readily mimics the positively charged center of acetylcholine or protonated nicotine, a phenyl group is typically a poor substituent for pyridine or acetyl as the putative H-bond acceptor.

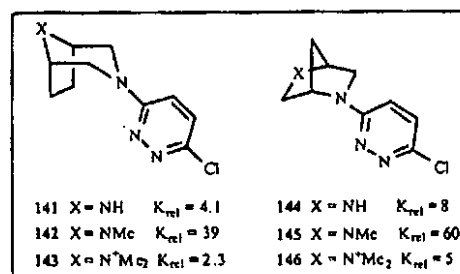


Likewise, the aniline nitrogen of 127 seems ill-suited for this purpose [13]. Nevertheless, DMPP is a relatively potent agonist across a variety of neuronal nAChR subtypes (see Table), and serves as an alternative scaffold on which to construct nAChR ligands. A detailed SAR investigation of substituted DMPP analogs has been reported [151]. *meta*-Substituents, particularly those capable of forming a hydrogen bond, enhance binding affinity (128-130), while placement in the *ortho*- or *para*- positions is not nearly as effective. An interesting feature of this series that sets it apart from others discussed here is that only the quaternary salts, with a permanent positive charge, exhibit potent nAChR binding. The tertiary (*N*-methyl) and secondary (NH) piperazines are typically at least 100-fold weaker. Since it is unlikely that the quaternary compounds will distribute effectively into the CNS, replacement of phenyl with better H-bond acceptors was evaluated in order to identify secondary and tertiary amines that might have improved potency. *N*-Acetyl (131) and 2-pyridinyl (132) are not effective, but 3- and 4-pyridinyl (133 and 134, respectively)

substitution restores respectable binding affinity [44] for the *N*-methyl derivatives. Ring expansion of the diamine to homopiperazine results in a significant enhancement of potency compared to piperazine for the NH analogs [152, 153], but the two series exhibit striking differences in the effect of *N*-methylation on binding affinity [154]. For the piperazines, a modest increase in potency is achieved by conversion of the NH analog 135 to the tertiary *N*-methyl amine 136. A more significant boost in potency accompanies the second methylation to the quaternary salt 137. In contrast, it is the NH (non-methylated) homopiperazine 138 that has the highest binding affinity, and mono (139) or dimethylation (140) results in substantially weaker ligands. This difference holds as well for other examples in the series [153], and implies a change in the binding mode of the 'cationic' nitrogen between piperazine and homopiperazine triads.

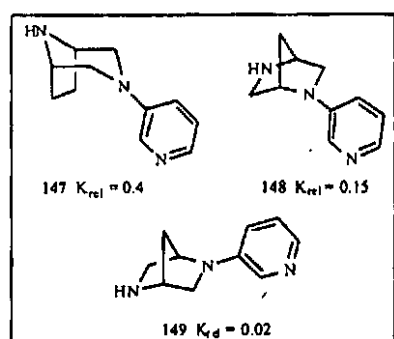


Interestingly, the bridged piperazines 141 and 144 display a different trend, where the monomethyl amine is distinctly less potent than the parent secondary amine, but activity is restored by dimethylation to the quaternary salt [154].

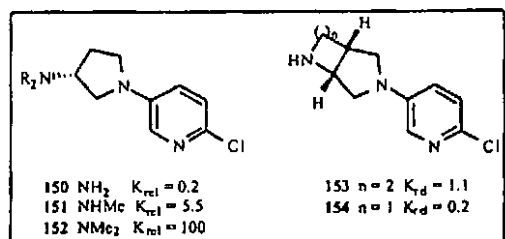


The bridged piperazine DBO-83 (141) is a full agonist with little selectivity between $\alpha 4\beta 2$ and $\alpha 3\beta 4^*$ nAChRs (EC_{50} 24 and 12 μ M, respectively) [155]; the potent analgesic activity of DBO-83 is clearly mediated by activation of central nAChRs [156]. Likewise, analogs of 144 have shown good analgesic activity in the mouse hot plate model [157]. Replacement of the pyridazine ring of 141 or 144 by 3-pyridinyl (but not 2-pyridinyl) affords even more potent compounds (147). The 2,5-diazabicycloheptane analogs are chiral, and the (*R,R*)-enantiomers (e.g., 149) are typically more potent at $\alpha 4\beta 2$ receptors than the (*S,S*)-isomers (148) [157]. 5-Aryloxy analogs of 148 have been disclosed as potent $\alpha 4\beta 2$ ligands [158], and 6-aryl derivatives are reported to be potent $\alpha 7$ compounds [159]. Related bridged bicyclic diamines also serve as useful

scaffolds for construction of potent nAChR ligands [157,160,161].

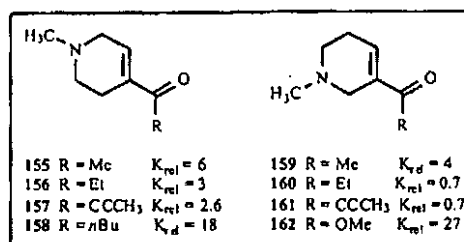


Disconnection of the aza-bridge in 148 affords aminopyrrolidines like 150-152 [162], while fused diamines like 153-154 result from a formal reattachment process [163]. Both series provide potent nAChR ligands that are effective analgesic agents in the mouse hot plate assay, suggesting that these compounds activate central nAChRs. The series 150-152 illustrates the trend, observed for the bridged diamines, that increased substitution at the basic nitrogen center causes attenuation of binding affinity. When the *N*-alkyl group of 151 is tied back as in 153, potency is improved. Moreover, ring contraction to the azetidine (154) results in a boost in potency, reminiscent of the pyridyl ethers described above.

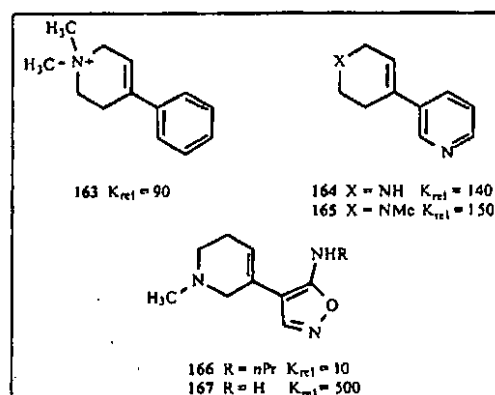


Electronic conjugation in the nitrogen-linked aryl derivatives should favor a coplanar, sp^2 -hybridized arrangement [154], and in some cases the isosteric replacement of the nitrogen link with a vinylic carbon is effective. Although the piperazine acetamide (131) is a poor ligand for the $\alpha 4\beta 2$ nAChR, the sp^2 -carbon linked analog isoarecolone (155) seems to have much greater affinity for CNS receptors [164-166]². Modest enhancement of nAChR potency is achieved in the ethyl and propynyl ketones (156-157); the isomeric arecolones (159-161) exhibit somewhat higher activity at nAChRs [164]. Interestingly, while all of the ketones (156-161) are selective for interaction with nicotinic versus muscarinic receptors, the ester arecoline (162) has the opposite selectivity. *N,N*-Dimethyl quaternary analogs of

² The reported potencies of isoarecolone range from 6 [164], 47 [163], and 250-fold [166] less potent than nicotine. Since most of the available SAR is based on the $K_m = 6$, that value is used here.



isoarecolone are potent activators of the NMJ receptors [167], and also more potent at the $\alpha 4\beta 2$ nAChR [165].



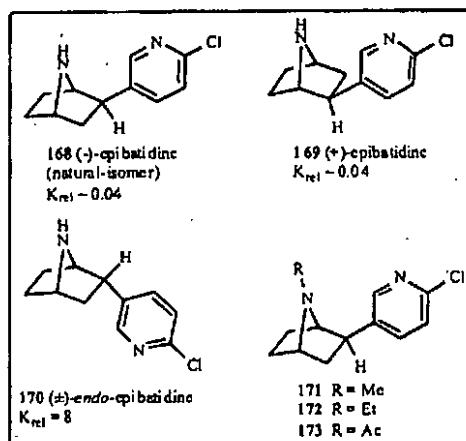
In contrast to 155, aryl-substituted analogs are less potent than the corresponding piperazines. Thus, the quaternary salt of MTPT (163) binds to the $\alpha 4\beta 2$ nAChR with 3-fold lower affinity than DMPP (127), and the 3-pyridyl versions (164 and 165) are likewise less potent than the piperazine analog (128) [151]. In fact, there is little difference in binding affinity between the olefinic compounds (163-165) and their saturated counterparts, suggesting that hybridization/conjugation at the point of ring attachment is not important for these. Isoxazolyl analogs of arecolone have been reported [168], and rather strict boundaries are evident in the SAR. The *N*-propylaminoisoxazole 166 is most potent at nAChRs, and also exhibits highest selectivity versus muscarinic receptors. Nicotinic potency and selectivity are much reduced in the dealkylated version 167.

EPIBATIDINE SAR

Interest in targeting neuronal nAChRs for the development of novel analgesic compounds has been largely fueled in the last decade by the characterization of the exceptionally potent frog toxin epibatidine (168) [169]. This toxic alkaloid, which is 200-fold more potent than morphine at producing analgesia in mice, is the most potent naturally occurring nicotinic agonist isolated to date. Epibatidine activates numerous nAChR subtypes and exhibits efficacy in many pharmacological and behavioral assays at potencies several hundred-fold greater than that of (-)-nicotine [170]. Unfortunately, this lack of nicotinic receptor subtype

selectivity is ultimately manifested in an extremely poor therapeutic index, which prohibits the development of epibatidine as a broad-spectrum analgesic agent. The successful discovery and development of any nicotinic-mediated analgesic compound will likely need to surmount these selectivity hurdles. The unverified assumption is that subtype-selective compounds, particularly those that selectively activate $\alpha 4\beta 2$ nAChRs, might retain the beneficial analgesic properties displayed by epibatidine without the accompanying toxicity due to activation of peripheral ganglionic and neuromuscular nAChRs. In spite of its inherent lack of selectivity, epibatidine has served as a structural template in the design of many new nicotinic ligands since Daly and coworkers reported its structure and incredibly potent biological activity in 1992 [169].

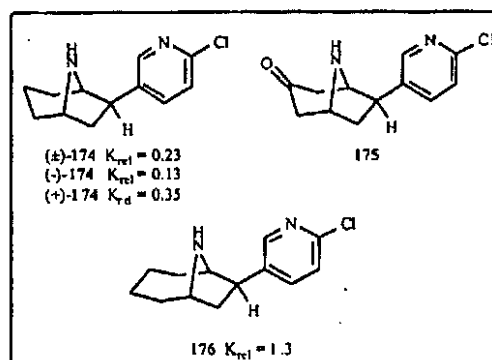
The enantiomers of epibatidine (168 and 169) have essentially equivalent biological activity with respect to their binding, functional and *in vivo* profiles [171]. The *exo*-orientation of the chloropyridine substituent is critically important for biological potency as the *endo*-diastereomer 170 binds with 180-fold lower affinity than 168 [172] and lacks analgesic activity even at relatively high doses [173]. Despite the fact that equivalent picomolar binding affinity is retained in both of the *N*-methyl epibatidine enantiomers 171 [171,174], the (-)-*N*-methyl isomer is approximately 10- to 30-fold more potent than its (+)-antipode at activating rat $\alpha 4\beta 2$, $\alpha 3\beta 4$ and $\alpha 7$ nAChRs [175]. This illustrates the unpredictability frequently associated with relating binding affinities and functional potencies. Larger *N*-substituents significantly decrease activity as exemplified by the binding affinity of the racemic *N*-ethyl derivative 172, which is several hundred-fold weaker than epibatidine [174]. Nicotinic activity is essentially abolished in the *N*-acetyl epibatidine 173, which underscores the requirement of a basic nitrogen in the azanorbomane framework [173].



MODIFICATIONS OF 7-AZANORBORNANE RING SYSTEM

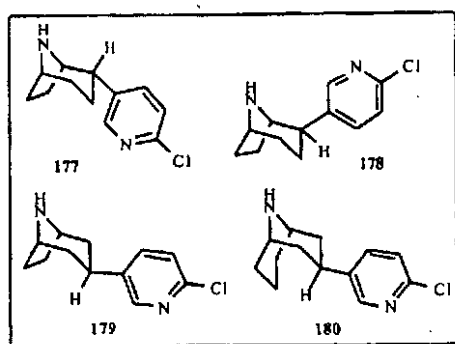
Numerous epibatidine analogs have been prepared in which the rigid 7-azabicyclo[2.2.1]heptane scaffold has been modified. The tropane-derived homoepipatidine derivative

174, in which the two-carbon bridge has been enlarged by one insertion of a methylene unit, exhibits subnanomolar binding affinity [176,177], and elicits an analgesic response in the mouse hot-plate assay at doses only 4-fold higher than that of ($\pm$)-168 [178]. Although little enantioselectivity is observed with respect to binding, (+)-homoepipatidine 174 was significantly more active than (-)-174 in functional assays with $\alpha 4\beta 2$ and $\alpha 3\beta 4$ nAChRs, exhibiting potency and efficacy similar to that of epibatidine [179]. However, like epibatidine, (+)-homoepipatidine 174 does not discriminate between $\alpha 4\beta 2$ or $\alpha 3\beta 4$ receptors, and therefore, it is predicted that this compound may not have the requisite safety window needed for its advancement as a therapeutically useful analgesic. The *N*-methyl and *N*-isopropyl analogs of 174 are also active in the mouse hot plate (no *in vitro* data published), although the *N*-isopropyl derivative is much less potent [178]. The tropane compound 175, however, does not produce any antinociceptive activity. Expansion of the ethano bridge of epibatidine to four-carbons affords compound 176, which has binding affinity similar to nicotine [176].

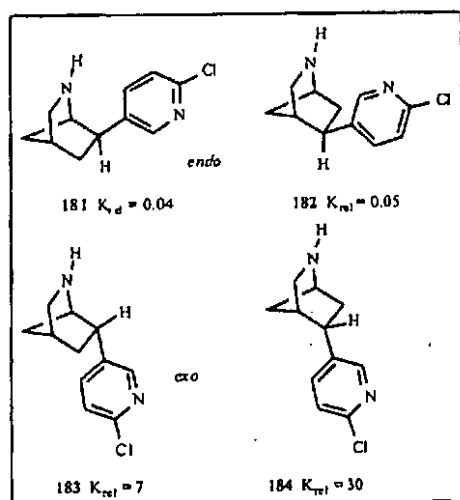


Expansion of the bridge containing the pendant chloropyridine substituent of epibatidine has also been explored. Compounds 177 and 178, prepared in enantiomerically pure form from cocaine, exhibit weak binding affinity for Torpedo receptors and are 30- to 100-fold less potent than epibatidine at stimulating rat mean arterial blood pressure [180]. No data have been reported for the activity of 177 or 178 at $\alpha 4\beta 2$ or $\alpha 7$ nAChRs. The symmetrical azabicyclo-[3.2.1]octane 179 is an interesting hybrid between epibatidine and analogs in the 3,8-diazabicyclo[3.2.1]octane series (exemplified by 147), but no biological data have been reported for this compound [181,182]. The 9-azabicyclo-[3.3.1]nonane analog 180 has also been claimed in a patent application filed by Neurosearch [183].

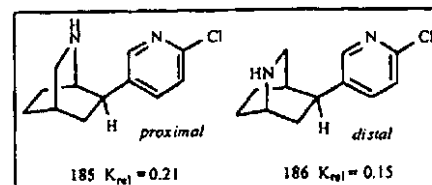
Another simple structural modification of the azaskeleton of epibatidine involves translocation of the apical ring nitrogen from the 7- to the 2-position of the rigid bicyclo[2.2.1]heptane framework. The *endo*-substituted compounds 181 and 182 are structurally similar to epibatidine and possess binding affinity for $\alpha 4\beta 2$ and $\alpha 7$ nAChRs that is comparable to that observed for the frog toxin [76,177]. The enantiomers of 181 have been resolved and preliminary electrophysiological studies indicate that the (+)-enantiomer is much more active than the (-)-antipode [184]. Racemic 181 is nearly equipotent and as efficacious as epibatidine in



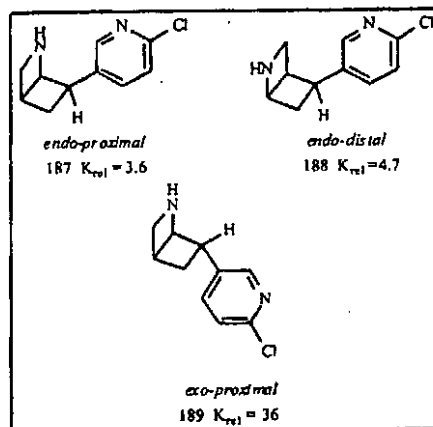
the mouse hot-plate assay, but like epibatidine, this 2-aza analog is also very toxic and causes overt side effects such as seizures at doses only slightly higher than those that induce antinociceptive responses [185]. Meanwhile, the *exo*-substituted 2-azabicycloheptanes, 183 and 184, are much less potent analogs and exhibit affinity that is comparable to the structurally related *endo*-epimer of epibatidine (170).



Expansion of the aza-bridge of epibatidine by insertion of a methylene group affords homoepipatidine analogs 185 and 186 in which the chloropyridine substituent is either proximal or distal to the nitrogen atom [186,187]. Isoquinuclidines 185 and 186 both possess subnanomolar affinity for $\alpha 4 \beta 2$ nAChRs, and produce analgesia in the mouse tail-flick assay, although significant potency differences were noted. The proximal analog 185 is efficacious at doses only 4-fold higher than (-)-168, whereas the distal derivative 186 is 140-fold less potent. The *in vivo* effects of 185 were partially blocked by pretreatment with the nicotinic antagonist mecamylamine, but opioid and muscarinic antagonists had no effect. Unfortunately, both 185 and 186 exhibit marked side effects that were concomitant with the observed analgesic activity.



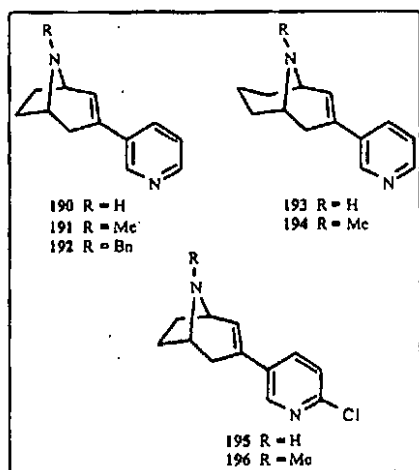
Replacement of the methano-bridge of the 2-azanorbornane series (181-184) with a zero-atom bridge affords fused azabicyclo[2.2.0]hexane analogs (187-189). The *endo*-derivatives, 187 and 188, exhibit low nanomolar binding affinity and the *exo*-substituted compound 189 is 10-fold weaker [188]. Both proximal isomers were only partial agonists at ganglionic-like $\alpha 3 \beta 4^*$ receptors expressed in IMR-32 cells, whereas 188 was more efficacious. However, the 5-*endo* isomer 188 failed to produce an analgesic response in the mouse hot-plate assay at relatively high doses.



Unsaturated analogs of 179 and 180 have been prepared, exemplified by compounds 190-196, and are reasonably potent nicotinic ligands [152,189,190]. Compounds 190 and 193 exhibit potent binding affinity with IC_{50} s only 4.5- and 3-fold less potent than A-85380 [152], which has affinity for $\alpha 4 \beta 2$ nAChRs similar to epibatidine [110]. The *N*-methyl (191 and 194) and *N*-benzyl (192) derivatives are only 3- to 4-fold weaker than the corresponding secondary amines [181,183]. Chloropyridine analogs 195 and 196 also show good binding affinity for $\alpha 4 \beta 2$ receptors [189] and both compounds elicit analgesic responses in the mouse hot-plate and acetic acid-induced writhing assays [190], although overt toxicity and was also noted.

SUBSTITUTED-PYRIDINE ANALOGS OF EPIBATIDINE

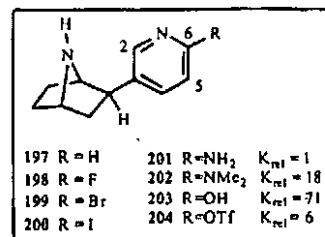
Numerous substituted-pyridine analogs of epibatidine have been synthesized, and several retain the exquisite binding affinity for $\alpha 4 \beta 2$ receptors displayed by the natural product. Included among these are racemic compounds 197-



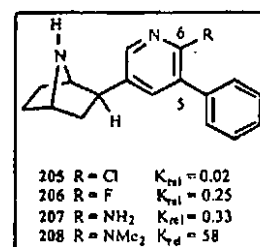
200, in which the chloro substituent has been replaced with a hydrogen, fluorine, bromine, or iodine, respectively [174, 191, 192]. The binding affinity of the amino-substituted analog 201 is similar to that of nicotine, whereas compounds 202-204 have much lower affinity. The *N*-methyl derivatives of 198 and 199 also possess picomolar affinity [174].

Subtype selectivity has also been assessed (for rat $\alpha 2\beta 2$, $\alpha 2\beta 4$, $\alpha 3\beta 2$, $\alpha 3\beta 4$, $\alpha 4\beta 2$, and $\alpha 4\beta 4$ nAChRs expressed in oocytes) in this series, and compounds generally display higher binding affinities for $\beta 2$ -containing receptors over $\beta 4$ -containing receptors [193]. Deschloro-epibatidine (197) and the fluoro-substituted analog (198) were particularly impressive in this regard and exhibit several hundred- to several thousand-fold selectivity for the $\beta 2$ -containing receptors. Unfortunately, the intriguing level of selectivity that was noted in the equilibrium binding assays was not achieved in the corresponding functional assays. For example, although 198 displays >200-fold higher affinity for $\alpha 4\beta 2$ over $\alpha 3\beta 4$ receptors in the competitive binding experiments, nearly identical potency and efficacy are observed in the activation of the $\alpha 4\beta 2$ and $\alpha 3\beta 4$ receptor subtypes.

A similar lack of correlation between ligand binding affinity and functional activity has been observed between epibatidine and deschloro derivative 197 [179]. Removal of the chloro-substituent of epibatidine does not influence affinity for $\alpha 4\beta 2$ as 168 and 197 have equivalent binding potency. However, a dramatic reduction in $\alpha 4\beta 2$ functional activity is manifested. Moreover, this reduced agonist activity at $\alpha 4\beta 2$ receptors was more pronounced for the (-)-enantiomer of 197. In this instance, the decreased functional activity of 197 appears to translate into decreased analgesic activity [194]. The results from these investigations exemplify the disparities commonly observed between binding affinities and functional potencies for receptor activation.

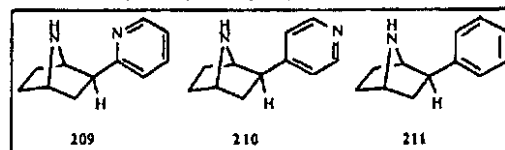


Introduction of a 5-phenyl group into epibatidine (205) does not alter $\alpha 4\beta 2$ binding affinity [192]. However, the *in vivo* potency of 205 is decreased 100-fold relative to 168 in mouse models of analgesia. The enantiomers of the 5-phenyl-6-fluoro analog 206 exhibit equivalent binding affinity, which is approximately 10-fold weaker than 168 [192]. The 5-phenyl-6-amino derivative 207 also has subnanomolar binding affinity, but the 5-phenyl-6-dimethyl-amino compound 208 is much weaker. Of particular interest is the ability of 206 to block the antinociceptive effects of nicotine in the mouse tail-flick and hot-plate assays, with the (-)-enantiomer of 206 being significantly more potent. A selective $\alpha 4\beta 2$ nAChR antagonist that penetrates the blood-brain barrier would be an extremely useful pharmacological tool. Whether or not compound 206 is a selective $\alpha 4\beta 2$ nAChR antagonist remains to be ascertained.

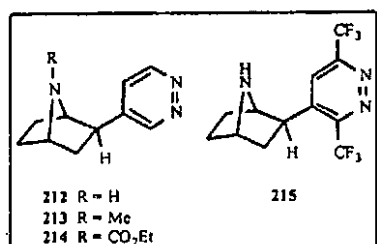


3-PYRIDINE REPLACEMENTS

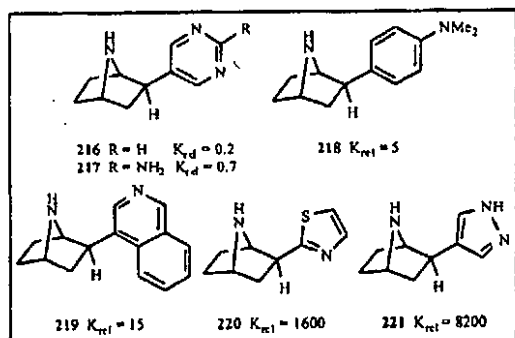
The functional activity of 2-pyridyl (209) and 4-pyridyl (210) analogs of deschloroepibatidine (197), and the *exo*-phenyl-substituted 7-azanorbornane compound 211 have been explored in $\alpha 4\beta 2$, $\alpha 3\beta 4$, and $\alpha 7$ nAChRs (no binding data reported) [179]. $\alpha 4\beta 2$ activity is essentially abolished in all three derivatives indicating that a 3-pyridyl-linked heterocycle is optimal for achieving good functional activity at this subtype. The enantiomers of 209 differentially modulate the activity of $\alpha 7$ receptors. The (+)-2-pyridyl enantiomer is a partial agonist (80% efficacy vs acetylcholine), in contrast to the (-)-enantiomer of 209, which blocks the response of acetylcholine at $\alpha 7$ nAChRs [179, 195]. Both 210 and 211 elicit significant inward currents in oocytes expressing $\alpha 3\beta 4$ receptors.



4-Pyridazines have been examined as bioisosteric replacements for the chloropyridine substituent of epibatidine [196]. The unsubstituted 4-pyridazinyl analog **212** and its *N*-methyl derivative **213** are more potent than nicotine, but somewhat weaker than epibatidine at activating rat $\alpha 4\beta 2$, $\alpha 3\beta 4$, $\alpha 7$ and $\alpha 1$ receptors expressed in *Xenopus* oocytes. The ethyl carbamate **214** and the 3,6-bis(trifluoromethyl)-substituted pyridazine **215** fail to show any agonist activity. Pyridazines **212** and **213** show modest levels of functional selectivity between $\alpha 4\beta 2$ and $\alpha 3\beta 4$ receptor subtypes, 8- and 25-fold, respectively, which is an improvement over the nonselective ligand epibatidine.

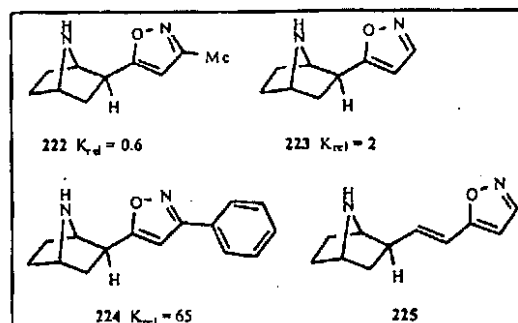


A variety of aryl groups have been utilized as potential bioisosteres for the chloropyridine substituent of epibatidine as illustrated by compounds **216-221**. These isosteric replacements provide analogs having a wide range of binding affinities, all less potent than epibatidine. The 5-pyrimidinyl derivative **216** and the 2-amino analog **217** have subnanomolar binding affinity, whereas dimethylaniline **218** and the 4-isoquinolinyl compound **219** are less active (100- and 300-fold less potent than 168, respectively). Meanwhile, the 2-thiazole and 4-pyrazole are poor surrogates for the chloropyridine group since **220** and **221** possess only micromolar binding affinity [197].

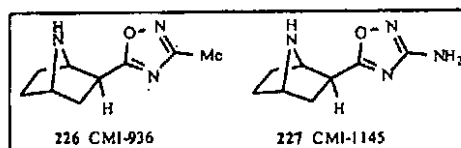


Based on the precedent of ABT-418 relative to (*S*)-nicotine, substituted isoxazoles have been utilized as bioisosteric replacements for the chloropyridyl ring of epibatidine. Isoxazole **222**, termed ($\pm$)-epiboxidine by Daly and coworkers, is approximately 10-fold less potent than epibatidine with respect to $\alpha 4\beta 2$ receptor binding affinity and a similar reduction in potency was noted in the mouse hot plate assay [198]. The unsubstituted isoxazole **223** binds with 3-fold weaker affinity relative to **222**, whereas the phenyl-substituted analog **224** is 110-fold less potent [199]. Another

isoxazole derivative **225** ($K_i = 80$ nM, no data reported for standard nicotinic ligands), recently described by Targacept scientists, contains an olefinic linker between the aromatic heterocycle and the azanorbomane skeleton [200].

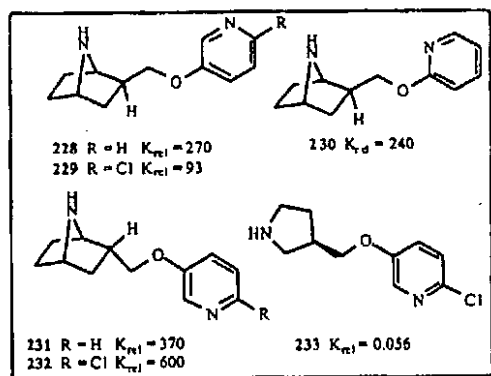


The 1,2,4-oxadiazole analogs **226** and **227** produce potent antinociception in the mouse tail-flick assay, but this effect has been shown to be mediated by activation of muscarinic M_4 receptors and not nAChRs [194,201]. *In vitro* data have not been published for these derivatives, although the (+)-*N*-methyl enantiomer of oxadiazole **226** reportedly displaces [³H]-cytisine from rat brain preparations with a $K_i = 1,400$ nM [194]. Thus a complete change in pharmacology from nicotinic to muscarinic was realized between isoxazole **222** and oxadiazole **226**, by simply replacing CH with N in the isoxazole heterocycle.

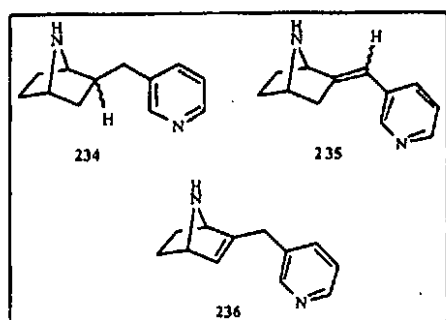


A series of *endo*- and *exo*-pyridyl ether analogs of epibatidine, compounds **228-232**, has been synthesized and $\alpha 4\beta 2$ receptor binding affinity has been evaluated [202]. The most potent ligand, *exo*-chloropyridyl ether **229**, has >5,000-fold weaker binding affinity for $\alpha 4\beta 2$ nAChRs than epibatidine. The 2-pyridyl analog **230** exhibits binding potency similar to the 3-pyridyl derivative **228**. The *endo*-substituted analogs **231** and **232** are somewhat less potent. In this series, the pyridinyloxymethyl substituent is clearly not a good bioisosteric replacement for the chloropyridine heterocycle of epibatidine. The weak activity of these epibatidine pyridyl ethers stands in stark contrast to many incredibly potent and efficacious pyridyl ether ligands, such as A-85380 (78) and ABT-594 (87). Another striking example is illustrated by removal of the ethano-bridge of **229**, which affords the isonicotine pyridyl ether analog **233**, which has picomolar affinity comparable to epibatidine [127].

A recent Targacept patent application has highlighted potential nicotinic ligands in which the aromatic heterocycle is linked to the azabicyclic system through an alkylene or alkylidene unit [203]. Compounds **234-236** are examples of 3-pyridyl alkylene bridged 7-azanorbomane analogs that are

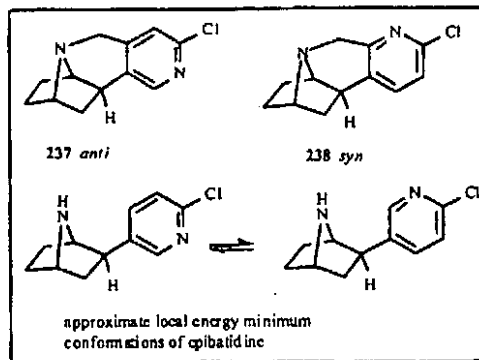


specifically claimed. The saturated pyridylmethyl homoeipibatidine analog 234 reportedly has moderate binding affinity for central nicotinic receptors ($K_i = 43$ nM, no binding data provided for any standard nicotinic ligands).



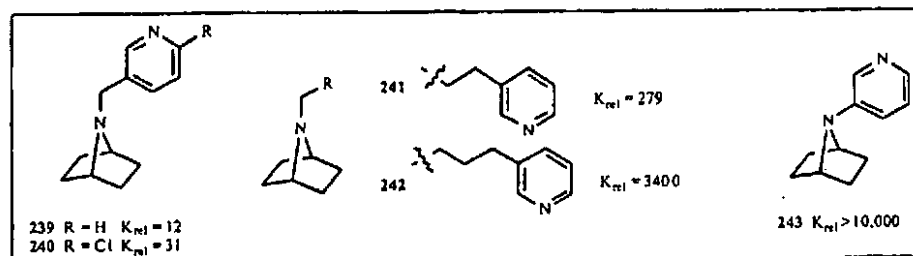
CONFORMATIONALLY CONSTRAINED EPIBATIDINE ANALOGS

Two conformationally locked analogs of epibatidine have been synthesized [204]. The structures of 237 and 238 are comparable to the two low energy conformations of epibatidine that differ by roughly 180° in the rotation of the chloropyridine substituent. Although no numerical data were reported, these compounds apparently failed to show high affinity for $\alpha 4\beta 2$ nicotinic receptors.



A series of *N*-arylalkyl-substituted 7-azabicyclo[2.2.1]heptanes have been evaluated as potential nicotinic ligands [205]. The methylene-linked 3-pyridyl analogs 239 and 240 are the most potent analogs in the series, approximately 12- and 31-fold weaker than (-)-nicotine, respectively, and equipotent to the structurally related pyrrolidine 114. A significant reduction in activity was noted with the 2- or 4-pyridyl analogs. Likewise, homologation of the methylene linker to ethyl (241) or *n*-propyl (242) causes a decrease in binding affinity relative to 239. Removal of the linker and direct attachment of the 3-pyridyl heterocycle to the 7-azanorbomane nitrogen affords analog 243, which fails to show any detectable interaction with $\alpha 4\beta 2$ receptors. Both 239 and 240 produce dose-dependent analgesia upon subcutaneous administration in the tail-flick and hot-plate assays, but fatal seizures are observed at doses only slightly above those required for antinociception.

As illustrated by the discussion above, many alterations of the 7-azabicyclo[2.2.1]heptane skeleton or modifications of the chloropyridine heterocycle of epibatidine afford compounds that possess potent $\alpha 4\beta 2$ receptor binding affinity, and several of these derivatives induce antinociceptive responses in rodent models of pain. However, it is unfortunate that the *in vivo* profiles of these potent epibatidine analogs generally appear to mimic the natural product in that significant toxicity is observed at or just above analgesic doses. It is interesting to note that although the enantiomers of epibatidine are equally active with respect to nicotinic receptor binding and function, some simple modifications of the 7-azanorbomane core provide analogs, such as homoeipibatidine 171 and 2-azaepibatidine 181, that display significant levels of enantioselectivity. The search for analogs of epibatidine that possess high levels of receptor

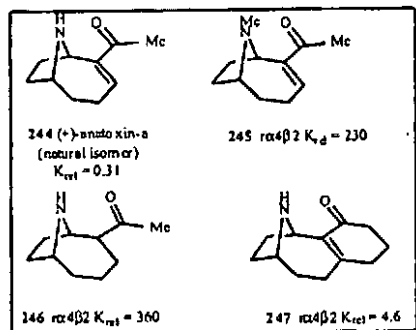


subtype selectivity, particularly for activation of $\alpha 4\beta 2$ over ganglionic receptors, and ultimately have good *in vivo* activity with a large safety window is ongoing.

ANATOXIN AND RELATED ANALOGS

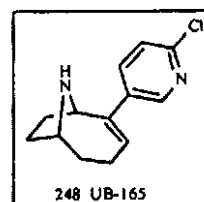
(+)-Anatoxin-a (244), originally described as the very fast death factor (VFDF), is a potent nicotinic agonist isolated from specific strains of freshwater blue-green algae [206]. Ingestion of the neurotoxin by livestock, waterfowl, and other wildlife species can ultimately result in death due to potent muscle depolarization and subsequent respiratory paralysis. (+)-Anatoxin-a is generally 10- to 50-fold more potent than (-)-nicotine in a variety of assays assessing function at neuromuscular, peripheral, and central ($\alpha 4\beta 2$ and $\alpha 7$) nicotinic receptors [207-209]. A fairly wide range of values has been reported for the binding affinity of 244 to $\alpha 4\beta 2$ nAChRs [210,211]. In our unpublished binding studies on the fumarate salt of (+)-anatoxin-a, we determined a K_i of 0.31 nM for displacement of [^{3} H]-cytisine from rat brain membranes.³ It is interesting to note that unlike most of the highest affinity nicotinic ligands, which contain a 3-pyridyl heterocycle as a critical structural component (i.e. epibatidine and A-85380), anatoxin-a simply possesses a methyl ketone substituent.

Several structure activity studies of anatoxin-a have been reported, although, until the discovery of UB-165 (*vide infra*), none of these investigations provided analogs that were more active than the natural product. The natural (+)-enantiomer of anatoxin-a (244) is significantly more active than the (-)-antipode (82), as demonstrated by its >1,000-fold higher affinity for rat brain $\alpha 4\beta 2$ and >200-fold potency at $\alpha 7$ receptors [211]. N-Methylation (245) results in a >700-fold decrease in $\alpha 4\beta 2$ binding affinity, while reduction of the olefin (246) only diminishes potency 10-fold relative to 244 [212]. Homologation of the ketone alkyl group from methyl to ethyl, n-propyl, or isopropyl provides derivatives with comparable activity, although the n-propyl analog has 20-fold higher affinity than 244 for $\alpha 7$ receptors [211]. Other modifications of the methyl ketone functionality, such as replacement with a carboxylic acid, ester, amide, aldehyde, or oxime, lead to a decrease in potency relative to anatoxin-a [212]. A constrained analog has been synthesized in an attempt to mimic the presumed bioactive conformer of anatoxin-a [213]. The fused *s-trans* analog 247 exhibits approximately 10-fold weaker $\alpha 4\beta 2$ affinity than 244 [3].



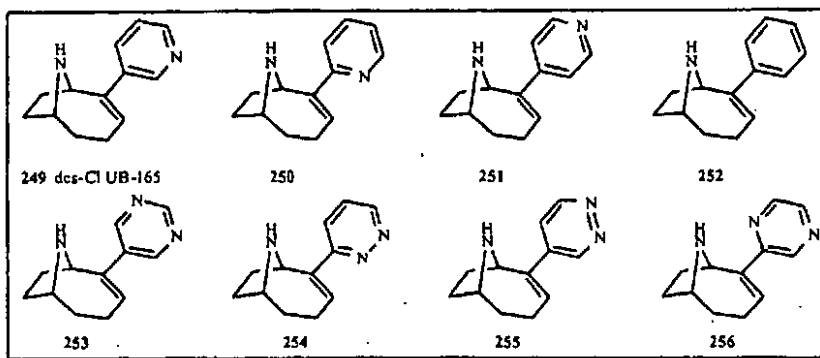
³ Dan, M. J.; Anderson, D. J. Unpublished observation.

UB-165 (248) is a novel, rationally designed hybrid of the exceptionally potent nicotinic ligands anatoxin-a and epibatidine, in which the methyl ketone functionality of anatoxin-a has been replaced with a chloropyridine substituent that is present in epibatidine [214]. It possesses affinity for $\alpha 4\beta 2$, $\alpha 7$, and $\alpha 3\beta 4$ binding sites that is intermediate between the values of the parent molecules [215,216]. UB-165 has been reported to displace (-)-[^{3} H]-nicotine and ($\pm$)-[^{3} H]-epibatidine binding from rat brain preparations with K_i values 0.17 nM [214] and 0.04 nM [216], respectively. The absolute configuration of UB-165 corresponds to that of natural (+)-anatoxin-a and, like anatoxin-a, a high degree of enantioselectivity is observed with respect to affinity for $\alpha 4\beta 2$ receptors (UB-165 is at least 20-fold more potent than its enantiomer) [214]. This compound fully activates a variety of nicotinic receptors, including the $\alpha 7$, $\alpha 3\beta 4$, $\alpha 3\beta 2$, and $\alpha 4\beta 4$ subtypes, but is only a very weak partial agonist at $\alpha 4\beta 2$ nAChRs [217].

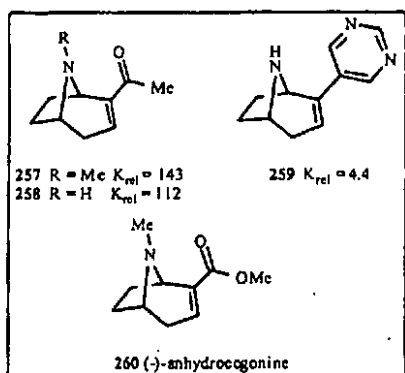


Deschloro-UB-165 (249) retains the potent $\alpha 4\beta 2$ binding affinity of 248, but exhibits a >10-fold increase in binding potency relative to UB-165 at $\alpha 7$ receptors [215,216]. However, $\alpha 4\beta 2$ binding affinity is diminished by several orders of magnitude in the 2-pyridyl (250), 4-pyridyl (251) [215] and phenyl (252) [218] analogs, demonstrating the importance of the 3-pyridyl nitrogen that is generally required for potent nicotinic activity. Several diazine derivatives have also been synthesized. The 5-pyrimidine 253 and 3-pyridazine 254 analogs are approximately 10-fold less potent than 248 with respect to $\alpha 4\beta 2$ affinity. In addition, 254 exhibits particularly high binding to $\alpha 7$ receptors that is comparable to epibatidine and deschloro-UB-165 [215]. The 4-pyridazine and 2-pyrazine heterocycles proved to be poor bioisosteres for the 3-pyridyl substituent of UB-165, since the binding affinity of 255 and 256 for $\alpha 4\beta 2$ receptors was reduced several hundred-fold [215,216].

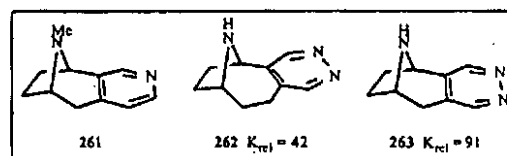
The natural product (+)-ferruginine is a potent plant-derived neurotoxin [219,220]. Its (-)-enantiomer 257, which is readily prepared from (-)-cocaine, has been shown to have some agonist activity at nicotinic receptors. Ferruginine and anatoxin-a are structurally related as removal of a methylene unit from the 9-azabicyclo[4.2.1]nonene skeleton of (+)-anatoxin-a (244) affords the tropene derivative (-)-norferruginine (258). (-)-Ferruginine (257) and its desmethyl analog 258 are relatively weak ligands having approximately 100-fold reduced affinity for $\alpha 4\beta 2$ receptors relative to (-)-nicotine [221]. Several diazine-containing bioisosteres of (-)-ferruginine have been prepared, but only the 5-pyrimidine 259 has good $\alpha 4\beta 2$ affinity [221]. The methyl ester of (-)-ferruginine, a tropane derivative known as anhydroecgonine (260) that has comparable $\alpha 4\beta 2$ affinity to 257 [222], is the major pyrolysis product formed by elimination of benzoic



acid from (-)-cocaine while free base smoking [223]. In direct analogy with UB-165, it would be of interest to evaluate the nicotinic activity of ferruginine analogs having a 3-pyridyl bioisosteric substituent in place of the acyl moiety.



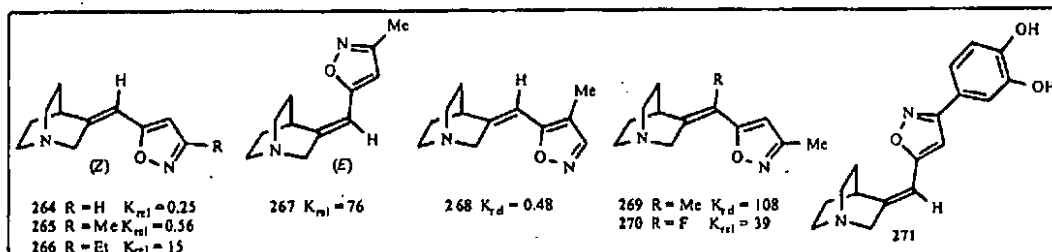
Pyrido[3,4-*b*]homotropane (PHT, 57) represents a conformationally restricted structural hybrid of normicotine (3) and anatoxin-a (244). The analogous conformationally constrained bioisosteric variant of ferruginine has also been synthesized (261) [94], but no biological data have been reported. Several novel diazine analogs of PHT and 261 have recently been evaluated for their binding to $\alpha 4\beta 2$ nAChRs and the most potent derivatives, 262 and 263, interact with only moderate affinity [224].



NOVO NORDISK QUINUCLIDINES AND RELATED COMPOUNDS

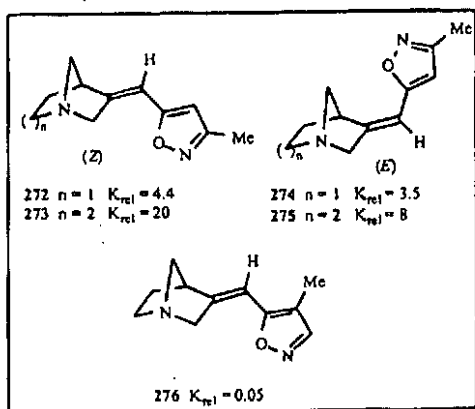
Novo Nordisk has actively pursued several series of 1-azabicyclic compounds linked to an aromatic heterocycle through an alkylidene bridge, which are exemplified by the (isoxazole)methylene quinuclidines 264-270 [225-227]. In the (*Z*)-olefinic series, only small substituents, such as hydrogen (264) or methyl (265), are readily accommodated on the isoxazole heterocycle, and compounds with larger substituents (266) display a marked decrease in affinity. The (*Z*)-isomers are much more potent than the corresponding (*E*)-analogs (compare 265 and 267) in this quinuclidine series. Replacement of the methyl substituent of (*E*)-isomer 267 with larger groups such as ethyl, propyl or methoxymethyl affords analogs with IC_{50} values $>1,000$ nM [226]. The 4-methylisoxazoly derivative 268 exhibits high affinity that is comparable to the 3-methyl isomer 265. Substitution of the methyldiene bridge with methyl (269) or fluoro (270) results in significantly decreased binding potency.

A related series described by Koh and coworkers contains substituted 3-(3-phenylisoxazoly)methylene-quinuclidines,



in which the nicotinic or muscarinic pharmacology of these ligands is largely dependent upon the phenyl ring substituents [228]. Although binding affinities were generally low, in general, small polar substituents, such as hydroxy and amino, tended to favor binding to $\alpha 4 \beta 2$ nAChRs, while large hydrophobic groups favored binding to M_1 muscarinic receptors. The most potent nicotinic ligand was the catechol derivative 271, which has 40-fold reduced affinity relative to 265.

Modifications of the quinuclidine ring to the 1-azabicyclo[2.2.1]heptane and 1-azabicyclo[3.2.1]octane skeletons have also been reported [225,226]. With these two azabicycloalkane scaffolds, olefin geometry does not appear to play a major role in determining binding potency (compare 272/274 and 273/275), in contrast to the corresponding quinuclidines (265/267). The 4-methylisoxazole 276 is the most potent derivative analyzed and possesses extremely high binding affinity that is equivalent to epibatidine and 10-fold more potent than quinuclidine 268.



The 3-pyridyl heterocycle and methylisoxazole substituent are known bioisosteres with respect to nicotinic

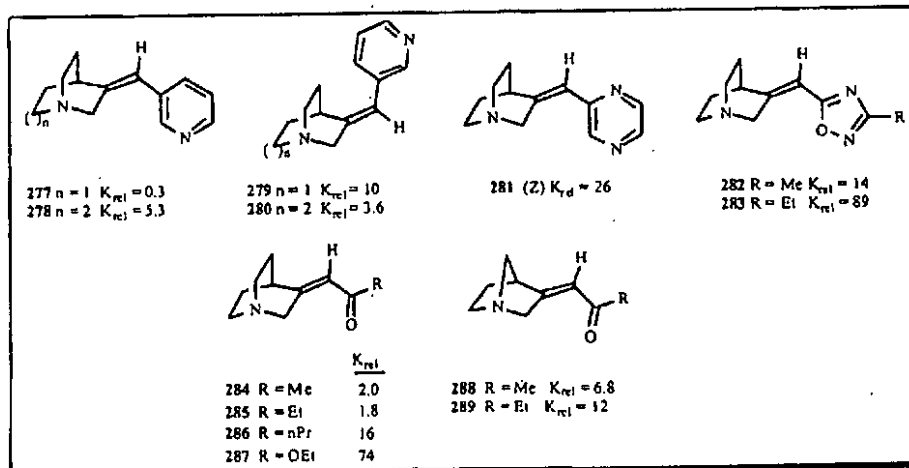
activity [79,198], and this trend is preserved in the methylidene 1-azabicycloalkane series [227]. The most active 3-pyridyl derivative is quinuclidine 277, which is equipotent with isoxazole analogs 264 and 265. The corresponding (*E*)-isomer (279) has 33-fold lower affinity. The 2- and 4-pyridyl analogs possessed IC_{50} values of $>1,000$ nM. The (*Z*)- and (*E*)-isomeric azabicyclo[3.2.1]octane derivatives 278 and 280 displayed roughly equal affinity which was >12 -fold less potent than 277. A dramatic reduction in binding affinity (85-fold) was noted upon replacement of the 3-pyridyl substituent of 277 with a 2-pyrazinyl heterocycle to give 281. Likewise, addition of a nitrogen to isoxazole 265 to provide 3-methyl[1,2,4]oxadiazole 282 also resulted in diminished binding potency. A further decrease in binding affinity was noted upon extension of the oxadiazole methyl substituent to ethyl (283).

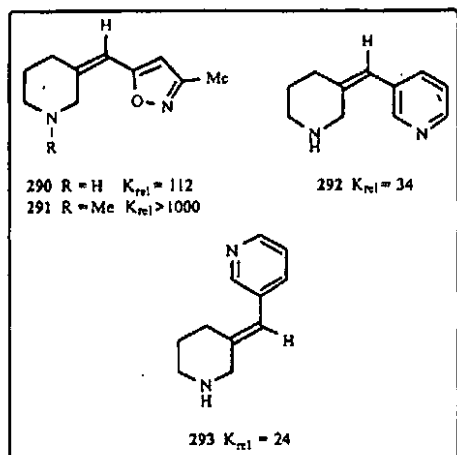
Small acyl substituents serve as useful bioisosteric replacements for the 3-pyridyl or methylisoxazole substituents in the methylidene-linked quinuclidine series. The α, β -unsaturated methyl and ethyl ketones 284 and 285 maintain good binding affinity, which decreases roughly 8-fold in the *n*-propyl derivative 286. Meanwhile, the ethyl ester 287 is much less potent. The two ketone derivatives in the 1-azanobornane series, 288 and 289, are somewhat weaker than quinuclidines 284 and 285, but exhibit comparable binding affinity to their corresponding isoxazole counterpart 272.

Removal of an ethano bridge from quinuclidine 265 affords piperidine 290, which interacts with $\alpha 4 \beta 2$ receptors with 200-fold lower affinity. The *N*-methyl analog 291 is significantly weaker than 290. The pyrrolidine, azetidine and (*E*)-olefinic piperidine analogs of 290 all have IC_{50} values of $>1,000$ nM. The 3-pyridyl derivative 292 and 293 are approximately 100-fold less active than quinuclidine 277.

CYTISINE SAR

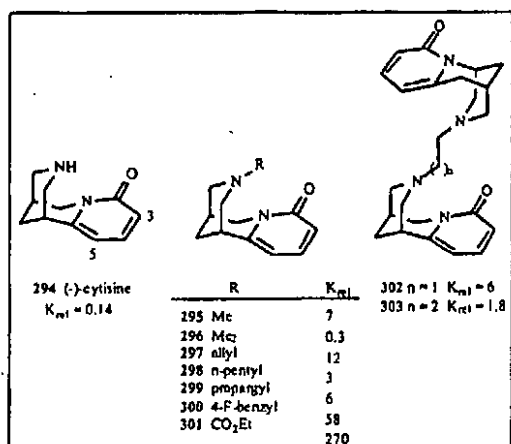
(-)-Cytisine (294) is a toxic alkaloid found in many plants or seeds of the Leguminosae family. Its peripheral actions were described over 90 years ago as being indistinguishable from nicotine [229], although cytisine is a more potent





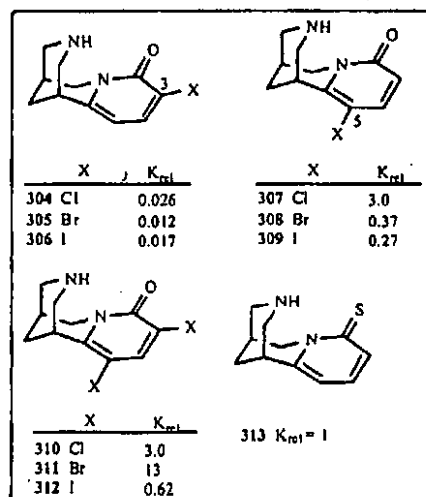
ganglionic stimulant [230]. The main structural features of 294 include a bispidine skeleton fused to a 2-pyridone heterocycle. (-)-Cytisine displays potent subnanomolar affinity for $\alpha 4\beta 2$ receptors [231-233], but is a poor agonist with only weak efficacy at this subtype [41,234]. Structure activity studies have concentrated on substitution of the secondary amine and halogenation of the pyridone ring.

N-Methyl cytosine (cauliphylline, 295) has 50-fold lower affinity for $\alpha 4\beta 2$ receptors than cytosine, but cauliphylline methiodide (296) retains subnanomolar affinity [235]. Numerous cytosine analogs possessing a variety of *N*-substituents have been evaluated (i.e. 297-301), but all are significantly less active than the parent [236,237]. Analogs with relatively small *N*-alkyl substituents (297-299) are roughly 20- to 100-fold less potent, whereas, compounds with larger substituents, such as the fluorobenzyl derivative 300, exhibit a further reduction in binding affinity. Ethyl carbamate 301 also has low binding affinity, again demon-



strating the requirement of a basic amine functionality for a nicotinic ligand to have potent activity. A couple of interesting dimeric compounds, joined by linkage of two cytosine molecules through an ethyl or propyl chain (302 and 303), have similar affinity as some of the *N*-alkyl cytosine analogs.

Halogenation of the pyridone ring of (-)-cytosine provides a series of analogs with generally high $\alpha 4\beta 2$ binding affinity [234,235]. The 3-halo derivatives (304-312) are particularly potent and have binding affinity roughly equivalent to epibatidine. Like (-)-cytosine, the 3-bromo analog 305 is a full agonist at recombinant human $\alpha 7$ nAChRs (40-fold more potent than 294) and only a partial agonist at $\alpha 4\beta 2$ and $\alpha 4\beta 4$ receptors [234]. Interestingly, both 305 and (-)-cytosine appear to functionally discriminate between two apparently distinct receptor subform populations each of $\alpha 4\beta 2$ [238-241] and $\alpha 4\beta 4$. Meanwhile, the 5-bromo and 3,5-dibromo cytosine analogs 308 and 311 are only partial agonists at $\alpha 7$ and $\alpha 4\beta 4$ nAChRs and fail to elicit inward currents in oocytes expressing $\alpha 4\beta 2$ receptors. Disparate binding values have been reported for the 3,5-dibromo compound 311. In one report, 311 has 90-fold weaker $\alpha 4\beta 2$ binding affinity relative to 294 [235], whereas a 2.5-fold increased binding potency compared to (-)-cytosine was determined in another investigation [234]. Replacement of the lactam carbonyl oxygen of 294 with a sulfur affords thiocytosine 313, which binds with 7-fold weaker affinity than (-)-cytosine.



LOBELINE

The properties of the naturally-occurring alkaloid lobeline (314), a constituent of the Indian tobacco plant (*Lobelia inflata*), resemble those of nicotine in many respects [242,243]. Its affinity for the rat brain cytosine binding site is roughly the same as that of nicotine ($K_{rel} = 2.5$) [233,244], although it does not activate $\alpha 4\beta 2$ receptors expressed in oocytes [242]. Both nicotine and lobeline produce bradycardia and hypotension, in anesthetized rats [245], and both cause hyperalgesia when administered into

the rat brainstem [246]. Lobeline mimics the performance of nicotine in certain behavioral assays in rodents [247,248], however the effects in these assays are not blocked by the nicotinic antagonist mecamylamine [43], suggesting the involvement of an alternative mechanism of action. In contrast to nicotine, chronic treatment of rats with lobeline does not promote central nAChR upregulation [249], nor does the response of animals conditioned to nicotine generalize to lobeline [250]. Thus, the actions of lobeline are pharmacologically complex and distinct from that of nicotine. Recent evidence suggests that lobeline may exert some of its *in vivo* effects by a mechanism involving inhibition of dopamine uptake and stimulation of DA release from synaptic vesicles within dopaminergic terminals [243]. Lobeline and its analogs have been investigated for the aid of smoking cessation, and are claimed for the treatment of CNS disorders and for drug addiction [251-253].

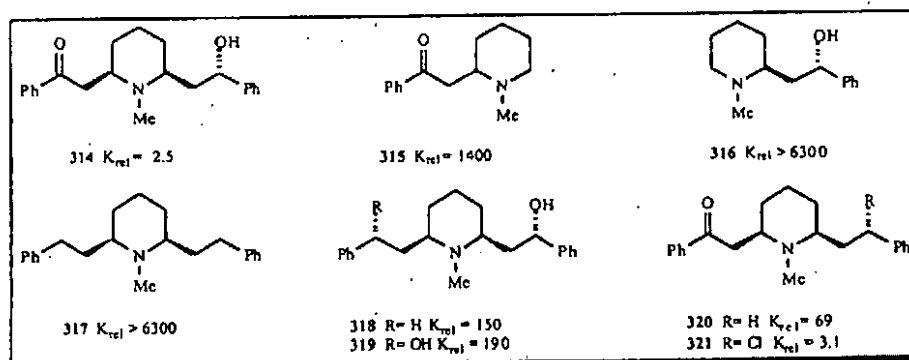
Structurally, lobeline bears little resemblance to nicotine and does not easily fit the classical nicotinic pharmacophore model. It has been demonstrated that the piperidine ring is required for strong binding affinity, and it is tempting to overlay the basic amine with the pyrrolidine portion of nicotine, and allow one of lobeline's oxygens to assume the putative hydrogen-bonding role of the pyridine nitrogen [254]. If this were valid, then truncated analogs such as 315 or 316 might be expected to retain much of the high nAChR affinity of the natural product. However, it has been shown that the ketone derivative 315 is >500-fold weaker than lobeline and the reduced variant 316 has even poorer affinity [255]. Clearly, both side-chains are required for a strong interaction with the receptor. Furthermore, at least one must be oxygenated, as illustrated by the observation that the symmetrical amine 317 (lobelan) lacking both oxygen functionalities is a poor ligand for the receptor, whereas addition of either the hydroxyl (318) or ketone (320) restores some affinity. The symmetrical diol 319 does not appear to gain any advantage over 318 from the additional hydroxyl substituent, yet the chloroketone 321 is nearly equipotent with lobeline itself. In spite of these and other medicinal chemistry efforts [256,257], no lobeline analogs have yet been reported that improve on the potency of the parent compound.

$\alpha 7$ nAChR LIGANDS

The $\alpha 7$ nAChR is increasingly the target of therapeutic intervention. Possible indications overlap to some extent with those associated with the $\alpha 4\beta 2$ subtype, including the treatment of cognitive deficiencies associated with Alzheimer's disease and other dementias, ADHD, pain, depression, and Parkinson's disease, in addition to schizophrenia and neuroprotection. A recent report delineates a role for $\alpha 7$ in the inflammatory response, suggesting a new direction for research in this arena [258]. The $\alpha 7$ receptor has unique biophysical properties, including high calcium permeability and rapid desensitization kinetics, and there is a correspondingly unique SAR surrounding ligands for this subtype. In contrast to the $\alpha 4\beta 2$ situation, high selectivity for $\alpha 7$ over other nicotinic subtypes has been fairly easy to achieve. Indeed, interference from non-nicotinic pharmacologies (5-HT₂, muscarinic, etc.) has sometimes been a more vexing problem [259-261]. Nevertheless, several highly selective $\alpha 7$ ligands have recently become available which may serve to further elucidate the function of these receptors in native systems.

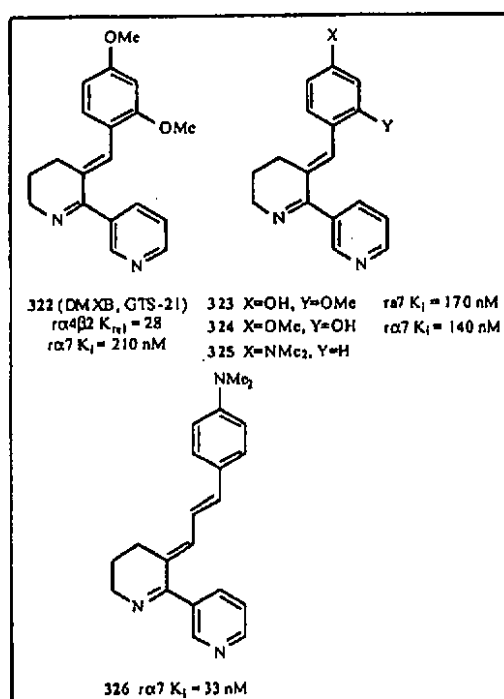
ANABASEINE/GTS-21

The worm alkaloid anabaseine (10) is a weak partial agonist at the $\alpha 4\beta 2$ nAChR subtype, but it is substantially more efficacious than either nicotine or acetylcholine at rat $\alpha 7$ receptors expressed in oocytes [262]. Its inherent subtype bias has positioned anabaseine as a template for the construction of novel $\alpha 7$ -selective agonists [263]. SAR investigations revealed that conjugated aryl substituents at the piperidine C-3 position magnify anabaseine's intrinsic $\alpha 7$ preference, primarily by attenuating activity across all subtypes, but to a lesser extent at $\alpha 7$ than at the others. The (2,4-dimethoxybenzylidene)anabaseine derivative 322 (DMXB, GTS-21) in particular has emerged as an important compound for the characterization of $\alpha 7$ function *in vivo* [262]. Despite its weak potency and low maximal efficacy at the rat $\alpha 7$ nAChR ($EC_{50} = 81 \mu M$, 50% of ACh response) [264], GTS-21 has demonstrated effectiveness in rodent models of cognition and memory [265,266]. Significantly, it does not act as a nicotine cue, suggesting that $\alpha 7$ receptors play at most a limited role in the addiction liabilities of nicotinic compounds [267]. Although GTS-21 is even less



active at the human receptor ($<20\%$, $EC_{50} = 109 \mu\text{M}$) [264], it has shown some encouraging hints of efficacy in human trials for cognition enhancement [263]. The primary metabolite of GTS-21, the 4-hydroxy analog 323, has 4-fold better potency and greater efficacy at the human $\alpha 7$ nAChR ($EC_{50} = 26 \mu\text{M}$, 40% of ACh) than the parent compound, and may therefore be a strong contributor to the observed *in vivo* effects [264]. On the other hand, it was recently reported that although GTS-21 does not activate the mouse 5-HT_{1A} receptor, 323 is a weak agonist, and other metabolites such as 324 are even more potent [259].

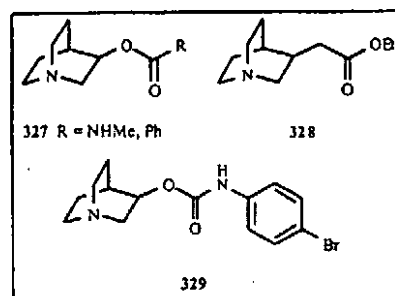
Further optimization studies in the benzylidene anabaseine series have identified several analogs with improved $\alpha 7$ affinity. The *p*-dimethylamino variant 325 is only slightly more potent than GTS-21, however the extended cinnamylidene analog 326 shows a much more substantial improvement [262]. A number of additional analogs have been prepared and evaluated at the cockroach nAChR, but none were as potent as the well-known nicotinic imidaclopride.



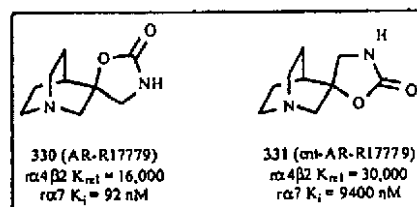
QUINUCLIDINES

Quinuclidine (1-azabicyclo[2.2.2]octane) has been a popular scaffold for the construction of novel nicotinic ligands, with most efforts in this area directed towards the $\alpha 7$ nAChR subtype. Simple quinuclidinol esters and carbamates such as 327, which are essentially constrained analogs of the endogenous ligand ACh, are reported to have comparable affinity for both nicotinic and muscarinic rat

brain receptors [244]. The same holds true for the methylene-linked analog 328 [270,271]. However, *N*-aryl carbamates such as 329 are selective nAChR agonists, particularly for the $\alpha 7$ subtype [272]. Although no corroborating data have been published, compounds such as 329 are claimed for their potential utility in the treatment of a broad variety of psychotic conditions, cognitive deficits, and pain. Analogs containing alternative azabicyclic ring systems have also been prepared, but they are allegedly inferior to the quinuclidine analogs.

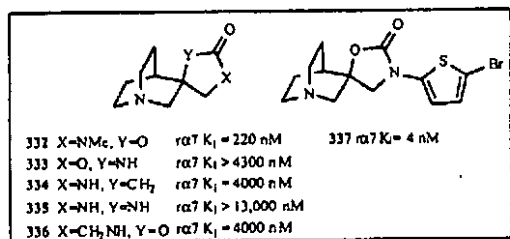


In an effort to further reduce conformational flexibility in the carbamate structures, the AstraZeneca group prepared a series of spirocyclic quinuclidine derivatives. The prototypical example is the spirooxazolidinone 330 (AR-R17779) which is a full agonist ($EC_{50} = 21 \mu\text{M}$, 96% relative to ACh) at the rat $\alpha 7$ nAChR in oocytes with excellent selectivity *in vitro* for $\alpha 7$ versus the $\alpha 4\beta 2$, $\alpha 4\beta 4$, and $\alpha 3\beta 4$ nAChR subtypes [273,274]. A growing body of evidence suggests a link between $\alpha 7$ activation and a variety of biological effects, yet *in vivo* studies with this selective compound have failed to show very impressive efficacy. AR-R17779 has been shown to enhance learning and memory function in rats (radial arm maze model) [275], but it fails to improve performance in a model of attention [276]. Although a genetic link has been established between mutations at $\alpha 7$ and sensory deficits in schizophrenics [277], AR-R17779 has no effect on auditory gating using a pre-pulse inhibition paradigm [278]. It has been suggested that 330 possesses poor pharmacokinetic properties [11], which may help to explain the observed lack of *in vivo* efficacy, and may be expected to limit the usefulness of this compound therapeutically.



Efforts to map the SAR around AR-R17779 have revealed that its $\alpha 7$ activity is sensitive to even minor structural changes in the spirooxazolidinone region [273]. The enantiomer of AR-R17779 (331) is essentially inactive

at $\alpha 7$ (<10% in oocytes), and transposition of N and O of the oxazolidinone (333) abolishes activity. *N*-Methylation (332) causes a modest attenuation of $\alpha 7$ potency, but it significantly erodes selectivity by enhancing $\alpha 4\beta 2$ binding affinity. Replacing the spirooxazolidinone ring oxygen with either CH₂ or NH affords the lactam 334 or urea 335 analogs, respectively, both of which lack significant affinity for the $\alpha 7$ nAChR. Ring expansion of AR-R17779 to the six-membered ring urethane analog 336 likewise produces an inactive compound. On the other hand, a series of *N*-aryl and *N*-heteroaryl analogs of AR-R17779 have been prepared by Welfide [279] and are purported to show enhanced affinity for the $\alpha 7$ nAChR. Bromothiophene 337, for example, has particularly high $\alpha 7$ affinity ($K_i = 4$ nM) and is 500-fold selective versus $\alpha 4\beta 2$. It might be anticipated that the addition of the more lipophilic side-chain would improve the pharmacokinetic characteristics relative to AR-R17779, but no information on this issue is available.

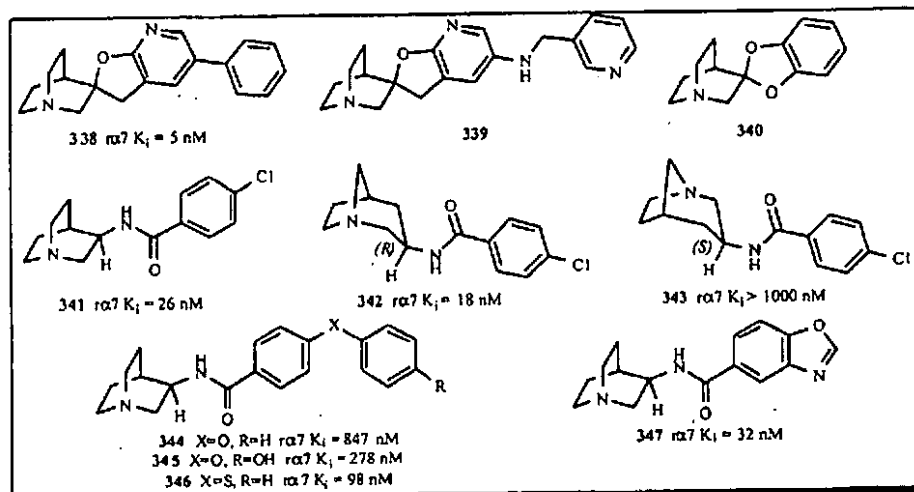


Further SAR explorations of the spirocyclic quinuclidine motif have led to a novel series of highly selective dihydrofuropyridines exemplified by PSAB-OPF (338) [261,280-282]. This compound shows a >15-fold boost in binding affinity relative to AR-R17779 (330), with comparable intrinsic functional activity (83%, $EC_{50} = 2.2$ μ M), and furthermore it retains the excellent nAChR subtype selectivity profile of AR-R17779. Confounding any efforts to ascribe *in vivo* effects of 338 to $\alpha 7$ activation is the fact

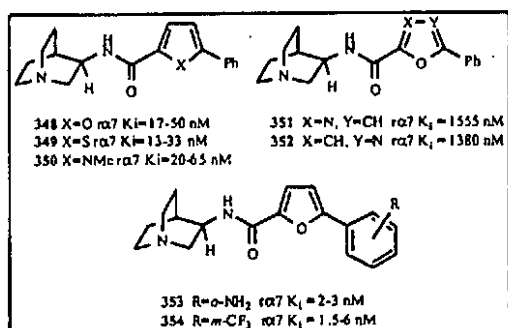
that the compound is essentially equipotent at $\alpha 7$ and the 5-HT₃ receptor [261], an ion channel with high homology to $\alpha 7$, particularly in the ligand binding domain. Fortunately, it has been reported that the cross reactivity at the 5-HT₃R can be abolished simply by replacing the phenyl group on the furopyridine portion of 338 with a pyridylmethylamine substituent (339) [11,283]. Another entry in the spirocyclic class comes from NeuroSearch, who has disclosed quinuclidine ketals exemplified by 340 that are moderately selective for $\alpha 7$ ($IC_{50} = 0.19$ μ M versus α -bungarotoxin) vs. $\alpha 4\beta 2$ ($IC_{50} = 30$ μ M versus cytisine) [284].

The past three years have seen a flurry of patent activity describing substituted aryl-, biaryl-, and heteroaryl amide derivatives of 3-aminoquinuclidine as selective $\alpha 7$ ligands (both agonists and antagonists). Even very simple analogs like the *p*-chlorobenzamide 341, for instance, show impressively high binding affinity for the rat $\alpha 7$ receptor. Although only limited data have been revealed, it is clear that a wide variety of aryl groups can be accommodated, and that a generally high degree of enantioselectivity exists favoring (3*R*)-aminoquinuclidine amides over the (3*S*)-antipodes. The *exo* isomers of related azabicycles 342 and 343 mirror this dramatic stereochemical preference, as the (3*R*)-enantiomer 342 binds in the nanomolar range whereas the (3*S*)-enantiomer 343 shows no affinity for the rat $\alpha 7$ nAChR [285]. Both AstraZeneca [286] and Pharmacia [287-289] have staked claims to more elaborate aryl amides exemplified by the phenyl ether 344 that presumably utilize additional hydrophobic interactions with the $\alpha 7$ receptor to improve selectivity. Substituting a *p*-hydroxyl on the terminal aromatic ring (345) results in a 3-fold enhancement in potency, and the thioether analog 346 boosts affinity by another factor of three. Further improvements were realized by the preparation of fused ring analogs like the benzoxazole 347 [290], which is nearly equipotent with the *p*-chlorobenzamide 341.

In terms of affinity for the rat $\alpha 7$ receptor, the best quinuclidine amide analogs reported to date are derived from

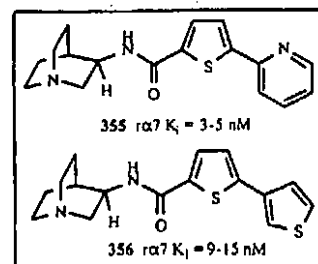


heterocyclic carboxylic acids. Furan (348), thiophene (349), and *N*-methylpyrrole (350) analogs bearing phenyl groups or other aromatic moieties at C-5 generally bind to the $\alpha 7$ nAChR in the low nanomolar range [291-293]. Additional heteroatom replacements in the five-membered ring, as in the oxazole analogs 351 and 352, attenuate affinity for $\alpha 7$ by at least an order of magnitude. The effects of modifications to the terminal aromatic ring do not follow obvious trends, but a couple of generalizations are possible. For instance, small hydrophobic substituents (chloro, methyl) are allowed at any position, and polar groups at C-2 (e.g. 353) appear to improve affinity for the $\alpha 7$ subtype. A single fluorine substituent anywhere on the terminal phenyl weakens binding by at least a factor of two, with additional fluoro groups decreasing affinity even more dramatically. An interesting exception is the *m*-trifluoromethyl analog 354, which is reported to bind at 1.5-6 nM, nearly 70 times as potent as the *o*-CF₃ version.

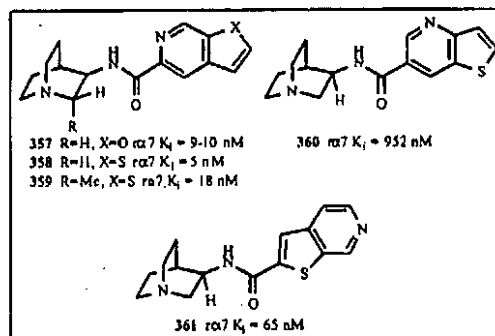


Two analogs in this series have been more extensively profiled by a group at Eli Lilly [11,281,282]. The 2-pyridyl compound 355 and the 3-thienyl analog 356 are exceptionally potent full agonists at human $\alpha 7$ receptors (EC_{50} = 14 nM and EC_{50} = 37 nM, respectively) with no functional activity at other nAChRs as determined by FLIPR analysis in recombinant cell lines. Notably, neither has activity at muscarinic receptors (M_1 - M_5) nor at the human 5-HT₂R, and both allegedly possess good pharmacokinetic properties in rats. Evaluation of 355 in a battery of rat behavioral assays including the radial arm maze, the forced swim test, the pre-pulse inhibition model, and the formalin assay, as well as others, failed to show any efficacy attributable to the compound [282]. Furthermore, exposure of rats to 355 (either chronically or acutely) did not produce a neuroprotective effect in response to 6-hydroxydopamine infusion. The authors concluded that rapid desensitization of the $\alpha 7$ receptor, which is observed at low agonist concentrations *in vitro*, translates into functional antagonism at $\alpha 7$ receptors *in vivo*.

A set of novel furopyridine and thienopyridine amides has recently been revealed by the Pharmacia group [295]. The sulfur-containing analogs (such as 358) are typically more potent than the corresponding furans (357), and the thieno[2,3-*c*]pyridine-5-carboxylate isomer (358) is preferred to alternative heterocycle arrangements like 360 and 361.

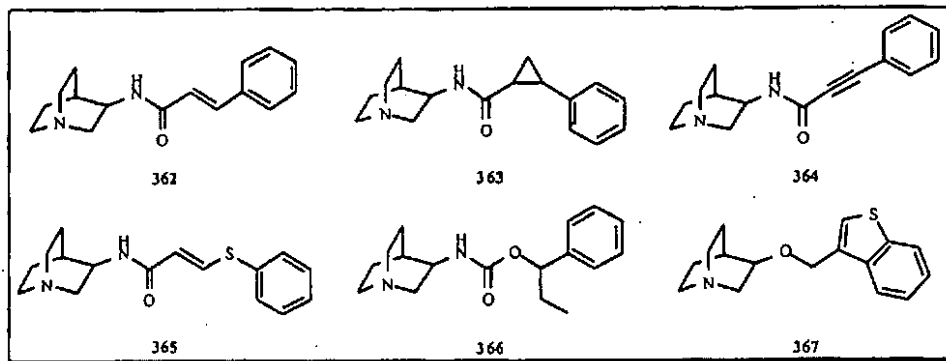


Introduction of a 2-methyl substituent on the quinclidine core (359) causes a 3-fold loss of affinity for $\alpha 7$. Analogs of 358 with additional substituents in the thiophene region have also been prepared but no data on these compounds have been published.

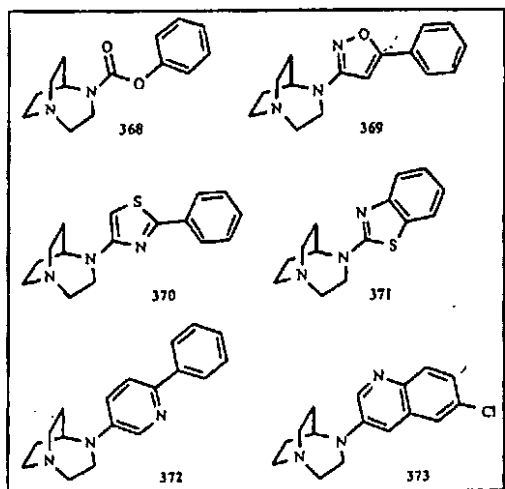


A number of related $\alpha 7$ ligands have been disclosed in the patent literature without data on specific compounds being provided. Some representative examples are shown below. In essence, all of these structures represent alternative strategies for connecting the common pharmacophore elements of a 3-quinclidine and a lipophilic fragment. For instance, amides 362-364 [296] replace the heteroaryl element of 348 with simple olefinic, cyclopropyl, or acetylenic linkers. Compound 365 [297] is similar, but a heteroatom (sulfur) is incorporated into the tether. Benzylic carbamates exemplified by 366 [298] comprise another class, in which the orientation of the carbamate is reversed from that illustrated by 329. Finally, simple ether analogs such as 367 [299] have recently been claimed. These oxygen linked compounds might be expected to be more flexible than their amide counterparts, in addition to lacking potential hydrogen-bonding sites in the amide linker. The importance of these modifications cannot be ascertained due to the absence of published data.

A unique feature of quinclidine is the presence of the bridgehead nitrogen atom, which renders this amine unusually compact and enforces a well-defined orientation with respect to protonation of the nitrogen. These characteristics are also present in the 1,4-diazabicyclo-[3.2.2]nonane skeleton, and the development of this core toward selective $\alpha 7$ nicotinic ligands has proceeded in



parallel with the quinuclidines. Several representative examples include 368-373 [300-307]. Although the diamine derivatives clearly bear a resemblance to the $\alpha 7$ -selective quinuclidines discussed above, they have the advantage of being achiral, so the question of enantioselectivity disappears. In addition, by incorporating the non-bridgehead nitrogen atom into the bicyclic framework, some of the flexibility of the parent 3-aminoquinuclidine system is removed. At this time, however, no relevant biological data have been divulged to enable a meaningful comparison of the two structural classes.



CONCLUSION

Drug discovery efforts have greatly expanded the range of known nAChR agonists. Several new structure types have been introduced, and elements of selectivity with respect to the best-characterized receptor subtypes are beginning to emerge. There is a long way still to go. The nAChR family is large and complex, and it is simply not yet possible to assess selectivity beyond a few of the best-characterized subunit combinations. Indeed, the vast majority of the available data pertains only to binding affinities, which do not correlate

well with potencies for receptor activation. Nevertheless, ligands that achieve good levels of selectivity for specific subtypes (e.g., $\alpha 7$ vs $\alpha 4\beta 2$, $\alpha 4\beta 2$ vs $\alpha 3\beta 4$ and $\alpha 7$) have been identified. In the last decade, several nicotinic agonists with improved therapeutic potential relative to nicotine have been identified and advanced to clinical trials. It seems only a matter of time before some of these emerge to the market as effective drugs. Likewise, approaches aimed at identifying selective nAChR antagonists, as well as development of allosteric modulators, can be expected to expand the therapeutic potential of nAChRs.

The issue of subtype selectivity is certainly not solved, but some progress has been made in designing compounds that have different selectivities for major subunit combinations compared to nicotine, and concomitantly improved therapeutic indices in preclinical models. We can expect that further progress to understand the structural features of ligands that confer subtype selectivity will result in even better therapeutic compounds. Importantly, such studies will also provide exquisitely selective pharmacological tools, currently unavailable, that will be required to deconvolute the complex set of behavioral effects elicited by non-selective agonists like nicotine.

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Selective $\alpha 7$ Nicotinic Acetylcholine Receptor Ligands

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Abstract: Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand gated ion channels of broad distribution and structural heterogeneity. Their functional diversity demonstrated involvement in a variety of neuronal processes (e.g., sensory gating and cognitive function) and generated great interest in them as targets for therapeutic intervention in a number of neuropathological conditions and diseases. In order to control distinct nicotinic functions pharmacologically, it is important to design ligands that selectively interact with distinct receptor subtypes in such a way as to maximize the therapeutic effect and minimize the adverse effects. The $\alpha 7$ nAChR, a CNS subtype, has been the most intensively studied nAChR in recent years. Selective $\alpha 7$ nAChR agonists have been developed as potential candidates for the treatment of schizophrenia, cognitive disorders (including Alzheimer's disease), and inflammation. Despite early concerns that the rapid desensitization property of the $\alpha 7$ nAChR would limit their therapeutic potential, several have already been advanced to clinical trials (e.g., PH-399733, Pfizer; MEM 3454, Memory Pharmaceuticals/Roche). Further development of allosteric modulators and pharmaceutically relevant antagonists might expand the therapeutic potential of compounds that target $\alpha 7$ nAChRs. In this review we briefly describe the structure and function of the $\alpha 7$ nAChR and its *in vitro* and *in vivo* pharmacology, discuss the clinical relevance of these efforts, and review the current progress in $\alpha 7$ ligand development.

Keywords: $\alpha 7$ neuronal nicotinic acetylcholine receptors (nAChRs), $\alpha 7$ selective nicotinic ligand, quinuclidine, allosteric modulator, radiolabeled imaging ligand.

1. INTRODUCTION

Neuronal nicotinic acetylcholine receptors (nAChRs) exist as several subtypes and are involved in a variety of disorders. Their putative functional diversity stimulated the development of subtype-selective ligands as potential therapeutics for the treatment of neuropathological conditions and diseases. In order to be able to control distinctive nicotinic functions pharmacologically, it is important to design ligands that selectively interact with a distinct receptor subtype in such a way as to maximize the therapeutic effect and minimize the adverse effects.

The $\alpha 7$ subtype has been the most intensively studied nAChR in recent years. Structural and pharmacologic examination and the design of ligands for this receptor are areas of expanding effort. Over 1000 manuscripts describing $\alpha 7$ research have published in the last five years, and a growing number of patent applications for $\alpha 7$ nAChR ligands, allosteric modulators and uses thereof demonstrates interest by the pharmaceutical industry. The advancement of some of these agents into clinical trials (e.g., PH-399733, Pfizer; MEM 3454, Memory Pharmaceuticals/Roche) provides further encouragement that the $\alpha 7$ target is worthy of pursuit.

2. MOLECULAR BIOLOGY

2.1. Structure and Ligand Interaction

nAChRs are pentameric ligand-gated ion channels. The $\alpha 7$ nAChR is homomeric, consisting of five $\alpha 7$ subunits [1]. The $\alpha 7$ subunit has also been shown to form functional

receptors *in vitro* with $\beta 2$, however we will limit our discussion to homomeric $\alpha 7$ receptors. The human $\alpha 7$ subunit is approximately 50kD in size and composed of 502 amino acids, including a 22 amino acid signal peptide (Fig. 1). The molecule can be subdivided into an N-terminal domain and a transmembrane domain. The N-terminal domain is extracellular; composed of approximately 200 amino acids that form ten β strands, and is involved in forming the ligand-binding domain (LBD, Fig. 2a). The transmembrane domain is composed of four α helices that cross the lipid bilayer and form the ion channel.

The subunits interact at multiple sites. In the extracellular domain, the positive face of one subunit interacts with the negative face of an adjacent subunit to form the ligand-binding pocket. On the positive face, loops A, B and C have been shown to be involved in forming the LBD (Fig. 2b). On the negative face, loops D, E and F contribute to the binding region [2]. The pentameric architecture of $\alpha 7$ presents five potential ligand-binding sites.

Recent studies with the acetylcholine binding protein (AChBP) from the snail *Lymnaea stagnalis* have provided atomic scale resolution of the protein's structure and a framework to model the $\alpha 7$ nAChR extracellular domain [2]. Using the AChBP framework and defining residues within 5 Å of N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES) seen in the AChBP crystal, Chou [3] has defined the $\alpha 7$ specific residues that are in close proximity to the ligand binding region and that may interact with bound ligands. On the positive face of the LBD, the residues are Tyr⁹³ in loop A, Ser¹⁴⁸, Trp¹⁴⁹ and Ser¹⁵⁰ in loop B and Tyr¹⁸⁸, Cys¹⁹⁰, Cys¹⁹¹ and Tyr¹⁹⁵ in loop C. Contributing from the negative face of the LBD are Trp⁵⁵ from loop D and Val¹⁰⁸, Val¹¹⁰, Gln¹¹⁷, Tyr¹¹⁸ and Leu¹¹⁹ from loop E (Fig. 2b).

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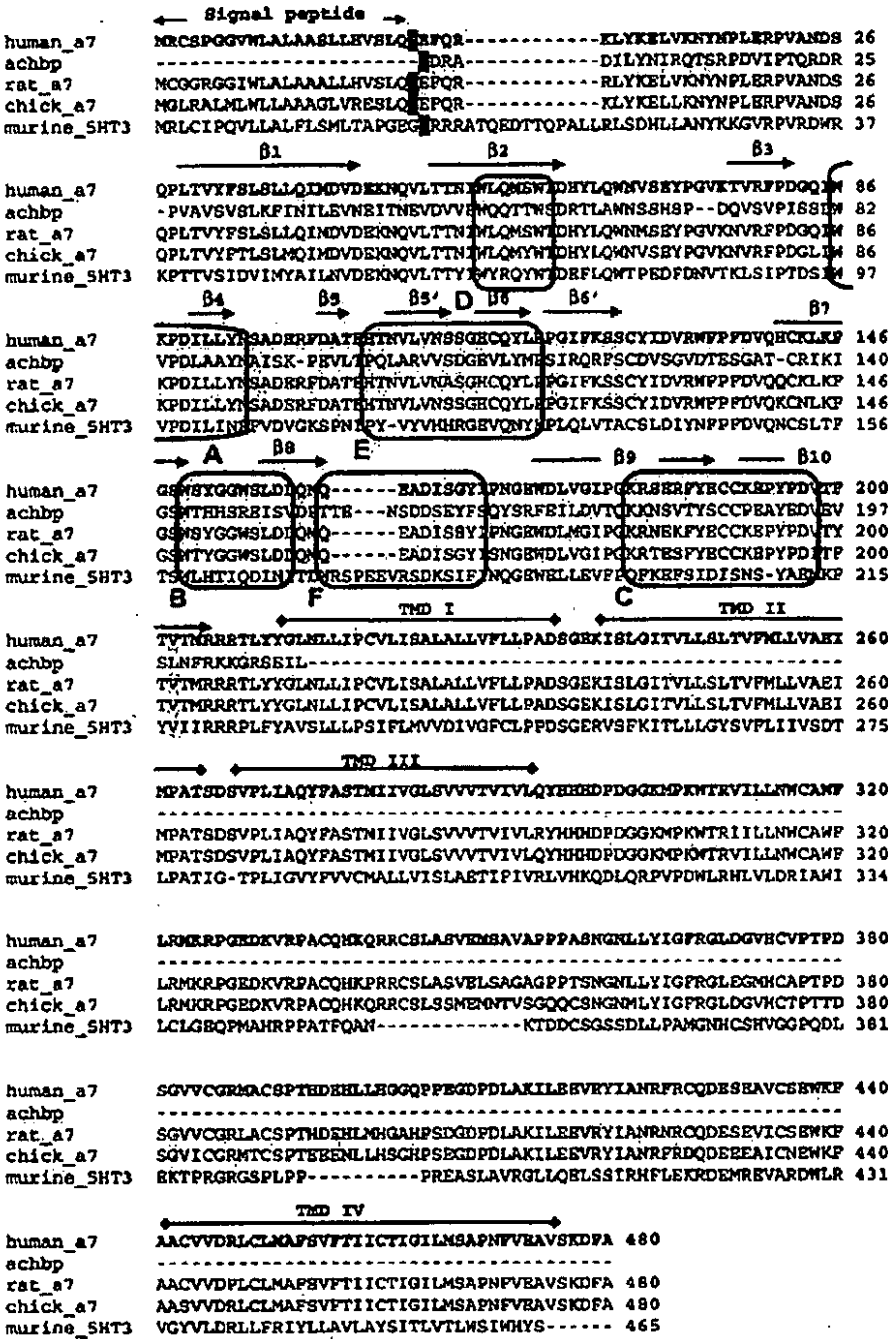


Fig. (1). Sequence of the human α7 nAChR compared with the α7 sequences from rat and chicken, and the sequence of the AChBP and murine 5HT₃ receptor. All are members of the ligand gated ion channel (LGIC) superfamily. The sequence alignments are based on a multi-sequence ClustalX alignment [187]. The first numbered residue in each sequence is highlighted in green. The putative binding loops, β strands and transmembrane domains are indicated. Loops A, B and C from the positive face of the LBD are indicated in red, and loops D, E and F from the negative face are indicated in blue. The β strands are numbered according to the AChBP crystal structure determination [2]. The conserved valine at position 202 is highlighted in yellow.

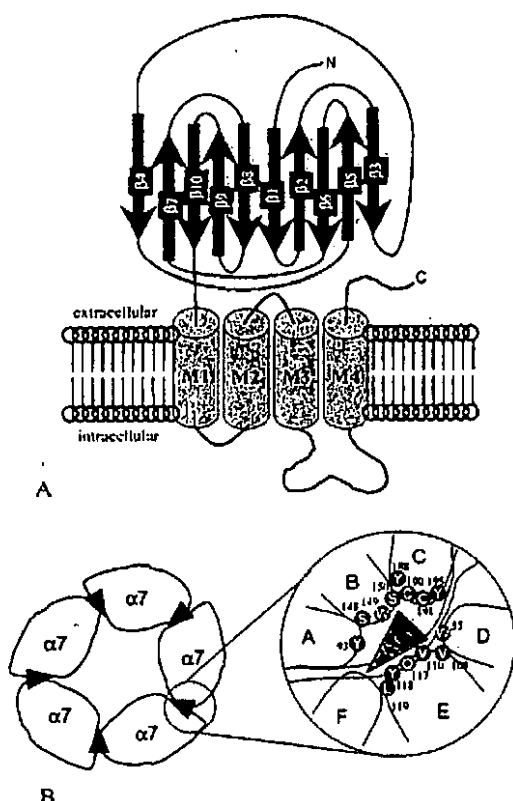


Fig. (2). Putative organization of the $\alpha 7$ nicotinic receptor. (A) The extracellular domain is composed of ten β strands [2]. Four α -helical bundles that cross the cellular membrane constitute the transmembrane domain. (B) Schematic of the pentameric architecture of the $\alpha 7$ receptor. The interfaces of adjacent subunits form five potential ligand binding sites. The exploded detail shows the residues in loops A, B and C from the positive face and loops D, E and F from the negative face that may interact with ligands in the binding domain [3].

Celie *et al.* [4] have recently presented the structures of AChBP with nicotine and carbamylcholine bound, defining the residues that interact with these ligands in the binding site. From this study, it is clear that there are differences in residue contributions depending upon the ligand bound. The loop C Tyr¹⁸⁵ (AChBP sequence numbering) has limited contact with nicotine but contributes aromatic contacts with carbamylcholine, and Arg¹⁰⁴ in loop E only interacts with carbamylcholine. Future studies should continue to elucidate $\alpha 7$ -specific interactions and define specific differences due to affinity and activation (agonism or antagonism).

The transmembrane domains form the ion channel pore. The M2 segments from each of the five subunits line the aqueous lumen. The other transmembrane segments encircle the M2 segments, protecting them from the lipid bilayer [5,6]. The ion channel contains an extracellular charged ring involved in ion conductance, an intracellular charged ring

defined as the charge selectivity filter, and a constriction at the middle of the membrane that forms a hydrophobic gate (Fig. 3a) [6-8]. The negatively charged residues at the intra- and extracellular vestibules selectively interact with and conduct cations through the pore. Upon agonist binding, changes occur leading to a quaternary symmetrical twist that opens the hydrophobic gate and allows the passage of ions (Fig. 3b) [6, 9-11].

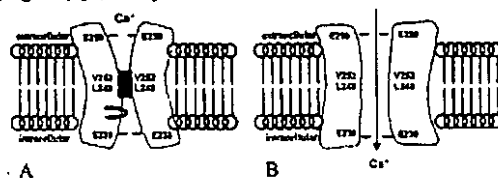


Fig. (3). The ion channel pore of the $\alpha 7$ receptor. (A) The charged residues in the extracellular ring involved in ion conductance, and those that form the ion selectivity filter on the intracellular side are labeled. Valine-252 and leucine-248 form the hydrophobic gate at the middle of the membrane. (B) Upon activation, a quaternary twist of the subunits opens the gate and allows the passage of cations.

2.2. Activation and Desensitization

The nicotinic receptors are cation channels. Classically, the nAChRs have been defined as sodium channels. Upon activation, the ion pore conducts monovalent Na^+ ions into the cell while allowing an efflux of K^+ ions. The narrowness of the channel appeared to limit the flux of divalent cations. However, the $\alpha 7$ nAChR has been shown to be highly permeable to Ca^{2+} , defining $\alpha 7$ as a calcium channel [12-14].

The Monod-Wyman-Changeux (MWC) model was proposed forty years ago to describe the mechanistic states of receptor ligand interactions [15]. Over time, this model has been adapted to explain the mechanistic states of membrane bound receptors. Fig. 4 shows the reaction scheme, defining four potential allosteric states of the receptor. The receptor is initially in a resting or basal (B) state. Antagonists such as α -bungarotoxin (α -BGT) or methyllycaconitine (MLA, 92, see chapter 5) stabilize this state in $\alpha 7$ nAChR. Agonists have a higher affinity for the active state (A) of the receptor. The symmetric quaternary twist, previously described, opens the pore and allows ion conductance in the active state. Upon activation, the $\alpha 7$ nAChR moves rapidly to an intermediate (I), closed state and then to the desensitized state (D) [16-18].

Desensitization is a loss of the biological response due to agonist stimulation. Upon washout of ligand, receptors return to the basal allosteric state. The $\alpha 7$ nAChR displays fast (millisecond) onset of desensitization upon agonist application [16]. The rapid inactivation of the receptor caused by ligand-induced desensitization is often described negatively. However, desensitization plays an important physiological role in preventing cell death by excitotoxicity. Hyperfunctional $\alpha 7$ receptors have been designed by mutations in the M2 domain. Expression of such hyperfunctional $\alpha 7$ receptors causes extensive apoptosis and toxicity in the neuron-like cells SH-SY5Y and IMR-32 [19] and leads to death within one day of birth, when the receptors are expressed *in vivo* in mice [20].

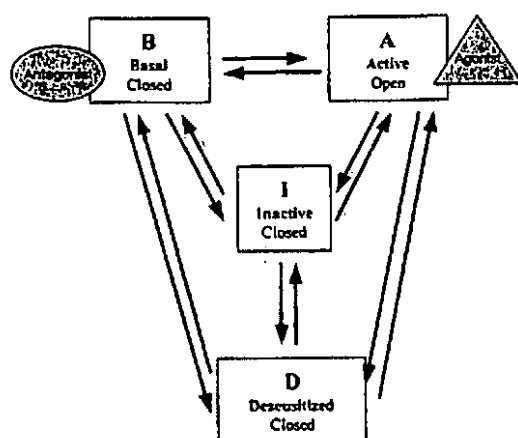


Fig. (4). Allosteric states of the $\alpha 7$ nAChR. The potential transitions between the four allosteric states of the $\alpha 7$ receptor are represented. The resting state of the receptor is defined as the basal (B) state. Binding of agonists causes the transition to the active (A) state. Once activated, the $\alpha 7$ receptor moves quickly to the closed, inactive (I) state and then to the desensitized (D) state. Upon ligand washout, the receptor returns to the basal state. Antagonists display affinity for the basal state, preventing transition of the receptor to the active state.

3. PHARMACOLOGY: CHARACTERIZATION OF $\alpha 7$ NICOTINIC LIGANDS

3.1. Radioligand Binding

The $\alpha 7$ nicotinic receptors were initially defined as the low affinity nicotinic receptors in the CNS [21]. Better characterization of this receptor was made possible with the discovery of α -bungarotoxin (α -BGT) as a selective ligand for the $\alpha 7$ nAChR in the CNS. ^{125}I - α -BGT, displaying a K_d of approximately 1 nM for rat and human $\alpha 7$ homomers [22-25], allowed the localization of $\alpha 7$ receptors in the brain and provided a labeled ligand for competitive binding studies.

Such receptor binding studies provide the primary means to examine the interaction of potential ligands with the $\alpha 7$ receptor. The high expression level of $\alpha 7$ in the rat hippocampus, 30-40 fmol/mg, has made it the tissue source of choice for binding assays [26-28]. The human neuroblastoma lines SH-SY5Y and IMR-32, and the rat pheochromocytoma line PC12 natively express $\alpha 7$ and have been used in binding, as well as functional studies. However, these lines also express $\alpha 3$ and $\beta 4$ nicotinic subunit containing receptors, complicating their usage for studying $\alpha 7$ [24,29-31]. Development of recombinant cell lines expressing functional $\alpha 7$ receptors has been limited and shown to be host cell dependent [32-34]. Recent evidence points to the need for expression of the RIC-3 gene product for proper transport or cell surface assembly of functional $\alpha 7$ receptors [35,36]. With co-expression of $\alpha 7$ and RIC-3, functional $\alpha 7$ receptors have now been expressed in the non-neuronal line HEK-293 [37]. SH-SY5Y and GH4CI cells, which were previously successfully transfected with $\alpha 7$ nAChRs, were shown to natively express RIC-3. This finding may open the way for the development of cell lines

expressing pmol/mg levels of $\alpha 7$, on a nicotinic null background, to aid in nicotinic ligand characterization.

The discovery and characterization of methyllycaconitine (MLA), the principal toxin from the seeds of *Delphinium brownii*, as an $\alpha 7$ -selective ligand has provided another means for studying $\alpha 7$ receptors [23, 38]. MLA, radiolabeled with tritium, binds to α -BGT-sensitive sites in the brain and displays a K_d of 2 nM for the rat $\alpha 7$ receptor [39].

3.2. Functional Assays

Binding defines only the affinity of a ligand for a single state of the receptor and provides no information on the agonism or antagonism of the compound. To elucidate the effect of avid compounds upon the receptor, they must be examined in functional assays.

3.2.1. Ion Flux Assays

Ion flux assays using radioisotopic ions, such as $^{86}\text{Rb}^+$ as a surrogate for K^+ , have been applied to examine functional responses at $\alpha 4\beta 2$, $\alpha 3\beta 4$ and muscle-type nicotinic receptors [40]. More readily adaptable to high throughput screening are assays using fluorescent ion indicators to measure real-time ion flux. However, even though the $\alpha 7$ receptor is highly permeable to calcium, the rapid kinetics of the receptor limit the utility of these assay formats for studying $\alpha 7$ functional responses. To circumvent this issue, mutant and chimeric $\alpha 7$ subunits have been engineered to increase the open probability of the receptor and produce more readily measurable responses. Benrard and colleagues generated a gain-of-function chick $\alpha 7$ mutant by replacing the leucine at position 247 with a threonine [41,42]. Along with increasing the open probability of the receptor, the response to ligands was altered, limiting the use of this mutant to predict the function of ligands at the wildtype receptor. The L247T mutation converts serotonin from an antagonist to an agonist, and the classical nicotinic antagonists DH β E, hexamethonium and (+)-tubocurarine were shown to activate the mutant receptor expressed in oocytes [42-44]. In an attempt to correlate the functional properties of ligand gated ion channels to defined structural domains, Eisele and colleagues [45] constructed a chimeric receptor composed of the N-terminal portion of chick $\alpha 7$ and the C-terminal part of the murine 5HT $_3$ subunit, connecting them at the conserved valine at position 202. In oocytes, the response to agonist has been shown to be similar between the chimera and wildtype $\alpha 7$ [45, 46]. However, the chimera was blocked by calcium ions, whereas wildtype $\alpha 7$ is highly permeable to Ca^{2+} [45]. Further work with the equivalent human $\alpha 7$ /murine 5HT $_3$ chimera, using a defined isotonic, low sodium buffer that enhances the calcium flux, has shown that compounds behave similarly at the chimeric and wildtype receptors [47]. Recently, this same group has described the engineering of a dual mutant $\alpha 7$ receptor. By mutating the threonine at position 208 to a proline, and the cysteine at position 219 to a serine, the increased open probability seen with the chimeric receptor was maintained. Use of the dual mutant $\alpha 7$ has been described in characterization of $\alpha 7$ agonists and positive allosteric modulators (PAM) [47,48]. The mutant may be stably expressed in SH-EP1 cells and utilized in a calcium flux assay. Attempts by the same group to express wildtype $\alpha 7$ in SH-EP1 cells were unsuccessful.

3.2.2. Electrophysiological Assays

Due to the rapid activation and desensitization kinetics of the $\alpha 7$ receptor, electrophysiology has been the gold standard for examination of $\alpha 7$ function. Oocytes are harvested from female *Xenopus laevis* frogs, injected with cRNA for transient receptor expression and examined using two electrode voltage clamp recordings. This system is advantageous in allowing the examination of functional receptors of defined subunits. For the examination of cellular responses and elucidation of the physiological role of $\alpha 7$ nAChRs, whole cell electrophysiological techniques may be utilized with tissue slices or dissociated neurons *in vitro*, or in defined cell layers *in vivo*. Electrophysiological studies with slices and dissociated neurons have shown nicotinic enhancement of glutamatergic currents in the hippocampus, auditory cortex, lateral geniculate, olfactory bulb and cerebellum [49-54]. Blockade of this enhancement by $\alpha 7$ -specific antagonists (α -BGT or MLA) implicates $\alpha 7$ in this nicotinic response.

$\alpha 7$ receptors have also been implicated in long-term potentiation (LTP). LTP is an increase in synaptic strength that may last for hours or even days [55], and that has been correlated with memory formation. LTP is studied using electrophysiological techniques with tissue slices or in discrete brain regions in the intact animal. LTP in the hippocampus and ventral tegmental area (VTA) has been shown to be dependent upon $\alpha 7$ activation [56-60].

3.2.3. Neurotransmission Assays

In addition to electrophysiology, *in vitro* neurotransmission and *in vivo* microdialysis have been invaluable in elucidating the role of $\alpha 7$ nAChRs. Using cortical and striatal synaptosomes, $\alpha 7$ receptor activation has been shown to enhance depolarizing stimulus evoked [^{3} H]-D-aspartate release [61,62]. Application of choline alone, an $\alpha 7$ -specific agonist, also induced [^{3} H]-D-aspartate release from rat frontal cortex synaptosomes. The role of $\alpha 7$ was confirmed by the α -BGT blockade of the choline-induced responses [62]. Slice preparations, which better maintain the integrity of the neuronal circuitry, have shown that an $\alpha 7$ component is involved in [^{3} H]-dopamine release in striatal slices induced by high concentrations of nicotine [63]. Blockade by an ionotropic glutamate antagonist implicated $\alpha 7$ -mediated glutamate release in this effect.

Schilstrom and colleagues have used *in vivo* microdialysis to identify the role of $\alpha 7$ in the VTA. Initial work showed that dopamine release in the nucleus accumbens, in response to systemic nicotine, may be blocked by infusion of MLA in the VTA [64]. Subsequent microdialysis studies in the VTA confirmed nicotinic enhancement of glutamate and aspartate release in this area [65].

3.3. Behavioral Characterization

Use of non-selective nicotinic agonists and $\alpha 7$ -selective antagonists has allowed detailed characterization of $\alpha 7$ nAChR at the receptor and cellular level. *In vivo*, infusion of MLA in the ventral hippocampus or basolateral amygdala causes significant working memory deficits in rats as observed in radial arm maze tasks [66-68]. However, further

elucidation of the role of $\alpha 7$ nAChR in behavior has necessitated the development of $\alpha 7$ -specific agonists.

GTS-21 (1), which shows functional selectivity for $\alpha 7$ nAChR, enhances cognition in several behavioral models using multiple species. This compound displays effectiveness in passive avoidance in lesioned rats [69], in the aged rat in active avoidance, the Lashley III maze, and a 17-arm radial maze [70], eye-blink conditioning in the aged rabbit [71] and delayed-matching task in a non-human primate [Briggs *et al*, 1997]. GTS-21, though defined as an $\alpha 7$ selective agonist, displays 100-fold greater binding affinity for the $\alpha 4\beta 2$ receptor, toward which it acts as an antagonist, and induces dopamine release from rat striatal slices [72]. *In vivo* efficacy of other nicotinic compounds, that display improved $\alpha 7$ -specificity over GTS-21, is needed to confirm the usefulness of these models in the development of $\alpha 7$ ligands.

Schizophrenics display a sensory processing deficit that can be measured by auditory evoked potentials. This deficit may be modeled in DBA/2 mice, fimbria-fornix lesioned rats and isolation reared rats. GTS-21 improves auditory gating deficits in these animal models, and blockade by α -BGT confirmed the $\alpha 7$ mechanism of the GTS-21 effects [73-76]. Recently, sensory gating has been used to show the *in vivo* efficacy of other $\alpha 7$ -specific compounds 62 and 98 [48, 77] (see chapter 5, Table 2).

In auditory gating, measurements of the evoked potentials are made using electrodes implanted in the CA3 region of the hippocampus. Another paradigm used to model the sensory deficits of schizophrenia is Prepulse Inhibition (PPI). In this model, auditory pulses are used, and physical startle measured by the magnitude of deflection of the animal in a startle chamber. Cilia and colleagues [78] recently demonstrated the effectiveness of an $\alpha 7$ selective agonist 77 in reversing PPI deficits in isolation-reared rats.

The high level of expression of $\alpha 7$ nAChRs in the hippocampus, the involvement of the hippocampus in memory formation and the effect of MLA blockade on working memory suggest that $\alpha 7$ nAChR agonists should have memory enhancing potential. AR-R17779 (29, see chapter 5), an $\alpha 7$ selective agonist, improves scopolamine-induced deficits in social recognition, and also shows improvement when employing a 24-hour retention interval in unimpaired animals [79]. MLA reversed the AR-R17779 improvement in unimpaired animals. In an 8-arm radial maze model, repeated doses of AR-R17779 improved long-term learning and attenuated working memory deficits in rats [80].

Recently, Sanofi reported the effectiveness of another $\alpha 7$ selective agonist, SSR180711A, in several cognitive models. This agonist displayed effectiveness in object recognition paradigms for long-term and chemically-induced (MK801 or PCP hypersensitization) short-term episodic memory [81,82]. SSR180711A (9, see chapter 5) also displayed effectiveness in Morris Water maze models for reference memory (scopolamine-induced deficit) and working memory (MK-801-induced deficit).

The effectiveness of $\alpha 7$ -selective agonists in the aforementioned behavioral models supports the therapeutic potential of $\alpha 7$ agonists for memory impairment and sensory

deficits associated with schizophrenia. If the recent rate of $\alpha 7$ -related publications is indicative, the number of $\alpha 7$ -selective ligands will continue to rapidly increase. Studies on these compounds will provide further verification of the relevance of the current models for $\alpha 7$ nAChR compound development, and, hopefully, illuminate new animal models indicative of the therapeutic potential of $\alpha 7$ -selective ligands.

4. THERAPEUTIC POTENTIAL

4.1. Cognitive Impairment

Alzheimer's disease produces a devastating decline in mental function, with profound effects on learning and memory. Early consequences of the disease include the specific loss of cholinergic neurons in brain, diminished cholinergic signaling, and the accumulation of β -amyloid peptide in neuritic plaques. Of the nicotinic acetylcholine receptors at risk, the most critical may be those containing the $\alpha 7$ subtype of nAChRs, because they are widespread, have a high relative permeability to calcium, and regulate numerous cellular events in the nervous system. Because $\alpha 7$ nAChRs in the central nervous system are thought to have numerous functions and recently have been implicated in learning and memory, impaired receptor function in this case may contribute to cognitive deficits associated with Alzheimer's disease [83]. A novel $\alpha 7$ agonist TC-1698, has been shown to provide neuroprotection against $A\beta$ through an effect on the JAK2/PI-3K cascade [84].

The involvement of nAChRs and particularly the $\alpha 7$ subtype in cognitive impairment in schizophrenia has been suggested based on a few observations:

- The high incidence of smokers among schizophrenic patients [85,86].
- The decrease in $\alpha 7$ nAChR in postmortem hippocampus of schizophrenic subjects in comparison with controls [87].
- Schizophrenic patients have high levels of nAChR antibodies, which may contribute to the nicotinic deficit [88,89].
- Diminished auditory sensory gating in schizophrenia is thought to reflect a genetic abnormality in the cholinergic system; the P50 gating deficit has been linked to a locus at the $\alpha 7$ nAChR gene (CHRNA-7) [90].
- Compared to most other strains of mice, the DBA/2 mouse has genetically decreased levels of $\alpha 7$ nAChR in the CA3 region and impaired auditory gating [76].
- The most effective medication in the treatment of refractory schizophrenia, clozapine, normalizes auditory gating in both people with schizophrenia and animal models, where an $\alpha 7$ nicotinic mechanism has been demonstrated [91].

4.2. Inflammation

Recent reports define the $\alpha 7$ nAChR as an essential regulator of inflammation [92]. $\alpha 7$ nAChR full agonists have been proposed for treatment of inflammatory diseases via

reduction of tumor necrosis factor (TNF- α) release from macrophages [93]. *In vivo* treatment with nicotine has been reported to modulate an inflammatory pathway through the $\alpha 7$ -stimulated suppression of HMGB1 secretion, thereby improving survival in models of sepsis [94]. These results suggest that selective nicotinic agonists for the $\alpha 7$ nAChR might have therapeutic potential for the treatment of sepsis.

4.3. Fibromyalgia Syndrome

Agonists at $\alpha 7$ nAChRs have been proposed for treatment of fibromyalgia syndrome, a complex chronic condition that causes widespread muscular pain and profound fatigue [95]. The concept has been built on the discovery that the 5-HT₃ receptor antagonist tropisetron is a potent and selective partial $\alpha 7$ nAChR agonist [96]. Tropisetron is available for the treatment of emesis and displayed pharmacological effects different from other 5-HT₃ receptor antagonists. Reported statistically significant reduction in symptoms of fibromyalgia patients after treatment with tropisetron has been associated with the $\alpha 7$ nAChR.

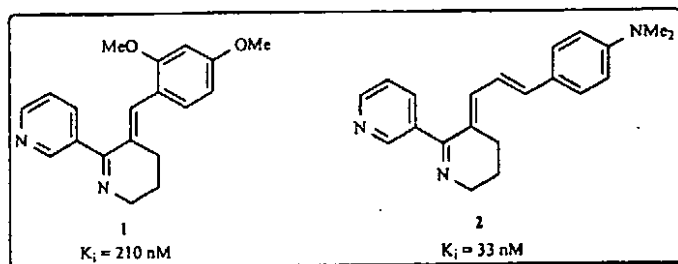
4.4. Miscellaneous

Recent experiments with choline demonstrates that activation of $\alpha 7$ nAChRs elicits antinociceptive effects both in an acute thermal pain model [97] and in an inflammatory pain model [98]. Synergistic antinociceptive interaction was obtained from coadministration of choline and morphine. These observations might rationalize development of potential non-addictive analgesic and anti-inflammatory drugs. $\alpha 7$ nAChR appears to play no role in the abuse liability of nicotine. The $\alpha 7$ receptors are not involved in the hyperlocomotor and rewarding effects of nicotine [99]. Agonists at the $\alpha 7$ subtype have been proposed for treatment of glaucoma, macular degeneration and diabetic retinopathy through a neuroprotective and antiangiogenic nicotinic mechanism [100].

5. MEDICINAL CHEMISTRY STRATEGIES

For many years the research on and development of ligands for nAChRs were largely focused on selectivity for $\alpha 4\beta 2$, the most abundant in the CNS subtype. Recent increasing interest in the $\alpha 7$ nAChR has fueled development of novel selective ligands for this subtype as well. In contrast to difficulty often encountered in the search for selective $\alpha 4\beta 2$ ligands, high nAChR subtype selectivity has been relatively easily achieved in $\alpha 7$ ligands.

Analogues of the marine worm toxin anabaseine [101,102] were reported as the first "functionally" selective agonists for the $\alpha 7$ subtype since these compounds partially activate the rodent $\alpha 7$ nicotinic receptor and are antagonists at all other nAChRs. The therapeutic potential of this series is compromised by the lack of binding selectivity. For example, 3-(2,4-dimethoxybenzylidene)anabaseine (GTS-21, 1; $\alpha 4\beta 2$ K_i = 19 nM, $\alpha 7$ K_i = 210 nM) [72] possesses a higher affinity for the $\alpha 4\beta 2$ receptor, toward which it acts as an antagonist [103] and failed to show a satisfactory pharmacokinetic profile in the areas of bioavailability and brain permeability. Regardless of its weak potency (EC_{50} = 81 μ M) and low efficacy (E_{max} = 50%), GTS-21 was effective in rodent cognitive models. Experiments with GTS-21 have revealed the lack of its effect upon the nicotine cue, suggesting $\alpha 7$ agonists are not involved in the addiction



liabilities of nicotinic compounds [104]. Extension of the conjugated system in position 3 of the tetrahydropyridine ring along with incorporation of a *p*-dimethylamino group (2) significantly improved affinity [101].

5.1. Pharmacophoric Elements of Selective $\alpha 7$ nAChR Ligands

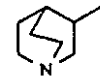
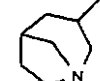
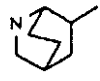
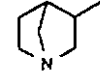
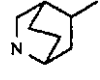
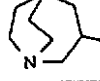
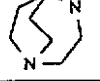
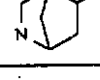
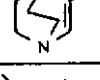
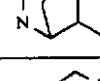
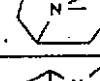
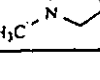
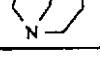
A pharmacophore is generally considered to be the minimum ensemble of molecular elements necessary for binding to the receptor. nAChR pharmacophore models [105] typically identify three pharmacophoric elements, a cationic center, a hydrogen bond acceptor and a hydrophobic group. The binding of many relatively unselective ligands can be rationalized using a rather simplistic model composed of one of each of these elements. However, this model is less

helpful in explaining subtype selectivity, particularly in the case of $\alpha 7$ ligands. In this review, the traditional three element framework will be used as an organizing tool, but the diversity, which exists in each of these three elements, will be examined separately, in an attempt to identify relationships leading to selectivity (see Table 1).

5.1.1. Cationic Centers

Acetylcholine receptors are typically divided into two receptor families: muscarinic and nicotinic acetylcholine receptors. Various synthetic muscarinic ligands mimic the cationic quaternary ammonium part of acetylcholine with quinuclidine (1-azabicyclo[2.2.2]octane). Quinuclidine has been the most abundant scaffold for the construction of nicotinic ligands as well, with significant emphasis on $\alpha 7$

Table 1. Representative Groups in the $\alpha 7$ nAChR Pharmacophore: CC-Cationic Center, HA-Hydrogen Bond Acceptor, HP-Hydrophobic Element

CC		HA		HP	
					
					
					
					
					
					
					

nAChRs. Its basic nitrogen, occupying a bridgehead position within an azabicyclic system, allocates maximal electrostatic interaction combined with minimal steric demand. Owing to the strong basicity of quinuclidine derivatives ($pK_a \sim 10-11$), $\alpha 7$ ligands exist in physiological conditions in cationic form with a well-defined orientation of the proton.

The 3-substituted 1-azabicyclo[2.2.2]octane moiety has proved to be a valuable scaffold for generation of potent $\alpha 7$ nAChR ligands [106] with stereochemical preference at the 3-position for the *R*-enantiomer [107]. The stereospecificity of binding to $\alpha 7$ nAChRs is dramatically evident in the example of an enantiomeric pair of 1-azabicyclo[3.2.1]octane-based ligands (3 and 4) [108], the 1-azabicyclo[3.2.1]octane ring system being isomeric with quinuclidine. Generally, incorporation of small (methyl) (6) [108] or large (benzyl) (8) groups [109] in position 2 of the azabicyclic ring is detrimental to affinity.

Numerous azabicyclic systems have been disclosed in the patent literature [110-116] as alternatives to quinuclidine (Table 1). However, some of those require multi-step synthesis and create additional stereogenic centers. Invariably the azabicyclic provides not only the cationic center, but also some spacer (typically two or three carbon atoms) between the protonated nitrogen and point of attachment of the hydrogen bond acceptor and/or hydrophobic fragment. This characteristic is also present in the 1,4-diazabicyclo[3.2.2]nonane scaffold, which can be obtained from commercially available quinuclidin-3-one in three steps via Beckmann rearrangement. Absence of a stereogenic center makes its derivatives advantageous for development. 4-(*p*-Bromophenylloxycarbonyl)-1,4-diazabicyclo[3.2.2]nonane (9), the partial agonist SSR180711A ($\alpha 7 K_i = 50$ nM, $\alpha 7 K_i = 78$ nM, $EC_{50} = 0.8$ μ M, $E_{max} = 38\%$), potentiates glutamatergic transmission in mouse hippocampus [117], up-regulates $\alpha 7$ nAChR (increases [^3H]- α -BGT binding sites *in vitro* and *in vivo*) [118] and is active in experimental models predictive of therapeutic activity on cognitive symptoms of Alzheimer's disease [82] and schizophrenia [81] (Table 2).

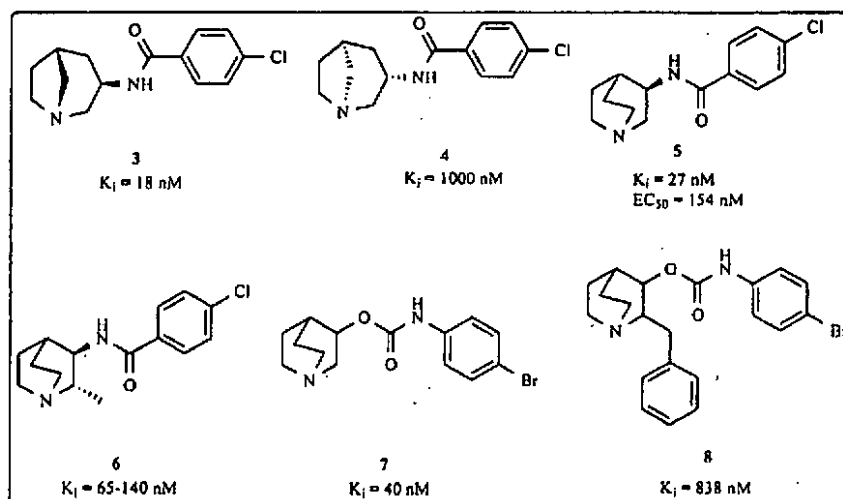
Table 2. Selective $\alpha 7$ nAChR Agonists Effective in Behavioral Models

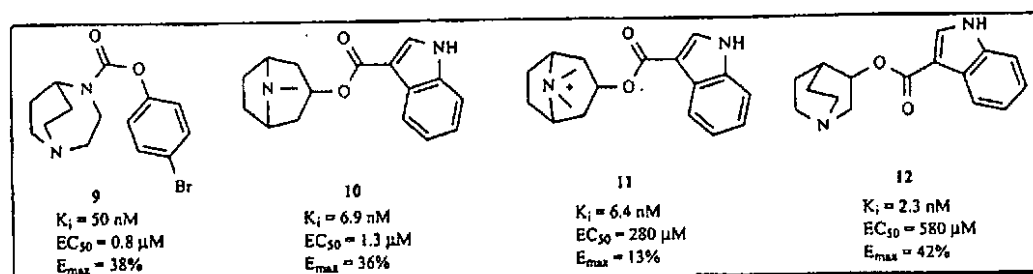
$\alpha 7$ nAChR Agonist	Behavioral Model	Ref.
9	Object Recognition Task in rats	[81, 82]
9	Morris Water Maze in mice	[81, 82]
9	Selective Attention Task in rats	[81]
29	Radial Arm Maze Model in rats	[80]
29	Social Recognition Test in rats	[79]
54	Auditory Sensory Gating Model in rats	[138]
66	Auditory Sensory Gating Model in rats	[77]
77	Prepulse Inhibition	[78]
98*	Auditory Sensory Gating Model in rats	[48]

*positive allosteric modulator.

A comparison of $\alpha 7$ nAChR ligands, which differ only in the cationic part of the molecules, has been reported by Macor *et al.* [96]. Quaternization of tropisetron (10) results in reduction of efficacy for its derivative 11, while replacement of 8-azabicyclo[3.2.1]heptane with 1-azabicyclo[2.2.2]octane afforded an $\alpha 7$ nAChR agonist 12 with greater intrinsic agonist activity. The 5-HT₃ receptor antagonist tropisetron was studied extensively in human clinical trials and is available on the market for the treatment of emesis. Its safety profile [119] can be interpreted as demonstration of the safety of an $\alpha 7$ nAChR agonist as well.

Incorporation of an endocyclic double bond weakens basicity of the nitrogen in 1-azabicyclo[2.2.2]oct-2-ene ($pK_a \sim 8-9$). Nevertheless, the modification of the ring does not reduce interaction with the receptor: the affinity of 3-(naphth-2-yl)quinuclidine (13) is almost three fold less than its unsaturated analog 14 [120]. Tropene derivative 15 [121] with the double bond remote from cationic center exhibits





good affinity as well. The effect of quaternization on affinity varies, as can be seen in the cases of 1-azabicyclo[2.2.2]oct-2-ene- and tropene-based ligands. N-Methylation of 1-azabicyclo[2.2.2]oct-2-ene derivative 16 increases the IC_{50} more than three times (compound 17). However, addition of second methyl group to the tropene scaffold of 18 augments the affinity by two orders of magnitude (compound 19) [122].

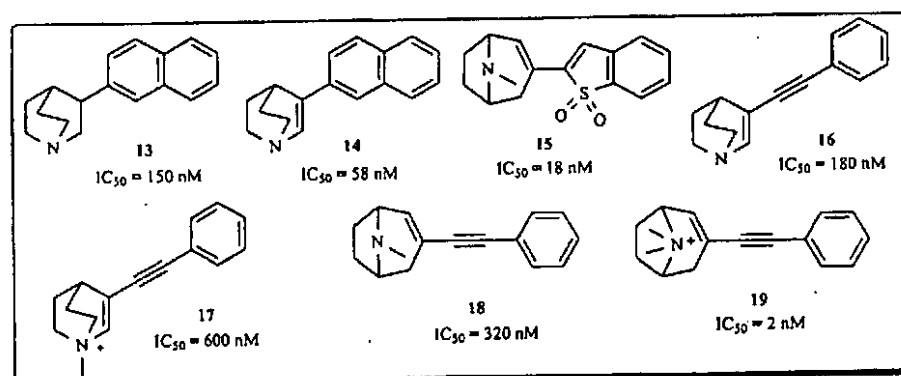
In contrast to $\alpha 7$ nAChR ligands containing an azabicyclic system as a cationic center, the corresponding azacyclic (pyrrolidine, piperidine) analogs display much lower affinity [123].

oxygen drastically diminishes affinity (compound 22), probably, because of obstruction of interaction between the hydrogen bond acceptor (oxygen atom) and the receptor.

5.1.2. Hydrogen Bond Acceptors

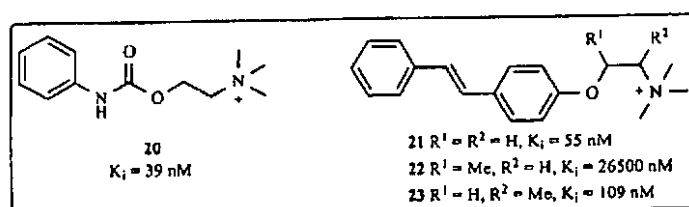
Oxygen-containing hydrogen bond acceptor moieties (Table 1) are common, if not essential, structural elements in selective $\alpha 7$ nAChR ligands.

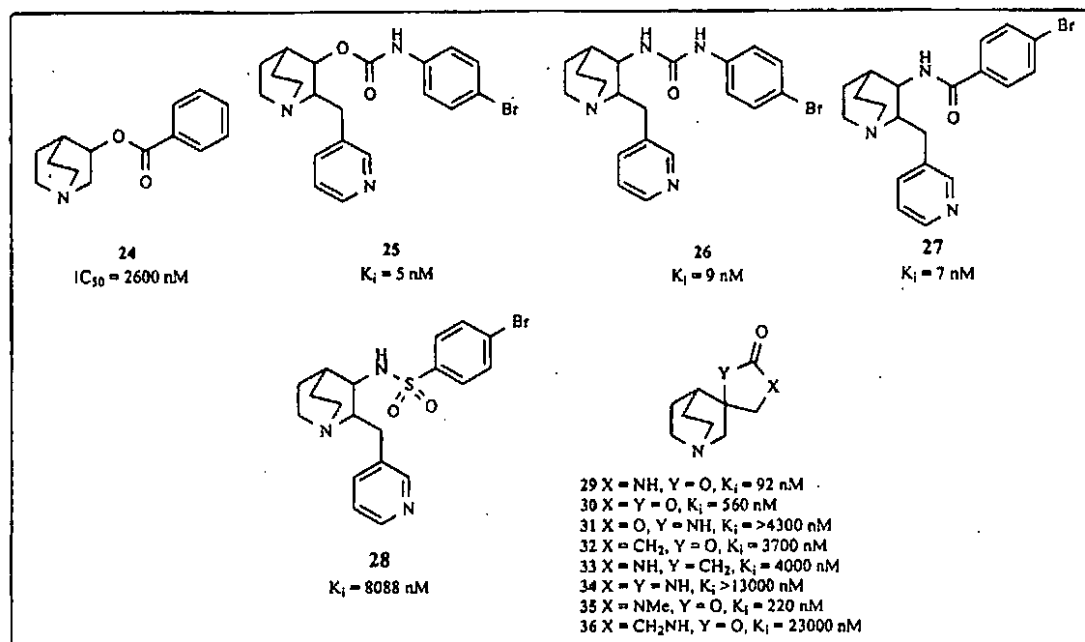
Tropisetron (10) containing ester moiety is described as a partial $\alpha 7$ nAChR agonist, and quinuclidin-3-yl benzoate (24) [125,126] has been reported as a ligand for both nicotinic and muscarinic rat brain receptors. Comparative



Choline as a cationic site has been explored by Gotti *et al.* [124] and Gündisch *et al.* [123]. Binding of choline phenylcarbamate (20), containing a positively charged quaternary ammonium fragment, is comparable with quinuclidine carbamate 7. Aryl ether 21 has shown excellent selectivity for $\alpha 7$ nAChR over the $\alpha 4\beta 2$ subtype ($\alpha 4\beta 2$ $K_i/\alpha 7$ $K_i = 180$). Methylation of the carbon adjacent to quaternary nitrogen decreases affinity only modestly (compound 23), while methylation at the position next to

study of various hydrogen bond acceptor groups in the series of carbamate 25, urea 26, amide 27, and sulfonamide 28 has been undertaken by Mazurov *et al.* [109]. Results of binding assays revealed little difference between the first three selective $\alpha 7$ nAChR ligands suggesting a putative contribution of the carbonyl moiety in interaction with the receptor. The bulky sulfonamide group impedes approach of the molecule to the binding site resulting in diminution of affinity.





The conformationally restricted carbamate 29 (AR-R17779), first reported by Gordon *et al.* [127], was reported by Mullen *et al.* [128] to be the first full agonist (EC₅₀ = 21 μ M, E_{max} = 96%) at the rat $\alpha 7$ nAChR subtype in oocytes with selectivity for the $\alpha 7$ (K_i = 92 nM, [¹²⁵I] α -bungarotoxin binding assay) versus the $\alpha 4\beta 2$ subtype. Compound 29 also lacks noncompetitive antagonist activity.

Eli Lilly laboratories have disclosed results for AR-R17779 obtained using human recombinant nAChR subunit combinations expressed in GH4 cells [129]. In terms of potency, 29 (K_i = 420 nM, MLA radioligand binding assay; EC₅₀ = 420 nM, E_{max} = 93%) is comparable to GTS-21 (1). In contrast to the series of 25, 26, 27 (with acyclic carbonyl functionalities), attempts to modify the cyclic carbonyl functionality cause substantial reduction or loss in $\alpha 7$ receptor affinity. Transformation of carbamate 29 into carbonate 30, reversed carbamate 31, lactone 32, lactam 33, or urea 34 provides compounds ranging from moderate affinity (560 nM) to completely inactive. Incorporation of a methyl group into carbamate 29 reduces its binding to $\alpha 7$ and turns it into a non-selective ligand (35) with equal affinity towards both $\alpha 7$ and $\alpha 4\beta 2$ nAChRs. Expansion of the 5-membered ring to the corresponding 6-membered (36) turned out to be deleterious as well. A clear stereogenic preference is observed for the (-) (29) versus the (+) enantiomer, which binds with more than one hundred times less affinity.

Despite the poor pharmacokinetic profile [129] and mediocre efficacy, 29 served as invaluable tool for *in vivo* assessment of $\alpha 7$ nAChR function validating a link between its activation and a variety of biological effects. AR-R17779 (29) has been shown to enhance learning and memory function in rats in a radial arm maze model [80] and social

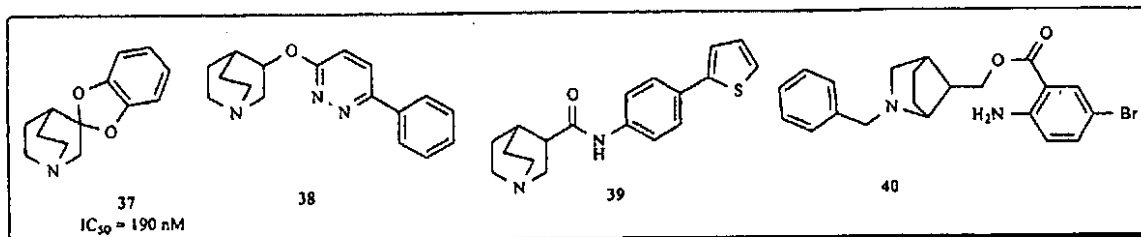
recognition test [79], but has failed to improve performance in a model of attention [130]. AR-R17779 (29) and GTS-21 (1) have no effect on auditory gating using a prepulse inhibition of the acoustic startle response (relevant to sensory deficits in schizophrenic patients [131]), while epibatidine predominantly augmented it [132].

In contrast to attempts to modify spiro-lactam 29 by replacement of nitrogen with oxygen in compounds 30 and 31, it has been shown that the spiro-oxazolidinone moiety, as hydrogen bond acceptor, can be replaced with a 1,3-dioxolane fragment to give compound 37 [133]. Further optimization of quinuclidine ketals might be limited by potential metabolic instability and poor synthetic yield.

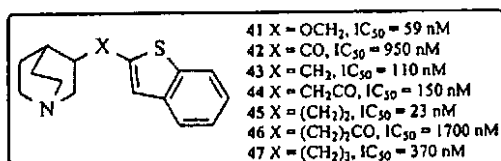
Linear oxygen-containing linkers are more amenable to adjustment, providing a diverse set of selective $\alpha 7$ nAChR ligands as 38 [134], 9, 39 [135], 40 [136].

The length and nature of the linker between the quinuclidine and hydrophobic part of a series of $\alpha 7$ nAChR ligands have been studied by Tatsumi *et al.* [126]. Two-atom spacers were found to provide optimal binding to the receptor. However, ether (S)- 41 has shown poor bioavailability, and both enantiomers 45 demonstrated slight and no agonistic activity. Compound (+)-44, on the other hand, shows dose-dependent agonistic effects, which are more potent than those for GTS-21 (1). Obtained data suggest an essential role of the carbonyl moiety as a potential hydrogen bond acceptor for efficacy of selective nAChR ligands.

The effect of a second hydrogen bond acceptor site has been demonstrated by diversification around a combination of 2-position and 3-position substitutions of the quinuclidine scaffold [137].



2-Heteroaryl methyl quinuclidines **25**, **48** and **49** containing a β -nitrogen atom are favored over benzyl (**8**) derivatives, and steric constraints restrict substitution on the heteroaryl ring. The contribution of this second hydrogen bond acceptor moiety is significant (one-two orders of magnitude increase of affinity), as can be seen by incorporation of pyridin-3-ylmethyl into *p*-chlorobenzamide **5**, resulting in improvement from $K_i = 27$ nM to $K_i = 0.28$ nM (for **50**). The presence of the second hydrogen bond acceptor does not adversely affect agonistic activity, the 3-(pyridin-3-ylmethyl)quinuclidine amides being selective $\alpha 7$ nAChR agonists, as exemplified by **51**.



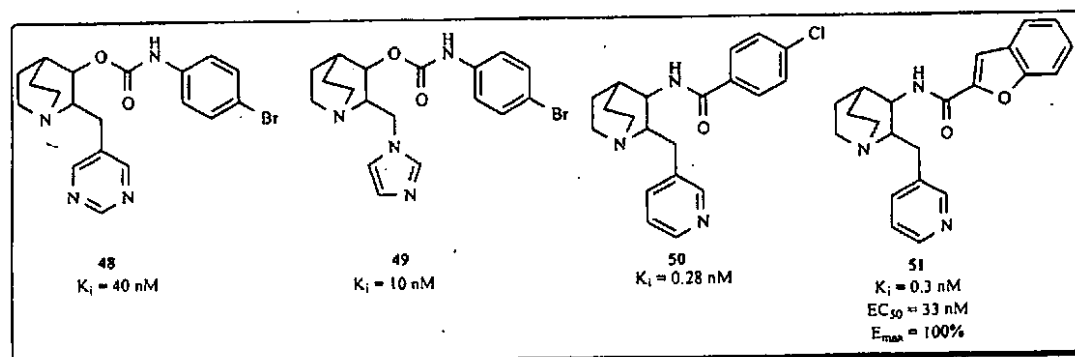
5.1.3. Hydrophobic Elements

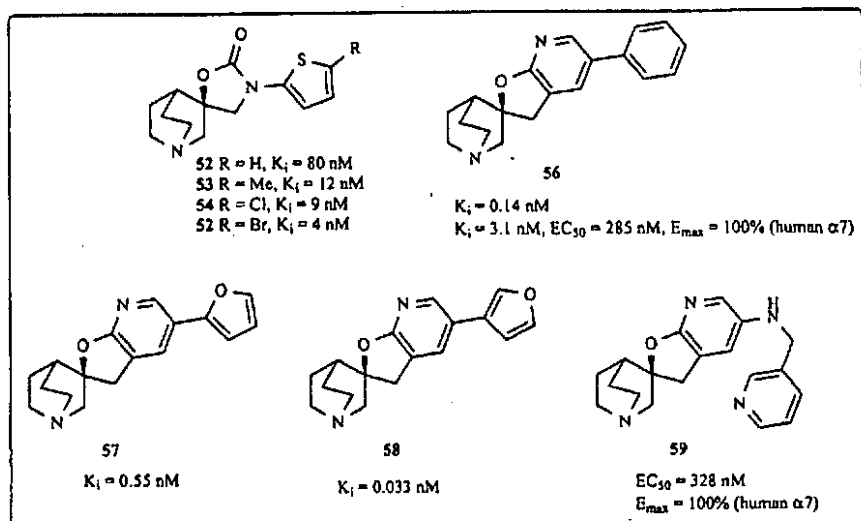
Incorporation of a hydrophobic moiety, which is either directly attached to the cationic nitrogen containing moiety or attached *via* a hydrogen bond acceptor, enhances binding properties of $\alpha 7$ nAChR ligands. Although N-methylation of spirooxazolidinone **29** has led to disappearance of its selectivity and has reduced binding affinity, substitution with a 5-bromothiophene ring results in compound **55** with $K_i = 4$ nM [138]. A clear stereogenic preference can be seen for the (R) versus the (S) enantiomer in this series. Pharmacokinetic profiling of compound **54** in rats indicates good oral bioavailability (52%) and high brain permeability ($C_p/C_f = 59$ at 10 mg/kg po). It also reduces MK-801-induced auditory gating deficit.

Lipophilic analogs **56-58** [139,140] of AR-R17779 (**29**) maintain $\alpha 7$ nAChR selectivity and demonstrate excellent improvement in affinity. Extension of the hydrophobic fragment modulates interaction with the receptor, which was optimized by synthesis of dihydrofuropyridine **58**. Compound **56** is equipotent at human recombinant $\alpha 7$ receptors [141] and 5-HT₂ receptors, ligand gated ion channels with approximately 30% homology with the $\alpha 7$ receptor. The 5-HT₂ cross-reactivity has been circumvented [129] by replacement of phenyl ring in **56** with aminomethylpyridine fragment to obtain **59** [142].

Distribution of electron density in the hydrophobic part of $\alpha 7$ nAChR ligand is essential for π -interaction with the corresponding binding site. This can be optimized by two methods: variation of substituents in aromatic rings or alteration of the heteroatom position in heteroaromatic systems. Among substituted N-quinuclidinyl benzamides [107], *o*-derivatives are very poor agonists (**61**) or practically inactive (**62**). *m*-Substituted amides, as **63** and **65**, have shown improvement in a functional high-throughput screening assay using SHEP cells expressing the $\alpha 7$ -5-HT₂ chimera. The *p*-substituted analogues **64**, **66** are the most active. Compound **66** (PNU-282987) restores amphetamine-induced sensory gating deficit, determined by auditory-evoked potentials in the hippocampal CA3 region, by systemic administration in chloral hydrate-anesthetized rats. Hajós *et al.* [77] proposed that PNU-282987 improves auditory gating *via* modulating/enhancing hippocampal GABAergic neurotransmission.

Substitution of the *p*-chlorophenyl in **66** with benzoxazole provides a compound **67** with similar affinity [143]. The K_i for benzodioxine derivative **68** [144] has not been disclosed. Replacement of the phenyl moiety with heteroaromatic mono- or bicyclic systems is advantageous especially when a carbonyl group is adjacent to the heteroatom as exemplified by compounds **69** [145] **70** [146],





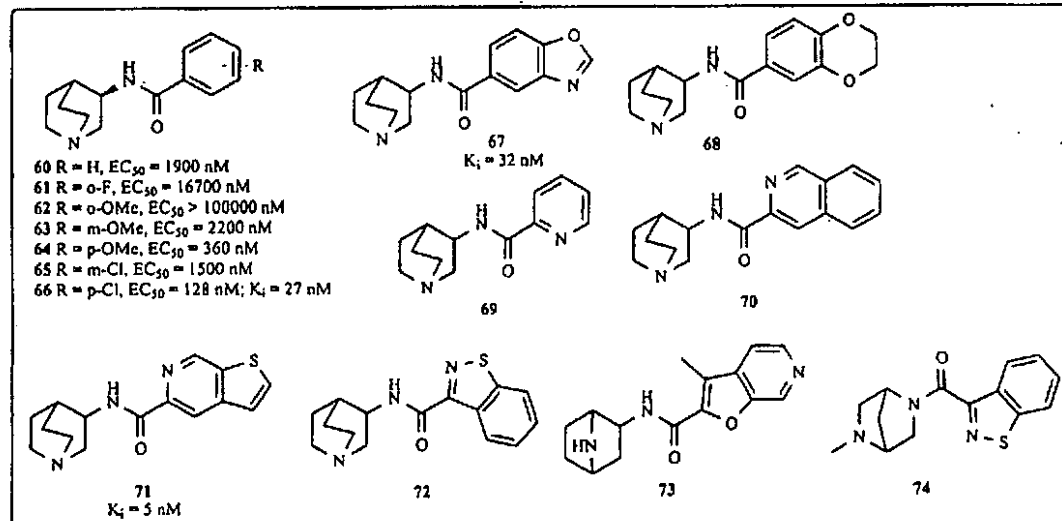
71 [147], 72 [148], 73 [149], 74 [116]. In general, the aromatic or heteroaromatic ring is conjugated with the hydrogen bond acceptor, although, there are several examples where the pharmacophoric elements are bridged via one (75) [150] or more (45, 47) [126] sp^3 -hybridized carbon atoms. Again, extension of the hydrophobic moiety by addition of a second π -electronic system yields a series of high affinity quinuclidine amides: 76, 77, 78 [151,152], 79 [153], and 80 [111]. Noteworthy, the evaluation of 77 and 78 [154,155] in behavioral assays, including the formalin assay, the prepulse inhibition model, the forced swim test, the radial arm maze, has failed to produce any positive data. However, Cilia *et al.* [78] recently demonstrated the effectiveness of an $\alpha 7$ selective agonist-77 in reversing prepulse inhibition deficits in isolation-reared rats.

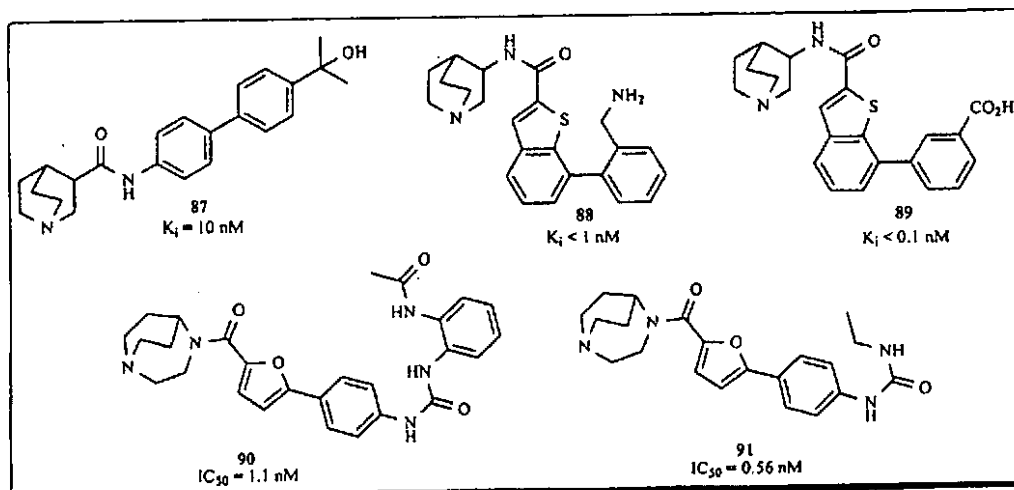
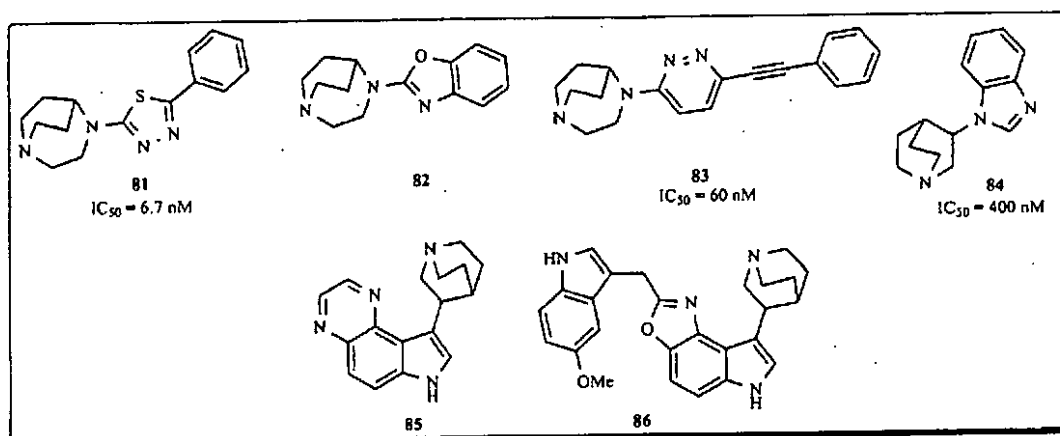
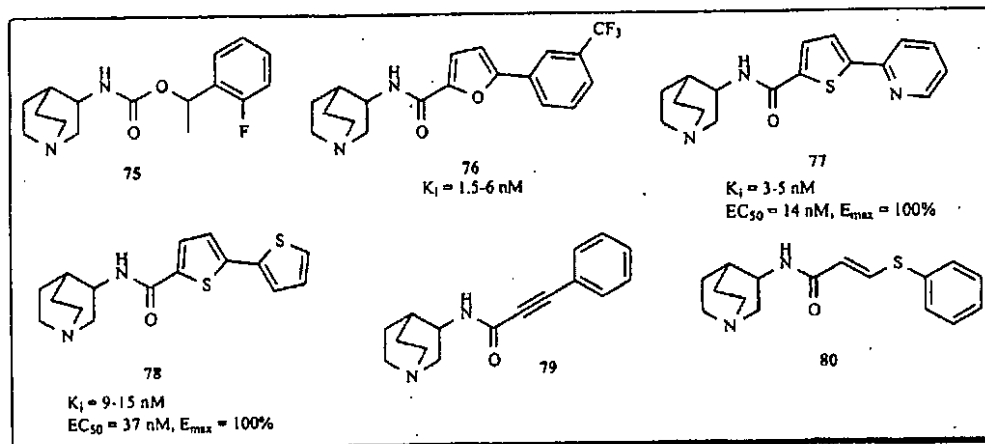
There are a few $\alpha 7$ nAChR ligands, 81 [156], 82 [157] 83 [158], 84 [159], in which a cationic fragment is directly

connected to a heteroaromatic hydrophobic system, described in the literature. The aromatic heteroatom (nitrogen or oxygen) of these might contribute a hydrogen bond acceptor interaction with the receptor.

3-(Indol-3-yl)quinuclidine derivatives are known serotonergic ligands. Compounds 85 [160] and 86 [161] have been claimed as mixed ligands for nAChR, 5-HT₃ and 5-HT₆ receptors.

Inclusion of what would typically be described as a hydrogen bond donor group, at the appropriate position in the hydrophobic part of an $\alpha 7$ nAChR ligand, can lead to high affinity. Compounds, containing terminal hydroxyl (87) [135], amino (88), carboxyl (89) [162] or urea (90, 91) [163] moieties, have been claimed as $\alpha 7$ ligands, although no information about their functional activity was provided.





5.2. Selective Antagonists

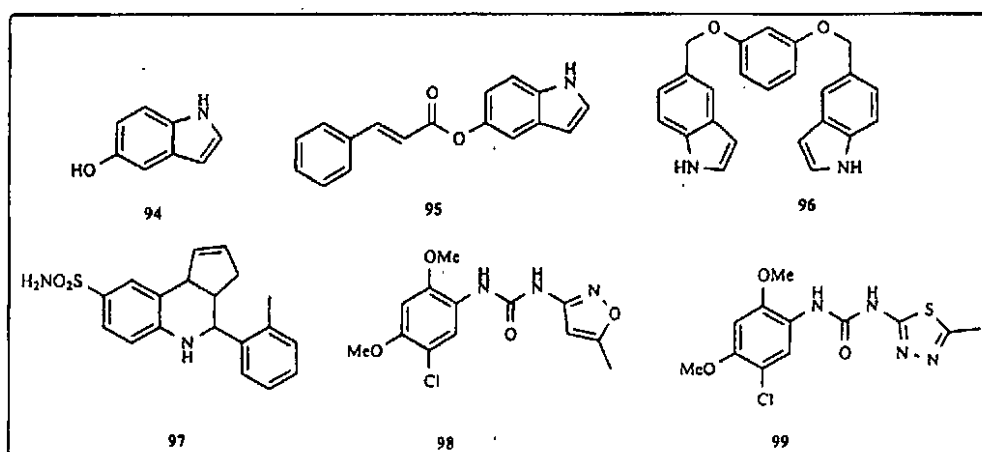
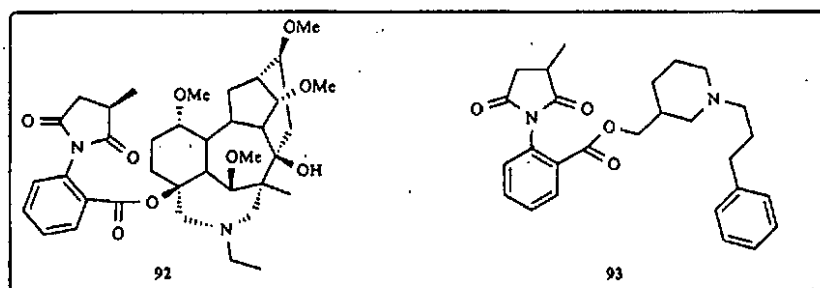
Although selective $\alpha 7$ nAChR full and partial agonists have been quite well explored, especially in the pharmaceutical industry, selective $\alpha 7$ nAChR antagonists would be valuable as pharmacological tools and may also prove to have therapeutic value. Many $\alpha 7$ nAChR agonists rapidly desensitize the receptor at much lower concentrations than is required for activation. Therefore, the compounds could perform as functional antagonists [129]. It might be expected that a desensitizing $\alpha 7$ nAChR agonist and an $\alpha 7$ nAChR antagonist would have similar pharmacology.

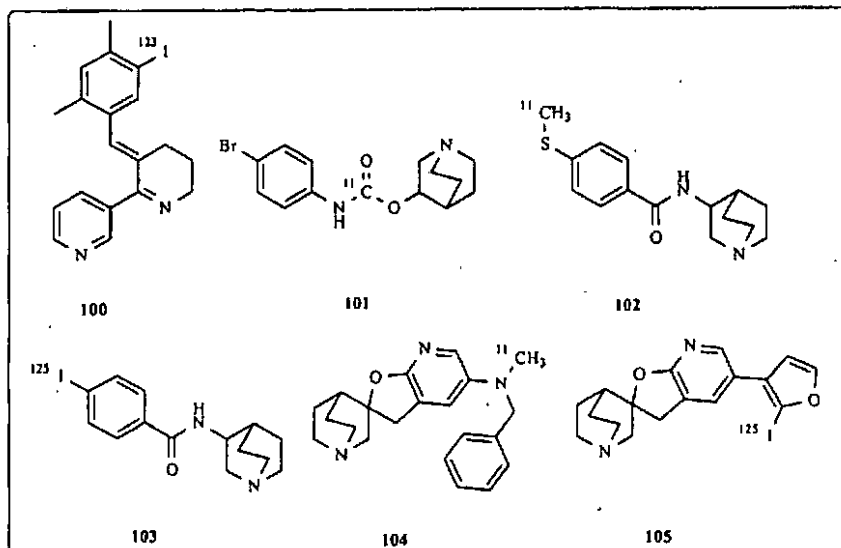
Methyllycaconitine (MLA) (92), a diterpenoid alkaloid isolated from *Delphinium brownie*, and α -bungarotoxin, a 75 amino acid peptide isolated from venom of *Bungarus multicinctus*, are potent and selective natural $\alpha 7$ nAChR antagonists. Efforts to modify MLA have been undertaken with the purpose of investigating the structural basis of its potency and subtype selectivity [164-167]. Obtained results define the norditerpenoid core of MLA as the major determinant of $\alpha 7$ nAChR selectivity, and establishes the prime importance of the 2-(methylsuccinimido)benzoyl substituent for nicotinic potency. A structurally less complex analog of MLA, 93 [168] has been identified by replacement of the hexacyclic structural fragment with simple N-substituted piperidine. Compound 93 inhibits nicotine-stimulated catecholamine release at micromolar concentration, being almost as efficacious as MLA.

5.3. Positive Allosteric Modulators

Under conditions of continuous exposure, such as chronic drug treatment, selective $\alpha 7$ nAChR agonists may have a relatively narrow effective dose range, limited to the region of overlap in which the receptors are activated but not fully desensitized [48]. A different treatment strategy would be to administer a nicotinic positive allosteric modulator that can reinforce the endogenous cholinergic neurotransmission without its direct stimulation [169]. Unlike agonists, allosteric modulators do not directly activate or desensitize ligand-gated receptors but enhance the sensitivity and/or efficacy of the receptor during agonist activation, probably by stabilization of its activated conformation.

Several positive allosteric modulators of $\alpha 7$ nAChR have been described, including 5-hydroxyindole (94) [53], and its derivatives, as 95 [170] and 96 [171], cyclopentanoquinoline analogues (97) [172], ivermectin [173], serum albumins [174], secreted mammalian Ly-6/uPAR-related protein [175], and a peptide fragment of acetylcholinesterase [176]. All of these compounds potentiate the peak agonist-evoked response mediated by the $\alpha 7$ nAChR. Piotrowski *et al.* [177] and Hurst *et al.* [48] reported the novel synthetic positive allosteric modulator PNU-120596 (98), which not only increases the potency and efficacy of agonists but also dramatically prolongs the duration of agonist-evoked response. It increases the frequency of acetylcholine-evoked GABAergic postsynaptic currents measured in pyramidal





neurons, an effect which is suppressed by TTX, suggesting that 98 modulates the function of $\alpha 7$ nAChRs located on the somatodendritic membrane of hippocampal interneurons. PNU-120596 has no detectable activity when applied alone. Prominently, systemic administration of PNU-120596 improves auditory gating in rats impaired by amphetamine. Similar to 98, ureas (99) have been claimed by Piotrowski *et al.* [178].

6. RADIOLABELED IMAGING LIGANDS

$\alpha 7$ nAChRs are implicated in a wide variety of pathologic states including Alzheimer's disease [179] and schizophrenia [180]. These observations prompted exploration of $\alpha 7$ nAChR radioligands, which would be applied in positron emission tomography (PET) and single photon emission computed tomography (SPECT) to visualize the receptors in human brain during treatment of patients. Though the number of $\alpha 4\beta 2$ nAChRs is decreased in Alzheimer's disease, the number of $\alpha 7$ nAChRs remains largely intact and therefore available for binding new therapeutic agents [179].

A few radiolabeled selective $\alpha 7$ nAChR ligands have been described in the literature. ^{125}I - α -bungarotoxin [181] and ^3H -methyllycaconitine [182] have been utilized most commonly for *in vitro* and *ex vivo* characterization of the receptor. The "functionally"-selective anabaseine derivative 100 [183] has not demonstrated regionally selective binding *in vivo*. Navarro *et al.* [184] have shown encouraging *in vivo* biodistribution studies of ^{125}I -methyllycaconitine in rats, despite the rapid clearance and poor brain penetration. Quinuclidine carbamate 101 [185] has exhibited a relatively good brain uptake (0.8-1.2% I.D./g tissue in various brain regions), but without preferential concentration in brain regions rich in $\alpha 7$ nAChR (e.g. hippocampus, pons and colliculi). Recently, Pomper *et al.* [186] have published four potential imaging ligands 102-105. Only compound 105 has shown promising results with brain uptake (2.5%), higher uptake in the hippocampus and superior colliculus than in

nontarget sites, and definite site-selective blockade. Unfortunately, it is not entirely clear whether it was the $\alpha 7$ nAChR or the 5-HT₂ receptor binding sites that were blocked because of the low binding selectivity of 105 for $\alpha 7$ over 5-HT₂.

7. CONCLUSION

In less than a decade, the $\alpha 7$ nAChR has become the most intensely studied nicotinic acetylcholine receptor subtype. A series of selective nAChR agonists have been developed as potential candidates for the treatment of schizophrenia, cognitive disorders (including Alzheimer's disease), and inflammation. Despite early skepticism centered around the rapid desensitization of the $\alpha 7$ nAChR and the hypothesis that agonists acting might not be functional agonists *in vivo*, some of them have already been advanced to clinical trials. Further development of allosteric modulators and pharmaceutically relevant antagonists might expand the therapeutic potential of $\alpha 7$ nAChRs.

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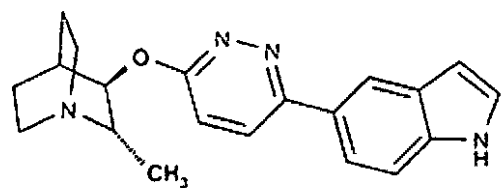
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ANEXO 3

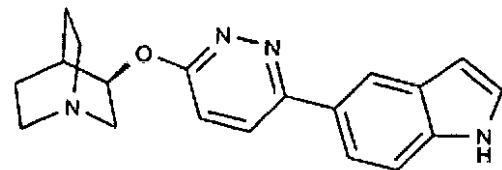
Biological Data to support European Patent Application No. 05 758 825.3

The affinity and efficacy of a typical compound of the invention (Example No. 3k) and of the corresponding prior art compound (Example 9 of US2005/0137183 / US2005/0137204) in activating the nicotinic acetylcholine receptor nAChR $\alpha 7$ was assessed in a functional assay.

Structure of compounds:



Example 3k of the invention



Prior art example

Results:

Compound	nAChR $\alpha 7$ (EC ₅₀ nM)	SEM	nAChR $\alpha 7$ efficacy (%) ¹	SEM
Example 3k	13	0.1	89	5%
Prior art example	16	0.2	93	14%

¹ Efficacy compared to epibatidine (which is a full nAChR $\alpha 7$ agonist; D Feuerbach et al, 2005, see below)

Assay description

To assess nAChR $\alpha 7$ activity, a functional assay was employed using GH3 cells that recombinantly expressed human nAChR $\alpha 7$. 5 x 10⁴ cells per well were seeded 72 h prior to the experiment on black 96-well plates (Costar) and incubated at 37°C in a humidified atmosphere (5 % CO₂/95 % air). On the day of the experiment, medium was removed by flicking the plates and replaced with 100 μ l growth medium containing 2 mM Fluo-4, (Molecular Probes) in the presence of 2.5 mM probenecid (Sigma). The cells were incubated at 37°C in a humidified atmosphere (5 % CO₂/95 % air) for 1 h. Plates were flicked to remove excess of Fluo-4, washed twice with Hepes-buffered salt solution (in mM: NaCl 130, KCl 5.4, CaCl₂ 2, MgSO₄ 0.8, NaH₂PO₄ 0.9, glucose 25, Hepes 20, pH 7.4; HBS) and refilled with 100 μ l of HBS containing antagonist when appropriate. The incubation in the presence of the antagonist lasted 3-5 minutes.

Plates were placed in the cell plate stage of a FLIPR device (fluorescent imaging plate reader, Molecular Devices, Sunnyvale, CA, USA). After recording of the baseline (laser: excitation 488 nm at 1 W, CCD camera opening of 0.4 seconds) the agonists (50 μ l) were added to the cell plate using the FLIPR 96-tip pipettor while simultaneously recording the fluorescence. Calcium kinetic data were normalized to the maximal fitted response induced by epibatidine, which is a full agonist at nAChR $\alpha 7$. Four parameter Hill equations were fitted to the concentration-response. Values of Emax (maximal effect in % compared to the epibatidine response) and EC50 (concentration producing half the maximal effect in μ M) were derived from this fit. Assay described in: D Feuerbach et al, Neuropharmacology (2005), 48, 215-227.