

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
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DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 1 REGISTRO/DEPOSITO/CONCESION/DEPOSITO
TRA: 2 PATENTES DE INVENCION	FOLIOS: 7
ACT: 519 PRESENTACION	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
NATALIA CABRERA MARTINEZ

Asunto: Radicación: 12-69442- -4
 Trámite: 2
 Evento: 1
 Actuación: 519
 Oficio: 23155
 Folios: 7

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional	X				
Vía PCT (Fase Nacional)		Capítulo I		Capítulo II	
No. Solicitud Internacional					
Fecha de Presentación Internacional					

1 . Documentos estudiados

Descripción:	Radicado N° 12-069442-00000-0000	27/04/2012
Reivindicaciones:	Radicado N° 12-069442-00000-0000	27/04/2012
Prioridad:	11/0134775 INPI-Paris SP 34	02/05/2011

2. Análisis de la Prioridad

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

3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del Capítulo Primero del Título X de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada.

Título de la solicitud: “NUEVA ASOCIACIÓN ENTRE LA 4-{3-[CIS-HEXAHIDROCICLOPENTA[C]PIRROL-2(1H)-IL]PROPOXI}BENZAMIDA Y UN INHIBIDOR DE LA ACETILCOLINESTERASA Y LAS COMPOSICIONES FARMACÉUTICAS QUE LA CONTIENEN”

El problema planteado en esta solicitud consiste en la necesidad de proporcionar una

combinación alternativa útil en el tratamiento de los trastornos cognitivos asociados al envejecimiento cerebral y a las enfermedades neurodegenerativas

Para resolver este problema técnico la solicitud en estudio proporciona una asociación entre la 4-{3-[cis-hexahidrociclopenta[c]pirrol-2(1h)-il]propoxi}benzamida de fórmula (I) o una de sus sales de adición a un ácido o una base farmacéuticamente aceptable y un inhibidor de la acetilcolinesterasa y las composiciones farmacéuticas que la contienen

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07D259/52 // 403/12

4 . Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El uso de las reivindicaciones 7, 8 y 10 - 15 no es patentable, de acuerdo con el Art 14.

5 . De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

Las reivindicaciones 1 y 6 no son claras cuando reclaman una asociación entre la 4-{3-[cis-hexahidrociclopenta[c]pirrol-2(1h)-il]propoxi}benzamida y un inhibidor de la acetil colinesterasa. Esta manera de reclamar el segundo componente no es válida puesto que no define un componente como tal. Se sugiere restringirse a las asociaciones y composiciones sustentadas en la descripción.

Las reivindicaciones 7 y 8 indican el uso que puede darse a la composición farmacéutica aunque las reivindicaciones estén encabezadas en términos de “Composición farmacéutica”, la materia objeto de las reivindicaciones 7 y 8 ya se encuentra contemplada en la reivindicación 6. Se sugiere eliminar estas reivindicaciones del pliego reivindicatorio.

Las reivindicaciones 12 y 13 indican el uso que puede darse a la asociación reclamada aunque las reivindicaciones estén encabezadas en términos de “Asociación”, la materia objeto de las reivindicaciones 12 y 13 ya se encuentra contemplada en las reivindicaciones 1 a 5. Se sugiere eliminar estas reivindicaciones del pliego reivindicatorio.

Las reivindicaciones 10, 11, 14 y 15 se refieren a usos que se le pueden dar al compuesto lo cual no es patentable según la legislación aplicable. La materia objeto de las reivindicaciones 10, 11, 14 y 15 ya se encuentra contemplada en las reivindicaciones 1 a 5. Se sugiere eliminar estas reivindicaciones del pliego reivindicatorio.

6 . Unidad de invención (Art 25 D 486)

La solicitud no cumple con el requisito de unidad de invención porque se refiere a 3 grupos inventivos.

Los grupos inventivos están definidos por las siguientes características esenciales:

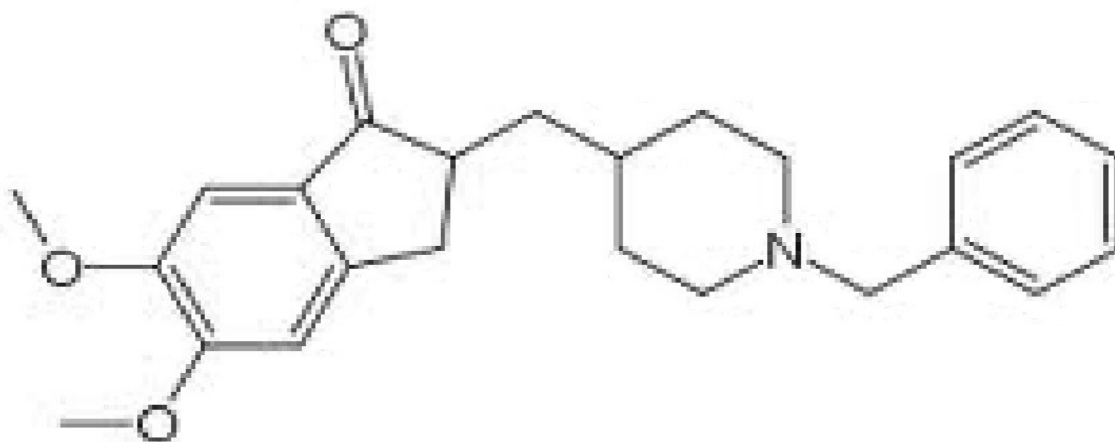
- 1° Asociación del compuesto 4-{3-[cis-hexahidrociclopenta[c]pirrol-2(1h)-il]propoxi}benzamida de fórmula (I) y donepezil
- 2° Asociación del compuesto 4-{3-[cis-hexahidrociclopenta[c]pirrol-2(1h)-il]propoxi}benzamida de fórmula (I) y rivastigmina
- 3° Asociación del compuesto 4-{3-[cis-hexahidrociclopenta[c]pirrol-2(1h)-il]propoxi}benzamida de fórmula (I) y galantamina

No hay unidad de invención entre los tres grupos inventivos ya que el concepto común inventivo, que corresponde al compuesto 4-{3-[cis-hexahidrociclopenta[c]pirrol-2(1h)-il]propoxi}benzamida

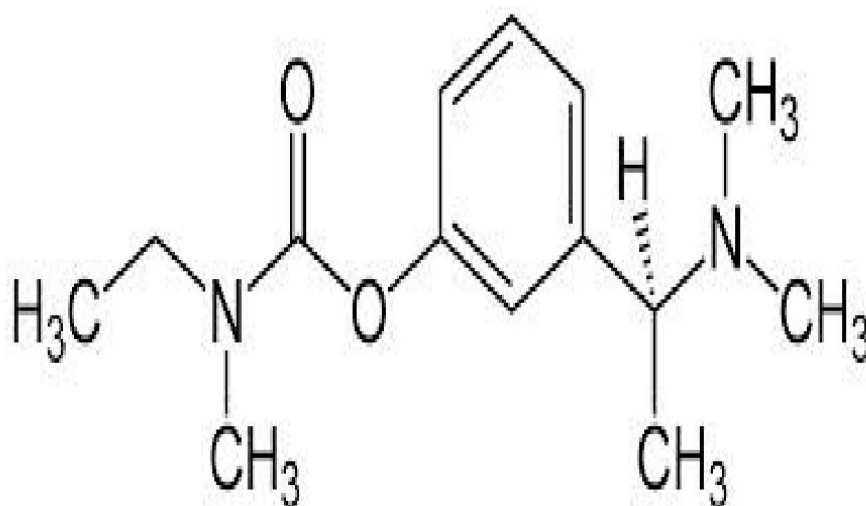
de fórmula (I), no cumple con el requisito de novedad de acuerdo con el documento WO2010043787 que divulga completamente la estructura de dicho compuesto (Págs. 1 a 3) (Ver análisis de patentabilidad).

Adicionalmente se observa que con respecto a los tres fármacos con los que se pretende reclamar una asociación con el compuesto 4-{3-[cis-hexahidrociclopenta[c]pirrol-2(1h)-il]propoxi}benzamida, es decir donepezil, rivastigmina y galantamina; si bien es cierto presentan el mismo mecanismo de acción como lo es inhibir la acetilcolinesterasa, sus estructuras químicas son completamente diferentes, como se puede observar en las siguientes gráficas. Por lo tanto el único concepto común inventivo corresponde al compuesto 4-{3-[cis-hexahidrociclopenta[c]pirrol-2(1h)-il]propoxi}benzamida y al este encontrarse completamente divulgado en el estado de la técnica, esta oficina considera que la solicitud carece de unidad de invención.

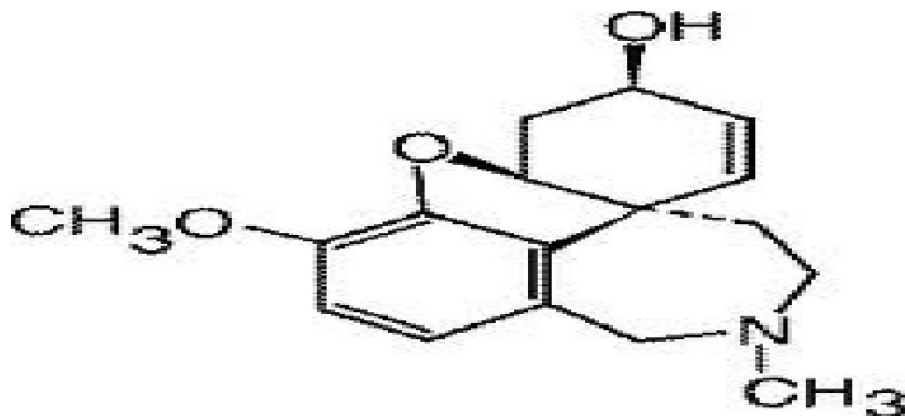
Donepezil



Rivastigmina



Galantamina



En el examen de patentabilidad se estudiara uno de los grupos inventivos el cual será la asociación entre el compuesto 4-{3-[cis-hexahidrociclopenta[c]pirrol-2(1h)-il]propoxi}benzamida de fórmula (I) y donepezil

Se le sugiere al solicitante restringir la materia a patentar en un solo concepto común inventivo especificando el avance tecnológico sobre el estado de la técnica. Esto se logra entre otras formas definiendo las características esenciales de la invención y mostrando las otras características particulares como dependientes, pero sin perjuicio de la concisión de las nuevas reivindicaciones tanto independientes como dependientes.

El solicitante también puede realizar una o más de las divisionales de acuerdo a los grupos de conceptos técnicos inventivos presentados en esta solicitud, esto claro, sin ampliar el objeto inicial de la invención.

7. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de la cual se invoca prioridad, es decir, el 02 de mayo de 2011.

Consultadas las fuentes de información con que se cuenta, se encontró (encontraron) el (los) siguiente(s) documento(s) que anticipa(n) el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1 ^[1]	WO2010043787	"USE OF 4-(3-[HEXAHYDROCYCLOPENTA[C]PYRROL-1(1H)-YL]PROPOXY}BENZAMIDE FOR MAKING DRUGS FOR TREATING SLEEP DISORDERS"	22/Abril/2010
D2 ^[2]	NEUROLOGY 2000;55:870-871	"TREATMENT OF REM SLEEP BEHAVIOR DISORDER WITH DONEPEZIL: A REPORT OF THREE CASES"	Septiembre/2000

El documento D1 divulga el compuesto 4-{3-[cis-hexahidrociclopenta[c]pirrol-2(1H)-il]propoxi}benzamida útil en el tratamiento de trastornos del sueño (Págs. 1 a 3) útil en el tratamiento de trastornos del sueño y de enfermedades neurodegenerativas.

El documento D2 divulga el efecto del donepezil en el tratamiento de trastornos del sueño (Págs. 1 a 3)

1[URL: [http://worldwide.espacenet.com/publicationDetails/originalDocument?](http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=WO&NR=2010043787A1&KC=A1&FT=D&ND=3&date=20100422&DB=worldwide.espacenet.com&locale=en_EP)

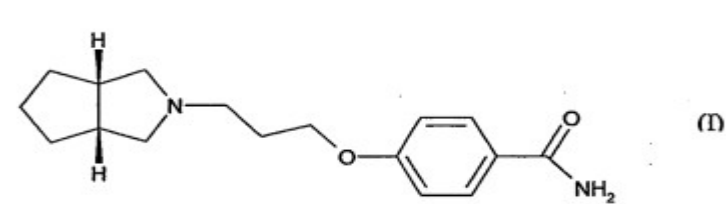
CC=WO&NR=2010043787A1&KC=A1&FT=D&ND=3&date=20100422&DB=worldwide.espacenet.com&locale=en_EP

2[URL: <http://www.neurology.org/content/55/6/870.abstract>

8 . Examen de Patentabilidad (Art 14 D486)

Nivel Inventivo (Art18 D486)

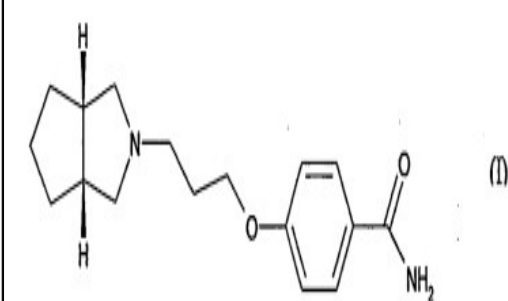
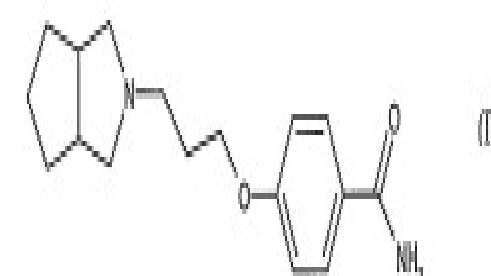
La reivindicación 1 consiste en la asociación de la 4-{3-[cis-hexahidrociclopenta[c]pirrol-2(1H)-il]propoxi}benzamida de fórmula (I)



o una de sus sales de adición o una base farmacéuticamente aceptable y un inhibidor de la acetilcolinesterasa.

Se estudiara específicamente el grupo inventivo en el cual el inhibidor de acetilcolinesterasa corresponde a donepezil.

Comparación de las características técnicas esenciales, con las del estado de la técnica:

SOLICITUD	D1	D2
Asociación entre  y un inhibidor de acetilcolinesterasa	Clorhidrato del compuesto  como inhibidor del receptor de histamina H₃ útil en el tratamiento de trastornos del sueño y neurodegenerativos (Págs. 1 a 3; Págs. 7 y 8, reivindicaciones 1 a 8)	Donepezil (fármaco inhibidor de acetilcolinesterasa) administrado a una dosis de 10 mg diarios en el tratamiento de trastornos del sueño (Págs. 1 a 3)

El documento D1 divulga el compuesto 4-{3-[cis-hexahidrociclopenta[c]pirrol-2(1H)-il]propoxi}benzamida útil en el tratamiento de trastornos del sueño (Págs. 1 a 3)

La diferencia entre la invención, definida en la reivindicación 1 y el compuesto del documento D1 consiste en que este documento no divulga que el compuesto 4-{3-[cis-hexahidrociclopenta[c]pirrol-2(1h)-il]propoxi}benzamida se pueda utilizar en una asociación con un inhibidor de acetilcolinesterasa.

El documento D2 divulga efecto del donepezil en el tratamiento de trastornos del sueño (Págs. 1 a 3)

La diferencia entre la invención, definida en la reivindicación 1 y el compuesto del documento D2 consiste en que este documento no divulga que el donepezil se pueda utilizar en una asociación con el compuesto 4-{3-[cis-hexahidrociclopenta[c]pirrol-2(1h)-il]propoxi}benzamida

El efecto que logra el asociar el compuesto 4-{3-[cis-hexahidrociclopenta[c]pirrol-2(1H)-il]propoxi}benzamida con un inhibidor de acetilcolinesterasa (en este caso el donepezil) es que proporciona un tratamiento alternativo para trastornos cognitivos asociados al envejecimiento cerebral y a las enfermedades neurodegenerativas.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo modificar el compuesto conocido en el documento D1 para proporcionar un tratamiento alternativo para trastornos cognitivos asociados al envejecimiento cerebral y a las enfermedades neurodegenerativas”.

Sin embargo, para una persona normalmente versada en la materia sería obvio realizar una combinación entre los compuestos conocidos en el estado de la técnica con efecto en el tratamiento de para trastornos cognitivos asociados al envejecimiento cerebral y a las enfermedades neurodegenerativas esperando se mantenga dicho efecto, presentándose un efecto aditivo o sinérgico; más aún cuando en el estado de la técnica ya se sugiere la combinación de inhibidores del receptor de histamina H₃ con inhibidores de acetilcolinesterasa^[3]

En consecuencia, la persona del oficio normalmente versada en la materia, fácilmente esperaría que una combinación entre el fármaco inhibidor del receptor de histamina H₃ 4-{3-[cis-hexahidrociclopenta[c]pirrol-2(1H)-il]propoxi}benzamida utilizando sus diversas formas enantioméricas teniendo en cuenta que estas corresponden a propiedades intrínsecas del compuesto y el fármaco inhibidor de acetilcolinesterasa donepezil, presentara un efecto aditivo útil en el tratamiento para trastornos cognitivos asociados al envejecimiento cerebral y a las enfermedades neurodegenerativas dada la eficacia comprobada de los dos compuestos en tratamiento de trastornos del sueño; los cuales se encuentran relacionados con procesos de envejecimiento cerebral y aparición de enfermedades neurodegenerativas según las enseñanzas proporcionadas en los documentos D1 y D2 para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia. Adicionalmente una persona normalmente versada en la materia utilizaría dichos compuestos en forma de las sales ampliamente reconocidas en el campo farmacéutico como lo son clorhidratos, oxalatos y tartratos entre otras.^[4]

En conclusión la reivindicación 1 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

Puesto que las reivindicaciones dependientes 2 – 6 y 9 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

En consecuencia las reivindicaciones 1 – 6 y 9 cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

9. Conclusión

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

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO2010043787	1 – 6 y 9	Nivel Inventivo
D2	TREATMENT OF REM SLEEP BEHAVIOR DISORDER WITH DONEPEZIL: A REPORT OF THREE CASES	1 – 6 y 9	Nivel Inventivo

3[]:http://www.sciencedirect.com/science/article/pii/S0968089607011170

4[] Handbook of Pharmaceutical Analysis Chapter 1 “Form selection of Pharmaceutical compounds”, III Salt Selection.

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

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

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



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2013-12-24

Elaboró: Rodolfo Cadena Amaya
Revisó: Gloria Jacqueline Alfonso Roza

Treatment of REM sleep behavior disorder with donepezil: A report of three cases

Article abstract—Three patients with REM behavior disorder whose nocturnal symptoms were markedly improved by treatment with the acetylcholinesterase inhibitor donepezil are reported. Donepezil may have a role in the treatment of REM behavior disorder, possibly through its actions on cholinergic pathways in the brainstem.

NEUROLOGY 2000;55:870–871

J.M. Ringman, MD; and J.H. Simmons, MD

REM sleep behavior disorder (RBD) is a parasomnia characterized by loss of the atonia normally accompanying REM sleep.¹ This results in the acting out of dream content, with disruptive and potentially violent behavior. There is a body of convergent data suggesting the involvement of brainstem cholinergic neurons in the regulation of REM sleep and its atonia (see below).^{2,3} Dysfunction of these neurons might therefore be implicated in the etiology of RBD. We report three patients with parasomnias consistent with RBD who had diminution of their RBD symptoms after treatment with donepezil, an acetylcholinesterase inhibitor.

Case reports. *Patient 1.* Patient 1 is a 72-year-old man referred for a 3-year history of progressive behavioral changes and difficulty with memory.⁴ Medical history included a suprasellar craniopharyngioma resection 18 years prior. After the surgery he began having a sleep disorder characterized by somnolism and threatening dreams consisting of cars driving toward him and wild animals attacking him. These episodes might culminate with the patient's jumping out of bed to avoid the perceived threat. Although these events occurred every night, no workup had been performed. Examination revealed no evidence of parkinsonism. Evaluation for cognitive loss was performed and the patient was given the diagnosis of possible AD. He was begun on donepezil 5 mg every day, increased to 10 mg 1 month later. After a week of being on 10 mg donepezil, the patient and his wife stated that there was a marked decrease in his dreams and agitation, although a mild degree of somnolism persisted. The patient refused further evaluation for his sleep disorder.

Patient 2. Patient 2 is a 29-year-old man who reported sleep behaviors originating during early childhood. These consisted of talking and occasionally walking and manipulating items in his bedroom. He recalled vivid dream content associated with these behaviors. On average he had about one spell per week. Polysomnography demonstrated phasic increases in electromyographic activity in association with the eye movements of REM sleep. He was placed on donepezil 5 mg every night at bedtime without benefit.

The dose was then increased to 10 mg, which produced a decline in the frequency of the spells to about one per month. The parasomnic activity, when present, was described as minimal, consisting only of mild somnolism and small movements. He no longer strayed from his bed. This benefit had been maintained for over a year at the time of the last clinic visit.

Patient 3. Patient 3 is a 56-year-old man who presented with a 4-year history of parasomnias consisting of shouting, screaming, and thrashing behaviors during sleep. The patient occasionally remembered vivid, usually violent, dream content associated with these episodes. These spells occurred to some degree almost nightly, with severe episodes occurring approximately three times per week. Polysomnography was performed, demonstrating phasic increases in electromyographic activity in association with REM sleep. The patient was placed on clonazepam and titrated up to 1.5 mg each bedtime and there were almost no parasomnic episodes reported at follow-up.

As the patient experienced fatigue and memory impairment from clonazepam, it was tapered 2 years later and oral donepezil 10 mg each bedtime was started. Once off clonazepam, the parasomnic events returned, occurring almost nightly. The dose of donepezil was increased to 15 mg, which resulted in a decrease in the frequency of the parasomnic spells to about once every one to two weeks, consisting only of minimal somnolism. This degree of benefit has been maintained for over 1 year.

Discussion. Our three cases provide evidence that symptoms of RBD can be ameliorated by an intervention that enhances cholinergic neurotransmission. Our first patient likely had AD with an RBD-like parasomnia developing in conjunction with basal skull surgery. However, considering the association of RBD with neurodegenerative diseases¹ such as Lewy body dementia,⁵ the possibility of RBD arising as a result of the degenerative process can not be ruled out. Although Patient 1 did not undergo polysomnography, he fit minimal diagnostic criteria for RBD as defined by the International Classification of Sleep Disorders,⁶ that is, movement of limbs or body associated with dream content. The other two patients had idiopathic RBD demonstrated polysomnographically.

There is considerable evidence from animal studies that neurons located in the upper pontine tegmentum play a major role in producing REM sleep and the related atonia.¹ Lesions of this area in cats produce a model closely resembling human RBD, in

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which loss of atonia and excessive motor activity during paradoxical sleep is seen.³ The identity of the specific cell groups in the mesopontine tegmentum most involved in RBD is not clear. Neurons in the pedunculopontine nuclei, the midbrain extrapyramidal area, and in the retrorubral field overlap in distribution and may all play a role in regulating REM sleep.⁷ These nuclei receive afferents from the basal ganglia, the locus coeruleus, and the dorsal raphe nucleus. They exert their cortical activating influence in REM sleep via ascending projections to both specific and nonspecific thalamic nuclei and on REM atonia via descending projections to the nuclei reticularis magnocellularis in the medulla. These medullary neurons in turn project to spinal motoneurons where they employ glycine postsynaptically as an inhibitory neurotransmitter, inducing REM-related atonia. RBD could potentially arise from disruption of this circuit at any point.

Human and animal pharmacologic studies implicate a number of different neurotransmitter systems in REM sleep and RBD. RBD is effectively treated with clonazepam 0.5 to 1.5 mg at bedtime.¹ This effect is well documented though the exact mechanism whereby benzodiazepines improve RBD is not clear. γ -Aminobutyric acid (GABA)-ergic pallidal outputs to the mesopontine tegmentum may have a role as might GABA-containing neurons intrinsic to the pedunculopontine nuclei. RBD in the setting of PD has been reported to improve after treatment with L-dopa.⁸ This implicates dopaminergic neurons in RBD, either those in the basal ganglia upstream from the aforementioned pallidal output or in the mesopontine tegmentum itself. Pathways involving acetylcholine are known to be involved in the regulation of REM sleep and therefore likely relate to the pathology of RBD.^{2,3} Infusion of cholinergic agonists such as carbachol directly into the brainstem of cats induces cortical desynchronization, pontogeniculo-occipital waves, REM, and cataplexy comparable to the atonia of REM. This effect can be blocked by the administration of cholinergic antagonists such as atropine. Further evidence for the involvement of acetylcholine in the atonia of REM sleep is that administration of cholinergic agonists to narcoleptic dogs induces cataplexy whereas the administration of muscarinic receptor blockers delays these epi-

sodes. In humans it has been demonstrated that acetylcholinesterase inhibitors can shorten REM latency and increase REM density.⁹ On the other hand, cholinesterase inhibitors were reported to induce RBD in a patient with AD.¹⁰ REM sleep involves multiple neurotransmitters acting at multiple sites and RBD may arise from diverse perturbations of the system. Cholinergic pathways, however, appear to play a central role.

It is only relatively recently that centrally active cholinesterase inhibitors have come into widespread use in humans. Our cases support an ameliorative effect of donepezil on RBD and a potential new use for the drug. Whether the effect is mediated by increased REM-related atonia or through some other effect on REM sleep is not known. A controlled trial of cholinergic agonists in RBD with pre- and post-treatment polysomnography would be helpful both in elucidating the nature of the condition and in helping patients unresponsive to or intolerant of treatment with clonazepam.

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Lead identification of acetylcholinesterase inhibitors–histamine H₃ receptor antagonists from molecular modeling

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Abstract—Currently, the only clinically effective treatment for Alzheimer's disease (AD) is the use of acetylcholinesterase (AChE) inhibitors. These inhibitors have limited efficacy in that they only treat the symptoms and not the disease itself. Additionally, they often have unpleasant side effects. Here we consider the viability of a single molecule having the actions of both an AChE inhibitor and histamine H₃ receptor antagonist. Both histamine H₃ receptor antagonists and AChE inhibitors improve and augment cholinergic neurotransmission in the cortex. However, whereas an AChE inhibitor will impart its effect everywhere, a histamine H₃ antagonist will raise acetylcholine levels mostly in the brain as its mode of action will primarily be on the central nervous system. Therefore, the combination of both activities in a single molecule could be advantageous. Indeed, studies suggest an appropriate dual-acting compound may offer the desired therapeutic effect with fewer unpleasant side effects [*CNS Drugs* **2004**, *18*, 827]. Further, recent studies² indicate the peripheral anionic site (PAS) of AChE interacts with the β -amyloid (β A) peptide. Consequently, a molecule capable of disrupting this interaction may have a significant impact on the production of or the aggregation of β A. This may result in slowing down the progression of the disease rather than only treating the symptoms as current therapies do. Here, we detail how the use of the available crystal structure information, pharmacophore modeling and docking (automated, manual, classical, and QM/MM) lead to the identification of an AChE inhibitor–histamine H₃ receptor antagonist. Further, based on our models we speculate that this dual-acting compound may interact with the PAS. Such a dual-acting compound may be able to affect the pathology of AD in addition to providing symptomatic relief.
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1. Introduction

Alzheimer's disease is the most common form of neurodegenerative dementia.³ It accounts for approximately 50–60% of the overall cases of dementia among individuals over the age of 65 years.⁴ Unfortunately, the therapeutic options for Alzheimer's disease are limited. No true disease-modifying therapies are known and at best physicians can only hope to alleviate the cognitive deficits that characterize this disease with symptomatic treatments. The most frequently prescribed anti-Alzheimer's drugs are the acetylcholinesterase (AChE) inhibitors, which promote memory function and delay the

cognitive decline without altering the underlying pathology.⁵ These compounds, however, leave much to be desired, both in efficacy and tolerability. Their efficacy is limited; the preferred target patient population suffers a mild to moderate cognitive impairment.⁵ Additionally, they are associated with unpleasant mechanism-based side effects such as gastrointestinal discomfort and risk of bradycardia, especially the nonselective AChE inhibitors, which inhibit AChE as well as butyrylcholinesterase.⁶

Clinical studies suggest combination therapies can provide synergistic benefits in some instances.¹ An alternative approach taken by several pharmaceutical companies is to combine⁷ AChE inhibition with other mechanisms targeted at symptoms of Alzheimer's disease using a single molecular entity. For instance, inhibition of the serotonin reuptake transporter is expected to

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bestow antidepressant efficacy, which may alleviate some of the psychiatric symptoms observed in Alzheimer's populations. Dual-acting compounds of AChE and the serotonin transporter have been synthesized.⁸ The argument in favor of the combination of an AChE inhibitor and histamine H₃ receptor antagonist can be separated into a neurochemical and a procognitive argument. Neurochemically it is believed the decline in cognitive function in normal aging, mild cognitive impairment, Alzheimer's disease, and related conditions is caused by a decrease in cholinergic neurotransmission in the cortex. Both AChE inhibitors and histamine H₃ receptor antagonists improve and augment cholinergic neurotransmission in the cortex, albeit by different mechanisms. Therefore, the combination of both activities in a single molecule may lead to a potentiation of cholinergic neurotransmission. Procognitive benefits are expected from both AChE inhibitors and histamine H₃ receptor antagonists as both exhibit memory-enhancing effects. The combination of both activities in a single molecule might be expected to have significant synergistic or additive effects on cognitive function and memory.

Inhibition of AChE increases the synaptic levels of acetylcholine and thereby promotes cholinergic neurotransmission. The effect of AChE inhibitors is thus to stabilize and prolong the biological half-life of acetylcholine. In contrast, histamine H₃ receptor antagonists increase cholinergic neurotransmission via a distinct mechanism. The presynaptic inhibitor–histamine H₃ receptor, when activated, decreases the release of acetylcholine from cholinergic neurons. This has been shown both in the gastrointestinal⁹ and central nervous system.¹⁰ Consequently, when a histamine H₃ receptor antagonist is present, an increased release of acetylcholine is observed. Thus, both mechanisms contribute to the same neurochemical end result (increased synaptic levels of acetylcholine) in different ways. The histamine H₃ receptor antagonists increase the amount of acetylcholine molecules entering the synaptic space, and AChE inhibitors prolong their survival time and hence the likelihood of their interacting with a postsynaptic cholinergic neurotransmitter and eliciting a biological effect.

In order to achieve a specific, cognition-enhancing level of cholinergic neurotransmission, a lower dose of an AChE inhibitor–histamine H₃ receptor antagonist compound will likely be required than of either an AChE inhibitor or histamine H₃ receptor antagonist alone; a combination molecule will have a higher potency for increasing acetylcholine levels than either an AChE inhibitor alone or histamine H₃ receptor antagonist alone. It is likely reduced compound requirements for efficacy will then improve the side effect profile. At present, not much is known about the side effects of H₃ antagonists. For AChE inhibitors, on the other hand, the major side effects are nausea and gastrointestinal discomfort.⁶ By decreasing the amount of acetylcholine released in the periphery through augmentation of its release centrally via histamine H₃ receptor antagonism, fewer side effects may result.

Further, there is evidence for some AChE inhibitors exhibiting therapeutic benefits beyond influencing cholinergic transmission. AChE inhibitors able to interact with the peripheral anionic site (PAS) are of particular relevance. In addition to their ability to stimulate the cholinergic system, these inhibitors may be able to disrupt the interactions between AChE and the β -amyloid (β A) peptide and thereby be capable of slowing the progression of the disease by inhibiting the production or aggregation of the β A promoted by AChE.³

We describe herein the molecular modeling efforts that lead to the identification of an AChE inhibitor–histamine H₃ receptor antagonist. The use of available crystal structure information, pharmacophore modeling, classical, and quantum calculations lead us to the discovery. Our models also predict this dual-acting compound can interact with the PAS thus possibly having the potential to directly impact the disease itself unlike current therapies.

2. The acetylcholin esterase enzyme

The ability to study AChE by molecular modeling methods¹¹ (in particular by structure-based design) is benefited by the numerous crystal structures available both of the apo and complexed forms. The three-dimensional structure of *Torpedo californica* AChE was initially solved at a resolution of 2.8 Å¹² and later refined to 2.5 Å.¹³ For our modeling purposes we used the structure of *T. californica* (PDB ID: 1EVE) complexed with the AChE inhibitor Aricept® (donepezil).¹⁴ Reference to residue numbers will follow from this structure. The enzyme monomer is an α/β serine hydrolase consisting of 537 residues with a 12-stranded mixed β sheet surrounded by 14 α helices.¹² The active site is striking in that it is a deep, narrow tunnel approximately 20 Å long. This tunnel penetrates halfway into the enzyme and widens out at the end. Fourteen highly conserved residues cover a substantial portion of the tunnel. Crystallographic and theoretical analyses suggest approximately 20 water molecules occupy the tunnel^{15,16} and indeed, the presence of water is necessary for the deacylation of acetylcholine. Approximately 4 Å above the bottom of the tunnel is the catalytic machinery: a catalytic triad consisting of Ser 200, His 440, and Glu 327. AChE is the first serine protease discovered to contain a Glu rather than an Asp as a catalytic member. It is in this area of the active site that deacylation of acetylcholine occurs. The electrophilic carbon on the ester group of acetylcholine undergoes nucleophilic attack from Ser 200 to form a tetrahedral intermediate followed by acylation of the enzyme and displacement of choline. Subsequent hydrolysis restores the enzyme with the generation of acetic acid. Proximal to the catalytic Ser 200 are the residues Gly 118, Gly 119, and Ala 201, which comprise the oxyanion hole. The amide protons of these residues aid in the deacylation process by forming hydrogen bonds to the carbonyl oxygen of acetylcholine. This orients the electrophilic carbon for nucleophilic attack by Ser 200. Directly across from the catalytic triad is Trp 84, which binds the quaternary

amine of acetylcholine through a cation- π interaction. (This region is known by the unfortunate name of ‘anionic’ site, AS, since it was initially believed that binding in this region occurred due to a collection of negative charges.) This finding was rather unexpected but is further substantiated by theoretical calculations showing the large stability of cation- π interactions.^{17,18} There is also the peripheral ‘anionic’ site (PAS)¹⁹ corresponding to Trp 279. The role of this site (with regard to the substrate) appears to be to attract, via cation- π and/or hydrophobic interactions with the quaternary amine moiety, acetylcholine into the active site. One can imagine Trp 279 attracting acetylcholine into the active site and assisting in aligning it into the correct orientation for subsequent deacylation. The PAS has gained much interest in the design of newer drugs for AD. Recent data suggest inhibitors interacting with the PAS could prevent the β A peptide from interacting with AChE. This could result in the elimination of AChE’s role in the fibril formation process.²⁰ Consequently, it is believed AChE inhibitors interacting with the PAS could have an impact on the pathology of AD, rather than just treating the symptoms as current therapies do.

3. Molecular modeling

Our goal was to design an AChE inhibitor–histamine H₃ receptor antagonist capable of interacting with the PAS of AChE. Our initial efforts focused on virtual screening our in-house library. We sought to use the abundance of crystal structure data on AChE, our knowledge of the three-dimensional pharmacophore features of the histamine H₃ receptor²¹ and modeling to optimize this effort. Our review of the crystal structure data focused on Aricept® (donepezil),¹⁴ tacrine,²² galantamine,²³ rivastigmine,²⁴ and decamethonium.²² Decamethonium was of particular interest to us.

Shown in Figure 1 is the crystal structure of decamethonium²² in the active site of AChE. The crystal structure

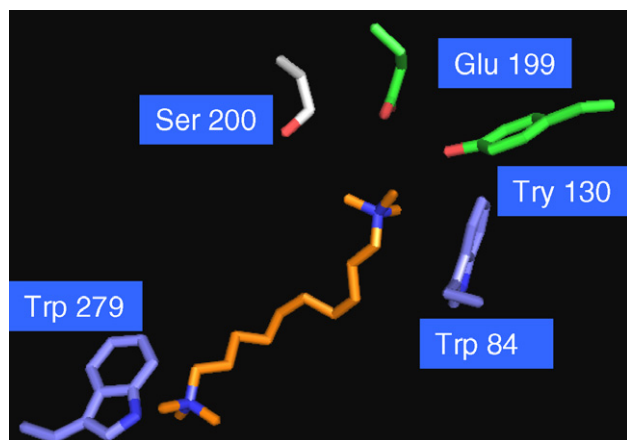


Figure 1. The crystal structure binding mode of decamethonium in the active site of AChE. The most notable interactions are those of the quaternary amines of decamethonium with the protein. One quaternary amine interacts with Trp 84 (the AS) and the other with Trp 279 (the PAS).

reveals two sets of key interactions between the protein and the ligand. One set of interactions is between the Trp 84 (the AS), located at the base of the gorge, and one of the quaternary amines of decamethonium. The other important interaction occurs between the Trp 279 (the PAS), located at the opening of the tunnel, and the other quaternary amine on the ligand. Both of these are cation- π interactions.^{17,18} In Figure 2 is the proposed three-dimensional pharmacophore for histamine H₃ based on our previous work²¹ along with a compound (**1** in Table 1) known to have good activity for the histamine H₃ receptor. The pharmacophore modeling was carried out with Catalyst²⁵ using the Hip-Hop option. This procedure takes three-dimensional conformations for each training molecule, identifies common features, and overlays them in space.²⁶ We used the automatic conformer generation feature employing the flexible option. Three active compounds we felt captured all of the features required for activity, while providing significant structural variation as well, were used to define the three-dimensional pharmacophore model. The three-dimensional pharmacophore model consists of three features: two positive ionic centers and an aromatic ring forming the center of the molecule. The model well explains known experimental data.²⁷

For the most part, decamethonium fits this H₃ pharmacophore rather well with its two quaternary amines and hydrophobic alkyl chain acting as a replacement for the aromatic center. However, we expected an aromatic center would indeed be favored over a general hydrophobic for two reasons: first, we concluded from our previous work²¹ the central aromatic ring can make π -stacking interactions with the residues Tyr 115 and Trp 371 in the histamine H₃ receptor; AChE contains several aromatic residues in its active site (the gating residues)²⁸ capable of π -stacking interactions. We hypothesized this pharmacophore model may well describe inhibitors of AChE (capable of interactions with the PAS) and histamine H₃ receptor antagonists. Starting from this premise we performed similarity and substructure searches around **1**. In conjunction with this effort we also performed manual docking²⁹ of **1** in the active site of AChE using Insight II version 2005²⁵ and the consistent valence force field (CVFF),³⁰ which is expected to be sufficient for our system. All modeling requiring a protein structure used *T. californica* (PDB ID: 1EVE) complexed with Aricept® (donepezil).¹⁴ While this effort provided useful information, the force field is not parameterized to handle the important cation- π interactions between the basic amines and the key tryptophans (Trp 84 and Trp 279). To build off this original effort and reconcile the issue with the cation- π interactions we further performed automated docking with Glide³¹ followed by QM/MM^{32–34} calculations using QSite.³¹ All Glide dockings were done in extra precision mode. The docking grid was generated with the default size using the aforementioned structure. All QM/MM were performed with the protein fixed using B3LYP/6-31G**. The QM region contained the following residues: Ser 200, Glu 327, His 440 (the catalytic triad); Trp 84 (the AS); Trp 279 (the PAS); Tyr 121, Phe 290, Phe

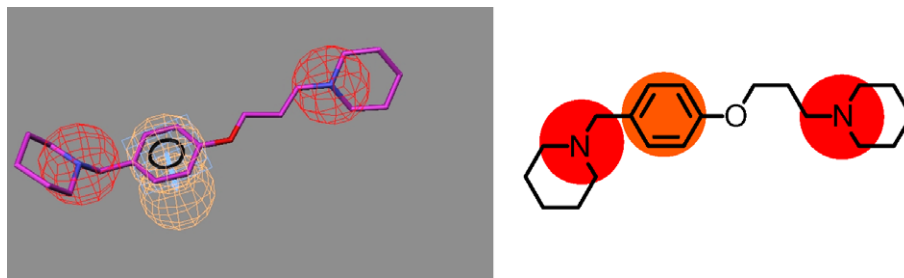
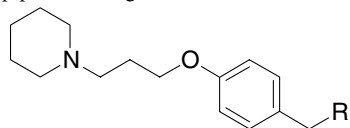
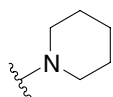
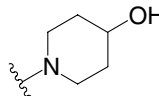
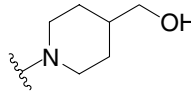
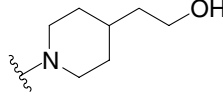


Figure 2. The proposed three-dimensional pharmacophore for histamine H_3 with **1**. The main features are two positive ionic centers and an aromatic ring forming the center of the molecule.

Table 1. Data for hydroxyl linkers of various lengths in the four position of the piperidine ring

			
Compound	R	AChE IC ₅₀ (nM)	H ₃ (nM)
1		3200 ± 400	0.53 ± 0.49
7		2300 ± 100	0.72 ± 0.19
2		1100	1.30 ± 0.60
6		350 ± 10	0.98 ± 0.21
Decamethonium		5900 ± 1400	820 ± 170
Donepezil		160 ± 30	350 ± 70

330, Phe 331, Tyr 334 (the gating residues) and Gly 117, Glu 199, Tyr 130 (other residues showing the potential for interaction). For these residues only the side chains were chosen except for Gly 117 where we chose the whole residue since the important interaction was with the carbonyl backbone. All other settings for the QM/MM were the defaults chosen by QSite. The automated docking with Glide allowed us to get a more robust sampling of the possible binding modes for **1** in AChE. Clearly, it is not feasible to perform QM/MM on all the binding modes returned by Glide. However, one mode stood out among the rest and was followed up with QM/MM. This mode scored high in Glide, was similar to the initial binding mode from the manual docking with Insight II and, upon overlaying the Glide binding mode and the binding mode of decamethonium from the crystal structure, showed excellent overlap of their pharmacophore features. This was especially true for the positively charged nitrogens.

4. Results and discussion

Figure 3 shows the QM/MM binding mode for **1** overlaid with the crystal structure binding mode of decamethonium (the residues shown are of the crystal structure of decamethonium²²). There is excellent overlap of the pharmacophore features with the most notable being between the basic amines of **1** and the quaternary nitrogens of decamethonium. The central aryl ring of **1** is also coincident with the alkyl chain of decamethonium. From the results of the QM/MM binding mode and from the shown overlay we suggest **1** is making cation- π interactions between the basic amines and the key tryptophans (Trp 84 and Trp 279). Also notable in **Figure 3** are the residues Tyr 130 (green), Glu 199 (green), and the catalytic serine Ser 200 (gray). It was speculated these nearby residues could interact favorably with pendant substituents on the proximal piperidine ring of **1** and thus improve the affinity of **1** for AChE. The QM/MM binding mode for **6** is shown in **Figure 4** (the residues shown are from the crystal structure of *T. californica* (PDB ID: 1EVE) complexed with donepezil).¹⁴ As with **1**, **6** also makes cation- π interactions with the key tryptophans. Additionally the hydroxyl group is well aligned to make hydrogen bond interactions with Glu 199 and Ser 200 and possibly Tyr 130. **Table 1** shows the data for hydroxyl linkers of various lengths in the

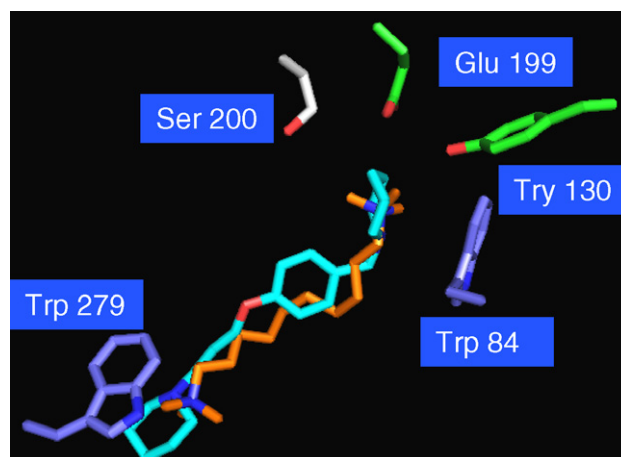


Figure 3. The QM/MM binding mode for **1** overlaid with the crystal structure binding mode of decamethonium in the active site of AChE. The residues shown are from the crystal structure of decamethonium. Note the near perfect overlay of the basic amines with the quaternary nitrogens of decamethonium. Further, the central aryl ring of **1** is coincident with the alkyl chain of decamethonium.

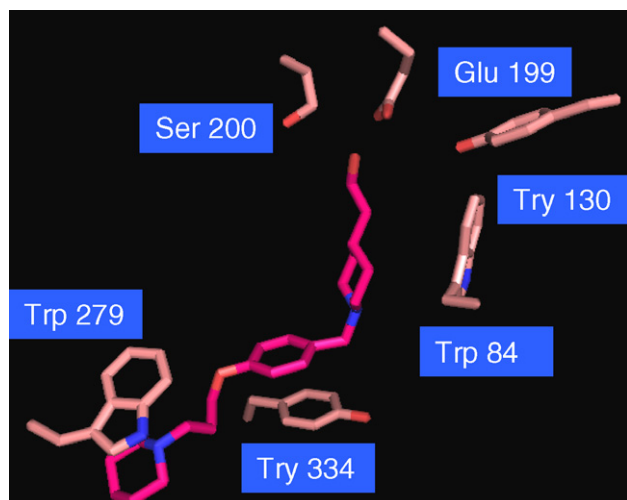


Figure 4. The QM/MM binding mode of compound **6**. The hydroxyl group is positioned to make hydrogen bond interactions with Ser 200 and Glu 199 and possibly Tyr 130. Further, the central aromatic ring appears well situated to make a π -stacking interaction with Tyr 334. The residues shown are from the protein of *Torpedo californica* (available from the PDB) complexed with donepezil.

four position of the piperidine ring. Docking calculations suggest the hydroxyethyl moiety provides the minimum linker length needed to have the opportunity to make interactions with Glu 199, Ser 200, and Tyr 130 and indeed, **6** is the most potent compound of the three analogs shown in Table 1. Finally, the central aromatic ring of **6** appears to be well positioned for π -stacking interactions with Tyr 334. We believe the central aromatic ring of **6** makes π -stacking interactions in the active sites of both AChE and histamine H_3 receptor (in histamine H_3 receptor the residues Tyr 115 and Trp 371 are most likely providing this interaction).

5. Conclusions

Currently, AChE inhibitors represent the most common therapeutic option for AD. Their procognitive and memory-enhancing effects are well known in the literature.³⁵ In addition, there is a considerable body of experimental work suggesting antagonism of the histamine H_3 receptor translates into increased cognitive performance.^{36–38}

Herein we demonstrated how the use of available crystal structure information, pharmacophore modeling, docking (automated, manual, classical, and QM/MM) lead us to a compound (**6**) (and potential series) with excellent activity at both AChE and the histamine H_3 receptor. In addition, our models suggest such a dual-acting compound may interact with the PAS and, based on current thinking, could potentially slow the progression of AD.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.12.048.

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