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# Anticonvulsant and procognitive properties of Novel Histamine H<sub>3</sub> Receptor Antagonists in male adult rats

Ali Kassem Saad

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ANTICONVULSANT AND PROCOGNITIVE PROPERTIES OF  
NOVEL HISTAMINE H3 RECEPTOR ANTAGONISTS IN MALE  
ADULT RATS

Ali Kassem Saad

This thesis is submitted in partial fulfillment of the requirements for the degree of  
Master of Medical Sciences (Pharmacology and Toxicology)

Under the Supervision of Dr. Bassem Sadek

April 2016

### **Declaration of Original Work**

I, Ali Kassem Saad, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled “*Anticonvulsant and Procognitive Properties of Novel Histamine H3 Receptor Antagonists in Male Adult Rats*”, hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Dr. Bassem Sadek, in the College of Medicine and Health Sciences at UAEU. This work has not previously been presented or published, or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

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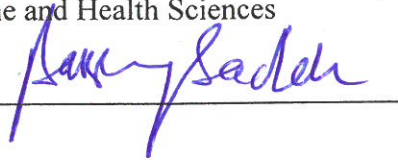
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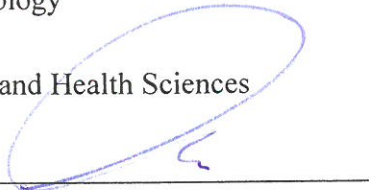
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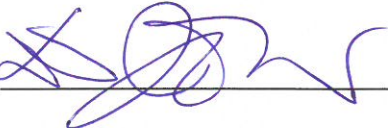


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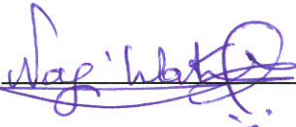
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## Abstract

To determine the potential of histamine H3 receptor (H3R) ligands as new antiepileptic and procognitive drugs, aromatic ether derivatives (**1-12**) belonging to the nonimidazole class of ligands, with high *in-vitro* binding affinity at human H3R, were tested for their *in-vivo* anticonvulsive activity in maximal electroshock (MES)-, pentylenetetrazole (PTZ)-, and strychnine (STR)-induced seizure models in male adult rats having phenytoin (PHT) and valproic acid (VPA) as the reference antiepileptic drugs, pitolisant (PIT) as the standard H3R antagonist/inverse agonist, and donepezil (DOZ) as a reference procognitive drug. Among the H3R ligands (**1-12**) tested in the current project, H3R antagonist **4** showed significant and dose-dependent reduction in the duration of tonic hind limb extension (THLE) subsequent to acute systemic administration (5, 10, and 15 mg/kg, i.p.). Importantly, the protective action observed for H3R antagonist **4** in MES-induced seizure was comparable to that of the reference antiepileptic drug phenytoin (PHT), and was also reversed when rats were pretreated with the CNS penetrant pyrilamine (PYR) (10 mg/kg, i.p.), or with the selective H3R agonist *R*-( $\alpha$ )-methylhistamine (RAMH) (10 mg/kg, i.p.). Furthermore, the procognitive studies indicate that acute pre-training systemic administration of H3R antagonist **4** (2.5 mg/kg, i.p.) facilitated acquisition, whereas pre-testing acute administration of **4** (5 and 10 mg/kg, i.p.) improved retrieval. Interestingly, the procognitive effect of **4** on retrieval was completely abrogated when rats were pretreated with the centrally-acting H2R antagonist zolantidine (ZOL) but not the centrally acting H1R antagonist PYR, indicating that histaminergic pathways through activation of H2Rs appear to be participating in neuronal circuits involved in the retrieval processes. Taken together, our results show that H3R antagonist **4** demonstrates anticonvulsant properties in the MES-induced



seizure model and improves cognitive performance through actions on different memory stages. Therefore, H3Rs may have implications for the treatment of degenerative disorders associated with impaired memory function and may represent a novel therapeutic pharmacological target to tackle cognitive problems associated with the chronic use of antiepileptic drugs.

**Keywords:** Histamine H3 receptors, Antagonists, Anticonvulsant, Learning, Memory, Passive avoidance test, *R*-( $\alpha$ )-methyl-histamine, Pyrilamine, Zolantidine

## Title and Abstract (in Arabic)

### دراسة فعالية عدد من مثبطات مستقبلات الهستامين-3 المستخلقة حديثاً على نماذج بحثية مختلفة للصرع وضعف الذاكرة في فصيلة معينة من القوارض

#### الملخص

الصرع هو مرض مزمن ويعد من احد الأمراض العصبية الأكثر انتشاراً. يعتبر العلاج الدوائي أهم وسائل المعالجة لهذا المرض. لكن أدوية الصرع المتوفرة حالياً فعالة لدى 70-80% من المرضى عوضاً عن أن 20-30% من الحالات مازالت مستعصية بالإضافة للأعراض الجانبية المصاحبة لهذه الأدوية خاصة تلك التي تصيب الذاكرة. تم إجراء الكثير من الدراسات مؤخراً حول صنف جديد من العلاجات الدوائية يسمى مثبطات مستقبلات الهستامين-3 (H3R antagonists) لاستكشاف تأثيرها على كثير من الأمراض العصبية والسلوكية. (Pitolisant) يعتبر من أول مثبطات مستقبلات الهستامين-3 وصولاً للدراسات السريرية، وقد تخطى مراحل متقدمة في كثير من هذه الدراسات بما فيها الصرع، وضعف الذاكرة. لذلك قمنا في دراستنا هذه باستخدام مركبات مختلفة (1-12) ومشتقة من (Pitolisant) ولها نسبة ارتباط تثبيطي عالية بمستقبلات الهستامين-3 كما تشير الدراسات المخبرية. الأهداف: هدف هذه الرسالة البحثية هو دراسة فعالية هذه المركبات المستخلقة حديثاً على عدة نماذج بحثية (models) للصرع وضعف الذاكرة على ذكور الجرذان البالغة من فصيلة (Wistar). عوضاً عن ذلك ولغرض المقارنة، قمنا أيضاً بفحص تأثير مركبات مستخدمة حالياً من قبل مرضى الصرع أو ضعف الذاكرة وهي (Phenytoin)، (Valproic Acid) و (Donepezil). بداية قمنا بدراسة تأثير هذه المركبات، باستخدام ثلاث نماذج بحثية مختلفة، على نوبات الصرع الناتج عن الصدمات الكهربائية أو (Maximal electric shock) أو تلك الناتجة عن مواد كيميائية وهي إما (Strychnine) أو (Pentylenetetrazol). اعتماداً على فعاليتها في تقليل أو منع هذه النوبات، تم اختيار أفضل هذه

المركبات لفحص تأثيرها على ضعف الذاكرة باستخدام (Passive avoidance test). النتائج:  
 لاحظنا اختلاف تأثير هذه المركبات على نماذج الصرع البحثية المختلفة. لكن المركب 4 تميز  
 بتأثيره القوي إحصائياً على نوبات الصرع الناتجة عن الصدمات الكهربائية بحيث شابه تأثيره  
 تأثير (Phenytoin) ولكن كان أشد فاعلية إحصائياً مقارنة ب (Pitolisant) عند الجرعة ( 10  
 ميليجرام/ كيلوجرام). هذا التأثير اعتمد على الهستامين كناقل عصبي حيث تم إبطال هذا  
 المفعول باستخدام كل من منشط مستقبلات الهستامين-3 (R- $\alpha$ -3) methylhistamine) ومثبط  
 مستقبلات الهستامين-1 (Pyrilamine). اختلف تأثير هذا المركب على مراحل التعلم أو الذاكرة.  
 فالجرعة (2.5 ميليجرام/ كيلوجرام) حسنت إحصائياً وبشكل كبير أحد مراحل الذاكرة المسماة  
 بالاكْتساب (acquisition). هذا التأثير لم يكن معتمداً على الهستامين كناقل عصبي حيث أن كلاً  
 من مثبط مستقبلات الهستامين-1 (Pyrilamine) ومثبط مستقبلات الهستامين-2 (Zolantidine)  
 فشل في إبطال مفعول هذه الجرعة من المركب 4. في المقابل، كان لكل من الجرعتين (5 و 10  
 ميليجرام/ كيلوجرام) فاعلية كبيرة إحصائياً في تقوية مرحلة أخرى من الذاكرة تسمى  
 بالاسترجاع (Retrieval) وتم إبطال مفعول الجرعة (10 ميليجرام/ كيلوجرام) بواسطة  
 (Zolantidine). تأسيساً على ذلك، يمكن القول بأن تأثير هذه الجرعة اعتمد على الهستامين  
 كناقل عصبي تحديداً من خلال تفاعل الهستامين مع مستقبلاته العصبية-2. الخاتمة: في  
 المحصلة، نتائجا أظهرت أن المركب 4 له خصائص مضادة لنوبات الصرع الناتجة عن  
 الصدمات العصبية هذا بالإضافة لقدرته على تحسين الذاكرة من خلال تأثيره على مراحل  
 مختلفة منها. لذلك فإن مثبطات مستقبلات الهستامين-3 قد تكون لها تطبيقات مستقبلية في علاج  
 مرض الصرع تحديداً لتجنب أو معالجة مشاكل ضعف الذاكرة المصاحبة لكثير من أدوية  
 الصرع المستخدمة حالياً أو المصاحبة لمرض الصرع في حد ذاته.

**مفاهيم البحث الرئيسية:** مثبطات مستقبلات الهستامين-3، نوبات الصرع، ضعف الذاكرة

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Last of all, special thanks go to my parents and friends who helped me along the way. I am sure they suspected it was endless.

## **Dedication**

*To my beloved parents and family*

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## List of Abbreviations

AA	Arachidonic Acid
ACT	Active Avoidance Test
AD	Alzheimer's Disease
ADHD	Attention-Deficit Hyperactivity Disorder
AED	Antiepileptic Drugs
Akt	Also known as Protein Kinase B
cAMP	Cyclic Adenosine Mono-Phosphate
CREB	cAMP Response Element-Binding
DOZ	Donepezil
ED <sub>50</sub>	Median effective dose
EEG	Electroencephalography
$\alpha$ -FMH	$\alpha$ -Fluromethylhistidine
GPCR	G-protein Coupled Receptors
GSK3 $\beta$	Glycogen Synthase Kinase 3 $\beta$
H1R	Histamine H1 Receptor
H2R	Histamine H2 Receptor
H3R	Histamine H3 Receptor
H4R	Histamine H4 Receptor
HDC	Histidine Decarboxylase
i.c.v.	Intracerebroventricular
IP3	Inositol Triphosphate
$K_i$	Inhibitory constant (binding affinity)
KO	Knockout

LTD	Long Term Depression
LTP	Long Term Potentiation
MAPK	Mitogen-Activated Protein Kinase
MES	Maximal Electric Shock
OFT	Open Field Test
PAT	Passive Avoidance Test
PET	Positron Emission Tomography
PHT	Phenytoin
PIT	Pitolisant
PKA	Protein Kinase A
PKC	Protein Kinase C
$pK_i$	$-\log [K_i]$
PLA2	Phospholipase A2
PTZ	Pentylentetrazole
PYR	Pyrilamine
RAMH	( <i>R</i> )- $\alpha$ -Methylhistamine
RT-PCR	Real Time- polymerase chain reaction
STL	Step-Through Latency
STR	Strychnine
TBPS	<i>t</i> -Butylbicyclophosphorothionate
THLE	Tonic Hind Limb Extension
TMN	Tubero-mammillary Nucleus
VPA	Valproic Acid
VLPO	Ventro-lateral pre-optic

VMAT-2 Vesicular Monoamine Transporter-2

ZOL Zolantidine

## Chapter 1: Introduction

### 1.1 Histamine as a central neurotransmitter

Brain histamine (Figure 1) is a story that dates back to the early 20<sup>th</sup> century when first-generation antihistamines were introduced at the time even before histamine H1 or H2 receptors (H1-2Rs) were known. Sedation, a central side effect associated with early antihistamines, raised speculation about a role for histamine in the brain, particularly in regard to wakefulness. Kwiatkowski (1943) was the first to isolate histamine from brain extract, mainly from the cortex. Later, electrophysiological studies in several brain regions and behavioural studies with intracerebroventricular (i.c.v.) infusion of histamine demonstrated a potential new neurotransmitter to be added to the existing neurotransmitter systems (Haas et al., 2008). Furthermore, activity of neuronal histidine decarboxylase (HDC), histamine synthesizing enzyme, which was known to exist in nerve endings, decreased in many brain regions after lesions of the lateral hypothalamus (Garbarg et al., 1976, Schwartz et al., 1976).

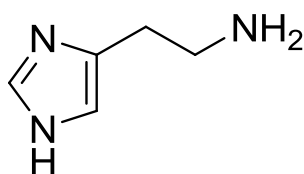


Figure 1: Chemical structure of histamine: 2-(1*H*-imidazol-4-yl) ethanamine

The breakthrough in the field of neuronal histamine was the production of antibodies against HDC and histamine that were used to draw the first map of neuronal histamine pathways utilizing histochemical methods (Watanabe et al., 1983,

Panula et al., 1984). There are two storage compartments of brain histamine, one in the synaptic vesicles of neuronal endplates and one in the mast cells (Verdiere et al., 1975). Both compartments equally contribute to the levels of brain histamine since  $W/W^V$  knockout mice, in which mast cells are absent, lack around 50% of brain histamine compared to their wild counterparts (Maeyama et al., 1983).  $W/W^V$  knockout studies and administration of 48/48 (Verdiere et al., 1975), a selective histamine releaser from mast cell granules, can be used to differentiate between the two histamine pools. Through their contact with the brain side of the blood brain barrier, brain mast cells gate the interplay between the nervous, vascular and immune systems. Their amounts decrease with age and vary between species, sex, and pathological conditions (Silver and Curley, 2013).

## **1.2 Localization and projections of histaminergic neurons**

Cell bodies of histamine neurons are confined in one area of the posterior hypothalamus called tuberomammillary nucleus (TMN), i.e. between the mammillary bodies and the optic chiasm of the tuber cinereum. Unlike other aminergic neurons with separate nuclei, histaminergic cell bodies send projections to different brain areas without a topographical pattern of their projections as confirmed with retrograde tracers. One perikarya can send descending and ascending extensions (Brown et al., 2001, Haas et al., 2008). Histamine neurons are heterogeneous demonstrating distinct control mechanisms and innervating different brain regions (Blandina et al., 2012). Histamine neurons are heterogeneous de Human brain contains 64000 histamine neurons (Haas et al., 2008), 16 times higher than that in rats, 4000 neurons (Ericson et al., 1987). Histaminergic neurons send their ascending and descending pathways to the cerebrum, brain stem and spinal cord,



respectively (Figure 2). Through their extensions, histaminergic neurons rarely form the classical synaptic contact with their targets. Instead, histamine is released from varicosities, i.e. swellings along the axon, and spreads to the surrounding area like a local hormone (Figure 3) affecting neuronal and non-neuronal components (Brown et al., 2001, Haas et al., 2008).

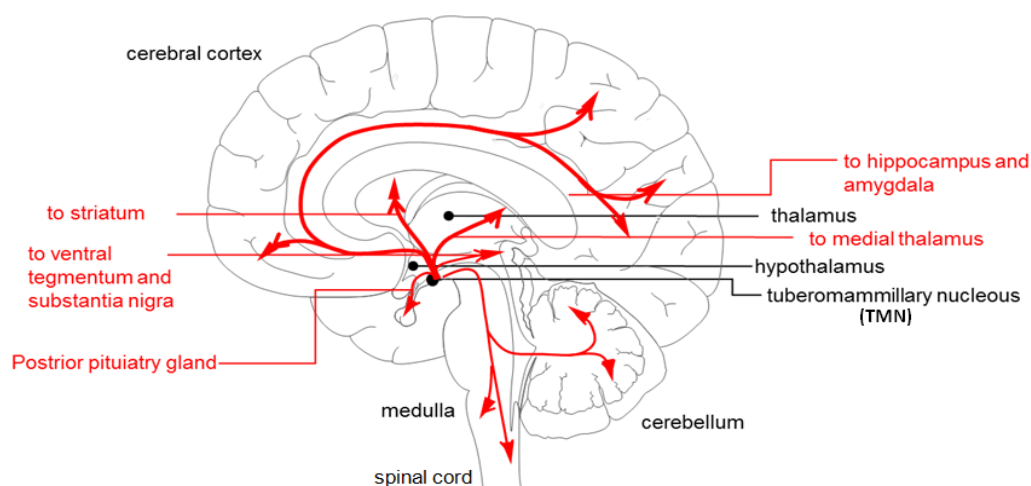


Figure 2: Projections of TMN histamine neurons. Two ascending pathways: 1) ventral part to the hypothalamus and septum, 2) dorsal part to the thalamus, hippocampus, amygdala, and forebrain. One descending pathway to the brain stem, cerebellum and spinal cord. Adopted from (Die Entwicklung von. (2010). Histamin-H3-Rezeptorliganden als potenzielle Arzneistoffe und bildgebende pharmakologische Werkzeuge (unpublished doctoral thesis). Institute for Pharmaceutical Chemistry, Medicinal Chemistry Division, Goethe University, Frankfurt/Main, Germany).

Brain regions differ in amount of histaminergic fibers that they receive. Ventrally, histaminergic neurons highly innervate the hypothalamus, diagonal band, septum and olfactory tubercle. Amygdala, periventricular nuclei of the hypothalamus and hippocampal subiculum, and the dentate gyrus receive the largest amount of histaminergic fibers through the dorsal pathway. Other areas, like the cerebral cortex, striatum, nucleus accumbens, tend to be moderately innervated. The Substantia nigra and ventral tegmentum have more projections compared to the locus coeruleus and raphe nuclei. Generally, the descending pathway incorporates the least fiber densities (Brown et al., 2001).

### 1.3 Biosynthesis, release, and metabolism of central histamine

Histamine cannot pass through the blood brain barrier; thus it has to be biosynthesized *in situ*. A dietary amino acid precursor of histamine is L-histidine can pass through L-amino acid transporters (Figure 3) to the cerebrospinal fluid (CSF) pool and the cytoplasm of neurons. Therefore, histamine biosynthesis depends on the presence of this amino acid. Accordingly, L-histidine is converted to histamine through oxidative removal of a carboxyl group by a specific enzyme, HDC, EC 4.1.1.22. At high concentration, histidine can be a substrate for nonspecific decarboxylase (Prell et al., 1996). The nonspecific synthesis pathways can be revealed with alpha-fluoromethyl histidine ( $\alpha$ -FMH), an inhibitor of specific HDC. Once synthesized, histamine is packed in vesicles in both soma and varicosities through vesicular monoamine transporter-2 (VMAT-2). Upon depolarization of the histamine neuron, calcium-dependent release of histamine occurs (Verdiere et al., 1975). Histamine biosynthesis and release in the CNS is modulated by the histamine H3 auto-receptors (H3Rs) through cAMP/PKA-dependent pathways (section 1.4.3) (Bongers et al., 2007). Moreover, firing of central histaminergic neurons and release of histamine is also controlled by inputs and receptors of other systems including acetylcholine, norepinephrine, serotonin, and orexin among others (Brown et al., 2001). Unlike other aminergic neurotransmitters, histamine has no known re-uptake mechanism (Brown et al., 2001). However, the central action of histamine is terminated via methylation by histamine *N*-methyltransferase (HNMT) in the extracellular space to yield  $N^{\text{tele}}$ -methylhistamine which is then degraded by monoamine oxidase B to t-methyl-imidazole acetic acid (Prell et al., 1996, Brown et al., 2001, Haas et al., 2008).

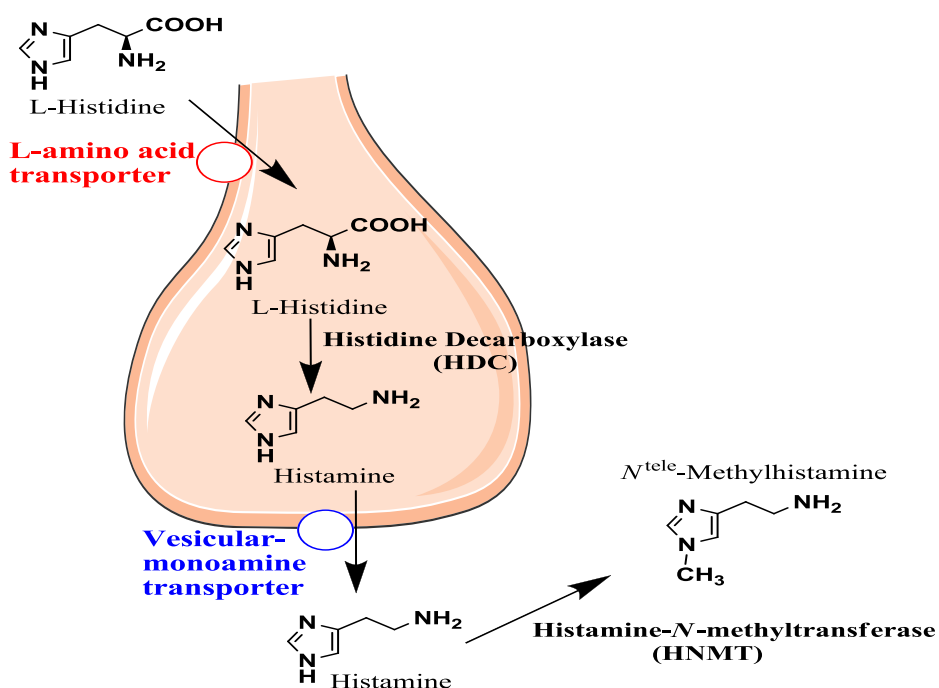


Figure 3: Neuronal histamine biosynthesis, release and metabolism in the CNS. Adopted from (Haas et al., 2008).

#### 1.4 Histamine receptors

The precise effects of histamine are exerted through stimulation of four different G-protein coupled receptor (GPCR) subtypes, namely H1-H4R (Figure 4) (Arrang et al., 1988; Arrang et al., 1987; Arrang et al., 1983, 1985a; Hill, 1990; Hill et al., 1997; Lovenberg et al., 1999). All histamine receptors, to varying levels, display constitutive activity (Bakker et al., 2000), i.e. have an active conformation state that is independent of any ligand binding. Whereas H1-3Rs are highly expressed in the brain. H4Rs have low expression, if any, in the CNS (see Schneider and Seifert, 2015).

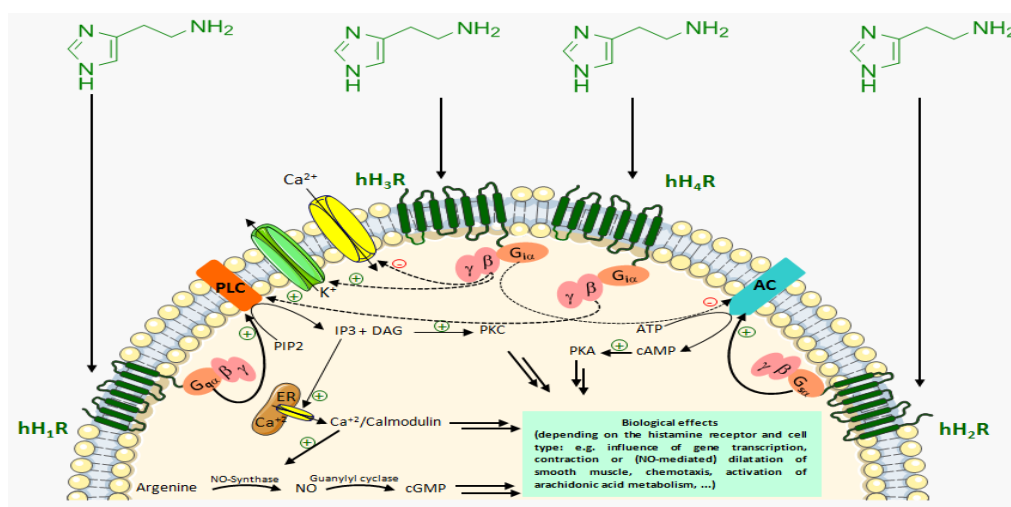


Figure 4: Schematic illustration of main signaling pathways of human histamine receptors hH1-4R. Modified after Strasser et al., 2015 (Sadek and Stark, 2015).

### 1.4.1 Histamine H1Rs in the Central Nervous System

H1R is coupled to the Gq/11 signaling pathway and has mainly excitatory effects. However, inhibitory effects through calcium-induced activation of potassium conductance are also possible. Furthermore, H1R is associated with formation of arachidonic acid (AA) and nitric oxide (NO) that can act as a retrograde messenger controlling activity of pre-synaptic neurons however, the signaling pathway is yet to be clarified (Brown et al., 2001, Haas et al., 2008).

H1Rs are primarily localized in brain regions involved in arousal and cognition including the thalamus, cortex, cholinergic nuclei in the mesopontine tegmentum and basal forebrain, aminergic nuclei in the locus coeruleus and raphe nuclei, septal nuclei, hippocampus, and amygdala. Also, H1Rs exist in high density in the nucleus accumbens, molecular layer of the cerebellum, nuclei of the cranial nerves, area postrema, and nucleus tractus solitarius (Brown et al., 2001).

Interestingly, experimental studies with H1R KO mice have elucidated abnormal circadian rhythm of locomotor activity (i.e. more active at light cycle),

reduced exploratory activity in a novel environment, reduced anxiety and aggressiveness, tapered pain sensitivity in different tests, selective changes in different memory tests. The latter observations shed light on the importance of histaminergic neurotransmission through central H1Rs. (Inoue et al., 1996, Yanai et al., 1998, Mobarakeh et al., 2000, Dai et al., 2007b, Dere et al., 2008).

#### **1.4.2 Histamine H2Rs in the central nervous system**

The H2R is coupled to a Gs protein and is thus involved in the cAMP/PKA/cAMP response element-binding protein (CREB) signaling pathway producing excitatory effects like those observed in the CA1 region of the hippocampus, pyramidal cells and thalamic neurons (Figure 4).

The H1- and H2Rs are colocalized in hippocampal pyramidal and granule cells, and aminergic nuclei (locus coeruleus, raphe nuclei, substantia nigra, ventral tegmental area). Further, H2Rs are present in high density in the basal ganglia and amygdala. However, the receptor distribution is limited in the septal nuclei and the hypothalamus where H1Rs predominate (Brown et al., 2001). Previous studies have shown that H2R KO mice suffered test-specific cognitive dysfunction accompanied by impaired hippocampal long-term potentiation (LTP) (Dai et al., 2007b).

#### **1.4.3 Histamine H3Rs in the central nervous system**

The H3R was discovered in 1983 in Paris using traditional pharmacology (Schwartz, 2011). Its molecular structure was revealed almost a decade later in efforts to identify an orphan GPCR that has widespread expression in the CNS (Lovenberg et al., 1999). Employing genetic information from human H3R and RT-PCR technology, many isoforms are obtained from alternative splicing of the human

H3R gene as well as other species. These isoforms displayed heterogeneous pharmacology, signal transduction, and distribution in the CNS (Drutel et al., 2001, Hancock et al., 2003, Esbenshade et al., 2008), and will be further discussed below. Human H3R (hH3R) protein sequence displays high homology to hH4R [38-58%] (Liu et al., 2001, Morse et al., 2001, Zhu et al., 2001), however, very low homology (21-22%) compared to hH1- and hH2Rs (Lovenberg et al., 1999).

As observed with other GPCRs *in vitro*, H3Rs display constitutive activity. This activity was noted *in vivo* at normal physiological levels, unlike other GPCRs (Morisset et al., 2000). GPCRs exist in equilibrium between their inactive and active conformations. The quantity of the active conformations and how effectively they couple to G-protein dictates the level of constitutive activity of the receptor. Inverse agonists are agents that stabilize the inactive state and thus inhibit the constitutive activity.

Although H3Rs do exist in the periphery (Ichinose et al., 1990, Taylor and Kilpatrick, 1992, Imamura et al., 1995, Sugimoto et al., 2004, Hough and Rice, 2011), it has predominant, heterogeneous expression in the CNS (Lovenberg et al., 1999). High densities of the H3Rs were found in the rostral part of the rat cerebral cortex, hippocampus, amygdala, nucleus accumbens, striatum, olfactory tubercles, cerebellum, substantia nigra, and brain stem. In addition, rat H3Rs were moderately expressed in the hypothalamus and the cell bodies of histamine neurons (Panula et al., 2015). In humans, H3Rs are found mainly in the basal ganglia, globus pallidus, hippocampus and cortex (Martinez-Mir et al., 1990). This distribution is in concert with their function in controlling the firing of histamine neurons and modulating the synthesis and release of histamine as well as the release of other neurotransmitters

including dopamine, serotonin, norepinephrine, acetylcholine,  $\gamma$ -aminobutyric acid (GABA) and glutamate (Panula et al., 2015).

The H3R is a member of the GPCR family. Through the  $\alpha$ -subunit of the Gi/o protein (Gi/o $\alpha$ ), H3Rs are negatively coupled to adenylate cyclase (Figure 4 and 5) (Hancock et al., 2003, Bongers et al., 2007, Panula et al., 2015). In addition, H3Rs

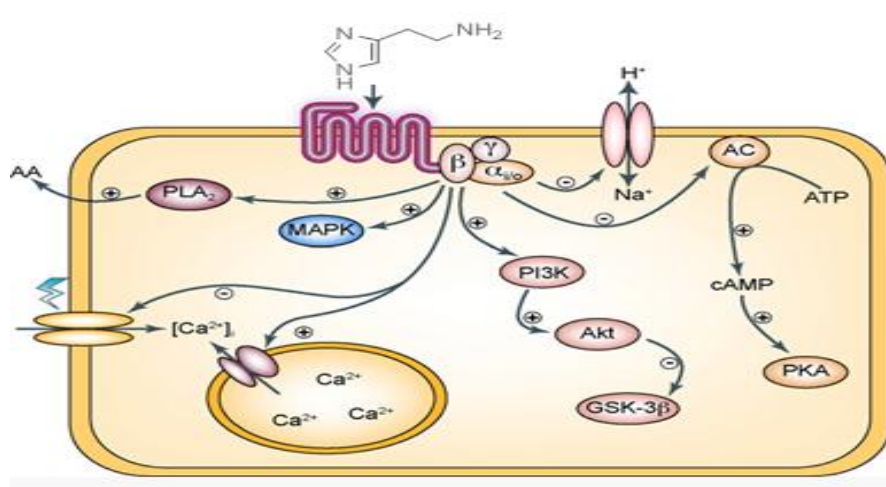


Figure 5: Different possible signaling pathways of H3Rs. Adopted from (Bongers et al., 2007).

were found to reduce intracellular calcium by Gi/o $\alpha$ -mediated suppression of N- and P-type calcium channels in dissociated TMNs (Takeshita et al., 1998). There is a lack of evidence supporting the coupling of H3R to inwardly rectifying potassium channels as is the case for other autoreceptors. Therefore, the aforementioned Gi/o $\alpha$ -mediated reduction in calcium influx seems to explain the observed histamine-induced inhibition of its own as well as other neurotransmitter release (Brown et al., 2001, Hancock et al., 2003, Panula et al., 2015).

Also, reduced intracellular calcium was postulated to be the direct effector in H3R agonist-induced reduction of norepinephrine release from noradrenergic nerve terminals in the myocardium (Silver et al., 2002). The G-protein  $\beta\gamma$ -subunit of H3Rs,

with possible complex interactions with other GPCRs, can activate other signaling pathways including mitogen-activated protein kinase (MAPK), Akt/ glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) and phospholipase A2 (PLA2) (Figure 5) that are involved in neuronal plasticity and brain disorders (Bongers et al., 2007, Panula et al., 2015).

In contrast to H1- and H2R genes, H3R gene has many “species-distinct” splice variants that result in the formation of a large number of isoforms (20 in human) (Esbenshade et al., 2008). The isoforms include the supersized form [e.g. hH3R (453) or rH3R (449)], the wild-type [445 amino acid] and the rest have shorter protein sequences [e.g. hH3R (453), hH3R (220)] (Hancock et al., 2003). Eight of the 20 isoforms (in human) have been shown to be functional in heterogeneous expression systems including [hH3R (445), (453), (415), (413), (409), (373), (365) and (329)] (Esbenshade et al., 2008). Other H3R isoforms are either unstudied or expected to be non-functional since they lack important domains essential for ligand binding, carry a detrimental stop codon, or couldn't assemble to a tertiary structure. However, these functionally inert isoforms could have a physiological role by altering the expression of other isoforms or changing the activity of other receptors or proteins (Hancock et al., 2003, Bongers et al., 2007).

Different domains of H3R receptor (or any other GPCRs) have roles in affinity to H3R ligands, efficacy in coupling to a variety of signal transduction pathways or constitutive activity (Hancock et al., 2003, Arrang et al., 2007). Shorter H3R isoforms, with deletion in various domains of the receptor, could have dissimilar affinity or response to different H3R ligands. These dissimilarities are augmented with differential distribution of these receptors in different brain regions as indicated by real time- polymerase chain reaction (RT-PCR) studies (Bongers et



al., 2007, Esbenshade et al., 2008). For example, compared to shorter isoforms, rat H3R (rH3R) (445) appears to have more effective coupling to MAP kinase pathways, which are important for synaptic plasticity. Interestingly, the same isoform has a preferential localization in the hippocampus, a memory consolidation center (Drutel et al., 2001). Thus, selective H3R (445) ligands would be more applicable in cognitive disorders, like Alzheimer's disease (AD).

Knockout studies have demonstrated Electroencephalography (EEG)-confirmed enhanced vigilance and increased arousal during behavioral tests including environmental change, locomotion and motivation tests (Gondard et al., 2013), and general reduction of locomotor activity (Toyota et al., 2002) in H3R KO mice. These H3R KO mice displayed sleep restriction (Gondard et al., 2013), and loss of sensitivity to waking action of H3R antagonists and to memory impairing effects of scopolamine (Toyota et al., 2002). Total absence of H3R was advantageous in terms of cognition and reduced anxiety in response to avoidable stimuli (Rizk et al., 2004).

#### **1.4.4 Histamine H4Rs**

Similar to H3Rs, these receptors are coupled to Gi/o protein yet, unlike H3Rs, they are primarily expressed in the periphery (Zhu et al., 2001). Homology between H3- and H4Rs, as mentioned above, results in pharmacological similarities between the two receptors mainly with imidazole-containing compounds (Lim et al., 2005, Haas et al., 2008). H4Rs are largely engaged in immunomodulation and their expression predominates in cells and tissues of the immune system and by alteration of their levels in response to inflammatory stimuli (Zampeli and Tiligada, 2009).

## **1.5 Brain histamine in physiological and pathological conditions**

In agreement with widespread projection of its neurons and several signaling pathways coupled to its receptors, neuronal histamine is involved in multiple CNS actions. Brain histamine modulates several homeostatic functions like thermoregulation, fluid balance, energy metabolism and stress. Histamine, also, orchestrates biological rhythms, different behavioral states and higher brain activities like cognition (Brown et al., 2001, Haas et al., 2008). In this section, some selected histamine functions are described.

### **1.5.1 Epilepsy**

Seizures are clinical manifestations of excessive excitation and abnormally enhanced synchrony of neurons in a restricted region or in different parts of the brain (Fisher et al., 2005). Epilepsy is a group of disorders characterized by a high predisposition to recurrent seizures. The etiology of epilepsy can be due to genetic, congenital, metabolic factors or secondary to stroke, trauma, tumors, drugs, alcohol or medications. Mechanistically, these change intrinsic (e.g.; pumps, ion channels, transporters) activity, alter synaptic transmission or cause “re-wiring” of neurons resulting in reduced seizure threshold.

One of the main available strategies of pharmacotherapy for epilepsy is to correct the imbalance between excitatory, glutamate, and inhibitory, GABA, neurotransmission (Meldrum, 1995, Loscher, 2002, Stephen and Brodie, 2011). The central histaminergic system in many studies has been shown to reduce or protect from seizures. In preclinical studies, i.c.v. histamine prevented different types of seizures (Table 1). Inhibition of *N*-methyltransferase, the histamine metabolizing

enzyme in the CNS, by metoprine increased brain histamine content and reduced seizure susceptibility (Tuomisto and Tacke, 1986, Onodera et al., 1992a, Kakinoki et al., 1998, Yawata et al., 2004). Conversely,  $\alpha$ -FMH, that diminishes histamine synthesis, increased seizure activity (Zhang et al., 2003b). Similarly, HDC KO mice were more prone to seizures (Hirai et al., 2004).

H1Rs seem to be the key mediators of histamine-induced elevation of seizure threshold since centrally-acting H1R antagonists were shown to induce a proconvulsant effect in several models (Yokoyama et al., 1996, Zhang et al., 2003b). The proconvulsant action of H1R antagonists was prevented by histidine not physostigmine indicating the involvement of histaminergic mechanisms (Kamei et al., 2000). H1R KO mice showed faster responses to amygdaloid and pentylene-tetrazole (PTZ)-induced kindling (Chen et al., 2003, Hirai et al., 2004). Clinically, children with febrile seizures had lower CSF histamine than those with fever but no convulsions, suggesting an anti-seizure effect of histamine (Kiviranta et al., 1995, Takano et al., 2010). Moreover, it has been found that H1R antagonists increased the incidence of seizures in children (Miyata et al., 2011).

Table 1: Preclinical studies of H3R antagonists in different epilepsy models

	<b>Electrically-induced seizures</b>	<b>Chemically-induced seizures</b>	<b>Genetically-encoded seizures</b>
Histamine (i.c.v.)	↓ Amygdaloid-kindled in rats <sup>b</sup>	↓ PTZ-induced in mice <sup>a</sup>	
Thiopentamide	↓ Amygdaloid-kindled and maximum electric-shock in rats <sup>d,e</sup>	↓ PTZ-induced in mice <sup>c</sup>	↓ EL mice <sup>f</sup>
Clobenpropit	↓ Amygdaloid-kindled in	↓ PTZ-induced in rats <sup>g</sup>	

	<b>Electrically-induced seizures</b>	<b>Chemically-induced seizures</b>	<b>Genetically-encoded seizures</b>
	rats <sup>d,h</sup>		
Pitolisant	↓ Maximum electric-shock in mice <sup>i</sup>	↓ KA-induced hippocampal discharges (EEG) in mice <sup>i</sup>	↓spike-and-wave discharges (EEG) in the GAERS* <sup>i</sup>
ABT-239		↓KA-induced in mice <sup>j</sup>	

↓= Inhibit, reduce or delay seizures. PTZ= pentylenetetrazole, KA = Kianic Acid. \*GAERS = genetic absence epilepsy rats from Strasbourg. <sup>a</sup>Yokoyama et al. (1994). <sup>b</sup>Kamei (2001). <sup>c</sup>Vohora et al. (2000). <sup>d</sup>Kakinoki et al. (1998). <sup>e</sup>Harada et al. (2004). <sup>f</sup>Yawata et al. (2004). <sup>g</sup>Zhang et al. (2003a). <sup>h</sup>Kamei and Okuma (2001). <sup>i</sup>Kasteleijn-Nolst Trenite et al. (2013). <sup>j</sup>Bhowmik et al. (2014).

Furthermore, abnormal levels of HIRs around the focus of epileptic injury in complex partial seizures were observed in human positron emission tomography (PET) studies (Tuomisto et al., 2001).

### 1.5.2 Arousal and sleep-wake cycle

Histaminergic neurons follow circadian rhythm in their firing and histamine release. Their activity peaks at high vigilance state and diminishes during sleep (Brown et al., 2001, Haas et al., 2008). TMN histaminergic neurons have a mutual inhibitory interaction with the ventrolateral preoptic nuclei (VLPO), which is involved in sleep regulation (Williams et al., 2014). The sedative effect of general anesthetics is postulated to depend on GBAB-mediated inhibitory pathways from VLPO to TMN neurons (Nelson et al., 2002, Moore et al., 2012). Histaminergic neurons also receive excitatory input (through orexin and glutamate as a co-transmitter) from orexin neurons, which are involved in arousal (Schone et al., 2014). Narcoleptic dogs with mutant orexin receptor-2 have lower histamine levels in their thalami and cortices (Nishino et al., 2001). Furthermore, TMN neurons have

reciprocal interaction with other aminergic nuclei (involved in cortical activation). Bilateral removal of the posterior hypothalamus leads to hypersomnia (Brown et al., 2001). Interestingly, lower CSF histamine levels are observed in narcoleptic patients (Nishino et al., 2009).

Pharmacologically, i.c.v. administration of histamine, H1R agonists, and oral thioperamide (causes increase in histamine levels) promotes wakefulness or EEG desynchronization. Their action is blocked by H1R antagonists. This suggests an H1R dependence of this function supported by a lack of effect of selective H2R antagonists in the sleep-wake cycle. Depletion of brain histamine by HDC inhibitor,  $\alpha$ -FMH, increased sleeping time (Brown et al., 2001, Haas et al., 2008). Similarly, HDC KO displayed inability to stay awake in a new environment, a decreased EEG activation during waking, and an increased paradoxical sleep (Parmentier et al., 2002).

### **1.5.3 Cognition**

Cognition is not just learning and memorizing. It, also, includes several higher brain functions including attention, reasoning, problem solving and decision making. The cognitive process is not controlled by certain parts of the brain but rather it involves complex interactions between cortical and subcortical neuronal pathways. Cognitive impairment may involve one or more aspects of cognition. For instance, AD, a neurodegenerative disorder, is characterized by memory deficit while attention is impaired in attention-deficit hyperactivity disorder (ADHD). Schizophrenia is associated with impairment of executive function, i.e. cognitive inflexibility (Esbenshade et al., 2008).

In order to evaluate cognitive processes, many tests have been developed to analyze specific domains of cognition in animals as well as in clinical trials. In memory tests, animals are trained or habituated to a certain environment or behavior (utilizing fear or reward) during training sessions. Later during test sessions, animals are reintroduced to the same environment and observed for signs of recall (test session). For example, in contextual fear conditioning (CFC), animals receive a foot-shock in a novel environment during training sessions. During test sessions, animals are returned to the same environment and a characteristic freezing behavior appears as an indication of recall. Active (AVT) and passive (PAT) avoidance tests use foot-shock as an unpleasant consequence associated with a stimulus (light/sound) or an intrinsic dark preference, respectively. PAT and ACT assess long-term memory. Social recognition and object recognition tasks are short term memory tests that rely on the animals ability to recall familiar olfactory cues (social interaction) or test objects, respectively. In spatial memory tasks, animals are conditioned to link an object, reward or punishment to a particular place (e.g., Barnes maze, water maze and radial maze). Attentional set-shifting assays examine the animals ability to learn and re-learn (cognitive flexibility). Attention is analyzed by 5-trial inhibitory avoidance (5-trial IA) and 5-choice serial reaction time (5-CSRT) tests (Esbenshade et al., 2008). Some behavioral tests in clinical trials share similarities to these animal studies but take into account the complexity of the human brain. For pharmacological testing, agents or lesions may be applied to induce deficiency in a region or a system of the brain and to produce a memory deficit model. Scopolamine for example induces memory impairment through an effect on cholinergic transmission (Flood and Cherkin, 1986). Memory deficit can be introduced in animal strains through

genetic manipulation as is the case for transgenic APPTg2576 mice, a model of AD (McGowan et al., 2006).

Several preclinical studies indicate a role for histamine neurotransmission in the cognitive process. In a previous study, inhibition of histamine synthesis in the brain by  $\alpha$ -FMH was found to reduce social recognition in rats (Prast et al., 1996). In contrast, histamine (i.c.v.) facilitates memory in several tasks (Table 2). Moreover, impairment of performance in several memory tests has been observed in mice lacking H1- or H2Rs (Dai et al., 2007b, Dere et al., 2008). The selective H1R agonist 2-(3-(trifluoromethyl)-phenyl)histamine, through i.c.v. administration, enhanced object recognition (Malmberg-Aiello et al., 2003). Clinically, the H1R antagonist negatively influenced attention (Okamura et al., 2000). H1R binding was shown to progressively decline in AD patients compared to their old, normal counterparts (Higuchi et al., 2000). Similarly, postmortem AD brains had low histamine content in the hypothalamus, hippocampus and temporal cortex (Mazurkiewicz-Kwilecki and Nsonwah, 1989, Panula et al., 1998). H3R KO mice had enhanced place recognition in the Barnes maze (Rizk et al., 2004). These mice were also resistant to scopolamine-induced amnesia in PAT (Toyota et al., 2002). In parallel, the selective H3R agonist RAMH increased forgetfulness in different memory models (Table 2).

Table 2: Preclinical behavioral characterization of H3R agonists and antagonists

	Recognition memory	Spatial memory	Working memory	Long-term memory (fear memory)	Executive memory	Attention/impulsivity
Histamine (i.c.v.)	↑ rats <sup>1,a</sup>	↑ rats <sup>2,b</sup>	↑ rats <sup>3,c</sup>	↑ in rats <sup>1,d</sup>		

RAMH	↓ in rats <sup>1,e</sup>			↓ in rats <sup>1,e</sup>		
Thioperamide	↑ rats <sup>1,4,a,f</sup>	↑ rats and mice <sup>2,b,g</sup>	↑ rats <sup>h</sup>	↑ mice <sup>1,2,3,4,f,i,j</sup>		↑ SHR pups <sup>k</sup>
Ciproxifan	↑ rats <sup>1,2,l</sup>	↑ in mice <sup>2,g</sup>				↑ SHR pups and rats <sup>1,m,n</sup>
Pitolisant	↑ mice <sup>1,2,o</sup>			↑ mice <sup>1,3,p</sup>		
GSK189254	↑ rats <sup>1,q</sup>	↑ in aged rats <sup>1,q</sup>		↑ rats <sup>1,q</sup>	↑ rats <sup>1,q</sup>	

SHR = spontaneously hypertensive rats (attention deficit). ↑= increase or improve, ↓= decrease or worsen. <sup>1</sup>Naïve animals, <sup>2</sup>scopolamine-induced memory deficit, <sup>3</sup>Dizocilpine-induced memory deficit, <sup>4</sup>PTZ-induced memory deficit. <sup>a</sup>(Prast et al., 1996). <sup>b</sup>(Chen and Kamei, 2000). <sup>c</sup>(Huang et al., 2003). <sup>d</sup>(de Almeida and Izquierdo, 1986). <sup>e</sup>(Blandina et al., 1996). <sup>f</sup>(Jia et al., 2006). <sup>g</sup>(Komater et al., 2005). <sup>h</sup>(Orsetti et al., 2002). <sup>i</sup>(Meguro et al., 1995). <sup>j</sup>(Charlier et al., 2013). <sup>k</sup>(Komater et al., 2003). <sup>l</sup>(Pascoli et al., 2009). <sup>m</sup>(Fox et al., 2002). <sup>n</sup>(Day et al., 2007). <sup>o</sup>(Ligneau et al., 2007). <sup>p</sup>(Brabant et al., 2013). <sup>q</sup>(Medhurst et al., 2007).

#### 1.5.4 Synaptic plasticity

In the process of memory formation, molecular and cellular changes occur to enforce synapses that are essential for memory. These changes are called synaptic plasticity and involve activation or inhibition of receptors, ion channels and proteins with consequent alternation in the size and number of synapses (Middei et al., 2014). Long-term potentiation (LTP) and long-term depression (LTD) are forms of synaptic plasticity. The hippocampus is a brain structure that is highly involved in memory consolidation and the most studied region in this regard.

Histamine was found to induce LTP in the CA1 region of the hippocampus *in vitro* in the absence of any external tetanus stimulation that is usually employed in such experiments (Selbach et al., 1997). On the other hand, impairment of electrically-induced LTP was demonstrated *in vitro* in the CA1 region of H1 and H2 KO mice (Dai et al., 2007b). Such facilitation of synaptic activity could be attributed



to: 1) activation of kinases like PKA (through H2R and potentiated by H1R) and PKC (through H1R) and subsequent activation of other proteins, 2) mobilization of intracellular  $\text{Ca}^{+2}$  stores by inositol triphosphate (IP3) (through H1R), 3) H1-mediated removal of  $\text{Mg}^{+2}$  block from NMDA receptors and subsequent  $\text{Ca}^{+2}$  influx and 4) H2R-mediated blockage of leak  $\text{K}^{+}$  conductance and increase of burst discharges in CA3 with enhancement of its inputs to the CA1 (Brown et al., 2001, Haas et al., 2008).

### **1.5.5 Locomotor activity and exploratory behavior**

An important component of animal activity is locomotion, i.e. movement from one place to another. It can be affected by many factors including external stimuli (like introduction to a novel environment) or internal physiological characteristics such as the circadian rhythm (Bevins and Besheer, 2001, Tosini, 2007). Impairment of locomotor activity is associated with several disorders including Parkinson's and Huntington' diseases and hyperactivity syndrome. CNS drugs can affect locomotion which can influence other behavioral parameters including memory.

In several studies, histamine was found to enhance locomotion. Depletion of brain histamine through pharmacological tools using  $\alpha$ -FMH in ICR mice or by genetic methods (HDC-KO) reduced novelty-induced or spontaneous locomotor activity, respectively (Onodera et al., 1992b, Sakai et al., 1992, Kubota et al., 2002). Sakai et al. (1992) demonstrated that higher brain histamine is associated with increased locomotion. H1R KO mice showed impaired spontaneous activity (dark), exploration, and rearing in a new environment (Inoue et al., 1996). Recently, it has been shown that selective activation of histamine neurons increases locomotion (Yu

et al., 2015). Moreover, previous results demonstrated that some H3R antagonists increased the release of histamine and tendency to increased locomotion (Ghi et al., 1998, Mohsen et al., 2014).

Contrary, depletion of histamine by  $\alpha$ -FMH (in ICR mice but at higher dose: 100 mg/kg) was reported to increase locomotor activity and rearing (at night time) which was accompanied by an 85% decrease in brain GABA (Sakai et al., 1995). In addition, the imidazole-based H3R antagonist thioperamide was shown to decrease, to various degrees, amphetamine/apomorphine/cocaine-induced hyperactivity, an effect that was inhibited by the selective H3R agonist, namely (*R*)- $\alpha$ -methylhistamine RAMH (Clapham and Kilpatrick, 1994). Indeed, the methamphetamine's hyperactivity effects were more pronounced in HDC-KO mice (Kubota et al., 2002). Moreover, H1R antagonism increased locomotor activity with involvement of an opioid-dependent mechanism (Leza et al., 1991). Histamine (i.c.v.) in rats had a biphasic action: a transient augmentation then a reduction in locomotion (Haas et al., 2008). Furthermore, H3R KO mice displayed reduced spontaneous locomotor activity (Toyota et al., 2002).

## **1.6 Histamine H3R antagonists**

### **1.6.1 Drug Development**

Thioperamide (Figure 6) was the first potent H3R antagonist to be developed as a research tool to explore the function of H3Rs. However, this agent was associated with hepatotoxicity and the search began for more tolerable candidates (Schwartz, 2011).

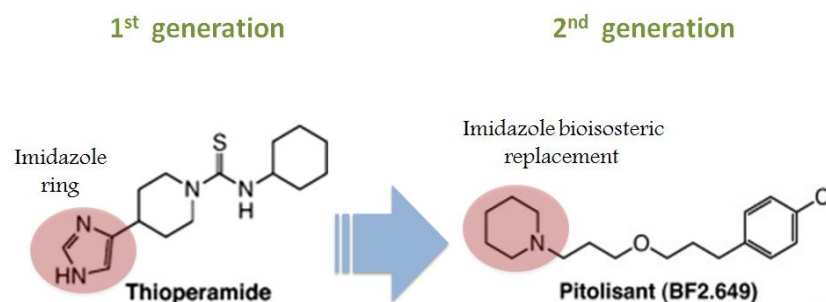


Figure 6: Structural development of H3R antagonists by replacement of the disadvantageous imidazole group.

Later, several compounds, like ciproxifan and clobenpropit, that share the basic imidazole ring with thioperamide, were developed (McLeod et al., 2003). For the latter imidazole-based antagonists, however, it has been shown that numerous metabolic interactions may develop due to the ability of the imidazole heterocycle to potently inhibit CYP450 isoenzymes. Other class-related drawbacks of imidazole-based agents include relatively poor CNS penetration and incidence of off-target activity at H4R or other receptors (Berlin et al., 2011, Panula et al., 2015). Therefore, further chemical modulation, by replacing imidazole (Figure 6) with piperidine or tertiary amine, resulted in the development of numerous non-imidazole H3R antagonists which has attracted the main focus of the H3R antagonists design work in recent years. Thus, the newer H3R antagonists, e.g. pitolisant, are seen as bioisosteric alternatives to the imidazole-based group. Extensive structure-activity relationship efforts in the field of drug design and development led to an understanding of the general structural pattern (Figure 7) consisting of the most required pharmacophores for a given compound to exert histamine H3R antagonistic affinity. The general structural pattern (Figure 7) consists of a western basic moiety, that is connected via an alkyl spacer to a hydrophilic (hetero-atom or -cycle) central core and an eastern

part that is important for H3R antagonistic affinity and is only limited by the overall molecular size.

### 1.6.2 H3 antagonists as candidates for the treatment of epilepsy and cognitive disorders

Epilepsy is a serious neurological disorder that affects around 70 million people worldwide from all age groups. The mortality rate is 3 times higher than the normal population and the risk increases with uncontrolled cases. Pharmacological intervention with antiepileptic drugs (AEDs) represents the chief strategy for the

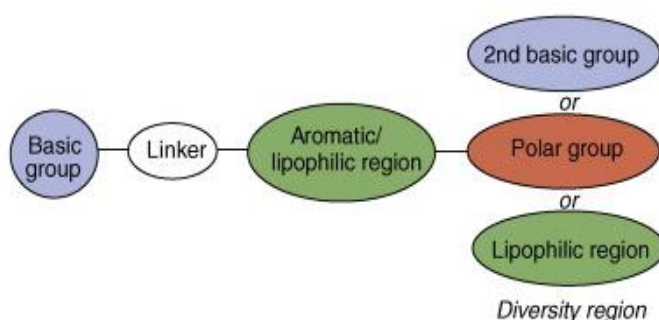


Figure 7: General structural pattern of non-imidazole H3R antagonists. Adopted from (Gemkow et al., 2009)

management of this ailment. Around 60-70 % of patients respond to treatment, however, resistance to mono-therapy is not uncommon and drug combination is often inevitable (Brigo et al., 2013). Available AEDs do not bear tolerable safety profiles and, with poly-therapy, side effects are further augmented (Schmidt, 2002).

Cognitive impairment accompanies some types of epilepsy at onset and worsens with chronic, poorly controlled seizures particularly in the developing brain (Elger et al., 2004). Appropriate, early management of seizures removes the insult on the brain cognitive function, though many AEDs themselves can negatively alter

cognition (Hermann et al., 2010). Therefore, more effective, safer pharmacological approaches that remove seizure risk and improve the quality of life are warranted.

Given their prominent expression and widespread distribution in the CNS, in addition to their regulatory function on the release of histamine as well as other neurotransmitters (refer to section 1.4.3), H3Rs are an attractive target for the treatment of several CNS diseases including epilepsy and cognitive disorders. The presence of different isoforms of the receptor with differential distribution in the brain (refer to section 1.4.3) could permit the design of selective ligands with further reduction of side effects.

#### **1.6.2.1 H3R antagonists in epilepsy**

As illustrated in Table 2, H3R antagonists attenuated or delayed convulsions in several electrical, chemical or genetic models of epilepsy. This effect seems to largely depend on H1R activation since it was reversed by CNS-penetrant H1R antagonists (Kakinoki et al., 1998, Zhang et al., 2003a, Sadek et al., 2014). These findings are supported by preclinical and clinical studies with H3R antagonists as discussed in section 1.5.3. Furthermore, bicuculline, a GABA<sub>A</sub> receptor antagonist, significantly diminished clobenpropit inhibition of amygdaloid kindling (Ishizawa et al., 2000). Thus an association between H3R antagonist anticonvulsive action and GABA transmission has been suggested. Accordingly, two scenarios are possible, either 1) H3R antagonists inhibit autoreceptors and increase release of histamine that in turn activates H1R and consequently excites GABAergic neurons and/or 2) H3R antagonists directly increase GABA release through inhibition of H3R heteroreceptors (Figure 8).

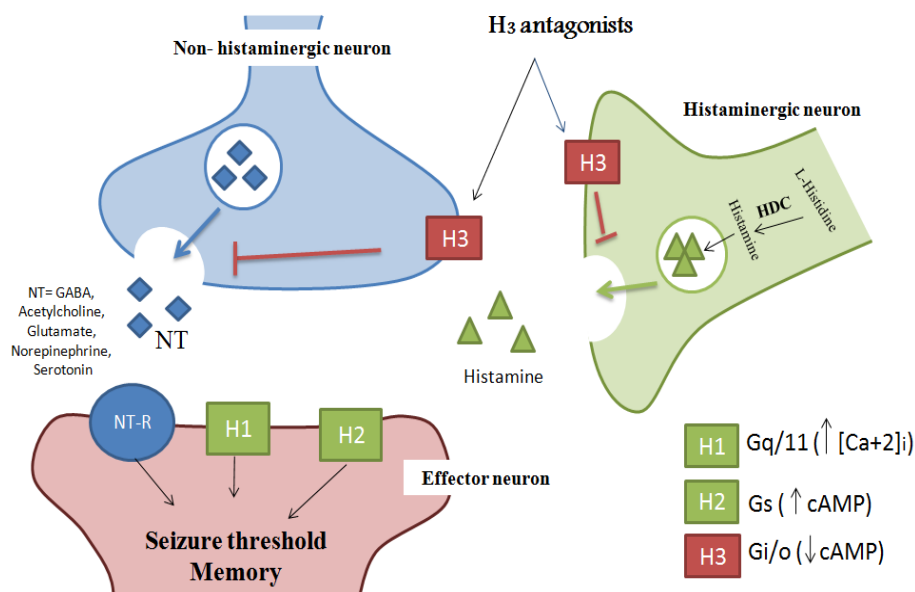


Figure 8: Schematic illustration of possible mechanisms by which H3R antagonists affect seizures and cognition.

In addition, clobenpropit and iodophenpropit, H3R antagonists, exert noncompetitive antagonism at the NR1/NR2B subunits of the NMDA receptors with submicromolar potency (Hansen et al., 2010). NR2 (A, B, C or D) subunits, unlike NR1, of NMDA receptors possess differential distribution and pharmacology (Vicini et al., 1998). NR2A/B subunits are expressed abnormally in dysplastic neurons of epileptic human cortex (Ying et al., 1999). Furthermore, selective NR1/NR2B NMDA antagonists like ifenprodil, have anticonvulsant activity with no cognitive side effects compared to non-selective NMDA antagonists (Chizh et al., 2001, Mares and Mikulecka, 2009). Thus a synergistic action at H3R and NMDA receptors of clobenpropit and iodophenpropit could explain their antiepileptic properties. Such observations can be utilized to create more potent dual-target agents that can be used for the management of epilepsy. Notably, H3R antagonists are progressing well in clinical trials in the field of epilepsy. Recently, pitolisant showed promising outcomes in proof-of-concept trials in a human photosensitivity model of epilepsy

(Kasteleijn-Nolst Trenite et al., 2013). In this model, photosensitive, epileptic patients are exposed to intermittent flashes of light at different frequencies before and after test drug administration. These flashes at variable frequencies induce subclinical epileptiform discharges on EEG (photo-paroxysmal responses) in photosensitive patients. This model is an early “informative indicator” of AED’s effectiveness and will help in dose selection for future phase 2/3 clinical trials (Yuen and Sims, 2014).

Excessive excitation of neurons can lead to excitotoxicity and neuronal death. Widespread neuronal necrosis can arise due to status epilepticus, prolonged or rapidly recurrent seizures, even at its clinically-silent, non-convulsive stage (Wasterlain et al., 1993). The neuronal damage that can occur during the status epilepticus and thereafter can impair normal brain functions including cognitive impairment, and potentiate further seizure activity, i.e. epileptogenesis (Walker, 2007). Therefore, early intervention to prevent seizures is the first-line of protection against seizure-associated neurotoxicity. Antiepileptic agents should also provide neuroprotection and preserve normal brain activity (Walker, 2007). In previous studies, the neuroprotective effects of H3R antagonists, e.g. thioperamide and ABT-239, have been revealed in *in vitro* and *in vivo* kainic acid- or 3-nitropropionic acid (3-NP)-induced neurotoxicity models (Kukko-Lukjanov et al., 2006, Bhowmik et al., 2014). It has been observed that H3R up regulation and H1R down-regulation coincided with excessive neuronal damage and behavioral effects as a consequence of 3-NP-induced neurotoxicity in hamster and rat (Canonaco et al., 2005). Moreover, the H3R antagonist clobenpropit prevented NMDA-induced neurotoxicity of cultured cortical neurons by increasing GABA release mediated by cAMP/PKA (Dai et al., 2007a). Furthermore, a previous study showed that the H3R antagonist ABT-

239 protected against seizures and neuronal damage induced by kainic acid through augmentation of PKA/CREB and Akt/GSK3 $\beta$ ser9 signaling pathways that regulate neuronal survival and inhibit excitotoxicity. Interestingly, these signaling pathways are also involved in learning, memory and neuronal plasticity. In summary, H3R antagonists have advantages over other available AEDs of possessing disease modifying properties and may ameliorate cognitive decline that is associated with epilepsy especially in the developing brain (Elger et al., 2004). Indeed, thioperamide and JNJ-5207852 prevented different memory deficits induced by PTZ kindling in weanling mice (Jia et al., 2006). Notably, H3R antagonists have cognitive enhancing properties in other models as reviewed below.

#### **1.6.2.2 H3R antagonists in cognition**

Many H3R antagonists have been shown to facilitate learning and memory as shown in Table 1. First possible etiology stems from their wake-promoting properties in preclinical and clinical studies (Parmentier et al., 2007, Lin et al., 2011, Dauvilliers et al., 2013). The arousal effect is combined with enhanced cortical fast rhythms that are correlated with higher brain functions including alertness, attention and cognition (Parmentier et al., 2007, Lin et al., 2011). Secondly, H3R antagonists increase the synthesis and release of histamine and its associated memory enhancement (Table 1). In addition, H3R antagonists can increase the release of other neurotransmitters involved in cognition. Moreover, H3R antagonists increase acetylcholine release in the prefrontal cortex, anterior cingulate cortex and hippocampus (Mochizuki et al., 1994, Fox et al., 2005, Medhurst et al., 2007), dopamine release in the prefrontal cortex and the anterior cingulated cortex (Fox et al., 2005, Medhurst et al., 2007), and norepinephrine in the anterior cingulate cortex



(Medhurst et al., 2007). The mechanism by which H3R antagonists influence other neurotransmitter release is complex. It involves 1) excitation of the neurotransmitter neuron through H1/H2, 2) excitation or inhibition of a “third-party” excitatory or inhibitory neuron that in turn modulates neurotransmitter release and 3) direct increase of the neurotransmitter release through inhibition of H3R heteroreceptors (Figure 8 above).

Several H3R antagonists have entered phase 2 clinical trials to evaluate their effectiveness against cognitive deficits associated with Schizophrenia, AD, and ADHD. Two separate studies with the H3R antagonist GSK239512 have indicated its beneficial effects in attention and memory in patients with mild-moderate AD (Nathan et al., 2013, Grove et al., 2014). However, further larger scale testing is required to clarify its effectiveness (Nathan et al., 2013). Interestingly, an essential component or prerequisite of cognition is arousal and vigilance, both of which are modulated by H3R neurotransmission. Wake-promoting effects have been demonstrated with the H3R antagonists pitolisant (Inocente et al., 2012) and MK-7288 (Sun et al., 2013). In addition, enhancement of cortical fast rhythms on EEG has been observed with pitolisant (Kasteleijn-Nolst Trenite et al., 2013).

Supported by the evidence discussed above, the current study aimed to investigate the activity of newly developed H3R antagonists (**1-12**) in several *in vivo* epilepsy models, including MES-, PTZ- and strychnine (STR)-induced seizures. In addition, selected H3R antagonist in terms of seizure control was nominated for further cognitive *in vivo* tests in naïve animals utilizing PAT. The newly synthesized H3R antagonists (**1-12**) belong to the non-imidazole class of compounds and possess *in vitro* antagonistic binding properties in the nanomolar range at the human histamine H3R.

## Chapter 2: Aims and Objectives

Previous studies have revealed the anticonvulsant and procognitive effects of the imidazole-based H3R antagonists. Since the effects of non-imidazole based H3R antagonists on memory and seizures in the same species are still unknown. Our main aim was to investigate the effects of newly synthesized non-imidazole H3R antagonists (**1-12**) of high *in vitro* antagonist affinities for hH3Rs, on their *in vivo* anticonvulsant in MES-, (PTZ)-, and (STR)-induced seizures and procognitive effects in passive avoidance paradigm in male adult rats. The novel non-imidazole H3R antagonists (**1-12**) were synthesized based on structure-activity relationship studies of pitolisant and related derivatives and incorporated different alkyl spacers (3, 5, or 6 methylene groups) and various aromatic substitutions (see Material & Methods section). The detailed objectives of the experiments are as follows:

A. Investigate anticonvulsant effects of H3R antagonists **1-12** on MES-, (PTZ)-, and (STR)-induced seizure models.

B. Dose-dependency of anticonvulsant effects provided by a selected H3R antagonist in terms of seizure control in MES, (PTZ), and/or (STR) seizure models

C. Abrogation study of anticonvulsant effects provided by the selected H3R antagonist in MES-, (PTZ)-, and/or (STR)-induced seizure models.

D. Memory-enhancing effects of a selected H3R antagonist offering the greatest, histamine-dependent seizure control on acquisition, consolidation, and retrieval stages in an inhibitory avoidance paradigm.

E. Abrogation study of memory-enhancing effect provided by most effective H3R antagonist on the level of acquisition, consolidation, and/or retrieval in an inhibitory avoidance paradigm.

## Chapter 3: Materials and Methods

### 3.1 Animals

Male Wistar rats 180-200 g, bred at the Central Animal Facility of the UAE University, were used. In the study, animals were maintained in an air-conditioned room with controlled temperature ( $24\pm 2$  C) and humidity ( $55\pm 15\%$ ) under a 12-h light/dark cycle (lights on at 07:00 h). The animals were given free access to food and water. Experiments were conducted between 9:00 and 15:00 h, and all procedures were performed in accordance with the guidelines of the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were approved by the Institutional Animal Ethics Committee of the College of Medicine and Health Sciences, United Arab Emirates University (A9-14) for epilepsy and (A30-13) for precognitive study.

### 3.2 Drugs

Phenytoin (PHT), valproic acid (VPA), *R*-( $\alpha$ )-methyl-histamine (RAMH), the brain-accessible H1R antagonist pyrilamine (PYR) and H2R antagonist zolantidine (ZOL), donepezil hydrochloride (DOZ), pentylenetetrazole (PTZ), and strychnine (STR) were purchased from Sigma-Aldrich (St Louis, Missouri, USA). The test compounds **1** and **2** were synthesized at the Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University, Düsseldorf, Germany. The standard H3R antagonist pitolisant (PIT) and the test compounds, namely the H3R antagonists (**3-12**) were synthesized in the Department of Technology and Biotechnology of Drugs, Kraków/Poland as described previously (Lazewska et al., 2006) (Figure 9). The test compounds were dissolved in either 0.9% saline or

isotonic 10% DMSO and administered intraperitoneally (i.p.) at a volume of 1 ml/kg. Doses of test compounds **1-12** as well as reference compounds were expressed in terms of the free base.

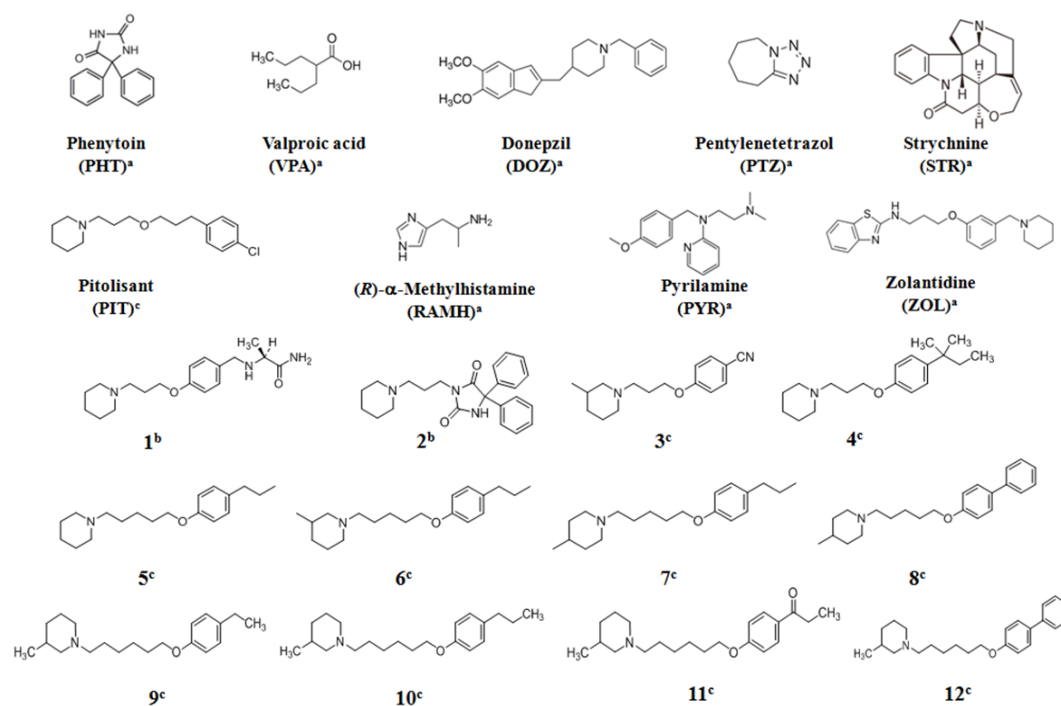


Figure 9: Chemical structures of compounds used in this study. <sup>a</sup>Compounds were purchased from Sigma-Aldrich (St Louis, Missouri, USA). <sup>b</sup>H3R antagonists were synthesized in the Institute of Pharmaceutical and Medicinal Chemistry, Heinrich Heine University, Düsseldorf, Germany. <sup>c</sup>H3R antagonists were synthesized in the Department of Technology and Biotechnology of Drugs, Kraków, Poland.

### 3.3 *In vivo* models of seizures

#### 3.3.1 Maximal electric shock

MES is a model of generalized tonic-clonic seizures arising from the brain stem on exposure to auricular stimulation. Through ear electrodes, an alternating current with 50 Hz frequency and 120 mA intensity for 1-sec was applied to each rat. Duration of tonic hind limb extension (THLE), tonic phase, was taken as a measure

of seizures. Reduction in duration or complete absence of THLE was regarded or defined as protection from seizures.

Animals were divided into different groups (a saline treated group and groups receiving test compounds) each containing 7-8 rats. Saline, PHT 10 mg/kg (a standard antiepileptic drug), PIT 10 mg/kg (a standard H3R antagonist), and test compounds (**1-12**) each at dose of 10 mg/kg were injected i.p. 30 minutes before shock application. H3R test compounds with significant protection against THLE were then tested at other doses (5 and 15 mg/kg, i.p.) applying the same protocol to explore dose dependency of the protective effect provided by the respective test compound. In further experiments, rats were injected with the selected H3R antagonist (test compound) offering the highest protection in combination with CNS-penetrant HR1 antagonist (PYR, 10 mg/kg, i.p.) to clarify the mechanism of seizure protection.

### **3.3.2 Chemically induced seizures**

PTZ, a GABAA antagonist, and STR, a glycine receptor antagonist, were the two proconvulsant compounds used in the current study. Immediately after i.p. administration of PTZ 60 mg/kg or STR 3.5 mg/kg (Loscher, 2011, Sowemimo et al., 2011, Branco Cdos et al., 2013, Lopes et al., 2013), seizure activity was recorded for 30 minutes. Graded scores (0-5) were utilized to evaluate the resulting behavioral phenomena (seizures) as the following: 0 = no seizures, 1 = eye or facial twitches, 2 = convulsive waves across the body, 3 = myo-clonic jerks or rearing, 4 = turn over onto one side position, and 5 = turn over onto back position, generalized tonic-clonic seizures, or die during the experiment period. Maximum seizure scores were evaluated at 10-min intervals (10 min, 20 min and 30 min). Significant reduction of

the maximum score at each time interval was regarded or defined as protection from seizures at that interval.

Animals were divided into groups of 6-8 rats each according to the test compounds. Saline, VPA 100 mg/Kg (in PTZ-induced seizure model), VPA 300 mg/kg (in STR-induced seizure model), PIT 10 mg/kg and test compounds (**1-12**) each at 10 mg/kg were injected i.p. 30 min. before the administration of either PTZ or STR. Test compounds that showed beneficial anti-seizure activity as defined above were further tested at other doses ( 5 and 15 mg/kg, i.p.) against PTZ or STR-induced seizures to explore the dose dependency of their protective effect. In a further experiment, the test compounds with the highest protection were co-injected with PYR (10 mg/kg, i.p.) to clarify the mechanism of their anti-seizure effect in the respective model (PTZ- or STR-induced seizure model).

### **3.4 Behavioral tests**

#### **3.4.1 Open Field Test**

The open field test is a prerequisite for memory studies that require animal movement and normal anxiety level. Any effect of compounds on the locomotor activity or anxiety of animals could alter or confound their activity in the memory test. Locomotor activity and anxiety levels were examined in an open field test (OFT). A rectangular 112x56x56 cm<sup>3</sup> (length/width/height) box that is open from above provides the testing arena (Figure 10). The floor of the box was divided into 32 equal squares (14 cm in diameter). The 4 central squares were regarded as the “center” of the field. At the beginning of the test, animals were transferred to the arena only by their tails and placed in the center where they are allowed to move freely for 3 minutes. During this time animal activity was recorded by a digital

camera placed above the arena. Video recordings were utilized for assessment. When a rat moved from one square to another with its four paws, one line crossing was noted and a distance of 14 cm is recorded. The following parameters were manually scored: the number of lines crossed and time spent in the center of the arena during a 3-minute test (Charlier et al., 2013). More time spent in the peripheral area was usually taken as a measure of a higher level of anxiety and vice versa. After each test, the arena was sprayed with 70% ethanol and wiped thoroughly to remove any

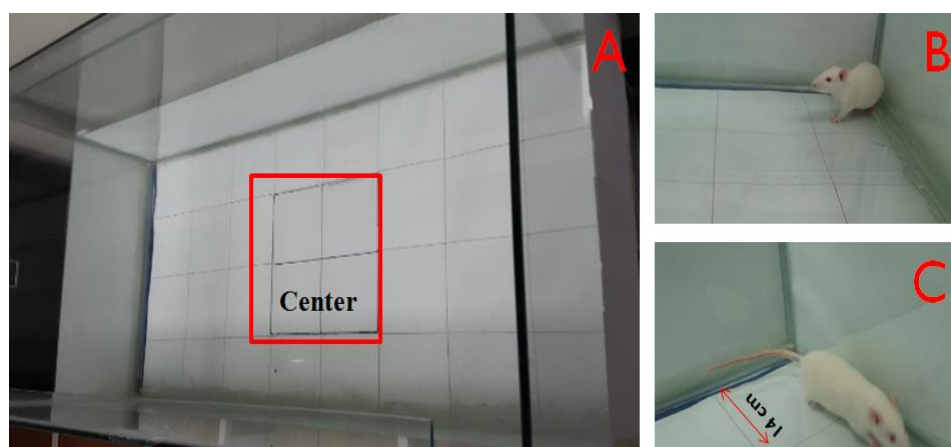


Figure 10: Open field arena utilized to explore locomotion and anxiety levels. (A) Central area surrounded by a red square, when an animal stays in the center it means low level of anxiety and vice versa. (B) No locomotion, animal is not crossing any line or staying within a square. (C) Animal is about to cross a line, i.e. 14 cm distance is recorded.

residual odor. Animals were divided into groups of 7 rats each. 30 minutes prior to the test, animals were injected i.p. with saline, PIT 10 mg/kg, DOZ 1 mg/kg or H3R test compound **4** at various doses (2.5, 5 and 10 mg/kg, i.p.). Doses of H3R antagonist **4** that didn't significantly alter locomotor or anxiety levels were used for subsequent memory tests.

### 3.4.2 Passive Avoidance Test (step-through)

The test was performed in a Passive Avoidance Apparatus (Step-through Cage, 7550, Ugo Basile, Comerio, Italy). The apparatus consisted of two equal size



compartments (51 x 25 x 24 cm each) with grid floors which can be electrified separately. The walls of the first compartment are painted white, and the second compartment is dark with walls painted black. An automated guillotine door separates the two compartments (Figure 11).



Figure 11: Passive avoidance test apparatus utilized in this study.

PAT is a long-term memory test. It consists of two types of session, namely a training session and a test session separated usually by a 24 hour interval. This test exploits the fact that rodents prefer a dark environment. Each rat was introduced to the white compartment for 30 seconds to explore then the door separating the dark chamber was automatically opened. Within a minute or less, rats should move to the dark or otherwise would be excluded from the study. When animals reached the dark compartment with all four paws, the door was closed within 3 seconds and an inescapable foot shock (intensity = 0.5 mA, duration= 3 seconds) was administered. This concluded the training session and 70 % ethanol was used to clean the compartments to remove olfactory cues after each experiment. In the same manner and 24 hr later the animals were again placed in the white compartment and 300 seconds as a cut-off time was provided while the door was open. Once the rats moved to the dark compartment, the test was ended without any foot shock to be

delivered. The latency to move to the dark compartment (step-through latency) was utilized to evaluate the memory of the animals. Higher latency indicated better recall.

To investigate the effect of test compound **4** on several components of memory formation, H3R antagonist **4** was injected in separate groups of animals at different time points (Figure 12). Drugs were given 30 minutes before the training session (time point 1), directly after the training (time point 2) or 30 minutes before the test session (time point 3). These three separate experiments were employed to explore their activity in memory acquisition, consolidation or retrieval, respectively.

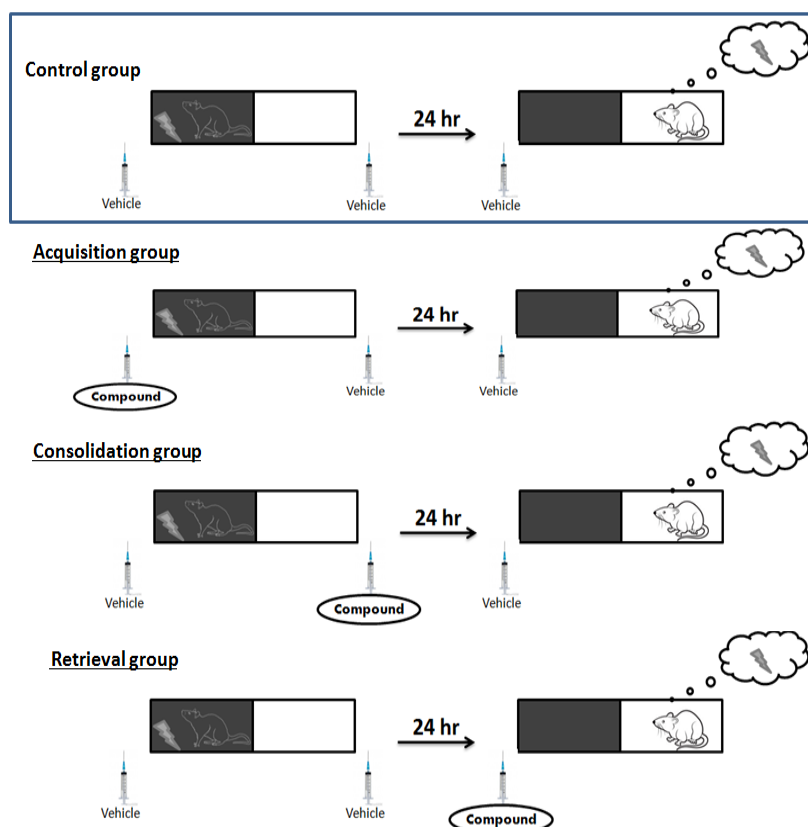


Figure 12: Schematic description of passive avoidance test protocol and time points of injections to test effect of a compound in several aspects of memory: acquisition, consolidation and retrieval.

To reduce the number of animals and even out experience of injection among all animals, all the groups received saline injection at all three time points, except when

the compound was given, (Figure 12) and one control group (only saline treated at all time points) was used for comparison.

Any memory-improving effect of the selected H3R antagonist **4** was then further investigated by abrogation studies that included co-administration of the most effective dose of H3R antagonist **4** and zolantidine (ZOL, 10 mg/kg, i.p.) or pyrilamine (PYR, 10 mg/kg, i.p.). Doses of ZOL and PYR were selected according to previous studies (Orsetti et al., 2001, Sadek et al., 2013, Sadek et al., 2014).

### **3.5 Statistical analysis**

For statistical comparisons, the software package SPSS 20.0 (IBM Middle East, Dubai, UAE) was used. All data were expressed as the means  $\pm$  SEM. All the results were analyzed using one-way ANOVA analysis of variance (ANOVA) with Treatment (vehicle or test compound) and Dose (test compound) as the between-subjects factor. In case of a significant effect, post hoc comparisons were performed with Bonferroni's test. The criterion for statistical significance was set at a *P* value of less than 0.05.

## Chapter 4: Results

### 4.1 Effect of H3R antagonists 1-12 on MES-induced seizures

Figure 13 shows the protective effects of acute systemic administration of test compounds **1-12** on MES-induced seizures in rats. One-way analysis of variance showed that treatment with PHT, PIT, and H3R ligands 1-12 (10 mg/kg, i.p.) exerted a significant protective effect against MES-induced convulsions [ $F_{(14,90)} = 34.422$ ;  $P < 0.05$ ]. Among the compounds tested **4**, **8**, **10**, **11**, and **12** provided the best protection against MES-induced convulsion in rats pretreated with 10 mg/kg, i.p. when compared with the saline-treated group [ $F_{(1,12)} = 235.86, 16.52, 53.04, 25.99,$

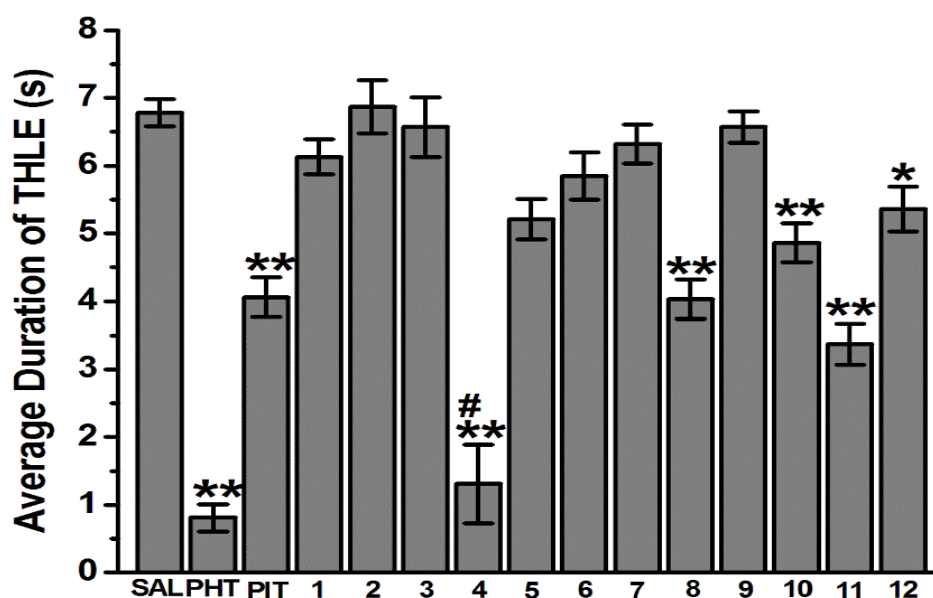


Figure 13: Protective effect of an intraperitoneal injection of H3R antagonists **1-12** on maximal electroshock-induced seizure. The figure shows the protective effects of phenytoin (PHT, 10 mg/kg, i.p.), pitolisant (PIT, 10 mg/kg, i.p.), and H3R antagonists **1-12** (10 mg/kg, i.p.) on the duration of tonic hind limb extension (THLE) induced in the maximal electroshock (MES) model in rats. Data are expressed as mean  $\pm$  SEM (n=7). \* $P < 0.05$  vs. the saline-treated group. \*\* $P < 0.001$  vs. saline-treated, group. # $P < 0.001$  vs. PIT (10 mg)-treated group.

77.37, and 11.66, respectively,  $P < 0.05$ ]. When compared with the group pretreated with PIT (10 mg/kg, i.p.), only H3R antagonist **4** showed significantly higher protective effect on MES-induced seizures [ $F_{(1,12)} = 41.65$ ,  $P < 0.05$ ]. Also, the protective effect observed for H3R antagonist **4** (10 mg/kg, i.p.) was comparable to those observed with PHT-treated group [ $F_{(1,12)} = 1.66$ ,  $p = 0.22$ , NS].

#### **4.2 H3R antagonist 4 dose-dependently reduced MES-induced seizures**

Figure 14 shows the protective and dose-dependent effects of acute administration of H3R antagonist **4** (5, 10 or 15 mg/kg, i.p.) on MES-induced seizures in rats. One-way analysis of variance showed that pretreatment with PHT (10 mg/kg, i.p.), PIT (10 mg/kg, i.p.), and **4** (5, 10, and 15 mg/kg, i.p.) exerted significant protective effects against MES-induced convulsions [ $F_{(5,36)} = 83.05$ ;  $P < 0.05$ ]. Moreover, significant increases in protective effects were observed after pretreatment with 10 and 15 mg/kg of **4** when compared with 5 mg/kg of the same compound [ $F_{(1,12)} = 74.59$  and  $F_{(1,12)} = 92.86$ ;  $P < 0.05$ ], respectively.

Also, the protection observed for H3R antagonist **4** (5, 10 and 15 mg/kg, i.p.) was significant when compared with the saline-treated group [ $F_{(1,12)} = 31.35$ ,  $F_{(1,12)} = 235.87$ , and  $F_{(1,12)} = 257.19$ , respectively,  $P < 0.05$ ]. The results, also, show that H3R antagonist **4** exhibited the highest protection against MES-induced convulsion in rats pretreated with 10 and 15 mg/kg, i.p. and were similar to the protective effects observed for the standard AED PHT (10 mg/kg, i.p.) [ $F_{(1,12)} = 1.66$ , and  $F_{(1,12)} = 1.96$ , respectively, all  $P < 0.05$ ].

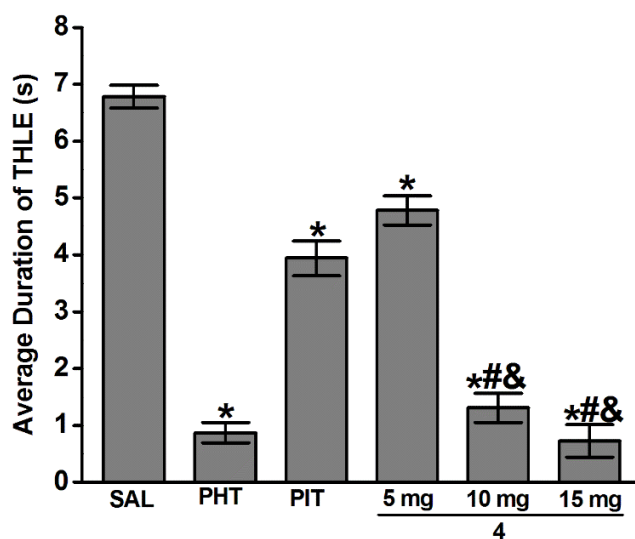


Figure 14: Dose-dependent protective effects of H3R antagonist **4** against maximal electroshock (MES)-induced seizures. The figure shows the protective effects of phenytoin (PHT, 10 mg/kg, i.p.), pitolisant (PIT, 10 mg/kg, i.p.), and H3R antagonist **4** (5, 10, 15 mg/kg, i.p.) on the duration of tonic hind limb extension (THLE) induced in MES model in rats. Data are expressed as mean  $\pm$  SEM (n=7). \* $P < 0.001$  vs. the saline-treated group. # $P < 0.001$  vs. 5 mg H3R antagonist **4**. & $P < 0.001$  vs. PIT-treated group.

### 4.3 H3R antagonist **4**-induced shortening of THLE duration was abrogated by PYR and RAMH in MES-induced seizures

Figure 15 shows reversal of the protective effects of H3R antagonist **4** by pretreatment with 10 mg/kg of the CNS penetrant histamine H1R antagonist pyrilamine (PYR) 30 min, and with 10 mg/kg of RAMH 15 min before MES challenge with [ $F_{(1,12)} = 12.38$ , for the comparison between **4** + PYR and **4** alone], and [ $F_{(1,12)} = 66.67$ , for the comparison between **4** + RAMH and **4** alone].

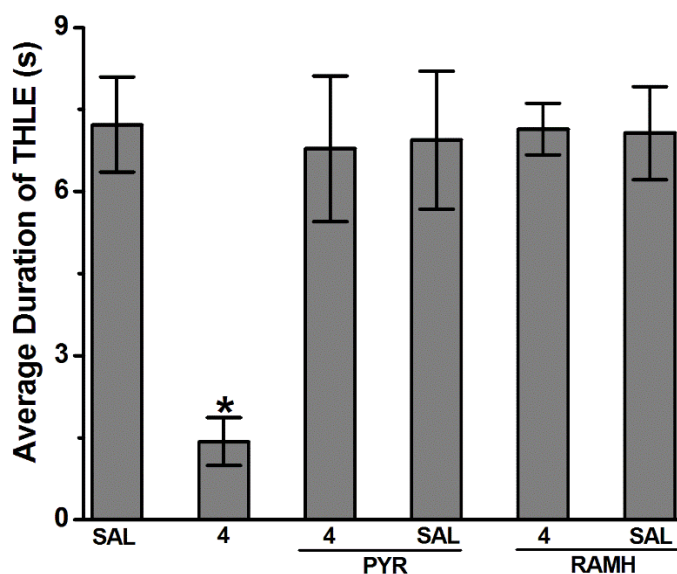


Figure 15: Effect of PYR and RAMH pretreatment on the protection by H3R antagonist **4** on maximal electroshock (MES)-induced convulsions in rats. Saline, H3R antagonist **4** (10 mg/kg), H3R antagonist **4** (10 mg/kg) + PYR (10 mg/kg), saline + PYR (10 mg/kg), H3R antagonist **4** (10 mg/kg) + RAMH (10 mg/kg), and saline + RAMH (10 mg/kg) were injected 30 min before electroshock. Effects shown are expressed as the duration of tonic hind limb extension (THLE) induced in the MES model in rats. Data are expressed as mean  $\pm$  SEM ( $n = 7$ ). \* $P < 0.001$  vs. the saline-treated group.

#### 4.4 Effect of H3R antagonists 1-12 on PTZ-induced seizures

Figure 16 shows the effects of H3R ligands **1-12** (10 mg/kg, i.p.) and the standard H3R antagonist PIT in comparison to the protective effects of the reference antiepileptic drug VPA in PTZ-induced seizures in rats. In PTZ-induced seizure, H3R ligands **5**, **6**, and **12** provided significant protective effects relative to the saline-treated group after 10, 20, and 30 min of observation [ $P < 0.05$ ]. Among the most effective compounds, only H3R ligand **6** provided full protection when compared with saline-treated group after 10, 20, and 30 min with [ $F_{(1,12)} = 101.4$ ;  $P < 0.05$ ], [ $F_{(1,12)} = 44.74$ ;  $P < 0.05$ ], and [ $F_{(1,12)} = 16.8$ ;  $P < 0.05$ ], respectively. However, H3R ligand **4** (10 mg/kg, i.p.) did not provide significant protective effect relative to the saline-treated after 10, 20, and 30 min [ $F_{(1,12)} = 0.10$ , 3.68, and 0.021, respectively, all NS]. Similarly, PIT failed to provide protection in the PTZ-induced seizure model after

10, 20, and 30 min [ $F_{(1,12)} = 0.03, 0.01, \text{ and } 0.50$ , respectively, all NS]. However, VPA (VPA 100 mg/kg) provided significant protection when compared with saline-treated group after 10, 20, and 30 min with [ $F_{(1,12)} = 101.4; P < 0.005$ ], [ $F_{(1,12)} = 44.74; P < 0.05$ ], and [ $F_{(1,12)} = 16.8; P < 0.05$ ], respectively.

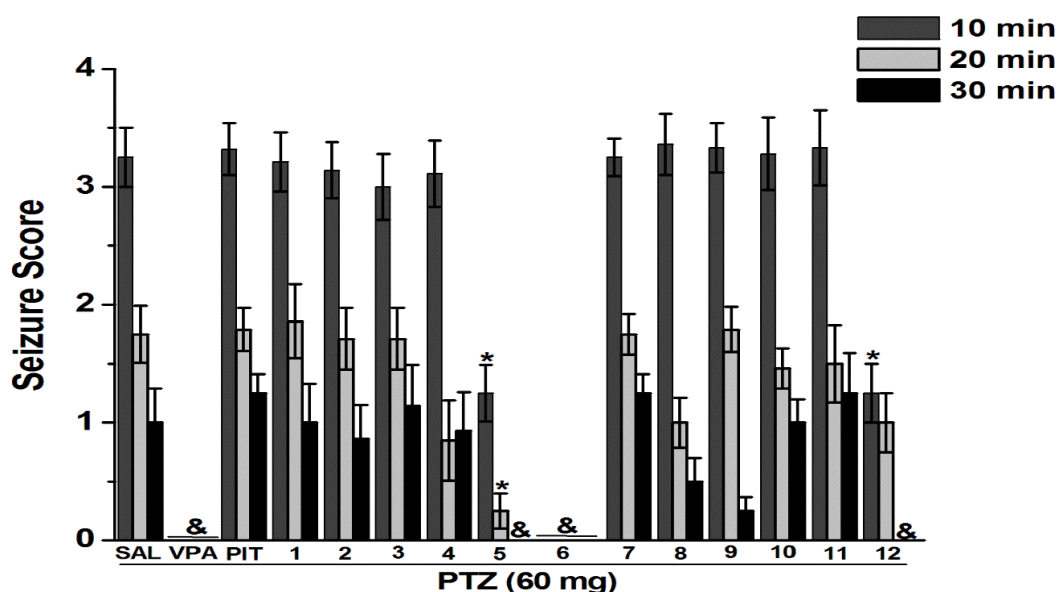


Figure 16: Protective effect of pretreatment with H3R antagonists **1-12** on pentylenetetrazole (PTZ)-induced seizures in rats. Valproic acid (VPA, 100 mg/kg, i.p.) as a reference AED in PTZ-induced seizure (Serdiuk and Gmiro, 2014), 10 mg of pitolisant (PIT), and 10 mg of the respective H3R antagonist were injected i.p. 30 min before PTZ (60 mg/kg, i.p.) treatments. Effects shown are expressed as score of seizures after 10 min, 20 min and 30 min of PTZ injection. Data are expressed as the mean  $\pm$  SEM ( $n=7$ ). \* $P < 0.05$  vs. the saline-treated group. & Full protection for VPA (100 mg/kg, i.p.), H3R antagonists **5** (after 30 min), **6** (after 10, 20, and 30 min), and **12** (after 30 min).

#### 4.5 Dose-dependent effects of H3R antagonist **6** on PTZ-induced seizures

Figure 17 shows the protective and dose-dependent effects of acute administration of H3R ligand **6** (5, 10 or 15 mg/kg, i.p.) on PTZ-induced seizures in rats. One-way analysis of variance showed that pretreatment with reference antiepileptic drug VPA and H3R ligand **6** (5, 10, and 15 mg/kg, i.p.) exerted a significant protective effect against PTZ-induced convulsions with [ $F_{(3,24)} = 53.55; P < 0.05$ ], [ $F_{(3,24)} = 24.13; P < 0.05$ ], and [ $F_{(3,24)} = 10.50; P < 0.05$ ] for 10, 20, and 30



min observation, respectively. The results showed that significant and full protective effect was provided at 10, 20, and 30 min after pretreatment with 10 and 15 mg/kg of H3R antagonist **6**. Comparable full protection was observed with 5 mg/kg of H3R antagonist **6** only at 30 min [ $F_{(1,12)} = 14.00$ ;  $P < 0.05$  for 30 min], yet significant protective effect of lesser magnitude was recorded at 10 and 20 min [ $P < 0.05$  for 10 and 20 min].

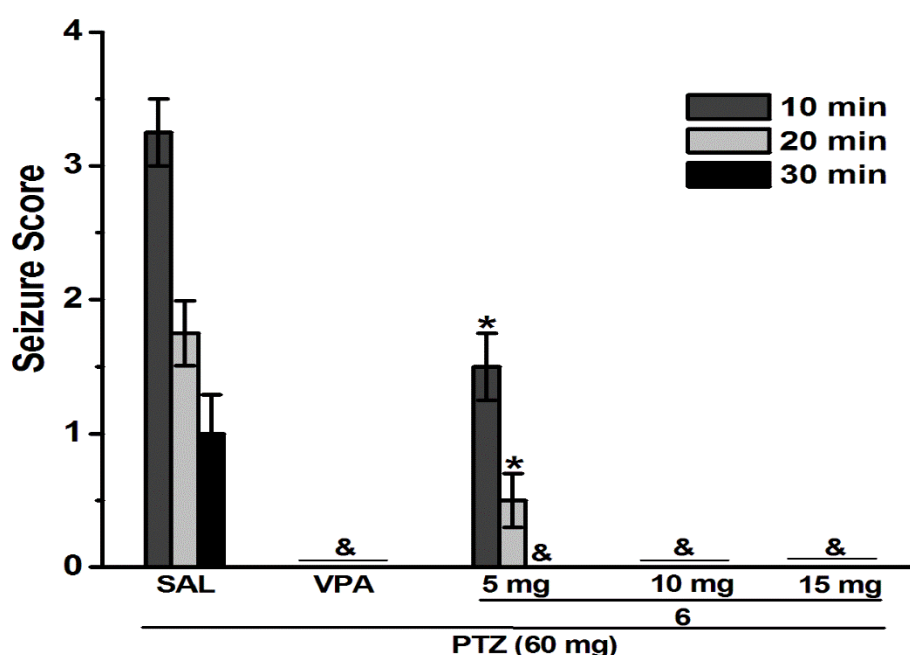


Figure 17: Dose-dependent protective effects of H3R antagonist **6** against pentelenetetrazole (PTZ)-induced seizures. The figure shows the protective effects of Valproic acid (VPA, 100 mg/kg, i.p.) as a reference AED and H3R antagonist **6** (5, 10, 15 mg/kg, i.p.) on the score of PTZ-induced seizure model in rats. Effects shown are expressed as score of seizures after 10 min, 20 min and 30 min of PTZ injection. Data are expressed as mean  $\pm$  SEM (n=7). \* $P < 0.001$  vs. the saline-treated group. &Full protection.

#### 4.6 H3R antagonist **6**-provided protection was partially abrogated by PYR in PTZ-induced seizures

Figure 18 shows the protective effect of H3R ligand **6** when co-administered with 10 mg/kg of the CNS penetrant histamine H1R antagonist pyrilamine (PYR) 30 min before PTZ challenge [ $F_{(1,12)} = 37.75$  ( $P < 0.05$ ) and 6.50 ( $P < 0.05$ ) for the

comparison between H3R ligand **6** + PYR and H3R ligand **6** alone for 10 min and 20 min of observation, respectively; all  $P < 0.05$ ]. However, and only after 30 min of observation, the protective effect of H3R ligand **6** was reversed by pretreatment with pyrilamine (PYR) [ $F_{(1,12)} = 0.08$ ;  $P=0.78$  for the comparison between H3R ligand **6** + PYR and H3R ligand **6** alone for 30 min of observation].

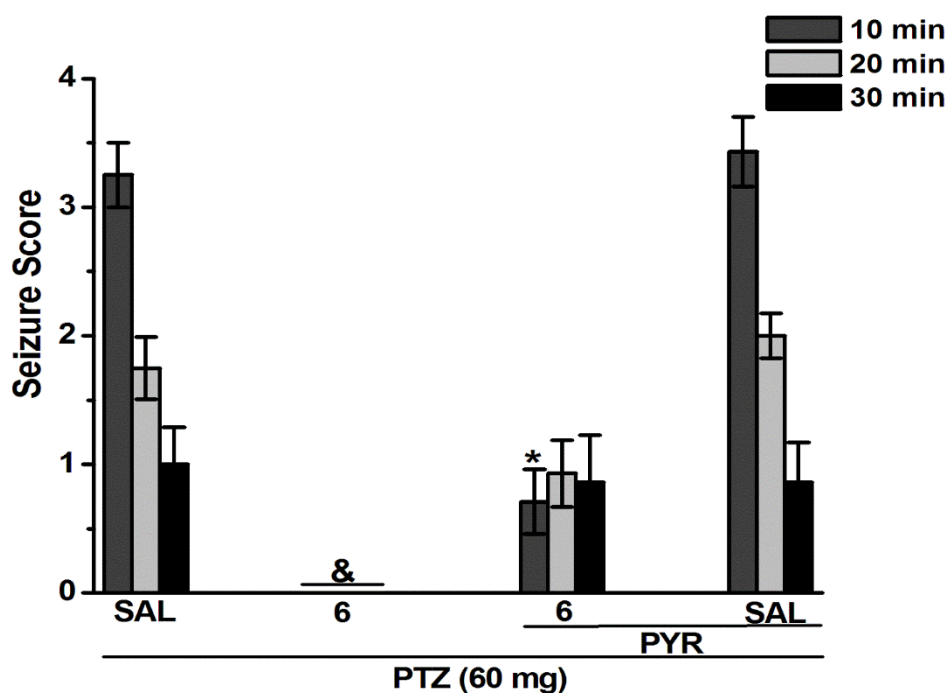


Figure 18: Effect of PYR pretreatment on the protection by H3R antagonist **6** against pentelenetetrazole (PTZ)-induced seizures in rats. Saline, H3R antagonist **6** alone, H3R antagonist **6** (10 mg/kg) + PYR (10 mg/kg) or saline + PYR (10 mg/kg) were administered 30 min before PTZ injection. Effects shown are expressed as score of seizures after 10 min, 20 min and 30 min of PTZ injection. Data are expressed as mean  $\pm$  SEM (n=7). \* $P < 0.05$  for seizure scores vs. the saline-treated group. &Full protection.

#### 4.7 Effect of H3R antagonists 1-12 on STR-induced seizures

Figure 19 shows the effects of H3R ligands **1-12** (10 mg/kg, i.p.) and the standard H3R antagonist PIT in comparison to the protective effects of reference antiepileptic drug VPA in STR-induced seizures in rats. In STR-induced seizure, H3R ligands **8** and **11** provided significant protective effects relative to the saline-

treated group after 10, 20, and 30 min of observation [ $P < 0.05$ ]. The results showed that H3R ligand **8** provided significant protection when compared with saline-treated group after 10, 20, and 30 with [ $F_{(1,12)} = 24.08$ ;  $P < 0.05$ ], [ $F_{(1,12)} = 40.33$ ;  $P < 0.05$ ], and [ $F_{(1,12)} = 20.74$ ;  $P < 0.05$ ], respectively.

Also, H3R antagonist **11** (10 mg/kg, i.p.) provided significant protective effect relative to the saline-treated after 10, 20, and 30 min of observation [ $F_{(1,12)} = 12.10$ ;  $P < 0.05$ ,  $F_{(1,12)} = 5.113$ ;  $P < 0.05$ ,  $F_{(1,12)} = 19.09$ ;  $P < 0.05$ , respectively. Moreover, the standard H3R ligand PIT (10 mg/kg, i.p.) failed to provide protection in STR-induced seizure model after 10, 20, and 30 min [ $F_{(1,12)} = 0, 3.32$ , and  $3.50$ ; all NS], respectively. However, VPA (VPA 300 mg/kg, i.p.) provided significant protection when compared with saline-treated group after 10, 20, and 30 with [ $F_{(1,12)} = 41.82$ ;  $P < 0.05$ ], [ $F_{(1,12)} = 144.15$ ;  $P < 0.05$ ], and [ $F_{(1,12)} = 81.82$ ;  $P < 0.05$ ], respectively.

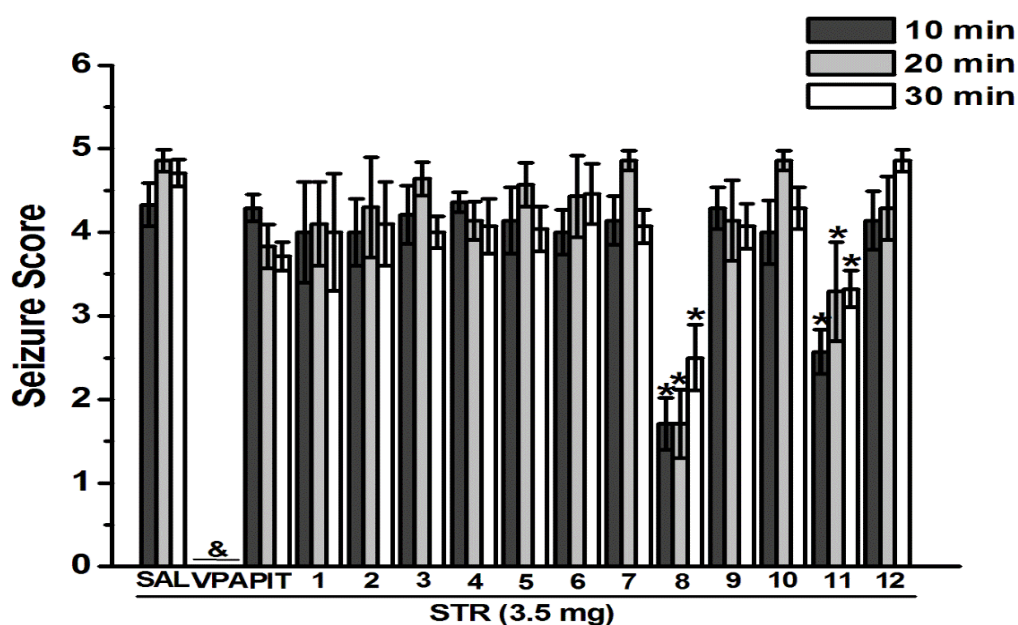


Figure 19: Protective effect of pretreatment with H3R antagonists **1-12** on strychnine (STR)-induced seizures in rats. Valproic acid (VPA, 300 mg/kg, i.p.) as a reference AED in STR-induced seizure (Serdiuk et al., 2014), 10 mg of pitolisant (PIT), and 10 mg of the respective H3R antagonist **1-12** were injected i.p. 30 min before STR (3.5 mg/kg, i.p.) treatments. Effects shown are expressed as score of seizures after 10 min, 20 min and 30 min of STR injection. Data are expressed as the mean  $\pm$  SEM (n=7). \* $P < 0.05$  vs. the saline treated group. &#x26A0;Full protection.

#### 4.8 Effects of H3R antagonist 4 on acquisition

Figure 20 shows no significant effects of H3R antagonist 4 (5 and 10 mg/kg, i.p.), PIT (10 mg/kg, i.p.), and DOZ (1 mg/kg, i.p.) on memory acquisition in the one trial inhibitory avoidance task [ $F_{(5,40)} = 1.75$ ;  $P=0.14$ ]. However, only rats pretreated

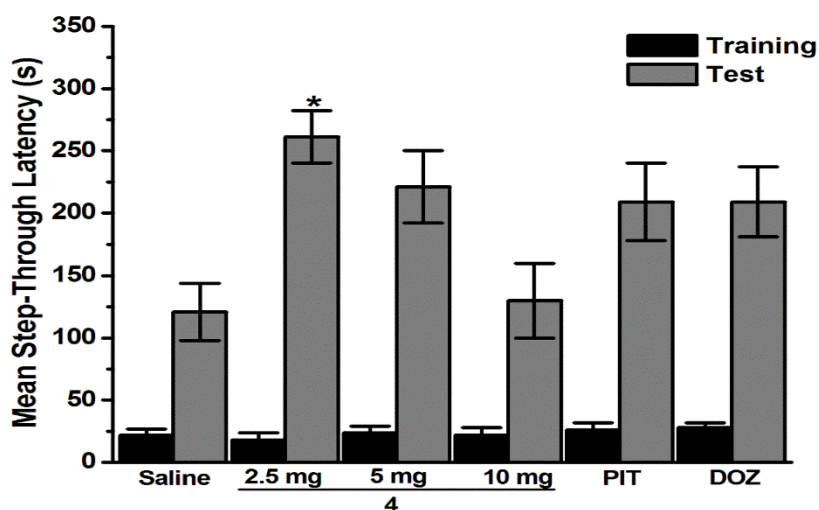


Figure 20: Effect of pre-training administration of H3R antagonist 4 on acquisition in an inhibitory avoidance conditioned response in adult male rats. Gray columns represent the mean step-through latencies measured during the retention test (test latencies) and black columns the mean step-through latencies measured during the training trial before the delivery of the foot-shock (pre-shock latencies). Rats were injected with H3R antagonist 4 (2.5, 5 or 10 mg/kg, i.p.), PIT (10 mg/kg, i.p.), or donepezil (DOZ, 1 mg/kg, i.p.) 30 min before the training session. Memory retention was measured 24 h after training. \* $P < 0.005$  for mean step-through latencies vs. the value of the saline group. Data are expressed as mean  $\pm$  SEM ( $n=7$ ).

in the pre-training session with H3R antagonist 4 (2.5 mg/kg, i.p.) showed a significant effect on step-through latencies when compared with the saline-treated with [ $F_{(1,12)} = 12.09$ ;  $P < 0.05$ ]. Surprisingly, higher doses of H3R ligand 4 (5 and 10 mg/kg, i.p.), PIT (10 mg/kg, i.p.), and DOZ (1 mg/kg, i.p.) failed to improve performance of rats treated in the pretraining session with [ $F_{(1,13)} = 4.26$ ,  $F_{(1,13)} = 0.04$ ,  $F_{(1,12)} = 3.46$ , and  $F_{(1,14)} = 3.87$ ; all NS], respectively.

#### 4.9 Effect of PYR and ZOL on the H3R antagonist **4**-provided memory improvement of acquisition in an inhibitory avoidance conditioned response

Figure 21 shows the results of the experiment testing whether the H3R antagonist **4** (2.5 mg/kg)-induced improvement of acquisition was mediated through histaminergic neurotransmission. For this purpose, a group of rats ( $n=7$  for each

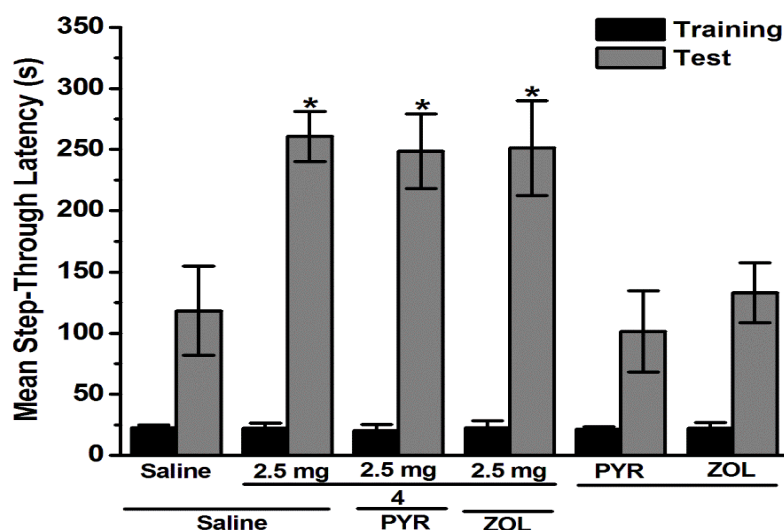


Figure 21: Effect of PYR and ZOL on acquisition improvement of H3R antagonist **4** in an inhibitory avoidance conditioned response in rats. Gray columns represent the mean STLs measured during the retention test (test latencies) and black columns the mean STLs measured during the training trial before the delivery of the foot-shock (pre-shock latencies). Rats were injected with Saline + **4** (2.5 mg/kg), **4** (2.5 mg/kg) + PYR (10 mg/kg), **4** (2.5 mg/kg) + ZOL (10 mg/kg), Saline + PYR (10 mg/kg), or Saline + ZOL (10 mg/kg) 30 min before the test session. \* $P < 0.05$  for mean STLs vs. the value of the (Saline)-treated group. Data are expressed as mean  $\pm$  SEM ( $n=7$ ).

group) was injected with Saline + **4** (2.5 mg/kg), Saline + **4** (2.5 mg/kg) + PYR (10 mg/kg), or **4** (2.5 mg/kg) + ZOL (10 mg/kg) 30 min before the test session. As depicted in Figure 21, one-way ANOVA repeated measure with Treatment as the between-subject factor indicated a significant effect of this factor on latency time [ $F_{(5,36)} = 5.13$ ;  $P < 0.05$ ]. Pairwise comparisons reported that acute systemic injection of **4** (10 mg/kg) improved STL time when compared to the (Saline)-treated group [ $F_{(1,12)} = 9.90$ ;  $P < 0.05$ ]. This **4**-induced improvement of latency time was not

reversed following ZOL (10 mg/kg) injection [ $F_{(1,12)} = 0.04$ ;  $p=0.84$ : Saline + **4** vs. **4** + ZOL]. Also, systemic injection of PYR (10 mg/kg) failed to reverse the **4**-induced improvement of latency time when compared to the **4** (10 mg/kg)-treated group with [ $F_{(1,12)} = 1.08$ ;  $P=0.32$ ]. Also, neither Saline + Saline vs. PYR + Saline, nor Saline + Saline vs. ZOL + Saline differences were significant ( $p=0.77$  and  $p=0.76$ , respectively).

#### 4.10 Effects of H3R antagonist **4** on consolidation

Figure 22 shows the effects of H3R antagonist **4** (2.5, 5, and 10 mg/kg, i.p.), PIT (10 mg/kg, i.p.), and DOZ (1 mg/kg, i.p.) on memory consolidation in the one-trial inhibitory avoidance task. One-way analysis of variance showed that treatment

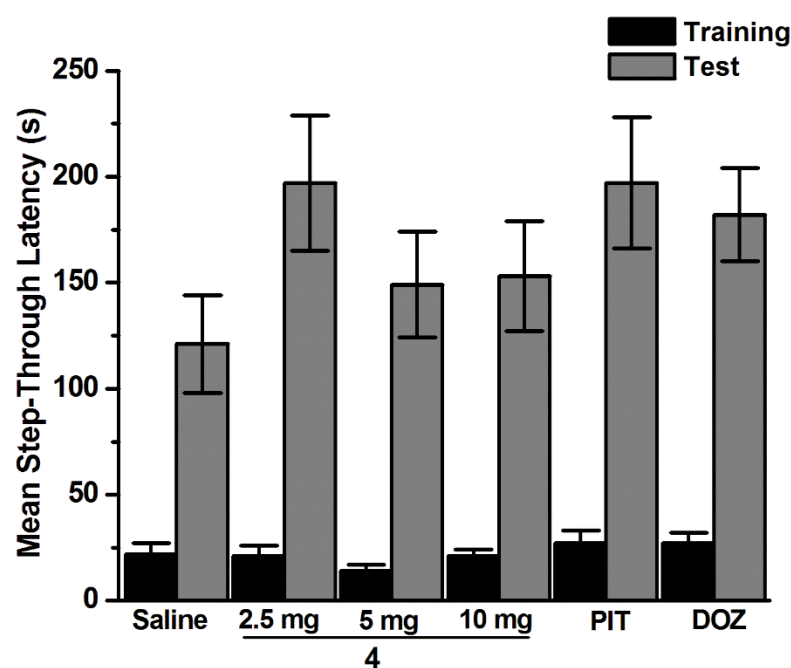


Figure 22: Effect of post-training administration of H3R antagonist **4** on consolidation in an inhibitory avoidance conditioned response in male adult rats. Gray columns represent the mean step-through latencies measured during the retention test (test latencies) and black columns the mean step-through latencies measured during the training trial before the delivery of the foot-shock (pre-shock latencies). Rats were injected with **4** (2.5, 5 or 10 mg/kg, i.p.), PIT (10 mg/kg, i.p.), or donepezil (DOZ, 1 mg/kg, i.p.) immediately after the training trial. Memory retention was measured 24 h after training. Data are expressed as mean  $\pm$  SEM ( $n=7$ ).

with PIT (10 mg/kg, i.p.), DOZ (1 mg/kg, i.p.), and H3R antagonist **4** (2.5, 5, and 10 mg/kg, i.p.) injected immediately after the training session, did not enhance the effects on step-through latencies [ $F_{(5,41)} = 0.50$ ;  $P=0.78$ ] measured 24 h later. Among the compounds tested, rats pretreated with **4** (2.5, 5, and 10 mg/kg, i.p.), PIT (10 mg/kg, i.p.), and DOZ (1 mg/kg, i. p.) showed no significant effect on mean step-through latencies when compared with the saline-treated group with [ $F_{(1,13)} = 2.51$ ,  $F_{(1,13)} = 0.43$ , and  $F_{(1,13)} = 0.57$ ,  $F_{(1,14)} = 2.54$ , and  $F_{(1,12)} = 2.57$ ; all NS], respectively.

#### **4.11 Effects of H3R antagonist 4 on retrieval**

Figure 23 displays the effects of H3R ligand **4** (2.5, 5, and 10 mg/kg, i.p.), PIT (10 mg/kg, i.p.), and DOZ (1 mg/kg, i.p.) on the retrieval process. One-way analysis of variance showed that treatment **4** (5 and 10 mg/kg, i.p.) and DOZ (1 mg/kg, i.p.) injected before the retention test exerted a significant effect on step-through latencies with [ $F_{(1,12)} = 5.11$ ,  $F_{(1,12)} = 9.08$ , and  $F_{(1,15)} = 2.59$ ; all  $P < 0.05$ ], respectively. However, the step-through latencies of rats injected with **4** (2.5 mg/kg, i.p.), and 10 mg/kg PIT were not significantly different from saline-treated with [ $F_{(1,12)} = 3.46$ ;  $p=0.09$ ] and [ $F_{(1,14)} = 0.19$ ;  $p=0.67$ ], respectively, all NS.

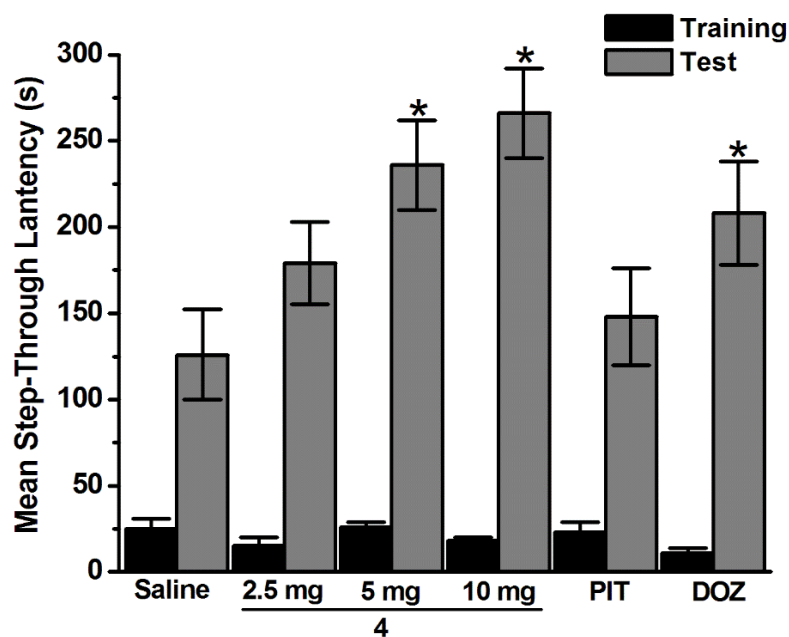


Figure 23: Effect of pre-retrieval administration of H3R antagonist **4** on retrieval in an inhibitory avoidance conditioned response in male adult rats. Gray columns represent the mean step-through latencies measured during the retention test (test latencies) and black columns the mean step-through latencies measured during the training trial before the delivery of the foot-shock (pre-shock latencies). Rats were injected with **4** (2.5, 5 or 10 mg/kg, i.p.), PIT (10 mg/kg, i.p.), or donepezil (DOZ, 1 mg/kg, i.p.) 30 min before the retention test. \* $P < 0.05$  for mean step-through latencies vs. the value of the saline group. Data are expressed as mean  $\pm$  SEM (n=7).

#### 4.12 Effect of PYR and ZOL on the H3R antagonist **4**-provided memory

##### improvement of retrieval in an inhibitory avoidance conditioned response

Figure 24 shows the results of the experiment in which it was tested whether the H3R antagonist **4** (10 mg/kg)-induced improvement of retrieval was mediated through histaminergic neurotransmission. For this purpose, a group of rats (n=7 for each group) was injected with Saline + **4** (10 mg/kg), with Saline + **4** (10 mg/kg) + PYR (10 mg/kg), or with **4** (10 mg/kg) + ZOL (10 mg/kg) 30 min before the test session.

As depicted in Figure 24, one-way ANOVA repeated measure with treatment as the between-subject factor indicated a significant effect of this factor indicated a



significant effect of this factor on latency time [ $F_{(5,36)} = 4.42$ ;  $P < 0.05$ ]. Pairwise comparisons reported that acute systemic injection of **4** (10 mg/kg) improved STL time when compared to the (Saline)-treated group [ $F_{(1,12)} = 7.86$ ;  $P < 0.05$ ]. This **4**-

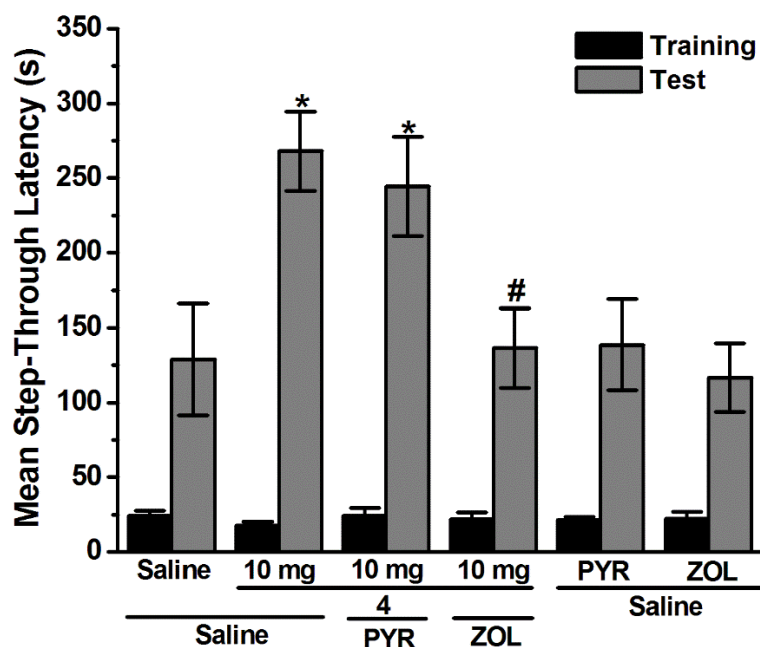


Figure 24: Effect of PYR and ZOL on retrieval improvement of H3R antagonist **4** in an inhibitory avoidance conditioned response in rats. Gray columns represent the mean STLs measured during the retention test (test latencies) and black columns the mean STLs measured during the training trial before the delivery of the foot-shock (pre-shock latencies). Rats were injected with Saline + **4** (10 mg/kg), **4** (10 mg/kg) + PYR (10 mg/kg), **4** (10 mg/kg) + ZOL (10 mg/kg), Saline + PYR (10 mg/kg), or Saline + ZOL (10 mg/kg) 30 min before the test session. \* $P < 0.05$  for mean STLs vs. the value of the (Saline)-treated group. # $P < 0.05$  for mean STLs of **4** (10 mg/kg) + ZOL (10 mg/kg)-treated group vs. the value of the Saline + **4** (10 mg/kg)-treated group. Data are expressed as mean  $\pm$  SEM ( $n=7$ ).

induced improvement of latency time was reversed following ZOL (10 mg/kg) injection [ $F_{(1,12)} = 0.02$ ;  $p=0.88$ : Saline + Saline vs. **4** + ZOL]. However, systemic injection of PYR (10 mg/kg) failed to reverse the **4**-induced improvement of latency time when compared to the **4** (10 mg/kg)-treated group with [ $F_{(1,12)} = 0.19$ ;  $p=0.67$ ]. Also, neither Saline + Saline vs. PYR + Saline, nor Saline + Saline vs. ZOL + Saline differences were significant ( $p=0.85$  and  $p=0.80$ , respectively).

### 4.13 Effects of H3R antagonist 4 on locomotor activity and anxiety levels

We used the OFT to rule out possible intrinsic impairment of spontaneous locomotor activity or anxiety measures. Locomotor activity and anxiety levels were measured by the number of line crossings in the arena and time spent in the center or periphery in the arena. One-way analysis of variance showed that, compared with vehicle, H3R antagonist 4 (2.5, 5, and 10 mg/kg, i.p.), PIT (10 mg/kg, i.p.), and DOZ (1 mg/kg, i.p.) had no effect on number of line crossings, [ $F_{(5,36)} = 0.60$ ,  $p=0.70$ ] (Figure 25A). Similarly, no significant treatment differences were found with regard

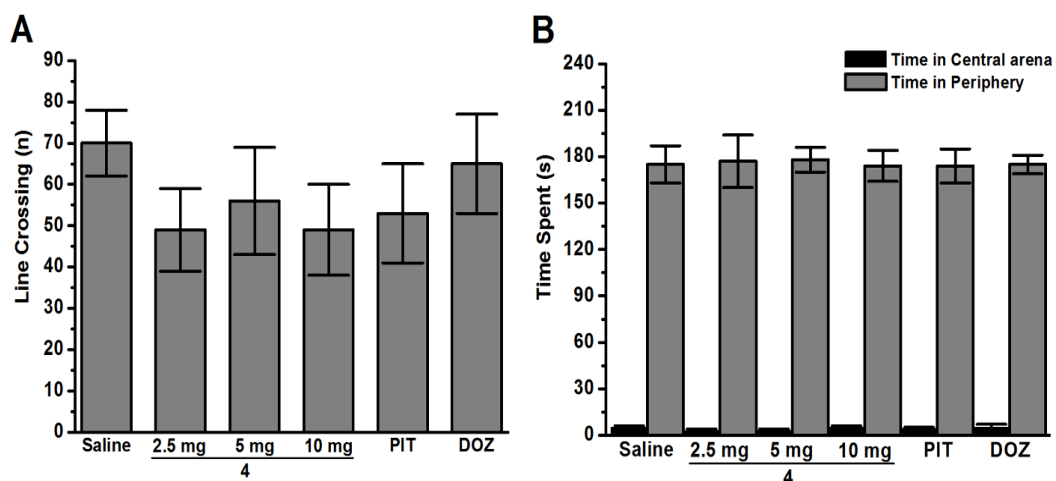


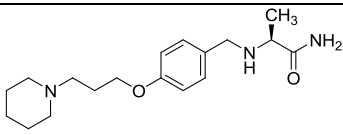
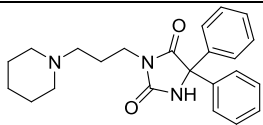
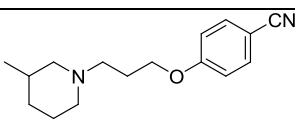
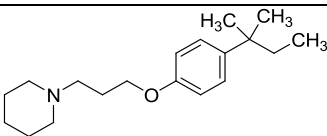
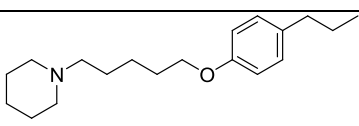
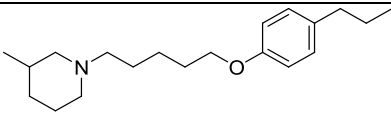
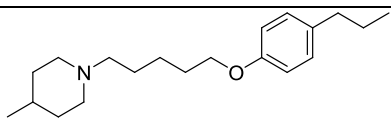
Figure 25: Number of line crossing (A) and time spent in center and periphery (B) in total area of an open field following acute systemic administration of H3R antagonist 4. In the open field test, H3R antagonist 4 (2.5, 5 or 10 mg/kg, i.p.) had no effect on number of line crossing (A) and time spent in center or periphery (B). Data are expressed as mean  $\pm$  SEM (n=7).

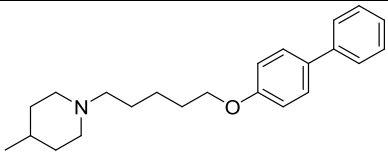
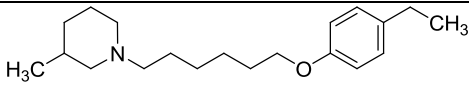
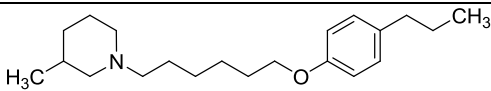
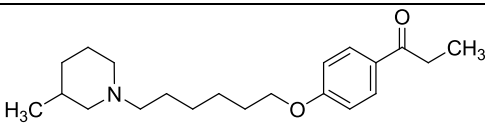
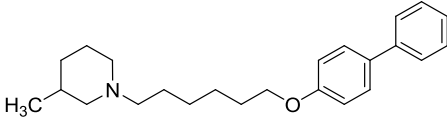
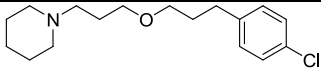
to time spent in the central area or periphery (i.e. level of anxiety) with [ $F_{(5,36)} = 0.85$ ,  $p=0.52$ ] and [ $F_{(5,36)} = 0.02$ ,  $p=0.99$ ], respectively (Figure 25B).

## Chapter 5: Discussion

Examined compounds **1-12** differed in their chemical structures in the linker or spacer lengths (Figure 7) being three, five, or six methylene groups as well as in the lipophilic substitution being various groups of known AEDs (Table 3). All compounds chosen for the current study have, as previously confirmed, high *in vitro* hH3R affinities with observed  $pK_i$  values in the range 6.22 to 8.08 (Table 3).

Table 3: Chemical structures and *in vitro* antagonist affinities of test compounds **1-12** for human histamine H3Rs stably expressed in CHO-K1 cells.

Compound	Structure	MWt <sup>a</sup> [g/mol]	hH3R $pK_i^b$
1		499.50*	7.37
2		467.25	6.25
3		348.41	6.46
4		379.51	8.08
5		379.49	7.32
6		393.52	7.42
7		393.52	6.81

Compound	Structure	MWt <sup>a</sup> [g/mol]	hH3R pK <sub>i</sub> <sup>b</sup>
<b>8</b>		427.53	7.06
<b>9</b>		393.52	7.13
<b>10</b>		407.54	7.07
<b>11</b>		421.53	7.41
<b>12</b>		441.56	6.83
<b>Pitolisant</b>		385.21	7.57

<sup>a</sup>Molecular weight calculated as hydrogen oxalate. <sup>b</sup>[<sup>125</sup>I]Iodoproxyfan binding assay at human H3R stably expressed in CHO-K1 cells, n = 3. \*Molecular weight calculated as dioxalate.

In the current study, all experiments were conducted with PHT (10 mg/kg, i.p), VPA (100 mg/kg, i.p.), or (300 mg/kg, i.p.) in MES-, PTZ- and STR-induced seizure models, respectively. These were the minimal doses of PHT and VPA that protected animals against MES-, PTZ-, and STR-induced seizures without mortality. The test compounds (**1-12**) varied in their level of protection in the three seizure models used in the current study. In MES-induced experiments, H3R antagonists **4**, **8**, **10**, **11**, and **12** showed the best protection against MES-induced (Figure 13) convulsion in rats pretreated with 10 mg/kg, i.p. when compared with the saline-treated group. Accordingly, the anticonvulsant effects obtained were expressed in the order of increasing *in vivo* potency (**1** ~ **2** ~ **3** ~ **5** ~ **6** ~ **7** ~ **9** - group I) < (**7** ~ **10** ~

**11** ~ PIT – group II) < (**4** - group III), whereas values for *in-vitro* H3R affinity measured with  $pK_i$  values were **2** < **3** < **7** < **12** < **8** < **10** < **9** < **5** < **1** < **11** < **6** < PIT < **4**. It was found that the introduction of a three methylene-spacer together with the *tert*-pentyl moiety significantly increased the lipophilic properties as observed for H3R antagonist **4** (c.f. compound **4** vs. **1-3** and **5-12**). Such an effect was, also, beneficial for the protective *in-vivo* anticonvulsant effect of H3R antagonist **4** was comparable to that observed for the standard H3R antagonist PIT. Interestingly, PIT and H3R antagonist **4** incorporate the same structural pharmacophores, namely a piperidinyloxy moiety, known to be responsible for H3R antagonist affinity (Figure 26).

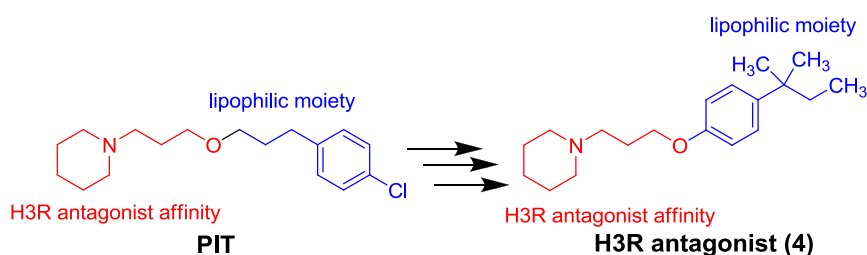


Figure 26: Structural similarities (red) and differences (blue) between H3R antagonist **4** and the standard H3R antagonist PIT.

In contrast, introduction of longer alkyl spacers (5 or 6 methylene groups), e.g. H3R antagonists **5-12**, failed to further increase *in vitro* antagonist affinity for hH3Rs as well as to provide higher *in vivo* protective effects in MES-induced seizure model. Nonetheless, it may be concluded that H3R ligands with the piperidinyloxy moiety (e. g. PIT and H3R antagonist **4**) as a common structural feature showed the best protection among the compounds **1-12**.

The results observed in the MES model indicated that H3R antagonist **4** offered the best protection against MES-induced convulsions when rats were pretreated with 10 and 15 mg/kg i.p., as compared with standard H3R antagonist PIT

(10 mg/kg, i.p.), the saline-treated group, and the H3R antagonist **4**-treated group at a dose of 5 mg/kg, i.p (Figure 14). Accordingly, the observed results indicated a dose-response relationship of the protection observed and the presence of a ceiling for H3R antagonist **4** in MES-induced seizures. Notably, the protective effect observed for H3R antagonist **4** (10 or 15 mg/kg, i.p.) was comparable to that observed in the group pretreated with the reference drug PHT (10 mg/kg, i.p.). Interestingly, these results are in agreement with recent studies that described the dose-dependent anticonvulsant effect of H3R antagonists in MES-induced seizures in animal models and PIT in a photosensitivity seizure model in adult patients (Kasteleijn-Nolst Trenite et al., 2013; Sadek et al., 2014a, 2014b, 2013). Further experiments showed that the protective effect of H3R antagonist **4** was reversed when rats were pretreated with 10 mg/kg i.p., of the CNS penetrant histamine H1R antagonist PYR 30 min, or with the selective H3R agonist *R*-( $\alpha$ )-methyl-histamine (RAMH) (10 mg/kg, i.p.) 15 min before MES challenge (Figure 15). Importantly, PYR (10 mg/kg) and RAMH (10 mg/kg) administered alone did not show either a protective or an epileptogenic effect in rats challenged by the MES-induced seizure. These findings suggest that the protective action of H3R antagonist **4** in the MES-induced seizure is mediated, at least partially, through blockade of H3Rs and interactions of released histamine with postsynaptically located histamine H1Rs, and these findings are in agreement with the previously observed protective effects for H3R antagonists (Kakinoki et al., 1998; Sadek et al., 2014a). H3Rs are auto-receptors located on presynaptic histaminergic terminals with an inhibitory action on the synthesis and release of histamine (Arrang et al., 1983). Blockade of these receptors by selective H3R antagonists, such as compound **4**, would lead to an enhanced neuronal histamine release in the brain, resulting in an anticonvulsant effect. Similar effects of

imidazole-based as well as non-imidazole-based H3R ligands have been reported previously to be reversed either by H3R agonists or by H1R antagonists, but not by H2R antagonists, signifying an interaction of the H3R antagonism-released histamine with H1Rs on postsynaptic neurons (Bhowmik et al., 2012; Kakinoki et al., 1998; Sadek et al., 2014a, 2014b, 2013).

H3R antagonist **4**, on the other hand, failed to offer significant protection against chemically (PTZ or STR)-induced seizures (Figure 16 and 19) Notably, PIT (10 mg/kg i.p.) had no significant effect in chemically-induced seizures. This could be due to differences in the mechanism or type of seizures in each model. Accordingly, MES is a model of generalized tonic-clonic seizures (Loscher et al., 1991a), while PTZ (60 mg/kg, i.p.) induces generalized myoclonic and/or tonic-clonic seizures (Loscher et al., 1991b). Moreover, electrically-induced seizures result from stimulation of all neuronal pathways and the insult only lasts for a short time (one second). However, in chemically-induced seizures, the trigger remains until the elimination of the proconvulsant, e.g. PTZ or STR. Furthermore, chemical agents affect only some neuronal pathways, e.g. PTZ provides blockage of t-butylbicyclophosphorothionate (TBPS) site at GABAA receptors (Olsen, 1981) and reduces GABA neurotransmission (Corda et al., 1991). It is noteworthy that most marketed antiepileptic drugs were not effective in all seizure models. For instance, carbamazepine, oxcarbazepine and PHT are highly effective in the MES-induced model, however, they lack any protective effects against seizures induced by PTZ, STR or picrotoxin (Swinyard et al., 1986, White, 1999). In contrast, ethosuximide and tiagabine, which provide high protection in chemically-induced seizure models, are inactive in the MES-induced model when used at nontoxic doses (White, 1999). These characteristic preclinical activities explain the effectiveness of PHT,

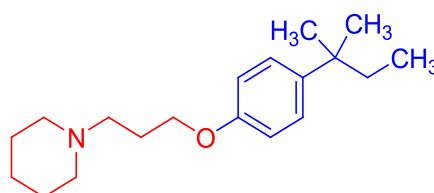
carbamazepine and oxcarbazepine but not ethosuximide or tiagabine in patients with generalized tonic-clonic seizures. Similarly, H3R antagonist **6** that has no pronounced effect in MES test or STR-induced seizures dose dependently suppressed PTZ-induced seizures (Figure 17). Notably, H3R antagonist **6** completely abolished seizures after acute systemic pretreatment with 10 and 15 mg/kg, and the protection observed was similar to that provided by the standard AED VPA 100 mg/kg. Interestingly, the protection observed for H3R antagonist **6** in PTZ-induced seizure model was partially reversed (Figure 18) upon acute systemic co-administration of PYR indicating that part of this effect was H1R-dependent. Other neurotransmitters like GABA may be involved in the protective mechanism. It can, also, be hypothesized that H3R antagonists could also block histamine H3 heteroreceptors that control the release of other neurotransmitters including GABA (Brown et al., 2001, Haas et al., 2008). In a previous study the H3R antagonist clobenpropit was found to increase GABA release and to protect against NMDA-induced excitotoxicity in rat cultured cortical neurons (Dai et al., 2007a).

In a further experiment, H3R antagonists **8** and **11** offered some protection in the STR-induced seizure model (Figure 19), however, not as effective as standard AED VPA (300 mg/kg, i.p). It is well known that STR is a competitive antagonist of the inhibitory amino acid glycine. Consequently, the inability of H3R antagonist **4** to protect against STR-induced seizure suggests little or no effect on the glycine receptors, since the mechanism underlying STR-induced convulsions is thought to be attributed to its blocking effect on glycine receptors in brain as well as in spinal cord (Sowemimo et al., 2011).

Several AEDs negatively influence memory (Hermann et al., 2010). Therefore, it is essential to probe such troublesome side effects in novel antiepileptic



treatment. Histamine, agents that increase brain histamine (e.g. L-histidine), and H3R antagonists have been found to improve different measures of cognition in a plethora of preclinical behavioral studies (Table 1) (reviewed by Esbenshade et al., 2008, Zlomuzica et al., 2015). Increment of wakefulness and cortical fast rhythms are aspects of cognition that have been approved with PIT in the clinics (Dauvilliers et al., 2013, Kasteleijn-Nolst Trenite et al., 2013). Several H3R antagonists including PIT are currently under clinical investigation for their efficacy in the treatment of cognitive disorders (Brioni et al., 2011, Zlomuzica et al., 2015). Since the antiepileptic effect observed for H3R antagonist **4** in the MES model depends on its histamine neurotransmission modulating effect, the question remained whether such central histamine modulation has any impact on memory processes in the same animal species.



**H3R antagonist (4)**

$ED_{50}^a = 2.1 \pm 0.2$  mg/kg, p.o.;

$pK_i(\text{hH3R})^b = 8.08$ ;  $pK_i(\text{hH4R})^c = 4.31$ ;  $pK_i(\text{hH1R})^d = 6.18$ ;  $pK_i(\alpha 2R)^e = 2.10$

Figure 27: Chemical structure, *in vitro* affinities, and *in vivo* potency of H3R antagonist **4**. <sup>a</sup>Central histamine H3R assay *in vivo* after p.o. administration to mice, n=3 (Garbarg et al., 1989, Garbarg et al., 1992, Ligneau et al., 1998, Lazewska et al., 2006). <sup>b</sup>[<sup>125</sup>I]Iodoproxyfan binding assay at human H3R stably expressed in CHO-K1 cells, n=3 (Ligneau et al., 1994, Ligneau et al., 2000, Lazewska et al., 2006). <sup>c</sup>[<sup>3</sup>H]Histamine binding assay performed with cell membrane preparation of Sf9 cells transiently expressing the human histamine H4R and co-expressed with G<sub>ai2</sub> and β<sub>1γ2</sub> subunits n=3 (Meier et al., 2001, Amon et al., 2007, Isensee et al., 2009, Tomasch et al., 2013). <sup>d</sup>[<sup>3</sup>H]Pyrilamine binding assay performed with cell membrane preparation of CHO-hH1R cells stably expressing the human H1R; n=3 (Schibli and Schubiger, 2002, van Staveren and Metzler-Nolte, 2004). <sup>e</sup>The affinity for α(2)-adrenoceptors was evaluated by radioligand binding assays to rats cortex membrane using [<sup>3</sup>H]clonidine (Barbaro et al., 2002, Betti et al., 2002, Handzlik et al., 2008).

Therefore, the non-imidazole-based H3R antagonist **4** with high H3R *in vitro* affinity, excellent *in vitro* selectivity profile, and *in vivo* antagonist potency (Figure 27) has in the current project been investigated for its behavioral effects on acquisition, consolidation and retrieval processes in a one-trial inhibitory avoidance paradigm in rats. Given that motor activity could mask the effects of H3R antagonist **4** on learning and memory, we also used an open field test (OFT) to evaluate activity and anxiety in the same animals. Memory is often considered to be a process that has several stages including acquisition, consolidation, and retrieval (Abel and Lattal, 2001). Usually, tasks that have been designed to modulate memory processes consist of a training session followed, after 24 or more hours, by a test session. In the present study, acute systemic administration with 2.5 mg/kg of H3R antagonist **4** enhanced memory acquisition when compared with the saline-treated group. However, higher doses (5 and 10 mg/kg, i.p.) of H3R antagonist **4** did not affect acquisition, indicating that the effect is dose-dependent (Figure 20). The observed dose-dependent facilitating effect of H3R antagonist **4** on acquisition is in agreement with a previous study in which the reference H3R antagonist GT-2331 showed dose-dependent memory-enhancing effects on repeated acquisition in an inhibitory avoidance paradigm (Fox et al., 2002). In their study, rats treated with GT-2331 (1 mg/kg, i.p.) performed significantly better than those injected with a higher dose (30 mg/kg, i.p.). Nonetheless, further behavioral tests should be conducted to investigate whether the improving effect of H3R antagonist **4** (2.5 mg/kg) on acquisition is mediated through neurotransmission other than histamine, since PYR and ZOL failed to reverse the effects of H3R antagonist **4** (Figure 21). In contrast, our results showed that acute systemic administration of H3R antagonist **4** in the post-training session failed to facilitate consolidation (Figure 22). These results are in disagreement with previous

findings highlighting the critical role of H3Rs in consolidation as a stage of memory processing (de Almeida and Izquierdo, 1986). Nonetheless, in the latter study different animal species and step-down inhibitory avoidance procedures were used which could explain the discrepancy between the studies (Bernaerts et al., 2004, Charlier et al., 2013). Our results also showed that systemic pre-testing administration of H3R antagonist **4** (5 and 10 mg/kg, i.p.) improved memory (Figure 23), which indicates that this compound when administered at higher doses facilitates dose-dependently the retrieval stage. These results for H3R antagonist **4** are in agreement with previous studies in which systemic administration of various imidazole-based H3R antagonists were found to enhance memory retrieval in a dose-dependent manner in an object recognition task in rats (Orsetti et al., 2002, Pascoli et al., 2009, Bardgett et al., 2010). Interestingly, the procognitive activity of H3R antagonist **4** (10 mg/kg, i.p.) on retrieval was in a another experiment completely reversed when rats were pretreated with the centrally acting H2R antagonist zolantidine (ZOL, 10 mg/kg, i.p.) but not the centrally acting H1R antagonist (PYR, 10 mg/kg, i.p.), indicating that histaminergic pathways through activation of H2Rs appear to be participating in neuronal circuits involved in the retrieval processes (Figure 24).

Importantly, the results observed in the current study for H3R antagonist **4** at doses of 2.5, 5, and 10 mg/kg, i.p., did not affect spontaneous locomotor activity and anxiety levels of male Wistar rats (Figure 25). This finding is important, since improved performance in an inhibitory avoidance task can be the result of numerous variables unrelated to memory acquisition such as alterations in spontaneous locomotor activity or emotional response (McGaugh and Roozendaal, 2009, Charlier et al., 2013). Therefore, it is unlikely that H3R antagonist **4** administered before the

conditioning session increased retention through a nonspecific effect during the training session.

## Chapter 6: Conclusion

In conclusion (Figure 28), acute systemic administration of novel and highly affine H<sub>3</sub>R antagonists (**1-12**) provided different protective effects against in a variety of rat seizure models. Through histaminergic-neurotransmission, H<sub>3</sub>R antagonist **4** dose-dependently displayed the greatest anticonvulsant activity in MES-induced seizures. However, H<sub>3</sub>R antagonists **6**, **8**, and **11** provided the highest protection in PTZ-, and STR-induced seizure models, respectively. Moreover, acute systemic administration of H<sub>3</sub>R antagonist **4** dose-dependently improved different aspects of memory, especially at the level of retrieval. These results enhance further

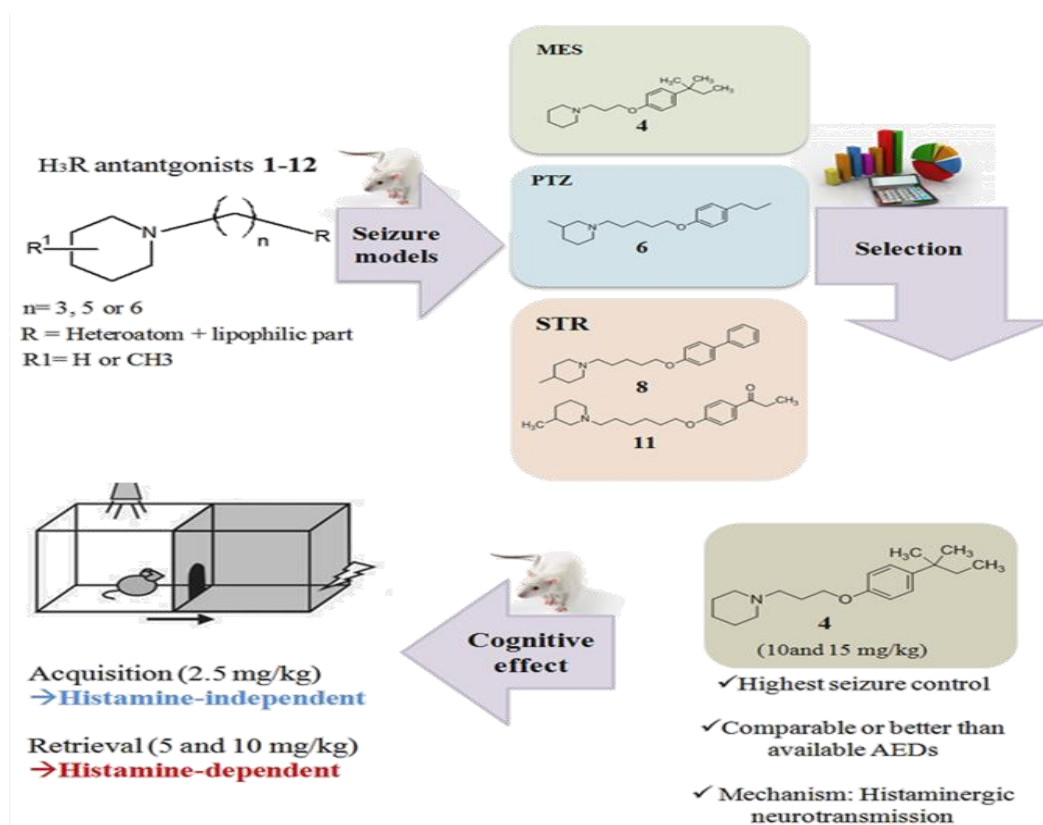


Figure 28: Schematic simplified illustration of the study including the most essential points regarding theory, objectives, experiments and concluding remarks.

the potential utility of histamine H3R antagonists in the treatment of epilepsy and other neuropsychiatric disorders typified by cognitive deficits.

### **6.1 Limitations of the study**

- No further experiment were conducted to explain other mechanisms by which H3R antagonist **6** protect against PTZ-induced seizures other than the partial contribution of H1 receptors. However, possible mechanisms can be deduced from the available literature with other H3R antagonists or histamine.
- No additional mechanistic experiments were done to understand the pathway by which H3R antagonist **4** improve acquisition at 2.5 mg/kg.
- Based on the main objectives of the study, only one compound of the tested H3R antagonists was selected depending on its seizure control and histamine-driven mechanism of action. The memory effect of other less effective anti-seizure H3R antagonist can be extrapolated or assumed from H3R antagonist **4** effects.
- Due to current unavailability of other optimized memory rat models in our laboratory, cognitive effects of H3R antagonist **4** was analyzed based on passive avoidance test only.

### **6.2 Future direction**

- Explore the behavioral activity of H3R antagonist **4** in other memory models for example spatial memory utilizing the Y-maze or the Place recognition test.
- Test H3R antagonist **4** or other effective compounds like **6**, **8** and **11** in other seizure models especially those that represent resistant forms of epilepsy to expand the possible therapeutic utility of these compounds and other H3R antagonists.

- Explore the effects of the most effective anticonvulsant H3R antagonists **1-12** against low-magnesium- or chemically-induced epileptiform discharges at CA1/CA3 regions of hippocampus at the level of brain slices with easier and reliable access of compounds. Through this method possible mechanisms of beneficial effects can be thoroughly investigated.
- Further analyze the mechanisms of anticonvulsant activity of H3R antagonists through patch-clamp or whole-cell recording in individual isolated CA1/CA3 neurons.

## Bibliography

- Abel T, Lattal KM (2001) Molecular mechanisms of memory acquisition, consolidation and retrieval. *Curr Opin Neurobiol* 11:180-187.
- Amon M, Ligneau X, Camelin JC, Berrebi-Bertrand I, Schwartz JC, Stark H (2007) Highly potent fluorescence-tagged nonimidazole histamine H3 receptor ligands. *ChemMedChem* 2:708-716.
- Arrang JM, Morisset S, Gbahou F (2007) Constitutive activity of the histamine H3 receptor. *Trends Pharmacol Sci* 28:350-357.
- Bakker RA, Wieland K, Timmerman H, Leurs R (2000) Constitutive activity of the histamine H(1) receptor reveals inverse agonism of histamine H(1) receptor antagonists. *Eur J Pharmacol* 387:R5-7.
- Barbaro R, Betti L, Botta M, Corelli F, Giannaccini G, Maccari L, Manetti F, Strappaghetti G, Corsano S (2002) Synthesis and biological activity of new 1,4-benzodioxan-arylpiperazine derivatives. Further validation of a pharmacophore model for alpha(1)-adrenoceptor antagonists. *Bioorg Med Chem* 10:361-369.
- Bardgett ME, Points M, Kleier J, Blankenship M, Griffith MS (2010) The H3 antagonist, ciproxifan, alleviates the memory impairment but enhances the motor effects of MK-801 (dizocilpine) in rats. *Neuropharmacology* 59:492-502.
- Berlin M, Boyce CW, Ruiz Mde L (2011) Histamine H3 receptor as a drug discovery target. *J Med Chem* 54:26-53.
- Bernaerts P, Lamberty Y, Tirelli E (2004) Histamine H3 antagonist thioperamide dose-dependently enhances memory consolidation and reverses amnesia induced by dizocilpine or scopolamine in a one-trial inhibitory avoidance task in mice. *Behav Brain Res* 154:211-219.
- Betti L, Botta M, Corelli F, Floridi M, Giannaccini G, Maccari L, Manetti F, Strappaghetti G, Tafi A, Corsano S (2002) Alpha(1)-adrenoceptor antagonists. 4. Pharmacophore-based design, synthesis, and biological evaluation of new imidazo-, benzimidazo-, and indoloarylpiperazine derivatives. *J Med Chem* 45:3603-3611.
- Bevins RA, Besheer J (2001) Individual differences in rat locomotor activity are diminished by nicotine through stimulation of central nicotinic acetylcholine receptors. *Physiol Behav* 72:237-244.



- Bhowmik M, Saini N, Vohora D (2014) Histamine H3 receptor antagonism by ABT-239 attenuates kainic acid induced excitotoxicity in mice. *Brain Res* 1581:129-140.
- Blandina P, Giorgetti M, Bartolini L, Cecchi M, Timmerman H, Leurs R, Pepeu G, Giovannini MG (1996) Inhibition of cortical acetylcholine release and cognitive performance by histamine H3 receptor activation in rats. *Br J Pharmacol* 119:1656-1664.
- Blandina P, Munari L, Provensi G, Passani MB (2012) Histamine neurons in the tuberomammillary nucleus: a whole center or distinct subpopulations? *Front Syst Neurosci* 6:33.
- Bongers G, Bakker RA, Leurs R (2007) Molecular aspects of the histamine H3 receptor. *Biochem Pharmacol* 73:1195-1204.
- Brabant C, Charlier Y, Tirelli E (2013) The histamine H(3)-receptor inverse agonist pitolisant improves fear memory in mice. *Behav Brain Res* 243:199-204.
- Branco Cdos S, Scola G, Rodrigues AD, Cesio V, Laprovitera M, Heinzen H, Dos Santos MT, Fank B, de Freitas SC, Coitinho AS, Salvador M (2013) Anticonvulsant, neuroprotective and behavioral effects of organic and conventional yerba mate (*Ilex paraguariensis* St. Hil.) on pentylenetetrazol-induced seizures in Wistar rats. *Brain Res Bull* 92:60-68.
- Brigo F, Ausserer H, Tezzon F, Nardone R (2013) When one plus one makes three: the quest for rational antiepileptic polytherapy with supraadditive anticonvulsant efficacy. *Epilepsy Behav* 27:439-442.
- Brioni JD, Esbenshade TA, Garrison TR, Bitner SR, Cowart MD (2011) Discovery of histamine H3 antagonists for the treatment of cognitive disorders and Alzheimer's disease. *J Pharmacol Exp Ther* 336:38-46.
- Brown RE, Stevens DR, Haas HL (2001) The physiology of brain histamine. *Prog Neurobiol* 63:637-672.
- Canonaco M, Madeo M, Alo R, Giusi G, Granata T, Carelli A, Canonaco A, Facciolo RM (2005) The histaminergic signaling system exerts a neuroprotective role against neurodegenerative-induced processes in the hamster. *J Pharmacol Exp Ther* 315:188-195.
- Charlier Y, Brabant C, Serrano ME, Lamberty Y, Tirelli E (2013) The prototypical histamine H3 receptor inverse agonist thioperamide improves multiple aspects of memory processing in an inhibitory avoidance task. *Behav Brain Res* 253:121-127.

- Chen Z, Kamei C (2000) Facilitating effects of histamine on spatial memory deficit induced by scopolamine in rats. *Acta Pharmacol Sin* 21:814-818.
- Chen Z, Li Z, Sakurai E, Izadi Mobarakeh J, Ohtsu H, Watanabe T, Watanabe T, Inuma K, Yanai K (2003) Chemical kindling induced by pentylenetetrazol in histamine H(1) receptor gene knockout mice (H(1)KO), histidine decarboxylase-deficient mice (HDC(-/-)) and mast cell-deficient W/W(v) mice. *Brain Res* 968:162-166.
- Chizh BA, Headley PM, Tzschentke TM (2001) NMDA receptor antagonists as analgesics: focus on the NR2B subtype. *Trends Pharmacol Sci* 22:636-642.
- Clapham J, Kilpatrick GJ (1994) Thioperamide, the selective histamine H3 receptor antagonist, attenuates stimulant-induced locomotor activity in the mouse. *Eur J Pharmacol* 259:107-114.
- Corda MG, Orlandi M, Lecca D, Carboni G, Frau V, Giorgi O (1991) Pentylenetetrazol-induced kindling in rats: effect of GABA function inhibitors. *Pharmacol Biochem Behav* 40:329-333.
- Dai H, Fu Q, Shen Y, Hu W, Zhang Z, Timmerman H, Leurs R, Chen Z (2007a) The histamine H3 receptor antagonist clobenpropit enhances GABA release to protect against NMDA-induced excitotoxicity through the cAMP/protein kinase A pathway in cultured cortical neurons. *Eur J Pharmacol* 563:117-123.
- Dai H, Kaneko K, Kato H, Fujii S, Jing Y, Xu A, Sakurai E, Kato M, Okamura N, Kuramasu A, Yanai K (2007b) Selective cognitive dysfunction in mice lacking histamine H1 and H2 receptors. *Neurosci Res* 57:306-313.
- Dauvilliers Y, Bassetti C, Lammers GJ, Arnulf I, Mayer G, Rodenbeck A, Lehert P, Ding CL, Lecomte JM, Schwartz JC, group HIs (2013) Pitolisant versus placebo or modafinil in patients with narcolepsy: a double-blind, randomised trial. *Lancet Neurol* 12:1068-1075.
- Day M, Pan JB, Buckley MJ, Cronin E, Hollingsworth PR, Hirst WD, Navarra R, Sullivan JP, Decker MW, Fox GB (2007) Differential effects of ciproxifan and nicotine on impulsivity and attention measures in the 5-choice serial reaction time test. *Biochem Pharmacol* 73:1123-1134.
- de Almeida MA, Izquierdo I (1986) Memory facilitation by histamine. *Arch Int Pharmacodyn Ther* 283:193-198.
- Dere E, Zlomuzica A, Viggiano D, Ruocco LA, Watanabe T, Sadile AG, Huston JP, De Souza-Silva MA (2008) Episodic-like and procedural memory impairments in histamine H1 Receptor knockout mice coincide with changes in acetylcholine esterase activity in the hippocampus and dopamine turnover in the cerebellum. *Neuroscience* 157:532-541.

- Drutel G, Peitsaro N, Karlstedt K, Wieland K, Smit MJ, Timmerman H, Panula P, Leurs R (2001) Identification of rat H3 receptor isoforms with different brain expression and signaling properties. *Mol Pharmacol* 59:1-8.
- Elger CE, Helmstaedter C, Kurthen M (2004) Chronic epilepsy and cognition. *Lancet Neurol* 3:663-672.
- Ericson H, Watanabe T, Kohler C (1987) Morphological analysis of the tuberomammillary nucleus in the rat brain: delineation of subgroups with antibody against L-histidine decarboxylase as a marker. *J Comp Neurol* 263:1-24.
- Esbenshade TA, Browman KE, Bitner RS, Strakhova M, Cowart MD, Brioni JD (2008) The histamine H3 receptor: an attractive target for the treatment of cognitive disorders. *Br J Pharmacol* 154:1166-1181.
- Fisher RS, van Emde Boas W, Blume W, Elger C, Genton P, Lee P, Engel J, Jr. (2005) Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia* 46:470-472.
- Flood JF, Cherkin A (1986) Scopolamine effects on memory retention in mice: a model of dementia? *Behav Neural Biol* 45:169-184.
- Fox GB, Esbenshade TA, Pan JB, Radek RJ, Krueger KM, Yao BB, Browman KE, Buckley MJ, Ballard ME, Komater VA, Miner H, Zhang M, Faghieh R, Rueter LE, Bitner RS, Drescher KU, Wetter J, Marsh K, Lemaire M, Porsolt RD, Bennani YL, Sullivan JP, Cowart MD, Decker MW, Hancock AA (2005) Pharmacological properties of ABT-239 [4-(2-{2-[(2R)-2-Methylpyrrolidinyl]ethyl}-benzofuran-5-yl)benzotrile]: II. Neurophysiological characterization and broad preclinical efficacy in cognition and schizophrenia of a potent and selective histamine H3 receptor antagonist. *J Pharmacol Exp Ther* 313:176-190.
- Fox GB, Pan JB, Esbenshade TA, Bennani YL, Black LA, Faghieh R, Hancock AA, Decker MW (2002) Effects of histamine H(3) receptor ligands GT-2331 and ciproxifan in a repeated acquisition avoidance response in the spontaneously hypertensive rat pup. *Behav Brain Res* 131:151-161.
- Garbarg M, Arrang JM, Rouleau A, Ligneau X, Tuong MD, Schwartz JC, Ganellin CR (1992) S-[2-(4-imidazolyl)ethyl]isothiourea, a highly specific and potent histamine H3 receptor agonist. *J Pharmacol Exp Ther* 263:304-310.
- Garbarg M, Barbin G, Bischoff S, Pollard H, Schwartz JC (1976) Dual localization of histamine in an ascending neuronal pathway and in non-neuronal cells

- evidenced by lesions in the lateral hypothalamic area. *Brain Res* 106:333-348.
- Garbarg M, Pollard H, Trung Tuong MD, Schwartz JC, Gros C (1989) Sensitive radioimmunoassays for histamine and tele-methylhistamine in the brain. *J Neurochem* 53:1724-1730.
- Gemkow MJ, Davenport AJ, Harich S, Ellenbroek BA, Cesura A, Hallett D (2009) The histamine H3 receptor as a therapeutic drug target for CNS disorders. *Drug Discov Today* 14:509-515.
- Ghi P, di Carlo G, Molinengo L (1998) Effects of thioperamide on locomotor activity and on memory processes. *Prog Neuropsychopharmacol Biol Psychiatry* 22:387-395.
- Gondard E, Anaclet C, Akaoka H, Guo RX, Zhang M, Buda C, Franco P, Kotani H, Lin JS (2013) Enhanced histaminergic neurotransmission and sleep-wake alterations, a study in histamine H3-receptor knock-out mice. *Neuropsychopharmacology* 38:1015-1031.
- Grove RA, Harrington CM, Mahler A, Beresford I, Maruff P, Lowy MT, Nicholls AP, Boardley RL, Berges AC, Nathan PJ, Horrigan JP (2014) A randomized, double-blind, placebo-controlled, 16-week study of the H3 receptor antagonist, GSK239512 as a monotherapy in subjects with mild-to-moderate Alzheimer's disease. *Curr Alzheimer Res* 11:47-58.
- Haas HL, Sergeeva OA, Selbach O (2008) Histamine in the nervous system. *Physiol Rev* 88:1183-1241.
- Hancock AA, Esbenshade TA, Krueger KM, Yao BB (2003) Genetic and pharmacological aspects of histamine H3 receptor heterogeneity. *Life Sci* 73:3043-3072.
- Handzlik J, Maciag D, Kubacka M, Mogilski S, Filipek B, Stadnicka K, Kiec-Kononowicz K (2008) Synthesis, alpha 1-adrenoceptor antagonist activity, and SAR study of novel arylpiperazine derivatives of phenytoin. *Bioorg Med Chem* 16:5982-5998.
- Hansen KB, Mullasseril P, Dawit S, Kurtkaya NL, Yuan H, Vance KM, Orr AG, Kvist T, Ogden KK, Le P, Vellano KM, Lewis I, Kurtkaya S, Du Y, Qui M, Murphy TJ, Snyder JP, Brauner-Osborne H, Traynelis SF (2010) Implementation of a fluorescence-based screening assay identifies histamine H3 receptor antagonists clobenpropit and iodophenpropit as subunit-selective N-methyl-D-aspartate receptor antagonists. *J Pharmacol Exp Ther* 333:650-662.

- Harada C, Hirai T, Fujii Y, Harusawa S, Kurihara T, Kamei C (2004) Intracerebroventricular administration of histamine H3 receptor antagonists decreases seizures in rat models of epilepsy. *Methods Find Exp Clin Pharmacol* 26:263-270.
- Hermann B, Meador KJ, Gaillard WD, Cramer JA (2010) Cognition across the lifespan: antiepileptic drugs, epilepsy, or both? *Epilepsy Behav* 17:1-5.
- Higuchi M, Yanai K, Okamura N, Meguro K, Arai H, Itoh M, Iwata R, Ido T, Watanabe T, Sasaki H (2000) Histamine H(1) receptors in patients with Alzheimer's disease assessed by positron emission tomography. *Neuroscience* 99:721-729.
- Hirai T, Okuma C, Harada C, Mio M, Ohtsu H, Watanabe T, Kamei C (2004) Development of amygdaloid kindling in histidine decarboxylase-deficient and histamine H1 receptor-deficient mice. *Epilepsia* 45:309-313.
- Hough LB, Rice FL (2011) H3 receptors and pain modulation: peripheral, spinal, and brain interactions. *J Pharmacol Exp Ther* 336:30-37.
- Huang YW, Chen Z, Hu WW, Zhang LS, Wu W, Ying LY, Wei EQ (2003) Facilitating effect of histamine on spatial memory deficits induced by dizocilpine as evaluated by 8-arm radial maze in SD rats. *Acta Pharmacol Sin* 24:1270-1276.
- Ichinose M, Belvisi MG, Barnes PJ (1990) Histamine H3-receptors inhibit neurogenic microvascular leakage in airways. *J Appl Physiol* (1985) 68:21-25.
- Imamura M, Seyedi N, Lander HM, Levi R (1995) Functional identification of histamine H3-receptors in the human heart. *Circ Res* 77:206-210.
- Inocente C, Arnulf I, Bastuji H, Thibault-Stoll A, Raoux A, Reimao R, Lin JS, Franco P (2012) Pitolisant, an inverse agonist of the histamine H3 receptor: an alternative stimulant for narcolepsy-cataplexy in teenagers with refractory sleepiness. *Clin Neuropharmacol* 35:55-60.
- Inoue I, Yanai K, Kitamura D, Taniuchi I, Kobayashi T, Niimura K, Watanabe T, Watanabe T (1996) Impaired locomotor activity and exploratory behavior in mice lacking histamine H1 receptors. *Proc Natl Acad Sci U S A* 93:13316-13320.
- Isensee K, Amon M, Garlapati A, Ligneau X, Camelin JC, Capet M, Schwartz JC, Stark H (2009) Fluorinated non-imidazole histamine H3 receptor antagonists. *Bioorg Med Chem Lett* 19:2172-2175.

- Ishizawa K, Chen Z, Okuma C, Sugimoto Y, Fujii Y, Kamei C (2000) Participation of GABAergic and histaminergic systems in inhibiting amygdaloid kindled seizures. *Jpn J Pharmacol* 82:48-53.
- Jia F, Kato M, Dai H, Xu A, Okuda T, Sakurai E, Okamura N, Lovenberg TW, Barbier A, Carruthers NI, Iinuma K, Yanai K (2006) Effects of histamine H<sub>3</sub> antagonists and donepezil on learning and mnemonic deficits induced by pentylenetetrazol kindling in weanling mice. *Neuropharmacology* 50:404-411.
- Kakinoki H, Ishizawa K, Fukunaga M, Fujii Y, Kamei C (1998) The effects of histamine H<sub>3</sub>-receptor antagonists on amygdaloid kindled seizures in rats. *Brain Res Bull* 46:461-465.
- Kamei C (2001) Involvement of central histamine in amygdaloid kindled seizures in rats. *Behav Brain Res* 124:243-250.
- Kamei C, Ohuchi M, Sugimoto Y, Okuma C (2000) Mechanism responsible for epileptogenic activity by first-generation H<sub>1</sub>-antagonists in rats. *Brain Res* 887:183-186.
- Kamei C, Okuma C (2001) [Role of central histamine in amygdaloid kindled seizures]. *Nihon Yakurigaku Zasshi* 117:329-334.
- Kasteleijn-Nolst Trenite D, Parain D, Genton P, Masnou P, Schwartz JC, Hirsch E (2013) Efficacy of the histamine 3 receptor (H<sub>3</sub>R) antagonist pitolisant (formerly known as tiprolisant; BF2.649) in epilepsy: dose-dependent effects in the human photosensitivity model. *Epilepsy Behav* 28:66-70.
- Kiviranta T, Tuomisto L, Airaksinen EM (1995) Histamine in cerebrospinal fluid of children with febrile convulsions. *Epilepsia* 36:276-280.
- Komater VA, Browman KE, Curzon P, Hancock AA, Decker MW, Fox GB (2003) H<sub>3</sub> receptor blockade by thioperamide enhances cognition in rats without inducing locomotor sensitization. *Psychopharmacology (Berl)* 167:363-372.
- Komater VA, Buckley MJ, Browman KE, Pan JB, Hancock AA, Decker MW, Fox GB (2005) Effects of histamine H<sub>3</sub> receptor antagonists in two models of spatial learning. *Behav Brain Res* 159:295-300.
- Kubota Y, Ito C, Sakurai E, Sakurai E, Watanabe T, Ohtsu H (2002) Increased methamphetamine-induced locomotor activity and behavioral sensitization in histamine-deficient mice. *J Neurochem* 83:837-845.
- Kukko-Lukjanov TK, Soini S, Taira T, Michelsen KA, Panula P, Holopainen IE (2006) Histaminergic neurons protect the developing hippocampus from

- kainic acid-induced neuronal damage in an organotypic coculture system. *J Neurosci* 26:1088-1097.
- Lazewska D, Ligneau X, Schwartz JC, Schunack W, Stark H, Kiec-Kononowicz K (2006) Ether derivatives of 3-piperidinopropan-1-ol as non-imidazole histamine H3 receptor antagonists. *Bioorg Med Chem* 14:3522-3529.
- Leza JC, Lizasoain I, Lorenzo P (1991) Effects of antihistaminics on locomotor activity in mice. Comparison with opiate and amphetamine-induced hyperactivity. *Gen Pharmacol* 22:293-296.
- Ligneau X, Garbarg M, Vizuete ML, Diaz J, Purand K, Stark H, Schunack W, Schwartz JC (1994) [<sup>125</sup>I]iodoproxyfan, a new antagonist to label and visualize cerebral histamine H3 receptors. *J Pharmacol Exp Ther* 271:452-459.
- Ligneau X, Lin J, Vanni-Mercier G, Jouvret M, Muir JL, Ganellin CR, Stark H, Elz S, Schunack W, Schwartz J (1998) Neurochemical and behavioral effects of ciproxifan, a potent histamine H3-receptor antagonist. *J Pharmacol Exp Ther* 287:658-666.
- Ligneau X, Morisset S, Tardivel-Lacombe J, Gbahou F, Ganellin CR, Stark H, Schunack W, Schwartz JC, Arrang JM (2000) Distinct pharmacology of rat and human histamine H(3) receptors: role of two amino acids in the third transmembrane domain. *Br J Pharmacol* 131:1247-1250.
- Ligneau X, Perrin D, Landais L, Camelin JC, Calmels TP, Berrebi-Bertrand I, Lecomte JM, Parmentier R, Anaclet C, Lin JS, Bertaina-Anglade V, la Rochelle CD, d'Aniello F, Rouleau A, Gbahou F, Arrang JM, Ganellin CR, Stark H, Schunack W, Schwartz JC (2007) BF2.649 [1-{3-[3-(4-Chlorophenyl)propoxy]propyl}piperidine, hydrochloride], a nonimidazole inverse agonist/antagonist at the human histamine H3 receptor: Preclinical pharmacology. *J Pharmacol Exp Ther* 320:365-375.
- Lim HD, van Rijn RM, Ling P, Bakker RA, Thurmond RL, Leurs R (2005) Evaluation of histamine H1-, H2-, and H3-receptor ligands at the human histamine H4 receptor: identification of 4-methylhistamine as the first potent and selective H4 receptor agonist. *J Pharmacol Exp Ther* 314:1310-1321.
- Lin JS, Sergeeva OA, Haas HL (2011) Histamine H3 receptors and sleep-wake regulation. *J Pharmacol Exp Ther* 336:17-23.
- Liu C, Ma X, Jiang X, Wilson SJ, Hofstra CL, Blevitt J, Pyati J, Li X, Chai W, Carruthers N, Lovenberg TW (2001) Cloning and pharmacological characterization of a fourth histamine receptor (H(4)) expressed in bone marrow. *Mol Pharmacol* 59:420-426.

- Lopes KS, Rios ER, Lima CN, Linhares MI, Torres AF, Havt A, Quinet YP, Fonteles MM, Martins AM (2013) The effects of the Brazilian ant *Dinoponera quadriceps* venom on chemically induced seizure models. *Neurochem Int* 63:141-145.
- Loscher W (2002) Current status and future directions in the pharmacotherapy of epilepsy. *Trends Pharmacol Sci* 23:113-118.
- Loscher W (2011) Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. *Seizure* 20:359-368.
- Loscher W, Fassbender CP, Nolting B (1991a) The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. II. Maximal electroshock seizure models. *Epilepsy Res* 8:79-94.
- Loscher W, Honack D, Fassbender CP, Nolting B (1991b) The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. III. Pentylentetrazole seizure models. *Epilepsy Res* 8:171-189.
- Lovenberg TW, Roland BL, Wilson SJ, Jiang X, Pyati J, Huvar A, Jackson MR, Erlander MG (1999) Cloning and functional expression of the human histamine H3 receptor. *Mol Pharmacol* 55:1101-1107.
- Maeyama K, Watanabe T, Yamatodani A, Taguchi Y, Kambe H, Wada H (1983) Effect of alpha-fluoromethylhistidine on the histamine content of the brain of W/W<sup>v</sup> mice devoid of mast cells: turnover of brain histamine. *J Neurochem* 41:128-134.
- Malmberg-Aiello P, Ipponi A, Blandina P, Bartolini L, Schunack W (2003) Pro-cognitive effect of a selective histamine H1-receptor agonist, 2-(3-trifluoromethylphenyl)histamine, in the rat object recognition test. *Inflamm Res* 52 Suppl 1:S33-34.
- Mares P, Mikulecka A (2009) Different effects of two N-methyl-D-aspartate receptor antagonists on seizures, spontaneous behavior, and motor performance in immature rats. *Epilepsy Behav* 14:32-39.
- Martinez-Mir MI, Pollard H, Moreau J, Arrang JM, Ruat M, Traiffort E, Schwartz JC, Palacios JM (1990) Three histamine receptors (H1, H2 and H3) visualized in the brain of human and non-human primates. *Brain Res* 526:322-327.
- Mazurkiewicz-Kwilecki IM, Nsonwah S (1989) Changes in the regional brain histamine and histidine levels in postmortem brains of Alzheimer patients. *Can J Physiol Pharmacol* 67:75-78.



- McGaugh JL, Roozendaal B (2009) Drug enhancement of memory consolidation: historical perspective and neurobiological implications. *Psychopharmacology (Berl)* 202:3-14.
- McGowan E, Eriksen J, Hutton M (2006) A decade of modeling Alzheimer's disease in transgenic mice. *Trends Genet* 22:281-289.
- McLeod RL, Rizzo CA, West RE, Jr., Aslanian R, McCormick K, Bryant M, Hsieh Y, Korfmacher W, Mingo GG, Varty L, Williams SM, Shih NY, Egan RW, Hey JA (2003) Pharmacological characterization of the novel histamine H3-receptor antagonist N-(3,5-dichlorophenyl)-N'-[[4-(1H-imidazol-4-ylmethyl)phenyl]-methyl]-urea (SCH 79687). *J Pharmacol Exp Ther* 305:1037-1044.
- Medhurst AD, Atkins AR, Beresford IJ, Brackenborough K, Briggs MA, Calver AR, Cilia J, Cluderay JE, Crook B, Davis JB, Davis RK, Davis RP, Dawson LA, Foley AG, Gartlon J, Gonzalez MI, Heslop T, Hirst WD, Jennings C, Jones DN, Lacroix LP, Martyn A, Ociepka S, Ray A, Regan CM, Roberts JC, Schogger J, Southam E, Stean TO, Trail BK, Upton N, Wadsworth G, Wald JA, White T, Witherington J, Woolley ML, Worby A, Wilson DM (2007) GSK189254, a novel H3 receptor antagonist that binds to histamine H3 receptors in Alzheimer's disease brain and improves cognitive performance in preclinical models. *J Pharmacol Exp Ther* 321:1032-1045.
- Meguro K, Yanai K, Sakai N, Sakurai E, Maeyama K, Sasaki H, Watanabe T (1995) Effects of thioperamide, a histamine H3 antagonist, on the step-through passive avoidance response and histidine decarboxylase activity in senescence-accelerated mice. *Pharmacol Biochem Behav* 50:321-325.
- Meier G, Apelt J, Reichert U, Grassmann S, Ligneau X, Elz S, Leurquin F, Ganellin CR, Schwartz JC, Schunack W, Stark H (2001) Influence of imidazole replacement in different structural classes of histamine H(3)-receptor antagonists. *Eur J Pharm Sci* 13:249-259.
- Meldrum BS (1995) Neurotransmission in epilepsy. *Epilepsia* 36 Suppl 1:S30-35.
- Middei S, Ammassari-Teule M, Marie H (2014) Synaptic plasticity under learning challenge. *Neurobiol Learn Mem* 115:108-115.
- Miyata I, Saegusa H, Sakurai M (2011) Seizure-modifying potential of histamine H1 antagonists: a clinical observation. *Pediatr Int* 53:706-708.
- Mobarakeh JI, Sakurada S, Katsuyama S, Kutsuwa M, Kuramasu A, Lin ZY, Watanabe T, Hashimoto Y, Watanabe T, Yanai K (2000) Role of histamine H(1) receptor in pain perception: a study of the receptor gene knockout mice. *Eur J Pharmacol* 391:81-89.

- Mochizuki T, Okakura-Mochizuki K, Horii A, Yamamoto Y, Yamatodani A (1994) Histaminergic modulation of hippocampal acetylcholine release in vivo. *J Neurochem* 62:2275-2282.
- Mohsen A, Yoshikawa T, Miura Y, Nakamura T, Naganuma F, Shibuya K, Iida T, Harada R, Okamura N, Watanabe T, Yanai K (2014) Mechanism of the histamine H(3) receptor-mediated increase in exploratory locomotor activity and anxiety-like behaviours in mice. *Neuropharmacology* 81:188-194.
- Moore JT, Chen J, Han B, Meng QC, Veasey SC, Beck SG, Kelz MB (2012) Direct activation of sleep-promoting VLPO neurons by volatile anesthetics contributes to anesthetic hypnosis. *Curr Biol* 22:2008-2016.
- Morisset S, Rouleau A, Ligneau X, Gbahou F, Tardivel-Lacombe J, Stark H, Schunack W, Ganellin CR, Schwartz JC, Arrang JM (2000) High constitutive activity of native H3 receptors regulates histamine neurons in brain. *Nature* 408:860-864.
- Morse KL, Behan J, Laz TM, West RE, Jr., Greenfeder SA, Anthes JC, Umland S, Wan Y, Hipkin RW, Gonsiorek W, Shin N, Gustafson EL, Qiao X, Wang S, Hedrick JA, Greene J, Bayne M, Monsma FJ, Jr. (2001) Cloning and characterization of a novel human histamine receptor. *J Pharmacol Exp Ther* 296:1058-1066.
- Nathan PJ, Boardley R, Scott N, Berges A, Maruff P, Sivananthan T, Upton N, Lowy MT, Nestor PJ, Lai R (2013) The safety, tolerability, pharmacokinetics and cognitive effects of GSK239512, a selective histamine H(3) receptor antagonist in patients with mild to moderate Alzheimer's disease: a preliminary investigation. *Curr Alzheimer Res* 10:240-251.
- Nelson LE, Guo TZ, Lu J, Saper CB, Franks NP, Maze M (2002) The sedative component of anesthesia is mediated by GABA(A) receptors in an endogenous sleep pathway. *Nat Neurosci* 5:979-984.
- Nishino S, Fujiki N, Ripley B, Sakurai E, Kato M, Watanabe T, Mignot E, Yanai K (2001) Decreased brain histamine content in hypocretin/orexin receptor-2 mutated narcoleptic dogs. *Neurosci Lett* 313:125-128.
- Nishino S, Sakurai E, Nevsimalova S, Yoshida Y, Watanabe T, Yanai K, Mignot E (2009) Decreased CSF histamine in narcolepsy with and without low CSF hypocretin-1 in comparison to healthy controls. *Sleep* 32:175-180.
- Okamura N, Yanai K, Higuchi M, Sakai J, Iwata R, Ido T, Sasaki H, Watanabe T, Itoh M (2000) Functional neuroimaging of cognition impaired by a classical antihistamine, d-chlorpheniramine. *Br J Pharmacol* 129:115-123.

- Olsen RW (1981) The GABA postsynaptic membrane receptor-ionophore complex. Site of action of convulsant and anticonvulsant drugs. *Mol Cell Biochem* 39:261-279.
- Onodera K, Tuomisto L, Tacke U, Airaksinen M (1992a) Strain differences in regional brain histamine levels between genetically epilepsy-prone and resistant rats. *Methods Find Exp Clin Pharmacol* 14:13-16.
- Onodera K, Yamatodani A, Watanabe T (1992b) Effects of alpha-fluoromethylhistidine on locomotor activity, brain histamine and catecholamine contents in rats. *Methods Find Exp Clin Pharmacol* 14:97-105.
- Orsetti M, Ferretti C, Gamalero R, Ghi P (2002) Histamine H<sub>3</sub>-receptor blockade in the rat nucleus basalis magnocellularis improves place recognition memory. *Psychopharmacology (Berl)* 159:133-137.
- Orsetti M, Ghi P, Di Carlo G (2001) Histamine H<sub>3</sub>-receptor antagonism improves memory retention and reverses the cognitive deficit induced by scopolamine in a two-trial place recognition task. *Behav Brain Res* 124:235-242.
- Panula P, Chazot PL, Cowart M, Gutzmer R, Leurs R, Liu WL, Stark H, Thurmond RL, Haas HL (2015) International Union of Basic and Clinical Pharmacology. XCVIII. Histamine Receptors. *Pharmacol Rev* 67:601-655.
- Panula P, Rinne J, Kuokkanen K, Eriksson KS, Sallmen T, Kalimo H, Relja M (1998) Neuronal histamine deficit in Alzheimer's disease. *Neuroscience* 82:993-997.
- Panula P, Yang HY, Costa E (1984) Histamine-containing neurons in the rat hypothalamus. *Proc Natl Acad Sci U S A* 81:2572-2576.
- Parmentier R, Anaclet C, Guhenec C, Brousseau E, Bricout D, Giboulot T, Bozyczko-Coyne D, Spiegel K, Ohtsu H, Williams M, Lin JS (2007) The brain H<sub>3</sub>-receptor as a novel therapeutic target for vigilance and sleep-wake disorders. *Biochem Pharmacol* 73:1157-1171.
- Parmentier R, Ohtsu H, Djebbara-Hannas Z, Valatx JL, Watanabe T, Lin JS (2002) Anatomical, physiological, and pharmacological characteristics of histidine decarboxylase knock-out mice: evidence for the role of brain histamine in behavioral and sleep-wake control. *J Neurosci* 22:7695-7711.
- Pascoli V, Boer-Sacomani C, Hermant JF (2009) H<sub>3</sub> receptor antagonists reverse delay-dependent deficits in novel object discrimination by enhancing retrieval. *Psychopharmacology (Berl)* 202:141-152.
- Prast H, Argyriou A, Philippu A (1996) Histaminergic neurons facilitate social memory in rats. *Brain Res* 734:316-318.

- Prell GD, Hough LB, Khandelwal J, Green JP (1996) Lack of a precursor-product relationship between histamine and its metabolites in brain after histidine loading. *J Neurochem* 67:1938-1944.
- Rizk A, Curley J, Robertson J, Raber J (2004) Anxiety and cognition in histamine H3 receptor-/- mice. *Eur J Neurosci* 19:1992-1996.
- Sadek B, Kuder K, Subramanian D, Shafiullah M, Stark H, Lazewska D, Adem A, Kiec-Kononowicz K (2014) Anticonvulsive effect of nonimidazole histamine H3 receptor antagonists. *Behav Pharmacol* 25:245-252.
- Sadek B, Shehab S, Wiecek M, Subramanian D, Shafiullah M, Kiec-Kononowicz K, Adem A (2013) Anticonvulsant properties of histamine H3 receptor ligands belonging to N-substituted carbamates of imidazopropanol. *Bioorg Med Chem Lett* 23:4886-4891.
- Sadek B, Stark H (2015) Cherry-picked ligands at histamine receptor subtypes. *Neuropharmacology*.
- Sakai N, Onodera K, Maeyama K, Yanai K, Watanabe T (1992) Effects of (S)-alpha-fluoromethylhistidine and metoprine on locomotor activity and brain histamine content in mice. *Life Sci* 51:397-405.
- Sakai N, Sakurai E, Onodera K, Sakurai E, Asada H, Miura Y, Watanabe T (1995) Long-term depletion of brain histamine induced by alpha-fluoromethylhistidine increases feeding-associated locomotor activity in mice with a modulation of brain amino acid levels. *Behav Brain Res* 72:83-88.
- Schibli R, Schubiger PA (2002) Current use and future potential of organometallic radiopharmaceuticals. *Eur J Nucl Med Mol Imaging* 29:1529-1542.
- Schmidt D (2002) The clinical impact of new antiepileptic drugs after a decade of use in epilepsy. *Epilepsy Res* 50:21-32.
- Schneider EH, Seifert R (2015) The histamine H-receptor and the central and peripheral nervous system: A critical analysis of the literature. *Neuropharmacology*.
- Schone C, Apergis-Schoute J, Sakurai T, Adamantidis A, Burdakov D (2014) Coreleased orexin and glutamate evoke nonredundant spike outputs and computations in histamine neurons. *Cell Rep* 7:697-704.
- Schwartz JC (2011) The histamine H3 receptor: from discovery to clinical trials with pitolisant. *Br J Pharmacol* 163:713-721.

- Schwartz JC, Barbin G, Garbarg BM, Pollard H, Rose C, Verdiere M (1976) Neurochemical evidence for histamine acting as a transmitter in mammalian brain. *Adv Biochem Psychopharmacol* 15:111-126.
- Selbach O, Brown RE, Haas HL (1997) Long-term increase of hippocampal excitability by histamine and cyclic AMP. *Neuropharmacology* 36:1539-1548.
- Serdiuk SE, Gmiro VE, Veselkina OS (2014) [Stimulation of gastric mucosa afferents by phenylephrine potentiates anticonvulsive and eliminates sedative action of sodium valproate in the pentylenetetrazol kindling model in rats]. *Russ Fiziol Zh Im I M Sechenova* 100:96-104.
- Silver R, Curley JP (2013) Mast cells on the mind: new insights and opportunities. *Trends Neurosci* 36:513-521.
- Silver RB, Poonwasi KS, Seyedi N, Wilson SJ, Lovenberg TW, Levi R (2002) Decreased intracellular calcium mediates the histamine H<sub>3</sub>-receptor-induced attenuation of norepinephrine exocytosis from cardiac sympathetic nerve endings. *Proc Natl Acad Sci U S A* 99:501-506.
- Sowemimo AA, Adio O, Fageyinbo S (2011) Anticonvulsant activity of the methanolic extract of *Justicia extensa* T. Anders. *J Ethnopharmacol* 138:697-699.
- Stephen LJ, Brodie MJ (2011) Pharmacotherapy of epilepsy: newly approved and developmental agents. *CNS Drugs* 25:89-107.
- Sugimoto Y, Iba Y, Nakamura Y, Kayasuga R, Kamei C (2004) Pruritus-associated response mediated by cutaneous histamine H<sub>3</sub> receptors. *Clin Exp Allergy* 34:456-459.
- Sun H, MacLeod C, Mostoller K, Mahon C, Han L, Renger JJ, Ma J, Brown KR, Schulz V, Kay GG, Herring WJ, Lines C, Rosen LB, Murphy MG, Wagner JA (2013) Early-stage comparative effectiveness: randomized controlled trial with histamine inverse agonist MK-7288 in excessive daytime sleepiness patients. *J Clin Pharmacol* 53:1294-1302.
- Swinyard EA, Sofia RD, Kupferberg HJ (1986) Comparative anticonvulsant activity and neurotoxicity of felbamate and four prototype antiepileptic drugs in mice and rats. *Epilepsia* 27:27-34.
- Takano T, Sakaue Y, Sokoda T, Sawai C, Akabori S, Maruo Y, Taga T, Ohno M, Takeuchi Y (2010) Seizure susceptibility due to antihistamines in febrile seizures. *Pediatr Neurol* 42:277-279.

- Takeshita Y, Watanabe T, Sakata T, Munakata M, Ishibashi H, Akaike N (1998) Histamine modulates high-voltage-activated calcium channels in neurons dissociated from the rat tuberomammillary nucleus. *Neuroscience* 87:797-805.
- Taylor SJ, Kilpatrick GJ (1992) Characterization of histamine-H3 receptors controlling non-adrenergic non-cholinergic contractions of the guinea-pig isolated ileum. *Br J Pharmacol* 105:667-674.
- Tomasch M, Schwed JS, Paulke A, Stark H (2013) Bodilisant-a novel fluorescent, highly affine histamine h3 receptor ligand. *ACS Med Chem Lett* 4:269-273.
- Tosini G (2007) Locomotor activity in rodents. *Methods Mol Biol* 362:95-101.
- Toyota H, Dugovic C, Koehl M, Laposky AD, Weber C, Ngo K, Wu Y, Lee DH, Yanai K, Sakurai E, Watanabe T, Liu C, Chen J, Barbier AJ, Turek FW, Fung-Leung WP, Lovenberg TW (2002) Behavioral characterization of mice lacking histamine H(3) receptors. *Mol Pharmacol* 62:389-397.
- Tuomisto L, Lozeva V, Valjakka A, Lecklin A (2001) Modifying effects of histamine on circadian rhythms and neuronal excitability. *Behav Brain Res* 124:129-135.
- Tuomisto L, Tacke U (1986) Is histamine an anticonvulsive inhibitory transmitter? *Neuropharmacology* 25:955-958.
- van Staveren DR, Metzler-Nolte N (2004) Bioorganometallic chemistry of ferrocene. *Chem Rev* 104:5931-5985.
- Verdiere M, Rose C, Schwartz JC (1975) Synthesis and release of histamine studied on slices from rat hypothalamus. *Eur J Pharmacol* 34:157-168.
- Vicini S, Wang JF, Li JH, Zhu WJ, Wang YH, Luo JH, Wolfe BB, Grayson DR (1998) Functional and pharmacological differences between recombinant N-methyl-D-aspartate receptors. *J Neurophysiol* 79:555-566.
- Vohora D, Pal SN, Pillai KK (2000) Thioperamide, a selective histamine H3 receptor antagonist, protects against PTZ-induced seizures in mice. *Life Sci* 66:PL297-301.
- Walker M (2007) Neuroprotection in epilepsy. *Epilepsia* 48 Suppl 8:66-68.
- Wasterlain CG, Fujikawa DG, Penix L, Sankar R (1993) Pathophysiological mechanisms of brain damage from status epilepticus. *Epilepsia* 34 Suppl 1:S37-53.
- Watanabe T, Taguchi Y, Hayashi H, Tanaka J, Shiosaka S, Tohyama M, Kubota H, Terano Y, Wada H (1983) Evidence for the presence of a histaminergic

- neuron system in the rat brain: an immunohistochemical analysis. *Neurosci Lett* 39:249-254.
- White HS (1999) Comparative anticonvulsant and mechanistic profile of the established and newer antiepileptic drugs. *Epilepsia* 40 Suppl 5:S2-10.
- Williams RH, Chee MJ, Kroeger D, Ferrari LL, Maratos-Flier E, Scammell TE, Arrigoni E (2014) Optogenetic-mediated release of histamine reveals distal and autoregulatory mechanisms for controlling arousal. *J Neurosci* 34:6023-6029.
- Yanai K, Son LZ, Endou M, Sakurai E, Nakagawasai O, Tadano T, Kisara K, Inoue I, Watanabe T, Watanabe T (1998) Behavioural characterization and amounts of brain monoamines and their metabolites in mice lacking histamine H1 receptors. *Neuroscience* 87:479-487.
- Yawata I, Tanaka K, Nakagawa Y, Watanabe Y, Murashima YL, Nakano K (2004) Role of histaminergic neurons in development of epileptic seizures in EL mice. *Brain Res Mol Brain Res* 132:13-17.
- Ying Z, Babb TL, Mikuni N, Najm I, Drazba J, Bingaman W (1999) Selective coexpression of NMDAR2A/B and NMDAR1 subunit proteins in dysplastic neurons of human epileptic cortex. *Exp Neurol* 159:409-418.
- Yokoyama H, Onodera K, Iinuma K, Watanabe T (1994) 2-Thiazolyethylamine, a selective histamine H1 agonist, decreases seizure susceptibility in mice. *Pharmacol Biochem Behav* 47:503-507.
- Yokoyama H, Sato M, Iinuma K, Onodera K, Watanabe T (1996) Centrally acting histamine H1 antagonists promote the development of amygdala kindling in rats. *Neurosci Lett* 217:194-196.
- Yu X, Ye Z, Houston CM, Zecharia AY, Ma Y, Zhang Z, Uygun DS, Parker S, Vyssotski AL, Yustos R, Franks NP, Brickley SG, Wisden W (2015) Wakefulness Is Governed by GABA and Histamine Cotransmission. *Neuron* 87:164-178.
- Yuen ES, Sims JR (2014) How predictive are photosensitive epilepsy models as proof of principle trials for epilepsy? *Seizure* 23:490-493.
- Zampeli E, Tiligada E (2009) The role of histamine H4 receptor in immune and inflammatory disorders. *Br J Pharmacol* 157:24-33.
- Zhang L, Chen Z, Ren K, Leurs R, Chen J, Zhang W, Ye B, Wei E, Timmerman H (2003a) Effects of clobenpropit on pentylentetrazole-kindled seizures in rats. *Eur J Pharmacol* 482:169-175.

Zhang LS, Chen Z, Huang YW, Hu WW, Wei EQ, Yanai K (2003b) Effects of endogenous histamine on seizure development of pentylenetetrazole-induced kindling in rats. *Pharmacology* 69:27-32.

Zhu Y, Michalovich D, Wu H, Tan KB, Dytko GM, Mannan IJ, Boyce R, Alston J, Tierney LA, Li X, Herrity NC, Vawter L, Sarau HM, Ames RS, Davenport CM, Hieble JP, Wilson S, Bergsma DJ, Fitzgerald LR (2001) Cloning, expression, and pharmacological characterization of a novel human histamine receptor. *Mol Pharmacol* 59:434-441.

Zlomuzica A, Dere D, Binder S, De Souza Silva MA, Huston JP, Dere E (2015) Neuronal histamine and cognitive symptoms in Alzheimer's disease. *Neuropharmacology*.



### List of Publications

- Sadek B, Saad A et al. (2015) Anticonvulsant and Procognitive Properties of the Non-imidazole Histamine H3 Receptor Antagonist DL77 in Male Adult Rats. *Neuropharmacology*. In press.
- Khan N, Saad A et al. (2015) The dual-acting H3 receptor antagonist and AChE inhibitor UW-MD-71 dose-dependently enhances memory retrieval and reverses dizocilpine-induced memory impairment in rats. *Behavioral Brain research* 297: 155–164