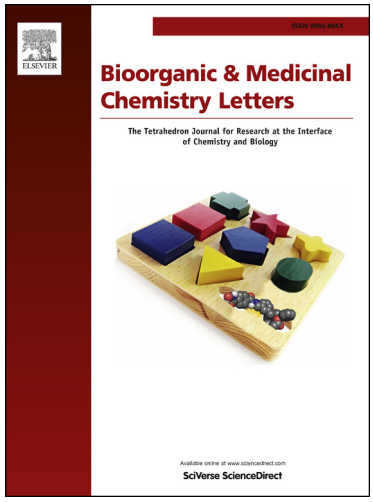

Anticonvulsant properties of histamine H_3 receptor ligands belonging to *N*-substituted carbamates of imidazopropanol

Bassem Sadek, Safa Shehab, Małgorzata Więcek, Dhanasekaran Subramanian, Mohamed Shafiullah, Katarzyna Kieć-Kononowicz, Abdu Adem

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Graphical Abstract

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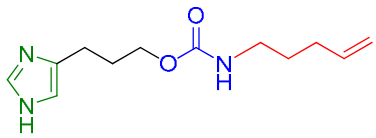

3-(*1H*-imidazol-4-yl)propylpent-4-enylcarbamate (**4**)

In-vivo H3 antagonist activity: ED_{50} = 2.3 ± 0.6 [mg/kg p.o.];
In-vitro H3 antagonist affinity: *h*H3R pK_i = 6.85;

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Anticonvulsant properties of histamine H_3 receptor ligands belonging to *N*-substituted carbamates of imidazopropanol

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ABSTRACT

Ligands targeting central histamine H_3 receptors (H_3Rs) for epilepsy might be a promising therapeutic approach. Therefore, the previously described and structurally strongly related imidazole-based derivatives belonging to carbamate class with high H_3R *in-vitro* affinity, *in-vivo* antagonist potency, and H_3R selectivity profile were investigated on their anticonvulsant activity in maximal electroshock (MES)-induced and pentylenetetrazole (PTZ)-kindled seizure models in Wistar rats. The effects of systemic injection of H_3R ligands **1-13** on MES-induced and PTZ-kindled seizures were screened and evaluated against the reference antiepileptic drug (AED) Phenytoin (PHT) and the standard histamine H_3R inverse agonist/antagonist Thioperamide (THP) to determine their potential as new antiepileptic drugs. Following administration of the H_3R ligands **1-13** (5, 10 and 15 mg/kg, i.p.) there was a significant dose dependent reduction in MES-induced seizure duration. The protective action observed for the pentenyl carbamate derivative **4**, the most protective H_3R ligand among **1-13**, was significantly higher (P<0.05) than that of standard H_3R antagonist THP, and was reversed when rats were pretreated with the selective H_3R agonist *R*-( )-methyl-histamine (RAMH) (10 mg/kg), or with the CNS penetrant H_1R antagonist Pyrilamine (PYR) (10 mg/kg). In addition, subeffective dose of H_3R ligand **4** (5 mg/kg, i.p.) significantly potentiated the protective action in rats pretreated with PHT (5 mg/kg, i.p.), a dose without appreciable protective effect when given alone. In contrast, pretreatment with H_3R ligand **4** (10 mg/kg i.p.) failed to modify PTZ-kindled convulsion, whereas the reference drug PHT was found to fully protect PTZ-induced seizure. These results indicate that some of the investigated imidazole-based H_3R ligands **1-13** may be of future therapeutic value in epilepsy.

The excessive discharge of neurons with incidences of 5‰□7‰ is a main characteristic for epilepsy which is defined as a neurological disorder with recurrent seizures.[1,2] Besides suffering from seizures, many epilepsy patients have cognitive problems.[3-6] For example, children with epilepsy were more likely to experience mental and developmental health problems, and with the most mutual cognitive problem being memory impairment.[2,3,7] While the origins of impaired memory in patients with epilepsy have not yet been fully explained, the types and frequencies of seizures and the side-effects of antiepileptic drugs at therapeutic doses have been proposed as underlying etiology of epilepsy. [9,10] Thus, the ideal antiepileptic drug should modify epileptogenesis, suppress seizures, and ameliorate the concomitant cognitive impairment.

The role of brain histaminergic system being involved in epilepsy is driven by mounting evidences of experimental findings that lead to the inference that histamine regulates seizure susceptibility and, thus acts as an anticonvulsant neurotransmitter in electrically as well as chemically induced seizure models in animals.[11,12] In addition, histidine, the precursor of histamine, has been found to decrease chemically-induced seizure in rats, since it activates central histaminergic system and increases seizure threshold and decreases seizure predisposition mediated by hisamine H_1 receptors.[13] Similar results were also observed in mice deficient of histidine decarboxylase and histamine H_1 receptors.[14] Therefore, the central histamine H_1 receptor complex has been usually associated with seizure development.[15,16]

Moreover, it has been found that high doses of several centrally acting H_1 receptor (H_1R) antagonists used as anti-allergic drugs occasionally promote the development of convulsions in healthy young children, especially those taking antihistamines for long time.[17-21] Also, the same findings were

observed in animal seizure models, and further reinforce the association of neuronal histaminergic system with epileptic seizure pathophysiology.[16] Histamine mediates its effects through binding to four, so far known histamine receptor subtypes belonging to the family of G-protein-coupled receptors, and designated H_1 to H_4 receptors (H_1R–H_4R), and the H_3R initially described in 1983 was found to be a constitutively active receptor mostly expressed in the brain and was evaluated pharmacologically to negatively regulate histamine synthesis and release, acting as presynaptic auto-receptors.[22,23] Therefore, histamine receptor ligands capable of increasing histamine levels, such as histidine and histamine *N*-methyl transferase (HNMT) inhibitors (e.g metoprine), reduce seizures in epileptic patients through H_1R stimulation.[24-26] Consequently, pharmacological profile of H_3R antagonists/inverse agonists with respect to their anticonvulsive potential has begun to receive increased attention as mounting experimental proofs from both acute and chronic models of epilepsy indicate effectiveness of H_3R antagonists.[27-30]

Encouraged by the aforementioned results in experimental epilepsy, many academic and industrial units have begun extensive research for the possible efficacy of H_3R antagonists in clinical epilepsy. So far the only success is in the form of pitolisant which showed protective effects in the human photosensitivity model of epilepsy.[31]

Given their localization and their ability to affect multiple neurotransmitter systems,[25,29] the development of new H_3R ligands targeting central H_3Rs might affect multi-neurotransmitter disorders including epilepsy. Therefore, the previously described and structurally strongly related imidazole-based carbamate derivatives (**1-13**, **Table 1**) with high H_3R *in-vitro* affinity, *in-vitro* selectivity, and *in-vivo* antagonist potency have in the current study been investigated to test their anticonvulsant effects in MES-induced and PTZ-kindled seizure models in rats.[34,48]

Examined compounds differed in their chemical structure only in the lipophilic part of molecules being *N*-alkyl, alkenyl, cycloalkyl or arylalkyl carbamates of 3-(1*H*-imidazol-4-yl)propanol (**Table 1**). All compounds chosen for the current study were previously confirmed in their high *in vitro* hH_3R affinities with observed pK_i values in the range from 6.71 to 7.89, high inverse agonist/antagonist *in vivo* potency with ED_{50} values ranging from 0.56 to 5.8 mg/kg peroral (p.o), and good H_3R selectivity among histamine H_1-H_4Rs (**Table 1**). General procedure for the synthesis of investigated compounds is described in Supplementary materials (**Scheme 1 and 2**).

Since no further increase in protective effect observed by intraperitonial injection (i.p.) of 10 and 15 mg/kg reference drug PHT, and a dose of 5 mg/kg PHT was inactive, all further experiments were carried out at a dose of 10 mg/kg PHT, which was the minimal dose of PHT that protected animals against spread of MES-induced seizures without mortality in rats. Our data observed for H_3R standard THP and H3R ligands **1-13** in MES model showed that ligands **1**, **3**, **4**, **5**, **9**, **11**, and **13** significantly exhibited the most promising protection against seizure in rats pretreated with 10 mg/kg i.p. when compared with standard H3R antagonist/inverse agonist THP (10 mg/kg) or saline-treated group (P<0.05), however the protective effects observed by these compounds (**1**, **3**, **4**, **5**, **9**, **11**, **13**) were significantly lower than that of active control group pretreated with PHT (10 mg/kg i.p.) as a reference drug (P<0.05) and comparable with that of H3R standard antagonist THP (**Figure 1**). In a second experiment, four H3R ligands, namely **1**, **3**, **4**, and **9**, representing structural diversity of substitutions present at carbamate moiety, were chosen to further establish a dose-response relationship of protective effect observed. Interestingly, our findings indicated that among **1**, **3**, **4**, and **9**, the protective effect of H_3R ligand **4** was intensely dependent on the dose administered; hence rats pretreated with **4** in a dose of 15 mg/kg were fully protected against spread of MES-induced seizure (**Figure 2**). Noticeably, H_3R ligands **1**, **3**, **9**, standard antagonist/inverse agonist THP, and reference drug PHT in a dose of 10 and 15 mg/kg i.p exhibited significantly higher (P<0.05) protection than in 5 mg/kg i.p., however, no significant difference in protective effect was observed between individual doses of 10 and 15 mg/kg (**Figure 2**).

Interestingly, our major findings for anticonvulsant activity of the investigated H_3R ligands did not directly correlate with their H_3R *in vitro* affinity and *in vivo* potency. Accordingly, the obtained anticonvulsant properties were expressed in the order of decreasing potency **13**<**5**~**THP**<**11**<**3**~**1**<**9**<**4**, while *in vitro* H_3R affinity measured with pK_i values were **9**<**11**<**13**<**THP**<**3**<**5**<**4**<**1** and *in vivo* ED_{50} values were **9**<**11**<**3**<**4**<**13**<**1**<**THP**<**5**. The correlation with physicochemical properties expressed with calculated logP values did not exist either (**Table 2**). However, lessons learned suggest that differences between histamine receptor species orthologues should be considered, in particular with respect to potential studies in translational animal models.[36,37] Nevertheless, it may be concluded that differences in H_3R activity, especially in observed ED_{50} values, were not in agreement with observed anticonvulsant activity for the current series **1-13**.

The findings allowed further explanations of the protective profile observed by ligand **4**, since the protection for **4** was significantly higher (P<0.05) than those exhibited by **1**, **3**, **9**, and **THP**. Therefore, ligand **4** was selected to further examine whether its effect is through interaction with H_3Rs. Results indicated that the protective effect observed for ligand **4** was reversed by pretreatment with 10 mg/kg i.p. of histamine H_3R agonist *R*-( )-methyl-histamine (RAMH) 15 min before MES challenge (**Figure 3**).[38-41] Interestingly, RAMH alone did not offer a protective effect in rats challenged by MES-induced seizure model (**Figure 3**). These findings suggested that H_3R antagonism plays a suppressive role in MES-induced seizure, and more importantly, our results were in agreement with previous studies that demonstrated a dose-dependent protective action of H_3R antagonists and its reversal by RAMH in mice.[42]

Our results also showed that the protective effect of ligand **4** was blocked when the Wistar rats were pretreated with 10 mg/kg i.p. of the CNS penetrant histamine H_1R antagonist Pyrilamine (PYR) 15 min before MES challenge (**Figure 3**).[43] Interestingly, PYR administered alone (10 mg/kg) did not offer either a protective or epileptogenic effect in rats challenged by MES-induced seizure model (**Figure 3**). The findings further profile the protective effect of ligand **4**.

These findings indicate that the protective role of ligand **4** in MES-induced seizure is mediated through interactions of released histamine with postsynaptically located histamine H_1Rs. H_3Rs are autoreceptors located on presynaptic histaminergic terminals with an inhibitory action on the synthesis and release of histamine.[22] Blockade of these receptors by selective H_3R ligands, e.g. **4**, would lead to an enhanced neuronal histamine release in the brain, resulting in anticonvulsant effect. Notably, comparable protective effects of imidazole-based H_3R ligands, were reported to be reversed either by H_3R agonists or by H_1R antagonists but not by H_2R antagonists, suggesting an interaction of the H_3R-antagonisim-released histamine with H_1Rs on the postsynaptic neurons.[28,43]

H_3Rs are also considered as heteroreceptors in both CNS and peripheral nervous system, since they modulate not only the release of histamine but also of other neurotransmitter, e.g. Ach,[32] NE,[33] DA,[34] 5-HT,[35] and GABA.[36] However, the heteroreceptor function of H_3R ligands in the regulation of monoaminergic activity was proposed to be minor rather than their function in modulating histaminergic activity.[35]

Interestingly, our initial dose-response studies showed that i.p. administration of PHT (5 mg/kg) demonstrated no protection, whereas ligand **4** (5 mg/kg) offered only moderate protective effect in MES model (**Figure 4**). The combined efficacy with the subeffective dose of ligand **4** and ineffective dose of reference drug PHT is shown in **Figure 4**. PHT, when combined with **4** and administered 30 min before MES challenge, significantly reduced the average duration of THLE phase as compared to compound **4** or PHT treatment alone ($^{*}P<0.05$ versus saline group; $^{\#}P<0.05$ versus control groups pretreated with ligand **4** or PHT, respectively). These findings indicate synergism between PHT and ligand **4** albeit through different mechanisms. However, more investigations are needed to validate the observed synergy between PHT and ligand **4** (Figure **4**).

Previous reports suggested that H_3R ligands protected animals in PTZ-kindled seizure.[28] In the present study, the effects of H_3R ligand **4** and PHT were monitored in PTZ-kindled rats. To this end, PTZ was applied three times a week to develop the kindling model since recurrent administration of PTZ has been confirmed as more appropriate than daily injections.[51] In contrast, results obtained showed low protection for ligand **4** in PTZ-kindled rat model (**Figure 5**). The protection observed for ligand **4** after 5 and 10 min was significantly higher ($P<0.05$) than control group, however, significantly lower than that pretreated with the reference drug PHT (10 mg/kg i.p.) which exhibited full protection after 30 min of time observation. The discrepancy in the effects of ligand **4** could be explained by the different levels histamine release as a result of the seizure in different models. In this respect, a significant increase was found in brain following MES seizures, whereas a tendency towards decrease in histamine levels was found following PTZ-induced clonic convulsions.[35]

It can be concluded that these results supported the concept of H_3Rs being involved in altering MES-induced seizure. Data observed suggest that H_3R ligands enhance histamine release, and that the released histamine depresses MES-induced seizures via activation of histamine H_1Rs located on postsynaptic neurons. Future experiments will continue to examine the multiplicity of the histaminergic connection with other monoaminergic signaling and, hence blockade of the H_3R should be included in studying the mechanisms involved in MES-induced seizures.

On the other hand, histamine H_3R ligands, like THP, have been demonstrated to improve learning and memory in several cognition-deficit animal models.[44] Given the pro-cognitive effects of H_3R ligands, and considering mounting clinical data that proved cognitive deficits, e.g. mental side-effects, such as depression, and memory or attention problems especially in children taking AEDs over long time, the results of our present experiments with selective H_3R antagonists provide additional evidence that H_3R ligands can be of therapeutic value in epilepsy, or at least a viable approach that can be combined with AEDs, since the concept of such combination therapy has been previously projected for imidazole-based H_3R ligands.[24]

Much work however is still needed to fully comprehend the mechanisms of the histamine interaction in epilepsy and to test the potential use of H_3R ligands in therapeutic options on epilepsy. Moreover, additional new H_3R ligands belonging to the carbamate class as well as further pro-cognitive experiments are needed to clarify the role of H_3R ligands in altering epilepsy and to afford multi-target ligands combining pro-cognitive property of H_3R ligands with anticonvulsant effect.

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Supplementary Material

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Legends to Figures

Figure 1. Protective effect of i.p. injection of H_3R ligands **1-13** on MES-induced seizure.

Protective effects of Phenytoin (PHT, 10 mg/kg, i.p.) (THP, 10 mg/ kg, i.p.) and test compounds **1-13** (10 mg/kg) on duration of tonic hind limb extension (THLE) induced in MES model in rats. Each value represents mean ± S.E.M. (n = 8). **P* < 0.05 vs. saline-treated group. ***P* < 0.01 vs. saline-treated group. $^{\#}P$ < 0.05 PHT (10 mg)- vs. THP, **1**, **3**, **5**, **9**, **11**, and **13** (10 mg) -treated groups. $^{\&}P$ < 0.05 vs. THP-treated group.

Figure 2. Dose-dependent protective effects of selected H_3R ligands **1**, **3**, **4**, and **9** against MES seizures.

H_3R ligands **1**, **3**, **4**, and **9**, as well as reference drug PHT and standard H_3R inverse agonist/antagonist THP (5, 10, and 15 mg/kg, i.p.) on duration of tonic hind limb extension (THLE) induced in MES model in rats. Each value represents mean ± S.E.M. (n = 8). **P* < 0.05 vs. saline-treated group. ***P* < 0.01 vs. saline-treated group. $^{\#}P$ < 0.05 vs. 5 mg of the same compound. $^{\#\#}P$ < 0.01 vs. 5 mg of the same compound. $^{\&}$Full protection for ligand **4** (15 mg/kg, i.p.).

Figure 3. Effect RAMH or PYR pretreatment on the protection of H_3R ligand **4** against MES seizures.

Compound **4** (Cpd **4**, mg/kg i.p.) 30 min before MES challenge, Cpd **4** (10 mg/kg i.p.) with RAMH (10 mg/kg i.p., 15 min before MES challenge), RAMH (10 mg/kg) alone, Cpd **4** (10 mg/kg i.p.) with Pyrilamine (PYR 10 mg/kg i.p., 10 min before MES challenge), and PYR (10 mg/kg) alone on duration of tonic hind limb extension (THLE) induced in MES model in rats. Each value represents mean ± S.E.M. (n = 5). **P* < 0.05 vs. saline-treated group. $^{\#}P$ < 0.05 vs. Cdp **4** (5 mg)+RAMH (5 mg) or PYR (10 mg)-treated groups.

Figure 4. Interaction of subeffective doses of H_3R ligand **4** and phenytoin on MES-induced convulsions in rats.

Protective effects of compound **4** (Cpd **4**, 5 mg/kg i.p.), reference drug Phenytoin (PHT, 5 mg/kg i.p.), and Cpd **4** (5 mg/kg i.p.) with PHT (5 mg/kg i.p.) on duration of tonic hind limb extension (THLE) induced in MES model in rats. Each value represents mean ± S.E.M. (n = 5). **P* < 0.05 vs. saline-treated group. $^{\#}P$ < 0.05 vs. Cdp **4**(5 mg)- or PHT(mg)-treated group.

Figure 5. Protective effect of H_3R ligand **4** pretreatment on PTZ-kindled seizure in rats**.**

Effect of phenytoin (PHT) and compound **4** (Cpd **4**) on pentylenetetrazole (PTZ)- kindled seizures in rats. PHT (10 mg/kg kg, i.p.) and Cpd **4** were injected 30 min before PTZ (35 mg /kg, i.p.) treatments.[60] Each value was expressed as the mean ± S.E.M. (n = 8). **P* < 0.05 vs. PTZ-saline-treated group. ***P* < 0.01 vs. PTZ-saline-treated group. $^{\$}$Full protection for PHT (10 mg/kg, i.p.).

Table 1. Structures and pharmacological results for compounds **1-13**

[a]Central H_3R test *in vivo*: measurement of *N*-methylhistamine in brain after oral administration to mouse mean ± S.E.M.[53] [b]Affinity of the test compounds determined by displacement of [^{125}I]iodoproxyfan binding to membranes of CHO-K1 cells expressing the human H_3R.[54] [c] Functional H_1R assay on guinea-pig ileum.[55] [d] Functional H_2R assay on guinea-pig atrium.[54] [e]Affinity of the test compounds determined by displacement of [^{3}H]histamine binding to membranes of Sf9 cells expressing the human H_4R, co-expressed with G_{i2} and G_{12} subunits, mean ± SD of at least three independent experiments.[56,57] * ND; not determined.

Table 2. Structures and calculated logP values for H_3R ligands **1-13.**

The logP values of the tested compounds **1-13** were calculated using: a,b ChemDraw® Ultra version 7.0.0., CambridgeSoft, (2001), c Pallas 3.0 , CompuDrug Chemistry Ltd (1994, 95)**.**

Figure 1

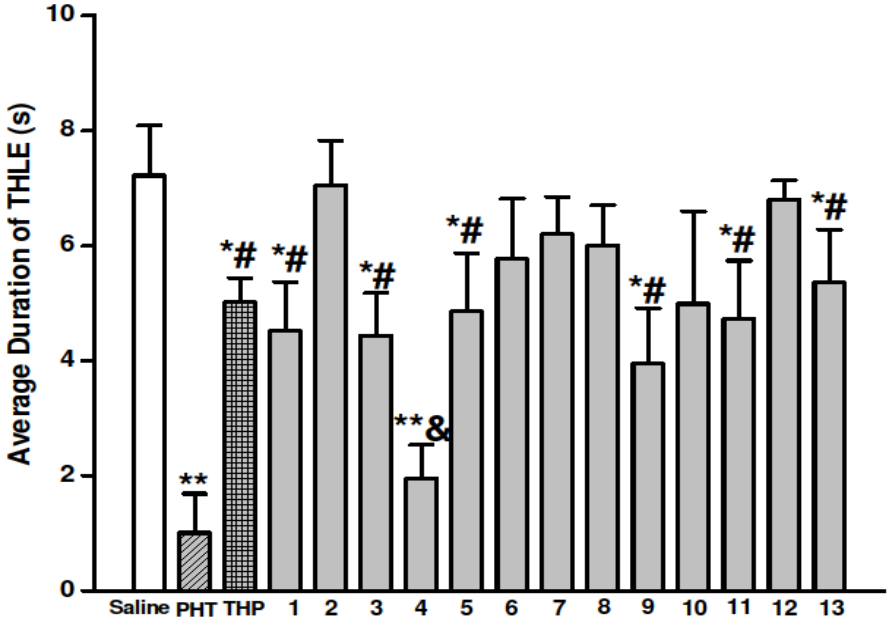


Figure 2

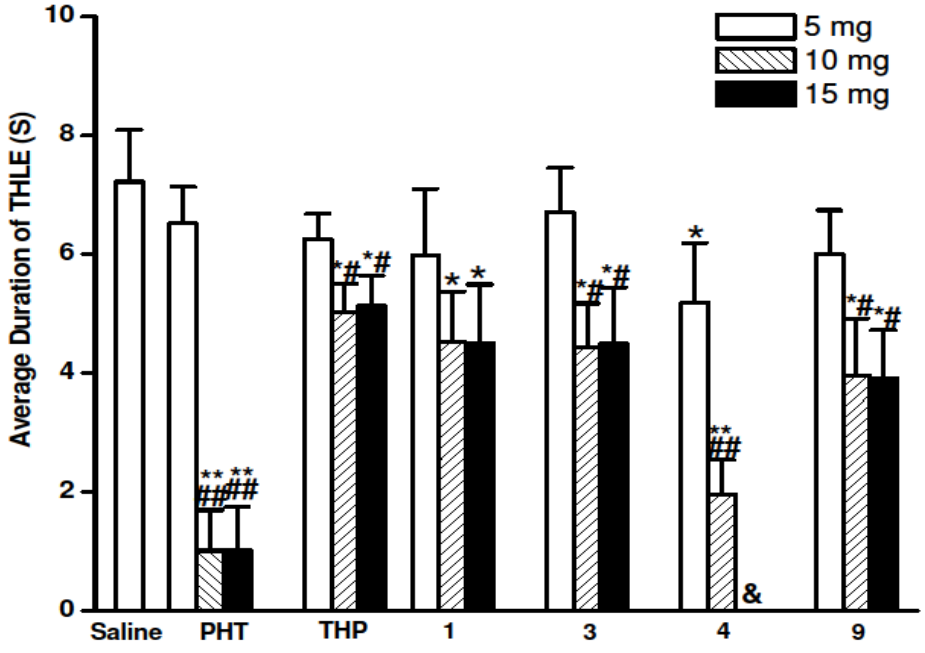


Figure 3

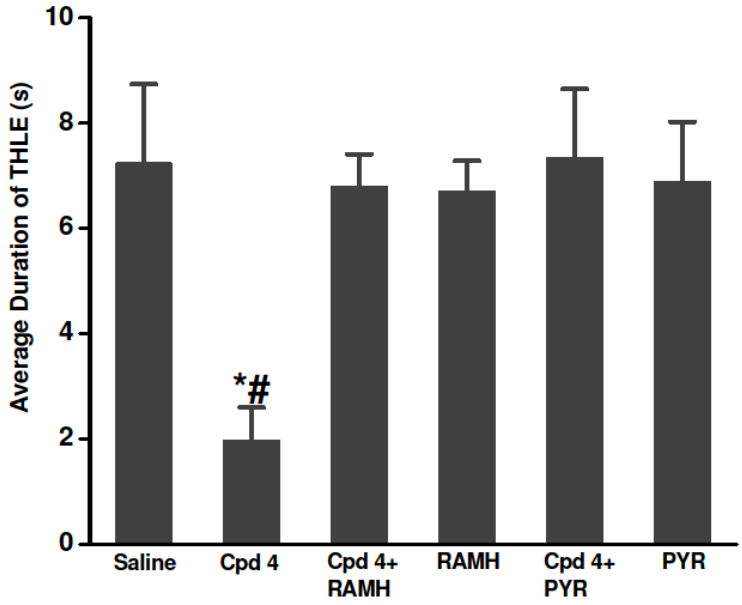


Figure 4

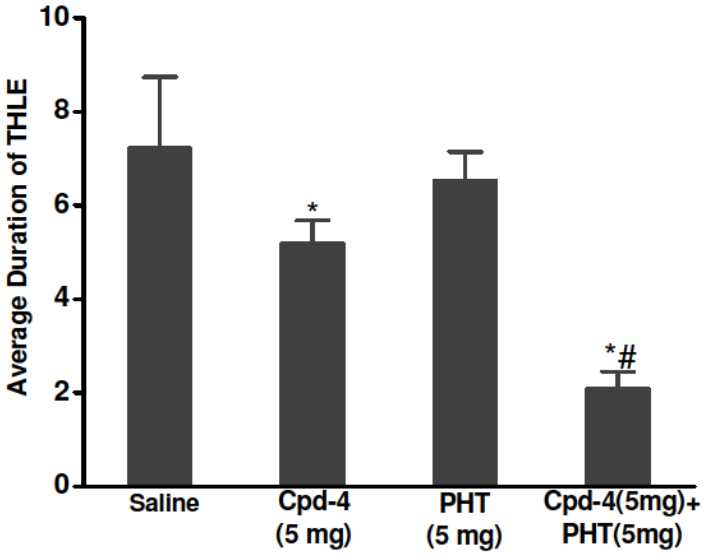


Figure 5

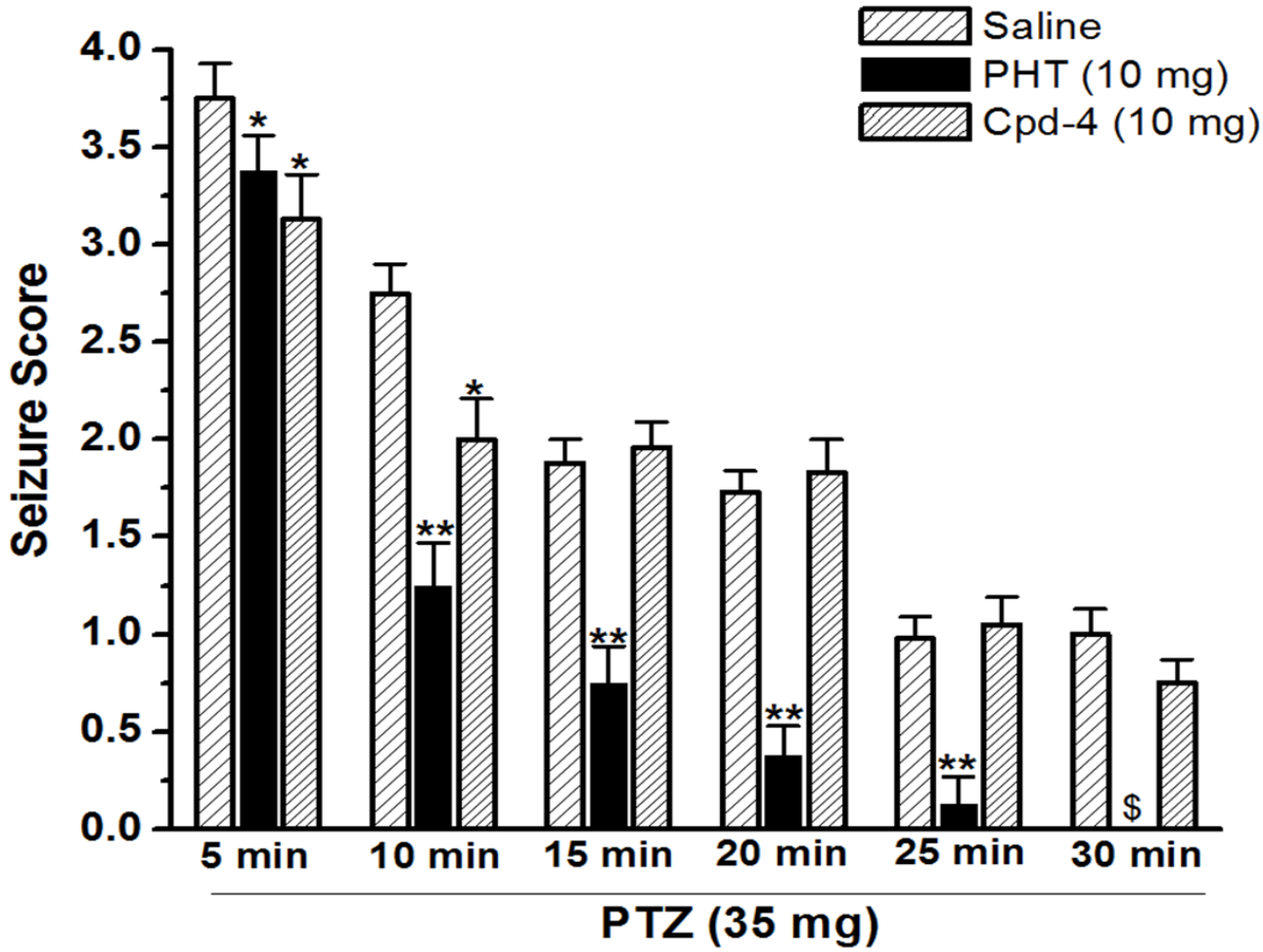
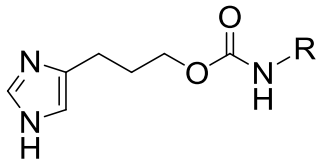


Table 1.



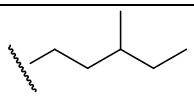
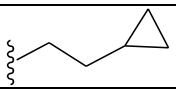
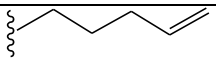
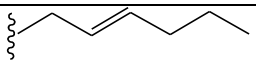
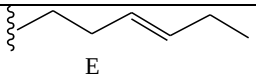
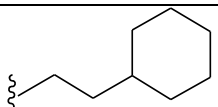
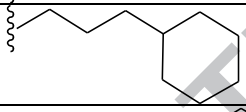
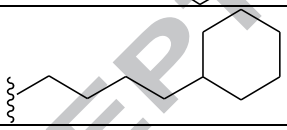
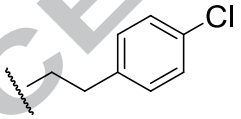
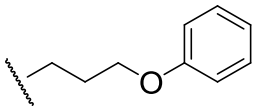
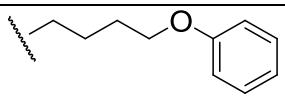
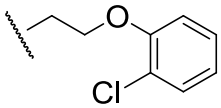
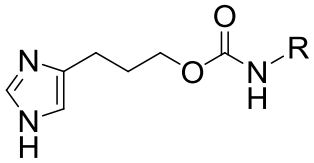
Compds	R	ED_{50}[a] [mg/kg p.o.]	*h*H_3R pK_i[b]	H_1R pK_B[c]	H_2R pK_B[d]	*h*H_4R pK_i[e]
1		2.0 ± 0.3	6.89	ND*	ND*	5.91
2		1.4 ± 0.8	6.77	3.8	~3.5	5.81
3		2.7 ± 1.0	6.26	<4.0	<4.0	5.42
4		2.3 ± 0.6	6.85	3.9	3.6	4.38
5	 E	0.56 ± 0.06	6.62	ND*	ND*	5.15
6	 E	1.9 ± 0.8	6.59	ND*	ND*	4.88
7		3.5 ± 1.4	6.60	3.96	4.49	5.81
8		4.6 ± 2.2	6.07	4.97	4.94	5.35
9		4.7 ± 1.6	5.71	<5.52	4.61	5.30
10		3.1 ± 1.0	6.37	4.82	4.41	5.17
11		4.1 ± 1.4	5.89	4.34	4.26	<4.0
12		5.8 ± 0.5	5.98	4.27	4.78	<4.0
13		2.2 ± 0.7	6.15	ND*	ND*	4.79
	Thioperamide	1.0	6.22[44]	<5	<5	6.37

Table 2.



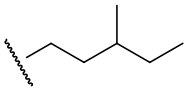
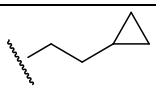
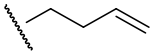
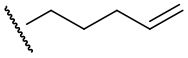
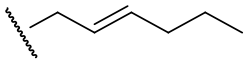
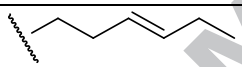
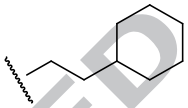
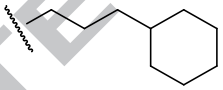
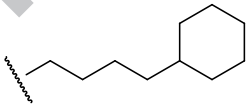
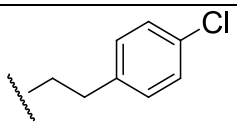
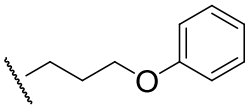
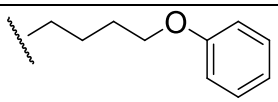
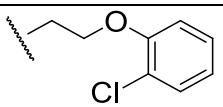
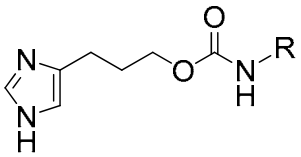
Compound	R	logP[a]	clogP[b]	logP[c] (Pallas)
Phenytoin	-	2.14	2.08	1.65
Thioperamide	-	2.16	3.00	1.83
1		1.87	2.43	2.51
2		0.95	1.42	1.56
3		0.85	1.02	1.35
4		1.27	1.55	1.86
5		1.77	2.28	2.27
6		1.64	2.08	2.27
7		2.20	3.10	3.13
8		2.62	3.62	3.64
9		3.04	4.15	4.15
10		2.45	2.73	3.01
11		1.65	2.29	2.24
12		2.10	2.34	2.75
13		2.10	2.53	2.60

Table 1.



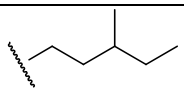
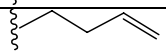
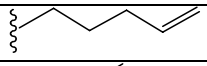
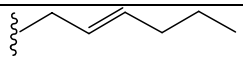
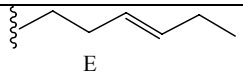
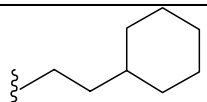
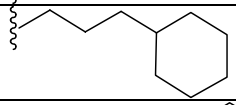
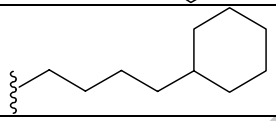
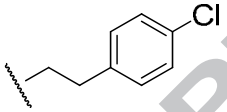
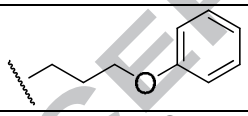
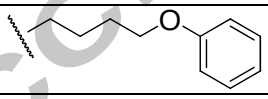
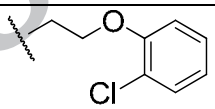
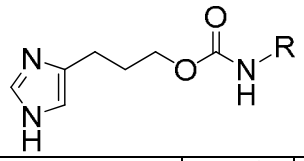
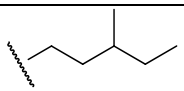
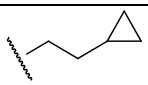
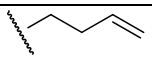
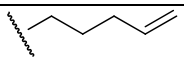
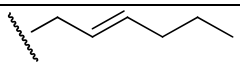
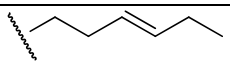
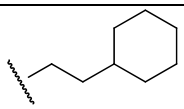
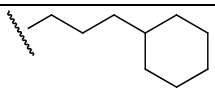
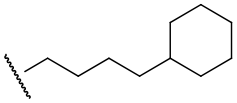
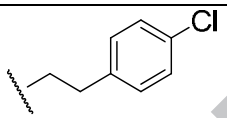
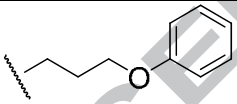
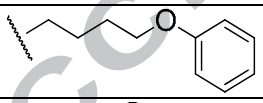
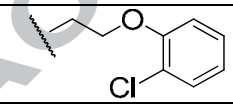
Compds	R	ED_{50}[a] [mg/kg p.o.]	*h*H_3R pK_i[b]	H_1R pK_B[c]	H_2R pK_B[d]	*h*H_4R pK_i[e]
1		2.0 ± 0.3	6.89	ND*	ND*	5.91
2		1.4 ± 0.8	6.77	3.8	~3.5	5.81
3		2.7 ± 1.0	6.26	<4.0	<4.0	5.42
4		2.3 ± 0.6	6.85	3.9	3.6	4.38
5	 E	0.56 ± 0.06	6.62	ND*	ND*	5.15
6	 E	1.9 ± 0.8	6.59	ND*	ND*	4.88
7		3.5 ± 1.4	6.60	3.96	4.49	5.81
8		4.6 ± 2.2	6.07	4.97	4.94	5.35
9		4.7 ± 1.6	5.71	<5.52	4.61	5.30
10		3.1 ± 1.0	6.37	4.82	4.41	5.17
11		4.1 ± 1.4	5.89	4.34	4.26	<4.0
12		5.8 ± 0.5	5.98	4.27	4.78	<4.0
13		2.2 ± 0.7	6.15	ND*	ND*	4.79
	Thioperamide	1.0	6.22[44]	<5	<5	6.37

Table 2.



Compound	R	logP[a]	clogP[b]	logP[c] (Pallas)
Phenytoin	-	2.14	2.08	1.65
Thioperamide	-	2.16	3.00	1.83
1		1.87	2.43	2.51
2		0.95	1.42	1.56
3		0.85	1.02	1.35
4		1.27	1.55	1.86
5		1.77	2.28	2.27
6		1.64	2.08	2.27
7		2.20	3.10	3.13
8		2.62	3.62	3.64
9		3.04	4.15	4.15
10		2.45	2.73	3.01
11		1.65	2.29	2.24
12		2.10	2.34	2.75
13		2.10	2.53	2.60