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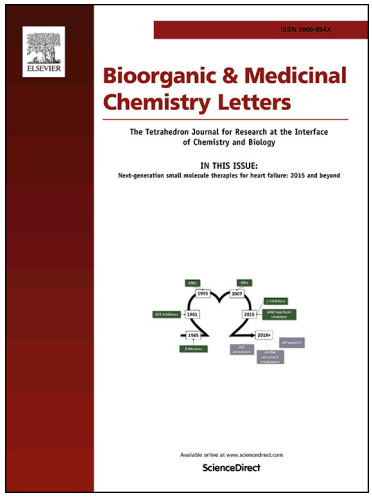

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4-*Tert*-pentylphenoxyalkyl derivatives – histamine H_3 receptor ligands and monoamine oxidase B inhibitors

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Abstract

The synthesis and biological activity of 4-*tert*-pentylphenoxypropyl derivatives are described in this manuscript. All compounds (except one) showed human histamine H_3 receptor affinity with K_i values below 760 nM. The inhibitory activity toward human monoamine oxidase B (hMAO B) was evaluated using a fluorometric Amplex-Red assay, and most of the compounds were effective in the submicromolar range. Among them, 1-(3-(4-*tert*-pPentylphenoxy)propyl)pyrrolidine (**5**) exhibited hMAO B inhibitory activity with an IC_{50} value of 4.5 nM. In addition, hMAO B inhibition by **5** was shown to be non-competitive and reversible. Further, recently described potent histamine H_3 receptor ligands – 4-*tert*-pentylphenoxyalkyl derivatives (with a 4-8 carbon spacer) – were evaluated for hMAO B inhibitory activity, and some of them displayed activity in the submicromolar range. Selected compounds were also tested for human MAO A (hMAO A) inhibitory potencies and exhibited no activity. Moreover, molecular modeling studies were carried out for tested compounds to explain their molecular mechanism of hMAO B inhibition and the selectivity of compounds for hMAO B over hMAO A.

Monoamine oxidase B (MAO B), the predominant isoform in the human brain, catalyzes the oxidative deamination of β-phenylethylamine – an amine which stimulates the release of dopamine and inhibits its neuronal reuptake. Moreover, oxidative deamination causes release of hydrogen peroxide (H_2O_2), which contributes to the production of reactive oxygen species (ROS) and participates in oxidative stress in the brain. In the human brain, MAO B activity increases with age and is elevated in some degenerative diseases such as Parkinson's (PD), Alzheimer's (AD) and Huntington's.[1] MAO B inhibition enhances the activity of endogenous and exogenous dopamine and could prevent neurodegeneration.

Currently, two MAO B inhibitors – selegiline (**1**; **Figure 1**) and rasagiline (**2**; **Figure 1**) – are used for symptomatic treatment of PD. Both of them are irreversible MAO B inhibitors. Recently, a reversible inhibitor – safinamide (**3**; **Figure 1**) –was approved by the EMA and FDA as an anti-Parkinson drug.[2]

Histamine H_3 receptors (H_3Rs) are widely expressed in the brain, particularly in the area involved in cognitive processes and arousal. Pharmacological studies have suggested the utility of histamine H_3R antagonists/inverse agonists in the treatment of various human disorders, e.g. AD, ADHD, PD, schizophrenia, narcolepsy and allergy.[3] Ten years ago, in medicinal chemistry has appeared the idea of designing multitarget-directed ligands (MTDLs) as a new way to treat multifactorial diseases such as neurodegenerative disorders: AD or PD.[4]

In 2017, the first potent H_3R and MAO B ligand – contilisant (**4**; **Figure 1**) – was described by Bautista-Aguilera *et al.*[5] This compound showed not only high affinity for human H_3R (K_i = 11 nM) and human MAOs (hMAO A IC_{50} = 145 nM; hMAO B IC_{50} = 78 nM), but also showed inhibitory activity at human cholinesterases (hAChE IC_{50} = 530 nM; hBuChE IC_{50} = 1690 nM). Simultaneously modulation of the targets which contribute in the development of AD or PD could bring better therapeutic effect in the treatment of these multifactorial disorders.[6]

Some time ago we described 1-(3-(4-*tert*-pentylphenoxy)propyl)piperidine, named **DL77 (Figure 1)**.[7] This compound showed high affinity for human H_3R (hH_3R) *in vitro* (K_i = 8.4 nM) and *in vivo* (ED_{50} = 2.1 ± 0.2 mg/kg; p.o. mice). Moreover, further tested *in vivo*, it showed procognitive effects in rats[8,9], anticonvulsant activity in rats[8], and reduced both ethanol intake and preference in mice[10]. Recently, we have described the synthesis of **DL77** analogues with a longer alkyl chain (from 4-8 carbons) that also proved to be potent histamine H_3R ligands (9 nM ≤ K_i < 330 nM).[11] Preliminary screening of our database of H_3R ligands for inhibitory activity toward hMAO B showed high inhibition of this enzyme by **DL 77** (IC_{50} = 19 nM). Encouraged by these results, we have designed new analogues of **DL77** – 4-*tert*-pentylphenoxypropyl derivatives (**Scheme 1**) with different amine moieties. Herein, we report the synthesis of these compounds, their histamine H_3R affinity and hMAO B inhibition. Moreover, 4-*tert*-pentylphenoxyalkyl derivatives previously described by Kuder *et al*.[11] were also evaluated for hMAO B activity in the present study.

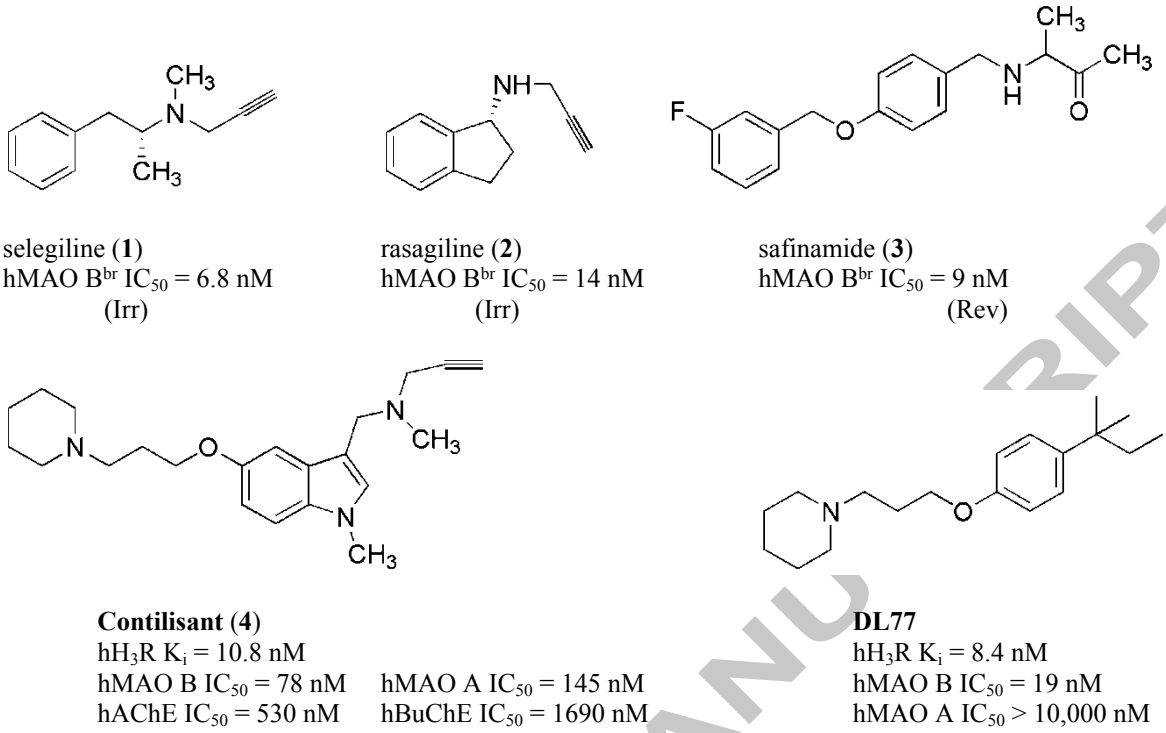
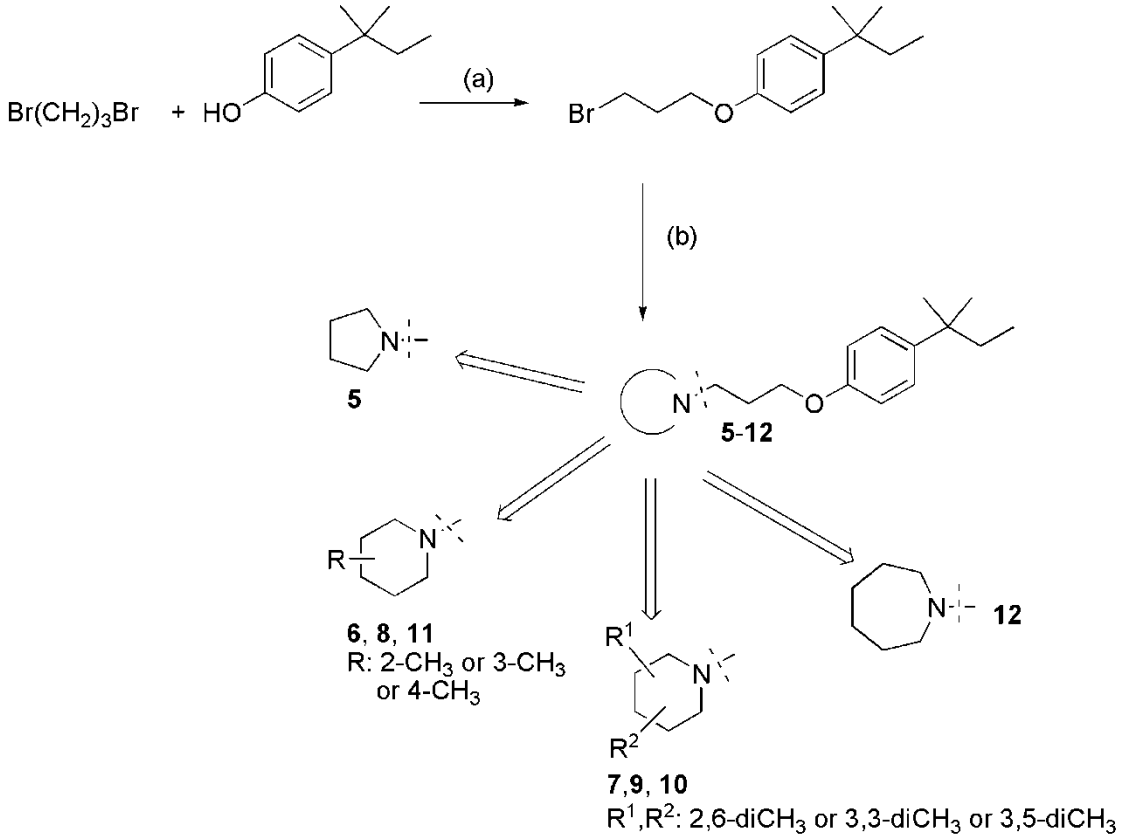


Figure 1. Structures of known MAO B inhibitors, **contilisant** and **DL77**. Irr – irreversible; Rev - reversible

Compounds **5-12** were synthesized as shown in **Scheme 1** with moderate yields (24-47%). 1-(3-Bromopropoxy)-4-*tert*-pentylbenzene was prepared from 4-*tert*-pentylphenol and dibromopropane in freshly prepared sodium propanolate (50 mL; 1.15 g, 0.05 mol Na). The final compounds were obtained by *N*-alkylation in a mixture of ethanol and water (21:4) in the presence of potassium carbonate and potassium iodide as described previously.[12] Final oily compounds were transformed into solid hydrogen oxalates (except **7**). All compounds were fully characterized by NMR spectroscopy (^{1}H and ^{13}C), mass spectroscopy (LC/EST-MS) and elemental analysis. The purity of compounds (HPLC-UV) was 100% (except **7** – 98%). Synthesis of compounds **13-32** was described previously.[11]

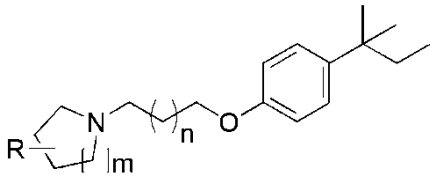
Scheme 1. Synthetic pathway of compounds **5-12**.[(a)]



[(a)]Reagents and conditions (a) C_3H_7ONa, 60^0C -3h, reflux - 3h (b) amine, K_2CO_3, KI, C_2H_5OH:H_2O (21:4), reflux, 20-48 h.

The affinity of new compounds (**5-12**) for hH_3R was evaluated in a radioligand binding assay with *N*-methylhistamine as a radioligand in CHO K1 cells stably expressing hH_3R. Results are presented as K_i values in **Table 1**. Generally, all tested compounds (**5-12**) except one (**9**) showed hH_3R affinities in the nanomolar range (K_i < 800 nM), most of them with moderate strength (100 nM < K_i< 300 nM). The exchange of the amine moiety (piperidine in **DL77**) for other amines (pyrrolidine, substituted piperidines, azepane) not only did not improve the affinity but even caused its decline (e.g. **DL77** vs **9**). The most potent compound (**5**) showed a K_i value of 63 nM.

Table 1. Pharmacological activity of compounds **5-32** and **DL77**.



Cmpd.	R	m	n	human histamine H_3 receptor[a] K_i [nM]	human MAO B[b] IC_{50} [nM]	human MAO A[b] (inhibition%

					(inhibition % at 1 µM)	at 10 µM)
5	**---**	**1**	**1**	63 ± 6.2	4.5 ± 0.4	(5%)
DL77	**---**	**2**	**1**	8.4 ± 1.3[c] 37 ± 5	19 ± 7	(14%)
6	**2-CH_3**	**2**	**1**	437 ± 27	20 ± 2	(5%)
7	**2,6-diCH_3**	**2**	**1**	140 ± 15	22 ± 2	(5%)
8	**3-CH_3**	**2**	**1**	100 ± 11	428 ± 45	(11%)
9	**3,3-diCH_3**	**2**	**1**	>1000	417 ± 43	(7%)
10	**3,5-diCH_3**	**2**	**1**	753 ± 58	962 ± 89	(7%)
11	**4-CH_3**	**2**	**1**	147 ± 4	2070 ± 360	nt[f]
12	**---**	**3**	**1**	298 ± 17	65 ± 23	(12%)
13	**---**	**2**	**2**	140 ± 47[d]	379 ± 84	(10%)
14	**3-CH_3**	**2**	**2**	46 ± 22[d]	1228 ± 191	nt[f]
15	**4-CH_3**	**2**	**2**	238 ± 95[d]	> 1000 (47%)	nt[f]
16	**---**	**3**	**2**	68 ± 20[d]	609 ± 165	nt[f]
17	**---**	**2**	**3**	8.8[e]	> 1000 (28%)	nt[f]
18	**3-CH_3**	**2**	**3**	13.6[e]	> 1000 (29%)	nt[f]
19	**4-CH_3**	**2**	**3**	23.4[e]	> 1000 (48%)	nt[f]
20	**---**	**3**	**3**	21.4[e]	1128 ± 240	nt[f]
21	**---**	**2**	**4**	46.5[e]	> 1000 (21%)	nt[f]
22	**3-CH_3**	**2**	**4**	33.8[e]	> 1000 (46%)	nt[f]
23	**4-CH_3**	**2**	**4**	60.5[e]	> 1000 (37%)	nt[f]
24	**---**	**3**	**4**	41.2[e]	390 ± 55	nt[f]
25	**---**	**2**	**5**	55[e]	> 1000 (18%)	nt[f]
26	**3-CH_3**	**2**	**5**	36.6[e]	> 1000 (35%)	nt[f]
27	**4-CH_3**	**2**	**5**	64.4[e]	> 1000 (35%)	nt[f]
28	**---**	**3**	**5**	128.3[e]	1495 ± 420	nt[f]
29	**---**	**2**	**6**	325.9[e]	> 1000 (22%)	nt[f]
30	**3-CH_3**	**2**	**6**	120.7[e]	> 1000 (33%)	nt[f]
31	**4-CH_3**	**2**	**6**	308.1[e]	> 1000 (37%)	nt[f]
32	**---**	**3**	**6**	182.5[e]	> 1000 (40%)	nt[f]
	rasagiline (**2**)			nt[f]	25.0 ± 6.5 14[g]	710[g]
	safinamide (**3**)			nt[f]	7.7 ± 1.2 7.7 ± 1.8[h]	nt[f]

[a] [^{3}H] N^{α}-Methylhistamine binding assay performed with cell membrane preparation of CHO-K1 cells stably expressing the human H_3 receptor; mean value of two independent experiments ± SEM

[b] fluorometric Amplex™ Red MAO assay.[13]

[c] [^{125}I] Iodoproxyfan binding assay performed with cell membrane preparation of CHO-K1 cells stably expressing the human H_3 receptor; mean value of two independent experiments ± SEM[7]

[d] N^{α}-Methylhistamine binding assay performed with cell membrane preparation of HEK293 cells stably expressing the human H_3 receptor; mean value of triplicate independent experiments; data from Kuder *et al.*[11]

[e] [^{125}I] Iodoproxyfan binding assay performed with cell membrane preparation of CHO-K1 cells stably expressing the human H_3 receptor; data from a single experiment with each concentration tested at least in triplicate; data from Kuder *et al.*[11]
[f] not tested
[g] human brain MAO B or MAO A; data from Ref.[14]
[h] data from Ref.[15]

Inhibition of MAO B was evaluated using Amplex Red® Monoamine Oxidase kits.[13] Rasagiline, pargyline and safinamide were used as reference inhibitors. The inhibitory activity of compounds was evaluated by measuring their effects on the production of H_2O_2 by MAO-B during the oxidative deamination of *para*-tyramine. First, compounds were tested at one concentration (1 μM), then those with at least 50% inhibitory activity against a fully inhibited enzyme were selected for further testing (IC_{50} evaluation). Data are collected in **Table 1**. The exchange of amine moiety (piperidine in **DL77**) for others (see **Table 1**) strongly influenced hMAO B activity: increased it (e.g. **5**; IC_{50} = 4.5 nM), kept it on the same level (e.g. **6**,**7**; IC_{50}: 20-22 nM respectively) or decreased it (e.g. **8-12**; $IC_{50} \geq 65$ nM). Enlargement of the amine ring caused a decrease in activity in the order: pyrrolidine (**5**) > piperidine (**DL77**) > azepane (**12**). It appears that the introduction of a methyl substituent into the ortho position of piperidine (e.g. **6** and **7**) did not influence hMAO B inhibition. However, the least effective was the 4-methylpiperidine moiety (**11**). This compound was preliminarily screened twice and despite inhibition ≤ 50%, was fully evaluated for IC_{50}. The obtained data (IC_{50} = 2070 nM) confirmed its low inhibitory activity. In order to check the influence of alkyl chain length on hMAO B inhibition, we tested 4-*tert*-pentylphenoxyalkyl derivatives (**13-32**) with different amine moieties (piperidine, 3-methylpiperidine, 4-methylpiperidine and azepane) that have recently been described by Kuder *et al.*[11] Generally, elongation of a carbon chain resulted in activity decrease (e.g. **DL77** vs **13** vs **17** vs **21** vs **25** vs **29**) with one exception – **24**. This hexyl azepane derivative (IC_{50} = 390 nM) showed higher activity than pentyl (**20**; IC_{50} = 1128 nM) and butyl (**16**; IC_{50} = 609 nM) derivatives. To sum up, not only the kind of amine moiety but also the length of the carbon chain has an influence on hMAO B activity. There was no correlation observed between the kind of amine moiety and the influence on hH_3R affinity and hMAO B inhibition. However, two of the most potent hH_3R compounds (**5**, **DL77**) are also among the strongest hMAO B inhibitors (pyrrolidine and piperidine derivatives, respectively).

Then, selected compounds (**DL77**, **5-10**, **12**, **13**) were screened (at concentration of 10 μM) for monoamine oxidase A inhibition using Amplex Red® Monoamine Oxidase kits[13] and showed the percentage of inhibition < 15%.

Experiments of the reversibility of hMAO B inhibition by 4-*tert*-pentylphenoxy derivatives were performed with compounds: **DL77**, **5**, **6** and **7**. hMAO B was first incubated with inhibitors (at concentrations representing the IC_{80} value) in the presence of 10 μM *para*-tyramine. After 22 minutes of incubation, the concentration of *para*-tyramine was increased to 1 mM. Fluorescence was measured every minute for 5 hours.[14] Results are collected in **Table 2** and shown in **Figure 2**.

Table 2. Investigation of the reversibility of reference hMAO B inhibitors and **DL77**, **5**, **6**, **7**.

compound	reversibility
rasagiline	irreversible
safinamide	reversible
DL77	reversible
5	reversible
6	reversible
7	reversible

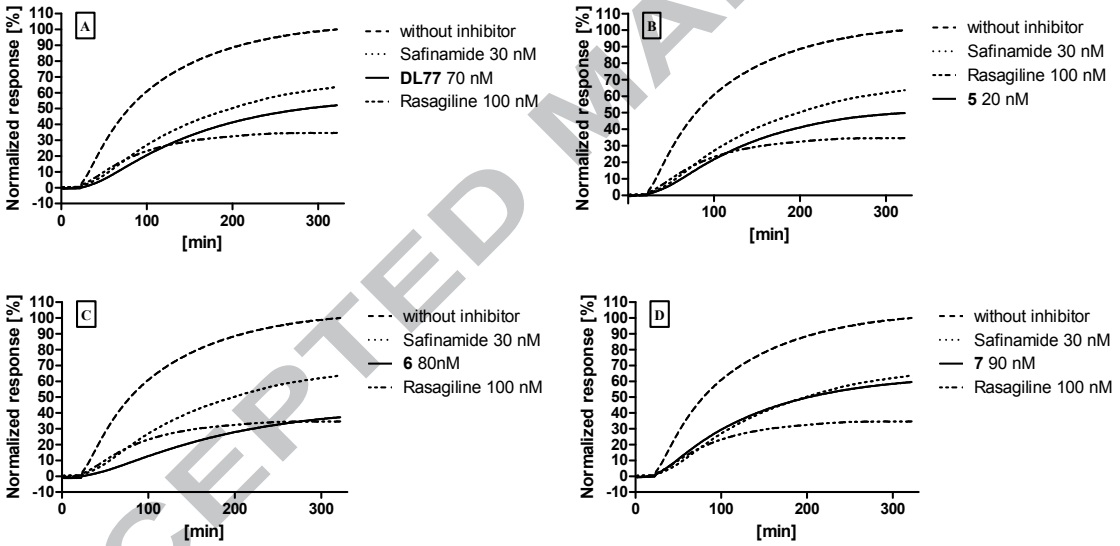


Figure 2. Investigation of reversibility of tested compounds **DL77** (**A**), **5** (**B**), **6** (**C**) and **7** (**D**).

To investigate the modality of reversible inhibition of 4-*tert*-pentylphenoxy derivatives on hMAO B, compound **5** was chosen for kinetic studies. For this purpose, the hMAO-B activity was measured for various concentrations of **5** (0 nM, 0.2 nM, 0.5 nM and 20 nM) in the presence of six concentrations of *para*-tyramine (0.05, 0.1, 0.5, 1.0, 1.5 and 2.0 mM). Saturation curves and Lineweaver-Burk plots were prepared. As illustrated in **Figure 3,** compound **5** displayed non-competitive inhibition (i.e. a decrease in V_{max} values; all lines of the plot intersect at the same point on the x-axis).

Figure 3. Lineweaver-Burk plot of **5**.

To investigate the binding mode of the obtained compounds and to explain the reasons of diverse activity as well as selectivity molecular modeling studies were performed. All derivatives were docked into the active site of hMAO A and hMAO B as described in **Supplementary data**. With respect to MAO-B all derivatives revealed similar general orientation within the active site of enzyme. The differences in details were responsible for varied activities (see **Figure A** in **Supplementary data)**. The heterocyclic moieties of ligands were located near cofactor (FAD) and interacted with Tyr398, Tyr435, Phe343 and Tyr60. The protonated amine group of pyrrolidine, piperidine and azepane created hydrogen bond with Gln206. Introduction of methyl groups into the position 3 or 4 of piperidine ring (*e.g.* compounds **8-11**) led to the occurrence of steric hindrance and usually decreased the activity. The linker always occurred in extended conformation. However, the shorter chains (e.g. compounds **5-7, DL77**) provided the better fit of aromatic part of ligands to the active site. Moreover, in case of 3-carbon linker hydrogen bond between oxygen atom of compounds and hydroxyl group of Tyr326 might be observed. The 4-*tert*-pentylphenyl moiety of compounds with three methylene groups in the chain was located in the lipophilic pocket, created by Trp119, Leu167, Phe168, Leu171, Cys172, Ile198, Ile199, Ile316 and Tyr326. In case of compounds with longer linkers the previously mentioned hydrogen bond with Tyr326 was not observed. Moreover, the 4-*tert*-pentylphenyl group was moved toward the edge of lipophilic pocket. Such changes in the binding mode led to significant decrease in the activity. The binding mode of the most potent compound **5** is presented in **Figure 4**. Comparing the results of docking to MAO-B with docking to MAO-A, the selectivity of ligands toward MAO B could be easily judged. Replacement of Tyr326 and Ile199 from MAO-B by Ile335 and Phe208, respectively in MAO-A led to significant change of shape and size of the active site of enzyme. This was responsible for essential steric clashes when the tested compounds were docked into MAO A and resulted in the lack of the activity (**Figure 5**).

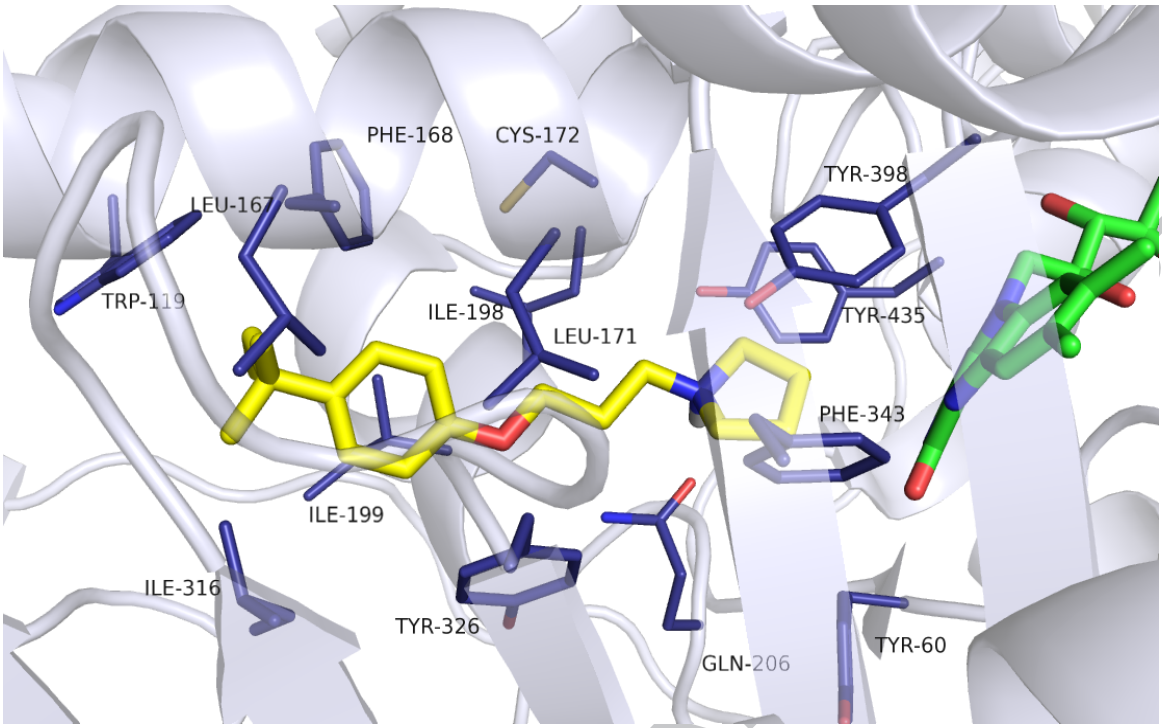


Figure 4. The binding mode of the most active compound **5** within the active site of monoamine oxidase B. The most important amino acid residues are shown as navy blue sticks while cofactor (FAD) as green ones.

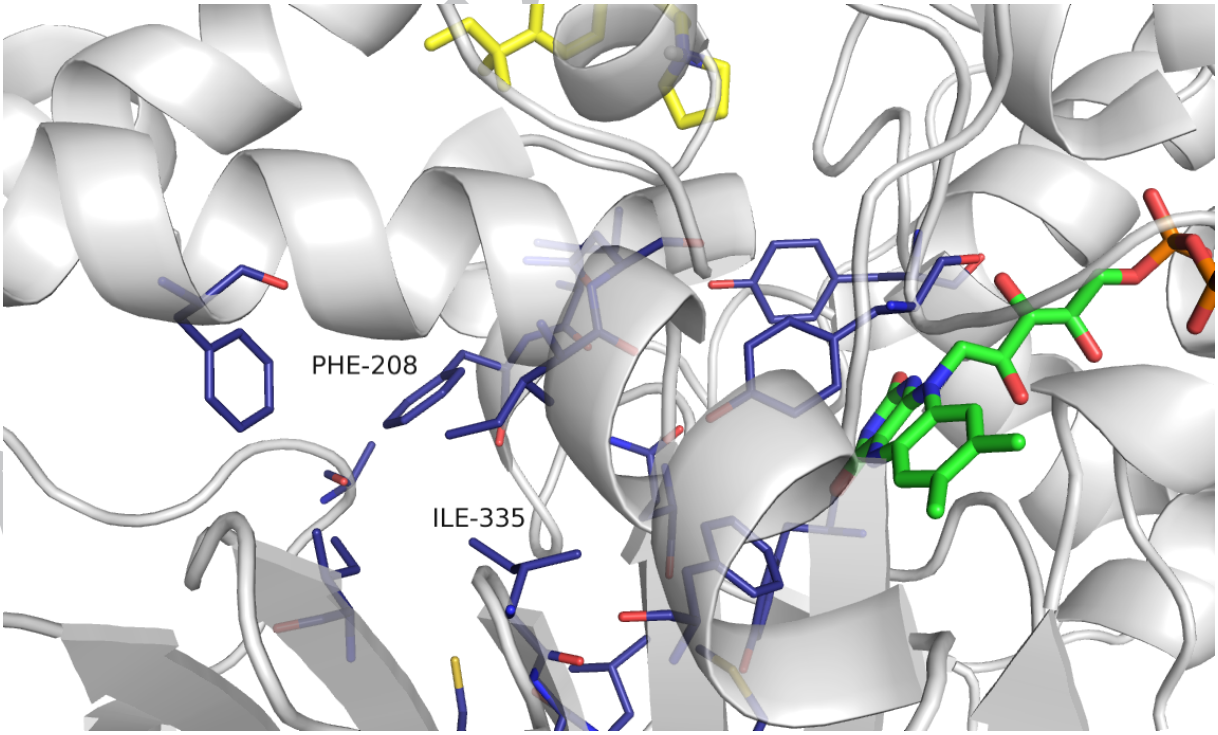


Figure 5. The top-ranked pose of compound **5** (yellow) was located outside of the active site when docked into monoamine oxidase A. The replacement of Tyr326 and Ile199 from MAO-B by Ile335 and Phe208 in MAO-A changed the size and shape of the pocket, leading to the steric clashes and disabling the entry of compound to the

active site. The most important amino acid residues are shown as navy blue sticks while cofactor (FAD) as green ones.

To sum up, the designed compounds, based on the H_3R ligand - **DL77**, were synthesized and evaluated *in vitro* for hH_3R affinity and hMAO B inhibition. The performed experiments confirmed the good affinity for hH_3R (K_i < 1000 nM) and high hMAO B inhibitory properties of a number of compounds. Among this series (**5-12**), three compounds exhibited excellent hMAO B inhibition in the lower micro-molar range ($IC_{50} \leq 20$ nM). These potencies are comparable to the reference inhibitor rasagiline, which, under identical conditions, inhibited hMAO B with an IC_{50} of 25 nM. The most potent compound in this series - **5** (IC_{50} = 4.5 nM) showed non-competitive and reversible mode of the enzyme inhibition. None of tested compounds showed any inhibitory activity for hMAO A. Molecular modeling studies helped to identify interactions between compounds and enzymes as well as understand the biological activity.

In conclusion, the presented 4-*tert*-pentylphenoxy scaffold is a new structural feature in hMAO B inhibitors as contilisant, like many compounds, contains in its structure a propargylamine moiety, which is considered to be responsible for MAO B inhibitory activity. Moreover, these results encourage us for further exploration of this class of compounds as potential active CNS agents as so far, not many nonimidazole hH_3R ligands have been described as hMAO B inhibitors.

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Supplementary data

Figure A, synthetic procedures and analytical data of compounds, assays of biological evaluations, and molecular modeling studies.

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- A series of 4-tertpentylphenoxy alkyl derivatives was synthesized and biologically evaluated
- Compounds tested *in vitro* showed human histamine H_3 receptor affinity and human monoamine oxidase B inhibitory activity
- Compound **5** displayed affinity for hH_3R with a K_i of 63 nM and inhibited hMAO B activity with an IC_{50} value of 4.5 nM
- Non-competitive inhibition of hMAO B for compound **5** in the enzyme kinetic study was determined
- For the most potent compounds investigation of the reversibility of hMAO B inhibition was performed