Search for MAO B Inhibition in Known Histamine H_3 Receptor Ligands

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Summary

Dual-targeting ligands (histamine H_3 receptor and MAO B inhibitors) are reported to be a promising approach for the treatment of Parkinson's Disease. [1] We chose the most active dual-target ligands from our compounds library for more detailed MAO-B inhibition studies. For this purpose, the reversibility and modality of the inhibition were examined, and results were compared with the reference MAO B inhibitor(s) showing a similar inhibition pattern.

Enzyme inhibitors evaluation flowchart

Initial IC_{50} evaluation → reversibility → modality of reversible inhibition [2].

Conclusions

Tested ligands inhibited MAO B in a reversible manner. Reversible inhibitors lead to less side effects connected with blockade of the enzyme, but they need to be more active than irreversible inhibitors (in nanomolar range) to achieve inhibition of at least 90 % of MAO B in the body (needed for clinical effectiveness) [1,8].

Tested ligands showed similarities to safinamide in reversibility and the mode of inhibition. IC_{50} of inhibitors can be compared only when they belong to the same group (e.g., irreversible to irreversible, reversible competitive to reversible competitive)[1-3] therefore safinamide can be compared and used as the reference with our ligands during *in-vitro* and *in-vivo* tests.

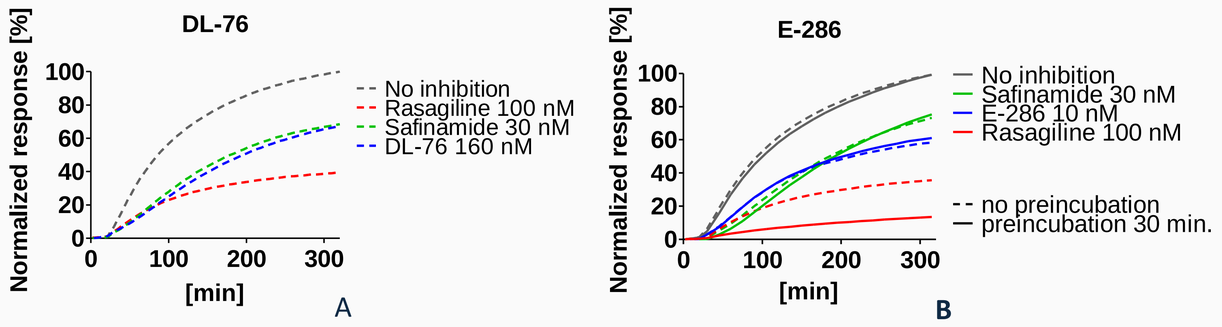


Fig. 1. Examples of curves from testing of MAO B reversibility.

Reversibility

•All compounds were tested in the concentration corresponding to their IC_{80} along with reference reversible (safinamide) and irreversible (rasagiline) inhibitors using testing protocol A, or A and B [4-6]:

A) Inhibitors and substrate were added to the enzyme solution simultaneously

B) inhibitors were preincubated with the enzyme for 30 minutes then substrate was added

• All tested compounds showed behaviour similar to safinamide (Fig. 1 A and B, Tab. 1) therefore were considered reversible.

• Compounds **safinamide**, **E-286, E-270, E-288, E-293, WS-24** tested in both A and B protocols showed no difference between preincubated and not preincubated curves suggesting that only non-covalent bonds were formed between enzyme and inhibitors. Curves for irreversible **rasagiline** showed difference between preincubated and non-preincubated (rasagiline needs to be activated by enzyme and to form covalent bond with the active centre [1] (Fig, 1 B)).

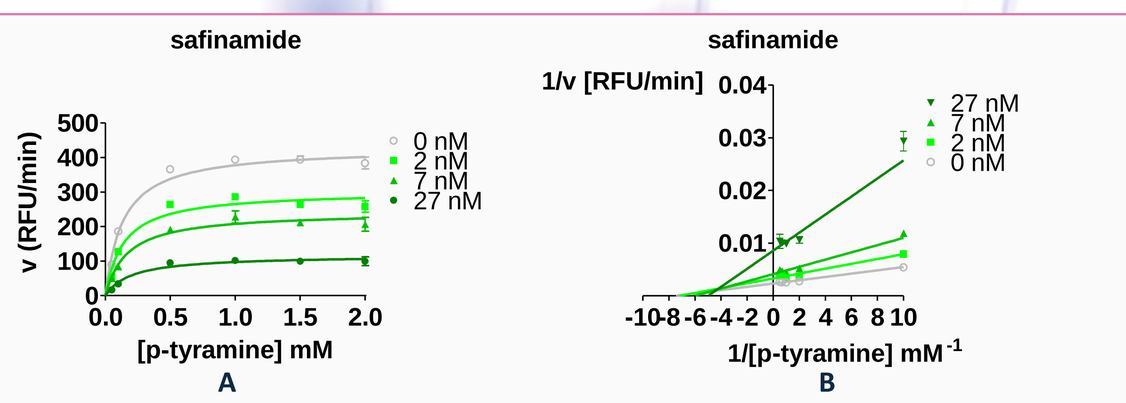


Fig. 2. Michaelis-Menten curves (A) and Lineweaver-Burk plot (B) for **Safinamide**. B: lines intersecting to the left of y axis suggest mixed inhibition, above x axis indicates subtype of mixed inhibition where affinity to the free enzyme is higher than to the enzyme-substrate complex. RFU – relative fluorescence unit, v - initial velocity.

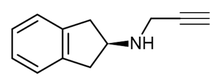
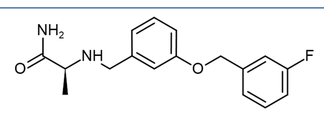
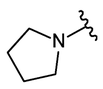
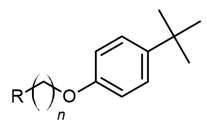
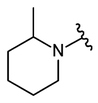
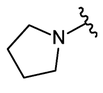
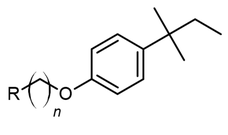
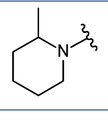
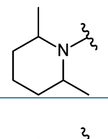
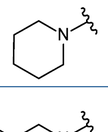
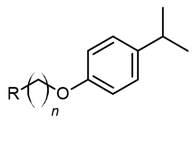
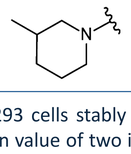
Modality

•Selected compounds **(safinamide, DL-76, E-270, E-289)** were tested in three concentrations (IC_{20}, IC_{50}, IC_{80}) using 6 concentrations of p-tyramine (0.05 mM, 0.1 mM, 0.2 mM, 0.5 mM, 1.5 mM, 2 mM) [4-6].

• Michaelis-Menten curves and Lineweaver-Burk plots were prepared from the data (Figs. 2+ 3).

• From apparent K_M, apparent V_{max} (calculated from Michaelis-Menten curves) and from apparence on Lineweaver-Burk plots we concluded that **safinamide** and all our compounds showed mixed inhibition mode (inhibitor binds to free enzyme and enzyme-substrate complex) with higher affinity towards the free enzyme (Tab. 1) [2,3].

Tab. 1. Structures of tested compounds and results from H_3R binding studies, and extended MAO-B inhibition studies.

No	Name	Structure			hH_3R affinity[a,b] K_i [nM] [95%CI] or ± SEM	hMAO B[c] inhibition		
		R	n	East side		IC_{50} [nM] ± SEM	Reversibility	Modality
1	**Rasagiline**				nt[d]	**16.9** ± 2.7 [e]	irreversible	nt [d]
2	**Safinamide**				nt[d]	**6.7** ± 1.1 [e]	reversible	mixed
3	**E-286**		3		**371** [a] [136;1009]	**2.7** ± 0.4 [f]	reversible	nt [d]
4	**DL-76**		3		**38** [a] [8;181] 22 ± 3 [h]	**48.1** ± 15.4 [f]	reversible	mixed
5	**E-270**		3		**69** [a] [49;96]	**10.8** ± 0.88 [f]	reversible	mixed
6	**WS-13**		3		**1110** ± 45 [g]	**15.4** ± 3.86	reversible	nt [d]
7	**E-289**		3		**63** ± 6.2 [b]	**4.5** ± 0.4[i]	reversible	mixed
8	**DL-77**		3		**8.4** ± 1.3 [h] **37** ± 5 [b]	**19** ± 7[i]	reversible	nt [d]
9	**E-293**		3		**437** ± 27 [b]	**20** ± 2[i]	reversible	nt [d]
10	**E-292**		3		**140** ± 15 [b]	**22** ± 2[i]	reversible	nt [d]
11	**WS-24**		3		**52** [24;113] [j]	**21** ± 3	reversible	nt [d]
12	**E-288**		3		**106** [69;162] [j]	**214** ± 19.1	reversible	nt [d]

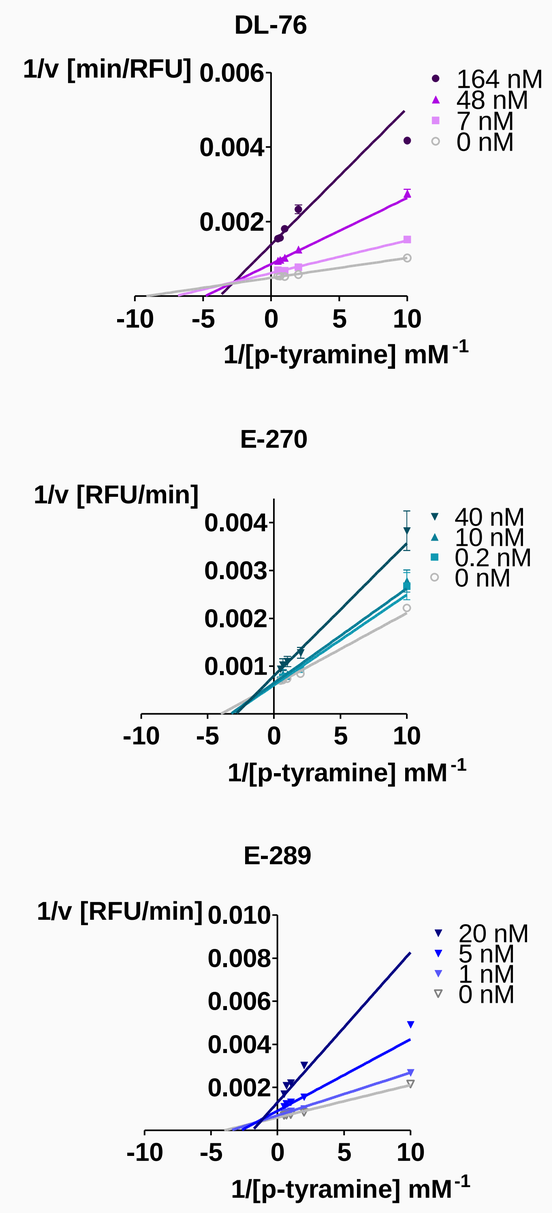


Fig. 3 Lineweaver-Burk plot for tested dual-ligands: **DL-76, E-270, E-289**. Lines intersect to the left of y axis (indicates mixed mode) and above the x axis (indicates higher affinity to the free enzyme). RFU – relative fluorescence unit, v - initial velocity.

a - Cell membrane preparation of HEK293 cells stably expressing *h*H_3R, radioligand [^{3}H]*N*$^{\alpha}$-Methylhistamine. Data presented as mean value within the 95% confidence interval [CI]. Data from [6]; **b**- Cell membrane preparation from CHO-K1 cells stably expressing hH_3R, radioligand [^{3}H]*N*$^{\alpha}$-Methylhistamine. Data presented as mean value of two independent experiments ± SEM. Data from [5]; **c** MAO-B inhibition was measured using human recombinant MAO B (Sigma Aldrich) and *p*-tyramine as substrate (described [5,6]) **d** – not tested; **e** – data for reference compounds are means from all experiments till present therefore may be different than the data presented in our publications; **f** – data from [6]; **g**- data from Agata Siwek, PhD. Data presented as mean ± SEM; **i** – data from [5]; **j** - data from Stark Lab. Cell membrane preparation of HEK-293 cells stably expressing hH_3R, radioligand [^{3}H]*N*$^{\alpha}$-Methylhistamine. Data presented as mean within the 95% confidence interval [CI;]; **h** - cell membrane preparation from CHO-K1 cells stably expressing hH_3R, radioligand [^{125}I] Iodoproxyfan. Data presented as mean value of two independent experiments ± SEM, from: [7].

Reference: [1] Ramsay et al. Front. Neurosci. 2016, 10, doi:10.3389/fnins.2016.00375. [2] Copeland, R.A.; John Wiley & Sons, Inc.: Hoboken, New Jersey, 2005; ISBN 0-471-68696-4.[3] Copeland, R.A. Willey-VCH: New York, 2000; ISBN 0-471-22063-9. [4] Stößel, A. et al. J. Med. Chem. 2013, 56, 4580–4596. [5] Łażewska, D et al. Bioorganic Med. Chem. Lett. 2018, 28, 3596–3600, doi:10.1016/j.bmcl.2018.10.048. [6] Łażewska, D.et al. Int. J. Mol. Sci. 2020, 21, doi:10.3390/ijms21103411.[7] Łażewska, D. et al. Bioorg. Med. Chem. 2006, 14, 3522–3529, doi:10.1016/j.bmc.2006.01.013. .[8] Ramsay, R.R. Prog. Neuro-Psychopharmacology Biol. Psychiatry 2016, 69, 81–89, doi:10.1016/j.pnpbp.2016.02.005.

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