*Supplementary Materials S2*

# Dual target ligands with 4-*tert*-butylphenoxy scaffold as histamine $H_3$ receptor antagonists and monoamine oxidase B inhibitors

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*hMAO B Kinetic studies*

The $\alpha$ values for different modes of reversible inhibition as well as their diagnostic signature on double-reciprocal plot are shown in Table S1. The $\alpha$ value for compound **9** was calculated by GraphPad Prism software from the nonlinear regression curves using following equation for mixed-model of inhibition:

$$v = V_{max}*[S] / [S]*(1+ [I]/ \alpha K_i) + K_M*(1+ [I]/K_i) \quad (1)$$

v – velocity of enzyme reaction. $V_{max}$ - maximum velocity (at infinite substrate concentration); $K_i$ – dissociation constant for Enzyme-Inhibitor complex (in the enzymology literature other symbols can be used such as: $K_i$, $K_{ii}$ , $K_{EI}$ etc.); $\alpha K_i$ – dissociation constant for Enzyme-Substrate-Inhibitor complex (in the literature also under the symbols: $K_i'$, $K_{is}$ and $K_{ES}$); $K_M$ – Michaelis-Menten constant; [I]– concentration of the inhibitor; [S] – concentration of the substrate [Ref S1].

**Table S1.** Relation between inhibition modality, $\alpha$ value, and diagnostic signature on the double-reciprocal plots (i.e Lineweaver-Burk plot) [S2].

| $\alpha$ | Inhibition modality | Diagnostic signature on double-reciprocal plot |
|---|---|---|
| $\alpha = 1$ | **noncompetitive** (inhibitor binds to free enzyme and enzyme-substrate complex with equal affinity) | lines converge at the x-axis |
| $\alpha > 1$ | **mixed mode inhibitor** (inhibitor can bind to free enzyme and enzyme-substrate complex unequally), inhibitor's affinity is higher for free enzyme | lines converge to the left of the y-axis and above the x-axis |
| $\alpha < 1$ | **mixed mode inhibitor** (inhibitor can bind to free enzyme and enzyme-substrate complex unequally), inhibitor's affinity is higher for the enzyme-substrate complex) | lines converge to the left of the y-axis and below the x-axis |
| $\alpha \to \infty$ | **competitive** (inhibitor and substrate compete for the same site of binding) | lines converge at the y-axis |
| $\alpha \to 0$ **and** $\alpha > 0$ | **uncompetitive** (inhibitor binds only to the enzyme-substrate complex) | parallel lines |

References:

S1. Copeland, R.A. Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis.; 2nd ed.; Willey-VCH: New York / Chichester / Weinheim / Brisbane / Singapore / Toronto, 2000; ISBN 0-471-22063-9.

S2. Copeland, R.A. Evaluation of Enzyme Inhibitors in Drug Discovery. A Guide for Medicinal Chemists and Pharmacologists, John Wiley & Sons, Inc., Hoboken, New Jersey, **2005.**