Neurotransmitter selection by monoamine oxidase isoforms, dissected in terms of function groups by novel thermodynamic cycles

L. Hudspith, F. Shmam.C. Dalton, A. Princivalle and S. M. Turega

Supporting information

Experimental

Materials and Methods. Chemicals used were bought from Sigma Aldrich, or Fisher Scientific. 3-Methoxyphenethylamine was purchased from Fluorochem Ltd., Glossop, UK. Human MAOA and Human MAOB were purchased though Sigma Aldrich with product codes of M7316 and M7441 respectively. Molecular grade DMSO was used in all kinetic experiments

Buffers for substrates and enzymes. Phosphate buffer solution (50 mM, pH 7.47, ionic strength 154 mM) was prepared by dissolving $NaH_2PO_4.2H_2O$ (7.8 g, 156 g/mol) and KCl (1.54 g, 74.6 g/mol) in 900 mL of de-ionised water. The pH was altered using NaOH (1 M) and HCl (1 M) to 7.51 at 20 °C. The volume was made up to 1 L with de-ionised water.

Stock solutions of substrates(0.06-32 mM) were made in Phospate buffer containg 10% v/v. Phosphate buffer solution (50 mM, pH 7.47, ionic strength 154 mM, 10.0 % v/v DMSO) was prepared by dissolving $NaH_2PO_4.2H_2O$ (7.8 g, 156 g/mol), KCl (1.54 g, 74.6 g/mol) and Molecular Biology grade DMSO (100 mL) in 800 mL of de-ionised water. The pH was altered using NaOH (1 M) and HCl (1 M) to 7.51 at 20 °C. The volume was made up to 1 L with de-ionised water.

Stock solutions of MAOA and MAOB - MAOA and MAOB enzymes (0.5 mL, 5 mg/mL) in a solution of potassium phosphate (100 mM, pH 7.4, 0.25 mM sucrose, 0.1 mM EDTA, 5% glycerol) were defrosted on ice upon arrival and aliquotted to produce working solutions (3.8 active units, 720 µL) upon dilution. The aliquots were then quickly frozen to −80 °C. Before use the aliquots were defrosted over ice and diluted with phosphate buffer (50 mM, pH 7.47, ionic strength 154 mM).

Chromogenic solution. Amplex Red® solution - The working solution (1 mM, 20 mL) was prepared by dissolving Amplex red® (5 mg, 257.25 g/mol) in molecular grade DMSO (1 mL) which was subsequently diluted with phosphate buffer (9 mL, 55 mM, pH 7.47, ionic strength 154 mM) and phosphate buffer (10 mL, 50 mM, pH 7.47, ionic strength 154 mM, 10.0 % v/v DMSO). The solution was then aliquotted and stored away from light at -20 °C.

Horseradish peroxidase (HRP) solution - The working solution (20 mM, 100 mL) of HRP was prepared by dissolving HRP (8 mg) into phosphate buffer (100 mL, 50 mM, pH 7.47, ionic strength 154 mM, 0.0 % v/v DMSO). This was aliquotted and stored at -20 °C.

The chromogenic solution was prepared from phosphate buffer solution (1.4 mL, 50 mM, pH 7.47, ionic strength 154 mM), HRP solution (700 µL, 20 mM) and Amplex Red solution (700 µL 1mM). The solution was made fresh each day and stored at 4 °C away from light.

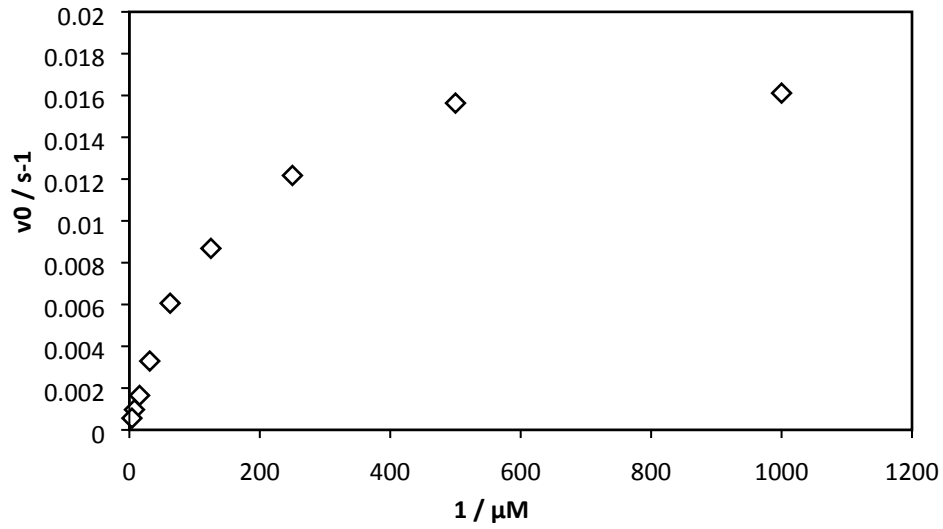
MAO substrate binding assays. All analyses were performed using a BMGtech CLARIOstar plate reader with Nunc black, 384 well, flat bottomed, non-binding plates incubated at 37 °C. Resorufin was excited at 580 nm and the fluorescence monitored at 620 nm. The reaction mixtures consisted of 5 µL MAO solution, 20 µL chromogenic solution and 25 µL dilute substrate solution. Kinetics data acquisition began immediately after initiation with the MAO enzyme, and was obtained over 15 minutes. Reaction solutions contained MAO (0.38 U ml^{-1}), substrate (0.03 - 16 mM), HPR 2.5 U ml^{-1} and Amplex Red® (0.05 mM). Values of FU were converted in to molar values using a H_2O_2 standard curve.

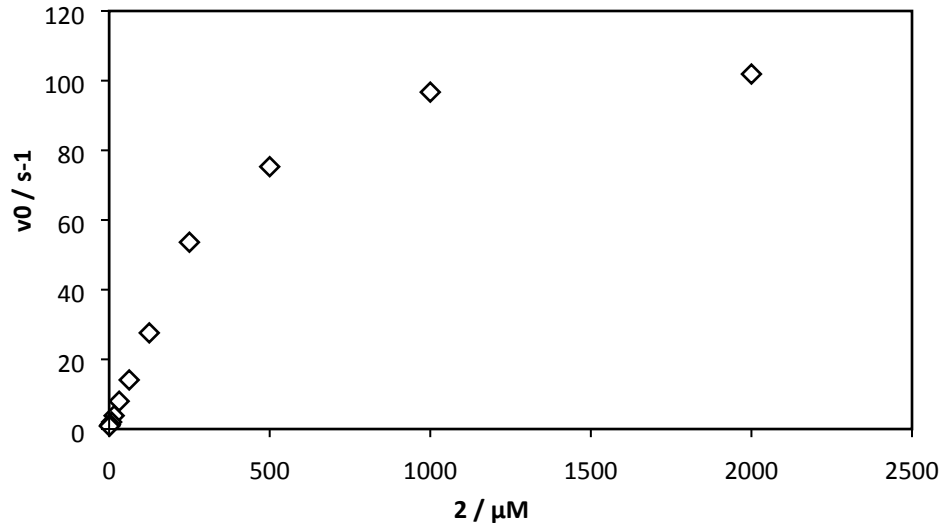
Running Gold. The docking studies in this work used GOLD 5.4.1 running on a Windows PC. Crystal structures of MAOA (PDB code: 2Z5X) and MAOB (PDB code: 3PO7) were retrieved from the Protein Data Bank, the non-covalent inhibitor ligands and majority of water molecules were removed, the MAOA model retained seven active site waters identified in the literature, the MAOB model retained ten waters identified in the literature.[1,2] In both models the three water molecules in the aromatic cage of the active site were set to be "on" in all dockings, other water molecules set to toggle "on" or "off". The models were tested by docking the non-native conformations of the non-covalent inhibitor ligands found in the original Protein Data Bank structures. Substrates were docked into the active sites of MAOA and MAOB allowing the observation of conformations containing hydrogen bonds between substrates **1**, **2, 4** and active sites while the distances between the cofactor and N-atom of the substrate correlated with published studies.[3]

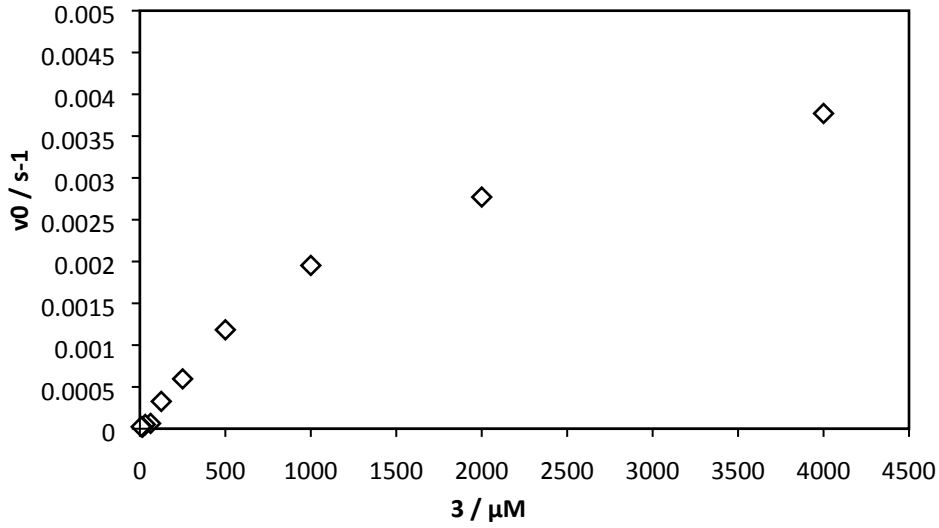
MM curves

Sample Michaelis Menten curves for the MAOA and MAOB enzymes with substrates 1 to 7

MAOA



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0.0025
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1000
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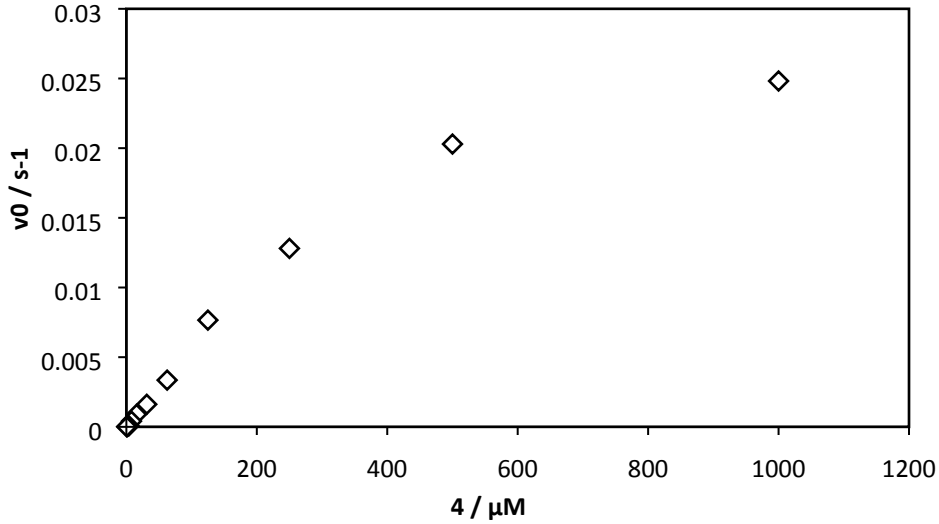
3000
3500
4000
4500
3 / µM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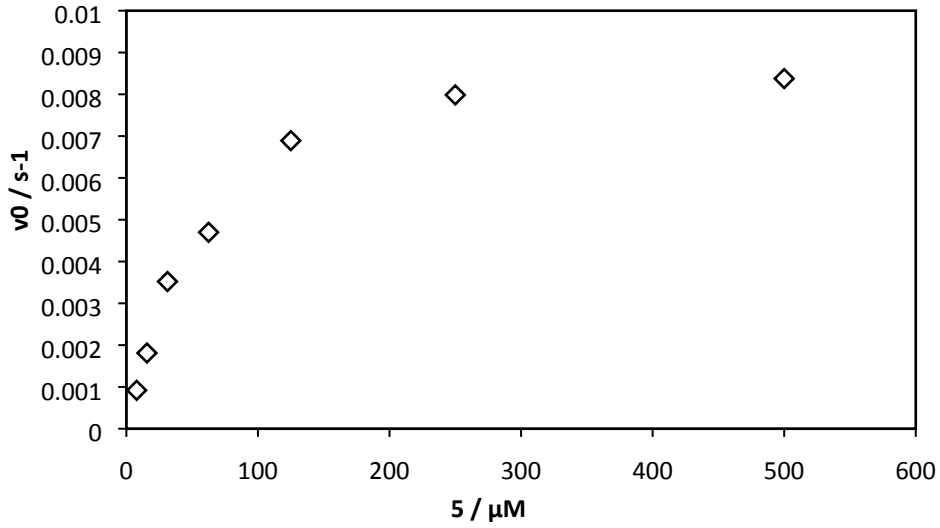
4 / µM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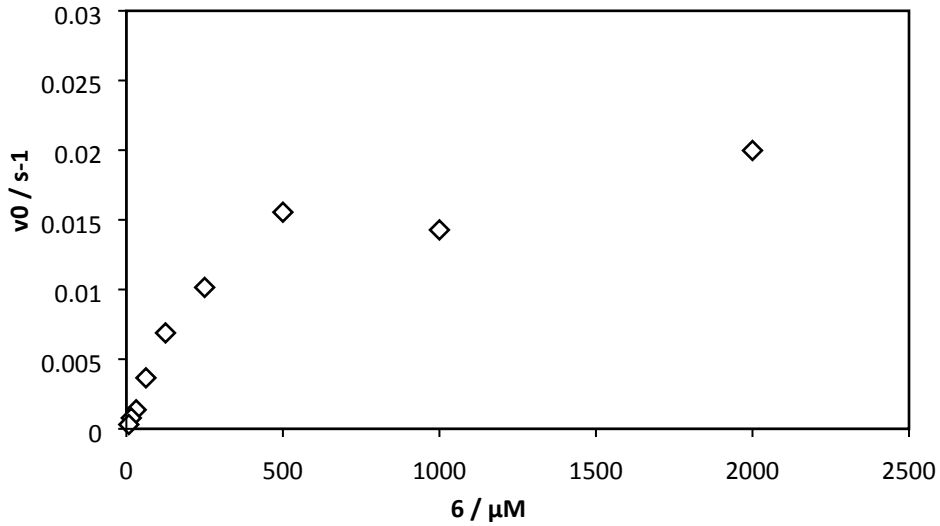
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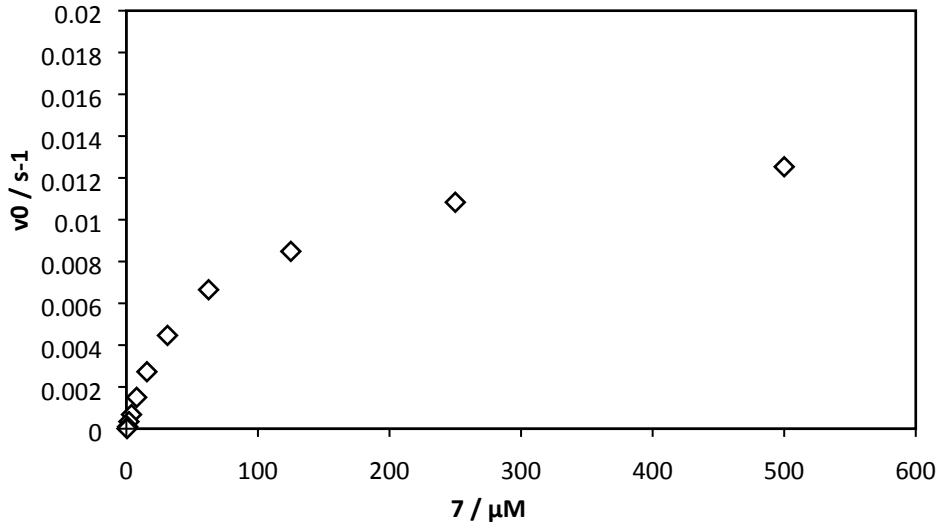
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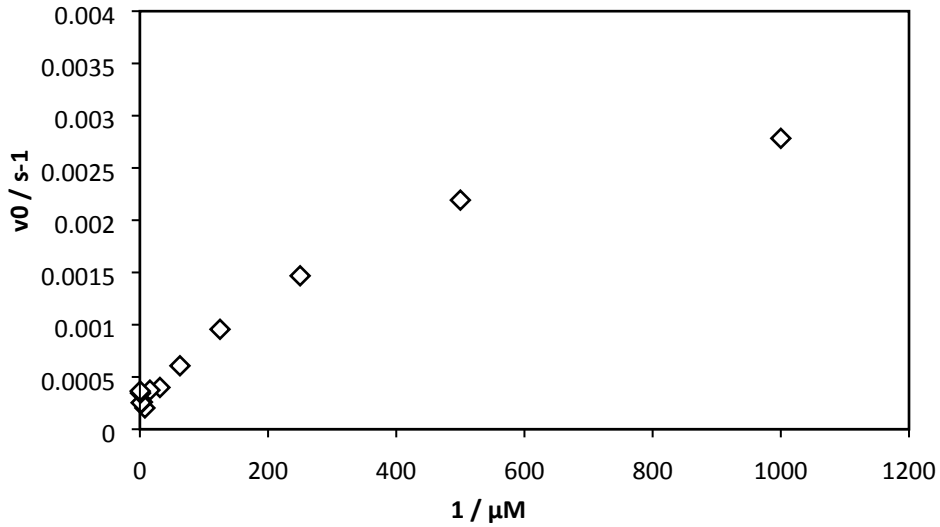
MAOB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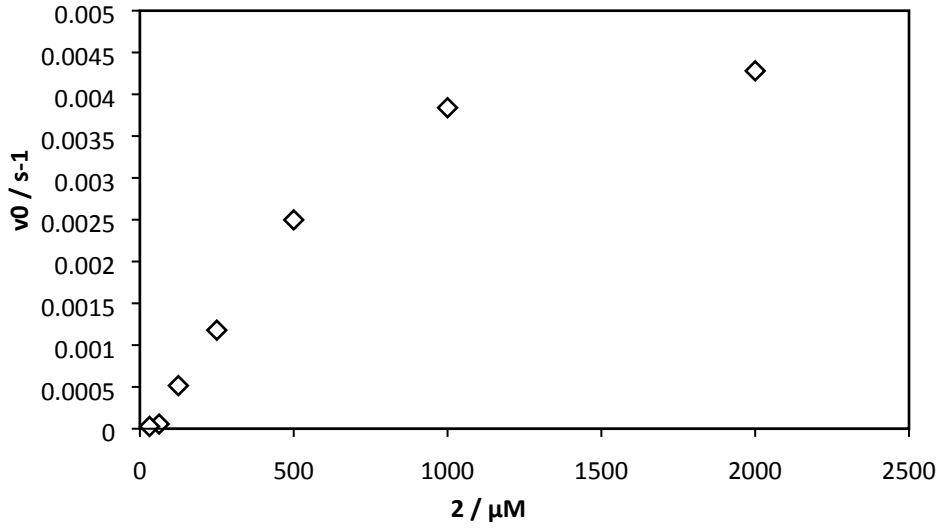
1 / µM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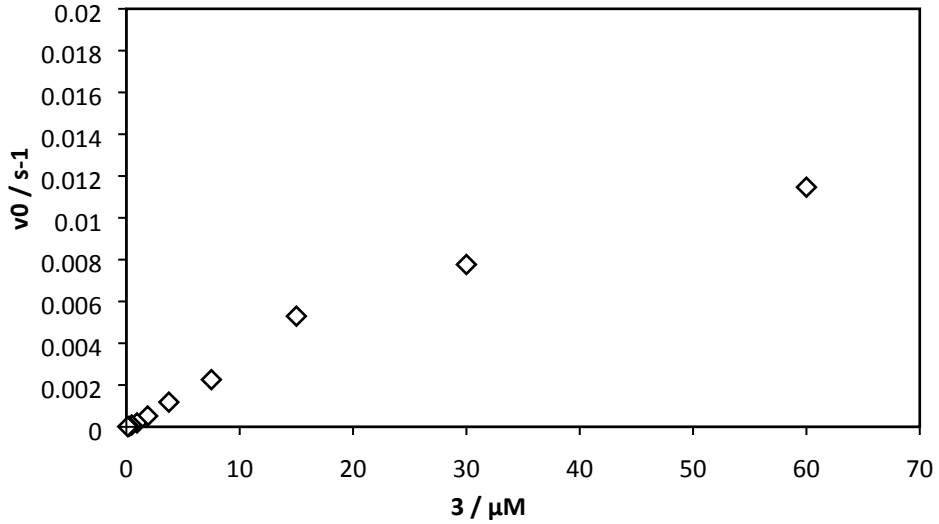
2 / µM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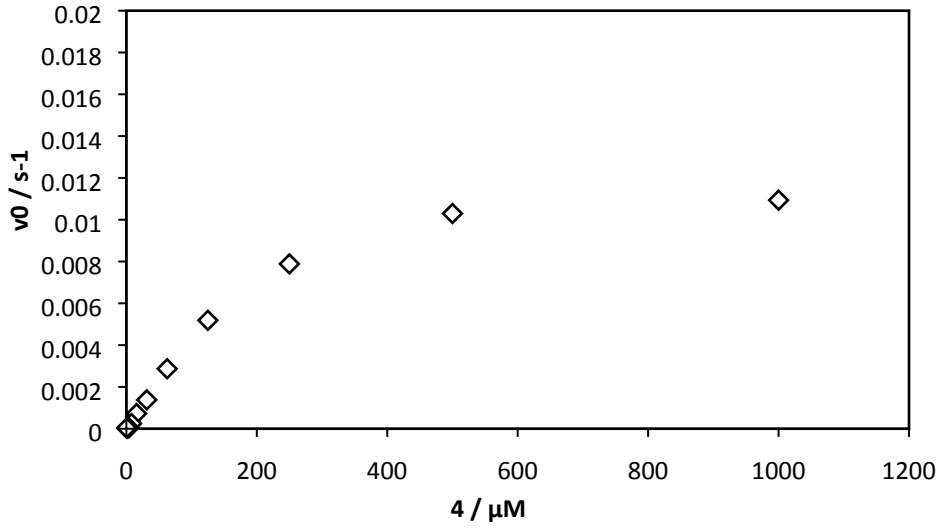
3 / µM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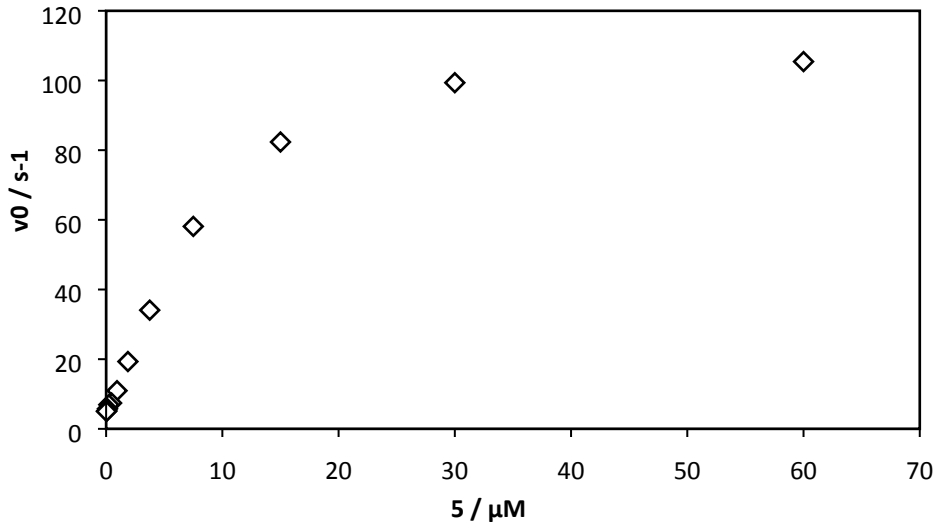
4 / µM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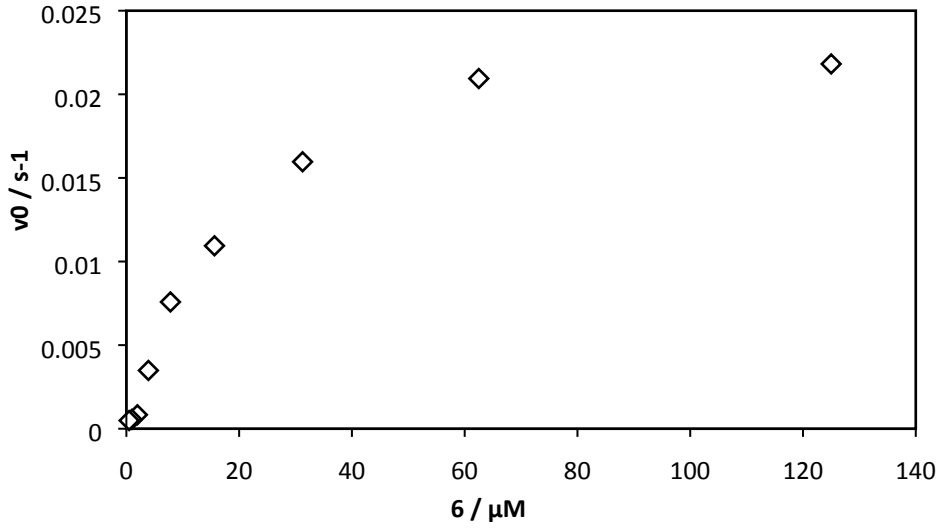
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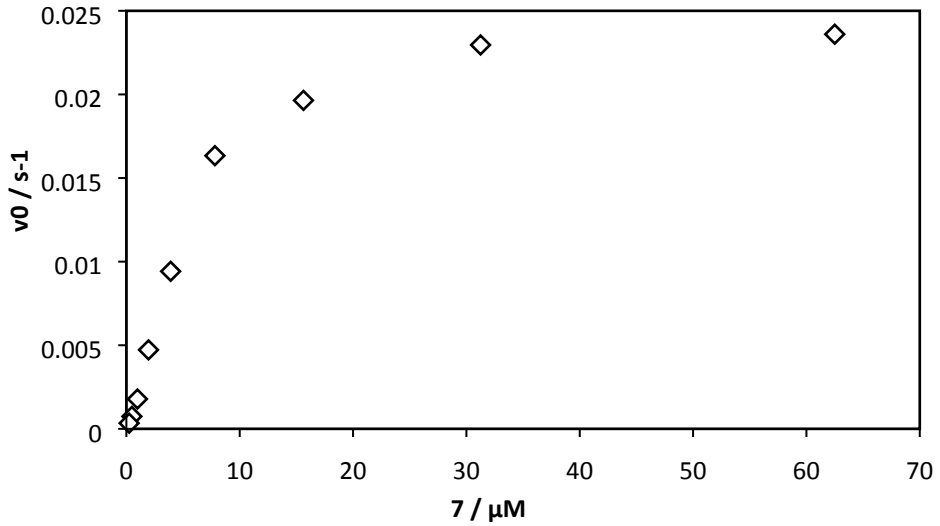
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7 / µM

Additional figures

TableS1. Values for ΔG substrate binding for compounds 1-7 measured 310 K with both MAOA and MAOB.

Substrate	MAOA $\Delta G_{substrate\ binding}$ / kJ mol^{-1}	MAOB $\Delta G_{substrate\ binding}$ / kJ mol^{-1}
1	-21 ±0.1	-20 ±0.7
2	-17 ±0.1	-17 ±0.1
3	-16 ±0.5	-25±0.2
4	-20 ±0.1	-22 ±05.
5	-26 ±0.2	-27 ±0.2
6	-21 ±0.2	-28 ±0.3
7	-24 ±2	-30 ±0.5

Table S2. Single arm ΔΔG for the substitution of R1 for R2 that dissect the molecular recognition of dopamine **1** in a series of stepwise substitutions, measured at 310K.

R1	**R2**	**MAO**	**ΔΔG / kJ mol^{-1}**	**MAO**	**ΔΔG / kJ mol^{-1}**
1	**7**	**A**	**-3.5 ±0.2**	**B**	**-10.3 ±0.6**
2	**1**	**A**	**-4.7 ±0.2**	**B**	**-3.5 ± 1**
2	**5**	**A**	**-9.2 ±0.1**	**B**	**-10.3 ±0.2**
3	**2**	**A**	**-0.9 ±0.6**	**B**	**8.6 ±0.6**
3	**4**	**A**	**-4.1 ±0.6**	**B**	**3.6 ±0.8**
4	**1**	**A**	**-1.6 ±0.2**	**B**	**1.5 ±0.7**
4	**6**	**A**	**-1.2 ±0.2**	**B**	**-6.8 ±0.3**
5	**7**	**A**	**1.0 ±0.2**	**B**	**-3.6 ±0.6**

Table S3. Change in free energy for substrate binding for all possible the substitution of R2 for R1 for MAOA and MAOB individually measured at 310 KΔΔG

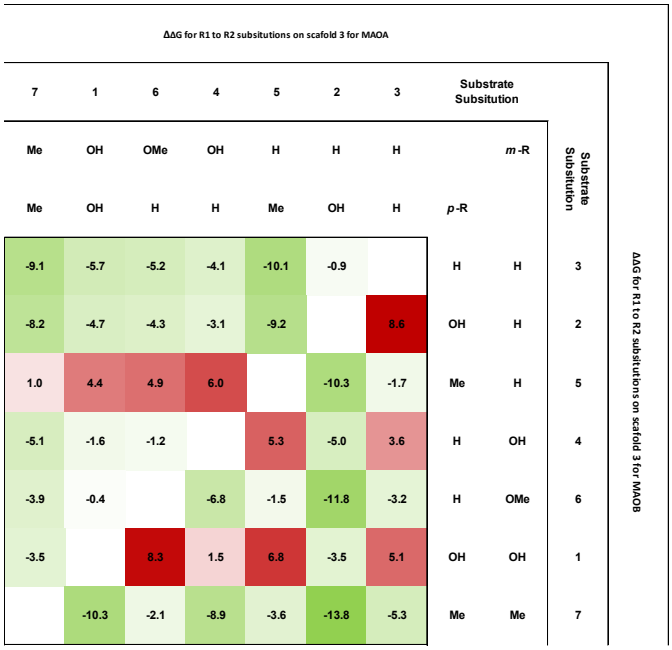
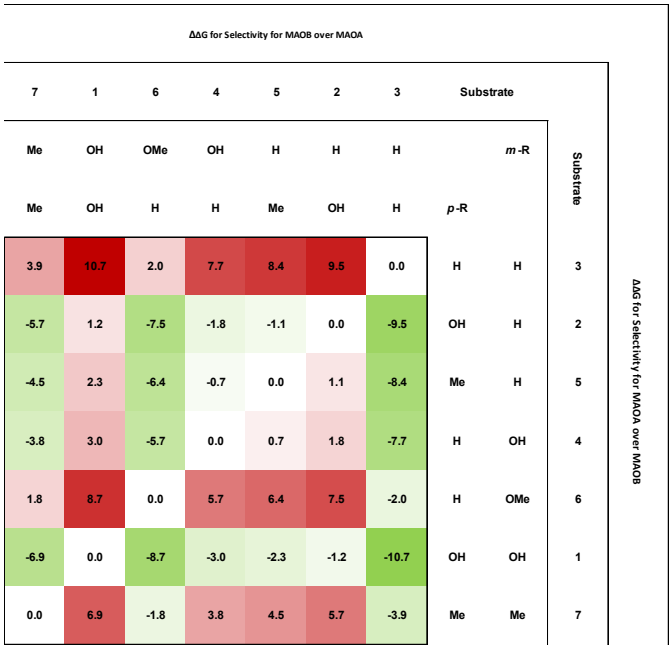
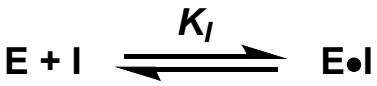


Table S4. Measured ΔΔG for the DMC in built from all possible combinations of single and double functional group substitutions using substrates **1** to **7**, at 310K.



Michaelis-Menten mechanism



Competitive (non-covalent) reversible inhibition

Competitive (covalent) irreversible inhibition

Figure S1. Mechanistic details describing the binding step Ks of the Michaelis-Menten mechanism, the binding step KI for competitive (non-covalent) inhibition the Ki binding step for competitive (covalent) irreversible inhibition.

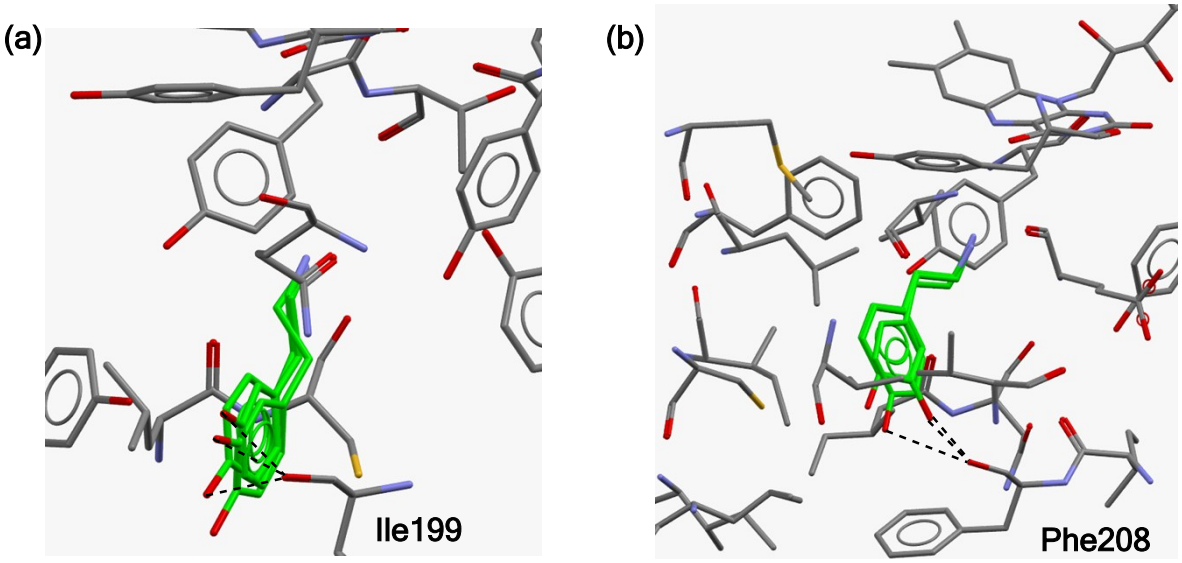


Figure S2. GOLD docking conformations of substrates **1**, **2** and **4** with the active sites of MAOB and MAOA, substrates show hydrogen bonds (dashed line) between active site residues (a) Ile199 in MAOB and (b) Phe208 in MAOA.

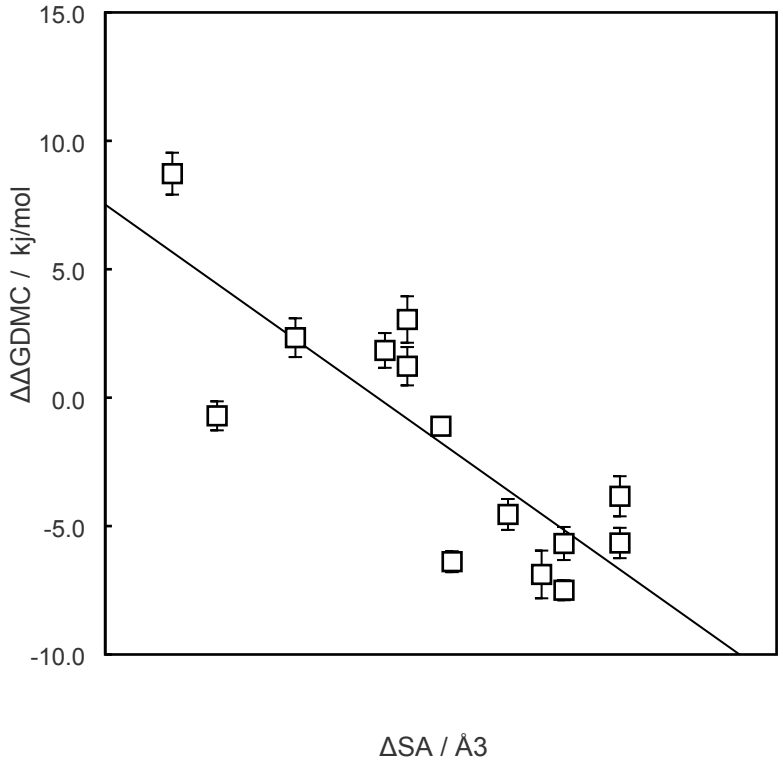


Figure S3, The plot of change in surface area (ΔAS / Å^2) against ΔΔG for the DMC in built from all possible combinations of single and double functional group substitutions using substrates **1** to **7**, the linear trend line has $R^2 = 0.663$.

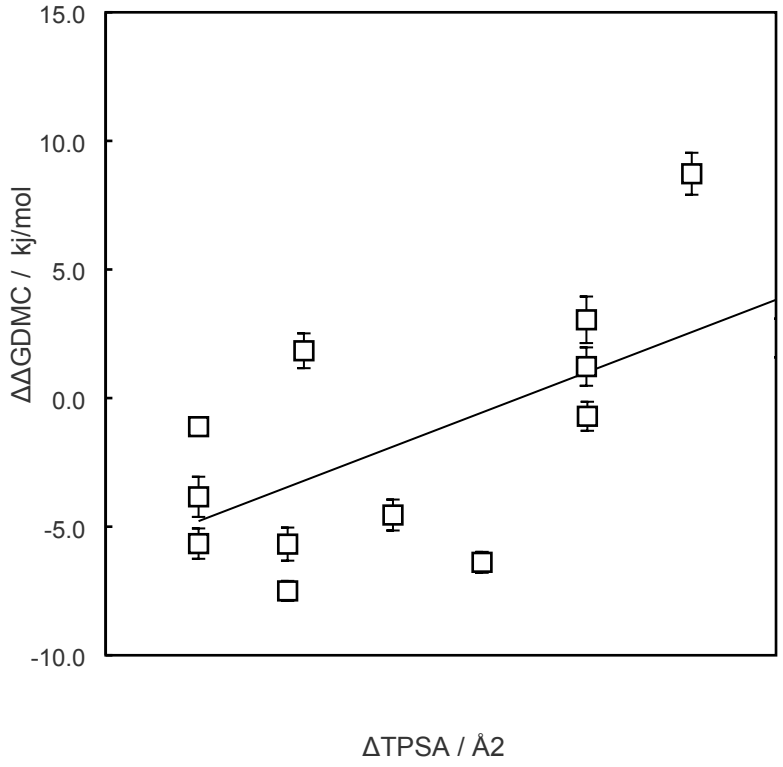


Figure S4, The plot of change in total polar surface area (ΔTPSA / Å^2) against ΔΔG for the DMC in built from all possible combinations of single and double functional group substitutions using substrates **1** to **7**, the linear trend line has $R^2 = 0.490$.

1 S. Son, J. Mat, Y. Kondou, M. Yoshimura, E. Yamashita and T. Tsukihara, *Proc. Natl. Acad. Sci. U. S. A.,* 2008, **105**, 5739.
2 C. Binda, M. Aldeco, A. Mattevi and D. E. Edmondson, *J. Med. Chem.,* 2011, **54**, 909
3 A. Maršavelski and R. Vianello, *Chemistry – A European Journal,* 2017, **23**, 2915-2925