SUPPORTING INFORMATION

for

4-(3-Aminoazetidin-1-yl)pyrimidin-2-amines as High-Affinity Non-imidazole Histamine H_3 Receptor agonists with In Vivo Central Nervous System Activity

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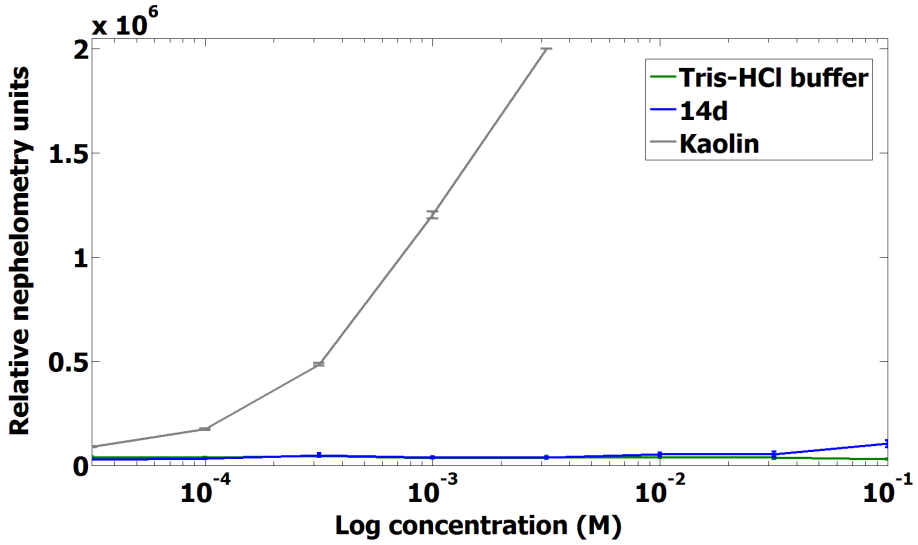


Figure S1. Representative plot for nephelometry measurements at different concentrations of **14d** (blue). A kaolin dispersion was used as a positive control (grey) and Tris-HCl buffer as negative control (green). Experiments were performed in triplicate and data points represent the mean ± SEM of a representative experiment.

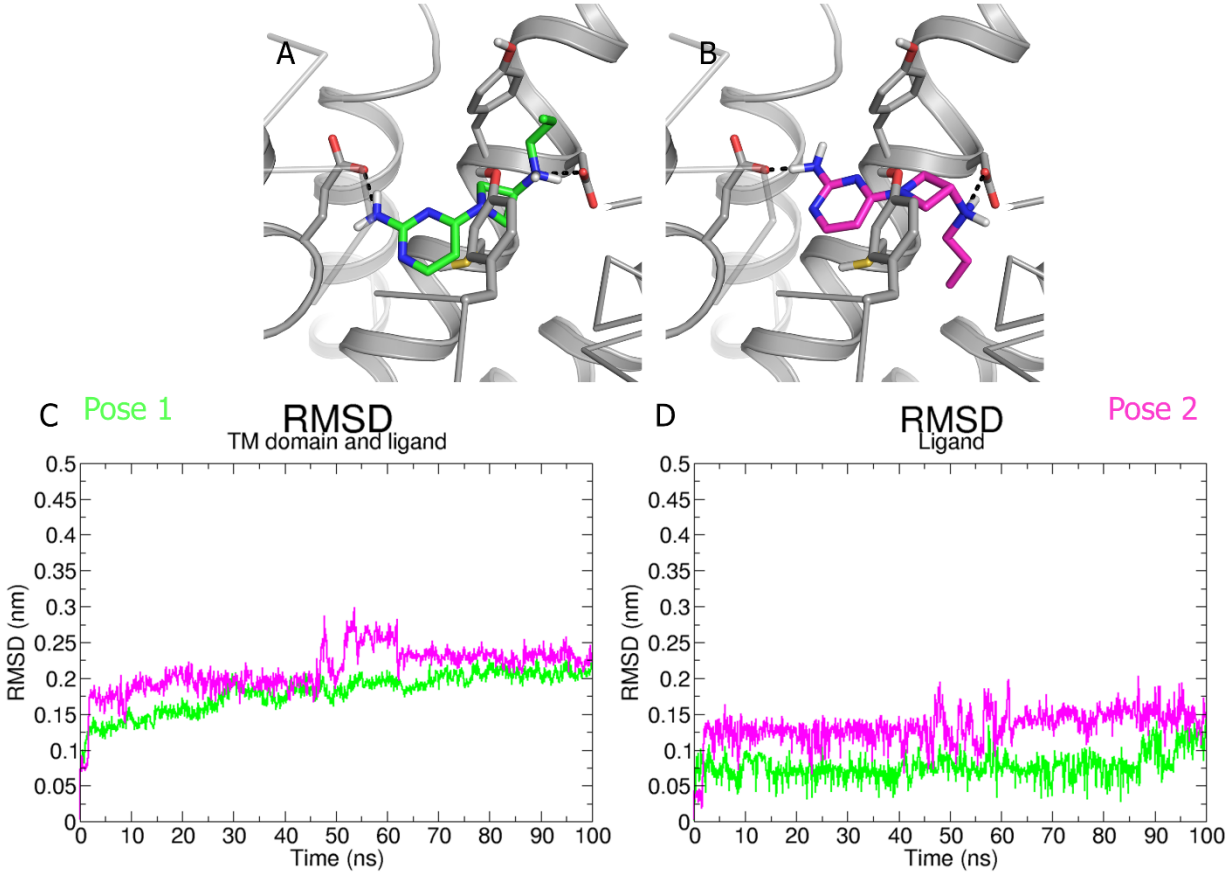


Figure S2. Best-scored docking poses of **14d**. Ligand **14d** shows a different positioning of the linear propyl moiety at the R^2 position: (**A**) towards the extracellular surface of the receptor or (**B**) towards the intracellular site. (**C**) RMSD in nm of the transmembrane domain-ligand complex during the 100 ns of simulation time. (**D**) RMSD in nm of the ligand during the 100 ns of simulation time.

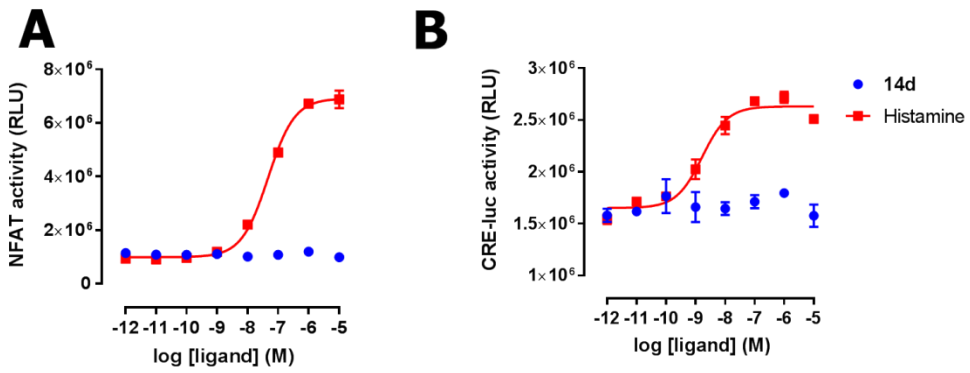


Figure S3. Ligand-induced (**A**) H_1R-mediated NFAT-luc activity and (**B**) H_2R-mediated CRE-luc activity. Representative graphs of at least three experiments performed in triplicate are shown

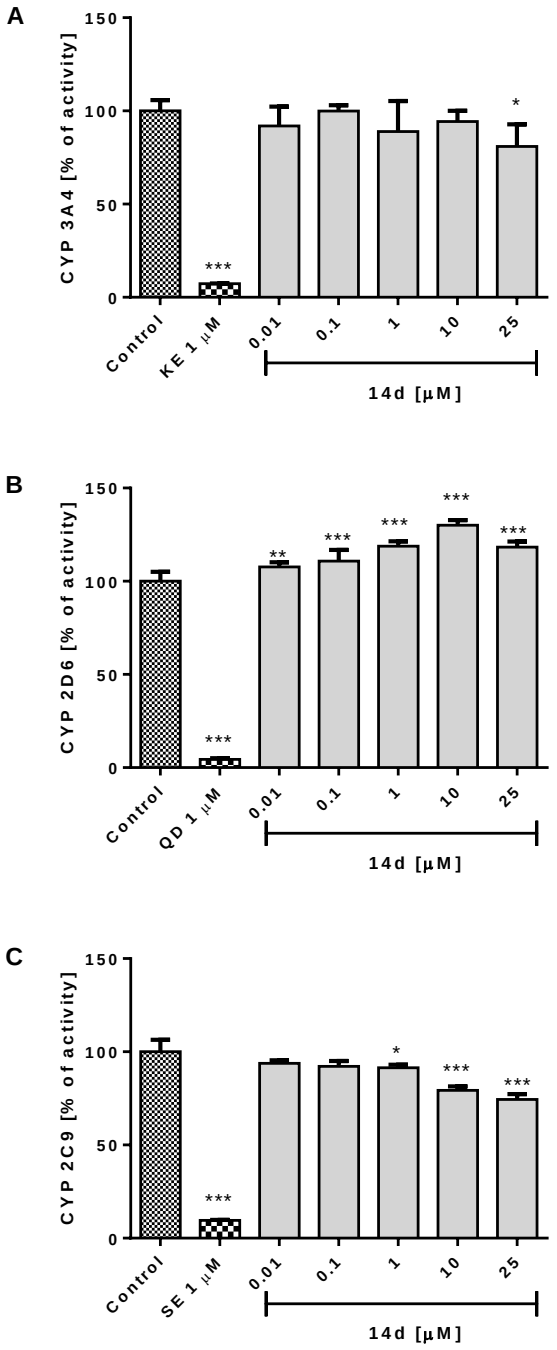


Figure S4. Effect of **14d** on (**A**) CYP3A4, (**B**) CYP2D6 and (**C**) CYP2C9 activity. Statistical significance was evaluated by one-way ANOVA, followed by Bonferroni's comparison test (*$p<0.05$, **$p<0.01$, ***$p < 0.001$). Reference inhibitors: KE – ketoconazole, QD – quinidine, SE –sulfaphenazole.

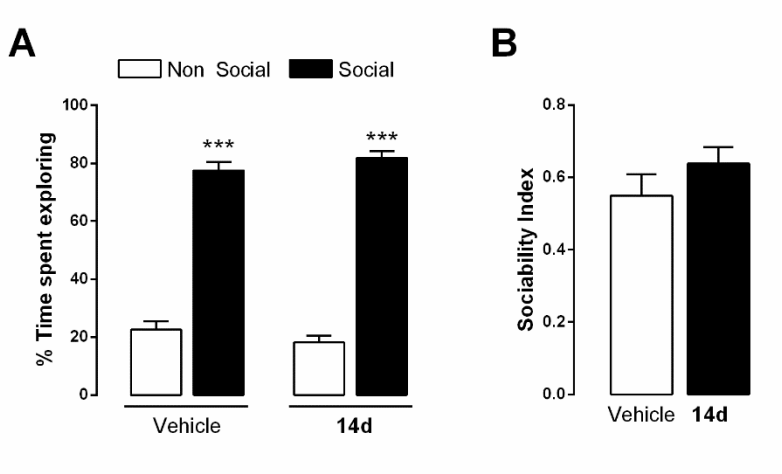
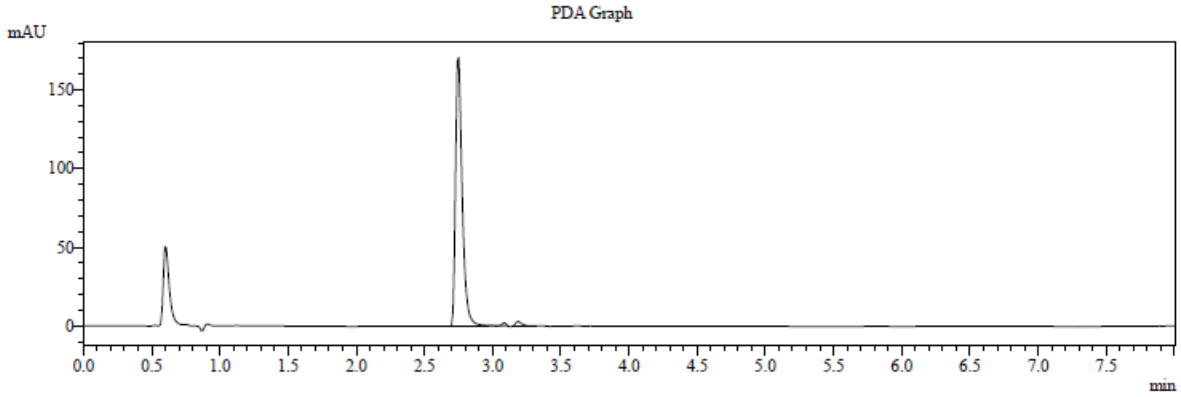
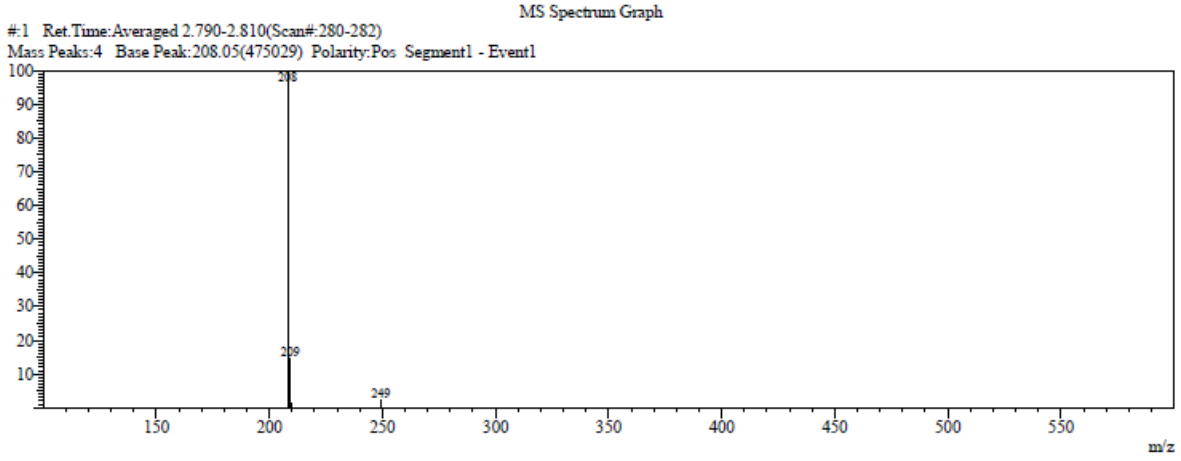


Figure S5. Compound **14d** does not affect animals' sociability. **A**) Results are calculated as means of individual percentage of time spent exploring non-social (empty, white columns) and social (containing a juvenile mouse, black columns) cups. ***P<0.001 vs. respective non-social subject (Two-way ANOVA and Bonferroni's MCT). **B**) Sociability index calculated according to the formula tS-tNS/tS+tNS. Shown are means±S.E.M. of 10-11 animals per experimental group.



PDA Ch1 254nm 4nm

Peak#	Name	Ret. Time	Area	Area %
1		2.742	583706	97.924
2		3.079	3610	0.606
3		3.182	8767	1.471



MS Spectrum Table

#:1 Ret.Time:
BG Mode:Calc 2.690<->3.050(270<->306)
Mass Peaks:4 Base Peak:208.05(475029) Polarity:Pos Segment1 - Event1

#	m/z	Abs.Inten.	Rel.Inten.	Charge	Polarity	Monoisotopic	#	m/z	Abs.Inten.	Rel.Inten.	Charge	Polarity	Monoisotopic
1	208.05	475029	100.00				3	210.10	5354	1.13			
2	209.10	68078	14.33				4	249.15	10801	2.27			

Figure S6. HPLC-MS chromatogram of compound **14d**. The peak at 0.6 min represents the fumarate counterion.

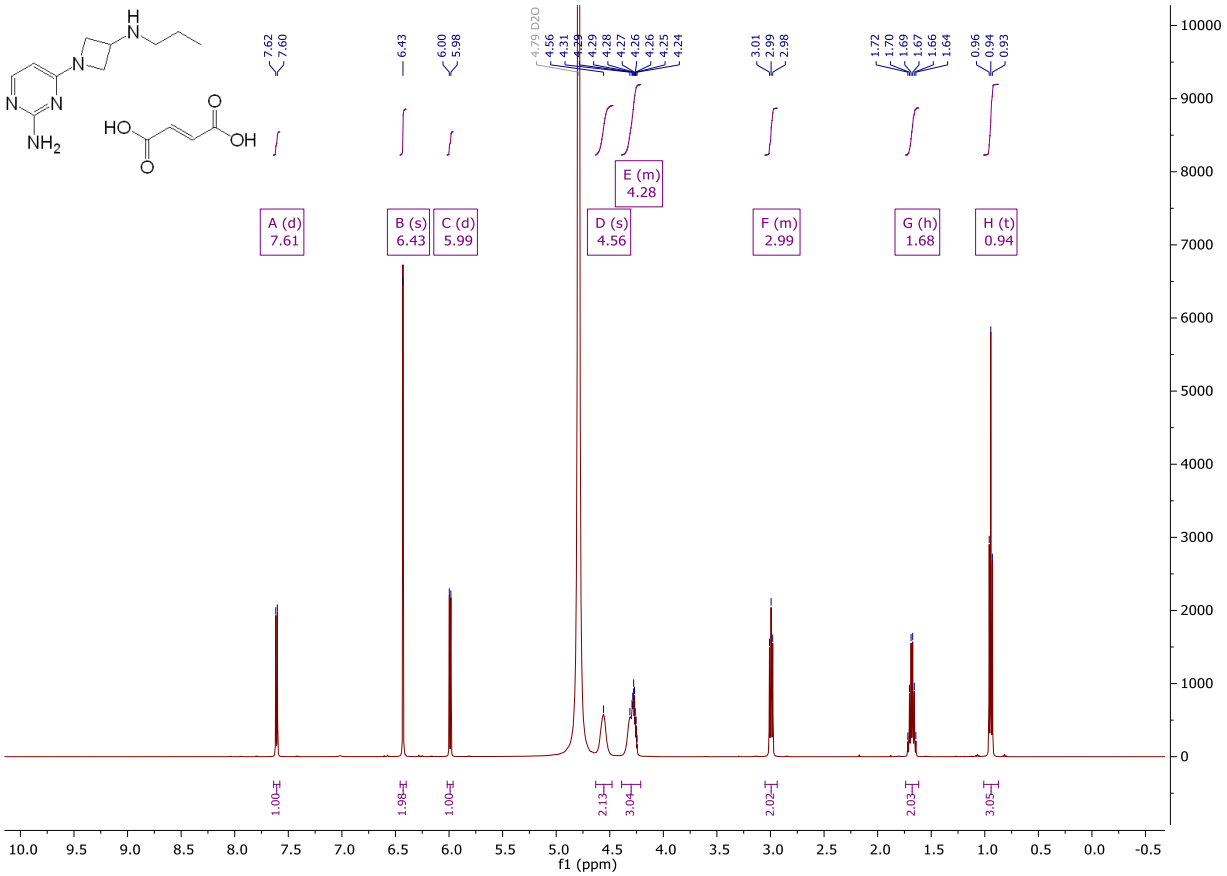


Figure S7. ^{1}H NMR spectrum of compound **14d** in D_2O.

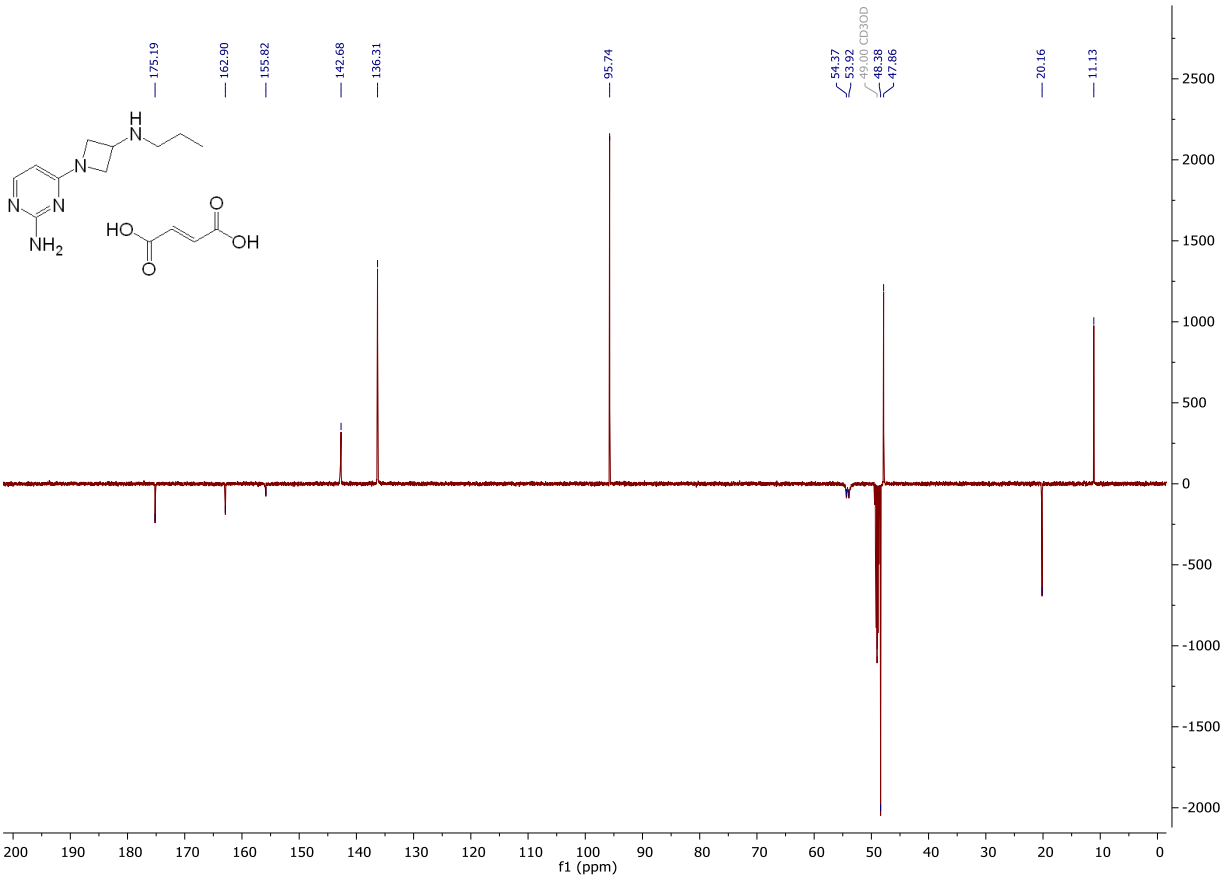


Figure S8. ^{13}C NMR spectrum of compound **14d** in CD_3OD/D_2O.

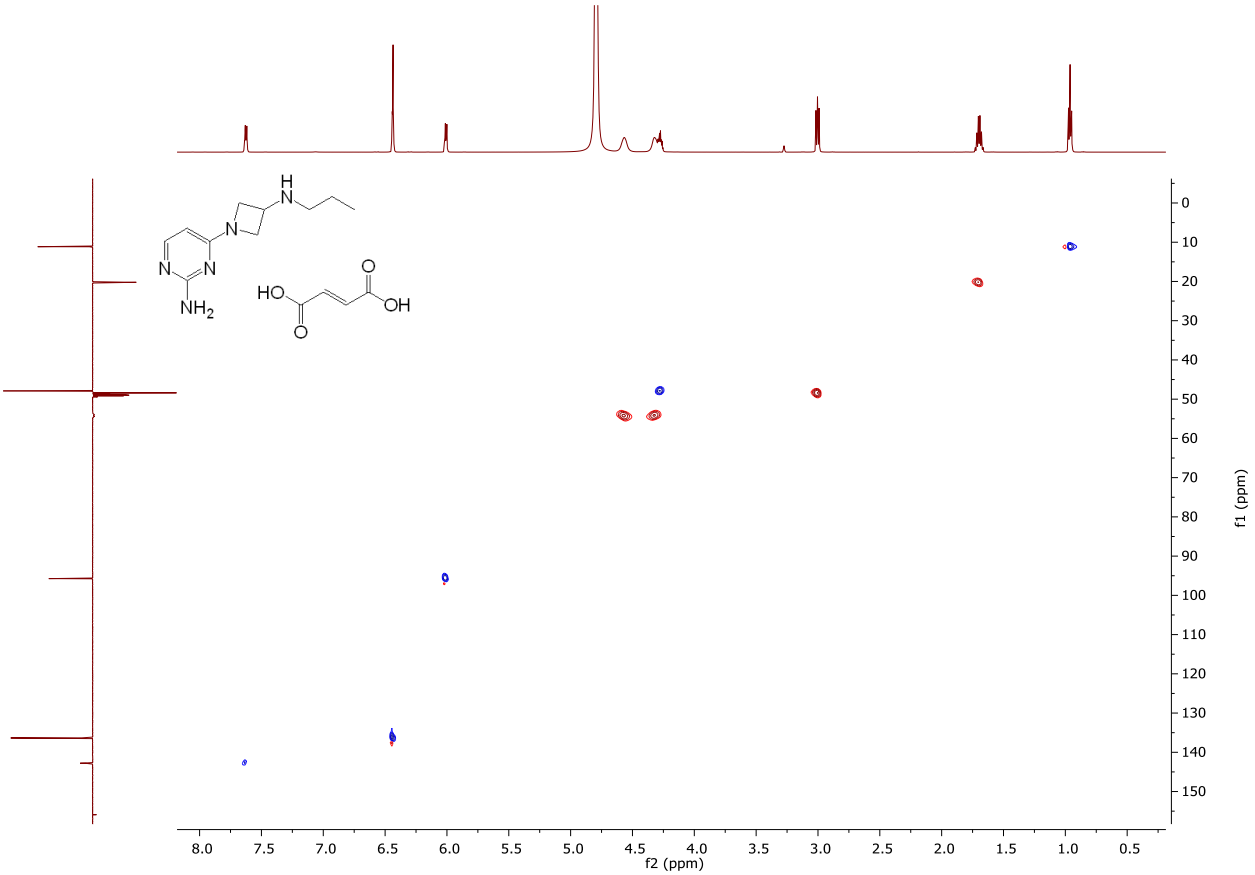


Figure S9. HSQC NMR spectrum of compound **14d** in CD_3OD/D_2O.

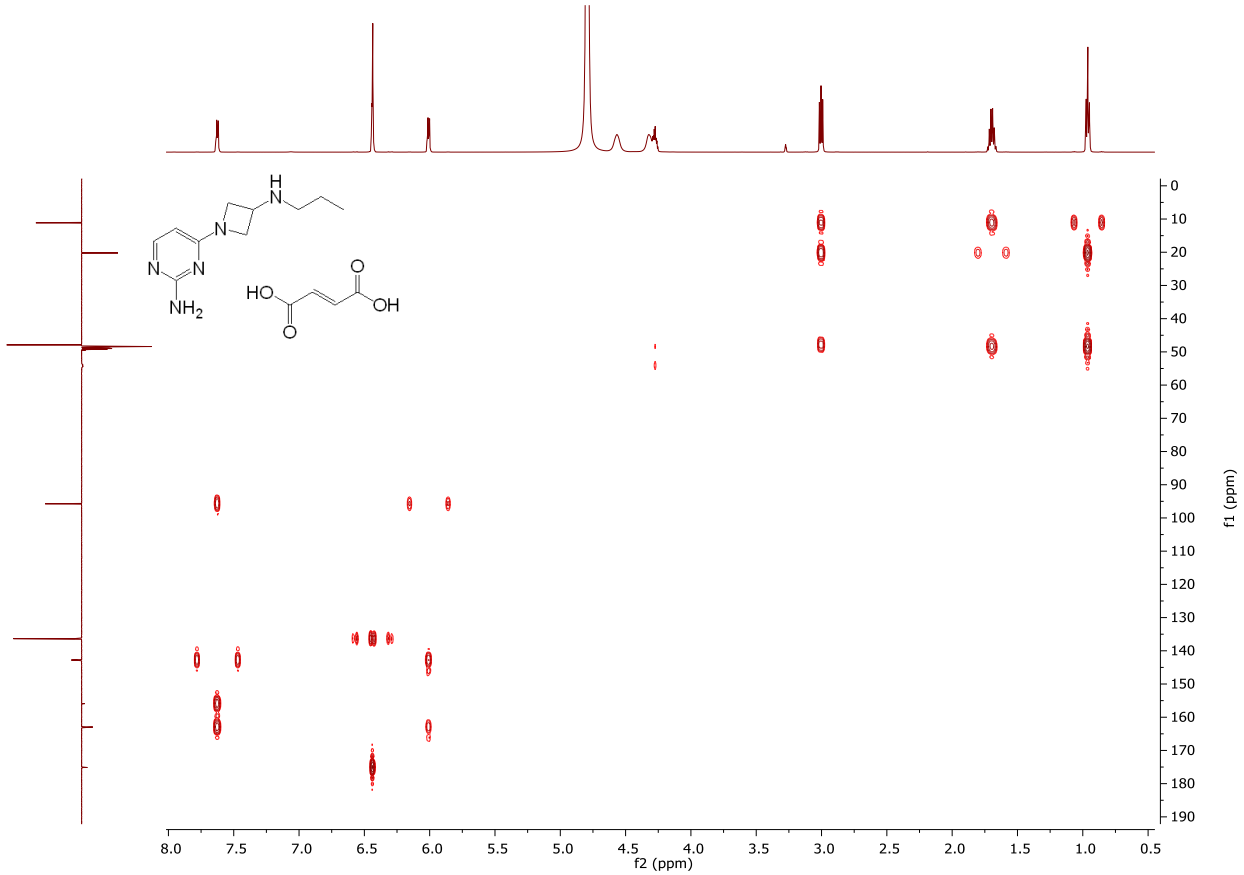


Figure S10. HMBC NMR spectrum of compound **14d** in CD_3OD/D_2O.