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Synthesis, Characterization and *in vitro* anti-Cholinesterase Screening of Novel Indole Amines

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The present study involved targeted synthesis and characterization of novel indole amines with anti-acetylcholinesterase profiling. A series of proposed indole amines was virtually screened against human acetylcholinesterase. A few indole amines (**26**, **30** and **31**) showing strong enzyme binding in the *in silico* studies, were synthesized in the laboratory and characterized with spectroscopic (IR, UV, NMR, SC XRD) and spectrometric (EIMS, HR EIMS) methods. The indole amine **26** was crystallized from EtOH and analyzed with SC-XRD. These ligands were found to interact with PAS site in the enzyme and their binding may disrupt the activity. The *in vitro* acetylcholinesterase inhibition studies exhibits that indole amines (**30** and **31**) show comparable IC_{50} values (4.28 and 4.66 μM, respectively) to galantamine (4.15 μM) and may be studied further as cost-effective acetylcholinesterase inhibitors.

Keywords: Indoles, Indole amines, Anti-cholinesterase potential, Alzheimer's disease, Inhibitory action

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Table S1: Binding energies (kcal/mol units), dissociation constant (nanomolar units) and active sites of targeted indole amines (**19**-**34**) through *in silico* study.

Indole Amine	B.E.	D.C.	Active Site
25	-13.37	0.158	Tyr72, Asp74, Trp86, Gly121, Trp286, Leu289, Glu292, Val294, Phe295, Phe297, Tyr337, Phe338, Tyr341
26	-12.81	0.4	Tyr72, Asp74, Gly121, Gly122, Tyr124, Trp286, Leu289, Glu292, Ser293, Val294, Phe295, Arg296, Tyr337, Phe338, Tyr341
24	-12.43	0.769	Tyr72, Asp74, Gly121, Ser125, Trp286, Leu289, Glu292, Ser293, Val294, Phe295, Phe297, Tyr337, Phe338, Tyr341
22	-12.39	0.83	Tyr72, Asp74, Leu76, Tyr124, Trp286, Glu292, Ser293, Val294, Phe295, Phe297, Phe338, Tyr341
21	-12.34	0.903	Tyr72, Asp74, Trp86, Gly121, Gly122, Tyr124, Ser125, Trp286, His287, Leu289, Gln291, Glu292, Ser293, Val294, Phe295, Arg296, Phe297, Tyr337, Phe338, Tyr341
20	-12.19	1.17	(Tyr72, Asp74, Thr75, Leu76, Tyr77, Thr83, Tyr124, Ser125, Trp286, Leu289, Gln291, Glu292, Ser293, Val294, Phe295, Arg296, Phe297, Tyr337, Phe338, Tyr341, Gly342
19	-11.67	2,81	Tyr72, Asp74, Gly121, Gly122, Tyr124, Ser125, Trp286, His287, Leu289, Gln291, Glu292, Ser293, Val294, Phe295, Arg296, Phe297, Tyr337, Phe338, Tyr341
23	-11.67	2.81	Tyr72, Asp74, Gly121, Gly122, Tyr124, Ser125, Trp286, His287, Leu289, Gln291, Glu292, Ser293, Val294, Phe295, Arg296, Phe297, Tyr337, Phe338, Tyr341
33	-10.3	28.02	Tyr72, Asp74, Thr75, Leu76, Tyr77, Thr83, Trp286, Leu289, Ser293, Val294, Phe295, Arg296, Phe297, Tyr337, Phe338, Tyr341
30	-10.21	32.58	Tyr72, Asp74, Trp86, Gly121, Gly122, Ser125, Ser203, Phe295, Phe297, Tyr337, Phe338, Tyr341, His447
34	-9.93	52.58	Tyr72, Asp74, Gly121, Gly122, Ser203, Trp286, Ser293, Val294, Phe295, Arg296, Phe297, Asp333, Tyr337, Phe338, Tyr341, His447
29	-9.85	60.13	Tyr72, Asp74, Thr83, Trp86, Gly121, Gly122, Ser125, Ser203, Ala204, Trp236, Trp286, Leu289, Ser293, Val294, Phe295, Arg296, Phe297, Tyr337, Phe338, Tyr341, His447
32	-9.57	97.32	Gln71, Tyr72, Asp74, Trp86, Asn87, Gly120, Gly121, Gly122, Ser125, Gly126, Leu130, Ser203, Trp286, Leu289, Ser293, Val294, Phe295, Arg296, Phe297, Tyr337, Phe338, Tyr341, His447, Gly448
28	-9.14	198.53	(Chain B) Tyr72, Asp74, Thr75, Leu76, Trp86, Gly120, Gly121, Gly122, Ser125, Glu202, Trp286, Val294, Phe295, Arg296, Phe297, Tyr337, Phe338, Tyr341, His447, Gly448
27	-8.72	405.14	(Chain B)Tyr72, Asp74, Gly121, Gly122, Tyr124, Ser125, Trp286, Ser293, Val294, Phe295, Arg296, Phe297, Tyr337, Phe338, Tyr341

Table S2: Selected crystallographic data of *N*-phenyl (4,5,6-trimethoxy-2,3-diphenyl-1*H*-indol-7-yl) methanimine.

Important crystallographic parameters of imine	
Molecular Formula	$C_{30}H_{26}N_2O_3$
Molecular mass [amu]	462.53
Crystal System	Triclinic
a, b, c [Å]	6.7431(13), 10.7860(19), 17.370(3)
α, β, γ [º]	94.936(10), 92.093(11), 98.257(11)
Volume of crystal [Å^3]	1244.1(4)
Z	2
Density of crystal (calc.) [g/cm^3]	1.235
μ (MoKa) [/mm]	0.080
F (000)	488

Bond Angles (º)		Bond Distances (Å)	
C^{20}-O^{1}-C^{21}	117.52	O^{1} -C^{20}	1.3648
C^{9}-C^{14}-C^{13}	121.25	O^{1}-C^{21}	1.4394
C^{7}-N^{1}-C^{16}	108.17	C^{17}-C^{18}	1.3959
C^{24}-N^{2}-C^{25}	116.44	C^{17}-C^{18}	1.3959
N^{1}-C^{16}-C^{17}	127.55	N^{1}-C^{7}	1.3936
C^{7}-N^{1}-H^{1A}	126.00	N^{1}-C^{16}	1.3684
N^{1}-C^{16}-C^{15}	108.51	N^{2}-C^{24}	1.2981
C^{15}-C^{16}-C^{17}	123.92	N^{2}-C^{25}	1.4234
C^{17}-C^{18}-C^{19}	122.42	N^{1}-H^{1A}	0.8600
C^{5}-C^{6}-C^{7}	119.63	C^{25}-C^{26}	1.3746

Table S3: Selected crystallographic data of 4,5,6-trimethoxy-2,3-diphenyl-7-phenylaminomethyl-1*H*-indole.

Important crystallographic parameters of indole amine	
Molecular Formula	$C_{30}H_{28}N_2O_3$
Molecular mass [amu]	464.54
Crystal System	Monoclinic
a, b, c [Å]	9.5472(6), 11.8512(8), 21.3627(17)
α, β, γ [º]	90, 94.605(4), S90
Volume of crystal [$Å^3$]	2409.3(3)
Z	4
Density of crystal (calc.) [g/cm^3]	1.281
μ (MoKa) [/mm]	0.083
F (000)	984

Bond Angles (º)		Bond Distances (Å)	
C^{20}-O^{1}-C^{21}	117.52	O^{1} -C^{20}	1.3802
C^{9}-C^{14}-C^{13}	121.25	O^{1}-C^{21}	1.4258
C^{7}-N^{1}-C^{16}	108.17	C^{17}-C^{18}	1.3781
C^{24}-N^{2}-C^{25}	116.44	C^{15}-C^{16}	1.4031
N^{1}-C^{16}-C^{17}	127.55	N^{1}-C^{7}	1.3904
C^{7}-N^{1}-H^{1A}	126.00	N^{1}-C^{16}	1.3634
N^{1}-C^{16}-C^{15}	108.51	N^{2}-C^{24}	1.4416
C^{15}-C^{16}-C^{17}	123.92	N^{2}-C^{25}	1.4198
C^{17}-C^{18}-C^{19}	122.42	N^{1}-H^{1A}	0.8800
C^{5}-C^{6}-C^{7}	119.63	N^{2}-H^{2A}	1.2100

Table S4: ADMET parameters of the 2,3-diphenylindole amines.

Indole amines	**25**	**24**	**21**	**20**
Physiochemical Properties				
MW*	509.55	509.55	479.53	479.53
NRB^	9	9	8	8
HBA+	5	5	4	4
HBD++	2	2	2	2
LogP	5.20	5.17	5.16	5.17
TPSA[1]	101.33	101.33	92.10	92.10

MR^2		151.26	151.26	144.77	144.77
Log S		-7.06 Poorly soluble	-7.06 Poorly soluble	-6.99 Poorly soluble	-6.99 Poorly soluble
Adsorption					
Water solubility (log mol/L)		-3.479	-3.471	-3.967	-3.944
Caco2 permeability (log Papp in 10^{-6} cm/s)		0.621	0.613	0.568	0.554
Intestinal absorption (human) (% Absorbed)		100	100	91.784	93.464
Skin Permeability (log Kp)		-2.735	-2.735	-2.735	-2.735
P-glycoprotein substrate		Yes	Yes	Yes	Yes
P-glycoprotein I inhibitor		Yes	Yes	Yes	Yes
P-glycoprotein II inhibitor		Yes	Yes	Yes	Yes
Distribution					
VDss[a] (human) (log L/kg)		-1.585	-1.587	-1.487	-1.491
Fraction unbound (human)		0.312	0.314	0.283	0.285
BBB[b] permeability (log BB)		-0.1 (No)	-0.117 (No)	0.037 (No)	0.02 (No)
CNS[c] permeability (log PS)		-1.935	-1.919	-1.71	-1.699
Metabolism					
CYP2D6 substrate		No	No	No	No
CYP3A4 substrate		Yes	Yes	Yes	Yes
CYP1A2 inhibitor		Yes	Yes	Yes	Yes
CYP2C19 inhibitor		Yes	Yes	Yes	Yes
CYP2C9 inhibitor		Yes	Yes	Yes	Yes
CYP2D6 inhibitor		No	No	No	No
CYP3A4 inhibitor		No	No	No	No
Excretion					
Total Clearance (log ml/min/kg)		0.62	0.627	0.68	0.688
Renal OCT2 substrate	No	No	No	No	
Toxicity					
AMES toxicity	Yes	Yes	Yes	Yes	
Max. tolerated dose (human) (log mg/kg/day)	0.436	0.438	0.474	0.475	
hERG I inhibitor	No	No	No	No	
hERG II inhibitor	Yes	Yes	Yes	Yes	
Oral Rat Acute Toxicity (LD50) (mol/kg)	2.934	2.933	3.145	3.146	
Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day)	1.582	1.364	1.805	1.6	
Hepatotoxicity	Yes	Yes	Yes	Yes	
Skin Sensitisation	No	No	No	No	
T.Pyriformis toxicity (log ug/L)	0.285	0.285	0.285	0.285	
Minnow toxicity (log mM)	-3.944	-3.745	-2.877	-2.694	

*Molecular weight (g/mol), ^Non-rotatable bond, +Hydrogen bond acceptor, ++Hydrogen bond doner, [1]Topological Polar Surface Area (Å²), [2]Molar refractivity, [a]Volume of Distribution, [b]BBB (Blood-brain Barrier), [c]CNS (Central Nervous System)

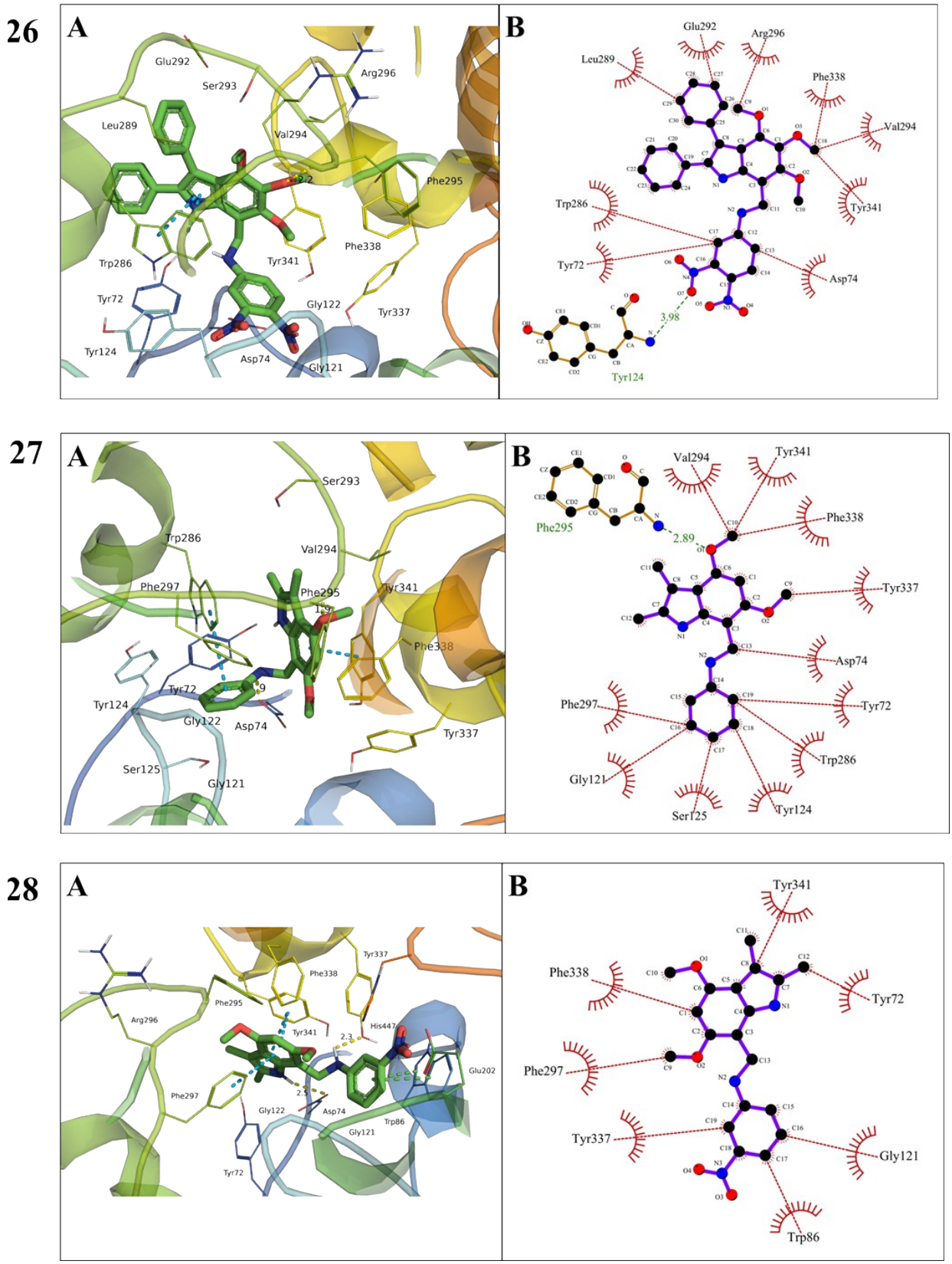

Figure S1: Binding mode with AChE (4M0E). **A:** 3D interaction of key residues of 4M0E, where pi-pi stacking (blue dotted line), hydrogen bond (yellow dotted line) and pi-cation (green dotted line) are represented. **B**: 2D interaction of key residues of 4M0E, where hydrophobic interactions (red lines) and hydrogen bond (green lines) are represented.

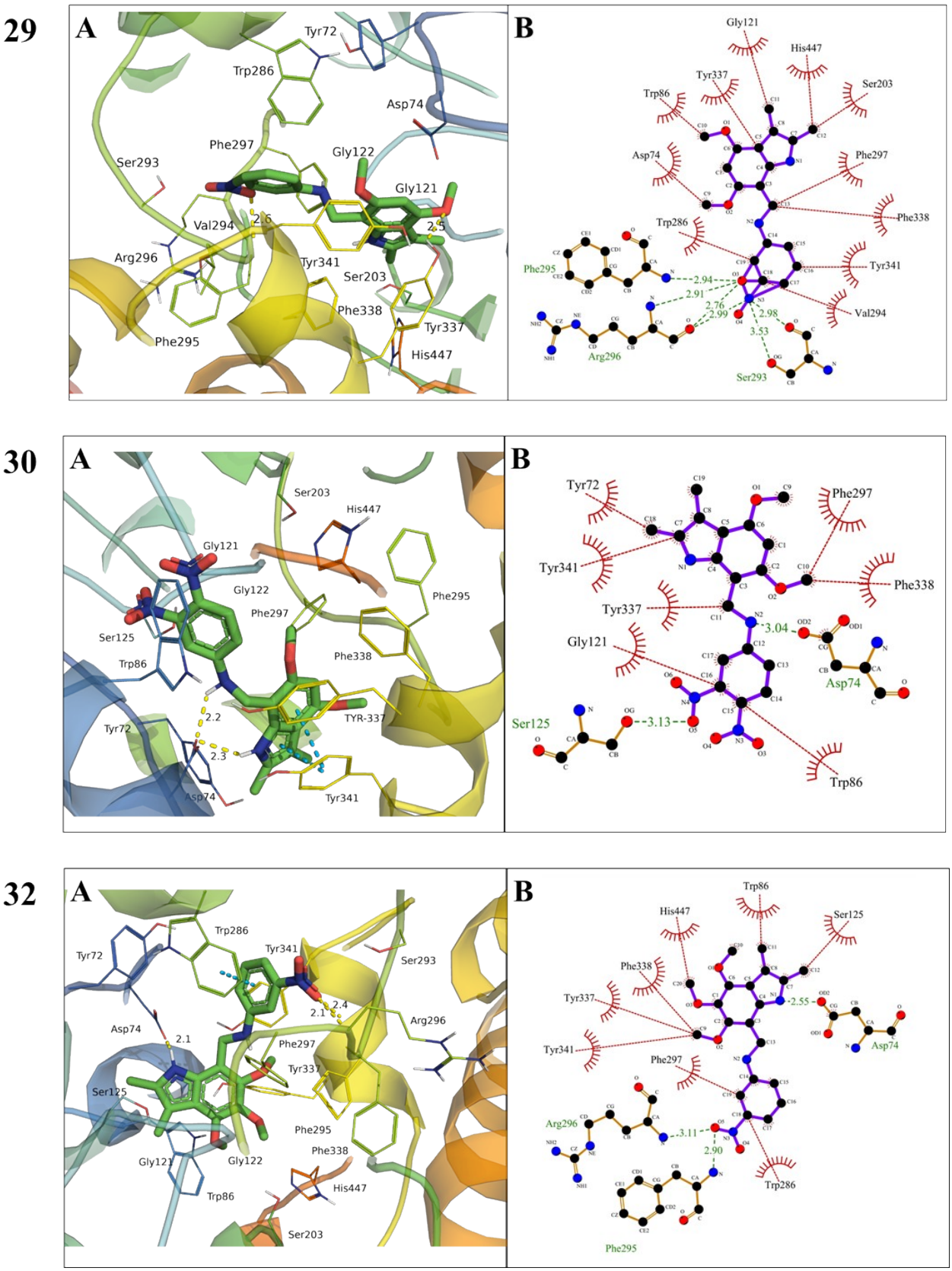

Figure S2: Binding mode with AChE (4M0E). **A:** 3D interaction of key residues of 4M0E, where pi-pi stacking (blue dotted line), hydrogen bond (yellow dotted line) and pi-cation (green dotted line) are represented. **B**: 2D interaction of key residues of 4M0E, where hydrophobic interactions (red lines) and hydrogen bond (green lines) are represented.

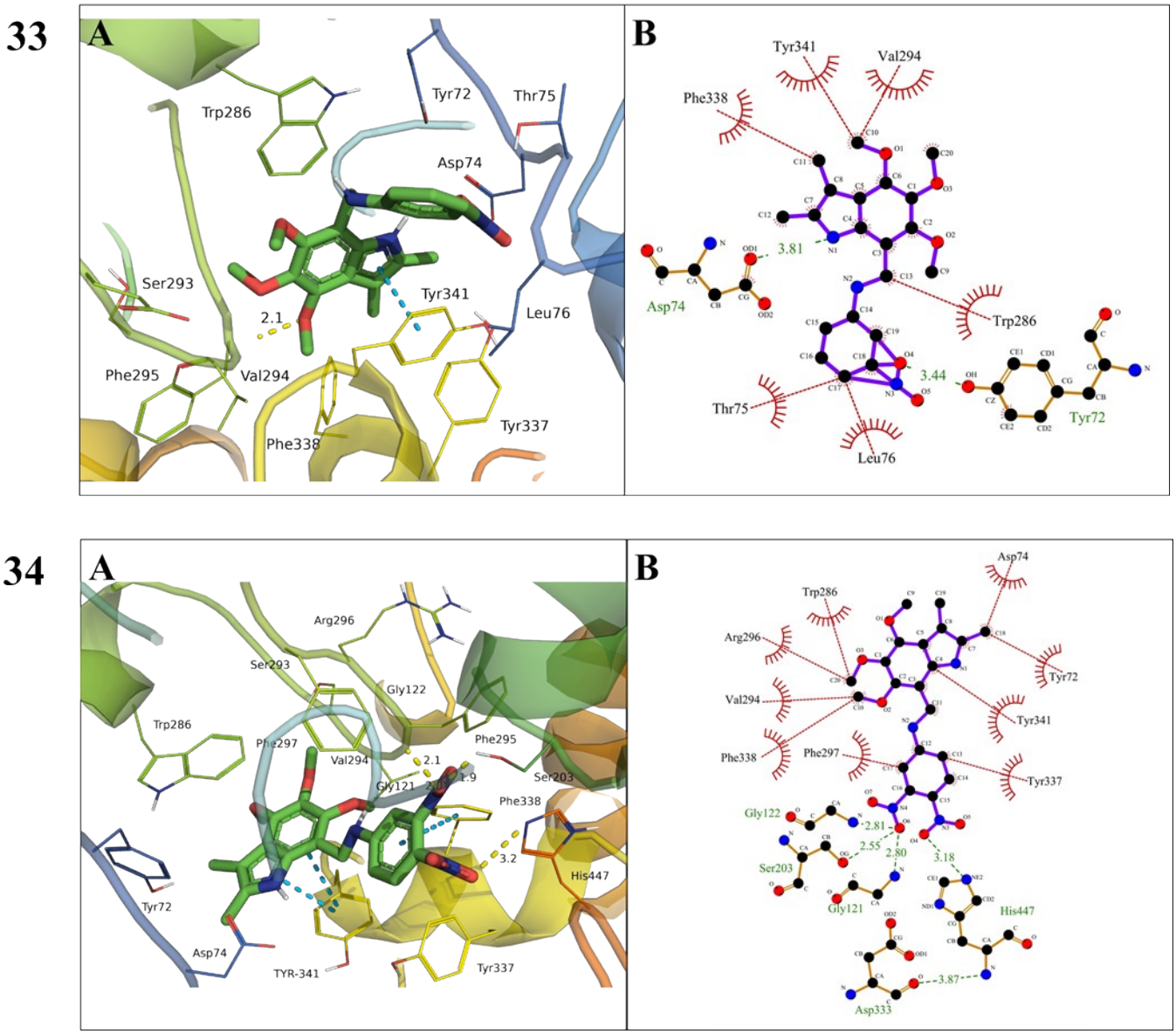

Figure S3: Binding mode with AChE (4M0E). **A:** 3D interaction of key residues of 4M0E, where pi-pi stacking (blue dotted line), hydrogen bond (yellow dotted line) and pi-cation (green dotted line) are represented. **B**: 2D interaction of key residues of 4M0E, where hydrophobic interactions (red lines) and hydrogen bond (green lines) are represented.