Supplementary Material

Multiple 3D-QSAR modeling, e-pharmacophore, molecular docking, and *in vitro* study to explore novel AChE inhibitors

Srabanti Jana, Ankit Ganeshpurkar, and Sushil Kumar Singh*

Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutical Engineering & Technology, Indian Institute of Technology (Banaras Hindu University), Varanasi-221005, India.

***Corresponding Author:**

Phone: +91-542-6702736/49; fax: +91-542-2368428; e-mail: sksingh.phe@iitbhu.ac.in.

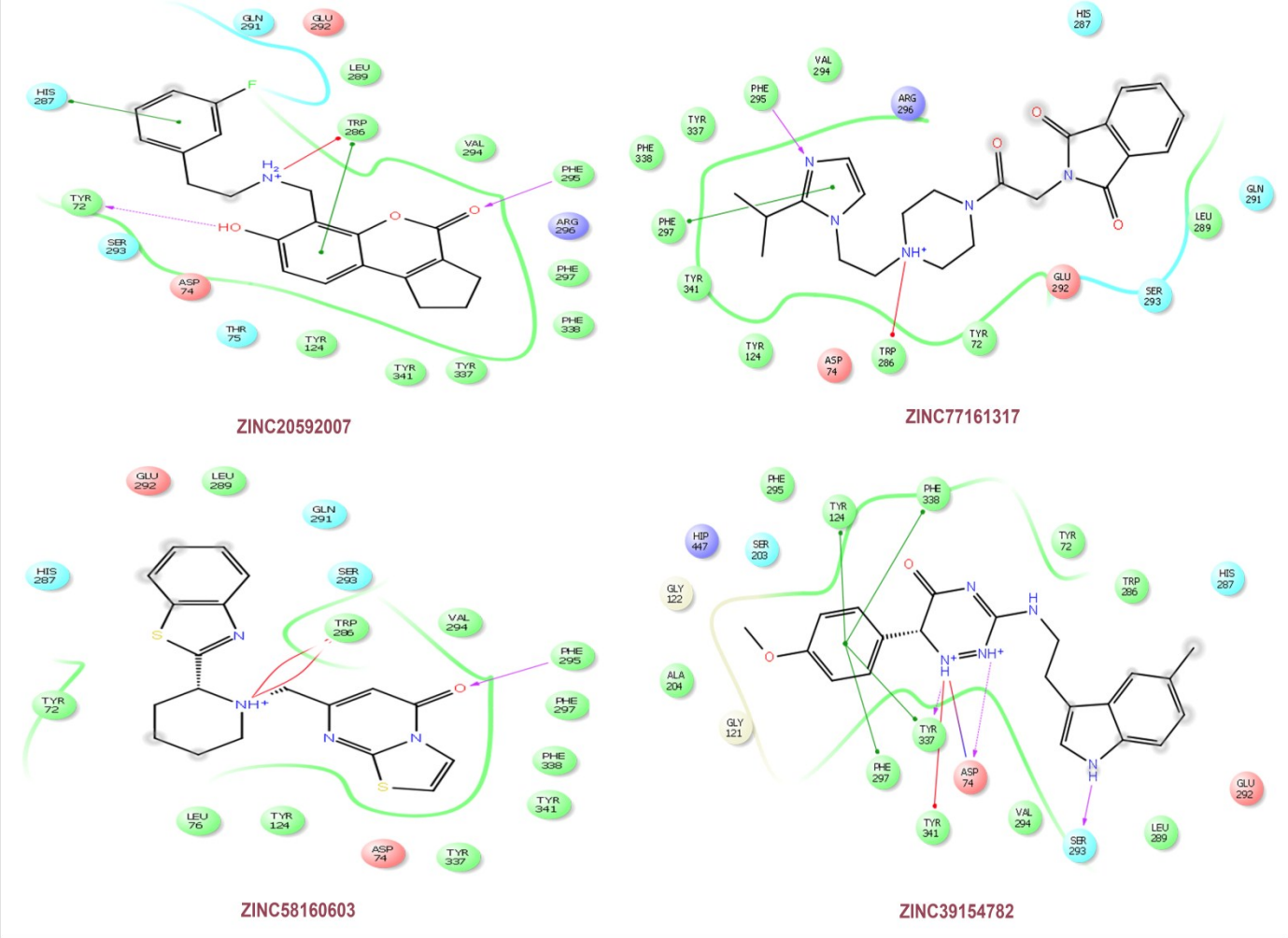


Figure S 1. Docking pose of ZINC72451013, ZINC20649934, ZINC05354646, and ZINC79331983 with 4M0E crystal structure using AutoDock.

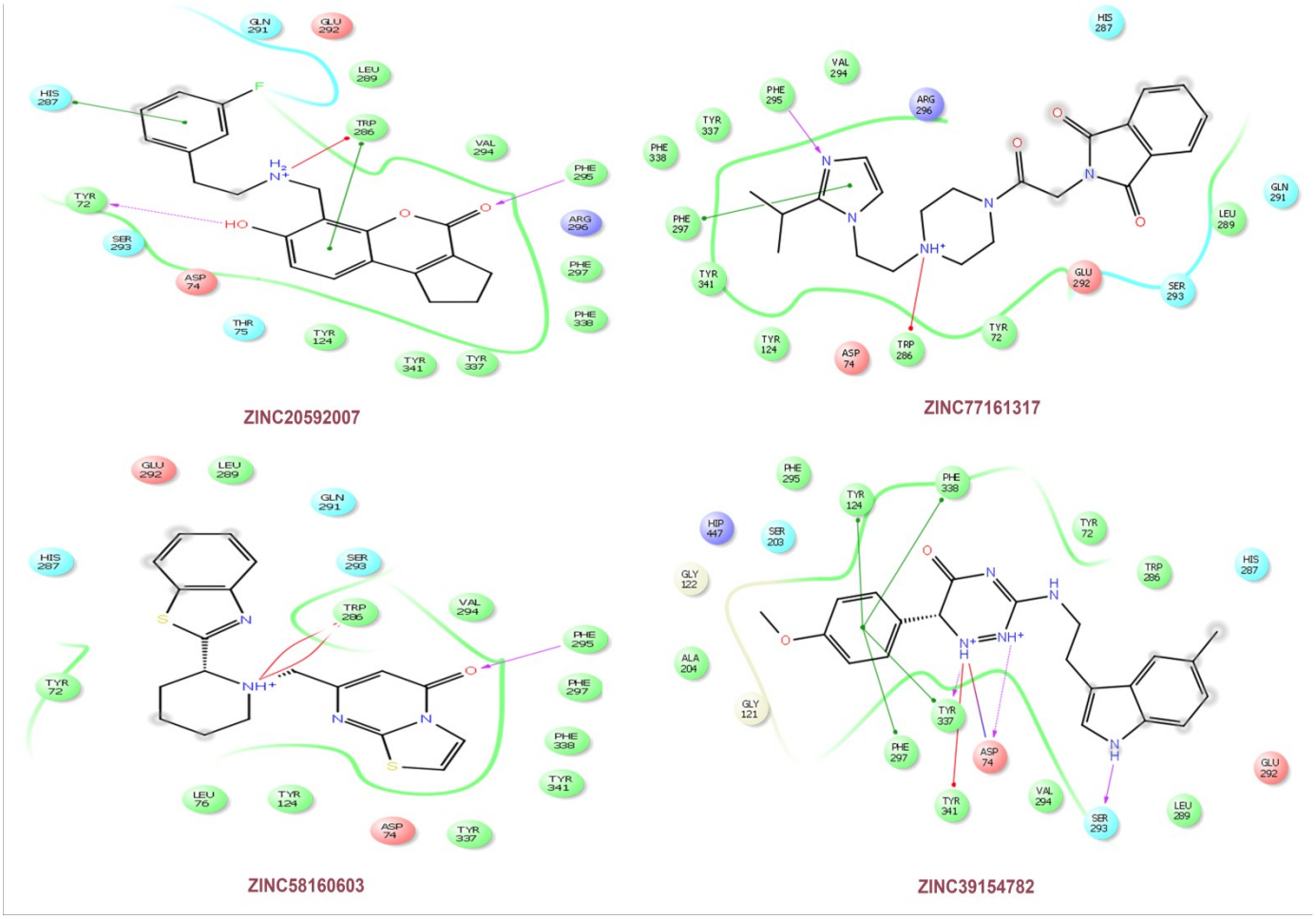


Figure S 2. Docking pose of ZINC20592007, ZINC77161317, ZINC58160603, and ZINC39154782 with 4M0E crystal structure using AutoDock

Table S 1. Final hits outcome from respective pharmacophore models.

Hits	Pharmacophore model
ZINC72451013, and ZINC20649934	4M0F
ZINC05354646, and ZINC20592007	4M0E
ZINC79331983, ZINC58160603, and ZINC39154782	3D-QSAR
ZINC77161317	4EY7

Table S 2. Calculated DFT properties of hit molecules.

Hits	HOMO	LUMO	HOMO-LUMO gap	ESP mean(kcal/mol)
ZINC72451013	-0.205	-0.069	-0.137	-0.13
ZINC20649934	-0.229	-0.079	-0.150	1.38
ZINC05354646	-0.21455	-0.047	-0.168	1.51
ZINC79331983	-0.199	-0.021	-0.177	1.88
ZINC20592007	-0.218	-0.048	-0.171	0.96
ZINC77161317	-0.214	-0.078	-0.136	1.12
ZINC58160603	-0.222	-0.054	-0.168	-0.07
ZINC39154782	-0.20	-0.094	-0.105	-0.22

Table S 3. Hits with Glide energy and docking score against crystal of BuChE with AChE

Compounds	Docking score (AChE)	Docking score (BuChE)	Glide energy (BuChE)
ZINC72451013	-12.87	-6.52	-39.459
ZINC20649934	-12.65	-5.85	-39.436
ZINC05354646	-11.93	-5.66	-30.077
ZINC79331983	-11.40	-8.78	-46.851
ZINC20592007	-11.26	-8.5	-42.257
ZINC77161317	-11.02	-7.31	-52.366
ZINC58160603	-10.93	-7.35	-49.362
ZINC39154782	-10.74	-9.34	-53.435

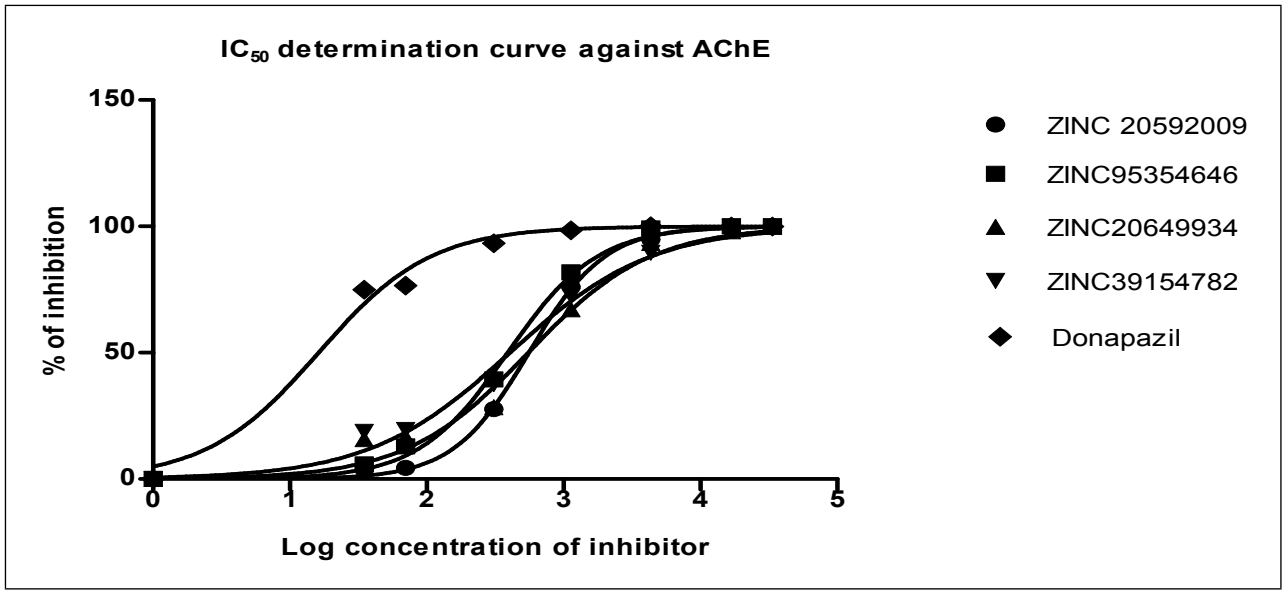


Figure S3. Dose response curve of selected hits with donepezil

Table S 4. The V_{max}, and K_m values of selected hits at various concentrations

Conc of ZINC 20592009	**0µM**	**0.25 µM**	**0.5 µM**	**1 µM**
V_{max}	0.003854	0.02164	0.01371	0.01009
K_m	0.8966	1.352	0.9819	1.119
Conc of ZINC95354646	**0µM**	**0.25 µM**	**0.5 µM**	**1 µM**
V_{max}	0.003854	0.0184	0.01514	0.01218
K_m	0.8966	0.8523	0.7853	0.898
Conc of ZINC20649934	**0µM**	**0.5 µM**	**1 µM**	**2 µM**
V_{max}	0.003879	0.03073	0.02251	0.01666
K_m	0.9066	1.31	1.034	1.268
Conc of ZINC39154782	**0µM**	**0.5 µM**	**1 µM**	**2 µM**
V_{max}	0.003854	0.03091	0.02595	0.01913
K_m	0.8966	1.692	1.577	1.537

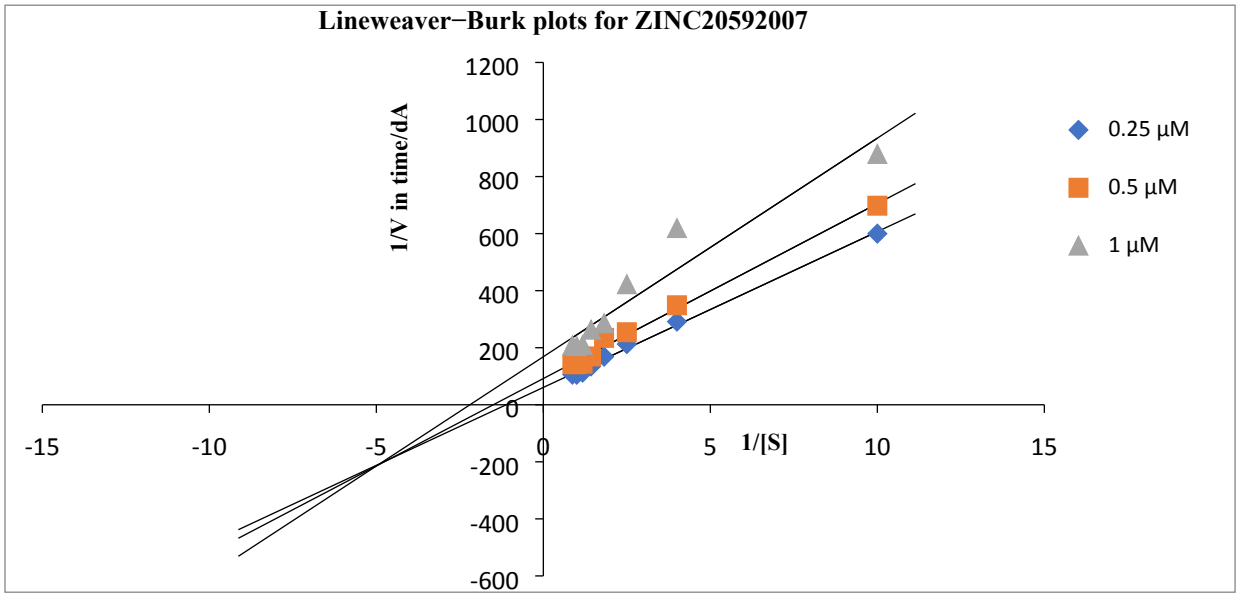


Figure S4. Lineweaver−Burk plots from the substrate-velocity curves of AChE activity with different substrate concentrations (0.15-1.15µM) in the absence and presence of 0.25, 0.5 and 1 µM ZINC20592007

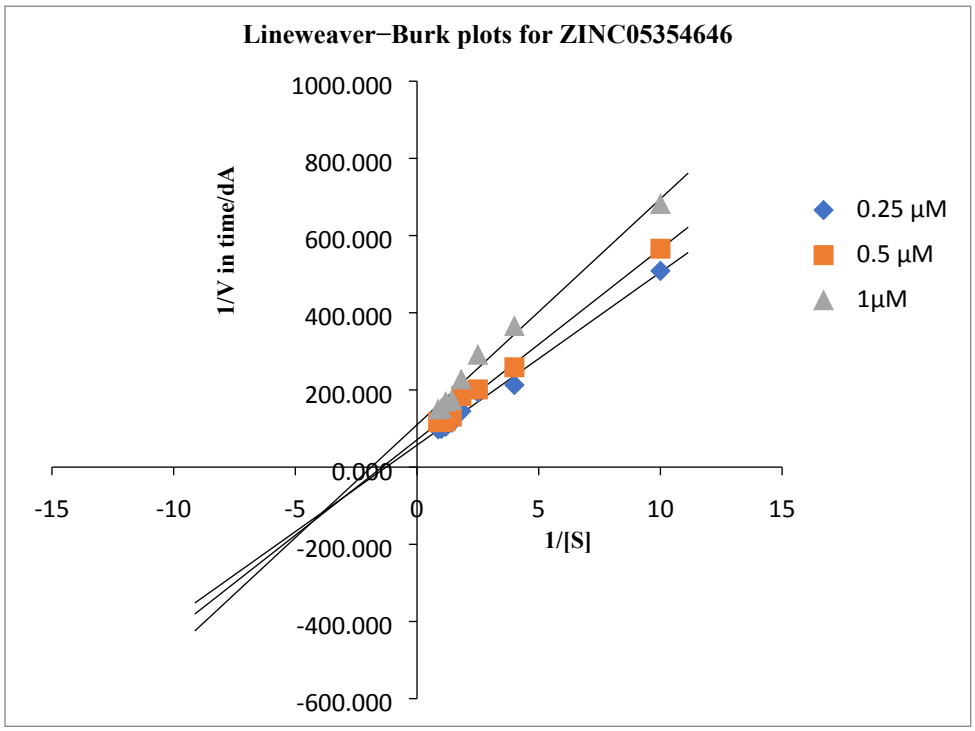


Figure S5. Lineweaver−Burk plots from the substrate-velocity curves of AChE activity with different substrate concentrations (0.15-1.15µM) in the absence and presence of 0.25, 0.5 and 1 µM ZINC05354646

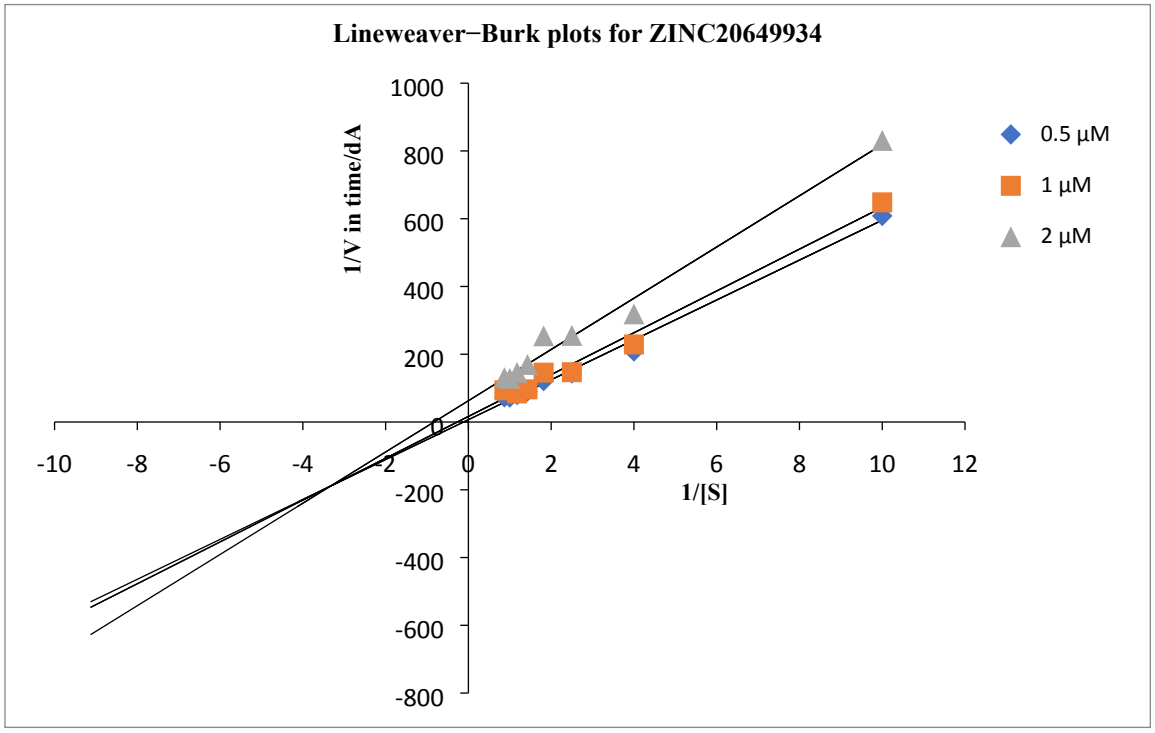


Figure S6. Lineweaver−Burk plots from the substrate-velocity curves of AChE activity with different substrate concentrations (0.15-1.15µM) in the absence and presence of 0.5, 1 and 2 µM of ZINC20649934

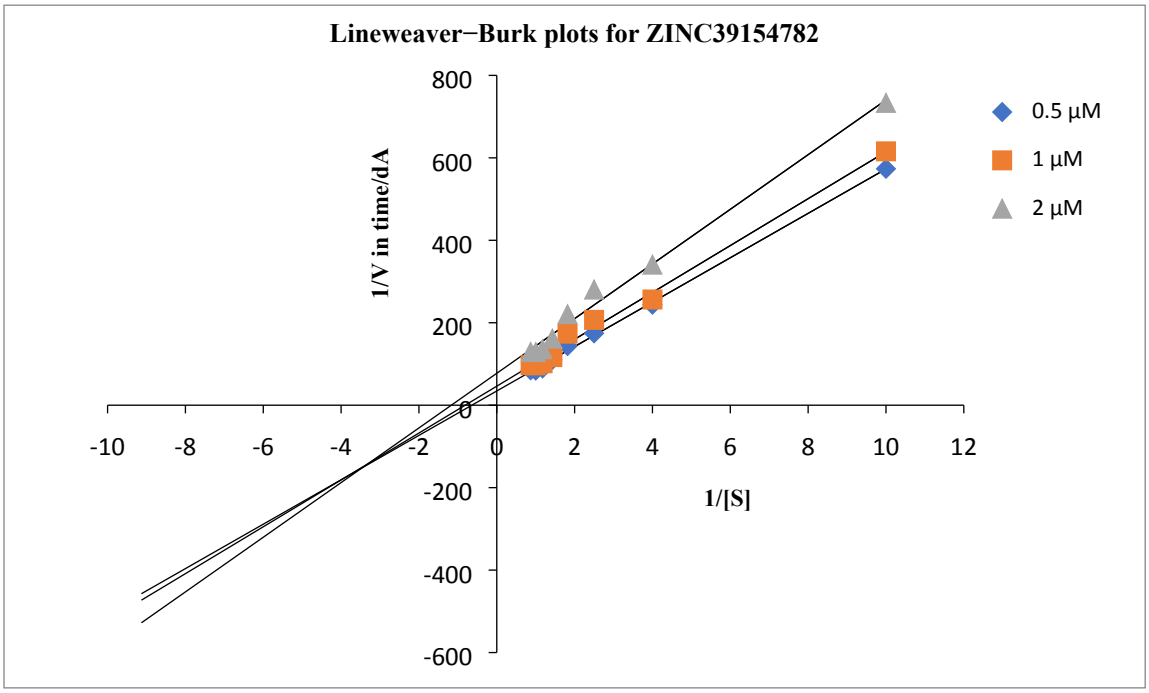


Figure S7. Lineweaver−Burk plots from the substrate-velocity curves of AChE activity with different substrate concentrations (0.15-1.15µM) in the absence and presence of 0.5, 1 and 2 µM of ZINC39154782

Table S 5. Permeability (Pe 10^{-6} cm s^{-1}) of nine commercial drugs in the PAMPA-BBB assay used for the validation of the experiment

Commercial drugs	Reference value Pe (10^{-6})[a]	Experimental value Pe (10^{-6})[b]
Verapamil	16	20.81±1.2
Diazepam	16	19.9±0.5
Progesterone	9.3	15.79±1.4
Atenolol	0.8	0.68±0.4
Dopamine	0.2	0.17±0.1
Lomefloxacin	1.1	0.31±0.3
Alprazolam	5.4	4.6±0.7
Chlorpromazine	6.5	5.9±0.6
Oxazepam	10	8.7±1.1

[a]Taken from literature. [b]Data are expressed as mean ± SEM of three independent experiments.

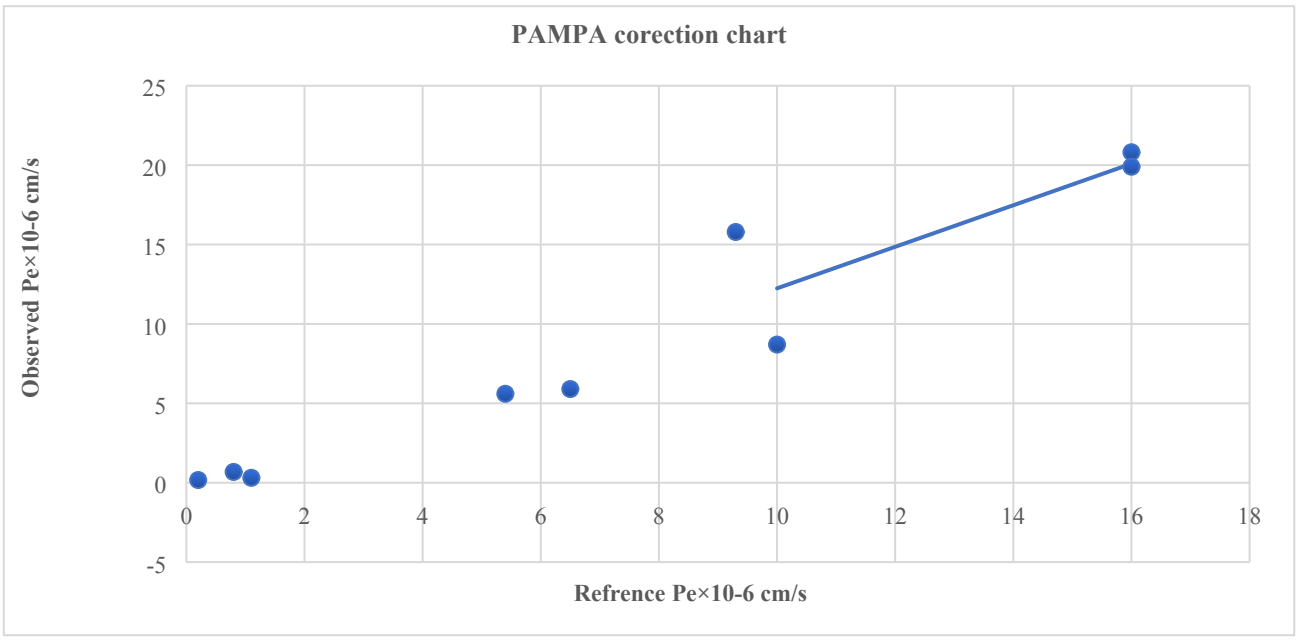
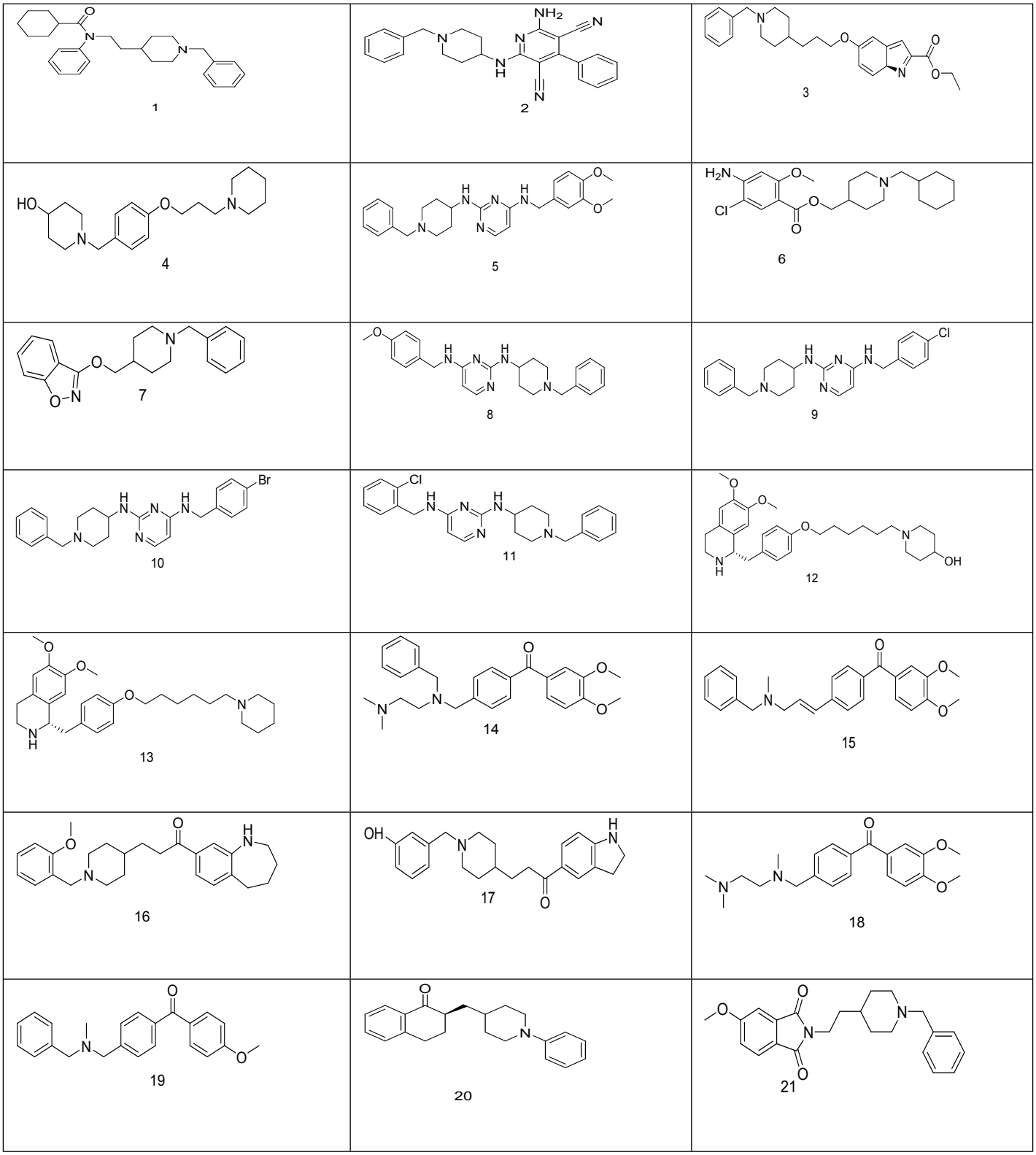


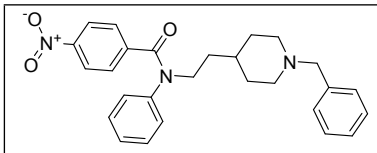
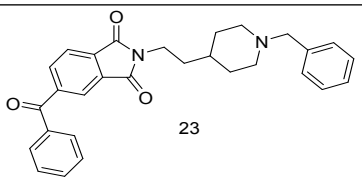
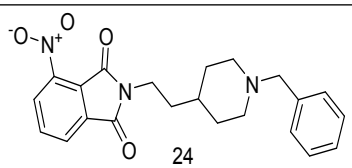
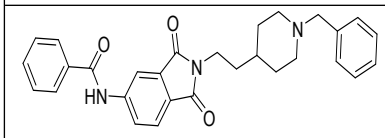
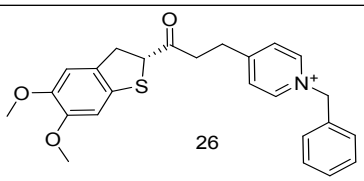
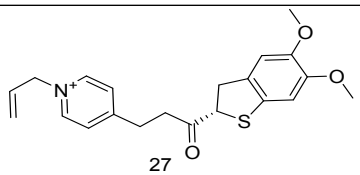
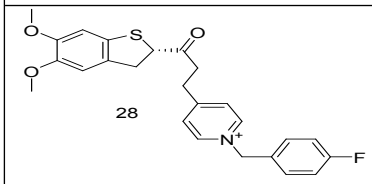
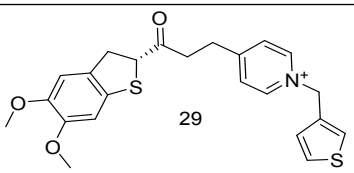
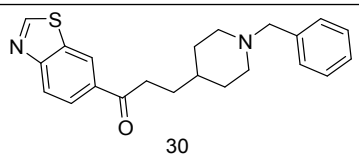
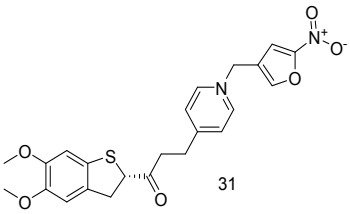
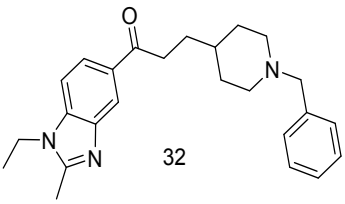
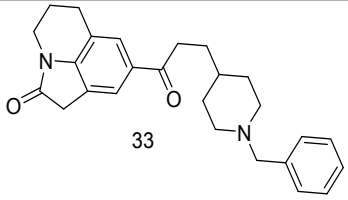
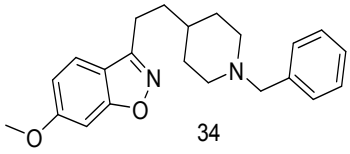
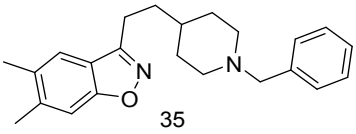
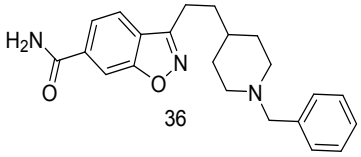
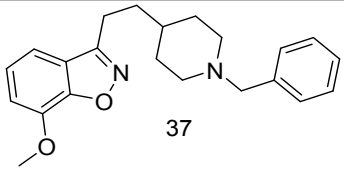
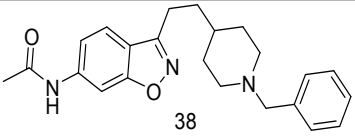
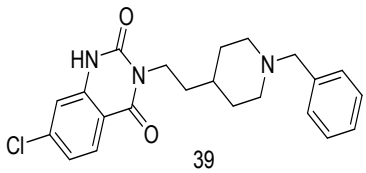
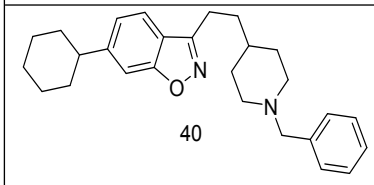
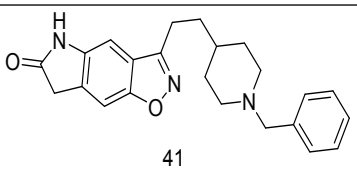
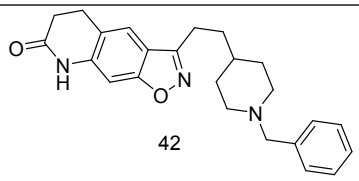
Figure S8. Linear correlation between experimental and reported permeability of nine commercial drugs using PAMPA-BBB assay.

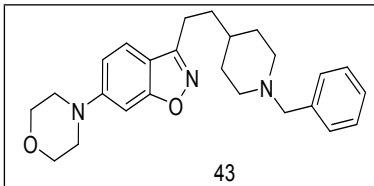
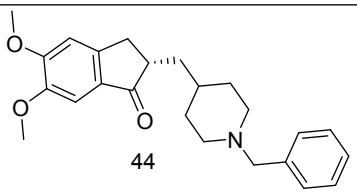
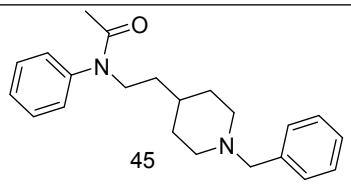
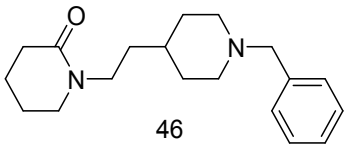
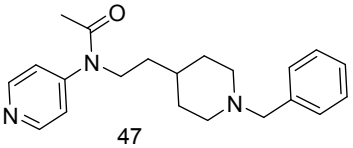
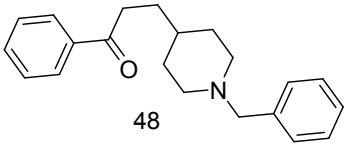
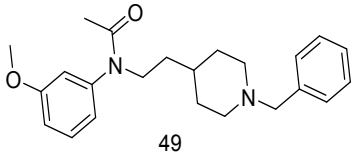
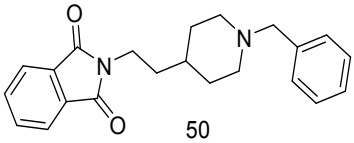
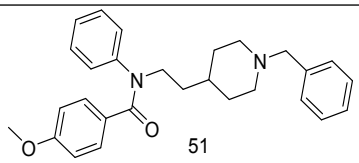
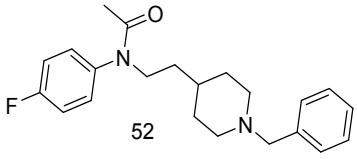
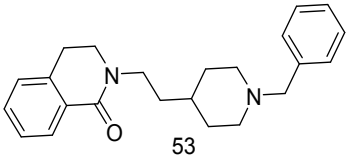
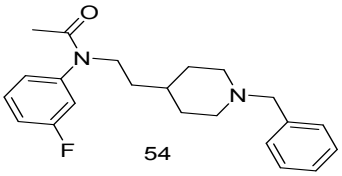
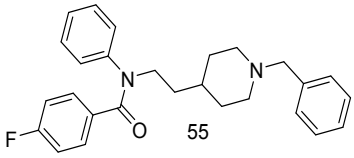
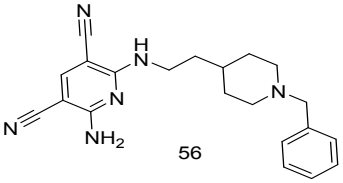
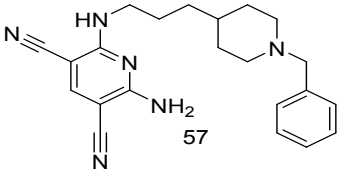
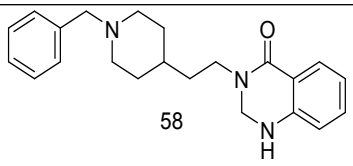
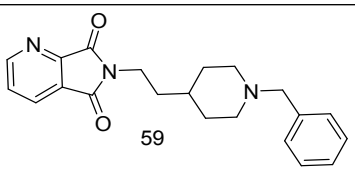
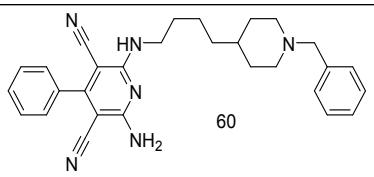
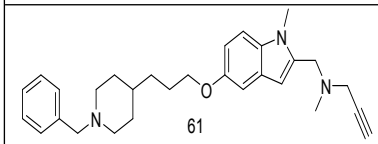
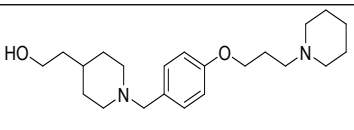
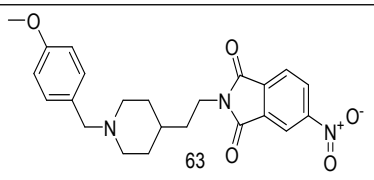
Table S 6. Ranges of permeability (Pe, 10-6 cm s-1) of PAMPA-BBB assay using equation [Pe(exp) = 1.308Pe(ref) + 0.839 (R2 = 0.931)] and literature

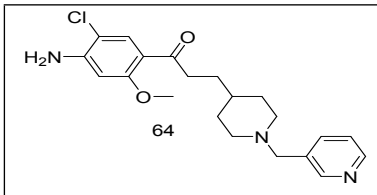
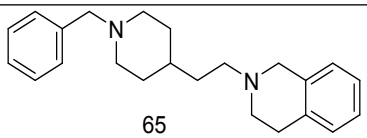
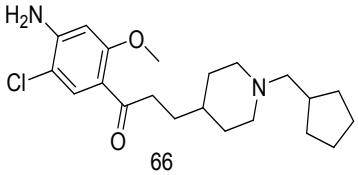
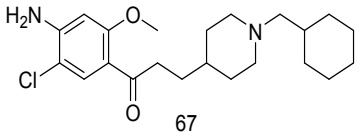
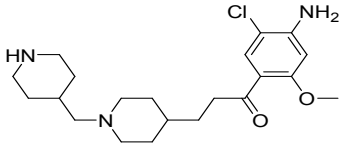
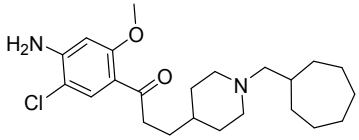
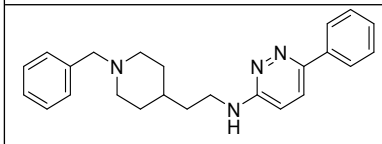
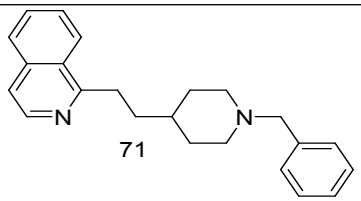
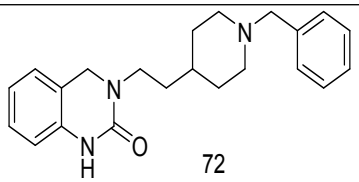
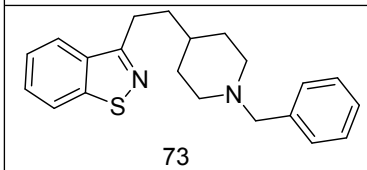
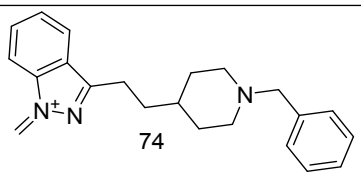
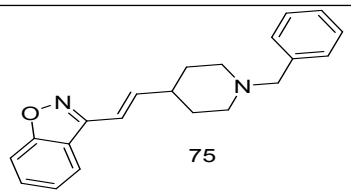
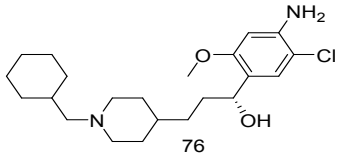
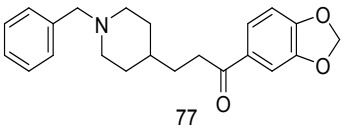
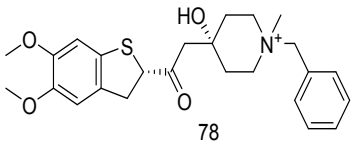
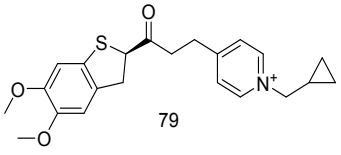
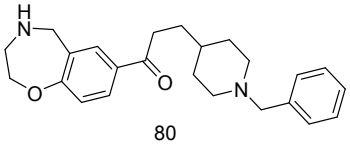
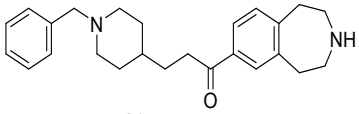
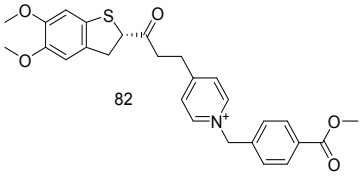
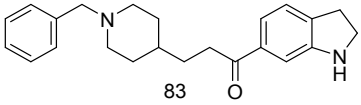
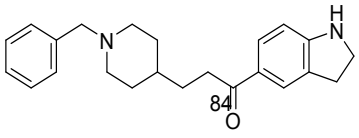
Permeability prediction in PBS	Permeability (Pe, 10^{-6} cm s^{-1})
Compounds of high BBB permeation (CNS+)	Pe (exp) >4.39
Compounds of uncertain BBB permeation (CNS+/-)	4.39> Pe(exp) > 1.78
Compounds of low BBB permeation (CNS-)	Pe (exp) <1.78

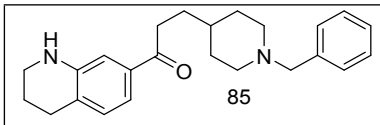
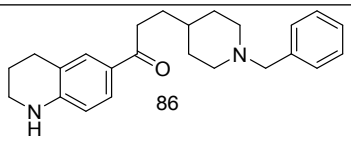
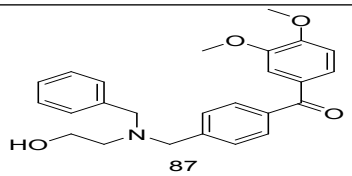
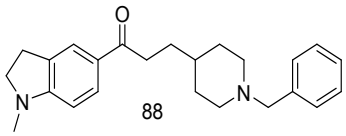
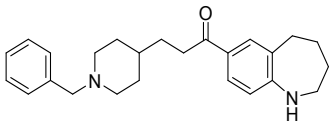
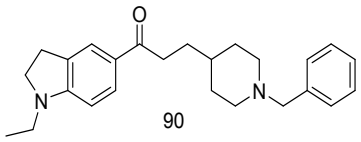
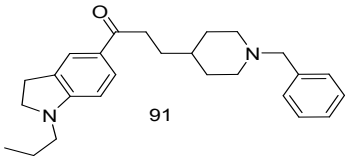
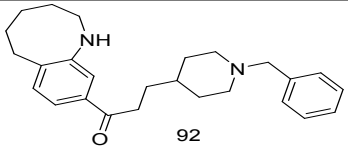
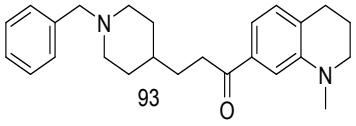
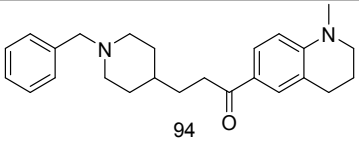
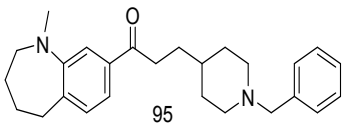
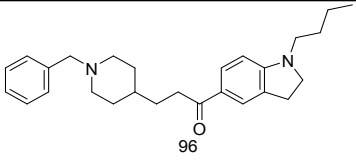
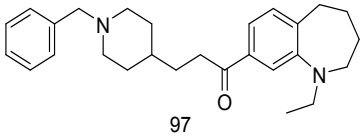
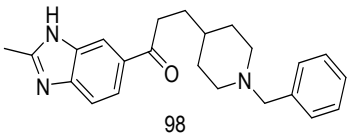
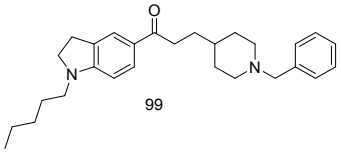
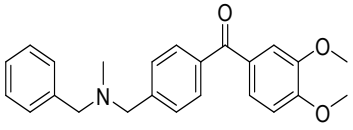
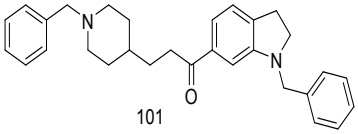
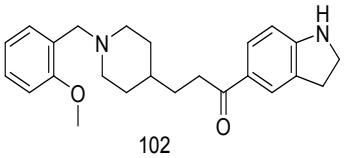
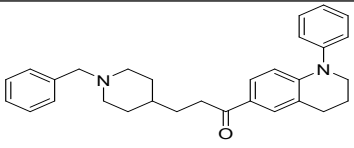
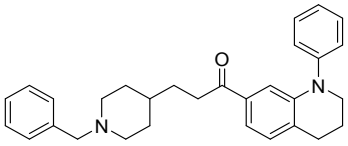
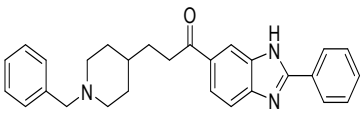
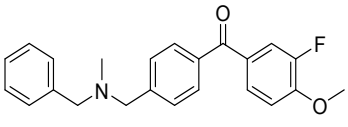
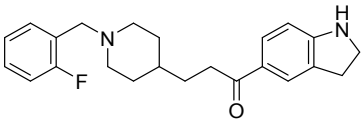
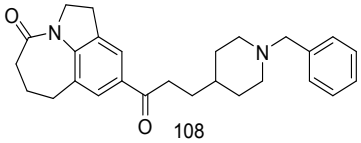
Figure S9. List of AChE Inhibitors used for development of 3D-QSAR model

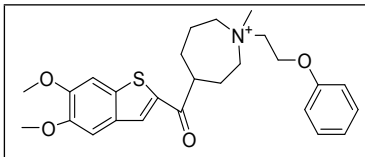
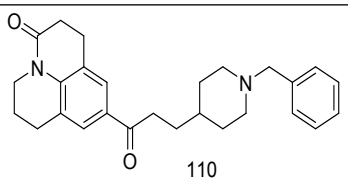
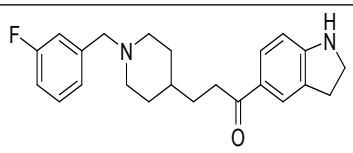
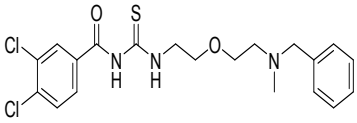
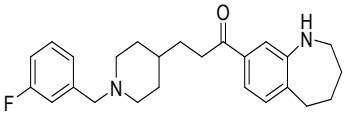
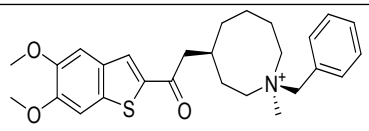
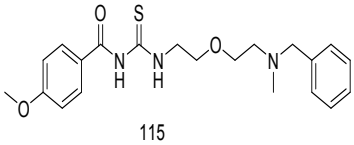
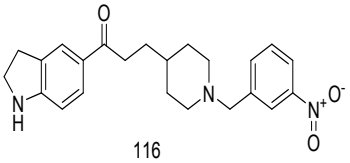
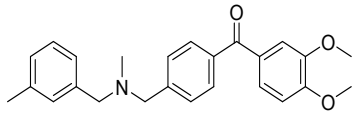
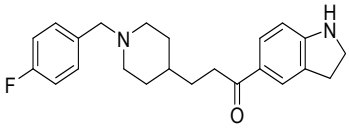
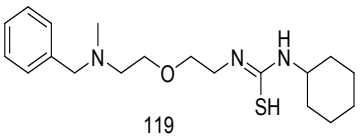
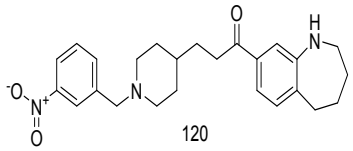
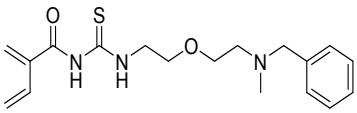
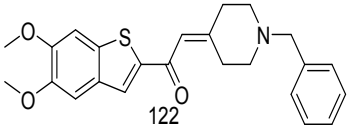
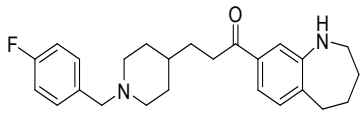
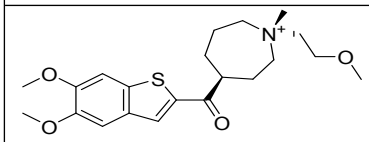
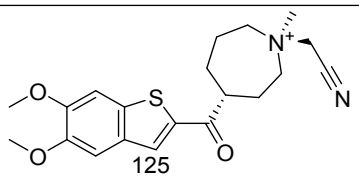
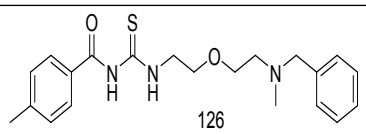
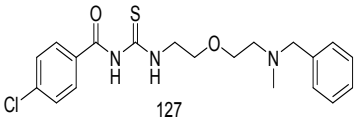
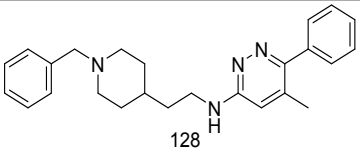
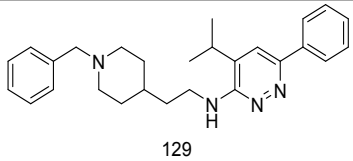


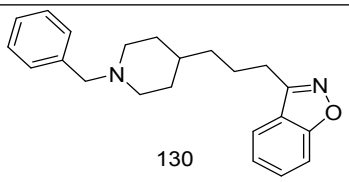
 22	 23	 24
 25	 26	 27
 28	 29	 30
 31	 32	 33
 34	 35	 36
 37	 38	 39
 40	 41	 42

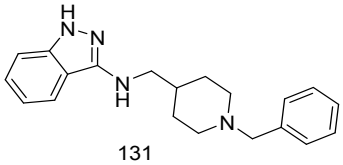
 43	 44	 45
 46	 47	 48
 49	 50	 51
 52	 53	 54
 55	 56	 57
 58	 59	 60
 61	 62	 63

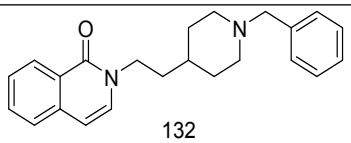
 64	 65	 66
 67	 68	 69
 70	 71	 72
 73	 74	 75
 76	 77	 78
 79	 80	 81
 82	 83	 84

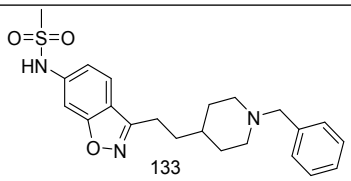
 85	 86	 87
 88	 89	 90
 91	 92	 93
 94	 95	 96
 97	 98	 99
 100	 101	 102
 103	 104	 105
 106	 107	 108

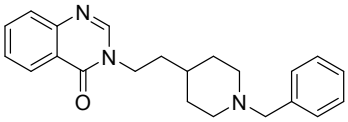
 109	 110	 111
 112	 113	 114
 115	 116	 117
 118	 119	 120
 121	 122	 123
 124	 125	 126
 127	 128	 129

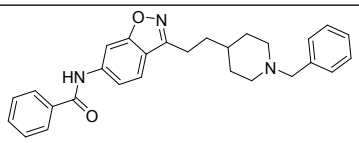

130

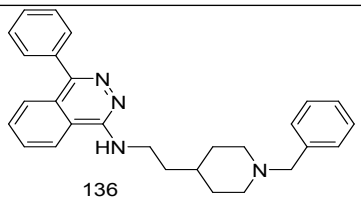

131

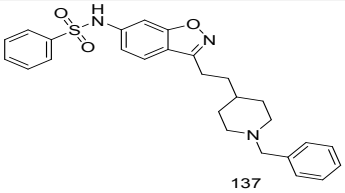

132

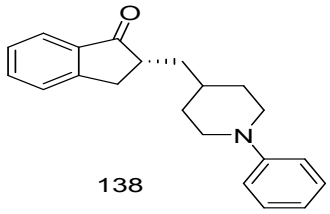

133

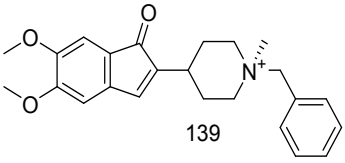

134

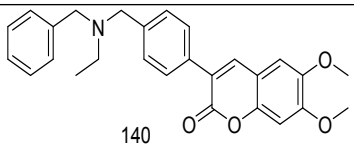

135

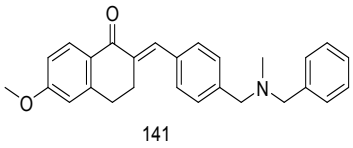

136


137


138


139


140


141

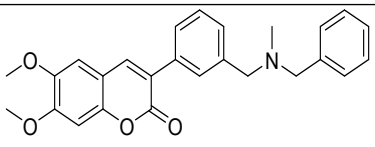

142

Table S 7. List of AChE inhibitors with observed and predicted pIC_{50}, fitness, and errors

Compound no.	Mol. Wt.	Canvas Similarity	Fitness	Phase QSAR set	pIC_{50}	Predicted pIC_{50}	Error
1	433.558	0.155	1.365	test	5.00	5.93	0.93
2	322.411	0.134	2.078	test	5.59	5.99	0.40
3	467.573	0.125	1.986	test	8.92	8.44	-0.48
4	426.581	0.153	1.503	test	8.22	8.38	0.16
5	350.465	0.146	2.915	test	8.08	8.09	0.01
6	348.492	0.14	3	test	8.24	8.01	-0.23
7	363.463	0.139	2.787	test	8.06	7.96	-0.10
8	377.491	0.124	2.805	test	8.55	8.27	-0.28
9	300.448	0.162	2.106	test	6.57	6.39	-0.18
10	348.449	0.161	2.12	test	7.10	7.67	0.57
11	428.579	0.161	1.869	test	6.23	6.41	0.18
12	354.472	0.16	1.996	test	6.69	6.98	0.29
13	354.472	0.151	2.105	test	7.19	6.51	-0.68
14	416.543	0.151	1.699	test	7.74	6.97	-0.77
15	360.466	0.144	1.456	test	6.66	6.58	-0.08
16	387.913	0.195	1.561	test	7.35	7.14	-0.21
17	393.961	0.146	1.793	test	6.93	7.27	0.34
18	407.001	0.146	1.674	test	7.24	6.7	-0.54
19	336.503	0.133	2.85	test	7.01	7.25	0.24
20	318.422	0.123	1.541	test	6.68	7.15	0.47
21	376.547	0.156	1.88	test	7.60	6.76	-0.84
22	362.519	0.151	2.167	test	7.27	7.32	0.05
23	362.519	0.149	0.83	test	8.00	7.33	-0.67
24	390.574	0.146	0.67	test	6.90	6.68	-0.22
25	376.547	0.146	1.515	test	7.44	7.08	-0.36
26	390.574	0.144	1.085	test	7.09	7.05	-0.04
27	375.472	0.136	1.569	test	6.34	6.48	0.14
28	438.618	0.129	1.053	test	7.27	7.27	0.00
29	363.436	0.122	1.758	test	5.80	5.69	-0.11
30	366.483	0.117	0.961	test	7.37	6.83	-0.54
31	454.613	0.113	1.295	test	6.41	6.3	-0.11
32	416.568	0.113	1.845	test	7.80	7.28	-0.52
33	401.532	0.108	1.516	test	7.59	7.52	-0.07
34	366.483	0.105	2.167	test	7.05	7.08	0.03
35	421.544	0.103	2.088	test	6.41	6.49	0.08
36	422.571	0.102	1.952	test	6.28	6.58	0.30
37	394.537	0.101	1.872	test	6.41	7.07	0.66
38	405.95	0.1	1.758	test	7.52	7.01	-0.51
39	386.545	0.144	2.122	test	7.68	7.58	-0.10
40	320.441	0.128	2.078	test	6.92	6.27	-0.65
41	305.424	0.286	1.772	test	6.82	6.19	-0.63
42	378.496	0.144	1.243	test	6.60	6.59	-0.01
43	404.601	0.153	1.686	training	5.03	5.09	0.06
44	408.51	0.121	1.75	training	5.06	5.02	-0.04
45	420.557	0.115	1.942	training	5.02	4.58	-0.44

46	332.49	0.1	1.065	training	5.64	5.68	0.04
47	394.946	0.143	1.49	training	5.57	5.41	-0.16
48	403.532	0.134	1.191	training	5.03	4.95	-0.08
49	407.95	0.124	0.876	training	5.06	4.96	-0.10
50	452.406	0.124	0.862	training	5.00	4.78	-0.22
51	407.95	0.124	1.543	training	5.11	5.02	-0.09
52	482.669	0.122	1.698	training	5.02	5.22	0.20
53	466.67	0.106	1.699	training	5.67	5.48	-0.19
54	432.568	0.148	0.803	training	5.06	5.02	-0.04
55	401.51	0.131	1.197	training	5.68	5.74	0.06
56	406.573	0.126	2.111	training	5.58	5.86	0.28
57	378.519	0.125	2.156	training	5.57	6.13	0.56
58	356.469	0.113	1.076	training	5.63	5.6	-0.03
59	345.445	0.1	1.76	training	5.74	5.72	-0.02
60	319.451	0.176	1.229	training	5.68	5.56	-0.12
61	378.475	0.176	2.06	training	8.10	7.66	-0.44
62	443.55	0.144	1.951	training	8.27	8.16	-0.11
63	452.558	0.132	1.965	training	8.62	8.8	0.18
64	393.446	0.127	2.121	training	8.05	8.14	0.09
65	420.555	0.178	1.551	training	8.34	8.5	0.16
66	370.494	0.164	1.625	training	8.20	8.25	0.05
67	438.545	0.155	1.693	training	8.59	8.38	-0.21
68	378.54	0.143	1.43	training	8.17	8.2	0.03
69	455.514	0.142	1.537	training	8.35	8.42	0.07
70	389.545	0.132	1.367	training	8.37	8.72	0.35
71	402.541	0.11	2.127	training	8.44	7.92	-0.52
72	350.465	0.138	2.757	training	8.15	7.92	-0.23
73	397.908	0.117	1.875	training	8.35	7.96	-0.39
74	402.585	0.116	2.71	training	9.10	8.59	-0.51
75	375.475	0.116	2.825	training	9.02	8.56	-0.46
76	389.502	0.115	2.892	training	9.24	9.02	-0.22
77	405.545	0.114	2.732	training	9.10	8.75	-0.35
78	379.504	1	1.774	training	8.24	8.18	-0.06
79	336.481	0.166	2.005	training	7.28	6.89	-0.39
80	337.469	0.162	2.006	training	6.97	6.97	0.00
81	307.44	0.179	2.188	training	6.52	6.35	-0.17
82	366.508	0.161	1.952	training	7.34	7.22	-0.12
83	348.492	0.153	1.923	training	6.00	6.07	0.07
84	374.493	0.144	1.987	training	8.03	8.21	0.18
85	349.48	0.142	1.988	training	6.10	6.17	0.07
86	349.436	0.137	1.778	training	7.89	7.98	0.09
87	464.619	0.126	0.889	training	6.92	7.04	0.12
88	443.638	0.125	0.73	training	6.42	6.51	0.09
89	360.544	0.103	1.355	training	6.46	6.45	-0.01
90	423.473	0.101	2.046	training	6.36	6.5	0.14
91	334.509	0.151	1.958	training	5.80	5.96	0.16
92	378.946	0.147	1.665	training	7.16	7.28	0.12

93	392.974	0.146	1.677	training	7.80	7.61	-0.19
94	372.518	0.139	1.738	training	6.92	6.63	-0.29
95	330.477	0.137	2.686	training	6.66	6.78	0.12
96	349.48	0.136	1.707	training	7.89	8.04	0.15
97	332.473	0.128	1.863	training	6.47	6.3	-0.17
98	394.989	0.103	1.767	training	5.99	5.95	-0.04
99	351.449	0.172	2.176	training	7.52	7.02	-0.50
100	442.602	0.169	1.624	training	7.05	7.1	0.05
101	384.521	0.163	1.653	training	6.96	7.14	0.18
102	378.519	0.161	2.145	training	7.40	7.3	-0.10
103	478.592	0.155	1.69	training	6.00	5.83	-0.17
104	348.492	0.154	2.187	training	6.95	6.79	-0.16
105	348.492	0.154	0.732	training	7.28	6.89	-0.39
106	362.519	0.151	2.167	training	6.79	6.8	0.01
107	405.498	0.149	1.696	training	5.85	6.05	0.20
108	376.547	0.148	2.14	training	6.90	7.06	0.16
109	376.547	0.148	0.757	training	7.96	8.06	0.10
110	390.574	0.147	1.057	training	7.66	7.49	-0.17
111	376.547	0.146	0.524	training	7.62	7.67	0.05
112	404.601	0.144	0.354	training	7.54	7.31	-0.23
113	404.601	0.143	0.348	training	6.42	6.39	-0.03
114	361.491	0.143	2.112	training	7.92	7.6	-0.32
115	418.628	0.141	1.102	training	7.33	7.33	0.00
116	438.618	0.135	0.084	training	6.07	5.64	-0.43
117	378.519	0.131	0.79	training	6.09	6.41	0.32
118	438.618	0.128	2.025	training	5.83	5.93	0.10
119	423.563	0.123	1.815	training	7.48	7.85	0.37
120	416.568	0.113	0.045	training	7.28	7.02	-0.26
121	366.483	0.111	1.929	training	7.80	7.7	-0.10
122	440.395	0.111	1.738	training	7.26	7.16	-0.10
123	394.537	0.108	2.116	training	6.84	6.63	-0.21
124	452.641	0.108	0.855	training	6.96	6.88	-0.08
125	393.49	0.106	2.13	training	7.19	6.81	-0.38
126	389.499	0.105	1.754	training	5.78	5.71	-0.07
127	349.542	0.103	1.569	training	5.85	5.73	-0.12
128	371.505	0.103	1.007	training	6.92	7.08	0.16
129	392.542	0.101	1.578	training	7.28	7.18	-0.10
130	373.498	0.1	1.616	training	6.00	5.78	-0.22
131	385.532	0.1	1.525	training	7.11	7.11	0.00
132	414.599	0.139	1.986	training	6.37	6.4	0.03
133	334.465	0.133	1.777	training	6.05	5.88	-0.17
134	346.476	0.128	2.302	training	5.96	6.24	0.28
135	413.543	0.122	2.735	training	7.85	8.02	0.17
136	347.464	0.122	2.27	training	5.92	6.39	0.47
137	439.562	0.119	2.669	training	8.03	8.23	0.20
138	422.578	0.117	1.667	training	6.36	6.19	-0.17
139	475.614	0.111	2.669	training	7.85	7.88	0.03
140	429.521	0.11	1.217	training	7.74	7.95	0.21

141	397.522	0.105	1.087	training	6.96	6.94	-0.02
142	415.493	0.101	1.916	training	6.69	6.86	0.17

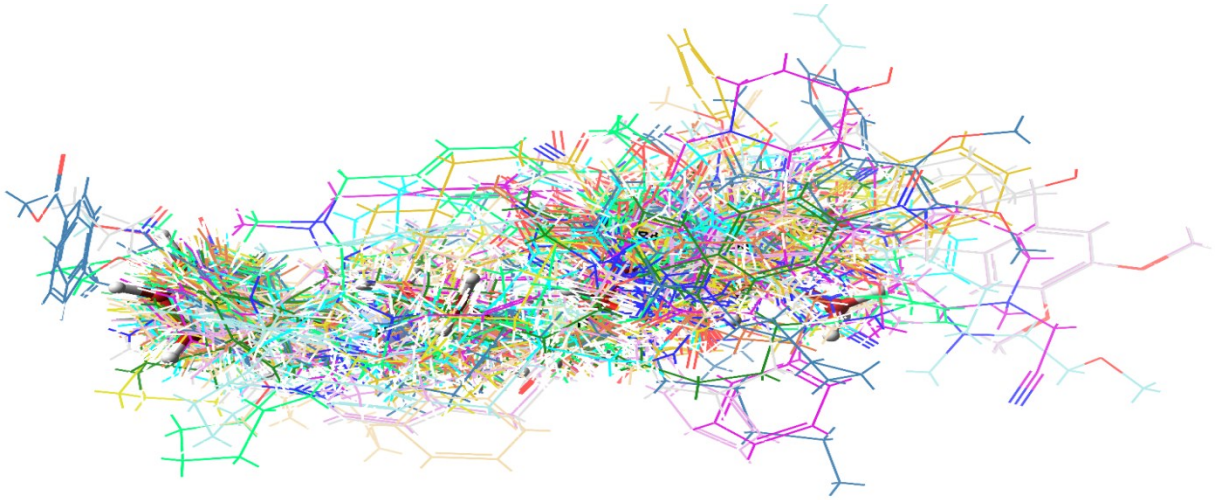


Figure S10. Alignment of all 142 AChE inhibitors with developed Pharmacophore hypothesis