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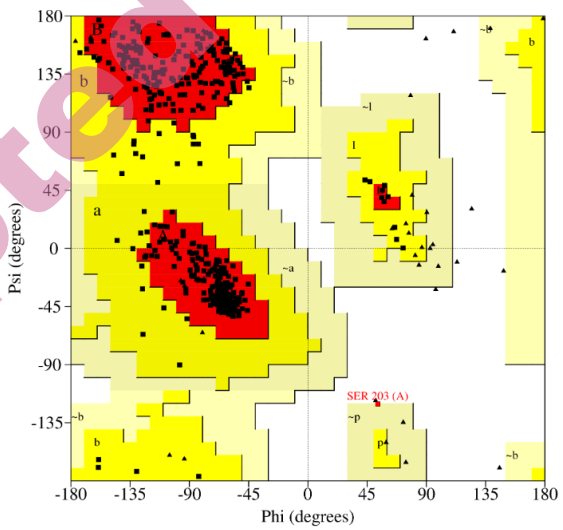
SUPPLEMENTARY MATERIAL TO

Selected phytochemicals as potent acetylcholinesterase inhibitors: An *in silico* prediction

RAM LAL SWAGAT SHRESTHA[1,2,3], PRABHAT NEUPANE[1], SUJAN DHITAL[1], NIRMAL PARAJULI[1], BINITA MAHARJAN[1,2], TIMILA SHRESTHA[1,2], SAMJHANA BHARATI[1,2], BISHNU PRASAD MARASINI[3,4] AND JHASHANATH ADHIKARI SUBIN[5]*

[1]Department of Chemistry, Amrit Campus, Tribhuvan University, Lainchour, Kathmandu 44600, Nepal, [2]Kathmandu Valley College, Syuchatar Bridge, Kalanki, Kathmandu 44600, Nepal, [3]Institute of Natural Resources Innovation, Kalimati, Kathmandu 44600, Nepal, [4]Nepal Health Research Council, Ramshah Path, Kathmandu 44600, Nepal, and [5]Bioinformatics and Cheminformatics Division, Scientific Research and Training Nepal P. Ltd., Bhaktapur 44800, Nepal.



Plot statistics

Residues in most favoured regions [A,B,L]	387	90.0%
Residues in additional allowed regions [a,b,l,p]	42	9.8%
Residues in generously allowed regions [~a,~b,~l,~p]	1	0.2%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	430	100.0%
Number of end-residues (excl. Gly and Pro)	5	
Number of glycine residues (shown as triangles)	49	
Number of proline residues	44	
Total number of residues	528	

Fig. S5. Ramachandran plot with statistics (SAVES v6.0 server. Procheck program).

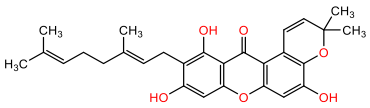
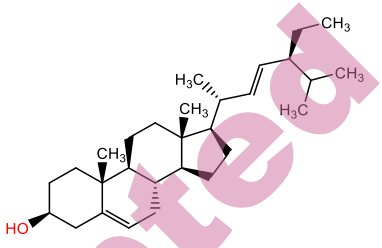
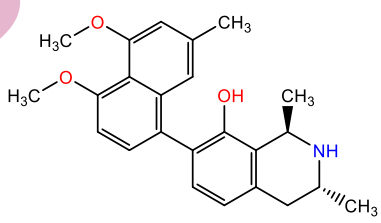
* Corresponding author. E-mail: subinadhikari2018@gmail.com

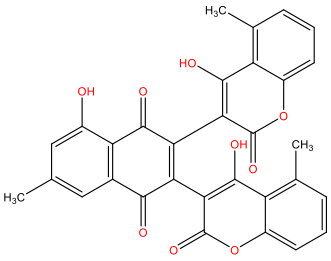
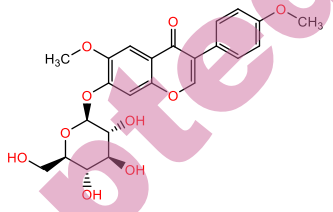
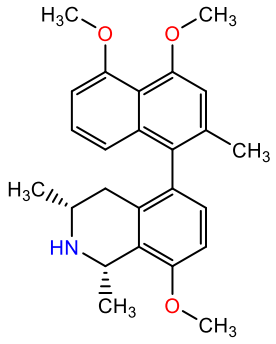
TABLE S-3: Binding affinity (kJ/mol) of the docked ligands with protein (PDB ID: 7E3H)

S. N	Ligands	PubChem CID	Binding affinity (kJ/mol)
1	Allanxanthone B	11328706	-50.651
2	Stigmasterol	5280794	-49.446
3	5′-O-methyldioncophylline D	132542154	-48.400
4	Durallone	1023565	-48.216
5	Simplexin	119045	-47.990
6	Ismailin	135454728	-47.977
7	Wistin	10095770	-47.839
8	Dioncophylline C2	132500912	-47.417
9	Asphodelin	182665	-47.325
10	Sungucine	189778	-47.237
11	Rotenone	6758	-46.944
12	Azadirone	10906239	-46.814
13	Native	1150567	-46.789
14	Niloticin	14021529	-46.651
15	Strychnogucine C	5321534	-46.505
16	7,2′-dimethoxy-4′,5′-methylenedioxyisofavone	343083	-46.496
17	Mellerin B	102317133	-46.061
18	Dioncophylline F	132500908	-46.032
19	Hispidol B	13967183	-45.923
20	Isoacteoside	6476333	-45.835
21	Piscidinol A	12004524	-45.814
22	Isoknipholone	11729754	-45.781
23	Seco-tiaminic acid A	122178985	-45.697
24	Stachannin	44258461	-45.501
25	Jamaicin	12304682	-45.203
26	Microdontin A	101629131	-45.053
27	Maximaisoflavone B	44257236	-44.986
28	Salaterpene A	102583505	-44.969
29	Retusin	5352005	-44.915
30	Busseihydroquinone C	60201022	-44.911
31	Microdontin B	101629132	-44.777
32	Tetrahydropalmatine	5417	-44.764
33	Quercetin-3-O-β-dglucopyranoside	5280804	-44.689
34	Deguelin	107935	-44.685
35	Volkensiflavone	5480834	-44.584
36	Montrifoline	157014	-44.555
37	5-Demethyltangeretin	96539	-44.517
38	Pectolinarigenin-7-glucoside	44258439	-44.488
39	Tephrodin	44257635	-44.475
40	Calopogoniumisoflavone A	354119	-44.404
41	Kaempferol 3-O-beta-D-glucoside	5282102	-44.379
42	Mzikonone	14109464	-44.367

43	Ekeberin A	101843374	-44.061
44	Busseihydroquinone D	60201023	-43.994
45	Dianellin	422284	-43.798
46	Ursolic acid	64945	-43.722
47	Lupeol	259846	-43.706
48	Macluraxanthone	5281646	-43.672
49	Maximaisoflavone J	177731	-43.643
50	Betulin	72326	-43.559
51	Aloin A	12305761	-43.538
52	Acteoside	5281800	-43.526
53	11-Epi-toonacilin	73348892	-43.496
54	Azadironolide	10814144	-43.400
55	Aloin B	14989	-42.743
56	Rhamnazin	5320945	-42.639
57	3-Epi-oleanolic acid	11869658	-42.513
58	Quercetin-3,4′-dimethyl ether	5380905	-42.404
59	Lysicamine	122691	-42.103
60	Proceranolide	23258999	-41.350
61	Maximaisoflavone H	13873188	-41.346
62	Salaterpene B	102583506	-41.342
63	Skimmiamine	6760	-40.258
64	3-Epioleanolic acid	11869658	-39.714
65	Busseihydroquinone B	70688905	-39.655
66	Montanin	442060	-39.463
67	Darienine	130672	-39.199
68	Heliparvifoline	5281846	-38.630
69	Maculine	68232	-38.225
70	Bisnordihydrotoxiferine	6440874	-37.049
71	Bergenin	66065	-36.342
72	Gadenine	436042	-32.605
73	Cyclombandakamine A2	132608472	-13.275
74	Cyclombandakamine A1	132608471	-11.844

TABLE S-2: Chemical structures of top candidates, their binding affinities and type of interactions with amino acid residues of AChE

Lig.	Structure	Types of interactions	Active site residues (Distance Å)
Allanxanthone B		Hydrogen Bond	ASP74 (2.55)
		Pi-Pi Stacked	TRP286 (5.95, 6.19), TYR337 (5.38), TYR341 (3.46, 3.99, 4.48)
		Alkyl	LEU289 (5.33)
		Pi-Alkyl	TRP86 (4.11, 4.33), TRP286 (4.44, 4.66), TYR337 (4.49), PHE338 (5.02), TYR341 (5.39), **HIS447 (4.74)**
		van der Waals	TYR72, GLY121, GLY120, TYR124, SER125, GLY126, LEU130, TYR133, GLU202, **SER203**, ALA204, SER293, VAL294, PHE295, ARG296, GLY342, GLY448
Stigmasterol		Pi-Donor Hydrogen Bond	TRP86 (2.67)
		Pi-Alkyl	TRP286 (3.75, 4.55), PHE297 (4.88, 5.25), TYR337 (3.43, 5.26), PHE338 (4.69, 4.71), TYR341 (4.56, 4.67, 4.84), **HIS447 (4.82)**
		van der Waals	TYR72, ASP74, LEU76, GLY121, TYR124, SER125, GLU202, **SER203**, LEU289, SER293, VAL294, PHE295, ARG296, GLY342, GLY448
5'-O-methyl dioncophylline D		Hydrogen Bond	**HIS447 (3.08)**
		Carbon-hydrogen bond	PHE338 (2.89), **HIS447 (3.14, 3.41)**
		Pi-Donor Hydrogen Bond	TYR124 (2.78)
		Pi-Pi Stacked	TRP286 (5.33), TYR337 (5.04), TYR341 (3.88, 4.92)
		Alkyl	VAL294 (3.81)
		Pi-Alkyl	TYR72 (4.35), TYR124 (4.74), TRP286 (4.25, 6.16), TYR337 (3.64), PHE338 (4.26), TYR341 (4.52, 4.72, 4.80, 5.38), **HIS447 (4.79, 5.29)**
		van der Waals	ASP74, LEU76, GLY121, GLU202, **SER203**, PHE295, PHE297, GLY448

Ismailin		Hydrogen Bond	PHE295 (2.66)
		Pi-Cation	ASP74 (2.75, 3.18)
		Pi-Donor Hydrogen Bond	TYR124 (2.20), TYR341 (2.68)
		Pi-Pi Stacked	TRP286 (4.62, 5.06), TYR341 (5.49)
		Pi-Sigma	TYR341 (3.65)
		Pi-Lone Pair	TYR124 (2.36)
		Alkyl	VAL294 (5.15)
		Pi-Alkyl	TRP86 (4.69), TYR337 (3.61, 5.06), PHE338 (3.80), TYR341 (5.37)
		van der Waals	TYR72, THR83, ASN87, GLY121, SER125, **SER203**, SER293, ARG296, PHE297, GLY342, **HIS447**
Wistin		Hydrogen Bond	ASP74 (2.55), SER293 (2.59)
		Carbon-hydrogen bond	SER125 (2.70), SER293 (2.94)
		Pi-Pi Stacked	TYR337 (5.56), TYR341 (3.92, 4.24)
		Alkyl	LEU289 (5.49)
		Pi-Alkyl	TRP86 (4.47), TRP286 (5.48)
		van der Waals	TYR72, THR83, ASN87, GLY121, TYR124, GLY126, LEU130, TYR133, GLU202, **SER203**, VAL294, PHE295, ARG296, PHE297, PHE338, **HIS447**, GLY448
Dioncophylline C2		Carbon Hydrogen Bond	TYR341 (2.90, 3.05)
		Pi-Sigma	TRP86 (2.32)
		Pi-Pi Stacked	TRP86 (5.61), TYR124 (5.15), TRP286 (4.94), TYR337 (4.78), TYR341 (3.29, 4.26)
		Alkyl	VAL294 (4.07)
		Pi-Alkyl	TRP86 (3.33), TRP286 (4.42), PHE295 (4.75), PHE297 (3.90), TYR337 (5.09), PHE338 (3.26, 4.88), TYR341 (4.27, 5.23)
		van der Waals	TYR72, ASP74, THR83, GLY121, SER125, **SER203**, SER293, ARG296, **HIS447**, GLY448, TYR449

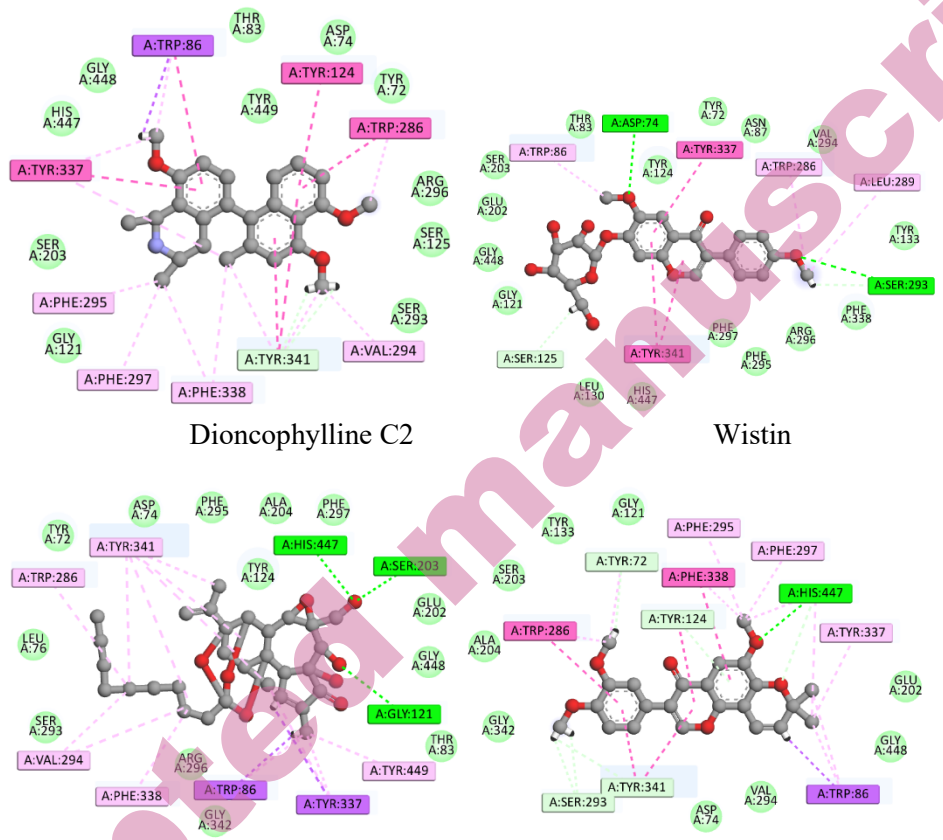


Fig. S6. 2D interaction of dioncophylline C2, wistin, simplexin and durallone with amino acid residues of the receptor

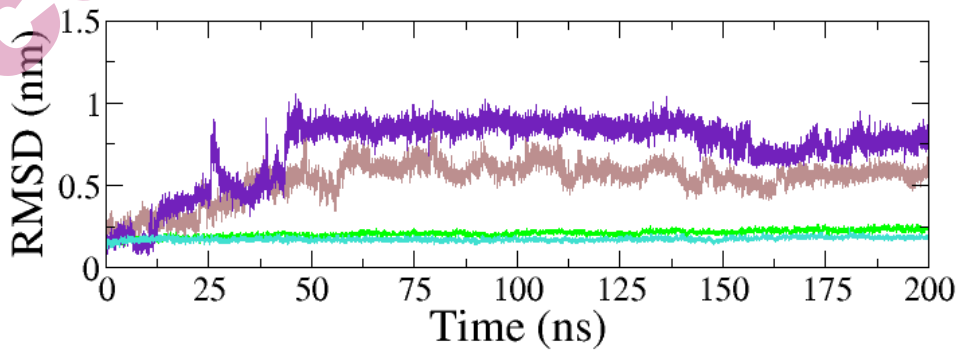


Fig. S7. RMSD curves of simplexin (brown) and durallone (violet) with RMSD curve of protein backbone of simplexin (green) and durallone complex (turquoise)

Spatial analysis of ligand and protein

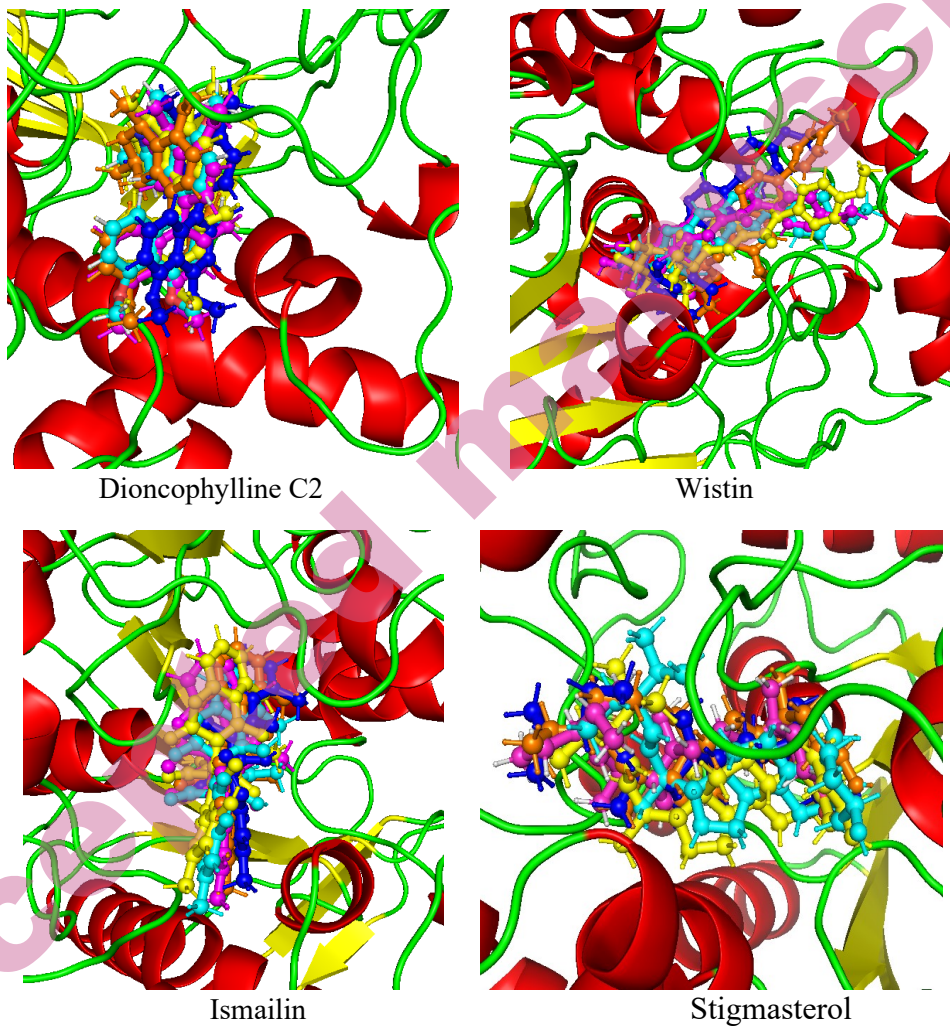
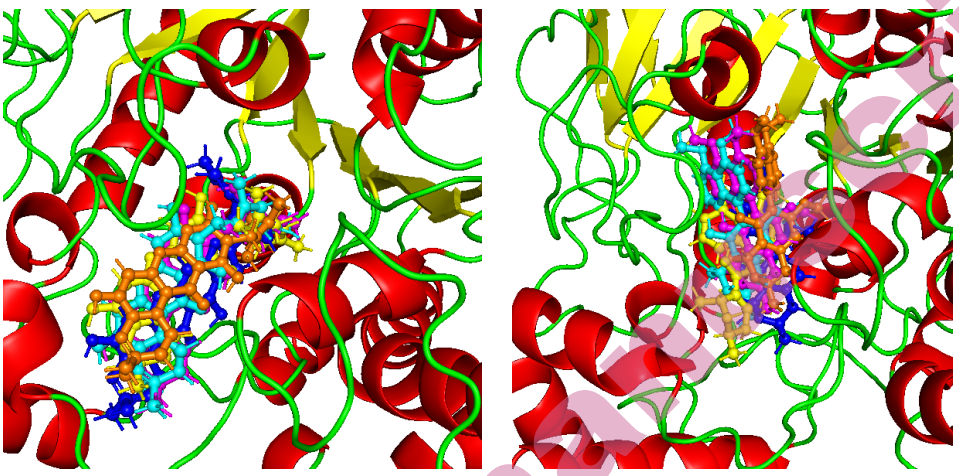


Fig. S4. Snapshots of protein-ligand interactions in three complexes captured at various time points (1 ns in blue, 50 ns in orange, 100 ns in magenta, 150 ns in cyan, and 200 ns in yellow) during MDS



Allanxanthrone B 5'-O-methyldioncophylline D

Fig. S4. Snapshots of protein-ligand interactions in three complexes captured at various time points (1 ns in blue, 50 ns in orange, 100 ns in magenta, 150 ns in cyan, and 200 ns in yellow) during MDS

TABLE-S3: Binding free energy changes (ΔG_{BFE}) along with its components in complexes

Complexes	$\Delta E_{VDWAALS}$	ΔE_{EL}	ΔE_{PB}	ΔE_{NPOLAR}	ΔG_{GAS}	ΔG_{SOLV}	ΔG_{BFE}
Allanxanthone B	-237.90 ± 11.29	-49.70 ± 20.25	216.52 ± 23.05	-23.55 ± 0.75	-287.60 ± 23.97	192.96 ± 22.71	-94.64 ± 19.49
Stigmasterol	-205.89 ± 11.50	-39.83 ± 13.13	136.23 ± 16.98	-21.79 ± 0.54	-245.72 ± 18.45	114.39 ± 16.94	-131.33 ± 18.24
5′-O-methyldioncophylline D	-182.79 ± 11.71	-21.12 ± 13.17	133.80 ± 21.25	-19.83 ± 0.71	-203.92 ± 16.31	113.93 ± 21.04	-89.99 ± 18.49
Ismailin	-225.93 ± 10.33	-71.63 ± 16.73	242.42 ± 22.04	-22.46 ± 0.62	-297.56 ± 20.12	219.95 ± 21.92	-77.61 ± 17.07
Wistin	-196.77 ± 11.58	-120.75 ± 42.04	246.06 ± 28.36	-22.13 ± 0.50	-317.52 ± 43.17	223.96 ± 28.24	-93.59 ± 29.32
Dioncophylline C2	-210.58 ± 12.46	-99.66 ± 13.80	222.71 ± 22.00	-20.83 ± 0.54	-310.20 ± 20.04	201.83 ± 22.75	-108.36 ± 18.66

TABLE-S4: ADMET properties of six hit candidates

ADMET parameters	Compounds					
	Allanxanthone B	Stigmasterol	5′-O-methyldioncophylline D	Ismailin	Wistin	Dioncophylline C2
Caco2 permeability	Low	High	High	Low	Low	Low
Intestinal absorption	High	High	High	High	High	High
Skin Permeability	High	High	High	High	High	High
BBB permeability	-1.1	0.771	-0.314	-1.126	-1.478	-0.132
CNS permeability	-1.767	-1.652	-1.563	-3.154	-3.784	-1.341
CYP2D6 substrate	No	No	Yes	No	No	No
CYP3A4 substrate	Yes	Yes	Yes	Yes	No	Yes
CYP1A2 inhibitor	No	No	Yes	No	No	Yes
CYP2C19 inhibitor	Yes	No	No	Yes	No	Yes
CYP2C9 inhibitor	Yes	No	No	Yes	No	No
CYP2D6 inhibitor	No	No	Yes	No	No	Yes
CYP3A4 inhibitor	Yes	No	Yes	No	No	Yes
Total clearance	-0.213	0.618	0.8	0.21	0.162	0.743
AMES toxicity	No	No	Yes	No	No	Yes
hERG I inhibitor	No	No	No	NO	No	Yes
hERG II inhibitor	Yes	Yes	Yes	Yes	No	Yes
Hepatotoxicity	Yes	No	Yes	Yes	No	Yes
Skin sensitisation	No	No	No	No	No	No

TABLE-S5: ADMET properties of native and reference drugs

ADMET parameters	Compounds		
	Donepezil (native)	Rivastigmine	Galantamine
Caco2 permeability	High	High	High
Intestinal absorption	High	High	High
Skin Permeability	High	High	High
BBB permeability	0.157	0.508	-0.081
CNS permeability	-1.464	-2.255	-2.511
CYP2D6 substrate	Yes	No	No
CYP3A4 substrate	Yes	No	Yes
CYP1A2 inhibitor	No	No	No
CYP2C19 inhibitor	No	No	No
CYP2C9 inhibitor	No	No	No
CYP2D6 inhibitor	Yes	Yes	No
CYP3A4 inhibitor	Yes	No	No
Total clearance	0.987	0.557	0.991
AMES toxicity	No	No	No
hERG I inhibitor	No	No	No
hERG II inhibitor	Yes	No	Yes
Hepatotoxicity	Yes	No	Yes
Skin sensitisation	No	No	No