

Supporting Information

Design, Synthesis, and Evaluation of Multitarget-Directed Resveratrol Derivatives for the Treatment of Alzheimer's Disease

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Table of Contents

- SI1. Docking study of compounds to MAO-B (S2)
- SI2. Docking study of compounds to AChE (S3)
- SI3. In Vitro Blood-Brain Barrier Permeation Assay (S4-S5)
- SI4. Metal-chelating properties of CQ (S6)
- SI5. Association constant study (S6-S7)
- SI6. HPLC chromatograms of target compounds (S8-S17)

SI1: Docking study of compounds to MAO-B

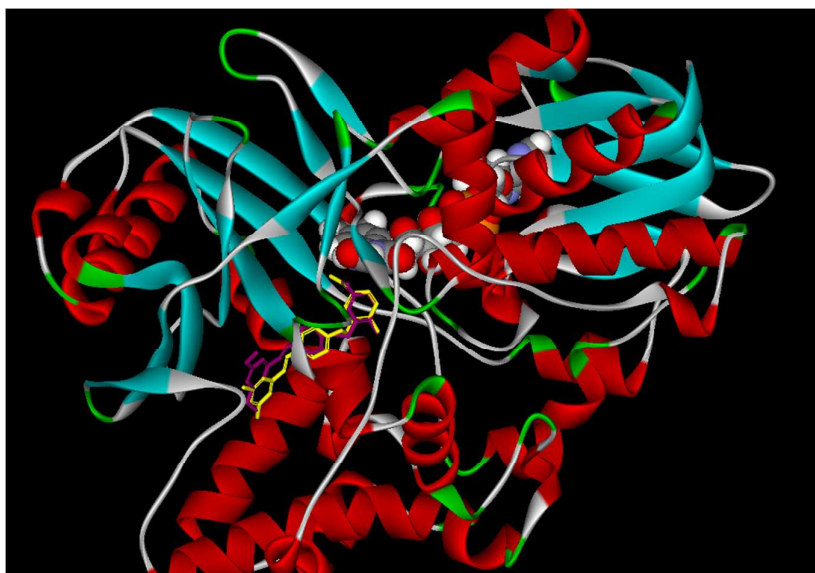


Figure S1. Predicted positions of **5d** (purple red) and **10d** (yellow) into *h*MAO-B catalytic sites. Compounds and the FAD cofactor were depicted using stick and space fill representation, respectively.

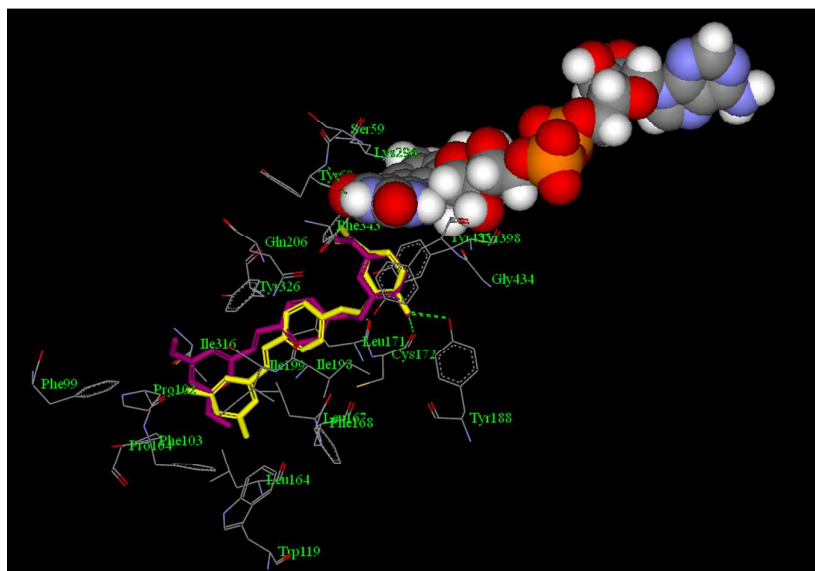


Figure S2. Representation of compounds **5d** (purple red) and **10d** (yellow) docked into the binding site of MAO-B highlighting the protein residues that form the main interactions with the inhibitors. FAD cofactor were depicted using space fill representation. Hydrogen-bonding interaction between ligands and residues are shown with the green line.

SI2: Docking study of compounds to AChE



Figure S3. Predicted positions of **5d** (purple red) and **10d** (yellow) into *TcAChE* catalytic sites. Compounds and protein were depicted using stick and solid ribbon, respectively.

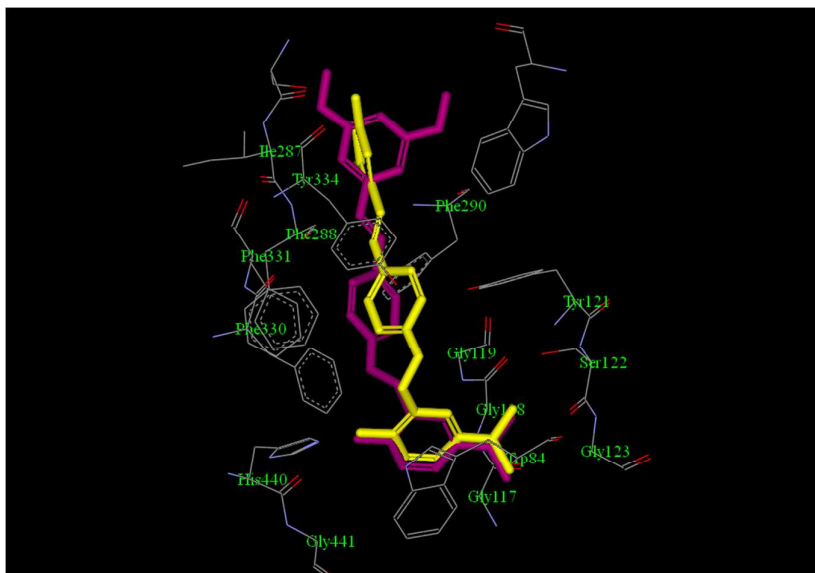


Figure S4. Representation of compounds **5d** (purple red) and **10d** (yellow) docked into the binding site of *TcAChE* highlighting the protein residues that form the main interactions with the inhibitors.

SI3. In Vitro Blood-Brain Barrier Permeation Assay

Table S1. Permeability ($P_e \times 10^{-6} \text{ cm s}^{-1}$) in the PAMPA-BBB Assay for 13 commercial drugs used in the Experiment Validation.

Commercial drugs	Bibl ^a	PBS:EtOH(70:30) ^b
testosterone	17	22.3 ± 1.4
verapamil	16	21.2 ± 1.9
desipramine	12	16.4 ± 1.2
progesterone	9.3	17.7 ± 1.2
promazine	8.8	14.3 ± 0.5
chlorpromazine	6.5	6.0 ± 0.3
clonidine	5.3	5.1 ± 0.3
piroxicam	2.5	0.24 ± 0.01
hydrocortisone	1.9	0.65 ± 0.01
lomefloxacin	1.1	0.37 ± 0.02
atnolol	0.8	0.78 ± 0.02
ofloxacin	0.8	0.37 ± 0.02
theophylline	0.1	0.26 ± 0.01

^a Taken from ref. ¹

^b Data are the mean \pm SD of three independent experiments

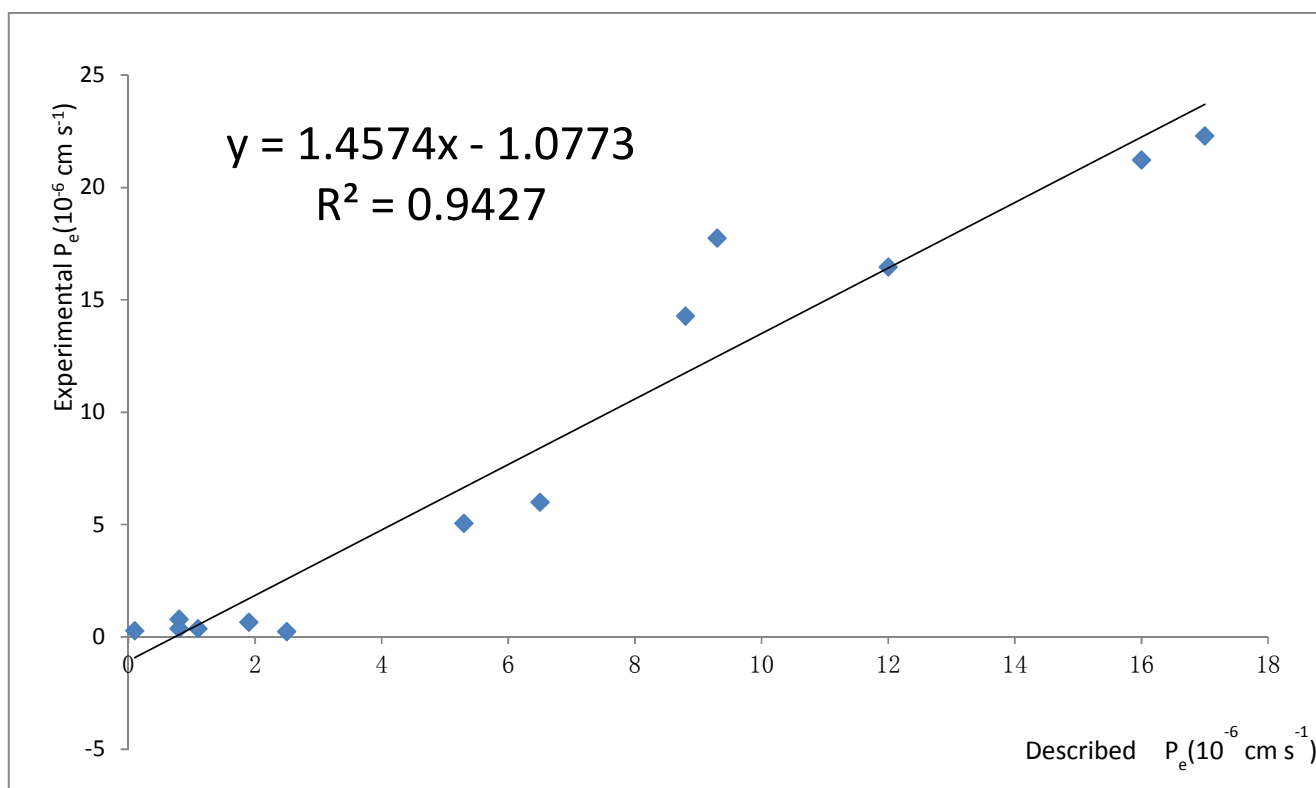
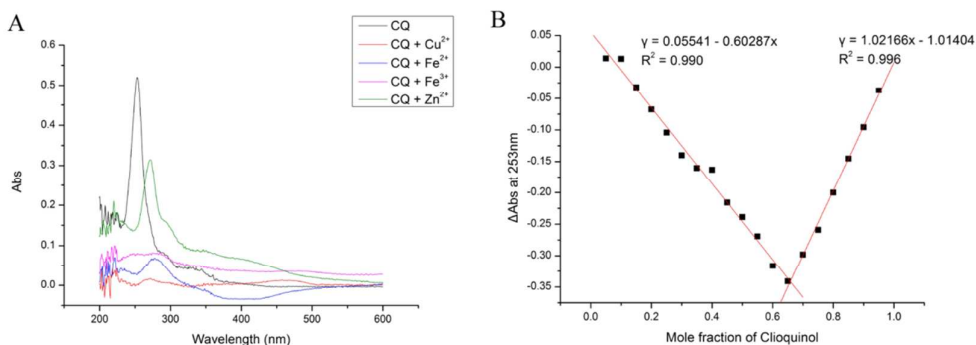


Figure S5. Lineal correlation between experimental and reported permeability of commercial drugs using the PAMPA-BBB assay. $P_e \text{ (exp.)} = 1.4574P_e \text{ (bibl.)} - 1.0773$ ($R^2 = 0.9427$)

Table S2. Ranges of Permeability of PAMPA-BBB Assays (P_e , $10^{-6} \text{ cm s}^{-1}$)

Compounds of high BBB permeation (CNS+)	$P_e > 4.7$
Compounds of uncertain BBB permeation (CNS+/-)	$4.7 > P_e > 1.8$
Compounds of low BBB permeation (CNS-)	$P_e < 1.8$

SI4. Metal-chelating properties of CQ



(A) UV spectrum of compound **CQ** (20 μM) alone and in the presence of CuSO₄ (40 μM), FeSO₄ (40 μM), FeCl₃ (40 μM) or ZnCl₂ (40 μM) in 20% (v/v) ethanol/buffer (20 mM HEPES, 150 mM NaCl, pH 7.4). (B) Determination of the stoichiometry of complex CQ-Cu(II) by Job's method.

The conditional stability constant for Cu(CQ)₂ is $1.2 \times 10^{10} \text{ M}^{-2}$ as literature report.²

SI5. Association constant study

The studies were conducted in 20% (v/v) ethanol/buffer (20 mM HEPES, 150 mM NaCl, pH 7.4). Solutions were obtained with the condition that the concentrations of **5d** or **10d** was constant in all samples (20 μM), but the proportions of copper ion varied between 0 and 300%. The spectrum was recorded after 30 min incubation at room temperature. The association constant were calculated using nonlinear least-square analysis.

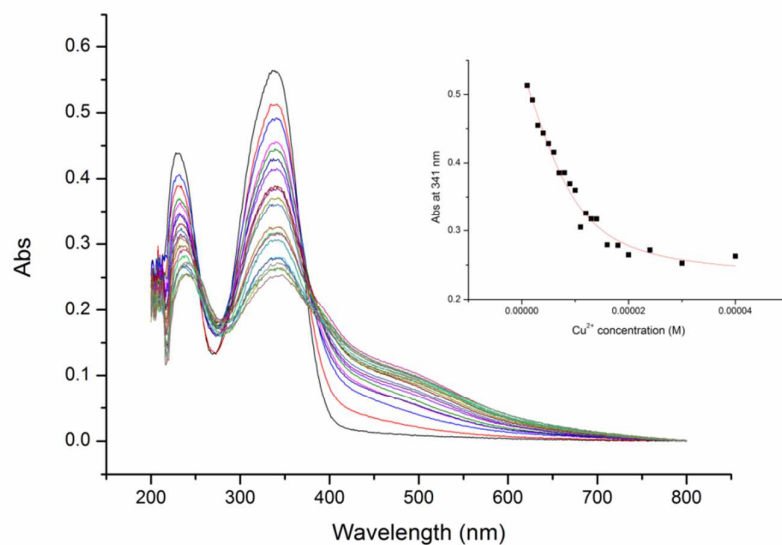


Fig S7. Spectra of **5d** (20 μM) upon addition of Cu^{2+} (0–40 μM) in 20% (v/v) ethanol/buffer (20 mM HEPES, 150 mM NaCl, pH 7.4). Inset: Absorption at 341 nm of **5d** (20 μM) as a function of Cu^{2+} .

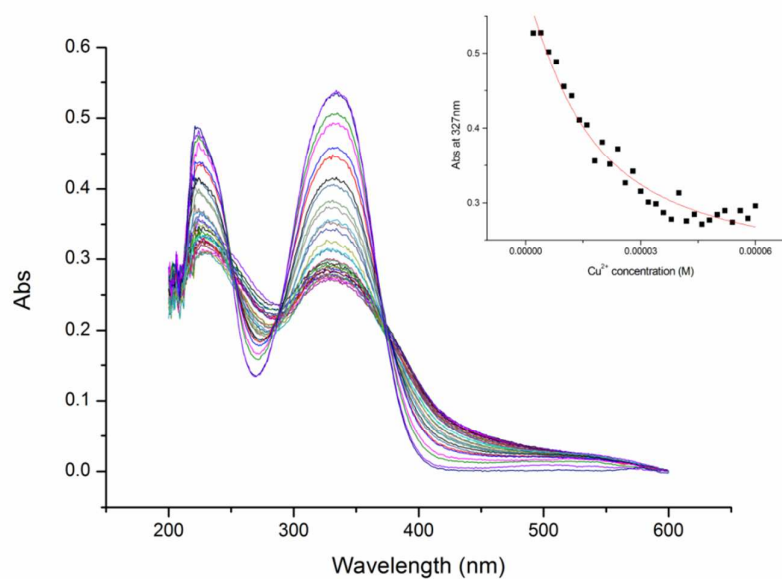
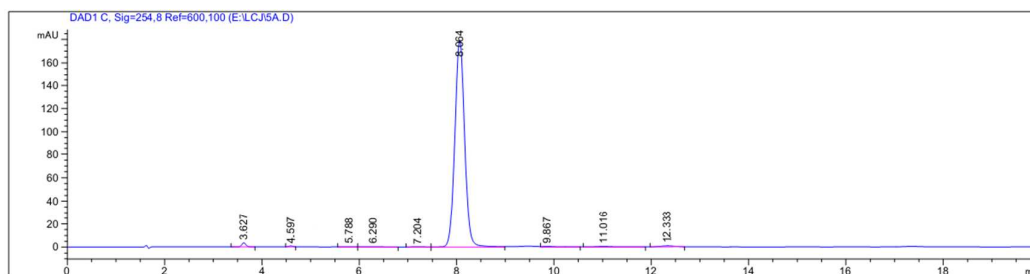


Fig S8. Spectra of **10d** (20 μM) upon addition of Cu^{2+} (0–60 μM) in 20% (v/v) ethanol/buffer (20 mM HEPES, 150 mM NaCl, pH 7.4). Inset: Absorption at 327 nm of **10d** (20 μM) as a function of Cu^{2+} .

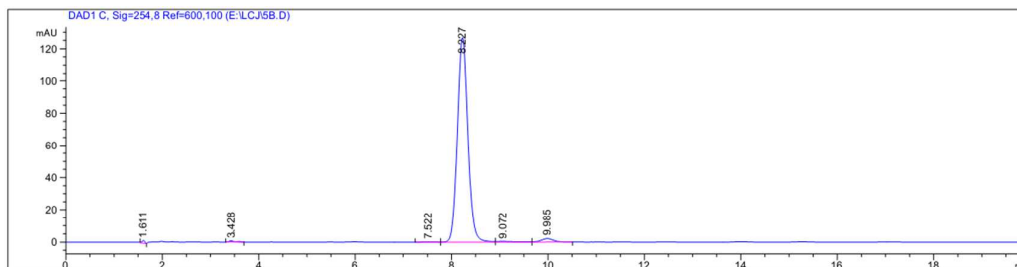
SI6. HPLC chromatograms of target compounds

HPLC chromatograms of **5a**



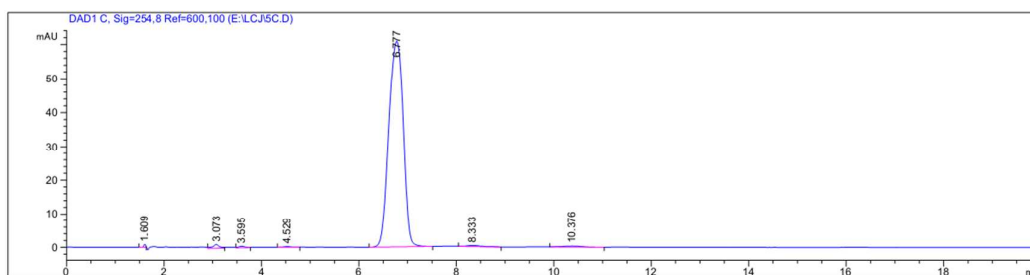
#	[min]		[min]	[mAU*s]	[mAU]	%
1	3.627	BB	0.0953	20.70368	3.38793	0.7950
2	4.597	MM	0.1246	5.91027	7.90761e-1	0.2270
3	5.788	BV	0.1987	2.77632	1.79236e-1	0.1066
4	6.290	VB	0.3124	6.47548	3.04170e-1	0.2487
5	7.204	BV	0.2644	2.80996	1.33051e-1	0.1079
6	8.064	VB	0.2172	2507.47461	179.27007	96.2884
7	9.867	VB	0.3726	16.74744	5.80486e-1	0.6431
8	11.016	BV	0.5759	25.70781	5.72534e-1	0.9872
9	12.333	MM	0.3397	15.52500	7.61658e-1	0.5962

HPLC chromatograms of **5b**



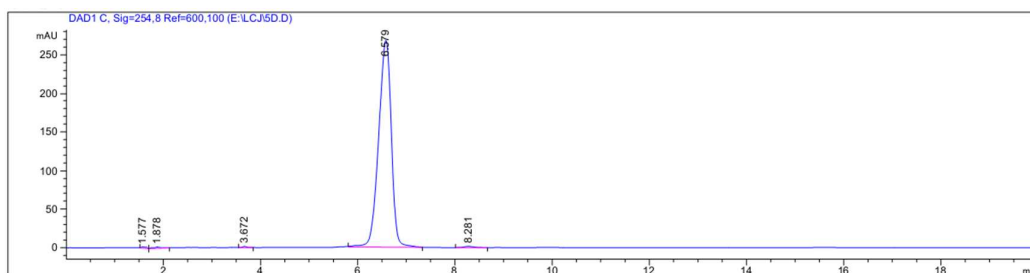
#	[min]		[min]	[mAU*s]	[mAU]	%
1	1.611	BV	0.0678	6.76398	1.58502	0.3414
2	3.428	MM	0.1497	6.77063	7.53708e-1	0.3417
3	7.522	BV	0.2191	2.25709	1.39235e-1	0.1139
4	8.227	VV	0.2325	1917.02808	126.68177	96.7503
5	9.072	VB	0.3430	11.36791	4.58866e-1	0.5737
6	9.985	BB	0.2691	37.23132	2.11807	1.8790

HPLC chromatograms of **5c**



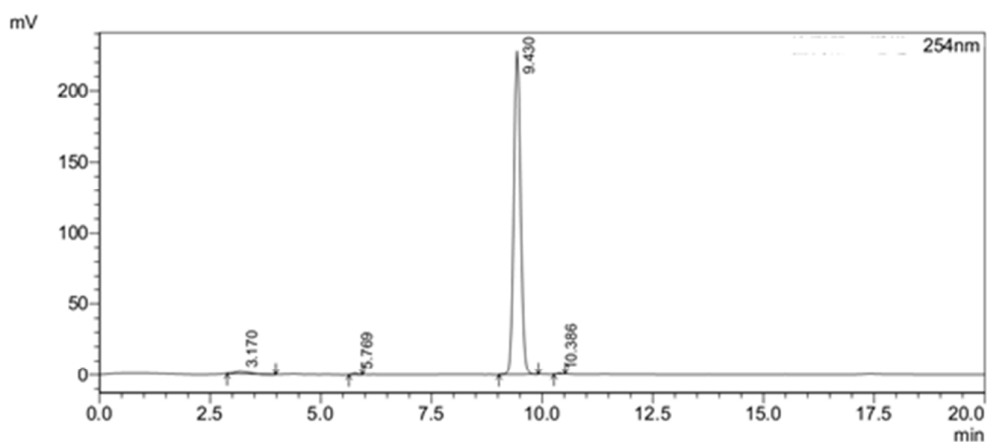
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1	1.609	MM	0.0460	2.38470	8.64449e-1	0.1847
2	3.073	VB	0.1400	11.44960	1.15131	0.8867
3	3.595	BB	0.1419	4.06998	4.09689e-1	0.3152
4	4.529	BB	0.1782	1.73461	1.44376e-1	0.1343
5	6.777	BB	0.3431	1257.42859	60.85807	97.3793
6	8.333	BB	0.3229	6.89660	3.08374e-1	0.5341
7	10.376	BB	0.3135	7.30514	3.16280e-1	0.5657

HPLC chromatograms of **5d**



#	[min]		[min]	[mAU*s]	[mAU]	%
1	1.577	BV	0.0973	13.81524	1.89044	0.2809
2	1.878	VB	0.1611	21.26019	1.68564	0.4323
3	3.672	BB	0.1025	9.70051	1.47901	0.1973
4	6.579	BB	0.2814	4850.00146	267.70364	98.6239
5	8.281	BB	0.2294	22.89616	1.55865	0.4656

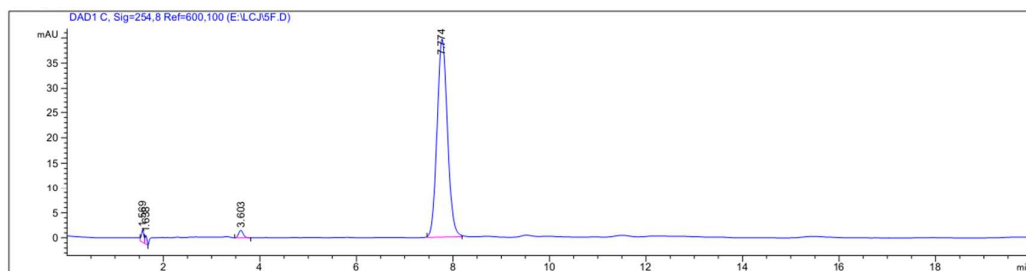
HPLC chromatograms of **5e**



254nm

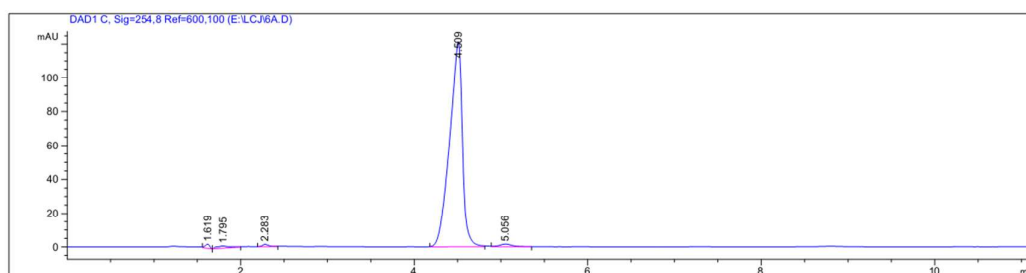
#	min	mAU*s	mAU	%
1	3.170	37342	1647	1.508
2	5.769	7879	1111	0.318
3	9.430	2426467	227770	97.994
4	10.386	4444	525	0.179

HPLC chromatograms of **5f**



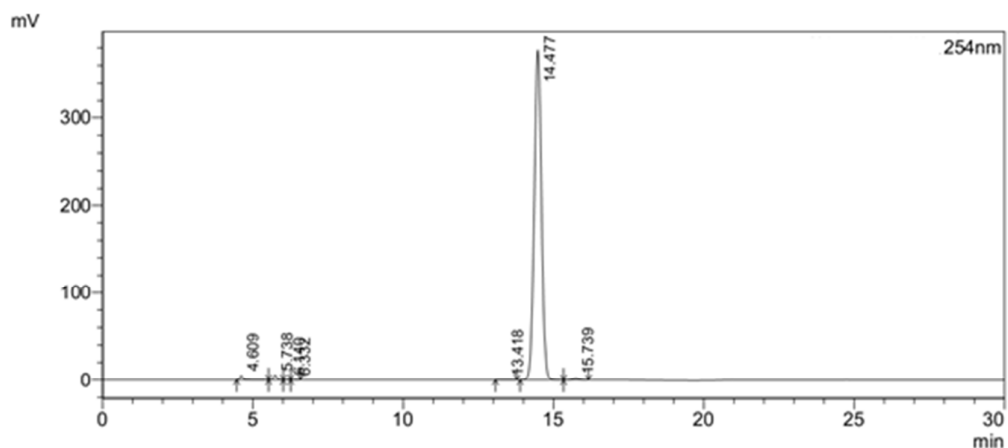
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1	1.569	BV	0.0537	8.87826	2.48395	1.4402
2	1.638	VV	0.0402	4.43376	1.71167	0.7192
3	3.603	BB	0.1026	9.77267	1.48857	1.5853
4	7.774	BB	0.2307	593.36365	39.61987	96.2552

HPLC chromatograms of **6a**



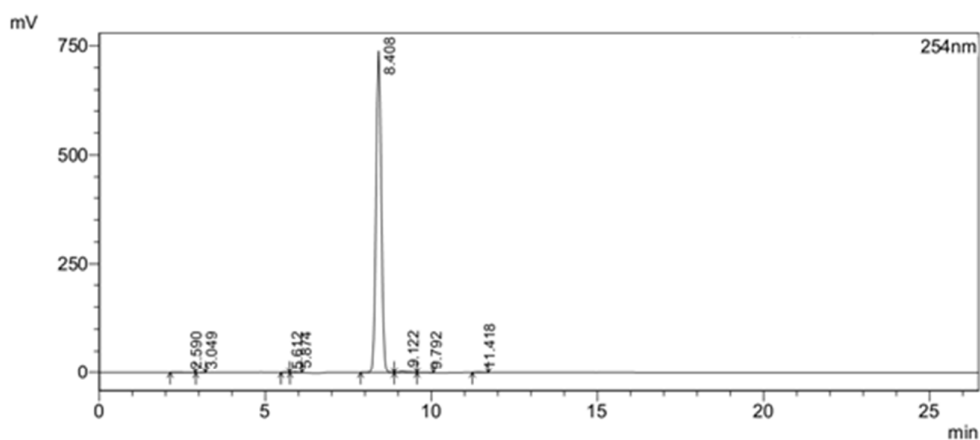
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1	1.619	BV	0.0569	9.03975	2.45866	0.7034
2	1.795	VB	0.1435	15.07959	1.35805	1.1734
3	2.283	BB	0.0812	7.07955	1.30651	0.5509
4	4.509	BB	0.1469	1237.80078	121.48688	96.3144
5	5.056	BB	0.1529	16.16717	1.56053	1.2580

HPLC chromatograms of **6b**



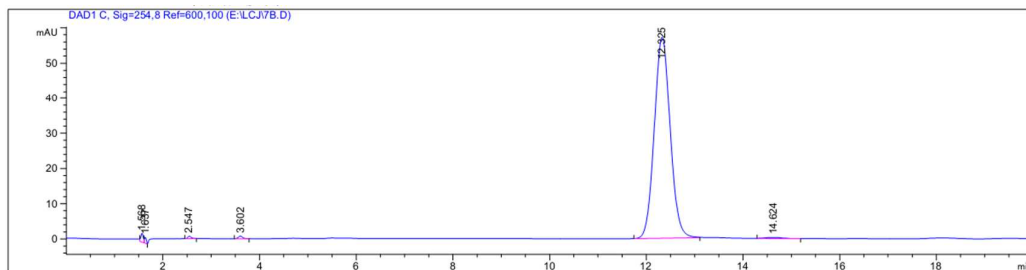
#	min	mAU*s	mAU	%
1	4.609	36797	5047	0.575
2	5.738	43458	5649	0.679
3	6.140	2999	345	0.047
4	6.332	1764	174	0.028
5	13.418	5034	288	0.079
6	14.477	6276927	377757	98.102
7	15.739	31370	1736	0.490

HPLC chromatograms of **7a**



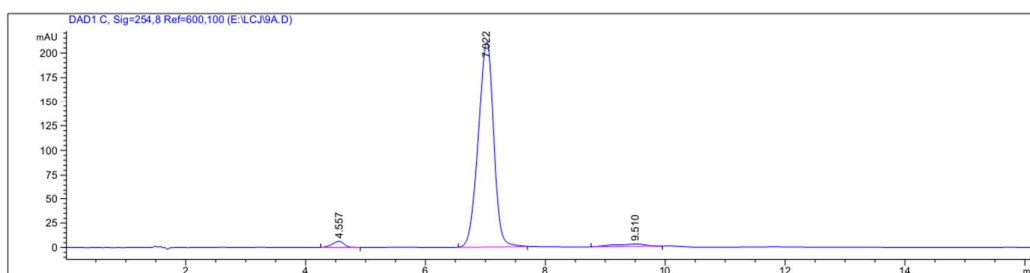
#	min	mAU*s	mAU	%
1	2.590	13736	503	0.167
2	3.049	5074	672	0.062
3	5.612	2913	396	0.036
4	5.874	12008	1414	0.146
5	8.408	8116773	737819	98.936
6	9.122	45848	2957	0.559
7	9.792	3475	230	0.042
8	11.418	4274	313	0.052

HPLC chromatograms of **7b**



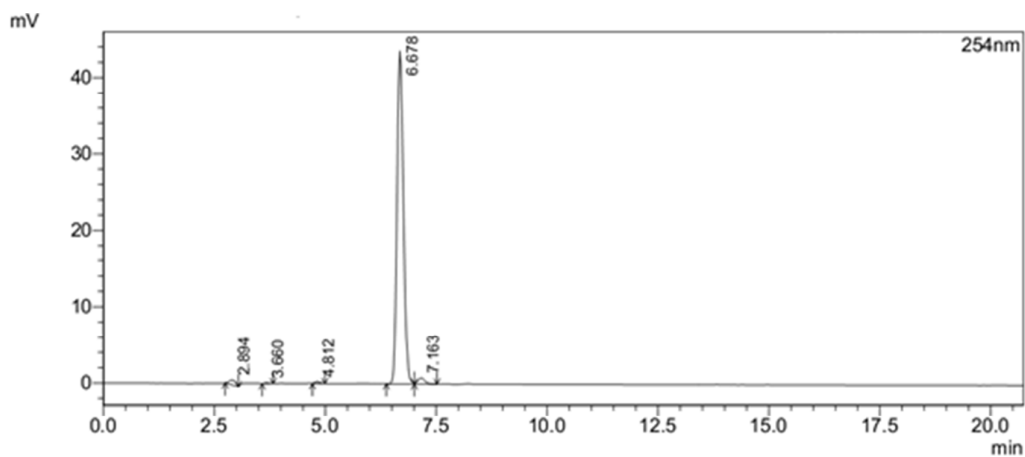
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1	1.568	BV	0.0526	8.90930	2.55847	0.6664
2	1.637	VV	0.0407	4.43640	1.68641	0.3318
3	2.547	BB	0.0838	3.93517	6.97407e-1	0.2943
4	3.602	BB	0.1065	5.17681	7.50078e-1	0.3872
5	12.325	BB	0.3553	1306.87329	57.04456	97.7537
6	14.624	BB	0.2838	7.57304	3.22713e-1	0.5665

HPLC chromatograms of **9a**



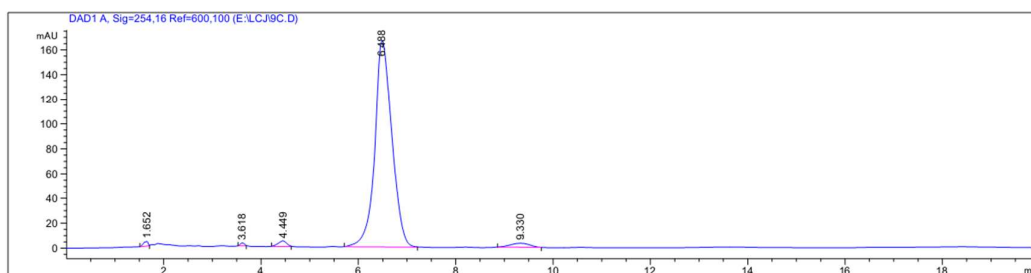
#	[min]		[min]	[mAU*s]	[mAU]	%
1	4.557	BB	0.2066	86.24985	6.26660	2.1872
2	7.022	BB	0.2765	3766.79858	210.73233	95.5218
3	9.510	MM	0.6166	90.34515	2.44210	2.2911

HPLC chromatograms of **9b**



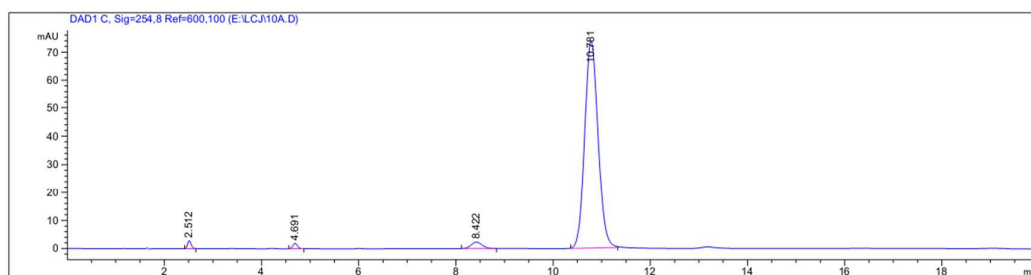
#	min	mAU*s	mAU	%
1	2.894	6452	627	1.421
2	3.660	934	121	0.206
3	4.812	2151	272	0.474
4	6.678	435998	43607	96.038
5	7.163	8449	792	1.861

HPLC chromatograms of 9c



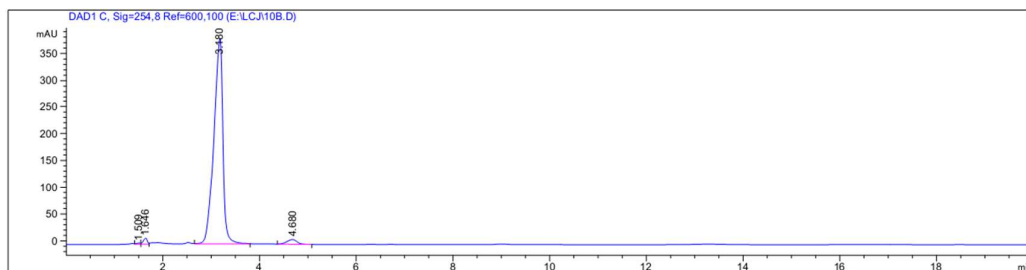
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1	1.652	MM	0.1107	24.69381	3.71800	0.5814
2	3.618	MM	0.0991	13.93231	2.34350	0.3281
3	4.449	MM	0.2111	57.37321	4.52964	1.3509
4	6.488	BB	0.3627	4065.77808	166.63039	95.7342
5	9.330	MM	0.4469	85.16888	3.17606	2.0054

HPLC chromatograms of 10a



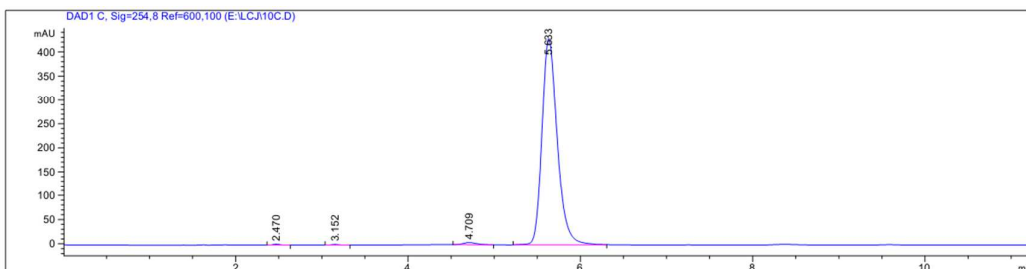
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1	2.512	BB	0.0798	14.85433	2.89998	1.0101
2	4.691	BB	0.1052	12.94543	1.90778	0.8803
3	8.422	BB	0.2480	36.41845	2.28455	2.4765
4	10.781	BB	0.2962	1406.31958	73.83119	95.6330

HPLC chromatograms of **10b**



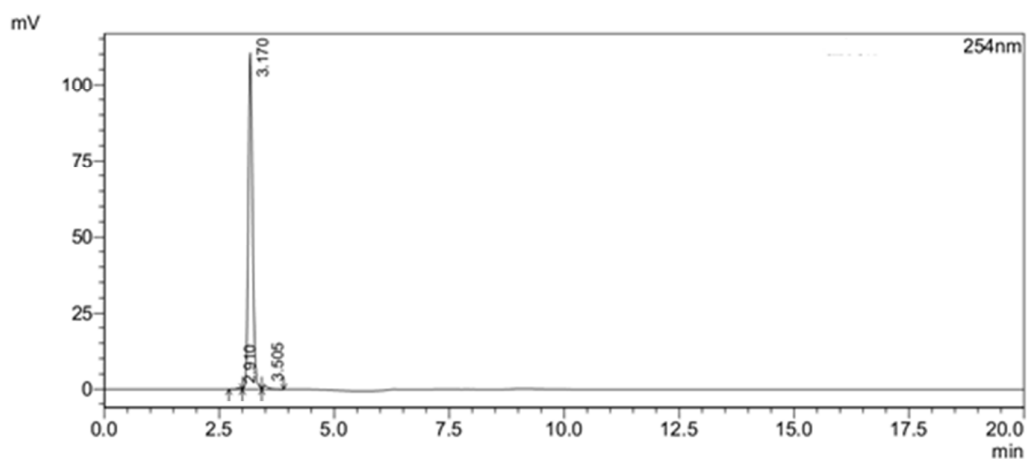
#	[min]		[min]	[mAU*s]	[mAU]	%
1	1.509	VV	0.0846	13.14340	2.23640	0.2532
2	1.646	VV	0.0787	61.75870	11.14065	1.1898
3	3.180	VB	0.1977	4990.39453	383.89471	96.1384
4	4.680	BB	0.2264	125.54990	8.69971	2.4187

HPLC chromatograms of **10c**



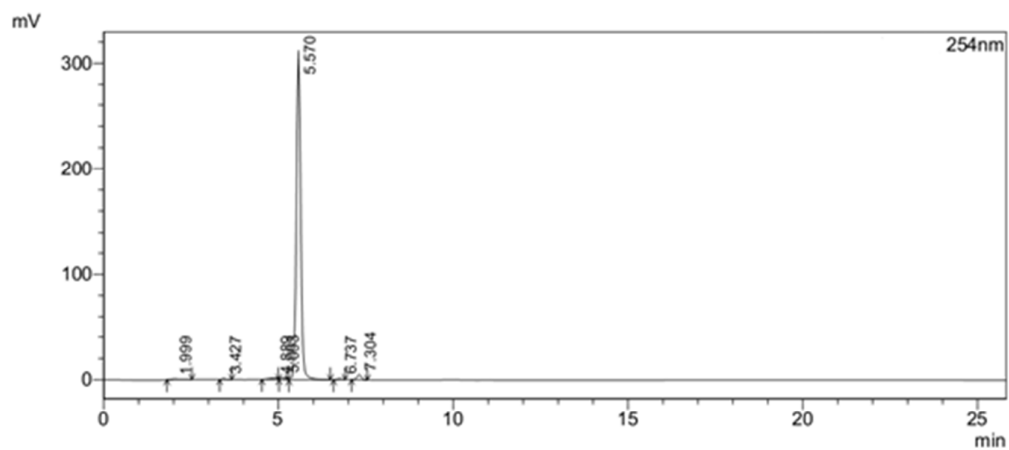
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1	2.470	BB	0.0838	10.06742	1.84031	0.1880
2	3.152	BB	0.0902	5.84540	1.00066	0.1092
3	4.709	BB	0.1633	48.82671	4.54764	0.9120
4	5.633	BB	0.1897	5289.01855	429.33203	98.7908

HPLC chromatograms of **10d**



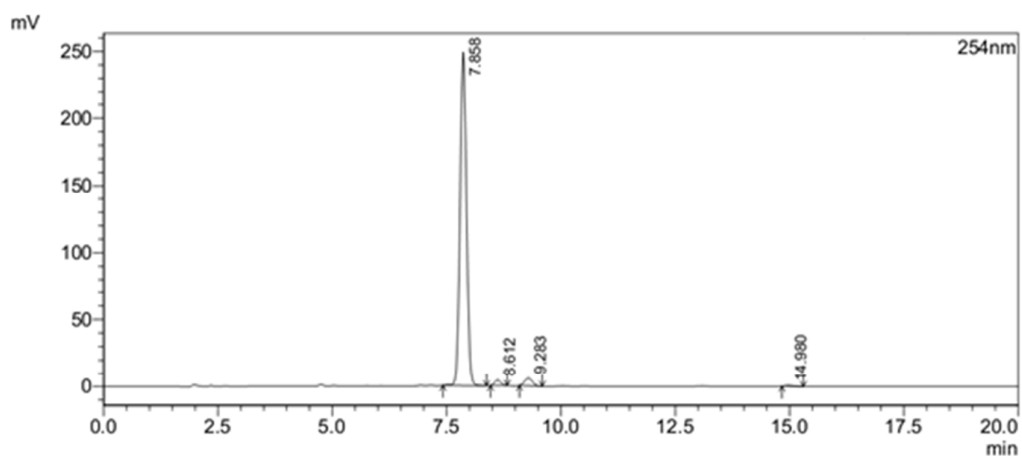
254nm				
#	min	mAU*s	mAU	%
1	2.910	3570	408	0.492
2	3.170	712221	110466	98.170
3	3.505	9705	1138	1.338

HPLC chromatograms of **10e**



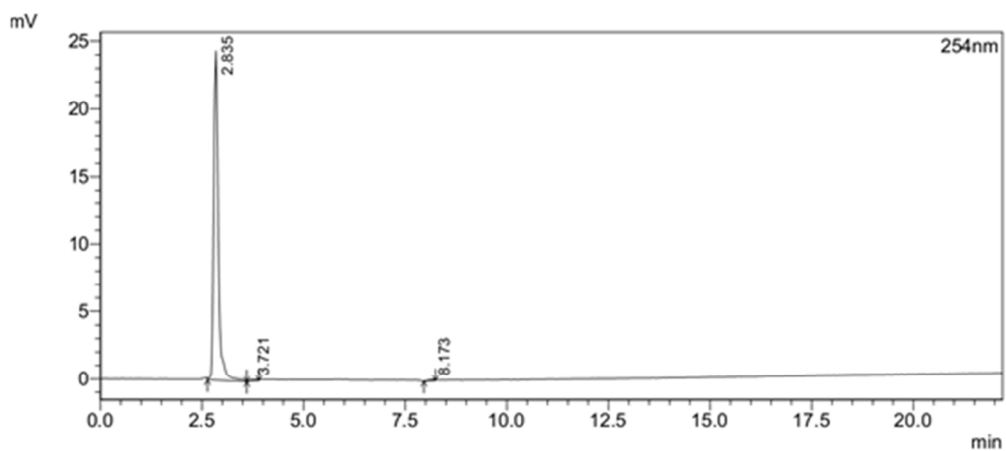
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#	min	mAU*s	mAU	%
1	1.999	9790	581	0.350
2	3.427	5412	897	0.193
3	4.889	23939	1311	0.855
4	5.093	19629	1804	0.701
5	5.570	2691423	312071	96.098
6	6.737	7530	899	0.269
7	7.304	42983	4698	1.535

HPLC chromatograms of **12**



254nm				
#	min	mAU*s	mAU	%
1	7.858	2427528	248949	95.446
2	8.612	38910	4111	1.530
3	9.283	69517	5816	2.733
4	14.980	7406	595	0.291

HPLC chromatograms of **14**



254nm				
#	min	mAU*s	mAU	%
1	2.835	194165	24390	98.645
2	3.721	1651	131	0.839
3	8.173	1015	83	0.516

REFERENCES

1. Di, L.; Kerns, E. H.; Fan, K.; McConnell, O. J.; Carter, G. T. High throughput artificial membrane permeability assay for blood–brain barrier. *Eur. J. Med. Chem.* **2003**, *38*, 223-232.

2. Ferrada, E.; Arancibia, V.; Loeb, B.; Norambuena, E.; Olea-Azar, C.; Pablo Huidobro-Toro, J. Stoichiometry and conditional stability constants of Cu(II) or Zn(II) clioquinol complexes; implications for Alzheimer's and Huntington's disease therapy. *NeuroToxicology* **2007**, 28, 445–449.