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Supporting information for

Novel huperzine A based NMDA antagonists: insights from molecular docking, ADME/T and molecular dynamics simulation studies

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Contents

Protein sequence alignment:

Figure S1. Sequence alignment of GluN1 from *Homo sapiens* (UniProt Q05586) and *Xenopus laevis* (PDB ID: 5UN1).

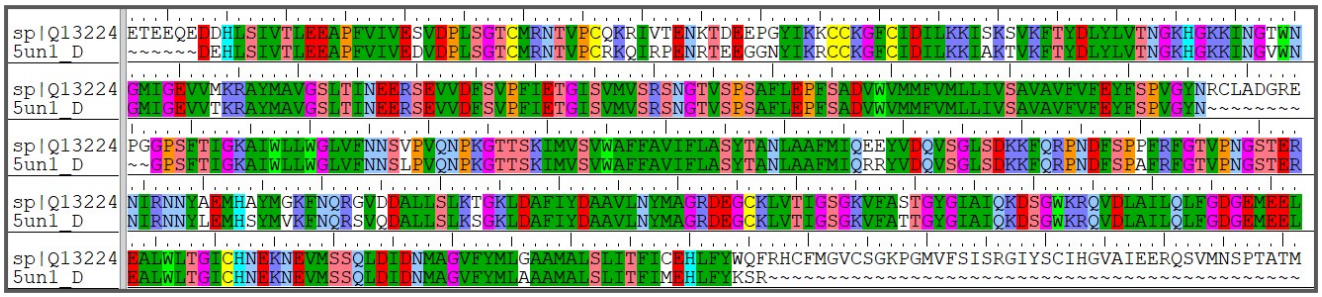


Figure S2. Sequence alignment of GluN2B from *Homo sapiens* (UniProt Q13224) and *Xenopus laevis* (PDB ID: 5UN1).

Binding site evaluation:

Table S1. Average SiteMap values across NMDA receptor

S. No	Site name	Site score	D score	Size	Hydrophobic	Volume
1	**Site-I**	**1.125**	**1.194**	**2211**	**1.920**	**4920.335**
2	Site-II	1.038	1.037	488	0.590	1299.970
3	Site-III	1.122	1.150	342	1.585	648.613
4	Site-IV	1.057	1.102	333	1.101	1005.333
5	Site-V	1.111	1.159	316	1.666	728.189

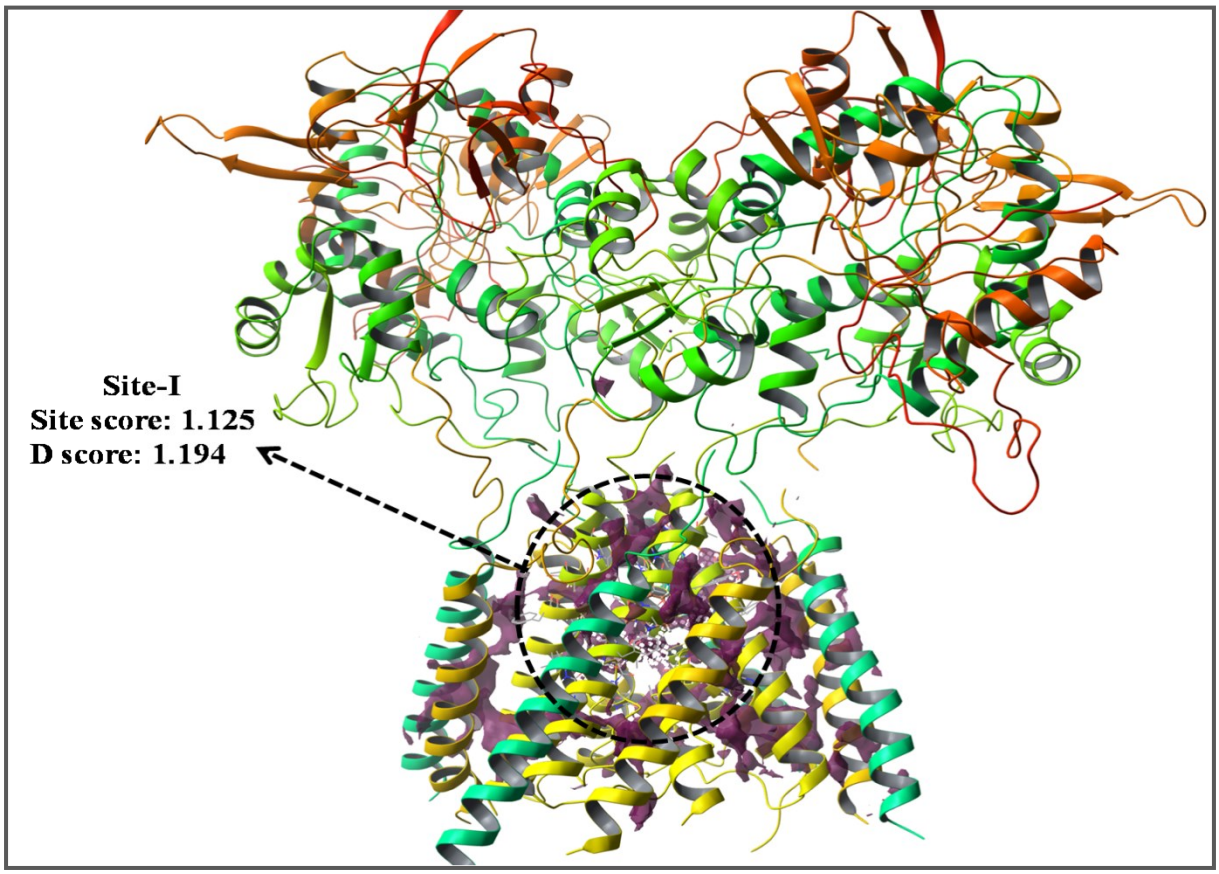


Figure S3. Site points (white spheres) and SiteMap surface (maroon colour contour) for Site-I binding site of NMDA receptor.

Derivatized compounds of Huperzine A:

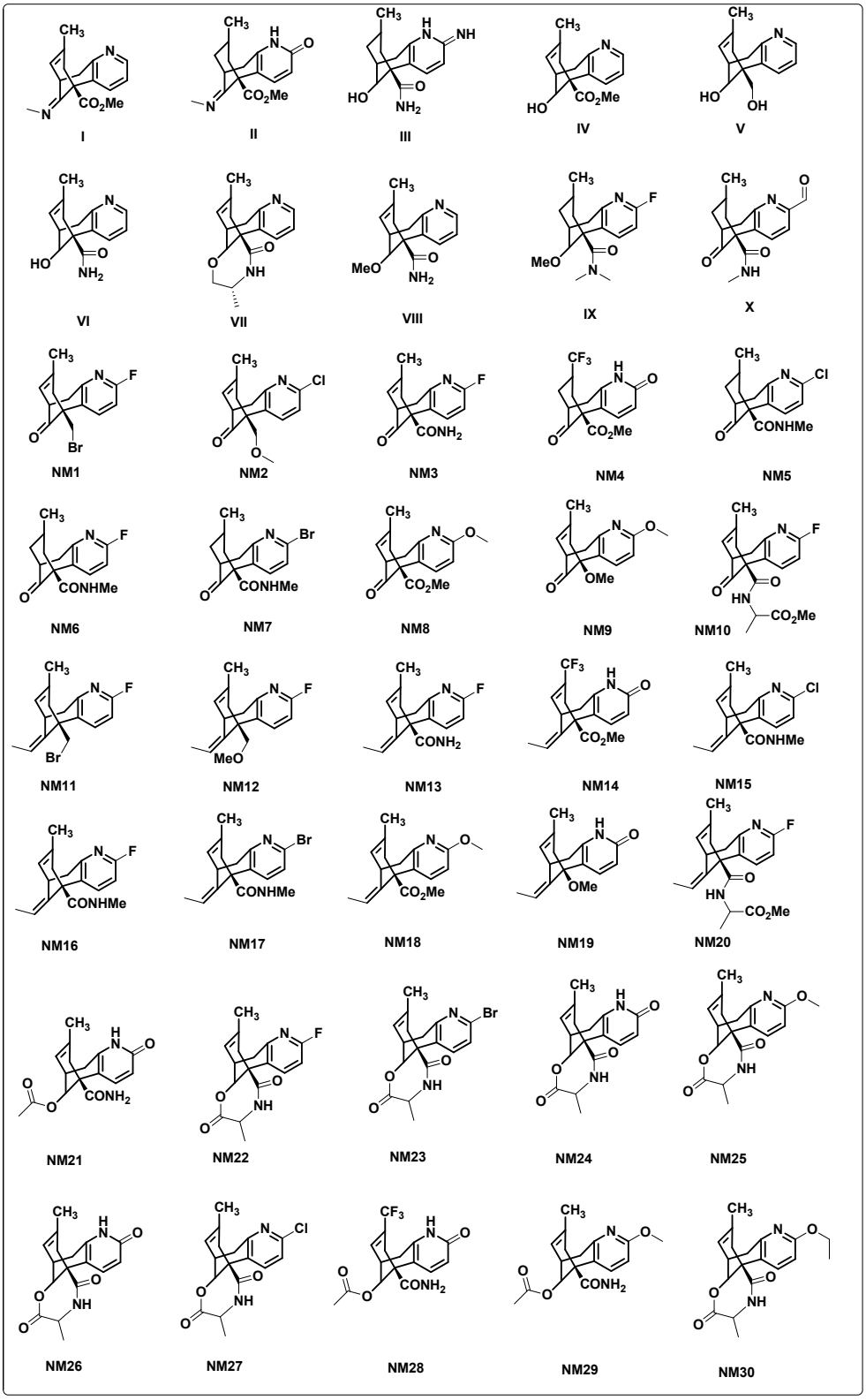


Figure S4. The derivatized compounds of Huperzine A.

Molecular docking protocol validation:

The docking protocol was validated by redocking of the bound ligand MK-801 in the active site of NMDA receptor using GLIDE module of Schrödinger software. The bound and docked conformations of MK-801 (RMSD 0.0806 Å) showed similar interactions and binding pose at the binding site. This analysis establishes the acceptability of docking protocol and thus can be extended to dock investigated molecules in the active site of NMDA receptor.

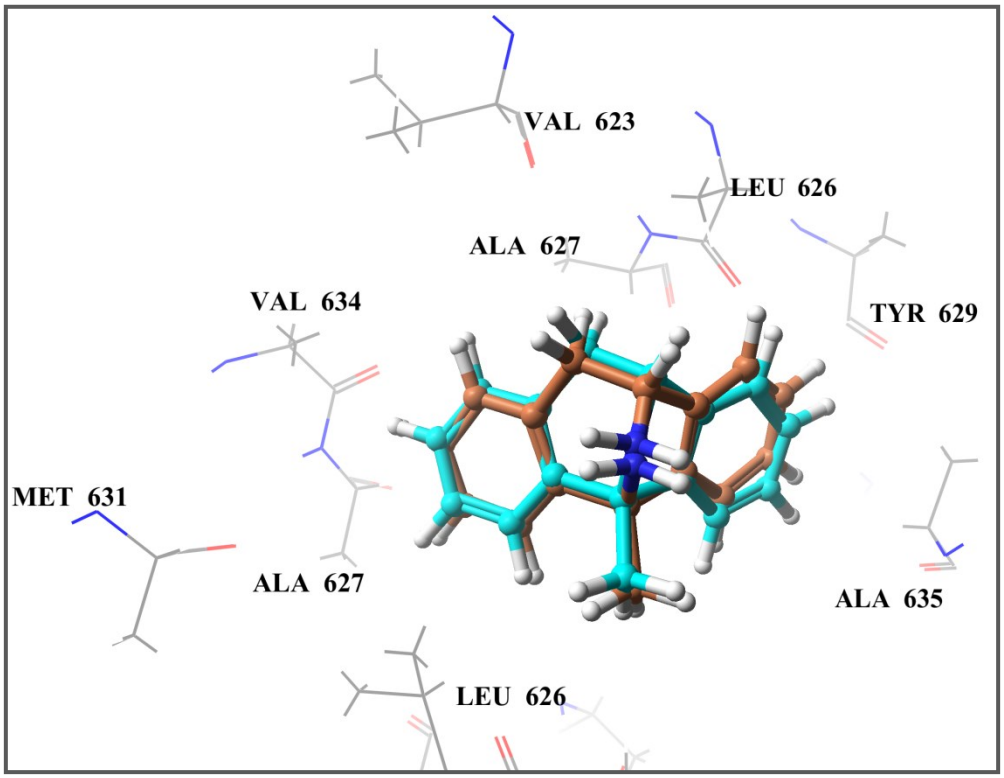
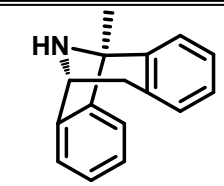
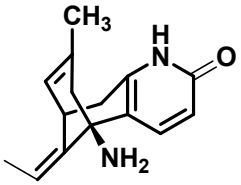
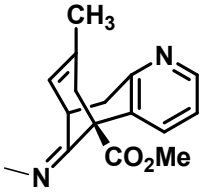
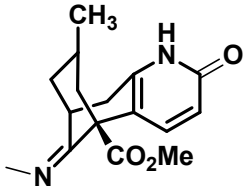
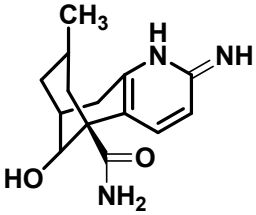
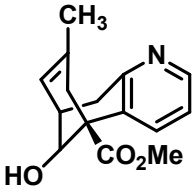
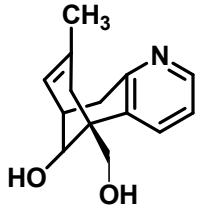
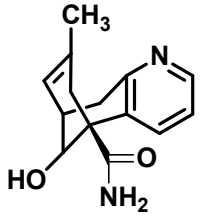
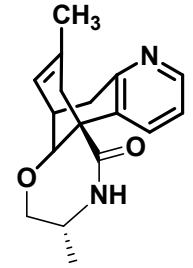
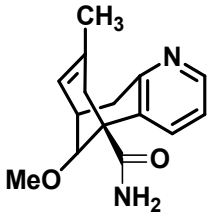
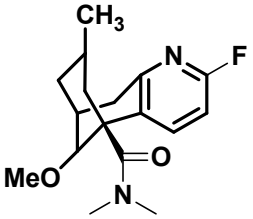
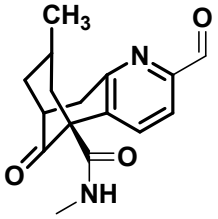
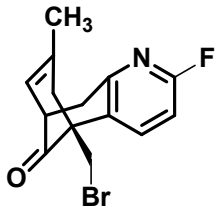
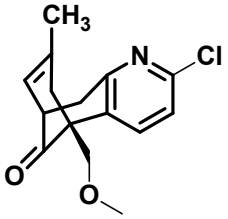


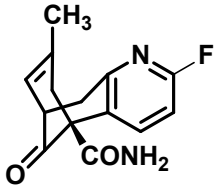
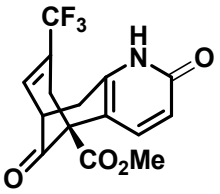
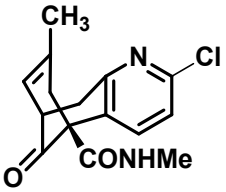
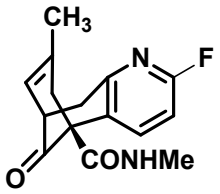
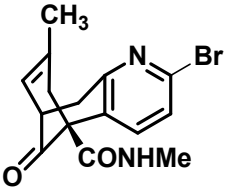
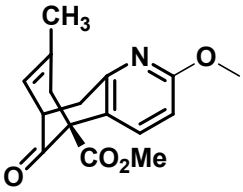
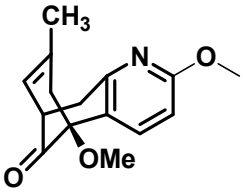
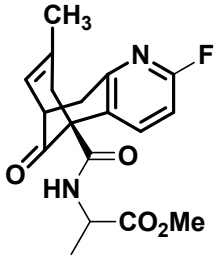
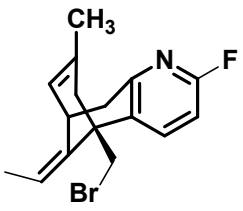
Figure S5 . Superimposition of bound co-crystal (cyan colour) and docked conformation of co-crystal (burnt orange colour) in the binding site of NMDA receptor (PDB ID: 5UN1).

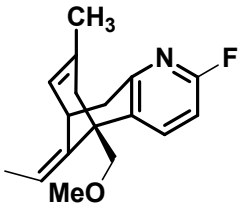
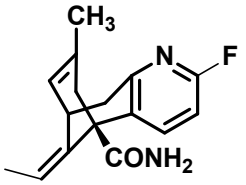
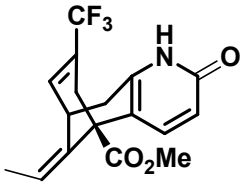
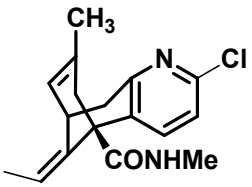
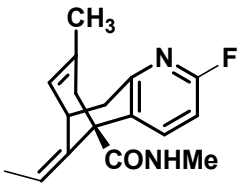
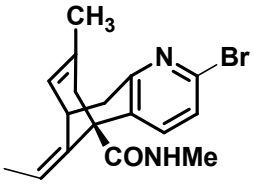
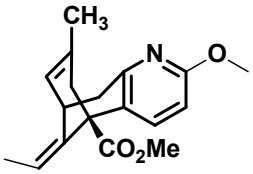
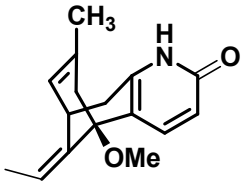
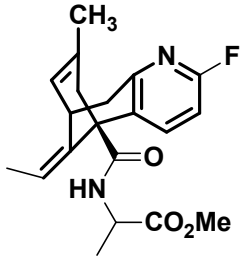
Molecular docking analysis:

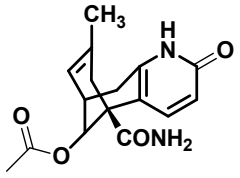
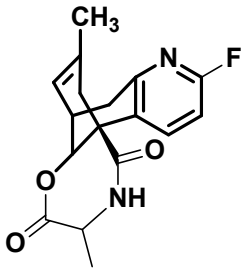
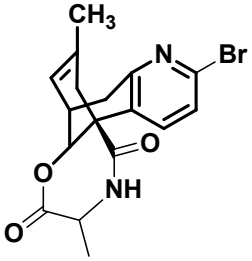
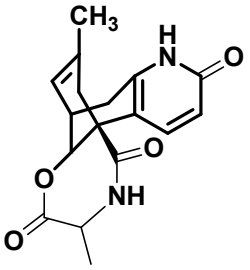
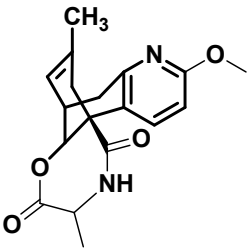
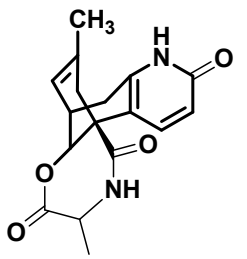
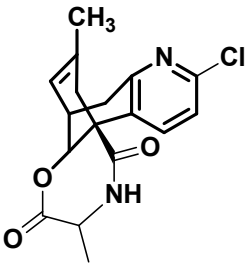
Table S2. GLIDE docking results for designed Huperzine A analogues at active site of NMDA receptor (PDB ID: 5UN1).

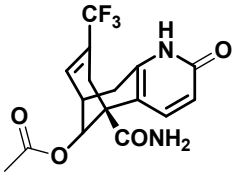
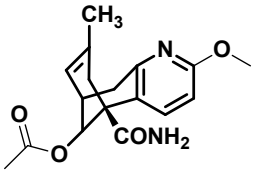
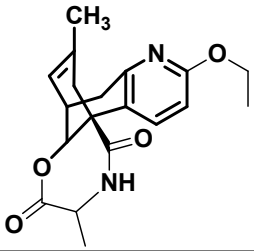
S. no	Ligand name	Ligand structure	Docking score	Interactions	
				Hydrophobic	H- bonds
1	**MK-801**		**-7.365**	**Met631(A), Val634(A), Ala635(A), Leu626(B), Ala627(B), Tyr629(B), Val623(D), Leu626(D), Ala627(D)**	**-**
2	**Huperzine A**		**-6.970**	**Met631(A), Val634(A), Ala635(A), Phe597(B), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Ala635(C), Ala622(D), Val623(D), Leu626(D), Ala627(D)**	**-**
3	**I**		**-7.006**	**Met631(A), Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Val623(D), Leu626(D), Ala627(D)**	**-**
4	**II**		**-6.684**	**Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Ala630(C), Met631(C), Val634(C), Ala635(C), Tyr637(C), Phe597(D), Leu626(D), Ala627(D)**	**-**
5	**III**		**-6.903**	**Met631(A), Val634(A), Ala635(A), Phe597(B), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Val623(D), Leu626(D), Ala627(D)**	**-**
6	**IV**		**-6.546**	**Met631(A), Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Ala635(C), Val623(D), Leu626(D), Ala627(D)**	**-**

7	**V**		**-6.289**	**Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Val634(C), Ala635(C), Val623(D), Leu626(D), Ala627(D)**	**Thr630(D)**
8	**VI**		**-6.614**	**Met631(A), Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Val623(D), Leu626(D), Ala627(D)**	**-**
9	**VII**		**-6.761**	**Met631(A), Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Met631(C), Val634(C), Ala635(C), Val623(D), Leu626(D), Ala627(D)**	**-**
10	**VIII**		**-6.960**	**Met631(A), Val634(A), Ala635(A), Leu626(B), Ala627(B), Val634(C), Ala635(C), Val623(D), Leu626(D), Ala627(D)**	**-**
11	**IX**		**-6.441**	**Met631(A), Val634(A), Ala635(A), Phe597(B), Val623(B), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Ala635(C), Val623(D), Leu626(D), Ala627(D)**	**-**
12	**X**		**-7.311**	**Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Ala630(C), Met631(C), Val634(C), Ala635(C), Phe597(D), Leu626(D), Ala627(D)**	**-**
13	**NM1**		**-7.173**	**Met631(A), Val634(A), Ala635(A), Leu626(B), Ala627(B), Tyr629(B), Ala622(D), Val623(D), Leu626(D), Ala627(D)**	
14	**NM2**		**-6.612**	**Met631(A), Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Ala635(C), Val623(D), Leu626(D), Ala627(D)**	

15	**NM3**		**-7.365**	**Met631(A), Val634(A), Ala635(A), Leu626(B), Ala627(B), Tyr629(B), Val623(D), Leu626(D), Ala627(D)**	
16	**NM4**		**-7.148**	**Val634(A), Ala635(A), Leu626(B), Tyr629(B), Val634(C), Ala635(C), Val623(D), Leu626(D), Ala627(D)**	
17	**NM5**		**-7.102**	**Met631(A), Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Ala622(D), Val623(D), Leu626(D), Ala627(D)**	
18	**NM6**		**-7.298**	**Met631(A), Val634(A), Ala635(A), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Ala635(C), Ala622(D), Val623(D), Leu626(D), Ala627(D)**	
19	**NM7**		**-7.011**	**Met631(A), Val634(A), Ala635(A), Phe597(B), Val623(B), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Val623(D), Leu626(D), Ala627(D)**	**Leu626(B)**
20	**NM8**		**-6.495**	**Met631(A), Val634(A), Ala635(A), Phe597(B), Leu626(B), Ala627(B), Met631(C), Val634(C), Ala635(C), Leu626(D), Ala627(D)**	
21	**NM9**		**-6.246**	**Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Tyr629(B), Ala630(C), Met631(C), Val634(C), Ala635(C), Val623(D), Leu626(D), Ala627(D)**	
22	**NM10**		**-7.304**	**Met631(A), Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Met631(C), Val634(C), Ala635(C), Val623(D), Leu626(D), Ala627(D)**	
23	**NM11**		**-5.721**	**Met631(A), Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Tyr629(B), Ala622(D), Val623(D), Leu626(D), Ala627(D)**	

24	**NM12**		**-7.331**	**Met631(A), Val634(A), Ala635(A), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Ala635(C), Ala622(D), Val623(D), Leu626(D), Ala627(D)**
25	**NM13**		**-7.193**	**Met631(A), Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Ala622(D), Val623(D), Leu626(D), Ala627(D)**
26	**NM14**		**-7.475**	**Met631(A), Val634(A), Ala635(A), Phe597(B), Val623(B), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Val623(D), Leu626(D), Ala627(D)**
27	**NM15**		**-7.388**	**Met631(A), Val634(A), Ala635(A), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Ala635(C), Val623(D), Leu626(D), Ala627(D), Ala631(D)**
28	**NM16**		**-7.542**	**Met631(A), Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Val623(D), Leu626(D), Ala627(D)**
29	**NM17**		**-7.178**	**Met631(A), Val634(A), Ala635(A), Phe597(B), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Ala635(C), Val623(D), Leu626(D), Ala627(D)**
30	**NM18**		**-6.404**	**Met631(A), Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Tyr629(B), Ala630(C), Met631(C), Val634(C), Ala635(C), Phe597(D), Val623(D), Leu626(D), Ala627(D)**
31	**NM19**		**-6.839**	**Met631(A), Val634(A), Ala635(A), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Ala635(C), Val623(D), Leu626(D), Ala627(D)**
32	**NM20**		**-7.722**	**Met631(A), Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Ala622(D), Val623(D), Leu626(D), Ala627(D)**

33	**NM21**		**-7.123**	**Met631(A), Val634(A), Ala635(A), Val623(B), Leu626(B), Tyr629(B), Met631(C), Val634(C), Ala635(C), Val623(D), Leu626(D), Ala627(D)**	
34	**NM22**		**-7.558**	**Met631(A), Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Val634(C), Ala635(C), Val623(D), Leu626(D), Ala627(D)**	
35	**NM23**		**-7.036**	**Met631(A), Val634(A), Ala635(A), Leu626(B), Tyr629(B), Met631(C), Val634(C), Ala635(C), Val623(D), Leu626(D), Ala627(D)**	
36	**NM24**		**-7.386**	**Met631(A), Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Ala622(D), Val623(D), Leu626(D), Ala627(D)**	**Leu626(B)**
37	**NM25**		**-7.273**	**Met631(A), Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Ala635(C), Ala619(D), Leu595(D), Ala622(D), Val623(D), Leu626(D), Ala627(D)**	
38	**NM26**		**-7.208**	**Met631(A), Val634(A), Ala635(A), Val623(B), Leu626(B), Ala627(B), Val634(C), Ala635(C), Val623(D), Leu626(D), Ala627(D)**	
39	**NM27**		**-7.365**	**Met631(A), Val634(A), Ala635(A), Leu626(B), Tyr629(B), Met631(C), Val634(C), Ala635(C), Val623(D), Leu626(D), Ala627(D)**	

40	**NM28**		**-7.830**	**Val634(A), Phe597(B), Val623(B), Leu626(B), Val634(C), Val623(D), Leu626(D), Ala627(D)**
41	**NM29**		**-7.324**	**Met631(A), Val634(A), Ala635(A), Val623(B), Leu626(B), Tyr629(B), Met631(C), Val634(C), Ala635(C), Val623(D), Leu626(D), Ala627(D)**
42	**NM30**		**-7.152**	**Leu605(A), Met631(A), Val634(A), Ala635(A), Leu626(B), Ala627(B), Tyr629(B), Val634(C), Ala635(C), Ala619(D), Leu595(D), Ala622(D), Val623(D), Leu626(D), Ala627(D)**

Molecular dynamics simulation:

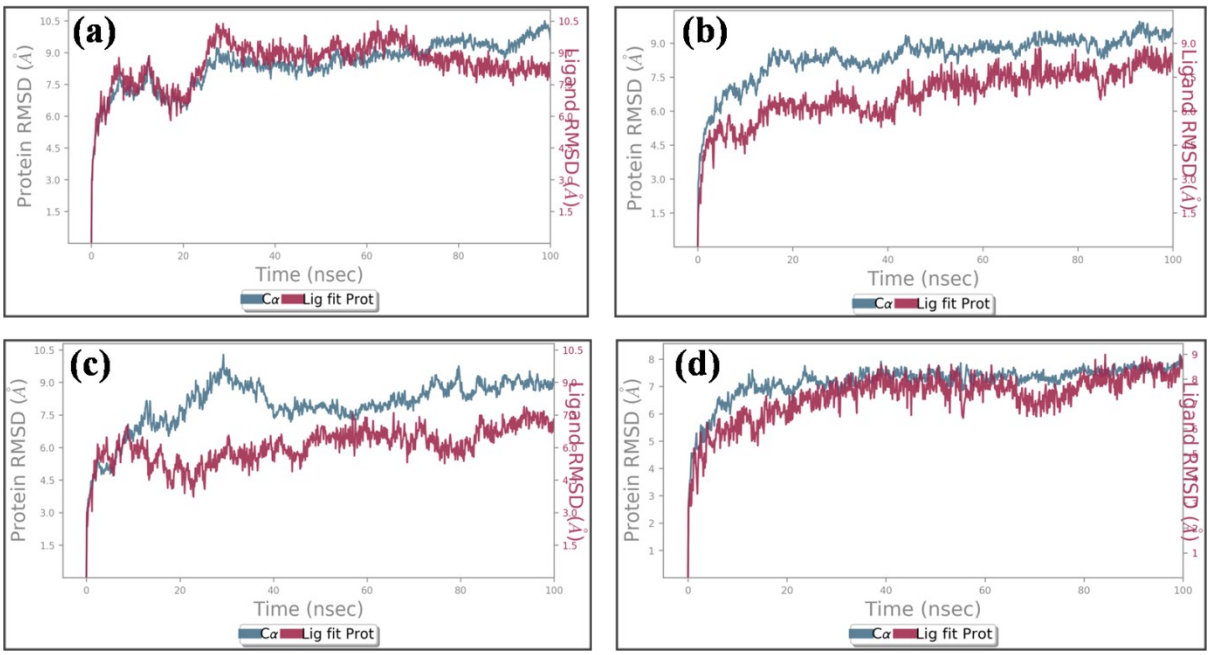


Figure S6. Average RMSD of C-alphas of protein and ligand complexes (a) **NM14**-5UN1, (b) **NM16**-5UN1, (c) **NM20**-5UN1 and (d) **NM22**-5UN1 during molecular dynamics simulation (100 ns).

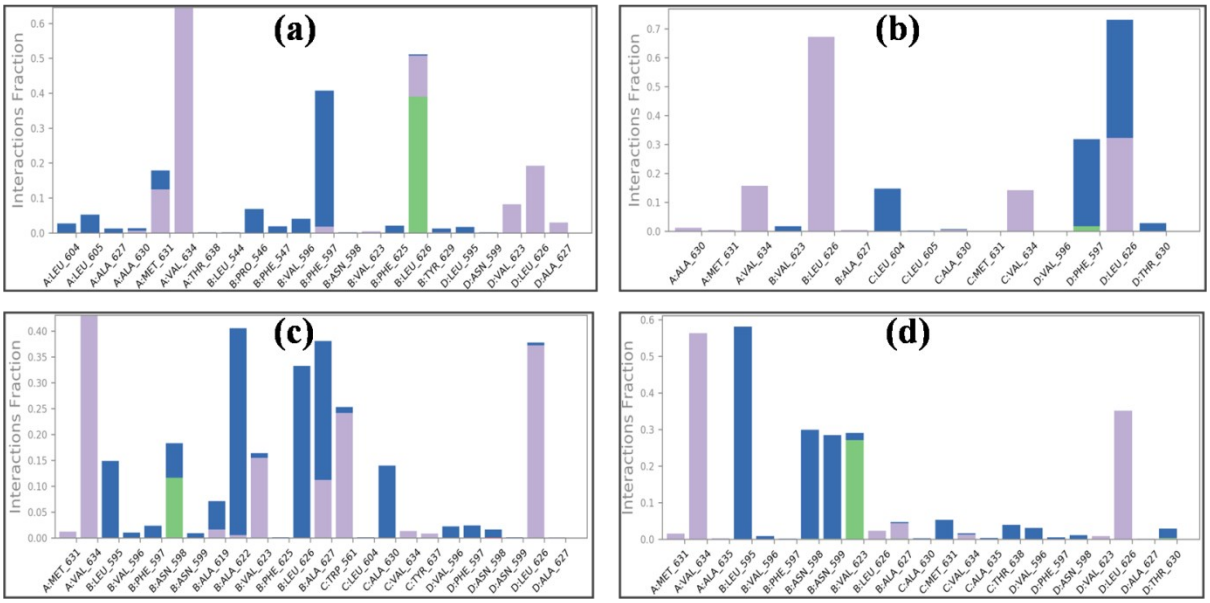


Figure S7. Bar-chart representation of protein-ligand contacts of (a) **NM14**-5UN1, (b) **NM16**-5UN1, (c) **NM20**-5UN1 and (d) **NM22**-5UN1 complexes during molecular dynamics simulation.

Simulation quality analysis:

Table S3. Thermodynamic properties of **NM28**-5UN1 complex observed during molecular dynamics simulation.

Properties	Time duration	Average	Standard deviation	Slope (ps^{-1})
Total energy (kcal/mol)	0ps	-553039.708	490.896	-0.002
	100ps	-553039.643	142.450	-0.002
	1000ps	-553039.564	103.576	-0.002
	10000ps	-553035.673	95.499	-0.003
	50000ps	-553039.666	75.035	-0.002
Potential energy (kcal/mol)	0ps	-673674.854	411.323	-0.002
	100ps	-673674.836	129.568	-0.002
	1000ps	-673674.774	102.579	-0.002
	10000ps	-673670.487	95.859	-0.003
	50000ps	-673674.816	75.081	-0.002
Temperature	0ps	298.742	0.838	0.000

(K)	100ps	298.741	0.145	0.000
	1000ps	298.740	0.047	0.000
	10000ps	298.739	0.013	0.000
	50000ps	298.740	0.005	0.000
Pressure (bar)	0ps	1.402	62.664	0.000
	100ps	1.400	6.996	0.000
	1000ps	1.362	2.272	0.000
	10000ps	1.359	1.075	0.000
	50000ps	1.393	0.699	0.000
Volume (Å^3)	0ps	2002998.364	2049.943	-0.006
	100ps	2002998.083	479.333	-0.006
	1000ps	2002995.069	345.851	-0.006
	10000ps	2002990.303	290.735	-0.008
	50000ps	2002998.555	213.145	-0.006

Table S4. Thermodynamic properties of MK-801-5UN1 complex observed during molecular dynamics simulation.

Properties	**Time duration**	**Average**	**Standard deviation**	**Slope (ps^{-1})**
Total energy (kcal/mol)	0ps	-552851.770	495.571	-0.002
	100ps	-552851.703	148.515	-0.002
	1000ps	-552850.964	111.829	-0.003
	10000ps	-552845.658	104.093	-0.003
	50000ps	-552851.732	72.174	-0.002
Potential energy (kcal/mol)	0ps	-673548.262	416.462	-0.003
	100ps	-673548.153	137.449	-0.003
	1000ps	-673547.412	111.276	-0.003
	10000ps	-673541.869	105.255	-0.003
	50000ps	-673548.225	74.052	-0.002
Temperature (K)	0ps	298.746	0.841	0.000
	100ps	298.744	0.145	0.000
	1000ps	298.744	0.044	0.000
	10000ps	298.743	0.016	0.000
	50000ps	298.745	0.009	0.000
Pressure (bar)	0ps	1.121	62.977	0.000
	100ps	1.116	6.844	0.000
	1000ps	1.127	2.148	0.000
	10000ps	1.041	0.845	-0.000
	50000ps	1.113	0.370	0.000
Volume (Å^3)	0ps	2004076.296	2077.442	-0.010
	100ps	2004076.806	566.144	-0.010
	1000ps	2004080.431	456.372	-0.010
	10000ps	2004094.839	438.579	-0.012
	50000ps	2004076.464	298.533	-0.008

Table S5. Thermodynamic properties of HupA-5UN1 complex observed during molecular dynamics simulation.

Properties	Time duration	Average	Standard deviation	Slope (ps^{-1})
Total energy (kcal/mol)	0ps	-552862.922	491.736	-0.003
	100ps	-552862.841	147.724	-0.003
	1000ps	-552861.736	109.590	-0.003
	10000ps	-552855.143	97.376	-0.003
	50000ps	-552862.894	90.088	-0.003
Potential energy (kcal/mol)	0ps	-673542.711	413.991	-0.003
	100ps	-673542.623	134.380	-0.003
	1000ps	-673541.715	108.047	-0.003
	10000ps	-673535.021	97.891	-0.003
	50000ps	-673542.684	90.341	-0.003
Temperature (K)	0ps	298.742	0.839	0.000
	100ps	298.741	0.147	0.000
	1000ps	298.741	0.041	0.000
	10000ps	298.740	0.015	0.000
	50000ps	298.741	0.006	0.000
Pressure (bar)	0ps	0.927	62.715	0.000
	100ps	0.922	7.325	0.000
	1000ps	0.919	2.541	0.000
	10000ps	0.917	1.109	0.000
	50000ps	0.919	0.783	0.000
Volume ($Å^3$)	0ps	2003742.042	2031.195	-0.005
	100ps	2003741.933	480.800	-0.005
	1000ps	2003743.950	351.596	-0.005
	10000ps	2003744.956	288.420	-0.007
	50000ps	2003742.215	151.515	-0.004

Table S6: RMSD values of free protein (5UN1) with that of the bound proteins.

S. No.	Ligand-Protein Complex ID	RMSD value (Å)
1	Co-crystal (MK-801)/ 5UN1	2.2654
2	HupA/5UN1	3.8548
3	NM14/5UN1	3.7380
4	NM16/5UN1	3.4747
5	NM20/5UN1	3.5872
6	NM22/5UN1	3.8196
7	NM28/5UN1	3.7960

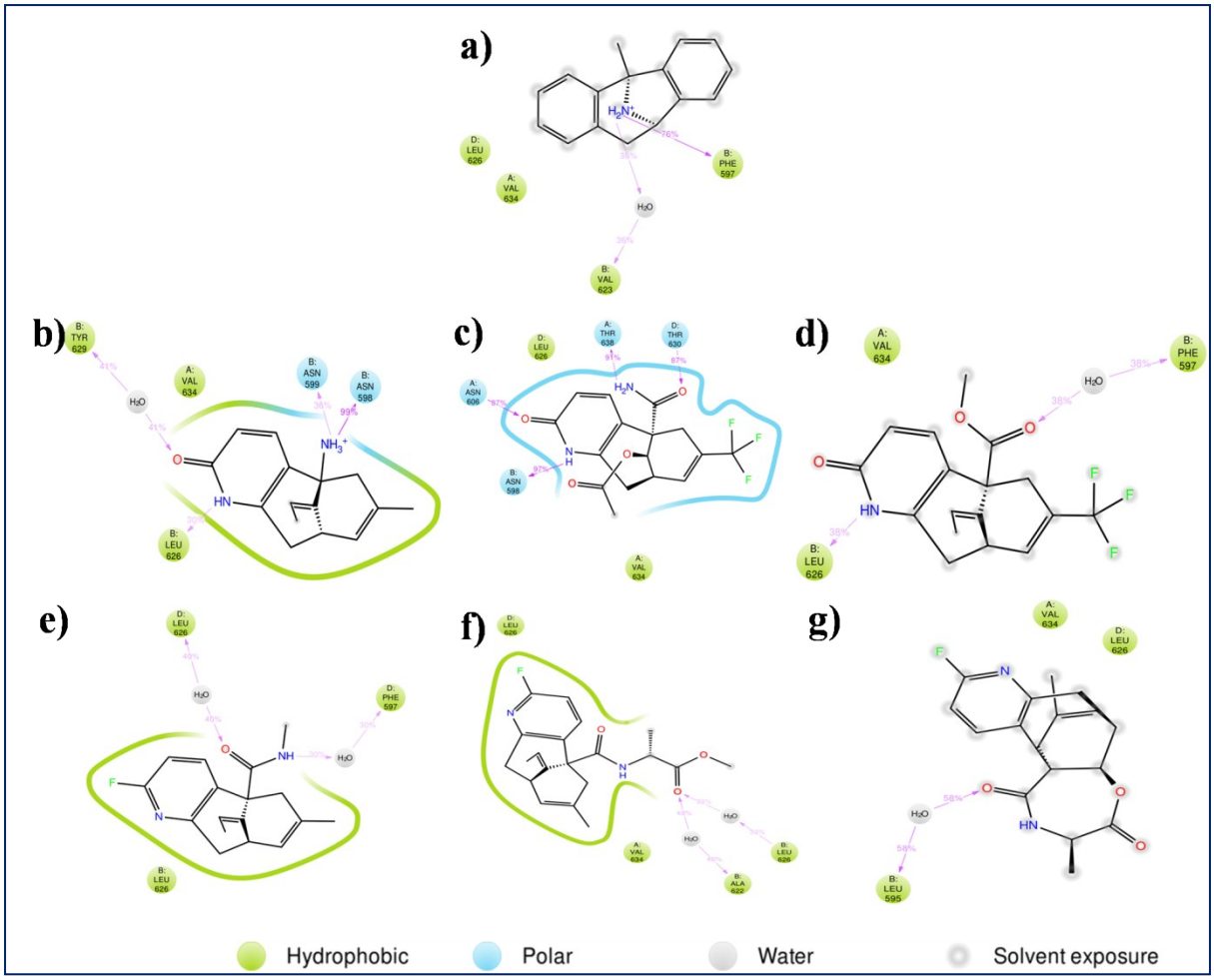


Figure S8. Simulation Interactions Diagram (SID) of compound **co-crystal (a), HupA (b), NM28 (c), NM14 (d), NM16 (e), NM20(f)** and **NM22 (g)** with NMDA receptor.