Supporting Information

Discovery of Novel Positive Allosteric Modulators Targeting GluN1/2A NMDAR as Anti-Stroke Therapeutic Agents

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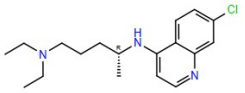
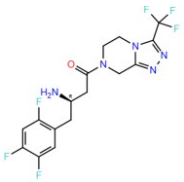
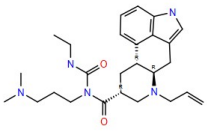
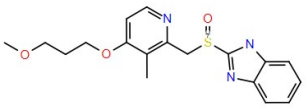
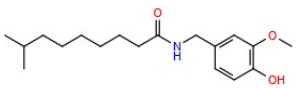
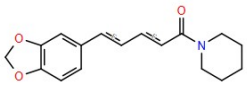
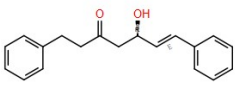
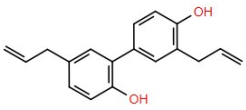
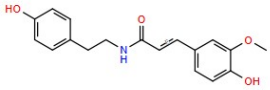
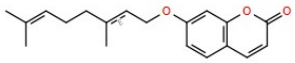
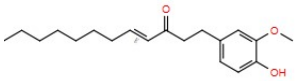
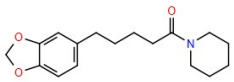
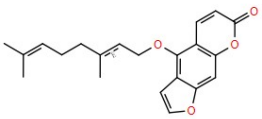
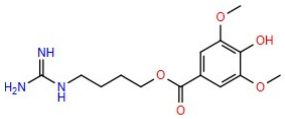
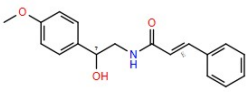
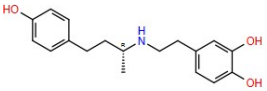
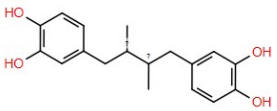
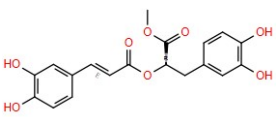
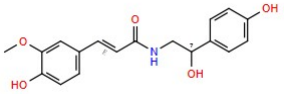
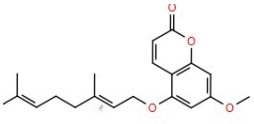
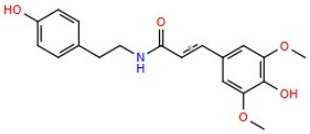
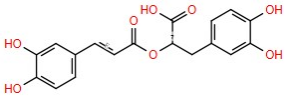
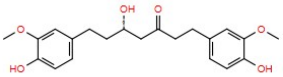
Table S1. Information of 23 selected compounds.

NO.	Compound name	Structure	Docking score	Glide gscore
1	Chloroquine		-7.457	-7.493
2	Sitagliptin		-8.641	-8.644
3	Cabergoline		-7.334	-7.567
4	Rabeprazole		-7.575	-7.580
5	Dihydrocapsaicin		-7.525	-7.525
6	Piperine		-7.532	-7.532
7	5-Hydroxy-1,7-diphenyl-6-hepten-3-one		-8.701	-8.701
8	Honokiol		-7.504	-7.505

9	Feruloyltyramine		-7.360	-7.429
10	Auraptene		-7.843	-7.843
11	[8]-Shogaol		-7.310	-7.310
12	TETRAHYDROPIPERINE		-7.297	-7.297
13	Bergamottin		-7.102	-7.102
14	Leonurine		-7.263	-7.282
15	Aegeline		-7.408	-7.408
16	Dobutamine hydrochloride		-8.675	-8.675
17	Nordihydroguaiaretic acid		-7.798	-7.798
18	Methyl rosmarinate		-8.388	-8.388

19	N-Feruloyloctopamine		-7.417	-7.484
20	5-Geranoxy-7-methoxycoumarin		-7.428	-7.428
21	N-trans-Sinapoyltyramine		-7.094	-7.163
22	rosmarinate acid		-8.300	-8.300
23	Hexahydrocurcumin		-8.950	-8.950

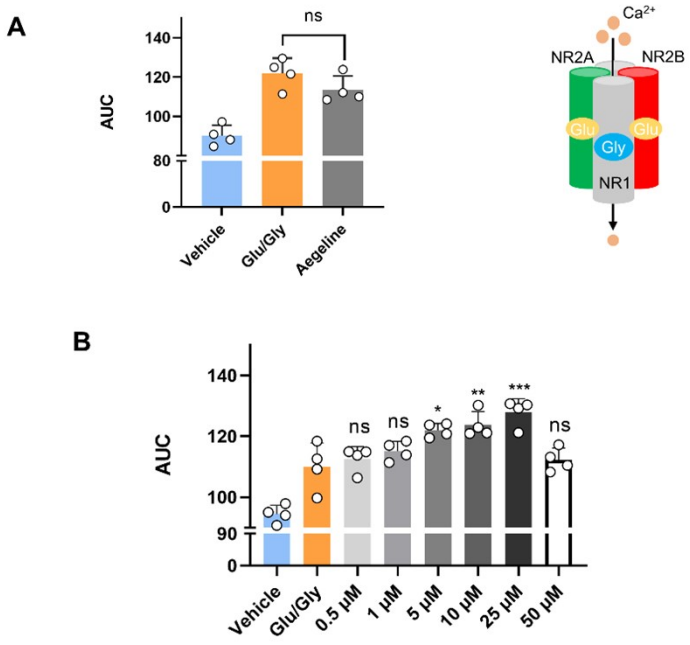


Figure S1. (A) Compound **15** (Aegeline) has no effect on agonist-induced triheteromeric NMDARs calcium influx. The modulating effect of compound **15** (Aegeline), co-applied with Glu/Gly, was detected in cell lines expressing GluN1/GluN2A/GluN2B. (B) Compound **15** at different concentrations increased agonist-induced intracellular calcium concentrations in cell lines expressing GluN1/2A. Data are presented as the mean ± SD from at least three independent experiments, and *** $P < 0.005$, ** $P < 0.01$, * $P < 0.05$, ns no significance, test groups compared to the Glu/Gly group.

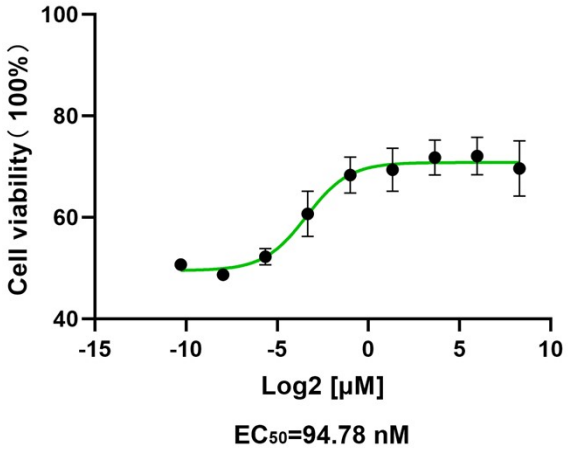


Figure S2. EC_{50} of the neuroprotective effect of compound **15** (Aegeline) on the model of glutamate-induced excitotoxicity in PC-12 cells. Data are expressed as the mean ± SEM (n = 4).

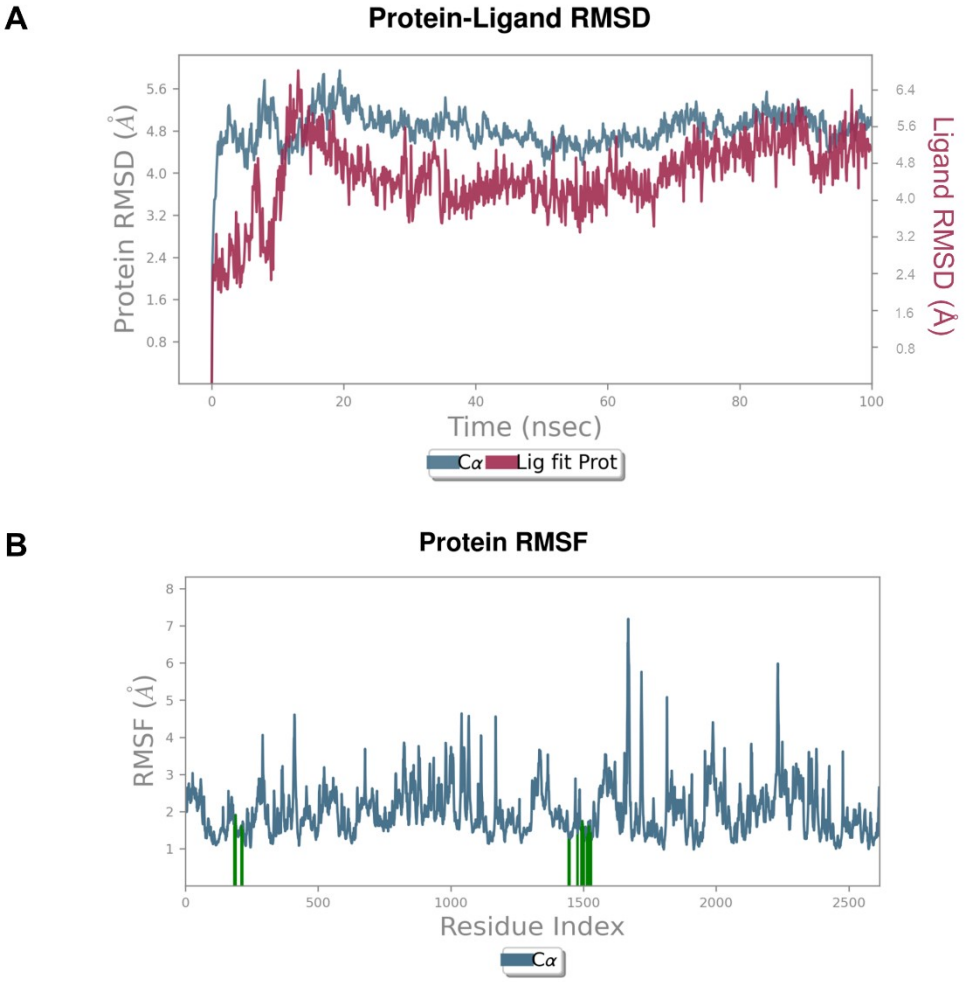


Figure S3. (A) RMSD of the complex formed by 7EOS and compound **15** (Aegeline).

The protein backbone is represented in teal color and the ligand is shown in purple. (A) The Root Mean Square Fluctuation (RMSF) is used for characterizing local changes along the protein chain. Protein residues that interact with the ligand are marked with green-colored vertical bars.

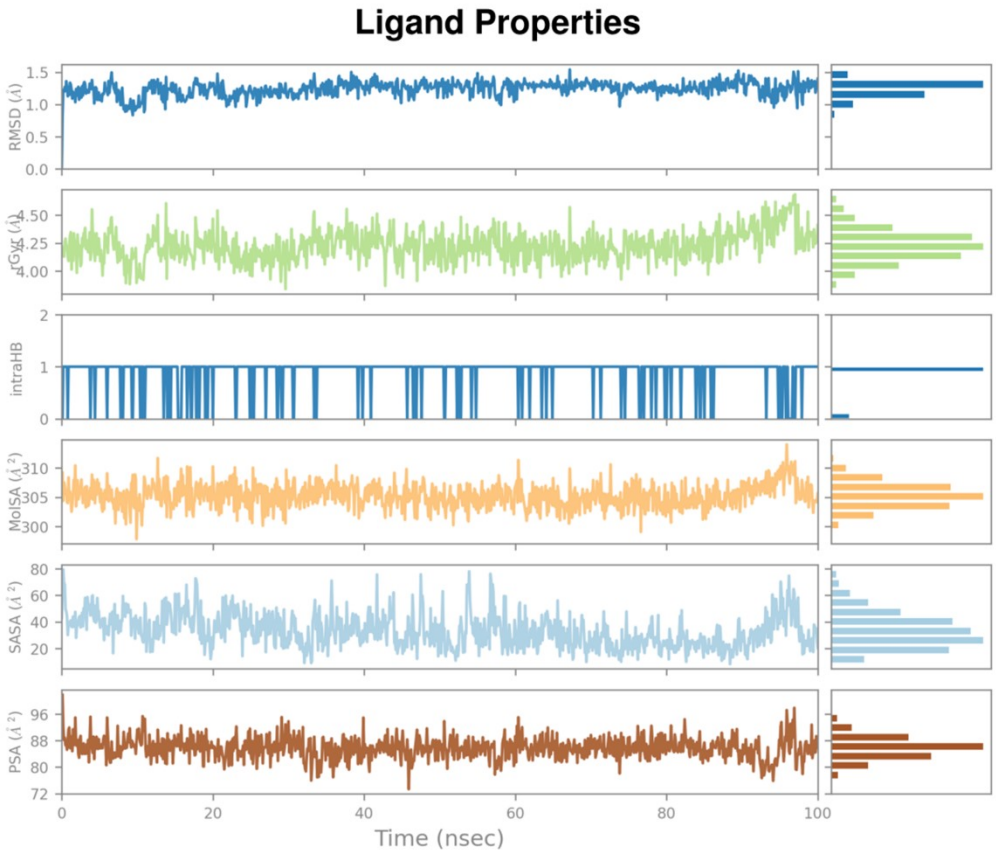


Figure S4. Variation properties of compound **15** (Aegeline) during 100 ns molecular dynamics simulation. RMSD indicates the RMSD of compound **15** with respect to the reference conformation (the first frame); Radius of Gyration (rGyr) is used to measure the ligand extendedness which is equivalent to its principal moment of inertia; MolSA indicates the molecular surface area calculated with 1.4 Å probe radius, this value is

equivalent to a van der Waals surface area; SASA illustrates surface area of a molecule accessible by a water molecule; PSA can explain that the solvent accessible surface area in a molecule contributed only by oxygen and nitrogen atoms.

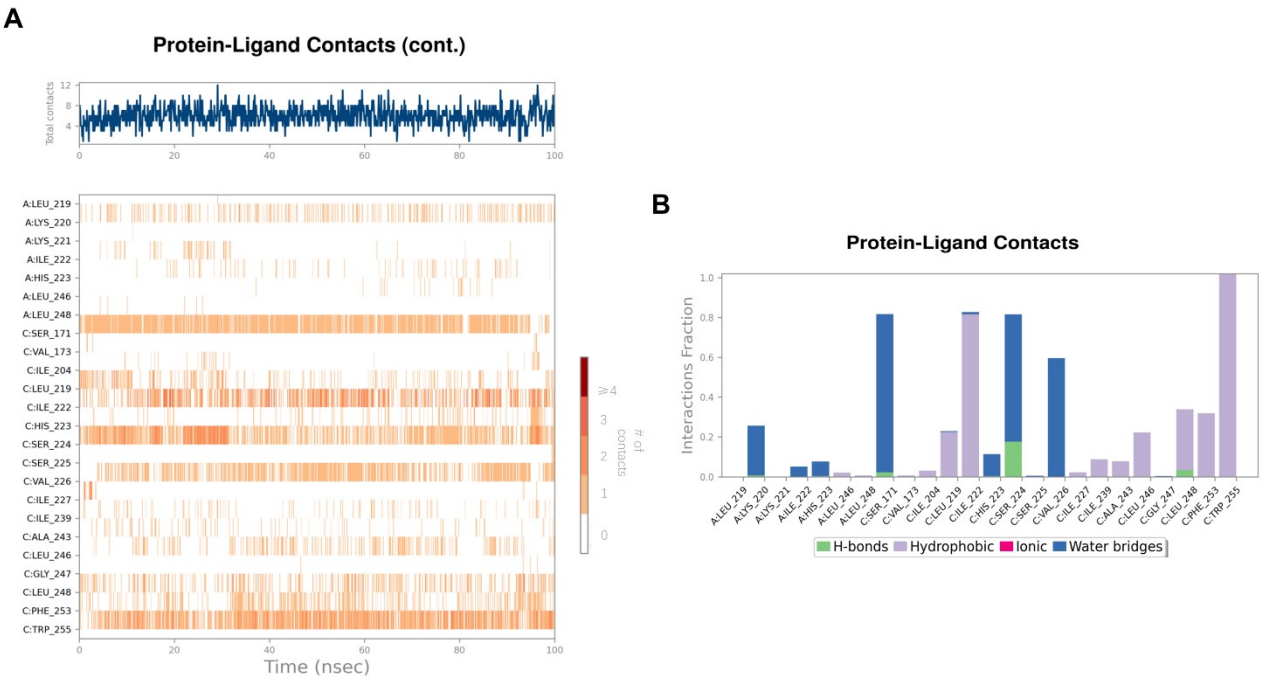


Figure S5. Binding interactions of compound **15** (Aegeline) with 7EOS through the MD simulation (A) A timeline representation of the interactions and contacts (H-bonds, Hydrophobic, Ionic, Water bridges). The top panel shows the total number of specific contacts the protein had with the ligands throughout the trajectory. The panel below shows which residues interacted with the ligand during the simulation time. Some residues make more than one specific contact with the ligand, which is shown in a

darker shade of orange depending on the ratio on the right. (B) Protein-ligand interactions were monitored in 100 ns MD simulations: normalized stacked bar charts representing interactions and contacts on the tracks. Protein-ligand interactions can be categorized into four types: Hydrogen Bonds, Hydrophobic, Ionic and Water Bridges.