Supplementary Information

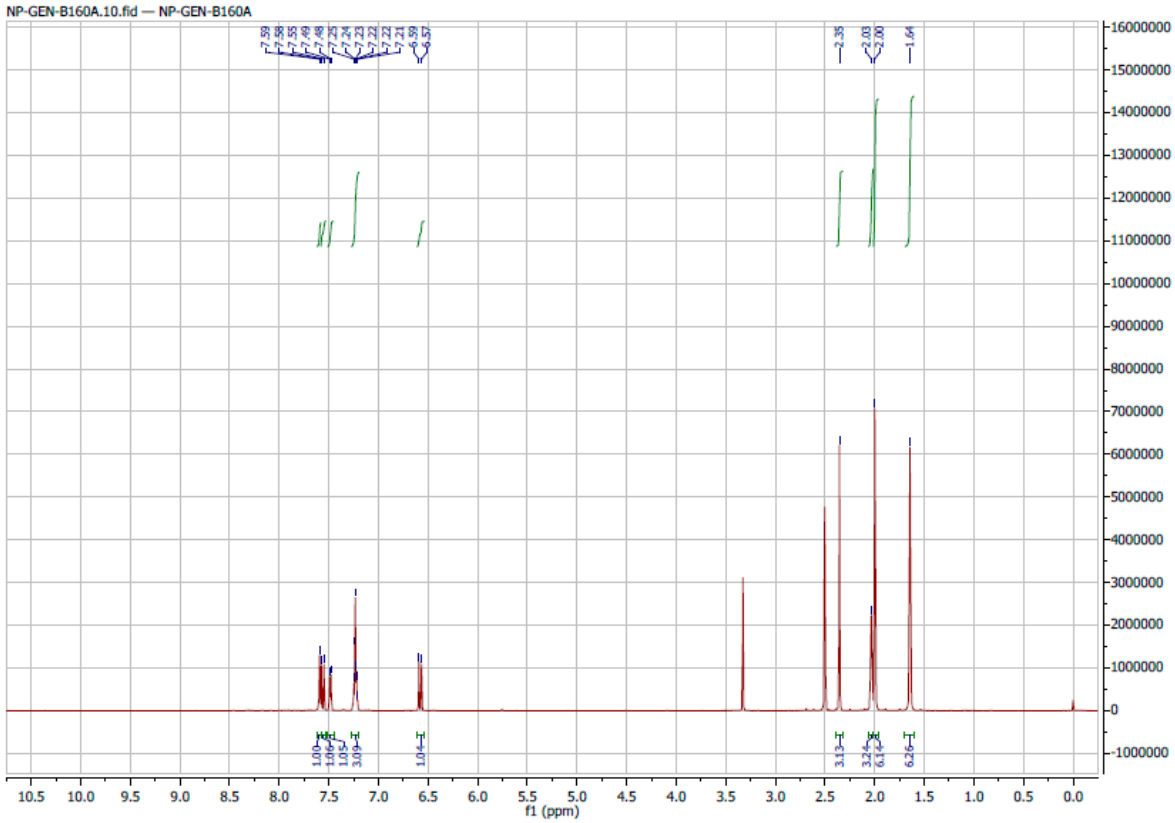
2-Methylcinnamic Acid Amide: Synthesis, Molecular Docking, and Neuroprotective Effect in the MPTP Mouse Model of Parkinson's disease

Maya Chochkova[1,*], Rusi Rusew[2], Reni Kalfin[3], Lyubka Tancheva[3], Maria Lazarova[3], Hrisitna Sbirkova-Dimitrova[2], Andrey Popatanasov[3], Krasimira Tasheva[4], Boris Shivachev[2], Nejc Petek[5], Martin Štícha[6]

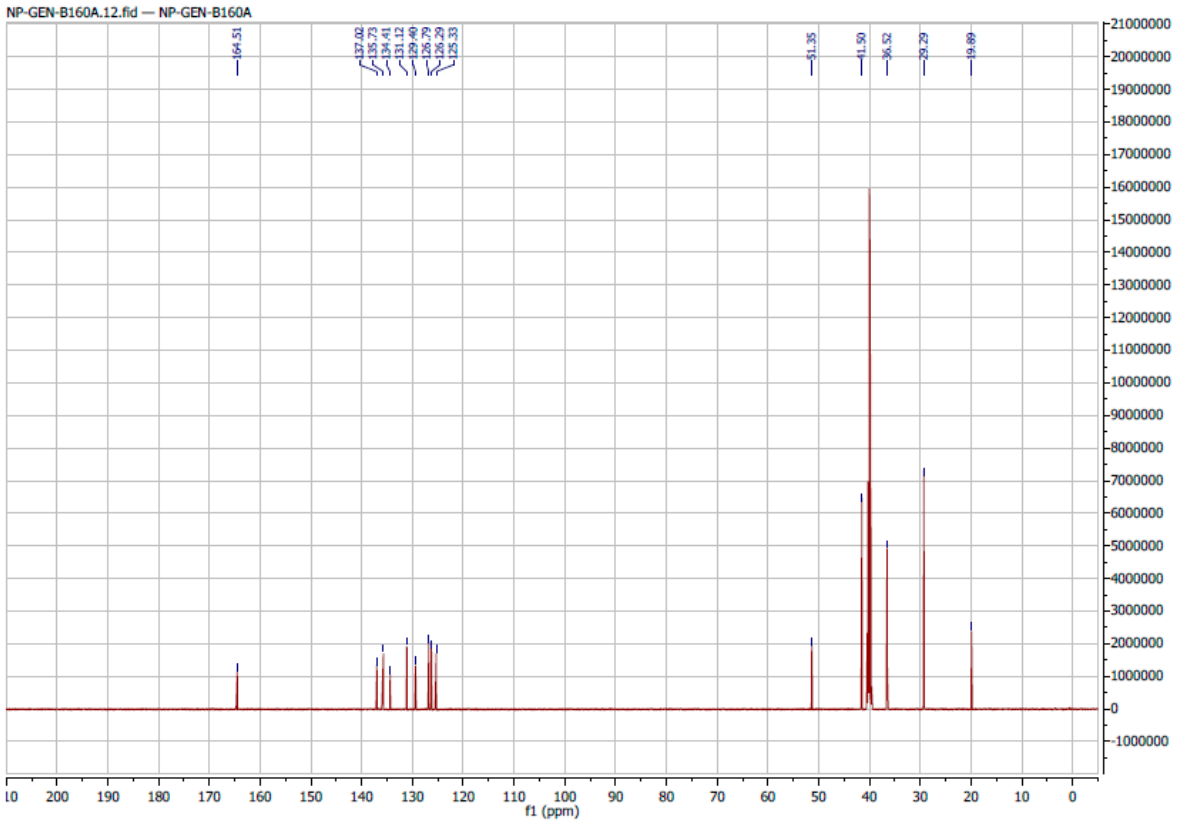
[1] Faculty of Mathematics and Natural Sciences, South-West University ''Neofit Rilski'', 66, Ivan Mihailov Str., 2700, Blagoevgrad, Bulgaria
[2] Institute of Mineralogy and Crystallography "Akad. Ivan Kostov", Bulgarian Academy of Sciences, Acad. G. Bonchev bl. 107, Sofia, Bulgaria
[3] Institute of Neurobiology, Bulgarian Academy of Sciences, Acad. G. Bonchev Bl.23, 1113 Sofia, Bulgaria
[4] Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
[5] Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, 1000 Ljubljana, Slovenia
[6] Charles University, Faculty of Science, Section of Chemistry, Hlavova 2030/8, 12843, Prague 2, Czechia
* Correspondence: mayabg2002@yahoo.com

Contents

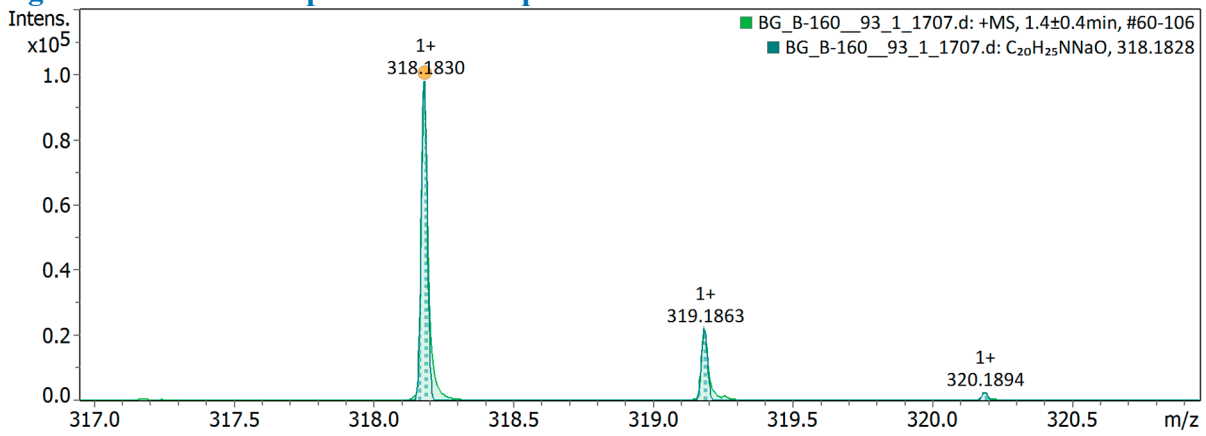
^{1}H NMR spectrum of compound 3



^{13}C NMR spectrum of compound 3



High resolution Mass spectrum of compound 3



Molecular docking and validation

The validation of the docking has been carried out against crystal structure of 7SAD that incorporates memantine. We ran Procheck and/or Coot to check for AA outliers and obtaining the Ramachandran plot. The Procheck and Coot data slightly differ but both pass the verification criteria (Fig. S4). The RMSD of 0.51 of the original vs docked protein was obtained using the Structural RMSD alignment tool incorporated in Molegro Virtual Docker. The value is below the largely accepted threshold of 2.0 Å. The alignment of the memantine and redocked ligand is shown below (Fig. S2) and discloses the similar orientation. The validation of the docking in 1H1D, 3EML and 2C65 followed the same approach: Ramachandran check, RMSD and similarity of the docked ligand vs the original in the crystals structure. The RMSD scores for proteins 1H1D, 3EML and 2C65 are 1.25, 1.48 and 0.8 Å respectively. The overlay original and redocked ligands of 1H1D, 3EML and 2C65 is shown on (Fig. S5).

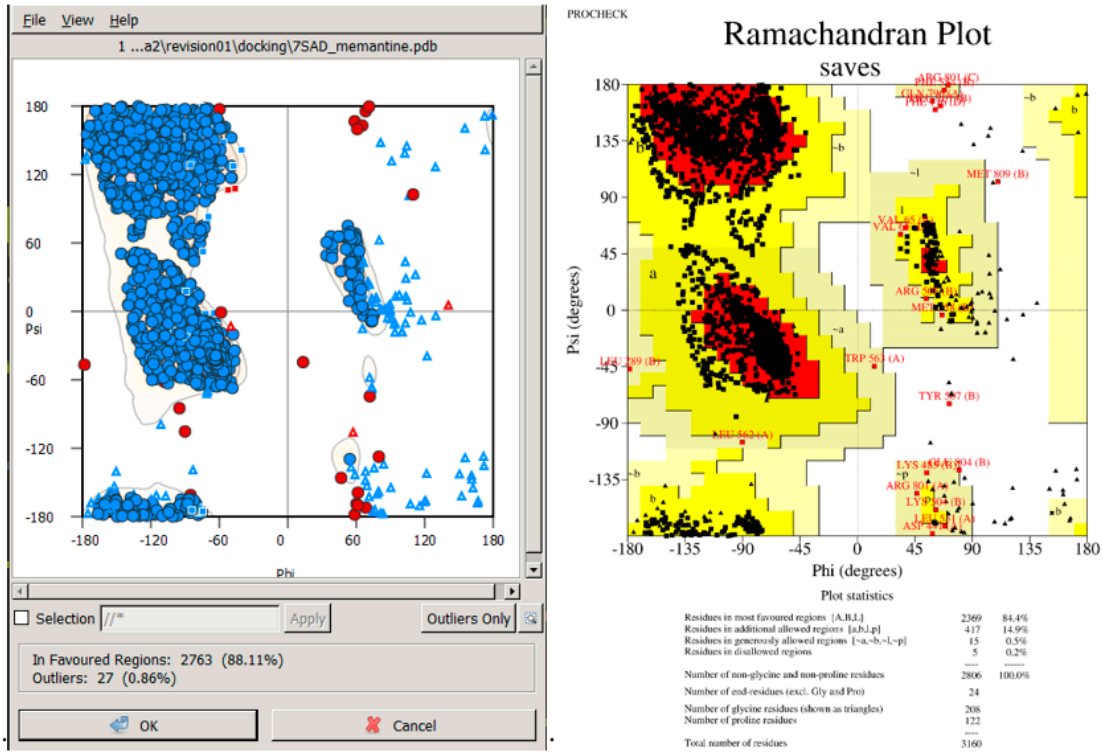


Figure S4. **Procheck and Coot Ramachandran plots for 7SAD with docked (*E*) – *N*-(2-methylcinnamoyl)-amantadine.**

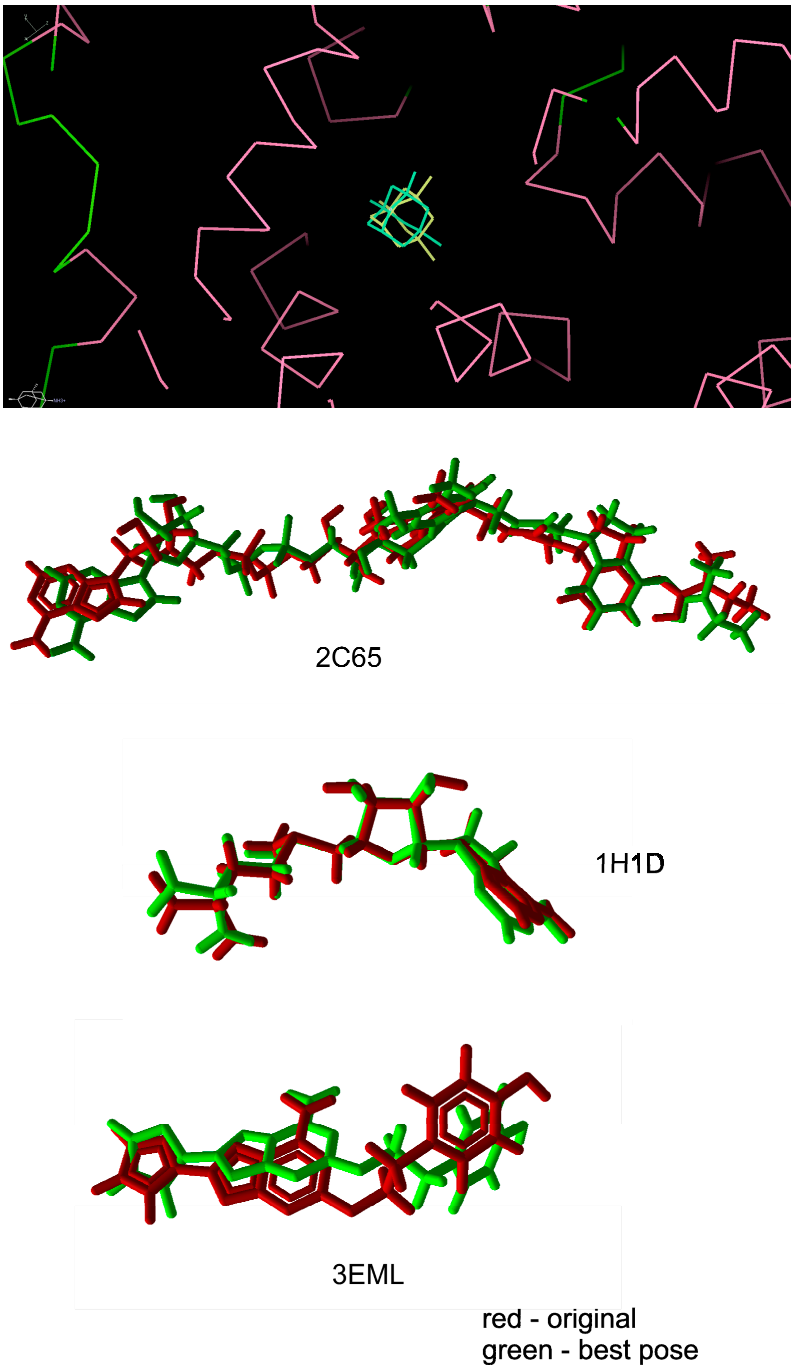


Figure. S5. Validation of docking protocol by superimposing the native ligand (green, memantine) to redocked ligands (memantine in yellow) in the respective 7SAD, 1H1D, 3EML and 2C65 structures.

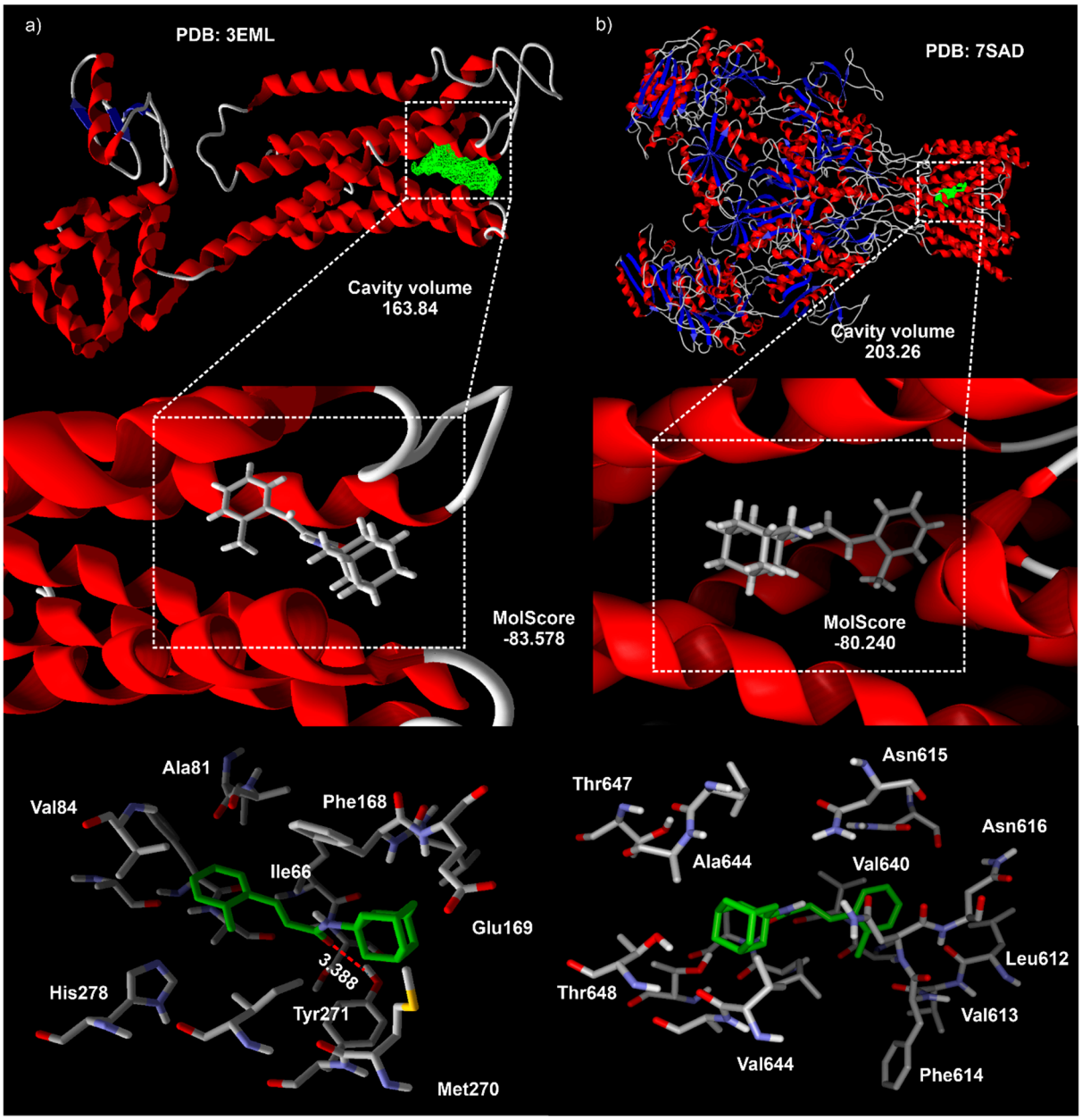


Figure S7. Visualization of the docking studies and molecular interaction of (E) – N-(2-methylcinnamoyl)-amantadine with a) A2aAR and b) NMAD; the hydrogen bonding interaction O-H…O=C is shown in as red dashed line with the A…D distance of 3.388 Å.

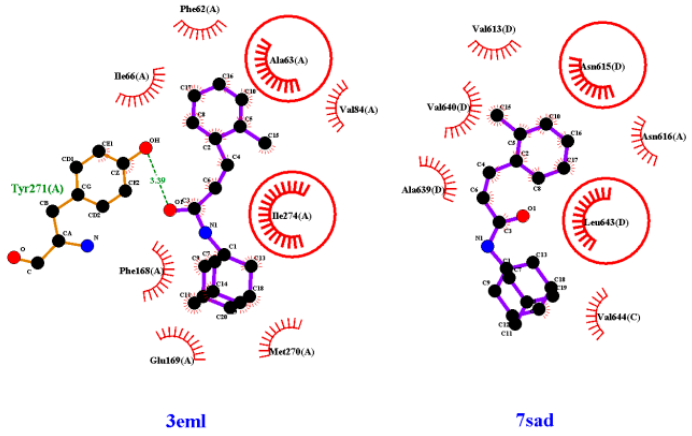


Figure S8. Observed interactions after docking of (*E*) – *N*-(2-methylcinnamoyl)-amantadine into the active site of two putative PD targets a) NMAD (7SAD) and b) A2aAR (3EML); the hydrophobic contacts are shown as for enzyme and with for ligand; the similar residues for both enzymes are shown as .