Supplementary file

Antipsychotic clozapine binding to alpha-2-macroglobulin protects interacting partners against oxidation and preserves the anti-proteinase activity of the protein

Miloš Šunderić[a]*, Tamara Vasović[b], Miloš Milčić[b], Čedo Miljević[c], Olgica Nedić[a], Milan R. Nikolić[b], Nikola Gligorijević[a]

[a]*Institute for Application of Nuclear Energy, University of Belgrade, Banatska 31b, 11080 Belgrade, Serbia*

[b]*Department of Biochemistry & Center of Excellence for Molecular Food Sciences, University of Belgrade - Faculty of Chemistry, Studentski trg 12-16, 11000 Belgrade, Serbia*

[c]*Institute of Mental Health, University of Belgrade - Faculty of Medicine, Milana Kašanina 3, 11000 Belgrade, Serbia*

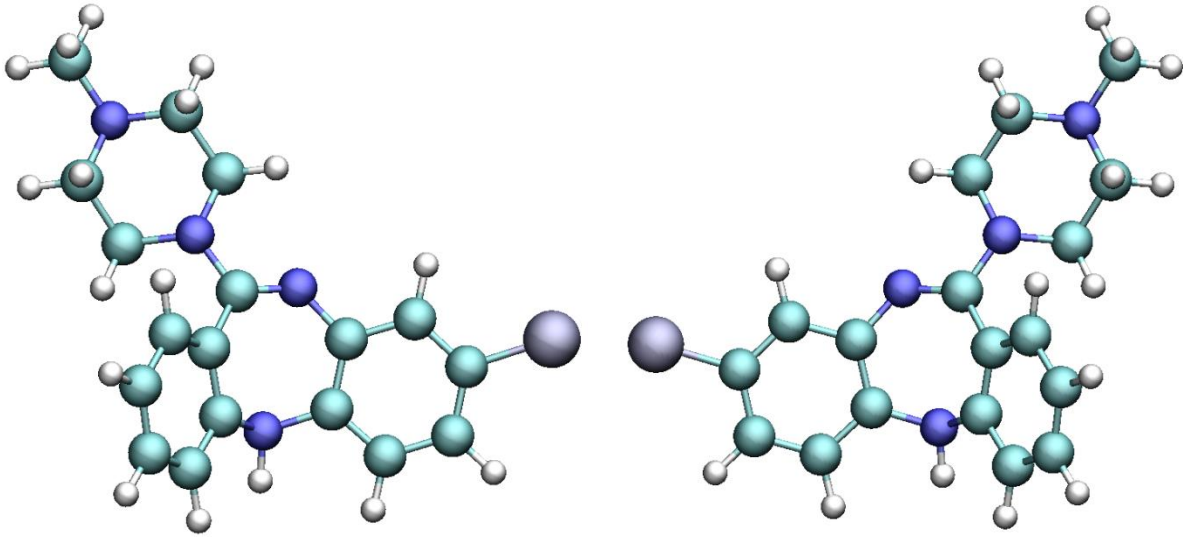

Figure S1. Optimized structures of two atropisomers of the clozapine molecule. Left: *P* (plus) atropisomer. Right: *M* (minus) atropisomer. Two atropisomers are also enantiomers.

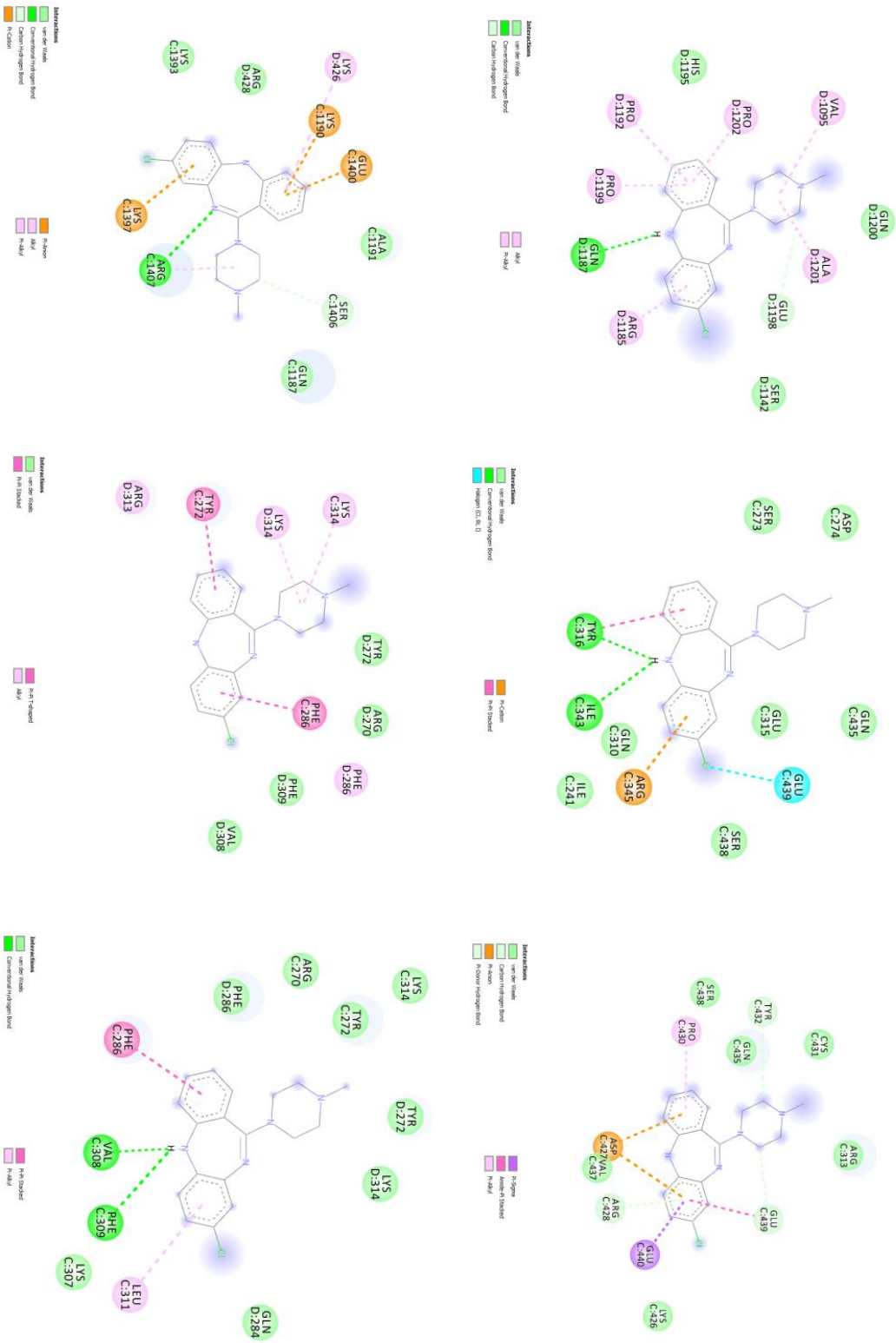

Figure S2. The 2D protein-ligand interaction diagram for the selected binding sites in clozapine binding pocket (Note: the residue numbers correspond to residue numbers in 4ACQ).

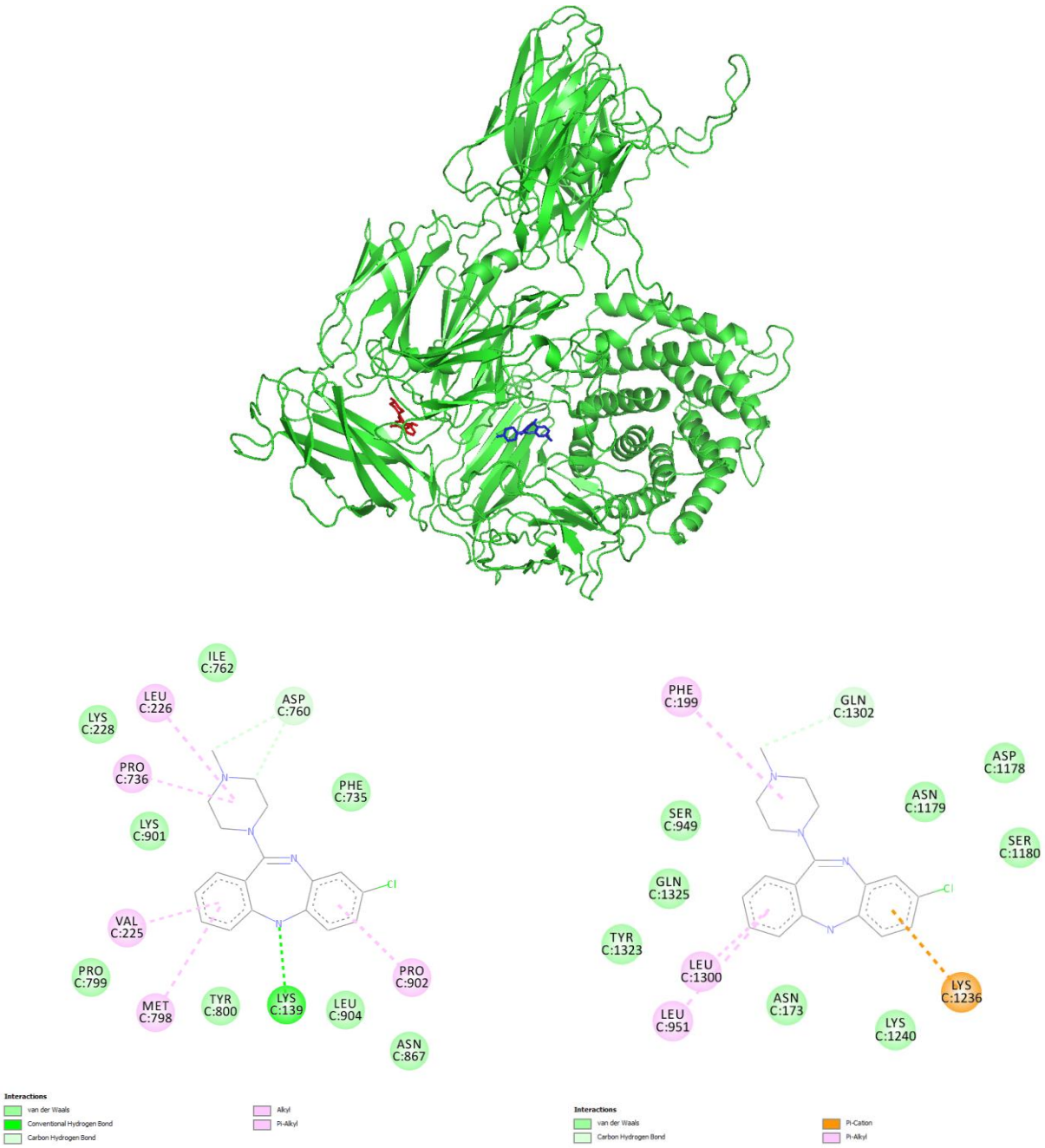

Figure S3. Two binding sites found in chain C by docking experiment. **Left and red**: binding site with binding energy 8.2 kcal/mol; **Right and blue**: binding site with binding energy 7.7 kcal/mol (Note: the residue numbers correspond to residue numbers in 4ACQ).

The two highest energy binding sites that can accommodate both atropisomers of clozapine found in a single chain docking simulation are presented in **Figure S2**. The first binding site (colored red in upper panel, left image in lower panel) has binding energy of 8.2 kcal/mol and is

located inside the protein. Ligand is interacting with amino acids from two beta sheets (867-875, 897-906) and surrounding loops. The dominant types of interactions are π-alkyl and alkyl-alkyl, and there is hydrogen bond between amino group from 1,4-diazepine ring and Lys139.

The second binding site is located between two beta sheets (945-951 and 1322-1332) and small α helix (1225-1240). Binding energy in this site is 7.7 kcal/mol. Once again, hydrophobic π-alkyl and alkyl-alkyl interactions are the most abundant. Also, there is a cation-π interaction between chlorobenzene ring and Lys1236.

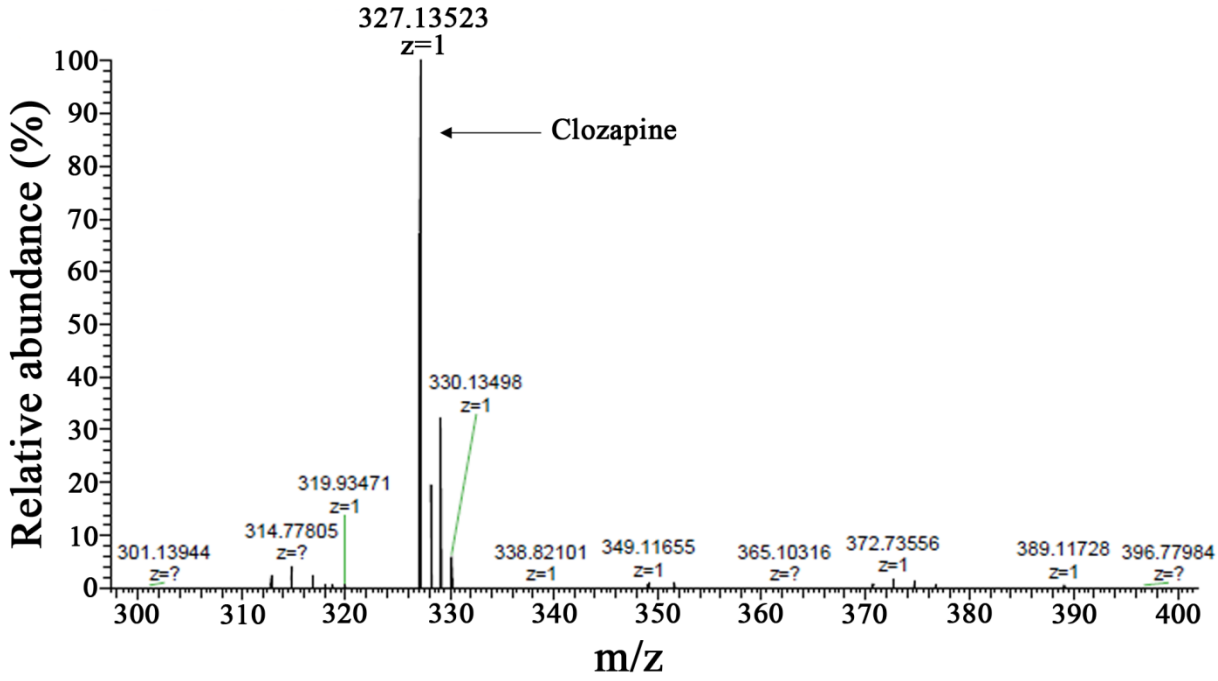

Figure S4. Mass spectrum of clozapine.