Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Analyzing Atomic Scale Structural Details and Nuclear Spin Dynamics of Four Macrolide Antibiotics: Erythromycin, Clarithromycin, Azithromycin, and Roxithromycin

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S1. Exploring the interactions of Ribosomal Protein L4 with Erythromycin, Azithromycin, and Clarithromycin through Molecular Dynamics Simulations:

The molecular dynamics simulations of protein L4 with azithromycin and protein L4 with clarithromycin were performed in the same way as those performed in MD simulations of protein L4 with erythromycin and roxithromycin. The physiological environment created for each of the antibiotics with protein L4 for MD simulation is illustrated in Figure S1.

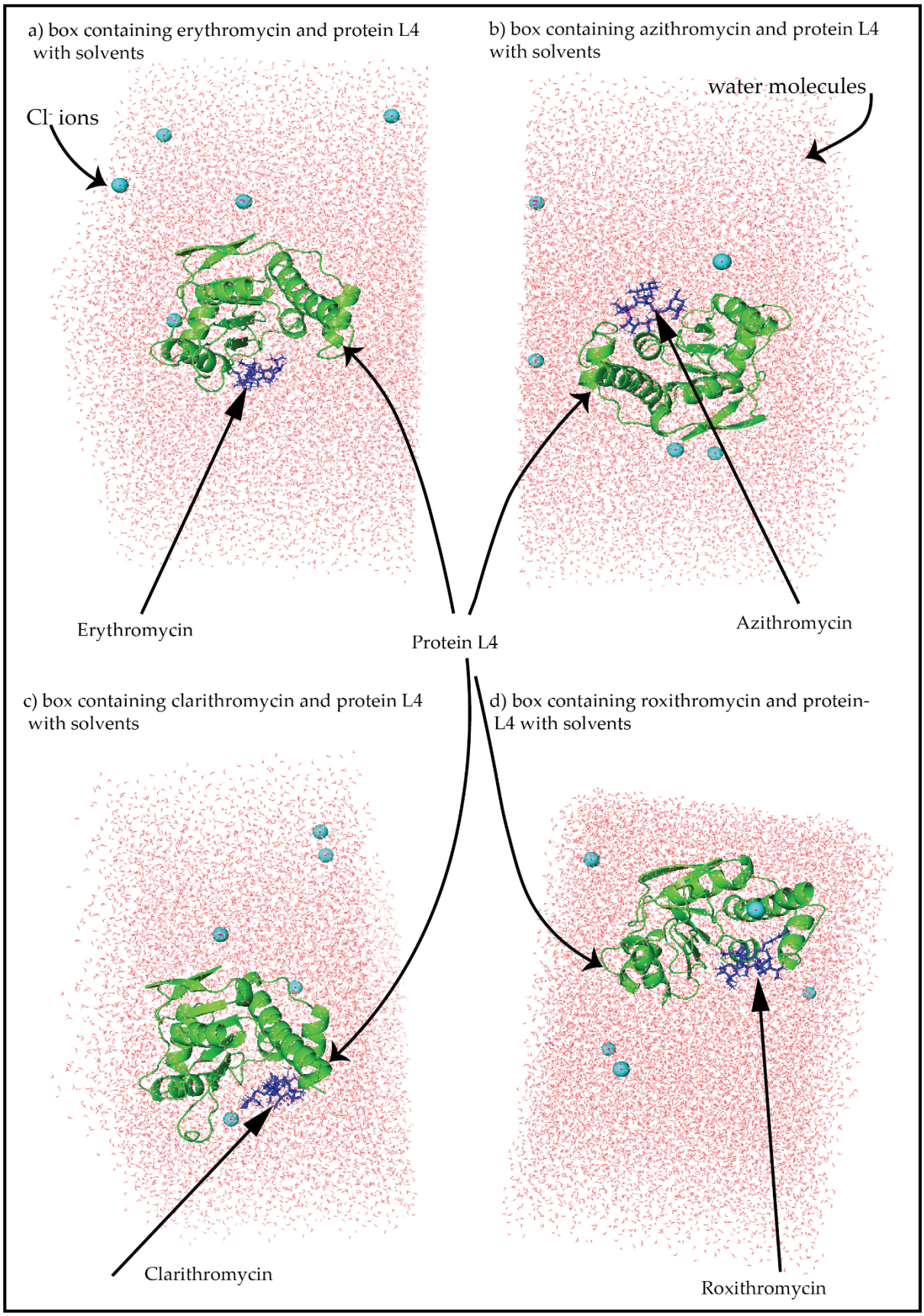


Figure S1: Physiological environment created for each antibiotic with protein L4 prior to conducting MD simulations (a) setup for erythromycin and protein L4 MD simulation, (b) setup

for azithromycin and protein L4 MD simulation, (c) setup for clarithromycin and protein L4 MD simulation, and (d) setup for roxithromycin and protein L4 MD simulation.

S1.1 Molecular Dynamics Simulation insights into Protein L4 interactions with Azithromycin Verses Erythromycin:

a) RMSD analysis:

In Figure S2(a), we present the temporal variations in the root mean square deviations (RMSD) of protein-L4 atoms during its interaction with erythromycin and azithromycin. Notably, our analysis reveals that RMSD of atoms present within protein L4, when interacts with azithromycin, varies approximately the same way as it interacts with erythromycin for the initial 40 ns of simulation. Within this time frame, the RMSD values of protein L4 range from 1.5 Å to 2.8 Å. However, after 40 ns to nearly 80 ns time frame, it starts fluctuating with lower RMSD valueswhen interacts with azithromycin than during its interaction with erythromycin. After 80 ns, dynamics of protein L4 stabilize with an average RMSD value of approximately 2.6 Å, showcasing heightened dynamics compared to its interaction with erythromycin.

b) RMS Fluctuation analysis:

Upon analysing the results derived from MD simulations of protein L4 interacting with erythromycin and azithromycin, as depicted in Figure S2(b), it is observed that the majority of the protein L4 atoms exhibit similar fluctuation patterns during interactions with both azithromycin and erythromycin, with exceptions noted for residues numbered 300 to 1000 and 1800 to 2000. Specifically, residues numbered 300 to 1000 demonstrate greater fluctuation during interaction with azithromycin compare to their interaction with erythromycin. Conversely, residues numbered 1800 to 2000 exhibit reduced fluctuation during the protein-azithromycin interaction compared to the protein-erythromycin interaction. This different fluctuation pattern among specific protein residues suggests a response of distinct chemical properties and binding modes of azithromycin and erythromycin.

c) Radius of gyration analysis:

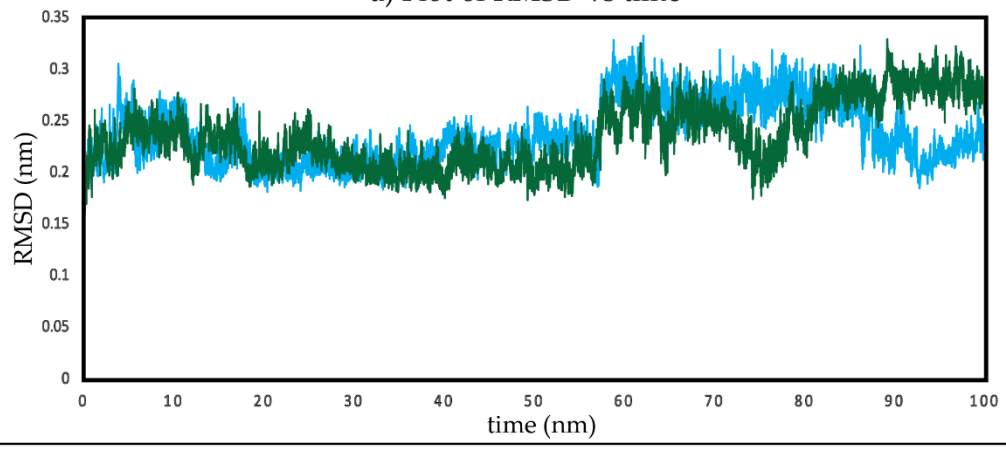
The plot of radius of gyration against time, illustrated in Figure S2(c), reveals that within the first 10 ns of simulation, protein L4 exhibits a lower radius of gyration when interacting with azithromycin as compare tothat of protein L4 when interact with erythromycin. This initial phase suggests a degree of compactness in the protein structure, indicative of a potential binding

event with azithromycin. However, beyond this initial timeframe, a distinct shift occurs. protein L4 begins to display a higher radius of gyration compared to its interaction with erythromycin. This shift implies a change in the conformational dynamics of protein over time. Specifically, it suggests that protein L4 may initially adopt a more compact conformation in the presence of azithromycin, but as time progress, the protein structure becomes more expanded or flexible as compare to that in presence of erythromycin.

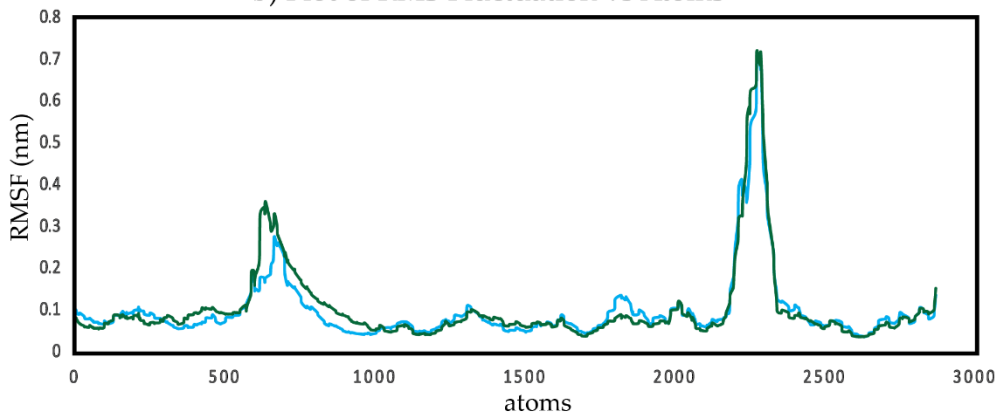
d) Hydrogen bond analysis:

The plot of number of hydrogen bond verses time, as given in Figure S2(d), reveals a significant distinction in the interactions between azithromycin and protein L4 compared to the interaction between erythromycin and protein L4. Throughout the entire simulation period, it is evident that the number of hydrogen bonds formed between azithromycin and protein L4 is notably lower than those formed between erythromycin and protein L4. This observation underscores a crucial aspect of interactions: azithromycin exhibits considerably fewer interactions with protein L4 via hydrogen bonding, suggests a diminished degree of interaction and, consequently, a less stable structural configuration. In contrast, the higher number of hydrogen bonds formed between erythromycin and protein L4 indicates a more extensive and likely stronger interaction, contributing a more stable complex.

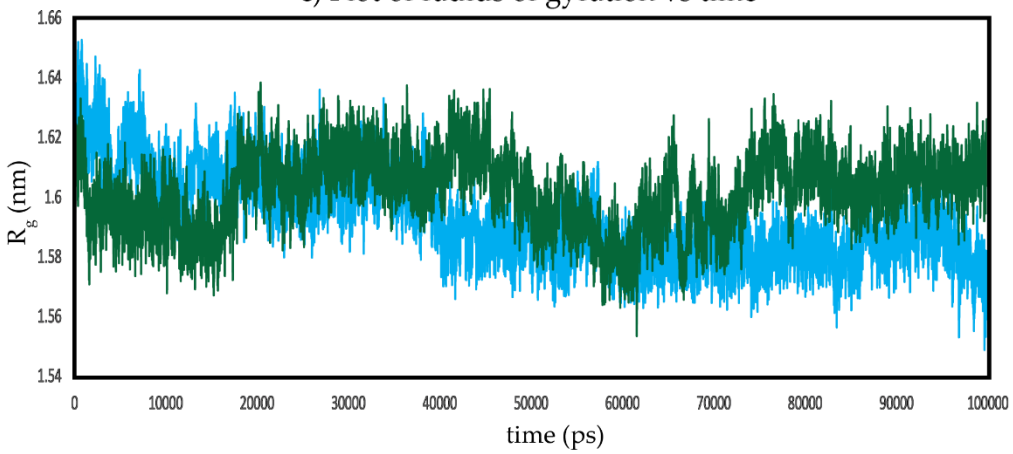
a) Plot of RMSD vs time

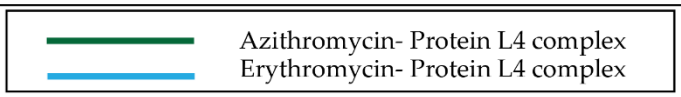


b) Plot of RMS Fluctuation vs Atoms



c) Plot of radius of gyration vs time





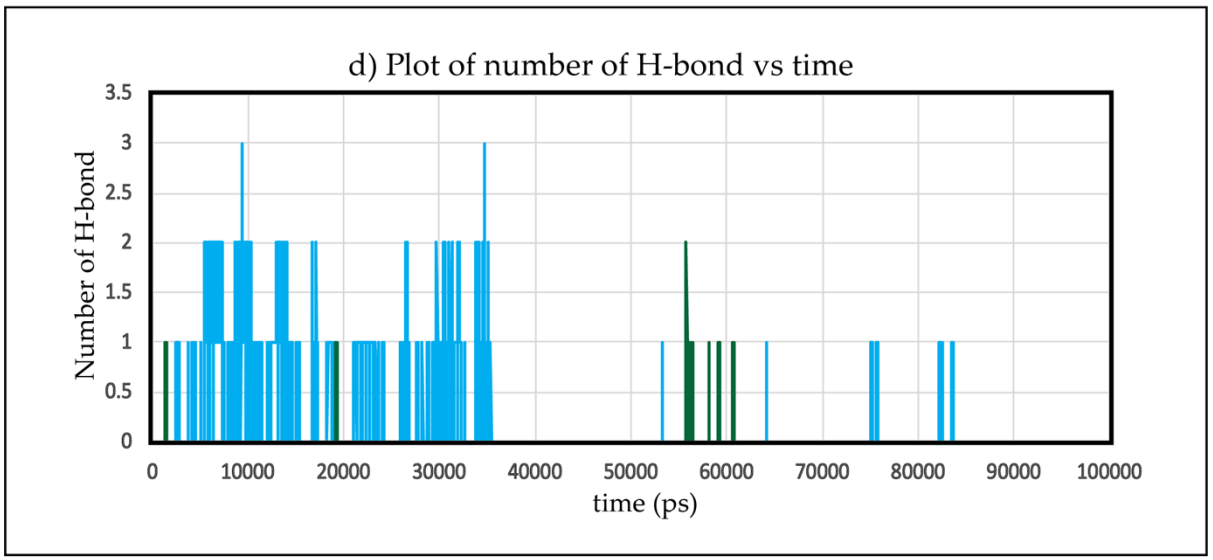


Figure S2: Plot of (a) RMSD verses time, (b) RMSF verses atoms, (c) radius of gyration verses time, and (d) number of hydrogen bond verses time for two complexes: erythromycin-protein L4 complex and roxithromycin-protein L4 complex.

S1.2 Analysing the differences: Molecular Dynamics Simulation insights into Protein L4 interactions with Azithromycin Verses Erythromycin:

a) RMSD analysis:

Examining Figure S3(a), we observed that protein L4 initiates its interaction with both erythromycin and clarithromycin with a similar RMSD value of approximately 1.5 Å. Over the entire 100 ns simulation period, protein L4 maintains an average RMSD value of 2.2 Å, exhibiting comparatively less fluctuation when interacting with clarithromycin. Notably, a distinct difference emerges between the two interactions around the 60-90 ns timeframe. During this period, protein L4 displays a greater RMSD value when interacting with erythromycin compared to clarithromycin. This increased RMSD values in this region indicates a greater dynamics or instability in the protein- ligand complex compared to its interaction with clarithromycin.

b) RMSF analysis:

The root mean square fluctuation (RMSF) of residues of protein backbone during the interaction with both the antibiotics are shown in Figure S3(b). Upon analysing the plot, it becomes evident that the fluctuations of protein L4 residues are generally comparable in both cases. However, specific residues with number 1800 to 2000 and 2200 to 2300, exhibit slightly greater fluctuation when interacting with erythromycin compared to the other antibiotic. This

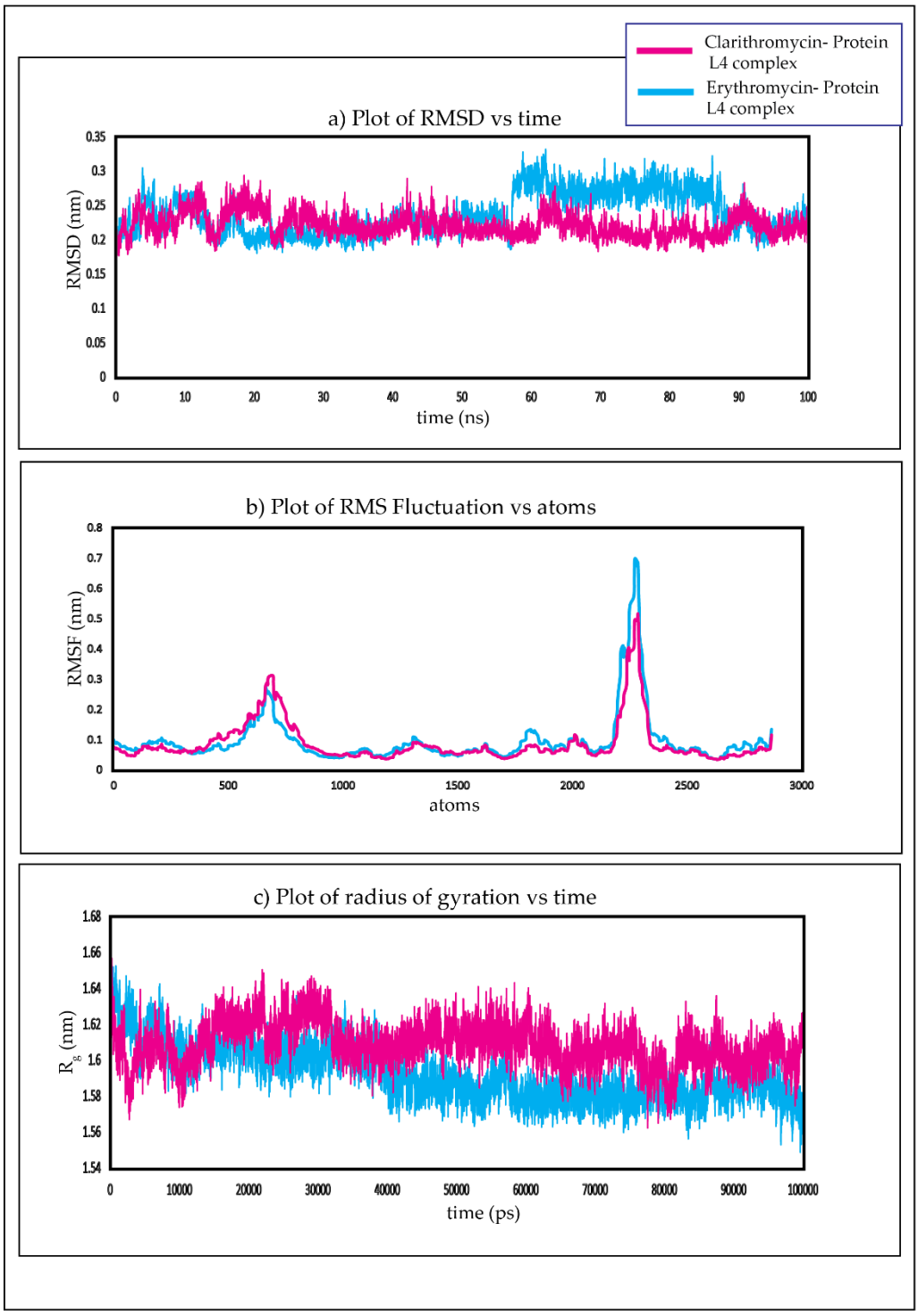
increased fluctuation of certain residues in the presence of erythromycin may indicate a unique response of these regions to the binding of this antibiotic.

c) Radius of gyration analysis:

Upon examining the radius of gyration analysis depicted in Figure S3(c), it is noticed that starting from 0 ns to 15 ns, protein L4 exhibits a reduced radius of gyration when interacting with clarithromycin as compare to the radius of gyration when interacting with erythromycin. This suggests that during the initial timeframe, the structure of protein L4 becomes more compact in presence of clarithromycin compared to when it interacts with erythromycin. However, beyond the 15 ns of simulation, and throughout the entire simulation period, protein L4 displays higher values of radius of gyration when interacting with clarithromycin compared to its interaction with erythromycin. This observation implies a divergence in structural dynamics between the two interactions, with clarithromycin leading to a less compact conformation of protein L4 over an extended period.

d) Hydrogen bond analysis:

From the plot of hydrogen bond interaction of antibiotics with protein L4 with the variation of time, illustrated in Figure S3(d), it is observed that, across the entire md simulation timeframe, clarithromycin makes a greater hydrogen bonding interaction with protein-L4 as compare to other antibiotic erythromycin. It implies clarithromycin forms more frequent and potentially stronger hydrogen bonds, underscores its potential efficacy on targeting protein L4.


Clarithromycin- Protein L4 complex
Erythromycin- Protein L4 complex
a) Plot of RMSD vs time
RMSD (nm)
time (ns)
b) Plot of RMS Fluctuation vs atoms
RMSF (nm)
atoms
c) Plot of radius of gyration vs time
R_g (nm)
time (ps)

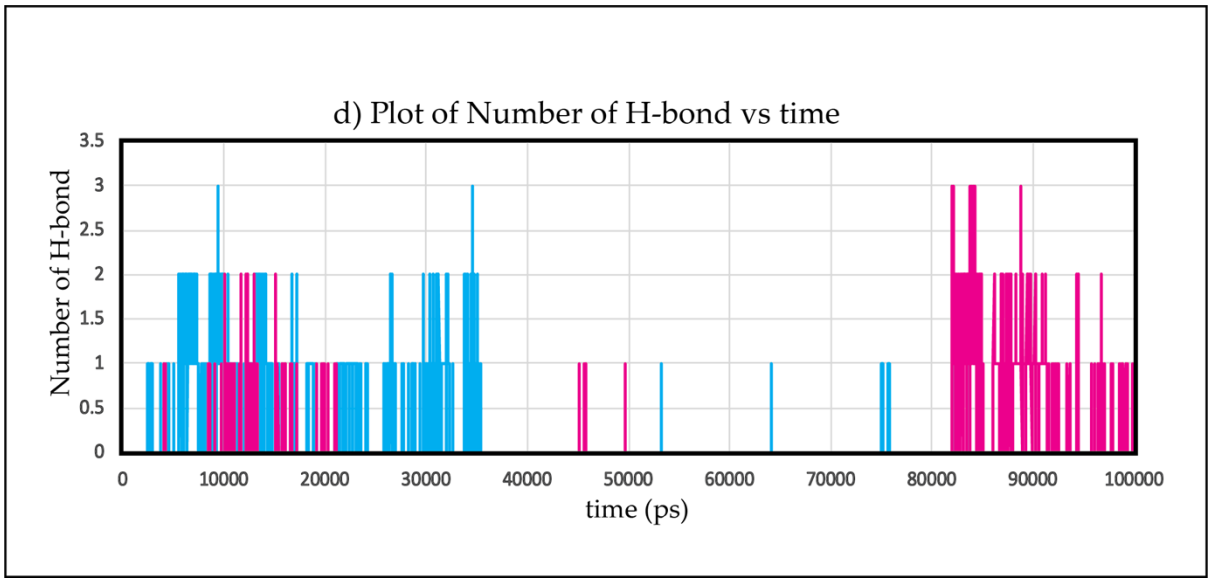
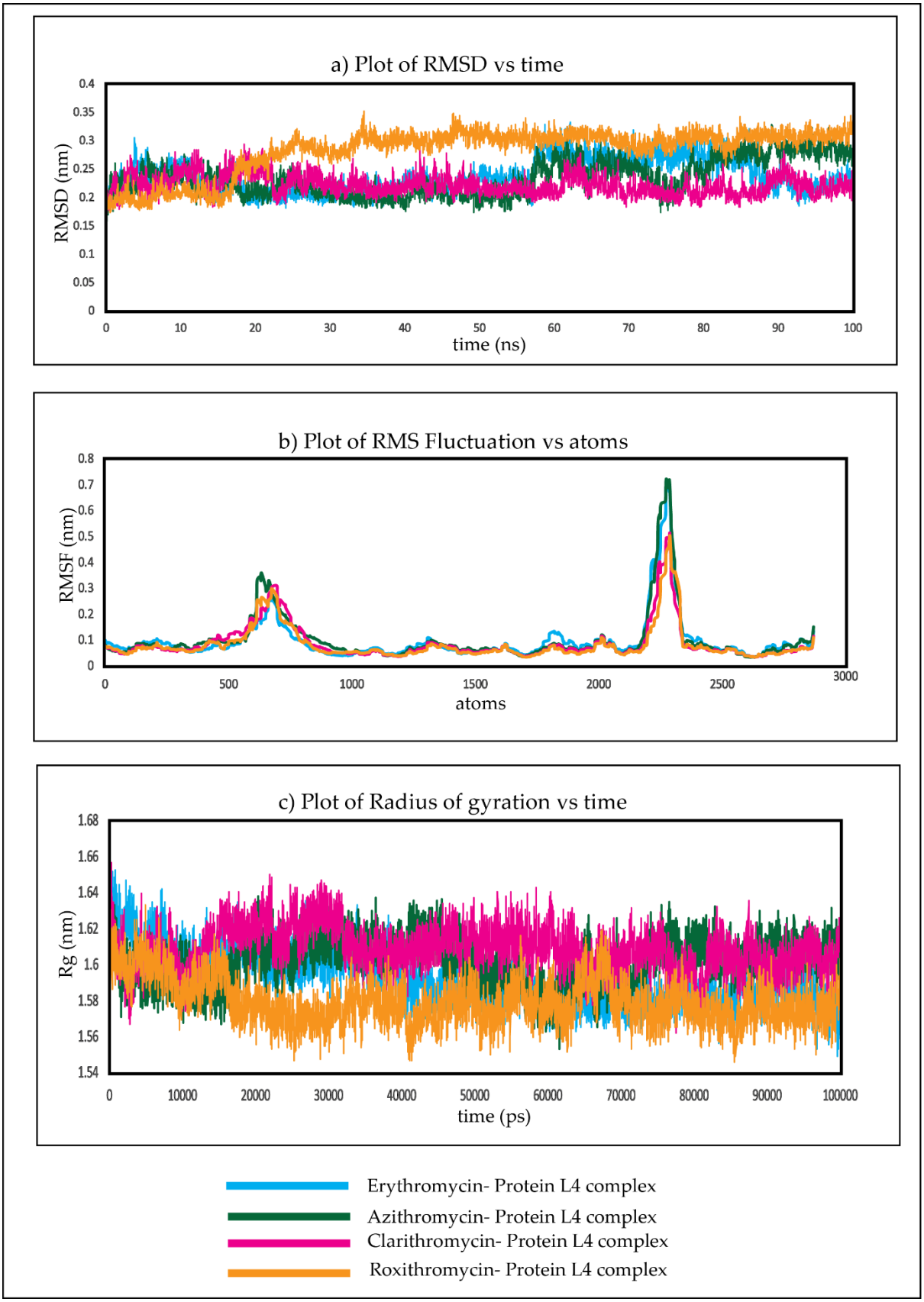


Figure S3: Plot of (a) RMSD verses time, (b) RMSF verses atoms, (c) radius of gyration verses time, (d) number of hydrogen bond verses time for two complexes: erythromycin-protein L4 complex, and clarithromycin-protein L4 complex.

The result of md simulations of each antibiotics with protein L4 those plotted in one frame is shown in Figure S4.


a) Plot of RMSD vs time
RMSD (nm)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
10
20
30
40
50
60
70
80
90
100
time (ns)
b) Plot of RMS Fluctuation vs atoms
RMSF (nm)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
500
1000
1500
2000
2500
3000
atoms
c) Plot of Radius of gyration vs time
Rg (nm)
1.68
1.66
1.64
1.62
1.6
1.58
1.56
1.54
0
10000
20000
30000
40000
50000
60000
70000
80000
90000
100000
time (ps)
Erythromycin- Protein L4 complex
Azithromycin- Protein L4 complex
Clarithromycin- Protein L4 complex
Roxithromycin- Protein L4 complex

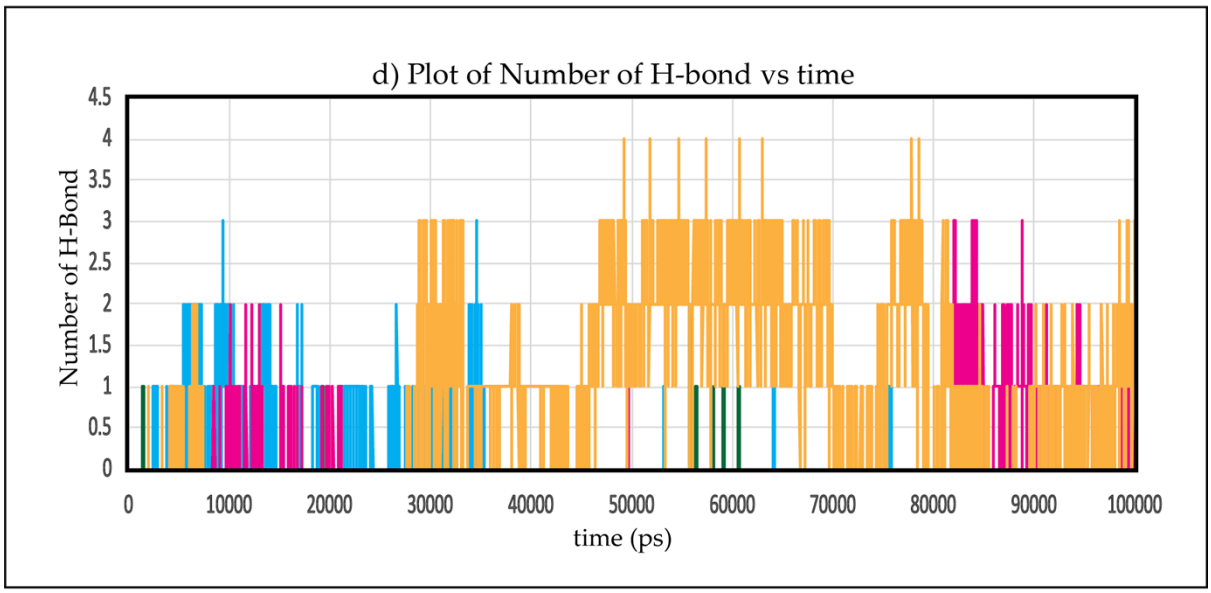


Figure S4: Plot of (a) RMSD verses time, (b) RMSF verses atoms, (c) radius of gyration verses time, (d) number of hydrogen bond verses time for four complexes: erythromycin-protein L4 complex, azithromycin-protein L4 complex, clarithromycin-protein L4 complex, and roxithromycin- protein L4 complex.

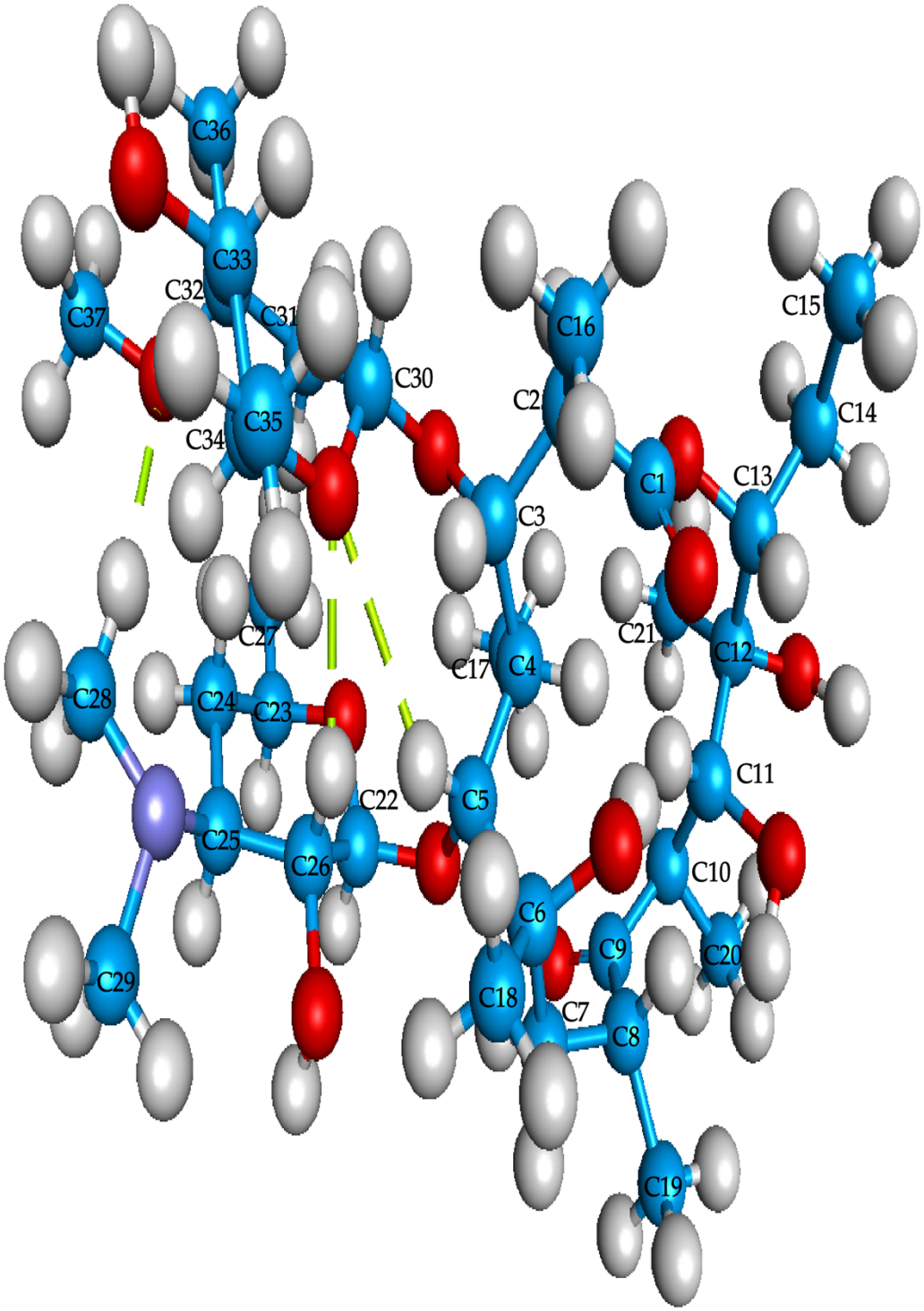


Figure S5: Chemical structure of Erythromycin.

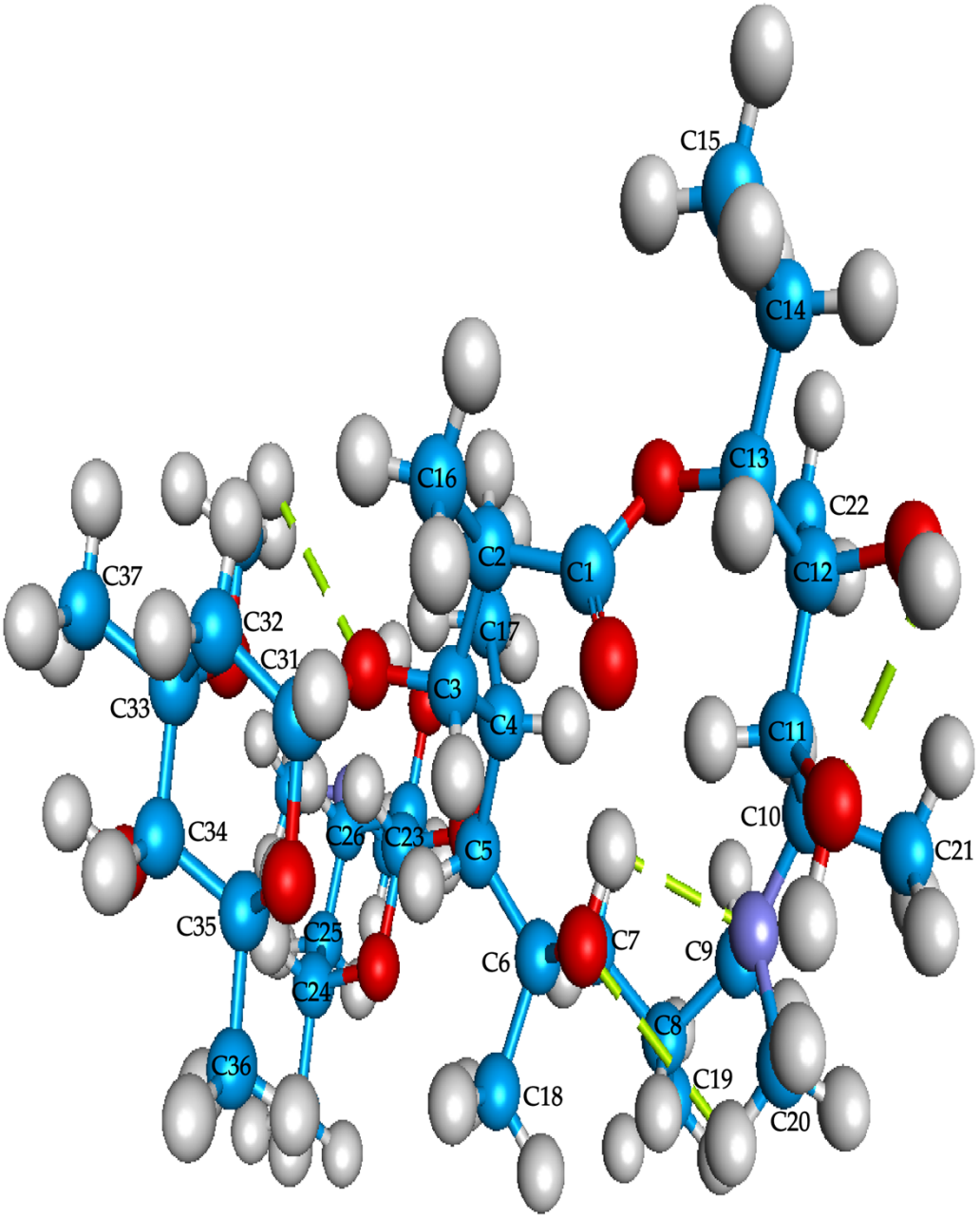


Figure S6: Chemical structure of azithromycin.

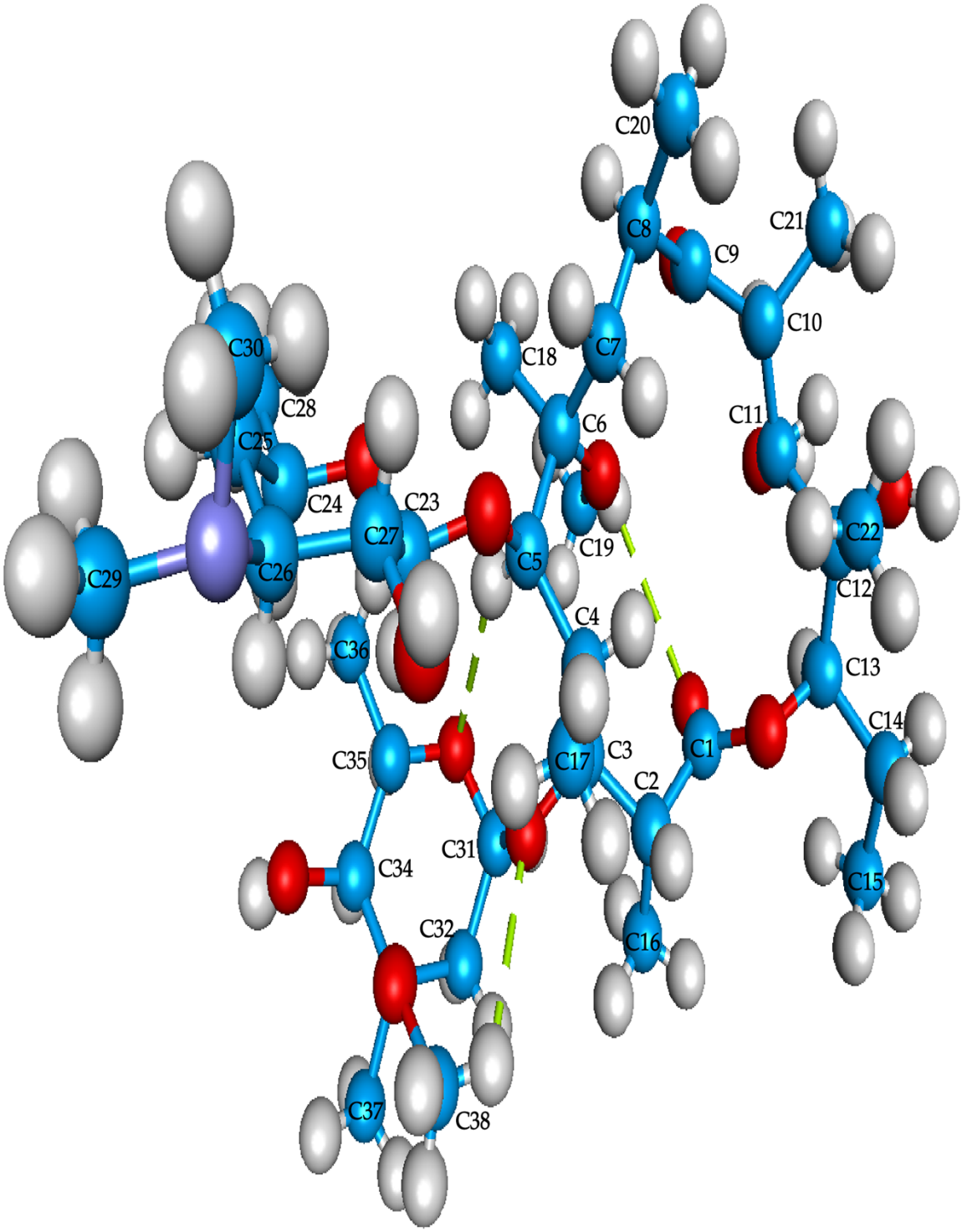


Figure S7: Chemical structure of clarithromycin.

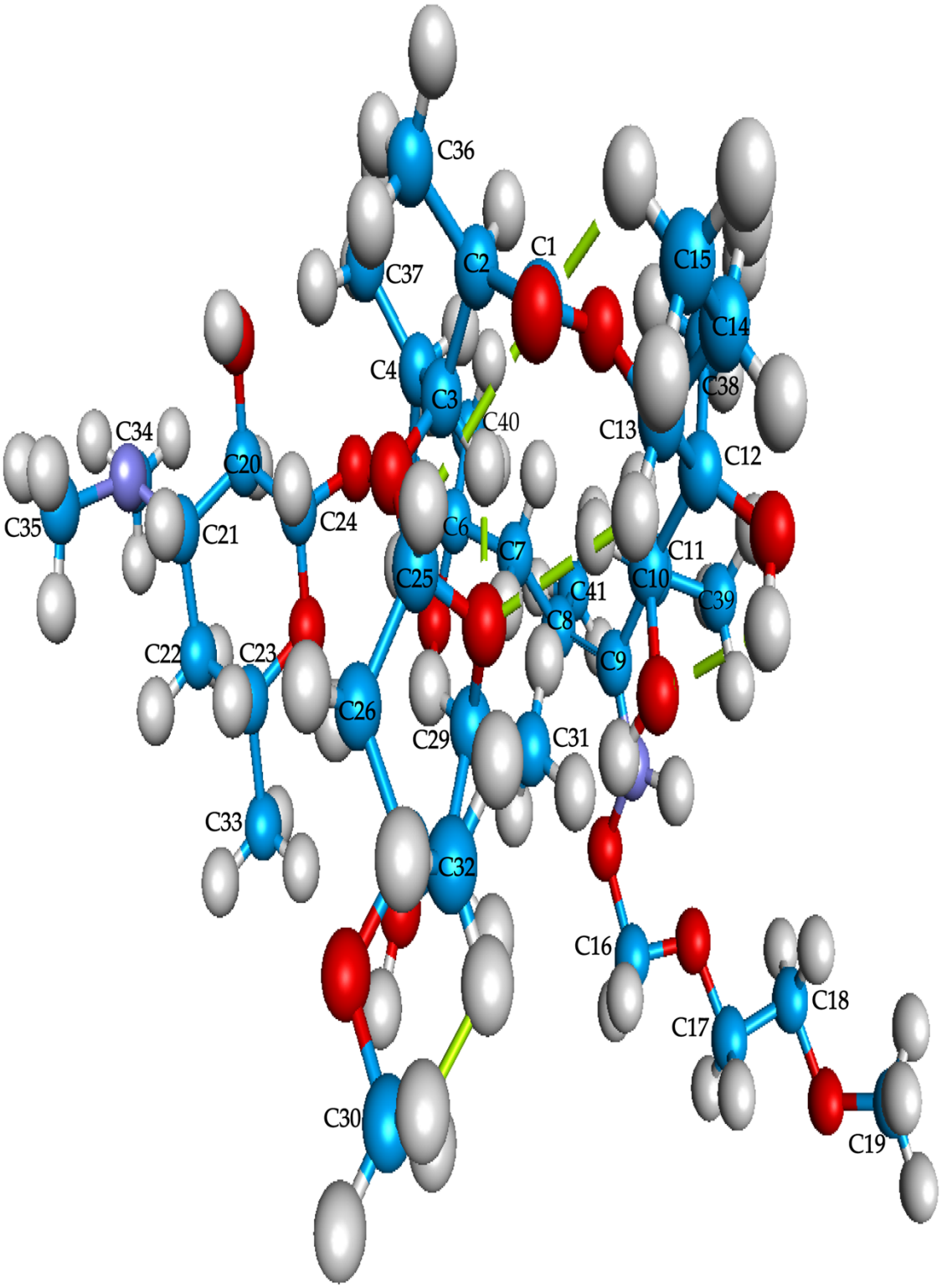


Figure S8: Chemical structure of roxithromycin.

S2. Docking of three antibiotics erythromycin, azithromycin, and roxithromycin with motilin receptor:

The docking of three antibiotics namely erythromycin, azithromycin, and roxithromycin with motilin receptor were carried out by using MGL Tool Auto Dockvina version 1.5.7 (https://vina.scripps.edu/). Here we have taken three antibiotics (erythromycin, azithromycin, and roxithromycin) as ligand and motilin receptor as target to perform molecular docking.

The crystal structure of motilin receptor and three mentioned antibiotics were achieved from RCSB protein data bank (PDB) (https://www.rcsb.org/) in the form of complexes. The cleaning and preparation of receptor and ligand files were performed by Discovery Studio Visualizer version 2021 (https://www.3ds.com/products/biovia/discovery-studio/visualization). The pdbqt files and the grid box of corresponding ligands and receptor were generated by using Auto Dockvina tools. After this dockings of erythromycin, azithromycin, and roxithromycin with motilin receptor were performed one after one. The obtained docking complexes with different binding modes were visualised by using PyMol (https://pymol.org).

Motilin receptors are the specific proteins those located on the surface of cells within the gastrointestinal tract. They are responsible for binding with motilin, a hormone produced in the small intestine. Motilin receptors serve as targets for motilin signaling and play a vital role in regulating gastrointestinal motility. Gastrointestinal motility is essential for the digestion, absorption, and elimination process that support overall digestive function and nutrient uptake in the body. Erythromycin and its derivatives are well known as motilin receptor agonists, the drugs that bind to and activate the motilin receptor, mimicking the action of the natural hormone motilin.[1,2,3]

While docking with each ligand, nine different binding poses were obtained. The best poses of interactions of the above macrolides with motilin are shown in Figure S9. The erythromycin was found to be interacts with motilin receptor with maximum negative binding affinity ranging from -8.1 kcal/mol to -7.2 kcal/mol in nine best modes of interactions. While performing docking of azithromycin with motilin receptor, the best binding modes were found with higher negative binding affinity ranging from -8.8 kcal/mol to -7.8 kcal/mol. Similarly, roxithromycin was found to be bound to motilin with maximum negative binding affinity ranging from -9.3 kcal/mol to -8.4 kcal/mol. The table of different mode of interactions with different binding affinity is shown in Table S1.

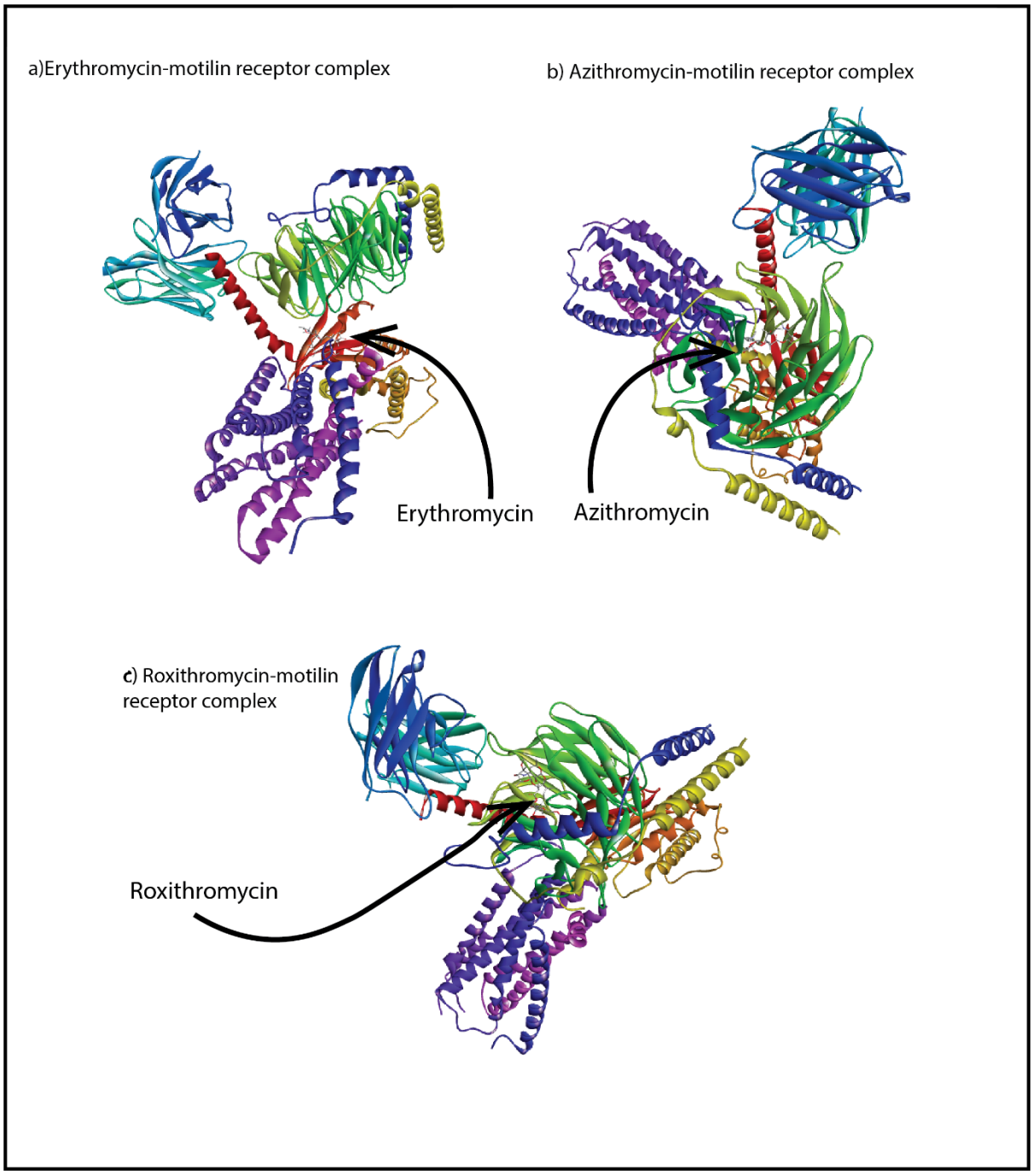


Figure S9: The interactions of three macrolide antibiotics : (a) Erythromycin with Motilin receptor, (b) Azithromycin with Motilin receptor and (c) Roxithromycin with receptor Motilin.

Table S1: Details of different modes of interactions of antibiotics with motilin receptor with different binding affinity

mode	Affinity of erythromycin (kcal/mol)	Affinity of azithromycin (kcal/mol)	Affinity of roxithromycin (kcal/mol)

1	-8.1	-8.8	-9.3
2	-8.0	-8.3	-9.0
3	-7.6	-8.2	-8.7
4	-7.6	-8.2	-8.5
5	-7.5	-7.9	-8.5
6	-7.4	-7.9	-8.5
7	-7.4	-7.9	-8.5
8	-7.2	-7.8	-8.4
9	-7.2	-7.8	-8.4

The two dimensional interactions of the above mentioned antibiotics with motilin receptor with a more negative binding affinity are shown below in Figure S10, Figure S11, and Figure S12 as more negative binding affinity indicates a stronger binding between ligand and receptor.

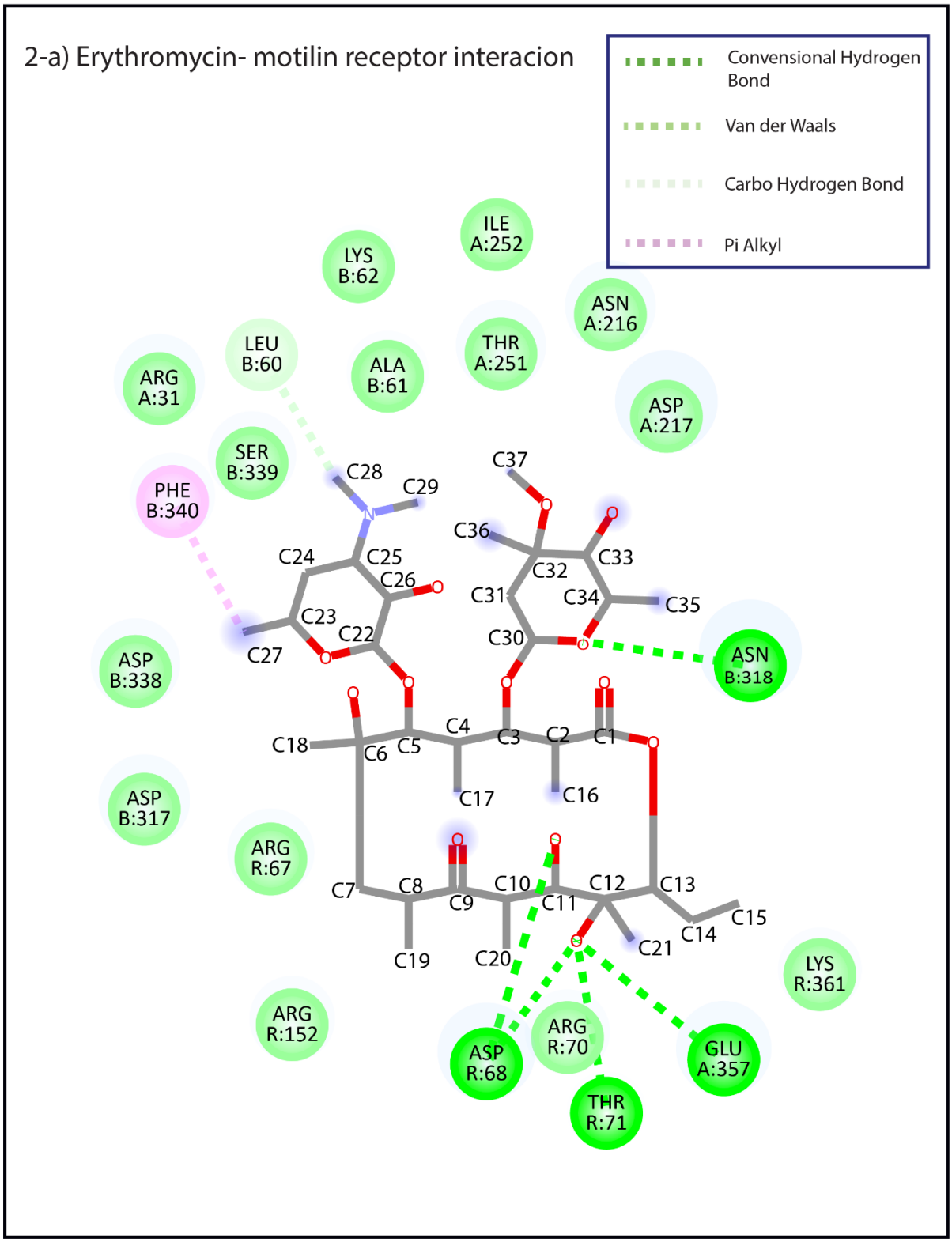


Figure S10: Two dimensional interactions of (2-a) Erythromycin-motilin receptor.

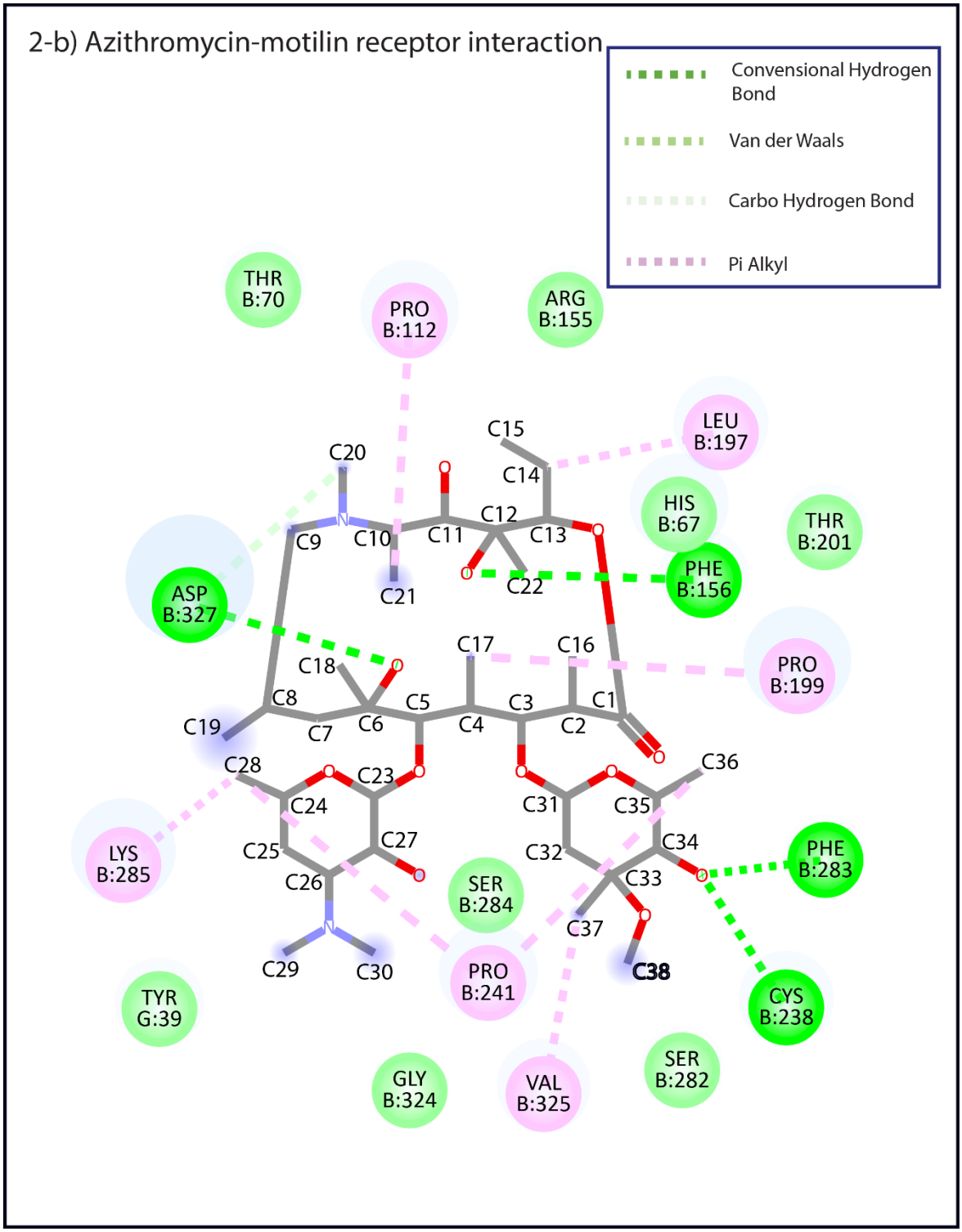


Figure S11: Two dimensional interactions of (2-b) Azithromycin-motilin receptor.

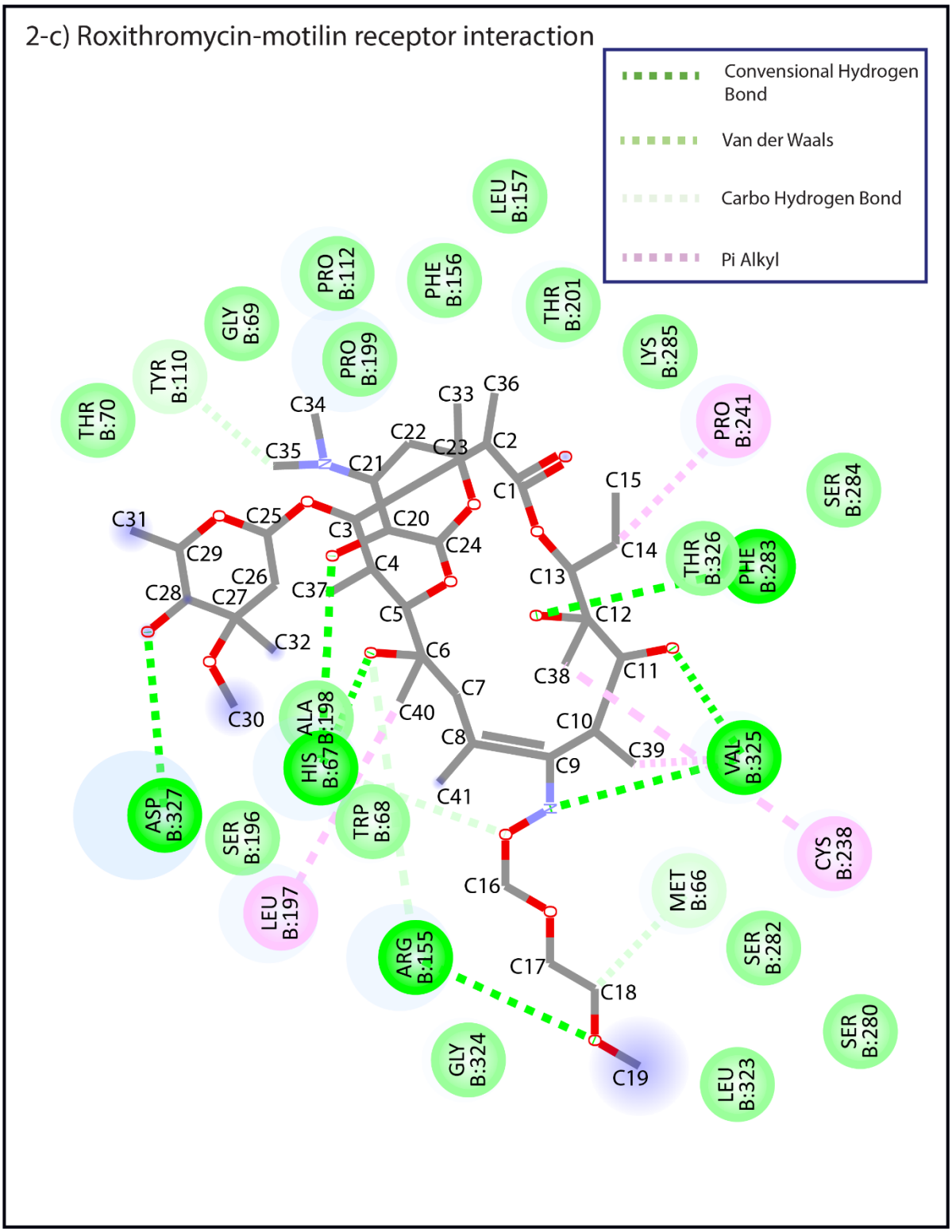


Figure S12: Two dimensional interactions of (2-b) Roxithromycin-motilin receptor.

The docking analysis of erythromycin, azithromycin and roxithromycin with motilin receptor revealed distinct binding affinity and interactions. From the docking result of erythromycin and motilin receptor, it is observed that the carbon atoms present in the position of C27 and C28 are

responsible for key interactions between the erythromycin and residues of different amino acids present in motilin receptor through alkyl bonds and carbohydrate bonds. Additionally, oxygen atoms attached to the carbon atoms at positions C34, C11 and C12 are found to form conventional hydrogen bonds with receptor residues. In case of azithromycin- motilin receptor complex, it is found that the carbon atoms present at the positions C14, C21, C17, C20, C28, C36, C37 are involved in interaction with residues of motilin receptor by means of alkyl bonds and carbohydrate bonds, where the oxygen atoms attached to carbon atom at positions C6, C12, C34 are participated in conventional hydrogen bonding interactions with the receptor. Similarly, from the roxithromycin-motilin receptor interaction it is observed that, the specific carbon atoms at positions C14, C40, C41, C39, C38, C35, C18 interacts to motilin receptor by $\pi -$ alkyl bonds and carbohydrate bonds, while the oxygen atoms and nitrogen atoms attached to the carbon atoms at positions C6, C28, C20, C11, C12, C18 and C16 respectively form conventional hydrogen bonds with the motilin receptor residues. The presence of many van der waals interactions are also shown in Figure S10, Figure S11 and Figure S12 in each ligand-target complexes. In conclusion, it is analysed that among the three macrolide antibiotics, the derivatives of erythromycin such as roxithromycin and azithromycin interacts more with motilin receptor as a target with a greater binding affinity. This finding implies that roxithromycin and azithromycin may have a more pronounced biological effect on motilin receptor activity and consequently on gastrointestinal motility compared to erythromycin itself.

References:

1. Broad, J., & Sanger, G. J. (2013). The antibiotic azithromycin is a motilin receptor agonist in human stomach: comparison with erythromycin. *British journal of pharmacology*, *168*(8), 1859-1867.
2. SALAT, P.,& PARIKH, V. (1999). Motilin receptor agonists as novel gastrointestinal prokinetic agents. *Indian Journal of Pharmacology*, *31*(5), 333-339.
3. Faghih, R., Nellans, H. N., & Plattner, J. J. (1998). Motilides and motilactides: design and development of motilin receptor agonists as a new class of gastrointestinal prokinetic drugs. *Drugs of the Future*, *23*(8), 861-872.