[PHAR02] Analysis of functional groups in the binding of erythromycin A and its derivatives by molecular docking technique

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Introduction

Erythromycin A is an old drug of macrolide antibiotic. It is a main fermentation product of *Streptomyces erythreus*, and represents the first generation of a 14-membered macrolactone ring namely erythronolide A (Omura, 2002). Two sugar moieties, are attached to this ring, cladinose and desosamine at position C3 and C5 respectively. The fermentation process that mainly produces erythromycin A also produces small quantities of erythromycin B, C, D, E and F, (Kanfer *et. al.*, 1998; Omura, 2002) as well as 3-mycarosyl erythronolide B also been produced. In acidic environment especially in the stomach, the erythromycin A is degraded into erythromycin A anhydrate and 5-desosaminyl erythronolide A. In case of erythromycin B, it is degraded into 5-desosaminyl erythronolide B (Omura, 2002).

Erythromycin exhibited unique and superior biological properties *in vivo* and *in vitro*, such as its activity, safety, and mode of action in inhibiting selective bacterial protein synthesis (Omura, 2002). However, erythromycin showed several disadvantages, such as instability in acidic environment, weak activity against Gram-negative bacteria, induction of macrolide resistance, a bitter taste and showed a low serum concentration. These have been the major problems to be conquered by chemical modification. Various semi synthetic have been synthesized as derivatives to improve their acid stability, intestinal absorption, and serum concentration. The new semi synthetic derivatives of erythromycin A are clarithromycin, roxithromycin and azithromycin. These include the second generation of semi synthetic macrolide antibiotic namely ketolide which is telithromycin (HMR-3467) and cethromycin (ABT-773).

The macrolide antibiotic exerts their antibacterial activity by preventing bacterial protein biosynthesis by binding to the 50S subunit (Mazzei *et. al.*, 1993). A molecular docking employing AutoDock version 3.0 was used to study and analyse the interaction between various derivatives of erythromycin A and their target macromolecule. The motivation of AutoDock is to provide a computational tool to assist researchers in the determination of bio-molecular complexes. In addition, the AutoDock program package can predict the binding energy and inhibition value of each derivative. In order to compute and compare the energy levels, AutoDock utilizes three programs namely Autotors, Autogrid and AutoDock. Autotors facilitates the input of ligand coordinates and definition of rotatable bonds, AutoGrid calculates a three dimensional grid of interaction energy based on macromolecular coordinates, and AutoDock performs the docking simulation.

Methods

Preparation of macromolecule

The crystal structure of 50S subunit of *Deinococcus radiodurans* in complex with erythromycin A (PDB entry 1JZY) was retrieved from the Brookhaven Protein Databank (PDB). In order to calculate the binding energy with automated docking, all water molecules, ions, and inhibitor need to be removed from the original PDB file. The original file was separated into two files, one containing the 50S subunit, while the other consists of the ligand. All bonds were modified automatically and all missing hydrogen atoms were added using Biopolymer module in InsightII molecular modeling program (Accelrys, USA). The partial atomic charges were assigned using Consistent Valence Force Field (CVFF). Lastly, the atomic fragmental volume and the atomic solvation parameters for every atom in the 50S subunit of *Deinococcus radiodurans* was assigned using Addsol program.

Ligand setup

Twelve ligands from a class of macrolide antibiotics were studied i.e erythromycin A, B and C, clarithromycin, roxithromycin, azithromycin, telithromycin, cetromycin, 3-mycarosyl erythronolide B, 5-desosaminyl erythronolide A, 5-desosaminyl erythronolide B and erythromycin A anhydrate. The PDB code for clarithromycin, azithromycin, roxithromycin, telithromycin (HMR 3647) and cethromycin (ABT 773) were 1JZY, 1J5A, 1NWY, 1JZZ, 1P9X, and 1NWX, respectively. Structures of other ligands were modified and generated using Builder. All bonds were modified automatically and hydrogen atoms were added to all atoms in the ligand to correct their valences. The setup followed by assigning partial charges using CVFF and the resulting structures were optimized using Discover module in InsightII. Finally, Autotors, a utility in AutoDock was used to define rotatable torsion angles for each ligand.

Docking Simulation

In order to run AutoDock, grid maps have to be calculated using AutoGrid. For consistency, all receptor-ligand interactions were prepared using the same parameters; (i) number of grid points were set to 90 Å × 90 Å × 90 Å in x, y and z dimension, (ii) spacing between grid points was set to the default value of 0.375 Å, and (iii) a grid center was chosen slightly off the center axis of the crystal structure coordinates of erythromycin A. In this study, the Lamarckian Genetic Algorithm (LGA) was selected to identify the binding conformations of the macrolide inhibitors. The step size was set to 0.2Å for translation and 5° for orientation and torsion. The other important parameters for LGA calculations were reasonably set; (i) an initial population of random individuals with a size of 50; (ii) a maximum number of 1.5×10^6 energy evaluations; (iii) a maximum number of generations of 27000; (iv) an elitism value of 1 for surviving the step into the next generation; (v) a mutation rate of 0.02, which was the probability that a gene would undergo a random change; and (vi) a crossover rate of 0.80, which was the probability proportional selection. The pseudo-Solis and Wets local search method was applied, having a maximum of 300 iterations per local search;

the probability of performing local search on an individual in the population was 0.06; the maximum number of successes or failures was 4, in both cases; and the termination criterion for the local search, was 0.01.

Results and Discussion

Interacting model with ribosomal inhibitor's conformation

The AutoDock predicted conformation of 50S subunit with erythromycin A complex is shown in Figure 1, superimposed with the X-ray crystallographic conformation structure. The root mean square deviation (RMSD) between the two conformations is ~1.928Å. This indicates that the parameter set for running AutoDock simulation is reasonable to reproduce the X-ray structure. Thus, it could be extended to search the binding conformations for other macrolide. Figure 2 represents the 3D model of twelve inhibitors of macrolide extracted from the AutoDock results. Although the macrolides differ slightly in their structures, their binding location is composed at the same segment of 50S subunit and is consistent with other findings (Garza-Ramos *et. al.*, 2001; Schlunzen *et al.*, 2001).
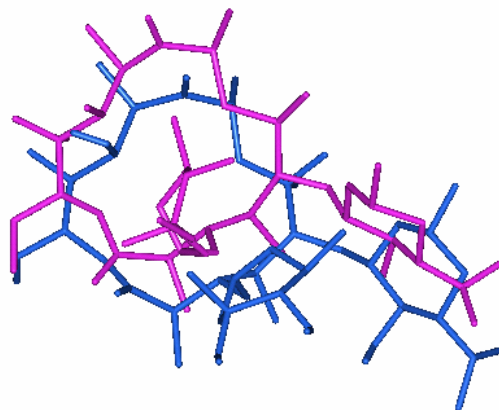

FIGURE 1 Conformational comparison of erythromycin A from the crystal structure (blue) and that from the AutoDock result (purple), produced by the InsightII program.

Hydrogen interaction

Hydrogen bonding is one important characteristic of the interaction between the ligands and macromolecular target. By definition, hydrogen bonds are formed between two electronegative atoms sharing a
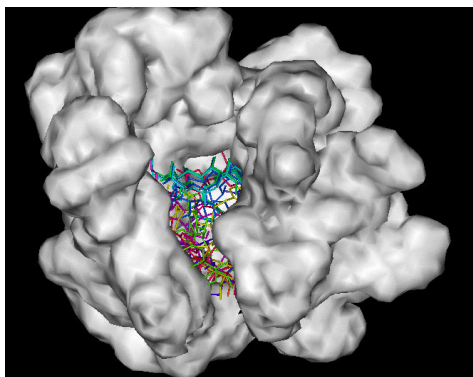
FIGURE 2 Three dimensional structural model of twelve macrolide antibiotics with 50S subunit of *Deinococcus radiodurans* complex, rendered by the InsightII.

proton between them, where one of the participants is the donor and the other is acceptor of the proton (Pauling, 1960). In order to define hydrogen bond, a superset of possible hydrogen bonds is assigned based on the following geometric (McDonald and Thorton, 1983; Stickle *et. al.*, 1992) criteria; the donor–acceptor distance, $d \leq 3.6$ Å, the hydrogen-acceptor distance, $r \leq 2.6$ Å, and the donor–hydrogen-acceptor angle, θ, falls between 90° and 180° (Fig. 3).
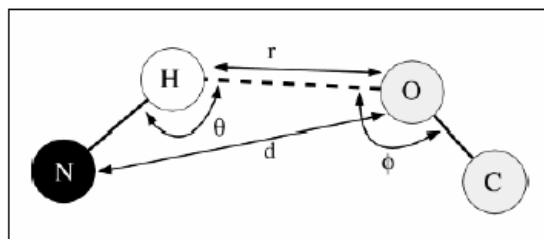

FIGURE 3 Geometry used in the hydrogen bond energy potential. θ is the donor–hydrogen–acceptor angle; Φ is the hydrogen–acceptor–base angle, where base is the atom (C, in this case) covalently bonded to the acceptor; d is the donor–acceptor distance; r is the hydrogen–acceptor distance; and φ (not shown) is the angle between the normal of the planes defined by the covalent bonds of the donor and base atoms (e.g., the planes defined by the two sp2 centers, N and C, in this case). (Figure is taken from Jacobs *et. al.*, 2001).

Figure 4 generally shows the interacting model of twelve ligands with ribosomal subunit. The reactive groups of the macrolactone ring and cladinose sugar mediate the hydrogen-bond interactions of erythromycin A and its derivatives with the ribosomal target. The ligand with the most

hydrogen bonds appeared to be Erythromycin C (4bonds), roxithromycin, telithromycin and cethromycin (3 bonds) and followed by erythromycin A and B, clarithromycin, 3-mycarosyl erythronolide B, 5desosaminyl erythronolide A and B (2bonds) and azithromycin (1bond). Erythromycin A anhydrate, inactive analogoue of erythromycin A, do not involve in this interaction.

The desosamine sugar was not involved in hydrogen bond interactions with the 50S subunit, however the cladinose and mycarose appears to form hydrogen bonds mainly with two bases, guanine of A2484 and urasil of A2485, respectively, except for azithromycin, telithromycin, cethromycin and erythromycin A anhydrate. Telithromycin and cethromycin do not have any sugar molecule molecule at C-3 position in the erythronolide ring, while azithromycin has a ring expansion. With respect to erythromycin A anhydrate, the erythronolide ring has a major modification due to the internal bridges between C6-C9 and C9-C12.

The bases of A2484 and A2485 positions are targeted by the sugar moiety of the macrolides. The importance of A2484 nucleotide position has been established previously. The A2484 was shown to be protected from chemical modification on macrolide binding (Moazed and Noller, 1987; Rodriguez-Fonseca *et. al.*, 1995) , no mutation on A2484 conferring resistance to these antibiotics has been reported (Schlunzen *et. al.*, 2001). In addition, A2484 is also one of the nucleotides protected on binding of peptidyl-tRNA and the identify of this nucleotide is important for inhibiting protein synthesis. It is believed that cladinose and mycarose group plays important criteria when designing a new derivative. The semi synthetic analogues showed additional hydrogen bonding via the erythronolide ring. This may be the important reason why the semi synthetic derivative analogue could shows a better activity against Gram-positive bacteria compared with erythromycin A.

Hydrophobic Interactions

Hydrophobic interaction is another important characteristic of the interaction between the macrolide and ribosomal subunit. Hydrophobic interaction is defined as a cluster of non-polar groups given water removed from the volume of the cluster (Torshin, 1999)
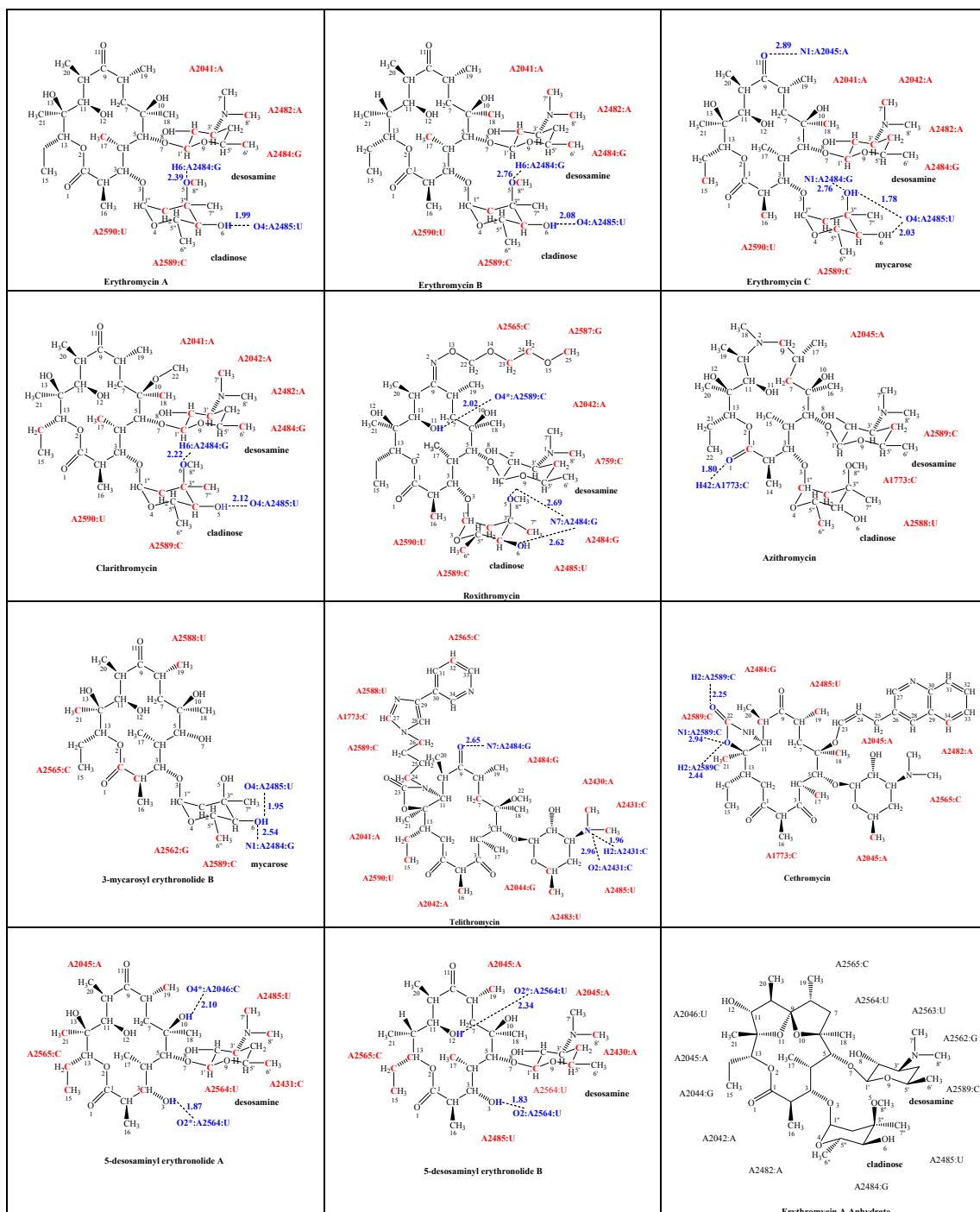
FIGURE 4 Two dimensional representation for the interacting mode of macrolide compound with the active site of 50S subunit of *Deinococcus radiodurans*. Residues and atoms in blue and red are involved in hydrogen bonding and hydrophobic interaction, respectively. Black dotted lines are the hydrogen bond distances in angstrom.

and was determined as carbon-carbon interactions within a distance ≤4.0 Å (Mandel-Gutfreund and Mangalit, 1998; Nobeli *et. al.*, 2000). There are several hydrophobic interactions were observed between macrolide compounds and ribosomal target as depicted in Figure 4. In all ligand, telithromycin appeared to have the most hydrophobic interaction with 13 residues, followed by roxithromycin (8 residues), cethromycin (7 residues), erythromycin C, clarithromycin and 5-desosaminyl erythronolide B (6 residues),

erythromycin A and B and 5-desosaminyl erythronolide A (5 residues) and azithromycin and 3-mycarosyl erythronolide B (4 residues).

Erythromycin A anhydrate do not posses any hydrophobic interaction with the macromolecular target. Hydrophobic interaction was observed between urasil A2590 and the erythronolide A ring in all the active analogues except in azithromycin and 3-mycarosyl eythronolide B. With respect to hydrophobic interactions, sugars of the active analogues interactions mainly are due to the bases of adenine of A2482 and guanine of A2484.

Conclusion

This study has shown that majority of hydrogen and hydrophobic interactions involved between the sugar molecules and the bases of A2482, A2484, A2485 and A2590. In order to design a better analogue, new derivatives should have a bioisostere that could produced more interactions with the 50S subunit target molecule.

Acknowledgements

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