Binding Studies of Erythromycin A and its Analogues using Molecular Docking Technique

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ABSTRACT: Interaction of twelve erythromycin A analogues with 50S ribosomal subunit were studied employing AutoDock 3.0.5. Results showed that all active macrolides bound at the same binding site with erythromycin A in contrast to the inactive analogues which bound at location slightly different than erythromycin A. The binding site showed consistency with the X-ray data from the perspectives of hydrogen bonding and hydrophobic interactions formed by erythromycins, roxithromycin, azithromycin, cethromycin and telithromycin with the ribosome. The inactive derivatives of erythromycin A anhydride showed higher binding free energy, while 5-desosaminyl erythronolides A and B even though having quiet similar values of binding free energy with the active analogues, docked at binding sites which are quiet different than the active analogues. These results suggest the molecular docking technique can be used in predicting the binding of erythromycin A analogues to their ribosomal target.

1 INTRODUCION

Erythromycin A is a macrolide antibiotic that was discovered in 1952 from the fermentation of *Streptomyces erythreus* [1]. It binds to 50S bacterial ribosome and can be used to combat bacterial resistant by inhibiting the elongation step of protein biosynthesis [2, 3]. It contains cladinose and desosamine sugar moieties at positions C3 and C5 of the macrolactone ring. Beside erythromycin A, the fermentation broth also produced small quantities of erythromycins B,C,D,E and F [1,2].

The major disadvantages of erythromycin A is its extreme acid sensitivity which leads to degradation in the stomach, poor absorption after oral administration and short serum half-life time. Erythromycin A is degraded into erythromycin A anhydride, 5-desosaminyl erythronolide B and erythromycin A enol ether [2,3,4,5,]. Erythromycin B is degraded into 5-desosaminyl erythronolide B and erythromycin B enol ether [6]. Since Kurath and co-workers [7] reported the acid degradation pathway of erythromycin A, the roles of the functional groups of it macrolactone ring and its sugar moieties have become important subjects of study.

Modification of the macrolide structure has been shown to affect the quantitative activity and, in particular, in *in vitro* and *in vivo* activities against bacterial pathogens. The modification involves the sugar moieties and the macrolactone ring. With respect to the sugar moieties, the presence of desosamine and cladinose is essential for retaining the antibacterial activity but later has been revised [1,2]. As for the lactone ring, the attention has been focused mainly to functional groups attached to C6, C8, C9, C11 and C12 which could to be modified. An expansion of the macrolactone ring has also been carried out, that results is the design and synthesis of azithromycin, which is formed by the inclusion of a methyl-substitued nitrogen at position C9. Semi synthetic analogues of erythromycin A, i.e. the ketolides, have remarkable antibacterial activity against macrolide-resistant strains. The ketolides, interesting to note in the structure that the cladinose moiety is replaced by a keto function, even though this sugar was thought to be essential for bacterial activity.

Macrolides bind to 50S bacterial ribosome and can be used to combat bacteria by inhibiting the elongation of nascent peptide chain during the protein biosynthesis [8,9]. The interaction studies between antibiotic and ribosome complex using experimental data have been reported, but the interaction study of macrolide-ribosome particularly erythromycin A at atomic and molecular level using molecular docking technique is not available. In this study, AutoDock3.0 was used to investigate the interaction of erythromycin A analogues at the 50S ribosomal subunit from *Deinococcus radiodurans.*

2 METHODS

The crystal structure of 50S ribosomal subunit of D. radiodurans in complex with erythromycin A was retrieved from the Brookhaven Data Bank (PDB; entry code 1JZY). All water molecules, ions, and inhibitor were removed and missing hydrogen atoms were added using Builder module in InsightII (Accelrys Inc., USA). The partial atomic charges were assigned using Consistent Valence Force Field (CVFF) and the atomic fragmental volume and the atomic solvation parameters for macromolecule was assigned using Addsol. The structure of the various analogues were modified and generated using Builder module in InsightII. Only the structure of erythromycin A anhydride was generated using HyperChem® molecular modelling package (Hypercube Inc., 2002). All the missing hydrogen atoms were added and of the ligand was then geometry optimized using Discover module in InsightII. An appropriate partial atomic charges were assigned using CVFF. Autotors was used to define rotatable torsion angles for each ligand.

The AutoDock programme package [8-14] was used to dock various erythromycin A analogues to their ribosomal target. The grid maps for each atom type in the ligands studied here were calculated using AutoGrid 3.0 with the

number of grid points set to 90 Å × 90 Å × 90 Å in x, y and z dimension and the spacing between grid points was set to 0.375 Å. The grid centre was set up at the centre axis of the crystal structure coordinates of erythromycin A. The Lamarckian Genetic Algorithm (LGA) was used to identify the binding conformations of the macrolide inhibitors. Important parameters for LGA calculations were reasonably set up to an initial population of random individuals with a population size of 50 individuals, a maximum number of 1,500,000 energy evaluations and a maximum number of generations of 27000, which was the set up number to terminate the docking simulation. An elitism value was set at 1, which was the number of top individuals that are guaranteed to survive into the next generation. The probability that a particular "gene" would undergo crossover and mutation was set to 0.80 and 0.02, respectively. A proportional selection was used, where the average of the worst energy in the current population was calculated over a window of the previous 10 generations.

3 RESULT AND DISCUSSION

Docking of erythromycin A to *D. radiodurans*' 50S ribosomal subunit showed that the ligand bound within the same binding site and in almost the same binding mode as the crystallographic conformation (Figure 1) with a root mean square deviation, rmsd of 1.9 Å. This value is less than 3.0 Å, which is considered acceptable for docking [15-19]. This also implied that Autodock is able to reproduce crystallography data with acceptable level of accuracy.

Table 1 shows the estimated free energy of binding (ΔG) and the predicted inhibition constant of the highly populated cluster for erythromycin A and its analogues. ΔG of erythromycin A was -9.16kcal/mol. The estimated binding free energy obtained from the binding of erythromycins B and C with the ribosome were -9.10 and -9.49 Kcal/mol, respectively. This indicates that both erythromycins B and C have similar activity to erythromycin A. This is not surprising as the results also show that erythromycins B and C also bound at the same binding site as erythromycin A (Figure 2).

Figure 2 also illustrates the binding of erythromycin A and its analogues with *D. radiodurans* 50S ribosomal subunit. Consistent to the experimental observation [20-22], it was also observed in this study that the active analogues of erythromycins B and C, the semi synthetics and the ketolides bind at the same binding region of erythromycin A. However, even though ΔG of the degradation products (5-desosaminyl erythronolide A and 5-desosaminyl erythronolide B), are more negative than erythromycin A, these compounds bound at a distance further away from the binding site of erythromycin A. Another analogue, erythromycin A anhydride did not bind at the ribosome. It is worth noting here that ΔG of erythromycin A anhydride was +11.3 kcal/mol thus supporting the evidence that this molecule is not active against bacteria [23].

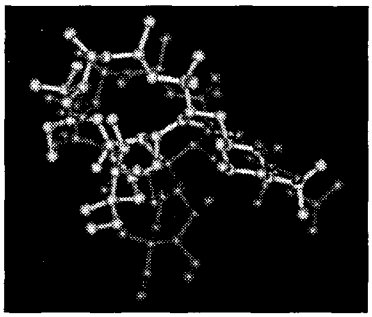


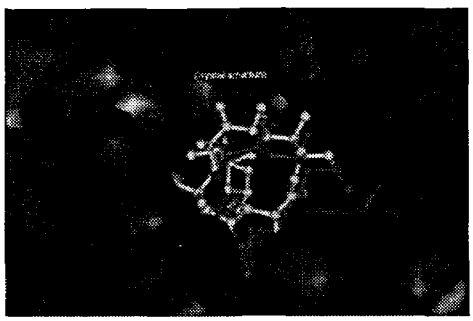
Figure 1: Binding model for erythromycin and ribosome complex. The crystal structure (from X-ray) coloured in yellow and docked conformation (from AutoDock) in red. Both conformations rendered as ball and stick.

The estimated binding free energies (Table 1) for the semi-synthetic analogues when compared to free energy of erythromycin A could be ranked as roxithromycin < azithromycin < clarithromycin < 3-mycarosyl erythronolide B < erythromycin A. This suggests that the semi synthetic analogues fit the active site more tightly than erythromycin A, which is also support the finding that these compounds have better antibacterial activity than erythromycin A [1,2,6].

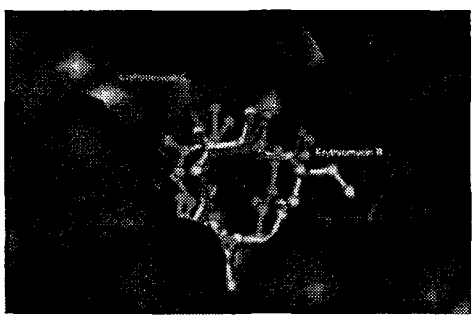
The ketolides generally showed more negative lowest free energy of binding compared with erythromycin A and the other analogues. ΔG for telithromycin is -14.76 kcal/mol, while the ΔG calculated for cethromycin is -11.10 kcal/mol. Thus, telithromycin would be expected to form a more stable complex with ribosome compared to the complex formed by erythromycin A and other analogues. This also supports the claims that the ketolides are generally more effective antibiotics than erythromycin A and the semi-synthetic analogues.

Hydrogen bonding and hydrophobic interactions made by the ligands and their targets were also investigated in this study. Figure 3 shows the schematic representation of erythromycins A B and C at the ribosomal target. The docked conformation of erythromycins A and B, mediated the hydrogen bonds through cladinose sugar, while erythromycin C through its mycarose sugar. The C9-ketone of erythromycin C forms hydrogen bonding with N1 of A2045. Residue A2045 involved in hydrogen bonding was consistent with the X-ray data [21]. The hydrophobic interactions appeared at the macrolactone ring and all sugar moieties such as desosamine, cladinose and mycarose.

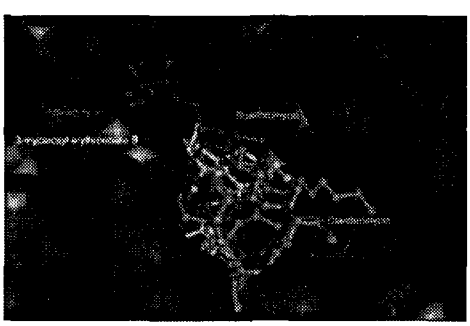
The hydrogen bond and hydrophobic interactions of semi synthetic analogues of erythromycin A are shown in Figure 4. Docking results showed that the desosamine sugar was not involved in hydrogen bond interactions with the ribosomal target. Clarithromycin, roxithromycin and 3-mycarosyl erythronolide B form hydrogen bonds through cladinose and mycarose sugars mainly with two bases, G2484 and U2485. The 11-OH of roxithromycin forms hydrogen bonding with O4 of C2589. However, the X-ray data showed that 11-OH interacts through hydrogen bonding with U2588 [21]. The C1-keto group forms hydrogen bonding that was only observed in azithromycin.



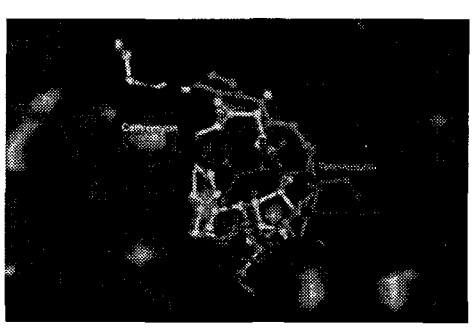
(a) Crystal and docked conformations of erythromycin A.



(b) Docked conformations of erythromycins A, B and C

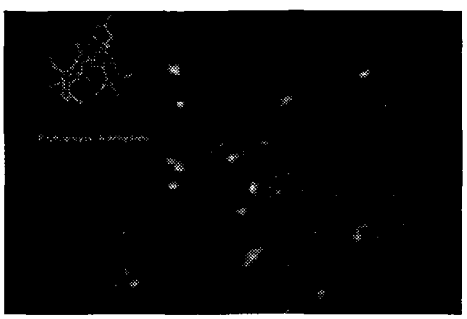


(c) Docked conformations of erythromycin A and its semi synthetic analogues of clarithromycin, roxithromycin, azithromycin and 3-mycarosyl erythronolide B



(d) Docked conformations of erythromycin A and the ketolide compounds of telithromycin and cethromycin

(e) Docked conformations of erythromycin A, 5-desosaminyl erythronolide A, 5-desosaminyl erythronolide B



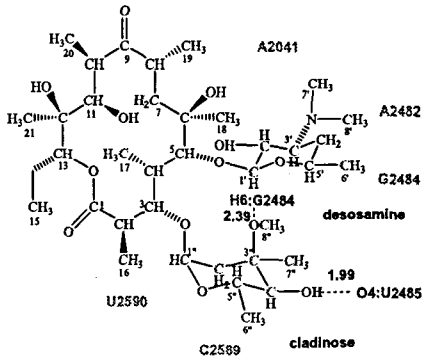
(f) Docked conformations of erythromycin A and erythromycin A anhydride

Figure 2: Surface representation of binding models for macrolides-ribosome complex at the 50S ribosomal subunit. Ligands are rendered as ball and stick.

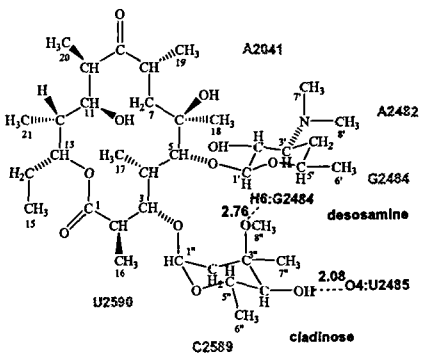
Ligand	Number of atoms	Number of torsions	EFEB (Kcal/mol)
Erythromycin A	118	12	-9.16
Erythromycin B	117	11	-9.10
Erythromycin C	115	12	-9.49
Clarithromycin	121	12	-9.41
Roxithromycin	136	19	-10.48
Azithromycin	124	12	-9.90
3-mycarosyl erythronolide B	91	9	-8.93
Telithromycin	123	12	-14.76
Cethromycin	114	10	-11.10
5-desosaminyl erythronolide A	95	9	-10.33
5-desosaminyl erythronolide B	93	8	-10.33
Erythromycin A anhydride	115	10	+11.30

EFEB = Estimated free energy of binding

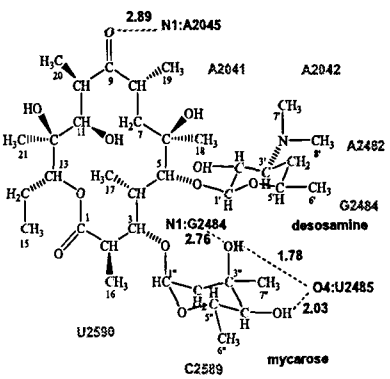
Table 1: Comparison of estimated free energy of binding, ΔG of the various erythromycin A analogues.



(a) Erythromycin A



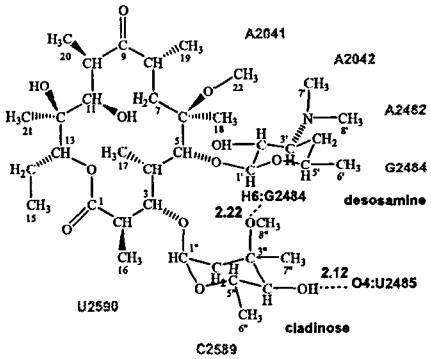
(b) Erythromycin B



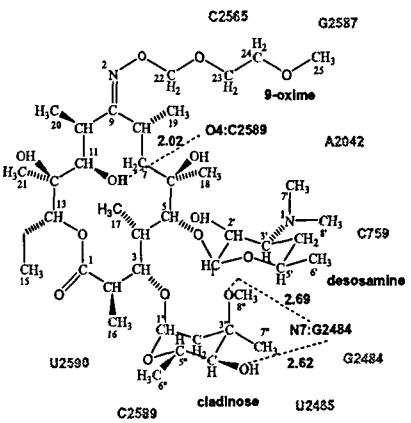
(c) Erythromycin C

Figure 3: Two-dimensional of the interacting mode of erythromycins A, B and C at the 50S of *D.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.

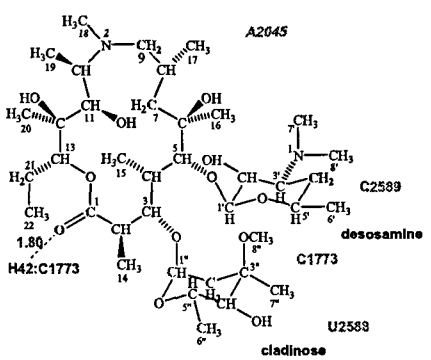
With respect to the hydrophobic interaction, the macrolactone ring, sugars and 9-oxime chain formed a network of hydrophobic interactions with the ribosomal target. Azithromycin was shown to interact with A2045 and U2588 via hydrophobic interactions and it was in agreement with X-ray data [22]. In addition, both crystal and docking data observed that the nitrogen atom at the macrolactone ring does not involve in the interaction with the ribosomal target.



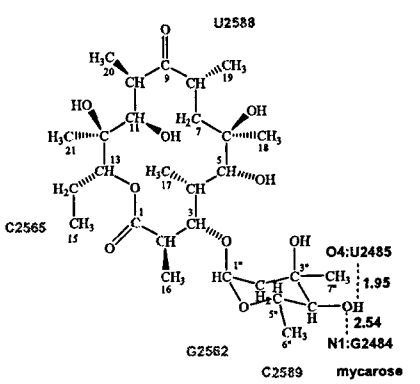
(a) Clarithromycin



(b) Roxithromycin



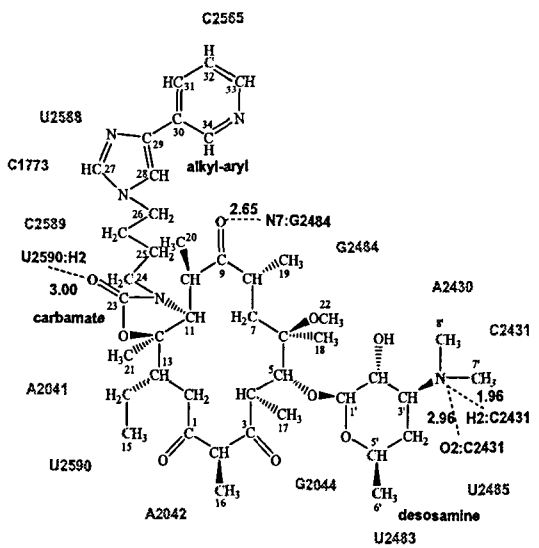
(c) Azithromycin



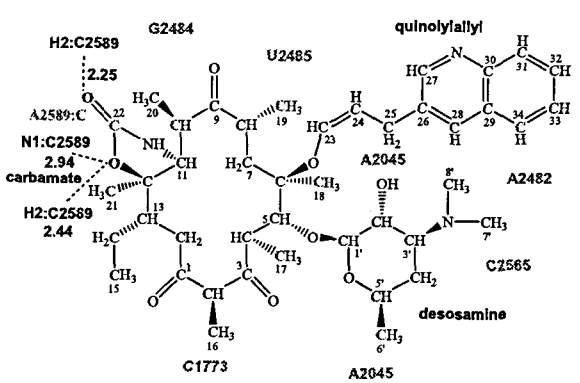
(d) 3-mycarosyl erythronolide B

Figure 4: Two dimensional of the interacting mode of semi synthetic analogues at the 50S of *D.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.

Figure 5 illustrates the interaction of telithromycin and cethromycin at the 50S ribosomal target. Telithromycin docked conformation forms four hydrogen bonds through desosamine, carbamate and 9-keto groups. While, twelve carbon atoms of its macrolactone, desosamine and alkyl-aryl groups are involved in hydrophobic interactions with residues of C1773, A2041, A2042, G2044, A2430, C2431, U2483, G2484, U2485, C2563, U2588, C2589. Cethromycin forms three hydrogen bonds and hydrophobic interactions through its carbamate group with C2589 and the results are slightly different from X-ray data as U2588 contributes to hydrogen and hydrophobic interactions [22]. Ligand with the lowest free energy of binding was scored by ketolide group with telithromycin has ΔG of -14.76 Kcal/mol, while cethromycin scored ΔG of -11.10 kcal/mol. Thus, telithromycin can be expected to form more stable complex with ribosome compared to the complex formed by erythromycin A and other analogues.



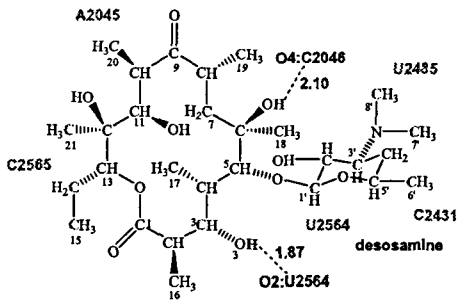
(a) Telithromycin



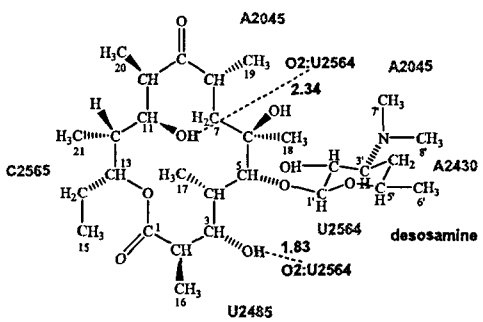
(b) Cethromycin

Figure 5: Two-dimensional of the interacting mode of second generation of semi synthetic analogues of erythromycin A at the 50S of *D.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.

The interaction of 5-desosaminyl erythronolide A and 5-desosaminyl erythronolide B are shown in Figure 6. The replacement of cladinose sugar with hydroxyl group make these compounds bind quite far from the binding site of erythromycin A (refer Figure 2). Both ligands form hydrogen bonding through the hydroxyl group on macrolactone ring mostly with the residue of U2564 and hydrophobic interactions through the macrolactone ring and desosamine sugar.



(a) 5-desosaminyl erythronolide A



(b) 5-desosaminyl erythronolide B

Figure 6: Two dimensional of the interacting mode of degradation products of erythromycins A and B at the 50S of *D.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.

4 CONCLUSION

The results from these studies have shown that the docking simulations not only agree with the experimental observations but are also able to generate insight as to how macrolide antibiotics might works at an atomic level. The present study provides a clear view as to how erythromycin A and its analogues interact with the bacterial ribosome. With the increased understanding of the mechanism of action of the antibiotics, so provided it could be hoped that more drugs might be designed to combat bacterial infections.

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