MOLECULAR DOCKING OF Δ^6-ANHYDROERYTHROMYCIN TO rRNA 23S *Deinococcus radiodurans* AND THE PREDICTION OF ITS ANTIBIOTIC POTENCY

Winarto Haryadi[1*], Umar Anggara Jenie[2], Retno Sunarminingsih Sudibyo[2], Harno Dwi Pranowo[1], and Fajar Rakhman Wibowo[3]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Gadjah Mada University, Yogyakarta*

[2]*Faculty of Pharmacy, Gadjah Mada University, Yogyakarta*

[3]*Departmen of Chemistry, Faculty of Mathematics and Natural Sciences, Sebelas Maret State University, Surakarta*

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ABSTRACT

Δ^6-anhidroeritromisin-A is a new derivative of erythromycin which is synthesized through biosynthetic engineering technique. The molecular docking in rRNA 23S Deinoccocus radiodurans are accomplished to determine the model and strength of binding to the target macromolecule. The molecular docking of erythromycin-A and 6-deoksieritromisin-A to the same macromolecule is used as a control. The docking result of the Δ^6-anhidroeritromisin-A shows that it occupies the same cavity as of the experimental erythromycin-A in the same macromolecule. The binding position of Δ^6-anhidroeritromisin-A is not exactly same as erythromycin-A and 6-deoksieritromisin-A due to the presence of Δ^6 unsaturated double bond. However the hydroxyl group(OH) at C-6 does not have an apparent effect on the binding model to rRNA23S D. radiodurans.

Keywords: *Δ^6-anhidroeritromisin-A, rRNA 23S D. radiodurans, molecular docking, antibiotic potency*

INTRODUCTION

Erythromycin is a broad spectrum macrolide antibiotic. This compound can be used to substitute penicillin because of the resistence of penicillin to microbes and its sensitivity to some patients. The disadvantage of the usage of erythromycin is the instability in stomach acid [1] omura. The instability is caused by internal nucleophilic attack of hydroxyl group(C-6) to protonated carbonyl (C-9) in macrolide ring [2]. This reaction causes decomposition of erythromycin and lack of its activity. The decomposition can be prevented by structural modifications, because these modifications can prevent the internal nucleophilic attack. Some erythromycin derivative antibiotics produced by structural modification are clarithromycin, roxythromycin and azythromycin [3].

Macrolide antibiotics able to inhibit the elongation of oligopeptide synthesis in the cavity of rRNA 23S named peptide exit tunnel [4]. The complex structure of macrolide-rRNA 23S *D. radiodurans* has been found through X-ray crystallography method [5]. Active site of some macrolides (erythromycin-A, roxythromycin and chlarythromycin) in macromolecule rRNA 23S was obtained from X-ray crystallography data [5]. The molecular docking of these molecules are accomplished to determine the mode and strength of binding to rRNA 23S. As references, molecular docking of chlarythromycin, roxythromycin, 6-deoxyerithromycin-A,

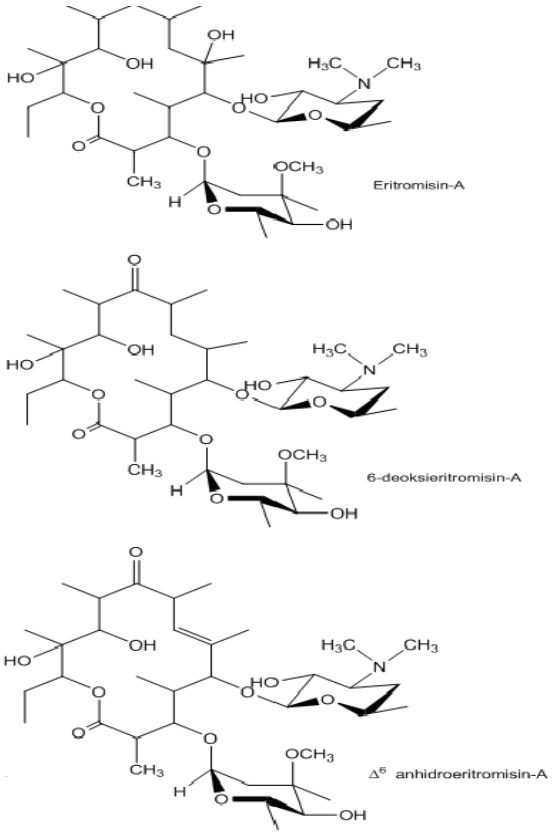

Figure 1. Molecular structure of Erythromycin-A, 6-deoksieritromisin-A, and Δ^6-anhidroeritromisin-A

* Corresponding author. Tel/Fax : +62-274-545188
Email address : wnrt_ haryadi@yahoo.com

spiramycin and chloramphenicol have also been conducted to the active site of erythromisin to rRNA 23S *D. radiodurans.*

Δ^6-anhidroeritromisin-A is synthesized using biosynthetic engineering technique. This technique comprises of addition of isonizide into *Sac. erythraea* fermentation [6-9]. The Δ^6-anhidroerythromycin-A molecule itself differs from of erythromycin-A due to the presence of Δ^6 double bond and unavailability of hydroxyl molecule (OH) at C-6 in erytronoid ring. The unavailability of hydroxyl molecule (OH) at C-6 is accustomed to increase its stability in acidic solution due to the hydroxyl molecule (OH) at C-6 which is an initiator of erythromycin decomposition [1]. The Δ^6-anhidroeritromisin-A molecule is experimentally proven to be stable and active until pH 3 [6].

The molecular docking of Δ^6-anhidroerythromycin-A against rRNA 23S is accomplished to determine the model and the strength of binding to rRNA 23S *D. radiodurans* macromolecule. In order to evaluate the effect of Δ^6 double bond existence and unavailability of hydroxyl molecule (OH) at C-6 in Δ^6-anhidroeritromisin-A molecule, a comparison model using erythromycin-A and 6-deoxyerythromycin-A molecules were experimented to evaluate the docking model. The binding positions of these molecules resulting from the docking are compared with the site of attachment of erythromycin-A produced by X-ray crystallography based on RMSD (root mean square deviation) calculations. The strength of binding of these molecules from the docking to rRNA 23S macromolecule could be computated based on the free energy of bonding.

EXPERIMENTAL SECTION

Material

Software which is used in this research are: CentOS, Molden, Gaussian98, Chimera, Rasmol and Autodock Tools version 4.01., Protein data bank site (PDB) www.RCSB.org and other sites which are relevant.

Procedure

The complex structure of erytromycin-A with rRNA 23S *Deinococcus radiodurans* macromolecule is obtained through Protein Data Bank searching via www.RCSB.org. The erythromycin-A structure is seperated from antibiotic macromolecule with the use of CHIMERA programme and saved in PDB format. The structure of Δ^6anhidroeritromisin-A and 6-deoksieritromisin-A are drawn using MOLDEN programme and optimalized with *ab initio* method using Gaussian98 software. The docking of erythromycin-A, Δ^6anhidroeritromisin-A and 6-deoxyerythromycin-A to rRNA 23S macromolecule using Autodock version 4.0. The docking parameters used in this research are *Lamarckian Genetic Algorithm*, *grid box* 60 and total energy evaluation amounted to 2,5 x 10^8. The *Genetic Algoritm* parameter amongst others are 150 initial population, 27.000 maximum generation, cross over rate of 0.8, and mutation rate of 0.02. Local search Solis parameter and Wet including 300 iterations of local search, consecutive successes before changing and 4 consecutive failures before changing.

RESULT AND DISCUSSION

The docking model of Δ^6-anhidroerythromycin-A, erythromycin-A and 6 deoxyerythromycin-A molecules are executed ten times at the active site of 23S macromolecule by X-ray crystallography [5]. The molecules' relative position of RMSD resulting from docking into erythromycin-A (X-ray) and free energy of binding are shown in table 1.

Based on RMSD calculations, erythromycin-A result of 8th docking(5,83 Å), 6-deoxyerythromycin-A

Table 1. RMSD and free energy of binding through docking of erythromycin-A, Δ^6-anhidroerytromycin-A and $\tilde{6}$deoxyerythromycin-A.

Docking number (ligand)	erytromycin -A	6-deoxy-erytromycin-A	Δ^6–anhidro-erytromycin -A
	RMSD (Å)	RMSD (Å)	RMSD (Å)
1	8.30	8.25	6.25
2	8.85	9.06	7.85
3	8.27	8.24	7.85
4	8.25	**5.98**	7.35
5	8.85	10.37	**5.87**
6	7.68	8.60	8.20
7	6.92	8.59	8.38
8	**5.83**	8.25	9.92
9	8.84	10.38	9.70
10	8.24	10.36	8.20

Table 2. RMSD of Δ^6-anhidroerythromycin-A (docking-5) with erythromycin-A (dock-8) and 6-deoxyerythromycin-A (dock-4) resulting from their docking.

Atom	Distance between compiling atom in molecule (Å)		
	Eri-A(8)–Deoksi (4)	Eri-A(8)-Aneri-A(5)	Aneri-A(5)–deoksi(4)
C1	0.76	2.08	2.11
C3	0.72	1.85	1.66
C5	0.76	2.46	1.83
C6	0.79	2.40	1.73
C7	0.76	1.76	1.01
C9	0.78	2.31	1.82
C11	0.74	1.94	1.73
C12	0.75	2.55	2.44
O(C-4")	0.80	3.83	4.30
O(C-3")	0.80	2.23	2.89
O(C-2')	0.85	6.93	6.10
N(C-3')	0.89	9.06	8.21
RMSD	0.78	3.28	2.99

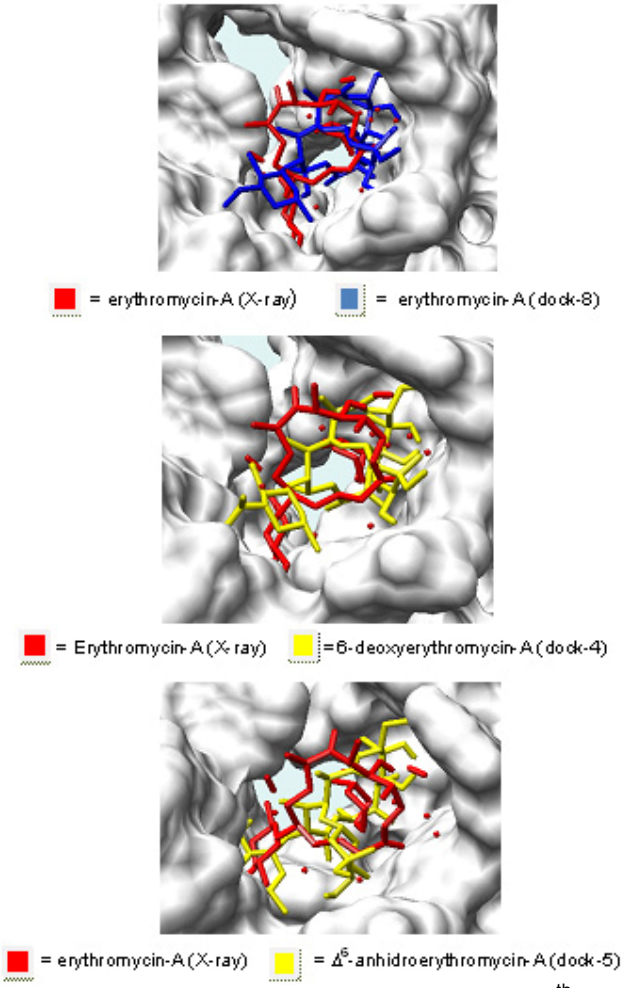

Figure 2. Relative position of erythromycin-A 8th docking (a), 6-deoxyerythromycin-A 4th docking (b) and Δ^6-anhidroerythromycin-A 5th docking (c) to erythromycin-A resulting X-ray crystallography into rRNA 23S *D. radiodurans* active site.

result of 4th docking (5,98 Å) and Δ^6-anhidroeritromisin-A result of 5th docking (5,87 Å), are docking molecules (ligand) which occupies the closest position to experimental erythromycin-A. The relative position of the three molecules against erythromycin-A from X-ray crystallography in the active site of RNA 23S macromolecule is shown in figure 2.

From figure 2 it is clear that 6-deoxyerythromycin-A (dock-4) and Δ^6-anhidroerythromycin-A (dock-5) molecules' position are not an exact match with erythromycin-A from X-ray crystallography, even tough through RMSD less than 6 Å these molecules are probably occupying peptide exit tunnel of rRNA 23S similar to experimental erythromycin-A. In figure 2, the macrolide ring of Δ^6-anhidroerythromycin-A and 6-deoxyerythromycin-A are situated very close to the macrolide ring of erythromycin-A. From this fact, it could be said that both these molecules has the capability of closing the peptide exit tunnel aperture and inhibit polypeptide synthesis elongation as in erythromycin-A.

The relative position of Δ^6-anhidroerythromycin-A (dock-5), 6-deoxyerythromycin-A (dock-4) and erythromycin-A (dock-8) are determined with RMSD. RMSD calculation is based on the distance between 12 similar atoms compiling these three molecules. The relative positions of these three molecules are used to analyze the effect of structural difference of the three molecules with their respective binding patterns. RMSD of the three molecules resulting from their docking are shown in table 2.

From the RMSD data it is shown that the relative position of erythromycin-A is 0.78 Å meanwhile the relative position of Δ^6-anhidroerythromycin-A is significantly further than of erythromycin-A and 6-deoxyerythromycin-A. This shows that the structural

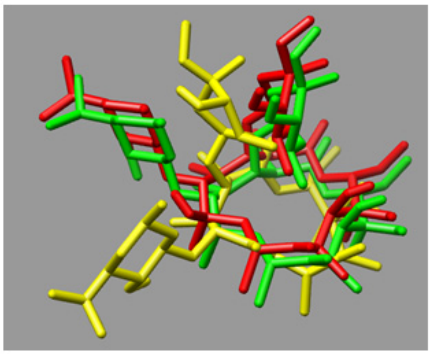

Figure 3. Relative position between Δ^6-anhidroerythromycin-A (dock-5), erythromycin-A (dock-8), and 6-deoxyerythromycin-A (dock-4).

difference between erythromycin-A and 6-deoxyerythromycin-A does not constitute to their binding patterns. Meanwhile the structural difference of Δ^6-anhidroerythromycin-A is significantly effects the binding pattern making it different from the binding pattern of erythromycin-A. Figure 3 shows the relative position between erythromycin-A molecule (dock-8), 6-deoxyerythromycin-A (dock-4) and Δ^6-anhidroerythromycin-A (dock-5).

Based on the RMSD calculation data shown in table 2 and relative position shown by figure 3, it is evident that the result of docking position of Δ^6-anhidroerythromycin-A differs from docking results of erythromycin-A and 6-deoxyerythromycin-A. This difference in binding occurs due to difference in the structure chamber in Δ^6-anhidroerythromycin-A molecule than that of erythromycin-A and 6-deoxyerythromycin-A. This difference in chamber structure of Δ^6-anhidroerythromycin-A with the other two molecules are caused by the presence of Δ^6 double bond. This Δ^6 double bond occurs due to sp^2 orbital hybrid at C-6 and C-7 in Δ^6-anhidroerythromycin-A molecule meanwhile erythromycin-A has sp^3 orbital hybrid. The sp^2 orbital hybrid in Δ^6-anhidroerythromycin-A molecule has made the structural conformation of atom C-5, C-6, C-7 and C-8 to be planar, meanwhile the similar atoms in erythromycin-A and 6-deoxyerythromycin-A does not exist in one domain. The binding position of 6-deoxyerythromycin-A is similar to that of erythromycin-A. This is apparent that the inexistence of a hydroxyl molecule (C-6) in 6-deoxyerythromycin-A molecule does not hamper the binding capabilities with rRNA 23S, as it is not the case with erythromycin-A. As with that, the presence of hydroxyl molecule at C-6 does cause an effect of any nature towards the pharmacological properties of macrolide antibiotic.

Table 3. Calculation of free binding energy of Δ^6-anhidroerythromycin-A against rRNA 23S through molecular docking method.

Ligand Docking	free binding energy (Δ G) (kkal/mol)		
	Erythromycin-A	6-deoxyery-thromycin-A	Δ^6-anhidro-erythromycin-A
1	-8.78	-9.02	-14.34
2	-9.41	-9.08	-13.05
3	-9.20	-9.02	-13.53
4	-9.19	-7.71	-14.33
5	-9.40	-10.75	-12.91
6	-8.66	-8.94	-15.34
7	-8.25	-8.94	-14.86
8	-8.14	-9.02	-15.17
9	-9.41	-10.68	-15.18
10	-9.17	-10.74	-14.34
Mean	-8.96	-9.39	-14.31

The difference in binding position between kladinose against erythromycin-A and kladinose against Δ^6-anhidroerythromycin-A could probably influence the strength of binding with rRNA 23S. By that even though both molecules are capable of inhibiting elongation of polypeptide chain their respective binding strength differs. The strength of binding could be studied based on free molecule binding energy estimation of Δ^6-anhidroerythromycin-A to rRNA 23S through molecular docking method.

From table 3 it is clear that the mean free binding energy from ten times of docking erythromycin-A molecule is similar to free binding energy of 6-deoxyerythromycin-A because of the difference in the mean of free binding energy is only 0.43. The difference in free binding energy is not significant in case of value less than 2 kkal/mol [10]. The free binding energy of Δ^6-anhidroerythromycin-A is lower than of erythromycin-A with the mean ratio of 5.35 kkal/mol. This signifies that Δ^6-anhidroerythromycin-A based its free binding energy to enhance the strength on binding into peptide exit tunnel rRNA 23S than of erythromycin-A. Therefore, Δ^6-anhidroerythromycin-A is predictably a higher potential microlide than erythromycin-A.

CONCLUSION

Based on docking results, Δ^6-anhidroerythromycin-A is a new derivative of erythromycin which occupies binding aperture similar to experimental erythromycin-A. Binding position of Δ^6-anhidroerythromycin-A is not an exact match with binding position of erythromycin-A due to the presence

of Δ^6 double bond and hydroxyl molecules (C-6) which does not cause an effect on its binding against rRNA 23S. Based on docking molecule, Δ^6-anhidroerythromycin-A which has lower binding energy compared to erythromycin-A therefore it is predicted to have a better antimicrobial potential than of erythromycin-A.

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