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Research Article

BETWEEN ARTEMISININ AND DERIVATIVES WITH NEURAMINIDASE: A DOCKING STUDY INSIGHT

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ABSTRACT

Objectives: This study aims to find the relationship between artemisinins and neuraminidase (NA) with molecular docking study and also to determine the most potent NA inhibitor from artemisinin and derivatives.

Methods: All ligands were sketched and optimized using Gaussian 03W with Hartree-Fock method basis sets 6-311G. Molecular docking was performed using AutoDock 4.2.3 toward NA in complexes with oseltamivir as co-crystal ligand. The main parameters used were the free energy of binding (ΔG) and dissociation constant (K_i) as affinity marker.

Results: Artesunate provided most negative free ΔG and lowest K_i toward NA with −9.55 kcal/mol and 100.66 nM, respectively. Artesunate shows higher affinity than oseltamivir with interactions between artesunate and amino acids at position 246 had important influences on artesunate affinity toward NA from H5N1.

Conclusion: *In silico* molecular docking results indicated that artesunate could be considered as NA inhibitor and should be potential to be developed as anti-influenza particularly to H5N1 with oseltamivir resistance.

Keywords: Artemisinin, Artesunate, Anti-influenza, H5N1, Neuraminidase.

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INTRODUCTION

Although avian influenza is no longer a frightening disease due to the development of H5N1 antivirus including oseltamivir and zanamivir, mutations of H5N1 could increase the resistance of influenza antiviral currently available [1]. Several H5N1 mutations even not only cause resistance to common neuraminidase inhibitor (NAI) but also cause transmission of avian influenza become airborne [2]. Sooner or later, avian influenza resistance toward known influenza antivirus will spread, and the need for new antiviral could be inevitable [3].

Compared with hemagglutinin, NA is a more ideal target for the development of anti-influenza compounds. Like oseltamivir and zanamivir, early developed NAIs considered to be effective for treatment and prophylaxis of avian influenza with minor side effects [4,5]. Several mutations in NA could reduce NAIs sensitivity [6]. However, several mutations only affect specific NAIs. For example, H274Y mutation caused resistance to oseltamivir (754-fold increase) and peramivir (260-fold increase), indicated that NA was still had potency as anti-influenza targets therapy [7].

Discovery of NAIs with oseltamivir-based compounds resulting in the development of several known NAIs with more effectiveness example like laninamivir, a long action NAI [8]. However, since almost every known NAIs share similar molecular structure, the potency of cross-resistances between NAIs is still high [9,10]. Discovery of secondary metabolites from medicinal plants with antiviral especially anti-influenza activity is a key to obtain new NAI with high effectiveness. Some herbs extract shows anti-influenza activity and appear to be safe for human consumption [11]. One of the traditional medicinal plants with potent antiviral activity was *Artemisia annua* or qinghaosu. Artemisinins, its main secondary metabolites was known for having several antiviral activities [12,13]. However, to date still, no researches linking between artemisinin and derivatives with anti-influenza properties toward NA inhibition. In this study, we conducted *in silico* study to determine the most potent artemisinin derivatives as NAI with molecular docking method. We have selected seven artemisinin derivatives as test ligands that already used as antimalarial therapy or still in preclinical phase. Our purpose is to obtain information about other therapeutics activity that could be developed as drug repurposing from these compound class.

METHODS

Preparation of ligands and receptor

Structures of artemether, artemisinin, artemisone, artemotil, artelinic acid, artesunate, and dihydroartemisinin were sketched using GaussView 3.08 Software from Gaussian, Inc. All structures were geometry optimized by Hartree-Fock method basis sets 6-311G with Gaussian 03 W Software from Gaussian, Inc. Geometry optimization provided the most ideal conformation of following compounds that approaching conformation of these compounds in nature [14]. Optimized structures format changed from .log to .pdb using Open Babel 2.3.2 Software [15]. Docking program used in this study was AutoDock 4.2.3 from the Scripps Research Institute [16]. The molecular structure of NA H5N1 in complexes with oseltamivir (protein data bank [PDB] ID 2HU4) was obtained from website of PDB www.rscb.org with oseltamivir binding site chosen as active site since this site already known for the development of NAI [17].

Validation of docking process

The method used for molecular docking validation was pose selection using co-crystal structure by redocking it into active site of NA protein. Thus, redocking was performed with oseltamivir on NA active site. The parameters observed in validation is root-mean-square deviation (RMSD) of each ligand co-crystal at selected binding site [18]. Docking

programs are preferred to predict results from experimental poses with RMSD no more than 2 Å. Smaller RMSD indicate that position of redocking ligand was closer to crystallography ligand [19,20].

Docking studies

Molecular docking for all test ligand performed in same way as validation process using with similar grid box size and position [21]. The main parameter used in docking process were free energy of binding (ΔG), dissociation constant (K_i), amino acids residues, and number of hydrogen bonds. Ligand affinity to receptor in docking method was determined by ΔG and K_i scores. More negative ΔG and lower K_i indicated higher ligand affinity toward active site of used receptor [22-24]. Test ligand with the highest affinity was compared with validation result of co-crystal ligand of active site to determine the potency of test ligand as NAI [14].

RESULTS AND DISCUSSION

Docking validation was done with redocking method using AutoDock 4.2.3. Validation was performed on the entire binding site using co-crystal ligand of selected receptor. Using of entire binding site was purposed to identify any other potential active site at NA receptor. However, redocking result showed that oseltamivir as cocrystal ligand docked into similar position like crystallography result [14].

Redocking results from this study were provided RMSD value wand almost at stacked position with crystallography results (Fig. 1), indicated that receptor 2HU4 was valid for molecular docking purpose [20]. Other parameters observed in validation was ΔG, K_i, amino acids residues, and number of hydrogen bonds of cocrystal ligand as shown in Table 1.

Test ligands were sketched and performed geometry optimization. Hartree-Fock method was used with basis set 6-311G for geometry optimization (Table 2). This method was *ab initio* approximation with relatively high confidence rate for *in silico* analysis [14].

Docking was performed using AutoDock 4.2.3 at active site of NA receptor with 100 genetic algorithms runs to improve accuracy of docking result [16]. For each test ligand, one poses with most negative ΔG and lowest K_i was selected as representatives of test ligand [14]. The docking results data of seven ligands to NA were compared each other as shown in Table 3. Compared to other ligands, artesunate had the most negative ΔG and lowest K_i, thus had the highest affinity toward NA than other artemisinin derivatives. All ligands also provided negative score of ΔG, indicated that interaction between NA receptor active site and ligands will occur spontaneously [19].

Comparison of amino acids residues and number of hydrogen bonds between docking results of oseltamivir as co-crystal ligand and artesunate as test ligand then performed to analyzed similarities and differences between docking results of two compounds. Comparison results shown in Table 4 indicated slight differences in amino acids residues and number of hydrogen bonds between oseltamivir and artesunate docking results. Interestingly, artesunate had slight higher affinity than oseltamivir.

Whether artesunate had the same activity with oseltamivir or not was still unclear. However, since most amino acids residues which interacted with oseltamivir also interacted with artesunate (8 out of 10), there was possible that artesunate also inhibits NA like oseltamivir [25]. Since NA inhibition was one of primary target for influenza therapy, this study results indicated that artesunate had potency as NAI.

There is only one amino acid residue that forms in docking result of artesunate in position 246, which do not occurs in other ligands docking result. As comparison, docking result of artemisone had many similarity with docking result of artesunate, the differences occurs only in 3 out of 13 amino acids residues despite artesunate gave much number of hydrogen bonds than artemisone. However, compared to artesunate, artemisone gave lower affinity toward NA receptor active site (Table 3). This result proved that presence interaction at amino acid residue in position 246 had important influence on artesunate affinity toward NA receptor.

More observation conducted to reveal interaction between artesunate and NA receptor active site as shown in Fig. 2. Artesunate was docked into cleavage between set of β-strand peptides (yellow in left Fig. 2) in N-terminal lobes of tyrosine kinase domain. On the other hand, interactions between artesunate and amino acids residues dominated with hydrophilic interactions with five hydrogen bonds formed (right Fig. 2), even more than oseltamivir (Table 4). We also compared position of artesunate with oseltamivir directly which obtained from

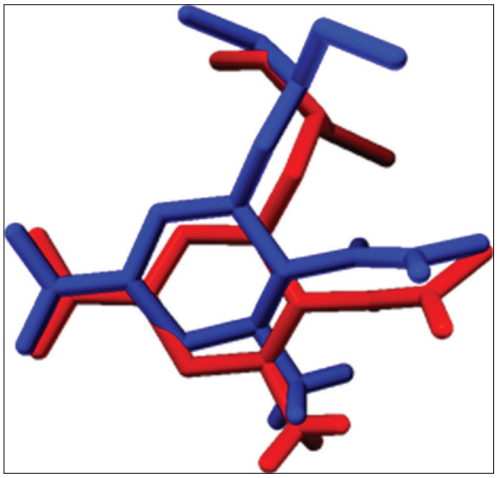


Fig. 1: Results of validation from oseltamivir and neuraminidase; root-mean-square deviation=1.183 Å (Red: Oseltamivir redocking result; Blue: Oseltamivir crystallography result)

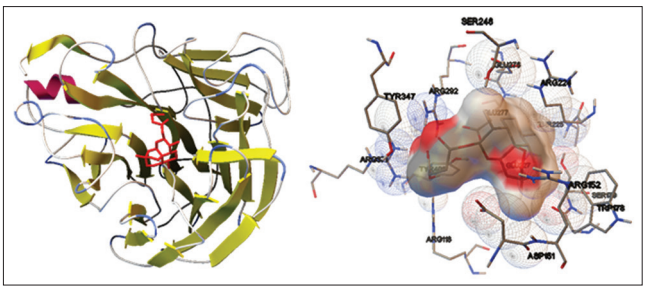


Fig. 2: Docking results of artesunate and neuraminidase (NA) (Left: Artesunate position in NA; Right: Interactions between artesunate and amino acids residues of NA receptor active site)

Table 1: Validation results of NA receptors PDB ID 2HU4 with co-crystal ligand oseltamivir

Receptor	Ligand	RMSD (Å)	ΔG (kcal/mol)	K_i (μM)	Amino acids residues	Number of hydrogen bonds
NA	Oseltamivir	1.183	−9.26	0.16253	118-Arg, 119-Glu, 151-Asp, 152-Arg, 178-Trp, 224-Arg, 277-Glu, 292-Arg, 371-Arg, 406-Tyr	4

NA: Neuraminidase, PDB: Protein data bank, RMSD: Root-mean-square deviation, ΔG: Energy of binding, K_i: Dissociation constant

Table 2: 2D and 3D structure of ligands

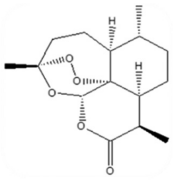
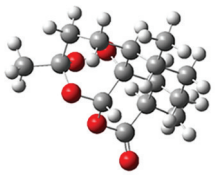
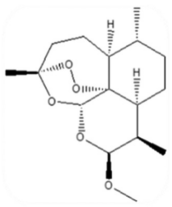
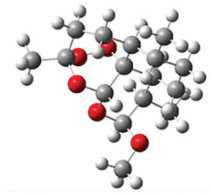
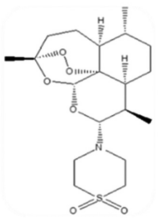
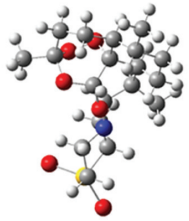
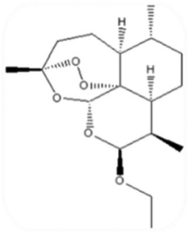
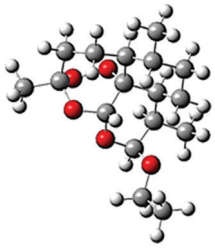
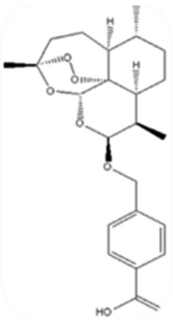
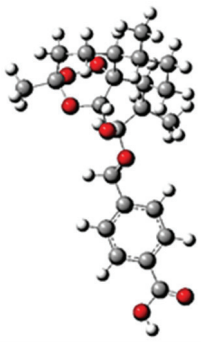
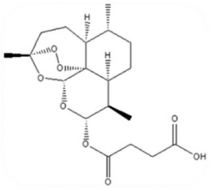
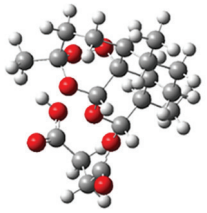
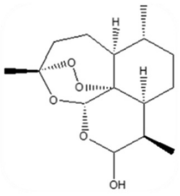
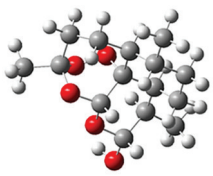
Compounds	2D Structure	3D Structure
Artemisinin (ART)		
Artemether (ARM)		
Artemisone (ARO)		
Artemotil (ARL)		
Artelinic acid (ARA)		
Artesunate (ARS)		
Dihydroartemisinin (DHA)		

Table 3: Docking results of artemisinin derivatives at NA receptor

Parameters	ART	ARM	ARO	ARL	ARA	ARS	DHA
ΔG (kcal/mol)	−6.85	−6.10	−7.84	−6.31	−7.82	−9.55	−6.98
K_i (μM)	9.56	33.54	1.78	23.51	1.87	0.10066	7.65
Amino acids residues	-	-	118-Arg	-	118-Arg	118-Arg	-
	-	-	-	-	-	-	-
	-	-	-	-	-	-	-
	-	151-Asp	151-Asp	151-Asp	-	151-Asp	151-Asp
	152-Arg	152-Arg	-	152-Arg	152-Arg	152-Arg	152-Arg
	-	-	-	-	-	-	-
	178-Trp	178-Trp	178-Trp	178-Trp	-	178-Trp	178-Trp
	179-Ser	179-Ser	179-Ser	179-Ser	179-Ser	179-Ser	-
	-	-	-	-	222-Ile	-	-
	224-Arg	224-Arg	224-Arg	224-Arg	-	224-Arg	224-Arg
	225-Thr	225-Thr	225-Thr	225-Thr	-	225-Thr	-
	227-Glu	227-Glu	227-Glu	227-Glu	-	227-Glu	-
	-	-	-	-	-	246-Ser	-
	276-Glu	-	276-Glu	-	-	276-Glu	-
	277-Glu	277-Glu	277-Glu	277-Glu	277-Glu	277-Glu	277-Glu
	-	-	292-Arg	292-Arg	292-Arg	-	-
	-	-	-	-	-	-	-
	-	-	347-Tyr	-	347-Tyr	347-Tyr	-
	-	-	371-Arg	371-Arg	371-Arg	371-Arg	-
	-	-	406-Tyr	406-Tyr	406-Tyr	406-Tyr	-
Number of hydrogen bonds	1	1	1	1	3	5	3

ART: Artemisinin, ARM: Artemether, ARO: Artemisone, ARL: Artemotil, ARA: Artelinic acid, ARS: Artesunate, DHA: Dihydroartemisinin, NA: Neuraminidase, ΔG: Energy of binding, K_i: Dissociation constant

Table 4: Comparison of docking results between oseltamivir and artesunate

Parameters	Oseltamivir	Artesunate
ΔG (kcal/mol)	−9.26	−9.55
K_i (nM)	162.53	100.66
Amino acids residues	118-Arg	118-Arg
	119-Glu	-
	-	-
	151-Asp	151-Asp
	152-Arg	152-Arg
	-	-
	178-Trp	178-Trp
	-	179-Ser
	-	-
	224-Arg	224-Arg
	-	225-Thr
	-	227-Glu
	-	246-Ser
	-	276-Glu
	277-Glu	277-Glu
	292-Arg	-
	-	-
	-	347-Tyr
	371-Arg	371-Arg
	406-Tyr	406-Tyr
Number of hydrogen bonds	4	5

ΔG: Energy of binding, K_i: Dissociation constant

redocking method. As result, we overlay both docking results structure as shown in Fig. 3.

Both artesunate and oseltamivir docked into similar position at the active site of NA receptor active site. However, endoperoxide chain of artesunate had different behavior with side chain of oseltamivir. Aside from other artemisinins, interactions of artesunate and NA were dominated by interactions at amino acid's in position 224-277 (Table 4). Total six interactions were formed in artesunate compared with two in oseltamivir. This results could lead in conclusion that artesunate had higher affinity toward NA than oseltamivir caused by more interactions occurred at those position. Hence, interactions at amino acids residues number 224-277 could have important

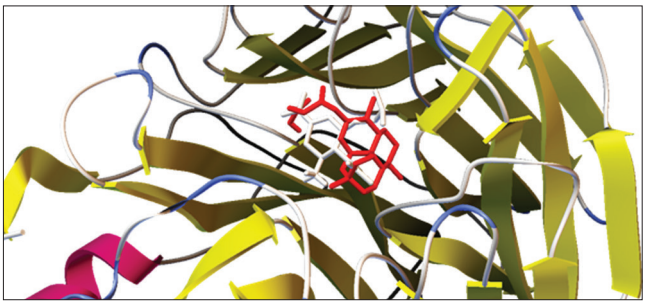


Fig. 3: Comparison between docking results of artesunate and oseltamivir (Red: Artesunate; White: Oseltamivir)

influences toward activity as NAI, primarily amino acid serine at position 246.

CONCLUSION

This study was successfully described linking between artemisinin and derivatives with NA, even gave interesting result where artesunate had higher affinity than oseltamivir at NA active site. Artesunate provided ΔG and K_i -9.55 kcal/mol and 100.66 nM, respectively. These result open up opportunities to develop artesunate as potent anti-influenza especially one with oseltamivir resistance. More researches should be done to optimize the interactions mainly in amino acid position 224-277, especially with 246-serine. Designing novel NAI derives from artesunate should be focus at those amino acids residues. Thus, this study clearly indicates a promising potential of artemisinin and derivatives to be develop as NAI for anti-influenza therapy.

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