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FRAGMENT-BASED DRUG DESIGN TO INHIBIT DNA METHYLTRANSFERASE 1 (DNMT1) FOR BREAST CANCER THERAPY

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ABSTRACT: Breast cancer is the most substantial cancer among women in the world. The uncontrollably high DNA Methyltransferase-1 (DNMT1) activity which leads to abnormal gene expression is one of the primary cause of breast cancer. Therefore, DNMT1, as an essential enzyme in epigenetic regulation, is considered as a potential therapeutic target for breast cancer treatment. In this research, the inhibitors of DNMT1 were designed through fragment-based drug design. About 168,646 natural products from PubChem database were used as fragment candidates. Initial screening based on toxicity and Lipinski's Rule of Three was performed to obtain 2,601 favorable fragments. Pharmacophore-based rigid and flexible molecular docking simulation was employed with DNMT1 as the target protein. The selected fragments from docking simulation underwent fragment linking modification and second toxicity screening, generating 23 ligands. Subsequently, the newly designed ligands were subjected to pharmacophore-based flexible molecular docking simulation. Two ligands, HAMI 9 and HAMI 14, with Gibbs free binding energy of -11.6095 and -11.5904 kcal/mol, respectively, are considered as a promising inhibitor of DNMT1. The pharmacological properties of the ligands were analyzed using DataWarrior v04.07.02, Toxtree v2.6.13, SwissADME, admetSAR, and Molinspiration. The ligands show not only superior affinity and molecular interaction to DNMT1 but also have advantageous pharmacological properties compared to the standards. Additional in silico as well as in vivo experiments are needed to further assess the potency of HAMI 9 and HAMI 14 as drug candidates against breast cancer.

Keywords: Breast cancer, DNA methyltransferase, natural products, fragment-based drug design, molecular docking simulation.

1. INTRODUCTION

Breast cancer is the biggest evidence of cancer among women and the second prominent cause of cancer mortality after lung cancer in the world [1], [2]. Breast cancer occurs due to genetic aberration such as gene deletions, point mutations, chromosomal rearrangements and epigenetic misregulation [1]. DNA methylation pattern guides epigenetic regulation. DNA methylation plays an essential role in the regulation of the gene expression and the structure of chromatin, which leads to the manifestation of diseases in humans, such as various types of cancer [3]-[6].

DNA methylation pattern managed by the DNA methyltransferase (DNMT) enzymes, which will catalyze the transfer of methyl groups from S-Adenosyl-L-Methionine (SAM) to the C5 position of cytosine residues in CpG dinucleotides. DNMT in human is classified into three families: DNMT1, DNMT2, and DNMT3 [7].

DNMT1 is an enzyme composed of 1,616 amino acids in humans and is the ubiquitous methyltransferase in humans. DNMT1 maintained the methylation pattern of the DNA parent strand to the new DNA daughter strand and expressed during the S phase [5], [8]. The misregulation of DNMT1 initiates to hypermethylation in DNA promoter gene and hypomethylation which lead to the abnormal growth of cancer cells [5]. In breast cancer, the DNMT1 is overexpressed; it has uncontrollably high activity [8]. Therefore, the inhibition of the DNMT1 enzyme, which keeping its activity under control, is a promising method of epigenetic therapy for the treatment of breast cancer [5], [6], [8].

The natural products have long been used by in silico method for drugs discovery and development in various diseases, such as cancer, because of its pharmacological activity and bioavailability in living organisms [9]. The natural products can be used as a fragment library for lead compound discovery through fragment-based drug design. The fragment-based drug design is an in a silicon method in drug discovery which employed to construct the ligands by linking, merging, or growing the ligands from small fragments to improve its interaction with target protein binding site [10]. In this research, the natural products are selected as the source of fragments which then modified by fragment-based drug design to inhibit DNMT1 protein. Thus, obtaining the inhibitor of DNMT1 for breast cancer therapy.

2. METHODS

In silico method were used in this research to obtain the compounds that have high affinity and advantageous pharmacological properties. The compounds through in silico method were used for drug discovery in human disease, and the compounds would be used to inhibit DNMT1 protein. In this research, the in silico method were performed through Molecular Operating Environment (MOE) v2014.09, DataWarrior v04.07.02, Toxtree v2.6.13, SwissADME, AdmetSAR, and Molinspiration software.

2.1 Preparation of DNMT1 Protein

The three-dimensional (3D) structures of DNMT1 protein were acquired from RCSB Protein Data Bank with PDB ID: 3AV5, 3AV6, 3SWR, 3PTA, and 4WXX. The DNMT1 proteins were acquired from RCSB as one of protein structures source for molecular docking. Afterward, the protein structures were prepared and optimized by protonation and energy minimization using the LigX function in MOE v2014.09 with R-field solvation and AMBER 10: EHT as a forcefield. Finally, the DNMT1 proteins were stored in .moe format.

2.2 Protein-Ligand Interaction Fingerprints (PLIF) and Pharmacophore Selection of DNMT1 Protein

The pharmacophore construction of DNMT1 proteins was performed through PLIF method using MOE v2014.09 software with Amber10: EHT as a forcefield, and R-field solvation. The PLIF method used to determine interaction fingerprints between ligand−protein based on surface contacts according to the residues, hydrogen bonds, and ionic interactions [11]. Afterward, the proteins were performed superpose to compare three-dimensional (3D) structure of the protein and to superimpose the protein structures that differ based on protein sequence, size or shape [12].

Afterward, the pharmacophore feature that commonly applied in drug discovery were used in this research [13]. The pharmacophore feature is used to determine the interaction site between the ligands and the protein target. Therefore, the molecules that potentially trigger the desired biological effect were obtained [14], [15]. The pharmacophore site was stored in .ph4 format.

2.3 Preparation of The Fragments

The natural products from PubChem database were used as fragment library and were stored in sdf format. The fragment library was screened based on toxicity prediction test (tumorigenic, mutagenic, irritant, reproductive effect predictions) and Lipinski's Rule of Three using DataWarrior v04.07.02 to obtain favorable fragments that can be used for molecular docking [16], [17].

Afterward, all of the fragments underwent preparation and energy minimization by MOE v2014.09 software with MMFF94x modified as a forcefield. Three ligands, namely S-Adenosyl-L-Methionine (SAM), S-Adenosyl-L-Homocysteine (SAH), and sinefungin (SFG), were selected as standard compounds for the experiment. Finally, the fragment library was stored in .mdb format.

2.3 Molecular Docking Simulation and Fragment Linking Method

The fragments underwent pharmacophore-based rigid and flexible molecular docking with DNMT1 protein using MOE v2014.09 software with AMBER 10: EHT as the forcefield and R-field solvation. The fragments were eliminated if their root-mean-square deviation (RMSD) value lower than 2.0 Å and have higher Gibbs free binding energy ($\Delta G_{binding}$) than the standards.

Afterward, the fragments were linked to developing new ligands through fragment linking method. Fragment linking is one of fragment-based drug design method, the fragment linking method applied to acquire compounds that have a higher affinity to bind with the pocket [18]. The new ligands were acquired from the fragments that do not overlap and have been linked. Afterward, the new ligands were docked with DNMT1 protein by pharmacophore-based rigid and flexible molecular docking using retain 100 through MOE v2014.09 software.

2.4 Analysis of Pharmacological Properties

The pharmacological properties of the best ligands were analyzed through some software. The tumorigenic, mutagenic, and drug-likeness properties were identified by DataWarrior v04.07.02 [19]. The ligands mutagenicity and carcinogenicity properties were predicted by Toxtree v2.6.13 [20]. The physicochemical, pharmacokinetics and drug-likeness of ligands were evaluated by SwissADME (http://www.swissadme.ch/) [21]. The admetSAR (http://lmmd.ecust.edu.cn/admetsar1) used to predict absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of ligands [22]. The bioactivity properties of ligands were analyzed through Molinspiration software [23].

3. RESULTS AND DISCUSSIONS

3.1 Preparation of DNMT1 Protein

The DNMT1 protein, which responsible for DNA methylation, is a promising target for the treatment of breast cancer [7], [8]. The water, metal, and unnecessary molecules in DNMT1 proteins from RCSB Protein Data Bank were removed because can influence interactions between the protein and ligand. The binding site of DNMT1 protein can be determined based on site finder feature in MOE v2014.09 software.

3.1 Protein-Ligand Interaction Fingerprints (PLIF) and Pharmacophore Selection of DNMT1 Protein

The interactions between DNMT1 proteins and ligands were compared through PLIF method. Five proteins of DNMT1 (3AV5, 3AV6, 3SWR, 3PTA, 4WXX) have similarity interactions. The protein of DNMT1 with PDB ID: 4WXX was chosen because it has an excellent 3D structure resolution (2.622 Å) and is originated from a human. The pharmacophore of DNMT1 protein was analyzed and validated using standard ligands. Finally, there are three pharmacophore site namely HydA, Don and Acc indicated in green, pink, and blue respectively (Fig. 1). The 3D structures and the pharmacophore site of the DNMT1 protein can be seen in Fig. 1.

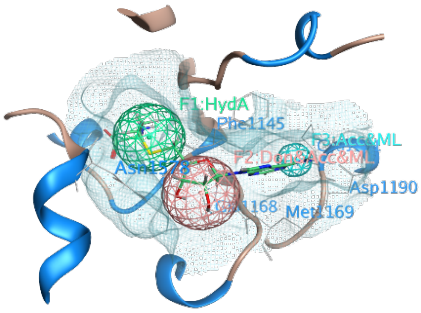


Fig. 1 The pharmacophore site and SAH in the binding pocket of DNMT1 protein (4WXX).

3.2 Preparation of the Fragments

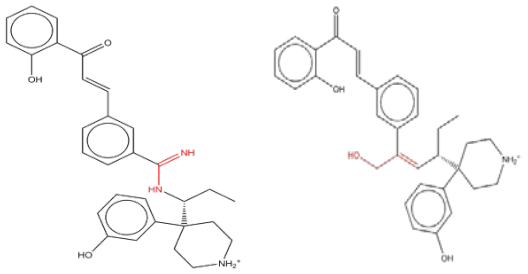
About 168,646 natural products from PubChem database were used in this research. All of the natural products were screened through DataWarrior v04.07.02 to find the potential fragment as an inhibitor candidate of the DNMT1 protein. The natural products were screened based on toxicity prediction test to predict and to eliminate the compounds that have tumorigenic, mutagenic, reproductive effect and irritant characteristics. The natural products also were screened based on Lipinski's Rule of Three, which consisted of three parameters: (1) mass of the molecules less than 300 Da, (2) the hydrogen donor and acceptor molecules up to three, and (3) the calculated clogP of molecules less than three [24]. After screening by DataWarrior v04.07.02, about 2,601 fragments obtained.

3.3 Molecular Docking Simulation and Fragment Linking Method

The 2,601 fragments were docked with DNMT1 protein based on Acc and HydA pharmacophore site and also based on Hyd and Don pharmacophore site. The molecular docking simulation was performed twice in rigid docking simulations using retain 30 in the first simulation and retain 100 in the second simulation with AMBER10: EHT as a forcefield. After docked using retain 100, there are 11 ligands based on Acc and HydA pharmacophore points, and also there are 76 ligands based on Hyd and Don pharmacophore site.

In this research, the linker was generated from three ligands which did not overlap to each other so they were eligible to create linker through fragment linking. About 31 ligands were acquired from the fragments and the linkers screened by DataWarrior v04.07.02. Afterward, 23 linkers were obtained after screened and were docked with DNMT1 protein based on Acc, HydA and Don pharmacophore site by pharmacophore-based rigid and flexible molecular docking simulation using retain 100 with AMBER10: EHT as a forcefield. Finally, two best compounds were potential can inhibit DNMT1 refer to standard RMSD value, $\Delta G_{binding}$, and pharmacological properties.

The two of best compounds structure, namely HAMI 9 and HAMI 14, can be seen in Fig. 2.



(A) (B)

Fig. 2 The structure of (A) HAMI 9 and (B) The structure of HAMI 14

The HAMI 9 and HAMI 14 are the best drug candidate because they have RMSD value lower than 2.0 Å and have lower $\Delta G_{binding}$ energy compared to the standard ligands. The properties of the standards and all of the best ligands are appropriate with the Lipinski's Rule of Five and Veber's rule, even though the HAMI 14 has a logP value higher than five (Table 1).

Table 1. Molecular Properties of the Ligands

Ligand Name	Flexible Docking		MW	LogP	H-Don	H-Acc	TPSA	Rotatable Bond
	ΔGbinding	RMSD						
*SAM	-11.2605	1.3306	399.451	-3.9384	4	11	187.08	7
*SFG	-10.9262	1.8644	382.470	-3.9574	5	12	214.72	7
*SAH	-11.2323	1.6747	384.400	-3.7275	4	11	212.38	7
HAMI 9	-11.6094	1.7746	484.618	3.8948	5	6	110.02	8
HAMI 14	-11.5903	1.5264	498.641	5.1464	4	5	94.37	9

Note: The meaning of the asterisk symbol is the standard compound.

The HAMI 9 interacts with 22 amino acid residues DNMT1 protein and has four hydrogen bond interactions with Asparagine 1578, Glutamine 1223, and Cysteine 1148. On the other hand, HAMI 14 interacts with 21 amino acid residues and has two hydrogen bond interactions with Asparagine 1578, Alanine 1579 (Fig. 3 and 4).

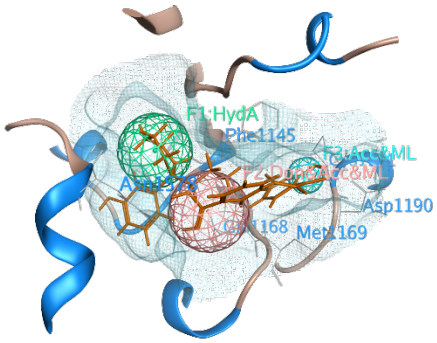


Fig. 3 The HAMI 9 molecular interactions.

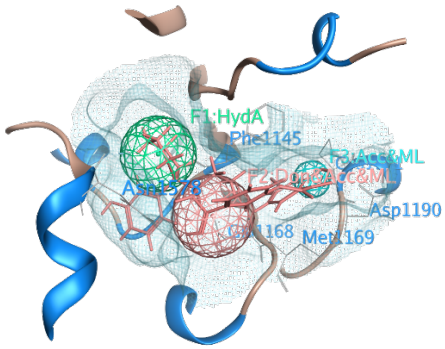


Fig. 4 The HAMI 14 molecular interactions

3.4 Analysis of Pharmacological Properties

Both ligands have similar molecular properties. However, they show different characteristics in the pharmacological tests through SWISSADME, Molinspiration, and ADMETSAR. The results of pharmacological tests can be seen in Table 2. All best ligands are better than Sinefungin as the standard because they have high gastrointestinal (GI) absorption. The HAMI 9 and HAMI 14 have subcellular localization in mitochondria**.**

The kinase and protease inhibitors of the ligands have a different value; the score higher than 0.00 indicates the ligand has high activity, while a score between 0.00 to −0.50 indicates that the ligand has moderate activity, and score less than −0.50 indicates the ligand does not have activity [25]. HAMI 9 is the best drug candidate based on kinase and protease inhibitor value because of its moderate activity as a kinase inhibitor and high activity as a protease inhibitor. Protein kinases are essential for proliferation, metabolism, and apoptosis cells. The molecule that inhibits kinases cause decreased cellular proliferation and increased apoptosis cells. The inhibition of protein kinase is one strategy of cancer therapy such as breast cancer therapy [26]. HAMI 9 also has high activity as a protease inhibitor that can be used against cancer. Metastases and cancer progression are highly dependent on nutrient and oxygen supply, which are affected by various proteases in the tumor and organs. The proteases are essential for cell death, cell differentiation, gene expression, cancer growth and metastases [27]. The protease inhibitors have activity as anti-cancer because they can inhibit proteolytic activity in cancer development and metastases [28]. Therefore, HAMI 9 has an activity to interfere with the development of cancer.

HAMI 9 and HAMI 14 are not organic cation transporter (SLC22A2) and CYP inhibitor. It indicates that they are able to translocate the molecules, have proper drug elimination, and does not lead unwanted effects because of the accumulation of the drug [21], [29]. The ligands are both none in carcinogens and AMES toxicity test. Therefore, the ligands are non-carcinogens and non-mutagenic [30] (Table 2).

The best ligands have good drug-likeness properties refer to Veber's and Egan's Rule. The bioavailability of the best ligands is at moderate level because their bioavailability score is 0.55. All of the best ligands are more readily synthesized than the standard because it has lower synthetic accessibility value than standard ligands [21]. All of the best ligands do not have PAINS alerts, but the HAMI 9 as the best ligand has 3 BRENK alerts (Table 3).

Table 2 ADME-Tox prediction all of the ligands using admetSAR, Molinspiration, and SwissADME software.

ADME-Tox	Properties	Ligands				
		*SAM	*SFG	*SAH	HAMI 9	HAMI 14
Absorption	GI Absorption	High	Low	High	High	High
Distribution	Sub Localization	Nucleus	Nucleus	Nucleus	Mitochondria	Mitochondria
	Kinase Inhibitor	0.56	0.58	0.57	-0.04	-0.14
Metabolism	Protease Inhibitor	0.38	0.10	0.23	0.19	-0.04
	CYP Inhibitor	No	No	No	No	No
Excretion	SLC22A2	No	No	No	No	No
	AMES Toxicity	No	No	No	No	No
Toxicity	Carcinogens	No	No	No	No	No
	Biodegradation	No	No	No	No	No

Table 3 The drug-likeness and medicinal chemistry properties all of the ligands using SwissADME software.

Ligand Name	Druglikeness			Medicinal Chemistry		
	Veber	Egan	Bioavailability score	PAINS	Brenk	Synthetic accessibility
*SAM	Yes	No	0.55	0 alert	1 alert	4.94
*Sinefungin	Yes	No	0.55	0 alert	0 alert	4.78
*SAH	Yes	No	0.55	0 alert	0 alert	4.69
HAMI 9	Yes	Yes	0.55	0 alert	3 alert	4.31
HAMI 14	Yes	Yes	0.55	0 alert	1 alert	4.44

Note: The meaning of the asterisk symbol is the standard compound.

4. CONCLUSION

The in the silicon method is essential in drug discovery, especially in the identification of new lead compounds as drug candidate through fragment-based drug design. The new lead compounds namely HAMI 9 and HAMI 14 derived from the natural products. The HAMI 9 and HAMI 14 can be used as a DNMT1 inhibitor for breast cancer therapy because of its excellent molecular properties, pharmacological properties to inhibit DNMT1 protein. Based on the result, the HAMI 9 is the best drug candidate to inhibit DNMT1 because HAMI 9 has the lowest Gibbs free binding energy, has the most significant molecular interaction, and has the best pharmacological properties. Therefore, the HAMI 9 is the promising drug candidates against breast cancer.

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