DOCKING STUDIES OF CURCUMIN AS A POTENTIAL LEAD COMPOUND TO DEVELOP NOVEL DIPEPTYDYL PEPTIDASE-4 INHIBITORS

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ABSTRACT

Interaction of curcumin to dipeptydyl peptidase-4 (DPP-4) has been studied by employing docking method using Molecular Operating Environment (MOE) and AutoDock as the docking software applications. Although MOE can sample more conformational spaces that represent the original interaction poses than AutoDock, both softwares serve as valid and acceptable docking applications to study the interactions of small compound to DPP-4. The calculated free energy of binding ($\Delta G_{binding}$) results from MOE and AutoDock shows that curcumin is needed to be optimized to reach similar or better $\Delta G_{binding}$ compare to the reference compound. Curcumin can be considered as a good lead compound in the development of new DPP-4 inhibitor. The results of these studies can serve as an initial effort of the further study.

Keywords: *curcumin, docking, molecular operating environment (MOE), AutoDock, dipeptydyl peptidase-4 (DPP-4)*

INTRODUCTION

Dipeptydyl peptidase-4 (DPP-4) becomes an attractive target of drug discovery and development [1, 2], since it has served as the target of the discovery and development of sitagliptin (Januvia®; Merck) (**1**) and vildagliptin (Galvus®; Novartis) (**2**) (Figure 1) [3-11]. As DPP-4 inhibitors, the drugs prevent the inactivation of glucagon-like peptide-1 (GLP-1), which then enhance and prolong the action of the endogenously released incretin hormone, and therefore insulin secretion is stimulated [8]. Both sitagliptin and vildagliptin open new opportunity and new strategy in the therapy for type 2 diabetes mellitus (T2DM) [4, 8]. More than 200 million adults are reported to have T2DM (with an estimated 70 million in the United States and Europe) [4]. An epidemiology study in 2002 showed that the prevalence of diabetes in Indonesia was 1.2–2.3% among people over 15 years and the prevalence was predicted to explode in the coming years [12]. In order to provide cheaper and easily available T2DM drugs for Indonesian, development of drugs based on its own natural resources by employing similar strategy can serve as an alternative approach.

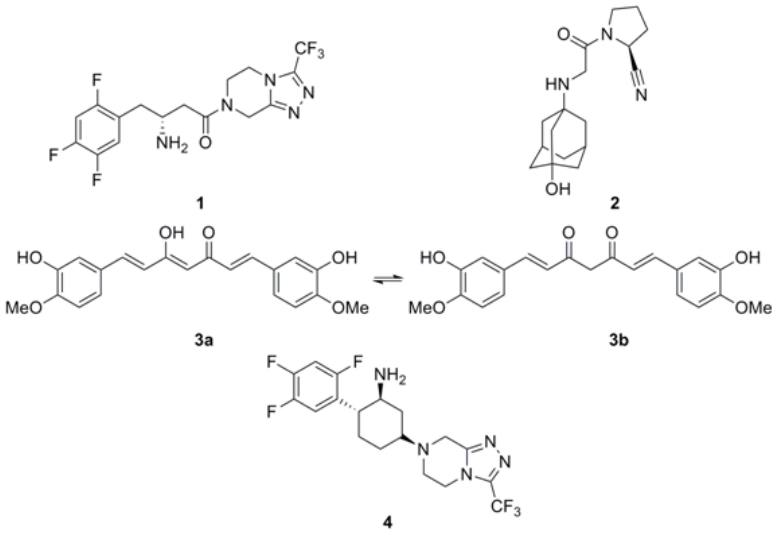

Figure 1. Structures of sitagliptin (**1**), vildagliptin (**2**), the tautomer enol of curcumin (**3a**), the tautomer keto of curcumin (**3b**), and (1S,2R,5S)-5-[3-(trifluoromethyl)-5,6-dihyro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-2-(2,4,5-trifluorophenyl)-cyclohexanamine (**4**).

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Curcumin (**3a** (enol form) and **3b** (keto form)) (Figure 1), the yellow pigment of turmeric (*Curcuma longa*, Linn.), has been reported to have some biological activities, *e.g.* antimicrobial, antiviral, antifungal, cholekinetic, anti-inflammatory, and chemopreventive [13]. Turmeric has widely used in Indonesia as an ingredient of some traditional medicines [14]. Curcumin itself has served as a lead compound which led to the discovery some patented analogs whose better anti-inflamatory activity [15, 16 benzylidene cyclopentanone, and benzylidene acetone, and therapeutic uses thereof #16 benzylidene cyclopentanone, and benzylidene acetone, and therapeutic uses thereof #16]. Recently, an epidemiology study reported positive correlations of curcumin intake with the treatment of T2DM [17]. This raises a question about the molecular mechanism of curcumin in the T2DM treatment. Since some DPP-4 inhibitors has been approved as drugs for the T2DM therapy [4, 8], the fact that curcumin has positive correlation in the treatment of T2DM [17] leads to a hypothesis that curcumin can inhibit DPP-4. Curcumin, therefore, has a possibility to serve as a lead compound in the development of new DPP-4 inhibitors.

Studies of the molecular interaction of drug and its target have been widely performed by employing docking method and lead to some successes in the medicinal chemistry field of research [18]. In this article, docking studies of curcumin to DPP-4 by employing Molecular Operating Environment (MOE) [19] and AutoDock [20] software applications are described. The results open chances to perform further virtual screening for some previously developed curcumin analogs [15, 16 benzylidene cyclopentanone, and benzylidene acetone, and therapeutic uses thereof #16 benzylidene cyclopentanone, and benzylidene acetone, and therapeutic uses thereof #16].

EXPERIMENTAL SECTION

Material

Structure of DPP-4 submitted by Biftu *et al.* to the protein data bank website (http://www.pdb.org/; PDB code: 2P8S) [1] was obtained and employed as the virtual target.

Instruments

Molecular Operating Environment (MOE) version 2007.0902 (developed by Chemical Computing Group Inc, Canada) [19] and AutoDock version 4 (developed by The Scripps Research Institute, USA) [20] were employed to perform the docking procedures. AutoDockTools (ADT) version 1.5.2 was employed as the graphical-user interface (GUI) to perform AutoDock [21]. PyMol release 0.99 (developed by DeLano Scientific LLC, USA) was used to prepare some input files and to produce pictures [22]. As long as no further explanation is stated, the default settings of each application were used. All computational simulations were performed on a Linux (Ubuntu 8.04 LTS Hardy Heron) machine with Intel Core 2 Duo (@ 2.5 GHz) as the processors and 3.00 GB of RAM.

Procedure

Method validation and benchmarking

PyMol was employed to select and separate the protein (DPP-4) from the ligand ((1S,2R,5S)-5-[3-(trifluoromethyl)-5,6-dihyro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-2-(2,4,5-trifluorophenyl)cyclohexanamine (**4**); (Figure 1)) in the initial structure obtained from the protein data bank [1]. Each structure was restored separately as a pdb file.

The pdb files written by PyMol were employed as input files in MOE docking procedure. Hydrogens were added to the compounds and partial charges were assigned to each atom in the molecules. Docking module using "Affinity dG" as the scoring function in MOE was then employed.

AutoDockTools (ADT) was employed to perform docking simulation using AutoDock. This procedure was done as the benchmarking method for the docking simulation performed in MOE. The pdb files written by PyMol were employed as input files. The macromolecule (DPP-4) and the ligand (compound **4**) were prepared by adding hydrogens and assigning charges. The ligands were also subjected for torsion rotation settings. The used grid box parameters are the following: 1. The number of points in x,y,z-dimensions were 40; 2. the spacing was 0.603 Å; and 3. the Cartesian coordinate of the center grid box was (42.385, 51.723, 36.551). The used docking parameters are the following: 1. The search parameter was genetic algorithm (GA); 2. the number of GA run was 30; and 3. the output was chosen to be written as Lamarckian GA.

The results from MOE and AutoDock were then subject for analysis and comparison. The method is considered as valid and acceptable if the root mean square deviation (rmsd) of the best suggested pose to the original pose is less than or equal to 2.00 Å [23].

Molecular docking of curcumin to DPP-4

Curcumin has two tautomeric forms, the enol form (**3a**) and the keto form (**3b**) [24]. Each tautomer was drawn in MOE using Molecule Builder module and stored as a moe file. The structures were subjected to conformational search in MOE using Conformations Stochastic Search module. The global minimum conformation of each tautomer was minimized in MOE by using Energy Minimize module. AM1 semi empirical method with gradient of 0.001 was employed. The minimized structures were stored as both moe and pdb

files. Similar procedures described in the previous subsection were performed using these files as the ligand input files. The moe files were use as the ligands input files for docking procedure in MOE while the pdb files were use as the ligands input files for docking procedure in ADT.

RESULT AND DISCUSSION

Method validation and benchmarking

Biftu *et al.* suggested two different binding modes of compound **4** to DPP-4 (Figure 2) and put those two conformations together in the submitted crystal structure [1]. This makes MOE fails to read the pdb files and results in a distorted structure of compound **4**. PyMol can distinguish those two conformations and therefore was employed to separate the structures which in this article are assigned as conformer A and conformer B (Figure 2). The pdb file of the macromolecule (DPP-4) was also prepared using PyMol. Both ligands and macromolecule were obtained from Chain A of the crystal structure [1]. MOE and AutoDock are chosen to be the docking applications since MOE provides most of common methods in molecular modeling and start to be used widely in medicinal chemistry field of study [19], while AutoDock has served as the most docking application used so far and has provided some successes in the drug discovery research [25, 26]. In fact, AutoDock has proven as a useful tool in the discovery of the first clinically-approved HIV integrase inhibitor, raltegavir (Isentress®; Merck) [27].

The molecular docking results of compound **4** to DPP-4 obtained from MOE and AutoDock are presented in Table 1. The best docking poses obtained from MOE can reproduce the original poses both for conformers A (rmsd = 1.98 Å) and B (rmsd = 1.76 Å), while AutoDock can reproduce the original pose for conformer A (rmsd = 1.87 Å) but not for conformer B (rmsd = 2.34 Å). This indicates that conformer A is suggested to be the preferred conformation of the interaction of compound **4** to DPP-4. This also indicates that MOE can sample more conformational spaces that represent the original interaction poses of compound **4** to DPP-4 than AutoDock. Unfortunately, it is not possible to compare the calculated free energy of binding ($\Delta G_{binding}$) to the experimental data since Biftu *et al.* [1] provide only the IC_{50} data. Due to the fact that the conformer A of compound **4** can be reproduced by both MOE and AutoDock while conformer B can only be reproduced by MOE, further studies are focused on the conformer A as the reference structure.

Molecular docking of curcumin to DPP-4

The molecular docking results of tautomers **3a** and **3b** to DPP-4 obtained from MOE and AutoDock are presented in Table 2. In MOE, the calculated $\Delta G_{binding}$ of tautomer **3a** (-5.41 kcal/mol) is slightly better than the calculated $\Delta G_{binding}$ of tautomer **3b** (-5.34 kcal/mol). The similar phenomenon is found in the docking results from AutoDock, the calculated $\Delta G_{binding}$ of tautomer **3a** (-6.94 kcal/mol) is slightly better than the calculated$\Delta G_{binding}$ of tautomer **3b**(-5.94 kcal/mol).

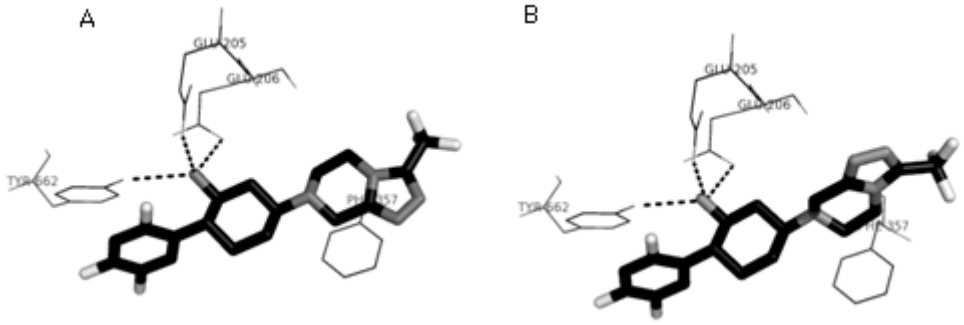

Figure 2. Two conformations of compound **4** in the binding pocket of DPP-4 proposed by Biftu *et al.* [1]. Only residues which interact with the ligand are presented here. Carbon atoms are presented in black, hydrogen atoms are not shown, fluorine atoms are presented in white, nitrogen atoms are presented in dark grey, and oxygen atoms are presented in light grey; A) Conformer A: The triazole moiety is stacked over the side chain of Phe357; and B) Conformer B: The piperazine moiety is making a side-to-face hydrophobic interaction with Phe357.

Table 1. The molecular docking results of the reference compound (compound **4**) to DPP-4

No.	Ligand	MOE		AutoDock	
		RMSD (Å)	Calculated $\Delta G_{binding}$ (kcal/mol)	RMSD (Å)	Calculated $\Delta G_{binding}$ (kcal/mol)
1.	Conformer A	1.98	-5.97	1.87	-9.67
2.	Conformer B	1.76	-6.14	2.34	-9.50

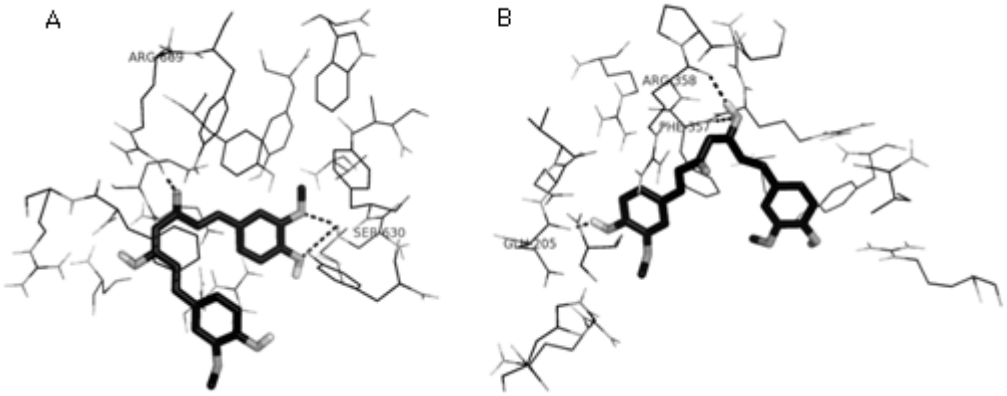

Figure 3. The interactions of curcumin in the enol form (**3a**) to the binding pocket of DPP-4 (Binding pocket is defined as the residues within 4.5 Å from the ligand). Carbon atoms are presented in black, polar hydrogen atoms are presented in white, nitrogen atoms are presented in light grey, and oxygen atoms are presented in dark grey; A) The binding pose suggested by docking studies using MOE: The oxygen carbonyl of curcumin interacts with the side chain of ARG 669, while in the one aromatic moiety of curcumin, both of the oxygen atoms interact with the side chain of SER 630; and B) The binding pose suggested by docking studies using AutoDock: The hydrogen atom from the hydroxyl group in the enol moiety of curcumin interacts with the backbone of PHE 357 and ARG 358, while in the one aromatic moiety of curcumin, the hydrogen atom from the hydroxyl group interacts with the backbone of GLU 205.

Table 2. The molecular docking results of curcumin (**3**) to DPP-4

No.	Ligand	Calculated $\Delta G_{binding}$ (kcal/mol)	
		MOE	AutoDock
1.	Tautomer enol (3a)	-5.41	-6.94
2.	Tautomer keto (3b)	-5.34	-5.94

Based on these results, it can be concluded that curcumin prefers to have the enol tautomer to interact with DPP-4. This conclusion is in line with the result of the curcumin tautomerization studies by Istyastono *et al.* [24].

The binding poses of curcumin in the enol form (**3a**) based on the docking results both from MOE and AutoDock are presented in Figure 3A and 3B, respectively. In Figure 3A can be seen that curcumin forms hydrogen bonds with the side chain of ARG 669 and the side chain of SER 630 from DPP-4. The binding pose suggested by the docking studies using AutoDock (Figure 3B) shows that curcumin interacts with the backbone of GLU 205, PHE 357, and ARG 358. Despite the different system in each docking applications, one additional hydrogen bond in the result from AutoDock explains the better calculated $\Delta G_{binding}$ compare to the result from MOE. However, when the calculated $\Delta G_{binding}$ results of the interaction of curcumin (**3a**) to DPP-4 from each docking application (Table 2) compare to those of the interaction of compound **4** to DPP-4 (Table 1), it shows clearly that curcumin is needed to be optimized in order to obtain better affinity. Since it falls into the definition of a lead compound proposed by Oprea *et al.* [28], shows relevant interactions with DPP-4, and is still able to be optimized, curcumin can serve as a good lead compound in the development of new DPP-4 inhibitor. Notably, some curcumin analogs have been patented and developed [15, 16 benzylidene cyclopentanone, and benzylidene acetone, and therapeutic uses thereof #16 benzylidene cyclopentanone, and benzylidene acetone, and therapeutic uses thereof #16]. The results of the studies presented in this article can serve as an initial effort of the further study in the development of curcumin analogs as new DPP-4 inhibitors.

CONCLUSION

Both MOE and AutoDock serve as valid and acceptable docking applications to study the interactions of small compound to DPP-4. Notably, MOE can sample more conformational spaces that represent the original interaction poses than AutoDock. Though curcumin exists in two tautomers, the docking results show that curcumin prefers to have the enol tautomer to interact with DPP-4. The best docking pose result from MOE shows that the oxygen carbonyl of curcumin interacts with the side chain of ARG 669, while in the one aromatic moiety of curcumin, both of the oxygen atoms interact with the side chain of SER 630. On the other hand, the binding pose suggested by docking studies using AutoDock shows that the hydrogen atom from the hydroxyl group in the enol moiety of curcumin interacts with the backbone of PHE 357 and ARG 358, while in the one aromatic moiety of curcumin, the hydrogen atom from the hydroxyl group interacts with the backbone of GLU 205. The calculated $\Delta G_{binding}$ results from MOE and AutoDock shows that

curcumin is needed to be optimized to reach similar or better $\Delta G_{binding}$ compare to compound **4**. Curcumin can serve as a good lead compound in the development of new DPP-4 inhibitor. The results of these studies can serve as an initial effort of the further study.

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