Blind Docking Study of Compound D and Dimethoxyphenylbutadiene (DMPBD) Against Cyclooxygenase-2

Prasan Tangyuenyongwatana* and Maneerat Sombutaree

Graduate Program, College of Oriental Medicine, Rangsit University, Phathumthani, 12000, Thailand
*Corresponding author, E-mail: prasan.t@rsu.ac.th

Abstract

In this study, blind docking method was applied to discriminate against the ligand-protein binding of 4-(3,4-dimethoxyphenyl)but-1,3-diene (DMPBD) and (*E*)-(3,4-dimethoxyphenyl)but-3-en-1-ol (compound D) at the active site of cyclooxygenase-2 (COX-2). These two compounds can bind flawlessly in the COX-2 active site with nearly the similar docking energies with conventional docking method, but only DMPBD was the active compound (IC_{50} = 20.68 μM). Blind docking was achieved for the finding of the possible binding site of ligand by skimming the entire surface of COX-2 protein. Cyclooxygenase-2 (PDB ID: 1CX2, 1PXX, 3NTG) crystal structures were used in this research, and the blind docking was run by PyRx 0.8 program. The experimental results of DMPBD at exhaustiveness 4 showed the positive binding ratio of DMPBD/compound D at the active site of three enzymes (1CX2, 1PXX, 3NTG) in the ratio values of 1.63, 2.08, and 2.43, respectively, meaning that DMPBD was more appropriate than compound D in binding with enzymes. These results demonstrated that there was some specificity of DMPBD to bind with the enzymes which may due to the low rotational bond and rigidity of DMPBD structure, making it easily reach lower energy of the active site. In contrast, compound D that has more flexible and more polar structure was unable to give good conformation for the lowest energy at exhaustiveness 4. These blind docking outcomes were compatible with the *in-vitro* test result. However, blind docking can work well with some enzymes. This technique has to be studied more about the parameters that concern with the searching.

Keywords: *Blind docking, Cyclooxygenase-2, DMPBD, Compound D, PyRx*

1. Introduction

Compound D ((*E*)-(3,4-dimethoxyphenyl)but-3-en-1-ol) and DMPBD (4-(3,4-dimethoxyphenyl) but-1,3-diene) are well-known active compounds derived from rhizomes of *Zingiber cassumunar* Roxb, a Thai tradition medicinal plant. The *Z. cassumunar* rhizomes also express various biological activities (Kiatyingungsulee, Wangmud, Swasdimongkol, & Mokkhasmit, 1979) such as anti-inflammatory (Panthong, Kanjanapothi, Niwatananun, Tuntiwachwuttikul, & Reutrakul, 1990; Jeenapongsa, Yoovathaworn, Sriwatanakul, Pongprayoon, & Sriwatanakul, 2003) uterus, intestine, and stomach relaxant (Anantasan, 1982). Researchers from Korea studied on inhibition effect of phenylbutanoids from rhizomes of *Z. cassumunar* (Han, Kim, Jeong, Lee, & Seo, 2005). Two phenylbutanoid dimers, (±)-trans-3-(4-hydroxy-3-methoxyphenyl)-4-[(E)-3,4-dimethoxystyryl]cyclohex-1-ene and (±)-trans-3-(3,4-dimethoxyphenyl)-4-[(*E*)-3,4-dimethoxystyryl]cyclohex-1-ene were found for exhibiting COX-2 enzyme with IC_{50} values of 3.64 and 2.71 μM, respectively. Furthermore, the other two small phenylbutanoids, which were DMPBD and Compound D (Figure 1) exhibited weakened inhibition activity (IC_{50} 20.68 and >50 μM). Nevertheless, a later compound that was believed to have COX-2 activity and the structure a little different from DMPBD were inactive to COX-2 active site at IC_{50} over 50 μM. The *in-vitro* testing result made some unclear to the previous study of the inflammatory test of compound D in animal (Kiatyingungsulee et al., 1979; Panthong et al., 1990). Because of the small size of both molecules, molecular weights of compound D and DMPBD are 208 and 190.2. The lipophilicity of both compounds was calculated and obtained log P of 1.95 and 2.96, respectively. These two structures have nearly the same structural conformation when performed the energy optimization. The objective of this experiment is to find out the reasons that make compound D inactive.

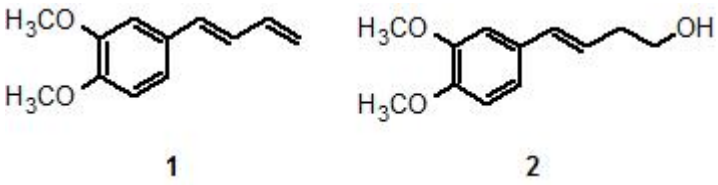


Figure 1 Structure of DMPBD (**1**) and Compound D (**2**)

The most influential technique in drug discovery is molecular docking. This technique focuses on the location and alignment of a ligand in the target protein active site. Most active compounds are a small molecule that can specifically bind to a receptor. Most of the drug discovery recognizes the pocket site of the target enzyme, and it can proceed with the regular molecular docking, which focuses on a small part of the protein. (McAdam, Catella-Lawson, Mardini, Kapoor, & Fitzgerald, 1999). When ligand-protein binding, many forces are used in the molecular interaction such as weak force (hydrophobic, dispersion or van der Waals) and stronger interaction (hydrogen bonding, and electrostatic force) These forces can occur together for stability of ligand-protein complex (Willoughby, Tomlison, Gilroy, & Willis, 1996). In the area of docking technique, there are two kinds of docking approaches, the focused and the blind docking methods. The focus method is the conventional docking method that active site is assigned at a specific location. Next, when the information of the active site of protein is unclear, the blind docking is performed to find the possible active sites (Sehgak, Tahir, Hammad Mirza, & Mir, 2018). The docking algorithm will pursuit the whole surface of the enzyme for the active site. This alternative docking method is practiced to unravel many complications such as the modeling of peptide inhibition of T-cell adhesion (Cohen, 1996), determining substrate-binding pose (Ghersi & Sanchez, 2008) and comparison of a microtubule-stabilizing agent such as taxol and colchicine with laulimalide and peloruside (Pineda, Farras, Maccari, Manetti, Botta, & Vilarrasa, 2004). In this work, the blind docking technique was applied to explain the protein-ligand binding of COX-2, DMPBD and compound D.

2. Objectives

The objective of this research was to solve the ligand-protein binding of DMPBD and compound D with three COX-2 proteins using blind docking technique.

3. Materials and Methods

Software for this research composed of PyRx 0.8 virtual screening tool (http://pyrx. sourceforge.net.), ChemSketch program (http://www.acdlabs.com /resources /freeware /chemsketch/), Avogrado package (http:// avogadro. cc/ wiki/Main_Page) and Discovery Studio Visualizer (https://www.3dsbiovia.com/ products/ collaborativescience/ bioviadiscoverystudio/ visualizationdownload. php). Three cyclooxygenase-2 (PDB ID: 1CX2, 1PXX, 3NTG) proteins, were obtained from RCSB Protein Data Bank. The ligands in cyclooxygenase-2 x-ray crystal structures were used as positive control compounds.

3.1 Ligand structures building

The DMPBD and compound D ligand structures were drawn in 2D using ChemSketch program. After that, they were converted to 3D structures together with energy minimization in Avogrado program. For the lipophilicity of each compound, Molinspiration engine v2016.10 (http://www.molinspiration.com) was used to calculated log P and number of rotational bonds.

3.2 Blind-docking studies

The ligand-free chain A of cyclooxygenase-2 crystal structures (PDB ID: 1CX2, 1PXX, 3NTG) were loaded in PyRx program in pdbqt format. This program used AutoDock Vina as an engine for docking (Trott & Olson, 2010). The ligand also was added into the protocol in pdbqt format. Then the ligand and protein were chosen, the grid box mechanically displayed, and the co-ordinate of the center of the active site

was marked together with the dimensions (Table 1). The exhaustiveness value was set equal to 4. Then click run for stating the blind docking for 100 runs repeating three times. The positive results were obtained by review each docking pose ligand comparing with x-ray crystal structure in cyclooxygenase-2 receptors using Discovery Studio. SC558, which is 1CX2 ligand, was overlaid with the docking result.

Table 1 The co-ordination and box dimensions in PyRx 0.8 for blind docking of each protein.

PDB ID	Co-ordination	Box Dimensions	X-ray structure resolution (A°)
1CX2	X = 27.9479 Y = 25.8694 Z = 8.3232	X = 56.4552 Y = 70.0910 Z = 59.4676	3.00
1PXX	X = 30.1828 Y = 27.0212 Z = 7.3387	X = 56.0285 Y = 70.7634 Z = 61.0023	2.99
3NTG	X = 28.6012 Y = 25.9978 Z = 8.3100	X = 58.6000 Y = 72.5171 Z = 63.9160	2.19

3.3 Focused docking study

The ligand-free chain A of COX-2 crystal structures (PDB ID: 1CX2, 1PXX, 3NTG) were loaded in PyRx program in pdbqt format. After that, the ligand was imported into the protocol in pdbqt format. Then both of them were chosen, the grid box was mechanically display, and the co-ordinate of the active site was marked for 1CX2 (X =23.9644, Y = 21.5188, Z = 15.2493), 1PXX (X =26.6973, Y = 23.3497, Z = 14.2468), 3NTG (X =26.8443, Y = 21.5152, Z = 17.2699) with the exhaustiveness 8. The dimensions of the searching area were set to 25 × 25 × 25 Å. Then click run for stating the focused docking. The positive results were obtained by review each docking pose ligand comparing with x-ray crystal structure in COX-2 protein using Discovery Studio.

4. Results and Discussion

For amino acids in the active site of COX-2 which are ARG120, TYR385, VAL434, ARG513, PHE518, VAL523, and SER530, the most important one is position 523, this position is different in one methyl group, which in COX-1 is isoleucine and in COX-2 is valine. This difference of a one methyl group is satisfactory to let an inhibitor to entry a side pocket in COX-2 (Botting & Botting, 2004). According to focused docking, DMPBD and compound D were docked into the assigned active site of 1PXX protein with the docking energy of -6.6 and -6.4 Kcal/mol. For 1PXX, these two compounds exhibited almost the same conformation. The result showed slightly different in the interaction between amino acids, inactive sites, and ligands (Figure 2 and 3). SC558 was chosen as a ligand for cross-docking to check the side pocket (VAL523) of COX-2 in 1PXX. DMPBD interacted with the crucial amino acids TYR355, VAL523, ALA527, VAL349 that specific to COX-2 activity when compared with SC558 while compound D showed one more amino acid interaction with the 1PXX than DMPBD. For this focused docking information, there were no ligands selective of COX-2 inhibitor between DMPBD and compound D because both compounds interacted with almost the same set of amino acids. This result showed some confusing why compound D was inactive in the enzyme inhibition experiment test.

In this study, an alternative approach, blind docking was performed. The crucial notion behind the technique is to search for the whole protein surface. The location of the compound after blind docking was assessed comparing to x-ray ligand to see that the compound location placed in the correct site or not (Figure 4). After 300 runs, the average positive docking results of DMPBD and compound D with three enzymes at exhaustiveness equalling to 4 were obtained. The variation of exhaustiveness values is previously studied on phenylbutanoid dimers which it plays a crucial part in positive or negative docking results (Sapjaroen &Tangyuenyongwatana, 2019). From the experimental docking results, both ligands presented the correct docking position where DMPBD and compound D placed in the pocket of COX-2 (Figure 3) with different

percentages of binding (Table 2). Table 3 showed the blind docking binding ratios results of compounds with different COX-2. These three COX-2 enzymes were chosen to test the blind docking experiment in which there were different environments of the active site, which open a wide opportunity for test compounds to bind.

Table 2 The average positive docking results of DMPBD and compound D with three enzymes at exhaustiveness equalling to 4* compared to the inhibitory activity of COX-2 enzyme from Han et al., (2008)

Enzymes	DMPBD (%)	Compound D (%)	IC_{50} DMPBD	IC_{50} Compound D
1CX2	26.7 ± 1.2	16.3 ± 2.1	20.68 µM	> 50 µM
1PXX	24.3 ± 3.3	16.3 ± 2.1	20.68 µM	> 50 µM
3NTG	24.3 ± 3.3	16.0 ± 2.0	20.68 µM	> 50 µM

*Each value was calculated from three times and each time made 100 runs for 3 times.

Table 3 The ratios of DMPBD binding to right position over compound D at exhaustiveness equaling to 4

Enzymes	DMPBD/Compound D
1CX2	1.63
1PXX	2.08
3NTG	2.43

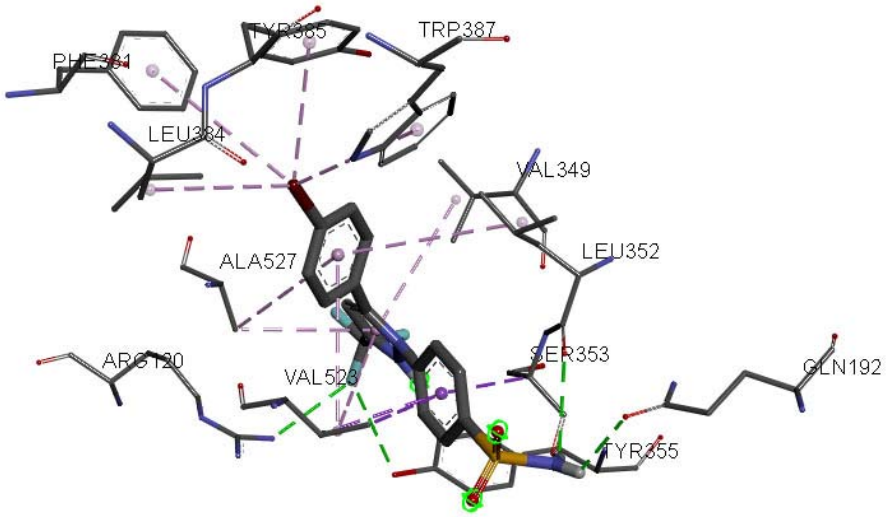
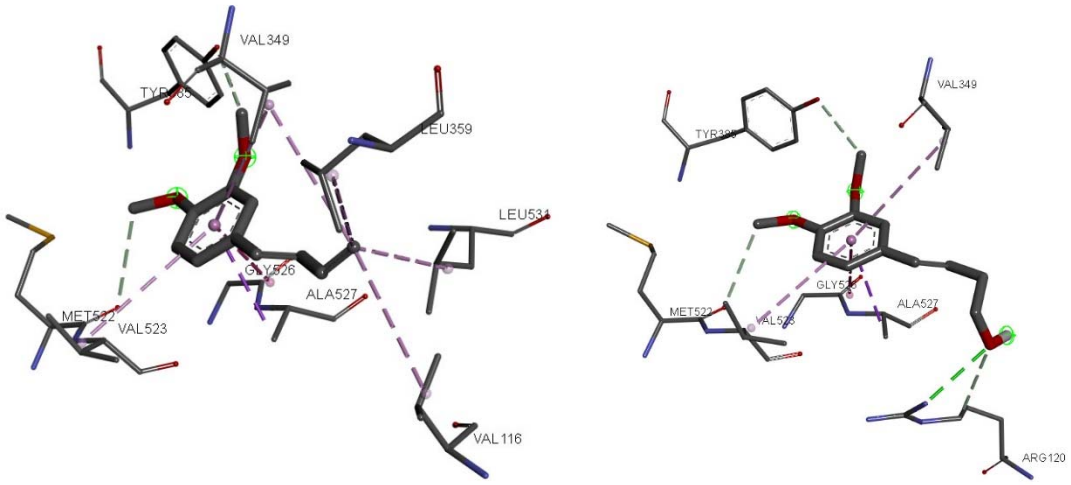


Figure 2 SC558 interacted with amino acids in 1PXX active site



(a) (b)

Figure 3 (a) DMPBD interacted with amino acids especially TYR355, VAL523, ALA527, VAL349 while compound D (b) showed slightly different interactions with an active site with TYR355, VAL523, ALA527, VAL349, ARG120.

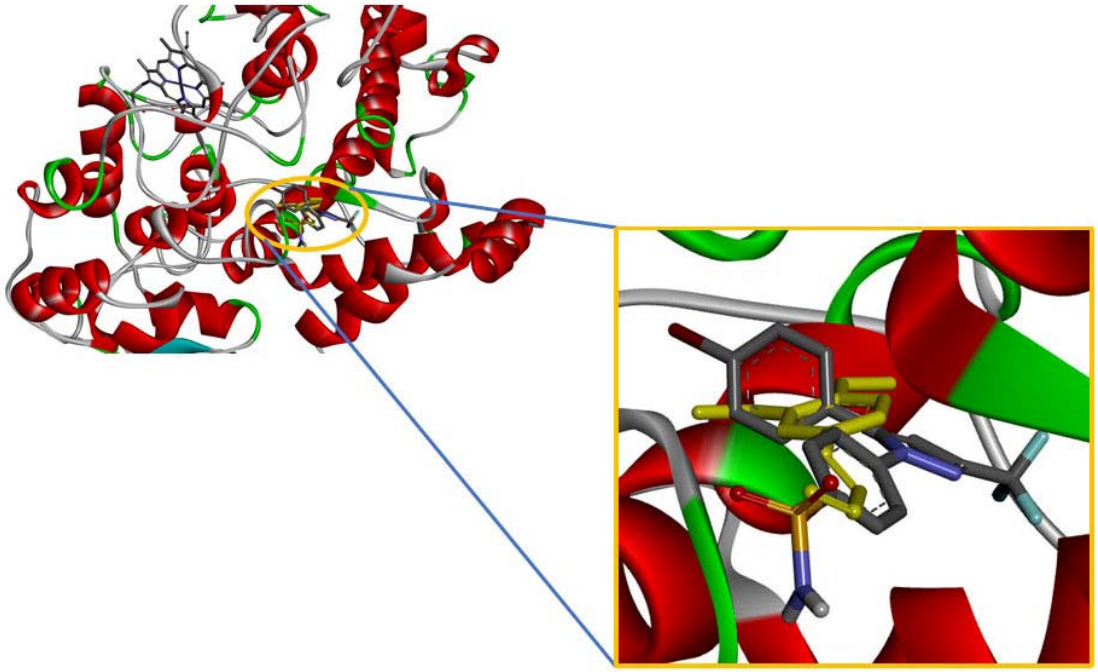


Figure 4 DMPBD (yellow) inserted in the active site of cyclooxygenase-2 (1PXX)

The explanation for blind docking solving this selectivity problem was that DMPBD and compound D are small molecules. They can travel around the big box searching area in the blind docking protocol. AutoDock Vina calculates the energy of the protein to locate the minimum energy sites around the protein and the ligand try to reach to the minimum local site that closes to it. The low exhaustiveness value equalling to 4 was used to give a chance for a more stable structure to reach the local minimum active site quickly. So, the positive docking results are quite low, but they can differentiate DMPBD from Compound D. These

results demonstrated that there was some specificity of DMPBD to bind with the enzymes which may due to the low rotational bond and rigidity of DMPBD structure making it can easily reach to lower energy of the active site. While compound D which has more flexible and more polar structure was unable to give good conformation for lowest energy at exhaustiveness 4. The negative binding result may appear at some low energy sites or some unidentified space. If we use exhaustiveness equalling to 8 or more, the different structures will have enough time for varying their structural conformation to lower minimum energy that they will be no different in binding with the active site. That means the high exhaustiveness value cannot discriminate DMPBD and Compound D binding to COX-2 protein. For the consistency of the experimental results, AutoDock Vina will give some consistency which depends on how many runs the experiment carry on. The higher the number of runs, the more consistency of the result is. Also, it will not give the same number, poses or same binding energy for each run because in its' algorithm every new run will start with the random conformation of ligand.

5. Conclusion

In this study, the focused docking gave nearly the same result of both DMPBD and compound D in binding with COX-2 proteins. The blind docking technique was used to try the docking of both ligands with COX-2 enzymes. The method used exhaustiveness value of 4 that could discriminate DMPBD and compound D binding with COX-2. The experiment found that DMPBD over compound D insertion ratios were in the range of 1.63-2.43 times. These results gave a chance to explain the blind docking results matching with the *in-vitro* test result and help us to understand compound D that it is not a good substrate of COX-2.

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7. References

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