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Docking and Molecular Dynamics Simulation of Carbonic Anhydrase II Inhibitors from Phenolic and Flavonoid Group

Reza Aditama, Didin Mujahidin, Yana Maolana Syah, Rukman Hertadi*

Department of Chemistry, Bandung Institute of Technology, Jalan Ganeca no 10 Bandung, 40132, Indonesia

Abstract

Carbonic Anhydrase II (CAII) has role in pH regulation, water transport and hydration of CO_2. In addition, CAII is also related to many diseases, including glaucoma, tumours, epilepsy, diabetes and osteopetrosis. Various inhibitors for CAII have been developed and commercialized as a drug. Recent development of CAII inhibitors drive the invention of novel inhibitors based on natural product structures and their derivatives. This research aim to screen potential inhibitors from phenolic and flavonoid groups by *in silico* approach. The screening of natural products compounds was performed by a molecular docking method. The best ligand derived from the molecular docking selection was further refined with a molecular dynamics simulation and the resulted structure was used to evaluate the stability of CAII-ligand complex. By using the upper mentioned procedures, fisetin (Fic) and 6-(3,4-dihydroxyphenyl)-5,6,7,8-tetrahydronaphthalene-1,3,7-triol (Afr3) were strongly suggested to be a potent inhibitor for CAII.

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Keywords: docking; flavonoid; carbonic anhydrase inhibitor; molecular dynamics simulation

1. Introduction

Carbonic anhydrase II (CAII) has physiological function to catalyze the hydration reaction of carbon dioxide into bicarbonate and *vice versa*. In addition, this enzyme also plays an important role in regulating the body's pH, water transport, and maintain electrolyte balance in the body. This enzyme has several isozymes, namely CAI, II, III, VII

* Corresponding author. Tel.: +62 222-502103 ; fax: +62 222-504154.
E-mail address: rukman@chem.itb.ac.id

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(contained in cytosol), CAIV, IX, XII, XIV (located in cell membrane), CAV (mostly in mitochondria) and CA VI, which is typically found in saliva[1]. Almost every isozyme associated to certain diseases, such as cancer, diabetes, glaucoma, etc. Diseases related directly to CAII are diabetes and glaucoma[2].

CAII inhibitors that have been invented to date are still questionable in terms of safety usage. Inhibitors that have been commercially available, such as acetazolamide, methazolamide, and exthozolamide[3,4,5] have strong inhibition power for CAII, but they were not specific. Those compounds are not only inhibits CAII but also other CA isozymes. Therefore, the use of the above compounds may cause side effects, such as numbness, tingling, depression, fatigue, weight loss, gastro-intestinal irritation, metabolic acidosis, and transient myopia[3,5]. The other commercial inhibitor, such as topiramate is still quite weak to inhibit CAII activity[1]. This motivate many researchers to search novel compound for CA inhibitors. One way to obtain such inhibitor is by screening from the database of natural products and their derivatives. The reason of choosing the natural product is to minimize the unwanted side effect, thereby improving the safety of the inhibitor. Natural products from the phenolic group compounds have been prooved to have strong inhibition power for CAII[6,7]. Therefore, it open the way to design novel inhibitors from the phenolic-based natural product.

The biggest obstacle in finding a potential drug compound is the vast number of variants that must be constructed and tested individually in the selection phase. Currently, the flow of information and the need of new drugs is increasing. This lead to the development of drugs solely rely on the experimental approach will not likely pursue the existing demands. Fast improvement of computer hardwares and softwares make it possible to carry out high-throughput srceening, thereby enhancing the speed of discovery for new potential drugs. Molecular docking is one of the popular tool for the high-throughput screening to get a novel potential candidate for drugs. It has become a routine protocol in the process of drug design in the pharmaceutical industry and academia to minimize the effort, time and cost required for the invention of new potential drug candidates.[8]

Molecular docking algorithm is developed mostly to find best pose of a ligand molecule inside the binding site of a receptor. The best pose is selected based on the lowest value of interaction energy among the ligand and the residues inside the binding site. Molecular docking method typically consider a protein as a rigid molecule. Some molecular docking program add induced-fit feature but on a limited basis. Therefore, a protein-ligand complex generated by the molecular docking is typically further refined by using a molecular dynamic (MD) simulation. The combine methods between molecular docking and MD simulaton is well-known as the rational drug design. The approach is expected to increase the odds of discovery of a new potential drug.[8]

2. Methods

Molecular structure of each inhibitors was prepared and minimized using Marvin Sketch program with MMFF94 Force Field. Molecular docking was carried out by using GOLD[9] employing genetic algorithm for searching best pose of ligand inside the binding site of CAII structure. GoldScore were used to calculate the interaction energy that display as a docking score. Initial structure of CAII was obtained from protein data bank (PDB) with accession code 2Q38. Grid box used in docking simulation was 14 x 20 x 20 $Å^3$. Schematic representation of interaction among a ligand and residues inside the binding site of the receptor was generated by LigPlot programs[10]. Binding posed generated by molecular docking was used as an initial coordinate for MD simulation. Each set of protein's atoms was parameterized by AMBER force fields 2012. Parameters for Zinc ion and the catalytic site of CAII were generated by cationic dummy atom[12] while parameters for a ligand were generated by ANTECHAMBER. CAII-inhibitor complex was then solvated by 45,405 TIP3P water molecules. MD simulation protocol was first began with the energy minimization step to relieves bad contacts in the structure. The minimized structure was then heated gradually started from 0 K to 310 K with NVT ensamble while preserving the backbone atom position by applying a harmonic constraint with the magnitude of 10 kkal/mol. After the heating process, the system was bring to the equilibration state by using NPT ensamble for 250 ps at 310 K and 1 atm and the applied harmonic constraint was removed gradually in the course of this step. The equilibrated system was finally used for MD production run for 2 nano second (ns). Potential energy for non-bonding interactions was calculated within the cut off of 12 Å. In this MD simulation, potential energy for long-range electrostatic interactions were calculated by particle-mesh Ewald method[12]. All MD simulations were performed by using AMBER version 12.0[13]. VMD 1.9.2[14] was used to visualize and analyze the simulation trajectory.

3. Results and discussion

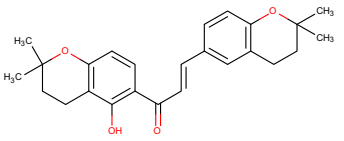
Initial stage prior molecular docking experiment was the optimization of docking parameters by using the original inhibitor found in the initial structure of CAII-LSA503 complex. LSA503 molecule was taken out from the structure then was used for testing the docking parameters until the generated pose of the ligand by the docking closed to the original pose in the crystal structure. The optimized parameters for the docking in this experiment gave the root mean-square deviation (RMSD) about 0.78 Å between the predicted ligand pose and the original one. These parameters were then used for docking other ligands in this study.

Forty phenolic and flavonoid molecules (40 ligand isolated from 10 different Indonesian plants by Natural Products Research Group of Institut Teknologi Bandung) have been docked into CAII active site using GOLD software. The chemical structures of ligands are shown in Fig 1. Binding affinity of those ligand samples to CAII were evaluated according to their fitness score, which vary from -218.22 to 51.46 (**Table 1**). Fitness score reveal the binding affinity of each ligand samples to the receptor, thereby the higher of the fitness score the better of the binding affinity In this experiment, we found fisetin (Fic) from *Dipterocarpaceae* and 6-(3,4-dihydroxyphenyl)-5,6,7,8-tetrahydronaphthalene-1,3,7-triol (Afr3) from *A. fretessi* have the highest fitness score, which was 51.46 and 51.31, respectively. These results are in accordance with the previous experiment carried out by Sahin et.al[7] showing that compounds from polyphenols and flavonoid group, including fisetin, have strong inhibitory effects towards CAII activity with IC_{50} about 0.76 μg/mL.

Table 1. Fitness score resulted from docking phenolic and flavonoid molecules into CAII binding site.

No	Sources	Molecules	Fitness Score
1	A. bracteata	Abr1	50.69
2	A. bracteata	Abr2	50.06
3	A. champeden	Ach1	50.89
4	A. champeden	Ach2	42.11
5	A. champeden	Ach3	42.56
6	A. champeden	Ach4	51.03
7	A. champeden	Ach5	50.71
8	A. champeden	Ach6	49.79
9	A. champeden	Ach7	50.85
10	A. champeden	Ach8	45.96
11	A. fretessi	Afr1	46.92
12	A. fretessi	Afr2	41.36
13	A. fretessi	Afr3	51.31
14	A. gomezianus	Ago	50.81
15	A. lanceifolius	Ala1	34.56
16	A. lanceifolius	Ala2	44.57
17	A. lanceifolius	Ala3	39.28
18	A. lanceifolius	Ala4	35.93
19	A. lanceifolius	Ala5	38.28
20	A. lanceifolius	Ala6	39.36
21	A. maingayii	Ama	36.16
22	A. rotunda	Aro1	46.37
23	A. rotunda	Aro2	51.17
24	A. schortechinii	Asc	49.15
25	A. teysmannii	Ate	44.20
26	Dipterocarpaceae	Dip1	-59.29
27	Dipterocarpaceae	Dip2	45.43
28	Dipterocarpaceae	Dip3	42.63
29	Dipterocarpaceae	Dip4	41.93

30	Dipterocarpaceae	Dip5	-218.22
31	Dipterocarpaceae	Dip6	8.77
32	Dipterocarpaceae	Dip7	0.99
33	Dipterocarpaceae	Dip8	36.36
34	Dipterocarpaceae	Dip9	41.03
35	Dipterocarpaceae	Dip10	13.8
36	Dipterocarpaceae	Fic	51.46
37	M. Pruinosa	Mpr	50.84
38	M. Recuvarta	Mre	45.31
39	M. Rhizinoides	Mrh	44.24
40	Macaranga andenoceras	Mac	43.61



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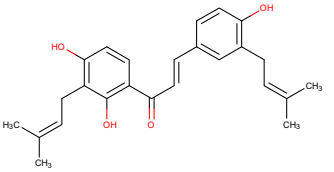

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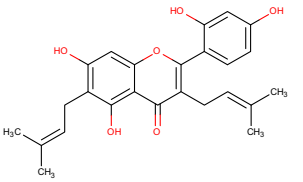

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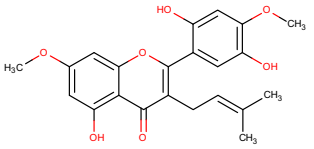

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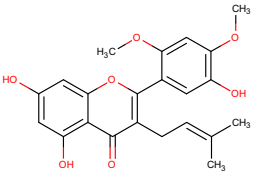

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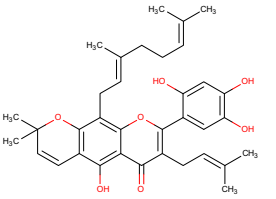

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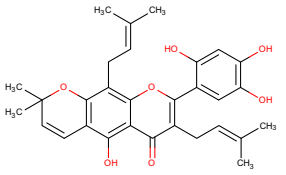

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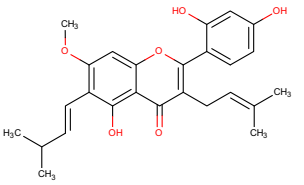

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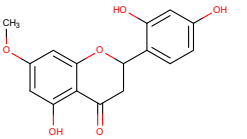

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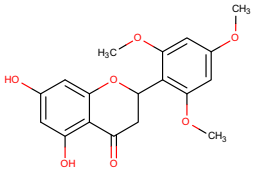

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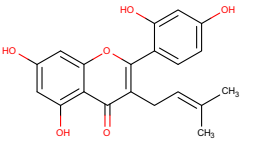

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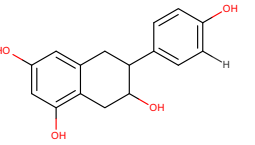

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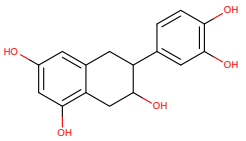

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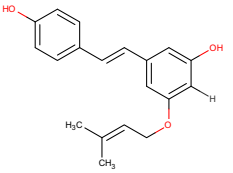

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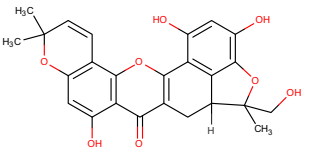

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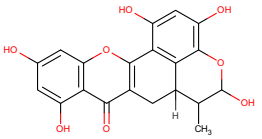

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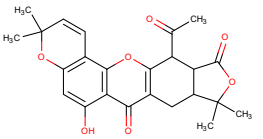

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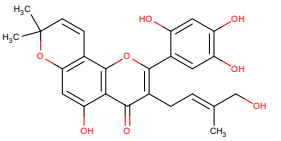

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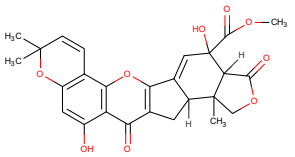

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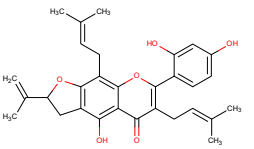

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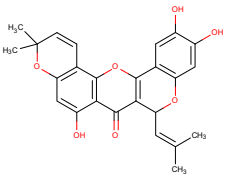

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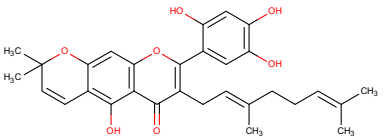

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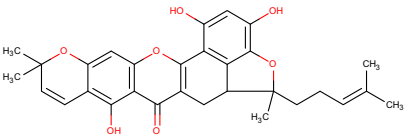

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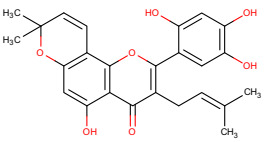

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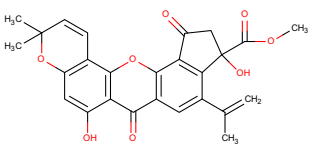

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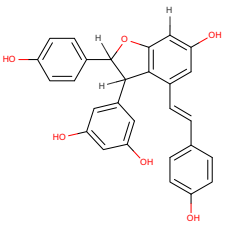

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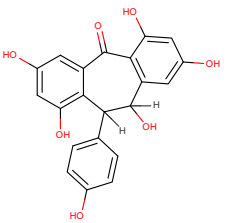

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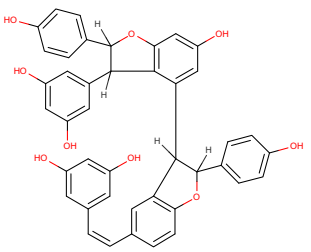

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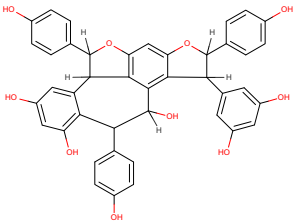

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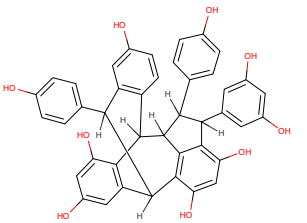

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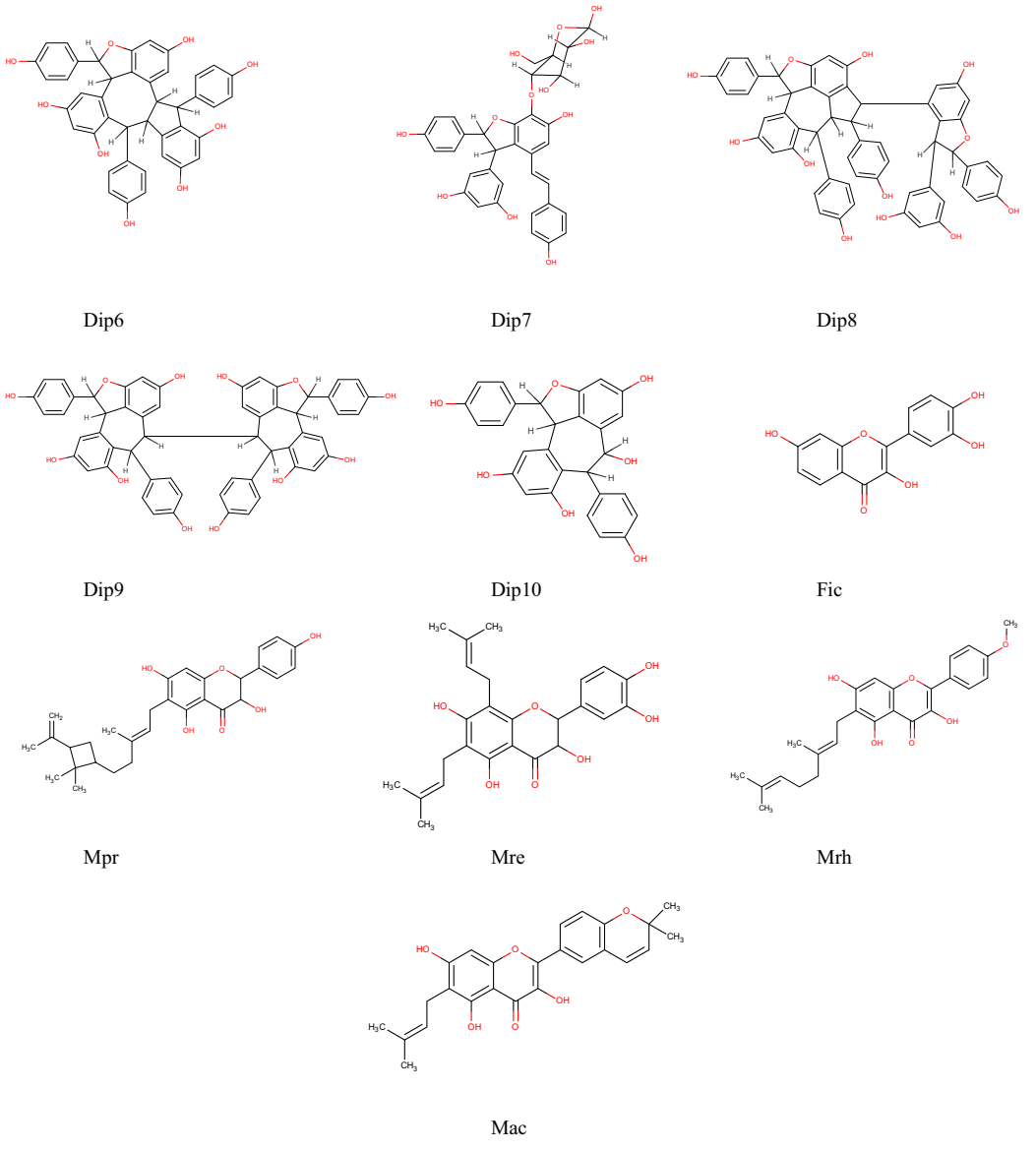


Fig. 1. Molecular structures of inhibitor

Further analysis by using LigPlot progam to evaluate interactions of both Fic and Afr3 ligands inside the binding site of CAII revealed some interacting residues and zinc ion involved in the binding with both ligands (Fig. 2). We noticed that both ligands have the same interaction mode with CO_2 (the substrate molecule of CAII), in which both Fic and Afr3 molecules are replacing the position of CO_2 molecule to make coordination bond with Zn^{2+} ion that also covalently bound with tetrahedral conformation to His94, His96, and His119 residues. In addition, the position of both ligand molecules inside the binding site of CAII were also stabilized by hydrogen bonds with surounding residues. in which for Fic molecule, it made hydrogen bonds with Asn67, Thr 199 and Thr 200 residues, while for Afr3 with Asn62, Gln92 and Thr199. We also noticed that CAII binding site have some non-polar residues that may contribute to the stability of the ligand binding via hydrophobic interactions. Although the type of interactions stabilizing both ligands are similar but Fic molecule has better fitness score compare to Afr3. This can be explained

by the difference in the closest distance of zinc ion to Fic and Afr3 molecules, which were 2.64 Å and 3.12 Å, respectively (Fig. 2).

(a) (b)

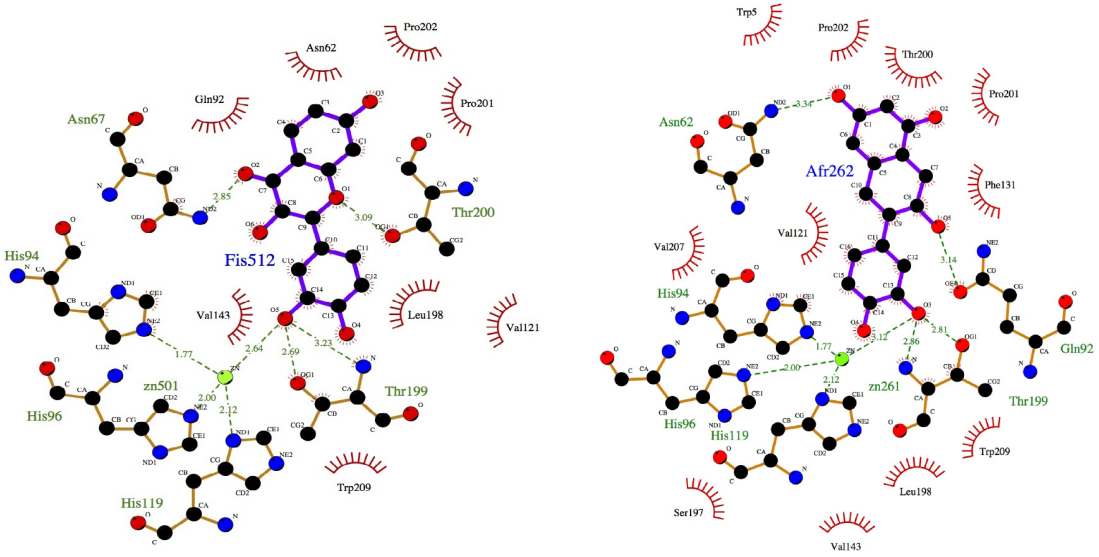
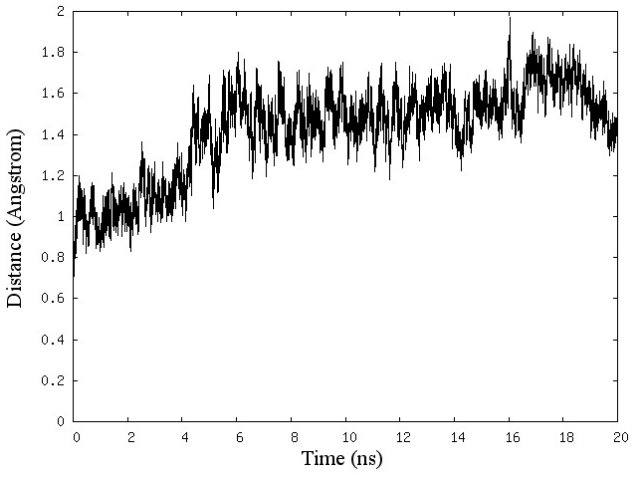


Fig. 2. Schematic representation of Fic (a) and Afr3 (b) interaction with CAII

Although docking method is very useful tools to predict the interaction between a ligand and a receptor, but it still has limitation due to the simplification of this method. In this work, we performed molecular dynamics simulation to refine the docking result. Fisetin which has the highest fitness score were parameterized by Cationic Dummy Atom approach. The MD results showed that the CAII-Fic complex was stable in the course of 20 ns simulation period as revealed by the time course of RMSD value, which was below 2 Å along the simulation period. (Fig. 3a). The interaction between zinc ion with His94, His96, His119 residues, and Fic molecule were still intact in the course of simulation. Simulation results also showed Fic molecule no longer attached to the Asn67, Thr 199 and Thr 200. The interactions are replaced by Thr198 via hydrogen bond (Fig. 3b). The different result between Molecular dynamics and docking simulation is perceivable due to the limitation of docking method. Molecular dynamics simulation is expected to give us more accurate binding prediction of CAII-Fic complex.

(a)



(b)

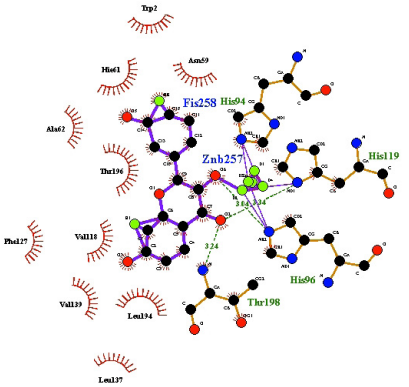


Fig. 3. RMSD (a) and schematic representation (b) of CAII-Fic complex in the course of 20 ns simulation time

4. Conclusion

This research suggested that both Fic and Afr3 molecules are good candidate to be used as CAII inhibitor. The binding mode of Fic and Afr3 with CAII binding site are similar to CO_2 as original ligand of this enzyme. The binding distance between zinc ion with both ligands significantly affect the affinity of both ligand. Both Fic and Afr3 molecule can further be used as structural basis to design new inhibitors for CAII.

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