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Original Article

# *IN SILICO* INVESTIGATION OF XANTHONE DERIVATIVE POTENCY IN INHIBITING CARBONIC ANHYDRASE II (CA II) USING MOLECULAR DOCKING AND MOLECULAR DYNAMICS (MD) SIMULATION

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**ABSTRACT**

**Objective:** Hypertension is the leading contributor to all-cause death and disability worldwide. One of the most well-known first-line antihypertensive drugs is chlorthalidone which treats hypertension through carbonic anhydrase (CA) II inhibition. However, due to the high number of cases of hypertension, a more potent medication is still needed. Xanthone is a potential candidate for the compound group for its potency in inhibiting CA II. Therefore, this research aims to evaluate around 500 xanthones' potency as a better oral antihypertensive drug than chlorthalidone.

**Methods:** 507 xanthones were analyzed for their potency using *in silico* method. Xanthone's structures were retrieved from the PubChem website or built using Avogadro software, while the CA II receptor was retrieved from The RCSB website. Then molecular docking, ADME evaluation, and toxicity test were evaluated from selected ligands. Finally, a molecular dynamics simulation was conducted to evaluate the stability of the potential ligand as the inhibitor of CA II protein.

**Results:** This research found that globulixanthone c is considered to be a better CA II inhibitor compared to chlorthalidone. It is due to its lower binding affinity compared to chlorthalidone and its stable binding to CA II's important inhibition sites with low fluctuation. It also has the potential to be consumed orally because it fulfills all of Lipinski's rule of five standards and its toxicity is on the moderate level.

**Conclusion:** Globulixanthone c, a type of prenylated xanthones group, showed the best potential activity as the inhibitor of CA II protein to treat hypertension among other xanthones.

**Keywords:** Carbonic anhydrase (CA) II, Hypertension, Xanthone, Molecular docking, Molecular dynamics


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## INTRODUCTION

Systemic arterial hypertension (or hypertension) is a condition characterized by persistently high blood pressure (BP) in the systemic arteries. BP is often stated as the ratio of the systolic BP (that is, the pressure that blood exerts on the arterial walls when the heart contracts) and the diastolic BP (the pressure when the heart relaxes) [1]. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC) 7 categorizes the disease as a condition when one's systolic/diastolic pressure exceeds 140/90 mmHg [2].

Hypertension becomes the most common risk factor for cardiovascular disease (CVD), chronic kidney disease (CKD), and cognitive impairment. It is the single leading contributor to all-cause death and disability worldwide [1]. According to WHO (2021), 1.13 of 7.8 billion of the world's population are diagnosed with hypertension, and the disease itself is estimated to cause 7.5 million deaths (which is 12.8% of total deaths) worldwide [3, 4]. The high level of hypertension cases and deaths caused by it make hypertension treatment become crucial to lower the world's morbidity level and increase global life expectancy. Some researchers conducted research on targeting certain proteins involved in hypertension using natural products and a standard drug to elaborate the activity and potential pathway inhibition. Demir (2019) revealed the potential inhibitor of some antihypertension drugs to PON1 protein that links with another disease [5]. In addition, Demir (2020) also showed the potential of quinones as an antihypertensive agent [6].

One of the most common first-line antihypertensive medications is thiazide diuretics with chlorthalidone as the most commonly used drug in the group [7]. In lowering blood pressure, chlorthalidone's important mechanism is carbonic anhydrase (CA) inhibition [8]. Carbonic anhydrase (EC 4.2.1.1) is a group of metalloenzymes that catalyzes the hydration of $CO_2$ and $H_2O$ into bicarbonate and hydrogen ions [9]. This enzyme plays a role in various physiological processes in various organisms, including humans, therefore, the abnormal level or activity of this enzyme can trigger diseases [9, 10]. Agree with that, inhibition of this enzyme becomes crucial to treat some diseases and to examine the new potential drug for clinical applications [11]. In the human body, there are 13 catalytically active CA isozymes that spread in various concentrations and locations [8]. These are classified according to crucial properties such as inhibitor sensitivity, catalytic activity, and subcellular location [12]. Amongst these isozymes, isozymes I, II, III, IV, V, IX, XII, and XIV have relevance in cardiovascular regulation. From them, CA II is counted as a very potent drug target to lower blood pressure because of its high activity (105-6/sec) and its various locations–red cells, kidney, lung, heart, brain, vascular smooth muscle, and endothelium. However, some research revealed the side effects of thiazide diuretics, especially from chlorthalidone treatment, such as hypokalemia, hyperglycemia, myocardial infarction, hospitalization for heart failure, ischemic or hemorrhagic stroke, and a composite cardiovascular disease [13, 14]. Therefore, the necessity of looking for a new hypertension treatment is still required.

Xanthones as a group of secondary metabolites are normally found in a restricted assembly of higher plants (mostly family Clusiaceae and Gentianaceae), fungi, and lichens. This compound group has a symmetrical parent compound–9H-xanthen-9-one and was classified into six groups–simple xanthones, glycosylated xanthones, prenylated xanthones, xanthonolignoids, bis-xanthones, and miscellaneous

xanthones [15]. Xanthones and their derivatives have broad biological activity, especially in the medical field [16]. Their antioxidant activity is good for antimicrobial agents [17]. Furthermore, Abuzaid *et al.* [18] revealed xanthone's benefit for preventing obesity and metabolic syndrome. Some xanthone derivatives were found to be good inhibitors of CA II. One of them is mangiferin, a type of glycosylated xanthones, which was found by Saleem *et al.* to be a good inhibitor of CA II due to its good inhibition concentration of the enzyme using both molecular docking and *in vitro* methods [19]. Furthermore, Davis *et al.* [20] showed that xanthones extraction from microfungus of the genus Xylaria had potential inhibitors for carbonic anhydrase enzymes and had better inhibition activity than phenol extract. This finding made xanthone deserve to be researched further, corresponding to its potential to be a better CA II inhibitor.

At present, around 500 xanthones have been reported in the previous research. However, there is no research found that compares the ability among around 500 xanthones to act as the best CA II protein inhibitor. Therefore, this study investigated 507 xanthone compounds' molecular interaction and dynamics against CA II by molecular docking and molecular dynamics (MD) simulations approach using chlorthalidone as the standard inhibitor.

## MATERIALS AND METHODS

### Ligand retrieval and preparation

The 507 xanthone compounds and standard ligand in the 3D structure were gathered. 332 of the xanthone compounds and chlorthalidone (PubChem ID: 2732) 3D structures were retrieved in 3D. sdf format from PubChem (https://pubchem.ncbi.nlm.nih.gov/) while 175 of the rest were made using Avogadro software. The ligands retrieved from PubChem were given CHARMm force field and MMFF94 partial charge and converted to a. pdb file using Discovery Studio 2020 (BIOVIA). On the other hand, the ligands made using Avogadro software were given MMFF94 force field and saved in. sdf format. Later, the ligands made manually were opened in Discovery Studio 2020 (BIOVIA), given CHARMm force field and MMFF94 partial charge, and saved in. pdb format. These ligands were then brought further into the next screening.

### Protein retrieval and preparation

The protein used as the target for this study is CA II. The crystal structure of CA II was retrieved from Protein Data Bank (PDB) (https://www.rcsb.org/) with the PDB ID: 1I91. The retrieved crystal structure was then prepared using Discovery Studio 2020 (BIOVIA) by removing water molecules, ligand molecules, and ions.

**Table 1: Protein target, their binding sites, their grid settings for docking**

| Protein target | Center | Dimension (Å) |
|---|---|---|
| CA II (PDB ID: 1I91) | X: –7.107 | X: 25 |
| | Y: –0.669 | Y: 25 |
| | Z: 12.624 | Z: 25 |

### Ligand filtering using lipinski's rule of five

Ligand filtering was done based on the ligands' fulfillment of Lipinski's rule of five (Ro5). The Absorption, Distribution, Metabolism, and Excretion (ADME) properties of the 507 ligands were firstly retrieved from the SwissADME (http://www.swissadme.ch/) web tool by inputting the SMILES string of the ligands. The SMILES strings of the 332 ligands from PubChem were also obtained from the site. Whereas the SMILES strings of the manually made 175 ligands were obtained from ChemBD (http://chemdb.ics.uci.edu/cgibin/BabelWeb.py) web tool by inputting the. sdf format of the ligands. After obtaining the SMILES strings of the ligands, the strings were then inputted into SwissADME. Only the ligands that pass all of the Ro5 would be brought to the next screening.

### Molecular docking validation

Before molecular docking was done, the docking method was previously validated. If the RMSD is below 3 Å, then the method is acceptable [21]. The validation was done by re-docking the crystal native ligand (6-[n-(3-hydroxy-phenyl)-3-(morpholin-4-ylmethyl)-2h-thieno[3,2-E]-1,2-thiazine-1,1-dioxide]-sulfonamide) to the prepared protein CA II using the PyRx 0.9.8 software [22]. The native ligand's post-docking conformation as compared to its crystal structure conformation RMSD using Pymol software.

### Molecular docking

After the docking method validation, the prepared protein and ligands were loaded to PyRx 0.9.8 and then prepared to be docked in the built-in AutoDock VINA in the PyRx program [22]. The docking done in this research was specific-site docking with grid selection parameters as shown in table 1 and the rest of the setting was left as default. The binding affinity result table and the best model of each protein-ligand interaction with the five most negative binding affinities were saved to be visualized. Docking visualization was done using Discovery Studio 2020 (BIOVIA) to see the 2D and 3D interactions of each protein-ligand's best models. Docking analysis and visualization were done in Microsoft Windows PC with Intel® Core™ i5-8265U CPU @1.60GHz 1.80 GHz, 8 GB RAM with NVIDIA GeForce MX230 ver. 462.31.

### Ligand toxicity test

Ligands with the five most negative binding affinities of each protein were then evaluated, corresponding to their toxicity score. The toxicity test used in this research is based on oral rat $LD_{50}$ score using ADMETlab (https://admet.scbdd.com/calcpre/calc_cf_single_mol/#) web tool by inputting the ligands' SMILES strings into the site.

### Conserved amino acid analysis

To analyze the conserved amino acid region, the Consurf web server was used (https://consurf.tau.ac.il/). PDB ID 1I91 was uploaded to the server and Multiple Sequence Alignment (MSA) was used for this analysis. The conserved region was displayed using a gradation color scale from 1 as the variable region to 9 as the conserved region.

### Molecular dynamics simulation

The best ligand from the previous filtering then simulated molecular dynamically using GROMACS and followed the MD simulation [23]. The ligand-protein post-dock conformation was firstly prepared as input. The preparation started by separating the ligand-protein file into protein and ligand files and saving them in the. pdb format. Then, the topologies and post-processed. gro files of both files were made. To create the protein topology and post-processed. gro file, the addition of CHARMM36 force field and TIP3P water model and ignoration of H atoms were done to the protein. pdb file. On the other hand, to create the ligand topology and post-processed. gro file, the addition of H atoms and CGenFF force field were done to the ligand. pdb file. After the system's energy was minimized, the system was equilibrated. The equilibration was done in two phases–NVT and NPT phases. After setup, the system was then equilibrated in the NVT, then NPT phase, for 500 ps for each phase. In the NVT phase, the system was equilibrated to reach 309.5 degrees Celsius. After that, in the NPT phase, the system was equilibrated to reach a stable density.

## RESULTS AND DISCUSSION

### Ligand filtering using lipinski's rule of five (Ro5)

After 507 of the ligands, 3D structures and SMILES were collected and filtered. In this filtering, it is found that only 412 ligands fulfill all Lipinski's rule of five–100% (146 of 146 ligands) from simple xanthones, 5% (3 of 60 ligands) from glycosylated xanthones, 91.81% (258 of 281 ligands) from prenylated xanthones, 100% (2 of 2 ligands) from xanthonolignoids, 0% (0 of 11 ligands) from bis-xanthones, and 42.9% (3 of 7 ligands) from miscellaneous xanthones (table S1).

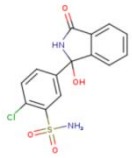
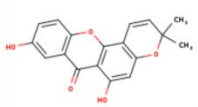
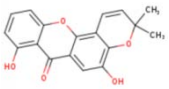
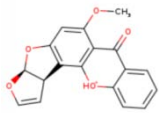
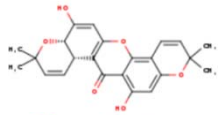
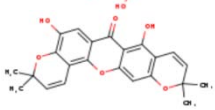
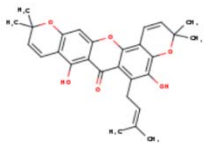
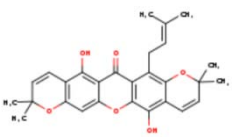
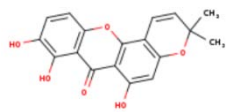
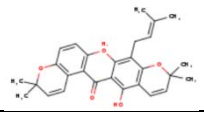
Lipinski's rule of five (Ro5) is a methodology to set the drugability properties for drug formulation [24]. Compounds that fulfill Lipinski's rule of five are predicted to have favorable oral bioavailability and drug-like characteristics [25]. Among four rules in the Ro5, molecular weight (MW) and H-bond donor/acceptor

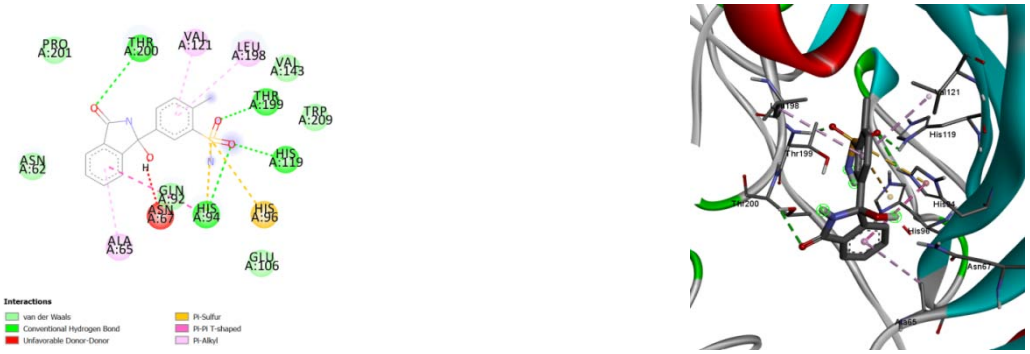
were the main challenges in filtering the xanthone compounds (table S1). Molecular weight is important in drug development as higher MW will tend to have higher lipophilicity characteristics [26]. Furthermore, a proper balance between lipophilicity and hydrophilicity is also crucial in drug design. Excessing hydrogen donor/acceptor can disrupt the hydrophilicity balance by decreasing the affinity of the hydrophobic region [27]. According to table 2, simple xanthones and prenylated xanthones groups were the xanthone group that most fulfil Ro5 requirements. Simple xanthones only consist of simple substituents such as hydroxy, methoxy, or methyl group with around three benzene rings as the main structure, similar to prenylated xanthones. Conversely, Bis-xanthones are the xanthone group that consists of more than one cluster benzene ring with more hydroxyl and carbonyl residue [15]. It brings to the more complex structure with excess MW and hydrogen donor/acceptor requirements from Ro5.

**Table 2: Ligands with 0 lipinski's rule of five violation**

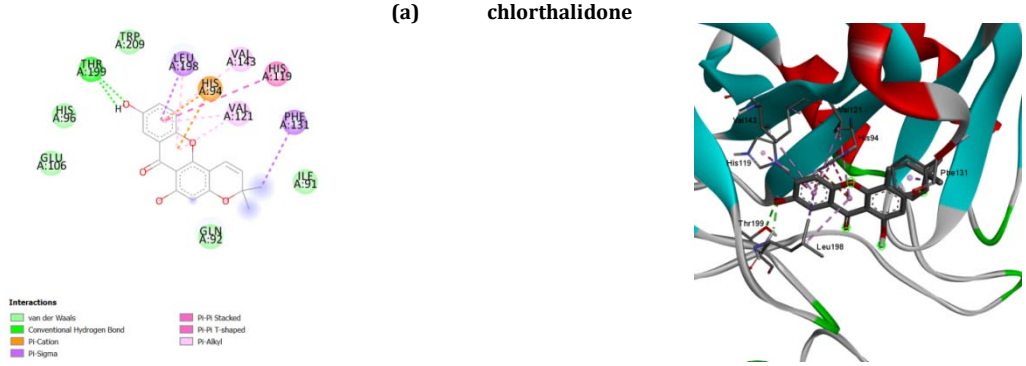
| Xanthone group | Total ligands | Total ligands with 0 lipinski's rule of five violation |
|---|---|---|
| Simple xanthones | 146 | 146 |
| Glycosylated xanthones | 60 | 3 |
| Prenylated xanthones | 281 | 258 |
| Xanthonolignoids | 2 | 2 |
| Bis-xanthones | 11 | 0 |
| Miscellaneous Xanthones | 7 | 3 |
| Total | 507 | 412 |

**Table 3: Ligands with 5 lowest docking binding affinity with CA II**

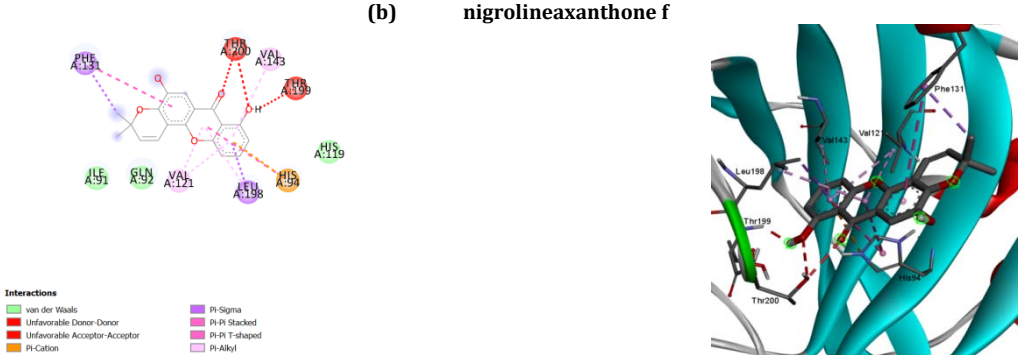
| Ligand | 2D structure | Binding affinity (kcal/mol) |
|---|---|---|
| Chlorthalidone (standard ligand) |  | –8.2 |
| Nigrolineaxanthone F |  | –9 |
| Nigrolineaxanthone H |  | –9 |
| 7-Deoxysterigmatocystin |  | –9.1 |
| Brasilixanthone A |  | –9.1 |
| Nigrolineaxanthone I |  | –9.2 |
| Mangostenone A |  | –9.2 |
| Garcimangosone A |  | –9.5 |
| Globulixanthone C |  | –9.5 |
| Calophinone |  | –9.8 |



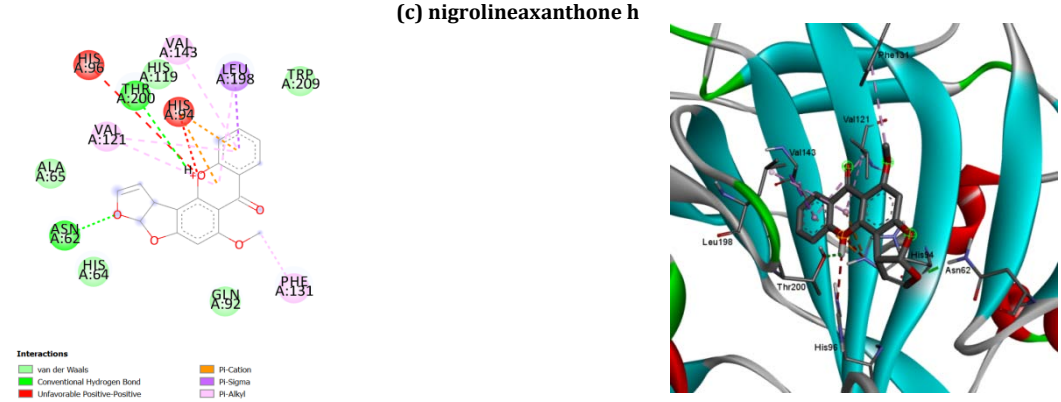

**(a) chlorthalidone**




**(b) nigrolineaxanthone f**



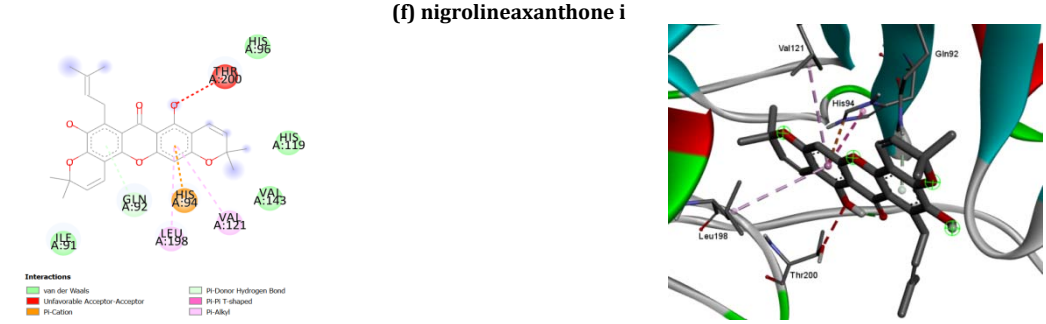

**(c) nigrolineaxanthone h**



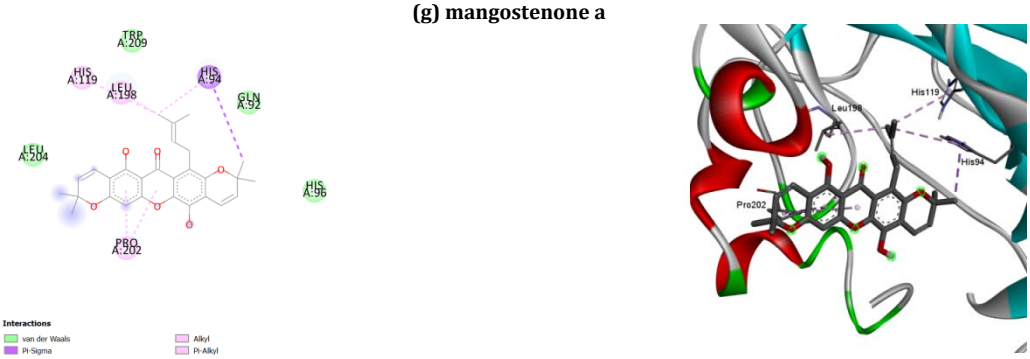

**(d) 7-deoxysterigmatocystin**


**(e) brasilixanthone a**

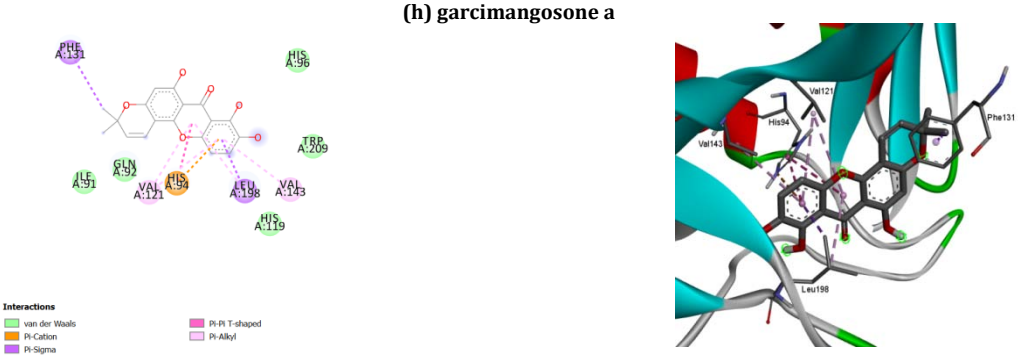

**(f) nigrolineaxanthone i**




**(g) mangostenone a**




**(h) garcimangosone a**




**(i) globulixanthone c**


**(j) calophinone**

**Fig. 3: Post-dock 2D and 3D interactions of (a) chlorthalidone, (b) nigrolineaxanthone f, (c) nigrolineaxanthone h, (d) 7-deoxysterigmatocystin, (e) brasilixanthone a, (f) nigrolineaxanthone i, (g) mangostenone a, (h) garcimangosone a, (i) globulixanthone c, (j) calophinone with CA II**

**Molecular docking**

Molecular docking validation was conducted to validate the docking method. The result of RMSD was 2,6 Å and it was acceptable to continue the docking process (fig. S1) [21]. The docking of the 412 ligands that fulfill all of the Ro5 and the standard ligand–chlorthalidone–to CA II was done. The binding site for specific docking and the grid settings were set up as shown in table 1.

After the 412 ligands were docked to CA II, it was found that 85 of the ligands have a more negative binding affinity than chlorthalidone, which indicates that the ligands then have a better binding to CA II than chlorthalidone. Chlorthalidone's binding affinity itself is–8.2 kcal/mol, while the binding affinity range of the ligands with lower binding affinity than chlorthalidone is–8.3 kcal/mol to–9.8 kcal/mol (table S2)

The 85 ligands were then filtered further and the ligands with the 5 lowest binding affinities were chosen to be evaluated more and brought to the next screening. Nine (9) ligands were found to have the 5 lowest docking binding affinity with CA II shown in table 3–nigrolineaxanthone f (–9 kcal/mol), nigrolineaxanthone h (–9 kcal/mol), 7-deoxysterigmatocystin (–9.1 kcal/mol), brasilixanthone a (–9.1 kcal/mol), nigrolineaxanthone i (–9.2 kcal/mol), mangostenone a (–9.2 kcal/mol), garcimangosone a (–9.5 kcal/mol), globulixanthone c (–9.5 kcal/mol), and calophinone (–9.8 kcal/mol).

As we can observe in fig. 3, chlorthalidone makes hydrogen bonding with His94, His119, Thr199, and Thr200, pi interactions with Ala65, Val121 and Leu198 (pi-alkyl), His94 and His96 (pi-sulfur), and His94 (pi-pi T-shaped), and unfavorable interaction with Asn67 (donor-donor). Hydrogen bonding is a strong non-covalent bond that occurs when hydrogen that is covalently bonded with a very electronegative atom (N, O, or F) is attracted by the electrons of another atom nearby it. Whereas pi interaction is relatively weaker than the hydrogen bond that occurs between molecules with a pi system from conjugated molecules like benzene.

The same hydrogen bonding sites as chlorthalidone were found to be interacting with some of the test ligands, namely nigrolineaxanthone f (with Thr199 two times), 7-deoxysterigmatocystin (with Thr200), and nigrolineaxanthone i (with Thr200). Besides, the same pi-alkyl interaction sites were also found to interact in pi-alkyl interaction, alkyl or pi-sigma with the test ligands–nigrolineaxanthone f (with Val121 two times and Leu198 (pi-alkyl) and with Leu198 (pi-sigma)), nigrolineaxanthone h (with Val121 two times and Leu198 (pi-alkyl) and with Leu198 (pi-sigma)), 7-deoxysterigmatocystin (with Val121 two times and Leu198 (pi-alkyl) and with Leu198 (pi-sigma)), brasilixanthone a (with Leu198 (pi-sigma)) nigrolineaxanthone i (with Val121 and Leu198 (pi-alkyl)), mangostenone a (with Val121 and Leu198 (pi-alkyl) and with Leu198 (alkyl)), globulixanthone c (with Val121 two times and Leu198 (pi-alkyl) and with Leu198 (pi-sigma)), and calophinone (with Leu198 two times (pi-alkyl) and with Val121 and Leu198 (alkyl)).

Other pi interaction sites in chlorthalidone–pi-sulfur interaction sites (His94 and His96)–were not found to interact in pi-sulfur interaction with the test ligands. However, His94 (which is also the site of pi-pi T-shaped interaction with chlorthalidone) makes other forms of pi interactions with the test ligands, that is, nigrolineaxanthone f (in pi-pi T-shaped and pi-cation interactions (two times both), nigrolineaxanthone h (in pi-pi T-shaped (two times) and pi-cation interactions), 7-deoxysterigmatocystin (in pi-cation interactions (two times)), brasilixanthone a (in pi-sigma interaction), nigrolineaxanthone i (in pi-pi T-shaped and pi-cation interactions), nigrolineaxanthone i (in pi-pi T-shaped and pi-cation interactions), mangostenone a (in pi-pi T-shaped and pi-cation interactions), garcimangosone a (in pi-sigma interaction), globulixanthone c (in pi-pi T-shaped (two times) and pi-cation interactions) and calophinone (in pi-sigma interaction)

According to the previous research [28-30] Asn62, Ala65, His94, His96, Val121, Phe131, Leu141, Val143, Leu198, Thr199, Thr200, Val207, Trp209 were the important residues to stabilize CAII inhibitor. Chlorthalidone and 7-deoxysterigmatocystin were built with 9 important residues out of 13 in total. They interacted with various chemical bondings such as hydrogen, hydrophobic, and van der waals interactions. Furthermore, other ligands are only built with 3 to 7 important residues with almost similar chemical bonding interactions. Simone *et al.* [31] stated that strong CAII inhibition was correlated with liposolubility characteristics. Agree with that, all 9 potential ligands showed high liposolubility based on the MLOGP value on the Ro5 [table S1]. Interestingly, the important residues in the CAII protein show a hydrophobicity region (fig. S2) and it will increase the chance to interact with the nonpolar molecular surfaces of the potential ligands. Freitas *et al.* [32] also stated that hydrophobic interaction was crucial to developing high-efficiency ligands. Hydrophobic interaction is shown as alkyl, pi-alkyl, pi-pi stacked, pi-pi t shaped, and pi-sigma interaction. Based on table 3, all potential ligands showed various hydrophobic interactions.

**Ligand oral rat $LD_{50}$ toxicity test**

After the selected ligands were docked to CA II, the oral rat $LD_{50}$ toxicity test was done on the selected ligands. This toxicity test states that a compound with a certain amount of its dose is categorized into some level of toxicity if it causes the death of one-half of a group of test animals (rats) that consumed the compound orally. The oral rat $LD_{50}$ score of a compound is often expressed as mg/kg, with mg stating the amount of the compound and the kg stating the weight of the consumer.

According to Hodge and Sterner's scale of toxicity, a compound is categorized into some levels of toxicity, that is, extremely toxic if its oral rat LD50 score is<1 mg/kg, highly toxic if its score is 1–5 mg/kg, moderately toxic (50–500 mg/kg), slightly toxic (500–5,000 mg/kg), practically non-toxic (5,000–15,000 mg/kg), and relatively harmless (>15,000 mg/kg).

The selected ligands were found to have toxicity levels around moderately toxic and slightly toxic. As shown in table 5, among the selected ligands, only nigrolineaxanthone h is found to be labeled with a slightly toxic toxicity level. The rest of the ligands are all categorized as moderately toxic toxicity. For further analysis, globuloxanthone C was selected according to the binding affinity score, important residues interaction, and toxicity result (table 6).

**Table 5: Selected xanthone's oral rat $LD_{50}$ toxicity score and their oral rat $LD_{50}$ toxicity level according to hodge and sterner**

| Ligand | Oral rat $LD_{50}$ toxicity score (mg/kg) | Hodge and sterner's oral rat LD50 toxicity level |
|---|---|---|
| Chlorthalidone | 2,264.35 | slightly toxic |
| Nigrolineaxanthone F | 462.15 | moderately toxic |
| Nigrolineaxanthone H | 509.1 | slightly toxic |
| 7-Deoxysterigmatocystin | 246.81 | moderately toxic |
| Brasilixanthone A | 424.58 | moderately toxic |
| Nigrolineaxanthone I | 486.1 | moderately toxic |
| Mangostenone A | 251.32 | moderately toxic |
| Garcimangosone A | 247.87 | moderately toxic |
| Globulixanthone C | 465.18 | moderately toxic |
| Calophinone | 225.88 | moderately toxic |

**Table 6: Selected ligand parameters**

| Ligands | Parameters | | | |
|---|---|---|---|---|
| | Binding affinity (kcal/mol) | Important residues interaction | Hydrophobic interaction | Toxicity result (mg/kg) |
| Chlorthalidone (control) | -8.3 | 9 | 3 | 2264 |
| Nigrolineaxanthone F | -9 | 7 | 4 | 462 |
| Nigrolineaxanthone H | -9 | 5 | 4 | 509 |
| 7-Deoxysterigmatocystin | -9.1 | 9 | 4 | 246 |
| Brasilixanthone A | -9.1 | 3 | 2 | 424 |
| Nigrolineaxanthone I | -9.2 | 6 | 2 | 486 |
| Mangostenone A | -9.2 | 6 | 2 | 251 |
| Garcimangosone A | -9.5 | 3 | 4 | 247 |
| Globulixanthone C | -9.5 | 7 | 4 | 464 |
| Calophinone | -9.8 | 7 | 7 | 225 |

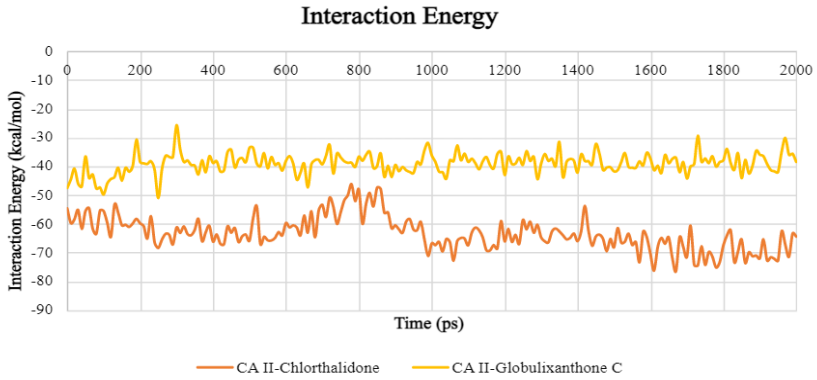



**Fig. 7: CA II-chlorthalidone and CA II-globulixanthone c interaction energy fluctuation in 2 ns simulation**

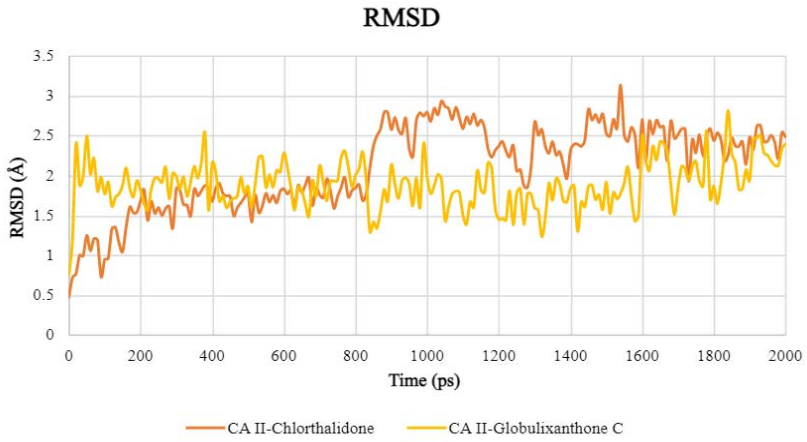



**Fig. 8: CA II-chlorthalidone and CA II-globulixanthone c RMSD fluctuation in 2 ns simulation**

**Molecular dynamics simulation**

Globulixanthone c, as the selected ligand, was then simulated in MD simulation with CA II. After the systems were equilibrated, they both run in the simulation for 2 ns. The interaction energy, RMSD, important interactions, and RMSF were evaluated. The interaction energy used in this analysis is the total of Lennard-Jones and Coulombic energy of the protein and the ligand binding. Therefore, this interaction energy is not enough to depict the overall binding of the ligand to the protein. However, this interaction energy can be used to see how the ligand poses and binding to the protein changes over time.

CA II-chlorthalidone's average interaction energy is–63.39 kcal/mol while for CA II-globulixanthone c is–39.04 kcal/mol (fig. 7). As depicted in fig. 7, the CA II-globulixanthone c complex's binding is more stable than the standard chlorthalidone with CA II complex. The binding stability can also be seen from the Root Mean Square Deviation (RMSD) fluctuation of the complexes. RMSD is a measurement of how the ligand's position in binding to its target changes along simulation compared to its starting structure [33, 34]. CA II-globulixanthone c's average RMSD is shown to be lower–1.88 Å–than CA II-chlorthalidone's average RMSD–2.11 Å. CA II-globulixanthone c's RMSD fluctuation is also more stable compared to CA II-chlorthalidone's fluctuation. This indicates that in the interaction energy and RMSD, globulixanthone c could be a better inhibitor for CA II compared to the standard drug chlorthalidone.

We also evaluate the CA II-chlorthalidone and CA II-globulixanthone c complexes for Root Mean Square Fluctuation (RMSF) result. RMSF is a measurement of how much each of the protein residues fluctuates within the simulation. The larger the RMSF score means the higher the flexibility and instability of the residue. While the

smaller it is, then the lower the flexibility and instability of the residue [35]. From the RMSF result, both complexes showed an almost similar pattern suggesting that globulixanthone c has good potential for CAII inhibitor.

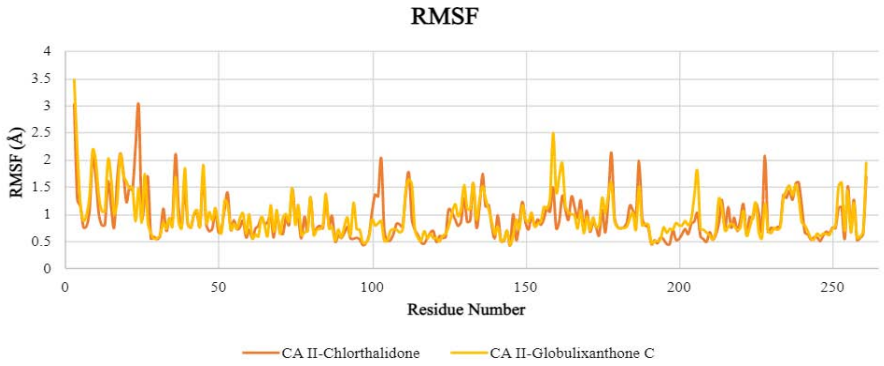



**Fig. 9: CA II-chlorthalidone and CA II-globulixanthone c RMSF in 2 ns simulation**

**Table 8: RMSF values of important CA II residues when simulated with chlorthalidone and globulixanthone c**

| Residues | RMSF (Å) | |
|---|---|---|
| | CA II-chlorthalidone | CA II-globulixanthone c |
| Asn62 | 0.7 | 0.6 |
| Ala65 | 0.8 | 0.8 |
| His94 | 0.6 | 1.2 |
| His96 | 0.5 | 0.7 |
| Val121 | 0.5 | 0.6 |
| Phe131 | 0.9 | 1.1 |
| Leu141 | 1.0 | 0.8 |
| Val143 | 0.5 | 0.5 |
| Leu198 | 0.7 | 0.7 |
| Thr199 | 0.5 | 0.8 |
| Thr200 | 0.5 | 0.8 |
| Val207 | 0.6 | 0.7 |
| Trp209 | 0.5 | 0.7 |

For deeper analysis, RMSF evaluation was also conducted on the important residues of CA II–Asn62, Ala65, His94, His96, Val121, Phe131, Leu 141, Val 143, Leu198, Thr199 and Thr200, Val207, Trp209 when simulated to globulixanthone c compared to chlorthalidone. The RMSF of the residues are shown in table 8 and the result showed that the important residues are in a similar range from both complexes and it was still considered stable for the RMSFs as the value was around 1 Å. Furthermore, the important residues were also analyzed for their conservation level. Amitai *et al.* [36] and Buyong *et al.* [37] stated that the important residues play a crucial role in drug development, especially for ligand binding. Fig. S3 showed that these residues reveal a high conservation scale compared to other organisms.

## CONCLUSION

Globulixanthone c, a type of prenylated xanthone, is considered to be a potential CA II inhibitor candidate due to its lower binding affinity (–9.5 kcal/mol) than the standard drug chlorthalidone (–8.2 kcal/mol) from molecular docking result and its stable binding to CA II's important inhibition sites from MD simulation. It also has the potential to be consumed orally because it fulfills all of Lipinski's rule of five and its toxicity is at a moderate level.

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## AUTHORS CONTRIBUTIONS

All authors have contributed equally.

## CONFLICT OF INTERESTS

Among the authors have no conflict of interest

## REFERENCES

1. Oparil S, Acelajado MC, Bakris GL, Berlowitz DR, Cifkova R, Dominiczak AF. Hypertension. Nat Rev Dis Primers. 2018 Mar 22;4:18014. doi: 10.1038/nrdp.2018.14, PMID 29565029.
2. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr. The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report. JAMA. 2003 May 21;289(19):2560-72. doi: 10.1001/jama.289.19.2560, PMID 12748199.
3. World Health Organization. Hypertension; Aug 25 2021. Available from: https://www.who.int/news-room/fact-sheets/detail/hypertension. [Last accessed on 14 May 14 2022]
4. World Health Organization. Blood pressure/hypertension. Available from: https://www.who.int/data/gho/indicator-metadata-registry/imr-details/3155. [Last accessed on 14 May 2022]
5. Demir Y. The behaviour of some antihypertension drugs on human serum paraoxonase-1: an important protector enzyme against atherosclerosis. J Pharm Pharmacol. 2019 Oct;71(10):1576-83. doi: 10.1111/jphp.13144, PMID 31347707.
6. Demir Y. Naphthoquinones, benzoquinones, and anthraquinones: molecular docking, ADME and inhibition studies on human serum paraoxonase-1 associated with cardiovascular diseases. Drug Dev Res. 2020 Aug;81(5):628-36. doi: 10.1002/ddr.21667, PMID 32232985.
7. Wright JM, Musini VM, Gill R. First-line drugs for hypertension. Cochrane Database Syst Rev. 2018;4:CD001841. doi: 10.1002/14651858.CD001841.pub3. PMID 29667175.
8. Swenson ER. New insights into carbonic anhydrase inhibition, vasodilation, and treatment of hypertensive-related diseases.

Curr Hypertens Rep. 2014 Sep;16(9):467. doi: 10.1007/s11906-014-0467-3, PMID 25079851.

9. Hoff E, Zou D, Schiza S, Demir Y, Grote L, Bouloukaki I. Carbonic anhydrase, obstructive sleep apnea and hypertension: effects of intervention. J Sleep Res. 2020 Apr;29(2):e12956. doi: 10.1111/jsr.12956, PMID 31808986.
10. Sever B, Turkes C, Altıntop MD, Demir Y, Beydemir S. Thiazolyl-pyrazoline derivatives: *in vitro* and *in silico* evaluation as potential acetylcholinesterase and carbonic anhydrase inhibitors. Int J Biol Macromol. 2020 Nov 15;163:1970-88. doi: 10.1016/j.ijbiomac.2020.09.043. PMID 32931834.
11. Kaya Y, Erçağ A, Zorlu Y, Demir Y, Gülçin I. New Pd(II) complexes of the bisthiocarbohydrazones derived from isatin and disubstituted salicylaldehydes: synthesis, characterization, crystal structures and inhibitory properties against some metabolic enzymes. J Biol Inorg Chem. 2022 Mar;27(2):271-81. doi: 10.1007/s00775-022-01932-9, PMID 35175415.
12. Caglayan C, Taslimi P, Demir Y, Kucukler S, Kandemir FM, Gulçin I. The effects of zingerone against vancomycin-induced lung, liver, kidney and testis toxicity in rats: the behavior of some metabolic enzymes. J Biochem Mol Toxicol. 2019 Oct;33(10):e22381. doi: 10.1002/jbt.22381, PMID 31454121.
13. Carter BL, Einhorn PT, Brands M, He J, Cutler JA, Whelton PK. Thiazide-induced dysglycemia: call for research from a working group from the National Heart Lung and Blood Institute. Hypertension. 2008 Jul;52(1):30-6. doi: 10.1161/hypertensionaha.108.114389, PMID 18504319.
14. Hripcsak G, Suchard MA, Shea S, Chen R, You SC, Pratt N. Comparison of cardiovascular and safety outcomes of chlorthalidone vs hydrochlorothiazide to treat hypertension. JAMA Intern Med. 2020 Apr 1;180(4):542-51. doi: 10.1001/jamainternmed.2019.7454, PMID 32065600.
15. Vieira LM, Kijjoa A. Naturally occurring xanthones: recent developments. Curr Med Chem. 2005;12(21):2413-46. doi: 10.2174/092986705774370682, PMID 16250871.
16. Bedi P, Gupta R, Gupta R, Pramanik T, Pramanik T. Synthesis and biological properties of pharmaceutically important xanthones and benzoxanthone analogs: a brief review. Asian J Pharm Clin Res 2018;11(2). doi: 10.22159/ajpcr.2018.v11i2.22426.
17. Chansakaow S, Sirisa Ard P, Khonkarn R. Preparation, characterization and antioxidant activity of xanthone-loaded making (hodgsonia heteroclita) microemulsions. Int J Pharm Pharm Sci. 2017;9(3):262-7. doi: 10.22159/ijpps.2017v9i3.16584.
18. Abuzaid AS, Sukandar EY, Kurniati NF, Adnyana IK. Prevention of obesity and development of metabolic syndrome by mangosteen (Garcinia mangostana L.) pericarp ethanolic extract in male Wistar rats fed with high-fat diet. Int J Pharm Pharm Sci. 2018;8(5):372-8.
19. Saleem M, Hareem S, Khan A, Naheed S, Raza M, Hussain R. Dual inhibitors of urease and carbonic anhydrase-II from Iris species. Pure Appl Chem. 2019 Jul 18;91(10):1695-707. doi: 10.1515/pac-2019-0407.
20. Davis RA, Hofmann A, Osman A, Hall RA, Mühlschlegel FA, Vullo D. Natural product-based phenols as novel probes for mycobacterial and fungal carbonic anhydrases. J Med Chem. 2011 Mar 24;54(6):1682-92. doi: 10.1021/jm1013242, PMID 21332115.
21. Ramirez D, Caballero J. Is it reliable to take the molecular docking top scoring position as the best solution without considering available structural data? Molecules. 2018 Apr 28;23(5):1038. doi: 10.3390/molecules23051038, PMID 29710787.
22. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010 Jan 30;31(2):455-61. doi: 10.1002/jcc.21334, PMID 19499576.
23. Berendsen HJC, Van der Spoel D, Van Drunen R. GROMACS: A message-passing parallel molecular dynamics implementation. Comput Phys Commun. 1995;91(1-3):43-56. doi: 10.1016/0010-4655(95)00042-E.
24. Benet LZ, Hosey CM, Ursu O, Oprea TI. BDDCS, the rule of 5 and drug ability. Adv Drug Deliv Rev. 2016 Jun 1;101:89-98. doi: 10.1016/j.addr.2016.05.007. PMID 27182629.
25. Sever B, Altıntop MD, Demir Y, Turkes C, Ozbaş K, Ciftci GA. A new series of 2,4-thiazolidinediones endowed with potent aldose reductase inhibitory activity. Open Chem. 2021;19(1):347-57. doi: 10.1515/chem-2021-0032.
26. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 2001 Mar 1;46(1-3):3-26. doi: 10.1016/s0169-409x(00)00129-0, PMID 11259830.
27. Coimbra JTS, Feghali R, Ribeiro RP, Ramos MJ, Fernandes PA. The importance of intramolecular hydrogen bonds on the translocation of the small drug piracetam through a lipid bilayer. RSC Adv. 2020;11(2):899-908. doi: 10.1039/d0ra09995c, PMID 35423709.
28. Sangkaew A, Samritsakulchai N, Sanachai K, Rungrotmongkol T, Chavasiri W, Yompakdee C. Two flavonoid-based compounds from Murraya paniculata as novel human carbonic anhydrase isozyme II inhibitors detected by a resazurin yeast-based assay. J Microbiol Biotechnol. 2020 Apr 28;30(4):552-60. doi: 10.4014/jmb.1910.10037, PMID 31893608.
29. Ghorab MM, Alsaid MS, Ceruso M, Nissan YM, Supuran CT. Carbonic anhydrase inhibitors: synthesis, molecular docking, cytotoxic and inhibition of the human carbonic anhydrase isoforms I, II, IX, XII with novel benzenesulfonamides incorporating pyrrole, pyrrolopyrimidine and fused pyrrolopyrimidine moieties. Bioorg Med Chem. 2014 Jul 15;22(14):3684-95. doi: 10.1016/j.bmc.2014.05.009. PMID 24878360.
30. Abuelizz HA, Dib RE, Marzouk M, Anouar EH, A Maklad Y, N Attia H. Molecular docking and anticonvulsant activity of newly synthesized quinazoline derivatives. Molecules. 2017 Jun 30;22(7):1094. doi: 10.3390/molecules22071094, PMID 28665338.
31. De Simone G, Scozzafava A, Supuran CT. Which carbonic anhydrases are targeted by the antiepileptic sulfonamides and sulfamates? Chem Biol Drug Des. 2009 Sep;74(3):317-21. doi: 10.1111/j.1747-0285.2009.00857.x. PMID 19703035.
32. Ferreira de Freitas R, Schapira M. A systematic analysis of atomic protein-ligand interactions in the PDB. MedChemComm. 2017 Oct 1;8(10):1970-81. doi: 10.1039/c7md00381a, PMID 29308120.
33. Martinez L. Automatic identification of mobile and rigid substructures in molecular dynamics simulations and fractional structural fluctuation analysis. Plos One. 2015 Mar 27;10(3):e0119264. doi: 10.1371/journal.pone.0119264. PMID 25816325.
34. Ramirez D, Caballero J. Is it reliable to take the molecular docking top scoring position as the best solution without considering available structural data? Molecules. 2018 Apr 28;23(5):1038. doi: 10.3390/molecules23051038, PMID 29710787.
35. Cheng X, Ivanov I. Molecular dynamics. Methods Mol Biol. 2012;929:243-85. doi: 10.1007/978-1-62703-050-2_11, PMID 23007433.
36. Amitai G, Shemesh A, Sitbon E, Shklar M, Netanely D, Venger I. Network analysis of protein structures identifies functional residues. J Mol Biol. 2004 Dec 3;344(4):1135-46. doi: 10.1016/j.jmb.2004.10.055. PMID 15544817.
37. Ma B, Elkayam T, Wolfson H, Nussinov R. Protein-protein interactions: structurally conserved residues distinguish between binding sites and exposed protein surfaces. Proc Natl Acad Sci USA. 2003 May 13;100(10):5772-7. doi: 10.1073/pnas.1030237100, PMID 12730379.