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RESEARCH ARTICLE

In Silico Study of Anti-Inflammatory Potential of 1.8-Cineole against Cox-2 and TLR-2

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Abstract

Objective: To determine the anti-inflammatory activity of 1.8-cineole against COX-2 and TLR-2 using an *in silico* approach. Methods: *In silico* studies were conducted using the Molegro Virtual Docker (MolDoc) program to predict the anti-inflammatory potency of 1.8-cineole against COX-2 and TLR-2 receptors. Results: MolDoc score showed that 1, 8-cineole (-49.1236) could be predicted to be an anti-inflammatory ligand demonstrating lower activity than diclofenac or COX-2 (-106.8010) ligand. 1, 8-cineole (-42.7930) can also be predicted to be an anti-inflammatory ligand with lower activity than TLR-2 / CAS_673 ligand (-56.5640). Conclusion: 1.8-cineole possesses anti-inflammatory potential against COX-2 and TLR-2 receptors based on an *in silico* predictive study.

Keywords: *1.8-cineole, In silico, COX-2, TLR-2.*

Introduction

1,8-cineole ($C_{10}H_{18}O$) is a natural organic compound in the form of a colorless liquid, a cyclic ether, or a saturated monoterpene which is found in various plant species including; *Eucalyptus, Croton, Hyptis, Pectis, Rosamarinus* and *Salvia* [1]. In 1870, it was established that 1,8-cineole constitutes a prominent element within *Eucalyptus globulus* oil [2].

Pure 1,8-cineole is a clear liquid at room temperature with a melting point of 1.5°C, a flash point of 49°C [3], water solubility of 3.5 g/l (21°C), a density of 0.925/cm^3 (20°C), a boiling point between 174 - 177°C, and a molar mass of 154.25 g/mol [4].

1,8-cineole is a compound showing considerable promise as a treatment for such conditions since its anti-inflammatory and antioxidant effects have been confirmed [5]. Moreover, it has been widely applied in cases of respiratory infection such as common colds and bronchitis, in addition to be an adjunctive therapy for sinusitis. This material is capable of improving the ciliary beat frequency of mucous membranes and demonstrates secretolytic and bronchospasmolytic properties [6]. *In silico* study is a relatively new approach in the field of research that is becoming more widely adopted in predicting the manner through which drugs and pathogens interact inside the body.

One technique applied during *in silico* studies is that of molecular modeling [7] which plays a very important role in medicinal chemistry through the design, discovery and optimizing of bioactive compounds in the drug development process [8]. *In silico* study, often performed prior to in vivo study, should be conducted in order to determine the anti-inflammatory activity of 1, 8 -cineole as the active ingredient of *Eucalyptus globulus*.

Diclofenac is used as a ligand of an anti-inflammatory patent drug. *In silico* constitutes a method of predicting the

chemical properties of drug molecular physics and of understanding the description of compounds interacting with receptors.

In this case, COX-2 and TLR-2 play a role as receptors in inflammation. The method of conducting an *in silico* test involves docking the molecule to predict its activity in the selected target cell. Docking constitutes an attempt to harmonize those ligands which are small molecules with the target cells consisting of large protein molecules [9].

The *in silico* test produces a bond energy value or Rerank Score (RS) which equals the amount of energy required to form bonds between ligands and receptors. This study was undertaken to analyze and predict anti-inflammatory 1, 8-cineole activity in relation to COX-2 and TLR-2 receptors *in silico*.

Material and Methods

This study predicts the potential anti-inflammatory activity of 1, 8-cineole against TLR-2 and COX-2 receptors. The patent drugs, diclofenac and S_ (dimethylarsaneyl) cysteine (CAS_673), were used as comparative ligands, ChemBioOffice Ultra 12.0 (Cambridge Soft Co.) was used to create 2-dimensional structures, while ChemBio3D 12.0 (Cambridge Soft Co.) was employed to construct 3-dimensional structures. Molegro Virtual Docker (MVD) 5.5 (CLC Bio, Aarhus, Denmark) was employed to dock and analyze amino acids. The computer utilized had the following specifications: Intel® Core™ i7, 860@ 2, 8 GHz, RAM 12 GB, 64-bit type Operating System, Windows 10. Chem Bio Office Ultra 12.0 and a Molegro Virtual Docker (MVD) 5.5 license issued on behalf of Siswandono Siswodihardjo.

The creation of a 2-dimensional (2-D) structure of 1, 8-cineole involved the application of a ChemBioOffice Ultra 12.0 program. The 2-D 1,8-cineole structure was transformed into a 3-dimensional (3-D) shape using a ChemBio3D 12.0 program which enabled observation of the stereo chemical form of compounds and the production of the most stable form of compounds by minimizing energy.

The method applied in this process was that of MMFF94 [10]. After the structural energy of the compound had been minimized, it was stored in the form of an SYBYL.mol2 file in order that it could be read by the Molegro Virtual Docker (MVD) 5.5 program and utilized for the docking process [11]. In order to analyze the interaction of 1, 8-cineole with CoX-2 receptors, a docking process was undertaken, all stages of which used the 3-D illustration form.

The first step consisted of downloading the cyclooxygenase-2 receptor that binds to the diclofenac ligand (PDB code: 1PXX) from the Protein Data Bank (PDB) internet website. It was a prerequisite that the downloaded receptor must contain ligands, the diclofenac variety of which was chosen because it constitutes a patent drug for treating inflammation.

Furthermore, detection was undertaken of receptor structure cavities where the ligands would be bound (interacted). The structure of the 1, 8-cineole compounds was subsequently deposited in the selected cavity, by aligning it to the molecule in the Molegro Virtual Docker program which attached three atoms in the compound to their counterparts in the ligand of the receptor.

The atoms selected were those of the pharmacophore group. The subsequent step consisted of observing the location of the compound in the receptor cavity, followed by docking the compound to the receptor automatically using the Molegro Virtual Docker program (CLC Bio, Aarhus, Denmark). During this process, it was also noted the pharmacophore group of compounds were supported in the cavity in which the compounds would interact.

The parameters measured in the docking process, using the Mol Dock Score and Rerank Score were the energy values involved. To analyze the interaction between 1, 8-cineole and the TLR-2 receptor, TLR-2 receptors which were bound to ligand S_ (dimethylarsaneyl) cysteine (CAS_673) were downloaded from the PDB site (PDB code: 1FYW).

Results

The chemical structures of the 1, 8-cineole compound and the diclofenac compound were first sought. Figure 1 and 2 contain 2-dimensional and 3-dimensional structures of the compound respectively.

Figure 1: 2D and 3D structures of 1,8-cineole

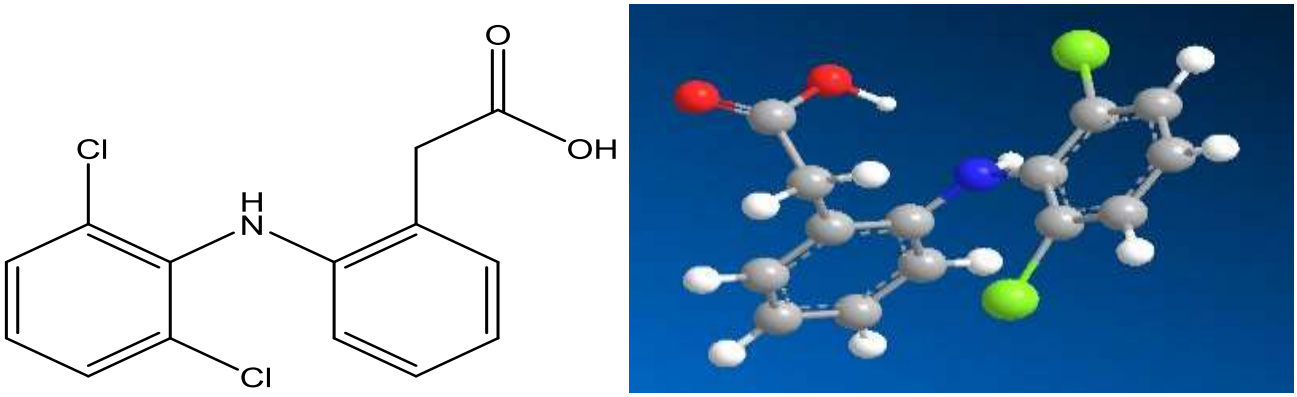

Figure 2: 2D and 3D structures of diclofenac

Five cavities were found to be present in the structure of 1 PXX (COX-2 receptors that were bound to diclofenac ligands) and diclofenac ligands were detected in cavity 5 (see Figure 3).

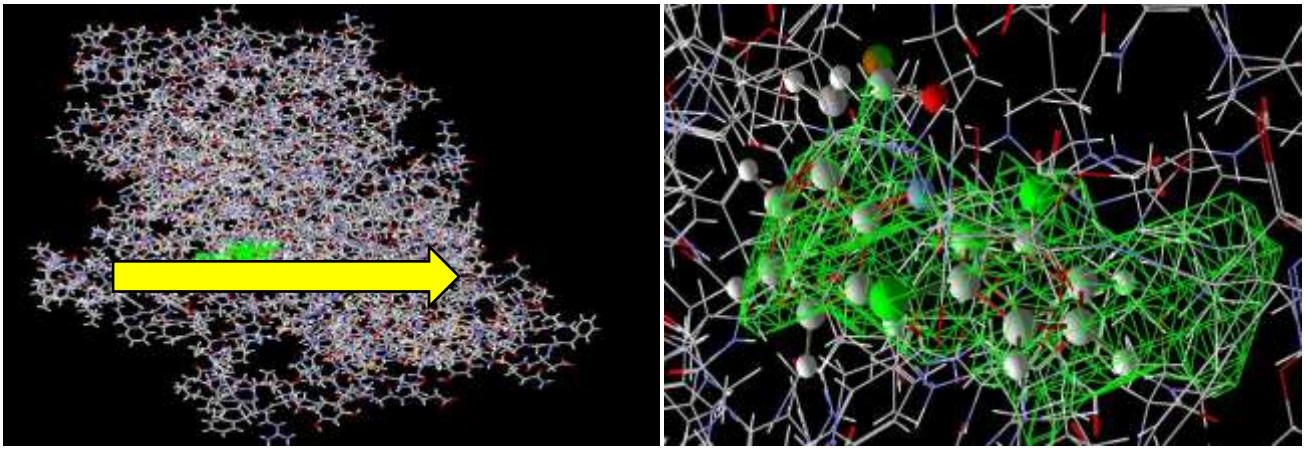

Figure 3: Cavity 5 on the cyclooxygenase-2 (COX-2) PDB code receptor: 1PXX with diclofenac ligands

Cavity 5 constituted a location where 1,8-cineole interacts with COX-2 amino acid receptors through two hydrogen bonds; Tyr385 and Ser 530, and six steric bonds; Tyr 385, Phe 381, Ser 530, Leu 352, Tyr 348, and Trp 387 (see Figure 4).

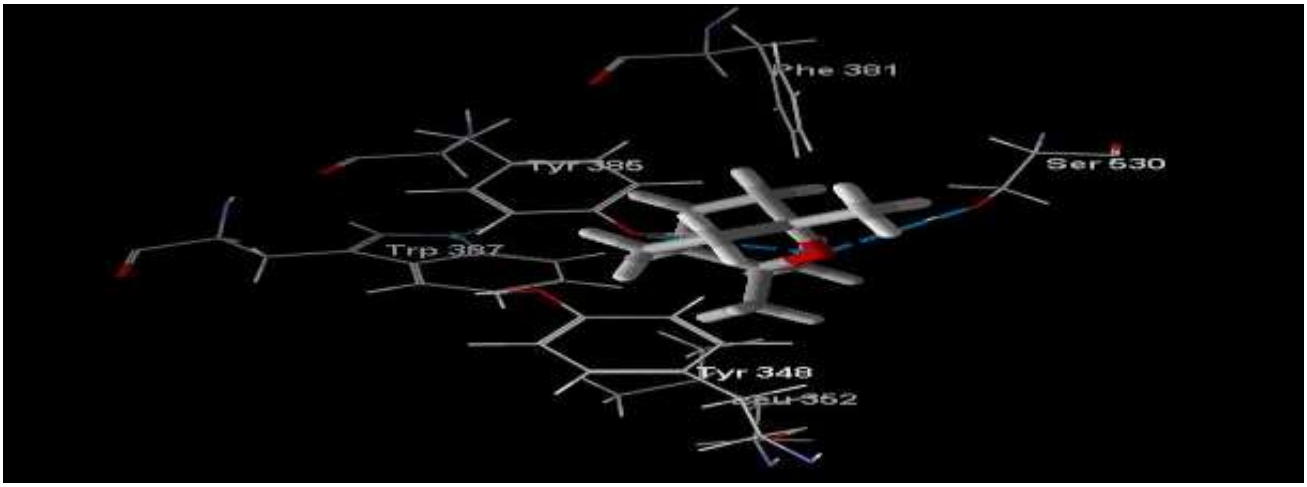

Figure 4: Image of 3-D interaction of 1,8-cineole with the amino acid cyclooxygenase-2 (COX-2) receptor (PDB code: 1PXX) via hydrogen bonds and steric interactions

The interaction of diclofenac with COX-2 receptor amino acids is through two hydrogen bonds; Tyr385 and Ser 530 and three steric bonds Tyr 385, Ser 530, and Gly 526 (Figure 5).

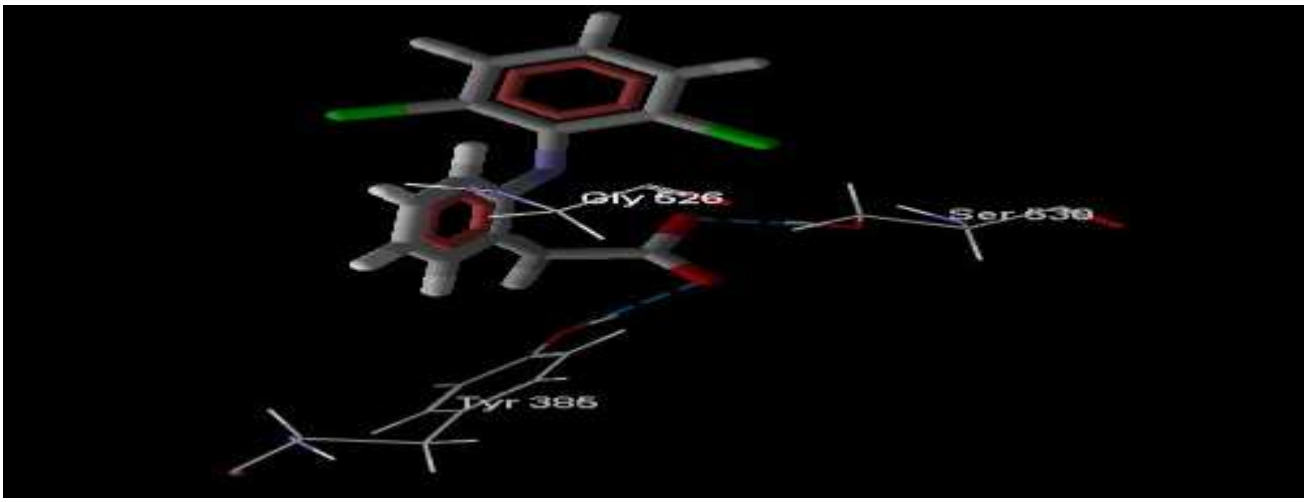

Figure 5: A 3-D picture of the diclofenac interaction with the amino acid cyclooxygenase-2 (COX-2) receptor (PDB code: 1PXX) via hydrogen bonds and steric interactions

Five cavities were found in the Toll Like Receptor-2 (TLR-2) structure (PDB code: 1FYW), while a CAS_673 (A) ligand was detected in cavity 3 (Figure 6). Cavity 3 represented a location where 1,8-cineole interacts with TLR-2 receptor amino acids through hydrogen bonds and steric bonds. There were two hydrogen bonds; Val 645 (A) and Ala 643 (A) and six steric bonds; Leu 674 (A), Leu 672 (A), Ala 643 (A), Tyr 641 (A), Ser 692 (A) and Val 645 (A) (Figure 7).

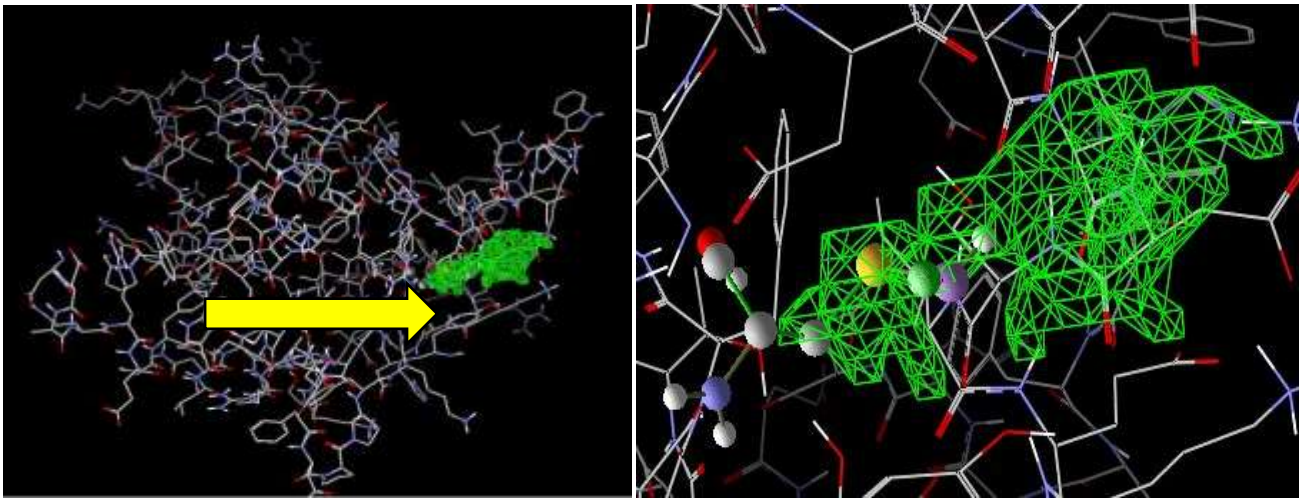

Figure 6: Cavity 3 in the Toll Like Receptor-2 (TLR-2) (PDB code: 1FYW) with CAS_673 (A) ligand

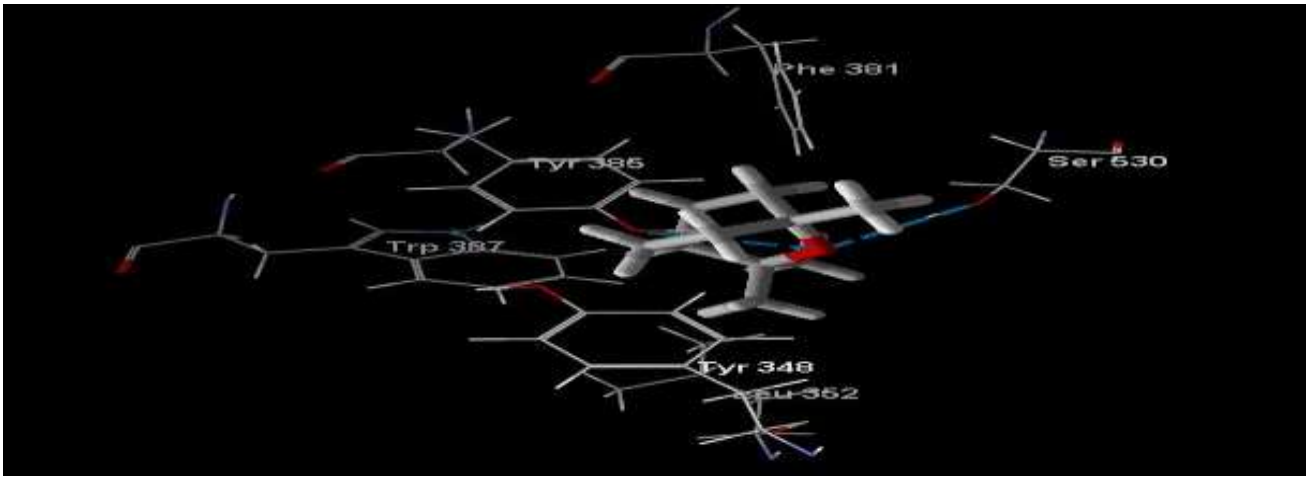

Figure 7: Figure 3-D interaction of 1, 8-cineole with the amino acid and Toll Like Receptor-2 (TLR-2) (PDB code: 1FYW) through hydrogen bonds and steric interactions

The interaction between CAS_673 and the amino acids Toll like Receptor-2 (TLR-2) (PDB code: 1FYW) was by means of hydrogen bonds and steric bonds.

There were two hydrogen bonds; Ala 643 (A) and Val 645 (A) and seven steric bonds; Ala 643 (A), Leu 672 (A), Leu 674 (A), His 675 (A), Val 645 (A), Ser 692 (A), and Tyr 641 (A) (Figure 8).

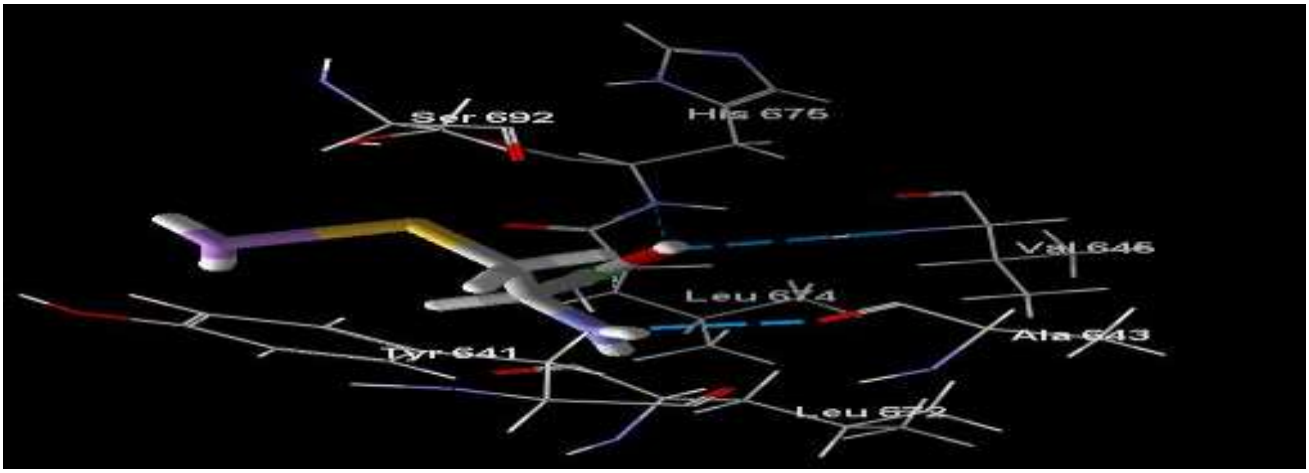

Figure 8: Overview of 3-D interaction of CAS_673 ligands with amino acids Toll Like Receptor-2 (TLR-2) (PDB code: 1FYW) through hydrogen bonds and steric interactions

The illustration of interaction between 1, 8 cineole and COX-2 and interaction of 1, 8-cineole and TLR2 in 2-D and 3-D can be seen in Figures 9 and 10.

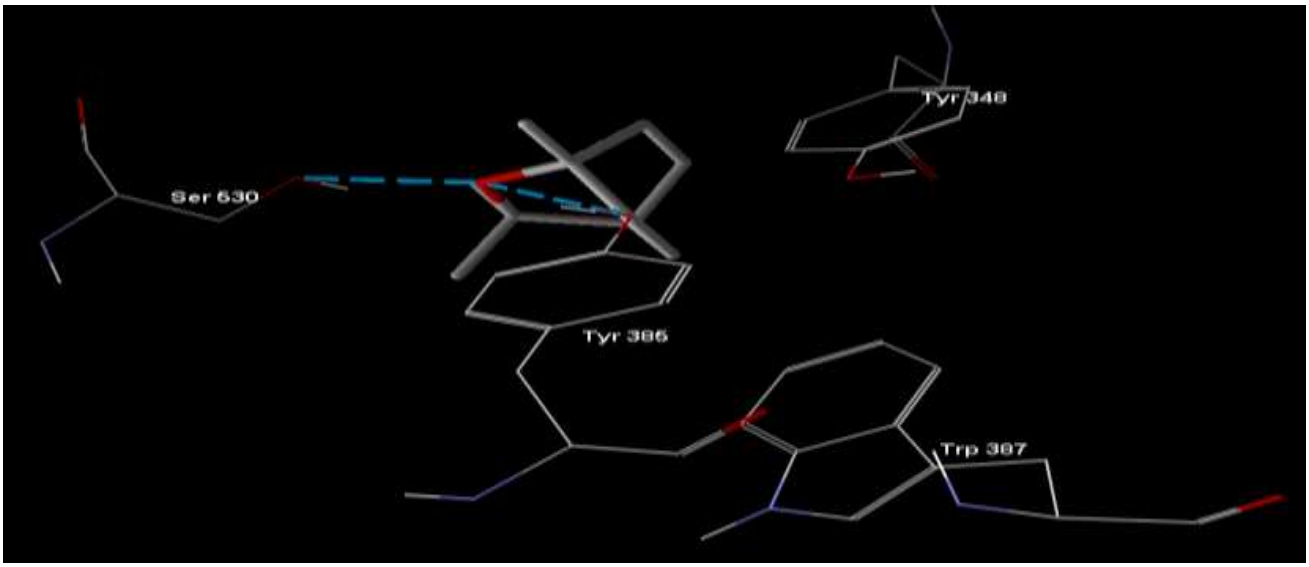

Figure 9: 3-D image of Hydrogen and steric bond of 1, 8-cineole and COX-2

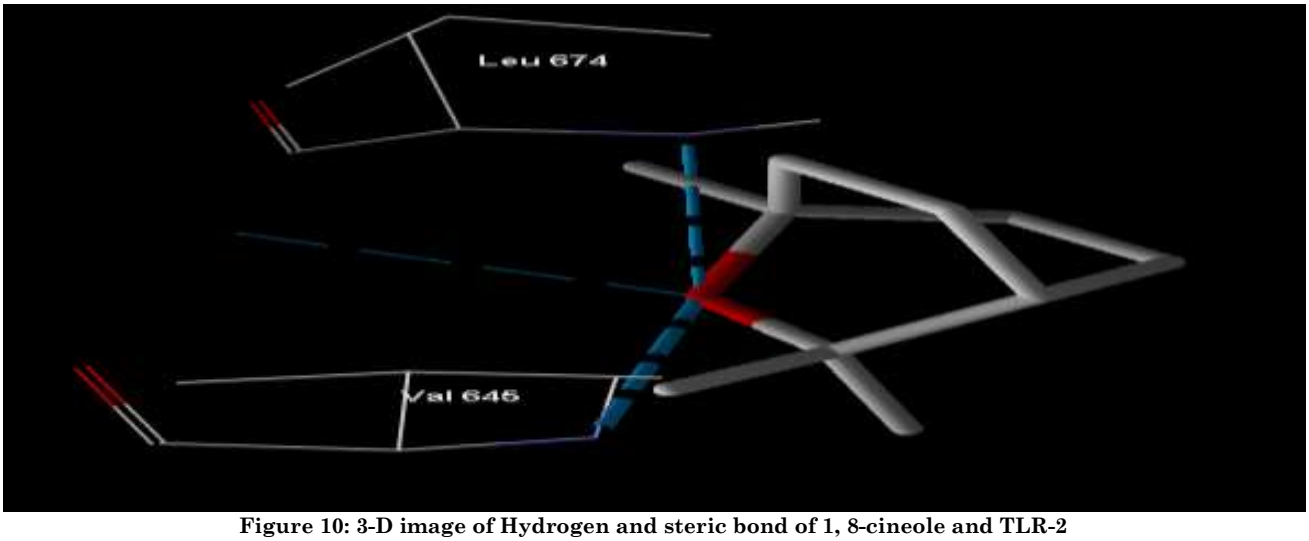

Figure 10: 3-D image of Hydrogen and steric bond of 1, 8-cineole and TLR-2

Table 1: Docking results of 1, 8-cineole and diclofenac receptors ligands (PDB code: 1PXX) using the Molegro Virtual Docker 5.5 program.

No.	Compound	Moldock Score	Rerank Score
1.	1,8-cineole	-49.1236	-46.2926
2.	Diclofenac	-106.8010	-89.6848

Table 2: Docking results of 1, 8-cineole, TLR-2 ligand (CAS_673) (PDB code: 1FYW) using the Molegro Virtual Docker 5.5 program

No.	Compound	Moldock Score	Rerank Score
1.	1,8-cineole	-42.7930	-42.0850
2.	CAS_673	-56.5640	-47.7661

The MolDoc score results showed that 1, 8-cineole (-49.1236) could be predicted to be an anti-inflammatory ligand with a lower activity level than diclofenac/COX-2 ligand (-106.8010) (see Table 1). 1, 8-cineole (-42.7930) could also be predicted to be an anti-inflammatory ligand with a lower activity level than TLR-2/CAS_673 (56.5640) ligand (see Table 2)

Discussion

Molecular modeling or *in silico* tests play a crucial role in the field of medicinal chemistry through a framework of designing, discovering and optimizing bioactive compounds during the process of drug development [8]. Through bioinformatics, the design of a new drug can be achieved cheaply, and rapidly [12]. This process can be simulated, and an accurate calculation arrived at with the help of a computer.

This *in silico* study was conducted to quantify the minimum energy from the molecular ligand of 1, 8-cineole predicted to have the property of inhibiting COX-2 and TLR-2. There are several databases including Zinc Data Base, ChEMBL, Chemspider, Ligand Protein Data Base (LPDB), Protein Data Bank (PDB), TTD and STITCH which contain chemical compounds together with information regarding their functions or bioassay results [12].

The docking results in the form of bond energy were illustrated by the Rerank Score (RS) or MolDoc score. RS quantifies the energy required during the process of ligand-receptor interaction. From this value, the anti-inflammatory activity of compounds across the 1, 8-cineole barrier which is presented by the cyclooxygenase-2 (COX-2) and TLR-2 receptor targets can be predicted. The bond energy constitutes the amount of energy required to form the bond between the ligand and the receptor. The lower the bond energy, the greater its stability.

It can also be predicted that the more stable the ligands binding to the receptor, the greater the activity [13]. A compound with a small RS value has a stable ligand-receptor bond whose activity is predicted to be considerable. The MolDoc score results showed that 1, -cineole (-49.1236) could be predicted as an anti-inflammatory ligand which had lower activity than a diclofenac / COX-2 ligand (-106.8010). 1, 8-cineole (-42.7930) could also be predicted as an anti-inflammatory ligand which demonstrated lower activity than a TLR-2/CAS_673 (-56.5640) ligand. From the RS values and Mol Doc scores (see Table 1) it can be concluded that the bond between diclofenac (RS = -89.6848; Mol Doc score = -106.8010) as COX-2 receptor target is more stable than 1, 8-cineole compounds (RS = -46.2926; MolDoc score = -49.1236).

The diclofenac bond is supported by two hydrogen bonds (Tyr385 and Ser 530) and three steric bonds (Tyr 385, Ser 530, and Gly 526), while 1,8-cineole compound is supported by two hydrogen bonds (Tyr385 and Ser 530) and six steric bonds (Tyr 385, Phe 381, Ser 530, Leu 352, Tyr 348, and Trp 387). From the RS value and MolDoc score data (see Table 2), it can be concluded that the bond between CAS_673 (RS = -47.7661; MolDoc score = -56.5640) as a TLR-2 receptor target is slightly more stable than the 1, 8-cineol compound (RS = -42.0850; MolDoc score = -42.7930). The bond between CAS_673 is supported by two hydrogen bonds (Ala 643 (A) and Val 645 (A)) and seven steric bonds (Ala 643 (A), Leu 672 (A), Leu 674 (A), His 675 (A), Val 645 (A), Ser 692 (A), and Tyr 641 (A)).

1, 8-cineole compounds are supported by two hydrogen bonds (Val 645 (A) and Ala 643 (A)) and six steric bonds (Leu 674 (A), Leu 672 (A), Ala 643 (A), Tyr 641 (A), Ser 692 (A) and Val 645 (A)). The anti-inflammatory potential of 1,8-cineole indicated by this study supports the results of that conducted by Juergens (2014) which shows that 1,8-cineole has potential as a drug with therapeutic activity with the most likely effects to be anti-inflammatory and anti-asthmatic [14].

Conclusion

1.8-cineole possesses anti-inflammatory potential against COX-2 and TLR-2 receptors based on an *in silico* predictive study.

Acknowledgement

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