MOLECULAR DOCKING OF BENZIMIDAZOLE DERIVATIVE COMPOUNDS AS XANTHIN OXIDASE INHIBITOR

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Abstract

Modifying the structure of a compound can increase the potency and selectivity of a compound. In designing a compound that is potent and selective, this can be done by: several methods, one of which is molecular docking. Benzimidazole, a special organic compounds with N-heterocyclic ring systems show various biological activities through effective binding to enzyme receptor sites. xanthin oxidase is one of the target proteins which is an enzyme. This study aimed to predict benzimidazole derivate compounds as xanthin oxidase inhibitor using molecular docking and predict their physicochemical properties. Molecular docking menggunakan Molegro Virtual Docker, for physicochemical properties using SwissADME. The results of the research showed that the activity of Benzimidazole derivative compounds is better than Allopurinol, whic is shown from the Rerank Score value which is lower than allupurinol so that the activity of the Benzimidazole derivative compound as a Xanthin Oxidase receptor inhibitor is better and more stable as a new drug candidate.

***Keyword**: benzimidazole, xanthin oxidase inhibitor, allopurinol. docking*

Background

Xanthin oxidase is an enzyme complex consisting of protein molecules, each molecule of which consists of 2 moles of FAD, 2 moles of Mo atoms, and 8 moles of Fe atoms. This enzyme is found in the liver and muscles in the human body, and plays an important role in purine catabolism, namely oxidizing hypoxanthine to xanthine and then to uric acid (Figure). One unit of the xanthine oxidase enzyme can convert one µmol of substrate (xanthine) into uric acid every minute at optimum pH (pH 7.5) and optimum temperature (25^{o}C) (Umamaheswari *et. al*, 2009).
Xanthin oxidase is the target protein of allopurinol. Allopurinol is a synthetic drug that is commonly used for gout therapy with the mechanism of inhibiting the activity of the enzyme xanthine oxidase to inhibit the formation of uric acid. Indonesia is one of the countries that commonly uses allopurinol for gout therapy (Price and Wilson, 2005). Allopurinol is used as a competitive inhibitor of the xanthine oxidase enzyme because it has a structure that is almost the same as xanthine. Allopurinol can form a combination of covalent, hydrogen and electrostatic bonds at the active site of the xanthine oxidase enzyme. Allopurinol's affinity for the xanthine oxidase enzyme is also stronger than xanthine. The following is the mechanism for inhibiting xanthine oxidase by allopurinol (Kostic, 2015).
Benzimidazole is a heterocyclic compound that is widely used in medicinal compounds, because it shows a wide spectrum of pharmacological activity. Benzimidazoles are structural isosters of naturally occurring nucleotides that enable them to bind only to enzymes of biological systems due to their wide range of biological activities (Kankeaw and Ratchaneeporn, 2015)
Structure modification can increase the potency and selectivity of a compound. In designing a Structure that is potent and selective, several methods can be used, one of which is molecular docking. Molecular docking is a computational method that aims to imitate the interaction of a ligand molecule with its target protein in an in-vitro test (*Katsila et.al, 2016).* In this research, an in silico study was carried out on benzimidazole derivatives as xanthin oxidase receptor inhibitors

Methods

In this research, the equipment needed is software ChemDraw, Chem3D, Molegro Virtual Docker, pkCSM Tool, ProTox II, RCSB programs. Meanwhile, the materials needed are downloading the Xanthine Oxidase receptor ligand in RCSB and obtaining the 3NVY receptor ligand, 2D and 3D structures of the test compounds (Allopurinol and 20 Benzimidazole derivative compounds)

Ligand Preparation

Preparation of the 2D structure of the test compound was carried out by creating a structure in the ChemDraw program by drawing the structure manually and then stabilizing the stereochemical shape of the compound by "clean up structure". The structure is saved in .cdx format, then 3D structure preparation is carried out using the Chem3D program and then the structure is stabilized by selecting the configuration with the minimum energy using "Calculations → MMFF94 → MMFF94 Performance Minimization". The structure is saved in the form SYBYL.mOl2

Docking Analysis

The docking process is carried out with the Molegro Virtual Docker (MVD) program.
- The first step is to download the ligand-receptor involved in the reaction that occurs for the drug's target action on the RCSB PDB site, in this case the receptor required is 3NVY. This receptor is imported into the MVD, the appropriate native ligand is selected, the cavities and interactions between the bonds are seen.
- The second step is to detect cavities, detect the position of the receptor where the drug will bind, namely in the form of holes (cavities) in the ligand-receptor.
- The third step is the docking wizard, the structure of the test compound is imported into MVD as well as the ligand of the test compound, which is then looked at for interactions between bonds

(ligand map), then detects the pharmacophore group and determines the 3 points (compound atoms) where the drug is located mwill interact (bind) with the ligand-receptor. The parameters measured in the docking process are the energy values involved, in the form of MolDock Score, Rerank Score, and Hbond, as well as the RMSD (Root Mean Square Deviation) value. To measure the strength of drug-receptor bonds, the parameter that is often used is the Rerank Score (RS) value.

Physicochemical Properties Prediction

Prediction of the physicochemical properties of the test compounds is carried out by entering the smiles structures of the test compounds one by one into the SWISS ADME program, from which complete data can be obtained regarding the predicted physicochemical properties of each compound and absorption percentage is predicted use PkcsmTool

Result and Discussion

Docking Result

Based on the docking results that have been carried out between the control compound, in this case Allopurinol and 20 Benzimidazole derivative compounds with the Xanthin Oxidase (3NVY) receptor, the lowest Rerank Score value was obtained, namely for the 20th compound or N-(4-acetylphenyl)-1H-benzo [d]imidazole-4-carboxamide with a value of -99,353 kcal/mol and the highest Rerank Score value is the 1st compound or Benzimidazole with a value of -62,277 kcal/mol, while Allopurinol as a control compound only has a value of -72,600 kcal/mol with a value The Rerank Score of the native ligand is -105.081 kcal/mol. This docking had previously been validated on the original ligand and obtained an RMSD (Root Mean Square Deviation) value of less than 2 angstrom.

Table 1. Result of Docking Validation on 3NVY receptor with allopurinol as native ligand

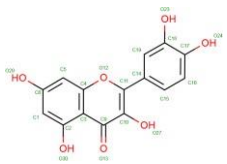
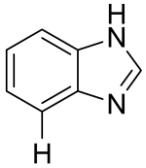
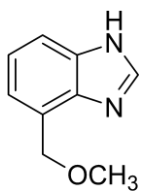
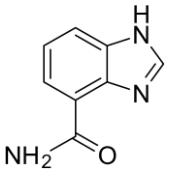
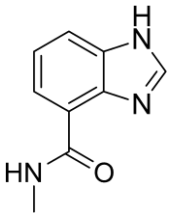
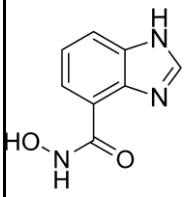
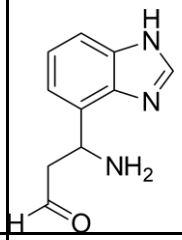
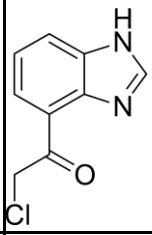
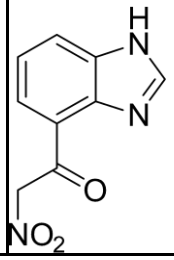
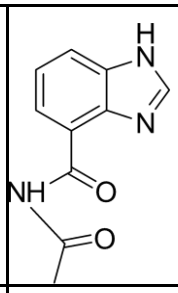
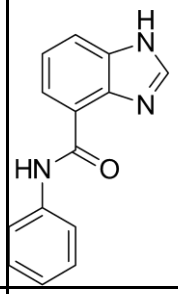
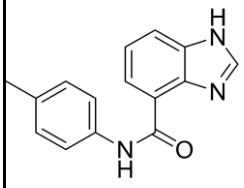
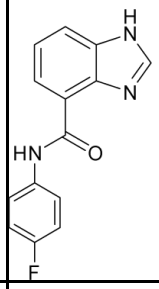
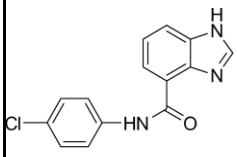
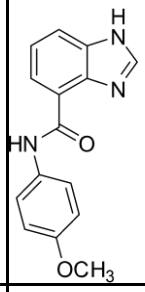
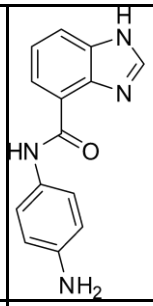
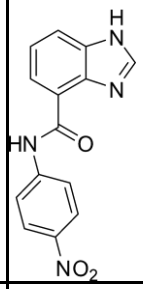
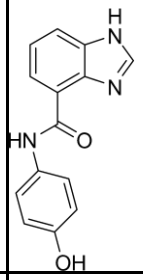
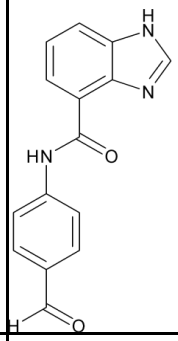
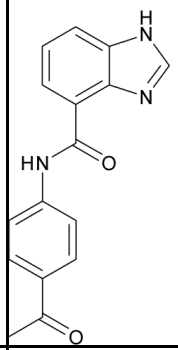
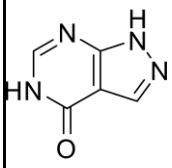
Compound	Struktur	Structure Name	Rerank Score	RMSD (Å)
Native ligand		3,5,7,3',4'-Pentahydroxyflavone	-105,081	1,064

Table 2. Results of docking analysis on 3NVY receptor from benzimidazole derivates

Compound	Structure	Structure Name	Rerank Score
B1		1*H*-benzo[*d*]imidazole	-62,277
B2		4-(methoxymethyl)-1*H*-benzo[*d*]imid	-77,031

B3		4-(2,2,2-trifluoroethyl)-1*H*-benzo[*d*]imidazole	-76,902
B4		1*H*-benzo[*d*]imidazole-4-carboxa	-78,015
B5		*N*-methyl-1*H*-benzo[*d*]imidazole-4-carboxamide	-81,347
B6		*N*-hydroxy-1*H*-benzo[*d*]imidazole-4-carboxar	-82,179
B7		3-amino-3-(1*H*-benzo[*d*]imidazol-4-yl)propa	-82,510
B8		1-(1*H*-benzo[*d*]imidazol-4-yl)-chloroethanone	-82,667
B9		1-(1*H*-benzo[*d*]imidazol-4-yl)-2-nitroethan	-83,543

B10		*N*-acetyl-1*H*-benzo[*d*]imidazole-carboxamide	-89,895
B11		*N*-phenyl-1*H*-benzo[*d*]imidazole-carboxamide	-93,134
B12		*N*-(*p*-tolyl)-1*H*-benzo[*d*]imidazole-4-carboxar	-84,053
B13		*N*-(4-fluorophenyl)-1*H*-benzo[*d*]imidazole carboxamide	-92,375
B14		*N*-(4-chlorophenyl)-1*H*-benzo[*d*]imidazole-4-carboxar	-92,066
B15		*N*-(4-methoxyphenyl)-1*H*-benzo[*d*]imidazole carboxamide	-94,746
B16		*N*-(4-aminophenyl)-1*H*-benzo[*d*]imidazole-4-carboxa	-92,890

			
B17		*N*-(4-nitrophenyl)-1*H*-benzo[*d*]imidazole-4-carboxar	-97,318
B18		*N*-(4-hydroxyphenyl)-1*H*-benzo[*d*]imidazole-4-carboxar	-92,622
B19		*N*-(4-formylphenyl)-1*H*-benzo[*d*]imidazol carboxamide	-96,282
B20		*N*-(4-acetylphenyl)-1*H*-benzo[*d*]imidazole-4-carboxar	-99,353
Allopurinol		***1,5-Dihydro-4H-pyrazolo[3,4- d]pyrimidin-4-one***	-72,600

Based on these results, it can be said that the test compound N-(4-acetylphenyl)- 1H-benzo[d]imidazole-4-carboxamide has the highest XO receptor inhibitor activity compared to the other test compounds because it has the lowest Rerank Score value which illustrates that Compounds require less binding energy to form ligand-receptor bonds so that the compound's activity will be better and more stable. Judging from the Rerank Score value of other benzimidazole derivatives, it is in the range of -62.2 to -99.35 kcal/mol which has an average value lower than the control compound, namely Allopurinol, at -72.6 kcal/mol but not lower than the native ligand. This can be interpreted that the benzimidazole derivative compound has better activity than the Allopurinol and benzimidazole compounds themselves. Based on interactions with receptors, it is known that imidazole derivatives form the same hydrogen bonds with allopurinol as xanthin oxidase inhibitors, namely in the amino acids arginine 880 and threonine 1010. Therefore, they have the potential to have the same mechanism apart from a better rerank score.

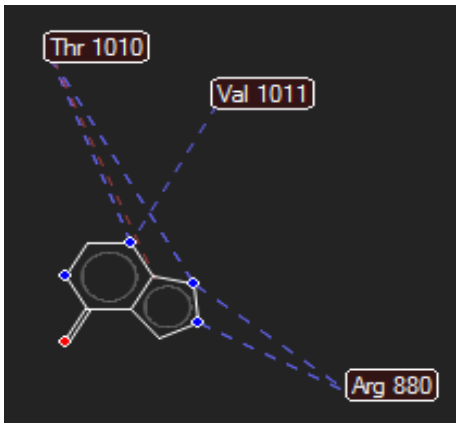


Figure 1. interaction allopurinol with 3NVY receptor

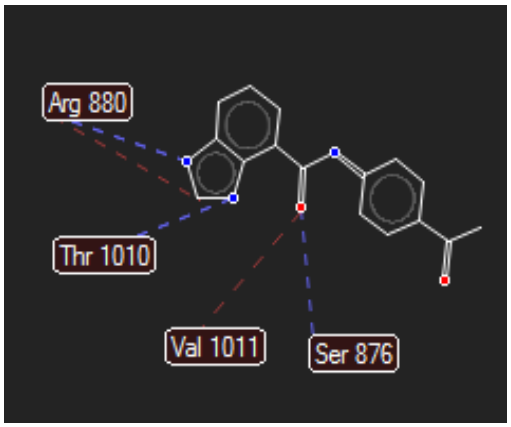


Figure 2. interaction benzimidazole derivates with 3NVY receptor

The xanthin oxidase receptor plays a role in purine metabolism. Compounds that inhibit xanthin oxidase receptors can reduce the production of uric acid so they can be indicated as hyperuricemia drugs. Based on the results of docking analysis, the test compounds have a mechanism that can bind the active site of the purine compound binding site, so that it can increase the excretion of uric acid from the body or suppress the production of uric acid and reduce hyperuricemia levels (Pacher P, Nivorozhkin A, Szabó C 2006). It can be concluded that based on the rerank score results, benzimidazole derivative compounds can be developed into potential hyperuricemia drug candidates.

Table 3. Intestinal Absorption (%Absorbed) Prediction

Compound	Intestinal Absorption (%Absorbed)	Lipinski's Rule of Five (Lipinski, 2004)
Allopurinol	94.141	*yes*
B1	89.806	*yes*
B2	88.491	*yes*
B3	84.723	*yes*
B4	84.518	*yes*
B5	89.765	*yes*
B6	89.359	*yes*
B7	67.531	*yes*
B8	85.994	*yes*
B9	73.147	*yes*
B10	87.475	*yes*
B11	99.822	*yes*
B12	88.852	*yes*
B13	88.35	*yes*
B14	87.393	*yes*

B15	89.797	*yes*
B16	86.545	*yes*
B17	86.894	*yes*
B18	86.371	*yes*
B19	90.143	*yes*
B20	89.754	*yes*

Based on the prediction of percent absorption, the 20 hit compounds resulting from virtual screening using the pkCSM Tool had a percentage of human intestinal absorption in humans ranging from 60-90%, which indicates that these compounds can be absorbed well through the intestines. A compound is said to have good absorption if the absorption value is > 80% and is said to have a poor absorption value if the absorption value is > 30% (Chander *et al*, 2017).
The results of the predicted physicochemical properties show that all benzimidazole derivatives fulfill Lipinski's 5th law. The provisions of Lipinski's 5th law indicate the compound's ability to be absorbed and have the potential to be used as an oral drug (Kesuma *et.al*, 2018).

Conlusion

In this research it can be concluded that benzimidazole derivatives work in a similar mechanism to allopurinol as Xanthin Oxidase inhibitors so they have potential as drugs. In addition, based on the prediction of percent absorption and physicochemical parameters, benzimidazole derivatives can be administered as oral drugs

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