

*In silico* exploration for aldose reductase (AR) inhibitorsMuhammad Arba<sup>1</sup> , Arfan Arfan<sup>1</sup>, Nuralifah<sup>1</sup>, Wa Ode Haerunnisah<sup>2</sup>, Laode Kadidae<sup>2</sup><sup>1</sup>Faculty of Pharmacy, Universitas Halu Oleo, Kendari, Indonesia, 93232<sup>2</sup>Faculty of Mathematics and Natural Sciences, Universitas Halu Oleo, Kendari, Indonesia, 93232\*corresponding author e-mail address: [muh.arba@uho.ac.id](mailto:muh.arba@uho.ac.id) | Scopus ID [57155296400](https://orcid.org/0009-0001-9000-0000)**ABSTRACT**

Aldose reductase plays a role in glucose metabolism in the polyol pathway responsible for complications of diabetes mellitus. In this study, pharmacophore modeling and molecular docking were conducted to identify hit compounds as inhibitors of aldose reductase (AR). The pharmacophore feature consists of two hydrogen-bond acceptors, four hydrophobics and one aromatic ring feature with Area Under Curve of Receiver Operating Characteristics (AUC-ROC) is 0.53 and the Goodness of Hit (GH) value is 0.76. Screening in the ZINC database generated 1,225 hit compounds, were subjected to molecular docking to determine their binding energy and interactions with AR. The range of binding energy (E) of all hit compounds is -8.81 to -14.22 kcal/mol and there are four best hit compounds namely Lig\_234, Lig\_873, Lig\_1, and Lig\_902, when compared to native ligands (3NA, E = -12.98 kcal/mol) based on their binding energy and orientation, which indicate their potential as new AR inhibitors.

**Keywords:** *aldose reductase; diabetes; molecular docking; pharmacophore; virtual screening.*

**1. INTRODUCTION**

Diabetes mellitus is a disease caused by metabolic disorders characterized by an increase in glucose levels in the blood [1]. The disease arises as a result of not produced insulin due to autoimmune of  $\beta$ -cells (type 1 diabetes), or insulin secretion defected in the body (type 2 diabetes) [2]. One of the serious problems in diabetes is complications of diseases that may be caused such as nephropathy, diabetic neuropathy, retinopathy, and cardiovascular disease [3]. Some evidence shows a correlation between increased polyol pathway activation in hyperglycemic conditions to diabetes complications [4].

Aldose reductase (AR) is an enzyme that plays a role in the polyol pathway that converts glucose into sorbitol which will then be metabolized into fructose [5], [6]. Accumulation of sorbitol and fructose can cause intracellular osmotic stress and support the development of diabetes complications [7], [8]. The inhibition of aldose reductase has been an important concern for the last decades as a promising target for diabetes complications [9].

**2. MATERIALS AND METHODS**

The PDB aldose reductase structure was downloaded from the Brookhaven Protein Data Bank (<http://www.rcsb.org/pdb/>) with PDB ID 1Z3N. Interaction of 3NA with aldose reductase was used to develop pharmacophore model employing LigandScout Advanced 4.3 [16]. The model validation was achieved using 220 actives and 9131 decoys, taken from a Directory of Useful Decoys: Enhanced (DUD-E) (<http://www.dude.docking.org/>) [17]. Screening against PubChem database was performed by employing Pharmit (<http://pharmit.csb.pitt.edu>) [18].

**3. RESULTS**

The 3NA consisted of twelve pharmacophore features, i.e. six hydrophobics, two aromatic rings, and four hydrogen bond acceptors. Figure 1 displays the pharmacophore features of 3NA.

AR inhibitors such as zopolrestat, sorbinil, and ranirestat have the disadvantage of being poor in penetration into tissues, causing skin irritation and toxic reactions to the liver [10], [11]. Epalrestat is one of the AR inhibitors have been invented and used today [12]. Some drugs are still in clinical trials and some are withdrawn because of safety concerns [13]. This study focuses on identifying potential molecules that can inhibit aldose reductase by utilizing virtual screening methods.

Virtual screening is one of the methods in computer-aided drug design that plays a role in the discovery of new hit compounds. Computer-aided drug design consists of a structure-based and ligand-based drug design [14], [15]. In this study, a pharmacophore modeling which is a ligand-based drug design was developed to build a pharmacophore model that can be used to screen new hit compounds against a database containing a large number of compounds, as well as a molecular docking study was performed to determine their binding energy and orientation to aldose reductase.

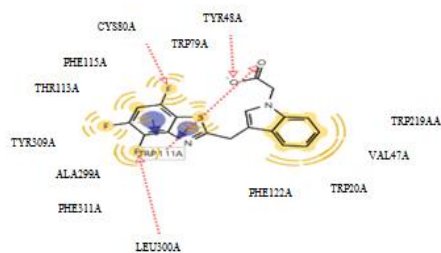
The structure preparation consisted of assigning Kollman charge and removing water molecules, which was conducted using AutoDockTools 1.5.6 software [19], [20]. Molecular docking was performed by using iDock software [21], while visualization was performed using Discovery Studio Visualizer.

The grid box of active site of the aldose reductase was built by following the 3NA conformation (center x = 21.245, center y = -5.686, center z = 24.865) with 40 x 40 x 40 width on the X Y Z grid point spacing 0.375 Å.

Using those features, several pharmacophore models were built. Table 2 lists the pharmacophore models with the Area Under

Curve of Receiver Operating Characteristics (AUC-ROC) above 0.5.

Among the thirteen models above, one model with the highest AUC score was selected, i.e. ALR-13 with GH score 0.76 and the values of AUC<sub>100%</sub> 0.53. Table 1 shows the parameter and GH score obtained for the pharmacophore model ALR-13.

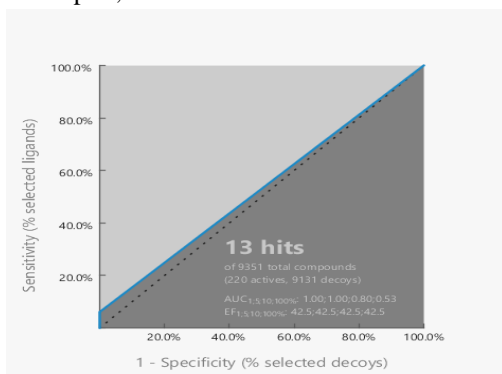


**Figure 1.** The pharmacophore features of 3NA, in which hydrophobic, aromatic ring, and hydrogen bond acceptors were assigned as yellow, violet, and red colors, respectively.

**Table 1.** The parameter and GH score obtained for pharmacophore model ALR-13.

Parameter	Values
Total molecules in a database (D)	9351
Actives in a database (A)	220
The obtained hits (Ht)	12
The obtained actives (Ha)	12
% actives [(Ha/Hit)*100]	100
% actives to total molecules [(Ha/A)*100]	5.45
Enrichment factor (E) [(Ha*D)/(Hit*A)]	42.50
False negatives [A-Ha]	208
False positives [Ht-Ha]	0
Goodness of Hit Score (GH)*	0.76

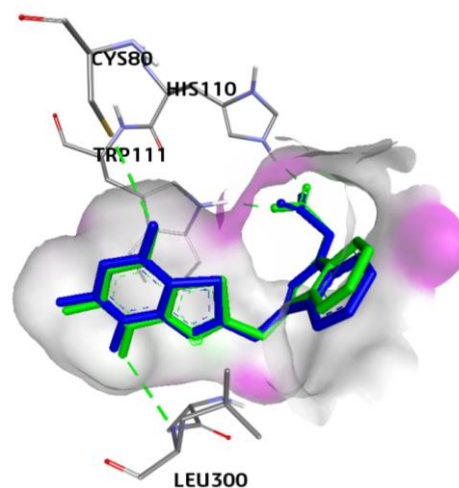
The high GH score and Area Under Curve of Receiver Operating Characteristic curve (AUC-ROC) implied that the model was valid and be able to identify the actives from decoys. Figure 2 shows the ROC plot, in which the AUC value was 0.53.



**Figure 2.** The Area Under Curve (AUC) of Receiver Operating Characteristic plot (ROC) of ALR-13.

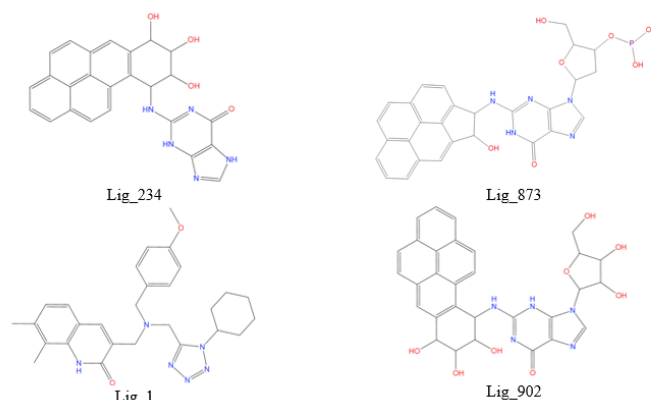
Further, virtual screening against PubChem database was performed using the validated pharmacophore model employing Pharmit webserver and 1225 hits were retrieved. Molecular docking on every 1225 hits including 3NA was performed to explore their binding interactions with aldose reductase. The validation of the docking protocol by redocking of 3NA resulted in the binding energy of  $-12.98$  kcal/mol, with the value of root mean square deviation (RMSD)  $0.195$  Å, indicating that the docking protocol was acceptable [22]–[24]. The docked conformation of 3NA consisted of four hydrogen bonding interactions with Trp111, Leu300, Cys80 and His110. The hydrophobic interactions with

Ala299, Phe311, Tyr309, Thr113, and Phe115, as shown in Figure 3. Those interactions were also confirmed in the X-ray crystallographic pose [25].

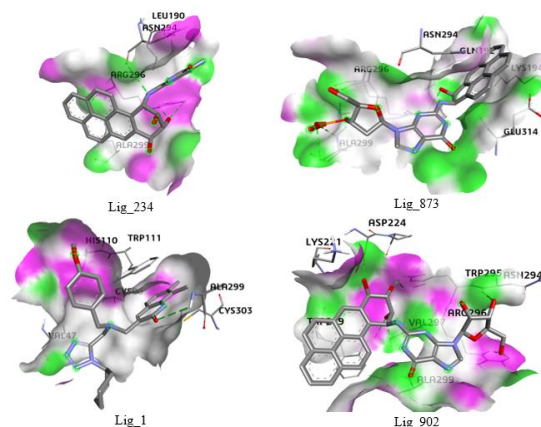


**Figure 3.** Superimposed of 3NA between X-ray (green) and docked (blue) conformations with RMSD  $0.195$  Å.

Meanwhile, molecular docking to 1225 ligands resulted in conformations and binding energies between  $-8.81$  and  $-14.22$  kcal/mol. The four best hit molecules in terms of binding orientation and binding energy were Lig\_234 ( $E=-14.22$  kcal/mol), Lig\_873 ( $E=-14.14$  kcal/mol), Lig\_1 ( $E=-13.90$  kcal/mol), and Lig\_902 ( $E=-13.64$  kcal/mol). All the four hits had the binding energy lower than that of 3NA. The structures of best hits are displayed in Figure 4.



**Figure 4.** The molecular structures of best-docked hit molecules.



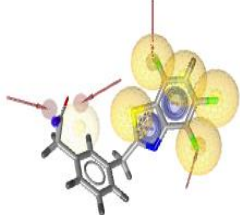
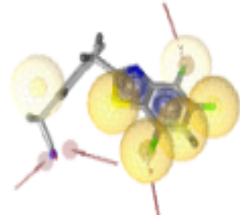
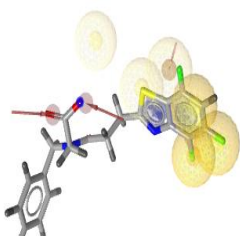
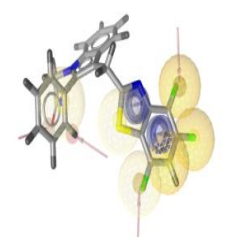
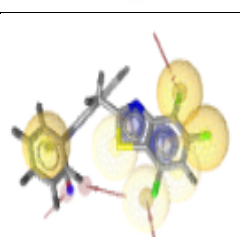
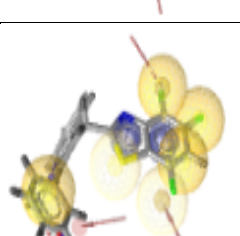
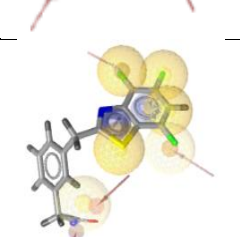
**Figure 5.** The interactions of Lig\_234, Lig\_873, Lig\_1, and Lig\_902, each with aldose reductase.

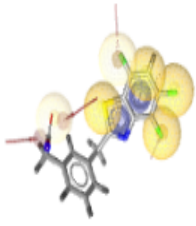
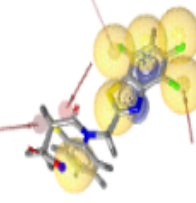
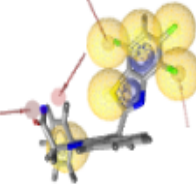
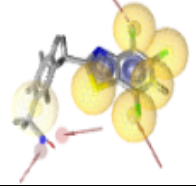
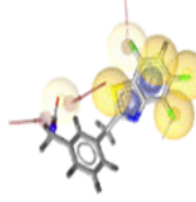
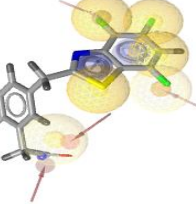
The interaction of hit molecules occurred in the active site of aldose reductase through hydrogen bond (Hbond) and hydrophobic interactions. Lig\_234 and Lig\_902 formed Hbond

interaction with Asn294 and Arg296. In addition, hydrophobic interactions of Lig\_234 were observed with Phe311, Tyr309, Trp295, Gln192 and Glu314. Hydrogen bonding was also observed at Lig\_873 with Ala299, Val297, Arg296 and Gln192 residues.

Interactions with Tyr48, His110, Trp111, Leu300, Cys330 and Thr 113 residues were observed at Lig\_1 as in the crystallographic conformation [25]. Figure 5 shows the interaction of Lig\_234, Lig\_873, Lig\_1, and Lig\_902, with aldose reductase.

**Table 2.** The pharmacophore models

ID	Pharmacophore Model	Features of pharmacophore	Goodness of Hit (GH)	AUC <sub>100%</sub>
ALR 1		5 hydrophobics, 2 aromatic rings, 4 hbond acceptors	0.76	0.52
ALR 2		4 hydrophobics, 2 aromatic rings, 4 hbond acceptors	0.76	0.53
ALR 3		3 hydrophobics, 1 aromatic ring, 1 hbond acceptor	0.3	0.53
ALR 4		3 hydrophobics, 2 aromatic rings, 1 hbond acceptor	0.4	0.54
ALR 5		3 hydrophobics, 2 aromatic rings, 4 hbond acceptors	0.75	0.50
ALR 6		4 hydrophobics, 2 aromatic rings, 4 hbond acceptors	0.75	0.50
ALR 7		4 hydrophobics, 1 aromatic ring, 2 hbond acceptors	0.76	0.53

ID	Pharmacophore Model	Features of pharmacophore	Goodness of Hit (GH)	AUC <sub>100%</sub>
ALR 8		4 hydrophobics, 2 aromatic rings, 2 hbond acceptors	0.76	0.53
ALR 9		6 hydrophobics, 2 aromatic rings, 3 hbond acceptors	0.75	0.51
ALR 10		6 hydrophobics, 2 aromatic rings, 3 hbond acceptors	0.75	0.50
ALR 11		5 hydrophobics, 2 aromatic rings, 4 hbond acceptors	0.76	0.52
ALR 12		4 hydrophobics, 2 aromatic rings, 2 hbond acceptors	0.76	0.53
ALR 13		4 hydrophobics, 1 aromatic ring, 2 hbond acceptors	0.76	0.53

#### 4. CONCLUSIONS

In this study, pharmacophore modeling was carried out successfully identifying the potential hit compounds to inhibit aldose reductase. The validated pharmacophore model was used to screen new hit compounds in the Pubchem database which contained more than 93 million compounds. The best four hit

compounds obtained (Lig\_234, Lig\_873, Lig\_1, and Lig\_902) had a better binding affinity compared to native ligands (3NA), which can be used in further experimental studies to prove their potential as aldose reductase inhibitors.

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## 6. ACKNOWLEDGEMENTS

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