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Original Article

Virtual Screening of Indonesian Herbal Database as Murine Double Minute-2 (MDM2) Inhibitor

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ABSTRACT

Background: Murine Double Minute-2 (MDM2) overexpression causes the p53 deficiency, so the role p53 as a cell regulator does not work in the case of cancer. **Methods:** In this study, virtual screening of Indonesian herbal database to discover MDM2 inhibitors was carried out. Autodock and Autodock Vina validated with Directory of Useful Decoy-Enhanced (DUD-E). Validation parameters were performed with Enrichment Factor, Receiver Operating Characteristics, and Area Under Curve. **Results:** The validation with the grid box 70x70x70 on Autodock resulting AUC value 0.72, while in Autodock Vina 0.43. Autodock Vina did not fulfilll the standard value but still used for comparison. Based on the virtual screening result, top ten compounds from Autodock are Nimolicinol, Jacoumaric acid, Isoarborinol, Lantic acid, Diosgenin, Theasaponin E1, Taraxasterol, Leucadenone C, Simiarenol, and Alpha-Amyrin were found to have strong interaction with MDM2, with binding energy (ΔG) ranging from -8.83 to -9.65 kcal/mol. The Autodock Vina screening resulted in the identification of Yuehchukene, Morusin, Cyanidin, Leucadenone C, Roxburghine-B, Ocidentoside, Beta-sitosterol, Curine, Withangulatin, and Jacoumaric acid as potential inhibitors with binding energy (ΔG) ranging from -8.7 to -9.4 kcal/mol. **Conclusion:** Jacoumaric acid and Leucadenone C were shown to interact with the active site in MDM2 at residues Leu54, Ile61, Met62, and Ile99.

Key words: Cancer, Docking, Virtual Screening, Indonesian Herbal, Inhibitor, MDM2.

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INTRODUCTION

Chemotherapy treatment has a high level of toxicity so in the treatment of cancer looking for a new approach regarding the development of anticancer that selectively kill cancer cells by triggering apoptosis without damaging healthy cells in the body.[1] In this research, virtual screening is performed to search for compounds *in silico* from Indonesian herbal database which has the potential activity to inhibit MDM2 (Murine double minute- 2 homolog) or also known as E3 ubiquitin-protein ligase. Indonesian herbal database is selected as an alternative treatment to exploit the potential of Indonesia's natural wealth, especially in the treatment of cancer and with appropriate development methods, this potential can be utilized as much as possible in the medical world. Virtual screening is used as a complementary method of *in vivo* and *in vitro* screening that needs more cost and time.

Indonesian Herbal Database

Indonesian herbal potential can be found in herbaldb.farmasi.ui.ac.id which is an Indonesian herbal database that already gathers 3810 species with total 6776 compounds. Indonesian herbal database is a small example of Indonesian herbal potential that can use to get the maximum result.

Cell cycle

The cell cycle is a sequence of events experienced by a cell during its lifetime. The speed of a cell through a cell cycle depends on growth factors, hormones, and chemical factors. The cell cycle is separated into two parts which are interphase and mitosis.[2]

Cancer

Cancer is a disease caused by abnormal growth of body tissue cells that turn into cancer cells. Cancer cells can attack healthy cells around them and can affect everyone in every limb and all age groups. Treatment of cancer by radiotherapy, hormonal therapy, chemotherapy, and biological treatment aimed at increasing the life expectancy of cancer patients.[3]

p53 and Murine Double Minute-2 (MDM2)

p53 protein level under natural conditions exists at low concentrations in cells, but if stimulated, p53 protein level may increase. This situation is also a stimulation for MDM2 that plays a role in inactivating p53 by binding to complex or degrading p53 so that p53 level returns to normal.[4] MDM2 overexpression causes p53 cell deficiency in many cases of cancer.[5] The selection of MDM2 as a target in cancer cases by way of inhibition can return the level of p53 to the optimum conditions so that its function as a regulator of cell growth can normally work.[6]

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MDM2 inhibitors have a purpose of preventing MDM2 binding with p53. MDM2 interaction with p53 occurred on the hydrophobic side of MDM2 and three hydrophobic residues p53 which are Phe19, Trp23, and Leu26. The existence of the MDM2-p53 binding site enables small molecules of inhibitor compounds to inhibit this interaction.[7] Therapies targeting MDM2 in clinical development include small inhibitor compounds derived from Cis-imidazoline, Spiro-oxindole, Imidazothiazole, Dihydroquinolinone, Piperidines, Piperidone, and Pyrrolidine which have passed the phase 1 clinical trial.[8] The collected MDM2 inhibitor is summarized as a positive control compound in the study.

METHODS

Preparation of three-dimensional structure

MDM2 three-dimensional structure downloaded from RCSB PDB with ID 5LN2. The structure has a resolution of 1.58 Å. The 1400 ligands used were obtained from an Indonesian herbal database which is accessed from herbaldb.farmasi.ui.ac.id in the form of three dimensions compounds.[28]

Preparation of Three Dimensional Protein Structure

The search for the macromolecule structure used as the target of the docking is then separated from the residue that can disturb the process so that it will produce suitable docking process. Macromolecular structure preparation starts from searching, selecting, downloading, separating from the unwanted small molecule, and adding of hydrogen atoms and charge. The three-dimensional structure preparation target was done with Protein Data Bank (PDB) and Autodock Tools (ADT).

Docking Optimization

The optimization of molecular docking is carried out by cocrystal redocking on the target molecule. Docking parameter is Root Mean Square Deviation (RMSD) is lower than 2 Å. Inclusion criteria are an MDM2 macromolecule from human-derived, and exclusion criteria were proteins that had a resolution of more than 2.5 Å. Macromolecules in this research are MDM2 which has a PDB ID: 5LN2.

Virtual Screening Using Autodock and Autodock Vina

Screening of compounds from HerbalDB with Autodock and Autodock Vina software. Screening starts with the docking of the compounds in the database on the cocrystal binding site.

Analysis and Visualization of Protein-Ligand Interaction

The visualization process using PoseView and LigandScout. Visualization is done to see the interaction between ligand and amino acid residues on the macromolecule.

RESULTS AND DISCUSSION

In docking preparation with Autodock Tools, macromolecule added with hydrogen atoms, merge nonpolar hydrogen, adding Gasteiger charge, then saved in *.pdbqt format. The next step is to separate the cocrystal ligand (6ZT) with the 5LN2 macromolecule so can be used for the docking process. The grid box size determination obtained from LigandScout will be used as a reference later for redocking process performed in Autodock. From the result of docking with LigandScout, achieved the size of grid box 60 x 50 x 52 with the center coordinates obtained from the Autodock Tools with the option center on ligand is x= -9.442; y= -10.504; and z= 0.238

Negative controls are obtained from the Database of Useful Decoy – Enhanced (DUD-E) by accessing dude.docking.org and entering the two-dimensional file of a positive control as shown in Table 1, in the format *.smi. The positive control compound files included in DUD-E total is 19 compounds with a total of 1100 decoys of compounds. The positive and negative control files then minimized using OpenBabel by setting the force field with mmff94 and storing a total 1119 compounds in the *.pdbqt format which are ready for the validation process with Autoodock and Autodock Vina.

Grid Box Optimization with Autodock

The optimization step is performed by cocrystal (6ZT) redocking with the macromolecule (5LN2) which have been cleaned from the cocrystal, then looking at the Root Mean Square Deviation (RMSD) value and the binding energy (ΔG). The data from the redocking result, the lowest RMSD and the highest ΔG is used as the parameters in determination of the optimum grid. Grid optimization is done in various grid size with the grid size reference obtained from LigandScout. At first stage of redocking done with Autodock using various grid box size from 30 x 30 x 30 to 100 x 100 x 100 and repeated ten times (ga_run10) which then will be done the next stage with repetition 100 times (ga_run100).From the redocking result, as shown in the Table 2, the grid box size 70 x 70 x 70 was chosen as the best grid box size and used for the grid box in the virtual screening process. The value of RMSD is considered can be used if it has a value below 2Å.[29]

Validation of Virtual Screening Methods with Autodock Enrichment Factor (EF) dan Receiver Operating Characteristics (ROC)

A high value of EF 1%-2% signifies a positive control compound that has the high binding energy value in the virtual screening, so it is in the upper section in the order of binding energy value.[30] Based on the docking result, the best data is on the 70 x 70 x 70 grid box. This result is seen from the emergence of the first positive control data on the first 56 ligands (EF 5%) so that EF values start to increase in EF 10% and EF 20%. The value of EF 1% has value 0 in all grid sized indicates that positives control do not have a solid interaction compared to ligand decoys.

The docking result in Autodock shows that four grid box sizes have a value above the random line which is worth 0.5. The highest Area Under Curve (AUC) is owned by the 70 x 70 x 70 grid box with AUC 0.7206, and it is closest to 1, which is the ideal AUC value. The Area Under Curve Receiver Operating Characteristics (AUROC) > 0.7 signifies the data is valid to use for validation.[31]

The residues shown are the hydrophobic residues of MDM2 which are Leu54, Leu57, Ile 61, Met62, Tyr67, Val75, Phe86, Phe91, Val93, Ile99, and Ile103 that play a role in the interaction of MDM2 and p53.[32] The value of EF 1% in grid box 70 x 70 x 70 in Autodock is 0. In the Table 3 shown three negative control compounds derived from DUD-E and can be seen the interaction of all the decoys compared with cocrystal 6ZT. All the compounds have highest binding energy but have incomplete interaction to MDM2 macromolecule compared with the cocrystal 6ZT.

Validation of Virtual Screening Methods with Autodock Vina Enrichment Factor (EF) dan Receiver Operating Characteristics (ROC)

Based on the docking result in Autodock Vina, the best data is in the grid box size 15 x 15 x 15 which is equivalent to grid box 40 x 40 x 40 in Autodock. The appearance of the first positive control data on the first 112 ligands (EF 10%). EF 1% and EF 5% value is 0 in all grid size which shows that the positive control does not have a stronger binding energy than the decoys.

The docking result in Autodock Vina obtained one grid box size has an AUC value that passes through the random line. The best AUC value is owned by the grid box 15 x 15 x 15 with AUC value of 0.5053 as shown in the Table 4. The AUC value is not so close to the ideal AUC value and does not pass the value of 0.7 as a parameter of valid data. Although EF and ROC value does not pass the parameter, virtual screening in Autodock Vina still will be done with the grid box size equivalent of the optimum grid box in Autodock as a comparison.

Table 1: Positive control compounds

No	Compound	IC_{50}	Information
1	RG7112	18nM[9]	Phase one clinical trial in hematological cancer and liposarcoma[8], Inhibits interaction between MDM2-p53.[10]
2	SAR405838	0.092 μM[11]	Phase one clinical trial[8], Inhibits interaction between MDM2-p53 in malignant and neoplasm tumor10, an analog of MI-888.[12]
3	AMG-232	0.6 nM[13]	Phase one clinical trial[8], Inhibits interaction between MDM2-p53 in malignant and neoplasm tumor.[10]
4	RG7388	6 nM[14]	Phase one clinical trial8, Inhibits interaction between MDM2-p5315, targeting malignant and neoplasm tumor.[10]
5	TDP-665759	0.5 μM[16]	Phase one clinical trial, Inhibits interaction between MDM2-p5316
6	Caylin-1	20 μM[17]	Inhibits interaction between MDM2-p53.[17]
7	Caylin-2	8 μM (Cayman)	-
8	RO8994	5nM[14]	Preclinical phase, inhibits interaction between MDM2-p53.[14]
9	RO2468	15nM[18]	Preclinical phase, inhibits interaction between MDM2-p53.[18]
10	R5C3	Ki 0.1 μM[19]	Inhibits interaction between MDM2-p53.[19]
11	Nutlin	20 μM[20]	Inhibits interaction between MDM2-p53.[21]
12	Nutlin 3a	90 nM[20]	Inhibits interaction between MDM2-p53.[20]
13	Nutlin 3b	0.09 μM[21]	Inhibits interaction between MDM2-p53.[21]
14	NSC-66811	Ki 120nM[22]	Inhibits interaction between MDM2-p53.[22]
15	MX69	44.6 nM[23]	MX69 downregulates MDM2 through induction of MDM2 degradation.[24]
16	MI-888	6.8 nM[23]	-
17	MI-219	1.4 μM[11]	Disturb interaction between MDM2 – p53, and selectively inhibits the cell growth in lung cancer.[25]
18	JNJ-26854165	0.25-3 μM[26]	Phase one clinical trial[8], inhibition target to E3 ubiquitin ligase in advanced solid tumor.[10]
19	CGM-097	1.7 nM[27]	Phase one clinical trial, Inhibits interaction between MDM2-p53 in advanced solid tumor.[10]

Table 2: Grid box size optimization.

Grid box size	Binding energy/ ΔG (kcal/mol)	RMSD (Å)
40 x 40 x 40	-10.30	1.47
70 x 70 x 70	-10.48	1.39

Table 3: Table Interaction between negative control with MDM2 macromolecule.

		Residues									
Compounds		LEU	LEU	ILE	MET	TYR	VAL	PHE	VAL	ILE	ILE
		54	57	61	62	67	75	86	93	99	103
6ZT	LS	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	PV	✓	-	✓	✓	-	-	-	✓	✓	-
Decoy 1	LS	✓	-	✓	-	-	-	-	-	✓	✓
	PV	✓	-	✓	-	-	-	-	✓	-	-
Decoy 2	LS	✓	✓	✓	-	-	-	-	-	✓	✓
	PV	-	-	-	-	-	-	-	-	-	-
Decoy 3	LS	-	-	-	-	-	-	-	-	-	-
	PV	-	-	✓	✓	-	-	-	✓	-	-

Table 4: Validation of EF and ROC value with Autodock Vina.

Grid box	EF 1%	EF 5%	EF 10%	EF 20%	Area Under Curve (AUC)
15 x 15 x 15	0	0	0.52	1.31	0.5053
18.75 x 18.75 x 18.75	0	0	0	0.52	0.4550
22.5 x 22.5 x 22.5	0	0	0	0.52	0.4245
26.25 x 26.25 x 26.25	0	0	0	0.78	0.4303

Table 5: Top 10 Virtual Screening Result with Autodock and Autodock Vina.

	Autodock		Autodock Vina	
Rankings	Compound	ΔG (kcal/mol)	Compound	ΔG (kcal/mol)
1	Nimolicinol	-9.65	Yuehchukene	-9.4
2	Jacoumaric acid	-9.24	Morusin	-9.0
3	Isoarborinol	-9.23	Cyanidin	-8.8
4	Lantic acid	-9.12	Leucadenone C	-8.8
5	Diosgenin	-9.01	Roxburghine B	-8.8
6	Theasaponin E1	-9.01	Occidentoseide	-8.7
7	Taraxasterol	-8.96	Beta Sitosterol	-8.7
8	Leucadenone C	-8.92	Curine	-8.7
9	Simiarenol	-8.85	Withangulatin	-8.7
10	Alpha-Amyrin	-8.83	Jacoumaric acid	-8.7

Table 6: Slice of Virtual Screening by Autodock and Autodock Vina.

		Autodock	Autodock Vina
Compound code	Compound	ΔG (kcal/mol)	ΔG (kcal/mol)
M00037357	Jacoumaric acid	-9.24	-8.7
M00014276	Leucadenone C	-8.92	-8.8

Table 7: Interaction of MDM2 amino acid residues with Jacoumaric acid.

			Residue										
	Compound		LEU	LEU	ILE	MET	TYR	VAL	PHE	PHE	VAL	ILE	ILE
			54	57	61	62	67	75	86	91	93	99	103
6ZT	Autodock	LS	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
		PV	✓		✓	✓	-	-	-	-	✓	✓	
	Autodock	LS	✓	✓	✓	✓	✓	-	✓	✓	-	✓	✓
	Vina	PV	✓	-	✓	✓	-	-	-	-	-	✓	-
Jacoumaric	Autodock	LS	✓	-	✓	✓	-	✓	-	-	✓	✓	-
		PV	✓	-	-	-	-	-	-	-	✓	-	-
Acid	Autodock	LS	✓	-	✓	✓	-	-	-	✓	✓	✓	✓
	Vina	PV	✓	-	-	-	-	-	-	-	-	-	-

Table 8: Interaction of MDM2 amino acid residues with Leucadenone C

	Compound		Residue										
			LEU	LEU	ILE	MET	TYR	VAL	PHE	PHE	VAL	ILE	ILE
			54	57	61	62	67	75	86	91	93	99	103
6ZT	Autodock	LS	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓
		PV	✓		✓	✓	-	-	-	-	✓	✓	
	Autodock	LS	✓	✓	✓	✓	✓	-	✓	✓	✓	✓	✓
	Vina	PV	✓	-	✓	✓	-	-	-	-	-	✓	-
Leucadenone C	Autodock	LS	✓	✓	✓	✓	✓	✓	-	✓	✓	✓	-
		PV	✓		-	✓	-	-	-	-	D	-	-
	Autodock	LS	✓	-	✓	-	-	-	-	✓	✓	✓	✓
	Vina	PV	-	-	-	-	-	-	-	-	-	-	-

Virtual Screening with Autodock and Autodock Vina

Table 5 show the result of Autodock and Autodock Vina virtual screening with HerbalDB. Table 6 show the intersection of both virtual screening resulted in two potential compounds as MDM2 inhibitors. Several studies that have been conducted which states the eficacy of both compounds that play a role in inhibiting cancer directly or indirectly Jacoumaric acid comes from the plant *Psidium guajava* (*jambu klutuk*) and useful as anticancer.[33] Leucadenone C is obtained from a *kayu gelang* (*Melaleuca leucadendron*). This plant has four main compounds that efficacious as antioxidants.[34] Antioxidant-related research as a prevention and treatment of cancer has been done before in an attempt to deal with the impact of cancer on society.[35]

As shown in the Table 7 summary of the amino acid residues that play important role in the MDM2 interaction between cocrystal 6ZT and ligand that appear on the Autodock and Autodock Vina virtual screening processes (Jacoumaric acid). Residues are shown more specific to MDM2 hydrophobic residues which are Leu54, Leu 57, Ile61, Met62, Tyr67, Val75, Phe86, Phe91, Val93, Ile99, and Ile103 that play a role in the interaction of MDM2 and p53.[32] The cocrystal 6ZT interaction with MDM2 macromolecule is a hydrophobic bond mainly on Leu54, Ile61, Met62, and Ile99. Due to interference on one of those binding sites can interrupt the p53 and MDM2 bonds.[36]

Jacoumaric acid is obtained from species *Psidium guajava*, family Myrtaceae which is used as anticancer.[33] The largest number of interactions that can be seen in visualization results is with Leu54 residue on MDM2. MDM2 interactions with p53 occur on the hydrophobic side of MDM2 and three p53 residues. The presence of the MDM2-p53 binding sites enables small molecules of inhibitor to inhibit this interaction.[7] Jacoumaric acid has a molecular weight of 618.855 g/mol equivalent to 618.855 Dalton. The small molecule inhibitor with the molecular weight between 500-900 Dalton is recommended in the treatment of cancer.[37] This characteristic supports the potential of Jacoumaric acid as an MDM2 inhibitor.

Leucadenone C is obtained from *kayu gelang* plant (*Melaleuca leucadendron*) family Myrtaceae. Leucadenone A, Leucadenone B, Leucadenone C, Leucadenone D were derived from *Melaleuca leucadendron* leaf extract.[38] The Table 8 shows the interaction between cocrystal 6ZT and MDM2 macromolecule mainly a hydrophobic bond on Leu54, Ile61, Met62, and Ile99 residues. The most substantial number of interaction in visualization result is with Leu54 residue on MDM2. Leucadenone C has a hydrophobic bonding interaction with Leu54 residue. Leucadenone C molecular weight is 540.612 g/mol equivalent to 540.612 Dalton. The molecular weight between 500-900 Dalton is recommended in use for cancer treatment.[37] This characteristic supports the potential of Leucadenone C as an MDM2 inhibitor.

CONCLUSION

Validation results that match the criteria for positive and negative control using Autodock software with AUC value 0.72, but not for Autodock Vina with AUC 0.43. Virtual screening of the Indonesian herbal database on 5LN2 macromolecule using Autodock and Autodock Vina obtained the top-ten lists which are: nimolicinol, jacoumaric acid, isoarborinol, lantic acid, diosgenin, theasaponin E1, taraxasterol, leucadenone C, simiarenol, alpha-amyrin; and yuehchukene, morusin, cyanidin, leucadenone C, roxburghine B, occidentoside, beta-sitosterol, curine, withangulatin, jacoumaric acid, respectively. The results of virtual screening in Autodock and Autodock Vina are jacoumaric acid and leucadenone C where the interaction is on Leu54, Ile61, Met62, and Ile99 residues. It is necessary to simulate the molecular dynamics to inspect the bond strength stability of each recommended compound. Furthermore, *in vivo* and *in vitro* tests are required for recommended compounds.

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CONFLICT OF INTEREST

The authors are declare that there is no conflict of interest.

ABBREVIATIONS

MDM2: Murine Double Minute-2; **PDB:** Protein Data Bank; **ADT:** AutoDock Tools; **DUD-E:** Database of Useful Decoy – Enhanced; **AUC:** Area Under Curve; **ROC:** Receiver Operating Characteristics; **AUROC:** Area Under Curve Receiver Operating Characteristics; **EF:** Enrichment Factor; **RMSD:** Root Mean Square Deviation; **RMSF:** Root Mean Square Fluctuation; **PV:** PoseView, **LS:** LigandScout.

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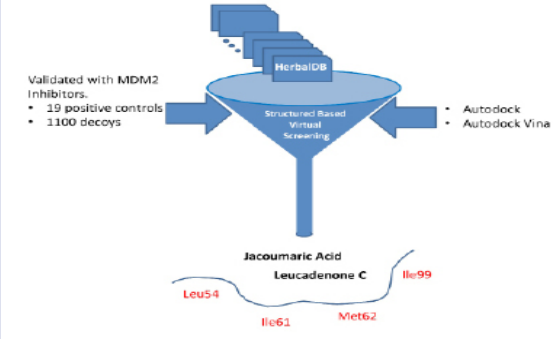
GRAPHICAL ABSTRACT



SUMMARY

- Virtual screening of Indonesian herbal database to discover MDM2 inhibitors was carried out using Autodock and Autodock Vina.
- Top ten virtual screening result compounds from Autodock are Nimolicinol, Jacoumaric acid, Isoarborinol, Lantic acid, Diosgenin, Theasaponin E1, Taraxasterol, Leucadenone C, Simiarenol, and Alpha-Amyrin were found to have strong interaction with MDM2, with binding energy (ΔG) ranging from -8.83 to -9.65 kcal/mol.
- Top ten virtual screening result compounds from Autodock are Yuehchukene, Morusin, Cyanidin, Leucadenone C, Roxburghine-B, Ocidentoside, Beta-sitosterol, Curine, Withangulatin, and Jacoumaric acid as potential inhibitors with binding energy (ΔG) ranging from -8.7 to -9.4 kcal/mol.
- Jacoumaric acid and Leucadenone C were shown to interact with the active site in MDM2 at residues Leu54, Ile61, Met62, and Ile99.

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