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Research Article

Study of Molecular Docking, Molecular Dynamic and Toxicity Prediction of Several Quinoline Alkaloid Derivatives as a Bruton Tyrosine Kinase Inhibitor as Anti-Leukemia

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Abstract

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Quinoline alkaloid and its derivatives play a vital role in the development of new therapeutic agents. Cinnoline structure has similarities with quinoline alkaloid compound and has the potential to inhibit Bruton's Tyrosine Kinase (BTK) in leukemia treatment. This research aims to study the interaction of several quinoline alkaloids with BTK and to predict the toxicity to ensure their safety. This study was carried out using computational studies, including molecular docking, molecular dynamics simulation, and toxicity prediction, to assess the compound's activity towards BTK and their toxicity. Molecular docking simulations showed that ten compounds (S1, S2, S4, S5, S8, S13, S14, S16, S17, and S20) had the best affinity to BTK. Molecular dynamics simulations to these ten compounds showed that only seven compounds (S1, S5, S8, S13, S16, S17, and S20) could stabilize the interaction towards BTK with RMSD and RMSF value of $0.5 \pm 2 \text{ \AA}$ and $0.5 \pm 6, 5 \text{ \AA}$, respectively. Toxicity prediction results showed that these quinoline alkaloids had various toxicity characteristics, but most were not carcinogens and mutagens (S4, S5, S6, S7, S8, S10, S11, S12, S14, and S15). It can be concluded that Yukositrin (S8) has the most potential affinity towards BTK, which can be used as anti-leukemia with low toxicity.

Keywords: anti-leukemia, Bruton Tyrosine Kinase, docking, MD, quinoline alkaloids

INTRODUCTION

Cancer is a disease with a high prevalence and is the second-highest disease in the world after heart disease. In Indonesia, cancer is a dreaded disease because it is difficult to cure and causes many deaths. Cancer risk factors such as genetics, carcinogens, and lifestyle behaviors lead to nearly 1.4%, or about 347,792 of all cancer cases ¹. In the previous research, we found some potential anticancer drug candidate compounds ². Some of the leukemia treatments currently used are surgery and drug treatment. The death rate from leukemia reaches 9.8%, and leukemia recurrence approaches 5.3%. This is due to the lack of available treatments. One of the leukemia treatments is the use of ibrutinib ³. Ibrutinib is the primary choice in chronic lymphocytic leukemia treatment and is one of the potent drugs in leukemia treatment that acts as a Bruton's Tyrosine Kinase (BTK) inhibitor ^{4,5}. BTK plays an essential role in the B cell signaling process, especially in the B leukocytes cell proliferase ^{6,7}. Some studies suggest that other synthetic derivatives can inhibit BTK, namely GDC-0834. GDC-0834 has the same

bonding position as ibrutinib (PCI-32765), occupying three inhibition positions on BTK receptor ⁸. Another study suggests that the presence of small molecules, called cinnoline, is known to have the potential to inhibit BTK by occupying one position of inhibitory bonding ⁹. Cinnoline structure has similarities with quinoline alkaloid compound, based on applying the Structure-Activity Relationship (SAR) principle ¹⁰. In this study, we conducted in silico study of some quinoline alkaloid derivatives as Bruton's Tyrosine Kinase inhibitor by docking and MD for the development of antileukemia drugs.

MATERIALS AND METHODS

Docking, molecular dynamic, and ADMET studies were performed for all designed compounds by Gaussian 09, AutoDock4 and AutoDockTools4, GROMACS, and ADMET PredictorTM installed in a single machine running on a 3.4 GHz Intel (R) Core(TM) i7-6700 processor with 16 GB RAM, 4 GB VGA, and 500 GB hard disk with dual-boot Microsoft Windows 7 Pro 64 bit and Linux Ubuntu as the operating system.

Macromolecule preparation

Crystal structure of Bruton's Tyrosine Kinase was obtained from the Protein Data Bank with PDB ID 4ZLY¹¹. From the

macromolecule, the small molecule (ligand) and water molecule were removed, and polar hydrogens and Kollman charges were added (Figure 1).resulting

Figure 1: (a) Chemical structure of ibrutinib¹² (b) interaction of GDC-0834 into the crystal structure of Bruton's tyrosine (PDB ID 4ZLY)⁸.

Ligand preparation

The structures of twenty quinoline alkaloid derivative compounds¹³⁻¹⁵ were built using the ChemOffice suite¹⁶. Geometry optimization and density functional theory (DFT) calculations were performed in Gaussian¹⁷ using the Becke three-parameter Lee-Yang-Parr (B3LYP) functional at the 6-31G basis sets.

Determination of Physicochemical Properties Parameters

After the structure of the compounds in 2D and 3D was made, these compounds were measured for physicochemical properties parameters, including analysis of RO5 (BM, Log P, H-bond and Rotatable Bond) and determining the similarity of the structure of the compound with overlaying.

Molecular Docking

Each ligand molecule was prepared for docking using AutoDock Tools 1.5.6¹⁸. Hydrogen atoms were added, and partial charges of each atom were determined from the DFT calculations and incorporated. Grid maps were created by centering the grid box at the position of the natural ligand of each macromolecule with a spacing of 1.85 Å and size covering the binding cavity of each target. Lamarckian genetic algorithm and 100 docking runs were used for each simulation.

Molecular Dynamics Simulations

Ten ligands with the best docking score were chosen for further molecular dynamics (MD) study. MD simulations were carried out using the Gromacs 5.1.1 suite¹⁹. The Amber99sb-ildn force field and the general AMBER force field (GAFF) were used to parameterize the atoms of the macromolecules and ligands, respectively. Energy minimization was carried out on the macromolecules in vacuum using the steepest descent algorithm. Then, the macromolecules were solvated with TIP3P water molecules in an octahedron box. Positive and negative ions were added to the system at a concentration of 0.15 N to neutralize all charges. Energy minimization was again performed on the macromolecule/solvent/ion system to release strains resulted from the solvation procedure; the steepest descent

algorithm was used again. Next, the system was carefully heated to 310 K and pressurized to 1 atm using the constant-volume, constant-temperature (NVT) and constant-pressure, constant-temperature (NPT) ensembles. The Berendsen thermostat coupling was used to maintain the system temperature and pressure²⁰. The Particle Mesh Ewald (PME) method²¹ with a cut-off value of 5.0 Å was used to compute long-range interactions. The system's stability was evaluated by analyzing the root mean square deviation (RMSD) and root mean square fluctuation (RMSF) of the protein backbones. A production simulation run was carried out on each macromolecule for ten ns. Analysis of the stability of ligand-protein interactions was performed by calculating the RMSD and RMSF values of the atoms at the protein binding sites throughout the simulation.

Toxicity Prediction

Toxicity prediction to ensure the safety of drug candidates was performed using ADMET Predictor 8.1²² from SimulationsPlus.inc including endocrine toxicity, maximum recommended therapeutic dose (MRTD), carcinogenicity and genotoxicity, cardiac toxicity, human liver adverse effects, acute rat toxicity, allergenic skin and respiratory sensitization, and reproductive toxicity.

RESULTS

Geometry Optimization

Geometry optimization is a way to obtain structures with minimum or lowest energy from a molecule. The structure with the lowest energy represents the most stable state²³. In geometry optimization, the priority is how to find the most stable structure with low energy²⁴. The lowest energy and geometry structure obtained can be used as a comparison in an experiment²⁵. The geometry optimization was carried out using the Gaussian09 software to achieve the minimum energy and the most suitable/stable position for the molecular test compound for docking at the MCL-1 receptor. The method used in this study was the Density Functional Theory (DFT) method since this method has a high accuracy value, and the basis set used was the 6-31G with the hybrid functional B3LYP.

Bonding Energy (HOMO-LUMO)

The calculation of the HOMO-LUMO energy of the ligands (Table 1) showed the HOMO-LUMO energy values vary in

each ligand. Then, the GAP or the difference between HOMO energy and LUMO energy was determined and was used as an indicator of stability.

Table 1: HOMO – LUMO energy and energy gap

No	Compound	Code	HOMO	LUMO	Energy gap
1	Candidin	S1	-0.1915	-0.1039	0.0876
2	Berberine	S2	-0.3178	-0.2079	0.1099
3	N-Methylalaphilinine	S3	-0.1798	-0.0587	0.1211
4	Evoxine	S4	-0.1776	-0.0539	0.1237
5	Phenyl Kuinolin	S5	-0.1833	-0.0506	0.1327
6	Citracridon	S6	-0.1843	-0.0501	0.1342
7	Triptanthin	S7	-0.2413	-0.1064	0.1349
8	Glikofolinin	S8	-0.1717	-0.0339	0.1377
9	Akrifolin	S9	-0.1924	-0.0521	0.1403
10	Yukositrin	S10	-0.1849	-0.0387	0.1462
11	Rutaekarpin	S11	-0.2046	-0.0577	0.1470
12	Roxiamin A	S12	-0.1980	-0.0488	0.1492
13	Roxiamin C	S13	-0.2017	-0.0523	0.1494
14	Dionkopolin B	S14	-0.1789	-0.0226	0.1564
15	Vebilosin	S15	-0.1993	-0.0407	0.1586
16	Diktiolamid A	S16	-0.1927	-0.0198	0.1729
17	Diktiolamid B	S17	-0.1898	-0.0167	0.1731
18	Dyctyolomide C	S18	-0.2097	-0.0349	0.1748
19	Galipinine	S19	-0.1763	0.0004	0.1767
20	Tetrahydro Berberine	S20	-0.1852	0.0181	0.2032

Molecular Docking

Validation of Molecular Docking Methods

Previously, the validation of the docking method was conducted by re-docking between natural ligands and the

Bruton's Tyrosine target receptor (PDB ID 4ZLY). The Grid Box settings used were X = 40, Y = 40, and Z = 40. While Grid Spacing was 0.375 with Grid Center X = 20.060; Y = 5.856; and Z = 1.280.

a

b

Figure 2: (a) analysis of amino acid residue bonds (b) visualization of redocking results. green color = natural ligand; pink = ligands from re-docking.

Molecular Docking Simulation

The compounds were subsequently docked at the Bruton Tyrosine (PDB ID 4ZLY) target receptor with parameters

adjusted to those obtained at the validation stage. The binding free energy values and inhibition constant values (Ki) were obtained for each compound (Table 2).

TABLE 2: BINDING FREE ENERGY VALUES, INHIBITION CONSTANT VALUES (K_i) DAN BOND INTERACTION FOR EACH COMPOUND IN THE BRUTON'S TYROSINE RECEPTOR TARGET (PDB ID 4ZLY)

No	Compound	Code	BE (kcal/mol)	KI (nM)	Residue	Distance	Atom	
							Ligand	Receptor
1	Candidin	S1	-11.46	3.99	MET477	2.9704	O18	N
2	Vebilosin	S2	-10.69	14.48	MET477	2.8898	O16	N
					SER538	3.1870	O15	OG
3	Glikofolinin	S3	-10.5	20.08	LYS430	3.0190	O23	NZ
					THR474	3.1906	O16	OG1
					MET477	2.6318	O15	N
					LEU408	2.1810	O	H29
					GLU475	2.3410	O	H28
4	Evoxine	S4	-9.44	119.69	LYS430	2.3116	O18	NZ
					SER538	2.68696	O21	OG
					SER538	2.1734	OG	H40
5	Triptanthin	S5	-9.42	123.85	MET477	2.8262	O18	N
6	Yukositrin	S6	-9.42	124.28	MET477	2.9062	O15	N
					LEU408	2.1759	O	H29
7	N-Methylatala philinine	S7	-9.22	174.23	MET477	3.1673	O26	N
					MET477	2.1161	O	H48
					LEU408	2.2194	O	H44
8	Citracridon	S8	-8.93	284.80	LYS430	2.8775	O26	NZ
					SER538	3.1130	O26	OG
					MET477	2.0129	O	H39
9	Roxiamin A	S9	-8.51	582	ASP426	2.8708	O2	N
					MET477	3.1384	N7	N
					MET477	3.0284	O11	N
10	Akriofolin	S10	-8.44	649.01	LYS430	2.9317	O24	NZ
					SER538	2.9556	O24	OG
					SER538	2.2056	OG	H39
					MET477	2.0886	O	H38
11	Roxiamin C	S11	-8.12	1.11	ASP426	2.8708	O2	N
					MET477	3.1368	N7	N
					MET477	2.8977	O11	N
12	Dionkopolin B	S12	-7.83	1.81	THR474	2.7010	O26	OG1
					MET477	2.6769	O24	N
					ARG525	2.0740	O	H35
					THR410	1.6759	O	H49
13	Rutaekarpin	S13	-6.74	11.39	LYS430	3.0870	O22	NZ
14	Phenyl Kuinolin	S14	-6.73	11.57	MET477	2.1745	O	H24
15	Berberine	S15	-6.19	28.82	LYS430	3.0140	O13	NZ
					THR474	2.7367	O11	OG1
16	Galipinin	S16	-5.96	42.84	MET477	2.8267	O20	N
17	Dyctyolomide C	S17	-5.6	77.99	MET477	2.7638	O20	N
18	Diktiolamid A	S18	-3.8	1.65	MET477	3.0607	O21	N
19	Diktiolamid B	S19	-2.8	8.81	LYS430	2.4219	O16	NZ
20	Tetrahidro berberin	S20	-2.06	31.01	LYS430	2.8417	O2	NZ
					LYS430	3.1929	O5	NZ
					CYS481	2.7259	O22	N

Molecular Dynamic Simulation

Molecular Dynamics is the most recognized simulation method and is considered very close to the original simulation. In various fields of molecular modeling, MD simulations show the flexibility of both ligands and proteins more effectively than other algorithms. The search strategy of new drug compounds by using random search to identify ligands' conformations, followed by MD simulations, is

believed to ease researchers in developing new drugs. Molecular Dynamic was carried out using the Gromacs application. After topography preparation, the output file was obtained, which was then analyzed for several parameters. The time used was 10 ns, with the main parameters analyzed were the RMSD and RMSF values. In this test, it can be compared between the RMSD and RMSF values from before and after docking the compounds into the receptor (Table 3).

Table 3: RMSD and RMSF MD parameter analysis of the test compound

Compound	RMSD	RMSF
S1
S2
S3
S4
S5

S6

S7

S8

S9

S10

Toxicity Prediction

Toxicity prediction was carried out using ADMET Predictor ver 8.1 software which included endocrine toxicity, maximum recommended therapeutic dose (MRTD),

carcinogenicity and genotoxicity, acute toxicity to mice, cardiac toxicity, side effects on the human liver, skin allergenic and respiratory sensitivity, and toxicity to the reproductive system. The results of toxicity analysis per test compound can be seen as follows:

Table 4: Summary of toxicity predictions analysis

No.	Compound	Toxicity Prediction								
		KG	M	E	J	K	P	R	T_R	MRTD
1	Candidin	√				√	√	√	2.4	
2	N-Methylatalaphilinine		√	√		√			2	√
3	Dionkopolin B		√		√	√			1.5	√
4	Vebilosin		√						1	
5	Citracridon		√			√			1	√
6	Berberin		√			√		√	1	√
7	Galipinin					√		√	0	√
8	Yukositrin		√						1	
9	Roxiamin C		√					√	1	
10	Diktiolamid B					√	√		0	

Note: (KG) carcinogenicity, (M) mutagenicity, (E) endocrine, (J) heart, (K) skin, (P) respiratory, (R) reproduction, (T_R) risk of toxicity, (MRTD) maximum recommended therapeutic dose.

DISCUSSION

Parameters of physicochemical properties

From the analysis, it can be seen that the violations carried out were in the Rotatable-bond parameter whereby compound 17 had a value of 8; this would not be too influential because, based on the research, Rotatable-bond values of more than 10 correlates with a decrease in oral bioavailability in mice ²⁶. The mechanistic basis for the rotatable bond parameters was unclear because the number of rotatable bonds did not correlate with the in vivo clearance testing results in mice. However, the in-vitro experimental results allowed it since the average ligand affinity drops 0.5 kcal for each of the two rotatable bonds. Thus, the results of the physicochemical analysis based on the Lipinski Rule of Five found that all the compounds can be used as drug candidates.

Determination of chemical structure similarity

From overlaying process, the actual value obtained was less than 1 Angstrom (<1Å). This showed that the compounds had similarities with natural ligands of receptors. Thus 20 ligands of these compounds were expected to occupy the same active site at the same receptor. Likewise, the biological activity of the compounds was expected to have similar activities as biological activity possessed by natural ligands when occupying the active site of the same receptor.

Bonding Energy (HOMO-LUMO)

The result showed that the highest GAP value was possessed by Tetrahydro Berberine was 0.2032, and the lowest GAP value was possessed by Candidin, which was 0.0876. The large HOMO-LUMO gap indicated high kinetic stability and low chemical reactivity. This is attributed to the reactivity to interact with other compounds and more to the analysis of the formation and breaking of covalent bonds with the test compounds

Molecular Docking

Validation of Molecular Docking Methods

The validation results of the docking method obtained a valid value with the RMSD value that met the requirements of 1.03 Å and was considered valid because the RMSD value was less

than 2 Å ²⁷. From the results of docking validation, the binding free energy values and inhibition constant values (Ki) were -11.35 kcal/mol and 0.00483 μM, respectively. The reference value of RMSD and the lowest free energy of the natural ligands that were redocked to the target receptor Bruton's Tyrosine (PDB ID 4ZLY) were -14.13 kcal/mol and 1.85, respectively. The RMSD value obtained showed that the Docking validation method was considered valid because the RMSD value had fulfilled the requirements of <2Å, and the Docking method parameter could be used for the Docking process of the compounds. Apart from the RMSD values, the following analyzed was amino acid residues that interact with natural ligands (Figure 2a).

Molecular Docking Simulations

The compounds were subsequently docked at the Bruton Tyrosine (PDB ID 4ZLY) target receptor with parameters adjusted to those obtained at the validation stage. The binding free energy values and inhibition constant values (Ki) were obtained for each compound (Table 2). The interactions formed between natural ligands and receptors were the bonds of amino acid residues, namely MET477, GLU475, LEU408, and THR474. Although the interaction of the compounds had a slightly different bond pattern with amino acid residues, it showed overall interaction patterns similar to natural ligands. From this docking stage, ten compounds were selected for the next MD stage. The selection of these ten compounds was based on the same interaction pattern with natural ligands and had a Ki value smaller than natural ligands, so it was suspected that these compounds could stabilize and have better biological activity among other test compounds. The ten compounds were S1, S2, S3, S4, S5, S6, S7, S8, S9, and S10.

Molecular Dynamic Simulations

Molecular Dynamics is the most recognized simulation method and is considered *very close* to the *original simulation*. In various fields of molecular modeling, MD simulations show the flexibility of both ligands and proteins more effectively than other algorithms. The search strategy of new drug compounds by using random search to identify ligands' conformations, followed by MD simulations, is believed to ease researchers in developing new drugs. Molecular Dynamic was carried out using the Gromacs application. After topography preparation, the output file

was obtained, which was then analyzed for several parameters. The time used was 10 ns, with the main parameters analyzed were the RMSD and RMSF values. RMSD analysis describes changes or shifts of a molecular conformation. RMSD shows the stability of a molecule based on time. If the RMSD receptor value is higher than the RMSD of the compounds, it is estimated that the bond of the compounds is more stable. It can be concluded that the compounds S1, S4, S5, S6, S8, S9, and S10 are more stable than natural ligands with the RMSD value range is 0.075-0.5) (Table 3).

RMSE

Root Mean Square Fluctuation (RMSF) analysis describes a change or shift from each atom/receptor amino acid residue that will produce flexibility from the receptor. The RMSF curve of the compounds was lower than the RMSF receptor curve. Thus, it can be stated that the compounds are better. To observe the fluctuations in the movement, the interaction of each atom was analyzed and then visualized according to the atomic analysis, where it was obtained the interaction movement per atom and visualized per residue that interacted just with the receptor graph and the receptor with

the docking compound. It produced several compounds proven at the atomic level can stabilize receptors, including S1, S2, S5, S6, S8, S9, and S10, with fluctuations values in the range of 0.05-0.1.

Toxicity Prediction

Toxicity prediction was carried out using ADMET Predictor version 8.1 software which included endocrine toxicity, *maximum recommended therapeutic dose* (MRTD), carcinogenicity and genotoxicity, acute toxicity to mice, cardiac toxicity, side effects on the human liver, skin allergenic and respiratory sensitivity, and toxicity to the reproductive system. The results of toxicity analysis per test compound can be seen as follows:

From the analysis of the results in Table 4, it can be seen that each compound has a different risk of toxicity, which can help researchers to develop new drugs by modifying existing structures to reduce the possible side effects. This data can be used as a reference if the effects have not been detrimental to the host in the long term, then the compounds can still potentially be developed into a new Leukemia drug.

Figure 3: Docking Visualization, RMSD, and RMSF of S8.

After analyzing the prediction of physicochemical properties, molecular docking, molecular dynamics, and toxicity prediction, it can be concluded that the compound, Yukositrin (S8), is a potent candidate compound to be developed to the next stage to become a Bruton's Tyrosine Kinase inhibitor (Figure 3).

CONCLUSION

1. The results of molecular docking of 20 test compounds revealed that some compounds could be used as candidates for Bruton Tyrosine Kinase Inhibitors,

including Candidine (S1), N-Methylaphinine (S2), Vebilocine (S4), Citracridone (S5), Yukocitrine (S8) compounds, Acrifoline (S13), Glycofolinine (S14), Roxiamine A (S16), Evoxine (S17) and Triphthantin (S20) which were predicted to have a higher potential than other compounds in inhibiting B cells signaling (MR).

2. The results of molecular dynamics of 10 compounds obtained seven compounds that have lower RMSD and RMSF values , namely Candidine, Vebilocene, Citracridone, Yukocitrine, Acrifoline, Roxiamine A. and

Triptanthin. Thus it can be stated that the compounds have the best stability.

3. The compound, Yukositrin (S8), was a potential candidate to be developed to the next stage to become a Bruton's Tyrosine Kinase inhibitor.

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