

Structure-Based Drug Design of Quercetin and Its Derivatives against HMGB1

KRISTINA SIMANJUNTAK¹, JUSMAN E. SIMANJUNTAK²,
ROSMALENA³ and VIVITRI D. PRASASTY^{4*}

¹Faculty of Medicine, Pembangunan Nasional "Veteran"

University of Indonesia Jalan RS Fatmawati, 12450, Jakarta Selatan, Indonesia.

²Faculty of Health Science, Pembangunan Nasional "Veteran" University of Indonesia
Jalan RS Fatmawati, 12450, Jakarta Selatan, Indonesia.

³Department of Medical Chemistry, Faculty of Medicine, University of Indonesia,
Jalan Salemba Raya 6, Jakarta 10430, Indonesia.

⁴Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia
Jalan Jenderal Sudirman 51, Jakarta 12930, Indonesia.

*Corresponding author E-mail: vivitri.dewi@atmajaya.ac.id

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ABSTRACT

High mobility group box 1 (HMGB1) is known as non-histone nuclear protein which has many biological functions, and it plays a significant role in many diseases inhibition, such as inflammatory and cancer diseases. HMGB1 in cancer cells could induce cell proliferation, cell differentiation, carcinogenesis, and tumorigenesis. In addition, HMGB1 function and location highly depends on the redox states. Our work focuses on molecular interaction studies of quercetin and its derivatives with HGMB1 protein target. Early reports showed that quercetin could inhibit tumors and cancers in different experimental examinations. However, clinical studies of quercetin showed low performance in its bioavailability. Therefore, structural modification of quercetin is needed to enhance its pharmacological properties. Here, we found 9 molecules of quercetin derivatives (QD) which have favorable interaction with HGMB1 and improved pharmacological properties. These findings indicate that QD might be the potential candidates against HMGB1 as therapeutic target for anticancer.

Keywords: HMGB1, Quercetin Derivatives, Drug Design, Molecular docking, Anticancer.

INTRODUCTION

High mobility group box 1 (HMGB1) is a non-histone chromosomal and highly conserved protein that serves various roles in intracellular and extracellular systems. This group of proteins was appointed as the "high mobility group," due to

their rapid mobility properties in polyacrylamide gel electrophoresis. HMGB1 is discovered firstly as a chromatin-associated protein, extensive studies of this group have directed to the identification of many critical functions of HMGB1. In eukaryotic organisms, HMGB1 protein is mainly found in the nucleus, where its function is chaperoning DNA molecules



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alleviating replication, recombination, transcription, DNA repair, and nucleosome formation stabilization by forming the DNA helical structure and promoting the binding of regulatory complexes to DNA. HMGB1 takes part in regulating autophagy and maintaining balance between autophagy and apoptosis within the cytoplasm compartment ^{1,2}.

HMGB1 is also implicated as an important mediator in cancer. HMGB1 expression is high in many cancer cells, especially in colon cancer, breast cancer, cervical cancer, lung cancer, prostate cancer, and pancreatic cancer ³⁻⁶. Previous studies exhibited that there is a correlation between HMGB1 and various malignant cancer mechanisms. The principle behind this connection may be due to the role of extracellular HMGB1 in angiogenesis, cell migration, and macrophages recruitment, which could contribute to tumor growth and metastasis. HMGB1 has an elaborated role in carcinogenesis which could suppress tumorigenesis by interacting with tumor suppressor genes such as p53, p73 and RB. Moreover, HMGB1 has mixed effects on the superiorities of cancer including unlimited replicative potential, ability to blood vessels development (angiogenesis), deflection of programmed cell death (apoptosis), self-sufficiency in growth signals, insensitivity to inhibitors of growth, tissue invasion and metastasis which cause inflammation ^{7,8}. This exhibits that HMGB1 is a potential target for cancer treatment.

Suppression of HMGB1 is important and it becomes prospective strategy for treating various cancers. Recently, various synthetic and natural products have been used to inhibit HMGB1 expression. Quercetin is a secondary metabolite compound which has polyphenol groups, and it belongs to the flavonol class of flavonoids. The broad range of biochemical and pharmacological properties of quercetin and its metabolites are due to the relative substitution of various functional groups on the flavonol molecule ⁹. Phytochemical investigations of various plant extracts have revealed that quercetin can exist in a free state as an aglycone, or as its derivative by conjugating with: carbohydrates as quercetin glycosides ¹⁰; alkyls as quercetin methyl or ethyl ^{11,12}; hydroxyl group as quercetin ethers ¹³⁻¹⁵; and a sulfate group as quercetin derived-sulfates ¹⁶. Our research aim is to investigate the interaction binding between quercetin derivatives (QD) and HMGB1 through molecular docking analysis. Furthermore, the best docking hit of QD were analysed the ADMET (absorption, distribution, metabolism, excretion and toxicity) prediction ^{17,18} to evaluate its pharmacological properties of QD in different mechanisms in the body. Here, we report the highlight molecular nature of QD action mechanisms with various pharmacological properties as HMGB1 inhibitors.

METHOD

Protein Preparation

The protein sequence of human High

Table 1: Pharmacophore properties following Lipinski rule of five with ACD/I-LAB

No.	Compound Name	HBA	HBD	MW	Log P	Rotatable Bonds	TPSA	Violation of Ro5
1	Quercetin	7	5	302.24	2.07	1	127.45	0
2	Quercetin, 3-glucuronide	13	8	478.36	2.10	4	223.67	3
3	Quercetin, 3-O-glucuronide methyl ester	13	7	492.39	1.73	5	212.67	3
4	Isoquercetin glucose	12	8	464.38	1.75	4	206.6	3
5	Isoquercetin galactose	12	8	464.38	1.75	4	206.6	3
6	Quercetin, 3-sulfate	10	5	382.3	1.66	3	179.2	0
7	Quercetin, 3-sodium sulfate	10	1	382.3	1.66	7	135.2	0
8	Quercetin, 3'-methyl ether	7	4	316.26	1.76	2	116.45	0
9	Quercetin, 3'-ethyl ether	7	4	330.29	2.36	3	116.45	0

mobility group box 1 (UNIPROT ID: P09429) was retrieved from Protein Sequence Databank: Uniprot (<http://www.uniprot.org/>). The three-dimensional structure of human High mobility group box 1 was built by I-TASSER (Iterative Threading ASSEMBly Refinement) ¹⁹. The coordinates of hHGMB1 only consists of amino acid residues in .pdb format file.

Ligand Preparation

The structure of the ligands were obtained from PubChem ^{20, 21} in .sdf format files (Fig. 1). Ligands were converted into .pdb format using GUI OpenBabel ²². Some of ligand structures were drawn and the 3D structures were optimized by using ChemDraw ²³. Pharmacokinetic properties of all the

ligands were analysed by using SwissADME ¹⁷.

Molecular Docking Analysis

Molecular docking was conducted by using Autodock Vina ^{24, 25}. The grid box site parameters was set up on xyz center coordinates at 73x68x73, and grid box size at (40x40x40) Å. Before running docking, receptor and ligands were prepared using AutoDock Tools ²⁶ with activated Polygon Stipples and visualized by PyMOL Viewer ²⁷. All receptor and ligand files were saved as pdbqt. Binding energies were obtained from molecular docking. Furthermore, these values were calculated to have inhibitor constant (Ki) values by using equation below ²⁸:

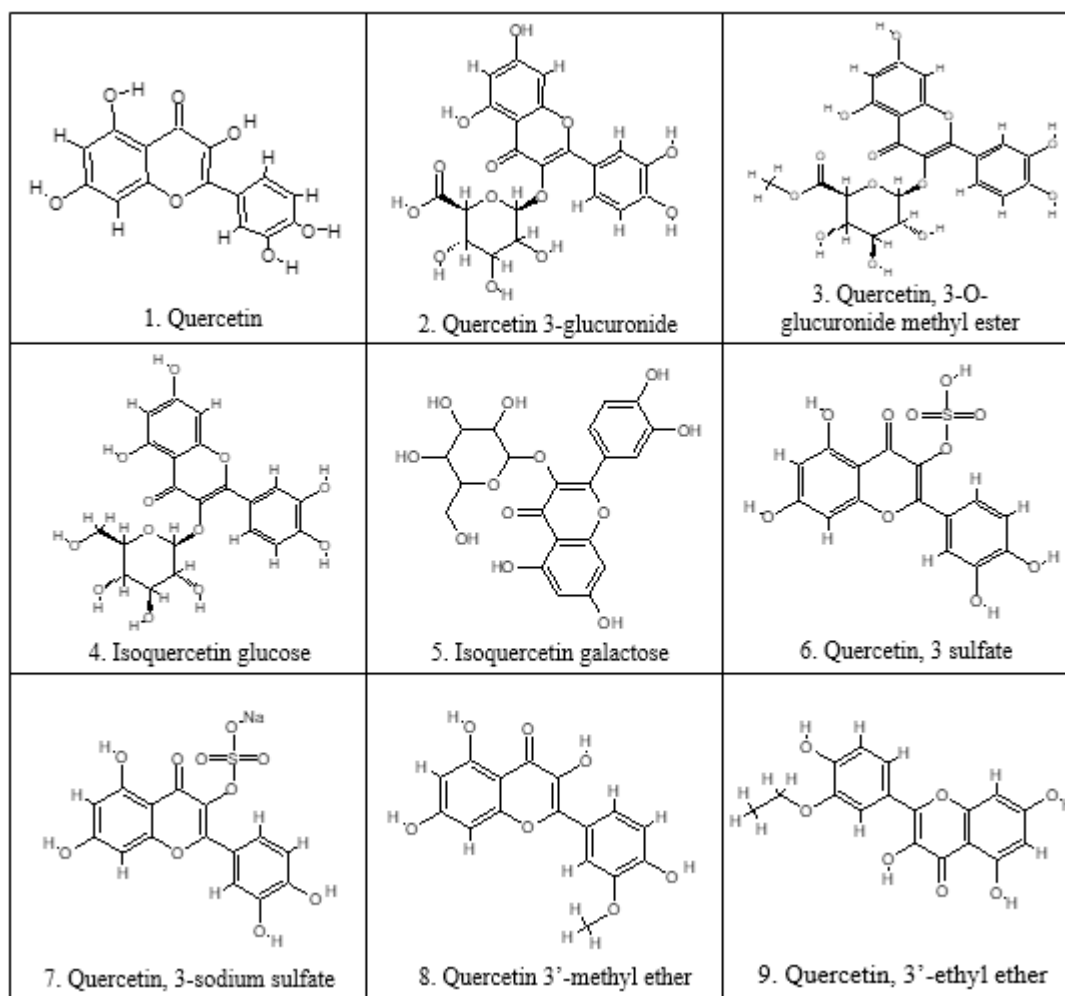


Fig. 1: Structure of quercetin and its derivatives

$$\Delta G = -RT \ln K_A; \quad K_A = K_i^{-1} = \frac{[E]}{[E][I]}$$

$$K_i = e^{\left(\frac{\Delta G}{RT}\right)}$$

ADMET Prediction

Absorption, distribution, metabolism,

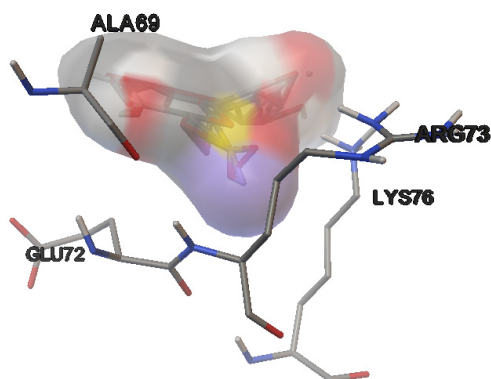


Fig. 2: Molecular docking interaction between Quercetin, 3 sodium sulfate with human HGMB1

excretion and toxicity (ADMET) prediction for the top docking of quercetin derivatives were predicted using ACD/I-Lab¹⁸. This platform predicts physicochemical descriptors as well as to predict ADMET parameters. ACD/I-Lab provides particular molecule's properties with 95% of known drugs. Evaluation of ADMET properties, were based on absorption involved maximum passive absorption through intracellular and paracellular pathways, Volume distribution (Vd) was also involved in this work. Plasma Protein Binding percentage (%PPB) and P-gp specificity were used to evaluate metabolism and excretion profiles, respectively. Additionally, LD50 mouse and probability of health effects predictions of QD were calculated using ACD/I-Lab which is a web-based service that provides instant access to spectral and chemical databases, ADME predictions, and toxicity characteristics. A comparative analysis was performed for mouse LD50 (intraperitoneal, oral, intravenous, subcutaneous) and probability of health effect of blood, cardiovascular system, gastrointestinal system, kidney, liver and lung by QD's biological activities.

Table 2: Molecular Docking Analysis

No.	Compound Name	Affinity Binding (kcal/mol)	Ki(M)	Hydrogen Bond	Residues involved in Hydrophobic interaction
1	Quercetin	-6.8	1.02x10 ⁻⁵	1	Arg97, Ala148, Lys152, Lys180
2	Quercetin, 3-glucuronide	-11.1	7.16x10 ⁻⁹	0	Ala69, Glu72, Arg73, Lys76, Glu203, Glu204, Asp211
3	Quercetin, 3-O-glucuronide methyl ester	-7.5	3.14x10 ⁻⁶	0	Met63, Ala66, Lys65, Arg70, Glu210, Asp212, Asp213, Asp214, Glu215
4	Isoquercetin glucose	-7.2	5.20x10 ⁻⁶	2	Lys65, Ala66, Asp67, Glu210, Asp211, Asp212, Asp213, Asp213, Glu215
5	Isoquercetin galactose	-6.6	1.43x10 ⁻⁵	2	Lys68, Glu72, Met75, Lys76, Glu201, Asp211, Glu215
6	Quercetin, 3-sulfate	-7.7	2.24x10 ⁻⁶	2	Arg97, Ala148, Ala149, Lys152, Tyr155
7	Quercetin, 3-sodium sulfate	-11.2	6.04x10 ⁻⁹	0	Ala69, Glu72, Arg73, Lys76
8	Quercetin, 3'-methyl ether	-9.8	6.44x10 ⁻⁸	0	Lys96, Pro95, Arg97
9	Quercetin, 3'-ethyl ether	-7.3	4.40x10 ⁻⁶	0	Ala69, Glu72, Met75, Lys76, Glu215

RESULTS

The derivatives of Quercetin have been successfully built for further *in silico* analysis. The structures of Quercetin derivatives are based on the variation of their side group, as depicted in Fig. 1.

The pharmacophore properties showed no violation Lipinski's rule of five on Quercetin; Quercetin, 3-sulfate; Quercetin, 3-sodium sulfate; Quercetin, 3'-methyl ether; and Quercetin, 3'-ethyl ether. Meanwhile, Quercetin, 3-glucuronide; Quercetin,

3-O-glucuronide methyl ester; Isoquercetin glucose; and Isoquercetin galactose showed 3 violations following Lipinski's rule of five (Table 1).

Molecular docking analysis showed that Quercetin, 3 sodium sulfate showed the best interaction with HMGB1, based on its affinity energy (-11.2 kcal/mol) and K_i value (6.04×10^{-9} M). This interaction does not involve hydrogen bond, but it involves hydrophobic interaction with the residues in HMGB1 binding site, including: Ala69, Glu72, Arg73, and Lys76 (Table 2, Fig. 2)

Table 3: ADME Prediction with ACD/I-LAB

No.	Compound Name	Absorption ^a (%)	Distribution ^b (L/kg)	Metabolism ^c (%)	Excretion Probability ^d
1	Quercetin	100	0.60	93.43	2.5
2	Quercetin, 3-glucuronide	0	0.25	88.35	5
3	Quercetin, 3-O-glucuronide methyl ester	12	0.62	85.36	0.5
4	Isoquercetin glucose	7	0.61	82.9	0.5
5	Isoquercetin galactose	7	0.61	82.9	0.5
6	Quercetin, 3-sulfate	1	0.25	99.82	5
7	Quercetin, 3-sodium sulfate	2	0.25	99.36	5
8	Quercetin, 3'-methyl ether	100	0.62	94.9	2.5
9	Quercetin, 3'-ethyl ether	100	0.62	94.9	2.5

^aMaximum passive absorption

^bVolume of distribution (Vd)

^c%Plasma Protein Binding (%PPB)

^dP-gp specificity (AB/logP), value between 2.5-5 is possibly inhibitor

Table 4: Toxicity Prediction with ACD/I-LAB

No.	Compound Name	LD ₅₀ (mg/Kg)			
		Intraperitoneal	Oral	Intravenous	Subcutaneous
1	Quercetin	450	670	350	160
2	Quercetin, 3-glucuronide	470	3600	1800	1900
3	Quercetin, 3-O-glucuronide methyl ester	390	3300	1200	740
4	Isoquercetin glucose	730	1200	740	310
5	Isoquercetin galactose	730	1200	740	310
6	Quercetin, 3-sulfate	760	1500	270	730
7	Quercetin, 3-sodium sulfate	1100	1600	210	1500
8	Quercetin, 3'-methyl ether	570	550	290	120
9	Quercetin, 3'-ethyl ether	560	570	270	120

Table 5: Health Effects Prediction with ACD/I-LAB

No.	Compound Name	Probability of Health Effects					
		Blood	Cardiovas- cular	Gastroin- testinal	Kidney	Lung	Liver
1	Quercetin	0.69	0.27	0.45	0.54	0.38	0.09
2	Quercetin, 3-glucuronide	0.96	0.9	0.68	0.75	0.81	0.82
3	Quercetin, 3-O-glucuronide methyl ester	0.95	0.98	1	0.68	0.9	0.77
4	Isoquercetin glucose	0.96	0.93	0.99	0.62	0.80	0.84
5	Isoquercetin galactose	0.96	0.87	0.88	0.55	0.80	0.74
6	Quercetin, 3-sulfate	0.19	0.27	0.2	0.56	0.2	0.27
7	Quercetin, 3-sodium sulfate	0.79	0.48	0.99	0.51	0.23	0.38
8	Quercetin, 3'-methyl ether	0.6	0.64	0.44	0.5	0.37	0.27
9	Quercetin, 3'-ethyl ether	0.29	0.61	0.41	0.49	0.4	0.34

ADME and toxicity predictions of Quercetin derivatives showed various results (Table 3 and Table 4). These analyses were conducted by using ACD/I-LAB program.

DISCUSSION

Quercetin is categorized by a benzo-(γ)-pyrone skeletal structure with C6-C3-C6 carbon form, consisting of two benzene rings, A and B, linked by three carbons pyrone ring C as shown in Fig. 1. Quercetin is referred to pentahydroxy flavonol due to the presence of five hydroxyl groups on its flavonol skeletal framework at 3, 30, 40, 5, and 7 carbons²⁹⁻³¹. The broad range of biochemical and pharmacological properties of quercetin and its derivatives are due to functional group substitutions of flavonol molecule³². However, glycosylation of other hydroxyl groups has also been found from plants^{33, 34}. The sugar moieties could be in the form of monosaccharides, disaccharides, or polysaccharides. The most common monosaccharides substituents are glucose, galactose, rhamnose, and xylose. Isoquercetin glycosides also has been reported having radical scavenging properties^{35, 36}.

Quercetin (3,31,41,5,7-pentahydroxy flavone) is natural flavonoid and its common derivative as flavone (2-phenylchromen-4-one). Quercetin contains five hydroxyl groups that are responsible for its biological activities and derivative diversification. Flavonoids generally consist of two benzene rings linked by pyran or pyrone rings^{30, 32}.

In addition, the conformational analysis of quercetin showed the presence of 12 conformations of this molecule having Gibbs energies in the range of 0 to 5.33 kcal/mol^{37,38}. Furthermore, quercetin exhibits strong intramolecular Hydrogen bond interactions, which displaying its biological multi-functional activities and renders its ability to form strong complex interactions, including with metals, affecting its bioavailability and transport system in the cells⁽³⁹⁻⁴¹⁾. Among these H-bonds, two bonds are with carbonyl groups and third one is between hydroxyl groups^(42, 43). Moreover, the derivatives of quercetin glycosides may also contain acyl and sulfur substituents becomes sugar moieties. In the case of quercetin derivatives, the hydroxyl groups of quercetin are attached with alcohols via ether bonds. Although quercetin is lipophilic, the glycosylation of quercetin derivatives can increase the hydrophilicity and enable the molecules to transport through all parts of the plant^(44, 45).

Pharmacophore properties of quercetin and its derivatives showed various properties according to Lipinski's rule of five (Ro5). Ro5 consist of HBA/HBD value up to 10 and 5, respectively; MW less than 500, Log P value less than 5 and total polar surface area (TPSA) value less than 140 Å²⁽⁴⁶⁾. From the results showed that there was no violation of Ro5 for quercetin; Quercetin, 3-sulfate; Quercetin, 3-sodium sulphate; Quercetin, 3'-methyl ether; and Quercetin, 3'-ethyl ether, while the 4 other derivatives showed 3 violations of Ro5 as shown on Table 1.

Analysis of molecular docking was performed by using Autodock Vina. The best docking is Quercetin, 3-sodium sulfate with Affinity binding -11.2 kcal/mol. This favorable binding was contributed by hydrophobic interactions from four amino acid residues from HGMB1 protein including Ala69, Glu72, Arg73, and Lys76 (Fig.2). The inhibition constant (Ki) of Quercetin, 3-sodium sulfate is 6.04×10^{-9} M. The result of docking interaction, Ki value, and HGMB1 residues involved in hydrophobic interaction is shown on Tabel 2.

Prediction of ADME properties of quercetin and its derivatives were done by using ACD/I-Lab platform as shown on Table 3. Maximum passive absorptions of Quercetin, Quercetin, 3'-methyl ether and Quercetin, 3'-methyl ether were found 100%. Meanwhile, other quercetin derivatives were found less than 15%. Volume distribution rates of quercetin and its derivatives we found within 0.25 – 0.65 L/kg. Plasma binding protein ability rate in metabolisms prediction from quercetin and its derivatives were within 85.36 – 99.82. Excretion probabilities by involving P-gp specificity (AB/logP) from quercetin and its derivatives had value between 2.5-5 were possibly inhibitor, meanwhile P-gp specificity value less than 2.5 indicated were not inhibitors as well.

Moreover, toxicity analyses of quercetin and its derivatives were done by using ACD/I-Lab platform as shown on Table 4 and health effects prediction on Table 5. Quercetin, 3-sodium sulfate was found as acceptable ADME value, meanwhile toxicity of Quercetin, 3-sodium sulfate seems high on oral system with $LD_{50} = 1600$ mg/kg, whatsoever other derivatives gave toxicity prediction values in various deposition in the body tissues. To evaluate health effect in the body system responses, we

also analysed the health effect prediction in blood, cardiovascular, gastrointestinal, kidney, lung, and liver. Quercetin, 3-sodium sulfate might give high effect to gastrointestinal which causes dysfunction of gastrointestinal.

CONCLUSION

The molecular docking studies with quercetin and its derivatives into the binding cavity of human HGMB1 inducible showed the derivatives having more favorable interaction than quercetin with docking score involving hydrogen bond and ligand-protein interaction energy compared to quercetin. As earlier reported in literature, quercetin is known for having anticancer property and inhibiting the HGMB1 protein, the derivatives were docked at the binding cavity could have also possess some sort of anticancer property as it is 95% similar to quercetin retrieved from the NCBI PubChem database. The docked compounds used in the present study do not violate the Lipinski rule of five parameters. Also, from the ADME-Toxicity prediction using ACD/I-Lab revealed the docked compounds are in the acceptable range of various pharmacological parameters and they have similar behaviour of health effects and LD50 compared to quercetin. Therefore, we conclude that quercetin derivative compounds could be potential candidate molecules and could support for experimental testing against HGMB1 protein as anticancer agents.

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