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In Silico Prediction of Isoliquiritigenin and Oxyresveratrol Compounds to BCL-2 dan VEGF-2 Receptors

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Abstract

Isoliquiritigenin and oxyresveratrol are compounds that have been reported to have anticancer activities. This study aimed to predict cytotoxic activity, toxicity and physicochemical properties of the compounds isoliquiritigenin and oxyresveratrol. Prediction of physicochemical properties referred to Lipinski rules of five using the pkCSM online tool. Prediction of compounds toxicity using Protox II online tool while ligand interaction with receptors using Molegro Virtual Docker (MVD). Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) (PDB: 2RL5) and B Cell Lymphoma BCL-2 (PDB: 4AQ3) were used as target cancer receptor proteins. In silico predictive results showed that oxyresveratrol and isoliquiritigenin complied with Lipinski rules of five, predictive values of LD_{50} between 500-2000 mg/kg respectively 1560 mg/kg and 1048 mg/kg. The docking result was in the form of bound energy described by Rerank Score (RS). A compound having a small RS value was predicted to have greater activity. RS of oxyresveratrol on 2RL5: -73.0413 and 4AQ3: -87.9985, while isoliquiritigenin on 2RL5: -68.0282 and 4AQ3: -78.5041. The cytotoxic activity of oxyresveratrol was also shown by hydrogen bonds in active amino acids (2RL5: Cys 919 in 4AQ3: Tyr 67). From docking results of both compounds, oxyreveratrol had greater activity than isoliquiritigenin to both target cancer receptor proteins and complied Lipinski rules of five and have a low toxicity.

Keywords : *cytotoxicity, toxicity, isoliquiritigenin, oxyresveratrol, in silico*

INTRODUCTION

Cases of cervical cancer in the world are estimated to have more than 570,000 with 83% caused by Human Papilloma Virus (HPV) and two-thirds of these cases occur in developing countries (Martel, *et al.*, 2017). Chemotherapy and radiotherapy are used as therapies for cervical cancer (Liu, 2018). The resistance of chemotherapy drugs including cisplatin has become an important problem in the treatment of cervical cancer (Wang, *et al.*, 2015). So that it can be found and developed anti-cancer drugs from natural ingredients (Kinghorn, 2008). Isoliquiritigenin and oxyresveratrol are compounds that have been isolated from *Eleutherine palmifolia* (L.) Merr, *Glycyrrhiza uralensis*,

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Sinofranchetia chinensis, *Dalbergia odorifera* and *Glycine max* (L.) Merr. Both of these compounds have been reported to have anticancer activities in vitro (Minggarwati, 2018, Ayeka, *et al*., 2016; Cao, *et al.*, 2004; Pan, *et al.*, 2000; Chan, *et al.,* 1998; Kape, *et al.*, 1992). The development of anticancer drugs that have specific molecular targets is one of the strategies to overcome cancer. The two main targets in this study are apoptosis induction cancer cell and antiangiogenic. Apoptosis is programmed cell death which plays an important role in maintaining the body's homeostasis. Failure of apoptosis is a major factor in cancer cell malignancy. The approach to treating cancer through the mechanism of apoptosis induction has been known to be able to prevent promotion, progression and re-emergence of cancer (Rastogi and Sinha, 2009). Implementation of apoptosis clinical activities produces the effects of chemotherapy and chemopreventive (Sun, *et al.*, 2004). Apoptosis in the mitochondrial pathway has two pathways, the extrinsic pathway and intrinsic pathway. The extrinsic pathway involves Fas, while the intrinsic pathway involves cytochrome c (cyt-c) released from mitochondria (Kumar, 2005). The intrinsic pathway is mediated by the Bcl-2 family. This apoptotic regulation is carried out by antiapoptotic (Bcl-2 and Bcl-xl) and pro-apoptotic (Bax and Bak) proteins. These proteins play a role in the regulation of apoptosis through regulation of release cyt-c. The expression of Bcl-2 or Bcl-xl prevents the release of cyt-c from mitochondria. Direct addition of Bax to mitochondrial isolates can induce the release of cyt-c (Igney and Krammer, 2002). In the cytosol cyt-c will form a complex with apaf1 (Apoptotic Protease Factor-1), ATP and procaspase-9. This complex is called the apoptosome. Cancer growth is also often associated with expression of Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) as a proangiogenic pathway to increase the angiogenesis stage including vascular permeability, endothelial cell survival, proliferation, migration or invasion of surrounding tissues, and formation capillary blood vessels (Ferrara, 2009). The pathway mechanism VEGFR-2 is blockade of activation of tyrosine kinase (Hoi, *et al.*, 2014). Receptor tyrosine kinases can mediate Angiogenesis (Jeltsch, *et al*., 2013). In this study, we performed the prediction of cytotoxic activity, toxicity and physicochemical properties of the two compounds to VEGFR-2 and BCL-2 receptors.

METHODS

Software

Chem Bio Ultra 12.0, pkCSM on line tool, Protox II online tool, Molegro Virtual Docker 5.5, Molegro Data Modeller 3.0.

Target and Template Selection

PDB of BCL-2: 4AQ3 and VEGFR-2: 2RL5 taken from Protein Data Bank (https://www.rcsb.org). The SMILES code is taken from PubChem Compound (https://pubchem.ncbi.nlm.nih.gov/).

Prediction of Physicochemical Properties and Toxicity

Physicochemical properties and toxicity prediction used the SMILES format and pkCSM online tool (Pires, 2015), then categorized into Lipinski rules of five. Toxicity predictions used the Protox II Online tool anad categorized as toxicity classes (Drwal, 2014).

Molecular Docking Study

Docking is done using Molegro Virtual Docker software 5.5. Identification of ligand bonds is carried out by evaluating repeated identification and estimating bond energy with a macromolecule (CLCbio, 2013). Isoliquiritigenin and oxyresveratrol compounds drawn 2D first then transferred into a 3D shape using Ultra Chem 12.0. Validation of methods done by ligands already present in proteins VEGFR-2 (PDB: 2RL5) and BCL-2 (PDB: 4AQ3) and performed the docking process. Programs that can return poses below RMSD value less than 2 Å is considered to have performed successfully (Hev-

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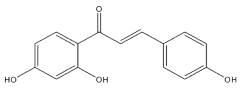
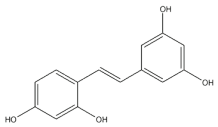
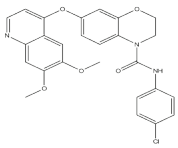
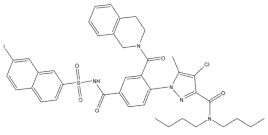
ener, *et al.,* 2009). Furthermore, the existing ligand are replaced with isoliquiritigenin and oxyresveratrol by conducting alignment and docking simulations carried out for each of these compounds. Protein 2RL5 has a chain protein and 4AQ3 has six chains and each of which is filled with ligand. The results obtained are the value of root mean square deviation (RMSD) and rerank score (RS), which is the bond energy required in the ligand-receptor interaction process (Siswandono, 2016). Bond energy indicated the amount of energy needed to form bonds between ligands and receptors. The lower the bond energy, the more stable the bond. The more stable the ligand bond with the receptor, the higher the activity (Hardjono, 2012).

RESULTS

Prediction of Physicochemical Properties and Toxicity

The results of absorption and permeability prediction to oxyresveratrol and isoliquiritigenin compounds using Lipinski rules of five showed that the four physicochemical properties were Log *p*<5, Molecular Weight <500, H bond donor <5 and H bond acceptor <10. Based on these results, two compound meet the Lipinski rules of five requirements so it can be predicted that the compounds will be easily absorbed and have high permeability (Lipinski, *et al.,* 1997). Physicochemical Properties prediction is presented in Table 1.

Table 1. Prediction of physicochemical properties.

Compound	Physicochemical Properties								Toxicity			Lipinski Rules of Five
	A*	B*	C*	D*	E*	F*	G*	H*	I*	J**	K**	
 *Isoliquiritigenin*	256.257	2.6995	3	3	4	109.438	No	No	No	1048	IV	Yes
 *Oxyresveratrol*	244.246	2.6794	2	4	4	103.706	Yes	No	No	1560	IV	Yes
 *2,3-dihydro-1, 4-benzoxazine inhibitor*	491.931	6.1286	5	1	6	206.494	No	Yes	No	5000	V	No
 *Phenylacylsulfonamide inhibitor*	866.222	7.9515	12	1	7	328.24	No	Yes	No	370	IV	No

Description: A: Molecular Weight, B: Partition Coefficient, C: Number of Rotatable Bonds (Torsion), D: Hydrogen Bond Donors (HBD), E: Hydrogen Bond Acceptors (HBA), F: Polar Surface Activity, G: AMES Mutagenic Test, H: Toxic to the liver, I: Skin sensitization, J: LD_{50} (mg/kg), K: Toxicity classes. class IV= harmfull is swallow (300-2000) Class IV = maybe harmful swallow (2000-5000). *pkCSM Online Tool **Protox II Online Tool.

The results of toxicity prediction show that the test compounds of isoliquiritigenin and oxyresveratrol and the comparative compound phenylacylsulfonamide inhibitor belong to category four toxicity, namely slightly toxic (lethal dose $(LD)_{50}$ 300-2000 mg/kg body weight (BW)). Comparative compounds (2,3-dihydro-1, 4-benzoxazine inhibitors) are included in category five toxicity, namely practically nontoxic (LD_{50} 2000-5000 mg/kg BW). The toxicity prediction results of isolquiritigenin, oxyresveratrol, and comparative compounds are presented in Table 1. Based on Table 1, isoliquiritigenin compound was not mutagenic in the AMES mutagenic test, no hepatotoxic in hepatotoxicity tests and no cause skin irritation in skin sensitization tests. Oxyresveratrol compound showed that is mutagenic in AMES mutagenic test, but did not cause hepatotoxicity and skin sensitization. Both comparative compounds could be predicted to be hepatotoxic but no mutagenic and no toxic to the skin.

Validation of Receptor

The RMSD values between the generated poses and the bound native ligand of crystal structure VEGFR-2 (2RL5) and BCL-2 (4AQ3) are shown in Table 2. Based on Table 2 Validation results are shown with the RMSD value. Valid RMSD values lass then 2 Å (Hevener. *et al*., 2009). It was found that poses receptors with native ligand has valid. RSMD in VEGFR-2 show value 1.18875 Å and RMSD in BCL-2 chain E is lower than all chain with value RMSD 0.780526 Å.

Docking Molecular and Interaction

The result of molecular docking and interactions get the best position between ligands and receptors. The best position that has been done was where the ligand attaches to the receptor. The results of the best position of the test ligands and native ligands on the VEGFR-2 and BCL-2 Chain E receptors are shown in Figure 1. VEGFR-2 showed hydrogen bond and steric in-

Table 2. Validation receptor and RMSD values.

Receptor	VEGFR-2	BCL-2 Chain A	BCL-2 Chain B	BCL-2 Chain C	BCL-2 Chain D	BCL-2 Chain E	BCL-2 ChainF
RMSD values	1.18875	4.43089	11.3424	2.76257	10.8581	0.780526	3.42542

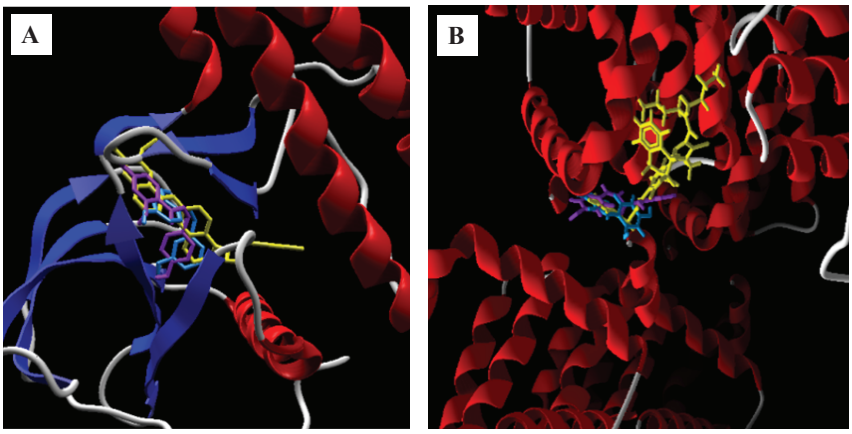


Figure 1. The two-dimensional interactions are shown between the test compounds against the VEGFR-2 (A) and BCL-2 Chain E (B) receptors with their respective native ligands.

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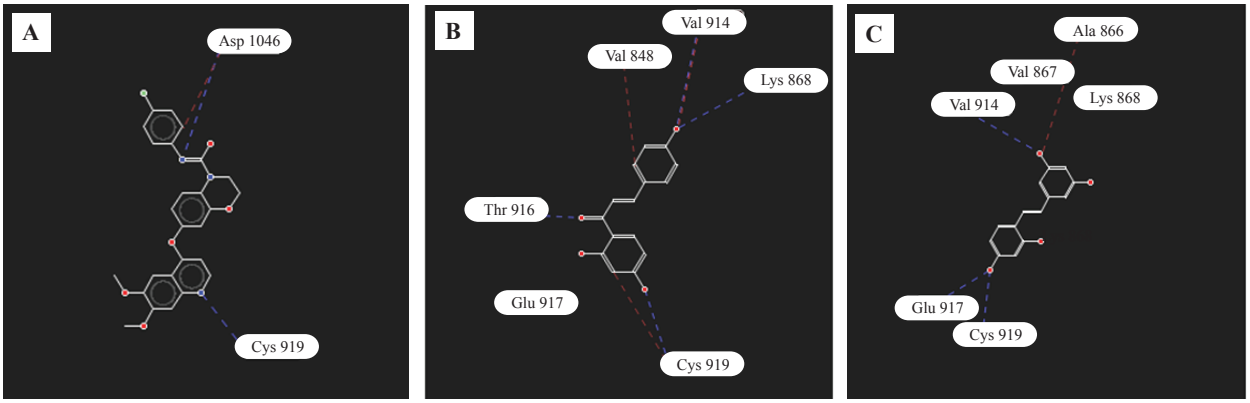

Figure 2. Two dimensions form of hydrogen and steric bonds between (A) 2,3-dihydro-1, 4-benzoxazine inhibitors (B) Isoliquiritigenin and (C) Oxyresveratrol with VEGFR-2 receptors (2RL5); blue lines as hydrogen bonds and red lines as steric bonds.

teraction that occurs between amino acid receptors and ligands. The ligand isoliquiritigenin had hydrogen bonds with amino acids Thr 916, Cys 919, Lys 868, Val 914 and steric bonds Cys 919, Val 848, Val 914. The ligand oxyresveratrol had hydrogen bonds with amino acids Glu 917, Cys 919, Lys 868, Val 914 and steric bond Ala 866. The native ligand 2,3-dihydro-1,4-benzoxazine inhibitors had hydrogen bonds with amino acids Cys 919, Asp 1046, and steric bonds Asp 1046. The bond results with the amino acid VEGFR-2 are presented in Figure 2. BCL-2 showed hydrogen bond and steric interaction that occurs between amino acid receptors and ligands. The ligand of isoliquiritigenin had hydrogen bonds with amino acids Arg 66 (E), Asp 62 (A), Arg 105 (E), Tyr 67 (E) and steric bonds Gly 104 (E),Arg 66 (E). The ligands of oxyresveratrol had hydrogen bonds with amino acids Leu 160 (A), Ala 59 (E), Gln 58 (A), Tyr 67 (E). The native ligands had hydrogen bonds with amino acids Gly 162 (A), Gln 58 (A), Tyr 67 (E) and steric interaction Phe 63 (E), Leu 96 (E), Glu 95 (E). The bond results between the amino acid and BCL-2 are shown in Figure 3.

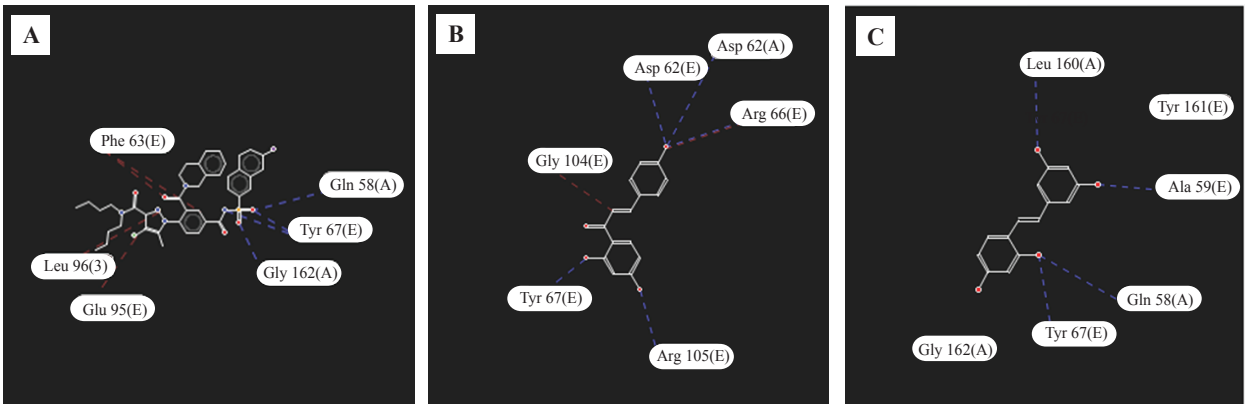

Figure 3. Two dimensions from of hydrogen bonds between (A) Phenyl-acyl-sulfonamide inhibitor (B) Isoliquiritigenin and (C) Oxyresveratrol with receptor BCL-2 (4AQ3); blue lines as hydrogen bonds and red lines as steric interaction.

The docking results between isoliquiritigenin and oxyresveratrol compounds to both VEGFR-2 receptor (2RL5) and native ligand 2,3-dihydro-1,4-benzoxazine inhibitors, then to both BCL-2 Chain E receptors (4AQ3-E) and native ligands phenylacylsulfonamide inhibitors had a ranging of RS from -68.0282 to -123,998. The two test ligands had a RS greater than the native ligand of each receptor. The docking results show that each receptor has the same amino acid at the VEGFR-2 receptor, Cys 919 and BCL-2, Tyr 67. The docking results are presented in Table 3.

DISCUSSION

This study aims to predict the physicochemical properties, toxicity and cytotoxic activity through in silico test of isoliquiritigenin and oxyresveratrol compounds. In both compounds test, isoliquiritigenin and oxyresveratrol fulfill the Lipinski rules of five with the meaning that it can be absorbed well and had good permeability while the comparative compounds did not meet the Lipinski rules of five because it have a molecular weight of ≥ 500 and HBA ≥ 10. All compounds have LD_{50} value between 500-2000 mg/kg BW with V and IV grade toxicity which showed relatively low toxicity. The higher the LD_{50} value, the lower the toxicity (Supandi, *et al*., 2018). The compound will be difficult to absorb and its permeability is low if it has: its molecular weight is ≥ 500, the log value of the partition coefficient is octanol/water (log P) ≥ +5; donor H-bond (HBD), expressed by the number of OH and NH groups, ≥ 5; and H-receptor bonds (HBA) expressed by the number of atoms O and N, ≥ 10 (Lipinski, *et al.,* 1997). Through fulfilling the Lipinski rules of five criterias, showed that isoliquiritigenin and oxyresveratrol compounds had good absorption and permeability.

Native ligands of 2RL5 and 4AQ3 coordinates were used for all docking experiments. In ordered to validate the scoring function, before redocking molecules for selecting prospective hits, we preparation into 2RL5 and 4AQ3 protein structure in MVD. there is one chain of VEGFR-2 protein in 2RL5 were each charged with its ligand and There are 6 chains of BCL-2 protein in 4AQ3 were each charged with its ligand. Subsequently, we compared the conformation and position with the bound ligand conformation measured regarding the root-mean square deviation (RMSD). Isoliquiritigenin and oxyresveratrol structures are docked to the receptor at the same place and coordinates previously.

In Table 3, the RS of VEGFR-2 receptor with an oxyresveratrol ligand had a smaller than ligand isoliquiritigenin, but the two test ligands are still higher when compared to native ligands 2,3-dihydro-1,4-benzoxazine inhibitors. This might be caused the hydrogen bond in the native ligand was more on the active side. The RS of BCL-2 receptor and oxyresveratrol ligand had a smaller value than the isoliquiritigenin ligand but the two ligands are not smaller than the native ligand (phenylacylsulfonamide inhibitor). A low RS can be attributed to the interaction of active amino acid bonds and also a less strong bond distance. RS or bond energy was the total calculation of all existing bonds. Bond energy stated the amount of energy needed to carry out interactions between ligands and receptors (Nugroho, 2014). The smaller RS indicates more stable bonds and results in increased activity (Thomsen, *et al*., 2006; Hincliffe, 2008; Kusumaningrum, *et al.*, 2014; Hardjono, 2012).

The similarity of Cys 919 amino acid residue involved in the binding process of the VEGFR-2 receptor will cause the compound to inhibit the receptor activity by competitive inhibitors (Figure 2). This mechanism occured through the blockade of tyrosine kinase activation from VEGFR-2 (Hoi, *et al*., 2014). It was reported in previous studies that the interaction of ligands with amino acids Cys 919 can be observed as the active side (La, *et al.*, 2008). Amino acid Lys 868 can be responsible as the active side and increase receptor bond energy (Ebadi, *et al*., 2012). The role of Glutamic Acid (Glu) in hydrogen bonds has been explained to have

Table 3. Rerank score, hydrogen bond and steric interaction.

Receptors	Compounds	Hidrogen Bound and Distance (Å)	Steric Interaction and Distance (Å)	Rerank Score
2RL5	2,3-dihydro-1,4-benzoxazine inhibitor	Cys 919 (2.87) Asp 1046 (2.70)	Asp 1046 (3.15)	-123.998
	Isoliquiritigenin	Thr 916 (3.16) Cys 919 (3.06) Lys 868 (2.93) Val 914 (2.18)	Cys 919 (3.04) Val 848 (3.02) Val 914 (2.99)	-68.0282
	Oxyresveratrol	Glu 917 (3.01) Cys 919 (2.94) Lys 868 (3.14) Val 914 (3.14)	Ala 866 (3.07)	-73.0413
4AQ3-E	Phenylacylsulfonamide inhibitor	Gly 162 (A) (3.11) Gln 58 (A) (2.81) Tyr 67 (E) (3.10) (3.19)	Phe 63(E) (3.15) Leu 96(E) (3.07) Glu 95(E) (3.14)	-123.174
	Isoliquiritigenin	Arg 66 (E) (3.04) Asp 62 (A) (2.74) Arg 105 (E) (2.63) Tyr 67 (E) (3.05)	Gly 104(E) (2.98) Arg 66 (E) (3.06)	-78.5041
	Oxyresveratrol	Leu 160 (A) (3.06) Ala 59 (E) (2.58) Gln 58 (A) (3.09) Tyr 67 (E) (3.12)		-87.9985

a role in inhibiting tumor development by suppressing the process of angiogenesis and permeability through the VEGFR-2 pathway (Baek, *et al.*, 2017).

The similarity of tyrosine (Tyr) 67 (E) amino acid residue in both ligand and native ligand inhibitors test found that these amino acids were the active side of the BCL-2 receptor (4AQ3) (Figure 3). The Tyr 67 (E) amino acid residue is found in the BH-3 domain of BCL-2 (Kennedy, *et al.*, 2015). This bond resulted in the activation of BH-3 as pro-Apoptosis. Pro-apoptosis will compete with anti-apoptosis, so when more apoptosis will mediate the release of cytochrome-C from mitochondria. After cytochrome-C exits from the mitochondria cytochrome-C will be bound by Apaf-1 (Apoptosis Activating Factor), then it will be bound and form a CARD domain (Caspase Recruitment Domain) and form Apoptosome. Apoptosome will activate caspase 9 then activate caspase 3. Caspase 3 is a mediator of cell death (apoptosis) (Chipuk, *et al.*, 2010).

CONCLUSION

Isoliquiritigenin and oxyresveratrol compounds complied Lipinski rules of five and have a low toxicity. Oxyresveratrol compound had greater activity than isoliquiritigenin caused had lower rerank score. The activity of oxyresveratrol was also shown by hydrogen bonds in active amino acids (2RL5: Cys 919 in 4AQ3: Tyr 67).

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