

# TYROSINASE INHIBITORY ACTIVITIES AND QSAR MODELS OF KOJYL THIOETHER DERIVATIVES

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## TYROSINASE INHIBITORY ACTIVITIES AND QSAR MODELS OF KOJYL THIOETHER DERIVATIVES

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### ABSTRACT

Quantitative Relationship of Structures and Activities (QSAR) models on the kojyl thioether derivative compounds are performed to determine the relationship between the properties of the compound molecules (descriptors) and their biological activity as tyrosinase inhibitors. In this research, we obtain a valid QSAR equation which is then used to design new compounds based on kojyl thioether properties. Descriptor calculations are performed using a hyperchem 8.0 application with a PM3 semi-empirical geometry optimization method. The best QSAR equation model is:  $pIC_{50} = -922,517 + 15,872 * ELUMO - 436,654 * qC4 - 209,509 * qC5 + 1,0008 e^{-5*} E_{int}$  n = 14; m = 4; r = 0.949; R<sup>2</sup> = 0.901; PRESS = 3.0469; q<sub>2</sub> = 0.8246. Based on the QSAR equation, 15 out of 19 compounds derived from kojic acid containing thioether can be proposed to be analogs of new tyrosinase inhibitors. All compounds were not hepatotoxic and have better predicted inhibitory activity values (pIC<sub>50</sub>) as tyrosinase inhibitors than the leading compound. They did not cause AMES toxicity neither cause skin sensitivity. To determine the interaction between compounds and tyrosinase enzymes, docking was done using Molegro Virtual Docker. Results show that all compounds have hydrogen bonds and steric interactions in the amino acid residue His263.

**Keywords:** Tyrosinase, QSAR, Docking, Kojyc Acid, Kojyl Thioether.

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### INTRODUCTION

Skin hyperpigmentation is a disorder of skin color caused by excessive melanin production due to increased melanocytes and tyrosinase activity.<sup>1</sup> To overcome this problem, usually people will use skin lightening products. There are already plenty of whale-whitening ingredients in which the main mechanism of action of the active substance is to reduce the amount of melanin. The amount of melanin affects the darkness of a person's skin color.

One way to reduce the amount of melanin is by inhibiting the action of the enzyme tyrosinase. Examples of active substances with tyrosinase inhibitor mechanism in skin whitening ingredients such as hydroquinone, kojic acid, arbutin, azelaic acid, and vitamin C.<sup>2</sup> But hydroquinone, retinoic acid, azelaic acid, and vitamin C can cause irritation, cytotoxics, contact dermatitis, redness with a rather slow therapeutic response and low activity.<sup>3</sup>

The discovery of quantum mechanics which has emerged in the field computational chemistry minimizes trial and error processes in drug discovery. Research costs thus can be reduced and research time can be shortened. Computational chemistry uses the results of theoretical chemistry that is put into a computer program so that it can calculate the properties of molecules and their changes. The properties of drug

molecules are closely related to drug activity. Computational chemistry can also simulate large systems in the form of gases, liquids, solids or liquid crystals.<sup>4</sup>

In this research, we perform QSAR models on kojyl thioether derivative compounds to determine the relationship between the properties of the compound molecules (descriptors) and their biological activity as tyrosinase inhibitors. The equation of QSAR is then used to design new compounds based on the properties of kojyl thioether. The design of compounds are then essential to further study the interaction of derivatives with the target protein (tyrosinase enzyme) through molecular docking analysis.

## EXPERIMENTAL

### Compound Optimization and Descriptor Calculations

All 14 compounds were geometry optimized using hyperchem 8.0 software with the parameterized Semiempirical Model 3 (PM3) method using the polak-ribiere algorithm, rms gradient 0.01 kcal / mol. The results of the descriptor calculations are total energy (PM3\_E), dipole moment (PM3\_Dipol), atomic net charge energy (PM3\_q), formation heat (PM3\_HF), bond energy (PM3\_Eik), atomic isolation energy (PM3\_Eis), electronic energy (PM3\_Eele), and formation heat (PM3\_HF), bond energy (PM3\_Eik), atomic isolation energy (PM3\_Eis), electronic energy (PM3\_Eele), and heat formation (PM3\_HF). Core interactions (PM3\_Eint) are obtained from the .log file when geometry optimization, HOMO energy (PM3\_HOMO) and LUMO energy (PM3\_LUMO) are obtained in the orbitals menu while mass (BM), partition coefficient (logP) and van der waals volume (Vol) are obtained through qsar property menu. The selection of descriptors already represents electronic, steric and hydrophobic parameters.

### Build the QSAR Model

Multilinear regression analysis was performed to obtain the QSAR equation with the help of SPSS 25 software using the stepwise, backward and enter methods. The independent variable used was the compound descriptor while the dependent variable was the tyrosinase inhibitor activity of the kojyl thioether derivative expressed in pIC<sub>50</sub>. The HKSA equation was chosen based on statistical criteria such as correlation coefficient (r), coefficient of determination (R<sup>2</sup>), F value and standard error (SE). In addition, the leave one out (LOO) cross validation test is also carried out to ensure the validity of the selected equation by looking at the largest value of the cross validation coefficient (q<sup>2</sup>) (≥ 0.5). LOO is done by means of each compound in the fitting data being eliminated then a new HKSA equation is built and the activity of the compound is predicted using a new equation without the compound. This was repeated for 14 compounds.<sup>5,6,7</sup>

### New Compound Design and Molecular Tethering

Based on the QSAR equation, the structure modification is done to obtain new thioether kojyl derivatives which have better activity. Modification is done by adding benzene groups to be modified again by adding hydroxy, methoxy, and halogens. Molecular tethering is performed using Molegro Virtual Docker (MVD) software with an enzyme of a 2y9x pdb code. Molecular tethering process is carried out on the active side with native ligands, namely position X = -0.45, Y = 26.5 and Z = -44.78 with a grid radius of 15 and a resolution of 0.3 Å.

### Inhibitory Activity Assay

A 80 μL phosphate buffer is placed on the microplate. The sample solution is put into each well until a concentration of 0.1 is obtained; 1; 10; 100; and 1000 μM for all 14 compounds. L-Tyrosine substrate solution was added to the microplate by 5 μM. The mixture was then incubated for 10 minutes at room temperature (± 27°C). MTE was put into each of the 80 microplate wells. The mixture is then homogenized and re-incubated at the same temperature for 30 minutes. Each test concentration was repeated three times (triplo) and made control (containing phosphate buffer, 5 μM L-Tyrosine, and 20 U

MTE). The test compound was then measured for absorbance at a wavelength of 490 nm using a microplate reader,  $\frac{\mu}{\text{mL}}$ .

### IC<sub>50</sub> and Data Analysis

The data obtained is plotted on the graph using Microsoft Excel 2013©. The graph is plotted between absorbance against concentration and percentage inhibition against concentration. Linearity curves are calculated and graphs between the two test subjects are compared. IC<sub>50</sub> is then calculated based on the results obtained. The percentage of inhibition is calculated using the formula:

$$\% \text{ Inhibitory} = \frac{\text{Control Absorbance} - \text{Samples Absorbance}}{\text{Control Absorbance}} \times 100\%$$

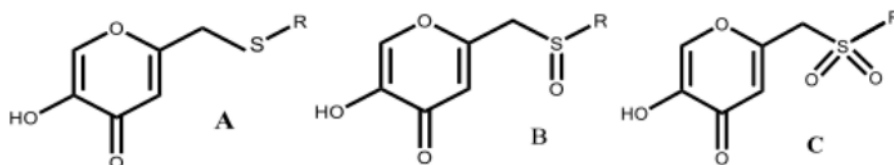
## RESULTS AND DISCUSSION

### *In vitro* inhibitory Activities (IC<sub>50</sub>) and *In silico* inhibitory Activities (pIC<sub>50</sub>) of Compounds

Data on the structure and activity of 14 kojyl thioether derivatives as tyrosinase inhibitors obtained can be seen in Figure 1 and Table 1. Inhibitory activities in the form of IC<sub>50</sub> all compounds were converted to pIC<sub>50</sub> (-log IC<sub>50</sub>). Compound A5 has the best IC<sub>50</sub> and pIC<sub>50</sub> values, thus is considered to be the leading compound (Table 1).

**Table 1** Derivatives of kojyl thioether compounds and their activities.

No	Compound	Side chain	IC <sub>50</sub> (μM)	pIC <sub>50</sub>
1	A1	R = -CH <sub>3</sub>	11.05	-1.0433
2	A2	R = -C <sub>3</sub> H <sub>7</sub>	8.93	-0.9509
3	A3	R = -C <sub>4</sub> H <sub>9</sub>	12.68	-1.1031
4	A4	R = -C <sub>5</sub> H <sub>11</sub>	15.16	-1.1806
5	A5	R = -C <sub>6</sub> H <sub>15</sub>	0.087	1.0604
6	A6	R = -C <sub>7</sub> H <sub>15</sub>	12.33	-1.0909
7	A7	R = -C <sub>8</sub> H <sub>17</sub>	40.18	-1.6040
8	A8	R = -C <sub>6</sub> H <sub>11</sub>	59.41	-1.7738
9	B1	R = -C <sub>5</sub> H <sub>11</sub>	2.24	-0.3502
10	B2	R = -C <sub>6</sub> H <sub>13</sub>	18.09	-1.2574
11	B3	R = -C <sub>6</sub> H <sub>11</sub>	1.68	-0.2253
12	C1	R = -C <sub>5</sub> H <sub>11</sub>	70.11	-1.8457
13	C2	R = -C <sub>6</sub> H <sub>13</sub>	72.63	-1.8611
14	C3	R = -C <sub>6</sub> H <sub>11</sub>	77.19	-1.8875



**Figure 1.** Basic structure of kojyl thioether.

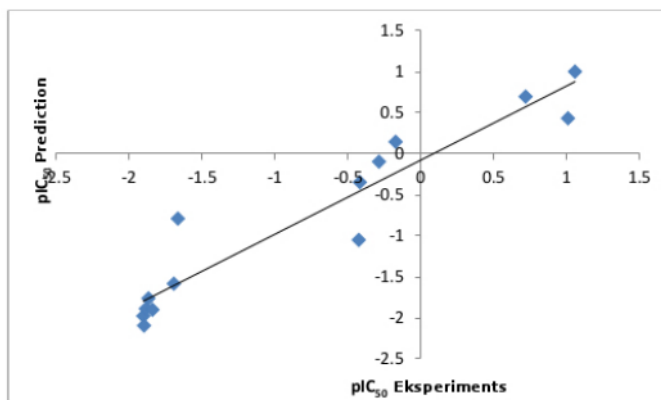
### QSAR models

QSAR begins with the geometry optimization of all compound fittings using Hyperchem 8.0 software to get the descriptor values of these compounds. The purpose of geometry optimization is to make the surface potential energy of the compound close to 0. Multilinear regression analysis is performed to obtain the HKSA equation. HKSA equations obtained are then ranked based on statistical parameters such as r, R<sup>2</sup>, F and SE values. The 5 best equations were selected which were then tested for validation using LOO

cross validation to see the value of  $q^2$ . Obtained the best HKSA equation with parameters  $r = 0.946$ ,  $R^2 = 0.901$ ,  $F_{hit} / F_{tab} = 5.6$ ,  $SE = 0.436$  and  $q^2 = 0.8246$  which means valid.  $pIC_{50} = -922,517 + 15,872 * ELUMO - 436,654 * qC4 - 209,509 * qC5 + 1,0008 e^{-5} * E_{int} [1]$

**Table 2.** Predictors used and the best HKSA Equation statistical data.

Descriptor	r	R2	Fhit / Ftab	SE	q2
PM3_ELUMO, PM3_qC4, PM3_qC5, PM3_Eint	0.949	0901	5.6	0.436	0.8246



**Figure 2.** Relationship between  $pIC_{50}$  experiments Vs  $pIC_{50}$  predictions of thioether kojyl derivatives.

Based on the QSAR model, electronic parameters are the only parameters that affect the activity of tyrosinase inhibitors of kojyl thioether derivatives. The bond between receptor-compounds is usually influenced by steric and electronic parameters but electronic parameters have a greater role. Hydrophobic parameters only affect the distribution of compounds and the biological membrane penetration process while the activity data obtained are only in vitro tests that do not require the biological membrane penetration process but rather the binding of the compound-receptors. The greater ELUMO and Eint will increase tyrosinase inhibitor activity. Conversely, even a small amount of qC4 and qC5 will increase its activity

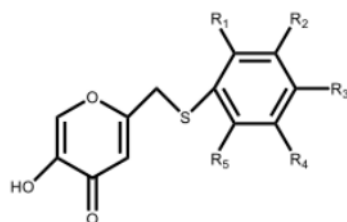
### Molecular Tethering

Modification of the kojyl thioether compound was not carried out on the structure of pyranone or its enol because modifying the part would eliminate tyrosinase activity. From the fitting data, the best compound is A5 which has a side chain in the form of cyclohexil. This compound should be used as a guiding compound in structural modification. Chemically, a new compound can be proposed as a tyrosinase inhibitor if possible to be synthesized. So that the structure of cyclohexil is replaced with the structure of benzene which is then modified by the addition of OH, OCH<sub>3</sub>, Cl, Br and I.

Fifteen out of nineteen compounds (compounds 4 to 18) have better activities than the guiding compound B2. They are also less toxic. ([http://biosig.unimelb.edu.au/pkcsdm/admet\\_prediction](http://biosig.unimelb.edu.au/pkcsdm/admet_prediction)). Toxicity needs to be predicted because the parameter is required in clinical trials. If the compounds are toxic, they cannot be proposed to be candidate of new drugs. Compound KT 1,3,5 OCH<sub>3</sub> has the biggest prediction  $pIC_{50}$ , almost 8x bigger than compound A5 which is 8.01739. Data on all predictive activity and toxicity of modified compounds is presented in Table 3.

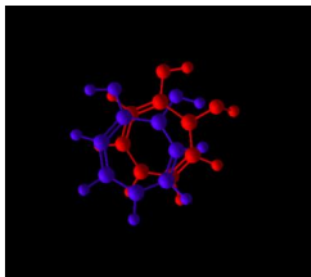
**Table 3.** Predictive activity and toxicity of modified compounds.

No.	Code	Compound	pIC <sub>50</sub> (μM)	AMES toxicity	Hepatotoxic	Skin sensitisation
1	KT 1 OH	R1 = OH, R2 = R3 = R4 = R5 = H	4.85177	yes	no	no
2	KT 3 OH	R3 = OH, R1 = R2 = R4 = R5 = H	6.3425	yes	no	no
3	KT 1,2 OH	R1 = R2 = OH, R3 = R4 = R5 = H	3,59918	yes	no	no
4	KT 1,3 OH	R1 = R3 = OH, R4 = R5 = H	6.06145	no	no	no
5	KT 1,4 OH	R1 = R4 = OH, R2 = R3 = R5 = H	3,32983	no	no	no
6	KT 1,2,3 OH	R1 = R2 = R3 = OH, R4 = R5 = H	4.73196	no	no	no
7	KT 1,2,4 OH	R1 = R2 = R4 = OH, R3 = R5 = H	2,32285	no	no	no
8	Kt 1,3,4 OH	R1 = R3 = R4 = OH, R2 = R5 = H	4,47875	no	no	no
9	2nd Floor OCH <sub>3</sub>	R2 = OCH <sub>3</sub> , R1 = R3 = R4 = R5 = H	6.65894	no	no	no
0	3rd place OCH <sub>3</sub>	R3 = OCH <sub>3</sub> , R1 = R2 = R4 = R5 = H	6.90195	no	no	no
11	1,2 OCH <sub>3</sub> Ct	R1 = R2 = OCH <sub>3</sub> , R3 = R4 = R5 = H	5.39492	no	no	no
12	Kt 1,4 OCH <sub>3</sub>	R1 = R4 = OCH <sub>3</sub> , R2 = R3 = R5 = H	7.6404	no	no	no
13	Kt 1,5 OCH <sub>3</sub>	R1 = R5 = OCH <sub>3</sub> , R2 = R3 = R4 = H	2.19696	no	no	no
14	Kt 1,2,4 OCH <sub>3</sub>	R1 = R2 = R4 = OCH <sub>3</sub> , R3 = R5 = H	6,27738	no	no	no
15	Kt 1,2,5 OCH <sub>3</sub>	R1 = R2 = R5 = OCH <sub>3</sub> , R3 = R4 = H	7.13981	no	no	no
16	Ct 2,3,4 OCH <sub>3</sub>	R2 = R3 = R4 = OCH <sub>3</sub> , R1 = R5 = H	5.79247	no	no	no
17	Kt 1,3,4 OCH <sub>3</sub>	R1 = R3 = R4 = OCH <sub>3</sub> , R2 = R5 = H	5.67855	no	no	no
18	Kt 1,3,5 OCH <sub>3</sub>	R1 = R3 = R5 = OCH <sub>3</sub> , R2 = R4 = H	8,01739	no	no	no
19	Kt 1,2 I	R1 = I, R2 = R3 = R4 = R5 = H	1,36975	no	yes	no



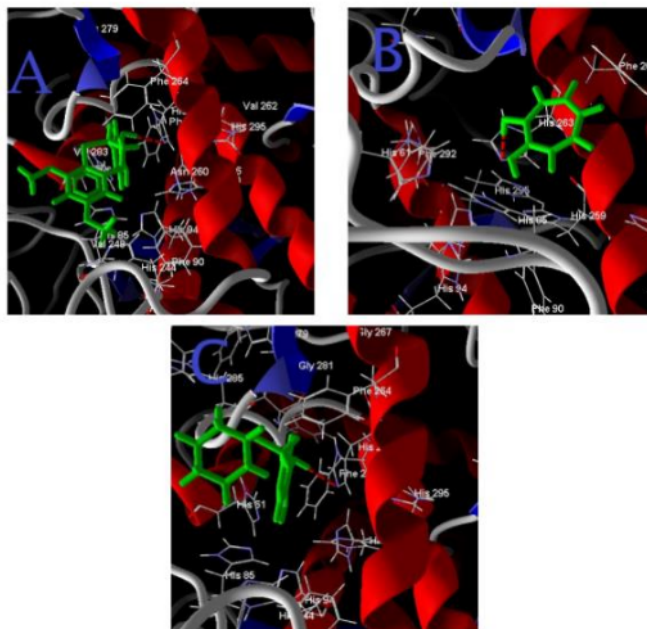
**Figure 3.** The structure of parent compounds for structural modification

Molecular tethering is carried out with the enzyme tyrosinase (pdb code: 2y9x) to see the interaction between compounds and amino acid residues of the enzyme. The validation is performed by redocking native ligands with the tyrosinase enzyme, which results in the RMSD values of  $1.6 \text{ \AA} (\leq 2 \text{ \AA})$  (Figure 4). This result indicated that the simulation can be continued to the molecular docking stage for each compound.



**Figure 4.** 2y9x molecular docking validation (blue = native ligand, red = redocking ligand).

There is at least one hydrogen bond between all compounds with an amino acid residues of the tyrosinase enzyme. The amino acid residue is His263 with a hydrogen bond distance of  $1.63\text{-}3.44 \text{ \AA}$ . In steric interactions of all compounds, His263 amino acid residue is also involved. Compound A5 has one hydrogen bond with His263 residue at a distance of  $3.43 \text{ \AA}$ , and has a steric interaction on both amino acids His263 and Phe264. Compound KT 1,3,5 OCH<sub>3</sub> has the largest value of pIC<sub>50</sub>. It shares the same hydrogen bond as the compound A5. However, the steric interaction of compound KT 1,3,5 OCH<sub>3</sub> has additional two ammonia acid residues (Val248 and Asn260). Visualization of hydrogen compound bonds and the interaction of all compounds can be seen in Figure 5.



**Figure 5.** Visualization of interactions between compounds and amino acid residues His263 (native ligand (A), Compound A5 (B), Compound KT 1,3,5 OCH<sub>3</sub> (C), blue line is hydrogen bond).

## CONCLUSION

In this research, QSAR equation models have been built to link structures of kojyl thioether compounds with their activities as tyrosinase inhibitors. Results exhibited that 15 out of 19 compounds have the potential to be new tyrosinase analog inhibitors. They are also less toxic. Compound KT 1,3,5 OCH<sub>3</sub> has the largest value of pIC<sub>50</sub> and has an IC<sub>50</sub> value eight times greater than the leading compound. The most important amino acid residue for hydrogen bonding and steric interactions is His263.

## ACKNOWLEDGEMENT

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