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Synthesis and biological activity of oxadiazole and triazolothiadiazole derivatives as tyrosinase inhibitors

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ABSTRACT

A series of 16 oxadiazole and triazolothiadiazole derivatives were designed, synthesized and evaluated as mushroom tyrosinase inhibitors. Five derivatives were found to display high inhibition on the tyrosinase activity ranging from 0.87 to 1.49 μM. Compound **5** exhibited highest tyrosinase inhibitory activity with an IC_{50} value of 0.87 ± 0.16 μM. The in silico protein–ligand docking using AUTODOCK 4.1 was successfully performed on compound **5** with significant binding energy value of −5.58 kcal/mol. The docking results also showed that the tyrosinase inhibition might be due to the metal chelating effect by the presence of thione functionality in compounds **1**–**5**. Further studies revealed that the presence of hydrophobic group such as cycloamine derivatives played a major role in the inhibition. Piperazine moiety in compound **5** appeared to be involved in an extensive hydrophobic contact and a 2.9 Å hydrogen bonding with residue Glu 182 in the active site.

Tyrosinase is a copper containing enzyme which acts as a catalyst in two different reactions involving the hydroxylation of monophenols to *o*-diphenols and the oxidation of the *o*-diphenols to *o*-quinones.[1] This class of enzyme is widely distributed in plant, animal and microorganism kingdoms.[2] Studies have shown that this enzyme was not only responsible for the browning of fruits and vegetables but also caused some dermatological problems such as flecks, melasma and ephelide due to overproduction of melanin.[1,3] Several known inhibitors such as hydroquinone, kojic acid and arbutin were used in the treatment of skin diseases but recently they were found to be unsafe to human health.[4–6] Recently, Khan et al. reported that 1,3,4-oxadiazole, to be active in inhibiting the activity of tyrosinase enzyme.[7] 1,3,4-Oxadiazole was also found to show a broad range of biological activities including anti-microbial, antitumoral, anti-inflammatory and analgesic activities.[8–13] In this Letter, we describe the synthesis and tyrosinase inhibitory activity of oxadiazole and triazolothiadiazole derivatives. Compounds **1**–**5** were found actively inhibiting the tyrosinase activity with compound **5** exhibited the highest inhibition with an IC_{50} values of 0.87 ± 0.16 μM. Molecular docking was then performed using AUTODOCK 4.1 to examine the binding of the active compound into the pocket site of the tyrosinase enzyme.

The synthesis of title compounds was achieved by the reactions in Scheme 1. The general method of preparing oxadiazole and triazolothiadiazole derivatives were based on the work of Mathew et al. and Almajan et al.[14,15] The intermediates involved for the synthesis of compounds **1**–**16** were 1,3,4-oxadiazole and 4-amino-5-mercapto-1,2,4-triazole. 1,3,4-Oxadiazole was prepared in a stepwise manner involving esterification of the carboxylic group followed by the hydrazinolysis to give aroyl hydrazides. Further reaction with carbon disulfide and potassium hydroxide in ethanol afforded the potassium dithiocarbazates salts. 1,3,4-Oxadiazole was obtained subsequently from acid hydrolysis while reaction with hydrazine monohydrate afforded 4-amino-5-mercapto-1,2,4-triazole. Both of these intermediates were used to prepare compounds **1**–**16**. Compounds **1**–**5** were prepared by the one-pot Mannich reaction consisting of oxadiazole condensation with secondary amine in the presence of formaldehyde. On the other hand, preparation of compounds **10**–**16** required strong dehydrating agent such as phosphorus oxychloride to complete the reaction. Compounds **6**–**9** required additional steps, first by nucleophilic substitution reaction with ethane dibromides followed by the addition of secondary amine such as piperidine and morpholine. The final products obtained in Scheme 1 were in satisfactory yields. The structures of the synthesized compounds were determined by spectroscopic techniques, including ^{1}H NMR, ^{13}C NMR, EIMS, CHN elemental analysis and IR (see Scheme 1).

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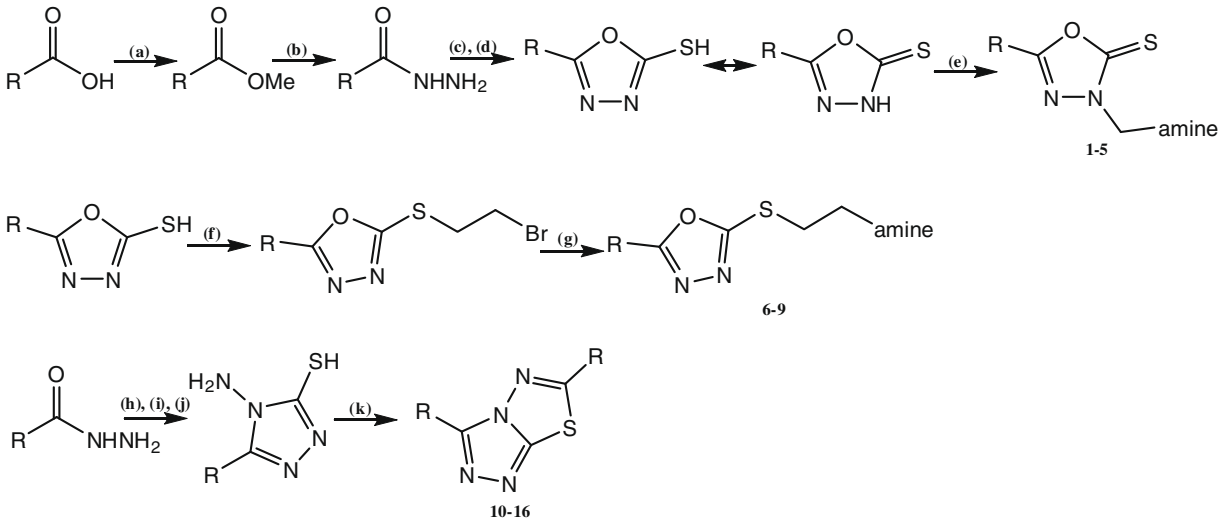


Scheme 1. Synthesis of oxadiazole and triazolothiadiazole analogues. Reagents and reaction conditions: (a) methanol, H_2SO_4, reflux, 4 h; (b) $NH_2NH_2{\cdot}H_2O$, acetic acid, ethanol, reflux, 4 h; (c) CS_2, KOH, ethanol, reflux, 4–6 h; (d) HCl, cold distilled water; (e) respective secondary amines, formaldehyde, dioxane, ethanol; (f) 1,2-dibromoethane, K_2CO_3, acetonitrile; (g) piperidine/morpholine, mixture of ethanol and acetone, rt, 4 h; (h) CS_2, KOH, ethanol, reflux, 4–6 h; (i) $NH_2NH_2{\cdot}H_2O$, H_2O, reflux, 4 h; (j) HCl, cold distilled water; (k) respective carboxylic acid derivatives, $POCl_3$, reflux 6 h.

Table 1
The docking and free energies calculated by using AUTODOCK 4.1 and comparison between the experimental and calculated IC_{50} values of tyrosinase inhibitory activities

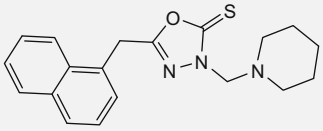
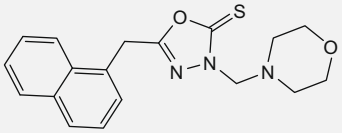
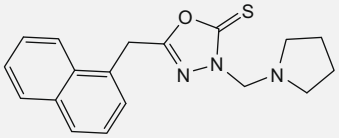
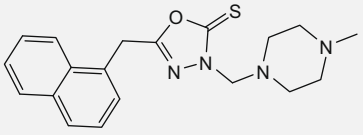
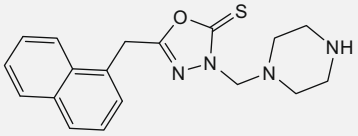
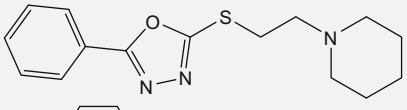
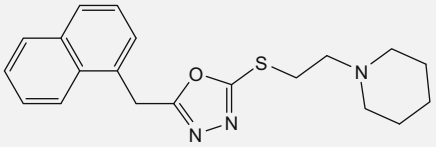
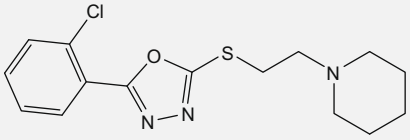
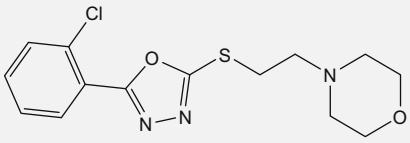
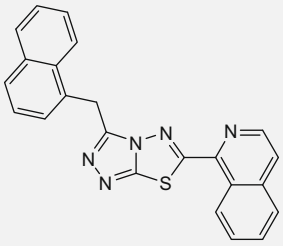
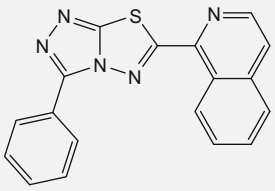
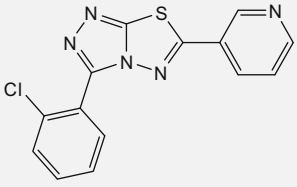
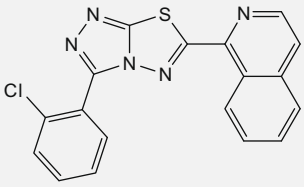
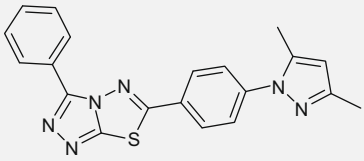
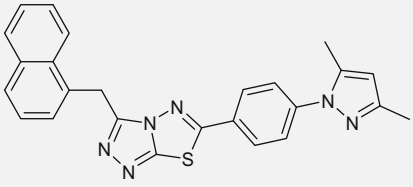
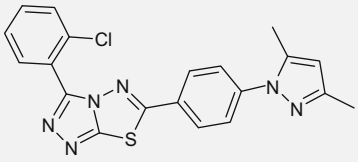
Compound	Analogues	IC_{50} (μM) (Mean ± SEM[a]) Experiment	Free binding energy[b] (kcal/mol)
1		0.97 ± 0.01	−4.91
2		1.34 ± 0.14	−4.80
3		1.33 ± 0.18	−4.56
4		1.49 ± 0.17	−2.32
5		0.87 ± 0.16	−5.58
6		NA[c]	NA[d]
7		NA[c]	NA[d]

Table 1 (*continued*)

Compound	Analogues	IC_{50} (μM) (Mean ± SEM[a]) Experiment	Free binding energy[b] (kcal/mol)
8		NA[c]	NA[d]
9		NA[c]	NA[d]
10		NA[c]	NA[d]
11		NA[c]	NA[d]
12		NA[c]	NA[d]
13		NA[c]	NA[d]
14		NA[c]	NA[d]
15		NA[c]	NA[d]
16		NA[c]	NA[d]
	Kojic Acid[e]	9.18 ± 0.28	ND[d]

[a] SEM: standard error of the mean.
[b] Free binding energy: values of binding energy generated by AUTODOCK 4.1.
[c] NA: not active at 50 μM concentration.
[d] ND: not determined.
[e] Kojic acid: positive control used in the assay.

Inhibition activities of the synthesized oxadiazole and triazolothiadiazole analogues were tested on mushroom tyrosinase according to the method reported by Hearing with slight modification.[16] The activities were compared to that of kojic acid as positive inhibitor. The IC_{50} value of the compounds examined was summarized in Table 1. Our results showed that compounds **1**–**5** exhibited potent inhibitory on mushroom tyrosinase with the highest tyrosinase inhibitory activity with an IC_{50} value of 0.87 ± 0.16 μM, compared to kojic acid which recorded IC_{50} of 9.18 ± 0.28 μM. The decrease in tyrosinase inhibitory activity of this group of compounds, could be related to the nature of the cycloamine moiety attached at N-3 of the oxadiazole ring (piperazine > piperidine > pyrrolidine > morpholine > methylpiperazines). Compounds **6**–**16** did not show any significant activities at 50 μM concentration. The discovery of compounds **1**–**5** as tyrosinase inhibitor has encouraged us to further investigate the ligand–receptor interaction of this group of compounds on mushroom tyrosinase (see Table 1).

Tyrosinase is also known as polyphenol oxidase and contained a type-3 copper center with a coupled dinuclear copper active site in the catalytic core. Protein crystal structure with PDB code 1wx2 was obtained from the protein data bank followed by docking studies using AUTODOCK 4.1.[17] The ligands were constructed and energy minimized using MOPAC program with MMFF94 force field calculation. The caddie protein (ORF 378) and water molecules were removed before polar hydrogen atoms were added and Kollman united atomic charges were assigned using the AUTODOCK 4.1. The grid points were set as 40 × 40 × 40 with the spacing valued at 0.375 to the catalytic site of the tyrosinase while the grid center were placed at −13.175, −14.234 and 16.952. The solvation parameters were added by the ADDSOL program. In default mode, parameters were not given for the dicopper ions which were essentially important for our docking studies. Therefore, the solvation of copper ions was assigned to '0' and the copper van der Waals parameters were assigned based on the Quanta 3.0 Parameters Handbook. The type M equilibrium separation between the nucleuses of the two copper ions, R_{ij} was set as 1.27 Å while the pairwise potential energy eps_{ij} was adjusted to 0.06.[18] Finally ADT, Chimera and LIGPLOT software were used to evaluate the docking of ligand in the active site of tyrosinase.[19–21]

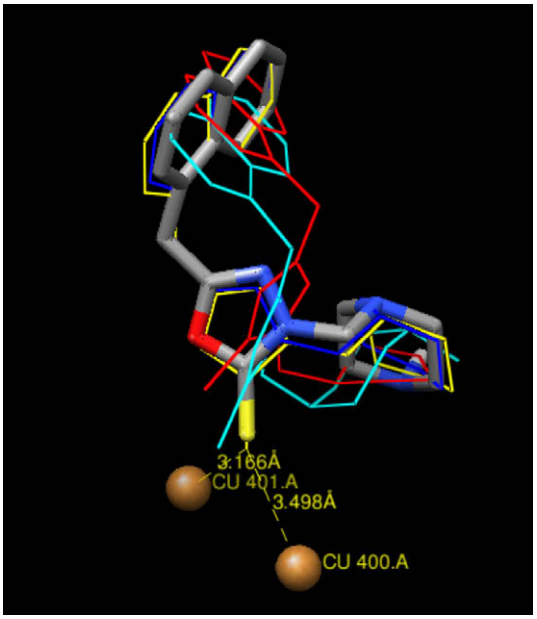


Figure 1. Superimposition of five docking compounds (compound **5** in ball and stick diagram, **1** = blue, **2** = yellow, **3** = red, **4** = cyan). Diagram above shows the position of sulfur atom of the active compounds with the dicopper ions in the active site.

As shown in Figure 1, apparently the tyrosinase inhibition of compounds **1–5** depended on the competency of the sulfur atom to chelate with the dicopper nucleus in the active site. In fact, Kim et al. proposed that the hydroxyl groups in flavonoids might inhibit the tyrosinase enzyme by participating in a metal–ligand binding interaction with the dicopper nucleus.[22] Recent studies found that higher number of hydroxyl groups in the benzene ring of flavonoids played an important role in enzyme inhibition, while methylated and glycosylated flavonoids exhibited less inhibitory effects.[23] Generally, these agreed well with our hypothesis that compound **5** might display the same mode of inhibitory mechanism through the metal chelating effects. In the absence of the phenolic hydroxyl group, compound **5** was replaced by the thione moiety which capable of acting as metal chelator. This was further strengthened by the published article of tyrosinase crystal structure, 1 bug revealing the mechanism of phenylthiourea inhibitor, where the sulfur atom was found to coordinate with both the copper ions, and further increased the metal–metal separation between the ions through the sulfur ligand atom.[24]

The present docking studies revealed the fact that thione group of compound **5**, which resided 3.2–3.5 Å adjacent to the dicopper nucleus are exposed to potential metal–ligand interaction. The LIGPLOT analyses were conducted to understand in depth interaction pattern between the docked ligands and the active site residues through hydrophobic interaction as well as hydrogen bonding pattern.[21] Figures 2 and 3 showed that compound **5** was involved in an extensive hydrophobic contact with the nearby residues. The

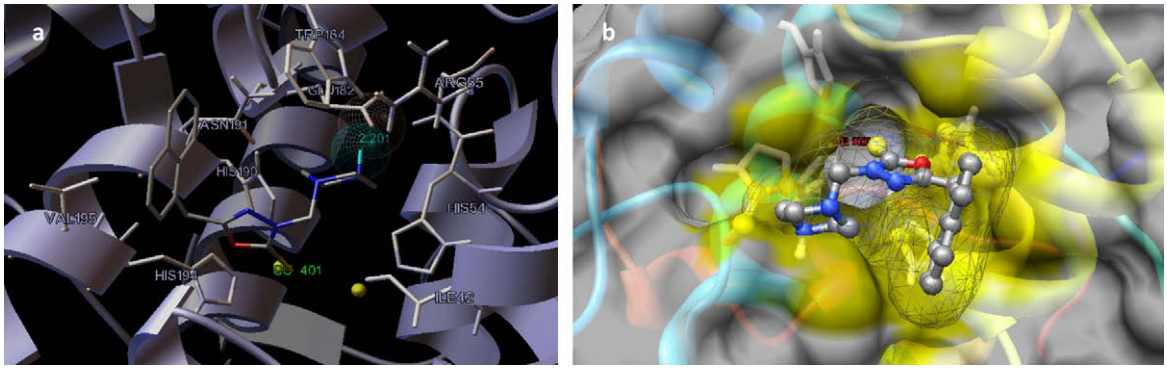


Figure 2. (a) Binding mode of compound **5** into the binding site of tyrosinase (PDB code 1wx2).[17] The amine group was involved in a hydrogen bonding with the uncharged side chain carboxylate of Glu 182 with estimated free binding energy of −5.58 kcal/mol. (b) Interatomic contacts (yellow) of the ligand with the residues in active site based on the van der Waals radii generated by Chimera software.[20]

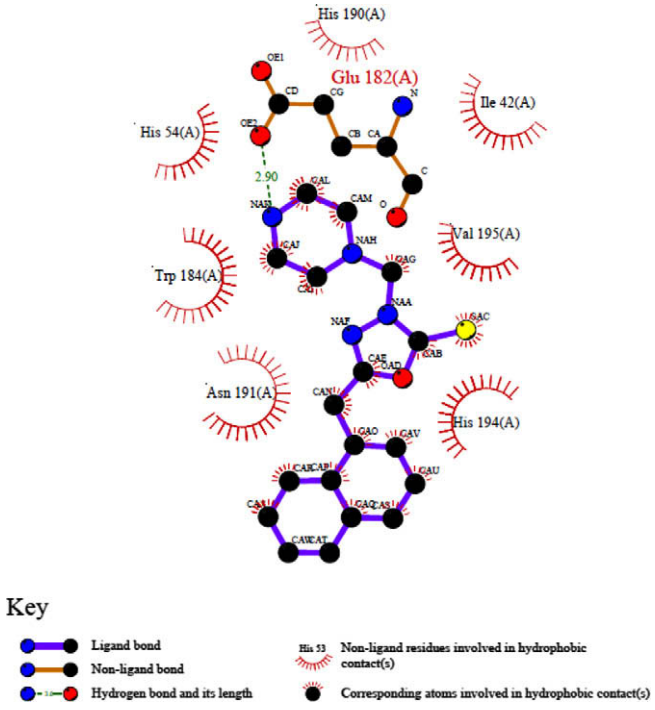


Figure 3. The 2D representation docking result of compound **5** with the residues in the active site of tyrosinase (PDB code 1wx2) showed by LIGPLOT.[17,21]

role of the secondary amine in hydrogen bonding interaction and hydrophobic contact was further explored by substituting different cycloamine derivatives including piperazine, piperidine, pyrrolidine, morpholine and *N*-methylpiperazine. From the docking results, the piperazine moiety was found to well occupy the pocket consisting His 54, Trp 184, His 190 and Asn 191 residues, while the piperazine side chain further formed a 2.9 Å hydrogen bonding with the carboxylate side chain of Glu 182 (see Fig. 3). Replacing the piperidine moiety with pyrrolidine, slightly reduced the inhibitory activity which could explain the importance of the hydrophobic interaction surrounding the active site. Chelation between the sulfur atom and the metal nucleus could act as a bridge to link the naphthyl and aminyl moieties with the hydrophobic pockets surrounding the active site. In Figure 3, the naphthalene ring was within close contact range with the adjacent residues of Val 195 and Asn 191. Compounds **6**–**16** did not show inhibitory effects on the tyrosinase activity. Structurally these compounds lacked the thione functionality and the shielding effects surrounding the thioether group might be the reason for its inactivity. Based on the bioactivity results and this structural observation, the presence of thione is necessary as metal chelator (see Figs. 2 and 3). Previous studies of tyrosinase inhibitors by Khatib et al. showed that the performance of tyrosinase inhibitors could be predicted theoretically through the binding energy related to interactions between the inhibitor and the amino acid residues in the active site.[18] In our study, a good correlation between their IC_{50} of compounds **1**–**5** and AUTODOCK binding free energy was exhibited.

In conclusion, the present data showed promising initial results in the rational design of anti-tyrosinase inhibitor. The results suggested the capability of the thione group to chelate the dicopper nucleus of the enzyme and the substitution pattern of cycloamine moiety are the main contributors to the activity, indicate the significance of the hydrophobic interaction surrounding the active site. The information derived from the docking study of compound **5** provides a good starting platform for further structural modifications and synthesis of potent tyrosinase inhibitor.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.067.

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