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Antioxidant and tyrosinase docking studies of heterocyclic sulfide derivatives containing a thymol moiety

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Abstract

Fourteen heterocyclic sulfide derivatives (**4-17**) containing a thymol moiety and oxadiazole, thiadiazole, triazole, oxazole, thiazole, imidazole, pyridine or pyridine heterocycles were synthesized in three steps. The cupric, Cu(II), ion reducing antioxidant capacity of the compounds was examined, and molecular docking studies were performed to determine whether the sulfur, thymol or heterocyclic moieties interact with the Cu ions in tyrosinase, a type 3 copper enzyme. Using the CUPRAC assay, eight compounds (**5-8, 10, 15-17**) showed equal or better Cu (II) reducing capacity than trolox at neutral pH, with trolox equivalent antioxidant capacity (TEAC) coefficients ranging between 1.00 and 1.48. The compounds containing a thiadiazole moiety were most effective with the methyl thiadiazole derivative (**8**), having the highest Cu(II) reducing capacity. Molecular docking studies of the sulfide derivatives with tyrosinase revealed that there were no direct interactions between the sulfur atom and the active site copper ions. However, the compounds displayed two different binding interactions with the histidine-Cu catalytic center. For compounds **4-13**, the thymol portion was embedded in the active site cavity, while for compounds **14-17** the heterocyclic portion of the molecule approached the cavity.

Keywords: Thymol; heterocyclic sulfides; antioxidant; copper (II) ion reduction; tyrosinase docking studies

1. Introduction

Endogenous sulfur containing molecules such as glutathione, thioredoxin and glutaredoxin play an important role in the body. They act as antioxidants and reduce the amount of excess reactive oxygen species (ROS), which are causative agents in oxidative stress-related diseases such as inflammation, cancer, cardiovascular and neurodegenerative diseases [1]. Synthetic sulfur containing compounds, as well as those derived from natural sources, have also displayed a wide range of biological properties [2-6]. In particular, sulfides have found applications as treatment for cancer, bacterial infections, inflammation, Alzheimer's, Parkinson, tuberculosis, and HIV diseases [7]. However, unlike thiols which have been widely studied as antioxidants [8-9], the role of sulfides as antioxidant agents, particularly reduction of transition metal ions such as copper and iron, is largely understudied [10].

Melanogenesis is regulated by tyrosinase, a metalloenzyme which is responsible for the oxidation of tyrosine to dopaquinone, via L-Dopa as an intermediate. Tyrosinase is a type 3 copper enzyme, in which the active site contains two copper ions associated with histidine residues, which play a vital role in the oxidative mechanism of the enzyme [11-12]. While melanin serves a protective role against skin damage by UV light, overproduction of melanin can lead to undesirable hyperpigmentation disorders, such as solar lentigines, freckles, and melasma. Tyrosinase is also responsible for the browning in fruit and vegetables. In order to reduce these undesirable effects, several agents that target tyrosinase function directly and indirectly have been developed. Inhibition of tyrosinase function occurs by a variety of mechanisms, including copper ion chelation, reversible inhibition, irreversible inactivation, dopamine scavenging and reduction of dopaquinone [11-12].

Many compounds incorporating N, O and S heterocyclic moieties have been reported to possess both strong antioxidant [13] and tyrosinase inhibitory [14] activities. These include

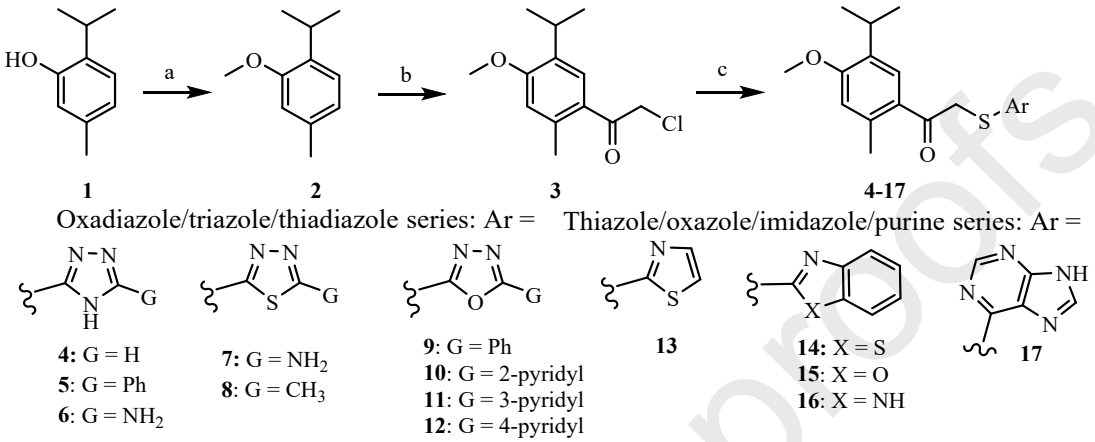
oxadiazole, thiadiazole, triazole, imidazole, thiazole, oxazole, pyridine and purine moieties. In recent years, our lab has been focused on the development of derivatives of carvacrol and thymol, the primary monoterpenoids in thyme and oregano, and evaluation of their antioxidant and tyrosinase inhibitory activities. We have reported the tyrosinase inhibitory activities of alkyl oxobutanoate derivatives of carvacrol and thymol [15] and the cupric ion reducing capacity of N, O, S heterocyclic sulfide derivatives containing a carvacrol core [16]. As an extension of those studies, we herein report the cupric ion reducing capacities of N, O, S heterocyclic thioether derivatives containing a thymol core, and evaluation of their binding affinity for the mushroom tyrosinase enzyme (PDB: 2Y9X). We examined two factors, 1) Given that sulfur has a high affinity for copper, does the exocyclic sulfur atom show an interaction with the Cu ions in the tyrosinase active site, and 2) does the structure of the heterocycle affect the binding mode.

2. Results and discussion

2.1 Chemistry

The mono and bicyclic heterocyclic sulfide derivatives of thymol (**4-17**) were prepared using the method previously described for heterocyclic carvacrol derivatives [16], with slight modifications (Scheme 1). Methyl thymol (**2**) was obtained in 88% yield by treating thymol (**1**) with cesium carbonate and methyl iodide in DMF. The yield and spectroscopic data of **2** were in good agreement with those reported by Silva, et al. [17]. Friedel-Crafts acylation of the methyl thymol in the presence of chloroacetyl chloride and aluminum chloride in dichloromethane afforded compound (**3**) in 48% yield. The heterocyclic sulfides were obtained by nucleophilic displacement of the chloro group with a heterocyclic aromatic thiol in the presence of potassium iodide and potassium iodide in acetonitrile. The products were obtained in 45-96 yield, after purification by

silica gel column chromatography using ethyl acetate-hexane solvent mixtures. The products were characterized by IR and NMR spectroscopic data, together with HRMS data (See experimental section).



Reagents and conditions: a) Cs_2CO_3, CH_3I, dry DMF, heat, 88%; b) Chloroacetyl chloride, $AlCl_3$, dry CH_2Cl_2, 0°C to rt, 48%; c) Ar-SH, K_2CO_3, KI, dry CH_3CN, rt, 45-96%.

Scheme 1. Synthesis of heterocyclic thioether derivatives of thymol.

*2.2 CUPRAC assay of compounds **4-17***

Exposure of Cu(II) ions to neocuproine (2,9-dimethyl-1,10-phenathroline) leads to the formation of a green bis-neocuproine Cu(II) complex. In the presence of an antioxidant, Cu(II) is reduced to Cu(I) by an outer sphere single electron transfer to form a yellow-orange charge-transfer neocuproine-Cu(I) complex. The cupric, Cu(II), ion reducing antioxidant capacity (CUPRAC) assay is used to measure the extent of reduction of Cu(II) to Cu(I) and gives a measure of the strength of the antioxidant [18]. The charge-transfer complex displays an absorption maximum at 450 nm, thus, increased absorbance at 450 nm indicates increased antioxidant activity. CUPRAC data are reported as trolox equivalent antioxidant capacity (TEAC) coefficients, which represents the ratio of the molar absorptivity (obtained from calibration plots using varying concentrations) of the antioxidant sample to that of trolox (TEAC = $\varepsilon_{sample}/\varepsilon_{trolox}$).

As previously reported, when the antioxidant is a sulfide, the sulfur atom is thought to undergo a one-electron oxidation to a radical cation, which is the subsequently oxidized by molecular oxygen to the corresponding sulfone (Fig. 1) [16, 19]. We also demonstrated that the sulfur atom was the primary group involved in Cu(II) reduction of heterocyclic sulfide derivatives based on carvacrol, because conversion to the corresponding sulfone led to elimination of Cu(II) ion reducing capacity [16].

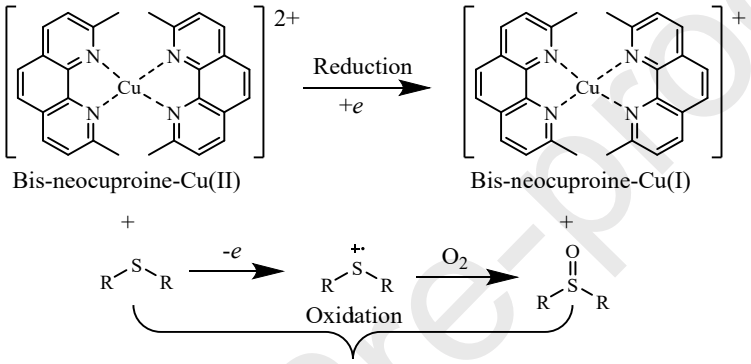
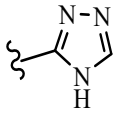
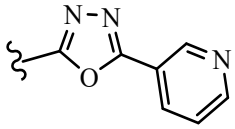
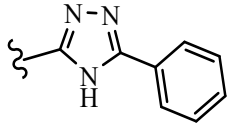
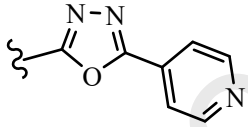
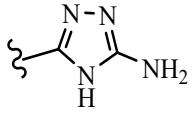
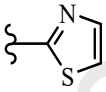
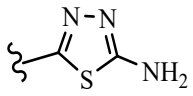
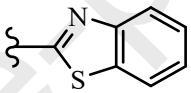
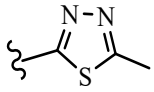
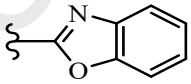
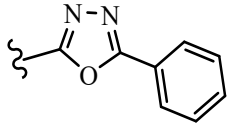
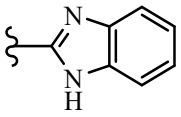
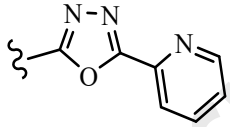
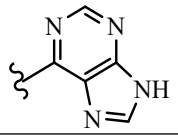


Fig. 1. Bis-neocuproine-Cu(II) reduction by sulfides

With the foregoing in mind, our goal was to determine the influence of the type of heterocyclic moiety on the Cu(II) ion reducing capacity of the thymol containing heterocyclic sulfide derivatives (**4-17**), using ascorbic acid and the parent thymol as additional standards. The compounds are divided into two groups: 1) oxadiazole/triazole/thiadiazole series (**4-12**) and thiazole/oxazole/imidazole/purine series (**13-17**). TEAC coefficients are shown in Table 1.

Table 1. Trolox equivalent antioxidant capacity (TEAC) and tyrosinase binding energy for compounds **4-17.**

Cpd	Ar	TEAC	Tyr BE (kcal/mol)	Cpd	Ar	TEAC	TBE (kcal/mol)
4		0.73	-6.1	**11**		0.50	-7.9
5		1.00	-6.8	**12**		0.84	-7.4
6		1.14	-6.4	**13**		0.91	-5.9
7		1.25	-6.3	**14**		0.64	-6.5
8		1.48	-6.5	**15**		1.08	-6.5
9		ND	-7.8	**16**		1.20	-6.7
10		1.25	-7.8	**17**		1.06	-6.7
KA		NA	-5.6	THY		0.86	-5.7
AA		0.85	ND				

Trolox: ε = 16,100 M^{-1} cm^{-1}; r^2 = 0.9999

KA: Kojic acid ; AA: Ascorbic acid; THY: Thymol

NA: No activity; ND: Not determined

The triazole derivative (**4**) showed a lower antioxidant capacity than trolox, with a TEAC coefficient of 0.73, and was also less effective than ascorbic acid and thymol with TEAC coefficients of 0.85 and 0.86 respectively. Incorporation of an electron donating phenyl group at position 5 on the triazole

ring led to the phenyl triazole derivative (**5**), which is equipotent to trolox, with a TEAC coefficient of 1.00. Replacement of the phenyl group with an amino group led to a slight increase in reducing capacity, with the amino triazole derivative (**6**) having a TEAC coefficient of 1.14. Isosteric replacement of the NH in the triazole ring of compound **6** with sulfur (**7**), led to a further increase in reducing capacity, with **7** showing slightly enhanced activity over **6**. Conversion of the amino thiadiazole derivative to the corresponding methyl derivative (**8**) gave a compound with approximately 1.5 fold increase in activity over trolox.

The TEAC coefficient of phenyl oxadiazole (**9**) was not determined owing to insolubility in the assay medium. The pyridyl oxadiazole derivatives, **10-12**, exhibited a range of TEAC values between 0.50 and 1.25, with the 2-pyridyl derivative (**10**) having the highest reducing capacity and the 3-pyridyl derivative (**11**) having the lowest reducing capacity. These data are consistent with those observed for the corresponding carvacrol derivatives [16]. Furthermore, the 3-pyridyl derivative has the lowest reducing capacity of all the compounds tested.

The thiazole derivative (**13**) showed comparable antioxidant activity to trolox, with a TEAC coefficient of 0.91. However, the incorporation of a fused ring in benzothiazole (**14**) resulted in lowered activity (0.64). The benzoxazole (**15**) and benzimidazole (**16**) derivatives, showed 1.7 and 1.9 fold increase in activity, respectively, relative to the benzothiazole. The increase in antioxidant activity among this series trends similarly to the basicity of the heteroatom. Finally, purine derivative (**17**) showed slightly less activity than benzimidazole **16**, likely due to the electron-withdrawing pyrimidine ring of **17** being closer to the sulfur atom.

*2.3 Molecular docking analysis of compounds **4-17** with mushroom tyrosinase*

Given the copper reducing activities of the heterocyclic sulfide derivatives of thymol, and the high affinity of sulfur for copper ions, it was envisaged that the sulfur atom may interact with the copper ions in the active site of the tyrosinase enzyme. Thus, the compounds were subjected to *in silico* docking studies with mushroom tyrosinase (PDB ID: 2Y9X), using Autodock Vina to determine their binding affinity for the enzyme. Docking experiments were performed in triplicate, where all residues on tyrosinase were rigid, and all rotatable bonds on the test compound were made flexible. The charges on the copper atoms in tyrosinase were set to 2.00, in agreement with recent literature [20]. The search volume was defined as a 15 Å × 15 Å × 15 Å cube containing the two-Cu atom active site in the center. All other parameters were left at their default values.

Docking experiments using kojic acid and thymol, known tyrosinase inhibitors, were performed for comparison with the synthesized derivatives. Kojic acid has been shown to inhibit tyrosinase by multiple mechanisms, including copper-chelation and by embedding itself into the tyrosinase active site [21]. As demonstrated in Fig. 2, the hydroxymethyl group of kojic acid is oriented close to the active site copper atoms, while the phenol is associated with a histidine residue. The observed docking store of -5.6 kcal/mol for kojic acid is in good agreement with the reported docking affinity of -5.5 kcal/mol [22]. Molecular docking of thymol revealed that thymol was embedded into the active site in a similar conformation as kojic acid, with their phenol groups oriented in a similar fashion. However, thymol sits further outside the active site than kojic acid, likely due to the bulkier isopropyl group (Fig. 2). The docking score of thymol (-5.7 kcal/ mol) is similar to that of kojic acid. A study by da Silva, using the MolDock program, also showed that kojic acid and thymol had similar binding affinity to the tyrosinase enzyme, although the docking scores were different than

those observed using the AutoDock Vina program [23]. Interestingly, unlike thymol, kojic acid showed no activity in the CUPRAC assay.

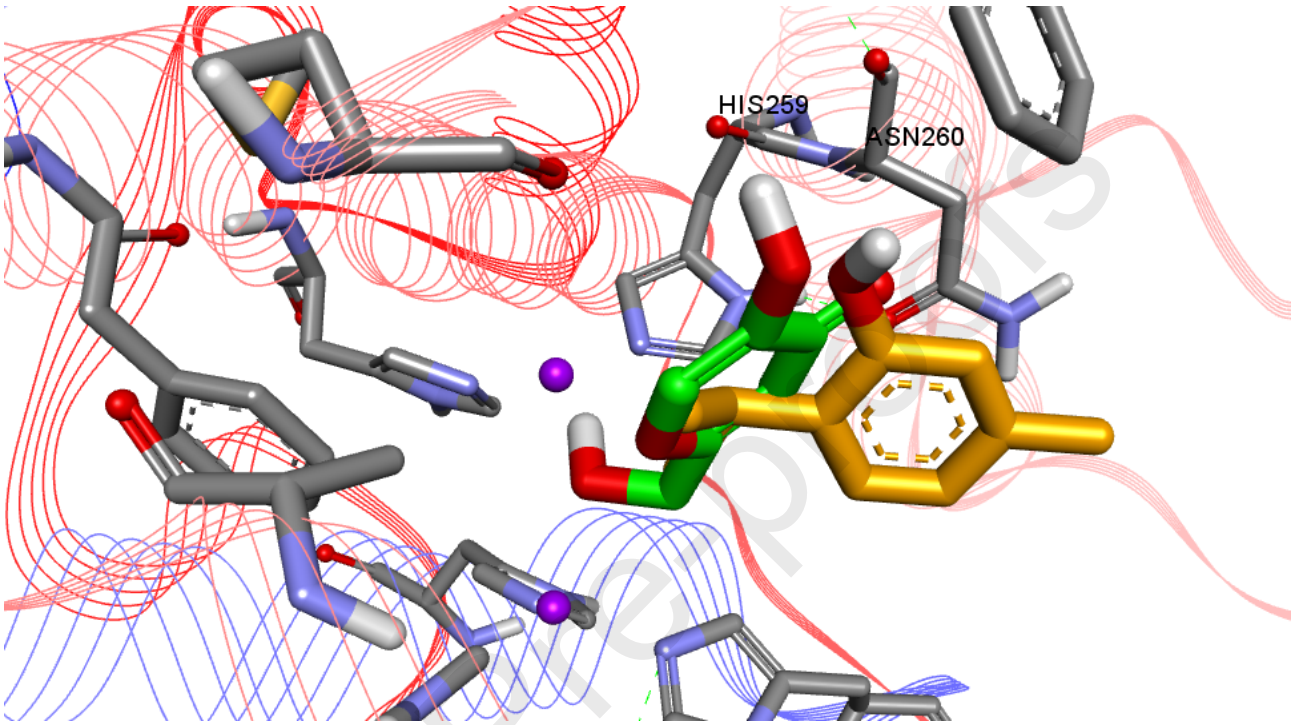


Fig. 2. Docking conformations of kojic acid (green) and thymol (orange).

The heterocyclic sulfide derivatives adopted one of two general conformations within the tyrosinase active site: **4-13** assumed conformations in which the thymol moiety was oriented toward the copper-histidine catalytic center, whereas for derivatives containing fused rings, **14-17**, the heterocyclic moiety was oriented toward the catalytic center. Triazoles **4-6** and thiazole **13** interact with tyrosinase similarly, as shown in Fig. 3. Each compound is stabilized by nonpolar interactions between the hydrophobic thymol region and residues Ala286, Phe264, and Val283. Thiazole **13** had the weakest docking score of all derivatives (-5.9 kcal/mol), due the absence of hydrogen bonding interactions with the site residues. Triazole **4** showed increased binding affinity due to hydrogen bonding between the triazole and the backbone of Val 283. Addition of the phenyl substituent in **5**

lead to a significant increase in docking affinity (-6.8 kcal/mol), due to hydrogen bonding with Asn260 and numerous hydrophobic interactions. Substitution of the phenyl group for an amino as in **6** also led to a slight increase in docking affinity relative to triazole **4** (6.4 kcal/mol), due to hydrogen bonding to His85 via the amino group.

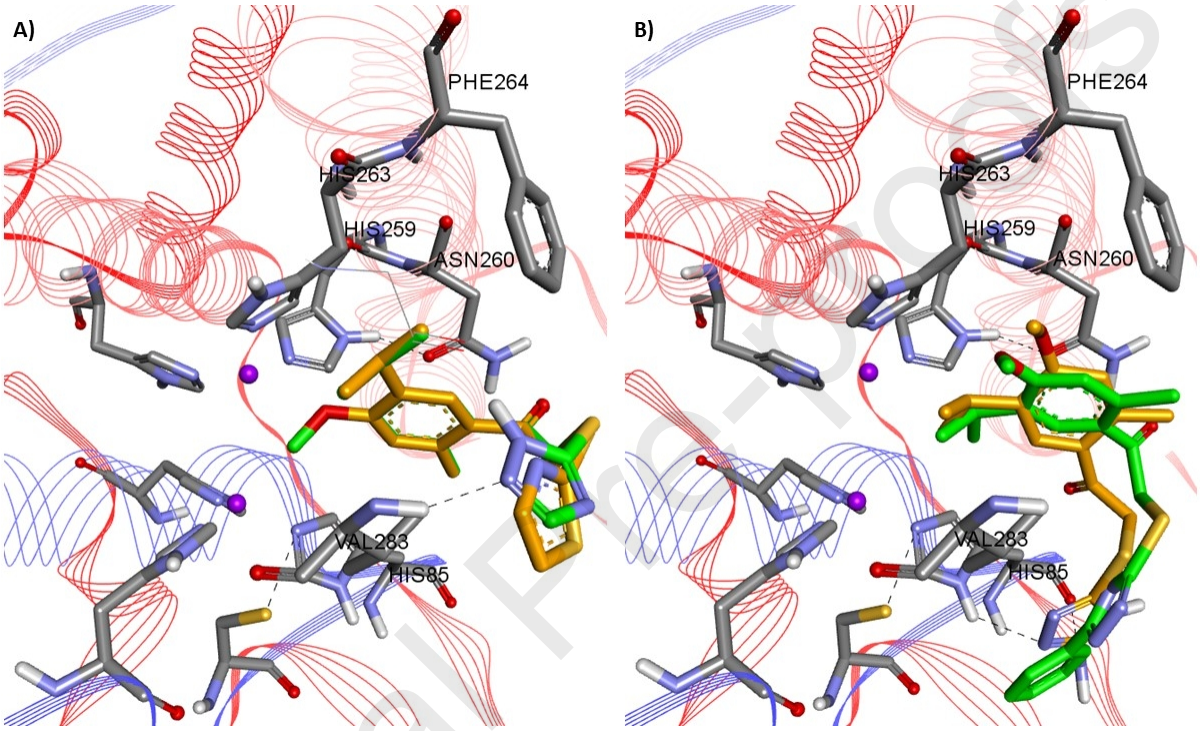


Fig. 3. A) Docking conformations of triazole **4** (green) and thiazole **13** (orange). B) Conformations of triazole derivatives **5** (green) and **6** (orange). Copper atoms are shown in purple.

Thiadiazole derivatives **7** and **8** displayed similar docking affinity to the amino triazole (**6**). Both compounds are stabilized by hydrogen bonding between the thiadiazole N atoms and His85 (Fig. 4). Compound **7** is further stabilized by hydrogen bonding between the amino group and His85. Both structures also interact with Val283 and Asn260, as well as show pi-sigma interactions with Phe264 and His263. Surprisingly, methyl derivative **8** has a slightly more favorable binding

energy (-6.5 kcal/mol) than amino derivative **7** (-6.3 kcal/mole), even though the amino group of **7** is involved in hydrogen bonding.

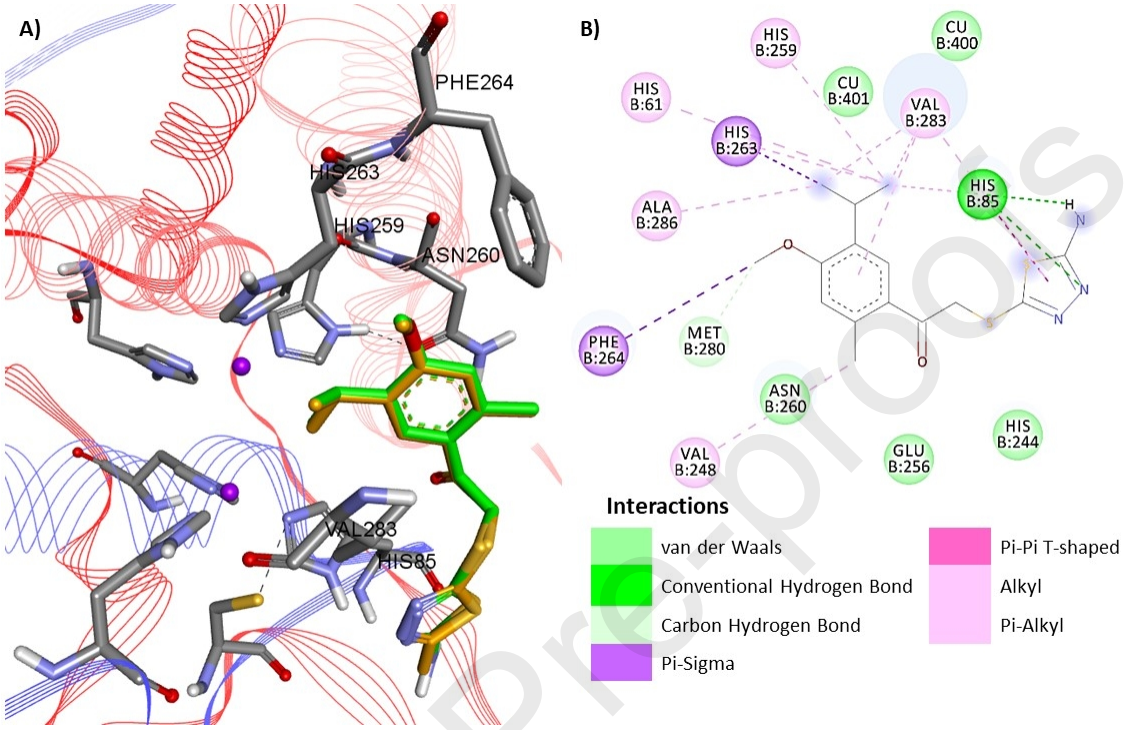


Fig. 4. A) Docking conformations of thiadiazoles **7** (green) and **8** (orange). B) Ligand-protein interaction map of thiadiazole **7**.

The oxadiazole derivatives containing an additional aromatic ring (**9-12**) showed binding affinities markedly stronger than any other class of compounds (Table 1, Fig. 5). Among these compounds, 3-pyridyl oxadiazole **11** showed the highest affinity (-7.9 kcal/mol), while 4-pyridyl oxadiazole **12** showed the lowest affinity (-7.4 kcal/mol). The increased stability of **11**, and to a lesser degree, the 2-pyridyl derivative **10** (-7.8 kcal/mol), is attributable to the capability of the pyridyl group to hydrogen bond to Asn81 and His85. Phenyl oxadiazole **9** also showed significant binding affinity, likely due to pi-type interactions with His 85.

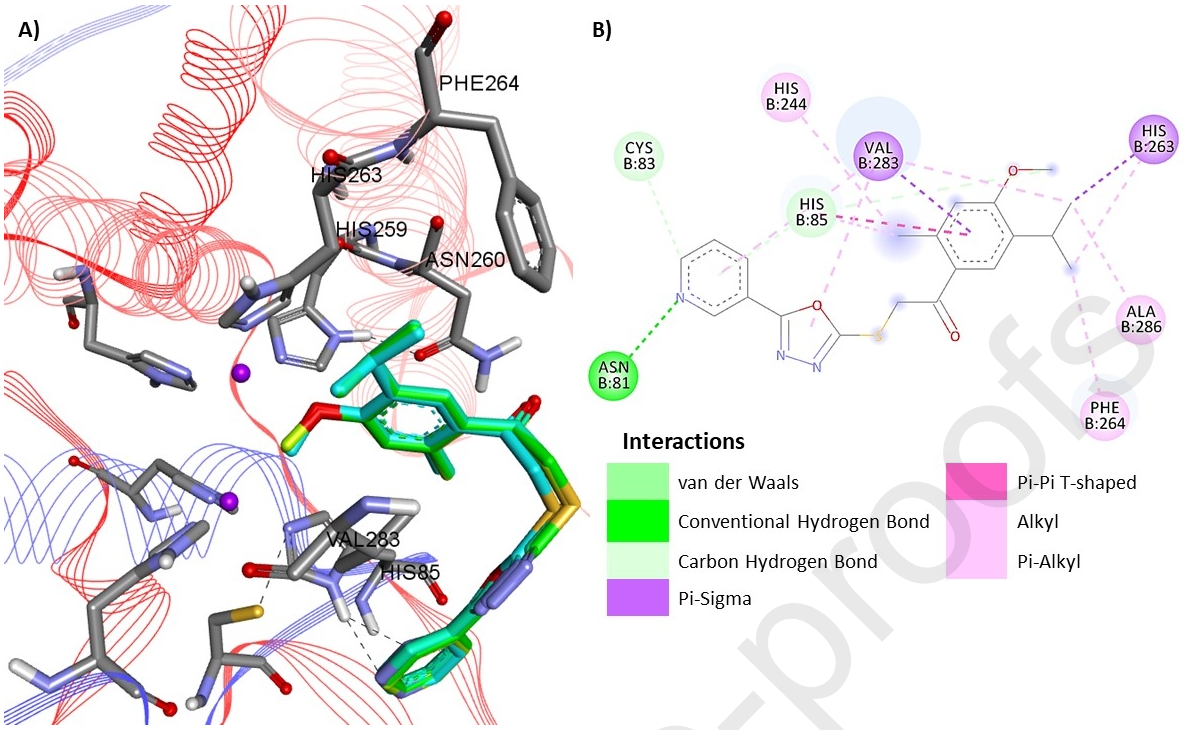


Fig. 5. A) Docking conformations of oxadiazoles **9** (green), **10** (light green), **11** (light blue-green), and **12** (light blue). B) Ligand-protein interaction map for nicotinic oxadiazole **11**.

The fused heterocyclic derivatives **14-17** interacted with the tyrosinase active site by embedding the heterocyclic portion into the binding pocket, in contrast to all other derivatives (Fig. 6). The four derivatives can be categorized by docking affinity: Benzoxazole and benzothiazole **14** and **15** both showed a binding affinity of -6.5 kcal/mol, stabilized by pi-pi stacking with His263 and pi-sigma interactions with Val283. Benzimidazole **16** and purine **17** showed more favorable docking scores (-6.7 kcal/mol), due to the presence of the N-H group, which serves as a hydrogen bond-donor. Both **16** and **17** interact with Val283 and His263; however, benzimidazole **16** is further stabilized by hydrogen bonding with Asn260, while purine **17** is stabilized by hydrogen bonding with Met280.

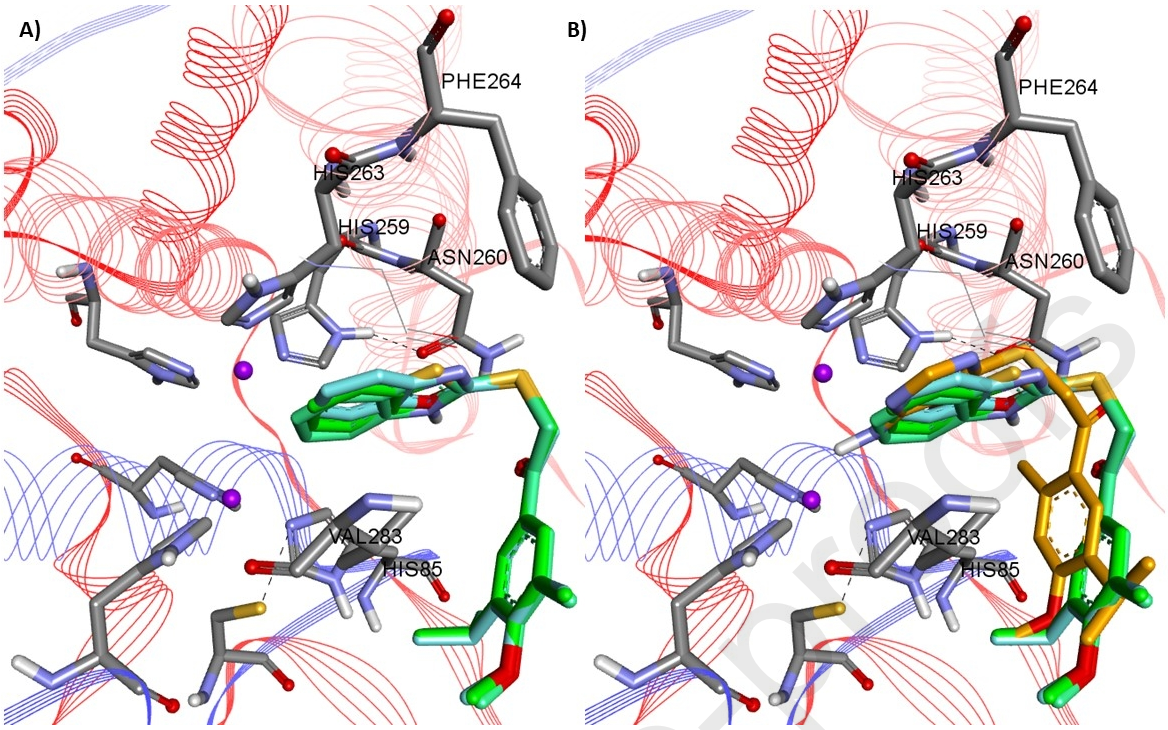


Fig. 6. A) Docking conformations of fused rings **14** (green), **15** (blue-green), and **16** (light blue) (16. B) Docking conformations of **14-16**, with purine **17 (**orange).

Based on the favorable binding affinity data for compounds **4-17**, *in vitro* tyrosinase inhibitory experiments were explored. However, even the most polar compounds gave poor solubility in the assay medium, and thus those studies were not completed.

3. Conclusions

In summary, fourteen new heterocyclic sulfide derivatives of thymol (**4-17**) have been synthesized in three steps and evaluated for their ability to reduce Cu(II) to Cu(I) using the CUPRAC assay. Eight compounds (**5-8, 10, 15-17**) showed greater or equal reducing capacity relative to trolox. For the tyrosinase docking studies, all the derivatives showed lower binding energy than kojic acid and thymol, implying greater affinity toward the binding site. The oxadiazole derivatives (**9-12**),

containing an appended aromatic ring, have the largest binding affinities for the active site. This study reveals that the structure of the heterocyclic moiety has an influence on the Cu(II) reducing capacity of the heterocyclic sulfides, and that they are effective Cu(II) antioxidants. However, there is no conclusive correlation of Cu(II) reducing capacity and tyrosinase binding affinity. Furthermore, the sulfur atom does not appear to play a significant role in the binding affinity of the derivatives to the tyrosinase enzyme.

4. Experimental section

4.1 Materials and instrumentation

The chemicals and solvents were obtained from TCI Chemicals, Sigma-Aldrich, Fisher Scientific. Dichloromethane and acetonitrile were dried according to standard procedures. Column chromatography was performed using Teledyne Isco Rf-Gold prepacked silica gel columns (20-40 μm). Melting point data were acquired on a Thomas-Hoover capillary melting point apparatus. Ultraviolet-Visible and Infrared spectra were recorded using a Varian Cary 4000 spectrometer and a Thermo Scientific Nicolet iS50 FT-IR spectrometer, respectively. NMR data were recorded using a JEOL ECZ 400S spectrometer (^{1}H NMR, 400 MHz; ^{13}C NMR, 100 MHz), using $CDCl_3$ or DMSO-d6 as solvents and TMS as internal standard. HR-ESIMS data were acquired on a Waters SYNAPT G2-S QTOFMS system.

4.2 Synthesis

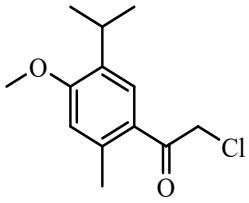
*4.2.1 Synthesis of methyl thymol, 1-isopropyl-2-methoxy-4-methylbenzene (**2**)*

A mixture of thymol **1**, (8.0 g, 53 mmol), iodomethane (5.0 mL, 80 mmol), and cesium carbonate (26.0 g, 80 mmol) in anhydrous DMF (30 mL) was heated for 17 h. After cooling, the mixture was poured into water (150 mL) and extracted with EtOAc (3 × 50 mL). The EtOAc solution was washed with brine (60 mL), dried with Na_2SO_4, filtered and concentrated *in vacuo*. Column chromatography of the residue afforded the methyl ether (**2**) as a colorless oil (7.69 g, 88%). The NMR data for compound **2** was in good agreement with literature data [17].

*4.2.2 Synthesis of 2-chloro-1-(5-isopropyl-4-methoxy-2-methylphenyl)ethan-1-one (**3**)*

Chloroacetyl chloride (4.1 mL, 47 mmol) was added slowly via syringe to an ice-cooled mixture of methyl thymol, **2**, (6.05 g, 37 mmol) and $AlCl_3$ (6.44 g, 48 mmol) in anhydrous CH_2Cl_2 (180 mL) under nitrogen, with stirring. The mixture was allowed to warm to room temperature and stirring was continued for 72 hours before pouring into crushed ice (~400 mL). After separation of the layers, the CH_2Cl_2 solution was washed with10% aqueous NaHCO3 (3 × 100 mL), followed by saturated NaCl (100 mL). The solution was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography, eluting with 5% ethyl acetate-hexanes to afford compound **3** (4.25 g).

Off white solid (48%); M.p. 50-53 °C; IR (ATR), cm^{-1}: 1691 (C=O).



^{1}H NMR ($CDCl_3$): δ 7.53 (1H, s, C*H*=C-C=O), 6.70 (1H, s, C*H*=C-O), 4.65 (2H, s, CH_2Cl), 3.87 (3H, s, CH_3O), 3.26 (1H, septet, *J* = 6.8 Hz, C*H*[CH_3]$_2$), 2.56 (3H, s, CH_3Ar), 1.20 (6H, d, *J* = 6.8 Hz, ([CH_3]$_2$CH); ^{13}C NMR ($CDCl_3$): δ 192.3 (*C*=O), 160.0 (*C*-O), 140.9 (*C*-CH_3), 134.3 (*C*-CH[CH_3]$_2$), 128.0 (*C*H=C-C=O), 126.2 (*C*-C=O), 114.1 (*C*H=C-O), 55.5 (*C*H_3O), 47.9 (*C*H_2Cl), 26.6 (*C*H[CH_3]$_2$), 22.6 ([*C*H_3]$_2$CH), 22.4 (*C*H_3Ar).

HRMS (ESI): *m/z* 241.0984 $[M+H]^+$; calcd. for $C_{13}H_{18}O_2Cl$, 241.0989

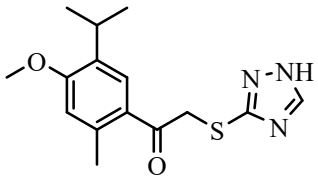
*4.2.3 General synthesis of β-keto sulfide derivatives of methyl thymol (**4-17**)*

KI (1.2 equiv) was added to a stirred solution of compound **3** (150 mg, 0.62 mmol) in dry acetonitrile (20 mL) at room temperature. After 5 minutes, the aromatic thiol (1.05 equiv) was added to the resulting cloudy mixture, followed by K_2CO_3 (1.5 equiv). The mixture was stirred at room temperature for 4 to 24 hours, followed by removal of the acetonitrile *in vacuo*. Column chromatography of the residue using ethyl acetate mixtures (100% hexanes to 60% ethyl acetate-

hexanes; 12g column; flow rate 4 mL/min) gave the target compounds in 45-96% yields, based on compound **3**. For benzimidazole, amino thiadiazole, and amino triazole, only one equivalent of K_2CO_3 was used and the solvent included 20% of water. The mercapto-oxadiazole nucleophiles used in the synthesis of compounds **10-12** were obtained by treatment of the corresponding pyridine hydrazides with carbon disulfide under basic conditions, as previously reported [24].

2-((1H-1,2,4-triazol-3-yl)thio)-1-(5-isopropyl-4-methoxy-2-methylphenyl)ethan-1-one ***(4)***

White solid (88%); M.p. 147-148 °C; IR (ATR), cm^{-1}: 1670 (C=O).

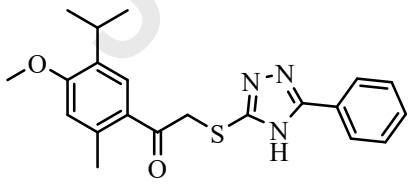


^{1}H NMR ($CDCl_3$): δ 8.11 (1H, s, C*H*=N), 7.64 (1H, s, C*H*=C-C=O), 6.67 (1H, s, C*H*=C-O), 4.57 (2H, s, CH_2S), 3.86 (3H, s, CH_3O), 3.24 (1H, septet, *J* = 6.8 Hz, C*H*[CH_3]$_2$), 2.51 (3H, s, CH_3Ar), 1.19 (6H, d, *J* = 6.8 Hz, ([CH_3]$_2$CH); ^{13}C NMR ($CDCl_3$): δ 195.8 (*C*=O), 160.1 (*C*-O), 140.9 (*C*-CH_3), 134.4 (*C*-CH[CH_3]$_2$), 128.5 (*C*H=C-C=O), 127.2 (*C*-C=O), 114.1 (*C*H=C-O), 55.6 (*C*H_3O), 41.4 (*C*H_2S), 26.8 (*C*H[CH_3]$_2$), 22.5 (*C*H_3Ar), 22.5 ([*C*H_3]$_2$CH). *Triazole carbons did not show on the spectrum.*

HRMS (ESI): *m/z* 306.1267 [M+ NH] $^+$; calcd. for $C_{15}H_{20}N_3NO_2S$, 306.1276.

1-(5-isopropyl-4-methoxy-2-methylphenyl)-2-((5-phenyl-4H-1,2,4-triazol-3-yl)thio)ethan-1-one ***(5)***

White solid (45 %); M.p. 212-214 °C; IR (ATR), cm^{-1}: 1641 (C=O).



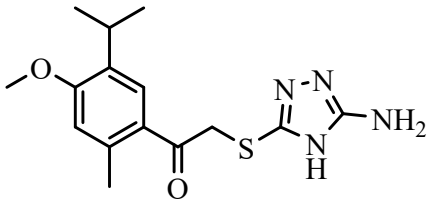
^{1}H NMR (DMSO-d6): δ 7.89 (2H, d, *J* = 8.0 Hz, C*H*=CH, *ortho*),7.72 (1H, s, C*H*=C-C=O), 7.44-7.45 (3H, m, C*H*=C*H*-C*H*),6.86 (1H, s, C*H*=C-O), 4.71 (2H, s, CH_2S), 3.82 (3H, s, CH_3O), 3.17 (1H, septet, *J* = 7.2 Hz, C*H*[CH_3]$_2$), 2.37 (3H, s, CH_3Ar), 1.14 (6H, d, *J* = 7.2 Hz,

([C*H*$_3$]$_2$CH); ^{13}C NMR (DMSO-d6): δ 196.2 (*C*=O), 159.3 (*C*-O), 139.0 (*C*-CH$_3$), 133.6 (*C*-CH[CH$_3$]$_2$), 130.4 (*C*-C=N), 129.5 (*C*H=*C*H-*C*H), 128.8 (*C*-C=O), 128.3 (*C*H=C-C=O), 126.4 (*C*H=*C*H-*C*H), 114.5 (*C*H=C-O), 56.2 (*C*H$_3$O), 41.6 (*C*H$_2$S), 26.6 (*C*H[CH$_3$]$_2$), 22.9 ([*C*H$_3$]$_2$CH), 21.7 (*C*H$_3$Ar).). *The triazole carbons did not show on the spectrum.*

HRMS (ESI): *m/z* 382.1593 [M+H]$^+$; calcd. for $C_{21}H_{24}N_3O_2S$, 382.1583

2-((5-amino-4H-1,2,4-triazol-3-yl)thio)-1-(5-isopropyl-4-methoxy-2-methylphenyl)ethan-1-one ***(6)***

White solid (94%); M.p. 166-169 °C; IR (ATR), cm^{-1}: 1663 (C=O).

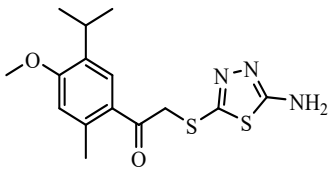


^{1}H NMR (DMSO-d6): δ 11.88 (1H, s, N*H*), 7.66 (1H, s, C*H*=C-C=O), 6.84 (1H, s, C*H*=C-O), 6.01 (2H, s, N*H*$_2$), 4.46 (2H, s, C*H*$_2$S), 3.81 (3H, s, C*H*$_3$O), 3.16 (1H, septet, *J* = 7.2 Hz, C*H*[CH$_3$]$_2$), 2.37 (3H, s, C*H*$_3$Ar), 1.14 (6H, d, *J* = 7.2 Hz, ([C*H*$_3$]$_2$CH); ^{13}C NMR (DMSO-d6): δ 196.6 (*C*=O), 159.2 (*C*-O), 157.9 (*C*-NH$_2$), 155.9 (*C*-S), 139.0 (*C*-CH$_3$), 133.5 (*C*-CH[CH$_3$]$_2$), 128.7 (*C*H=C-C=O), 128.4 (*C*-C=O), 114.5 (*C*H=C-O), 56.1 (*C*H$_3$O), 40.9 (*C*H$_2$S), 26.6 (*C*H[CH$_3$]$_2$), 22.9 ([*C*H$_3$]$_2$CH), 21.8 (*C*H$_3$Ar).

HRMS (ESI): *m/z* 321.1392 [M+H]$^+$; calcd. for $C_{15}H_{21}N_4O_2S$, 321.1385

2-((5-amino-1,3,4-thiadiazol-2-yl)thio)-1-(5-isopropyl-4-methoxy-2-methylphenyl)ethan-1-one ***(7)***

Off-white solid (91%); M.p. 118-119 °C; IR (ATR), cm^{-1}: 1662 (C=O).



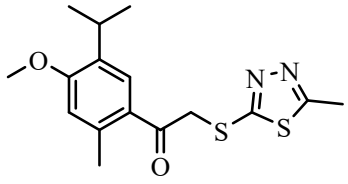
^{1}H NMR (DMSO-d6): δ 7.68 (1H, s, C*H*=C-C=O), 7.25 (2H, s, N*H*$_2$), 6.86 (1H, s, C*H*=C-O), 4.66 (2H, s, C*H*$_2$S), 3.82 (3H, s, C*H*$_3$O), 3.17 (1H, septet, *J* = 6.8 Hz, C*H*[CH$_3$]$_2$), 2.39 (3H, s, C*H*$_3$Ar), 1.14 (6H, d, *J* = 6.8

Hz, ([C*H*$_3$]$_2$CH); ^{13}C NMR (DMSO-d6): δ 195.6 (*C*=O), 170.3 (*C*-NH$_2$), 159.5 (*C*-O), 150.0 (*C*-S), 139.5 (*C*-CH$_3$), 133.7 (*C*-CH[CH$_3$]$_2$), 128.7 (*C*H=C-C=O), 128.2 (*C*-C=O), 114.5 (*C*H=C-O), 56.2 (*C*H$_3$O), 44.1 (*C*H$_2$S), 26.6 (*C*H[CH$_3$]$_2$), 22.9 ([*C*H$_3$]$_2$CH), 22.0 (*C*H$_3$Ar).

HRMS (ESI): *m/z* 338.0986 [M+H]$^+$; calcd. for $C_{15}H_{20}N_3O_2S_2$, 338.0997

1-(5-isopropyl-4-methoxy-2-methylphenyl)-2-((5-methyl-1,3,4-thiadiazol-2-yl)thio)ethan-1-one ***(8)***

White solid (87%); M.p. 102-105 °C; IR (ATR), cm^{-1}: 1662 (C=O).

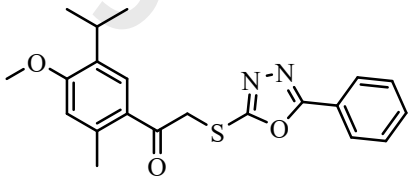


^{1}H NMR (CDCl$_3$): δ 7.69 (1H, s, C*H*=C-C=O), 6.69 (1H, s, C*H*=C-O), 4.91 (2H, s, C*H*$_2$S), 3.87 (3H, s, C*H*$_3$O), 3.26 (1H, septet, *J* = 6.8 Hz, C*H*[CH$_3$]$_2$), 2.71 (3H, s, C*H*$_3$C=N), 2.55 (3H, s, C*H*$_3$Ar), 1.22 (6H, d, *J* = 6.8 Hz, ([C*H*$_3$]$_2$CH); ^{13}C NMR (CDCl$_3$): δ 193.7 (*C*=O), 165.3 (CH$_3$*C*=N), 164.8 (*C*-S), 160.1 (*C*-O), 140.7 (*C*-CH$_3$), 134.3 (*C*-CH[CH$_3$]$_2$), 128.4 (*C*H=C-C=O), 127.3 (*C*-C=O), 114.9 (*C*H=C-O), 55.5 (*C*H$_3$O), 44.0 (*C*H$_2$S), 26.8 (*C*H[CH$_3$]$_2$), 22.6 ([*C*H$_3$]$_2$CH), 22.5 (*C*H$_3$Ar), 15.7 (*C*H$_3$C=N).

HRMS (ESI): *m/z* 337.1051 [M+H]$^+$; calcd. for $C_{16}H_{21}N_2O_2S_2$, 337.1038.

1-(5-isopropyl-4-methoxy-2-methylphenyl)-2-((5-phenyl-1,3,4-oxadiazol-2-yl)thio)ethan-1-one ***(9)***

White solid (95%); M.p. 156-159 °C; IR (ATR), cm^{-1}: 1665 (C=O).



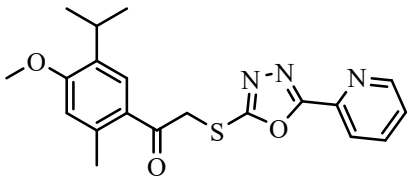
^{1}H NMR (CDCl$_3$): δ 8.01 (2H, d, *J* = 6.4 Hz, C*H*=CH, *ortho*), 7.71 (1H, s, C*H*=C-C=O), 7.49-7.52 (3H, m, C*H*=C*H*-C*H*), 6.71 (1H, s, C*H*=C-O), 4.95 (2H, s, C*H*$_2$S), 3.89 (3H, s, C*H*$_3$O), 3.28 (1H, septet, *J* = 7.2 Hz, C*H*[CH$_3$]$_2$), 2.59 (3H, s, C*H*$_3$Ar), 1.23 (6H, d, *J* = 7.2 Hz, ([C*H*$_3$]$_2$CH); ^{13}C NMR

($CDCl_3$): δ 192.9 (*C*=O), 165.9 (N=*C*-C), 164.3 (*C*-S), 160.3 (*C*-O), 141.0 (*C*-CH_3), 134.6 (*C*-$CH[CH_3]_2$), 131.8 (CH=*C*H-CH, *para*), 129.1 (*C*H=CH, *meta*), 128.5 (*C*H=C-C=O), 126.8 (*C*-C=O), 126.8 (*C*H=CH, *ortho*), 123.7 (*C*-C=N), 114.2 (*C*H=C-O), 55.6 (CH_3O), 43.9 (CH_2S), 26.9 ($CH[CH_3]_2$), 22.7 (CH_3Ar), 22.6 ($[CH_3]_2CH$).

HRMS (ESI): *m/z* 383.1418 $[M+H]^+$; calcd. for $C_{21}H_{23}N_2O_3S$, 383.1423

1-(5-isopropyl-4-methoxy-2-methylphenyl)-2-((5-(pyridin-2-yl)-1,3,4-oxadiazol-2-yl)thio)ethan-1-one ***(10)***

White solid (84%); M.p. 159-160 °C; IR (ATR), cm^{-1}: 1668 (C=O).

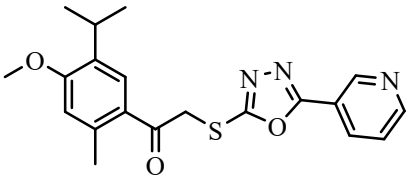


^1H NMR ($CDCl_3$): δ 8.74 (1H, d, *J* = 4.8 Hz, C*H*=N), 8.16 (1H, d, *J* = 8.0 Hz, C*H*=C-N), 7.85 (1H, t, *J* = 8.0 Hz, C*H*=CH-CH=N), 7.69 (1H, s, C*H*=C-C=O), 7.44 (1H, dd, *J* = 8.0, 4.8 Hz, CH=C*H*-CH=N), 6.69 (1H, s, C*H*=C-O), 4.97 (2H, s, CH_2S), 3.86 (3H, s, CH_3O), 3.26 (1H, septet, *J* = 6.8 Hz, C*H*$[CH_3]_2$), 2.56 (3H, s, CH_3Ar), 1.22 (6H, d, *J* = 6.8 Hz, ($[CH_3]_2CH$); ^{13}C NMR ($CDCl_3$): δ 192.8 (*C*=O), 165.9 (N=*C*-C), 165.1 (*C*-S), 160.3 (*C*-O), 150.4 (*C*H=N), 143.3 (*C*-C=N), 141.0 (*C*-CH_3), 137.3 (*C*H=CH-CH=N), 134.6 (*C*-$CH[CH_3]_2$), 128.6 (*C*H=C-C=O), 126.7 (*C*-C=O), 125.9 (*C*H=C-N), 122.9 (CH=*C*H-CH=N), 114.2 (*C*H=C-O), 55.6 (CH_3O), 44.0 (CH_2S), 26.9 ($CH[CH_3]_2$), 22.7 (CH_3Ar), 22.6 ($[CH_3]_2CH$).

HRMS (ESI): *m/z* 384.1366 $[M+H]^+$; calcd. for $C_{20}H_{22}N_3O_3S$, 384.1376.

1-(5-isopropyl-4-methoxy-2-methylphenyl)-2-((5-(pyridin-3-yl)-1,3,4-oxadiazol-2-yl)thio)ethan-1-one ***(11)***

White solid (46%); M.p. 169-172 °C; IR (ATR), cm^{-1}: 1657 (C=O).

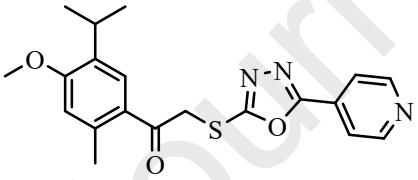


^{1}H NMR ($CDCl_3$): δ 9.22 (1H, s, N=C*H*-C), 8.75 (1H, d, *J* = 4.8 Hz, CH=C*H*-N), 8.28 (1H, d, *J* = 8.0 Hz, C=C*H*-CH), 7.69 (1H, s, C*H*=C-C=O), 7.44 (1H, dd, *J* = 8.0, 4.8 Hz, C*H*=CH-N), 6.70 (1H, s, C*H*=C-O), 4.96 (2H, s, CH_2S), 3.87 (3H, s, CH_3O), 3.27 (1H, septet, *J* = 6.8 Hz, C*H*[CH_3]$_2$), 2.57 (3H, s, CH_3Ar), 1.23 (6H, d, *J* = 6.8 Hz, ([CH_3]$_2$CH); ^{13}C NMR ($CDCl_3$): δ 192.6 (*C*=O), 165.3 (N=*C*-C), 163.8 (*C*-S), 160.4 (*C*-O), 152.4 (C-*C*H=N), 147.7 (CH=*C*H-N), 141.1 (*C*-CH_3), 134.6 (*C*-CH[CH_3]$_2$), 134.0 (C=*C*H-CH), 128.5 (*C*H=C-C=O), 126.7 (*C*-C=O), 123.9 (CH-*C*H=CH), 120.2 (*C*-CH=N), 114.2 (*C*H=C-O), 55.6 (*C*H_3O), 43.9 (*C*H_2S), 26.9 (*C*H[CH_3]$_2$), 22.7 (*C*H_3Ar), 22.6 ([*C*H_3]$_2$CH).

HRMS (ESI): *m/z* 384.1373 [M+H]$^+$; calcd. for $C_{20}H_{22}N_3O_3S$, 384.1376.

1-(5-isopropyl-4-methoxy-2-methylphenyl)-2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)ethan-1-one ***(12)***

White solid (51%); M.p. 136-118 °C; IR (ATR), cm^{-1}: 1661 (C=O).

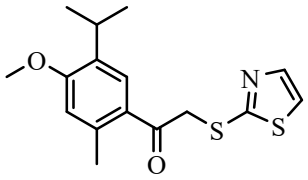


^{1}H NMR ($CDCl_3$): δ 8.79 (2H, d, *J* = 4.8 Hz, N-C*H*=CH), 7.85 (2H, d, *J* = 4.8 Hz, N-CH=C*H*), 7.70 (1H, s, C*H*=C-C=O), 6.71 (1H, s, C*H*=C-O), 4.97 (2H, s, CH_2S), 3.89 (3H, s, CH_3O), 3.28 (1H, septet, *J* = 6.8 Hz, C*H*[CH_3]$_2$), 2.58 (3H, s, CH_3Ar), 1.24 (6H, d, *J* = 6.8 Hz, ([CH_3]$_2$CH); ^{13}C NMR ($CDCl_3$): δ 192.5 (*C*=O), 166.0 (N=*C*-C), 164.1 (*C*-S), 160.4 (*C*-O), 151.0 (N-*C*H=CH), 141.1 (*C*-CH_3), 134.7 (*C*-CH[CH_3]$_2$), 130.7 (*C*-C=N), 128.5 (*C*H=C-C=O), 126.6 (*C*-C=O), 120.2 (N=CH-*C*H), 114.2 (*C*H=C-O), 55.6 (*C*H_3O), 43.9 (*C*H_2S), 26.9 (*C*H[CH_3]$_2$), 22.7 (*C*H_3Ar), 22.6 ([*C*H_3]$_2$CH).

HRMS (ESI): *m/z* 384.1379 [M+H]$^+$; calcd. for $C_{20}H_{22}N_3O_3S$, 384.1376.

1-(5-isopropyl-4-methoxy-2-methylphenyl)-2-(thiazol-2-ylthio)ethan-1-one ***(13)***

Yellow solid (91%); M.p. 110-112 °C; IR (ATR), cm^{-1}: 1668 (C=O).

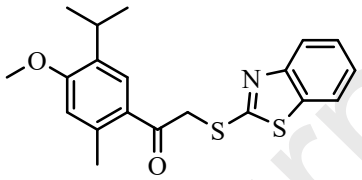


^{1}H NMR ($CDCl_3$): δ 7.67 (1H, s, C*H*=C-C=O), 7.63 (1H, d, *J* = 3.2 Hz, CH=C*H*-N), 7.19 (1H, d, *J* = 3.2 Hz, CH=C*H*-S), 6.68 (1H, s, C*H*=C-O), 4.72 (2H, s, CH_2S), 3.86 (3H, s, CH_3O), 3.26 (1H, septet, *J* = 7.2 Hz, C*H*[CH_3]$_2$), 2.53 (3H, s, CH_3Ar), 1.21 (6H, d, *J* = 7.2 Hz, ([CH_3]$_2$CH); ^{13}C NMR ($CDCl_3$): δ 194.5 (*C*=O), 163.7 (*C*-S), 159.8 (*C*-O), 142.6 (CH=*C*H-N), 140.4 (*C*-CH_3), 134.2 (*C*-CH[CH_3]$_2$), 128.3 (*C*H=C-C=O), 127.7 (*C*-C=O), 119.3 (CH=*C*H-S), 114.0 (*C*H=C-O), 55.5 (*C*H_3O), 43.6 (*C*H_2S), 26.8 (*C*H[CH_3]$_2$), 22.6 ([*C*H_3]$_2$CH), 22.3 (*C*H_3Ar).

HRMS (ESI): *m/z* 322.0937 [M+H]$^+$; calcd. for $C_{16}H_{20}NO_2S_2$, 322.0935.

2-(benzo[d]thiazol-2-ylthio)-1-(5-isopropyl-4-methoxy-2-methylphenyl)ethan-1-one ***(14)***

White solid (96%); 129-130 °C; IR (ATR), cm^{-1}: 1661 (C=O).

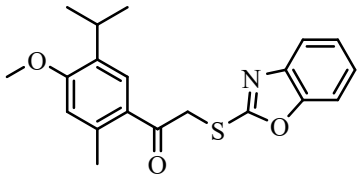


^{1}H NMR ($CDCl_3$): δ 7.82 (1H, d, *J* = 8.0 Hz, C*H*=C-N), 7.75 (1H, s, C*H*=C-C=O), 7.74 (1H, d, *J* = 8.0 Hz, C*H*=C-S), 7.39 (1H, t, *J* = 8.0 Hz, C*H*-CH=C-N), 7.28 (1H, t, *J* = 8.0, C*H*-CH=C-S), 6.70 (1H, s, C*H*=C-O), 4.86 (2H, s, CH_2S), 3.87 (3H, s, CH_3O), 3.27 (1H, septet, *J* = 6.8 Hz, C*H*[CH_3]$_2$), 2.55 (3H, s, CH_3Ar), 1.20 (6H, d, *J* = 6.8 Hz, ([CH_3]$_2$CH); ^{13}C NMR ($CDCl_3$): δ 194.3 (*C*=O), 165.8 (*C*-S), 159.8 (*C*-O), 153.0 (*C*-N), 140.4 (*C*-CH_3), 135.6 (*C*-S), 134.3 (*C*-CH[CH_3]$_2$), 128.3 (*C*H=C-C=O), 127.8 (*C*-C=O), 126.1 (*C*H-CH=C-N), 124.4 (*C*H-CH=C-S), 121.6 (*C*H=C-N), 121.1 (*C*H=C-S), 114.0 (*C*H=C-O), 55.5 (*C*H_3O), 42.8 (*C*H_2S), 26.8 (*C*H[CH_3]$_2$), 22.6 ([*C*H_3]$_2$CH), 22.3 (*C*H_3Ar).

HRMS (ESI): *m/z* 372.1967 [M+H]$^+$; calcd. for $C_{20}H_{22}NO_2S_2$, 372.1086

2-(benzo[d]oxazol-2-ylthio)-1-(5-isopropyl-4-methoxy-2-methylphenyl)ethan-1-one ***(15)***

Off-white solid (72%); M.p. 135-138 °C; IR (ATR), cm^{-1}: 1660 (C=O).

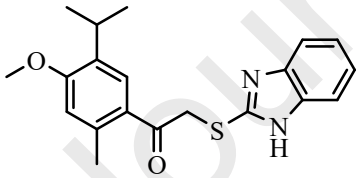


^{1}H NMR ($CDCl_3$): δ 7.77 (1H, s, C*H*=C-C=O), 7.57 (1H, d, *J* = 8.0 Hz, C*H*=C-N), 7.43 (1H, d, *J* = 8.0 Hz, C*H*=C-O), 7.27 (1H, t, *J* = 8.0 Hz, C*H*-CH=C-N), 7.25 (1H, t, *J* = 8.0, C*H*-CH=C-O), 6.70 (1H, s, C*H*=C-O), 4.86 (2H, s, CH_2S), 3.88 (3H, s, CH_3O), 3.28 (1H, septet, *J* = 6.8 Hz, C*H*[CH_3]$_2$), 2.56 (3H, s, CH_3Ar), 1.24 (6H, d, *J* = 6.8 Hz, ([CH_3]$_2$CH); ^{13}C NMR ($CDCl_3$): δ 193.7 (*C*=O), 164.5 (*C*-S), 160.1 (*C*-O), 152.1 (*C*-O-C=N), 141.9 (*C*-N), 140.7 (*C*-CH_3), 134.4 (*C*-CH[CH_3]$_2$), 128.5 (*C*H=C-C=O), 127.4 (*C*-C=O), 124.4 (*C*H-CH=C-N), 124.0 (*C*H-CH=C-O), 118.5 (*C*H=C-N), 114.1 (*C*H=C-O), 110.1 (*C*H=C-O-C=N), 55.6 (*C*H_3O), 42.8 (*C*H_2S), 26.9 (*C*H[CH_3]$_2$), 22.6 ([*C*H_3]$_2$CH), 22.4 (*C*H_3Ar).

HRMS (ESI): *m/z* 356.1326 [M+H] $^+$; calcd. for $C_{20}H_{22}NO_3S$, 356.1314.

2-((1H-benzo[d]imidazol-2-yl)thio)-1-(5-isopropyl-4-methoxy-2-methylphenyl)ethan-1-one ***(16)***

Yellow solid (60%); M.p. 158 °C (decomposed); IR (ATR), cm^{-1}: 1655 (C=O).

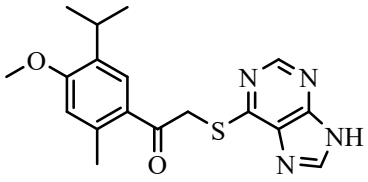


^{1}H NMR (DMSO-d6): δ 7.82 (1H, s, C*H*=C-C=O), 7.38 (2H, dd, *J* = 6.0, 3.2 Hz, C*H*=C-N), 7.08 (2H, dd, *J* = 6.0, 3.2 Hz, C*H*-CH=C-N), 6.86 (1H, s, C*H*=C-O), 4.85 (2H, s, CH_2S), 3.82 (3H, s, CH_3O), 3.18 (1H, septet, *J* = 6.8 Hz, C*H*[CH_3]$_2$), 2.37 (3H, s, CH_3Ar), 1.13 (6H, d, *J* = 6.8 Hz, ([CH_3]$_2$CH); ^{13}C NMR (DMSO-d6): δ 195.9 (*C*=O), 159.4 (*C*-O), 150.3 (*C*-S), 139.1 (*C*-CH_3), 133.6 (*C*-CH[CH_3]$_2$), 128.8 (*C*-C=O), 128.5 (*C*H=C-C=O), 122.0 (broad, *imidazole aromatic*), 114.5 (*C*H=C-O), 56.2 (*C*H_3O), 41.5 (*C*H_2S), 26.6 (*C*H[CH_3]$_2$), 22.9 ([*C*H_3]$_2$CH), 21.8 (*C*H_3Ar).

HRMS (ESI): *m/z* 355.1478 $[M+H]^+$; calcd. for $C_{20}H_{23}N_2O_2S$, 355.1474

2-((9H-purin-6-yl)thio)-1-(5-isopropyl-4-methoxy-2-methylphenyl)ethan-1-one ***(17)***

Pale yellow solid (45%); M.p: 181-184 °C; IR (ATR): 1661 (C=O ketone),



^{1}H NMR (DMSO-d6): δ 8.55 (1H, s, N-C*H*=N), 8.41 (1H, s, NH-C*H*=N), 7.81 (1H, s, C*H*=C-C=O), 6.86 (1H, s, C*H*=C-O), 4.84 (2H, s, CH_2S), 3.82 (3H, s, CH_3O), 3.19 (1H, septet, *J* = 6.8 Hz, C*H*[CH_3]$_2$), 2.36 (3H, s, CH_3Ar), 1.12 (6H, d, *J* = 6.8 Hz, ([CH_3]$_2$CH); ^{13}C NMR (DMSO-d6): δ 195.9 (*C*=O), 159.2 (*C*-O), 158.4 (*C*-S), 151.7 (N-*C*H=N), 149.8 (N-*C*-NH), 143.6 (NH-*C*H=N), 138.8 (*C*-CH_3), 133.6 (*C*-CH[CH_3]$_2$), 130.7 (*C*-C-S), 129.2 (*C*H=C-C=O), 128.1 (*C*-C=O), 114.4 (*C*H=C-O), 56.2 (*C*H_3O), 38.3 (*C*H_2S), 26.6 (*C*H[CH_3]$_2$), 22.9 ([*C*H_3]$_2$CH), 21.6 (*C*H_3Ar).

HRMS (ESI): *m/z* 357.1387 $[M+H]^+$; calcd. for $C_{18}H_{21}N_4O_2S$, 357.1385

4.3 CUPRAC assay [16,18]

Test solutions for the CUPRAC assay were prepared as follows: 6 tubes each containing 1 mL each of MilliQ H_2O, 7.5 mM neocuproine in absolute ethanol, 1 M aqueous NH_4Ac in and 10 mM aqueous $CuCl_2$ solutions. Additional reagents were placed in the tubes as follows, using 5 mM stock solutions of test samples (TS), prepared in 95% ethanol or 85:15 ethanol:DMSO: Tube 1, Blank (100 µL H_2O); Tube 2 (10 µL TS, 90 µL H_2O); Tube 3 (20 µL TS, 80 µL H_2O); Tube 4 (30 µL TS, 70 µL H_2O); Tube 5 (40 µL TS, 60 µL H_2O); Tube 6 (50 µL TS, 50 µL H_2O) to give final concentrations of test samples ranging from 12.2-60.9 µM. The samples were vortexed for 30 seconds, and allowed to sit at room temperature for 30 minutes. During this period, the blue green color of the test solution was changed to various shades of yellow and orange, depending on the reducing power of each

compound. trolox, ascorbic acid, and carvacrol were used as positive controls. The measurements were performed in triplicate for each concentration, and average absorbance values were used to generate the calibration plots. The Trolox Equivalent Antioxidant Capacity (TEAC) coefficient for this assay was determined by relating the molar absorptivity, ε (obtained from the slopes of the calibration plots), of the test samples to that of trolox as follows: ε Test samples/ε Trolox.

*Note: For the benzoxazole derivative (**15**), the calibration plot was linear up to 36.6 µM (30 µL TS), thus the data from tubes 5 (40 µL TS), and 6 (50 µL TS) was not used in determining the slope.*

4.4 Tyrosinase docking studies

To determine whether the heterocyclic sulfide derivatives possessed tyrosinase inhibitory activity, compounds **4-17**, thymol and kojic acid were docked onto mushroom tyrosinase (PDB ID: 2Y9X) using Autodock Vina. To prepare tyrosinase 2Y9X for docking, all water molecules and tropolone ligands were removed, and the protein was saved as a rigid .pdbqt file. This .pdbqt file was opened in a text editor to manually change the charge of each copper atom to 2.00. The binding site was centered around the copper-containing active site of the B chain, encompassing a volume 15 Å × 15 Å × 15 Å. All ligands were sketched in Avogadro, and their geometries were optimized using a steepest-descent algorithm. The ligand files were saved as .pdb before torsions were selected in Python Molecule Viewer and exported as .pdbqt files. The prepared ligands were docked to tyrosinase over three consecutive runs, using an exhaustiveness value of 8, and the most stable conformation was recorded (Table 1). The lowest-energy docking poses were visually inspected for interactions between the active-site copper atoms and ligand sulfur.

Declaration of Competing Interests

The authors declare no competing conflicts of interest.

Acknowledgments

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Ressler: Investigation, Writing, Editing

Parks: Investigation, Editing

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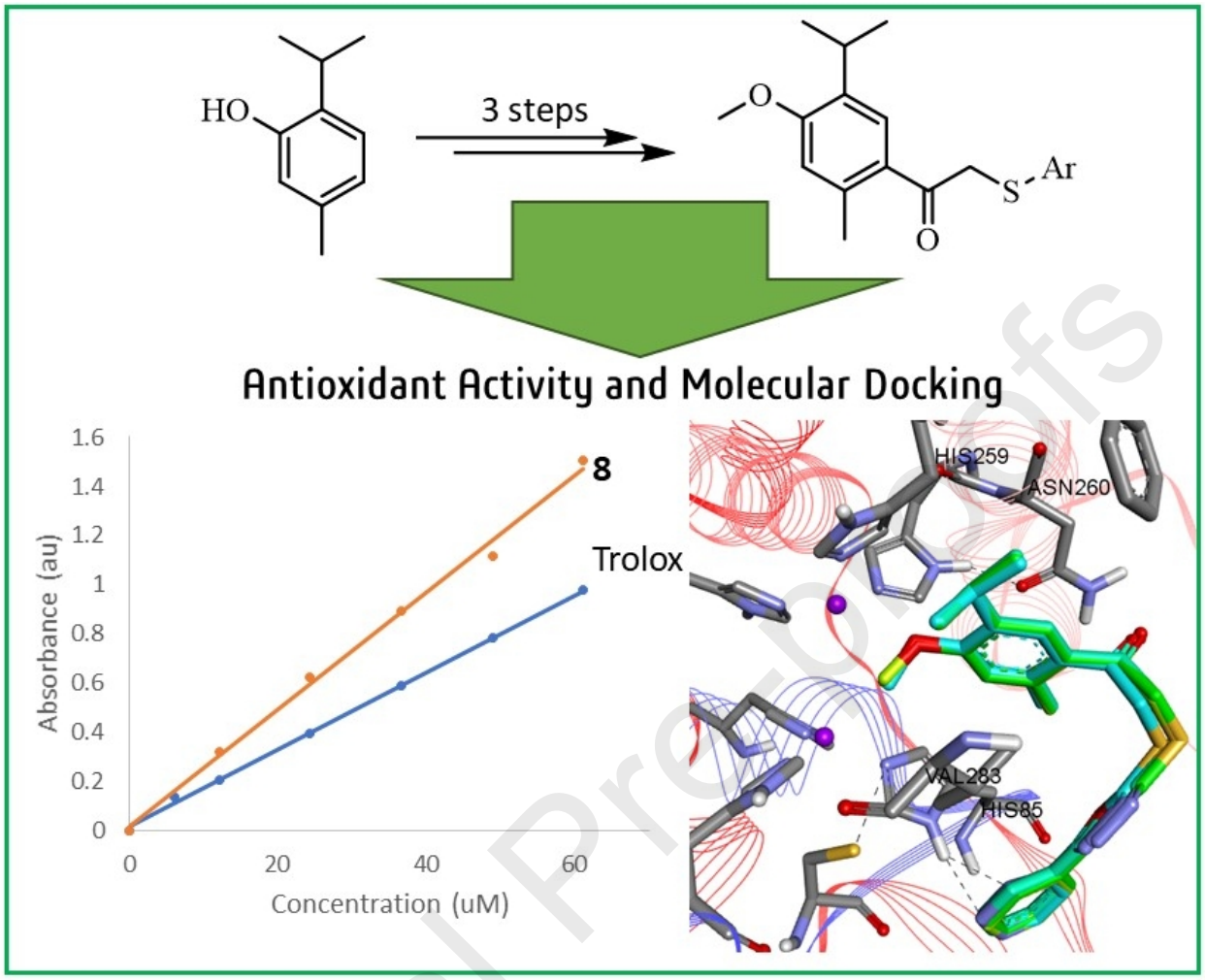
Seeram: Investigation, Funding Acquisition, Editing

Declaration of Competing Interests

The authors declare no competing conflicts of interest.



Highlights

- Fourteen heterocyclic sulfide derivatives containing a thymol moiety were synthesized and characterized by spectroscopic methods.
- The cupric ion reducing antioxidant capacity of the compounds was examined, and more than half of the compounds showed better or comparable reducing capacity to Trolox.
- Docking studies of the derivatives in the mushroom tyrosinase active site revealed superior binding affinity than kojic acid, a known tyrosinase inhibitor.