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Microwave-assisted synthesis and biological evaluation of new thiazolylhydrazone derivatives as tyrosinase inhibitors and antioxidants

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Abstract

In this work, we have synthesized a series of 2-thiazolylhydrazone derivatives (**1–27**) and investigated their biological activities as tyrosinase inhibitors and antioxidants. Some compounds showed potent tyrosinase inhibitory activities and 4-(2-(2-(1-(4-Aminophenyl)ethylidene)-hydrazinyl)thiazol-4-yl) phenol (**26**) showed more potent inhibitory effect than the standard tyrosinase inhibitor kojic acid (IC_{50}: 9.8 μM vs. 23.6 μM). Compounds **2**, **14**, and **26** exhibited high antioxidant activities in 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. The structure–activity relationship (SAR) indicated that the substitutions of bromine, hydroxyl group, and amino groups cause great effect to the inhibition effect against tyrosinase. The mechanism and kinetic studies demonstrated that the inhibitory effect of compound **26** on the tyrosinase by acting as the reversible and uncompetitive inhibitor. Docking studies suggests that compound **26** interacts strongly with mushroom tyrosinase via hydrogen bonding.

1 | INTRODUCTION

Tyrosinase (monophenol or *o*-diphenol, oxygen oxidoreductase, EC 1.14.18.1, syn. Polyphenol oxidase) is a multifunctional copper containing oxidase, which is widely distributed in mammals, plants, bacteria, and fungi.[1,2] Tyrosinase enables to catalyze the hydroxylation of monophenols to *o*-diphenols (monophenolase activity) and the oxidation of *o*-diphenols to *o*-quinone (diphenolase activity).[3,4] The dopaquinone could transform to melanin pigment in melanin biosynthesis pathway,[5] which can block ultraviolent light rays and protect skin cells from harmful ultraviolent light radiation.[6,7] However, overproduction and accumulation of melanin can cause a number of skin diseases, such as melasma, freckles, ephelide, and solar lentigines.[8] Tyrosinase has been identified to be able to oxidize dopamine to form melanin in the brain. Excessive production of dopaquinone by oxidation of dopamine results in neuronal damage and cell death, and thus tyrosinase is implicated in the pathogenesis of Parkinson's disease and other neurodegenerative disease processes.[9,10] The browning of fruits, vegetables, and beverages poses serious threat to agro-economic countries. Tyrosinase is one of the main factors for most fruits and vegetables quality loss during post-harvest handling and processing, leading to fast degradation and the shortening of storage life.[11] Thus, how to prevent this unfavorable browning reaction remains challenge in food science. Tyrosinase is also associated with three biochemical processes of insects, including sclerotization of cuticle, defensive encapsulation and melanization of foreign organism and wound healing.[12,13] These processes provide potential targets for developing safe and effective tyrosinase inhibitors as insecticides. These phenomena encourage researchers to develop new tyrosinase inhibitors using in medicinal, agricultural, food sciences, and cosmetic industries.[14,15] By far, some natural and synthetic tyrosinase inhibitors have been reported, for example, hydroquinone, ascorbic acid,

arbutin, kojic acid, aromatic aldehydes, aromatic acids, aromatic alcohols, tropolone, and polyphenols.[16–19] However, only few compounds such as kojic acid, arbutin, tropolone, and 1-phenyl-2-thiourea (PTU) have been used as cosmetic products and therapeutic agents.[20,21] Moreover, it has been reported that arbutin decomposes at room temperature (10% decomposition at 20°C for 15 days).[22] Hydroquinone, a widely used skin-lightening agent, is still controversial with respect to its biosafety owing to its cytotoxic and mitogenic properties.[23] Kojic acid as the commonly used tyrosinase inhibitor has been banned in many countries due to its serious side effects and unstable property.[23] Hence, there is still an urgent need for developing novel tyrosinase inhibitors with high activities and low side effects. Klabunde et al. reported that phenyl thioureas, thiazole ring, and alkyl thioureas exhibited weak to moderate inhibition of tyrosinase activity.[24] Similarly, thiosemicarbazide derivatives and curcumin showed potential ability to coordinate the two copper ions in the active site of tyrosinase.[25,26] Lately, our group described that thiosemicarbazide derivatives exhibited potent inhibitory activities against mushroom tyrosinase and the lead compounds could potentially bind to the binuclear active site of tyrosinase to inhibit its activity.[27,28] In addition, we found that 1,3,4-Thiadiazole-2(3H)-thiones and 1,3,4-thiadiazole Schiff base derivatives performed potent inhibitory activities against mushroom tyrosinase.[29,30] Inspired by the literatures and our previous work, here we rationally design and synthesize novel tyrosinase inhibitors.

Nitrogen heterocyclic compounds are an important class in medicinal chemistry. Thiazoles and their derivatives have attracted continuing interest over the years because of the compounds with thiazole structures impose a broad spectrum of biological activities such as anticonvulsant, antiinflammatory, analgesic, antituberculosis, antiviral, pesticidal, antimicrobial, anticancer, antitumor, and enzyme inhibition activities.[31,32] This heterocyclic system has broad application in drug development for the treatment of allergies, hypertension, inflammation, schizophrenia, bacterial, HIV infections, hypnotics, and pain.[33] Hydrazinylthiazoles are an important class of compounds of profound interest to medicinal and industrial chemists because compounds bearing the hydrazinylthiazolyl moieties exhibit diverse biological properties such as antioxidant, antitubercular, adenosine receptor antagonist, xanthine oxidase inhibitors, antimicrobial, anti-inflammatory, and antiplasmodial activity.[34–36] To the best of our knowledge, the tyrosinase inhibitors with hydrazinylthiazolyl moiety have never been reported. In this work, we are going to design and synthesize some hydrazone derivatives bearing thiazole and phenyl moieties, and evaluate their inhibitory activities against mushroom tyrosinase and antioxidative performances. We further analyze the structure–activity relationships (SARs) of as-synthesized compounds and tyrosinase by enzymatic kinetic assays and docking calculations.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

As shown in Scheme 1, a series of 2-thiazolylhydrazone derivatives (**1–27**) have been synthesized using the general procedure. A mixture of different aromatic aldehyde or aromatic ketone with thiosemicarbazide in ethanol was refluxed for 24 h catalyzed by acetic acid under microwave irradiation, affording the corresponding thiosemicarbazone compounds after recrystallization. Under microwave irradiation, the 2-thiazolylhydrazone derivatives were obtained using equimolar of the as-prepared thiosemicarbazone and α-bromo acetophenones in moderate to high yields. The microwave assisted organic syntheses (MAOS) shows highly yielding and eco-friendly. All the target compounds were characterized by chemical and spectral methods.

2.2 | Tyrosinase inhibitory activity assay

According to the reported protocol,[30] the compounds **1–27** were subjected to tyrosinase inhibition assay with L-DOPA as substrate. The tyrosinase inhibitory activity of kojic acid was selected as a reference substance. The IC_{50} values of the thiazolylhydrazone derivatives against tyrosinase were summarized in Table 1.

Most of the 2-thiazolylhydrazone derivatives displayed potent inhibition activities against mushroom tyrosinase with IC_{50} values ranged from 9.80 to 98.16 μM. Compared to the reference inhibitor kojic acid, compound **3** showed a comparable tyrosinase inhibitory activity. Particularly, compound **26** exhibited the most potent tyrosinase inhibitory activity with an IC_{50} value of 9.80 μM, that performs better than the reference inhibitor kojic acid. From Table 1, the results suggested that the substitution pattern of the benzene ring and substitution position on the benzene ring of 2-thiazolylhydrazone derivatives cause significant influence to the tyrosinase inhibitory activity. Considering the tyrosinase inhibitory activities of compounds **1–12** originating from aromatic aldehydes, compound **3** exhibited better inhibitory activity than compounds **1**, **2**, and **4**. The similar phenomena were observed for compound **7** versus compounds **5**, **6**, **8**, and compound **11** versus compounds **9**, **10**, **12**. These results

indicate that the type of substitution group (R^3) at the benzene ring might modulate the activities and bromine atom (R^3) might be an important active group to target the enzyme active site or amino acid residues. Moreover, the IC_{50} value of the compound **3** was 28.08 μM that performed better inhibitory activity than compounds **7** and **11**. However, compared to compounds **1–12** bearing R^3 as hydroxyl group caused decreased inhibitory activity. This result suggests that hydroxyl group (R^1) in 2-thiazolylhydrazone derivatives cause great effect to the inhibitory activity. Therefore, there were different effects on inhibitory activities when the hydroxyl groups were introduced in different benzene ring. Hydroxyl group (R^1) might be beneficial to increasing the inhibitory activity and it could interact with the enzyme active site or amino acid residues. However, Hydroxyl group (R^3) could not interact with the enzyme active site or amino acid residues. For the compounds **13–27** from aromatic ketones, the corresponding inhibitory activities were certainly enhanced once the hydroxyl group (R^1) was replaced by bromine atom (R^1). Among the compounds **16**, **20**, **24**, and **25**, compound **25** (R^1 = methyl group) exhibited the lowest potent tyrosinase inhibitory activity with IC_{50} value of more than 100 μM. These results indicate that bromine atom positively affects the tyrosinase inhibitory activity yet methyl group causes adverse influences to the tyrosinase inhibitory activities. Compound **26** (R^1 is amino group) showed more potent inhibitory activity than compounds **14**, **18**, and **22**. This data suggest that amino group might play an important role to the inhibitory owing the lone pair electrons of amino group. From Table 1, we also can found that tyrosinase inhibitory activity of compound synthesized from aromatic aldehyde is better than the tyrosinase inhibitory activity of corresponding compound synthesized by aromatic ketone.

2.3 | Antioxidant activity

DPPH assays and ABTS assays were employed to examine the antioxidant activities of the 2-thiazolylhydrazone derivatives. As shown in Table 2, most of the 2-thiazolylhydrazone derivatives showed promising antioxidant activities. In DPPH assay, compared to BHT with IC_{50} of 55.35 μM, some compounds (**1**, **2**, **3**, **6**, **10**, **14**, **15**, **26**) exhibited higher antioxidant activities and compound **2** showed the best performance of antioxidant activity with IC_{50} of 12.53 μM. In ABTS assay, compounds **2**, **14**, and **26** exhibited potent activity with IC_{50} values of 1.06, 2.35, and 1.82 μM, respectively, that showed better performances than that of standard of BHT (IC_{50} = 3.12 μM). Notably, all of the compounds with potent antioxidant activities contain phenolic hydroxyl groups in phenyl ring, indicating that phenolic hydroxyl group could enhance the radical scavenging activity. However, the compound bearing NO_2 group on phenyl ring (**4**, **8**, **12**, **16**, **20**, **24**, **25**) always showed lower radical scavenging ability. Concerning the effect of the R^2 group, compounds **13–24** showed less activity compare with the corresponding compounds **1–12**, indicating that the R^2 = methyl group might exert negative effect to the antioxidant activity. Additionally, compounds with halogen atoms in phenyl ring possessed moderate radical scavenging ability compared with BHT.

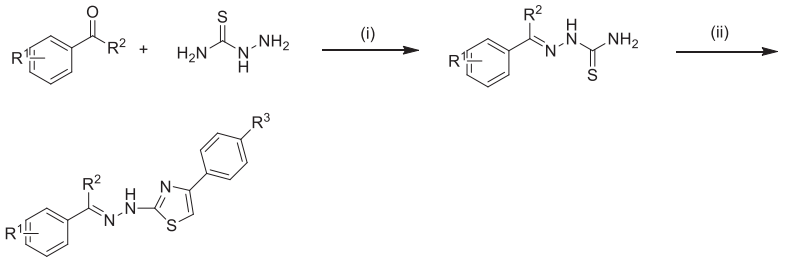
2.4 | Inhibitory mechanism on tyrosinase

The inhibition mechanism of compound **26** on mushroom tyrosinase for the oxidation of L-DOPA was explored. Figure **1** shows the relationship between compound **26** at different concentrations and the corresponding enzymatic activities. With compound **26** at any concentration, the plots showed linear profiles. With increasing the concentration of compound **26**, the decreased slope was obtained, indicating that the inhibition of compound **26** on mushroom tyrosinase was reversible.

To further get insight into the inhibition mechanism, the inhibitory type of the compound **26** on mushroom tyrosinase for the oxidation of L-DOPA was fitted to the Line Weaver–Burk double reciprocal plots. Figure **2** shows the double-reciprocal plots of the enzymatic kinetics of mushroom tyrosinase using compound **26**. The result displayed that the plots of 1/V versus 1/[S] gave a family of parallel straight lines with the same slopes. Meanwhile, As the concentration of compound increasing, the values of both Km and Vm decreased, but the ratio of Km/Vm remained unchanged. This result indicated that compound **26** was the uncompetitive tyrosinase inhibitor.

2.5 | In silico docking between tyrosinase and the compound 26

The binding affinities of the inhibitor to the receptor tyrosinase were calculated using computational docking studies. The binding energy between compound **26** and tyrosinase was −5.06 kcal/mol, suggesting that compound **26** was bound tightly to tyrosinase. As shown in Figure 3, amino group of compound **26** formed hydrogen bonds with Ala246 (1.87 Å) and Glu322 (2.91 Å). Nitrogen atom of thiazole ring formed hydrogen bond with Asn260 with a distance of 3.01 Å, and the nitrogen atom of hydrazone formed hydrogen bond with His244 with a distance of 2.43 Å. Thiazole ring exhibited a Pi–S



SCHEME 1 Synthesis routes of 2-thiazolylhydrazone derivatives. Reagents and conditions: (i) thiosemicarbazide, ethanol, reflux, or microwave; (ii) substituted α-bromo acetophenone, 2-propanol, r.t, or microwave

1. R^1 = 4-OH, R^2 = H, R^3 = Cl; 2. R^1 = 4-OH, R^2 = H, R^3 = OH;
3. R^1 = 4-OH, R^2 = H, R^3 = Br; 4. R^1 = 4-OH, R^2 = H, R^3 = NO_2;
5. R^1 = 3-Cl, R^2 = H, R^3 = Cl; 6. R^1 = 3-Cl, R^2 = H, R^3 = OH;
7. R^1 = 3-Cl, R^2 = H, R^3 = Br; 8. R^1 = 3-Cl, R^2 = H, R^3 = NO_2;
9. R^1 = 4-Br, R^2 = H, R^3 = Cl; 10. R^1 = 4-Br, R^2 = H, R^3 = OH;
11. R^1 = 4-Br, R^2 = H, R^3 = Br; 12. R^1 = 4-Br, R^2 = H, R^3 = NO_2;
13. R^1 = 4-OH, R^2 = CH_3, R^3 = Cl; 14. R^1 = 4-OH, R^2 = CH_3, R^3 = OH;
15. R^1 = 4-OH, R^2 = CH_3, R^3 = Br; 16. R^1 = 4-OH, R^2 = CH_3, R^3 = NO_2;
17. R^1 = 3-Cl, R^2 = CH_3, R^3 = Cl; 18. R^1 = 3-Cl, R^2 = CH_3, R^3 = OH;
19. R^1 = 3-Cl, R^2 = CH_3, R^3 = Br; 20. R^1 = 3-Cl, R^2 = CH_3, R^3 = NO_2;
21. R^1 = 4-Br, R^2 = CH_3, R^3 = Cl; 22. R^1 = 4-Br, R^2 = CH_3, R^3 = OH;
23. R^1 = 4-Br, R^2 = CH_3, R^3 = Br; 24. R^1 = 4-Br, R^2 = CH_3, R^3 = NO_2;
25. R^1 = 4-CH_3, R^2 = CH_3, R^3 = NO_2; 26. R^1 = 4-NH_2, R^2 = CH_3, R^3 = OH;
27. R^1 = 4-NH_2, R^2 = CH_3, R^3 = NO_2

interaction with Phe264 with a distance of 5.37 Å. Benzene ring involved in Pi–Alkyl with Val283 (5.37 Å), His85 (5.49 Å), and His263 (4.93 Å), respectively. From the docking results, no interaction between inhibitor and the copper atom active center of tyrosinase was observed, indicating that a distinct inhibition type exists between tyrosinase and compound **26**.

3 | CONCLUSIONS

In summary, we have synthesized a series of 2-thiazolylhydrazone derivatives (**1–27**) for biological investigations. Some compounds show potent tyrosinase inhibitory activities and the lead compound **26** is the best tyrosinase inhibitor with an IC_{50} value of 9.80 μM. Most of the compounds exhibit promising antioxidant activities in DPPH and ABTS assays. Compound **2** is found to be the most active antioxidative compound with IC_{50} values of 12.53 μM and 1.06 μM in DPPH and ABTS assays, respectively. Structure–activity relationship (SAR) analysis indicates that (1) bromine atom (R^3) might be an important active group of this kind of compounds synthesized by aromatic aldehyde; (2) hydroxyl group (R^1) may be beneficial to increasing the inhibitory activity; (3) There are different effects on inhibitory activities when the hydroxyl groups were introduced into different benzene ring; (4) amino group (R^1) might play an important role in the increase of inhibitory activity. Moreover, the inhibition mechanism and kinetic studies reveal that the compound **26** acts as the reversible and uncompetitive inhibitor to tyrosinase by acting as the reversible and uncompetitive inhibitor. Docking studies suggest that compound **26** interact strongly with mushroom tyrosinase where amino group forms hydrogen bonds with Ala246 and Glu322 and the thiazole ring and amino acid residues interact with each other.

4 | EXPERIMENTAL

Melting points were measured on SGW-X4 melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 300 at 25°C in DMSO-d_6 using tetramethyl silane (TMS) as an internal standard. Infrared (IR) spectra were obtained on a Nicolet is 5 spectrophotometer (KBr disks). Mass (MS) spectra were reported in m/z using the LCMS-2010A. Tyrosinase, L-3,4-dhydroxyphenylalanine (L-DOPA), and kojic acid were purchased from Sigma-Aldrich Chemical Co. (Shanghai, China). Other chemicals were obtained from commercial suppliers and used without further purification.

TABLE 1 Tyrosinase inhibitory activities of the synthesized compounds

Compounds	CLog *P*[a]	IC_{50} (μM)[b]
1	5.645	40.53
2	4.461	55.15
3	5.795	28.08
4	4.686	60.83
5	6.244	98.16
6	5.065	>100
7	6.394	71.72
8	5.281	79.39
9	6.394	>100
10	5.215	92.73
11	6.544	76.56
12	5.431	>100
13	4.742	>100
14	3.562	>100
15	4.892	46.26
16	3.779	73.13
17	6.122	>100
18	4.942	>100
19	6.272	82.27
20	5.159	95.28
21	6.272	43.13
22	5.092	88.18
23	6.422	82.72
24	5.309	62.57
25	4.945	>100
26	3.002	9.80
27	3.219	>100
Kojic acid	—	23.6

[a]Value of CLog *P* was obtained by ChemBioDraw Ultra 12.0.
[b]Values were determined from logarithmic concentration–inhibition curves (at least eight points) and are given as means of three experiments.

TABLE 2 Antioxidant activities of the synthesized compounds

Compounds	DPPH IC_{50} (μM)	ABTS IC_{50} (μM)
1	52.31	6.87
2	12.53	1.06
3	45.34	6.32
4	67.89	26.91
5	91.68	78.36
6	38.16	62.18
7	97.15	58.21
8	98.65	55.28
9	94.45	89.15
10	41.78	53.10
11	95.17	46.37
12	117.25	88.49
13	58.06	16.26
14	19.17	2.35
15	50.19	34.67
16	96.55	63.14
17	126.15	128.08
18	74.52	51.33
19	123.07	99.45
20	148.65	94.75
21	138.26	95.36
22	69.13	55.76
23	>200	82.77
24	>200	152.68
25	>200	>200
26	18.93	1.82
27	58.16	64.25
BHT	55.15	3.02

4.1 | General procedure for the synthesis of 2-thiazolylhydrazone derivatives 1–27

4.1.1 | Method 1

The procedure was adapted from the literature reported.[37] To a solution of appropriate aldehyde or ketone (10 mmol) in anhydrous ethanol (20 mL), thiosemicarbazide (10 mmol), and acetic acid (0.5 mL) were added. The reaction mixture was refluxed for 24 h. The reaction progress was monitored by thin layer chromatography (TLC). After the reaction was completed, the reaction mixture was cooled to room temperature. The appearing precipitate was filtered and recrystallized from 95% alcohol to obtain the corresponding thiosemicarbazone compounds.

Equimolar of the as-prepared thiosemicarbazone (10 mmol) and substituted α-bromo acetophenone (10 mmol) were dissolved in 2-propanol (20 mL). The mixture was stirred at room temperature until the thiosemicarbazone was disappeared on TLC. The precipitate was filtered and dried to give the compounds **1–27** in moderate to high yields.

4.1.2 | Method 2

To a solution of substituted acetophenone (1.0 mmol) or benzaldehyde (1.0 mmol) in anhydrous ethanol (10 mL), thiosemicarbazide (1.0 mmol), and acetic acid (catalytic

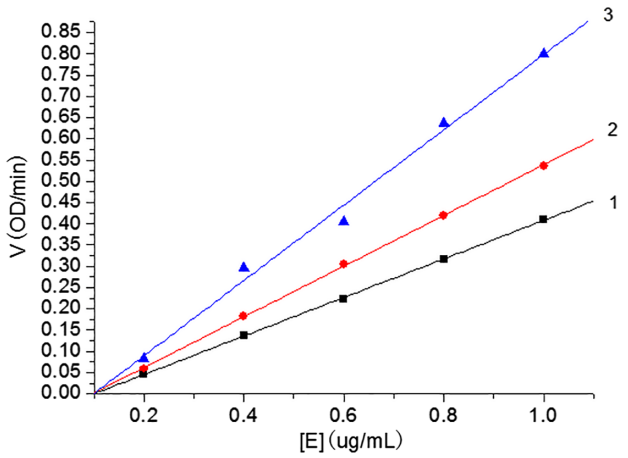


FIGURE 1 Determination of the inhibitory effect of compound **26** on mushroom tyrosinase for the oxidation of L-DOPA. The concentrations of compound **26** for curves 1–3 were 12 μM, 8 μM, and 0 μM, respectively [Color figure can be viewed at wileyonlinelibrary.com]

amount) were added. The reaction mixture was put in a round-bottomed flask and placed in a microwave reactor under irradiation at 800 W for 5–10 min. After the reaction was completed, the reaction mixture was cooled to room temperature. The appearing precipitate was filtered and recrystallized from 95% alcohol to obtain the corresponding thiosemicarbazone compounds.

A mixture of thiosemicarbazone (1.0 mmol) and substituted α-bromo acetophenone (1.0 mmol) was added in 2-propanol (10 mL). The reaction mixture was placed in a microwave reactor and irradiated at 200 W for 8–15 min. After the reaction was finished, the reaction mixture was cooled to room temperature. The resulted precipitate was filtered and washed with ethanol. The crude product was recrystallized from 95% ethanol to afford the compounds **1–27**.

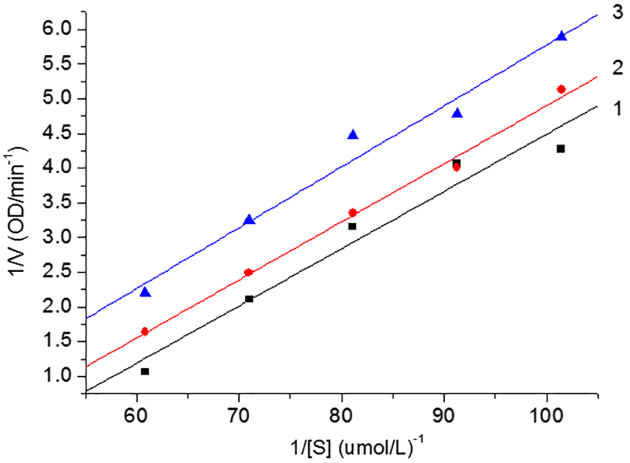
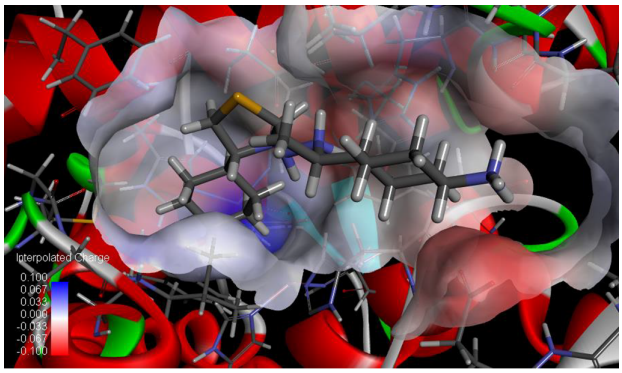


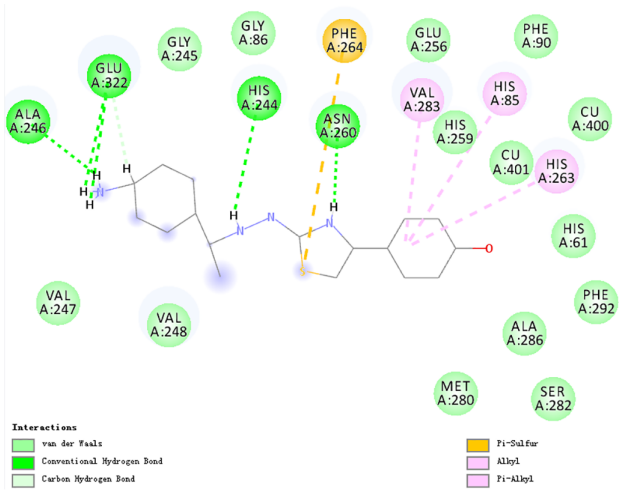
FIGURE 2 Kinetic inhibition assay of compound **26** against the oxidation of L-DOPA by tyrosinase. The mode of inhibition type exhibited through Lineweaver–Burk plot with different concentrations of compound **26**. The concentrations of compound **26** for curves 1–3 were 0 μM, 8 μM, and 12 μM, respectively [Color figure can be viewed at wileyonlinelibrary.com]

4.2 | 4-((2-(4-(4-chlorophenyl)thiazol-2-yl)hydrazono)methyl)phenol (1)

White solid; Yield: 74.49%; mp: >280°C; IR (KBr, ν/cm^{-1}) 1623, 1516, 1215, 1159, 1096. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.25 (s, 1H, =CH), 7.87 (d, J = 8.6 Hz, 2H, ph-H), 7.49–7.42 (m, 4H, ph-H), 7.36 (s, 1H, thiazol-H), 6.35 (d, J = 8.6 Hz, 2H, ph-H); ^{13}C NMR (DMSO-d_6,



A



B

FIGURE 3 A, the simulated docking model of the interactions of compound **26** with the active site of mushroom tyrosinase by AutoDock4.2. B, the schematic representation of the interactions of compound **26** in the binding pocket of mushroom tyrosinase derived from the docking model. Dashed lines represent bond distances between interacting functionalities of the ligand and receptor. The legends inset represent the type of interaction between the ligand atoms and the amino acid residues of the protein [Color figure can be viewed at wileyonlinelibrary.com]

75 MHz) δ 168.0, 160.1, 157.7, 143.6, 132.0, 128.6, 128.2, 127.3, 111.4, 107.9, 103.8, 102.4; ESI-MS, $m/z = 329$ $(M\text{-}H)^-$.

4.3 | 4-(2-(2-(4-hydroxybenzylidene) hydrazinyl)thiazol-4-yl)phenol (2)

Yellow solid; Yield: 87.76%; mp: 267.5–270.3°C; IR (KBr, ν/cm^{-1}) 3329, 1628, 1509, 1310, 1243. ^1H NMR (DMSO-d_6, 300 MHz) δ 8.30 (s, 1H, =CH), 7.65 (d, $J = 8.7$ Hz, 2H, ph-H), 7.46 (d, $J = 8.7$ Hz, 2H, ph-H), 7.03 (s, 1H, thiazol-H), 6.83 (d, $J = 8.7$ Hz, 2H, ph-H), 6.36 (d, $J = 8.7$ Hz, 2H, ph-H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 167.7, 158.0, 157.5, 143.2, 128.4, 127.2, 115.3, 111.3, 107.9, 102.4, 100.1, 99.5; ESI-MS, $m/z = 328$ $(M + NH_4)^+$.

4.4 | 4-((2-(4-(4-bromophenyl)thiazol-2-yl)hydrazono)methyl)phenol (3)[38]

Yellow solid; Yield: 89.23%; mp: 253.5–255.9°C; IR (KBr, ν/cm^{-1}) 1619, 1495, 1312, 1214. ^1H NMR (DMSO-d_6, 300 MHz) δ 8.23 (s, 1H, =CH), 7.81 (d, $J = 8.7$ Hz, 2H, ph-H), 7.62 (d, $J = 8.7$ Hz, 2H, ph-H), 7.44 (d, $J = 9.0$ Hz, 2H, ph-H), 7.37 (s, 1H, thiazol-H), 6.36 (d, $J = 8.7$ Hz, 2H, ph-H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 167.7, 160.1, 157.7, 141.6, 133.5, 131.5, 128.2, 127.6, 120.6, 111.5, 107.8, 103.7; ESI-MS, $m/z = 390$ $(M + K)^+$.

4.5 | 4-((2-(4-(4-nitrophenyl)thiazol-2-yl) hydrazono)methyl)phenol (4)

Yellow solid; Yield: 96.07%; mp: >280°C; IR (KBr, ν/cm^{-1}) 1631, 1502, 1343, 1108. ^1H NMR (DMSO-d_6, 300 MHz) δ 8.29 (s, 1H, =CH), 8.26 (d, $J = 7.8$ Hz, 2H, ph-H), 8.11 (d, $J = 7.8$ Hz, 2H, ph-H), 7.67 (s, 1H, thiazol-H), 7.45 (d, $J = 8.1$ Hz, 1H, ph-H), 6.36 (d, $J = 8.7$ Hz, 2H, ph-H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 168.4, 160.1, 157.6, 148.5, 146.1, 141.3, 128.1, 126.3, 124.1, 111.5, 107.9, 102.4; ESI-MS, $m/z = 357$ $(M + NH_4)^+$.

4.6 | 3-((2-(4-(4-chlorophenyl)thiazol-2-yl)hydrazono)methyl)phenol (5)

Yellow solid; Yield: 97.99%; mp: 191.5–194.5°C; IR (KBr, ν/cm^{-1}) 1620, 1487, 1323, 1089. ^1H NMR (DMSO-d_6, 300 MHz) δ 8.03 (s, 1H, =CH), 7.91–7.86 (m, 2H, ph-H), 7.70 (s, 1H, thiazol-H), 7.64–7.61 (m, 1H, ph-H), 7.56 (d, $J = 9.0$ Hz, 1H, ph-H), 7.49–7.44 (m, 4H, ph-H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 168.1, 149.2, 140.8, 139.7, 136.5, 133.6, 132.0, 130.7, 129.5, 128.6, 127.2, 125.5, 124.9, 104.8; ESI-MS, $m/z = 348$ $(M + H)^+$.

4.7 | 3-((2-(4-(4-hydroxyphenyl)thiazol-2-yl)hydrazono)methyl)phenol (6)

Yellow solid; Yield: 27.58%; mp: 143.9–146.8°C; IR (KBr, ν/cm^{-1}) 3213, 1624, 1513, 1362, 1276, 1215. ^1H NMR (DMSO-d_6, 300 MHz) δ 8.02 (s, 1H, =CH), 7.70–7.61 (m, 4H, ph-H), 7.46 (d, $J = 7.5$ Hz, 2H, ph-H), 7.08 (s, 1H, thiazol-H), 7.68 (d, $J = 8.7$ Hz, 2H, ph-H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 168.1, 160.2, 157.1, 148.2, 143.0, 133.6, 131.2, 130.7, 128.6, 126.9, 125.4, 124.9, 115.3, 100.9; ESI-MS, $m/z = 329$ $(M\text{-}H)^-$.

4.8 | 3-((2-(4-(4-bromophenyl)thiazol-2-yl)hydrazono)methyl)phenol (7)

White solid; Yield: 66.67%; mp: 262.8–265.5°C; IR (KBr, ν/cm^{-1}) 1623, 1484, 1108, 1071, 1005. ^1H NMR (DMSO-d_6, 300 MHz) δ 8.03 (s, 1H, =CH), 7.83 (d, $J = 8.1$ Hz, 2H, ph-H), 7.69–7.59(m, 4H, ph-H, thiazol-H), 7.47–7.42(m, 3H, ph-H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 168.1, 149.2, 140.9, 139.7, 136.5, 133.6, 131.5, 130.7, 128.9, 127.5, 125.5, 124.9, 120.6, 104.9; ESI-MS, $m/z = 392$ $(M\text{-}H)^-$.

4.9 | 3-((2-(4-(4-nitrophenyl)thiazol-2-yl) hydrazono)methyl)phenol (8)

White solid; Yield: 93.59%; mp: 265.5–266.7°C; IR (KBr, ν/cm^{-1}) 1641, 1513, 1341, 1109, 1056. ^1H NMR (DMSO-d_6, 300 MHz) δ 8.30 (d, $J = 9.0$ Hz, 2H, ph-H), 8.13 (d, $J = 9.0$ Hz, 2H, ph-H), 8.05 (s, 1H, =CH), 7.76 (s, 1H, ph-H), 7.71 (s, 1H, thiazol-H), 7.65–7.62(m, 1H, ph-H), 7.47–7.45(m, 1H, ph-H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 168.4, 148.5, 146.2, 140.5, 140.0, 136.5, 133.6, 130.7, 129.0, 126.3, 125.5, 125.0, 124.1, 108.9; ESI-MS, $m/z = 359$ $(M + H)^+$.

4.10 | 2-(2-(4-bromobenzylidene) hydrazinyl)-4-(4-chlorophenyl)thiazole (9)[39]

Yellow solid; Yield: 63.15%; mp: 197.8–201.2°C; IR (KBr, ν/cm^{-1}) 1617, 1558, 1491, 1093, 1010, 830. ^1H NMR (DMSO-d_6, 300 MHz) δ 8.22 (s, 1H, =CH), 7.82 (d, $J = 8.4$ Hz, 2H, ph-H), 7.71 (d, $J = 8.4$ Hz, 2H, ph-H), 7.55–7.52 (m, 4H, ph-H), 7.31 (s, 1H, thiazol-H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 170.1, 153.6, 143.6, 134.8,

132.4, 131.1, 129.8, 128.9, 128.2, 127.3, 125.6, 103.4; ESI-MS, $m/z = 394$ $(M + H)^+$.

4.11 | 4-(2-(2-(4-bromobenzylidene) hydrazinyl)thiazol-4-yl)phenol (10)[40]

Yellow solid; Yield: 55.89%; mp: 224.6–227.1°C; IR (KBr, ν/cm^{-1}) 3326, 1609, 1514, 1275, 1233, 1121, 832. ^1H NMR (DMSO-d_6, 300 MHz) δ 7.85 (d, $J = 8.7$ Hz, 2H, ph-H), 7.71 (d, $J = 8.7$ Hz, 2H, ph-H), 7.58 (d, $J = 8.7$ Hz, 2H, ph-H), 7.46 (d, $J = 8.7$ Hz, 2H, ph-H), 7.36 (s, 1H, thiazol-H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 164.7, 158.1, 154.7, 134.2, 133.3, 131.8, 129.7, 128.6, 128.2, 127.2, 122.7, 105.3; ESI-MS, $m/z = 373$ $(M-H)^-$.

4.12 | 2-(2-(4-bromobenzylidene) hydrazinyl)-4-(4-bromophenyl)thiazole (11)[39]

Yellow solid; Yield: 63.82%; mp: 227.6–229.1°C; IR (KBr, ν/cm^{-1}) 1603, 1508, 1488, 1207, 1096, 1007, 829. ^1H NMR (DMSO-d_6, 300 MHz) δ 8.23 (s, 1H, =CH), 7.81 (d, $J = 8.7$ Hz, 2H, ph-H), 7.72 (d, $J = 8.7$ Hz, 2H, ph-H), 7.64 (d, $J = 8.7$ Hz, 2H, ph-H), 7.52 (d, $J = 8.7$ Hz, 2H, ph-H), 7.37 (s, 1H, thiazol-H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 167.7, 160.1, 157.7, 141.6, 133.5, 131.5, 128.2, 127.6, 120.6, 111.5, 107.8, 103.7; ESI-MS, $m/z = 438$ $(M + H)^+$.

4.13 | 2-(2-(4-bromobenzylidene) hydrazinyl)-4-(4-nitrophenyl)thiazole (12)

Yellow solid; Yield: 73.91%; mp: 261.5–263.2°C; IR (KBr, ν/cm^{-1}) 1624, 1594, 1507, 1339, 1139, 1070, 1049, 829. ^1H NMR (DMSO-d_6, 300 MHz) δ 8.32 (s, 1H, =CH), 8.15 (d, $J = 7.8$ Hz, 2H, ph-H), 8.06 (d, $J = 8.1$ Hz, 2H, ph-H), 7.72 (d, $J = 8.1$ Hz, 2H, ph-H),7.67 (s, 1H, thiazol-H), 7.58 (d, $J = 8.1$ Hz, 2H, ph-H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 169.8, 161.1, 156.6, 143.3, 139.1, 132.7, 131.7, 128.5, 126.2, 125.4, 124.4, 105.1; ESI-MS, $m/z = 421$ $(M + NH_4)^+$.

4.14 | 4-(1-(2-(4-(4-chlorophenyl)thiazol-2-yl)hydrazono)ethyl)phenol (13)[41]

White solid; Yield: 86.08%; mp: 263.4–265.2°C; IR (KBr, ν/cm^{-1}) 3386, 1617, 1490, 1196, 1171.^1H NMR (DMSO-d_6, 300 MHz) δ 7.90 (d, $J = 8.4$ Hz, 2H, ph-H), 7.64 (d, $J = 7.2$ Hz, 2H, ph-H), 7.48 (d, $J = 8.4$ Hz, 2H, ph-H), 7.37 (s, 1H, thiazol-H), 6.81 (d, $J = 7.2$ Hz, 2H, ph-H), 2.26 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 170.0, 158.3, 147.2, 143.1, 134.5, 131.9, 129.3, 128.6, 128.3, 127.2, 115.3, 104.6, 14.0; ESI-MS, $m/z = 342$ $(M-H)^-$.

4.15 | 4-(2-(2-(1-(4-hydroxyphenyl) ethylidene)hydrazinyl)thiazol-4-yl)phenol (14)

White solid; Yield 71.38%; mp: 239.5–240.7°C; IR (KBr, ν/cm^{-1}) 3327, 3128, 1621, 1523, 1269, 1242. ^1H NMR (DMSO-d_6, 300 MHz) δ 7.84 (d, $J = 8.4$ Hz, 2H, ph-H), 7.66 (d, $J = 7.2$ Hz, 2H, ph-H), 7.60 (d, $J = 8.4$ Hz, 2H, ph-H), 7.39 (s, 1H, thiazol-H), 6.83 (d, $J = 7.2$ Hz, 2H, ph-H), 2.28 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 170.1, 158.8, 147.2, 142.0, 132.7, 131.0, 129.2, 127.9, 127.4, 117.8, 115.4, 106.4, 14.1; ESI-MS, $m/z = 324$ $(M-H)^-$.

4.16 | 4-(1-(2-(4-(4-bromophenyl)thiazol-2-yl)hydrazono)ethyl)phenol (15)

White solid; Yield 96.13%; mp: 283.2–285.6°C; IR (KBr, ν/cm^{-1}) 1610, 1571, 1279, 1169. ^1H NMR (DMSO-d_6, 300 MHz) δ 7.84 (d, $J = 8.4$ Hz, 2H, ph-H), 7.70 (d, $J = 7.2$ Hz, 2H, ph-H), 7.51 (d, $J = 8.4$ Hz, 2H, ph-H), 7.08 (s, 1H, thiazol-H), 6.85 (d, $J = 7.2$ Hz, 2H, ph-H), 2.28 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 170.2, 158.5, 147.6, 143.8,133.4, 131.3, 129.3, 128.4, 128.3, 127.2, 115.1, 111.3, 14.0; ESI-MS, $m/z = 387$ $(M-H)^-$.

4.17 | 4-(1-(2-(4-(4-nitrophenyl)thiazol-2-yl)hydrazono)ethyl)phenol (16)

Yellow solid; Yield 88.71%; mp: 263.4–265.8°C; IR (KBr, ν/cm^{-1}) 2987, 1617, 1541, 1345, 1190. ^1H NMR (DMSO-d_6, 300 MHz) δ 8.30 (d, $J = 9.0$ Hz, 2H, ph-H), 8.15 (d, $J = 9.0$ Hz, 2H, ph-H), 7.71 (s, 1H, thiazol-H), 7.65 (d, $J = 8.7$ Hz, 2H, ph-H), 6.83 (d, $J = 8.7$ Hz, 2H, ph-H), 2.28 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 170.3, 158.3, 147.5, 146.1,140.8, 131.3, 128.6, 127.2, 126.3, 124.1, 115.2, 108.7, 14.0; ESI-MS, $m/z = 355$ $(M + H)^+$.

4.18 | 4-(4-chlorophenyl)-2-(2-(1-(3-chlorophenyl)ethylidene) hydrazinyl)thiazole (17)

Yellow solid; Yield 72.65%; mp: 193.1–194.9°C; IR (KBr, ν/cm^{-1}) 1607, 1488, 1195, 1124, 1090, 828. ^1H NMR (DMSO-d_6, 300 MHz) δ 7.97 (d, $J = 7.5$ Hz, 2H, ph-H), 7.91 (s, 1H, ph-H), 7.36–7.69 (m, 5H, ph-H), 7.05 (s, 1H, thiazol-H), 2.60 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 163.9,

154.2, 149.6, 138.5, 133.6, 133.3, 132.8, 130.7, 129.1, 127.7, 126.8, 125.9, 116.4, 115.3, 18.5; ESI-MS, $m/z = 361$ $(M\text{-}H)^-$.

4.19 | 4-(2-(2-(1-(3-chlorophenyl) ethylidene)hydrazinyl)thiazol-4-yl)phenol (18)

Yellow solid; Yield76.76%; mp: 194.6–195.9°C; IR (KBr, ν/cm^{-1}) 3116, 1591, 1514, 1397, 1275, 1042. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.90 (s, 1H, ph-H), 7.83 (d, $J = 8.1$ Hz, 1H, ph-H), 7.60 (d, $J = 8.1$ Hz, 1H, ph-H), 7.46 (d, $J = 9.3$ Hz, 2H, ph-H), 7.29 (s, 1H, thiazol-H), 7.25 (d, $J = 9.3$ Hz, 2H, ph-H), 6.82–6.77 (m, 1H, ph-H), 2.30 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 169.7, 149.0, 145.7, 137.5, 136.2, 133.7, 132.8, 131.5, 130.2, 128.9, 127.5, 127.0, 123.2, 120.3, 18.5; ESI-MS, $m/z = 342$ $(M\text{-}H)^-$.

4.20 | 4-(4-bromophenyl)-2-(2-(1-(3-chlorophenyl)ethylidene) hydrazinyl)thiazole (19)

Yellow solid; Yield 79.61%; mp: 180.7–182.0°C; IR (KBr, ν/cm^{-1}) 1606, 1482, 1366, 1110, 1071, 835. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.99 (s, 1H, ph-H), 7.83 (d, $J = 8.1$ Hz, 1H, ph-H), 7.76–7.21 (m, 7H, ph-H, thiazol-H), 2.60 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 167.0, 165.4, 140.0, 138.5, 133.3, 132.8, 132.2, 130.6, 129.9, 129.2, 128.3, 127.7, 126.8, 120.8, 16.3; ESI-MS, $m/z = 407$ $(M + H)^+$.

4.21 | 2-(2-(1-(3-chlorophenyl) ethylidene)hydrazinyl)-4-(4-nitrophenyl) thiazole (20)

Yellow solid; Yield 77.74%; mp: 245.7–247.9°C; IR (KBr, ν/cm^{-1}) 1609, 1598, 1521, 1341, 1112. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.99 (s, 1H, ph-H), 7.88 (d, $J = 8.1$ Hz, 1H, ph-H), 7.83–7.34 (m, 7H, ph-H, thiazol-H), 2.61 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 176.2, 165.3, 151.2, 145.1, 132.8, 132.4, 131.3, 130.7, 130.2, 129.4, 128.1, 127.7, 126.8, 125.5, 16.3; ESI-MS, $m/z = 373$ $(M + H)^+$.

4.22 | 2-(2-(1-(4-bromophenyl) ethylidene)hydrazinyl)-4-(4-chlorophenyl) thiazole (21)[42]

Yellow solid; Yield 76.66%; mp: 247.3–248.5°C; IR (KBr, ν/cm^{-1}) 1612, 1490, 1370, 1113, 1093. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.73 (d, $J = 7.8$ Hz, 2H, ph-H), 7.66 (d, $J = 9.0$ Hz, 2H, ph-H), 7.65 (d, $J = 8.7$ Hz, 2H, ph-H), 7.07 (s, 1H, thiazol-H), 6.73 (d, $J = 8.7$ Hz, 2H, ph-H), 2.28 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 170.2, 157.2, 146.8, 136.8, 136.5, 130.6, 128.0, 127.2, 124.9, 122.1, 115.1, 106.6, 13.9; ESI-MS, $m/z = 387$ $(M\text{-}H)^-$.

4.23 | 4-(2-(2-(1-(4-Bromophenyl) ethylidene)hydrazinyl)thiazol-4-yl)phenol (22)

Yellow solid; Yield 61.59%; mp: 282.3–283.1°C; IR (KBr, ν/cm^{-1}) 3126, 1606, 1509, 1397, 1271, 1080. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.91 (d, $J = 8.7$ Hz, 2H, ph-H), 7.74 (d, $J = 8.7$ Hz, 2H, ph-H), 7.64 (d, $J = 8.7$ Hz, 2H, ph-H), 7.49 (d, $J = 8.7$ Hz, 2H, ph-H), 7.42 (s, 1H, thiazol-H),, 2.30 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 169.7, 168.5, 145.5, 137.0, 133.5, 131.9, 129.5, 128.6, 128.4, 127.7, 122.1, 105.0, 13.8; ESI-MS, $m/z = 405$ $(M\text{-}H)^-$.

4.24 | 4-(4-Bromophenyl)-2-(2-(1-(4-bromophenyl)ethylidene) hydrazinyl)thiazole (23)[43]

Yellow solid; Yield 85.14%; mp: 247.7–249.3°C; IR (KBr, ν/cm^{-1}) 1623, 1487, 1398, 1110, 1073. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.86 (d, $J = 8.7$ Hz, 2H, ph-H), 7.74(d, $J = 8.7$ Hz, 2H, ph-H), 7.64–7.60 (m, 4H, ph-H), 7.43 (s, 1H, thiazol-H), 2.30 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 169.7, 168.5, 145.5, 137.0, 133.8, 131.3, 129.8, 127.6, 122.1, 121.4, 120.5, 105.1, 14.0; ESI-MS, $m/z = 450$ $(M\text{-}H)^-$.

4.25 | 2-(2-(1-(4-Bromophenyl) ethylidene)hydrazinyl)-4-(4-nitrophenyl) thiazole (24)[42]

Yellow solid; Yield 93.05%; mp: 245.1–247.7°C; IR (KBr, ν/cm^{-1}) 1617, 1600, 1514, 1343, 1081. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.70–7.61 (m, 4H, ph-H), 7.46–7.43 (m, 2H, ph-H), 7.08 (s, 1H, thiazol-H), 6.81 (d, $J = 8.7$ Hz, 2H, ph-H), 2.30 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 169.9, 168.6, 157.1, 137.0, 133.6, 130.7, 129.8, 126.9, 125.4, 124.9, 115.3, 100.9, 13.8; ESI-MS, $m/z = 418$ $(M + H)^+$.

4.26 | 4-(4-Nitrophenyl)-2-(2-(1-(p-tolyl) ethylidene)hydrazinyl)thiazole (25)[44]

Yellow solid; Yield 96.59%; mp: 267.6–269.7°C; IR (KBr, ν/cm^{-1}) 3340, 1654, 1507, 1344, 1137, 842, 717; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.31 (d, $J = 9.0$ Hz, 2H, ph-H),

8.15 (d, $J = 9.0$ Hz, 2H, ph-H), 7.96 (s, 1H, -NH), 7.72 (s, 1H, thiazol-H), 7.70 (d, $J = 8.1$ Hz, 2H, ph-H), 7.25 (d, $J = 8.1$ Hz, 2H, ph-H), 2.90 (s, 3H, CH_3), 2.30 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 170.3, 162.3, 148.6, 147.0, 146.1, 140.8, 138.3, 135.0, 129.0, 126.3, 124.1, 108.9, 20.8, 14.0; ESI-MS, $m/z = 353$ $(M + H)^+$.

4.27 | 4-(2-(2-(1-(4-Aminophenyl) ethylidene)hydrazinyl)thiazol-4-yl)phenol (26)

White solid; Yield 97.53%; mp: 260.6–262.2°C; IR (KBr, ν/cm^{-1}) 3316, 1621, 1513, 1271, 1184, 844, 738; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.89 (d, $J = 8.4$ Hz, 2H, ph-H), 8.15 (d, $J = 8.7$ Hz, 2H, ph-H), 7.38 (d, $J = 8.7$ Hz, 2H, ph-H), 7.08 (s, 1H, thiazol-H), 6.85 (d, $J = 8.4$ Hz, 2H, ph-H), 2.34 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 169.4, 157.1, 149.9, 145.9, 136.2, 133.8, 130.4, 126.9, 122.1, 115.3, 113.6, 101.1, 14.0; ESI-MS, $m/z = 324$ $(M + H)^-$.

4.28 | 4-(1-(2-(4-(4-Nitrophenyl)thiazol-2-yl)hydrazono)ethyl)aniline (27)

Yellow solid; Yield: 88.38%; mp: >280°C; IR (KBr, ν/cm^{-1}) 3319, 1617, 1571, 1335, 1142, 859, 730; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.32 (d, $J = 8.4$ Hz, 2H, ph-H), 8.16 (d, $J = 8.7$ Hz, 2H, ph-H), 7.90 (d, $J = 8.1$ Hz, 2H, ph-H), 7.77 (s, 1H, thiazol-H), 7.41 (d, $J = 8.4$ Hz, 2H, ph-H), 2.36 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 170.1, 148.6, 146.1, 140.7, 136.5, 133.4, 130.4, 127.0, 126.3, 124.1, 122.4, 109.1, 14.1; ESI-MS, $m/z = 355$ $(M + H)^-$.

4.29 | Tyrosinase activity assay

Tyrosinase inhibition assays were performed from our reported procedure.[30] Briefly, thiazolylhydrazone derivatives were screened for diphenolase inhibitory activity of tyrosinase using L-DOPA as substrate. The compounds **1–27** and kojic acid (used as control) were respectively dissolved in DMSO to make their final concentration at 2.0%. Phosphate buffer (50 mM, pH 6.8) was used to dilute the DMSO stock solution of tested compounds. Thirty units of mushroom tyrosinase (0.5 mg/mL) were firstly pre-incubated with the compounds in phosphate buffer (50 mM, pH 6.8), for 10 min at 25°C. Then, the L-DOPA (0.5 mM) was added to the reaction mixture and the enzyme reaction was continuously monitored by measuring the UV absorbance at 475 nm of formation of the DOPA chrome for 1 min. The measurement was performed in triplicate for each concentration and averaged before further calculation. IC_{50} values were determined by interpolation of the dose–response curves and the results are listed in Table 1.

4.30 | Kinetic assay of the inhibition of tyrosinase

The assay of inhibition mechanism and inhibitory type of selected compound **26** on mushroom tyrosinase was carried out according to the protocol as previously reported.[30] Compound **26** (0, 8, 12 μM) and L-DOPA (60–150 μM) were used in the kinetic studies. Pre-incubation and measurement time were the same as discussed in mushroom tyrosinase inhibition assay protocol. The inhibition type on the enzyme was evaluated by Lineweaver–Burk plots of inverse of velocities (1/V) versus inverse of substrate concentration 1/[S] μM^{-1}.

4.31 | In silico docking simulation of tyrosinase with compound 26

For docking simulations of tyrosinase to the compound **26**, the AutoDock 4.2 program was employed according to the reported procedure.[30] Crystal structure of tyrosinase (PDB code: 2Y9X) was obtained from the Protein DataBank (http://www.rcsb.org, Rutgers, and UCSD/SDSC). To prepare the compound for the docking simulation, the following steps were performed: (1) conversion of 2D structures into 3D structures, (2) calculation of charges, and (3) addition of hydrogen atoms using the ChemOffice program (http://www.cambridgeoft.com).

4.32 | Free radical scavenging activity assay of DPPH

The antioxidation effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined adapting from the reported method.[45] Reaction mixtures consisted of 100 μL of DPPH (150 μM), 20 μL of increasing concentration of test compounds, and the volume was adjusted to 200 μL in each well with DMSO. The above solution was incubated at room temperature (25°C) for 30 min in the dark. After completion of the incubation, the UV absorbance at 517 nm was measured using a micro plate reader. All of the experiments were triply conducted using DMSO as control and BHT as antioxidant standard. The DPPH radical scavenging activity was calculated followed by the formula:

DPPH radical scavenging activity (%) = (Absorbance of standard−absorbance of sample) /Absorbance of standard × 100.

4.33 | Radical cation scavenging activity assay of ABTS

2,2′-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) free radical scavenging activity assay was performed according to the literature.[45] Test solution of ABTS decolorization assay was prepared by mixing 2.45 mM potassium persulfate and 7 mM ABTS at 8:12 volume/volume ratio. The working solution was kept in the dark at room temperature for 16 to 18 h. Before ABTS radical scavenging test, the ABTS test solution was diluted with DMSO to an absorbance of 0.7 at 734 nm. One milliliter of diluted ABTS test solution was added to 10 μL of DMSO or tested sample solutions. After the working solution was kept in the dark at room temperature for 4 min, the UV absorbance was measured at 734 nm. The measurement was repeated triply with DMSO as a control and BHT as an antioxidant standard. The absorbance of test samples was calculated by following formula.

ABTS scavenging ability (%) = (Absorbance of standard−Absorbance of sample) /Absorbance of standard × 100%.

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