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1-(1-Arylethylidene)thiosemicarbazide derivatives: A new class of tyrosinase inhibitors

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Abstract—A series of 1-(1-arylethylidene)thiosemicarbazide compounds and their analogues were synthesized and characterized by ^{1}H NMR, MS. Their tyrosinase inhibitory activities were investigated by an assay based on the catalyzing ability of tyrosinase for the oxidation of L-DOPA, comparing with 4-methoxycinnamic acid and arbutin. The results showed that (1) all the synthesized compounds could perform a significant inhibitory activity for tyrosinase; (2) for these compounds, the main active moiety interacting with the center of tyrosinase would be thiosemicarbazo group; (3) the inhibitory activity was close related with thiosemicarbazide moieties and the groups attached on the aromatic ring.

1. Introduction

Tyrosinase(monophenol or *o*-diphenol, oxygen oxidoreductase, EC 1.14.18.1), also known as polyphenol oxidase (PPO), is a copper-containing monooxygenase that is widely distributed in microorganisms, animals, and plants.[1–6] Tyrosinase could catalyze two distinct reactions involving molecular oxygen[7–13] in the hydroxylation of monophenols to *o*-diphenols (monophenolase) and in the oxidation of *o*-diphenols to *o*-quinones (diphenolase). Due to the high reactivity, quinones could polymerize spontaneously to form high molecular weight brown-pigments (melanins) or react with amino acids and proteins to enhance brown color of the pigment produced. In addition, tyrosinase is known to be involved in the molting process of insect, and adhesion of marine organisms.[14–17]

Therefore, there is an increasing significance to discover effective tyrosinase inhibitors for clinical medicines, cosmetic products, food industry as well as agricultural purposes.[18–24] Up to now, although a large number of tyrosinase inhibitors were reported, most of them could not be used now[25–35] because of their lower individual activities or side effect. For example, 2-(4-hydroxyphenoxy)tetrahydro-6-(hydroxylmethyl)-2H-pyran-3,4,5-triol (arbutin or arbutoside)[36] (IC_{50} = 30 mM) and kojic acid[37] (IC_{50} = 23 μM) (Fig. 1) were not demonstrated as their clinically efficient.[38] So far tropolone is one of the most strong tyrosinase-inhibitors reported (Fig. 1) (IC_{50} = 0.4 μM),[39] but the serious side effect limited its use as medicine.

On the other hand, it was reported that phenyl thioureas[40] and alkyl thioureas[41] could exhibit weak to moderate depigmenting activity and Ley and Bertram[28] reported that benzaldoximes and benzaldehyde-*o*-alkyloximes possess higher tyrosinase inhibitory ability. Based on these reports, we hoped that condensation products of acetophenone homologues and thiosemicarbazide could show a high inhibition activity, as sulfur atom and nitrogen atom are able to complex the two copper atoms in the active site of tyrosinase.

In order to look for highly potent tyrosinase inhibitors, a series of 1-(1-arylethylidene)thiosemicarbazide derivatives were synthesized and their inhibitory activities against mushroom tyrosinase were evaluated using arbutin and 4-methoxycinnamic acid as comparing substances. Meanwhile, the structure–activity relationships of these compounds were also primarily discussed.

Keywords: Tyrosinase inhibitors; 1-(1-Arylethylidene)thiosemicarbazide; Analogues.
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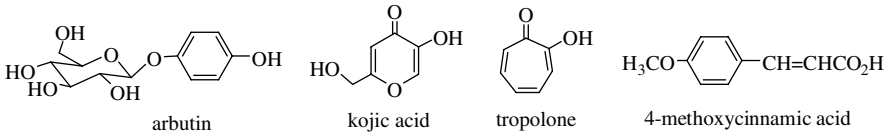


Figure 1. Structures of some known tyrosinase inhibitors.

2. Results and discussion

2.1. Chemistry

According to the general procedure shown in Scheme 1, the synthesis of a series of 1-(1-arylethylidene)thiosemicarbazide compounds and their analogues could be carried out easily in anhydrous alcohol, using acetic acid as catalyst, just by the condensation of methyl ketone or aldehyde with thiosemicarbazide in the molecular ratio of 1:1 or 1:2. The reaction mixture was refluxed for 24 h and then cooled to room temperature, the formed precipitate was separated by filtration. If necessary, the target compounds could be purified by recrystallization from 95% alcohol. The yields for these reactions are from fair to good (65–86%), and all the compounds were characterized by ^{1}H NMR, MS.

2.2. Inhibiting activity

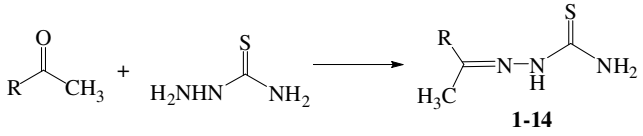
For evaluating the tyrosinase inhibitory activity, all the synthesized compounds were subjected to tyrosinase inhibition assay with L-DOPA as substrate, according to the typical assay protocol developed by Hearing.[42] The tyrosinase inhibitory activity of arbutin and 4-methoxycinnamic acid was ever reported,[6,36] therefore, they were selected as comparing substances. The IC_{50} values of 1-(1-arylethylidene)thiosemicarbazide compounds and their analogues against tyrosinase were summarized in Table 1.

The IC_{50} value of 4-methoxycinnamic acid, determined in this work, is 0.41 mM, the reported IC_{50} value of 4-methoxycinnamic acid is 0.34 mM and 0.43 mM;[6,43] therefore, both the values reported and determined in this work are close to each other, which means that the protocol employed in this work could be reasonable.

Tropolone (in Fig. 1), as tyrosinase inhibitor, could show an IC_{50} value of 0.4 μM.[39] It could be found from Table 1 that compounds **1**, **2**, **3**, **6**, **9**, **11**, and **17** all showed a little higher inhibiting ability against tyrosinase than tropolone. Meantime, the IC_{50} values of compounds **4**, **7**, **13**, **14** are close to that of tropolone. Therefore, it is worth for these synthesized compounds to be investigated further.

For the relationships between the structure and the activity of these synthesized compounds, some results could be obtained from the data listed in Table 1:

(1) For the 1-(1-phenylethylidene)thiosemicarbazide compounds, the substituents attached on the benzene ring could change the inhibition activity. Methyl, hydro-

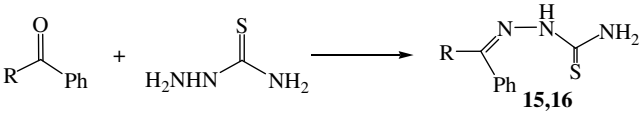


R =: **1**, phenyl; **2**, 4-methylphenyl; **3,** 4-hydroxyphenyl; **4**, 2,4-dihydroxyphenyl;

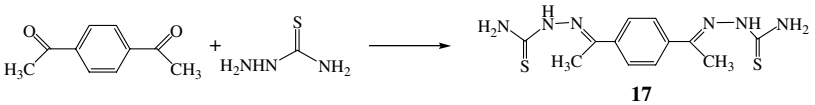
5, 2,4,6-trihydroxyphenyl; **6**, 4-fluorophenyl; **7**, 4-bromophenyl;

8, 4-isopropylphenyl; **9**, 4-methoxyphenyl; **10**, 2-pyrazinyl; **11**, 2-thiophenyl;

12, 3-pyridinyl; **13**, (4-methoxyphenyl)methyl; **14**, 2-(4-hydroxyphenyl)ethyl.



R =: **15**, hydroxy(phenyl)methyl; **16**, benzoyl.



Scheme 1. Synthesis of 1-(1-arylethylidene)thiosemicarbazide compounds and their analogues.

Table 1. Inhibitory activity of 1-(1-arylethylidene)thiosemicarbazide against tyrosinase

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
IC_{50} (μM)	0.34	0.27	0.31	0.58	22.0	0.17	0.52	1.0
Compound	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**17**
IC_{50} (μM)	0.11	0.88	0.14	0.82	0.42	0.54	55.5	0.15
Compound	**16**[a]	Thiosemicarbazide[b]		1-(Thiophen-2-yl)-ethanone			Acetophenone	
IC_{50} (mM)	0.10[a]	2.00[b]		0.15			0.85	
Compound	1-(4-Fluorophenyl)-ethanone		1-(4-Methoxyphenyl)-ethanone[c]		4-Methoxycinnamic acid[d]		Arbutin[e]	
IC_{50} (mM)	0.99		2.00[c]		0.41[d]		10.4[e]	

[a] The concentration of 0.10 (mM) corresponding to inhibition percentage, determined in this work, is 47%.
[b] The concentration of 2.00 (mM) corresponding to inhibition percentage, determined in this work, is 39.9%.
[c] The concentration of 2.00 (mM) corresponding to inhibition percentage, determined in this work, is 47.2%.
[d] The reported IC_{50} values of 4-methoxycinnamic acid is 0.34–0.43 mM.[6,43]
[e] For arbutin, the concentration of 10.40 (mM) corresponding to inhibition percentage, determined in this work, is 30%. The reported IC_{50} value of arbutin was more than 30 mM.[36]

xyl, fluoro, and methoxy on the 4-positon of benzene ring could enhance the activity, but bromo and isopropyl could decrease the activity. Although hydroxyl on 4-position could enhance the activity, with the increase of the number of hydroxyl on benzene ring, the activity of 1-(1-phenylethylidene)thiosemicarbazide compounds would decrease.

(2) The increase in the number of thiosemicarbazido group in one molecule could not obviously enhance the activity, although the thiosemicarbazido group could be the main active position in these synthesized compounds. For example, the IC_{50} value against tyrosinase for **1** is 0.34 μM, for **17** is 0.15 μM.

(3) When the benzene ring of phenylethylidenethiosemicarbazide molecule was changed by heterocyclic ring, the obtained ethylidenethiosemicarbazide compounds could still show a stronger activity against tyrosinase. For example, the IC_{50} value for **11** is 0.14 μM.

(4) When thiosemicarbazido group and benzene ring are separated by one or two methene groups, the change on the inhibitory activity of the obtained ethylidenethiosemicarbazide compounds is not significant.

(5) The inhibitory activity of corresponding compounds would be decreased obviously when the methyl of 1-(1-phenylethylidene)thiosemicarbazide compounds was changed into hydroxy(phenyl)methyl or benzoyl.

2.3. Inhibiting mechanism

It was reported that the structure of tyrosinase was determined[44] (shown in Fig. 2), there are two copper ions in the active center of tyrosinase and it was deduced that there is a lipophilic long-narrow gorge near to the active center.[45]

There are sulfur atom and nitrogen atom in the molecule of arylethylidenethiosemicarbazide compounds and their analogues, and sulfur atom and nitrogen atom could exhibit strong affinity for copper ion. Therefore, it could be supposed that complexes would be formed

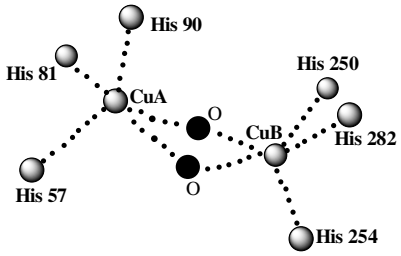


Figure 2. The active center structure of tyrosinase.

between arylethylidenethiosemicarbazide compounds and tyrosinase when both substrates were mixed together in solution. In addition, sulfur atom and nitrogen atom, especially sulfur atom, of arylethylidenethiosemicarbazide compounds and copper ion of tyrosinase could be the center of complexation. The structures of the complexes are suggested as shown in Figure 3, based on the active center structure of tyrosinase.

In the complex, the active center of tyrosinase could coordinate with two arylethylidenethiosemicarbazide molecules at the same time in two opposite directions. It is suggested that the intramolecule hydrogen bond was formed between hydrogen atom on 3-N and nitrogen atom (2-N) in arylethylidenethiosemicarbazide molecule, which are beneficial to decreasing molecular energy state.

According to the structure of arylethylidenethiosemicarbazide molecules, there would be two forms (Fig. 3, form **A** and form **B**) to form complexes with tyrosinase. When the coordination between copper ion and sulfur atom of arylethylidenethiosemicarbazide molecule occurred, the intermolecule hydrogen bond would be formed between hydrogen atom on 3-N (form **A**) or on 2-N (form **B**) and two oxygen atoms located between two copper ions, which would make the coordination between copper ion and sulfur atom become more tight, and consequently, which could make the free oxygen molecule decrease its reaction ability and even unable to take part in the hydroxylation with monophenols

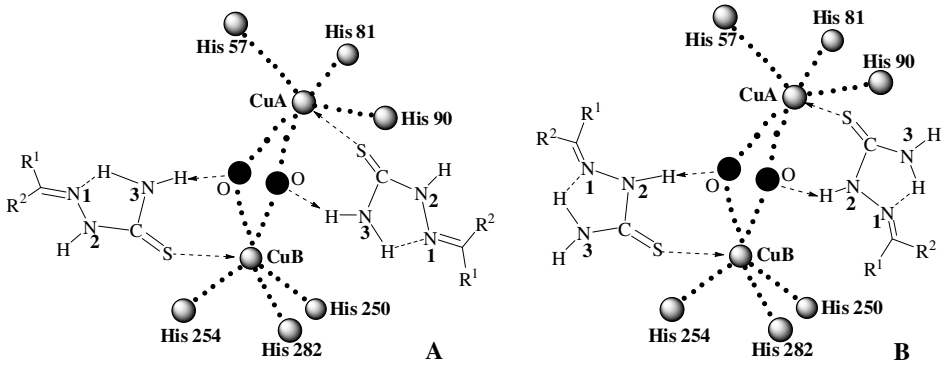


Figure 3. The proposed structure of the formed complexes between tyrosinase and synthesized compounds.

and in the oxidation with *o*-diphenols, as the free oxygen molecule was surrounded closely by two copper ions from tyrosinase and two hydrogen atoms from two arylethylidenethiosemicarbazide molecules. Therefore, tyrosinase would lose its catalyzing ability.

As for the complex form **A** and form **B** (Fig. 3), which one is the best beneficial form? Based on the molecular energy state, form **A** should be the best one. For the form **A**, comparing with form **B**, larger substituted-benzene ring (R^1) and methyl (or H) (R^2) could depart from tyrosinase for a longer distance, and consequently, the bulk inhibition from R^1 and R^2 would decrease, which would decrease the energy of the complex and which is just beneficial to the formation of the complexes between arylethylidenethiosemicarbazide and tyrsoinase.

In order to confirm this conclusion, four compounds, acetophenone, 1-(4-fluorophenyl)ethanone, 1-(4-methoxyphenyl)ethanone, and 1-(thiophen-2-yl)-ethanone, which are corresponding to the starting materials of compounds **1**, **6**, **9**, and **11** were selected and their inhibiting abilities against tyrosinase determined at the same condition, their IC_{50} value are all more than 0.15 mM. At the same time, when the concentration of thiosemicarbazide is 2.00 mM, the corresponding inhibition percentage, determined in this work, is only 39.9%.

On the other hand, there is a sulfur atom in molecule of 1-(thiophen-2-yl)-ethanone, and this sulfur atom should exhibit strong affinity for copper atom of tyrosinase. However, 1-(thiophen-2-yl)ethanone just expressed a weaker inhibiting ability against tyrosinase at the same condition, which means that the sulfur atom of 1-(thiophen-2-yl)ethanone possesses a weaker affinity or a weaker coordination ability than that of thiosemicarbazido group of compounds **11**, 1-(1-(thiophen-2-yl)-ethylidene)thiosemicarbazide, although both sulfur atoms of compounds **11** could possess chance to take part in the complexation at the same time. The reason could be that there is a larger bulk inhibition for the coordination between copper ion and sulfur atom of thiophene; meantime, there is a strong competition from the coordination between copper ion and sulfur atom of thiosemicarbazido group. Therefore, the thiosemicarbazido group could be the important active position in the molecule of arylethylidenethiosemicarbazide compounds as tyrosinase inhibitor, and the major form of the complexation of tyrosinase with compound **11** is supposed as **B**, instead of **A** (Fig. 4).

As to the lower IC_{50} value of thiosemicarbazide, comparing with that of arylethylidenethiosemicarbazide compounds and their homologues, the following two main reasons could be suggested here: (1) Because thiosemicarbazide was hydrophilic and tyrosinase was lipophilic and all the tyrosinase inhibition assays were determined almost in phosphate buffer, there was a less chance for thiosemicarbazide and tyrosinase to

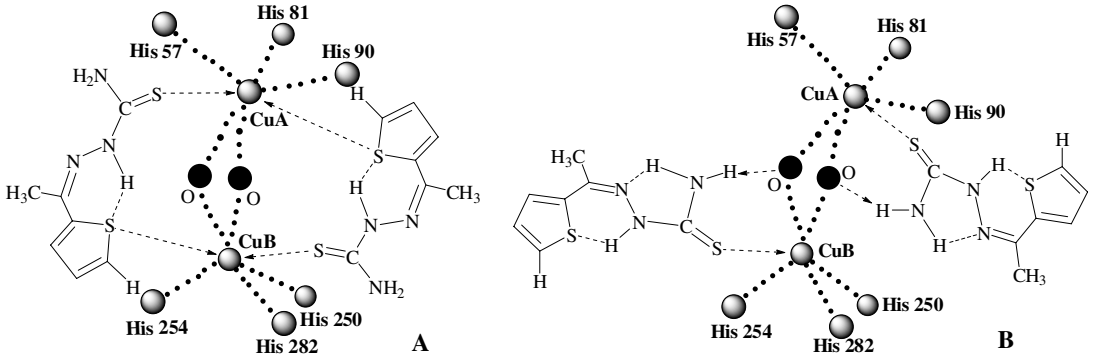


Figure 4. The proposed structures of the complexation of tyrosinase with arylethylidenethiosemicarbazide compounds and 1-(1-(thiophen-2-yl)ethylidene)thiosemicarbazide.

interact to form complex; (2) The presence of substituted-aromatic ring in arylethylidenethiosemicarbazide compounds would be the main beneficial reason, because it was deduced that there is a lipophilic long-narrow gorge near to the active center[45] and the lipophilic aromatic ring of arylethylidenethiosemicarbazide compounds could easily get close to the gorge, which would make the thiosemicarbazido group of arylethylidenethiosemicarbazide compounds get close to the active center of tyrosinase much more easily. In other words, lipophilic moiety of arylethylidenethiosemicarbazide compounds could bring the thiosemicarbazido group to the site close to the active center of tyrosinase, which was beneficial for the coordination between the active center of tyrosinase and the thiosemicarbazido group of thiosemicarbazide compounds. It is the stronger coordination between the active center of tyrosinase and arylethylidenethiosemicarbazide compounds than that between the active center of tyrosinase and thiosemicarbazide, arylethylidenethiosemicarbazide compounds would show a stronger inhibiting ability than thiosemicarbazide against tyrosinase.

3. Conclusions

In this study, a series of 1-(1-arylethylidene)thiosemicarbazide compounds and their analogues were synthesized and characterized by ^{1}H NMR, MS. Their inhibitory activities against tyrosinase were studied based on the catalyzing ability of tyrosinase for the oxidation of L-DOPA, using arbutin and 4-methoxycinnamic acid as comparing substrate. The following conclusions would be obtained: (1) 1-(1-arylethylidene)thiosemicarbazide compounds could perform a significant inhibitory activity for tyrosinase; seven compounds could show stronger inhibiting ability than reported tropolone, four compounds showed nearly same IC_{50} values with tropolone. (2) The form of the interaction between these compounds and tyrosinase could be supposed as in Figure 3 (form **A**) in which the formation of hydrogen bond between hydrogen atom on 3-N (form **A**) and free oxygen molecule located between two copper ions of tyrosinase would make the free oxygen molecule decrease its reaction ability and even unable to take part in the hydroxylation and in the oxidation. (3) For these investigated compounds, it was suggested that the main active moiety interacting with the center of tyrosinase would be thiosemicarbazido group. (4) The liposoluble group of arylethylidenethiosemicarbazide compounds would make the interaction between the active center of tyrosinase and thiosemicarbazido moiety become much easier, which would make tyrosinase lose its catalyzing ability. (5) Although thiosemicarbazido group could be the main active part, the increase in the number of thiosemicarbazido group could not obviously increase their activity. (6) The changes of the substituent on benzene ring would exert an influence on the inhibitory activity. Meanwhile, when benzene was replaced by heterocyclic ring, the corresponding activity would have little change.

4. Experimental

4.1. Equipments and reagents

Melting points (mp) were determined with WRS-1B melting point apparatus and are uncorrected. NMR spectra were recorded on Mercury-Plus300 spectrometers at 25 °C using $CDCl_3$ or DMSO-d_6 as a solvent. All chemical shifts (δ) are quoted in ppm downfield from TMS and coupling constants (J) are given in Hz. LC–MS spectra were recorded using the LCMS-2010A. All reactions were monitored by TLC (Merck Kieselgel 60 F254) and the spots were visualized under UV light. The appropriate aldehyde or ketone, thiosemicarbazide, and 4-methoxycinnamic acid were purchased from Darui Chemical Co. (ShangHai, China), arbutin was obtained from Brillian Biochemical Co. (Beijing, China). The other commercially available reagents and solvents were used without further purification. Mushroom tyrosinase and L-DOPA were purchased from Sigma Chemical Co. (Sigma-Aldrich China, Beijing, China).

4.2. Synthesis of thiosemicarbazides

4.2.1. The general procedures for the synthesis of 1-(1-arylethylidene)thiosemicarbazide compounds. The appropriate aldehyde or ketone (10 mmol) was dissolved in anhydrous ethanol(10 mL), thiosemicarbazide (10 mmol) and acetic acid (0.5 mL) were added to the above solution. The reaction mixture was refluxed for 24 h and then was cooled to room temperature. The appearing precipitate was filtered and recrystallized from 95% alcohol to obtain the corresponding 1-(1-arylethylidene)thiosemicarbazide compounds.

4.2.2. 1-(1-Phenylethylidene)thiosemicarbazide (1). Yield 86%; mp 132–134 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.95 (1H, br s, NH), 7.70 (2H, d, J = 1.2 Hz, phH), 7.48 (1H, br s, NH_2), 7.41 (3H, m, J = 1.2 Hz, phH), 6.53 (1H, br s, NH_2), 2.31 (3H, s, CH_3). MS (ESI): m/z (100%) = 194 (M+1).

4.2.3. 1-(1-*p*-Tolylethylidene)thiosemicarbazide (2). Yield 78%; mp 158–160 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.77 (1H, br s, NH), 7.61 (2H, d, J = 7.5 Hz, phH), 7.36 (1H, br s, NH_2), 7.21 (2H, d, J = 7.5 Hz, phH), 6.42 (1H, br s, NH_2), 2.39 (3H, s, $COCH_3$), 2.29 (3H, s, ph-CH_3). MS (ESI): m/z (100%) = 208 (M+1).

4.2.4. 1-(1-(4-Hydroxyphenyl)ethylidene)thiosemicarbazide (3). Yield 73%; mp 208–209 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.02 (1H, br s, NH), 9.69 (1H, s, OH), 8.13 (1H, br s, NH_2), 7.77 (2H, d, J = 8.7 Hz, phH), 7.72 (1H, br s, NH_2), 6.75 (2H,d, J = 8.7 Hz, phH), 2.22 (3H, s, CH_3). MS (ESI): m/z (100%) = 208 (M−1).

4.2.5. 1-(1-(2,4-Dihydroxyphenyl)ethylidene)thiosemicarbazide (4). Yield 68%; mp 186–187 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.57 (1H, s, OH), 10.57 (1H, s, OH), 9.72 (1H, br s, NH), 7.74 (1H, s, NH_2), 7.70 (1H, s, NH_2), 7.36 (H, d, J = 8.4 Hz, phH), 6.37

(1H, d, J = 8.4 Hz, phH), 6.23 (1H, s, phH), 2.25 (3H, s, CH_3). MS (ESI): m/z (100%) = 224 (M−1).

4.2.6. 1-(1-(2,4,6-Trihydroxyphenyl)ethylidene)thiosemicarbazide (5). Yield 65%; mp 211–213 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.18 (2H, s, OH), 10.32 (1H, s, OH), 9.72 (1H, br s, NH), 7.73 (1H, br s, NH_2), 7.36 (1H, br s, NH_2), 5.78 (2H, s, phH), 2.54 (3H, s, CH_3). MS (ESI): m/z (100%) = 240 (M−1).

4.2.7. 1-(1-(4-Fluorophenyl)ethylidene)thiosemicarbazide (6). Yield 82%, mp 154–156 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.17 (1H, br s, NH), 8.24 (1H, br s, NH_2), 7.98 (2H, d, J = 6.9 Hz, phH), 7.78 (1H, br s, NH_2), 7.20 (2H,d, J = 6.9 Hz, phH), 2.28 (3H, s, CH_3). MS (ESI): m/z (100%) = 212 (M+1).

4.2.8. 1-(1-(4-Bromophenyl)ethylidene)thiosemicarbazide (7). Yield 79%, mp 190–192 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.08 (1H, br s, NH), 8.28 (1H, br s, NH_2), 7.97 (1H, br s, NH_2), 7.89 (2H, d, J = 7.8 Hz, phH), 7.55 (2H, d, J = 7.8 Hz, phH), 2.27 (3H, s, CH_3). MS (ESI): m/z (100%) = 273 (M+1).

4.2.9. 1-(1-(4-Isopropylphenyl)ethylidene)thiosemicarbazide (8). Yield 68%, mp 99–100 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.77 (1H, br s, NH),7.64 (2H,d, J = 8.1 Hz, phH), 7.37 (1H, br s, NH_2), 7.25 (2H, d, J = 8.1 Hz, phH),6.47 (1H, br s, NH_2), 2.94 (m, 1H, CH), 2.29 (3H, s, CH_3), 1.28 (d, 6H, $2CH_3$). MS (ESI): m/z (100%) = 236 (M+1).

4.2.10. 1-(1-(4-Methoxyphenyl)ethylidene)thiosemicarbazide (9). Yield 83%, mp 175–176 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.04 (1H, br s, NH),7.57 (2H, d, J = 8.7 Hz, phH), 7.27 (1H, br s, NH_2), 7.26 (1H, br s, NH_2), 7.25 (2H, d, J = 8.7 Hz, phH), 3.67 (3H, s, OCH_3), 2.16 (3H, s, CH_3). MS (ESI): m/z (100%) = 224 (M+1).

4.2.11. 1-(1-(Pyrazin-2-yl)ethylidene)thiosemicarbazide (10). Yield 67%, mp 190–191 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.43 (1H, br s, NH), 9.62 (1H, s, pyrazine proton), 8.58 (2H, d, pyrazine protons), 8.44 (1H, br s, NH_2), 8.28 (1H, br s, NH_2), 2.36 (3H, s, CH_3). MS (ESI): m/z (100%) = 196 (M+1).

4.2.12. 1-(1-(Thiophen-2-yl)ethylidene)thiosemicarbazide (11). Yield 65%, mp 146–147 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.86 (1H, br s, NH), 7.35 (1H, d, thiophen), 7.31 (1H, d, thiophene proton), 7.28 (1H, br s, NH_2), 7.04 (1H, t, thiophene proton), 6.59 (1H, br s, NH_2), 2.31 (3H, s, CH_3). MS (ESI): m/z (100%) = 200 (M+1).

4.2.13. 1-(1-(Pyridin-3-yl)ethylidene)thiosemicarbazide (12). Yield 70%, mp 212–214 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.29 (1H, br s, NH), 9.08 (1H, s, pyridine proton), 8.54 (1H, d, pyridine proton), 8.31 (1H, d, pyridine proton), 8.29 (1H, br s, NH_2), 8.05 (1H, br s, NH_2), 7.40 (1H, t, pyridine proton), MS (ESI): m/z (100%) = 195 (M+1).

4.2.14. 1-(1-(4-Methoxyphenyl)propan-2-ylidene)thiosemicarbazide (13). Yield 88%, mp 122–123 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.50 (1H, br s, NH), 7.26 (1H, br s, NH_2),7.10 (2H, d, J = 8.7 Hz, phH), 6.86 (2H, d, J = 8.7 Hz, phH), 6.34 (1H, br s, NH_2), 3.80 (3H, s, OCH_3), 3.50 (2H, s, CH_2), 1.84 (3H, s, CH_3). MS (ESI): m/z (100%) = 238 (M+1).

4.2.15. 1-(4-(4-Hydroxyphenyl)butan-2-ylidene)thiosemicarbazide (14). Yield 71%, mp154–155 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.87 (1H, br s, NH), 8.50 (1H, s, OH), 7.12 (1H, br s, NH_2), 7.02 (2H, d, J = 6.3 Hz, phH), 6.77 (2H, d, J = 6.3 Hz, phH), 6.22 (1H, br s, NH_2), 2.80 (2H, t, CH_2), 2.60 (2H, t, CH_2), 1.89 (3H, s, CH_3). MS (ESI): m/z (100%) = 236 (M−1).

4.2.16. 1-(2-Hydroxy-1,2-diphenylethylidene)thiosemicarbazide (15). Yield 58%, mp 178–179 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.44 (1H, br s, NH), 8.40 (1H, br s, NH_2), 7.97 (1H, br s, NH_2), 7.90 (t, 2H, phH), 7.36 (m, 8H, phH), 7.29 (s, 1H, OH), 6.27 (d, 1H, CH). MS (ESI): m/z (100%) = 286 (M+1).

4.2.17. 1-(2-Oxo-1,2-diphenylethylidene)thiosemicarbazide (16). Yield 62%, mp 203–204 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.87 (1H, br s, NH), 8.20 (1H, br s, NH_2), 7.39 (m, 10H, phH), 6.75 (1H, br s, NH). MS (ESI): m/z (100%) = 284 (M+1).

4.2.18. 1-(1,4-Diacetylphenyl)dithiosemicarbazide (17). Yield 82%, mp > 280 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.22 (2H, br s, NH), 8.28 (2H, br s, NH_2), 7.94 (2H, br s, NH_2), 2.30 (6H, s, 2× CH_3). MS (ESI): m/z (100%) = 309 (M+1).

4.3. Tyrosinase assay

Tyrosinase inhibition assays were performed according to the method developed by Hearing with slight modification.[42] Briefly, all the synthesized compounds were screened for the o-diphenolase inhibitory activity of tyrosinase using L-DOPA as substrate. All the active inhibitors from the preliminary screening were subjected to IC_{50} studies. All the synthesized compounds were dissolved in DMSO to a concentration of 2.5%. Phosphate buffer, pH 6.8, was used to dilute the DMSO stock solution of test compound. Thirty units of mushroom tyrosinase (28 nM) was first pre-incubated with the compounds, in 50 nM phosphate buffer (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 monitored by measuring the change in absorbance at 475 nm of formation of the DOPAchrome for 10 min. Dose–response curves were obtained by performing assays in the presence of increasing concentrations of inhibitors (0, 1.6, 3.2, 6.3, 12.5, 25, 50, 100, 200 μM). IC_{50} value, a concentration giving 50% inhibition of tyrosinase activity, was determined by interpolation of the dose–response curves. The percent of inhibition of tyrosinase reaction was calculated as follows:

$$\text{percent inhibition } (\%) = [(B - S)/B] \times 100.$$

Here, the *B* and *S* are the absorbances for the blank and samples, respectively. 4-Methoxycinnamic acid and arbutin were used as standard inhibitors for the tyrosinase.

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