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Design and synthesis of 3,5-diaryl-4,5-dihydro-1*H*-pyrazoles as new tyrosinase inhibitors

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ABSTRACT

In this study, twenty 3,5-diaryl-4,5-dihydro-1*H*-pyrazole derivatives with hydroxyl(s) (**1a**–**1p**, **2a**–**2d**) were synthesized and their inhibitory activity on mushroom tyrosinase was examined. The results showed that among these compounds, 1-(5-(3,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone **1d** was found to be the most potent tyrosinase inhibitor with IC_{50} value of 0.301 μM. Kinetic study revealed that these compounds were competitive inhibitors of tyrosinase and their structure–activity relationships were investigated in this article.

1. Introduction

Tyrosinase (EC 1.14.18.1), which is also referred to as polyphenoloxidase, is a multifunctional copper-containing enzyme widely exists in plants and animals. It catalyzes two steps in the conversion of tyrosine to melanin. This process proceeds via 3,4-dihydroxy phenylalanine (DOPA), which is formed by tyrosinase monophenolase activity on tyrosine. Then DOPA was oxidized into DOPA quinine, which is a process catalyzed by active diphenolase. These quinines can polymerize spontaneously to form melanins, which are brown pigments of high molecular weight and determine the color of mammalian skin and hair.[1] However, the accumulation of an excessive level of epidermal pigmentation causes melasama, age spots and other dermatological disorders.[2] Therefore, tyrosinase inhibitors have become increasingly important for medicinal and cosmetic products that may be used to prevent or treat pigmentation disorders.[3] Tyrosinase also play a vital role in undesirable enzymatic browning reactions of some vegetables and fruits.[4] Moreover, tyrosinase is one of the most important key enzymes in the insect molting process, investigation of its inhibitors may be important in finding alternative insect control agents.[5] These phenomena have encouraged researchers to seek new potent tyrosinase inhibitors for use in foods, cosmetics and insect control.

Many efforts have been addressed to the search and synthesis for effective and safe tyrosinase inhibitors. Polyphenol derivatives, such as flavanones,[6] chalcones,[7] resveratrol and it analogs,[8] *N*-benzylbenzamides,[9] and benzoate ester derivatives[10] are a class of tyrosinase inhibitors that had been investigated intensively. They often contain two hydroxylated benzene rings separated by three atoms and often constructed with one of two distinct substructures: a 4-substituted resorcinol or catechol moiety. Introduction of a heterocycle in the compounds, including oxazolones,[11] pyrazoles,[12] oxadiazoles,[13] thiazoles,[14] and carbazoles,[15] the compounds show more potent tyrosinase inhibition.

Based on these studies, we designed new 3,5-diaryl-4,5-dihydro-1*H*-pyrazole with hydroxyls in order to discover effective tyrosinase inhibitors (Fig. 1). At first, 3,5-diaryl-4,5-dihydro-1*H*-pyrazole ethanones were synthesized by our designed route. We found that these ethanones derivatives are also potent tyrosinase inhibitors. Based on the results of ethanones, we modified the reaction conditions and successfully synthesized four compounds of polyhydroxyl 3,5-diaryl-4,5-dihydro-1*H*-pyrazoles. We carried out on their inhibitory effect (inhibitory activity and inhibitory kinetics), and investigated the structure–activity relationship on the enzyme inhibitory activity. Mushroom tyrosinase was used throughout our studies. This research aimed at discovering and filtering effective compounds as tyrosinase inhibitors, which can have potential application in cosmetic careers and other fields to inhibit enzymatic browning.

2. Results and discussion

2.1. Chemistry

Polyhydroxyl 3,5-diaryl-4,5-dihydro-1*H*-pyrazole derivatives were synthesized according to the routes in Scheme 1. Chalcones

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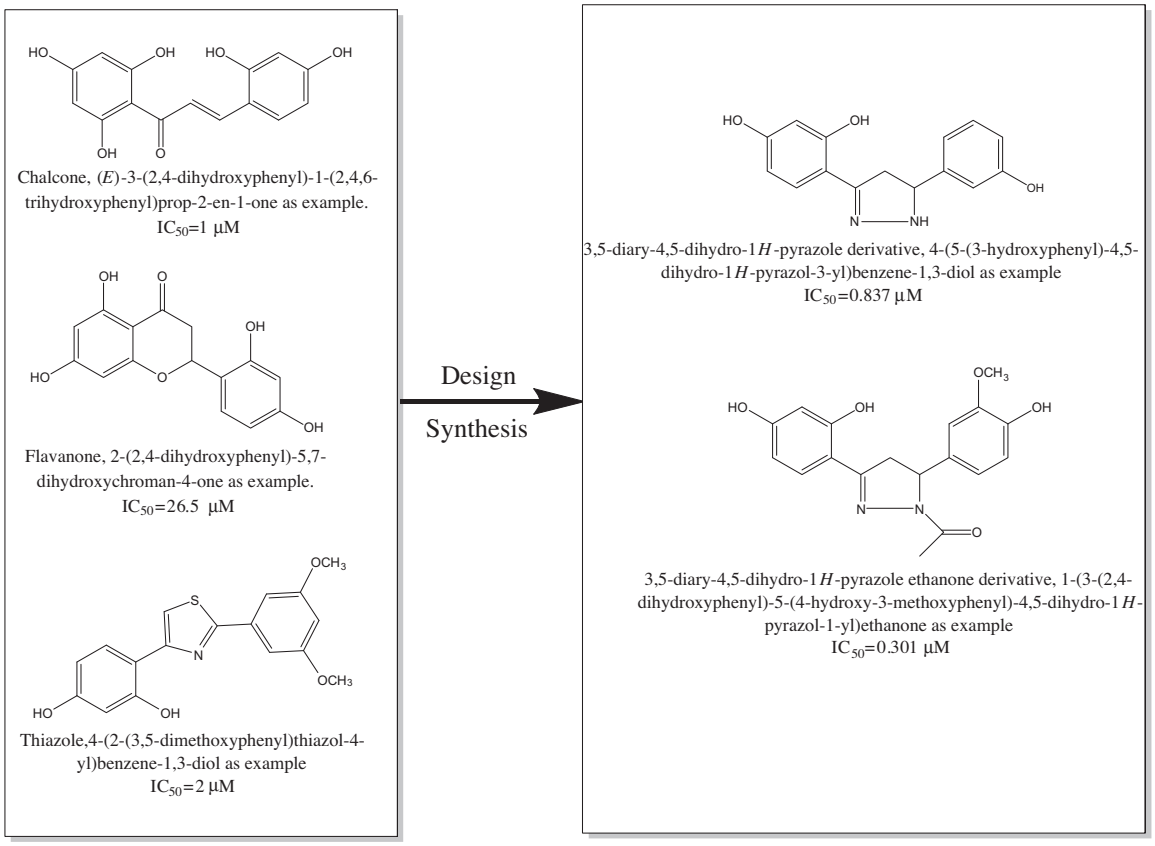


Figure 1. Design and synthesis of compounds in this article.

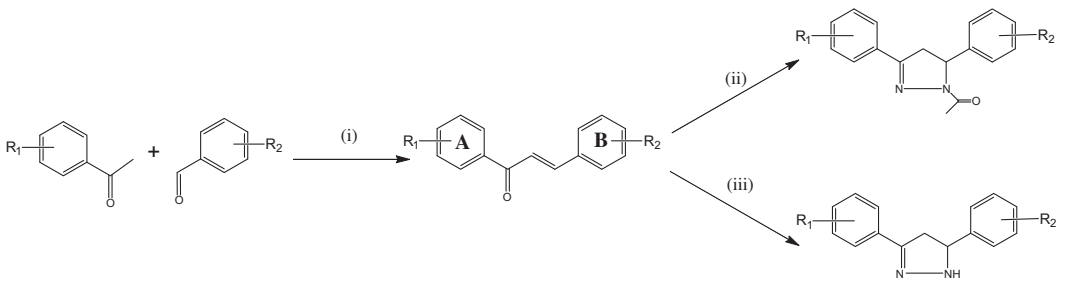
were synthesized through the Claisen–Schmidt condensation of the corresponding acetophenones and benzaldehydes under alkaline condition, as described in synthesis of isoliquiritigenin (2,4,4′-trihydroxychalcone).[16] Polyhydroxylchalcones can also be synthesized under acidic condition,[17] or using protection and deprotection on hydroxyl group,[5] but these methods suffered from more reaction steps, prolonged reaction time, and toxic reagents. In terms of economization and time-saving, we conducted the reactions under alkaline condition. The reactions were processed in presence of solid phase (neutral Al_2O_3) and KOH catalyst under microwave irradiation. After irradiation, the mixture was treated with water, then the resulting solution was acidified and the precipitate was filtered to afford chalcone. Attempts to synthesis 2,4,2′,4′-tetrahydroxychalcone by this method resulted in the formation of polymeric products.

Chalcones were purified and dissolved in ethanol, then hydrazine hydrate was added and the mixture was heated under reflux to afford polyhydroxyl 3,5-diaryl-4,5-dihydro-1*H*-pyrazoles. When the reaction was processed in acetic acid, the active hydrogen of 4,5-dihydro-1*H*-pyrazole was acetylated by the solution, and yielded 3,5-diaryl-4,5-dihydro-1*H*-pyrazole ethanones.

2.2. 3,5-Diaryl-4,5-dihydro-1*H*-pyrazole derivatives on the diphenolase activity of mushroom tyrosinase

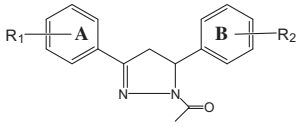
Kojic acid, a widely used skin-whitening material for its high inhibitory activity against tyrosinase[18] and synthesized compounds **1a**–**1p**, **2a**–**2d** were tested for their enzymatic inhibitory activities against tyrosinase. The two hydroxylated benzene rings in the compounds were asymmetric; therefore, different position of hydroxyl group(s) on ring A and B may result in different inhibitory effect on tyrosinase. The results were shown in Table 1. Eleven of the synthesized compounds show IC_{50} lower than kojic acid, indicating these compounds were more effective than kojic acid when used as tyrosinase inhibitor.

The position and number of hydroxyl substituted on ring A had a great influence on the inhibitory activity against tyrosinase. Compounds **1a**–**1d** and **2a**–**2c** with a 2,4-dihydroxyphenyl moiety on

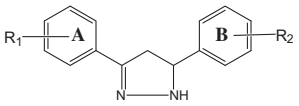


Scheme 1. Reagents and conditions: (i) KOH, neutral Al_2O_3, microwave irradiation, 30–70 s; (ii) acetic acid, hydrazine hydrate, reflux, 50–90 min; (iii) ethanol, hydrazine hydrate, reflux, 5 h.

Table 1
Inhibition effect of 3,5-diaryl-4,5-dihydro-1*H*-pyrazole derivatives on mushroom tyrosinase activities



1a-1p



2a-2d

Compounds	R^1	R^2	IC_{50} (μM)
1a	2,4-OH	3′,4′-OH	2.74 ± 0.21
1b	2,4-OH	4′-OH	1.69 ± 0.03
1c	2,4-OH	3′-OH	0.863 ± 0.102
1d	2,4-OH	3′-OMe, 4′-OH	0.301 ± 0.090
1e	2,4-OH	3′-NO_2, 4′-OH	>200
1f	4-OH	3′,4′-OH	1.07 ± 0.14
1g	4-OH	4′-OH	3.51 ± 0.04
1h	4-OH	3′-OH	147 ± 5
1i	4-OH	3′-OMe, 4′-OH	>200
1j	2-OH	3′,4′-OH	>200
1k	2-OH	4′-OH	13.1 ± 0.8
1l	2-OH	3′-OH	>200
1m	2-OH	3′-OMe, 4′-OH	13.4 ± 0.3
1n	2-OH	2′-OMe, 4′-OH	>200
1o	3,5-OH	4′-OH	161 ± 16
1p	3,5-OH	3′-OH	>200
2a	2,4-OH	4′-OH	0.882 ± 0.057
2b	2,4-OH	3′-OH	0.837 ± 0.148
2c	2,4-OH	3′-OMe, 4′-OH	2.43 ± 0.41
2d	2-OH	4′-OH	11.8 ± 1.5
Kojic acid			18.3 ± 0.7

ring A were all very active members of the synthesized compounds, even better than the standard inhibitor, kojic acid. Compound **1d** showed most potent tyrosinase inhibition and the IC_{50} is 0.301 μM. When there was only hydroxy substituted at C-3′ and C-4′, compounds exhibited slightly lower inhibition. For compounds **1f**–**1i** with a *p*-hydroxy on ring A, most compounds exhibited lower inhibitory activity against tyrosinase compared to those with 2,4-dihydroxy substituent. Only compound **1f** with a 3,4-dihydroxyphenyl moiety on ring B showed higher potency than corresponding **1a** with 2,4-dihydroxyphenyl moiety. Similarly, compounds **1j**–**1m** possessing an 2-dihydroxyphenyl on ring A also exhibited lower inhibition potency, and even lower than those with 4-dihydroxyphenyl moiety on ring A. Compounds **1o**, **1p** with a 3,5-dihydroxyphenyl moiety demonstrated practically low activity. The results were in agreement with previous studies on chalcones, suggesting that compounds possessing 2, 4-dihydroxyphenyl moiety on ring A exhibited better inhibitory activity against tyrosinase than other compounds. These results were in good agreement with previous studies, which showed that 4-resorcinol moiety is the key substituted group in exerting potent inhibitory activity.[19]

In contrast, the effects caused by different position of substituted groups attached on ring B were not as conspicuous as on ring A. According to earlier studies, a 3′,4′-dihydroxyphenyl moiety was required on ring B for successful diphenolase inhibition in order to make the structure of the inhibitor molecule similar to L-DOPA. This leads to the competitive displacement of L-DOPA from the active site of the cofactor in a lock and key model. 3,5-Diaryl pyrazole derivatives with nucleophilic methoxy groups or halogen groups such as fluoro, chloro, and bromo along with the phenolic skeleton also recognized as tyrosinase inhibitor.[12,20] In the present study, addition of methoxy greatly influenced the inhibitory activity of the compounds, when compared the inhibitory activity of compound **1d** and **1k** with methoxy at C-3′ and hydroxy at C-4′ and compound **1a** and **1j** with a 3′,4′-dihydroxyphenyl moiety on ring B, compound **1d** and **1m** shows more potent inhibitory activity. However, compound **1i** exhibited practically no activity and compound **1f** was a very active inhibitor against tyrosinase with IC_{50} at 1.07 μM. The substitution position of methoxy was also of importance, as compound **1n** with a methoxy at C-2′ exhibits no inhibitory activity, while compound **1m** was active. And, addition of a $-NO_2$ at C-3′ (compound **1e**) diminished the inhibitory activity. These results indicated that different several manners of inhibition mechanism on tyrosinase from various sources could exist.

The chemical groups between the two hydroxylated benzene rings were another major factor that influenced the inhibition activity. As shown in Figure 2 and Table 2, where compounds with similar 4-substituted resorcinol and same number of atoms between two hydroxylated benzene rings were taken into comparison, when a ring such as thizole and 4,5-dihydro-1*H*-pyrazole was introduced between the the two hydroxylated benzene rings, the compounds are more effective inhibitor of tyrosinase than those with a linear chain that separates the two hydroxylated benzene rings like chalcones and benzyl benzoates.[21] These results

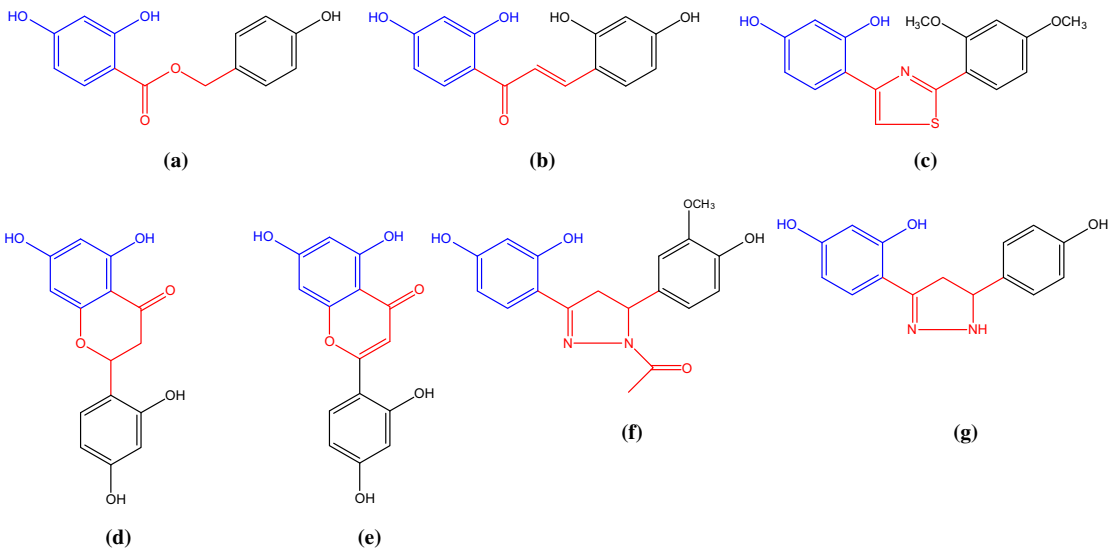


Figure 2. Comparison between tyrosinase inhibitors with different chemical groups (red) which separated two hydroxylated benzene rings. (a) Benzyl benzoate; (b) chalcone; (c) bis-resorcinol-substituted thiazole; (d) flavanone; (e) flavone; (f) 3,5-diaryl-4,5-dihydro-1*H*-pyrazole ethanone; (g) 3,5-diaryl-4,5-dihydro-1*H*-pyrazole.

Table 2
Comparison between compounds different chemical groups which separated two hydroxylated benzene rings

Compound structure	IC_{50} (μM)	Inhibitory type
Benzyl benzoate	4.69	Competitive
Chalcone	5	Competitive
Bis-resorcinol-substituted thiazole	2	—[a]
Flavanone	26.5	Competitive
Flavone	>200	—[a]
3,5-Diaryl-4,5-dihydro-1*H*-pyrazole ethanone	0.301	Competitive
3,5-Diaryl-4,5-dihydro-1*H*-pyrazole	0.837	Competitive

[a] No data afforded from the article.

indicated that a hydrophobic site exists in tyrosinase and a hydrophobic ring of proper size allowed compounds possess a stronger interaction with tyrosinase, thus enhance their inhibitory activity. A different type of ring closure, flavanone for example, resulted in decrease in inhibitory activity.

In order to get a better understanding of influence of pyrazole ring and acetyl group, based on the results of compounds with an acetyl group (**1a**–**1p**), and previous studies on tyrosinase inhibition, compounds without an acetyl group (**2a**–**2d**) were synthesized and evaluated. Compounds **2a**, **2b** and **2d** showed a similar structure–activity relationship with their corresponding acetylated compounds while exerting more potent inhibitory activity. However, to our surprise, **2c** exhibited diminish in inhibitory activity when compared to corresponding **1d**. This result indicated that the influence of acetyl group on the pyrazole ring and methoxy group on ring B might be complicate. Generally the acetyl group on the pyrazole ring diminish the inhibitory activity, possible due to its steric hindrance. Further studies are needed to get a better understanding about the influence of the chemical groups between the two hydroxylated benzene rings, as well as the role of acetyl group and methoxy group.

2.3. Inhibitory types of 3,5-diaryl-4,5-dihydro-1*H*-pyrazole derivatives on mushroom tyrosinase

Kinetic behavior of the oxidation of L-DOPA catalyzed by tyrosinase of compounds **1a**, **1b**, **1c**, **1d**, **2a** and **2b**, which showed exceptional tyrosinase inhibition, were further studied by method steady-state kinetic analysis. Inhibition data were then analyzed using Lineweaver–Burk plot shown in Figure 3 (compounds **1d** and **2b** as an example). The plots of $1/V$ versus $1/[S]$ were characterized by a family of straight lines with different slopes, which presented different fixed substrate concentrations. All these lines were intersected each other at vertical axis, indicating that the inhibitory type of **1d** was competitive manner with L-DOPA as a substrate. This data suggested that compound **1d** to be an effective inhibitor by binding to active site of the enzyme. Identical research was carried out on **1a**, **1b**, **1c**, **2a** and **2b**, and same conclusions were drawn that the five compounds were competitive inhibitors.

3. Conclusions

In summary, series of polyhydroxyl 3,5-diaryl-4,5-dihydro-1*H*-pyrazole derivatives were synthesized and tested. The results showed that polyhydroxyl 3,5-diaryl pyrazole represent a new class of tyrosinase inhibitors. Judging from the results, position of hydroxyl moiety remarkably influenced their inhibitory activity on tyrosinase. When compared the synthesized compounds to compounds with similar structure, it can be concluded that chemical groups between the two hydroxylated benzene rings of the compounds also influenced the inhibitory activity on tyrosinase, addition of a heterocycle could increase inhibitory activity. Among the synthesized compounds, compound **1d** presented the most potent inhibition against the enzyme tyrosinase. These compounds will be of potential use for cosmetic, clinical applications, and other fields which need to inhibit over activity of tyrosinase. The results also provide guidance for further studies on tyrosinase and design of its effective inhibitors.

4. Experimental

Tyrosinase was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). 3,4-Dihydroxyphenylalanine (L-DOPA), 3,4-dihydroxybenzaldehyde, 3-hydroxybenzaldehyde, 2,4-dihydroxyactetophenone were purchased from Alfa Aesar. 4-Hydroxybenzaldehyde, 4-hydroxyactetophenone, 2-hydroxyactetophenone, hydrazine hydrate were purchased from Aladdin reagent Co., Shanghai, China. Other reagents were purchased from commercial suppliers and were dried and purified when necessary. Water used was re-distilled and ion-free.

All UV–vis absorbance were measured on a SHIMADZU UV-2501PC spectrometer without correction. IR spectra were recorded as KBr flakes on a Thermo Nicolet 330FT-IR spectrometer. ^{1}H NMR

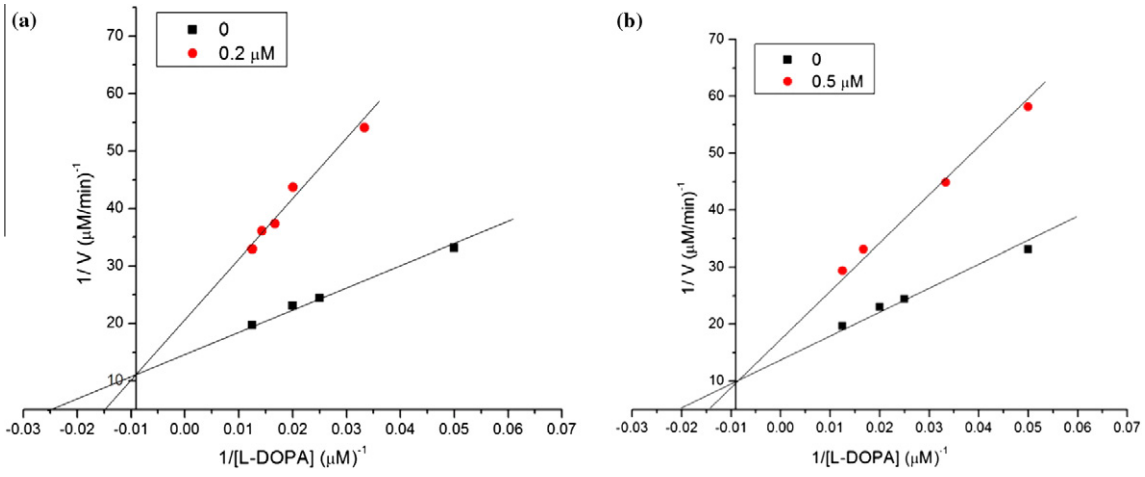


Figure 3. (a) Lineweaver–Burk plot of mushroom tyrosinase inhibition by compound **1d**. Enzyme activity was measured at 475 nm in the presence of 0.2 μM compound **1d** while the substrate. L-DOPA, varied from 20 to 80 μM. (b) Lineweaver–Burk plot of mushroom tyrosinase inhibition by compound **2b**. Enzyme activity was measured at 475 nm in the presence of 0.5 μM compound **2b** while the substrate. L-DOPA, varied from 20 to 80 μM.

and ^{13}C NMR spectra were recorded with a Varian Mercury-Plus 300 instrument (^{1}H, 300 MHz), (^{13}C, 75 MHz) in DMSO-d_6. Melting points were determined on a SGW X-4 melting point apparatus and were uncorrected. Reactions were monitored by TLC on silica gel plates in either iodine chamber or in UV chamber.

4.1. Assay of the diphenolase activity

All the synthesized compounds were screened for diphenolase inhibitory activity of tyrosinase, using L-DOPA as substrate. The inhibitors were dissolved in DMSO and prepared at concentrations of 10 mmol/L. 20 units of mushroom tyrosinase (2000 U/mL), 10 μL of DMSO and 900 μL of phosphate buffer (pH 6.8) were mixed and pre-incubated for 20 min at 30 °C. Then, the L-DOPA (2 mg/mL) was added into this blending and the reaction was monitored for 1 min by measuring the change in absorbance at 475 nm, due to the formation of DOPAchrome. In subsequent experiments, DMSO was replaced by equivalent inhibitors, whose concentration would be decreased from 200 μM until the inhibition was less than 50%. The extent of inhibition by the addition of the sample was expressed as the percentage necessary for 50% inhibition (IC_{50}). As a control, the IC_{50} of kojic acid was also measured.

4.2. General procedure for the preparation of polyhydroxychalcones

Polyhydroxyactetophenone (2 mmol) and polyhydroxybenzaldehyde (2 mmol) was mixed with neutral Al_2O_3 (1.6 g) and grinded in mortar, then KOH (0.8 g) was added and mixed. The obtained mixture was irradiated at 400 W under domestic microwave oven. After 30–70 s, the reaction mixture had become reddish orange. Upon completion, monitored by TLC, the reaction mixture was cooled to room temperature and then treated with water. The mixture was filtered and the resulting solution was acidified by 1 N HCl. The resultant precipitate was filtered off, washed with water, and purified by recrystallization or column chromatography.

4.3. General procedure for the preparation of compounds 1a–1p

To a stirred solution of polyhydrochalcone (1 mmol) in acetic acid, hydrazine hydrate (80% aqueous solution, 5 mmol) was added. The mixture was then refluxed for a period of 50–90 min, monitored by TLC. Upon completion, the solution was cooled to room temperature. Saturated Na_2CO_3 solution was added to neutralize the solution. The resultant precipitate was filtered and recrystallized from methanol to afford compound **1a**–**1p**.

4.3.1. 1-(3-(2,4-Dihydroxyphenyl)-5-(3,4-dihydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (1a)

Yield 26%, light brown powder, mp 259–261 °C, IR(KBr): ν (cm^{-1}): 3256(ν_{O-H}), 1672($\nu_{C=O}$), 1614($\nu_{C=N}$), 1565, 1514, 1480(ν_{as}), 1363(ν_{C-N}). ^{1}H NMR (DMSO-d_6): δ (ppm): 11.06(s, 1H, OH), 10.94(s, 1H, OH), 9.30(s, 1H, OH), 9.13(s, 1H, OH), 7.84(d, 2H, ArH), 6.96(m, 4H, ArH), 5.37(dd, 1H, CH, J = 11.49, 3.43 Hz), 3.80(dd, 1H, H_M, J = 11.67, 17.90 Hz), 3.19(1H, H_A, d, merged & appeared as doublet), 1.90(s, 3H, $COCH_3$).

4.3.2. 1-(3-(2,4-Dihydroxyphenyl)-5-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (1b)

Yield 42%, yellow brown powder, mp 233–235 °C, IR(KBr): ν (cm^{-1}): 3276(ν_{O-H}), 1672($\nu_{C=O}$), 1626($\nu_{C=N}$), 1515, 1475, 1419(ν_{as}), 1248(ν_{C-N}). ^{1}H NMR (DMSO-d_6): δ (ppm): 10.40(s, 1H, OH), 9.90(s, 1H, OH), 9.14(s, 1H, OH), 6.89(m, 7H, ArH), 5.34(dd, 1H, CH, J = 11.41, 3.54 Hz), 3.80(dd, 1H, H_M, J = 11.43, 17.76 Hz), 3.14(dd, 1H, H_A, J = 18.16, 3.78 Hz), 1.78(s, 3H, $COCH_3$).

4.3.3. 1-(3-(2,4-Dihydroxyphenyl)-5-(3-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (1c)

Yield 49%, light brown powder, mp 193–194 °C, IR(KBr): ν (cm^{-1}): 3211(ν_{O-H}), 1670($\nu_{C=O}$), 1618($\nu_{C=N}$), 1462, 1420 (ν_{as}), 1301(ν_{C-N}). ^{1}H NMR (DMSO-d_6): δ (ppm): 10.84(s, 2H, OH), 9.31(s, 1H, OH), 7.58–6.17(m, 7H, ArH), 5.31(dd, 1H, CH, J = 11.57, 3.28 Hz), 3.83(dd, 1H, H_M, J = 10.34, 17.73 Hz), 3.16(dd, 1H, H_A, J = 17.73, 3.67 Hz), 1.77(s, 3H, $COCH_3$).

4.3.4. 1-(3-(2,4-Dihydroxyphenyl)-5-(4-hydroxy-3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (1d)

Yield 45%, light brown powder, mp 228–230 °C, IR(KBr): ν (cm^{-1}): 3437(ν_{O-H}), 1674($\nu_{C=O}$), 1624($\nu_{C=N}$), 1597, 1577, 1512, 1428(ν_{as}), 1315(ν_{C-N}). ^{1}H NMR (DMSO-d_6): δ (ppm): 9.73(s, 2H, OH), 9.03(s, 1H, OH), 7.33–6.23(m, 6H, ArH), 5.37(dd, 1H, CH, J = 11.47, 3.90 Hz), 3.65(s, 3H, OCH_3), 3.89(dd, 1H, H_M, J = 11.50, 17.88 Hz), 3.16(dd, 1H, H_A, J = 18.38, 4.04 Hz), 1.75(s, 3H, $COCH_3$). ^{13}C NMR (DMSO-d_6): δ (ppm): 174.01, 160.31, 159.53, 156.87, 150.01, 148.02, 131.57, 129.89, 121.34, 117.98, 117.31, 114.61, 113.82, 110.54, 70.07, 57.75, 41.29, 25.82.

4.3.5. 1-(3-(2,4-Dihydroxyphenyl)-5-(4-hydroxy-3-nitrophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl) (1e)

Yield 68%, yellow powder, mp 275–276 °C, IR(KBr): ν (cm^{-1}): 3227(ν_{O-H}), 1673($\nu_{C=O}$), 1629($\nu_{C=N}$), 1597, 1535, 1488, 1421 (ν_{as}), 1317(ν_{C-N}). ^{1}H NMR (DMSO-d_6): δ (ppm): 11.35(s, 1H, OH), 11.21(s, 2H, OH), 7.41–6.19(m, 6H, ArH), 5.37(dd, 1H, CH, J = 11.68, 4.13 Hz), 3.80(dd, 1H, H_M, J = 11.55, 18.20 Hz), 3.11(H_A, d, merged & appeared as doublet), 1.83(s, 3H, $COCH_3$).

4.3.6. 1-(5-(3,4-Dihydroxyphenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (1f)

Yield 40%, light yellow powder, mp 216–217 °C, IR(KBr): ν (cm^{-1}): 3247(ν_{O-H}), 1670($\nu_{C=O}$), 1611($\nu_{C=N}$), 1574, 1521, 1485, 1441(ν_{as}), 1364(ν_{C-N}). ^{1}H NMR (DMSO-d_6): δ (ppm): 10.03(s, 1H, OH), 9.43(s, 2H, OH), 8.22–6.77(m, 7H, ArH), 5.29(dd, 1H, CH, J = 11.39, 3.75 Hz), 3.82(dd, 1H, H_M, J = 11.55, 17.85 Hz), 3.03(H_A, d, merged & appeared as doublet), 1.93(s, 3H, $COCH_3$).

4.3.7. 1-(3,5-Bis(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (1g)

Yield 53%, orange powder, mp 171–173 °C, IR(KBr): ν (cm^{-1}): 3319(ν_{O-H}), 1655($\nu_{C=O}$), 1609($\nu_{C=N}$), 1572, 1516, 1441(ν_{as}), 1362(ν_{C-N}). ^{1}H NMR (DMSO-d_6): δ (ppm): 9.63(s, 2H, OH), 7.59(d, 2H, ArH), 7.00–6.40(m, 4H, ArH), 5.37(dd, 1H, CH, J = 11.32, 3.73 Hz), 3.83(dd, 1H, H_M, J = 11.56, 17.54 Hz), 3.13(H_A, d, merged & appeared as doublet), 1.81(s, 3H, $COCH_3$).

4.3.8. 1-(5-(3-Hydroxyphenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (1h)

Yield 57%, light yellow powder, mp 134–135 °C, IR(KBr): ν (cm^{-1}): 3241(ν_{O-H}), 1653($\nu_{C=O}$), 1604($\nu_{C=N}$), 1518, 1458(ν_{as}), 1365(ν_{C-N}). ^{1}H NMR (DMSO-d_6): δ (ppm): 10.02(s, 1H, OH), 9.55(s, 1H, OH), 7.80–6.35(m, 8H, ArH), 5.43(dd, 1H, CH, J = 11.49, 3.93 Hz), 3.12(dd, 1H, H_M, J = 10.23, 17.90 Hz), 2.91(dd, 1H, H_A, J = 17.89, 4.19 Hz), 1.85(s, 3H, $COCH_3$).

4.3.9. 1-(5-(4-Hydroxy-3-methoxyphenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (1i)

Yield 51%, light yellow powder, mp 216–217 °C, IR(KBr): ν (cm^{-1}): 3438(ν_{O-H}), 1665($\nu_{C=O}$), 1610($\nu_{C=N}$), 1596, 1576, 1514, 1433(ν_{as}), 1364(ν_{C-N}). ^{1}H NMR (DMSO-d_6): δ (ppm): 9.98(s, 1H, OH), 9.64(s, 1H, OH), 7.85–6.41(m, 7H, ArH), 5.37(dd, 1H, CH, J = 11.50, 3.81 Hz), 3.71(s, 3H, OCH_3), 3.82(dd, 1H, H_M, J = 11.58, 18.01 Hz), 3.03(dd, 1H, H_A, J = 17.86, 4.28 Hz), 1.83(s, 3H, $COCH_3$).

4.3.10. 1-(5-(3,4-Dihydroxyphenyl)-3-(2-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (1j)

Yield 45%, light yellow powder, mp 119–120 °C, IR(KBr): ν (cm^{-1}): 3233(ν_{O-H}), 1659($\nu_{C=O}$), 1612($\nu_{C=N}$), 1563, 1494, 1447(ν_{as}), 1287(ν_{C-N}). ^1H NMR (DMSO-d_6): δ (ppm): 10.53(s, 1H, OH), 9.05(s, 2H, OH), 7.87–6.27(m, 7H, ArH), 5.30(dd, 1H, CH, J = 11.45, 3.80 Hz), 3.38(dd, 1H, H_M, J = 11.61, 17.59 Hz), 3.18(dd, 1H, H_A, J = 18.39, 4.29 Hz), 1.74(s, 3H, $COCH_3$).

4.3.11. 1-(3-(2-Hydroxyphenyl)-5-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (1k)

Yield 50%, yellow powder, mp 242–243 °C, IR(KBr): ν (cm^{-1}): 3346(ν_{O-H}), 1655($\nu_{C=O}$), 1608($\nu_{C=N}$), 1592, 1564, 1513, 1448(ν_{as}), 1263(ν_{C-N}). ^1H NMR (DMSO-d_6): δ (ppm): 11.31(s, 1H, OH), 9.14(s, 1H, OH) 8.85–6.25(m, 8H, ArH), 5.30(dd, 1H, CH, J = 11.32, 3.62 Hz), 3.88(dd, 1H, H_M, J = 11.44, 17.92 Hz), 3.15(H_A, d, merged & appeared as doublet), 1.90(s, 3H, $COCH_3$).

4.3.12. 1-(3-(2-Hydroxyphenyl)-5-(3-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (1l)

Yield 55%, light brown powder, mp 177–178 °C, IR(KBr): ν (cm^{-1}): 3173(ν_{O-H}), 1681($\nu_{C=O}$), 1636($\nu_{C=N}$), 1589, 1461(ν_{as}), 1311(ν_{C-N}). ^1H NMR (DMSO-d_6): δ (ppm): 10.33(s, 1H, OH), 9.49(s, 1H, OH), 7.60–6.66(m, 8H, ArH), 5.38(dd, 1H, CH, J = 11.61, 3.74 Hz), 3.89(dd, 1H, H_M, J = 11.80, 17.25 Hz), 3.18(dd, 1H, H_A, J = 18.36, 4.23 Hz) 1.78(s, 3H, $COCH_3$).

4.3.13. 1-(5-(4-Hydroxy-3-methoxyphenyl)-3-(2-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (1m)

Yield 49%, light brown powder, mp 211–213 °C, IR(KBr): ν (cm^{-1}): 3430(ν_{O-H}), 1677($\nu_{C=O}$), 1630($\nu_{C=N}$), 1569, 1507, 1440(ν_{as}), 1345(ν_{C-N}). ^1H NMR (DMSO-d_6): δ (ppm): 11.30(s, 1H, OH), 9.89(s, 1H, OH), 7.54–6.14(m, 7H, ArH), 5.35(dd, 1H, CH, 11.63, 3.90 Hz), 3.76(s, 3H, OCH_3), 3.84(dd, 1H, H_M, J = 11.91, 17.60 Hz), 3.09(H_A, d, merged & appeared as doublet), 1.82(s, 3H, $COCH_3$).

4.3.14. 1-(5-(4-Hydroxy-2-methoxyphenyl)-3-(2-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (1n)

Yield 42%, light brown powder, mp 175–176 °C, IR(KBr): ν (cm^{-1}): 3263(ν_{O-H}), 1694($\nu_{C=O}$), 1657($\nu_{C=N}$), 1598, 1560, 1519, 1428(ν_{as}), 1309(ν_{C-N}). ^1H NMR (DMSO-d_6): δ (ppm): 9.64(s, 1H, OH) 8.54(s, 1H, OH), 7.45–6.50(m, 7H, ArH), 5.33(dd, 1H, CH, J = 11.21, 3.45 Hz), 3.63(s, 3H, OCH_3), 3.88(dd, 1H, H_M, J = 11.41, 17.32 Hz), 3.14(H_A, d, merged & appeared as doublet), 1.79(s, 3H, $COCH_3$).

4.3.15. 1-(3-(3,5-Dihydroxyphenyl)-5-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (1o)

Yield 59%, orange powder, mp 264–265 °C, IR(KBr): ν (cm^{-1}): 3346(ν_{O-H}), 1661($\nu_{C=O}$), 1610($\nu_{C=N}$), 1591, 1565, 1448(ν_{as}), 1303(ν_{C-N}). ^1H NMR (DMSO-d_6): δ (ppm): 9.55(s, 3H, OH), 6.93(d, 2H, ArH), 6.66(d, 2H, ArH), 6.60(d, 2H, ArH), 6.28(t, 1H, ArH), 5.36(dd, 1H, CH, J = 11.57, 4.12 Hz), 3.83(dd, 1H, H_M, J = 11.65, 17.13 Hz), 3.14(dd, H_A, J = 18.13, 3.31 Hz), 1.74(s, 3H, $COCH_3$).

4.3.16. 1-(3-(3,5-Dihydroxyphenyl)-5-(3-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone (1p)

Yield 63%, light brown powder, mp 165–167 °C, IR(KBr): ν (cm^{-1}): 3288(ν_{O-H}), 1662($\nu_{C=N}$), 1588, 1463(ν_{as}), 1288(ν_{C-N}). ^1H NMR (DMSO-d_6): δ (ppm): 9.38(s, 2H, OH), 9.20(s, 1H, OH), 7.30–6.30(m, 7H, ArH), 5.37(dd, 1H, CH, J = 11.33, 4.23 Hz), 3.82(dd, 1H, H_M, J = 11.54, 17.45 Hz), 2.96(dd, 1H, H_A, J = 17.79, 3.90 Hz), 1.75(s, 3H, $COCH_3$).

4.4. General procedure for the preparation of compounds 2a–2d

To a stirred solution of polyhydrochalcone (1 mmol) in ethanol, hydrazine hydrate (80% aqueous solution, 10 mmol) was added. The mixture was then refluxed for a period of 6 h, monitored by TLC. Upon completion, the solution was cooled to room temperature. Water was added to the reaction mixture. The products were extracted from the one-third concentrated solution of reaction mixture using chloroform. The organic layer was then evaporated to yield the required crude 3,5-diaryl pyrazole derivatives. Residues obtained were purified by column chromatography to afford pure 3,5-diaryl pyrazole derivatives.

4.4.1. 4-(5-(4-Hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)benzene-1,3-diol (2a)

Yield 19%, orange powder, mp 225–226°C, IR(KBr): ν (cm^{-1}): 3323(ν_{O-H}), 1657($\nu_{C=N}$), 1573, 1559, 1509, 1436(ν_{as}), 1314(ν_{C-N}). ^1H NMR (DMSO-d_6): δ (ppm): 11.43(s, 1H, NH), 10.06(s, 1H, OH), 9.92(s, 1H, OH), 9.68(s, 1H, OH), 7.70–6.40(m, 7H, ArH), 4.92(dd, 1H, CH, J = 11.82, 11.02 Hz), 3.56(dd, 1H, HM, J = 16.45, 10.87 Hz), 3.18(dd, 1H, HA, J = 13.91, 13.70 Hz).

4.4.2. 4-(5-(3-Hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)benzene-1,3-diol (2b)

Yield 26%, orange powder, mp 179–181 °C, IR(KBr): ν (cm^{-1}): 3287(ν_{O-H}), 1650($\nu_{C=N}$), 1579, 1511, 1452(ν_{as}), 1330(ν_{C-N}). ^1H NMR (DMSO-d_6): δ (ppm): 10.92(s, 1H, NH), 10.38(s, 1H, OH), 10.01(s, 1H, OH), 9.41(s, 1H, OH), 7.80–6.30(m, 7H, ArH), 5.12(dd, 1H, CH, J = 10.12, 9.43 Hz), 3.33(dd, 1H, H_M, J = 14.92, 10.23 Hz), 3.06(dd, 1H, H_A, J = 14.72, 12.00 Hz). ^{13}C NMR (DMSO-d_6): δ (ppm): 160.01, 159.42, 156.96, 144.19, 131.47, 130.02, 122.17, 122.48, 118.32, 117.77, 110.65, 109.12, 100.66, 69.89, 41.50.

4.4.3. 4-(5-(4-Hydroxy-3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)benzene-1,3-diol (2c)

Yield 22%, brown powder, mp 126–127°C, IR(KBr): ν (cm^{-1}): 3309(ν_{O-H}), 1661($\nu_{C=N}$), 1591, 1556, 1434(ν_{as}), 1351(ν_{C-N}). ^1H NMR (DMSO-d_6): δ (ppm): 11.23(s, 1H, NH), 10.45(s, 1H, OH), 9.82(s, 1H, OH), 9.32(s, 1H, OH), 7.40–6.20(m, 6H, ArH), 4.91(dd, 1H, CH, J = 9.62, 8.49 Hz), 3.72(s, 3H, OCH3), 3.62(dd, 1H, HM, J = 16.15, 12.09 Hz), 3.15(dd, 1H, HA, J = 16.16, 10.09 Hz). ^{13}C NMR (DMSO-d_6): δ (ppm): 160.92, 160.18, 149.41, 146.23, 145.88, 128.69, 126.34, 122.48, 118.74, 116.33, 115.51, 114.70, 112.37, 70.23, 58.19, 41.52

4.4.4. 2-(5-(4-Hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)phenol (2d)

Yield 33%, brown powder, mp 232–234 °C, IR(KBr): ν (cm^{-1}): 3271(ν_{O-H}), 1663($\nu_{C=N}$), 1569, 1506, 1452(ν_{as}), 1387(ν_{C-N}). ^1H NMR (DMSO-d_6): δ (ppm): 12.10(s, 1H, NH), 11.13(s, 1H, OH), 10.32(s, 1H, OH), 7.30–6.50(m, 8H, ArH), 5.03(dd, 1H, CH, J = 9.83, 8.62 Hz), 3.62(dd, 1H, H_M, J = 16.31, 10.45 Hz), 3.15(dd, 1H, H_A, J = 16.34, 8.31 Hz).

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Supplementary data

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These data include MOL files and InChiKeys of the most important compounds described in this article.

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