The Synthesis of Imine Compounds with Polyphenol Structure and their Tyrosinase Inhibitory Activity

Yang Wang, Yudong Du, Zhaoming Liu, Yafei Fu, Xiao Sheng*, and Shouxin Wang

School of Pharmaceutical Sciences, Jining Medical University, Rizhao, Shandong Province, China

*Corresponding author: Xiao Sheng

Keywords: tyrosinase inhibitors; polyphenol; imine; synthesis; kinetics

Abstract: In this study, 12 imine compounds were synthesized by the condensation reaction of tyramine and 4-hydroxybenzylamine with 6 aromatic aldehydes; their structures were confirmed by ^{1}H NMR, ^{13}C NMR and MS. Using L-DOPA as the substrate, the tyrosinase inhibition activity and mechanism of the target compounds were determined by measuring the rate of oxidation of DOPA in mushroom tyrosinase and the enzyme inhibition kinetic experiment. All compounds have different degrees of tyrosinase inhibition activity, among which the activity of aromatic aldehydes tyramine compound 4a - 4f is significantly higher than that of the corresponding aromatic aldehydes 4-hydroxy benzylamine 3a - 3f. The compound 4c has the strongest tyrosinase inhibition activity; the IC_{50} value is 19.30 μmol·L^{-1}, which is significantly better than the control drug kojic acid (IC_{50} =77.58 μmol·L^{-1}). The results of inhibition mechanism and inhibition kinetics show that the inhibitory effect of 4c on tyrosinase is reversible and competitive; the K_I value is 14.61 μmol·L^{-1}. The aromatic aldehydes tyramine compounds synthesized in this study have good tyrosinase inhibitory activity, which is worthy of further study.

1. Introduction

Tyrosinase (TYR) (EC 1.14.18.1), also known as polyphenol oxidase, is a kind of multi-functional binuclear copper ion metal enzyme, which widely exists in animals, plants and microorganisms, and plays an important role in a variety of physiological and pathological processes. [1-2] TYR is the key enzyme in melanin biosynthesis. The over-expression of TYR will cause melanin accumulation, which can lead to a series of pigmentation skin diseases and even malignant melanoma. [3] The high level of TYR activity is also related to neurodegenerative diseases such as Parkinson. [4] In addition, the enzymatic browning of fruits and vegetables, the molting of insects and the healing of wounds are also directly related to the activity of TYR. [5-6] Therefore, the search for specific, efficient and safe TYR inhibitors has attracted much attention in the fields of medicine, cosmetics, agriculture and food industry. In recent years, a large number of natural and synthetic TYR inhibitors have been reported in the literature. [7-8] However, due to the weak activity or safety problems, only a few compounds such as kojic acid and arbutin are put into practical application. Therefore, it is still necessary to continuously seek and develop ideal TYR inhibitors.

At present, polyphenols are a kind of TYR inhibitors which been studied. It was found that polyphenols have good TYR inhibitory activity because they can bind to the binuclear active center of TYR or chelate the copper ion in enzyme. [9] For example, resveratrol, a natural stilbene polyphenol, as a TYR inhibitor, can effectively reduce the synthesis of melanin. [10] In recent years, researchers have modified the structure of resveratrol and synthesized several series of resveratrol analogues containing imine [11], azo [12] and coumarin [13], some of which have excellent activity of inhibiting TYR. These analogues mainly replace or modify the carbon-carbon double bond and the aromatic ring in resveratrol. In this study, we intend to change the carbon-carbon double bond in resveratrol and increase the length of the chain between the two benzene rings to investigate the impact of changes in substituent group and chain length on the activity of compounds.

In order to find and develop more polyphenol TYR inhibitors with high activity, 12 imines containing polyphenol structure were synthesized by condensation reaction of tyramine and

  DOI: 10.25236/swcas.2019.024

4-hydroxybenzylamine with 6 kinds of aromatic aldehydes respectively (see Figure 1 for synthetic route and Table 1 for structure). Their structures were characterized by ^{1}H NMR, ^{13}C NMR and MS; their TYR inhibitory activity, kinetics and structure-activity relationship were studied. The preliminary study provides a reference for the further research and development of polyphenol TYR inhibitors.

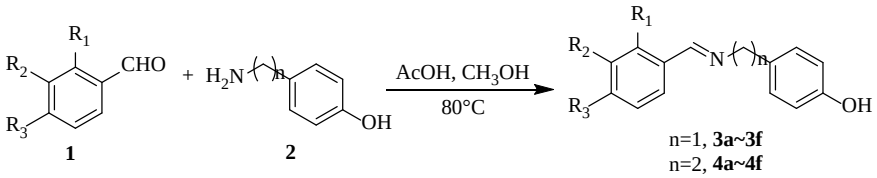

Figure 1. Synthetic Routes of Target Compounds

2. The Experiment

2.1 Instruments and reagents

The X-4 digital display micro melting point detector was produced by the Henan Gongyi Yingyu Yuhua Instrument Company; the thermometer was uncorrected. The Bruker AVANCE III 400 nuclear magnetic resonance meter was produced by the Bruker company, Switzerland. TMS was adopted as the internal standard; the DMSO-d_6 was the solvent. The Xevo G2 Q-TOF hygroplasm spectrometer was produced by Waters company, USA. The DNM-9602G enzyme standard analyzer was produced by the Beijing Perlong New Technology Company. The BSA124S electronic analytical balance was produced by the Sartorius Company, Germany. The N-1100D-WD rotary evaporator was produced by Tokyo Institute of Physical and Chemical Equipment Company, Japan. Tyramine, 4-hydroxybenzylamine, L-dopa, 3-methyl-2-benzothiazolinone hydrazone (MBTH) and various substituted benzaldehyde were all purchased from SAIN Chemical Technology (Shanghai) Company. Other reagents were all commercially available and analytical pure.

2.2 Experiment method

2.2.1 Synthesis of target compounds 3a - 3f and 4a - 4f

The synthesis of salicylaldehyde tyramine (3a) is taken as an example. The researcher needed to add 0.14 g (1 mmol) of 2, 4-dihydroxybenzaldehyde, 0.14 g (1 mmol) of tyramine and 10 mL of dry methanol into a 100 mL round bottom flask, heat and stir the solution, and promote the reaction in an oil bath of 80 ^{o}C. The reaction solution was clear initially; solid precipitated out on the wall of the flask about 1 hour after the reaction. TLC monitored the process (the developer is petroleum ether: ethyl acetate = 1:2) until the raw material reaction finished. Then the solution was then cooled to room temperature. After filtering and washing, the cold methanol became dry to get pure product. If there was no product out of the reaction solution, it should be concentrated and frozen in the refrigerator overnight.

(*E*)-2-(((4-hydroxybenzyl)imino)methyl)phenol (3a): yellow solid, yield coefficient 86.2%, m.p. 162.1 - 163 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.55(s, 1H), 9.38(s, 1H), 8.65(s, 1H),7.46(d, 1H, *J*=7.6 Hz), 7.32(t, 1H, *J*=7.8Hz), 7.13(d, 2H, *J*=8.2 Hz), 6.90(d, 1H, *J*=7.5 Hz), 6.86(d, 1H, *J*=8.4 Hz), 6.75(d, 2H, *J*=8.0Hz), 4.68(s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ:165.84, 160.66, 156.58, 132.34, 131.71, 129.14, 128.68, 118.67, 118.54, 116.46, 115.31, 61.55; ESI-MS (m/z) Calcd. for $C_{14}H_{14}NO_2$ $[M+H]^+$: 228.1019, Found 228.1042.

(E)-4-(((4-hydroxybenzyl)imino)methyl)phenol (3b): yellow solid, yield coefficient 76.5%, m.p. 101.1 - 102.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.39(s, 2H), 8.28(s, 1H), 7.57(d, 2H, *J*=8.5 Hz), 7.08(d, 2H, *J*=8.4Hz), 6.80(d, 2H, *J*=8.5Hz), 6.71(d, 2H, *J*=8.4 Hz), 4.56(s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ:160.36, 159.81, 156.15, 130.08, 129.64, 129.04, 127.45, 115.42, 115.04, 63.59; ESI-MS (m/z) Calcd. for $C_{14}H_{14}NO_2$ $[M+H]^+$: 228.1019, Found 228.1043.

(*E*)-4-(((4-hydroxybenzyl)imino)methyl)benzene-1,3-diol (3c): yellow solid, yield coefficient 88.4%, m.p. 113.5 - 114.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.86(s, 1H), 10.00(s, 1H), 9.42(s, 1H), 8.44(s, 1H), 7.20(d, 1H, *J*=8.5 Hz), 7.11(d, 2H, *J*=8.5Hz), 6.74(d, 2H, *J*=8.4Hz), 6.27(dd, 1H,

J=2.2 Hz, *J*=8.4 Hz), 6.14(d, 1H, *J*=2.2Hz), 4.58(s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ:164.96, 164.82, 161.86, 156.58, 133.47, 129.06, 128.92, 115.31, 111.23, 106.88, 102.62, 60.14; ESI-MS (m/z) Calcd. for $C_{14}H_{14}NO_3$ $[M+H]^+$: 224.0968, Found 224.0996.

(*E*)-2-(((4-hydroxybenzyl)imino)methyl)-5-methoxyphenol (3d): yellow solid, yield coefficient 84.5%, m.p. 153.6 - 154.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.98(s, 1H), 9.38(s, 1H), 8.49(s, 1H), 7.29(d, 1H, *J*=8.6 Hz), 7.13(d, 2H, *J*=8.4Hz), 6.75(d, 2H, *J*=8.4Hz), 6.38(dd, 1H, *J*=2.4 Hz, *J*=8.6 Hz), 6.30(d, 1H, *J*=2.2 Hz), 4.61(s, 2H), 3.74(s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ:165.97, 164.64, 163.38, 156.63, 133.25, 129.80, 128.62, 115.31, 111.91, 105.81, 101.04, 59.58, 55.23; ESI-MS (m/z) Calcd. for $C_{15}H_{16}NO_3$ $[M+H]^+$: 258.1125, Found 258.1146.

(*E*)-4-(((4-hydroxybenzyl)imino)methyl)-2-methoxyphenol (3e): yellow solid, yield coefficient 82.4%, m.p. 205.5 - 206.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.34(s, 2H), 8.27(s, 1H),7.34(d, 1H, *J*=1.6 Hz), 7.13(dd, 1H, *J*=1.6Hz, *J*=8.1Hz), 7.09(d, 2H, *J*=8.4Hz), 6.82(d, 1H, *J*=8.1 Hz), 6.72(d, 2H, *J*=8.4 Hz), 4.57(s, 2H), 3.78(s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ:160.54, 156.19, 149.27, 147.88, 129.99, 127.88, 122.75, 115.18, 115.06, 109.82, 63.52 55.43; ESI-MS (m/z) Calcd. for $C_{15}H_{16}NO_3$ $[M+H]^+$: 258.1125, Found 258.1147.

(E)-4-(((4-hydroxybenzyl)imino)methyl)-3-methoxyphenol (3f): yellow solid, yield coefficient 78.3%, m.p. 221 °C decompose. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.37(s, 2H), 8.55(s, 1H),7.65(d, 1H, *J*=8.5 Hz), 7.07(d, 2H, *J*=8.4 Hz), 6.71(d, 2H, *J*=8.4 Hz), 4.55(s, 2H), 6.36(d, 2H, *J*=8.5 Hz), 4.57(s, 2H), 3.78(s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ:160.16, 158.11, 156.17, 155.52, 130.15, 129.47, 128.98, 115.04, 108.42, 106.49, 98.85, 63.60, 55.34; ESI-MS (m/z) Calcd. for $C_{15}H_{16}NO_3$ $[M+H]^+$: 258.1125, Found 258.1097.

(E)-2-(((4-hydroxyphenethyl)imino)methyl)phenol (4a): yellow solid, yield coefficient 79.8 %, m.p. 146.5 - 147.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.53(s, 1H), 9.17(s, 1H), 8.45(s, 1H), 7.36(d, 1H, *J*=7.8 Hz), 7.32-7.28(m, 1H), 7.03(d, 2H, *J*=8.3 Hz), 6.86(t, 2H, *J*=7.3 Hz), 6.67(d, 2H, *J*=8.3Hz), 3.77(t, 2H, *J*=7.0Hz) , 2.82(t, 2H, *J*=7.0Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ:165.79, 160.77, 155.60, 132.17, 131.53, 129.65, 129.34, 118.53, 118.34, 116.47, 115.07, 60.03, 35.86; ESI-MS (m/z) Calcd. for $C_{15}H_{16}NO_2$ $[M+H]^+$: 242.1176, Found 242.1201.

(*E*)-4-(2-((4-hydroxybenzylidene)amino)ethyl)phenol (4b): yellow solid, yield coefficient 88.1%, m.p. 229.2 - 230.3 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.83(s, 1H), 9.17(s, 1H), 8.10(s, 1H), 7.52(d, 2H, *J*=8.5 Hz), 7.01(d, 2H, *J*=8.3 Hz), 6.79(d, 2H, *J*=8.5 Hz), 6.65(d, 2H, *J*=8.3 Hz), 3.66(t, 2H, *J*=7.3 Hz), 2.76(t, 2H, *J*=7.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ:160.18, 159.64, 155.44, 130.04, 129.67, 129.50, 127.53, 115.38, 114.96, 62.46, 36.34; ESI-MS (m/z) Calcd. for $C_{15}H_{16}NO_2$ $[M+H]^+$: 242.1176, Found 242.1197.

(*E*)-4-(((4-hydroxyphenethyl)imino)methyl)benzene-1,3-diol (4c): yellow solid, yield coefficient 85.4%, m.p. 176.5 - 177.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.76(s, 1H), 9.39(s, 2H), 8.23(s, 1H), 7.10(d, 1H, *J*=8.5 Hz), 7.01(d, 2H, *J*=8.4 Hz), 6.66(d, 2H, *J*=8.4 Hz), 6.22(dd, 1H, *J*=2.2 Hz, *J*=8.4 Hz), 6.13(d, 1H, *J*=2.2 Hz), 3.67(t, 2H, *J*=7.0 Hz), 2.78(t, 2H, *J*=7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ:165.33, 164.68, 161.84, 155.63, 133.28, 129.65, 129.29, 115.07, 111.05, 106.67, 102.67, 58.48, 35.99; ESI-MS (m/z) Calcd. for $C_{15}H_{16}NO_3$ $[M+H]^+$: 258.1125, Found 258.1148.

(*E*)-2-(((4-hydroxyphenethyl)imino)methyl)-5-methoxyphenol (4d): yellow solid, yield coefficient 80.7 %, m.p. 161.2 - 162 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.91(s, 1H), 9.18(s, 1H), 8.27(s, 1H),7.19(d, 1H, *J*=8.6 Hz), 7.02(d, 2H, *J*=8.4Hz), 6.66(d, 2H, *J*=8.4Hz), 6.32(dd, 1H, *J*=2.4 Hz, *J*=8.6 Hz), 6.27(d, 1H, *J*=2.3 Hz), 3.73(s, 3H), 3.70(t, 2H, *J*=7.0 Hz), 2.80(t, 2H, *J*=7.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ:166.63, 164.56, 163.39, 155.65, 133.14, 129.67, 129.13, 115.08, 111.71, 105.59, 101.11, 57.85, 55.16, 35.85; ESI-MS (m/z) Calcd. for $C_{16}H_{18}NO_3$ $[M+H]^+$: 272.1281, Found 272.1302.

(*E*)-4-(((4-hydroxyphenethyl)imino)methyl)-2-methoxyphenol (4e): yellow solid, yield coefficient 90.3%, m.p. 225.1 - 225.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.29(s, 2H), 8.09(s, 1H),7.31(d, 1H, *J*=1.5 Hz), 7.07(dd, 1H, *J*=1.6Hz, *J*=8.1Hz), 7.02(d, 2H, *J*=8.4Hz), 6.80(d, 1H, *J*=8.0 Hz), 6.66(d, 2H, *J*=8.4 Hz), 3.80(s, 3H), 3.67(t, 2H, *J*=7.4 Hz), 2.77(t, 2H, *J*=7.4 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ:160.38, 155.47, 149.20, 147.86, 130.00, 129.67, 127.94, 122.52, 115.17, 114.98, 109.81, 62.42, 55.43, 36.35; ESI-MS (m/z) Calcd. for C16H18NO3 [M+H] + :

272.1281, Found 272.1306.

(E)-4-(((4-hydroxyphenethyl)imino)methyl)-3-methoxyphenol (4f): yellow solid, yield coefficient 85.3 %, m.p. 212.5 - 213.4 °C. 1H NMR (400 MHz, DMSO-d6) δ: 9.84(s, 1H), 9.19(s, 1H), 8.36(s, 1H),7.62(s, 1H), 7.01(d, 2H, *J*=7.4Hz), 6.65(d, 2H, *J*=7.3 Hz), 6.36(s, 2H), 3.75(s, 3H), 3.64(t, 2H, *J*=6.8 Hz), 2.74(t, 2H, *J*=6.9 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.44, 155.37, 129.95, 129.65, 114.95, 98.86, 55.28, 36.40; ESI-MS (m/z) Calcd. for $C_{16}H_{18}NO_3$ $[M+H]^+$: 272.1281, Found 272.1257.

2.2.2 TYR activity inhibition experiment

According to the method reported in literature [11], the inhibitory activity of 12 target compounds on TYR of mushroom was tested. L- DOPA was used as substrate and kojic acid was the positive control drug. Finally, the inhibition rate of TYR was calculated.

TYR inhibition rate = $[1- (A_1 - A_2)/(A_3 - A_4)]\times100\%$.

A1: the absorbance measured by the mixture with inhibitor and enzyme; *A2*: the absorbance measured by the mixture with inhibitor but without enzyme; *A3*: the absorbance measured by the mixture with enzyme but without inhibitor; *A4*: the absorbance measured by the mixture without inhibitor or enzyme. Each experiment was repeated for three times; the IC_{50} value of the compound was calculated by the software of graphpad prism 6.

2.2.3 Determination of the inhibition mechanism of TYR activity

The concentration of L- DOPA was fixed; TYR was diluted to 4 concentrations of 2.7, 5.4, 8.1 and 10.7 μg·mL^{-1}. The inhibitor was diluted to solutions with the final concentration of 5, 10, 20 and 40 μmol· L^{-1} respectively. By measuring the relationship between the speed of enzymatic reaction and the mass concentration of enzyme, as well as the effect of inhibitors with different molar concentrations on the enzymatic reaction (catalytic oxidation of L- DOPA), and then mapping with the mass concentration of enzyme as the x-axis and enzyme activity as the Y-axis, we can determine the type of inhibition mechanism of chemical inhibitors on enzyme.

2.2.4 Determination of TYR inhibition activity type

The concentration of the enzyme was fixed as 10.7 μg·mL^{-1}; the compound 4c was diluted to solutions with the final concentration of 5, 10, 15 and 20 μmol·L^{-1} respectively. The L- DOPA was prepared to solutions with the concentration of 0.5, 0.75, 1, 1.5 and 2 mmol·L^{-1}. The effect of inhibitors with different molar concentrations on TYR activity was determined. The inhibition type of inhibitors was determined by the method of Lineweaver-Burk double reciprocal graph. The obtained slope and intercept of the straight line were used for secondary mapping of the sample concentration, and the corresponding inhibition constants were calculated through the data of slope and intercept in the second mapping.

3. Results and Discussion

3.1 Synthesis structural characterization

The target compounds were synthesized by the conventional method of imine synthesis. The amount of tyramine, 4-hydroxybenzylamine and aromatic aldehydes were mixed; the catalytic amount of acetic acid was added, and then refluxed in methanol. Most of the target compounds can be directly separated from the reaction liquid, and the pure products can be obtained after cooling, filtration, washing and drying. Compounds 3a, 3e, 4a and 4e cannot be separated from the reaction solution directly. The reaction solution was concentrated and frozen overnight in the refrigerator. The target compounds 3a - 3f and 4a - 4f all obtained the unique imine products with (*E*) - configuration; the structures were confirmed by ^{1}H NMR, ^{13}C NMR and MS analysis, which was consistent with the spectral data of similar compounds in literature. [14]

3.2 The inhibition TYR activity of target compounds and the relationship between their structures and activity

From the results in Table 1, it is not difficult to find that the TYR inhibitory activity of aromatic aldehydes tyramine compound 4a - 4f is significantly higher than that of the corresponding aromatic aldehydes 4-hydroxy benzylamine 3a - 3f, indicating that the chain length between the two benzene rings has great effects on the activity of target compounds. After adding a carbon atom, the activity of the compounds can be enhanced obviously. One possible reason is that the structure of compound 4a - 4f is close to that of L-DOPA, the substrate of TYR, and can competitively chelate with copper ion in the active center of TYR. Among them, compounds 4a, 4c and 4d have strong inhibitory activity on TYR, which is superior to the control group of kojic acid. It shows that the activity of the target compound can be effectively enhanced when there are hydroxyl groups in the 2-position of aromatic aldehyde, and that the activity is the strongest when there are hydroxyl groups in the 2,4-position. The activity of 4-hydroxybenzylamine 3a - 3f was weaker than that of kojic acid, and the activity of compound 3b was the strongest when there were para hydroxyl groups in aromatic aldehydes.

Table 1. Structures of Target Compounds and Results of TYR Inhibition Activity

Compd.	n	R_1	R_2	R_3	IC_{50} ($\mu mol \cdot L^{-1}$)	Compd.	n	R_1	R_2	R_3	IC_{50} ($\mu mol \cdot L^{-1}$)
3a	1	OH	H	H	297.50	4a	2	OH	H	H	68.27
3b	1	H	H	OH	143.12	3b	2	H	H	OH	34.87
3c	1	OH	H	OH	307.71	4c	2	OH	H	OH	19.30
3d	1	OH	H	OCH_3	575.21	4d	2	OH	H	OCH_3	32.51
3e	1	H	OCH_3	OH	>1000	4e	2	H	OCH_3	OH	147.7
3f	1	OCH_3	H	OH	456.91	4f	2	OCH_3	H	OH	56.83
Kojic acid					77.58						

3.3 Determination of the TYR inhibition mechanism of compound 4c

In order to investigate the mechanism of TYR inhibition activity of target compounds, we selected the compound 4c with the best activity, and measured its effect on the activity of enzyme catalyzed oxidation of L-DOPA at different concentrations. The concentration of TYR was mapped with enzyme activity, and a group of straight lines were obtained through the origin, as shown in Figure 2. With the increase of 4c concentration in the system, the slope of the straight line decreased gradually, indicating that the binding of compound 4c to TYR is a reversible process. The decrease of enzyme activity is not caused by the decrease of TYR amount, but by the inhibition of compound 4c on TYR.

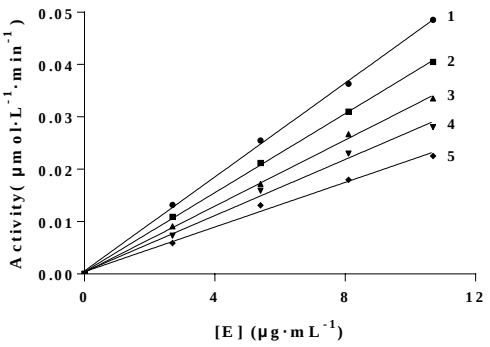

Figure 2. Curve of the Inhibition Mechanism of Compound 4c; Concentrations of Curve 1-5 are 0, 5, 10, 20 and 40 $\mu mol \cdot L^{-1}$ respectively

In order to further investigate the inhibition type of the target compound on TYR activity, in the activity measurement system, the concentration of TYR was fixed. The concentration of L-DOPA

was changed to determine the influence of different concentrations of compound 4c on the enzyme activity. The Lineweaver-burk double reciprocal was used as the diagram to determine the inhibition type of compound 4c on tyrosinase. The results are shown in Figure 3A. In the figure, a group of straight lines intersect a point on the y-axis; the intercept between the straight line and the y-axis does not change with the change of the concentration of compound 4c; the slopes show an upward trend. This shows that the Michaelis constant (K_m) increases with the increase of the concentration of compound 4c; the maximum reaction speed (V_m) of enzymatic reaction remains unchanged. The above results show that the inhibition type of compound 4c to tyrosinase is competitive inhibition. Comparing the structure of 4c and L- DOPA, we can find that they have some similarity in structure. Therefore, it is speculated that compound 4c can compete with substrate L- DOPA and reversibly bind to TYR, reduce the binding probability of substrate and enzyme active center, and effectively inhibit the activity of enzyme. Figure 3B is obtained by plotting the concentration of compound 4c. With the slope of the straight line, we can get the inhibition constant K_I of 14.61 μmol·L^{-1}.

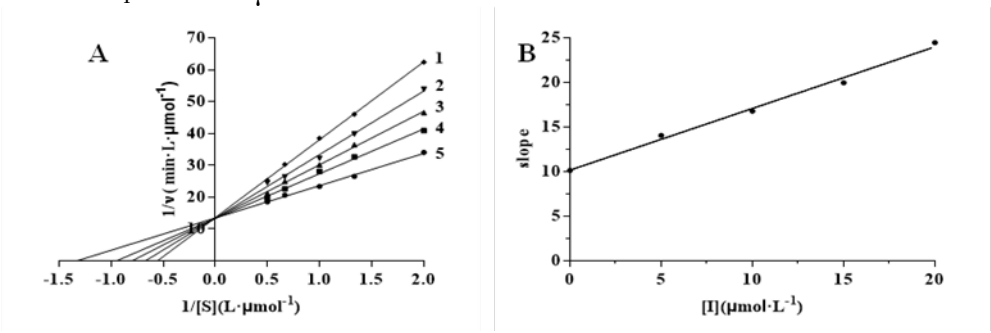

Figure 3. (A) inhibition type of compound 4c; (B) the effect of compound 4c concentration on Michaelis constant. In figure A, the concentrations of curves 1-5 are 20, 15, 10, 5 and 0 μmol·L^{-1} respectively.

4. Conclusion

In this experiment, 12 imine compounds were synthesized by the condensation reaction of tyramine and 4-hydroxybenzylamine with 6 aromatic aldehydes. The preliminary activity test results show that the TYR inhibitory activity of aromatic aldehydes tyramine compound 4a - 4f is significantly higher than that of the corresponding aromatic aldehydes 4-hydroxy benzylamine 3a - 3f, indicating that the chain length between the two benzene rings has great effects on the activity of the target compounds. After adding a carbon atom, the activity of the compound can be enhanced obviously. The compound 4c has the strongest inhibitory activity on tyrosinase, which is obviously superior to the control drug, kojic acid. The preliminary study on mechanism and kinetic shows that the inhibitory effect of 4c on tyrosinase is reversible competitive inhibition. In conclusion, the aromatic aldehydes tyramines and imines have good inhibitory activity on TYR; they can be used as the new lead compound of TYR inhibitors.

Acknowledgement

In this paper, the research was sponsored by the Foundation for Projects of the National Undergraduate Training Program for Innovation and Entrepreneurship; the Project Number is 201610443008. It was sponsored by the Foundation for Jining Medical University Students' Research Project; the Project Number is JYXS2017KJ024. It was also sponsored by the Foundation for Projects of the Jining Medical University Students' Innovation Training Program; the Project Number is cx2016008.

References

[1] Casanola-Martin G M, Le-Thi-Thu H, Marrero-Ponce Y, Castillo-Garit J A, Torrens F,

Rescigno A, Abad C, Khan M T. Tyrosinase Enzyme: 1. An overview on a Pharmacological Target [J]. Curr Top Med Chem. 2014 (14): 1494-1501.

[2] Lai X, Wichers H J, Soler-Lopez M, Dijkstra B W. Structure and Function of Human Tyrosinase and Tyrosinase-related Proteins [J]. Chem-Eur J. 2018 (24): 47-55.

[3] Buitrago E, Hardré R, Haudecoeur R, Jamet H, Belle C. Boumendjel A, Bubacco L, Réglier M. Are human Tyrosinase and related proteins suitable targets for melanoma therapy? [J]. Curr Top Med Chem. 2016 (16): 3033-3047.

[4] Bizzarri B M, Martini A, Serafini F, Aversa D, Piccinino D, Botta L, Berretta N, Guatteo E, Saladino R. Tyrosinase Mediated Oidative Functionalization in the Synthesis of DOPA-derived Peptidomimetics with Anti-Parkinson Activity [J]. RSC Adv. 2017 (7): 20502-20509.

[5] Shao L L, Wang X L, Chen K, Dong X W, Kong L M, Zhao D Y, Hider R C, Zhou T. Novel Hydroxypyridinone Derivatives Containing an Oxime Ether Moiety: Synthesis, Inhibition on Mushroom Tyrosinase and Application in Anti-browning of Fresh-cut Apples [J]. Food Chem. 2018 (242): 174-181.

[6] Sugumaran M. Comparative Biochemistry of Eumelanogenesis and the Protective Roles of Phenoloxidase and Melanin in Insects [J]. Pigment Cell Res. 2002 (15): 2-9.

[7] Zolghadri S, Bahrami A, Hassan Khan M T, Munoz-Munoz J, Garcia-Molina F, Garcia-Canovas F, Saboury A A. A Comprehensive Review on Tyrosinase Inhibitors [J]. J Enzyme Inhib Med Chem. 2019 (34): 279-309.

[8] Lee S, Baek N, Nam T. Natural, Semisynthetic and Synthetic Tyrosinase Inhibitors [J]. J Enzyme Inhib Med Chem. 2016 (31): 1-13.

[9] Akhtar M N, Sakeh N M, Zareen S, Gul S, Lo K M, Ul-Haq Z, Shah S A A, Ahmad S. Design and Synthesis of Chalcone Derivatives as Pot Z. ent Tyrosinase Inhibitors and Their Structural Activity Relationship [J]. J Mol Struct. 2015 (1085): 97-103.

[10] Park J, Boo Y C. Isolation of Resveratrol from Vitis Viniferae Caulis and its Potent Inhibition of Human Tyrosinase [J]. Evid Based Complement Alternat Med. 2013 (2013).

[11] Bae S J, Ha Y M, Park Y J, Park J Y, Song Y M, Ha T K, Chun P, Moon H R, Chung H Y. Design, Synthesis, and Evaluation of (E)-N-substituted Benzylideneeaniline Derivatives as Tyrosinase Inhibitors [J]. Eur J Med Chem. 2012 (57): 383-390.

[12] Song Y M, Ha Y M, Kim J A, Chung K W, Uehara Y, Lee K J, Chun P, Byun Y, Chung H Y, Moon H R. Synthesis of Novel Azo-resveratrol, Azo-oxyresveratrol and their Derivatives as Potent Tyrosinase Inhibitors [J]. Bioorg Med Chem Lett. 2012 (22): 7451-7455.

[13] Fais A, Corda M, Era B, Fadda M B, Matos M J, Quezada E, Santana L, Picciau C, Podda G, Delogu G. Tyrosinase Inhibitor Activity of Coumarin-resveratrol Hybrids [J]. Molecules. 2009 (14): 2514 - 2520.

[14] Youn S W. Development of the Pictet-Spengler Reaction Catalyzed by AuCl3/AgOTf [J]. J Org Chem. 2006 (71): 2521-2523.