Egypt. J. Chem. **Vol. 68**, No. 2 pp.39 – 46 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Synthesis and Bioactivity Study of Mannich Base Derivatives of Ferulic Acid as a Tyrosinase Inhibitor and Antioxidant

Lin Hofa Nurul Alifah[a], Haezel Michel Chandra[a], Rafael Rafael[a], Catur Jatmika[a], Hayun Hayun[a*]

[a] *Faculty of Pharmacy, Universitas Indonesia, Depok 16424, West Java, Indonesia*

Abstract

Ferulic acid is reported to have antioxidant and anti-tyrosinase. However, it tends to change its colour because of undergoes autooxidation, decarboxylation, and dimerization. Structural modification through Mannich base substitution could change the compound's physicochemical properties and biological activity. This study aims to synthesize novel Mannich base derivatives of ferulic acid (**3a-f**) and evaluate them for their tyrosinase inhibitory and antioxidant activity. The study indicated that all the compounds exhibited tyrosinase inhibitor and antioxidant activity. Compound **3b** has the highest tyrosinase inhibitory activity (IC_{50}: 312.60 µM), higher than ferulic acid (IC_{50}: 345.95 µM) but lower than a standard used (kojic acid, IC_{50}: 46.34 µM). Compound **3e** has the highest antioxidant activity (IC_{50}:15.98 µM), higher than ferulic acid (IC_{50}: 22.04 µM), and equal to ascorbic acid used as standard (IC50: 15.26 µM).

Keywords: Ferulic acid, Mannich base, Antioxidant, DPPH, Tyrosinase inhibitor, Anti-tyrosinase.

1. Introduction

Melanin is a pigment used to colour the eye, skin, and hair and as a barrier against harmful UV radiation [1]. UV exposure encourages melanin formation in the skin and causes inflammation, freckles, brown patches, and age spots. Melanin is produced in melanocytes by oxidizing L-DOPA to dopaquinone catalyzed by tyrosinase enzyme [2]. The most popular method to terminate melanin production is by blocking tyrosinase activity. In addition, based on a previous report, the mechanism of melanogenesis is related to radical oxidative stress [3]. Thus, the antioxidant properties compound could be a potential anti-tyrosinase.

Ferulic acid is well known as an antioxidant. In addition to being a free radical scavenger, ferulic acid also increases the activity of scavenger enzymes and inhibits the enzymes that cause the production of free radicals [4]. It has a unique structure containing phenyl with o-methoxy and p-hydroxyl, ethylenic chain, and carboxylic group [5-6] Its distinctive structure contributes significantly to its activity as an antioxidant. The structure of ferulic acid contains a hydrogen-donating group that can produce stable phenol radicals [5]. The advantages of these structural characteristics are utilized in cosmetic preparation formulas as sunscreen agents to absorb UV light at a wavelength of 290-320 nm, anti-aging, anti-wrinkle, and skin-lightening agents because of inhibition of the enzyme tyrosinase activity and melanocyte proliferation [5,7].

The physicochemical properties of ferulic acid are less favourable. It has low bioavailability and poor water stability [8]. Exposure to moisture, oxygen, high temperatures, and light during storage causes ferulic acid to change colour and develop an unpleasant odour. It undergoes autooxidation [9], decarboxylation, and dimerization [7], forming dimer anions with lower pharmacological activity. Providing nitrogen gas or adding other antioxidants to ferulic acid preparations does not prevent the degradation process [10-13]

The stability improvements of ferulic acid have been made by modifying the structure, including through encapsulation with poly(anhydride-ester) [7], esterification [14-15] and amidation [16-18]. However, the results were not optimal, and the ester product showed a cytotoxic effect.

Mannich base derivatives of phenolic compounds thermodynamically show better stability due to intramolecular hydrogen bonds between the hydroxy group and N of the amine [19]. However, the Mannich base substitution may affect their biological activity. For example, the Mannich derivatives of the vanillin-dopamine compound had better antioxidant activity than standard BHT and, as a tyrosinase inhibitor, was more effective than kojic acid [20]. Likewise, the anti-tyrosinase activity of several kojic acid derivative compounds substituted with Mannich bases was more effective than unsubstituted kojic acid [21].

To further study the effect of Mannich base substitution on biological activity, we report here the synthesis and bioactivity study of Mannich base derivatives of ferulic acid as a tyrosinase inhibitor and antioxidant.

*Corresponding author e-mail: hayun@farmasi.ui.ac.id; (Hayun Hayun)
Receive Date: 06 April 2024, Revise Date: 29 June 2024, Accept Date: 15 July 2024
DOI: 10.21608/ejchem.2024.281898.9561
©2025 National Information and Documentation Center (NIDOC)

2. Experimental

2.1. General

Ferulic acid was prepared by Knoevenagel condensation, as reported earlier [22]. The chemicals and reagents were analytical grades purchased from commercial suppliers (Sigma Aldrich and Merck). A purity test and reaction monitoring were performed using the TLC method. The melting points' products were measured using melting point equipment (Stuart Scientific). The IR and NMR spectra were taken by FTIR Spectrophotometer (Shimadzu 8400S) and NMR Spectrometer (JEOL JNM 500), respectively. Mass spectra were determined by LC-MS/MS with the ESI+ method (Xevo G2-S QToF, Waters, USA).

*2.2. Synthesis of Vanillin Mannich Base Derivatives (**2a-f**)*

The compounds (**2a-f**) were synthesized by amino alkylation of vanillin reported earlier [23]. Paraformaldehyde (1.5 eq) and sec-amine (1.2 eq) were mixed in ethanol (20 mL) and refluxed at 80ºC for 1 h. Then vanillin (1 eq) was added, refluxed, and stirred until a complete reaction for 2-8 h. After that, the solvent was reduced to 1/3 volume, refrigerated overnight, filtered the precipitate, and washed with ethanol to afford pure **2a-f**.

4-Hydroxy-3-methoxy-5-(morpholinomethyl) benzaldehyde (2a). Pinkish-white powder; 56,95% yield (143.1 mg); mp 98-100ºC; FTIR (cm^{-1}): 2949 (CH Al), 2837 (CHO), 1651 (HC=O), 1593 & 1471 (C=C Ar), 1274 (C-O), 1144 (C-N), 1062 (C_{Ar}-O). ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm = 9.79 (s, 1H, CHO), 7.36 (d, *J* = 1.5 Hz, 1H, ArH), 7.18 (d, *J* = 1.5 Hz, 1H, ArH), 3.95 (s, 3H, CH_3-O), 3.82 (s, 2H, ArCH_2-N), 3.78 (s, 4H, -CH_2-O); 2.63 (4H, -CH_2-N).

3-((2,6-Dimethylmorpholino)methyl)-4-hydroxy-5-methoxybenzaldehyde (2b). White powder, 44.51% yield (124.3 mg), mp 120-122ºC, FTIR (cm^{-1}): 2938 (CH Al), 2878 (CHO), 1677 (HC=O), 1586 & 1431 (C=C Ar), 1279 (C-O), 1143 (C-N), 1070 (C_{Ar}-O). ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm = 9.79 (s, 1H, CHO), 7.36 (d, *J* = 1.7 Hz, 1H, ArH), 7.17 (d, *J* = 1.4 Hz, 1H, ArH), 3.95 (s, 3H, CH_3-O), 3.88 (ArCH_2-N), 3.75-3.69 (m, 2H, 2 -CH-O), 2.85-2.83 (d, 2H, -CH_2-N), 1.95-1.91 (t, 2H, -CH_2-N), 1.18 (d, 6H, C-CH_3) [23].

3-((Dimethylamino)methyl)-4-hydroxy-5-methoxy benzaldehyde (**2c)**. Pale-white powder, 75.43% yield (157.8 mg), mp 138-140ºC, FTIR (cm^{-1}): 2941 (CH Al), 2832 (CHO), 1641 (HC=O), 1589 & 1431 (C=C Ar), 1265 (C-O), 1144 (C-N), 1039 (C_{Ar}-O).). ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm = 9.77 (s, 1H, CHO), 7.35 (d, *J* = 1.7 Hz, 1H, ArH), 7.15 (d, *J* = 0.9 Hz, 1H, ArH), 3.94 (s, 3H, CH_3-O), 3.76 (s, 2H, ArCH_2-N), 2.38 (s, 6H, (N-CH_3)$_2$.

3-((Diethylamino)methyl)-4-hydroxy-5-methoxy benzaldehyde (2d). Yellow powder, 64.99% yield (118.2 mg), mp 73-75ºC, FTIR (cm^{-1}): 2940 (CH Al), 2832 (CHO), 1647 (HC=O), 1589 & 1452 (C=C Ar), 1273 (C-O), 1144 (C-N), 1038 (C_{Ar}-O). ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm = 9.76 (s, 1H, CHO), 7,33 (d, *J* = 1.7 Hz, 1H, ArH), 7.15 (d, *J* = 1.7 Hz, 1H, ArH), 3.93 (s, 3H, CH_3-O), 3.88 (s, 2H, ArCH_2-N), 2.68 (m, 4H, N-(CH_2-C)$_2$, 1.15 (t, 6H, (C-CH_3)$_2$.

4-Hydroxy-3-methoxy-5-(pyrrolidine-1-ylmethyl) benzaldehyde (2e). Orange powder, 61.34% yield (144.3 mg); mp 130-132ºC, FTIR (cm^{-1}): 2985 (CH Al), 2857 (CHO); 1672 (HC=O), 1588 &1435 (C=C Ar), 1221 (C-O), 1137 (C-N), 1085 (C_{Ar}-O); ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm = 9.76 (s, 1H, CHO), 7.33 (d, *J* = 1.5 Hz, 1H, ArH), 7.16 (d, *J* = 0.5 Hz, 1H, ArH), 3.95 (s, 2H, ArCH_2-N), 3.94 (s, 3H, CH_3-O); 2.70 (m, 4H, -CH_2-N-CH_2-), 1.89 (m, 4H, (-CH_2-CH_2-).

4-Hydroxy-3-methoxy-5-((4-methylpiperazin-1-yl) methyl)benzaldehyde (2f). White powder, 57.08% yield (150.8 mg), mp 116-118ºC, FTIR (cm^{-1}): 2985 (CH Al), 2810 (CHO), 1672 (HC=O), 1459 (C=C Ar), 1282 (C-O), 1151 (C-N), 1071 (C_{Ar}-O). ^{1}H-NMR (500 MHz, $CDCl_3$): δ/ppm = 9.78 (s, 1H, CHO), 7.35 (d, *J* = 1.7 Hz, 1H, ArH), 7.17 (d, *J* = 1.6 Hz, 1H, ArH), 3.94 (s, 2H, ArCH_2-N), 3.83 (s, 3H, CH_3-O), 2.40-3.20 (m, 8H, (-CH_2-N-CH_2-)$_2$), 2.31 (s, 3H, N-CH_3).

*2.3. Synthesis of Ferulic Acid Mannich Base Derivatives (**3a-f**)*

The compounds (**3a-f**) were synthesized by adapting the Ferulic acid method synthesis [22]. A mixture of vanillin Mannich base derivatives (**2a-f)** (1 eq), malonic acid (1.2 eq), and ammonium bicarbonate (0.4 eq) dissolved in a minimum volume of solvent (ethanol or ethyl acetate). The solvent was removed by vacuum oven at 40ºC. The solid reaction was heated at 90ºC for 2 h, diluted in methanol, and cooled in a refrigerator. The precipitate gained was filtered and purified by recrystallization or column chromatography afforded pure **3a-f**.

I-3-(4-Hydroxy-3-methoxy-5-(morpholinome-thyl)phenyl)acrylic acid (3a). Pale-white powder, 36.85% yield (43.01 mg), mp 148-150ºC**.** FTIR (cm^{-1}): 3371 (OH, COOH), 2987 & 2869 (CH Al), 1642 (C=O, COOH), 1597 & 1428 (C=C Ar), 1284 (C-O), 1156 (C-N), 1054 (C_{Ar}-O). ^{1}H-NMR (500 MHz, DMSO-d_6): δ/ppm = 7.47 (d, *J* = 15.9 Hz, 1H, C=CH-Ar), 7.24 (d, *J* = 1.2 Hz, 1H, ArH), 7.05 (d, *J* = 1.3 Hz, 1H, ArH); 6.38 (d, *J* = 15.9 Hz, 1H, C=CH-C=O), 3.81 (s, 3H, CH_3-O), 3.62 (s, 2H, ArCH_2-N), 3.60 (s, 4H, (CH_2-O)$_2$, 2.45 (s, 4H, (-CH_2-N)$_2$. ^{13}C-NMR (125 MHz, DMSO-d_6): δ/ppm = 168.46 (C=O, COOH), 148.79, 148.15, 145.01, 125.39, 123.66, 122.71, 116.23, 110.33, 66.52, 58.73, 56.20, 53.08. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ calcd for $C_{15}H_{20}NO_5$ 294.1341; found 294.1359; Error 0.0018 m/z unit.

I-3-(3-((2,6-Dimethylmorpholino)methyl)-4-hydroxy-5-methoxyphenyl)acrylic acid (3b). Yellow powder, 28.74% yield (33.06 mg), mp 194-196ºC. FTIR (cm^{-1}): 3213 (OH, COOH); 2972 & 2877 (CH Al), 1655 (C=O, COOH), 1575 & 1430 (C=C Ar), 1296 (C-O), 1157 (C-N), 1053 (C_{Ar}-O). ^{1}H-NMR (500 MHz, DMSO-d_6): δ/ppm = 7.48 (d, *J* = 15.9 Hz, 1H, C=CH-Ar), 7.24

(d, *J* = 1 Hz, 1H, ArH), 7.03 (d, *J* = 1 Hz, 1H, ArH), 6.38 (d, *J* = 15.9 Hz, 1H, C=CH-C=O), 3.81 (s, 3H, CH_3-O), 3.60 (s, 2H, $ArCH_2$-N), 3.54-3.58 (m, 2H, $(-CH-O)_2$, 2.74-276 (d, 2H, $-CH_2$-N), 1,73-1.77 (t, 2H, $-CH_2$-N), 1.04-1.05 (d, 6H, C-CH_3) [24]. ^{13}C-NMR (125 MHz, DMSO-d_6): δ/ppm = 168.47 (C=O, COOH), 148.90, 148.14, 145.01, 125.39, 122.67, 116.22, 110.31, 71.45, 62.59, 58.57, 56.15, 19.33. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ calcd for $C_{17}H_{24}NO_5$ 322.1654; found 322.1646; Error 0.0008 m/z unit.

I-3-(3-((Dimethylamino)methyl)-4-hydroxy-5-methoxyphenyl)acrylic acid (3c). Brown powder, 21.13% yield (25.56 mg), mp 146-148ºC. FTIR (cm^{-1}): 3246 (OH, COOH), 2922 & 2853 (CH Al), 1638 (C=OOH), 1595 & 1431 (C=C Ar), 1288 (C-O), 1161 (C-N), 1086 (C_{Ar}-O). ^{1}H-NMR (500 MHz, DMSO-d_6): δ/ppm = 7.38 (d, *J* = 15.8 Hz, 1H, C=CH-Ar), 7.16 (d, *J* = 2.1 Hz, 1H ArH), 6.95 (d, *J* = 2.1 Hz, 1H, ArH), 6.35 (d, *J* = 15.9 Hz, 1H, C=CH-C=O), 3.79 (s, 3H, CH_3-O), 3.59 (s, 2H, $ArCH_2$-N), 2.24 (s, 6H, $(N-CH_3)_2$. ^{13}C-NMR (125 MHz, DMSO-d_6): δ/ppm = 169.41 (C=O, COOH), 149.24, 148.15, 143.28, 125.68, 123,25, 122.52, 118.45, 110.49, 60.73, 56.14, 44.58. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ calcd for $C_{13}H_{18}NO_4$ 252.1236; found 252.1243; Error 0.0007 m/z unit.

I-3-(3-((Diethylamino)methyl)-4-hydroxy-5-me-thoxyphenyl)acrylic acid (3d). Yellowish-brown powder, 14.88% yield (17.49 mg), mp 162-164ºC. FTIR (cm^{-1}): 3331 (OH, COOH), 2969 & 2838 (CH Al), 1638 (C=O, COOH), 1559 & 1429 (C=C Ar), 1289 (C-O), 1161 (C-N), 1082 (C_{Ar}-O). ^{1}H-NMR (500 MHz, DMSO-d_6): δ/ppm = 7.39 (d, *J* = 15.9 Hz, 1H, C=CH-Ar), 7.15 (d, *J* = 1.9 Hz, 1H, ArH), 6.96 (d, *J* = 1.8 Hz, 1H, ArH), 6.34 (d, *J* = 15.9 Hz, 1H, C=CH-C=O), 3.76 (s, 3H, CH_3-O), 3.74 (s, 2H, $ArCH_2$-N), 2.56 (m, 4H, $-CH_2$-N), 1,03 (t, 6H, CH_3-C). ^{13}C-NMR (125 MHz, DMSO-d_6): δ/ppm = 168.62 (C=O, COOH), 150.32, 148.20, 144.69, 125.11, 123.16, 122.57, 116.38, 110.68, 56.13, 55.55, 46.16, 11.41. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ calcd for $C_{15}H_{22}NO_4$ 280.1549; found 280.1559; Error 0.001 m/z unit.

I-3-(4-Hydroxy-3-methoxy-5-(pyrrolidine-1-ylme-thyl)phenyl)acrylic acid (3e). Yellow powder, 26,64% yield (31.4 mg), mp 156-158ºC. FTIR (cm^{-1}): 3340 (OH, COOH); 2985 & 2958 (CH Al), 1650 (C=OOH), 1497 & 1428 (C=C Ar), 1278 (C-O), 1154 (C-N), 1080 (C_{Ar}-O). ^{1}H-NMR (500 MHz, DMSO-d_6): 7.45 (d, *J* = 14 Hz, 1H, C=CH-Ar), 7.21 (d, *J* = 1.5 Hz,1H, ArH), 7.02 (d, *J* = 1.5 Hz, 1H, ArH), 6.35 (d, *J* = 15 Hz, 1H, C=CH-C=O), 3.80 (s, 3H, CH_3-O), 3.79 (s, 2H, $ArCH_2$-N), 2.58 (m, 4H, $-CH_2$-N-CH_2-), 1.76 (m, 4H, $(-CH_2-CH_2-)$. ^{13}C-NMR (125 MHz, DMSO-d_6): δ/ppm = 167.98 (C=O, COOH), 148.89, 147.56, 144.32, 122.23, 124.53, 122.93, 115.65, 109.88, 55.88, 55.57, 52.83, 23.02. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ calcd for $C_{15}H_{20}NO_4$ 278.1392; found 278.1398; Error 0.0006 m/z unit.

I-3-(4-Hydroxy-3-methoxy-5-((4-methylpipera-zin-1-yl)methyl)phenyl)acrylic acid (3f). Yellow powder, 52,8% yield (61.21 mg), mp 166-168ºC. FTIR (cm^{-1}): 3250 (OH, COOH), 2873 and 2810 (CH Al), 1672 (C=O, C=OOH), 1592 & 1493 (C=C Ar), 1282 (C-O), 1152 (C-N), 1072 (C_{Ar}-O). ^{1}H-NMR (500 MHz, DMSO-d_6): 7.47 (d, *J* = 15.9 Hz, 1H, C=CH-Ar), 7.21 (d, *J* = 1.5 Hz, 1H, ArH), 7,01 (d, *J* = 1.3 Hz, 1H, ArH), 6.36 (d, *J* = 15.9 Hz, 1H, C=CH-C=O), 3.83 (s, 2H, $ArCH_2$-N); 3.79 (s, 3H, CH_3-O), 3.64 (m, 3H, CH_3-N); 2.23-2.53 (m. 8H, $(-CH_2-N-CH_2-)_2$. ^{13}C-NMR (125 MHz, DMSO-d_6): δ/ppm = 168.59 (C=O, COOH), 149.07, 148.19, 144.86, 125.40, 123.18, 122.71, 116.40, 110.33, 58.78, 56.14, 54.76, 52.18, 45.63. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ calcd for $C_{16}H_{23}N_2O_4$ 307.1658; found 307.1669; Error 0.0011 m/z unit.

2.4. Anti-tyrosinase Activity Assay

The method previously reported [25] with kojic acid as a standard and mushroom tyrosinase as an enzyme was used to evaluate the tyrosinase inhibitory activity of the synthesized compounds (**3a-f**) and ferulic acid. In 96 well plates contain mixtures of phosphate buffer (50 mM, pH 6.5) (80 µL), tested or standard solution (40 µL), L-DOPA solution (3 mM) (40 µL), and tyrosinase enzyme solution (175 U/mL) (40 µL); the above mixture without tyrosinase enzyme as sample control; the mixture of phosphate buffer (50 mM, pH 6.5) (120 µL), L-DOPA solution (4 mM) (40 µL), and tyrosinase enzyme solution (75 U/mL) (40 µL) as a blank; and a blank without tyrosinase enzyme as a blank control. The well plates were incubated (25˚C, 30 min), then measured their absorbance at 490 nm, and calculated the inhibition (%) by the formula:

$$\text{Inhibition}(\%) = \frac{(A-B)-(C-D)}{(A-B)} x100$$

A = blank solution's absorbance,
B = blank control solution's absorbance
C = sample solution's absorbance
D = sample control solution's absorbance.

The values of 50% inhibitory activity (IC_{50}) were determined by plotting the inhibition (%) versus concentration.

2.5. Antioxidant Activity Assay

The free-radical 1,1-diphenyl-2-pycrylhydrazine (DPPH) scavenger method, as reported previously, with slight modification [25-27] was used to evaluate the antioxidant activity of the synthesized compounds (**3a-f**), ferulic acid, and ascorbic acid (standard). A series concentration of tested or standard solution was mixed with DPPH solution in methanol (100 µg/ml), stirred, incubated (30 min, 25ºC, protected from light), and then measured the absorbance at 517 nm against the blank. The percent of scavenging and the IC_{50} were then calculated.

3. Result and Discussion

3.1. Chemistry

The Mannich base derivatives of Ferulic Acid (**3a-f**) were prepared in two steps (**Figure 1**). The amino-alkylation of vanillin (1) by Mannich base reaction using formaldehyde and sec-amine (morpholine, 2,6-dimethylmorpholine, dimethylamine, diethylamine, pyrroliidine, and N-methylpiperazine) afforded vanillin Manncih base derivatives (**2a-f**). Knoevenagel condensation of **2a-f** and malonic acid utilizing ammonium carbonate as a catalyst in solvent-free reaction afforded the title compounds (**3a-f**).

The IR spectra of **2a-f** indicated the appearance of aliphatic C-N as a strong band at 1000-1250 cm^{-1} and OH phenolic peak disappearance due to hydrogen bond formation between the OH and N atom of the Mannich base [28]. The ^{1}H-NMR spectra exhibited the presence of two methylene protons detected as a singlet peak at δ 3.96-3.76 ppm connecting the aromatic ring and the amine group [29] and proton aldehyde as a singlet peak at δ 9.76-9.79 ppm. The data confirmed the successful preparation of vanillin Mannich base derivatives.

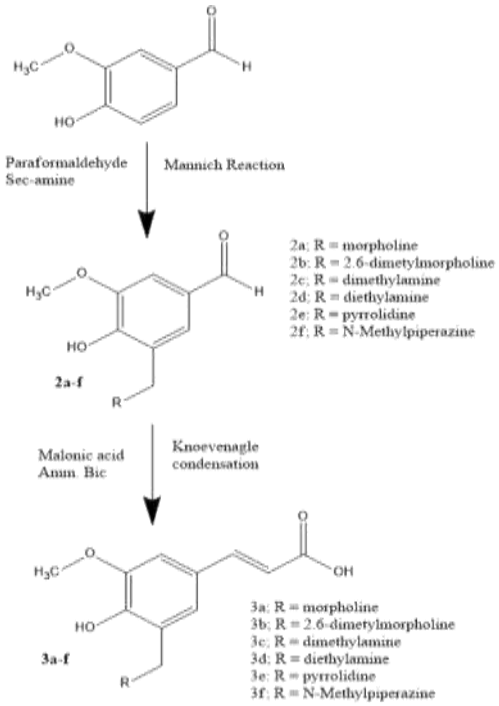

Figure 1. Synthesis of Ferulic acid Mannich base derivatives.

The IR spectra of **3a-f** indicated the appearance of OH and C=O bands of the carboxylic group at 3213-3371 and 1637-1672 $cm^{-1.}$ The ^{1}H-NMR spectra showed the ethylenic chains at δ 6.34-6.38 and 7.38-7.48 ppm. There is no OH proton peak on both aromatic rings on carboxylic groups in the ^{1}H-NMR spectra observed because of its inclination to exchange with the deuteriums of D_2O [30]. The occurrence of carboxylic groups and ethylenic chains was strengthened with the appearance of peaks at δ 169.41–167.56, 145.01-143.26, and 122.71–122.23 ppm in the ^{13}C-NMR spectra. The data were also completed with mass spectra, confirming their suitability with the targeted structures.

3.2. Anti-tyrosinase Activity

The newly synthesized compounds (**3a-f**) were screened for their anti-tyrosinase activity in vitro. The inhibition activity displayed a relation with their concentration (**Figure 2**). The activities were concentration-dependent, and all the synthesized compounds have anti-tyrosinase (IC_{50}: 312.60 – 380.75 μM). Their activity was lower than kojic acid (IC_{50}: 46.34 μM), but **3a**, **3b**, and **3f** were a little higher than ferulic acid (IC_{50}: 345.95 μM) (**Table 1**). The IC_{50} value for kojic acid is not too different from the result of the previous study [31-32].

The anti-tyrosinase activity of the phenolic compounds might be caused by the formation of hydrogen bond interaction between hydroxyl groups and the active site of the enzyme, changing conformation and leading to the inhibition of the enzymatic activity [33]. The introduction of the Mannich base to phenolic compounds results in an intramolecular hydrogen bond between the OH

and N of Mannich bases. However, the strength of the hydrogen bond is different between the compounds, leading to various inhibition effects. The lower basicity of N Mannich base on morpholine, 2,6-dimethylmorpholine, and N-methylpiperazine (compounds **3a**, **3b,** and **3f**) (**Table 1**) might lower intermolecular hydrogen interaction and, on the contrary, the Mannich group enhance the strength interaction with the enzyme and enhance the inhibitory activity of the compounds, and also vice versa.

3.3. Antioxidant Activity

The compounds' antioxidant activity was tested using the DPPH radical scavenging method. **Figure 3** presents the relation between the concentration of the compounds and their activity. The activities were concentration-dependent. All the compounds **3a-f** exhibited potent antioxidants (IC_{50}: 15.98 – 27.77 µM) (**Table 1**). The compound (**3e**) showed the best antioxidant activity in this series. The activity was similar to ascorbic acid, which was used as a standard.

The antioxidant activity of compounds **3c**, **3d**. and **3e**, having dimethylamine, diethylamine, and pyrroline Mannich base groups, was higher than Ferulic acid, in contrast with compounds **3a**, **3b**, and **3f**, having morpholine, 2,6-dimethylmorpholine, and N-methylpiperazine Mannich base groups. This finding is in line with previous research on the Mannich base of cyclovalone [34]. It demonstrated that active compounds with larger pKa values will enhance their antioxidant activity more [35].

The antioxidant activity of ferulic acid is due to its ability to make stable phenoxyl radicals, which inhibit the formation of new free radicals, or its ability to transfer hydrogen directly to the radicals [36]. In addition, the ethylenic chains shield the aromatic ring from the electron-withdrawing effect of the carboxyl groups [37].

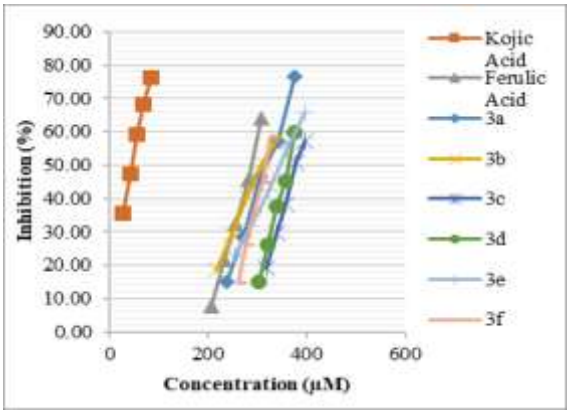

Figure 2. Tyrosinase Inhibition (%) vs Concentrations Curve of Ferulic acid Mannich base derivatives.

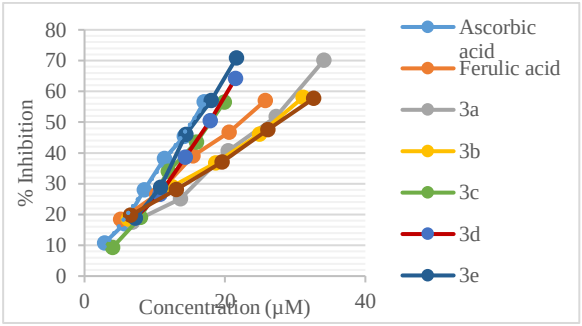

Figure 3. Radical Scavenging (%) vs Concentrations Curve of Ferulic acid Mannich base derivatives.

Table 1. Anti-tyrosinase and Antioxidant Activity

Compounds	N basicity*	IC_{50} (µM)	
		Anti-tyrosinase	Antioxidant
3a	6.89	318.55 ± 1.25	25.08 ± 0.10
3b	7.51	312.60 ± 1.59	26.64 ± 0.33
3c	8.50	380.75 ± 1.10	17.53 ± 0.08
3d	9.81	361.81 ± 0.92	17.81 ± 0.06
3e	9.72	345.95 ± 1.57	15.98 ± 0.13
3f	7.89	315.31 ± 0.80	27.77 ± 0.33
Ferulic Acid	-	345.95 ± 1.57	22.04 ± 0.17
Ascorbic acid	-	46.34 ± 0.27	15.26 ± 0.18

*N basicity of Mannich base. Calculated using MarvinSketch [38].

The structure of the majority of tyrosinase inhibitors is like that of tyrosine. The activity of phenolic compounds is closely related to the antioxidant activity. In this study, inconsistent results were obtained. All synthesized compounds showed anti-tyrosinase and antioxidant activity. However, compounds with higher anti-tyrosinase activity (**3a**, **3b**, and **3f**) had lower antioxidant capacity and vice versa for compounds **3c**, **3d**, and **3e**. The result is more in line with the anti-tyrosinase activity of p-aminophenol, as reported earlier [39].

4. Conclusions

Ferulic acid Mannich base derivatives have been synthesized successfully through Knoevenagel condensation using vanillin Mannich base derivatives as starting materials. The compounds **3a**, **3b**, and **3f,** having morpholine, 2,6-dimethylmorpholine, and N-methylpiperazine Mannich base substitutions exhibited higher antityrosinase activity than ferulic acid. While compounds **3c**, **3d**, and **3e,** having diethylamine, dimethylamine, and pyrrolidine Mannich base substitutions, exhibited higher antioxidant activity than ferulic acid.

Conflicts of interest

There are no conflicts to declare.

Acknowledgments

We thank the Research and Development Directorate Universitas Indonesia for supporting this study by providing a Postgraduate PUTI Grant (NKB-033/UN2.RST/HKP.05.00/2023), ILRC Universitas Indonesia for providing FTIR and NMR facilities, and Forensic Laboratory Centre, Indonesian National Police Headquarters for providing LC-MS/MS facilities.

5. References

[1] Ghannam, M. M., Awad, B., Al-Ayed, M.S., Aly, A.A. Improving the Physical Properties of Polyvinyl Alcohol (PVA)-Melanin Doping, *Egypt. J. Chem*., **64**(7), 3665 - 3670 (2021). https://doi.org/10.21608/ejchem.2021.46441.3061

[2] Hsu, K.D., Chan, Y.H., Chen, H.J., Hsu, KD., Chan, Y.H., and Chen, H.J., Tyrosinase-based TLC Autography for anti-melanogenic drug screening. *Sci. Rep.*, **8**(401), 1-10 (2018). https://doi.org/10.1038/s41598-017-18720-0

[3] Chaiprasongsuk A., Onkoksoong T., Pluemsamran T., Limsaengurai S., and Panich, U. Photoprotection by dietary phenolics against melanogenesis induced by UVA through Nrf2-dependent antioxidant responses," *Redox. Biol.*, **8**, 79–90 (2016). https://doi.org/10.1016/j.redox.2015.12.006.

[4] Gawish, R. A., Samy, E. M., and Aziz, M. M., Ferulic acid protects against gamma-radiation induced liver injury via regulating JAK/STAT/Nrf2 pathways., *Arch Biochem Biophys*, 109895, (2024). https://api.semanticscholar.org/CorpusID:267059603

[5] Zduńska, K., Dana, A., Kolodziejczak, A., and Rotsztejn, H., Antioxidant properties of ferulic acid and its possible application. In *Skin Pharmacol. Physiol.*, **31** (6), 332–336 (2018). https://doi.org/10.1159/000491755.

[6] Alifah, L.H.N., Jatmika, C., and Hayun, H., Exploration of Ferulic Acid and Its Derivatives as Potent Anti-Tyrosinase: A Systematic Review, *Egypt. J. Chem*. **67**(3). 257 - 271 (2024). https://doi.org/10.21608/EJCHEM.2023.229107.8427

[7] Ouimet, M. A., Griffin, J., Carbone-Howell, A. L., Wu, W. H., Stebbins, N. D., Di, R., and Uhrich, K. E., Biodegradable ferulic acid-containing poly(anhydride-ester): Degradation products with controlled release and sustained antioxidant activity. *Biomacromolecules*, **14**(3), 854–861 (2013). https://doi.org/10.1021/bm3018998

[8] Sweed, N. M., Dawoud, M. H. S., Aborehab, N. M., and Ezzat, S. M., An approach for an enhanced anticancer activity of ferulic acid-loaded polymeric micelles via MicroRNA-221 mediated activation of TP53INP1 in caco-2 cell line, *Sci Rep.* **14**(1), Dec. (2024). https://doi.org/10.1038/s41598-024-52143-y.

[9] Wu, Y., Farrag, H. N., Kato, T., Li, H., and Ikeno, S. Design and synthesis of novel peptides to protect ferulic acid against ultraviolet radiation based on domain site of bovine serum albumin. *Biomolecules*, **11**(9), 1285 (2021). https://doi.org/10.3390/biom11091285

[10] Carlotti, M. E., Sapino, S., Ugazio, E., Peira, E., Vione, D., and Minero, C., Photostability of ferulic acid and its antioxidant activity against linoleic acid peroxidation. *J. Dispers. Sci. Tech.*, **29**(5), 629–640 (2008). https://doi.org/10.1080/01932690701757766

[11] Wang, Q.-J., Gao, X., Gong, H., Lin, X.-R., Didier S-L, & Senee, J. (2011). Chemical-Stability-Degradation-Mechanisms-Ferulic-Acid (1). *J. Cosmet. Sci*, **62**, 483–503 (2011). https://pubmed.ncbi.nlm.nih.gov/22152493/

[12] Razboršek, M.I.; Ivanović, M.; Kolar, M. Validated Stability-Indicating GC-MS Method for Characterization of Forced Degradation Products of Trans-Caffeic Acid and Trans-Ferulic Acid. *Molecules*, **26**, 2475 (2021). https://doi.org/10.3390/molecules26092475

[13] Pueknang, J., and Saewan, N., Stability and Anti-Aging of Encapsulated Ferulic Acid in Phosphorylated Rice Starch. *Molecules*, **27**(11), 3463 (2022). https://doi.org/10.3390/molecules27113463

[14] Obregón-Mendoza, M. A., Estévez-Carmona, M. M., Alvarez-Ricardo, Y., Meza-Morales, W., Escobedo-Martínez, C., Soriano-García, M., & Enríquez, R. G., Crystal Structure, Synthesis and Biological Activity of Ether and Ester

<i>Trans</i>-Ferulic Acid Derivatives. *Int. J. Org. Chemi.*, **08**(04), 359–377 (2018). https://doi.org/10.4236/ijoc.2018.84028

[15] Tang, K., Jiang, Y., Zhang, H., Huang, W., Xie, Y., Deng, C., Xu, H., Song, X., & Xu, H. (2021). Design, synthesis of Cinnamyl-phenol derivatives with 1, 3-Dioxypropyl as link arm and screening of tyrosinase inhibition activity in vitro. *Bioorganic. Chemistry*, ***106***, 104512 (2021). https://doi.org/10.1016/j.bioorg.2020.104512.

[16] Fan, Q., Jiang, H., Yuan, E. D., Zhang, J. X., Ning, Z. X., Qi, S. J., & Wei, Q. Y., Tyrosinase inhibitory effects and antioxidative activities of novel cinnamoyl amides with amino acid ester moiety. *Food Chem.*, ***134***(2), 1081–1087 (2012). https://doi.org/10.1016/j.foodchem.2012.03.021

[17] Ha, J.H., and Park, S.N. Dimeric cinnamo-ylamide analogues for regulation of tyrosinase activity in melanoma cells: A role of diamide-link chain length. *Bioorg. Med. Chem.*, **26**(23–24), 6015–6022 (2018). https://doi.org/10.1016/j.bmc.2018.10.036

[18] Wang, D., Zhu, J., Xu, J.R., and Ji, D.D., Synthesis of N-hydroxycinnamoyl amide derivatives and evaluation of their anti-oxidative and anti-tyrosinase activities. *Bioorg. Med. Chem.*, ***27***(20), 114918 (2019). https://doi.org/10.1016/j.bmc.2019.05.031

[19] Rivera, A., Quiroga, D., Ríos-Motta, J., Eigner, V., and Dušek, M. Single-step synthesis of a new series of meso di-Mannich bases from the cyclic aminal (2S,7R,11S,16R)-1,8,10,17-tetraazapentacyclo[8.8.1.1.8,170.2,7011,16]icosane and p-substituted phenols. *Chem. Central J.*, ***7***(100), 1-10 (2013). https://doi.org/10.1186/1752-153X-7-100

[20] A. Mani, A. Ahamed, D. Ali, S. Alarifi, and I. Mani, A., Ahamed, A., Ali, D., Alarifi, S., and Akbar, I., Dopamine-mediated vanillin multicomponent derivative synthesis via grindstone method: Application of antioxidant, anti-tyrosinase, and cytotoxic activities. *Drug Des. Devel. Ther.*, ***15***, 787–802 (2021). https://doi.org/10.2147/DDDT.S288389

[21] Karakaya, G., Türe, A., Ercan, A., Öncül, S., and Aytemir, M.D., "Synthesis, computational molecular docking analysis and effectiveness on tyrosinase inhibition of kojic acid derivatives, *Bioorg. Chem.*, **88** (2019). https://doi.org//10.1016/j.bioorg.2019.102950

[22] Van Schijndel, J., Canalle, L. A., Molendijk, D., and Meuldijk, J. The green knoevenagel condensation: Solvent-free condensation of benzaldehydes. *Green Chem. Lett. Rev.*, **10**(4), 404–411 (2017). https://doi.org/10.1080/17518253.2017.1391881

[23] Tokalı, F. S., Taslimi, P., Demircioğlu, İ. H., Şendil, K., Tuzun, B., and Gülçin, İ. Novel phenolic Mannich base derivatives: synthesis, bioactivity, molecular docking, and ADME-Tox Studies. *J. Iranian Chem. Soc.*, **19**(2), 563–577 (2022). https://doi.org/10.1007/s13738-021-02331-8

[24] Untung, J., Iskandarsyah, I. and Hayun, H., 2-[(2,6-Dimethylmorpholin-4-yl)methyl]-4-[(E)-2-{3[(E)-2-{3-[(2,6-dimethylmorpholin-4-yl)methyl]-4hydroxy-5-methoxyphenyl} ethenyl]-1H-pyrazol-5yl}ethenyl]-6-methoxyphenol, *Molbank* **2017**, M949, (2017). http://doi.org//10.3390/M949

[25] Wiliantari, S., Iswandana, R. and Elya, B., Total Polyphenols, Total Flavonoids, Antioxidant Activity and Inhibition of Tyrosinase Enzymes from Extract and Fraction of Passiflora ligularis Juss, *Pharmacog. J.*, **14**(3), 660–671 (2022) https://doi.org./10.5530/pj.2022.14.86.

[26] Mohammed, R.S., Hendawy, S.F., Omer, E.A., Egyptian Myrtus communis L. Essential oil Potential role as in vitro Antioxidant, Cytotoxic and α-amylase Inhibitor, *Egypt. J. Chem.*, **64**(6), 3005 - 3017 (2021). https://doi.org/10.21608/EJCHEM.2021.57354.3245

[27] Pallapati, R. K., Gugulothu, S., Vanga, U. R., Bollikolla, H. R., Bezafibrate Scaffold Derived Hydrazide-Hydrazones: Synthesis and Antioxidant Activities, *Egypt. J. Chem.* **63**(7). 2473 - 2482 (2020). https://doi.org/10.21608/ejchem.2020.20809.2251

[28] Jia, P., Zhang, M., Hu,L., Song, F., Feng, G.,and Zhou, Y., A Strategy for Nonmigrating Plasticized PVC Modified with Mannich base of Waste Cooking Oil Methyl Ester, *Sci. Rep.*, **8**, 1589, 1-8 (2018). https://doi.org/10.1038/s41598-018-19958-y

[29] Hayun, H., Gavrila, I., Silviana, S., Siahaan, A.EK., Vonna, R.F., and Latifah M.I.,, Synthesis and antioxidant activity study of new Mannich bases derived from vanillic acid, *Rasayan J. Chem.*, **13**(1), 131–138 (2020). https://doi.org/10.31788/RJC.2020.1315300

[30] Cseri, L., Kumar, S., Palchuber, P., and Szekely, G. "NMR Chemical Shifts of Emerging Green Solvents, Acids, and Bases for Facile Trace Impurity Analysis," *ACS Sustain Chem Eng*. **11**(14), 5696–5725 (2023). http://doi.org/10.1021/acssuschemeng.3c00244

[31] Chen, W.C., Tseng, T.S., Hsiao, N.W., Lin, Y.L., Wen, Z.H., Tsai, C.C., Lee, Y.C., Lin, H.H., and Tsai, K.C., Discovery of highly potent tyrosinase inhibitor, T1, with significant anti-melanogenesis ability by zebrafish in vivo assay and computational molecular modeling. *Sci. Rep.* **5**, 7995 (2015) https://doi.org/10.1038/srep07995

[32] Sindhu, R.J.A., Evaluation of in vitro antioxidant and Antityrosinase activities of Ixora coccinea Linn. Roots, *J. Pharmacog. Phytochem*., **11**(2), 144-150 (2022). https://doi.org/10.22271/phyto.2022.v11.i2b.14380.

[33] Alam, N., Yoon, K.N., and Lee, T.S., Evaluation of the antioxidant and antityrosinase activities of three extracts from Pleurotus nebrodensis fruiting bodies, *Afr. J. Biotechnol*.,**10**(11), 2978-2986 (2011). https://doi.org/10.5897/AJB10.2660.

[34] Hayun, H., Jatmika, C., Purwati, E.M. Salim, S., Kurniawan, R., Chandra, E.G., Fajriawan, A.A., and Nareswara, A.D., Synthesis and Free Radical-scavenging Activities of Di-mannich Bases of Cyclovalone Derivatives, *Orient. J. Chem*., **33**(6), 2742-2757 (2017). http://dx.doi.org/10.13005/ojc/330607

[35] Hu, Yi & Liang, Peiyi & Wang, Zhuxian & Jiang, CuiPing & Guo, Yinglin & Chen, Hongkai & Shen, Chunyan & Wu, Yufan & Liu, Li & Yi, Yankui & Liu, Qiang & Zhu, Hongxia. Exploring the Molecular Mechanism of the Antioxidant Activity of Medicine and Food Homology Licorice Flavonoids Based on Pharmacophore Theory and Quantum Calculations. *J. Food Biochem.*. **2023**. 1-16 (2023). http://doi.org/10.1155/2023/2801318.

[36] Zduńska-Pęciak, K., Dębowska, R., Kołodziejczak, A., and Rotsztejn, H., Ferulic acid – A novel topical agent in reducing signs of photoaging, *Dermatol. Ther*. **35**(7), e15543 (2022), http://doi.org/10.1111/dth.15543.

[37] Charlton, N. C., Mastyugin, M., Török, B., and Török, M., Structural Features of Small Molecule Antioxidants and Strategic Modifications to Improve Potential Bioactivity, *Molecules*, **28**(3), 1057 (2023). http://doi.org/10.3390/molecules28031057.

[38] MarvinSketch 20.8.0. Chemaxon Ltd. 1998 2020. Available online: http://www.chemaxon.com.

[39] Komori, Y., Imai, M., Yamauchi, T., Higashiyama, K., and Takahashi, N., Effect of p-aminophenols on tyrosinase activity, *Bioorg. Med. Chem*, **22**(15), 3994-4000 (2014). https://doi.org/10.1016/j.bmc.2014.05.073.