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ORIGINAL RESEARCH

Dopamine-Mediated Vanillin Multicomponent Derivative Synthesis via Grindstone Method: Application of Antioxidant, Anti-Tyrosinase, and Cytotoxic Activities

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Purpose: This study aimed to determine the extent of contribution of dopamine to antioxidant and anti-tyrosinase activities, by dopamine addition to vanillin. This study achieved the synthesis of dopamine-associated vanillin Mannich base derivatives prepared via a one-step reaction involving a green chemistry approach, and investigation of antioxidant and anti-tyrosinase activities.

Methods: Novel one-pot synthesis of Mannich base dopamine-connected vanillin (**1a-l**) derivatives can be achieved via green chemistry without using a catalyst. Newly-prepared compounds were characterised with FTIR and NMR (^{1}H and ^{13}C) spectra, mass spectra, and elemental analyses. In total, 12 compounds (**1a-l**) were synthesised and their antioxidant and anti-tyrosinase activities evaluated. Antioxidant activities of 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO), hydrogen peroxide (H_2O_2), and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and diammonium assays, $ABTS^{\bullet+}$ radical scavenging, and linoleic acid peroxidation were used to screen all synthesised compounds (**1a-l**) for anti-tyrosinase activities and cytotoxicity against MCF-7 and Vero cell lines;.

Results: The compound **1k** inhibited (IC_{50}:11.02µg/mL) the DPPH-scavenging activity to a greater extent than the standard BHT (IC_{50}:25.17µg/mL), and showed high activity in H_2O_2 and NO scavenging assays. Compound **1e** was more potent (96.21%) against ABTS and compound **1k** was more potent (95.28%) against 2,2′-azobis(2-amidinopropane)dihydrochloride antioxidant than the standard trolox. All synthesised compounds were screened for anti-tyrosinase inhibitory activity. Compound **1e** had higher activity against tyrosinase (IC_{50}=10.63 µg/mL), than kojic acid (IC_{50}=21.52µg/mL), and was more cytotoxic (GI_{50} 0.01µM) against MCF-7 cell line than the doxorubicin standard and other tested compounds.

Conclusion: In this study, all compounds were found to possess significant antioxidant and anti-tyrosinase activities. Compounds **1e** and **1k** performed well, compared with other compounds, in all assays. In addition, this study successfully identified several promising molecules that exhibited antioxidant and anti-tyrosinase activities.

Keywords: Mannich base, grindstone chemistry, antioxidant, anti-tyrosinase activity, cytotoxicity

Introduction

Tyrosinase inhibitors have natural, synthetic, and semi-synthetic sources,[1] such as tropolone, hydroquinone, kojic acid,[2] arbutin, and bibenzyl glycosides.[3] A drawback of these inhibitors is the low efficacy of designing the drug.[4,5] For example, tyrosine

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and dopamine of phenol hydroxyl (OH) compounds can inhibit the activity of tyrosinase,[6] whereas flavonoids of phenolic OH groups can have anti-tyrosinase and antioxidant activities.[7] The present study focused on dopamine with vanillin-containing compounds. Vanillin has anti-apoptotic, neuroprotective, antioxidative, and anticancer activities,[8,9] and mushroom tyrosinase active vanillin derivatives have been identified.[10]

The design and development of dopamine with vanillin derivatives via the Mannich condensation reaction based on Mannich base derivatives has been conducted previously using many different bioactive molecules, such as aminoalkyl derivatives,[11] chiral types, β-amino-carbonyl compounds, peptides, alkaloids, antibiotics, and vitamins.[12] Additionally, Mannich base bioactivity includes antioxidative,[13] antifungal,[14] anti-inflammatory,[15] analgesic,[16] anticancer,[17] vasorelaxing,[18] antimalarial,[19] and antitubercular[20] activities.

In particular, phenolic compounds of Mannich bases, such as chalcones, thymols, and flavanones, have antioxidant compounds.[21,22] Tyrosinase active Mannich base kojic acid derivatives have also been identified.[23] However, there have been no previous studies, to our knowledge, regarding biologically active dopamine connected to vanillin derivatives.

This study focused on the antioxidant activity of title compounds based on screening using various free-radical assays as oxidative stress may be the main cause of neurodegenerative diseases.[24] The brain's dependence on oxygen (O_2) and high consumption of glucose makes it highly susceptible to oxidative stress, as leaked O_2 has been implicated in the generation of free radicals, such as superoxide anions, hydrogen peroxide (H_2O_2), and OH.[25–28] Some molecules have both active antioxidant and tyrosinase activities, such as isoeugenol[29] (Figure 1). Designing antioxidant molecules using biosystems can protect inhibit tyrosinase enzymes and prevents related diseases. Flavonoids will consider the phenolic OH on the effetely antioxidant and tyrosinase activities.[30,31]

For example, phenolic hydroxyl on the ring catechins,[32,33] baicalein,[34] L-DOPA, and rosmarinic acid (Figure 1) can greatly enhance the tyrosinase activity. For the evidence, two phenolic hydroxyls can more effect the tyrosinase activity compared to one hydroxyl substitutions,[35,36] and other example phenol hydroxyl-containing tyrosine and dopamine have inhibited the tyrosinase enzyme.[6]

Tyrosinase inhibitors are used for various applications in the food,[37] cosmetics,[38] and medicinal industries, and tyrosinase is responsible for melanogenesis in mammals.[39,40]

However, very few inhibitors have been approved for clinical use or for use as skin-whitening agents, and there are limited rapid assays for the in vitro screening of tyrosinase inhibitors[41] hence, effective and low-cost methods need to be developed. Therefore, in this study, we selected the Grindstone method, which is a branch of green chemistry where solvent-free chemical reactions[42,43] can take place to produce a high yield in an inexpensive.[44] This method is used in the pharmaceutical industry with minimal environmental impact. The undertaking of reactions under solvent-free reaction conditions using a grinding technique is an alternative to other methods.[43,45]

Therefore, this study had two goals; to provide the best model of Mannich base vanillin-connected dopamine derivatives, and to test the obtained Mannich bases for possible anti-tyrosinase and antioxidative activities as well as provide a suitable mechanism. In addition, cytotoxic effects were investigated for the synthesis of Mannich bases against MCF-7 cancer cell lines.

Experimental

Synthesis

Spectrophotometer lambda 850 was used to check all bioactivities. FT-IR (4000–400 cm^{-1}) was recorded by Shimadzu 8201PC analysis. Bruker DRX-300 MHz, 75 MHz was used for the analysis of ^{1}H and ^{13}C NMR spectra. A Vario EL III organic element analyzer was used to analyze the percentage (%) elements (C, H, N and S) presents in synthesised compounds.

General Procedure for the Synthesis of Compound 1a-l

A reaction mixture consisting of dopamine (0.01 mol, 1.53 g), vanillin (0.01 mol, 1.52 g), and *N*-methylacetamide (0.01 mol, 0.730 g) was mixed in a mortar and ground for up to 15 min at 30°C. Subsequently, the powdered material was washed with water and filtered. The filtered final solid material was separated by column chromatography (ethyl acetate/hexane, 4:6) and recrystallized from suitable alcohol. The same method was followed for compounds **1b-l**.

3-((3,4-Dihydroxyphenethyl)amino)-3-(4-Hydroxy-3-Methoxyphenyl)-N-Methylpropan Amide (1a)

A pale yellow solid, yield 92%; MF = $C_{19}H_{24}N_2O_5$; MW = 360.40; m.p. = 152–154°C; IR (KBr) ν_{max}: 3415 (O-H, stretch), 3345, 2853, 1640, 1603, 1400, 1080, 1039 $cm^{-1;}$ ^{1}H NMR (DMSO-d_6) δ: 8.05 (s,

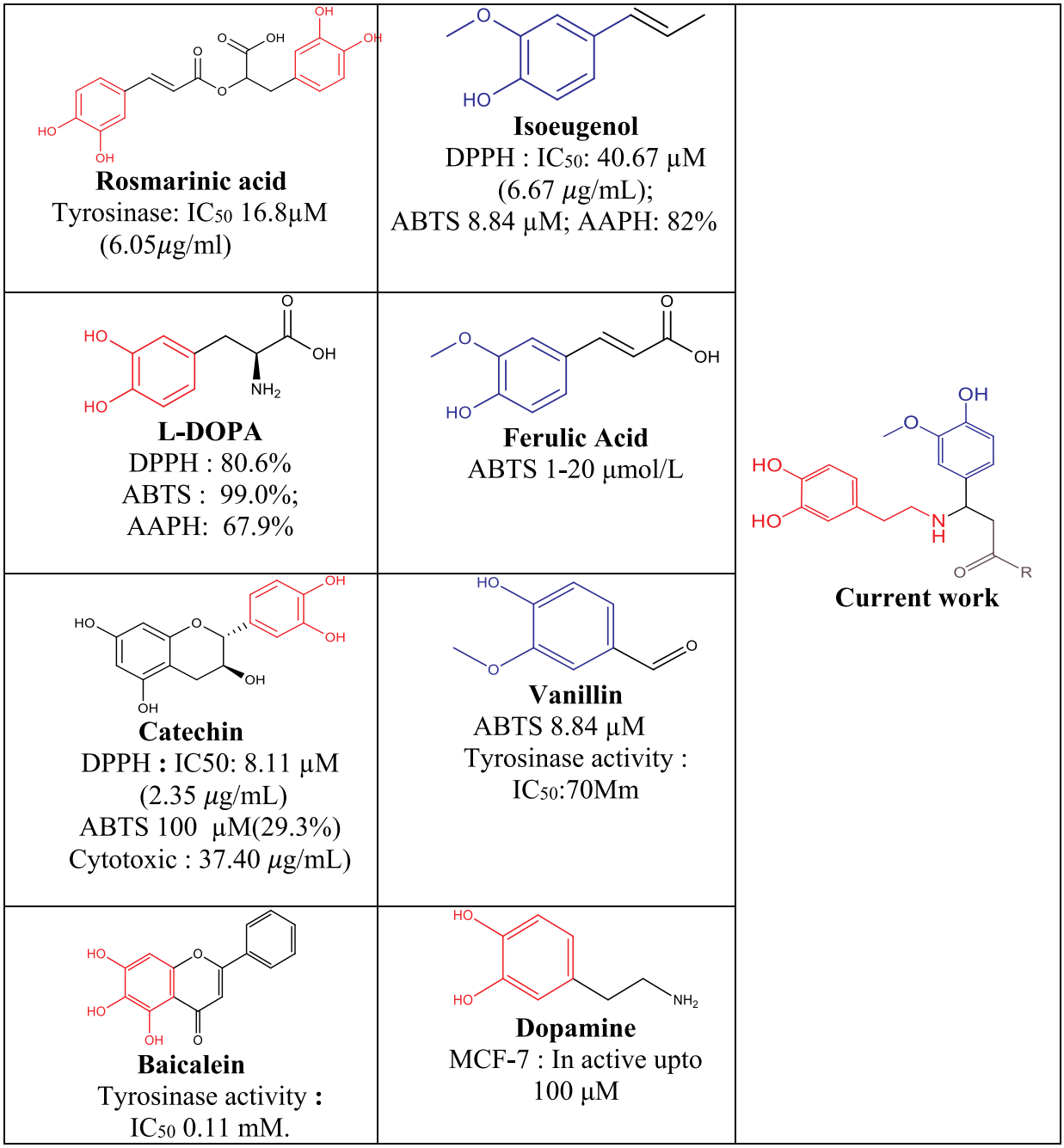


Figure 1 Deigning of target molecules.

1H), 6.98 (s,1H), 6.80 (s, 1H), 6.78 (d, J = 11.0 Hz, 1H), 6.68 (d, J = 11.12Hz, 1H), 6.69 (d, 1H), 6.66 (d, 1H), 5.33 (s, 3H, OH), 4.13 (dd, J=11.0 Hz, J = 11.2 Hz, 1H, CH), 3.81 (s, 3H), 3.06 (s, 3H), 2.85 (s, 2H), 2.71 (d, J = 11.0 Hz, 1H), 2.68 (2H, s, CH_2), 2.40 (1H, d, J=11.2 Hz), 2.08 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 172.1, 147.3, 147.0, 145.6, 144.5, 131.9, 131.6, 122.9, 120.3, 116.5, 115.8, 115.2, 111.2, 57.0, 56.6, 45.2, 42.5, 36.5, 26.9; EI–MS: m/z 360 $[M]^+$(20); HREIMS: m/z: calcd for $C_{19}H_{24}N_2O_5$: 360.17, found 360.21; Anal. calcd $C_{19}H_{24}N_2O_5$: C, 63.32; H, 6.71; N, 7.77; Found: C, 63.34; H, 6.74; N, 7.75.

3-((3,4-Dihydroxyphenethyl)amino)-N-Ethyl-3-(4-Hydroxy-3-Methoxyphenyl) propan Amide(1b)

Yellow solid, yield 90%; MF = $C_{20}H_{26}N_2O_5$; MW = 374.43; m.p. = 171–174°C; IR (KBr) ν_{max}: 3423, 3349,

2835, 1631, 1592, 1406, 1083, 1036 (-O-CH_3) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 8.05 (s, 1H), 6.92 (s, 1H), 6.84 ((s, 1H), 6.76 (d, 1H), 6.72 (d, 1H), 6.68 (d, 1H), 6.64 (d, J = 11.0 Hz, 1H,), 5.31 (3H, s, OH), 4.18 (1H, dd, CH), 3.84 (s, 3H), 3.10 (s, 2H), 2.87 (s, 2H), 2.69 (d, J = 11.0 Hz, 1H), 2.67 (s, 2H), 2.45 (d, J =11.0Hz, 1H), 1.14 (s, 3H), 2.10 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 175.1, 147.3, 146.8, 144.9, 143.8, 131.9, 131.6, 122.9, 120.3, 116.5, 115.8, 115.2, 111.2, 57.5, 56.1, 45.2, 42.5, 36.5, 34.2, 15.0; EI–MS: m/z 374[M]$^+$(24); HREIMS: m/z: calcd for $C_{20}H_{26}N_2O_5$: 374.43, found 374.40; Anal. calcd $C_{20}H_{26}N_2O_5$: C, 64.15; H, 7.00; N, 7.48; Found: C, 64.17; H, 7.02; N, 7.46.

4-((3,4-Dihydroxyphenethyl)amino)-4-(4-Hydroxy-3-Methoxyphenyl)butan-2-one (1c)

A pale yellow solid, yield 89%; MF = $C_{19}H_{23}NO_5$; MW = 345.39; m.p. = 160–162°C; IR (KBr) ν_{max}: 3441, 3332, 2831, 1630, 1595, 1404, 1080, 1021 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 6.98 (s, 1H), 6.80((s, 1H), 6.78 (d, J = 11.0 Hz, 1H), 6.70 (d, 1H, Ph-H), 6.69 (d, 1H, Ph-H), 6.66 (d, 1H), 5.30 (s, 3H, OH), 4.11 (dd, 1H), 3.81 (s, 3H), 2.95 (2H, d, J = 11.0 Hz), 2.85 (2H, s, CH_2), 2.73 (d, J = 11.0 Hz, 2H), 2.68 (s, 2H, CH_2), 2.31 (1H, s, -NH), 2.13 (3H, s); ^{13}C NMR (DMSO-d_6): δ: 210.6, 147.3, 146.0, 145.1, 143.8, 131.9, 131.6, 122.9, 120.3, 116.5, 115.8, 115.2, 111.2, 56.0, 55.1, 45.5, 42.2, 36.5; EI–MS: m/z 345 [M]$^+$(12); HREIMS: m/z: calcd for $C_{19}H_{23}NO_5$: 345.30, found 345.38; Anal. calcd $C_{19}H_{23}NO_5$: C, 66.07; H, 6.71; N, 4.06; Found: C, 66.09; H, 6.70; N, 4.04;.

3-((3,4-Dihydroxyphenethyl)amino)-3-(4-Hydroxy-3-Methoxyphenyl) propanamide (1d)

Yellow solid, yield 87%; MF = $C_{18}H_{22}N_2O_5$; MW = 346.38; m.p. = 149–151°C; IR (KBr) ν_{max}: 3452, 3339, 2821, 1715, 1621, 1590, 1408, 1078, 1019 cm^{-1}; ^{1}H NMR (DMSO-d_6)δ: 8.08 (s, 2H, NH_2), 6.98 (s, 1H), 6.80 (s,1H), 6.70 (d, 1H), 6.69 (d, 1H), 6.78 (d, J = 11.3Hz, 1H), 6.63 (d, 1H), 5.37 (s, 3H, OH), 4.10 (dd, J= 11.3Hz, J= 11.12Hz, 1H, CH), 3.81 (s, 3H), 2.85 (s, 2H), 2.71 (d, 2H, J= 11.30Hz, CH_2), 2.46 (d, J= 11.12Hz, 2H, CH_2), 2.67 (s, 2H), 2.03 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 174.3, 147.6, 147.3, 144.3, 140.1, 131.9, 131.6, 122.9, 120.3, 116.5, 115.8, 115.2, 111.2, 57.1, 53.3, 45.2, 44.5, 36.5; EI–MS: m/z 346 [M]$^+$(31); HREIMS: m/z: calcd for $C_{18}H_{22}N_2O_5$: 346.38, found 346.10; Anal. calcd $C_{18}H_{22}N_2O_5$: C, 62.42; H, 6.40; N, 8.09; Found: C, 62.44; H, 6.38; N, 8.07;

3-((3,4-Dihydroxyphenethyl)amino)-3-(4-Hydroxy-3-Methoxyphenyl)-N-Phenylpropan Amide (1e)

A pale yellow solid, yield 93%; MF = $C_{24}H_{26}N_2O_5$; MW = 422.47; m.p.130–133°C; 3463, 3323, 2819, 1626, 1580, 1412, 1070, 1001cm^{-1}, ^{1}H NMR (DMSO-d_6) δ: 8.0 (s, 1H), 7.61 (d, J = 10.1, 2H), 7.43 (d, J = 10.1, 2H), 7.19 (1H, d, J =10.1 Hz), 6.98 (s, 1H), 6.80((s, 1H), 6.70 (d, 1H), 6.78 (d, J = 11.0 Hz, 1H), 6.69 (d, 1H), 6.66 (d, 1H), 5.39 (s, 3H), 4.17 (dd, J=11.0Hz, J =11.2 Hz, 1H, CH), 3.81 (s, 3H, -CH_3), 2.85 (m, 2H CH_2), 2.71 (d, J =11.0Hz, 2H), 2.68 (d, 2H, CH_2), 2.52 (d, J = 11.2 Hz, 2H), 2.12 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 173.9, 147.3, 147.0, 146.2, 144.7, 138.5, 131.9, 131.6, 128.9, 128.1, 122.9, 121.6, 120.3, 116.5, 115.8, 115.2, 111.2, 57.2, 56.2, 45.2, 41.5, 36.5; EI–MS: m/z 422 [M]$^+$(08); HREIMS: m/z: calcd for $C_{24}H_{26}N_2O_5$: 422.17, found 422.19; Anal. Calcd $C_{24}H_{26}N_2O_5$: C, 68.23; H, 6.20; N, 6.63; Found: C, 68.25; H, 6.22; N, 6.66;

2-((3,4-Dihydroxyphenethyl)amino)-2-(4-Hydroxy-3-Methoxyphenyl)-1-(4-Methoxyphenyl) Ethanone (1f)

A pale yellow solid, yield 94%; MF = $C_{24}H_{25}NO_6$; MW = 423.17; m.p. = 165–167°C; IR (KBr) ν_{max}: 3512, 3312, 2889, 1621, 1582, 1410, 1121, 1012 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 6.98 (s, 1H), 6.80((1H, s), 6.69 (d, 1H), 6.70 (d, 1H), 6.66 (d, 1H), 6.78 (d, J = 11.0 Hz, 1H), 7.86 (d, J=10.23Hz, 2H), 7.06 (d, J=10.21Hz, 2H), 5.40 (s, 3H, OH), 4.43 (dd, J=10.34Hz, J=10.36Hz, 1H, CH), 3.81 (s, 6H, -CH_3), 3.08 (d, J=10.34Hz, 2H, CH_2), 2.85 (d, J=10.36Hz, 2H, CH_2), 2.88 (s, 2H), 2.64 (s, 2H), 2.14 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 201.36, 185.2, 147.3, 147.2, 145.3, 143.9, 131.9, 131.6, 129.8, 129.1, 114.5, 122.9, 120.3, 116.5, 115.8, 115.2, 111.2, 65.8, 56.2, 56.0, 45.2, 36.5, 32.8; EI–MS: m/z 360 [M]$^+$(20), 189 (100); HREIMS: m/z: calcd for $C_{24}H_{25}NO_6$: 423.17, found 423.10; Anal. calcd $C_{19}H_{24}N_2O_5$: C, 63.32; H, 6.71; N, 7.77; Found: C, 63.34; H, 6.74; N, 7.75.

1-(4-Bromophenyl)-3-((3,4-Dihydroxyphenethyl)amino)-3-(4-Hydroxy-3-Methoxyphenyl) Propan-1-one (1g)

A pale yellow solid, yield 89%; MF = $C_{24}H_{24}BrNO_5$; MW = 486.36; m.p. = 149–151°C; IR (KBr) ν_{max}: 3612, 3314, 2891, 1623, 1597, 1412, 1118, 1023 (-O-CH_3), 758 (C-Br)$cm^{-1;}$ ^{1}H NMR (DMSO-d_6) δ: 6.98 (1H, s, Ph-H), 6.80((1H, s, Ph-H), 6.69 (1H, d, Ph-H), 6.70 (1H, d, Ph-H), 6.66 (1H, d, Ar–H), 6.78 (1H, d, J = 11.0 Hz, Ph-H), 7.98 (d, J = 10.6 Hz, 2H), 7.65 (d, J = 10.9 Hz, 2H), 5.42 (3H, s, OH), 4.13 (dd, J = 11.0 Hz, J = 11.2 Hz, 1H, CH), 3.81 (s, 3H, -CH_3), 3.09 (2H, d, J = 11.0 Hz), 2.81 (d, J = 11.2 Hz, 2H), 2.85 (s, 2H), 2.68 (s, 2H), 2.05 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 200.65, 147.3, 146.8, 145.0, 144.1, 135.7, 131.5, 131.9, 131.6, 129.8, 127.6, 122.9, 120.3, 116.5, 115.8, 115.2, 111.2, 72.9, 57.3, 56.9, 45.2, 36.5; 131.6, 122.9, 120.3, 116.5, 115.8, 115.2, 111.2, 57.2, 45.2, 42.5, 36.5, 26.9; EI–MS: m/z 486 $[M]^+$(35), 189 (100); HREIMS: m/z: calcd for $C_{24}H_{24}BrNO_5$: 486.36, found 486.36; Anal. calcd $C_{24}H_{24}BrNO_5$: C, 59.27; H, 4.97; N, 2.88; Found: C, 59.29; H, 4.95; N, 2.87;

3-((3,4-Dihydroxyphenethyl)amino)-3-(4-Hydroxy-3-Methoxyphenyl)-1-(4-Nitrophenyl) Propan-1-one (1h)

A pale yellow solid, yield 90%; MF = $C_{24}H_{24}N_2O_7$; MW = 452.46; m.p. = 132–35°C; IR (KBr) ν_{max}: 3621, 3329, 2874, 1699, 1628, 1591, 1410, 1109, 1039 $cm^{-1;}$ ^{1}H NMR (DMSO-d_6) δ: 6.98 (1H, s, Ph-H), 6.80 (1H, s, Ph-H), 6.69 (1H, d, Ph-H), 6.70 (1H, d, Ph-H), 6.66 (d, 1H, Ar–H), 6.78 (1H, d, J = 11.0 Hz), 8.34 (d, J = 11.0 Hz, 4H), 5.44 (3H, s, OH), 4.15 (1H, dd, J = 11.0 Hz, J = 11.2 Hz, CH), 3.81 (3H, s, -CH_3), 3.01 (d, J = 11.0 Hz, 2H), 2.80 (2H, d, J = 11.2 Hz), 2.85 (s, 2H), 2.68 (s, 2H), 2.07 (s, 1H); ^{13}C NMR (DMSO-d_6) δ: 202.11, 147.3, 147.9, 145.1, 144.6, 135.7, 131.5, 131.9, 131.6, 129.8, 127.6, 122.9, 120.3, 116.5, 115.8, 115.2, 111.2, 72.9, 58.1, 56.2, 45.2, 36.5; EI–MS: m/z 452$[M]^+$(18), 189 (100); HREIMS: m/z: calcd for $C_{24}H_{24}N_2O_7$: 360.17, found 249.02; Anal. calcd $C_{24}H_{24}N_2O_7$: C, 63.71; H, 5.35; N, 6.19; Found: C, 63.70; H, 5.37; N, 6.17;

1-(4-Chlorophenyl)-3-((3,4-Dihydroxyphenethyl)amino)-3-(4-Hydroxy-3-Methoxyphenyl) Propan-1-one (1i)

A pale yellow solid, yield 87%; MF=$C_{24}H_{24}ClNO_5$; MW = 441.90; m.p.165–168 °C; IR (KBr) ν_{max}: 3512, 3341, 2873, 1620, 1612, 1593, 1412, 1119, 1031, 745 $cm^{-1;}$ ^{1}H NMR (DMSO-d_6) δ: 6.98 (1H, s, Ph-H), 6.80((1H, s, Ph-H), 6.69 (1H, d, Ph-H), 6.70 (1H, d, Ph-H), 6.66 (1H, d, Ar–H), 6.78 (1H, d, J =11.0 Hz, Ph-H), 7.94 (d, J =10.2 Hz, 2H), 7.44 (d, J=10.4 Hz, 2H), 5.31 (3H, s, OH), 4.16 (1H, dd, J = 11.0 Hz, J =11.2 Hz, CH), 3.81 (3H, s, -CH_3), 3.09 (2H, d, J=11.0 Hz), 2.85 (2H, s, CH_2), 2.81 (2H, d, J=11.2 Hz), 2.68 (2H, s, CH2), 2.10 (1H, s, -NH); ^{13}C NMR (DMSO-d_6) δ: 201.07, 147.3, 146.9, 145.1, 144.8, 138.7, 134.8, 131.5, 131.9, 130.8, 128.6, 122.9, 120.3, 116.5, 115.8, 115.2, 111.2, 72.9, 57.0, 56.1, 45.2, 36.5; EI–MS: m/z 441 $[M]^+$(37); HREIMS: m/z: calcd for $C_{24}H_{24}ClNO_5$: 441.90, found 441.87; Anal. calcd $C_{24}H_{24}ClNO_5$: C, 65.23; H, 5.47; N, 3.17; Found: C, 65.25; H, 5.45; N, 3.16;.

3-((3,4-Dihydroxyphenethyl)amino)-3-(4-Hydroxy-3-Methoxyphenyl)-1-(p-Tolyl)propan-1-one (1j)

Yellow solid, yield 91%; MF =$C_{25}H_{27}NO_5$; MW=421.49; m.p. = 165–167°C; IR (KBr) ν_{max}: 3485, 3379, 2870, 1614, 1636, 1589, 1484, 1402, 1145, 1036 $cm^{-1;}$ ^{1}H NMR (DMSO-d_6) δ: 7.31 (2H,d, J=, Ph), 6.98 (1H, s, Ph-H), 6.80((1H, s, Ph-H), 6.78 (1H, d, J =11.0 Hz, Ph-H), 6.72 (d, J = 11.0 Hz, 2H), 6.69 (d, 1H), 6.70 (d, 1H), 6.66 (d, 1H), 5.40 (3H, s, OH), 4.10 (1H, dd, J = 11.0 Hz, J = 11.2 Hz, CH), 3.81 (s, 3H), 3.09 (d, J = 11.0 Hz, 2H), 2.86 (d, J = 11.2 Hz, 2H), 2.83 (2H, s, CH_2), 2.68 (2H, s, CH_2), 2.34 (3H, s, CH_3), 2.09 (1H, s, -NH); ^{13}C NMR (DMSO-d_6) δ: 200.02, 147.9, 147.3, 145.0, 144.1, 138.7, 134.8, 131.9, 131.5, 130.8, 128.6, 122.9, 120.3, 116.5, 115.8, 115.2, 111.2, 72.9, 57.8, 56.3, 45.2, 36.5, 21.3; EI–MS: m/z 421 $[M]^+$(41), 189 (100); HREIMS: m/z: calcd for $C_{25}H_{27}NO_5$: 421.49, found 421.37; Anal. calcd $C_{25}H_{27}NO_5$: C, 71.24; H, 6.46; N, 3.32; Found: C, 71.26; H, 6.44; N, 3.30;

N-(4-Bromophenyl)-3-((3,4-Dihydroxyphenethyl)amino)-3-(4-Hydroxy-3-Methoxyphenyl) Propanamide (1k)

A pale yellow solid, yield 96%; MF = $C_{24}H_{25}BrN_2O_5$; MW = 501.37; m.p.141–143°C; IR (KBr) ν_{max}: 3409, 3361, 2865, 1646, 1618, 1581, 1482, 1404, 1140, 1032, 702 $cm^{-1;}$ ^{1}H NMR (DMSO-d_6) δ: 7.71 (d, J = 11.0 Hz, 2H), 7.56 (d, J = 11.0 Hz, 2H), 7.25 (1H, s, NH), 6.98 (1H, s, Ph-H), 6.80 (s, 1H), 6.78 (d, J = 11.0 Hz, 1H),

6.70 (d, 1H), 6.69 (d, 1H), 6.66 (d, 1H), 5.45 (s, 3H, OH), 4.13 (dd, J = 11.0 Hz, J = 11.2 Hz, 1H), 3.81 (s, 3H, -CH_3), 3.11 (d, J = 11.0 Hz, 2H), 2.85 (s, 2H, CH_2), 2.80 (d, J = 11.2 Hz, 2H), 2.68 (s, 2H, CH_2), 2.12 (s, 1H, -NH); ^{13}C NMR (DMSO-d_6) δ: 173.6, 147.3, 147.0, 145.6, 144.5, 137.6, 131.9, 131.8, 131.6, 122.9, 121.9, 121.5, 120.3, 116.5, 115.8, 115.2, 111.2, 57.2, 56.1, 45.2, 42.5, 36.5; EI–MS: m/z 360 $[M]^+$(20), 189 (100); HREIMS: m/z: calcd for $C_{24}H_{25}BrN_2O_5$: 501.37, found 501.32; Anal. calcd $C_{24}H_{25}BrN_2O_5$: C, 57.49; H, 5.03; N, 5.59;; Found: C, 57.47; H, 5.05; N, 5.61;

1-(4-(Tert-Butyl)phenyl)-3-((3,4-Dihydroxyphenethyl)amino)-3-(4-Hydroxy-3-Methoxy Phenyl)propan-1-one (1l)

A pale yellow solid, yield 96%; MF = $C_{28}H_{33}NO_5$; MW = 463.57; m.p. = 194–196°C; IR (KBr) ν_{max}: 3512, 3360, 2861, 1654, 1612, 1583, 1481, 1410, 1138, 1030 $cm^{-1;}$ ^{1}H NMR (DMSO-d_6) δ: 7.37 (d, J=10.23Hz, 2H), 6.98 (s, 1H, Ph-H), 6.80 (s, 1H, Ph-H), 6.69 (d, 1H, Ph-H), 6.78 (d, J = 11.0 Hz, 1H), 6.70 (d, J=11.23Hz, 1H), 6.66 (d, Ar–H, 1H), 6.87 (d, J=11.23Hz, 2H), 5.35 (s, 3H, OH), 4.17 (1H, dd, J = 11.0 Hz, J = 11.2 Hz, CH), 3.81 (3H, s, -CH_3), 3.05 (2H, d, J = 11.0 Hz), 2.84 (d, J = 11.2 Hz, 2H,), 2.85 (s, 2H), 2.68 (s, 2H), 2.15 (s, 1H, -NH), 1.36 (9H, s, CH_3); ^{13}C NMR (DMSO-d_6) δ: 200.02, 155.6, 147.3, 147.0, 146.6, 144.5, 138.7, 131.5, 131.9, 130.8, 126.4, 124.9, 122.9, 120.3, 116.5, 115.8, 115.2, 111.2, 72.9, 57.2, 45.2, 36.5,34.3, 31.3; EI–MS: m/z 463 $[M]^+$(24); HREIMS: m/z: calcd for $C_{28}H_{33}NO_5$: 463.57, found 463.55; Anal. calcd $C_{28}H_{33}NO_5$: C, 72.55; H, 7.18; N, 3.02;; Found: C, 72.52; H, 7.20; N, 3.04;.

Biological Activity

Antioxidant 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Scavenging Activity

DPPH antioxidant activity was screened for compounds (**1a-l**) following the methods of a previous study.[46] The detailed method is provided in the supplementary information section.

H_2O_2 Scavenging Activity

H_2O_2 scavenging activity was screened for all compounds (**1a-l**) following the methods of a previous study.[46] The detailed method is provided in the supplementary information section.

Nitric Oxide (NO) Scavenging Activity

The compounds (**1a-l**) were screened for NO scavenging activity following the methods of a previous study.[46] The detailed method is provided as a supplementary file in the experimental section.

2,2′-Azino-Bis(3-Ethylbenzothiazoline-6-Sulfonic Acid) (ABTS) Antioxidant Activity

The compounds (**1a-l**) were screened for the ABTS assay. The antioxidant $ABTS^{\bullet+}$ scavenging activity was checked with all compounds via spectrophotometric analysis according to the method previously described by Surendra kumar et al[47]. The detailed method is provided in the supplementary information section.

Inhibition of 2,2′-Azobis (2-Amidinopropane) Dihydrochloride (AAPH) Assay Free-Radical Analysis

A linoleic acid peroxidation assay was used to analyse all synthesised compounds (**1a-l**) following the methods of a previous study.[47] The detailed method is provided in the supplementary information section.

Anti-Tyrosinase Activity

All compounds (**1a-l**) were screened for anti-tyrosinase activities. The mushroom tyrosinase

(powder, ≥1000 unit/mg solid, EC 1.14.18.1) inhibitory activities were measured spectrophotometrically via a previously reported method.[48] The detailed method is provided in the supplementary information section.

Cell Lines and Cell Culture

The cell lines, MCF-7 and normal cell lines were obtained from the American Type Cell Collection (ATCC; Manassas, VA, USA). The cells were cultured at 37°C and 5% CO_2 environment to get 70–80% confluence in Dulbecco's Modified Eagle's Medium (DMEM; Gibco®, Thermo Fisher Scientific, Waltham, MA, USA) with 10% fetal bovine serum (FBS) (Gibco®).

Cytotoxic Screening

The newly synthesised compounds (**1a-l**) were tested for cytotoxicity following the methods of a previous study.[47]

The detailed method is provided in the supplementary information section.

Statistical Analysis

The mean of the results was calculated based on at least three independent evaluations and the standard deviations (SD) were also calculated using Microsoft Excel.

Result and Discussion

Chemistry

The one-pot dopamine-connected vanillin multicomponent derivatives were synthesised using the Mannich base method achieved via solvent-free green chemistry. The final solid material was recrystallised using a suitable alcohol to obtain a pure product, as shown in Scheme 1. The optimisation of the reaction conditions is presented in Scheme 2. Target compounds were analysed by FTIR, ^{1}H, and ^{13}C NMR spectra. The key assignments of the compounds showed significant bands at 3621–3409, 1039–1001, 3379–3312, and 1654–1620 cm^{-1} in the IR spectrum, conforming to the –OH, –O-CH_3, NH, and –CH_2-CO- groups, respectively. ^{1}H NMR showed signals at δ 5.45–5.30, 4.10–4.43, 3.11–2.69, 2.85–2.40, and 2.15–2.03 ppm, indicating –C-OH, CH, -CH-CH_2, -CH-CH_2, and NH protons. The ^{13}C NMR showed peaks at δ 210.6–200.2, 148.0–146.6, 146.2–144.3, 144.7–140.1, 58.1–56.0, and 56.9–53.3 ppm, which conformed to the –CH_2-CO, –C-HO, –C-HO, –C-HO, -CH-, and –O-CH_3 atoms. Mass spectroscopy and elemental analysis results were also consistent with the conformation of all compounds.

Biological Activity

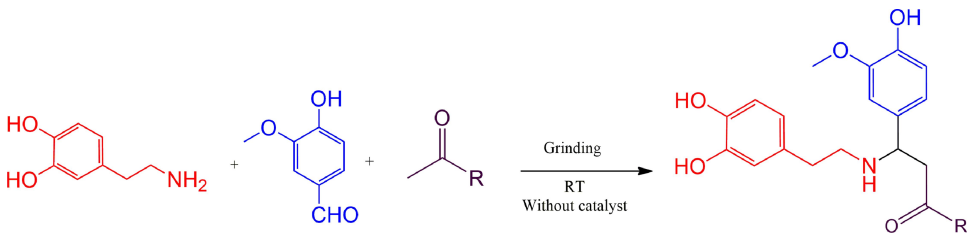
Antioxidant activity was tested using a UV-visible spectrophotometer for compounds (**1a-l**) via DPPH, H_2O_2, NO, ABTS, and AAPH assays. The compounds **1a-l** were screened for cytotoxic activity against MCF-7 and Vero cell lines.

DPPH free-radical scavenging activity increased with an increase in concentration, with compound **1k** showing a maximum of 100% activity at 50 µg/mL. The other compounds **1e**, **1f**, **1h**, and **1i** showed significant scavenging activity (IC_{50}: 14.97, 19.23, 14.56, and 15.28 µg/mL) compared with standard BHT (IC_{50}: 25.17 µg/mL). The DPPH free-radical scavenging activity results are presented in Table 1. Scheme 3 indicates that the mechanism of compound **1k** against the DPPH assay, which is attributed to the highly significant contributions of dopamine and vanillin, plays a major role in activity compared to standard BHT.

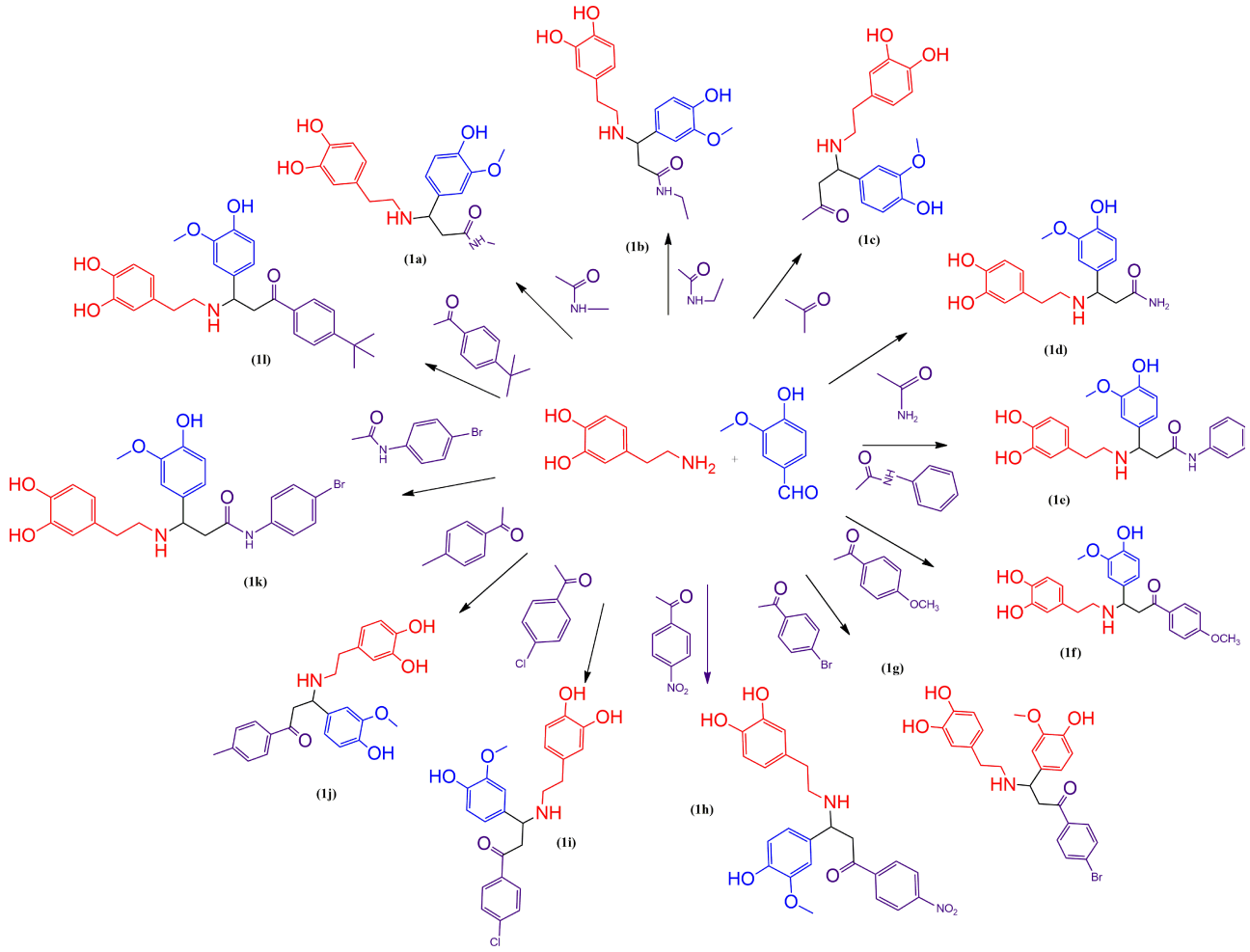
The dopamine-connected vanillin (**1a-l**) showed H_2O_2 scavenging activity between 10 and 100 µg/mL. Compounds **1e**, **1h**, **1i**, **1j**, **1k**, and **1L** showed high activity (100% activity at 100 µg/mL) compared with standard BHT (82.32%) at a concentration of 100µg/mL, with is IC_{50} values corresponding to 13.52, 11.82, 14.00, 13.27, 10.11, and 13.55 µg/mL. The values are shown in Table 2.

The antioxidant mechanism could be explained based on its chemical structures, which comparison with isoeugenol derivatives.[49] For example, compound **1k**, which bears an ortho-dihydroxy, can donate an H atom from its phenol group to DPPH to form the resonance-stabilized free-radical intermediate (Scheme 3). Furthermore, intermediate could react with a second DPPH to form an inactive anion, which on cleavage by protonation would give again quinone structures. Therefore, ortho-dihydroxylated (ie catechol) benzene ring system is generally known to be very efficient systems to delocalized electrons, but not for metadihydroxylated system (ie resorcinol).[50]

The NO radical reacted with Griess reagent to give formazon, which was measured spectrophotometrically by all synthesised compounds (**1a-l**). Compounds **1g**, **1h**, **1i**, **1k**, and **1L** were highly active (100% activity at 100 µg/mL) against standard (83.32% activity at 100 µg/mL)



Scheme 1 Synthesis of dopamine connected vanillin Mannich base derivatives (**1a-1l**).



Scheme 2 Optimization of reaction condition (**1a-1l**).

and other compounds. The IC_{50} values of **1g**, **1h**, **1i**, **1k**, and **1L** were 11.00, 10.36, 14.15, 9.94, and 12.56 µg/mL, respectively. However, compound **1k** was highly active, followed by standard compounds **1g**, **1h**, **1i**, and **1L**. The NO free-radical scavenging activity results are presented in Table 3.

Dopamine-connected vanillin (**1a-l**) was tested for the $ABTS^{\bullet+}$ assay. Compound **1e** (96.21%) was highly active compared with trolox (85.2%). Compounds **1f**, **1g**, **1h**, **1i**, **1j**, **1k**, and **1L** showed >90% more activity than trolox. The ABTS scavenging activity results are presented in Table 4.

The ABTS radical assay is based on a decolourization, with the stable blue/green $ABTS^{\bullet+}$ directly generated before its reaction. All compounds are highly active compounds >80 to 94% activity compared with standard trolox. Mechanism ABTS of activity was represented in scheme 4.

The compounds (**1a-l**) were screened using an AAPH assay for the conjugated diene hydroperoxide by the oxidation of linoleic acid at 234 nm, which was the formation of conjugated diene hydroperoxides caused by the hydrophilic AAPH initiator. The mechanism of activity is represented in Scheme 5. This assay was performed to characterise the antioxidant activity of the synthesised compounds, with **1k** being highly active at 95.28% at a concentration of 100 µg/mL.

The antioxidant action mechanism, mainly based on the inhibition of the formation of reactive O_2 species (ROS), can chelate with metal ions, such as Cu(II) or Fe(II).[51,52]

The compound **1k** was highly active against DPPH (IC_{50}:11.02µg/mL), H_2O_2 (IC_{50}: 10.11 µg/mL), and NO (IC_{50}:9.94 µg/mL) assays whereas low active (IC_{50} :12.11µg/mL) for anti-tyrosinase screening. The compound **1e** (IC_{50}:9.94 µg/mL) was highly active against

Table 1 DPPH-Scavenging Activity of Compounds (**1a-1l**)

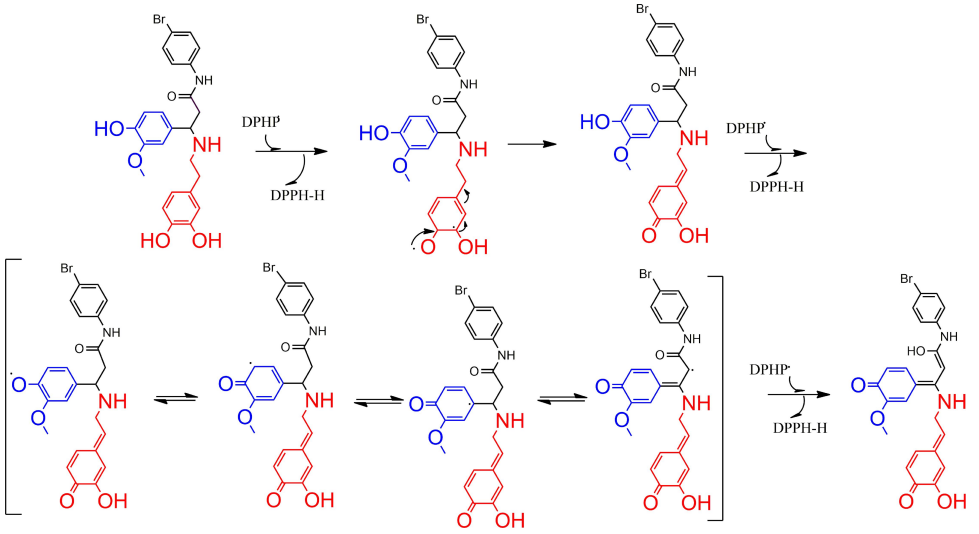
Compound Number	Concentration(μg/mL)[a], % Activity				IC_{50} (μg/mL)
	10 μg/mL	25 μg/mL	50 μg/mL	100 μg/mL	
1a	12.20 ± 0.10	28.21 ± 0.12	33.23 ± 0.15	43.01 ±0.37	>100
1b	14.60 ± 0.03	30.62 ± 0.01	41.01 ± 0.49	58.71 ± 0.12	69.15
1c	10.36 ± 0.15	25.22 ± 0.17	32.10 ± 0.82	51.02 ± 0.09	>100
1d	13.16 ± 0.02	29.10 ± 0.09	38.03 ± 0.01	55.26 ± 0.01	80.21
1e	36.11 ± 0.03	69.12 ± 0.04	84.52 ± 0.01	100 ± 0.00	14.97
1f	26.13 ± 0.03	60.63 ± 0.22	79.03 ± 0.85	100 ± 0.00	19.23
1g	37.03 ± 0.02	69.27 ± 0.14	77.12 ± 0.02	100 ± 0.00	15.22
1h	38.00 ± 0.27	71.10 ± 0.07	79.03 ± 0.21	100 ± 0.00	14.56
1i	37.14 ± 0.22	69.04 ± 0.12	76.11 ± 0.14	100 ± 0.00	15.28
1j	26.11 ± 0.43	61.12 ± 0.23	71.01 ± 0.16	88.71 ± 0.12	21.05
1k	47.22 ± 0.34	82.21 ± 0.10	100 ± 0.00	–	11.02
1l	35.90 ± 1.08	67.10 ± 0.13	81.03 ± 0.02	100 ± 0.11	15.55
BHT	22.08 ± 0.01	54.27 ± 0.22	70.30 ± 0.34	82.31 ± 0.25	25.17

Note: [a]Value expressed are means ± SD of three different experiments.

anti-tyrosinase activity whereas low active against DPPH (IC_{50}:14.97μg/mL), H_2O_2 (IC_{50}:13.52 μg/mL) assays.

Cytotoxicity activity **1e** (IC_{50}:0.16 μg/mL) was highly toxic compared with **1k** (IC_{50}: 0.51 μg/mL), since these activities are only present in concentration greater than 9.94 μg/mL, a concentration that is 100% toxic to MCF-7 and Vero cell lines. Therefore, the compounds **1e** and **1k** were observed highly active against antioxidant and anti-tyrosinase activities in cytotoxic concentrations for both cell lines (MCF-7 and Vero cell lines).

Figure 2 indicates that structure–activity relationship, the compound **1k** have acetamide with 4-bromophenyl group, which shows that it is high antioxidant activity than compound **1e** and **1g**, whereas the compound **1e** has acetamide without halogen, which shows that it is highly anti-tyrosinase activity compared with compounds **1k** and **1g**. The compound **1g** has acetophenone with halogens, which shows that high toxic (LC_{50}:0.30μg/mL) in MCF-7 cell line and twice the concentrations (LC_{50}:15.61 μg/mL) in Vero cell line, whereas it is low in active of antioxidant and anti-tyrosinase screening.



Scheme 3 DPPH-scavenging mechanism of compound **1k**.

Table 2 Hydrogen Peroxide (H_2O_2) Scavenging Activity of Compounds (**1a-1l**)

Extracts	Concentration (μg/mL)[a], % Activity				IC_{50} (μg/mL)
	10	25	50	100	
1a	2.10 ± 0.03	12.12± 0.06	20.21 ± 0.02	43.13 ± 0.02	>100
1b	26.61± 0.14	55.25 ± 0.01	72.01 ± 0.03	84.16 ± 0.15	22.57
1c	41.37 ± 0.09	68.47 ± 0.02	84.16 ± 0.01	92.13 ± 0.03	13.18
1d	4.10 ± 0.02	16.29 ± 0.35	23.11 ± 0.11	36.11 ± 0.03	>100
1e	42.01 ± 0.02	69.12 ± 0.04	84.52 ± 0.32	100 ± 0.00	13.52
1f	38.10 ± 0.16	61.62 ± 0.23	79.01 ± 0.16	92.71 ± 0.12	15.82
1g	40.17 ± 0.69	67.22 ± 0.19	74.12 ± 0.22	91.02 ± 0.21	14.40
1h	46.00 ± 1.27	74.10 ± 0.07	89.03 ± 0.21	100 ± 0.00	11.82
1i	41.04 ± 0.32	68.12 ± 0.12	82.52 ± 0.14	100 ± 0.00	14.00
1j	42.10 ± 0.13	71.62 ± 0.23	82.01 ± 0.16	100 ± 0.00	13.27
1k	51.02 ± 0.82	78.22 ± 0.10	85.00 ± 0.01	100 ± 0.00	10.11
1l	41.09 ± 0.11	71.10 ± 0.07	83.03 ± 0.21	100 ± 0.00	13.55
BHT	29.02 ± 0.03	59.01 ± 1.02	68.51 ± 0.02	82.17 ± 0.77	21.52

Note: [a]Value expressed are means ± SD of three different experiments.

Table 3 NO Scavenging Activity of Compounds (**1a-1l**)

Extracts	Concentration (μg/mL)[a], % Activity				IC_{50} (μg/mL)
	10	25	50	100	
1a	20.98 ± 0.02	40.29 ± 0.22	59.13 ± 0.07	74.39 ± 0.14	35.75
1b	27.60 ± 0.21	52.51 ± 0.21	67.16 ± 0.10	78.12 ± 0.16	24.90
1c	32.30 ± 0.55	61.01 ± 0.03	71.12 ± 0.64	81.11 ± 0.18	19.21
1d	21.13 ± 0.54	41.12 ± 0.05	67.09 ± 0.11	77.10 ± 0.12	31.53
1e	46.42 ± 0.01	79.12 ± 0.02	86.03 ± 0.01	96.20 ± 0.02	10.58
1f	41.00 ± 0.21	71.62 ± 0.20	81.23 ± 0.16	90.06 ± 0.10	12.75
1g	47.07 ± 0.09	79.22 ± 0.16	86.12 ± 0.22	100 ± 0.00	11.00
1h	49.70 ± 1.07	80.10 ± 0.04	91.13 ± 0.20	100 ± 0.00	10.36
1i	39.48 ± 0.82	69.12 ± 0.10	84.52 ± 0.14	100 ± 0.00	14.15
1j	44.19 ± 0.11	75.62 ± 0.18	86.07 ± 0.16	92.21 ± 0.19	11.13
1k	52.22 ± 0.02	86.21 ± 0.01	100 ± 0.00	–	9.94
1l	43.07 ± 0.46	73.10 ± 0.19	92.03 ± 0.21	100 ± 0.00	12.56
BHT	28.03 ± 0.02	53.16 ± 0.02	67.65 ±0.01	83.32 ± 0.51	23.58

Note: [a]Value expressed are means ± SD of three different experiments.

Compared with previous studies, rosmarinic acid was considered as competitive inhibitors[53,54] by mushroom tyrosinase with the IC_{50} values of 16.8 μM, respectively, which is less active than compared with compound **1e**. Another example, the ferulic acid[55] was less active against AAPH antioxidant assay (82%) than compound **1k**. The compound **1k** was compared with L-DOPA,[56] which is less active against DPPH (80.6%), ABTS (99.0%), and AAPH (67.9%) assays. Isoeugenol was also low active (82%) against AAPH assay[49] than compound **1e**. In comparison with vanillin, the vanillin is not active in the DPPH assay[57] and also vanillin was low active for tyrosinase inhibitory[58] than compound **1e**.

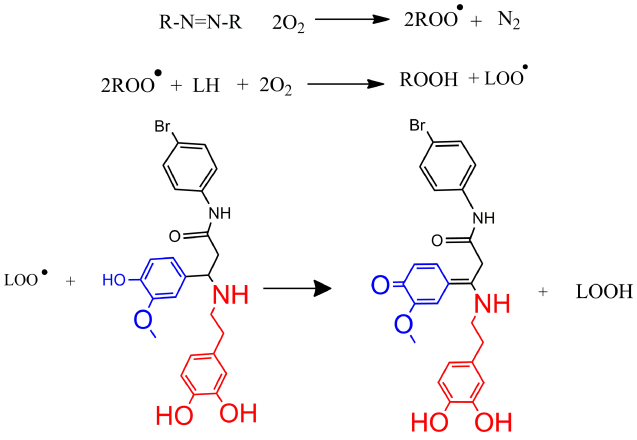
Dopamine was compared with compounds **1k** and **1e** against the MCF-7 and Vero cell lines, however, the dopamine was absolutely inactive up to 100 μM for all cell lines tested.[59]

To estimate the anti-tyrosinase inhibitory activities, the synthesized dopamine-connected vanillin (**1a-l**) was exposed to a tyrosinase inhibitor using L-DOPA as

Table 4 ABTS$^{\bullet+}$ and AAPH Activities of Compounds (**1a-1l**)

Compounds	Percentage of Activity (%)[a]	
	ABTS$^{\bullet+}$	AAPH
1a	87.60 ± 0.08	81.21± 0.11
1b	86.21 ± 0.32	80.21 ± 0.21
1c	81.23 ± 0.12	85.22 ± 0.34
1d	82.31 ± 0.53	80.12 ± 0.42
1e	96.21 ± 0.59	91.02 ± 0.01
1f	95.21 ± 0.19	92.32 ± 0.10
1g	93.25 ± 0.31	94.12 ± 0.42
1h	92.32 ± 0.36	92.01 ± 0.04
1i	91.51 ± 0.20	92.35 ± 0.15
1j	90.28 ± 0.10	94.29 ± 0.95
1k	94.28 ± 0.99	95.28 ± 0.25
1l	92.14 ± 0.17	92.19 ± 0.05
Trolox	85.28 ± 0.97	62.39 ± 0.35

Note: [a]Value expressed are means ± SD of three different experiments.



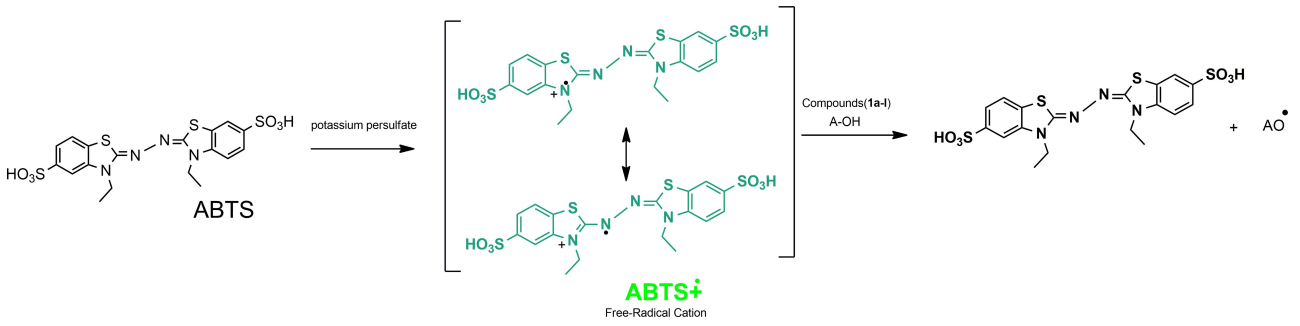
Scheme 5 Mechanism of lipid peroxidation and its inhibition **1k**.

a substrate. Kojic acid, which is used as a skin-whitening ingredient, was used as a reference. The inhibitory effects of the compounds (**1a-l**) are presented in Table 5. Compounds **1e** and **1k** bearing a dopamine-connected vanillin substituent showed better inhibitory activity with IC_{50} values of 10.63 and 12.11µg/mL, respectively, compared to other compounds and kojic acid with an IC_{50} value of 21.52 µg/mL.

Inhibition of dopamine-connected vanillin was tested using L-DOPA as a substrate. Kojic acid is used as a basic skin-whitening element, and was used as a reference compound in this study. The carboxyl and NH groups were present in compounds **1c-l** and kojic acid, which play a major role in this mechanism.[60] Compound **1e** showed the highest inhibition, among them, the mechanism of inhibition was represented in Scheme 6.

The kinetic behavior of the most active compound **1e** was studied with respect to the oxidation of L-DOPA by mushroom tyrosinase at different concentrations. As shown in Figure 3, Lineweaver -Burk plots of 1/V versus 1/[S] resulted in a family of straight lines with the same intercept on the vertical axis. The plots obtained indicated that compound **1e** is a competitive inhibitor and that its inhibitory activity decreases with increasing substrate concentration.

Antioxidant agents can form free-radical scavengers and inhibit enzymes, which are related to the design of chemical structures.[61–63] Target compounds (**1a-l**) can act as hydrogen donors, providing atoms directly to the radicals and preventing the formation of toxic OH radicals to the cell membrane peroxidation.[64]



Scheme 4 Reaction mechanism of ABTS$^{\bullet+}$ radical.

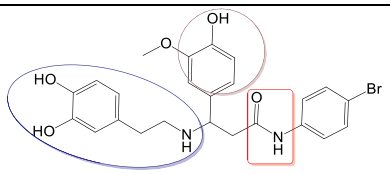
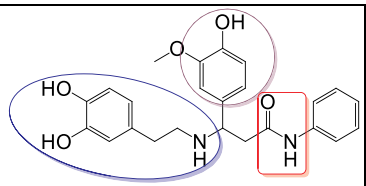
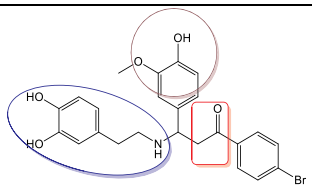
	 1K	 1e	 1g
Antioxidant Activity	DPPH= IC_{50}: 11.02 µg /mL	DPPH: IC_{50}:14.56 µg /mL	DPPH:IC_{50}:12.22 µg /mL
	H_2O_2=IC_{50}: 10.11 µg /mL	H_2O_2: IC_{50}:13.52 µg /mL	H_2O_2: IC_{50}:14.40 µg /mL
	NO: IC_{50}: 9.94 µg /mL	NO: 10.58 µg /mL	NO: IC_{50}:11.00 µg /mL
Tyrosinase activity	IC_{50}: 12.11 µg /mL	IC_{50}: 10.63 µg /mL	IC_{50}: 36.82 µg /mL
Cytotoxic Activity (MCF-7)	IC_{50}:0.51 µg /mL	IC_{50}: 0.16µg /mL	IC_{50}: 0.30 µg /mL

Figure 2 Structure–activity relationship and comparison of highly active compounds.

The compounds **1f**, **1g**, and **1h** were at close concentration range of (0.30 to 0.67 µm/mL) in MCF-7 cells with different activity such as DPPH (15.22 to 19.23, µm/mL), NO (10.36 to 12.75 µm/mL), H_2O_2 (11.82 to 14.40 µm/mL) assays, and anti-tyrosinase activity (25.47 to 36.59, µm/mL), whereas that are cytotoxic in VERO cells only at twice the concentrations.

The compound **1j**, and **1l** were equipotent activity against H_2O_2 antioxidant assay (13.27, and 13.55 µm/mL), closely related activity against NO antioxidant assay (11.13, and 12.56 µm/mL), and the closely related against anti-tyrosinase activity (24.76, and 28.63µm/mL), whereas that are cytotoxic in MCF-7 cell line (9.36, 4.49 µm/mL), and Vero cells (26.18, and 28.11µm/mL) concentrations, respectively.

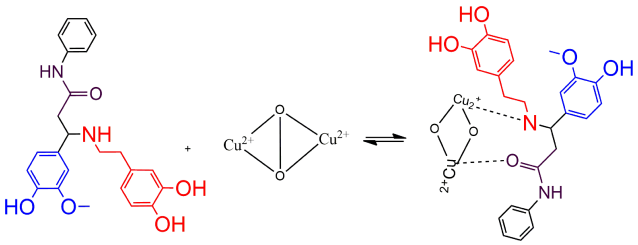
Further the activity of all effective compounds were tested against the normal cell line (VERO cell line) and it was concluded that most of compounds were obtained cytotoxic at twice the concentrations to normal cell compared than MCF-7 cell line.

The cytotoxic results of each test are reported as the growth of treated cells in Table 6. As a result,

Table 5 The Compounds (**1a-1l**) Tyrosinase Screening

Compound	Concentration (µg/mL)[a], % Activity				IC_{50} (µg/mL)
	10	25	50	100	
1a	0.0 + 0.00	3.12 ± 0.17	17.81 ± 0.19	36.74 ± 0.98	>100
1b	0.0 + 0.00	1.05 ± 0.26	12.84 ± 0. 12	26.52 ± 0.98	>100
1c	12.40 ± 0.14	28.70 ± 0.31	42.84 ± 0. 65	58.52 ± 0.57	68.09
1d	06.71 ± 0.19	18.70 ± 0.20	27.84 ± 0.43	48.52 ± 0.18	>100
1e	46.33 ± 0.03	78.63 ± 0.43	86.81 ± 0.29	96.02 ± 1.18	10.63
1f	21.05 ± 0.16	40.75 ± 0.66	59.84 ± 0.19	78.52 ± 0.63	33.59
1g	23.71 ± 0.44	44.75 ± 0.54	51.84 ± 0.13	72.52 ± 0.17	36.82
1h	27.79 ± 0.17	49.75 ± 0.47	63.84 ± 0.24	83.52 ± 0.05	25.47
1i	22.95 ± 0.48	42.75 ± 0.38	61.84 ± 0.29	80.52 ± 0.29	30.86
1j	28.55 ± 0.21	49.75 ± 0.23	64.84 ± 0.34	84.52 ± 0.88	24.76
1k	41.85 ± 0.17	74.75 ± 0.07	81.84 ± 0.10	92.02 ± 0.31	12.11
1l	22.99 ± 1.87	42.68 ± 0.87	69.15 ± 0.14	81.10 ± 0.12	28.63
Kojic acid	31.01 ± 0.98	55.60 ± 0.02	68.12 ± 0.10	84.12 ± 0.93	21.52

Note: [a]Value expressed are means ± SD of three different experiments.



Scheme 6 The binuclear active site of tyrosinase with reversible competitive binding of compound **1e**.

among the synthesized compounds evaluated, compound **1e**, **1f**, and **1g** were highest cytotoxic against MCF-7 cell line and low active against Vero cell line than that of doxorubicin. Moreover, the selectivity index (SI) of the compounds **1e**, **1k**, and **1g** (Vero and MCF-7) was equipotent than that of doxorubicin with SI values. The IC_{50} values and selectivity index (SI) that obtained from the MTT assay are presented in Table 6.

Conclusion

New dopamine-connected vanillin multicomponent derivatives (**1a-l**) were synthesised via the grindstone method in high yields (85–92%) via a one-pot Mannich base without using catalysis. This method is inexpensive and produces a high yield. We synthesised 12 dopamine-connected vanillin derivatives and evaluated their anti-tyrosinase and antioxidant activities as well as their cytotoxicity. Compound **1k** was highly active in DPPH, H_2O_2 scavenging, and NO scavenging. On the other hand, compounds **1e** and **1k** were highly active in $ABTS^{\bullet+}$ and AAPH assays compared with the trolox standard. Compounds **1e** and **1k** significantly inhibited tyrosinase activity compared with standard kojic acid, and compound **1e** (GI_{50} = 0.01 µM) showed higher cytotoxicity in the MCF-7 cancer cell line. Therefore, lead compounds **1e** and **1k** are the new class of most effective antioxidant and anti-tyrosinase agents, and further development is required.

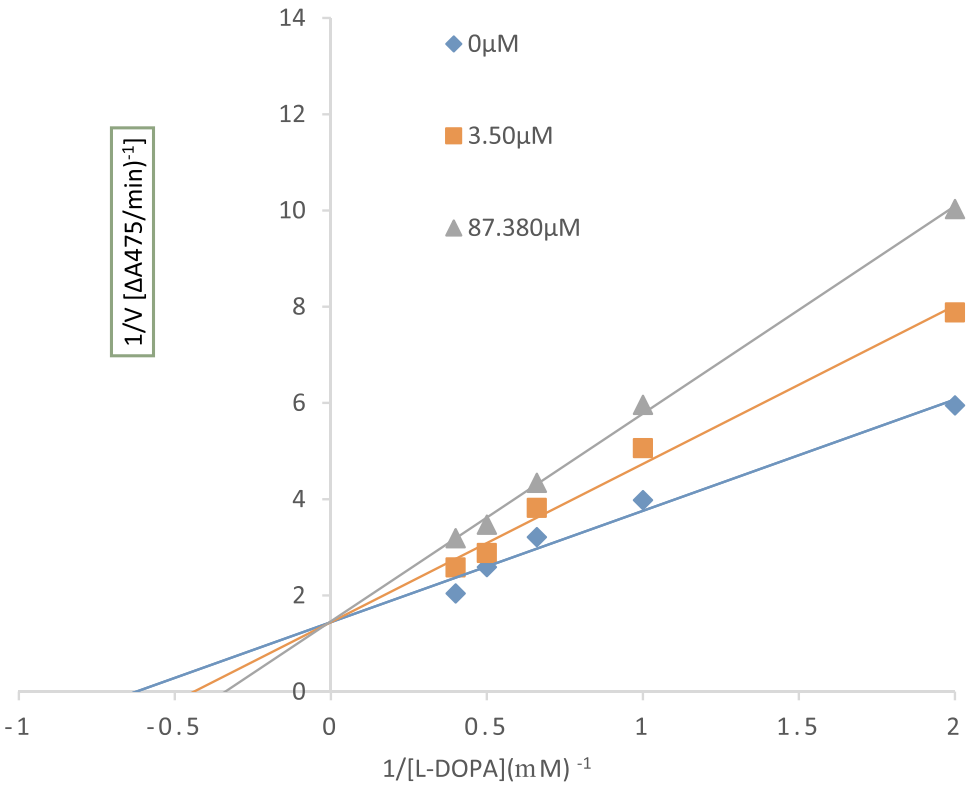


Figure 3 Inhibition of compound **1e** – Lineweaver-Burk plot.

Table 6 Cytotoxicity Activity of Compounds (**1a-j**)

Compounds	MCF-7 Cell Line			Vero	SI[b]
	GI_{50}(μM)[a]	TGI(μM)[a]	LC_{50}(μM)[a]/(μg/mL)	LC_{50}(μg/mL)[a]	
1a	12.10 ± 0.06	26.00 ± 0.09	48.15± 0.02/(17.35)	26.02 ± 0.11	1.49
1b	21.10 ± 0.14	44.90 ± 0.33	88.20 ± 0.11/(33.02)	35.23 ± 0.05	1.06
1c	22.40 ± 0.13	46.20 ± 0.42	87.00 ± 0.01/(30.04)	31.81 ± 0.15	1.05
1d	26.20 ± 0.03	52.00 ± 0.12	89.00 ± 0.77/(30.40)	33.36 ± 0.48	1.08
1e	0.01 ± 0.00	0.20 ± 0.01	0.40 ± 0.01/(0.16)	9.39 ± 0.86	55.62
1f	0.21 ± 0.09	0.46 ± 0.07	0.89 ± 0.04/(0.37)	16.16 ± 0.57	42.92
1g	0.02 ± 0.00	0.40 ± 0.04	0.62 ± 0.06/(0.30)	15.61 ± 0.76	61.62
1h	0.16 ± 0.41	0.29 ± 0.01	1.50 ± 0.15/(0.67)	19.96 ± 1.78	29.41
1i	1.09 ± 0.23	3.05 ± 0.02	6.56 ± 0.17/(2.89)	25.72 ± 1.05	8.87
1j	5.20 ± 0.19	10.10 ± 0.02	22.21 ± 0.22/(9.36)	26.18 ± 1.28	2.79
1k	0.05 ± 0.01	0.16 ± 0.01	1.02 ± 0.01/(0.51)	21.23 ± 1.11	41.52
1l	2.60 ± 0.04	4.24 ± 0.09	9.70 ± 0.08/(4.49)	28.11 ± 0.62	6.25
Doxorubicin	0.02 ± 0.00	0.21 ± 0.01	0.74 ± 0.01/(0.40)	21.85 ± 1.82	54.62

Notes: [a]Data represent the mean ± standard error of the mean values of three separate experiments. [b]SI, Selectivity Index; IC_{50} value normal cell/IC_{50} value cancer cell.

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Disclosure

The authors have no conflicts of interest to declare.

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