



Design, Synthesis and Biological Activities of Dihydroaurones

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To widen aurones applicability in achromatic food and cosmetic applications, a series of dihydroaurones were designed to mimic natural aurones as well as synthetic aurones. Dihydroaurones have been synthesized from the corresponding aurones by hydrogenation. These dihydroaurones and their corresponding aurones were screened for antioxidant, anti-inflammatory and tyrosinase enzyme inhibitory activity. Synthesized dihydroaurones (**3b-f**) displayed superior antioxidant activity in superoxide free radical scavenging assay than the standard gallic acid. Dihydroaurones (**3b-f**) also exhibited significant tyrosinase enzyme inhibitory activity and two dihydroaurones (**3h**, **3j**) showed promising 5-lipoxygenase inhibitory activity.

Keywords: Dihydroaurones, Aurones, Antioxidant, 5-Lipoxygenase, Tyrosinase.

INTRODUCTION

Dihydroaurones [2-benzylbenzofuran-3(2*H*)-ones] are hydrogenated derivatives of aurones [2-benzylidene benzofuran-3(2*H*)-ones]. Aurones are naturally occurring yellow pigments of plants and are structurally related to flavonoids. In addition to this pigmentation role, aurones have been described as phytoalexins [1], used by the plant as defense agents against various infections. Aurones exhibit wide range of pharmacological activities [2] such as antitumor/anticancer [3-6], antidiabetic [7], anti-inflammatory [8], antioxidant [9], antibacterial [10] anti-Alzheimer [11], insect antifeedant [12] and tyrosinase [13] *via* modulation of a variety of molecular targets. Unfortunately, biological activities of dihydroaurones are not studied so far.

Although yellow coloured aurones have been reported to be strong antioxidants, they cannot be used in the food and the like which shall not be coloured. So there is always a huge demand for the colourless strong antioxidants. Further, we thought that dihydroaurones might be colourless and finds use in achromatic food and cosmetic applications.

Free radicals play a major role in the progression of a wide range of pathological disturbances and lead to serious problems like cancer, Alzheimer's, Parkinson's and cardiovascular diseases. In food industry, free radicals have been found to be responsible

in the deterioration of foods during processing and storage. In view of this, considerable attention has been given to the addition of antioxidants in foods and supplementation of antioxidants to biological systems to scavenge free radicals [14,15].

Presently, there has been a tremendous surge in demand for non-steroidal, plant based anti-inflammatory agents. 5-Lipoxygenase is the key enzyme for the biosynthesis of leukotriene and 5(S)-HETE, the important mediators for inflammatory, allergic and obstructive process from arachidonic acid. 5-Lipoxygenase is the target enzyme for identifying inhibitors, which have potential to cope with a variety of inflammations and hypersensitivity-based human diseases including asthma, arthritis, bowel diseases such as ulcerative colitis and circulatory disorders such as shock and ischaemia [16].

Tyrosinase is a multifunctional membrane bound type-3 copper-containing glycoprotein located in the melanosome. Tyrosinase catalyzes the synthesis of melanin in a process called melanogenesis. Tyrosinase has also been linked in the quick degradation and browning of vegetables and fruits during post-harvest and handling process. Many natural and synthetic products like hydroquinone, arbutin, kojic acid, azelaic acid, L-ascorbic acid, ellagic acid and tranexamic acid, have been used as tyrosinase inhibitors [17,18]. All these products have certain defects like carcinogenicity, instability and poor bioavail-

ability. Thus, it is in great need of developing new tyrosinase inhibitors without side effects. Aurones are proved to be potent tyrosinase inhibitors [13] but the tyrosinase inhibitory activity of the corresponding dihydroaurones is not studied before. Due to our continuous interest on aurones and isoaurones [9, 19-21], herein we report the synthesis of dihydroaurones and their biological activities.

EXPERIMENTAL

Reagents and solvents were of analytical grade and used without further purification. Melting points were recorded on a Mel-Temp melting point apparatus using open capillaries and are uncorrected. ^1H NMR (400 MHz), ^{13}C NMR-DEPT (100 MHz) spectra were recorded on a Bruker AMX 400 MHz NMR spectrometer. In the ^{13}C NMR spectra, the nature of the carbons (C, CH, CH_2 or CH_3) was determined by DEPT-135 spectra. Mass spectra were recorded on Agilent 1100 LC/MSD. HRMS were recorded on Micromass Q-TOF spectrometer using electrospray ionization mode. Benzofuran-3(2H)-ones (**1**) was synthesized according to the known procedures [9].

Synthesis of aurones: A mixture of benzofuran-3(2H)-one (**1**, 2.0 mmol), aromatic aldehyde (**4**, 2.2 mmol) and water (5 mL) was stirred at reflux temperature for 6-10 h. Completion of the reaction was checked on TLC. Then the mixture was allowed to room temperature and stirred for 1 h. The precipitated solids were filtered, washed with water (2×5 mL) and dried to give the products.

(Z)-2-Benzylidenebenzofuran-3(2H)-one (2a): Pale yellow colour solid (380 mg, 86%), m.p.: 102-104 °C (lit. [22] m.p.: 110-111 °C). ^1H NMR (CDCl_3): δ 7.93 (2H, d, $J = 6.8$ Hz), 7.81 (1H, d, $J = 7.6$ Hz), 7.66 (1H, m), 7.43 (3H, m), 7.34 (1H, d, $J = 8.0$ Hz), 7.23 (1H, t, $J = 7.6$ Hz, 7.2 Hz) 6.90 (1H, s); ^{13}C NMR (CDCl_3): δ 184.7, 166.2, 146.9, 136.8, 132.4, 131.5, 129.9, 128.9, 124.7, 123.5, 121.7, 113.0, 112.9; LC-MS (positive ion mode): m/z 223 (M+H) $^+$.

(Z)-6-Hydroxy-2-(4-hydroxybenzylidene)benzofuran-3(2H)-one (2b, hispidol): Yellow colour solid (430 mg, 85%), m.p.: 288-290 °C (lit. [23] m.p.: 294-296 °C). ^1H NMR ($\text{DMSO}-d_6$): δ 11.11 (1H, br s), 10.12 (1H, br s), 7.82 (2H, d, $J = 8.8$ Hz), 7.6 (1H, d, $J = 8.4$ Hz), 6.89 (2H, d, $J = 8.8$ Hz), 6.78 (1H, d, $J = 1.6$ Hz), 6.73 (1H, s), 6.71 (1H, dd, $J = 8.8$ Hz, 2.0 Hz); ^{13}C NMR ($\text{DMSO}-d_6$): δ 181.2, 167.5, 166.1, 159.2, 145.7, 133.2, 125.6, 123.0, 116.0, 113.2, 112.8, 111.3, 98.5; LC-MS (negative ion mode): m/z 253 (M-H) $^-$.

(Z)-2-(3,4-Dihydroxybenzylidene)-6-hydroxybenzofuran-3(2H)-one (2c, sulfuretin): Yellow colour solid (440 mg, 81%), m.p.: 315-317 °C (lit. [24] m.p.: 315 °C). ^1H NMR ($\text{DMSO}-d_6$): δ 11.12 (1H, br s), 9.68 (1H, br s), 9.27 (1H, s), 7.61 (1H, d, $J = 8.4$ Hz), 7.46 (1H, d, $J = 1.6$ Hz), 7.25 (1H, dd, $J = 8.4$ Hz, 2.0 Hz), 6.84 (1H, d, $J = 8.0$ Hz), 6.76 (1H, d, $J = 2.0$ Hz), 6.71 (1H, dd, $J = 8.4$ Hz, 1.6 Hz), 6.65 (1H, s); ^{13}C NMR ($\text{DMSO}-d_6$): δ 181.1, 167.4, 166.1, 148.0, 145.6, 145.5, 125.7, 124.5, 123.4, 118.0, 116.0, 113.2, 112.8, 111.8, 98.3; LC-MS (negative ion mode): m/z 269 (M-H) $^-$.

(Z)-2-(3,4-Dihydroxybenzylidene)-6,7-dihydroxybenzofuran-3(2H)-one (2d, maritimetin): Yellow colour solid (460 mg, 80%), m.p.: 296-304 °C (lit. [9] m.p.: 286-288 °C). ^1H

NMR ($\text{DMSO}-d_6$): δ 10.66 (1H, br s), 9.67 (1H, br s), 9.50 (1H, br s), 9.22 (1H, br s), 7.44 (1H, s) 7.39 (1H, d, $J = 8.0$ Hz), 7.13 (1H, d, $J = 8.0$ Hz), 6.86 (1H, d, $J = 8.0$ Hz), 6.74 (1H, d, $J = 8.0$ Hz), 6.63 (1H, s); ^{13}C NMR ($\text{DMSO}-d_6$): δ 182.0, 155.0, 154.2, 147.9, 145.9, 145.4, 130.1, 124.5, 123.5, 118.4, 115.9, 115.2, 114.6, 112.5, 111.7; LC-MS (positive ion mode): m/z 287 (M+H) $^+$.

(Z)-2-(3,4-Dihydroxybenzylidene)-4,6-dihydroxybenzofuran-3(2H)-one (2e, aureusidin): Yellow colour solid (347 mg, 81%), m.p.: 264-268 °C (lit. [23] m.p.: > 260 °C). ^1H NMR ($\text{DMSO}-d_6$): δ 10.81 (2H, s), 9.52 (1H, s), 9.19 (1H, s), 7.39 (1H, d, $J = 0.8$ Hz), 7.18 (1H, d, $J = 8.4$ Hz), 6.81 (1H, d, $J = 8.0$ Hz), 6.45 (1H, s), 6.18 (1H, s), 6.07 (1H, s); ^{13}C NMR ($\text{DMSO}-d_6$): δ 179.0, 167.5, 166.9, 158.1, 147.4, 145.9, 145.4, 123.8, 123.6, 117.6, 115.9, 109.5, 102.8, 97.6, 90.3; LC-MS (negative ion mode): m/z 285 (M-H) $^-$.

(Z)-2-(4-Hydroxybenzylidene)-6-methoxybenzofuran-3(2H)-one (2f): Pale yellow colour solid (450 mg, 84%), m.p.: 212-214 °C (lit. [25] m.p.: 215-217 °C). ^1H NMR ($\text{DMSO}-d_6$): δ 10.17 (1H, s), 7.85 (2H, d, $J = 8.4$ Hz), 7.68 (1H, d, $J = 8.4$ Hz), 7.14 (1H, d, $J = 1.6$ Hz), 6.90 (2H, d, $J = 8.4$ Hz), 6.85 (1H, dd, $J = 8.8$ Hz, 2.0 Hz), 6.8 (1H, s), 3.93 (3H, s); ^{13}C NMR ($\text{DMSO}-d_6$): δ 181.3, 167.5, 167.0, 159.4, 145.6, 133.3, 125.2, 122.9, 116.1, 114.3, 112.4, 111.9, 97.0, 56.3; LC-MS (negative ion mode): m/z 267 (M-H) $^-$.

(Z)-2-(4-Methoxybenzylidene)-6-methoxybenzofuran-3(2H)-one (2g): Pale yellow colour solid (470 mg, 83%), m.p.: 130-132 °C (lit. [25] m.p.: 132-134 °C). ^1H NMR (CDCl_3): δ 7.85 (2H, d, $J = 8.8$ Hz), 7.69 (1H, d, $J = 8.4$ Hz), 6.96 (2H, d, $J = 8.4$ Hz), 6.8 (1H, s), 6.73 (2H, m), 3.92 (3H, s), 3.86 (3H, s); ^{13}C NMR (CDCl_3): δ 182.8, 168.2, 167.2, 160.8, 146.8, 133.1, 125.7, 125.2, 115.2, 114.4, 112.1, 111.9, 96.6, 55.9, 55.3; LC-MS (positive ion mode): m/z 283 (M+H) $^+$.

(Z)-2-(3,4-Dimethoxybenzylidene)-5-methoxybenzofuran-3(2H)-one (2h): Yellow colour solid (516 mg, 82%), m.p.: 160-162 °C. ^1H NMR ($\text{DMSO}-d_6$): δ 7.61 (2H, m) 7.50 (1H, d, $J = 8.8$ Hz), 7.38 (1H, dd, $J = 9.2$ Hz, 2.8 Hz), 7.24 (1H, d, $J = 2.8$ Hz), 7.10 (1H, d, $J = 8.8$ Hz), 6.92 (1H, s), 3.85 (3H, s), 3.84 (3H, s), 3.82 (3H, s); ^{13}C NMR ($\text{DMSO}-d_6$): δ 183.3, 160.1, 155.8, 150.8, 148.8, 146.0, 125.7, 125.6, 124.6, 121.4, 114.5, 114.1, 113.1, 112.0, 105.4, 55.9, 55.6, 55.5; LC-MS (positive ion mode): m/z 313 (M+H) $^+$. HRMS-(EI) (m/z): (M+H) $^+$ calcd. for $\text{C}_{18}\text{H}_{17}\text{O}_5$ 313.1076, found 313.1073.

(Z)-4,6-Dimethoxy-2-(4-methoxybenzylidene)benzofuran-3(2H)-one (2i): Yellow colour solid (491 mg, 79%), m.p.: 164-166 °C (lit. [26] 168-169 °C). ^1H NMR ($\text{DMSO}-d_6$): δ 7.89 (2H, d, $J = 8.4$ Hz) 7.05 (2H, d, $J = 8.4$ Hz), 6.69 (1H, s), 6.68 (1H, s), 6.33 (1H, s), 3.92 (3H, s), 3.89 (3H, s), 3.83 (3H, s); ^{13}C NMR ($\text{DMSO}-d_6$): δ 178.8, 168.7, 168.0, 160.3, 158.8, 146.1, 132.7, 124.6, 114.5, 109.7, 104.2, 94.3, 89.8, 56.4, 56.1, 55.3; LC-MS (positive ion mode): m/z 313 (M+H) $^+$.

(Z)-2-(4-(Dimethylamino)benzylidene)-6-hydroxybenzofuran-3(2H)-one (2j): Orange red colour solid (264 mg, 47%), m.p.: 258-262 °C (lit. [27] m.p.: 235-237 °C). ^1H NMR ($\text{DMSO}-d_6$): δ 11.03 (1H, br s), 7.80 (2H, d, $J = 8.8$ Hz), 7.58 (1H, d, $J = 8.4$ Hz), 6.80 (2H, d, $J = 9.2$ Hz), 6.77 (1H, d, $J = 2.0$ Hz), 6.72 (1H, s), 6.70 (1H, dd, $J = 8.4$ Hz, 2.0 Hz), 3.01

(6H, s); ^{13}C NMR (DMSO- d_6): δ 180.7, 167.0, 165.7, 151.0, 145.0, 132.9, 125.4, 119.1, 113.6, 112.6, 112.4, 112.0, 98.4, 39.58; LC-MS (negative ion mode): m/z 280 (M-H) $^-$.

General procedure for the synthesis of dihydroaurones:

To a solution of aurone (**2**, 5 mmol) in a mixture of ethyl acetate: methanol (1:1, 70 mL) was added palladium-charcoal (10%, 200 mg). The reaction mixture was stirred under hydrogen atmosphere for 1 h. The catalyst was removed by filtration and the solvent was evaporated under reduced pressure. The residue was chromatographed over silica gel column to give dihydroaurones.

2-Benzylbenzofuran-3(2H)-one (3a): White colour solid (900 mg, 80%), m.p.: 42-44 °C. ^1H NMR (DMSO- d_6): δ 7.68 (1H, t, J = 8.4 Hz, 7.2 Hz) 7.60 (1H, d, J = 7.6 Hz), 7.23 (6H, m), 7.11 (1H, t, J = 7.6 Hz, 2.0 Hz), 5.09 (1H, dd, J = 8.0 Hz, 4.0 Hz), 3.27 (1H, dd, J = 14.4 Hz, 4.0 Hz), 2.98 (1H, dd, J = 14.8 Hz, 8.0 Hz); ^{13}C NMR (DMSO- d_6): δ 200.4, 171.9, 138.3, 136.1, 129.3, 128.1, 126.6, 123.7, 121.9, 120.6, 113.4, 85.1, 36.3; LC-MS (positive ion mode): m/z 225 (M+H) $^+$. HRMS-(EI) (m/z): (M+H) $^+$ calcd. for $\text{C}_{15}\text{H}_{13}\text{O}_2$ 225.0915, found 225.0910.

6-Hydroxy-2-(4-hydroxybenzyl)benzofuran-3(2H)-one (3b, dihydrohispidol): Off-white colour powder (1.13 g, 88%), m.p.: 152-154 °C. ^1H NMR (DMSO- d_6): δ 10.87 (1H, s), 9.20 (1H, s), 7.40 (1H, d, J = 8.4 Hz), 7.03 (2H, d, J = 8.4 Hz), 6.63 (2H, d, J = 8.0 Hz) 6.51 (1H, dd, J = 8.4 Hz, 1.6 Hz), 6.42 (1H, d, J = 1.6 Hz), 4.89 (1H, dd, J = 8.0 Hz, 4.0 Hz), 3.1 (1H, dd, J = 14.8 Hz, 4.0 Hz), 2.80 (1H, dd, J = 14.8 Hz, 8.0 Hz); ^{13}C NMR (DMSO- d_6): δ 197.6, 174.3, 166.9, 155.9, 130.2, 126.2, 125.2, 114.9, 112.7, 111.7, 98.0, 86.0, 35.8; LC-MS (negative ion mode): m/z 255 (M-H) $^-$. HRMS-(EI) (m/z): (M+H) $^+$ calcd. for $\text{C}_{15}\text{H}_{13}\text{O}_4$ 257.0813, found 257.0820.

2-(3,4-Dihydroxybenzyl)-6-hydroxybenzofuran-3(2H)-one (3c, Dihydrosulfuretin): Off-white colour powder (1.1 g, 81%), m.p.: 200-204 °C. ^1H NMR (DMSO- d_6): δ 10.87 (1H, s), 8.73 (1H, s), 8.67 (1H, s), 7.40 (1H, d, J = 8.4 Hz), 6.63 (1H, d, J = 2.0 Hz), 6.59 (1H, d, J = 8.0 Hz), 6.52 (1H, dd, J = 8.4 Hz, 1.6 Hz), 6.49 (1H, dd, J = 8.4 Hz, 1.6 Hz), 6.43 (1H, d, J = 2.0 Hz), 4.85 (1H, dd, J = 8.0 Hz, 4.0 Hz), 3.04 (1H, dd, J = 14.8 Hz, 4.0 Hz), 2.73 (1H, dd, J = 14.8 Hz, 8.0 Hz); ^{13}C NMR (DMSO- d_6): δ 197.6, 174.3, 166.8, 144.8, 143.8, 126.9, 125.2, 120.0, 116.7, 115.3, 112.7, 111.6, 98.0, 86.0, 36.0; LC-MS (negative ion mode): m/z 271 (M-H) $^-$. HRMS-(EI) (m/z): (M+H) $^+$ calcd. for $\text{C}_{15}\text{H}_{13}\text{O}_5$ 273.0763, found 273.0758.

2-(3,4-Dihydroxybenzyl)-6,7-dihydroxybenzofuran-3(2H)-one (3d, dihydromaritimetin): Off-white colour powder (1.21 g, 84%), m.p.: 198-200 °C. ^1H NMR (DMSO- d_6): δ 10.35 (1H, s), 9.05 (1H, s), 8.75 (1H, s), 8.69 (1H, s), 6.93 (1H, d, J = 8.4 Hz), 6.66 (1H, s), 6.56 (3H, m), 4.87 (1H, dd, J = 7.6 Hz, 3.6 Hz), 3.04 (1H, dd, J = 14.8 Hz, 3.6 Hz), 2.74 (1H, dd, J = 14.8 Hz, 8.0 Hz); ^{13}C NMR (DMSO- d_6): δ 198.5, 162.3, 154.2, 144.8, 143.8, 130.1, 127.1, 120.0, 116.8, 115.3, 114.5, 113.9, 111.6, 86.1, 36.2; LC-MS (negative ion mode): m/z 287 (M-H) $^-$. HRMS-(EI) (m/z): (M+H) $^+$ calcd. for $\text{C}_{15}\text{H}_{13}\text{O}_6$ 289.0712, found 289.0705.

2-(3,4-Dihydroxybenzyl)-4,6-dihydroxybenzofuran-3(2H)-one (3e, dihydroaureusidin): White colour powder (1.24 g, 86%), m.p.: 258-260 °C. ^1H NMR (DMSO- d_6): δ 10.54

(2H, s), 8.73 (1H, s), 8.66 (1H, s), 6.63 (1H, d, J = 1.6 Hz) 6.60 (1H, d, J = 8.0 Hz), 6.49 (1H, dd, J = 8.0 Hz, 1.6 Hz), 5.87 (1H, s), 5.85 (1H, s), 4.7 (1H, dd, J = 8.0 Hz, 3.6 Hz), 2.98 (1H, dd, J = 14.8 Hz, 3.6 Hz), 2.66 (1H, dd, J = 14.8 Hz, 8.0 Hz); ^{13}C NMR (DMSO- d_6): δ 194.6, 174.1, 167.6, 157.5, 144.8, 143.8, 127.3, 120.0, 116.7, 115.3, 102.4, 96.1, 89.9, 85.7, 36.2; LC-MS (negative ion mode): m/z 287 (M-H) $^-$. HRMS-(EI) (m/z): (M+H) $^+$ calcd. for $\text{C}_{15}\text{H}_{13}\text{O}_6$ 289.0712, found 289.0705.

2-(4-Hydroxybenzyl)-6-methoxybenzofuran-3(2H)-one (3f): Off-white colour powder (1.1 g, 82%), m.p.: 118-120 °C. ^1H NMR (DMSO- d_6): δ 9.20 (1H, s), 7.47 (1H, d, J = 8.4 Hz), 7.05 (2H, d, J = 8.0 Hz), 6.75 (1H, d, J = 1.6 Hz), 6.65 (3H, m), 4.96 (1H, dd, J = 8.0 Hz, 4.0 Hz), 3.84 (3H, s), 3.13 (1H, dd, J = 14.8 Hz, 4.0 Hz), 2.83 (1H, dd, J = 14.8 Hz, 8.0 Hz); ^{13}C NMR (DMSO- d_6): δ 197.9, 174.5, 167.9, 156.0, 130.2, 126.1, 124.8, 114.9, 113.7, 111.4, 96.5, 86.3, 56.1, 35.8; LC-MS (positive ion mode): m/z 271 (M+H) $^+$. HRMS-(EI) (m/z): (M+H) $^+$ calcd. for $\text{C}_{16}\text{H}_{15}\text{O}_4$ 271.0970, found 271.0963.

6-Methoxy-2-(4-methoxybenzyl)benzofuran-3(2H)-one (3g): Off-white colour powder (1.2 g, 84%), m.p.: 62-64 °C. ^1H NMR (DMSO- d_6): δ 7.48 (1H, d, J = 8.8 Hz), 7.19 (2H, d, J = 8.0 Hz), 6.82 (2H, d, J = 8.4 Hz), 6.76 (1H, d, J = 1.6 Hz), 6.65 (1H, dd, J = 8.8 Hz, 1.6 Hz), 5.00 (1H, dd, J = 8.0 Hz, 4.0 Hz), 3.84 (3H, s), 3.70 (3H, s), 3.18 (1H, dd, J = 14.8 Hz, 4.0 Hz), 2.88 (1H, dd, J = 14.4 Hz, 8.0 Hz); ^{13}C NMR (DMSO- d_6): δ 197.8, 174.4, 167.9, 158.0, 130.3, 128.0, 124.8, 113.7, 113.6, 111.4, 96.5, 86.1, 56.1, 54.9, 35.6; LC-MS (positive ion mode): m/z 285 (M+H) $^+$. HRMS-(EI) (m/z): (M+H) $^+$ calcd. for $\text{C}_{17}\text{H}_{17}\text{O}_4$ 285.1126, found 285.1124.

2-(3,4-Dimethoxybenzyl)-5-methoxybenzofuran-3(2H)-one (3h): Off-white colour powder (1.26 g, 80%), m.p.: 94-96 °C. ^1H NMR (DMSO- d_6): δ 7.31 (1H, dd, J = 8.8 Hz, 2.4 Hz), 7.18 (1H, d, J = 8.8 Hz), 7.03 (1H, d, J = 2.4 Hz), 6.88 (1H, s), 6.79 (2H, m), 5.06 (1H, dd, J = 7.6 Hz, 4.0 Hz), 3.75 (3H, s), 3.69 (6H, s), 3.20 (1H, dd, J = 14.8 Hz, 4.0 Hz), 2.90 (1H, dd, J = 14.8 Hz, 8.0 Hz); ^{13}C NMR (DMSO- d_6): δ 200.6, 167.2, 154.4, 148.4, 147.6, 128.4, 127.5, 121.4, 120.7, 114.4, 113.2, 111.7, 104.2, 85.9, 55.8, 55.5, 55.4, 36.0; LC-MS (positive ion mode): m/z 315 (M+H) $^+$. HRMS-(EI) (m/z): (M+H) $^+$ calcd. for $\text{C}_{18}\text{H}_{19}\text{O}_5$ 315.1232, found 315.1228.

4,6-Dimethoxy-2-(4-methoxybenzyl)benzofuran-3(2H)-one (3i): Off-white colour powder (1.4 g, 89%), m.p.: 98-100 °C. ^1H NMR (DMSO- d_6): δ 7.17 (2H, d, J = 8.4 Hz), 6.83 (2H, d, J = 8.4 Hz), 6.32 (1H, d, J = 1.6 Hz), 6.13 (1H, d, J = 1.6 Hz), 4.88 (1H, dd, J = 8.0 Hz, 4.0 Hz), 3.83 (3H, s), 3.81 (3H, s), 3.71 (3H, s), 3.13 (1H, dd, J = 14.8 Hz, 4.0 Hz), 2.83 (1H, dd, J = 14.8 Hz, 8.4 Hz); ^{13}C NMR (DMSO- d_6): δ 194.6, 174.8, 169.4, 158.3, 158.0, 130.3, 128.1, 113.6, 103.8, 92.8, 89.2, 85.9, 56.2, 55.8, 54.9, 35.8; LC-MS (positive ion mode): m/z 315 (M+H) $^+$. HRMS-(EI) (m/z): (M+H) $^+$ calcd. for $\text{C}_{18}\text{H}_{19}\text{O}_5$ 315.1232, found 315.1228.

2-(4-(Dimethylamino)benzyl)-6-hydroxybenzofuran-3(2H)-one (3j): Off-white colour powder (1.2 g, 85%), m.p.: 158-160 °C. ^1H NMR (DMSO- d_6): δ 10.88 (1H, br s), 7.40 (1H, d, J = 8.4 Hz), 7.06 (2H, d, J = 8.8 Hz), 6.61 (2H, d, J = 8.4 Hz), 6.52 (1H, dd, J = 8.4 Hz, 1.6 Hz), 6.43 (1H, d, J = 1.6 Hz), 4.89 (1H, dd, J = 7.6 Hz, 3.6 Hz), 3.10 (1H, dd, J =

14.8 Hz, 4.0 Hz), 2.83 (6H, s), 2.80 (1H, dd, $J = 14.8$ Hz, 8.0 Hz); ^{13}C NMR (DMSO- d_6): δ 197.6, 174.3, 166.8, 149.2, 129.8, 125.2, 123.5, 112.7, 112.3, 111.6, 98.1, 86.1, 40.1, 35.7; LC-MS (positive ion mode): m/z 284 (M+H) $^+$. HRMS-(EI) (m/z): (M+H) $^+$ calcd. for $\text{C}_{17}\text{H}_{18}\text{NO}_3$ 284.1286, found 284.1281.

Antioxidant activity

DPPH free radical scavenging activity assay: The (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of dihydroaurones (**3a-j**) and aurones (**2a-j**) was measured based on the reduction of methanolic solution of the coloured DPPH. Free radical scavenging ability of the test compounds in ethanol added to the methanolic solution of DPPH is inversely proportional to the difference in initial and final absorption of DPPH solution at 517 nm. Drug activity is expressed as the 50% inhibitory concentration (IC_{50}). The reaction mixture contained 20 mL of test compound and 280 mL DPPH solution was incubated for 50 min and then absorbance was noted at 517 nm using X-mark Micro plate spectrophotometer. Percentage inhibition was determined by comparing the absorbance values of test and control tubes. The IC_{50} values were obtained from the plot, drawn for concentration in mg *versus* percentage inhibition.

Superoxide free radical scavenging activity assay: The superoxide free radical scavenging activity of dihydroaurones (**3a-j**) and aurones (**2a-j**) was determined by the nitro blue tetrazolium (NBT) method. The reaction mixture contained EDTA (6.6 mM), NaCN (3 mg), riboflavin (2 mM), NBT (50 mM), various concentrations of the test drug in ethanol and a phosphate buffer (58 mM, pH 7.8) in a final volume of 3 mL. Optical density was measured at 560 nm. The test tubes were uniformly illuminated with an incandescent lamp for 15 min, after which the optical density was measured again at 560 nm. The percentage inhibition of superoxide radical generation was measured by comparing mean absorbance values of the control and those of the test substances $[(A-B/A)] \times 100$. Here A = absorbance of control sample; B = absorbance of test sample. The IC_{50} values were obtained from the plot drawn of concentration in mg *versus* percentage inhibition.

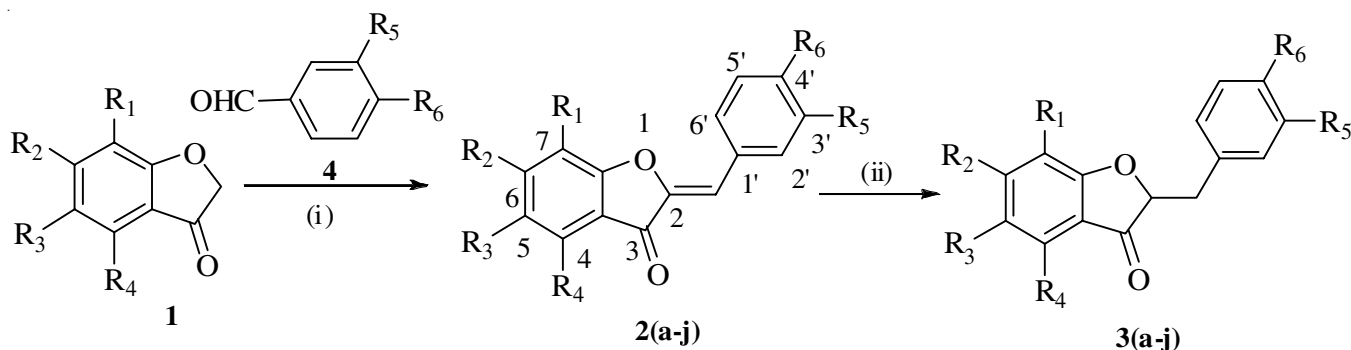
5-Lipoxygenase enzyme inhibitory activity assay: Dihydroaurones (**3a-j**) and aurones (**2a-j**) were screened for their 5-lipoxygenase inhibitory potential using colorimetric method. The assay mixture contained 50 mM phosphate buffer (pH 6.3), 5-lipoxygenase, various concentrations of test subst-

ances in DMSO and linoleic acid in a total volume of 0.5 mL. After 20 min incubation of above reaction mixture, 0.5 mL ferric xylenol orange reagent was added and optical density was measured after 2 min at 595 nm using spectrophotometer. Controls were run along with test in a similar manner except using vehicle instead of test substance solution. Percent inhibition was calculated by comparing absorbance of test solution with that of control.

Tyrosinase enzyme inhibitory activity assay: Tyrosinase enzyme inhibitory activity of dihydroaurones (**3a-j**) and aurones (**2a-j**) was evaluated using potato tyrosinase. The enzyme was obtained as the supernatant after centrifugation of an extract of potato (*Solanum tuberosum*) in phosphate buffer (50 mM, pH 6.0). The activity of the enzyme solution was determined spectrophotometrically by the formation of dopachrome at 30 °C. Prepared tyrosinase were stored at 4 °C and the tyrosinase enzyme inhibitory activity was evaluated by a known method with minor modifications [22,28]. The reaction mixture was prepared by addition of 175 μL of 0.1 M citrate buffer (pH 4.8), 5 μL test item, 30 μL enzyme and 90 μL L-Dopa (8 mM). The reaction mixture was incubated at room temperature for 10 min and the absorbance was measured at 475 nm. An IC_{50} value was determined as the concentration that elicits the half maximal response. Tyrosinase activity is calculated by the formula $[(A-B)/A] \times 100$. Where A = difference in absorbance of control sample between samples with and without tyrosinase; B = difference in absorbance of test sample between samples with and without tyrosinase.

RESULTS AND DISCUSSION

Synthesis: Recently, a green method is developed for the synthesis of aurones by condensation of benzofuranone with aromatic aldehyde in neat water under reflux condition [21]. A series of ten aurones (**2a-j**) were synthesized by the above green method including the naturally available high potent antioxidants, hispidol (**2b**), sulfuretin (**2c**), maritimetin (**2d**) and aureusidin (**2e**). Dihydroaurones (**3a-j**) were synthesized from aurones (**2a-j**) by hydrogenation using hydrogen gas in presence of 10% Pd/C catalyst (**Scheme-I**). Ethyl acetate-methanol mixture was found to be the better solvent for the above reaction as it completely prevents the formation of side products. Thus, Pd/C catalyzed hydrogenation of aurones using hydrogen gas in ethyl acetate-methanol mixture produced



Scheme-I: Reagents and conditions: (i) aromatic aldehyde (**4**), water, reflux, 6-10 h; (ii) H₂ (g), 10% Pd/C (catalyst), ethyl acetate-methanol, room temperature, 1 h, 80-90% yield

dihydroaurones cleanly in good yields and the results are shown in Table-1. Aurones are generally dark yellow coloured compounds, whereas the dihydroaurones are colourless compounds (Fig. 1). Hence, it is easy to monitor the progress of the reaction by the disappearance of colour.

Antioxidant activity: There are known potent antioxidants in aurone class like hispidol, sulfuretin, maritimetin and aureusidin, but their use in the food and cosmetic industry is very limited owing to their dark yellow colour. On the other hand dihydroaurones are colourless in nature and will be useful in non-coloured food and cosmetic applications that currently employ synthetic antioxidants (Fig. 1). To compare antioxidant activity of dihydroaurones and aurones, two commonly used antioxidant evaluation methods, DPPH free radical scavenging and superoxide radical scavenging, were chosen. Both methods measure the efficacy of a hydrogen atom transfer from a phenol to a radical.

DPPH radical scavenging activity: DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of dihydroaurones (**3a-j**) and aurones (**2a-j**) was determined by the method described by Lamaisan *et al.* [29] and Matsuda *et al.* [30] based on the reduction of methanolic solution of the coloured DPPH

radical. From the IC₅₀ values (Table-2), it was observed that both dihydroaurones and aurones with more than one hydroxyl group as substituents were found to exhibit significant DPPH free radical scavenging assay in comparison with standard vitamin C. Among the two groups, dihydroaurones were found to be more potent than their corresponding aurones. It is remarkable to note that dihydrosulfuretin (**3c**), dihydromaritimetin (**3d**) and dihydroaureusidin (**3e**), show higher antioxidant activity than their corresponding known potent antioxidant aurones (Table-1, entries 3-5). Other substituent groups like methoxy, monohydroxy and dimethylamine have no effect on the increase of DPPH radical scavenging assay.

Superoxide radical scavenging activity: Superoxide radicals were generated *in vitro* by non-enzymatic system and determined spectrophotometrically (560 nm) by nitro blue tetrazolium (NBT) photo reduction method of McCord & Fridovich [31,32]. The antioxidant activity of the dihydroaurones (**3a-j**) and aurones (**2a-j**) was expressed as 50% inhibitory concentration (IC₅₀ in mM) and the values are shown in Table-2. From the superoxide radical scavenging activity data, it was found that dihydroaurones with more than one hydroxyl group as substituents exhibit higher super oxide radical scavenging assay

TABLE-1
SYNTHESIS OF DIHYDROAURONES FROM AURONES

S. No.	Aurone (2)	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	Dihydroaurone (3)	
								Comp. No.	Yield (%) ^a
1	2a	H	H	H	H	H	H	3a	80
2	2b	H	OH	H	H	H	OH	3b	88
3	2c	H	OH	H	H	OH	OH	3c	81
4	2d	OH	OH	H	H	OH	OH	3d	84
5	2e	H	OH	H	OH	OH	OH	3e	86
6	2f	H	OCH ₃	H	H	H	OH	3f	82
7	2g	H	OCH ₃	H	H	H	OCH ₃	3g	84
8	2h	H	H	OCH ₃	H	OCH ₃	OCH ₃	3h	80
9	2i	H	OCH ₃	H	OCH ₃	H	OCH ₃	3i	89
10	2j	H	OH	H	H	H	N(CH ₃) ₂	3j	85

^aIsolated yields



Fig. 1. Advantage of colour and antioxidant activity of dihydroaurones over aurones

TABLE-2
ANTIOXIDANT ACTIVITY OF DIHYDROAURONES AND AURONES

Entry	Comp. No	DPPH [IC ₅₀ (µg/mL)]		NBT [IC ₅₀ (µg/mL)]	
		Dihydroaurone (3)	Aurone (2)	Dihydroaurone (3)	Aurone (2)
1	a	>100	>100	>100	>100
2	b	>100	>100	0.25	>100
3	c	10.81	20.15	0.20	0.36
4	d	5.15	5.30	0.36	2.50
5	e	7.71	8.86	0.57	3.27
6	f	>100	>100	1.2	>100
7	g	>100	>100	>100	>100
8	h	>100	>100	>100	>100
9	i	>100	>100	>100	>100
10	j	>100	>100	0.52	>100
11	Vitamin C	3.79			
12	Gallic acid			1.34	

in comparison with the standard gallic acid. Likewise the substitution of mono hydroxyl or dimethylamine in ring B makes the dihydroaurones as strong superoxide radical inhibitors than the standard gallic acid (Table-2, entry 6 and 10). Surprisingly the same substitution pattern didn't result any activity in aurones. It is noteworthy that dihydrohispidol (**3b**), dihydrosulfuretin (**3c**), dihydromaritimetin (**3d**) and dihydroaureusidin (**3e**), show higher superoxide radical inhibition than their corresponding known potent aurones. These results further suggest that dihydroaurones with strong electron releasing substituents are potent superoxide inhibitors than their corresponding aurones and the standard gallic acid (Fig. 1).

5-Lipoxygenase enzyme inhibitory activity: Dihydroaurones (**3a-j**) and aurones (**2a-j**) were screened for their 5-lipoxygenase inhibitory potential using colorimetric method [33,34]. From the inhibitory values (Table-3), it is evident that the substitution of methoxy, hydroxyl and dimethylamine groups on the tested compounds didn't follow a particular pattern in enhancing the 5-lipoxygenase enzyme inhibition. Aureusidin (**3e**) shows a higher 5-lipoxygenase inhibitory activity (IC₅₀: 7.61 µg/mL) and hispidol (**3b**) shows significant activity (IC₅₀: 9.89 µg/mL) in comparison with the standard curcumin (IC₅₀: 8.07 µg/mL). Unfortunately, the corresponding dihydroaurones of the above two didn't show any 5-LOX inhibition. On contrary, 2-(3,4-dimethoxybenzyl)-5-methoxybenzofuran-3(2H)-one

TABLE-3
ANTI-INFLAMMATORY (5-LIPOXYGENASE
ENZYME INHIBITORY) ACTIVITY

Entry	Compd. No.	IC ₅₀ (µg/mL)	
		Dihydroaurone (3)	Aurone (2)
1	a	>100	>100
2	b	>100	9.89
3	c	>100	>100
4	d	>100	>100
5	e	>100	7.61
6	f	>100	>100
7	g	>100	>100
8	h	7.67	>100
9	i	>100	>100
10	j	9.69	14.47
11	Curcumin	8.07	

(**3h**) (Table-3, entry-8) exhibits potent 5-lipoxygenase enzyme inhibition (IC₅₀: 7.67 µg/mL) than the standard curcumin. The substitution of mono hydroxyl in ring-A and dimethylamine in ring-B (Table-3, entry-10) results in significant activity (IC₅₀: 9.69 µg/mL and 14.47 µg/mL) in both dihydroaurone and aurone.

Tyrosinase enzyme inhibitory activity: Dihydroaurones (**3a-j**) and aurones (**2a-j**) were tested for tyrosinase enzyme inhibition on potato tyrosinase using spectrophotometric method and the results are presented in Table-4. Dihydroaurones and aurones with more than one hydroxyl group as substituents (**3b-e** and **2b-e**) displayed significant tyrosinase inhibition in comparison with the standard kojic acid. The substitution of monohydroxyl, methoxy and dimethylamine on both aurones and dihydroaurones resulted in substantial improvement of their tyrosinase enzyme inhibitory activity [35,36]. It is interesting to note that all the dihydroaurones are competent to their corresponding aurones in tyrosinase inhibition.

TABLE-4
TYROSINASE ENZYME INHIBITORY ACTIVITY

Entry	Compd. No.	Inhibition (%)	
		Dihydroaurone (3)	Aurone (2)
1	a	2.77	3.64
2	b	17.67	18.32
3	c	15.63	16.18
4	d	19.11	18.95
5	e	18.91	19.49
6	f	10.78	11.25
7	g	14.98	15.83
8	h	16.79	17.62
9	i	11.28	12.52
10	j	11.96	11.58
11	Kojic acid	29.36	

Conclusion

In conclusion, the designed dihydroaurones were synthesized easily from aurones in excellent yields. Dihydroaurones were found to be colourless unlike their yellow coloured aurone precursors. Dihydroaurones show potent antioxidant activity than their corresponding aurones in both DPPH free radical scavenging and superoxide radical scavenging assays. Both

dihydroaurones and aurones exhibit significant to moderate tyrosinase enzyme inhibitory activity. Being colourless and having potent antioxidant and tyrosinase enzyme inhibitory activity, dihydroaurones will find uses in several applications. Few synthesized dihydroaurones also exhibited promising 5-Lox enzyme inhibitory activity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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