In vitro Antioxidant Activity and Scavenging Effects of Some Synthesized 4'-Aminochalcones

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A new series of substituted 4'-aminochalcones were synthesized by Claisen-Schmidt condensation of 4-aminoacetophenone with various substituted aromatic/heteroaromatic aldehydes. The antioxidant activity for all these compounds were studied on various reactive oxygen species assays containing superoxide anion, hydroxyl radical, ABTS cation radical scavenging and inhibition of lipid peroxidation assays. Chalcones **9** and **10** made up of 3,4,5-trimethoxyphenyl and 4-dimethylaminophenyl moieties as ring-B of chalcones respectively exhibited maximum activity in various antioxidant assays, which may be due to the free radical scavenging activity. The present study revealed that an electron releasing group at position 4 of the ring B of chalcone is essential for exhibiting significant antioxidant activity.

Key Words: 4'-Aminochalcones, Lipid peroxidation, Antioxidant activity, Scavenging effects.

INTRODUCTION

Several diseases caused by free radicals have been reported such as atherosclerosis, cancer, liver cirrhosis, cancer, aging, diabetes, *etc.*¹ and the scavenging effects of these free radicals by the chemical compounds have great potential in ameliorating these disease progresses². Because of the human body has an inherent mechanism to reduce free radical induced injury by endogenous enzymes such as superoxide dismutase, glutathione peroxidase, catalase and others such as ascorbic acid (vitamin C) *etc.* When compared to the damage produced to the body, these protective mechanisms are sometimes found not to be sufficient. Hence, the exogenous antioxidants are required to overcome the injuries produced by free radicals and lipid peroxidation.

Flavonoids widely distributed in nature, possessing high degree of antioxidant activity are found to be useful in this type of injuries³. An antioxidant which is capable of attacking the reactive oxygen species along with inhibiting lipid peroxidation process is of immense value in the effective therapy of various diseases resulting from oxidative damage, *e.g.* curcumin, a popular and natural antioxidant⁴. As chalcones (1,3-diaryl-2-propen-1-one, Fig. 1) are the precursors for flavonoids, it has been hypothesized to have antioxidant activity. The role of the chalcones as antipromoters becomes evident. Isoliquiritigenin (Fig. 2), a trihydroxy chalcone has been shown to inhibit TPA induced tumor promotion in mice⁵.

Chalcones are also reported to display a number of interesting biological activities such as antimalarial⁶, antimicrobial⁷, antitubercular⁸, antiviral⁹, antileishmanial¹⁰, cytotoxic¹¹, anticancer¹², antioxidant¹³, antiinflammatory¹⁴, analgesic¹⁵, antihyperglycemic¹⁶, antimutagenic¹⁷ and phosphodiesterase inhibitory activities¹⁸. The discovery of this class of compounds provides an outstanding case history of modern drug development and also points out the unpredictability of biological activity from structural modification of a prototype leading molecule. As part of our continuing efforts in this area, a series of some new 4'-aminochalcones have been synthesized and evaluated for their antiinflammatory and analgesic activities^{19,20}. The present paper was aimed to investigate the free radical scavenging and antioxidant activities of synthesized 4'-aminochalcones.

Fig. 1.

Fig. 2.

EXPERIMENTAL

Melting points were determined with an electrothermal capillary melting point apparatus and are reported uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer BXF1 FT-IR spectrophotometer using KBr disc and the values are expressed in cm^{-1} . ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AMX 400 and 100 spectrophotometer respectively with tetramethylsilane (TMS) as internal standard using CDCl_3 as solvent and the values are expressed in δ ppm. Mass [EI-MS (70 eV)] spectra were recorded on Agilent 1100 EI-Mass spectrophotometer. The elemental analyses of the synthesized compounds were recorded on Carlo Erba 1108 elemental analyzer and were within $\pm 0.4\%$ of the theoretical values, unless otherwise noted. Analytical TLC was performed on Silica Gel F₂₅₄ plates (Merck) with visualization by UV (254 nm) chamber with protective filters. 4-Aminoacetophenone, substituted aromatic and/or heteroaromatic aldehydes were purchased from E. Merck (India) Ltd., Mumbai, India. All other materials used were of analytical reagent grade.

Chalcones (**1** to **16**) were obtained as following the procedure given under **Scheme-I** by the reaction of 4-aminoacetophenone with various substituted aromatic/heteroaromatic aldehydes in the presence of aqueous alkali. The absorption bands in IR spectra for primary amino group ($3350\text{--}3300\text{ cm}^{-1}$), carbonyl group ($1650\text{--}1600\text{ cm}^{-1}$) and olefinic group ($1600\text{--}1550\text{ cm}^{-1}$) indicated the formation of the chalcones. The ^1H NMR spectra (400 MHz, CDCl_3) showed a broad singlet between δ 4.15–4.22 for NH_2 group, two doublets in between δ 6.50–7.50 for olefinic protons ($J \approx 16.0$ Hz) indicates that E-geometrical isomers and the aromatic protons appeared in between δ 6.00–9.00. In the ^{13}C NMR spectrum exhibited characteristic peaks between δ 190–180 for carbonyl group, δ 145–135 and δ 125–120 for olefinic carbons confirming the chalcones structure. The mass spectra showed the corresponding molecular ion peak $[\text{M}^+]$ as the base peak and the fragmentation patterns was characteristic of respective chalcones. The elemental analyses of all the newly synthesized compounds confirmed their structures.

Scheme-I: Synthesis of 4'-aminochalcones

Synthesis of chalcones, (1-16)⁷: Potassium hydroxide (10 % aqueous solution, 1 mL) was added to a stirred solution of 4-aminoacetophenone (1 mmol) and a substituted aromatic/heteroaromatic aldehyde (1 mmol) in methanol (10 mL) (**Scheme-I** and Table-1). Stirring was carried out for at least 12–16 h at room temperature and each hour the reaction

mixture was analyzed by TLC. Then the reaction mixture was poured into ice water, if necessary acidified with dil. HCl and extracted with ethyl acetate ($3 \times 50\text{ mL}$). The combined organic layer was washed with water, dried and concentrated *in vacuo*. The residue was purified on a column of silica gel using hexane-ethyl acetate (4:1) as eluant affording pure chalcones **1-16** in 37 to 81 % yield.

TABLE-1
SUBSTITUTED 4'-AMINOCHALCONES

Comp.	Ar	Comp.	Ar
1	3-Bromophenyl	9	3,4,5-Trimethoxyphenyl
2	4-Methylphenyl	10	4-Dimethylaminophenyl
3	2-Chlorophenyl	11	9-Anthracenyl
4	4-Chlorophenyl	12	4-Nitrophenyl
5	2,4-Dichlorophenyl	13	3-Pyridinyl
6	4-Fluorophenyl	14	2-Pyridinyl
7	4-Methoxyphenyl	15	4-Pyridinyl
8	3,4-Dimethoxyphenyl	16	2-Quinolinylnyl

1-(4'-Aminophenyl)-3-(3''-bromophenyl)-2-propen-1-one (1): Orange crystals, yield 81 %, m.p. 168–171 °C. IR (KBr, ν_{max} , cm^{-1}): 3414, 3326, 1652, 1626, 1304, 1177. ^1H NMR (CDCl_3 , 400 MHz, δ ppm): 4.20 (2H, br s, NH_2), 6.72 (1H, d, $J = 16.0$ Hz, CO-CH=), 7.27 (2H, d, $J = 10.2$ Hz, C-3' and 5'-H), 7.57–7.49 (3H, m, C-4'', 5'' and 6''-H), 7.70 (1H, d, $J = 16.0$ Hz, Ar-CH=), 7.80 (1H, s, C-2''-H), 7.95 (2H, d, $J = 10.0$ Hz, C-2' and 6'-H). ^{13}C NMR (CDCl_3 , 100 MHz, δ ppm): 187.59, 151.31, 141.26, 137.63, 132.79, 131.19, 130.65, 130.39, 128.43, 127.11, 123.50, 123.06 and 114.01. EI-MS, m/z : 302 [$[\text{M}+\text{H}]^+$]. Anal. calcd. for $\text{C}_{15}\text{H}_{12}\text{NOBr}$: C, 59.66; H, 4.00. Found: C, 59.60; H, 3.97.

1-(4'-Aminophenyl)-3-(4''-methylphenyl)-2-propen-1-one (2): Yellow crystals, yield 65.78 %, m.p. 162–165 °C. IR (KBr, cm^{-1}): 3471, 3348, 1628 and 1602. ^1H NMR (CDCl_3 , 400 MHz, δ ppm): 2.42 (3H, s, C-4''-CH₃), 4.20 (2H, br s, NH_2), 6.81 (2H, d, $J = 8.0$ Hz, C-3' and 5'-H), 7.05 (1H, d, $J = 15.8$ Hz, CO-CH=), 7.23 (2H, d, $J = 7.6$ Hz, C-2'' and 6''-H), 7.53 (2H, d, $J = 8.0$ Hz, C-3'' and 5''-H), 7.73 (1H, d, $J = 16.0$ Hz, Ar-CH=), 8.03 (2H, d, $J = 10.0$ Hz, C-2' and 6'-H). ^{13}C NMR (CDCl_3 , 100 MHz, δ ppm): 191.75, 159.05, 148.45, 142.81, 139.49, 136.23, 134.06, 131.17, 130.29, 129.68, 121.48 and 63.54. EI-MS, m/z : 238 [$[\text{M}+\text{H}]^+$]. Anal. calcd. for $\text{C}_{16}\text{H}_{15}\text{NO}$: C, 81.08; H, 6.32. Found: C, 81.01; H, 6.32.

1-(4'-Aminophenyl)-3-(2''-chlorophenyl)-2-propen-1-one (3): Yellow crystals, yield 79.64 %, m.p. 108–111 °C. IR (KBr, ν_{max} , cm^{-1}): 3384, 3332, 1647, 1607, 1340 and 1178. ^1H NMR (CDCl_3 , 400 MHz, δ ppm): 4.09 (2H, br s, NH_2), 6.62 (2H, d, $J = 8.8$ Hz, C-3' and 5'-H), 7.26–7.21 (2H, m, C-4'' and 5''-H), 7.37–7.34 (1H, m, C-6''-H), 7.41 (1H, d, $J = 16.0$ Hz, CO-CH=), 7.67–7.64 (1H, m, C-3''-H), 7.85 (2H, d, $J = 8.4$ Hz, C-2' and 6'-H), 8.05 (1H, d, $J = 15.8$ Hz, Ar-CH=). EI-MS, m/z : 258 [$[\text{M}+\text{H}]^+$]. Anal. calcd. for $\text{C}_{15}\text{H}_{12}\text{NOCl}$: C, 69.97; H, 4.70. Found: C, 69.90; H, 4.66.

1-(4'-Aminophenyl)-3-(4''-chlorophenyl)-2-propen-1-one (4): Orange crystals, yield 66 %, m.p. 158–161 °C. IR (KBr, ν_{max} , cm^{-1}): 3459, 3341, 1645, 1629, 1346 and 1176. ^1H NMR (CDCl_3 , 400 MHz, δ ppm): 4.17 (2H, br s, NH_2), 6.72 (2H, d, $J = 10.0$ Hz, C-3' and 5'-H), 7.22 (1H, d, $J = 16.0$ Hz,

CO-CH=), 7.38 (2H, d, $J = 8.0$ Hz, C-2" and 6"-H), 7.73 (2H, d, $J = 8.8$ Hz, C-3" and 5"-H), 7.93 (2H, d, $J = 10.0$ Hz, C-2' and 6'-H), 8.02 (1H, d, $J = 15.8$ Hz, Ar-CH=). EI-MS, m/z : 258 [$\{M+H\}^+$]. Anal. calcd. for $C_{15}H_{12}NOCl$: C, 69.97; H, 4.70. Found: C, 69.90; H, 4.66.

1-(4'-Aminophenyl)-3-(2'',4''-dichlorophenyl)-2-propen-1-one (5): Creamy yellow crystals, yield 87.73 %, m.p. 178-180 °C. IR (KBr, $\nu_{\max}$, cm^{-1}): 3436, 3362, 1651, 1609, 1343 and 1180. 1H NMR ($CDCl_3$, 400 MHz, δ ppm): 4.20 (2H, br s, NH_2), 6.71 (1H, d, $J = 15.8$ Hz, CO-CH=), 7.31 (1H, d, $J = 8.6$ Hz, C-6"-H), 7.47 (2H, d, $J = 10.0$ Hz, C-3' and 5'-H), 7.56 (1H, d, $J = 8.2$ Hz, C-5"-H), 7.70 (1H, m, C-3"-H), 7.93 (1H, d, $J = 16.0$ Hz, Ar-CH=), 8.06 (2H, d, $J = 8.0$ Hz, C-2' and 6'-H). EI-MS, m/z : 292 (M^+). Anal. calcd. for $C_{15}H_{11}NOCl_2$: C, 61.70; H, 3.79. Found: C, 61.64; H, 3.77.

1-(4'-Aminophenyl)-3-(4''-fluorophenyl)-2-propen-1-one (6): Orange crystals, yield 66 %, m.p. 140-143 °C. IR (KBr, $\nu_{\max}$, cm^{-1}): 3460, 3340, 1628, 1603, 1345 and 1223. 1H NMR ($CDCl_3$, 400 MHz, δ ppm): 4.20 (2H, br s, NH_2), 6.62 (2H, d, $J = 8.4$ Hz, C-3' and 5'-H), 7.03 (2H, d, $J = 8.8$ Hz, C-2" and 6"-H), 7.38 (1H, d, $J = 16.0$ Hz, CO-CH=), 7.53 (2H, d, $J = 10.0$ Hz, C-3" and 5"-H), 7.66 (1H, d, $J = 16.0$ Hz, Ar-CH=), 7.85 (2H, d, $J = 8.2$ Hz, C-2' and 6'-H). EI-MS, m/z : 242 [$\{M+H\}^+$]. Anal. calcd. for $C_{15}H_{12}NOF$: C, 74.76; H, 5.01. Found: C, 74.68; H, 4.97.

1-(4'-Aminophenyl)-3-(4''-methoxyphenyl)-2-propen-1-one (7): Yellow crystals, yield 64.87 %, m.p. 108-111 °C. IR (KBr, $\nu_{\max}$, cm^{-1}): 3467, 3329, 1631, 1598, 1342 and 1230. 1H NMR ($CDCl_3$, 400 MHz, δ ppm): 3.85 (3H, s, C-4"-OCH₃), 4.20 (2H, br s, NH_2), 6.71 (1H, d, $J = 16.0$ Hz, CO-CH=), 6.93 (2H, d, $J = 10.0$ Hz, C-3' and 5'-H), 7.43 (2H, d, $J = 9.0$ Hz, C-3" and 5"-H), 7.60 (2H, d, $J = 8.8$ Hz, C-2" and 6"-H), 7.76 (1H, d, $J = 16.0$ Hz, Ar-CH=), 7.94 (2H, d, $J = 9.6$ Hz, C-2' and 6'-H). EI-MS, m/z : 253 (M^+). Anal. calcd. for $C_{16}H_{15}NO_2$: C, 75.96; H, 5.97. Found: C, 75.88; H, 5.92.

1-(4'-Aminophenyl)-3-(3'',4''-dimethoxyphenyl)-2-propen-1-one (8): Orange crystals, yield 72.43 %, m.p. 146-149 °C. IR (KBr, $\nu_{\max}$, cm^{-1}): 3445, 3351, 1641, 1597, 1317 and 1260. 1H NMR ($CDCl_3$, 400 MHz, δ ppm): 3.84 (3H, s, C-3"-OCH₃), 3.86 (3H, s, C-4"-OCH₃), 4.21 (2H, br s, NH_2), 6.71 (2H, d, $J = 8.0$ Hz, C-3' and 5'-H), 7.13-6.79 (3H, m, C-2'',5" and 6"-H), 7.30 (1H, d, $J = 15.8$ Hz, CO-CH=), 7.64 (1H, d, $J = 15.8$ Hz, Ar-CH=), 7.84 (2H, d, $J = 8.4$ Hz, C-2' and 6'-H). EI-MS, m/z : 283 (M^+). Anal. calcd. for $C_{17}H_{17}O_3N$: C, 72.15; H, 6.03. Found: C, 72.08; H, 6.00.

1-(4'-Aminophenyl)-3-(3'',4'',5''-trimethoxyphenyl)-2-propen-1-one (9): Yellow crystals, yield 70.55 %, m.p. 160-162 °C. IR (KBr, $\nu_{\max}$, cm^{-1}): 3469, 3344, 1630, 1604, 1316 and 1219. 1H NMR ($CDCl_3$, 400 MHz, δ ppm): 3.90 (3H, s, C-4"-OCH₃), 3.93 (6H, s, C-3" and 5"-OCH₃), 4.19 (2H, br s, NH_2), 6.71 (2H, d, $J = 10.0$ Hz, C-3' and 5'-H), 6.86 (2H, s, C-2" and 6"-H), 7.43 (1H, d, $J = 16.0$ Hz, CO-CH=), 7.72 (1H, d, $J = 15.8$ Hz, Ar-CH=), 7.94 (2H, d, $J = 8.0$ Hz, C-2' and 6'-H). EI-MS, m/z : 313 (M^+). Anal. calcd. for $C_{18}H_{19}O_4N$: C, 69.07; H, 6.12. Found: C, 69.00; H, 6.07.

1-(4'-Aminophenyl)-3-(4''-dimethylaminophenyl)-2-propen-1-one (10): Orange red crystals, yield 66.50 %, m.p. 168-170 °C. IR (KBr, $\nu_{\max}$, cm^{-1}): 3474, 3432, 1620, 1597, 1346 and 1303. 1H NMR ($CDCl_3$, 400 MHz, δ ppm): 3.05

(6H, s, C-4"-NMe₂), 4.19 (2H, br s, NH_2), 6.70 (2H, d, $J = 10.0$ Hz, C-3' and 5'-H), 6.88 (2H, d, $J = 8.8$ Hz, C-3" and 5"-H), 7.39 (1H, d, $J = 15.6$ Hz, CO-CH=), 7.57 (2H, d, $J = 8.0$ Hz, C-2" and 6"-H), 7.76 (1H, d, $J = 15.4$ Hz, Ar-CH=), 7.94 (2H, d, $J = 8.0$ Hz, C-2' and 6'-H). EI-MS, m/z : 267 [$\{M+H\}^+$]. Anal. calcd. for $C_{17}H_{18}N_2O$: C, 76.76; H, 6.82. Found: C, 76.69; H, 6.76.

1-(4'-Aminophenyl)-3-(9''-anthracenyl)-2-propen-1-one (11): Orange red crystals, yield 62.08 %, m.p. 173-176 °C. IR (KBr, $\nu_{\max}$, cm^{-1}): 3469, 3332, 1650, 1633 and 1356. 1H NMR ($CDCl_3$, 400 MHz, δ ppm): 4.20 (2H, br s, NH_2), 7.22 (1H, d, $J = 16.0$ Hz, CO-CH=), 7.53-7.45 (4H, m, Ar-H-anthracenyl), 7.57 (2H, d, $J = 8.0$ Hz, C-3' and 5'-H), 8.03 (1H, d, $J = 15.8$ Hz, Ar-CH=), 8.15-8.07 (2H, m, Ar-H-anthracenyl), 8.30 (2H, d, $J = 8.2$ Hz, C-2' and 6'-H), 8.88-8.45 (3H, m, Ar-H-anthracenyl). EI-MS, m/z : 324 [$\{M+H\}^+$]. Anal. calcd. for $C_{23}H_{17}NO$: C, 85.52; H, 5.30. Found: C, 85.44; H, 5.26.

1-(4'-Aminophenyl)-3-(4''-nitrophenyl)-2-propen-1-one (12): Orange crystals, yield 60 %, m.p. 182-184 °C. IR (KBr, $\nu_{\max}$, cm^{-1}): 3484, 3389, 1636, 1610, 1506 and 1341. 1H NMR ($CDCl_3$, 400 MHz, δ ppm): 4.20 (2H, br s, NH_2), 6.68 (2H, d, $J = 10.0$ Hz, C-3' and 5'-H), 6.72 (1H, d, $J = 16.0$ Hz, CO-CH=), 7.66 (2H, d, $J = 10.0$ Hz, C-2" and 6"-H), 7.80 (1H, d, $J = 16.2$ Hz, Ar-CH=), 7.95 (2H, d, $J = 9.8$ Hz, C-2' and 6'-H), 8.28 (2H, d, $J = 10.0$ Hz, C-3" and 5"-H). EI-MS, m/z : 269 [$\{M+H\}^+$]. Anal. calcd. for $C_{15}H_{12}N_2O_3$: C, 67.22; H, 4.51. Found: C, 67.16; H, 4.47.

1-(4'-Aminophenyl)-3-(3''-pyridinyl)-2-propen-1-one (13): Orange red crystals, yield 62.06 %, m.p. 160-163 °C. IR (KBr, $\nu_{\max}$, cm^{-1}): 3437, 3354, 1636, 1595 and 1342. 1H NMR ($CDCl_3$, 400 MHz, δ ppm): 4.13 (2H, br s, NH_2), 6.63 (2H, d, $J = 8.8$ Hz, C-3' and 5'-H), 7.26 (1H, d, $J = 8.0$ Hz, C-5"-H), 7.52 (1H, d, $J = 15.6$ Hz, CO-CH=), 7.68 (1H, d, $J = 16.0$ Hz, Ar-CH=), 7.86 (2H, d, $J = 8.4$ Hz, C-2' and 6'-H), 8.54-8.52 (1H, m, C-4"-H), 8.78 (2H, d, $J = 9.0$ Hz, C-2" and 6"-H). ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 185.58, 154.01, 150.39, 149.93, 137.83, 134.72, 131.21, 130.98, 125.15, 124.48, 123.80 and 112.76. EI-MS, m/z : 225 [$\{M+H\}^+$]. Anal. calcd. for $C_{14}H_{12}N_2O$: C, 75.06; H, 5.39. Found: C, 75.00; H, 5.35.

1-(4'-Aminophenyl)-3-(2''-pyridinyl)-2-propen-1-one (14): Light orange crystals, yield 60.88 %, m.p. 146-150 °C. IR (KBr, $\nu_{\max}$, cm^{-1}): 3487, 3399, 1646, 1585, 1432 and 1336. 1H NMR ($CDCl_3$, 400 MHz, δ ppm): 4.12 (2H, br s, NH_2), 6.62 (2H, d, $J = 8.8$ Hz, C-3' and 5'-H), 7.22-7.19 (1H, m, C-5"-H), 7.38 (1H, d, $J = 15.0$ Hz, CO-CH=), 7.65 (1H, m, C-3"-H), 7.68 (1H, d, $J = 8.0$ Hz, C-4"-H), 7.93 (2H, d, $J = 10.0$ Hz, C-2' and 6'-H), 8.06 (1H, d, $J = 8.8$ Hz, C-6"-H), 8.60 (1H, d, $J = 15.5$ Hz, Ar-CH=). EI-MS, m/z : 225 [$\{M+H\}^+$]. Anal. calcd. for $C_{14}H_{12}N_2O$: C, 75.06; H, 5.39. Found: C, 75.00; H, 5.35.

1-(4'-Aminophenyl)-3-(4''-pyridinyl)-2-propen-1-one (15): Light orange yellow crystals, yield 55.38 %, m.p. 172-174 °C. IR (KBr, $\nu_{\max}$, cm^{-1}): 3439, 3334, 1643, 1593, 1414 and 1336. 1H NMR ($CDCl_3$, 400 MHz, δ ppm): 4.16 (2H, br s, NH_2), 6.63 (2H, d, $J = 8.4$ Hz, C-3' and 5'-H), 7.38 (2H, d, $J = 18.0$ Hz, CO-CH= and C-3"-H), 7.60 (2H, d, $J = 8.0$ Hz, C-2" and 6"-H), 7.85 (2H, d, $J = 8.4$ Hz, C-2' and 6'-H), 8.60 (2H, d, $J = 18.6$ Hz, Ar-CH= and C-5"-H). EI-MS, m/z : 224 (M^+).

Anal. calcd. for $C_{14}H_{12}N_2O$: C, 75.06; H, 5.39. Found: C, 75.00; H, 5.35.

1-(4'-Aminophenyl)-3-(2"-quinolinyl)-2-propen-1-one (16): Yellowish red crystals, yield 48.54 %, m.p. 162-165 °C. IR (KBr, $\nu_{\max}$, cm^{-1}): 3470, 3357, 1638, 1605, 1524, 1357. ^1H NMR (CDCl_3 , 400 MHz, δ ppm): 4.15 (2H, br s, NH_2), 6.73 (2H, d, $J = 8.0$ Hz, C-3' and 5'-H), 7.70 (1H, d, $J = 16.4$ Hz, CO-CH=), 7.93 (1H, d, $J = 16.2$ Hz, Ar-CH=), 8.06 (1H, d, $J = 10.0$ Hz, C-2' and 6'-H), 8.40-7.61 (6H, m, Ar-H-quinolinyl). EI-MS, m/z : 275 [$\text{M}+\text{H}$] $^+$. Anal. calcd. for $C_{18}H_{14}N_2O$: C, 78.90; H, 5.15. Found: C, 78.83; H, 5.10.

Pharmacology: 2-Deoxy-D-ribose was purchased from Sigma chemical Co. (St. Louis, MO, USA). 2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was purchased from Sigma-Aldrich, Hyderabad, India. Nitroblue tetrazolium (NBT) was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. L-ascorbic acid and riboflavin were purchased from Loba Chemicals, Mumbai, India. Thiobarbituric acid was purchased from BDH Chemicals, Poole, UK. All other materials and solvents used were of analytical reagent quality.

Determination of superoxide scavenging activity²¹

Riboflavin photo reduction method: Superoxide scavenging activity of the compounds was determined by McCord and Fridovich method, which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitrobluetetrazolium. The assay mixture contained different quantities of the compounds and ethylene diamine tetraacetic acid (6 μM containing 0.061 μM NaCN), nitrobluetetrazolium (50 μM), riboflavin (2 μM) and phosphate buffer (58 mM, pH 7.8) to give a total volume of 3 mL. The tubes were uniformly illuminated with an incandescent light (40 w) for 15 min and thereafter the optical density was measured at 560 nm. The percentage inhibition of superoxide production was evaluated by comparing the absorbance values of control and experimental tubes. The inhibitory effects of samples on the generation of superoxide anion were estimated by the equation:

$$\text{Inhibitory ratio} = [(A_0 - A_1) \times 100] / A_0$$

where, A_0 is the absorbance with no addition of sample, A_1 is the absorbance with addition of sample. IC_{50} (the concentration necessary for 50 % reduction) values were calculated from the percentage of inhibition, which was calculated from the control where no test compound was added.

Determination of hydroxyl radical scavenging activity²²

Deoxyribose degradation method: Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the compounds for the hydroxyl radical generated from the Fe^{3+} -ascorbate-EDTA- H_2O_2 system. The hydroxyl radical attacks deoxyribose and eventually results in thiobarbituric acid reacting substances (TBARS) formation. The reaction mixture containing deoxyribose (2.8 mM), ferric chloride (0.1 mM), EDTA (0.1 mM), H_2O_2 (1 mM), ascorbate (0.1 mM), phosphate buffer (20 mM, pH 7.4) and various quantities of the compounds in a final volume of 1 mL was incubated for 1 h at 37 °C. Thiobarbituric acid reacting substances method was proposed to measure deoxyribose degradation. IC_{50} values were calculated from the percentage

of inhibition, which was calculated from the control where no test compound was added.

Determination of ABTS $^+$ radical cation scavenging activity: The ABTS $^+$ radical cation scavenging activity of test compounds, ascorbic acid (as standard) was determined according to Re *et al.*²³. Briefly, 5 mL of 7.0 mM ABTS was reacted with 88.0 μL of 140 mM potassiumpersulfate overnight in the dark to yield the ABTS $^+$ radical cation. Prior to use in the assay, the ABTS $^+$ radical cation diluted with 50 % ethanol for an initial absorbance of ≈ 0.700 (1:88 ratio) at 734 nm, with temperature control set at 30 °C. Free radical scavenging activity was assessed by mixing 1.0 mL diluted ABTS $^+$ radical cation with 10 μL of test antioxidant and monitoring the change in absorbance at 0, 0.5, 1 min and again at 5 min intervals until a steady state was achieved. The antioxidant capacity of tested compounds were calculated and expressed as IC_{50} .

Determination of lipid peroxidation inhibitory activity: Inhibition of lipid peroxidation was determined by the thiobarbituric acid method²⁴. Different quantities of the compounds were incubated at 37 °C with 25 % (w/v) rat liver homogenate (0.1 mL) containing Tris-Buffer (40 mM, pH 7.0), KCl (30 mM), ascorbic acid (0.06 mM) and ammonium ferrous sulphate (0.16 mM) in a total volume of 0.5 mL for 1 h. At the end of the incubation period, 0.4 mL of the reaction mixture was treated with 0.2 mL of sodium dodecyl sulphate (8.1 %), 1.5 mL thiobarbituric acid (0.8 %) and 1.5 mL of acetic acid (20 %, pH 3.5). The total volume was then made up to 4 mL by adding distilled water and kept in an oil bath at 95 °C for 1 h. After the mixture had been cooled, 1 mL of distilled water and 5 mL of butanol-pyridine mixture (15:1 v/v) was added. Following vigorous shaking, the tubes were centrifuged and the absorbance of the upper layer containing the chromophore was read at 532 nm. The percentage inhibition of lipid peroxidation was determined by comparing the absorbance values of the control and experimental tubes and calculated IC_{50} values of all tested chalcones and standard from their percentage inhibition values.

The IC_{50} values of chalcones tested for their antioxidant activity against reactive oxygen species and inhibition of lipid peroxidation are shown in Table-2.

RESULTS AND DISCUSSION

Chalcones were prepared by Claisen-Schmidt condensation, starting from the reaction of 4-aminoacetophenone with various substituted aromatic and heteroaromatic aldehydes under basic conditions in the presence of alcohol to furnish 4'-aminochalcones **1** to **16**, in good yield (37-81 %). A number of α,β -unsaturated ketones possessed preferential reactivity towards thiols²⁵, one of the identified mechanisms is the driving force for the present study. Chalcones possessing such α,β -unsaturated carbonyl entity, capable of acting as a soft electrophile would attract only soft nucleophiles like thiols but unlikely to react with hard nucleophiles like amino and hydroxyl groups present on nucleic acids.

Antioxidant activity: The *in vitro* antioxidant activity and scavenging effects of the sixteen chalcones were evaluated by using different reactive oxygen species assays containing

TABLE-2
IC₅₀ VALUES OF 4'-AMINOCHALCONES (1 TO 16) AND ASCORBIC ACID

Compound	Ar	IC ₅₀ (µg/mL)			
		Superoxide radical ^a	Hydroxyl radical ^b	ABTS ⁺ cation radical ^c	Lipid peroxidation ^d
1	3-Bromophenyl	85.65 ± 1.30	204.33 ± 1.53	74.45 ± 0.96	600.85 ± 2.38
2	4-Methylphenyl	86.02 ± 1.69	192.15 ± 2.18	64.85 ± 0.34	587.25 ± 2.32
3	2-Chlorophenyl	88.25 ± 1.33	185.14 ± 1.62	78.86 ± 1.56	612.12 ± 2.31
4	4-Chlorophenyl	78.84 ± 1.99	170.18 ± 1.43	55.87 ± 0.63	547.57 ± 3.50
5	2,4-Dichlorophenyl	82.54 ± 1.57	207.21 ± 1.33	73.45 ± 1.69	650.32 ± 1.54
6	4-Fluorophenyl	79.73 ± 1.58	175.71 ± 1.61	61.06 ± 1.78	552.18 ± 2.98
7	4-Methoxyphenyl	80.09 ± 1.21	179.01 ± 1.58	56.91 ± 0.68	575.97 ± 1.80
8	3,4-Dimethoxyphenyl	80.65 ± 1.58	168.63 ± 1.55	52.89 ± 1.31	550.47 ± 1.97
9	3,4,5-Trimethoxyphenyl	78.32 ± 1.59	158.18 ± 1.63	50.06 ± 1.41	547.18 ± 2.01
10	4-Dimethylaminophenyl	78.87 ± 1.32	151.48 ± 1.09	50.36 ± 1.22	423.08 ± 2.26
11	9-Anthracenyl	89.25 ± 1.99	198.22 ± 1.69	76.32 ± 2.66	652.25 ± 1.44
12	4-Nitrophenyl	85.31 ± 1.30	194.22 ± 1.76	62.32 ± 1.33	612.46 ± 2.99
13	3-Pyridinyl	88.97 ± 1.53	189.63 ± 2.18	77.22 ± 1.38	617.74 ± 2.32
14	2-Pyridinyl	89.65 ± 2.01	191.35 ± 2.03	75.36 ± 2.31	604.47 ± 1.29
15	4-Pyridinyl	88.31 ± 1.34	185.21 ± 1.35	77.01 ± 2.06	624.46 ± 1.92
16	2-Quinoliny	88.87 ± 1.78	197.87 ± 1.48	77.98 ± 2.52	656.54 ± 1.75
	Ascorbic acid	69.89 ± 0.82	113.50 ± 0.91	45.42 ± 1.24	488.62 ± 1.02

All values are expressed as mean ± SD of 3 determinations

TABLE-3
% INHIBITION OF SUPEROXIDE RADICAL USING RIBOFLAVIN PHOTO REDUCTION METHOD

Compound	Quantity (µg)				
	5	10	25	50	100
1	13.23 ± 1.25	18.56 ± 0.57	25.32 ± 1.21	38.54 ± 0.67	50.89 ± 0.52
2	15.70 ± 0.23	22.60 ± 0.20	30.26 ± 1.30	41.76 ± 0.17	52.87 ± 0.12
3	13.21 ± 0.54	21.35 ± 0.26	27.59 ± 1.23	37.58 ± 0.52	50.01 ± 1.32
4	11.76 ± 0.34	19.48 ± 0.15	29.77 ± 0.21	41.54 ± 0.21	53.30 ± 0.53
5	8.69 ± 0.18	16.60 ± 0.57	26.08 ± 0.35	41.89 ± 0.38	52.17 ± 0.23
6	8.98 ± 0.35	17.22 ± 0.44	28.46 ± 0.46	39.32 ± 0.55	50.93 ± 0.47
7	10.25 ± 1.02	17.64 ± 0.51	29.31 ± 0.87	40.28 ± 0.69	51.05 ± 1.05
8	11.19 ± 0.33	18.05 ± 0.38	29.24 ± 0.47	38.26 ± 1.35	51.98 ± 0.53
9	16.72 ± 0.23	22.77 ± 0.54	34.87 ± 0.74	41.99 ± 0.48	51.60 ± 0.69
10	17.46 ± 1.11	23.62 ± 0.75	35.95 ± 0.75	44.51 ± 0.55	53.08 ± 0.59
11	6.58 ± 1.04	12.54 ± 0.63	25.32 ± 0.78	30.52 ± 1.33	40.63 ± 0.87
12	16.88 ± 0.61	23.50 ± 0.75	33.44 ± 0.63	39.40 ± 0.54	53.31 ± 0.59
13	7.82 ± 0.53	15.32 ± 1.52	30.64 ± 0.54	41.55 ± 0.54	51.25 ± 0.58
14	5.48 ± 0.83	10.54 ± 0.74	23.20 ± 0.86	39.66 ± 0.79	51.89 ± 0.54
15	8.89 ± 0.64	21.60 ± 0.68	33.47 ± 0.63	44.06 ± 0.59	52.54 ± 0.79
16	7.32 ± 0.54	14.26 ± 0.52	30.25 ± 1.03	43.23 ± 0.23	51.05 ± 0.58
Ascorbic acid	0.54 ± 1.01	0.78 ± 0.52	0.91 ± 0.63	9.32 ± 0.21	17.60 ± 0.48

superoxide anion radical, hydroxyl radical, ABTS⁺ cation radical and inhibition of lipid peroxidation.

Superoxide scavenging activity: All the chalcones were found to scavenge the superoxides generated by photoreduction of riboflavin, when each tested at five dose levels (5, 10, 25, 50 and 100 µg). However, compounds 4, 6, 7, 9 and 10 showed a dose dependent inhibition of superoxide radicals. Ascorbic acid, the known antioxidant is employed in this study for comparing the results. Compound 9 having 3, 4, 5-trimethoxyphenyl ring was the best among all the tested chalcones. The mean values of inhibition for each compound at each concentration level are presented in Table-3.

Hydroxyl scavenging activity: Degradation of deoxyribose mediated by hydroxyl radicals generated by Fe³⁺/ascorbate/EDTA/H₂O₂ system was found to be inhibited by the compounds tested. Table-4 displays the hydroxyl scavenging activity of the compounds when tested by the deoxyribose method and

results are expressed as percentage inhibition of hydroxyl radical in relation to a control. All compounds in general showed good hydroxyl radical scavenging activity, when each tested at six dose levels (5, 10, 25, 50, 100 and 250 µg) and in particular compounds 4, 6, 7, 8, 9 and 10 exhibited dose dependant inhibition, which is comparable to that of the standard drug ascorbic acid. Compound 10 carrying dimethylaminophenyl ring as ring B of chalcone showed highest activity.

ABTS⁺ radical cation scavenging activity: The antioxidant ability to scavenge the ABTS⁺ radical has been compared to the standard ascorbic acid and is an excellent tool for determining the antioxidant activity of hydrogen donating antioxidants and of chain breaking antioxidants. The percentage scavenging of ABTS⁺ by the compounds at different concentrations were shown in Table-5. Even though all compounds showed a dose dependent scavenging activity, compounds 4, 6, 7, 8, 9 and 10 showed remarkable activity, comparable to

TABLE-4
 % INHIBITION OF HYDROXYL RADICAL USING DEOXYRIBOSE DEGRADATION METHOD

Compound	Quantity (μg)					
	5	10	25	50	100	250
1	16.31 $\pm$ 0.65	20.62 $\pm$ 1.05	28.65 $\pm$ 0.63	39.27 $\pm$ 0.62	41.23 $\pm$ 1.21	48.47 $\pm$ 0.34
2	17.22 $\pm$ 0.37	23.94 $\pm$ 0.66	29.83 $\pm$ 0.82	40.33 $\pm$ 0.57	44.95 $\pm$ 0.88	50.83 $\pm$ 0.30
3	15.45 $\pm$ 0.42	21.11 $\pm$ 0.87	28.47 $\pm$ 1.63	38.12 $\pm$ 1.05	40.19 $\pm$ 0.33	48.32 $\pm$ 0.57
4	23.68 $\pm$ 0.42	26.84 $\pm$ 0.52	33.46 $\pm$ 0.84	36.96 $\pm$ 0.72	44.35 $\pm$ 0.84	52.52 $\pm$ 0.46
5	20.24 $\pm$ 0.92	29.75 $\pm$ 1.20	33.47 $\pm$ 1.35	42.14 $\pm$ 1.19	45.46 $\pm$ 1.23	50.82 $\pm$ 0.85
6	23.72 $\pm$ 0.75	28.81 $\pm$ 0.89	32.62 $\pm$ 0.66	42.37 $\pm$ 0.47	48.72 $\pm$ 1.02	53.81 $\pm$ 0.94
7	24.21 $\pm$ 0.54	28.97 $\pm$ 1.21	34.55 $\pm$ 1.45	41.98 $\pm$ 0.55	48.04 $\pm$ 0.26	54.03 $\pm$ 0.55
8	24.59 $\pm$ 0.98	29.83 $\pm$ 0.81	35.08 $\pm$ 0.82	42.74 $\pm$ 0.82	46.77 $\pm$ 0.82	54.42 $\pm$ 0.62
9	20.67 $\pm$ 0.81	23.39 $\pm$ 0.63	35.32 $\pm$ 0.88	39.44 $\pm$ 0.84	43.57 $\pm$ 0.80	53.66 $\pm$ 0.95
10	26.54 $\pm$ 1.05	34.49 $\pm$ 1.18	40.69 $\pm$ 1.06	45.34 $\pm$ 1.08	50.77 $\pm$ 1.07	56.02 $\pm$ 1.04
11	10.47 $\pm$ 1.21	20.52 $\pm$ 0.52	29.58 $\pm$ 2.10	35.54 $\pm$ 0.68	42.97 $\pm$ 0.64	46.87 $\pm$ 0.87
12	19.02 $\pm$ 0.88	28.74 $\pm$ 0.93	36.43 $\pm$ 1.05	46.96 $\pm$ 1.47	51.01 $\pm$ 0.66	52.63 $\pm$ 0.99
13	10.54 $\pm$ 1.33	18.67 $\pm$ 0.54	29.31 $\pm$ 1.55	34.33 $\pm$ 0.71	41.87 $\pm$ 0.54	56.35 $\pm$ 1.04
14	8.34 $\pm$ 0.45	15.56 $\pm$ 1.72	27.50 $\pm$ 0.71	33.98 $\pm$ 0.98	41.50 $\pm$ 0.98	57.31 $\pm$ 1.26
15	12.68 $\pm$ 0.86	21.26 $\pm$ 1.16	31.71 $\pm$ 1.12	37.31 $\pm$ 0.94	42.53 $\pm$ 0.93	54.85 $\pm$ 1.09
16	10.32 $\pm$ 1.24	15.45 $\pm$ 1.08	20.55 $\pm$ 0.54	31.11 $\pm$ 0.52	38.54 $\pm$ 0.21	49.51 $\pm$ 1.14
Ascorbic acid	2.43 $\pm$ 0.61	3.85 $\pm$ 1.05	4.33 $\pm$ 0.50	5.22 $\pm$ 1.52	15.47 $\pm$ 1.00	42.12 $\pm$ 2.68

 TABLE-5
 % INHIBITION OF ABTS⁺ CATION RADICAL SCAVENGING ACTIVITY OF TEST COMPOUNDS

Compound	Quantity (μg)				
	5	10	25	50	100
1	17.65 $\pm$ 1.21	20.91 $\pm$ 0.76	27.44 $\pm$ 1.65	36.15 $\pm$ 0.45	57.93 $\pm$ 0.67
2	18.86 $\pm$ 1.56	22.39 $\pm$ 1.78	30.54 $\pm$ 0.99	39.24 $\pm$ 0.44	62.43 $\pm$ 0.66
3	13.83 $\pm$ 0.98	18.54 $\pm$ 1.57	24.97 $\pm$ 0.68	35.24 $\pm$ 1.26	58.75 $\pm$ 0.54
4	17.67 $\pm$ 1.32	20.85 $\pm$ 0.68	27.35 $\pm$ 1.02	36.56 $\pm$ 0.78	56.47 $\pm$ 0.99
5	15.23 $\pm$ 0.56	18.98 $\pm$ 0.87	25.35 $\pm$ 1.22	32.32 $\pm$ 0.58	50.98 $\pm$ 1.68
6	18.92 $\pm$ 0.76	22.27 $\pm$ 1.02	28.21 $\pm$ 0.89	32.26 $\pm$ 1.25	47.91 $\pm$ 0.99
7	19.35 $\pm$ 1.65	23.35 $\pm$ 0.55	27.47 $\pm$ 0.68	34.24 $\pm$ 0.43	50.35 $\pm$ 1.98
8	15.70 $\pm$ 0.56	19.02 $\pm$ 0.72	25.64 $\pm$ 1.26	34.48 $\pm$ 0.38	56.55 $\pm$ 1.57
9	17.56 $\pm$ 0.65	21.53 $\pm$ 1.33	28.62 $\pm$ 2.31	34.84 $\pm$ 0.58	51.58 $\pm$ 1.26
10	10.52 $\pm$ 0.35	12.35 $\pm$ 1.24	19.26 $\pm$ 0.56	27.02 $\pm$ 0.65	46.64 $\pm$ 1.52
11	5.65 $\pm$ 1.25	14.24 $\pm$ 0.69	26.36 $\pm$ 1.55	30.21 $\pm$ 0.57	38.21 $\pm$ 1.35
12	20.70 $\pm$ 0.54	25.56 $\pm$ 1.02	35.55 $\pm$ 1.57	48.73 $\pm$ 1.21	65.21 $\pm$ 0.38
13	13.33 $\pm$ 1.88	16.69 $\pm$ 0.34	20.23 $\pm$ 0.53	25.54 $\pm$ 1.39	37.52 $\pm$ 1.76
14	11.71 $\pm$ 0.99	17.22 $\pm$ 1.22	21.40 $\pm$ 0.91	26.87 $\pm$ 0.67	40.90 $\pm$ 1.17
15	15.13 $\pm$ 1.68	17.22 $\pm$ 2.06	21.40 $\pm$ 0.91	26.97 $\pm$ 0.78	33.93 $\pm$ 2.11
16	5.65 $\pm$ 1.24	15.35 $\pm$ 1.33	19.48 $\pm$ 0.55	20.14 $\pm$ 1.11	30.35 $\pm$ 2.31
Ascorbic acid	12.35 $\pm$ 0.59	23.46 $\pm$ 0.44	27.86 $\pm$ 0.65	35.58 $\pm$ 2.13	57.53 $\pm$ 0.78

that of the standard drug ascorbic acid. Compound **9** and **10** showed maximum activity.

Inhibition of lipid peroxidation: Lipid peroxides generated by the induction of Fe²⁺/ascorbate on rat liver homogenate were found to be inhibited by the addition of synthesized chalcones when tested by thiobarbituric acid method, using ascorbic acid as standard. Table-6 exhibited the percentage inhibition of lipid peroxidation of the compounds tested. Compounds **4**, **6**, **7**, **9** and **10** showed dose dependent inhibitions when each tested at different dose levels (50, 100, 200, 300, 500 and 750 μg). Again compound **9** and **10** found to be the most potent.

From the results of the antioxidant activity studies it is evident that the compound **9** and **10** possessed significant activity either in reactive oxygen species assays or in inhibiting the lipid peroxidation, which suggest electron releasing pharmacophores like methoxy or dimethylamino group may be essential for activity and compounds when synthesized and

tested with such type of functional groups as substituent may further conform this observation. Such observation is also consistent with the earlier reports. As superoxides and peroxide radicals are inevitable participants in the process of tumor promotion²⁶.

It could be inferred from this study that substitution of electron donating groups at the *ortho* and/or *para* positions of the benzene ring could increase the antioxidant activity of the chalcones.

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TABLE-6
 % INHIBITION OF LIPID PEROXIDATION USING THIOBARBITURIC ACID METHOD

Compound	Quantity (μg)					
	50	100	200	300	500	750
1	15.22 $\pm$ 0.68	18.51 $\pm$ 1.21	22.34 $\pm$ 0.65	28.66 $\pm$ 0.87	39.38 $\pm$ 1.22	49.74 $\pm$ 0.34
2	17.33 $\pm$ 1.12	21.37 $\pm$ 1.37	27.41 $\pm$ 0.86	32.25 $\pm$ 0.90	43.14 $\pm$ 1.54	54.43 $\pm$ 1.41
3	8.66 $\pm$ 0.57	16.57 $\pm$ 1.31	20.94 $\pm$ 0.52	30.23 $\pm$ 0.98	41.05 $\pm$ 1.22	50.95 $\pm$ 0.38
4	5.46 $\pm$ 0.39	15.43 $\pm$ 0.96	24.21 $\pm$ 1.12	32.42 $\pm$ 1.13	44.53 $\pm$ 0.98	54.29 $\pm$ 1.19
5	10.48 $\pm$ 0.57	20.59 $\pm$ 1.10	29.21 $\pm$ 0.92	39.32 $\pm$ 1.23	45.62 $\pm$ 1.17	54.68 $\pm$ 1.68
6	14.74 $\pm$ 1.39	19.92 $\pm$ 1.21	28.28 $\pm$ 1.22	43.42 $\pm$ 1.21	48.60 $\pm$ 1.15	54.97 $\pm$ 0.85
7	10.58 $\pm$ 1.64	19.34 $\pm$ 0.55	28.65 $\pm$ 0.67	41.14 $\pm$ 0.59	48.24 $\pm$ 1.11	53.97 $\pm$ 1.34
8	8.58 $\pm$ 1.01	19.02 $\pm$ 0.89	26.49 $\pm$ 0.92	39.17 $\pm$ 0.89	48.50 $\pm$ 0.82	54.85 $\pm$ 1.12
9	18.90 $\pm$ 0.91	21.84 $\pm$ 0.91	33.19 $\pm$ 0.92	38.23 $\pm$ 1.04	48.31 $\pm$ 0.95	55.04 $\pm$ 1.12
10	12.86 $\pm$ 0.84	22.07 $\pm$ 0.72	32.77 $\pm$ 1.07	39.83 $\pm$ 0.96	47.30 $\pm$ 0.88	53.94 $\pm$ 1.18
11	8.65 $\pm$ 0.57	17.85 $\pm$ 0.87	22.45 $\pm$ 0.58	30.54 $\pm$ 1.52	41.21 $\pm$ 0.22	52.14 $\pm$ 0.68
12	3.57 $\pm$ 0.49	19.44 $\pm$ 0.55	27.38 $\pm$ 1.06	35.71 $\pm$ 0.82	43.65 $\pm$ 0.83	55.15 $\pm$ 1.15
13	5.87 $\pm$ 0.87	18.74 $\pm$ 1.14	30.28 $\pm$ 0.67	33.38 $\pm$ 0.97	40.56 $\pm$ 0.57	55.25 $\pm$ 0.54
14	5.26 $\pm$ 0.63	16.22 $\pm$ 0.86	29.38 $\pm$ 1.08	32.01 $\pm$ 1.10	42.54 $\pm$ 0.93	55.26 $\pm$ 0.67
15	6.45 $\pm$ 0.81	20.27 $\pm$ 1.09	30.41 $\pm$ 0.91	34.56 $\pm$ 1.13	41.93 $\pm$ 0.89	55.75 $\pm$ 0.94
16	4.25 $\pm$ 1.32	9.67 $\pm$ 1.04	19.35 $\pm$ 0.87	25.07 $\pm$ 1.55	31.32 $\pm$ 0.74	40.47 $\pm$ 0.85
Ascorbic acid	20.46 $\pm$ 3.70	37.01 $\pm$ 0.83	51.65 $\pm$ 0.58	59.00 $\pm$ 0.35	74.33 $\pm$ 1.73	88.51 $\pm$ 1.28

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