Synthesis and antioxidant evaluation of novel 4-aryl-hexahydroquinolines from lignin

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DOI: http://dx.doi.org/10.3998/ark.5550190.0012.a27

Abstract
A series of 4-aryl-hexahydroquinolines were prepared by Hantzsch reaction using aromatic aldehydes obtained from lignin, 1,3-cyclohexanediones, \( \beta \)-ketoesters and ammonium carbonate. The antioxidant properties of compounds 5a-c and 5g-i were evaluated by two methods: scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and scavenging effect on 2,2'-azino-bis(3-ethylbenzothiazoline-sulfonic acid) diammonium salt (ABTS\(^+\)) radical. The results show that the compounds containing a methoxy moiety exhibit good activities. The study suggests that some synthesized compounds may serve as promising leads for the treatment against tumors or other free radical-related diseases.

Keywords: Polyhydroquinoline, microwave irradiation, aromatic aldehyde, lignin, antioxidant activity

Introduction
Free radicals play an important role in a number of biological processes, some of which are necessary for life, such as the intracellular killing of bacteria by phagocytic cells, but excessive amounts of free radicals can lead to damage to biomolecules such as lipids, proteins, enzymes, and DNA in cells and tissues, which may result in cancer, diabetes, cardiovascular diseases, autoimmune diseases, myocardial infarction, neurodegenerative disorders, aging, and other variety of diseases because of their excellent reactivity. The antioxidants can minimize or inhibit the oxidative damage through interrupting the free-radical formation or terminating the...
chain reaction. Thus, identification and development of novel antioxidants to prevent radical-induced damage have attracted the attention of chemists and medical scientists.

Quinolines are an important class of pharmaceutical compounds, which occur predominately in nature among the various heterocyclic compounds,6,7 and exhibit a broad spectrum of biological activities such as antioxidant,8 antiproliferation,9 antiinflammation,10 and anticancer.11 The polyhydroquinolines possessing a bioactive 1,4-dihydropyridine moiety also have important biological properties.12-14 Although the antioxidant activities of quinolines and 1,4-dihydropyridines15,16 have been extensively studied, the polyhydroquinolines are not well explored. The synthesis and antioxidant evaluation of new polyhydroquinolines, therefore, is included as part of our medicinal chemistry project aiming to identify a new class of antioxidants.

It is well-known that lots of natural and synthesized antioxidants possessing phenolic hydroxyl groups can improve their antioxidant activities by reacting with free radicals.17 Vanillin and syringaldehyde, two phenolic hydroxyl containing compounds obtained from lignin, are widely used as additives in food, pharmaceutical and cosmetic industries due to their antioxidant activity besides the fragrant odor and non-toxicity.18-20 The synthesis of polyhydroquinolines with vanillin and syringaldehyde as starting materials is expected to afford better antioxidant properties. Starting from these considerations, we report herein the synthesis and in vitro antioxidant properties of 4-aryl-hexahydroquinolines using the aromatic aldehydes from lignin via an efficient, catalyst-free, and four-component MCR (multi-component-reactions) Hantzsch reaction under microwave irradiation.

Results and Discussion

Our study started from the preparation of the aromatic aldehydes from lignin according to the previously reported procedure18-20 with nitrobenzene oxidation as the standard method, providing vanillin and syringaldehyde as the major compounds (Scheme 1).

![Scheme 1. Preparation of aromatic aldehydes from lignin.](image)
Scheme 2. Microwave-assisted synthesis of 4-aryl-hexahydroquinolines.

Table 1. Microwave-assisted synthesis of polyhydroquinoline derivatives 5a-l through Hantzsch reaction in water

| Entry | Product | R<sup>1</sup> | R<sup>2</sup> | R<sup>3</sup> | R<sup>4</sup> | Yield (%)<sup>a</sup> |
|-------|---------|--------------|--------------|--------------|--------------|---------------------|
| 1     | 5a      | OMe          | OMe          | H            | Et           | 96                  |
| 2     | 5b      | OMe          | H            | H            | Et           | 94                  |
| 3     | 5c      | H            | H            | H            | Et           | 95                  |
| 4     | 5d      | OMe          | OMe          | H            | Me           | 94                  |
| 5     | 5e      | OMe          | H            | H            | Me           | 95                  |
| 6     | 5f      | H            | H            | H            | Me           | 91                  |
| 7     | 5g      | OMe          | OMe          | Me           | Et           | 93                  |
| 8     | 5h      | OMe          | H            | Me           | Et           | 94                  |
| 9     | 5i      | H            | H            | Me           | Et           | 91                  |
| 10    | 5j      | OMe          | OMe          | Me           | Me           | 93                  |
| 11    | 5k      | OMe          | H            | Me           | Me           | 89                  |
| 12    | 5l      | H            | H            | Me           | Me           | 90                  |

<sup>a</sup>Isolated yield.

Due to the biological importance of polyhydroquinolines, several procedures for their synthesis have been reported with good yields comprising the use of reflux,<sup>21</sup> ionic liquids,<sup>22</sup> microwaves,<sup>23</sup> I<sub>2</sub>,<sup>24</sup> ceric ammonium nitrate (CAN),<sup>25</sup> organo-catalysts,<sup>26</sup> etc. If they could avoid the use of long reaction times, high temperatures, catalysts, or organic solvents, they would be applied extensively. Recently, the progress in the field of reactions in aqua media is gaining significance because of their operational simplicity and environmentally benign processes. Herein, we wished to develop a new eco-friendly procedure<sup>27-29</sup> with our continuation to the use
of the microwave technology for MCR for efficient and catalyst-free synthesis of polyhydroquinolines in water (Schemes 2). According to green chemistry concept, water was used as the solvent. Ammonium carbonate was chosen as a solid ammonia source because it has low toxicity (LD50 1497 mg/kg) and melting point (58 °C) and resulted in high yields compared with ammonium acetate, ammonium chloride, or ammonium nitrate etc.\(^{28}\) The microwave irradiation can accelerate reaction rate, shorten reaction time, and improve product yields by transferring energy directly to the reactive species.\(^{30,31}\) Thus, the four-component reactions between the aromatic aldehydes, 1,3-cyclohexanediones, β-ketoesters, and ammonium carbonate were conducted in water under microwave irradiation without any catalysts in a short time providing the desired polyhydroquinolines in high yields. The workup procedure was very simple and gave the products after a single filtration in good purity.

As indicated in Table 1, the reactions were performed in water at 60 °C within 5 min under microwave irradiation without catalysts and all of them yielded well (≥ 89%). All the compounds were characterized by IR, \(^1\)H NMR, \(^13\)C NMR, mass spectra and elemental analysis.

In conclusion, we designed and developed a convenient, efficient, economically and environmentally benign procedure for the synthesis of polyhydroquinolines which will be studied below for their antioxidation activities.

**In vitro biological evaluation.**

The \(N,N\)-diphenyl-\(N\)′-picrylhydrazyl (DPPH) assay is a well-known method for determining antioxidant activity of plant flavonoids. So compounds 5a-c and 5g-i were first evaluated for antioxidant activity by DPPH assay. The reduction rate constant of DPPH radical for compounds 5a, 5b, 5g and 5h was found good to moderate compared to the standard Trolox, while that for 5c and 5i was found very poor (Figure 1).

![Figure 1. Radical scavenging activity of compounds 5a-c, 5g-i and Trolox in \(N,N\)-diphenyl-\(N\)′-picrylhydrazyl (DPPH) assay. Results were expressed as reduction rate constant \((k)\) of DPPH±S.D. Significance was calculated according to the Student’s \(t\)-test, \(p<0.05\).](image)
After the good reduction rate constant was observed for compounds 5a, 5b, 5g and 5h, the 50% inhibitory concentrations (IC₅₀) against DPPH and ABTS⁺ radicals were tested (Table 2). The values of IC₅₀ for compounds 5a and 5g were lower than that for Trolox, while those for the compounds 5b and 5h were higher in reaction with DPPH radical. The IC₅₀ of compounds 5b, 5g, and 5h were lower than that of Trolox, while the compound 5a showed almost the same IC₅₀ as Trolox in the ABST⁺ radical cation scavenging assays.

Table 2. Radical scavenging activity of compounds 5a-c, 5g-i and Trolox in DPPH and ABST⁺ assays

| Sample | IC₅₀ (μM) | DPPH | ABST⁺ |
|--------|----------|------|-------|
| 5a     | 14.89 ± 0.68 | 17.4 ± 0.07 |       |
| 5b     | 65.86 ± 2.45 | 0.76 ± 0.04 |       |
| 5c     | —        | —     | —     |
| 5g     | 7.48 ± 0.43 | 0.87 ± 0.02 |       |
| 5h     | 41.50 ± 1.56 | 0.74 ± 0.02 |       |
| 5i     | —        | —     | —     |
| Trolox | 37.35 ± 1.76 | 1.56 ± 0.05 |       |

aNo test.

As demonstrated in Figure 1 and Table 2, 5a and 5g showed the best antioxidation activities, and 5b and 5h provided moderate values, while 5c and 5i were proven very poor. Obviously, one could realize that the substituents of polyhydroquinolines on the aromatic ring have a major influence on their antioxidation activities. The presence of methoxy groups in aromatic groups increased their antioxidation properties. Without such methoxy group on the aromatic ring, 5c and 5i did not exhibit good activity; the antioxidation activity increased with one methoxy group in 5b and 5h and further enhanced when there are two methoxy groups in compounds 5a and 5g. The strong antioxidant activities of these compounds indicated their potential application in the drug development, and also provided an effective approach for value-added application of lignin.

Conclusions

A series of novel 4-aryl-hexahydroquinolines were obtained from lignin with a very environmentally benign procedure in good to excellent yields. Some compounds were screened for their in vitro biological activities showing good DPPH and ABST⁺ radical scavenging activities. Compounds 5a and 5g showed the best antioxidant activities against DPPH and ABST⁺ which suggest further studies to explore the potential chemotherapeutic treatment against tumors or other free radical induced diseases. In addition, it was realized that the methoxy groups
at the aryl substituents linked at the C-4 of the 4-aryl-hexahydroquinolines increased the antioxidation activities.

Experimental Section

General. Vanillin, syringaldehyde and p-hydroxybenzaldehyde were synthesized according to previously reported methods.\textsuperscript{18-20} Other reagents were obtained from Sinopharm Chemical Reagent Co, Ltd. All reactions were conducted in MAS (II) Sineo microwave reactors using external surface sensors. All synthesized compounds were characterized by infra-red (IR), \textsuperscript{1}H NMR, \textsuperscript{13}C NMR, mass spectrometry (MS) and elemental analysis (EA). Melting point was determined by SGW X-4 Micro Melting Point Apparatus. IR spectra were recorded with a Nicolet Magna-IR 550 spectrometer. Mass spectra were recorded on WATERS Q-TOF Premier Mass Spectrometer using electrospray ionization (ESI). \textsuperscript{1}H and \textsuperscript{13}C NMR spectra were recorded with a Bruker DRX-300 Advance spectrometer at 300 MHz and 75 MHz, respectively. Elemental analyses (C, H, N, S) were conducted using a PE-2400 (II) Elemental Analyser, and results were found to be in good agreement (± 0.2%) with the calculated values.

Typical procedure to synthesize 4-aryl-4,6,7,8-tetrahydroquinolines

A stirred aqueous mixture of aromatic aldehydes (5 mmol), 1,3-cyclohexanediones (5 mmol), \(\beta\)-ketoesters (5 mmol) and anhydrous ammonium carbonate (5 mmol) was placed into an open microwave oven (300 W) at 60 °C for 5 min. The reaction mixture was cooled to room temperature to afford the product as a precipitate. The solid residue was filtered, washed with water and 5 mL of 50% ethanol, and then recrystallized from ethyl acetate/petroleum ether (50:50, v/v) to give products.

Ethyl \(4\)-\((4\)-hydroxy-3,5-dimethoxyphenyl\))-2-methyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (5a). (1.86 g, 96%). Mp: 227–229 °C. IR (KBr) \(\nu/cm\): 3358, 3283, 3218, 2947, 1679, 1606, 1482, 1461, 1379, 1219, 1183, 1112, 1079, 733; \textsuperscript{1}H NMR (300 MHz, DMSO-\(d_6\)) \(\delta/ppm\): 9.08 (s, 1H, NH), 8.04 (s, 1H, OH), 6.36 (s, 2H, Ar-H), 4.83 (s, 1H, H4), 4.02 (quart, \(J\ 8.1\ Hz\), 2H, OCH\(\textsubscript{2}\)CH\(_3\)), 3.66 (s, 6H, 2×OCH\(_3\)), 2.50 (s, 3H, CH\(_3\)), 1.70-2.51 (m, 6H, 3×CH\(_2\)), 1.19 (t, \(J\ 6.3\ Hz\), 3H, OCH\(_2\)CH\(_3\)); \textsuperscript{13}C NMR (75 MHz, DMSO-\(d_6\)) \(\delta/ppm\): 194.8, 167.1, 151.3, 147.5, 144.3, 138.0, 133.9, 111.0, 105.0, 103.9, 59.0, 55.9, 36.8, 35.0, 26.2, 21.0, 18.2, 14.3; MS (ESI) \(m/z\): 388.1 [M+H]\(^+\), 410.1 [M+Na]\(^+\), 426.1 [M+K]\(^+\); Anal. Calcd for C\(_{21}\)H\(_{25}\)NO\(_6\): C, 65.10; H, 6.50; N, 3.62; found: C, 65.29; H, 6.59; N, 3.51.

Ethyl \(4\)-(4-hydroxy-3-methoxyphenyl)-2-methyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (5b). (1.68 g, 94%). Mp: 236–238 °C. IR (KBr) \(\nu/cm\): 3392, 3297, 2997, 1679, 1478, 1377, 1286, 1224, 1183, 1161, 1124, 1078, 726, 686; \textsuperscript{1}H NMR (300 MHz, DMSO-\(d_6\)) \(\delta/ppm\): 9.07 (s, 1H, NH), 8.63 (s, 1H, OH), 6.71 (s, 1H, Ar-H), 6.58 (d, \(J\ 8.1\ Hz\), 1H, Ar-H), 6.48 (d, \(J\ 8.1\ Hz\), 1H, Ar-H), 4.80 (s, 1H, H4), 4.00 (quart, \(J\ 8.1\ Hz\), 2H, OCH\(_2\)CH\(_3\)), 3.68 (s, 3H, OCH\(_3\)), 2.48 (s, 3H, CH\(_3\)), 1.62-2.29 (m, 6H, 3×CH\(_2\)), 1.16 (t, 3H, \(J\ 8.1\ Hz\), OCH\(_2\)CH\(_3\)); \textsuperscript{13}C
NMR (75 MHz, DMSO-d$_6$) $\delta$/ppm: 194.7, 167.1, 151.1, 146.7, 144.5, 144.3, 139.0, 119.4, 115.0, 111.9, 111.2, 103.9, 58.9, 55.4, 36.8, 34.7, 26.1, 20.9, 18.2, 14.2; MS (ESI) m/z: 358.1 [M+H]$^+$, 380.1 [M+Na]$^+$, 396.1 [M+K]$^+$; Anal. Calcd for C$_{29}$H$_{33}$NO$_5$: C, 67.21; H, 6.49; N, 3.92; found: C, 67.29; H, 6.44; N, 3.98.

**Ethyl 4-(4-hydroxyphenyl)-2-methyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (5c).** (1.55 g, 95%). Mp: 243–245 °C. IR (KBr) $\nu$/cm$^{-1}$: 3440, 3282, 2960, 1677, 1605, 1481, 1373, 1217, 1179, 1122, 1017, 837, 688; $^1$H NMR (300 MHz, DMSO-d$_6$) $\delta$/ppm: 9.04 (s, 2H, NH, OH), 6.92 (d, J 8.1 Hz, 2H, Ar-H,), 6.56 (d, J 8.1 Hz, 2H, Ar-H), 4.78 (s, 1H, H4), 3.98 (quart, J 6.9 Hz, 2H, OCH$_2$CH$_3$), 2.46 (s, 3H, CH$_3$), 1.62-2.26 (m, 6H, 3×CH$_2$), 1.13 (t, J 6.9 Hz, 3H, OCH$_2$CH$_3$); $^{13}$C NMR (75 MHz, DMSO-d$_6$) $\delta$/ppm: 194.6, 167.0, 155.2, 151.0, 144.2, 138.5, 128.2, 114.5, 111.4, 104.1, 58.9, 36.7, 34.5, 26.1, 20.8, 18.2, 14.1; MS (ESI) m/z: 328.2 [M+H]$^+$, 350.1 [M+Na]$^+$, 366.1 [M+K]$^+$; Anal. Calcd for C$_{19}$H$_{21}$NO$_4$: C, 69.71; H, 6.47; N, 4.28; found: C, 69.82; H, 6.40; N, 4.40.

**Methyl 4-(4-hydroxy-3,5-dimethoxyphenyl)-2-methyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (5d).** (1.76 g, 94%). Mp: 252–256 °C. IR (KBr) $\nu$/cm$^{-1}$: 3413, 3275, 3208, 2947, 1680, 1607, 1480, 1376, 1226, 1185, 1106, 770, 734; $^1$H NMR (300 MHz, DMSO-d$_6$) $\delta$/ppm: 9.09 (s, 1H, NH), 8.03 (s, 1H, OH), 6.35 (s, 2H, Ar-H), 4.84 (s, 1H, H4), 3.66 (s, 6H, 2×OCH$_3$), 3.58 (s, 3H, CO$_2$CH$_3$), 1.70-2.28 (m, 9H, CH$_3$, 3×CH$_2$); $^{13}$C NMR (75 MHz, DMSO-d$_6$) $\delta$/ppm: 194.8, 167.6, 151.2, 147.5, 144.6, 137.8, 134.0, 111.0, 104.8, 104.3, 55.9, 50.6, 36.8, 34.8, 26.1, 20.9, 18.1; MS (ESI) m/z: 374.1 [M+H]$^+$, 396.1 [M+Na]$^+$, 412.1 [M+K]$^+$; Anal. Calcd for C$_{20}$H$_{23}$NO$_6$: C, 64.33; H, 6.21; N, 3.75; found: C, 64.52; H, 6.29; N, 3.64.

**Methyl 4-(4-hydroxy-3-methoxyphenyl)-2-methyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (5e).** (1.63 g, 95%). Mp: 248–251 °C. IR (KBr) $\nu$/cm$^{-1}$: 3302, 2954, 1681, 1612, 1515, 1476, 1432, 1380, 1274, 1229, 1187, 1080, 729; $^1$H NMR (300 MHz, DMSO-d$_6$) $\delta$/ppm: 9.06 (s, 1H, NH), 8.58 (s, 1H, OH), 6.70 (s, 1H, Ar-H), 6.57 (d, J 8.1 Hz, 1H, Ar-H), 6.46 (d, J 8.1 Hz, 1H, Ar-H), 4.81 (s, 1H, H4), 3.68 (s, 3H, OCH$_3$), 3.55 (s, 3H, CO$_2$CH$_3$), 1.68-2.28 (m, 9H, CH$_3$, 3×CH$_2$); $^{13}$C NMR (75 MHz, DMSO-d$_6$) $\delta$/ppm: 194.7, 167.5, 151.0, 146.8, 144.6, 138.9, 119.2, 115.1, 111.8, 111.3, 103.6, 55.5, 50.5, 36.8, 34.6, 26.1, 20.8, 18.1; MS (ESI) m/z: 344.1 [M+H]$^+$, 366.1 [M+Na]$^+$, 382.1 [M+K]$^+$; Anal. Calcd for C$_{19}$H$_{21}$NO$_5$: C, 66.46; H, 6.16; N, 4.08; found: C, 66.54; H, 6.31; N, 4.01.

**Methyl 4-(4-hydroxyphenyl)-2-methyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (5f).** (1.43 g, 91%). Mp: 256–257 °C. IR (KBr) $\nu$/cm$^{-1}$: 3498, 3303, 3216, 2949, 1650, 1590, 1437, 1334, 1231, 1199, 1139, 1079, 980, 833; $^1$H NMR (300 MHz, DMSO-d$_6$) $\delta$/ppm: 9.03 (s, 1H, NH,), 8.99 (s, 1H, OH), 6.90 (d, J 8.4 Hz, 2H, Ar-H), 6.55 (d, J 8.4 Hz, 2H, Ar-H), 4.79 (s, 1H, H4), 3.53 (s, 3H, CO$_2$CH$_3$), 1.68-2.27 (m, 9H, CH$_3$, 3×CH$_2$); $^{13}$C NMR (75 MHz, DMSO-d$_6$) $\delta$/ppm: 194.6, 167.3, 155.3, 150.9, 144.6, 138.3, 128.0, 114.6, 111.5, 103.9, 50.5, 36.8, 34.3, 26.1, 20.8, 18.2; MS (ESI) m/z: 314.1 [M+H]$^+$, 336.1 [M+Na]$^+$, 352.0 [M+K]$^+$; Anal. Calcd for C$_{18}$H$_{19}$NO$_4$: C, 68.99; H, 6.11; N, 4.47; found: C, 69.08; H, 6.01; N, 4.36.

**Ethyl 4-(4-hydroxy-3,5-dimethoxyphenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (5g).** (1.94 g, 93%). Mp: 238–240 °C. IR (KBr) $\nu$/cm$^{-1}$: 3546, 3275,
3199, 3075, 2960, 1692, 1606, 1486, 1391, 1281, 1215, 1118, 1071, 1032, 778; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$/ppm: 8.98 (s, 1H, NH), 8.00 (s, 1H, OH), 6.37 (s, 2H, Ar-H), 4.77 (s, 1H, H4), 4.02 (quart, $J$ 6.9 Hz, 2H, OCH$_2$CH$_3$), 3.65 (s, 6H, 2xOCH$_3$), 1.97-2.47 (m, 7H, CH$_3$, 2xCH$_2$), 1.19 (t, $J$ 6.9 Hz, 3H, OCH$_2$CH$_3$), 0.93, 1.03 (2s, 6H, 2xCH$_3$); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$/ppm: 194.3, 167.0, 149.4, 147.4, 144.3, 138.1, 134.0, 109.9, 105.2, 104.0, 59.0, 55.9, 50.3, 35.3, 32.0, 29.3, 26.3, 18.2, 14.2; MS (ESI) $m/z$: 416.2 [M+H]$^+$; Anal. Calcd for C$_{23}$H$_{32}$NO$_6$: C, 66.49; H, 7.04; N, 3.37; found: C, 66.40; H, 7.16; N, 3.31.

**Ethyl 4-(4-hydroxy-3-methoxyphenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (5h).** (1.81 g, 94%). Mp: 235–237 °C. IR (KBr) v/cm$^{-1}$: 3368, 3283, 3237, 2952, 1697, 1586, 1477, 1392, 1378, 1268, 1203, 1146, 1068, 1030, 861, 783; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$/ppm: 8.95 (s, 1H, NH), 8.57 (s, 1H, OH), 6.70 (s, 1H, Ar-H), 6.57 (d, $J$ 8.1 Hz, 1H, Ar-H), 6.50 (d, $J$ 8.1 Hz, 1H, Ar-H), 4.75 (s, 1H, H4), 3.99 (quart, $J$ 6.9 Hz, 2H, OCH$_2$CH$_3$), 3.67 (s, 3H, OCH$_3$), 1.95-2.44 (m, 7H, CH$_3$, 2xCH$_2$), 1.16 (t, $J$ 6.9 Hz, 3H, OCH$_2$CH$_3$), 0.88, 1.01 (2s, 6H, 2xCH$_3$); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$/ppm: 194.3, 167.0, 149.2, 146.7, 144.5, 144.3, 139.0, 119.6, 114.9, 112.1, 110.2, 104.1, 58.9, 55.5, 50.3, 35.0, 32.1, 29.2, 26.4, 18.2, 14.2; MS (ESI) $m/z$: 386.1 [M+H]$^+$, 408.1 [M+Na]$^+$, 424.1 [M+K]$^+$; Anal. Calcd for C$_{22}$H$_{27}$NO$_5$: C, 68.55; H, 7.06; N, 3.63; found: C, 68.41; H, 7.22; N, 3.46.

**Ethyl 4-(4-hydroxy-3-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (5i).** (1.62 g, 91%). Mp: 224–225 °C. IR (KBr) v/cm$^{-1}$: 3435, 3274, 3205, 2966, 1676, 1608, 1484, 1387, 1319, 1150, 1078, 852, 769; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$/ppm: 9.00 (s, 1H, NH), 8.94 (s, 1H, OH), 6.69 (d, $J$ 8.4 Hz, 2H, Ar-H), 6.55 (d, $J$ 8.4 Hz, 2H, Ar-H), 4.74 (s, 1H, H4), 3.97 (quart, $J$ 6.9 Hz, 2H, OCH$_2$CH$_3$), 1.93-2.42 (m, 7H, CH$_3$, 2xCH$_2$), 1.13 (t, $J$ 6.9 Hz, 3H, OCH$_2$CH$_3$), 0.86, 1.00 (2s, 6H, 2xCH$_3$); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$/ppm: 194.2, 167.0, 155.2, 149.1, 144.3, 138.4, 128.3, 114.4, 110.3, 104.1, 58.9, 50.3, 34.8, 32.1, 29.1, 26.5, 18.2, 14.1; MS (ESI) $m/z$: 356.1 [M+H]$^+$; Anal. Calcd for C$_{21}$H$_{25}$NO$_4$: C, 70.96; H, 7.09; N, 3.94; found: C, 70.90; H, 7.17; N, 3.81.

**Methyl 4-(4-hydroxy-3,5-dimethoxyphenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (5j).** (1.87 g, 93%). Mp: 228–231 °C. IR (KBr) v/cm$^{-1}$: 3379, 3300, 2956, 1684, 1604, 1479, 1379, 1314, 1218, 1093, 1042, 920, 779; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$/ppm: 9.02 (s, 1H, NH), 8.02 (s, 1H, OH), 6.36 (s, 2H, Ar-H), 4.78 (s, 1H, H4), 3.65 (s, 6H, 2xOCH$_3$), 3.58 (s, 3H, CO$_2$CH$_3$), 1.97-2.47 (m, 7H, CH$_3$, 2xCH$_2$), 0.92, 1.02 (2s, 6H, 2xCH$_3$); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$/ppm: 194.4, 167.5, 149.4, 147.5, 144.6, 137.9, 133.9, 109.9, 104.9, 103.7, 55.9, 50.5, 50.3, 35.1, 32.0, 29.3, 26.2, 18.2; MS (ESI) $m/z$: 402.1 [M+H]$^+$, 424.1 [M+Na]$^+$, 440.1 [M+K]$^+$; Anal. Calcd for C$_{22}$H$_{25}$NO$_4$: C, 65.82; H, 6.78; N, 3.49; found: C, 65.71; H, 6.70; N, 3.63.

**Methyl 4-(4-hydroxy-3-methoxyphenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (5k).** (1.65 g, 89%). Mp: 254–256 °C. IR (KBr) v/cm$^{-1}$: 3291, 3242, 2967, 1702, 1587, 1482, 1273, 1215, 1130, 1074, 861, 783; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$/ppm: 9.01 (s, 1H, NH), 8.61 (s, 1H, OH), 6.69 (s, 1H, Ar-H), 6.57 (d, $J$ 8.1 Hz, 1H, Ar-H), 6.48 (d, $J$ 8.1 Hz, 1H, Ar-H), 4.76 (s, 1H, H4), 3.66 (s, 3H, OCH$_3$), 3.55 (s, 3H, CO$_2$CH$_3$), 1.96-2.47
Methyl 4-(4-hydroxyphenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate (5l). (1.54 g, 90%). Mp: 288–290 °C. IR (KBr) ν/cm⁻¹: 3395, 3276, 3197, 2959, 1685, 1608, 1483, 1377, 1218, 1113, 1082, 849, 768; ¹H NMR (300 MHz, DMSO-d₆) δ/ppm: 9.01 (s, 1H, NH), 8.98 (s, 1H, OH), 6.91 (d, J 8.4 Hz, 2H, Ar-H), 6.55 (d, J 8.4 Hz, 2H, Ar-H), 4.75 (s, 1H, H₄), 3.52 (s, 3H, CO₂CH₃), 1.94–2.37 (m, 7H, CH₃, 2×CH₂), 0.84, 1.03 (2s, 6H, 2×CH₃); ¹³C NMR (75 MHz, DMSO-d₆) δ/ppm: 195.3, 167.7, 155.0, 149.8, 144.7, 138.5, 128.1, 114.6, 110.2, 104.0, 55.4, 50.7, 34.6, 32.0, 29.0, 26.2, 18.1; MS (ESI) m/z: 342.2 [M+H]⁺, 364.2 [M+Na]⁺, 380.1 [M+K]⁺; Anal. Calcd for C₂₀H₂₃NO₄: C, 70.36; H, 6.79; N, 4.10; found: C, 70.30; H, 6.70; N, 4.22.

In vitro biological evaluation
The compounds were evaluated for their in vitro free radical scavenging activity by the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-sulfonic acid) diammonium salt (ABTS⁺) radical scavenging method.¹⁶,³²⁻³⁵

DPPH radical scavenging assay
This assay is based on the measurement of the scavenging ability of compounds towards the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and performed according to the reported method.¹⁶,³²,³³ Briefly, an ethanol solution (200 μL) of 100 μM diphenyl-picrylhydrazide (Aldrich, USA) was incubated at 30 °C with 1 μL of compound or Trolox solutions in deionized H₂O. The final concentration of compounds was 100 μM. A decrease in absorbance was measured at 517 nm (Perkin Elmer Lambda 2 Spectrophotometer). The rate constant was calculated as the average value of 5 to 6 time-points (intervals) until the absorbance diminished by 50%. The radical scavenging activity was expressed as the reduction rate constant (k) of DPPH radical and calculated according to the Equation 1, where [DPPH]₀ is the starting concentration and [DPPH]ₜ is the concentration at the time ‘t’.

\[
k (M⁻¹S⁻¹) = ([DPPH]₀[DPPH]ₜ)/([DPPH]₀[DPPH]ₜ)
\]  

The percentage of scavenged DPPH radical was plotted versus the concentration of antioxidants and the concentration of antioxidant required obtaining 50% inhibition (50% inhibition concentration, i. e., IC₅₀) was obtained from the graph.

ABST⁺ radical cation scavenging assay
The concentration of 2,2′-azino-bis(3-ethylbenzothiazoline-sulfonic acid) diammonium salt (ABST⁺) remaining after reaction with the antioxidants was determined according to previously
The procedure to prepare the ABST\(^+\) stock solution was modified slightly. Sufficient amounts of the diammonium salts of ABTS\(^+\) (Sigma, USA) and \(\text{K}_2\text{S}_2\text{O}_8\) (Sigma, USA) were dissolved in 2.0 mL water to achieve concentrations of 4.00 and 1.41 mM, respectively. This solution was kept in the dark for at least 16 h to form ABST\(^+\) radical, then diluted to 100 mL with 80% ethanol so that the solution had an absorbance value \((\text{Abs}_{\text{ref}})\) of 0.70 ± 0.05 at 734 nm (Perkin Elmer Lambda 2 Spectrophotometer). Various concentrations of compounds (10 \(\mu\)L) were added to ABST\(^+\) solution (200 \(\mu\)L) at ambient temperature to reach a stable absorbance \((\text{Abs}_{\text{detect}})\). The percentage of ABST\(^+\) radical scavenged was calculated using Equation 2.

\[
\text{scavenging ABST}^+ (\%) = \left(1 - \frac{\text{Abs}_{\text{detect}}}{\text{Abs}_{\text{ref}}} \right) \times 100
\]

The percentage of scavenged ABST\(^+\) radical was plotted versus the concentration of antioxidants and the concentration of antioxidant required obtaining 50% inhibition (50% inhibition concentration, i.e., IC\(_{50}\)) was obtained from the graph.

**Acknowledgements**

This work was supported by Key Projects in the National Science & Technology Pillar Program in the Eleventh Five-year Plan Period (2006BAD18B10) and the President Foundation of the Chinese Academy of Forestry (CAFYBB2008009).

**References**

1. Pacher, P.; Beckman, J. S.; Liaudet, L. *Physiol. Rev.* 2007, 87, 315.
2. Karalti, N.; Güzel, Ö.; Özsoy, N.; Özbey, S.; Salman, A. *Eur. J. Med. Chem.* 2010, 45, 1068.
3. Karthikeyan, R.; Manivasagam, T.; Anantharaman, P.; Balasubramanian, T.; Somasundaram, S. T. *J. Appl. Phycol.* 2011, 23, 257.
4. Ratnam, D. V.; Ankola, D. D.; Bhardwaj, V.; Sahana, D. K.; Ravi Kumar, M. N. V. *J. Control Release* 2006, 113, 189.
5. Süzen, S. *Top. Heterocycl. Chem.* 2007, 11, 145.
6. Nordell, P.; Lincoln, P. *J. Am. Chem. Soc.* 2005, 127, 9670.
7. Barraja, P.; Diana, P.; Montalbano, A.; Dattolo, G.; Cirrincione, G.; Viola, G.; Edaldi, D.; Acqua, F. D. *Bioorg. Med. Chem.* 2006, 14, 8712.
8. Chung, H. S.; Woo, W. S. *J. Nat. Prod.* 2001, 64, 1579.
9. Sedik, M.; Poznici, M.; Gehrig, P.; Scott, M. *Mol. Cancer Ther.* 2008, 7, 2121.
10. El-Sayed, O. A.; Al-Turki, T. M.; Al-Daffiri, H. M.; Al-Bassam, B. A.; Hussein, M. E. *Boll. Chim. Farm.* 2004, 143, 227.
11. Gakhari, G.; Ohira, T.; Shi, A.; Hua, D. H.; Nguyen, T. A. Drug Dev. Res. 2009, 69, 526.
12. Chen, Y. L.; Fang, K. C.; Sheu, J. Y.; Hsu, S. L.; Tseng, C. C. J. Med. Chem. 2001, 44, 2374.
13. Roma, G.; Braccio, M. D.; Grossi, G.; Chia, M. Eur. J. Med. Chem. 2000, 35, 1021.
14. Maguire, M. P.; Sheets, K. R.; McVety, K.; Spada, A. P.; Zilberstein, A. J. Med. Chem. 1994, 37, 2129.
15. Heravi, M. M.; Behbahani, F. K.; Oskooie, H. A.; Shoar, R. H. Tetrahedron Lett. 2005, 46, 2775.
16. Borovic, S.; Tirzitis, G.; Tirzite, D.; Cipak, A.; Khoschshorur, G. A.; Waeg, G.; Tatzber, F.; Scukanec-Soljar, M.; Zarkovic, N. Eur. J. Pharmacol. 2006, 537, 12.
17. Murias, M.; Jäger, W.; Handler, N.; Erker, T.; Horvath, Z.; Szekeres, T.; Nohl, H.; Gille, L. Biochem. Pharmacol. 2005, 69, 903.
18. Sun, R.; Lawther, J. M.; Banks, W. B. Ind. Crops Prod. 1995, 4, 241.
19. Wang, Z. J.; Chen, K. F.; Li, J.; Wang, Q. Q.; Guo, J. Clean–Soil, Air, Water 2010, 38, 1074.
20. Ibrahim, M. N. M.; Nadiah, M. Y. N.; Norliyana, M. S.; Sipaut, C. S.; Shuib, S. Clean–Soil, Air, Water 2008, 36, 287.
21. Margarita, S.; Estael, O.; Yamila, V.; Beatriz, P.; Lourdes, M.; Nazario, M.; Margarita, Q.; Carlos, S.; Jose, L. S.; Hector, N.; Norbert, B.; Oswald, M. P. Tetrahedron 1999, 55, 875.
22. Legay, J. C.; Goujon, J. Y.; Eynede, J. J. V.; Toufet, L.; Bazureau, J. P. J. Comb. Chem. 2006, 8, 829.
23. Agarwal, A.; Chauhan, P. M. S. Tetrahedron Lett. 2005, 46, 1345.
24. Ko, S.; Sastry, M. N. V.; Lin, C.; Yao, C. F. Tetrahedron Lett. 2005, 46, 5771.
25. Ko, S. K.; Yao, C. F. Tetrahedron 2006, 62, 7293.
26. Kumar, A.; Maurya, R. A. Tetrahedron 2007, 63, 1946.
27. Kumar, S.; Sharma, P.; Kapoor, K. K.; Hundal, M. S. Tetrahedron 2008, 64, 536.
28. Tamaddon, F.; Razmi, Z.; Jafari, A. A. Tetrahedron Lett. 2010, 51, 1187.
29. Mekheimer, R. A.; Hameed, A. A.; Sadek, K. U. Green Chem. 2008, 10, 592.
30. Pomerai, D. I.; Smith, B.; Dawe, A.; North, K.; Smith, T.; Archer, D. B.; Duce, I. R.; Jones, D.; Candido, E. P. M. FEBS Lett. 2003, 543, 93.
31. Hayes, B. L. Aldrichim. Acta 2004, 37, 66.
32. Abdalla, A. E.; Tirzite, D.; Tirzitis, G.; Roozen, J. P. Food Chem. 1999, 66, 189.
33. Yang, Y.; Song, Z. G.; Liu, Z. Q. Free Radical Res. 2011, 45, 445.
34. Miller, N.; Rice-Evans, C.; Davies, M. J.; Gopinathan, V.; Milner, A. Clin. Sci. 1993, 84, 407.
35. Cos, P.; Rajan, P.; Vedernikova, I.; Calomme, M.; Pieters, L.; Vlietinck, A. J.; Augustyns, K.; Haemers, A.; Berghe, D. V. Free Radical Res. 2002, 36, 711.