Electronic Supplementary Information

Synthesis of chromenoindole derivatives from *Robinia pseudoacacia*

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Experimental section

(1) Isolation of compound 1 from the root of *Robinia pseudoacacia*

Roots of *Robinia pseudoacacia* (typically 3.0 kg per batch) were collected freshly from trees found in the Botanical Garden of Al-Azhar University, Nasr City, Cairo, Egypt. They were cleaned and air dried for 1-2 weeks, and subsequently ground down manually. The powder (usually around 850.0 g) was extracted 5 times with 4 L of 70% ethanol. The ethanolic extracts were combined and the solvent was removed at 40°C under vacuum to dryness. 95.0 g of ethanolic extract were obtained from 3.0 kg of wet root material. The extract was re-dissolved in H₂O and the insoluble material was removed by filtration. The water-soluble fraction (62.0 g) was then washed three times with petroleum ether to remove any fatty acids and subsequently extracted three times with ethyl acetate and then with n-butanol, respectively. The solvent was removed at 40°C under vacuum to yield 5.0 g of ethyl acetate extract and 8.5 g of n-butanol extract. These extracts were dissolved in methanol, combined and applied to silica gel chromatography (CH₂Cl₂:CH₃OH, 80:20) to yield five fractions, of which three contained material in excess of 100.0 mg. These fractions were purified further individually by Sephadex LH-20 chromatography (using methanol as running solvent), ultimately resulting in five chemically pure compounds. The latter were characterized by one- and two-dimensional ¹H- and ¹³C-NMR and mass spectrometry. Ultimately, these compounds were identified as one known dihydroflavone, 7,4’-dihydroxy-dihydroflavone (a compound found in many plants¹), three chalcones, *i.e.* 2’-hydroxy-chalcone, 4,2’,4’-tri hydroxychalcone and 2’-hydroxy-2,3-dimethoxy-chalcone, and the hitherto unknown compound 1.

(2) Chemical synthesis

1-[4-(Benzyloxy)-2-hydroxyphenyl]acetophenone (14):

A solution of 2, 4-dihydroxyacetophenone (3.04 g, 20.0 mmol) and K₂CO₃ (3.44 g, 24.0 mmol) in acetonitrile (100 mL) was heated to reflux. Benzyl chloride (2.5 mL, 22.0 mmol) was added dropwise and the mixture was refluxed for 16 h. After cooling to r.t., H₂O (50 mL) was added and the aqueous layer was extracted with EtOAc (3 × 50 mL). The organic layer was washed with sat. NaHCO₃ (2 × 50 mL) and 1 M HCl (2 × 50 mL) and dried with MgSO₄. After concentration under reduced pressure, the crude product was obtained as light brown solid (4.96 g, 100%).

¹H NMR: (CDCl₃): δ = 12.72 (s, 1 H, OH), 7.64 (dd, J = 8.3, 1.0 Hz, 1 H), 7.43–7.38 (m, 5 H), 6.51 (dd, J = 9.5, 2.5 Hz, 1 H), 6.50 (s, 1 H), 5.09 (s, 2 H), 2.55 (s, 3 H).


1-(4-(Benzyloxy)-2-hydroxyphenyl)-3-(dimethylamino)prop-2-en-1-one (15):

The crude acetophenone 14 (4.96 g, 20.0 mmol) was heated at 90°C for 15 h together with N,N-dimethylformamide dimethyl acetal (5.4 mL, 40.0 mmol). After cooling to r.t., the precipitate was filtered off and washed with EtOAc to afford 15 as yellow solid (4.58 g, 77 %), mp. 141-142°C

MS calcd. for C₁₈H₁₉NO₃ 297.1365 (HR-MS) found 297.1358.

¹H NMR: (CDCl₃): δ = 14.44 (s, 1 H, OH), 7.84 (d, J = 12.2 Hz, 1 H), 7.61 (d, J = 8.9 Hz, 1 H), 7.44–7.33 (m, 5 H), 6.49 (d, J = 2.6 Hz, 1 H), 6.45 (dd, J = 8.9, 2.6 Hz, 1 H), 5.68 (d, J = 12.2 Hz, 1 H), 5.07 (s, 2 H), 3.17 (s, 3 H, NCH₃), 2.95 (s, 3 H, NCH₃).

¹³C NMR: (CDCl₃): δ = 190.6, 165.5, 163.5, 154.0, 136.4, 129.7, 128.6, 128.1, 127.6, 114.1, 106.9, 102.1, 89.85, 69.99.
7-(Benzyloxy)-3-iodo-4\textit{H}-chromen-4-one (16):

To the propenone 15 (3.78 g, 12.7 mmol) in CHCl\textsubscript{3} (50 mL) was added iodine (2.40 g, 17.1 mmol) and the solution was stirred at r.t. for 15 h. The mixture was washed with sat. Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} (50 ml), the aqueous layer was extracted with CH\textsubscript{2}Cl\textsubscript{2} (3 × 50 mL) and dried with MgSO\textsubscript{4}. The crude material was purified by flash chromatography (SiO\textsubscript{2}, n–hexane, EtOAc 1:1) and afforded the product as light yellowish solid (2.31 g, 48 %), mp 121-122°C.

**MS** calcd. for C\textsubscript{16}H\textsubscript{11}IO\textsubscript{3} 377.9753 (HR-MS) found 377.9774

\textsuperscript{1}H NMR: (CDCl\textsubscript{3}): \(\delta= 8.20\ (s,\ 1\ H), 8.15\ (d,\ J = 8.8\ Hz,\ 1\ H), 7.44–7.38\ (m,\ 5\ H), 7.08\ (dd,\ J = 8.8, 2.3\ Hz,\ 1\ H), 6.91\ (d,\ J = 2.3\ Hz,\ 1\ H), 5.16\ (s,\ 2\ H).

\textsuperscript{13}C NMR: (CDCl\textsubscript{3}): \(\delta= 172.6, 163.4, 157.9, 157.2, 135.5, 128.8, 128.5, 128.2, 127.5, 115.8, 101.2, 87.15, 70.67.

7-(Benzyloxy)-3-(4-methoxy-2-nitrophenyl)-4\textit{H}-chromen-4-one (18):

The chromenone 16 (2.31 g, 6.1 mmol) was stirred for 1.5 h with 4-bromo-3-nitroanisole (2.81 g, 12.2 mmol), Cu (3.91 g, 61.0 mmol) and Pd\textsubscript{2}(dba)\textsubscript{3} (271 mg, 0.3 mmol) in DMSO (20 mL) at 70°C. After cooling to r.t., the mixture was filtered through a pad of silica, eluting with EtOAc. After concentration in vacuo the crude product was purified by flash chromatography (SiO\textsubscript{2}, n-hexane, EtOAc, CH\textsubscript{2}Cl\textsubscript{2} 3:1:2) to obtain a light yellowish solid (1.53 g, 62 %), mp 161°C

**MS** calcd. for C\textsubscript{23}H\textsubscript{17}NO\textsubscript{6} 403.1056 (HR-MS) found 403.0934.

\textsuperscript{1}H NMR: (CDCl\textsubscript{3}): \(\delta= 8.16\ (d,\ J = 8.8\ Hz,\ 1\ H), 8.09\ (dd,\ J = 8.1, 1.2\ Hz,\ 1\ H), 7.97\ (s,\ 1\ H), 7.66\ (dt,\ J = 8.1, 1.2\ Hz,\ 1\ H), 7.57\ (dt,\ J = 8.1, 1.5\ Hz,\ 1\ H), 7.45–7.36\ (m,\ 5\ H), 7.07\ (dd,\ J = 9.0, 2.4\ Hz,\ 1\ H), 6.96\ (d,\ J = 2.4\ Hz,\ 1\ H), 5.19\ (s,\ 2\ H), 3.91\ (s,\ 3H, OCH\textsubscript{3}).

\textsuperscript{13}C NMR: (CDCl\textsubscript{3}): \(\delta= 174.3, 163.4, 158.1, 151.7, 149.8, 135.6, 133.2, 132.0, 129.5, 128.8, 128.5, 128.1, 127.5, 126.9, 124.8, 124.5, 117.8, 115.3, 101.4, 70.62, 55.99.
9-Methoxy-6,11-dihydrochromeno[4,3-b]indol-3-ol (2):

A hydrogenation vessel was charged with 7-(benzyloxy)-3-(4-methoxy-2-nitrophenyl)-4H-chromen-4-one (18) (1.44 g, 3.60 mmol), 175 mg 10% Pt/C and 490 mg Pd(OH)$_2$/C in 20 ml THF. The tube was charged 3 times with hydrogen and the mixture was stirred 3 days at r.t. The mixture was filtered through a pad of silica and washed with EtOAc. Removing of the solvent yielded the pure product as light yellow solid (882 mg, 92%).

mp: >300°C (dec.)

MS calcd. for C$_{16}$H$_{13}$NO$_3$ 267.0895 (HR-MS) found 267.0887.

$^1$H NMR: (DMSO-d$_6$): $\delta$ = 11.30 (s, 1 H, NH), 9.53 (s, 1 H, OH), 7.33 (d, $J$ = 8.3 Hz, 1 H), 7.27 (d, $J$ = 8.5 Hz, 1 H), 6.87 (d, $J$ = 2.3 Hz, 1 H), 6.65 (dd, $J$ = 8.5, 2.3 Hz, 1 H), 6.40 (dd, $J$ = 8.3, 2.3 Hz, 1 H), 6.35 (d, $J$ = 2.3 Hz, 1 H), 5.47 (s, 2 H), 3.76 (s, 3 H, OCH$_3$).

$^{13}$C NMR: (DMSO-d$_6$): $\delta$ = 157.8, 155.5, 154.2, 144.9, 137.8, 128.5, 121.6, 118.9, 118.2, 109.7, 108.9, 108.3, 103.6, 102.1, 94.83, 65.15, 55.21.

9-Methoxy-6,6a,11,11a-tetrahydrochromeno[4,3-b]indol-3-ol (1):

To a suspension of the indole 2 (240 mg, 0.90 mmol) in HOAc (6 mL) was added NaBH$_3$CN (63.0 mg, 2.70 mmol) in small portions. After stirring at r.t. for 30 min, the mixture was basified with NH$_4$OH to pH 8 and extracted with CH$_2$Cl$_2$ (3 x 20 mL). The crude product was purified by flash chromatography (SiO$_2$, n-hexane, EtOAc 2:1) to afford the product as light yellow solid (189 mg, 78%), mp: >300°C (dec.)

MS calcd. for C$_{16}$H$_{15}$NO$_3$ 269.1052 (HR-MS) found 269.1082.

$^1$H NMR: (CDCl$_3$): $\delta$ = 7.10 (d, $J$ = 8.3 Hz, 1 H), 7.07 (d, $J$ = 8.0 Hz, 1 H), 6.47 (dd, $J$ = 8.3, 2.5 Hz, 1 H), 6.38 (d, $J$ = 2.5 Hz, 1 H), 6.32 (dd, $J$ = 8.0, 2.3 Hz, 1 H), 6.26 (d, $J$ = 2.3 Hz, 1 H), 4.80 (d, $J$ = 7.5 Hz, 1 H), 4.16–4.12 (m, 1 H), 3.82 (t, $J$ = 10.5 Hz, 1 H), 3.75 (s, 3 H, OCH$_3$), 3.44 (ddd, $J$ = 10.5, 7.5, 4.9 Hz, 1 H).

$^{13}$C NMR: (CDCl$_3$): $\delta$ = 160.7, 156.1, 155.9, 151.6, 130.6, 124.9, 120.6, 115.7, 109.3, 104.3, 103.9, 97.01, 65.78, 56.55, 55.38, 39.41.
Table S1. Correlation of the NMR data for compound 1

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<th>Position</th>
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<th>δ C [ppm]</th>
<th>DEPT</th>
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<td>CH</td>
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<tr>
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<td>6.47, dd, J = 8.3, 2.5</td>
<td>109.3</td>
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<tr>
<td>3</td>
<td>-</td>
<td>160.7</td>
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</tr>
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<td>103.9</td>
<td>CH</td>
</tr>
<tr>
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<td>-</td>
<td>156.1</td>
<td>C</td>
</tr>
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<td>6</td>
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<tr>
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<td>CH</td>
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<tr>
<td>6b</td>
<td>-</td>
<td>120.6</td>
<td>C</td>
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<td>124.9</td>
<td>CH</td>
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<td>8</td>
<td>6.32, dd, J = 8.0, 2.3</td>
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<td>CH</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
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<td>6.26, d, J = 2.3</td>
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<tr>
<td>10a</td>
<td>-</td>
<td>151.6</td>
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<td>3.75, s</td>
<td>55.38</td>
<td>CH₃</td>
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</table>

¹H NMR of compound 1:

![NMR Spectrum](image)

² The spectroscopic data of the synthetic sample are identical with these of the isolated compound (H. Mohammed, PhD thesis, Saarbrücken 2009)

$^1$H NMR of compound 1 (detail):

$^{13}$C NMR of compound 1:
HH COSY of compound 1:

CH COSY of compound 1:
(3) **In vitro activity assays** and activity against *Steinernema feltiae*

(a) Antioxidant activity

The DPPH assay is a standard assay measuring certain aspects of antioxidant activity, such as radical scavenging activity. It has been performed according to literature.\(^4\)

(b) The aromatase inhibition assay is used widely to establish possible pharmaceutically interesting properties associated with suspected phyto-estrogens. This assay has been performed as described previously.\(^5\)

(c) The assay based on the nematode *S. feltiae* is used routinely to screen for possible nematicidal activity. This assay is fairly robust, easy to perform and also indicative of activity against an agriculturally relevant model nematode (*S. feltiae* itself is not an agricultural pest and hence not a direct target). This assay has been performed as described by us in a recent publication.\(^6\)

(d) Cell culture studies

As a distinct cytotoxicity of medicarpin against lymphocytes has been reported,\(^7\) human promyelocytic leukemia (HL-60) cells were initially selected in order to obtain a first indication of biological activity. These cells have been cultured according to standard procedures. Compound 1 was dissolved in DMSO, added to the culture, and cell proliferation/survival was measured at distinct time intervals using the standard full name (MTT) assay.


Once compound 1 and its analogue 2 had been obtained by chemical synthesis, HCT116 cells were used for more detailed studies:

p53-Positive HCT116 cells were maintained at 37°C and 5 % CO₂ in McCoy’s 5A medium (PromoCell, Heidelberg, Germany) with 10 % fetal calf serum (FCS). Organic compounds were dissolved in DMSO to a 100 mM stock solution which was freshly prepared before use.

Evaluation of cell viability:

In order to determine the effect of the organic compounds on HCT116 cells were seeded at 1×10⁴ cells per well to a final volume of 500 µL in a 24-well plate and incubated overnight. Cells were then incubated in various concentrations of the organic compounds for 24 h. Viability of the cells was determined by a colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma) assay according to the manufacturer’s instructions. One hour before the end of treatment, 50 µL MTT (5 mg/mL PBS) were added. The enzymatic reaction took place at 37°C in a humified atmosphere. Following 1 h MTT treatment, medium was disposed off and cells solubilized by adding 500 µL solubilizing solution (0.05% (w/v) SDS in DMSO and 0.01 % acetic acid) to each well and allowing the crystals to completely dissolve. The spectrophotometrical absorbance of the purple–blue formazan dye was determined in an ELISA reader at 595 nm.