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 grinded 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 H_2O 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 (CH_2Cl_2:CH_3OH, 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 ^{1}H- and ^{13}C-NMR and mass spectrometry. Ultimately, these compounds were identified as one known dihydroflavone, 7,4'-dihydroxy-dihydroflavone (a compound found in many plants[11]), three chalcons, *i.e.* 2'-hydroxy-chalcone, 4,2',4'-trihydroxychalcone and 2'-hydroxy-2,3-dimethoxy-chalcone, and the hitherto unknown compound **1**.

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[1] [1]M.J.C. Falcao, Y.B.M. Pouliquem, M.A.S. Lima, N.V. Gramosa, L.V. Costa-Lotufo, G.C.G. Militao, C. Pessoa, M.O. De Moraes, E.R. Silveira, *J. Nat. Prod.* **2005**, 68, 423-426.

(2) Chemical synthesis

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

A solution of 2, 4-dihydroxyacetophenone (3.04 g, 20.0 mmol) and K_2CO_3 (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., H_2O (50 mL) was added and the aqueous layer was extracted with EtOAc (3 × 50 mL). The organic layer was washed with sat. $NaHCO_3$ (2 × 50 mL) and 1 M HCl (2 × 50 mL) and dried with $MgSO_4$. After concentration under reduced pressure, the crude product was obtained as light brown solid (4.96 g, 100 %).

^{1}H NMR: ($CDCl_3$): 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).

^{13}C NMR: ($CDCl_3$): δ= 202.6, 165.2, 135.9, 132.4, 128.7, 128.3, 127.5, 114.1, 108.2, 101.9, 70.22, 26.33.

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 $C_{18}H_{19}NO_3$ 297.1365 (**HR-MS**) found 297.1358.

^{1}H NMR: ($CDCl_3$): 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, NCH_3), 2.95 (s, 3 H, NCH_3).

^{13}C NMR: ($CDCl_3$): 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*H*-chromen-4-one (16):

To the propenone **15** (3.78 g, 12.7 mmol) in $CHCl_3$ (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. $Na_2S_2O_3$ (50 ml), the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL) and dried with $MgSO_4$. The crude material was purified by flash chromatography (*SiO_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 $C_{16}H_{11}IO_3$ 377.9753 (**HR-MS**) found 377.9774

^{1}H NMR: ($CDCl_3$): δ= 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).

^{13}C NMR: ($CDCl_3$): δ= 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*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 $Pd_2(dba)_3$ (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_2*, *n*-hexane, EtOAc, CH_2Cl_2 3:1:2) to obtain a light yellowish solid (1.53 g, 62 %), mp 161°C

MS calcd. for $C_{23}H_{17}NO_6$ 403.1056 (**HR-MS**) found 403.0934.

^{1}H NMR: ($CDCl_3$): δ= 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_3).

^{13}C NMR: ($CDCl_3$): δ= 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): δ= 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): δ= 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 $NaBH_3CN$ (63.0 mg, 2.70 mmol) in small portions. After stirring at r.t. for 30 min, the mixture was basified with NH_4OH to pH 8 and extracted with CH_2Cl_2 (3 × 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.

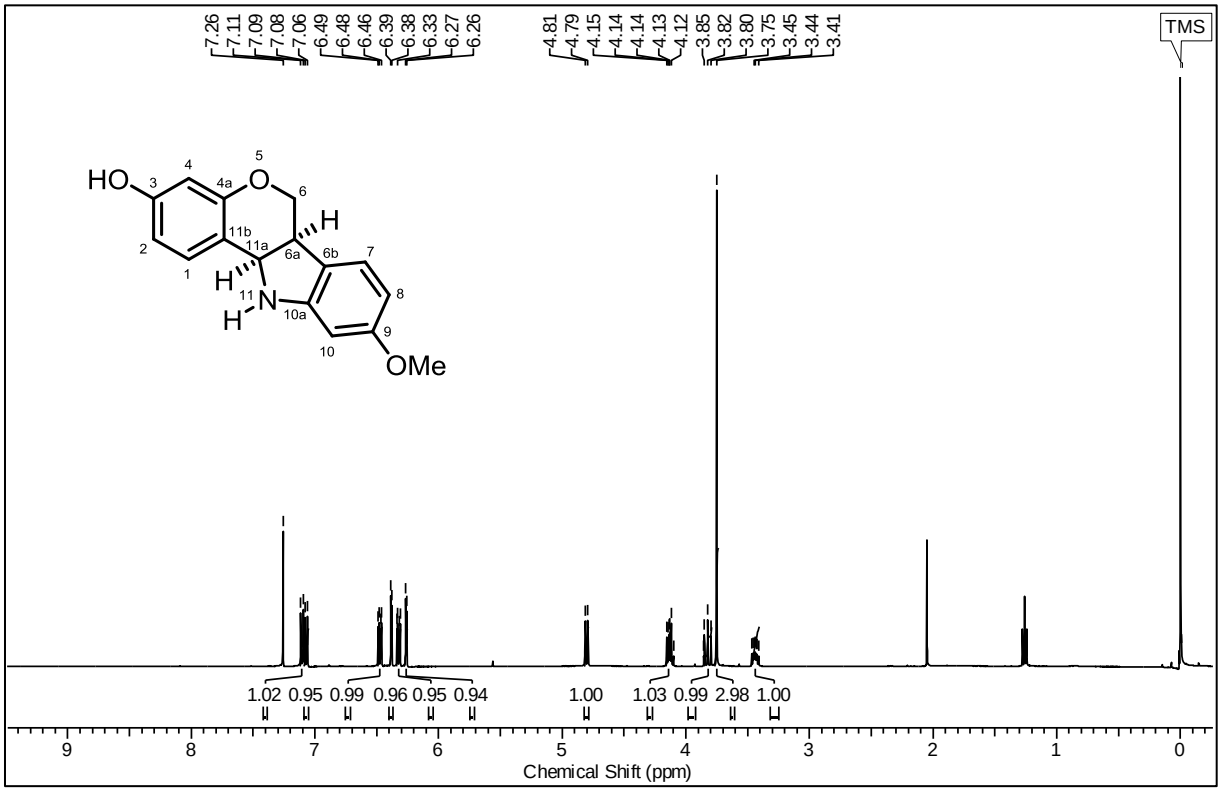
^{1}H NMR: ($CDCl_3$): δ= 7.10 (d, *J* = 8.3 Hz, 1 H), 7.07 (d, *J* = 8.0 Hz, 1 H), 6.47 (dd, *J* = 8.3, 2.5 Hz, 1 H), 6.38 (d, *J* = 2.5 Hz, 1 H), 6.32 (dd, *J* = 8.0, 2.3 Hz, 1 H), 6.26 (d, *J* = 2.3 Hz, 1 H), 4.80 (d, *J* = 7.5 Hz, 1 H), 4.16–4.12 (m, 1 H), 3.82 (t, *J* = 10.5 Hz, 1 H), 3.75 (s, 3 H, OCH_3), 3.44 (ddd, *J* = 10.5, 7.5, 4.9 Hz, 1 H).

^{13}C NMR: ($CDCl_3$): δ= 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**[2]

Position	**δ H** [ppm], ***J*** [Hz]	**δ C** [ppm]	**DEPT**
1	7.10, d, *J* = 8.3	130.6	CH
2	6.47,dd, *J* = 8.3, 2.5	109.3	CH
3	-	160.7	C
4	6.38, d, *J* = 2.5	103.9	CH
4a	-	156.1	C
6	3.82, t, *J* = 10.5, ax-H, 4.16–4.12, m, eq-H	65.78	CH_2
6a	3.44, ddd, *J* = 10.5, 7.5 (*cis*),[3] 4.9	39.41	CH
6b	-	120.6	C
7	7.07, d, *J* = 8.0	124.9	CH
8	6.32,dd, *J* = 8.0, 2.3	104.3	CH
9	-	155.9	C
10	6.26, d, *J* = 2.3	97.01	CH
10a	-	151.6	C
11 (N-H)	-	-	-
11a	4.80, d, *J* = 7.5 (*cis*)[3]	56.55	CH
11b	-	115.7	C
O-CH_3	3.75, s	55.38	CH_3

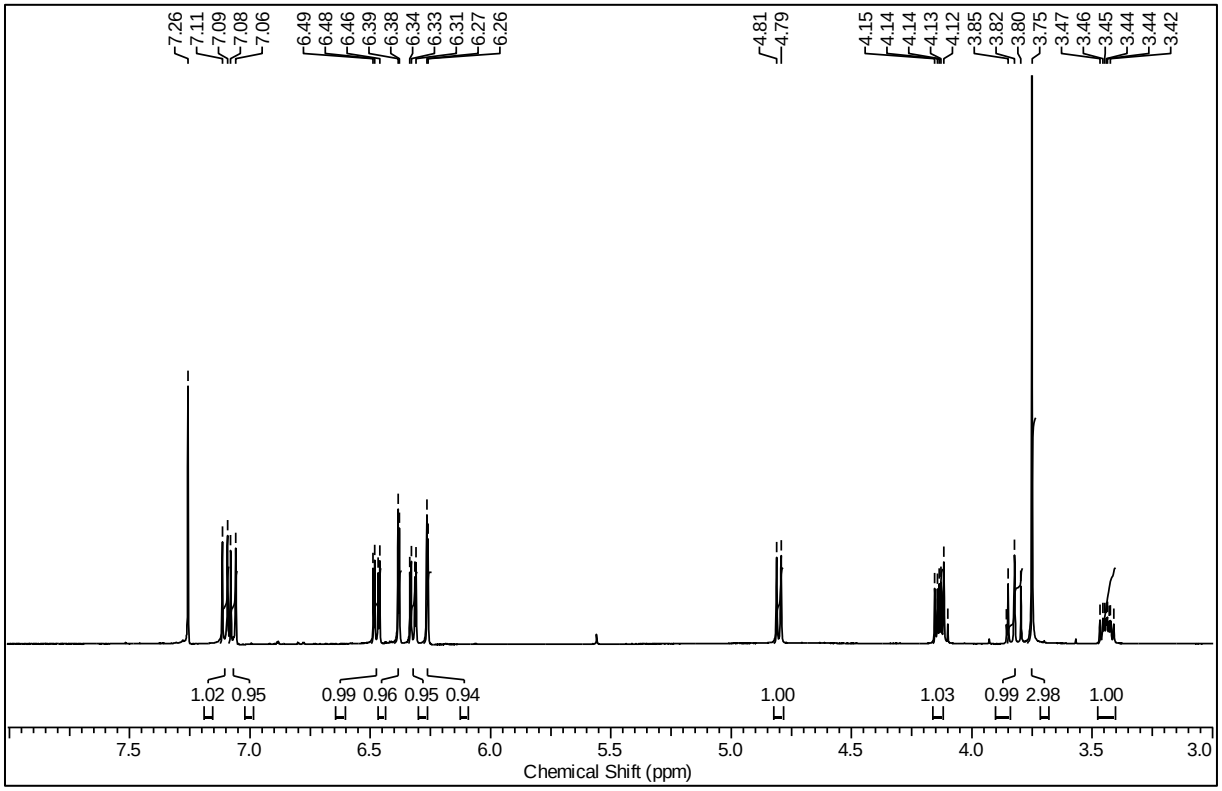
^{1}H NMR of compound **1**:



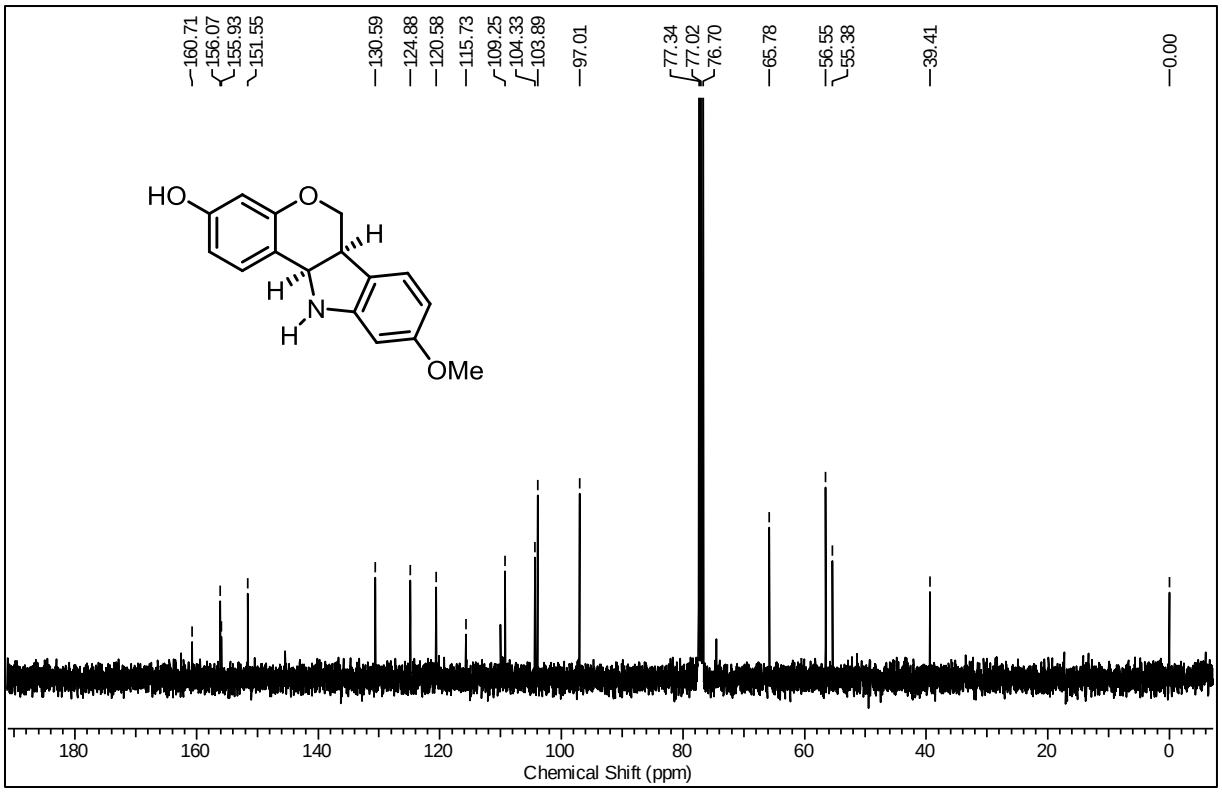
[2] The spectroscopic data of the synthetic sample are identical with these of the isolated compound (H. Mohammed, PhD thesis, Saarbrücken **2009**)

[3] T. G. van Aardt, H. van Rensburg, D. Ferreira, *Tetrahedron* **2001**, *57*, 7113-7126.

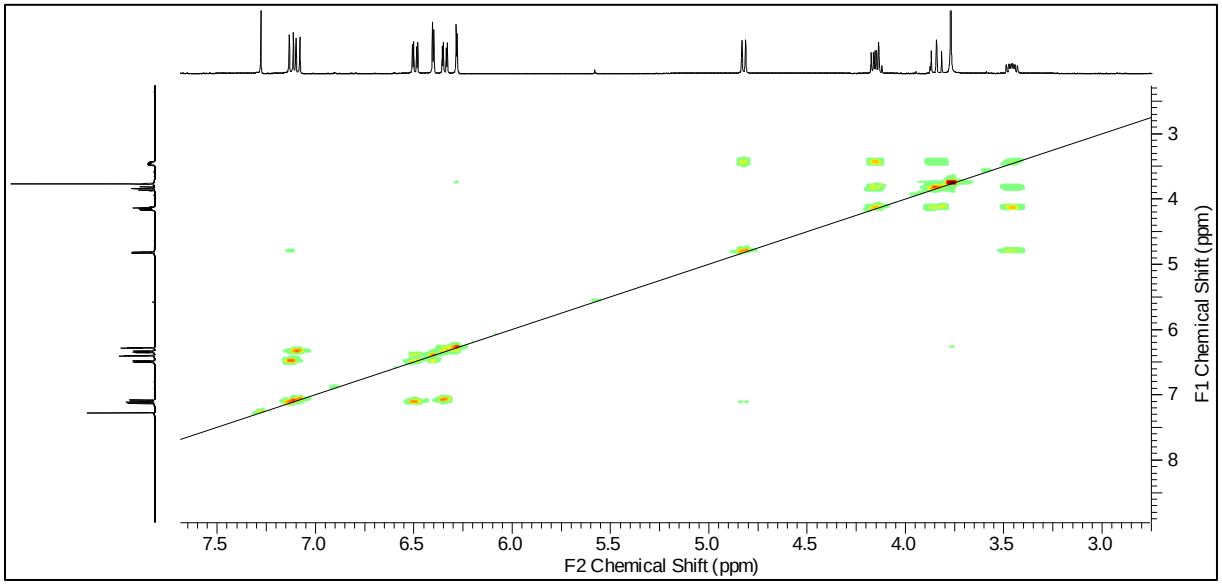
^{1}H NMR of compound **1** (detail):



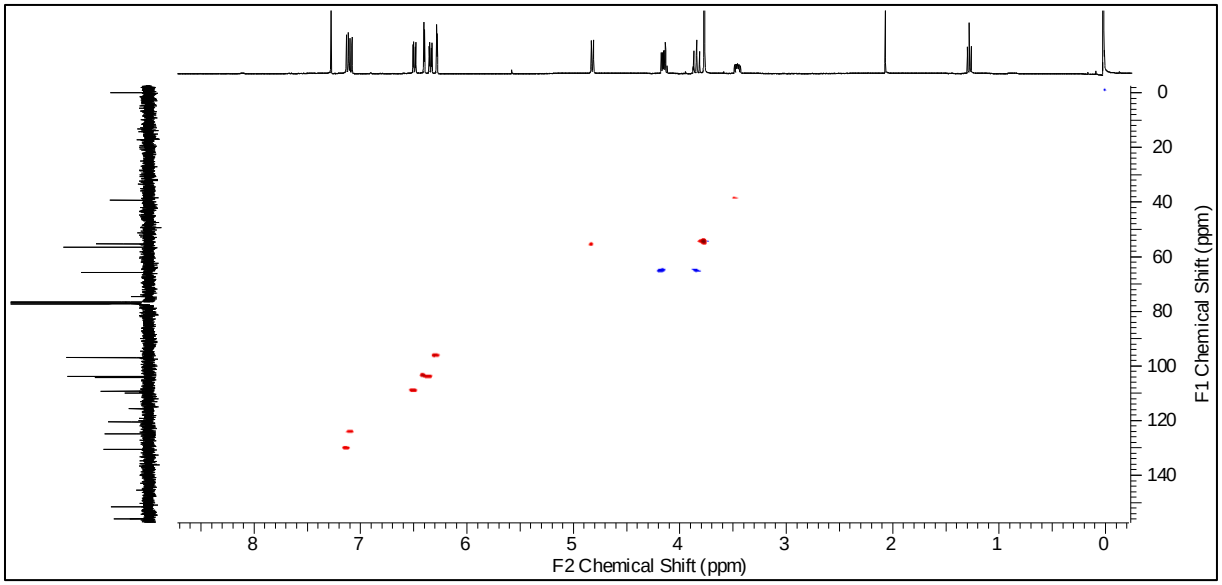
^{13}C NMR of compound **1**:



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.

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[4] M. S. Blois, *Nature* **1958**, *181*, 1199-1200.

[5] E. A. Thompson Jr., P. K. Siiteri, J. Biol. Chem. **1974**, *249*, 5364-5372; R. W. Hartmann, C. Batzl, *J. Med. Chem.* **1986**, *29*, 1362-1369.

[6] B. Czepukojc, U. M. Viswanathan, A. Raza, S. Ali, T. Burkholz, C Jacob, *Phosphorus, Sulfur Silicon Relat. Elem.* **2013**, DOI: 10.1080/10426507.2012.746691.

[7] Z.-L. Liu, S. Tanaka, H. Horigome, T. Hirano, K. Oka, *Biol. Pharm. Bull.* **2002**, *25*, 37-41.

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 % CO_2 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 1×10^4 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.