SUPPLEMENTARY MATERIAL

Cytotoxic New Furoquinoline Alkaloid Isolated from *Ammi majus* L. Growing in Egypt

Magdy M. D. Mohammed^{1,2,*}, Eman R. El-Sharkawy^{3,4}

¹Nucleic Acid Center, Institute of Physics, Chemistry and Pharmacy, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark.

²Pharmacognosy Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, Dokki-12622, Cairo, Egypt.

³Ecology Department, Desert Research Center, Mathef El-Mataria, 15753, Egypt

⁴Department of Chemistry, Science Faculty for Girls, Northern Border University-Arar, North Region, Saudi Arabia.

* Correspondence to: E-mail: melhenawy111@gmail.com

Abstract

Two alkaloids of the furoquinoline-type were isolated from *Ammi majus* L.; a new one and was identified as 4-hydro-7-hydroxy-8-methoxyfuroquinoline (**1**), and the other was isolated for the second time from nature and was identified as 4-hydro-7-hydroxy-8-prenyloxyfuroquinoline (**2**). The structures of the isolated compounds were established and confirmed by 1D and 2D NMR spectroscopy including ¹H-, ¹³C-NMR, COSY, HSQC & HMBC, while the exact masses were confirmed by HRESI/MS. The cytotoxic activity of the isolated compounds (**1** & **2**) was evaluated against HepG-2, PC-3, A-549 and MCF-7 and the obtained results suggested selective antiproliferative and cytotoxic effects, with IC₅₀ = 230.2 and 326.5 μ M against HepG-2 and MCF-7 respectively, for compound (**1**). While, compound (**2**) recorded IC₅₀ = 234.2 μ M against MCF-7.

Keywords: *Ammi majus* L.; Umbelliferae; furoquinoline alkaloids; cytotoxicity; SAR.

S1: Compound (1) ^1H -NMR spectrum
S2: Compound (1) ^{13}C -NMR spectrum
S3: Compound (1) COSY spectrum
S4: Compound (1) DEPT-135 spectrum
S5: Compound (1) HSQC spectrum
S6: Compound (1) HMBC spectrum
S7: Compound (1) HR-ESI/MS spectrum

S8: Compound (2) ^1H -NMR spectrum
S9: Compound (2) ^{13}C -NMR spectrum
S10: Compound (2) COSY spectrum
S11: Compound (2) DEPT-135 spectrum
S12: Compound (2) HSQC spectrum
S13: Compound (2) HMBC spectrum
S14: Compound (2) HR-ESI/MS spectrum

S15: The Cytotoxicity test and the resulted data

Table S16: The ^1H -, ^{13}C -NMR, DEPT, HSQC and HMBC assignment for compounds (1 & 2)

Figure S17: Full Correlations of COSY and HMBC of the furoquinoline alkaloids (1 & 2).

Acquisition Time (sec)	1.9923	Comment	5 mm PABBO BB-1H0 Z-GRD Z1086180050	Date	28 Sep 2012 15:49:52
Date Stamp	28 Sep 2012 15:49:52				
File Name	D:\8- Projects\0- Denmark\Analysis of Samples\Full NMR Data Denmark 2012\mmid 5 mmid 5				
Nucleus	¹ H	Number of Transients	128	Origin	spec1
Owner	nmr	Points Count	16384	Pulse Sequence	zgpg30
SW(cyclical) (Hz)	8223.88	Solvent	CHLOROFORM-d	Spectrum Offset (Hz)	1994.6145
Temperature (degree C)	23.960			Sweep Width (Hz)	8223.18

mmid_5_1H.010.esp

S1: Compound (1)_1H-NMR spectrum

Acquisition Time (sec)	1.3831	Comment	5 mm PABBO BB-1HJD Z-GRD Z106818/0050	Date	16 Oct 2012 12:48:32
Date Stamp	16 Oct 2012 12:48:32				
File Name	D:\8- Projects\0- Denmark\Analysis of Samples\Full NMR Data Denmark 2012\mmid 5 mmid 5 13C\10MId			Frequency (MHz)	100.61
Nucleus	13C	Number of Transients	256	Original Points Count	32768
Owner	nmr	Points Count	32768	Receiver Gain	203.00
SW(cyclical) (Hz)	24,038.46	Solvent	CHLOROFORM-d	Sweep Width (Hz)	24037.73
Temperature (degree C)	25.060				

mmid_5_13C.010.esp

mmid_5_COSY_010.001.2tr.asp

S3: Compound (1)_COSY spectrum

Acquisition Time (sec)	1.3831	Comment	5 mm PABBO BB-1H/D Z-GRD Z100618/0050	Date	18 Oct 2012 11:03:28
Date Stamp	18 Oct 2012 11:03:28				
File Name	D:\2. Publications\Full Professor Publications\10- Animi melius\NMR Data\mmd_5 DEPT-135\mmd_5 DEPT-135_0100000hd				
Frequency (MHz)	100.62	Nucleus	13C	Number of Transients	256
Original Points Count	32768	Owner	nmr	Points Count	32768
Receiver Gain	203.00	SW (cycles) (Hz)	24036.46	Solvent	CHLOROFORM-d
Sweep Width (Hz)	24037.73	Temperature (degree C)	24.650	Pulse Sequence	deg135
				Spectrum Offset (Hz)	100514932

mmd_5_DEPT-135_0100000hd

S4: Compound (1)_DEPT-135 spectrum

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name D:\Data\PKH\AAO 904_RA1_01_12833.d
Method standard pos low.m
Sample Name AAO 904
Comment

Acquisition Date 12/7/2012 12:16:25 PM

Operator BDAL@DE
Instrument / Ser# microTOF-Q II 10205

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Not active	Set Capillary	4200 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	300.0 Vpp	Set Divert Valve	Source

S7: Compound (1)_HR-ESI/MS spectrum

Acquisition Time (sec)	1.9923	Comment	5 mm PABBO BB-1H-D Z-GRD Z108618/0050	Date	28 Sep 2012 13:17:52
Date Stamp	28 Sep 2012 13:17:52				
File Name	D:\8- Fellowships\The Fellowship at Denmark 2012\Analysis of Samples\Maody new\ao_901\ao_1H_901_010000f1d				
Frequency (MHz)	400.12	Nucleus	1H	Origin	spec1
Original Points Count	16384	Owner	nmr	Points Count	2030
Receiver Gain	101.00	SW (cycles) (Hz)	8223.68	Pulse Sequence	zgpg30
Sweep Width (Hz)	8223.18	Temperature (degree C)	24.080	Solvent	CHLOROFORM-d
				Spectrum Offset (Hz)	1994.1124

ao_1H_901_010000f1d.esp

S8: Compound (2) ¹H-NMR spectrum

Acquisition Time (sec)	1.3831	Comment	5 mm PABBO BB-1HVD Z-GRD Z108818/0050	Date	15 Oct 2012 08:29:52
Date Stamp	15 Oct 2012 08:29:52				
File Name	D:\8- Fellowships\The Fellowship at Denmark 2012\Analysis of Samples\Maody new\mmd_2nmmd_2_13C_01000001d				
Frequency (MHz)	100.62	Nucleus	13C	Origin	spect
Original Points Count	32768	Owner	nmr	Points Count	256
Receiver Gain	203.00	SW/cycle(s) (Hz)	24038.46	Pulse Sequence	zgpg30
Sweep Width (Hz)	24037.73	Temperature (degree C)	25.280	Spectrum Offset (Hz)	10056.0225

mmd_2_13C_01000001d.esp

S9: Compound (2)_13C-NMR spectrum

Acquisition Time (sec)	1.3831	Comment	5 mm PABBO BB-1H/D Z-GRD Z1088180050	Date	15 Oct 2012 08:44:48
Date Stamp	15 Oct 2012 08:44:48				
File Name	D:\2- Publications\Full Professor Publications\10- Animi melius\NMR Data\mmd_2 DEPT\mmd_2 DEPT_01000001d				
Frequency (MHz)	100.62	Nucleus	¹³ C	Number of Transients	256
Original Points Count	32768	Owner	nmf	Points Count	32768
Receiver Gain	203.00	SW(cyclical) (Hz)	24038.48	Solvent	CHLOROFORM-d
Sweep Width (Hz)	24037.73	Temperature (degree C)	24.760	Pulse Sequence	dept135
				Spectrum Offset (Hz)	10056.0894

mmd_2_DEPT_01000001d.esp

S11: Compound (2)_DEPT-135 spectrum

Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name D:\Data\PKH\AAO 901_RA4_01_12701.d
Method standard pos low.m
Sample Name AAO 901
Comment

Acquisition Date 12/3/2012 3:42:01 PM

Operator BDAL@DE
Instrument / Ser# micrOTOF-Q II 10205

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.3 Bar
Focus	Not active	Set Capillary	4200 V	Set Dry Heater	180 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set Collision Cell RF	300.0 Vpp	Set Divert Valve	Source

S14: Compound (2)_HR-ESI/MS spectrum

S15: The cytotoxic test and the resulted data.

Samples	HepG-2	PC-3	A549	MCF-7
	IC ₅₀ μ M			
Compound (1)	230.2	-	-	326.5
Compound (2)	-	-	-	234.5
Adriamycin	37.8	41.1	48.8	45.02

LC₅₀: Lethal concentration of the sample which causes the death of 50 % of cells in 48 hrs.

HepG-2: Human hepatocellular carcinoma cell line

PC-3: human prostate cell line

A549: Lung carcinoma cell line

MCF-7: Human Caucasian breast adenocarcinoma

1- Cytotoxic effect on human cell lines.

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan (Mosmann,1983).

Procedure:

All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in RPMI 1640 medium [(for HePG2 and MCF7 – DMEM for A549 and PC3)], 1% antibiotic-antimycotic mixture (10,000U/ml Potassium Penicillin, 10,000 μ g/ml Streptomycin Sulfate and 25 μ g/ml Amphotericin B) and 1% L-glutamine at 37 °C under 5% CO₂.

Cells were batch cultured for 10 days, then seeded at concentration of 10×10^3 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37 °C for 24 h under 5% CO₂ using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of sample to give a final concentration of (100 – 50 – 25 – 12.5 – 6.25 – 3.125 – 0.78 and 1.56 μ g/ml). After 48 h of incubation, medium was aspirated, 40 μ l MTT salt (2.5 μ g/ml) were added to each well and incubated for further four hours at 37°C under 5% CO₂. To stop the reaction and dissolving the formed crystals, 200 μ L of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37°C. A positive control which composed of 400 μ M was used as a known cytotoxic natural agent who gives 100% lethality under the same conditions (Thabrew, *et al.*, 1997, Menshawi *et al.*, 2010).

The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595nm and a reference

wavelength of 620nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula:

$$((\text{Reading of extract} / \text{Reading of negative control}) - 1) \times 100$$

A probit analysis was carried for IC₅₀ determination using SPSS 11 program.

References:

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Table S16: The ^1H -, ^{13}C -NMR, DEPT, HSQC and HMBC assignment for compounds (1 & 2)

No.	Compound 1				Compound 2			
	^1H	^{13}C	DEPT & HSQC	HMBC H $\rightarrow$ C	^1H	^{13}C	DEPT & HSQC	HMBC H $\rightarrow$ C
2	7.67 (<i>d</i> , <i>J</i> = 2.0 Hz)	146.57	CH	C-3, C-3a	7.69 (<i>d</i> , <i>J</i> = 2.4 Hz)	146.57	CH	C-3, C-3a
3	6.80 (<i>d</i> , <i>J</i> = 2.0 Hz)	106.66	CH	C-2, C-4, C-3a	6.81 (<i>d</i> , <i>J</i> = 2.4 Hz)	106.67	CH	C-2, C-4, C-3a
3a		126.07	C			125.83	C	
4	7.33 (<i>s</i>)	112.86	CH	C-3, C-3a, C-5, C-4a, C-8a	7.36 (<i>s</i>)	113.12	CH	C-3, C-3a, C-5, C-4a, C-8a
4a		116.37	C			116.44	C	
5	7.75 (<i>d</i> , <i>J</i> = 9.6 Hz)	144.34	CH	C-4, C-4a, C-6, C-7, C-8a	7.76 (<i>d</i> , <i>J</i> = 9.6 Hz)	144.22	CH	C-4, C-4a, C-6, C-7, C-8a
6	6.34 (<i>d</i> , <i>J</i> = 9.6 Hz)	114.56	CH	C-4a, C-5, C-7	6.36 (<i>d</i> , <i>J</i> = 9.6 Hz)	114.63	CH	C-4a, C-5, C-7
7		139.40	C			143.76	C	
8		145.04	C			148.56	C	
8a		132.64	C			131.61	C	
9a		160.46	C			160.52	C	
OCH ₃	4.27 (<i>s</i>)	61.21	CH ₃	C-8				
1'					5.00 (<i>d</i> , <i>J</i> = 6.8 Hz)	70.12	CH ₂	C-8, C-2', C-3'
2'					5.60 (<i>t</i> , <i>J</i> = 6.8 Hz)	119.72	CH	C-4', C-5'
3'						139.70	C	
4'					1.71 (brs)	25.77	CH ₃	C-3', C-5'
5'					1.74 (brs)	18.08	CH ₃	C-3', C-4'

Figure S17: Full Correlations of COSY and HMBC of the furoquinoline alkaloids (1 & 2).