

SUPPLEMENTARY MATERIAL

Unguiculins A-C: cytotoxic bis-guanidine alkaloids from the French Polynesian sponge, *Monanchora* n. sp.

Amr El-Demerdash ^{a,b}, Céline Moriou ^a, Marie-Thérèse Martin ^a, Sylvain Petek ^c, Cécile Debitus ^{c*} and Ali Al-Mourabit ^a

^a*Institut de Chimie des Substances Naturelles, CNRS UPR 2301, Univ. Paris-Sud, Université Paris-Saclay, 1, av. de la Terrasse, 91198 Gif-sur-Yvette, France*

^b*Organic Chemistry Division, Chemistry Department, Faculty of Science, Mansoura University, Mansoura, 35516-Egypt.*

^c*LEMAR, IRD, IFREMER, CNRS, Univ. Bretagne Occidentale, IUEM, rue Dumont d'Urville, 29280 Plouzané, France*

Abstract: Two new acyclic bis-guanidine alkaloids, unguiculins B-C (**2-3**), were isolated from a French Polynesian sponge *Monanchora* n. sp. together with the known compound unguiculin A (**1**). Their structures were established by spectroscopic data interpretation and comparison with the literature. Unguiculins A-C displayed antiproliferative and cytotoxic efficacy against several human cancer cells with IC₅₀ values in the micromolar range.

Keywords: *Monanchora*, Crambeidae, unguiculin, guanidine, spermidine alkaloid

Spectral data (UV and IR) of unguiculin A	3
Figure S1: ¹H-NMR of unguiculin A (1) in CD₃OD, 500 MHz	3
Figure S2: ¹³C-NMR of unguiculin A (1) in CD₃OD, 125 MHz	3
Figure S3: COSY of unguiculin A (1) in CD₃OD, 500 MHz	4
Figure S4: HSQC of unguiculin A (1) in CD₃OD, 500 MHz	4
Figure S5: HMBC of unguiculin A (1) in CD₃OD, 500 MHz	5
Figure S6: NOESY of unguiculin A (1) in CD₃OD, 500 MHz	5
Figure S7: HRESIMS+ of unguiculin A (1)	6
Figure S8: ¹H-NMR of unguiculin B (2) in CD₃OD, 500 MHz	6
Figure S9: ¹³C-NMR of unguiculin B (2) in CD₃OD, 125 MHz	7
Figure S12: HMBC of unguiculin B (2) in CD₃OD, 500 MHz	8
Figure S13: NOESY of unguiculin B (2) in CD₃OD, 500 MHz	9
Figure S14: General key COSY and HMBC for unguiculin B	9
Figure S15: HRESIMS+ of unguiculin B (2)	10
Table S1: 1 and 2D NMR data for unguiculin B (2)	10
Figure S16: ¹H-NMR of unguiculin C (3) in CD₃OD, 500 MHz	11
Figure S17: ¹³C-NMR of unguiculin C (3) in CD₃OD, 125 MHz	11
Figure S18: COSY of unguiculin C (3) in CD₃OD, 500 MHz	12
Figure S19: HMBC of unguiculin C (3) in CD₃OD, 500 MHz	13
Figure S20: General key COSY and HMBC for unguiculin C (3)	13
Figure S21: HRESIMS+ of unguiculin C (3)	14
Table S2: 1 and 2D NMR data for unguiculin C (3)	14
Table S3: comparison of ¹H NMR & ¹³C NMR (500 and 125 MHz) data for unguiculin B-C (2-3) in CD₃OD.	15
Figure S21: ¹H-NMR comparison between unguiculin A-C (1-3), 500 MHz in CD₃OD	16

Spectral data (UV and IR) of unguiculine A

UV (MeOH) λ_{\max} (log ϵ) 208.2 (0.85).

IR (film) ν_{\max} 3352, 3171, 2923, 2853, 1666, 1605, 1465, 1375, 1201, 1177, 1132, 1020, 834, 800, 720 cm^{-1} .

Figure S1: $^1\text{H-NMR}$ of unguiculin A (**1**) in CD_3OD , 500 MHz

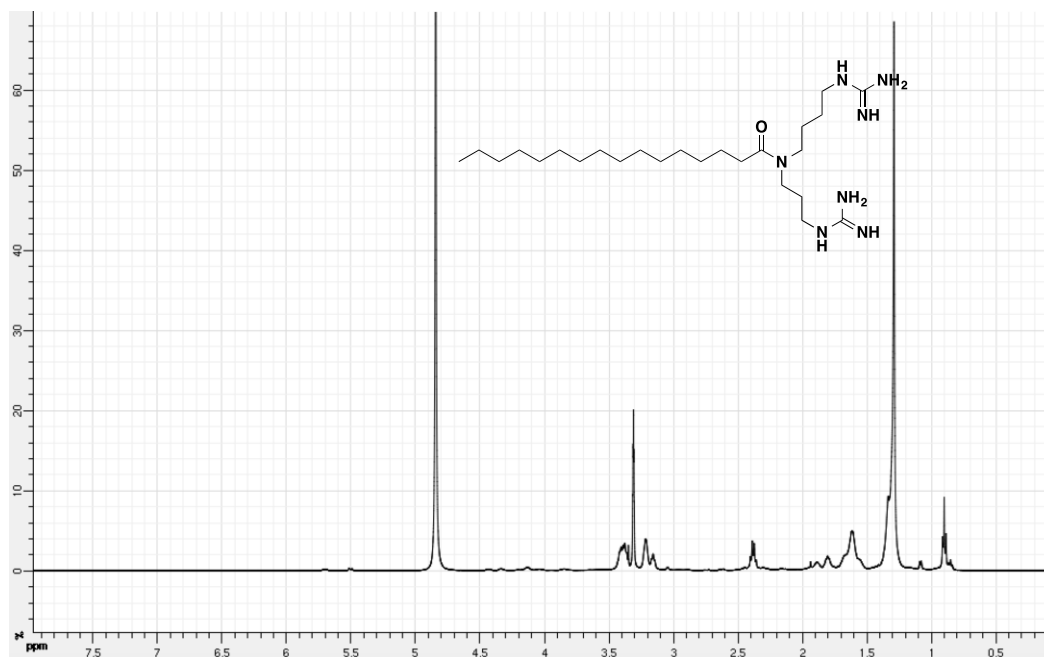


Figure S2: $^{13}\text{C-NMR}$ of unguiculin A (**1**) in CD_3OD , 125 MHz

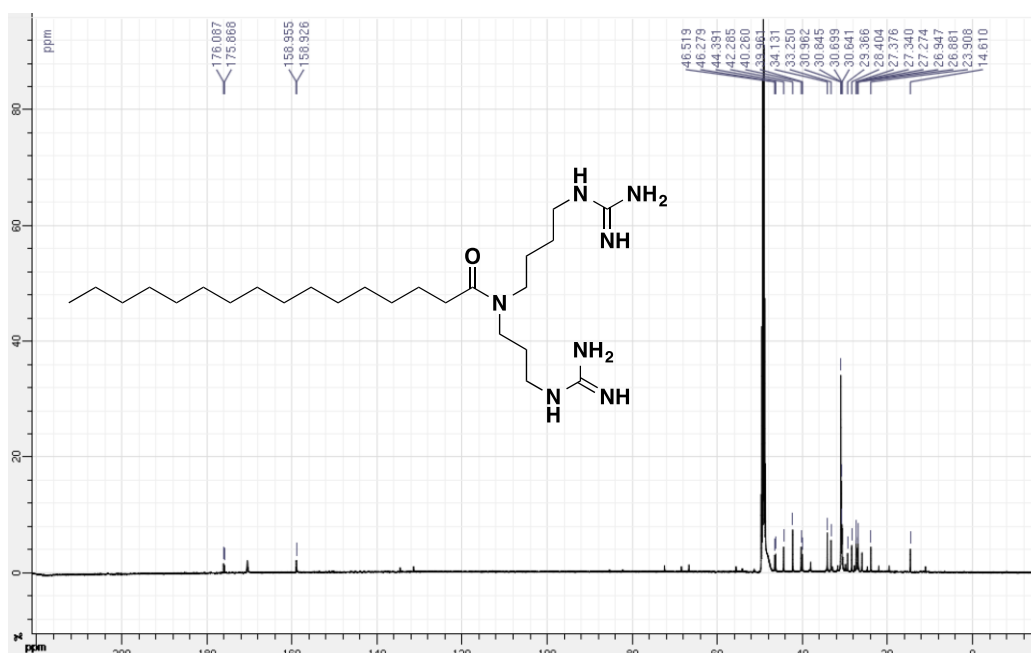


Figure S3: COSY of unguiculin A (1) in CD₃OD, 500 MHz

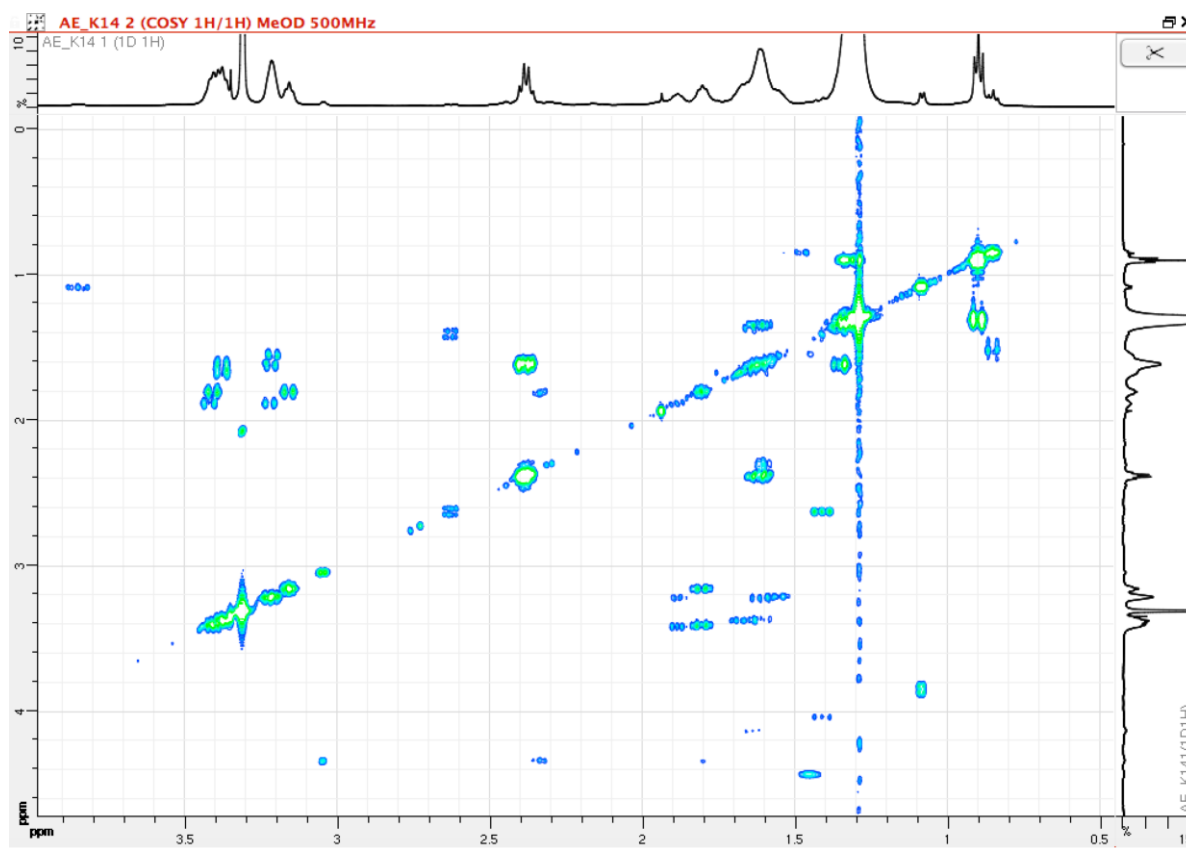


Figure S4: HSQC of unguiculin A (1) in CD₃OD, 500 MHz

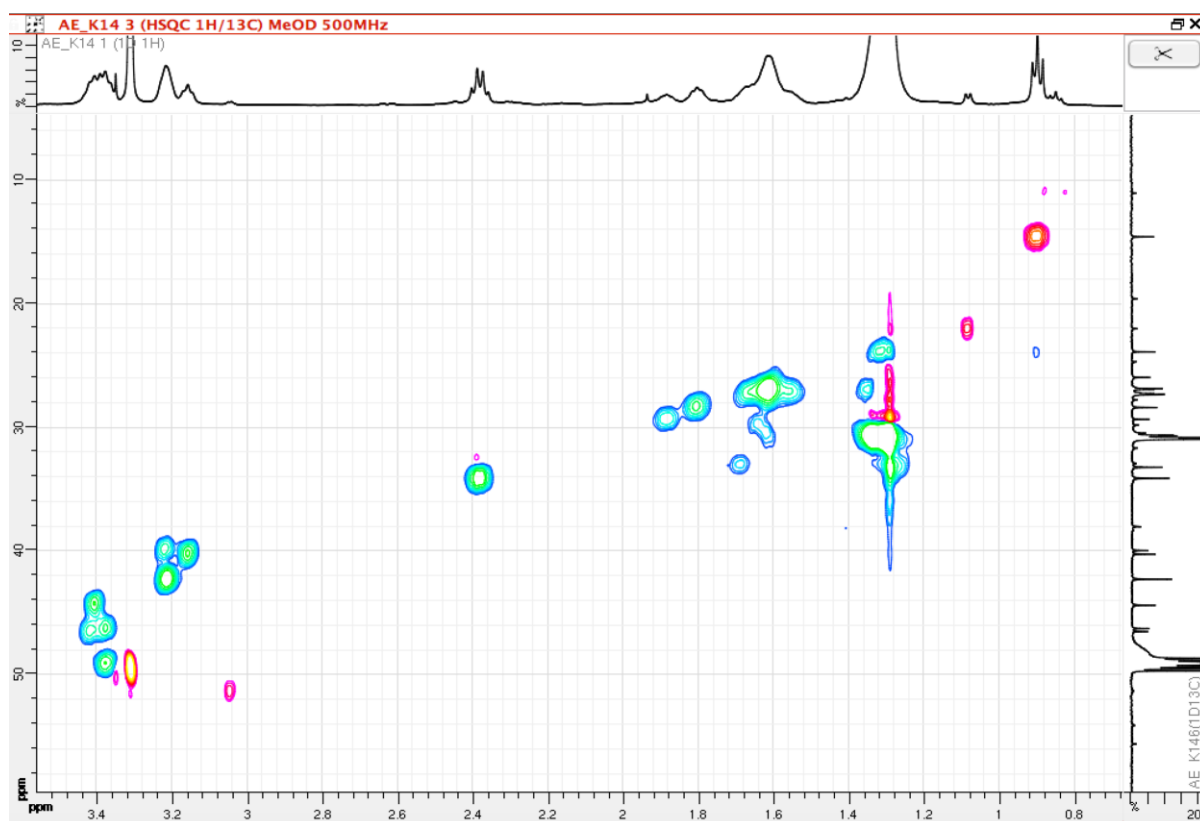


Figure S5: HMBC of unguiculin A (**1**) in CD₃OD, 500 MHz

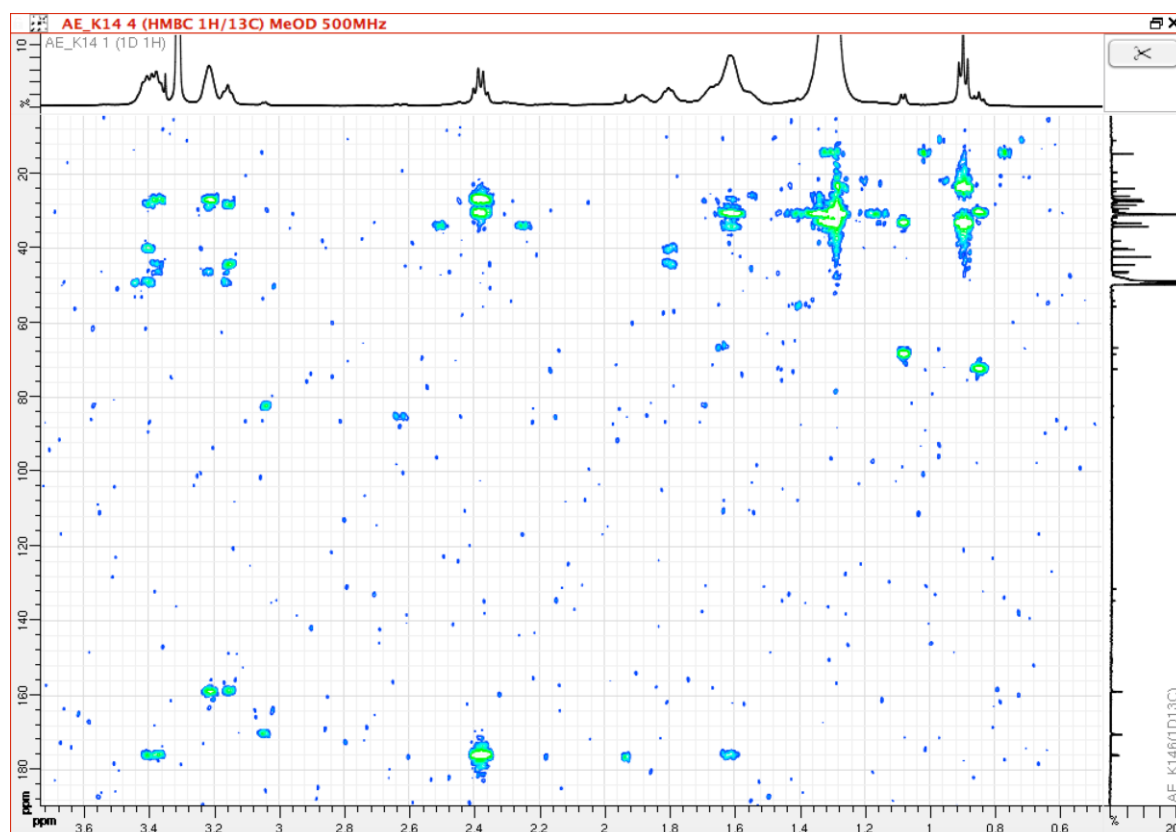


Figure S6: NOESY of unguiculin A (**1**) in CD₃OD, 500 MHz

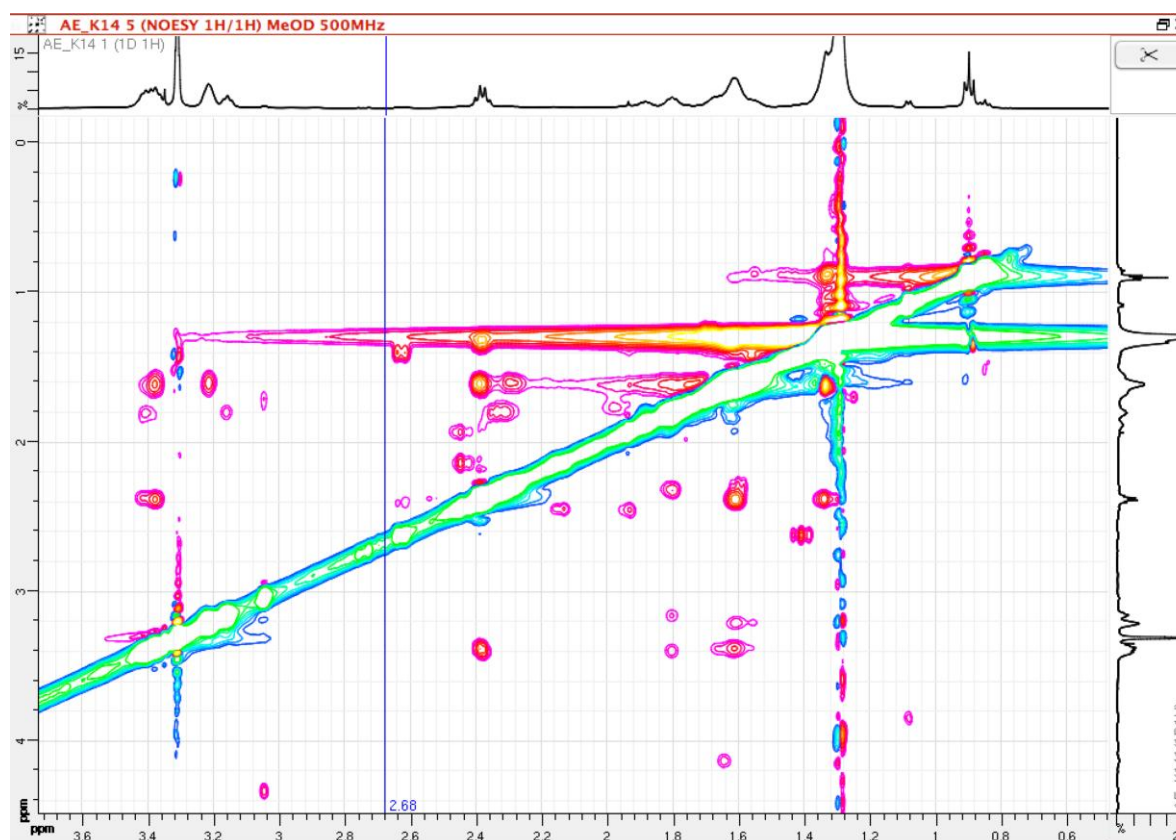


Figure S7: HRESIMS+ of unguiculin A (1)

Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions

188 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)

Elements Used:

C: 0-100 H: 0-100 N: 0-7 O: 0-3

31-Jan-2013 6:9:6

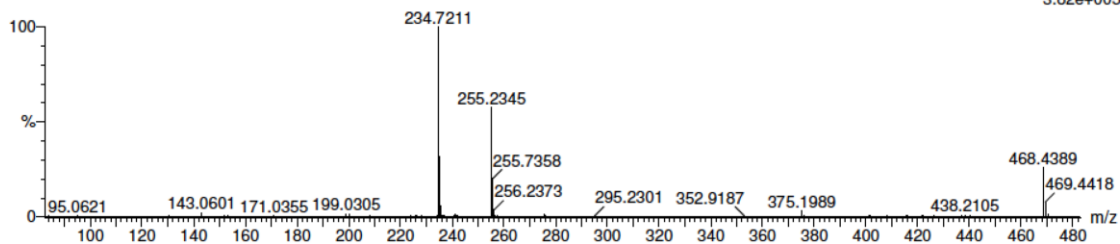
ALMOURABIT_ahmed45-1 491 (2.327) Cm (476:513)

AE_K14

LCT Premier XE KE483

1: TOF MS ES+

3.82e+005



Minimum:

Maximum: 5.0 5.0 -1.5 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
------	------------	-----	-----	-----	-------	--------------	---------

468.4389	468.4390	-0.1	-0.2	2.5	1471.6	0.0	C25 H54 N7 O
----------	----------	------	------	-----	--------	-----	--------------

Figure S8: ¹H-NMR of unguiculin B (2) in CD₃OD, 500 MHz

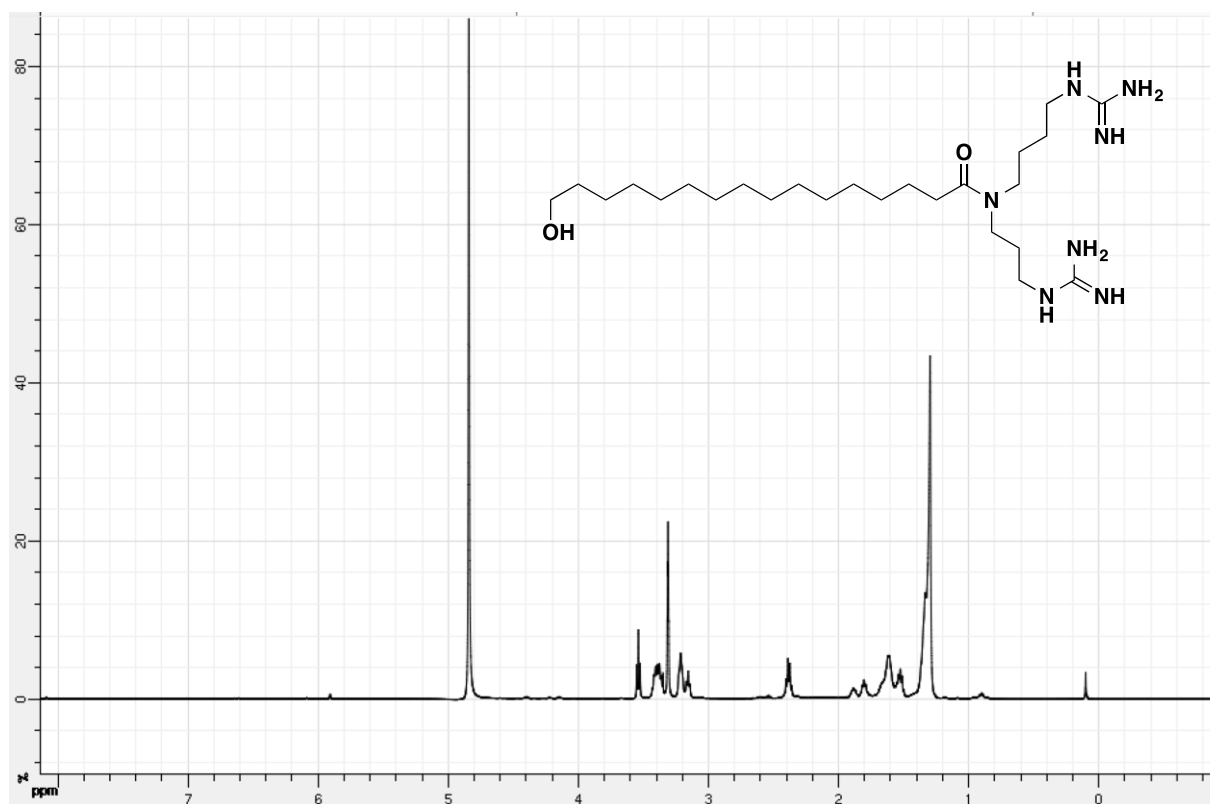


Figure S9: ^{13}C -NMR of unguiculin B (**2**) in CD_3OD , 125 MHz

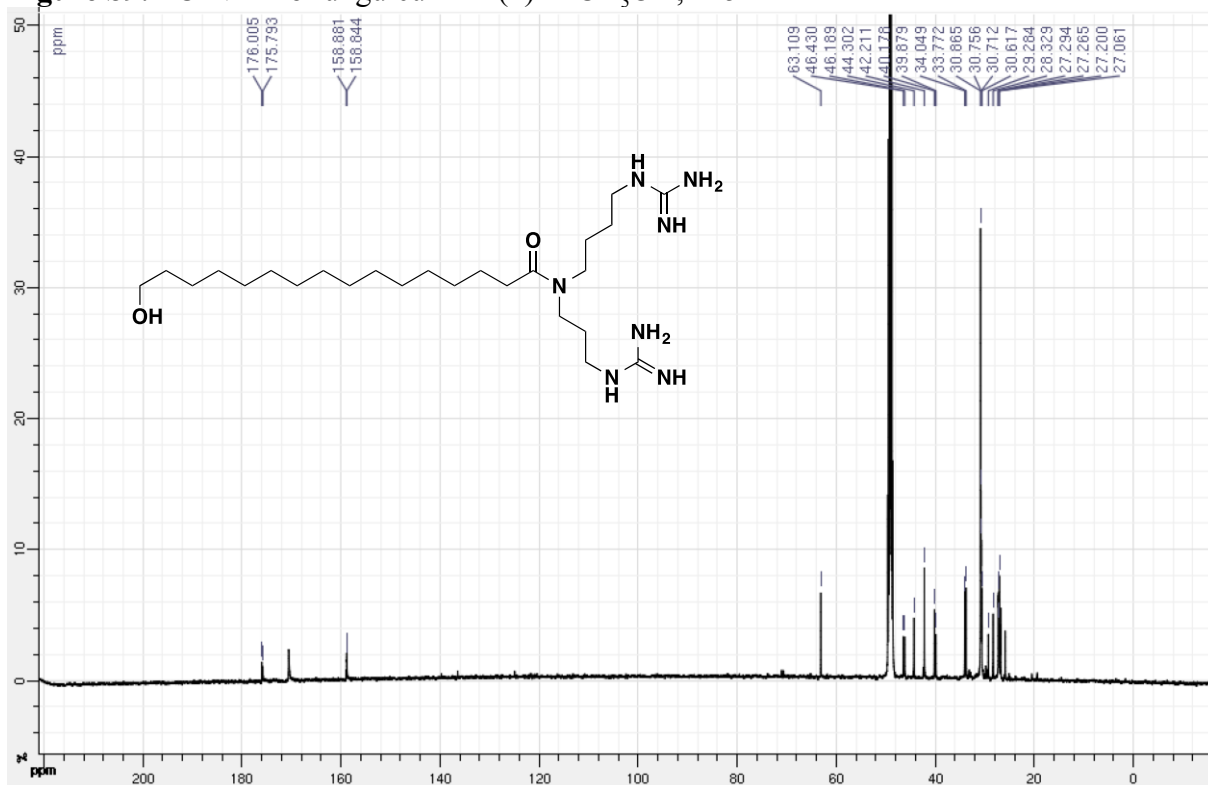


Figure S10: COSY of unguiculin B (**2**) in CD_3OD , 500 MHz

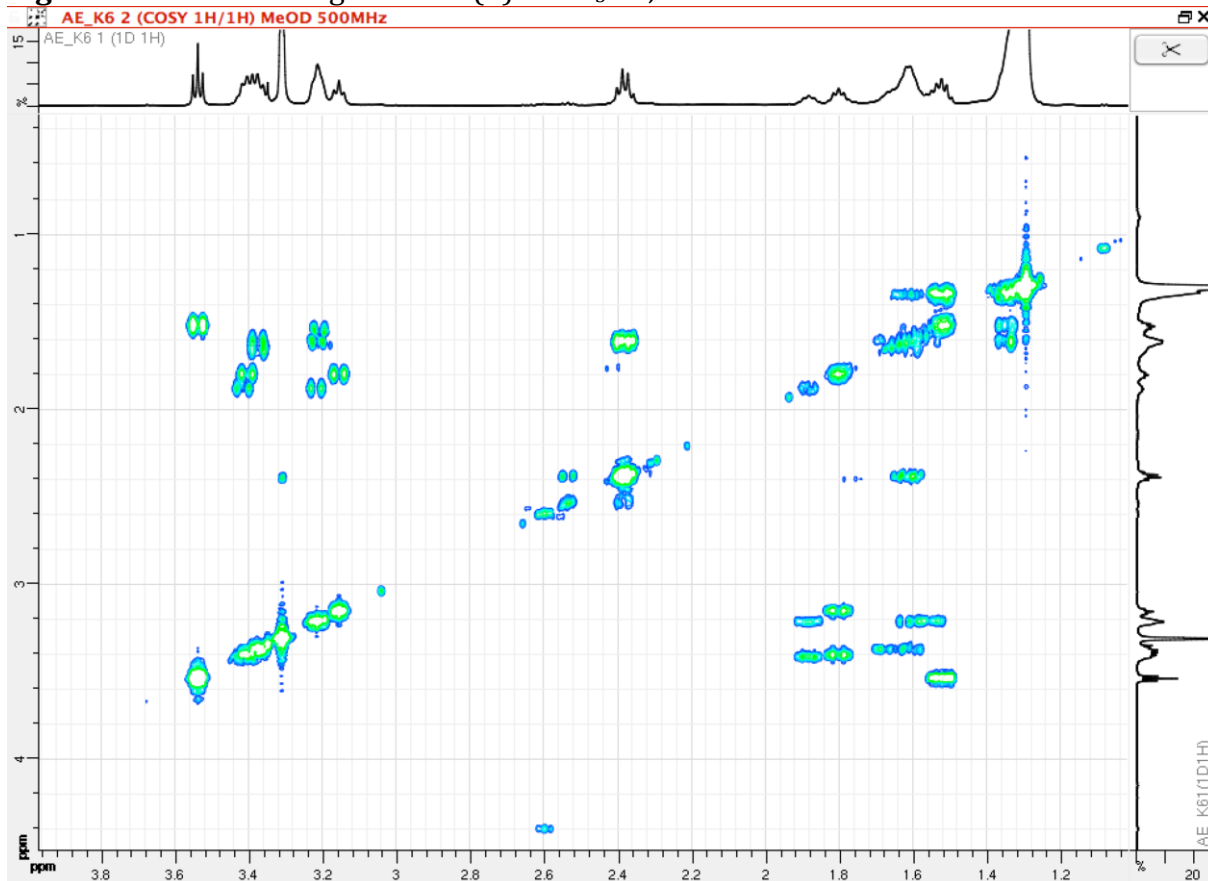


Figure S11: HSQC of unguiculin B (2) in CD₃OD, 500 MHz



Figure S12: HMBC of unguiculin B (2) in CD₃OD, 500 MHz

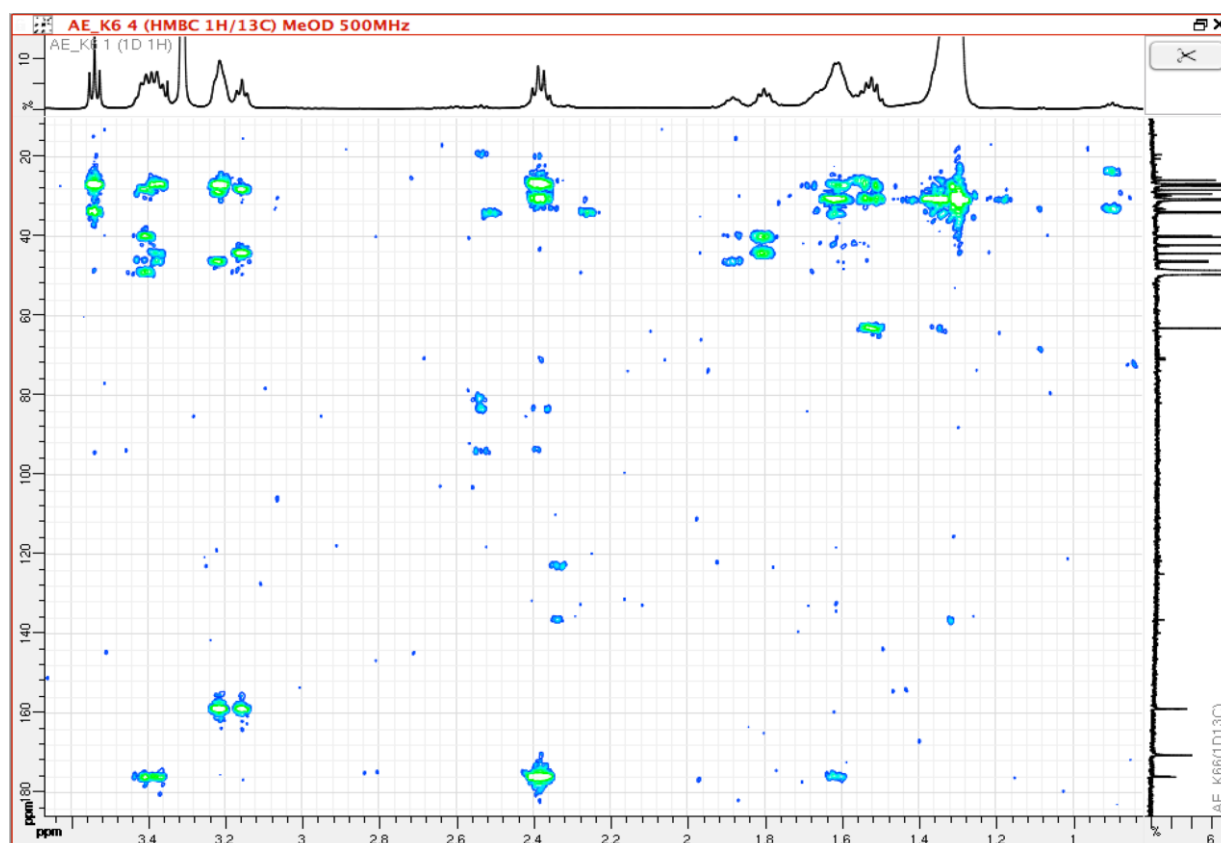


Figure S13: NOESY of unguiculin B (**2**) in CD₃OD, 500 MHz

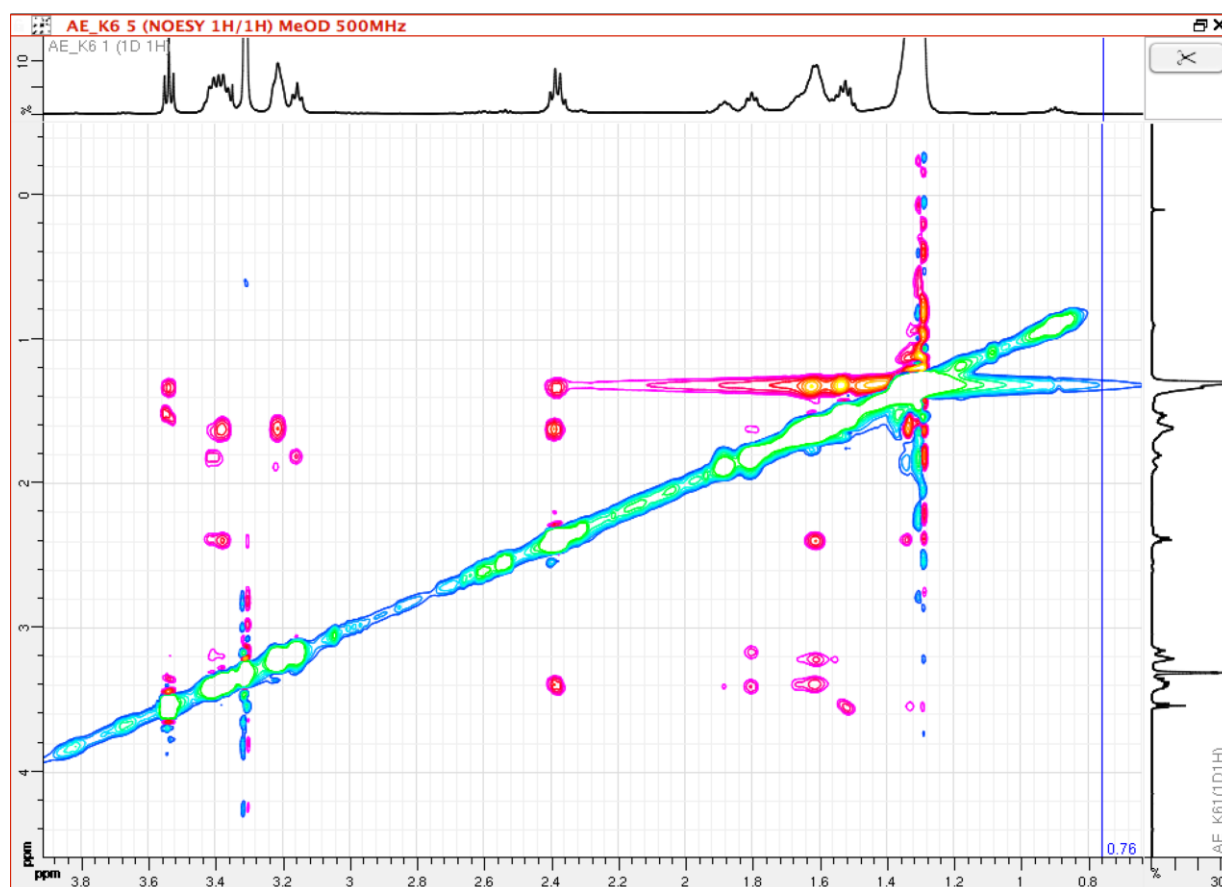


Figure S14: General key COSY and HMBC for unguiculin B

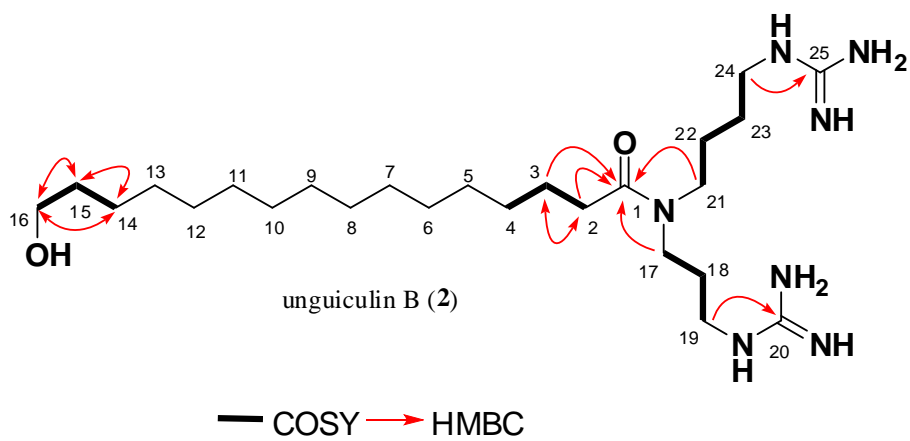


Figure S15: HRESIMS+ of unguiculin B (2)

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Odd and Even Electron Ions

193 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)

Elements Used:

C: 0-100 H: 0-100 N: 0-7 O: 0-3

31-Jan-2013 6:6:6

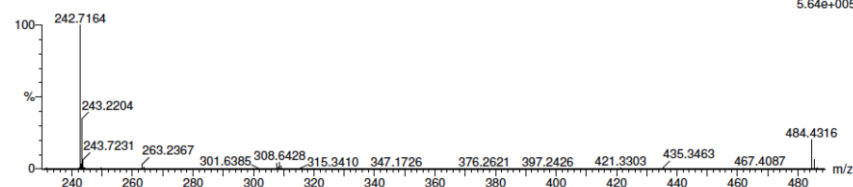
ALMOURABIT_ahmed43-1 363 (1.758) Cm (354:375)

AE_K6

LCT Premier XE KE483

1: TOF MS ES+

5.64e+005



Minimum: -1.5
Maximum: 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
484.4316	484.4339	-2.3	-4.7	2.5	834.5	0.0	C25 H54 N7 O2

Table S1: 1 and 2D NMR data for unguiculin B (2)

Position	$\bar{\delta}_c$, Type	$\bar{\delta}_H$ (J in Hz)	(^1H - ^1H) COSY	(^1H - ^{13}C) HMBC
1	175.8/176.0, C	-	-	-
2	34.0, CH ₂	2.38, m	3,4	1, 3
3	27.0, CH ₂	1.61, m	2	1, 2
4-12	30.6-30.9, CH ₂	1.30-1.33, m	-	-
13	30.6-30.9, CH ₂	1.30-1.33, m	14, 15, 16	-
14	27.1, CH ₂	1.34, m	13, 15, 16	15, 16
15	33.8, CH ₂	1.53, m	13, 14, 16	14, 16
16	63.1, CH ₂	3.54, t, 7	13, 14, 15	14, 15
17	44.3, CH ₂ (a)	3.40, m (a)	18, 19	1, 18, 19, 21
	46.4 CH ₂ (b)	3.41, m (b)		
18	28.3, CH ₂ (a)	1.80, m (a)	17, 19	17, 19
	29.3, CH ₂ (b)	1.88, m (b)		
19	40.2, CH ₂ (a)	3.16, m (a)	17, 18	17, 18, 20
	39.9, CH ₂ (b)	3.21, m (b)		
20	158.8/158.9	-	-	-
21	46.2, CH ₂ , (a)	3.37, m (a)	22, 23, 24	1, 17, 23
	49.1, CH ₂ , (b)	3.37, m (b)		
22	27.3, CH ₂	1.62, m	21, 23, 24	1, 23
23	27.1, CH ₂	1.57, m	21, 22, 24	24
24	42.2, CH ₂	3.21, m	21, 22, 23	22, 25
25	158.8/158.9	-	-	-

Figure S16: $^1\text{H-NMR}$ of unguiculin C (**3**) in CD_3OD , 500 MHz

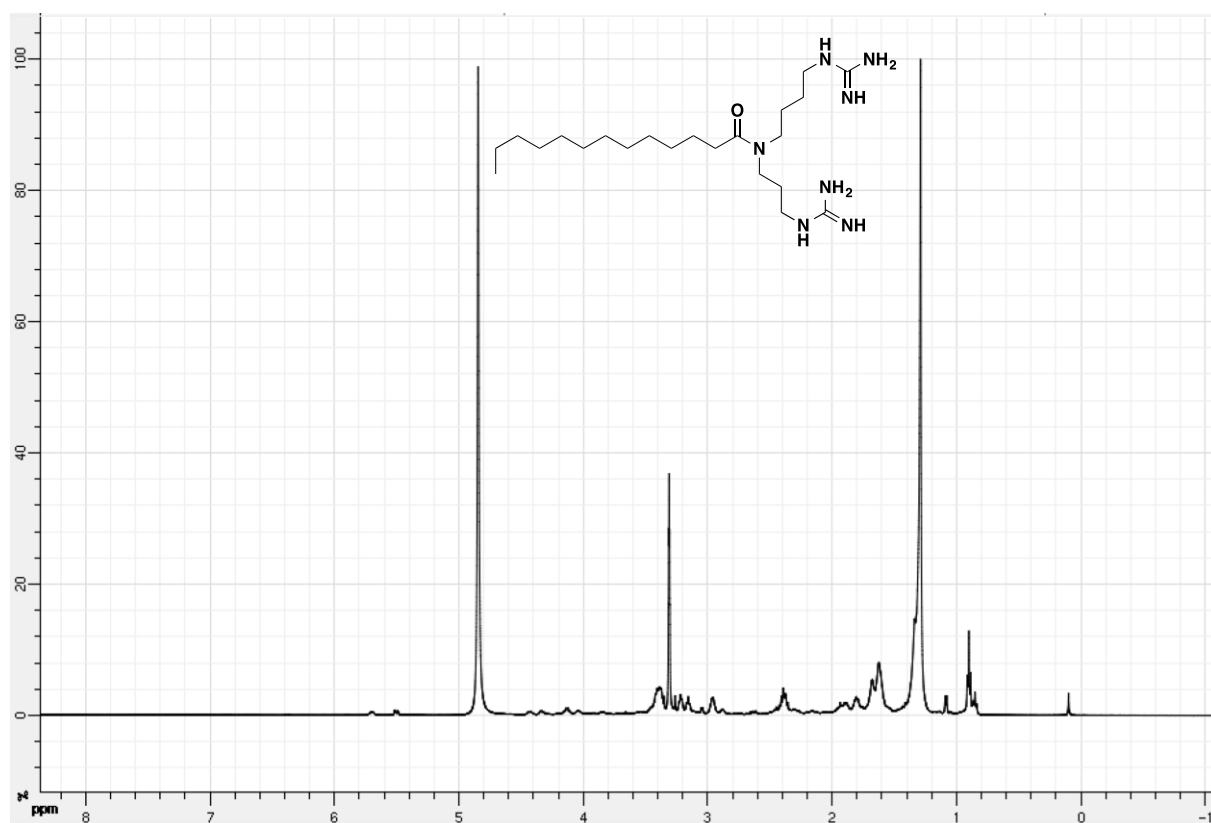


Figure S17: $^{13}\text{C-NMR}$ of unguiculin C (**3**) in CD_3OD , 125 MHz

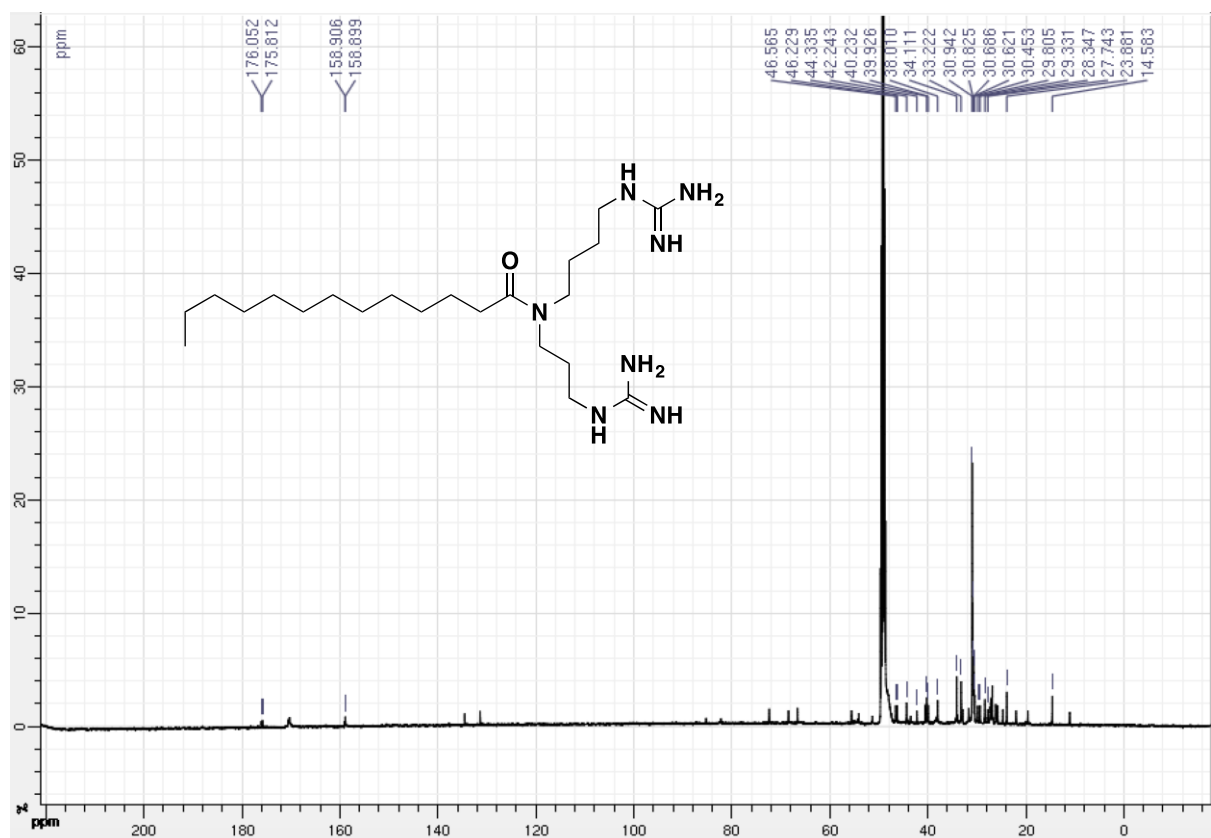


Figure S18: COSY of unguiculin C (**3**) in CD₃OD, 500 MHz

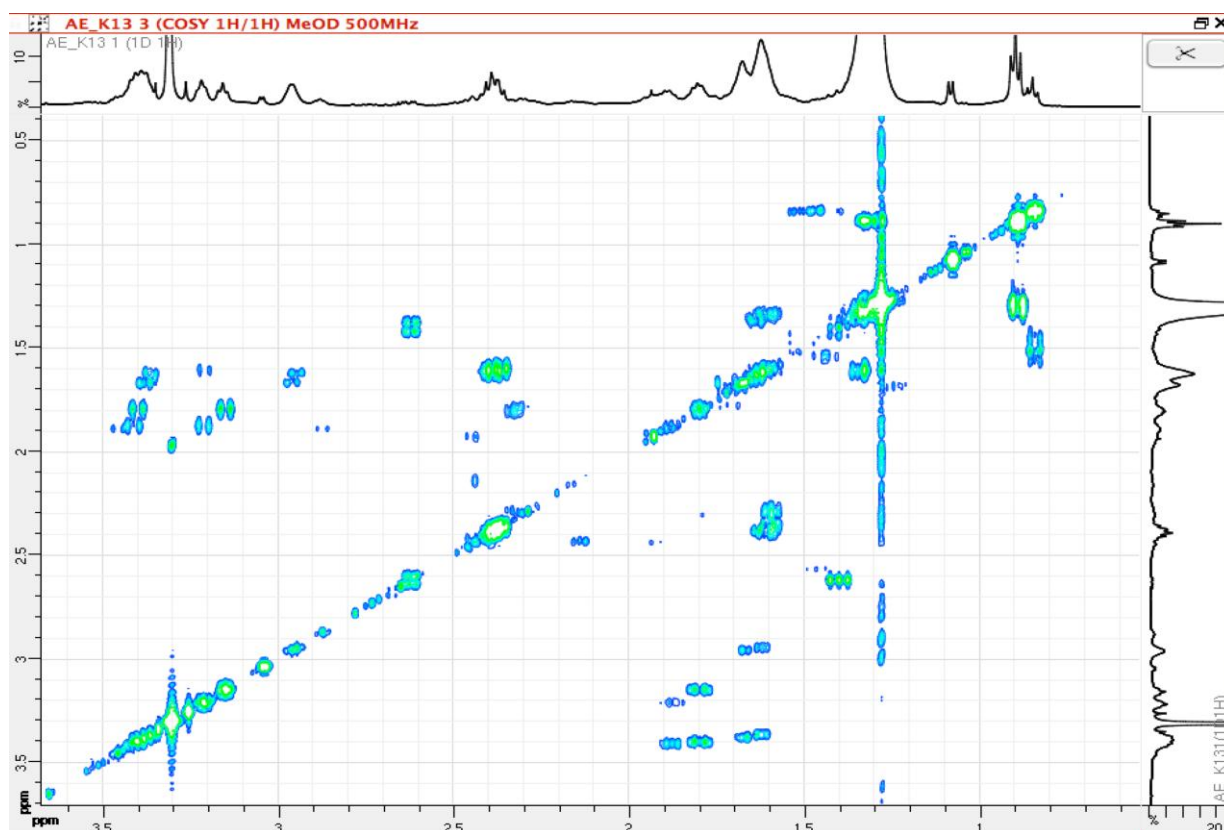


Figure S17: HSQC of unguiculin C (**3**) in CD₃OD, 500 MHz

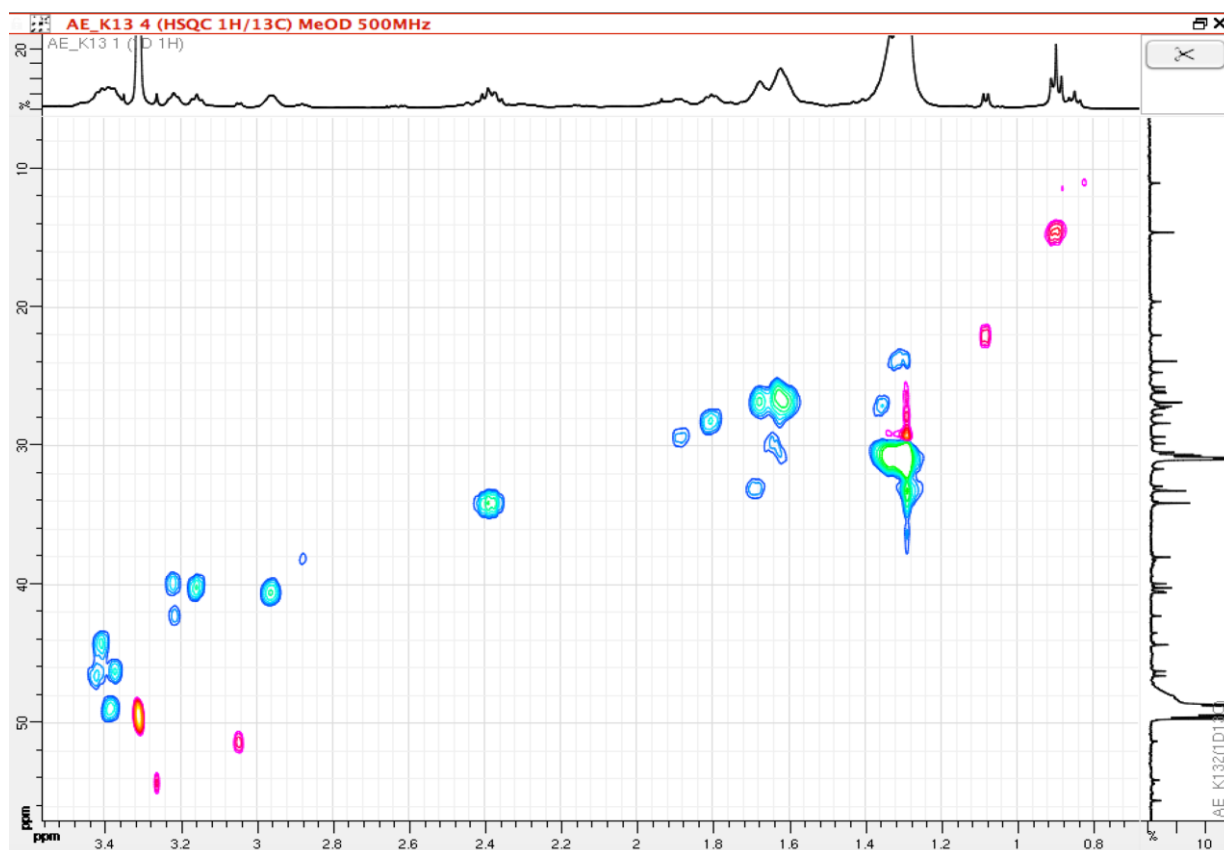


Figure S19: HMBC of unguiculin C (**3**) in CD₃OD, 500 MHz

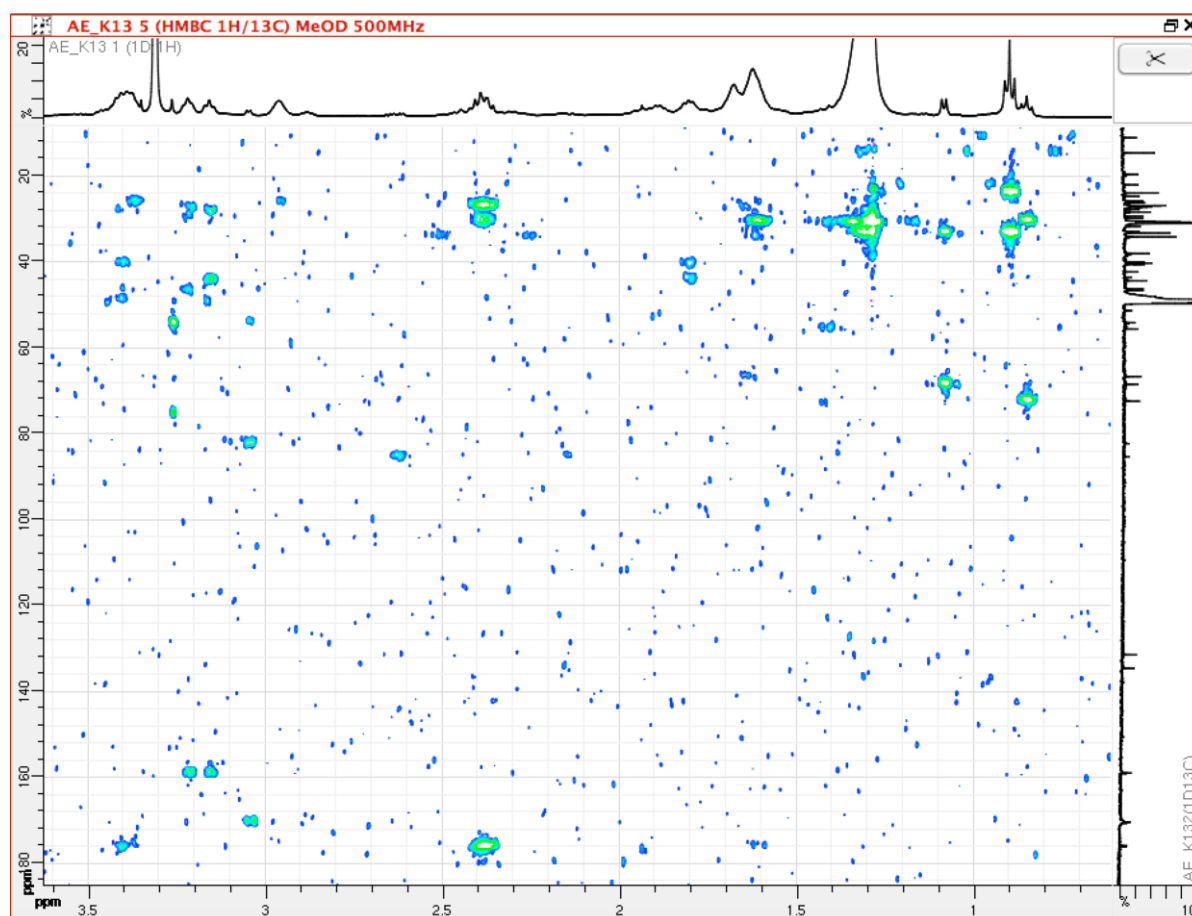


Figure S20: General key COSY and HMBC for unguiculin C (**3**)

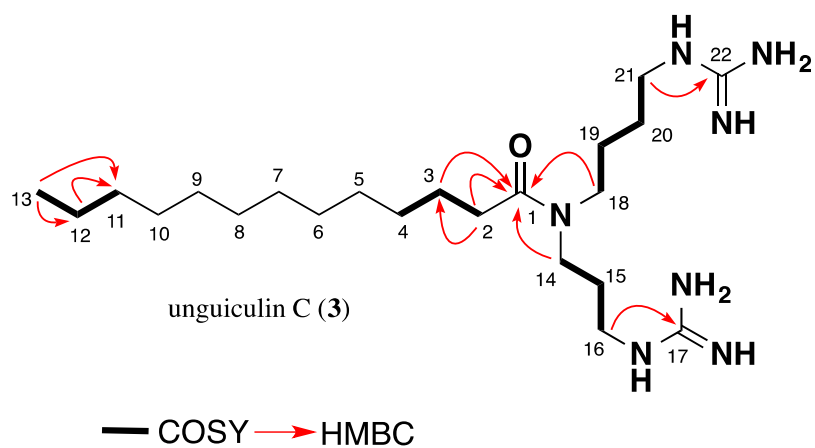


Figure S21: HRESIMS+ of unguiculin C (3)

Elemental Composition Report

Page 1

Single Mass Analysis

Tolerance = 70.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions

88 formula(e) evaluated with 5 results within limits (up to 50 best isotopic matches for each mass)

Elements Used:

C: 0-100 H: 0-100 N: 0-7 O: 0-1

26-Jan-2013 3:5:7

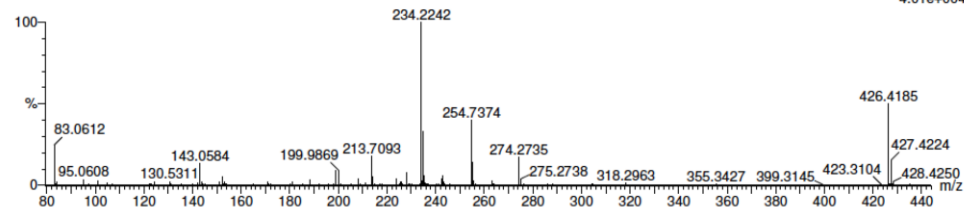
ALMOURABIT_ahmed16-1 487 (2.313) Cm (476:494)

AE_K13

LCT Premier XE KE483

1: TOF MS ES+

4.01e+004



Minimum:

Maximum: 5.0 70.0 -1.5

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
426.4185	426.4172	1.3	3.0	1.5	908.2	0.5	C24 H52 N5 O
	426.4423	-23.8	-55.8	0.5	908.6	0.9	C26 H56 N3 O
	426.4284	-9.9	-23.2	1.5	911.7	4.0	C23 H52 N7
	426.3920	26.5	62.1	2.5	915.8	8.2	C22 H48 N7 O

Table S2: 1 and 2D NMR data for unguiculin C (3)

Position	δ_c , Type	δ_H (J in Hz)	(¹ H- ¹ H) COSY	(¹ H- ¹³ C) HMBC
1	176.1/175.8, C	-	-	-
2	34.1, CH ₂	2.39, m	3	1, 3
3	26.9, CH ₂	1.61, m	2	1
4-11	30.5-30.2, CH ₂	1.29-1.34, m	-	-
12	23.9, CH ₂	1.31, m	13	13
13	14.6, CH ₃	0.90, t, 7	12	12
14	44.3, CH ₂ (a)	3.41, m (a)	15, 16	1, 16
	46.6, CH ₂ (b)	3.42, m (b)		
15	28.4, CH ₂ (a)	1.81, m (a)	14, 16	14, 16
	29.3, CH ₂ (b)	1.89, m (b)		
16	40.2, CH ₂ (a)	3.16, t, 7 (a)	14, 15	14, 15, 17
	39.9, CH ₂ (b)	3.22, m (b)		
17	158.9, C	-		-
18	46.2, CH ₂ (a)	3.37, m (a)	19, 20, 21	1, 20
	49.0, CH ₂ (b)	3.38, m (b)		
19	27.2, CH ₂	1.62, m	18, 20, 21	1
20	26.8, CH ₂	1.60, m	18, 19, 21	-
21	42.2, CH ₂	3.22, m	18, 19, 20	18, 22
22	158.9, C	-	-	-

Table S3: comparison of ^1H NMR & ^{13}C NMR (500 and 125 MHz) data for unguiculin B-C (2-3) in CD_3OD .

Unguiculin B (2)			Unguiculin C (3)		
Position	δ_{C} , type	δ_{H} mult, (J in Hz)	Position	δ_{C} , type	δ_{H} mult, (J in Hz)
1	175.8/176.0, C	-	1	176.1/175.8, C	-
2	34.0, CH_2	2.38, m	2	34.1, CH_2	2.39, m
3	27.0, CH_2	1.61, m	3	26.9, CH_2	1.61, m
4-12	30.6-30.9, CH_2	1.30-1.33, m	4-12	30.5-30.2, CH_2	1.29-1.34, m
13	30.6-30.9, CH_2	1.30-1.33, m	13	14.6, CH_3	0.90, t, 7
14	27.1, CH_2	1.34, m			
15	33.8, CH_2	1.53, m			
16	63.1, CH_2	3.54, t, 7			
17	44.3, CH_2 (a)	3.40, m (a)	14	44.3, CH_2 (a)	3.41, m (a)
	46.4 CH_2 (b)	3.41, m (b)		46.6, CH_2 (b)	3.42, m (b)
18	28.3, CH_2 (a)	1.80, m (a)	15	28.4, CH_2 (a)	1.81, m (a)
	29.3, CH_2 (b)	1.88, m (b)		29.3, CH_2 (b)	1.89, m (b)
19	40.2, CH_2 (a)	3.16, m (a)	16	40.2, CH_2 (a)	3.16, t, 7 (a)
	39.9, CH_2 (b)	3.21, m (b)		39.9, CH_2 (b)	3.22, m (b)
20	158.8/158.9	-	17	158.9, C	-
21	46.2, CH_2 , (a)	3.37, m (a)	18	46.2, CH_2 (a)	3.37, m (a)
	49.1, CH_2 , (b)	3.37, m (b)		49.0, CH_2 (b)	3.38, m (b)
22	27.3, CH_2	1.62, m	19	27.2, CH_2	1.62, m
23	27.1, CH_2	1.57, m	20	26.8, CH_2	1.60, m
24	42.2, CH_2	3.21, m	21	42.2 CH_2	3.22, m
25	158.8/158.9, C	-	22	158.9, C	-

Figure S21: $^1\text{H-NMR}$ comparison between unguiculin A-C (1-3), 500 MHz in CD_3OD

