SUPPLEMENTARY MATERIAL

Contortamide, a new anti-colon cancer cerebroside and other constituents from *Tabernaemontana contorta* Stapf (Apocynaceae).

Guy R. Ebede[a,d], Joseph T. Ndongo[b*], Joséphine N. Mbing[a], Hector C. M. Kenfack[c], Dieudonné E. Pegnyemb[a] and Christian G. Bochet[d].

[a] *Department of Organic Chemistry, Faculty of Science, University of Yaoundé I, P.O Box 812, Yaoundé, Cameroon.*

[b] *Department of Chemistry, Higher Training College, University of Yaoundé I, P.O Box 47, Yaoundé, Cameroon.*

[c] *Department of Biochemistry, Faculty of Science, University of Dschang I, P.O Box 67, Dschang, Cameroon.*

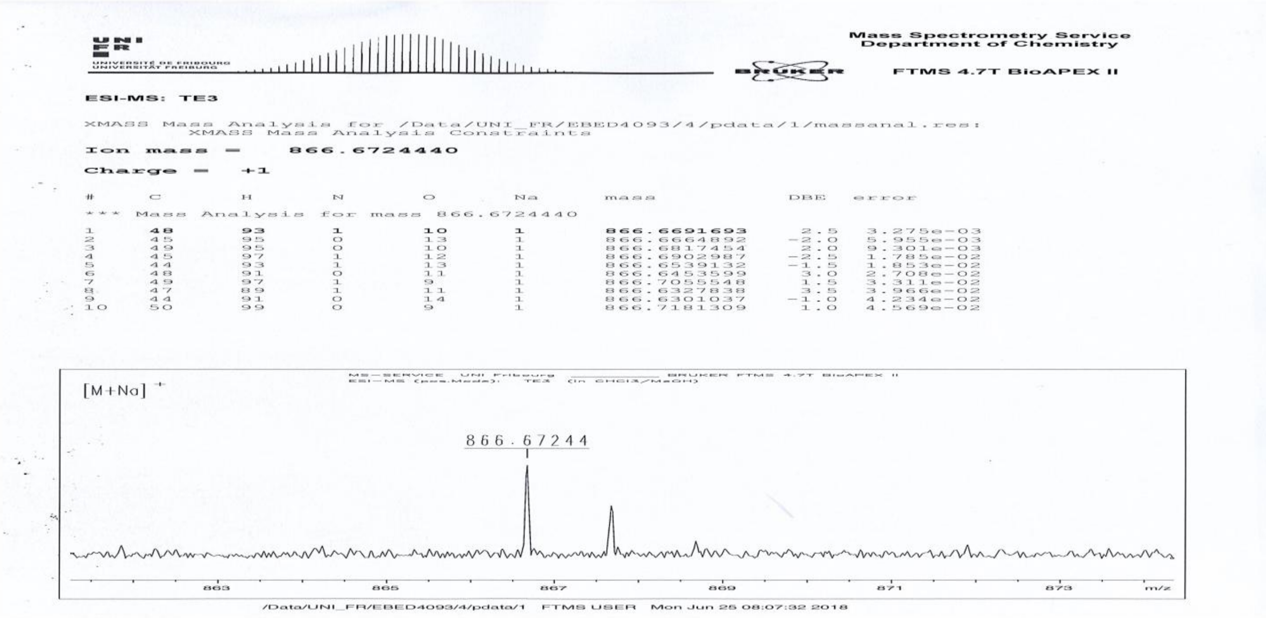
[d] *Department of Chemistry, University of Fribourg, Chemin du Musée 9, 1700 Fribourg, Switzerland.*

*Corresponding author: Tel: +237 690 22 47 80; E-mail: thierry.ndongo@ens.cm (J. T. Ndongo).

Abstract

Tabernaemontana contorta Stapf, a flowering plant, belongs to the family Apocynaceae. In Cameroon, its leaves are used to prevent keloids formation and as antiseptic (Burkill, 1985). A new cerebroside, Contortamide (**1**) together with nine know compounds spegatrine (**2**), affinisine (**3**), N_{b-}methylaffinisine (**4**), ursolic acid (**5**), α-amyrin (**6**), bauerenol acetate (**7**), Lupeol (**8**), betulinic acid (**9**), β-sitosterolglycoside (**10**) were isolated from the bark of trunk of *Tabernaemontana contorta* Stapf. The new compound **1** showed significant activity against Caco-2 colon cancer cells with the MTT method. Compounds **1**, **2**, **3, 4**, **6**, **7**, **8** and **9** were isolated for the first time from this species.

Keywords: Contortamide; *Tabernaemontana contorta* Stapf; Apocynaceae; Colon Cancer.


Mass Spectrometry Service
Department of Chemistry

UNIVERSITÉ DE FRIBOURG
UNIVERSITÄT FREIBURG

BRUKER FTMS 4.7T BioAPEX II

ESI-MS: TE3

XMASS Mass Analysis for /Data/UNI_FR/EBED4093/4/pdata/1/massanal.res:
XMASS Mass Analysis Constraints

Ion mass = 866.6724440

Charge = +1

*** Mass Analysis for mass 866.6724440

#	C	H	N	O	Na	mass	DBE	error
1	48	93	1	10	1	866.6691693	2.5	3.275e-03
2	45	95	0	13	1	866.6664892	-2.0	5.955e-03
3	49	95	0	10	1	866.6817454	2.0	9.301e-03
4	45	97	1	12	1	866.6902987	-2.5	1.785e-02
5	44	93	1	13	1	866.6539132	-1.5	1.853e-02
6	48	91	0	11	1	866.6453599	3.0	2.708e-02
7	49	97	1	9	1	866.7055548	1.5	3.311e-02
8	47	89	1	11	1	866.6327838	3.5	3.966e-02
9	44	91	0	14	1	866.6301037	-1.0	4.234e-02
10	50	99	0	9	1	866.7181309	1.0	4.569e-02

Figure S1. HRESI-MS spectrum of compound **1**.

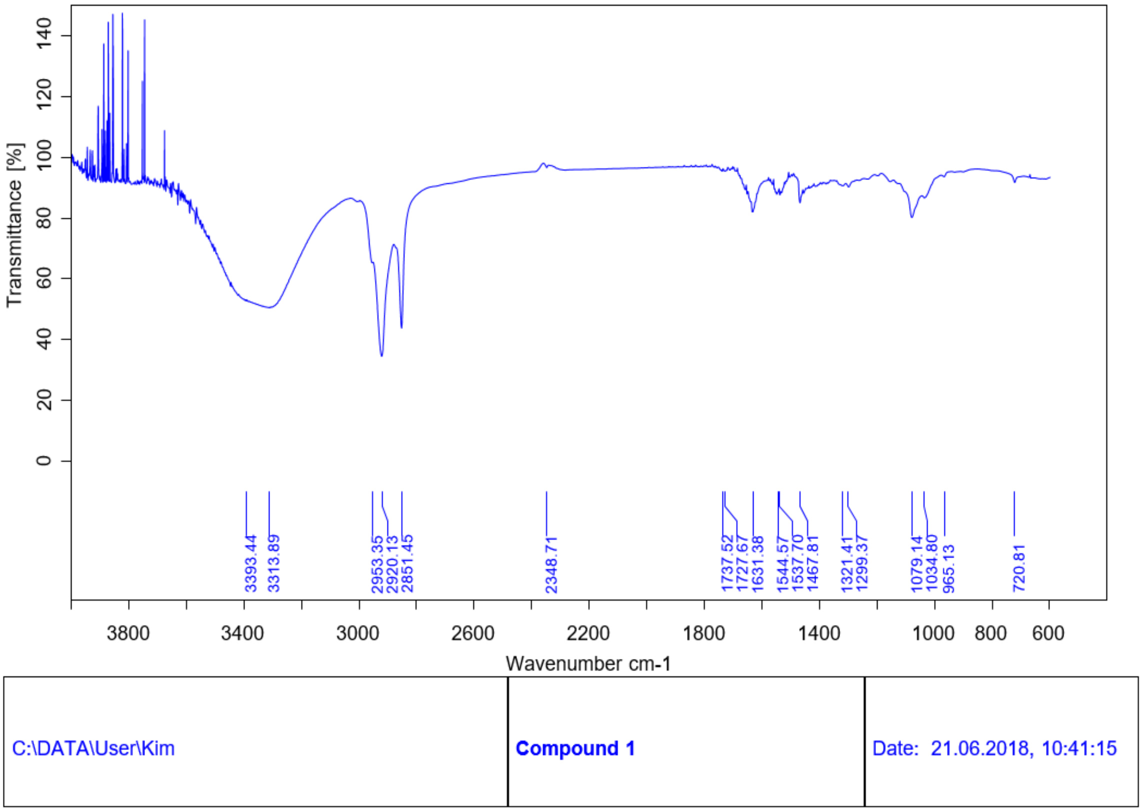


Figure S2. IR spectrum of compound **1**.

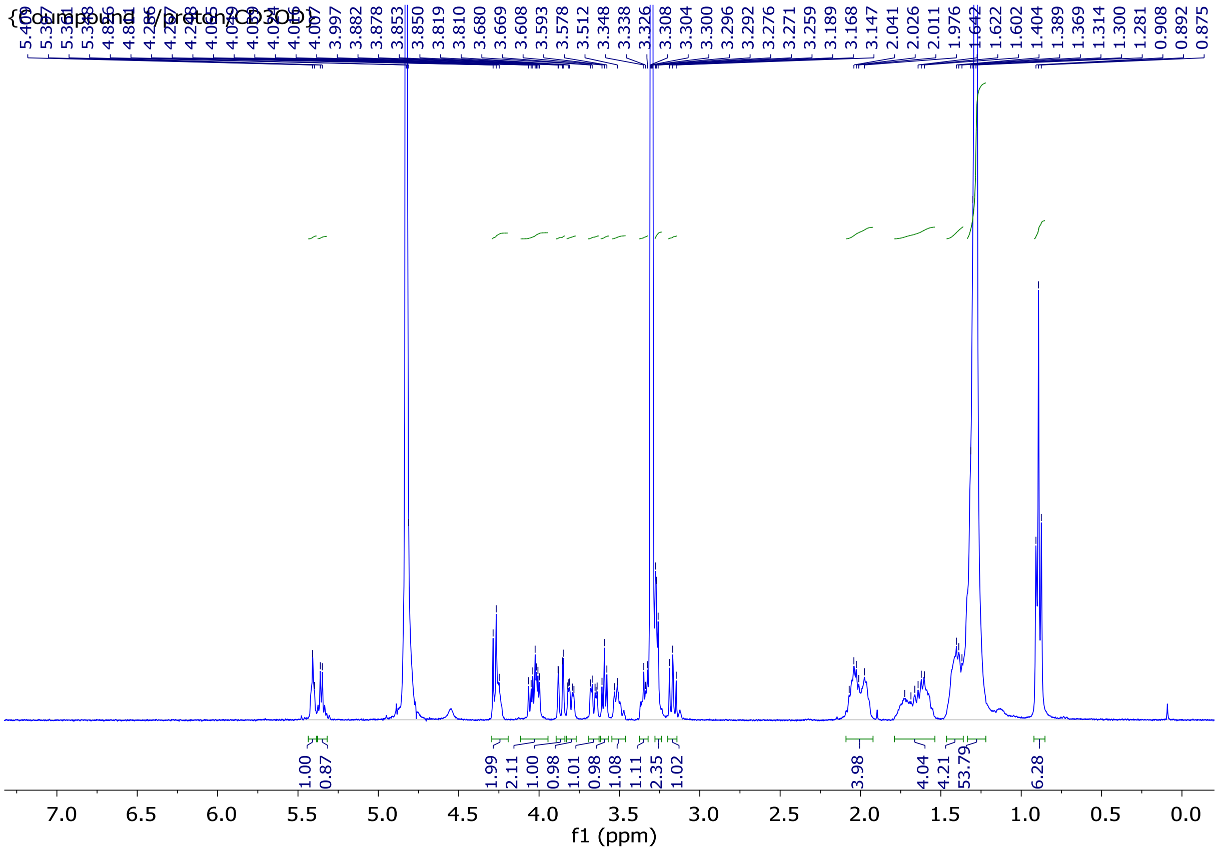


Figure S3. ^{1}H NMR spectrum (CD_3OD, 400 MHz) of compound **1**.

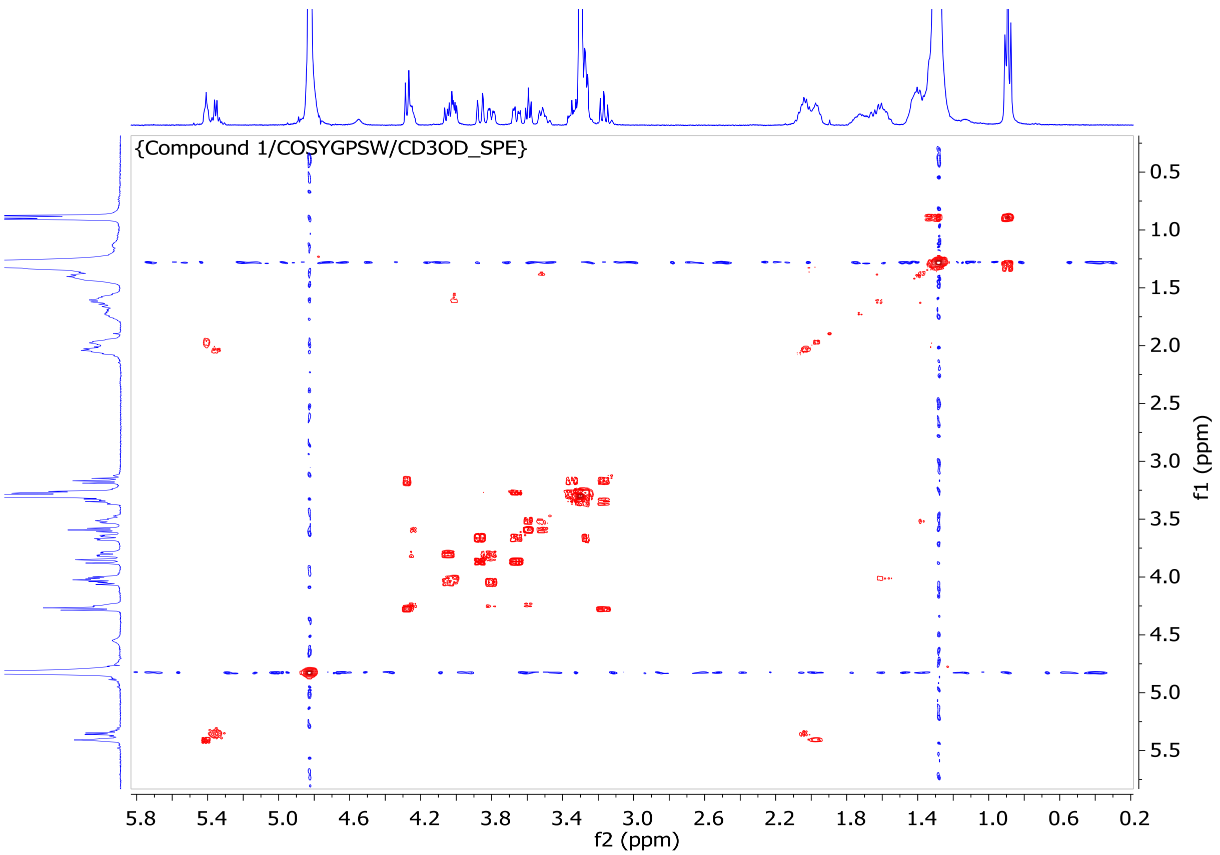


Figure S4. COSY spectrum (CD_3OD, 400 MHz) of compound **1**.

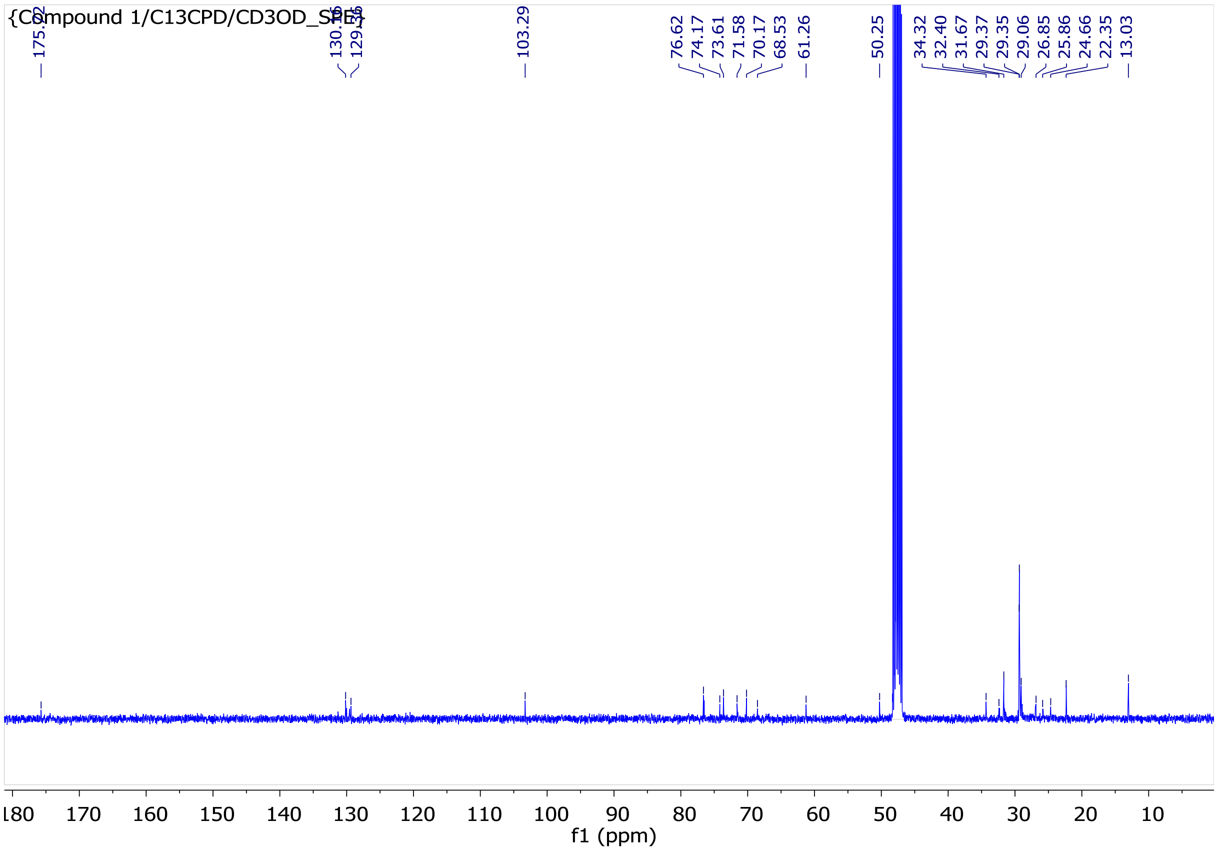


Figure S5. ^{13}C NMR spectrum (CD_3OD, 100 MHz) of compound **1**.

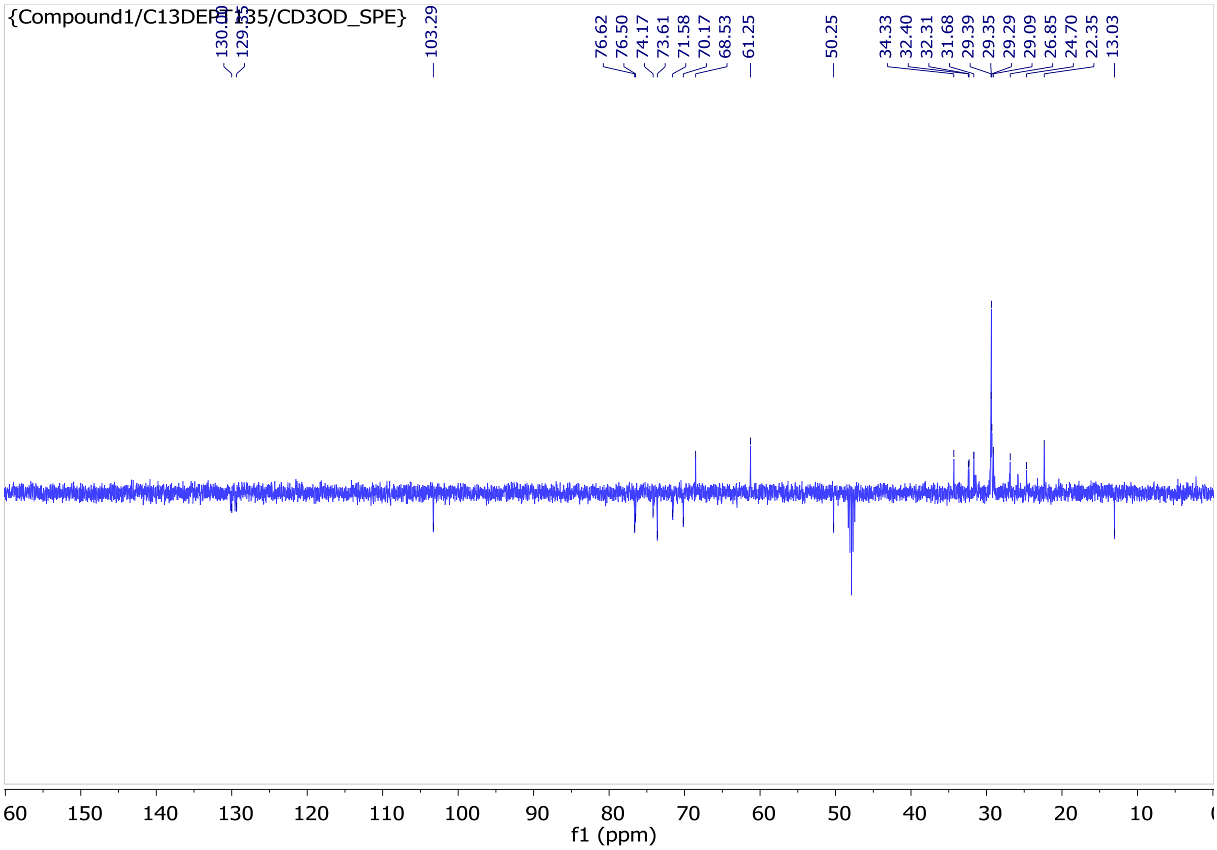


Figure S6. DEPT 135 spectrum (CD_3OD, 100 MHz) of compound **1**.

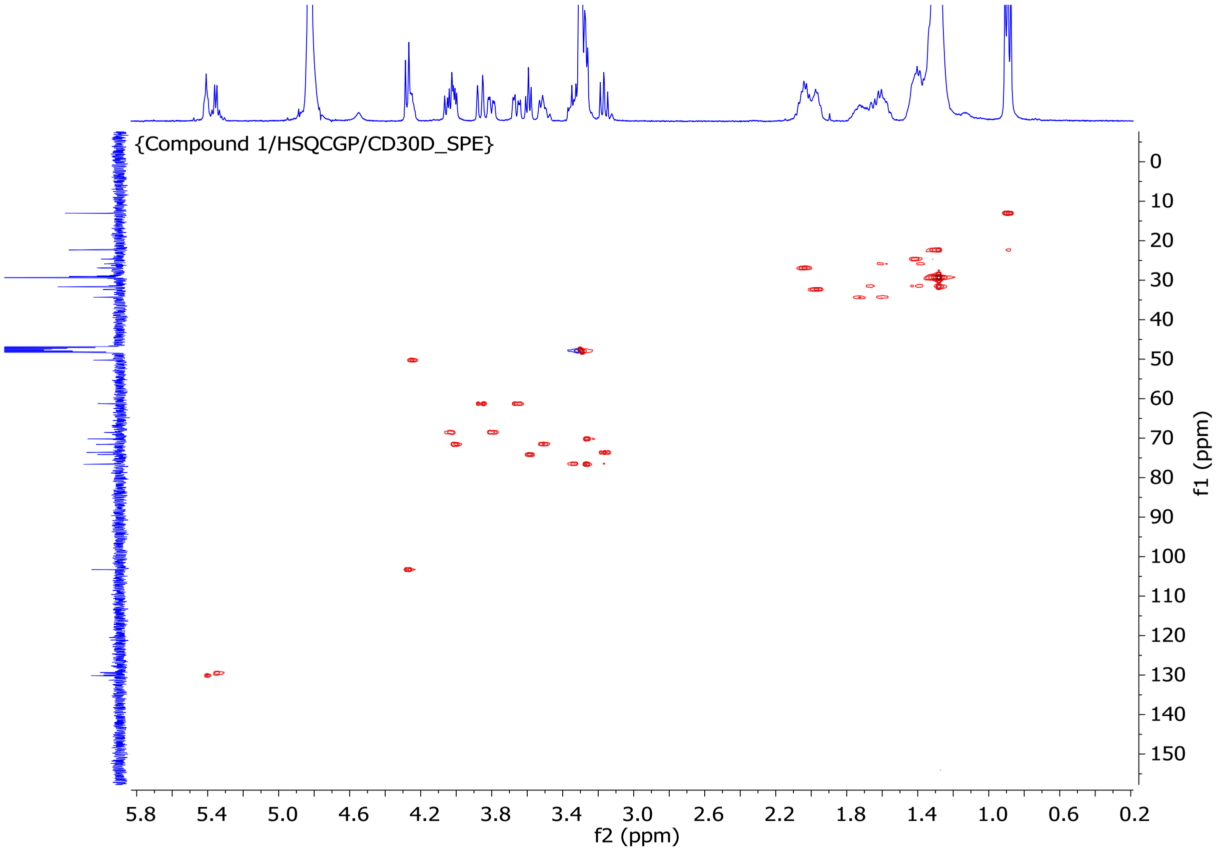


Figure S7. HSQC spectrum (CD_3OD, 400 MHz) of compound **1**.

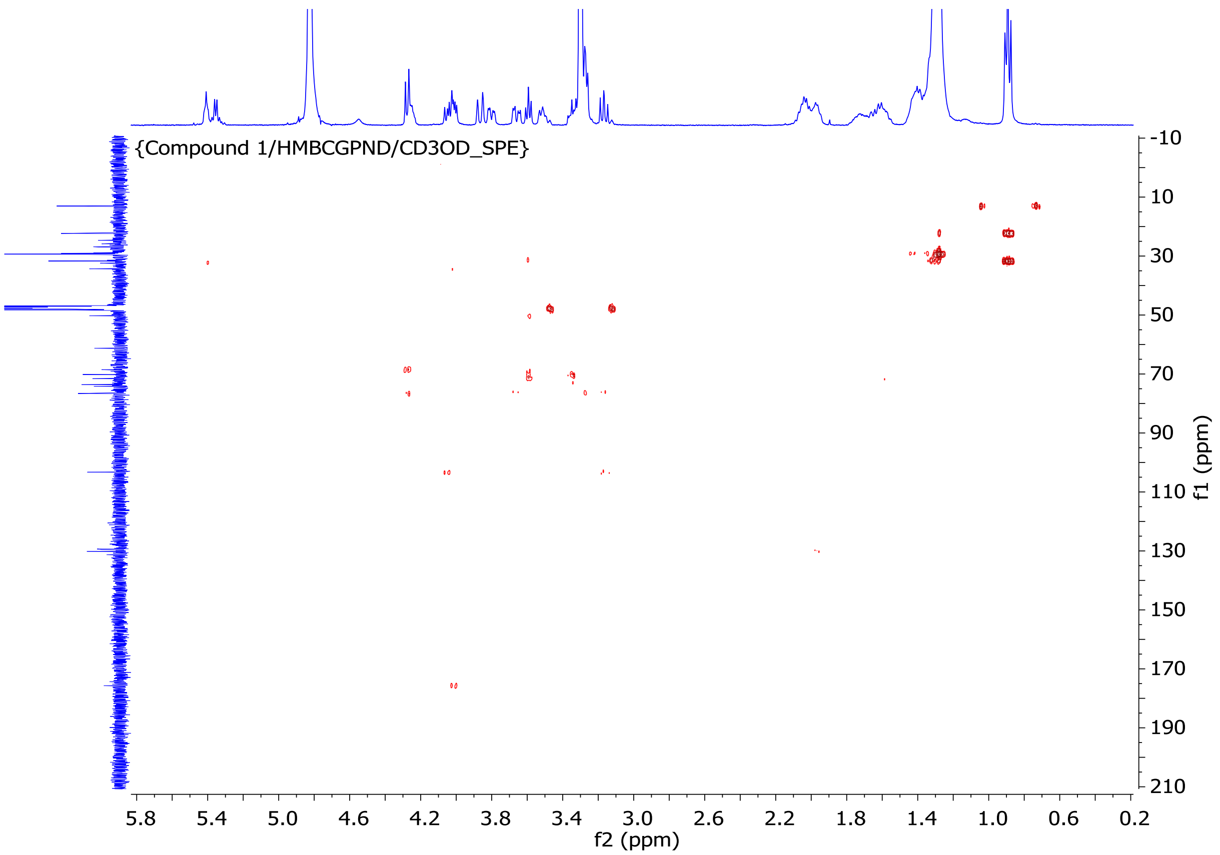


Figure S8. HMBC spectrum (CD_3OD, 400 MHz) of compound **1**.

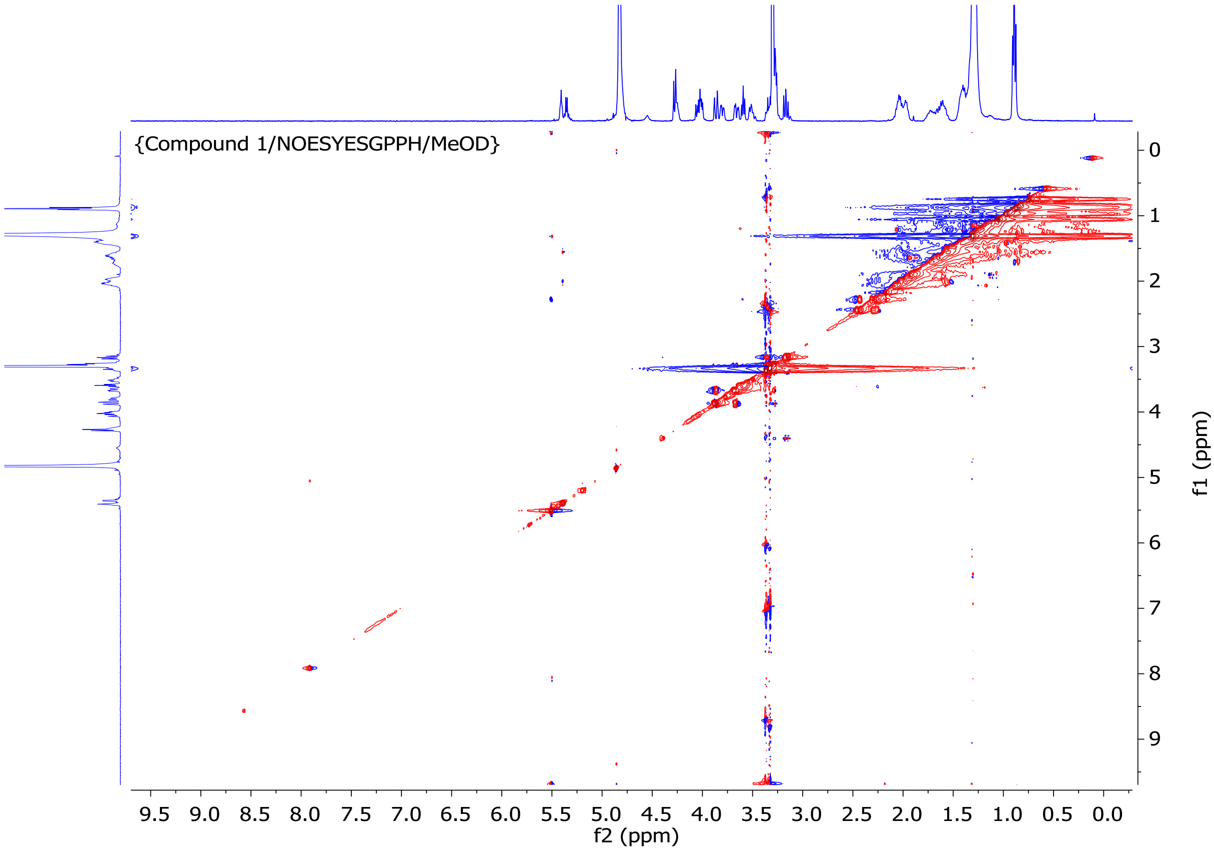


Figure S9. NOESY spectrum (CD_3OD, 400 MHz) of compound **1**.

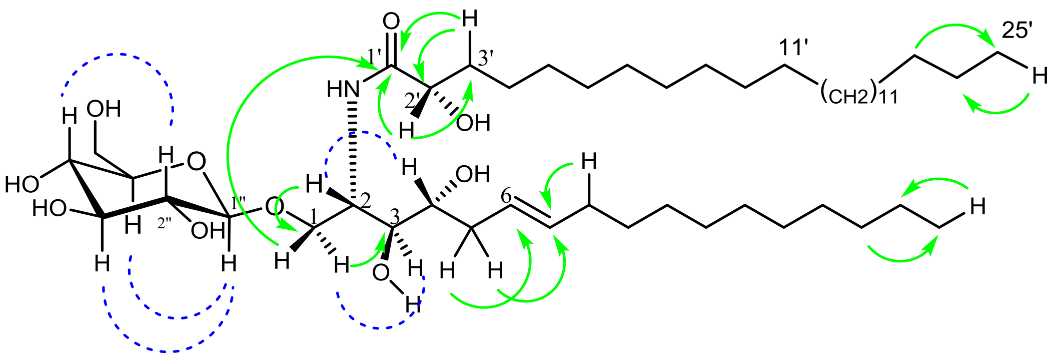


Figure S10. Selected HMBC (→) and NOESY(⋯⋯) correlations for compound **1**.

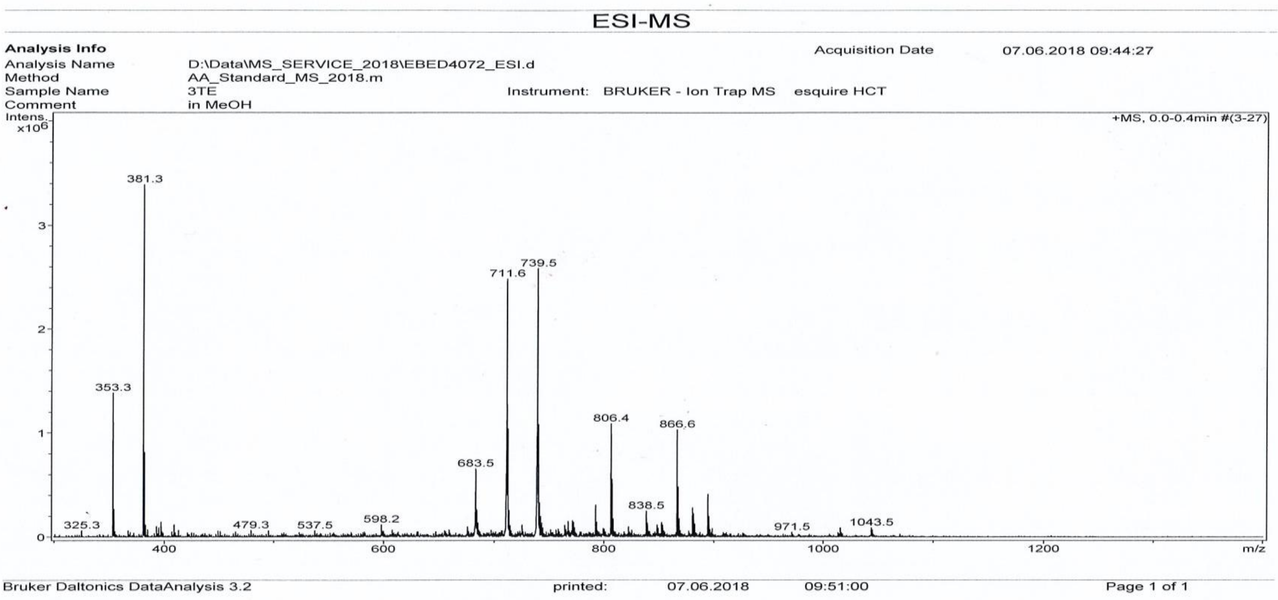


Figure S11. ESI-MS Spectrum of compound **1**.

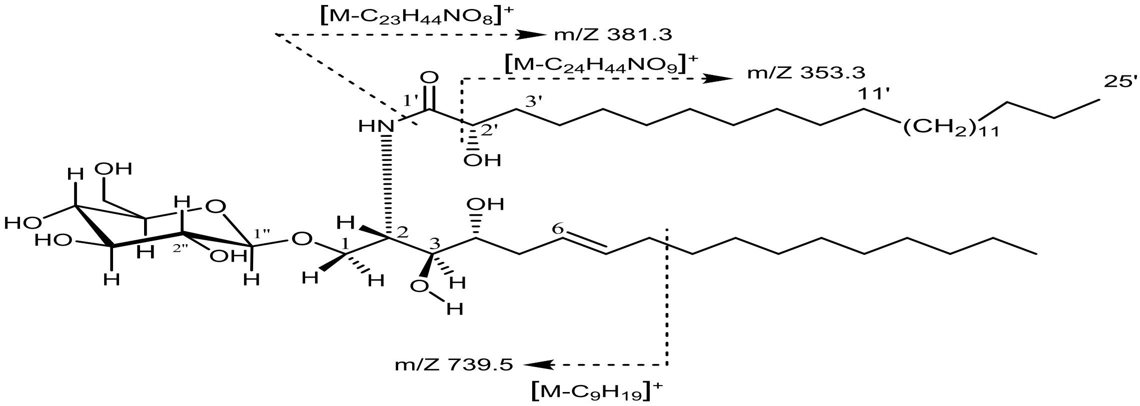


Figure S12. Mass fragmentation pattern of compound **1**.

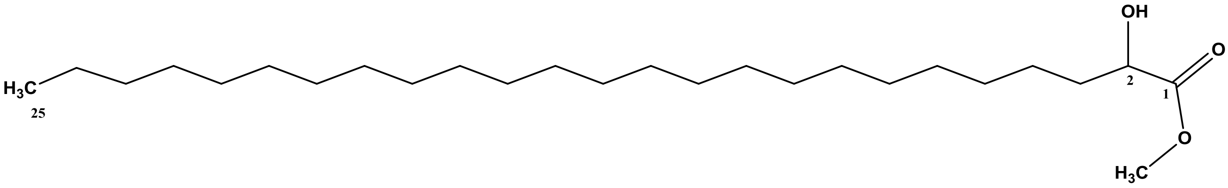


Figure S13. Chemical structure of fatty acid (Methyl-2-hydroxypentacosanoate).

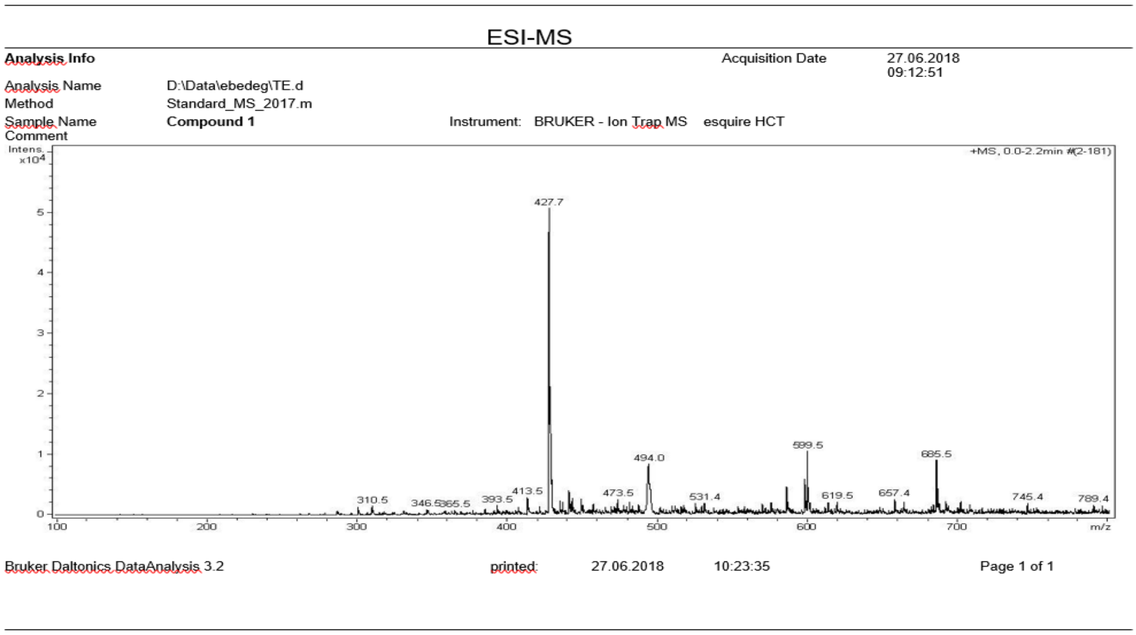


Figure S14. ESI-MS of fatty acid (Methyl-2-hydroxypentacosanoate).

(a) Caco-2 cell line control **(b)** Inhibition of Caco-2 cell line by TEM (**1**)

Figure S15. Observation of Caco-2 cancer cells under a microscope.

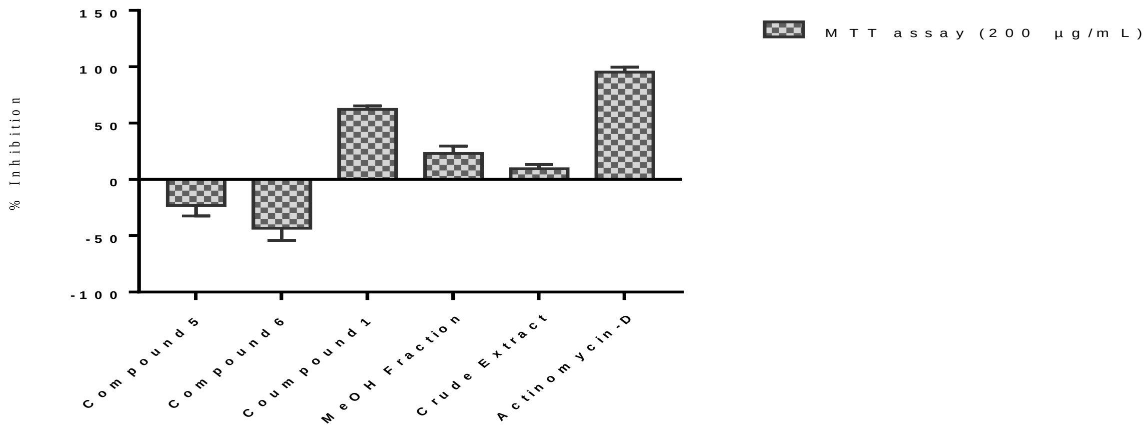


Figure S16. A comparison of pure compounds, fraction, extract and standard drug actinomycin D growth inhibitory activities against Caco-2. All the results are represented as mean ± SD (n =3).

Table S1. [1]H (CD_3OD, 400 MHz) and [13]C (CD_3OD, 100 MHz) NMR spectral data and HMBC correlations of compound **1**.

Position	δ_H ppm (J in Hz)	δ_C ppm	**HMBC** (H→C)	$^1H-^1H$ **COSY**
1a	4.02 (m)	68.5	C-1', C-1''	H-2
1b	3.79 (dd, 7.2; 14.8)	68.5	C-1', C-1'', C-3	H-2
2	4.24 (m)	50.3	C-1	H-1, H-3
3	3.57 (t, 6.1)	74.4	C-2, C-4, C-5	H-2, H-4
4	3.50 (m)	71.4	-	H-3, H-5
5a	1.98 (m)	32.4	C-6, C-7	H-4
5b	2.01 (m)	32.4	C-6, C-7	H-4
6	5.40 (dt)	129.4	C-5	H-5
7	5.36 (dt)	130.4	C-8	H-8
8	1.64 (m)	32.0	C-7	H-7
9 - 16	1.28-1.40 (brs)	22.2-31.7		H-8
17	0.89 (t, 6.7)	13.1	C-16	H-17
1'		176.2	-	
2'	4.00 (m)	71,5	C-1', C-3'	H-3'
3'a	1.73 (m)	34.4	C-2'	H-2'
3'b	1.63 (m)	34.4	C-2'	H-4'
4' – 24'	1.28-1.40 (brs)	22.2-31.7		H-5'
25'	0.89 (t, 6.7)	13.1	C-25'	H-25'
Glucose				
1''	4.26 (d, 7.8)	103.3	C-1, C-2'', C-3''	H-2''
2''	3.16 (dd, 8; 16.8)	73.6	C-1, C-3''	H-1'', H-3''
3''	3.33 (m)	76.5	C-4''	H-2'', H-4''
4''	3.26 (dd, 4;12.3;)	70.2	C-3'', C-5''	H-3'', H-5''
5''	3.27 (m)	76.6	C-3''	H-4'', H-6''
6''a	3.85 (dd, J = 11,2)	61.3	C-5''	H-5''
6''b	3.64 (dd, J = 4.4; 11.2)	61.3	C-5''	H-5''