3-[(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)oxy]propyl 9-hydroxy-5a,5b,8,8,11a-pentamethyl-1-(prop-1-en-2-yl)icosahydro-3a*H*-cyclopenta[*a*]chrysene-3a-carboxylate

Jiang Ai [1], Margrate Anyanwu [2], Leong Ka Fai [1], Li Jinxin [1], Alessandra Gianoncelli [2], Paolo Coghi [1,*] and Giovanni Ribaudo [2,*]

[1] School of Pharmacy, Macau University of Science and Technology, Macau, China; 1809853jpa11002@student.must.edu.mo (J.A.); 1809853apa11006@student.must.edu.mo (L.K.F.); 17098537@student.must.edu.mo (L.J.)
[2] Department of Molecular and Translational Medicine, University of Brescia, 25121 Brescia, Italy; margrate.anyanwu@unibs.it (M.A.); alessandra.gianoncelli@unibs.it (A.G.)
* Correspondence: giovanni.ribaudo@unibs.it (G.R.); coghips@must.edu.mo (P.C.); Tel.: +853-68145596 (P.C.)

Supplementary Material

Table of content

NMR spectrometry

Figure S1. ^{1}H NMR spectrum ($CDCl_3$, 600 MHz) of Betulinic acid (**BA**).

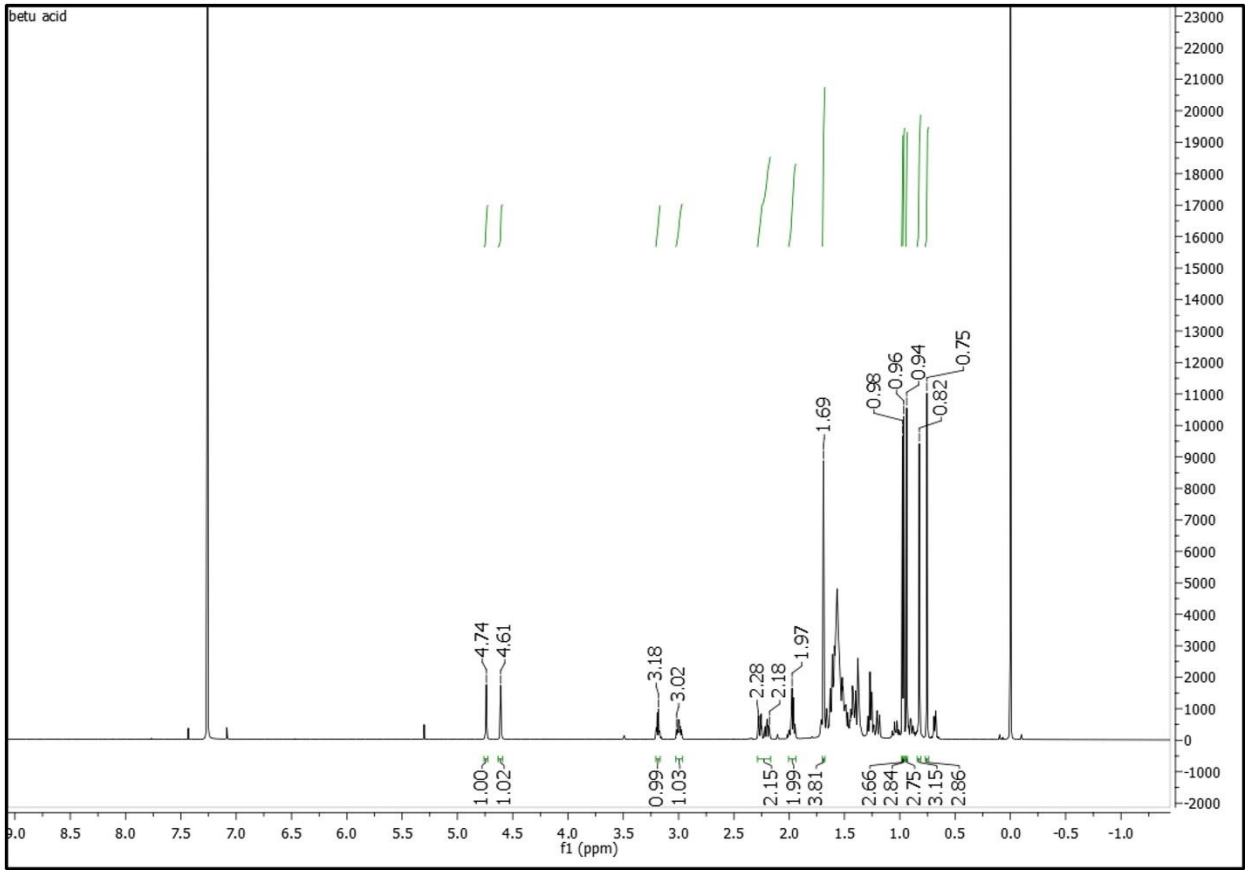


Figure S2. ^{13}C NMR spectrum ($CDCl_3$, 150 MHz) of Betulinic acid (**BA**).

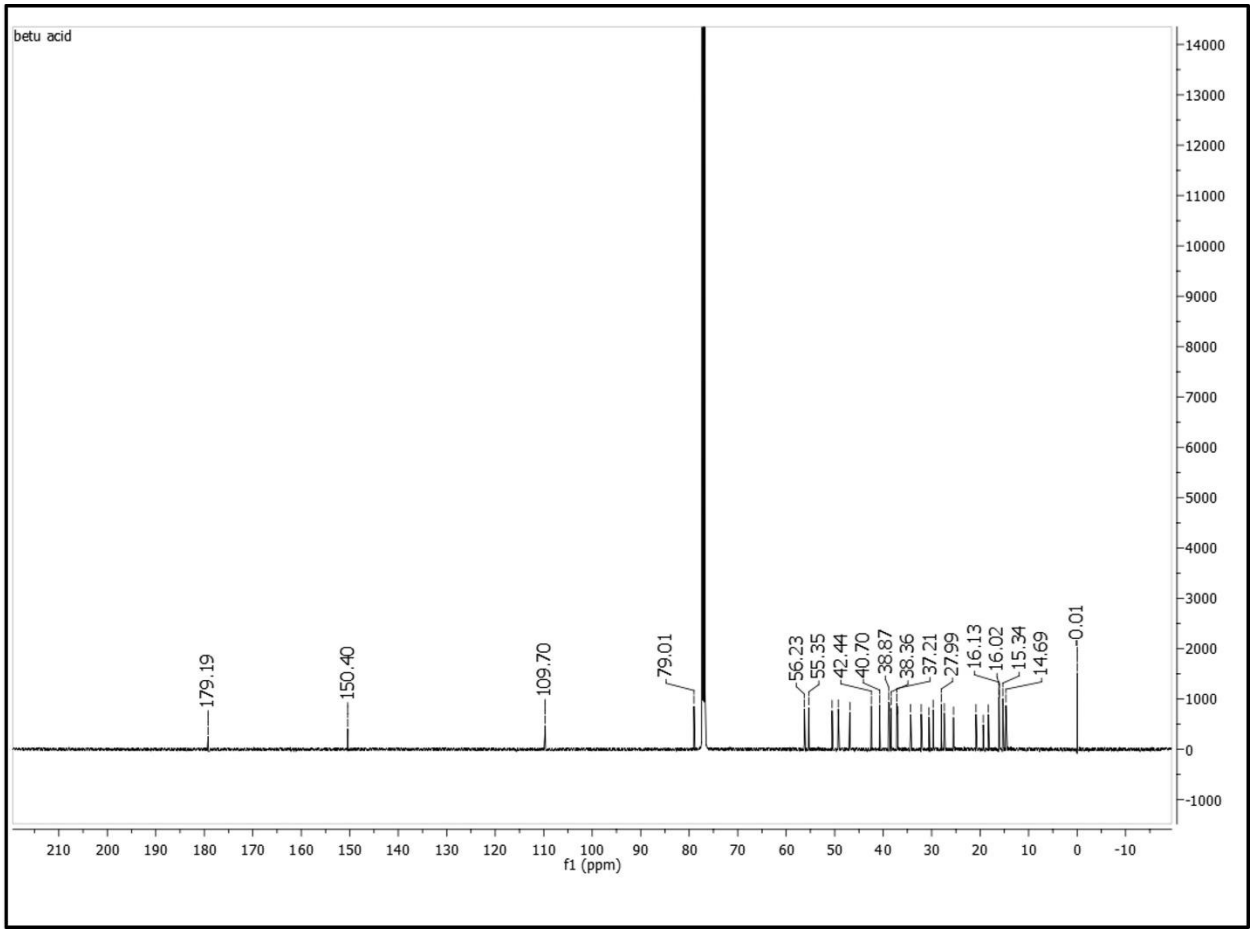


Figure S3. ^{1}H NMR spectrum ($CDCl_3$, 600 MHz) of compound **2**.

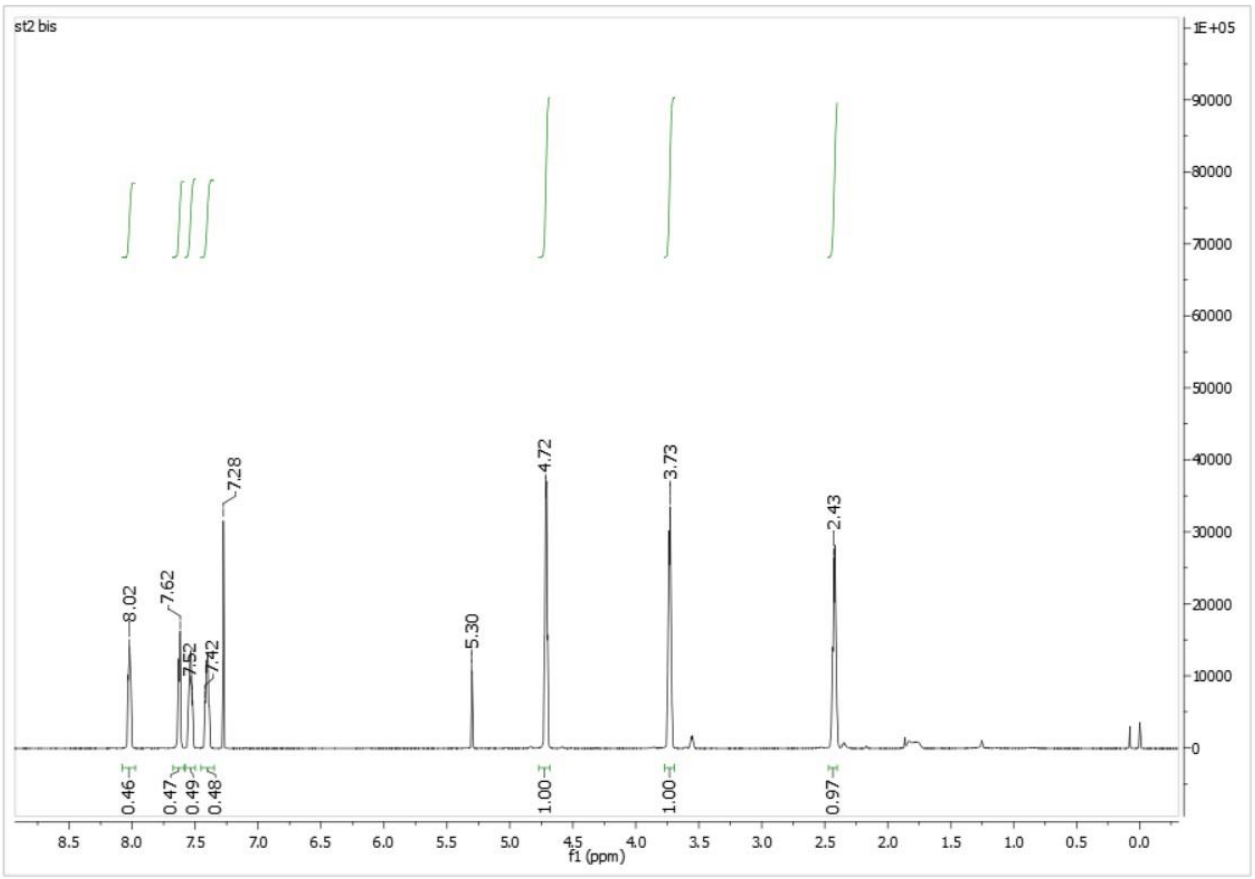


Figure S4. ^{13}C NMR spectrum ($CDCl_3$, 150 MHz) of compound **2**.

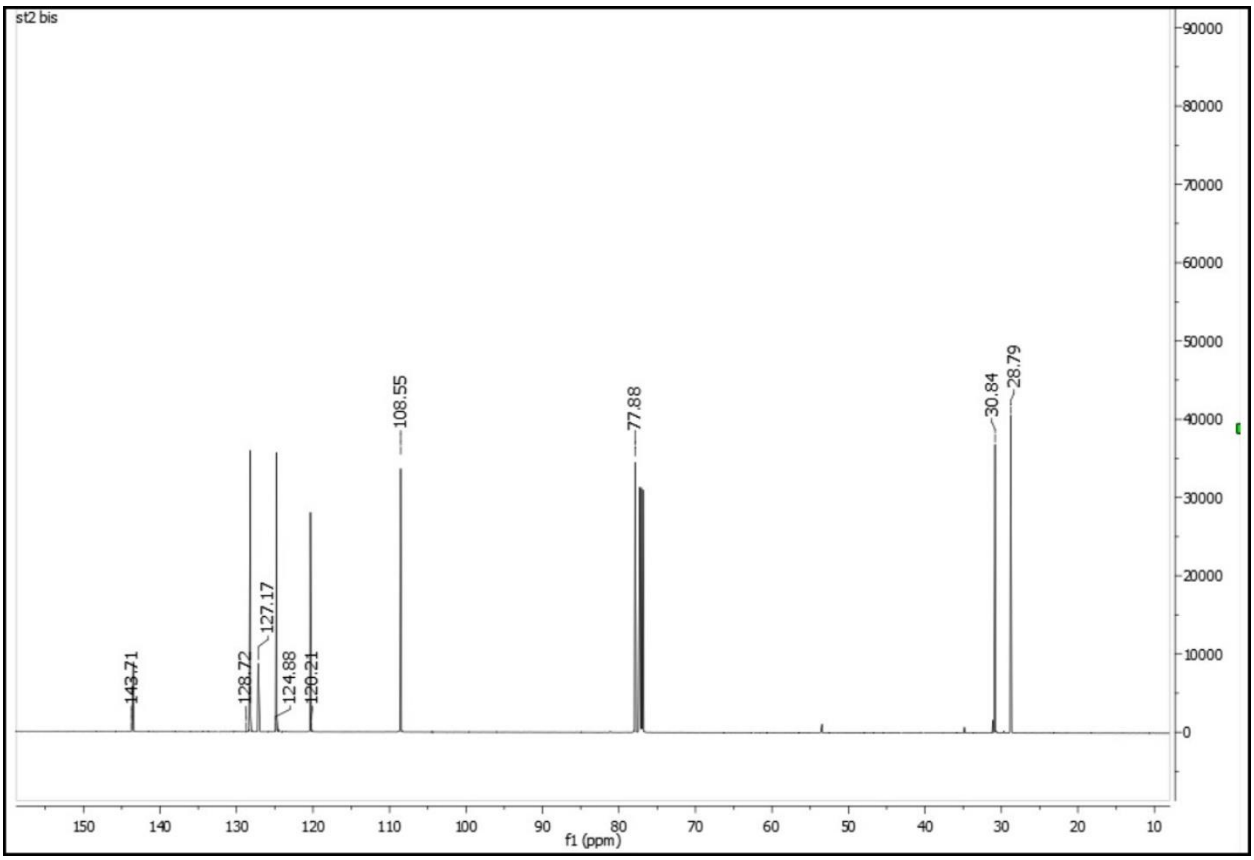
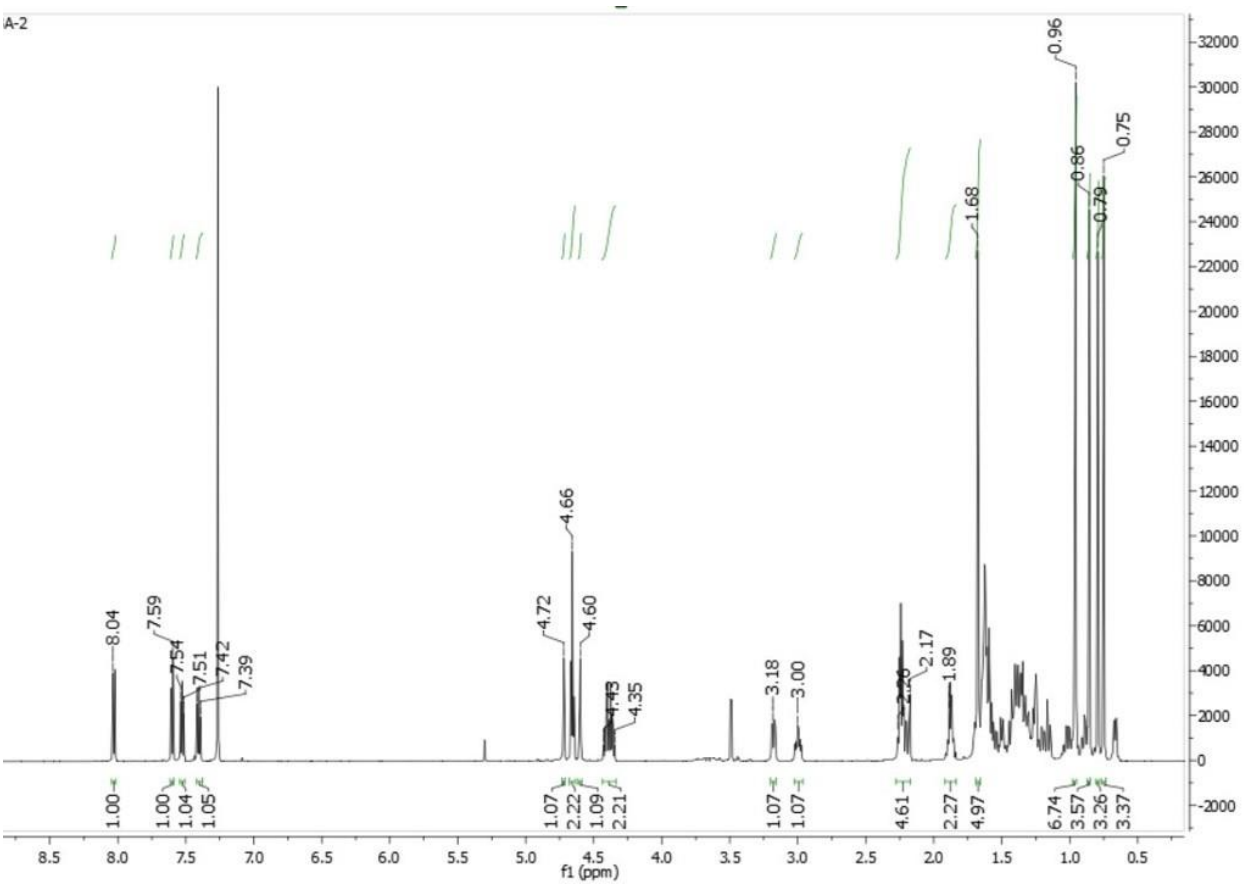


Figure S5. ^{1}H NMR spectrum ($CDCl_3$, 600 MHz) of compound **3**.



(Traces: MeOH at δ 3.5, water at δ 1.6, CH_2Cl_2 δ 5.3)

Figure S5a. Expanded view of ^{1}H NMR spectrum ($CDCl_3$, 150 MHz) of compound **3**.

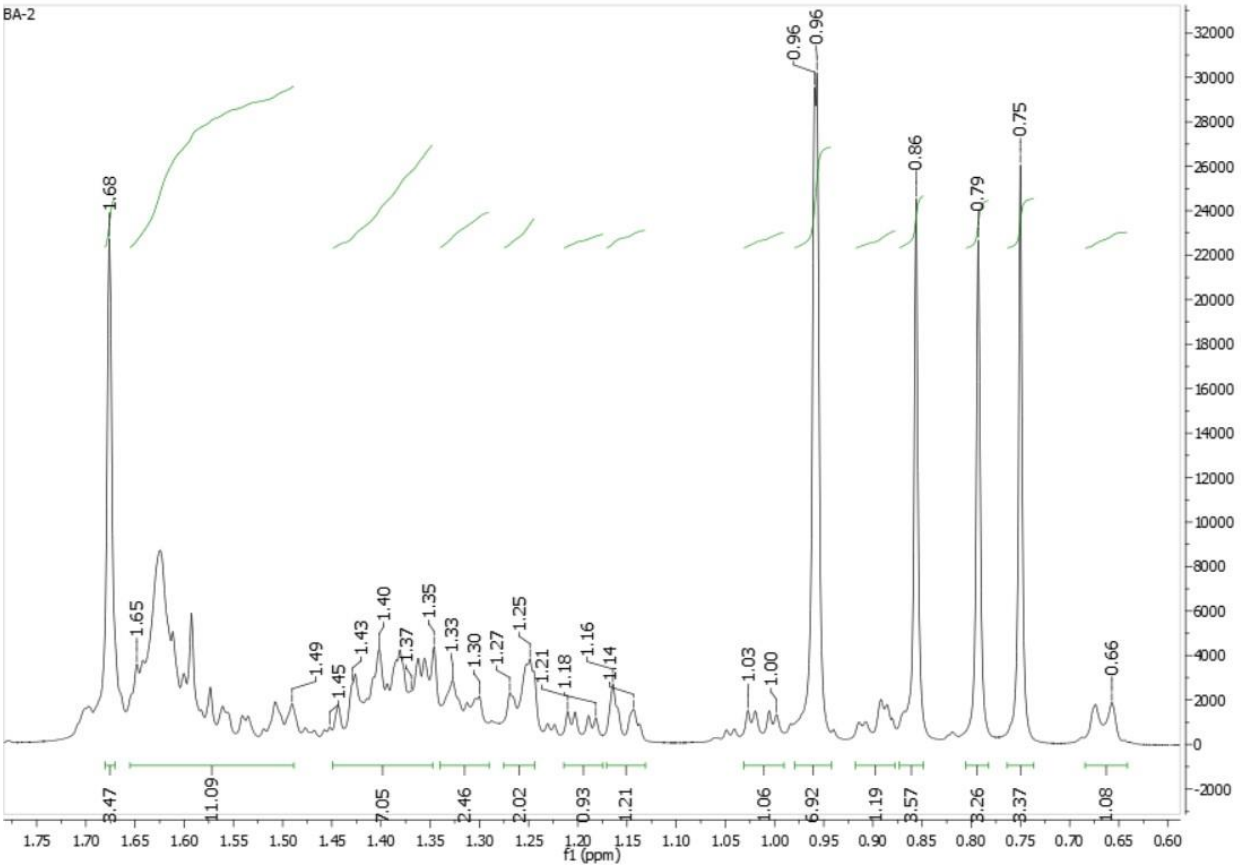


Figure S6. ^{13}C NMR spectrum ($CDCl_3$, 150 MHz) of compound **3**.

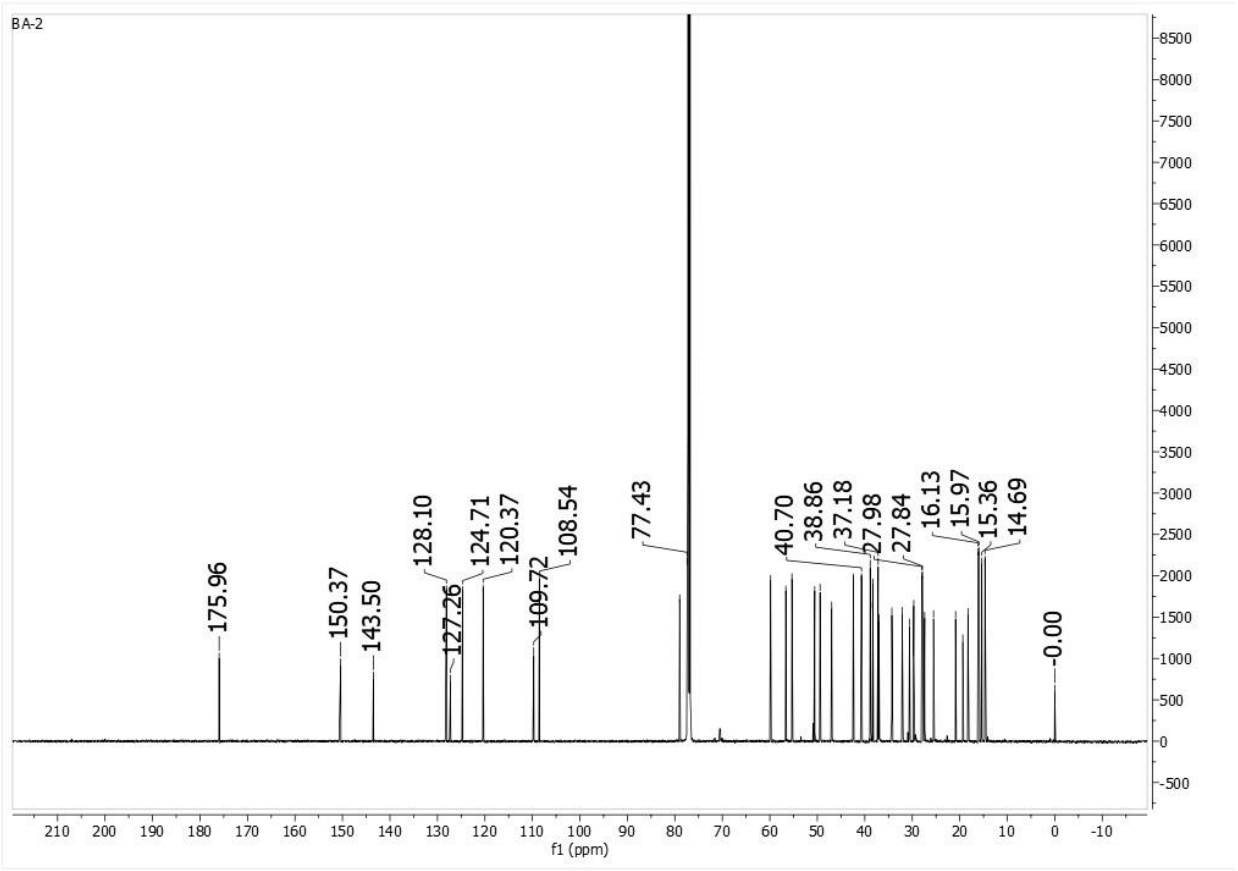


Figure S6a. Expanded view of ^{13}C NMR spectrum ($CDCl_3$, 150 MHz) of compound **3**.

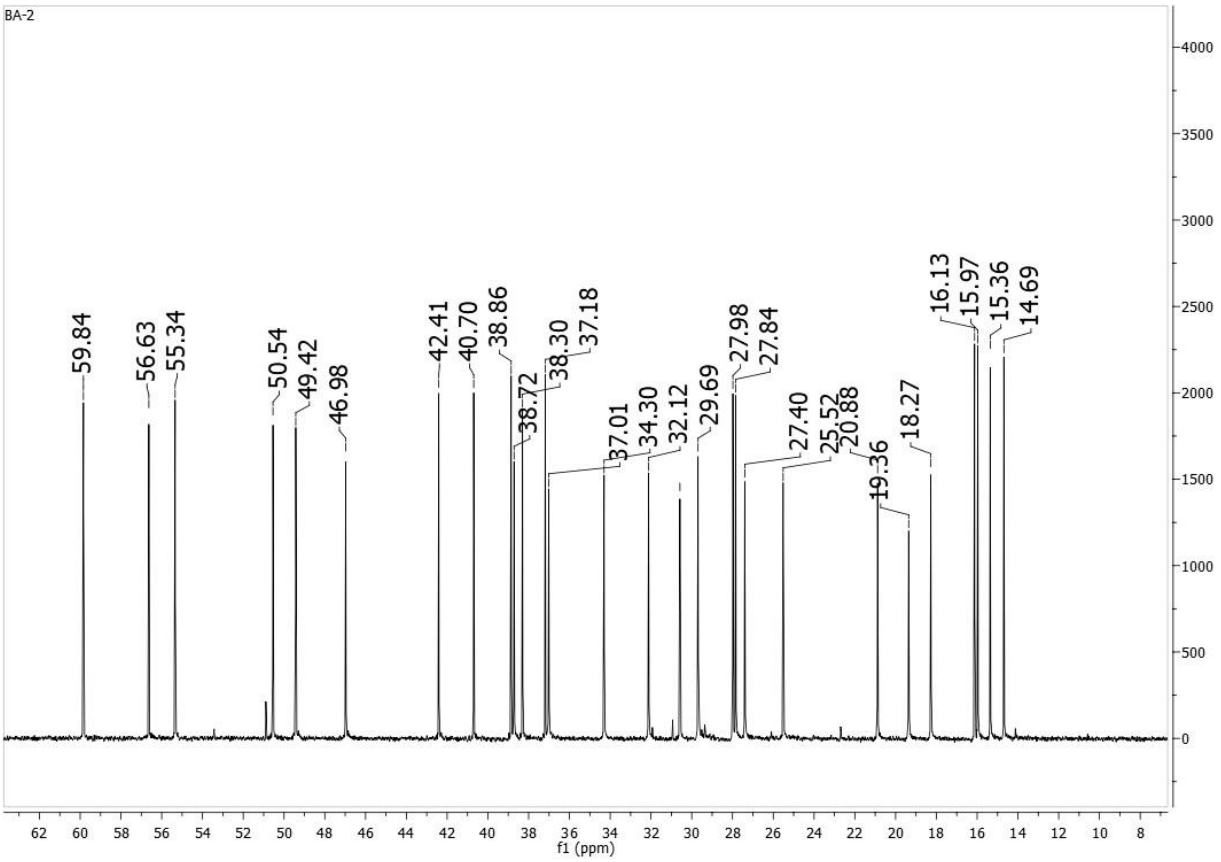


Figure S7. HSQC spectrum ($CDCl_3$, 600 MHz) of compound **3**.

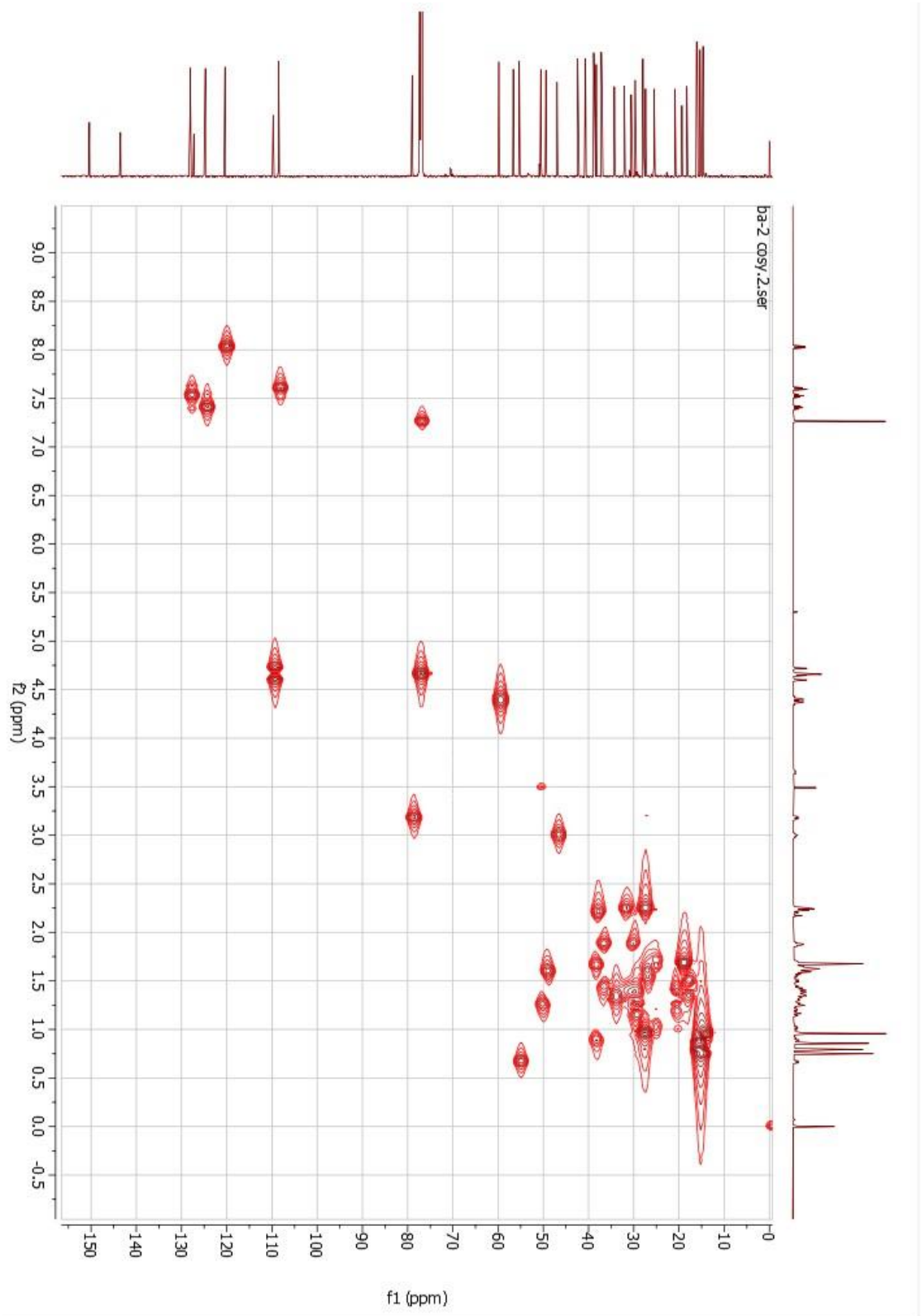


Figure S7a. Expanded view of HSQC spectrum ($CDCl_3$, 600 MHz) of compound **3**.

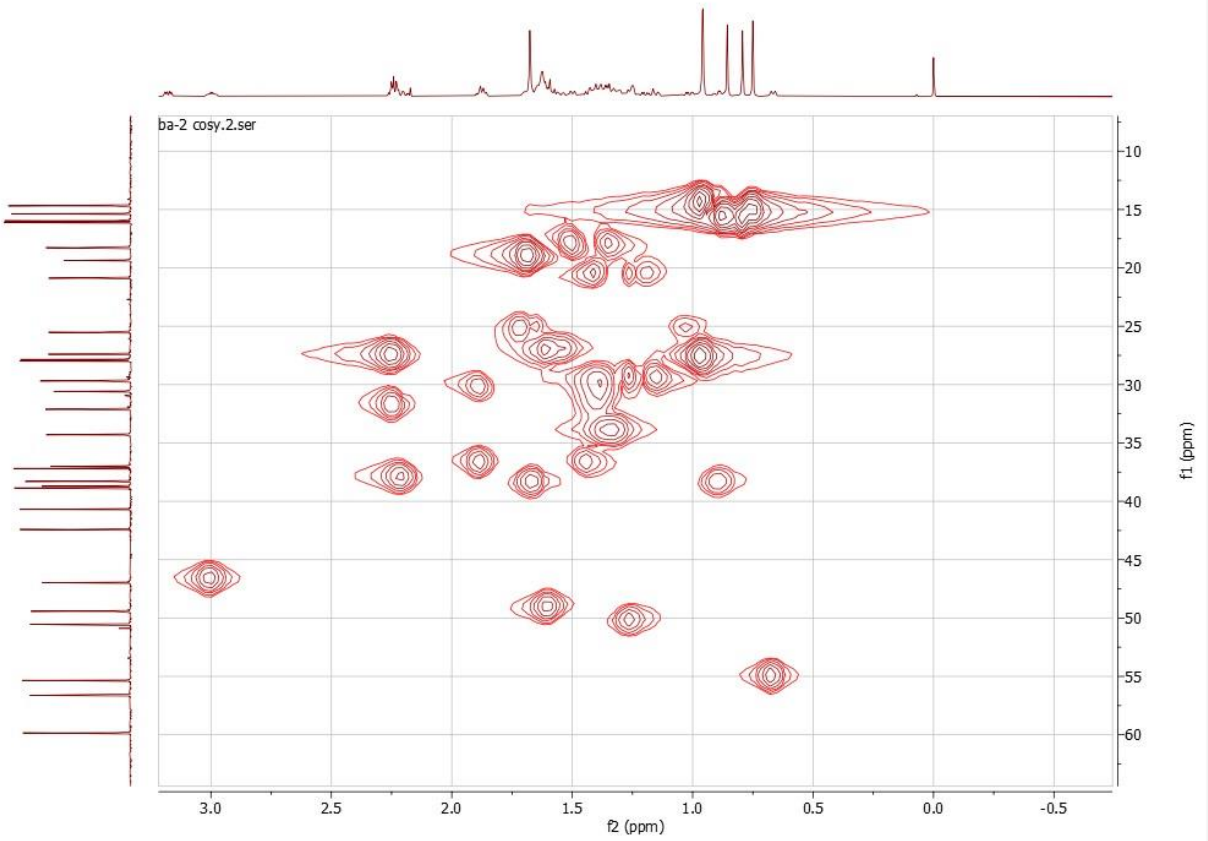
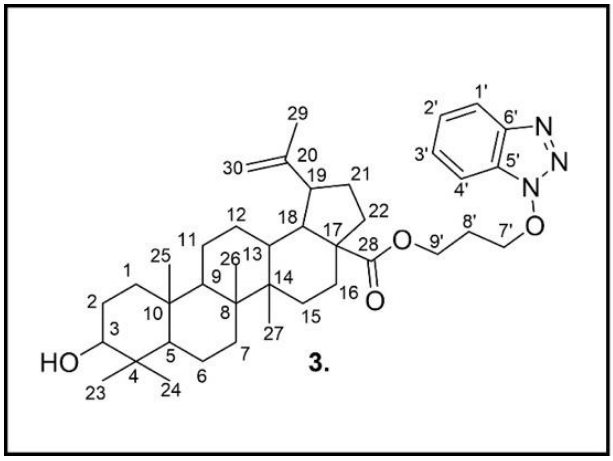
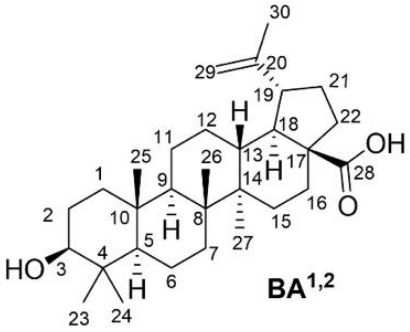


Table S1. ^{1}H and ^{13}C-nuclear magnetic spectroscopy (NMR) chemical shifts and the structure of **3**. [1,2]





^{1}H **3**	^{13}C **3**	Assignment **3**	^{1}H **BA**[2]	^{13}C **BA**[1]
0.96	14.6	C-27 (CH_3)	0.96	15
0.75	15.3	C-24 (CH_3)	0.73	15.5
0.79	15.9	C-25 (CH_3)	0.80	16.4
0.86	16.1	C-26 (CH_3)	0.91	16.7
1.35 - 1.37	18.2	C-6 (CH_2)	1.50 (6a)	18.4
1.68	19.4	C-29 (CH_3)	1.67	19.6
1.38	20.8	C-11 (CH_2)	1.37 (11a)	21
1.03	25.5	C12 (CH_2)	1.04 (12a)	25.7
1.49	27.4	C-2 (CH_2)	1.52 (2a)	27.6
2.22	27.8	C-8' (CH_2)	---	---
0.95	27.9	C-23 (CH_3)	0.95	27.9
1.14 – 1.16	29.7	C-15 (CH_2)	1.14 (15a)	30.2
1.84 - 1.91	30.6	C-21 (CH_2)	1.99 (21a)	30.6
1.25 - 1.27	32.1	C-16 (CH_2)	1.27	32.9
1.30 -1.33	34.3	C-7 (CH_2)	1.35 (7a)	34.5
1.89	37	C-22 (CH_2)	1.96 (22a)	37
----	37.1	C-10	---	37.3
2.18-2.21	38.3	C-13 (CH)	2.20	38.1
1.64	38.7	C-1 (CH_2)	1.64 (1a)	39
----	38.8	C-4	---	39.1
----	40.7	C-8	---	40.8
----	42.4	C-14	---	42.5
2.97 – 3.02	46.9	C-19 (CH)	3	49.2
1.62	49.4	C-18 (CH)	1.61	48.1
1.25	50.5	C-9 (CH)	1.25	50.7
0.66	55.3	C-5 (CH)	0.66	55.5
----	56.6	C-17	---	47.1
4.35 - 4.43	59.8	C-7'(CH2)	---	---
4.66	77.4	C-9'(CH2)	---	---
3.18	78.9	C-3 (CH-OH)	3.17	78.2
7.59	108.5	C-4'(H_{aromat})	---	---
4.60 br - 4.72 br	109.7	C-30 (CH_2=)	4.58 – 4.61	108.9
8.02	120.3	C-1'(H_{aromat})	---	---
7.39	124.7	C-3' (H_{aromat})	---	---
----	127.2	C-5' ($_{aromat}$)	---	---
7.51	128.1	C-2' (H_{aromat})	---	---
----	143.5	C-6' ($_{aromat}$)	---	---
----	150.3	C-20 (C=)	---	150.1
----	175.9	CO (C28)	---	180.2(COOH)

Figure S8. DEPT-135 spectrum ($CDCl_3$, 600 MHz) of compound **3**.

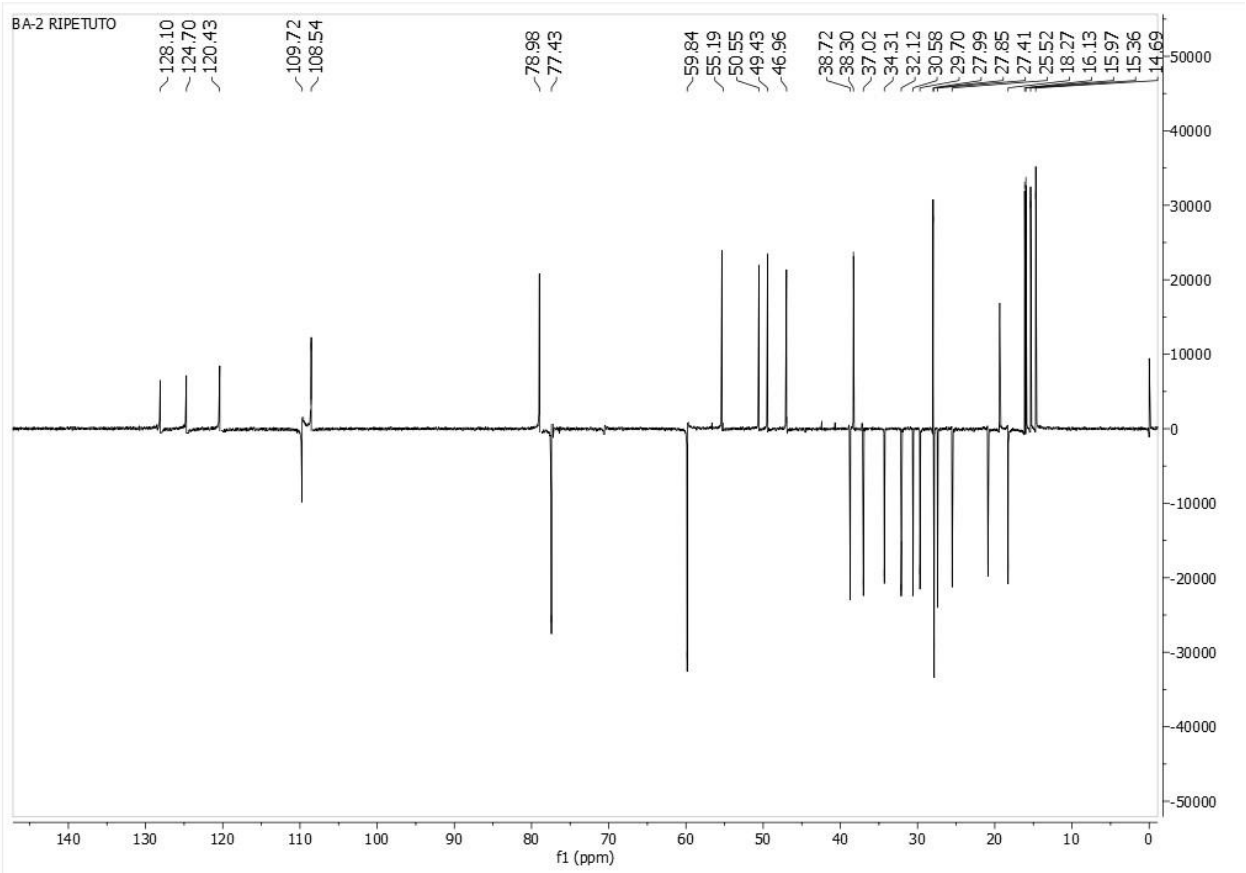
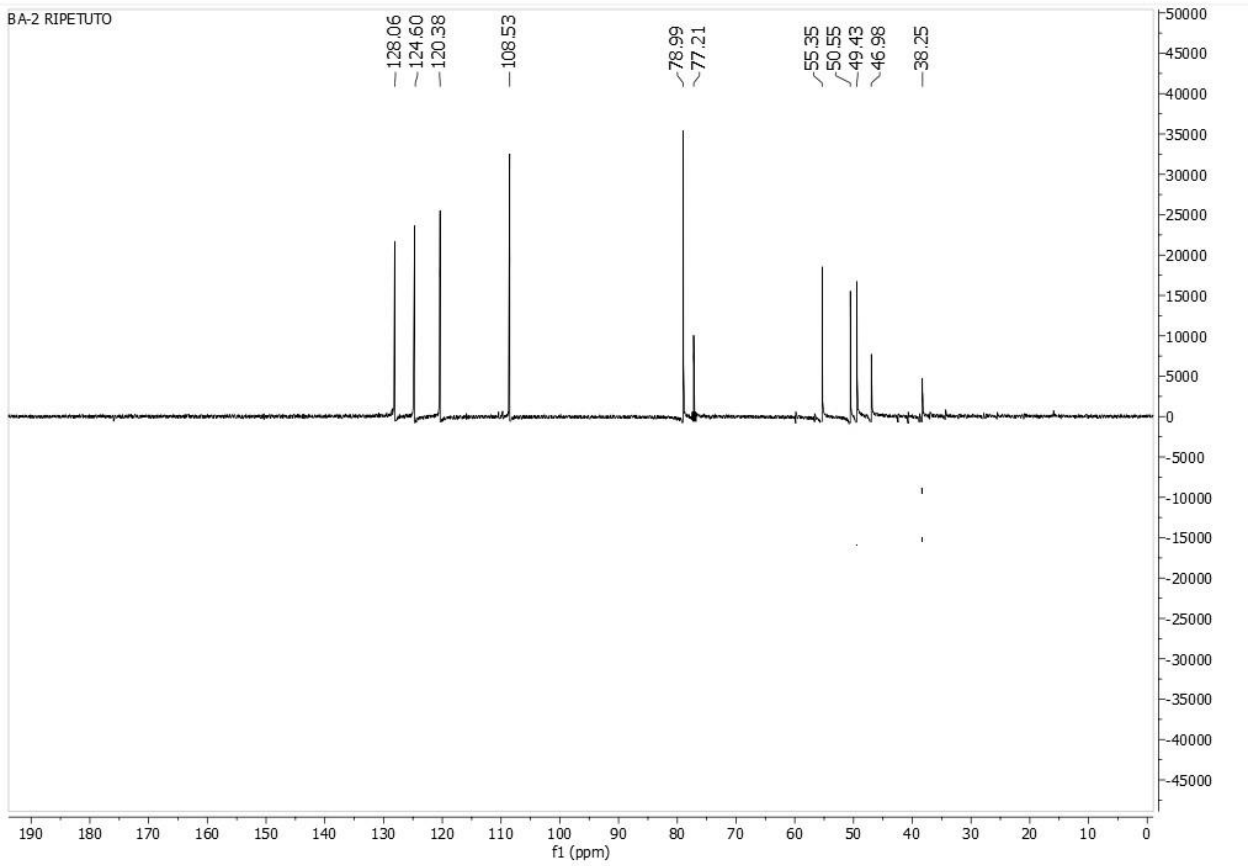
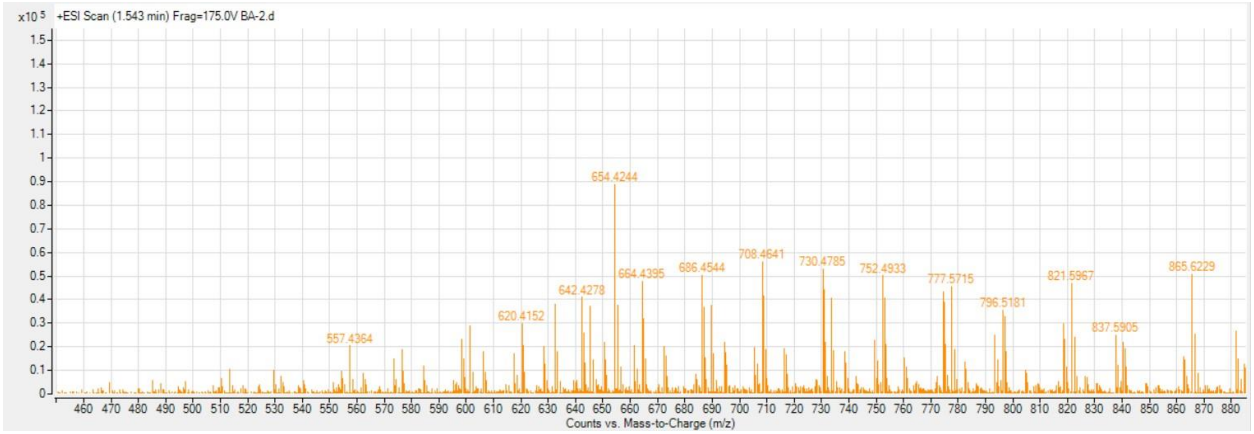


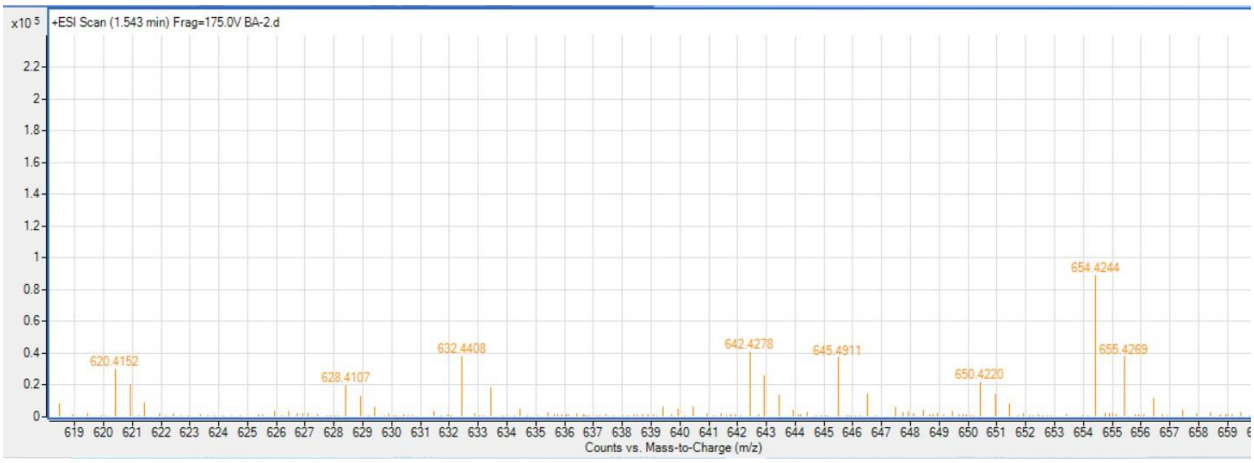
Figure S9. DEPT-90 spectrum ($CDCl_3$, 600 MHz) of compound **3**.

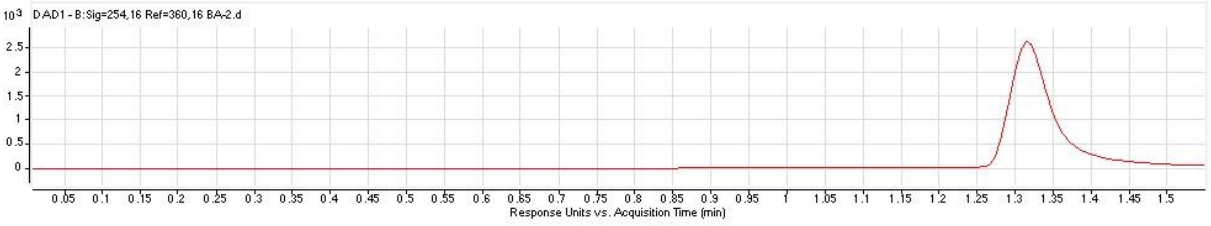


Mass spectrometry

Figure S10. Mass spectrum and UPLC-UV chromatogram (254 nm) of compound **3.**



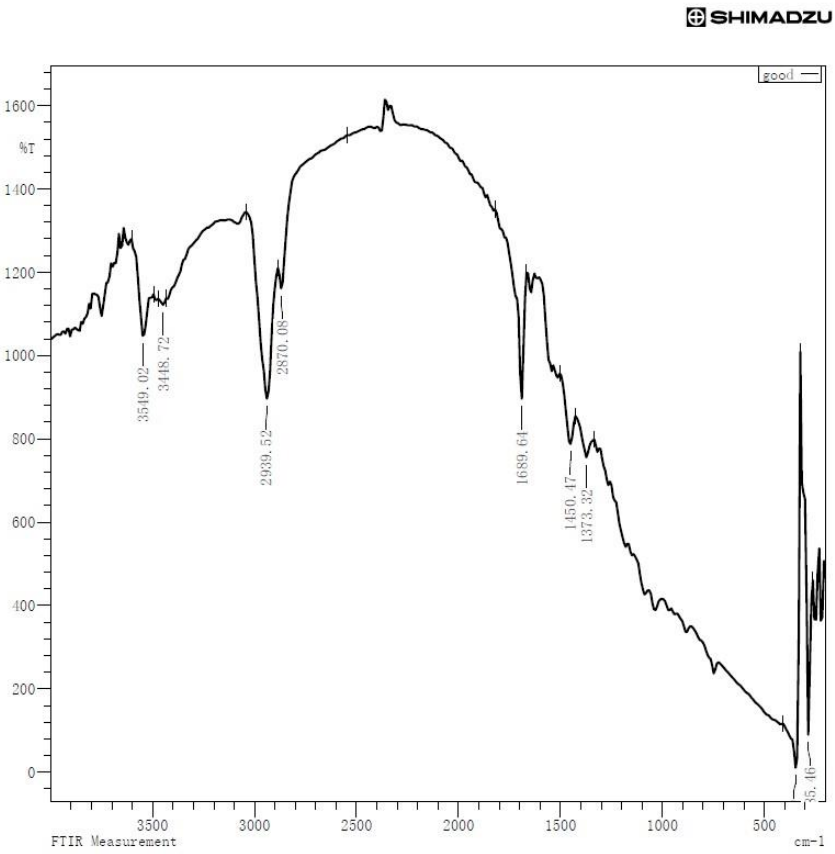




Mass spectrum and UPLC-UV chromatogram (254 nm) of compound **3**. The spectrum was recorded in positive ionization mode (ESI). Analysis was performed using a solvent mixture containing acetonitrile/water at a flow rate of 0.5 mL/min. The mobile phase was isocratic (water + 0.01% TFA; CH_3CN).

IR spectrometry

Figure S11. IR spectrum (KBr) of compound **3**.



Peak Pick Table | Point Pick Table | Pause Record

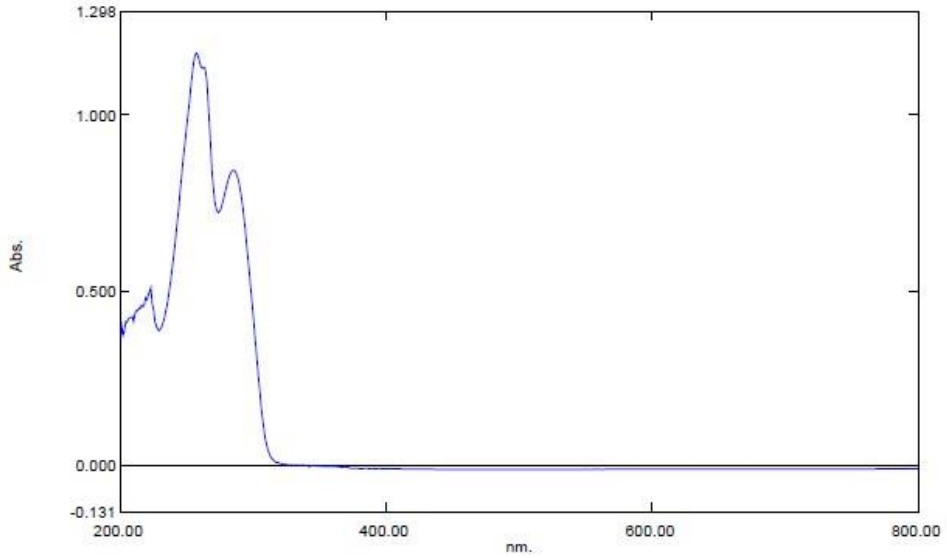
No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Area color	Comment
1	285.46	89.77	576.34	324.04	262.32	-23909.630	15263.344	Auto	
2	347.19	10.41	755.12	408.91	324.04	-3441.928	35829.170	Auto	
3	1373.32	755.75	66.07	1427.32	1334.74	-64856.682	2401.745	Auto	
4	1450.47	787.98	97.09	1504.48	1427.32	-58748.637	3401.013	Auto	
5	1689.64	896.30	324.51	1820.80	1666.50	-168804.006	12424.150	Auto	
6	2870.08	1161.45	63.27	2885.51	2546.04	-455266.402	-24275.586	Auto	
7	2939.52	896.47	360.74	3039.81	2885.51	-157318.701	24351.645	Auto	
8	3448.72	1121.83	15.15	3471.87	3433.29	-39694.826	302.169	Auto	
9	3549.02	1047.86	165.47	3603.03	3495.01	-113152.742	7102.857	Auto	

UV-VIS spectrometry

Figure S12. UV spectrum of compound **3** (range 200-400 nm in CH_2Cl_2)

Spectrum Peak Pick Report 06/02/2022 04:47:35 PM

Data Set: betulinic acid derivative - RawData



nm.

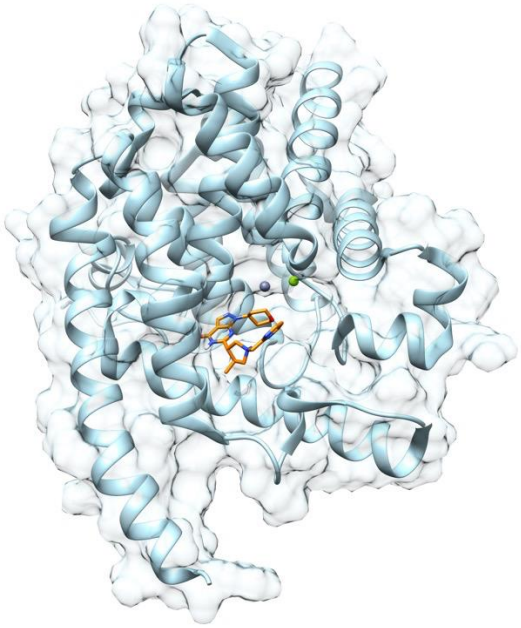
No.	P/V	Wavelength	Abs.	Description
1	T	285.00	0.843	
2	T	257.00	1.178	
3	V	274.00	0.723	
4	V	229.00	0.385	

Computational studies

Figure S13. Interaction of PF-04447943 with PDE9A

Artworks were made with Chimera [3].

(a)



(b)

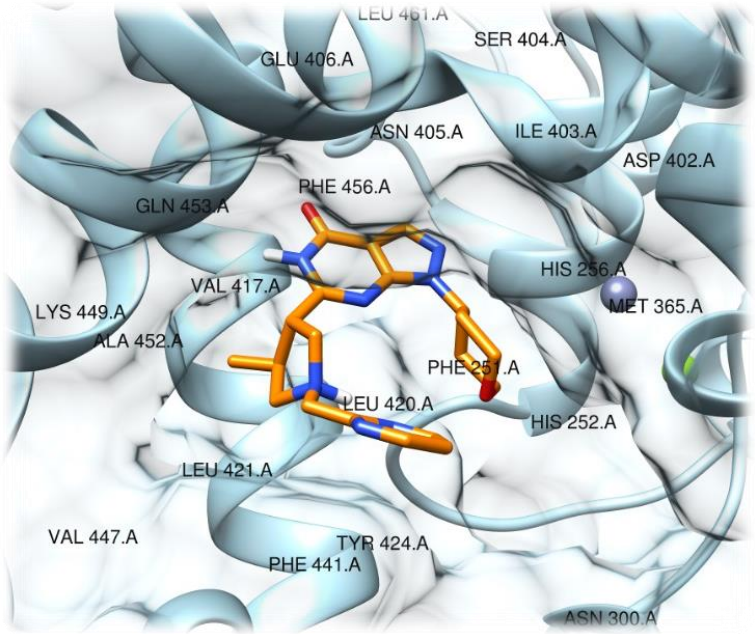
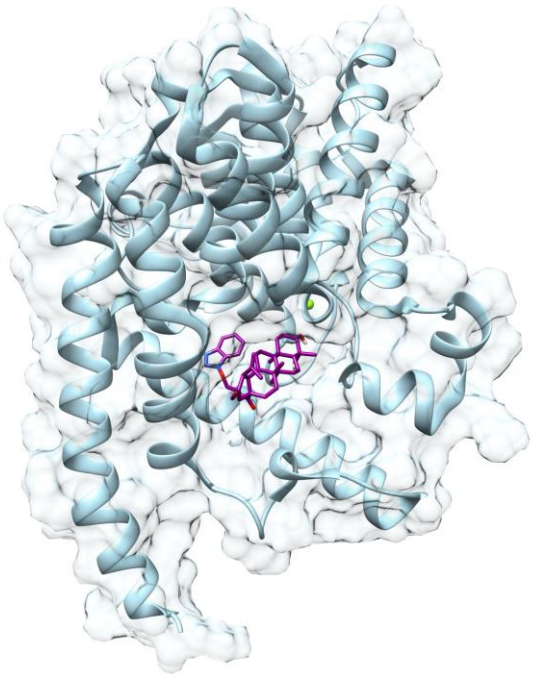


Figure S14. Interaction of compound **3** with PDE9A

Artworks made with Chimera [3].

(a)



(b)

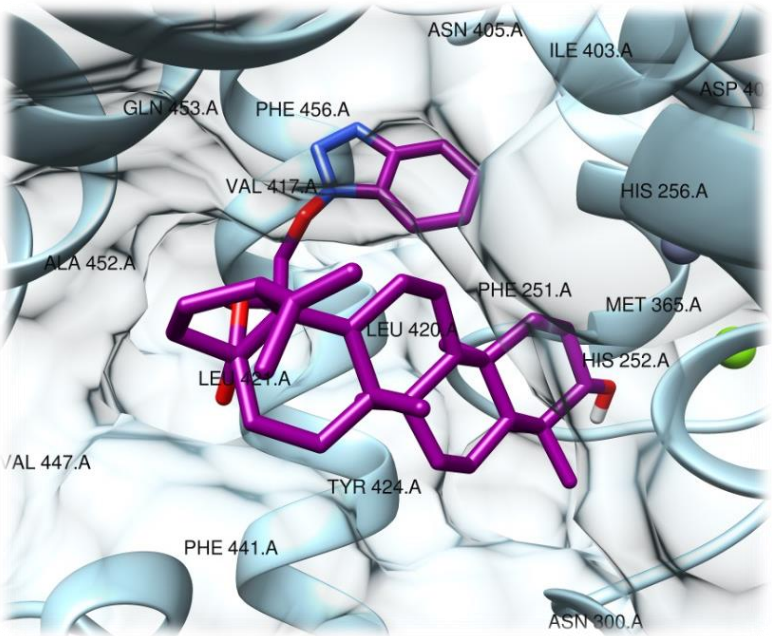


Figure S15. Residues interacting with compound 3

Interactions are depicted with different colours, as reported in the following: π- π interactions (green dashed line), hydrogen bond (blue line), and hydrophobic interaction (grey dotted line) [4].

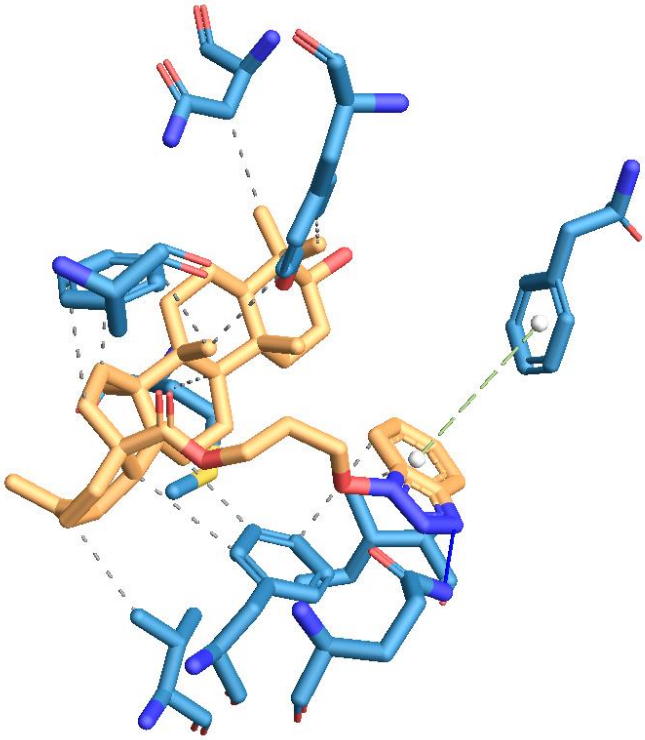


Table S2. Interactions of PF-04447943 and compound 3 with PDE9A

Interactions achieved with Protein-Ligand Interaction Profiler tool (PLIP) [4].

	Hydrophobic interactions	**Pi-stacking interactions (centdist)**	**Hydrogen bonds**
PF-04447943	Ile403 (3.95 Å) Val417 (3.87 Å) Phe456 (3.99 Å)	Phe441 (3.93 Å) Phe456 (3.85 Å)	Tyr424 (2.37/2.98Å) Gln453 (2.06/3.01 Å)
compound 3	Asn300 (3.42 Å) Met365 (3.66 Å) Ile403 (3.63 Å) Tyr424 (3.98 Å) Tyr424 (3.76 Å) Phe441 (3.83 Å) Phe441 (3.65 Å) Phe441 (3.72 Å) Phe456 (3.69 Å) Phe456 (3.12 Å) Phe456 (3.88 Å) Val460 (3.66 Å)	Phe251 (5.24 Å)	Gln453 (2.91/3.86 Å)

Table S3. Physico-chemical properties of compound 3 calculated by SwissADME

Physico-chemical properties of compound **3** were obtained with SwissADME [5].

	MW	HBA	HBD	TPSA	nRtB
Compound 3	631.89 g/mol	6	1	86.74 Å	8

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5. Daina, A.; Michielin, O.; Zoete, V. SwissADME: A Free Web Tool to Evaluate Pharmacokinetics, Drug-Likeness and Medicinal Chemistry Friendliness of Small Molecules. *Sci Rep* **2017**, *7*, 42717, doi:10.1038/srep42717.