SUPPLEMENTARY MATERIAL

Actinobacteria associated with stingless bee biosynthesize bioactive polyketides against bacterial pathogen

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Table S1. 16S rRNA identification of actinobacteria isolated from *Mellipona scutellaris* colony.

Isolates	Isolation source	NCBI accession number	Closest relative (accession number)
ICBG1307 - *Streptomyces* sp.	*Mellipona scutellaris* nurse bee	MK608316	*Streptomyces kunmingensis* (AB184597)
ICBG1313 - *Streptomyces* sp.	*M. scutellaris* nurse bee	MK608317	*Streptomyces drozdowiczii* (AB249957)
ICBG1318 - *Streptomyces* sp.	*M. scutellaris* nurse bee	MK608318	*Streptomyces albiaxialis* (AY999901)
ICBG1319 - *Streptomyces* sp.	*M. scutellaris* nurse bee	MK608319	*Streptomyces xylanilyticus* (LC128341)
ICBG1321 - *Micromonospora* sp.	*M. scutellaris* nurse bee	MK608320	*Micromonospora tulbaghiae* (Jgi.1058868)
ICBG1323 - *Streptomyces* sp.	*M. scutellaris* foraging bee	MK608321	*Streptomyces olivaceus* (JOFH01000101)
ICBG1324 - *Streptomyces* sp.	*M. scutellaris* foraging bee	MK608322	*Streptomyces rhizosphaerihabitans* (HQ267983)

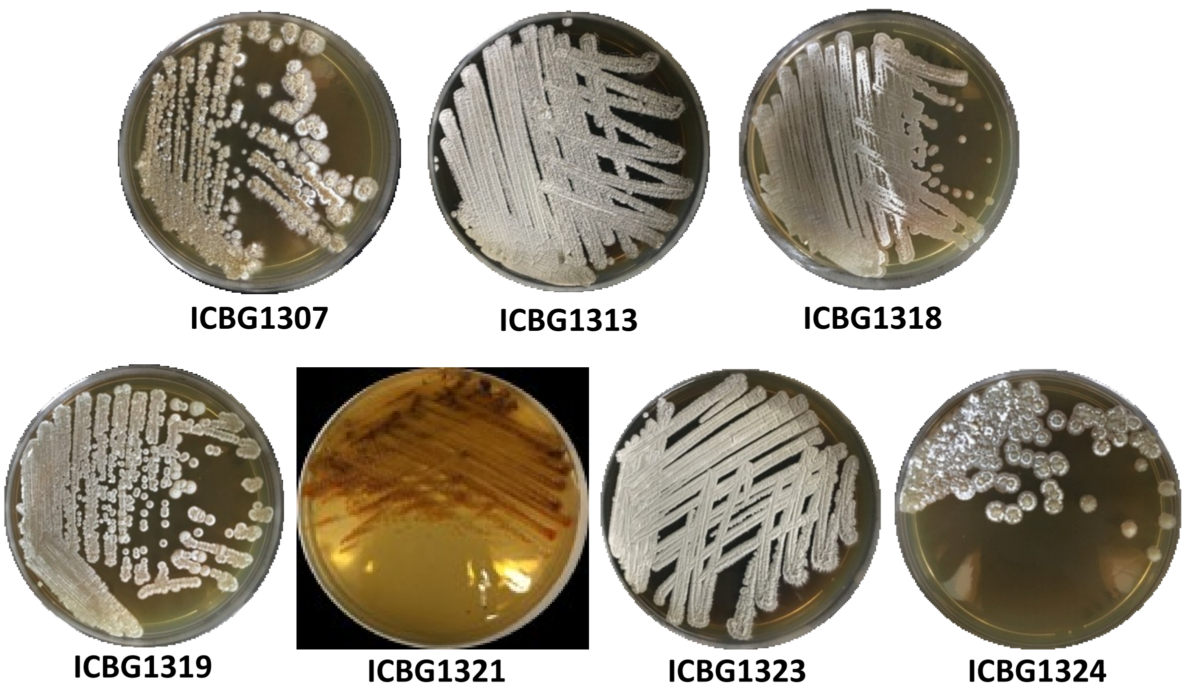


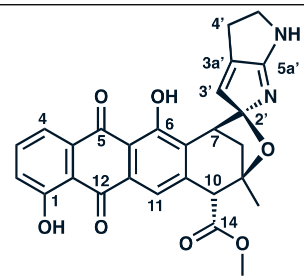
Fig. S1 Macroscopic characteristics of actinobacterial strains isolated from *M. scutellaris*, cultured in ISP-2 agar during 10 days at 30 °C (*Streptomyces* sp. ICBG1307, *Streptomyces* sp. ICBG1313, *Streptomyces* sp. ICBG1318, *Streptomyces* sp. ICBG1319, *Streptomyces* sp. ICBG1323, *Streptomyces* sp. ICBG1324, and *Micromonospora* sp. ICBG1321).

Table S2. Visual depiction of antagonist assay between isolated actinobacteria against *P. larvae* ATCC 9545, *M. anisopliae* and *B. bassiana*.

Strains	*P. larvae*	*M. anisopliae*	*B. bassiana*
Streptomyces sp. ICBG1307	0	0	0
Streptomyces sp. ICBG1313	0	0	0
Streptomyces sp. ICBG1319	0	0	0
Streptomyces sp. ICBG1324	0	0	0
Streptomyces sp. ICBG1318	3	0	0
Streptomyces sp. ICBG1323	3	0	0
Micromonospora sp. ICBG1321	3	0	0

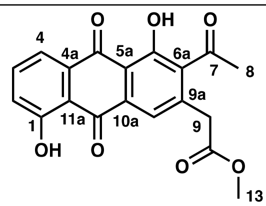
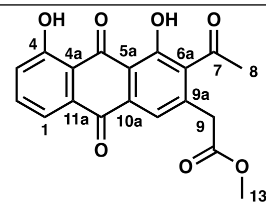
Inhibition	
3	***High*** ↑
2	
1	
0	***Low***

Table S3. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound **8** (CD_3OD).



	Compound 8	
#	**^{13}C NMR**	**^{1}H NMR (m, *J* = Hz)**
1	163.8 (C)	-
2	125.8 (CH)	7.34 (d, 8.1)
3	137.9 (CH)	7.76 (dd, 7.5; 8.1)
4	120.12 (CH)	7.82 (d, 7.4)
4a	134.67 (C)	-
5	188.8 (C)	-
5a	115.1 (C)	-
6	159.8 (C)	-
6a	134.14 (C)	-
7	44.18 (CH)	4.25 (d, 4.0)
8A	36.01 (CH_2)	2.76 (d, 12.7)
8B		2.47 (dd, 12.7; 4.0)
9	85.1 (C)	-
10	58.14 (CH)	4.07 (s)
10a	142.9 (C)	-
11	120.03 (CH)	7.66 (s)
11a	134.67 (C)	
12	188.27 (C)	-
12a	116.7 (C)	-
13	23.63 (CH_3)	1.58 (s)
14	172.1 (C)	-
15	53.05 (CH_3)	3.82 (s)
2'	116.8 (C)	-
3'	134.8	5.72 (t, 2.1)
3a'	141.4 (C)	-
4'	21.67 (CH_2)	2.85 (m)
5'	54.16 (CH_2)	4.17 (m)
5a'	168.8 (C)	-

Table S4. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for compound **13** and their isomer reported by KUNNARI et al. (1999) ($CDCl_3$).

				
	Compound 13		Kunnari et al., 1999	
#	**^{13}C NMR**	**^{1}H NMR (m, *J* = Hz)**	**^{13}C NMR**	**^{1}H NMR (m, *J* = Hz)**
1	162.8 (C)	-	119.8 (CH)	7.85 (dd, 7.4; 1.2)
1-OH	-	11.93 (s)	-	-
2	124.7 (CH)	7.34 (d, 8.4)	132.7 (CH)	7.73 (dd, 8.5; 7.4)
3	137.4 (CH)	7.73 (dd, 8.4; 7.5)	124.6 (CH)	7.34 (dd, 8.5; 1.2)
4	120.2 (CH)	7.85 (d, 7.5)	162.1 (C)	-
4-OH	-	-	-	11.94 (s)
4a	133.3 (C)	-	114.8 (C)	-
5	180.9 (C)	-	180.4 (C)	-
5a	115.6 (C)	-	115.1 (C)	-
6	160.1 (C)	-	159.5 (C)	-
6-OH	-	12.53 (s)	-	12.54 (s)
6a	135.9 (C)	-	135.5 (C)	-
7	202.8 (C)	-	202.2 (C)	-
8	31.5 (CH_3)	2.68 (s)	31.2 (CH_3)	2.96 (s)
9	38.4 5 (CH_2)	3.87 (s)	38.3 (CH_2)	3.88 (s)
9a	142.5 (C)	-	141.1 (C)	
10	122.3 (CH)	7.70 (s)	121.9 (CH)	7.71 (s)
10a	Not observed	-	132.7 (C)	-
11	181.2 (C)	-	192.1 (C)	-
11a	116.0 (C)	-	133.0 (C)	-
12	170.9 (C)	-	169.7 (C)	-
13	52.7 (CH_3)	3.71 (s)	51.9 CH_3)	3.72 (s)

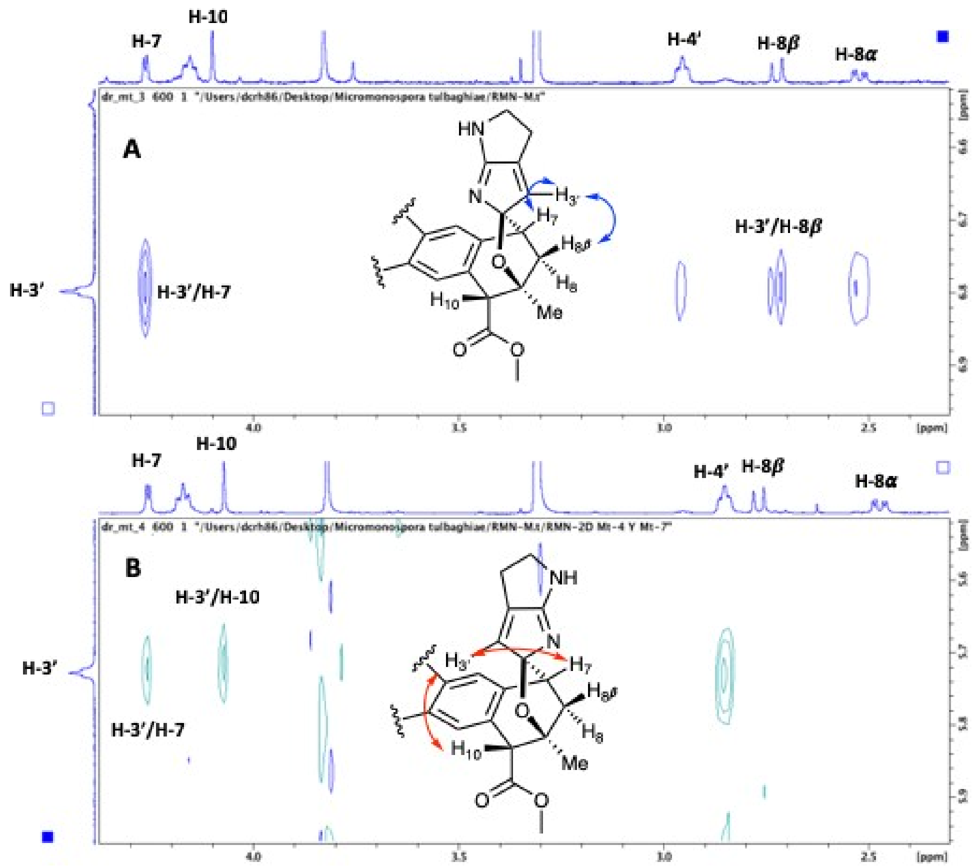


Fig. S2. NOESY correlations. A) compounds **7**; B) compound **8**.

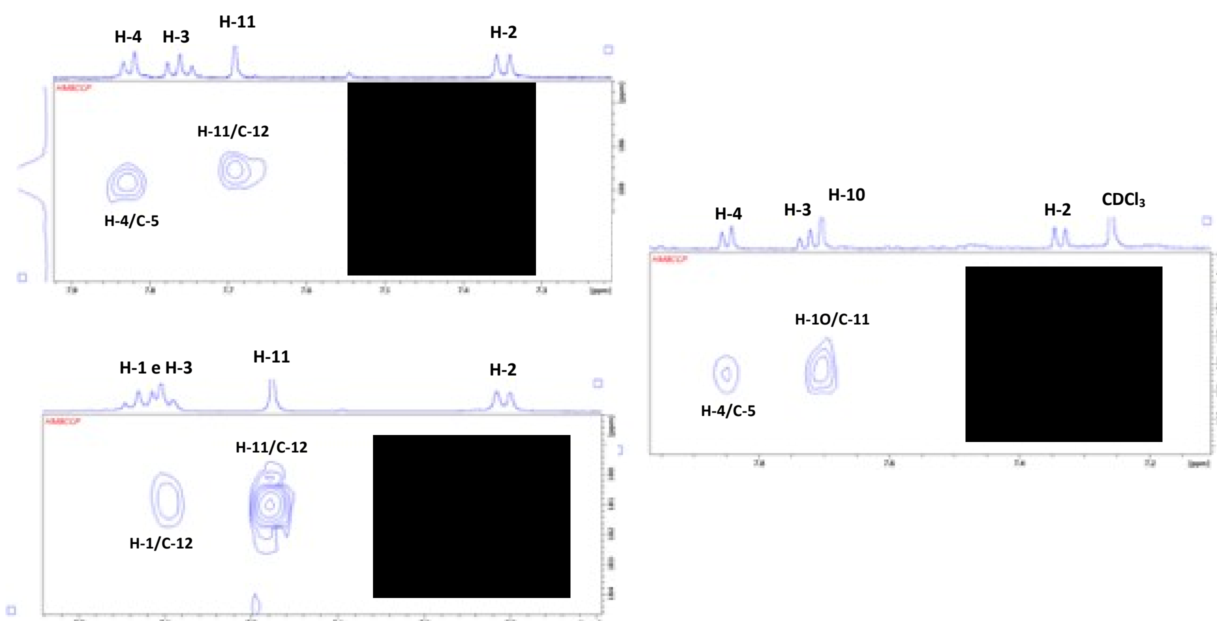


Fig. S3. *g*HMBC correlations of compounds **7**, **9** and **13**.

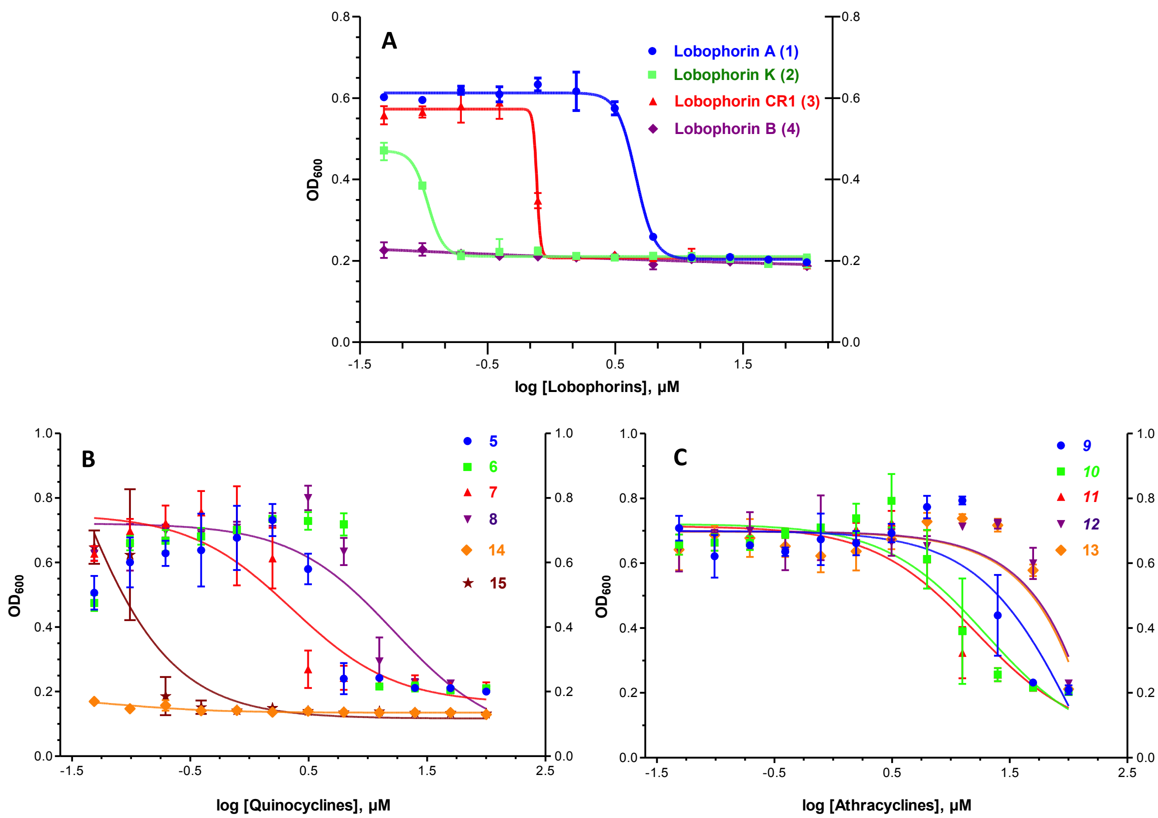


Figure S4. Dose-response curve using the isolated compounds (**1**-**15**) against *P. larvae* ATCC 9545. A) Lobophorins (**1**-**4**); B) Quinocyclines (**5**-**8** and **14**-**15**) and C) Anthracyclines (**9**-**13**). The dose-response curves log (Concentration of compounds **1**-**15**) versus OD_{600} values of *P. larvae* ATCC 9545 were obtained using GraphPad Prism 5.0 software, through of nonlinear regression analysis and were performed in triplicate.

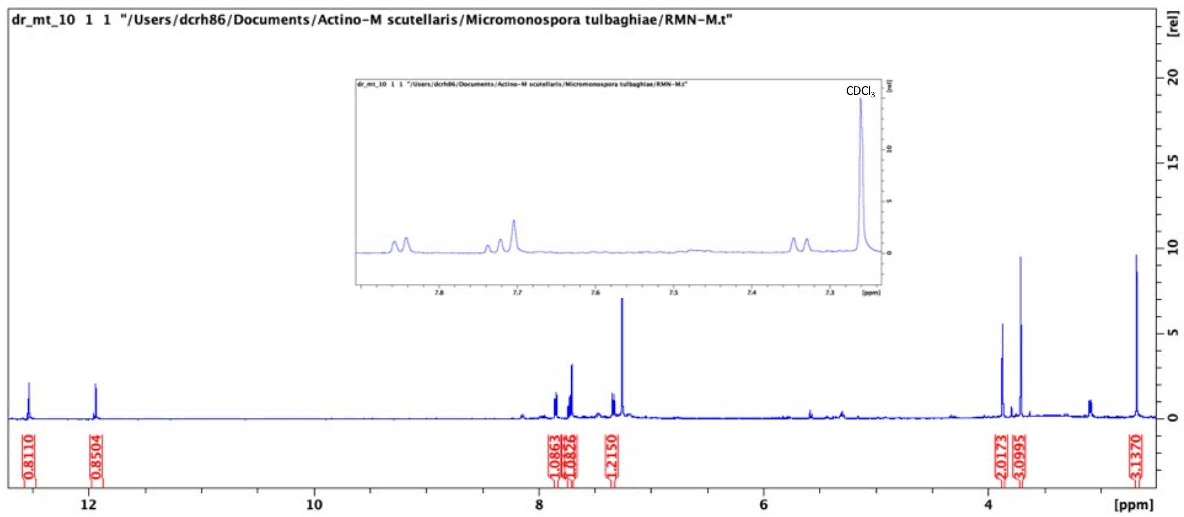


Fig. S5. ^{1}H NMR spectrum of **13** (500 MHz, $CDCl_3$).

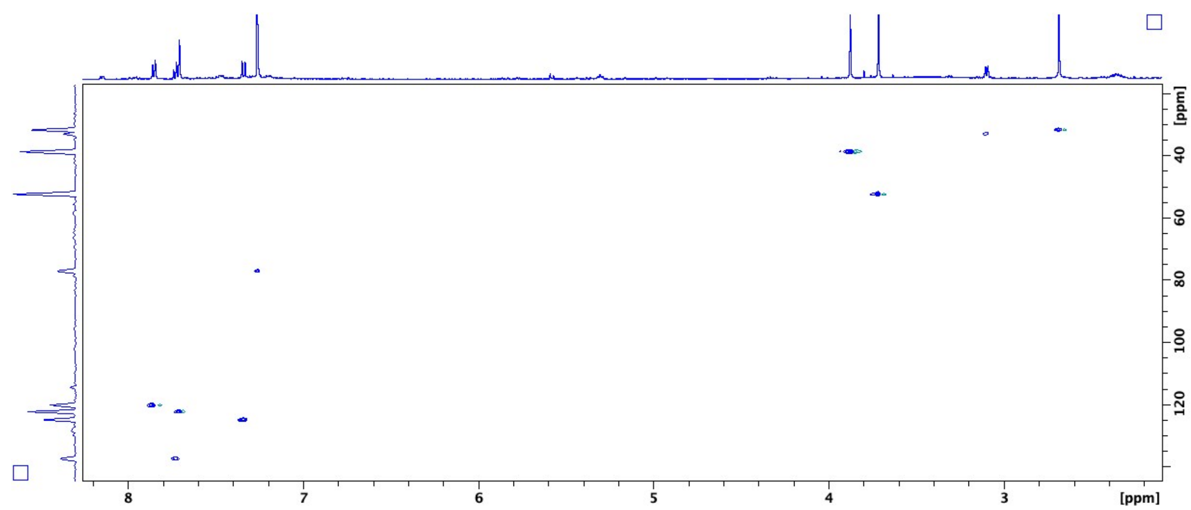


Fig. S6. *g*HSQC spectrum of **13** in $CDCl_3$.

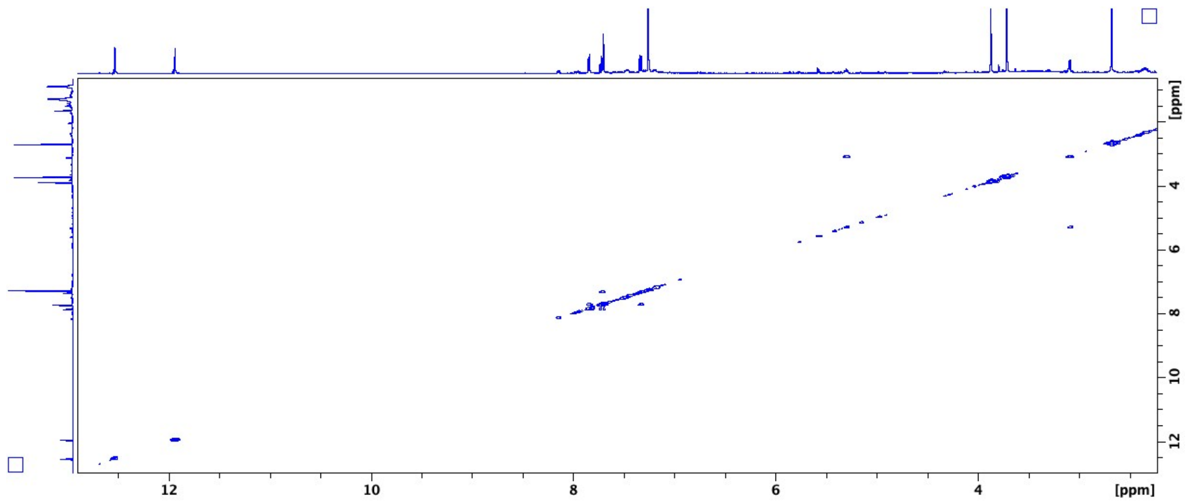


Fig. S7. *g*COSY spectrum of **13** in $CDCl_3$.

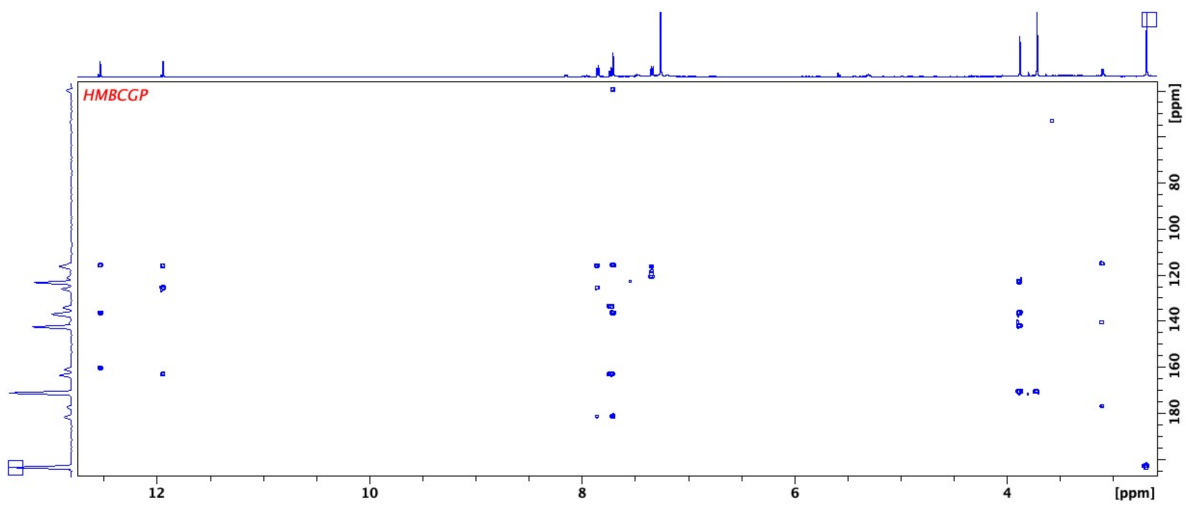


Fig. S8. *g*HMBC spectrum of **13** in $CDCl_3$.

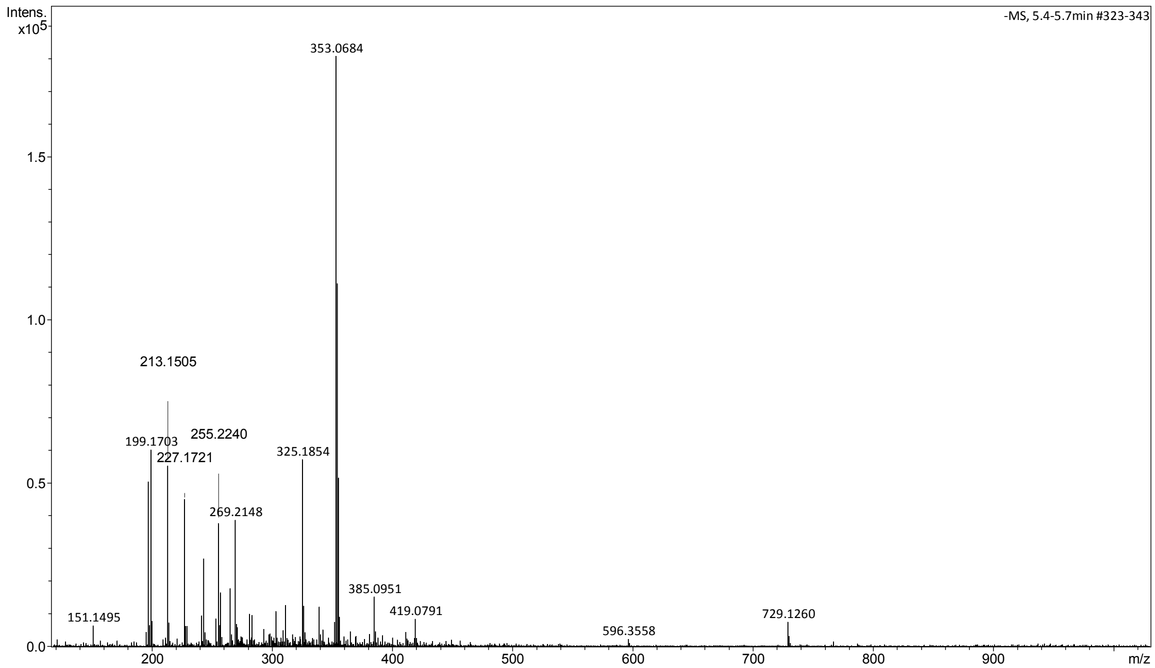


Fig. S9. The HRESIMS $[M-H]^-$ spectrum of **13**, *m/z* (experimental mass) = 353.0684 ($[M-H]^-$), *m/z* (calculated mass [calcd.]) = 353.0667 ($[M-H]^-$) consistent with the molecular formula $C_{19}H_{14}O_7$).

Notes and references

T. Kunnari, J. Kantola, K. Ylihonko, K. D. Klika, P. Mäntsälä, J. Hakala, J. Chem. Soc. Perkin. Trans., 1999, 2, 1649-1652.