SUPPLEMENTARY MATERIAL

A new procyanidin B from *Campylospermum zenkeri* (Ochnaceae) and antiplasmodial activity of two derivatives of (±)-serotobenine.

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Abstract

Phytochemical investigation of the stem bark of *Campylospermum zenkeri* led to the isolation of five known compounds: (*Z,Z*)-9,12-octadecadienoic acid (**1),** serotobenine (**2**), agathisflavone (**3**), lophirone A (**4**) and lophirone F (**5**) together with a new derivative of procyanidin B3, a catechin dimer named zenkerinol (**6**). Serotobenine (**2**) is structurally related to decursivine which shows moderate activity against D6 and W2 strains of *Plasmodium falciparum*. For a better understanding of structure-activity relationships three new semi-synthetic derivatives of serotobenine (**2**) have been prepared. These are: serotobenine monopropionate (**2a**), serotobenine monopivalate (**2b**), and serotobenine cyclohexyl ether (**2c**) respectively. Two of them (**2a)** and **(2b)**, were evaluated for their antiplasmodial activity against *Plasmodium falciparum* 3D7 strain in a parasite lactate-dehydrogenase (pLDH) assay. Compound **2b** was more active than compound **2a** based on their IC_{50} values (36.6 and 123 μM respectively).

Keywords: *Campylospermum zenkeri*; Ochnaceae; zenkerinol, flavonoids, serotobenine, hemisynthesis, antiplasmodial activity

Results and discussion (section)

Description of semisynthetic compounds

Compound **2a** was obtained as a white solid (m.p. 250-252°C), soluble in acetone. The HR-ESI-MS exhibited quasi-molecular ion peak $[M-H]^-$ at m/z 405.4307 suggesting a molecular formula of $C_{23}H_{22}N_2O_5$ (calcd. 405.4303). In the IR spectum, absorption bands at 1755, 1486, 1425, 1187 cm^{-1} were respectively attributed to C=O (ester), C-H (methyl group), C-H (methylene group), and C-O (ester) elongations. The large band at 3374 cm^{-1} usually observed for an O-H (phenol group) elongation in serotobenine IR spectra was replaced by ester elongation described above. Preliminary inspection of ^{1}H-NMR spectrum of compound **2a** showed a triplet at δ_H 1.20 and quadruplet at δ_H 2.58 ppm strongly deshielded, suggesting its attachment to a carbonyl group. The HMBC spectrum confirmed this connection between H-2″ (δ_H 2.58) and C-1″ (δ_C 172.6) as well as C-3″ (δ_C 9.4), indicating the attachment of propanoyl moiety to the free hydroxyl group of serotobenine (**2**) (Fig.2). The ^{1}H-NMR spectrum in DMSO (Table S1) also showed an ABX type of aromatic proton at δ_H 6.72, 7.25 and 7.27 ppm. That spectrum showed also in the indole moiety, an AB type of aromatic proton at δ_H 7.07 and 7.09 ppm. The proton of the pyrrole group was observed at δ_H 7.18 ppm. Apart from the signals of the propanoyl moiety, these other ones are in agreement with

those reported for serotobenine (Sato *et al*., 1985). The ^{13}C-NMR spectrum (Table S1) showed carbon atoms with resonance of amide and ester groups at δ_C 171.4 and 172.6 ppm respectively, resonances of the indole moiety at δ_C 124.9, methoxyl group at δ_C 56.3, three methylene groups at δ_C 27.6, 30.7 and 41.5 and, and a methyl group at δ 9.4 ppm belonging to the propanoyl moiety. The correlations observed in the HMBC spectrum and ^{1}H-NMR as well as ^{13}C-NMR confirmed the proposed structure of **2a** (Fig. S1 and S2) identified as a new derivative of serotobenine (**2**).

Compound **2b** was obtained as a white solid (m.p. 269-272°C) soluble in acetone. The HR-ESI-MS exhibited quasi-molecular ion peak $[M-H]^-$ at m/z 433.4845 suggesting a molecular formula of $C_{25}H_{26}N_2O_5$ (calcd. 433.4833). Its IR spectum displayed absorption bands at 1738 (C=O, ester), 1157 (C-O, ester) and 1346 (C-H, *tert*-butyl group) cm^{-1}. Compared to compound **2**, the ^{1}H-NMR spectrum of compound **2b** showed a signal at δ_H 1.35 (*s*) attributable to a *tert*-butyl unit; the latter was confirmed by a chemical shift at δ_C 27.3 ppm from the ^{13}C-NMR spectrum which also showed peaks of one quaternary carbon at δ_C 39.4 and of an ester group at δ_C 176.4 (Table S1). Selected HMBC correlations between H-3″ (δ_H 1.35) and C-2″ (δ_C 39.4), H-3″ and C-1″ (δ_C 176.4), and the methoxyl proton (δ_H, 3.82) and C-3' (δ_C 153.5) suggest the occurrence of a *tert*-butylmethanoate group in serotobenine skeleton indicating the proposed structure of **2b** (Fig. S1).

Compound **2c** was obtained as a white solid, soluble in acetone. The HR-ESI-MS exhibited pseudo-molecular ion peak $[M+Na]^+$ at m/z 455.4987 suggesting a molecular formula of $C_{26}H_{28}N_2O_4$ (calcd. 455.5001). As compound **2**, compound **2c** showed additional cyclohexyl protons signals between δ_H 1.39 and 3.50 from ^{1}H-NMR spectrum, and between δ_C 24.0 – 69.2 from the ^{13}C-NMR giving emphasis to the occurrence of a cyclohexyl unit together with an ether group (Table S1). The correlations observed in the HMBC spectrum (Fig. S1) between H-1″ (δ_H 3.50 ppm) respectively with C-4′ (δ_C 134.0) and C-2″ (δ_C 28.2) confirmed the proposed structure of **2c** recognised as another new derivative of serotobenine (**2**) (Fig. S1).

Experimental section (Annex)

Hemisynthesis reactions

Esterification of serotobenine with propionic anhydride

rac-Serotobenine **2** (5 mg, 14.29 µmol) was dissolved in pyridine (0.83 mL) and propionic anhydride (0.75 mL) at 0°C (Koizumi *et al.,* 2008). The solution was allowed to warm to 22°C overnight. Then, an aqueous hydrochloric acid (1M, 5 mL) was added, layers were separated and the aqueous layer was extracted with tert-butylmethyl ether (2x10 mL). The combined extracts were washed with sodium hydrogen carbonate (1M, 10 mL) and brine (10 mL). The organic layer was dried with magnesium sulfate, filtered and the solvent was removed at evaporator under reduced pressure. The residue was purified by CC SiO_2 ($CHCl_3$/MeOH :15:1), giving a white solid compound named serotobenine monopropionate **2a** (5.5 mg, 95%) (scheme 1)

Esterification of serotobenine with pivalic anhydride

rac-Serotobenine **2** (5 mg, 14.29 µmol) was dissolved in pyridine (0.83 mL) and *pivalic* anhydride (1.00 mL) at 0°C. Thereafter, 4-diethylaminopyridine (0.17 mg, 1.429 µmol) was added as a catalyst. The solution was allowed to stirr at room temperature (22°C) overnight. Then, an aqueous hydrochloric acid (1M, 5 mL) was added, layers were separated and the aqueous layer was extracted with tert-butylmethyl ether (2x10 mL). The combined extracts were washed with sodium hydrogen carbonate (1M, 10 mL) and brine (10 mL). The organic layer was dried with magnesium sulfate, filtered and the solvent was removed under reduced pressure. The residue was purified by CC SiO_2 ($CHCl_3$/MeOH: 15:1) to afford a white solid compound named serotobenine monopivalate **2b** (5.8 mg, 93%) (Scheme 1).

Etherification of serotobenine with cyclohenanol via a Mitsunobu reaction

rac-Serotobenine **2** (10 mg, 28.56 µmol) was added to a solution of cyclohexanol (5.72 mg, 57.12 µmol), tributylphosphine (9.5 µL, 57.12 µmol) and in anhydrous THF under N_2 atmosphere at 0°C. The resulting solution was treated with DIAD (11.2 µL, 57.12 µmol) and the reaction mixture was continuously stirred at room temperature up to completion of the reaction. The solvent was evaporated and the residue dissolved in tert-butylmethyl ether (2x3

mL). The combined extracts were washed with sodium hydrogen carbonate (1M, 2 mL) and brine (3 mL). The organic layer was dried with magnesium sulfate, filtered and the solvent was removed under reduced pressure. The residue was purified by CC SiO_2 ($CHCl_3$/MeOH: 50:1) to afford a white solid compound, serotobenine cyclohexyl ether (2.5 mg, 20 %). (Scheme 1)

Table S1: ^{1}H and ^{13}C-NMR spectroscopic data of compounds **2, 2a, 2b** and **2c** (500, and 125 MHz in acetone d_6) δ in ppm.

	2			**2a**			**2b**			**2c**		
N°	δ_C	δ_H ; m	HMBC (C→H)	δ_C	δ_H ; m ; J	HMBC (C→H)	δ_C	$\delta_{H,}$ m	HMBC (C→H)	δ_C	δ_H, m	HMBC (C→H)
1		10.17			10,17			10,21			10,28	
2	125.6	7.17 ; s		124,9	7,18 ; s		124,9	7,19 ; s		125,6	7,17 ; s	
3	112.2			112,3			112,2			112,2		
3a				125,9			123,7			123,8		
4	114.4		H-C(8′)	115,0		H-C(8′)	114,7		H-C(8′’)	115,0		
5	148.8			152,4			152,2			151,9		
6	111.2	7.27 ; d		123,6	7,07 ; d ; 7.0		123,4	7,08 ; d		119,6	7,00 ; d	
7	104.5	6.70 ; d		119,1	7,09 ; d ; 7.0		118,9	7,10 ; d		117,6	7,13 ; d	
7a	134.4			144,8			140,6			147,8		
8a	30,7	3,04-3,09; m		30,7	3,05-3,10; m ;		30,5	3,12-3,09 ; m		29,0	3,10-3,05 ; m	
8b	30,7	3,14-3,19; m		30,7	3,15-3,18 ; m		30,5	3,16-3,15 ; m		29,0	3,18-314 ; m	
9a	41,5	3,55-3.59 ; m		41,5	3,58 ; m		41,3	3,60-3,57 ; m		41,4	3,51-3,49 ; m	
9b	41,5	4.10-4.18; m		41,5	4,17 ; m		41,3	4,17-4,12 ; m		41,4	4,16-4,11 ; m	
10		8.17 ; 1H			8.01 ; 1H			8,03 ; 1H			8,02, 1H	

1′	147.2		H-C(5′)	141,9		H-C(8′)	141,6		H-C(6′) ; H-C(2′)	136,2		H-C(8′)
2′	110.7	7.12 ; d		111,3	7,25 ; d		111,1	7,26 ; d		111,7	7,25 ; d	
3′	153.7			153,6		MeOH	153,5			153,7		MeOH
4′	133.9		H-C(5′)	134,1		H-C(5′)	133,9		H-C(5′)	134,0		
5′	120.1	6.85d		105,4	6,72 ; d ; 9.3		105,3	6,74, d		105,4	6,69, d	
6′	115.1	6.95 ; dd		125,6	7,27 ; dd ; 9.3		125,7	7,29 ; dd		125,8	7,26 ; dd	
7′	85.4	6.32 ; d	H-C(8′) ; H-C(2′)	85,0	6,40 ; d ; 10.5	H-C(6′)	84,9	6,41 ; d	H-C(8′) ; H-C(6′)	85,3	6,34 ; d	
8′	55.2	4.73 ; d		55,4	4,78 ; d ; 10.5		55,2	4,78 ; d		55,2	4,78 ; d	
9′	171.5		H-C(8′) ; H-C(7′)	171,4		H-C(8′)	171,3			171,5		H-C(8′)
1″				172,6		H-C(2″)	176,4		H-C(3″)	69,2	3,50 ; m	
2″				27,6	2,58 ; q	H-C(3″)	39,4		H-C(3″)	28,2	1,67-1,61 ; m	H-C(4″)
3″				9,4	1,20 ; t	H-C(2″)	27,3	1,35 ; s		23,8	1,50-1,54 ; m	H-C(4″)
4″										24,0	1,45-1,39 ; m	
O-CH_3	56.2	3.83 ; s		56,3	3,82 ; s		56,1	3,83 ; s		56,2	3,80, s	

Table S2: ^{1}H and ^{13}C-NMR spectroscopic data of compound **6** (500 and 125 MHz in DMSO) δ in ppm

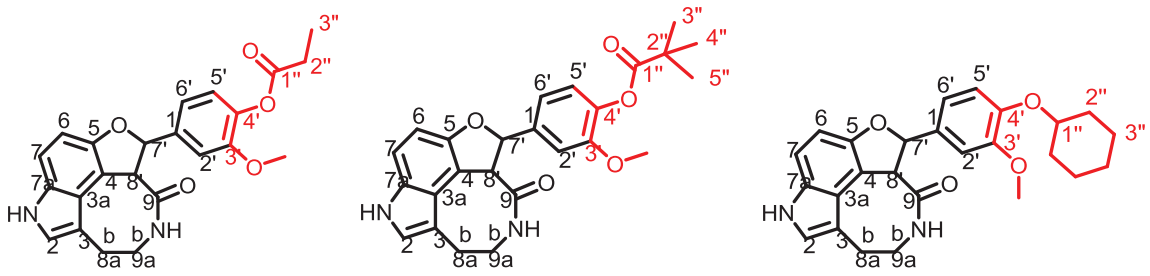
Position	δ_C	δ_H (-OH) ; m ; J (Hz)	HMBC (C→H)
1	/	/	
2	140 .0	/	H-C(2′)
3	133.1	/	H-C(4)
4	48.8	3.18 ; s	OH-C(3)
4a	99.9	/	H-C(3); H-C(6); H-C(8)
5	156.5*	/	H-C(6)
6	95.4	5.88 ; d ; 2.3	/
7	156.5*	(8.90) OH	H-C(6); HO-C(7)
8	94.4	5.71 ; d ; 2.3	H-C(6)
8a	156.0*	/	H-C(8); OH-C(7)
1′	120.2	/	H-C(2′)
2′	115.2	6.89 ; d ; 1.7	H-C(6′) ; OH-C(3′)
3′	144.8	(8.80) OH	H-C(2′) ; OH-C(3′)
4′	144.7	(8.74) OH	H-C(5′) ; OH-C(4′)
5′	115.2	6.65 ; d ; 7.2	H-C(6′) ;OH-C(4′)
6′	118.2	6.66 ; d ; 7.2; 1.7	H-C(2′) ;H-C(5′)
1″	/	/	/
2″	78.3	4.65 ; d ; 9.5	H-C(2‴)
3″	65.1	3.91 ; m ; 9.5; 4.2	H-C(4β″) ;H-C(4α″)
4″	28.5	2.46 ; dd ; 16.7 ; 4.2 ; 2.66 ; dd ; 16.7 ; 5.9	H-C(2″)
4a″	99.7	/	H-C(4β″) ;H-C(4α″)
5″	156.8	(9.06) OH	H-C(4β″) ;H-C(4α″); OH-C(5″)
6″	98.8	6.18 ; s	OH-C(5″)
7″	156.0*	/	H-C(6″) ; OH-C(4)
8″	102.4	/	H-C(4) ; H-C(6″)
8a″	156.5*	/	/
1‴	130.9	/	H-C(2″); H-C(5‴)

2‴	115.2	6.89 ; d ; 1.7	H-C(2″) ; H-C(6‴)
3‴	144.8	(8.80) OH	H-C(2‴) ; OH-C(3‴)
4‴	144.7	(8.74) OH	H-C(5‴) ; OH-C(3‴) ; OH-C(4‴)
5‴	115.2	6.65 ; d ; 7.2	H-C(6‴) ; OH-C(4‴)
6‴	118.2	6.66 ; d ; 7.2; 1.7	H-C(2‴) ; H-C(5‴)

* Signals can be interchanged.

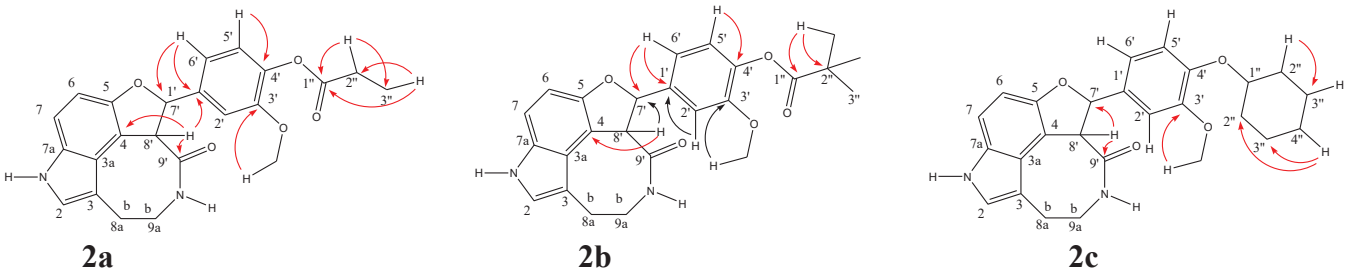
Table S3. Inhibition-values of **2a** and **2b** against *P. falciparum*. Inhibition is shown in %. SD is the standard deviation. IC_{50} values of compounds **2a** and **2b** are respectively 123.2 µM and 36.6 µM.

Substance	**2a**		**2b**	
Conc [µM]	Mean	SD	Mean	SD
100	45.4	13.03	85.8	11.74
10	0.0	11.12	4.7	7.544
1	4.3	6.783	4.4	9.432
0.1	6.1	3.759	3.6	8.462
0.01	6.8	2.012	3.0	7.66



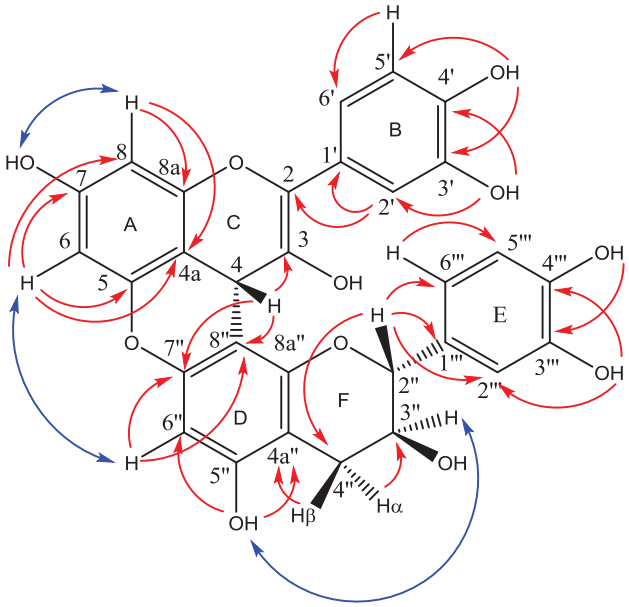
2a **2b** **2c**

Figure S1: Chemical structures of hemisynthetic derivatives of serotobenine (**2a-2c**)



2a **2b** **2c**

Figure S2: Selected HMBC correlations of compounds **2a**, **2b** and **2c.**



Sub-structure I

Sub-structure II

Figure S3: Selected HMBC (⟶) and NOESY (⟷) correlations of compound **6**

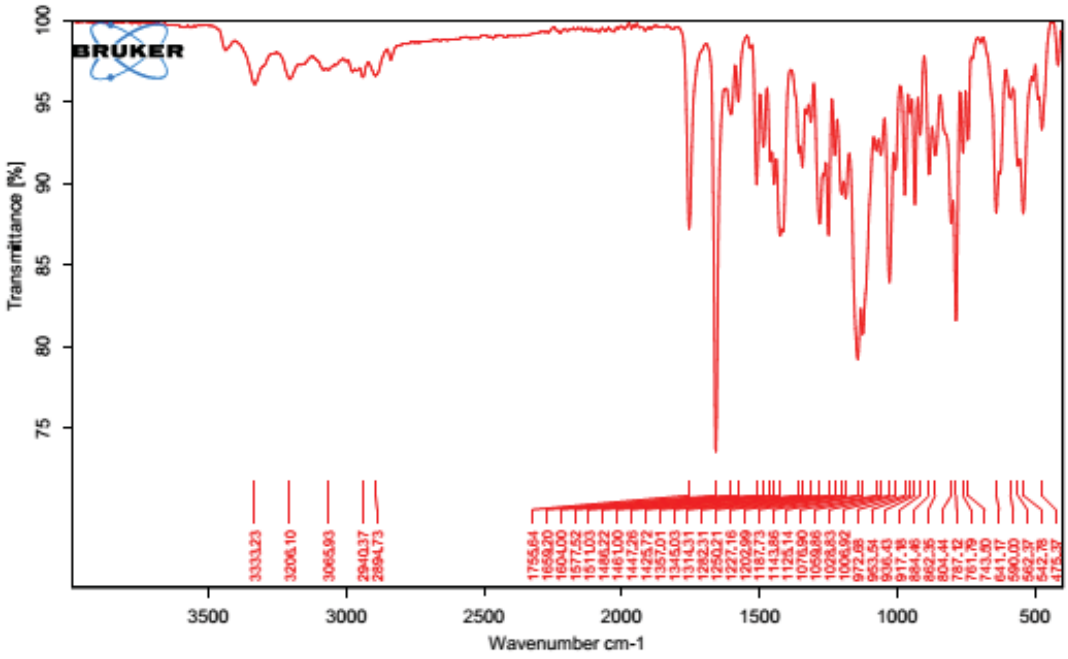


Figure S4: IR spectra of compound **2a**

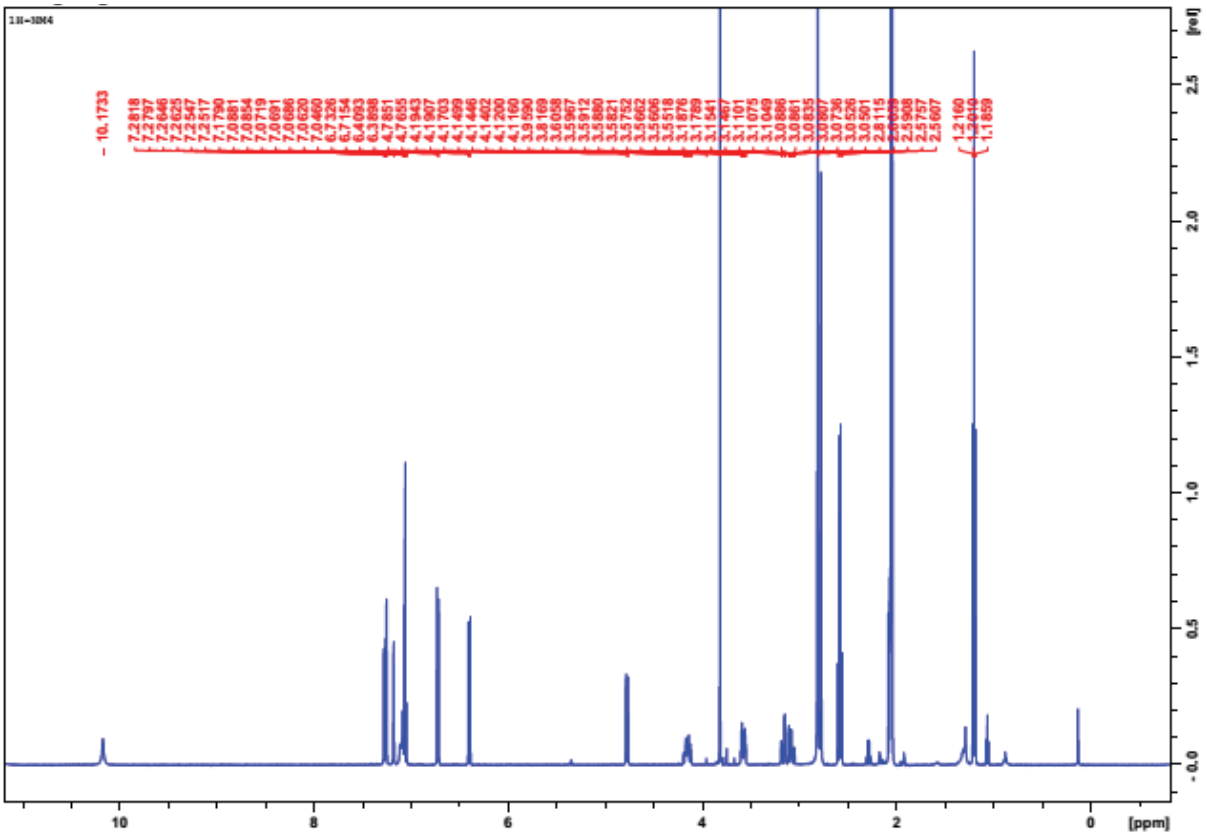


Figure S5: ^{1}H NMR of compound **2a**

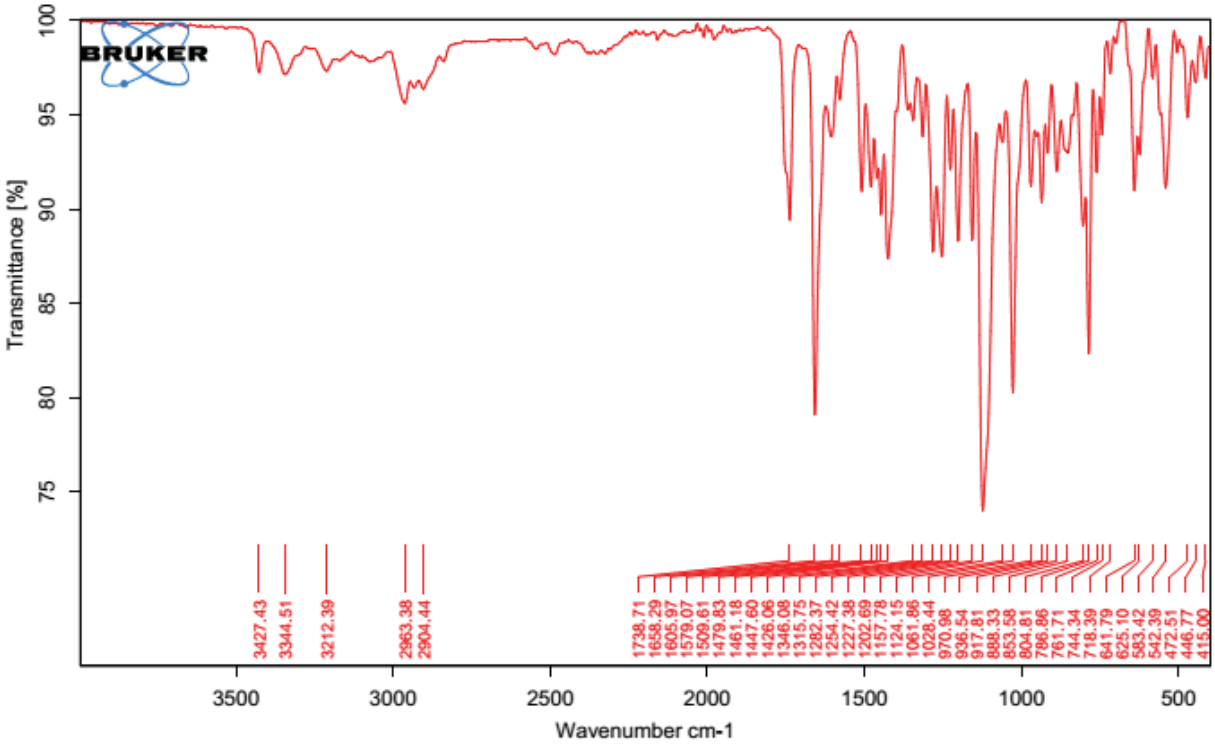


Figure S6: IR spectra of compound **2b**

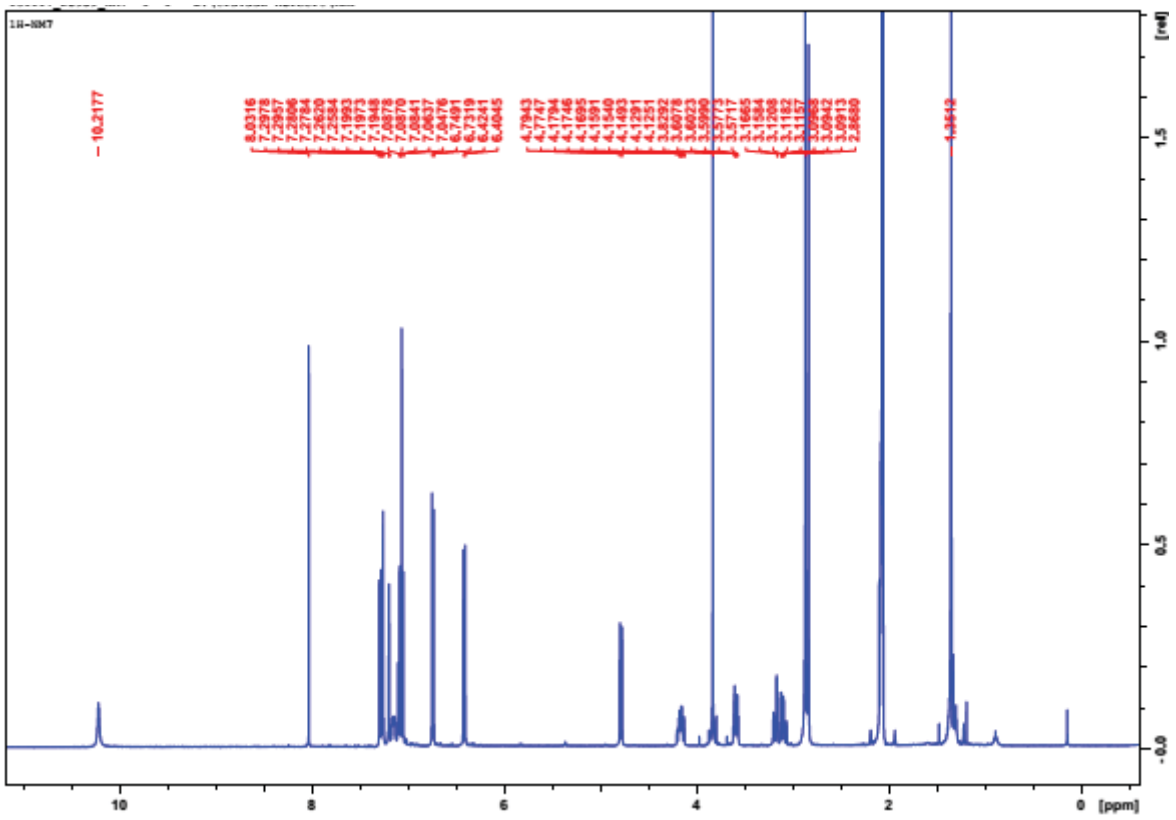


Figure S7: ^{1}H NMR of compound **2b**

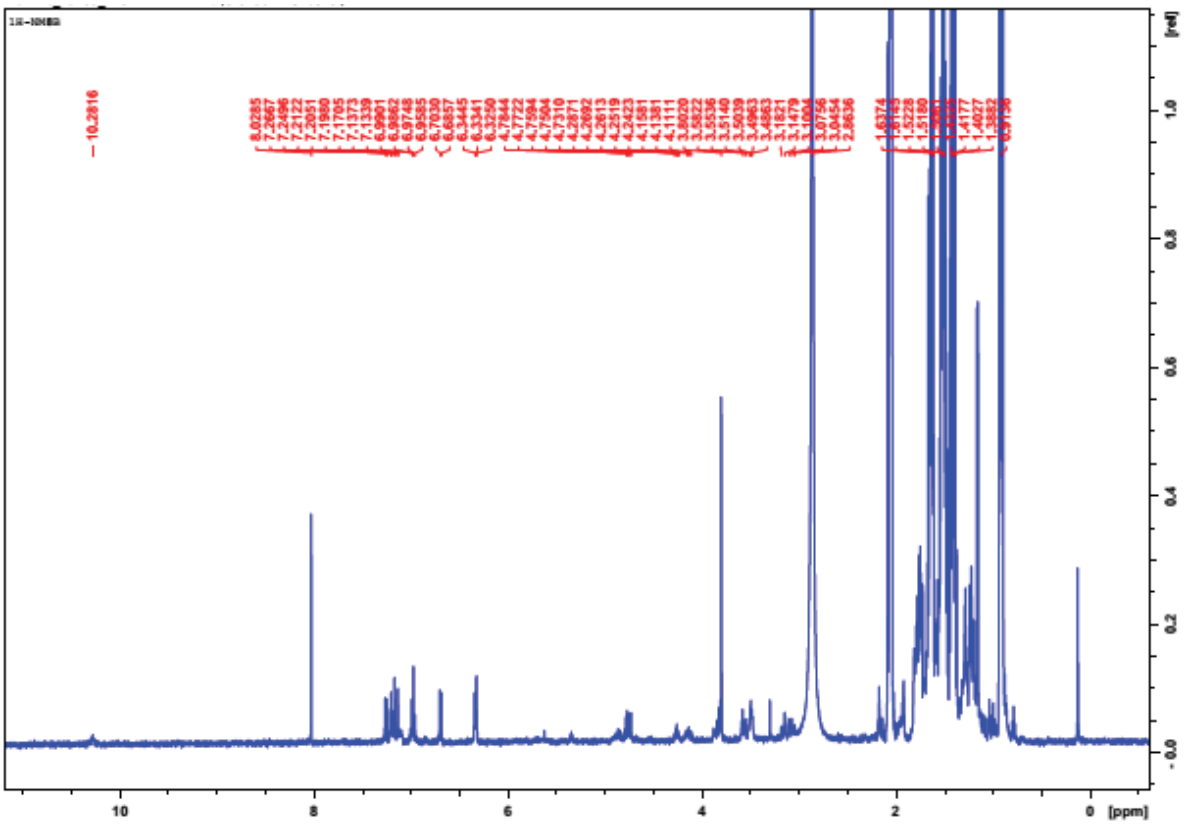


Figure S8: ^{1}H NMR of compound **2c**

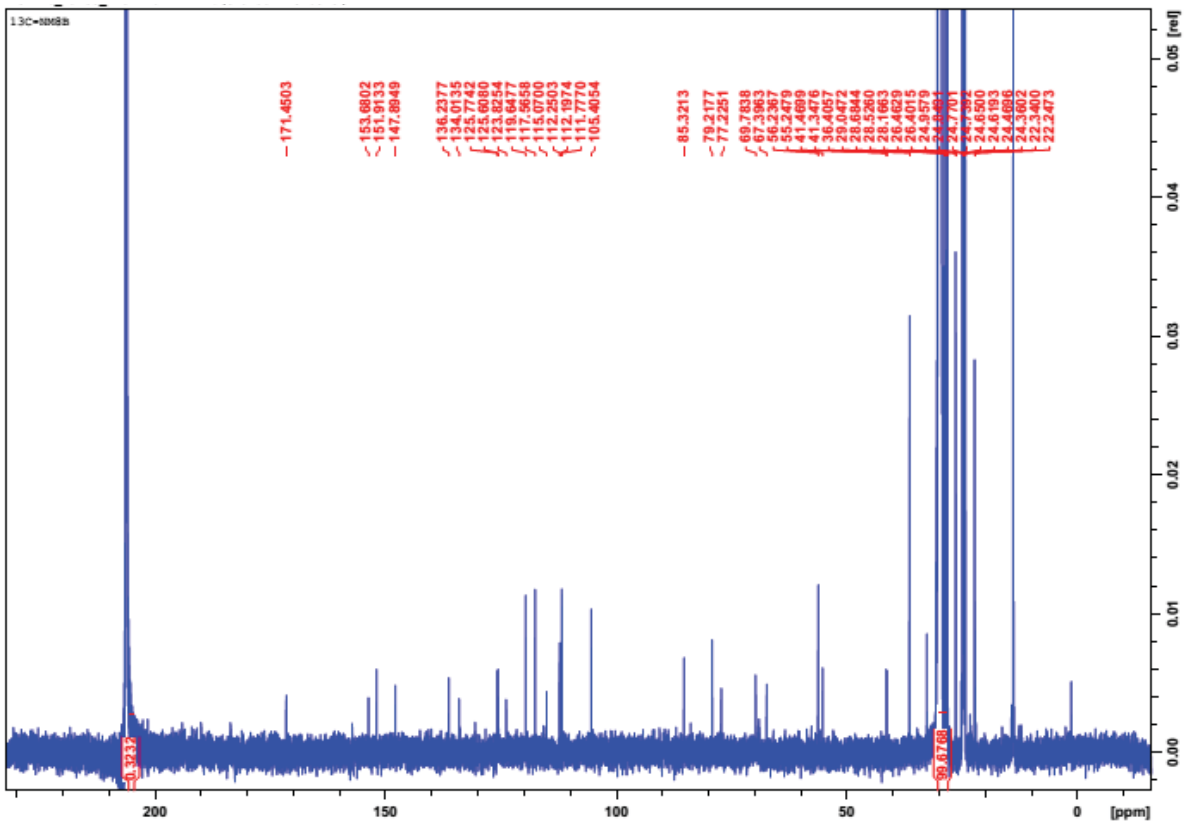


Figure S9: ^{13}C NMR spectrum of compound **2c**

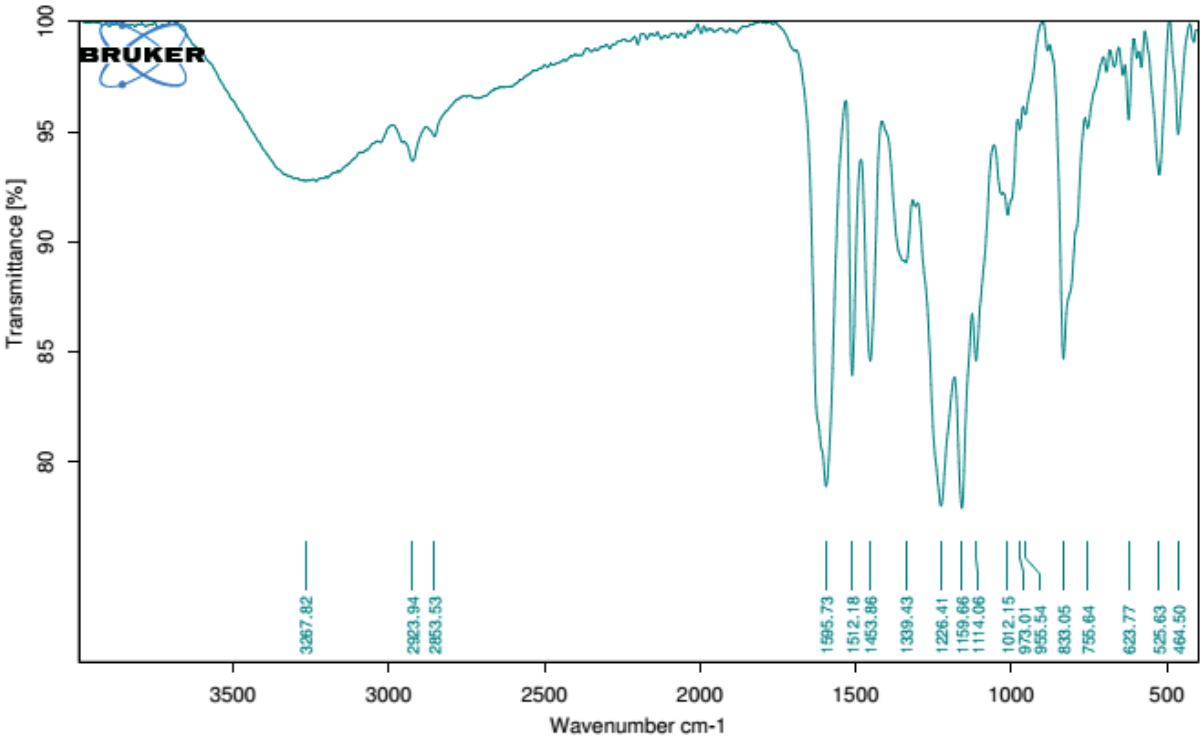


Figure S10: IR spectra of compound **6**

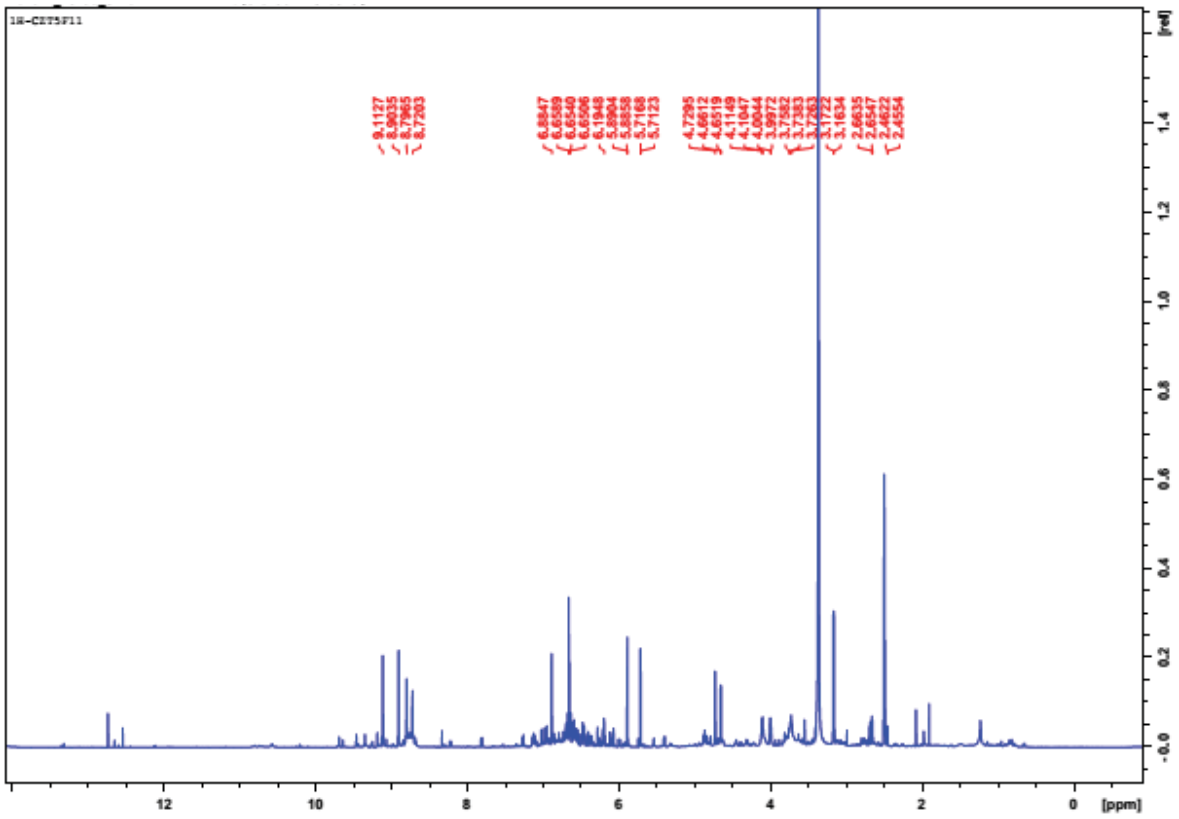


Figure S11: ^{1}H NMR spectrum of compound **6**

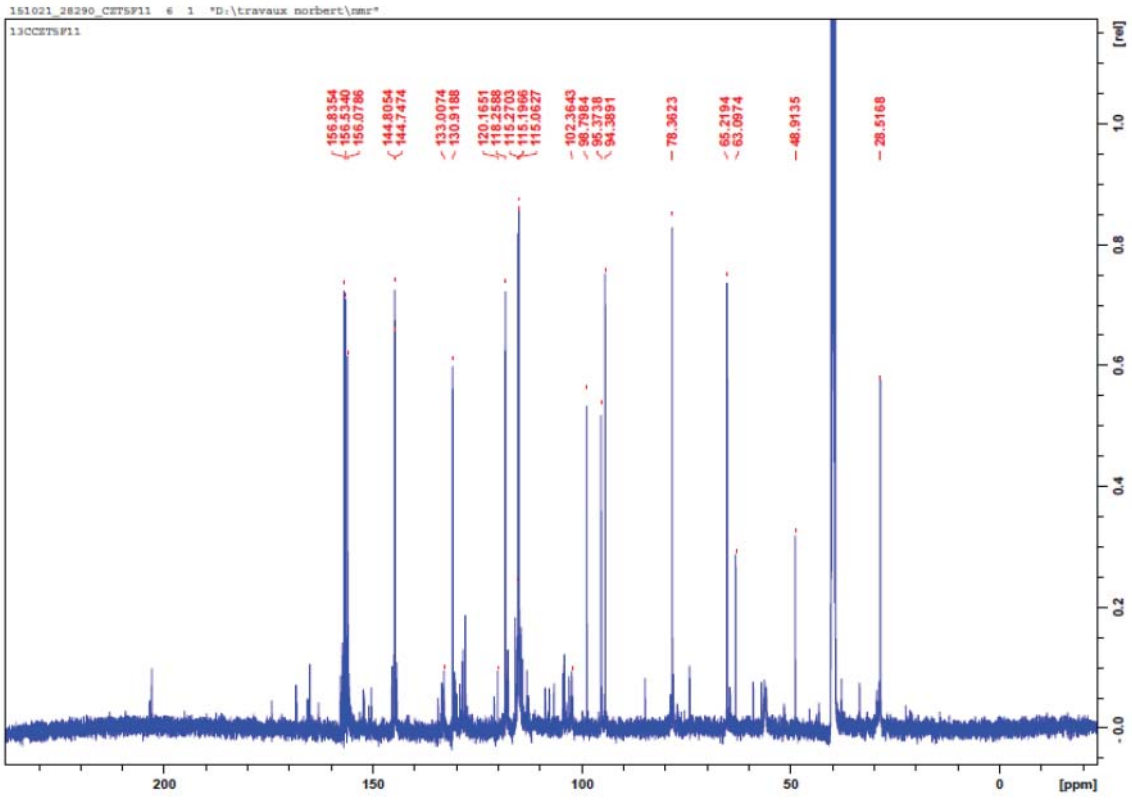


Figure S12: ^{13}C NMR spectrum of compound **6**

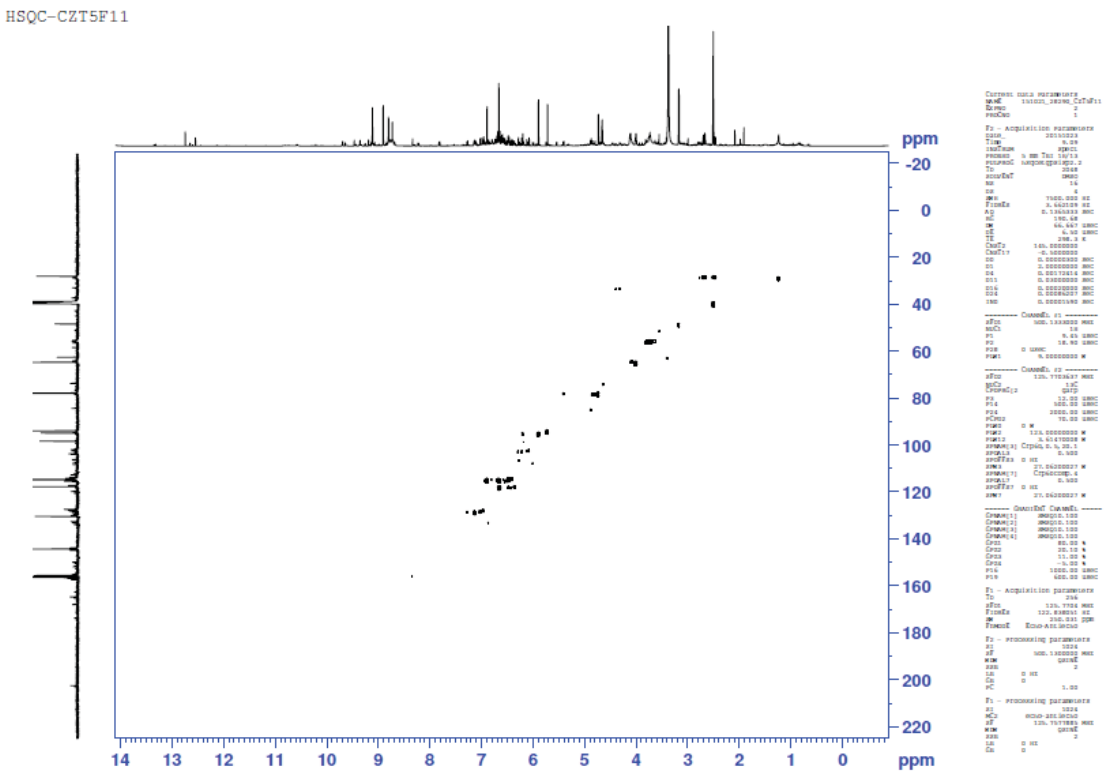


Figure S13: HSQC spectrum of compound **6**

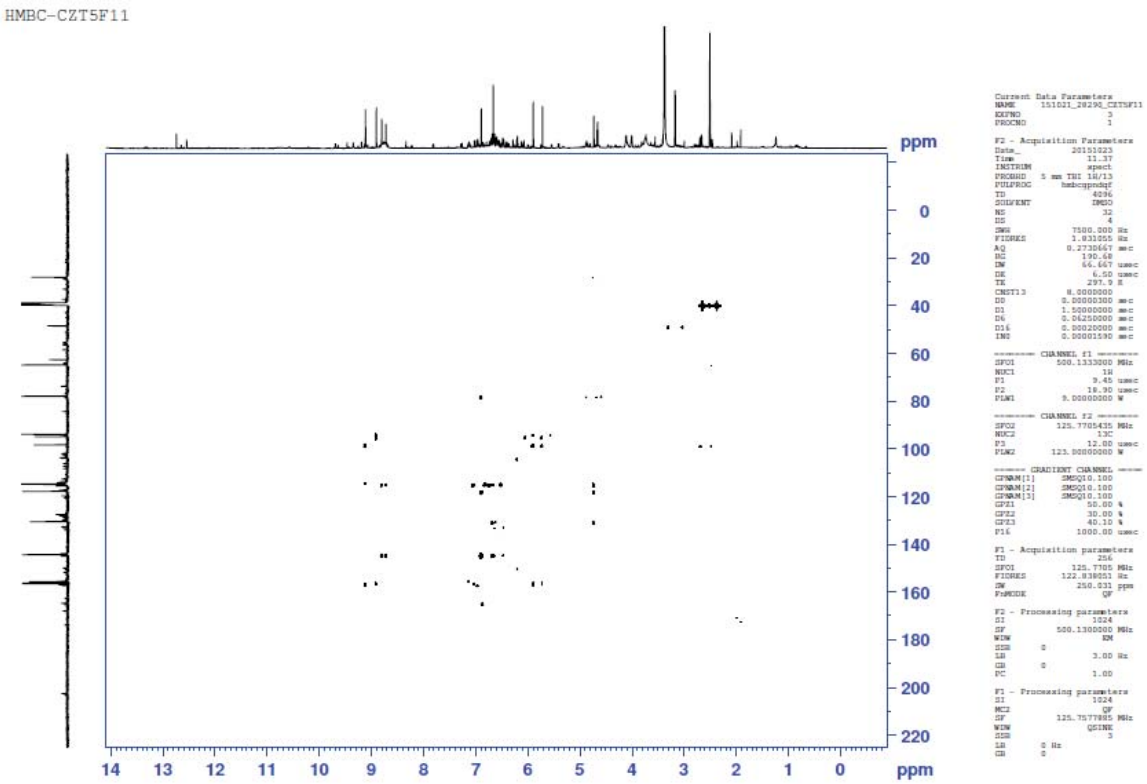
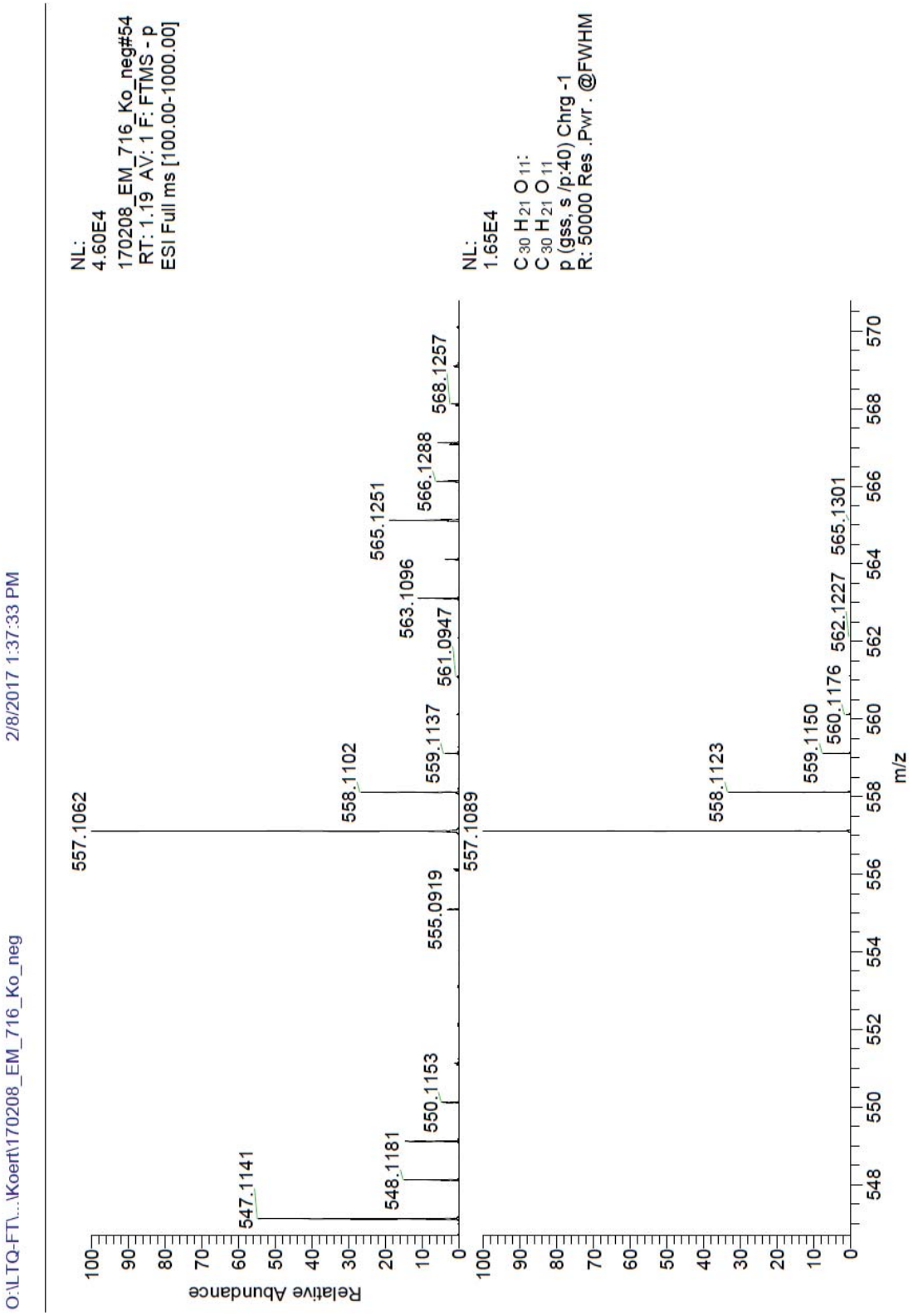


Figure S14: HMBC spectrum of compound **6**

Figure S15: HR-ESI-MS of compound **6**



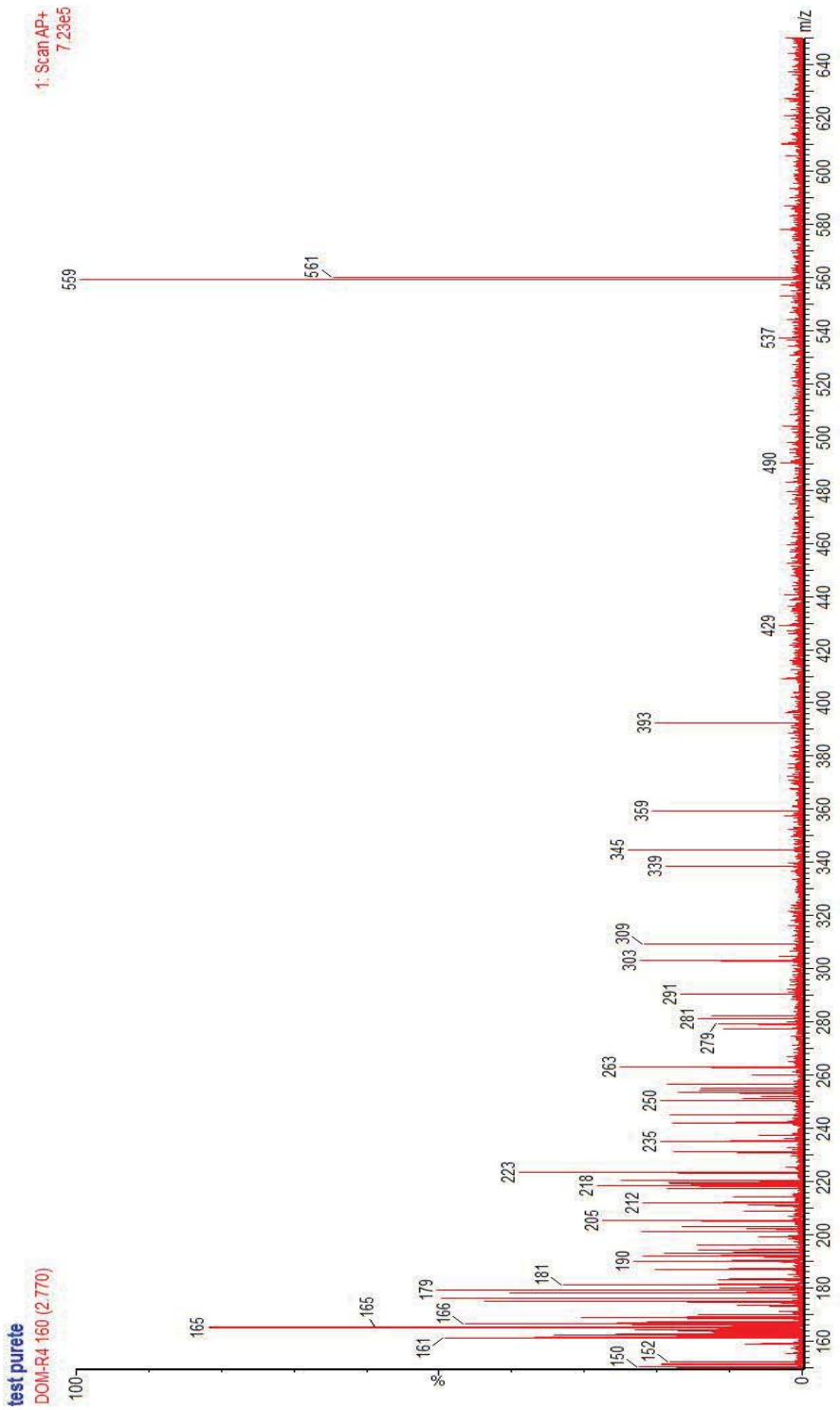


Figure S16: LC-MS of compound **6**

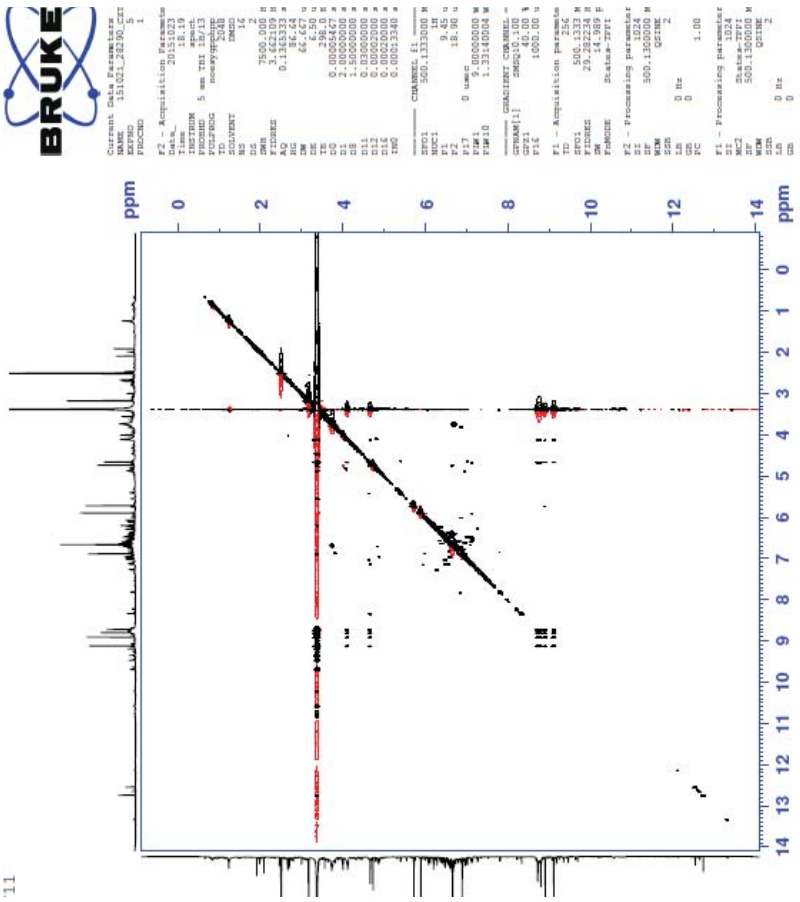


Figure S17: NOESY spectrum of compound **6**

Reference

Koizumi Y, Kobayashi H, Wakimoto T, Furata T, Fukuyama T, Kan T. 2008. Total synthesis of (-)-serotobenine. *J Am Chem Soc.* 130(50): 16854-16855.