SUPPLEMENTARY MATERIAL

A new flavonol derivative and other compounds from the leaves of *Bauhinia thonningii* Schum with activity against multidrug-resistant bacteria

Guy Raphael Sado Nouemsi[a], Jean-Bosco Jouda[b], Peron Bosco Leutcha[a], Valaire Yemene Matieta[c], Virginie Flaure Tsague Tankeu[a], Jenifer Reine Ngnouzouba Kuete[d], İlhami Çelik[e], Victor Kuete[c]; Norbert Sewald[f], Alain Meli Lanang[b,g,*]

ABSTRACT

The Investigation of the leaves of *Bauhinia thonningii* Schum led to the isolation and identification of a new flavonol derivative, 6-C-methylquercetin-3,4'-dimethyl ether (**1**) together with eleven known compounds including nine flavonoids (**2**-**10**), one pentacyclic triterpenoid (**11**) and one steroid glycoside (**12**). Their structures were established by extensive spectroscopic analyses, like 1D and 2D NMR, and HR-ESI-MS. The antibacterial activity of compound **1** as well as the reference antibiotic, ciprofloxacin was tested on Gram-negative multidrug-resistant bacteria overexpressing active efflux pumps, and against methicillin-resistant strains of *Staphylococcus aureus* (MRSA). Samples were tested alone and in combination with an efflux pump inhibitor (EPI), phenylalanine-arginine-*β*-naphthylamide (PAßN). Results show that when compound **1** was tested alone, its inhibitor effects were obtained on 7/10 tested bacteria with the highest MIC value of 128 µg/mL whilst in the presence of EPI, this activity significantly increase in all the 10 bacteria, with MIC ranging from 8-4 µg/mL. An interesting antibacterial activity was obtained with compound **1** against *Klebsiella pneumoniae* ATCC11296 (MIC of 4 µg/mL), KP55 and *Staphylococcus aureus* MRSA6 (MIC of 8 µg/mL) in the presence of the PAßN.

Key words: *Bauhinia thonningii*, flavonoids, antibacterial activity.

[*] Corresponding Author: Alain Meli Lannang, PO Box 454 Ngaoundere, E-mail address: alainmeli@yahoo.com

Table of contents

Figure S26. ^{1}H-NMR spectrum (600 MHz, Pyridine-d_5) of compound **12**

Figure S27. ^{13}C-NMR spectrum (150 MHz, Pyridine-d_5) of compound **12**

Figure S28. ^{1}H-NMR spectrum (600 MHz, DMSO-d_6) of compound **13**

Figure S29. ^{13}C-NMR spectrum (150 MHz, DMSO-d_6) of compound **13**

Figure S30. ^{1}H-^{1}H COSY and HMBC correlations of compound **1**.

Table S1: ^{13}C (150 MHz) and ^{1}H NMR (CD_3OD, 600 MHz) [*J* (Hz), δ (ppm)] data for compound **1**

	1	
Pos	$\boldsymbol{\delta_C}$	$\boldsymbol{\delta_H}$ (nH, mult., *J*)
2	156.2	-
3	138.2	-
4	178.6	-
5	158.4	-
6	107.4	-
7	162.5	-
8	92.5	6.45 (1H, *s*)
9	154.8	-
10	104.4	-
1'	121.6	-
2'	111.5	7.70 (d, *J* = 2.1 Hz)
3'	147.5	-
4'	149.4	-
5'	115.2	6.96 (1H, d, *J* = 8.4 Hz)
6'	122.3	7.63 (1H, dd, *J* = 8.4, 2.1 Hz)
6-Me	6.4	2.09 (3H, *s*)
3-OMe	55.4	3.79 (3H, *s*)
4'-OMe	59.5	3.95 (3H, *s*)

Table S2. Minimal inhibitory concentrations (MIC) of compound **1** and ciprofloxacin alone and in the presence of an efflux pump inhibitor.

Bacterial strains	**Samples, MIC values (in µg/mL) and fold increase of activity**					
	1 alone	**1+PAβN**	**Fold increase**	**CIP alone**	**CIP+PAβN**	**Fold increase**
Escherichia coli						
ATCC10356	128	64	2	**8**	**8**	1
AG102	>128	64	>2	128	**2**	64
Klebsiella pneumoniae						
ATCC11296	32	**4**	8	64	**2**	32
KP55	64	**8**	8	16	**1**	16
Pseudomonas aeruginosa						
PA124	>128	32	>4	64	**4**	16
PA01	128	32	4	64	**2**	32
Providencia stuartii						
PS2636	>128	128	>1	128	**4**	32
NEA16	128	32	4	32	**2**	16
Staphylococcus aureus						
MRSA6	128	**8**	16	128	**4**	32
MRSA3	128	128	1	128	**8**	16

1: 6-C-methylquercetin-3,4'-dimethyl ether; CIP: ciprofloxacin; PAβN: phenylalanine-arginine-*β*-naphthylamide (an efflux pump inhibitor); Samples were tested alone or in the presence of PAβN at 20 µg/mL; Fold increase of activity was determined as the ratio of the MIC of Sample alone versus the sample in the presence of PAβN; MIC value in bold are significant antibacterial activity (Kuete, 2010).

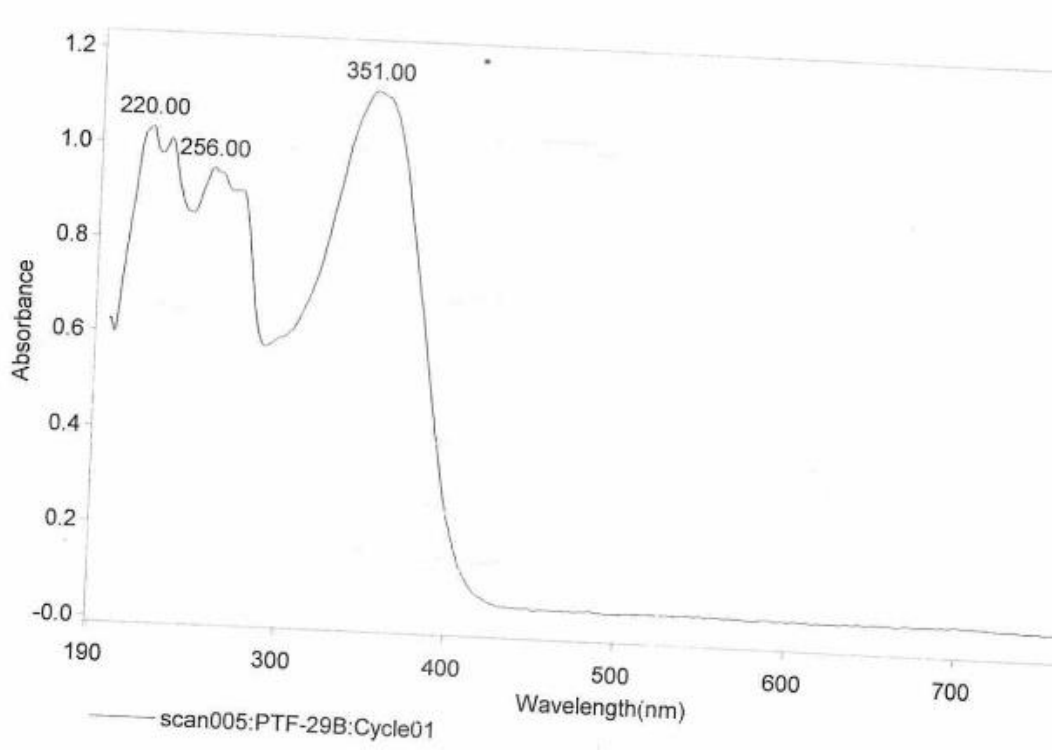

Figure S1. UV spectrum of 6-C-methylquercetin-3,4'-dimethyl ether **(1)**

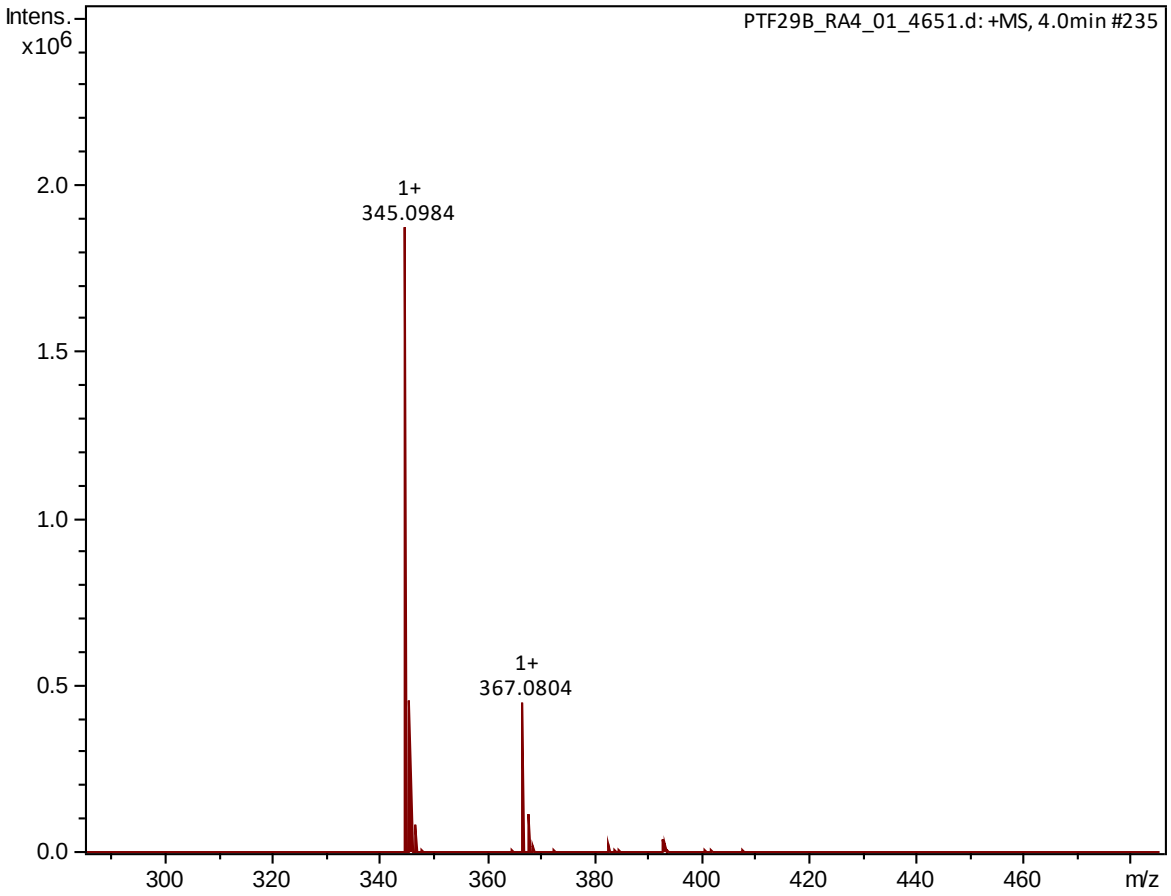

Figure S2. HRESI-MS (+) of 6-C-methylquercetin-3,4'-dimethyl ether **(1)**

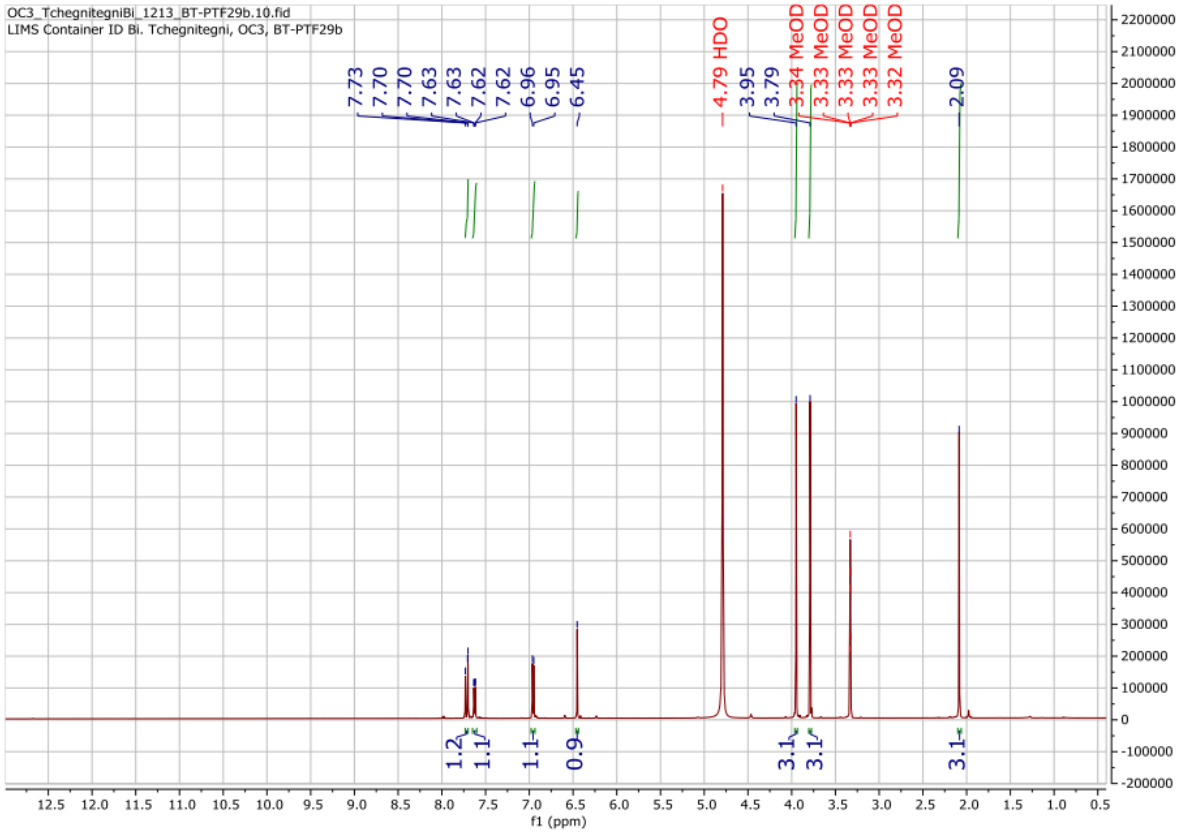

Figure S3. ^{1}H-NMR spectrum (600 MHz, CD_3OD) of 6-C-methylquercetin-3,4'-dimethyl ether **(1)**

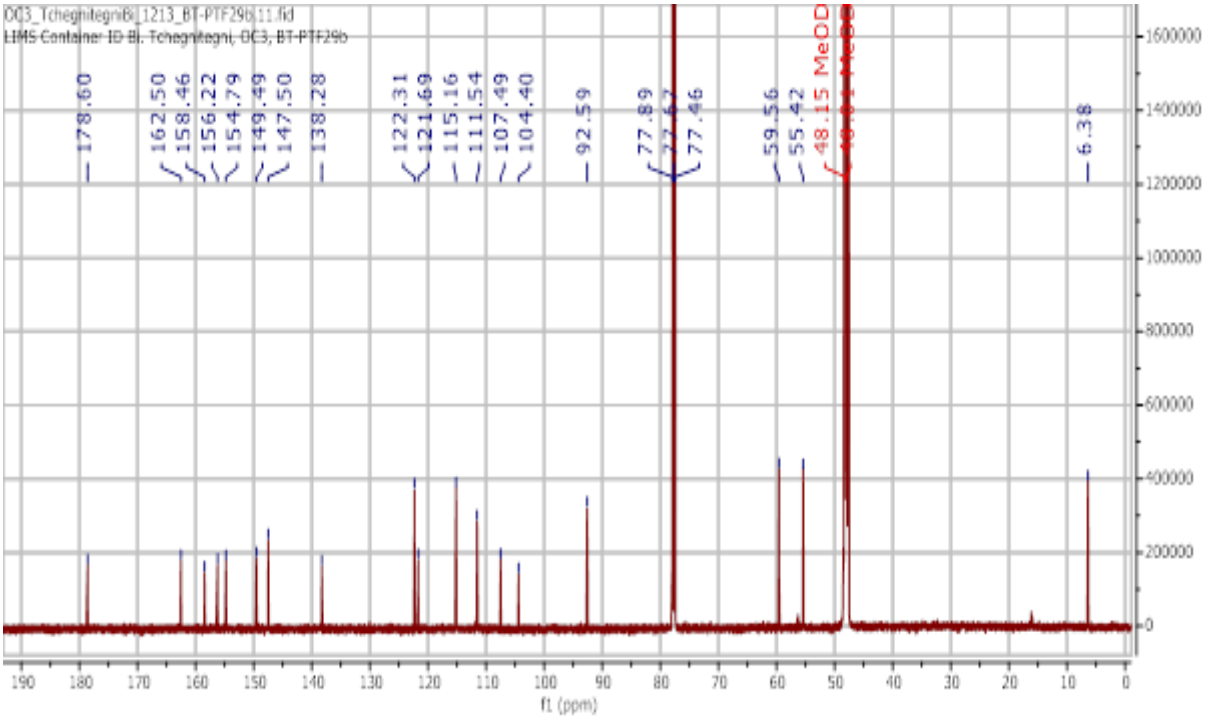

Figure S4. ^{13}C-NMR spectrum (150 MHz, CD_3OD) of 6-C-methylquercetin-3,4'-dimethyl ether **(1)**

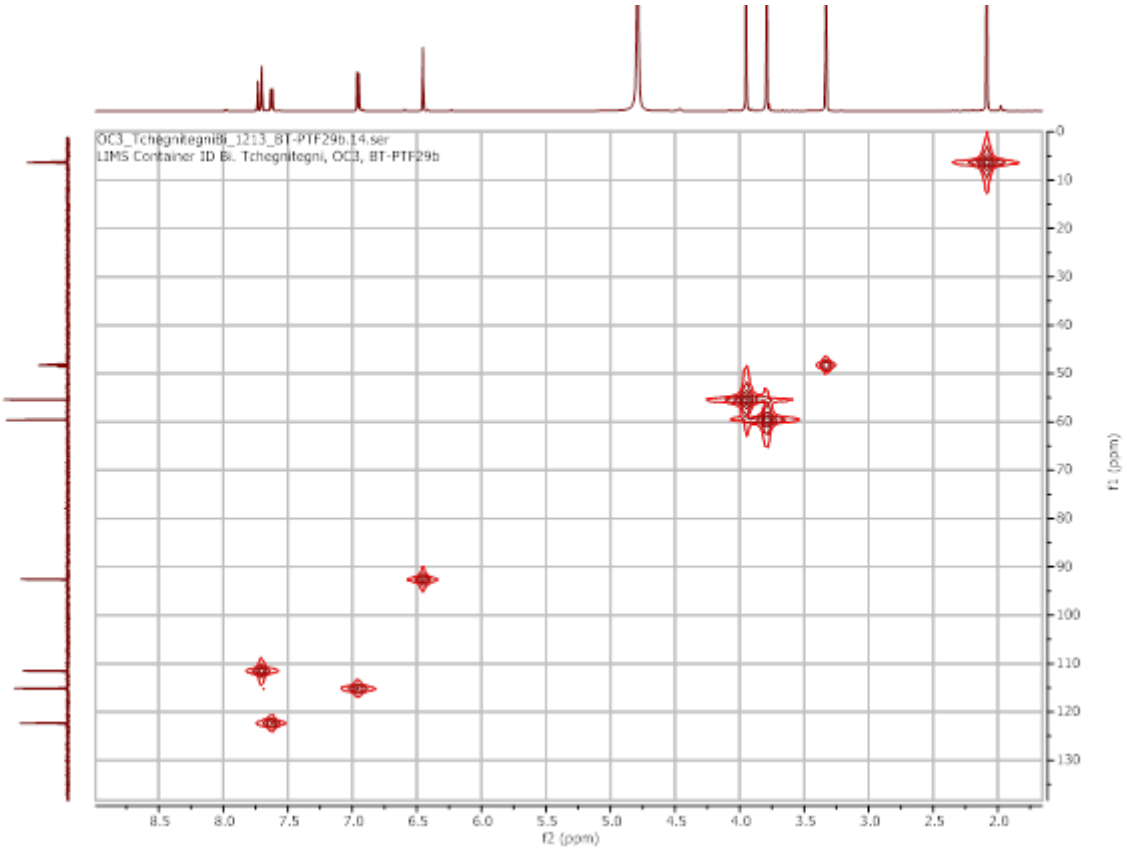

Figure S5. HSQC spectrum of 6-C-methylquercetin-3,4'-dimethyl ether **(1)**

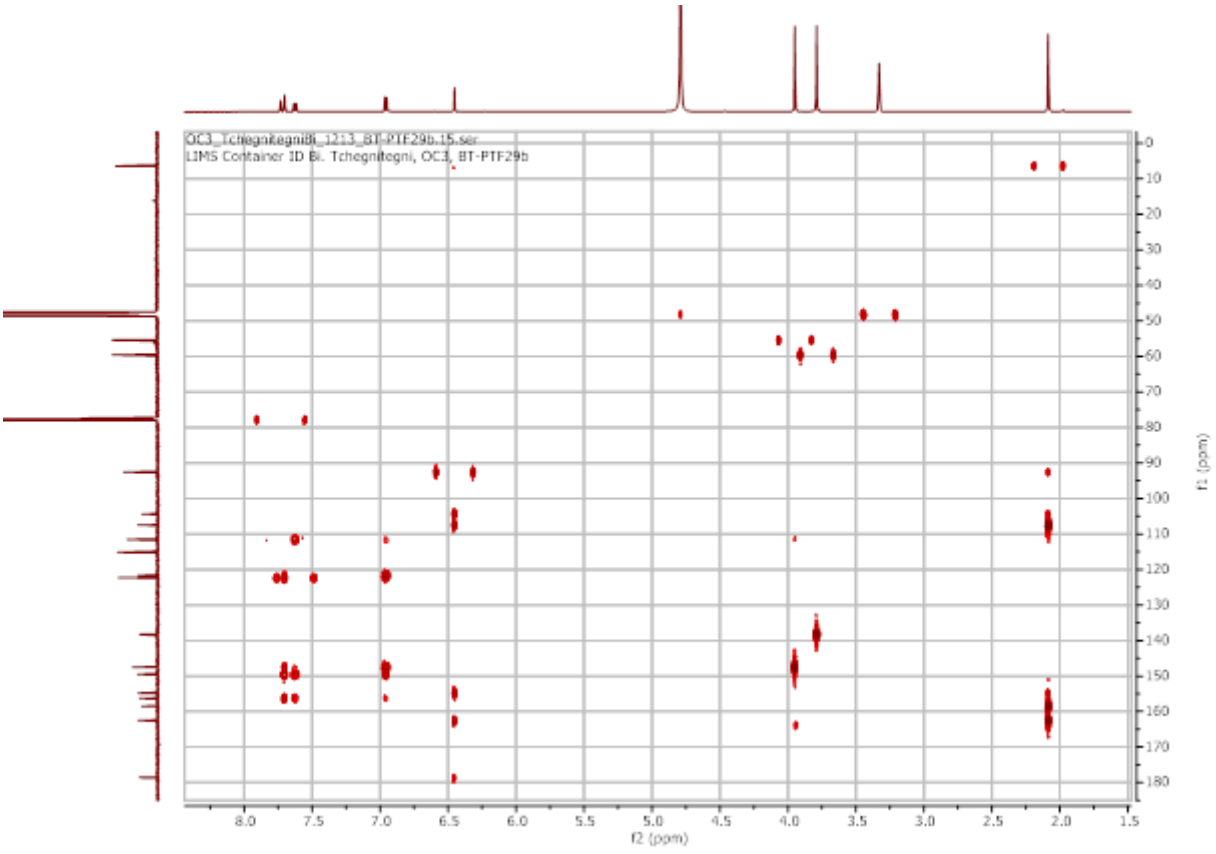

Figure S6. HMBC spectrum of 6-C-methylquercetin-3,4'-dimethyl ether **(1)**

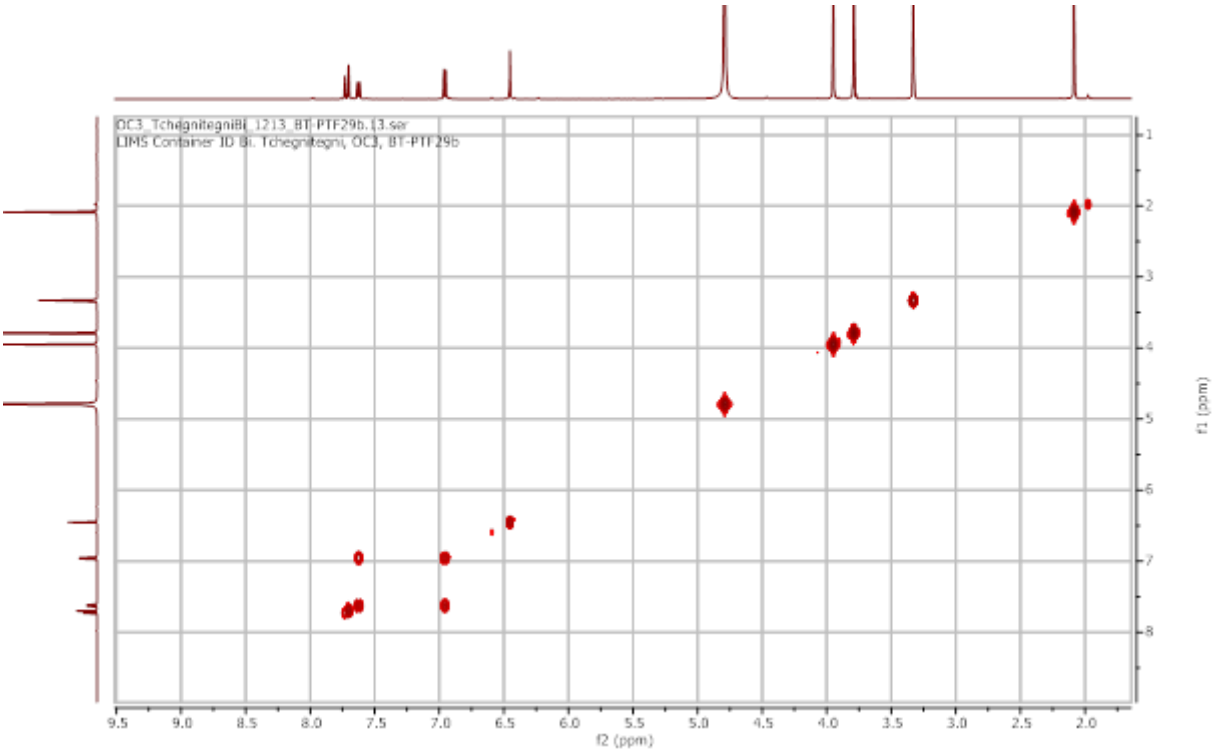

Figure S7. COSY ^{1}H-^{1}H spectrum of 6-C-methylquercetin-3,4'-dimethyl ether **(1)**

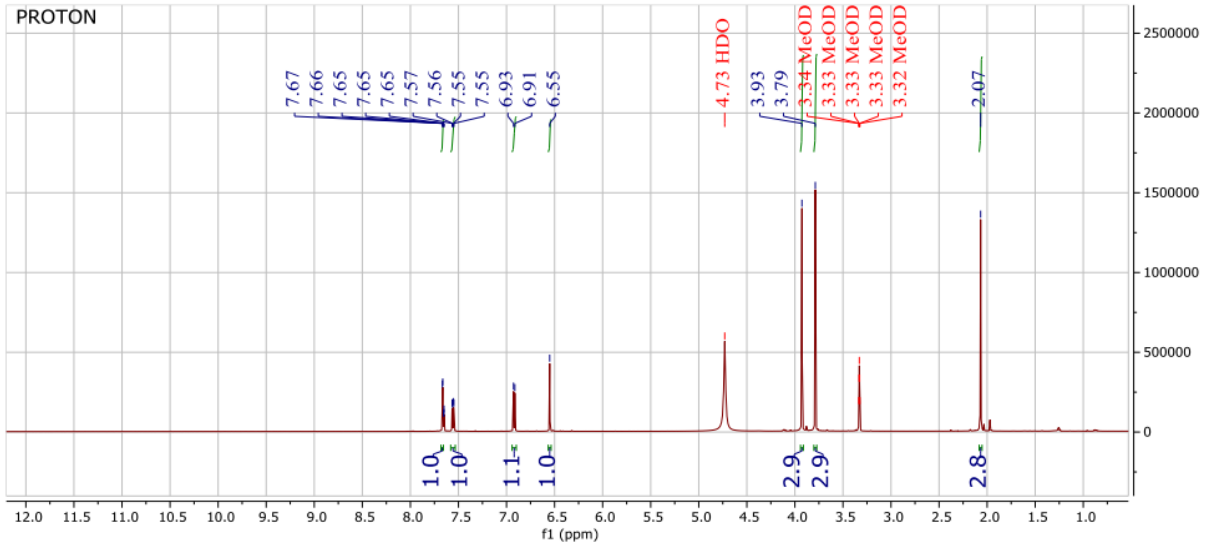

Figure S8. ^{1}H-NMR spectrum (600 MHz, CD_3OD) of compound **2**

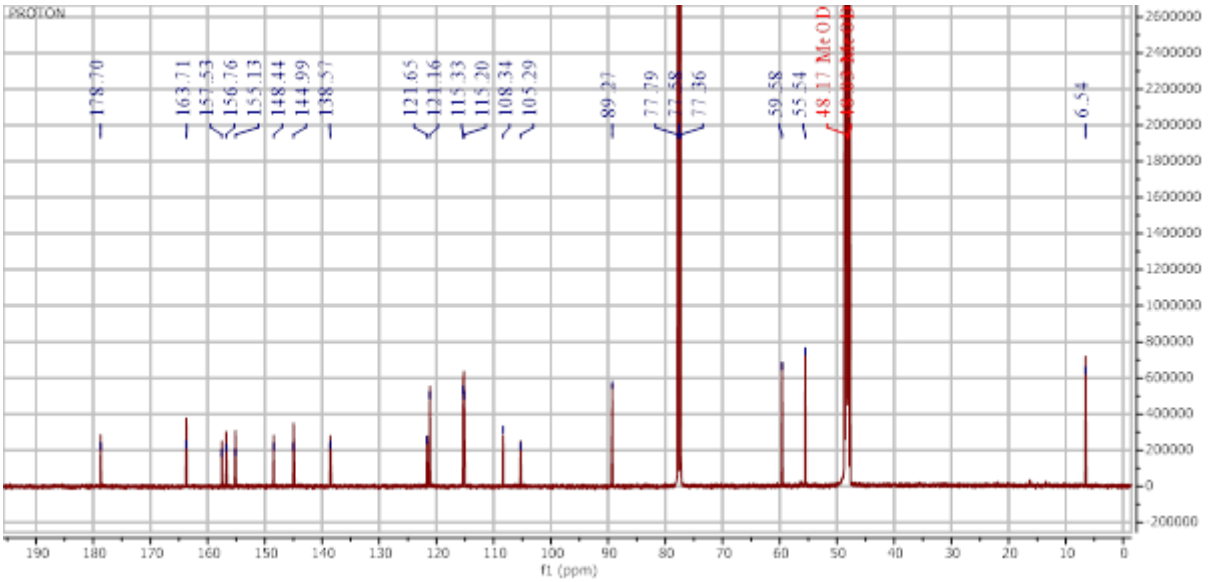

Figure S9. ^{13}C-NMR spectrum (150 MHz, CD_3OD) of 6 compound **2**

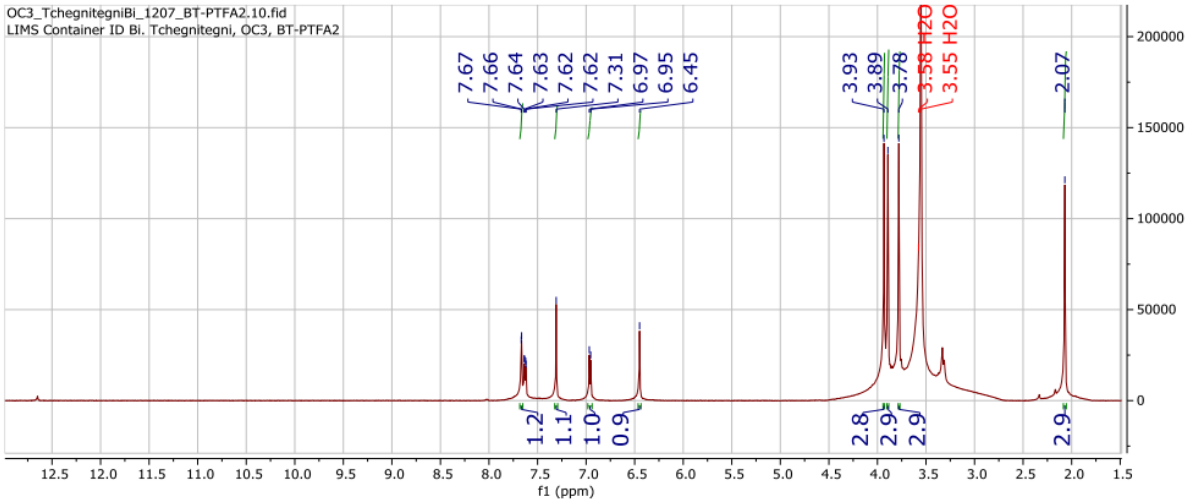

Figure S10. ^{1}H-NMR spectrum (600 MHz, Acetone-d_6) of compound **3**

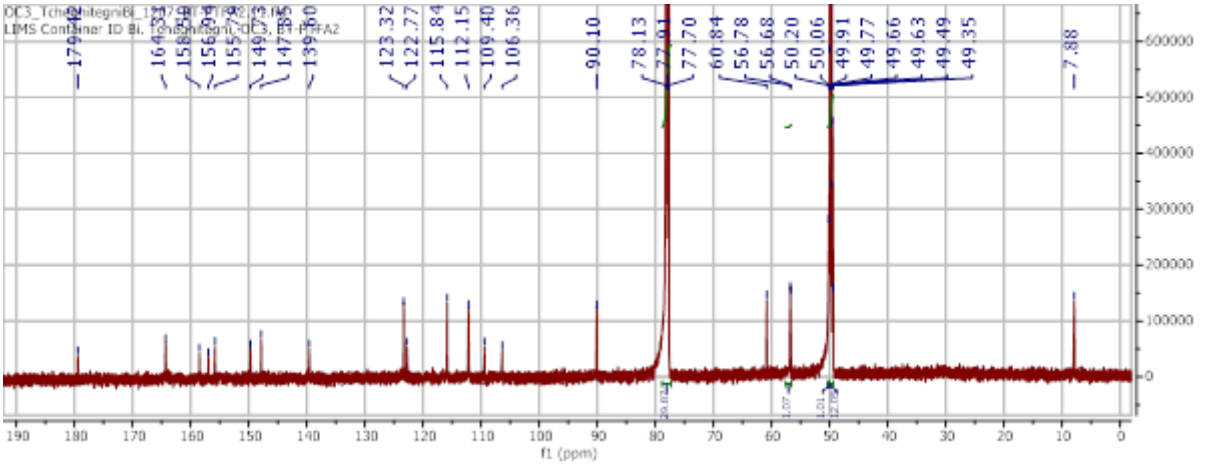

Figure S11. ^{13}C-NMR spectrum (150 MHz, Acetone-d_6) of compound **3**

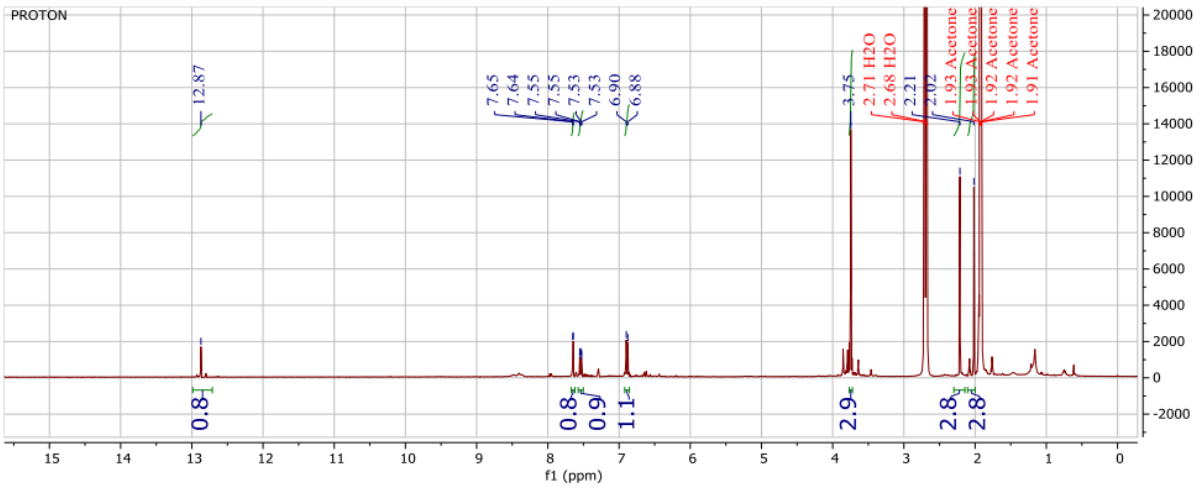

Figure S12. ^{1}H-NMR spectrum (600 MHz, Acetone-d_6) of compound **4**

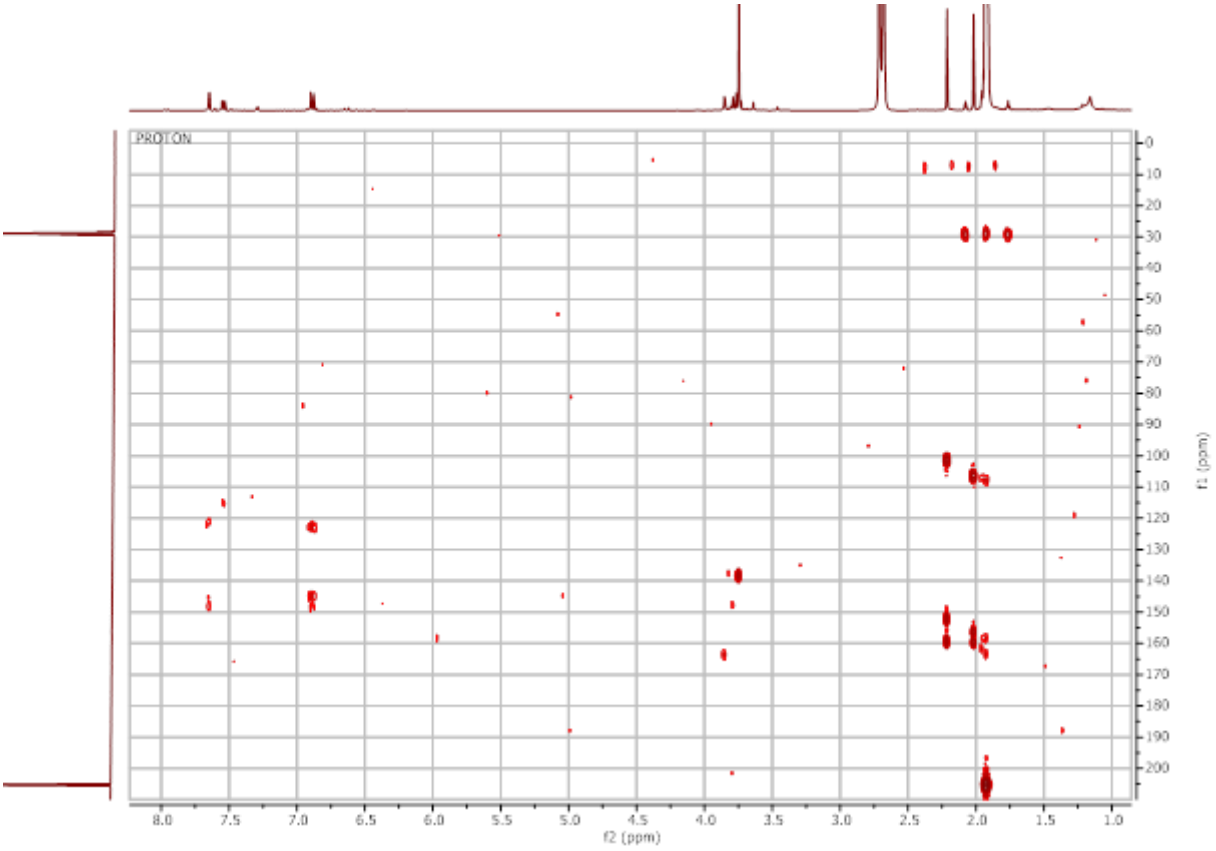

Figure S13. HMBC spectrum of compound **4**

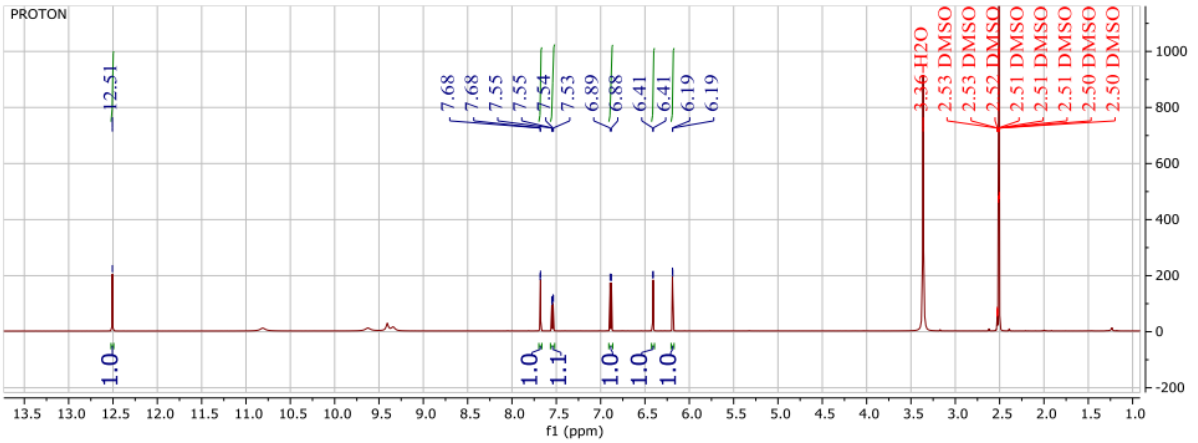

Figure S14. ^{1}H-NMR spectrum (600 MHz, DMSO-d_6) of compound **5**

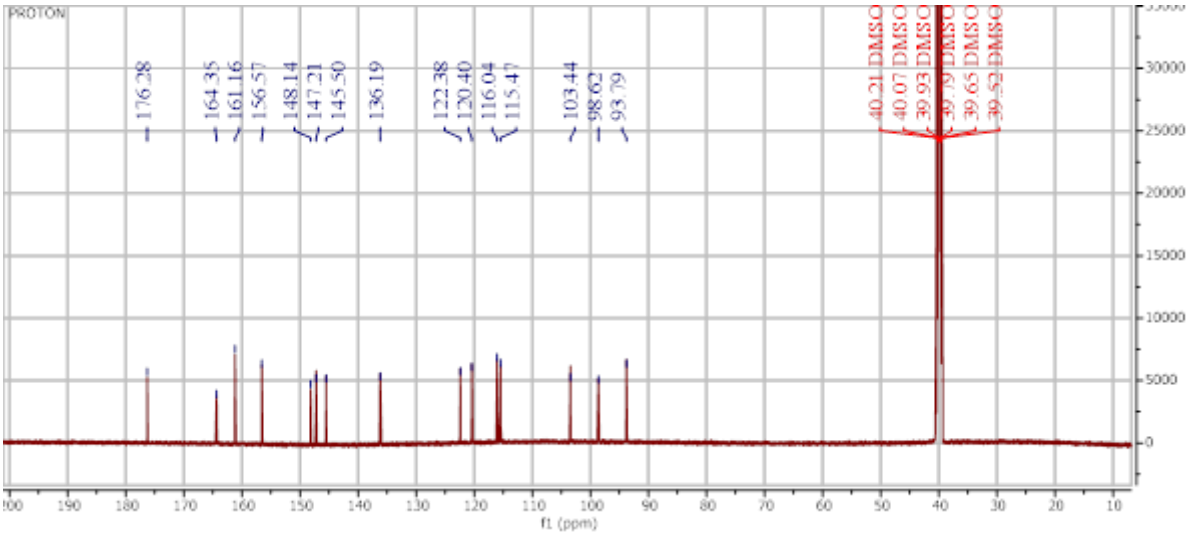

Figure S15. ^{13}C-NMR spectrum (150 MHz, DMSO-d_6) of compound **5**

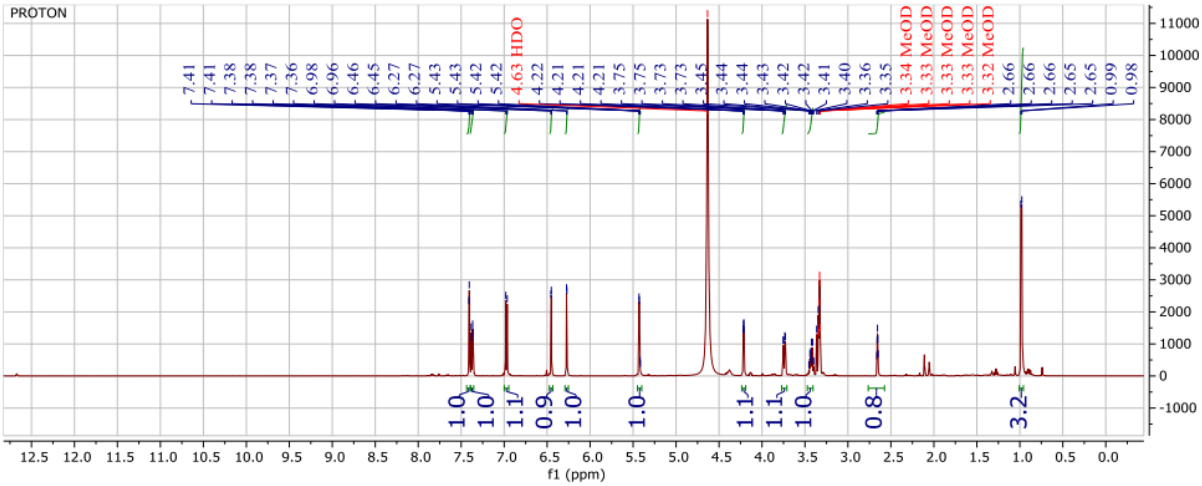

Figure S16. ^{1}H-NMR spectrum (500 MHz, CD_3OD) of compound **6**

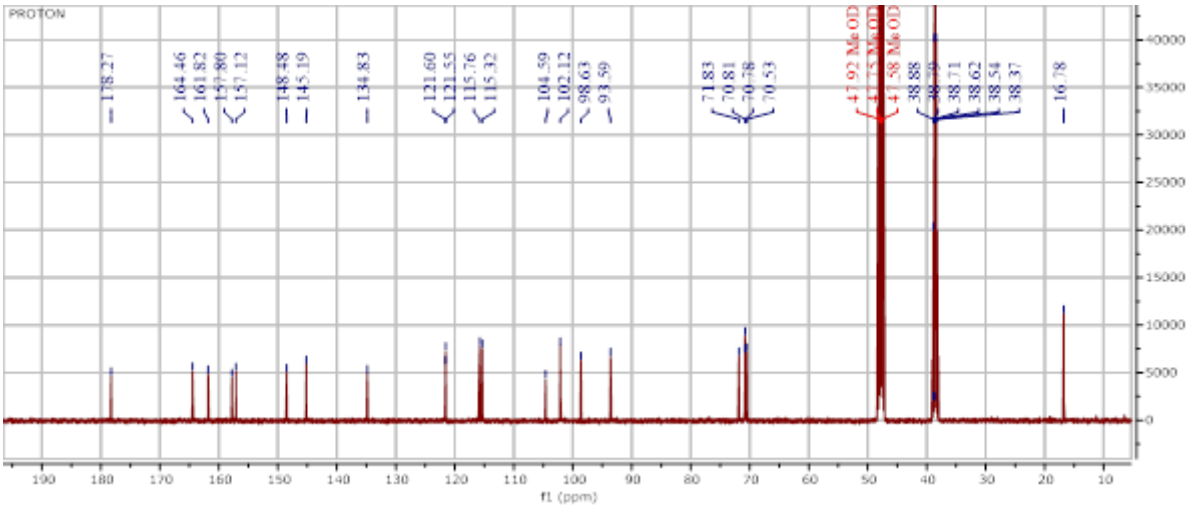

Figure S17. ^{13}C-NMR spectrum (125 MHz, CD_3OD) of compound **6**

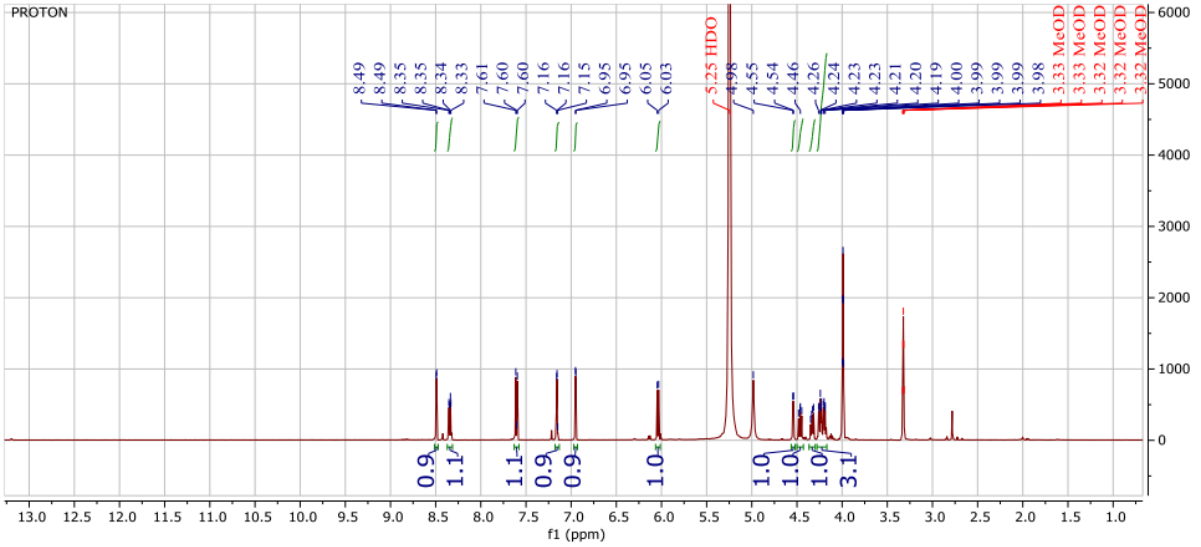

Figure S18. ^{1}H-NMR spectrum (500 MHz, CD_3OD) of compound **7**

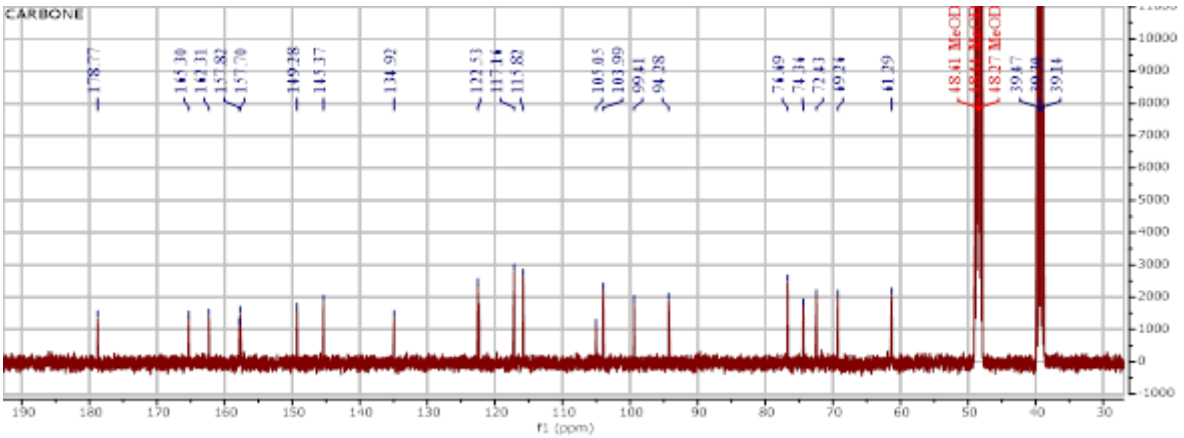

Figure S19. ^{13}C-NMR spectrum (125 MHz, CD_3OD) of compound **7**

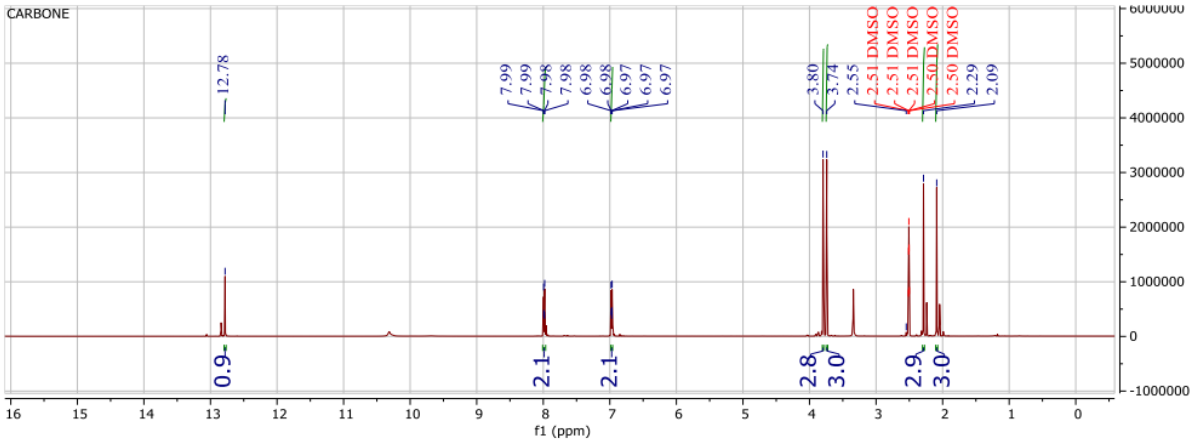

Figure S20. ^{1}H-NMR spectrum (600 MHz, DMSO-d_6) of compound **8**

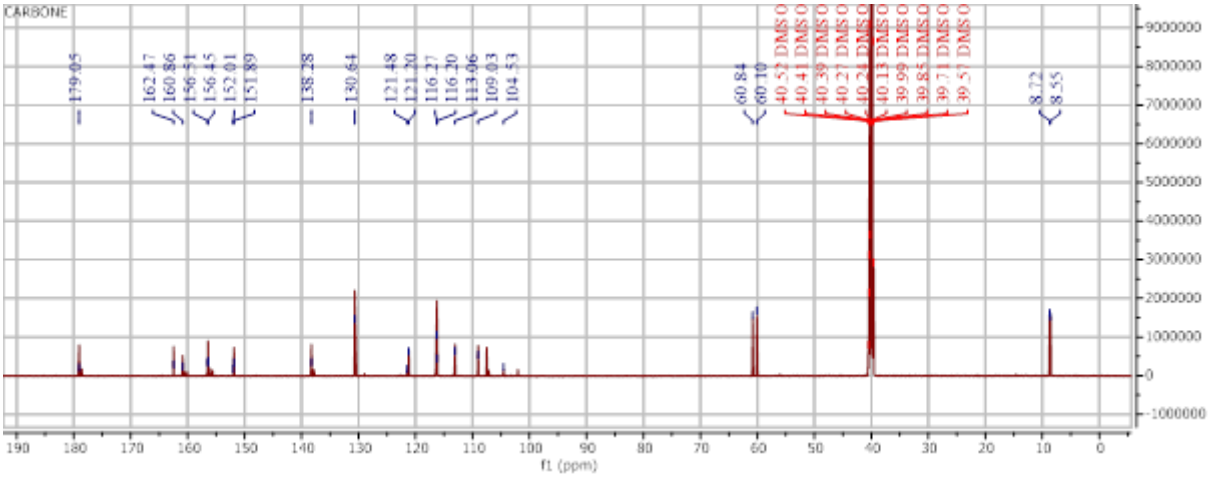

Figure S21. ^{13}C-NMR spectrum (150 MHz, DMSO-d_6) of compound **8**

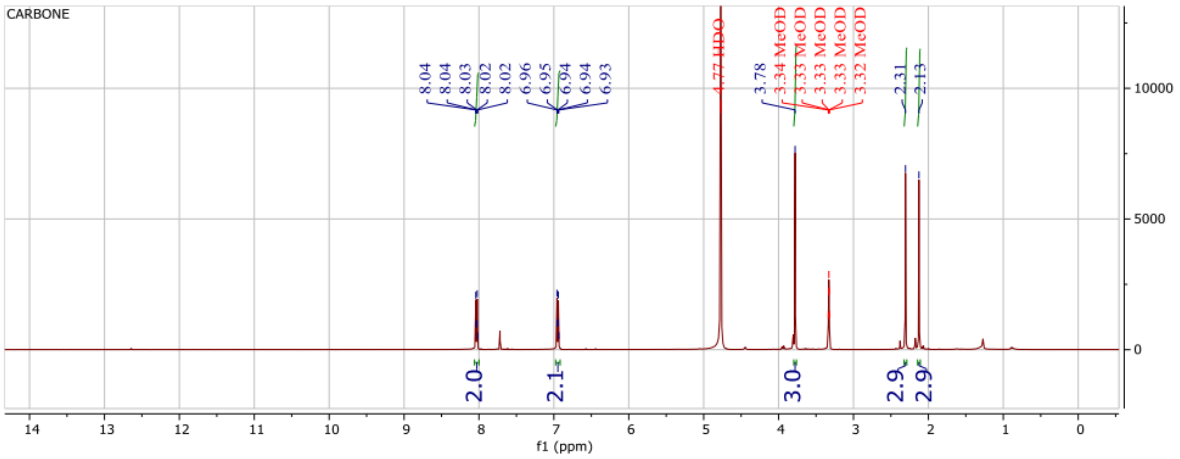

Figure S22. ^{1}H-NMR spectrum (600 MHz, CD_3OD) of compound **9**

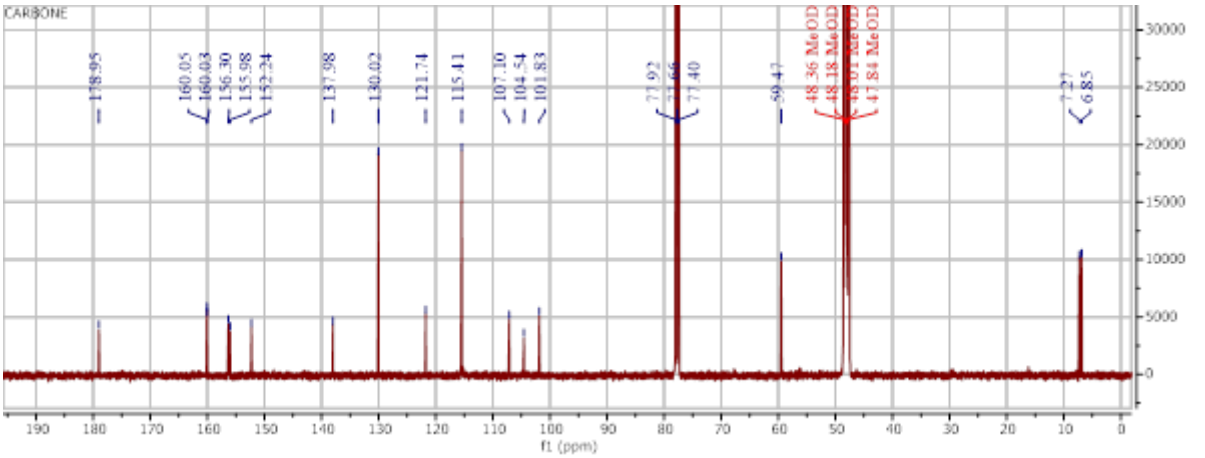

Figure S23. ^{13}C-NMR spectrum (150 MHz, CD_3OD) of compound **9**

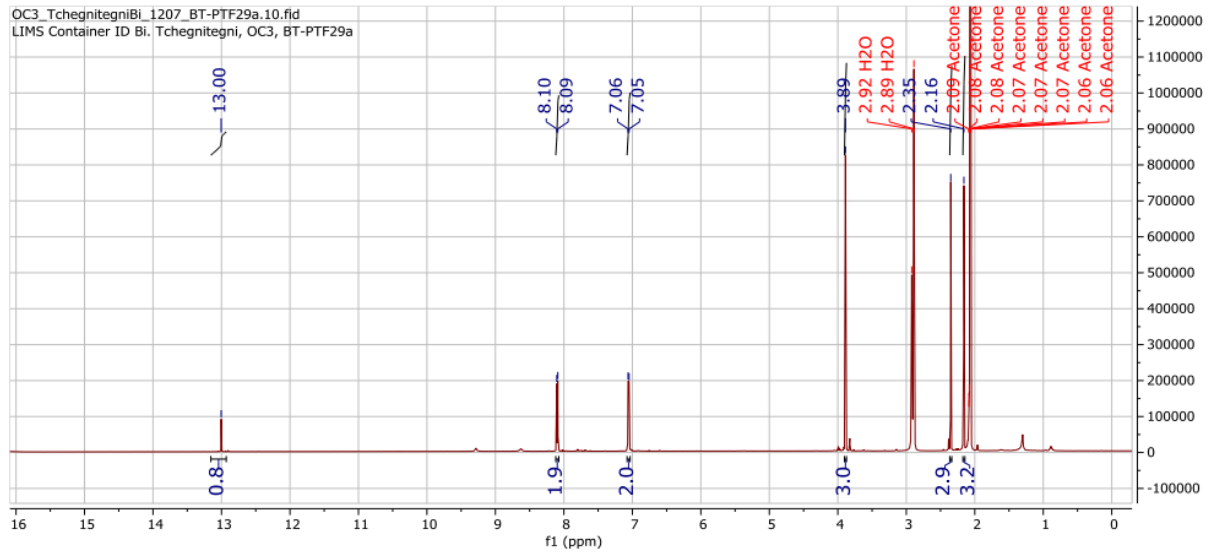

Figure S24. ^{1}H-NMR spectrum (600 MHz, Acetone-d_6) mixture of compounds **10** and **11**

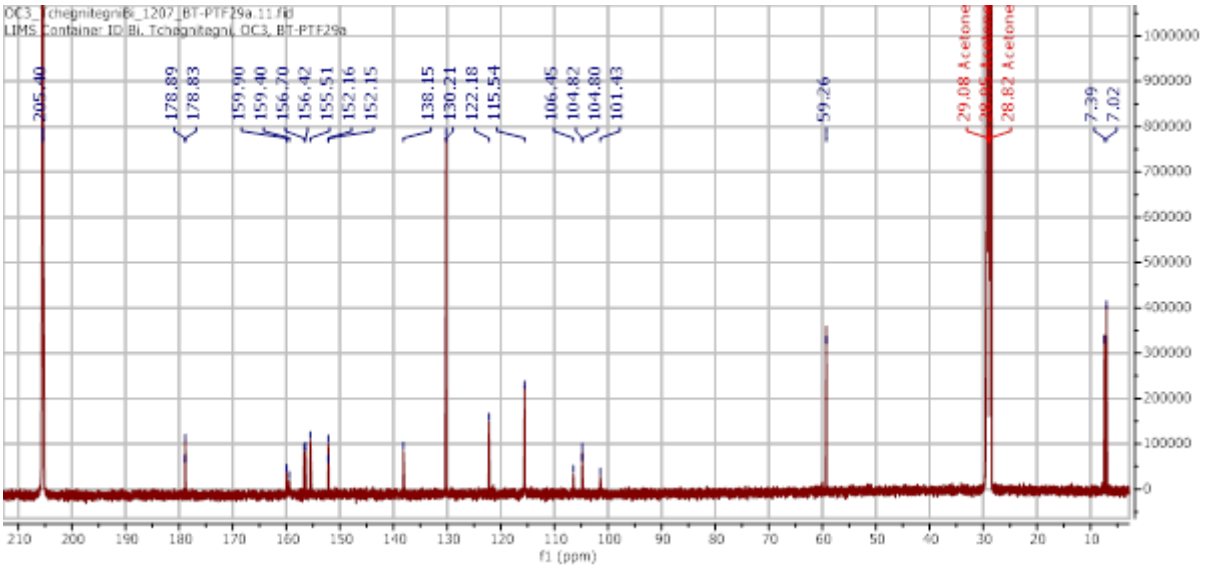

Figure S25. ^{13}C-NMR spectrum (150 MHz, Acetone-d_6) mixture of compounds **10** and **11**

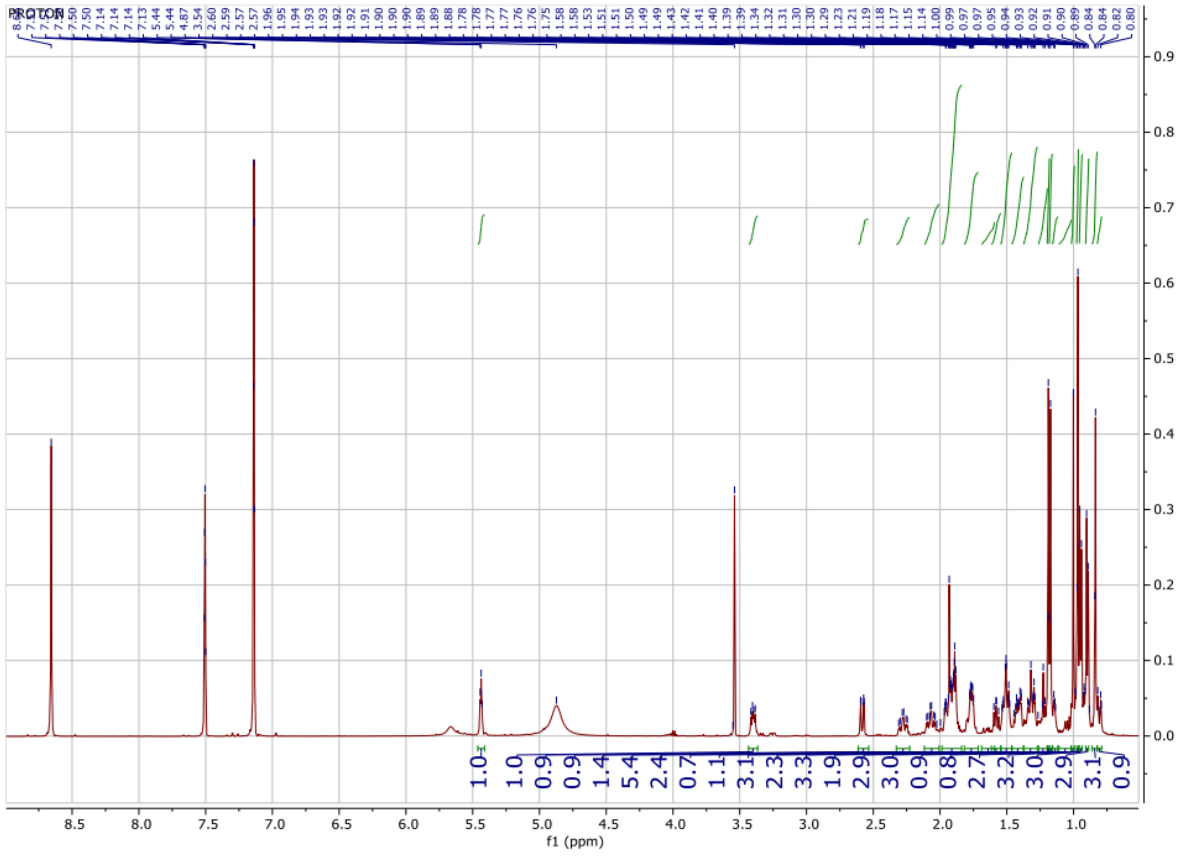

Figure S26. ^{1}H-NMR spectrum (600 MHz, Pyridine-d_5) of compound **12**

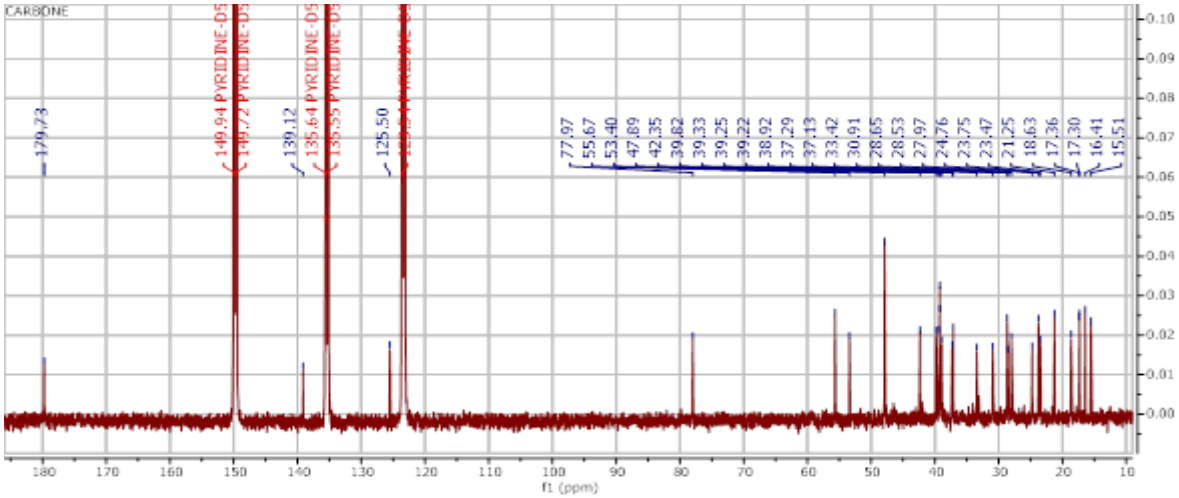

Figure S27. ^{13}C-NMR spectrum (150 MHz, Pyridine-d_5) of compound **12**

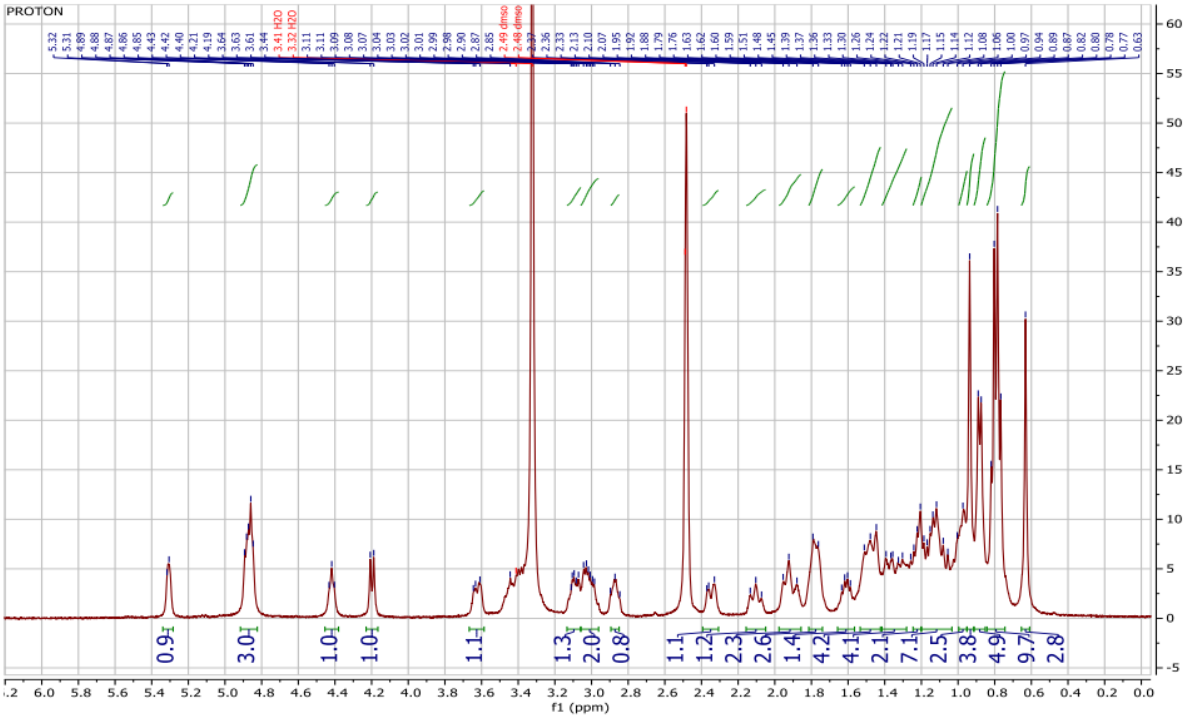

Figure S28. ^{1}H-NMR spectrum (600 MHz, DMSO-d_6) of compound **13**

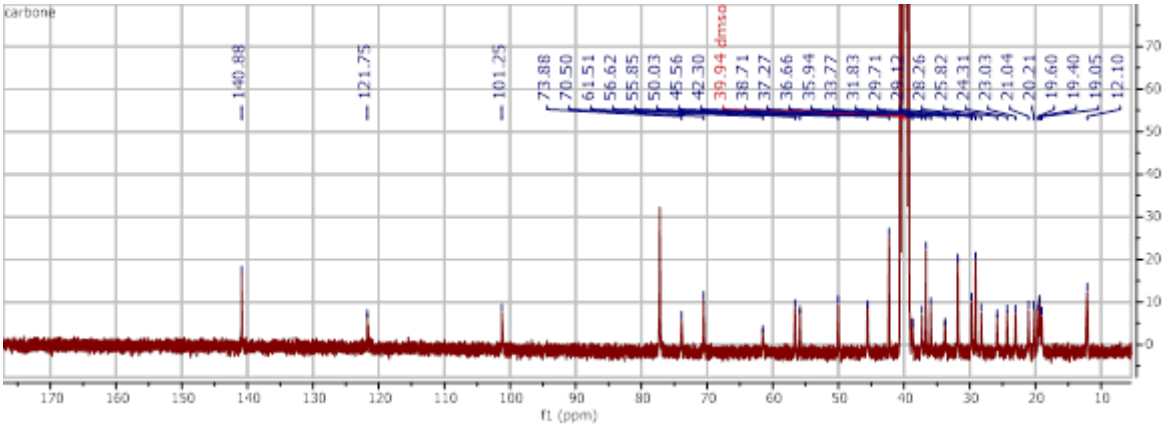

Figure S29. ^{13}C-NMR spectrum (150 MHz, DMSO-d_6) of compound **13**

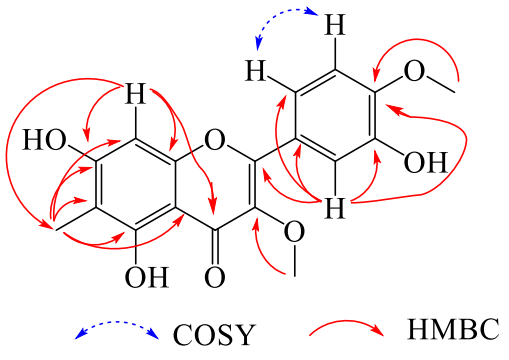

Figure S30. ^{1}H-^{1}H COSY and HMBC correlations of compound **1**.

References

Kuete V. 2010. Potential of Cameroonian plants and derived products against microbial infections: a review. Planta Med. 76(14):1479-1491.