



Supporting Information

for

Isolation and characterization of new phenolic siderophores with antimicrobial properties from *Pseudomonas* sp. UIAU-6B

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Additional analytical and experimental information

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EOE17 #179 RT: 4.78 AV: 1 NL: 4.27E7
F: FTMS - p ESI Full ms [100.00-2000.00]

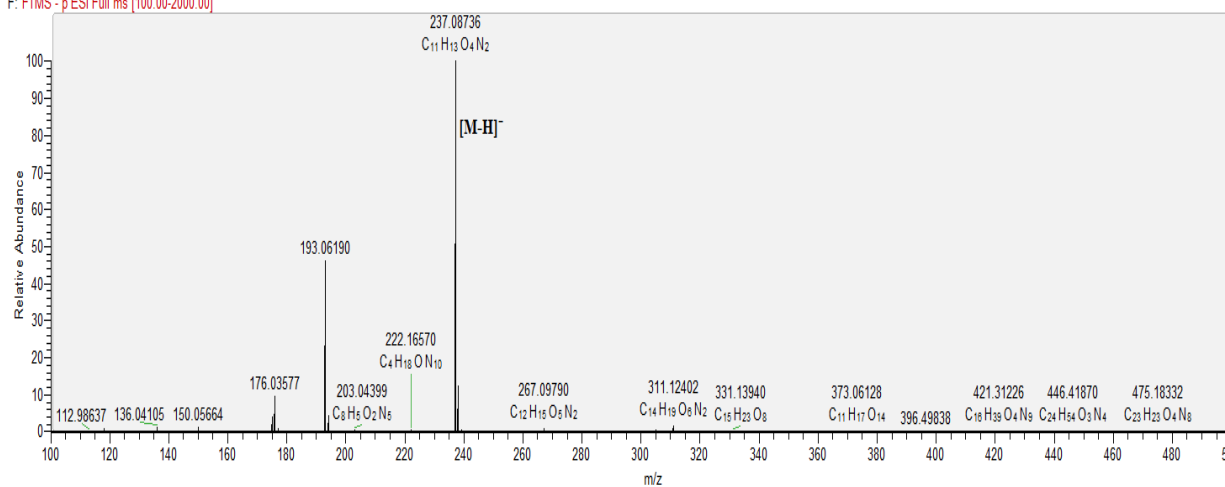


Figure S1. (+)-HR-ESIMS data of **1**

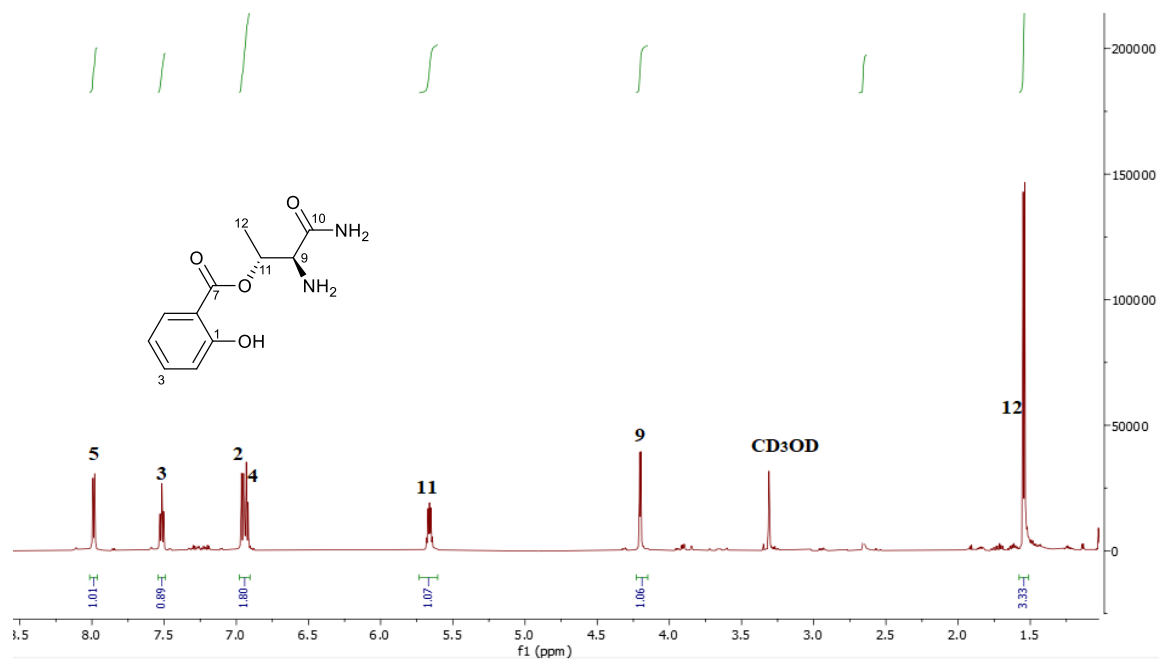


Figure S2. 1H NMR spectrum of **1**

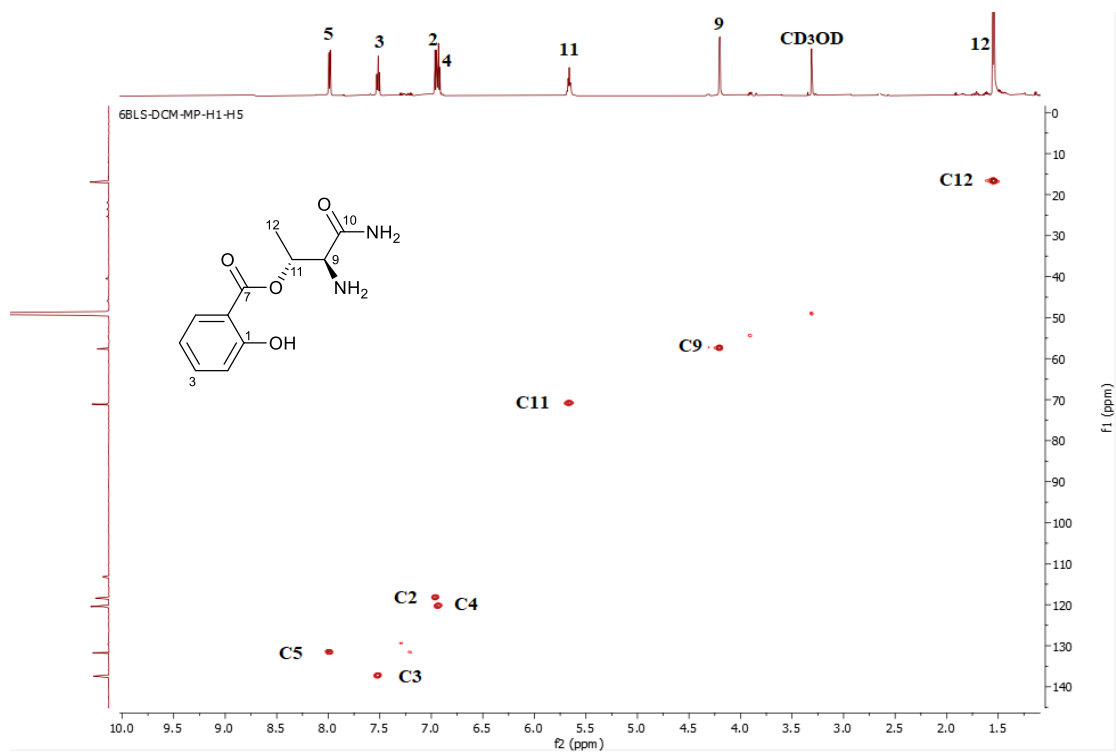


Figure S3. HSQC NMR spectrum of **1**

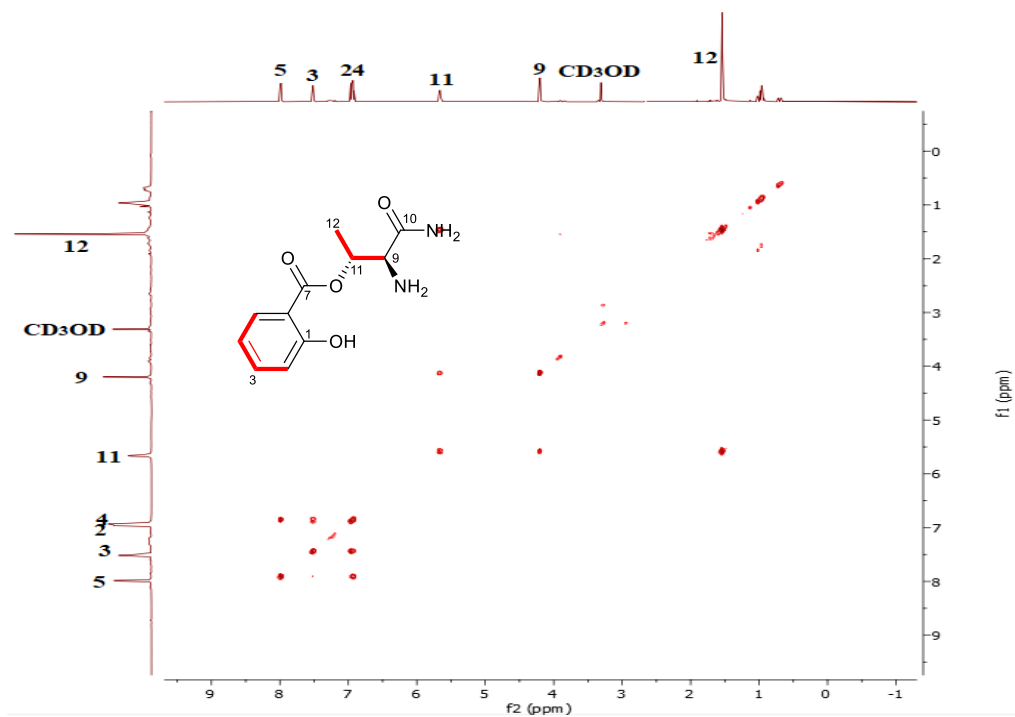


Figure S4. COSY NMR spectrum of **1**

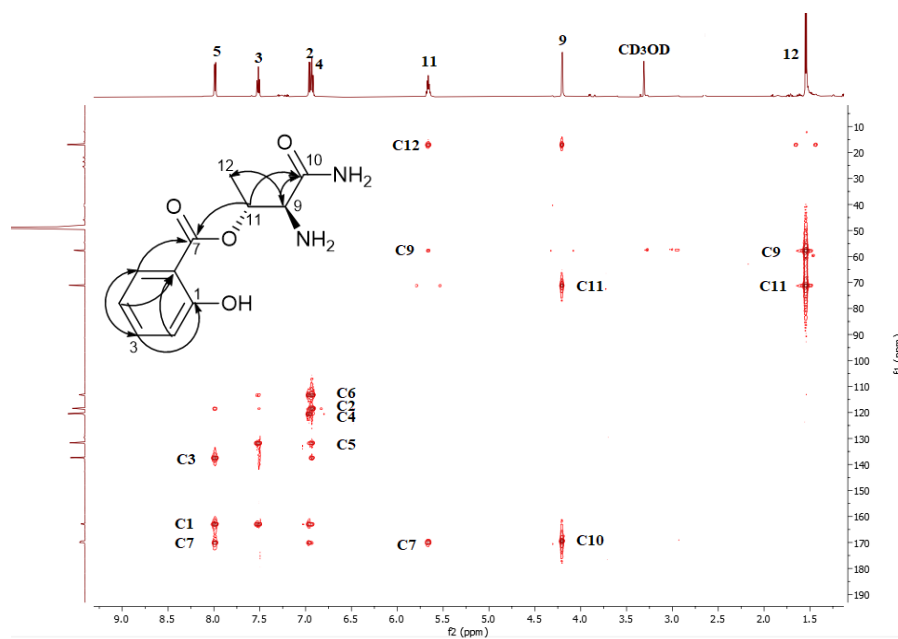


Figure S5. HMBC NMR spectrum of **1**

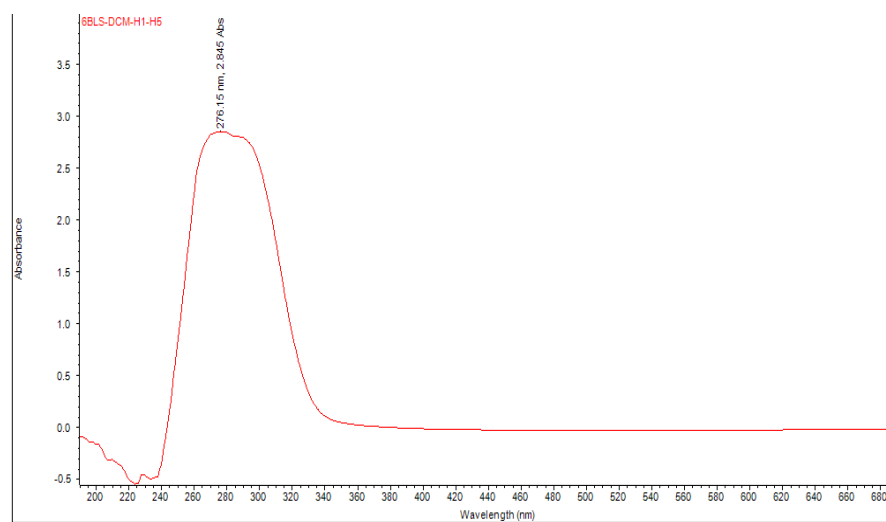


Figure S6. UV data of **1**

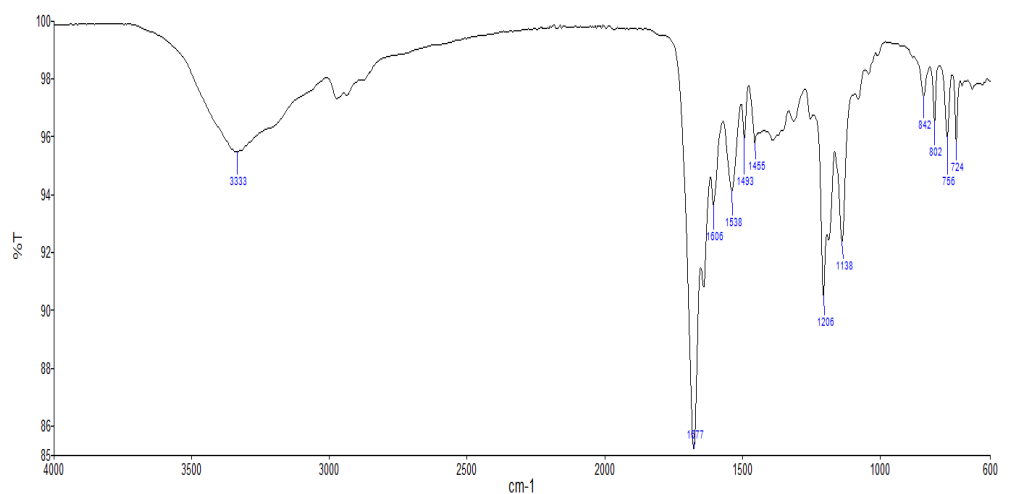


Figure S7. IR data of **1**

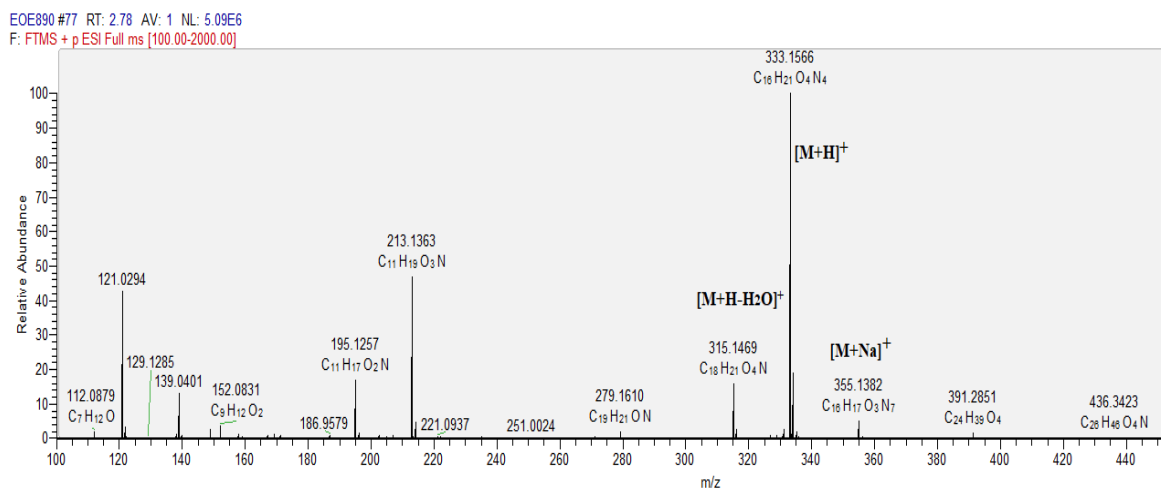


Figure S8. (+)-HR-ESIMS data of **2**

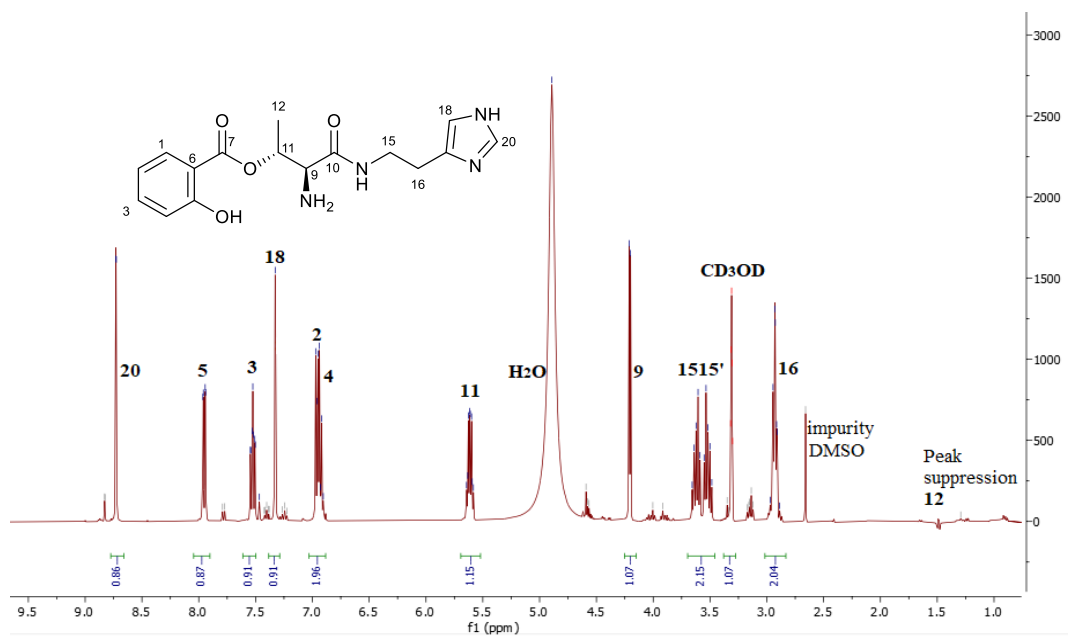


Figure S9. ^1H NMR spectrum of **2**

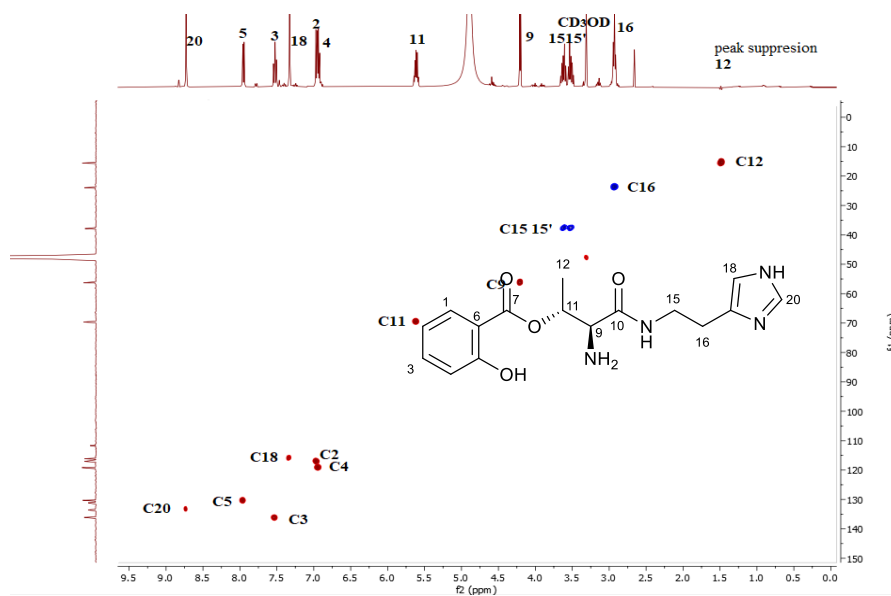


Figure S10. HSQC NMR spectrum of **2**

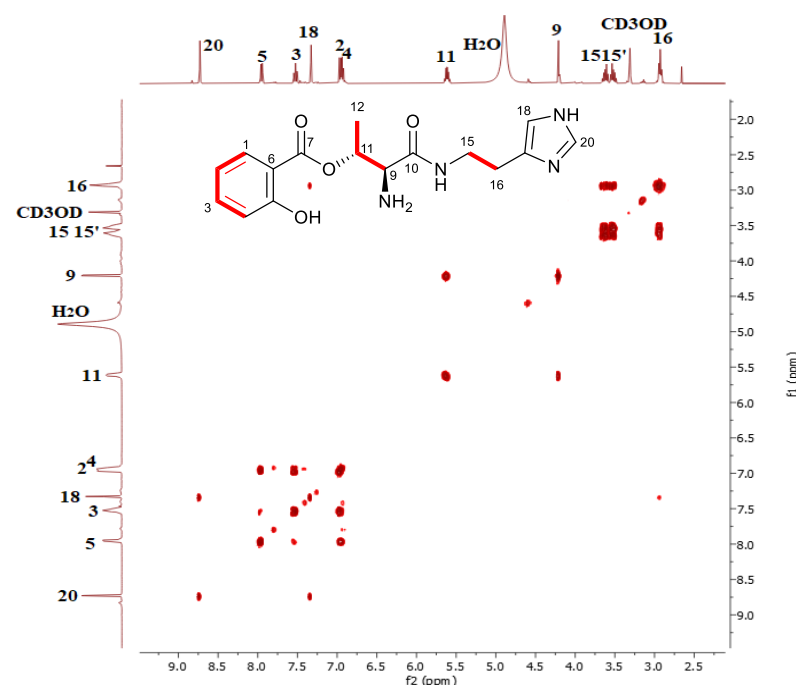


Figure S11. COSY NMR spectrum of **2**

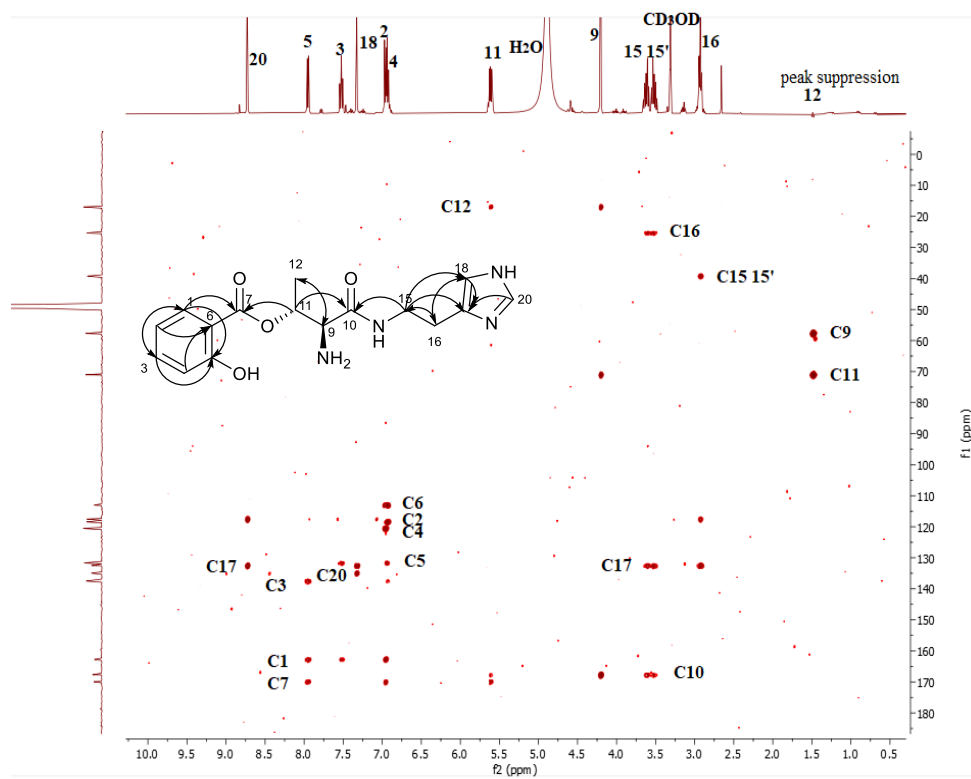


Figure S12. HMBC NMR spectrum of **2**

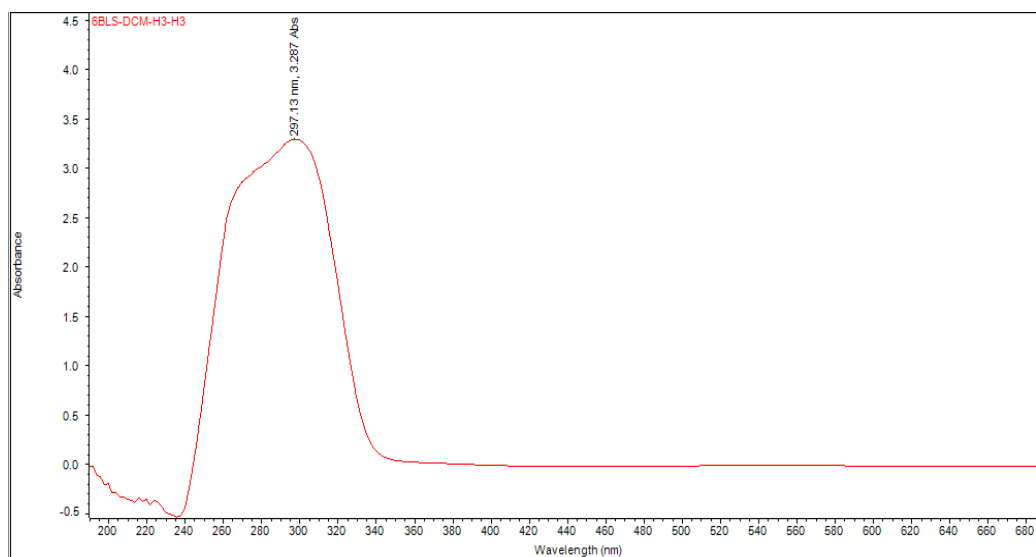


Figure S13. UV data of **2**

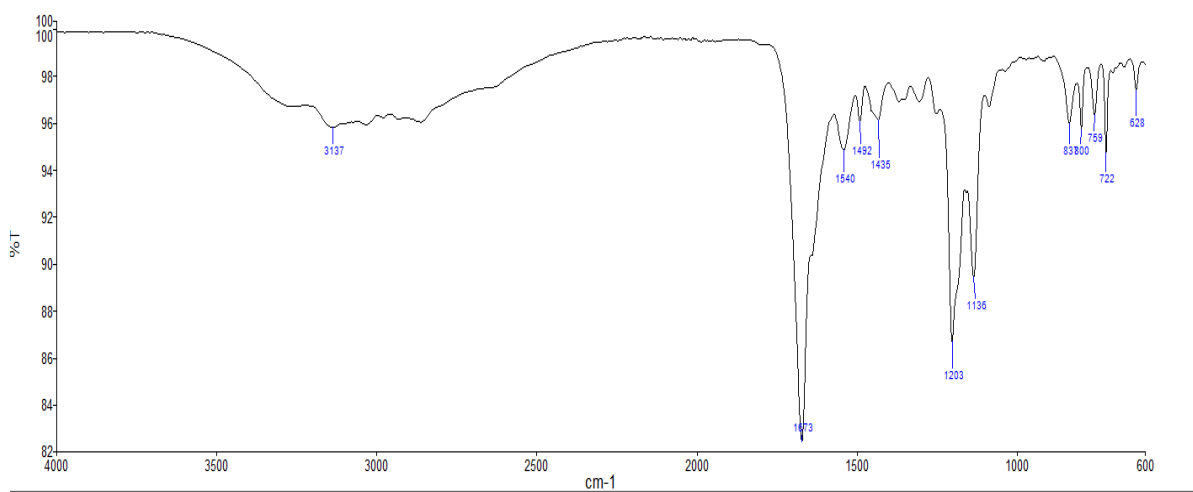


Figure S14. IR data of **2**

EOE928 #171 RT: 8.18 AV: 1 NL: 1.42E8
F: FIMS + p ESI Full ms [100.00-2000.00]

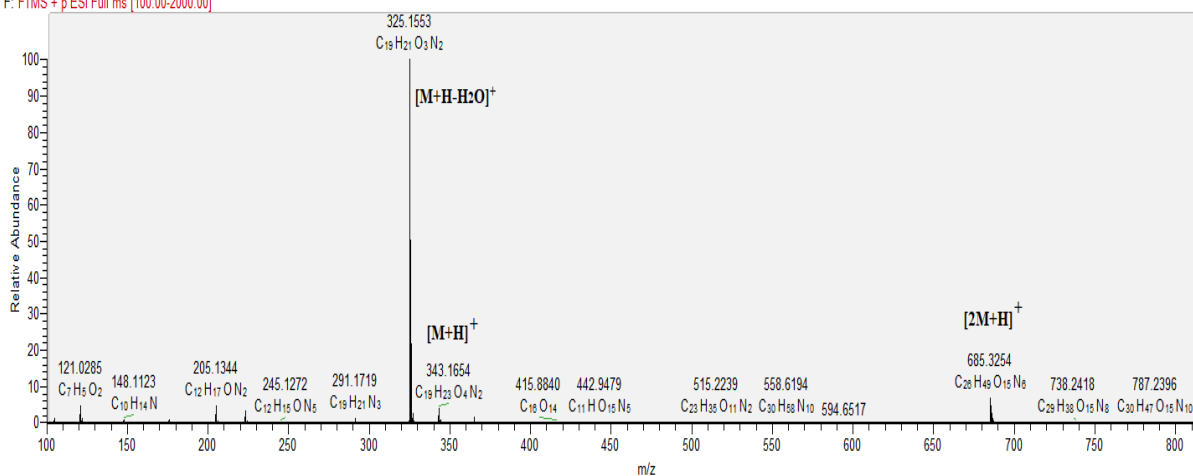


Figure S15. (+)-HR-ESIMS data of **3**

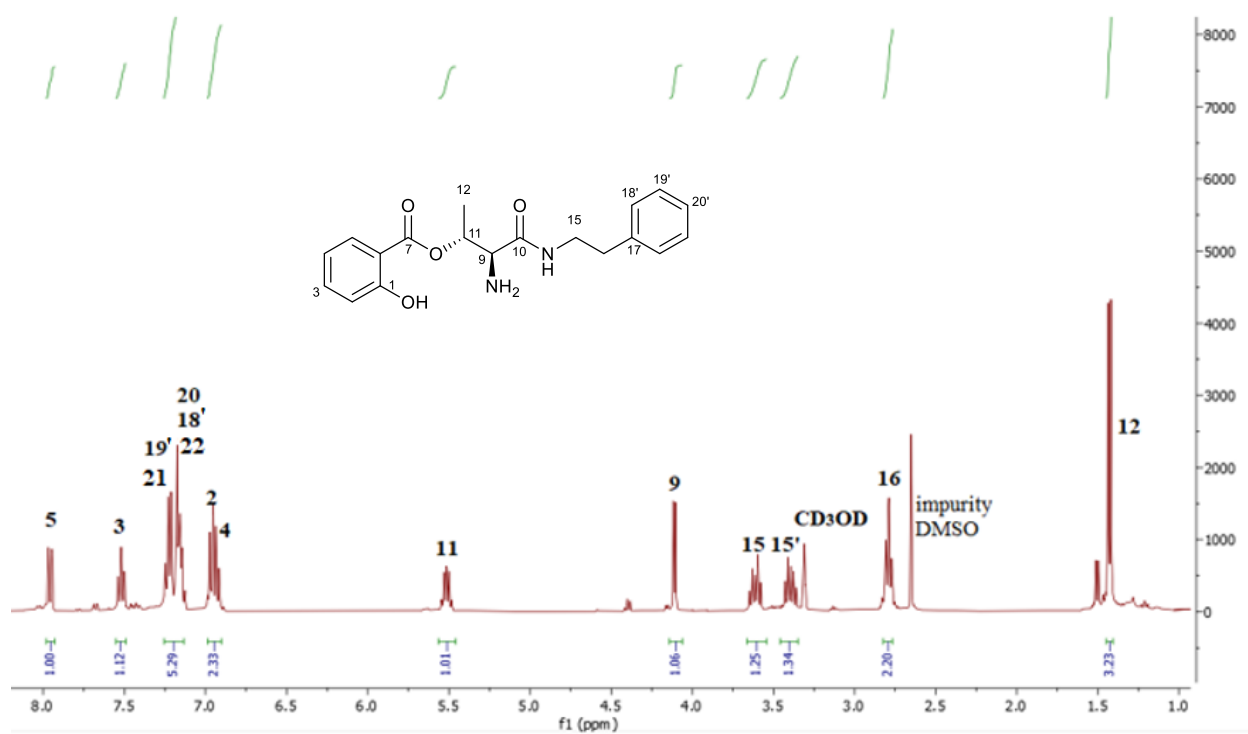


Figure S16. ¹H NMR spectrum of **3**

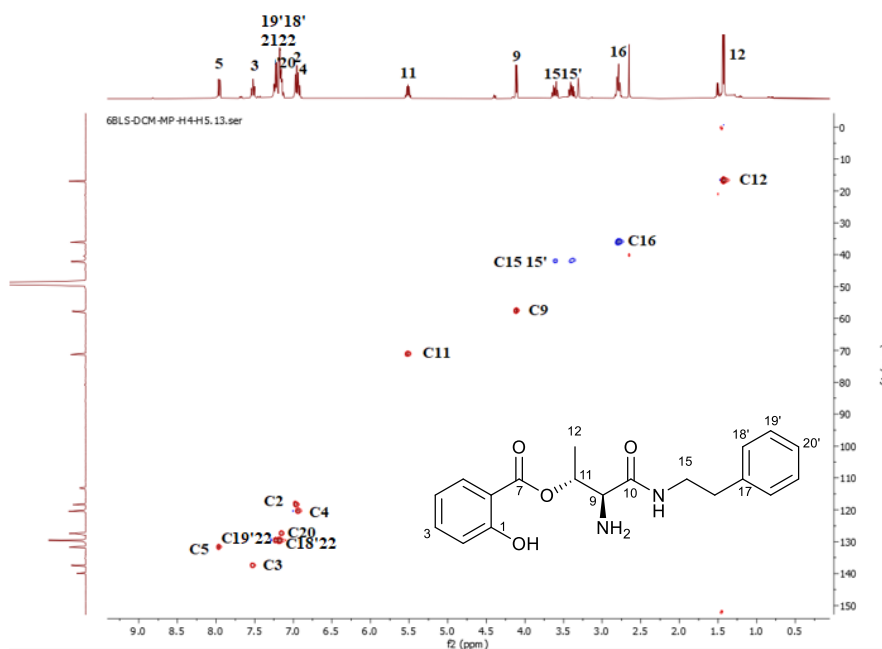


Figure S17. HSQC NMR spectrum of **3**

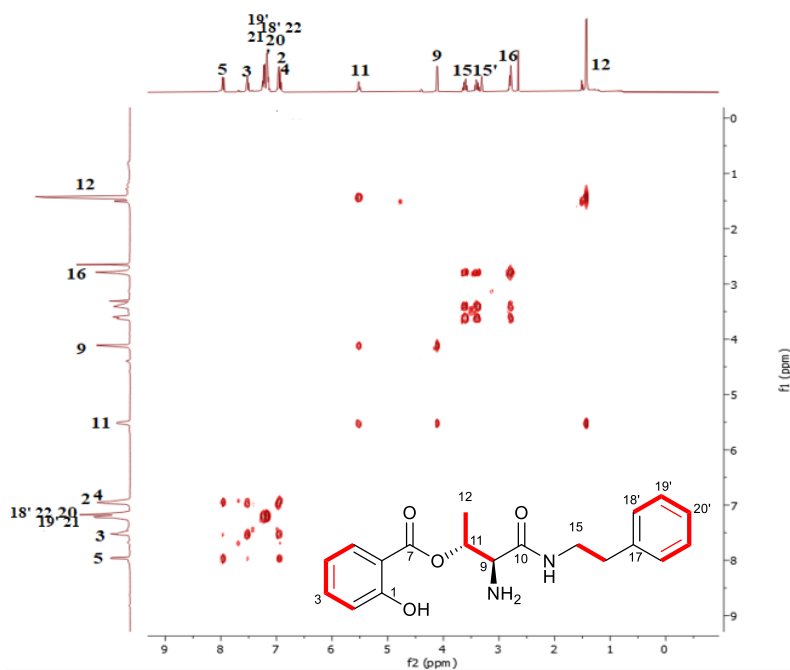


Figure S18. COSY NMR spectrum of **3**

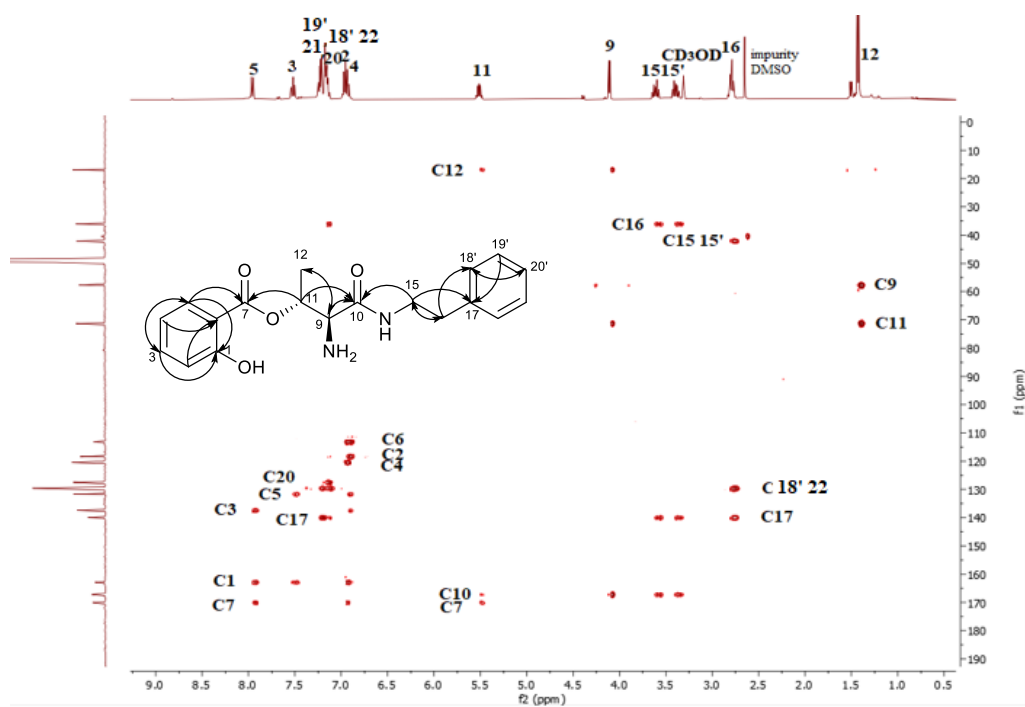


Figure S19. HMBC NMR spectrum of **3**

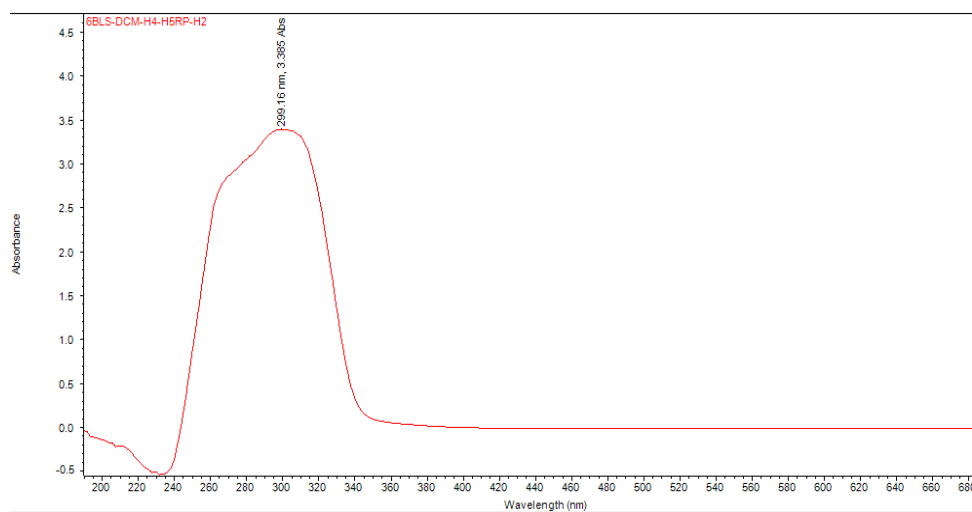


Figure S20. UV data of **3**

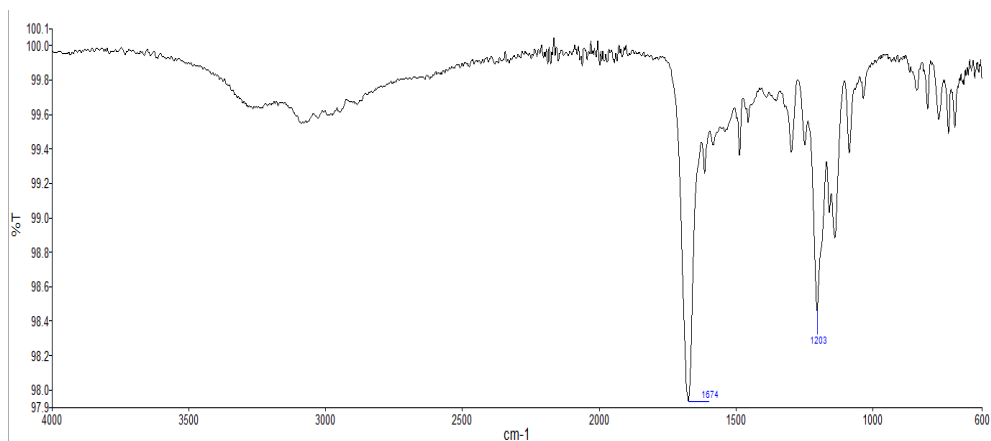


Figure S21. IR data of **3**

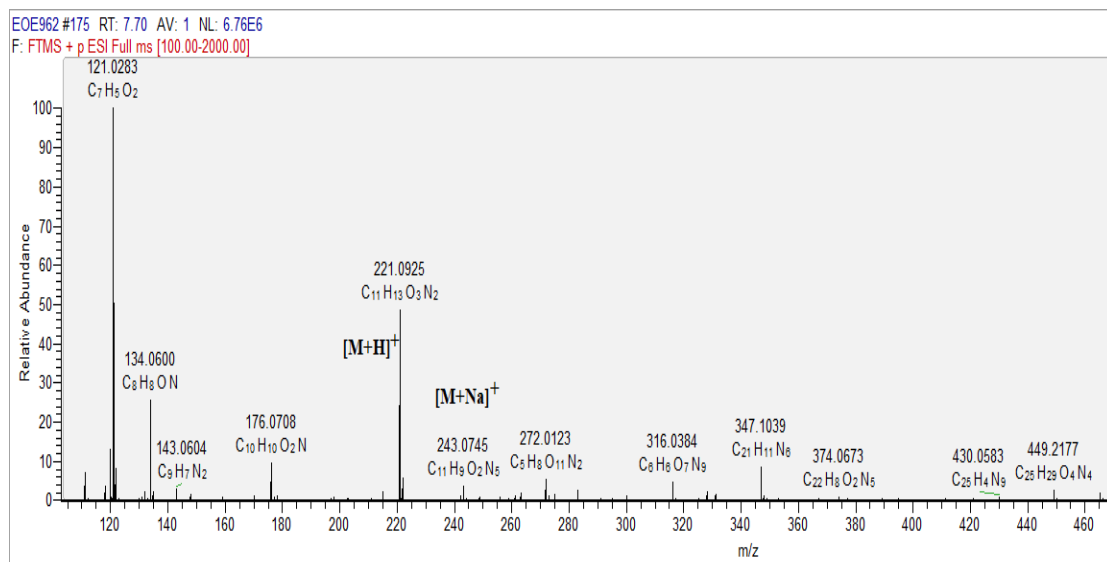


Figure S22. (+)-HR-ESIMS data of **4**

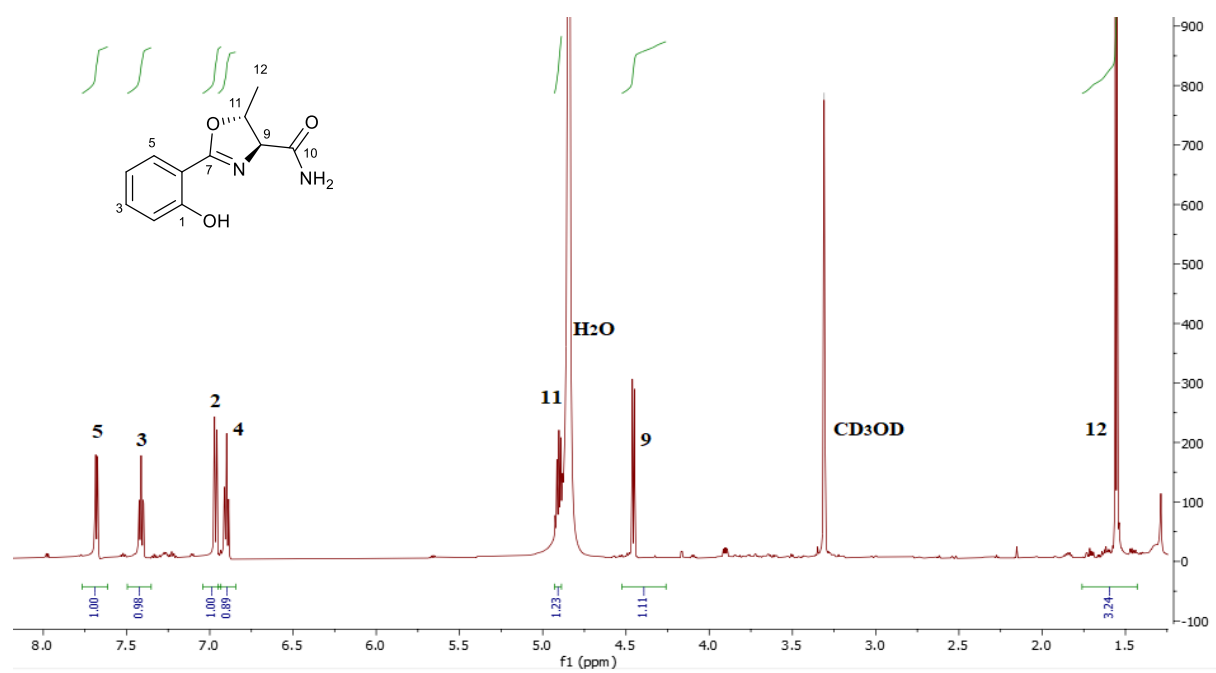


Figure S23. ¹H NMR spectrum of 4

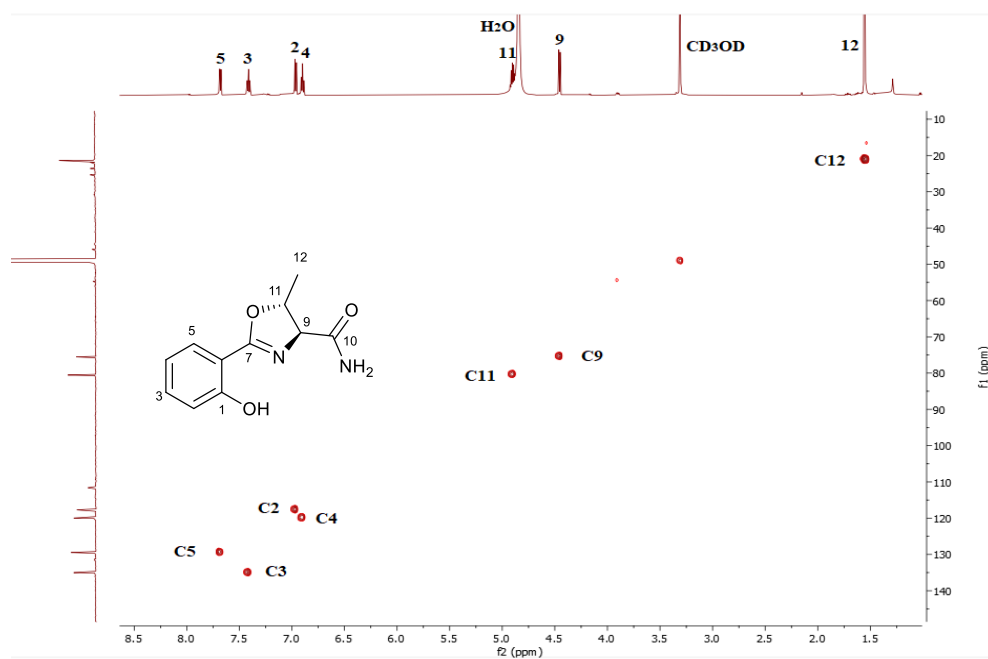


Figure S24. HSQC NMR spectrum of 4

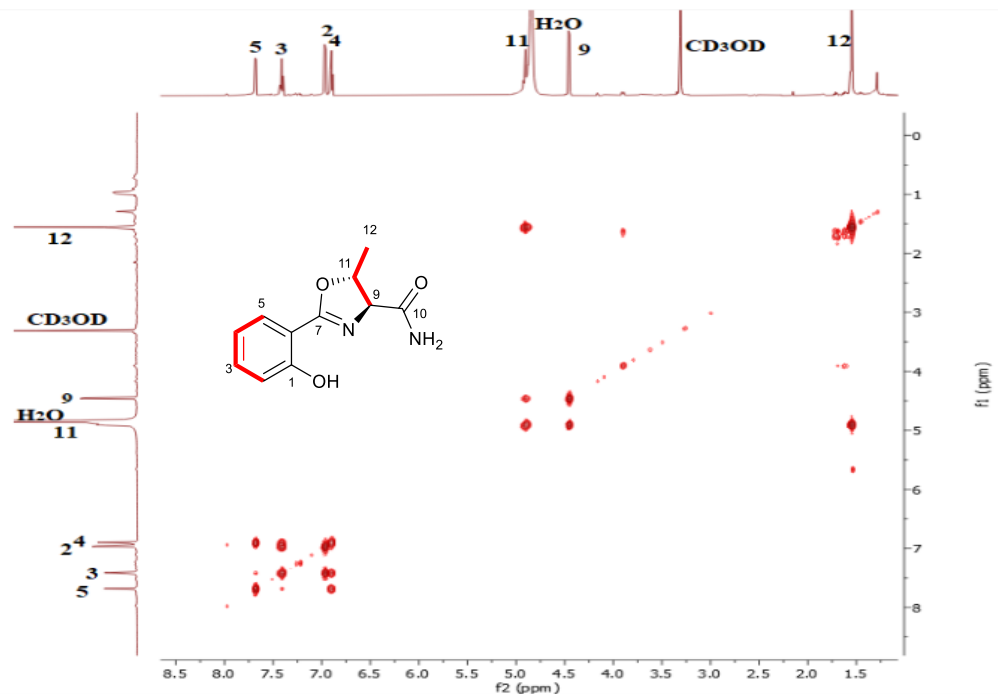


Figure S25. COSY NMR spectrum of **4**

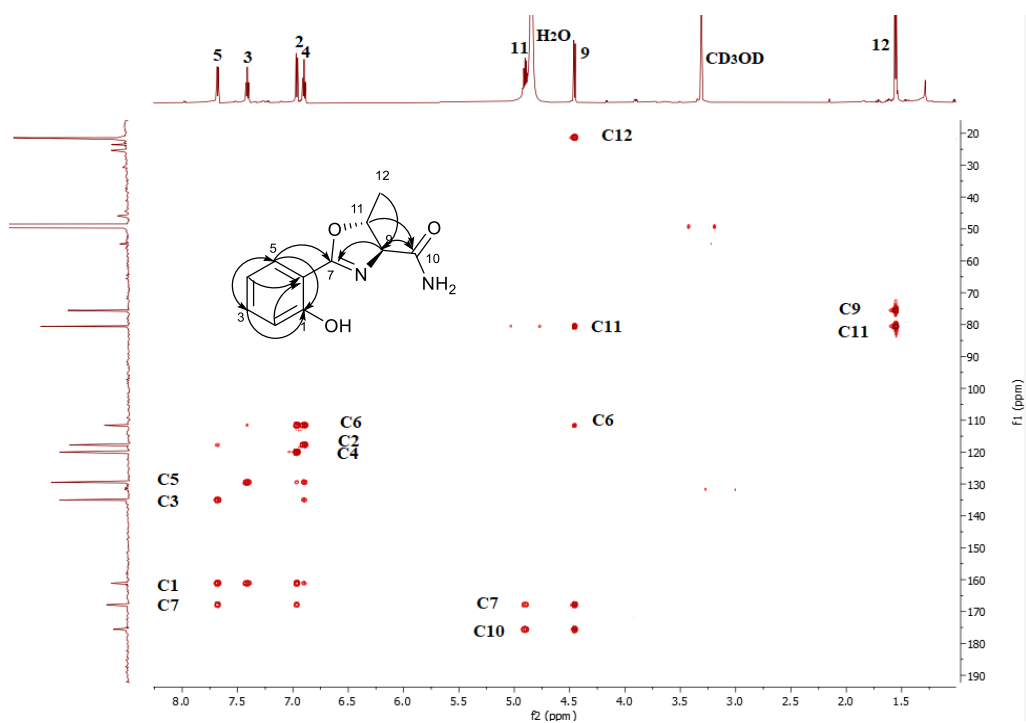


Figure S26. HMBC NMR spectrum of **4**

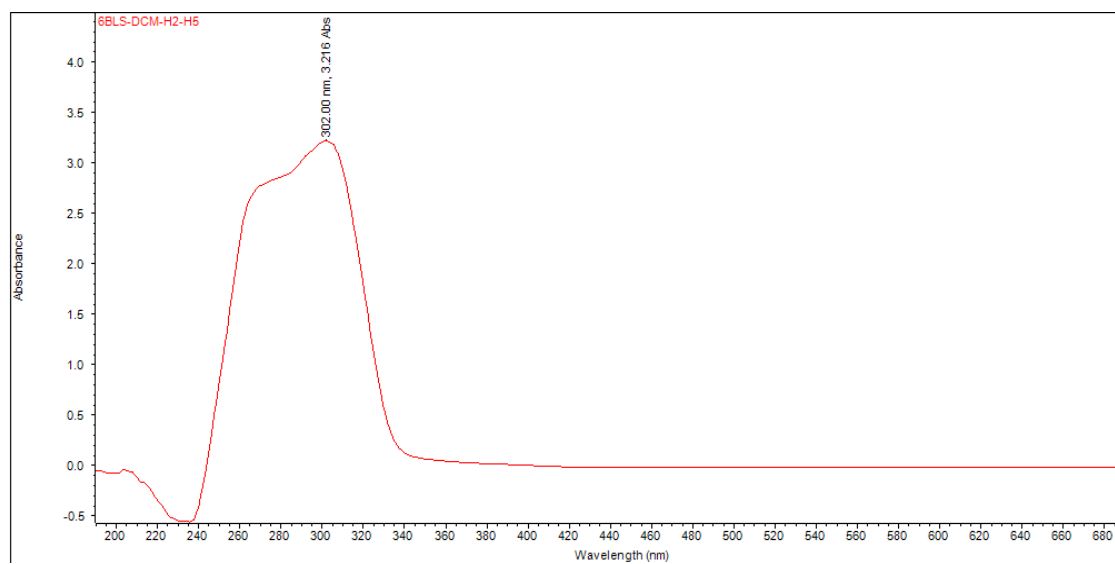


Figure S27. UV data of **4**

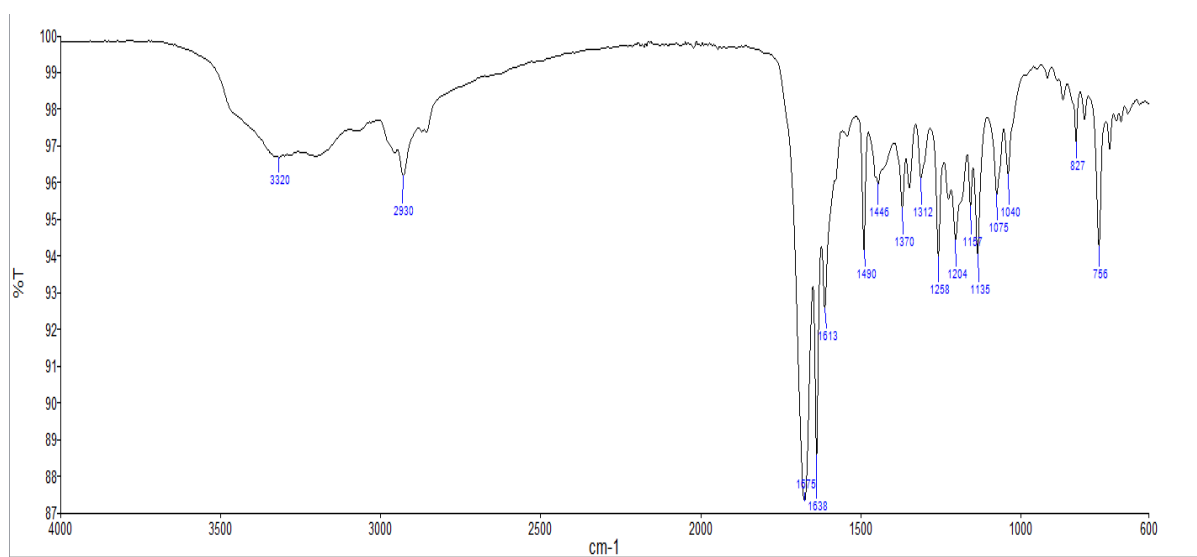


Figure S28. IR data of **4**

EOE928 #325 RT: 14.06 AV: 1 NL: 3.93E7
F: FTMS + p ESI Full ms [100.00-2000.00]

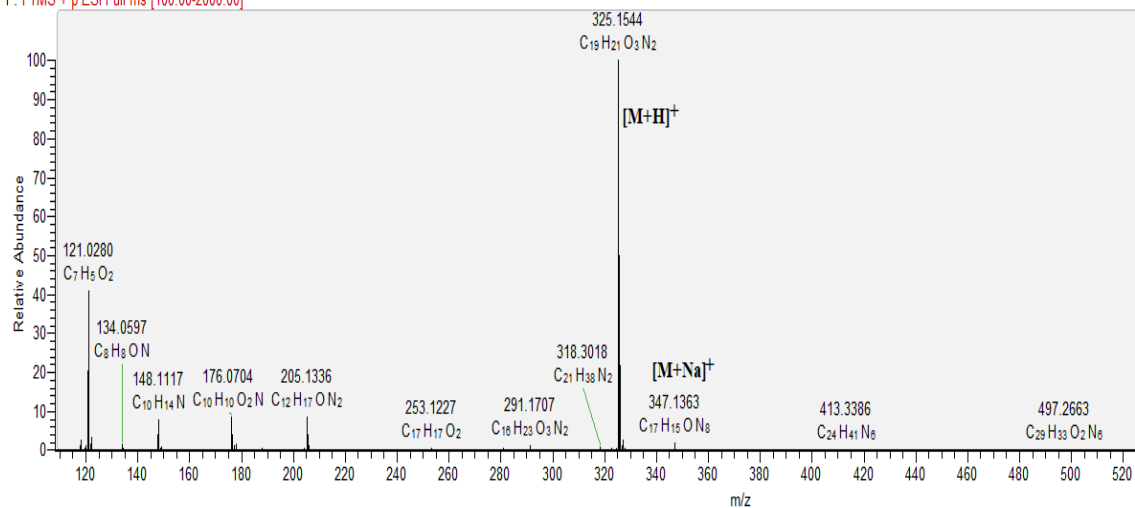


Figure S29. (+)-HR-ESIMS data of **5**

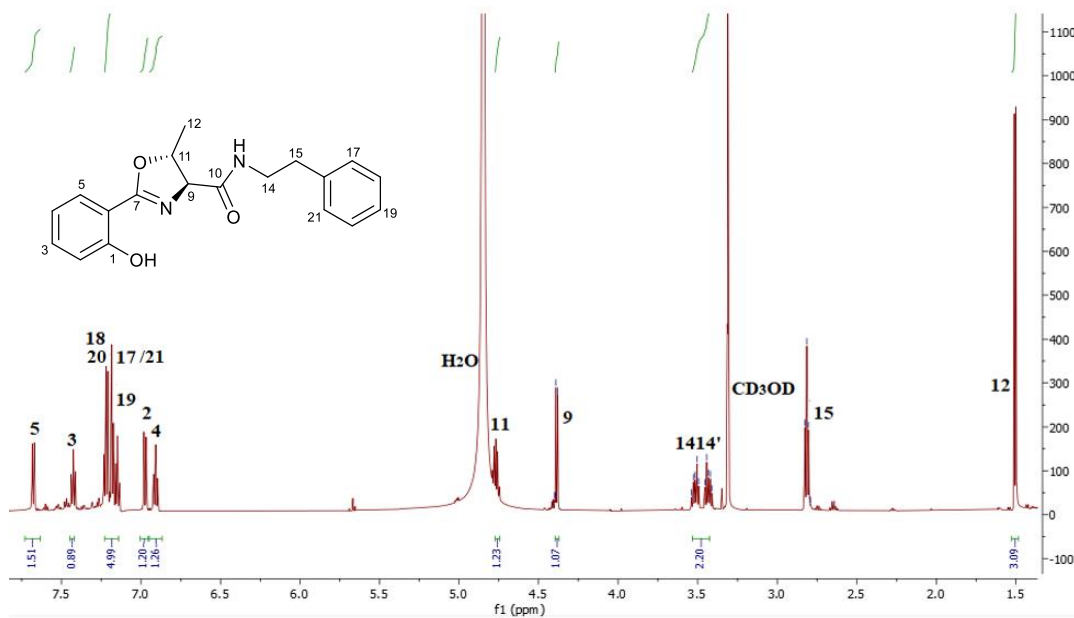


Figure S30. ^1H NMR spectrum of **5**

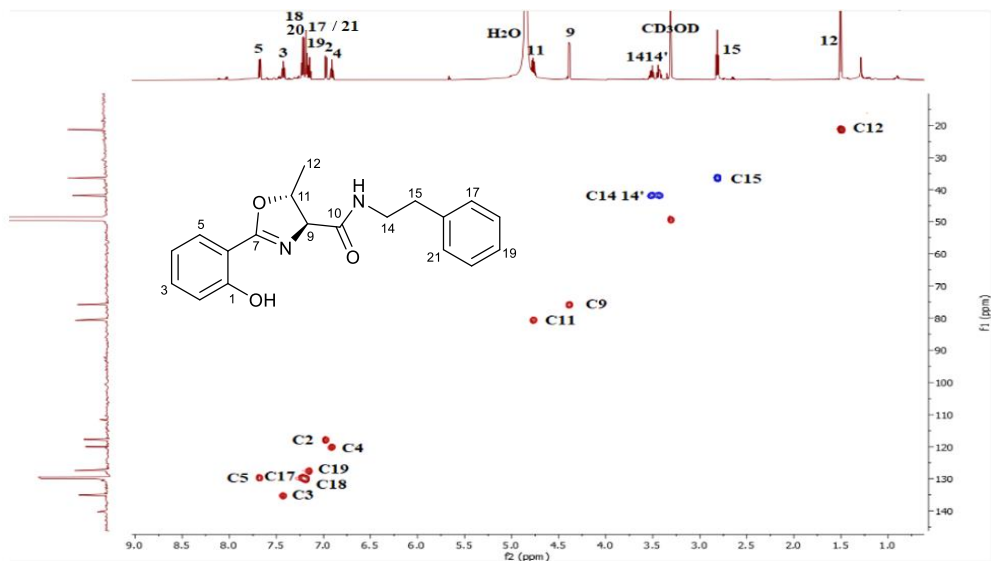


Figure S31. HSQC NMR spectrum of **5**

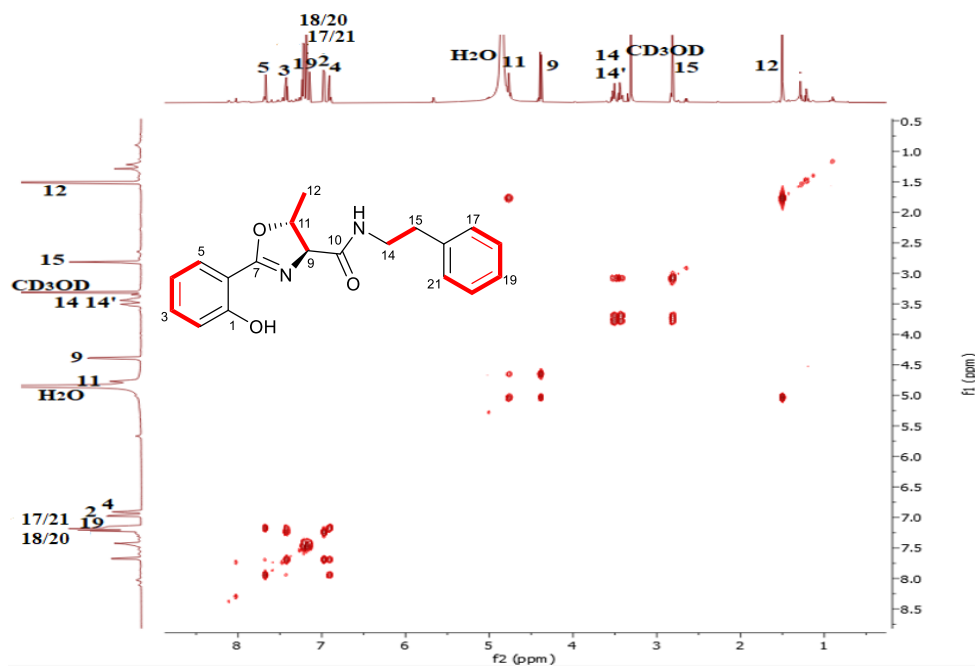


Figure S32. COSY NMR spectrum of **5**

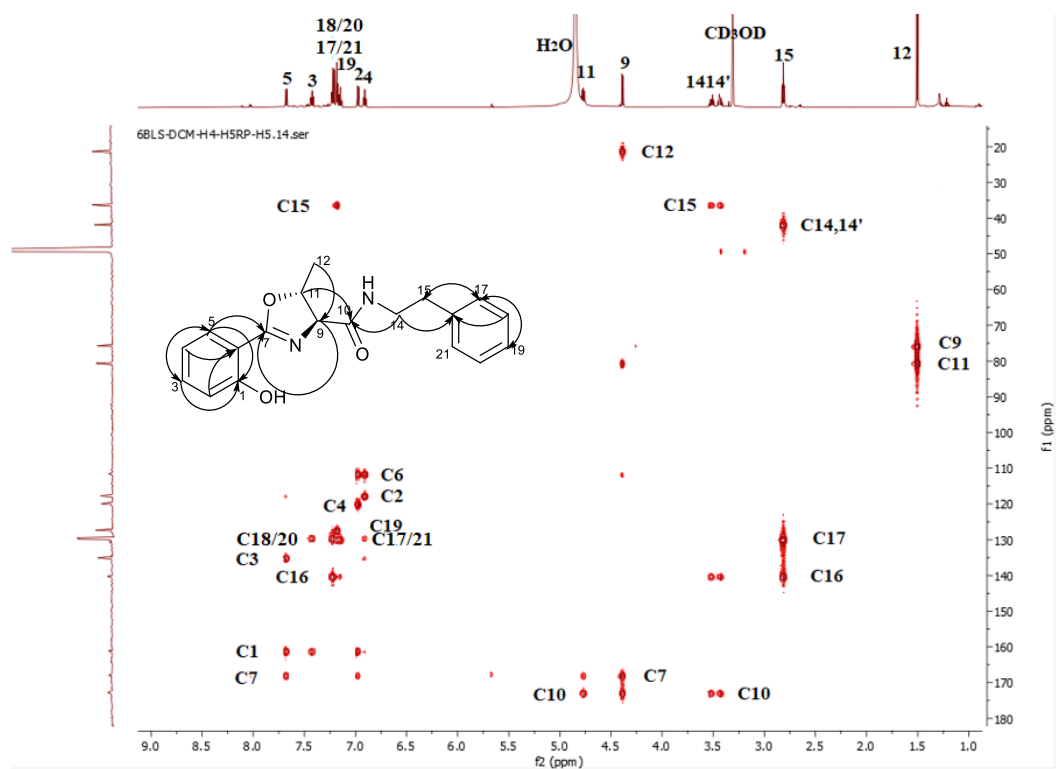


Figure S33. HMBC NMR spectrum of **5**

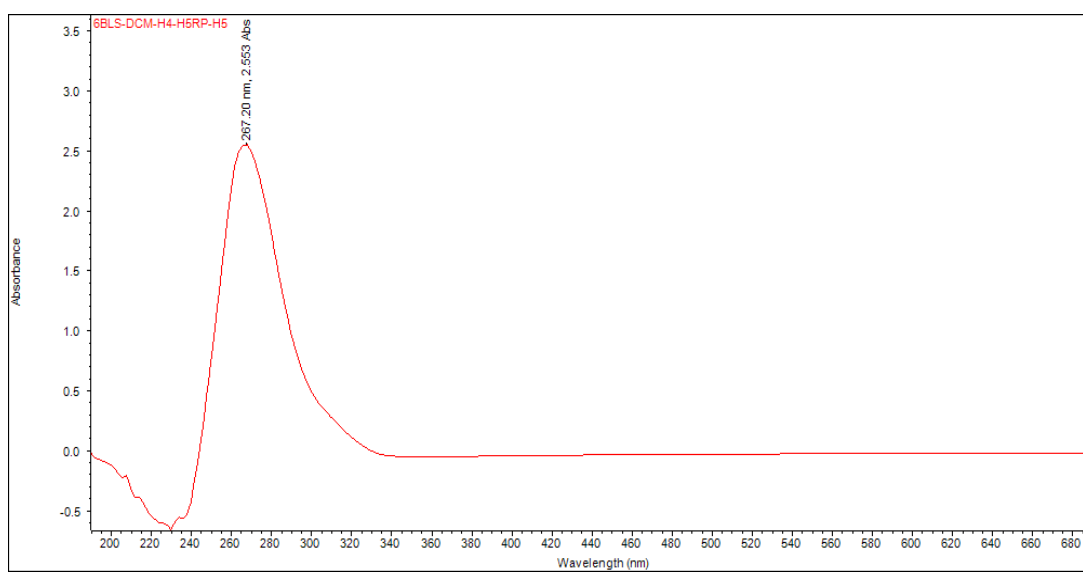


Figure S34. UV data of **5**

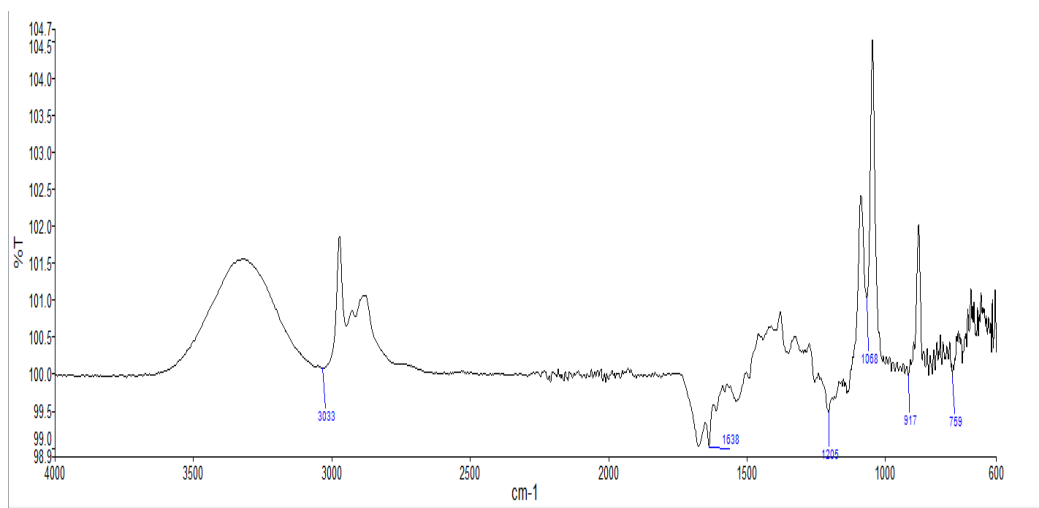


Figure S35. IR data of **5**

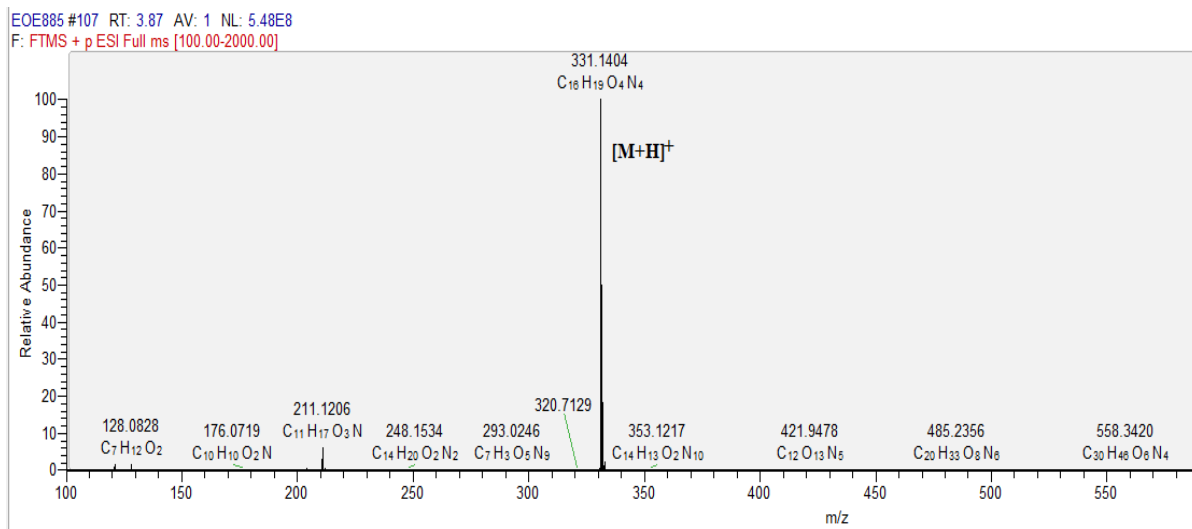


Figure S36. (+)-HR-ESIMS data of **6**

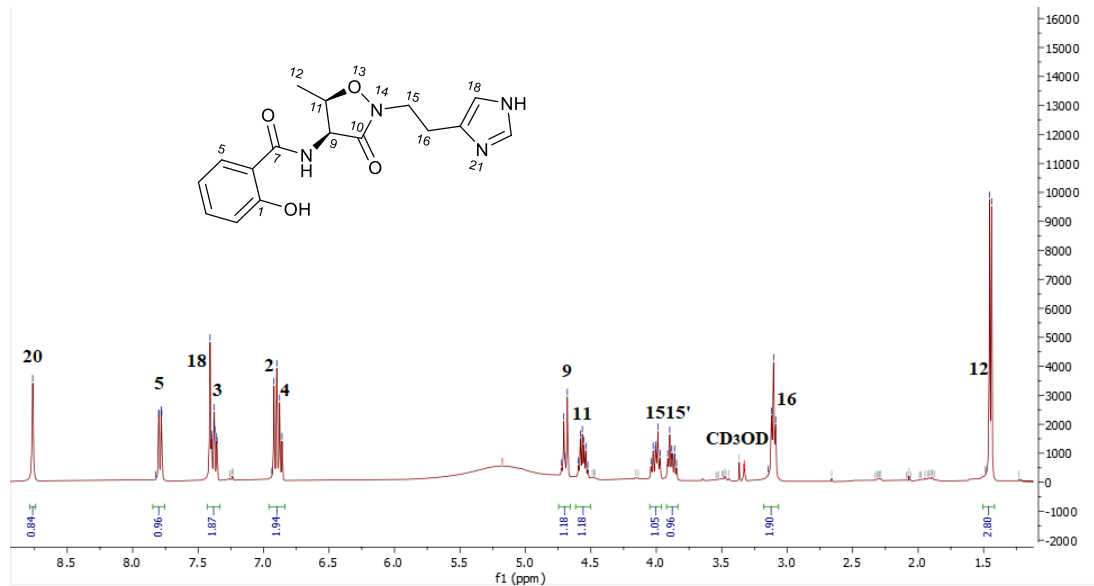


Figure S37. ^1H NMR spectrum of **6**

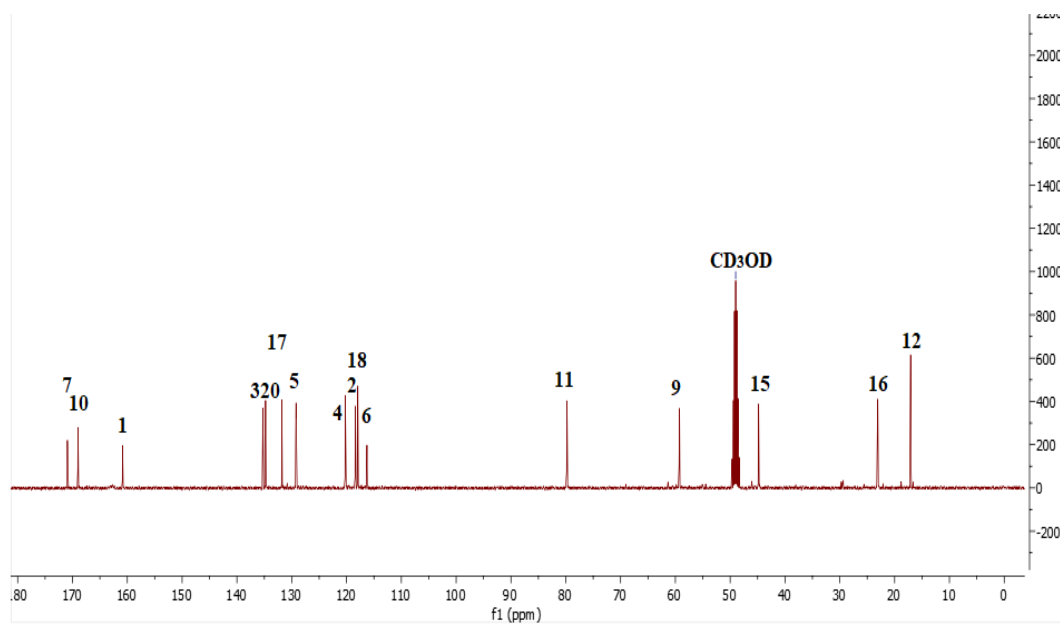


Figure S38. ^{13}C NMR spectrum of **6**

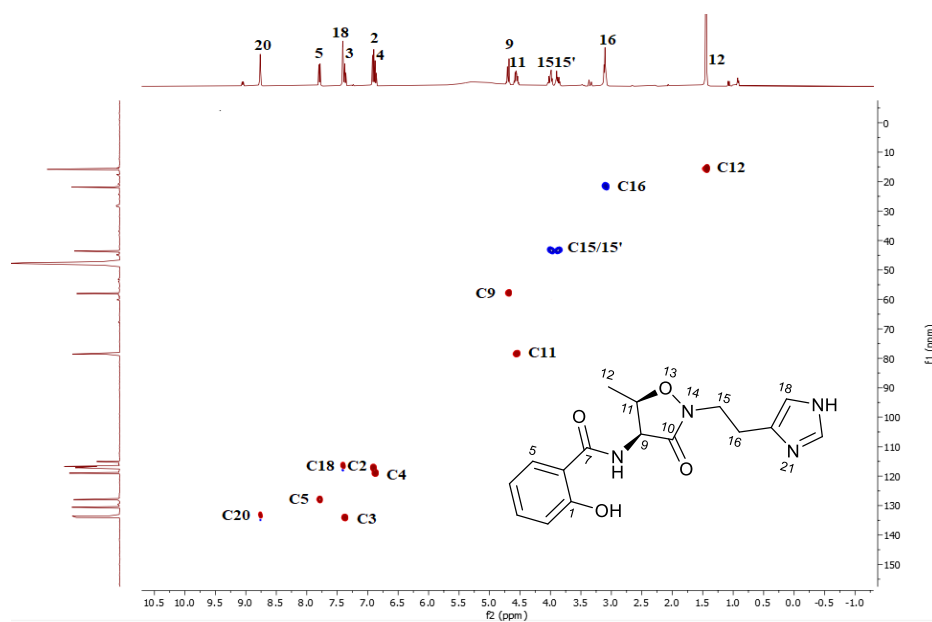


Figure S39. HSQC NMR spectrum of **6**

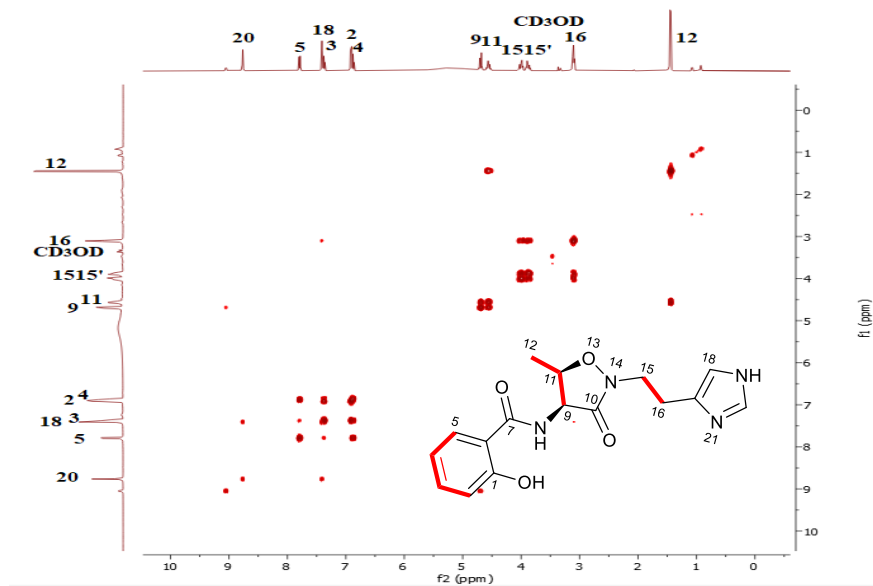


Figure S40. COSY NMR spectrum of **6**

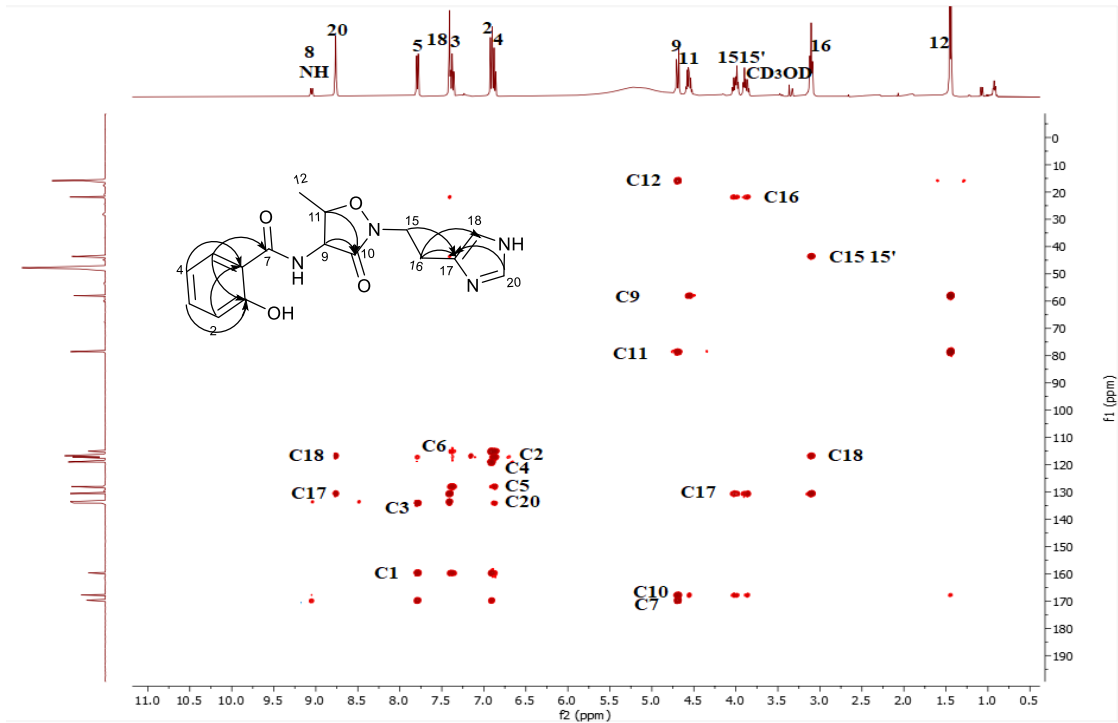


Figure S41. HMBC NMR spectrum of **6**

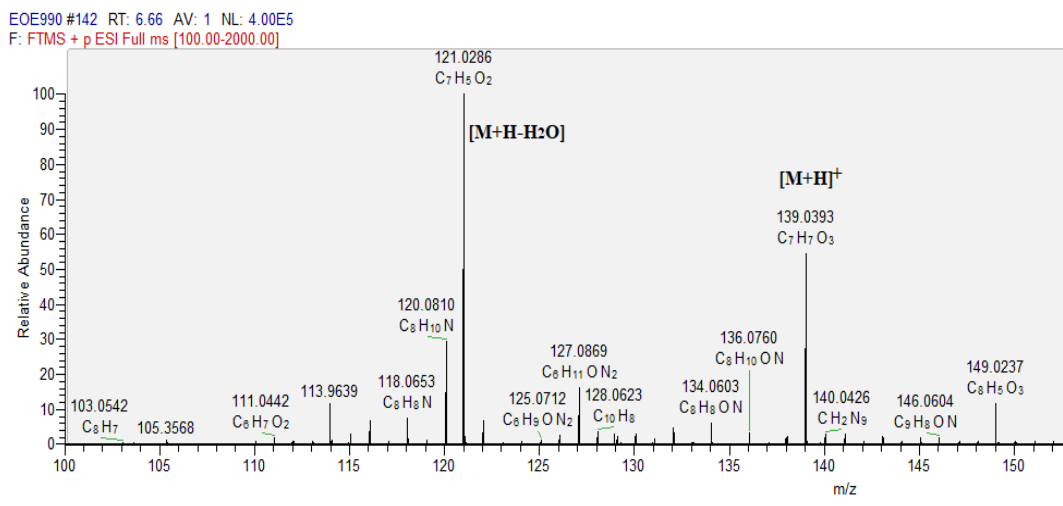


Figure S42. (+)-HR-ESIMS data of **7**

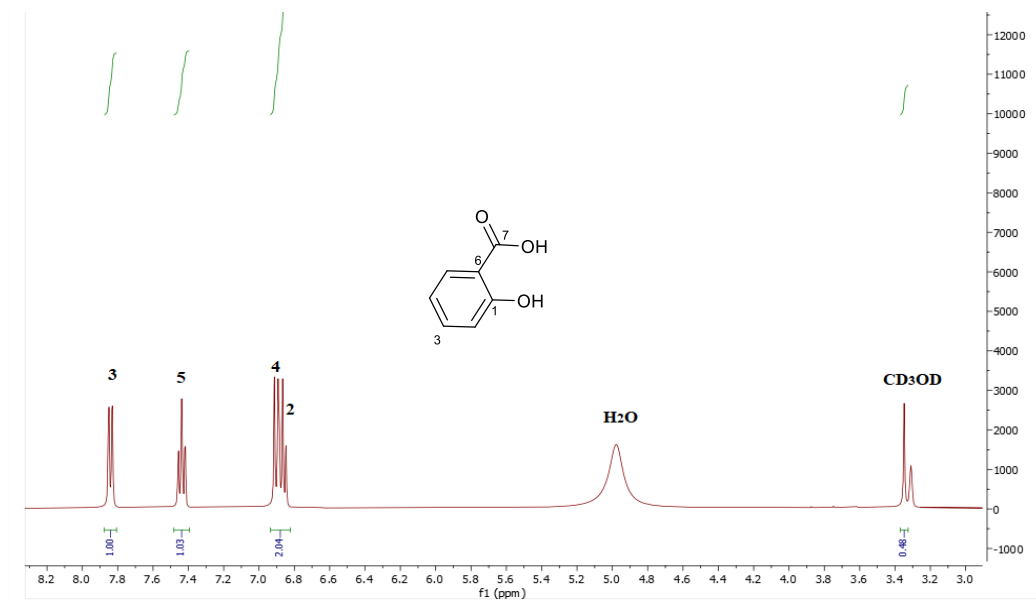


Figure S43. ^1H NMR spectrum of 7

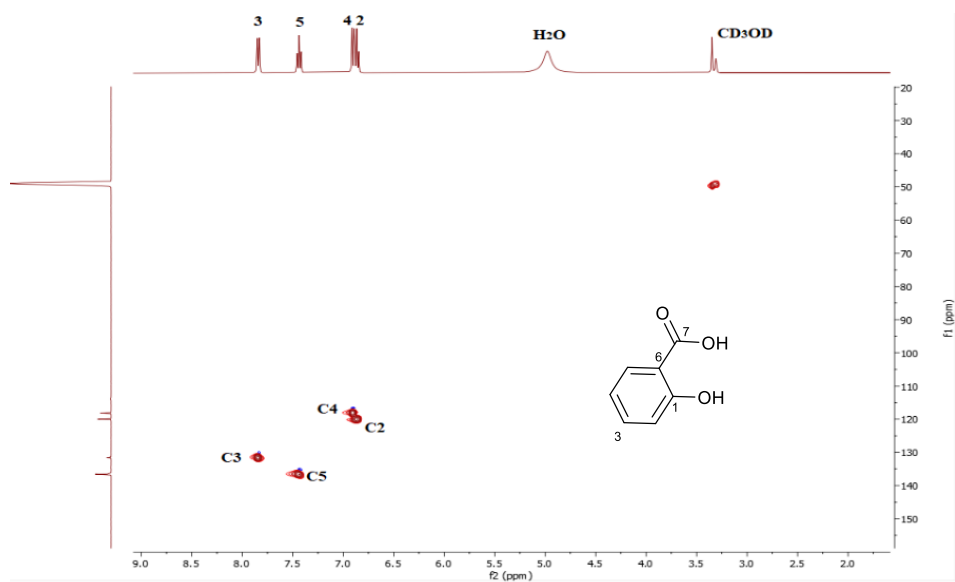


Figure S44. HSQC NMR spectrum of 7

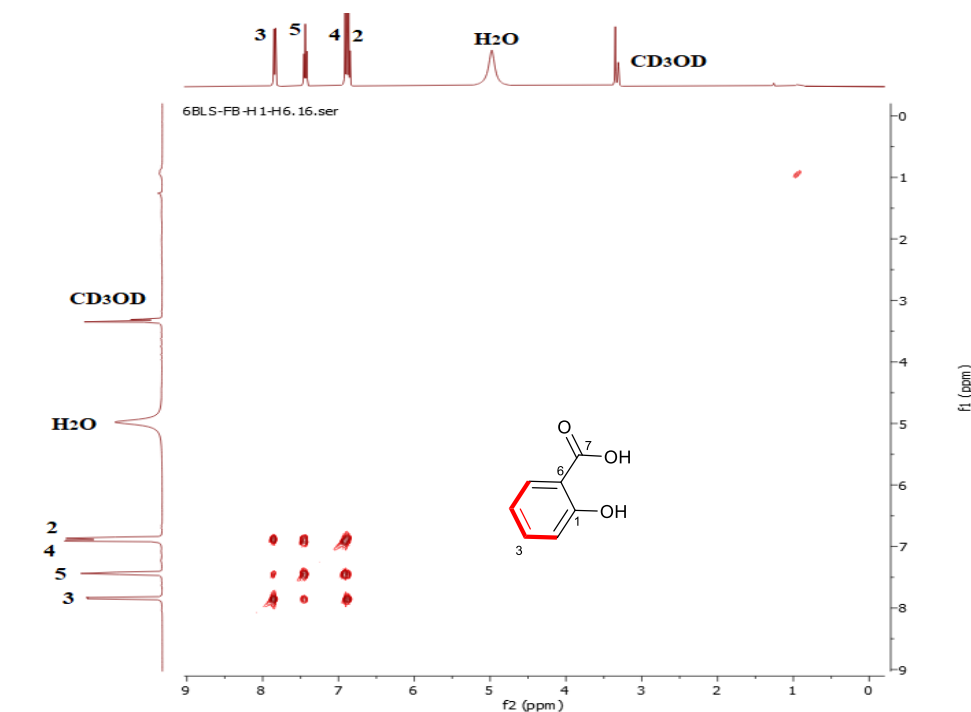


Figure S45. COSY NMR spectrum of **7**

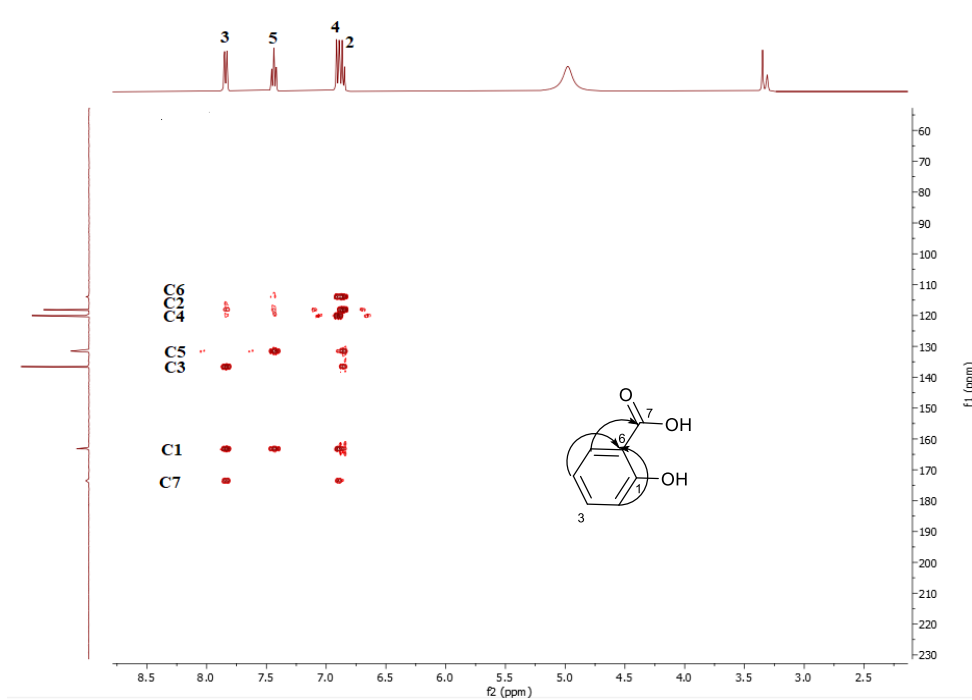


Figure S46. HMBC NMR spectrum of **7**

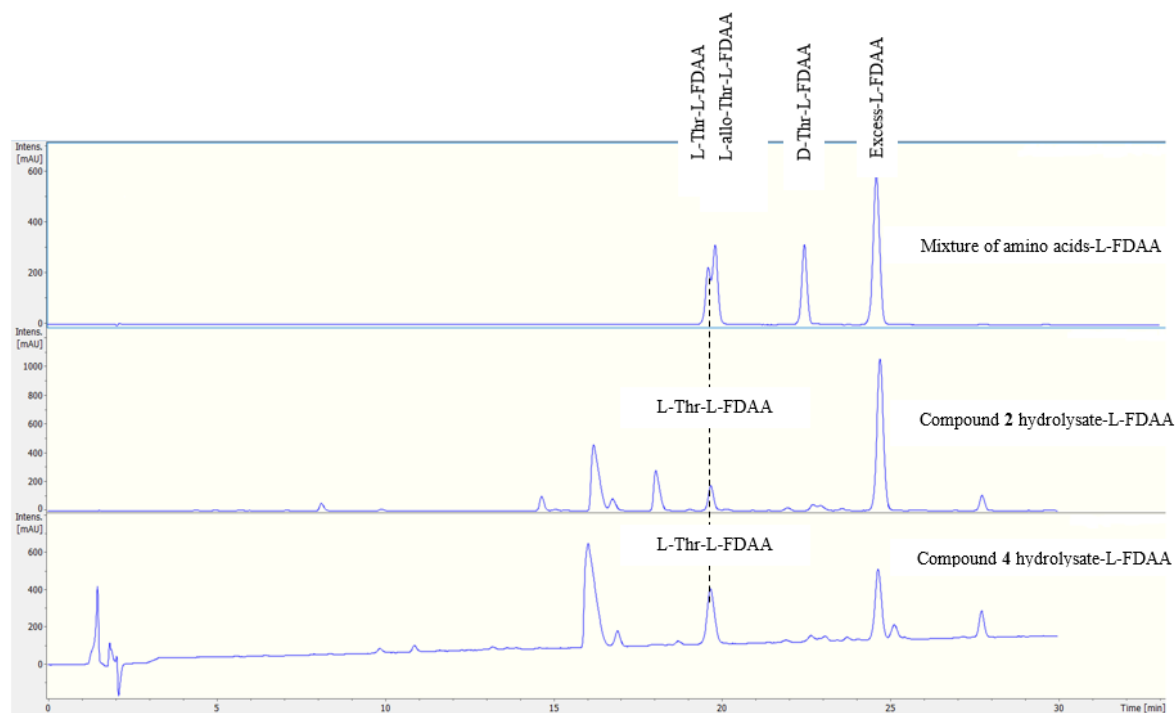
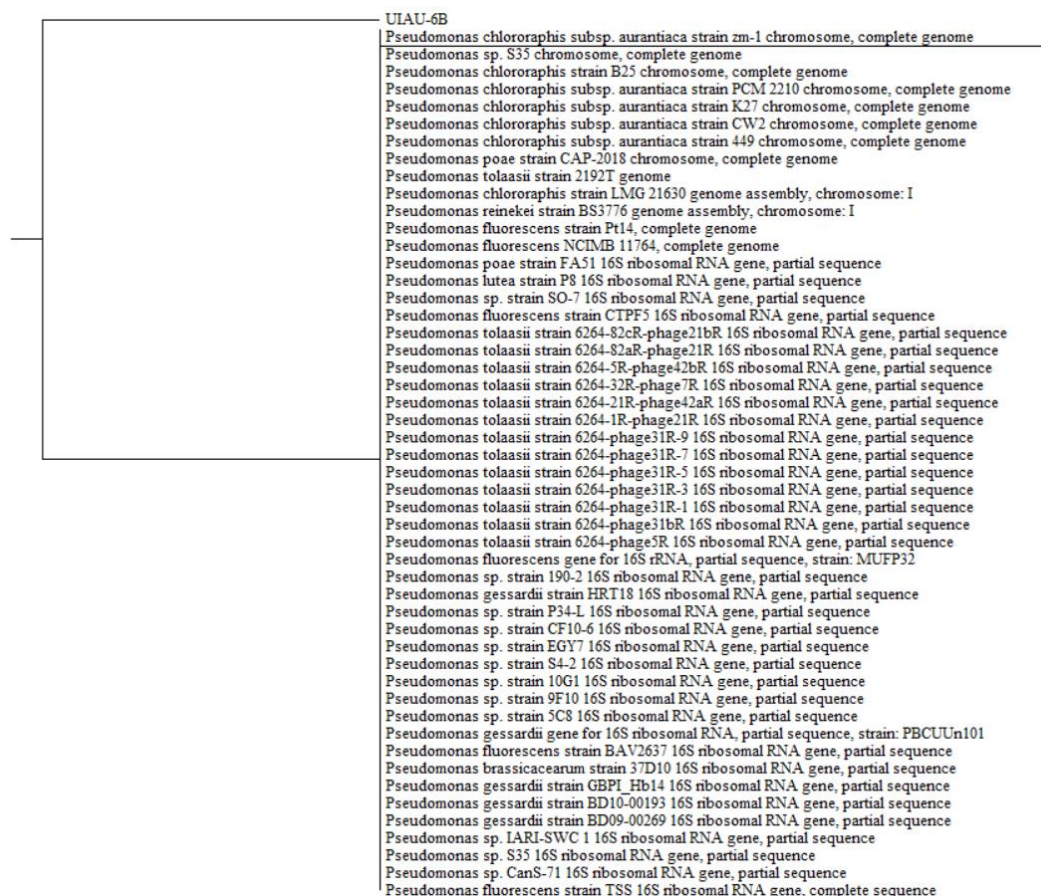


Figure S47 Chromatographic profiles of the compound (2) and (4) derivatized with L-FDAA and the standard amino acids with L-FDAA.

CCTANATCTTGAAAACAACTTCACCCAGTCATGAATCACACCGTGGTAACCGTCCTCCCGA
 AGGTTAGACTACCTACTTCTGGTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAG
 GCCCGGAACGTATTCACCGCGACATTCTGATTGCGGATTACTAGCGATTCCGACTTCACGC
 AGTCGAGTTGCAGACTGCGATCCGGACTACGATCGGTTTTATGGGATTAGCTCCACCTCGCG
 GCTTGGCAACCCTCTGTACCGACCATTGTAGCACGTGTGTAGCCCAGGCCGTAAGGGCCATG
 ATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTACCCGGCAGTCTCCTTAGAGTGCCAC
 CATAACGTGCTGGTAACTAAGGACAAGGGTTGCGCTCGTTACGGGACTTAACCCAACATCTC
 ACGACACGAGCTGACGACAGCCATGCAGCACCTGTCTCAATGTTCCCGAAGGCACCAATCT
 ATCTCTAGAAAGTTCATTGGATGTCAAGGCCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAA
 ACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCG
 TACTCCCCAGGCGGTCAACTTAATGCGTTAGCTGCGCCACTAAAAGCTCAAGGCTTCCAACG
 GCTAGTTGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCT
 TTCGCACCTCAGTGTGAGTATTAGTCCAGGTGGTCGCCTTCGCCACTGGTGTTCTTCCTATA
 TCTACGCATTTACCGCTACACAGGAAATTCCACCACCCTCTACCATACTCTAGTCAGTCAG
 TTTTGAATGCAGCTCCCAGGTTGAACCCGGGGATTTCACATCCAACCTTATCATACCACCTAC
 GTGCGCTTTACGCCATAATTCCGATCAACCCTTGCACCCTCTNATTTACCATG

Figure S48 DNA sequence of the bacterial strain UIAU-6B

A



B



Figure S49 (A) Phylogenetic tree showing *Pseudomonad spp.*. **(B)** Photo of *Pseudomonad spp* growing on ISP2 an agar plate

Table S1 NMR data for **1** (600 MHz, CD₃OD)

Position	δ_C , mult.	δ_H , mult. (<i>J</i> in Hz)	COSY	HMBC H→C
1	162.9, C			
2	118.2, CH	6.96, dd (8.7, 1.8)	3	1, 4, 5w, 6, 7
3	137.2, CH	7.52, td (8.7, 1.8)	2, 4	1, 2w, 4w, 5, 6w
4	120.2, CH	6.94, td (8.7, 1.8)	3, 5	1, 2, 3, 5, 6, 7w
5	131.6, CH	7.99, dd (8.7, 1.8)	4	1, 2w, 3, 7
6	113.2, C			
7	170.0, C			
9	57.6, CH	4.20, d (6.5)	11	10, 11, 12
10	169.4, C			
11	71.1, CH	5.67, qd (6.6, 6.5)	9, 12	7, 10, 12
12	16.7, CH ₃	1.54, d (6.6)	9	9, 10

w= weak HMBC intensity.

Table S2 NMR data for **2** (600 MHz, CD₃OD)

Position	δ_C , mult.	δ_H , mult. (<i>J</i> in Hz)	COSY	HMBC H→C
1	162.8, C			
2	118.4, CH	6.96, d (8.7)	3	1, 4, 7
3	137.5, CH	7.52, td (8.7, 1.7)	2, 4	1, 2, 5, 6, 7
4	120.6, CH	6.94, dd (8.7, 1.7)	3, 5	1, 2
5	131.7, CH	7.95, dd (8.7, 1.7)	4	3, 6
6	113.1, C			
7	169.9, C			
9	57.7, CH	4.21, d (6.5)	11	11, 12, 10
10	167.7, C			
11	71.0, CH	5.61, qd (6.6, 6.5)	9, 12	7, 10, 12
12	17.0, CH ₃	1.49, d (6.1)	11	9, 11
15,15'	39.3, CH ₂	3.62, dt (13.8, 6.8); 3.52, dt (13.8, 6.8)	16	10, 16, 17
16	25.3, CH ₂	2.92, m	15	15, 17, 18
17	132.5, C			
18	117.5 CH	7.33, d (0.6)	20	17, 20
20	135.0, CH	8.73, d (0.6)	18	17, 18

Table S3 NMR data for **3** (600 MHz, CD₃OD)

Position	δ_C , mult.	δ_H , mult. (<i>J</i> in Hz)	COSY	HMBC H→C
1	162.8, C			
2	118.4, CH	6.98, m	3	1, 4, 6, 7
3	137.4, CH	7.54, td (8.7, 1.8)	2, 4	1, 5
4	120.5, CH	6.95, m	3, 5	2, 3, 5, 6
5	131.7, CH	7.98, dd (8.7, 1.8)	4	1, 3, 7
6	113.2, C			
7	170.0, C			
9	57.8, CH	4.13, d (6.5)	11	10, 11, 12
10	167.2, C			
11	71.3, CH	5.53, dq (6.5, 6.5)	9, 12	7, 10, 12
12	16.9, CH ₃	1.43, d (6.5)	11	9, 11
15	42.1, CH ₂	3.44, dt (13.7, 7.2) 3.64, dt (13.7, 7.2)	16	10, 16, 17
16	36.1, CH ₂	2.79, m	15, 15'	15, 17, 18', 22
17	140.0, C			
18', 22	129.5, CH	7.16 (m)	19', 21	17, 20', 22
19', 21	129.6, CH	7.22(m)	18', 22, 20'	17, 21
20'	127.5, CH	7.14 (m)	19, 21	17, 18', 22

Table S4 NMR data for **4** (600 MHz, CD₃OD)

Position	δ_C , mult.	δ_H , mult. (<i>J</i> in Hz)	COSY	HMBC → H C
1	161.1, C			
2	117.7, CH	6.96, d (8.3)	3	1, 4, 5, 6, 7
3	135.0, CH	7.41, ddd (8.3, 1.7)	2, 4	1, 2, 4w, 5, 6
4	119.9, CH	6.90, td (7.4, 1.7)	3, 5	1, 2, 3, 5, 6, 7
5	129.5, CH	7.68, dd(7.4, 1.7)	4	1, 2, 3, 7
6	111.6, C			
7	167.8, C			
9	75.5, CH	4.46, d (7.3)	11	5w, 6, 7, 10, 11, 12,
10	175.6, C			
11	80.6, CH	4.90, qd (6.3, 7.3)	9, 12	7, 9, 10
12	21.4, CH ₃	1.57, d (6.3)	11	9, 11

w= weak HMBC intensity.

Table S5 NMR data for **5** (600 MHz, CD₃OD)

Position	δ_C , mult.	δ_H , mult. (<i>J</i> in Hz)	COSY	HMBC H $\rightarrow$ C
1	161.1, C			
2	117.7, CH	6.99, d (8.3)	3	1, 4, 6, 7
3	135.1, CH	7.43, td (8.3, 1.7)	2, 4	1, 5, 6
4	120.0, CH	6.92, t (7.4, 1.7)	3, 5	2, 3, 5, 6
5	129.5, CH	7.67, dd (7.4, 1.7)	4	1, 2, 3, 7
6	111.6, C			
7	168.0, C			
9	75.8, CH	4.38, d (7.3)	11	6, 7, 10, 11, 12
10	172.9, C			
11	80.6, CH	4.78, dq (6.3, 7.3)	9, 12	7, 10
12	21.3, CH ₃	1.51, d (6.3)	11	9, 11
14	41.9, CH ₂	3.44, dt (13.6, 7.2), 3.51, dt (13.6, 7.2)	15	10, 15, 16
15	36.3, CH ₂	2.82, t (7.2)	14	14, 16, 17, 21
16	140.2, C			
17/21	129.9, CH	7.20 (m)	18, 20	15, 21, 19
18/20	129.5, CH	7.22 (m)	17, 21, 19	16, 20
19	127.3, CH	7.15 (m)	18, 21	16, 17/21

Table S6 NMR data for **6** (600 MHz, CD₃OD)

Position	δ_C , mult.	δ_C ¹⁹	δ_H , mult. (<i>J</i> in Hz)	HMBC H $\rightarrow$ C
1	160.9, C	161.1		
2	118.4, CH	118.5	6.88, t (8.0)	1, 4, 7, 6
3	135.3, CH	135.3	7.36, td (8.0, 1.6)	1, 5, 6
4	120.2, CH	120.3	6.89, dd (8.0, 1.6)	1w, 2, 3, 5, 6
5	129.2, CH	129.2	7.77, dd (8.0, 1.6)	1, 2w, 3, 7
6	116.3, C	116.5		
7	171.1, C	171.1		
9	59.25, CH	59.5	4.68, d (10.8)	7, 10, 11, 12
10	168.6, C	169.1		
11	79.9, CH	79.9	4.54, dq (10.8, 6.1)	9, 10
12	17.2, CH ₃	17.2	1.45, d (6.1)	9, 11
15 15'	44.8, CH ₂	45.1	3.99, dt (14.6, 6.5) 3.86, dt (14.6, 6.5)	10, 16, 17
16	23.1, CH ₂	23.6	3.09, t (6.5)	15, 17, 18
17	131.8, C	132.6		
18	117.9, CH	118.2	7.39, s	17, 20
20	134.8, CH	135.3	8.75, s	17, 18

¹⁹ Literature reference for **6** ¹³C NMR. NMR solvents used for reported **6** in the literature was CD₃OD at 600 MHz w= weak HMBC intensity.

Table S7 NMR data for **7** (600 MHz, CD₃OD)

Position	δ_C , mult.	δ_C ³⁰	δ_H mult. (<i>J</i> in Hz)
1	163.2, C	161.7	
2	118.1, CH	116.8	6.91, d (8.3)
3	136.6, CH	135.5	7.43, td (8.3, 7.1)
4	120.0, CH	118.7	6.86, m
5	131.5, CH	129.9	7.83, dd (7.4, 1.7)
6	113.9, C	111.9	
7	173.5, C	171.4	

³⁰ Literature reference for **7** ¹³C NMR. NMR solvents used for the reported in the literature was in CD₃OD at 300 MHz

Experimental procedures

Antibacterial assays

For the antibacterial tests, thawed stock inocula suspensions from cryovials of each microorganism are streaked onto MHII agar plates (Muller Hinton II) and incubated at 37 °C overnight to obtain isolated colonies. Single colonies of each microorganism are inoculated in 10 mL of MHII broth and incubated overnight at 37 °C with shaking at 220 rpm and then diluted in order to obtain assay inocula of approximately $5\text{--}6 \times 10^5$ CFU/mL.

For the assay 90 μ L/well of the diluted inoculum are mixed with 1.6 μ L/well of each compound (**1–7**) dissolved in DMSO and 8.4 μ L/well of MHII medium. Absorbance at 612 nm is measured with a ENVISION, Perkin Elmer™ spectrophotometer at *T*₀ (zero time) and immediately after that, plates are statically incubated at 37 °C for 20 h. After this period, the assay plates are shaken using the DPC Micromix-5 and once more the absorbance at OD 612 nm is measured at *T*_f (final time). Percentage inhibition of growth is calculated using the following normalization:

$$\% \text{ Inhibition} = [1 - ((T_f\text{Sample} - T_0\text{Sample}) - (T_f\text{Blank} - T_0\text{blank})) / (T_f\text{Growth} - T_0\text{Growth}) - (T_f\text{Blank} - T_0\text{blank})] \times 100.$$

Each compound is serially diluted in DMSO with a dilution factor of 2 to provide 10 concentrations starting at 64 µg/mL for all the assays. The MIC is defined as the lowest concentration of an antimicrobial or antifungal compound that inhibited $\geq 90\%$ of the growth of a microorganism after overnight incubation. The data are analyzed using the Genedata Screener program (Genedata AG, Switzerland). In all experiments performed in this work the RZ' factor obtained was between 0.85 and 0.95.

Antimycobacterium Tuberculosis assay

The minimum inhibitory concentration (MIC) values were determined using the previously reported standard broth microdilution method [1]. A 10 mL culture of *M. tuberculosis* H37Rv [2] was grown to an absorbance (OD600) of 0.6–0.7 in Middlebrook 7H9 medium (Difco) supplemented with 0.2% glucose, Middlebrook 7H9 supplemented with 0.03% casitone, 0.4% glucose, and 0.05% tyloxapol. Cultures grown in this medium were diluted 1:500 prior to inoculation of the MIC assay. The new compounds **1–7** were reconstituted in DMSO to a concentration of 10 mM and 2-fold serial dilution duplicates were prepared across a 96-well microtiter plate, in a volume of 50 µL. Diluted *M. tuberculosis* cultures (50 µL) were added to each well of the plate (including control wells) to obtain a final volume of 100 µL. Rifampicin (at $2 \times \text{MIC}$) was used as assay control at a minimum growth control (rifampicin), and maximum growth control (5% DMSO). The microtiter plates were sealed in a secondary container and incubated at 37 °C with 5% CO₂ and humidification. Alamar Blue reagent was added to each well of the assay plate 24 h prior to fluorescence reading. Relative fluorescence (excitation at 485 nm; emission 520 nm) was measured using a plate reader (FLUOstar OPTIMA, BMG LABTECH) at day 14. The raw fluorescence data was archived and analyzed using the CDD Vault from Collaborative Drug Discovery, in which data was normalized to the minimum and maximum inhibition controls to generate a dose–response curve (percentage inhibition), using the Levenberg–Marquardt damped least squares method, from which the MIC was calculated [3]. The lowest concentration of the drug that inhibited growth by more than 90% of the mycobacterial population was considered the MIC.

Table S8. Antimicrobial activities (MIC in µg/ mL) of compounds **1–7**

Compound ID	MRSA	MSSA	<i>E.coli</i>	<i>A.b</i>	<i>E.f</i> (VanA)	<i>E. f</i> (VS)	<i>T. B</i>
1	>64	>64	>64	>64	>64	>64	>125
2	>64	>64	>64	>64	>64	>64	7.8
3	>64	>64	>64	>64	>64	16	15.6
4	>64	>64	>64	>64	>64	8-16	>125
5	>64	>64	>64	>64	>64	32	125
6	>64	>64	>64	>64	>64	>64	>64
7	>64	>64	>64	>64	>64	>64	>64
Rifampicin	-	-	-	-	-	-	0.02
Vancomycin	1-2	2-4	-	-	>128	8	-
Aztreonam	-	-	0.25-0.5	8	-	-	-

A.b (*A. baumannii*), *E.f* VanA (vancomycin-resistant *E. faecium* VanA15167) and *E.f* VS (Vancomycin-sensitive *E. faecium* VS144754) and *T.B* (*mycobacterium tuberculosis*).

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