# Taxonomic Characterization and Secondary Metabolite Analysis of NEAU-wh3-1: an *Embleya* strain with Antitumor and Antibacterial Activity

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**Table S1.** GenBank accession numbers of the sequences used in MLSA.

| Strain | Type strain | Whole genome | *atp*D | *gyr*B | *rec*A | *rpo*B | *trp*B |
|---|---|---|---|---|---|---|---|
| *Streptomyces radiciphilus* | NEAU-wh3-1 | – | MT039411 | MT039412 | MT039413 | MT039414 | MT039415 |
| *Streptomyces hyalinus* | MB891-A1$^{T}$ | BIFH01000000 | – | – | – | – | – |
| *Embleya scabrispora* | DSM 41855$^{T}$ | MWQN00000000 | – | – | – | – | – |
| *Streptomyces qinglanensis* | 172205$^{T}$ | LJGV01000022 | – | – | – | – | – |
| *Streptomyces glebosus* | CGMCC 4.1873$^{T}$ | BLIO00000000 | – | – | – | – | – |
| *Streptomyces durbertensis* | NEAU-S1GS20$^{T}$ | – | MH534864 | MH534865 | MH534866 | MH534867 | MH534868 |
| *Streptomyces ramulosus* | NRRL B-2714$^{T}$ | – | MF581785 | MF188173 | MF581787 | MF581789 | MF581783 |
| *Streptomyces barkulensis* | RC 1831$^{T}$ | PGSG00000000 | – | – | – | – | – |
| *Streptacidiphilus bronchialis* | DSM 106435$^{T}$ | CP031264 | – | – | – | – | – |
| *Kitasatospora xanthocidica* | NBRC 13469$^{T}$ | QVIG00000000 | – | – | – | – | – |
| *Streptomyces griseoplanus* | NRRL B-3064$^{T}$ | LIQR00000000 | KT384582 | KT384931 | JQ806239 | JQ806243 | JQ806247 |
| *Streptomyces cattleya* | NRRL 8057$^{T}$ | JWDF00000000 | KT384775 | KT385123 | KT385477 | KT389095 | KT389444 |
| *Streptomyces rubrisoli* | FXJ1.725$^{T}$ | – | KC137297 | KC137293 | KC137289 | KC137285 | KC137281 |
| *Streptomyces rubidus* | 13C15$^{T}$ | MDCQ00000000 | KT384713 | KT385063 | KT385414 | KT389034 | KT389382 |
| *Streptomyces niger* | NBRC 13362$^{T}$ | JOFQ00000000 | FJ406130 | FJ406186 | FJ406242 | FJ406298 | FJ406353 |
| *Streptomyces sparsogenes* | ATCC 25498$^{T}$ | ASQP00000000 | KT384724 | KT385073 | KT385425 | DQ241995 | KT389393 |
| *Streptomyces hiroshimensis* | NBRC 3839$^{T}$ | – | KT384596 | KT384945 | KT385294 | AY280780 | KT389265 |
| *Streptomyces mobaraensis* | NBRC 13819$^{T}$ | VOKX00000000 | KT384648 | KT384997 | KT385348 | KT388968 | KT389317 |
| *Streptomyces abikoensis* | NBRC 13860$^{T}$ | – | FJ406150 | FJ406206 | FJ406262 | FJ406318 | FJ406373 |
| *Streptomyces sclerotialus* | NRRL ISP-5269$^{T}$ | JOBC00000000 | FJ406181 | FJ406237 | FJ406293 | FJ406348 | FJ406404 |
| *Streptomyces pratens* | BK 138$^{T}$ | – | KP890254 | KP890257 | KP890260 | KP890263 | KP890266 |
| *Streptomyces monomycini* | NRRL B-24309$^{T}$ | JNYL00000000 | FJ406180 | KT384998 | KT385349 | FJ406347 | FJ406403 |
| *Streptomyces rimosus* subsp. *rimosus* | ATCC 10970$^{T}$ | LGCS00000000 | FJ406141 | FJ406197 | FJ406253 | KJ996628 | FJ406364 |
| *Streptomyces olivaceiscleroticus* | DSM 40595$^{T}$ | – | FJ406164 | FJ406220 | FJ406276 | FJ406332 | FJ406387 |
| *Streptomyces catenulae* | NRRL B-2342$^{T}$ | JODY00000000 | FJ406152 | FJ406208 | FJ406264 | KJ996525 | FJ406375 |
| *Streptomyces sioyaensis* | NRRL B-5408$^{T}$ | SDIF00000000 | MG881213 | FJ406188 | MG881215 | MG881217 | MG881219 |
| *Streptomyces decoyicus* | NRRL 2666$^{T}$ | LGUU00000000 | FJ406159 | FJ406215 | FJ406271 | FJ406327 | FJ406382 |
| *Streptomyces caniferus* | NBRC 15389$^{T}$ | BLIN00000000 | KT384499 | KT384848 | KT385196 | KT388818 | KT389168 |
| *Streptomyces libani* subsp. *rufus* | LMG 20087$^{T}$ | BLIQ00000000 | FJ406165 | FJ406221 | FJ406277 | FJ406333 | FJ406388 |
| *Streptomyces hygroscopicus subsp. glebosus* | NBRC 13786$^{T}$ | – | FJ406158 | FJ406214 | FJ406270 | FJ406326 | FJ406381 |
| *Streptomyces platensis* | JCM 4662$^{T}$ | MIGA00000000 | GU383329 | FJ406219 | KJ469294 | GU383753 | KT389356 |

**Table S2**. MLSA distance values for selected strains in this study.

Strains: 1, NEAU-wh3-1; 2, *Streptomyces platensis* JCM 4662$^T$; 3, *Streptomyces hygroscopicus* subsp. *glebosus* NBRC 13786$^T$; 4, *Streptomyces glebosus* CGMCC 4.1873$^T$; 5, *Streptomyces libani* subsp. *rufus* LMG 20087$^T$; 6, *Streptomyces ramulosus* NRRL B-2714$^T$; 7, *Streptomyces caniferus* NBRC 15389$^T$; 8, *Streptomyces decoyicus* NRRL 2666$^T$; 9, *Streptomyces catenulae* NRRL B-2342$^T$; 10, *Streptomyces sioyaensis* NRRL B-5408$^T$; 11, *Streptomyces pratens* BK 138$^T$; 12, *Streptomyces abikoensis* NBRC 13860$^T$; 13, *Streptomyces hiroshimensis* NBRC 3839$^T$; 14, *Streptomyces sparsogenes* ATCC 25498$^T$; 15, *Streptomyces mobaraensis* NBRC 13819$^T$; 16, *Streptomyces rimosus* subsp. *rimosus* ATCC 10970$^T$; 17, *Streptomyces sclerotialus* NRRL ISP-5269$^T$; 18, *Streptomyces monomycini* NRRL B-24309$^T$; 19, *Streptomyces olivaceiscleroticus* DSM 40595$^T$; 20, *Streptomyces niger* NBRC 13362$^T$; 21, *Streptomyces durbertensis* NEAU-S1GS20$^T$; 22, *Streptomyces rubrisoli* FXJ1.725$^T$; 23, *Streptomyces qinglanensis* 172205$^T$; 24, *Streptomyces rubidus* 13C15$^T$; 25, *Kitasatospora xanthocidica* NBRC 13469$^T$; 26, *Streptacidiphilus bronchialis* DSM 106435$^T$; 27, *Streptomyces griseoplanus* NRRL B-3064$^T$; 28, *Streptomyces cattleya* NRRL 8057$^T$; 29, *Streptomyces barkulensis* RC 1831$^T$; 30, *Embleya hyalina* MB891-A1$^T$; 31, *Embleya scabrispora* DSM 41855$^T$.

| Strain | MLSA (Kimura 2-parameter) distance | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
| 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2 | 0.526 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3 | 0.472 | 0.053 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 4 | 0.412 | 0.558 | 0.490 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 5 | 0.481 | 0.077 | 0.030 | 0.505 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 6 | 0.565 | 0.389 | 0.369 | 0.670 | 0.370 | | | | | | | | | | | | | | | | | | | | | | | | | |
| 7 | 0.464 | 0.097 | 0.047 | 0.522 | 0.067 | 0.375 | | | | | | | | | | | | | | | | | | | | | | | | |
| 8 | 0.464 | 0.085 | 0.040 | 0.522 | 0.061 | 0.375 | 0.043 | | | | | | | | | | | | | | | | | | | | | | | |
| 9 | 0.470 | 0.129 | 0.079 | 0.564 | 0.100 | 0.316 | 0.081 | 0.073 | | | | | | | | | | | | | | | | | | | | | | |
| 10 | 0.475 | 0.112 | 0.063 | 0.533 | 0.087 | 0.371 | 0.064 | 0.067 | 0.088 | | | | | | | | | | | | | | | | | | | | | |
| 11 | 0.477 | 0.165 | 0.132 | 0.561 | 0.144 | 0.380 | 0.136 | 0.135 | 0.136 | 0.131 | | | | | | | | | | | | | | | | | | | | |
| 12 | 0.464 | 0.168 | 0.129 | 0.574 | 0.126 | 0.365 | 0.115 | 0.126 | 0.106 | 0.123 | 0.138 | | | | | | | | | | | | | | | | | | | |
| 13 | 0.513 | 0.178 | 0.168 | 0.633 | 0.161 | 0.376 | 0.168 | 0.173 | 0.155 | 0.175 | 0.187 | 0.116 | | | | | | | | | | | | | | | | | | |
| 14 | 0.536 | 0.204 | 0.193 | 0.673 | 0.180 | 0.416 | 0.183 | 0.195 | 0.203 | 0.197 | 0.215 | 0.170 | 0.109 | | | | | | | | | | | | | | | | | |
| 15 | 0.458 | 0.161 | 0.120 | 0.556 | 0.116 | 0.366 | 0.114 | 0.117 | 0.107 | 0.111 | 0.117 | 0.076 | 0.133 | 0.168 | | | | | | | | | | | | | | | | |
| 16 | 0.459 | 0.135 | 0.096 | 0.554 | 0.116 | 0.352 | 0.092 | 0.087 | 0.081 | 0.104 | 0.123 | 0.120 | 0.168 | 0.188 | 0.117 | | | | | | | | | | | | | | | |
| 17 | 0.475 | 0.162 | 0.123 | 0.577 | 0.137 | 0.393 | 0.116 | 0.123 | 0.130 | 0.112 | 0.091 | 0.132 | 0.173 | 0.204 | 0.125 | 0.118 | | | | | | | | | | | | | | |
| 18 | 0.475 | 0.133 | 0.097 | 0.560 | 0.117 | 0.352 | 0.094 | 0.089 | 0.087 | 0.105 | 0.131 | 0.118 | 0.171 | 0.183 | 0.121 | 0.026 | 0.128 | | | | | | | | | | | | | |
| 19 | 0.456 | 0.137 | 0.098 | 0.559 | 0.117 | 0.361 | 0.095 | 0.092 | 0.080 | 0.094 | 0.134 | 0.110 | 0.159 | 0.190 | 0.120 | 0.085 | 0.112 | 0.089 | | | | | | | | | | | | |
| 20 | 0.478 | 0.180 | 0.144 | 0.581 | 0.146 | 0.395 | 0.138 | 0.146 | 0.150 | 0.142 | 0.158 | 0.135 | 0.185 | 0.207 | 0.124 | 0.141 | 0.155 | 0.144 | 0.153 | | | | | | | | | | | |
| 21 | 0.508 | 0.355 | 0.308 | 0.620 | 0.313 | 0.120 | 0.307 | 0.309 | 0.327 | 0.311 | 0.329 | 0.330 | 0.398 | 0.412 | 0.316 | 0.319 | 0.331 | 0.319 | 0.330 | 0.325 | | | | | | | | | | |
| 22 | 0.471 | 0.180 | 0.139 | 0.587 | 0.132 | 0.400 | 0.129 | 0.135 | 0.138 | 0.139 | 0.147 | 0.114 | 0.176 | 0.188 | 0.118 | 0.135 | 0.153 | 0.141 | 0.133 | 0.125 | 0.349 | | | | | | | | | |
| 23 | 0.640 | 0.941 | 0.885 | 0.327 | 0.907 | 1.020 | 0.901 | 0.912 | 0.907 | 0.896 | 0.932 | 0.929 | 0.962 | 0.995 | 0.914 | 0.919 | 0.945 | 0.922 | 0.938 | 0.956 | 0.979 | 0.933 | | | | | | | | |
| 24 | 0.459 | 0.179 | 0.145 | 0.570 | 0.144 | 0.381 | 0.135 | 0.143 | 0.136 | 0.140 | 0.134 | 0.118 | 0.165 | 0.196 | 0.107 | 0.129 | 0.141 | 0.128 | 0.142 | 0.118 | 0.321 | 0.131 | 0.927 | | | | | | | |
| 25 | 0.715 | 0.384 | 0.366 | 0.968 | 0.378 | 0.675 | 0.363 | 0.366 | 0.354 | 0.345 | 0.360 | 0.369 | 0.384 | 0.387 | 0.360 | 0.365 | 0.367 | 0.356 | 0.364 | 0.383 | 0.661 | 0.373 | 0.749 | 0.375 | | | | | | |
| 26 | 0.700 | 0.376 | 0.357 | 0.963 | 0.372 | 0.674 | 0.358 | 0.363 | 0.355 | 0.345 | 0.349 | 0.350 | 0.364 | 0.366 | 0.339 | 0.354 | 0.354 | 0.346 | 0.353 | 0.371 | 0.655 | 0.363 | 0.739 | 0.367 | 0.105 | | | | | |
| 27 | 0.449 | 0.186 | 0.150 | 0.579 | 0.161 | 0.408 | 0.154 | 0.159 | 0.152 | 0.152 | 0.159 | 0.150 | 0.210 | 0.225 | 0.146 | 0.145 | 0.162 | 0.146 | 0.152 | 0.159 | 0.362 | 0.159 | 0.957 | 0.144 | 0.363 | 0.311 | | | | |
| 28 | 0.460 | 0.167 | 0.125 | 0.564 | 0.126 | 0.368 | 0.120 | 0.127 | 0.122 | 0.129 | 0.143 | 0.109 | 0.164 | 0.184 | 0.122 | 0.115 | 0.131 | 0.121 | 0.120 | 0.116 | 0.317 | 0.097 | 0.916 | 0.116 | 0.361 | 0.350 | 0.146 | | | |
| 29 | 0.527 | 0.289 | 0.272 | 0.554 | 0.268 | 0.305 | 0.274 | 0.282 | 0.273 | 0.275 | 0.273 | 0.262 | 0.268 | 0.282 | 0.253 | 0.259 | 0.278 | 0.267 | 0.272 | 0.240 | 0.357 | 0.265 | 0.861 | 0.234 | 0.515 | 0.524 | 0.299 | 0.245 | | |
| 30 | 0.186 | 0.519 | 0.499 | 0.392 | 0.503 | 0.555 | 0.493 | 0.508 | 0.502 | 0.508 | 0.508 | 0.495 | 0.510 | 0.526 | 0.492 | 0.498 | 0.517 | 0.507 | 0.495 | 0.521 | 0.536 | 0.515 | 0.457 | 0.499 | 0.614 | 0.607 | 0.496 | 0.489 | 0.475 | |
| 31 | 0.195 | 0.524 | 0.505 | 0.483 | 0.513 | 0.567 | 0.503 | 0.512 | 0.498 | 0.493 | 0.512 | 0.497 | 0.513 | 0.526 | 0.486 | 0.505 | 0.515 | 0.508 | 0.500 | 0.520 | 0.551 | 0.522 | 0.548 | 0.507 | 0.607 | 0.605 | 0.498 | 0.493 | 0.545 | 0.108 |

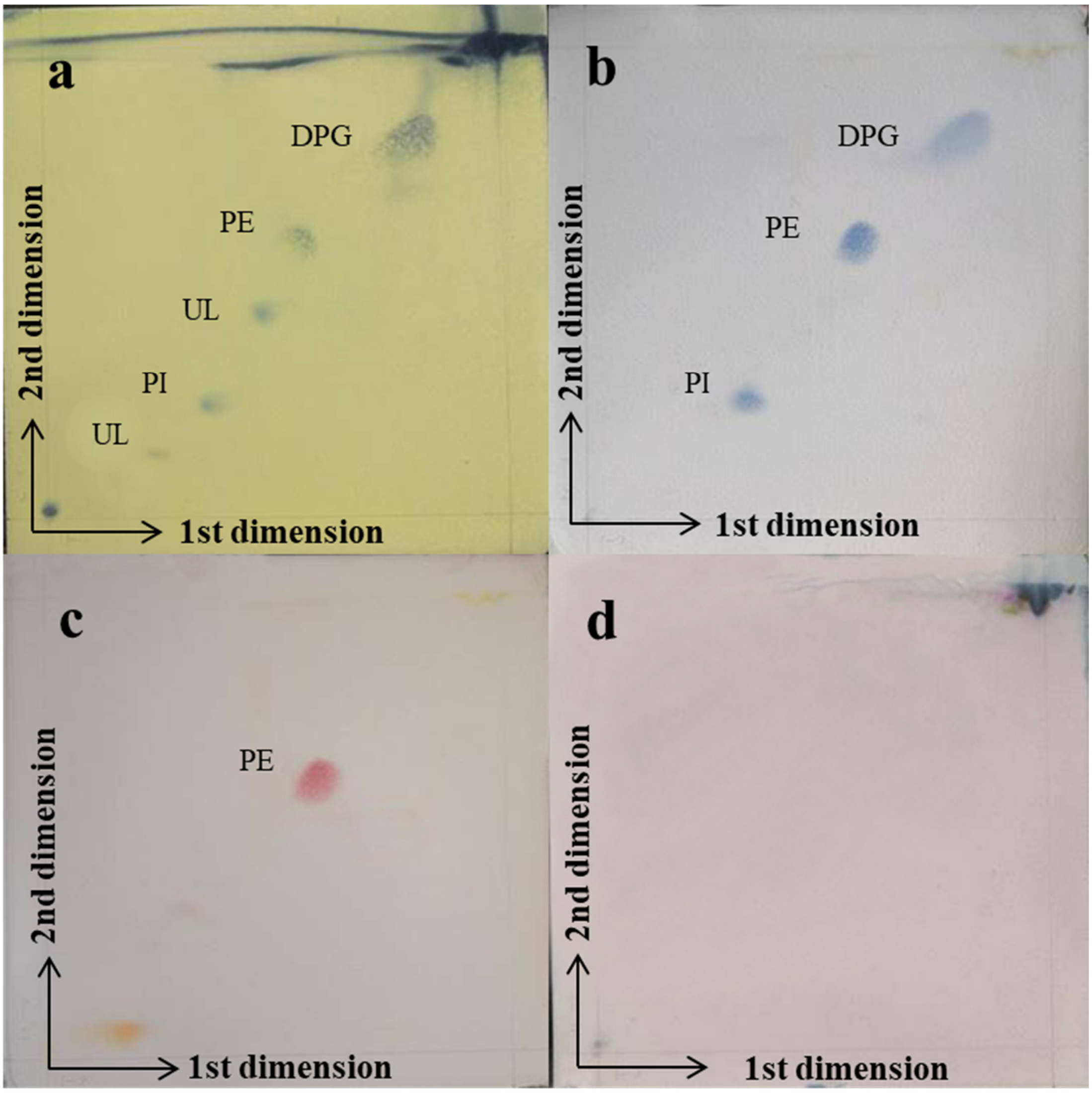


**Figure 1.** The polar lipids of strain NEAU-wh3-1. a, using molybdophosphoric acid reagent; b, molybdenum blue reagent; c, using ninhydrin reagent, d, using anisaldehyde reagent. Diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and unidentified lipid (UL); 1st dimension: chloroform: methanol: water (65: 25: 4, v/v); 2nd dimension: chloroform: acetic acid: methanol: water (80: 18: 12: 5, v/v).

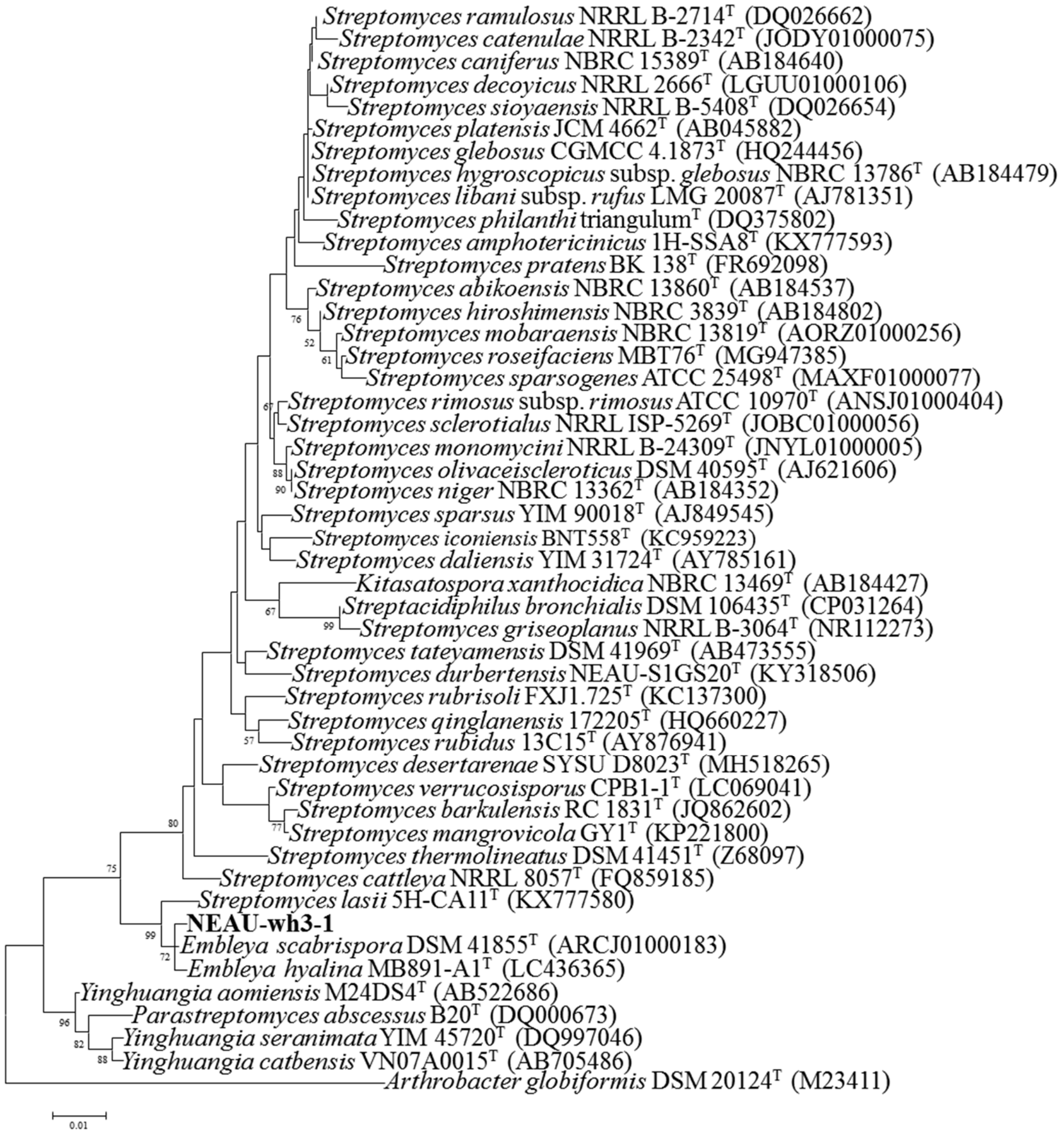


**Figure S2.** Maximum-likelihood tree based on 16S rRNA gene sequences showing relationship between strain NEAU-wh3-1 and related taxa based on 16S rRNA gene sequences. Only bootstrap values above 50 % (percentages of 1000 replications) are indicated. *Arthrobacter globiformis* DSM 20124$^T$ (M23411) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

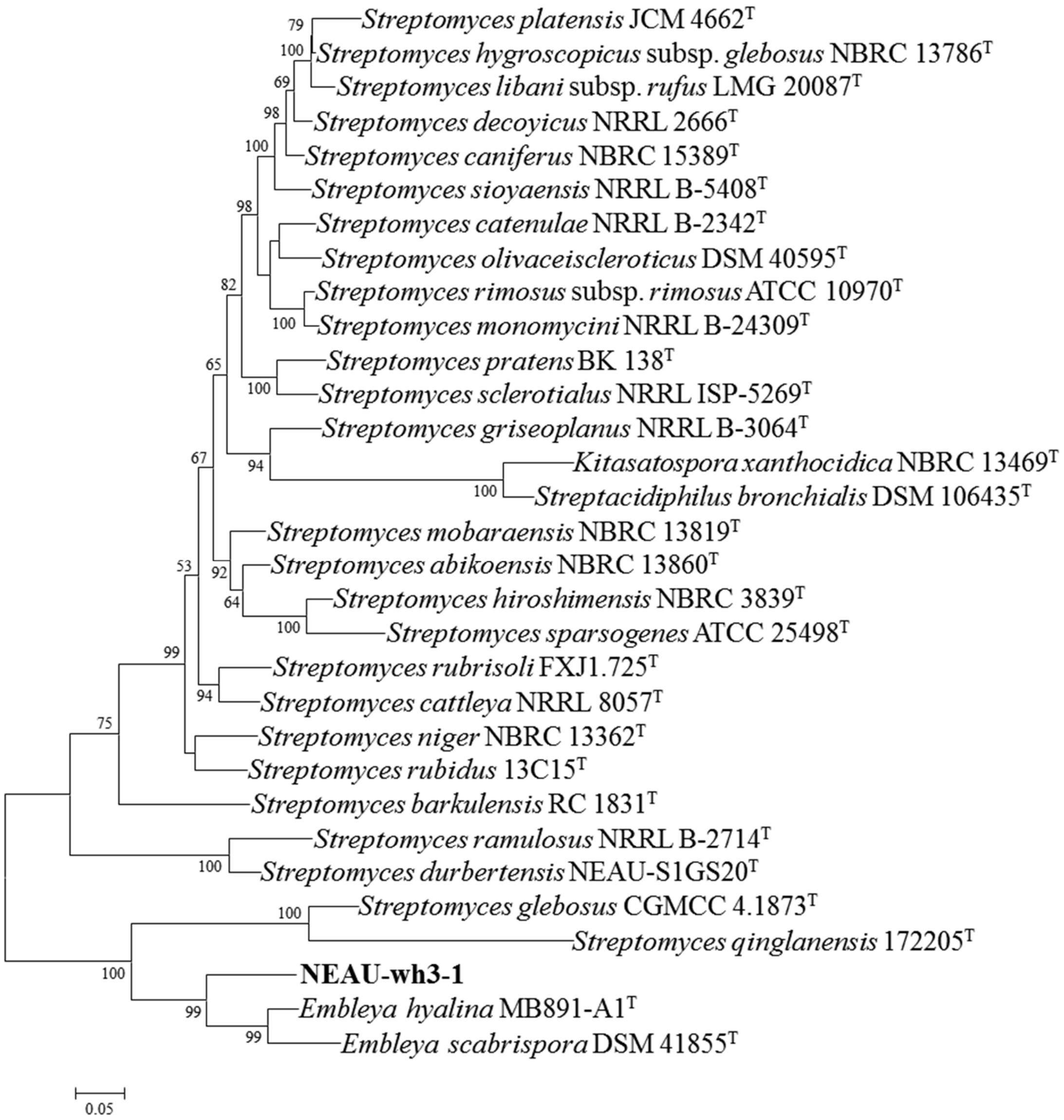


**Figure S3** Maximum-likelihood tree based on multilocus sequence analysis (MLSA) analysis of the concatenated partial sequences (1979 bp) from five housekeeping genes (*atp*D, *gyr*B, *rec*A, *rpo*B, and *trp*B) of strain NEAU-wh3-1 (in bold) with related taxa. Only bootstrap values above 50% (percentages of 1000 replications) are indicated. Bar, 0.05 substitutions per nucleotide position.

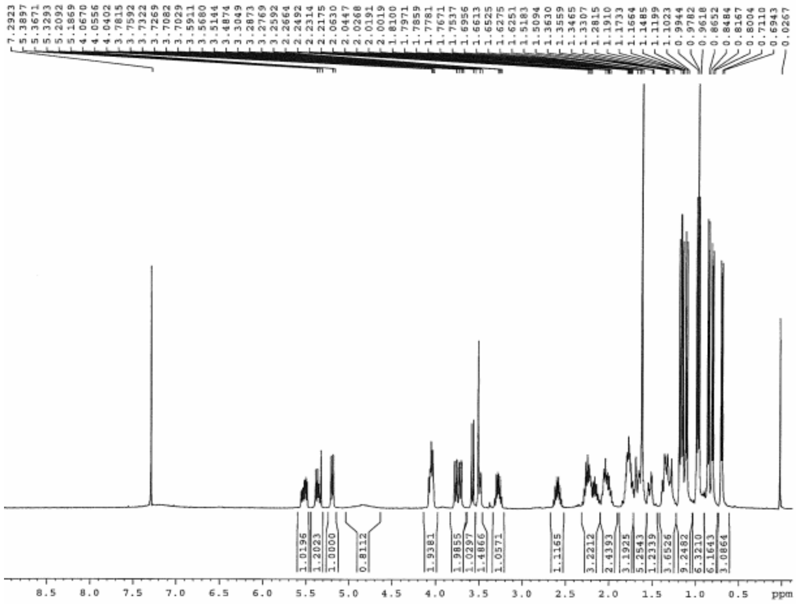


**Figure. S4.** $^1$H NMR (400 MHz) spectrum of compound **1** in $CDCl_3$

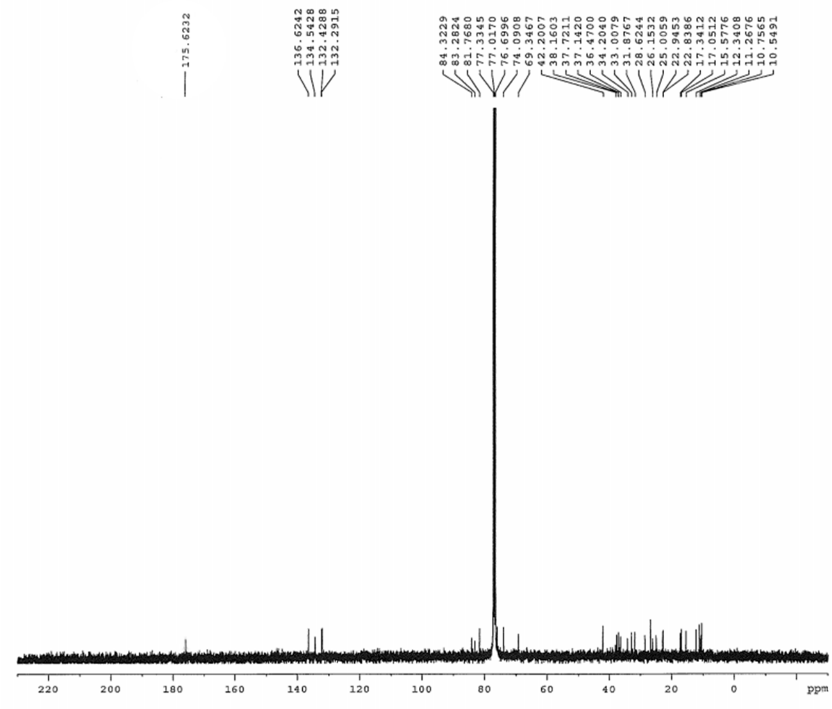


**Figure S5.** $^{13}$C NMR (150 MHz) spectrum of compound **1 (**in $CDCl_3$)

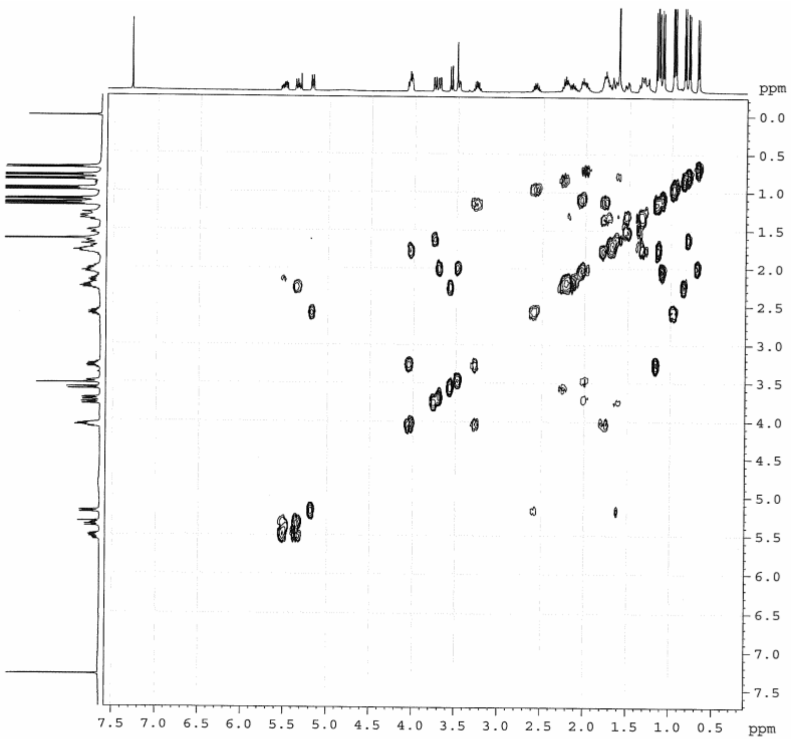


**Figure S6.** $^1$H-$^1$H COSY spectrum (400 MHz) of compound **1** (in $CDCl_3$)

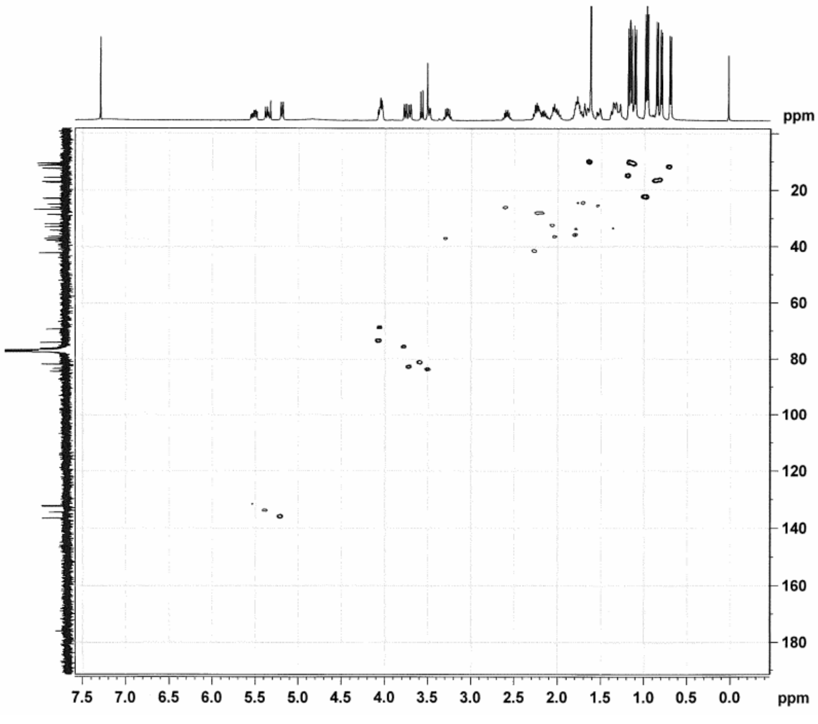


**Figure S7.** HSQC spectrum (400 MHz) of compound **1** (in $CDCl_3$)

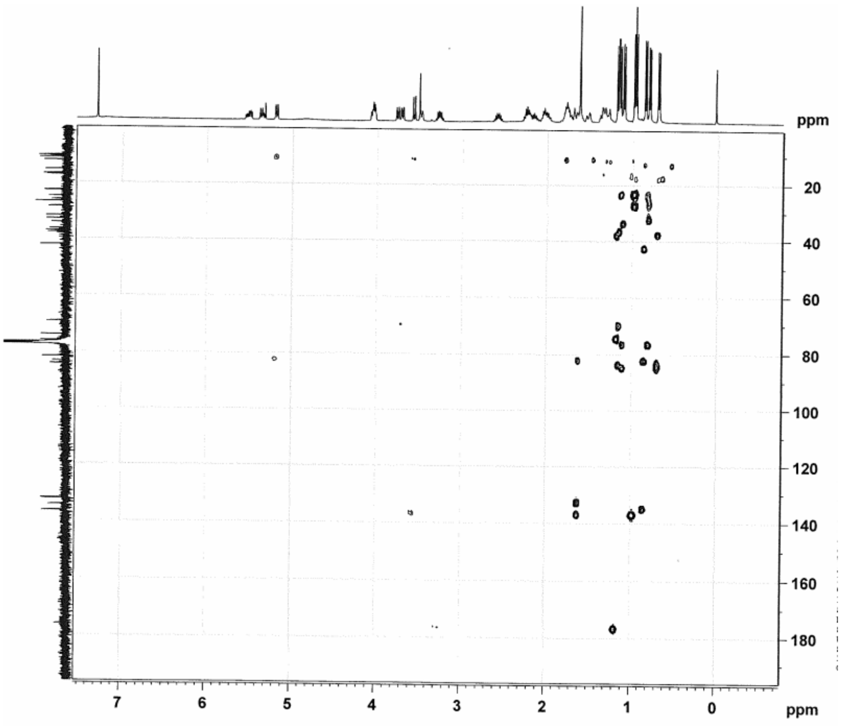


Figure S8. HMBC spectrum (400 MHz) of compound **1** (in CDCl3)

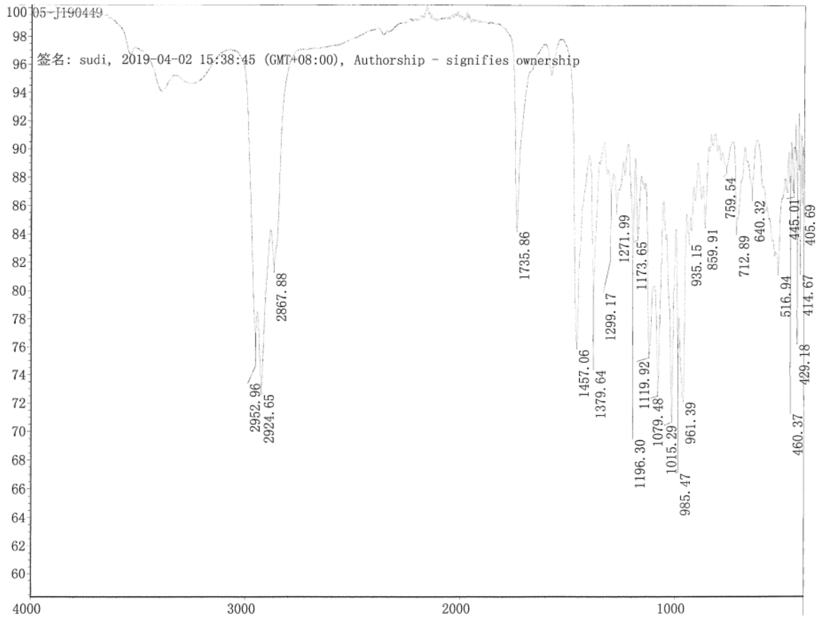
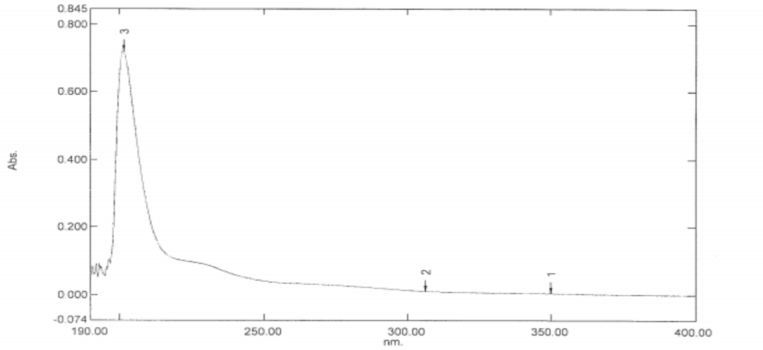


**Figure S9.** IR spectrum of compound **1** （in EtOH）



[Measurement Properties]
Wavelength Range (nm.): 190.00 to 400.00
Scan Speed: Fast
Sampling Interval. 0.2
Auto Sampling Interval: Enabled
Scan Mode: Single

[Instrument Properties]
Instrument Type: UV-2600 Series
Measuring Mode: Absorbance
Slit Width: 1.0
Accumulation time: 1.0 sec.
Light Source Change Wavelength: 360.0 nm
Detector Unit: Direct
S/R Exchange: Normal
Stair Correction: ON

[Attachment Properties]
Attachment: None

[Operation]
Threshold: 0.0010000
Points: 4
InterPolate: Disabled
Average: Disabled

[Sample Preparation Properties]
Weight: 25.04mg
Volume: 25ml
Dilution: 0.5ml-100ml
Path Length:
Additional Information:

| No. | P/V | Wavelength | Abs. | Description |
|---|---|---|---|---|
| 1 | ↑ | 350.00 | 0.005 | |
| 2 | ↑ | 306.20 | 0.011 | |
| 3 | ↑ | 201.60 | 0.721 | |

**Figure S10**. UV spectrum of compound **1** (in EtOH)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Sample Name** | NF813B-17 | **Position** | Vial 1 | **Instrument Name** | Instrument 1 | **User Name** | CAY-JSA2-04\y |
| **Inj Vol** | 0.1 | **InjPosition** | | **SampleType** | Sample | **IRM Calibration Status** | Success |
| **Data Filename** | NF813B-17-2.d | **ACQ Method** | MS.m | **Comment** | | **Acquired Time** | 3/26/2019 1:4 |

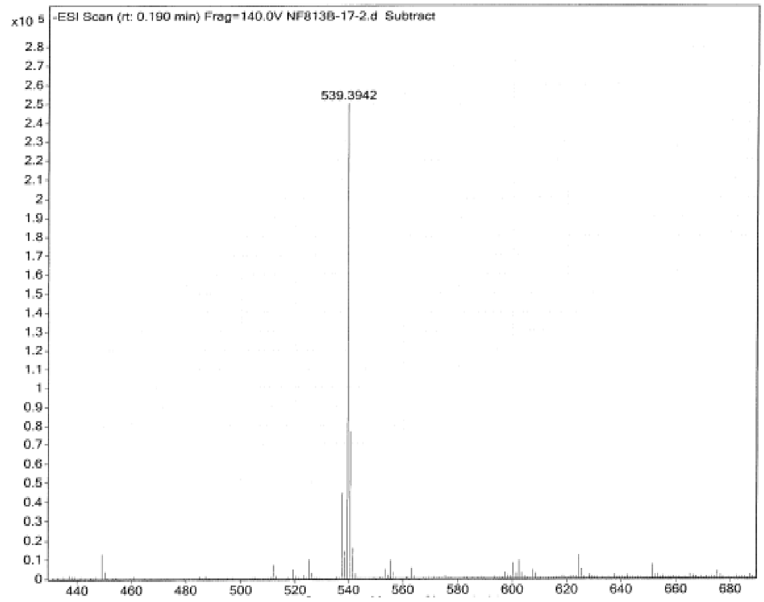


**Figure S11**. The HRESIMS spectrum of compound **1**

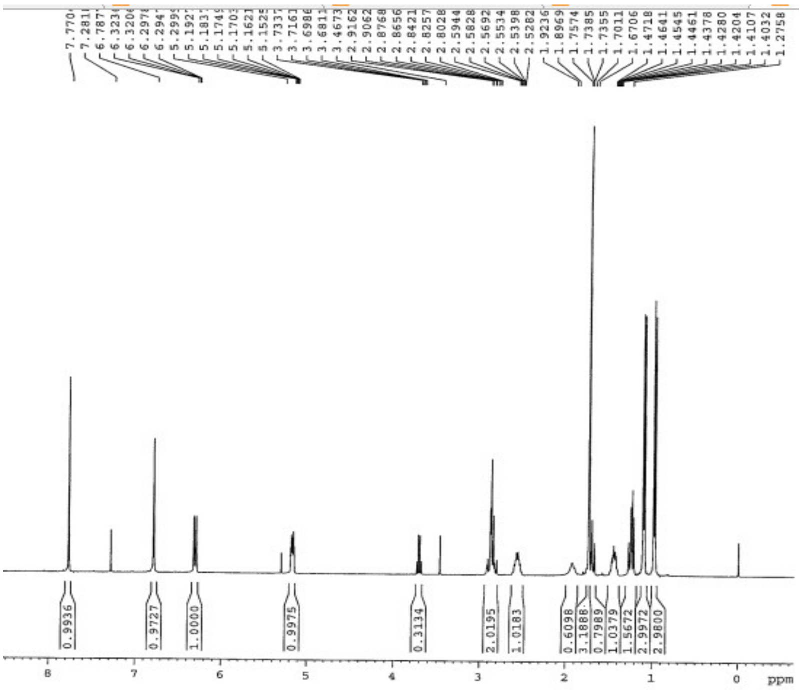


**Figure S12**. $^1H$ NMR (400 MHz) spectrum of compound **2** in $CDCl_3$

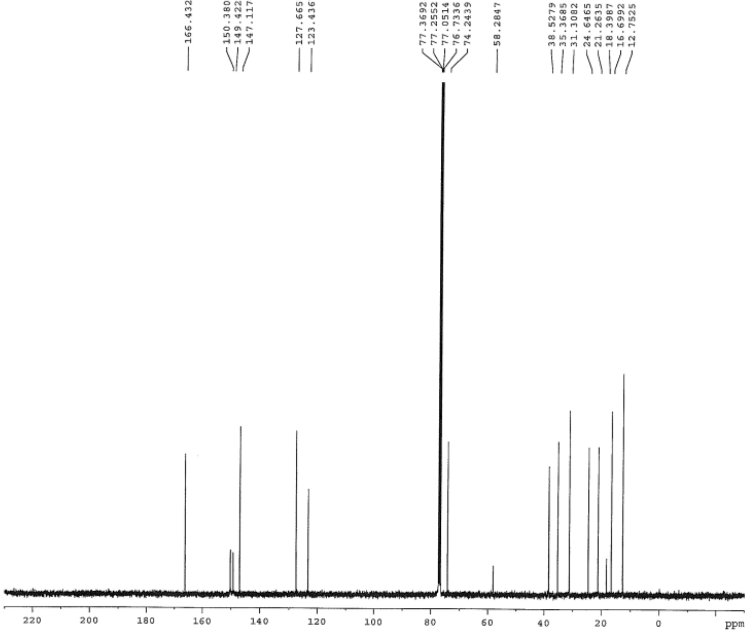


**Figure S13**. $^{13}C$ NMR (150 MHz) spectrum of compound **2** in $CDCl_3$

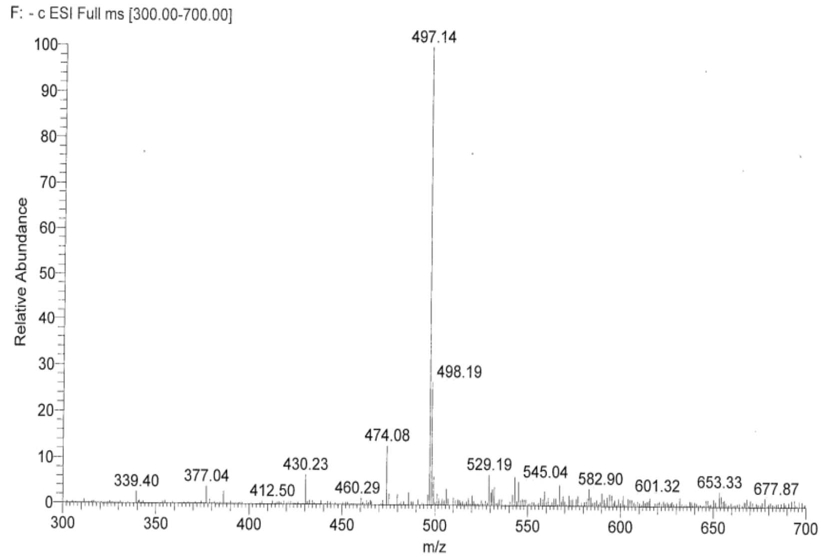


**Figure S14**. The ESI-MS spectrum of compound **2**

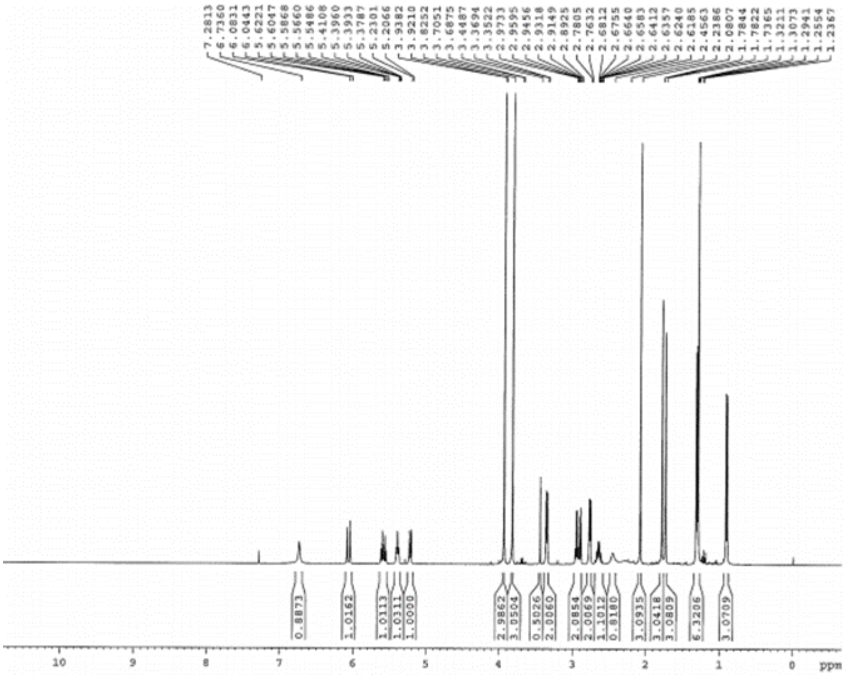


**Figure S15**. $^1$H NMR (400 MHz) spectrum of compound **3** in $CDCl_3$

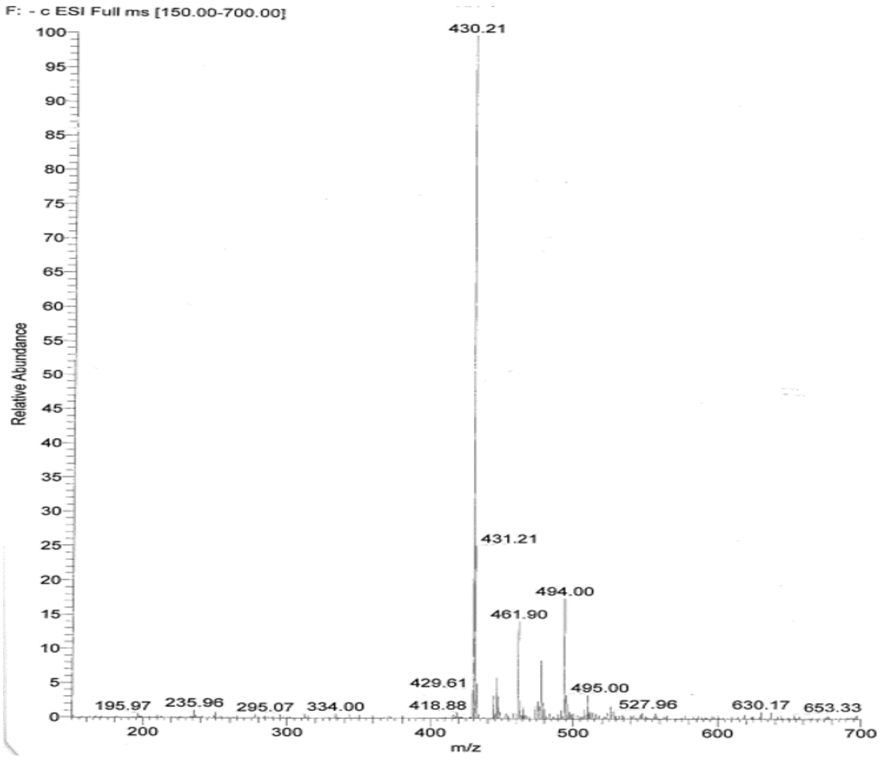


Figure S16. The ESI-MS spectrum of compound **3**

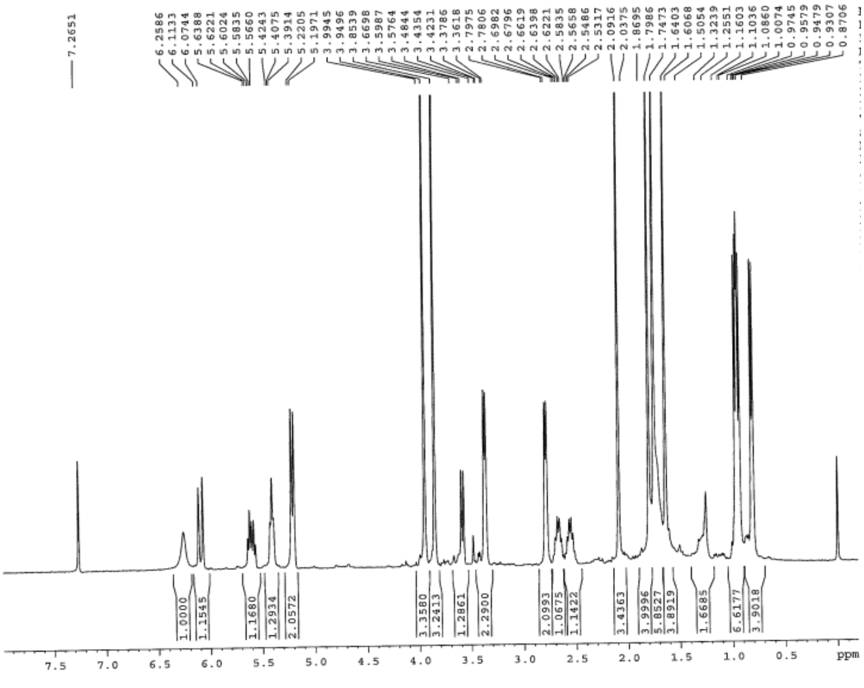


**Figure S17**. $^1$H NMR (400 MHz) spectrum of compound **4** in $CDCl_3$

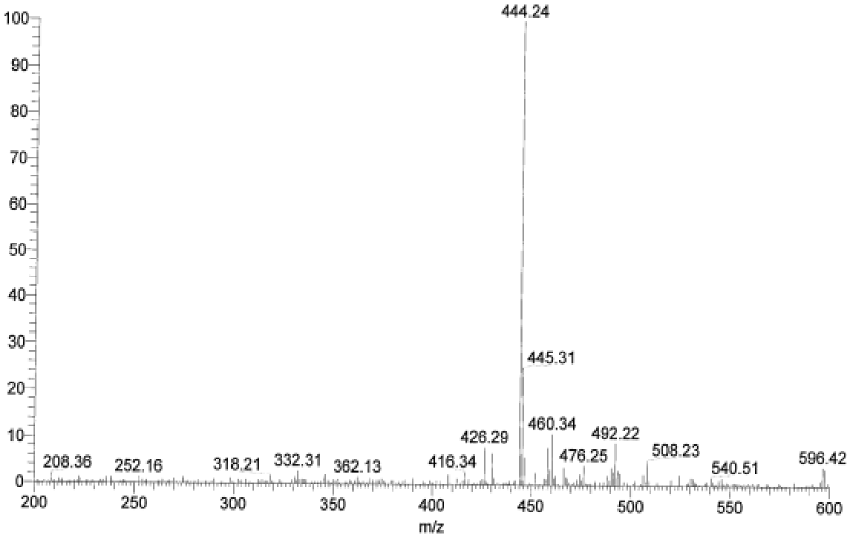


**Figure S18**. The ESI-MS spectrum of compound **4**

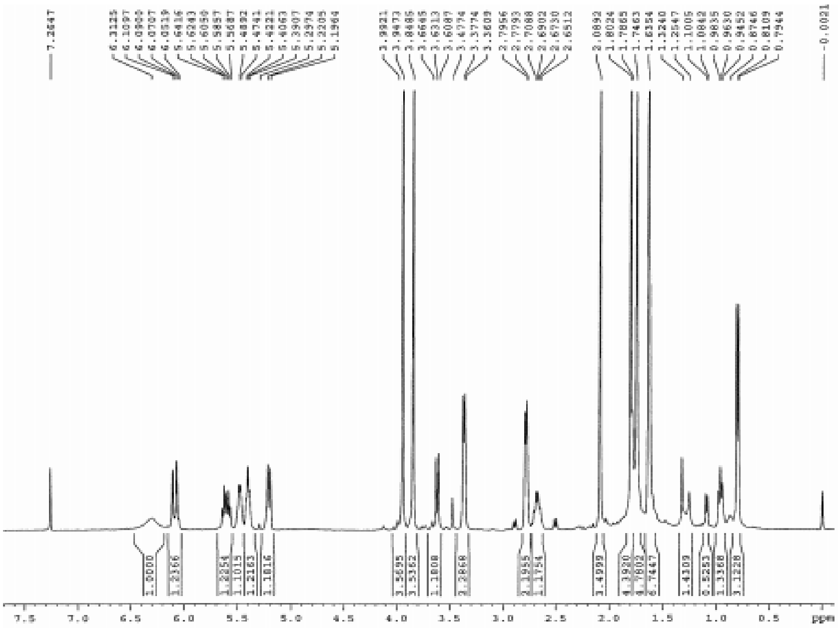


**Figure S19**. $^{1}$H NMR (400 MHz) spectrum of compound **5** in $CDCl_3$

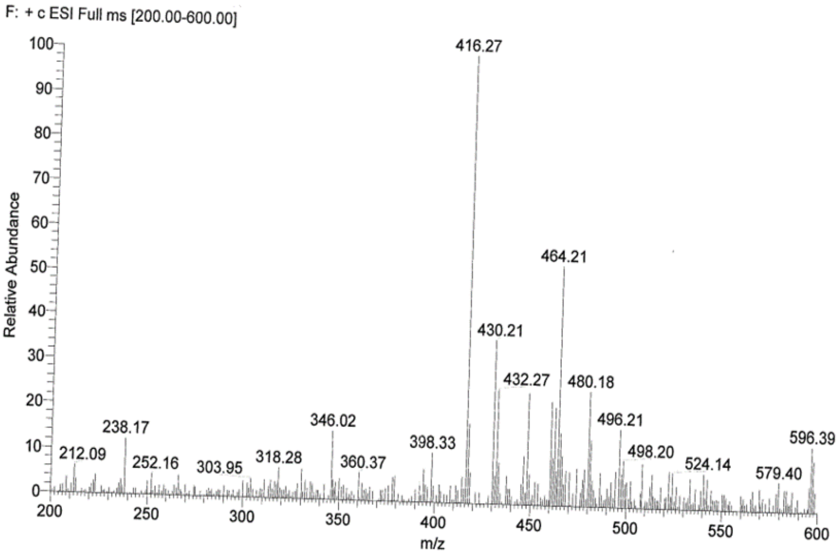


**Figure S20**. The ESI-MS spectrum of compound **5**

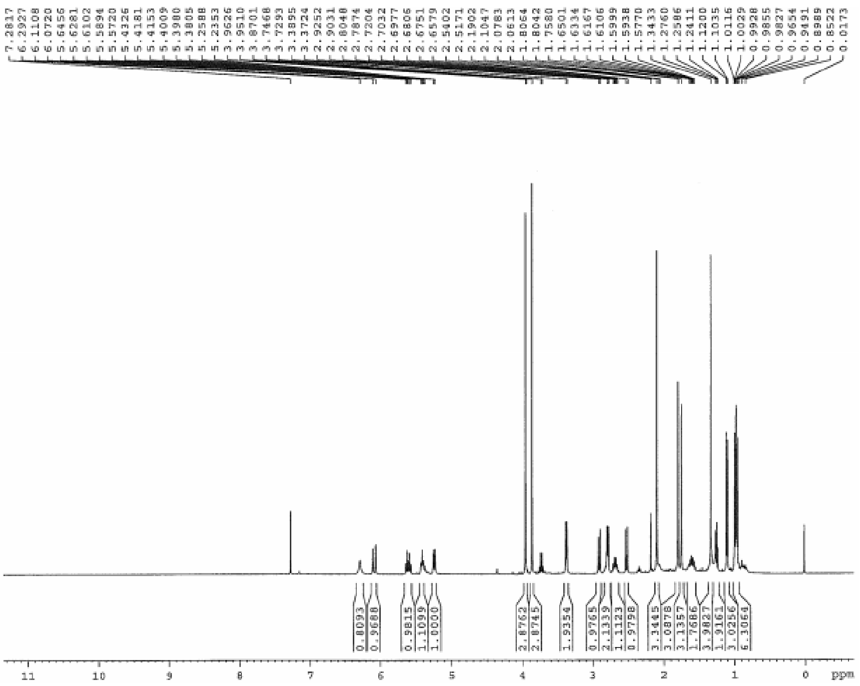


**Figure S21**. ${}^1$H NMR (400 MHz) spectrum of compound **6** in $CDCl_3$

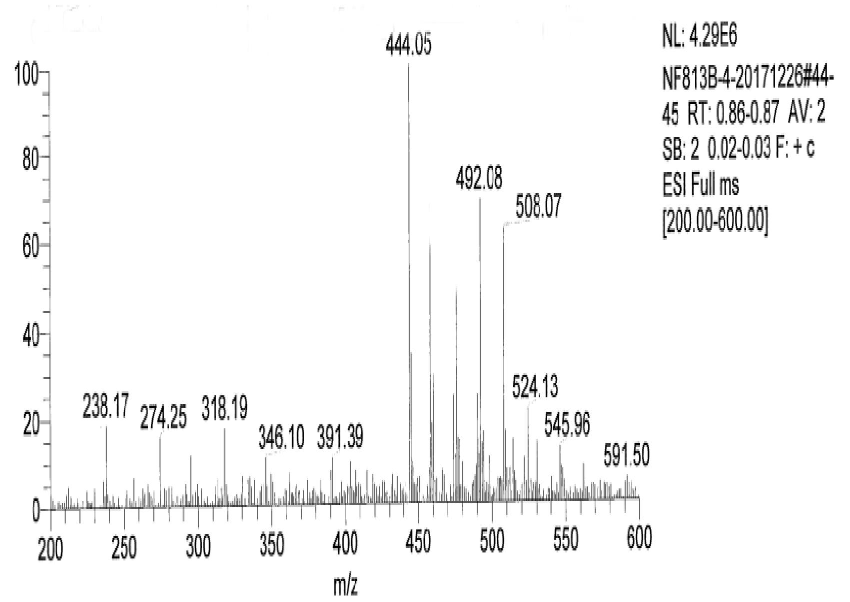


**Figure S22**. The ESI-MS spectrum of compound **6**

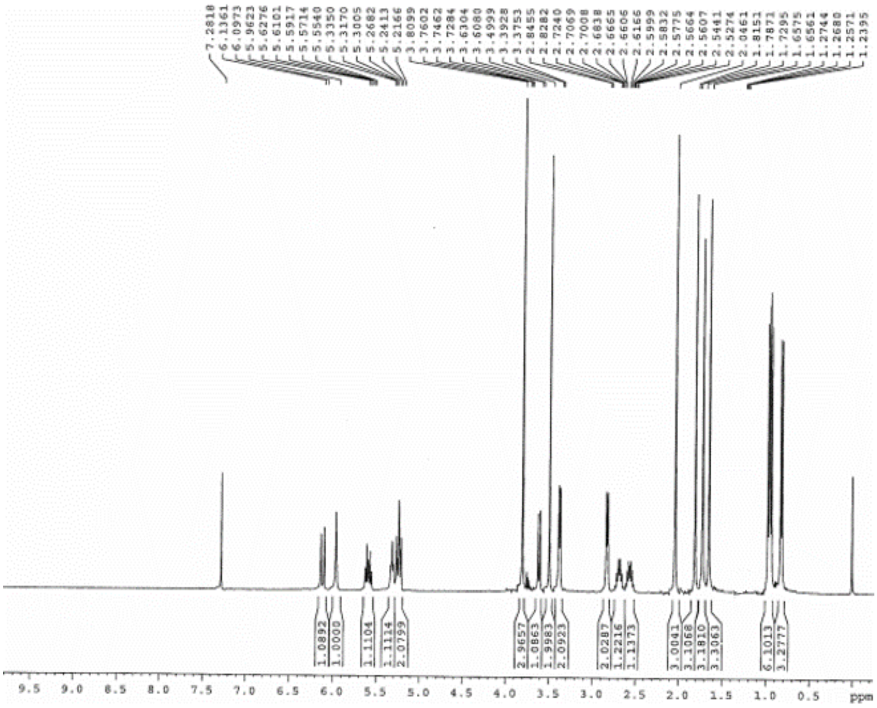


**Figure S23**. $^1$H NMR (400 MHz) spectrum of compound **7** in $CDCl_3$

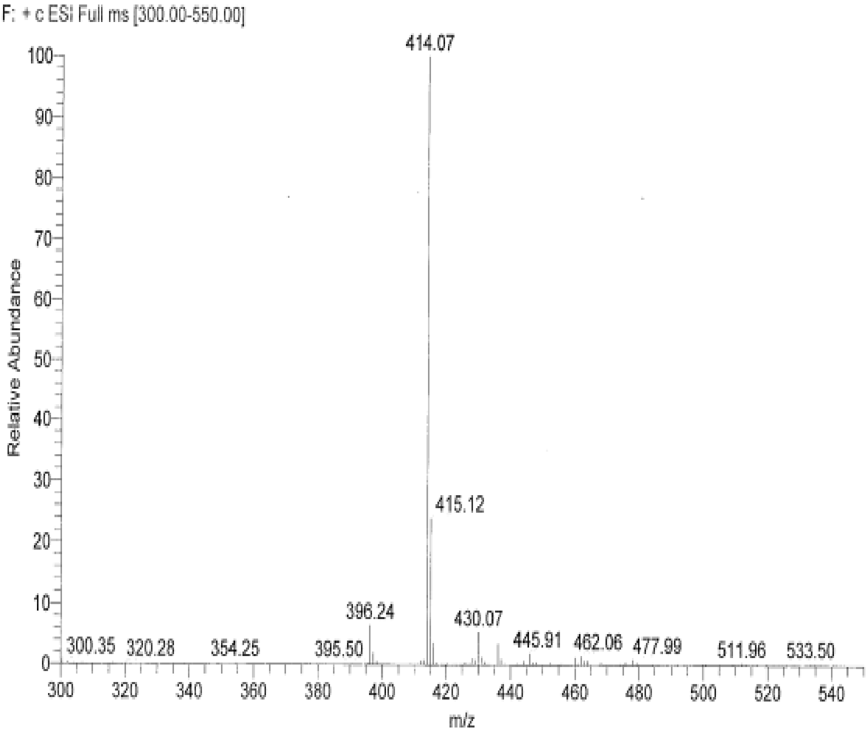


**Figure S24**. ESI-MS spectrum of compound of compound **7**

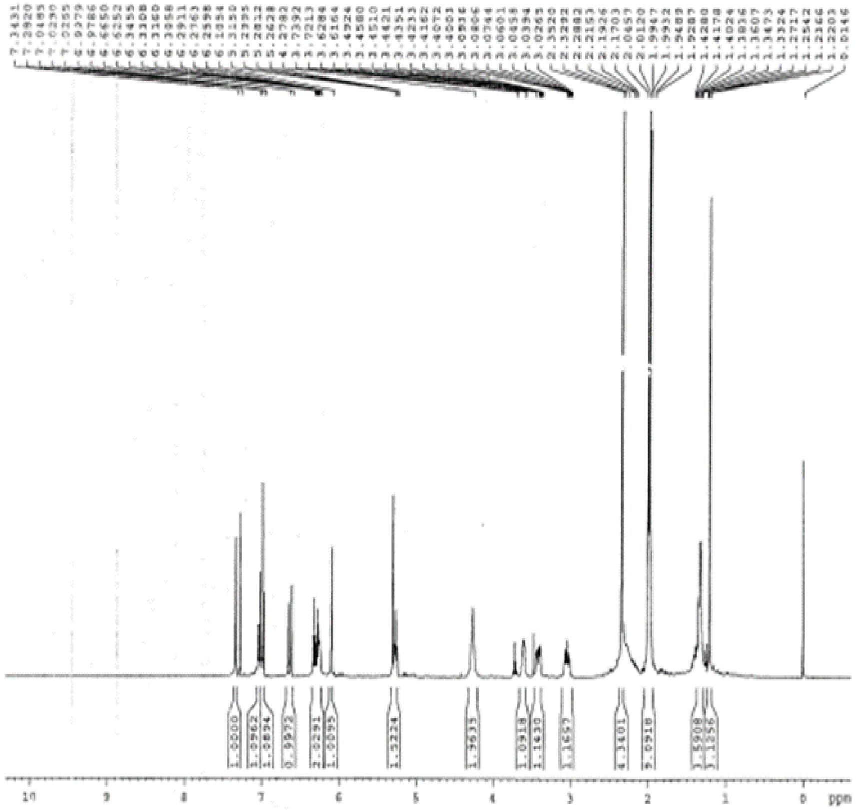


**Figure S25**. $^1$H NMR (400 MHz) spectrum of compound **8** in $CDCl_3$

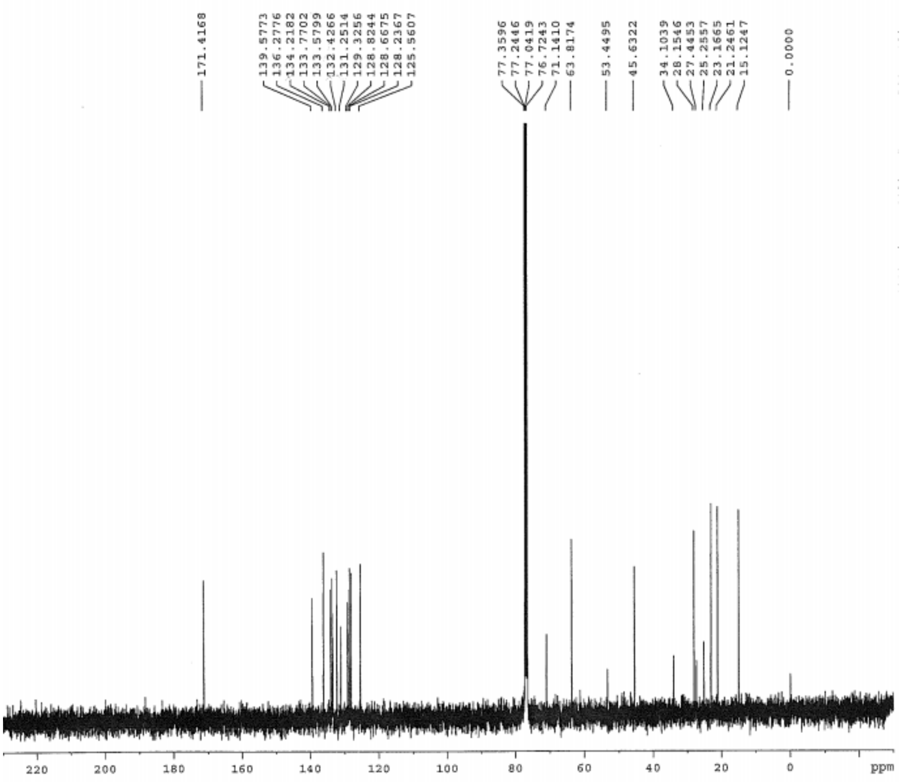


**Figure S26**. $^{13}$C NMR (150 MHz) spectrum of compound **8** in $CDCl_3$

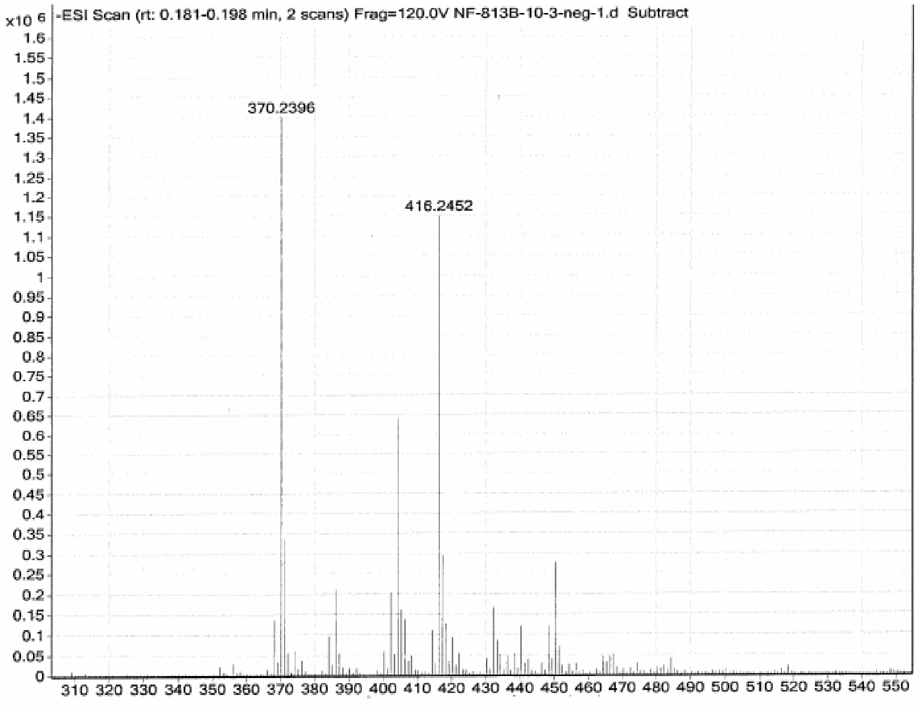


**Figure S27**. The HRESIMS spectrum of compound **8**