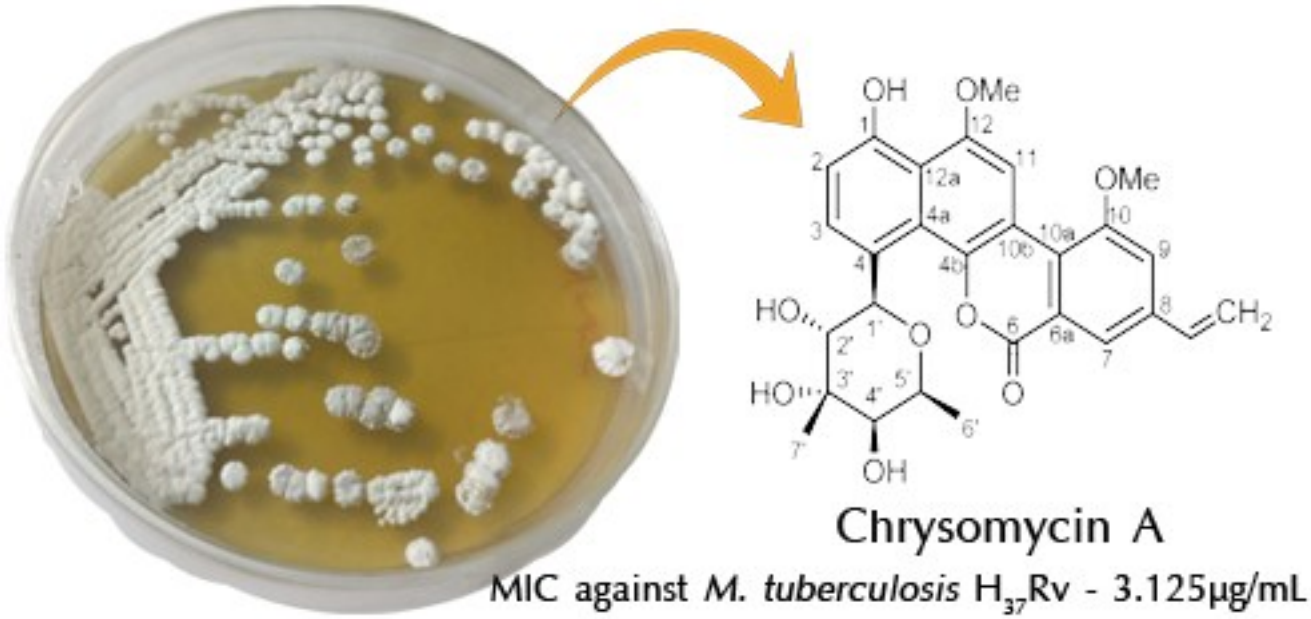
Electronic Supplementary Material (ESI) for RSC Advances.


**Figure S1:**




## Supplementary Data

### Table of Contents

**1.** Table S1, shows different solvent extracts and their activity; ethyl acetate extract had the best activity against *Mycobacterium tuberculosis* H37Rv using REMA.

| **Solvent used for Extraction** | **MIC against *M.tb* H37Rv (in µg/mL)** |
|---|---|
| Ethyl acetate | 30 |
| Chloroform | No activity |
| Methanol | 60 |
| Acetone | 120 |

**2.** The zone of clearance indicated with red circle is the active principle. At left is the fractionated AME on TLC silica plate with Hexane:acetone::1:1. This was overlaid with soft agar containing *M.smegmatis* and incubated for 24-48hrs. The red circles on both the image shows the AMF (Antimycobacterial Fraction).

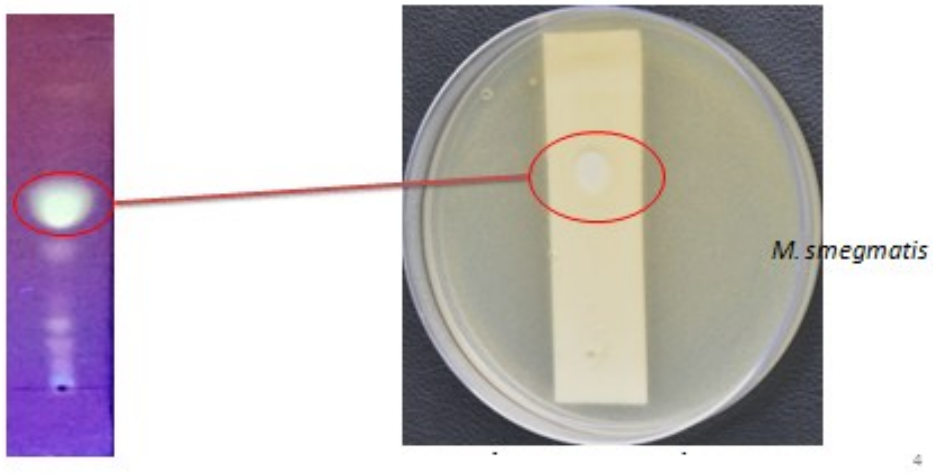



**Figure S2:** Bioautography

**3.**

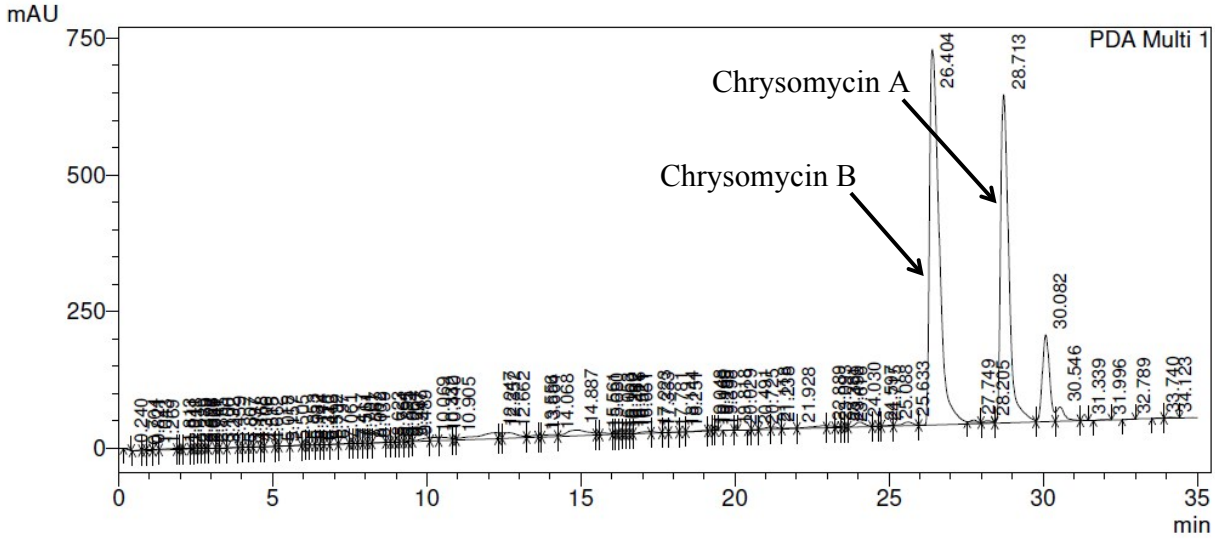
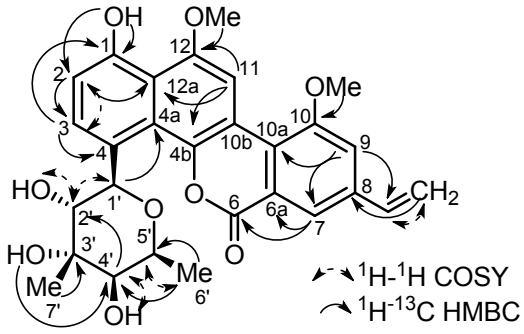



**Figure S3:** HPLC profile of compound mixture observed at $\lambda_{max}$: 254 nm

4. Elucidation of structure of the isolated compounds

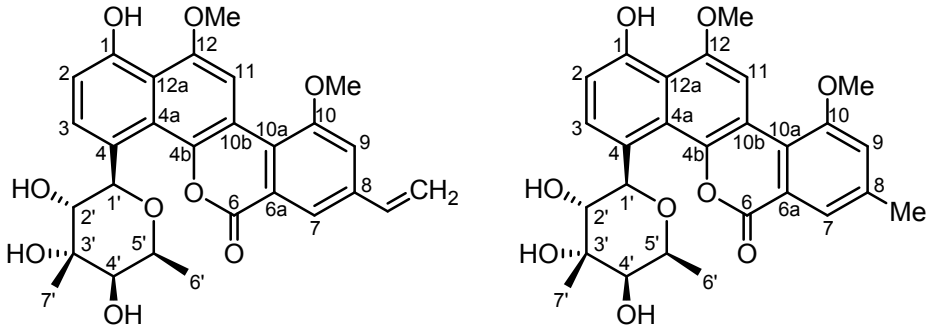
**Table S2.** NMR data for Chrysomycin A (**1**) and Chrysomycin B (**2**) in $(CD_3)_2CO$

| **Carbon No**. | Compound **1** | | Compound **2** | |
|---|---|---|---|---|
| | **$^1$H** | **$^{13}$C** | **$^1$H** | **$^{13}$C** |
| 1 | - | 154.8 | - | 154.9 |
| 2 | 6.96 (d, *J* = 8.5) | 113.2 | 6.78 (d, *J* = 8.5) | 112.9 |
| 3 | 8.03 (d, *J* = 8.5) | 130.1 | 7.83 (d, *J* = 8) | 130.0 |
| 4 | - | 126.2 | - | 126.2 |
| 4a | - | 129.8 | - | 129.6 |
| 4b | - | 143.9 | - | 143.5 |
| 6 | - | 161.2 | - | 161.3 |
| 6a | - | 124.5 | - | 123.1 |
| 7 | 8.08 (d, *J* = 1) | 120.2 | 7.61 (s) | 122.3 |
| 8 | - | 140.0 | - | 141.3 |
| 9 | 7.76 (d, *J* = 1) | 115.3 | 7.22 (s) | 119.4 |
| 10 | - | 158.7 | - | 158.2 |
| 10a | - | 123.5 | - | 122.4 |
| 10b | - | 114.4 | - | 114.6 |
| 11 | 8.61 (s) | 102.7 | 8.31 (s) | 102.8 |
| 12 | - | 153.1 | - | 153.0 |
| 12a | - | 116.2 | - | 116.0 |
| -CH= | 6.97 ( dd, *J* = 7, 18) | 136.3 | - | - |
| $=CH_2$ | 5.52 (d, *J* = 11) | 117.0 | - | - |
| | 6.15 (d, *J* = 17.5) | | - | |
| | - | | | |
| 1’ | 6.41 (d, *J* = 9.5) | 76.2 | 6.21 (d, *J* = 9.5) | 76.3 |
| 2’ | 3.87 (dd, *J* = 7, 9.5) | 76.1 | 3.70 (dd, *J* = 7, 9.5) | 76.1 |
| 3’ | - | 74.5 | - | 74.5 |
| 4’ | 3.37 (d, *J* = 8.5) | 77.5 | 3.21 (d, *J* = 8) | 77.5 |
| 5’ | 4.64 (q, *J* = 6.5) | 72.5 | 4.48 (q, *J* = 6.5) | 72.5 |
| 6’ | 1.24 (d, *J* = 6.5) | 17.3 | 1.05 (d, *J* = 6.5) | 17.3 |
| 7’ | 1.45 (s) | 24.2 | 1.28 (s) | 24.2 |
| 1(-OH) | 9.84 (s) | - | 9.64 (s) | - |
| 10($-OCH_3$) | 4.28 (s) | 57.0 | 3.98 (s) | 56.9 |
| 12($-OCH_3$) | 4.26 (s) | 56.9 | 4.03 (s) | 56.8 |
| 8($-CH_3$) | - | - | 2.34 (s) | 21.5 |
| 2’(-OH) | 3.67 (d, *J* = 7) | - | 3.50 (d, *J* = 7) | - |
| 3’(-OH) | 4.00 (s) | | 3.86 (s) | - |
| 4’(-OH) | 3.79 (d, *J* = 8.5) | - | 3.63 (d, *J* = 8.5) | - |




Chrysomycin A (**1**)

**Figure S4a.** COSY and HBMC correlations for chrysomycin A (**1**)




Chrysomycin A (**1**) Chrysomycin B (**2**)

**Figure S4b.** Structures of Chrysomycin A (**1**) and Chrysomycin B (**2**)

Table S2 shows the $^{1}H$ and $^{13}C$ NMR spectral data of the isolated compounds. The pure compound **1** was yellow crystalline in nature, and by HR-ESI-MS (High-resolution electrospray ionization mass spectrometry) the molecular formula was determined as $C_{28}H_{28}O_9$ which exhibited an ion peak at *m/z* 531.1625 $[M+Na]^+$. Presence of eleven quaternary carbons in the aromatic region as indicated by the DEPT (Distortionless Enhancement by Polarization Transfer) spectra along with five aromatic protons implied a fused polyaromatic framework. Chemical shifts at $\delta_H$ 3.87 (H-2'), $\delta_H$ 3.37 (H-4'), $\delta_H$ 4.64 (H-5') along with the presence of three hydroxyl protons at $\delta_H$ 3.67, $\delta_H$ 3.79, $\delta_H$ 4.00 which did not exhibit HSQC correlations clearly indicated the presence of a carbohydrate ring. There was also a hydroxyl proton at $\delta_H$ 9.84 indicating the presence of phenolic group. Presence of COSY correlation between $\delta_H$ 6.41 (H-1') and $\delta_H$ 3.87 (H-2') along with HMBC correlation of H-1' with C-3 ($\delta_C$ 130.1) and C-4a ($\delta_C$ 129.8) indicated H-1' as a benzylic carbon and established the connectivity between the carbohydrate ring and the

aromatic group as a C-glycoside. Furthermore, HMBC correlation between H-2 ($\delta_H$ 6.96) and C-3; phenolic proton ($\delta_H$ 9.84) and C-2 ($\delta_C$ 113.2) unambiguously confirmed the carbohydrate ring attachment to the phenolic portion of the polyaromatic moiety. HMBC correlations of H-2 and H-11 ($\delta_H$ 8.61) with C-12a ($\delta_C$ 116.2); H-11 with C-10a ($\delta_C$ 123.5); H-9 ($\delta_H$ 7.76) with C-10a; H-9 with C-7 ($\delta_C$ 120.2); H-7 ($\delta_H$ 8.08) with C-6 ($\delta_C$ 161.2, C=O) along with H-11 with C-4b ($\delta_C$ 143.9) indicated the fused nature of the polyaromatic framework. COSY, HMBC correlations (Figure S4a) along with coupling constants suggested the identity of the carbohydrate ring as a virenoside. The $^1$H NMR showed the presence of a vinyl group in the compound at C-8. This and the above-discussed NMR data, together strongly suggested that the isolated compound **1** is chrysomycin A, a naptho-coumarin (Figure S4b) which was further confirmed by comparison of the NMR data with the published literature.[1-4]

The molecular formula of compound **2** was determined as $C_{27}H_{28}O_9$ by HR-ESI-MS which exhibited an ion peak at *m/z* 519.1638 $[M+Na]^+$. The $^1$H and $^{13}$C NMR of compound **2** (Table S2) was similar to chrysomycin A (**1**) and HRMS data showed a mass difference of 12 da indicating that these two compounds belongs to the same chemical class. The $^1$H NMR showed the presence of methyl group in compound **2** at C-8 which confirms chrysomycin B (Figure S4b).

Initially, partial gene sequence (NCBI accession number, KX364007) of polyketide synthase II alpha subunit (PKS IIα) of the isolate OA161 showed 86% homology to the PKS IIα gene of the chrysomycin biosynthetic gene cluster of *Streptomyces albaduncus*[5]. This suggested that the backbone of the active principle produced by our isolate could be a naptho-coumarin.

References


1. F. Strelitz, H. Flon and I. N. Asheshov, *Journal of Bacteriology*, 1955, 69, 280-283.
2. G. T. Carter, A. A. Fantini, J. C. Jjames, D. B. Borders and R. J. White, *The Journal of antibiotics*, 1985, 38, 242-248.
3. S. K. Jain, A. S. Pathania, R. Parshad, C. Raina, A. Ali, A. P. Gupta, M. Kushwaha, S. Aravinda, S. Bhushan and S. B. Bharate, *RSC Advances*, 2013, 3, 21046-21053.
4. U. Weiss, K. Yoshihira, R. Highet, R. White and T. Wei, *The Journal of antibiotics*, 1982, 35, 1194-1201.
5. Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) "Basic local alignment search tool." J. Mol. Biol. 215:403-410.

**5**.$^{1}$H and $^{13}$C NMR spectra of Compound **1** (Chrysomycin A) and Compound **2** (Chrysomycin B) in $(CD_3)_2CO$

**Figure S5a**. $^{1}$H -NMR spectrum of Compound **1** (Chrysomycin A) in $(CD_3)_2CO$

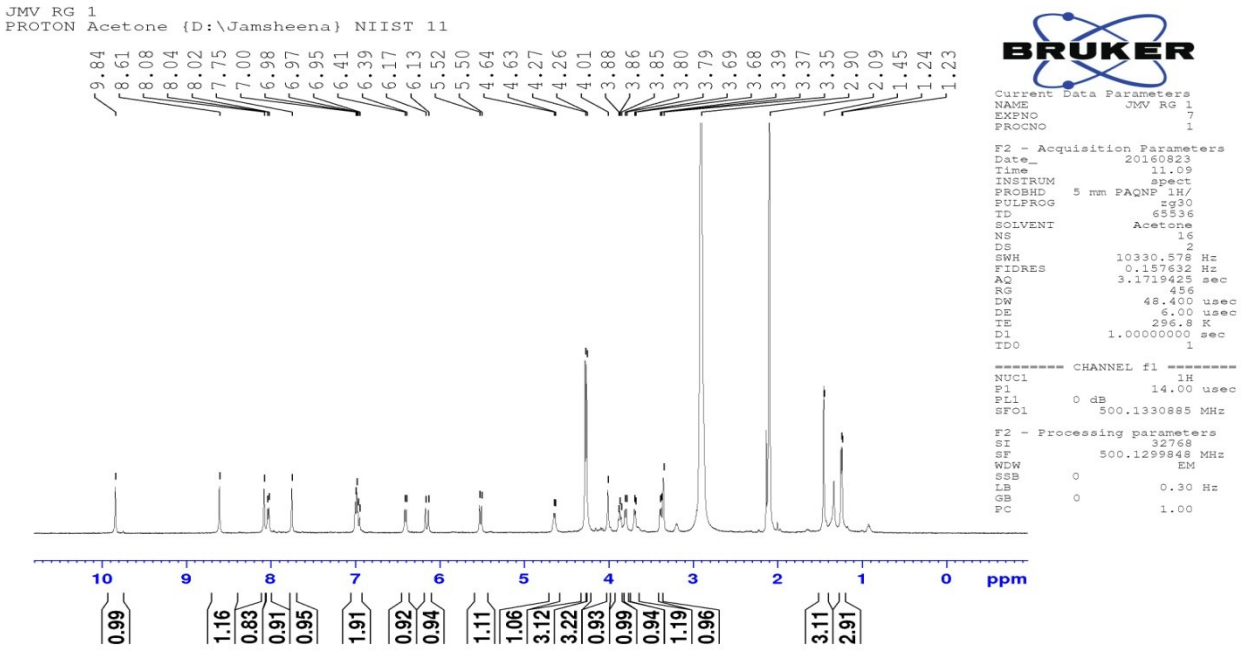



**Figure S5b**. $^{13}$C -NMR spectrum of Compound **1** (Chrysomycin A) in $(CD_3)_2CO$

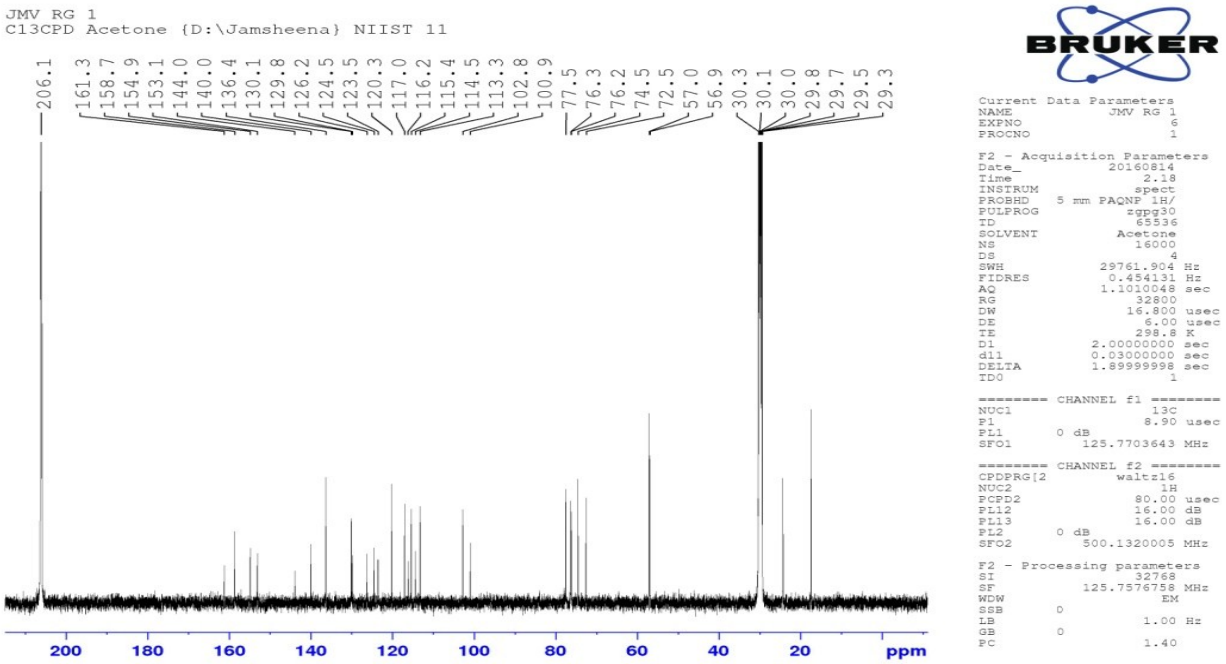


**Figure S5c**. $^{1}H$ -NMR spectrum of Compound **2** (Chrysomycin B) in $(CD_3)_2CO$

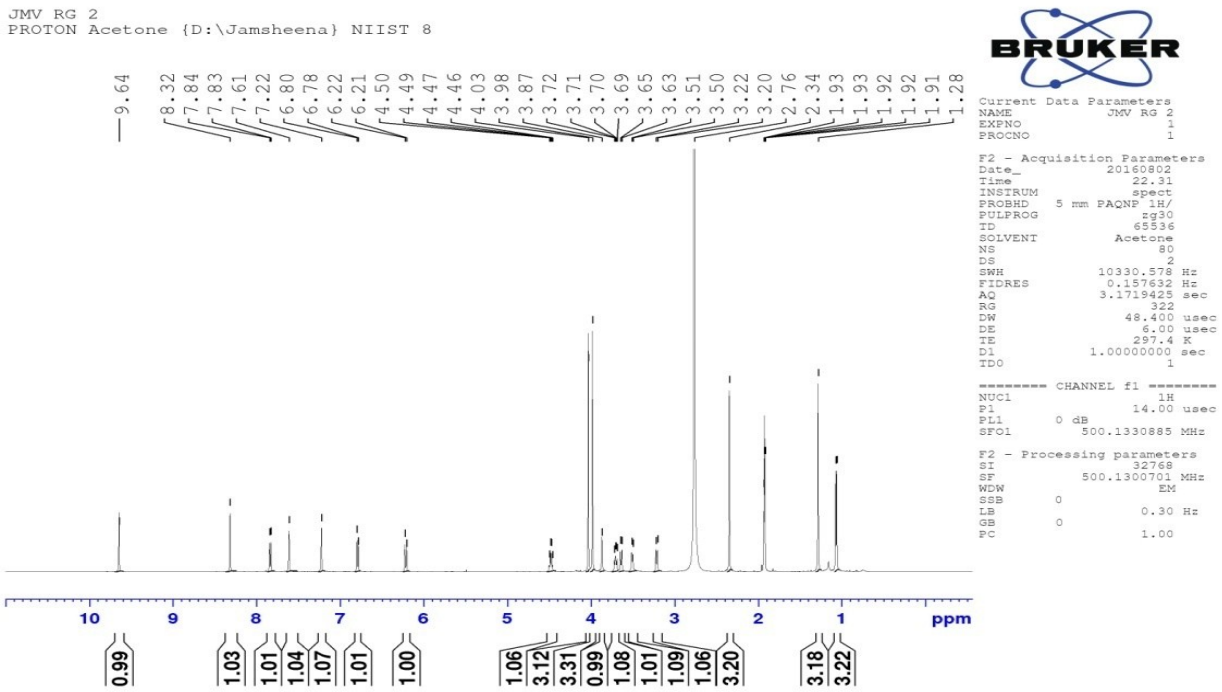
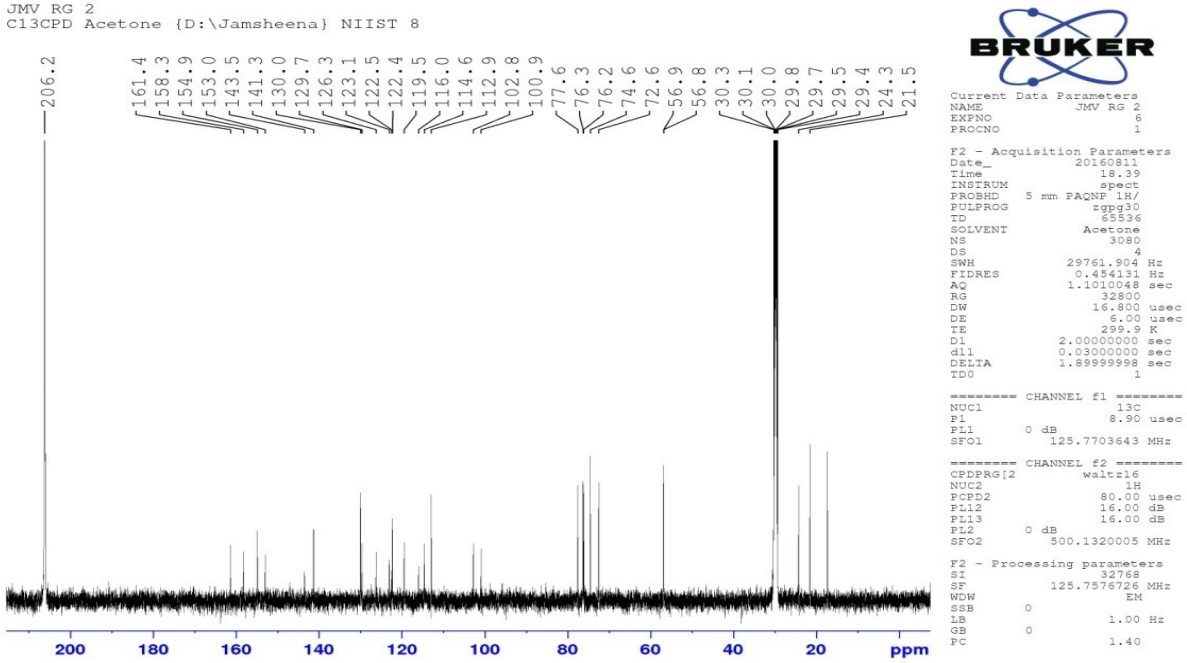



**Figure S5d**. $^{13}C$ -NMR spectrum of Compound **2** (Chrysomycin B) in $(CD_3)_2CO$



**6**. HRMS spectra of Compound **1** and Compound **2**

**Figure S6a**. HRMS spectrum of Compound **1** (Chrysomycin A)

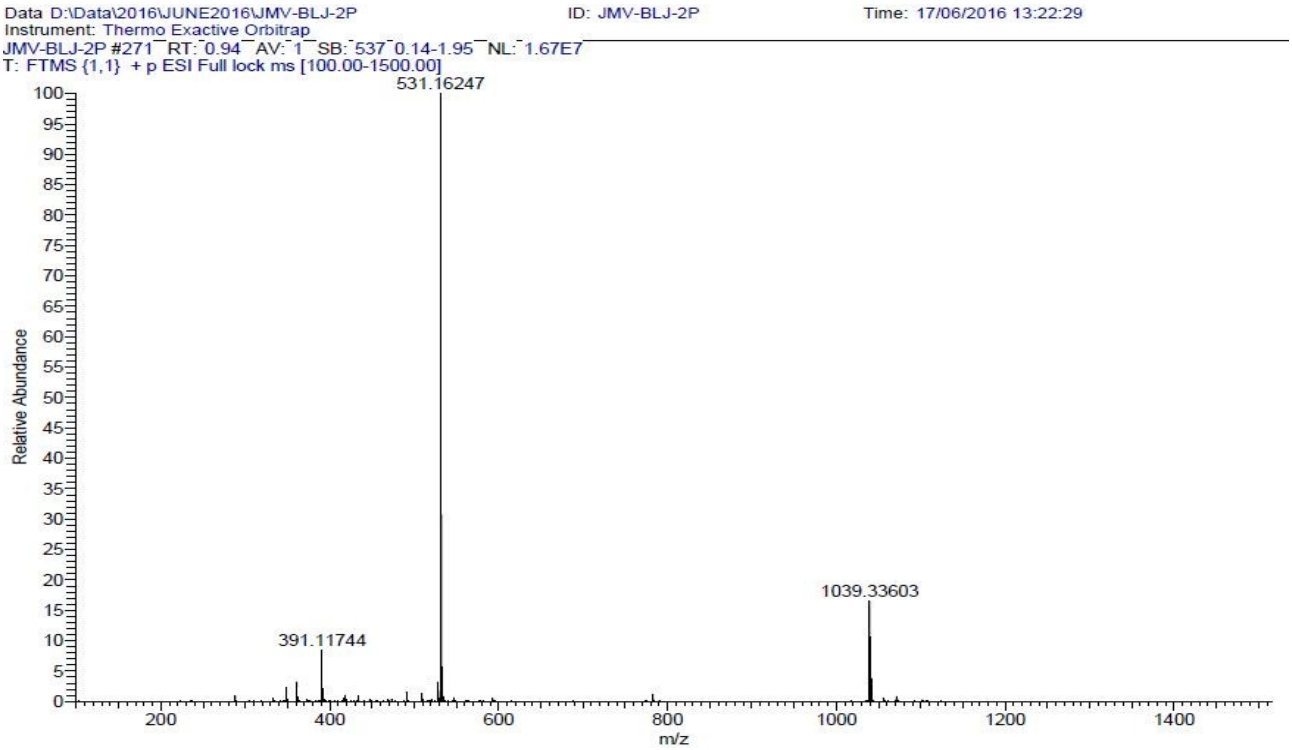
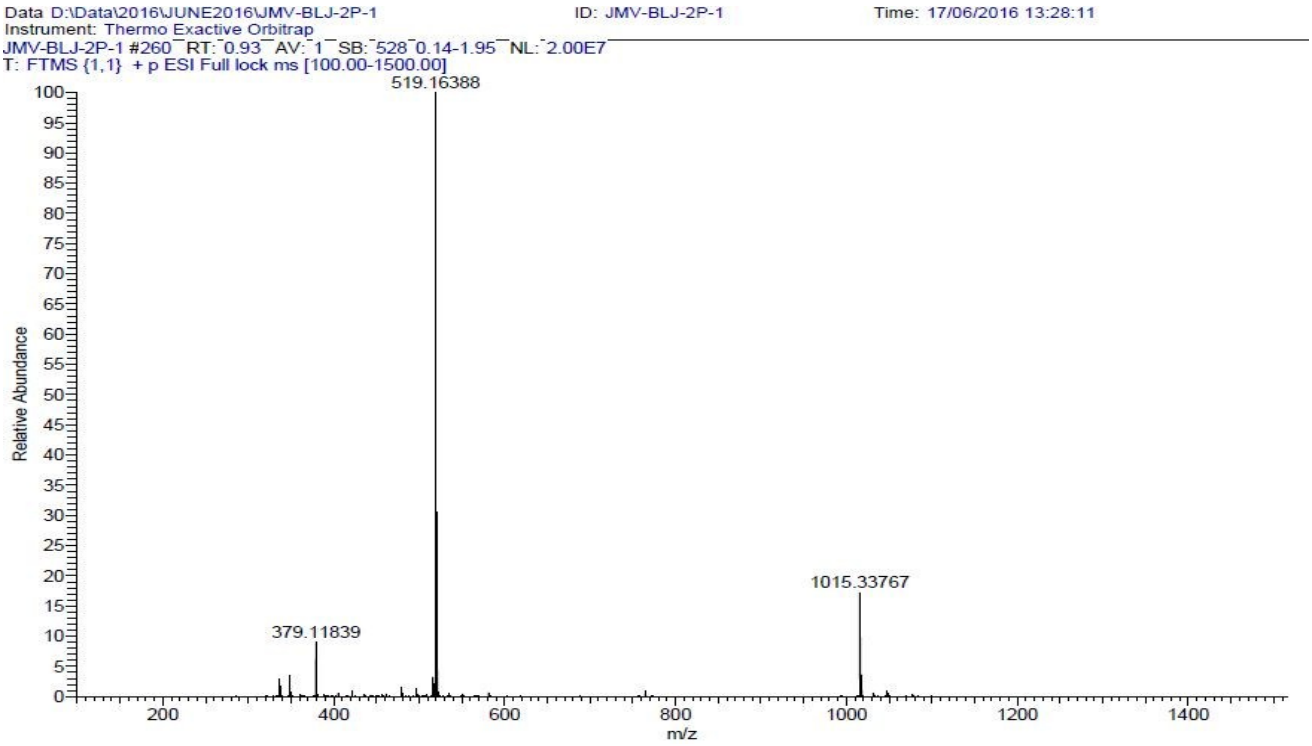
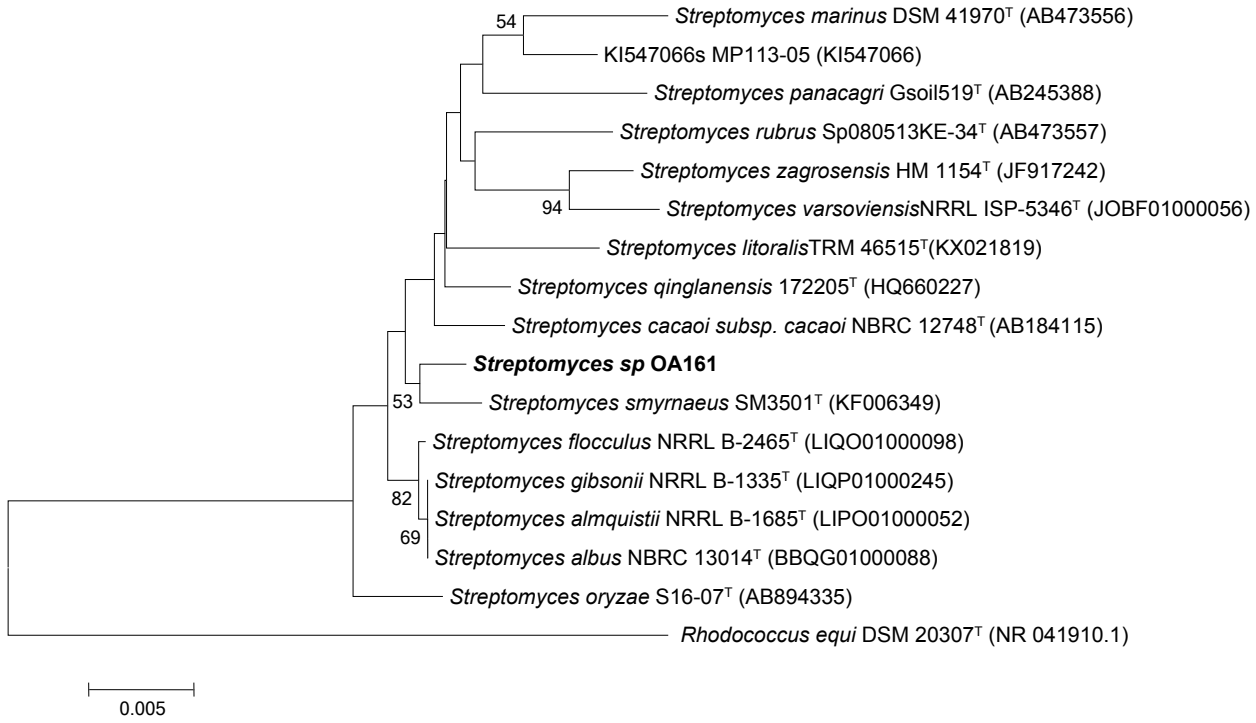



**Figure S6b.**HRMS spectrum of Compound **2** (Chrysomycin B)



**7.** Phylogentic tree constructed using the 16S rRNA gene sequence.




Supplementary data. 7: Evolutionary relationships of taxa. The optimal tree with the sum of branch length = 0.19121665 is shown. The tree is constructed with MEGA 7 software and drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbor-joining analysis of the datasets. only values >50 % are shown. NCBI accession numbers are at the end of the species name. Bar 0.005 denote nucleotide substitutions per site. *Rhodococcus eui* was selected as the outgroup.

**8.** List of Primers used in this study

| S.No | Primers | Used for Amplificatiion(Amp)/Sequencing(Seq) |
|---|---|---|
| 1 | 5’-GAGTTTGATCCTGGCTCAG-3’ (9F) | 16S rRNA gene Amplification and sequencing |
| 2 | 5’-AAGGAGGTGATCCAGCC-3’ (1541R) | 16S rRNA gene Amplification and sequencing |
| 3 | 5’-TSGCSTGCTTGGAYGCSATC-3’ (F) | Partial PKS II alpha gene amplication and sequencing |
| 4 | 5’-TGGAANCCGCCGAABCCTCT-3’ (R) | Partial PKS II alpha gene amplication |

| | | |
|---|---|---|
| | | and sequencing |
| 5 | 5’-GGTGGCGAAGGCGGA-3 ’(F) | 16S rRNA gene Amplification and sequencing |
| 6 | 5’- GAACTGAGACCGGCTTTTGA-3’ (R) | 16S rRNA gene Amplification and sequencing |

‘F’ and ‘R’ represents forward and reverse primer, respectively. The numerals before the F/R represents the nucleotide position with respect to *E.coli* 16S rRNA gene.