Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.


# Inhibition and Eradication of *Pseudomonas aeruginosa* biofilms by secondary metabolites of *Nocardiopsis lucentensis* EMB25

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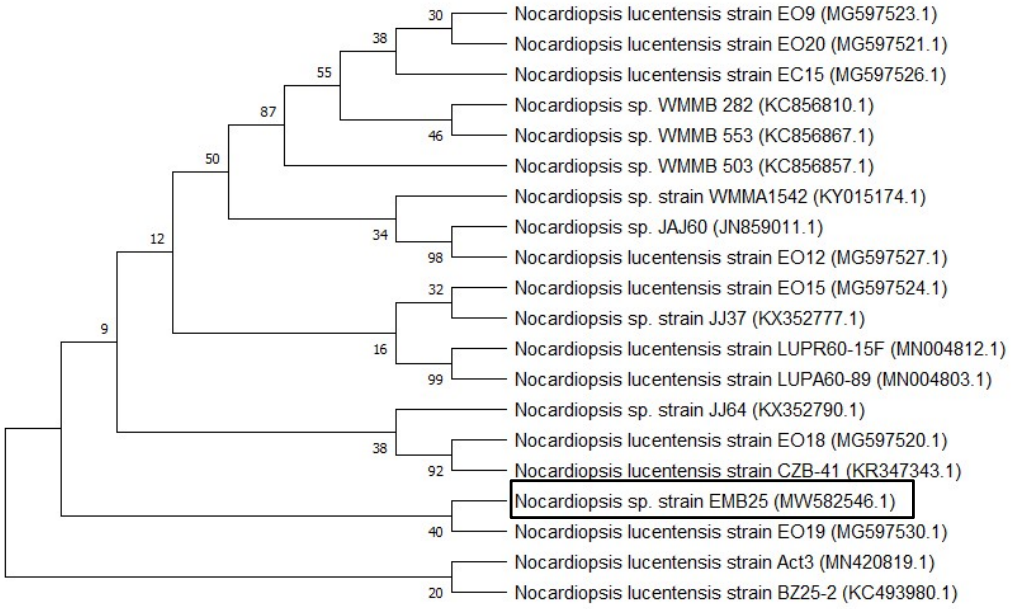


**Fig. S1:** Phylogenetic tree based on 16S rRNA sequencing of selected strain EMB25 constructed using the neighbor-joining method performed in MEGA 7.0 software. The number of bootstrap replications was set to 1000. The strain used in this study is highlighted in box.

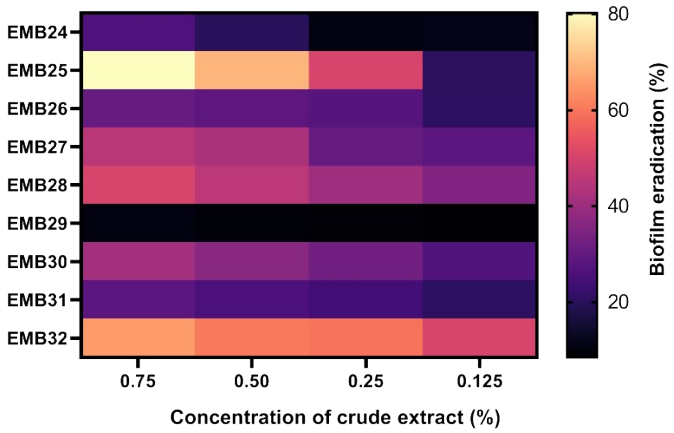


**Fig. S2:** Effect of cell-free extract of various actinobacteria isolated in the lab at concentration 0.75 %, 0.5 %, 0.25 %, and 0.125 % (v/v) on 48 h biofilm of *P. aeruginosa*. As inferred using heat map, EMB25 displayed maximum biofilm eradication and was used in further study.

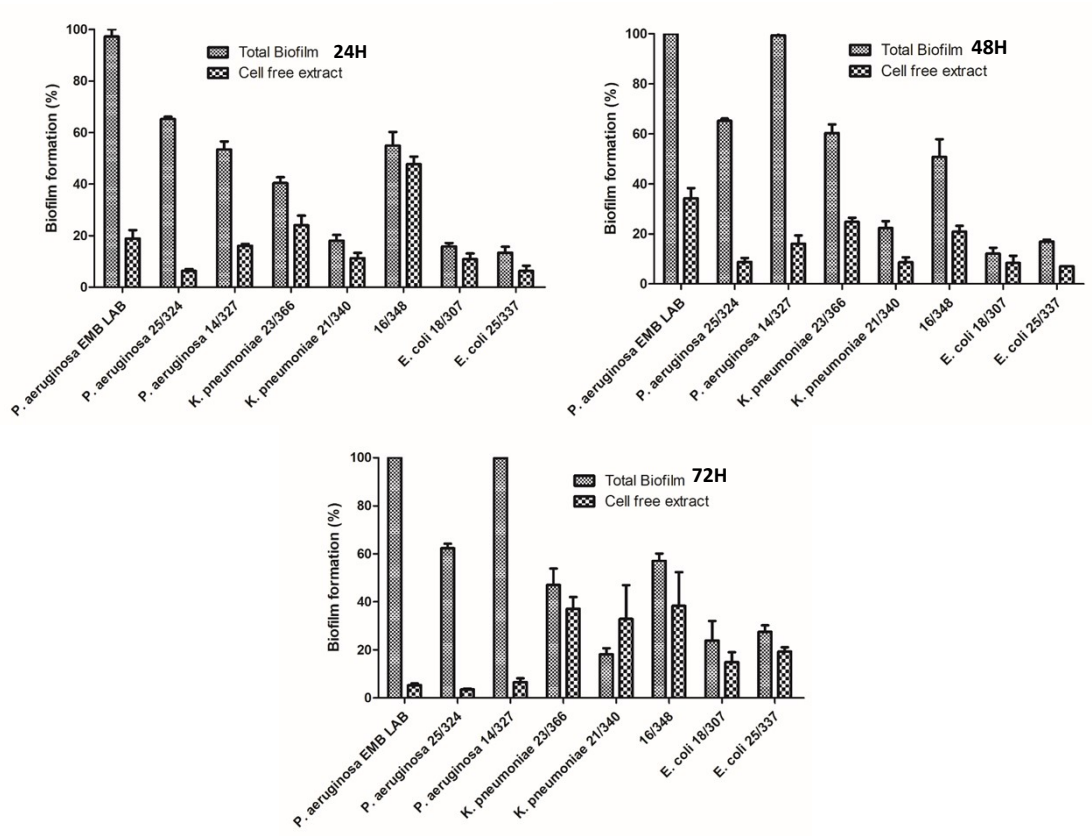


**Fig. S3:** a) The effect of cell-free extract of EMB25 was checked on preformed biofilm of various clinical strains namely, *P. aeruginosa*, *K. pneumoniae*, and *E. coli* A) 24 h preformed biofilm B) 48 h preformed biofilm C) 72 h preformed biofilm, with replenishment of media at every 24 h. The EMB25 extract has removed biofilm at every stage of the clinical strains used in the study. The crystal assay was used to quantify the biofilm performed on polystyrene flat-bottom 96 well plate.

## Table S1: Metabolites obtained from GC-MS

| S. No. | Comp No. | Retention Time | Area% | Name of the compounds identified |
|---|---|---|---|---|
| 1 | C 1 | 4.928 | 0.92 | Acetic acid, phenylmethyl ester |
| 2 | C 2 | 5.313 | 0.18 | 1-dodecene |
| 3 | C 3 | 5.470 | 4.65 | Dodecane |
| 4 | C 4 | 8.353 | 0.21 | Tridecane, 5-methyl- |
| 5 | C 5 | 6.188 | 0.97 | 4,5-Heptadien-2-one, 3,3,6-trimethyl- |
| 6 | C 6 | 8.713 | 0.20 | 2-Bromo dodecane |
| 7 | C 7 | 9.163 | 0.90 | 3-hexadecene, (z)- |
| 8 | C 8 | 9.372 | 10.1 | Tetradecane |
| 9 | C 9 | 10.292 | 0.30 | 2,4-heptadienal, 2,4-dimethyl- |
| 10 | C 10 | 9.518 | 0.30 | Benzene, 1,1'-oxybis- |
| 11 | C 11 | 11.314 | 0.19 | Pentadecane |
| 12 | C 12 | 10.870 | 0.06 | Cyclododecane |
| 13 | C 13 | 12.314 | 0.77 | Tetradecane, 5-methyl- |
| 14 | C 14 | 12.692 | 0.20 | Pentadecane, 3-methyl- |
| 15 | C 15 | 11.605 | 0.28 | Phenol, 3,5-bis(1,1-dimethylethyl)- |
| 16 | C 16 | 12.970 | 0.42 | (2-Methyl-1-cyclohexenyl) methanol, TMS derivative |
| 17 | C 17 | 13.132 | 1.36 | 3-octadecene, (e)- |
| 18 | C 18 | 13.336 | 10.5 | Hexadecane |
| 19 | C 19 | 13.391 | 0.72 | 5-octadecene, (e)- |
| 20 | C 20 | 14.001 | 0.21 | 1,2,4-Trioxolane, 3,3,5-triphenyl- |
| 21 | C 21 | 14.231 | 0.10 | 3-acetoxydodecane |
| 22 | C 22 | 15.017 | 0.07 | 2-propenoic acid, isodecyl ester |
| 23 | C 23 | 15.134 | 0.07 | Tricosane |
| 24 | C 24 | 15.924 | 0.38 | Heptadecane, 7-methyl- |
| 25 | C 25 | 16.419 | 0.56 | Heptadecane, 3-methyl- |
| 26 | C 26 | 16.530 | 1.52 | 3-Methyl-1-phenyl-5-[(trimethylsilyl)oxy]-1H-pyrazole |
| 27 | C 27 | 16.823 | 0.77 | 1-heptadecene |
| 28 | C 28 | 16.989 | 13.3 | Heneicosane |
| 29 | C 29 | 17.132 | 0.77 | Undecane, 4-cyclohexyl- |
| 30 | C 30 | 16.112 | 0.13 | Benzene, 1,1'-(1,2-cyclobutanediyl) bis-, trans- |
| 31 | C 31 | 18.553 | 0.36 | Phenoxyethanol, TMS derivative |
| 32 | C 32 | 19.464 | 0.79 | Octadecane, 2-methyl- |
| 33 | C 33 | 19.734 | 13.42 | 1,2-benzenedicarboxylic acid, dibutyl este |
| 34 | C 34 | 20.199 | 0.60 | 1-heptadecene |
| 35 | C 35 | 20.328 | 4.44 | Eicosane |
| 36 | C 36 | 20.570 | 2.58 | 2,6-Dimethyl-1-nonen-3-yn-5-ol, TMS derivative |
| 37 | C 37 | 21.071 | 8.45 | Palmitic Acid, TMS derivative |
| 38 | C 38 | 22.592 | 0.38 | Eicosane, 2,4-dimethyl- |

| | | | | |
|---|---|---|---|---|
| 39 | C 46 | 23.262 | 0.54 | Pentadecanoic acid |
| 40 | C 47 | 23.653 | 0.81 | Decane, 4-cyclohexyl- |
| 41 | C 48 | 23.962 | 0.55 | Octadecanoic acid, trimethylsilyl ester |
| 42 | C 49 | 24.649 | 0.36 | 1-Octanamine, N,N-dioctyl- |
| 43 | C 50 | 26.509 | 0.53 | Dodecane, 2-cyclohexyl- |
| 44 | C 54 | 28.413 | 0.94 | Hexadecanoic acid, 4-[(trimethylsilyl) oxy] butyl ester |
| 45 | C 55 | 28.773 | 0.81 | 1-Monopalmitin, 2TMS derivative |
| 46 | C 56 | 29.524 | 0.46 | Tridecane, 4-cyclohexyl- |
| 47 | C 58 | 26.774 | 0.53 | Benzonitrile, m-phenethyl- |
| 48 | C 59 | 27.145 | 0.20 | Octadecanoic acid, 3-oxo-, ethyl ester |
| 49 | C 60 | 30.764 | 0.48 | Hexatriacontane |
| 50 | C 61 | 28.249 | 2.20 | (2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, trans- |
| 51 | C 62 | 31.826 | 0.51 | Dodecane, 4-cyclohexyl- |
| 52 | C 63 | 32.352 | 0.30 | Ricinolsaeure |
| 53 | C 64 | 32.658 | 0.72 | Ricinoleic acid, 2TMS derivative |

## Table S2: Residues within 4 Å of the docked compounds

| S. No. | Metabolites | Residues within 4 Å of the docked compounds |
|---|---|---|
| Docking in LasR | | |
| C 30 | Benzene, 1,1'-(1,2-cyclobutanediyl)bis-, trans- | Leu36, Leu38, Leu39, Leu40, Tyr47, Ala50, Ile52, Tyr56, Arg61, Tyr64, Asp65, Ala70, Asp73, Thr75, Val76, Cys79, Thr115, Gly126, Ala127, Leu128, Ser129 |
| C 15 | Phenol, 3,5-bis(1,1-dimethylethyl)- | Leu36, Leu38, Leu40, Tyr47, Ala50, Ile52, Tyr56, Trp60, Arg61, Tyr64, Ala70, Asp73, Thr75, Val76, Cys79, Thr115, Gly126, Ala127, Leu128, Ser129 |
| C 12 | Cyclododecane | Leu36, Phe37, Leu38, Leu39, Leu40, Tyr47, Ala50, Ile52, Tyr56, Arg61, Tyr64, Ala70, Asp73, Thr75, Val76, Cys79, Thr115, Gly126, Ala127, Ser129 |
| C 58 | Benzonitrile, m-phenethyl | Leu36, Phe37, Leu38, Leu39, Tyr47, Ala50, Ile52, Tyr56, Trp60, Arg61, Tyr64, Ala70, Asp73, Val76, Ala127, Ser129 |
| C 5 | 4,5-Heptadien-2-one, 3,3,6-trimethyl | Leu36, Leu38, Ile52, Tyr56, Trp60, Arg61, Tyr64, Asp73, Thr75, Val76, Trp88, Phe101, Ala105, Leu110, Thr115, Ala127, Ser129 |
| C 61 | 2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans- | Leu36, Phe37, Leu38, Leu39, Leu40, Tyr47, Glu48, Ala50, Ile52, Tyr56, Trp60, Arg61, Tyr64, Asp65, Ala70, Asp73, Thr75, Val76, Cys79, Phe101, Thr115, Gly126, Ala127, Ser129 |
| Docking in RhlR | | |
| C 58 | Benzonitrile, m-phenethyl | Ala44, Thr58, Val60, Tyr64, Trp68, Leu69, Tyr72, Asp81, Ala83, Ile84, Trp96, Phe101, Leu107, Trp108, Ala111, Leu116, Thr121, Ser135 |
| C 15 | Phenol, 3,5-bis(1,1-dimethylethyl)- | Ala44, Tyr45, Gly46, Val60, His61, Tyr64, Trp68, Leu69, Tyr72, Glu73, Tyr77, Asp81, Ala83, Ile84, Trp96, Thr121, Val133, Ser135 |
| C 5 | 4,5-Heptadien-2-one, 3,3,6-trimethyl | Tyr64, Trp68, Tyr72, Asp81, Ala83, Ile84, Trp96, Leu107, Trp108, Glu108, Glu110, Ala111, Arg112, Trp114, Leu116, Thr121, Ser135, Ala137 |
| C 30 | Benzene, 1,1'-(1,2-cyclobutanediyl)bis-, trans- | Ala44, Tyr45, Gly46, Arg48, Thr58, Val60, His61, Tyr64, Trp68, Leu69, Tyr72, Gly78, Asp81, Ala83, Ile84, Trp96, Phe101, Leu107, Ala111, Leu116, Thr121, Leu131, Val133, Leu134, Ser135 |
| C 12 | Cyclododecane | Tyr64, Trp68, Tyr72, Asp81, Ala83, Trp96, Phe101, Leu107, Trp108, Glu110, Ala111, Arg112, Trp114, Leu116, Thr121, Ser135, Ala137 |
| C 10 | BENZENE, 1,1'-OXYBIS- | Ala44, Val60, Tyr64, Trp68, Tyr72, Asp81, Ile84, Trp96, Phe101, Leu107, Trp108, Ala111, Arg112, Leu116, Thr121, Ser135 |

**Table S3: ADME properties of compounds showing docking score better than -6**

| S. No. | Metabolites | Molecular Weight | QPlogPw (a) | QPlogS (b) | QPlogBB (c) | QPPCaco (d) | QPlogHERG (e) | QPloKhsa (f) | Lipinski Rule of five (g) |
|---|---|---|---|---|---|---|---|---|---|
| C 5 | 4,5-Heptadien-2-one, 3,3,6-trimethyl | 152.236 | 2.152 | -2.444 | 0.003 | 4255.95 | -3.218 | -0.097 | 0 |
| C 10 | BENZENE, 1,1'-OXYBIS- | 170.21 | 4.184 | -3.965 | -0.037 | 9906.04 | -4.755 | 0.225 | 0 |
| C 12 | Cyclododecane | 168.322 | 5.947 | -6.154 | 1.023 | 9906.04 | -2.589 | 0.704 | 1 |
| C 15 | Phenol, 3,5-bis(1,1-dimethylethyl)- | 206.327 | 3.708 | -3.979 | -0.068 | 3011.19 | -3.386 | 0.584 | 0 |
| C 30 | Benzene, 1,1'-(1,2-cyclobutanediyl)bis-, trans- | 208.302 | 5.619 | -6.063 | 0.427 | 9906.04 | -5.168 | 0.918 | 1 |
| C 58 | Benzonitrile, m-phenethyl | 207.274 | 3.513 | -4.353 | -0.337 | 2055.58 | -5.607 | 0.374 | 0 |
| C 61 | 2,3-Diphenylcyclo propyl)methylphenyl sulfoxide, trans | 332.459 | 5.154 | -4.096 | 0.3 | 284.39 | -6.641 | 0.625 | 1 |

a. *Predicted water/gas partition coefficient (acceptable range is −2.0–6.5).*
b. *Predicted aqueous solubility, S in mol/L (acceptable range is−6.5–0.5).*
c. *Predicted brain/blood partition coefficient, default is −3.0 to + 1.2.*
d. *Predicted Caco-2 cell permeability in nm/s (>500, great).*
e. *Predicted IC50 value for blockage of HERG K+ channels (> −5).*
f. *Predicted value of binding to human serum albumin (range is −1.5 to 1.5*
g. *Lipinski = MM ≤ 500; Log Po/w ≤ 5; H-bond donors ≤ 5; H-bond acceptors ≤ 10*

## Table S4: Primers used in RT-PCR

| Gene | Sequence of primers | Amplicon size |
|---|---|---|
| LasA_FP | CGACCTCGAGGCCTACCT | 102 |
| LasA_RP | AACACTTTCGGGTTGATGCT | |
| LasB_FP | GTCATCGACGCCAAGACC | 162 |
| LasB_RP | GTTGCCGTCGTCCATCTC | |
| RhlA_FP | CATTTCAACGTGGTGCTGTT | 173 |
| RhlA_RP | AGCAGCGTGGAGATACCG | |
| RhlB_FP | ATCGCTCACGAGAAGTACGG | 161 |
| RhlB_RP | TTGAAGCGCTCGATGCAG | |
| PqsA_FP | CCTGTTCTTTCCCTGGTTCA | 169 |
| PqsA_RP | CACGCTGCTCAACAGCTC | |
| rpoD_FP | AGAAGGCCCTGAAGAAGCAC | 182 |
| rpoD_RP | CACGCAGAGCTGCATGAT | |