Target Accumulation of Selective Anticancer Depsipeptides by Reconstructing Precursor Supply in Neoantimycins Biosynthetic Pathway

Lin Zhou
Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital
Yaoyao Shen
Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital
Nannan Chen
Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital
Wanlu Li
Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital
Hou-wen Lin
Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital
Yongjun Zhou (zhouyongjun66@163.com)
Shanghai Jiao Tong University School of Medicine Affiliated Renji Hospital https://orcid.org/0000-0002-3005-2843

Research

Keywords: anticancer, depsipeptides, natural products biosynthesis, 3-hydroxybenzoate, 3-formamidosalicylate, chorismic acid

Posted Date: March 5th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-269613/v1

Abstract

Background

Neoantimycins are a group of 15-membered ring depsipeptides isolated from streptomycetes with a broad-spectrum of anticancer activities. Their biosynthesis is directed by the hybrid multimodular megaenzymes of non-ribosomal peptide synthetase and polyketide synthase. We have previously discovered a new neoantimycin analogue unantimycin B, which was demonstrated with selective anticancer activities and was produced from neoantimycins biosynthetic pathway with a starter unit of 3-hydroxybenzoate, instead of the 3-formamidosalicylate for neoantimycins. However, the low fermentation yield and tough isolation procedure have been hindering in-depth pharmacology investigation of unantimycin B as anticancer agents.

Results

In the work, we genetically constructed two unantimycin B producer strains with neoantimycins production destroyed by removing natO and natJ-L genes essential for 3-formamidosalicylate biosynthesis and therefore facilitated chromatographic separation of unantimycin B from the complex fermentation extract. Based on the △natO mutant, we improved unantimycin B

production by two times, reaching to approximate 12.8 mg/L, by feeding 3-hydroxybenzoate in fermentation. Further, the production was improved by more than six times, reaching to approximate 40.0 mg/L, in the △natO strain introduced with a chorismatase gene highly expressed under a strong promoter for over-producing 3-hydroxybenzoate endogenously.

Conclusion

The work gives a case of targeting accumulation and significant production improvement of medicinally interesting natural products via genetically manipulation of precursor biosynthesis in streptomycetes, the talented producers of pharmaceutical molecules.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the latest manuscript can be downloaded and accessed as a PDF.

Figures

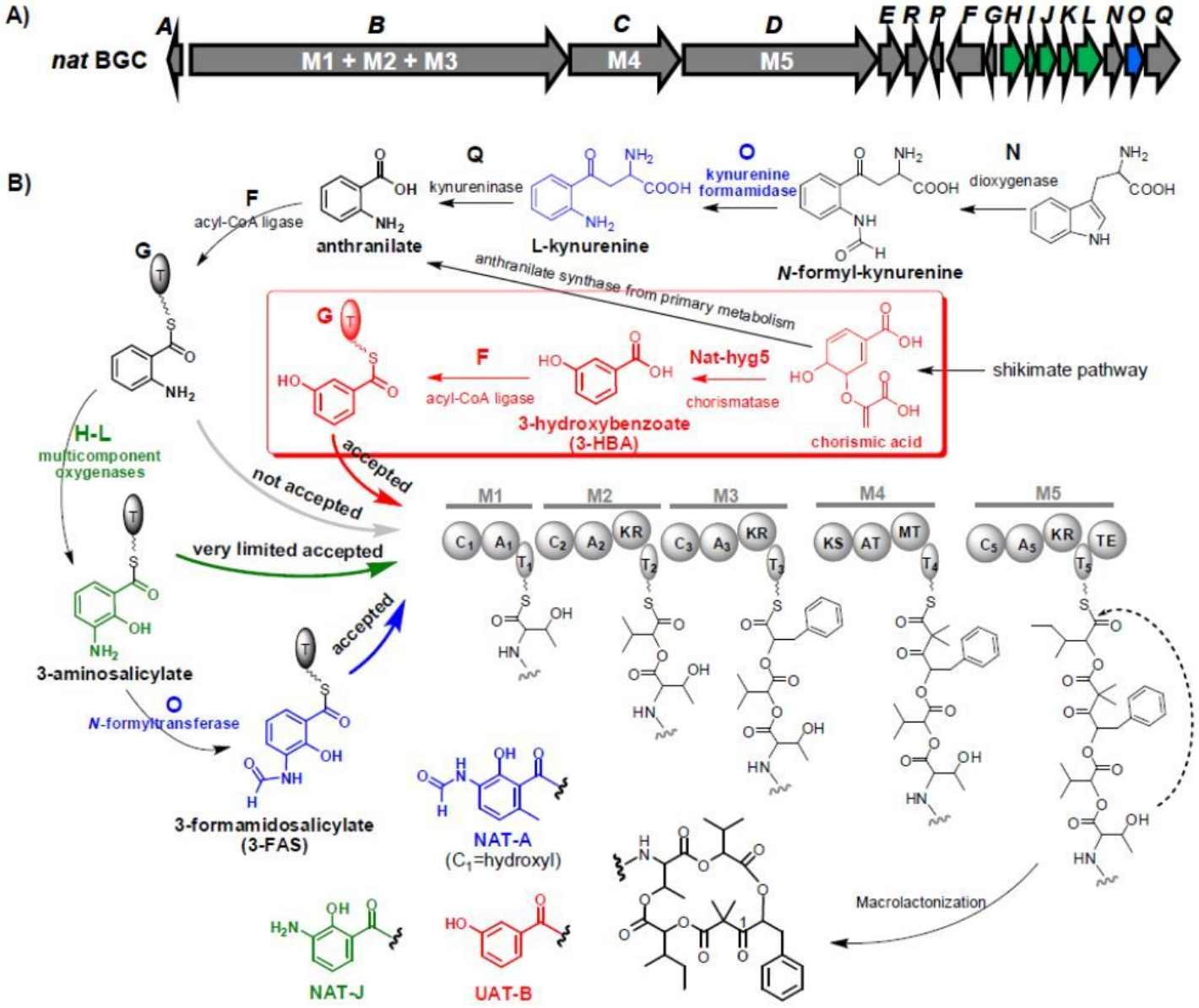

Figure 1

The gene organization of NATs biosynthetic gene cluster (nat BGC) (A) and the biosynthetic routes of starting precursor for NATs NRPS-PKS assembly line (B). Green and blue colors are respectively used to mark the natH-L and natO genes and their related products. The bio-reaction schemes of 3-hydroxybenzoate (3-HBA) generation and loading processes are highlighted with redness. A: adenylation; T: thiolation; C: condensation; KR: ketoreductase; KS: ketosynthase; AT: acyltransferase; MT: methyltransferase; ACP: acyl-carrier protein; TE: thioesterase.

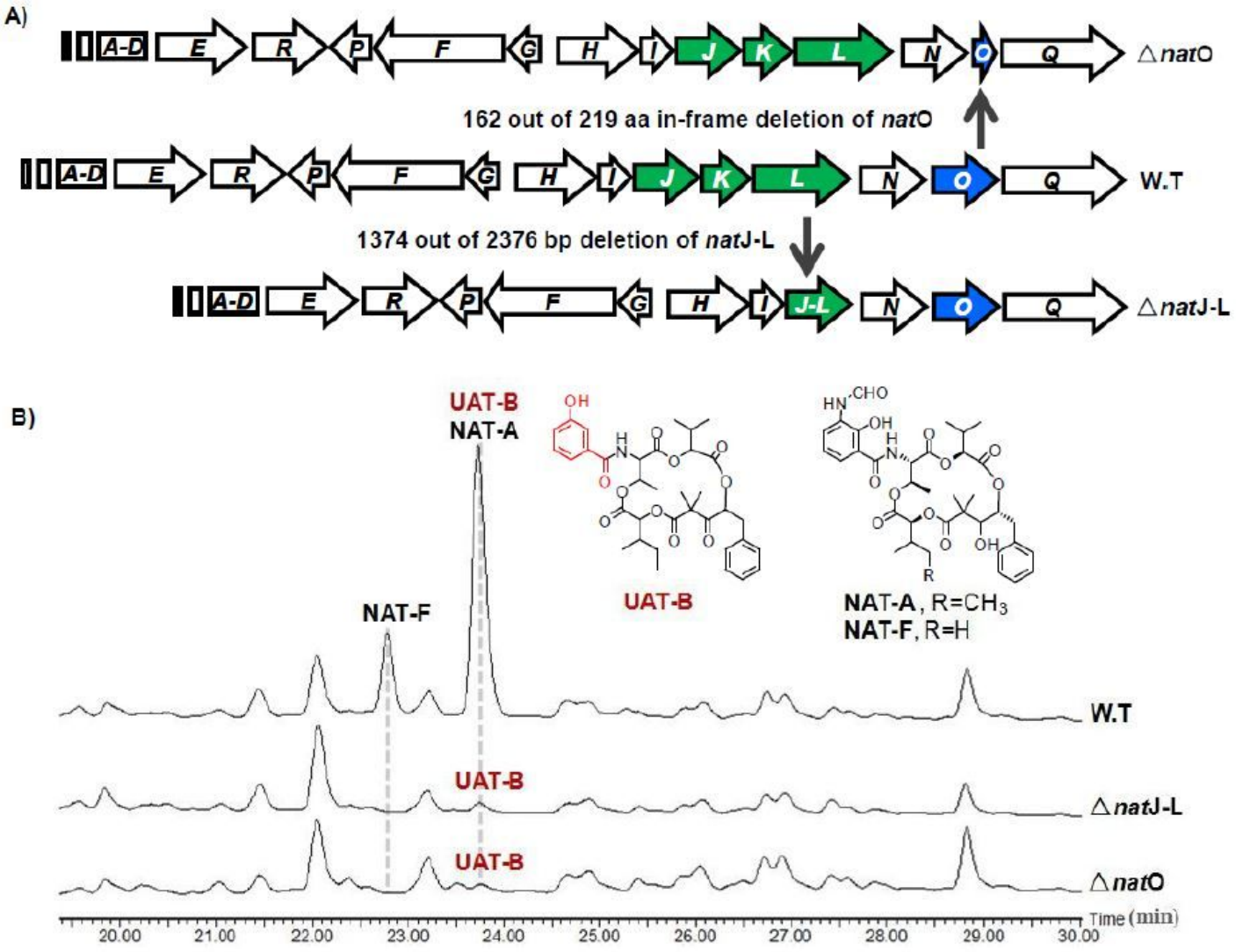

Figure 2

The schematic diagram of △natO and △natJ-L mutation generations (A) and HPLC analysis of UAT-B, NAT-A and NAT-B from the fermentation extracts of W.T (RJ2) strain and the mutants △natO and △natJ-L (B). The data is displayed with UV320 extraction.

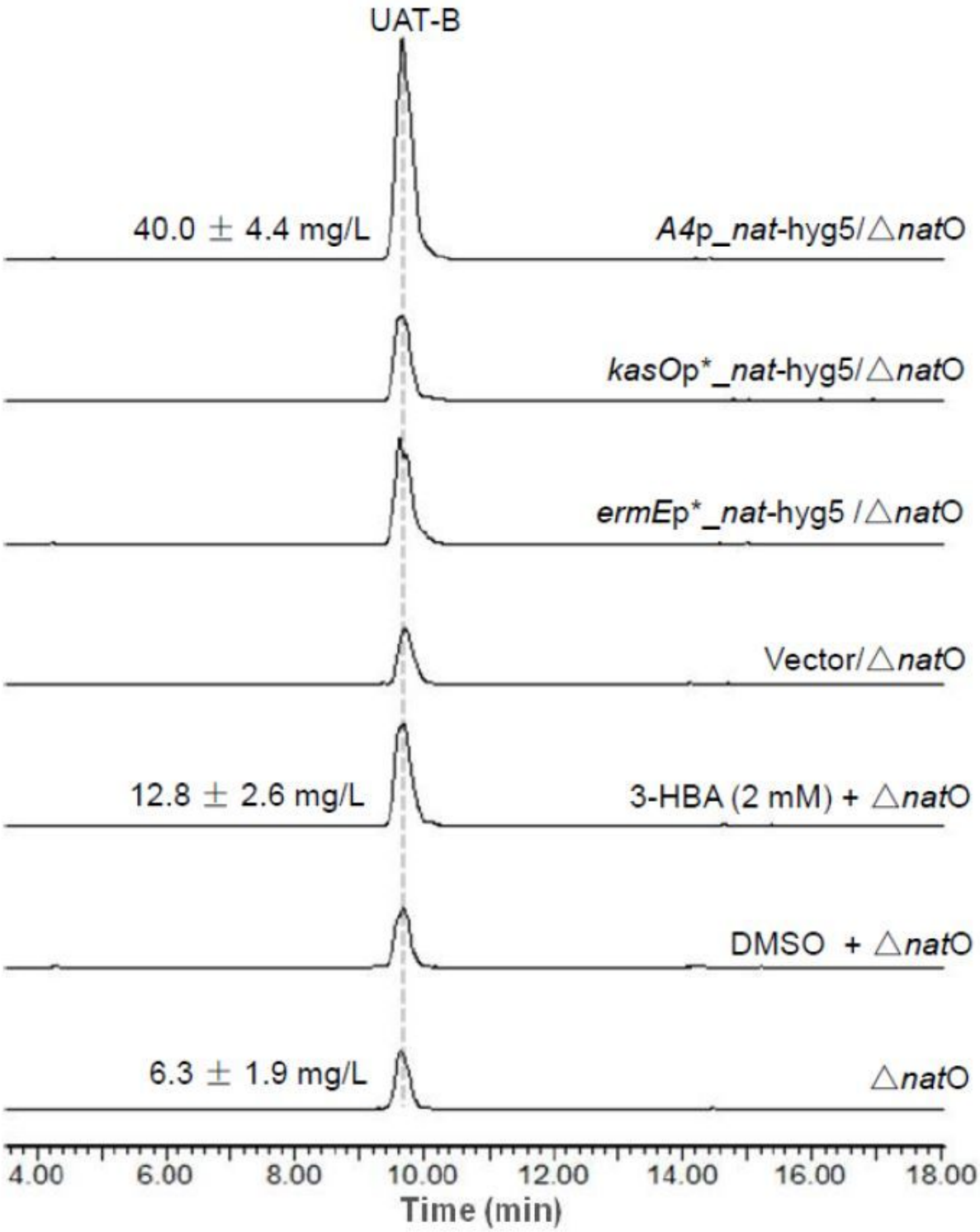

Figure 3

HPLC-MS analysis of UAT-B production in the fermentation extracts of △natO strain fed with 3-HBA or containing different nat-hyg3 gene expression cassette: A4p_nat-hyg5, kasOp*_nat-hyg5 or ermEp*_nat-hyg5. The HPLC-MS data is displayed with the mass extraction of [M+Na]+ m/z 676.4 for UAT-B. The exact UAT-B yields of selected samples were given by using UPLC-QqQ/MS analysis.

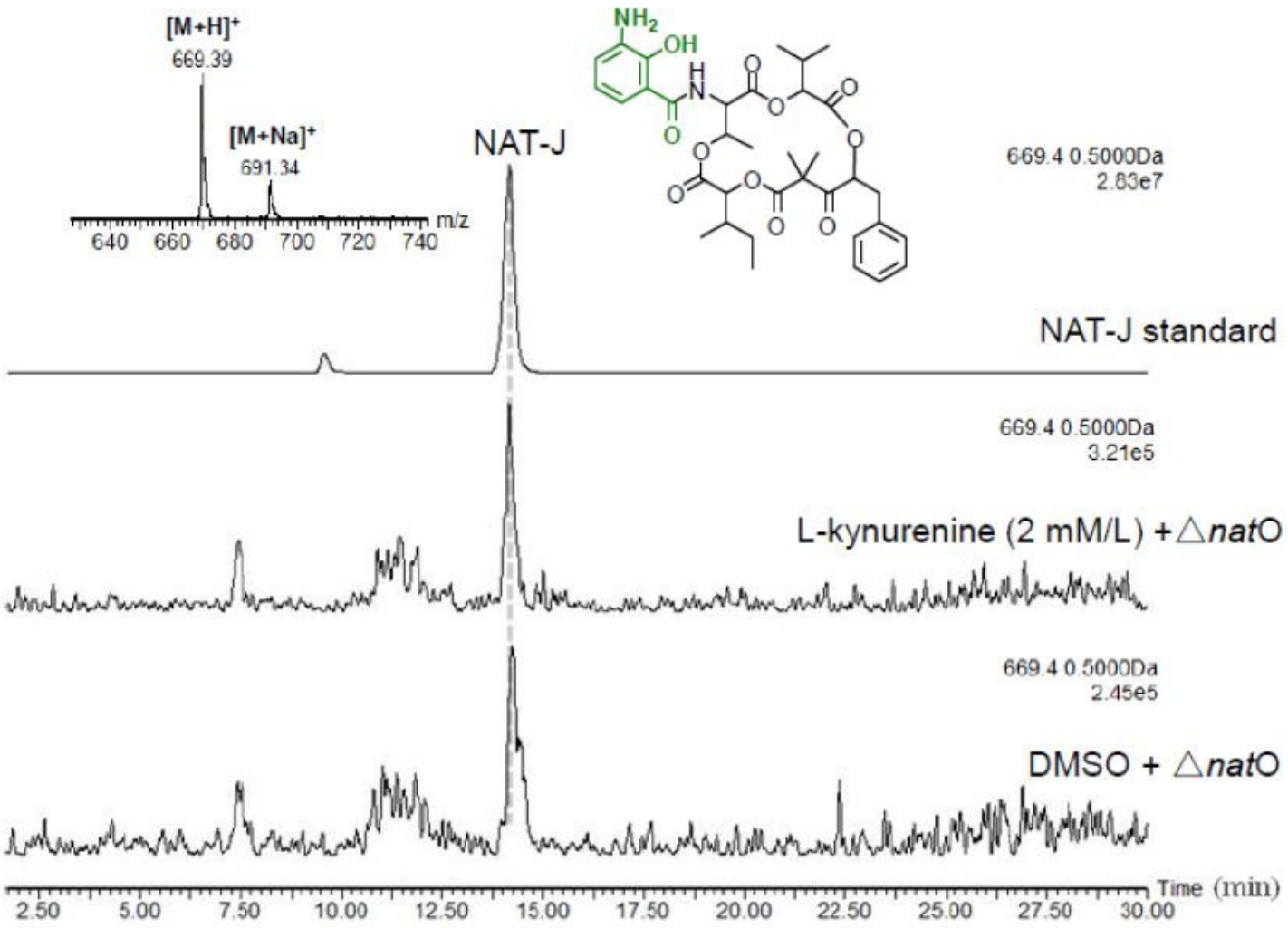

Figure 4

HPLC-MS analysis of NAT-J production in the fermentation extracts of △natO strain fed with L-kynurenine (2 mM) or DMSO. The HPLC-MS data is displayed with the mass extraction of [M+H]+ m/z 669.4 for NAT-J.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.pdf