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MOLECULAR ENGINEERING OF NOGALAMYCINES

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Actinomycetes are used to produce the majority of antibiotics applied in human and veterinary medicine and agriculture, as well as anti-parasitic agents, herbicides, pharmacologically active. Here we focused on biosynthesis of nogalamycin an anthracycline-like antitumor drug which is produced by *Streptomyces nogalater* Lv65. More specifically, genetic approaches have been used in order to produce modified nogalamycines which share the same activity as the parent compound at a more efficient level, with less toxicity, absence of cross resistance or activity against bacteria insensitive to the parent drugs.

Genes *snogM*, *snogL* and *snogY* disruption in *S. nogalater* chromosome was carried on and *S. nogalater* MI, LI and YI strains were generated. The gene replacement events were verified by Southern hybridization. Three novel compounds were isolated from M1, L1 and Y1 and their structures were elucidated using NMR spectroscopy and mass spectrometry. Furthermore, the mass spectra data reveal also the presence of three, two, and one deoxysugar moiety, respectively. Regarding the O-methyltransferases of the nogalamycin biosynthesis, it was found that methyltransferases SnogM, SnogL, SnogY were responsible for the nogalose moiety modification. Treatment with new modifications of nogalamycines led to stronger stimulation of stress pathways indicated by upregulation of P53, Cleavage PARP, Cleavage Caspase 3 and Cleavage Caspase 7. Especially at lower drug concentrations (5 nM), the new forms of nogalamycines increased the apoptosis-inducing potential comparing to doxorubicin (Dox) in HCT116 cells, Dox in such low concentration did not lead to enhanced cell death induction. Modificated nogalamycines have shown their effectively against P-gp and MRP1 types of cells drug-resistance.

Molecular biological engineering of nogalamycin is a promising tool for the rational design of natural products, and a number of new “unnatural” antibiotics have been generated by these attempts.