Metabolic profiling and biological potential of the marine sponge-associated *Nocardiopsis* sp. UR67 along with docking studies

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Abstract

This work was performed to dig into the phytochemical composition and bioactivities of *Nocardiopsis* sp. UR67 associated with the marine sponge *Callyspongia* sp. It was fermented in suspension and immobilized in calcium alginate bead cultures. The ethyl acetate extracts, afforded from the broth in each case named EG-49 and J-48g, respectively, revealed 16 chemical principles mostly belonging to polyketides, macrolides, and peptides. EG-49 and J-48g displayed anti-*Candida albicans* activity with IC_{50} values of 8.1 and 8.3 µg/mL, and a substantial cytotoxic effect against lung adenocarcinoma H1650 at IC_{50} 12.6 and 13.7 µg/mL, respectively. However, only EG-49 exhibited a noteworthy anti-trypanosomal activity at 7.5 µg/mL. Molecular docking of the characterized compounds against *Trypanosoma brucei* trypanothione reductase demonstrated the highest binding models of griseochelin-methyl ester (**9**) and filipin-II (**11**), which drew considerable significance of the metabolites derived from *Nocardiopsis* sp. UR67 developing potential *T. brucei* trypanothione reductase inhibitors.

Keywords: *Nocardiopsis*; marine sponges; metabolomics; anti-infective; cytotoxicity

1. Introduction

The rise of drug resistance of numerous pathogens beside the newly emerging infectious diseases together with the toxic effects of some currently used anti-tumor drugs entails searching for novel and more potent anti-infective and cytotoxic drugs (Woolhouse 2008). At least half of the antibiotics and anti-tumor agents approved by the Food and Drug Administration (FDA) have been natural products, derivatives of natural products or synthetic compounds inspired by natural product chemistry (Newman and Cragg 2020). In addition, microbial

fermentation continues to evolve and is now the preferred production method for many therapeutics, offering an optimal economic route for pharmaceutical companies (Lewis 2013).

Actinobacteria have shown intimate associations with diverse marine sources such as soft coral, sediments, sponges, fish, tunicates, seaweed, and seawater (Abdelmohsen et al. 2017, Chen et al. 2019, Shady et al. 2021, Sun et al. 2017, Sun et al. 2016, Tian et al. 2014, Xiang et al. 2020, Zhang et al. 2017, Zhao et al. 2019). In particular, Actinomycetes associated with marine sponges have shown enormous biodiversity indicating the opportunity to find new species and novel chemical scaffolds. Moreover, few studies have been performed to explore the actinobacterial communities from the Red Sea sponges (Abdelmohsen et al. 2015). Our previous investigation of this genus has led to the identification of a new cytotoxic cyclic hexapeptide, nocardiotide A, together with three known compounds tryptophan, kynurenic acid, and 4-amino-3-methoxy benzoic acid (Ibrahim, Attia, et al. 2018). Hence, the current study continues previous research and investigates the antimicrobial potential of the various extracts produced from different fermentation processes of *Nocardiopsis* sp. UR67, previously recovered from the marine sponge *Callyspongia* sp., against various pathogenic bacteria and fungi. It also examines their anti-trypanosomal and cytotoxic activities to best endorse its application in the treatment of various infections and appreciate its use as cytotoxic drugs. Additionally, metabolic profiling of these extracts, using LC–HR-ESI-MS, was carried out to explore the metabolites contributed to their biological activities along with in silico molecular docking of the characterized components as potential inhibitors of *Trypanosoma brucei* trypanothione reductase.

2. Results and Discussion

2.1.Metabolomic analysis

The dereplicated metabolites (**Fig. S1**) were identified by different databases by utilizing macros and algorithms that coupled MZmine with both in-house and commercial databases, particularly the DNP database along with high-resolution MS, RT, and UV data. The identified compounds have a large chemo-diversity, including mainly macrolides, peptides, and polyketides, in agreement with the isolated and identified compounds from that strain (Ibrahim, Desoukey, et al. 2018).

For instance, ansalactam A (**8**), a polyketide derivative, was corresponding to the mass ion peak at *m/z* 544.2698 $[M-H]^-$ for the predicted molecular formula $C_{33}H_{39}NO_6$ previously identified from *Streptomyces* sp. (Wilson et al. 2011), while 27-demethyl-25-desacetylrifamycin-SV (**12**), possessing antimicrobial activity, corresponded to a mass ion peak at *m/z* 642.2916 $[M+H]^+$ for the predicted molecular formula $C_{34}H_{43}NO_{11}$, previously identified from *Nocardia* sp. (Birner et al. 1972, Lancini and Sartori 1976). Moreover, other polyether derivatives were identified. The mass ion peak at *m/z* 583.4549 $[M+H]^+$ for the predicted molecular formula $C_{34}H_{62}O_7$ was dereplicated as griseochelin-methyl ester (**9**) obtained from *Streptomyces griseus* and had antiviral activity (Tonew et al. 1988).

Additionally, certain peptides were characterized, *i.e.*, the mass ion peak at *m/*z 427.3031 $[M+H]^+$ consistent with the molecular formula $C_{20}H_{38}N_6O_4$, which was dereplicated as leupeptin Ac (**7**), previously isolated from several *Streptomyces* sp. (Aoyagi et al. 1969), as well as, the mass ion peak at *m/z* 300.1344 $[M+H]^+$, in agreement with the molecular formula $C_{16}H_{17}N_3O_3$, was dereplicated as the peptide 3-*β*-hydroxy-cyclo-L-tryptophyl-L-proline (**3**), which was previously identified from *Streptomyces* sp. (Tang et al. 2001). On the other hand, the mass ion peak at *m/z* 639.4085 $[M+H]^+$ for the predicted molecular formula $C_{35}H_{58}O_{10}$, was dereplicated as the macrocyclic antifungal antibiotic filipin-II (**11**), which was isolated before from *Streptomyces filipinensis* (Bergy and Eble 1968, Richardson and Rychnovsky 1999) whereas, the mass ion peak at *m/z* 771.4875 $[M+H]^+$, corresponding to the predicted molecular formula $C_{41}H_{70}O_{13}$, was dereplicated as an anti-tumor substance, NK-154183A (**15**), obtained from *Streptomyces* sp. (Tsuchiya et al. 1996). Additionally, JBIR-67 (**2**), a benzastatin

derivative, was corresponding to mass ion peak at *m/z* 288.1598 $[M-H]^-$ for the predicted molecular formula $C_{17}H_{23}NO_3$ previously identified from *Streptomyces* sp. (Motohashi et al. 2011). Another mass ion peak at *m/z* 303.1341 $[M+H]^+$, corresponding to the molecular formula $C_{16}H_{18}N_2O_4$, was dereplicated as mitomycin-k (**4**), previously identified from *Streptomyces sandaensis* (Benbow et al. 1993) and possessed potent anti-tumor activity. It is worth mentioning that all these metabolites were characterized for the first time from the genus *Nocardiopsi*s. The chemical structures of various dereplicated metabolites of *Nocardiopsis* sp. UR67 are depicted in **Fig. 1**.

2.2.Anti-infective activities

Ethyl acetate (EtOAc) extracts resulted from the broth either follwing cultivation *Nocardiopsis sp*. UR67 either as planktonic suspension cultuture or immobilized in calcium alginate bead, named EG-49 and J-48 g, respectively, were tested against panel infective strains. Both extracts displayed antifungal activity against *Candida albicans* with IC_{50} values of 8.1 and 8.3 µg/mL, respectively. Lysocellin (**14**) (Liu et al. 1986), filipin-II (**11**) (Bittman et al. 1974), tetrangulol (**5**) (Vanga and Kaliappan 2012), having antifungal activity, were characterized by metabolomics analysis of *Nocardiopsis sp*. UR67. In addition, the EG-49 exhibited a significant anti-trypanosomal activity against *Trypanosoma brucei brucei* TC 221 with IC_{50} value of 7.5 µg/mL.

2.3.Cytotoxic activity

The results showed that both EG-49 and J-48 g demonstrated significant cytotoxic effect against lung adenocarcinoma H1650 cell line with the IC_{50} values of 13.7 and 12.6 µg/mL, respectively. Ansalactam A (**8**), dereplicated from *Nocardiopsis sp*. UR67 extract, belongs to ansamycins class of bacterial macrocyclic lactam polyketides (Wilson, Nam, Gulder, Kauffman, Jensen, Fenical and Moore 2011). The benzenic ansamycins as ansalactam A showed strong antitumor activities (Pacey et al. 2012), while naphthalenic ansamycins displayed strong antimicrobial activities (Wehrli and Staehelin 1971). Another dereplicated compound is NK-154183A (**15**) which has *in vitro* anti-tumor activity against the human colon adenocarcinoma (SW1116) and NIH Swiss mouse embryo cell (NIH3T3) (Tsuchiya, Kimura, Nishikawa, Harada, Nishikiori and Tatsuta 1996). In contrast, mitomycin-k (**4**), a member of the mitomycinoid class of natural products, exhibited potent cytotoxic activity (Benbow, McClure and Danishefsky 1993, Wilson and Danishefsky 2007).

2.4.Molecular docking

Molecular docking study of the 16 identified metabolites in the current study from *Nocardiopsis* sp. has been performed against *T. brucei* trypanothione reductase (TR) (Gamaleldin et al. 2020) present in *T. brucei brucei* to rationalize the obtained *in vitro* anti-trypanosomal activity against *T. brucei brucei*. The results of docking study scores are summarized in **Table S1**. In addition, the binding models were depicted for griseochelin-methyl ester (**9**) and filipin-II (**11**) in **Fig. S2** & **S3**, respectively.

3. Conclusion

Based on the aforementioned findings, LC-HR-MS-based analytical techniques allowed to study the chemical profile of marine sponge-derived *Nocardiopsis* sp. UR67, where the dereplicated metabolites were characterized for the first time from the genus. Moreover, fermentation of *Nocardiopsis* sp. UR67 by different methods demonstrated the potential to show anti-infective and cytotoxic activities. Hence, it unravels for new candidates treating resistant diseases.

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