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Total synthesis and biological evaluation of nannocystin analogues modified at the polyketide phenyl moiety

Yunfeng Tian, Yahui Ding, Xiaolong Xu, Yanju Bai, Yang Tang, Xin Hao, Weicheng Zhang *, Yue Chen *

The State Key Laboratory of Medicinal Chemical Biology, College of Pharmacy, and Tianjin Key Laboratory of Molecular Drug Research, Nankai University, Tianjin 300071, People's Republic of China

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ABSTRACT

Total synthesis of a focused set of 10 nannocystin analogues **3a**–**3j** modified at the polyketide phenyl moiety was reported. These compounds were evaluated against three cancer cell lines. Compared with the naturally occurring congener **3a**, the other synthetic variants either preserved or lose antiproliferative activity at varying degrees. Moreover, the potent analogues also displayed comparable levels of cytotoxicity toward two normal cell lines.

Nannocystin A (**1**) was initially isolated from the myxobacterial secondary metabolites [1] and later on reported in two side-by-side papers by Brönstrup et al. [2] and Hoepfner et al. [3] in 2015 (Fig. 1.). Its structure features a mixed polyketide-tripeptide scaffold bearing nine chiral centers, two continuous *E*-alkenes, and a unique α,β-epoxy amide. Biological studies [2,3] found strong and differential cytotoxicity of **1** across a broad range of cancer cell lines, with IC_{50} values ranging from sub-micromolar (0.5 μM) to low nanomolar (5 nM) levels. Through genetic and proteomic experiments, Hoepfner et al. deduced eukaryotic translation elongation factor 1A (eEF1A) as the most possible target [3]. This protein is primarily in charge of recruiting amino acyl-tRNA (aa-tRNA) in the presence of GTP to the ribosomal A site during the elongation step of protein synthesis [4]. Correct codon-anticodon recognition between the incoming aa-tRNA and the mRNA template activates eEF1A to catalyze the hydrolysis of GTP. The resulting GDP and eEF1A dissociate from the aa-tRNA-loaded ribosome, with subsequent regeneration of a new eEF1A/GTP complex for the binding and delivery of the next proper aa-tRNA. Didemnin B, a known inhibitor of eEF1A, was found by Hoepfner et al. to effectively displace its target protein eEF1A off the nannocystin-based affinity matrix with similar level of competition as **1**, which pointed to a common binding region on the target surface shared by the two different molecules [3]. Recently, this opinion was corroborated by Gago et al. through molecular dynamics calculations, who proposed detailed molecular modeling structures to rationalize the high-affinity binding of four structurally distinct compounds, namely didemnin B, ternatin, ansatrienin B, and **1** to eEF1A at the presumably overlapping binding site [5]. Nevertheless, so far there is no systematic study to clarify in detail whether nannocystins such as **1** target the intracellular eEF1A alone, the GTP-bound eEF1A as in the case of didemnin B [6], or the ternary complex of eEF1A/GTP/aa-tRNA as found in the ternatin derivatives [7]. Elaborate knowledge on this issue will facilitate direct characterization of the on-target inhibitory effects of nannocystins.

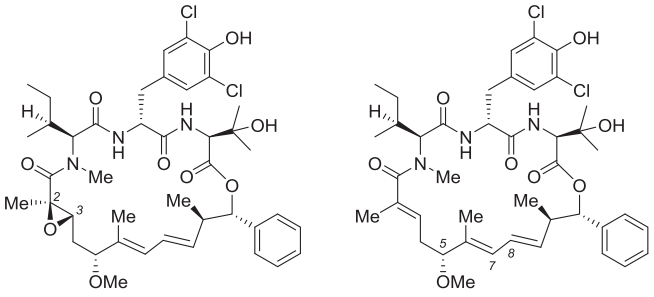

Fig. 1. Structures of naturally occurring nannocystin A (**1**) and 2,3-desoxy congener nannocystin Ax (**2**).

* Corresponding authors.
E-mail addresses: zhangweicheng@nankai.edu.cn (W. Zhang), yuechen@nankai.edu.cn (Y. Chen).

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Endowed with a novel structure and potentially intriguing mode of action [8], **1** immediately captured the attention of synthetic community. Indeed, soon after the two leading publications [2,3], several research teams have successfully achieved total synthesis of **1** or its 2,3-desoxy congener nannocystin Ax (**2**) via different routes [9–15]. These works have not only established a diversity of synthetic accesses to the natural nannocystins alongside the fermentation approach, but also enabled the indispensable tool of site-selective structure permutation in order to modulate ligand/protein interactions and, if necessary, optimize the various critical drug-like physicochemical properties. For instance, a "motif-oriented" strategy was adopted by Fürstner et al. to prepare a collection of **2** and other 10 non-natural nannocystins and probe the SAR of the trisubstitued 6,7-alkene and the proximate 5-methyl ether region [15]. While the alkene moiety was tolerant of moderate structural variation (the permissive site), the neighboring methyl ether had to be retained for high potency (the prohibitive site).

Parallel to Fürstner's work, we recently synthesized a focused set of 10 nannocystin analogues including **1**, **2**, and **3a** [16]. This study was aimed at understanding the possible binding role of the (2*R*,3*S*)-epoxide in **1**. Biological assay, in combination with variable temperature NMR experiments and computational studies, suggested that the epoxide unit is not likely to interact directly with the target binding site, but serves to control the macrocycle conformation. In the docking study [16], two pockets I and II were identified to accommodate the side chain of the β-OH-L-Val^3 residue and the polyketide C-11 phenyl group of **3a** respectively (Fig. 2). Pocket I appears to be hydrophobic in nature: mutation at the β-OH-L-Val^3 fragment with an L-valine had a marginal effect on potency [16], whereas substitution at this position for an L-serine caused a marked drop in activity [17]. Thus it is the two methyl groups rather than the hydroxyl group within the side chain of the β-OH-L-Val^3 segment that accounted for favorable binding to the target. Given the importance of hydrophobic interactions envisioned in the eEF1A-nannocystin binding process [3,5,16], it is a natural move for us to investigate the influence of the C-11 phenyl moiety, which was assumed to interact with the neighboring pocket II according to the docking analysis [16], on the cytotoxicity of nannocystins. Hence we chose the bromo-substituted natural congener **3a** as the reference compound [18] and further designed 9 non-natural nannocystins **3b**-**3j** modified at the C-11 phenyl part. Total synthesis of these compounds combined with biological evaluation at the cellular level would establish a structure–cytotoxicity correlation regarding this subunit, which could help with elucidating the nannocystin pharmacophore essential for its activity.

Syntheses of **3a**-**3j** were carried out in a straightforward manner following our previous total synthesis [11] of **1** (Scheme 1). Thus chiral *anti* aldols **5a**-**5j** were first prepared by converting optically pure *N*-propionylsultam **4** to an *O*-silyl-*N*,*O*-ketene acetal intermediate, followed by $TiCl_4$-mediated stereoselective aldol condensation with different aryl aldehydes [19]. Protection of the alcohols as TBS ethers, cleavage of the sultam auxiliary, and then Wittig olefination afforded terminal alkenes **8a**-**8j**, which were further deprotected and coupled to a common intermediate **13** [11] via Mitsunobu reaction leading to Boc-protected intermediates **10a**-**10j**, respectively. After removal of the Boc group, the resulting products **11a**-**11j** were condensed with another common intermediate **14** [16] and finally underwent intramolecular Heck macrocyclization to yield the designed analogues **3a**-**3j** respectively.

The cytotoxicity of **3a**-**3j** was examined in three typical cancer cell lines HCT116, HepG2, and PANC1. To assess the safety profile of these compounds, two normal cell lines FHC and LO2 were also subjected to the test. As shown in Table 1, differential activity was observed in all potent nannocystin analogues (**3a**-**3e**, **3h**-**3j**) across the three cancer cell lines, with the general order of potency being HCT116 > HepG2 > PANC1. In terms of selective cytotoxicity toward cancerous cells over normal cells, however, each of the potent analogues (**3a**-**3e**, **3h**-**3j**) also inhibited the growth of the noncancerous FHC and LO2 cell lines with comparable activity (IC_{50} values ranging from 2.13 nM to 16.95 nM for FHC, from 4.72 nM to 148.8 nM for LO2). This observation is reminiscent of an earlier study by Brönstrup et al., [2] wherein strong inhibitory activity of **1** was manifested against not only several cancer cell lines (IC_{50} values ranging from 1.0 nM to 12 nM) but also quiescent peripheral blood lymphocytes (IC_{50} 11 nM). These data collectively indicate nannocystins as strong antiproliferative compounds against both cancerous and normal cells, a result probably stemming from their underlying mechanism of action [3] that globally blocks the eukaryotic cell protein synthesis at the elongation stage. Hence much effort is in demand in the future to address the cytotoxic selectivity issue associated with nannocystins before they could be evolved into therapeutically useful antineoplastic agents [20].

Also found in the assay is a direct correlation between the structural permutation at the nannocystin C-11 phenyl moiety and the corresponding cytotoxicity toward each individual cancer cell line. With regard to the human colon carcinoma HCT116, for instance, a *para* fluoro (**3b**) or chloro (**3c**) substitution at the benzene ring gave equipotent activity (IC_{50} 1.00 nM for **3b**, 1.56 nM for **3c**) as compared with that of the control compound **3a** (IC_{50} 1.59 nM). Further increase in the bulkiness of the *para* substituent (Br for **3d**, CF_3 for **3e**), shuffling the fluoro or chloro group from *para* to *meta* position

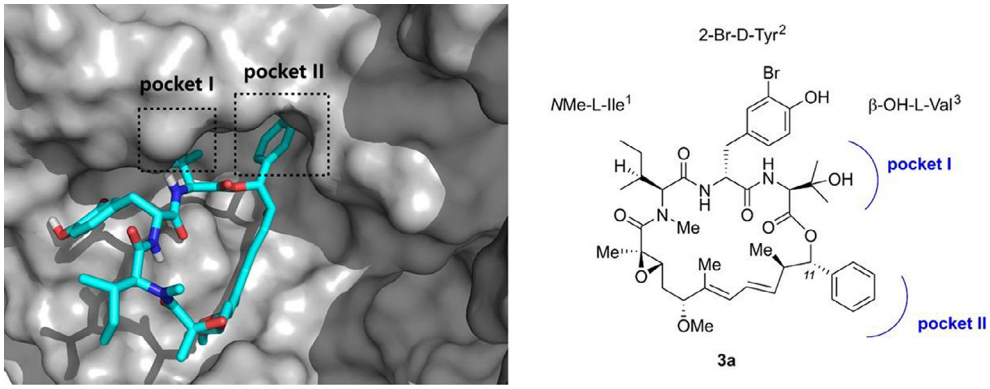

Fig. 2. Our previous study on in silico docking of the natural congener **3a** into the binding site of eEF1A identified two pockets I and II, which accommodate the side chain of the β-OH-L-Val^3 residue and the polyketide C-11 phenyl group respectively [16].