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Design and synthesis of a new class of cryptophycins based tubulin inhibitors

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ABSTRACT

Tubulin binding compounds represent one of the most attractive targets for anticancer drug development. They broadly fall into two categories viz., tubulin polymerization inhibitors, which block microtubule growth and destabilize microtubules like vinca alkaloids and cryptophycins, and the others, which polymerize microtubules into hyperstable forms represented by family of taxanes. In this context, we aimed at design and synthesis of cryptophycins based macrocyclic depsipeptides, which are synthetically more accessible, however have the basic information to target tubulins and establish structure activity relationship (SAR). Thus, a new class of cryptophycins based marocyclic depsipeptides with a truncated epoxide chain were synthesized as potential tubulin inhibitors. The resultant lead analogues **15a** and **16a** exhibited good anti-cancer activity, induced apoptosis, caused block/delay in cell cycle as well as significantly reduced the expression of α- and β-tubulins. Molecular modelling studies show that **15a** and **16a** bind in the same domain as that of cryptophycins.

1. Introduction

Cryptophycins, a group of macrocyclic depsipeptides produced by cyanobacteria of the genus *Nostoc* [1], like colchicine, combretastatins and vinca alkaloids inhibit the polymerization of tubulin by blocking microtubule growth and destabilizing them [2]. They are the most potent compounds known in the dynamics of tubulin and display remarkable cytotoxicity against multi drug resistance cancer cell lines. They had been a subject of extensive research in past, prompting myriad total synthesis [3,4] and structural modifications [5]. The result of these efforts was the development of cryptophycin-52 (C-52), a synthetic analogue of cryptophycins, which reached phase 2 clinical trials [6,7]. Studies continued to develop new cryptophycin analogues through synthetic or biotechnological methods [8,9] and recently Sanofi-Aventis published two patents on cryptophycin antibody-drug conjugates (ADCs) for treatment of lung cancer [10]. Notably, the cryptophycins-ADCs had IC_{50} value as high as 0.710 nM, which is way higher than the parent analogues having IC_{50} values as low as 10 pM. This implies that reducing the toxicities of these molecules is desirable; even if it means they have high IC_{50} values, as such molecules might be better option for targeted drug delivery and may well decipher new insights into their mechanism of action.

Thus, in our continued efforts to develop novel anti-cancer leads [11,12], we initiated a program directed toward the synthesis of macrocylic depsipeptides based on cryptophycins with an aim to find minimal structure for tubulin inhibition and hence, establish structure activity relationship (SAR). Though the SAR [5] of cryptophycins is largely known, but still to the best of our knowledge none of the studies have so far reported analogues with a truncated epoxide chain. However, the importance of epoxide moiety has been established by comparing its activity with analogues having aziridine, thiirane, olefin, chlorohydrin and dihydorxyl moieties instead [5]. Thus, we proposed two structural variants **1** and **2** sans epoxide chain. The structure **1** has a shorter carbon side chain (pruned epoxide moiety), whereas, **2** in addition has variable stereochemistry at C-10, to understand its effect on tubulin inhibition as naturally occurring cryptophycin-46 has L-tyrosine at C-10 position (Fig. 1). Retrosynthetic disconnection of **1** or **2** shows that they can be assembled from ester **7** (northern half) and peptide **11**

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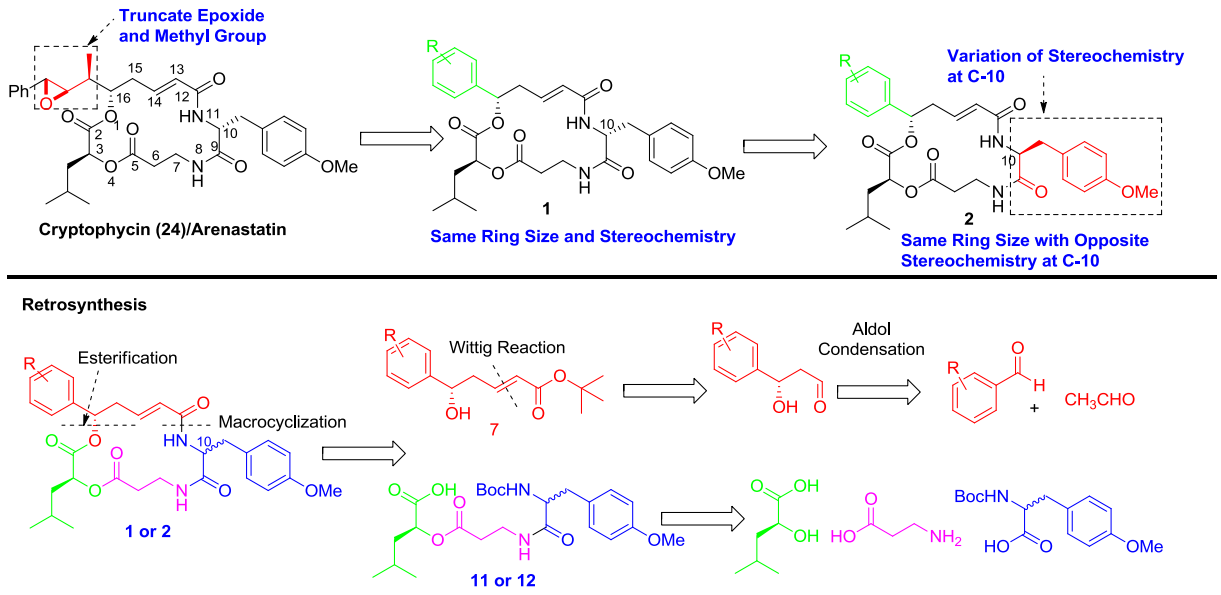

Fig. 1. Approach to simplified analogues of Cryptophycins.

or **12** (southern half). Further disconnection of **11** or **12** reveals that the fragment comprises of three segments *i.e.*, leucic acid, β-alanine and *D*-O-methyltyrosine. The ester **7** can be synthesized by Wittig reaction of β-hydroxy aldehyde. However, the bottleneck of the synthesis was the synthesis of β-hydroxy aldehyde, which as it turns out required the use of acetaldehyde in cross aldol condensation, deemed impossible until recently owing to large number of side product formations [13]. This may have been a reason that analogues without epoxide chain haven't been reported so far. We reasoned that our method employing vinyl acetate as a precursor to acetaldehyde to access β-hydroxy aldehyde could prove handy [14,15].

2. Results and discussion

2.1. Chemistry

Thus, our efforts commenced with the synthesis of northern half from cross aldol product of acetaldehyde (Scheme 1). To synthesize the enantioselective cross-aldol product we followed our methodology wherein the reaction involves lipase-catalysed *in situ* generation of acetaldehyde from vinyl acetate which in presence of organocatalyst (*R*-α,α-Bis[3,5-bis (trifluormethyl) phenyl]-2-pyrolidinemethanol) reacts with aromatic aldehydes to give highly enantiopure cross-aldol products **4a**–**f** [14,15]. The hydroxy groups of cross aldol products were protected as their TBS ethers using TBDMSCl and imidazole in DCM at 0 °C to give **5a**–**f**. The primary alcohols in **5a**–**f** were selectively deprotected using HF-Pyridine in THF, followed by oxidation with Dess-Martin periodinane in DCM to give **6a**–**f**, which were then subjected to Wittig reaction with (tert-butoxycarbonylmethylene) triphenylphosphorane in DCM, followed by deprotection of secondary alcohol using TBAF in THF resulting in synthesis of the northern halves **7a**–**f**.

We then focused on construction of southern half. Thus, we started with the synthesis of L-leucic acid from L-leucine using $NaNO_2$ and H_2SO_4, which was *O*-benzylated by treatment with Cs_2CO_3 in MeOH/H_2O (1:1) at room temperature for 30 min, followed by removal of the solvents and subsequent reaction with BnBr in DMF at 0 °C to room temperature for 12 h to give benzyl (−)-2-hydroxyisocaproate **9**. The compound **9** was coupled to N-Boc-β−alanine using EDCI and DMAP in DCM at room temperature for 12 h, resulting in the synthesis of diester **10**. The resultant diester **10** was deprotected using TFA in DCM to give the free amine, which was then condensed with N-Boc-O-methyl-D-tyrosine and N-Boc-O-methyl-L-tyrosine using EDCI, DMAP and HOBt followed by debenzylation via hydrogenation in the presence of 5% Pd/C under H_2 atmosphere in ethyl acetate to obtain southern halves **11** and **12** respectively. The coupling of **11** and **12** with **7a**–**f** were carried out through esterification using DIC and DMAP in DCM to give **13a**–**f** and **14a**–**f** respectively, thus setting the stage for final ring closure via macrolactamization. The Boc protecting group and tert-butyl ester of each coupled products **13a**–**f** and **14a**–**f** were cleaved in a single step with TFA and the resultant amines and carboxylic acids were macrocyclized using HBTU and Hunig's base in acetonitrile to afford macrocyclic depsipeptides 15a–f and 16a–f respectively (Scheme 1).

2.2. Biological evaluation

The synthesized depsipeptides were then screened for cell viability assay against human cancer cell lines; PC-3, HeLa and A549 (Table 1). Out of 12 molecules, two molecules, **15a** & **16a** displayed low IC_{50} value in A549 cells viz., 10 and 11 μM, respectively, whereas **15a** was found to be more cytotoxic when compared to **16a** in PC-3 and HeLa cells. Also, it was observed that the compounds show selective cytotoxicity against human cancer cell lines as their IC_{50} values in FR-2 (human normal breast epithelial) cells were considerably high. As both the compounds **15a** & **16a** showed relative low IC_{50} in A549 cell, so we chose this cell line for further studies at different concentrations Fig. 2.

Apoptotic bodies' formation is a characteristic feature of cells undergoing apoptosis [16]. These are membrane enclosed vesicles consisting of damaged organelles and DNA. Induction of apoptotic bodies by chemotherapeutic agents has always been a preferred choice in developing anti-cancer therapeutics. Apoptosis induction by **15a** and **16a** were analysed by visualizing cellular and nuclear morphology through phase contrast microscopy and Hoechst staining. Phase contrast microscopy of **15a** and **16a** treated cells showed cellular blebs, cell wall deformation, shrinkage of cell size, and formation of multiple apoptotic bodies, which were shown by