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Research paper

Synthesis and cytotoxicity of a novel series of saframycin-ecteinascidin analogs containing tetrahydro-β-carboline moieties

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ABSTRACT

A novel bistetrahydrocarboline heptacyclic skeleton and its twenty derivatives were prepared from L-tryptophan through a 15-step stereospecific route. The cytotoxicities of these compounds were tested against six human cancer cell lines including HCT-116, HepG2, BGC-823, MCF-7, A2780, and HT-29. Most of the derivatives with amide side chain exhibited the IC_{50} values at the level of 10^{-7} M, and a preliminary structure-activity relationship (SAR) was discussed. Both compound **30** with 2-pyridine amide side chain and compound **14** with phthalamide side chain showed the most potent and broad cytotoxicity towards all six cell lines with the IC_{50} values at the level of 10^{-8} M. Molecular docking of compound **30** indicated it bound to minor groove of DNA duplex.

1. Introduction

Natural products of the tetrahydroisoquinoline family and their simplified analogs have attracted considerable attention due to their unique structures and potent biological activities [1]. Ecteinascidin 743 (Et-743) displays the most potent cytotoxic activity against a variety of tumor cells [2,3] and has been used to treat soft tissue sarcoma [4] and ovarian cancer [5] in Europe. Pt-650, with simplified chemical structure, shows comparable antitumor activity to that of Et-743 [6] (see Fig. 1). The bistetrahydroisoquinoline skeleton with hemiaminal or aminonitrile at C-21 position is arguably believed to be the pharmacophore [7,8]. Besides, the topological configuration and the length of the molecular skeleton are thought to have great influence on their interaction with DNA and other biomacromolecules [1]. Indeed, studies show that the decrease of cytotoxic activities of the bistetrahydroisoquinoline analogs may be attributed to the changes of topological configuration and the length of the molecular skeleton [9–11].

The tetrahydro-β-carboline (THβC) ring system occurred in many indole alkaloids and showed widespread biological properties [12]. Furthermore, the tetrahydro-β-carboline (THβC) and the tetrahydroisoquinoline (THIQ) share the similar chemical structure and biosynthetic pathway (the Pictet-Spengler reaction) [1,13]. Thus, inspired by the plausible biosynthetic pathway of the bistetrahydroisoquinoline alkaloids, we decide to introduce two tetrahydro-β-carboline rings to replace the tetrahydroisoquinoline structure with the aim of elongating the pentacyclic skeleton of the natural products to a heptacyclic system, which will be helpful to further understand the mechanism and structure-activity relationship of the bistetrahydroisoquinoline alkaloids.

In this paper, a novel bistetrahydrocarboline skeleton and its twenty derivatives were prepared from L-tryptophan and the cytotoxicities of these compounds were tested against six human cancer cell lines including HCT-116, HepG2, BGC-823, MCF-7, A2780, and HT-29.

2. Chemistry

The synthetic route basically follows our previous one of synthesizing simplified ecteinascidin-saframycin analogs [7d]. L-tryptophan methyl ester **1** was subjected to Pictet–Spengler reaction using benzyloxyacetaldehyde in TFA at 0 °C to provide 1,3-*cis* 1,2,3,4-tetrahydrocarboline **2a** in 65% yield. The absolute configurations of *cis*- and *trans*-diastereoisomers were confirmed by NOE method. Obvious NOE enhancement was observed between H-1 (δ 4.43) and H-3 (δ 3.84), thus a *cis*-1,3-diaxial relationship was

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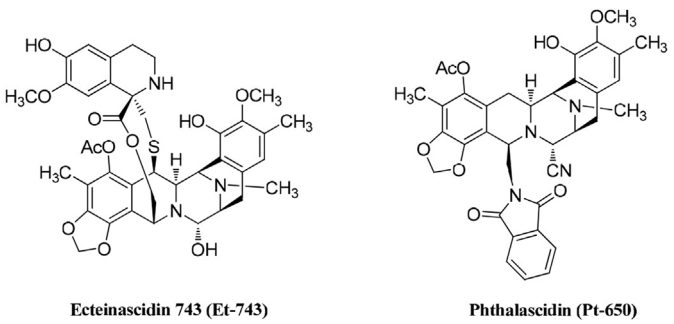


Fig. 1. Structures of Et-743, phthalascidin.

confirmed. The two diastereoisomers were separated by column chromatography, and the ratio between *cis*- and *trans*-diastereoisomers was about 2.7:1. Ester **2a** was reduced to the corresponding alcohol **3** by $LiAlH_4$ in 70% yield, which was subsequently silylated by tert-butyldimethylsilyl chloride (TBSCl) to afford the 1,3-*cis* 1,2,3,4-tetrahydrocarboline precursor **4** (Scheme 1). Compound **4** was then coupled with **5** through the action of bis(2-oxo-3-oxazolidinyl) phosphoric chloride (BOPCl) to afford amide **6**. Then, protection of the indolyl nitrogens of **6** by the Boc-group afforded **7**. The TBS ether of amide **7** was cleaved to provide the primary alcohol **8**. Compound **8** was oxidized to the corresponding hemiaminal **9** via Swern oxidation. The hemiaminal **9** was subjected to intramolecular Pictet–Spengler cyclization using CF_3CO_2H at room temperature, and the expected heptacyclic intermediate **10** was obtained in a moderate yield with three Boc-groups being removed simultaneously. The heptacyclic intermediate **10** possessed correct stereocenters as those of the natural products, which was confirmed by NOE method. Obvious NOE enhancement was observed between H-3 (δ 4.01) and H-14 (δ 4.33), thus the *syn* C3–C14 backbone stereochemical relationship was established. Reductive methylation of compound **10** with HCHO afforded compound **11**. The O-benzyl group of compound **11** was removed by catalytic hydrogenation. Then the lactam ring of **12** could be easily reduced through treatment with an excess of $LiAlH_4$ in THF at −17 °C and then 0 °C for l h to the corresponding cyclic hemiaminal intermediate, which upon exposure to TMSCN and BF_3-Et_2O in methylene chloride at −30 °C afforded the heptacyclic amino nitrile **13** as an enantiomerically pure product. Mitsunobu reaction was used to transform the primary alcohol to the corresponding amine through the intermediate **14**. Removal of the phthalimide of **14** with N_2H_4-H_2O in C_2H_5OH at 50 °C provided amine precursor **15**, which was acylated with different acids to afford the corresponding target amides **16**–**34** (Scheme 2). All the structures of the analogs were determined by ^1H, ^{13}C NMR, and FAB-MS.

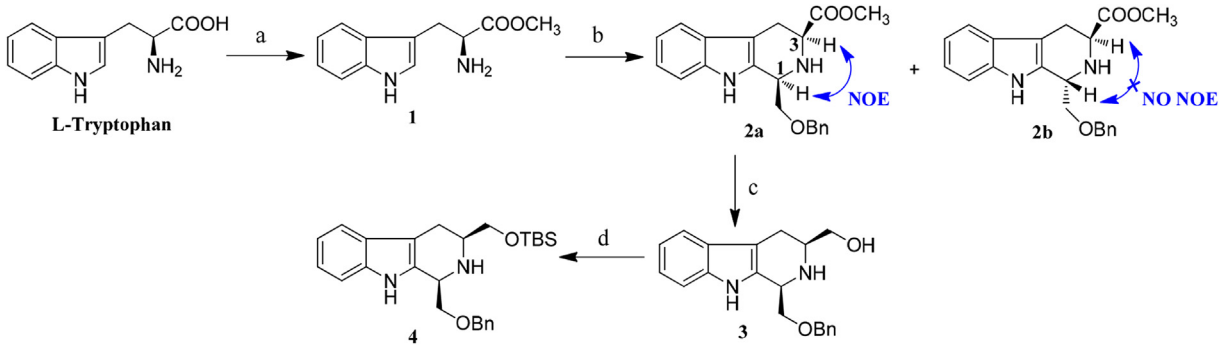
3. Cytotoxicity

Cytotoxicity of this series of bistetrahydrocarboline analogs was evaluated against six human cell lines: HCT-116 (human colon cancer cell line), HepG2 (human liver cancer cell line), BGC-823 (human gastric adenocarcinoma cell line), MCF-7 (human breast cancer cell line), A2780 (human ovarian cancer cell line), HT-29 (human colon adenocarcinoma cell line) by the standard MTT assay. The results are shown in Table 1.

4. Results and discussion

As shown in Table 1, most of the compounds exhibited considerable cytotoxicities to these six cell lines. Compounds **16**–**21** with different substituted benzamide side chain exhibited potent cytotoxicities with the IC_{50} values at the level of 10^{-7} M. There is no difference in inhibitory activity between benzoic acids with electron-donating groups or those with electron-withdrawing groups. In contrast, compounds **22** and **23** with sterically hindered aromatic amide side chain showed decreased cytotoxicity. Among the two five-membered heterocyclic aromatic acid derivatives (**28** and **29**), the inhibitory activities were similar to their corresponding 2-benzofuran and 2-benzothiophene derivatives (**26** and **25**), while compound **25** with 2-benzothiophene amide side chain showed strong selectivity towards A2780 with the IC_{50} value of 20 nM. Among all the twenty compounds, 2-pyridine derivative **30** exhibited broad and potent cytotoxic activity towards all six cell lines with the IC_{50} values at the level of 10^{-8} M, while 2-benzopyridine derivative **24** showed decreased cytotoxicity. Four compounds with cinnamic amide (**31**–**33**) and acetamide (**34**) side chain showed rather weak cytotoxicity against most of the cell lines. Compound **14** was found to be another one which had virtually identical cytotoxic potency with compound **30** against HCT-116, HepG2, A2780 and HT-29 with the IC_{50} values at the level of 10^{-8} M. The possible reason for their potent cytotoxicity seems to be the relatively strong hydrogen bond forming ability of compound **30** and **14** with the related biological targets because the C-22 pyridine and phthalimide moieties in these two compounds are good hydrogen bond acceptors. Besides, the low electronic density of the pyridine and phthalimide structures may be another structural reason for their potent cytotoxicity. Finally it is interesting to notice that compound **14** has the same C-22 phthalimide appendage as phthalascidin (Pt-650), which is a simplified analogue of Et-743 with very potent cytotoxicity).

The molecular simulation studies of compound **30** were shown



Scheme 1. Reagents and conditions: (a) $SOCl_2$, MeOH, reflux; (b) $BnOCH_2CHO$, TFA, CH_2Cl_2, 0 °C; (c) $LiAlH_4$, THF, 0 °C; (d) TBSCl, Imidazole, CH_2Cl_2, rt.