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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1282–1285

Synthesis and antitumor activity of simplified ecteinascidin–saframycin analogs

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Received 24 October 2005; revised 16 November 2005; accepted 18 November 2005
Available online 9 December 2005

Abstract—Two series of simplified analogs of the ecteinascidin–saframycin type alkaloids were prepared from L-DOPA. Their in vitro antitumor activity was tested against three human cancer cell lines (HCT-8 colon carcinoma, Bel-7402 liver carcinoma, and BGC-823 gastric carcinoma). Among these compounds, the ester analogs have stronger activities than those of amide analogs in general. Among them, 1-naphthalene carboxylate ester analog **31** has the strongest activity against BGC-823 cells.

Ecteinascidin 743 (Et 743, **1**), a highly promising, exceedingly potent antitumor agent, isolated from extracts of the marine tunicate *Ecteinascidin turbinate*,[1] is currently in phase II/III clinical trials.[2] The novel structure of Et 743 combined with its remarkable biological activities and the lack of availability from natural sources has made it an attractive synthetic target. The first total synthesis of Et 743 was accomplished by Corey et al.[3] In 2000, a semisynthesis of Et 743 from cyanosafracin B was described.[4] And in 2002, another total synthesis of Et 743 was accomplished by Fukuyama and coworkers.[5]

In addition to the natural product, several structurally simplified Et 743 analogs[6] were synthesized for the preliminary structure–activity relationship (SAR) study. One of them, phthalascidin (Pt 650, **2**), was found to exhibit comparable antitumor activity to that of Et 743 and may be a more practical therapeutic agent.[6] In view of the structural feature, the cores of both **1** and **2** are characterized by a bis(tetrahydroisoquinoline) pentacyclic system (ABCDE), which is believed to be the primary pharmacophore through the preliminary structure–activity study of Et 743 and its analogs (Fig. 1).[6]

While Et 743 shows a potent antitumor activity, it has a complex architecture and the available synthetic procedures are not practical enough to provide large amounts of the compound. Therefore, we felt that attempts toward designing a more practical synthetic route and synthesizing the simpler analogs of Et 743 are of considerable significance. As a result of a project aimed to synthesize Et 743 and its analogs, we have recently

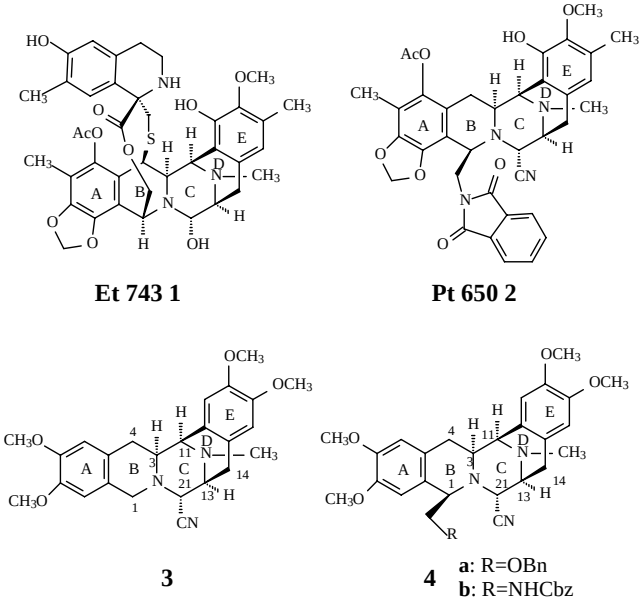

Figure 1.

Keywords: Et 743; Antitumor; Alkaloid; L-DOPA; Synthesis; Tetrahydroisoquinoline.
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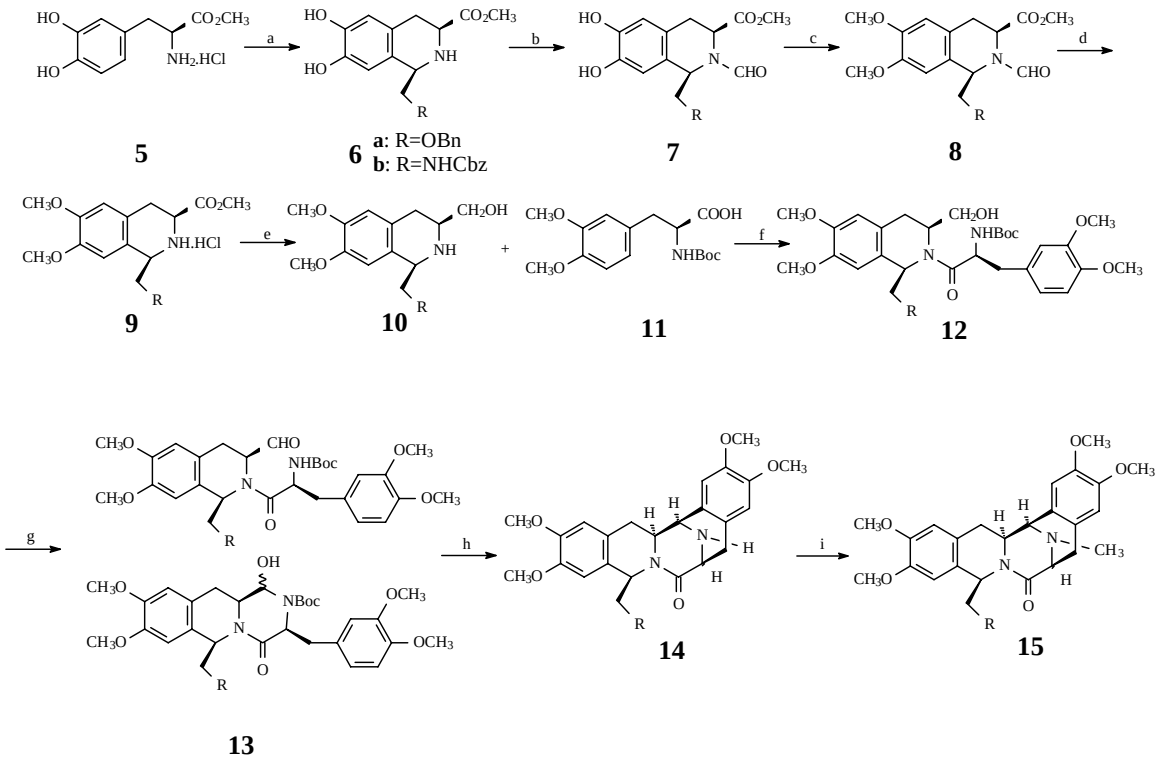
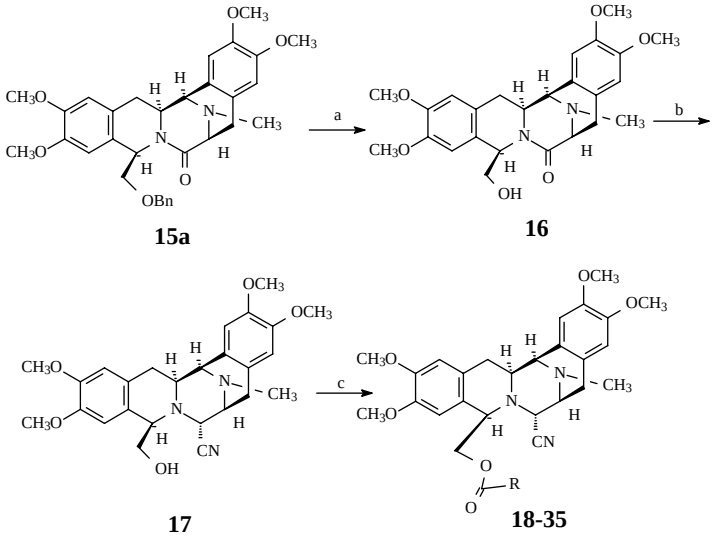
doi:10.1016/j.bmcl.2005.11.069

established a novel synthetic process for constructing the pentacyclic system of Et 743 **3**[7] and two functionalized pentacylic intermediates **4**. Herein, we reported the synthesis and the in vitro cytotoxic evaluation of two series of the simplified analogs of Et 743.

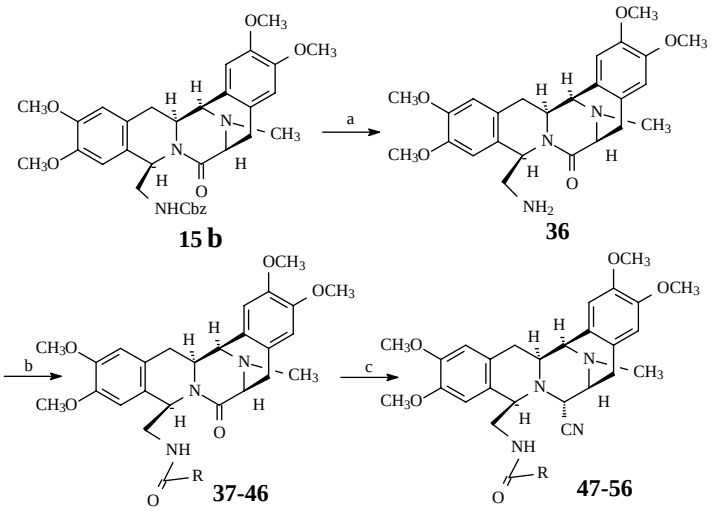
The synthesis of these analogs started from the readily available L-DOPA, which is thought to be the biosynthetic origin of Et 743.[8] L-DOPA methyl ester **5** was submitted to Pictet–Spengler reaction using the corresponding aldehydes in NaOAc/HOAc to afford *cis*-1-substituted tetrahydroisoquinoline-3-carboxylic acid esters **6** due to the 1,3-induction effect.[9] Then, protection of the nitrogen of **6** by the formyl group afforded **7**, which was transformed into the methylated product **8** upon treatment with Me_2SO_4/K_2CO_3 in acetone at reflux. Cleavage of the formyl group in HCl/CH_3OH provided **9**. Reduction of the ester group of **9** with $LiAlH_4$



Scheme 1. Reagents and conditions: (a) aldehyde, NaOAc/HOAc, rt, 20 h; (b) Ac_2O/HCO_2H, 4 h; then H_2O/CH_3OH, 87%; (c) Me_2SO_4, K_2CO_3, CH_3COCH_3, reflux, 90%; (d) HCl/CH_3OH, reflux, 4 h, 85%; (e) $LiAlH_4$, THF, 0 °C, 0.5 h, then rt 1.5 h, 83%; (f) BOP-Cl, Et_3N, CH_2Cl_2, 4 h, 80%; (g) $(COCl)_2$/DMSO, CH_2Cl_2, 30 min, then Et_3N, 5 min, −70 °C, 85%; (h) HCO_2H, 70 °C, 1 h, 67%; (i) $HCHO/HCO_2H$, 70 °C, 2 h, 95%.



Scheme 2. Reagents and conditions: (a) 10% Pd–C, CH_3CH_2OH, 70 °C, 50 psi; (b) $LiAlH_4$, THF, −17 °C, 0.5 h, then 0 °C, 1 h; KCN, phosphate buffer (pH 7), 2 h; (c) EDC, DMAP, acid, CH_2Cl_2, 5 h.



Scheme 3. Reagents and conditions: (a): HBr/HOAc, 1 h; (b) EDC, DMAP, acid, CH_2Cl_2, 5 h; (c) $LiAlH_4$, THF, −17 °C, 0.5 h, then 0 °C, 1 h; KCN, phosphate buffer (pH 7), 2 h.

in THF at 0 °C afforded the primary alcohol **10**, which was subsequently coupled with **11** through the action of bis(2-oxo-3-oxazolidinyl) phosphinic chloride (BOP-Cl) to afford the amide **12**. Compound **12** was oxidized to **13** via Swern oxidation.[10] Compound **13** was the key intermediate of our synthesis and existed as a mixture of amino aldehyde and hemiaminal, and when it was submitted to intramolecular Pictet–Spengler cyclization using CF_3CO_2H at room temperature, the expected pentacyclic intermediate **14** was obtained in a moderate yield with the Boc-group being removed simultaneously. It should be noted that a similar cyclization strategy had been used by two other research groups.[11] Reductive methylation of **12** with HCHO/HCO_2H at 70 °C for 2 h provided **15** (Scheme 1).

The *O*-benzyl group of compound **15a** was removed by catalytic hydrogenation. Then the lactam ring of **16** could be easily reduced through treatment with an excess of $LiAlH_4$ in THF at −17 °C and then 0 °C for 1 h to the corresponding cyclic hemiaminal, which upon exposure to KCN in phosphate buffer (pH 7) afforded the pentacyclic amino nitrile **17** as an enantiomerically pure product. Finally, the primary alcohol compound **17** was esterified with different acid to afford the corresponding ester analogs **18**–**35** (Scheme 2).

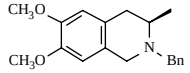
Removal of the *N*-Cbz group of **15b** with HBr/HOAc was followed by acylation of the amine **36** with different acid to afford the corresponding amides **37**–**46**. Finally, partial reduction of the lactam ring to the corresponding cyclic hemiaminal was followed by treatment with KCN to form the corresponding amide analogs **47**–**56** (Scheme 3). All the structures of the analogs were determined by ^1H, ^{13}C NMR, and FAB-MS.[13]

All the analogs were tested for their in vitro anticancer activities against HCT-8, Bel-7402, and BGC-823 cell lines by the MTT-based assay. The assays were performed in 96-well plates essentially as described by Mosmann.[12] The IC_{50} concentration represents the concentration which results in a 50% decrease in cell growth after six days of incubation. The given values are mean values of three experiments.

The pharmacological results are summarized in Table 1 for anti-HCT-8, anti-Bel-7402, and anti-BGC-823 cell

Table 1. Structure and in vitro cytotoxicity of the simplified analogs of Et 743 against the HCT-8, Bel-7402, and BGC-823 cell lines

Compound	R	IC_{50} (μM)		
		HCT-8	Bel-7402	BGC-823
18	Phenyl	0.44	0.59	0.015
19	Pyrazin-2-yl	3.48	3.50	1.69
20	Furan-2-yl	0.69	0.75	0.58
21	Pyridin-2-yl	5.15	2.02	1.44
22	Vinyl	2.14	0.76	0.46
23	Indol-2-yl	0.62	0.85	0.01
24	Pyridin-3-yl	3.28	2.10	1.02
25	Styryl	0.037	0.68	0.058
26	A	1.42	1.69	0.36
27	4-Methoxyl-benzyl	3.56	3.01	1.14
28	3-Chloro-phenyl	0.1	1.12	0.029
29	5-Bromo-piridin-3-yl	1.17	1.49	0.45
30	Thiophen-2-yl	1.60	0.82	0.21
31	1-Naphthyl	0.34	0.71	0.006
32	Methyl	0.31	0.34	0.15
33	4-Fluoro-benzyl	0.24	0.20	0.20
34	3-Phenyl-propyl	0.83	0.12	0.75
35	Ethyl	0.30	0.11	0.15
47	Phenyl	1.35	1.35	1.40
48	1-Naphthyl	1.72	2.08	1.86
49	3-Chloro-phenyl	1.54	0.95	1.01
50	4-Nitro-phenyl	1.23	1.22	1.37
51	Styrenyl	1.78	1.45	1.31
52	Thiophen-2-yl	1.28	0.79	1.16
53	Furan-2-yl	1.38	1.20	1.32
54	Acryl	2.71	1.87	2.00
55	2-Chloro-phenyl	1.85	1.87	1.91
56	Phthalimide	0.50	0.18	0.42

A= 

lines, respectively. As shown in Table 1, most of the compounds showed considerable cytotoxicities to these three cell lines, while the ester analogs (**18**–**35**) showed stronger cytotoxicity than that of amide analogs (**47**–**56**) in general. Although a general structure–activity relationship of the simplified analogs of Et 743 to anticancer effect could not be summarized from these data, the following points were noteworthy: compound **25** had better inhibitory activity to HCT-8 cells, compounds **18**, **23**, **25**, **28**, and **31** had stronger activities against BGC-823 cells, compound **31** showed the most significant activity.

In conclusion, we have synthesized a class of simplified analogs of Et 743. The preliminary structure–activity relationship study indicated that the ester analogs had stronger activity than that of the amide analogs, and 1-naphthalene carboxylate ester analog **31** showed the most significant activity against BGC-823 tumor cells.

Acknowledgments

We thank the National Natural Sciences Foundation of China (No. 30472077) and Beijing Municipal Natural Sciences Foundation (No. 7042041) for financial support.

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13. Compound **30**: mp: 111–113 °C, $[\alpha]_D^{20}$ +64.4 (*c* 0.59, $CHCl_3$); ^1H NMR (300 MHz, δ ppm, $CDCl_3$): 7.53 (d, 1H, $J = 4.2$ Hz, C═CH), 7.52 (d, 1H, $J = 4.8$ Hz, C═CH), 7.06 (t, 1H, $J = 4.2$, 4.8 Hz, C═CH), 6.61 (s, 2H, Ar-H), 6.44 (s, 1H, Ar-H), 6.43 (s, 1H, Ar-H), 4.28 (dd, 1H, $J = 4.2$, 10.8 Hz, 22-H), 4.17 (s, 1H, 21-H), 4.03 (t, 1H, $J = 3.6$ Hz, 1-H), 3.96 (dd, 1H, $J = 4.8$, 10.8 Hz, 22-H), 3.86 (s, 3H, CH_3O), 3.85 (s, 3H, CH_3O), 3.80 (s, 3H, CH_3O), 3.77 (s, 3H, CH_3O), 3.54 (s, 1H, 11-H), 3.38 (d, 1H, $J = 7.8$ Hz, 13-H), 3.32 (d, 1H, $J = 11.7$ Hz, 3-H), 3.04 (dd, 1H, $J = 7.8$, 17.7 Hz, 14-H), 2.55 (d, 2H, 4-H + 14-H), 2.42 (d, 1H, $J = 11.7$ Hz, 4-H), 2.33 (s, 3H, –NCH_3). ^{13}C NMR δ (300 MHz, $CDCl_3$, ppm): 161.54 (C═O), 148.00, 147.97, 147.61, 146.23, 133.40 (–CH–), 132.85, 132.34 (–CH–), 127.72 (–CH–), 127.47, 126.52, 124.46, 123.03, 118.17 (–CN), 112.14 (–CH–), 110.56 (–CH–), 110.28 (–CH–), 110.08 (–CH–), 68.50 (–CH_2–), 62.90 (–CH–), 60.83 (–CH–), 60.78 (–CH–), 56.59 (–CH–), 56.03 (–CH_3), 55.88 (–CH_3), 55.87 (–CH_3), 55.64 (–CH–), 55.56 (–CH_3), 41.69 (–CH_3), 32.83 (–CH_2–), 25.73 (–CH_2–). FABMS (*m/z*): 576 (M+1), 549 (M−CN), 434, 244, 204 (100%), 190, 111.