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Synthesis and inhibition of cancer cell proliferation of (1,3′)-bis-tetrahydroisoquinolines and piperazine systems

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Abstract—Some (1,3′)-bis-tetrahydroisoquinolines were reported as scaffold intermediates for the synthesis of pentacyclic piperazine core alkaloids and their cytotoxicity against cancerous cell lines was evaluated. The NMR and X-ray structural assignments revealed an *anti* C3–C11 backbone stereochemistry of piperazine structures. Inhibition of cancer cell proliferation of (1,3′)-bis-tetrahydroisoquinoline scaffolds and pentacyclic piperazine systems was assessed against three human cancer cell lines (K562 myelogenous leukemia, A549 lung carcinoma, MCF-7 breast adenocarcinoma) and both mouse tumor cell lines of blood (P388) and lymphocytic (L1210) leukemia with considerable activity against the latter. The cell cycle analysis was also studied by flow cytometry measurement on K562 cell line.

Our interest for the tetrahydroisoquinoline alkaloid, essentially aroused from their natural architectural complexity and their noteworthy biological properties as antitumor antibiotics.[1] The most active member of this family, Ecteinascidin 743 (**1**, Et 743), was isolated from the Caribbean tunicate *Ecteinascidia turbinate*[2] (Fig. 1) and displayed highly potent cytotoxic activity against a variety of tumor cancer cells in vitro and is currently undergoing phase II/III clinical trials.[3] The natural scarcity and potent medical use of Et 743 have attracted several groups to embark on its total synthesis.[4] With regard to the structural complexity of Et 743, a synthetic analogue, phthalascidin 650 (**2**, Pt 650), which exhibits similar biological activity to the natural product, has also been prepared (Fig. 1).[5] Since their discovery, interest in their biological activity has increased with several

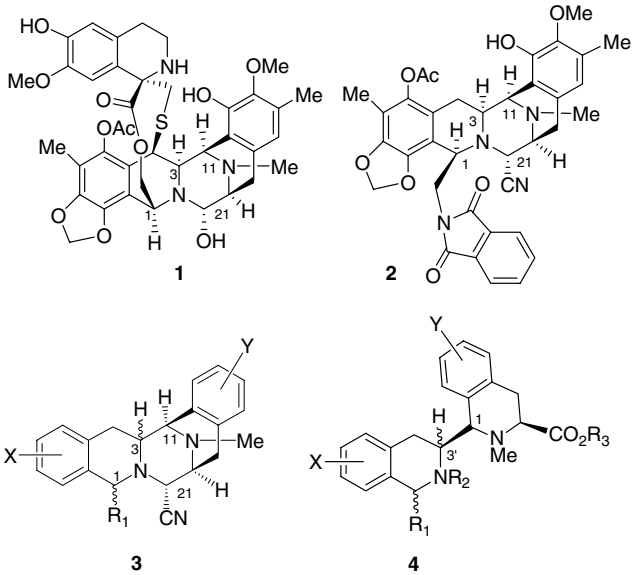

Figure 1. Et 743 (**1**), Pt 650 (**2**), pentacyclic piperazine systems (**3**), and (1,3′)-bis-tetrahydroisoquinolines (**4**).

Keywords: Piperazine systems; Synthesis; Cancer cell proliferation; Flow cytometry.
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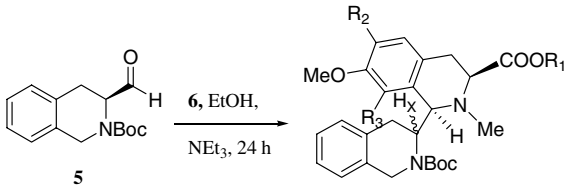
reports dealing with the synthesis and biological evaluation of piperazine systems **3** (Fig. 1).[6] Moreover, the structure-activity relationship study of synthetic compounds having an *anti* C3–C11 backbone stereochemistry in contrast with the *syn* relationship of their corresponding natural compounds has recently been investigated.[6e] It has been postulated that their ability to alkylate DNA in its minor groove could explain their highly antiproliferative activity against tumor cells.[7]

A program directed toward the synthesis of ecteinascidin and phthalascidin analogues by a novel synthetic approach was initiated in our laboratory, giving rise to the formation of reactive (1,3′)-bis-tetrahydroisoquinolines **4** (Fig. 1) and pentacyclic piperazine cores **3**.[8] This program also involved the biological evaluation of synthetic intermediates and simplified pentacyclic structures to study structure–activity relationship in order to establish pharmacophoric features. Herein, we report the synthesis and characterization of potentially reactive (1,3′)-bistetrahydroisoquinolines and piperazine pentacyclic systems, with respect to DNA alkylation and formation of covalent adducts. Inhibition of cancer cell proliferation of synthetic intermediates and modified pentacyclic systems is also discussed.

In a previous communication,[8] we have reported the synthesis of (1,3′)-bis-tetrahydroisoquinolines via a Pictet–Spengler reaction[9] between *N*-Boc-1,2,3,4-tetrahydroisoquinoline-3-carboxaldehyde **5** and L-3,4-dihydroxyphenylalanine methyl ester hydrochloride **6**, under non-acidic conditions (EtOH, NEt_3, rt),[10] with complete cis-stereoselectivity. Isomers **7** and **8** aroused from cyclization *para*- to the 3-phenolic group of **6** and **9** from cyclization *ortho*- to the same phenolic group obtained with an overall yield of 78% (Scheme 1). Structural elucidation of **7**–**15** was determined by 2D NMR (HSQC, COSY, and NOESY techniques). Moreover, ^{1}H NMR experiment in DMSO-d_6 at 80 °C confirmed the mixture composition of **8** and **9** (due to the presence of rotamers).

Depending on the reaction conditions, the optical integrity of the aldehyde stereocenter was sensitive to epimerization[11] and confirmed by X-ray analysis of **11** and **17** (Fig. 2).[12]

After trimethylation of **7**, **8**, and **9**, the corresponding methyl ethers **10**, **11**, and **12** were obtained (Scheme 2). A tunable amino acid functionality was then introduced in a two-step sequence. Hydrolysis of the ester moiety in **10**, **11**, and **12** to give **13**, **14**, and **15**, respectively (Scheme 2), followed by the *N*-Boc cleavage of **13** and **15** to allow cyclization, via an intramolecular peptide coupling, afforded lactams **16** and **17**(Scheme 3).



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7: H_X=H_β (1,3'-*trans*) R_1=Me, R_2=MeO, R_3=H 49%
8: H_X=H_α (1,3'-*cis*), R_1=Me, R_2=MeO, R_3=H and
9: H_X=H_β (1,3'-*trans*), R_1=Me, R_2=H, R_3=MeO 29%

Scheme 1. Synthesis of (1,3′)-bistetrahydroisoquinolines **7**, **8**, and **9**.

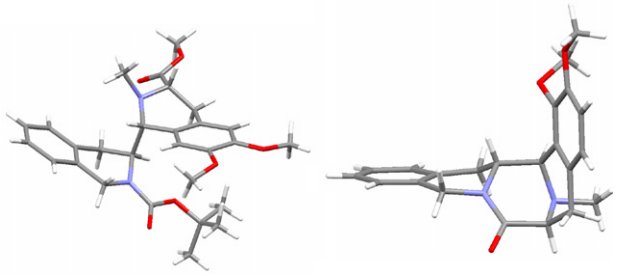
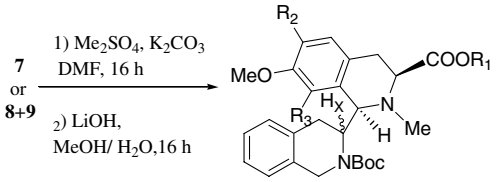


Figure 2. Single-crystal X-ray analysis of **11** and **17**.



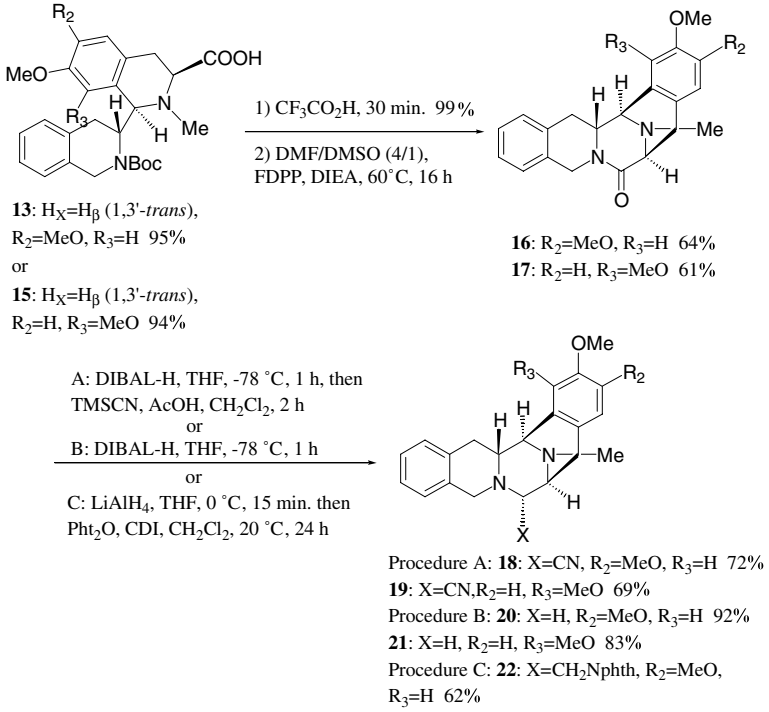
10: H_X=H_β (1,3'-*trans*), R_1=Me, R_2=MeO, R_3=H 85%
11: H_X=H_α (1,3'-*cis*), R_1=Me, R_2=MeO, R_3=H 39% and
12: H_X=H_β (1,3'-*trans*), R_1=Me, R_2=H, R_3=MeO 38%

13: H_X=H_β (1,3'-*trans*), R_1=H, R_2=MeO, R_3=H 95%
14: H_X=H_α (1,3'-*cis*), R_1=H, R_2=MeO, R_3=H 95%
15: H_X=H_β (1,3'-*trans*), R_1=H, R_2=H, R_3=MeO 94%

Scheme 2. Synthesis of esters **10**–**12** and acids **13**–**15**.

Complete structural characterization of **16** was achieved by NMR experiments, which displayed the same distinctive feature as **17** ($J_{H1–H3}$ = 0 Hz). Structural elucidation by single-crystal X-ray analysis of **17**[12] also revealed an *anti* C3–C11 stereochemistry formed by epimerization of the *N*-protected-α-aminoaldehyde during the Pictet–Spengler cyclization (Fig. 2). The dihedral angle observed between H1 and H3 by X-ray analysis of **17** (73.7°) correlates with the negligible coupling constant ($J_{H1–H3}$ = 0 Hz) found by ^{1}H NMR (Karplus correlation). Taking into account the postulated reasons for the antiproliferative activities of this alkaloid family through the DNA alkylation of a potent electrophilic iminium species, the reduction of the lactam ring of **16** and **17** was implemented.[7] Thus, the reduction of **16** and **17** was performed with 2.2 equiv of DIBAL-H in THF at −78 °C, affording the corresponding carbinolamine which was converted,

Under acidic conditions with trimethylsilyl cyanide, to α-amino nitrile **18** and **19** in 72% and 69% yield, respectively. On the other hand, reduction of **16** and **17** with seven equivalents of DIBAL-H in THF at −78 °C afforded the benzylamines **20** and **21** in good yields (Scheme 3). We then reduced the nitrile functionality of **18** with $LiAlH_4$ in THF to the corresponding primary amine. Subsequent functionalization with phthalic anhydride



13: H_X=H_β (1,3'-*trans*), R_2=MeO, R_3=H 95%
or
15: H_X=H_β (1,3'-*trans*), R_2=H, R_3=MeO 94%

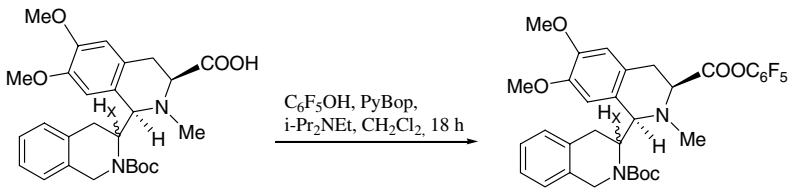
16: R_2=MeO, R_3=H 64%
17: R_2=H, R_3=MeO 61%

Procedure A: **18**: X=CN, R_2=MeO, R_3=H 72%
19: X=CN,R_2=H, R_3=MeO 69%
Procedure B: **20**: X=H, R_2=MeO, R_3=H 92%
21: X=H, R_2=H, R_3=MeO 83%
Procedure C: **22**: X=CH_2Nphth, R_2=MeO, R_3=H 62%

Scheme 3. Synthetic procedure for the pentacyclic systems **18**–**22**.

and carbonyldiimidazole afforded the phthalimide **22**. For this compound the phthalimide group is located at C21, and not at C1 like in the Pt 650 structure (Scheme 3). In our study, the compounds exhibited the opposite C3–C11 backbone stereochemistry recurrent in the natural alkaloid family which was confirmed by single-crystal X-ray analysis of **11** and **17** (Fig. 2).[12] After deprotection of the *N*-Boc linkage of **14**, the final cyclization did not occur with the established conditions. Due to the steric hindrance imposed by the activated acid intermediate, the intramolecular peptide coupling was inefficient. As previously observed by several groups, the fatal epimerization occurred at C3 through the phenolic Pictet–Spengler cyclization step.[6d,13] Based on the ability of escteinascidin to alkylate DNA in the minor groove, the synthesis of (1,3′)-bis-tetrahydroisoquinolines derivatives bearing a highly reactive leaving group was envisioned by carboxylic acid esterification with pentafluorophenol in presence of PyBoP®. This reaction produced the pentafluorophenyl esters **23** and**24** in moderate yields from **13** and **14**(Scheme 4). Starting from acid **15**, the corresponding ester proved unstable.

3-(4′,5′-Dimethylthiazol-2′-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay is widely used to assess the effect of potential anticancer agents on the proliferation of cancer cells in vitro. Cultured cancer cells were grown in the presence of the putative anticancer agent in 96-well plates using previously published procedures.[14] The amount of viable cells, before and after treatment, can be determined spectrophotometrically by measuring the optical density of the solutions using a UV–vis spectrophotometer (λ = 540 and 610 nm). This is based on the reduction of the yellow MTT by mitochondrial dehydrogenases of metabolically active cancer cells to a purple blue formazan after 3 h of incubation. The bioactivity is most often reported as its IC_{50}; that is, the concentration of drug resulting in inhibition of 50% of cell proliferation relative to an untreated control after five days of incubation. All compounds were evaluated against three human cancer cell lines (K562 mye-



13: H_X=H_β (1,3'-*trans*)
or
14: H_X=H_α (1,3'-*cis*)

23: 56% H_X=H_β, (1,3'-*trans*)
or
24: 53% H_X=H_α, (1,3'-*cis*)

Scheme 4. Synthesis of reactive (1,3′)-bis-tetrahydroisoquinolines.

logenous leukemia, A549 lung carcinoma, MCF-7 breast adenocarcinoma) and two mouse tumor cell lines of blood (P388) and lymphocytic (L1210) leukemia (Table 1). Compounds were tested in triplicate. All compounds described in this paper were first evaluated for their ability to inhibit cancer cell proliferation against the K562 cell line in vitro. The most active compounds were selected for further study. In agreement with reports concerning the ecteinascidin mode of action,[7] compounds **18** and **19** harboring an α-amino nitrile functionality proved substantially more cytotoxic in all cell lines. In absence of a leaving group at C21 (e.g., amide derivatives **16** and **17**, and benzylamines **20** and **21**) and as observed for some isolated saframycin, renieramycin, safracin, and ecteinascidin which lack a leaving group at C21,[1] cytotoxicities were less relevant.

The phthalimide derivative **22** displayed no antiproliferative activity confirming that the presence of a leaving group enabling the formation of an iminium species through dehydration of the C21 nitrile group is essential for bioactivity, presumably via DNA alkylation. Finally while the synthetic intermediates **8**–**12** displayed cytotoxicity in the micromolar range, compounds **23** and **24** displayed similar cytotoxicity to compounds **18** and **19**. The cytotoxicity may depend on the carboxylate functionalization as $RCO_2C_6F_5 > RCO_2Me$. This may be linked to the mode of action of the ecteinascidin family, by DNA alkylation of activated pentafluorophenyl ester. Interestingly, L1210 tumor cell lines displayed the highest sensitivity with all of the compounds.

Table 1. Inhibition of cancer cell proliferation of (1,3′)-bis-tetrahydroisoquinolines and pentacyclic piperazine systems

Compound	IC_{50} (μM)				
	K562	A549	MCF-7	P388	L1210
8, **9**	34.6	>100	56.6	12.4	12.1
10	44.3	62.0	37.4	57.0	6.4
11	20.8	67.5	37.5	24.6	10.0
12	40.2	55.6	37.9	19.6	15.8
16	>100	>100	>100	>100	>100
17	>100	>100	>100	>100	>100
18	24.7	33.4	21.7	11.6	1.4
19	15.2	19.4	38.6	9.3	1.1
20	79.8	>100	>100	11.1	5.1
21	>100	>100	>100	>100	6.4
22	>100	>100	>100	>100	>100
23	18.8	10.5	8.1	6.1	6.1
24	15.9	40.0	37.2	19.0	7.3

Table 2. Cell cycle distribution by flow cytometry in K562 cells treated for 24 h[a]

Compound	G0/G1 (%)	S phase (%)	G2/M (%)
Control	45.2	31.5	23.3
Cisplatin	60.5	24.1	15.4
18	55.4	29.2	15.4
19	54.8	35.1	10.1
23	51.2	26.5	22.3
24	55.4	36.3	8.3

[a] Percentage of cells in each phase of the cell cycle as measured by flow cytometry (according to previously described methods[15]).

Next flow cytometry was used to evaluate the effects of the most active compounds (**18**, **19**, **23**, and **24**) on the growth and division of K562 cells, by measuring the DNA content of eukaryotic cells. The results show the proportion of cells emitting a given level of fluorescent proportional to the DNA content.[15] Unlike antimitotic agents which accumulate their DNA content in the G_2/M phase of the cell cycle, the results in Table 2 show that three of the compounds (**18**, **19**, and **24**) cause significant arrest of the cell cycle at the G0/G1 and S phases (84.6–91.7% vs 76.7% for untreated control). This result is consistent with the behavior of a DNA-interfering agent such as cisplatin[16] and as previously described for Et 743.[17]

To conclude, an efficient synthesis of *anti* C3–C11 pentacyclic systems harboring the requisite function for the biological evaluation was realized through the synthesis of (1,3′)-bis-tetrahydroisoquinoline scaffolds. Preliminary biological evaluation of our compounds showed antiproliferative activity against a variety of cancer cell lines in the micromolar range in spite of the absence of complete aromatic functionalization and a propensity to promote the arrest of the cell cycle at the G0/G1 and S phases. Further biological evaluation is undergoing to evaluate the mode of action of these agents.

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Supplementary data

Supplementary data for new compounds associated with this article can be found, in the online version at doi: Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.01.108.

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12. Crystallographic data (excluding structure factors) for **11** and **17** in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 287225 and 286961. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].
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