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Synthesis and biological evaluation of novel benzoxazinic analogues of ellipticine

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ABSTRACT

Original 1,4-benzoxazine analogues of ellipticine were prepared using a general synthetic route that relied on an anionic ring annulation as the key transformation. The interest of this approach lies on the possibility of an easy entrance to a wide range of derivatives from the phenol intermediate. In addition, this synthetic strategy offers a smart access to diverse structurally related heteroaryl annulated carbazole analogues of ellipticine just by replacing the starting heterocyclic core. Antiproliferative activities of newly synthesized derivatives were evaluated toward tumor cell lines.

The alkaloids ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole) **1a** and 9-methoxyellipticine **1b** (Fig. 1) were first isolated in 1959 from the leaves of *Ochrosia elliptica* (*Apocynaceae* family).[1] These compounds and their derivatives are endowed with potent antitumor properties. They intercalate DNA and their high DNA binding affinity is thought to be responsible in part for these pharmacological properties.[2,3] More recent studies have also indicated anti-HIV activities.[4] The main reason for the interest in ellipticine and its derivatives for clinical purposes is their high affinity against several types of cancer with limited toxic side effects and total absence of hematological toxicity.[5] Ellipticine has proven to be a popular target for synthesis, and a wide variety of strategies have been reported.[6,7] Similarly, the structurally related aryl- and heteroarylannulated carbazoles have also received considerable synthetic attention.[8]

Given our interest in the synthesis of original heterocyclic systems with potential pharmacological value and in the aim to increase the aqueous solubility of ellipticine derivatives, we achieved the synthesis of original ellipticine analogue **2** by the replacement of the indole ring with a benzodioxine moiety (Fig. 1).[9] The latter exhibited potent in vitro cytotoxicity against B16 melanoma cells. In association with our work regarding the chemistry and the functionalization of benzoxazinic system,[10] we want now to describe the preparation of a novel analogue of ellipticine **I** (Fig. 2) as a new potent antitumor agent. Although benzoxazine moiety is rarely found in nature, it has been emerged as a valuable tool for the preparation of structurally diverse chemical libraries of drug-like heterocyclic compounds.[11]

We thus endeavored to prepare original 1,4-benzoxazine analogues of ellipticine **I** using a general synthetic route that relied on an anionic ring annulation as a key transformation.[12] The interest of this approach lies firstly on the possibility from the tetracyclic phenolic intermediate **II** to easily prepare a wide range of derivatives for biological evaluation. In addition, this synthetic strategy offers a smart access to diverse structurally related heteroaryl annulated carbazole analogues of ellipticine just by replacing the starting heterocyclic core **IV**.

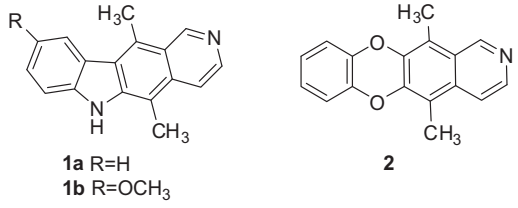

Figure 1. Ellipticine and analogues.

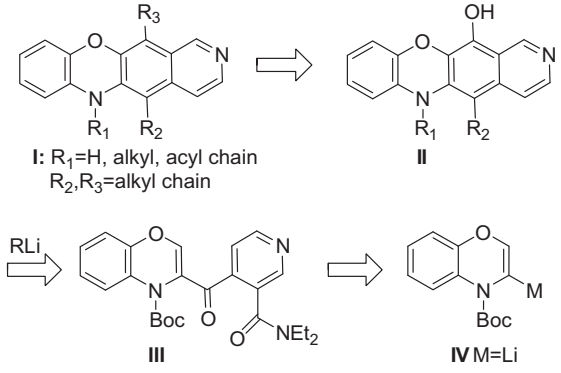

Figure 2. General retrosynthetic scheme via an anionic ring annelation.

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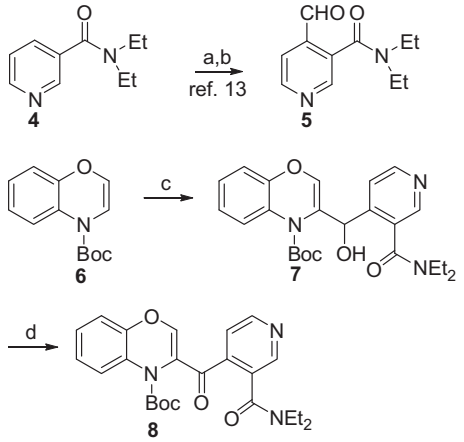
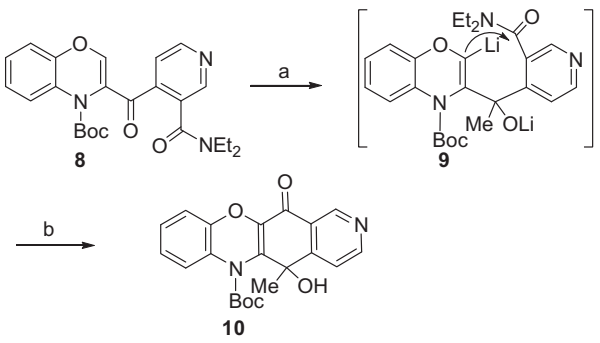


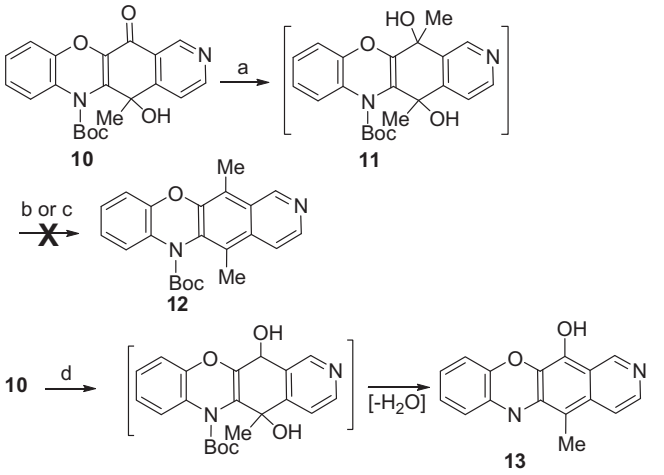
Scheme 1. Synthesis of 3-substituted benzoxazine **8**. Reagents and conditions: (a) *n*-BuMgCl (2.2 equiv), TMP (2.5 equiv), THF 0 °C, 1 h; (b) *N*-formylpiperidine (2.5 equiv), 0 °C, 3 h then citric acid (1 M) (55%, two steps); (c) *n*-BuLi (1 equiv), THF −78 °C, 35 min then **5** (0.5 equiv), −78 °C, 1 h (64%); (d) $(COCl)_2$ (1.3 equiv), DMSO (2.4 equiv), CH_2Cl_2, −78 °C, 1 h then Et_3N (5 equiv), −78 °C to rt, 1 h (80%).



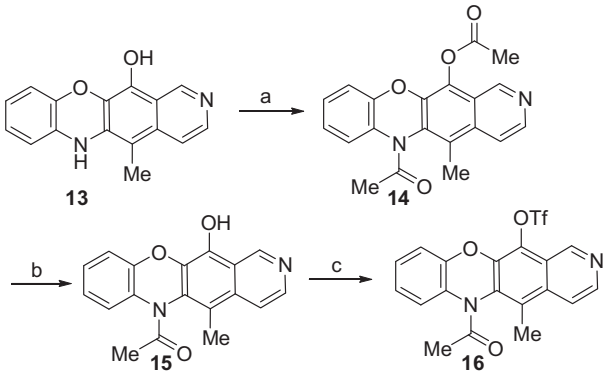
Scheme 2. Preparation of tetracycle **10** via a MeLi-mediated ring annulation. Reagents and conditions: (a) MeLi (3.5 equiv), THF −78 °C, 1 h; (b) H_3O^+ (100%, two steps).

Our initial task was to elaborate the acyclic precursor **8** (scheme 1). Treatment of *N*-Boc-benzoxazine **6** with *n*-BuLi afforded regioselectively the 3-lithioderivative which reacted with 4-formylpyridine **5**.[13] Resulting 3-substituted benzoxazine **7**, isolated in good yield, has been oxidized under Swern conditions to give the acyclic ketoderivative **8**.

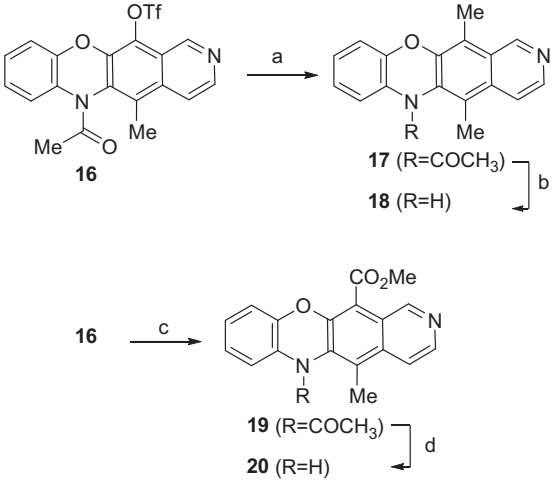
The synthesis of tetracyclic scaffold **10** was then expected via an anionic ring annulation in the presence of organolithium reagent (Scheme 2).[14] In fact, starting from ketoderivative **8**, an excess of MeLi allowed its nucleophilic addition onto the ketone function and also the concomitant deprotonation at the C-2 position of the benzoxazine ring. The newly formed lithio derivative **9** could then react via an intramolecular manner onto the amide function and provide the desired tetracyclic ketone **10**, isolated in high yield. Because of the noteworthy instability of compound **10**, the latter was directly engaged in the next step without any purification. In order to synthesize the tetracyclic core of the ellipticine analogue, our attention turned then to study the aromatization step. Taking into account Gribble's work,[15] we expected to obtain the desired tetracyclic aromatized compound **12** via a dehydration reaction from diol intermediate **11** (Scheme 3). In this manner, the carbonyl compound **10** was submitted to methyllithium in THF at low temperature, and the resulting not isolated diol **11** (TLC control) was thus directly engaged in a reduction step in the presence



Scheme 3. Synthesis of phenolic tetracycle **13** via a tandem reduction/dehydration sequence. Reagents and notes: (a) MeLi (3 equiv), THF −78 °C, 2 h (not isolated); (b) $NaBH_4$, EtOH, rflx or MeOH, rt; (c) $LiAlH_4$, THF 0 °C or EtOH rflx; (d) H_2, Pd/C (10%), MeOH, rt 30 min (100%).

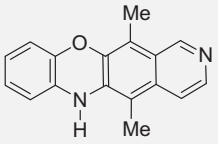
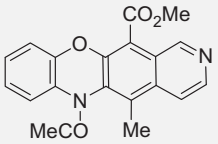
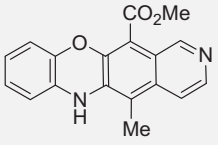


Scheme 4. Reagents and conditions: (a) Et_3N (4 equiv), Ac_2O (4 equiv), DMAP (0.4 equiv), CH_2Cl_2, rt 18 h (70%); (b) NaOH (4 equiv), H_2O, MeOH, rt 3 h (100%); (c) $PhNTf_2$ (1.3 equiv), CH_2Cl_2, −78 °C, 3 h (55%).



Scheme 5. Obtention of ellipticine analogues **17**–**20**. Reagents and notes: (a) Me_4Sn (3.5 equiv), LiCl (5 equiv), $PdCl_2(PPh_3)_2$ (15 mol %), DMF, rflx 2 h (77%); (b) NaOH (2 equiv), H_2O–MeOH, rt 3 h (87%); (c) CO (1 atm), $Pd(OAc)_2$ (10 mol %), d_{ppp} (10 mol %), Et_3N (2 equiv), DMSO, MeOH, 70 °C, 2 h (75%); (d) $NH_2(CH_2)_2N(C_2H_5)_2$, 100 °C, 3 h (90%).

Table 1
In vitro antiproliferative activities against a murine leukemia (L1210) and human colon adenocarcinoma cell line (HT29 and HCT116)[a]

Entry	Compound		IC_{50} L1210 (μM)	IC_{50} HCT116 (μM)	IC_{50} HT29 (μM)
1	Ellipticine		0.19	—	14.9
2		**18**	2.6	2.2	4.5
3		**19**	38.3	26.9	4.5
4		**20**	67.5	46.2	70.2

[a] Inhibition of L1210, HT29, and HCT116 cell proliferation measured by in vitro biological assay. IC_{50} values are concentrations that inhibit growth by 50% (IC_{50} values are the average of at least four determinations in triplicate obtained in independent experiments. Variation between replicates was less than 5%).

of $NaBH_4$ or $LiAlH_4$. Under these conditions, the desired derivative **12** could, unfortunately, not be isolated. Only degradation compounds were observed.

We therefore thought achieving the aromatization step via spontaneous dehydration after the reduction of the carbonyl function of **10**. By hydrogenolysis, the desired aromatic tetracycle **13** was thus successfully obtained in quantitative yield via a spontaneous aromatization step. An unexpected concomitant deprotection of the nitrogen atom was also observed. It is noteworthy that in this case, reduction with $LiAlH_4$ or $NaBH_4$ did not permit to isolate the desired aromatized compound **13**. Starting from this phenol intermediate **13**, a range of ellipticine analogues could easily be prepared by the replacement of the hydroxyl function.

To complete the synthesis, it was now necessary to introduce an alkyl chain onto the aromatic moiety. Toward this aim, we thought of transforming aryl alcohol **13** into the corresponding triflate **16** (Scheme 4) and submitting it to palladium cross-coupling reactions. For the preparation of the latter, the best results were obtained by using an acetamide function onto the nitrogen atom. With Boc-protecting group only decomposition occurred.

Thus, successive protection/deprotection steps[16] allowed us to isolate phenol **15** which was transformed in a good yield into triflate **16** by treatment with *N*-phenyltrifluoromethanesulfonimide. The latter was then submitted to palladium cross-coupling reaction in order to get the desired analogues (Scheme 5). Conversion of triflate **16** to the methyl derivative **17** was achieved in the presence of tetramethyltin by Stille cross-coupling as the key step (Scheme 5).[17] Hydrolysis of **17** in the presence of NaOH to remove the acetyl group attached to the nitrogen atom afforded **18** in good yield. Methoxycarbonylation of triflate **16** yielded the ester derivative **19**. The acetyl group was then removed by treatment of **19** with *N,N*-diethylethylendiamine to give **20** isolated in good yield.

Given that for some time we have been engaged in the design and the synthesis of new potential anticancer agents,[10,18] the cytotoxicity of these novel ellipticine analogues was evaluated. The newly synthesized derivatives **18**, **19**, and **20** were assayed in vitro for the ability to inhibit cell proliferation L1210 (murine leukemia), HT29, and HCT116 (human colon carcinoma).[19] The cytotoxicity of compounds was measured using a conventional microculture tetrazolium assay. The results expressed as IC_{50} values are listed in Table 1.

These compounds were found to possess moderate activity at micromolar concentration. It is important to point out that the methyl chain (**18**) (on the same side of the oxygen atom) confers greater activity for the inhibition of cell proliferation. Several enzymatic assays (topoisomerase and kinase) and DNA interaction are still in progress in order to elucidate the action of these new lead compounds.

In summary, we have described a novel and practical method for the synthesis of new tetracyclic hybrid molecule that includes a benzoxazinic substructure and the isoquinoline moiety of ellipticine. An anionic ring annulation was involved as the key transformation. Diversity in the synthesis could be introduced by changing the starting heterocycle or from the phenol intermediate. This procedure has the advantage of high yields, easy availability, and flexibility of starting materials. Encouraging biological results were then obtained. Experiments designed to explore the potentiality offered by this original heterocyclic scaffold will be described in due course.

Supplementary data

Supplementary data (experimental procedures and full spectroscopic data for new compounds) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.05.123.

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