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Inhibition of human DNA topoisomerase IIα by two novel ellipticine derivatives

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ABSTRACT

Ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole) is an antineoplastic agent that intercalates into DNA and alters topoisomerase II activity. Unfortunately, this compound displays a number of adverse properties. Therefore, to investigate new ellipticine-based compounds for their potential as topoisomerase II-targeted drugs, we synthesized two novel derivatives, *N*-methyl-5-demethyl ellipticine (ET-1) and 2-methyl-*N*-methyl-5-demethyl ellipticinium iodide (ET-2). As determined by DNA decatenation and cleavage assays, ET-1 and ET-2 act as catalytic inhibitors of human topoisomerase IIα and are both more potent than the parent compound. Neither compound impairs the ability of the type II enzyme to bind its DNA substrate. Finally, the potency of ET-1 and ET-2 as catalytic inhibitors of topoisomerase IIα appears to be related to their ability to intercalate into the double helix.

Type II topoisomerases have been important enzyme targets for medicinal and chemotherapeutic agents since the identification of amsacrine as a topoisomerase II-targeted anticancer drug in 1984.[1] Since that time, an increasing number of naturally occurring and synthetic compounds of pharmacological importance have been reported to exert their activities by interfering with DNA topoisomerase II function.[2–12] Although lower eukaryotic species encode only one type II topoisomerase, vertebrates encode two closely related isoforms of the enzyme, topoisomerase IIα and IIβ.[13–18] These enzymes play essential roles in a number of genetic processes, including DNA replication, transcription, and recombination, as well as chromosome segregation.[13–18] Type II topoisomerases resolve problems associated with the topological constraints of the genetic material (i.e., DNA under- or overwinding, knotting, and tangling) by transiently cleaving both strands of the double helix.[13–18]

Topoisomerase II-targeted agents act in one of two manners.[14,15,19–24] *Topoisomerase II poisons* kill cells by increasing levels of covalent topoisomerase II-cleaved DNA complexes. All of the clinically relevant topoisomerase II poisons examined to date do so by interfering with the ability of the enzyme to religate cleaved DNA molecules.[14,15,19,20,22–24] Alternatively, *topoisomerase II catalytic inhibitors* act by robbing the cell of the essential catalytic functions of the type II enzymes.[19–21,23] Catalytic inhibitors have been shown to act at a variety of steps of the topoisomerase II catalytic cycle, including DNA cleavage.[19–21,23]

Ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole), a natural product first isolated from the Australian evergreen tree, is an antineoplastic agent that intercalates into DNA and alters topoisomerase II activity.[1,25–32] The compound is a mild poison against topoisomerase II from *Drosophila* and *Saccharomyces cerevisiae*.[29,30] However, most studies report that ellipticine induces little, if any, DNA cleavage mediated by mammalian type II topoisomerases and inhibits enzyme activity at higher concentrations.[1,27–29] In contrast to the parent compound, several ellipticine derivatives are potent topoisomerase II poisons in mammalian systems and display anticancer activity against human breast cancer and other solid tumors.[26,32,33] Unfortunately, these compounds induce a number of adverse effects such as dry mouth, weight loss, hemolysis, and renal toxicity. Moreover, the parent compound, ellipticine, displays poor water solubility and target specificity, and drug resistance has been observed upon prolonged administration.[27,34–36]

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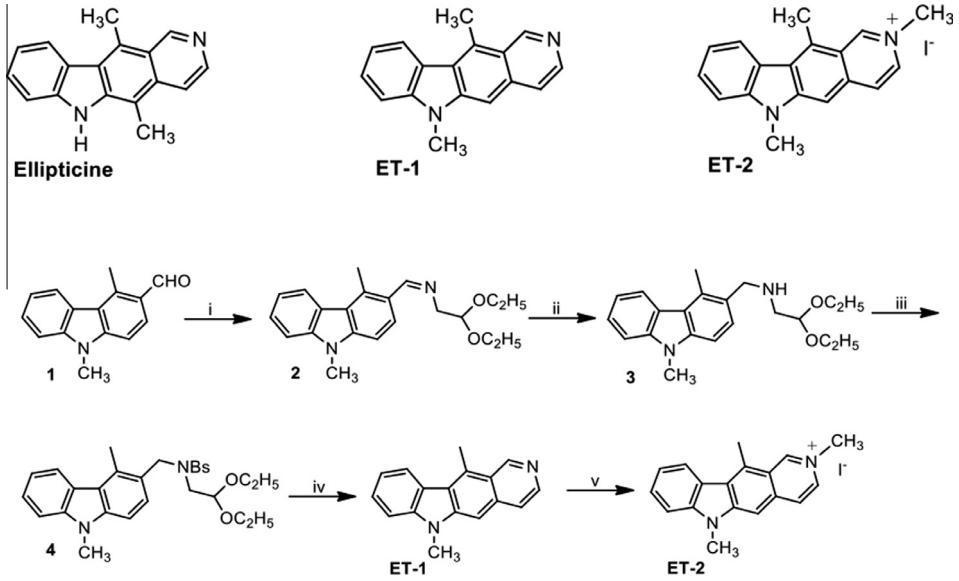

Figure 1. Structures and synthetic pathway of the compounds utilized in this study. (Top) The structures of ellipticine, *N*-methyl-5-demethyl ellipticine (ET-1), and 2-methyl-*N*-methyl-5-demethyl ellipticinium iodide (ET-2) are shown. (Bottom) The approach used to synthesize ellipticine derivatives ET-1 and ET-2 is shown. Reagents and conditions: (i) aminoacetaldehyde diethyl acetal, 100 °C, stirred, 4 h. (ii) $NaBH_4$, methanol, rt, stirred, 2 h. (iii) $N(C_2H_5)_3$, chloroform, benzenesulfonyl chloride, stirred, rt, 18 h. (iv) 6 N HCl, dioxane, N_2, reflux, 2 h. (v) CH_3I, DMF, stirred, rt, 5 h.

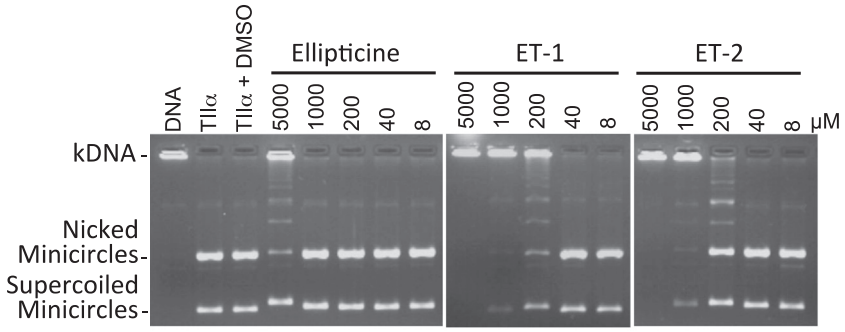

Figure 2. Effects of ellipticine, ET-1, and ET-2 (8-5000 μM) on DNA decatenation catalyzed by human topoisomerase IIα. Assays containing intact kDNA in the absence of topoisomerase IIα (DNA), or kDNA treated with topoisomerase IIα in the absence of ellipticine-based compounds (TIIα) or in the presence of compound diluent (TIIα + DMSO) are shown as controls. The positions of intact kDNA at the origin (kDNA), decatenated nicked kDNA minicircles, and decatenated supercoiled kDNA minicircles are indicated. Gels are representative of three independent experiments.

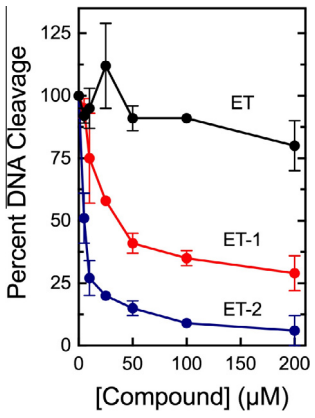

Figure 3. Effects of ellipticine, ET-1, and ET-2 on DNA cleavage mediated by human topoisomerase IIα. Results for ellipticine (ET; black), ET-1 (ET-1; red), and ET-2 (ET-2; blue) on the generation of enzyme-mediated double-stranded DNA breaks are shown. DNA cleavage levels were calculated relative to control reactions that contained no drug and were set to 100%. Error bars represent standard deviations for at least three independent experiments.

The activities of methylated, hydroxylated, and *N*-alkylated ellipticine analogs have been described previously.[27,32,33] However, the properties of C5 demethylated compounds have not been analyzed. Thus, in an effort to investigate new ellipticine-based compounds for their potential as topoisomerase II-targeted drugs, we synthesized two novel derivatives, *N*-methyl-5-demethyl ellipticine (ET-1) and 2-methyl-*N*-methyl-5-demethyl ellipticinium iodide (ET-2). Ellipticine derivatives were synthesized via a novel pathway shown in Figure 1. The detailed syntheses and physical and chemical characterizations of ET-1 and ET-2 are described in the accompanying Supplementary Data.

Briefly, ET-1 and ET-2 were generated using a nine-step synthetic pathway with a 12% overall yield (Fig. 1). First, 4,9-dimethyl-9*H*-carbazole-3-carbaldehyde (1) was synthesized in five steps, according to the literature, starting from Hagemann's ester (ethyl-2-methyl-4-oxocyclohex-2-enecarboxylate).[37,38] We then generated *N*-methyl-5-demethyl ellipticine (ET-1) and 2-methyl-*N*-methyl-5-demethyl ellipticinium iodide (ET-2) in four subsequent steps.[39] Aldehyde 1 was treated with aminoacetaldehyde diethylacetal in solvent-free conditions to yield imine 2. The imine was reduced with sodium borohydride to produce amine 3, which