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# DNA adduct formation by the anticancer drug ellipticine in human leukemia HL-60 and CCRF-CEM cells

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**Abstract**

Ellipticine induces formation of two DNA adducts in leukemia HL-60 and CCRF-CEM cells, identical with deoxyguanosine adducts generated by ellipticine metabolites 13-hydroxyellipticine and 12-hydroxyellipticine *in vitro* and *in vivo*. The ellipticine cytotoxicity to HL-60 ($IC_{50} = 0.64$ μM) and CCRF-CEM cells ($IC_{50} = 4.7$ μM) correlates with levels of DNA adducts. The different expressions of enzymes activating ellipticine in cells explain this finding. While cytochrome P450 1A1 and cyclooxygenase-1 are expressed in both cells, HL-60 cells express also high levels of another activator, myeloperoxidase. The results suggest the adduct formation as a new mode of antitumor action of ellipticine for leukemia.


*Keywords:* Ellipticine; Human leukemia HL-60 and CCRF-CEM cells; Peroxidases; Cytochromes P450; DNA adduct

## 1. Introduction

Ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole, Fig. 1), an alkaloid isolated from *Apocyanacea* plants, and several of its more soluble derivatives (9-hydroxyellipticine, 9-hydroxy-$N^2$-methyl-ellipticinium, 9-chloro-$N^2$-methyl-ellipticinium and 9-methoxy-$N^2$-methyl-ellipticinium) exhibit significant antitumor and anti-HIV activities (for a summary, see [1]). The main reason for the interest in ellipticine and its derivatives for clinical purposes is their high efficiencies against several types of cancer, their rather limited toxic side effects, and their complete lack of hematological toxicity [2]. Nevertheless, ellipticine is a potent mutagen.

Ellipticines are anticancer drugs, whose precise mechanisms of action have not yet been explained. It was suggested that the prevalent mechanisms of their antitumor, mutagenic and cytotoxic activities are (i) intercalation into DNA [3,4] and (ii) inhibition of DNA topoisomerase II activity [2,5–7]. Ellipticine and 9-hydroxyellipticine also cause selective inhibition of p53 protein phosphorylation

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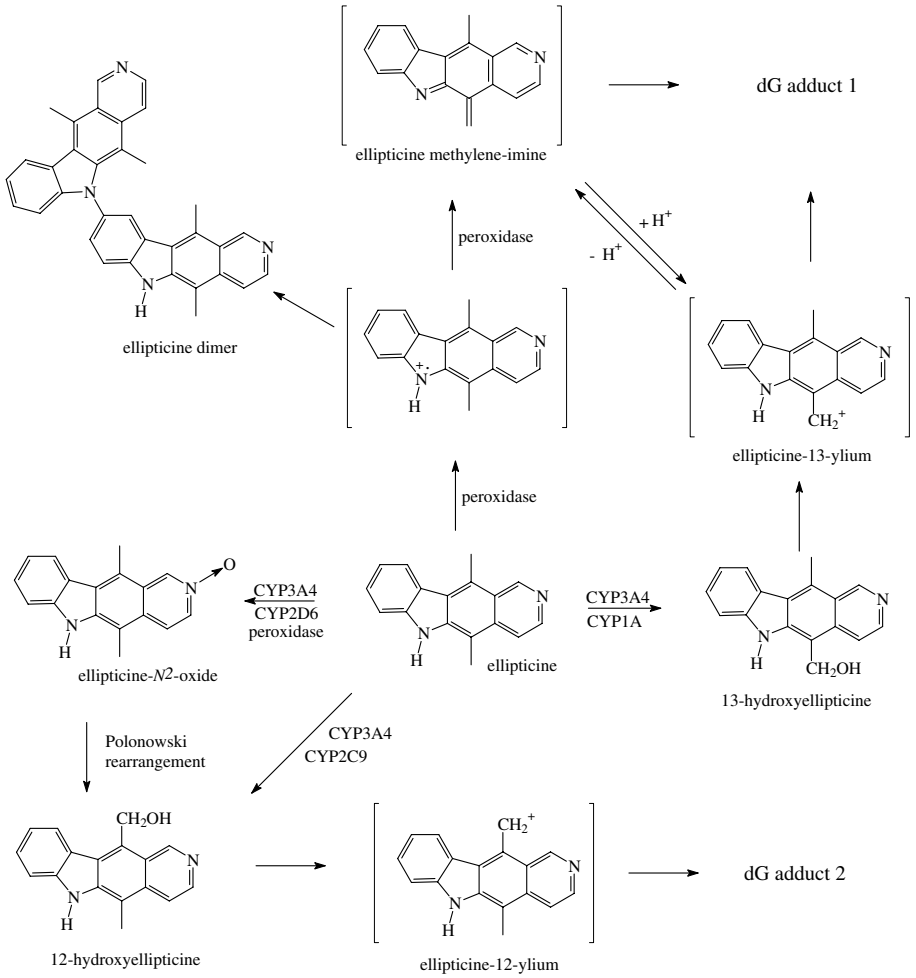


Fig. 1. Metabolism of ellipticine by peroxidases and human CYPs showing the characterized metabolites found to form DNA adducts.

in several human cancer cell lines [8,9], and this correlates with their cytotoxic activity. Ellipticines also uncouple mitochondrial oxidative phosphorylation [10] and thereby disrupt the energy balance of cells.

Recently, we found another mode of the ellipticine action [1,11,12]. We demonstrated that ellipticine covalently binds to DNA after being enzymatically activated with cytochromes P450 (CYP) or peroxidases. CYP3A4, 1A1 and 1B1 were found to be the most efficient enzymes activating ellipticine to form covalent DNA adducts *in vitro* [1]. Among the peroxidases, human cyclooxygenase (COX)-2, ovine COX-1, bovine lactoperoxidase (LPO), human myeloperoxidase (MPO) and plant horseradish peroxidase (HRP) efficiently generated ellipticine-derived DNA adducts [12,13]. Covalent DNA adducts were detected in human breast adenocarcinoma MCF-7 cells [14], in V79 Chinese hamster lung fibroblast cells transfected with human CYP3A4, 1A1 and 1A2 [15] and *in vivo* in rats exposed to this anticancer drug [16]. On the basis of these data, ellipticine might be considered a drug, whose pharmacological efficiency and/or genotoxic side effects are dependent on its enzymatic activation in target tissues.

In order to explain the mechanism of enzymatic activation of ellipticine, we have studied previously its oxidation by CYPs and peroxidases [11,17,18]. Not only the metabolites generated by both enzymatic systems, but those responsible for the formation of two major ellipticine-derived DNA adducts, were identified [12,17]. CYP enzymes oxidize ellipticine to metabolites containing one atom of oxygen, namely; 7-hydroxy-,