The Anticancer Drug Ellipticine Is a Potent Inducer of Rat Cytochromes P450 1A1 and 1A2, Thereby Modulating Its Own Metabolism

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ABSTRACT:

Ellipticine is an antineoplastic agent whose mode of action is based mainly on DNA intercalation, inhibition of topoisomerase II, and formation of covalent DNA adducts mediated by cytochromes P450 (P450s) and peroxidases. Here, this drug was found to induce CYP1A1 and/or 1A2 enzymes and their enzymatic activities in livers, lungs, and kidneys of rats treated (i.p.) with ellipticine. The induction is transient. In the absence of repeated administration of ellipticine, the levels and activities of the induced CYP1A decreased almost to the basal level 2 weeks after treatment. The ellipticine-mediated CYP1A induction increases the DNA adduct formation by the compound. When microsomal fractions from livers, kidneys, and lungs of rats treated with ellipticine were incubated with ellipticine, DNA adduct formation, measured by 32P-postlabeling analysis, was up to 3.8-fold higher in incubations with micromomes from pretreated rats than with controls. The observed stimulation of DNA adduct formation by ellipticine was attributed to induction of CYP1A1 and/or 1A2-mediated increase in ellipticine oxidative activation to 13-hydroxy- and 12-hydroxyellipticine, the metabolites generating two major DNA adducts in human and rat livers. In addition to these metabolites, increased formation of the excretion products 9-hydroxy- and 7-hydroxyellipticine was also observed in microsomes of rats treated with ellipticine. Taken together, these results demonstrate for the first time that by inducing CYP1A1/2, ellipticine increases its own metabolism, leading both to an activation of this drug to reactive species-forming DNA adducts and to detoxication metabolites, thereby modulating to some extent its pharmacological and/or genotoxic potential.

Ellipticine (5,11-dimethyl-6H-pyrido[4,3-b]carbazole; Fig. 1), an alkaloid isolated from Apocynaceae plants, exhibits significant antitumor and anti-human immunodeficiency virus activities (Auclair, 1987; Stiborová et al., 2001). The main reason for the interest in ellipticine and its derivatives for clinical purposes is their high efficacies against several types of cancer, their rather limited toxic side effects, and their complete lack of hematological toxicity (Auclair, 1987). Nevertheless, ellipticine is a potent mutagen (for a summary, see Stiborová et al., 2001).

Ellipticines are anticancer drugs whose precise mechanisms of action have not been explained yet. It was suggested that the prevalent mechanisms of their antitumor, mutagenic, and cytotoxic activities are 1) intercalation into DNA (Auclair, 1987; Singh et al., 1994), and 2) inhibition of DNA topoisomerase II activity (Auclair, 1987; Fosse et al., 1992; Froelich-Ammon et al., 1995). Ellipticine and its metabolite 9-hydroxyellipticine also cause selective inhibition of p53 protein phosphorylation in several human cancer cell lines (Sugikawa et al., 1999), and this correlates with their cytotoxic activity. Ellipticines also uncouple mitochondrial oxidative phosphorylation (Schwaller et al., 1995) and thereby disrupt the energy balance of cells.

We demonstrated that ellipticine covalently binds to DNA after being enzymatically activated with cytochromes P450 (P450s) or peroxidases (Stiborová et al., 2001, 2003c, 2004, 2007; Frei et al., 2002). Human and rat P450s of 1A and 3A subfamilies are the predominant enzymes catalyzing oxidation of ellipticine either to metabolites that are excreted from organisms (7-hydroxy- and 9-hydroxyellipticine) or form DNA adducts (12-hydroxy- and 13-hydroxyellipticine) (Stiborová et al., 2001, 2003c, 2004; Kotrbová et al., 2006). Of the peroxidases, human cyclooxygenase-2, ovine cyclooxygenase-1, bovine lactoperoxidases, human myeloperoxidase, and horseradish peroxidase efficiently generated ellipticine-doped microsomes.
derived DNA adducts (Fig. 1) (Stiborová et al., 2007). The same DNA adducts by ellipticine were also detected in human breast adenocarcinoma MCF-7 cells (Bořek-Dohalská et al., 2004); in V79 Chinese hamster lung fibroblasts transfected with human CYP3A4, 1A1, and 1A2 (Frei et al., 2002); in leukemia HL-60 and CCRF-CEM cells (Poljaková et al., 2007); and in vivo in rats exposed to this anticancer drug (Stiborová et al., 2003a). 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.

Because of the important role of P450 enzymes in ellipticine metabolism, expression levels of these enzymes are crucial for antitumor, cytostatic, and/or genotoxic activities of this drug in individual tissues. Indeed, we found recently that the formation of the major ellipticine-derived DNA adduct (adduct formed in deoxyguanosine from 13-hydroxyellipticine; Fig. 1) in different organs of rats exposed to ellipticine is dependent on expression levels of CYP3A1. This is also the most efficient rat P450 enzyme-activating ellipticine to form this DNA adduct in vitro (Stiborová et al., 2001, 2003a,c; Kotrbová et al., 2006). Likewise, the orthologous human enzyme CYP3A4 in human liver microsomes is also the most efficient enzyme activating ellipticine to 13-hydroxyellipticine and DNA adducts (Stiborová et al., 2004). In rats in vivo, however, not only CYP3A1 but also P450s of the 1A subfamily seem to be important for the formation of this adduct even though in in vitro incubations CYP1A enzymes are much less active than CYP3A1 (Stiborová et al., 2001, 2003a,c; Kotrbová et al., 2006). One of the reasons for this observed discrepancy might be the possibility of ellipticine-mediated induction of CYP1A enzymes in rats, which results in their higher protein levels and activities in vivo.

CYP1A1 and 1A2 enzymes are induced by a variety of compounds, of which dioxins such as 2,3,7,8-tetrachlorodibenzo-p-dioxin are the most effective (Couture et al., 1990). Dioxins and related compounds induce CYP1A by binding to the ligand-activated transcription factor, aryl hydrocarbon receptor (AhR). Binding of ellipticine to this transcription factor has already been described previously (Fernandez et al., 1988), suggesting that this drug may induce CYP1A expression (Cresteil et al., 1982; Gasiewicz et al., 1996). Indeed, the previous studies of Cresteil et al. (1982) have shown that ellipticine is a CYP1A inducer, although these authors did not determine which members of this subfamily were affected. The present study was aimed at elucidating this question. Because rats were found to be suitable models mimicking the fate of ellipticine in humans (Stiborová et al., 2003a,c), they were used as model organisms in this study. Furthermore, because the constitutive and induced expression of rat CYP1A is sexually dimorphic (Iba et al., 1999), rats of both sexes were used in some experiments.
microsomes of rabbits induced with phenobarbital (CYP2B4) or ethanol somal fractions from tissues of rats that had been pretreated with ellipticine. ticine or its metabolites by HPLC as described previously (Stiborová et al., had been pretreated with ellipticine were analyzed for the presence of ellip-

somes. Hepatic, renal, and pulmonary microsomal preparations from rats that
contained 0.17 and 1.19 nmol of P450/mg of protein, respectively. Using the

method of Omura and Sato (1964), no P450 was detectable in lung micro-

P450 with carbon monoxide. The total amounts of hepatic P450 are shown in

Table 1. Kidney microsomes of control and ellipticine-treated rats (40 mg/kg)

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Incubation mixtures used to study the ellipticine metabolites contained 50 mM potassium phosphate buffer, pH 7.4, 1 mM NADPH, pooled hepatic microsomal sample (0.5 mg of protein) from three male rats, either control or treated with 4, 40, and 80 mg/kg body weight of ellipticine, 100 μM ellipticine (dissolved in 7.5 μl of methanol), and 0.5 mg of calf thymus DNA in a final volume of 750 μl. The reaction was initiated by adding ellipticine. In incubations with lung and kidney microsomes, control microsomes were compared with microsomes from rats treated with 40 mg/kg body weight. Incubations were carried out at 37°C for 30 min; ellipticine-DNA adduct formation was found to be linear up to 30 min of incubation (Stiborová et al., 2004). After incubation, 5 μl of 1 mM phenacetine in methanol was added as an internal standard and the ellipticine metabolites were extracted twice with ethyl acetate (two times, 1 ml). Analyzes of ellipticine metabolites were performed by HPLC as described previously (Stiborová et al., 2004). Recoveries of ellipticine metabolites were around 95% in the presence of microsomes without a P450 cofactor (NADPH generation system).

Inhibition Studies. The following chemicals were used to inhibit the activation of ellipticine in hepatic microsomes of male rats: α-naphthoflavone (α-NF), which inhibits CYP1A1 and 1A2, being more efficient to inhibit CYP1A1 (Rendic and DiCarlo, 1997; Stiborová et al., 2005); furafylline, which inhibits CYP1A2; and troleandomycin and ketoconazole, which inhibit CYP3A (Rendic and DiCarlo, 1997). Inhibitors were dissolved in 7.5 μl of methanol to yield final concentrations of 100 μM in the incubation mixtures used to assess DNA adducts formed by ellipticine (see above). Mixtures were then incubated at 37°C for 10 min with NADPH before adding ellipticine and then incubated for a further 30 min at 37°C. After the incubation, DNA was isolated as mentioned above.

32P Postlabeling Analysis and HPLC Analysis of 32P-labeled 3′,5′-Deoxyribo nucleoside Bisphosphate Adducts. The 32P postlabeling of nucleotides using nuclease P1 enrichment procedure, found previously to be appropriate to detect and quantify ellipticine-derived DNA adducts formed in vitro (Stiborová et al., 2001, 2003a, 2004, 2007) and in vivo (Stiborová et al., 2003a), was used in the experiments. The thin-layer chromatography and HPLC analyzes were done as reported recently (Stiborová et al., 2001, 2003a,c, 2004, 2007).

Results

Effect of Ellipticine on P450 Expression. Western blots with chicken polyclonal antibodies raised against different P450s (CYP1A1, 2B4, 2E1, and 3A4) showed that the expression of hepatic
CYP1A1 and 1A2 was induced in male and female rats treated with ellipticine (Fig. 2). The expression of hepatic CYP2B, 2E1, and 3A was not altered by treating rats with ellipticine (data not shown).

Constitutive levels of CYP1A1 and 1A2 and their induction by ellipticine were sex-dependent; constitutive protein expression of CYP1A1 was higher in female rats than that of CYP1A2 in males (Fig. 2). The increase in hepatic CYP1A1 and 1A2 expression by ellipticine was dose-dependent in all cases (Fig. 2) and correlated significantly with all doses of ellipticine administered to male rats [correlation coefficients of 0.807 (p < 0.05, n = 4) for CYP1A1 and 0.896 (p < 0.05, n = 4) for CYP1A2] but only for doses of 4 and 40 mg/kg body weight administered to female rats [correlation coefficients of 0.966 (p < 0.01, n = 3) for CYP1A1 and 0.981 (p < 0.01, n = 3) for CYP1A2]. Lower induction of CYP1A1 in female rats treated with 80 mg/kg body weight may be caused by a higher sensitivity of female rats to the toxicity of the administered compound. Indeed, ellipticine at doses above 40 mg/kg was found to be toxic to rats (Cresteil et al., 1982). The dose of 80 mg/kg also caused a decrease in levels of total hepatic P450 in female rats but an increase in total P450 in males (Table 1). The EROD activity, a marker for CYP1A1 and 1A2, the MROD activity, a marker for CYP1A2, as well as the oxidation of Sudan I, a marker substrate of CYP1A1 (Stiborová et al., 2002b, 2005), increased in hepatic microsomes of rats treated with ellipticine (Table 2).

Ellipticine also induced CYP1A1 levels and its activities in kidneys and lungs (Fig. 3). Male rats were used in these experiments. Because of very low CYP1A1 protein levels and activities in kidneys and lungs of control animals, the ellipticine-mediated induction in these organs could not be quantified.

In addition to evaluating the effects of ellipticine on protein levels and enzyme activities of CYP1A1 and 1A2, modulation of their mRNA expression by this compound in male rats was also investigated. As shown in Table 3, treatment of male rats with 40 mg/kg body weight of ellipticine induced an increase in mRNA expression levels of CYP1A1 in livers, kidneys, and lungs, whereas no significant increase in CYP1A2 mRNA expression was observed.

**Ellipticine-Mediated CYP1A1 and 1A2 Induction Is Transient.** CYP1A1 and 1A2 protein expression was measured in livers of male rats 2 days and 2, 10, and 32 weeks after administration of a single i.p. dose of 80 mg of ellipticine/kg body weight. Maximum CYP1A1/2 expression levels in this organ were found 2 days after treatment, the earliest time point at which liver samples were collected. As shown in Fig. 4 a decline almost to the basal level of CYP1A1/2 protein expression and their enzymatic activities was detected 2 weeks after the dose.

**Oxidation of Ellipticine by Rat Hepatic, Renal, and Pulmonary Microsomes to DNA-Binding Metabolites.** We compared the formation of adducts in calf thymus DNA by ellipticine incubated with microsomes from the livers, kidneys, and lungs of treated and control male rats. The DNA adduct pattern generated by ellipticine consisted of at least two adducts (spots 1 and 2 in Fig. 5), which were identical to those formed in vitro in rats treated with ellipticine (Fig. 5C), in vitro incubations of DNA with ellipticine and human and rat hepatic microsomes (Stiborová et al., 2001, 2003a, 2004), and in those with 13-hydroxyellipticine (Fig. 5D) or 12-hydroxyellipticine (Fig. 5E). Lung and kidney microsomes were 22.4- and 32.7-fold less efficient than hepatic microsomes isolated from male rats (Fig. 6; Supplemental Tables S1 and S2).

Two additional minor ellipticine-derived DNA adducts (spots 6 and 7 in Fig. 5, A and B) were found in DNA incubated with ellipticine and rat hepatic microsomes. These adducts are also formed in vivo in rats treated with ellipticine (Fig. 5C), in vitro incubations of DNA with ellipticine and human and rat hepatic microsomes (Stiborová et al., 2001, 2003a, 2004), and in those with 13-hydroxyellipticine (Fig. 5D) or 12-hydroxyellipticine (Fig. 5E). Lung and kidney microsomes were 22.4- and 32.7-fold less efficient than microsomes isolated from livers (Fig. 6; Supplemental Tables S1 and S2).
the ellipticine-DNA adduct formation in hepatic microsomes of control rats was lower than those of CYP3A enzymes, in hepatic microsomes of rats pretreated with 40 mg/kg ellipticine, their contributions increased. This follows from the effect of inhibitors of P450 enzymes of both two subfamilies (Fig. 7). α-NF, an inhibitor of CYP1A1/2 with the predominant inhibitory effect on CYP1A1, and furafylline, an inhibitor of CYP1A2, decreased the levels of ellipticine-DNA adducts generated by hepatic microsomes of control rats by 62 and 50%, respectively, whereas troleandomycin and ketoconazole, selective inhibitors of CYP3A enzymes, were more effective. These two inhibitors decreased levels of ellipticine-DNA adducts by 75 to 92% (Fig. 7). This finding indicates that CYP3A enzymes play a major role in ellipticine-DNA adduct formation in livers of control (uninduced) rats. On the contrary, the effect of CYP1A inhibitors in hepatic microsomes of rats pretreated with ellipticine was up to 4 times higher than in those of control animals, being more pronounced for α-NF than for furafylline (Fig. 7, Supplemental Table S3).

The increase in levels of ellipticine-DNA adducts correlates with an increase in ellipticine oxidation by hepatic microsomes from the 40 mg of ellipticine/kg group (Figs. 6 and 8). Besides an increase in oxidation of ellipticine to 9-hydroxyellipticine and 7-hydroxyellipticine, which was expected, because these metabolites are predominantly formed by CYP1A1/2 (Stiborová et al., 2004; Kotrbová et al., 2006), up to a 2-fold increase in formation of 13-hydroxy- and 12-hydroxyellipticine, the metabolites generating DNA adducts 1 and 2, was found (Fig. 8).

**Discussion**

Ellipticine is an anticancer agent whose biological effects such as pharmacological efficiencies and its potential genotoxic side effects
ellipticine antagonize AhR activation by inducing ligands such as CYP1A1 by ellipticine results in an increase in constitutive activation (Puga, 1998), a decrease in oxidation of an endogenous substrate of ellipticine (Aimova and Stiborova, 2005). As shown by Chang and inhibition of the oxidation of other substrates of this enzyme has, until now, been limited.

In the present study, we have found that expressions of CYP1A1 and/or 1A2 proteins as well as their enzymatic activities were significantly induced by ellipticine in livers, lungs, and kidneys of Wistar rats treated with this drug. This induction might be a consequence of the ellipticine-binding to AhR described by several authors (Fernandez et al., 1988; Gasiewicz et al., 1996). Ellipticine binding allows the cytosolic AhR to translocate into the nucleus and to dimerize with AhR nuclear translocator (ARNT). The AhR-ARNT complex functions as a transcriptional activator by binding to the Ah-responsive element in the regulatory domains of CYP1A1 and CYP1A2 genes (Gasiewicz et al., 1996), thus stimulating their transcription (current study).

Another mechanism of CYP1A1 induction might result from the inhibition of the oxidation of other substrates of this enzyme by ellipticine (Aimova and Stiborová, 2005). As shown by Chang and Puga (1998), a decrease in oxidation of an endogenous substrate of CYP1A1 by ellipticine results in an increase in constitutive activation of AhR-ARNT transcriptional complexes. In addition, low levels of ellipticine antagonize AhR activation by inducing ligands such as 2,3,7,8-tetrachlorodibenzo-p-dioxin.

Differences in constitutive levels and inducibility of CYP1A1 and 1A2 by ellipticine were found in male and female rats. The induction of CYP1A1/2 proteins by ellipticine, when expressed as -fold over control values, is higher in males than in females. Similar results in constitutive levels and inducibility of CYP1A1/2 by β-naphthoflavone were found in Sprague-Dawley rats (Iba et al., 1999); males were more responsive than females to induction by β-naphthoflavone. However, the absolute levels of CYP1A1/2 protein at 40 mg/kg ellipticine seem similar in male and female rat liver (see immunodetection of these P450s shown in Fig. 2). Therefore, the relative induction of CYP1A depends on the level of the controls. The CYP1A induction by 80 mg/kg body weight in females was lower than that by the 40 mg/kg ellipticine dose. Whether a higher sensitivity of female rats to the toxicity of ellipticine (Cresteil et al., 1982) may be the reason for this observation remains to be answered. Another sex-dependent difference, which awaits further investigation, was found for the CYP1A-selective activities catalyzed by hepatic microsomes. Although there is a good agreement in -fold induction between CYP1A2 protein and MROD in males and females, this relationship holds only in females for CYP1A1 protein and Sudan I oxidation. In males, -fold induction is much greater for CYP1A1 protein, perhaps reflecting the lower accuracy of the measurement in the control animals.

The CYP1A induction by ellipticine resulted in up to 3.8-fold higher ellipticine-DNA adduct levels in incubations of ellipticine with microsomes from rats treated with this compound than in incubations with control microsomes. This increase corresponded to an increase in ellipticine oxidation to 13-hydroxy- and 12-hydroxyellipticine, the metabolites generating the two major DNA adducts. However, the increased ellipticine-DNA adduct formation caused by induction of CYP1A enzymes was 9 and 1.5 times lower than the increase in CYP1A1 and 1A2 protein levels, respectively, and was also lower than the increase in CYP1A1 and/or 1A2-mediated activities. These differences might be explained by the efficiencies of induced CYP1A1/2 to oxidize ellipticine not only to 13-hydroxy- and 12-hydroxyellipticine, but also to the metabolites that do not form DNA adducts, namely, to 9-hydroxy- and 7-hydroxyellipticine (Stiborová et al., 2004).

The results found in this study, demonstrating that ellipticine-mediated induction of CYP1A leads to an increase in ellipticine activation to species-forming DNA adducts, shed light on our previous data, showing the importance of CYP1A enzymes in ellipticine activation in vivo (Stiborová et al., 2003c). The importance of CYP1A in ellipticine-derived DNA adduct formation in vivo was initially rather surprising for us because it did not correspond to the in vitro situation (Stiborová et al., 2001, 2003a). 13-Hydroxyellipticine, the metabolite responsible for generation of adduct spot 1 due to the formation of the reactive species ellipticine-13-ylium (Fig. 1), was found to be formed by CYP1A1/2 in vitro only in low amounts (Stiborová et al., 2004). Here, we show that one of the reasons for the in vivo situation might be the induction of CYP1A with ellipticine; in hepatic microsomes from rats induced with ellipticine higher levels of ellipticine-DNA adducts were generated. Indeed, the relative contributions of CYP1A (predominantly CYP1A1) to the formation of ellipticine-DNA adducts were increased in hepatic microsomes of induced rats.

The induction of CYP1A by ellipticine is transient. In the absence of repeated administration of ellipticine, the amount and activities of the induced CYP1A decreased almost to the basal level 2 weeks after treatment.

The induction of CYP1A1 protein expression and enzymatic activities by ellipticine corresponded to elevated mRNA levels of this enzyme. Levels of CYP1A2 mRNA were, however, unaffected despite the increase in both protein and activity. Similar discrepancies between induction of CYP1A mRNAs and protein levels were observed previously (Chen et al., 1998; Degawa et al., 1998; Dickens, 2004; Stiborová et al., 2006). Detailed analyses of the time dependence of the expression levels of mRNAs and proteins of the tested enzymes were not performed in this study, but they might answer the questions whether the transient induction of the mRNAs of CYP1A, or the different half-lives for their mRNAs and proteins, and/or the effects of ellipticine on the stability of mRNAs and proteins of these enzymes are the rationale for our observation.
The rat was used as an experimental model on the basis that the results should provide some indication of what might occur in patients treated with the drug. However, it should be noted that the similarities as well as differences in the CYP1A systems and their induction exist in the two species. For example, in rats CYP1A1 is often more markedly induced in liver than CYP1A2, whereas the reverse is true for humans (Dickins, 2004). Furthermore, the specificity of rat and human CYP1A1 and 1A2 is known to differ, particularly for inhibitors (Guengerich and Shimada, 1991; Stiborová et al., 2001).

Recently, we have demonstrated that ellipticine is oxidatively activated to species binding to DNA also in human cancer cells such as the breast adenocarcinoma MCF-7 (Bořek-Dohalská et al., 2004) and leukemia HL-60 and CCRF-CEM cells (Poljaková et al., 2007). CYP1A1 is expressed in all these cells and was found to be one of the enzymes responsible for ellipticine-DNA binding and its cytotoxicity against these cancer cells (Bořek-Dohalská et al., 2004; Poljaková et al., 2007). Likewise, Rekha and Sladek (1997) demonstrated that the cytotoxic activity of ellipticine to MCF-7 cells depends on the levels of CYP1A enzymes. The cited authors show that MCF-7 cells treated with another CYP1A inducer, 3-methylcholanthrene, transiently expressed elevated levels of CYP1A and were transiently much more sensitive to ellipticine. The DNA adducts we have observed in MCF cells might be responsible for the higher sensitivity observed by the above authors.

Another important feature related to the expression of CYP1A and ellipticine toxicity was detected in MCF-7 cells selected for resistance to doxorubicin hydrochloride (Adriamycin) (AdrR MCF-7), exhibiting the phenotype of multidrug resistance (Ivy et al., 1988). Ivy et al. (1988) postulated that the resistance of AdrR MCF-7 cells involves several biochemical and genetic changes besides multidrug resistance. One of them is a regulatory defect at the level of CYP1A1 mRNA resulting in lower CYP1A1-mediated metabolism of xenobiotics in these cells. AdrR MCF-7 cells are cross-resistant to ellipticine (Ivy et al., 1988), which we would explain by a decrease in the CYP1A1-dependent activation of ellipticine.

Another P450 enzyme expressed in MCF-7 cells and breast cancer, CYP1B1 (Shehin et al., 2000), is known to activate ellipticine, and transcriptional activation of Cyp1b1 is believed to involve the AhR (Kerzee and Ramos, 2001). Therefore, its induction by ellipticine should also be evaluated. Taken together, the data in these studies indicate that the expression levels, activities, and induction of CYP1A1 and 1B1, the enzymes activating ellipticine, may be an important factor in the specificity and efficiency of ellipticine for breast cancer. In this context, it will be important to evaluate whether ellipticine induces CYP1A1 and 1B1 expression in MCF-7 cells. Therefore, the study investigating the induction of CYP1A1 and 1B1 expression and their activities by ellipticine and their efficiencies to activate this chemical in MCF-7 cells is under way in our laboratory.

In conclusion, the results of the present study show for the first time...
that ellipticine is capable of inducing CYP1A1, an enzyme that is involved in ellipticine biotransformation in both rats and humans, in rat livers, kidneys, and lungs. By such effects, ellipticine might modulate its own disposition, pharmacological efficiency, and/or genotoxic potential. Nevertheless, this feature has to be established in vivo.

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References


