Tyrosine kinase inhibitors vandetanib, lenvatinib and cabozantinib modulate oxidation of an anticancer agent ellipticine catalyzed by cytochromes P450 *in vitro*

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Running headline: Tyrosine kinase inhibitors influence the cytochrome P450-mediated metabolism of ellipticine

KEYWORDS

Tyrosine kinase inhibitors; Ellipticine; Cytochromes P450; Peroxidases; Ellipticine oxidation.

OBJECTIVES: Vandetanib, lenvatinib, and cabozantinib are tyrosine kinase inhibitors (TKIs) targeting VEGFR subtypes 1 and 2, EGFR and the RET-tyrosine kinase, thus considered as multiple TKIs. These TKIs have already been approved for treating patients suffering from thyroid cancer and renal cell carcinoma. Ellipticine, a DNA-damaging drug, is another anticancer agent that is effective against certain tumors of the thyroid gland, ovarian carcinoma, breast cancer and osteolytic breast cancer metastasis. Its anticancer efficiency is dictated by its oxidation with cytochrome P450 (CYP) and peroxidase enzymes. A number of studies testing the effectiveness of individual anticancer drugs, the pharmacological efficiencies of which are affected by their metabolism, alone or in a combination with other cytostatics demonstrated that such combination can have both positive and negative effects on treatment regimen. The aim of this study was to study the effect of vandetanib, lenvatinib and cabozantinib on oxidation of ellipticine which dictates its pharmacological efficiency.

METHODS: Ellipticine oxidation catalyzed by hepatic microsomes, recombinant CYP enzymes and peroxidases (horseradish peroxidase, lactoperoxidase and myeloperoxidase) and the effect of TKIs (vandetanib, lenvatinib and cabozantinib) on this oxidation were analyzed by HPLC used for separation of ellipticine metabolites and quantification of their amounts formed during oxidation.

RESULTS: The CYP enzymatic system oxidizes ellipticine up to five metabolites (9-hydroxy-, 12-hydroxy-, 13-hydroxy-, 7-hydroxyellipticine, and ellipticine N^2- oxide), while peroxidases form predominantly ellipticine dimer. Ellipticine oxidation catalyzed by rat and human hepatic microsomes was inhibited by vandetanib and cabozantinib, but essentially no inhibition was caused by lenvatinib. Of individual CYP enzymes catalyzing oxidation of ellipticine, TKIs inhibited oxidation of ellipticine catalyzed by CYP2D6 > 2D1 > 2C9 > 3A1 > 3A4, the CYP enzymes participating in ellipticine oxidation to metabolites increasing the ellipticine anticancer efficiency. On the contrary, they have essentially no inhibition effect on ellipticine oxidation catalyzed by CYP1A1 and 1A2, which are the enzymes that predominantly detoxify this drug. All tested TKIs had essentially no effect on oxidation of ellipticine by used peroxidases.

CONCLUSION: The results found demonstrate that TKIs vandetanib, lenvatinib and cabozantinib cause a decrease in oxidative activation of DNA-damaging drug ellipticine by several CYP enzymes *in vitro* which might lead to a decrease in its pharmacological efficiency. In contrast, they practically do not influence its detoxification catalyzed by CYP1A1, 1A2 and peroxidases. The present study indicates that tested TKIs seem not to have a potency to increase ellipticine anticancer efficiency.

Abbreviations

CYP - cytochrome P450

DMSO – dimethyl sulfoxide

HRP - horseradish peroxidase

EGFR - epidermal growth factor receptor

LPO - lactoperoxidase

MPO - myeloperoxidase

NADPH:CYP oxidoreductase - POR

ND – not determined

NQ – not quantified

RET – rearranged during transfection protooncogene

r.t. – retention time

TK – tyrosine kinase

TKI – tyrosine kinase inhibitor

VEGFR - vascular endothelial growth factor receptor

INTRODUCTION

Cancer treatment is one of the most difficult problems in clinic practice. The drugs utilized for cancer chemotherapy have usually a narrow therapeutic index, and often the produced responses are only palliative as well as unpredictable. Namely, although the drugs are directed toward certain biomacromolecules, they do not discriminate between rapidly dividing tumor *vs.* non-malignant cells [Heger et al., 2013]. In contrast, targeted therapy that has been introduced in recent years is directed against cancer-specific targets and signaling pathways, and thus provides more limited nonspecific mechanisms [Arora and Scholar, 2005]. One of the most promising targets are receptor tyrosine kinases (TKs), the enzymes that selectively phosphorylate the hydroxyl moieties of tyrosine residues on signal transduction molecules with a phosphate moiety from adenosine triphosphate [Reibenwein and Krainer, 2008; Hartmann et al., 2009]. Vandetanib, lenvatinib and cabozantinib are tyrosine kinase inhibitors (TKIs) targeting vascular endothelial growth factor receptor (VEGFR) subtypes 1 and 2, epidermal growth factor receptor (EGFR) and the RET

(rearranged during transfection)-tyrosine kinase, thus considered as multiple TKIs. These TKIs have already been approved for treating patients suffering from thyroid cancer and renal cell carcinoma, and further clinical trials are ongoing for prostate cancer and glioblastoma multiforme [Greenhill, 2017; Roviello et al., 2018; Abdelaziz and Vaishampayan, 2017].

Ellipticine (Fig. 1) and its derivatives are other anticancer agents that are effective against certain tumors of the thyroid gland (anaplastic thyroid carcinoma, medullary thyroid carcinoma), ovarian carcinoma, breast cancer and osteolytic breast cancer metastasis [Stiborova et al., 2001; 2011; Kumarasamy and Sun, 2017]. The predominant mechanisms of ellipticine's biological effects were suggested to be (i) intercalation into DNA [Garbett and Graves, 2004; Tmejova et al., 2014] and (ii) inhibition of topoisomerase II [Garbett and Graves, 2004; Stiborova et al., 2011; Kizek et al., 2012; Stiborova and Frei, 2014]. Further, ellipticine anticancer efficiencies are dependent on its metabolism leading both to the activation metabolites causing DNA damage (covalent DNA adducts) and their detoxification to products that are excreted. Ellipticine is oxidized by microsomal cytochrome P450 (CYP) enzymes and peroxidases. Its oxidative activation by CYP3A, 2C and 2D leads to formation of 12-hydroxy- and 13-hydroxyellipticine, reactive metabolites that are converted to ellipticine-12-ylium and ellipticine-13-ylium, binding to DNA, while formation 9-hydroxyellipticine and the ellipticine dimer catalyzed by CYP1A1/2 and peroxidases, respectively, are considered to be detoxification pathway of its metabolism (Fig. 1) [Stiborova et al., 2004; 2011; Stiborova and Frei, 2014].

Overall, in cancer chemotherapy, serious clinical consequences may occur from small alterations in drug metabolism affecting drug pharmacokinetics. Such alterations might be caused by several reasons, of them the drug-drug interactions

influencing their metabolism might be one of most important. A number of studies testing the effectiveness of individual anticancer drugs alone or in a combination with other cytostatics demonstrated that such combination can have additive and/or contradictory effects on treatment regimen [for a review, see Stiborova et al., 2012a]. In this context, ellipticine anticancer effects have been found to be increased by another drug, an histone deacetylase inhibitor valproic acid (VPA), which is mediated by its influence on ellipticine metabolism [Poljakova et al., 2011; Cerna et al., 2018]. The aim of this study was to investigate the effect of additional anticancer drugs, TKIs vandetanib, lenvatinib and cabozantinib, namely, their effects on oxidative metabolism of ellipticine dictating its pharmacological efficiency.

MATERIALS AND METHODS

Chemicals and material

Vandetanib, lenvatinib and cabozantinib were from LC Laboratories (Woburn, MA, USA), ellipticine, NADPH, horseradish peroxidase (HRP) type VI, bovine lactoperoxidase (LPO), human myeloperoxidase (MPO) and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) in ACS purity (purity meets the standards of American Chemical Society), unless noted otherwise. Rat microsomes were isolated from liver of male rats (Wistar) as described previously [Stiborova et al., 2001]. Male human hepatic microsomes (pooled sample) (sample LOT: 3043885), were from Gentest Corp. (Woburn, MA, USA). Human and rat recombinant enzymes were used in the forms of Supersomes™ that are microsomes isolated from insect cells transfected with a baculovirus construct containing cDNA of human and rat CYP enzymes (CYP1A1, 1A2, 2C9, 2D1/6, 3A1/4), and which also

express NADPH:CYP oxidoreductase (POR) and/or cytochrome b_5. They were purchased from Gentest Corp. (Woburn, MA, USA).

Oxidation of ellipticine by hepatic microsomes and CYP enzymes and the effect of TKIs on this oxidation

Unless stated otherwise, incubation mixtures used to study ellipticine metabolism contained the following in a final volume of 500 µl: 100 mM potassium phosphate buffer (pH 7.4), 1 mM NADPH, rat or human hepatic microsomes (0.25 mg protein), or rat or human recombinant CYPs in Supersomes™ (50 pmol) and 50 µM ellipticine dissolved in 5 µl dimethyl sulfoxide (DMSO). When the effect of TKIs vandetanib, lenvatinib and cabozantinib was investigated, the incubation mixtures also contained 50 µM TKIs dissolved in 5 µl DMSO. The reaction was initiated by adding ellipticine. In the control incubations, either microsomes or CYP or NADPH or TKIs or ellipticine were omitted. After incubation at 37°C for 20 min in open plastic Eppendorf tubes (ellipticine oxidation was linear up to 30 min of incubation [Kotrbova et al., 2006; Stiborova et al., 2006]) and 5 µl of 1 mM phenacetine in methanol was added as an internal standard, the reaction was stopped by extraction with ethyl acetate (twice with ethyl acetate, 2 x 1 ml). The extracts were evaporated, dissolved in 50 µl of methanol and ellipticine and its metabolites were separated by HPLC (5 mm Ultrasphere ODS Beckman, 4.6 x 250 mm preceded by a C-18 guard column); the eluent was 64% methanol plus 36% of 5 mM heptane sulfonic acid in 32 mM acetic acid in water with a flow rate of 0.7 ml/min, detection was at 296 nm. Ellipticine metabolites eluted by HPLC were characterized by mass spectroscopy and/or NMR as described [Stiborova et al., 2004; 2006]. Up to five ellipticine metabolites with the retention times of 5.8, 6.0, 6.8, 7.0 and 9.9 min, corresponding to 9-hydroxy-, 12-hydroxy-, 13-hydroxy-, 7-hydroxyellipticine and ellipticine N^2- oxide, were separated

[Stiborova et al., 2004; 2006]. Recoveries of ellipticine metabolites were approximately 95%. In the incubation mixture containing lenvatinib, one broad peak of this TKI eluting with a retention time (r.t.) of 5.8 min overlapped the peaks of 9-hydroxy- and/or 12-hydroxyellipticine. Therefore, their amounts could not been evaluated (determined).

Oxidation of ellipticine by peroxidases and the effect of TKIs on this oxidation

Incubation mixtures used to evaluate the oxidation of ellipticine by the studied peroxidases (HRP, LPO and MPO), in a final volume of 500 µl, consisted of 100 mM potassium phosphate buffer (pH 7.4), 10 µM ellipticine (dissolved in 1 µl DMSO), 1, 2 or 2 µg of HRP, LPO or MPO, respectively, and 50 µM hydrogen peroxide. When the effect of TKIs vandetanib, lenvatinib and cabozantinib was investigated, the incubation mixtures also contained 10 µM TKIs dissolved in 1µl DMSO. All reactions were initiated by adding ellipticine dissolved in DMSO. Control incubations were either without peroxidases, or without hydrogen peroxide, or without TKIs, or without ellipticine. Incubations were carried out at 37°C for 15 min. After incubations, 5 µl of 1 mM of phenacetine in methanol was added as an internal standard, and the ellipticine metabolites were extracted twice with ethyl acetate (2 × 1 ml) as described [Stiborova et al., 2007]. The extracts were evaporated and dissolved in 50 µl of methanol. Ellipticine and its metabolites were separated by HPLC. The column used was a 5 µm Ultrasphere ODS (Beckman, 4.6 × 250 mm) preceded by a C-18 guard column. The eluents were 45-90% methanol in 10 mM ammonium acetate (pH 2.8), with flow rate of 0.8 ml/min, detection was at 296 nm [Stiborova et al., 2004; 2007; Poljakova et al., 2005]. Recoveries of ellipticine metabolites were around 95% in the presence of enzymes without hydrogen peroxide. One product peak with r.t. of 16.0 min and unconverted ellipticine with r.t. of 11.8 min were separated by HPLC. The ellipticine

198 metabolite was identified by mass spectroscopy, NMR and/or cochromatography on
199 HPLC to be ellipticine dimer as described previously [Stiborova et al., 2007].
200 *Statistical analyses*
201 For statistical data analysis we used Student's *t*-test. All *P*-values are two-tailed and
202 considered significant at the 0.05 level.
203
204 **RESULTS AND DISCUSSION**
205 *The effect of vandetanib, lenvatinib and cabozantinib on ellipticine oxidation*
206 *catalyzed by rat and human hepatic microsomes*
207 In the study, liver microsomes of rats and humans were used as model *in vitro*
208 systems, because rats have been shown to mimic the metabolism of ellipticine in
209 humans, and the liver rich in enzymes biotransforming xenobiotics including drugs is
210 the major organ responsible for metabolism of these chemicals [Stiborova et al.,
211 2006; Stiborova and Frei, 2014]. Ellipticine was oxidized by rat and human hepatic
212 microsomes up to three metabolites (9-hydroxy-, 12-hydroxy- and 13-
213 hydroxyellipticine) (Fig. 2) that were separated by HPLC (see insert in Fig. 2A). Other
214 two metabolites (7-hydroxyellipticine and ellipticine N^2-oxide) (Stiborova et al., 2004;
215 2006) were formed at very low amounts (if any) and, therefore, they were not
216 quantified (NQ) (Fig. 2).
217 Of ellipticine reaction products formed in the systems, 9-hydroxyellipticine is
218 considered as a detoxification metabolic product, whereas 12-hydroxy- and 13-
219 hydroxyellipticine are the activation metabolites participating in an increase in
220 ellipticine anticancer efficiency due to the formation of covalent DNA adducts
221 [Stiborova et al., 2011; Stiborova and Frei, 2014]. The same HPLC method utilized
222 for separation of ellipticine metabolites was also used to examine the effect of TKIs

on ellipticine oxidation. Vandetanib and cabozantinib were eluted from the HPLC column at retention times different from those of ellipticine and its metabolites (data not shown). However, lenvatinib was eluted as a broad peak at retention time of 5.8 min, frequently overlapping the peaks of 9-hydroxy- and/or 12-hydroxyellipticine (see insert in Fig. 2B). Therefore, formation of these metabolites could not been determined (ND). Formation of several ellipticine metabolites in these microsomal systems was inhibited by the tested TKIs. Oxidation of ellipticine to its metabolites (9-hydroxy-, 12-hydroxy- and 13-hydroxyellipticine) by rat hepatic microsomes was inhibited by vandetanib and cabozantinib, while lenvatinib had no effect on ellipticine oxidation to 13-hydroxyellipticine catalyzed by this rat enzymatic system (Fig. 2A). In the case of human hepatic microsomes, tested TKIs did not inhibit the formation of an ellipticine detoxification product 9-hydroxyellipticine, while except of lenvatinib, they inhibited formation of the activation metabolites 12-hydroxy- and 13-hydroxyellipticine. In contrast, a slight, but non-significant (P = 0.1) increase in ellipticine oxidation to 13-hydroxyellipticine was produced by lenvatinib in human microsomes (Fig. 2B).

The effect of vandetanib, lenvatinib and cabozantinib on ellipticine oxidation catalyzed by rat and human recombinant CYPs

In order to evaluate the impact of TKIs on inhibition of ellipticine oxidation catalyzed by individual microsomal CYP enzymes, we investigated their effects on ellipticine oxidation by several CYP enzymes, especially those, which are known to be essential for its oxidation. Namely, again the CYPs important both for its oxidative detoxification to metabolites that are excreted from the body and for its activation to reactive metabolites responsible for formation of covalent DNA adducts leading to higher ellipticine anticancer efficiencies [for a review, see Stiborova et al., 2011;

Stiborova and Frei, 2014]. For such a study, rat and human recombinant CYPs were utilized. Of the CYP enzymes predominantly oxidizing ellipticine, rat and human CYP1A1/2 which mainly detoxify ellipticine, and human CYP2C9, rat CYP2D1 and its human orthologue CYP2D6, and rat CYP3A1 and its human orthologue CYP3A4, the enzymes which activate ellipticine to more reactive metabolites, were employed. Depending on individual CYPs, they oxidized ellipticine up to five metabolites, 9-hydroxy-, 12-hydroxy-, 13-hydroxy-, 7-hydroxyellipticine, and ellipticine N^2- oxide (Figs. 3 and 4).

Of the used CYPs, TKIs had essentially no inhibitory effect on ellipticine oxidation catalyzed by CYP1A1 and 1A2. These CYPs oxidized ellipticine up to four metabolites, 9-hydroxy-, 12-hydroxy-, 13-hydroxy- and 7-hydroxyellipticine, while no ellipticine N^2-oxide was detectable (Fig. 3). In contrast to no inhibition of ellipticine oxidation mediated by CYP1A1 and 1A2, an increase in levels of CYP1A1-mediated formation of 9-hydroxyellipticine and 7-hydroxyellipticine was mediated by cabozantinib (Fig. 3A). In the case of human CYP1A1, this TKI (cabozantinib) also stimulated oxidation of ellipticine to 12-hydroxyellipticine, while lenvatinib increased the formation of 13-hydroxyellipticine (Fig. 3B). Oxidation of ellipticine by rat CYP1A2 also resulted in formation of low amounts of 7-hydroxyellipticine, formation of which was increased by vandetanib and cabozantinib during reactions catalyzed by rat CYP1A2. Cabozantinib also stimulated oxidation of ellipticine to 9-hydroxyellipticine in this rat enzymatic system (Fig. 3C). TKIs did not inhibit any of the ellipticine metabolites formed by human CYP1A2. Of note, 12-hydroxyellipticine is formed at very low amounts by this human CYP (CY1A2), and therefore, it was not quantified (Fig. 3D).

In contrast to CYP1A1 and 1A2, TKIs inhibited oxidation of ellipticine catalyzed by CYP2D6 > 2D1 > 2C9 > 3A1 > 3A4, the CYP enzymes participating in ellipticine oxidation to metabolites increasing the ellipticine anticancer efficiency (Figs. 3 and 4). Concerning the degree of inhibition effects of TKIs on these ellipticine metabolites, the formation of ellipticine N^2-oxide predominantly catalyzed by human CYP2D6 (Fig. 4), which is also the major enzyme forming this ellipticine metabolite, was the most prominent inhibition caused by all analyzed TKIs. This human CYP (CYP2D6) and its rat orthologue (CYP2D1) did not oxidize ellipticine to 7-hydroxyellipticine (Fig. 4). The ellipticine N^2-oxide is the important activation metabolic product, because it forms 12-hydroxyellipticine (by Polonowski rearrangement) [Stiborova et al., 2004], which finally forms ellipticine-12-ylium generating DNA adducts (Fig. 1). Its formation by CYP2D1 was also inhibited by TKIs, but to a lower extent. On the contrary, no inhibition of production of this metabolite catalyzed by CYP3A4 (in the presence of cytochrome b_5) was observed (Fig. 4F).

Another activation metabolite, 12-hydroxyellipticine, formed by most analyzed CYPs was not inhibited by tested TKIs. This is unexpected finding when we compare the results showing the inhibition of 12-hydroxyellipticine formation in hepatic microsomes; a decrease in amounts of this metabolite formed in microsomes was produced by TKIs (see Fig. 2). We can speculate that this observed inhibition of 12-hydroxyellipticine formation (catalyzed by many CYPs in hepatic microsomes and also rearranged from ellipticine N^2-oxide) might result from inhibition of its primarily formed ellipticine N^2-oxide that therefore cannot be rearranged to 12-hydroxyellipticine and thus also not inhibited. This suggestion needs, however, to be investigated in further studies.

296 Oxidation of ellipticine to 13-hydroxyellipticine, the most important activation
297 metabolite generating DNA adducts, was inhibited by lenvatinib in the enzymatic
298 system of CYP3A4 and by all TKIs in the system of CYP3A1. But inhibition of 13-
299 hydroxyellipticine formation catalyzed by the CYP3A1 mediated by vandetanib and
300 lenvatinib was not significant (P = 0.06 and P = 0.08, respectively) (Fig. 4). The
301 CYP3A enzymes oxidized ellipticine up to four metabolites, 13-hydroxyellipticine
302 being the predominant oxidation product. Whereas 9-hydroxy-, 12-hydroxy-, 13-
303 hydroxyellipticine are formed by CYP3A1, the human orthologue CYP3A4 can also
304 generate ellipticine N^2-oxide, but only when cytochrome b_5 is present in the reaction
305 mixture. No 7-hydroxyellipticine was formed by CYP3A1/4 (Fig. 4D-F). The found
306 results indicate that inhibition of 13-hydroxyellipticine formation in hepatic
307 microsomes by vandetanib and cabozantinib might be attributed to CYP3A enzymes.
308 However, no inhibition of 13-hydroxyellipticine formation by lenvatinib, found in both
309 used subcellular enzymatic systems (rat and human hepatic microsomes) indicates
310 that the situation in these microsomal systems is more complex. Now, we can only
311 speculate on the reasons of these results. One of them can be the influence of
312 lenvatinib on 13-hydroxyellipticine formation catalyzed by CYP1A1 that stimulates 13-
313 hydroxyellipticine formation; namely, cytochrome b_5 that is the heme protein highly
314 expressed in hepatic microsomal system is known to increase the CYP1A1-mediated
315 formation of 13-hydroxyellipticine in microsomes [Kotrbova et al., 2011; Stiborova et
316 al., 2012b]. This feature might partially compensate the inhibition of its formation
317 caused by other CYP enzymes in microsomes. However again, such suggestion
318 needs to be evaluated in future study.
319 *The effect of vandetanib, lenvatinib and cabozantinib on ellipticine oxidation*
320 *catalyzed by peroxidases*

Oxidation of ellipticine to its major metabolite formed by peroxidases HRP, LPO and MPO, ellipticine dimer, which is considered to be a detoxification reaction product of peroxidase-mediated ellipticine oxidation [Stiborova et al., 2007], was utilized to investigate the effect of TKIs on reaction catalyzed by peroxidases. All tested peroxidases oxidized ellipticine to this metabolite, but to a different extent (Fig. 5). The tested TKIs had essentially no effect on formation of ellipticine dimer catalyzed by these peroxidases; only cabozantinib slightly increased oxidation of ellipticine by HRP while lenvatinib slightly inhibited the reaction catalyzed by LPO.

CONCLUSIONS

The results of this study demonstrate that oxidation of anticancer drug ellipticine mediated by CYP enzymes expressed in rat and human hepatic microsomal subcellular fractions, which determines its pharmacological (anticancer) efficiencies, is influenced by TKIs vandetanib, lenvatinib and cabozantinib. The combination effects of ellipticine with tested TKIs were investigated, because they are the drugs utilized for treatment of thyroid gland cancer, exhibiting specific efficiencies to the individual types of this cancer [Reibenwein et al., 2008; Hartmann et al., 2009; Stiborova and Frei 2014]. But, they act by different mechanisms; the DNA is target for ellipticine action, while TKIs regulate signaling of their enzymatic targets, TKs [Greenhill, 2017; Roviello et al., 2018; Abdelaziz and Vaishampayan, 2017]. What is however not known is whether they can influence the anticancer potency of them. Especially, it is not known whether TKIs can affect the metabolism of ellipticine, which dictate its DNA-damaging efficiency, thereby modulating its therapeutic effects when administered in combinations.

The data found demonstrate that TKIs vandetanib, lenvatinib and cabozantinib inhibit the *in vitro* oxidative activation of ellipticine catalyzed by several CYP enzymes and hepatic subcellular systems expressing these enzymes, which might lead to a decrease in ellipticine anticancer efficiency. In contrast, they practically do not influence its detoxification catalyzed by CYP1A1, 1A2 and peroxidases. All these results suggest that the TKIs might decrease the ellipticine-DNA-damaging effect mediated by the tested enzymes, thereby being ineffective to increase ellipticine anticancer efficiency. The *vice versa* effects, namely the influence of ellipticine on enzyme-mediated metabolism of the tested TKIs, which has not been unfortunately studied in details as yet [Martin et al., 2012; Lacy et al., 2015; Nguyen et al., 2015; Shumaker et al., 2015; Dubbelman et al., 2016], are the challenge of our future research.

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452 vivo and generated from 12-hydroxyellipticine and 13-hydroxyellipticine. *Int J*
453 *Cancer* 120: 243-251.
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462 Frei E (2012b). Ellipticine oxidation and DNA adduct formation in human
463 hepatocytes is catalyzed by human cytochromes P450 and enhanced by
464 cytochrome b_5. *Toxicology*. 302: 233-241.
465 29. Stiborova M and Frei E (2014) Ellipticines as DNA-targeted chemotherapeutics.
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Figure 1. Scheme of ellipticine metabolism catalyzed by CYPs and peroxidases showing the identified metabolites and those proposed to form DNA adducts. The compounds showed in brackets were not detected under the experimental conditions and/or not yet structurally characterized. The CYP enzymes predominantly oxidizing ellipticine shown in the figure were identified in our previous studies [Stiborova et al., 2004; 2008; 2012b; Kotrbova et al., 2011].

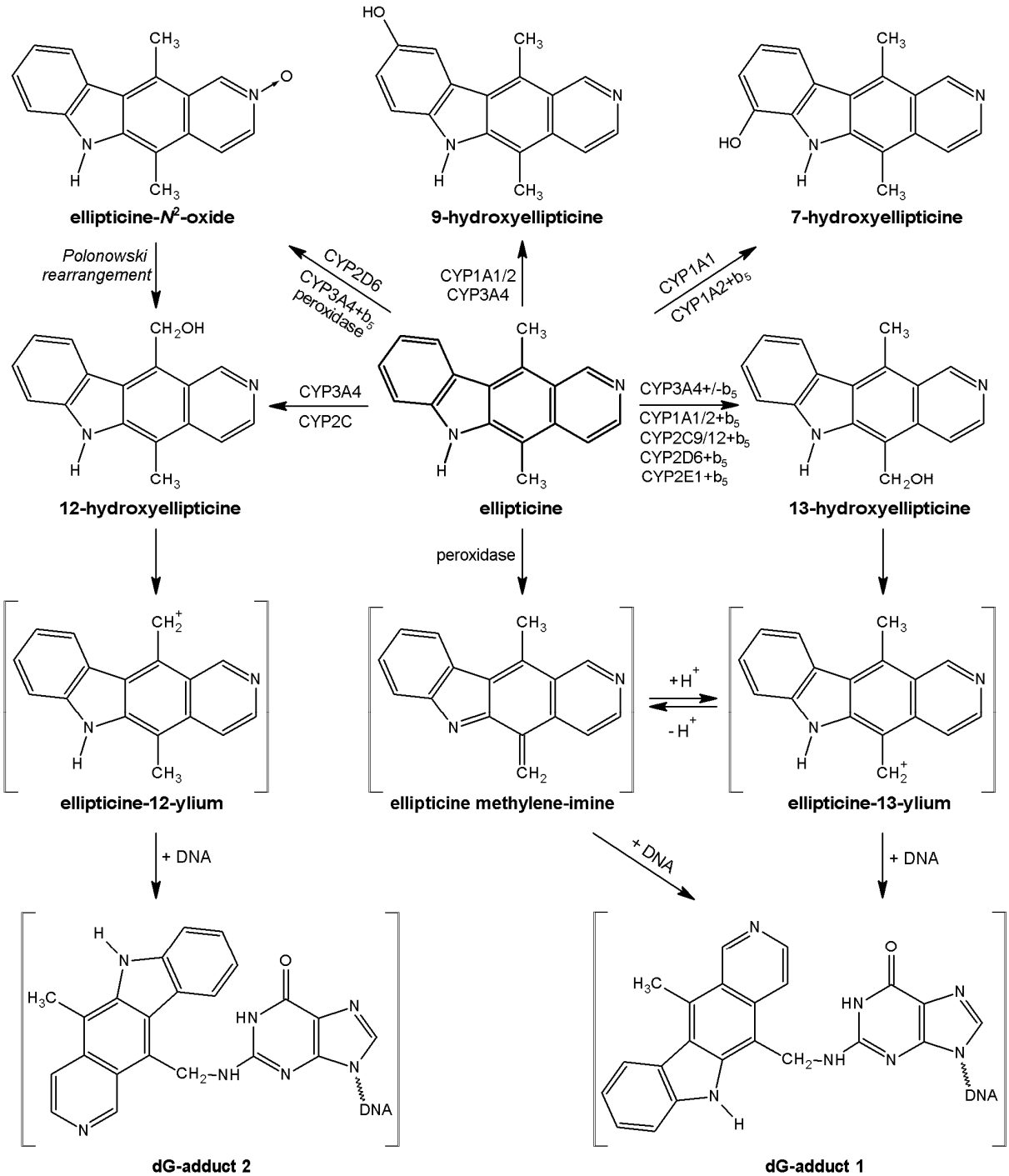
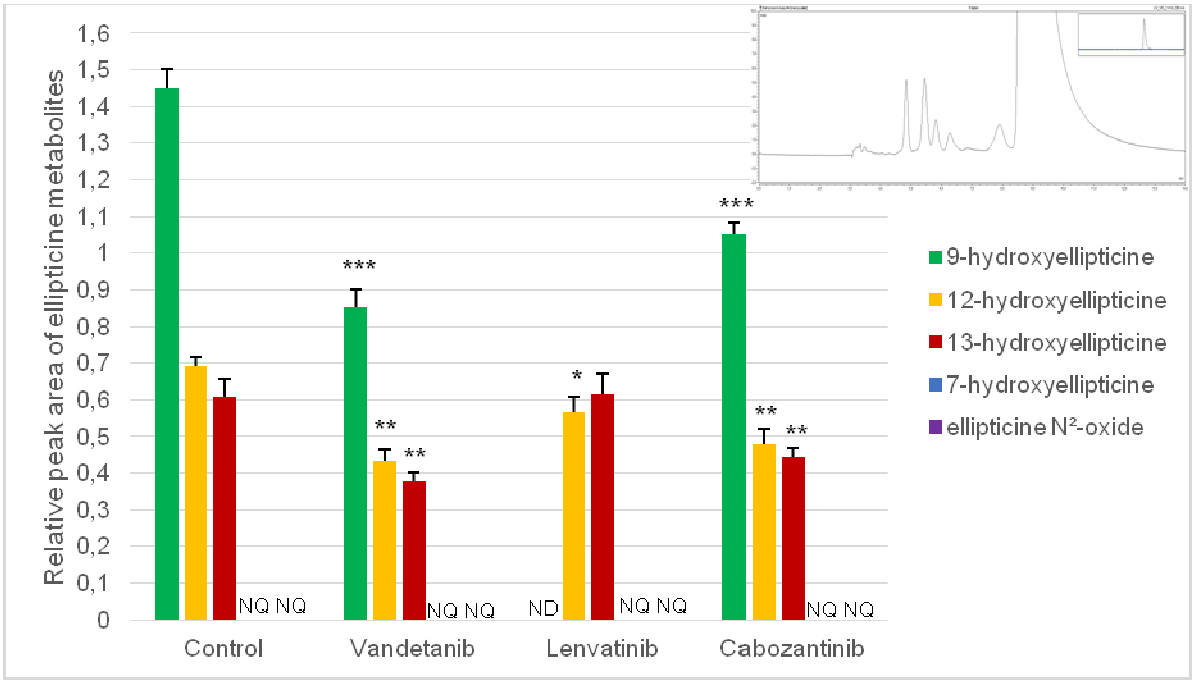


Figure 2. Oxidation of ellipticine by rat (**A**) and human (**B**) hepatic microsomes and the effect of vandetanib, lenvatinib and cabozantinib on this oxidation. The data are averages and standard deviations of three experiments. ***P<0.001; **P<0.01; *P<0.05 (Student's t-test), levels of ellipticine metabolites in the presence of TKIs significantly different from those generated without these inhibitors. NQ – not quantified, ND – not determined. *Insert* in 2A shows HPLC of ellipticine metabolites formed by rat hepatic microsmes. *Insert* in 2B shows HPLC of ellipticine metabolites formed by human microsomes from incubations in the presence of lenvatinib.

(**A**)



(B)

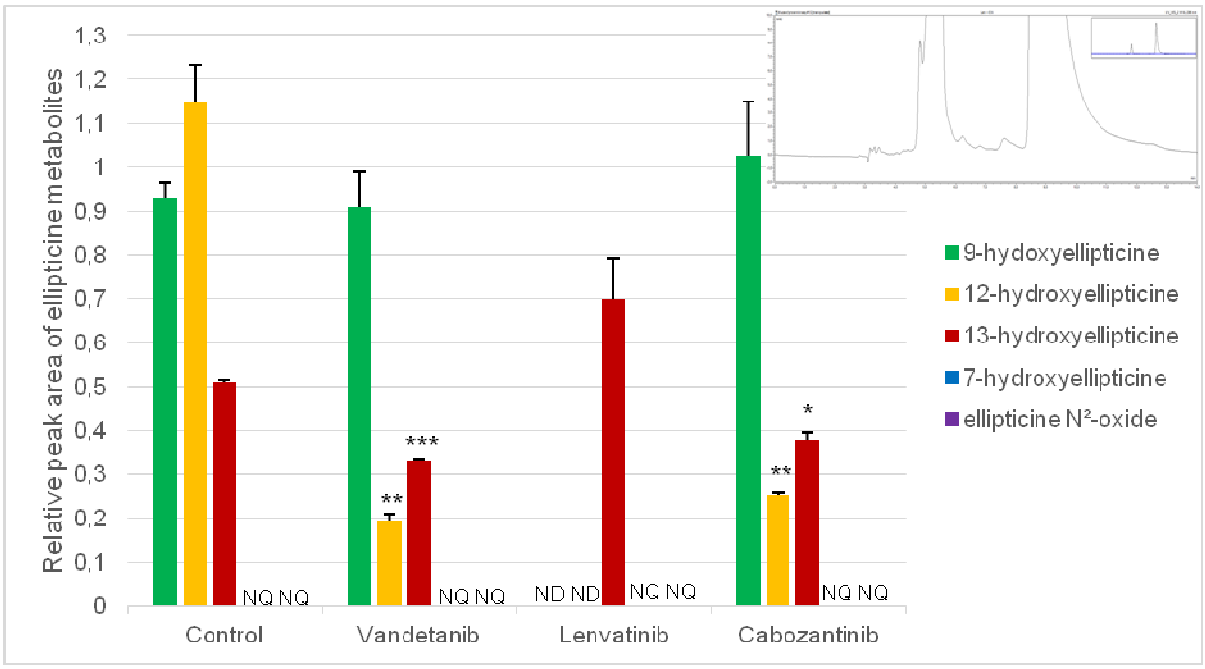
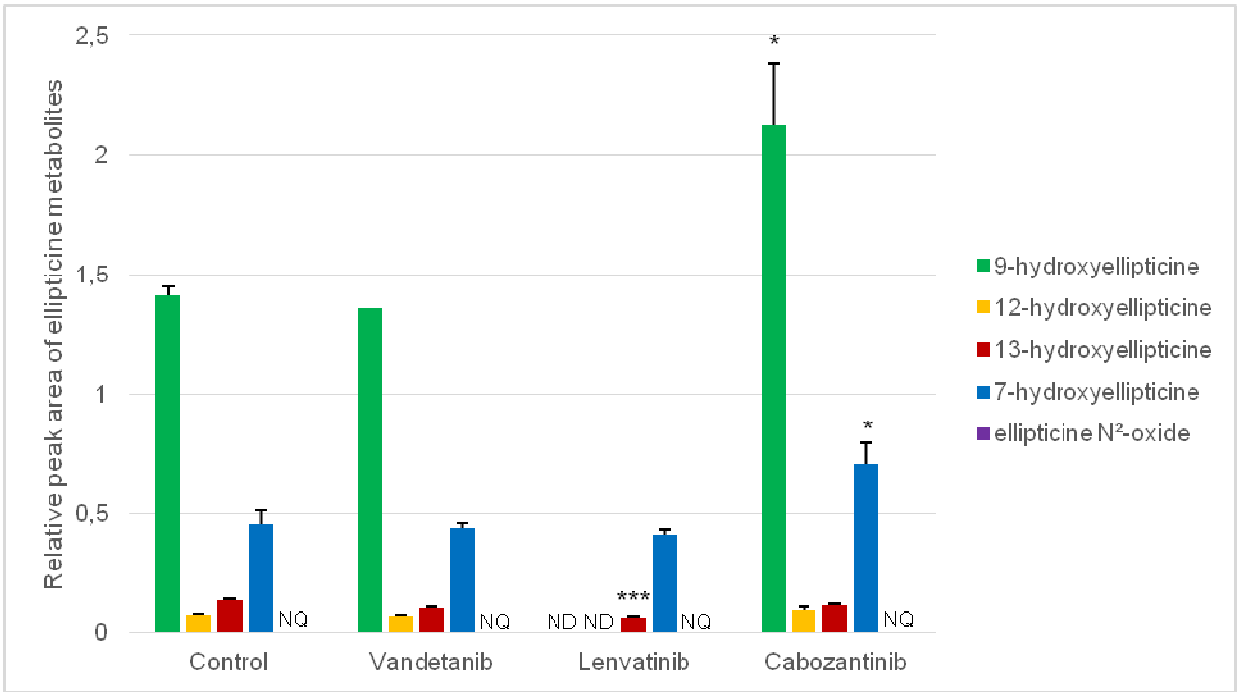


Figure 3. Oxidation of ellipticine by rat (**A**) and human (**B**) CYP1A1, rat (**C**) and human CYP1A2 (**D**) and the effect of vandetanib, lenvatinib and cabozantinib on this oxidation. The data are averages and standard deviations of three experiments. ***P<0.001; **P<0.01; *P<0.05 (Student's t-test), levels of ellipticine metabolites in the presence of TKIs significantly different from those generated without these inhibitors. NQ – not quantified, ND – not determined.

(**A**)



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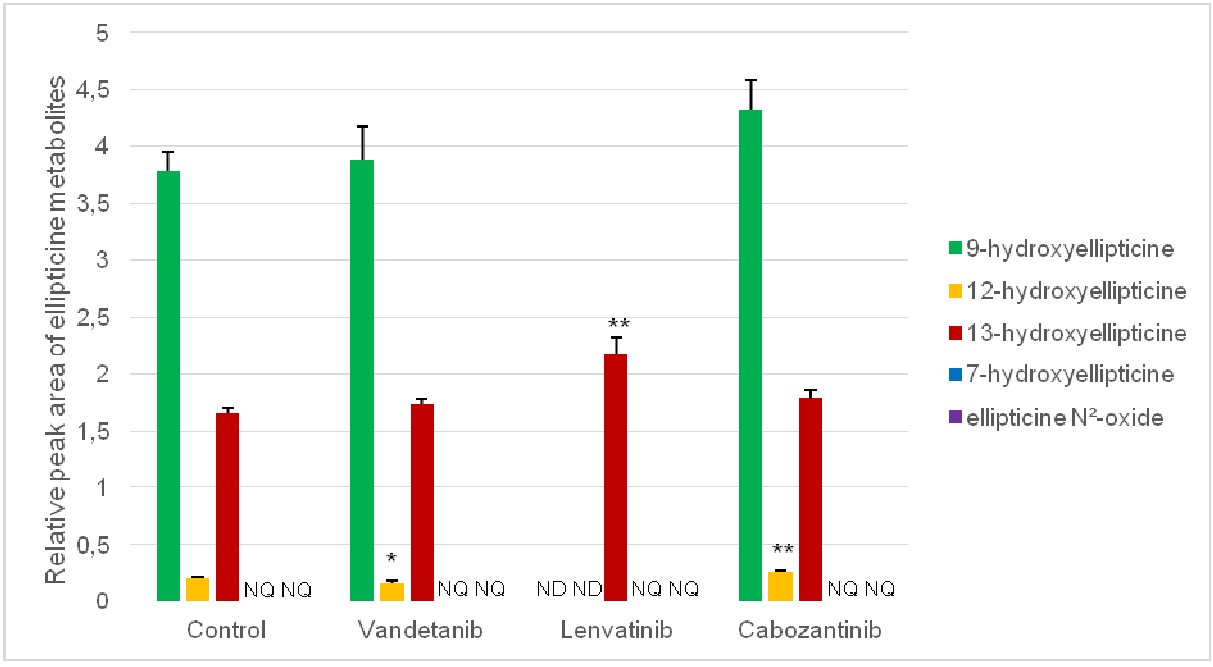
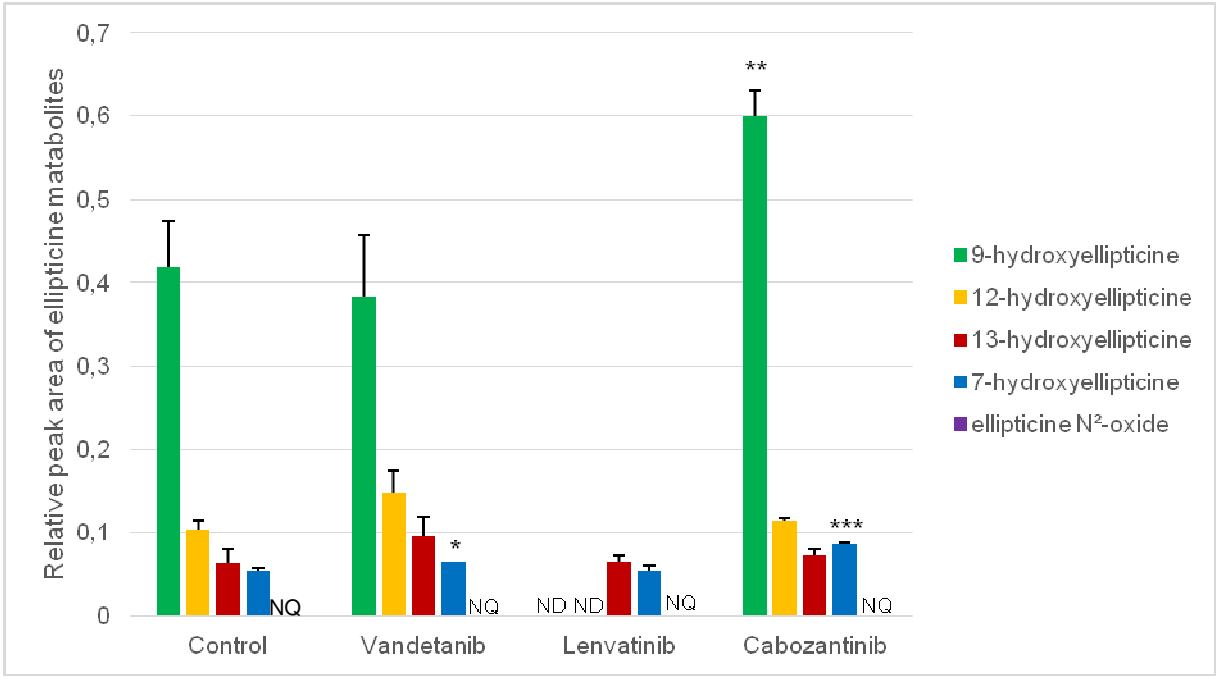


(C)


Relative peak area of ellipticine matabolites
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
**
*
***
NQ
ND ND
Control
Vandetanib
Lenvatinib
Cabozantinib
9-hydroxyellipticine
12-hydroxyellipticine
13-hydroxyellipticine
7-hydroxyellipticine
ellipticine N²-oxide

(D)

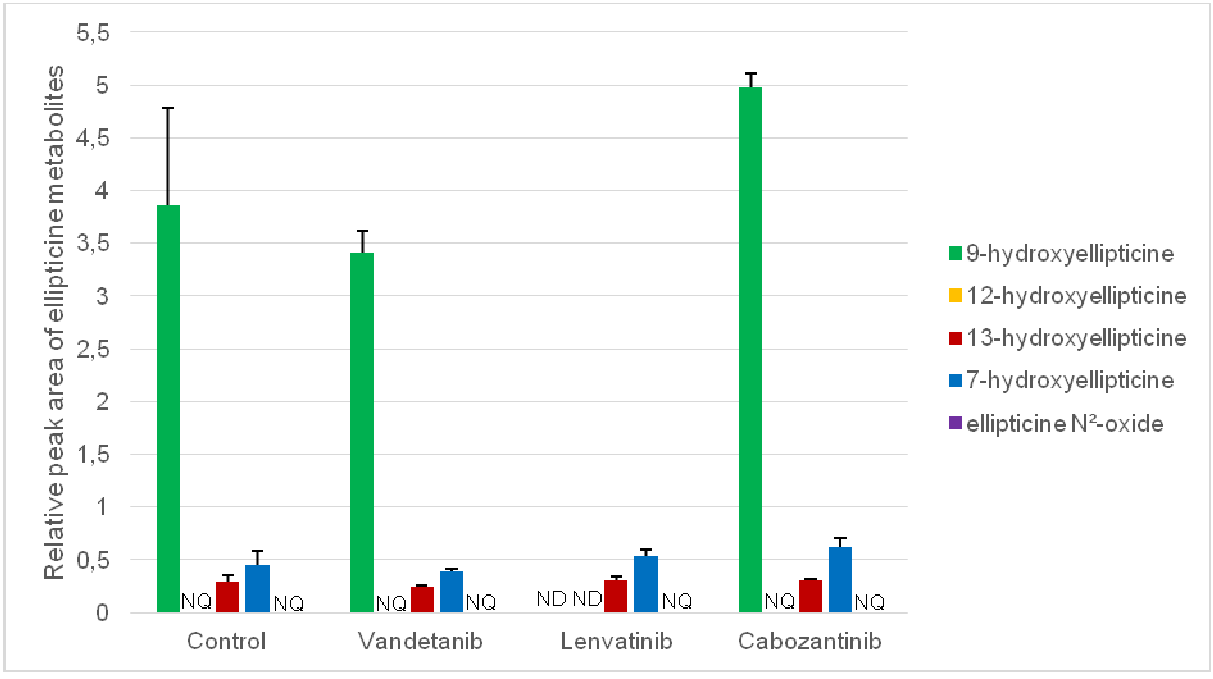
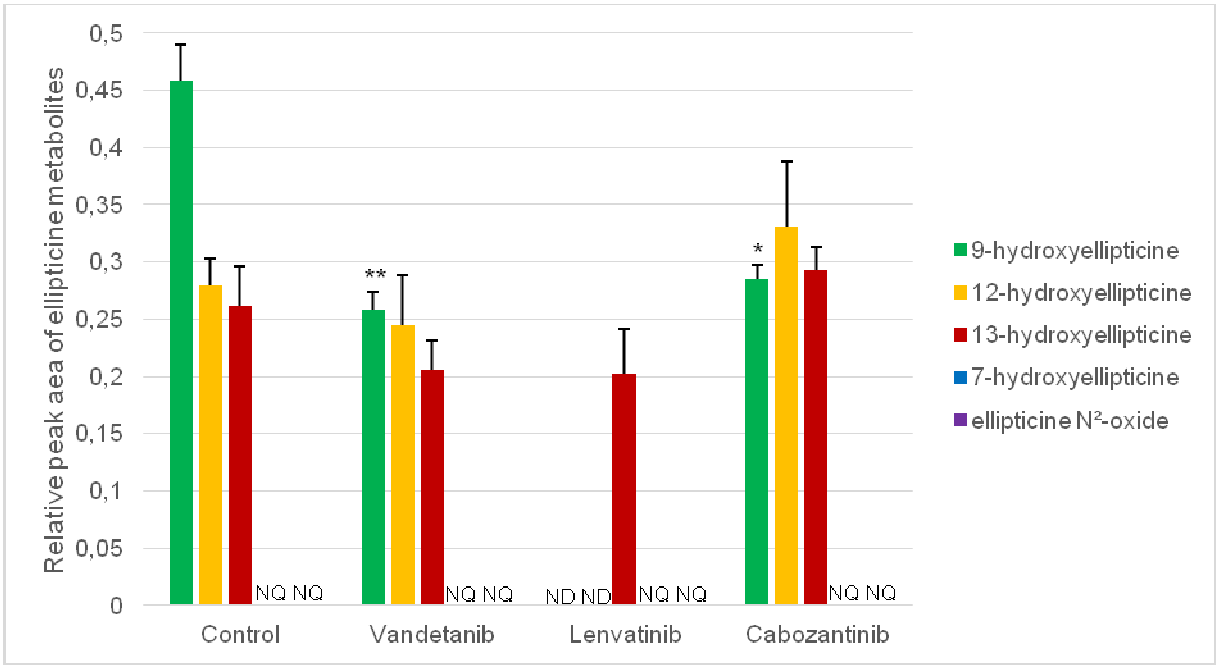
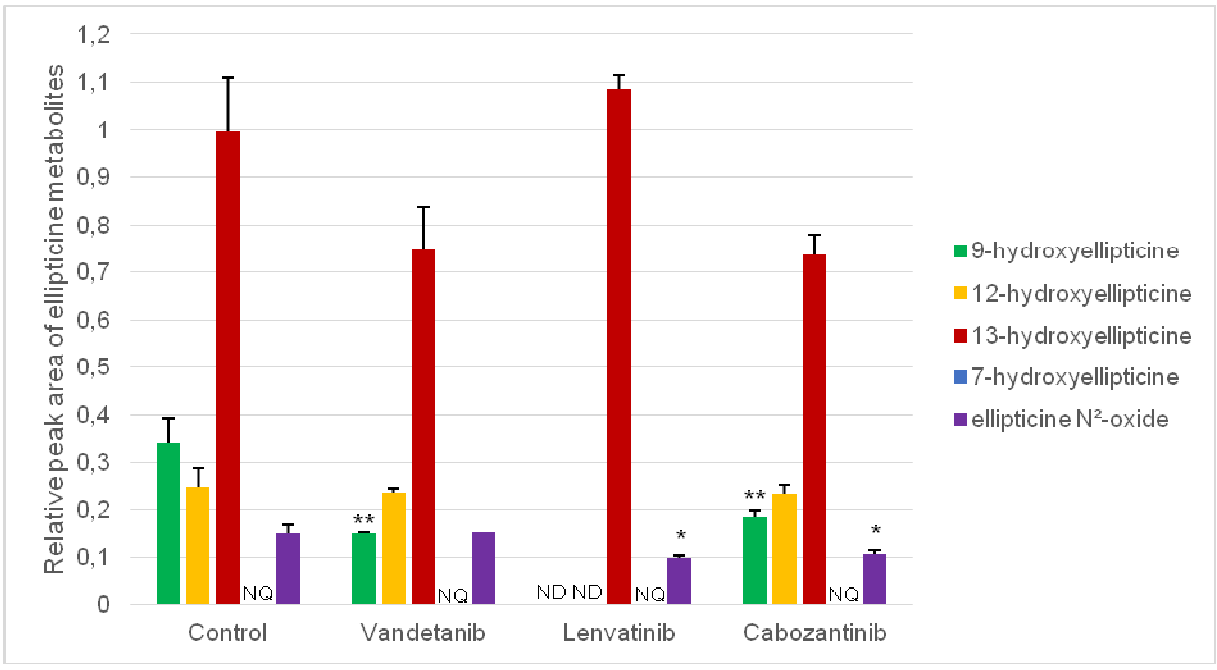

Relative peak area of ellipticine metabolites
5,5
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
NQ
ND ND
Control
Vandetanib
Lenvatinib
Cabozantinib
9-hydroxyellipticine
12-hydroxyellipticine
13-hydroxyellipticine
7-hydroxyellipticine
ellipticine N²-oxide

Figure 4. Oxidation of ellipticine by human CYP2C9 in the presence of cytochrome b_5 (**A**), rat CYP2D1 (**B**), human CYP2D6 (**C**), rat CYP3A1 in the presence of cytochrome b_5 (**D**), human CYP3A4 without (**E**) and in the presence of cytochrome b_5 (**F**) and the effect of vandetanib, lenvatinib and cabozantinib on this oxidation. The data are averages and standard deviations of three experiments. ***P<0.001; **P<0.01; *P<0.05 (Student's t-test), levels of ellipticine metabolites in the presence of TKIs significantly different from those generated without these inhibitors. NQ – not quantified, ND – not determined.

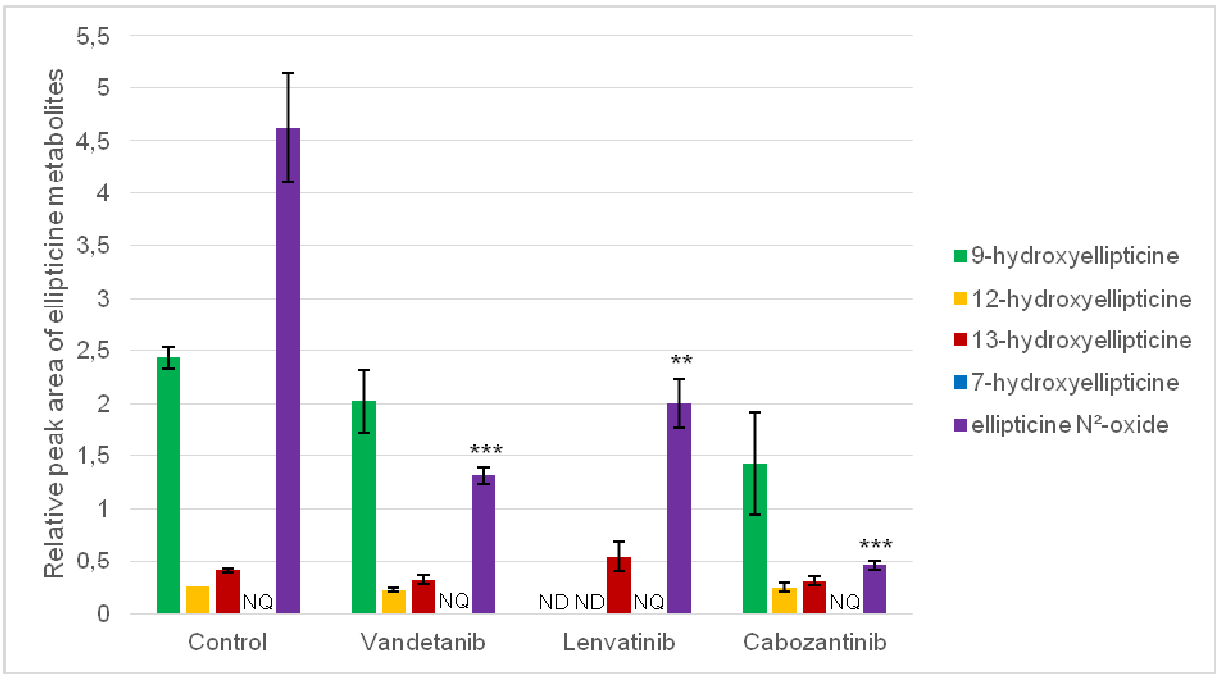
(A)



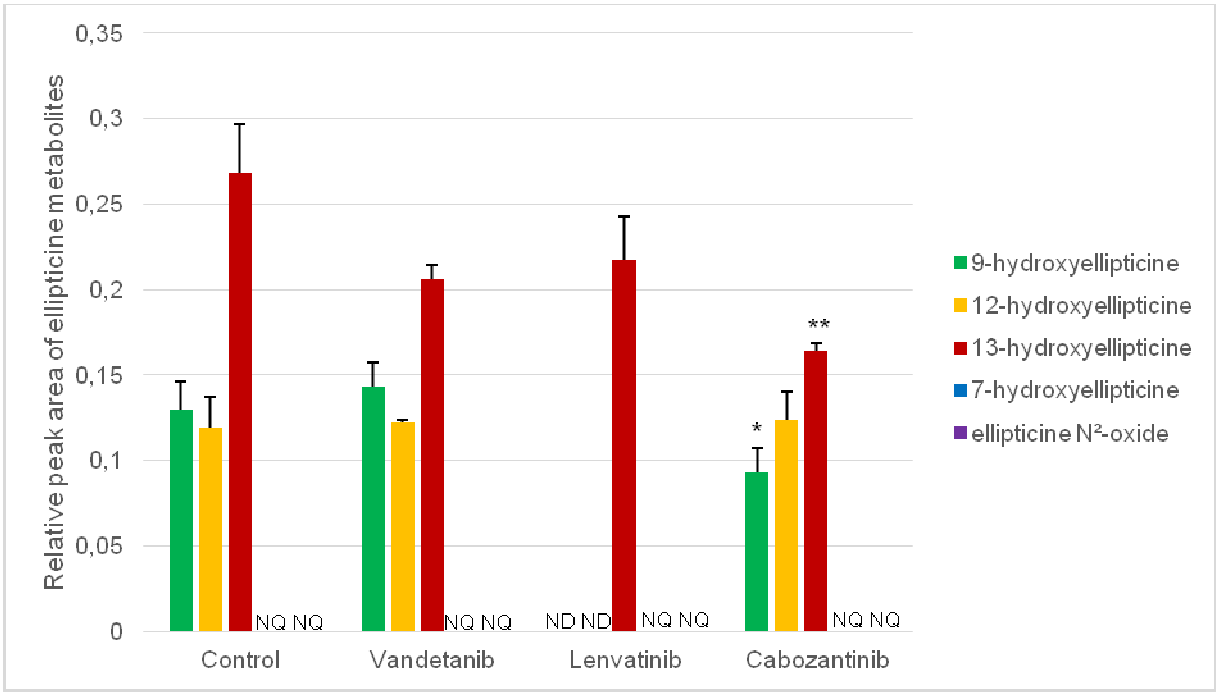
(B)



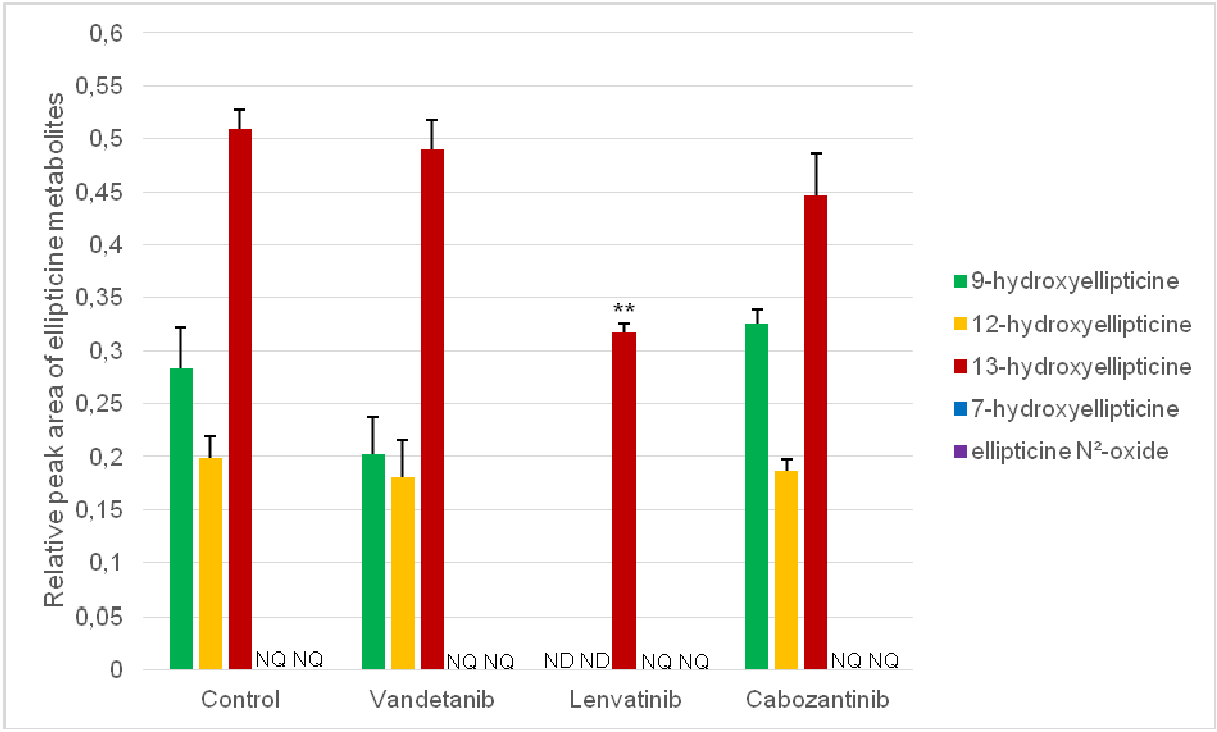
(C)


Relative peak area of ellipticine metabolites
5,5
5
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
NQ
NQ
ND ND
NQ
NQ
***
**
***
Control
Vandetanib
Lenvatinib
Cabozantinib
9-hydroxyellipticine
12-hydroxyellipticine
13-hydroxyellipticine
7-hydroxyellipticine
ellipticine N²-oxide

(D)


Relative peak area of ellipticine metabolites
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
NQ NQ
NQ NQ
ND ND
NQ NQ
NQ NQ
*
**
Control
Vandetanib
Lenvatinib
Cabozantinib
9-hydroxyellipticine
12-hydroxyellipticine
13-hydroxyellipticine
7-hydroxyellipticine
ellipticine N²-oxide

(E)


Relative peak area of ellipticine metabolites
0,6
0,55
0,5
0,45
0,4
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
**
NQ NQ
ND ND
NQ NQ
Control
Vandetanib
Lenvatinib
Cabozantinib
9-hydroxyellipticine
12-hydroxyellipticine
13-hydroxyellipticine
7-hydroxyellipticine
ellipticine N²-oxide

(F)

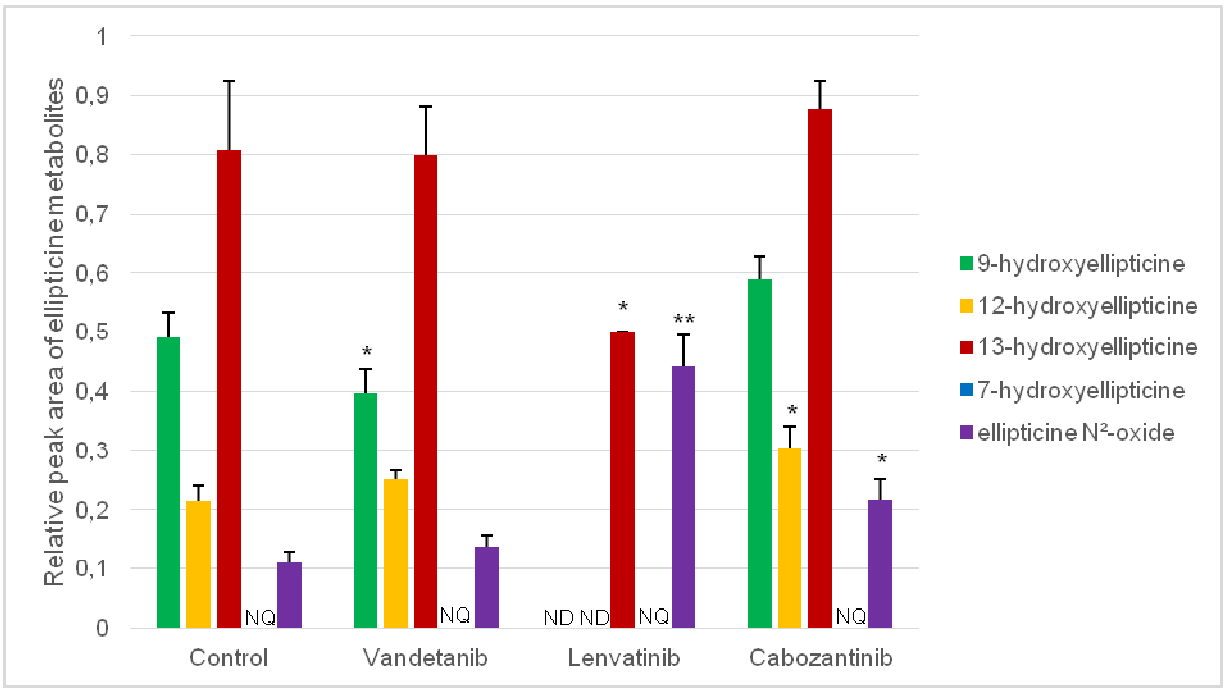
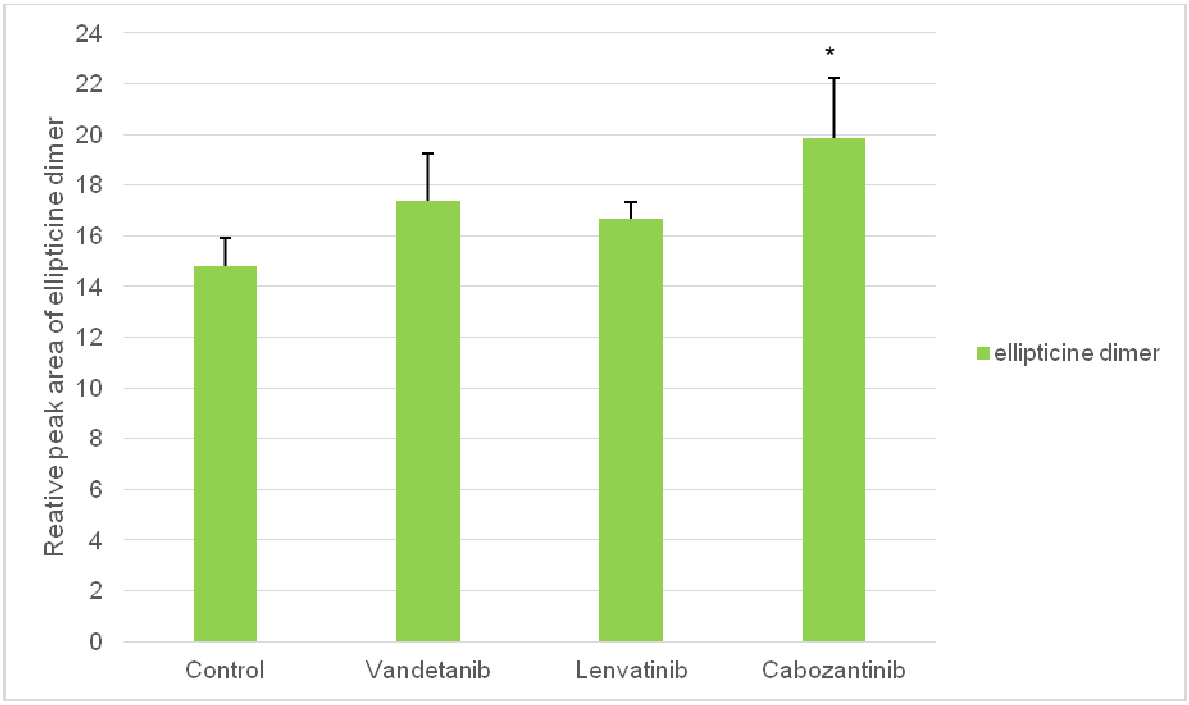

Relative peak area of ellipticine metabolites
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
*
**
NQ
ND ND
Control
Vandetanib
Lenvatinib
Cabozantinib
9-hydroxyellipticine
12-hydroxyellipticine
13-hydroxyellipticine
7-hydroxyellipticine
ellipticine N²-oxide

Figure 5. Oxidation of ellipticine by HRP (**A**), LPO **B**) and MPO (**C**) to ellipticine dimer and the effect of vandetanib, lenvatinib and cabozantinib on this oxidation. The data are averages and standard deviations of three experiments. ***P<0.001; **P<0.01; *P<0.05 (Student's t-test), levels of ellipticine metabolites in the presence of TKIs significantly different from those generated without these inhibitors.

(A)



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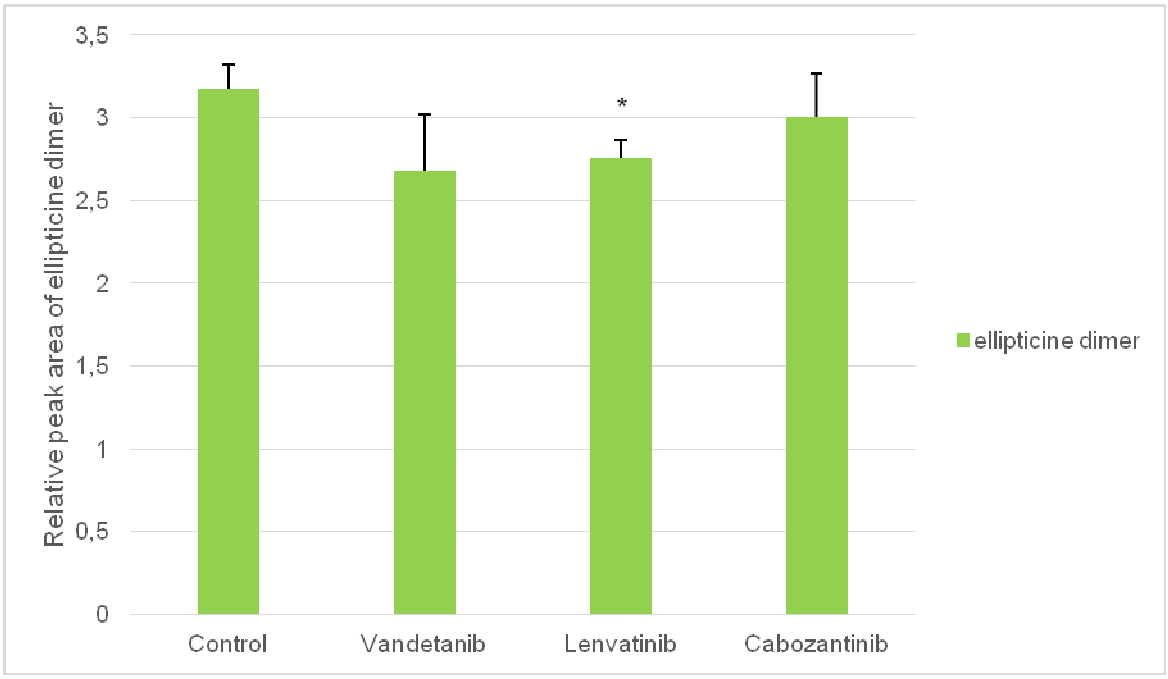
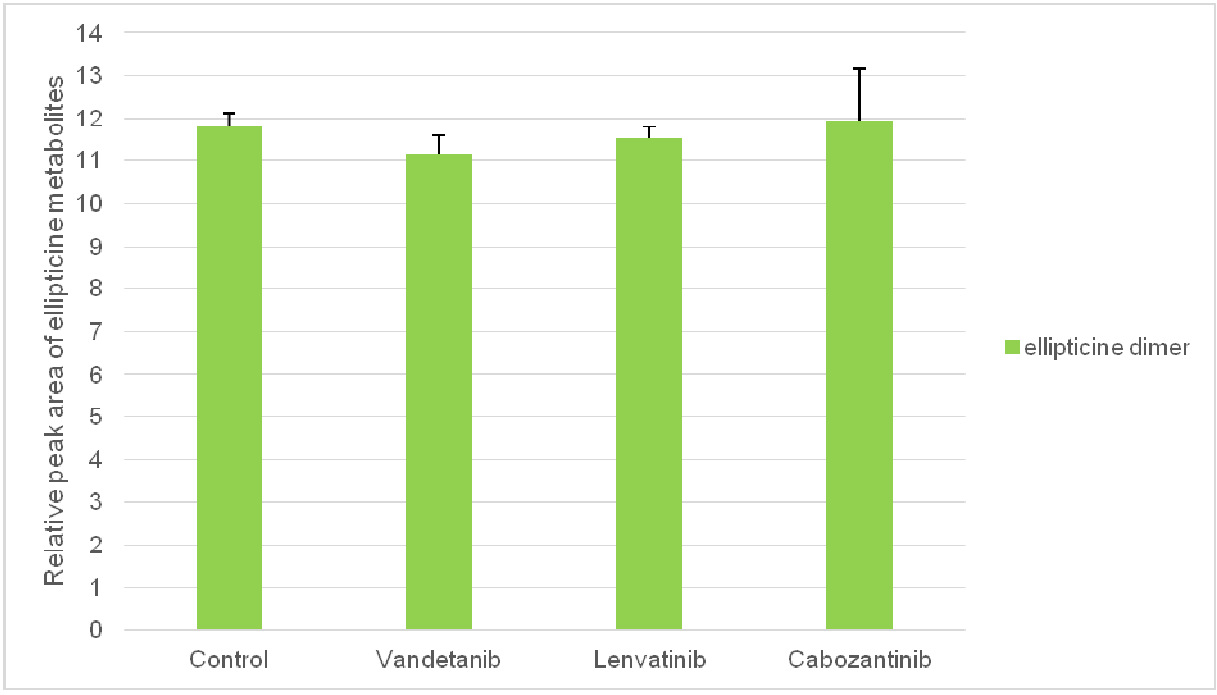


581 **(C)**



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