DMD Fast Forward. Published on November 12, 2018 as DOI: 10.1124/dmd.118.084657
This article has not been copyedited and formatted. The final version may differ from this version.

Short communication

Identification of 19-(S/R)Hydroxyeicosatetraenoic Acid as the First Endogenous Non-Competitive Inhibitor of Cytochrome P450 CYP1B1 with Enantioselective Activity

Sherif M. Shoieb, Ahmed A. El-Sherbeni and Ayman O.S. El-Kadi

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada (S.M.S., A.O.S.E.)

Faculty of Medicine, University of Toronto, 1 King's College Circle, Toronto, Ontario, Canada, M5S 1A8 (A.A.E)

Department of Clinical Pharmacy, Faculty of Pharmacy, Tanta University, 8130 Tanta, Egypt (A.A.E)

Running title

19-HETE enantiomers noncompetitively inhibit CYP1B1

Corresponding author

Ayman O.S. El-Kadi, PhD

Faculty of Pharmacy & Pharmaceutical Sciences

2142J Katz Group-Rexall Centre for Pharmacy and Health Research

University of Alberta, Edmonton, Alberta, Canada T6G 2E1

Phone: 780-492-3071

Fax: 780-492-1217

E-mail: aelkadi@ualberta.ca

Tables: 2

Text pages: 20

Figures: 3

References: 30

Abstract: 159 words

Introduction: 383 words

Results and Discussion: 1013 words

Abbreviations

19-HETE, 19-hydroxyeicosatetraenoic acid; AA, arachidonic acid; Ang II, angiotensin II; CYP, cytochrome P450; DMSO, dimethyl sulfoxide; EETs, epoxyeicosatrienoic acids; EROD, 7-ethoxyresorufin O-deethylation; NADPH, nicotinamide adenine dinucleotide phosphate.

Abstract

The overexpression of cytochrome P450 1B1 (CYP1B1) is a common characteristic of several diseases and conditions, such as inflammation, cancer and cardiac hypertrophy. CYP1B1 is believed to contribute to pathogenesis of these diseases by mediating the formation of toxic compounds, either from exogenous or endogenous origin. We recently reported that an arachidonic acid metabolite, 19(S/R-)hydroxyeicosatetraenoic (HETE) acid, protects from cardiac hypertrophy by inhibiting the formation of toxic compounds, midchain HETEs, known to be formed by CYP1B1. This raised the question whether 19(S/R)-HETE can directly inhibit CYP1B1. In the current study, we are reporting that 19(S/R)-HETE enantioselectively inhibits human recombinant CYP1B1 activity measured by 7-ethoxyresorufin O-deethylation assay. 19(S)-HETE is more potent than the R enantiomer (Ki = 37.3 and 89.1 nM, respectively). Non-competitive inhibition was identified as the mechanism of CYP1B1 inhibition, which underlines the potentially important physiological role of 19(S/R)-HETE as an endogenous CYP1B1 inhibitor; to our knowledge, 19(S/R)-HETE is the first inhibitor of its kind to be reported.

Introduction

Cytochrome P450 1B1 (CYP1B1) is a member of the heme-thiolate monooxygenase superfamily; a group of enzymes that are known to have the capacity of metabolizing a wide range of endobiotics and xenobiotics (Faiq *et al.*, 2014). Arachidonic acid (AA) and 17β-estradiol are examples of endogenous substances that are metabolized by human CYP1B1, while polycyclic aromatic hydrocarbons and dioxins represent some of its xenobiotic substrates (Shimada *et al.*, 1997a; Tsuchiya *et al.*, 2004). Several CYP1B1-dependent metabolic transformations result in toxic and/or carcinogenic metabolites. CYP1B1 mediates the formation of dihydroxy-epoxy-polycyclic aromatic hydrocarbons, which are very potent electrophiles attacking DNA to form adducts leading to mutation and neoplastic transformation. Also, CYP1B1 mediates the formation of mid-chain hydroxylated arachidonic acid, namely 5-, 8-, 9-, 11-, 12-, and 15-hydroxyeicosatetraenoic (HETE) acids (Choudhary *et al.*, 2004). Accumulating studies have reported the cardiotoxic effects of mid-chain HETEs and their involvement in the pathogenesis of hypertension, cardiac hypertrophy and heart failure (Dołęgowska *et al.*, 2009; Kayama *et al.*, 2009; Ahmed A. El-Sherbeni and El-Kadi, 2014). CYP1B1 is selectively expressed at low level in extrahepatic tissues and not in normal human liver. The expression of CYP1B1 only achieves high levels in abnormal tissues; CYP1B1 overexpression is actually regarded as a marker of malignancy phenotype, and was also reported in cardiovascular diseases (Jennings *et al.*, 2010; A. A. El-Sherbeni and El-Kadi, 2014; Maayah *et al.*, 2017). Therefore, considerable interests on CYP1B1 have been raised as a novel therapeutic target for cancer (Li *et al.*, 2015; Chang *et al.*, 2017; D'Uva *et al.*, 2018).

Another group of AA-associated metabolites that are formed by cardiac CYPs is subterminal HETEs exemplified as 16-, 17-, 18- and 19-HETE. Recently, we have demonstrated that both

enantiomers of 19-HETE (Figure 1) protect against Ang II-induced cardiac hypertrophy with preferential effect of 19(S)-HETE. One important finding of our study was that both enantiomers of 19-HETE inhibited the formation of mid-chain HETEs and the catalytic activity of CYP1B1 in two different cell lines; human ventricular cardiomyocytes RL-14 cells and rat cardiomyoblasts H9c2 cells (Shoieb and El-Kadi, 2018). In order to obtain a comprehensive understanding of the protective role of R- and S-enantiomers of 19-HETE in case of cardiac hypertrophy, in the current study we examined the inhibitory effect of both enantiomers on human recombinant CYP1B1 enzyme using 7-ethoxyresorufin O-deethylation (EROD) assay.

Materials and methods

Materials

19(R)- and 19(S)-HETEs were purchased from Cayman Chemical (Ann Arbor, MI). 7-ethoxyresorufin (7-ER) and nicotinamide adenine dinucleotide phosphate (NADPH) tetrasodium salt were purchased from Sigma Chemical Co. (St. Louis, MO). Human recombinant CYP1B1 microsomes supplemented with NADPH–cytochrome P450-oxidoreductase (Corning-Supersomes^{TM}) were obtained from Fisher Scientific (Hampton, NH); all other chemicals were purchased from Fisher Scientific (Toronto, ON).

Determination of CYP1B1-inhibition kinetics by 19-HETE enantiomers

The O-dealkylation rate of 7-ER by recombinant human CYP1B1 was measured in the absence and presence of 19(R)-HETE or 19(S)-HETE. Briefly, 96-well solid black polystyrene microplates were used to carry out the florescent assay. The reaction mixture containing 100 mM potassium phosphate (pH 7.4) buffer supplemented with 5 mM magnesium chloride hexahydrate and 1 pmol of human CYP1B1, was incubated with 10-100 nM of 7-ER. In addition, 0, 5, 10, 20, or 40 nM of either 19(R)-HETE or 19(S)-HETE was added to the reaction mixture. The volume of the reaction mixture was 100 µL. The reaction was initiated by the addition of 100 µL of 2 mM NADPH, the fluorescent signal related to the formation of resorufin was measured every min (excitation and emission wavelengths of 550 and 585 nm, respectively) for 30 min at 37°C using a BioTek Synergy H1 Hybrid Reader (BioTek Instruments, Inc.). The quantity of formed resorufin was measured by the construction of standard curve of 0-200 nM of resorufin dissolved in the same incubation buffer. The formation rate of resorufin in each well was determined over the first 9-min period using linear regression. The formation rates of resorufin at different 7-ER concentrations were fitted to Michaelis-Menten equation; while, 19(R)-HETE-CYP1B1 and 19(S)-HETE-CYP1B1

inhibition rates were fitted to competitive, noncompetitive, uncompetitive, and mixed models of enzyme inhibition. The most probable enzyme inhibition model was selected according to Akaike information criteria (AIC). The fitting was carried out using GraphPad Prism (version 5.01; GraphPad Software, Inc., La Jolla, CA).

Determination of 19(R)-HETE and 19(S)-HETE in-vitro stability

In order to assess the stability of 19-HETE enantiomers in solution under the same experimental conditions of the kinetic study, the stability experiment has been performed using 7-ER concentration corresponding to the Km of the reaction (86 nM). 19(R)-HETE and 19(S)-HETE were added to the reaction mixture at their Ki concentrations of 89.1 and 37.3 nM, respectively. After initiation of the reaction using 2 mM NADPH, 200 µL was taken from the mixture at the start of the reaction (T0), after 10, 20 and 30 min at 37°C. The reaction was stopped using 400 µL ice-cold acetonitrile and the extraction of 19-HETE enantiomers was performed using ethyl acetate and dried using speed vacuum (Savant, Farmingdale, NY, USA). The analysis of 19-HETE enantiomers was performed using liquid chromatography–electrospray ionization mass spectrometry (LC–ESI–MS) as previously described (Shoieb and El-Kadi, 2018).

Results and discussion

Inhibition of CYP1B1 is proposed as one of the strategies in the treatment of cardiovascular diseases and cancer chemoprevention (Mikstacka *et al.*, 2007). In this regard, we tested the capacity of both enantiomers of 19-HETE to inhibit the catalytic activity of human recombinant CYP1B1, based on our previous finding that 19(R)- and 19(S)-HETE protected against the Ang II-induced cellular hypertrophy via decreasing the level of cardiotoxic mid-chain HETEs; the major metabolites of CYP1B1 (Shoieb and El-Kadi, 2018). For that purpose, the inhibition kinetics of both enantiomers of 19-HETE on CYP1B1 activity were carried out using CYP1B1 substrate 7-ER, a compound that is metabolized via oxidative reaction by this enzyme (Shimada *et al.*, 1997b). The experimental conditions such as the concentrations of 7-ER, concentration of the recombinant enzyme in addition to the incubation time were all optimized before the addition of either 19(R)-HETE or 19(S)-HETE.

Inhibition of EROD is a well-reported assay carried out predominantly to examine the inhibitory potencies of CYP1B1 inhibitors (Androutsopoulos *et al.*, 2011). Inhibitory activity of 19(R)-HETE (Figure 2A) and 19(S)-HETE (Figure 2B) on CYP1B1 was studied. Foremost experiments involved the assessment of 7-ER-O-deethylase kinetics by determination of the rate of resorufin formation over time. Based on the nonlinear regression analysis and fitting to Michaelis-Menten model, the maximal EROD activity (V_{max}) in the control containing 0.1% DMSO was 16.7 pmol resorufin/pmol P450/min (R^2 =0.9888) (Table 1). The Michaelis-Menten constant (K_m) value for the current EROD reaction is 86.6 nM (Table 1). During the co-incubation of 10 nM of either 19-HETE enantiomers with different concentrations of 7-ER, we observed a significant inhibition of CYP1B1 enzymatic activity. This finding prompted us to test increasing concentrations (0-40 nM)

of either 19(R)-HETE or 19(S)-HETE with different concentrations (0-100 nM) of 7-ER in order to characterize the mode of inhibition by 19-HETE enantiomers.

Nonlinear regression analysis and comparisons showed that the mode of inhibition for 19(R)-HETE and 19(S)-HETE is noncompetitive inhibition of CYP1B1 enzyme. Dixon plots showed that 19(R)-HETE (Figure 3A) and 19(S)-HETE (Figure 3B) have Ki values of 89.1 and 37.3 nM, respectively (Table 1). The Ki values of both enantiomers showed that the S-enantiomer is more potent than the R-enantiomer by approximately 2.4 fold. This comes in agreement with our previous work demonstrating that the S-enantiomer of 19-HETE has a preferential effect over the R-enantiomer in inhibiting the formation of mid-chain HETEs (Shoieb and El-Kadi, 2018). In accordance with being noncompetitive inhibitors, the in vitro stability study showed that both enantiomers of 19-HETE are metabolically stable in solution during the 30 min-time course of the kinetic experiment as there was no significant difference in the level of both enantiomers during the duration of the experiment (Table 2, Supplemental Figure 1 & 2). Discovery of selective and potent inhibitors of CYP1B1 enables for the design of CYP1B1-targeted cardiovascular treatment and prevention in addition to cancer chemotherapy (Pingili *et al.*, 2017; Ware, 2017).

To date, more than fifty synthetic and natural compounds have been designed or recognized as inhibitors of CYP1B1 (Li *et al.*, 2017). Various CYP1B1 inhibitors with varying mode of inhibition have been identified. For instance, trans-resveratrol inhibited CYP1B1 enzymatic activity via mixed-type inhibitory mechanism and the Ki was 0.75 μM (Chang *et al.*, 2000). Also, myricetin and quercetin with Ki 230 and 120 nM, respectively exhibited mixed-type inhibition while apigenin with Ki 60 nM acted as a competitive inhibitor of CYP1B1 (Chaudhary and Willett, 2006). Moreover, tetramethoxystilbene (TMS) noncompetitively inhibited EROD activity of CYP1B1 with IC_{50} value of 2 nM when using 2 μM of 7-ER as a substrate (Chun *et al.*, 2009). In

the present study, both enantiomers of 19-HETE inhibited CYP1B1 enzymatic activity in the nanomolar range and more potently than some of the previously mentioned CYP1B1 inhibitors proposing them to be introduced as therapeutic and preventive modalities in CYP1B1-targeted therapies.

In the current study, there is a significant difference in the inhibitory activity between R- and S-enantiomers of 19-HETE. To the best of our knowledge, the current study is the first to characterize the enantioselective pattern of inhibition of CYP1B1 by enantiomers of 19-HETE. Enantioselective inhibition of CYP enzymes has been previously documented. S002-333, a novel and potent antithrombotic agent, inhibited CYP2B6-mediated bupropion 6-hydroxylation in a stereoselective manner. While the S-enantiomer showed strong inhibition with IC_{50} value of 5.28 μM, the R-enantiomer showed much less inhibition on CYP2B6 catalytic activity with IC_{50} value of more than 50 μM (Bhateria *et al.*, 2016).

It is noteworthy that CYP1B1 regulates several metabolic pathways. For example, AA is oxidized by CYP1B1 to produce the cardiotoxic mid-chain HETEs metabolites. In rat neonatal cardiomyocytes, 15-HETE has been reported to be capable of elevating isoproterenol-induced β-adrenergic response sensitivity. Moreover, 5-, 12-, and 15-HETE were capable of inducing cellular hypertrophy in RL-14 cells, human ventricular cardiomyocytes, via MAPK- and NF-κB-dependent pathways (Zhang *et al.*, 2014; Maayah and El-Kadi, 2016). Furthermore, CYP1B1 is responsible for some vital physiological processes in blood vessels, importantly the development and progression of hypertension. In Ang II-induced hypertension, CYP1B1 mediates cell migration, induces proliferation and increases protein synthesis in vascular smooth muscle cells through regulation of the metabolism of AA (Yaghini *et al.*, 2010). The fact that CYP1B1 expression is vastly enhanced in several tumors such as prostate, breast and colon cancers, suggests that

CYP1B1 inhibitors could be considered as an accepted therapeutic strategy in treatment and prevention of cancer (Gibson *et al.*, 2003; Tokizane *et al.*, 2005).

In summary, CYP1B1 is considered one of the important CYPs. It is involved in the metabolism of many endobiotics, xenobiotics and in the activation pathways of various procarcinogens. The findings of the current study show that both enantiomers of 19-HETE noncompetitively inhibited CYP1B1 enzymatic activity with higher potency of the S-enantiomer. The current study suggests that 19(R)-HETE and 19(S)-HETE could be considered as a novel therapeutic modality in the treatment of hypertension, cardiac hypertrophy and cancer. Moreover, given that a noncompetitive inhibitor might bind to the enzyme regulatory region, 19(R)-HETE and 19(S)-HETE could be considered to unravel the obscure mechanisms of CYP1B1 enzymatic reaction.

Authorship Contributions

Participated in research design: Shoieb, El-Kadi.

Conducted experiments: Shoieb.

Performed data analysis: Shoieb, El-Sherbeni and El-Kadi.

Wrote or contributed to the writing of the manuscript: Shoieb, El-Sherbeni and El-Kadi.

References

Androutsopoulos VP, Papakyriakou A, Vourloumis D, and Spandidos DA (2011) Comparative CYP1A1 and CYP1B1 substrate and inhibitor profile of dietary flavonoids. *Bioorg Med Chem* **19**:2842–2849, Pergamon.

Bhateria M, Ramakrishna R, Puttrevu SK, Saxena AK, and Bhatta RS (2016) Enantioselective inhibition of Cytochrome P450-mediated drug metabolism by a novel antithrombotic agent, S002-333: Major effect on CYP2B6. *Chem Biol Interact* **256**:257–265.

Chang I, Mitsui Y, Kim SK, Sun JS, Jeon HS, Kang JY, Kang NJ, Fukuhara S, Gill A, Shahryari V, Tabatabai ZL, Greene KL, Dahiya R, Shin DM, and Tanaka Y (2017) Cytochrome P450 1B1 inhibition suppresses tumorigenicity of prostate cancer via caspase-1 activation. *Oncotarget* **8**:39087–39100.

Chang TK, Lee WB, and Ko HH (2000) Trans-resveratrol modulates the catalytic activity and mRNA expression of the procarcinogen-activating human cytochrome P450 1B1. *Can J Physiol Pharmacol* **78**:874–81.

Chaudhary A, and Willett KL (2006) Inhibition of human cytochrome CYP1 enzymes by flavonoids of St. John's wort. *Toxicology* **217**:194–205, Elsevier.

Choudhary D, Jansson I, Stoilov I, Sarfarazi M, and Schenkman JB (2004) Metabolism of retinoids and arachidonic acid by human and mouse cytochrome P450 1b1. *Drug Metab Dispos* **32**:840–7.

Chun Y-J, Oh Y-K, Kim BJ, Kim D, Kim SS, Choi H-K, and Kim M-Y (2009) Potent inhibition of human cytochrome P450 1B1 by tetramethoxystilbene. *Toxicol Lett* **189**:84–89, Elsevier.

D'Uva G, Baci D, Albini A, and Noonan DM (2018) Cancer chemoprevention revisited: Cytochrome P450 family 1B1 as a target in the tumor and the microenvironment. *Cancer Treat Rev* **63**:1–18.

Dołęgowska B, Błogowski W, Kędzierska K, Safranow K, Jakubowska K, Olszewska M, Rać M, Chlubek D, and Ciechanowski K (2009) Platelets arachidonic acid metabolism in patients with essential hypertension. *Platelets* **20**:242–249.

El-Sherbeni AA, and El-Kadi AOS (2014) Alterations in cytochrome P450-derived arachidonic acid metabolism during pressure overload-induced cardiac hypertrophy. *Biochem Pharmacol* **87**:456–466.

El-Sherbeni AA, and El-Kadi AOS (2014) Characterization of Arachidonic Acid Metabolism by Rat Cytochrome P450 Enzymes: The Involvement of CYP1As. *Drug Metab Dispos* **42**:1498–1507.

Faiq MA, Dada R, Sharma R, Saluja D, and Dada T (2014) CYP1B1: a unique gene with unique characteristics. *Curr Drug Metab* **15**:893–914.

Gibson P, Gill JH, Khan PA, Seargent JM, Martin SW, Batman PA, Griffith J, Bradley C, Double JA, Bibby MC, and Loadman PM (2003) Cytochrome P450 1B1 (CYP1B1) is overexpressed in human colon adenocarcinomas relative to normal colon: implications for drug development. *Mol Cancer Ther* **2**:527–34.

Jennings BL, Sahan-Firat S, Estes AM, Das K, Farjana N, Fang XR, Gonzalez FJ, and Malik KU (2010) Cytochrome P450 1B1 contributes to angiotensin II-induced hypertension and associated pathophysiology. *Hypertens (Dallas, Tex 1979)* **56**:667–74.

Kayama Y, Minamino T, Toko H, Sakamoto M, Shimizu I, Takahashi H, Okada S, Tateno K, Moriya J, Yokoyama M, Nojima A, Yoshimura M, Egashira K, Aburatani H, and Komuro I (2009) Cardiac 12/15 lipoxygenase–induced inflammation is involved in heart failure. *J Exp Med* **206**:1565–1574.

Li C, Long B, Qin X, Li W, and Zhou Y (2015) Cytochrome P1B1 (CYP1B1) polymorphisms and cancer risk: A meta-analysis of 52 studies. *Toxicology* **327**:77–86, Elsevier.

Li F, Zhu W, and Gonzalez FJ (2017) Potential role of CYP1B1 in the development and treatment of metabolic diseases. *Pharmacol Ther* **178**:18–30.

Maayah ZH, Althurwi HN, El-Sherbeni AA, Abdelhamid G, Siraki AG, and El-Kadi AOS (2017) The role of cytochrome P450 1B1 and its associated mid-chain hydroxyeicosatetraenoic acid metabolites in the development of cardiac hypertrophy induced by isoproterenol. *Mol Cell Biochem* **429**:151–165.

Maayah ZH, and El-Kadi AO (2016) 5-, 12- and 15-Hydroxyeicosatetraenoic acids induce cellular hypertrophy in the human ventricular cardiomyocyte, RL-14 cell line, through MAPK- and NF-kappaB-dependent mechanism. *Arch Toxicol* **90**:359–373.

Mikstacka R, Przybylska D, Rimando AM, and Baer-Dubowska W (2007) Inhibition of human recombinant cytochromes P450 CYP1A1 and CYP1B1 by trans-resveratrol methyl ethers. *Mol Nutr Food Res* **51**:517–24.

Pingili AK, Davidge KN, Thirunavukkarasu S, Khan NS, Katsurada A, Majid DSA, Gonzalez FJ, Navar LG, and Malik KU (2017) 2-Methoxyestradiol Reduces Angiotensin II-Induced Hypertension and Renal Dysfunction in Ovariectomized Female and Intact Male Mice. *Hypertens (Dallas, Tex 1979)* **69**:1104–1112, NIH Public Access.

Shimada T, Gillam EM, Sutter TR, Strickland PT, Guengerich FP, and Yamazaki H (1997a) Oxidation of xenobiotics by recombinant human cytochrome P450 1B1. *Drug Metab Dispos* **25**:617–22.

Shimada T, Gillam EM, Sutter TR, Strickland PT, Guengerich FP, and Yamazaki H (1997b) Oxidation of xenobiotics by recombinant human cytochrome P450 1B1. *Drug Metab Dispos* **25**:617–22.

Shoieb SM, and El-Kadi AOS (2018) *S*- Enantiomer of 19-Hydroxyeicosatetraenoic Acid Preferentially Protects Against Angiotensin II-Induced Cardiac Hypertrophy. *Drug Metab Dispos* **46**:1157–1168.

Tokizane T, Shiina H, Igawa M, Enokida H, Urakami S, Kawakami T, Ogishima T, Okino ST, Li L-C, Tanaka Y, Nonomura N, Okuyama A, and Dahiya R (2005) Cytochrome P450 1B1 is overexpressed and regulated by hypomethylation in prostate cancer. *Clin Cancer Res* **11**:5793–801, American Association for Cancer Research.

Tsuchiya Y, Nakajima M, Kyo S, Kanaya T, Inoue M, and Yokoi T (2004) Human CYP1B1 is regulated by estradiol via estrogen receptor. *Cancer Res* **64**:3119–25.

Ware WR (2017) Natural Cancer Therapy and Prevention Targeted on Cancer Cells and Cancer Stem Cells Based on the Cytochrome P45O Enzyme CYP1B1: A Commentary. *Altern Ther Health Med* **23**:50–58.

Yaghini FA, Song CY, Lavrentyev EN, Ghafoor HUB, Fang XR, Estes AM, Campbell WB, and Malik KU (2010) Angiotensin II-Induced Vascular Smooth Muscle Cell Migration and Growth Are Mediated by Cytochrome P450 1B1-Dependent Superoxide Generation. *Hypertension* **55**:1461–1467.

Zhang L, Li Y, Chen M, Su X, Yi D, Lu P, and Zhu D (2014) 15-LO/15-HETE mediated vascular adventitia fibrosis via p38 MAPK-dependent TGF-beta. *J Cell Physiol* **229**:245–257.

Footnotes

This work was supported by a grant from the Canadian Institutes of Health Research [Grant 106665] to A.O.S.E. S.M.S. is the recipient of Antoine Noujaim Graduate Scholarship in Pharmaceutical Sciences.

Figure legends

Fig. 1. R- and S-enantiomers of 19-HETE

Both enantiomers represent an example of stereoisomerism of P450-mediated arachidonic acid metabolites. Incorporation of hydroxyl group in the carbon skeleton of arachidonic acid generates a chiral center, hence there are 2 enantiomers for each HETE metabolite.

Fig. 2. Inhibitory effect of 19(R)-HETE and 19(S)-HETE on EROD activity mediated by human recombinant CYP1B1

In 96-well solid black polystyrene plates, the reaction mixture containing 100 mM potassium phosphate (pH 7.4) buffer supplemented with 5 mM magnesium chloride hexahydrate and 1 pmol of human CYP1B1, was incubated with 10-100 nM of 7-ER. In addition, 0, 5, 10, 20, or 40 nM of either 19(R)-HETE (A) or 19(S)-HETE (B) was added to the reaction. The reaction was initiated by the addition of 100 µL of 2 mM NADPH, the fluorescent signal related to the formation of resorufin was measured every one minute (excitation and emission wavelengths of 550 and 585 nm, respectively) for 30 min at 37°C using a BioTek Synergy H1 Hybrid Reader (BioTek Instruments, Inc.). The quantity of formed resorufin was measured by the construction of standard curve of 0-200 nM of resorufin dissolved in the same incubation buffer. Each point represents the mean of 6 independent experiments ± SE.

Fig. 3. Dixon plots representing the inhibitory effect of 19(R)-HETE and 19(S)-HETE on EROD activity mediated by human recombinant CYP1B1

The Y-axis represents the reciprocal of EROD activity expressed in picomoles of resorufin/ pmol CYP1B1/min while the X-axis represents 19(R)-HETE (A) or 19(S)-HETE (B) concentrations in nM in the presence of increasing concentration of the substrate 7-ER (10-100 Nm). Each point represents the mean of 6 independent experiments ± SE.

DMD Fast Forward. Published on November 12, 2018 as DOI: 10.1124/dmd.118.084657
This article has not been copyedited and formatted. The final version may differ from this version.

Table 1. The mean values and standard error of kinetic parameters of resorufin formation by human recombinant CYP1B1, in the absence and presence of 19(R)-HETE or 19(S)-HETE

Km (nM)	**Vmax (pmol/pmol P450/min)**	**Ki (nM)**	
		19(R)-HETE	19(S)-HETE
86.6 ± 10.4	16.7 ± 1.1	89.1 ± 11.5	37.3 ± 5.5

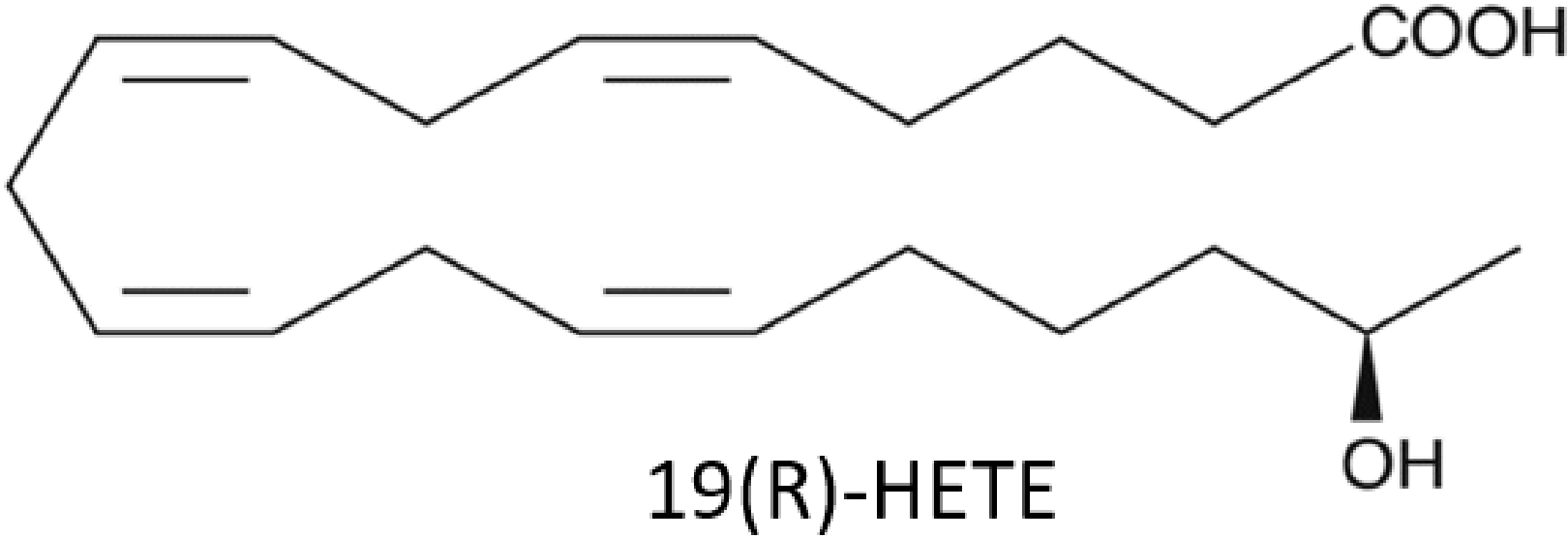
Results are presented as mean and SE, based on at least 3 individual experiments. Km, Vmax and Ki mean ± SE were determined by Enzyme Kinetics module from GraphPad Prism, version 5.01.

DMD Fast Forward. Published on November 12, 2018 as DOI: 10.1124/dmd.118.084657
This article has not been copyedited and formatted. The final version may differ from this version.

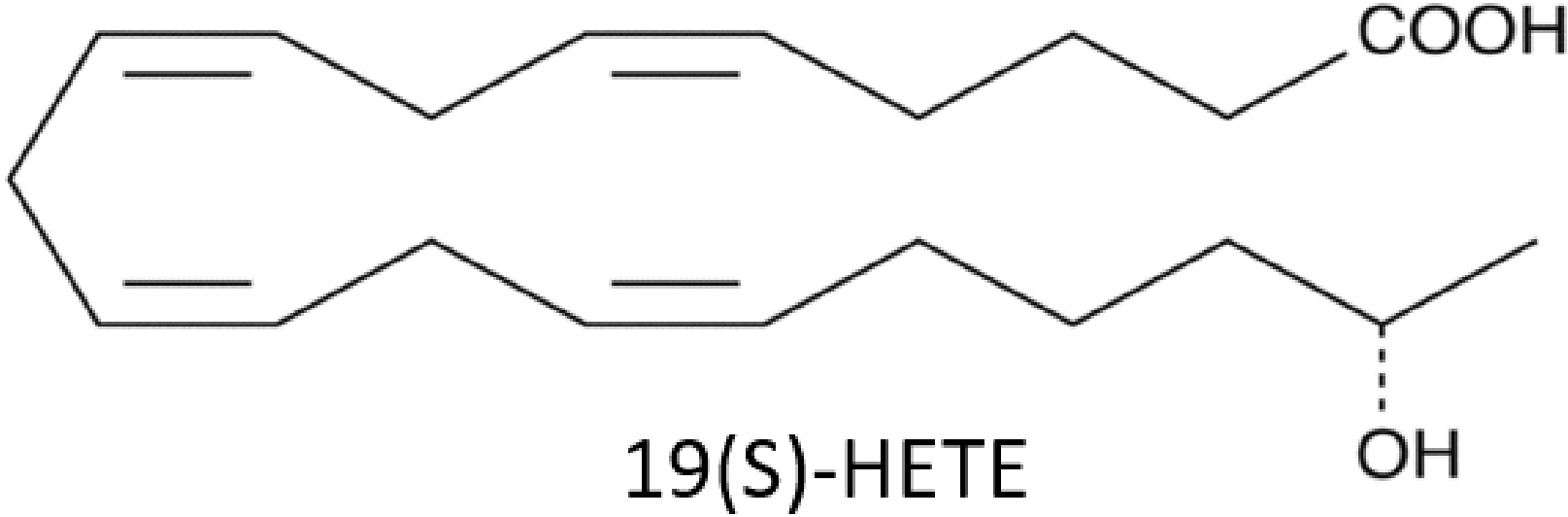
Table 2. The mean values and standard error of 19(R)-HETE or 19(S)-HETE levels calculated as a percentage of the metabolite level at the beginning of the experiment (T_0). Data are represented as mean ± SE where n=3.

	19(R/S)-HETE levels as a percentage of their levels at zero time (%)			
Time Interval (T_{min})	T_0	T_{10}	T_{20}	T_{30}
19(R)-HETE	100 ± 17.1	98.6 ± 7.8	96.3 ± 1.3	98.5 ± 8.9
19(S)-HETE	100 ± 5.9	99.7 ± 1.3	100.1 ± 0.9	98.9 ± 4.6

Figure 1



19(R)-HETE



19(S)-HETE

Figure 2

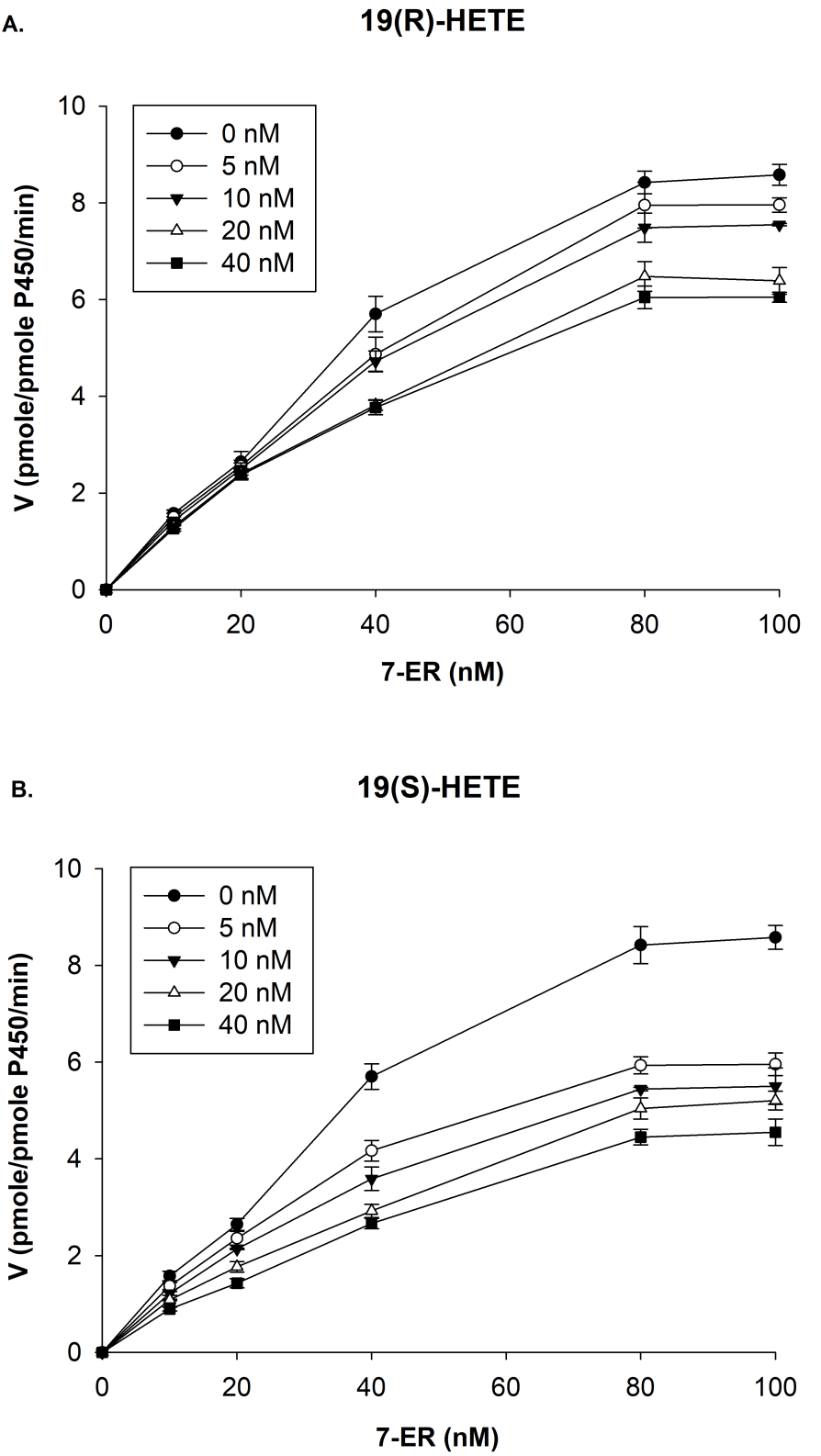
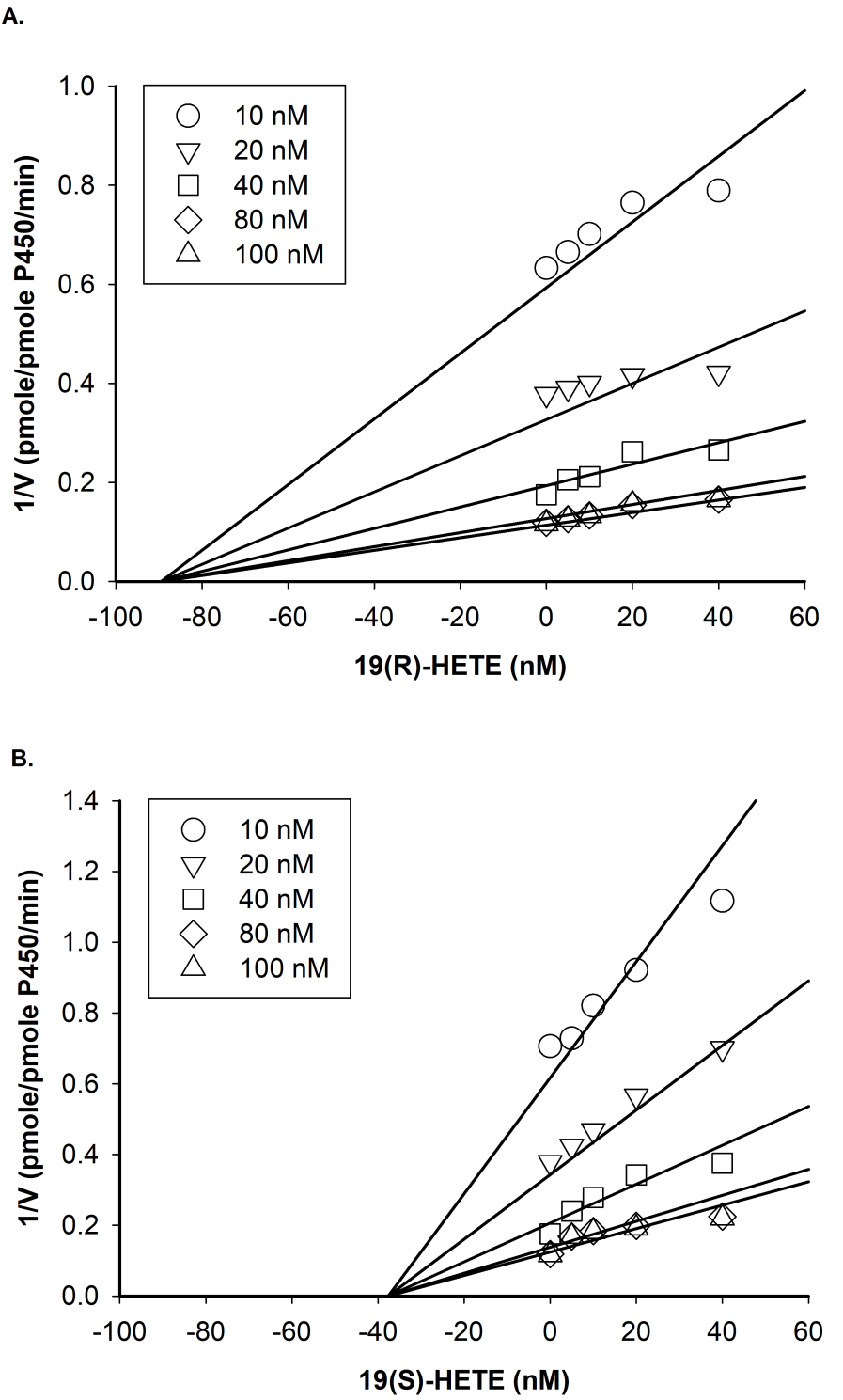

A.
19(R)-HETE
0 nM
5 nM
10 nM
20 nM
40 nM
V (pmole/pmole P450/min)
7-ER (nM)
B.
19(S)-HETE
0 nM
5 nM
10 nM
20 nM
40 nM
V (pmole/pmole P450/min)
7-ER (nM)

Figure 3


A.
1.0
0.8
0.6
0.4
0.2
0.0
1/V (pmole/pmole P450/min)
10 nM
20 nM
40 nM
80 nM
100 nM
-100
-80
-60
-40
-20
0
20
40
60
19(R)-HETE (nM)
B.
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1/V (pmole/pmole P450/min)
10 nM
20 nM
40 nM
80 nM
100 nM
-100
-80
-60
-40
-20
0
20
40
60
19(S)-HETE (nM)