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Research Article

Inhibition of human recombinant cytochromes P450 CYP1A1 and CYP1B1 by *trans*-resveratrol methyl ethers

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CYP1A1 and CYP1B1 are the inducible forms of cytochrome P450 expressed in extrahepatic tissues, which are responsible for the biotransformation of polycyclic aromatic hydrocarbons, heterocyclic amines and estradiol to the carcinogenic intermediates. The aim of our research was to determine and compare the inhibitory effect of naturally occurring analogues of *trans*-resveratrol on the catalytic activities of human recombinant CYP1A1 and CYP1B1. Pinostilbene (3,4′-dihydroxy-5-methoxystilbene), desoxyrhapontigenin (3,5-dihydroxy-4′-methoxystilbene), and pterostilbene (3,5-dimethoxy-4′-hydroxystilbene) appeared to be very potent inhibitors of CYP1A1 catalytic activity with K_i values of 0.13, 0.16 and 0.57 μM, respectively. Results from this study indicate that *trans*-resveratrol analogues in which the hydroxy groups are substituted by methoxy groups exhibit a remarkably stronger inhibitory effect towards CYP1A1 in comparison to the parent compound. On the contrary, the potency of pinostilbene, desoxyrhapontigenin and pterostilbene towards CYP1B1 with K_i values of 0.90, 2.06 and 0.91 μM, respectively, was comparable to that of resveratrol. It appears that between these analogues, inhibition of CYP1A1 and CYP1B1 catalytic activities does not vary much regardless of the number and position of methylether substitution. The results suggest that the *trans*-resveratrol analogues: pinostilbene, desoxyrhapontigenin and pterostilbene, which occur in some food plants, might be considered as promising chemopreventive agents.

Keywords: Cytochrome P450 / Desoxyrhapontigenin / Pinostilbene / Pterostilbene / *trans*-Resveratrol

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1 Introduction

Human cytochromes P450 (CYP), family 1 in particular, are involved in the metabolic activation of numerous chemical carcinogens such as polycyclic aromatic hydrocarbons (PAHs) and their dihydrodiol derivatives, aminoazo dyes, heterocyclic and aromatic amines, which are ubiquitous in human environment [1]. CYP1A1 and CYP1B1 beside oxidation of PAHs are also responsible for the metabolic activation of E_2 (β-estradiol), which has been suggested to be a major factor in mammary carcinogenesis. CYP1B1 primarily hydroxylates the C-4 position, while CYP1A1 exerts its activity at the C-2, C-6α and C-15α positions of estradiol [2, 3]. These P450 isoforms are found as constitutive forms in extrahepatic tissues and are overexpressed in a variety of tumours [4]. Because of the postulated significant role of both CYP1A1 and CYP1B1 in carcinogenesis, selective inhibitors of these P450s are sought in order to reduce the risk of mutagenesis and cancer. A favourable source would be the natural compounds derived from food plants.

trans-Resveratrol (3, 5,4′- trihydroxystilbene; Fig. 1) and its derivatives are phytoalexins. These compounds are synthesized and accumulated in leaves and fruits of numer-

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Abbreviations: CYP, cytochrome P450; **EROD,** 7-ethoxyresorufin-*O*-deethylase; **IC_{50},** the half maximal inhibitory concentration

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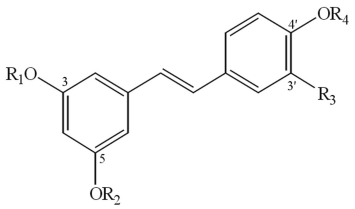

	R_1	R_2	R_3	R_4
1 *trans*- Resveratrol	H	H	H	H
2 Desoxyrhapontigenin	H	H	H	CH_3
3 Pinostilbene	H	CH_3	H	H
4 Pterostilbene	CH_3	CH_3	H	H

Figure 1. Chemical structures of resveratrol and its analogues tested in this study.

ous plants in response to stressors such as UV irradiation [5], aluminium chloride [6] and pathogens such as *Plasmopara viticola* (the causal agent for downy mildew) [7, 8] or *Botrytis cinerea* (the causal agent for grey mold) [9, 10]. *trans*-Resveratrol is the best known stilbene occurring in fruits such as grapes, peanuts, berries and some beverages derived from plants. It is believed to be a major contributor to the "French paradox", which refers to the health benefits associated with the moderate consumption of red wine [11, 12]. Grapes and blueberries are also significant sources of pterostilbene (3,5-dimethoxy-4′-hydroxystilbene; Fig. 1) [13], which was demonstrated to have cancer chemopreventive activity similar to *trans*-resveratrol [14]. Furthermore, pterostilbene was shown to have antihyperglycemic activity [15] and to be effective as a lipid/lipoprotein lowering agent in hamsters [16]. Monomethylated derivatives of resveratrol, pinostilbene (3,4′-dihydroxy-5-methoxystilbene; Fig. 1) and desoxyrhapontigenin (3,5-dihydroxy-4′-methoxystilbene; Fig. 1), are found in plants such as *Rumex bucephalophorus* (a common Red Dock) [17], and *Rheum undulatum* [18], which is known as Korean Rhubarb. Both compounds showed antioxidant activity [17]. In addition, desoxyrhapontigenin inhibited the lipid peroxidation of erythrocyte membrane induced by *tert*-butyl hydroperoxide [19]. Nevertheless, the information about the biological activities of the resveratrol analogues is still scarce, but promising, what should stimulate a further investigation.

Resveratrol, the most studied stilbene derivative, was shown to inhibit the growth of tumour cells in several *in vitro* and *in vivo* systems [20, 21] and was established as a chemopreventive agent in preclinical rodent models (for review, see [22, 23]). *trans*-Resveratrol's influence on the CYPs responsible for the procarcinogen biotransformation was also widely studied. It was reported that resveratrol inhibited human P450 1A1 activity in a competitive manner [24]. However, the half maximal inhibitory concentration (IC_{50}) of 23 μM was much higher than the value obtained for the well-known P450 family 1 inhibitors such as α-napthoflavone [25]. Resveratrol is a potent inhibitor of human recombinant CYP1B1 [26], CYP3A4 and CYP3A5 [27]. In addition, it is a mechanism-based inactivator of CYP1A2 [28] and CYP2E1 [29]. In spite of the broad activity of resveratrol towards numerous CYPs engaged in bioactivation of procarcinogens, the anticarcinogenic activity of this compound may be limited by its low bioavailability [30, 31]. There are still scarce data on the resveratrol bioavailability in humans, however, the available results demonstrate a high absorption but rapid metabolism of resveratrol given orally to human volunteers [32, 33]. That is one of the reasons why the current studies are focusing on the other members of stilbene family occurring in dietary products which might be as effective as a parent compound with regard to their potential usefulness in chemoprevention but demonstrating a slower metabolism. Since the inhibition of tumour initiation by resveratrol may be related to inhibition of CYP1A1 and CYP1B1, the more potent and selective inhibitors of these P450 isoforms among resveratrol analogues are searched.

In this regard, several hydroxystilbenes and their methyl ethers obtained from natural sources were evaluated. For example, it was shown that rhapontigenin (3,5,3′-trihydroxy-4′-methoxystilbene) was both a potent mechanism-based inactivator of CYP1A1 as well as a more potent competitive inhibitor than *trans*-resveratrol [34]. Our previous studies demonstrated that 3,4′,5-trimethoxy-*trans*-stilbene, pterostilbene, desoxyrhapontigenin and pinostilbene inhibited the activity of CYP1A2 with K_i values equal to 0.79, 0.39, 0.94 and 1.04 μM, respectively, while piceatannol was the least potent CYP1A2 inhibitor with K_i = 9.67 μM [35]. Thus, the resveratrol methyl ethers, particularly pterostilbene, were more efficient CYP1A2 inhibitors than *trans*-resveratrol with K_i value of 5.33 μM. It is apparent that substitution of the resveratrol hydroxy groups with methoxy groups increased the inhibition of CYP1A2 significantly, and the number and position of methylation were not restrictive. In contrast to CYP1A2 inhibition, desoxyrhapontigenin did not affect CYP2E1 and pinostilbene only moderately inhibited this enzyme (K_i = 42.6 μM), while resveratrol was demonstrated earlier to be a potent mechanism-based inhibitor of this P450 isoform [30].

There are several reports indicating that chemical modifications of the stilbene backbone of resveratrol enhance the biological activities of its analogues. These compounds appear to be concurrent or even superior to resveratrol as candidates for cardioprotective and chemopreventive agents exhibiting more potent antioxidant and cytotoxic effects [36–38]. The structure–activity relationship analysis presented in recent studies showed that both the antioxidant and proapoptotic activities of the analogues contain-

ing 3,4-dihydroxyl groups were significantly higher than those of the *trans*-resveratrol [36]. Moreover, it has been found that *trans*-stilbene compounds having 4′-hydroxy group, double bond and bearing *ortho*-diphenoxyl or *para*-diphenoxyl functionalities possess remarkably higher antioxidant activity than *trans*-resveratrol [39].

In the present study, we evaluated the inhibitory effect of pterostilbene and two monomethyl ethers of *trans*-resveratrol, desoxyrhapontigenin and pinostilbene, on human recombinant P450 family 1 members CYP1A1 and CYP1B1 and made an attempt to analyse the potential role of these analogues in the context of possible modulation of carcinogenesis process.

2 Materials and methods

2.1 Materials

Recombinant human CYP1A1 or CYP1B1 coexpressing nicotinamide adenine dinucleotide phosphate, reduced form (NADPH)-CYP reductase supersomes were obtained from Gentest (Woburn, MA, USA). The total CYP content was provided by the supplier. Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, nicotinamide adenine dinucleotide phosphate ($NADP^+$), Tris and other chemicals were obtained from Sigma (St. Louis, MO, USA). Stock solutions (10 mM) of stilbenes tested were prepared in DMSO (Sigma), while the 7-ethoxyresorufin (Sigma) for ethoxyresorufin-*O*-deethylase assay was prepared in methanol.

2.2 Stilbenes

Pinostilbene, desoxyrhapontigenin and pterostilbene were synthesized by partial methylation of *trans*-resveratrol using diazomethane. Diazomethane was freshly prepared, according to manufacturer's protocol, from the reaction of Diazald with potassium hydroxide using an Aldrich Mini Diazald apparatus (Sigma-Aldrich, St. Louis, MO, USA). To a solution of resveratrol (50 mg in 1 mL methanol), diazomethane was added dropwise and the synthesis of the methylether analogues was monitored by TLC using a silical gel plate (Merck Silica gel 60 F254; EMD Chemicals, Gibbstown, NJ, USA). The developing solvent for TLC was $CHCl_3$/MeOH (96:4) with 0.05% formic acid. The Rf values for pterostilbene, pinostilbene and desoxyrhapontigenin are 0.61, 0.28 and 0.20, respectively. These analogues were purified by preparative layer chromatography using the same developing solvent as in the TLC and identified from their ^{1}H-NMR spectra (Bruker 400 MHz; Bruker, Billerica, MA, USA) as well as from their mass spectra obtained through GC-MS using a JEOL (JEOL USA, Peabody, MA, USA) GCMate II system. For GC-MS, the temperature program was: initial temperature 120°C raised to 240°C at the rate of 15°C/min, then raised to 300°C at the rate of 6°C/min, then finally raised to 330°C at the rate of 30°C/min and held at this temperature for 2 min. The GC capillary column was DB-5 (0.25 mm id, 0.25 mm film thickness, 30 m length; J&W Scientific, Folsom, CA, USA). The carrier gas was ultra-high purity helium (Nex-Air, Batesville, MS, USA), at a flow rate of 1 mL/min. The inlet (splitless), GC interface and ion chamber temperatures were 250, 250 and 230°C, respectively. The volume of sample injected was 1 μL. The stilbenes were dissolved in chloroform and analysed underivatized. The retention time of pterostilbene, pinostilbene and desoxyrhapontigenin was 9.5, 10.2 and 10.4 min, respectively. The gas chromatograms and mass spectra of these analogues are shown in Fig. 2.

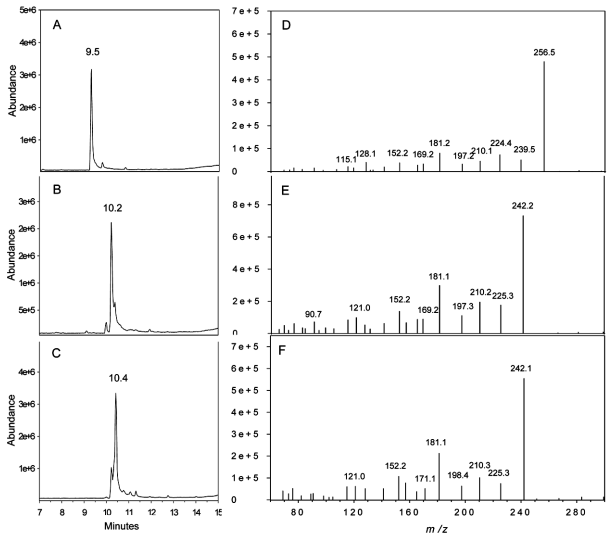

Figure 2. Gas chromatograms (A – C) and corresponding mass spectra (D – F) of pterostilbene (A and D), pinostilbene (B and E) and desoxyrhapontigenin (C and F); retention times 9.5, 10.2 and 10.4 min, respectively.

The ^{1}H-NMR data for pterostilbene, obtained as an oily substance, were similar to those published earlier [40, 41]. The ^{1}H-NMR data for pinostilbene and desoxyrhapontigenin, both obtained as brown powders, were also very similar to the published data [42].

2.3 7-Ethoxyresorufin-*O*-deethylation (EROD) assay

EROD, as an enzymatic biomarker of CYP1A1 and CYP1B1 activities, was measured according to the method of Burke *et al.* [43]. Reaction mixture (1 mL total volume) containing 1.25 pmol CYP1A1 or 5 pmol CYP1B1, 1.3 mM $NADP^+$, 3.3 mM glucose-6-phosphate, 0.5 U/mL glucose-6-phosphate dehydrogenase, 3.3 mM magnesium chloride and 2 μM 7-ethoxyresorufin in 100 mM potassium phosphate (pH 7.4) was incubated at 37°C for 15 min. The fluorescence of the product was determined on a HITACHI

Model F 2500 fluorescence spectrophotometer (λ_{ex}550 and λ_{em}585).

The quantitation of the deethylated metabolite was based on comparison of its fluorescence with resorufin as a standard. Control incubations did not contain the test compounds.

2.4 Enzyme inhibition kinetics

The enzyme kinetics for CYP1A1 and CYP1B1-catalysed EROD was measured at increasing concentrations (0.066, 0.1, 0.2, 0.5, 1.0 μM) of the substrate, 7-ethoxyresorufin. Recombinant supersomes were incubated at 37°C for 15 min in buffer with DMSO or one of the two concentrations (0.5 and 1 or 2 μM) of the tested stilbenes. Lineweaver–Burk plots were used for determination of the mode of inhibition. The apparent K_i values were determined from the *x*-intercept of a plot of apparent K_m/V_{max} (obtained from the slope of the Lineweaver–Burk plots) *versus* inhibitor concentration [44]. K_i value was calculated from the equation of linear regression using Excel software program. IC_{50} was determined graphically by plotting percent of control enzyme activity versus inhibitor concentration. In case of competitive inhibitors, K_i values were also calculated with the use of the Cheng–Prusoff equation: $K_i = IC_{50}/(1 + [S]/K_m)$, where [S] is the substrate concentration (2 μM) and K_m (affinity constant) values for CYP1A1 (0.105 ± 0.029 μM) and CYP1B1 (2.12 ± 0.93 μM) were calculated as means of 3 separate determinations from Lineweaver–Burk plots.

3 Results

Inhibitory activity on CYP1A1 and CYP1B1 of pinostilbene, desoxyrhapontigenin and pterostilbene compared to the corresponding parent compounds was studied. 7-Ethoxyresorufin was used as a substrate, as it has been demonstrated that both CYP1A1 and CYP1B1 are involved in the oxidative metabolism of this compound [45]. As shown in Fig. 3, pinostilbene, desoxyrhapontigenin and pterostilbene in the concentration range of 0.5–50 μM decreased EROD activity in a dose-dependent manner with IC_{50} values for CYP1A1: 2.2, 1.25 and 3.6 μM, and for CYP1B1: 0.8, 2.6 and 2.1 μM, respectively (Table 1). Maximal EROD activities in the controls containing <1% DMSO were 13.6 pmol resorufin/(min × pmol P450) and 4.77 pmol resorufin/(min × pmol P450) for the recombinant CYP1A1 and CYP1B1, respectively. To investigate the mode of CYP1A1 inhibition, enzyme kinetic experiments were performed with the concentration of the inhibitor equal to 0.5 and 1.0 μM and with the substrate concentration in the range of 0.066–1.0 μM. Kinetic data analysis of Lineweaver–Burk plots indicated that pinostilbene inhibited CYP1A1 in a mixed (competitive/noncompetitive) manner while desoxyrhapontigenin and pterostilbene appeared to be competitive inhibitors of the enzyme (Fig. 4). Parallel experiments carried out for CYP1B1 with the same substrate concentration range and the concentration of inhibitor ranging from 0.5 to 2.0 μM showed that the three tested *trans*-resveratrol derivatives competitively inhibited CYP1B1 (Fig. 5).

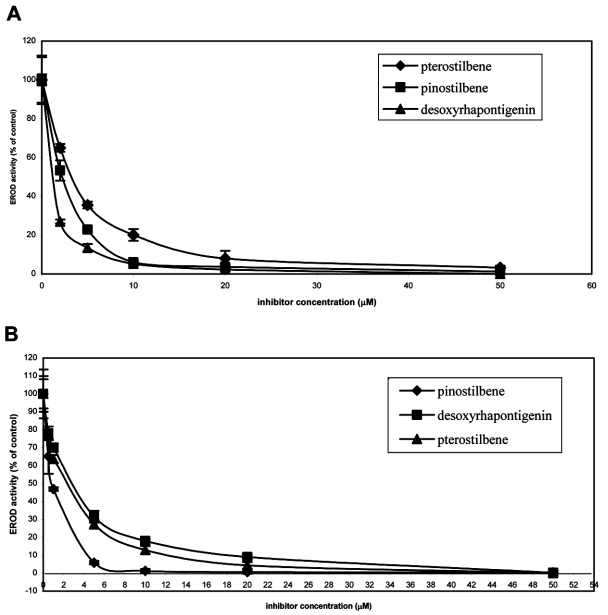

Figure 3. Effects of pinostilbene, desoxyrhapontigenin and pterostilbene on the catalytic activity of human recombinant CYP; (A) CYP1A1 and (B) CYP1B1. Each point represents the mean ± SD of triplicate sample.

The apparent K_i values for pinostilbene, desoxyrhapontigenin and pterostilbene determined in this study for CYP1A1 were 0.13, 0.16, 0.57 μM and for CYP1B1 0.9, 2.06 and 0.91 μM, respectively. The K_i values for the competitive inhibitors were also calculated using the Cheng–Prusoff equation, taking into account the ethoxyresorufin substrate concentration (2 μM) and the K_ms for each enzyme, which were 0.105 μM for CYP1A1 and 2.12 μM for CYP1B1 (K_i values are summarized in Table 1). Pinostilbene, desoxyrhapontigenin and pterostilbene were shown to be much more effective inhibitors of CYP1A1 activity than resveratrol; however, their potency towards CYP1B1 was similar to that of the parent compound.

4 Discussion

Inhibition of phase I enzymes concomitant with induction of phase II enzymes is considered as one of the strategies in cancer chemoprevention [46]. In this context, inhibitory effect of naturally occurring compounds on cytochrome P450 phase I enzymes involved in metabolic activation of procarcinogens is regarded as beneficial in cancer prophylaxy. Among phytochemicals *trans*-resveratrol is the most

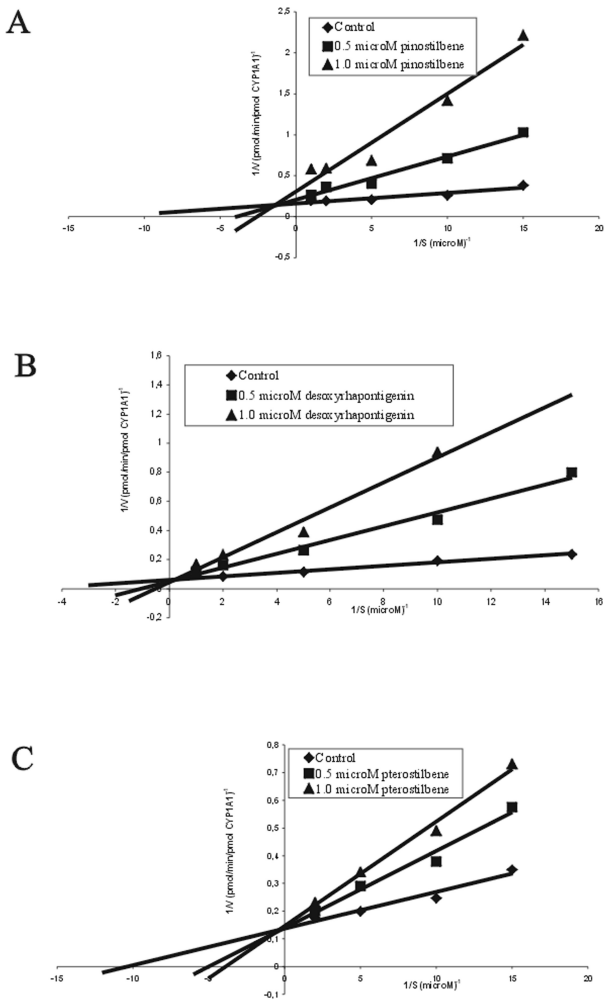

Figure 4. Lineweaver–Burk plots representing reciprocal of EROD activity expressed in picomoles of resorufin/min/pmol CYP1A1 *versus* reciprocal of 7-ethoxyresorufin concentration expressed in μM in the absence and presence of inhibitors: (A) pinostilbene; (B) desoxyrhapontigenin; (C) pterostilbene. Each point represents the mean of triplicate sample.

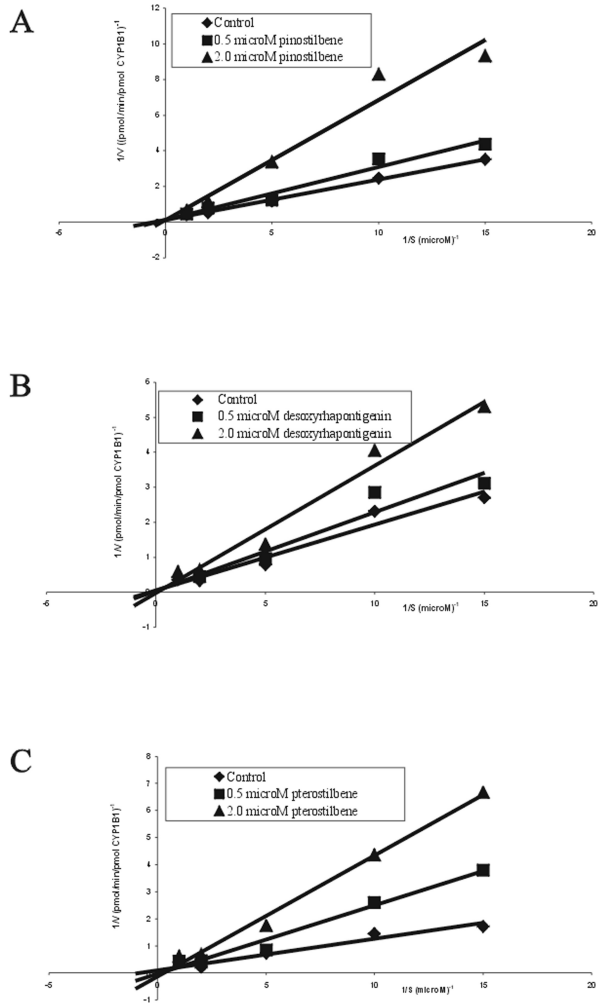

Figure 5. Lineweaver–Burk plots representing reciprocal of EROD activity expressed in picomoles of resorufin/min/pmol CYP1B1 *versus* reciprocal of 7-ethoxyresorufin concentration expressed in μM in the absence and presence of inhibitors: (A) pinostilbene; (B) desoxyrhapontigenin; (C) pterostilbene. Each point represents the mean of triplicate sample.

studied polyphenol, which has been demonstrated to have anticarcinogenic activity in a number of experimental systems (for review, see [23]). Resveratrol, however, shows some limitations in reference to its relatively low potency towards CYP1A1/2 and poor bioavailabity [30]. Thus, structural modifications of resveratrol – in order to increase its bioavailability are considered. In the present study, we provide the first comparative data on the inhibitory effects of three *trans*-resveratrol methyl ethers on human recombinant CYP1A1 and CYP1B1, isozymes of cytochrome P450, which are involved in the activation of numerous chemical procarcinogens. The tested stilbenes (pinostilbene, desoxyrhapontigenin and pterostilbene) occur in many fruits and traditional herbal remedies, thereby are constituents of the human diet and may be considered as potential chemopreventive agents.

Our study has shown that all tested resveratrol methyl ethers are very potent inhibitors of CYP1A1 catalytic activity, much more efficient than the parent compound (Fig. 3). Desoxyrhapontigenin and pinostilbene, which are the monomethyl ethers of resveratrol, were slightly more effective inhibitors with IC_{50} of 2.2 and 1.25 μM, respectively, in comparison to the dimethyl ether analogue, pterostilbene (IC_{50} = 3.6 μM). As summarized in Table 1, K_i values obtained for pinostilbene, desoxyrhapontigenin and pterostilbene confirmed the strong CYP1A1 inhibition by these compounds. Moreover, when compared with the data reported by Chun *et al.* [24] on CYP1A1 inhibition by *trans*-resveratrol (IC_{50} = 23 μM, K_i = 9 μM) the results of our present study show that there is a significant increase in the inhibitory effect towards CYP1A1 when hydroxy groups are replaced by the methoxy groups. The K_i values

Table 1. Effect of stilbenes on CYP1A1 and CYP1B1 catalytic activity

Inhibitor	CYP1A1			CYP1B1			K_i ratio (CYP1A1/CYP1B1)
	IC_{50} (μM)	K_i (μM)	Mode of inhibition	IC_{50} (μM)	K_i (μM)	Mode of inhibition	
trans-Resveratrol	23[a)]	9[a)]	Mixed type	1.4[b)]	0.8[b)]	Mixed type	11.25
Pinostilbene	2.2	0.13	Mixed type	0.8	0.90 (0.41)[c)]	Competitive	0.14
Desoxyrhapontigenin	1.25	0.16 (0.14)[c)]	Competitive	2.6	2.06 (1.34)[c)]	Competitive	0.08
Pterostilbene	3.6	0.57 (0.22)[c)]	Competitive	2.1	0.91 (1.08)[c)]	Competitive	0.62

a) Chun *et al.* [34].
b) Chang *et al.* [26].
c) K_i values in parenthesis were calculated according to the Cheng–Prusoff equation: $K_i = IC_{50}/(1 + [S]/K_m)$, where [S] is the substrate concentration (2 μM) and K_m (affinity constant) values were calculated for CYP1A1 (0.105 ± 0.029 μM) and CYP1B1 (2.12 ± 0.93 μM) by linear regression analysis.

obtained for CYP1A1 inhibition by desoxyrhapontigenin and pinostilbene are very close to those demonstrated in our previous study on CYP1A2 inhibition [35] indicating that a methoxy group increases the inhibitory effect, but not selectivity, towards one of these two CYPs. Chun *et al.* [34] have also studied the inhibitory effect of rhapontigenin (3,5,3′-trihydroxy-4′-methoxystilbene), another natural monomethylated analogue of *trans*-resveratrol on CYP1A1 activity and found it to be a very selective and an irreversible inactivator of CYP1A1, with IC_{50} value of 0.4 μM and K_i value of 0.09 μM [34]. Thus, while comparing with desoxyrhapontigenin that lacks the hydroxy group at 3′ position, it may be suggested that this substituent is a determinant of the strong inhibitory effect of rhapontigenin on CYP1A1.

The inhibitory action of *trans*-resveratrol on CYP1B1 (IC_{50} = 1.4 μM), reported by other authors [26], was similar to the effect of its methyl ethers evaluated in our present study. In the case of CYP1B1, there was no difference in the inhibitory effect of pinostilbene and pterostilbene (K_i values of 0.90 and 0.91 μM, respectively). The apparent K_i (2.06 μM) for CYP1B1 inhibition by desoxyrhapontigenin was about 2-fold higher than those of pterostilbene and pinostilbene. According to the IC_{50} values, pinostilbene (0.8 μM), desoxyrhapontigenin (2.6 μM) and pterostilbene (2.1 μM) exerted a comparable inhibitory effect. In summary, methylation of one or two hydroxy groups in resveratrol molecule did not change the affinity of the resveratrol analogues towards CYP1B1 active site.

On the basis of the results obtained for CYP1A1 and CYP1B1 it may be concluded that desoxyrhapontigenin with inhibition ratio CYP1A1/CYP1B1 of 0.08 (when K_i values were compared) is the most selective CYP1A1 inhibitor among the methoxylated resveratrol analogues tested in this study. However, Chun *et al.* [34] showed that rhapontigenin exhibited 23-fold selectivity for P450 1A1 over P450 1B1. It seems, therefore, that the 3′-hydroxy group might play a pivotal role in enhancing both the potency and selectivity of rhapontigenin as a CYP1A1 inhibitor. Studies using synthetic stilbenes led to the discovery of 2,4,3′,5′-tetramethoxystilbene, as a very potent and selective inhibitor of P450 1B1, with K_i of 3 nM [47, 48]. These data indicate that the presence of an additional methoxy group at the 2 position might be of crucial significance for increased inhibitory effect and selectivity; this methoxy substituent strongly increases the affinity of stilbene derivative towards CYP1B1 active site [48].

An analysis of the kinetics of enzyme inhibition by a double-reciprocal (Lineweaver–Burk) plot showed that pinostilbene functioned as a competitive inhibitor of CYP1B1 and a mixed-type one of CYP1A1, being able to compete for the substrate binding site and binding to region which is not, in fact, directly involved in substrate interaction. On the other hand, desoxyrhapontigenin and pterostilbene inhibited both enzymes CYP1A1 and CYP1B1 in a competitive manner. Therefore, these resveratrol analogues might effectively protect against CYP-dependent procarcinogen activation and contribute to the potential usefulness of plant products for cancer chemoprevention. Accordingly, pterostilbene seems to be particularly promising because, like resveratrol, it is found in grapes [49] and in numerous species of *Vaccinium* berries at levels of 99–520 ng/g dry sample [13]. It has been detected in fungus-infected grapes at levels of 0.2–4.7 μg/g fresh weight of skin [50]. Although the dietary intake may not provide plasma concentrations relevant to CYP inhibition, it should be emphasized that the protective action of phytochemicals as food components may give an additive and/or synergistic effect [51].

Biological activity of pterostilbene involves the pathways of cancer chemoprevention. Using a mouse mammary gland culture assay, pterostilbene has been shown to have a cancer chemopreventive activity similar to that of resveratrol [13]. It was found to be a potent but not selective inhibitor of cyclooxygenase-1 and cyclooxygenase-2 [52]. It also appears to be capable of down-regulating the expression of eukaryotic transcription factor nuclear factor κB (NF-κB) what may be suggested as a possible mechanism of its antitumor promoting action (Cichocki *et al.*, unpublished data). Additionally, substitution of a hydroxy with methoxy group

increases lipophilicity of resveratrol analogues, which may improve their bioavailability. This effect was demonstrated in the comparative studies of resveratrol and pterostilbene plasma levels [53]. The authors calculated a half-life of resveratrol and pterostilbene in mouse plasma after intravenous administration as ~10.2 and 77.9 min, respectively. In the first-time pilot studies on healthy human volunteers after 1 wk treatment with 450 mg per day of an extract of *Pterocarpus marsupium*, the highest pterostilbene concentration in plasma (0.2 μM) was reached after 7 days of treatment [54]. However, the interest in biological activity of resveratrol derivatives is growing, information on the bioavailability of monomethyl ethers is still lacking . Whether dietary resveratrol and its analogues *in vivo* will reach the desirable sites of action is still a matter of debate.

In summary, our study has revealed that the natural stilbene derivatives: pinostilbene, desoxyrhapontigenin and pterostilbene are potent inhibitors of CYP1A1 and CYP1B1 catalytic activities. Moreover, their inhibitory effect on CYP1A1 appeared to be much stronger than that reported for *trans*-resveratrol. With regard to structure–activity relationship, we demonstrated that the presence of a methoxy group was of considerable significance in case of CYP1A1 inhibition suggesting that a further modification of the chemical structure of polyhydroxystilbene may produce more specific and potent chemopreventive and/or chemotherapeutic agents. Natural methylether derivatives of *trans*-resveratrol may be considered as promising chemopreventive agents and important for dietary intervention approach. Although inhibition of carcinogen activation by modulation of key CYP may be important for chemopreventive activity, more studies are necessary to determine the *in vivo* bioavailability of these stilbenes as well as their action on multiple pathways involved in carcinogenesis.

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