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Research Article

Thiomethylstilbenes as inhibitors of CYP1A1, CYP1A2 and CYP1B1 activities

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Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) is a natural stilbene derivative occurring in grapes, peanuts and red wine. Its chemopreventive action has been established in studies on animal models. Recently, numerous classes of compounds with stilbene backbone have been investigated for their biological activity concerning cancer prevention; *e.g.* resveratrol methyl ethers appeared to be specific and potent inhibitors of cytochromes P450 (CYP) family 1 involved in the activation of procarcinogens. Since the replacement of the 4′-hydroxyl with a thiomethyl group is supposed to reduce toxicity of stilbene derivatives, the purpose of this study was the synthesis and evaluation of a series of 4-thiomethyl-*trans*-stilbene derivatives differing in a number and position of additional methoxy groups. Their inhibitory potency toward human recombinant CYPs: CYP1A1, CYP1A2 and CYP1B1 have been studied and compared with the effect of resveratrol and its analogues. Among compounds tested, 2-methoxy-4′-thiomethyl-*trans*-stilbene and 3-methoxy-4′-thiomethyl-*trans*-stilbene demonstrated the most potent and selective inhibitory effect on CYP1A1 and CYP1B1 activities. The results of our study indicate that modification of stilbene derivatives with thiomethyl group may influence the selectivity and inhibitory potency of these compounds toward P450 isozymes. Thus, it should be considered in developing new chemopreventive agents based on their mechanism of action.

Keywords: Cytochrome P450 / Thiomethylstilbenes / *trans*-Resveratrol

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1 Introduction

Cytochrome P450 (CYP) CYP1 family comprises CYP1A1, CYP1A2 and CYP1B1, which are involved in bioactivation of numerous procarcinogens, including polycyclic aromatic hydrocarbons, heterocyclic amines and 17β-estradiol (E2) to mutagenic and carcinogenic intermediates [1]. Because of the postulated significant role of CYP1B1 in carcinogenicity of E2, CYP1B1 is regarded as a target enzyme for blocking tumour initiation. Selective inhibition of CYP1B1 may prevent E2-related tumour formation, on the other hand, the use of CYP1B1 inhibitors might help to overcome anticancer drug resistance [2]. As selective inhibitors are important agents for toxicological and pharmacological applications, many types of compounds have been tested for their inhibitory effects on CYP1 dependent enzymes. *trans*-Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene; Fig. 1), one of the promising chemopreventive agents, represents widely investigated natural stilbenes. This polyphenol occurring in grapes, peanuts and red wine displays chemopreventive action established in numerous studies on animal models [3, 4], however, it exhibits several liabilities as poor stability in water solutions and low bioavailability [5]. Recently, numerous *trans*-resveratrol analogues have been investigated for their biological activity concerning cancer prevention; *e.g.* resveratrol methyl ethers appeared to be specific and potent inhibitors of CYPs family 1; CYP1A1, CYP1A2 and CYP1B1 [6–9]. The selectivity and potency of modified derivatives were strongly dependent on the positions of methoxy substituents. To examine the effect of structural modification on inhibitory selectivity, we have synthesized a series of methoxylated derivatives (Fig. 1) of 4′-thiomethyl-*trans*-stilbene (4′-MTS). As was observed by Yang *et al.* [10], substitution of 4′ oxygen atom with the less electronegative

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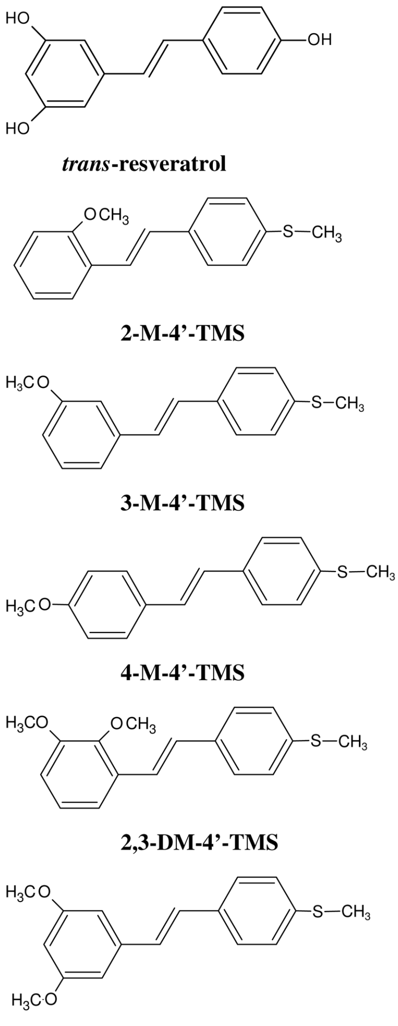
Abbreviations: **CYP,** cytochrome P450; **EROD,** 7-ethoxyresorufin-*O*-deethylase



***trans*-resveratrol**

2-M-4'-TMS

3-M-4'-TMS

4-M-4'-TMS

2,3-DM-4'-TMS

3,5-DM-4'-TMS

Figure 1. Chemical structures of tested compounds.

sulphur atom reduces toxicity to HEK 293 cells and enhances the ability of the compound to activate human SIRT1.

Hydrophobic 4′-thiomethyl group has been supposed to change the efficiency of stilbenes on CYPs inhibition. In the present study, the effect of a series of methoxy derivatives of 4′-methylthiostilbene on human recombinant CYP1A1, CYP1A2 and CYP1B1 activities has been investigated and compared to the inhibitory effect of *trans*-resveratrol and its methyl ethers.

2 Materials and methods

2.1 Materials

Supersomes, microsomes from baculovirus-infected insect cells coexpressing NADPH-CYP reductase and CYP1A1, CYP1A2 or CYP1B1, were purchased 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 ($NADP^+$), 7-ethoxyresorufin and *trans*-reveratrol were obtained from Sigma (St. Louis, MO, USA). *trans*-Resveratrol trimethyl ether was kindly gifted by Dr. Agnes Rimando (Natural Products Utilization Research Unit, US Department of Agriculture, University, MI, USA).

2.2 Compounds synthesis

2.2.1 General consideration

Commercially available reagents and solvents were used without further purification and were purchased from Sigma–Aldrich or Lancaster Synthesis.

Melting points were determined in an open glass capillary with a Stuart scientific SMP3 apparatus and are uncorrected. The structure of all compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR spectra (using Varian Gemini 300 VT apparatus) and MS spectra (using AMD 604 apparatus). Chemical shifts are reported in parts *per* million (ppm). *cis*/*trans*-Isomers of synthesized stilbenes were separated and purified using chromatographic column with silica gel (Merck Kieselgel 400 mesh ASTM) and chloroform/hexane (3:1) mixture as elution phase. TLC was carried out on Merck silica gel 60 F_{254} plates.

In the first step, 4-toluenesulphonyl chloride (6.6 g, 35 mmol) in 24 g (13 mL) sulphuric acid and 70 g ice was reduced by zinc dust 12.0 g. The reaction mixture was allowed to stir for 4 h at 0–5°C, and product – 4-thiocresol was isolated by steam distillation and purified by crystallization from EtOH (m.p. 43°C, lit. 42°C, yield: 3.5 g, 82%). *p*-Thiocresol (3,5 g, 29 mmol) was methylated by methyl iodide (4.1 g, 29 mmol) in toluene (20 mL) with triethylamine (2.9 g, 29 mmol) by heating under reflux for 2 h. After removing the solvent *in vacuo*, the oily residue was distilled under diminished pressure and allowed to afford 4-methylthiotoluene (b.p.$_{2\ \mathrm{mm\ Hg}}$: 61°C lit. b.p.$_{2\ \mathrm{mm\ Hg}}$: 61.5°C, yield 3.6, 82%) (Fig. 2). Chlorination of this product (3.6 g, 28 mmol) by sulphuryl chloride (7.5 g, 56 mmol) gave 4-methylthiobenzyl chloride yield (crude product) 2.9 g, 65%. This compound was very unstable, that is why it has been used immediately in the reaction with triphenylfosphine (4.3 g, 15 mmol) in 50 mL of toluene. The reaction mixture was carried on by heating under reflux for 4 h to afford 4-methylthiobenzyl-triphenylphosphonium chloride (5.1 g, 80% – based onto triphenylphosphine, m.p. 157°C), The structure of expected product was confirmed with the elementary analyses for $C_{26}H_{24}ClPS$ (Calcd. C 71.79, H 5.56, S 7.37; Found C 71.54, H 5.45, S 7.43) and HRMS (*m/z*): Calcd. 434.9603; Found 434.96071.

2.2.2 Representative procedure for the synthesis of target compounds

Stilbene backbones of title compounds were synthesized by liquid–liquid (CCl_4 and 0.3 M NaOH water solution) phase

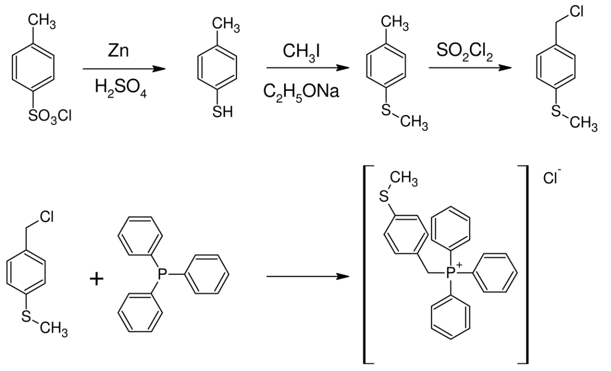


Figure 2. Synthesis of (4-thiomethyl-)benzyl-trifenylphosphonium salt.

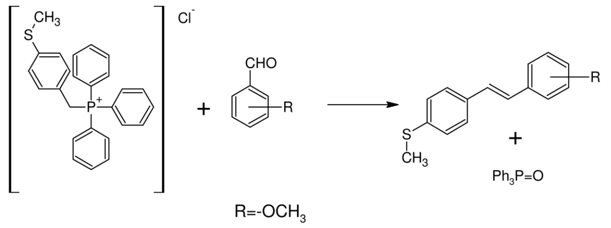


Figure 3. Wittig reaction in synthesis of stilbene 4′-thiomethyl derivatives.

transfer catalysed Wittig reaction [11, 12] (Fig. 3) of phosphonium salt and corresponding commercially available methoxybenzaldehydes (2-methoxybenzaldehyde; 3-methoxybenzaldehyde; anisaldehyde; 2,3-dimethoxybenzaldehyde and 3,5-dimethoxybenzaldehyde). 4-(Thiomethyl)benzyl-triphenylphosphonium chloride (1.4 g; 3.6 mmol), corresponding aldehyde (3.6 mmol), 50 mL CCl_4 and 50 mL of sodium hydroxide (water solution of 0.3 M NaOH) have been vigorously stirred for 6 h at room temperature. The organic layer was separated, washed with water and then CCl_4 was evaporated to give oily mixtures of *trans*/*cis* stilbenes and triphenylphosphine oxide. *trans*-Isomers were separated and purified using chromatographic column with silica gel and chloroform/hexane (3:1) as elution phase. All synthesized 4′-thiomethyl-*trans*-stilbene derivatives were identified by ^{1}H- and ^{13}C-NMR and HRMS spectra.

2.2.3 2-Methoxy-4′-thiomethyl-*trans*-stilbene (2-M-4′-TMS)

Yield 0.38 g, 41%, white crystals. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.51 (d, *J* = 8.1 Hz, 2H, C3′–**H**, C5′–**H**), 7.47 (d, *J* = 8.1 Hz, 2H, C2′–**H**, C6′–**H**), 7.23 (t, *J* = 8.1 Hz, 1H, C4–**H**), 7.13 (d, *J* = 8.1 Hz, 1H, C6–**H**), 7.09 (d, *J* = 16.4 Hz, 1H, Cvin′–**H**), 7.03 (d, *J* = 16.4 Hz, 1H, Cvin–**H**), 6.97 (t, *J* = 8.1 Hz, 1H, C5–**H**), 6.90 (d, *J* = 8.1 Hz, 1H, C3–**H**), 3.89 (s, 3H, –OC**H₃**), 2.50 (s, 3H, –SC**H₃**). ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm) = 156.86 (**C2**), 137.39 (**C4′**), 135.04 (**C1′**), 128.57 (**C4**), 128.42 (**Cvin**), 126.82 (**C2′**, **C6′**), 126.77 (**C3′**, **C5′**), 126.42 (**Cvin′**), 126.32 (**C1**), 122.92 (**C6**), 120.74 (**C5**), 110.94 (**C3**), 55.49 (C2–OCH_3), 15.91 (SCH_3). HRMS (*m*/*z*) Calcd. for $C_{16}H_{16}OS$: 256.3630; Found 256.3691.

2.2.4 3-Methoxy-4′-thiomethyl-*trans*-stilbene (3-M-TMS)

Yield 0.31 g, 34%, white crystals. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.44 (d, *J* = 8.1, 2H, C3′–**H**, C5′–**H**), 7.30–7.22 (m, *J* = 8.1 Hz, 4H, C5–**H**, C2′–**H**, C6′–**H**, C6–**H**), 7.15 (d, *J* = 16.4 Hz, Cvin′–**H**), 7.09 (d, *J* = 16.4 Hz, 1H, Cvin–**H**), 7.04 (s, 1H, C2–**H**), 6.82 (d, *J* = 8.1 Hz, 1H, C4–**H**), 3.85 (s, 3H, C3–OC**H₃**), 2.51 (s, 3H, –SC**H₃**). ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm) = 159.88 (**C3**), 138.76 (**C4′**), 137.91 (**C1**), 134.16 (**C1′**), 129.60 (**C5**), 128.31 (**Cvin**), 127.94 (**Cvin¢**), 126.89 (**C2**, **C6**), 126.67 (**C3**, **C5**), 119.13 (**C6**), 113.21 (**C2**), 111.65 (**C4**), 55.20 (C3–OCH_3), 15.76 (SCH_3). HRMS (*m*/*z*) Calcd. for $C_{16}H_{16}OS$: 256.3630; Found 256.3651.

2.2.5 4-Methoxy-4′-thiomethyl-*trans*-stilbene (4-M-4′-TMS)

Yield 0.42 g, 46%, white crystals. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.48 (d, *J* = 8.0 Hz, 2H, C3–**H**, C5–**H**), 7.28 (d, *J* = 8.0 Hz, 2H, C3′–**H**, C5′–**H**), 7.25 (d, *J* = 8.0 Hz, 2H, C2′–**H**, C6′–**H**), 7.09 (d, *J* = 16.3 Hz, 1H, Cvin′–**H**), 7.06 (d, *J* = 16.3 Hz, 1H, Cvin–**H**), 6.94 (d, *J* = 8.0 Hz, 2H, C2–**H**, C6–**H**), 3.81 (s, 3H, –OC**H₃**), 2.49 (s, 3H, –SC**H₃**). ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm) = 150.44 (**C4**), 136.26 (**C4′**), 132.44 (**C1′**), 129.37 (**C2′**, **C6′**), 128.76 (**C1**), 127.12 (**C2**, **C6**), 126.90 (**Cvin**), 126.61 (**Cvin′**), 123.49 (**C3′**, **C5′**), 114.56 (**C3**, **C5**), 55.24 (OCH_3), 15.28 (SCH_3). HRMS (*m*/*z*) Calcd. for $C_{16}H_{16}OS$: 256.3630; Found 256.3623.

2.2.6 2,3-Dimethoxy-4′-thiomethyl-*trans*-stilbene (2,3-DM-4′-TMS)

Yield 0.43 g, 42%, pale-brown crystals. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.37 (d, *J* = 8.0 Hz, 2H, C3′–**H**, C5′–**H**), 7.28 (d, *J* = 8.0 Hz, 2H, C2′–**H**, C6′–**H**), 7.09–6.96 (m, *J* = 8.0 Hz, 3H, C4–**H**, C5–**H**, C6–**H**), 6.90 (d, *J* = 16.3 Hz, 1H, Cvin′–**H**), 6.78 (d, *J* = 16.3 Hz, 1H, Cvin–**H**), 3.88 (s, 3H, C3–OC**H₃**), 3.82 (s, 3H, C2–OC**H₃**), 2.53 (s, 3H, –SC**H₃**). ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm) = 152.81 (**C2**), 147.15 (**C3**), 137.18 (**C4′**), 133.77 (**C1′**), 131.80 (**Cvin**), 130.12 (**C1**), 129.34 (**C2′**, **C6′**), 125.91 (**Cvin′**), 125.37 (**C6**), 123.59 (**C3′**, **C5′**), 121.91 (**C4**), 111.28 (**C5**), 60.72 (C2–OCH_3), 55.70 (C3–OCH_3), 15.53 (SCH_3). HRMS (*m*/*z*) Calcd. for $C_{17}H_{18}O_2S$: 286.3942; Found 286.3938.

2.2.7 3,5-Dimethoxy-4′-thiomethyl-*trans*-stilbene (3,5-DM-4′-TMS)

Yield 0.46 g, 45%, white crystals. ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.43 (d, *J* = 8.1 Hz, 2H, C3′–**H**, C5′–**H**), 7.24 (d, $J = 8.1$ Hz, 2H, C2′−**H**, C6′−**H**), 7.05 (d, $J = 16.3$ Hz, 1H, Cvin′−**H**), 6.98 (d, $J = 16.3$ Hz, 1H, Cvin−**H**) 6.67 (s, 2H, C2−**H**, C6−**H**), 6.40 (s, 1H, C4−**H**), 3.83 (s, 6H, C3−OC**H₃**, C5−OC**H₃**), 2.50 (s, 3H, −SC**H₃**). ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm) = 160.96 (**C3**, **C5**), 139.31 (**C4**), 137.99 (**C4′**), 134.04 (**C1′**), 128.51 (**Cvin**), 128.01 (**Cvin′**), 126.92 (**C2′**, **C6′**), 126.64 (**C3′**, **C5′**), 104.48 (**C2**, **C6**), 99.91 (**C4**), 55.32 (C3−O**C**H₃, C5−O**C**H₃), 15.73 (S**C**H₃). HRMS *m/z*: 286.1042. HRMS (*m/z*) Calcd. for $C_{17}H_{18}O_2S$: 286.3942; Found 286.3919.

2.3 7-Ethoxyresorufin-*O*-deethylation assay

To assess CYP1 enzyme activities 7-ethoxyresorufin-*O*-deethylase (EROD) activity was measured according to the method of Burke *et al.* [13].

Reaction mixture (1 mL total volume) containing 1.25 pmol CYP1A1, 5 pmol CYP1A2 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) were 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 7-ethoxyresorufin-*O*-deethylation 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 (control) or one of the following concentrations: 0.125, 0.25, 0.5 or 1.0 μM of the tested stilbenes in DMSO solution (the amount of DMSO in a reaction mixture did not exceed 1%). 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. K_i value was calculated from the equation of linear regression using the Microsoft Excel software. The concentrations of compounds required for 50% inhibition of catalytic activities (IC_{50}) were determined graphically by plotting percent of control enzyme activity *versus* inhibitor concentration.

3 Results and discussion

Table 1. IC_{50} values for EROD inhibition by stilbene analogues

	IC_{50} (μM)			Ratio	
	CYP1A1	CYP1A2	CYP1B1	CYP1A2/ CYP1A1	CYP1A2/ CYP1B1
Resveratrol	26	>100	11.2	>3.8	>8.9
RTE	10.2	32	13.5	3.1	2.4
2-M-4′-TMS	1.0	7.5	0.3	7.5	25
3-M-4′-TMS	1.1	11.5	0.5	10.5	23
4-M-4′-TMS	28	23	5.5	0.8	4.2
2,3-DM-4′-TMS	1.8	22.0	1.1	12.2	20
3,5-DM-4′-TMS	1.4	5.0	1.5	3.6	3.3

Table 2. K_i values for human recombinant CYP1A1 and CYP1B1 inhibition by monomethoxy-4′-methylthiostilbenes

	K_i (μM)	
	CYP1A1	CYP1B1
2-M-4′-TMS	0.033 ± 0.002	0.041 ± 0.013
3-M-4′-TMS	0.025 ± 0.006	0.030 ± 0.006

In our studies, the inhibitory potency of a series of 4′-thiomethylstilbene derivatives toward human recombinant CYP1A1, CYP1A2 and CYP1B1 has been investigated in the concentration range up to 100 μM (Fig. 4). IC_{50} determined for tested compounds was compared with the effect of *trans*-resveratrol and its 3,4′,5-trimethyl ether (RTE) on the CYP1A1, CYP1A2 and CYP1B1 activities (Table 1). Methoxy groups increase the affinity of resveratrol derivatives toward active sites of family CYP1 enzymes as it was proved previously in numerous studies [6–9]. In the present studies, methyl ethers of 4′-thiomethylstilbene appeared to be significantly stronger inhibitors of CYP1A1, CYP1A2 and CYP1B1 (except the effect of 4-M-4′-TMS on CYP1A1) in comparison with resveratrol and RTE (Table 2). *trans*-Resveratrol inhibits CYP1 enzymes by two distinct mechanisms: direct inhibition (CYP1A1 and CYP1B1) and mechanism-based inactivation (CYP1A2) [14]. Whether the methoxy derivatives of 4′-TMS are inactivators of CYP1A2 remains to be established.

Kinetic data analysis was performed using Lineweaver–Burk plots. A competitive mode of CYP1A1 and CYP1B1 inhibition was observed for 2-M-4′-TMS and 3-M-4′-TMS (Figs. 5 and 6). K_i values shown in the Table 2 demonstrate the exceptionally strong affinity of 2-M-4′-TMS and 3-M-4′-TMS to CYP1A1 and CYP1B1 active sites. For these two compounds, K_i values are of ten times smaller than corresponding values determined for natural resveratrol monomethyl ethers; pinostilbene (3-methoxy-5,4′-dihydroxy-*trans*-stilbene) and desoxyrhapontigenin (4′-methoxy-3,5-dihydroxy-*trans*-stilbene), which are considered as very potent inhibitors of CYP1A1 and CYP1B1 activities [9]. These derivatives were also the most selective inhibitors of CYP1B1 compared to CYP1A2; exhibiting the IC_{50} ratio

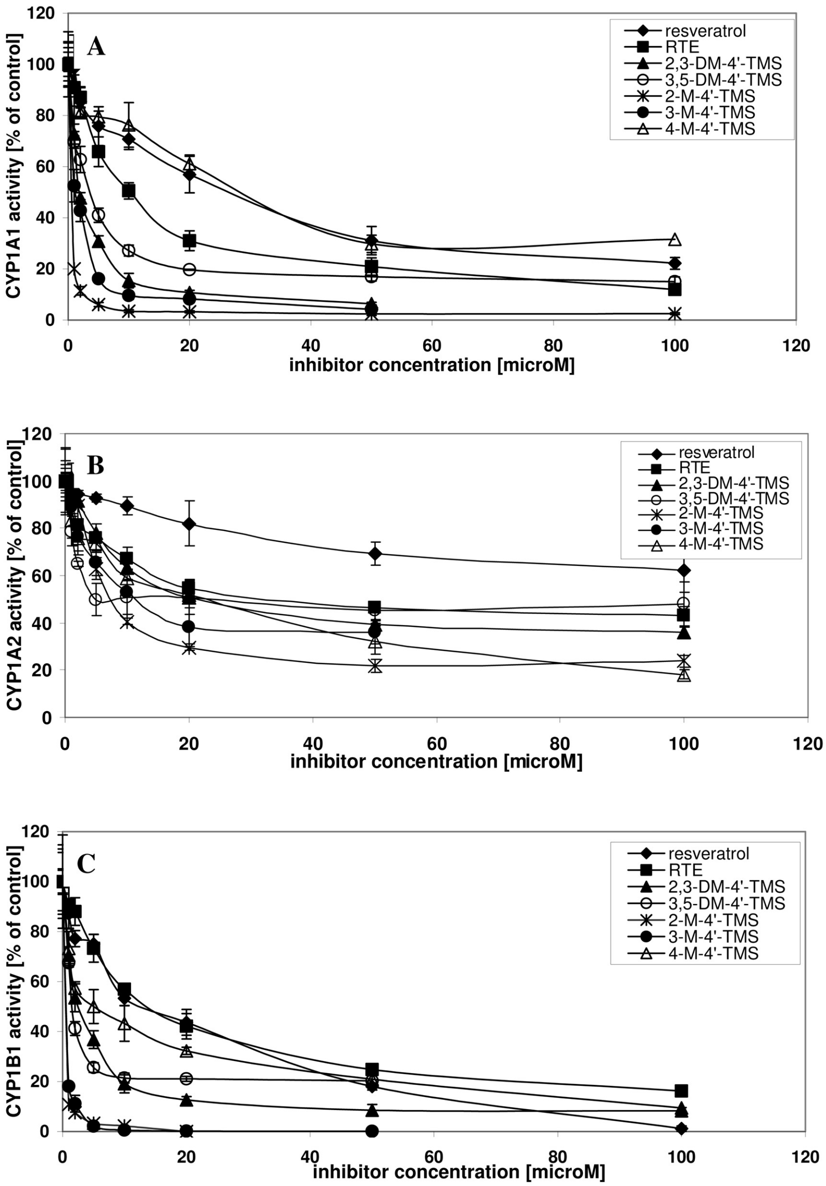


Figure 4. Effects of tested stilbenes on catalytic activity of human recombinant CYPs: (A) CYP1A1; (B) CYP1A2 and (C) CYP1B1. Each point represents the mean ± SD of triplicate sample.

CYP1A2/CYP1B1 equal 25 and 23, respectively (Table 1). In contrast, the inhibitory effect of 4-M-4′-TMS on CYP1 enzymes was weak and no selectivity of inhibition was observed. CYP1B1, an enzyme overexpressed in tumour cells, became recently a novel cancer therapeutic target. Several therapeutic strategies based on the presence and activity of CYP1B1 in tumour cells as CYP1B1-activated prodrugs and CYP1B1 inhibitors, are currently being developed [2]. In this context, the inhibitory activities of 2-M-4′-TMS and 3-M-4′-TMS seem to be very promising. Additionally, these compounds deserve more interest because of their anticipated activity in stimulation of Sir2/SIRT1 NAD^+ – dependent deacetylases [10, 15]. Recently, the role of STACs (sirtuin activating compounds) in delaying the onset of age-related diseases, such as cancer, diabetes and neurodegeneration, is widely investigated.

The preliminary analysis suggests that additional methoxy group at the position 2 or 3 may efficiently influence the interactions between inhibitors and the active site of enzyme, *e.g.* π–π stacking effect and hydrogen bonding, while methoxy group at the position 4 does not play significant role in the inhibitor–enzyme interactions. Surprisingly, in spite of the high homology of CYP1A1 and CYP1A2 structure, studied compounds, which potently inhibited CYP1A1 did not influence CYP1A2 activity to the comparable extent. The selective CYP1A1 inhibition by 2-M-4′-TMS, 3-M-4′-TMS, 2,3-DM-4′-TMS and 3,5-DM-4′-TMS with CYP1A2/CYP1A1 ratios of 7.5, 10.5, 12.2 and 3.6, respectively, was observed. Conversely, 3,5,4′-trimethoxy-*trans*-stilbene, an analogue of 3,5-DM-4′-TMS, was a potent but not selective inhibitor of CYP1A1, CYP1A2 and CYP1B1 [16]. Our experimental data might

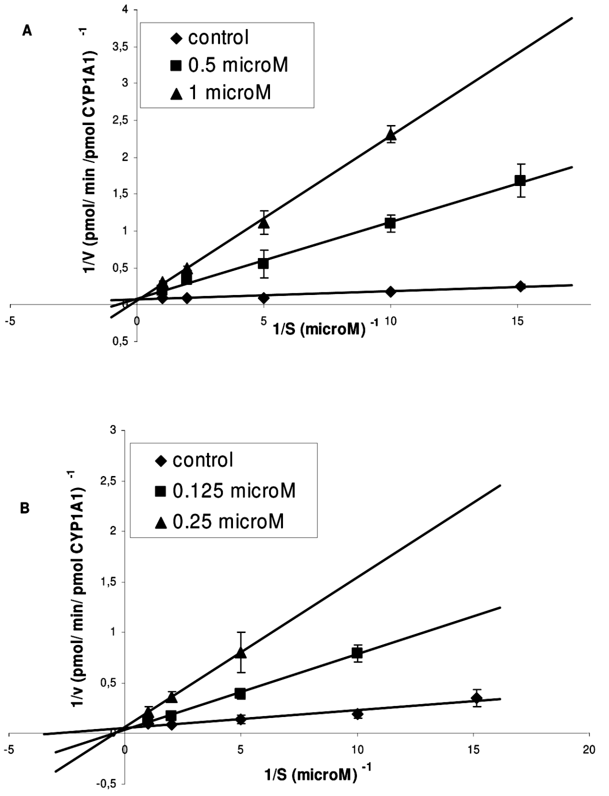


Figure 5. Lineweaver–Burk plots representing reciprocal of EROD activity expressed in picomoles of resorufin/min/pmol CYP1A1 *versus* reciprocal of 7-ethoxyresorufin concentration expressed in μM in the absence and presence of inhibitors: (A) 2-M-4′-TMS and (B) 3-M-4′-TMS. Each point represents the mean of triplicate sample.

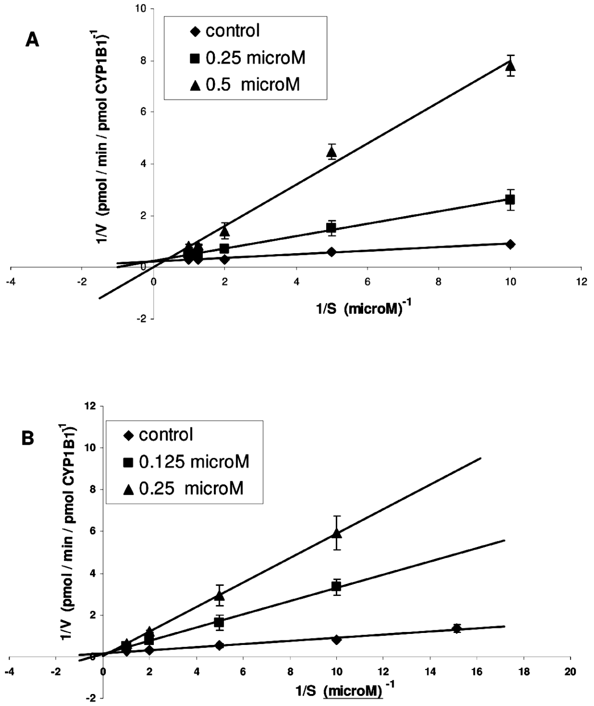


Figure 6. Lineweaver–Burk plots representing reciprocal of EROD activity expressed in picomoles of resorufin/min/pmol CYP1B1 *versus* reciprocal of 7-ethoxyresorufin concentration expressed in μM in the absence and presence of inhibitors: (A) 2-M-4′-TMS and (B) 3-M-4′-TMS. Each point represents the mean of triplicate sample.

be a subject of further molecular modelling based on the CYP1 models constructed by Lewis *et al.* [17].

Usefulness of compounds as chemopreventive agents depends strongly on their bioavailability. Substitution of hydroxy with methoxy groups is supposed to improve bioavailability of resveratrol derivatives by increasing their lipophilicity in comparison with a parent compound. Therefore, a half-life time of pterostilbene (3,5-dimethyl ether of resveratrol) in mouse plasma is nearly eight times longer than that of resveratrol [18]. In addition, the studies on natural flavonoids have proved that the methylation of hydroxy groups may protect dietary polyphenols from rapid hepatic metabolism consequently increasing their oral bioavailability [19]. It was suggested that methylated flavonoids might be attractive cancer chemopreventive/chemotherapeutic agents; as they display the inhibitory activity on benzo[a]-pyrene-activating enzymes what results in diminished DNA adduct formation [20]. Furthermore, methylated flavonoids show the ability to inhibit the proliferation of cancer cells. In the human oral SGG-9 cancer cells, 5,7-dimethoxyflavone and 5,7,4′-trimethoxyflavone were both ten times more potent inhibitors of cell proliferation than the corresponding unmethylated analogues chrysin and apigenin [21]. Finally, it should be emphasized, that further *in vivo* studies could confirm our results at the transcription level and significantly establish the potential role of stilbene derivatives in the inhibition of carcinogen bioactivation.

4 Concluding remarks

(i) Stilbene derivatives with thiomethyl group at the position 4′ exhibited high affinity toward active sites of CYP1 enzymes, in particular CYP1A1 and CYP1B1.

(ii) Among studied series of compounds, 2-M-4′-TMS and 3-M-4′-TMS were the most potent competitive inhibitors of CYP1A1 and CYP1B1 activities.

(iii) The methoxy substituents at the positions 2 and 3 displayed the decisive role in the inhibitory action of stilbene derivatives on CYP1A1 and CYP1B1 activities. The isomer 4-M-4′-TMS appeared to be the less potent inhibitor of studied CYP1 enzymes.

(iv) 3-Methoxy and 2-methoxy groups influenced the selective effect of stilbene derivatives on CYP1A1 and CYP1B1, however, in case of the compounds with two methoxy groups; 2,3-DM-4′-TMS and 3,5-DM-4′-TMS, reduced inhibitory efficiency toward CYP1B1 activity was observed.

(v) In summary, results of the study indicate that the modification of stilbene derivatives with thiomethyl and methoxy groups may increase the selectivity and inhibitory potency of these compounds toward P450 isozymes and should be considered in developing new chemopreventive agents based on their mechanism of action.

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The authors have declared no conflict of interest.

5 References

[1] Rendic, S., Summary of information on human CYP enzymes: Human P450 metabolism data. *Drug Metab. Rev.* 2002, *34*, 83–448.

[2] McFadyen, M. C., Murray, G. I., Cytochrome P450 1B1: A novel anticancer therapeutic target. *Future Oncol.* 2005, *1*, 259–263.

[3] Jang, M., Cai, G. O., Udeani, K. V., Slowing, C. F., *et al.*, Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* 1997, *275*, 218–220.

[4] Pervaiz, S., Resveratrol: From grapevines to mammalian biology. *FASEB* 2003, *17*, 1975–1985.

[5] Walle, T., Hsieh, F., DeLegge, M. H., Oatis, J. E., Jr., Walle, K. U., High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metab. Dispos.* 2004, *32*, 1377–1382.

[6] Chun, Y. J., Ryu, S. Y., Jeong, T. C., Kim, M. Y., Mechanism-based inhibition of human cytochrome P450 1A1 by rhapontigenin. *Drug Metab. Dispos.* 2001, *29*, 389–393.

[7] Kim, S., Ko, H., Park, J. E., Jung, S., *et. al.*, Design, synthesis, and discovery of novel *trans*-stilbene analogues as potent and selective human cytochrome P450 1B1. *J. Med. Chem.* 2002, *45*, 160–164.

[8] Mikstacka, R., Rimando, A. M., Szalaty, K., Stasik, K., Baer-Dubowska, W., Effect of natural analogues of *trans*-resveratrol on cytochromem P4501A2 and 2E1 catalytic activities. *Xenobiotica* 2006, *36*, 269–285.

[9] Mikstacka, R., Przybylska, D., Rimando, A. M., Baer-Dubowska, W., Inhibition of human recombinant cytochromes P450 CYP1A1 and CYP1B1 by *trans*-resveratrol methyl ethers. *Mol. Nutr. Food Res.* 2007, *51*, 517–524.

[10] Yang, H., Baur, J. A., Chen, A., Miller, C., Sinclair, D. A., Design and synthesis of compounds that extend yeast replicative lifespan. *Aging Cell* 2007, *6*, 35–43.

[11] Belluci, G., Chiappe, C., Lo Moro, G., Crown ether catalyzed stereospecific synthesis of Z- and E-stilbenes by Wittig reaction in a solid–liquid two-phases system. *Tetrahedron Lett.* 1996, *37*, 4225–4228.

[12] Hwang, J. J., Lin, R. L., Shieh, R. L., Jwo, J. J., Study of the Wittig reaction of benzyltriphenylphosphonium salt and benzaldehyde via ylide-mediated phase-transfer catalysis. Substituent and solvent effect. *J. Mol. Catal. A- Chem.* 1999, *142*, 125–139.

[13] Burke, M. D., Thompson, S., Elcombe, C. R., Halpert, J., *et al.*, Ethoxy-, pentoxy- and benzyloxyphenoxazones and homologues: A series of substrates to distinguish between different induced cytochromes P-450. *Biochem. Pharmacol.* 1985, *34*, 3337–3345.

[14] Chang, T. K. H., Chen, J., Lee, W. B. K., Differential inhibition and inactivation of human CYP1 enzymes by *trans*-resveratrol: Evidence for mechanism-based inactivation of CYP1A2. *JPET* 2001, *299*, 874–882.

[15] Howitz, K. T., Bitterman, K. J., Cohen, H. Y., Lamming, D. W., *et al.*, Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature* 2003, *425*, 191–196.

[16] Guengerich, F. P., Chun, Y.-J., Kim, D., Gillam, E. M. J., Shimada, T., Cytochrome P450 1B1: A target for inhibition in anticarcinogenesis strategies, *Mutat. Res.* 2003, *523–524*, 173–182.

[17] Lewis, D. F. V., Lake, B. G., Dickens, M., Quantitative structure-activity relationships within a homologous series of 7-alkoxyresorufins exhibiting activity towards CYP1A and CYP2B enzymem: Molecular modelling studies on key members of the resorufin series with CYP2C5-derived models of humans CYP1A1, CYP1A2, CYP2B6 and CYP3A4. *Xenobiotica* 2004, *34*, 501–513.

[18] Ferrer, P., Asensi, M., Segarra, R., Ortega, A., *et al.*, Association between pterostilbene and quercetin inhibits metastatic activity of B16 melanoma. *Neoplasia* 2005, *7*, 37–47.

[19] Wen, X., Walle, T., Methylated flavonoids have greatly improved intestinal absorption and metabolic stability. *Drug Metab. Dispos.* 2006, *34*, 1786–1792.

[20] Tsuji, P. A., Walle, T., Inhibition of benzo[a]pyrene-activating enzymes and DNA-binding in human bronchial epithelial BEAS-2B cells by methoxylated flavonoids. *Carcinogenesis* 2006, *27*, 1579–1585.

[21] Walle, T., Ta, N., Kawamori, T., Wen, X., *et. al.*, Cancer chemoprenetive properties of orally bioavailable flavonoids – methylated versus unmethylated flavones. *Biochem. Pharmacol.* 2007, *73*, 1288–1296.