MODIFIED APPROACH FOR PREPARING (*E*)-STILBENES RELATED TO RESVERATROL, AND EVALUATION OF THEIR POTENTIAL IMMUNOBIOLOGICAL EFFECTS

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Resveratrol and closely related stilbenoids belong to the most intensively studied biologically active compounds. This interest evoked several attempts to prepare such compounds in a convenient synthetic way. Our approach allowed obtaining largely methoxystilbenes, formed as *E*-isomers only (using Wittig–Horner synthesis as the key step), which were further demethylated by boron tribromide. The hydroxymethoxystilbenes (e.g. pterostilbene) were prepared using isopropyl protection, later selectively deprotected by boron trichloride. The method is suitable for preparing such compounds in a large amount. Effects of the obtained stilbene derivatives on immunobiological responses triggered by lipopolysacharide and interferon-γ were tested under in vitro conditions. Namely production of nitric oxide (NO) was investigated, and relation between the molecular structure and immunobiological activity was assessed.

Keywords: Stilbene; Resveratrol; Pinosylvin; Pterostilbene; Polyphenols; Antioxidants; Stilbenes; Nitric oxide (NO) bioassay; Immunobiological in vitro activity.

Resveratrol (3,4′,5-trihydroxystilbene (**1**)) is a phytoalexin found in various plant species, but mainly in grapevine (*Vitis vinifera* L.), as well as in wines prepared from resveratrol-containing grapevines[1,2]. Resveratrol belongs now to the most intensively studied biologically active compounds[3,4]. This interest evoked several attempts to prepare it in a synthetic way, too. Wittig–Horner synthesis is the key step of total synthesis for resveratrol and for other structurally related stilbenes. Our approach allowed stereoselective

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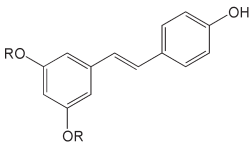
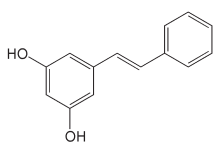
preparation of differently substituted hydroxy- and methoxystilbenes solely as *E*-isomers.

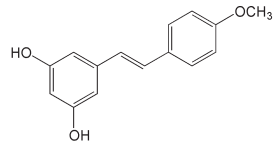
Resveratrol **1** was at the first time isolated and identified from the roots of *Veratrum grandiflorum* A. Gray (Liliaceae)[3,5]. Soon after, Späth and coworkers[6–9] synthesized a series of hydroxylated and methoxylated stilbenes, e.g. 4′-hydroxy-3,5-dimethoxystilbene (**2**), known as pterostilbene, identified as a phytoalexin together with resveratrol in leaves of *Vitis vinifera*, too[10]. They prepared also pterostilbene methyl ether, confirming the structures of both pterostilbene **2** and resveratrol **1**. Späth also directly synthesized resveratrol by condensation of sodium 4-hydroxyphenylacetate and 3,5-dihydroxybenzaldehyde followed by decarboxylation of the resulting carboxylic acid[8]. Pinosylvin **3** was prepared by Späth using the same synthetic strategy[9]. Later, also the 4′-methylresveratrol (**4**) was prepared[11].



1. R = H resveratrol
2. R = CH_3 pterostilben



3. pinosylvin



4. resveratrol-4′-methyl ether

However, the previously published synthetic methods[5–9], as well as the reinvestigated and upgraded methods published later[11–15] are convenient only for preparations low quantity of these compounds, or only as mixtures of isomers in various *E/Z* ratio[16,17]. Our current study of chemoecological[18] and pharmacological[19,20] properties of selected grapevine stilbenoids derived from the indicated basic stilbenes (**1**–**4**), evoked the necessity of access to their various synthetic analogues in a reasonable high quantity. This was available in our case by upgrading the method by a new more convenient approach[21].

A remarkable interest in biological effects of resveratrol has mainly emerged after the discovering its relation to the so called French paradox[1,22]. The initial interest to explain the role of resveratrol and its derivatives in wine, or in other natural sources (e.g. vegetables) was due to their antioxidative effect against reactive oxygen species, which may cause oxidative stress damages to biological units involved in lipid metabolism, in aging, inflammation and neurodegenerative disorders[3,23]. Resveratrol has been identified also as a potent anticancer agent[24]. Studies on anticarcinogenic mechanisms of action revealed multiple intracellular targets of

resveratrol[25]. Wine stilbenes are efficient also in cancer prevention[26], especially in chemoprevention of skin cancer[27]. Beneficial effect of resveratrol and related phenolics has been observed in brain health prevention[28] with therapeutic potential in neurodegenerative processes including Alzheimer's disease[29], Parkinson's desease, and stroke[28,29]. Resveratrol has been reported to be estrogenic (however with indistinct properties), as well as exhibiting antiviral and antiinflamatory effects[30]. In our study, functional groups modification of the resveratrol family stilbenes resulted in differentiation of their antioxidative properties, targeting mainly extracellular reactive oxygen species, which are responsible for tissue damage during chronic inflammation[19]. Our interest is focused on various types of plant secondary metabolites[31–33], including resveratrol and other structurally related stilbenes as modulators of inducible NO production[20] associated with the function of immune defense system[34].

RESULTS AND DISCUSSION

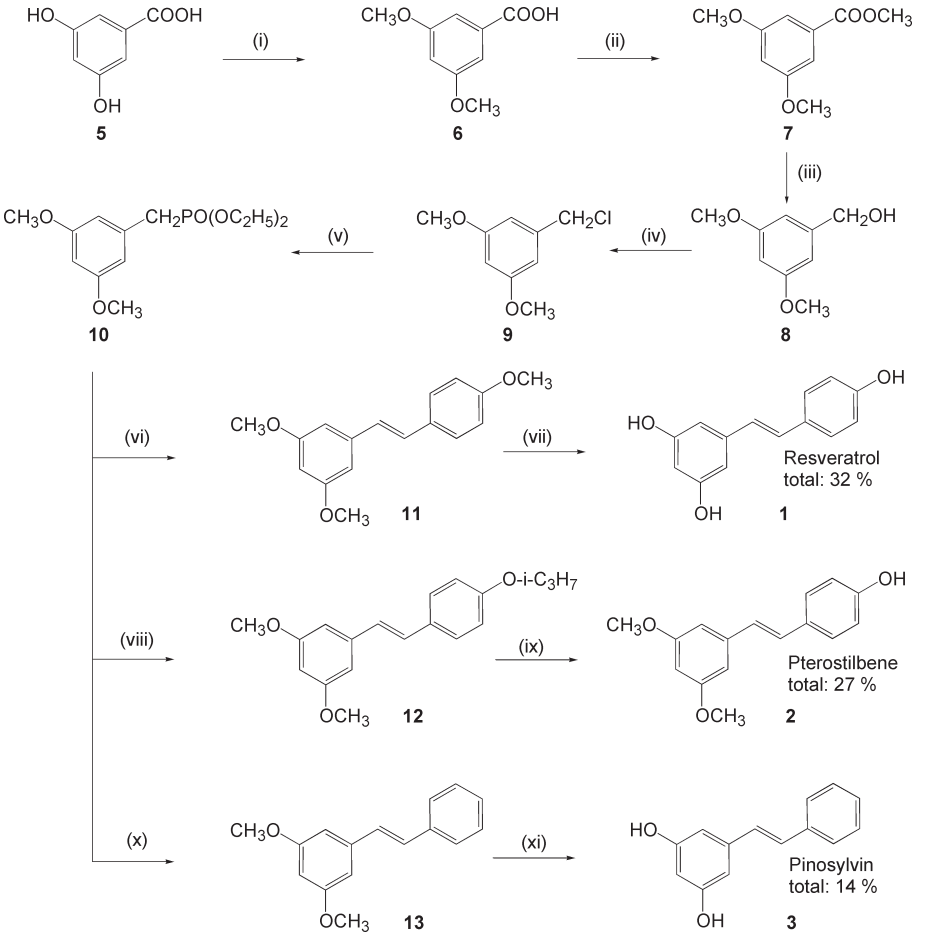
In our approach, the readily available starting 3,5-dihydroxybenzoic acid (**5**) was methylated to 3,5-dimethoxybenzoic acid (**6**), esterified to a methylester **7**, reduced to alcohol **8**, and transformed by thionyl chloride to the 3,5-dimethoxybenzylchloride (**9**) with a favorable high yield, and comparable quality (m.p. 42–44 °C, see e.g. ref.[35]) . Chloride **9** was subsequently converted into diethylphosphonate **10** by Arbuzov reaction (Scheme 1).

Afterwards, the phosphonate **10** was used in Wittig–Horner reaction together with 4-methoxybenzaldehyde to provide (*E*)-3,4′,5-trimethoxystilbene (**11**). Final demethylation by boron tribromide provided *trans*-resveratrol **1** in overall yield 32%. Dimethylphosphonate **10** was in the same way reacted with 4-isopropyloxybenzaldehyde to provide (*E*)-3,5-dimethoxy-4′-isopropoxystilbene (**12**), which was selectively dealkylated by boron trichloride to provide (*E*)-4′-hydroxy-3,5-dimethoxystilbene, i.e. pterostilben **2** in overall yield 27%. The same synthetic procedure was used to prepare and (*E*)-3,5-dihydroxystilbene (**13**) which, after demethylation, provided pinosylvin **3** in a total yield 14%.

A slightly modified way (Scheme 2) was used for the synthesis of (*E*)-3,5-dihydroxy-4′-methoxystilbene (**4**), however, obtained only in overall yield 4%.

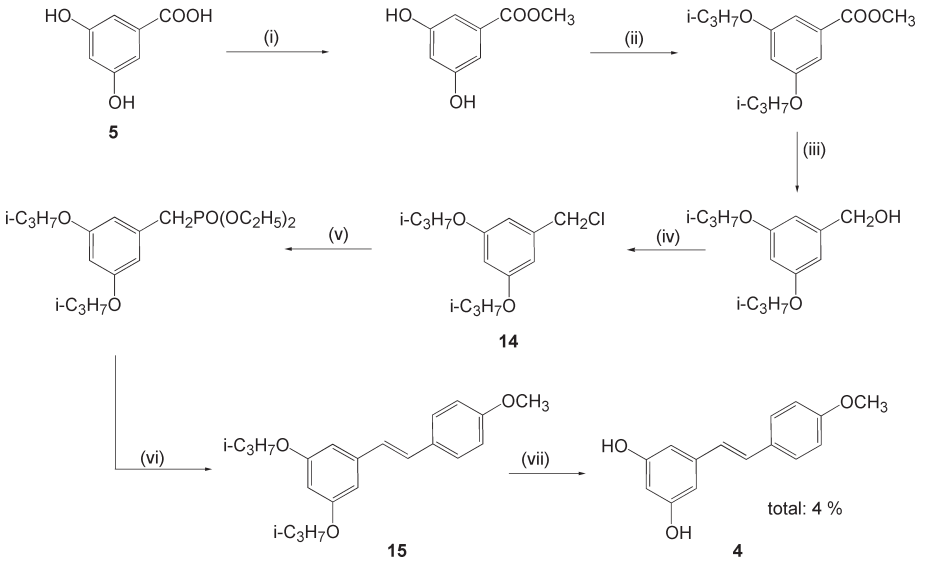
All tested compounds (**1–4**, **11**) have been found to suppress the immune-stimulated production of NO, with only marginal differences in the corresponding IC_{50} values estimates (Fig. 1a, Table I). The NO-inhibitory effects of resveratrol and related hydroxystilbenes are well recognized[36–38]. It

should be mentioned that in opposite to the inhibition of NO production, which depends on the activity of inducible NO synthase (iNOS), resveratrol in nanomolar concentration can activate endothelial NO synthase (eNOS), and thus, enhance production of NO by endothelial cells[39]. Our data are in agreement with the findings[40,41] showing that pterostilbene **2** is equally potent to reduce the iNOS-mediated NO production as resveratrol. To the



SCHEME 1

(i) $(CH_3O)_2SO_2$, NaOH, H_2O, 96%; (ii) CH_3OH, H_2SO_4, 98%; (iii) $LiAlH_4$, THF, 96%; (iv) $SOCl_2$, 82%; (v) $(C_2H_5O)_3P$; (vi) 4-methoxybenzaldehyde, NaH, 76% both steps; (vii) BBr_3, CH_2Cl_2, 56%; (viii) 4-isopropoxybenzaldehyde, NaH, 92% both steps; (ix) BCl_3, CH_2Cl_2, 39%; (x) benzaldehyde, NaH, 61% both steps; (xi) BBr_3, CH_2Cl_2, 32%



SCHEME 2

(i) CH_3OH, H_2SO_4, 89%; (ii) *i*-C_3H_7Br, KI, K_2CO_3, DMF, 82%; (iii) $LiAlH_4$, THF, 67%; (iv) $SOCl_2$, 32%; (v) $(C_2H_5O)_3P$; (vi) 4-methoxybenzaldehyde, NaH, 92% both steps; (vii) BCl_3, CH_2Cl_2, 28%

TABLE I

IC_{50} estimate values (in μM) of compounds **1**–**4** and **11** for their NO-inhibiting and cell viability (cytocidal) effects. The data are mean ± 95% limits of confidence (l.c.)

Compound	NO		Viability	
	IC_{50}	95% l.c.	IC_{50}	95% l.c.
Resveratrol **1**	33.7	30.3–37.6	100.1	85.0–117.8
Pterostilbene **2**	27.1	22.5–32.7	>200	
Pinosylvin **3**	29.9	24.3–36.7	115.8	102.4–131.1
4′-Methylresveratrol **4**	35.2	28.9–42.9	67.2	50.3–89.7
3,4′,5-Trimethoxystilbene **11**	44.4	38.9–50.7	112.9	102.3–124.5

best of our knowledge, reports on the interaction of pinosylin **3** and other compounds with biosynthesis of NO are missing.

The NO-inhibitory activity is obviously not due to the cytocidal effects (Fig. 1b) because, as determined by the estimated IC_{50} values, the compounds are several-fold less potent to kill the cells (Table I). The dissociation between the NO-inhibitory and cytotoxic activities is most clearly expressed by pterostilbene **2**. It inhibits NO with IC_{50} of 27 μM, while the

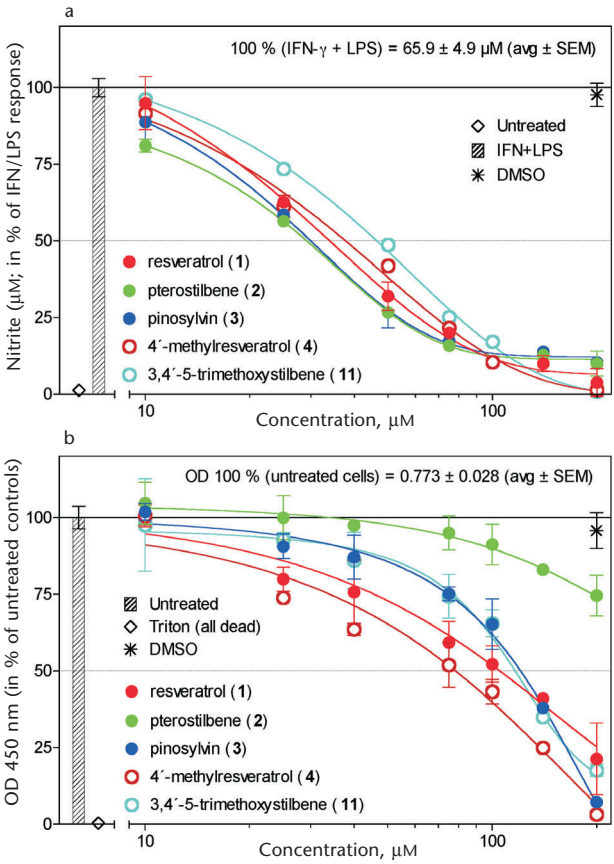


FIG. 1

Effects of compounds **1**–**4** and **11** on immunobiological responses triggered by lipopolysacharide (LPS) and interferon-γ (IFN-γ) under in vitro conditions using murine resident peritoneal macrophages. a Inhibitory effects of compounds on LPS + INF-γ induced nitric oxide (NO) production. b Effects of the compounds on viability of macrophages (cytocidal activity). The data are mean ± SEM and represent one of two identical experiments

IC_{50} for suppression of cell viability is >200 μM. Applied at the 50-μM concentration, pterostilbene **2** reduced the NO production by approximately 75%, while this concentration remained without any effect on the viability of cells.

The present data support the view that pterostilbene **2** is a promising candidate for further preclinical studies. It has been shown that its pharmacokinetic properties are more favorable than those of resveratrol[41,42], which has proved to possess antiinflammatory[19], hepatoprotective, cardiovascular protective, and cancer chemopreventive properties[43].

The synthetic *trans*-stilbenes (*E*-isomers) **1–4**, and their activities, served us for further photochemical and biochemical transformations performed for preparing various structural analogues (*Z*-isomers, cyclic isosters)[20], and other derivatives (as e.g. oxidative coupled oligomers)[4,44], targeted for assessing their immunobiological activities[33].

EXPERIMENTAL

The melting points were determined on a Boetius microblock and are uncorrected. The analytical samples were dried over phosphorus pentoxide at 14 Pa and room temperature or 60 °C for 6 h. Conversion of the compounds in each synthetic step and purity of the products during crystallizations were checked by TLC on silica gel plates (GF_{254} Merck, 0.25 mm). Plates were developed in solvent mixture of hexane–dichloromethane–propan-2-ol 1:1:0.1 (v/v/v) and detected by UV (254 nm). Purity of final products and of the compounds determined for biological assays were analyzed by reversed-phase HPLC using 4 × 250 mm column, packed with 5 mm size S5 ODS2 C-18 (Phase Separation). Elution was performed in gradient mode, with solvent system acetonitrile–water (2.5–70%)/60 min, 0.6 ml/mim, detected by UV at 220 nm.

^1H and ^{13}C NMR spectra were measured on Bruker AVANCE-600 instrument (^1H at 600.13 and ^{13}C at 150.9 MHz) in d_6-DMSO at 300 K. Homonuclear 2D-H,H-COSY and heteronuclear 2D-H,C-HSQC and 2D-H,C-HMBC spectra were used for the structural assignment of proton and carbon-13 signals. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) are given in Hz.

Mass spectra, including HR-MS, were recorded on a Thermo Scientific LCQ Fleet ion trap mass spectrometer (in negative ESI-MS mode, excepting compound **11**).

(*E*)-3,4′,5-Trihydroxystilbene (*trans*-Resveratrol; **1**)

A solution of 3,4′,5-trimethoxystilbene (**11**; 8.1 g, 30 mmol) in absolute dichloromethane (200 ml) was under continuous stirring cooled down to –5 °C, and a solution of boron tribromide (13 ml, 135 mmol) dissolved in absolute dichloromethane (130 ml) was stepwise added during 30 min. The reaction mixture under continuous stirring was let to warm-up to room temperature and stirred further 24 h. The obtained red solution was poured into a mixture of ice in water (500 ml), and after evaporating the dichloromethane, a grey suspension was obtained. The precipitate was filtered, washed with water, dried and, after crystallization from a benzene–ethanol mixture, pale-grey needles of resveratrol **1** (3.8 g, 56%)

were obtained, with m.p. 265–268 °C (according to ref.[8] m.p. 260–261 °C after sublimation). TLC detection at R_F 0.08, and RP-HPLC analysis indication at t_R 31.8 (both under conditions indicated above).

^{1}H NMR (600.13 MHz; d_6-DMSO): 9.57 (bs, 1 H, 4′-OH); 9.22 (bs, 2 H, 3-OH and 5-OH); 7.39 (m, 2 H, H-2′ and H-6′); 6.92 (dt, 1 H, J(7,8) = 16.4, J(7,2) = J(7,6) = 0.5, H-7); 6.81 (bd, 1 H, J(8,7) = 16.4, H-8); 6.75 (m, 2 H, H-3′ and H-5′); 6.38 (dd, 2 H, J(2,4) = J(6,4) = 2.2, J(2,7) = J(6,7) = 0.5, H-2 and H-6); 6.11 (t, 1 H, J(4,2) = J(4,6) = 2.2, H-4). ^{13}C NMR (150.9 MHz; d_6-DMSO): 158.72 (C-3 and C-5); 157.43 (C-4′); 139.48 (C-1); 128.27 (C-1′); 128.08 (C-8, C-2′ and C-6′); 125.84 (C-7); 115.68 (C-3′ and C-5′); 104.49 (C-2 and C-6); 101.95 (C-4).

Negative ESI-MS (in MeOH), *m/z* (%): 227.0 $[M - H]^-$ (100), 185 (95), 183 (42), 159 (29), 157 (27), 143 (14). HR ESI-MS, *m/z*: 227.0705 $[M - H]^-$ for $C_{14}H_{11}O_3$ required 227.0703.

(*E*)-4′-Hydroxy-3,5-dimethoxystilbene (Pterostilbene; **2**)

A solution of 4′-isopropoxy-3,5-dimethoxystilbene (**12**; 17.9 g, 60 mmol) in absolute dichloromethane (200 ml) was stirred, and a solution of 1 M boron trichloride (36 ml, 36 mmol) was added. Reaction was monitored by TLC, and after 8 h the reaction mixture did not contain any starting compound. The reaction mixture was poured in water (250 ml), the organic phase was separated and washed with water (2 × 200 ml). The organic phase was extracted with a solution of 2% NaOH (3 × 200 ml), and the combined alkaline extract was washed with dichloromethane (200 ml) and acidified by a solution of 5 M HCl (125 ml). The separated oil was extracted with diethylether (2 × 200 ml), and the combined extract was washed with water (2 × 200 ml), dried with anhydrous sodium sulfate and evaporated. The residue (16 g) was recrystallized (2×) from xylene, and the resulting pterostilbene **2** was obtained as pale yellow needles (6.0 g, 39%), with m.p. 86–88 °C (according to ref.[7] m.p. 87–88 °C from petroleum ether). TLC detection at R_F 0.38, and RP-HPLC analysis indication at t_R 49.6 (both under conditions indicated above).

^{1}H NMR (600.13 MHz; d_6-DMSO): 9.63 (bs, 1 H, OH); 7.41 (m, 2 H, H-2′ and H-6′); 7.16 (bd, 1 H, J(8,7) = 16.4, H-8); 6.94 (dt, 1 H, J(7,8) = 16.4, J(7,2) = J(7,6) = 0.4, H-7); 6.76 (m, 2 H, H-3′ and H-5′); 6.71 (dd, 2 H, J(2,4) = J(6,4) = 2.3, J(2,7) = J(6,7) = 0.4, H-2 and H-6); 6.37 (t, 1 H, J(4,2) = J(4,6) = 2.3, H-4); 3.76 (s, 6 H, 2 × OCH_3). ^{13}C NMR (150.9 MHz; d_6-DMSO): 160.84 (C-3 and C-5); 157.61 (C-4′); 139.82 (C-1); 129.17 (C-8); 128.14 (C-1′, C-2′ and C-6′); 125.34 (C-7); 115.78 (C-3′ and C-5′); 104.24 (C-2 and C-6); 99.48 (C-4); 55.38 (2 × OCH_3).

Negative ESI-MS (in MeOH), *m/z* (%): 255.0 $[M - H]^-$ (25), 240 (100), 239 (8). HR ESI-MS, *m/z*: 255.1018 $[M - H]^-$, for $C_{16}H_{15}O_3$ required 255.1016.

(*E*)-3,5-Dihydroxystilbene (Pinosylvin; **3**)

A solution of 3,5-dimethoxystilbene (**13**; 10.6 g, 50 mmol) in absolute dichloromethane (100 ml) was stirred and cooled to 5 °C, and a solution of boron tribromide (15.4 ml, 16 mmol) in absolute dichloromethane (500 ml) was added during 30 min. The reaction mixture was allowed to warm-up to room temperature and stirred for 24 h. Next day, the reaction mixture was refluxed for another 4 h. The red reaction mixture was cooled (with water and ice), water (250 ml) was added, and allowed to warm-up to room temperature. The organic phase was washed with a solution of 4% NaCl (4 × 250 ml), solution of 4% sodium hydrogen carbonate (250 ml) and once more with a solution of NaCl (250 ml), then dried with anhy-

drous sodium sulfate, and evaporated. The residue was crystallized from benzene as colorless needles (3.3 g, 32%), with m.p. 156–157 °C (according to ref.[10] m.p. 155–155.5 °C from benzene). TLC detection at R_F 0.19, and RP-HPLC analysis indication at t_R 41.6 (both under conditions indicated above).

^{1}H NMR (600.13 MHz; d_6-DMSO): 9.29 (bs, 2 H, 3-OH and 5-OH); 7.57 (m, 2 H, H-2′ and H-6′); 7.35 (m, 2 H, H-3′ and H-5′); 7.25 (m, 1 H, H-4′); 7.06 (d, 1 H, *J*(8,7) = 16.4, H-8); 7.03 (d, 1 H, *J*(7,8) = 16.4, H-7); 6.45 (d, 2 H, *J*(2,4) = *J*(6,4) = 2.2, H-2 and H-6); 6.16 (t, 1 H, *J*(4,2) = *J*(4,6) = 2.2, H-4). ^{13}C NMR (150.9 MHz; d_6-DMSO): 158.77 (C-3 and C-5); 138.95 (C-1); 137.23 (C-1′); 129.15 (C-7); 128.89 (C-3′ and C-5′); 128.05 (C-8); 127.76 (C-4′); 126.70 (C-2′ and C-6′); 104.91 (C-2 and C-6); 102.56 (C-4).

Negative ESI-MS (in MeOH), *m/z* (%): 211.1 $[M - H]^-$ (23), 169 (100), 167 (67), 152 (7). HR ESI-MS, *m/z*: 211.0758 $[M - H]^-$, for $C_{14}H_{11}O_2$ required 211.0754.

(*E*)-3,5-Dihydroxy-4′-methoxystilbene (Resveratrol-4′-methyl Ether; **4**)

Resveratrol-4′-methyl ether **4** was prepared from 3,5-diisopropoxy-4′-methoxystilbene (**15**) by identical method, differing only in using twice a higher amount of boron trichloride solution. Compound **4** was obtained as colorless needles (360 mg, 28%), with m.p. 172–175 °C (crystallized from ethyl acetate–benzene mixture). TLC detection at R_F 0.18, and RP-HPLC analysis indication at t_R 42.4 (both under conditions indicated above).

^{1}H NMR (600.13 MHz; d_6-DMSO): 9.25 (bs, 2 H, 3-OH and 5-OH); 7.51 (m, 2 H, H-2′ and H-6′); 6.98 (bd, 1 H, *J*(8,7) = 16.4, H-8); 6.92 (m, 2 H, H-3′ and H-5′); 6.89 (dt, 1 H, *J*(7,8) = 16.4, *J*(7,2) = *J*(7,6) = 0.5, H-7); 6.41 (dd, 2 H, *J*(2,4) = *J*(6,4) = 2.2, *J*(2,7) = *J*(6,7) = 0.5, H-2 and H-6); 6.13 (t, 1 H, *J*(4,2) = *J*(4,6) = 2.2, H-4); 3.76 (s, 3 H, OCH_3). ^{13}C NMR (150.9 MHz; d_6-DMSO): 159.10 (C-4′); 158.74 (C-3 and C-5); 139.32 (C-1); 129.87 (C-1′); 128.00 (C-2′ and C-6′); 127.70 (C-7); 126.86 (C-8); 114.34 (C-3′ and C-5′); 104.62 (C-2 and C-6); 102.14 (C-4); 55.35 (OCH_3).

Negative ESI-MS (in MeOH), *m/z* (%): 242 (16), 241.0 $[M - H]^-$ (100), 227 (3), 197 (1). HR ESI-MS, *m/z*: 241.0863 $[M - H]^-$, for $C_{15}H_{13}O_3$ required 241.0859.

(*E*)-3,4′,5-Trimethoxystilbene (**11**)

A mixture of 3,5-dimethoxybenzylchloride (**9**; 9.3 g, 50 mmol) and triethyl phosphite (8.3 g, 50 mmol) was stirred and heated under argon at 160 °C for 2 h. During the first hour, evolution of gas (ethyl chloride) was observed. After the reaction has been finished, the obtained crude 3,5-dimethoxybenzyl diethyl phosphonate (**10**) was cooled to room temperature. To a stirred absolute 1,2-dimethoxyethane (under argon), sodium hydride (80% dispersion in mineral oil, 2.1 g, 70 mmol) was added, then stepwise 4-methoxybenzaldehyde (6.8 g, 50 mmol), and finally the whole amount of the above acquired phosphonate **10**. The mixture was stirred and slowly heated up to 85 °C. Already at 70 °C, appearing evolution of gas (hydrogen) started and a semisolid material deposited. The reaction mixture was refluxed another 30 min, and then a half amount of the 1,2-dimethoxyethane solvent was distilled off. The residue was poured into water (500 ml) and the oily phase was extracted with diethyl ether (2 × 200 ml). The combined ether extract was dried with anhydrous calcium chloride, partly evaporated, and the residue was crystallized from ethanol. The final stilbene **11** was obtained in a form of colorless crystals (10.3 g, 76%), with m.p. 54–56 °C (according to ref.[7] m.p. 56–57 °C). TLC detection at R_F 0.7, and RP-HPLC analysis indication at t_R 60.1 (both under conditions indicated above).

^{1}H NMR (600.13 MHz; d_6-DMSO): 7.53 (m, 2 H, H-2′ and H-6′); 7.21 (bd, 1 H, *J*(8,7) = 16.4, H-8); 7.02 (dt, 1 H, *J*(7,8) = 16.4, *J*(7.2) = *J*(7,6) = 0.5, H-7); 6.94 (m, 2 H, H-3′ and H-5′); 6.74 (dd, 2 H, *J*(2,4) = *J*(6,4) = 2.2, *J*(2,7) = *J*(6,7) = 0.5, H-2 and H-6); 6.38 (t, 1 H, *J*(4,2) = *J*(4,6) = 2.2, H-4); 3.77 (s, 9 H, 3-, 5- and 4′-OMe). ^{13}C NMR (150.9 MHz; d_6-DMSO): 160.87 (C-3 and C-5); 159.26 (C-4′); 139.67 (C-1); 129.77 (C-1′); 128.80 (C-8); 128.09 (C-2′ and C-6′); 126.37 (C-7); 114.41 (C-3′ and C-5′); 104.38 (C-2 and C-6); 99.70 (C-4); 55.41 (3- and 5-OMe); 55.37 (4′-OMe).

Positive HR ESI-MS, *m/z*: 271.1325 $[M + H]^+$, for $C_{17}H_{19}O_3$ required 271.1329.

(*E*)-4-Isopropoxy-3,5-dimethoxystilbene (**12**)

Compound **12** was prepared from 3,5-dimethoxybenzylchloride (**9**) and 4-isopropyloxybenzaldehyde by identical method as for (*E*)-3,4′,5-trimethoxystilbene (**11**) as a glassy solid (92%), which crystallized afterwards reaching m.p. 35–38 °C. TLC detection at R_F 0.7 (under conditions indicated above).

^{1}H NMR (600.13 MHz; $CDCl_3$): 7.42 (m, 2 H, H-2′ and H-6′); 7.02 (d, 1 H, *J*(7,8) = 16.3, H-7); 6,89 (d, 1 H, *J*(8,7) = 16.3, H-8); 6.87 (m, 2 H, H-3′ and H-5′); 6.64 (d, 2 H, *J*(2,4) = *J*(6,4) = 2.3, H-2 and H-6); 6.37 (t, 1 H, *J*(4,2) = *J*(4,6) = 2.3, H-4); 4.55 (h, 1 H, *J* = 6.1, O-CH< (iPr)); 3.81 (s, 6 H, 3- and 5-OMe); 1.34 (d, 6 H, *J* = 6.1, 2 × CH_3 (iPr)). ^{13}C NMR (150.9 MHz; $CDCl_3$): 160.88 (C-3 and C-5); 157.66 (C-4′); 139.68 (C-1); 129.58 (C-1′); 128.74 (C-7); 127.77 (C-2′ and C-6′); 126.33 (C-8); 115.91 (C-3′ and C-5′); 104.22 (C-2 and C-6); 99.48 (C-4); 69.82 (O-CH< (iPr)); 55.28 (3- and 5-OMe); 21.99 (2 × CH_3 (iPr)).

Positive HR ESI-MS, *m/z*: 299.1641 $[M + H]^+$, for $C_{19}H_{23}O_3$ required 299.1642.

(*E*)-3,5-Dimethoxystilbene (**13**)

Compound **13** was prepared from 3,5-dimethoxybenzylchloride (**9**) and benzaldehyde by identical method as (*E*)-3,4′,5-trimethoxystilbene (**11**) as colorless needles (61%), with m.p. 55–56 °C (crystallized from propan-2-ol). TLC detection at R_F 0.7 (under conditions indicated above).

^{1}H NMR (600.13 MHz; $CDCl_3$): 7.50 (m, 2 H, H-2′ and H-6′); 7.35 (m, 2 H, H-3′ and H-5′); 7.26 (m, 1 H, H-4′); 7.08 (bd, 1 H, *J*(8,7) = 16.5, H-8); 7.03 (bd, 1 H, *J*(7,8) = 16.5, H-7); 6.67 (dd, 2 H, *J*(2,4) = *J*(6,4) = 2.3, *J*(2,7) = *J*(6,7) = 0.6, H-2 and H-6); 6.40 (t, 1 H, *J*(4,2) = *J*(4,6) = 2.3, H-4); 3.82 (s, 6 H, 3- and 5-OMe). ^{13}C NMR (150.9 MHz; $CDCl_3$): 160.92 (C-3 and C-5); 139.30 (C-1); 137.06 (C-1′); 129.15 (C-8); 128.65 (C-3′ and C-5′); 128.61 (C-7); 127.70 (C-4′); 126.54 (C-2′ and C-6′); 104.51 (C-2 and C-6); 99.92 (C-4); 55.32 (3- and 5-OMe).

Positive HR ESI-MS, *m/z*: 241.1222 $[M + H]^+$, for $C_{16}H_{17}O_2$ required 241.1223.

(*E*)-3,5-Diisopropoxy-4′-methoxystilbene (**15**)

Compound **15** was prepared from 3,5-diisopropoxybenzylchloride (**14**; Scheme 2) and 4-methoxybenzaldehyde by identical method as (*E*)-3,4′,5-trimethoxystilbene (**11**) as a colorless liquid (92%). TLC detection at R_F 0.7 (under conditions indicated above).

^{1}H NMR (500.13 MHz; $CDCl_3$): 7.43 (m, 2 H, H-2′ and H-6′); 7.00 (bd, 1 H, *J*(8,7) = 16.2, H-8); 6.89 (m, 2 H, H-3′ and H-5′); 6.87 (bd, 1 H, *J*(7,8) = 16.2, H-7); 6.61 (d, 2 H, *J*(2,4) = *J*(6,4) = 2.3, H-2 and H-6); 6.35 (t, 1 H, *J*(4,2) = *J*(4,6) = 2.3, H-4); 4.56 (h, 2 H, *J* = 6.1, 2 × O-CH< (iPr)); 3.82 (s, 3 H, 4′-OMe); 1.34 (d, 12 H, *J* = 6.1, 4 × CH_3 (iPr)).

HR EI-MS, *m/z*: 326.1897 $[M]^+$, for $C_{21}H_{26}O_3$ required 326.1882.

Nitric Oxide (NO) Bioassay

Isolation and cultivation of the murine peritoneal macrophages for the bioassay were performed in a routine way[32,33]. Macrophages (2×10^6/ml) were cultured 24 h in presence of test compounds, applied either alone or in the presence of NO-priming immune stimuli, i.e. murine recombinant interferon-γ (IFN-γ), and lipopolysaccharide (LPS). The concentration of nitrites in supernatants of cells was taken as a measure of NO production. It was detected in individual cell-free samples (50 µl) incubated 5 min at ambient temperature with an aliquot of a Griess reagent. The absorbance at 540 nm was recorded using a microplate spectrophotometer.

Cytotoxicity Assay

Viability of cells was determined using a colorimetric assay based on the cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenases in viable cells (Roche Diagnostics, Mannheim, Germany). The cells (1×10^6/ml) were cultured as described above. After the 24-h culture, the WST-1 was added and the cells were kept in the Heraeus incubator at 37 °C for additional 3 h. Optical density at 450–690 nm was evaluated.

The test compounds were dissolved in dimethylsulfoxide (DMSO). The final concentration of DMSO was lower than 0.02%. This amount was devoid of any effects on production of NO and on the cell viability.

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