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Original article

Synthesis of novel *trans*-stilbene derivatives and evaluation of their potent antioxidant and neuroprotective effects

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ABSTRACT

A convenient synthesis and the biological properties of new amides, esters and other derivatives of *trans*-stilbene are described. The key synthetic strategies involve the Wittig–Horner reaction of a phosphonium salt **9** and an aldehyde **10** to generate (*E*)- or (*Z*)-olefins and a coupling reaction of an acid **12** and various amines **13a–n** to give *trans*-stilbene derivatives **15a–n** in high yields. A amide derivative **15g** showed three times more *in vitro* free radical-scavenging activity than resveratrol, while another **15d** exhibited strong inhibitory activity against lipopolysaccharide (LPS)[a]-induced NO generation. Allylamide analogue **15a** showed the most potent neuroprotective activity in glutamate-induced primary cortical neuron cells.

1. Introduction

E-Resveratrol (3,4′,5-trihydroxy-*trans*-stilbene) is a phytoalexin found in grapes, berries, peanuts, and red wine, and its derivatives have been recognized to exhibit a wide range of biological and pharmacological properties [1–3]. They were shown to promote nitric oxide production [4,5], to inhibit platelet aggregation [6–8], and to increase high-density lipoprotein cholesterol [4,9,10] as well as to function as antitumor agents [11–14] and antioxidants [15,16]. The structural features of natural and synthetic resveratrol analogues consist of diaryl groups on either end of an active double bond to generate the stilbene skeleton. Although the presence of the double bond in resveratrol gives rise to *trans*- and *cis*-isomeric forms of resveratrol [(*E*)- and (*Z*)-diastereomers, respectively], the *trans*-form is the thermodynamically more stable form (Fig. 1) [17,18].

Recently, a great deal of interest has been generated by resveratrol and its analogues due to their antioxidative effects against reactive oxygen species (ROS), which cause oxidative damage to biological substances and are involved in aging and inflammation [19–22]. They exhibit biological activities that inhibit lipopolysaccharide (LPS)-induced production of PGE2 and NO in the mouse macrophage cell line RAW 264.7 [23,24]. As part of its anti-inflammatory action, resveratrol attenuates expression of the NF-kappaB-dependent inflammatory markers inducible nitric oxide synthase and IL-6 [25]. *trans*-Resveratrol has been shown to exert potent neuroprotective actions, which are attributed to its antioxidant properties [26]. Chronic administration of resveratrol to animal models of neurodegenerative injury and excitotoxic brain damage resulted in partial neuroprotective action [27]. Resveratrol also attenuated oxidized low density lipoprotein (oxLDL)-induced cytotoxicity in PC12 cells with neuroprotection [28]. These studies suggest that *trans*-resveratrol derivatives are the bioactive components that suppress NO generation in microglia cells, and that are responsible for the neuroprotective action in primary cortical neuron cells. In spite of extensive efforts, the mechanism of action related to the free radical-scavenging effects of *trans*-stilbene derivatives has not yet been established.

In a preliminary communication [29], we reported the design and synthesis of resveratrol derivatives based on lithospermic acid B as inhibitors of protein tyrosine phosphatase 1B. As part of our ongoing studies on the development of antioxidative and neuroprotective stilbene-derived drug candidates, we report in this paper the convenient synthesis of nineteen novel *trans*-stilbene derivatives and their *in vitro* free radical-scavenging effects,

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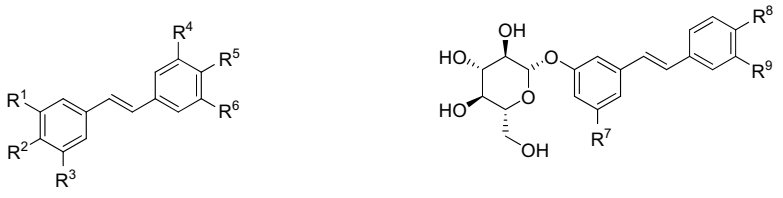


1 R^1=R^2=R^3=R^4=R^5=R^6=H, Stilbene
2 R^1=R^3=R^5=OH, R^2=R^4=R^6=H, Resveratrol
3 R^1=R^3=R^4=R^5=OH, R^2=R^6=H, Piceatannol
4 R^1=R^3=OH, R^2=R^4=R^6=H, R^5=OMe, 3,5-Dihydroxy-4'-methoxystilbene
5 R^7=R^8=OH, R^9=H, Piceid
6 R^7=R^9=OH, R^8=OMe, Rhaponticin
7 R^7=R^8=OH, R^9=OMe, Isorhaponticin
8 R^7=OH, R^8=OMe, R^9=H, Desoxyrhaponticin

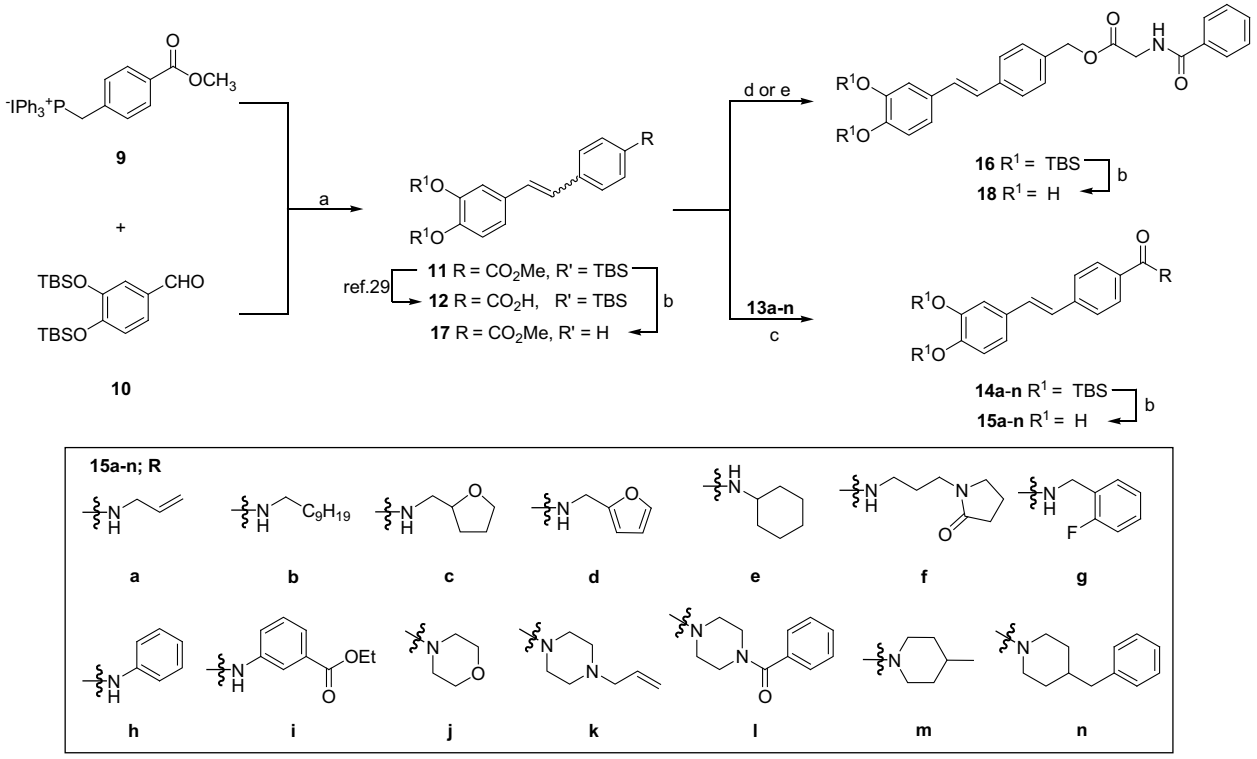
Fig. 1. Chemical structures of resveratrol and its analogues.

inhibition of LPS-induced NO generation, and neuroprotective effects.

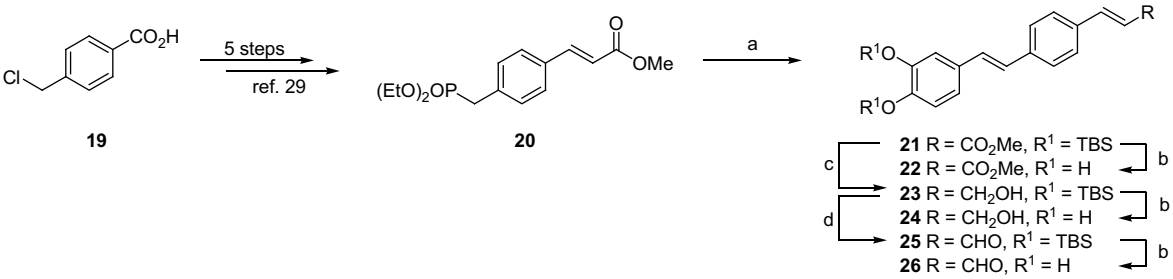
2. Results and discussion

2.1. Chemistry

A series of *trans*-stilbene analogues **15a–n**, **17–18**, **22**, **24**, and **26** were prepared from commercially available 4-(chloromethyl)benzoic acid and 3,4-dihydroxybenzaldehyde as starting materials. The phosphonium salt **9** was prepared from commercially available 4-(chloromethyl)-benzoic acid **19** in three steps in an overall 79% yield according to a previously described procedure [29]. A Wittig–Horner reaction of **9** with freshly protected *tert*-butyl dimethylsilanyloxy (TBS)-aldehyde **10** [30,31] gave ester **11** (3:2 ratio of *E*/*Z*, 86% combined yield), which was cleanly separated by column chromatography. In the proton NMR analyses, we found that the vinyl protons for the (*E*)-form of compound **11** showed at 7.13 ppm and 6.95 ppm as a doublet ($J = 16.0$ Hz), while the vinyl protons for the (*Z*)-form of compound **11** exhibited at 6.61 ppm and 6.51 ppm as a doublet ($J = 12.2$ Hz). According to a published procedure [29], *E*-olefin compound **11** was converted to the protected acid **12** in three steps in a 66% overall yield. The protected acid **12** was coupled with several amines **13a–n** [allyl amine, *n*-decyl amine, tetrahydrofurfuryl amine, furfuryl amine, cyclohexyl amine, (3′-aminopropyl)-2-pyrrolidinone, 2-fluorobenzylamine, aniline, ethyl-3-aminoethylbenzoate, morpholine, 1-allylpiperazine, 1-benzoylpiperazin, 4-methylpiperidine, 1-benzylpiperidine] to give amides **14a–n**, which were subjected to removal of a TBS group from the dihydroxy groups to yield new *trans*-stilbene derivatives **15a–n** in good yields (Scheme 1).



Scheme 1. Synthesis of new *trans*-stilbene derivatives **15a–n**. Reagents and conditions: (a) NaH (60% dispersion in paraffin liquid, 1.3 equiv), CH_2Cl_2, 0 °C, 16 h; (b) TBAF (2.5 equiv), THF, 0 °C, 30 min to 1 h. (c) **13a–n**, EDCI (3.0 equiv), HOBt (3.0 equiv), DMF, 1 h; (d) **11**, DIBAL-H (2.5 equiv), CH_2Cl_2, −78 °C, 1 h, and then EDCI (3.0 equiv), HOBt (3.0 equiv), hippuric acid (1.5 equiv), CH_2Cl_2, 40 °C, 36 h; (e) **17**, LAH (1.5 equiv), THF, −10 °C, 1 h, and then EDCI (3.0 equiv), HOBt (3.0 equiv), hippuric acid (1.5 equiv), CH_2Cl_2, 40 °C, 16 h.



Scheme 2. Synthetic route for the preparation of compounds **22**, **24** and **26**. Reagents and conditions: (a) **10**, NaH (1.3 equiv), CH_2Cl_2, 0 °C, 16 h; (b) TBAF (2.5 equiv), THF, 0 °C, 30 min; (c) DIBAL-H (2.5 equiv), CH_2Cl_2, −78 °C, 1 h; (d) TEMPO (0.3 equiv), $PhI(OAc)_2$ (3.0 equiv), CH_2Cl_2, rt, 7 h.

Treatment of ester **11** with 2.5 equiv of diisobutylaluminiumhydride (DIBAL-H) in dry CH_2Cl_2 led to the reduction of the ester group, and the resulting primary alcohol underwent coupling with 1.5 equiv of hippuric acid using ethyl (dimethylaminopropyl)carbodiimide (EDCI) and 1-hydroxy-benzotriazole (HOBt) to afford **16** in a 55% yield in two steps [32,33]. At this stage, coupling of the primary alcohol with hippuric acid was also accomplished by dicyclohexylcarbodiimide (DCC)/CH_2Cl_2 [34,35], 2-(7-aza-1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluraniumhexafluorophosphate (HATU)/CH_2Cl_2 [36,37], and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl) in CH_2Cl_2 [38,39]. Although these latter conditions were more convenient for handling, the HOBt/EDCI method afforded a superior yield. Removal of the TBS group of **16** was achieved using trifluoroacetic acid (TFA) or tetrabutylammonium fluoride (TBAF) conditions to give **18** in 87% or 90% yields, respectively. In addition, the deprotection of ester **11** was accomplished by a TBAF condition with 72% yield. Reduction of **17** using lithium aluminum hydride (LAH) in THF afforded the terminal alcohol, which was coupled with hippuric acid in the presence of HOBt and EDCI to give **18** in two steps with a 57% overall yield (Scheme 1).

Methyl-(*E*)-3-{4-[(diethoxyphosphinyl)methyl]phenyl}acrylate (**20**) was also prepared from commercially available 4-(chloromethyl)benzoic acid (**19**) in five steps in an overall 27% yield according to a previously published procedure [29]. Coupling of compound **20** with **10** in the presence of sodium hydride (NaH, 60% dispersion in paraffin liquid) in CH_2Cl_2 afforded **21** in 76% yield. Treatment of **21** with TBAF afforded **22** in 37% yield. Reduction of **21** with DIBAL-H in CH_2Cl_2 gave primary alcohol **23**, which was subsequently treated with TBAF to afford **24** in 70% yield. Treatment of **23** with $PhI(OAc)_2$ in the presence of 2,2,6,6-tetramethyl-1-piperidinyloxy, free radical (TEMPO) gave aldehyde **25** in 71% yield, and **25** was readily subjected to deprotection of the TBS group for the desired product **26** in 70% yield (Scheme 2).

Table 1
Rate (IC_{50} μM) of scavenging DPPH radical of resveratrol and *trans*-stilbene analogues **15a–n**, **17–18**, **22**, **24**, and **26** and resveratrol.

Compound	DPPH radical-scavenging activity (%)				IC_{50} (μM)
	10 μM	50 μM	100 μM	200 μM	
15a	15.00	29.01	41.70	74.13	98.06
15b	18.48	41.95	75.17	79.08	47.87
15c	16.73	17.99	26.46	31.74	>200
15d	21.95	35.06	47.99	79.17	69.0
15e	17.19	22.46	40.22	68.68	127.93
15f	12.88	22.16	28.45	44.03	>200
15g	29.82	42.23	52.32	87.98	43.59
15h	10.22	17.58	27.15	59.81	>200
15i	21.27	29.28	44.13	62.79	126.63
15j	5.70	10.19	19.14	29.40	>200
15k	24.76	31.18	40.05	62.37	140.96
15l	17.19	24.58	36.73	59.07	184.55
15m	15.07	21.34	31.49	48.63	>200
15n	4.75	10.12	17.41	25.80	>200
17	2.31	8.95	16.50	29.28	>200
18	21.22	28.15	33.17	46.91	>200
22	5.91	5.31	5.73	5.29	>200
24	6.82	8.64	11.48	17.28	>200
26	13.15	21.25	28.77	54.38	>200
Resveratrol[a]	9.45	8.44	28.39	33.79	>200

[a] Resveratrol is a reference material.

2.2. Chemical evaluation

2.2.1. Radical-scavenging activity

DPPH (diphenyl-1-picrylhydrazyl) radicals can be used in preliminary screening of compounds capable of scavenging reactive oxygen species, since these nitrogen radicals are much more stable and easier to handle than oxygen free radicals. The radical-scavenging activities of the new *trans*-stilbene analogues were evaluated by the published test method [40,41] over the concentration range of 10–200 μM (Table 1). Favorable scavenging ratios were found for almost all of compounds **15a–n**, **17–18**, **22**, **24**, and **26** ranging from 30 to 75% at the highest concentration, 200 μM (Fig. 2). To our surprise, most of the *trans*-stilbene derivatives showed good DPPH radical-scavenging activity compared with resveratrol, although compounds **15c**, **15j**, **15n**, **17**, **22**, and **24** were less effective than the reference material. Among these analogues, amide derivative **15g** exhibited tentatively potent radical-scavenging activity. Furthermore we found that acyclic amine moieties (**15a–15g**) showed better radical-scavenging activity than the cyclic amine moieties (**15h–15n**) as shown in Table 1.

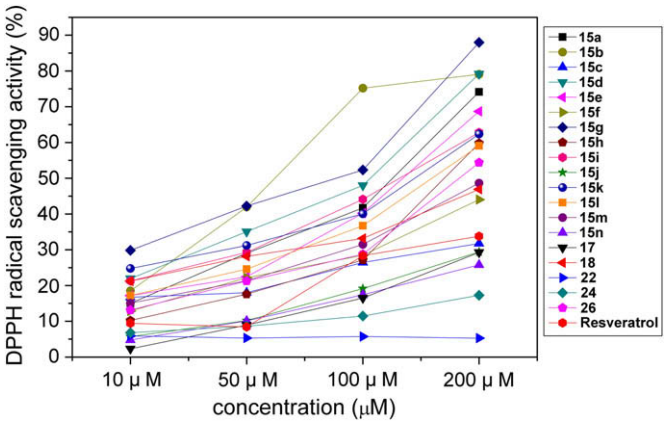


Fig. 2. DPPH bleaching kinetics in the presence of different concentrations (10, 50, 100, and 200 μM) of prepared novel *trans*-stilbene derivatives **15a–n**, **17–18**, **22**, **24**, and **26**.

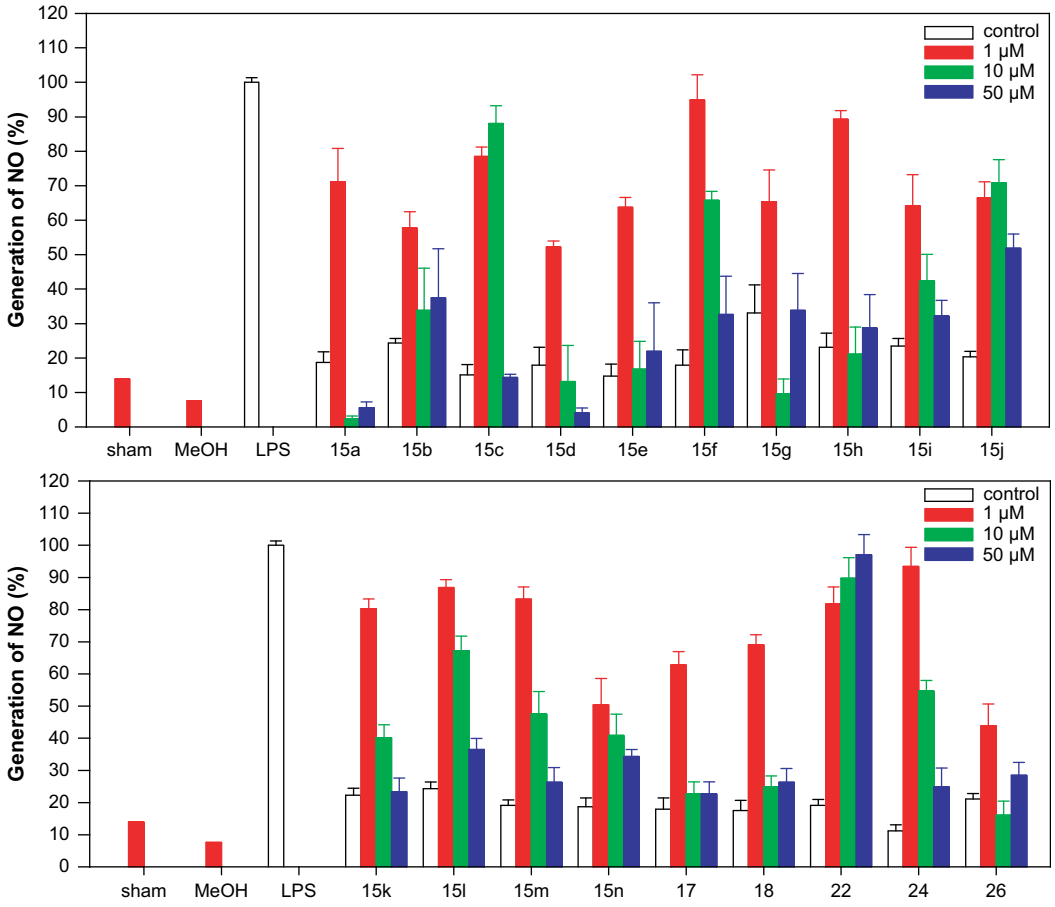


Fig. 3. Effect of *trans*-resveratrol derivatives **15a–n**, **17–18**, **22**, **24**, and **26** on nitrite production in LPS-stimulated BV-2 microglia cells. Cells were treated with 100 ng/mL LPS, then various concentrations of these compounds (1 μM, 10 μM, and 50 μM) were added for 24 h at 37 °C. Values indicate nitrite production from culture supernatants of LPS-treated cells with or without compounds. Data represent the mean ± standard deviation of three observations.

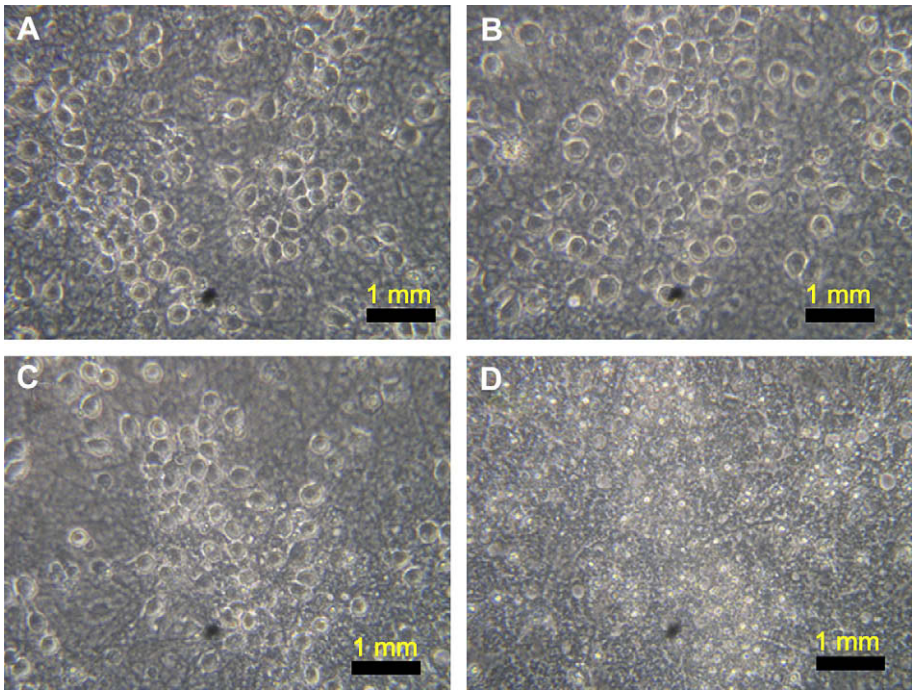


Fig. 4. Phase-contrast micrographs showing glutamate-induced neurotoxicity and neuroprotection of compound **26** in cultured cortical neurons. Neurons without compound treatment showed healthy shapes (A) and compound **26** alone did not induce neurotoxicity (B). Compound **26** (50 μM) was given for 24 h with glutamate (50 μM) at 37 °C and co-treatment showed neuroprotection (C), while glutamate alone (50 μM) induced neurotoxicity (D) (original magnification, 200). Bar = 1 mm.

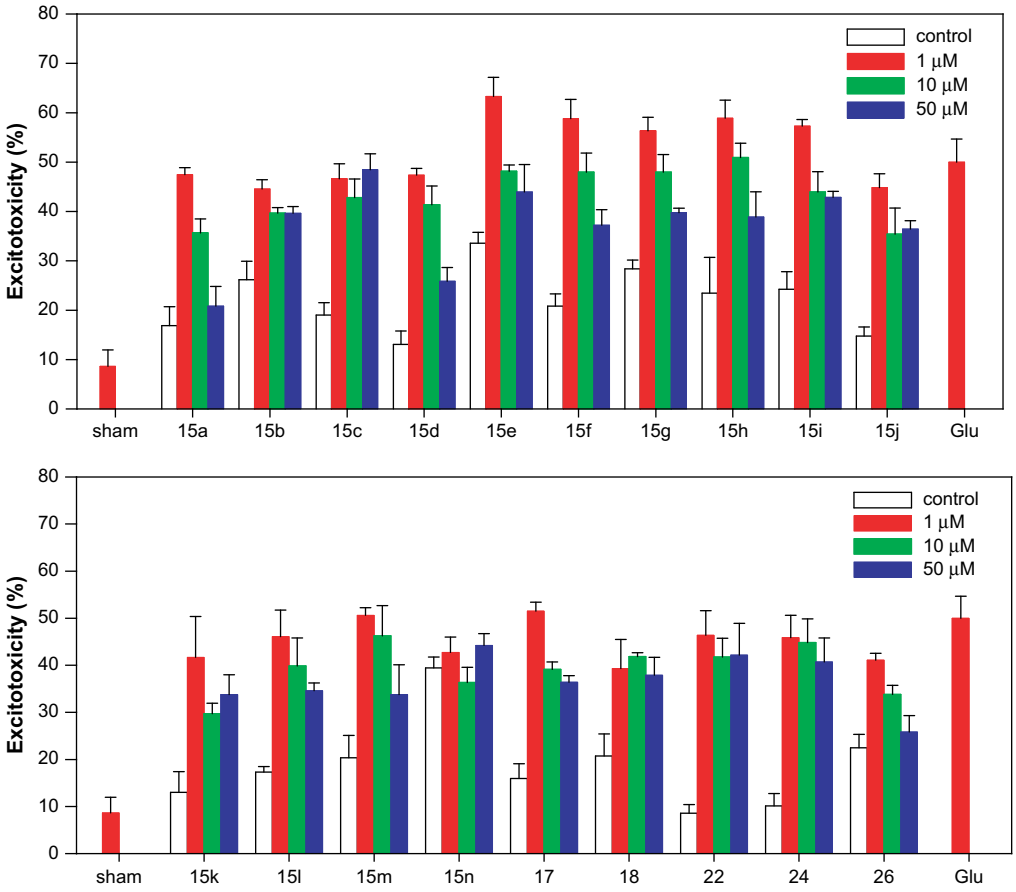


Fig. 5. Protection of glutamate-induced neurotoxicity in cultured cortical neurons for compounds **15a–n**, **17–18**, **22**, **24**, and **26**. Compounds were co-administered with glutamate (50 μM) for 24 h at 37 °C. Lactate dehydrogenase (LDH) was measured at 340 nm using a microplate spectrophotometer. Data represent the mean ± standard deviation of three observations.

2.3. Biological assays in cultured cortical neuron and BV-2 microglia cells

2.3.1. Nitric oxide assay

Nitrite was used as a measure of NO production. The *in vitro* suppression of LPS-induced NO generation of the prepared *trans*-stilbenes was evaluated by the published test method [21] and the results are summarized in Fig. 3. Most of the *trans*-stilbenes inhibited nitrite accumulation in LPS-stimulated microglia BV-2 cells, with amide derivative **15d** exhibiting the greatest inhibitory activity for LPS-induced NO generation. Interestingly, compound **15n** and propenylaldehyde analogue **26** effectively inhibited NO production at the lowest concentration of 1 μM, while compounds **15a** and **15d** exhibited considerable inhibition of NO production at 10–50 μM concentrations in LPS-stimulated BV-2 cells (Fig. 3). These results also showed that compounds **15a**, **15d**, **15n**, and **26** possessed good anti-inflammatory activity.

2.3.2. Lactate dehydrogenase (LDH) assay

Lactate dehydrogenases are valuable *in vitro* markers for cellular toxicity. In these studies, LDH activity was used as a measure of the neuroprotective effects of the novel *trans*-stilbene compounds. Compounds were added to the culture medium with glutamate, and neuroprotection was observed via microscopic images. Phase-contrast micrographs showed glutamate-induced neurotoxicity and neuroprotection in cultured cortical neurons for the tested compounds. Cultured neurons with neither glutamate nor compound showed healthy cellular shapes, while glutamate (50 μM) induced neurotoxicity (Fig. 4). Allylamide derivative **15a** showed the most potent neuroprotective activity in glutamate-induced primary cortical neuron cells (Fig. 5). Among the prepared *trans*-stilbene analogues, compounds **18** and **26** exhibited good anti-neurotoxicity activities at the lowest dose (1 μM), while compounds **15a**, **15d**, **15n**, and **26** showed favorable anti-neurotoxicity activity at higher doses (10–50 μM, Fig. 5). Excitotoxic neuronal death was prevented by inclusion of 1–50 μM of compounds **15a**, **15d**, and **26** in the culture media.

3. Conclusion

An efficient method for synthesis of new *trans*-stilbene analogues **15a–n**, **17–18**, **22**, **24**, and **26** has been described. Furthermore, all analogues were evaluated for free radical-scavenging activity, suppression of LPS-induced NO generation, and anti-excitotoxicity *in vitro*. Most of the *trans*-stilbene derivatives showed 2–3 times more radical-scavenging activity than resveratrol. They were also potent inhibitors of nitrite accumulation in LPS-stimulated microglia BV-2 cells.

Amide derivatives **15a**, **15d**, and **15n** (ED_{50}; 1.95, 0.82, 1.12 μM, respectively) and propenylaldehyde analogue **26** (ED_{50}, 1.51 μM) were especially effective in inhibiting nitrite production. In addition, excitotoxic neuronal death was prevented by inclusion of 1–50 μM of compounds **15a**, **15d**, and **26** (ED_{50}, 29.6, 58.3, 42.3 μM, respectively). Of these analogues, allylamide derivatives **15a** showed the most potent protective activity against glutamate-induced neurotoxicity and was a potent anti-inflammatory agent.

4. Experimental

4.1. Chemistry

All commercial reagents and solvents were used as received without further purification unless specified. Reaction solvents were distilled from calcium hydride for dichloromethane and from sodium metal and benzophenone for tetrahydrofuran. The reactions were monitored and the R_f values determined using analytical thin layer chromatography (TLC) with Merck silica gel 60 and F-254 precoated plates (0.25-mm thickness). Spots on the TLC plates were visualized using ultraviolet light (254 nm) and a basic potassium permanganate solution or cerium sulfate/ammonium dimolybdate/sulfuric acid solution followed by heating on a hot plate. Flash column chromatography was performed with Merck silica gel 60 (230–400 mesh). ^{1}H NMR spectra were recorded on Bruker DPX-250 or Varian Unity-Inova 500 spectrometers. Proton chemical shifts are reported in ppm (δ) relative to internal tetramethylsilane (TMS, δ 0.00) or with the solvent reference relative to TMS employed as the internal standard ($CDCl_3$, δ 7.26 ppm; d_4-CD_3OD, δ 3.31 ppm, d_6-DMSO, δ 2.50 ppm). Data are reported as follows: chemical shift {multiplicity [singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m)], coupling constants [Hz], integration}. ^{13}C NMR spectra were recorded on Bruker DPX-250 (63 MHz) or Varian Unity-Inova 500 (125 MHz) spectrometers with complete proton decoupling. Carbon chemical shifts are reported in ppm (δ) relative to TMS with the respective solvent resonance as the internal standard ($CDCl_3$, δ 77.0 ppm; d_4-CD_3OD, δ 49.0 ppm, d_6-DMSO, δ 39.5 ppm). Infrared (IR) spectra were recorded on a Nicolet Model Impact FT-IR 400 spectrometer. Data are reported in wave numbers (cm^{-1}). Mass spectra were recorded on a MALDI-TOF Voyager-DE STR (Applied Biosystems 4700 proteomics analyzer spectrometer, Palo Alto, CA) with an α-cyano-4-hydroxycinnamic acid (α-CHCA) matrix.

4.2. General procedure and spectral data of compounds **11** and **12**

4.2.1. trans-*3,4-Di-(*tert-*butyldimethylsilanyloxy)-4′-(methoxycarbonyl)stilbene*/cis-*3,4-Di-(*tert-*butyldimethylsilanyloxy)-4′-(methoxycarbonyl)stilbene (**11**)*

To a solution of phosphonium salt **9** (1.0 g, 3.6 mmol) in dry CH_2Cl_2 (10 mL) was added portionwise a suspension of NaH (0.16 g, 60% dispersion in paraffin liquid) in dry CH_2Cl_2 (10 mL) at 0 °C and the mixture was stirred at 0 °C for 30 min. The protected aldehyde **10** (1.0 g, 2.7 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise to the reaction mixture and the resulting mixture was stirred at 0 °C for 16 h. The reaction mixture was quenched by slow addition of water (20 mL) and extracted with ethyl acetate (2 × 30 mL). The combined organic phases were washed with $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (silica gel, hexanes/ethyl acetate, 20/1, v/v) to yield coupling products **11** (1.15 g, 86%, combined yield, $E/Z = 3{:}2$) as a white solid and a colorless syrup. (*E*)-form: $R_f = 0.41$ (hexanes/ethyl acetate, 10:1, v/v), IR ν_{max} ($CHCl_3$) 2941, 2854, 1729, 1672, 1601, 1515, 1464, 1429, 1281, 1179, 1113 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 8.02$ (d, 2H, $J = 8.2$ Hz, aromatic H), 7.55 (d, 2H, $J = 8.2$ Hz, aromatic H), 7.13 (d, 1H, $J = 16.0$ Hz, vinyl CH), 7.00 (s, 2H, aromatic H), 6.94 (d, 1H, $J = 16.0$ Hz, vinyl CH), 6.84 (d, 1H, $J = 8.7$ Hz, aromatic H), 3.91 (s, 3H, OCH_3), 1.01–0.99 (m, 18H, *tert*-butyl H), 0.23–0.22 (m, 12H, dimethyl H); ^{13}C NMR (63 MHz, $CDCl_3$) $\delta = 167.1, 147.7, 147.2, 142.3, 131.1, 130.6, 130.1, 128.6, 126.2, 125.7, 121.4, 119.6, 119.4, 52.4, 25.9, 25.4, 18.6, −3.8, −4.1$; *m*/*z* 499.2 ($M^+ + 1$). (*Z*)-form: $R_f = 0.48$ (hexanes/ethyl acetate, 10:1, v/v), IR ν_{max} ($CHCl_3$) 2955, 2929, 2858, 1511, 1471, 1422, 1297, 1254, 1228, 1123, 1007, 988, 838, 781 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 7.91$ (d, 2H, $J = 8.3$ Hz, aromatic H), 7.33 (d, 2H, $J = 8.3$ Hz, aromatic H), 6.70–6.67 (m, 3H, aromatic H), 6.60 (d, 1H, $J = 12.0$ Hz, vinyl CH), 6.50 (d, 1H, $J = 12.0$ Hz, vinyl CH), 3.90 (s, 3H, OCH_3), 0.98–0.89 (m, 18H, *tert*-butyl H), 0.20–0.04 (m, 12H, dimethyl H); ^{13}C NMR (63 MHz, $CDCl_3$) $\delta = 167.0, 146.7, 142.8, 132.1, 130.2, 129.8, 128.9, 128.4, 127.9, 122.5, 121.4, 120.9, 52.1, 26.0, 26.0, 18.6, 18.4, −3.9, −4.1$; *m*/*z* 499.2 ($M^+ + 1$).

4.2.2. trans-*3,4-Di-(*tert-*butyldimethylsilanyloxy)-4′-(carboxy)stilbene (**12**)*

Yield: 96%. $R_f = 0.26$ (hexanes/ethyl acetate, 2:1, v/v); IR ν_{max} ($CHCl_3$) 2928, 2855, 1681, 1605, 1595, 1564, 1513, 1421, 1286, 1251, 1177, 1126, 916, 837 cm^{-1}; ^{1}H NMR (250 MHz, DMSO-d_6) δ 12.8 (s, 1H, COOH), 7.98 (d, 2H, $J = 8.0$ Hz, aromatic H), 7.75 (d, 2H, $J = 8.1$ Hz, aromatic H), 7.41 (d, 1H, $J = 16.2$ Hz, vinyl CH), 7.23 (3H, m, aromatic H, vinyl CH), 6.94 (d, 1H, aromatic H), 1.02–1.01 (m, 18H, *tert*-butyl H), 0.25–0.24 (m, 12H, dimethyl H); ^{13}C NMR (63 MHz, DMSO-d_6) δ 167.6, 147.1, 146.8, 142.2, 131.3, 131.1, 130.2, 129.6, 126.7, 126.0, 121.5, 120.9, 119.9, 26.2, 18.7, −3.7; *m*/*z* 485.4 ($M^+ + 1$).

4.3. General procedure for the preparation of deprotected dihydroxy stilbenes (**15a**–**n**, **17**–**18**, **22**, **24**, and **26**)

TBAF (0.25 mmol, 1 M solution in THF) was added dropwise to a stirred solution of **11**, **14a**–**n**, **16**, **21**, **23**, and **25** (0.10 mmol) in dry THF (5 mL) at 0 °C under nitrogen atmosphere, and the mixture was stirred at room temperature for 30 min. The reaction mixture was then quenched with water and acidified with 10% HCl, followed by dilution with ethyl acetate (10 mL) and a brine wash (7 mL). The organic phase was separated, and the aqueous phase was extracted with ethyl acetate (2 × 10 mL). The combined organic phases were washed with brine (15 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to yield dihydroxy stilbenes, which were purified by flash column chromatography (silica gel, CH_2Cl_2/methanol, 15/1–10/1, v/v) to give **15a**–**n**, **17**–**18**, **22**, **24** and **26**.

4.3.1. trans-*3,4-Dihydroxy-4′-(*N-*allylaminocarbonyl)stilbene (**15a**)*

Yield: 64%. $R_f = 0.06$ (CH_2Cl_2/methanol, 20:1, v/v); IR ν_{max} ($CHCl_3$) 3326, 1728, 1634, 1601, 1539, 1524, 1441, 1373, 1288, 1189, 1112, 1041 cm^{-1}; ^{1}H NMR (250 MHz, CD_3OD) δ 7.82 (d, 2H, $J = 8.3$ Hz, aromatic H), 7.57 (d, 2H, $J = 8.2$ Hz, aromatic H), 7.20–6.89 (m, 4H, aromatic H, vinyl CH), 6.63 (d, 1H, $J = 8.2$ Hz, aromatic H), 6.01–5.86 (m, 1H, vinyl CH), 5.27–5.11 (m, 2H, vinyl CH), 4.00–3.98 (m, 2H, N–CH_2); ^{13}C NMR (63 MHz, CD_3OD) δ 169.7, 147.1, 146.6, 142.8, 135.6, 133.4, 132.1, 130.6, 128.7, 127.1, 125.5, 120.7, 116.4, 116.2, 114.1, 43.2; *m*/*z* 296.1 ($M^+ + 1$).

4.3.2. trans-*3,4-Dihydroxy-4′-(*N-*decylaminocarbonyl)stilbene (**15b**)*

Yield: 60%. $R_f = 0.47$ (CH_2Cl_2/methanol, 10:1, v/v); IR ν_{max} ($CHCl_3$) 3317, 2951, 2923, 2851, 1734, 1630, 1602, 1541, 1524, 1466, 1439, 1269, 963, 859 cm^{-1}; ^{1}H NMR (250 MHz, CD_3OD) δ 7.79 (d, 2H, $J = 8.3$ Hz, aromatic H), 7.58 (d, 2H, $J = 8.3$ Hz, aromatic H), 7.17 (d, 1H, $J = 16.3$ Hz, vinyl CH), 7.05–6.89 (m, 3H, aromatic H, vinyl CH), 6.77 (d, 1H, $J = 8.2$ Hz, aromatic H), 3.39–3.36 (m, 2H, N–CH_2), 1.62–1.59 (m, 2H, alkyl chain), 1.34–1.29 (m, 12H, alkyl chain), 1.00–0.87 (m, 5H, alkyl chain); ^{13}C NMR (63 MHz, CD_3OD) δ 169.7, 147.10, 146.59, 142.7, 133.8, 132.1, 130.2, 128.7, 127.0, 125.5, 120.7, 116.4, 114.1, 41.1, 33.1, 30.2, 30.5, 30.5, 28.1, 23.7, 14.5: *m*/*z* 396.3 ($M^+ + 1$).

4.3.3. trans-*3,4-Dihydroxy-4′-[*N-*(tetrahydrofuran-2-yl-methyl)aminocarbonyl]stilbene (**15c**)*

Yield: 60%. $R_f = 0.31$ ($CHCl_3$/methanol, 9:1, v/v); IR ν_{max} ($CHCl_3$) 3444, 2079, 1633, 1556, 1287 cm^{-1}; ^1H NMR (250 MHz, CD_3OD) $\delta = 7.80$ (d, 2H, $J = 8.3$ Hz, aromatic H), 7.58 (d, 2H, $J = 8.4$ Hz, aromatic H), 7.17 (d, 1H, $J = 16.3$ Hz, vinyl CH), 7.05–6.90 (m, 3H, aromatic H, vinyl CH), 6.77 (d, 1H, $J = 8.2$ Hz, aromatic H), 4.10–4.09 (m, 1H, tetrahydrofuran H), 3.91–3.88 (m, 1H, tetrahydrofuran H), 3.82–3.75 (m, 1H, tetrahydrofuran H), 3.50–3.39 (m, 2H, CH_2–C_4H_7O), 2.00–1.91 (m, 3H, tetrahydrofuran H), 1.71–1.63 (m, 1H, tetrahydrofuran H); ^{13}C NMR (63 MHz, CD_3OD) $\delta = 170.2$, 144.3, 143.8, 140.0, 129.4, 127.8, 126.7, 126.0, 124.2, 122.7, 117.9, 113.7, 111.3, 76.3, 66.3, 42.1, 27.1, 23.8; *m*/*z* 340.4 ($M^+ + 1$).

4.3.4. trans-*3,4-Dihydroxy-4′-[*N-*(furan-2-yl-methyl)aminocarbonyl]stilbene (**15d**)*

Yield: 73%. $R_f = 0.1$ (CH_2Cl_2/methanol, 20:1, v/v); IR ν_{max} ($CHCl_3$) 3431, 2924, 2958, 2853, 1726, 1634, 1601, 1523, 1442, 1289, 1189, 1112, 1043, 1101 cm^{-1}; ^1H NMR (250 MHz, CD_3OD) δ 7.81 (d, 2H, $J = 8.4$ Hz, aromatic H), 7.55 (d, 2H, $J = 8.4$ Hz, aromatic H), 7.42 (m, 1H, furfuran H), 7.15 (d, 1H, $J = 16.3$ Hz, vinyl CH), 7.05–6.88 (m, 3H, aromatic H, vinyl CH), 6.78 (d, 1H, $J = 8.2$ Hz, aromatic H), 6.35–6.28 (m, 2H, furfuran H), 4.55 (m, 2H, NCH_2); ^{13}C NMR (63 MHz, CD_3OD) δ 169.7, 153.2, 147.1, 146.6, 143.2, 142.8, 133.2, 130.6, 128.8, 127.0, 125.4, 120.7, 116.4, 114.1, 111.4, 108.1, 37.6: *m*/*z* 336.3 ($M^+ + 1$).

4.3.5. trans-*3,4-Dihydroxy-4′-(*N-*cyclohexylaminocarbonyl)stilbene (**15e**)*

Yield: 63%. $R_f = 0.28$ (CH_2Cl_2/methanol, 10:1, v/v); IR ν_{max} ($CHCl_3$) 3393, 2930, 2854, 1724, 1628, 1603, 1526, 1507, 1447, 1374, 1257 cm^{-1}; ^1H NMR (250 MHz, CD_3OD) δ 7.78 (d, 2H, $J = 8.3$, aromatic H), 7.55 (d, 2H, $J = 8.3$, aromatic H), 7.15 (d, 1H, $J = 16.3$ Hz, vinyl CH), 7.04–6.89 (m, 3H, aromatic H, vinyl CH), 6.78 (d, 1H, $J = 8.2$ Hz, aromatic H), 3.87–3.85 (m, 1H, NH–CH), 2.02–1.93 (m, 2H, cyclohexane), 1.79–1.66 (m, 4H, cyclohexane), 1.42–1.35 (m, 4H, cyclohexane); ^{13}C NMR (63 MHz, CD_3OD) δ 169.2, 147.1, 146.6, 142.6, 134.0, 132.0, 129.9, 128.7, 127.0, 125.5, 120.7, 116.5, 114.1, 50.5, 33.8, 26.6, 26.4; *m*/*z* 338.2 ($M^+ + 1$).

4.3.6. trans-*3,4-Dihydroxy-4′-{*N-*[3-(2-oxopyrrolidin-1-yl)propyl]aminocarbonyl}stilbene (**15f**)*

Yield: 66%. $R_f = 0.41$ (CH_2Cl_2/methanol, 10:1, v/v); IR ν_{max} ($CHCl_3$) 3444, 2924, 2958, 2853, 1732, 1646, 1455, 1383, 1242, 1123, 1086, 1025 cm^{-1}; ^1H NMR (250 MHz, CD_3OD) δ 7.82 (d, 2H, $J = 8.4$ Hz, aromatic H), 7.59 (d, 2H, $J = 8.4$ Hz, aromatic H), 7.17 (d, 1H, $J = 16.3$ Hz, vinyl CH), 7.05–6.89 (m, 3H, aromatic H, vinyl CH), 6.77 (d, 1H, $J = 8.2$ Hz, aromatic H), 3.51–3.46 (m, 2H, NH–CH_2), 3.40–3.29 (m, 4H, CH_2N–CH_2), 2.40–2.36 (m, 2H, pyrrolidone H), 2.11–2.02 (m, 2H, pyrrolidone H), 1.87–1.80 (m, 2H, CH_2); ^{13}C NMR (63 MHz, CD_3OD) δ 178.1, 169.8, 147.1, 146.6, 142.8, 133.5, 132.2, 130.6, 128.7, 127.1, 125.5, 120.7, 116.5, 114.1, 41.2, 38.1, 32.0, 30.8, 27.9, 18.8: *m*/*z* 381.6 ($M^+ + 1$).

4.3.7. trans-*3,4-Dihydroxy-4′-[*N-*(2-fluorobenzyl)aminocarbonyl]stilbene (**15g**)*

Yield: 64%. $R_f = 0.28$ (CH_2Cl_2/methanol, 10:1, v/v); IR ν_{max} ($CHCl_3$) 3349, 2924, 2853, 1726, 1636, 1601, 1541, 1505, 1456, 1374, 1270, 1190, 1108, 962, 757 cm^{-1}; ^1H NMR (250 MHz, CD_3OD) δ 7.83 (d, 2H, $J = 8.0$, aromatic H), 7.57 (d, 2H, $J = 8.6$ Hz, aromatic H), 7.42–7.23 (m, 3H, aromatic H), 7.15–6.89 (m, 4H, aromatic H, vinyl CH), 6.78 (d, 2H, $J = 8.1$ Hz, aromatic H), 4.63 (s, 2H, CH_2); ^{13}C NMR (63 MHz, CD_3OD) δ 170.0, 164.1 (C–F), 147.1, 146.6, 142.9, 133.3, 132.2, 130.6, 130.2, 130.0, 128.8, 127.1, 125.5, 125.3, 120.7, 116.5, 116.3, 115.9, 114.1, 38.3: *m*/*z* 364.3 ($M^+ + 1$).

4.3.8. trans-*3,4-Dihydroxy-4′-(*N-*phenylaminocarbonyl)stilbene (**15h**)*

Yield: 62%. $R_f = 0.26$ (CH_2Cl_2/methanol, 9:1, v/v), IR ν_{max} ($CHCl_3$) 3442, 1646, 1600, 1525, 1441 cm^{-1}; ^1H NMR (250 MHz, CD_3OD); $\delta = 7.93$–7.89 (d, 2H, $J = 8.4$ Hz, aromatic H), 7.71–7.60 (m, 4H, aromatic H), 7.41–7.33 (m, 3H, aromatic H), 7.20–7.17 (m, 2H, aromatic H, vinyl CH), 7.07–6.91 (m, 2H, aromatic H, vinyl CH), 6.78–6.75 (d, 1H, $J = 8.2$ Hz, aromatic H). ^{13}C NMR (63 MHz, CD_3OD) $\delta = 168.6$, 147.2, 146.6, 143.1, 139.9, 134.1, 132.4, 130.6, 129.8, 129.1, 127.1, 125.6, 125.5, 122.4, 120.7, 116.4, 114.1; *m*/*z* 322.3 ($M^+ + 1$).

4.3.9. trans-*3,4-Dihydroxy-4′-[*N-*(3-ethoxycarbonylphenyl)aminocarbonyl]stilbene (**15i**)*

Yield: 74%. $R_f = 0.26$ (CH_2Cl_2/methanol, 10:1, v/v); IR ν_{max} ($CHCl_3$) 3345, 2924, 2853, 1716, 1651, 1598, 1544, 1437, 1290, 1259, 1108, 1112, 1025, 1002, 957, 756 cm^{-1}; ^1H NMR (250 MHz, CD_3OD) δ 8.40–8.39 (m, 1H, aromatic H), 7.99–7.90 (m, 3H, aromatic H), 7.80 (d, 1H, $J = 8.3$ Hz, aromatic H), 7.63 (d, 2H, $J = 8.3$ Hz, aromatic H), 7.49 (t, 1H, $J = 7.9$ Hz, aromatic H), 7.20 (d, 1H, $J = 16.3$ Hz, vinyl CH), 7.06–6.90 (m, 3H, aromatic H, vinyl CH), 6.78 (d, 1H, $J = 8.2$ Hz, aromatic H), 4.41 (q, 2H, $J = 7.1$ Hz, O–CH_2), 1.42 (t, 3H, $J = 7.1$ Hz, CH_3); ^{13}C NMR (63 MHz, CD_3OD) δ 168.6, 167.8, 147.2, 146.6, 143.2, 140.4, 133.7, 130.5, 132.6, 132.2, 129.9, 129.1, 127.1, 126.5, 126.2, 125.4, 123.0, 120.8, 116.5, 114.1, 62.3, 14.6: *m*/*z* 404.7 ($M^+ + 1$).

4.3.10. trans-*3,4-Dihydroxy-4′-(*N-*morpholinecarbonyl)stilbene (**15j**)*

Yield: 66%. $R_f = 0.36$ (CH_2Cl_2/methanol, 15:1, v/v), IR ν_{max} ($CHCl_3$) 3375, 2964, 2925 2854, 1733, 1627, 1540, 1506, 1449, 1374, 1257, 1188, 1045, 857 cm^{-1}; ^1H NMR (250 MHz, CD_3OD); $\delta = 7.54$ (d, 2H, $J = 8.3$ Hz, aromatic H), 7.32 (d, 2H, $J = 8.3$ Hz, aromatic H), 7.12 (d, 1H, $J = 16.3$ Hz, vinyl CH), 7.12–6.81 (m, 3H, aromatic H, vinyl CH), 6.69 (d, 1H, $J = 8.1$ Hz, aromatic H), 3.52–3.43 (m, 8H, morpholine H); ^{13}C NMR (63 MHz, DMSO-d_6) $\delta = 168.6$, 145.5, 145.0, 138.5, 133.1, 129.8, 127.9, 127.2, 125.4, 123.6, 118.5, 115.3, 113.0, 65.7, 27.9; *m*/*z* 326.4 ($M^+ + 1$).

4.3.11. trans-*3,4-Dihydroxy-4′-[(4-allylpiperazinyl)carbonyl]stilbene (**15k**)*

Yield: 65%. $R_f = 0.33$ (CH_2Cl_2/methanol, 10:1, v/v); IR ν_{max} ($CHCl_3$) 3445, 2925, 2854, 1738, 1603, 1463, 1456, 1441, 1374, 1245, 1115, 1024 cm^{-1}; ^1H NMR (250 MHz, CD_3OD) $\delta = 7.60$ (d, 2H, $J = 8.0$ Hz, aromatic H), 7.40 (d, 2H, $J = 8.0$ Hz, aromatic H), 7.15 (d, 1H, $J = 16.3$ Hz, vinyl CH), 7.03–6.88 (m, 3H, aromatic H, vinyl CH), 6.77 (d, 1H, $J = 8.2$ Hz, aromatic H), 5.87–5.80 (m, 1H, vinyl CH), 5.24–5.20 (m, 2H, vinyl CH), 3.75–3.53 (m, 4H, piperazine H), 3.10 (d, 2H, $J = 6.0$ Hz, N–CH_2), 2.53–2.03 (m, 4H, piperazine H); ^{13}C NMR (63 MHz, CD_3OD) $\delta = 172.4$, 147.1, 146.6, 141.41, 135.1, 134.5, 131.8, 130.6, 128.7, 127.2, 125.5, 120.6, 119.5, 116.5, 114.1, 62.3, 54.1, 43.1; *m*/*z* 365.3 ($M^+ + 1$).

4.3.12. trans-*3,4-Dihydroxy-4′-[(4-benzoylpiperazinyl)carbonyl]stilbene (**15l**)*

Yield: 64%. $R_f = 0.46$ (CH_2Cl_2/methanol, 10:1, v/v); IR ν_{max} ($CHCl_3$) 3403, 2924, 2853, 1734, 1560, 1508, 1460, 1431, 1372, 1256, 1002 cm^{-1}; ^1H NMR (250 MHz, CD_3OD) δ 7.60 (d, 2H, $J = 8.1$ Hz, aromatic H), 7.46–7.39 (m, 7H, aromatic H), 7.15 (d, 1H, $J = 16.3$ Hz, vinyl CH), 7.03–6.88 (m, 3H, aromatic H, vinyl CH), 6.77 (d, 1H, $J = 8.1$ Hz, aromatic H), 3.91–3.61 (m, 8H, piperazine H); ^{13}C NMR (63 MHz, CD_3OD) δ 172.8, 147.1, 146.6, 141.6, 136.4, 134.2, 131.9, 131.4, 130.6, 129.8, 128.8, 128.2, 127.2, 125.4, 120.6, 116.4, 114.1, 30.8: *m*/*z* 329.2 ($M^+ + 1$).

4.3.13. trans-*3,4-Dihydroxy-4′-[*N*-(4-methylpiperidine)carbonyl]stilbene (**15m**)*

Yield: 65%. $R_f = 0.49$ (CH_2Cl_2/methanol, 10:1, v/v), IR ν_{max} ($CHCl_3$) 3408, 2957, 2924, 2854, 1736, 1601, 1508, 1446, 1373, 1273, 1251, 1197, 1114, 1045, 969 cm^{-1}; ^1H NMR (250 MHz, CD_3OD); δ 7.56 (d, 2H, $J = 8.0$ Hz, aromatic H), 7.35 (d, 2H, $J = 8.0$ Hz, aromatic H), 7.13–6.88 (m, 4H, aromatic H, vinyl CH), 6.77 (d, 1H, $J = 8.2$ Hz, aromatic H), 4.60–4.54 (m, 1H, piperidine H), 3.71–3.50 (m, 1H, piperidine H), 3.05–2.80 (m, 2H, piperidine H), 1.78–1.57 (m, 4H, piperidine H), 1.26 (m, 1H, piperidine H), 1.05 (d, 3H, $J = 6.6$ Hz, CH_3); ^{13}C NMR (63 MHz, CD_3OD) δ 172.4, 147.0, 146.6, 141.1, 135.3, 130.7, 131.7, 128.4, 127.1, 125.6, 120.6, 116.5, 114.0. 43.8, 35.8, 34.9, 32.2, 22.0: *m/z* 338.1 ($M^+ + 1$).

4.3.14. trans-*3,4-Dihydroxy-4′-[*N*-(4-benzylpiperidine)carbonyl]stilbene (**15n**)*

Yield: 68%. $R_f = 0.46$ (CH_2Cl_2/methanol, 10:1, v/v); IR ν_{max} ($CHCl_3$) 3395, 2923, 2851, 1593, 1558, 1445, 1838, 1237, 1086, 1021, 963 cm^{-1}; ^1H NMR (250 MHz, CD_3OD) δ 7.59 (d, 2H, $J = 8.2$ Hz, aromatic H), 7.37 (d, 2H, $J = 8.2$ Hz, aromatic H), 7.32–7.27 (m, 2H, aromatic H), 7.23–7.14 (m, 3H, aromatic H), 7.29–6.88 (m, 4H, aromatic H), 6.77 (d, 1H, $J = 8.2$ Hz, aromatic H), 4.37 (m, 1H, piperidine H), 3.57 (m, 1H, piperidine H), 2.71–2.60 (2H, piperidine H), 2.60 (d, 2H, $J = 6.6$ Hz, CH_2-Ph), 1.74–1.55 (m, 4H, piperidine H), 1.21–1.18 (m, 1H, piperidine H); ^{13}C NMR (63 MHz, CD_3OD) δ 168.8, 145.9, 145.5, 140.1, 138.7, 134.5, 130.1, 129.0, 128.4, 128.2, 127.3, 125.9, 124.1, 118.9, 115.7, 113.5, 42.1, 41.5, 37.6, 31.8–31.6: *m/z* 414.8 ($M^+ + 1$).

4.3.15. trans-*3,4-Dihydroxy-4′-(methoxycarbonyl)stilbene (**17**)*

Yield: 32%. $R_f = 0.10$ (hexanes/ethyl acetate, 2/1, v/v). IR ν_{max} ($CHCl_3$) 3423, 1678, 1629, 1595, 1525, 1438, 1296, 1231, 1115, 956 cm^{-1}; ^1H NMR (250 MHz, CD_3OD); $\delta = 7.92$ (d, 2H, $J = 8.3$ Hz, aromatic H), 7.67 (d, 2H, $J = 8.3$ Hz, aromatic H), 7.27 (d, 1H, $J = 16.4$ Hz, vinyl H), 7.04–6.90 (m, 3H, aromatic H), 6.77 (d, 1H, $J = 8.1$ Hz, aromatic H), 3.83 (s, 3H, OCH_3); ^{13}C NMR (63 MHz, CD_3OD) $\delta = 167.2$, 147.1, 146.4, 143.6, 132.9, 130.7, 129.3, 128.6, 127.2, 124.9, 120.4, 116.8, 114.6, 53.1; *m/z* 271.1 ($M^+ + 1$).

4.3.16. trans-*3,4-Dihydroxy-4′-[2-(benzamido)acetoxymethyl]stilbene (**18**)*

Yield: 90%. $R_f = 0.30$ ($CHCl_3$/methanol, 9:1, v/v), IR ν_{max} ($CHCl_3$) 3343, 2955, 2929, 2895, 2857, 1749, 1652, 1601, 1515, 1422, 1298, 1253, 1194, 982, 839, 781 cm^{-1}; ^1H NMR (250 MHz, CD_3OD) $\delta = 7.86$–7.83 (m, 2H, aromatic H), 7.55–7.44 (m, 5H, aromatic H), 7.36 (d, 2H, $J = 8.2$ Hz, aromatic H), 7.01–6.86 (m, 4H, aromatic H, vinyl CH), 6.76 (d, 1H, $J = 8.2$ Hz, aromatic H), 5.18 (s, 2H, CH_2O), 4.16 (s, 2H, CH_2N); ^{13}C NMR (63 MHz, $CDCl_3$) $\delta = 171.3$, 170.7, 146.7, 146.5, 139.5, 135.7, 135.0, 133.0, 130.9, 130.5, 129.7, 129.6, 128.4, 127.2, 126.1, 120.3, 116.4, 113.9, 67.8, 42.6; *m/z* 404.1 ($M^+ + 1$).

4.3.17. trans-*3,4-Dihydroxy-4′-(methoxycarbonyl-1-ethenyl)stilbene (**22**)*

Yield: 37%. $R_f = 0.47$ (hexanes/ethyl acetate, 1/1, v/v); IR ν_{max} ($CHCl_3$) 3413, 3020, 1634, 1439, 1251 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) $\delta = 7.72$ (d, 1H, $J = 16.0$ Hz, vinyl H), 7.60–7.55 (m, 4H, aromatic H), 7.20 (d, 1H, $J = 16.0$ Hz, vinyl H), 7.07 (s, 1H, aromatic H), 6.98 (d, 1H, $J = 16.5$ Hz, vinyl H), 6.94 (d, 1H, $J = 9.0$ Hz, aromatic H), 6.80 (d, 1H, $J = 8.5$ Hz, aromatic H), 6.55 (d, 1H, $J = 16.5$ Hz, vinyl H), 3.82 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, CD_3OD) $\delta = 169.4$, 154.7, 147.1, 146.0, 141.8, 134.2, 131.6, 130.8, 129.7, 127.6, 125.8, 120.7, 117.6, 116.5, 114.0, 52.2; *m/z* 297.3 ($M^+ + 1$).

4.3.18. trans-*3,4-Dihydroxy-4′-(3-hydroxyprop-1-enyl)stilbene (**24**)*

Yield: 70%. $R_f = 0.18$ (CH_2Cl_2/methanol, 15:1, v/v); IR ν_{max} ($CHCl_3$) 3326, 2924, 2853, 1728, 1634, 1601, 1538, 1525, 1441, 1374, 1289, 1112, 959, 853 cm^{-1}; ^1H NMR (250 MHz, CD_3OD) $\delta = 7.49$ (d, 2H, $J = 8.2$ Hz, aromatic H), 7.39 (d, 2H, $J = 8.3$ Hz, aromatic H), 7.08 (d, 1H, $J = 16.4$ Hz, vinyl CH), 6.99–6.84 (m, 3H, vinyl CH, aromatic H), 6.74 (d, 1H, $J = 8.11$ Hz, aromatic H), 6.56 (d, 1H, $J = 16.1$ Hz, vinyl CH), 6.41–6.37 (m, 1H, vinyl CH), 4.13–4.09 (m, 2H, CH_2O); ^{13}C NMR (63 MHz, DMSO-d_6) $\delta = 146.1$, 145.9, 136.9, 135.9, 130.8, 129.1, 129.0, 128.7, 126.9, 126.7, 125.1, 119.1, 116.2, 113.7, 62.0; *m/z* 291.2 ($M^+ + 1$).

4.3.19. trans-*3,4-Dihydroxy-4′-(2-formyl-1-ethenyl)stilbene (**26**)*

Yield: 70%. $R_f = 0.08$ (CH_2Cl_2/methanol, 30:1, v/v); IR ν_{max} ($CHCl_3$) 3395, 2923, 2958, 2855, 1673, 1620, 1594, 1382, 1129, 1019, 965 cm^{-1}; ^1H NMR (250 MHz, CD_3OD) $\delta = 10.07$ (d, 1H, $J = 7.8$ Hz, –COH), 8.22–8.01 (m, 5H, vinyl H, aromatic H), 7.66 (d, 1H, $J = 16.3$ Hz, vinyl H), 7.43 (m, 4H, vinyl H, aromatic H), 7.16 (d, 1H, $J = 8.1$ Hz, aromatic H); ^{13}C NMR (63 MHz, CD_3OD) $\delta = 193.8$, 152.4, 145.6, 144.8, 140.1, 132.0, 130.4, 128.8, 127.9, 127.2, 126.1, 123.6, 118.6, 115.2, 113.1; *m/z* 267.4 ($M^+ + 1$).

4.4. Chemical evaluation

4.4.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging effects

We used DPPH to assess free radical-scavenging activity [42,43]. DPPH is one of the few stable and commercially available organic nitrogen radicals and has a UV-vis absorption maximum at 515 nm. Upon reduction, the solution color fades; the reaction progress is conveniently monitored by a spectrophotometer [44]. To test free radical-scavenging effects using DPPH, compounds **15a–n**, **17–18**, **22**, **24**, and **26** were adjusted with methanol solution to final concentrations of 10–200 μM. Tris-base buffer (0.1 mM) was added, and after 5 min, a DPPH radical-ethanol solution (1 mL, 0.5 mM) was added. The mixture was warmed in a water bath for 25 min at 37 °C. After 20 min, absorbance was measured with a spectrophotometer (515 nm). The DPPH radical-scavenging rate of each sample and the 50% scavenging concentration based on the DPPH radical-scavenging rate were calculated using the following rate (%):

$$\text{DPPH radical-scavenging rate (\%)} = \left\{1 - \frac{A - C}{B}\right\} \times 100$$

where A is the absorbance of the sample (DPPH + compounds) when a blank was substituted for the tris-base buffer, B is the absorbance of the DPPH radical-ethanol solution when a blank was substituted for the tris-base buffer, and C is the absorbance of the sample (compounds) alone.

The IC_{50} values were calculated by linear regression of plots where *x*-axis represented the various concentrations (10–200 μM) of test *trans*-stilbenes while the *y*-axis represented the percentage of free radical-scavenging activities. The IC_{50} values of samples were compared against the standards, resveratrol, and the lower the IC_{50} of synthesized *trans*-stilbenes, the better it is as an antioxidant [45].

4.5. Biological evaluation

4.5.1. Cortical neuron and BV-2 microglia culture

Cortical cell cultures were prepared from embryos of ICR mice at a gestational age of 15 days. The cortex was dissected and kept in an ice-cold solution. The cortical tissues were dissociated to single cells by gentle suspension. The cell suspension was centrifuged at 1000 rpm for 5 min, and the resulting pellets were resuspended in minimal essential media (MEM), supplemented with 5% heat-inactivated fetal calf serum, mouse serum, glutamine, and glucose. The cells were plated on plates coated with poly-D-lysine and laminin at a density of 4.8×10^5 cells/well in 24-well cultured plates.

The cells were cultured in a CO_2 incubator (5% [v/v], 37 °C). Seven days after plating, cells were treated with 10 μM cytosine arabinofuranoside (Ara C) to reduce the growth of contaminating non-neuronal cells. After treatment for 48 h, cells were fed with fresh media (without fetal calf serum). The murine BV-2 microglia cell line was maintained in DMEM supplemented with 10% FBS and penicillin/streptomycin at 37 °C in a humidified incubator under 5% CO_2. For all experiments, cells were plated at a density of 1×10^5 cells/mL in 24-well plates and then treated with 100 ng/mL LPS alone or with various concentrations of compounds for 24 h at 37 °C.

4.5.2. Nitric oxide assay

The Griess reaction was used to perform nitrite assays [46–48]. Cells were incubated with LPS (lipopolysaccharide, 100 ng/mL) and various concentrations of *trans*-stilbene derivatives for 24 h at 37 °C. The culture media were then mixed with an equal volume of reagent (1 part 0.1% *N*-1-naphthylethylenediamine dihydrochloride, 1 part 1% sulfanilamide in 5% phosphoric acid) in 96-well plates. The absorbance was determined at 540 nm using a microplate reader. Data are reported as the mean ± the standard deviation of three observations.

4.5.3. Lactate dehydrogenase (LDH) assay

Lactate dehydrogenases are of great value as *in vitro* markers for cellular toxicity. Lactate dehydrogenases released into the culture medium were measured by monitoring the production of NAD^+ from NADH during the conversion of pyruvate to lactate. The cell supernatant (30 μL) was incubated in 120 μL of NADPH buffer (0.45 mg/mL), followed after 2 min by the addition of pyruvate (22 mM). The rate of NAD^+ formation was monitored for 5 min at 11-second intervals at 340 nm by spectrophotometer.

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Appendix. Supplementary data

General procedure and spectral data of compounds, **14a**–**14n**, **16**, **20**, **21**, **23**, and **25**. This material is available online at www.sciencedirect.com. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2009.03.011.

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Abbreviations

LPS: lipopolysaccharide
DPPH: diphenyl-1-picrylhydrazyl
LDH: lactate dehydrogenase
EDCI: ethyl(dimethylaminopropyl)carbodiimide
MEM: minimal essential media