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Design, synthesis and pharmacological evaluation of (*E*)-3,4-dihydroxy styryl sulfonamides derivatives as multifunctional neuroprotective agents against oxidative and inflammatory injury

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ABSTRACT

A novel class of (*E*)-3,4-dihydroxy styryl sulfonamides and their 3,4-diacetylated derivatives as caffeic acid phenethyl ester (CAPE) analogs was designed and prepared for improving stability and solubility of the lead compound. Their neuroprotective properties were assessed by several models. The results showed that target compounds displayed positive free radical quenching abilities, superior to that of CAPE. Compounds **6j–k** and **7j–k** demonstrated remarkable protection effects against damage induced by hydrogen peroxide which were apparently stronger than that of CAPE. Most of target compounds could inhibit nitric oxide production. Additionally, target compounds showed high blood–brain barrier permeability.

1. Introduction

Neurodegenerative disorders are a heterogeneous group of diseases of the nervous system, characterized by loss of nerve cells from brain and spinal cord, which leads either functional loss (ataxia) or sensory dysfunction (dementia).[1] Oxidative stress and inflammation have been linked to neuronal cell death and believed to be fundamental mechanisms of progressive neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's disease as well as amyotrophic lateral sclerosis.[2,3]

Compound **1** (Caffeic acid phenethyl ester, CAPE) (Fig. 1) derived from the propolis of honeybee hives has long been known to possess a wide spectrum of biological activities including antioxidant,[4] anti-inflammatory,[5] antiviral,[6] antibacterial,[7] anti-atherosclerotic,[8] immunostimulatory[9] and antitumor[10] properties. Compound **1** can significantly exhibit the antioxidant property by means of blocking production of reactive oxygen species and the xanthine/xanthine oxidase system,[11] and the anti-inflammatory property through reducing prostaglandin (PG) and leukotriene synthesis by inhibiting cyclooxygenase (COX) enzyme activity or the down-regulation of COX gene expression.[12,13] The antioxidant and anti-inflammatory properties of **1** is recognized to be beneficial for treatment of neurodegenerative disorders. Numerous recent studies have demonstrated effects of **1** against a variety of toxicities in different types of neuronal cells, including hydrogen peroxide (H_2O_2),[14] 6-hydroxydopamine[14] and glutamate.[15] It is also reported that **1** can block hypoxia–ischemic brain injury[16,17] and MPTP-induced dopaminergic neurodegeneration[18] in animal models. From the previous studies, it is confirmed that **1** is a promising neuroprotective agent with multiple targets effects.

Although many preclinical studies have demonstrated primary safety and biological activities of **1** in animal and in vitro models, pharmacokinetic studies demonstrate that **1** is easily hydrolyzed by plasmatic esterases in rats after the rapid oral absorption.[19,20] In addition, with two phenolic hydroxyl functions **1** does not have a good solubility in lipophilic environment and is difficult to pass through blood-brain barrier (BBB). The aim of this study, therefore, is to design and synthesize its analogs with better stability and higher BBB permeability, and to discuss structure–activity relationships of target compounds.

From the previous reported data, the structural feature responsible for the antioxidant activity of **1** was the *ortho*-dihydroxyl function in the catechol ring, and the unsaturated double bond of the side chain also maximized the stabilization of the phenolic radical.[21,22] Therefore, *E*-3,4-dihydroxy styryl sulfonamides reserving

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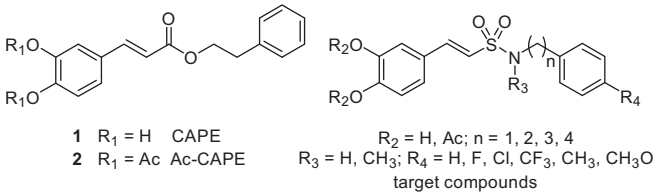


Figure 1. The Structures of **1**, **2** and target compounds.

(*E*)-3,4-dihydroxy styryl group were designed (Fig. 1). The sulfonamide group introduced to target compounds was more stability than the ester group of compound **1**. Meanwhile, the unsaturated sulfonamide function consists of skeleton structures of many promising neuroprotective agents including branched-chain amino acid aminotransferase inhibitors,[23] caspase-3 inhibitors[24] and NMDA receptor antagonists.[25] In order to improve BBB permeability, two phenolic hydroxyl functions of target compounds were acetylated rather than methylated to get corresponding high liposoluble compounds, because the methylation of phenolic hydroxyl functions may lead to the moderate loss of biological activity.[26] It is also reported that the acetylated phenolic compounds display the same or higher neuroprotective activity compared with the initial phenolic compounds.[27] To explore the structure–activity relationships, the compounds with various lengths of alky chains and with various substituted groups on the aromatic ring were designed too.

2. Results and discussion

2.1. Chemistry

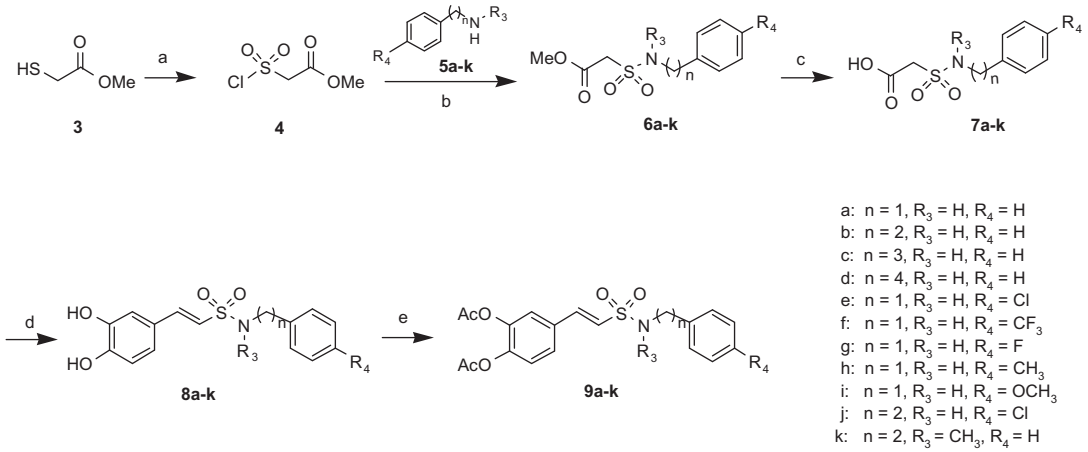
E-3,4-Dihydroxy styryl sulfonamides (**8a**–**k**) and their 3,4-diacetylated derivatives (**9a**–**k**) were synthesized as shown in Scheme 1. Commercially available mercaptoacetic acid methyl ester (**3**) served as starting material was oxidized at 0 °C with Cl_2 in the mixed solvent of CH_2Cl_2 and H_2O followed by pumping N_2 to expel Cl_2 to provide chlorosulfonyl-acetic acid methyl ester (**4**). The reaction of aromatic amines (**5a**–**k**) with chlorosulfonyl-acetic acid methyl ester (**4**) in THF in the presence of triethylamine produced the corresponding aromatic sulfamoyl-acetic acid methyl ester (**6a**–**k**) after chromatography in good yields (86–98%). These intermediates (**6a**–**k**) were hydrolyzed at room temperature with Na_2CO_3 as the catalyst followed by recrystallization purification to afford the aromatic sulfamoyl-acetic acids (**7a**–**k**). Then Knoevenagel condensation of the aromatic sulfamoyl-acetic acids (**7a**–**k**) with 3,4-dihydroxy benzaldehyde using the pyrrolidine and acetic acid as the catalysts in the refluxed THF followed by chromatographic purification obtained *E*-3,4-dihydroxy styryl sulfonamides (**8a**–**k**) in desired yields. Furthermore, to improve BBB permeability, *E*-3,4-dihydroxy styryl sulfonamides (**8a**–**k**) were acetylated by acetic anhydride with pyridine as the catalyst to afford the corresponding *E*-3,4-diacetyl styryl sulfonamides (**9a**–**k**) in the yields of 92–96%. The *E* geometry of the target compounds was confirmed by the coupling constants (J = 16 Hz).

2.2. Biological evaluation

The neuroprotective properties of *E*-3,4-dihydroxy styryl sulfonamides (**8a**–**k**) and their 3,4-diacetylated derivatives (**9a**–**k**) were assessed by way of several experimental pharmacological models in vitro, including 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity, neuronal protecting effect against damage induced by H_2O_2, nitric oxide production inhibiting effect and a parallel artificial membrane permeation assay for blood–brain barrier (PAMPA-BBB). The results showed that target compounds exhibited significant neuroprotective activities and higher BBB permeability compared with **1** and **2**, and the preliminary structure–activity relationship was also discussed.

2.2.1. Free radical scavenging capacity

DPPH, a stable free radical existing in vitro, can be used in preliminary screening of compounds capable of scavenging reactive free radical. The free radical scavenging capacities of the (*E*)-3,4-dihydroxy styryl sulfonamides (**8a**–**k**) were measured by the published test method[28,29] over the concentration range of 1–50 μM. The results are shown in Table 1. The compounds **1** and **8a**–**k** exhibit significant free radicals quenching abilities, comparable to that of the positive control Vitamin C. The reason of the result above is presumably that the conjugated system consisting of catechol group, double bond and sulfonamide group in compound 1 and **8a**–**k** can offer a higher electron delocalization than Vitamin C, and compounds **1** and **8a**–**k** as better hydrogen donors are easier to scavenge free radicals. It is observed that all target compounds show the similar or stronger free radical scavenging capacities than **1**. It is also noteworthy that the electron-withdrawing chloro, trifluoromethyl and fluoro substituted compounds (**8e**–**g**) exhibit more effective activities than electron-donating methyl and methoxyl substituted compounds



Scheme 1. Synthesis of *E*-3,4-dihydroxy styryl sulfonamides and their 3,4-diacetylated derivatives. Reagents and conditions: (a) Cl_2, H_2O, CH_2Cl_2, 0 °C, 92%; (b) $(CH_3CH_2)_3N$, THF, rt, 86–98%; (c) Na_2CO_3, MeOH, rt, 82–90%; (d) 3,4-dihydroxy-benzaldehyde, pyrrolidine, acetic acid, THF, reflux, 50–75%; (e) pyridine, acetic anhydride, rt, 92–96%.

Table 1
Free radical scavenging capacities of **1**, target compounds **8a–k** by the DPPH method[a]

Compound	IC_{50}[b] (μM)	Compound	IC_{50}[b] (μM)
8a	10.1 ± 0.4	**8h**	10.7 ± 0.5
8b	9.1 ± 0.3	**8i**	11.2 ± 0.2
8c	7.0 ± 0.6	**8j**	8.3 ± 0.4
8d	8.3 ± 0.2	**8k**	6.8 ± 0.5
8e	7.8 ± 0.4	**1**	12.1 ± 0.3
8f	7.2 ± 0.4	Vitamin C	25.7 ± 0.5
8g	8.3 ± 0.3		

[a] Data are expressed as the mean ± SD, $n = 3$.
[b] IC_{50}: the concentration that produces 50% inhibitory effect.

(**8h–i**). Compound **8k** ($IC_{50} = 6.8 \pm 0.5$ μM) exhibits prominent radical scavenging activity among the compounds, and its activity is superior to **1** and **8b** without *N*-substituent. The possible reason is that *N*-substituent compounds may possess higher free radical scavenging capacities, due to their tertiary amine groups liable to oxidation. Moreover, the compounds **2** and **9a–k** do not show detectable scavenging capacities under the concentration of 50 μM, which implies that two phenolic hydroxyl groups of target compounds play a significant role in free radical scavenging capacity.

2.2.2. Neuronal protecting effect against damage induced by H_2O_2

The neuroprotective capacities of target compounds against oxidative stress were assayed by the H_2O_2 model. H_2O_2 could injure nerve cells for the generation of exogenous free radicals. Rat pheochromocytoma (PC12) cells, commonly as a screening model for studies of neurodegenerative diseases,[30] were used in this experiment. The protection effect against H_2O_2 can be conveniently evaluated by the cell viability via MTT assay. The cell viabilities attributable to the protective efficiency of target compounds (**8a–k** and **9a–k**) against H_2O_2 at 5 μM are listed in Figure 2. All target compounds significantly exhibit protection effects against damage induced by H_2O_2 and compounds **8e–g**, **8j–k**, **9e–g** and **9j–k** display higher activities than **1**. It seems that chloro, trifluoromethyl and fluoro substituted compounds (**8e–g** and **9e–g**) exhibit more effective activities compared with unsubstituted (**8a** and **9a**) and methyl and methoxyl substituted compounds (**8h–i** and **9h–i**). And **8k** and **9k** with *N*-substituent display more potent than **8b** and **9b** without *N*-substituent. The results are similar to those of DPPH assay. In addition, compounds possessing longer alkyl chain (**8d** and **9d**) show weaker activities compared with shorter alkyl chain compounds (**8a–c** and **9a–c**). It is also observed that introducing two acetyl groups of target compounds **9a–k** leads to negligible improvement in protective effects against H_2O_2 induced injury compared with the corresponding un-acetylated compounds **8a–k**.

2.2.3. Nitric oxide production inhibiting effect in LPS-stimulated BV2 microglial cells

The microglial cells are believed to play an important role in the pathway that leads to inflammation-mediated neuronal cell death in a number of neurodegenerative diseases.[31] The uncontrolled activation of microglia cells may cause neuronal damage through the overproduction of proinflammatory substances, such as nitric oxide.[31,32] Therefore, inhibiting nitric oxide production is a useful strategy for treating neurodegenerative disorders. The inhibition effect on nitric oxide production of target compounds (**8a–k** and **9a–k**) can be measured by the Griess assay.[33] The results are summarized in Table 2. From the results, most of the target compounds inhibit nitric oxide production in LPS-stimulated BV2 microglial cells, although the activities of target compounds do not display highlighted activities compared with **1**. Almost all of acetylated compounds (**9a–k**) reveal the higher nitric oxide production inhibition effects compared with corresponding un-acetylated compounds (**8a–k**). The results above are presumably because that acetylated compounds possess higher lipophilicity, compared with un-acetylated compounds. Furthermore, it seems that electron-withdrawing groups substituted compounds (**8e–g** and **9e–g**) exhibit more effective activities compared with electron-donating groups substituted compounds (**8h–i** and **9h–i**), which is similar to the results of DPPH assay and H_2O_2 model. In addition, the selected concentrations of target compounds used in our experiment do not lead to any significant cytotoxicity even at a high concentration (80 μM) up to 24 h of incubation, and in all cases cell viability is above 90% by MTT assay, confirming that inhibition of nitric oxide production in LPS-stimulated BV2 microglial cells is not due to a cytotoxic action of target compounds.

2.2.4. In vitro evaluation of BBB permeability

Effectiveness of target compounds against neurodegenerative diseases greatly depends upon their BBB penetration capacities. To explore whether target compounds would be able to penetrate into BBB, a high throughput technique, Parallel Artificial Membrane Permeability Assay (PAMPA) was used. This simple and rapid model was described by Di et al.[34] and successfully applied by us to different compounds. The in vitro permeability (P_e) of target compounds (**8a–k** and **9a–k**) and control drugs (Verapamil, Hydrocortisone and Clonidine) through the porcine polar brain lipid (PBL) were determined using PBS as the solvent according to the method

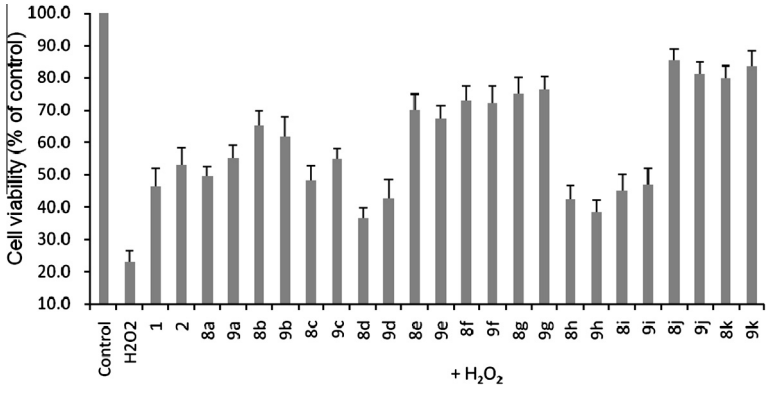


Figure 2. Protective effects of **1**, **2** and target compounds **8a–9k** against H_2O_2 induced injury in PC12 cells at 5 μM, respectively. PC12 cells were pretreated for 3 h. After 3 h, the cells were treated with 500 μM H_2O_2 for 5 h. Levels of cell viability were measured by the MTT assay. The viability of untreated control cells was defined as 100%. Data are expressed as the mean ± SD, $n = 3$.

Table 2
Nitric oxide suppression activities of **1**, **2** and target compounds **8a–9k** in LPS-stimulated BV2 microglial cells[a]

Compound	IC_{50}[b] (μM)	Compound	IC_{50}[b] (μM)
8a	21.5 ± 0.3	**9a**	19.5 ± 0.4
8b	39.2 ± 0.5	**9b**	30.8 ± 0.6
8c	17.5 ± 0.4	**9c**	15.6 ± 0.2
8d	23.8 ± 0.3	**9d**	20.8 ± 0.4
8e	13.7 ± 0.2	**9e**	11.0 ± 0.2
8f	12.6 ± 0.5	**9f**	9.3 ± 0.2
8g	18.9 ± 0.6	**9g**	15.5 ± 0.3
8h	43.9 ± 0.5	**9h**	32.0 ± 0.5
8i	26.5 ± 0.3	**9i**	26.3 ± 0.4
8j	9.8 ± 0.5	**9j**	8.6 ± 0.3
8k	17.0 ± 0.2	**9k**	10.2 ± 0.2
1	6.4 ± 0.4	**2**	6.2 ± 0.2

[a] Data are expressed as the mean ± SD, $n = 3$.
[b] IC_{50}: the concentration that produces 50% inhibitory effect.

Table 3
Permeability results of **1**, **2**, target compounds and control drugs from the PAMPA[a]

Compound	P_e (10^{-6} cm s^{-1})	Prediction	Compound	P_e (10^{-6} cm s^{-1})	Prediction
8a	12.2 ± 0.6	CNS+	**9a**	15.1 ± 0.5	CNS+
8b	8.2 ± 0.3	CNS+	**9b**	10.1 ± 0.3	CNS+
8c	5.7 ± 0.4	CNS+	**9c**	Nd[b]	
8d	3.8 ± 0.3	CNS+/−	**9d**	Nd[b]	
8e	2.5 ± 0.2	CNS+/−	**9e**	5.1 ± 0.2	CNS+
8f	2.8 ± 0.5	CNS+/−	**9f**	Nd[b]	
8g	6.0 ± 0.4	CNS+	**9g**	7.5 ± 0.3	CNS+
8h	6.7 ± 0.4	CNS+	**9h**	8.6 ± 0.5	CNS+
8i	4.7 ± 0.6	CNS+	**9i**	11.6 ± 0.3	CNS+
8j	3.4 ± 0.3	CNS+/−	**9j**	Nd[b]	
8k	4.8 ± 0.5	CNS+	**9k**	Nd[b]	
Hydrocortisone	1.8 ± 0.2	CNS−	**1**	1.8 ± 0.3	CNS−
Verapamil	16.9 ± 0.4	CNS+	**2**	Nd[b]	
Clonidine	5.2 ± 0.5	CNS+			

[a] Data are expressed as the mean ± SD, $n = 3$. PBS was used as solvent.
[b] Not determined due to poor solubility in PBS.

described by Di et al.[34] The results are summarized in Table 3. From the results, the P_e values of control drugs are consistent with the literature of Di et al. In this literature, compounds are classified according to following ranges for BBB permeation prediction: (a) 'CNS+' (high BBB permeation predicted), P_e (10^{-6} cm s^{-1}) > 4.0; (b) 'CNS−' (low BBB permeation predicted), P_e (10^{-6} cm s^{-1}) < 2.0; (c) 'CNS+/−' (BBB permeation uncertain), P_e (10^{-6} cm s^{-1}) from 4.0 to 2.0. Based on results and ranges above, we can consider that majority of target compounds are able to cross the BBB and only **8d**, **8e**, **8f** and **8j** exhibit weaker permeability. Compared to **1** ($1.8 \pm 0.3 \times 10^{-6}$ cm s^{-1}), all target compounds possess higher permeability. Especially, compound **8b** displays greater permeability than **1**, which demonstrates that the sulfonamide group instead of carboxylic ester group improves BBB permeability. Some acetylated compounds are not determined due to poor solubility in PBS. However, it can be still concluded that acetylated compounds possess greater permeability than corresponding un-acetylated compounds which are consistent with our prior design.

3. Conclusions

In summary, this study discloses that *E*-3,4-dihydroxy styryl sulfonamides and their 3,4-diacetylated derivatives demonstrate multifunctional neuroprotective activities against oxidative and inflammatory injury such as remarkable potency of scavenging reactive free radical as well as excellent protection effects against damage induced by H_2O_2, which are more potent than **1**. In addition, they display obvious inhibiting nitric oxide production effects, although their activities are lower than **1** and **2**. The effects of electron-donating versus electron-withdrawing group substituents on the aromatic ring as well as short versus long alkyl chain on the tested pharmacological activities are also discussed. The electron-withdrawing group substituted compounds exhibit more effective activities compared with unsubstituted and electron-donating group substituted compounds in all test models. The activities of compounds possessing various lengths of alky chains ($n = 1–4$) have no obvious regularity in the DPPH assay and NO model, however, compounds possessing longer alkyl chain, **8d** and **9d**, show weaker protection effects compared with shorter alkyl chain compounds in H_2O_2 model. Additionally, acetylated compounds reveal higher inhibiting effects on nitric oxide production and similar protection effects against damage induced by H_2O_2 compared to corresponding un-acetylated compounds. According to the PAMPA-BBB, both the acetoxyl group instead of the phenolic hydroxyl group and the sulfonamide group instead of carboxylic ester group improve BBB permeability which is consistent with our prior design. Besides, the superior activities of compounds **8k** and **9k** when compared to compounds **8b** and **9b** suggest that *N*-substituent compounds may possess higher antioxidant properties, because their tertiary amine groups are liable to oxidation. The *N*-substituent compounds of *E*-3,4-dihydroxy styryl sulfonamides should be emphasized in future studies. Above all, the findings reported in this study provide insights in future drug design and development for the treatment of neurodegenerative disorders.

4. Experimental

4.1. Chemistry

The structural characterization was done by NMR and high resonance mass spectrometer (HRMS). NMR spectra were recorded on a Bruker AVANCE III-400 spectrometer with tetramethylsilane (TMS) as an internal standard, and chemical shifts are reported in δ (ppm). High resolution mass spectrum (HRMS) was recorded on a Bruker Apex IV FTMS spectrometer. Melting points were measured on an X_4-type apparatus and left uncorrected. Thin-layer chromatography (TLC) was performed on precoated silica gel F_{254} plates. Detection was by iodine vapor staining and UV light irradiation (UV lamp, model UV-IIB). Column chromatography was carried out with Silica gel H (200–300 mesh or 500 mesh). Unless otherwise stated, all reagents were purchased from commercial sources. When necessary, they were purified and dried by standard methods. Organic solutions were dried over anhydrous sodium sulfate.

4.1.1. Synthesis of chlorosulfonyl-acetic acid methyl ester (4)

A solution of mercaptoacetic acid methyl ester (35 mmol) in CH_2Cl_2 (15 mL) was added dropwise to the ice water (10 mL) and Cl_2 was pumped in the solution. The solution was stirred at 0 °C until maintaining greenish, followed by pumping N_2 to expel Cl_2. After standing, the solution was separated. The organic fraction was concentrated under reduced pressure to obtain the title compound. Yellow liquid (92%). ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 3.75$ (s, 2H, SO_2CH_2), 3.68 (s, 3H, $COOCH_3$).

4.1.2. General procedure for the synthesis of aromatic sulfamoyl-acetic acid methyl ester (6a–k)

The triethylamine (1.2 mmol) was added to the solution of aromatic amines **5a–k** (1 mmol) and chlorosulfonyl-acetic acid methyl ester **4** (1 mmol) in THF (15 mL) and stirred at RT until absence of the aromatic amines (checked by TLC). The reaction mixture was concentrated in vacuo and diluted with H_2O_2, followed by

neutralization with 5% HCl to pH 7 and extracting with ethyl acetate. The combined organic fractions were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification of the crude residue by column chromatography (petroleum ether/ethyl acetate) afforded the title compound.

4.1.2.1. Benzylsulfamoyl-acetic acid methyl ester (6a). White solid (93%), mp 50–51 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.33–7.40 (m, 5H, Ar*H*), 5.17 (s, 1H, N*H*), 4.38 (s, 1H, $SO_2CH_2COOCH_3$), 4.37 (s, 1H, $SO_2CH_2COOCH_3$), 3.91 (s, 2H, $ArCH_2$), 3.80 (s, 3H, $COOCH_3$).

4.1.2.2. Phenethylsulfamoyl-acetic acid methyl ester (6b). White solid (90%), mp 63–64 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.23–7.35 (m, 5H, Ar*H*), 4.75 (s, 1H, N*H*), 3.92 (s, 2H, $SO_2CH_2COOCH_3$), 3.77 (s, 3H, $SO_2CH_2COOCH_3$), 3.47 (q, 2H, $ArCH_2CH_2SO_2$), 2.93 (t, 2H, $ArCH_2CH_2SO_2$). MS (ESI) *m*/*z*: 256.2142 [M−H]$^-$.

4.1.2.3. (3-Phenyl-propylsulfamoyl)-acetic acid methyl ester (6c). Yellow liquid (88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.20–7.33 (m, 5H, Ar*H*), 5.01 (s, 1H, N*H*), 4.01 (s, 2H, $SO_2CH_2COOCH_3$), 3.81 (s, 3H, $SO_2CH_2COOCH_3$), 3.19 (q, 2H, $ArCH_2CH_2CH_2SO_2$), 2.72 (t, 2H, $ArCH_2CH_2CH_2SO_2$), 1.94 (m, 2H, $ArCH_2CH_2CH_2SO_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 169.50, 145.79, 133.54, 133.39, 131.18, 59.97, 58.12, 48.16, 37.67, 36.53. MS (ESI) *m*/*z*: 270.2258 [M−H]$^-$.

4.1.2.4. (4-Phenyl-butylsulfamoyl)-acetic acid methyl ester (6d). Colorless liquid (86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.18–7.32 (m, 5H, Ar*H*), 4.93 (s, 1H, N*H*), 4.00 (s, 2H, $SO_2CH_2COOCH_3$), 3.80 (s, 3H, $SO_2CH_2COOCH_3$), 3.18 (q, 2H, $ArCH_2CH_2CH_2CH_2SO_2$), 2.66 (m, 2H, $ArCH_2CH_2CH_2CH_2SO_2$), 1.72 (m, 2H, $ArCH_2CH_2CH_2CH_2SO_2$), 1.64 (m, 2H, $ArCH_2CH_2CH_2CH_2SO_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 169.51, 146.77, 133.39, 130.92, 59.95, 58.09, 48.57, 40.27, 34.43, 33.18. MS (ESI) *m*/*z*: 284.2364 [M−H]$^-$.

4.1.2.5. (4-Chloro-benzylsulfamoyl)-acetic acid methyl ester (6e). White solid (95%), mp 85–86 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.32–7.37 (m, 4H, Ar*H*), 5.26 (s, 1H, N*H*), 4.34 (s, 1H, $SO_2CH_2COOCH_3$), 4.33 (s, 1H, $SO_2CH_2COOCH_3$), 3.95 (s, 2H, $ArCH_2$), 3.80 (s, 3H, $COOCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 164.53, 134.66, 134.16, 129.44, 129.07, 55.54, 53.21, 47.01. MS (ESI) *m*/*z*: 276.1536 [M−H]$^-$.

4.1.2.6. (4-Trifluoromethyl-benzylsulfamoyl)-acetic acid methyl ester (6f). White solid (95%), mp 108–109 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.50–7.65 (m, 4H, Ar*H*), 5.52 (s, 1H, N*H*), 4.43 (s, 1H, $SO_2CH_2COOCH_3$), 4.41 (s, 1H, $SO_2CH_2COOCH_3$), 3.99 (s, 2H, $ArCH_2$), 3.78 (s, 3H, $COOCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 164.45, 140.47, 128.20, 125.84, 125.81, 125.77, 55.74, 53.18, 46.99. MS (ESI) *m*/*z*: 310.1673 [M−H]$^-$.

4.1.2.7. (4-Fluoro-benzylsulfamoyl)-acetic acid methyl ester (6g). White solid (98%), mp 74–75 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.00–7.35 (m, 4H, Ar*H*), 5.65 (s, 1H, N*H*), 4.30 (s, 1H, $SO_2CH_2COOCH_3$), 4.29 (s, 1H, $SO_2CH_2COOCH_3$), 3.92 (s, 2H, $ArCH_2$), 3.74(s, 3H, $COOCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 169.45, 137.28, 137.26, 134.91, 134.83, 120.79, 120.57, 60.78, 58.11, 51.81. MS (ESI) *m*/*z*: 260.1888 [M−H]$^-$.

4.1.2.8. (4-Methyl-benzylsulfamoyl)-acetic acid methyl ester (6h). White solid (96%), mp 95–96 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.18–7.28 (m, 4H, Ar*H*), 5.22 (s, 1H, N*H*), 4.33 (s, 1H, $SO_2CH_2COOCH_3$), 4.31 (s, 1H, $SO_2CH_2COOCH_3$), 3.90 (s, 2H, $ArCH_2$), 3.79 (s, 3H, $COOCH_3$), 2.37 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 164.54, 138.07, 132.99, 129.56, 128.13, 55.49, 53.08, 47.54, 21.14. MS (ESI) *m*/*z*: 256.2158 [M−H]$^-$.

4.1.2.9. (4-Methoxy-benzylsulfamoyl)-acetic acid methyl ester (6i). White solid (93%), mp 82–83 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.89–7.31 (m, 4H, Ar*H*), 5.19 (s, 1H, N*H*), 4.31 (s, 1H, $SO_2CH_2COOCH_3$), 4.29 (s, 1H, $SO_2CH_2COOCH_3$), 3.89 (s, 2H, $ArCH_2$), 3.83 (s, 3H, $COOCH_3$), 3.79 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 164.56, 159.57, 129.58, 128.01, 114.26, 55.48, 55.33, 53.08, 47.30. MS (ESI) *m*/*z*: 272.2048 [M−H]$^-$.

4.1.2.10. [2-(4-Chloro-phenyl)-ethylsulfamoyl]-acetic acid methyl ester (6j). White solid (98%), mp 76–77 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.17–7.33 (m, 4H, Ar*H*), 4.82 (s, 1H, N*H*), 3.96 (s, 2H, $SO_2CH_2COOCH_3$), 3.79 (s, 3H, $SO_2CH_2COOCH_3$), 3.43 (q, 2H, $ArCH_2CH_2SO_2$), 2.90 (t, 2H, $ArCH_2CH_2SO_2$). MS (ESI) *m*/*z*: 290.1635 [M−H]$^-$.

4.1.2.11. (Methyl-phenethyl-sulfamoyl)-acetic acid methyl ester (6k). Colorless liquid (96%), mp 134–135°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.24–7.33 (m, 5H, Ar*H*), 3.83 (s, 2H, $SO_2CH_2COOCH_3$), 3.79 (s, 3H, $COOCH_3$), 3.49 (t, 2H, $ArCH_2CH_2SO_2$), 2.95 (s, 3H, NCH_3), 2.92 (t, 2H, $ArCH_2CH_2SO_2$).

4.1.3. General procedure for the synthesis of aromatic sulfamoyl-acetic acids (7a–k)

A solution of Na_2CO_3 (1 mmol) in MeOH (5 mL) was added dropwise to the solution of aromatic sulfamoyl-acetic acid methyl ester **6a–k** (1 mmol) in MeOH (10 mL) and the solution was stirred at RT until absence of the aromatic sulfamoyl-acetic acid methyl ester (checked by TLC). The reaction mixture was concentrated in vacuo and diluted with H_2O, followed by neutralization with 5% HCl to pH 7 and extracting with ethyl acetate. The combined organic fractions were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. The crude residue is purified by recrystallizing from petroleum ether and ethyl acetate to obtain the title compound.

4.1.3.1. Benzylsulfamoyl-acetic acid (7a). White solid (85%), mp 155–156 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.16 (s, 1H, COO*H*), 7.89 (s, 1H, N*H*), 7.26–7.35 (m, 5H, Ar*H*), 4.19 (s, 2H, SO_2CH_2 COOH), 4.04 (s, 2H, $ArCH_2$).

4.1.3.2. Phenethylsulfamoyl-acetic acid (7b). White solid (82%), mp 135–136 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.09 (s, 1H, COO*H*), 7.42 (s, 1H, N*H*), 7.21–7.32 (m, 5H, Ar*H*), 4.03 (s, 2H, SO_2CH_2COOH), 3.21 (q, 2H, $ArCH_2CH_2SO_2$), 2.78 (t, 2H, $ArCH_2CH_2SO_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 165.43, 139.29, 129.18, 128.80, 126.71, 55.91, 44.66, 36.27.

4.1.3.3. (3-Phenyl-propylsulfamoyl)-acetic acid (7c). White solid (82%), mp 125–126 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.07 (s, 1H, COO*H*), 7.40 (s, 1H, N*H*), 7.16–7.30 (m, 5H, Ar*H*), 4.04 (s, 2H, SO_2CH_2COOH), 2.99 (m, 2H, $ArCH_2CH_2CH_2SO_2$), 2.61 (q, 2H, $ArCH_2CH_2CH_2SO_2$), 1.75 (m, 2H, $ArCH_2CH_2CH_2SO_2$).

4.1.3.4. (4-Phenyl-butylsulfamoyl)-acetic acid (7d). White solid (87%), mp 117–118 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.13 (s, 1H, COO*H*), 7.17–7.29 (m, 6H, Ar*H*, N*H*), 4.02 (s, 2H, SO_2CH_2 COOH), 2.99 (q, 2H, $ArCH_2CH_2CH_2CH_2SO_2$), 2.60 (m, 2H, $ArCH_2CH_2CH_2CH_2SO_2$), 1.59 (m, 2H, $ArCH_2CH_2CH_2CH_2SO_2$), 1.47 (m, 2H, $ArCH_2CH_2CH_2CH_2SO_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 170.45, 147.54, 133.75, 133.70, 131.12, 61.78, 47.89, 40.11, 36.16, 34.54, 33.50. MS (ESI) *m*/*z*: 272.1094 [M+H]$^+$.

4.1.3.5. (4-Chloro-benzylsulfamoyl)-acetic acid (7e). White solid (83%), mp 153–154 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.19 (s, 1H, COO*H*), 7.97 (s, 1H, N*H*), 7.35–7.43 (m, 4H, Ar*H*), 4.20 (s, 1H, SO_2CH_2COOH), 4.18 (s, 1H, SO_2CH_2COOH), 4.08 (s, 2H, $ArCH_2$).

4.1.3.6. (4-Trifluoromethyl-benzylsulfamoyl)-acetic acid (7f). White solid (85%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.22 (s, 1H, COO*H*), 8.07 (s, 1H, N*H*), 7.58–7.74 (m, 4H, Ar*H*), 4.32 (s, 2H, SO_2CH_2COOH), 4.15 (s, 2H, $ArCH_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 165.39, 143.83, 128.62, 128.43, 128.11, 126.12, 125.67, 125.63, 57.26, 46.06.

4.1.3.7. (4-Fluoro-benzylsulfamoyl)-acetic acid (7g). White solid (83%), mp 171–172 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.17 (s, 1H, COO*H*), 7.92 (s, 1H, N*H*), 7.15–7.40 (m, 4H, Ar*H*), 4.19 (s, 1H, SO_2CH_2COOH), 4.18 (s, 1H, SO_2CH_2COOH), 4.06 (s, 2H, $ArCH_2$).

4.1.3.8. (4-Methyl-benzylsulfamoyl)-acetic acid (7h). White solid (86%), mp 157–158 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.14 (s, 1H, COO*H*), 7.84 (s, 1H, N*H*), 7.14–7.24 (m, 4H, Ar*H*), 4.15 (s, 1H, SO_2CH_2COOH), 4.14 (s, 1H, SO_2CH_2COOH), 4.02 (s, 2H, $ArCH_2$), 2.29(s, 3H, CH_3).

4.1.3.9. (4-Methoxy-benzylsulfamoyl)-acetic acid (7i). White solid (86%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.14 (s, 1H, COO*H*), 7.84 (s, 1H, N*H*), 7.14–7.24 (m, 4H, Ar*H*), 4.15 (s, 1H, SO_2CH_2COOH), 4.14 (s, 1H, SO_2CH_2COOH), 4.02 (s, 2H, $ArCH_2$), 2.29 (s, 3H, CH_3).

4.1.3.10. [2-(4-Chloro-phenyl)-ethylsulfamoyl]-acetic acid (7j). White solid (88%), mp 153–154 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.14 (s, 1H, COO*H*), 7.45 (s, 1H, N*H*), 7.26–7.37 (m, 4H, Ar*H*), 4.05 (s, 2H, SO_2CH_2COOH), 3.20 (q, 2H, $ArCH_2CH_2SO_2$), 2.77 (t, 2H, $ArCH_2CH_2SO_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 165.49, 138.35, 131.35, 131.16, 128.70, 56.78, 44.36, 35.39.

4.1.3.11. (Methyl-phenethyl-sulfamoyl)-acetic acid (7k). White solid (90%), mp 46–47 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 13.23 (s, 1H, COO*H*), 7.21–7.33 (m, 5H, Ar*H*), 4.09 (s, 2H, SO_2CH_2COOH), 3.35 (t, 2H, $ArCH_2CH_2SO_2$), 2.84 (t, 2H, $ArCH_2CH_2SO_2$), 2.09 (s, 3H, NCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 165.34, 139.02, 129.26, 128.86, 126.80, 54.40, 51.84, 35.24, 34.62, 31.17.

4.1.4. General procedure for the synthesis of *E*-3,4-dihydroxy styryl sulfonamides (8a–k)

The 3,4-dihydroxy benzaldehyde (2.2 mmol), the pyrrolidine (catalytic amount) and acetic acid (catalytic amount) were added to a solution of the aromatic sulfamoyl-acetic acid **7a**–**k** (2 mmol) solution in THF (15 mL) and heated to reflux until no longer reducing of the 3,4-dihydroxy benzaldehyde (checked by TLC). The reaction mixture was concentrated in vacuo, diluted with H_2O and extracted with ethyl acetate. The combined organic fractions were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification of the crude residue by column chromatography (petroleum ether/ethyl acetate) afforded the title compound.

4.1.4.1. 2-(3,4-Dihydroxy-phenyl)-ethenesulfonic acid benzylamide (8a). White solid (50%), mp 136–137 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.59 (s, 1H, *p*-ArO*H*), 9.13 (s, 1H, *m*-ArO*H*), 7.70 (t, 1H, N*H*), 6.71–7.69 (m, 10H, Ar*H*, Ar*CH*=*CH*SO_2), 4.09 (s, 1H, $ArCH_2SO_2$), 4.07 (s, 1H, $ArCH_2SO_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 148.65, 146.04, 140.19, 138.73, 128.11, 127.56, 124.62, 123.44, 121.64, 116.17, 115.40, 46.39. HRMS ESI$^+$ *m*/*z* 306.07946 [M+H]$^+$. Found: 306.07922 [M+H]$^+$.

4.1.4.2. 2-(3,4-Dihydroxy-phenyl)-ethenesulfonic acid phenethyl-amide (8b). White solid (60%), mp 158–159 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.61 (s, 1H, *p*-ArO*H*), 9.13 (s, 1H, *m*-ArO*H*), 6.71–7.30 (m, 11H, Ar*H*, Ar*CH*=*CH*SO_2, N*H*), 3.07 (q, 2H, $ArCH_2CH_2SO_2$), 2.76 (t, 2H, $ArCH_2CH_2SO_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 148.64, 146.04, 140.26, 139.39, 129.17, 128.79, 126.69, 124.60, 123.10, 121.71, 116.14, 115.43, 44.37, 36.07. HRMS ESI$^+$ *m*/*z* 320.09511 [M+H]$^+$. Found: 320.09518 [M+H]$^+$.

4.1.4.3. 2-(3,4-Dihydroxy-phenyl)-ethenesulfonic acid (3-phenyl-propyl)-amide (8c). White solid (59%), mp 126–127 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.57 (s, 1H, *p*-ArO*H*), 9.18 (s, 1H, *m*-ArO*H*), 6.94–7.26 (m, 10H, Ar*H*, Ar*CH*=*CH*SO_2), 4.55 (s, 1H, N*H*), 3.35 (m, 2H, $ArCH_2CH_2CH_2SO_2$), 2.86 (m, 2H, $ArCH_2CH_2CH_2SO_2$), 2.60 (m, 2H, $ArCH_2CH_2CH_2SO_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 148.65, 146.07, 141.97, 140.25, 128.77, 128.73, 126.21, 124.62, 123.22, 121.67, 116.19, 115.41, 68.98, 56.29, 42.30, 32.66, 32.56, 31.60, 30.07. HRMS ESI$^+$ *m*/*z* 334.11076 [M+H]$^+$. Found: 334.11075 [M+H]$^+$.

4.1.4.4. 2-(3,4-Dihydroxy-phenyl)-ethenesulfonic acid (4-phenyl-butyl)-amide (8d). White solid (68%), mp 129–130 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.59 (s, 1H, *p*-ArO*H*), 9.13 (s, 1H, *m*-ArO*H*), 6.73–7.27 (m, 10H, Ar*H*, Ar*CH*=*CH*SO_2), 2.86 (q, 2H, $ArCH_2CH_2CH_2CH_2SO_2$), 2.54 (m, 2H, $ArCH_2CH_2CH_2CH_2SO_2$), 1.58 (m, 2H, $ArCH_2CH_2CH_2CH_2SO_2$), 1.46 (m, 2H, $ArCH_2CH_2CH_2CH_2SO_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 148.61, 146.06, 142.47, 140.06, 128.73, 128.67, 126.11, 124.64, 123.32, 121.62, 116.18, 115.40, 42.58, 35.13, 29.44, 28.54. HRMS ESI$^+$ *m*/*z* 348.12641 [M+H]$^+$. Found: 348.12619 [M+H]$^+$.

4.1.4.5. 2-(3,4-Dihydroxy-phenyl)-ethenesulfonic acid 4-chlorobenzylamide (8e). White solid (61%), mp 154–155 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.61 (s, 1H, *p*-ArO*H*), 9.13 (s, 1H, *m*-ArO*H*), 7.75 (t, 1H, N*H*), 6.73–7.40 (m, 9H, Ar*H*, Ar*CH*=*CH*SO_2), 4.08 (s, 1H, $ArCH_2SO_2$), 4.06 (s, 1H, $ArCH_2SO_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 148.68, 146.04, 140.38, 137.91, 132.13, 129.93, 128.67, 124.57, 123.30, 121.65, 116.16, 115.45, 45.61. HRMS ESI$^+$ *m*/*z* 340.04048 [M+H]$^+$. Found: 340.04013 [M+H]$^+$.

4.1.4.6. 2-(3,4-Dihydroxy-phenyl)-ethenesulfonic acid 4-trifluoromethyl -benzylamide (8f). White solid (75%), mp 173–174 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.61 (s, 1H, *p*-ArO*H*), 9.12 (s, 1H, *m*-ArO*H*), 7.85 (t, 1H, N*H*), 6.75–7.70 (m, 9H, Ar*H*, Ar*CH*=*CH*SO_2), 4.19 (s, 1H, $ArCH_2SO_2$), 4.17 (s, 1H, $ArCH_2SO_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 148.71, 146.04, 143.83, 140.51, 128.73, 125.61, 125.54, 124.53, 123.43, 123.21, 121.63, 116.14, 115.49, 45.80. HRMS ESI$^+$ *m*/*z* 374.06684 [M+H]$^+$. Found: 374.06689 [M+H]$^+$, 391.09335 [M+NH_4]$^+$.

4.1.4.7. 2-(3,4-Dihydroxy-phenyl)-ethenesulfonic acid 4-Fluorobenzylamide (8g). White solid (65%), mp 155–156 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.62 (s, 1H, *p*-ArO*H*), 9.14 (s, 1H, *m*-ArO*H*), 7.72 (t, 1H, N*H*), 6.72–7.39 (m, 9H, Ar*H*, Ar*CH*=*CH*SO_2), 4.07 (s, 1H, $ArCH_2SO_2$), 4.06 (s, 1H, $ArCH_2SO_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 148.66, 146.04, 140.30, 134.96, 130.13, 130.05, 124.58, 123.35, 121.65, 116.15, 115.56, 115.40, 115.35, 45.61. HRMS ESI$^+$ *m*/*z* 324.07003 [M+H]$^+$. Found: 324.06985 [M+H]$^+$, 341.09666 [M+NH_4]$^+$, 346.05214 [M+Na]$^+$.

4.1.4.8. 2-(3,4-Dihydroxy-phenyl)-ethenesulfonic acid 4-Methyl-benzylamide (8h). White solid (61%), mp 116–117 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.60 (s, 1H, *p*-ArO*H*),

9.13 (s, 1H, *m*-ArO*H*), 7.64 (t, 1H, N*H*), 6.66–7.22 (m, 9H, Ar*H*, ArC*H*=C*H*SO_2), 4.04 (s, 1H, ArCH_2SO_2), 4.02 (s, 1H, ArCH_2SO_2), 2.26 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 148.61, 146.03, 140.65, 136.70, 135.61, 129.28, 128.14, 124.62, 123.49, 121.63, 116.14, 115.36, 46.21, 21.12. HRMS ESI$^+$ *m*/*z* 342.07705 [M+Na]$^+$. Found: 342.07713 [M+Na]$^+$.

4.1.4.9. 2-(3,4-Dihydroxy-phenyl)-ethenesulfonic acid 4-Methoxy-benzylamide (8i). White solid (63%), mp 161–162 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.59 (s, 1H, *p*-ArO*H*), 9.13 (s, 1H, *m*-ArO*H*), 7.60 (t, 1H, N*H*), 6.66–7.25 (m, 9H, Ar*H*, ArC*H*=C*H*SO_2), 4.01 (s, 1H, ArCH_2SO_2), 4.00 (s, 1H, ArCH_2SO_2), 3.71 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 158.90, 148.61, 146.03, 140.05, 130.52, 129.49, 124.63, 123.53, 121.60, 116.14, 115.38, 114.14, 55.51, 45.94. HRMS ESI$^+$ *m*/*z* 358.07196 [M+Na]$^+$. Found: 358.07176 [M+Na]$^+$.

4.1.4.10. 2-(3,4-Dihydroxy-phenyl)-ethenesulfonic acid [2-(4-chloro-phenyl)-ethyl]-amide (8j). White solid (65%), mp 153–154 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.61 (s, 1H, *p*-ArO*H*), 9.13 (s, 1H, *m*-ArO*H*), 6.71–7.34 (m, 10H, Ar*H*, ArC*H*=C*H*SO_2, N*H*), 3.07 (q, 2H, Ar $CH_2CH_2SO_2$), 2.76 (t, 2H, ArCH_2 CH_2SO_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 148.65, 146.05, 140.30, 138.43, 131.34, 131.13, 128.67, 124.59, 123.04, 121.65, 116.17, 115.48, 44.06, 35.23. HRMS ESI$^+$ *m*/*z* 354.05613 [M+H]$^+$. Found: 354.05614 [M+H]$^+$.

4.1.4.11. 2-(3,4-Dihydroxy-phenyl)-ethenesulfonic acid methyl-phenethyl-amide (8k). White solid (68%), mp 103–104 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.66 (s, 1H, *p*-ArO*H*), 9.09 (s, 1H, *m*-ArO*H*), 6.75–7.32 (m, 10H, Ar*H*, ArC*H*=C*H*SO_2), 3.26 (t, 2H, ArCH_2-CH_2SO_2), 2.85 (t, 2H, ArC$H_2CH_2SO_2$), 2.73 (s, 3H, NCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ = 148.86, 146.02, 142.45, 139.20, 129.25, 128.83, 126.75, 124.64, 121.99, 119.37, 116.07, 115.77, 51.49, 35.01, 34.34. HRMS ESI$^+$ *m*/*z* 334.11076 [M+H]$^+$. Found: 334.11082 [M+H]$^+$.

4.1.5. General procedure for the synthesis of *E*-3,4-diacetyl styryl sulfonamides (9a–k)

The *E*-3,4-dihydroxy styryl sulfonamide **8a**–**k** (1 mmol) was added to a solution of the pyridine (2.2 mmol) solution in acetic anhydride (3 mL) and stirred at RT until absence of the *E*-3,4-dihydroxy styryl sulfonamide (checked by TLC). The reaction mixture was diluted with H_2O and extracted with ethyl acetate. The combined organic fractions were washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification of the crude residue by column chromatography (petroleum ether/ethyl acetate) afforded the title compound.

4.1.5.1. 2-(3,4-Diacetoxy-phenyl)-ethenesulfonic acid benzylamide (9a). White solid (94%), mp 109–110 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.22–7.44 (m, 9H, Ar*H*, ArC*H*=C*H*SO_2), 6.63 (d, *J* = 15.2 Hz, 1H, ArC*H*=C*H*SO_2), 4.73 (t, 1H, N*H*), 4.28 (s, 1H, ArCH_2SO_2), 4.26 (s, 1H, ArCH_2SO_2), 2.34 (s, 6H, 2ArOCOCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 168.02, 167.91, 143.93, 142.55, 139.72, 136.43, 131.36, 128.90, 128.18, 128.12, 128.01, 126.58, 126.55, 124.15, 122.99, 47.18, 20.65. HRMS ESI$^+$ *m*/*z* 390.10058 [M+H]$^+$. Found: 390.10151 [M+H]$^+$, 412.08310 [M+Na]$^+$.

4.1.5.2. 2-(3,4-Diacetoxy-phenyl)-ethenesulfonic acid phenethyl-amide (9b). White solid (92%), mp 56–57°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.39 (d, *J* = 15.6 Hz, 1H, ArC*H*=C*H*SO_2), 7.19–7.35 (m, 8H, Ar*H*), 6.54 (d, *J* = 15.6 Hz, 1H, ArC*H*=C*H*SO_2), 4.37 (t, 1H, N*H*), 3.35 (q, 2H, ArCH_2CH_2SO_2), 2.89 (t, 2H, ArCH_2CH_2-SO_2), 2.34 (s, 3H, *p*-ArOCOCH_3), 2.33 (s, 3H, *m*-ArOCOCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 168.05, 167.93, 143.91, 142.55, 139.63, 137.64, 131.36, 128.87, 126.96, 126.62, 126.22, 124.15, 122.98, 44.12, 36.15, 30.95, 20.67, 20.63. HRMS ESI$^+$ *m*/*z* 404.11623 [M+H]$^+$. Found: 404.11678 [M+H]$^+$.

4.1.5.3. 2-(3,4-Diacetoxy-phenyl)-ethenesulfonic acid (3-phenyl-propyl)-amide (9c). White solid (96%), mp 104–105 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.42 (d, *J* = 15.2 Hz, 1H, ArC*H*=C*H*SO_2), 7.17–7.38 (m, 8H, Ar*H*), 6.68 (d, *J* = 15.2 Hz, 1H, ArC*H*=C*H*SO_2), 4.33 (t, 1H, N*H*), 3.09 (q, 2H, ArC$H_2CH_2CH_2SO_2$), 2.70 (m, 2H, ArC$H_2CH_2CH_2SO_2$), 2.34 (s, 6H, 2ArOCOCH_3), 1.93 (m, 2H, ArC$H_2CH_2CH_2SO_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 168.03, 167.90, 143.94, 142.60, 140.72, 139.79, 131.38, 128.57, 128.37, 126.57, 126.22, 124.19, 122.96, 42.52, 32.77, 31.49, 20.62. HRMS ESI$^+$ *m*/*z* 418.13188 [M+H]$^+$. Found: 418.13259 [M+H]$^+$, 440.11451 [M+Na]$^+$.

4.1.5.4. 2-(3,4-Diacetoxy-phenyl)-ethenesulfonic acid (4-phenyl-butyl)-amide (9d). White solid (93%), mp 70–71 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.42 (d, *J* = 15.6 Hz, 1H, ArC*H*=C*H*SO_2), 7.16–7.38 (m, 8H, Ar*H*), 6.68 (d, *J* = 15.6 Hz, 1H, ArC*H*=C*H*SO_2), 4.35 (t, 1H, N*H*), 3.07 (q, 2H, ArC$H_2CH_2CH_2CH_2SO_2$), 2.65 (m, 2H, ArC$H_2CH_2CH_2CH_2SO_2$), 2.34 (s, 6H, 2ArOCOCH_3), 1.68 (m, 2H, ArC$H_2CH_2CH_2CH_2SO_2$), 1.62 (m, 2H, ArC$H_2CH_2CH_2CH_2SO_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 168.05, 167.92, 143.91, 142.59, 141.73, 139.66, 131.41, 128.40, 126.55, 126.31, 125.93, 124.19, 122.97, 42.97, 35.28, 29.50, 28.29, 20.66. HRMS ESI$^+$ *m*/*z* 423.14753 [M+H]$^+$. Found: 432.14842 [M+H]$^+$.

4.1.5.5. 2-(3,4-Diacetoxy-phenyl)-ethenesulfonic acid 4-chlorobenzylamide (9e). White solid (92%), mp 109–110 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.40 (d, *J* = 15.2 Hz, 1H, ArC*H*=C*H*SO_2), 7.25–7.34 (m, 7H, Ar*H*), 6.62 (d, *J* = 15.2 Hz, 1H, ArC*H*=C*H*SO_2), 4.84 (t, 1H, N*H*), 4.23 (s, 1H, ArCH_2SO_2), 4.21(s, 1H, ArCH_2SO_2), 2.34 (s, 3H, *p*-ArOCOCH_3), 2.33 (s, 3H, *m*-ArOCOCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 168.09, 167.95, 143.99, 142.58, 139.92, 135.06, 133.97, 131.22, 129.39, 129.01, 126.63, 126.38, 124.23, 122.95, 46.45, 20.67. HRMS ESI$^+$ *m*/*z* 424.06161 [M+H]$^+$. Found: 424.06160 [M+H]$^+$, 441.08837 [M+NH_4]$^+$.

4.1.5.6. 2-(3,4-Diacetoxy-phenyl)-ethenesulfonic acid 4-trifluoromethyl-benzylamide (9f). White solid (96%), mp 115–116 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.25–7.63 (m, 8H, Ar*H*, ArC*H*=C*H*SO_2), 6.63 (d, *J* = 15.6 Hz, 1H, ArC*H*=C*H*SO_2), 4.92 (t, 1H, N*H*), 4.33 (s, 1H, ArCH_2SO_2), 4.31 (s, 1H, ArCH_2SO_2), 2.34 (s, 6H, 2ArOCOCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 168.09, 167.93, 144.06, 142.61, 140.65, 140.12, 131.11, 128.21, 126.63, 126.24, 125.82, 125.78, 124.24, 122.94, 46.55, 20.66, 20.62. HRMS ESI$^+$ *m*/*z* 458.08797 [M+H]$^+$. Found: 458.08860 [M+H]$^+$, 475.11516 [M+NH_4]$^+$.

4.1.5.7. 2-(3,4-Diacetoxy-phenyl)-ethenesulfonic acid 4-fluorobenzylamide (9g). White solid (94%), mp 99–100 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.43 (d, *J* = 15.2 Hz, 1H, ArC*H*=C*H*SO_2), 7.03–7.34 (m, 7H, Ar*H*), 6.63 (d, *J* = 15.2Hz, 1H, ArC*H*=C*H*SO_2), 4.71 (t, 1H, N*H*), 4.24 (s, 1H, ArCH_2SO_2), 4.23 (s, 1H, ArCH_2SO_2), 2.35 (s, 3H, *p*-ArOCOCH_3), 2.34 (s, 3H, *m*-ArOCOCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 168.07, 167.93, 143.99, 142.59, 139.89, 132.30, 132.27, 131.25, 129.83, 129.75, 126.63, 126.42, 124.22, 122.94, 115.91, 115.70, 46.46, 20.67. HRMS ESI$^+$ *m*/*z* 408.09116 [M+H]$^+$. Found: 408.09177 [M+H]$^+$, 425.11823 [M+NH_4]$^+$.

4.1.5.8. 2-(3,4-Diacetoxy-phenyl)-ethenesulfonic acid 4- Methyl -benzylamide (9h). White solid (95%), mp 98–99 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.38 (d, *J* = 15.6 Hz, 1H, ArC*H*=C*H*SO_2), 7.14–7.32 (m, 7H, Ar*H*), 6.59 (d, *J* = 15.6 Hz, 1H, ArC*H*=C*H*SO_2), 4.65 (t, 1H, N*H*), 4.23 (s, 1H, ArCH_2SO_2), 4.22 (s, 1H, ArCH_2SO_2), 2.34 (s,

3H, *p*-ArOCOCH_3), 2.33 (s, 3H, *m*-ArOCOCH_3), 2.32 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 168.04, 167.95, 143.87, 142.53, 139.55, 137.95, 133.36, 131.41, 129.54, 128.06, 126.63, 126.56, 124.13, 122.93, 46.99, 21.06, 20.67, 20.64. HRMS ESI$^+$ m/z 404.09818 $[M+H]^+$. Found: 404.11683 $[M+H]^+$, 426.09894 $[M+Na]^+$.

4.1.5.9. 2-(3,4-Diacetoxy-phenyl)-ethenesulfonic acid 4-methoxy-benzylamide (9i). White solid (94%), mp 96–97 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.37 (d, *J* = 15.6 Hz, 1H, ArC*H*=C*H*SO_2), 6.85–7.31 (m, 7H, Ar*H*), 6.58 (d, *J* = 15.6 Hz, 1H, ArC*H*=C*H*SO_2), 4.79 (t, 1H, N*H*), 4.20(s, 1H, Ar$CH_2CH_2SO_2$), 4.18 (s, 1H, Ar$CH_2CH_2SO_2$), 3.77 (s, 3H, OCH_3), 2.34 (s, 3H, *p*-ArOCOCH_3), 2.33 (s, 3H, *m*-ArOCOCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 168.07, 167.96, 159.41, 143.86, 142.53, 139.48, 131.42, 129.49, 128.45, 126.70, 126.57, 124.13, 122.93, 114.22, 55.31, 46.72, 20.67, 20.63. HRMS ESI$^+$ *m*/*z* 442.09309 $[M+Na]^+$. Found: 442.09358 $[M+Na]^+$.

4.1.5.10. 2-(3,4-Diacetoxy-phenyl)-ethenesulfonic acid [2-(4-chloro-phenyl)-ethyl]-amide (9j). White solid (94%), mp 92–93 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.39 (d, *J* = 15.2 Hz, 1H, ArC*H*=C*H*SO_2), 7.13–7.35 (m, 7H, Ar*H*), 6.53 (d, *J* = 15.2 Hz, 1H, ArC*H*=C*H*SO_2), 4.33 (t, 1H, N*H*), 3.32(q, 2H, Ar$CH_2CH_2SO_2$), 2.87 (q, 2H, Ar$CH_2CH_2SO_2$), 2.34 (s, 3H, *p*-ArOCOCH_3), 2.33 (s, 3H, *m*-ArOCOCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 168.09, 167.92, 143.97, 142.59, 139.81, 136.17, 132.79, 131.23, 130.26, 128.97, 126.62, 126.06, 124.23, 122.98, 43.99, 35.63, 20.66. HRMS ESI$^+$ *m*/*z* 438.07726 $[M+H]^+$. Found: 438.07768 $[M+H]^+$, 460.06001 $[M+Na]^+$.

4.1.5.11. 2-(3,4-Diacetoxy-phenyl)-ethenesulfonic acid methyl-phenethyl-amide (9k). White solid (95%), mp 104–105 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.35 (d, *J* = 15.2 Hz, 1H, ArC*H*=C*H*SO_2), 7.24–7.33 (m, 8H, Ar*H*), 6.33 (d, *J* = 15.2 Hz, 1H, ArC*H*=C*H*SO_2), 3.45 (t, 2H, Ar$CH_2CH_2SO_2$), 2.93 (t, 2H, Ar$CH_2CH_2SO_2$), 2.84 (s, 3H, NCH_3), 2.34 (s, 3H, *p*-ArOCOCH_3), 2.33 (s, 3H, *m*-ArOCOCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 168.08, 167.94, 143.83, 142.52, 139.97, 138.26, 131.53, 128.96, 128.68, 126.70, 126.65, 124.10, 123.91, 122.88, 51.52, 34.97, 34.60, 20.66, 20.62. HRMS ESI$^+$ *m*/*z* 418.13188 $[M+H]^+$. Found: 418.13228 $[M+H]^+$.

4.2. Biological assays

4.2.1. DPPH free radical scavenging activity

Free radical scavenging activity was studied using DPPH. The DPPH were bought from Sigma. The reaction was carried out in 96-well microplates. Test compounds and DPPH were dissolved in ethanol. Various concentrations of test solutions (100 μL) and DPPH (100 μL) were mixed in per well. The mixture of neat ethanol (100 μL) and DPPH (100 μL) was used as experimental control. The final concentration of DPPH was 0.1 mM. After 30 min of incubation at room temperature, the reduction in the number of free radical was measured by reading the absorbance at 517 nm. Vitamin C was used as reference free radical scavenger. For statistical viability, every sample was analyzed in three wells and in three independent runs. The percentage inhibition was calculated from the following formula: $[(A_0-A_c)/A_0] \times 100\%$, where A_0 is absorbance of control and A_c is absorbance of the test sample.

4.2.2. Protection effect against H_2O_2-induced cell Injury

The PC12 cells were bought from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences. Fetal bovine serum, trypsin–EDTA, penicillin streptomycin were obtained from Invitrogen. High-glucose DMEM and donor equine serum were obtained from Thermo Scientific. H_2O_2 was purchased from Sigma. MTT and DMSO were acquired from AMRESCO. PC12 cells were maintained in high-glucose DMEM supplemented with 10% fetal bovine serum, 5% horse serum, 100U mL^{-1} penicillin and 100U mL^{-1} streptomycin in a humidified atmosphere of 5% CO_2 at 37 °C. PC12 cells were seeded into 96-well microplates at a density of 4×10^5 cells mL^{-1} (100 μL per well). After 24 h of incubation to allow for cell attachment, the PC12 cells were preincubated with samples for 3 h, which were dissolved in DMSO and diluted with medium to the final concentrations. Then, 20 μL of H_2O_2 (diluted with medium to a final concentration of 500 μM) solution was added. After 5 h, the cell viability was measured with MTT assay. The absorbance was determined at 570 nm using a microplate reader. Compared with the normal cells, the viability of cells treated with drugs is calculated by the following formula: OD (drug-treated)/OD (normal cells) × 100%.

4.2.3. Inhibiting effect on nitric oxide production in LPS-stimulated BV2 microglial cells

BV2 microglial cells were purchased from Institute of Basic Medicine, Chinese Academy of Medical Sciences. LPS was bought from Sigma. BV2 microglial cells were maintained in high-glucose DMEM supplemented with 5% fetal bovine serum, 100 UmL^{-1} penicillin and 100U mL^{-1} streptomycin in a humidified atmosphere of 5% CO_2 at 37 °C. BV2 microglial cells were seeded into 96-well microplates at a density of 4×10^5 cells mL^{-1} (100 μL per well). After 24 h of incubation to allow for cell attachment, the BV2 microglial cells were preincubated with samples for 3 h, which were dissolved in DMSO and diluted with medium to the final concentrations. Then, 20 μL of LPS solution (diluted with medium to a final concentration of 100 nM) was added. After 24 h, the nitric oxide assay kit was used to perform nitrite assays. The nitric oxide assay kit was gained from Applygen. The culture media was mixed with an equal volume of reagent of the nitric oxide assay kit in 96-well plates. The absorbance was determined at 540 nm using a microplate reader. The release amount of nitric oxide was calculated by the offered linear equation of the nitric oxide assay kit. The percentage inhibition of nitric oxide was calculated from the following formula: $[(R_L - R_0 - R_c)/(R_L - R_0)] \times 100\%$, where R_L is the release amount of only LPS treated group, R_0 is the release amount of normal control and R_c is the release amount of the test compound and LPS treated group. The IC_{50} values of test compounds were calculated by linear regression of plots where the *x*-axis represented the various concentrations of test compounds while the *y*-axis represented the percentage inhibition of nitric oxide.

4.2.4. In vitro BBB permeability assay

Prediction of crossing BBB was evaluated using PAMPA. PBL (catalog no. 141101-p) was bought from Avanti Polar Lipids. Dodecane was obtained from Alfa Aesar. The 96-well filter plate (catalog no. MAIPN4550) and the donor plate (catalog no. MATRNPS50) were both from Millipore. Filter PDVF membrane units were from Symta. Verapamil, Hydrocortisone and Clonidine were bought from Sigma. Test compounds were dissolved in DMSO at 5 mg mL^{-1}. Ten microlitre of this compound stock solution was diluted in PBS to make secondary stock solution (final concentration 25 μg mL^{-1}) and these solutions were filtered. 300 mL of the secondary stock solution was added to the donor well. The filter membrane was coated with PBL in dodecane (selected empirically as 4 μL volume of 20 mg mL^{-1} PBL in dodecane) and the acceptor well was filled with 150 mL of PBS. The acceptor filter plate was carefully put on the donor plate to form a 'sandwich' (consisting of the donor with test compounds on the bottom, artificial lipid membrane in the middle, and the acceptor on the top). The 'sandwich' was incubated undisturbed at room temperature for 18 h while the permeation occurred. After incubation, the donor plate was removed. UV plate reader determined the concentration of tested compounds in the acceptor and reference solutions. Reference

solutions were prepared by diluting the sample secondary stock solution to the same concentration as that with no membrane barrier. Every sample was analyzed at three wavelengths, in three wells and in three independent runs. The permeability coefficient through the artificial membrane (P_e) was calculated using the following equation.[35,36]

$$P_e = -\frac{V_{dn} \times V_{ac}}{st(V_{dn} + V_{ac})} \ln\left(1 - \frac{[drug]_{ac}}{[drug]_{ref}}\right)$$

V_{dn} (mL) = volume of the donor compartment
V_{ac} (mL) = volume of the acceptor compartment
$[drug]_{ac}$ = optical density of the solution of the acceptor compartment
$[drug]_{ref}$ = optical density of the reference solution
s (cm^2) = membrane area
t (s) = incubation time

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Supplementary data

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