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Research Article

Design and Synthesis of Novel NO-Donor-Ferulic Acid Hybrids as Potential Antiatherosclerotic Drug Candidates

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Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT Novel NO-donor-ferulic acid hybrids were designed and synthesized through a symbiotic approach using ferulic acid and three different NO-donating groups, such as nitric ester, 4-hydroxyl-3-phenylfuroxan, and 4-hydroxymethyl-3-phenylsulfonylfuroxan. Antioxidant, nitric oxide release, and vasodilator properties studies showed that the target phenylsulfonylfuroxan 14, especially 14c, while keeping the antioxidant activity, showed more NO release activity and vasodilating activity than isosorbide dinitrate (ISDN). Thus, 14c may be considered a novel potent anti-atherosclerosis drug candidate. Drug Dev Res 72:405–415, 2011.

Key words: atherosclerosis; nitric oxide donor; ferulic acid; nitric oxide release

INTRODUCTION

Cardiovascular disease (CD), such as heart attack, stroke, and hypertension, is to blame for about 30% of all deaths worldwide, making it the leading cause of morbidity and mortality [Hennekens, 1998]. Atherosclerosis is

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Abbreviations used: CD, cardiovascular disease; DCC, dicyclohexylcarbodiimide; DEAD, diethyl azodicarboxylate; DMAP, 4-*N*,*N*-dimethylaminopyridine; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EDC, 1-ethyl-(3-dimethylaminopropyl)carbodiimide; EDRF, endothelial-derived relaxing factor; FA, ferulic acid; ISDN, isosorbide dinitrate; LDL, low-density lipoprotein; mp, melting point; ROS, reactive oxygen species; TBA, 2-thiobarbituric acid; TBARS, 2-thiobarbituric acid reactive substances.

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unquestionably the main underlying pathology of CD, among which the major risk factors for the development of atherosclerosis are increased levels of low-density lipoprotein (LDL), oxidative modification of LDL, and an impairment of endothelial derived relaxing factor (EDRF)-, nitric oxide (NO)-mediated bioactions [Steinberg et al., 1989; Napoli et al., 2006].

The accumulation of LDL and reactive oxygen species (ROS) in the subendothelial space induces a high degree of LDL oxidation. According to the oxidative hypothesis of atherosclerosis, this is an early event in a complex process leading to the formation of foam cells that constitute a fatty streak, a forerunner in the development of mature atherosclerotic plaques [Keaney and Vita, 1995]. An increasing number of studies indicate that the administration of exogenous antioxidants might decrease the impact of atherosclerosis in animals and humans through the regulation and protection of several aspects of endothelial function [Ogita and Liao, 2004; Cherubini et al., 2005; Praticò, 2005]. Endothelium-dependent vascular relaxation is mediated predominantly by NO. In the vessels affected by atherosclerosis, inactivation of NO is enhanced as a result of its reaction with the superoxide anion ($O_2^{-\cdot}$), leading to the formation of peroxynitrite ($ONOO^-$). As with decreasing the bioavailability of protective NO, the peroxynitrite formed in this reaction is a precursor for the highly reactive and very toxic hydroxyl radical ($OH^\cdot$). In addition, peroxynitrite is concerned to be of high cytotoxicity in its own right and can mediate lipid peroxidation reactions. Furthermore, a decrease in the production of NO by endothelial cells cannot be excluded, at least in advanced atherosclerotic disease [Ogita and Liao, 2004].

On this basis, some researchers have recently proposed and synthesized a new class of NO-donor hybrid drugs obtained by joining antioxidants, such as *p*-cresol, vitamin E, and vitamin C with appropriate NO-donor moieties as potential agents for the treatment of CD involving atherosclerotic vascular changes [Cena et al., 2004, 2008; Boschi et al., 2006]. In pursuing some anti-atherosclerosis agents, we report on the design and synthesis of another series of such compounds, and the preliminary results of a study on their capacity of inhibiting the ferrous salt/ascorbate-induced lipid peroxidation of membrane lipids of rat hepatocytes and on the in vitro vasodilating properties. As antioxidants, we considered ferulic acid (FA) **1** (Fig. 1), one of the most ubiquitous phenolic compounds in nature, especially as rich as an ester form in rice bran pitch [Graf, 1992], which has been largely used as a food preservative because of its ability to inhibit the autoxidation of oils [Dziedzic and Hudson, 1984]. It has been reported that FA inhibited the autoxidation of methyl linoleate and linoleic acid as well as the oxidation of liposomes induced by the water-soluble initiator 2,2-diphenyl-1-picrylhydrazyl (DPPH) [Son and Lewis, 2002; Medina et al., 2002]. Moreover, FA has been shown to reinforce the antioxidant capacity of lactoferrin by inhibiting the oxidation in liposomes and oil-in-water emulsions [Medina et al., 2002]. In addition, FA increased the antioxidant activity of plasma and the resistance of LDLs to oxidation [Ohta et al., 1997; Laranjinha et al., 1996; Andreasen et al., 2001]. As N-donor moieties, we chose the nitroxy function present in simple vasodilating nitric esters **2** (in **8**, **9**), the 3-phenylfuroxan-4-yloxy substructure present in the 4-hydroxyl-3-phenylfuroxan **3** (in **12**), and the 3-phenylsulfonylfuroxan-4-yloxy substructure present in 4-hydroxymethyl-3-phenylsulfonylfuroxan **4** (in **14**) (Fig. 1) as well. These reference compounds display extremely modulated in vitro NO-dependent vasodilator properties [Civelli et al., 1996].

MATERIALS AND METHODS

Synthesis

Reagents and solvents were purchased from commercial sources and used without further purification unless otherwise specified. Air- and moisture-sensitive liquids and solutions were transferred via syringe or stainless steel cannula. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.15–0.20-mm Yantai silica gel plates (RSGF 254) using ultraviolet (UV) light as the visualizing agent. Chromatography was performed on Qingdao silica gel (16–200 mesh) using petroleum ether (6–90) and ethyl acetate as the eluanting solvent.

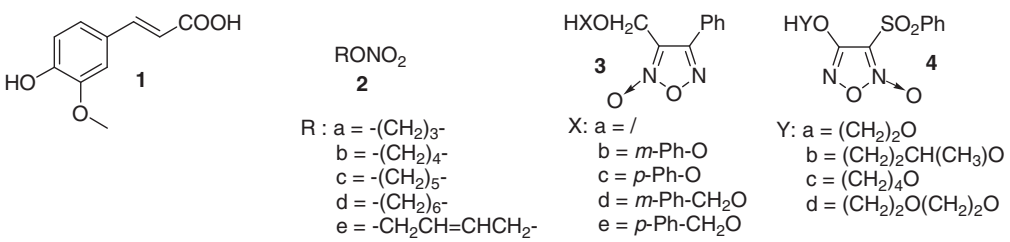

Fig. 1. Ferulic acid and reference NO-donor compounds.

The melting points (mp) were measured on a WRS-1B apparatus and were not corrected. 1H NMR spectra were obtained using a Bruker AV-300 (300 MHz). Chemical shifts were recorded in ppm downfield from tetramethylsilane. J-values were given in Hertz (Hz). Abbreviations used were s (singlet), d (doublet), t (triplet), q (quartet), b (broad), and m (multiplet). ESI-MS spectra were recorded on a Waters Synapt HDMS spectrometer.

The synthetic routes of nine nitrate/FA hybrids (**8a–8e** and **9a–9d**) are outlined in Figure 2. FA (**1**) was first treated with dibromoalkanes (**5a–5d**) bearing three to six carbons in the presence of Et_3N and acetone at 50°C to generate compounds **6a–6d** as the major products, and **7a–7d** were also obtained as by products no matter the stoichiometry between FA (**1**) and dibromoalkanes (**5a–5d**) were changed. In order to increase the diversity of target compounds, **6a–6d** or **7a–7d** were further converted to the corresponding nitrates **8a–8d** or **9a–9d**, respectively, with $AgNO_3$ in THF/CH_3CN. However, when FA (**1**) was treated with (E)-1,4-dibromobut-2-ene (**5e**), only **6e** was obtained, and then **6e** was further converted to the corresponding nitrates **8e** with $AgNO_3$ in THF/CH_3CN.

The other nine target compounds were synthesized through modifying the carboxyl group of FA with phenylfuroxan or phenylsulfonylfuroxan (Fig. 3). A total of five phenyl-substituted furoxans (**3**) and four phenylsulfonyl-substituted furoxans (**4**) were synthesized as described previously [Kenney et al., 1961; Farrar, 1964; Kelley et al., 1977; Gasco et al., 1991]. Direct esterification of FA with hydroxyl compound **3** or **4** was tried using many catalysts such as dicyclohexylcarbodiimide (DCC)/4-*N*,*N*-dimethylaminopyridine (DMAP), 1-ethyl-(3-dimethylaminopropyl)carbodiimide (EDC), BF_3/Et_2O, and diethyl azodicarboxylate (DEAD)/Ph_3P; unfortunately, no product was obtained, probably because of the weak acidity of the –COOH. Various protecting groups and conditions, such as Ac_2O and ethyl chlorformate, were then used to protect the phenolic hydroxyl group of FA; ethyl chlorformate was found to give optimum results when used in conjunction with NaOH in water to afford **10** in 90% yields. Subsequently, **10** was esterified with **3** or **4** in the presence of DCC and DMAP to generate compounds **11a–11e** and **13a–13d**. The ethoxycarbonyl groups in **11** and **13** were removed in ethanolamine solution, without the breakdown of other ester bonds, to produce target compounds **12a–12e** and **14a–14d**.

EXPERIMENTS

General Procedure for the Preparation of 6(a–e) and 7(a–d)

To a stirring mixture of FA (5.0 g, 25.8 mmol) and dibromo-alkane (100 mmol) in dry acetone (150 ml) at room temperature was added Et_3N (10 ml). The reaction mixture was refluxed gently for 4–6 h. The solution was cooled to room temperature and then

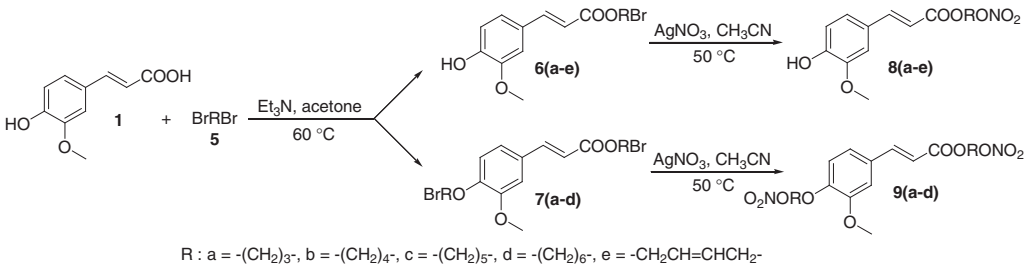

Fig. 2. Synthesis of nitric ester–ferulic acid hybrids.

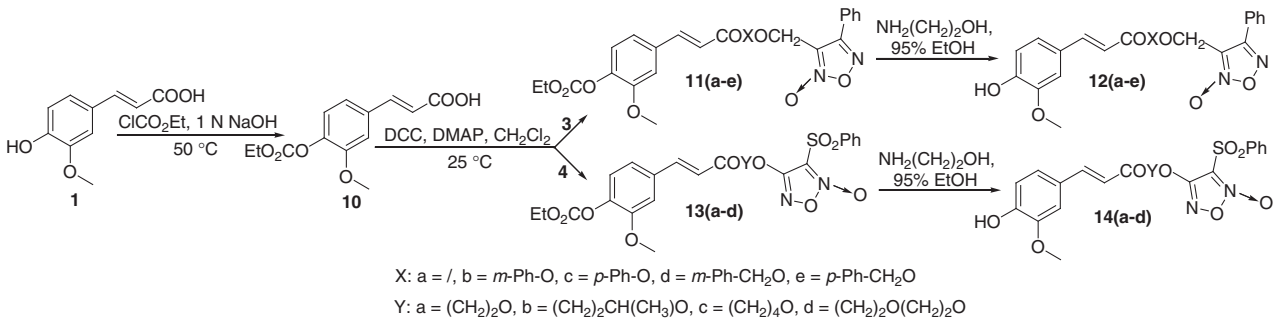

Fig. 3. Synthesis of 4-hydroxyl-3-phenylfuroxa–ferulic acid hybrids and 4-hydroxymethyl-3-phenylsulfonylfuroxa–ferulic acid hybrids.

filtered. Removal of the solvent under vacuo followed by silica gel column chromatographic purification of the residue using 20% ethyl acetate in petroleum ether afforded the compounds (yields 4.3–65.8%).

(E)-3-bromopropyl-3-(4-hydroxy-3-methoxyphenyl) acrylate (6a)

Dark red oil, 56% yield. 1H NMR ($CDCl_3$) δ 2.26 (m, 2H, CH_2), 3.52 (t, J = 3.7 Hz, 2H, CH_2Br), 3.94 (s, 3H, OCH_3), 4.34 (t, J = 3.7 Hz, 2H, $COOCH_2$), 5.83 (s, 1H, OH), 6.30 (d, J = 9.5 Hz, 1H, C=CH), 6.92 (d, 1H, Ar–H), 7.06–7.15 (m, 2H, Ar–H), 7.65 (d, J = 9.5Hz, 1H, CH=C). ESI-MS: 353 $[M+K]^+$.

(E)-4-bromobutyl-3-(4-hydroxy-3-methoxyphenyl) acrylate (6b)

White solid, 43% yield; mp 81.9–82.3°C. 1H NMR ($CDCl_3$) δ 1.87–2.07 (m, 4H, CH_2), 3.47 (t, J = 6.4 Hz, 2H, CH_2Br), 3.93 (s, 3H, OCH_3), 4.24 (t, J = 6.4 Hz, 2H, $COOCH_2$), 5.85 (s, 1H, OH), 6.28 (d, J = 15.9 Hz, 1H, C=CH), 6.92 (d, 1H, Ar–H), 7.03–7.10 (m, 2H, Ar–H), 7.62 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 351 $[M+Na]^+$.

(E)-5-bromopentyl-3-(4-hydroxy-3-methoxyphenyl) acrylate (6c)

White solid, 66% yield; mp 66.3–66.6°C. 1H NMR ($CDCl_3$) δ 1.58 (m, 2H, CH_2), 1.70 (m, 2H, CH_2), 1.90 (m, 2H, CH_2), 3.44 (m, 2H, CH_2Br), 3.92 (s, 3H, OCH_3), 4.20 (m, 2H, $COOCH_2$), 5.91 (s, 1H, OH), 6.28 (d, J = 15.9 Hz, 1H, C=CH), 6.91 (d, 1H, Ar–H), 7.03–7.09 (m, 2H, Ar–H), 7.61 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 365 $[M+Na]^+$.

(E)-6-bromohexyl-3-(4-hydroxy-3-methoxyphenyl) acrylate (6d)

Yellow solid, 53% yield; mp 50.9–51.4°C. 1H NMR ($CDCl_3$) δ 1.39–1.45 (m, 4H, CH_2), 1.65–1.70 (m, 2H, CH_2), 1.85–1.93 (m, 2H, CH_2), 3.41 (m, 2H, CH_2Br), 3.91 (s, 3H, OCH_3), 4.19 (m, 2H, $COOCH_2$), 5.94 (s, 1H, OH), 6.28 (d, J = 15.9 Hz, 1H, C=CH), 6.91 (d, 1H, Ar–H), 7.03–7.08 (m, 2H, Ar–H), 7.61 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 379 $[M+Na]^+$.

(E)-((E)-4-bromobut-2-enyl)-3-(4-hydroxy-3-methoxyphenyl)acrylate (6e)

Pale solid, 4% yield; mp 69.7–72.8°C. 1 H NMR ($CDCl_3$) δ 3.92 (s, 3H, OCH_3), 3.97 (d, J = 6.9 Hz, 2H, CH_2Br), 4.71 (d, J = 5.3 Hz, 2H, $COOCH_2$), 5.88 (s, 1H, OH), 5.92–6.05 (m, 2H, CH=CH), 6.30 (d, J = 15.9 Hz, 1H, C=CH), 6.91 (d, 1H, Ar–H), 7.03–7.08 (m, 2H, Ar–H), 7.63 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 349 $[M+Na]^+$.

(E)-3-bromopropyl-3-(4-(3-bromopropoxy)-3-methoxyphenyl)acrylate (7a)

Yellow solid, 11% yield; mp 63.2–63.8°C. 1H NMR ($CDCl_3$) δ 2.22–2.30 (m, 2H, CH_2), 2.34–2.43 (m, 2H, CH_2), 3.52 (t, J = 6.6 Hz, 2H, CH_2Br), 3.62 (t, J = 6.6 Hz, 2H, CH_2Br), 3.89 (s, 3H, OCH_3), 4.19 (t, J = 6.0 Hz, 2H, $ArOCH_2$), 4.35 (t, J = 6.0 Hz, 2H, $COOCH_2$), 6.31 (d, J = 15.9 Hz, 1H, C=CH), 6.90 (d, 1H, Ar–H), 7.05–7.11 (m, 2H, Ar–H), 7.64 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 457 $[M+Na]^+$.

(E)-4-bromobutyl-3-(4-(4-bromobutoxy)-3-methoxyphenyl)acrylate (7b)

Brown solid, 7% yield; mp 72.6–75.1°C. 1H NMR ($CDCl_3$) δ 1.85–1.93 (m, 2H, CH_2), 1.99–2.13 (m, 6H, CH_2), 3.45–3.52 (m, 4H, CH_2Br), 3.89 (s, 3H, OCH_3), 4.08 (t, J = 5.9 Hz, 2H, $ArOCH_2$), 4.24 (t, J = 6.2 Hz, 2H, $COOCH_2$), 6.30 (d, J = 15.9 Hz, 1H, C=CH), 6.85 (d, 1H, Ar–H), 7.05–7.10 (m, 2H, Ar–H), 7.62 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 485 $[M+Na]^+$.

(E)-5-bromopentyl-3-(4-(5-bromopentyloxy)-3-methoxyphenyl)acrylate (7c)

White solid, 8% yield; mp 52.9–53.6°C. 1 H NMR ($CDCl_3$) δ 1.55–1.76 (m, 6H, CH_2), 1.89–1.99 (m, 6H, CH_2), 3.42 (m, 4H, CH_2Br), 3.89 (s, 3H, OCH_3), 4.06 (t, J = 6.4 Hz, 2H, $ArOCH_2$), 4.21 (t, J = 6.4 Hz, 2H, $COOCH_2$), 6.30 (d, J = 15.9Hz, 1H, C=CH), 6.85 (d, 1H, Ar–H), 7.06–7.09 (t, 2H, Ar–H), 7.62 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 513 $[M+Na]^+$.

(E)-6-bromohexyl-3-(4-(6-bromohexyloxy)-3-methoxyphenyl)acrylate (7d)

Yellow oil, 28% yield. 1H NMR ($CDCl_3$) δ 1.42–1.48 (m, 8H, CH_2), 1.67–1.89 (m, 8H, CH_2), 3.41 (m, 4H, CH_2Br), 3.89 (s, 3H, OCH_3), 4.05 (t, J = 6.6 Hz, 2H, $ArOCH_2$), 4.20 (t, J = 6.6 Hz, 2H, $COOCH_2$), 6.30 (d, J = 15.9 Hz, 1H, C=CH), 6.85 (d, 1H, Ar–H), 7.06–7.09 (m, 2H, Ar–H), 7.62 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 541 $[M+Na]^+$.

General Procedure for the Preparation of 8(a–e) and 9(a–d)

To a stirring solution of **6** or **7** (0.7 mmol) in CH_3CN (20 ml) at room temperature was added $AgNO_3$ (0.6 g, 2.5 mmol); then the reaction mixture was stirred at 50°C in dark for 6–8 h. The solution was cooled to room temperature and then filtered. Removal of the solvent under vacuo followed by silica gel column chromatographic purification of the residue using 30% ethyl acetate in petroleum ether afforded the compounds (yields 17–96%).

(E)-3-(nitroxy)propyl-3-(4-hydroxy-3-methoxyphenyl) acrylate (8a)

The title compound was obtained in 30% as yellow oil starting from **6a**. 1H NMR ($CDCl_3$) δ 2.13–2.19 (m, 2H, CH_2), 3.94 (s, 3H, OCH_3), 4.33 (t, J = 6.1 Hz, 2H, $COOCH_2$), 4.59–4.64 (m, 2H, CH_2ONO_2), 5.91 (br, 1H, OH), 6.28 (d, J = 15.9 Hz, 1H, C=CH), 6.92 (d, 1H, Ar–H), 7.03–7.10 (m, 2H, Ar–H), 7.65 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 320 $[M+Na]^+$.

(E)-4-(nitroxy)butyl-3-(4-hydroxy-3-methoxyphenyl) acrylate (8b)

The title compound was obtained in 92% as white solid starting from **6b**; mp 58.1–62.7°C. 1H NMR ($CDCl_3$) δ 1.81–1.90 (m, 4H, CH_2), 3.93 (s, 3H, OCH_3), 4.24 (t, J = 5.9 Hz, 2H, $COOCH_2$), 4.52 (t, J = 6.2 Hz, 2H, CH_2ONO_2), 5.90 (s, 1H, OH), 6.28 (d, J = 15.9 Hz, 1H, C=CH), 6.92 (d, 1H, Ar–H), 7.03–7.10 (m, 2H, Ar–H), 7.62 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 334 $[M+Na]^+$.

(E)-5-(nitroxy)pentyl-3-(4-hydroxy-3-methoxyphenyl) acrylate (8c)

The title compound was obtained in 83% as yellow solid starting from **6c**; mp 51.3–53.7°C. 1H NMR ($CDCl_3$) δ 1.49–1.63 (m, 2H, CH_2), 1.71–1.84 (m, 4H, CH_2), 3.93 (s, 3H, OCH_3), 4.21 (t, J = 6.4 Hz, 2H, $COOCH_2$), 4.47 (t, J = 6.5 Hz, 2H, CH_2ONO_2), 5.91 (s, 1H, OH), 6.28 (d, J = 15.9Hz, 1H, C=CH), 6.92 (d, 1H, Ar–H), 7.03–7.09 (m, 2H, Ar–H), 7.61 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 348 $[M+Na]^+$.

(E)-6-(nitroxy)hexyl-3-(4-hydroxy-3-methoxyphenyl) acrylate (8d)

The title compound was obtained in 89% as white solid starting from **6d**; mp 64.1–66.6°C. 1 H NMR ($CDCl_3$) δ 1.45–1.49 (m, 4H, CH_2), 1.70–1.78 (m, 4H, CH_2), 3.92 (s, 3H, OCH_3), 4.20 (t, J = 6.5 Hz, 2H, $COOCH_2$), 4.46 (t, J = 6.6 Hz, 2H, CH_2ONO_2), 5.90 (s, 1H, OH), 6.28 (d, J = 15.9 Hz, 1H, C=CH), 6.91 (d, 1H, Ar–H), 7.04–7.09 (m, 2H, Ar–H), 7.61 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 362 $[M+Na]^+$.

(E)-((E)-4-(nitroxy)but-2-enyl)-3-(4-hydroxy-3-methoxy-phenyl)acrylate (8e)

The title compound was obtained in 46% as yellow solid starting from **6e**; mp 71.1–71.9°C. 1 H NMR ($CDCl_3$) δ 3.93 (s, 3H, OCH_3), 4.74 (d, J = 5.1 Hz, 2H, $COOCH_2$), 4.94 (d, J = 6.2 Hz, 2H, CH_2ONO_2), 5.92 (s, 1H, OH), 6.03–6.12 (m, 2H, CH=CH), 6.30 (d, J = 15.9 Hz, 1H, C=CH), 6.92 (d, 1H, Ar–H), 7.03–7.10 (m, 2 H, Ar–H), 7.64 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 332 $[M+Na]^+$.

(E)-3-(nitroxy)propyl-3-(3-methoxy-4-(3-(nitroxy) propoxy)phenyl)acrylate (9a)

The title compound was obtained in 22% as yellow solid starting from **7a**; mp 57.9–58.2°C. 1H NMR ($CDCl_3$) δ 2.15 (t, J = 6.1 Hz, 2H, CH_2), 2.27 (t, J = 6.1 Hz, 2H, CH_2), 3.89 (s, 3H, OCH_3), 4.15 (t, J = 6.0 Hz, 2H, $ArOCH_2$), 4.32 (t, J = 6.1 Hz, 2H, $COOCH_2$), 4.62 (t, J = 6.1 Hz, 2H, CH_2ONO_2), 4.70 (t, J = 6.4 Hz, 2H, CH_2ONO_2), 6.30 (d, J = 15.9 Hz, 1H, C=CH), 6.87 (d, 1H, Ar–H), 7.06–7.10 (m, 2H, Ar–H), 7.64 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 423 $[M+Na]^+$.

(E)-4-(nitroxy)butyl-3-(3-methoxy-4-(4-(nitroxy) butoxy)phenyl)acrylate (9b)

The title compound was obtained in 96% as white solid starting from **7b**; mp 58.1–58.6°C. 1 H NMR ($CDCl_3$) δ 1.82–1.86 (m, 4H, CH_2), 1.95–1.99 (m, 4H, CH_2), 3.89 (s, 3H, OCH_3), 4.09 (t, J = 5.9 Hz, 2H, $ArOCH_2$), 4.25 (t, J = 5.9 Hz, 2H, $COOCH_2$), 4.50–4.59 (m, 4H, CH_2ONO_2), 6.30 (d, J = 15.9 Hz, 1H, C=CH), 6.85 (d, 1H, Ar–H), 7.05–7.10 (m, 2H, Ar–H), 7.62 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 451 $[M+Na]^+$.

(E)-5-(nitroxy)pentyl-3-(3-methoxy-4-(5-(nitroxy) pentyloxy)phenyl)acrylate (9c)

The title compound was obtained in 86% as yellow solid starting from **7c**; mp 75.5°C. 1 H NMR ($CDCl_3$) δ 1.52–1.67 (m, 4H, CH_2), 1.71–1.93 (m, 8H, CH_2), 3.90 (s, 3H, OCH_3), 4.06 (t, J = 6.4 Hz, 2H, $ArOCH_2$), 4.22 (t, J = 6.4 Hz, 2H, $COOCH_2$), 4.48 (m, 4H, CH_2ONO_2), 6.30 (d, J = 15.9 Hz, 1H, C=CH), 6.85 (d, 1H, Ar–H), 7.07 (m, 2H, Ar–H), 7.62 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 479 $[M+Na]^+$.

(E)-6-(nitroxy)hexyl-3-(3-methoxy-4-(6-(nitroxy) hexyloxy)phenyl)acrylate (9d)

The title compound was obtained in 17% as yellow solid starting from **7d**; mp 55.8–56.8°C. 1H NMR ($CDCl_3$) δ 1.45–1.52 (m, 8 H, CH_2), 1.70–1.89 (m, 8H, CH_2), 3.89 (s, 3H, OCH_3), 4.05 (t, J = 6.6 Hz, 2H, $ArOCH_2$), 4.20 (t, J = 6.6 Hz, 2H, $COOCH_2$), 4.46 (t, J = 6.6 Hz, 4H, CH_2ONO_2), 6.30 (d, J = 15.9 Hz, 1H, C=CH), 6.85 (d, 1H, Ar–H), 7.07 (m, 2H, Ar–H), 7.62 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 507 $[M+Na]^+$.

(E)-3-(4-(ethoxycarbonyloxy)-3-methoxyphenyl) acrylic acid (10)

To a stirring solution of FA (11.6 g, 60 mmol) dissolved in 75 ml NaOH (1 mol/L) at room temperature was added ethyl chlorformate (7.1 ml, 75 mmol); then the reaction mixture was stirred at 50°C for 4 h. The solution was cooled to room temperature and then

poured into cold water, the solid obtained was filtered to give the target compound **10** in 90% yield as white solid that was used in the next step without purication; mp 177.4–178.4°C. 1H NMR ($CDCl_3$) δ 1.40 (t, J = 7.1 Hz, 3H, CH_3), 3.91 (s, 3H, OCH_3), 4.35 (q, J = 7.1 Hz, 2H, OCH_2), 6.48 (d, J = 15.9 Hz, 1H, C=CH), 7.15–7.19 (m, 3H, Ar–H), 7.82 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 289 $[M+Na]^+$.

General Procedure for the Preparation of 11(a–e) and 13(a–d)

To a stirring solution of **10** (2.1 mmol), DCC (433 mg, 2.1 mmol), and DMAP (25.5 mg, 0.21 mmol) in dry CH_2Cl_2 (40 ml) at room temperature was added **3** or **4** (2.1 mmol); then the reaction mixture was stirred at room temperature for 4–10 h, at last the reaction mixture was filtered. Removal of the solvent under vacuo followed by silica gel column chromatographic purification of the residue using 20% ethyl acetate in petroleum ether afforded the compounds (yields 36–83%).

(E)-(4-phenyl-1,2,5-oxadiazol-3-yl)methyl-3-(4-(ethoxycarbonyloxy)-3-methoxyphenyl)acrylate (11a)

The title compound was obtained in 83% yield as yellow solid starting from **10** and **3a**; mp 130.2–131.0°C. 1H NMR ($CDCl_3$) δ 1.39 (t, J = 7.1 Hz, 3H, CH_3), 3.88 (s, 3H, OCH_3), 4.32 (q, J = 7.1 Hz, 2H, OCH_2), 5.28 (s, 2H, CH_2), 6.38 (d, J = 15.9 Hz, 1H, C=CH), 7.09–7.18 (m, 3H, Ar–H), 7.53–7.58 (m, 3H, Ar–H), 7.66 (d, J = 15.9 Hz, 1H, CH=C), 7.73 (m, 2H, Ar–H). ESI-MS: 463 $[M+Na]^+$.

(E)-3-((4-phenyl-1,2,5-oxadiazol-3-yl)methoxy) phenyl-3-(4-(ethoxycarbonyloxy)-3-methoxyphenyl) acrylate (11b)

The title compound was obtained in 60% yield as yellow solid starting from **10** and **3b**; mp 109.2–111.2°C. 1H NMR ($CDCl_3$) δ 1.40 (t, J = 7.1 Hz, 3H, CH_3), 3.91 (s, 3H, OCH_3), 4.33 (q, J = 7.1 Hz, 2H, OCH_2), 5.11 (s, 2H, CH_2), 6.45 (d, J = 15.9 Hz, 1H, C=CH), 6.84–7.82 (m, 12H, Ar–H), 7.80 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 555 $[M+Na]^+$.

(E)-4-((4-phenyl-1,2,5-oxadiazol-3-yl)methoxy) phenyl-3-(4-(ethoxycarbonyloxy)-3-methoxyphenyl) acrylate (11c)

The title compound was obtained in 67% yield as yellow solid starting from **10** and **3c**; mp 92.7–93.6°C. 1H NMR ($CDCl_3$) δ 1.40 (t, J = 7.1Hz, 3 H, CH_3), 3.91 (s, 3H, OCH_3), 4.33 (q, J = 7.1 Hz, 2H, OCH_2), 5.08 (s, 2 H, CH_2), 6.57 (d, J = 15.9 Hz, 1H, C=CH), 6.76–7.82 (m, 12H, Ar–H), 7.81 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 555 $[M+Na]^+$.

(E)-3-((4-phenyl-1,2,5-oxadiazol-3-yl)methoxy) benzyl-3-(4-(ethoxycarbonyloxy)-3-methoxyphenyl) acrylate (11d)

The title compound was obtained in 55% yield as yellow oil starting from **10** and **3d**. 1H NMR ($CDCl_3$) δ 1.38 (t, J = 7.1 Hz, 3H, CH_3), 3.88 (s, 3H, OCH_3), 4.33 (q, J = 7.1 Hz, 2H, OCH_2), 5.15 (s, 2H, CH_2), 5.22 (s, 2H, Ar–CH_2), 6.45 (d, J = 15.9 Hz, 1H, C=CH), 6.90–7.92 (m, 12H, Ar–H), 7.71 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 569 $[M+Na]^+$.

(E)-4-((4-phenyl-1,2,5-oxadiazol-3-yl)methoxy) benzyl-3-(4-(ethoxycarbonyloxy)-3-methoxyphenyl) acrylate (11e)

The title compound was obtained in 55% yield as yellow solid starting from **10** and **3e**; mp 50.2–53.1°C. 1H NMR ($CDCl_3$) δ 1.38 (t, J = 7.1 Hz, 3H, CH_3), 3.87 (s, 3H, OCH_3), 4.32 (q, J = 7.1 Hz, 2H, OCH_2), 5.11 (s, 2H, CH_2), 5.19 (s, 2H, Ar–CH_2), 6.41 (d, J = 15.9 Hz, 1H, C=CH), 6.99–7.89 (m, 12H, Ar–H), 7.68 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 569 $[M+Na]^+$.

(E)-2-(4-(phenylsulfonyl)-1,2,5-oxadiazol-3-yloxy) ethyl-3-(4-(ethoxycarbonyloxy)-3-methoxyphenyl) acrylate (13a)

The title compound was obtained in 38% yield as yellow oil starting from **10** and **4a**. 1H NMR ($CDCl_3$) δ 1.39 (t, J = 7.1 Hz, 3H, CH3), 3.90 (s, 3H, OCH3), 4.33 (q, J = 7.1 Hz, 2H, OCH_2), 4.62 (m, 2H, $COOCH_2$), 4.71 (m, 2H, CH_2), 6.41 (d, J = 15.9 Hz, 1H, C=CH), 7.13–8.07 (m, 8H, Ar–H), 7.71 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 557 $[M+Na]^+$.

(E)-4-(4-(phenylsulfonyl)-1,2,5-oxadiazol-3-yloxy) butan-2-yl-3-(4-(ethoxycarbonyloxy)-3-methoxy-phenyl)acrylate (13b)

The title compound was obtained in 36% yield as white oil starting from **10** and **4b**. 1H NMR ($CDCl_3$) δ 1.40 (m, 6H, CH_3), 2.20 (m, 2H, CH_2), 3.89 (s, 3H, OCH_3), 4.30 (q, J = 7.1 Hz, 2H, OCH_2), 4.50 (t, J = 6.2 Hz, 2H, CH_2O), 5.24 (m, 1H, COOCH), 6.36 (d, J = 15.9 Hz, 1H, C=CH), 7.02–8.09 (m, 8H, Ar–H), 7.61 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 585 $[M+Na]^+$.

(E)-4-(4-(phenylsulfonyl)-1,2,5-oxadiazol-3-yloxy] butyl3-(4-(ethoxycarbonyloxy)-3-methoxyphenyl) acrylate (13c)

The title compound was obtained in 38% yield as white solid starting from **10** and **4c**; mp 107.5–108.0°C.

1H NMR ($CDCl_3$) δ 1.39 (t, J = 4.2 Hz, 3H, CH_3), 1.91 (m, 2H, CH_2), 2.03 (m, 2H, CH_2), 3.89 (s, 3H, OCH_3), 4.30 (m, 4H, OCH_2), 4.50 (t, J = 3.8 Hz, 2 H, CH_2O), 6.42 (d, J = 9.6 Hz, 1H, C=CH), 7.11–7.16 (m, 3H, Ar–H), 7.60–7.76 (m, 3H, Ar–H), 7.66 (d, J = 9.6 Hz, 1H, CH=C), 8.05–8.07 (m, 2H, Ar–H). ESI-MS: 585 $[M+Na]^+$.

(E)-2-(2-(4-(phenylsulfonyl)-1,2,5-oxadiazol-3-yloxy) ethoxy)ethyl3-(4-(ethoxycarbonyloxy)-3-methoxy-phenyl)acrylate 13d)

The title compound was obtained in 81% yield as white oil starting from **10** and **4d**. 1H NMR ($CDCl_3$) δ 1.41 (m, 3H, CH_3), 3.86 (s, 3H, OCH_3), 3.88–4.61 (m, 10H, OCH_2), 6.39 (d, J = 15.9 Hz, 1H, C=CH), 7.11–7.15 (m, 3H, Ar–H), 7.56–7.63 (m, 3H, Ar–H), 7.69 (d, J = 15.9 Hz, 1H, CH=C) 8.05–8.08 (m, 2 H, Ar–H). ESI-MS: 601 $[M+Na]^+$.

General Procedure for the Preparation of 12(a–e) and 14(a–d)

To a stirring solution of **11** or **13** (1.0 mmol) in 95% EtOH (20 ml) was added ethanolamine (0.26 ml, 4.0 mmol) at room temperature, after 30 min, 2 ml HCl (2 mol/L) was added to the reaction mixture; then the EtOH was removed under vacuo. The residue was diluted with water and extracted with EtOAc. The obtained organic layer was dried, filtered, and evaporated under vacuo. The crude product was purified by silica gel column chromatography using 30% ethyl acetate in petroleum ether afforded the compounds (yields 64–98%).

(E)-(4-phenyl-1,2,5-oxadiazol-3-yl)methyl 3-(4-hydroxy-3-methoxyphenyl) acrylate (12a)

The title compound was obtained in 89% yield as yellow solid starting from **11a**; mp 142.7–145.3°C. 1H NMR ($CDCl_3$) δ 3.92 (s, 3H, OCH_3), 5.26 (s, 2H, CH_2), 5.91 (s, 1H, OH), 6.27 (d, J = 15.9 Hz, 1H, C=CH), 6.91 (d, 1H, Ar–H), 7.00–7.08 (m, 2H, Ar–H), 7.52–7.57 (m, 3H, Ar–H), 7.63 (d, J = 15.9 Hz, 1H, CH=C), 7.73–7.77 (m, 2H, Ar–H). ESI-MS: 391 $[M+Na]^+$.

(E)-3-((4-phenyl-1,2,5-oxadiazol-3-yl)methoxy) phenyl-3-(4-hydroxy-3-methoxyphenyl)acrylate (12b)

The title compound was obtained in 64% yield as white solid starting from **11b**; mp 57.3–59.1°C. 1H NMR ($CDCl_3$) δ 3.95 (s, 3H, OCH_3), 5.10 (s, 2H, CH_2), 5.92 (s, 1H, OH), 6.45 (d, J = 15.9 Hz, 1H, C=CH), 6.84–7.86 (m, 12H, Ar–H), 7.80 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 483 $[M+Na]^+$.

(E)-4-((4-phenyl-1,2,5-oxadiazol-3-yl)methoxy) phenyl-3-(4-hydroxy-3-methoxyphenyl)acrylate (12c)

The title compound was obtained in 79% yield as yellow solid starting from **11c**; mp 123.2–126.7°C. 1H NMR ($CDCl_3$) δ 3.95 (s, 3H, OCH_3), 5.10 (s, 2H, CH_2), 5.93 (s, 1H, OH), 6.47 (d, J = 15.9 Hz, 1H, C=CH), 6.94–7.82 (m, 12H, Ar–H), 7.78 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 483 $[M+Na]^+$.

(E)-3-((4-phenyl-1,2,5-oxadiazol-3-yl)methoxy) benzyl-3-(4-hydroxy-3-methoxyphenyl)acrylate (12d)

The title compound was obtained in 73% yield as white solid starting from **11d**; mp 118.8–120.4°C. 1H NMR ($CDCl_3$) δ 3.92 (s, 3H, OCH_3), 5.13 (s, 2H, CH_2), 5.17 (s, 2H, $COOCH_2$), 5.91 (s, 1H, OH), 6.35 (d, J = 15.9 Hz, 1H, C=CH), 6.90–7.87 (m, 12H, Ar–H), 7.67 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 497 $[M+Na]^+$.

(E)-4-((4-phenyl-1,2,5-oxadiazol-3-yl)methoxy) benzyl-3-(4-hydroxy-3-methoxyphenyl)acrylate (12e)

The title compound was obtained in 91% yield as yellow oil starting from **11e**. 1H NMR ($CDCl_3$) δ 3.91 (s, 3H, OCH_3), 5.11 (s, 2H, CH_2), 5.18 (s, 2H, $COOCH_2$), 5.92 (b, 1H, OH), 6.31 (d, J = 15.9 Hz, 1H, C=CH), 6.89–7.83 (m, 12H, Ar–H), 7.64 (d, J = 15.9 Hz, 1H, CH=C). ESI-MS: 497 $[M+Na]^+$.

(E)-2-(4-(phenylsulfonyl)-1,2,5-oxadiazol-3-yloxy) ethyl-3-(4-hydroxy-3-methoxyphenyl)acrylate (14a)

The title compound was obtained in 82% yield as red solid starting from **13a**; mp 139.2–141.1°C. 1H NMR ($CDCl_3$) δ 3.94 (s, 3H, OCH_3), 4.61 (t, J = 2.4 Hz, 2H, CH_2O), 4.71 (t, J = 2.4 Hz, 2H, $COOCH_2$), 5.88 (s, 1H, OH), 6.31 (d, J = 15.9 Hz, 1H, C=CH), 7.05–7.12 (m, 3H, Ar–H), 7.63 (d, J = 15.9 Hz, 1H, CH=C), 7.54–7.73 (m, 3H, Ar–H), 8.06 (d, J = 7.3 Hz, 2H, Ar–H). ESI-MS: 485 $[M+Na]^+$.

(E)-4-(4-(phenylsulfonyl)-1,2,5-oxadiazol-3-yloxy) butan-2-yl-3-(4-hydroxy-3-methoxyphenyl)acrylate (14b)

The title compound was obtained in 98% yield as yellow solid starting from **13b**; mp 70.1–73.2°C. 1H NMR ($CDCl_3$) δ 1.40 (d, J = 6.2 Hz, 3H, CH_3), 2.22 (m, 2H, CH_2), 3.92 (s, 3H, OCH_3), 4.53 (t, J = 6.2 Hz, 2H, CH_2O), 5.26 (q, J = 6.2 Hz, 1H, COOCH), 5.90 (b, 1H, OH), 6.29 (d, J = 15.9 Hz, 1H, C=CH), 6.90–7.08 (m, 3H, Ar–H), 7.61 (d, J = 15.9 Hz, 1H, CH=C), 7.60–7.77 (m, 3H, Ar–H), 8.08 (m, 2H, Ar–H). ESI-MS: 513 $[M+Na]^+$.

(E)-4-(4-(phenylsulfonyl)-1,2,5-oxadiazol-3-yloxy) butyl-3-(4-hydroxy-3-methoxyphenyl)acrylate (14c)

The title compound was obtained in 92% yield as light brown solid starting from **13c**; mp 143.3–146.9°C. 1H NMR ($CDCl_3$) δ 1.88–2.05 (m, 4H, $2CH_2$), 3.91 (s, 3H, OCH_3), 4.30 (t, J = 6.2 Hz, 2H, CH_2O), 4.50 (t, J = 6.2 Hz, 2H, $COOCH_2$), 5.88 (b, 1H, OH), 6.29 (d, J = 15.9 Hz, 1H, C=CH), 6.91–7.09 (m, 3H, Ar–H), 7.62 (d, J = 15.9 Hz, 1H, CH=C), 7.62–7.77 (m, 3H, Ar–H), 8.06 (m, 2H, Ar–H). ESI-MS: 513 $[M+Na]^+$.

(E)-2-(2-(4-(phenylsulfonyl)-1,2,5-oxadiazol-3-yloxy) ethoxy)ethyl-3-(4-hydroxy-3-methoxyphenyl) acrylate (14d)

The title compound was obtained in 87% yield as white solid starting from **13d**; mp 115.5–115.8°C. 1H NMR ($CDCl_3$) δ 3.86 (t, J = 4.7 Hz, 2H, OCH_2), 3.91 (s, 3H, OCH_3), 3.93 (t, J = 3.5 Hz, 2H, CH_2O), 4.39 (t, J = 4.7 Hz, 2H, CH_2O), 4.59 (t, J = 3.5 Hz, 2H, $COOCH_2$), 5.89 (b, 1H, OH), 6.29 (d, J = 15.9 Hz, 1H, C=CH), 6.88–7.09 (m, 3H, Ar–H), 7.63 (d, J = 15.9 Hz, 1H, CH=C), 7.51–7.74 (m, 3H, Ar–H), 8.06 (m, 2H, Ar–H). ESI-MS: 529 $[M+Na]^+$.

BIOLOGICAL SCREENING

Antioxidant Assays by Evaluation of DPPH Radical Scavenging Activity and Inhibition of Lipid Peroxidation in Rat Liver Homogenate

DPPH assay measures the hydrogen-donating ability of antioxidants to convert the stable DPPH free radical into 1,1-diphenyl-2-picrylhydrazine [Brand-Williams et al., 1995]. The reaction is accompanied by a change in color from deep-violet to light-yellow and is monitored spectrophotometrically. FA serves as reference antioxidants in this assay. DPPH radical scavenging activity was determined using the method of Wang et al. [Wang et al., 2008] with minor modifications. The solution of the sample (10 μl) in ethanol was added to 90 μl of a 0.1 mM DPPH radical in ethanol in a 96-well plate. The sample solution refers to the tested compounds and the reference antioxidants at various concentrations, as well as ethanol as a control. The solutions of the tested compounds had concentrations ranging from 3 μg/ml to 1,000 μg/ml, whereas the concentrations of the reference compounds solutions varied from 0.1 μg/ml to 1,000 μg/ml. The reaction leading to the scavenging of DPPH radical was complete within 10 min at 25°C. The absorbance of the mixture was then measured at 517 nm using a microplate reader. The reduction of DPPH radical was expressed as a percentage:

$$\text{Scavenged DPPH (\%)} = (1 - A_{test}/A_{control}) \times 100, \quad (1)$$

where A_{test} is the absorbance of a sample at a given concentration after 10 min reaction time and $A_{control}$ is the absorbance recorded for 10 μl ethanol. The EC_{50} value is defined as the concentration of sample that causes 50% loss of the DPPH radical.

All the final compounds were assessed as inhibitors of ferrous salt/ascorbate induced peroxidation of membrane lipids of rat hepatocytes. FA was used for comparison. The 2-thiobarbituric acid (TBA) assay was used to follow the progress of the autooxidation. The procedure involves the detection of the final metabolites of the lipid autooxidation, namely 2-thiobarbituric acid reactive substances (TBARS) by visible spectroscopy [Buege and Aust, 1978]. Female Wister rats weighing 250 ± 20 g were starved overnight before cervical dislocation. Liver microsomes were prepared by tissue homogenization with ice-cold 0.25 M sucrose–0.01 M Tris buffer, pH 7.4, with 1 mM EDTA (STE buffer), in a motor-driven glass homogenizer. Microsomal fractions were isolated by the removal of nuclear faction at 8,000 *g* for 20 min and removal of mitochondrial fraction at 18,000 *g* for 10 min. The microsomal fraction was sedimented in a CP100MX ultracentrifuge at 105,000 *g* for 60 min, washed two times with 0.15 M KCl at 105,000 *g* for 30 min. The membranes, suspended in 0.1 M potassium phosphate buffer, pH 7.5, were stored in a deep freezer maintained at −20°C. Rat liver microsomes were incubated at 37°C in a Tris-HCl/KCl buffer (100 mM, 150 mM, pH 7.4), containing microsomal membranes (2 mg ml^{-1}), ascorbic acid (100 μM), and either ethanol solutions of the tested compounds or ethanol alone. Lipid peroxidation was initiated by the addition of $FeSO_4$ (2.5 μm). Aliquots were taken from the incubation mixture at 5, 15, and 30 min and were treated with trichloroacetic acid (TCA, 10% w/v) and 2-thiobarbituric acid (TBA, 2% w/v). Lipid peroxidation was assessed by spectrophotometric (λ = 532 nm) determination [25] of the TBARS consisting mainly of malondialdehyde (MDA), and TBARS concentrations (expressed in nmol mg^{-1} protein) were obtained by interpolation with an MDA standard curve. The antioxidant activity of tested compounds was evaluated the percentage inhibition of TBARS production with respect to control samples treated with ethanol alone. IC_{50} values were calculated by nonlinear regression analysis.

NO Release Assay In Vitro

The ability of the target compounds to release NO was indirectly evaluated through their capacity to

produce nitrite in physiological pH 7.4 buffered water and kept at 37°C for 2 and 4 h in the presence of a strong excess of cysteine (1:50). Nitrite was the most important product of the oxidation of NO in aerobic aqueous solution. Detection of nitrite can be used to infer the previous presence of NO. It was detected by Griess reaction, according to the procedure previously described [Sorba et al., 1997]. Isosorbide dinitrate (ISDN) was chosen as a positive control. A solution of the appropriate 80 µl furoxan (0.01 mol/L) in DMSO was added to 8 ml phosphate buffer (pH 7.4) containing 5 mmol/L L-cysteine. The final concentration of drug was 10^{-4} mol/L. After 2 h at 37°C, 100 µl of the reaction mixture was treated with 100 µl of the Griess reagent [sulfanilamide (4 g), *N*-naphthylethylenediamine dihydrochloride (0.2 g), and 85% phosphoric acid (10 ml) in distilled water (final volume: 100 ml)]. After 10 min at room temperature, the absorbance was measured at 550 nm; 6.25–200 µmol/ml sodium nitrite standard solutions were used for the calibration curve. Detection of nitrite can be used to infer the previous presence of NO.

Vasodilator Activity

The vasodilating activity of the target compounds was determined through an in vitro vascular relaxation assay (organ bath) using $PGF_{2\alpha}$-precontracted porcine pulmonary arteries. ISDN was chosen as positive controls. Pulmonary arteries were cut into rings (2 mm long, 1.5–2-mm diameter) [Klinger and Müller, 1974]. The rings were suspended between two L-shaped platinum hooks and mounted in a 10-ml organ bath filled with modified a Krebs–Henseleit solution. The solution was kept at 37°C and aerated with 95% O_2/5% CO_2. Preparations were connected to an isometric force transducer (BL-410, Chengdu Technology and Market, China) for continuous measurement of changes in tension. A resting tension of 20 mN was maintained throughout the experiment. After an equilibration period of 60 min, contractions were induced at intervals of 45 min. The ring segments were initially made to contract with KCl (45 mM) and subsequently with $PGF_{2\alpha}$ ($2–3 \times 3$ mM) until the contractions became constant. Endothelial integrity was assessed by the bradykinin (10 nM)-induced relaxation of $PGF_{2\alpha}$-precontracted vessels. In mechanically endotheliumdenuded arterial rings, pretreated with 0.2 mM L-NAME, the relaxation induced by bradykinin was less than 10%. The relaxation response to test compounds was studied after the second or third $PGF_{2\alpha}$-induced contraction had stabilized, by constructing a cumulative concentration-response curve. Results are expressed as $EC_{50} \pm SE$ (µM).

RESULTS AND DISCUSSION

Antioxidant Activity

Table 1 summarizes the results obtained in the DPPH and lipid peroxidation assay. The dual nitrates **9**, where both the hydroxyl groups were occupied by nitrates, were screened as side-products in the reaction and had no antioxidant activity. While the mono-nitrates **8**, phenylfuroxan nitrates **12** and phenylsulfonylfuroxan nitrates **14** had a higher antioxidant activity than the dual nitrates **9**, suggesting the FA phenolic hydroxyl group was required for free radical scavenging activity. In the series of mono-nitrates **8**, the saturated nitrates (**8a–8d**) showed a higher antioxidant activity than the unsaturated one (**8e**). Furthermore, the result showed that the antioxidant activity decreased as the number of atoms in the nitric esters increased, such as **8c** (IC_{50} 69.3 µM for DPPH, 67.2 µM for lipid peroxidation) and **8d** (IC_{50} 94.1 µM for DPPH, 99.7 µM for lipid peroxidation). In the series of phenylfuroxan nitrates **12**, no substituent between FA and phenylfuroxan **12a** (IC_{50} 98.3 µM for DPPH, 104.4 µM for lipid peroxidation) showed a much higher antioxidant activity than analogues with benzene ring substituent (**12b**, **12c**, **12e**), with **12d** as an exception (IC_{50} 85.6 µM for DPPH, 94.8 µM for lipid peroxidation). For the series of phenylsulfonylfuroxan nitrates **14**, the butyl ether derivatives **14b** (IC_{50} 83.4 µM for DPPH, 89.7 µM for lipid peroxidation) and **14c** (IC_{50} 66.9 µM for DPPH, 72.1 µM for lipid peroxidation) had a much higher antioxidant activity than the diethyl ether derivatives **14a** (IC_{50} 154.3 µM for DPPH, 165.4 µM for lipid peroxidation) and **14d** (IC_{50} 124.7 µM for DPPH, 130.4 µM for lipid peroxidation).

Nitric Oxide Release Assay In Vitro

Comparison of the data reported in Table 2 indicates that the mono-nitrates **8** generated the

TABLE 1. In Vitro Antioxidant Activity of NO-Donor-Ferulic Acid Hybrids (IC_{50} in µM) in DPPH and Lipid Peroxidation Assays

Compd.	DPPH (µM)	Lipid peroxidation (µM)	Compd.	DPPH (µM)	Lipid peroxidation (µM)
8a	31.3	39.1	**12a**	98.3	104.4
8b	54.9	56.9	**12b**	165.6	174.7
8c	69.3	67.2	**12c**	196.5	203.2
8d	94.1	99.7	**12d**	85.6	94.8
8e	258.3	260.4	**12e**	256.4	175.4
9a	>1,000	>1,000	**14a**	154.3	165.4
9b	>1,000	>1,000	**14b**	83.4	89.7
9c	>1,000	>1,000	**14c**	66.9	72.1
9d	>1,000	>1,000	**14d**	124.7	130.4
FA	16.2	17.6			

TABLE 2. NO Generation Properties and Vasodilating Potency of the Target Compounds

	NO_2^- (μM)				NO_2^- (μM)		
Compd.	2 h	4 h	EC_{50} (μM)	Compd.	2 h	4 h	EC_{50} (μM)
8a	1.63±0.24	2.61±0.48	13.49±1.09	**12a**	10.70±0.78	12.22±0.46	0.7147±0.0054
8b	1.00±0.25	1.34±0.28	16.85±1.56	**12b**	4.11±0.37	3.44±0.25	1.6438±0.0091
8c	1.05±0.39	1.24±0.26	14.96±1.17	**12c**	3.71±0.42	2.10±0.17	1.8342±0.0094
8d	1.10±0.30	0.96±0.20	14.38±1.27	**12d**	6.58±0.65	5.17±0.29	1.6253±0.0089
8e	1.79±0.38	2.27±0.23	12.67±1.43	**12e**	3.92±0.31	3.16±0.24	1.7214±0.0076
9a	9.95±1.25	8.13±0.83	0.7267±0.0088	**14a**	22.32±1.14	35.19±1.21	0.1754±0.0091
9b	11.27±1.24	8.37±0.83	0.5231±0.0059	**14b**	22.06±1.33	34.86±1.40	0.1143±0.0058
9c	10.45±0.76	9.83±0.60	0.6418±0.0068	**14c**	26.00±1.32	36.79±1.95	0.0928±0.0046
9d	10.52±0.45	9.52±0.52	0.5897±0.0082	**14d**	27.53±1.17	38.88±1.64	0.1039±0.0077
ISDN	12.14±1.17	14.23±0.94	0.1123±0.0067	FA	—	—	—

minimum amount of NO, and the introduction of different substituents between FA and nitrate does not modify the extent of the NO release ability induced by the thiol cofactor, such as **8a** and **8e**, the nitrites detected were 1.63 μM and 1.79 μM, respectively, after 2 h at 37°C when treated with 100 μl Griess reagent. The di-nitrates **9** showed a stronger NO release ability when compared with the mono-nitrates **8**, and were as potent as ISDN taken as reference, however, the NO release time of di-nitrates **9** was much shorter, such as **9a**, the nitrites detected were 9.95 μM after 2 h at 37°C, but they were reduced into 8.13 μM after 4 h at 37°C. The phenyl-substituted furoxans **12** showed a slightly stronger NO release ability and were 3–10 times stronger than mono-nitrates **8**; most of these series compounds showed a much shorter NO release time with **12a** as an exception, and the nitrites of **12a** detected were added from 10.70 μM after 2 h into 12.22 μM after 4 h. In the phenylsulfonyl series **14** all the compounds displayed good NO donating properties, and the nitrites detected were about two times more than that of the positive control ISDN after 2 h at 37°C, such as **14a**, the nitrites detected after 2 h were 22.32 μM, while the nitrites detected after 2 h from ISDN were only 12.14 μM. Furthermore, the substituents between FA and the phenylsulfonyl nitrate showed that the NO release activity increased with the side chain became much longer, such as **14a** and **14d**, with the ether group increased from ethyl ether into diethyl ether, the nitrites detected after 2 h increased from 22.32 μM into 27.53 μM. In the homologues **14b** and **14c**, the nitrites detected after 2 h increased from 22.06 μM into 26.00 μM with the side chain between FA and the phenylsulfonyl nitrate changed from isobutyl group into butyl group.

Vasodilating Activity

Analysis of EC_{50} values reported in Table 2 indicates that the compounds covered a wide range of vasodilating potency. The EC_{50} values followed the sequence **8**>**12**>**9**>**14**, and the most active compounds belonged to the phenylsulfonyl series **14**. **14b** (EC_{50} 0.1143 μM) and **14d** (EC_{50} 0.1039 μM) showed a roughly similar vasodilating activity to those of ISDN (EC_{50} 0.1123 μM). Importantly, Derivative **14c**, which showed the most potent antioxidant activity (72.1 μM for anti-lipid peroxidation), was more potent than ISDN in in vitro vascular relaxation assay (EC_{50} values were 0.0928 μM and 0.1123 μM, respectively).

Previous study has shown that FA exhibited the antioxidant activity of plasma and the resistance of LDLs to oxidation [Ohta et al., 1997; Laranjinha et al., 1996; Andreasen et al., 2001]. In the present study, we found that esterification of FA with phenylsulfonyl group through different side chains could afford some derivatives with high antioxidant, NO release, and vasodilating activity; one of the most important examples was **14c**, which could potentially be used for therapeutic intervention of atherosclerosis in the clinic.

CONCLUSIONS

In summary, we have designed and synthesized 18 novel ferulic acid hybrid compounds with three different NO-donating groups (e.g., nitric ester, 4-hydroxyl-3-phenylfuroxan, and 4-hydroxymethyl-3-phenylsulfonylfuroxan), via alkyl or benzyl spacer. The target phenylsulfonylfuroxan compounds **14**, in particular while maintaining antioxidant activity, showed more NO release activity and vasodilating activity compared with ISDN. Compound **14c** moderately relaxed the porcine pulmonary arteries in in vitro vasorelaxation experiments (organ bath), aided by the NO donor part of the molecule. The results suggest that these NO-donor-ferulic acid hybrids, especially compound **14c**, may be considered to be a novel more potent drug candidate for anti-atherosclerosis.

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