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Research Article

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Ligustrazine Derivatives. Part 4: Design, Synthesis, and Biological Evaluation of Novel Ligustrazine-based Stilbene Derivatives as Potential Cardiovascular Agents

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A series of novel stilbene derivatives containing ligustrazinyl moiety was designed, synthesized, and assayed for their protective effects on damaged endothelial cells. The results showed that most ligustrazinyl stilbene derivatives exhibited high protective effects on the human umbilical vascular endothelial cells (HUVECs) damaged by hydrogen peroxide in comparison with Ligustrazine. The stilbene derivatives A6, A9, A11, A21, A24, A25, and A27 exhibited high potency with low EC_{50} values ranged from 0.0249 μM to 0.0898 mM. Compound A27 displayed EC_{50} 0.0249 μM, which is 30 000 times higher than that of Ligustrazine, presenting a most promising lead for further investigation. Structure–activity relationships were briefly discussed.

Key words: cardiovascular activity, ligustrazine, stilbene derivatives, synthesis

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Substituted pyrazine derivatives with a broad spectrum of biological activities play a very important role in pharmaceutical applications [1,2], especially in the therapeutic effects on cardiovascular diseases, such as antihypertensive, vasodilating effects, and anti-cerebrocardiac ischemia. Currently, structurally based pyrazine drugs, such as Ligustrazine, amiloride, pyrazinoylguanidine, and their derivatives, have been widely used in the clinical for treatment of cardiovascular diseases [3–8].

Ligustrazine (Tetramethyl pyrazine, TMP, see Figure 1), a major efficient component from the Chinese traditional medicinal herb *Chuanxiong(Ligusticum wallichii Franchat)*, is widely used in China as a new kind of calcium antagonist for treatment of coronary atherosclerotic cardiovascular disease and ischemic cardiovascular disease [9–12]. However, pharmacokinetics studies have shown that Ligustrazine is rapidly absorbed when taken orally, but it is also rapidly excreted in the urine with a short half-life ($T_{1/2}$ = 2.89 h), displaying a low bioavailability [13]. To maintain high blood levels for keeping an effective plasma concentration and obtaining the desired effects, oral doses (100 mg or more each time) must be taken every few hours. Eventually, the accumulative toxicity often appeared in the patients and compromised the therapy [14]. Therefore, it is necessary to develop new generations of TMP class of cardiovascular drugs through molecular modification. In our previous work, we designed and synthesized a series of novel Ligustrazine derivatives by incorporation of Ligustrazine with pharmacophores or drug-like groups from active cardiovascular agents. Biological evaluation has discovered several lead compounds that are now under preclinical evaluation [15–17].

In continuation of our research work, a series of novel stilbene derivatives containing Ligustrazinyl moiety was designed based on the structure of natural product resveratrol (see Figure 1), which is one kind of active stilbenes that convey a number of health benefits to humans, for example, acting as vasodilator, free radical scavenger and antioxidant, anti-platelet aggregation, and anti-atherosclerosis agents, for prevention and treatment of cardiovascular disease and ischemia [18]. Holding the basic skeleton of the resveratrol as the model, the ligustrazinyl group was introduced to stilbene by replacement of the phenyl group, according to the bioisosteric replacement principle of medicinal chemistry. Totally, **27** novel Ligustrazine-based stilbene derivatives (**A,** see Figure 1) were synthesized to find more potent cardiovascular agents and obtain useful information for structure–activity relationship study.

Materials and Methods

Melting points were determined on a Gallenkamp capillary apparatus and are uncorrected. ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) spectra were obtained on a Bruker Avance-600 instrument in the indicated solvent. Chemical shifts are expressed in δ units with tetramethylsilane (TMS) as internal reference. Infrared spectra (IR) were recorded with a Nexus 470FT-IR Spectrometer. Mass spectra were recorded on a LC Autosampler Device: Standard

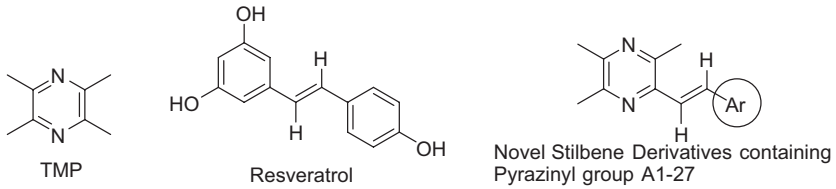


Figure 1: Structures of Ligustrazine, resveratrol and novel Ligustrazine-based stilbenes **A1**–**27**.

G1313A instrument. All compounds were routinely checked by thin-layer chromatography (TLC) on precoated silica gel G plates with fluorescent indicator at 254 nm, which were prepared in our laboratory. Developed plates were visualized by UV light. Solvents were of reagent grade and, when necessary, were purified and dried by standard methods. Concentration of the reaction solutions involved the use of rotary evaporator under reduced pressure.

General procedure for the synthesis of target compounds

(3,5,6-Trimethylpyrazin-2-yl)methanol (4)

Compound (**4**) was prepared according to our previously reported method by using one-pot reaction [15]. The crude product was purified by recrystallization with n-hexane to obtain yellow needles, mp 88–89°C, with total yeld 64%.

2-Chloromethyl-3,5,6-trimethylpyrazine (5)

Thionyl chloride (7.4 mL, 102 mmol) was added dropwise to (3,5,6-trimethylpyrazin-2-yl)methanol (**4**) (15.5 g, 102 mmol) in anhydrous CH_2Cl_2 (300 mL) at 0°C. The mixture was allowed to stand for 2.5 h, checking for product formation via TLC. The solvent was evaporated *in vacuo*, and the crude product 2-chloromethyl-3,5,6-trimethylpyrazine hydrochloride was obtained as a yellow solid (21.1 g, 100%), mp 102–105 °C. 5% KOH ethanol solution (100 mL) was added to the hydrochloride salt, and the mixture was stirred for 5 min and filtered. The filtrate was concentrated under reduced pressure to afford 2-chloromethyl-3,5,6-trimethylpyrazine (**5**).

3,5,6-trimethpyrazine-2-carbaldehyde (6)

Under vigorous stirring, anhydrous H_3PO_4 (0.51 g, 5 mmol) was added dropwise to the anhydrous DMSO solution containing (3,5,6-trimethylpyrazin-2-yl)methanol (**4**) (1.52 g, 10 mmol) and N,N′-dicyclohexylcarbodiimide (4.25 g, 20 mmol). When the mixture solution was cooled to room temperature, the solid was filtered off and washed by ethyl acetate. The combined filtrate was added with equal volume of water, and the aqueous layer was neutralized with sodium hydroxide solution (pH 9–10). The organic layer was separated and further extracted by ethyl acetate in three times. The combined organic layer was dried over anhydrous Na_2SO_4, and the solvent was removed under vacuum. The yellowish solid was recrystallized from *n*-hexane to obtain 3,5,6-trimethpyrazine-2-carbaldehyde (**6**) as white crystal (0.75 g, 50%), mp 79–80 °C. IR (KBr, cm^{-1}): 1598 ($\nu_{C=O}$), 1445, 1416 ($\nu_{C=N}$); ^{1}H-NMR ($CDCl_3$,δ ppm): 10.18(1H, s, -CHO), 2,83 (3H, s, -CH_3), 2.629 (3H, s, -CH_3), 2.626 (3H, s, -CH_3); ESI-MS: *m/z* 151.5 (M + 1). $C_8H_{10}N_2O$ (Exact Mass: 150.08).

General procedure for the preparation of stilbene derivatives containg pyrazinyl group (A1–17, A20–24 and A27, Method 1 of Scheme 1).

Freshly distilled triethyl phosphate (1.92 mL, 9.7 mmol) was added to 2-chloromethyl -3,5,6-trimethylpyrazine (**5**) (1.65 g, 9.7 mmol). The obtained mixture solution was heated under reflux till the reaction was finished (checked for product formation of diethyl benzylphosphonate *via* TLC). Subsequently, anhydrous tetrahydrofuran (THF) (10 mL) and 60% NaH (0.78 g, 19.4 mmol) were added in portion at −5 °C. After further stirring for 40 min, a solution of aromatic aldehyde (9.7 mmol) in anhydrous THF (25 mL) was slowly added. The resulting mixture was allowed to reach room temperature and kept to stir for 12 h. When the reaction was completed, the mixture was extracted with ethyl acetate, and the combined organic extracts were washed with water, followed by brine and then dried over Na_2SO_4. Evaporation of the solvent in vacuo gave the crude products (**A1–17, A20–24 and A27**), which were purified by flash column chromatography and recrystallized from methanol.

(E)-2,3,5-trimethyl-6-styrylpyrazine (A1)

Benzaldehyde was used as material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:10 and purified by recrystallization from methanol to give yellow crystals, mp 60–62 °C. Yield: 49%. IR (KBr, cm^{-1}): 3058 ($\nu_{=CH}$), 1626 ($\nu_{CH=CH}$), 1595, 1574, 1491, 1450 ($\nu_{CH=CH,}$ Ar), 1405, 1395 ($\nu_{C=N}$), 746, 691 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 7.78 (1H, d, =CH-, *J* = 15.7 Hz), 7.28 (1H, d, =CH-, *J* = 15.4 Hz,), 7.60 (2H, d, Ar-H, *J* = 7.3 Hz), 7.38 (2H, t, Ar-H, *J* = 7.5 Hz), 7.30 (1H, t, Ar-H, *J* = 7.3 Hz), 2.62 (3H, s, -CH_3), 2.54 (3H, s, -CH_3), 2.51 (3H, s, -CH_3); ^{13}C-NMR ($CDCl_3$, δppm): 149.53, 149.22, 147.04, 145.38 (pyrazine-C), 136.88, 134.03, 128.71, 128.37, 127.19, 122.89 (-CH=CH-Ar-C); 21.83 (-CH_3), 21.75 (-CH_3), 20.99 (-CH_3). ESI-MS: *m/z* 225.3(M + 1). $C_{15}H_{16}N_2$ (Exact Mass: 224.13).

(E)-2-(3-hydroxylstyryl)-3,5,6-trimethylpyrazine (A2)

3-Hydroxybenzaldehyde was used as material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane (1:5) and purified by recrystallization from methanol to obtain compound **A2** as white crystals, mp 202–204 °C. Yield: 40%. IR (KBr,cm^{-1}): 3055 ($\nu_{=CH}$), 1631 ($\nu_{CH=CH}$), 1607, 1578, 1489, 1468 ($\nu_{CH=CH,}$ Ar), 1407 ($\nu_{C=N}$), 872, 778, 687 ($\gamma_{=CH}$); ^{1}H-NMR (DMSO, δppm): 9.49 (1H, s, -OH), 7.59 (1H, d, =CH-, *J* = 15.6 Hz), 7.32 (1H,

d, =CH-, J = 15.6 Hz), 7.20 (1H, t, Ar-H, J = 7.8 Hz), 7.13 (1H, d, Ar-H, J = 7.7 Hz), 7.10 (1H, s, Ar-H), 6.72 (1H, d, Ar-H, J = 7.3 Hz), 2.56 (3H, s, -CH_3), 2.50 (3H, s, -CH_3), 2.44 (3H, s, -CH_3); ^{13}C-NMR (DMSO, δppm): 149.41, 178.67, 146.25, 137.63 (pyrazine-C), 157.54, 137.63, 133.02, 129.62, 122.77, 118.26, 115.51, 113.41 (-CH=CH-Ar-C), 21.31 (-CH_3), 21.25 (-CH_3), 20.40 (-CH_3); ESI-MS: *m/z* 241.4 (M + 1). $C_{15}H_{16}N_2O$ (Exact Mass: 240.13).

(E)-2-(3-chlorostyryl)-3,5,6-trimethylpyrazine (A3)

3-Chlorobenzaldehyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:5 and purified by recrystallization from methanol to give yellow crystals, mp 92–94 °C. Yield: 27%. IR (KBr, cm^{-1}): 3054 ($\nu_{=CH}$), 1631 ($\nu_{CH=CH}$), 1589, 1559, 1473 ($\nu_{CH=CH,}$ Ar), 1424, 1405 ($\nu_{C=N}$), 872, 799, 685 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$,δppm): 7.73 (1H, d, =CH-, J = 15.6 Hz), 7.29 (1H, d, =CH-, J = 15.7 Hz), 7.60 (1H, s, Ar-H), 7.47 (1H, d, Ar-H, J = 7.6 Hz), 7.32 (1H, t, Ar-H, J = 7.9 Hz), 7.29 (1H, d, Ar-H, J = 7.4Hz), 2.64 (3H, s, -CH_3), 2.56 (3H, s, -CH_3), 2.54 (3H, s, -CH_3); ^{13}C-NMR ($CDCl_3$,δppm): 150.62, 149.27, 147.29, 144.85 (pyrazine-C), 138.91, 134.72, 132.52, 129.93, 128.19, 126.84, 125.50, 124.39 (-CH=CH-Ar-C); 21.78, 20.96 (3-CH_3); ESI-MS: *m/z* 259.2 (M + 1). $C_{15}H_{15}ClN_2$ (Exact Mass: 258.09).

(E)-2-(4-fluorostyryl)-3,5,6-trimethylpyrazine (A4)

3-Fluorobenzaldehyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:5 and purified by recrystallization from methanol to give yellow crystal, mp 88–90 °C. Yield: 43%. IR (KBr,cm^{-1}): 3058 ($\nu_{=CH}$), 1635 ($\nu_{CH=CH}$), 1599, 1508 ($\nu_{CH=CH,}$ Ar), 1447, 1416 ($\nu_{C=N}$), 824 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 7.77 (1H, d, =CH-, J = 15.5 Hz), 7.20 (1H, d, =CH-, J = 15.4 Hz), 7.58 (2H, t, Ar-H, J = 8.3 Hz), 7.08 (2H, t, Ar-H, J = 8.4 Hz), 2.66 (3H, s, -CH_3), 2.57 (3H, s, -CH_3), 2.56 (3H, s, -CH_3); ^{13}C-NMR ($CDCl_3$, δppm): 149.31, 148.83, 146.70, 144.90 (pyrazine-C), 132.49, 130.23, 128.46, 122.46, 115.46, 115.31 (-CH_2=CH_2-Ar-C), 21.44 (-CH_3), 21.41 (-CH_3), 20.79 (-CH_3); ESI-MS: *m/z* 243.4 (M + 1). $C_{15}H_{15}FN_2$ (Exact Mass: 242.12).

(E)-2-(2,5-dimethoxylstyryl)-3,5,6-trimethylpyrazine (A5)

2,5-Dimethoxyl benzaldehyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:3 and purified by recrystallization from methanol to give yellow crystal, mp 124–126 °C. Yield: 40%. IR (KBr,cm^{-1}): 3036 ($\nu_{=CH}$), 1619 ($\nu_{CH=CH}$), 1604, 1495, 1462 ($\nu_{CH=CH,}$ Ar), 1443,1417 ($\nu_{C=N}$), 839, 810, 708 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 8.03 (1H, d, =CH-, J = 15.8 Hz), 7.34 (1H, d, =CH-, J = 15.8 Hz), 7.18 (1H, sd Ar-H, J = 2.8 Hz), 6.87 (2H, m, Ar-H), 3.88 (3H, s, -OCH_3), 3.84 (3H, s, -OCH_3), 2.62 (3H, s, -CH_3), 2.56 (3H, s, -CH_3), 2.52 (3H, s, -CH_3); ESI-MS: *m/z* 285.3 (M + 1). $C_{17}H_{20}N_2O_2$ (Exact Mass: 284.15).

(E)-2-(3-methoxyl-2-hydroxylstyryl)-3,5,6-trimethylpyrazine (A6)

3-Methoxyl-2-hydroxyl benzaldehyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:3 and purified by recrystallization from methanol to give yellow crystal, mp 187–188 °C.Yield: 35%. IR (KBr, cm^{-1}): 3052 ($\nu_{=CH}$), 1625 ($\nu_{CH=CH}$), 1602, 1581, 1477 ($\nu_{CH=CH,}$ Ar), 1441, 1399 ($\nu_{C=N}$), 717($\gamma_{=CH}$); ^{1}H-NMR (DMSO, δppm): 9.02 (1H, s, -OH), 8.02 (1H, d, =CH-, J = 15.8 Hz), 7.35 (1H, d, =CH-, J = 15.8 Hz), 7.32 (1H, d, Ar-H, J = 7.7 Hz), 6.92 (1H, d, Ar-H, J = 7.8 Hz), 6.80 (1H, t, Ar-H, J = 7.8 Hz), 3.83 (3H, s, -OCH_3), 2.54 (3H, s, -CH_3), 2.50 (3H, s, -CH_3), 2.49 (3H, s, -CH_3); ^{13}C-NMR (DMSO, δppm): 148.89, 148.03, 146.80, 145.14 (pyrazine-C), 149.21, 144.88, 128.37, 123.73, 122.36, 119.12, 118.81, 111.54 (-CH=CH-Ar-C), 56.02 (-OCH_3), 21.59 (-CH_3), 21.49 (-CH_3), 20.71 (-CH_3); ESI-MS: *m/z* 271.4 (M + 1). $C_{16}H_{18}N_2O_2$ (Exact Mass: 270.14).

(E)-2-(2-hydroxylstyryl)-3,5,6-trimethylpyrazine (A7)

2-Hydroxyl benzaldehyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:3 and purified by recrystallization from methanol to give yellow crystal, mp 221–222 °C.Yield: 35%. IR (KBr,cm^{-1}): 3068 ($\nu_{=CH}$), 1620 ($\nu_{CH=CH}$), 1601, 1539, 1498, 1458 ($\nu_{CH=CH,}$ Ar), 1408 ($\nu_{C=N}$), 742 ($\gamma_{=CH}$); ^{1}H-NMR (DMSO, δppm): 9.90 (1H, s, -OH), 7.97 (1H, d, =CH-, J = 15.8Hz), 7.39 (1H, d, =CH-, J = 15.8 Hz), 7.69 (1H, d, Ar-H, J = 7.8 Hz), 7.13 (1H, t, Ar-H, J = 8.3 Hz), 6.89 (1H, d, Ar-H, J = 8.1 Hz), 6.83 (1H, t, Ar-H, J = 7.6 Hz), 2.54 (3H, s, -CH_3), 2.47 (3H, s, -CH_3), 2.43 (3H, s, -CH_3); ^{13}C-NMR (DMSO, δppm): 149.10, 148.73, 146.79, 145.40 (pyrazine-C), 153.67, 129.05, 128.77, 127.82, 124.11, 120.66, 120.20, 115.93 (-CH=CH-Ar-C), 21.28 (-CH_3), 21.25 (-CH_3), 20.55 (-CH_3); ESI-MS: *m/z* 241.2 (M + 1). $C_{15}H_{16}N_2O$ (Exact Mass: 240.13).

(E)-2-(2,3-dimethoxylstyryl)-3,5,6-trimethylpyrazine (A8)

2,3-Dimethoxyl benzaldehyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:5 and purified by recrystallization from methanol to give yellow crystal, mp 87–88 °C.Yield: 25%. IR (KBr,cm^{-1}): 3069 ($\nu_{=CH}$), 1634 ($\nu_{CH=CH}$), 1576, 1559, 1477 ($\nu_{CH=CH,}$ Ar), 1455, 1429 ($\nu_{C=N}$), 787 ($\gamma_{=CH}$); ^{1}H-NMR (DMSO, δppm): 7.92 (1H, d, =CH-, J = 15.9 Hz), 7.43 (1H, d, =CH-, J = 15.8 Hz), 7.44 (1H, d, Ar-H, J = 7.9 Hz), 7.11 (1H, t, Ar-H, J = 7.8 Hz), 7.03 (1H, d, Ar-H, J = 8.2 Hz), 3.83 (3H, s, -OCH_3), 3.76 (3H, s, -OCH_3), 2.56 (3H, s, -CH_3), 2.48 (3H, s, -CH_3), 2.44 (3H, s, -CH_3); ESI-MS: *m/z* 285.4 (M + 1). $C_{17}H_{20}N_2O_2$ (Exact Mass: 284.15).

(E)-2-(4-nitrostyryl)-3,5,6-trimethylpyrazine (A9)

4-Nitrobenzaldehyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:5 and purified by recrystallization from methanol to give

yellow crystal, mp 175–177 °C.Yield: 35%. IR (KBr, cm^{-1}): 3073 ($\nu_{=CH}$), 1694 ($\nu_{CH=CH}$), 1591, 1490, 1447 ($\nu_{CH=CH,}$ Ar), 1413 ($\nu_{C=N}$), 1339 (ν_{NO2}), 818 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 8.27 (2H, d, Ar-H, J = 8.7 Hz), 7.87 (1H, d, =CH-, J = 15.6 Hz), 7.44 (1H, d, =CH-, J = 15.6 Hz), 7.75 (2H, d, Ar-H, J = 8.7 Hz), 2.69 (3H, s, $-CH_3$), 2.59 (3H, s, $-CH_3$), 2.57 (3H, s, $-CH_3$); ESI-MS: m/z 270.6 (M + 1). $C_{15}H_{15}N_3O_2$ (Exact Mass: 269.12).

(E)-2-(4-methoxylstyryl)-3,5,6-trimethylpyrazine (A10)

4-Methoxyl benzaldehyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:5 and purified by recrystallization from methanol to give yellow crystal, mp 101–102 °C. Yield: 45%. IR (KBr, cm^{-1}): 3058 ($\nu_{=CH}$), 1625 ($\nu_{CH=CH}$), 1602, 1510, 1447 ($\nu_{CH=CH,}$ Ar), 1418 ($\nu_{C=N}$), 808 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 7.77 (1H, d, =CH-, J = 15.5 Hz), 7.15 (1H, d, =CH-, J = 15.6 Hz), 7.57 (1H, d, Ar-H, J = 8.7 Hz), 6.94 (1H, d, Ar-H, J = 8.7 Hz), 3.86 (3H, s, $-OCH_3$), 2.64 (3H, s, $-CH_3$), 2.56 (3H, s, $-CH_3$), 2.54 (3H, s, $-CH_3$); ESI-MS: m/z 255.4 (M + 1). $C_{16}H_{18}N_2O$ (Exact Mass: 254.14).

(E)-2-(3,4-dimethoxylstyryl)-3,5,6-trimethylpyrazine (A11)

3,4-Dimethoxyl benzaldehyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:5 and purified by recrystallization from methanol to give yellow crystal, mp 121–123 °C. Yield: 36%. IR (KBr, cm^{-1}): 3060 ($\nu_{=CH}$), 1629 ($\nu_{CH=CH}$), 1598, 1515, 1459 ($\nu_{CH=CH,}$ Ar), 1417 ($\nu_{C=N}$), 841, 811, 766 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 7.74 (1H, d, =CH-, J = 15.6 Hz), 7.14 (1H, d, =CH-, J = 15.6 Hz), 7.20 (1H, dd, Ar-H, J = 8.2 Hz), 7.13 (1H, s, Ar-H), 6.90 (1H, d, Ar-H, J = 8.3 Hz), 3.97 (3H, s, $-OCH_3$), 3.93 (3H, s, $-OCH_3$), 2.64 (3H, s, $-CH_3$), 2.55 (3H, s, $-CH_3$), 2.52 (3H, s, $-CH_3$); ^{13}C-NMR ($CDCl_3$, δppm): 149.28, 148.72, 146.50, 145.31 (pyrazine-C), 148.80, 133.59, 129.81, 121.87, 120.79, 120.30, 110.96, 109.46 (-CH=CH-Ar-C), 55.65 ($-OCH_3$), 55.49 ($-OCH_3$), 21.47 ($-CH_3$), 21.39 ($-CH_3$), 20.91 ($-CH_3$); ESI-MS: m/z 285.5 (M + 1). $C_{17}H_{20}N_2O_2$ (Exact Mass: 284.15).

2,3,5-trimethyl-6-((1E,3E)-4-phenylbuta-1,3-dienyl)pyrazine (A12)

β-Phenylacrolein was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:10 and purified by recrystallization from methanol to give yellow crystal, mp 118–120 °C. Yield: 28%. IR (KBr, cm^{-1}): 3023 ($\nu_{=CH}$), 1615 ($\nu_{CH=CH}$), 1607, 1533, 1487, 1450 ($\nu_{CH=CH,}$ Ar), 1403 ($\nu_{C=N}$), 749 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 7.61, 7.05, 6.85 (4H, -CH=CH-CH=CH-, J = 14.4 Hz), 7.49 (2H, d, Ar-H, J = 7.6 Hz), 7.36 (2H, t, Ar-H, J = 7.5 Hz), 7.27 (1H, Ar-H); ^{13}C-NMR ($CDCl_3$, δppm): 148.98, 148.84, 146.66, 145.17 (pyrazine-C), 136.86, 135.20, 134.13, 128.43, 127.64, 126.62, 126.34 (-CH=CH-CH=CH-Ar-C), 21.47($-CH_3$), 21.39 ($-CH_3$), 20.91 ($-CH_3$); ESI-MS: m/z 251.6 (M + 1). $C_{17}H_{18}N_2$ (Exact Mass: 250.15).

(E)-2-(3-methoxylstyryl)-3,5,6-trimethylpyrazine (A13)

3-Methoxyl benzaldehyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:5 and purified by recrystallization from methanol to give yellow crystal, mp 87–89 °C. Yield: 41%. IR (KBr,cm^{-1}): 3051 ($\nu_{=CH}$), 1629 ($\nu_{CH=CH}$), 1602, 1577, 1485, 1455 ($\nu_{CH=CH,}$ Ar), 1436, 1401 ($\nu_{C=N}$), 873, 778, 686 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 7.80 (1H, d, =CH-, J = 15.6 Hz), 7.27 (1H, d, =CH-, J = 15.6 Hz), 7.32 (1H, t, Ar-H, J = 7.8 Hz), 7.23 (1H, d, Ar-H, J = 7.7 Hz), 6.90 (1H, dd, Ar-H, J = 8.1 Hz), 3.88 (3H, s, $-OCH_3$), 2.68 (3H, s, $-CH_3$), 2.58 (6H, s, 2-CH_3); ^{13}C-NMR ($CDCl_3$, δppm): 149.63, 149.17, 147.14, 145.32 (pyrazine-C), 159.94, 138.47, 133.98, 129.68, 123.40, 119.83, 113.97, 112.63 (-CH=CH-Ar-C), 55.32 ($-OCH_3$), 21.79 ($-CH_3$), 21.75 ($-CH_3$), 21.01 ($-CH_3$); ESI-MS: m/z 255.8(M + 1). $C_{16}H_{18}N_2O$ (Exact Mass: 254.14).

(E)-2-(2,4-dimethoxylstyryl)-3,5,6-trimethylpyrazine (A14)

2,4-Dimethoxyl benzaldehyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:5 and purified by recrystallization from methanol to give yellow crystal, mp 142–144 °C. Yield: 48%. IR (KBr,cm^{-1}): 3069 ($\nu_{=CH}$), 1604, 1574, 1499, 1453 ($\nu_{CH=CH,}$ Ar), 1438, 1399 ($\nu_{C=N}$), 879, 831 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 7.99 (1H, d, =CH-, J = 15.8 Hz), 7.26 (1H, d, =CH-, J = 15.7 Hz), 7.56 (1H, d, Ar-H, J = 8.5 Hz), 6.55 (1H, d, Ar-H, J = 8.5 Hz), 6.50 (1H, s, Ar-H), 3.91 (3H, s, $-OCH_3$), 3.86 (3H, s, $-OCH_3$), 2.61 (3H, s, $-CH_3$), 2.55 (3H, s, $-CH_3$), 2.52 (3H, s, $-CH_3$); ^{13}C-NMR ($CDCl_3$, δppm): 160.81, 158.60, 148.28, 146.09 (pyrazine-C), 129.79, 128.96, 128.52, 124.63, 121.46, 119.04, 104.71, 98.23 (-CH=CH-Ar-C), 55.19 ($-OCH_3$), 55.10 ($-OCH_3$), 21.46 ($-CH_3$), 21.36 ($-CH_3$), 20.74 ($-CH_3$); ESI-MS: m/z 285.3(M + 1). $C_{17}H_{20}N_2O_2$ (Exact Mass: 284.15).

(E)-2-(2-furyl)-vinyl-3,5,6-trimethylpyrazine (A15)

2-Furaldehyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:20 and purified by recrystallization from methanol to give yellow crystal, mp 56–58 °C. Yield: 50%. IR (KBr, cm^{-1}): 3112 ($\nu_{=CH}$), 1636 ($\nu_{CH=CH}$), 1565, 1486, 1443 ($\nu_{CH=CH,}$ Ar), 1398 ($\nu_{C=N}$), 745 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 7.58 (1H, d, =CH-, J = 15.4 Hz), 7.18 (1H, d, =CH-, J = 15.4 Hz), 7.44 (1H, s, furan-H), 6.46–6.44 (2H, m, furan-H, J = Hz), 2.60 (3H, s, $-CH_3$), 2.53 (3H, s, $-CH_3$), 2.51 (3H, s, $-CH_3$); ^{13}C-NMR ($CDCl_3$, δppm): 149.35, 149.12, 147.08, 145.14 (pyrazine-C), 153.16, 142.82, 121.07, 120.95, 111.94, 111.03 (-CH=CH-thiophene-C), 21.80 ($-CH_3$), 21.73 ($-CH_3$), 20.88 ($-CH_3$); ESI-MS: m/z 215.2 (M + 1). $C_{13}H_{14}N_2O$ (Exact Mass: 214.11).

(E)-2-(2-fluorostyryl)-3,5,6-trimethylpyrazine (A16)

2-Fluorobenzaldem2hyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash

column chromatography with eluent of ethyl acetate/cyclohexane = 1:10 and purified by recrystallization from methanol to give yellow crystal, mp 77–80 °C. Yield: 18%. IR (KBr, cm^{-1}): 3057 ($\nu_{=CH}$), 1629 ($\nu_{CH=CH}$), 1577, 1487, 1456 ($\nu_{CH=CH,}$ Ar), 1404, 1391 ($\nu_{C=N}$), 743 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 7.90 (1H, d, =CH-, J = 15.8Hz), 7.39 (1H, d, =CH-, J = 15.8 Hz), 7.63 (1H, t, Ar-H, J = 7.7 Hz), 7.27 (1H, Ar-H), 7.17 (1H, t, Ar-H, J = 7.6 Hz), 7.10, 7.27 (1H, Ar-H), 2.64 (3H, s, -CH_3), 2.56 (3H, s, -CH_3), 2.54 (3H, s, -CH_3); ^{13}C-NMR ($CDCl_3$, δppm): 161.52, 159.85 (pyrazine-C), 129.37, 128.17, 126.75, 125.08, 123.98, 115.79, 115.64 (-CH=CH-Ar-C); 21.51 (-CH_3); ESI-MS: m/z 243.5 (M + 1). $C_{15}H_{15}FN_2$ (Exact Mass: 242.12).

(E)-2-(2,3,4-trimethoxylstyryl)-3,5,6-trimethylpyrazine (A17)

2,3,4-Trimethoxyl benzaldehyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:10 and purified by recrystallization from methanol to give yellow crystal, mp 64–67 °C. Yield: 24%. IR (KBr, cm^{-1}): 1624 ($\nu_{CH=CH}$), 1592, 1493, 1463 ($\nu_{CH=CH,}$ Ar), 1415 ($\nu_{C=N}$), 792 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 7.92 (1H, d, =CH-, J = 15.8 Hz), 7.30 (1H, d, =CH-, J = 15.8 Hz), 7.35 (1H, d, Ar-H, J = 8.7 Hz), 6.73 (1H, d, Ar-H, J = 8.7 Hz), 3.96 (3H, s, -OCH_3), 3.92 (3H, s, -OCH_3), 3.91 (3H, s, -OCH_3), 2.62 (3H, s, -CH_3), 2.56 (3H, s, -CH_3), 2.52 (3H, s, -CH_3); ESI-MS: m/z 315.3(M + 1). $C_{18}H_{22}N_2O_3$ (Exact Mass: 314.16).

(E)-2-(4-chlorostyryl)-3,5,6-trimethylpyrazine (A20)

4-Chorobenzaldehyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:10 and purified by recrystallization from methanol to give yellow crystal, mp 79–81 °C. Yield: 29%. IR (KBr, cm^{-1}): 3061 ($\nu_{=CH}$), 1633 ($\nu_{CH=CH}$), 1591, 1566, 1490, 1443 ($\nu_{CH=CH,}$ Ar), 1409 ($\nu_{C=N}$), 810 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 7.75 (1H, d, =CH-, J = 15.7 Hz), 7.26 (1H, d, =CH-, J = 15.7 Hz), 7.54 (2H, d, Ar-H, J = 8.2 Hz), 7.37 (2H, d, Ar-H, J = 8.5 Hz), 2.63 (3H, s, -CH_3), 2.56 (3H, s, -CH_3), 2.53 (3H, s, -CH_3); ^{13}C-NMR ($CDCl_3$, δppm):149.83, 149.21, 147.14, 145.05 (pyrazine-C), 135.51, 133.99, 132.68, 128.9, 128.32, 123.57 (-CH=CH-Ar-C); 21.73 (-CH_3), 21.71 (-CH_3), 20.91 (-CH_3); ESI-MS: m/z 259.2 (M + 1). $C_{15}H_{15}ClN_2$ (Exact Mass: 258.09).

(E)-2-(3,4,5-trimethoxylstyryl)-3,5,6-trimethylpyrazine (A21)

3,4,5-Trimethoxyl benzaldehyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:10 and purified by recrystallization from methanol to give yellow crystal, mp 132–134 °C. Yield: 22%. IR (KBr, cm^{-1}): 1628 ($\nu_{CH=CH}$), 1578, 1504, 1454 ($\nu_{CH=CH,}$ Ar), 1419 ($\nu_{C=N}$), 810 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 7.73 (1H, d, =CH-, J = 15.6 Hz), 7.17 (1H, d, =CH-, J = 15.6 Hz), 6.83 (2H, s, Ar-H), 3.94 (6H, s, -OCH_3), 3.90 (3H, s, -OCH_3), 2.65 (3H, s, -CH_3), 2.56 (3H, s, -CH_3), 2.53 (3H, s, -CH_3); ^{13}C-NMR ($CDCl_3$, δppm): 149.12, 148.77, 146.63, 144.99 (pyrazine-C), 153.12, 133.78, 132.33, 122.12, 104.18 (-CH=CH-Ar-C); 60.63, 55.90 (-OCH_3), 21.43 (-CH_3), 21.38 (-CH_3), 20.74 (-CH_3); ESI-MS: m/z 315.3 (M + 1). $C_{18}H_{22}N_2O_3$ (Exact Mass: 314.16).

(E)-2-(2-thienyl)-vinyl-3,5,6-trimethylpyrazine (A22)

2-Thiophene carboxaldehyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:5 and purified by recrystallization from methanol to give yellow crystal, mp 142–144 °C. Yield: 48%. IR (KBr, cm^{-1}): 3065 ($\nu_{=CH}$), 1625 ($\nu_{CH=CH}$), 1540, 1517, 1444 ($\nu_{CH=CH,}$ Ar), 1402 ($\nu_{C=N}$), 726 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 7.92 (1H, d, =CH-, J = 15.4 Hz), 7.08 (1H, d, =CH-, J = 15.4 Hz), 7.27 (1H, d, thiophene-H, J = 2.2 Hz), 7.21 (1H, d, thiophene-H, J = 3.4 Hz), 7.05 (1H, thiophene-H, J = 3.6 Hz), 2.61 (3H, s, -CH_3), 2.54 (3H, s, -CH_3), 2.52 (3H, s, -CH_3); ^{13}C-NMR ($CDCl_3$, δppm): 149.09, 148.80, 146.59, 144.76 (pyrazine-C), 142.24, 127.48, 127.44, 126.54, 125.08, 122.07 (-CH=CH-thiophene-C), 21.43 (-CH_3), 21.40 (-CH_3), 20.52 (-CH_3); ESI-MS: m/z 231.2 (M + 1). $C_{13}H_{14}N_2S$ (Exact Mass: 230.09).

(E)-2-(3-methylstyryl)-3,5,6-trimethylpyrazine (A23)

3-Methyl benzaldehyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:10 and purified by recrystallization from methanol to give yellow crystal, mp 67–68 °C. Yield: 10%. IR (KBr, cm^{-1}): 3032 ($\nu_{=CH}$), 1630 ($\nu_{CH=CH}$), 1601, 1580, 1483, 1443 ($\nu_{CH=CH,}$ Ar), 1400 ($\nu_{C=N}$), 852, 788, 686 ($\gamma_{=CH}$); 1H-NMR ($CDCl_3$, δppm): 7.77 (1H, d, =CH-, J = 15.7 Hz), 7.26 (1H, d, =CH-, J = 17.5 Hz), 7.41 (1H, s, Ar-H,), 7.40 (1H, d, Ar-H, J = 7.9Hz), 7.28 (1H, t, Ar-H, J = 7.5 Hz), 7.14 (1H, d, Ar-H, J = 7.4 Hz), 2.65 (3H, s, -CH_3), 2.56 (3H, s, -CH_3), 2.54 (3H, s, -CH_3), 2.40 (3H, s, -CH_3); ^{13}C-NMR ($CDCl_3$, δppm): 149.12, 148.77, 146.72, 145.16 (pyrazine-C), 137.93, 136.63, 133.89, 128.86, 127.49, 128.27, 124.11, 122.50 (-CH=CH-Ar-C), 21.45(-CH_3), 21.40 (-CH_3), 20.70 (-CH_3), 21.10 (-CH_3); ESI-MS: m/z 239.3 (M + 1). $C_{16}H_{18}N_2$ (Exact Mass: 238.15).

(E)-2-[2-(3,5,6-trimethylpyrazinyl)-vinyl-3,5,6-trimethyl]pyrazine (A24)

3,5,6-Trimethpyrazine-2-carbaldehyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:5 and purified by recrystallization from methanol to give yellow crystal, mp 182–184 °C. Yield: 10%. IR (KBr, cm^{-1}): 1629 ($\nu_{CH=CH}$), 1444, 1408 ($\nu_{C=N}$), 826 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 7.96 (2H, s, -CH=CH-), 2.70 (6H, s, -CH_3), 2.57 (6H, s, -CH_3), 2.54 (6H, s, -CH_3); ESI-MS: m/z 269.5 (M + 1). $C_{16}H_{20}N_4$ (Exact Mass: 268.17).

(E)-2-(2-chlorostyryl)-3,5,6-trimethylpyrazine (A27)

2-Chlorobenzaldehyde was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash

column chromatography with eluent of ethyl acetate/cyclohexane = 1:10 and purified by recrystallization from methanol to give white crystal, mp 56–59 °C. Yield: 23%. IR (KBr, cm^{-1}): 3054 ($\nu_{=CH}$), 1623 ($\nu_{CH=CH}$), 1561, 1471, 1447 ($\nu_{CH=CH}$, Ar), 1406 ($\nu_{C=N}$), 758 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 8.15 (1H, d, =CH-, J = 15.6 Hz), 7.29 (1H, d, =CH-, J = 15.1 Hz), 7.73 (1H, d, Ar-H, J = 7.7 Hz), 7.51 (1H, Ar-H), 7.43 (1H, Ar-H), 7.37 (1H, Ar-H), 2.61 (3H, s, -CH_3), 2.55 (3H, s, -CH_3), 2.51 (3H, s, -CH_3); ESI-MS: m/z 259.2 (M + 1). $C_{15}H_{15}ClN_2$ (Exact Mass: 258.09).

General procedure for the preparation of stilbene derivatives containg pyrazinyl group (A18–19 and A25–26, Method 2 of Scheme 1).

Freshly distilled triethyl phosphate (1.92 mL, 9.7 mmol) was added to substituted benzylchloride (9.7 mmol). The mixture was heated under slight boiling condition, checking for product formation via TLC. Anhydrous THF (10 mL) and 60% NaH (0.78 g, 19.4 mmol) were added to the mixture, stirring for 40 min completely. Then the mixture of 3,5,6-trimethpyrazine- 2-carbaldehyde (**6**) (1.46 g, 9.7 mmol) and anhydrous THF (25 mL) was stirred for 12 h in ice-salt bath (<–5 °C), checking for product formation via TLC. The mixture was extracted by ethyl acetate and evaporated in vacuo. The final product was purified by flash column chromatography.

(E)-2-(2-methylstyryl)-3,5,6-trimethylpyrazine (A18)

2-Methyl benzylchloride was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:5 and purified by recrystallization from methanol to give yellow crystal, mp 142–144 °C. Yield: 46%. IR (KBr, cm^{-1}): 3058 ($\nu_{=CH}$), 1629 ($\nu_{CH=CH}$), 1598, 1571, 1484, 1445 ($\nu_{CH=CH,}$ Ar), 1404 ($\nu_{C=N}$), 745 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$,δppm): 8.02 (1H, d, =CH-, J = 15.5 Hz), 7.18 (1H, d, =CH-, J = 15.5 Hz), 7.66 (1H, Ar-H), 7.23 (3H, m, Ar-H), 2.62 (3H, s, -CH_3), 2.56 (3H, s, -CH_3), 2.53 (3H, s, -CH_3); ESI-MS: *m/z* 239.1 (M + 1). $C_{16}H_{18}N_2$ (Exact Mass: 238.15).

(E)-2-(4-methylstyryl)-3,5,6-trimethylpyrazine (A19)

4-Methyl benzylchloride was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:5 and purified by recrystallization from methanol to give yellow crystal, mp 76–79 °C. Yield: 23%. IR (KBr, cm^{-1}): 3058 ($\nu_{=CH}$), 1629 ($\nu_{CH=CH}$), 1571, 1510, 1484, 1446 ($\nu_{CH=CH,}$ Ar), 1404 ($\nu_{C=N}$), 808 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 8.03 (1H, d, =CH-, J = 15.5 Hz), 7.18 (1H, d, =CH-, J = 15.5 Hz), 7.66 (1H, Ar-H), 7.23 (3H, m, Ar-H), 2.62 (3H, s, -CH_3), 2.57 (3H, s, -CH_3), 2.53 (3H, s, -CH_3), 2.50 (3H, s, -CH_3); ESI-MS: *m/z* 239.4 (M + 1). $C_{16}H_{18}N_2$ (Exact Mass: 238.15).

(E)-2-(2-cyanostyryl)-3,5,6-trimethylpyrazine (A25)

2-Cyanobenzylchloride was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:10 and purified by recrystallization from methanol to give yellow crystal. Yield: 38%; mp 171–174 °C; IR (KBr, cm^{-1}): 3056 ($\nu_{=CH}$), 2222 (ν_{-CN}), 1632 ($\nu_{CH=CH}$), 1595, 1564, 1481, 1449 ($\nu_{CH=CH,}$ Ar), 1404 ($\nu_{C=N}$), 777 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 8.09 (1H, d, =CH-, J = 15.6 Hz), 7.52 (1H, d, =CH-, J = 15.7 Hz), 7.82 (1H, d, Ar-H, J = 8.0 Hz), 7.71 (1H, d, Ar-H, J = 7.7 Hz), 7.62 (1H, t, Ar-H, J = 7.7 Hz), 7.40 (1H, t, Ar-H, J = 7.6 Hz), 2.66 (3H, s, -CH_3), 2.58 (3H, s, -CH_3), 2.55 (3H, s, -CH_3); ^{13}C-NMR ($CDCl_3$, δppm): 144.07, 139.91, 131.74, 128.30 (pyrazine-C), 133.17, 132.43, 129.27, 127.80, 127.69, 126.40, 117.70, 111.29 (-CH=CH-Ar-C); 21.45 (-CH_3), 21.07 (-CH_3), 20.54 (-CH_3), 21.10 (-CH_3); ESI-MS: m/z 250.4 (M + 1). $C_{16}H_{15}N_3$ (Exact Mass: 249.13).

(E)-2-(3-cyanostyryl)-3,5,6-trimethylpyrazine (A26)

3-Cyanobenzylchloride was used as the material of Horner–Wadsworth–Emmons reaction. The crude product was separated by flash column chromatography with eluent of ethyl acetate/cyclohexane = 1:10 and purified by recrystallization from methanol to give yellow crystal. Yield: 50%; mp 149–151 °C; IR (KBr, cm^{-1}): 3056 ($\nu_{=CH}$), 2225 (ν_{-CN}), 1635 ($\nu_{CH=CH}$), 1596, 1576, 1479, 1443 ($\nu_{CH=CH,}$ Ar), 1412, 1391 ($\nu_{C=N}$), 856, 797, 681 ($\gamma_{=CH}$); ^{1}H-NMR ($CDCl_3$, δppm): 7.77 (1H, d, =CH-, J = 15.6 Hz), 7.33 (1H, d, =CH-, J = 15.6 Hz), 7.88 (1H, s, Ar-H), 7.81 (1H, d, Ar-H, J = 7.9 Hz), 7.59 (1H, d, Ar-H, J = 7.7 Hz), 7.50 (1H, t, Ar-H, J = 7.8 Hz), 2.65 (3H, s, -CH_3), 2.56 (3H, s, -CH_3), 2.54 (3H, s, -CH_3); ^{13}C-NMR ($CDCl_3$, δppm): 150.27, 149.12, 147.16, 144.06 (pyrazine-C), 137.94, 130.99, 130.11, 131.09, 129.23, 125.19, 118.37, 112.75 (-CH=CH-Ar-C), 118.37 (-CN), 21.62 (-CH_3), 21.46 (-CH_3), 20.60 (-CH_3); ESI-MS: m/z 250.4 (M + 1). $C_{16}H_{15}N_3$ (Exact Mass: 249.13).

Protective effects on ECV-304 cells damaged by hydrogen peroxide

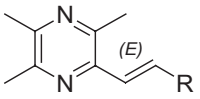
The human umbilical vascular endothelial cells (HUVECs, ECV-304 cells) were plated and grown for 24 h in cultured medium and then were switched to fresh medium in the presence of 0.025, 0.050, 0.100, 0.200 mM Ligustrazine and its derivatives. After 0.5-h incubation, 150 μM of hydrogen peroxide was added, and the cells were incubated for an additional 12 h. The results were expressed as the values of absorbance at 570 nm. The proliferation rates (P%) of damaged cells were calculated by $[OD_{570(Compd)}-OD_{570(H2O2)}]/[OD_{570(Control)}-OD_{570\ (H2O2)}] \times 100\%$, which was then used to obtain EC_{50} values (see Table 1), according to the equation: $-pEC_{50} = \log\ C_{max}$ -log2 ($\sum P$–0.75 + 0.25 P_{max} + 0.25 P_{min}),[18] where C_{max}, maximum concentration; $\sum P$, sum of proliferation rates; P_{max}, maximum value of proliferation rate; and P_{min}, minimum value of proliferation rate.

Result and Discussion

Chemistry

All the novel designed derivatives were synthesized via the route outline in Scheme 1. As the important intermediate, (3,5,6-trimethylpyrazin-2-yl)methanol **4** was prepared starting from tetramethylpyrazine

Table 1: The structure, maximum values of stimulating HUVCEs proliferation (P_{max} %), and EC_{50} for protecting damaged ECV-304 of Ligustrazine-based stilbene derivatives

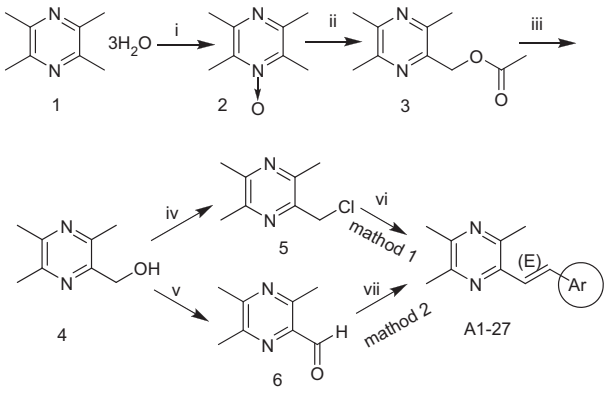


Compd.	R	Method	P_{max} (%)/C (mM)[a]	EC_{50} (mM)[b]
A1	Phenyl	1	12.4/0.05	14.7 ± 0.134
A2	3-Hydroxylphenyl	1	24.0/0.20	0.407 ± 0.0684
A3	3-Chlorophenyl	1	6.74/0.20	0.541 ± 0.0712
A4	4-Fluorophenyl	1	29.3/0.05	4.30 ± 0.946
A5	2,5-Dimethoxylphenyl	1	No[c]	32.7 ± 1.68
A6	2-Hydroxyl-3-methoxylphenyl	1	112/0.10	0.0289 ± 0.00451
A7	2-Hydroxylphenyl	1	No[c]	1.64 ± 0.542
A8	2,3-Dimethoxylphenyl	1	33.4/0.10	1.35 ± 0.147
A9	4-Nitrophenyl	1	103/0.10	0.0898 ± 0.0357
A10	4-Methoxylphenyl	1	No[c]	1.32 ± 0.976
A11	3,4-Dimethoxylphenyl	1	90.9/0.05	0.0622 ± 0.00319
A12	(E)-styryl	1	36.3/0.05	0.338 ± 0.00113
A13	3-Methoxylphenyl	1	105/0.025	1.42 ± 0.0129
A14	2,4-Dimethoxylphenyl	1	No[c]	3.55 ± 1.01
A15	2-Furyl	1	50.9/0.05	0.291 ± 0.0441
A16	2-Fluorophenyl	1	41.0/0.10	0.312 ± 0.0251
A17	2,3,4-Trimethoxylphenyl	1	0.934/0.05	2.68 ± 0.247
A18	2-Methylphenyl	2	34.5/0.025	0.793 ± 0.0849
A19	4-Methylphenyl	2	145/0.10	2.19 ± 0.998
A20	4-Chlorophenyl	1	No[c]	42.2 ± 3.51
A21	3,4,5-Trimethoxylphenyl	1	143/0.025	0.00576 ± 0.000369
A22	2-Thienyl	1	24.5/0.05	6.07 ± 1.22
A23	3-Methylphenyl	1	44.1/0.05	12.9 ± 0.868
A24	3,5,6-Trimethylpyrazinyl	1	104/0.20	0.0256 ± 0.00841
A25	2-Cyanophenyl	2	89.7/0.20	0.0436 ± 0.00121
A26	3-Cyanophenyl	2	8.55/0.10	2.54 ± 0.637
A27	2-Chlorophenyl	1	114/0.20	0.0000249 ± 0.00000971
Res			9.76/0.05	130 ± 2.46
Tetramethyl pyrazine			5.11/0.05	0.788 ± 0.0416

[a]Results were expressed as maximum values of stimulating HUVCEs proliferation (P_{max} %) at the corresponding concentration (C mM). P_{max} (%) = OD_{max}–☹ OD_a/OD_b–OD_a.

[b]EC_{50}: Effective concentration of compounds stimulating the damaged endothelial cells to proliferate by 50%.

[c]No P_{max} (%) value at the tested concentrations ranged from 0.025 to 0.20 mM.



Scheme 1: Reagents and conditions: (i) 30% H_2O_2/AcOH, 95 °C; (ii) Ac_2O/reflux 2 h; (iii) 20% NaOH; (iv) a:$SOCl_2$/anhydrous CH_2Cl_2; b:KOH/C_2H_5OH; (v) H_3PO_4 DCC/DMSO; (vi) Ar-CHO, Horner–Wadsworth–Emmons reaction; (vii) RCl, Horner–Wadsworth–Emmons reaction.

trihydrate **1** by the Boekelheide reaction [19] and followed by saponification with 20% NaOH. This process was modified by one-pot reaction according to our previous publication with 64% of the total yield [15]. 2-Chloromethyl-3,5,6-trimethyl-pyrazine **5** was prepared through chlorination of **4** with $SOCl_2$ in anhydrous CH_2Cl_2 with good yield. 3,5,6-Trimethylpyrazine-2-carbaldehyde **6** was prepared by the Pfitzner-Moffatt oxidation [20]. The intermediate **5** or **6** was converted to stilbene derivatives (**A1**–**27**) *via* Horner–Wadsworth–Emmons reaction [21]. The chemical structures of newly synthesized compounds were confirmed by IR, NMR, and ESI-MS.

Biological evaluation

Endothelial cells play a critical physiological role in maintaining normal vessel and organ function. Much evidence shows that vascular endothelial cells damage causes the alteration of endothelial permeability barrier and vascular tone, which is a major promoter of both atherogenesis and thrombosis and, consequently, cardiovascular events. In addition, oxidative stress is a cardiovascular risk

factor and contributes significantly to endothelial injury during atherogenesis [22]. Therefore, the protection of endothelial cells against damage caused by oxidative stress is a very important therapeutic strategy.

The newly synthesized Ligustrazine-based stilbene derivatives were evaluated for their protective effects on the HUVECs damaged by hydrogen peroxide (150 μM), and TMP was used as the control drug. The viability of normal and injured HUVECs was assessed by methyl thiazolyl tetrazolium (MTT) assay according to the references [23]. The structures and the maximum percentage of stimulating proliferation (P_{max} %) corresponding to the concentrations of the compounds, as well as the proliferation effective concentration (EC_{50}), were outlined in Table 1.

The results showed that the newly designed and synthesized Ligustrazine-based stilbene derivatives presented extraordinary strong activities in protection of the damaged HUVECs. Some new derivatives **A6, A9, A11, A21, A24, A25**, and **A27** exhibited high potency with low EC_{50} values ranged from 0.0000249 to 0.0898 mM in comparison with the positive control of Ligustrazine (EC_{50} = 0.788 mM). And also, these compounds displayed high P_{max} % values that exceeded to 100% (except for **A11**) at relatively low concentrations, indicating the tremendous stimulating effects of these compounds on damaged HUVECs proliferation, which has gone beyond the normal HUVECs proliferation.

Among these active compounds, **A27** was the most active one with EC_{50} values of 0.0000249 mM, which is 30 000 times higher than that of Ligustrazine, predicting a good lead compound for further study. Other derivatives such as **A2, A3, A12, A15, A16,** and **A18** also displayed good stimulating proliferation effect on the damaged cell, with similar order of EC_{50} values (0.4–0.7 mM) as that of TMP. However, some derivatives such as **A4**, **A7**, **A8**, **A10**, **A13**, **A14**, **A17**, **A19**, **A22,** and **A26** showed only moderate activity, and compounds **A5** and **A20** almost completely lost the activity. It is noteworthy that the fusion of ligustrazinyl fragment into the stilbene scaffold was endowed the new biological effect on protecting endothelial cells proliferation against oxidative damage, because resveratrol was found having no effect (CE_{50} = 130 mM) at the tested concentration ranges 0.025–0.20 mM, implying the possible other pathway that resveratrol takes effect in the prevention and treatment of cardiovascular diseases.

The polyphenol group plays a conspicuous role in clearing free radical and protecting against oxidative damage. The stilbene derivatives **A2** and **A6** containing hydroxyl groups at 3- and 2-phenyl moiety, respectively, exhibited high potency, especially **A6** (2-OH, 3-Me) with EC_{50} values at 0.0289 mM. However, this rule did not suit for **A7** (2-OH) with EC_{50} value of 1.64 mM. Derivatives **A3**, **A4**, **A16**, **A20,** and **A27** containing F and Cl groups are considered to be the bioisosters of those stilbenes containing hydroxyl groups, in which compound **A27** (2-Cl) displayed extremely high potency, others gave comparable activities, except for *para*-halogenated compounds **A4** (4-F, EC_{50} 4.3 mM) and **A20** (4-Cl, EC_{50} 42.2 mM), with reduced and almost lost activity. It seems that substituents at *ortho* position of phenyl moiety are more active than other positions in the same series. In chlorinated case, the active sequence of stilbene derivatives is 2-Cl >> 3-Cl > 4-Cl. 2-Methyl, and 2-cyano phenyl series also have shown the same trends as mentioned in the 2-hydroxyl and 2-halogen phenyl series. When mono-substitution at *ortho* position, there is an approximate tendency in activity: 2-Cl > 2-CN > 2-F > 2-Me > 2-OH.

Of the derivatives containing methoxyl groups, compound **A21** with 3,4,5-trimethoxyl group was the most active congeners with low EC_{50} value (0.00576 mM), which increased the activity above 136-fold in comparison with TMP. 3,4-Dimethoxyl stilbene (**A11**) also showed a good activity in stimulating the injured HUVECs proliferation. As a special event, derivative **A9** with 4-nitrophenyl group exhibited high potency with EC_{50} value lower than 0.09 mM.

In an attempt to expand the chemical diversity of the Ligustrazine-based stilbene analogs, some heterocyclic scaffolds such as 2-furyl (**A15**) and 2-thienyl (**A22**) were isosterically introduced by replacement of one phenyl moiety. Only **A15** exhibited good potency with EC_{50} value of 0.291 mM, which is better than that of the TMP. Compound (*E*)-1,2-bis(3,5,6-trimethylpyrazin-2-yl)ethene (**A24**), an interesting structure of diligustrazine stibene, showed a low EC_{50} value at 0.0256 mM, reflecting the combinational effects of two ligustrazinyl groups on the improved endothelial protection. This compound may be a promising prodrug of TMP and worthwhile to be further studied.

Conclusion

In conclusion, a series of novel stilbene derivatives containing ligustrazinyl moiety was designed and synthesized. The preliminary biological results have demonstrated that most of Ligustrazine-based stilbene derivatives exhibited good protective effects on the oxidatively damaged HUVECs in comparison with Ligustrazine. Some derivatives **A6**, **A9**, **A11**, **A21, A24**, **A25,** and **A27** presented high potency with low EC_{50} values ranged from 0.0000249 mM to 0.0898 mM. Among the active compounds, **A27** is the most active congener with EC_{50} 0.0000249 mM, which is 30 000 times higher than that of Ligustrazine, presenting a most promising lead for further investigation.

Structure–activity relationship analysis disclosed that substituents at *ortho* position of phenyl moiety seem to be more active than other positions in the same series, which has an approximate tendency in activity: 2-Cl > 2-CN > 2-F > 2-Me > 2-OH. In addition, the fusion of ligustrazinyl fragment into the stilbene scaffold endowed the new biological activity in contrast to the structure of resveratrol, which has also demonstrated that resveratrol might act by other mechanisms to convey its cardiovascular activity, rather than by rehabilitation of the damaged endothelial cell pathway.

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