Send Orders for Reprints to reprints@benthamscience.net

Medicinal Chemistry, **2014**, *10*, **81-89**

Ligustrazine Derivatives. Part 8: Design, Synthesis, and Preliminary Biological Evaluation of Novel Ligustrazinyl Amides as Cardiovascular Agents

Zhenyu Li[a], Fang Yu[a], Lei Cui[b], Wenmin Chen[a], Shouxun Wang[b], Peng Zhan[a], Yuemao Shen[a] and Xinyong Liu[a]*

[a]*Department of Medicinal Chemistry, Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Shandong University, No.44 Wenhuaxi Road, Jinan 250012, China;* [b]*Department of Biological Chemistry and Molecular Biology, School of Medicine, Weifang Medical University, No.7166, Baotongxi Road, Weifang 261053, China*

Abstract: A series of novel Ligustrazinyl amides was designed, synthesized and evaluated for their protective effect on the injured vascular endothelial cells. The preliminary results demonstrated that some compounds possessed more potent activities than that of Ligustrazine in stimulating replication of the injured human umbilical vascular endothelial cells (HUVECs) that is damaged by hydrogen peroxide. Among the active compounds, compounds **8i**, **8t** and **8u** exhibited the highest potency with low EC_{50} values of 0.037, 0.070 and 0.055 mM, respectively. Structure-activity relationships were briefly discussed.

Keywords: Ligustrazine, pyrazine amides, synthesis, cardiovascular disease.

INTRODUCTION

Pyrazine derivatives [1] have become of particular interest to chemists and biologists, because of their broad spectrum of biological activities and potential pharmacological applications [2]. For example, Pyrazinamide (PZA) is widely used in chemotherapy for tuberculosis. Amiloride, 3,5-diamino-6-chloropyrazinoyl-guanidine, is a potent Na^+/H^+ exchanger (NHE) inhibitor, and Ligustrazine (tetramethylpyrazine, TMP**)** is one of the calcium antagonists, both are clinically used for the treatment of cerebrocardiac vascular disease (CVD) (See Fig. **1**) [3-7].

Ligustrazine is an active component of Chinese traditional medicine herb Chuanxiong (*Ligusticum wallichii Franchat*), which is currently widely used in clinic for the treatment of coronary atherosclerotic cardiovascular disease and ischemic cerebrocardiac vascular disease [8]. However, pharmacokinetics studies found that Ligustrazine presented low bioavailability with a short half-life *in vivo* ($T_{1/2}$=2.89h), so accumulated toxicity often appeared in the patients for keeping an effective plasma concentration by the frequent administration [9]. Therefore, it is necessary to develop new generation of the Ligustrazine's cardiovascular drugs by molecular modification.

*Address correspondence to this author at the Department of Medicinal Chemistry, Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Shandong University, No.44 Wenhuaxi Road, Jinan 250012, China; Tel: +86 0531 88380270;
Fax: +86 0531 88382731; E-mail: xinyongl@sdu.edu.cn

In our previous work, we had designed and synthesized several series of Ligustrazine derivatives, such as Ligustrazinyl esters [9], Ligustrazinyl alkyl/acylpiperazines [10, 11] and Ligustrazinyl acylguanidines [12]. From biological evaluation, several promising lead compounds were discovered and exhibited good drugability. Among them, one of the representative Ligustrazinyl esters is the ferulic acid ester (Fig. **2**), which has potent activity in animal model for anticerebrocardiac vascular ischemia, but is prone to hydrolyze in gastrointestinal tract, subjecting to fast metabolic fate with short half-life.

In continuation of our research, we decided to undertake a study of the Ligustrazinyl amide series (Fig. **2**), because amides relatively have metabolic stability when compared to Ligustrazinyl esters. In the designed Ligustrazinyl amides, carbolic acid moieties were selectively used the active compounds in cardiovascular biology, such as nicotinic acid, salicylic acid, fumalic acid, cinnamic acid, gallic acid, salvianic acid, mandelic acid etc., in order to acquire the pharmacologically combinational effects based on the hybridization and prodrug principles [13, 14]. Herein, we report the preparation of the novel Ligustrazinyl amides, as well as their biological evaluation for protective effects on the injured vascular endothelial cells.

MATERIALS AND METHODS

Chemistry

Infrared spectra were measured using a nicolet nexus 470 FT-IR spectrometer using smear KBr crystal or KBr plate.

1875-6638/14 $58.00+.00

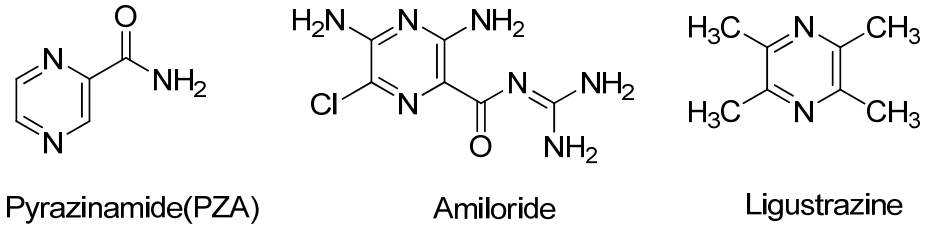


Fig. (1). Some pyrazine derivatives used in clinic.

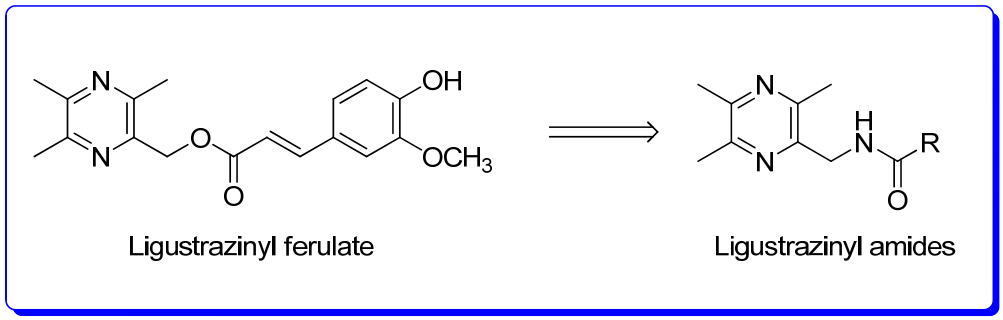


Fig. (2). Design strategy of Ligustrazinyl amide derivatives.

^{1}H-NMR spectra were recorded on a Bruker Avance (600 MHz) spectrometer; *J* values are in Hz. ^{13}C-NMR spectra were also recorded on the Bruker Avance spectrometer. Mass spectra were recorded on an electrospray ionization mass spectrometer as the value *m/z*. Thin-layer chromatography (TLC) was self-made silica gel (GF254) sheets. Flash chromatography was performed using 200-300 mesh silica gel. The yields were calculated by the last step reaction.

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

Compound **4** was prepared according to our previous reported method by using one-pot reaction [10, 15]. The crude product was purified by recrystallization with n-hexane to obtain yellow needles, mp: 88-89 °C, with total yield of 64%.

Synthesis of 2-Chloromethyl-3,5,6-trimethylpyrazine Hydrochloride (5)

To a solution of (3,5,6-trimethylpyrazin-2-yl)methanol (**4**) (15.50 g, 102 mmol) in anhydrous CH_2Cl_2 (300 mL), thionyl chloride (7.41 mL, 102 mmol) was added drop by drop at 0 °C. After finishing dropping, the mixture was stirred at room temperature for 2.5 h. The solvent was evaporated *in vacuo* and the product 2-chloromethyl-3,5,6-trimethylpyrazine hydrochloride (**5**) was obtained as yellow solid (21.11 g, 100%), mp: 102-105 °C [11].

Preparation of 2-aminemethyl-3,5,6-trimethylpyrazine (7)

To a solution of 2-chloromethyl-3,5,6-trimethylpyrazine (**5**) (20.7 g, 100 mmol) in DMF (100 mL), there was added a solution of phthalimide potassium salt (18.5 g, 150 mmol) in 60 mL of ethanol. After 1.5 h of stirring at 80 °C, the solids were filtered off. The filtrate was evaporated under reduced pressure to obtain the crude product, which was purified by recrystallization from ethanol affording the compound **6** as colorless crystals (20.8 g, 74.3%), mp: 154-156 °C. ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 7.91-7.84 (m, 4H, Ar-H), 4.92 (d, 2H, CH_2), 2.58 (s, 3H, CH_3), 2.54-2.53 (m, 6H, CH_3 x 2); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 168.32, 168.21 (C=O), 149.78, 147.85, 147.34, 144.81 (pyrazine-C), 134.46, 134,12, 128.64, 128.24, 127.17, 127.08 (benzene-C), 42.28 (CH_2), 21.33, 21.21, 20.08 (CH_3); ESI-MS: 282.2 $(M+H)^+$.

A solution of compound **6** (28.1 g, 100 mmol) and 50% hydrazine hydrate (10 mL, 100 mmol) in ethanol (100 mL) was refluxed for 2.5 h, and filtered. The filtrate was adjusted to pH 2-3 with concentrated hydrochloride and filtered. The solvent was evaporated off under reduced pressure, and then 40% sodium hydroxide (60 mL) was added. The organic layer was separated to give the product **7** as yellow oil (8.2 g, 54.2%). ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 8.70 (s, 2H, NH_2), 4.53 (d, 2H, CH_2), 2.56 (s, 3H, CH_3), 2.53-2.52 (m, 6H, CH_3 x 2); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 149.77, 147.90, 147.75, 144.63 (pyrazine-C), 41.72 (CH_2), 21.66, 21.42, 20.04 (CH_3); ESI-MS: 152.3 $(M+H)^+$.

General Procedure for the Preparation of Ligustrazinyl Amides (8a-8l, Method A of Scheme 1), Example of *N*-((3,5,6-trimethylpyrazin-2-yl)methyl)benzamide (8a)

A solution of 2-aminemethyl-3,5,6-trimethylpyrazine (**7**) (0.755 g, 0.005 mol) and pyridine (0.4 g, 0.0055 mol) in chloroform (30 mL), benzoyl chloride (0.0775 g, 0.0055 mol) was added drop by drop at 0 °C. After 2 h of stirring at room temperature, the mixture was washed with water (3 x 30 mL). The organic phase was dried over anhydrous sodium sulfate and the solvent evaporated *in vacuo*. The residue was purified by flash column chromatography and then recrystallized from ethanol to afford **8a** as white crystals (0.66 g, 52%), mp: 115-116 °C; IR (KBr, cm^{-1}): 3279.86 (NH), 1652.95 (C=O), 1530.02, 1486.59, 1446.19, 1416.15 (C=N, C=C); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 7.92 (s, 1H, NH), 7.90 (d, 2H, Ar-H, J = 7.2 Hz), 7.54 (dd, 1H, Ar-H, J_1 = 6.9 Hz, J_2 = 1.8 Hz), 7.49 (dd, 2H, Ar-H, J_1 = 7.0 Hz, J_2 = 7.1 Hz), 4.69 (d, 2H, CH_2, J = 4.1 Hz), 2.58 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 2.53 (s, 3H, CH_3); ^{13}C-NMR (150 MHz,

$CDC1_3$, δ ppm): 167.33 (C=O), 149.73, 147.92, 147.78, 144.85 (pyrazine-C), 134.46, 131.53, 128.62, 127.06 (benzene-C), 41.43 (CH_2) 21.56, 21.45, 20.06 (CH_3); ESI-MS: 256.0 $(M+H)^+$.

2-phenyl-N-((3,5,6-trimethylpyrazin-2-yl)methyl)acetamide (8b)

Flash column chromatography: ethyl acetate/cyclohexane = 4:1; yield 53%; yellow crystal; mp: 125-126 °C; IR (KBr, cm^{-1}): 3377.86 (NH), 1507.64, 1496.82, 1454.64 (C=N, C=C), 1662.40 (C=O); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 7.17 (s, H, NH), 7.40-7.33 (m, 5H, Ar-H), 4.43 (d, 2H, CH_2, J = 4.1 Hz), 2.46 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 2.34 (s, 3H, CH_3); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 171.13 (C=O), 144.57, 147.50, 147.73, 149.51 (pyrazine-C), 127.31, 128.97, 129.70, 134.91 (benzene-C), 41.02, 43.84 (CH_2), 19.91, 21.21, 21.34 (CH_3); ESI-MS: 270.5 $(M+H)^+$.

3-chloro-N-((3,5,6-trimethylpyrazin-2-yl)methyl)benzamide (8c)

Flash column chromatography: ethyl acetate/cyclohexane = 4:1; yield 45%; white crystal; mp: 110-111 °C; IR (KBr, cm^{-1}): 3406.14 (NH), 1523.23, 1565.89, 1523.23 (C=N, C=C), 1671.43 (C=O); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 7.98 (s, H, NH), 7.89 (t, H, Ar-H), 7.76 (dd, 1H, Ar-H, J_1 = 1.11 Hz, J_2 = 7.73 Hz), 7.50 (m, 1H, Ar-H), 7.40 (m, 1H, Ar-H), 4.67 (d, 2H, CH_2, J = 4.51 Hz), 2.56 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 2.53 (s, 3H, CH_3); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 165.99 (C=O), 144.61, 147.84, 148.00, 149.96 (pyrazine-C), 125.09, 127.55, 129.93, 133.14, 134.79, 136.15 (benzene-C), 41.41 (CH_2), 20.00, 21.40, 21.49 (CH_3); ESI-MS: 290.5 $(M+H)^+$.

N-((3,5,6-trimethylpyrazin-2-yl)methyl)nicotinamide (8d)

Flash column chromatography: ethyl acetate/cyclohexane = 4:1; yield 40%; white crystal; mp: 125-129 °C; IR (KBr, cm^{-1}): 3275.19 (NH), 1591.62, 1548.10, 1438.75 (C=N, C=C), 1642.60 (C=O), ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 8.23 (s, H, NH), 7.45 (m, H, Ar-H), 8.25 (dt, 1H, Ar-H, J_1 = 7.86 Hz, J_2 = 1.98 Hz), 8.75 (dd, 1H, Ar-H, J_1 = 4.81 Hz, J_2 = 1.63 Hz), 9.12 (m, 1H, Ar-H), 4.68 (d, 2H, CH_2, J = 4.08 Hz), 2.58 (s, 3H, CH_3), 2.54-2.53 (m, 6H, CH_3 x 2); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 165.36 (C=O), 123.60, 130.08, 135.27, 144.28, 147.71 (pyridine-C), 41.31 (CH_2), 20.00, 21.46, 21.54 (CH_3); ESI-MS: 257.6 $(M+H)^+$.

2-(((3,5,6-trimethylpyrazin-2-yl)methyl)carbamoyl)phenyl acetate (8e)

Flash column chromatography: ethyl acetate/cyclohexane = 4:1; yield 46%; yellow crystal; mp: 113-114 °C; IR (KBr, cm^{-1}): 3443.58 (NH), 1523.88, 1606.85, 1479.13 (C=N, C=C), 1758.85 (C=O), 1653.75 (C=O); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 8.11 (s, H, NH), 7.93 (dd, H, Ar-H, J_1 = 1.67 Hz, J_2 = 7.7 6Hz), 7.51 (dt, 1H, Ar-H, J_1 = 1.68 Hz, J_2 = 7.66 Hz), 7.35 (dt, 1H, Ar-H, J_1 = 1.06 Hz, J_2 = 7.60 Hz), 7.15 (dd, 1H, Ar-H, J_1 = 0.98 Hz, J_2 = 8.12 Hz), 4.66 (s, 2H, CH_2), 2.53 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 2.31 (s, 3H, $COCH_3$); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 165.34, 169.09 (C=O), 41.78 (CH_2), 20.09, 21.23, 21.37, 21.44 (CH_3); ESI-MS: 314.4 $(M+H)^+$.

(E)-3-(4-chlorophenyl)-N-((3,5,6-trimethylpyrazin-2-yl)methyl)acrylamide (8f)

Flash column chromatography: ethyl acetate/cyclohexane = 4:1; yield 50%; white crystal; mp: 169-171 °C; IR (KBr, cm^{-1}): 3264.29 (NH), 1619.16, 1543.28, 1493.85, 1442.58 (C=N, C=C), 1651.74 (C=O); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 7.32 (s, 1H, NH), 7.65 (d, H, C=CH, J = 15.60 Hz), 6.55 (d, 1H, C=CH, J = 15.60 Hz), 7.48 (d, 2H, Ar-H, J = 8.45 Hz), 7.35 (d, 2H, Ar-H, J = 8.44 Hz), 4.61 (s, 2H, CH_2); 2.53-2.51(m, 9H, CH_3 x 3); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 165.55 (C=O), 133.36, 135.50 (C=CH), 41.26 (CH_2), 20.08, 21.41, 21.45 (CH_3); ESI-MS: 316.3 $(M+H)^+$.

(E)-3-(4-fluorophenyl)-N-((3,5,6-trimethylpyrazin-2-yl)methyl)acrylamide (8g)

Flash column chromatography: ethyl acetate/cyclohexane = 4:1; yield 52%; white crystal; mp: 160-161 °C; IR (KBr, cm^{-1}): 3266.11 (NH), 1618.59, 1540.61, 1510.52, 1444.70 (C=N, C=C), 1652.47 (C=O); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 7.31 (s, 1H, NH), 7.65 (d, H, C=CH, J = 15.60 Hz), 6.51 (d, H, C=CH, J = 15.61 Hz), 7.54 (m, 2H, Ar-H), 7.07 (m, 2H, Ar-H), 4.62 (s, 2H, CH_2), 2.54(s, 3H, CH_3), 2.54(s, 3H, CH_3), 2.51(s, 3H, CH_3); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 165.71 (C=O), 139.90, 131.08 (C=CH), 41.22 (CH_2), 20.09, 21.38, 21.45 (CH_3), C: 163.53 ($^1J_{C\text{-}F}$ = 248.55 Hz), C: 120.27 ($^4J_{C\text{-}F}$ = 1.5 Hz), CH: 115.85 ($^2J_{C\text{-}F}$ = 21.5 Hz), CH: 129.64 ($^3J_{C\text{-}F}$ = 8.6 Hz); ESI-MS: 300.6 $(M+H)^+$.

(E)-3-(4-bromophenyl)-N-((3,5,6-trimethylpyrazin-2-yl)methyl)acrylamide (8h)

Flash column chromatography: ethyl acetate/cyclohexane = 4:1; yield 45%; white crystal; mp: 165-166 °C; IR (KBr, cm^{-1}): 3261.65 (NH), 1651.52, 1617.67, 1543.39, 1488.76 (C=N, C=C), 1757.39 (C=O); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 7.79 (d, H, C=CH, J = 15.92), 7.63 (d, H, C=CH, J = 15.59 Hz), 7.52 (d, 2H, Ar-H, J = 8.36 Hz), 7.40 (d, 2H, Ar-H, J = 8.36 Hz), 7.34 (s, H, NH), 4.62 (d, 2H, CH_2, J = 4.0 Hz), 2.63 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 2.53 (s, 3H, CH_3); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 165.54 (C=O), 117.28, 123.81 (C=CH), 41.23 (CH_2), 20.07, 21.33, 21.42 (CH_3); ESI-MS: 362.4 $(M+H)^+$.

N-((3,5,6-trimethylpyrazin-2-yl)methyl)isonicotinamide (8i)

Flash column chromatography: ethyl acetate/cyclohexane = 4:1; yield 38%; yellow crystal; mp: 119-120 °C; IR (KBr, cm^{-1}): 3275.81 (NH), 1591.52, 1547.44, 1478.53 (C=N, C=C), 1642.22 (C=O), ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 8.06 (s, H, NH), 7.45 (m, H, Ar-H), 8.25 (dt, 1H, Ar-H, J_1 = 7.86 Hz, J_2 = 1.98 Hz), 8.75 (dd, 1H, Ar-H, J_1 = 4.81 Hz, J_2 = 1.63 Hz), 9.12 (m, 1H, Ar-H), 4.68 (d, 2H, CH_2, J = 3.75 Hz), 2.54 (s, 9H, 3 × CH_3); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 165.34 (C=O), 123.59, 130.13, 135,26, 144.26, 147.73 (pyridine-C), 41.30 (CH_2), 19.99, 21.45, 21.51 (CH_3); ESI-MS: 257.4 $(M+H)^+$.

(E)-3-(4-methoxyphenyl)-N-((3,5,6-trimethylpyrazin-2-yl) methyl)acrylamide (8j)

Flash column chromatography: ethyl acetate/cyclohexane = 4:1; yield 48%; white crystal; mp: 185-187 °C; IR (KBr, cm^{-1}): 3285.87 (NH), 1615.61, 1603.50, 1576.22, 1567.12

(C=N, C=C), 1655.75 (C=O); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 7.65 (d, H, C=CH, J = 15.58 Hz), 7.51 (d, 2H, Ar-H, J = 8.68 Hz), 6.91 (d, 2H, Ar-H, J = 8.69 Hz), 6.46 (d, H, C=CH, J = 15.60 Hz), 4.63(s, H, CH_2), 3.85 (s, 3H, OCH_3), 2.55 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 2.53 (s, 3H, CH_3); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 166.24 (C=O), 129.95, 127.56 (C=CH), 41.19 (CH_2), 55.36 (OCH_3), 20.10, 21.32, 21.42 (CH_3); ESI-MS: 312.6 $(M+H)^+$.

(E)-3-p-tolyl-N-((3,5,6-trimethylpyrazin-2-yl)methyl)acrylamide (8k)

Flash column chromatography: ethyl acetate/cyclohexane = 4:1; yield 46%; white crystal; mp: 152-153 °C; IR (KBr, cm^{-1}): 3237.75 (NH), 1622.78, 1549.82, 1444.59 (C=N, C=C), 1667.90 (C=O); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 7.66 (d, H, C=CH, J = 15.60 Hz), 7.46 (d, 2H, Ar-H, J = 8.02 Hz), 7.20 (d, 2H, Ar-H, J = 7.94 Hz), 6.54 (d, H, C=CH, J = 15.60 Hz), 4.63(d, 2H, CH_2, J = 4.25 Hz), 2.54 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.19 (s, 3H, CH_3); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 166.07 (C=O), 132.13, 139.97 (C=CH), 41.25 (CH_2), 20.10, 21.37, 21.42, 21.43 (CH_3); ESI-MS: 296.6.

(E)-3-(4-hydroxyphenyl)-N-((3,5,6-trimethylpyrazin-2-yl) methyl)acrylamide (8l)

Flash column chromatography: ethyl acetate/cyclohexane = 4:1; yield 30%; white crystal; mp: 210-212 °C; IR (KBr, cm^{-1}): 3113.90 (NH), 3394.06 (OH), 1602.85, 1580.64, 1515.34, 1440.15 (C=N, C=C), 1650.06 (C=O); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 7.19 (s, H, NH), 6.40 (d, H, C=CH, J = 15.59 Hz), 6.86 (d, 2H, Ar-H, J = 8.52 Hz), 7.45 (d, 2H, Ar-H, J = 8.44 Hz), 7.62 (d, H, C=CH, J = 15.58 Hz), 4.63 (d, 2H, CH_2, J = 4.19 Hz), 2.55 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 2.53 (s, 3H, CH_3); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 166.28 (C=O), 118.13, 127.64 (C=CH), 41.26 (CH_2), 20.11, 21.35, 21.43 (CH_3); ESI-MS: 298.6 $(M+H)^+$.

General Procedure for the Preparation of Ligustrazinyl Amides (8m-8u, Method B in Scheme 1). Example of (*E*)-3-(2,5-dimethoxyphenyl)-*N*-((3,5,6-trimethylpyr-azin-2-yl) methyl) Acrylamide (8m)

A mixture of 2,5-dimethoxylcinnamic acid (1.04 g, 0.005 mol), HOBt (0.73 g, 0.0055 mol) and DCC (1.12 g, 0.0055 mol) in anhydrous THF (20 mL) was stirred at 0 °C for 5 h, and then the solids were filtered off. A solution of 2-aminemethyl-3,5,6-trimethylpyrazine (**7**) (0.755 g, 0.005 mol) in anhydrous THF (15 mL) was then added to the filtrate. After the mixture was stirred at room temperature overnight, the solvent was evaporated *in vacuo* and the residue was poured into ethyl acetate and washed with saturated sodium bicarbonate, 50% citric acid and brine. The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The crude product was purified by recrystallisation from 90-95% ethanol. yield 38%; white crystal; mp: 138-139 °C; IR (KBr, cm^{-1}): 3265.84 (NH), 1608.61, 1539.66, 1500.09 (C=N, C=C), 1645.42 (C=O); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 7.93 (d, H, HC=C, J = 15.81 Hz), 7.24 (s, H, NH), 7.06 (s, H, Ar-H), 6.89 (d, 2H, Ar-H, J = 8.95 Hz), 6.66 (d, H, C=CH, J = 8.89 Hz), 4.63(s, 2H, CH_2), 3.84 (s, 3H, CH_3), 3.81 (s, 3H, CH_3), 2.59 (s, 3H, CH_3); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 166.34 (C=O), 124.56, 132.56 (C=CH), 41.34 (CH_2), 55.82, 56.13 (OCH_3), 20.13, 21.18, 21.43 (CH_3); ESI-MS: 342.5 $(M+H)^+$.

(E)-3-(4-hydroxy-3-methoxyphenyl)-N-((3,5,6-trimethylpyr-azin-2-yl)methyl)acrylamide (8n)

Flash column chromatography: ethyl acetate/cyclohexane = 4:1; yield 35%; white crystal; mp: 204-206 °C; IR (KBr, cm^{-1}): 3324.79 (OH), 2996.19 (NH), 1599.66, 1583.17, 1583.17, 1559.39, 1510.43 (C=N, C=C), 1650.75 (C=O); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 7.22 (s, H, NH), 7.05 (s, H, Ar-H), 7.12 (d, H, Ar-H, J = 8.05 Hz), 6.93 (d, H, Ar-H, J = 8.14 Hz), 6.44 (d, H, C=CH, J = 15.53 Hz), 7.63 (d, H, C=CH, J = 15.52 Hz), 4.63 (d, 2H, CH_2, J = 3.51 Hz), 3.95 (s, 3H, OCH_3), 2.55 (s, 3H CH_3), 2.52 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 5.97 (s, H, OH); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 166.72 (C=O), 127.43, 141.14 (C=CH), 41.30 (CH_2), 55.97 (OCH_3), 20.10, 21.42, 21.44 (CH_3); ESI-MS: 328.6 $(M+H)^+$.

2-hydroxy-N-((3,5,6-trimethylpyrazin-2-yl)methyl)benzamide (8o)

Flash column chromatography: ethyl acetate/cyclohexane = 4:1; yield 41%; white crystal; mp: 152-153 °C; IR (KBr, cm^{-1}): 3381.89 (NH), 1595.08, 1523.00, 1487.51 (C=N, C=C), 1636.94 (C=O); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 12.49 (s, H, OH), 8.26 (s, H, NH), 7.57 (d, H, Ar-H, J = 2.2 Hz), 7.43 (t, H, Ar-H, J = 7.8 Hz), 7.01 (d, H, Ar-H, J=7.8 Hz), 6.62 (t, H, Ar-H, J = 7.8 Hz), 4.64 (s, 2H, CH_2), 2.56 (m, 9H, 3 × CH_3); ESI-MS: 342.4 $(M+H)^+$.

3,4,5-triacetoxy-N-((3,5,6-trimethylpyrazin-2-yl)methyl)benzamide (8p)

Flash column chromatography: ethyl acetate/cyclohexane = 4:1; yield 53%; white crystal; mp: 179-180 °C; IR (KBr, cm^{-1}): 3278.44 (NH), 1667.94, 1541.30, 1491.03 (C=N, C=C), 1777.84 (C=O); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 7.90 (s, H, NH), 7.66 (s, 2H, Ar-H), 4.65 (d, 2H, CH_2, J = 4.2 Hz), 2.54 (m, 9H, 3 × OCH_3), 2.32 (m, 9H, 3×CH_3); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 167.67, 166.62, 165.03 (C=O), 41.38 (CH_2), 20.01, 20.23, 20.67 (CH_3), 21.49 ($OCOCH_3$); ESI-MS: 430.6 $(M+H)^+$.

4-(((3,5,6-trimethylpyrazin-2-yl)methyl)carbamoyl)phenyl acetate (8q)

Flash column chromatography: ethyl acetate/cyclohexane = 4:1; yield 52%; white crystal; mp: 127-128 °C; IR (KBr, cm^{-1}): 3284.37 (NH), 1632.71, 1541.40, 1541.40, 1501.51 (C=N, C=C), 1752.65 (C=O), 1632.71 (C=O); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 7.93 (s, H, NH), 7.92 (s, 2H, Ar-H), 7.22 (m, 2H, Ar-H), 4.67 (d, 2H, CH_2, J = 3.6 Hz), 2.34 (s, 3H, $OCOCH_3$), 2.53 (m, 9H, 3 × CH_3); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 166.50 (C=O), 41.36 (CH_2), 20.03, 21.18, 21.44 (CH_3), 21.49 ($OCOCH_3$); ESI-MS: 314.5 $(M+H)^+$.

2-oxo-1-phenyl-2-((3,5,6-trimethylpyrazin-2-yl)methylamino)ethyl acetate (8r)

Flash column chromatography: ethyl acetate/cyclohexane = 4:1; yield 54%; white crystal; mp: 112-114 °C; IR (KBr, cm^{-1}): 3366.26 (NH), 1694.91, 1685.48, 1548.19 (C=N, C=C), 1717.79 (C=O), 1735.81 (C=O); ^{1}H-NMR (600 MHz,

$CDCl_3$, δ ppm): 7.93 (s, 1H, NH), 7.50 (m, 5H, Ar-H), 6.20 (s, H, CH-O), 4.50 (m, 2H, CH_2), 2.51 (m, 9H, 3 × CH_3), 2.26 (s, 3H, $COCH_3$); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 169.24, 168.38 (C=O), 40.61 (CH_2), 67.09 ($OCOCH_3$), 19.93, 21.04, 21.40 (CH_3); ESI-MS: 328.6 $(M+H)^+$

2-hydroxy-2-phenyl-N-((3,5,6-trimethylpyrazin-2-yl)methyl)acetamide (8s)

Flash column chromatography: ethyl acetate/cyclohexane = 4:1; yield 48%; white crystal; mp: 107-108 °C; IR (KBr, cm^{-1}): 3256.31 (NH), 1521.74, 1443.77, 1412.98, (C=N, C=C), 1653.10 (C=O); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 7.63 (s, 1H, NH), 5.17 (s, 1H, OH), 7.35 (m, H, Ar-H), 7.40 (t, 2H, Ar-H, J = 3.9 Hz), 7.51 (d, 2H, Ar-H, J = 8.4 Hz), 4.55 (d, 2H, CH_2, J = 4.2 Hz), 2.48 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.43 (s, 3H, CH_3); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 172.24 (C=O), 40.02 (CH_2), 67.09 ($OCOCH_3$), 19.92, 21.33, 21.37 (CH_3); ESI-MS: 286.5 $(M+H)^+$.

N-((3,5,6-trimethylpyrazin-2-yl)methyl)cinnamamide (8t)

Flash column chromatography: ethyl acetate/cyclohexane = 4:1; yield 56%; yellow crystal; mp: 142-144 °C; IR (KBr, cm^{-1}): 3308.13 (NH), 1618.19, 1540.95, 1445.92 (C=N, C=C), 1652.30 (C=O); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 7.32 (s, 1H, NH), 7.72 (d, 1H, C=CH, J = 15.6 Hz), 7.48 (m, 5H, Ar-H), 6.51 (d, 1H, C=CH, J = 15.4 Hz), 4.63 (d, 2H, CH_2, J = 4.1 Hz), 2.55 (s, 3H, CH_3), 2.53 (s, 3H, CH_3), 2.53 (s, 3H, CH_3); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 165.88 (C=O), 134.87, 129.7 (C=CH), 41.28 (CH_2), 20.11, 21.44, 21.47 (CH_3); ESI-MS: 282.5 $(M+H)^+$.

2-hydroxy-2-phenyl-N-((3,5,6-trimethylpyrazin-2-yl)methyl)acetamide (8u)

Flash column chromatography: ethyl acetate/cyclohexane = 4:1; yield 48%; white crystal; mp: 107-108 °C; IR (KBr, cm^{-1}): 3256.31 (NH), 1521.74, 1443.77, 1412.98, (C=N, C=C), 1653.10 (C=O); ^{1}H-NMR (600 MHz, $CDCl_3$, δ ppm): 7.63 (s, 1H, NH), 5.17 (s, 1H, OH), 7.35 (m, H, Ar-H), 7.40 (t, 2H, Ar-H , J = 3.9 Hz), 7.51 (d, 2H, Ar-H, J = 8.4 Hz), 4.55 (d, 2H, CH_2, J = 4.2 Hz), 2.48 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 2.43 (s, 3H, CH_3); ^{13}C-NMR (150 MHz, $CDC1_3$, δ ppm): 172.24 (C=O), 40.02 (CH_2), 67.09 ($OCOCH_3$), 19.92, 21.33, 21.37 (CH_3); ESI-MS: 286.5 $(M+H)^+$; $C_{16}H_{19}N_3O_2$.

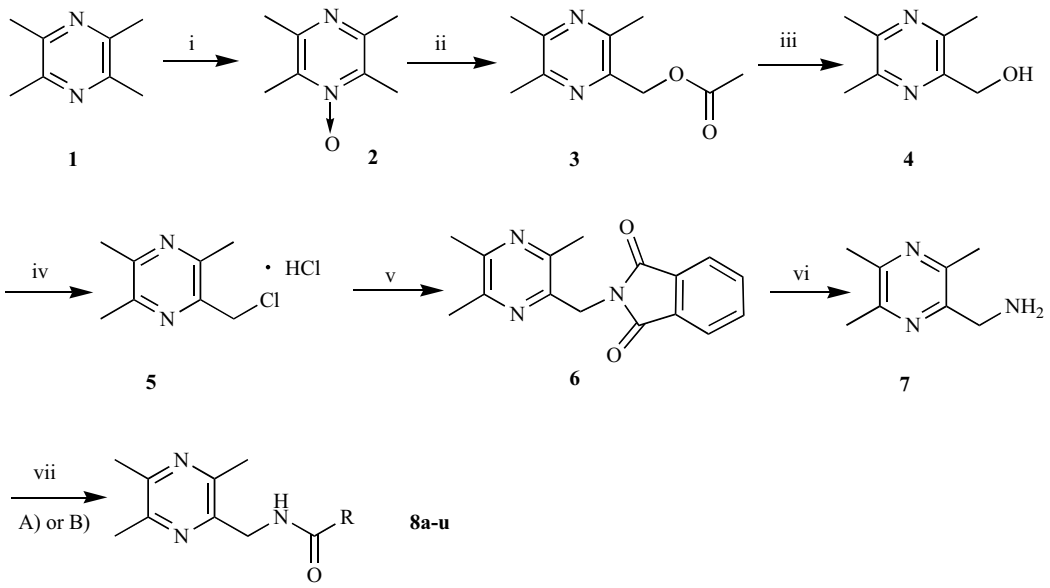
Biological Evaluation for Protective Effects on HUVECs Damaged by Hydrogen Peroxide [11, 16, 17]

The human umbilical vascular endothelial cells were plated and grown for 24 h in cultured medium, 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 $[OD_{570(Compd)}\text{-}OD_{570(H2O2)}]/[OD_{570(Control)}\text{-}OD_{570(H2O2)}] \times 100\%$, which was then used to obtain EC_{50} values (see Table **1**), according to the equation: $-pEC_{50} = \log C_{max} - \log 2 (\sum P - 0.75 + 0.25P_{max} + 0.25P_{min})$, where C_{max}, maximum concentration; $\sum P$, sum of proliferation rates; P_{max}, maximum value of proliferation rate; and Pmin, minimum value of proliferation rate.

RESULTS AND DISCUSSION

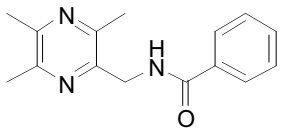
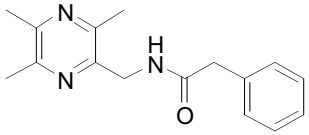
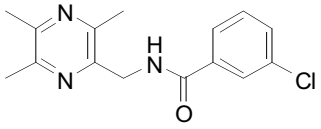
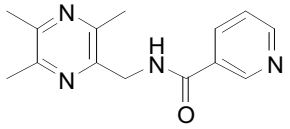
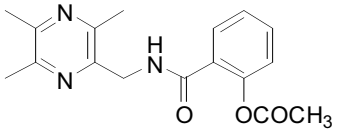
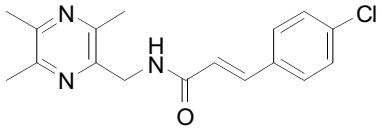
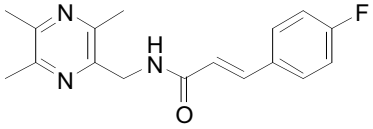
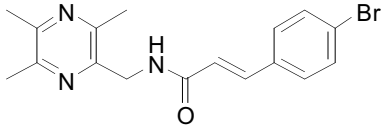
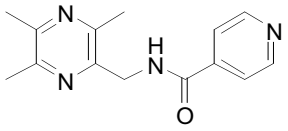
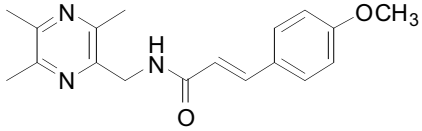
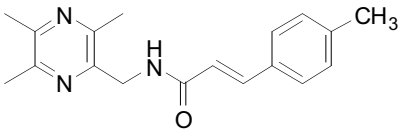
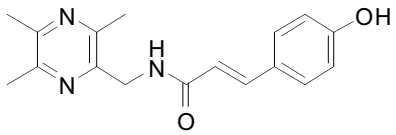
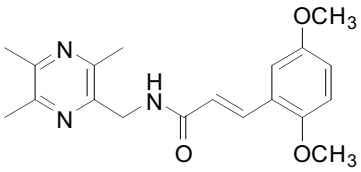
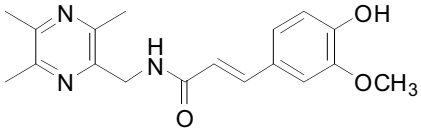
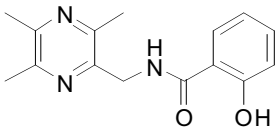
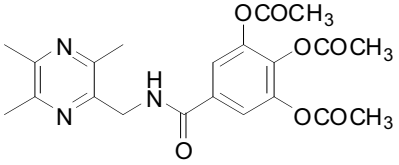
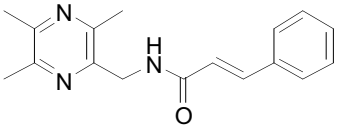
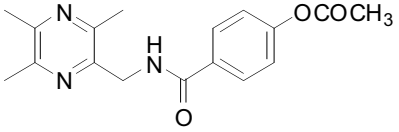
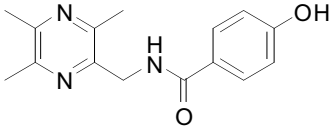
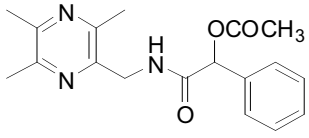
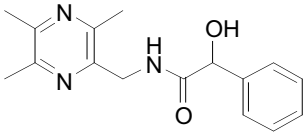
Synthesis of the Compounds 8a-8u

The important intermediate (3,5,6-trimethylpyrazin-2-yl)methanol (**3**) was prepared by the Boekelheide reaction [18] starting from Ligustrazine (**1**) and directly hydrolyzed with sodium hydroxide water solution to obtain hydroxyl Ligustrazine (HTMP, **4**). This process was used one-pot reaction according to our previous publication with 64% of the total yield (see Scheme **1**) [9]. The 2-chloromethyl-3,5,6-trimethylpyrazine hydrochloride (**5**) was synthesized by the chlorination of **4** with $SOCl_2$ in anhydrous CH_2Cl_2 [11]. 2-aminemethyl-3,5,6-trimethylpyrazine (**7**) was synthesized by typical Gabriel reaction through hydraziniolysis of the key



Scheme 1. Reagents: (i) 30% H_2O_2, AcOH; (ii) Ac_2O; (iii) 20% NaOH; (iv) $SOCl_2$, anhydrous CH_2Cl_2; (v) phthalimide, DMF; (vi) 50% hydrazine hydrate, ethanol; (vii) A) RCOCl, Py or B) HOBt, DCC, anhydrous THF.

Table 1. The Structures of the Synthesized Compounds

Compound	Structure	Compound	Structure
8a		**8b**	
8c		**8d**	
8e		**8f**	
8g		**8h**	
8i		**8j**	
8k		**8l**	
8m		**8n**	
8o		**8p**	
8q		**8r**	
8s		**8t**	
8u			

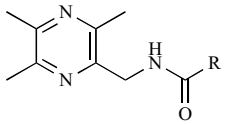
intermediate of *N*-2-((3,5,6-trimethylpyrazin-2-yl)methyl) phthalimide (**6**) [19]. Ligustrazinyl amides **8** were prepared from **7** in the following two methods. In method (**A**), the carboxylic acid was firstly transformed to acyl chloride, and then reacted with compound **7** in the presence of pyridine base. In method (**B**), the carboxylic acid and HOBt were firstly transformed to active ester in the presence of DCC, and then reacted with compound **7**, obtaining the target compounds **8**. All the newly synthesized compounds (Table **1**) were confirmed by IR, ^{1}H-NMR and MS spectrometries.

Biological Evaluation and Discussion

All newly synthesized Ligustrazinyl amide derivatives were evaluated for protecting effects on the HUVECs that is damaged by hydrogen peroxide. TMP was used as the control drug. The viability of normal and injured HUVECs was assessed by MTT assay [20]. The maximum percentage of stimulating proliferation (P_{max}%) corresponding to the concentrations of the compounds, and the proliferation effective concentration (EC_{50}) were listed in (Table **2**).

The results showed that some of the newly synthesized compounds significantly increased the viability of the damaged HUVECs (with low EC_{50} values) in comparison with the positive stimulator of TMP (EC_{50} = 0.600 mM). Among the active compounds, **8i** containing isonicotinoyl group was the most active one with the EC_{50} value at 0.037mM, which is 16 times higher than the reference drug TMP. The excellent potency of **8i** might be caused by the isonicotinoyl

Table 2. The EC_{50} for Protecting Damaged HUVECs of the Novel Ligustrazine Derivatives



No.	RCO-	Method	P_{max}%/C(mM)[a]	EC_{50}(mM)[b]
8a	Benzoyl	A	36.30/0.025	0.194±0.011
8b	Phenylacetyl	A	11.46/0.1	0.335±0.034
8c	3-Chlorobenzoyl	A	97.45/0.05	0.094±0.012
8d	Nicotinoyl	A	82.17/0.2	0.108±0.014
8e	Acetylsalicyloyl	A	-84.08/0.1	8.914±0.912
8f	4-Chlorocinnamoyl	A	12.74/0.025	0.370±0.024
8g	4-Fluorocinnamoyl	A	-103.82/0.05	23.873±1.452
8h	4-Bromocinnamoyl	A	-113.38/0.05	16.217±1.875
8i	Isonicotinoyl	A	98.73/0.1	0.037±0.004
8j	4-Methoxylcinnamoyl	A	84.71/0.05	0.109±0.017
8k	4-Methylcinnamoyl	A	31.85/0.1	2.520±0.321
8l	4-Hydroxylcinnamoyl	A	29.30/0.05	2.229±0.213
8m	2,5-Dimethylcinnamoyl	B	-51.81/0.025	2.618±0.324
8n	Ferulolyl	B	-38.55/0.2	2.170±0.223
8o	Salicyloyl	B	-36.14/0.05	4.004±0.342
8p	3,4,5-Triacetylgalloyl	B	-1.2/0.025	1.592±0.146
8q	Cinnamoyl	B	53.50/0.1	0.273±0.012
8r	4-Acetoxybenzoyl	B	-58.60/0.025	4.265±0.567
8s	4-Hydroxylbenzoyl	B	36.14/0.025	3.767±0.456
8t	2-acetoxy-2-phenylacetoyl	B	87.95/0.2	0.070±0.008
8u	Mandeloyl	B	106.02/0.2	0.055±0.004
TMP			10/0.05	0.600±0.073

[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]The activity of compounds was expressed as EC_{50}. The EC_{50} is defined as the effective concentration of compounds stimulating the damaged endothelial cells to proliferate by 50%. Values represent means ± SD of three individual experiments.

group, as the counterpart **8a** bearing benzoyl group showed less potency with the EC_{50} value at 0.194 mM. The analog **8d** (EC_{50} = 0.108 mM) containing nicotinoyl group showed less potent than **8i**, which revealed the significance of the position of the *N* atom.

In the phenylacetyl-substituted series, all the compounds exhibited excellent potency. The EC_{50} values of **8u**, **8t** and **8b** were 0.055, 0.070 and 0.335 mM, respectively, which were all potent than TMP. However, in the substituted benzoyl series, the compounds displayed moderate potency. Only compound **8c** manifested a particular high potency. The compounds **8e** and **8o** bearing salicyloyl groups did not present the pharmacologically additive or synergetic effects of ligustrazine with pharmacophores. The difference of potency between these two series reflected the importance of the $-CH_2-$ in the benzyl group, which may endow the compounds with appropriate lengths and suitable to bind to the target.

As expected, the introduction of cardiovascular activity pharmacophore to TMP indeed greatly improved their protective effect on the injured vascular endothelial cells (*i.e.* **8i**, **8t** and **8u**). However, this rule did not work in the cinnamoyl substituted series, only compound **8j** and **8q** presented a comparable potency (P_{max}%/C(mM) = 84.71/0.05, EC_{50} = 0.109 mM; P_{max}%/C(mM) = 53.50/0.1, EC_{50} = 0.273 mM, respectively). It is noteworthy that compound **8n**, the fusion of ferulic acid scaffold into ligustrazinyl fragment, showed lower potency (P_{max}%/C(mM) = -38.55/0.2, EC_{50} = 2.170 mM) than TMP (P_{max}%/C(mM) = 10/0.05, EC_{50} = 0.600 mM) on protecting HUVECs proliferation against oxidative damage. The P_{max} of the compound **8n** was negative value implying its toxicities on HUVECs, which could provide valuable information for further design novel analogues.

CONCLUSION

In conclusion, a series of novel Ligustrazinyl derivatives was designed and synthesized. Some Ligustrazinyl derivatives exhibited lower EC_{50} values for protective effects on the HUVECs damaged by hydrogen peroxide in comparison with Ligustrazine. The derivatives containing the isonicotinoyl pharmacophore (**8i**) exhibited the highest potency. Compound **8i** displayed most potential protective effects on the HUVECs damaged by hydrogen peroxide. Preliminary sructure–activity relationships were briefly discussed. Further modification on this family of Ligustrazinyl derivatives are undergoing in our laboratory.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The financial supports of this work by National Special Key Projects of New Drugs R&D (2009ZX09103-117), and Shandong Natural Science Foundation, Shandong Science and Technology Bureau Foundation (2006GG2202063) are gratefully acknowledged.

REFERENCES

[1] Müller, R.; Rappert, S. Pyrazines: occurrence, formation and biodegradation. *Appl. Microbiol. Biotechnol.,,* **2010**, *85*(5), 1315-1320.

[2] Corbett, J.W.; Rauckhorst, M.R.; Qian, F.; Hoffman, R.L.; Knauer, C.S.; Fitzgerald, L.W. Heteroatom-linked indanylpyrazines are corticotropin releasing factor type-1 receptor antagonists. *Bioorg. Med. Chem. Lett.,* **2007**, *17*(22), 6250-6.

[3] Liao, S.L.; Kao, T.K.; Chen, W.Y.; Lin, Y.S.; Chen, S.Y.; Raung, S.L.; Wu, C.W.; Lu, H.C.; Chen, C.J. Tetramethylpyrazine reduces ischemic brain injury in rats. *Neurosci. Lett.,* **2004**, *372*(1-2), 40-5.

[4] Masereel, B.; Pochet, L.; Laeckmann, D. An overview of inhibitors of Na(+)/H(+) exchanger. *Eur. J. Med. Chem.,* **2003**, *38*(6), 547-54.

[5] Laeckmann, D.; Rogister, F.; Dejardin, J.V.; Prosperi-Meys, C.; Geczy, J.; Delarge, J.; Masereel, B. Synthesis and biological evaluation of aroylguanidines related to amiloride as inhibitors of the human platelet Na(+)/H(+) exchanger. *Bioorg. Med. Chem.,* **2002**, *10*(6), 1793-804.

[6] Rogister, F.; Laeckmann, D.; Plasman, P.; Van Eylen, F.; Ghyoot, M.; Maggetto, C.; Liegeois, J.; Geczy, J.; Herchuelz, A.; Delarge, J.; Masereel, B. Novel inhibitors of the sodium-calcium exchanger: benzene ring analogues of N-guanidino substituted amiloride derivatives. *Eur. J. Med. Chem.,* **2001**, *36*(7-8), 597-614.

[7] Ren, M.; Dong, J.; Xu, Y.; Wen, N.; Gong, G. Synthesis of novel ligustrazine derivatives as NA+/H+ exchange inhibitors. *Chem. Biodivers,* **2010**, *7*(11), 2727-36.

[8] Cheng, X.C.; Liu, X.Y.; Xu, W.F. Recent advances in the structural modification of ligustrazine and cerebro/cardiovascular activity of ligustrazine derivatives. *Drug Future,* **2005**, *30*(10), 1059-1065.

[9] Liu, X.; Zhang, R.; Xu, W.; Li, C.; Zhao, Q.; Wang, X. Synthesis of the novel liqustrazine derivatives and Their protective effect on injured vascular endothelial cell damaged by hydrogen peroxide. *Bioorg. Med. Chem. Lett.,* **2003**, *13*(13), 2123-6.

[10] Cheng, X.C.; Liu, X.Y.; Xu, W.F.; Guo, X.L.; Ou, Y. Design, synthesis, and biological activities of novel Ligustrazine derivatives. *Bioorg. Med. Chem.,* **2007**, *15*(10), 3315-20.

[11] Cheng, X.C.; Liu, X.Y.; Xu, W.F.; Guo, X.L.; Zhang, N.; Song, Y.N. Ligustrazine derivatives. Part 3: Design, synthesis and evaluation of novel acylpiperazinyl derivatives as potential cerebrocardiac vascular agents. *Bioorg. Med. Chem.,* **2009**, *17*(8), 3018-24.

[12] Li, Z.; Yu, F.; Cui, L.; Zhan, P.; Wang, S.; Shen, Y.; Liu, X. Ligustrazine derivatives. Part 6: design, synthesis and evaluation of novel ligustrazinyl acylguanidine derivatives as potential cardiovascular agents. *Med. Chem.,* **2012**, *8*(5), 928-33.

[13] Erhardt, P.W.; Khupse, R.; Sarver, J.G.; Trendel, J.A., Prodrugs: Strategic deployment, metabolic considerations, and chemical design principles. Wiley Online Library: **2010**; pp. 103-150.

[14] Fu, X.Z.; Ou, Y.; Pei, J.Y.; Liu, Y.; Li, J.; Zhou, W.; Lan, Y.Y.; Wang, A.M.; Wang, Y.L. Synthesis, anti-HBV activity and renal cell toxicity evaluation of mixed phosphonate prodrugs of adefovir. *Eur. J. Med. Chem.,* **2012**, *49*(211-218.

[15] Deng, L.; Guo, X.; Zhai, L.; Song, Y.; Chen, H.; Zhan, P.; Wu, J.; Liu, X. Ligustrazine derivatives. Part 4: design, synthesis, and biological evaluation of novel ligustrazine-based stilbene derivatives as potential cardiovascular agents. *Chem. Biol. Drug Des.,* **2012**, *79*(5), 731-9.

[16] Chen, H.; Li, G.; Zhan, P.; Liu, X. Ligustrazine derivatives. Part 5: design, synthesis and biological evaluation of novel ligustrazinyloxy-cinnamic acid derivatives as potent cardiovascular agents. *Eur. J. Med. Chem.,* **2011**, *46*(11), 5609-15.

[17] Wang, Y.K.; Huang, Z.Q. Protective effects of icariin on human umbilical vein endothelial cell injury induced by H2O2 *in vitro*. *Pharmacol. Res.,* **2005**, *52*(2), 174-82.

[18] Klein, B.; Berkowitz, J.; Pyrazines, I. Pyrazine-N-oxides. Preparation and spectral characteristics. *J. Am. Chem. Soc.,* **1959**, *81*(19), 5160-5166.

[19] Khan, M.N. Suggested improvement in the Ing-Manske procedure and Gabriel synthesis of primary amines: Kinetic study on alkaline hydrolysis of N-Phthaloylglycine and acid hydrolysis of N-(o-

Carboxybenzoyl)glycine in aqueous organic solvents. *J. Org. Chem.,* **1996**, *61*(23), 8063-8068.

[20] Li, W.M.; Liu, H.T.; Li, X.Y.; Wu, J.Y.; Xu, G.; Teng, Y.Z.; Ding, S.T.; Yu, C. The effect of tetramethylpyrazine on hydrogen peroxide-induced oxidative damage in human umbilical vein endothelial cells. *Basic Clin. Pharmacol. Toxicol.,* **2010**, *106*(1), 45-52.

Received: December 31, 2012 Revised: September 03, 2013 Accepted: September 03, 2013