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# Synthesis of the Novel Liqustrazine Derivatives and Their Protective Effect on Injured Vascular Endothelial Cell Damaged by Hydrogen Peroxide

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**Abstract**—A series of novel 2-acyloxymethyl-3,5,6-trimethylpyrazine derivatives was designed and synthesized. Most compounds were found to be 1.5–4.5-fold higher potency than tetramethylpyrazine (TMP) in stimulating the proliferation of normal vascular endothelial cells and in protecting against hyperoxic acute injury. The most active one is the 2-nicotinoyl ester **5a** exhibiting the maximum proliferation rate ($P_{max}$) of 88.57% at the concentration of 0.1 mmol $L^{-1}$. Structure–activity relationships of these compounds were discussed.


Endothelial cells play a critical physiological role in maintaining normal vessel and organ function. Much evidence show that vascular endothelial cells damage that causes the alteration of endothelial permeability barrier and vascular tone is a major promoter of both atherogenesis and thrombosis and, consequently cardiovascular events. Oxidative stress is a cardiovascular risk factor and contributes significantly to endothelial injury during atherogenesis. Therefore, the protection of endothelial cells against damage caused by oxidative stress is a very important therapeutic strategy.[1]

Liqustrazine (tetramethyl pyrazine, TMP), one major efficient component from the Chinese traditional medicine herb *Chuanxiong* (*Ligusticum wallichii Franchat*), has long been reported to inhibit the platelet aggregation,[2] to cause negative chronotropic and inotropic responses on isolated atria,[3] to inhibit vasoconstriction in isolated vascular strips,[4] and to act as a vasodilator, a free radical scavenger, anti-thrombosis and anti-hypertension agent (Fig. 1).[5–7] Currently, TMP is widely used in China as a new kind of calcium antagonist for the treatment of coronary atherosclerotic cardiovascular disease and ischemic cerebrocardiac vascular disease.[8,9] More recently, it has been found to be more effective in protection against vascular endothelial cell injury.[10] However, pharmacodynamics studies found that TMP presented low bioavailability and to be metabolized fast in vivo with short half-life of $t_{1/2} = 2.89$ h, so accumulated toxicity often appeared in the patients for keeping an effective plasma concentration by the frequent administration.[11] Therefore, it is necessary to develop new generation of the cerebrocardiac vascular drugs from molecular modification of TMP.

Metabolism investigation of TMP found that 2-hydroxymethyl-3,5,6-trimethylpyrazine (HTMP) was the major product in the metabolic process in vivo.[12] HTMP had even been synthesized in our previous work[13] and found it possessed a better cardiovascular activity than TMP.[14] In the present paper, we will describe the preparation and biological effects of the acyl derivatives of the HTMP, in which some functional groups, like nicotinoyl, acetylsalicyloyl, ferulolyl, 2-(4-Cl-phenoxy)-2-methylpropionyl, cinnamoyl and 3,4,5-trimethoxylbenzoyl, were connected to the hydroxyl group for acquiring the pharmacologically combinational effect. Other acyl groups were also introduced in order to further expand our exploration and better

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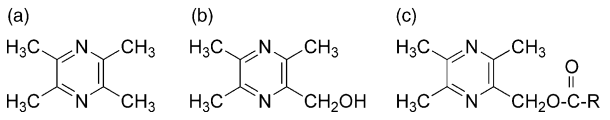



**Figure 1.** Structures of Liqustrazine (tetramethyl pyrazine, TMP) (a), HTMP (b) and newly synthesized derivatives (c).

understand the structure–activity relationships of TMP derivatives.

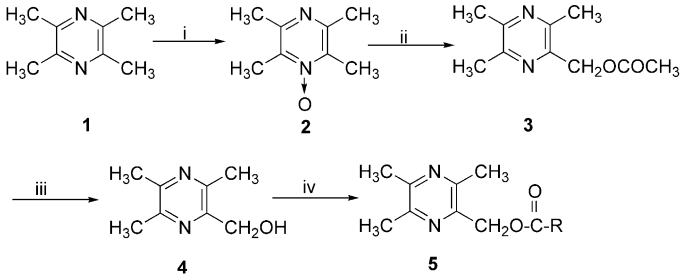
The titled compounds were prepared starting from TMP via four steps as shown in Scheme 1. The important intermediate HTMP **4** was prepared according to our previous work,[13] but with some modification in the workup, in which the crude compound of 2-acetyloxymethyl-3,5,6-trimethylpyrazine **3** was synthesized by the acetylation and rearrangement reaction of compound **2** with acetic anhydride, and directly hydrolyzed with sodium hydroxide water solution in one-pot reaction. HTMP was recrystallized from petroleum ether (30–60 °C) to obtain the pure material, 64% yield from TMP, mp 88–89 °C. Compounds **5** were prepared by the acylation of HTMP in the following two methods. In method (a), the corresponding carboxylic acid was straightly reacted with HTMP in the presence of DCC and DMAP. In method (b), the carboxylic acid was firstly transformed to acyl chloride, and then reacted with HTMP in the presence of pyridine as a base. Total 21 compounds derived from TMP (**5a**–**u**) were synthesized. All compounds were not reported in the literature before. Their chemical structures were confirmed by IR, $^1$H NMR and MS spectrometries, and microanalysis.[15]

All compounds were tested for stimulating the proliferation of cultured human umbilical vascular endothelial cells (HUVECs) and for protecting against acute injury damaged by hydrogen peroxide. TMP was used as the control drug. The viability of normal and injured HUVECs was assessed by methyl thiazolyl tetrazolium (MTT) assay according to ref 10. The structures and the maximum percentage of stimulating proliferation ($P_{max}$%) corresponding to the concentrations of the compounds were outlined in Table 1.

The results showed that all of the newly synthesized compounds significantly increased the normal HUVECs viability in comparison with the positive stimulator of TMP. Among the compounds, **5a**, **5c**, **5d**, **5g** and **5q** exhibited extraordinary high potency in stimulating HUVECs proliferation. All of their maximum values of proliferation rate ($P_{max}$%) were revealed over 80%. The most active compound was the nicotinoyl ester **5a**, which displayed 4 times higher in proliferation rate at 6 times lower concentration than the control drug TMP. As expected, the introduction of some functional groups, such as nicotinoyl **5a**, acetylsalicyloyl **5b**, ferulolyl **5c** and 2-(4-chlorophenoxyl)-2-methyl propionyl **5d**, which are active in cerebrocardiac vascular profiles, produced the remarkable combinational effects.

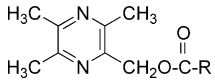
In the cinnamoyl substituted series, compounds **5c**, **5h**–**j** are potentially active but in decreasing order with increase of the substitution number of methoxy groups at the phenyl moiety. Replacement of the cinnamoyl group (**5h**) with dihydrocinnamoyl group (**5g**) improved the activity, and the values of $P_{max}$ increased from 77.32 to 80.54%. In the substituted or unsubstituted benzoic acid ester series (**5k**–**t**), compounds with methoxy substituents are moderately active and with chloro substituents seriously impaired the activity except for 4-chlorobenzoic acid ester (**5q**) presenting a particular high potency ($P_{max}$ = 82.55%, C = 0.1 mmol $L^{-1}$). 2-Iodobenzoic acid ester (**5t**) possessed a better activity than the corresponding 2-chloro compound (**5o**). Bromobenzoic acid esters **5r**–**s** completely lost the activity. In other analogues, dihydrocinnamic acid ester (**5g**) was more active than phenylacetic acid ester (**5f**), which might reflect the importance of alkyl chain length of the aralkyl carboxylic acid. All features described above should be considered in the design of novel TMP derivatives.

Protective effects of some active Liqustrazine derivatives were also tested on acute injured HUVECs damaged by hydrogen peroxide. The concentration of 75 μmol $L^{-1}$ of hydrogen peroxide was selected to induce cells damage identified by cells morphological examination.[10] The results listed in Table 2 showed that compounds **5a**, **5f**, **5g**, **5i** and **5q** were the better inhibitors than TMP against HUVECs proliferation suppression caused by hydrogen peroxide injury. At the concentration of 1.2 mmol $L^{-1}$, compounds **5f** and **5g** remarkably improved the proliferation rates of injured HUVECs exceeding to the normal rates (0.00%) of HUVECs viability, and at the concentration of 1.5 mmol $L^{-1}$ compound **5a** gave the similar result. The protective effects of the active compounds **5a**, **5f**, **5g**, **5i** and **5q** were in concentration-dependent at the tested concentration range of 0.1–1.5 mmol $L^{-1}$, which was paralleled with their properties of stimulating HUVECs proliferation. Interestingly, compounds **5b** and **5d** active in stimulating normal HUVECs proliferation, seemed to show no activity in the tested concentration for protecting the injured




**Scheme 1.** Reagents: (i) 30% $H_2O_2$, AcOH; (ii) $Ac_2O$; (iii) 20% NaOH; (iv) (a) RCOOH/DCC, DMAP or (b) RCOCl/Py.

**Table 1.** The structures and maximum values of stimulating HUVECs proliferation ($P_{max}$%) of the novel Liqustrazine derivatives **5a–u**



| Compd | RCO– | Method | $A_{0\ (570\ nm)}$[a] | $A_{570\ nm}$ | $P_{max}$%[c] | C (mM) |
|---|---|---|---|---|---|---|
| **5a** | Nicotinoyl | a | 0.831±0.05 | 1.56±0.03**[b] | 88.57 | 0.1 |
| **5b** | Acetylsalicyloyl | b | 0.831±0.05 | 1.420±0.03** | 70.88 | 0.1 |
| **5c** | Ferulolyl | a | 0.831±0.05 | 1.528±0.05** | 83.87 | 0.1 |
| **5d** | 2-(4-Cl-phenoxy)-2-methylpropionyl | a | 0.831±0.05 | 1.561±0.06** | 87.85 | 0.1 |
| **5e** | Benzoyl | b | 0.629±0.02 | 0.859±0.05* | 37.00 | 0.6 |
| **5f** | Penylacetyl | b | 0.745±0.04 | 1.312±0.05** | 76.11 | 0.1 |
| **5g** | Dihydrocinnamoyl | a | 0.745±0.04 | 1.345±0.02** | 80.54 | 0.1 |
| **5h** | Cinnamoyl | b | 0.882±0.05 | 1.564±0.04** | 77.32 | 0.6 |
| **5i** | 4-Methoxylcinnamoyl | a | 0.831±0.05 | 1.458±0.05** | 75.45 | 0.1 |
| **5j** | 2,4-Dimethoxylcinnamoyl | a | 0.831±0.05 | 1.414±0.06** | 70.16 | 0.1 |
| **5k** | 2-Methoxylbenzoyl | a | 0.745±0.04 | 1.281±0.05** | 71.95 | 0.1 |
| **5l** | 4-Methoxylbenzoyl | a | 0.745±0.04 | 1.237±0.04** | 66.04 | 0.1 |
| **5m** | 3,4-Dimethoxylbenzoyl | a | 0.882±0.05 | 1.349±0.03** | 52.95 | 0.1 |
| **5n** | 3,4,5-Trimethoxylbenzoyl | a | 0.882±0.05 | 1.286±0.03** | 45.80 | 0.1 |
| **5o** | 2-Chlorobenzoyl | b | 0.629±0.02 | 0.831±0.07 * | 32.10 | 0.6 |
| **5p** | 3-Chlorobenzoyl | b | 0.745±0.04 | 1.187±0.03** | 59.32 | 0.1 |
| **5q** | 4-Chlorobenzoyl | b | 0.745±0.04 | 1.36±0.112** | 82.55 | 0.1 |
| **5r** | 4-Bromobenzoyl | a | 0.629±0.02 | 0.565±0.03 | No[d] | 0.1–1.5 |
| **5s** | 2,4-Dibromobenzoyl | a | 0.629±0.02 | 0.561±0.01 | No | 0.1–1.5 |
| **5t** | 2-Iodobenzoyl | a | 0.629±0.02 | 0.858±0.08** | 61.36 | 1.5 |
| **5u** | 2-Furoyl | a | 0.882±0.05 | 1.494±0.04* | 69.39 | 0.6 |
| TMP | | | 1.433±0.06 | 1.729±0.06* | 20.66 | 0.6 |
| HTMP | 2-Hydrogen | | 1.433±0.06 | 1.835±0.06** | 28.03 | 0.6 |

*$p<0.01$,**$p<0.05$ compared with corresponding control value.
[a]The absorption values at 570 nm were measured using an ELISA plate reader, $A_{0\ (570\ nm)}$: absorption values of the blank control, $A_{570\ nm}$: absorption values of compounds.
[b]Mean±SEM, $n=6$ (well).
[c]Results were expressed as percentage of absorption value in vehicle-treated control culture well, $P(\%)=(A-A_0)/A_0$.
[d]Not active at the concentrations of 0.1–1.5 mmol $L^{-1}$.

**Table 2.** The protective effects of Liqustrazine derivatives on injured HUVECs proliferation

| Compd | Concentrations of compounds (mmol $L^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | 0[a] | 0.1 | 0.3 | 0.6 | 1.2 | 1.5 |
| **5a** | −49.8 | −46.7 | −36.4 | −13.9 | −12.0 | 3.79 |
| **5b** | −45.0 | −34.2 | −46.4 | −53.8 | −63.4 | ND[b] |
| **5c** | −49.8 | −43.2 | −76.7 | −79.7 | −60.6 | −15.6 |
| **5d** | −49.8 | −51.2 | −40.4 | −72.3 | −83.4 | −69.0 |
| **5f** | −45.0 | −29.1 | −24.4 | −12.2 | 6.01 | 4.43 |
| **5g** | −45.0 | −29.7 | −23.5 | −11.5 | 5.62 | 5.70 |
| **5i** | −45.0 | −37.6 | −33.3 | −21.2 | −25.1 | −28.5 |
| **5q** | −45.0 | −40.5 | −28.6 | −28.3 | −2.46 | −1.20 |
| TMP | −49.8 | −48.7 | −47.5 | −41.0 | −44.2 | ND |
| HTMP | −49.8 | −43.6 | −40.5 | −38.2 | −36.4 | −35.8 |

[a]Blank control: The proliferation rate ($P$%) of HUVECs injured by 75 μmol $L^{-1}$ of hydrogen peroxide without any compound addition. The proliferation rate (%) of the nomal HUVECs was designated as 0.00%.
[b]ND, not determined.

HUVECs. Compound **5c** displayed observable protective action while the concentration reached to 1.5 mmol $L^{-1}$. Further studies for the structure–activity relationships of these compounds on the profiles are needed.

In conclusion, a series of novel TMP derivatives was synthesized by way of the acylation reaction of HTMP. Some compounds were found better activity than TMP in stimulating the proliferation of normal vascular endothelial cells and in protecting against hyperoxic acute injury, as seen in 2-nicotinoyl ester **5a**, which exhibited $P_{max}$ value of 88.57% at the concentration of 0.1 mmol $L^{-1}$ in stimulating the normal HUVECs proliferation, and $P_{max}$ value of 3.79% at 1.5 mmol $L^{-1}$ in protecting the damaged HUVECs viability. Structure–activity relationships were briefly discussed. Further bioassay of these compounds on cerebrocardiac vascular activity in cell culture and on animal models is underway.

## Acknowledgements

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15. $^1$H NMR ($CDCl_3$, δ ppm), IR and MS(EI) data for representative compounds: **5a**: $^1$H NMR: 9.22–7.36 (m, 4H, Ar–H), 5.45 (s, 2H, $CH_2$), 2.58 (s, 3H, 6-$CH_3$), 2.53 (s, 3H, 5-$CH_3$), 2.47 (s, 3H, 3-$CH_3$); IR (KBr, $cm^{-1}$): 3085, 3068, 3044 ($\nu_{Py-H}$), 2983, 2949, 2854 ($\nu_{CH}$), 1706 ($\nu_{C=O}$), 1587, 1416 ($\nu_{C=C}$,), 1276, 1144 ($\nu_{C\text{-}O\text{-}C}$), 745 ($\gamma_{Ar-H}$); MS (*m*/*z*): M + 1: 258.9. **5b**: $^1$H NMR: 7.58–6.94 (m, 4H, Ar–H), 5.38 (s, 2H, $CH_2$), 2.56 (s, 3H, 6-$CH_3$), 2.52 (s, 3H, 5-$CH_3$), 2.51 (s, 3H, $CH_3COO$–Ar), 2.31 (s, 3H, 3-$CH_3$). IR (nujol, $cm^{-1}$): 2991, 2952, 2923 ($\nu_{CH}$), 1753 ($\nu_{C=O}$), 1615, 1469 ($\nu_{C=C}$), 1308, 1253 ($\nu_{C\text{-}O\text{-}C}$); MS (*m*/*z*): M + 1: 314.1. **5c**: IR (KBr, $cm^{-1}$): 3326 ($\nu_{NH}$), 2923, 2850 ($\nu_{CH}$), 1762, 1700 ($\nu_{C=O}$), 1629, 1509, 1423 ($\nu_{C=C}$), 1274, 1218 ($\nu_{C\text{-}O\text{-}C}$, $CH_2OCO$), 1235, 1155 ($\nu_{C\text{-}O\text{-}C}$, Ar-*O*-$CH_3$); MS (*m*/*z*): M + 1: 371.9. **5d**: $^1$H NMR: 7.11 (d, 2H, $C_2'$-H, $C_6'$-H, *J* = 8.7 Hz), 6.76 (d, 2H, $C_3'$-H, $C_5'$-H, *J* = 9.0 Hz), 5.26 (s, 2H, $CH_2$), 2.49 (s, 3H, 6-$CH_3$), 2.45 (s, 3H, 5-$CH_3$), 2.41(s, 3H, 3-$CH_3$), 1.60 (s, 6H, $CH_3$–C–$CH_3$); IR (nujol, $cm^{-1}$): 2992, 2939, 2924, 2857 ($\nu_{CH}$), 1739 ($\nu_{C=O}$), 1594, 1489 ($\nu_{C=C}$,), 1282, 1093 ($\nu_{C\text{-}O\text{-}C,}$ $CH_2OCO$), 1238, 1139 ($\nu_{C\text{-}O\text{-}C}$, CO–Ar). **5f**: $^1$H NMR: 7.32–7.27 (m, 5H, Ar–H), 5.21 (s, 2H, $CH_2O$), 3.68 (s, 2H, $CH_2$–Ar), 2.50 (s, 3H, 6-$CH_3$), 2.49 (s, 3H, 5-$CH_3$), 2.42 (s, 3H, 3-$CH_3$); IR (KBr, $cm^{-1}$): 3027, 2943, 2932, 2850 ($\nu_{CH}$), 1736 ($\nu_{C=O}$), 1495, 1412 ($\nu_{C=C}$), 1245 ($\nu_{C\text{-}O\text{-}C}$), 1128; MS (*m*/*z*): M + 1: 271.4. **5g**: $^1$H NMR: 7.27–7.16 (m, 5H, Ar–H), 5.19 (s, 2H, $CH_2O$), 2.97 (t, 2H, $CH_2$–Ar), 2.70 (t, 2H, CO–$CH_2$), 2.51 (s, 3H, 6-$CH_3$), 2.49 (s, 3H, 5-$CH_3$), 2.46 (s, 3H, 3-$CH_3$); IR (nujol, $cm^{-1}$): 3027, 2924, 2862 ($\nu_{CH}$), 1739 ($\nu_{C=O}$), 1496, 1415 ($\nu_{C=C}$), 1158 ($\nu_{C\text{-}O\text{-}C}$); MS (*m*/*z*): M + 1: 285.2. **5i:** $^1$H NMR: 7.67 (d, 1H, =CH–Ar, *J* = 16.2 Hz), 7.41 (d, 2H, $C_2'$-H, $C_6'$-H, *J* = 11.7 Hz), 6.89 (d, 2H, $C_3'$-H, $C_5'$-H, *J* = 9.0 Hz), 6.35 (d, 1H, CO–CH=, *J* = 16.2 Hz), 5.31 (s, 2H, $CH_2$), 3.83 (s, 3H, $OCH_3$), 2.57 (s, 3H, 5-$CH_3$), 2.52 (s, 6H, 5,6-$CH_3$); IR (KBr, $cm^{-1}$): 2972, 2960, 2923, 2836 ($\nu_{CH}$), 1704 ($\nu_{C=O}$), 1636 ($\nu_{C=C}$), 1602, 1512, 1412 ($\nu_{C=C}$), 1289, 1166 ($\nu_{C\text{-}O\text{-}C}$, $CH_2OCO$), 1248, 1026 ($\nu_{C\text{-}O\text{-}C}$, Ar-*O*-$CH_3$), 927 ($\gamma_{=CH}$); MS (*m*/*z*): M + 1: 313.4. **5q**: $^1$H NMR: 7.95 (d, 2H, $C_2'$ -H, $C_6'$-H), 7.36 (d, 2H, C3′ -H, C5′-H), 5.40 (s, 2H, $CH_2$), 2.54 (s, 3H, 6-$CH_3$), 2.52 (s, 3H, 5-$CH_3$), 2.48 (s, 3H, 3-$CH_3$); IR (KBr, $cm^{-1}$): 3056, 2985, 2956, 2920 ($\nu_{CH}$), 1724 ($\nu_{C=O}$), 1596, 1415 ($\nu_{C=C}$), 1274 ($\nu_{C\text{-}O\text{-}C}$); MS (*m*/*z*): M + 1: 291.