The preparation of novel ligustrazine derivatives as potential cerebrocardiac vascular agents

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A series of novel substituted cinnamoylpiperazinyl ligustrazine derivatives **7** has been prepared by alkylation of cinnamoylpiperazines by (chloromethyl)trimethylpyrazine (**5**) or by cinnamoylation of trimethyl(piperazinomethyl) pyrazine (**6**).

Keywords: ligustrazine, pyrazines, piperazines, cinnamic amides

Ligustrazine (tetramethylpyrazine, TMP, **1**, Fig. 1) is a major efficient component of the Chinese traditional medicinal herb *Ligusticum chuanxiong*, which currently is widely used in China for the treatment of coronary atherosclerotic cardiovascular and ischemic cerebrocardiac vascular disease.[1]

Ligustrazine has been reported to inhibit platelet aggregation,[2] to cause negative chronotropic and inotropic responses on isolated atria, to inhibit vasoconstriction in isolated vascular strips, and to act as a vasodilator, a free radical scavenger,[3] and an anti-thrombosis and anti-hypertensive agent.[4,5]

However, pharmacokinetic studies have shown ligustrazine to present low bioavailability and to be metabolised rapidly *in vivo* with a short half-life ($t_{1/2}$ = 2.89 h),[6] so accumulated toxicity often occurs in patients when an effective plasma concentration is maintained by frequent administration. Therefore, it is necessary to develop a new generation of cerebrocardiac vascular drugs derived from molecular modification of ligustrazine.

Structure-activity relationship studies have indicated that the pyrazine ring in the molecule of ligustrazine might largely be the determinant of its pharmacodynamics, while the methyl groups might primarily control its pharmacokinetics and toxicity.[7] According to the combination principles of medicinal chemistry, some drug-like groups and pharmacophores can be introduced into a methyl position of ligustrazine, it thereby acquiring pharmacologically additive or synergetic effects to improve pharmacokinetic properties.

Cinnamoyl and substituted cinnamoyl groups are the pharmacophores or drug-like groups of some cerebrocardiac vascular drugs, such as ferulic acid and Ozagrel. (**2**, Fig. 1) A piperazine moiety as a linker is a common character existing in some cerebrocardiac vascular drugs, such as Cinnarizine and Flunarizine (**3**), and it is considered as the functional group for keeping the drug potential. Based on the structures of ferulic acid, Ozagrel, Cinnarizine and Flunarizine, ligustrazine (**1**) can be modified by combination with a piperazine and various substituted cinnamoyl groups to form structures **4** (Fig. 1).[8]

In the synthesis, (3,5,6-trimethylpyrazin-2-yl)methanol (**4,** Scheme 1) was prepared *via* the Boekelheide reaction, but in an improved one-pot reaction, starting with the oxidation of tetramethylpyrazine trihydrate (**1**) by 30% hydrogen peroxide in acetic acid, to form tetramethylpyrazine mono-*N*-oxide (**2**), followed by acylation and rearrangement in refluxing acetic anhydride, producing (3,5,6-trimethylpyrazin-2-yl)methyl acetate (**3**). Without separation, the reaction mixture containing compound **3** was saponified with sodium hydroxide to obtain the crude product **4**, which was recrystallised from *n*-hexane to obtain the pure material.[9] The important intermediate 2-chloromethyl-3,5,6-trimethylpyrazine hydrochloride (**5**) was prepared by reaction of compound **4** with $SOCl_2$ in anhydrous CH_2Cl_2.[10] Amination of **5** with various N_1-(substituted cinnamoyl)piperazines gave compounds **7b**, **7c**, **7d**, while

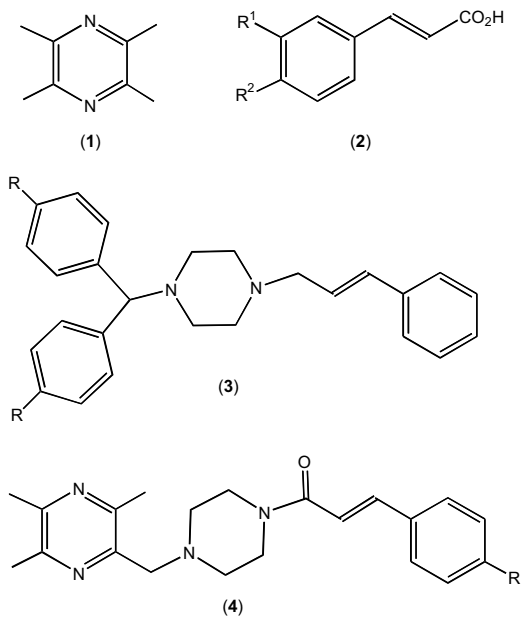

Fig. 1 (**1**) ligustrazine (tetramethylpyrazine, TMP); (**2**) ferulic acid (R^1 = CH_3O, R^2 = OH) and Ozagrel (R^1 = H, R^2 = (1H-imidazol-1-yl)methyl); (**3**) Cinnarizine (R = H) and Flunarizine (R = F); (**4**) cinnamoylpiperazinyl ligustrazine derivatives **7a**–**7f.**

amination with piperazine produced the intermediate 2,3,5-trimethyl-6-(piperazin-1-ylmethyl)pyrazine (**6**), which was then acylated with 4-substituted cinnamoyl chlorides, forming **7a**, **7e**, **7f**[11] (Scheme 1). The N_1-substituted cinnamoylpiperazines were prepared by acylation of piperazine in our laboratory.[12]

The newly synthesised compounds have not previously been reported, and their chemical structures were confirmed by IR, ^{1}H NMR and ESI–MS. In the IR spectra, the target compounds **7a**–**f** showed very strong peaks between 1644 and 1652 cm^{-1}, attributed to the C=O stretching frequency of the amide groups. A very strong peak of γ_{CH} was observed between 978 and 998 cm^{-1}, which indicated the *trans*-configuration of the double bond in the cinnamoyl group. In the ^{1}H NMR spectra, their chemical shifts (δ) were divided into three groups: the signals of CH_3 groups were at about 2.5 ppm, N–CH_2 groups were 2.3–4.0 ppm and the Ar–H and =CH between δ 6.5 – 8.0 ppm. The *trans*-configuration of the double bond in the cinnamoyl group was further proved by the coupling constants ($J_{CH=CH}$ = 15.4 Hz). In the ESI–MS spectra, the $[M + H]^+$ peak of each compound was well matched to its molecular weight.

In summary, six ligustrazine derivatives (**7a**–**f**) have been prepared and their chemical structures were confirmed by IR, ^{1}H NMR and ESI–MS. Screening tests for their cerebrocardiac vascular activities are under way.

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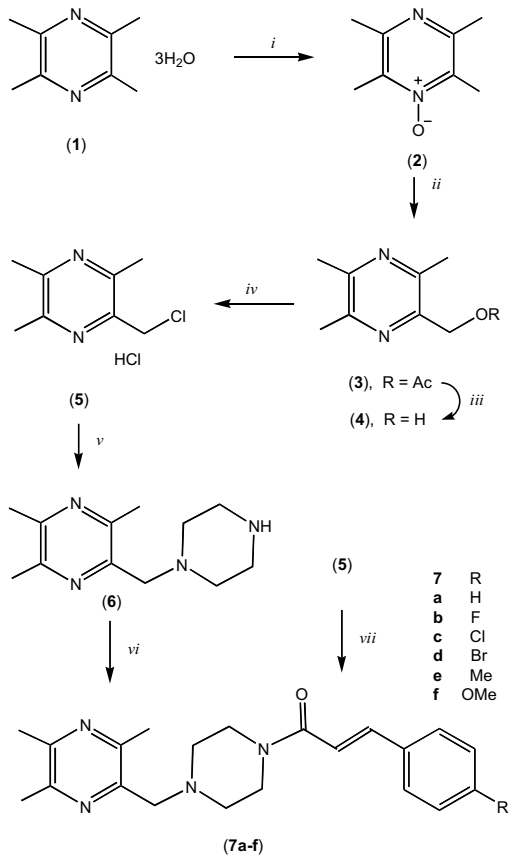

Scheme 1 *Reagents i* 30% H_2O_2, AcOH, 70°C; *ii* Ac_2O, 2 h reflux; *iii* 20% NaOH; *iv* $SOCl_2/CH_2Cl_2$, *v* anhydrous piperazine; *vi* cinnamoyl chlorides in CH_2Cl_2, Na_2CO_3, reflux 10 h, *vii* substituted cinnamoylpiperazines, NaI, Et_3N in toluene, reflux 10 h.

Experimental

Melting points were determined using a X-6 Microscope Melting Point Inspect instrument. Infrared spectra were recorded on a Nicolet Nexus 470 FT spectrometer. ^{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 API4000 mass spectrograph. Elemental analyses were performed on an Elementar Analysensysteme Vario EI instrument. Purification on silica gel refers to flash column chromatography on silica gel 60 (particles size 200-300 mesh).

(3,5,6-Trimethylpyrazin-2-yl)methanol (**4**): 2,3,5,6-Tetramethylpyrazine trihydrate (**1**) (30.40 g, 160 mmol) was heated with 30% hydrogen peroxide (18 ml, 160 mmol) in glacial acetic acid (40 ml) for 4 h at 70°C. Further 30% hydrogen peroxide (18 ml, 160 mmol) was then added and the heating was continued for 4 h. The solution was made alkaline with 50% sodium hydroxide and extracted with chloroform. The combined extracts were dried and evaporated *in vacuo*; tetramethylpyrazine mono-*N*-oxide (**2**) was obtained. To this, acetic anhydride (15.1 ml, 160 mmol) was added and the mixture was refluxed for 3 h, checking for product formation via TLC. The excess of acetic anhydride was evaporated and (3,5,6-trimethylpyrazin-2-yl)methyl acetate (**3**) was obtained, which was directly saponified with 20% NaOH (155 ml), and extracted with chloroform. The combined extracts were dried and the solvent removed. The residual oil was recrystallised from *n*-hexane; (3,5,6-trimethylpyrazin-2-yl)methanol (**4**) was obtained as yellow needles (15.50 g, 64%); m.p. 88–89°C (lit.[9] 88–89°C).

2-Chloromethyl-3,5,6-trimethylpyrazine hydrochloride (**5**): Thionyl chloride (7.41 ml, 102 mmol) was added dropwise to (3,5,6-trimethylpyrazin-2-yl)methanol (**4**) (15.50 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 (**5**) was obtained as a yellow solid (21.11 g, 100%); m.p. 102–105°C. Compound **5** was basified and then purified to give 2-chloromethyl-3,5,6-trimethylpyrazine as oil for spectral confirmation. IR: ν_{max} (KBr)/cm^{-1} 2993 (CH), 2952 (CH), 2923 (CH), 2856 (CH), 1548 (C=N). NMR: δ_H (600 MHz, $CDCl_3$) 4.68 (s, 2H, CH_2), 2.63 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 2.52 (s, 3H, CH_3); δ_C (150 MHz, $CDCl_3$) 151.6 (C=N), 149.3 (C=N), 149.0 (C=N), 146.3 (C=N), 44.8 (CH_2), 21.6 (CH_3), 21.4 (CH_3), 20.5 (CH_3). MS: *m/z* (ESI) 171 ([M + H]$^+$).

2,3,5-Trimethyl-6-(piperazin-1-ylmethyl)pyrazine (**6**): 2-Chloromethyl-3,5,6-trimethylpyrazine hydrochloride (**5**) (20.7 g, 100 mmol) in chloroform (100 ml) was added dropwise into anhydrous piperazine (49.88 g, 580 mmol) in chloroform (300 ml) at 0°C. The solution was left at room temperature for 5 h, checking for product formation via TLC. The mixture solution was washed with aqueous ammonia (4M), and the organic layers were dried. The solvent was evaporated *in vacuo* and the crude product was recrystallised from *n*-hexane to give 2,3,5-trimethyl-6-(piperazin-1-ylmethyl)pyrazine (**6**) as white crystals (11 g, 50%); m.p. 94°C. IR: ν_{max} (KBr)/cm^{-1} 3443, 3272 (NH), 2943 (CH), 1546 (C=N). NMR: δ_H (600 MHz, $CDCl_3$) 2.57–3.60 (m, 10H, 5 × CH_2), 2.49, 2.48 and 2.47 (3 × s, 9H, 3 × CH_3), 1.90 (s, 1H, NH); δ_C (150 MHz, $CDCl_3$) 153.6 (C=N), 150.4 (C=N), 148.7 (C=N), 147.2 (C=N), 56.6, 54.4, 54.1, 45.2 and 44.4 (5 × CH_2), 21.4, 21.0 and 20.1 (3 × CH_3). MS: *m/z* (ESI) 221 ([M + H]$^+$).

General procedure for the preparation of (E)-3-(4-substituted-phenyl)-1-[4-[(3,5,6-trimethylpyrazin-2-yl]methyl]piperazin-1-yl) prop-2-en-1-ones (**7b**, **7c** and **7d**)

2-Chloromethyl-3,5,6-trimethylpyrazine hydrochloride (**5**) (2.07 g, 10 mmol) and (*E*)-3-(4-substituted-phenyl)-1-(piperazin-1-yl)prop-2-en-1-one (10 mmol) were dissolved in toluene (70 ml). Triethylamine (3.46 ml, 25 mmol) and NaI (catalytic quantity) were added to the solution. The mixture solution was refluxed for 10 h until the reaction was complete (monitored by TLC). After cooling, the mixture was filtered and the filtrate was evaporated *in vacuo*. The final product was purified by flash column chromatography and recrystallisation from *n*-hexane.

(E)-3-(4-Fluorophenyl)-1-[4-[(3,5,6-trimethylpyrazin-2-yl) methyl]piperazin-1-yl]prop-2-en-1-one (**7b**): (2.25 g, 61%); m.p. 107–108°C. IR: ν_{max} (KBr)/cm^{-1} 2917 (CH), 1647 (C=O), 1601 (C =C), 1510 (C=N), 986 (=CH). NMR: δ_H (600 MHz, $CDCl_3$) 7.62 (d, 1H, =CH, *J* = 15.4 Hz), 7.49 (m, 2H, Ar–H), 7.05 (t, 2H, Ar–H, *J* = 8.6 Hz), 6.78 (d, 1H, =CH,*J* = 15.4 Hz), 2.50–3.72 (m, 10H, 5 × CH_2), 2.58, 2.53 and 2.49 (3 × s, 9H, 3 × CH_3). MS: *m/z* (ESI) 369 ([M + H]$^+$). Found: C, 68.73; H, 6.87; N, 15.15. $C_{21}H_{25}FN_4O$ requires C, 68.46; H, 6.84; N, 15.21%.

(E)-3-(4-Chlorophenyl)-1-(4-((3,5,6-trimethylpyrazin-2-yl)methyl) piperazin-1-yl)prop-2-en-1-one (**7c**): (1.19 g, 31%); m.p. 136–138°C. IR: ν_{max} (KBr)/cm^{-1} 2936 (CH), 1644 (C=O), 1602 (C =C), 1569 (C=N), 986 (=CH). NMR: δ_H (600 MHz, $CDCl_3$) 7.61 (d, 1H, =CH, *J* = 15.4 Hz), 7.44 (d, 2H, Ar–H,*J* = 7.5 Hz), 7.34 (d, 2H, Ar–H, *J* = 7.7 Hz), 6.84 (d, 1H, =CH, *J* = 15.4 Hz), 2.60–3.73 (m, 10H, 5 × CH_2), 2.53, 2.50 and 2.49 (3 × s, 9H, 3 × CH_3). MS: *m/z* (ESI) 385 ([M + H]$^+$). Found: C, 65.27; H, 6.57; N, 14.60. $C_{21}H_{25}ClN_4O$ requires C, 65.53; H, 6.55; N, 14.56%.

(E)-3-(4-Bromophenyl)-1-[4-[(3,5,6-trimethylpyrazin-2-yl) methyl]piperazin-1-yl]prop-2-en-1-one (**7d**): (1.72 g, 40%); m.p. 137–139°C. IR: ν_{max} (KBr)/cm^{-1} 2916 (CH), 1651 (C=O), 1600 (C =C), 1585 (C=N), 978 (=CH). NMR: δ_H (600 MHz, $CDCl_3$) 7.61 (d, 1H, =CH, *J* = 15.4 Hz), 7.51 (m, 2H, Ar–H), 7.39 (m, 2H, Ar–H), 6.86 (d, 1H, =CH, *J* = 15.4 Hz), 2.60–3.66 (m, 10H, 5 × CH_2), 2.59, 2.55 and 2.51 (3 × s, 9H, 3 × CH_3). MS: *m/z* (ESI) 429 ([M + H]$^+$). Found: C, 58.95; H, 5.89; N, 13.01. $C_{21}H_{25}BrN_4O$ requires C, 58.75; H, 5.87; N, 13.05%.

General procedure for the preparation of (E)-3-(4-substituted-phenyl)-1-[4-[(3,5,6-trimethylpyrazin-2-yl)methyl]piperazin-1-yl]prop-2-en-1-one (**7a**, **7e** and **7f**)

To a mixture of 2,3,5-trimethyl-6-(piperazin-1-ylmethyl)pyrazine (**6**) (2.2 g, 10 mmol) and Na_2CO_3 (3.18 g, 30 mmol) in anhydrous CH_2Cl_2 (100 ml), was added dropwise a 4-substituted cinnamoyl chloride (10 mmol) in anhydrous CH_2Cl_2 (100 ml) at room temperature. The mixture was refluxed for 10 h (checked by TLC), and the solvent was evaporated *in vacuo*. The final product was purified by flash column chromatography and recrystallisation from *n*-hexane.

(E)-3-Phenyl-1-[4-[(3,5,6-trimethylpyrazin-2-yl)methyl]piperazin-1-yl]prop-2-en-1-one (**7a**): (1.58 g, 45%); m.p. 60–62°C. IR: ν_{max} (KBr)/cm^{-1} 2918 (CH), 1650 (C=O), 1604 (C =C), 998 (=CH). NMR: δ_H (600 MHz, $CDCl_3$) 7.66 (d, 1H, =CH, *J* = 15.4 Hz), 7.51 (d, 2H, Ar–H, *J* = 6.7 Hz), 7.36 (m, 3H, Ar–H), 6.86 (1H, =CH, *J* = 15.4 Hz), 3.65–3.74 (m, 10H, 5 × CH_2), 2.59, 2.51 and 2.50

(3 × s, 9H, 3 × CH_3). MS: *m/z* (ESI) 351 ($[M + H]^+$). Found: C, 71.69; H, 7.45; N, 16.05. $C_{21}H_{26}N_4O$ requires C, 71.97; H, 7.48; N, 15.99%.

(E)-3-p-Tolyl-1-[4-[(3,5,6-trimethylpyrazin-2-yl)methyl] piperazin-1-yl]prop-2-en-1-one (**7e**): (2.33 g, 64%); m.p. 160–162°C. IR: ν_{max} (KBr)/cm^{-1} 2918 (CH), 1652 (C=O), 1600 (C =C), 1512 (C=N), 981 (=CH). NMR: δ_H (600 MHz, $CDCl_3$) 7.64 (d, 1H, =CH, *J* = 15.4 Hz), 7.41 (d, 2H, Ar–H, *J* = 8.1 Hz), 7.17 (d, 2H, Ar–H, *J* = 7.9 Hz), 6.81 (d, 1H, =CH, *J* = 15.4 Hz), 2.50–3.66 (m, 10H, 5 × CH_2), 2.59, 2.54, 2.50 and 2.36 (4 × s, 12H, 4 × CH_3). MS: *m/z* (ESI) 365 ($[M + H]^+$). Found: C, 72.21; H, 7.77; N, 15.43. $C_{22}H_{28}N_4O$ requires C, 72.50; H, 7.74; N, 15.37%.

(E)-3-(4-Methoxyphenyl)-1-[4-[(3,5,6-trimethylpyrazin-2-yl)methyl]piperazin-1-yl]prop-2-en-1-one (**7f**): (1.52 g, 40%); m.p. 100–101°C. IR: ν_{max} (KBr)/cm^{-1} 2937 (CH), 1644 (C=O), 1591 (C =C), 1574 (C=N), 1226 (C-O), 1176 (C–O), 984 (=CH). NMR: δ_H (600 MHz, $CDCl_3$) 7.63 (d, 1H, =CH, *J* = 15.4 Hz), 7.46 (d, 2H, Ar–H, *J* = 8.7 Hz), 6.89 (d, 2H, Ar–H, *J* = 8.7 Hz), 6.72 (d, 1H, =CH, *J* = 15.4 Hz), 3.84 (3H, OCH_3), 2.53–3.72 (m, 10H, 5 × CH_2), 2.59, 2.54 and 2.50 (3 × s, 9H, 3 × CH_3). MS: *m/z* (ESI) 381 ($[M + H]^+$). Found: C, 69.65; H, 7.41; N, 14.70. $C_{22}H_{28}N_4O_2$ requires C, 69.45; H, 7.42; N, 14.73%.

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