Synthesis of Ranolazine Metabolites and Their Anti-myocardial Ischemia Activities

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The anti-anginal drug Ranolazine, a partial fatty acid oxidation (pFOX) inhibitor, is thought to modulate the metabolism during myocardial ischemia by activating pyruvate dehydrogenase activity to promote glucose oxidation. Ranolazine and its five principal metabolites: CVT-2512, CVT-2513, CVT-2514, CVT-2738 and CVT-4786, were synthesized. The effect of Ranolazine and its metabolites on the ECG (electrocardiogram) of mice with myocardial ischemia induced by isoprenaline and their effect on alleviating the symptom of myocardial ischemia were tested and compared. The results showed that CVT-2738 and CVT-2513 could be protective against mice myocardial ischemia induced by isoprenaline. Within all the metabolites tested in this study, CVT-2738 exhibited the best potency, however, it was still less potent than Ranolazine.

Key words Ranolazine; metabolite; myocardial ischemia; R–R interval; T-wave

Angina pectoris is one of the most frequent clinical syndromes associated with ischemic heart diseases. The incidence of this disease is increasing with the improvement of living standard and the change of the mode of life. It has been generally accepted that an efficient therapeutic approach to ischemic heart diseases is to improve the myocardial oxygen balance between supply and demand in the ischemic heart, by either increasing coronary blood flow or decreasing cardiac mechanical function, or both. The traditional anti-anginal drugs are nitrates, β-blockers and calcium channel blockers, which are thought to improve the myocardial oxygen balance with changes in hemodynamic parameters. But these drugs also bring further injury to the weak cardiac function. So researchers are trying to find more efficient means with fewer side effects to prevent and treat myocardial ischemia.

Metabolism modulation is a novel way for the treatment of angina pectoris.[1)] Ranolazine (Fig. 1) is a piperazine derivative developed by Syntex Laboratories, which has been shown to have anti-ischemic action.[2—5)] It's a partial fatty acid oxidation (pFOX) inhibitor. Its mechanism has not been fully understood. The possible mechanism involves modulation of the metabolism of myocardial ischemia by activating pyruvate dehydrogenase activity to promote glucose oxidation.[2,6—9)] Ranolazine is thought to switch substrate utilization from fatty acids to glucose to improve the efficiency

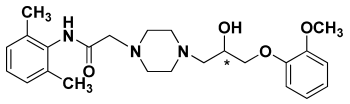

Fig. 1. Ranolazine

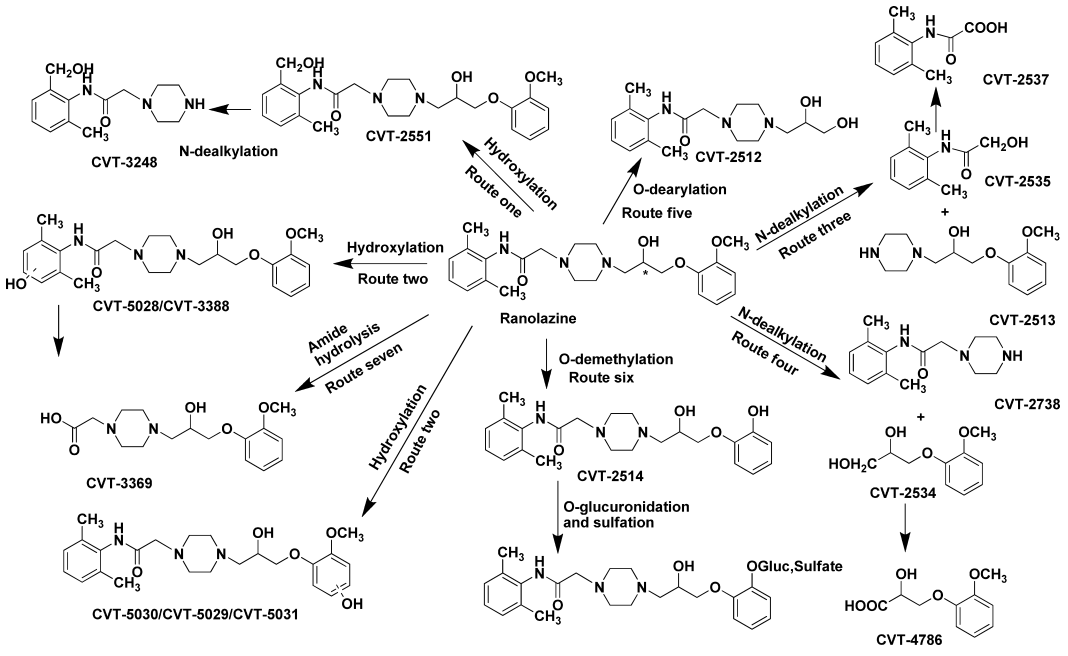

Fig. 2. Ranolazine Metabolic Way

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of oxygen utilization and limit the production of lactic acid.[6,10,11] Compared with the traditional anti-anginal drugs, the main advantage of Ranolazine is that it has little hemodynamic effect with minimal or no effect on blood pressure or heart rate.[12—14]

Ranolazine is almost completely metabolized after administration, and less than 5% of the administered dose is excreted unchanged.[15] Metabolism of Ranolazine is complex.[16,17] Cytochrome P450 (CYP) 3A (mainly in liver and small intestine) accounts for the major biotransformation of Ranolazine (70—75%). CYP 2D6 accounts for less than 20% of Ranolazine metabolism. Figure 2 shows the seven principal metabolic routes of Ranolazine.[18,19] Route one: hydroxylation of the methyl group in the 2,6-dimethylphenyl fragment to produce CVT-2551; route two: hydroxylation of the 2-methoxyphenyl or 2,6-dimethylphenyl group to produce CVT-5029/CVT-5030/CVT-5031 and CVT-5028/CVT-3388 respectively; route three and four: *N*-dealkylation of the piperazine ring to give the components CVT-2513, CVT-2535, CVT-2738, CVT-2534; route five: *O*-dearylation to produce CVT-2512; route six: *O*-demethylation to produce CVT-2514; route seven: hydrolysis of the amide group to produce CVT-3369. Among those metabolites, there are four metabolites should be paid more attention since their blood concentrations exceed 10% of the parent compound concentration. They are CVT-2738 (38%), CVT-2514 (26%), CVT-4786 (21%, a further metabolite of CVT-2534), and CVT-2512 (11%). Studies with human liver microsomes indicated that CVT-2738 and CVT-4786 were formed primarily through CYP3A; CVT-2514 was formed through CYP2D6; CVT-2512 was formed through both the pathways. Besides the four major metabolites, CVT-2537, CVT-2513, CVT-2535, CVT-3248, CVT-3388/CVT-5028, CVT-2551, and CVT-5030 have blood concentrations exceeding 1% of Ranolazine exposure.

In this study, Ranolazine and its five principal metabolites, including CVT-2512, CVT-2513, CVT-2514, CVT-2738 and CVT-4786 were synthesized and the effect of Ranolazine, CVT-2512, CVT-2513, CVT-2514, CVT-2738 and CVT-4786 on the ECG (electrocardiogram) of mice with myocardial ischemia induced by isoprenaline were tested and compared.

Experimental

General All commercially available solvents and reagents were used without further purification. Melting points were determined with a Bü chi capillary apparatus and were not corrected. ^{1}H- and ^{13}C-NMR spectra were recorded on an ACF 300Q Bruker, spectrometer in $CDCl_3$, with Me_4Si as the internal reference, or in DMSO-d_6. Low-and high-resolution mass spectra (LR-MS and HR-MS) were recorded in electron impact mode. Reactions were monitored by TLC on Silica Gel 60 F254 (Qindao Ocean Chemical Company, China) plates exposed to H_2SO_4 (10% in EtOH) spray followed by charring (≈50 °C). Column chromatography was performed with Silica Gel Geduran Si 60 (Qindao Ocean Chemical Company, China).

2-Chloro-*N*-(2,6-dimethylphenyl)acetamide (A1) Toluene (150 ml) and 2,6-dimethylaniline (9.25 ml, 75.09 mmol) were added to an aqueous solution of sodium bicarbonate (7.95 g in 75 ml water). Chloroacetyl chloride (7.15 ml, 89.87 mmol) was added slowly at 0 °C. Then the solution was stirred at room temperature for 2.5 h. The resulting solids were collected by filtration, washed with water and dried to give **A1** (13.18 g, 88.9%) as a white solid, mp 138—140 °C. The solid was used directly in the next step.

***N*-(2,6-Dimethyl phenyl)-1-piperazine Acetamide (CVT-2738)** A mixture of **A1** (8.0 g, 40 mmol) and piperazine (13.8 g, 160 mmol) in 100 ml of ethanol was stirred at 85 °C for 3 h. Then ethanol was removed under reduced pressure, the residue was neutralized with ammonia water. The mixture was extracted with dichloromethane, and the organic layer was washed with water, dried over Na_2SO_4 and concentrated. The residue was extracted with hot ether. Concentration of the ether layer gave CVT-2738 (26.2 g, 62%) as a white solid, mp 94—97 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.10 (s, 1H), 7.09 (s, 1H), 3.19 (s, 2H), 2.98 (t, 4H, J=5.27 Hz), 2.69 (t, 3H, J=4.77 Hz), 2.23 (s, 6H). ESI-MS *m*/*z*: 248.3 $[M+H]^+$. The analytical data of CVT-2738 was identical with the data reported in reference 20. CVT-2738 was dissolved in absolute ethanol and to the solution was added hydrochloric acid. Then the solvent was removed under reduced pressure and the residue was recrystallized with acetone to give hydrochloric acid salt of CVT-2738 as a white solid, mp 194—199 °C.

1-Methoxy-2-(oxiranyl methoxy)benzene (A2) 2-Methoxyphenol (6.73 ml, 61.2 mmol) and a solution of sodium hydroxide (5.8 g in 12 ml water) were added to 20 ml of dioxane and the mixture was heated to 45 °C. Then epichlorohydrin (13.5 ml) was added dropwise. The mixture was stirred at 45 °C for 3 h. The reaction mixture was cooled, diluted with ethyl acetate, filtered. The filtrate was washed with water (20 ml×3). The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography (SiO_2, petroleum ether : ethyl acetate=6 : 1) to give **A2** (5.83 g, 53.0%) as a colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.96—6.89 (m, 4H), 4.25—4.02 (m, 2H), 3.87 (s, 3H), 3.41—3.35 (m, 1H), 2.90—2.72 (m, 2H). EI-MS *m*/*z* (%): 180 (38), 124 (59), 109 (47), 95 (14), 77 (25), 58 (100). The analytical data of **A2** was identical with the data reported in ref. 20.

Ranolazine A mixture of CVT-2738 (2 g, 11.10 mmol) and **A2** (3 g, 11.1 mmol) in 20 ml of methanol and 40 ml of toluene was stirred at 65 °C for 9 h. The solvent was removed under reduced pressure. The crude product was purified by column chromatography (SiO_2, dichloromethane : methanol=15 : 1) to give Ranolazine (3.31 g, 69.8%) as a yellow oil. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 10.02 (s, 1H), 7.11—6.88 (m, 7H), 4.42 (m, 1H), 4.00 (s, 2H), 4.00—3.93 (m, 2H), 3.94—3.41 (m, 13H), 2.18 (s, 6H); ESI-MS *m*/*z*: 428.3 $[M+H]^+$. The analytical data of Ranolazine was identical with the data reported in ref. 20. Ranolazine was dissolved in absolute ethanol and to the solution was added hydrochloric acid. Then the solvent was removed under reduced pressure and recrystallization with acetone gave hydrochloric acid salt of Ranolazine as a white solid, mp 227—229 °C.

2-Benzyloxyphenol (A4) Catechol (2.2 g, 20 mmol), potassium carbonate (3.1 g, 22 mmol) and potassium iodide (0.04 g, 0.6 mmol) were added to 15 ml of acetone and heated to 50 °C. Then chlorobenzyl (2.36 ml, 22 mol) was added dropwise. The mixture was stirred for 3 h. The solvent was removed and to the residue was added 40 ml of cold water. The mixture was filtered, and the aqueous layer was neutralized with diluted hydrochloric acid and extracted with dichloromethane (60 ml×3). The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography (SiO_2, petroleum ether : ethyl acetate=15 : 1) to give **A4** (0.5 g, 12.5%) as a yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.42—7.37 (m, 5H), 6.94—6.83 (m, 4H), 5.64 (s, 1H), 5.10 (s, 2H); EI-MS *m*/*z* (%): 200 (3), 91 (100), 65 (10). The analytical data of **A4** was identical with the data reported in ref. 21.

1-(2-Benzyloxyphenoxy)-2,3-propylene Oxide (A5) The procedure was in accordance with the synthesis of A2. The product **A5** was a yellow oil (62.5%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.47—7.26 (m, 5H), 6.96—6.91 (m, 4H), 5.14 (s, 2H), 4.30—4.04 (dd, 2H, J=3.4, 5.4, 11.3 Hz), 3.40—3.37 (m, 1H), 2.90—2.75 (m, 2H); EI-MS *m*/*z* (%): 256 (10), 91 (100), 65 (8). The analytical data of **A5** was identical with the data reported in ref. 21.

1-[3-(2-Benzyloxyphenoxy)-2-hydroxypropyl]-4-[*N*-(2,6-dimethylphenyl)aminocarbonylmethyl]piperazine (A6) The procedure was in accordance with the synthesis of Ranolazine. The product **A6** was a yellow oil (74.7%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.67 (s, 1H), 7.48—6.92 (m, 12H), 5.11 (s, 2H), 4.16—4.01 (m, 3H), 3.19 (s, 2H), 2.71—2.46 (m, 10H), 2.25 (s, 6H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 168.3, 149.2, 149.1, 137.3, 134.9, 133.6, 128.4, 128.3, 127.8, 127.3, 127.1, 122.1, 121.8, 115.7, 115.1, 72.3, 71.2, 66.1, 61.6, 60.5, 53.8, 53.6, 53.4, 53.4, 18.6; IR (KBr) cm^{-1}: 3412, 3308, 2937, 2820, 1682, 1592, 1504, 1378, 1257, 1124, 1013, 831, 743, 698; ESI-MS *m*/*z*: 504.3 $[M+H]^+$, 526.2 $[M+Na]^+$; FAB-MS *m*/*z*: 503.2781 (Calcd for $C_{17}H_{27}N_3O_3$: 503.2784).

1-[3-(2-Hydroxylphenoxy)-2-hydroxypropyl]-4-[*N*-(2,6-dimethylphenyl)aminocarbonylmethyl]piperazine (CVT-2514) A suspension of **A6** and palladium on charcoal in ethyl acetate was stirred under a hydrogen atmosphere for 24 h. The reaction mixture was filtered and the ethyl acetate was removed under reduced pressure. The crude product was purified by column chromatography (SiO_2, dichloromethane : methanol=1 : 1) to give CVT-2514 (60.9%) as a yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.59 (s, 1H), 7.11—6.77 (m, 7H), 4.14—4.06 (m, 1H), 4.04—3.97 (m, 2H), 3.24 (s, 2H), 2.80—2.54 (m, 10H), 2.23 (s, 6H); ^{13}C-NMR (75 Hz, $CDCl_3$) δ: 168.2,

147.9, 146.1, 134.9, 133.6, 128.3, 127.2, 123.7, 119.9, 116.6, 116.1, 73.5, 65.9, 61.6, 60.1, 53.6, 18.6; IR (KBr) cm^{-1}: 3310, 2925, 2822, 1666, 1592, 1503, 1266, 1161, 1011, 834, 771, 745; ESI-MS *m/z*: 414.3 $[M+H]^+$, 436.2 $[M+Na]^+$; FAB-MS *m/z*: 413.2316 (Calcd for $C_{17}H_{27}N_3O_3$: 413.2315). CVT-2514 was dissolved in absolute ethanol and to the solution was added hydrochloric acid. Then the solvent was removed under reduced pressure and recrystallization with acetone gave hydrochloric acid salt of CVT-2514 as a white solid, mp 247—249 °C.

1-[(2,6-Dimethyl phenyl)aminocarbonylmethyl]-4-[2,3-dihydroxyl-propyl]piperazine (CVT-2512) A solution of concentrated sulfuric acid (0.2 g) and epichlorohydrin (1.8 ml) in 12 ml of water was heated to 85 °C, then heating was stopped. Another 6.1 ml of epichlorohydrin was added slowly while keeping the solution refluxing. After addition of epichlorohydrin the mixture was stirred at 105 °C for 2.5 h. The reaction mixture was cooled, neutralized with 30% sodium hydroxide aqueous solution. It was purified by vacuum distillation to give chlorohydrin (6.62 g, 60%) as a colorless oil (bp 110—114 °C/10 mmHg).

A mixture of CVT-2738 (1.00 g, 4 mmol), chlorohydrin (0.44 g, 4 mmol) and sodium bicarbonate (0.50 g, 6 mmol) in 25 ml of ethanol was stirred at 65 °C overnight. The solvent was removed and to the residue was added acetone. The mixture was filtered and the filtrate was concentrated to give a yellow solid (1.2 g). The crude product was purified by column chromatography (SiO_2, dichloromethane : methanol = 60 : 1) to give CVT-2512 (0.86 g, 66.2%) as a white solid, mp 128—131 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.58 (s, 1H), 7.10 (s, 3H), 3.90—3.74 (m, 2H), 3.62—3.50 (m, 1H), 3.23 (s, 2H), 2.80—2.43 (m, 10H), 2.23 (s, 6H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 168.2, 134.9, 133.6, 128.3, 127.2, 66.9, 64.8, 61.6, 60.2, 53.9, 53.5, 18.6; IR (KBr) cm^{-1}: 3395, 3308, 2819, 1655, 1509, 1453, 1338, 1151, 1040, 942, 830, 767, 534; ESI-MS *m/z*: 322.3 $[M+H]^+$; FAB-MS *m/z*: 321.2050 (Calcd for $C_{17}H_{27}N_3O_3$: 321.2052). CVT-2512 was dissolved in absolute ethanol and to the solution was added hydrochloric acid. Then the solvent was removed under reduced pressure and recrystallization with methanol/acetone gave hydrochloric acid salt of CVT-2512 as a white solid, mp 247—249 °C.

1-[3-(2-Methoxyphenoxy)-2-hydroxypropyl]piperazine (CVT-2513) A solution of **A2** (0.5 g, 2.8 mmol) and piperazine (1.0 g, 11.1 mmol) in 15 ml of absolute ethanol was stirred at 65 °C for 1.5 h. Then the solvent was removed and the residue was dissolved in 40 ml of dichloromethane. The organic layer was washed with water (25 ml×2), dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography (SiO_2, dichloromethane : methanol = 50 : 1) to give CVT-2513 (0.44 g, 59.5%) as a yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.95—6.87 (m, 4H), 4.17—4.13 (m, 1H), 4.02—3.97 (m, 2H), 3.85 (s, 3H), 2.91—2.42 (m, 10H); ESI-MS *m/z*: 267.1 $[M+H]^+$. The analytical data of CVT-2513 was identical with the data reported in ref. 22. CVT-2513 was dissolved in absolute ethanol and to the solution was added hydrochloric acid. Then the solvent was removed under reduced pressure and recrystallization with methanol/acetone gave hydrochloric acid salt of CVT-2513 as a white solid, mp 172—177 °C.

3-(2-Methoxyphenoxy)-1,2-propylene Glycol (A3) A mixture of **A2** (5.6 g, 30.9 mmol) and concentrated sulfuric acid (20 ml) in 100 ml of water was stirred at 70 °C for 2 h. The reaction mixture was extracted with dichloromethane (150 ml×3). The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography (SiO_2, dichloromethane : ethyl acetate = 1 : 1) to give **A3** (3.64 g, 59.1%) as a yellow solid, mp 75—78 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.01—6.89 (m, 4H), 4.20—4.06 (m, 3H), 3.87 (s, 3H), 3.81—3.77 (m, 2H); ESI-MS *m/z*: 199.2 $[M+H]^+$, 221.1 $[M+Na]^+$. The analytical data of **A3** was identical with the data reported in ref. 23.

2-Hydroxyl-3-(2-methoxyphenoxy)propionic Acid (CVT-4786) A3 (0.1 g, 0.5 mmol), TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy free radical, 5.5 mg, 0.035 mmol), 3 ml of acetonitrile, and 1.87 ml of 0.67 M sodium phosphate buffer (the sodium phosphate buffer consisted of a 1 : 1 mixture of 0.67 M NaH_2PO_4 and 0.67 M Na_2HPO_4, pH = 6.7) were added to a flask. The reaction mixture was heated to 35 °C with stirring and approximately 0.05 ml of sodium hypochlorite solution was added *via* one addition funnel followed by 0.1 ml of sodium chlorite solution *via* another funnel (the solution of sodium chlorite ($NaClO_2$) was prepared by dissolving 80% $NaClO_2$ (9.14 g, 80.0 mmol) in 40 ml of water and the solution of diluted sodium hypochlorite (NaOCl) was prepared by diluting a sodium hypochlorite solution (5.25%, 1.06 ml, *ca.* 2.0 mol%) with 19 ml of water). Other 0.4 ml of sodium chlorite solution and 0.2 ml of sodium hypochlorite solution were then added simultaneously over 3 h. The resulting mixture was stirred at 35 °C for 2 d. After cooling the reaction mixture, 5 ml of water was added and the solution was neutralized with sodium bicarbonate to pH = 8. Then the solution was washed with 10 ml of ethyl acetate, neutralized with hydrochloric acid to acidity and extracted with ethyl acetate (15 ml×3). The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography (SiO_2, petroleum ether : ethyl acetate = 10 : 1) to give CVT-4786 (40 mg, 37.4%) as a yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.99—6.86 (m, 4H), 4.54 (t, 1H, *J* = 4.31 Hz), 4.34—4.28 (m, 2H), 3.83 (m, 3H); ESI-MS *m/z*: 213.0 $[M+H]^+$, 235.1 $[M+Na]^+$. The analytical data of CVT-4786 was identical with the data reported in ref. 24.

Animal Experiments. Drugs and Reagents Drugs were dissolved in 5% CMC-Na (carboxymethyl cellulose sodium) to form clear solutions which were adjusted to the expected concentrations before use. Isoprenaline injection: Shanghai Harvest Pharmaceutical Co., Ltd., batch number: 6E20008, specification: 2 mg/ml. Composite Salvia Dropping Pills (produced by Tianshili Pharmaceutical Co., Ltd., batch number: 20040906) were dissolved in distilled water before use (concentration: 3 dropping pills/10 ml). Sodium chloride injection (0.9%) was provided by Shandong Hualu Pharmaceutical Co., Ltd., batch number: A07012001. Urethane (20%) was provided by Shanghai Jinxi Chemical Co., Ltd. The hydrochloric acid salts of Ranolazine and its metabolites: CVT-2738, CVT-2514, CVT-2512 and CVT-2513, and CVT-4786 in free acid form were used in the animal experiments.

Apparatus Electrocardiograph: ECG-6511, Shanghai Photoelectric Medical Electronic Instrument Co., Ltd. JA1003 electronic balance: Shanghai Balance Instrument Factory.

Experimental Animals Animal studies were performed in adherence with the guidelines established in the *Guide for the Care and Use of Laboratory Animals* (NIH publication 85-23, revised 1985). All the experiments were carried out with Kunming mice which were half male and half female weighing 20—30 g.

Statistic Analysis All date were represented as mean±standard error of mean. Data were analyzed by *t*-test for comparisons between groups. Values of $p<0.05$ and $p<0.01$ were considered statistically significant.

Effect on ECG (Electrocardiogram) of Myocardial Ischemic Mice Induced by Isoprenaline The animal experimentation was carried out based on the methodology described by Xu *et al.*[25] A total of 64 mice were used. The mice were divided into 8 groups with 8 mice in each group (4 male mice and 4 female mice). Group one was positive control group treated with composite Salvia Dropping Pills; Group two was model control group treated with equal volume of distilled water; Group three—eight were goups treated with Ranolazine, CVT-2512, CVT-2513, CVT-2514 and CVT-2738 and CVT-4786 (dose: 100 mg/kg) respectively. The medication administration volume of each group was 0.4 ml/20 g. 30 min after first intragastric administration of the drugs, the mice were anesthetized by intraperitoneal injection with 1.2 g/kg 20% ethyl carbamate. Then the mice were fixed on the breast position to record normal II lead ECG and observe R–R interval (heart rate), height of T-waves. Fifteen minutes after subcutaneous bolus injection of isoprenaline 0.5 ml/20 g, ECG was recorded again. *T*-test was applied to compare the difference values between pre and post administration of the drugs between groups.

Effect on Myocardial Water Content and Cardiac Index of Myocardial Ischemic Mice Induced by Isoprenaline Two hours after the injection of isoprenaline the mice were sacrificed and the hearts were separated. The hearts were weighed, and the wet hearts were baked for 8 h at 110 °C and then the dry hearts were weighed. The myocardial water content ((wet heart weight − dry heart weight)/wet heart weight) and cardiac index (wet heart weight/body weight) were calculated and compared among groups.

Results and Discussion

Chemistry As shown in Chart 1, Ranolazine, CVT 2738, and CVT 2512 were synthesized following a literature procedure.[20] 2,6-Dimethylaniline reacted with chloroacetyl chloride in the presence of sodium bicarbonate to afford 2-chloro-*N*-(2,6-dimethylphenyl) acetamide (**A1**), which could be used in the next step without further purification. Treatment of compound **A1** with excess piperazine formed compound CVT-2738. *N*-Alkylation of CVT-2738 with chlorohydrin in the presence of sodium bicarbonate gave compound CVT-2512. Condensation of CVT-2738 with epoxide **A2**, which could be easily accessible by treating 2-methoxyphenol with epichlorohydrin in the presence of sodium hydroxide, afforded Ranolazine.

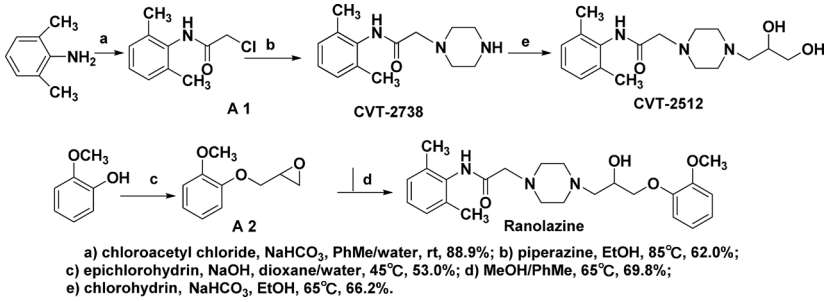


Chart 1. Synthesis of Compounds CVT-2512, CVT-2738, Ranolazine

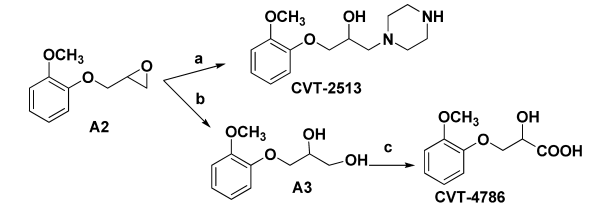


Chart 2. Synthesis of Compounds CVT-2513, CVT-4786

The syntheses of metabolites CVT-2513 and CVT-4784 are shown in Chart 2. Treatment of epoxide **A2** with piperazine in absolute ethanol afforded CVT-2513. In this step, excess piperazine should be added to avoid the double *N*-alkylation of the piperazine ring. Hydrolysis of **A2** in aqueous sulfuric acid solution provided glycol derivative **A3**. Selective oxidation of the terminal hydroxyl group of **A3** with sodium hypochlorite and TEMPO afforded CVT-4784.[26]

As shown in Chart 3, the preparation of metabolite CVT-2514 started from pyrocatechol. One hydroxyl group was selectively protected with benzyl to afford compound **A4**. Then **A6** was prepared according to the method for preparation of Ranolazine. Finally, deprotection of **A6** led to CVT-2514.

Anti-myocardial Ischemia Activities It is well-known that subcutaneous bolus injection of isoprenaline should introduce myocardial ischemia injury to mice so as to be mimicking the acute myocardial ischemia injury in human. We therefore employed isoprenaline-induced myocardial ischemia in mice as an animal model to evaluate the anti-myocardial ischemia activities of the test drugs.[27] Ischemic alterations such as T-waves elevation and R–R interval increasing are known to occur in ECG upon the injection of isoprenaline. Moreover, elevation of the myocardial water content and cardiac indexes are other alterations of myocardial ischemia. Therefore, T-waves, R–R interval, myocardial water content and cardiac indexes were recorded in this study to evaluate anti-myocardial ischemia activities of the test drugs.

Fifteen minutes after the subcutaneous bolus injection of isoprenaline, the height of T-waves, R–R interval, the myocardial water content and cardiac index significantly increased. Composite Salvia Dropping Pill was used as a positive control since it had been widely used in China as a plant medicine with anti-myocardial ischemic activity. In this study, the injection of Composite Salvia Dropping Pills could significantly inhibit the alternations induced by isoprenaline ($p<0.01$), indicating that the models were successfully established.

Table 1. Effect of the Test Drugs on the T-Waves

Groups	Dose (mg/kg)	T-wave		
		Pre-administration of isoprenaline (mV)	Post-administration of isoprenaline (mV)	Change rate
Model control		3.1000±0.2878	5.8625±0.8700	0.9026±0.3198
Positive control	6 pills/kg	2.9500±0.3546	4.0250±0.6756	0.3595±0.0993**
Ranolazine	100	3.2500±0.6279	3.4063±0.6951	0.0572±0.1506**
CVT-2512	100	3.2875±0.6446	4.8250±0.6296	0.5189±0.3416*
CVT-2513	100	2.9375±0.1996	4.000±0.5182	0.3644±0.1757**
CVT-2514	100	2.2130±0.4998	2.6375±1.6483	0.1880±0.7004*
CVT-2738	100	2.9000±0.2619	3.000±0.9442	0.0445±0.3437**
CVT-4786	100	2.2750±0.5365	2.725±1.9558	0.1812±0.7686*

* $p<0.05$, ** $p<0.01$, *vs.* model group.

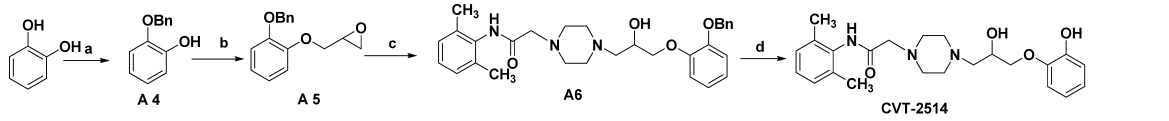
Table 2. Effect of the Test Drugs on the R–R Interval

Groups	Dose (mg/kg)	R–R interval		
		Pre-administration of isoprenaline (s)	Post-administration of isoprenaline (s)	Change rate
Model control		0.1343±0.0200	0.0985±0.0105	−0.2554±0.1143
Positive control	6 pills/kg	0.0868±0.0053	0.1048±0.0066	0.2119±0.1144**
Ranolazine	100	0.1060±0.0200	0.1023±0.0088	−0.0097±0.1749**
CVT-2512	100	0.1188±0.0141	0.1050±0.0101	−0.1052±0.1356*
CVT-2513	100	0.1063±0.0163	0.0963±0.0110	−0.0764±0.1718*
CVT-2514	100	0.1300±0.0156	0.0923±0.0111	−0.2839±0.0998***
CVT-2738	100	0.1023±0.0173	0.0915±0.0089	−0.0872±0.1549*
CVT-4786	100	0.1393±0.0304	0.0935±0.0083	−0.3021±0.1471***

* $p<0.05$, ** $p<0.01$, *** $p<0.05$, *vs.* model group.

The results of T-waves test (Table 1) showed that 15 min after the subcutaneous bolus injection of isoprenaline, Ranolazine, CVT-2513 and CVT-2738 could very significantly inhibit the elevation of T-waves ($p<0.01$), and on the other hand, CVT-2512, CVT-2514 and CVT-4786 could also remarkably inhibit the elevation of T-waves ($p<0.05$). When comparing CVT-2513 and CVT-2738 with Ranolazine, no remarkable difference was found between CVT-2738 and Ranolazine ($p>0.05$), but very remarkable difference was found between CVT-2513 and Ranolazine ($p<0.01$). According to the present data, the beneficial effect of CVT-2513 on T-waves is much less potent than that of Ranolazine.

The results of heart rate test (Table 2) showed that 15 min



a) $PhCH_2Cl$, K_2CO_3, KI, acetone, 50℃, 12.5%; b) epichlorohydrin, NaOH, dioxane/water, 45℃, 62.5%; c) CVT-2738, MeOH/PhMe, 65℃, 62.5%; d) Pd/C, AcOEt, rt, 60.9%.

Chart 3. Synthesis of Compound CVT-2514

Table 3. Effect of the Test Drugs on Myocardial Water Content and Cardiac Index

Groups	Dose (mg/kg)	Myocardial water content	Cardiac index
Model control		0.7867±0.0213	0.0045±0.0004
Positive control	6 pills/kg	0.5689±0.1095**	0.0020±0.0003**
Ranolazine	100	0.7791±0.0183***	0.0044±0.0005***
CVT-2512	100	0.7324±0.03823**	0.0038±0.0003**
CVT-2513	100	0.7667±0.0220***	0.0039±0.0003**
CVT-2514	100	0.7503±0.0142**	0.0040±0.0003**
CVT-2738	100	0.7707±0.0157***	0.0039±0.0004**
CVT-4786	100	0.7525±0.0377*	0.0037±0.0005**

* $p<0.05$, ** $p<0.01$, *** $p<0.05$, *vs.* model group.

after the subcutaneous bolus injection of isoprenaline, Ranolazine could inhibit the extension of R–R interval very remarkably ($p<0.01$), and CVT-2512, CVT-2513 and CVT-2738 could also inhibit the extension of R–R interval ($p<0.05$), but in a less extent. On the other hand, CVT-2514 and CVT-4786 had no remarkable effect ($p>0.05$). Therefore, CVT-2512, CVT-2513 and CVT-2738 had moderate effect on R–R interval, but less potent than that of Ranolazine.

Table 3 illustrates that CVT-2512 and CVT-2514 could inhibit the elevation of myocardial water content very remarkably ($p<0.01$), and CVT-4786 could inhibit the elevation of myocardial water content remarkably ($p<0.05$). On the other hand, Ranolazine, CVT-2738 and CVT-2513, which showed remarkable beneficial effect on T-waves and R–R interval, had no significant inhibition of the elevation of myocardial water content ($p>0.05$). While Ranolazine showed no remarkable effect on cardiac index ($p>0.05$), all of its metabolites had very remarkable effect on cardiac index ($p<0.01$).

Taken together, the test results of T-waves (Table 1) and heart rate (Table 2) indicate that CVT-2738 and CVT-2513 may have moderate anti-myocardial ischemia activity in myocardial ischemic mice induced by isoprenaline. Within all the metabolites tested in this study, CVT-2738 showed the best potency, however, it was still less potent than the mother drug Ranolazine.

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References and Notes

1) Chagas A. C. P., Dourado P. M. M., *Curr. Pharmaceut. Des.*, **14**, 2563—2571 (2008).
2) McCormack J. G., Barr R. L., Wolff A. A., Lopaschuk G. D., *Circulation*, **93**, 135—142 (1996).
3) Clarke B., Spedding M., Patmore L., McCormack J. G., *Br. J. Pharmacol.*, **109**, 748—750 (1993).
4) Gralinski M. R., Black S. C., Kilgore K. S., Chou A. Y., McCormack J. G., Lucchesi B. R., *Cardiovasc. Res.*, **28**, 1231—1237 (1994).
5) Gralinski M. R., Chi L., Friedrichs G. S., Tanhehco E. J., McCormack J. G., Lucchesi B. R., *J. Cardiovasc. Pharmacol. Ther.*, **1**, 141—148 (1996).
6) Clarke B., Wyatt K. M., McCormack J. G., *J. Mol. Cell. Cardiol.*, **28**, 341—350 (1996).
7) McCormack J. G., Stanley W. C., Wolff A. A., *Gen. Pharmacol.*, **30**, 639—645 (1998).
8) Collins-Nakai R. L., Barr R., *Can. J. Cardiol.*, **9**, Suppl. E, 33 (1933).
9) Gamble J., Collins-Nakai R. L., *Can. J. Physiol. Pharmacol.*, **72**, Suppl. I, 96 (1944).
10) Lopaschuk G. D., Wambolt R. B., Barr R. L., *J. Pharmacol. Exp. Ther.*, **264**, 135—144 (1993).
11) McCormack J. G., Stanley W. C., Wolff A. A., *Gen. Pharmacol.*, **30**, 639—645 (1998).
12) Pepine C. J., Wolff A. A., *Am. J. Cardiol.*, **84**, 46—50 (1999).
13) Zacharowski K., Blackburn B., Thiemermann C., *Eur. J. Pharmacol.*, **418**, 105—110 (2001).
14) Chaitman B. R., Skettino S. L., Parker J. O., Hanley P., Meluzin J., Kuch J., Wang W., Herbert D. A., Wolff A. A., *J. Am. Coll. Cardiol.*, **43**, 1375—1382 (2004).
15) Chu N., Lustig D., Wong S., *Drug Metab. Rev.*, **35**, 49 (2003).
16) Tafreshi M. J., Fisher B., *Ann. Pharmacother.*, **40**, 689—693 (2006).
17) Zerumsky K., Mcbride B. F., *Am. J. Health-Syst. Pharm.*, **63**, 2331—2338 (2006).
18) Herron W. J., Eadie J., Penmen A. D., *J. Chromatogr. A.*, **712**, 55—60 (1995).
19) Penman A. D., Eadie J., Herron W. J., Reilly M. A., Rush W. R., *Rapid Commun. Mass Spectrom.*, **9**, 1418—1430 (1995).
20) Lu W., Li Y., Zhao X., Guo C., Zhou K., *Chin. J. Med. Chem.*, **35**, 641—642 (2004).
21) Brimble M. A., Liu Y. C., Trzoss M., *Synthesis*, **9**, 1392—1402 (2007).
22) Gant T. G., Sarshar S., US2008312247, (2008).
23) Bredikhina Z. A., Novikova V. G., Zakharychev D. V., Bredikhin A. A., *Tetrahedron: Asymmetry*, **17**, 3015—3020 (2006).
24) Pickens C. L., Milliron A. R., *Urology*, **54**, 24—27 (1999).
25) Xu S., Bian R., Chen X., *Pharmacol. Test. Method*, **31**, 998—1022.
26) Li P., Evans C. D., Wu Y., Cao B., Hamel E., Joulfié M. M., *J. Am. Chem. Soc.*, **130**, 2351—2364 (2008).
27) Fan L., Yuan L., Chen Z., Ma C., *China Pharm.*, **16**, 176—177 (2005).