

Substituted beta-phenyl- alpha -hydroxy propanoic acid, synthesis method and use thereof

5 **Technical Field**

 The present invention relates to a substituted
 β -phenyl- α -hydroxyl-propionic acid derivative, a process for
 synthesizing the same, and use thereof for the manufacture of a
 medicament for treatment and prevention of cardiovascular and
10 cerebrovascular diseases.

Background Art

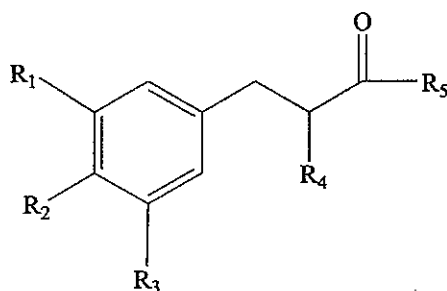
 Dan-shen Root (*Radix Salviae Militiorrhizae*) is a traditional
 Chinese medicine for treatment of cardiovascular and
 cerebrovascular diseases with definite therapeutic effects. At present,
15 it is generally presumed that salvianic acid (chemical name:
 β -(3,4-dihydroxyphenyl)- α -hydroxyl-propionic acid) is the main
 active ingredient in the water-soluble components of Dan-shen Root.
 It is demonstrated in Pharmacological tests that
 β -phenyl- α -hydroxylpropionic acid is the pharmacologically active
20 portion in propanoid acid, but its potency is not desirable. Thus, the
 substituted β -phenyl- α -hydroxylpropionic acid was modified, and
 the resulting modified derivatives might possess the same or greater
 potency than the parent compound, and might also exhibit an
 improved therapeutic effect in the treatment and prevention of
25 cardiovascular and cerebrovascular diseases. For example, borneol is
 capable of passing through cardiocerebral barrier, while propanoid
 acid is not prone to pass through cardiocerebral barrier. Therefore,
 propanoid acid may be modified in structure by incorporating the
 chemical structure of borneol.

30 **Disclosure of the Invention**

 One object of the present invention is to provide a substituted

β-phenyl-α-hydroxy-propionic acid derivative and a process for synthesizing the same, as well as use of the substituted β-phenyl-α-hydroxy-propionic acid derivative in the manufacture of a medicament for prevention and treatment of cardiovascular and cerebrovascular diseases.

In one aspect of the present invention a substituted β-phenyl-α-hydroxy-propionic acid derivative, specifically a compound of the formula (I), is provided:



(I)

Wherein, R₁, R₂, R₃ are each independently selected from the group consisting of H, OH, F, Cl, Br, methoxy and ethoxy; or alternatively, R₁ and R₂ together form -OCH₂O-, R₃ is selected from the group consisting of H, OH, methoxy, ethoxy and halogens;

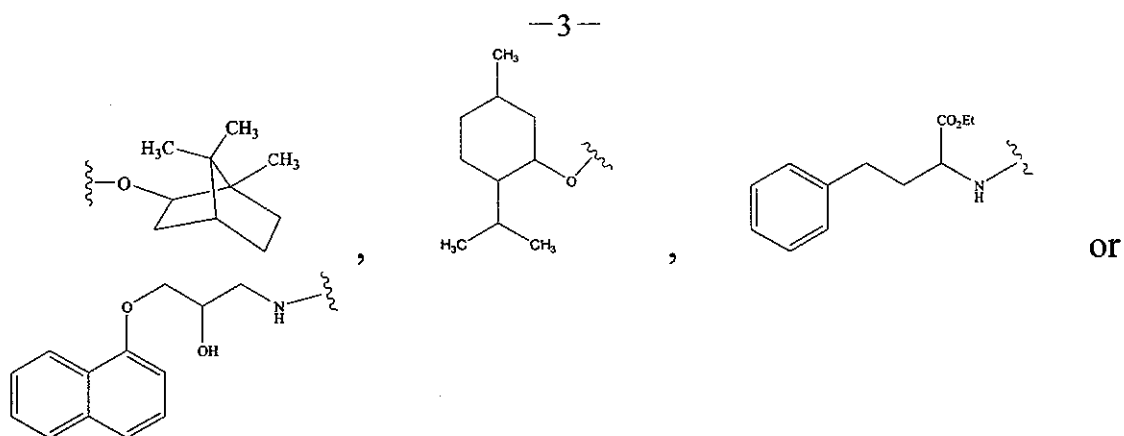
R₄ is OH or acyloxy;

R₅ is selected from the group consisting of cycloalkoxyl, amino and substituted amino, with the proviso that when R₅ is amino, then at least one of R₁, R₂ and R₃ is not H.

In one embodiment of the present invention, R₄ is OH.

In another embodiment of the present invention, R₄ is aroyloxy or heterocyclic radical-substituted acyloxy. Preferably, R₄ is *o*-acetoxybenzoyloxy, 3-pyridinylbenzoyloxy or 4-pyridinylbenzoyloxy.

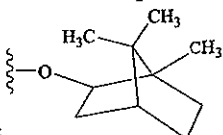
In a further embodiment of the present invention, R₅ is:

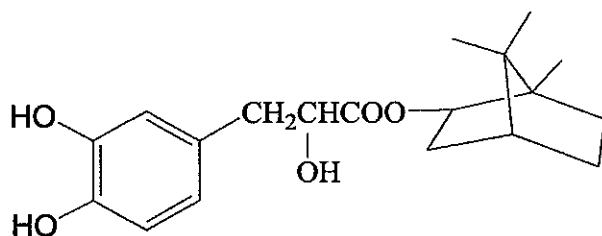


In a still further embodiment, R_1 and R_2 respectively are OH.

In a still further embodiment, R_1 and R_2 together form
 5 -OCH₂O-.

In a preferred embodiment, when R_1 and R_2 respectively are

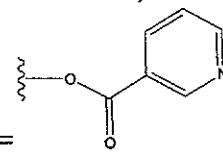
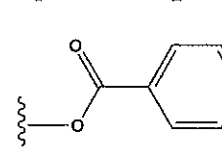
OH, then R_3 =H, R_4 =OH, and R_5 =, i.e., the compound is bornyl β -(3,4-dihydroxyphenyl)- α -hydroxypropionate ester as shown in formula (II).

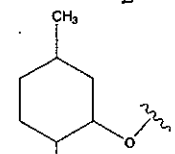
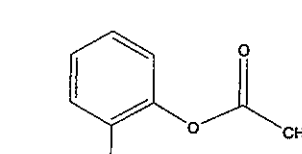


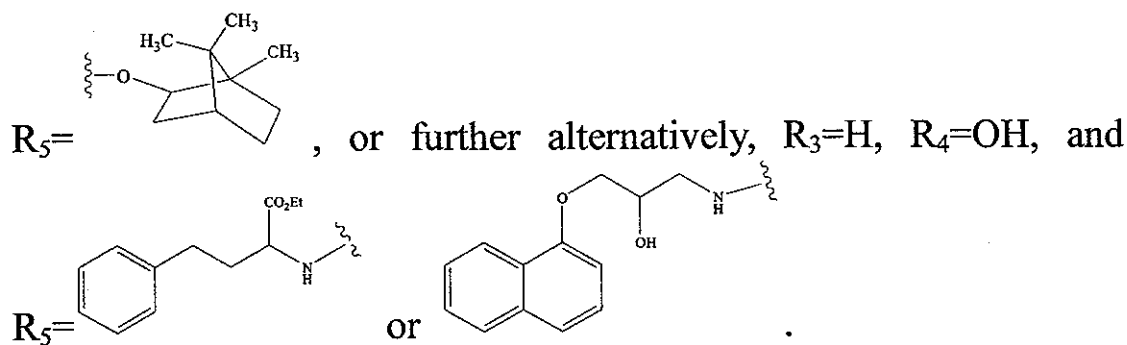
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(II)

In another preferred embodiment, when R_1 and R_2 together

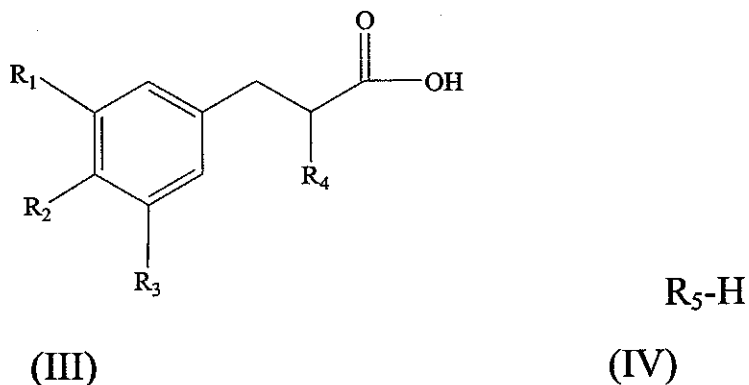
form -OCH₂O-, then R_3 =H, R_4 = or , and

 R_5 =H₃C-CH₃, or alternatively, R_3 =H, R_4 =, and



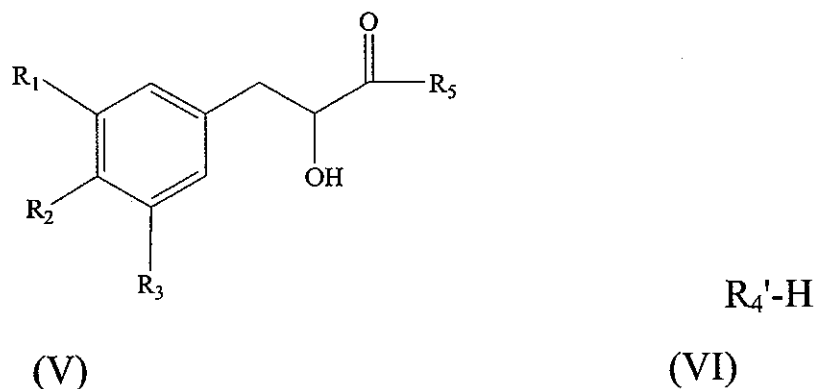
In another aspect of the present invention, a process for synthesizing a compound of the formula (I), is provided, which comprises: reacting a compound of the formula (III) with a

5 compound of formula (IV) or a hydrate thereof in the presence of a catalyst:



10 Wherein R_1 , R_2 , R_3 , R_4 and R_5 have the same meanings as defined above for the formula (I);

Or alternatively, said process comprises: reacting a compound of the formula (V) with a compound of the formula (VI) or a hydrate thereof in the present of a catalyst:



15 Wherein R_1 , R_2 , R_3 and R_5 have the same meanings as defined

above for the formula (I), and R_4' is acyloxy.

The said catalyst can be selected from concentrated H_2SO_4 , silicotungstic acid, phosphomolybdic acid, p-toluene sulfonic acid, $S_2O_8^{2-}/ZrO_2$, aluminum trichloride, zinc chloride and/or
5 magnesium chloride. Preferably, the said catalyst is p-toluene sulfonic acid, $S_2O_8^{2-}/ZrO_2$, aluminum trichloride and/or zinc chloride. It is particularly advantageous to use p-toluene sulfonic acid and/or $S_2O_8^{2-}/ZrO_2$.

The reaction molar ratio of the compound of the formula (III) to
10 the compound of the formula (IV) can be 1:0.8~1:1.5, preferably 1:1~1:1.5, more preferably 1:1.25~1:1.5, most preferably 1:1.5.

The reaction molar ratio of the compound of the formula (V) to the compound of the formula (VI) can be 1:0.8~1:1.5, preferably 1:1~1:1.5, more preferably 1:1.25~1:1.5, most preferably 1:1.5.

15 Optionally, the reaction is conducted in a solvent. The solvent may be selected from the group consisting of ethyl acetate, dichloromethane, tetrahydrofuran, acetone, toluene, 1,4-dioxane and *N,N*-dimethylformamide. Preferably, the solvent is selected from the group consisting of tetrahydrofuran, acetone, toluene, 1,4-dioxane,
20 and *N,N*-dimethylformamide. More preferably, the solvent is selected from tetrahydrofuran and acetone. Most preferably tetrahydrofuran is used as the solvent. These solvents can be used either alone or in any combination.

The reaction temperature can be varied depending on the
25 solvent being used. Advantageously, it is controlled within the range of 0°C~150°C. Preferably, the reaction temperature is 25°C~100°C. More preferably, the reaction temperature is 65°C.

The reaction duration can be 2h~24h, preferably 5h~15h, more preferably 8h~12h, most preferably 8h.

30 In one specific embodiment, there provided is a process for synthesizing a compound of the formula (II), which comprises:

reacting β -(3,4-dihydroxyphenyl)- α -hydroxypropionic acid with borneol in the presence of a catalyst. The catalyst can be a Lewis acid catalyst, such as toluene sulfonic acid, $\text{S}_2\text{O}_8^{2-}/\text{ZrO}_2$, aluminum trichloride and/or zinc chloride, preferably $\text{S}_2\text{O}_8^{2-}/\text{ZrO}_2$. In the said
5 process, the molar ratio of β -(3,4-dihydroxyphenyl)- α -hydroxypropionic acid to borneol can be 1:1~1:1.5, preferably 1:1.25~1:1.5, more preferably 1:1.5. The reaction is conducted in a solvent, which can be selected from the group consisting of tetrahydrofuran, toluene, 1,4-dioxane or *N,N*-dimethylformamide,
10 preferably tetrahydrofuran. The reaction temperature can be varied depending on the solvent being used, and is generally controlled within the range of 65°C~150°C, preferably at 65°C. The reaction duration can be 8h~12h, preferably 8h.

When $\text{S}_2\text{O}_8^{2-}/\text{ZrO}_2$ is used as the catalyst, the $\text{S}_2\text{O}_8^{2-}/\text{ZrO}_2$ can
15 be optionally prepared by the following process: adding aqueous ammonia to ZrOCl_2 solution at 0~10°C to reach a pH of 9-12, aging, washing the pellets to be free of Cl^- , heating in an oven to dryness, grinding, then adding into a solution of $(\text{NH}_4)_2\text{S}_2\text{O}_8$ for dipping, filtrating, drying, grinding, and then baking at 500~700°C for 2-5h,
20 resulting in $\text{S}_2\text{O}_8^{2-}/\text{ZrO}_2$.

In a further aspect of the present invention, there provided is use of the compound of the present invention in the manufacture of a medicament for the prevention and treatment of cardiovascular and cerebrovascular diseases, especially use of bornyl
25 β -(3,4-dihydroxyphenyl)- α -hydroxypropionate ester (the compound of the formula (II)) in the manufacture of a medicament for prevention and treatment of cardiovascular and cerebrovascular diseases.

Brief Description of the Drawings

30 Fig.1 shows a scheme for the synthesis of the compound of the formula (II) in Example 1, i.e., bornyl β -(3,4-dihydroxyphenyl)- α -hydroxypropionate ester.

Fig.2 shows the mass spectrum of the final product obtained in Example 1.

Fig.3 shows the infrared spectrum of the final product obtained in Example 1.

5 Fig.4 shows the ^1H NMR spectrum of the final product obtained in Example 1.

Fig.5 shows the ^{13}C NMR spectrum of the final product obtained in Example 1.

10 The present invention is further illustrated in conjunction with the following examples on synthesis and the pharmacodynamic tests. However, it should be understood that these examples are merely intended to illustrate, but by no way to limit, the present invention.

Example 1: Synthesis (I) of bornyl β -(3,4-dihydroxyphenyl)- α -hydroxypropionate ester

15 (1) Synthesis of acetyl glycine

Into a 250mL three-necked flask, 0.33mol glycine and 100mL distilled water were added and vigorously agitated until dissolution, and 0.67mol acetic anhydride was slowly added dropwise under stirring. The mixture was vigorously agitated continuously for 50
20 min, then sucking filtered. The pellets were washed and dried, resulting in a white crystal with a yield of 86.0%.

(2) Synthesis of 2-methyl-4-(3,4-diacetoxybenzylidene)-oxazolone

25 Into a 250mL three-necked flask, 0.20mol 3,4-dihydroxybenzaldehyde, 0.24mol acetyl glycine and 0.26mol anhydrous sodium acetate were added, then 189mL acetic anhydride was added, and mixed homogeneously by stirring. The reaction was performed for 4h in a 80°C water bath under stirring, then the temperature was elevated to 100°C and the reaction was continued
30 for 1h under stirring. The reaction mixture was cooled to room

temperature and then placed in a refrigerator for further cooling. 100mL water was added to the reaction mixture under stirring to precipitate a yellow crystal at the bottom. After sucking filtration, washing and drying, a yellow crystal was obtained in a yield of
5 75.0%.

(3) Synthesis of β -(3,4-diacetoxyphenyl)- α -acetamidoacrylic acid

Into a flask, 0.15mol 2-methyl-4-(3,4-diacetoxybenzylidene) oxazolone, 166mL acetone and 166mL distilled water were added,
10 then slowly heated to boiling, and refluxed by heating for 3h. The mixture was decolorized using active carbon. After filtration, the filtrate was placed for crystallization, then sucking-filtered, washed and dried resulting in an ecru crystalline powder in a yield of 72.9% was obtained.

15 (4) Synthesis of β -(3,4-dihydroxyphenyl)pyruvic acid

Into 0.25mol β -(3,4-diacetoxyphenyl)- α -acetamidoacrylic acid, 1500mL of $1\text{mol}\cdot\text{L}^{-1}$ hydrochloric acid was added. Then the mixture was heated for reflux under stirring for 8h. After decolorization using active carbon and sucking filtration, the filtrate was
20 concentrated to precipitate as a crystal. The mixture was sucking-filtered, washed and dried, resulting in a white loose crystal in a yield of 48.1%.

(5) Synthesis of β -(3,4-dihydroxyphenyl)- α -hydroxypropionic acid

25 Into 0.17mol β -(3,4-dihydroxyphenyl) pyruvic acid, 112g zinc amalgam and 1808mL of $1.4\text{mol}\cdot\text{L}^{-1}$ hydrochloric acid solution were added, and the reaction was performed under heating and refluxing for 8h. After filtration, the filtrate was extracted using ethyl acetate repeatedly, dried using anhydrous Na_2SO_4 . After
30 removal of ethyl acetate, β -(3,4-dihydroxyphenyl)- α -hydroxypropionic acid was obtained in a yield of 40.3%.

(6) Synthesis of bornyl β -(3,4-dihydroxyphenyl)- α -hydroxypropionate ester

Into a flask, 0.12mol β -(3,4-dihydroxyphenyl)- α -hydroxypropionic acid and 0.18mol borneol were added, then
5 0.86g p-toluene sulfonic acid or 2.00g of self-made $\text{S}_2\text{O}_8^{2-}/\text{ZrO}_2$ was added as catalyst, and 500mL tetrahydrofuran was added. The reaction was performed at 65°C for 8h. After the completion of the reaction, the catalyst, the solvent and the unreacted borneol were removed to obtain a brown viscous substance, which was further
10 separated using column chromatography to obtain a yellowish oil.

The catalyst $\text{S}_2\text{O}_8^{2-}/\text{ZrO}_2$ was prepared by a process comprising the following steps: preparing a solution of $1\text{mol}\cdot\text{L}^{-1}$ ZrOCl_2 with 0.025mol $\text{ZrOCl}_2\cdot 8\text{H}_2\text{O}$; stirring in an ice-water bath; adding slowly $6\text{mol}\cdot\text{L}^{-1}$ aqueous ammonia dropwise until pH reached 10; aging for
15 12h. suction filtrating; washing the filter cake with distilled water until no Cl^- could be determined (using $0.1\text{mol}\cdot\text{L}^{-1}$ AgNO_3 test); baking the filter cake at 110°C for 10h; comminuting; soaking in $0.5\text{mol}\cdot\text{L}^{-1}$ $(\text{NH}_4)_2\text{S}_2\text{O}_8$ solution for 12h; suction filtrating; drying; comminuting; and baking in a muffle furnace at 600°C for 3h to
20 obtain $\text{S}_2\text{O}_8^{2-}/\text{ZrO}_2$.

(7) The mass spectrum, infrared spectrum, ^1H NMR spectrum and ^{13}C NMR spectrum of the resulting yellowish oil

Fig.2 is the mass spectrum of the obtained yellowish oil, which shows 351.7 is the molecular ion peak of $(\text{M}+\text{H}_2\text{O})$, and the oil has
25 a molecular weight of 333.69;

Fig.3 shows the IR(KBr) ν/cm^{-1} : 3363.61(OH), 2953.12(CH_3), 2913.90(CH_2), 1725.51($\text{C}=\text{O}$), 1608.20, 1521.53, 1450.32(backbone of the benzene ring), 1281.36($\text{C}=\text{O}$ of the ester), 1114.39($\text{C}-\text{O}$ of the secondary hydroxyl), 885.71 and 805.68(1,2,4-trisubstituted
30 benzene ring);

Fig.4 shows the ^1H NMR(CD_3COCD_3 , 500MHz) δ : 6.57-7.64(m,

3H, Ar-H), 4.10-4.32(m, 1H, -CH(OH)-), 4.83(t, 1H, -CH-), 2.79-2.92(m, 2H, -CH₂-);

Fig.5 shows the ¹³CNMR(CDCl₃, 500MHz)δ: 174.790, 143.807, 143.056, 128.557, 121.549, 116.895, 115.488, 81.983, 71.646, 48.860, 47.881, 44.798, 39.871, 36.506, 27.918, 27.057, 19.653, 18.774, 13.501.

The above characterization data proved that bornyl β-(3,4-dihydroxylphenyl)-α-hydroxylpropionate ester was obtained by synthesis.

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Example 2: Synthesis (II) of bornyl β-(3,4-dihydroxylphenyl)-α-hydroxylpropionate ester

The synthesis was performed through the same procedure of Example 1, except that 0.12mol β-(3,4-dihydroxylphenyl)-α-hydroxylpropionic acid and 0.15mol borneol were added to the three-necked flask, then 0.86g p-toluene sulfonic acid as catalyst and 500mL tetrahydrofuran were added, and the reaction was performed at 65°C for 12h. After the completion of the reaction, the reaction solvent was removed by vacuum distillation, and the resulting viscous substance was treated by vacuum (1.3×10⁻³Pa) using an oil pump in a boiling-water bath to remove borneol, then 200mL ethyl acetate was added. The resulting solution was washed with saturated NaHCO₃ solution to remove unreacted β-(3,4-dihydroxylphenyl)-α-hydroxylpropionic acid and p-toluene sulfonic acid. The obtained ethyl acetate layer was concentrated under reduced pressure, resulting in a brown viscous substance, which was further separated by column chromatography, obtaining a yellowish oil. The resultant yellowish oil has the same mass spectrum and infrared spectrum as that in Example 1.

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Example 3: Synthesis (III) of bornyl β -(3,4-dihydroxyphenyl)- α -hydroxypropionate ester

The synthesis was performed through the same procedure of Example 1, except that 0.1mol β -(3,4-dihydroxyphenyl)- α -hydroxypropionic acid and 0.12mol borneol were added to the three-necked flask, then 1.33g $\text{S}_2\text{O}_8^{2-}/\text{ZrO}_2$ as catalyst and 400mL 1,4-dioxane were added, and the reaction was performed at 100°C for 8h. After the completion of the reaction, the catalyst $\text{S}_2\text{O}_8^{2-}/\text{ZrO}_2$ was removed by sucking filtration, and the solvent was removed by vacuum distillation, and the resulting viscous substance was treated by vacuum (1.3×10^{-3} Pa) using an oil pump in a boiling-water bath to remove borneol. The resultant black brown viscous substance was separated using column chromatography, obtaining a yellowish oil, which has the same mass spectrum and infrared spectrum as that in Example 1.

Example 4: Synthesis (IV) of bornyl β -(3,4-dihydroxyphenyl)- α -hydroxypropionate ester

The synthesis was performed through the same procedure of Example 1, except that 0.06mol β -(3,4-dihydroxyphenyl)- α -hydroxypropionic acid and 0.09mol borneol were added to the three-necked flask, then 0.60g aluminum trichloride as catalyst and 200mL *N,N*-dimethylformamide as solvent were added, and the reaction was performed at 150°C for 10h. After the completion of the reaction, the solvent was removed by vacuum distillation, and the resulting viscous substance was treated by vacuum (1.3×10^{-3} Pa) using an oil pump in a boiling-water bath to remove borneol. The resultant black brown substance was separated using column chromatography, obtaining a yellowish oil, which has the same mass spectrum and infrared spectrum as that of Example 1.

Example 5: Synthesis of bornyl β -(4-chlorophenyl)- α -hydroxypropionate ester

(1) 2-methyl-4-(4-chlorobenzylidene)oxazolone was synthesized in a way similar to Example 1(2), except that 5 4-chlorobenzaldehyde was used instead of 3,4-dihydroxybenzaldehyde. A brown crystal was obtained in a yield of 87.4%.

(2) Synthesis of β -(4-chlorophenyl)- α -acetamidoacrylic acid

0.10mol 2-methyl-4-(4-chlorobenzylidene)oxazolone, 110mL 10 acetone, 110mL water and 2mL concentrated hydrochloric acid were added to a flask, heated slowly to boil, and then kept on heating for reflux for 3h. After decolorization using active carbon and filtration, the filtrate was placed for crystalization, and an orange crystalline powder was obtained in a yield of 81.1% by sucking filtration, 15 washing and drying.

(3) Synthesis of β -(4-chlorophenyl) pyruvic acid

4.55g β -(4-chlorophenyl)- α -acetamidoacrylic acid, 91mL of 1mol·L⁻¹ hydrochloric acid solution and 45mL THF were added to a flask, and the mixture was heated for reflux for 10h. After 20 decolorization using active carbon and filtration, the filtrate was placed for crystalization, and an off-white crystalline powder was obtained in a yield of 77.3% by sucking filtration, washing and drying.

(4) Synthesis of β -(4-chlorophenyl)- α -hydroxypropionic acid

25 Into 15.00g β -(4-chlorophenyl) pyruvic acid, 98.00g Zn(Hg), 219mL of 2.5mol·L⁻¹ hydrochloric acid and 35mL THF solution were added, heated for refluxed for 10h. After the filtration was performed when the reaction mixture was hot, the filtrate was concentrated to reach 80mL and stood overnight. After sucking 30 filtration, washing, drying and recrystallizing in boiling-water, a white floccular crystal was obtained in a yield of 64.0%.

(5) Synthesis of bornyl β -(4-chlorophenyl)- α -hydroxypropionate ester

Into a three-necked flask, 0.12mol β -(4-chlorophenyl)- α -hydroxypropionic acid and 0.15mol borneol were added, then
5 0.86g p-toluene sulfonic acid as catalyst and 500mL tetrahydrofuran were added, and the reaction was performed at 65°C for 12h. After the completion of the reaction, the reaction solvent was removed by vacuum distillation, the obtained viscous substance was treated by vacuum (1.3×10^{-3} Pa) using an oil pump in a boiling-water bath to
10 remove borneol, then 200mL ethyl acetate was added to obtain a solution. The resulting solution was washed with saturated NaHCO₃ solution to remove unreacted β -(4-chlorophenyl)- α -hydroxypropionic acid and p-toluene sulfonic acid. The obtained organic phase was concentrated under vacuum,
15 resulting in a brown viscous substance, which was separated using column chromatography to obtain a yellowish oil.

IR(KBr) ν/cm^{-1} : 3461.45(OH), 2981.99(CH₃), 2935.46(CH₂), 1731.08(C=O), 1598.03, 1492.10, 1453.90(backbone of benzene ring), 1269.86(C=O of the ester), 1106.22(C-O of secondary
20 hydroxyl), 846.84(para-disubstituted);

¹HNMR(500MHz, CDCl₃) δ : 6.57-7.64(m, 3H, Ar-H), 4.10-4.32(m, 1H, -CH(OH)-), 4.83(t, 1H, -CH-), 2.79-2.92(m, 2H, -CH₂-), 1.205(t, 3H, -CH₃);

¹³CNMR(500MHz, CDCl₃) δ : 13.5, 19.5, 19.5, 23.3, 30.2, 32.5,
25 40.8, 45.4, 49.4, 50.6, 71.3, 82.4, 128.7, 128.7, 129.1, 129.1, 131.5, 137.5, 170.8.

Example 6: Synthesis of bornyl β -(3-methoxy-4-hydroxyphenyl)- α -hydroxypropionate ester

30 (1) 2-methyl-4-(3-methoxy-4-acetoxybenzylidene)oxazolone was synthesized in a way similar to Example 1(2), except that

3-methoxy-4-hydroxylbenzaldehyde was used instead of 3,4-dihydroxylbenzaldehyde. A yellow crystal was obtained in a yield of 73.5%.

(2) β -(3-methoxy-4-acetoxyphenyl)- α -acetamidoacrylic acid was synthesized in a way similar to Example 1(3), except that 2-methyl-4-(3-methoxy-4-acetoxybenzylidene)oxazolone was used instead of 2-methyl-4-(3,4-diacetoxybenzylidene)oxazolone. A ecru loose crystal powder was obtained in a yield of 71.6%.

(3) β -(3-methoxy-4-hydroxylphenyl)pyruvic acid was synthesized in a way similar to Example 1(4), except that β -(3-methoxy-4-acetoxyphenyl)- α -acetamidoacrylic acid was used instead of β -(3,4-diacetoxyphenyl)- α -acetamidoacrylic acid. A yellowish loose crystal powder was obtained in a yield of 64.2%.

(4) β -(3-methoxy-4-hydroxylphenyl)- α -hydroxylpropionic acid was synthesized in a way similar to Example 5(4), except that β -(3-methoxy-4-hydroxylphenyl)pyruvic acid was used instead of β -(3,4-dihydroxylphenyl)pyruvic acid. A yellowish oil or crystal was obtained in a yield of 77.8%.

(5) Bornyl β -(3-methoxy-4-hydroxylphenyl)- α -hydroxylpropionate ester was synthesized in a way similar to Example 5(5), except that β -(3-methoxy-4-hydroxylphenyl)- α -hydroxylpropionic acid was used instead of β -(3,4-dihydroxylphenyl)- α -hydroxylpropionic acid. A yellowish crystal was obtained in a yield of 59.8%.

IR(KBr) ν/cm^{-1} : 3363.61(OH), 2953.12(CH₃), 2913.90(CH₂), 1725.51(C=O), 1608.20, 1521.53, 1450.32(backbone of benzene ring), 1281.36(C=O of the ester), 1114.39(C-O of the secondary hydroxyl), 885.71, 805.68(1,2,4-trisubstituted benzene ring), 1237.58, 1027.61(aryl alkyl ether);

¹HNMR(400MHz, CD₃COCD₃) δ : 6.679-6.869(m, 3H, Ar-H), 4.920-4.983(m, 1H, -CH-), 4.257-4.286(t, 1H, -CH(OH)-), 3.819(s, 3H, -OCH₃), 2.804-2.978(m, 2H, -CH₂-);

¹³CNMR(500MHz, CD₃COCD₃) δ : 13.5, 19.5, 19.5, 23.3, 30.2,
5 32.5, 41.1, 45.4, 49.4, 50.6, 56.1, 71.3, 82.4, 113.1, 116.8, 121.4, 133.0, 142.9, 151.3, 170.8.

Example 7: Synthesis of menthyl β -(benzo[1,3]dioxol-5-yl)- α -(nicotinoyloxy)propionate ester

10 (1) 2-methyl-4-(benzo[1,3]dioxol-5-ylmethylene)-oxazolone was synthesized in a way similar to Example 1(2), except that benzo[1,3]dioxole-5-carbaldehyde was used instead of 3,4-dihydroxylbenzaldehyde. A yellow crystal was obtained in a yield of 76.5%.

15 (2) β -(benzo[1,3]dioxol-5-yl)- α -acetamidoacrylic acid was synthesized in a way similar to Example 1(3), except that 2-methyl-4-(benzo[1,3]dioxol-5-ylmethylene)-oxazolone was used instead of 2-methyl-4-(3,4-diacetoxybenzylidene)oxazolone. An ecru loose crystal powder was obtained in a yield of 78.7%.

20 (3) β -(benzo[1,3]dioxol-5-yl)pyruvic acid was synthesized in a way similar to Example 1(4), except that β -(benzo[1,3]dioxol-5-yl)- α -acetamidoacrylic acid was used instead of β -(benzo[1,3]dioxol-5-yl)- α -acetamidoacrylic acid. A yellowish loose crystal was obtained in a yield of 65.4%.

25 (4) β -(benzo[1,3]dioxol-5-yl)- α -hydroxylpropionic acid was synthesized in a way similar to Example 5(4), except that β -(benzo[1,3]dioxol-5-yl)pyruvic acid was used instead of β -(3,4-dihydroxylphenyl)pyruvic acid. A yellowish oil or crystal was obtained in a yield of 78.7%.

(5) Menthyl β -(benzo[1,3]dioxol-5-yl)- α -hydroxypropionate ester was synthesized in a way similar to Example 5(5), except that β -(benzo[1,3]dioxol-5-yl)- α -hydroxypropionic acid was used instead of β -(3,4-dihydroxyphenyl)- α -hydroxypropionic acid. A yellowish oil was obtained.

(6) Synthesis of menthyl β -(benzo[1,3]dioxol-5-yl)- α -(nicotinoyloxy) propionate ester

Into a three-necked flask, 0.12mol menthyl β -(benzo[1,3]dioxol-5-yl)- α -hydroxypropionate ester was dissolved in 15mL acetone, then an amount of the catalyst DCC/DMAP was added. A solution of 0.15mol nicotinic acid dissolved in 5mL acetone was added dropwise in an ice bath. The reaction was performed in the ice bath for 2h, then under room temperature for 1h. After the completion of the reaction, vacuum suction filtration was performed, the reaction solvent was removed by distillation, and 200mL ethyl acetate was added to the resultant viscous substance. The obtained solution was washed with a saturated NaHCO_3 solution to remove unreacted nicotinic acid and the catalyst. The organic phase was concentrated under vacuum to obtain a brown viscous substance, which was separated using column chromatography to obtain a yellowish oil, menthyl β -(benzo[1,3]dioxol-5-yl)- α -(nicotinoyloxy)propionate ester in a yield of 45.5%.

IR(KBr) ν/cm^{-1} : 3056.56(H-C=C), 2967.42(CH_3), 2940.54(CH_2), 1723.02(C=O), 1597.32, 1520.17, 1462.10(backbone of benzene ring), 1452.62, 1480.34, 1585(backbone of pyridine ring), 1268.53(C=O of ester), 1235.79, 1017.23(aryl alkyl ether), 1125.33(C-O of secondary hydroxyl), 884.43 and 798.62(1,2,4-trisubstituted);

$^1\text{HNMR}$ (400MHz, CD_3COCD_3) δ : 7.56-9.00(m, 4H, Pyridinio-H), 6.679-6.869(m, 3H, Ar-H), 6.06(s, 2H, $-\text{OCH}_2\text{O}-$), 5.10(m, 1H, $-\text{CH}(\text{O})-$), 4.920-4.983(m, 1H, $-\text{OCH}(\text{clcy})-$), 2.804-2.978(m, 2H, $-\text{CH}_2-$);

^{13}C NMR(500MHz, CD_3COCD_3) δ : 20.7, 21.0, 21.0, 22.3, 25.7, 28.5, 33.9, 37.8, 39.6, 47.1, 72.6, 75.6, 101.2, 112.7, 115.2, 121.0, 122.1, 126.0, 132.7, 136.4, 146.0, 148.7, 150.4, 151.4, 165.9, 170.8.

5 Example 8: Synthesis of menthyl β -(benzo[1,3]dioxol-5-yl)- α -(isonicotinoyloxy)propionate ester

The synthesis was performed in a way similar to Example 7, except that isonicotinic acid was used instead of nicotinic acid. A final product of yellowish oil was obtained, in a yield of 47.83%,
10 which was menthyl β -(benzo[1,3]dioxol-5-yl)- α -(isonicotinoyloxy)propionate ester.

IR(KBr) ν/cm^{-1} : 2966.27(CH_3), 2943.14(CH_2), 1720.82($\text{C}=\text{O}$), 1592.37, 1517.09, 1467.10(backbone of benzene ring), 1452.24, 1484.56, 1598.23(backbone of pyridine ring), 1267.67($\text{C}=\text{O}$ of ester),
15 1237.58, 1027.61(aryl alkyl ether), 1103.14($\text{C}-\text{O}$ of secondary hydroxyl), 880.43 and 795.81(1,2,4-trisubstituted);

^1H NMR(400MHz, CD_3COCD_3) δ : 7.56-9.00(m, 4H, Pyridinio-H), 6.679-6.869(m, 3H, Ar-H), 6.06(s, 2H, $-\text{OCH}_2\text{O}-$), 5.10(m, 1H, $-\text{CH}(\text{O})-$), 4.920-4.983(m, 1H, $-\text{OCH}(\text{clcy})-$),
20 2.804-2.978(m, 2H, $-\text{CH}_2-$);

^{13}C NMR(500MHz, CD_3COCD_3) δ : 20.7, 21.0, 21.0, 22.3, 25.7, 28.5, 33.9, 37.8, 39.6, 47.1, 72.6, 75.6, 101.2, 112.7, 115.2, 122.9, 122.9, 126.0, 132.7, 136.4, 146.0, 148.7, 150.3, 150.3, 165.9, 170.8.

25 Example 9: Synthesis of bornyl β -(benzo[1,3]-dioxol-5-yl)- α -(2-acetoxybenzoyloxy)propionate ester

The steps (1) to (4) of the synthesis were identical to the steps (1) to (4) in example 7.

(5) Bornyl β -(benzo[1,3]-dioxol-5-yl)- α -hydroxylpropionate
30 ester was synthesized in a way similar to Example 5(5), except that

borneol was used instead of menthol, and a yellowish oil was obtained.

(6) Bornyl β -(benzo[1,3]-dioxol-5-yl)- α -(2-acetoxy-benzoyloxy) propionate ester was synthesized in a way similar to Example 7(6), except that 2-acetoxybenzoic acid was used instead of nicotinic acid, and bornyl β -(benzo[1,3]-dioxol-5-yl)- α -hydroxylpropionate ester was used instead of menthyl β -(benzo[1,3]-dioxol-5-yl)- α -hydroxylpropionate ester. A light brown-yellow oil or crystal was obtained in a yield of 43.8%.

10 IR(KBr) ν/cm^{-1} : 2981.99(CH₃), 2935.46(CH₂), 1731.08 (C=O), 1598.03, 1492.10, 1453.90(backbone of benzene ring), 1269.86(C=O of ester), 1106.22(C-O of secondary hydroxyl), 880.43 and 795.81(1,2,4-trisubstituted), 746.84(ortho-disubstituted);

¹HNMR(400MHz, CD₃COCD₃) δ : 7.18-8.00(m, 4H, Ar-H), 6.679-6.869 (m, 3H, Ar-H), 6.06(s, 2H, -OCH₂O-), 5.10(m, 1H, -CH(O)-), 4.920-4.983(m, 1H, -OCH(cicy)-), 2.804-2.978(m, 2H, -CH₂-);

¹³CNMR(500MHz, CD₃COCD₃) δ : 13.5, 19.5, 19.5, 20.3, 23.3, 30.2, 32.5, 37.8, 45.4, 49.4, 50.6, 56.1, 72.6, 82.1, 112.7, 115.2, 120.9, 121.0, 121.5, 125.5, 130.3, 132.7, 133.5, 146.0, 148.7, 153.6, 165.9, 169.0, 170.8.

Example 10: Synthesis of β -(benzo[1,3]-dioxol-5-yl)- α -hydroxyl-*N*-(3-phenyl-1-ethoxycarbonyl-propyl)propionamide

25 The steps (1) to (4) of the synthesis were identical to the steps (1) to (4) in example 7.

(5) Synthesis of ethyl 2-amino-4-phenylbutyrate ester

Into 16.50g homophenylalanine, 350mL anhydrous ethanol was added, and dry HCl gas was fed under stirring. The feeding was stopped after 1.5hr, and the reaction apparatus was changed. The

reaction mixture was heated for reflux for 1.5h. After the completion of the reaction, most of ethanol was removed by distillation to precipitate a large amount of white crystal, and then 19.2g of white needle crystal was obtained after sucking filtration, washing and drying. The white crystal was dissolved in an aqueous solution, and the PH of the resultant solution was adjusted using a NaOH solution. The solution was extracted using ethyl ether. Then the solvent was removed, resulting in 14.92g colorless or yellowish liquid in a yield of 78.2%.

10 (6) Synthesis of β -(benzo[1,3]-dioxol-5-yl) - α -hydroxyl-*N*-(3-phenyl-1-ethoxycarbonyl-propyl)propionamide

Into a flask, 0.40g β -(benzo[1,3]-dioxol-5-yl)- α -hydroxyl-propionic acid and 12mL CH₃CN were added, and the flask was cooled exteriorly with a mixture of ice and water. 0.62g ethyl
15 2-amino-4-phenylbutyrate ester and 0.02g DMAP were added under agitation generated by a magnetic stirring bar. After the mixture was stirred to clarification, 0.45g DCC was added. The reaction temperature was elevated naturally to room temperature under stirring, and the reaction was performed at room temperature for 5h.
20 After the solvent was removed by vacuum distillation, ethyl acetate was added. The obtained solution in ethyl acetate was washed with NaHCO₃ solution, aqueous HCl solution, and water, then distilled under vacuum to obtain a crude product of the desired compound. After purification of the crude product by chromatography, 0.39g
25 white solid was obtained in a yield of 51.3%.

IR(KBr) ν /cm⁻¹: 3417.26(alcohol hydroxyl), 3255.79(NH), 2967.53(CH₃), 2934.21(CH₂), 1723.79(C=O), 1669.97(C=O of amide), 1593.37, 1515.19, 1463.13(backbone of benzene ring), 1239.98, 1026.76(aryl alkyl ether), 1111.35(C-O of secondary
30 hydroxyl), 884.45 and 792.17(1,2,4-trisubstituted); 698.69, 750.62(monosubstituted benzene ring);

¹HNMR(400MHz, CD₃COCD₃) δ : 6.18-7.50(m, 8H, Ar-H),

6.13(s, 2H, -OCH₂O-), 4.82(m, 1H, -CH(NH)-), 4.55(m, 1H, -CH(OH)-), 4.12(q, 2H, -OCH₂-), 2.804-2.978(m, 2H, -PhCH₂-), 2.30-2.54(m, 4H, -CH₂CH₂-), 1.31(t, 3H, -CH₃);

¹³CNMR(500MHz, CD₃COCD₃)δ: 14.1, 30.3, 32.3, 41.7, 52.7,
5 61.3, 73.3, 101.2, 112.7, 115.2, 121.0, 126.1, 128.1, 128.1, 128.9,
128.9, 132.7, 138.0, 146.0, 148.7, 171.5, 172.7.

Example 11: Synthesis of 2-hydroxyl-3-(benzo[1,3]-dioxol-5-yl)-*N*-[2-hydroxyl-3-(1-naphthoxy)-propyl]-propionamide

10 The steps (1) to (4) of the synthesis were identical to the steps (1) to (4) in example 7.

(5) Synthesis of 1-naphthyl epoxypropyl ether

Into a 500mL three-necked round bottom flask, 10.03g 1-naphthol, 3.1g NaOH, 20.4g epichlorohydrin (*S/R*) and 0.5g KI
15 were added, then 330mL ethanol was added. The flask was then placed in a microwave reactor. The reaction was performed at 30°C under stirring and 300W microwave irradiation for 12min. The reaction mixture was then removed and suction filtrated, and the filtrate was concentrated to obtain an oily substance. H₂O was added
20 to the oily substance, and the mixture was extracted with ethyl ether. The ethyl ether layers were combined and washed with a solution of NaOH, then washed with H₂O once. The ether layer was dried using anhydrous magnesium sulfate and concentrated to obtain 12.95g of the product in a yield of 93.2%.

25 (6) Synthesis of 1-amino-3-(1-naphthoxy)-2-propanol

450mL concentrated aqueous ammonia was placed in a reaction flask special for microwave, then 3.0g 1-naphthyl epoxypropyl ether was added, and the reaction was performed at 40°C under magnetic stirring and 300W microwave irradiation for 14min. After the
30 completion of the reaction, the reaction mixture was concentrated to

dryness, then ethyl acetate was added, and the pH was adjusted to acidic with concentrated hydrochloride. After sucking filtration, 1-amino-3-(1-naphthoxy)-2-propanol hydrochloride was obtained, and then dried to result in a white solid. The solid was dissolved in water by heating, and the pH was adjusted to alkaline. After cooling, a large amount of white solid was precipitated out. The precipitate was sucking filtrated and dried, resulting in 2.0g white solid in a yield of 63%.

(7) Preparation of 2-hydroxyl-3-(benzo[1,3]-dioxol-5-yl)-N-[2-hydroxyl-3-(1-naphthoxy)propyl]-propionamide

0.43g 1-amino-3-(1-naphthoxy)-2-propanol was dissolved in 15mL acetone then, 0.45g DCC and 0.10g DMAP were added, and 0.40g β -(benzo[1,3]-dioxol-5-yl)- α -hydroxylpropionic acid dissolved in 5mL acetone was added dropwise under magnetic stirring. The reaction was performed at room temperature for 1h, and a large amount of white solid was produced. After the completion of the reaction, the reaction mixture was suction filtrated, and the filtrate was concentrated to dry, then ethyl acetate was added to the resultant dry product and washed with NaHCO₃ solution. The ester layer was concentrated to dry, thereby obtaining a brown oily substance, which was purified via preparative liquid chromatography. 0.27g yellowish oil was obtained in a yield of 32.8%.

IR(KBr) ν/cm^{-1} : 3409.82(alcohol hydroxyl), 3251.72(NH), 2969.37(CH₃), 2944.74(CH₂), 1723.49(C=O), 1664.74(C=O of ester), 1591.77, 1519.90, 1469.21(backbone of benzene ring), 1235.78, 1029.63(aryl alkyl ether), 1101.15(C-O of secondary hydroxyl), 885.53 and 794.61(1,2,4-trisubstituted); 3050(backbone of naphthalene), 798.69, 780.62(monosubstituted naphthalene ring);

¹HNMR(400MHz, CD₃COCD₃) δ : 6.75-8.30(m, 10H, Ar-H), 6.13(s, 2H, -OCH₂O-), 3.55(m, 2H, -CH₂(NH)-), 4.55(m, 1H, -COCH(OH)-), 4.35(m, 1H, -CH(OH)-), 4.02(q, 2H, -OCH₂-), 2.90-3.07(m, 2H, -PhCH₂-);

^{13}C NMR(500MHz, CD_3COCD_3) δ : 41.7, 45.1, 68.5, 71.3, 73.3, 101.2, 104.3, 112.7, 115.2, 120.4, 121.0, 122.2, 125.4, 126.1, 126.6, 127.4, 127.6, 132.7, 134.5, 146.0, 148.7, 156.8, 172.7.

5 Example 12: Pharmacodynamic test

1. Effects of bornyl β -(3,4-dihydroxyphenyl)- α -hydroxypropionate ester (hereinafter briefly cited as “bornyl salvianate ester”) on blood flow of cerebral microcirculation in rats with middle cerebral artery occlusion

10 60 SD rats with a body weight of $220\pm 20\text{g}$ were randomly divided into normal control group, model control group, salvianic acid injection group (ip. 1mL/kg), and group of bornyl β -(3,4-dihydroxyphenyl)- α -hydroxypropionate ester at a small, middle and large dose respectively (ip. 5, 15, and 35mg/kg). The rats
15 of normal group and model group were administered with equal-volume of physiologic saline by ip. The rats were anesthetized by intraperitoneal injection of 1% pentobarbital sodium at an amount of 40mg/kg , then placed at supine position, head fixed, and cut skin along cervical middle line. Trachea cannula, was inserted into the rats
20 and they were allowed to spontaneous respire. Right common jugular vein and common carotid artery were isolated and a suture was introduced for later use. Animals were fixed on a rat stereotaxic apparatus, a cranial window with a size of $6\times 8\text{mm}$ was opened at the right temple with a dental drill, after hemostasis, cerebral dura mater
25 was cut open with scissors to expose cerebral pia mater. The window was covered and sealed with glass and dental cement, and the laser probe of a laser-doppler microcirculatory blood flow meter was fixed on the cranial window. Then the animals were fixed at lateral position, the right common carotid artery was lifted, ligated at the
30 proximal part and carefully cut with scissors, a nylon suture having a diameter of about 0.3mm was introduced into the artery. The distance between the artery cut and the paropia of the rats was

marked before the suture was introduced. When the nylon suture was introduced to approach the marked position, the speed for introducing the suture should slow down and the cerebral microcirculatory blood flow exhibited by the laser-doppler microcirculatory blood flow meter should be observed simultaneously. When the suture arrived at middle cerebral artery, an abrupt decrease of the microcirculatory blood flow would be observed. After the observation of a decrease in the microcirculatory blood flow, the suture was further penetrated by about 1mm, and the distal end of the cut and the suture in the artery were ligated securely. Spare suture was cut-off. After the end of the test, it was checked whether the nylon suture blocked the initiation site of the middle cerebral artery, and the data of any animal whose artery was not blocked were cancelled. The animals of the control group were not treated. After the cranial window was prepared, the probe of J I2200 type laser-doppler microcirculatory blood flow meter was fixed at the cranial window, and the probe was maintained without displacement and rotation during the whole test. The microcirculatory blood flows before ligation and 5, 15, 30, 45 and 60min after ligation were recorded, and the data of the animals in the drug-treatment groups were recorded at the same time points. The mean microcirculatory blood flow observed within 1min at each time point was designated as the microcirculatory blood flow of the respective time point.

Table 1. Effects on cerebral microcirculatory blood flow in rats with middle cerebral artery occlusion ($n=10$)

Group	Dose	Microcirculatory blood flow (mL)				
		5min	15min	30min	45min	60min
Control	/	65.3±5.7	67.4±7.4	67.8±6.8	65.1±5.9	64.2±8.5
Model	/	10.2±2.3	11.4±3.4	13.2±4.1	14.0±3.4	14.9±6.5
Salvianic	1mL/kg	10.8±3.6	20.1±6.9**	31.2±9.5**	33.0±6.8**	29.4±6.9**

acid						
Test Drug	5mg/kg	11.4±5.4	12.4±4.6	12.9±5.1	16.1±4.1	16.0±6.9
Test Drug	15mg/kg	9.4±3.7	12.8±5.6	20.7±3.2*	25.0±5.2**	24.4±4.8**
Test Drug	35mg/kg	10.8±2.7	19.4±5.1**	32.7±6.2**	35.4±8.2**	35.2±5.8**

In comparison with the model group: * $P < 0.05$, ** $P < 0.01$

The test results showed that, after the middle cerebral artery was blocked, the cerebral microcirculatory blood flow in blood-supply region (frontal and parietal lobe) decreased rapidly and kept at a relatively low level. Only after the artery was blocked for 30min did the cerebral microcirculatory blood flow increase slightly, which indicated that cerebral ischemia models were successfully established. Meanwhile the cerebral microcirculatory blood flow increased slightly 30 minutes or 15 minutes after the administration of bornyl β -(3,4-dihydroxylphenyl)- α -hydroxylpropionate ester at 15mg/kg and 35mg/kg respectively. These results suggest the dilatation of arteriole, and the increase of microcirculatory blood flow may have possible positive effects on ischemic cerebrovascular diseases, but the corresponding action mechanism needs to be investigated.

2. Protection effects of bornyl salvianate ester on cardiac ischemia reperfusion (I/R) injury

52 SD rats having a body weight of 220 ± 20 g were randomly divided into model control group, salviannic acid injection group (im 1mL/kg), and group of bornyl β -(3,4-dihydroxylphenyl)- α -hydroxylpropionate ester at a small, middle and large dose (ip 5, 15, 35mg/kg). The rats of the normal group and the model control group were ip administered with equal-volume of physiologic saline. The rats of all groups were administered for consecutive 5 days. When the rats were administered for the last time, they were simultaneously anesthetized with 1.5% pentobarbital sodium (ip 45mg/kg), then a catheter was

inserted into the right carotid artery, and connected via a transducer to an eight-edchanneled physiological recorder. Tracheal cannula was performed, and the ventilation rate was maintained at 60 times/min. The chest was opened, a 6/0 suture was used to form a
5 loop at a site 1~2mm from the root of the anterior descending coronary artery, and a plastic pipe was introduced through the loop, then the loop was tightened up. The change of electrocardiogram was observed. An increase or decrease of ST indicated the success of ligation. The color of the myocardial tissue below the ligation suture
10 became darker. 30 minutes later, the plastic pipe was pulled out to allow coronary artery blood flowing again and cause hyperemia of local tissue during reperfusion. For the groups subjected to ischemia for 30min and reperfusion for 30min, the myocardial infarction areas were recorded before the test, after ischemia for 1min and 30min,
15 and after reperfusion for 30min; for the groups subjected to ischemia for 30min and reperfusion for 2h, cardiac tissue samples were taken and fixed using 10% formalin, embedded with paraffin, serially sliced into sections with a thickness of 4 μ m, and separately subjected to immunohistochemistry tests; the rats of the sham
20 operation group were subjected only to the introduction of suture but their coronary arteries were not ligated.

Effects on myocardial infarct areas caused by cardiac muscle I/R

After the rats were subjected to ischemia and reperfusion for
25 30minutes, their anterior descending coronaries were ligated again, then they were sacrificed, their hearts were removed promptly, and 0.5mL of 1% evans blue was injected into heart chambers via aorta to distinguish the ischemic regions and the non-ischemic regions. After the heart atrium and right ventricle were cut-off, the heart was
30 refrigerated at -20°C for 30min, then placed in a tailor-made slice groove, and cut along the long axis to form 2mm slices. The slices were immersed in 1% TTC phosphoric acid buffer solution (pH7.4),

and incubated at 37°C for 30min to distinguish risky regions and necrotic regions. Then the slices were fixed with 10% formaldehyde for 24h to enhance staining color for contrast photography. After the above treatment, the myocardial tissue was divided into: normal myocardia in blue, ischemic myocardia in light red, and necrotic myocardion in gray. A computer image analysis software was used to calculate the percentage of the area of the infarct myocardial region (nec) based on the area of the risky myocardial region (aar, i.e., ischemic myocardia, including ischemic infarct region and ischemic non-infarct region)(nec/aar), and the percentage of the area of the infarct myocardial region based on the whole area of the myocardia(nec/lv) to indicate the degree of infarct, as well as the percentage of the area of the risky myocardia based on the area of the left ventricle(aar/lv).

15

Table 2. Effects on myocardial infarction areas caused by cardiac muscle I/R

Group	Dose	Animal Number	aar/lv(%)	nec/lv(%)	nec/aar(%)
Model control	/	12	67.10±11.40	50.52±15.65	64.10±13.03
Salvianic acid	1mL/kg	10	50.21±7.10*	41.41±5.49*	49.87±7.83*
Test drug	5 mg/kg	9	64.09±18.10	48.45±17.11	61.72±14.65
Test drug	15 mg/kg	10	63.02±15.98	48.23±15.34	62.63±13.82
Test drug	35 mg/kg	11	51.97±13.04*	40.21±12.65*	50.01±9.03*

20

In comparison with the model control group: * $P < 0.05$

The results showed that, in comparison with the model control group, the values of aar/lv, nec/lv and nec/aar in the large dose group respectively decreased by 22.5%, 20.4% and 22% ($P < 0.01$), suggesting that the area of the myocardial infarct caused by myocardia I/R can be lowered.

Effects on the protein expression of Bax, Bcl-2, caspase-3, MMP-2 and PPAR γ

Standard immunohistochemistry ABC and SP methods were employed for staining. Bax: anti-rabbit polyclonal antibody (Santa CruzBio. Inc.) at a dilution of 1:200; Bcl22: anti-rabbit polyclonal antibody (TBD Tianjin Biotechnological Center). At a dilution of 1:100; caspase-3: anti-rabbit polyclonal antibody (Normarkers Fromont, CA), at a dilution of 1:200; MMP-2: anti-murine monoclonal antibody (NormarkersFromont, CA) at a dilution of 1:200; PPAR γ : anti-caprine polyclonal antibody (Santa Cruz Bio. Inc.), at a dilution of 1:500. The specific procedures were performed in accordance with the instructions of the ABC and SP kits, DAB was used for color development, and neutral resin was used for mounting. PBS was used instead of the first antibody as a negative control. Cells with positive expression of the test polypeptides were of brown-yellow color, with MMP-2 protein being present in acytoplasm, Bcl-2 expressed in nuclear membrane and cytoplasm, Bax mainly in cytoplasm and partially in nucleus, and caspase-3 mainly in nucleus and partially in cytoplasm. CMIAS image analysis system was used for random selection of fields of slices and automatic selection of sites for analysis, and statistic analysis was performed by using mean optical density values or integral optical density values of the obtained myocardial tissue slices.

Table 3. Effects on the protein expression of Bax, Bcl-2, caspase-3, MMP-2, and PPAR γ ($n=10$)

Group	Dose	OD value
-------	------	----------

		Bax	Bcl-2	Caspase-3	MMP-2	PPAR γ
Model control	/	0.13 $\pm$ 0.04	0.14 $\pm$ 0.03	0.37 $\pm$ 0.11	0.16 $\pm$ 0.04	0.17 $\pm$ 0.02
Salvianic acid	1mL/kg	0.08 $\pm$ 0.02*	0.18 $\pm$ 0.04	0.21 $\pm$ 0.12**	0.12 $\pm$ 0.05	0.34 $\pm$ 0.08**
Test drug	5 mg/kg	0.12 $\pm$ 0.03	0.15 $\pm$ 0.03	0.35 $\pm$ 0.14	0.14 $\pm$ 0.05	0.16 $\pm$ 0.05
Test drug	15 mg/kg	0.09 $\pm$ 0.05	0.15 $\pm$ 0.06	0.32 $\pm$ 0.08	0.14 $\pm$ 0.03	0.18 $\pm$ 0.06
Test drug	35mg/kg	0.07 $\pm$ 0.03*	0.21 $\pm$ 0.04*	0.20 $\pm$ 0.07**	0.11 $\pm$ 0.03*	0.18 $\pm$ 0.10

In comparison with the model control group: * $P < 0.05$, ** $P < 0.01$

The results showed that the expression of Bcl-2 and Bax was indeed changed in the myocardial cells I/R, indicating that they participate in the regulation of cell apoptosis. Bornyl β -(3,4-dihydroxyphenyl)- α -hydroxylpropionate ester could reduce the expression of Bax and caspase-3 proteins and increase the expression of Bcl-2 protein, suggesting that bornyl β -(3,4-dihydroxyphenyl)- α -hydroxylpropionate ester could initiate the self-protective mechanism of cells against injury by inducing the expression of Bcl-2 and reducing the levels of Bax and caspase-3, reverse the cell apoptosis and necrosis processed stimulated by the myocardial I/R, thereby exhibit protection effects on myocardial cells.

MMP-2 is associated with cardiac muscle I/R injury, which is achieved by the cleavage of troponin I, which in turn would directly lead to cell apoptosis. MMP-2 specific inhibitor could improve the cardiac function of rats with myocardial I/R, and the test results indicated that bornyl β -(3,4-dihydroxyphenyl)- α -hydroxylpropionate ester could cause the decrease of MMP-2 protein, which could be another mechanism for bornyl β -(3,4-dihydroxyphenyl)- α -hydroxylpropionate ester to protect myocardia from I/R injury.

3. Effects of bornyl salvianate ester on blood pressure and left ventricular function of anesthetized rats

Rats were anesthetized by intraperitoneal injection of 20% urethane 5mL/kg and fixed; the skin of neck of rats were incised, anterior cervical muscles were isolated, trachea was exposed, and tracheal cannula was inserted; common carotid artery was isolated, a cardiac catheter was introduced through the common carotid artery into left ventricle, the left intraventricular pressure was measured with a pressure transducer (T-200) of RM-6000 multi-channel polygraph and the carrier amplifier (AP-601G) of RM-6000 multi-channel polygraph, then the signals of the left intraventricular pressure were input to the differential amplifier (ED-601G) of RM-6000 multi-channel polygraph to record the maximum rate of increasing and decreasing the left intraventricular pressure ($dp/dt_{\max}$ - $dp/dt_{\max}$); right femoral artery was isolated, the arterial blood pressure was measured by using cannula; a recording electrode of electrocardiograph was connected to record type II electrocardiogram. All data were input into PowerLab/8Sp data acquisition and processing system via RM-6000 multi-channel polygraph, and recorded, analyzed and processed by PowerLab/8Sp.

The abdomen was opened 1.5cm below xiphoid bone to isolate duodenum, a small incision was formed on the duodenum away from blood vessels by ophthalmic scissors, a catheter was inserted and the incision was fixed by suture for administration. After the end of operation and a further waiting period of 30min, normal data were recorded once the indexes to be monitored were stable.

Test drugs were duodenally administered via the catheter, and the indexes were monitored 5, 15, 30, 60, 90 and 120min after the administration. The change ratios of the indexes were calculated in accordance with the following formula, and were used for statistic analysis among groups.

$$\text{Change_ratio(\%)} = \frac{(\text{After_administration} - \text{Before_administration})}{\text{Before_administration}} \times 100$$

3.1 Effects on the heart rate of anesthetized rats

The test results indicated that the 4.5mg/kg, 9mg/kg and 18mg/kg doses of bornyl salvianate ester showed no significant effects on the heart rate of anesthetized rats, no significant difference can be observed as compared to the blank control group; while verapamil hydrochloride significantly reduced the heart rate and significant difference can be observed as compared to the model group ($P < 0.05$ or $P < 0.01$) (Table 4).

3.2 Effects on mean arterial pressure, systolic pressure and diastolic pressure of anesthetized rats

In the group at the does of 18mg/kg bornyl salvianate ester, the mean arterial pressure, systolic pressure and diastolic pressure of anesthetized rats significantly decreased after administration, and significant differences ($P < 0.05$ or $P < 0.01$) as compared to the blank control group were observed at the timepoints of 15, 60, 90 and 120 min; in the 9mg/kg group of bornyl salvianate ester, the mean arterial pressure, systolicsystolic pressure and diastolic pressure of anesthetized rats exhibited a decrease tendency, and significant differences ($P < 0.05$ or $P < 0.01$) from the blank control group were observed at 15 and 60 min; in the 4.5mg/kg group of bornyl salvianate ester, the mean arterial pressure, systolicsystolic pressure and diastolic pressure of anesthetized rats exhibited no significant change, and no significant difference from the blank control group were observed; while verapamil hydrochloride could significantly reduce the mean arterial pressure, systolicsystolic pressure and diastolic pressure of anesthetized rats, and significant differences ($P < 0.01$) from the model control group were observed at 5, 15, 30, 60, 90 and 120 min (Tables 5, 6 and 7).

3.3 Effects on left intraventricular pressure of anesthetized rats

In the 18mg/kg group of bornyl salvianate ester, the left

intraventricular pressure of anesthetized rats significantly decreased after administration, and significant differences ($P<0.05$ or $P<0.01$) from the blank control group were observed at 15, 30, 60, 90 and 120 min; in the 9mg/kg group of bornyl salvianate ester, the left intraventricular pressure of anesthetized rats exhibited a decrease tendency, and significant differences ($P<0.05$ or $P<0.01$) from the blank control group were observed at 60 min; in the 4.5mg/kg group of bornyl salvianate ester, the left intraventricular pressure of anesthetized rats was not significantly affected after administration, and no significant difference from the blank control group was observed; while verapamil hydrochloride significantly reduced the left intraventricular pressure of anesthetized rats, and significant differences ($P<0.01$) from the model control group were observed (Table 8).

3.4 Effects on dp/dt and $-dp/dt$ of anesthetized rats

In the 18mg/kg group of bornyl salvianate ester, the the dp/dt of anesthetized rats significantly decreased after administration, and significant differences ($P<0.05$ or $P<0.01$) from the blank control group were observed at 15, 30, 60, 90 and 120 min; in the 9mg/kg group of bornyl salvianate ester, the dp/dt of anesthetized rats exhibited a decrease tendency, and significant differences ($P<0.05$ or $P<0.01$) from the blank control group were observed at 60 and 120min; in the 4.5mg/kg group of bornyl salvianate ester, the dp/dt of anesthetized rats was not significantly affected after administration, and no significant difference from the blank control group was observed; while verapamil hydrochloride significantly reduced the dp/dt of anesthetized rats, and significant differences ($P<0.01$) from the model control group were observed.

In the 9mg/kg and 18mg/kg groups of bornyl salvianate ester, the $-dp/dt$ of anesthetized rats exhibited a decrease tendency, and significant differences ($P<0.05$ or $P<0.01$) from the blank control group were observed at 60 and 120min; in the 4.5mg/kg group of

bornyl salvianate ester, the $-dp/dt$ of anesthetized rats was not significantly affected after administration, and no significant difference from the blank control group was observed; while verapamil hydrochloride significantly reduced the $-dp/dt$ of
5 anesthetized rats, and significant differences ($P<0.01$) from the model control group were observed (Tables 9, 10).

The test results indicate that bornyl salvianate ester could reduce left intraventricular pressure, dp/dt and $-dp/dt$, which suggests that bornyl salvianate ester has effects of reducing the
10 negative cardiac efficiency of myocardial contractility, and the effects on negative cardiac efficiency could be the reason of the decrease of the mean arterial pressure, systolic pressure and diastolic pressure of anesthetized rats.

In the meantime, the test results showed that the dp/dt_{MAX}
15 decreased, but the heart rate did not change significantly, i.e., the dp/dt_{MAX} was not in direct correlation with the heart rate. The mechanism for these phenomena needs further studying.

4. Protective effects of bornyl salvianate ester on acute myocardial ischemia in rats

20 60 Male rats were randomly divided into sham operation group (0.5% Poloxamer, 10mL/kg), model control group (0.5% Poloxamer, 10mL/kg), verapamil group (verapamil tablet, 10mg/kg), and bornyl salvianate ester groups (10mg/kg, 20mg/kg and 40mg/kg). At 0.5h after the rats of these groups were administered intragastrically, the
25 standard II lead electrocardiogram (before modeling) was recorded, and the height of ST-T segment was measured. The rats of sham operation group were then subjected to coronary artery braid without ligation, and the rats of other groups were subjected to coronary artery ligation in accordance with the following method to establish
30 cute myocardial ischemia models. The rats were subjected to etherization, fixed at supine position, and their normal electrocardiograms (before modeling) were recorded. The skin of

left thorax was incised under aseptic conditions, the 4th intercostal muscles were isolated in blunt way, the heart was extruded by light pressure to the right thorax, the left anterior descending coronary artery was ligated at a position 2~3mm departing from the left coronary artery origin and between the pulmonary conus and the left auricle of heart, then the heart was sent back to thorax cavity immediately and sutured the incision. Penicillin was smeared topically for the prevention of infections. After operation, the electrocardiogram of postischemia was recorded immediately (at 0 min after modeling), and the height of ST-T segment was measured. At 24h after the animals of these groups underwent the operation, they were anesthetized by peritoneal injection of 20% urethane 5mL/kg, and the electrocardiogram was recorded again (at 24h after modeling), and the height of ST-T segment was measured; blood samples were drawn from abdominal aorta, sera were separated, and the activities of lactate dehydrogenase (LDH), creatine kinase (CK), creatine kinase isoenzyme (CK-MB) and superoxide dismutase (SOD) and the content of malondialdehyde (MDA) were measured; the heart was taken out by open chest and washed with cold physiological saline, the heart atrium was removed, the heart ventricle was cross cut to form 3~4 slices, the slices were dipped in 0.25% NBT solution and stained under 37°C water-bath for 10min, the infarct cardiac muscles were cut and weighed, and the weight percentage of the infarct cardiac muscles based on the cardiac muscles of the whole heart ventricle was calculated (Tables 11-14).

The results showed that in all the bornyl-salvianate-ester dosed groups, the proportion of the infarct cardiac muscles in the whole ventricle was reduced, in which relative potent effects were observed in the middle and large dose groups ($P<0.05$ or $P<0.01$); in all dose groups, the activities of lactate dehydrogenase (LDH), creatine kinase (CK), creatine kinase isoenzyme (CK-MB) decreased 24h later; the superoxide dismutase (SOD) in rats with acute myocardial

- ischemia increased, especially in the large dose group ($P<0.05$); in middle and large dose groups, a decrease tendency of the serum MDA content was observed, but no significant difference from the model control group was observed; at 24h after coronary artery
- 5 ligation, the ST-T segment elevation in the electrocardiogram was observed, especially in the large dose group ($P<0.05$). The results indicated that the bornyl salvianate ester could reduce the area of myocardial infarction in rats, and exhibit protective effects on acute myocardial ischemia in rats.

Table 4. Effects on heart rate (beat/min)

Group	Dose	Before administration	After administration				
			5'	15'	30'	60'	120'
Blank control (n=10)	2.5mL/kg	344.39±42.73	344.23±46.02	355.13±44.09	349.22±47.61	354.23±47.61	359.78±54.27
Tween control (n=10)	2.5mL/kg	322.67±58.28	326.83±50.06	328.47±46.72	337.91±49.80	332.98±55.18	327.03±57.06
	18mg/kg	357.28±36.29	358.56±44.88	356.00±40.82	348.07±39.21	357.22±47.10	357.68±53.26
Propanoid acid borneol ester (n=10)	9mg/kg	373.43±41.28	374.04±47.60	374.93±47.51	361.94±43.56	359.61±39.30	354.09±47.98
Propanoid acid borneol ester (n=10)	4.5mg/kg	329.43±43.77	337.41±47.67	347.56±47.18	348.91±52.11	365.43±32.77	360.36±51.93
Verapamil control (n=10)	11.4mg/kg	363.19±46.67	345.03±33.81	324.96±36.20	322.02±51.09	317.91±58.04	328.05±58.98
			-4.63±4.08**	-9.67±11.64**	-10.32±16.56*	-11.31±19.30*	-7.96±21.39

In comparison with the Tween control group: *p < 0.05, **p < 0.01

Table 5. Effects on contractive pressure (mmHg)

Group	Dose	Before administration	After administration				
			5'	15'	30'	60'	120'
Blank control (n=10)	2.5mL/kg	117.57±21.61	121.76±23.27	123.75±26.54	118.91±32.99	118.80±31.50	124.51±35.18
Tween control (n=10)	2.5mL/kg	124.41±26.13	122.64±26.97	124.75±24.75	121.92±23.44	120.80±29.92	119.48±25.05
	18mg/kg	128.25±23.86	128.34±28.96	120.90±26.54	117.76±22.90	106.97±32.22	113.34±24.47
Propanoid acid borneol ester (n=10)	9mg/kg	132.26±20.00	131.10±24.38	127.85±23.92	122.58±23.46	115.46±23.90	120.85±32.13
Propanoid acid borneol ester (n=10)	4.5mg/kg	129.06±22.13	126.54±25.94	128.80±24.39	129.90±23.86	135.97±22.80	128.62±28.30
Verapamil control (n=10)	11.4mg/kg	137.10±28.79	115.30±19.35	98.48±16.13	94.47±17.17	98.49±22.31	102.58±27.01
			-14.92±9.40**	-26.88±11.21**	-29.86±12.10**	-26.55±17.97**	-23.82±18.16**

In comparison with the Tween control group: *p < 0.05, **p < 0.01

Table 6. Effects on diastolic pressure (mmHg)

Group	Dose	Before administration	After administration				
			5'	15'	30'	60'	120'
Blank control (n=10)	2.5mL/kg	63.92±12.98	66.51±13.71	68.12±15.60	66.35±18.40	66.54±17.95	66.18±8.51
Tween control (n=10)	2.5mL/kg	67.56 ±14.29	4.05±6.32	6.28±10.50	2.60±16.02	3.59±15.55	3.24±17.92
Propanoid acid borneol ester (n=10)	18mg/kg	73.04±11.87	66.26±14.25	67.66±13.23	66.42±13.11	65.95±16.45	66.05±15.27
Propanoid acid borneol ester (n=10)	9mg/kg	69.21±15.19	-1.57±8.21	1.46±14.24	0.13±18.94	-1.21±21.94	-0.75±21.63
Propanoid acid borneol ester (n=10)	4.5mg/kg	68.47±11.97	72.55±16.58	68.87 ±14.57	66.68±11.95	60.98±16.13	60.29±15.88
Verapamil control (n=10)	11.4mg/kg	75.39±19.27	-1.00±14.79	-5.45±15.82	-7.68±16.90	-15.66±21.14	-16.74±18.37
			68.02±18.77	66.18±19.00	62.90±17.46	59.10±16.07	63.12±19.81
			-2.47±10.80	-5.33±9.64	-9.50±10.75	-14.30±13.75	-8.83±17.11
			68.68±15.82	70.87±15.08	71.78±15.67	76.78±14.34	74.71±18.13
			-0.08±12.81	3.38±12.17	4.78±14.39	12.13±9.39	8.47±17.66
			59.69±12.55	50.08±9.88	48.00±9.98	48.81±11.47	49.38±13.27
			-19.04±12.70	-31.18±15.04	-33.98±15.67	-32.54±19.02	-32.10±18.74
							-29.18±17.73

In comparison with the Tween control group: *p < 0.05, **p < 0.01

Table 7. Effects on mean arterial pressure (mmHg)

Group	Dose	Before administration	After administration				
			5'	15'	30'	60'	120'
Blank control (n=10)	2.5mL/kg	81.80±15.30	84.93±16.35	86.66±18.78	83.87±22.97	83.96±22.23	83.59±23.20
Tween control (n=10)	2.5mL/kg	86.51±18.19	3.81±6.04	5.64±9.78	1.26±15.60	1.82±12.68	1.51±15.15
Propanoid acid borneol ester (n=10)	18mg/kg	91.44±15.44	85.05±18.38	86.69±16.88	84.92±16.37	84.24±20.76	84.38±19.01
Propanoid acid borneol ester (n=10)	9mg/kg	90.23±16.25	-1.45±7.32	1.28±12.05	-0.32±16.30	-1.82±19.29	-1.26±19.14
Propanoid acid borneol ester (n=10)	4.5mg/kg	88.67±14.74	91.15±20.31	86.21±18.29	83.71±15.31	76.31±21.36	74.94 ±21.07
Verapamil control (n=10)	11.4mg/kg	95.96±22.22	-0.58±13.53	-5.52±14.99	-7.79±14.45	-16.01±20.44	-17.64±18.17
			89.05±20.26	86.74±20.25	82.79±18.98	77.89±18.11	82.38±23.18
			-1.79±8.69	-4.42±8.30	-8.42±9.56	-13.46±12.43	-8.77±16.66
			87.97±18.89	90.18±17.91	91.15±17.95	96.51±16.69	93.79±20.11
			-1.01±11.95	1.66±11.07	2.91±12.81	8.95±8.95	5.62±15.30
			78.22±14.31	66.21±11.57	63.49±11.93	65.37±14.57	65.97±16.34
			-17.07±11.03	-29.16±13.09	-32.04±13.81	-29.68±18.40	-29.33±17.82
							-26.65±17.62

In comparison with the Tween control group: *p < 0.05, **p < 0.01

Table 8. Effects on left intraventricular pressure (mmHg)

Group	Dose	Before administration	After administration					
			5'	15'	30'	60'	90'	120'
Blank control (n=10)	2.5mL/kg	124.84±14.88	127.99±16.77 2.48±4.20	128.87±18.64 3.05±5.46	126.10±22.02 0.51±8.27	126.30±21.69 0.75±7.10	125.79±22.94 0.27±8.10	128.12±24.71 2.02±10.87
Tween control (n=10)	2.5mL/kg	126.46±14.18	125.76±13.38 -0.41±4.07	127.22±12.98 0.87±6.22	125.33±14.26 -0.61±8.37	124.91±16.52 -1.07±9.12	124.32±15.43 -1.45±9.18	122.50±15.26 -2.83±9.80
Propanoid acid borneol ester (n=10)	18mg/kg	131.93±16.79	131.90±18.96 -0.01±7.29	126.28±18.51 -4.15±8.95*	124.81±18.94 -5.46±7.09	120.67±23.43 -8.71±11.64*	117.91±22.68 -10.81±11.60*	119.85±22.26 -9.13±13.16**
Propanoid acid borneol ester (n=10)	9mg/kg	128.29±18.38	128.87±25.63 -0.03±8.26	127.06±25.57 -1.37±9.10	123.59±25.06 -4.07±8.57	119.77±23.09 -6.84±8.75*	122.40±26.58 -4.88±11.37	121.85±26.36 -5.47±9.76
Propanoid acid borneol ester (n=10)	4.5mg/kg	128.68±20.18	128.67±18.24 0.38±6.72	130.13±18.12 1.51±6.25	129.67±17.48 1.22±7.07	134.39±19.12 4.73±6.55	130.91±17.22 2.39±9.41	129.15±18.53 0.79±7.76
Verapamil control (n=10)	11.4mg/kg	137.79±18.96	128.71±14.20 -6.24±4.79**	120.22±12.98 -12.21±7.42**	116.59±13.59 -14.73±8.91**	115.68±15.37 -15.14±12.14**	115.10±16.60 -15.59±12.52**	118.39±16.55 -13.34±11.53**

In comparison with the Tween control group: *p < 0.05, **p < 0.01

Table 9. Effects on the change-rate of left intraventricular pressure, MAX (mmHg/s)

Group	Dose	Before administration	After administration					
			5'	15'	30'	60'	90'	120'
Blank control (n=10)	2.5mL/kg	5121.1±940.7	5300.2±1071.0 3.5±8.2	5363.7±1193.1 4.6±10.9	5258.2±1366.8 1.7±13.5	5139.2±926.2 0.9±10.4	5094.3±946.1 0.2±12.7	5408.2±1342.3 5.1±14.8
Tween control (n=10)	2.5mL/kg	5246.5±14.18	5252.7±13.38 1.3±12.6	5408.4±12.98 4.5±18.3	5318.1±14.26 2.5±21.3	5269.2±16.52 1.6±21.5	5295.0±15.43 2.0±20.2	5205.3±15.26 0.2±21.8
Propanoid acid borneol ester (n=10)	18mg/kg	5695.0±1086.8	5694.7±1264.7 -0.3±9.3	5470.3±1416.7 -4.7±11.8*	5186.9±1177.3 -8.9±11.5*	4905.3±1047.7 -12.4±18.7*	4676.5±941.1 -16.7±15.5**	5012.6±777.5 -10.9±12.4**
Propanoid acid borneol ester (n=10)	9mg/kg	5435.8±1200.0	5388.9±1187.0 -0.7±6.7	5321.6±1133.0 -1.6±7.4	5179.1±1270.9 -5.0±7.8	4966.9±1297.4 -9.2±10.0*	5113.5±1356.4 -6.4±13.2	4959.8±1399.0 -9.7±11.2*
Propanoid acid borneol ester (n=10)	4.5mg/kg	5380.2±913.7	5429.3±1279.7 0.4±11.0	5435.9±1216.6 0.9±11.4	5408.8±1352.2 0.1±13.5	5706.1±1393.5 7.1±18.3	5706.1±1390.6 7.6±26.1	5512.7±1250.1 3.6±20.4
Verapamil control (n=10)	11.4mg/kg	5455.9±1418.1	4857.7±1002.4 -9.7±7.0**	4284.9±793.2 -19.6±11.8**	4152.8±901.5 -22.1±14.0**	3919.4±784.0 -25.4±17.3**	3759.0±814.5 -28.1±19.0**	4044.6±844.9 -23.1±18.4**

In comparison with the Tween control group: *p < 0.05, **p < 0.01

Table 10. Effects on the change-rate of left intraventricular pressure, -MAX (mmHg/s)

Group	Dose	Before administration		After administration							
				5'	15'	30'	60'	90'	120'		
Blank control (n=10)	2.5mL/kg	-3790.6±596.7		-3865.0±679.8 1.8±4.8	-3891.5±775.2 2.3±8.0	-3879.0±906.5 1.3±11.4	-3881.4±781.9 2.0±10.4	-3858.7±715.1 1.8±10.9	-3985.4±899.9 4.4±13.8		
Tween control (n=10)	2.5mL/kg	-3753.7±603.1		-3690.5±526.4 -1.4±2.7	-3767.3±551.9 0.9±8.5	-3692.4±698.4 -1.0±16.2	-3673.8±721.5 -1.6±16.5	-3658.5±653.0 -1.8±16.4	-3573.1±702.5 -4.4±15.4		
Propanoid acid borneol ester (n=10)	18mg/kg	-4092.0±905.4		-4103.4±981.2 0.0±7.8	-3991.9±948.6 -2.4±9.3	-3923.8±879.8 -3.9±8.2	-3744.3±851.5 -7.9±14.0*	-3659.3±806.3 -9.2±16.9	-3692.1±863.3 -8.8±16.9*		
Propanoid acid borneol ester (n=10)	9mg/kg	-3881.3±735.6		-3908.5±1013.0 0.0±11.2	-3792.8±979.5 -2.9±10.2	-3696.8±997.3 -5.5±10.8	-3569.5±837.8 -8.3±10.2*	-3629.7±1013.3 -7.2±13.3	-3541.7±917.7 -9.2±11.3*		
Propanoid acid borneol ester (n=10)	4.5mg/kg	-4246.1±669.3		-4152.9±764.8 -2.2±8.2	-4141.1±755.6 -2.4±9.1	-4090.5±704.6 -3.6±6.5	-4146.8±820.5 -2.4±10.2	-4156.0±947.7 -2.2±15.9	-3995.2±837.9 -5.6±14.3		
Verapamil control (n=10)	11.4mg/kg	-4215.6±1058.0		-3812.9±779.3 -8.5±6.6**	-3548.9±707.7 -14.5±9.9**	-3416.6±767.5 -17.8±11.8**	-3436.8±691.1 -16.2±16.0**	-3247.4±773.2 -20.9±16.9**	-3452.2±630.9 -16.3±11.4**		

In comparison with the Tween control group: *p < 0.05, **p < 0.01

Table 11. Effects on area of myocardial infarction in rats

Group	Dose (mg/kg)	Animal number	Ventricle weight(g)	Non-infarct region weight (g)	Infarct region weight (g)	Percentage (%)
Sham operation	/	10	0.66±0.03			
Model control	/	10	0.68±0.05	0.45±0.03	0.23±0.04	33.77±4.31
bornyl salvianate ester	10	10	0.68±0.09	0.47±0.07	0.20±0.05	30.09±5.69
bornyl salvianate ester	20	10	0.68±0.08	0.49±0.09	0.18±0.05*	25.73±6.35**
bornyl salvianate ester	40	10	0.63±0.07	0.45±0.06	0.18±0.04*	28.95±5.57*
Verapamil	10	10	0.65±0.09	0.46±0.08	0.18±0.04*	27.60±5.03**

In comparison with the model control group: *P<0.05, **P<0.01

Table 12. Effects on the activities of serum LDH and CK in rats with myocardial ischemia

5

Group	Dose (mg/kg)	Animal number	CK (U/L)	CK-MB (U/L)	LDH (U/L)
Sham operation	/	10	653.1±164.9**	565.3±122.2**	1091.7±259.7**
Model control	/	10	1058.5±150.0	1422.6±182.3	2351.4±220.83
bornyl salvianate ester	10	10	1214.2±160.2*	1267.0±274.4	1663.7±374.43**
bornyl salvianate ester	20	10	894.5±176.3*	1074.1±116.8**	2022.7±202.63**
bornyl salvianate ester	40	10	778.0±177.1**	906.3±99.9**	1712.5±209.1**
Verapamil	10	10	752.2±102.8**	776.6±127.6**	1160.8±145.0**

In comparison with the model control group: *P<0.05, **P<0.01

Table 13. Effects on the activities of serum SOD and MDA in rats with myocardial ischemia

Group	Dose (mg/kg)	Animal number	SOD(U/mL)	MDA(nmol/mL)
Sham operation	/	10	155.08±21.45**	6.86±5.67*
Model control	/	10	95.46±26.05	18.20±12.10
bornyl salvianate ester	10	10	117.66±31.97	21.69±27.94
bornyl salvianate ester	20	10	111.32±20.36	10.60±11.156
bornyl salvianate ester	40	10	119.63±23.53*	9.43±6.57
Verapamil	10	10	126.16±28.10*	11.86±5.93

In comparison with the model control group: *P<0.05, **P<0.01

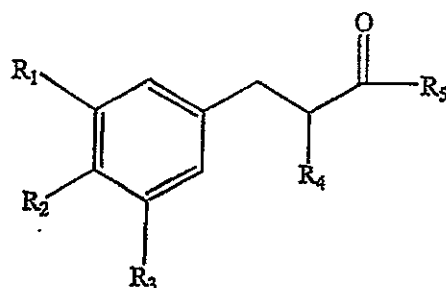
Table 14. Effects on the ST-T segment of ECG in rats with myocardial ischemia

Group	Dose (mg/kg)	Animal Number	Elevation of ST-T segment (mV)		
			Before modeling	0min after modeling	24h after modeling
Sham operation	/	10	0.18±0.08	0.15±0.25	0.12±0.06
			Change rate(%)	140.08±161.56	22.34±21.51**
Model control	/	10	0.20±0.09	0.19±0.41	0.23±0.10
			Change rate(%)	155.67±158.10	92.67±91.35
bornyl salvianate ester	10	10	0.21±0.06	0.24±0.24	0.17±0.09
			Change rate(%)	95.00±78.34	49.00±36.27
bornyl salvianate ester	20	10	0.17±0.07	0.13±0.16	0.11±0.10
			Change rate(%)	91.61±116.85	60.77±39.69
bornyl salvianate ester	40	10	0.21±0.06	0.36±0.17	0.18±0.07
			Change rate(%)	103.87±148.26	29.01±18.99*
Verapamil	10	10	0.23±0.04	0.23±0.12	0.18±0.04
			Change rate(%)	34.36±35.86*	19.17±20.94*

In comparison with the model control group: *P<0.05, **P<0.01

Claims:

1. A compound of the formula (I):



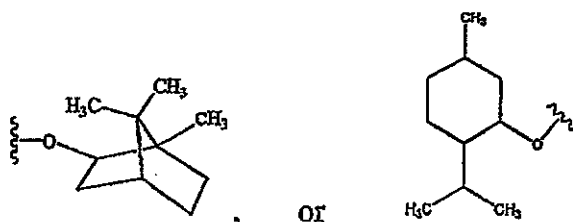
(I)

wherein R₁ and R₂ are OH, and R₃ is selected from the group consisting of H, OH, F, Cl, Br, methoxy and ethoxy; or alternatively, R₁ and R₂ together form -OCH₂O-, and R₃ is selected from the group consisting of H, OH, methoxy, ethoxy and halogens;

R₄ is OH or acyloxy; and

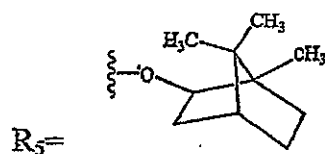
R₅ is cycloalkoxy.

2. The compound according to claim 1, wherein R₄ is OH.
3. The compound according to claim 1, wherein R₄ is aryloxy or heterocyclic radical-substituted acyloxy.
4. The compound according to claim 3, wherein R₄ is *o*-acetoxybenzoyloxy, 3-pyridinylbenzoyloxy or 4-pyridinylbenzoyloxy.
5. The compound according to any one of claims 1 to 4, wherein R₅ is:

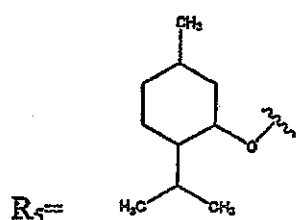
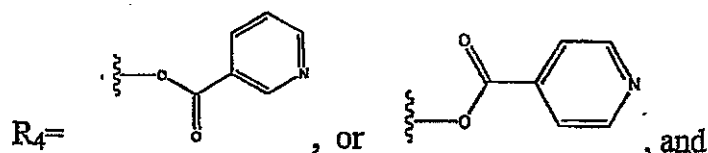


6. The compound according to any one of claims 1 to 5, wherein R₁ and R₂ together form -OCH₂O-.

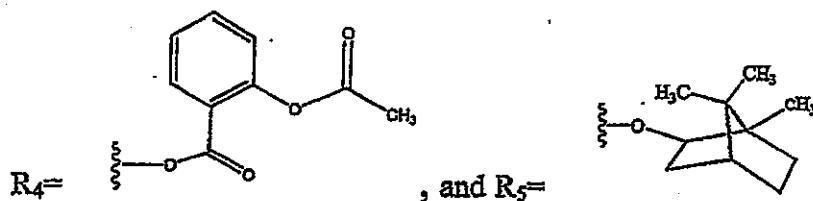
7. The compound according to claim 1 or claim 2, wherein $R_3=H$, $R_4=OH$, and



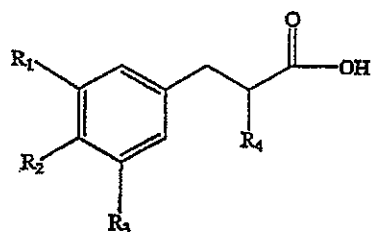
8. The compound according to claim 1, wherein $R_3=H$,



9. The compound according to claim 1, wherein $R_3=H$,



10. A process for synthesizing a compound according to any one of claims 1 to 9, which comprises reacting a compound of formula (III) with a compound of formula (IV) or a hydrate thereof in the presence of a catalyst:



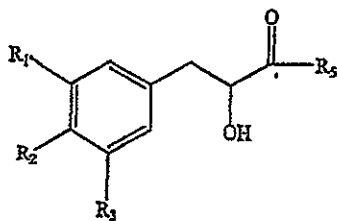
(III)

R_5-H

(IV)

wherein R_1 , R_2 , R_3 , R_4 and R_5 have the same meanings as in formula (I); or

which comprises reacting a compound of the formula (V) with a compound of formula (VI) or a hydrate thereof in the presence of a catalyst:



(V)

$R_4'-H$

(VI)

wherein R_1 , R_2 , R_3 , and R_5 have the same meaning as in formula (I), and R_4' is acyloxy.

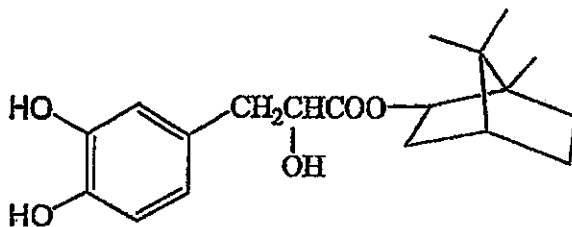
11. The process according to claim 10, wherein the catalyst is one or more of concentrated H_2SO_4 , silicotungstic acid, phosphomolybdic acid, p-toluene sulfonic acid, $S_2O_8^{2-}/ZrO_2$, aluminum trichloride, zinc chloride or magnesium chloride.

12. The process according to claim 10 or claim 11, wherein the reaction is performed in a solvent.

13. The process according to claim 12, wherein the solvent is ethyl acetate, dichloromethane, tetrahydrofuran, acetone, toluene, 1,4-dioxane or *N,N*-dimethylformamide, alone or in any combination.

14. Use of a compound according to any one of claims 1 to 9 in the manufacture of a medicament for the prevention or treatment of cardiovascular or cerebrovascular diseases.

15. Use according to claim 14, wherein the compound is a compound of the formula (II):



(II)