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Use of 3-carboxy-N-ethyl-N,N-dimethylpropan-1-aminium salts in the treatment of cardiovascular disease

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(54) Title: USE OF 3-CARBOXY-N-ETHYL-N,N-DIMETHYLPROPAN-1-AMINIUM SALTS IN THE TREATMENT OF CARDIOVASCULAR DISEASE

(57) Abstract: Salts of 3-carboxy-N-ethyl-N,N-dimethylpropan-1-aminium, method of preparation thereof and use in the treatment of cardiovascular disease.

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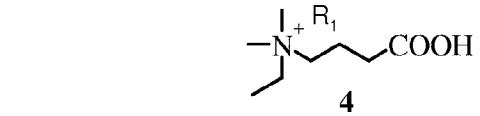
Description

Use of 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salts in the treatment of cardiovascular disease

Technical Field

The present invention relates to new compound 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salts, and to a method of preparation thereof (compound of formula 4)



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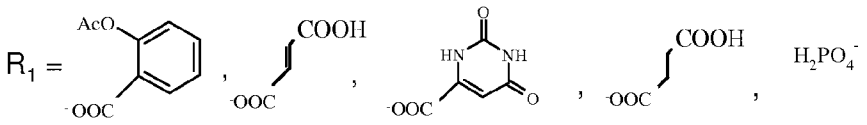

The present invention relates also to use of 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salts in the treatment of cardiovascular disease.

Background Art

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vesels.

An estimated 16.7 million - or 29.2% of total global deaths - result from the various forms of cardiovascular disease (CVD).

Myocardial infarction (heart attack) is a serious result of coronary artery disease. Myocardial infarction (MI) is the irreversible necrosis of heart muscle secondary to prolonged ischemia. A heart attack or myocardial infarction is a medical emergency in which the supply of blood to the heart is suddenly and severely reduced or cut off, causing the muscle to die from lack of oxygen. More than 1.1 million people experience a heart attack (myocardial infarction) each year, and for many of them, the heart attack is their first symptom of coronary artery disease. A heart attack may be severe enough to cause death or it may be silent. As many as one out of every five people have only mild symptoms or none at all, and the heart attack may only be discovered by routine electrocardiography done some time later.

A heart attack (myocardial infarction) is usually caused by a blood clot that blocks an artery of the heart. The artery has often already been narrowed by fatty deposits on its walls. These deposits can tear or break open, reducing the flow of blood and

releasing substances that make the platelets of the blood sticky and more likely to form clots. Sometimes a clot forms inside the heart itself, then breaks away and gets stuck in an artery that feeds the heart. A spasm in one of these arteries causes the blood flow to stop.

γ-Butyrobetaine, from which the mammalian organism synthesises carnitine, was primarily characterised as a toxic substance
which accelerates respiration, causes salivation and lacrimation, pupil dilation, vasoconstriction and heart stop in diastole **LINNEWEH, W.** Gamma-Butyrobetain, Crotonbetain und Carnitin im tierischen Stoffwechsel. *Hoppe-Seylers Zeitschrift für physiologische Chemie.* 1929, vol.181, p.42-53. At the same time, in later papers other authors ascertained that γ-butyrobetaine is extremely low toxic (LD50>7000 mg/kg, s.c.) **ROTZSCH, W.** Iber die Toxizitat des Carnitins und einiger verwandter Stoffe. *Acta biol. med. germ.*. 1959, vol.3, p.28-36.
In the literature data on nonsubstututed γ-butyrobetaine cardiovascular effects are missed, thought it was reported **HOSEIN, E.A.** Pharmacological actions of γ-butyrobetaine. *Nature.* 1959, vol.183, p.328-329. that γ-butyrobetaine is a substance similar to acetyl choline with a prolonged action. However, later the same authors reported that by an error the experiments involved, instead of γ-butyrobetaine, its methyl esther which in fact possesses cholinergic properties.
Contrary to the former γ-butyrobetaine was characterised as a pharmacologically inert substance **HOSEIN, E.A.** Isolation and probable functions of betaine esters in brain metabolism. *Nature.* 1960, vol.187, p.321-322.
As structurally related compounds to 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salts are disclosed in:

- **GB 1238868** A 14.07.1971 were disclosed betaines, such as 4-trimethylammoniobutanoate, used for polymers. Howerver no pharmacological propeties of these betaines weren't presented;
- **US 5973026** A (XEROX CORP) 26.10.1999 were disclosed 4-trimethylammoniobutanoate and 3-[diethyl(methyl)ammonio]propionate for using for ink compositions;
- **LLOYD ANDREW, et al.** A comparison of glycine, sarcosine, N,N-dimethylglycine, glycinebetaine and N-modified betaines as liposome cryoprotectants. *Journal of pharmacy and pharmacology.* 1992, vol.44, no.6, p.507-511 disclosed 2-[ethyl(dimethyl)ammonio]acetate used as cryoprotectants for liposomes;

- **DAVID B. , THOMAS, et al.** Synthesis, Characterization, and Aqueous Solution Behavior of Electrolyte- and pH-Responsive Carboxybetaine-Containing Cyclocopolymers. *Macromolecules.* 2003, vol.36, no.26, p.9710-9715 disclose 4-[diallyl(methyl)ammonio]butanoate and its synthesis starting from *N,N*-diallyl-*N*-methylaminiom and ethyl 4-bromobutanoate. The free acis is obtained from the ester in a second step using Amberlite ion exchange resin. The product is used as intermediate to synthesise polymers;
- *Prelog V.* 1930, vol.2, p.712-722 disclosed the synthesis of 4-trimethylammoniobutanoate starting from 4-dimethylammoniobutanoate and methyliodide;
- 4-Trimethylammoniobutanoate and its synthesis starting from trimethylamine and ethyl 4-bromobutanoate was described **JP 2009096766** A (KONAN GAKUEN) 07.05.2009. The free acid is obtained from the ester in a second step using Amberlite ion exchange resin;
- **WO 2008/055843** A (KALVINSH IVARS; CHERNOBROVIJS ALEKSANDRS; VARACHEVA LARISA; PUGOVICHS OSVALDS) 15.05.2008 was described 4-trimethylammoniobutanoate and synthesis, which started from the correspondin ester and using KOH-solution;
- **CA 2508094** A (VIVIER CANADA INC) 20.11.2006 was disclosed betaines, such as 4-trimethylammoniobutanoate, for use as medicament for accelerating collagen synthesis;
- **US 5965615** A (TAIHO PHARMACEUTICAL CO LTD; VALSTS ZINATNISKA IESTADE BEZP) 12.10.1999 was disclosed 4-trimethylammoniobutanoate as a medicament for the treatment of myocardial metabolic disorder, the same compound was disclosed in **US 2007191381** A (CONCERT PHARMACEUTICALS INC) 16.08.2007 for treatment of myocardial infarction.

3- (2,2,2-Trimethylhydrazinium) propionate dihydrate is known as compound with cardioprotective properties (this substance being known under its International Nonproprietary Name of Meldonium). 3- (2,2,2-Trimethylhydrazinium) propionate is disclosed in **US 4481218** (INST ORGANICHESKOGO SINTEZA) 06.11.1984 as well in **US 4451485** A (INSTITU ORCH SINTEZA AKADEMII) 29.05.1984.

It is well known that 3- (2, 2,2-trimethylhydrazinium) propionate as dihydrate is widely used for controlling carnitine and gamma-butyrobetaine concentration ratio and consequently the speed of fatty acid beta-oxidation in the body **DAMBROVA M., LIEPINSH E., KALVINSH I. I.** Mildronate: cardioprotective action through carnitine-lowering effect. *Trends in Cardiovascular Medicine,.* 2002, vol.12, no.6, p.275-279. Due to these properties, Meldonium is extensively applied in medicine as an anti-ischemic, stress-protective and cardioprotective drug in treating various cardio-vascular diseases and other pathologies involving tissue ischemia **KARPOV R.S., KOSHELSKAYA O.A., VRUBLEVSKY A.V., SOKOLOV A.A., TEPLYAKOV A.T., SKARDA I., DZERVE V., KLINTSARE D., VITOLS A., KALNINS U., KALVINSH I., MATVEYA L., URBANE D.** Clinical Efficacy and Safety of Mildronate in Patients With Ischemic Heart Disease and Chronic Heart Failure. *Kardiologiya.* 2000, no.6, p.69-74. In the treatment of cardiovascular diseases the mechanism of action of 3-(2,2,2-trimethylhydrazinium)propionate based on limitation of carnitine biosynthesis rate and related long-chain fatty acid transport limitation through mitochondria membranes **SIMKHOVICH B.Z., SHUTENKO Z.V., MEIRENA D.V., KHAGI K.B., MEZHAPUKE R.J., MOLODCHINA T.N., KALVINS I.J., LUKEVICS E.**

3-(2,2,2,-Trimethylhydrazinium)propionate (THP) – a novel gamma-butyrobetaine hydroxylase inhibitor with cardioprotective properties. *Biochemical Pharmacology.* 1988, vol.37, p.195-202. , **KIRIMOTO T., ASAKA N., NAKANO M., TAJIMA K., MIYAKE H., MATSUURA N.** Beneficial effects of MET-88, a γ-butyrobetaine hydroxylase inhibitor in rats with heart failure following myocardial infarction. *European Journal of Pharmacology.* 2000, vol.395, no.3, p.217-224.

A reference herein to a patent document or other matter which is given as prior art is not to be taken as an admission that that document or matter was known or that the information it contains was part of the common general knowledge as at the priority date of any of the claims.

Summary of invention

As it was known what Meldonium dihydrate has cardioprotective effect; however there are no data that γ-butyrobetaine itself has pronounced cardioprotective effect. In the patent **EP 0845986** B (KALVINSH IVARS, VEVERIS MARIS) 02.04.2003 is disclosed

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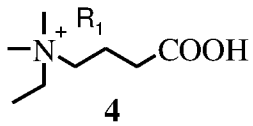
pharmaceutical composition of Meldonium dihydrate and γ-butyrobetaine for use in the treatment of cardiovascular diseases.

It would be desirable to provide a compound, which has pronounced cardioprotective effect.

The invention provides new compounds, 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salts (compound of formula 4), having a similar structure to Meldonium or γ-butyrobetaine.



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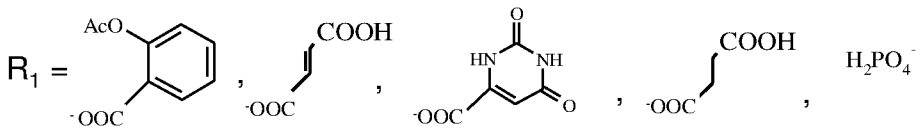
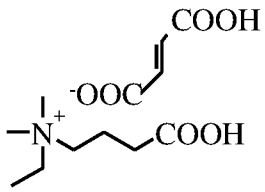
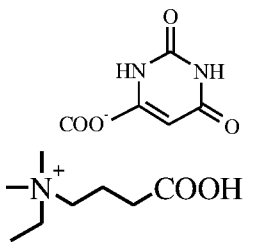


According to an embodiment of the invention, there is provided 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium (2*E*)-3-carboxyacrylate



According to another embodiment of the invention, there is provided 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium 2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate



According to another embodiment of the invention, there is provided 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium dihydrogen phosphate

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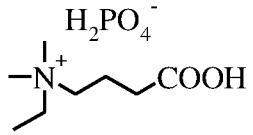


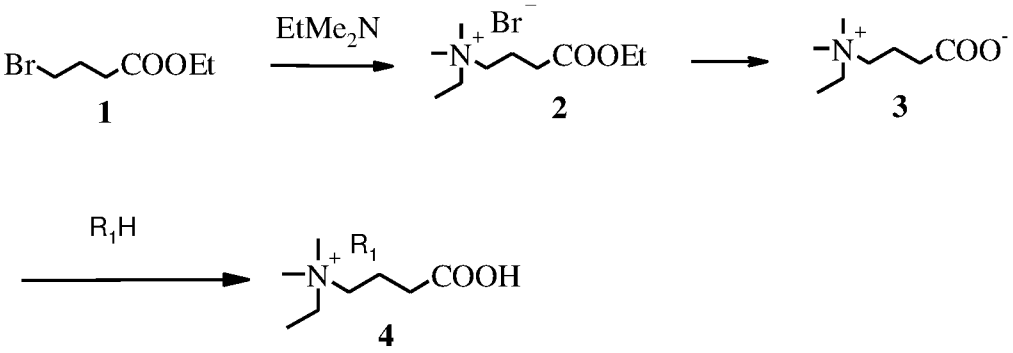
To our surprise 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salts possess pronounced cardioprotective effect and are more effective as Meldonium dihydrate *in vivo* myocardial infarction models, due to this property 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salts may be used in medicine. 3-Carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salts can be used as a solution for injection.

According to another embodiment of the invention, there is provided a 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salt, selected from the group consisting of 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium (2*E*)-3-carboxyacrylate, 2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate and dihydrogenphosphate for use as a medicament.

According to another embodiment of the invention, there is provided the use of a 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salt, selected from the group consisting of 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium, (2*E*)-3-carboxyacrylate and 2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate and dihydrogenphosphate for treatment of cardiovascular diseases.

The present invention is also directed to a method of preparation of the compound of formula **4**.

There is disclosed process, which can be used in purpose to prepare target compound 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salts of formula 4, see scheme bellow.



According to another embodiment of the invention, there is provided a process for preparing 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salt comprising:

a. adding *N,N*-dimethylethylamine to ethyl 4-bromobutanoate in appropriate solvent to obtain 4-ethoxy-*N*-ethyl-*N,N*-dimethyl-4-oxo-1-butanaminium bromide;
b. passing 4-ethoxy-*N*-ethyl-*N,N*-dimethyl-4-oxo-1-butanaminium bromide through ion exchange resin column to obtain 4-[ethyl(dimethyl)ammonio] butanoate;
c. adding acid selected from the group, consisting of fumaric acid, orotic acid and phosphoric acid in appropriate solvent to obtain the corresponding 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salt.

There is also disclosed a process for preparing 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salt of formula 4 involves the following process steps:

a) adding *N,N*-dimethylethylamine to ethyl 4-bromobutanoate (1) in appropriate solvent to obtain 4-ethoxy-*N*-ethyl-*N,N*-dimethyl-4-oxo-1-butanaminium bromide (2);
b) passing 4-ethoxy-*N*-ethyl-*N,N*-dimethyl-4-oxo-1-butanaminium bromide (2) through ion exchange resin column to obtain 4-[ethyl(dimethyl)ammonio] butanoate (3);
c) adding acid which is selected from 2-(acetyloxy)benzoic acid (4 a) or (*E*)-butenedioic acid (4 b) or succinic acid (4 c) or 2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylic acid monohydrate (4 d) or phosphoric acid (4 e) to 4-[ethyl(dimethyl)ammonio] butanoate (3) in appropriate solvent to obtain 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salt (4).

Throughout the description and claims of the specification, the word "comprise" and variations of the word, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps.

Description of embodiments

The present invention will be described in more detail by referring to the following non–limiting examples.

Preparation of 4-ethoxy-*N*-ethyl-*N,N*-dimethyl-4-oxo-1-butanaminium bromide (2)

Procedure A

To a solution of ethyl 4-bromobutanoate (**1**) (20.0 g, 102.5 mmol) in acetonitrile (70 ml) *N,N*-dimethylethylamine (15 ml, 139 mmol) was added and stirred at ambient temperature for 3 days. The reaction mixture was evaporated, the residue was triturated with acetone (50 ml), filtered, washed with ether, and dried to afford 26.051 g (94.8%) of the 4-ethoxy-*N*-ethyl-*N,N*-dimethyl-4-oxo-1-butanaminium bromide. LCMS (ESI$^+$, *m/z*): [M-Br$^-$]$^+$ 188, purity 98.9%.

[0001] ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.26 (t, J=7.2 Hz, 3H); 1.44 (t, J=7.4 Hz, 3H); 2.00-2.11 (m, 2H); 2.52 (t, J=6.6 Hz, 2H); 3.40 (s, 6H); 3.64-3.73 (m, 2H); 3.69 (q, J=7.4 Hz, 2H); 4.14 (q, J=7.2 Hz, 2H).

Procedure B

To a solution of ethyl 4-bromobutanoate (**1**) (19.5 g, 100 mmol) in acetone (70 ml) *N,N*-dimethylethylamine (15 ml, 139 mmol) was added and stirred at ambient temperature for 3 days. The reaction mixture was filtered; the solid material was washed with an acetone, ether, and dried to afford 24.19 g (90.2%) of the title compound **2**. The filtrate was evaporated; the residue (2.147 g) was triturated with ether and dried to give an extra batch (0.962 g, 3.6%) of the product **2** of the same quality as the main portion. The evaporation of the ether washings allowed recovering 0.956 g (4.9 mmol, 4.9%) of the starting material **1**. 4-ethoxy-*N*-ethyl-*N,N*-dimethyl-4-oxo-1-butanaminium bromide: LCMS (ESI$^+$, *m/z*): [M-Br$^-$]$^+$ 188, purity 98.4%. ^{1}H NMR ($CDCl_3$, HMDSO) δ: 1.26 (t, J=7.2 Hz, 3H); 1.44 (t, J=7.4 Hz, 3H); 2.00-2.11 (m, 2H); 2.52 (t, J=6.6 Hz, 2H); 3.40 (s, 6H); 3.64-3.73 (m, 2H); 3.69 (q, J=7.4 Hz, 2H); 4.14 (q, J=7.2 Hz, 2H).

Preparation of 4-[ethyl(dimethyl)ammonio]butanoate (3)

A solution of 4-ethoxy-*N*-ethyl-*N,N*-dimethyl-4-oxo-1-butanaminium bromide (2) (12.00 g, 44.7 mmol) in water (10 ml) was passed through Amberlite ® IRA-410 (OH) ion exchange resin column (250 ml) eluting slowly (*ca.* 10 drops/min) with ethanol (TLC control). The eluate was evaporated and the residue (12 g) was dissolved in water (50 ml). To this solution DOWEX® 50WX8 ion exchange resin (5 g) was added and stirred at ambient temperature for 0.5 h. The reaction mixture was filtered through celite (1 cm) and the eluate was evaporated. The residue was azeotropically dried with isopropanol, acetonitrile, and acetone. The obtained solid was triturated with acetone (10 ml) and the mixture was kept at 0°C for 2 h. The precipitate was filtered and dried *in vacuo* over P_2O_5 to give 4.65 g (65%) of the 4-[ethyl(dimethyl)ammonio]butanoate (3).

(DMSO-d_6, HMDSO) δ: 1.24 (t, J=7.3 Hz, 3H); 1.66-1.76 (m, 2H); 1.81 (t, J=6.4 Hz, 2H); 2.95 (s, 6H); 3.16-3.23 (m, 2H); 3.29 (q, J=7.3 Hz, 2H). LCMS (ESI$^+$, *m/z*): 160 $[M+H]^+$.

Anal. Calc. for $C_8H_{17}NO_2 \cdot 1.55\ H_2O$: C 51.34; H 10.82; N 7.48.

Found: C 51.36, H 11.40, N 7.34.

Preparation of 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium 2-(acetyloxy)benzoate (4 a)

3-Carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium 2-(acetyloxy)benzoate was prepared in a form of a water mixture. Thus, *ca.* 90% 4-[ethyl-(dimethyl)ammonio]butanoate (3) (2.20 g, 12.44 mmol) and 2-(acetyloxy)-benzoic acid (2.266 g, 12.57 mmol) were placed in a volumetric flask and diluted with water up to 100 ml. The content of the mixture dissolves by heating and precipitates by lowering of the temperature. According to ^{1}H-NMR, the precipitated solid material consists of almost pure 2-(acetyloxy)-benzoic acid.

Preparation of 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium (2*E*)-3-carboxyacrylate (4 b)

To a solution of 4-[ethyl(dimethyl)ammonio]butanoate (3) (2.0 g, 12.56 mmol) in anh. ethanol (10 ml) a hot (60°C) solution of (*E*)-butenedioic acid (1.46 g, 12.56 mmol) in ethanol (50 ml) was added. The reaction mixture was allowed to stand at ambient temperature for 2 h, the precipitated crystals were filtered and dried over

P_2O_5 to give 2.98 g (85%) of the 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium (2*E*)-3-carboxyacrylate. M.p. 122-123ºC.

^{1}H-NMR (D_2O, DSS) δ: 1.36 (tt, *J*=1.9, 7.3 Hz, 3H); 2.06 (m, 2H); 2.49 (t, *J*=7.1 Hz, 2H); 3.06 (s, 6H); 3.31 (m, 2H); 3.40 (q, *J*=7.3 Hz, 2H); 6.75 (s, 1.9H, CH=CH).

LCMS ESI$^+$ (*m/z*): 160 $[M+H]^+$. Titration assays: water content (Fisher) 0.13%, betaine content ($HClO_4$) 93.0%, (*E*)-butenedioic acid content 46.1%.

Anal. Calc. for $C_8H_{17}NO_2 \cdot 1.2\ C_4H_4O_4$ (46.7%): C 51.50, H 7.36, N 4.69.

Found: C 51.52, H 7.35, N 4.61.

Preparation of 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium 3-carboxypropanoate (4 c)

3-Carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium 3-carboxypropanoate was prepared in a form of a water solution. Thus, *ca.* 90% 4-[ethyl-(dimethyl)ammonio]butanoate (3) (2.20 g, 12.44 mmol) and succinic acid (1.49 g, 12.62 mmol) were placed in a volumetric flask and dissolved and diluted with water up to 100 ml.

Preparation of 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium 2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate (4 d)

To a solution of 4-[ethyl(dimethyl)ammonio]butanoate (3) (2.0 g, 12.56 mmol) in isopropanol (100 ml) 2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylic acid monohydrate (2.187 g, 12.56 mmol) was added and the reaction mixture was heated to reflux until all the carboxylic acid dissolved. The reaction mixture was allowed to cool to ambient temperature, the precipitated crystals were filtered, washed with isopropanol (5 ml) and diethyl ether (20 ml), and dried over P_2O_5 to give 3.238 g (97.4%) of the 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium 2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate. M.p. 150.7ºC.

^{1}H-NMR (D_2O, DSS) δ: 1.36 (tt, *J*=2.0, 7.3 Hz, 3H); 2.05 (m, 2H); 2.47 (t, *J*=7.0 Hz, 2H); 3.07 (s, 6H); 3.31 (m, 2H); 3.41 (q, *J*=7.3 Hz, 2H); 6.20 (s, 1H, C=CH).

LCMS ESI$^+$ (*m/z*): 160 $[M+H]^+$.

Anal. Calc. for $C_8H_{17}NO_2 \cdot C_5H_4N_2O_4$ (49.5%): C 49.52, H 6.71, N 13.33.

Found: C 49.59, H 6.69, N 13.26.

Preparation of 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium dihydrogen phosphate (4 e)

To a solution of 4-[ethyl(dimethyl)ammonio]butanoate **(3)** (6.4 g, 40 mmol) in water (10 ml) a solution of 85% aq. H_3PO_4 (4.73 g, 40 mmol) in acetone (10 ml) was added and the resulting solution was stirred at ambient temperature for 10 min. The reaction mixture was evaporated and azeotropically dried several times with acetone by rotary evaporator at 45°C. The obtained white crystalline substance was dried over P_2O_5 to give 9.82 g (95%) of the 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium dihydrogen phosphate. M.p. 110-135°C.

^{1}H-NMR (D_2O, DSS) δ: 1.36 (tt, J=1.8, 7.3 Hz, 3H); 2.06 (m, 2H); 2.50 (t, J=7.0 Hz, 2H); 3.06 (s, 6H); 3.32 (m, 2H); 3.41 (q, J=7.3 Hz, 2H). LCMS ESI$^+$ (*m/z*): 160 $[M+H]^+$. Titration assays: water content (Fisher) 0.356%, betaine content ($HClO_4$) – 95.682%.

Anal. Calc. for $C_8H_{17}NO_2 \cdot 0.052\ H_2O$ (0.356%) $\cdot$ 1.07 H_3PO_4 (39.6%): C 36.26; H 7.73; N 5.29.

Found: C 36.20, H 7.72, N 5.11.

The purity of the obtained 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium dihydrogen phosphate was increased by crystallization from methanol. Thus, the 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium dihydrogen phosphate (6.9 g) was crystallized from methanol (40 ml) to afford 5.326 g (77%) of the purified 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium dihydrogen phosphate with m.p. 139°C.

Calc. for $C_8H_{17}NO_2 \cdot H_3PO_4$ (38.1%): C 37.36; H 7.84; N 5.45.

Found: C 37.52, H 7.85, N 5.39

Cardioprotective activity

Fifty male, 10 weeks old Wistar rats weighing 200-250 g were housed under standard conditions (21-23°C, 12 h light-dark cycle) with unlimited access to food (R3 diet, Lactamin AB, Sweden) and water.

Rats were adapted to local conditions for two weeks before the start of treatment. Meldonium dihydrate at a dose of 20 mg/kg, gamma-butyrobetaine at a dose of 20 mg/kg and 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salts at dose of 20mg/kg were administered *p.o.* daily for 8 weeks. Control rats received water.

Isolated rat heart infarction study

The isolated rat heart experiment was performed essentially as described earlier (Liepinsh et al., *J. Cardiovasc. Pharmacol.* **2006**; 48(6):314-9). Twenty-four hours after the last drug administration hearts were excised and retrogradely perfused via

the aorta at a constant pressure with oxygenated Krebs-Henseleit buffer at 37°C. The heart rate, left ventricle end-diastolic pressure and left ventricle developed pressure were continuously recorded. Coronary flow was measured using an ultrasound flow detector (HSE) and the PowerLab 8 /30 system from ADInstruments. The hearts were perfused for 20 min to stabilize the hemodynamic functions and then occlusion was performed for 60 min by constricting threads through a plastic tube. Successful occlusion was confirmed by a coronary flow decrease of about 40 percent. Reperfusion was achieved by releasing the threads. At the end of the 150-min reperfusion period, the risk zone was delineated with 0.1% methylene blue. The hearts were then sectioned transversely from the apex to the base in five slices 2 mm in thickness and incubated in 1% triphenyltetrazolium chloride in phosphate buffer (pH 7.4, 37°C) for 10 min to stain viable tissue red and necrotic tissue white. Computerized planemetric analysis of Sony A900 photographs was performed using Image-Pro Plus 6.3 software to determine the area at risk and area of necrosis expressed as a % of the left ventricle. The obtained values were then used to calculate the infarct size (IS) as a % of risk area according to the formula:

Infarct Size = Area of Necrosis/Area at Risk x 100%.

Effects in isolated rat heart infarction model

The anti-infarction effect of examined substances was investigated in an isolated rat heart infarction model. During occlusion of left coronary artery, the coronary flow in all experimental groups was decreased for 40% (from 11 ml/min to 7 ml/min). Moreover, the drop of developed left ventricular pressure for 50% was observed. The heart rate during the occlusion period did not change significantly. In reperfusion stage, coronary flow, developed left ventricular pressure, ±dp/dt values were recovered till about 80% of control level. There were no significant differences between control and treatment groups.

Effects of Meldonium dihydrate (20 mg/kg), gamma-butyrobetaine (20 mg/kg) and 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salts (20 mg/kg) after 2 weeks of treatment on infarct size in the isolated rat heart infarction experiment are presented in *Table 1*, *Table 2, Table 3, Table 4, Table 5, Table 6*

Table 1

Effects of Meldonium dihydrate, gamma-butyrobetaine and 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium 2-(acetyloxy)benzoate on infarct size

	Infarct size, % of control
Control	100.0 ±5.9
Meldonium dihydrate 20 mg/kg	117.9 ±7.9
Gamma-butyrobetaine 20 mg/kg	87.6 ±11.4
3-Carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium 2-(acetyloxy)benzoate 20 mg/kg	61.6 ±6.7*,#,$

Table 2

Effects of Meldonium dihydrate, gamma-butyrobetaine and 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium (2*E*)-3-carboxyacrylate on infarct size

	Infarct size, % of control
Control	100.0 ±5.9
Meldonium dihydrate 20 mg/kg	117.9 ±7.9
Gamma-butyrobetaine 20 mg/kg	87.6 ±11.4
3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium (2*E*)-3-carboxyacrylate 20 mg/kg	46.5 ±7.0*,#,$

Table 3

Effects of Meldonium dihydrate, gamma-butyrobetaine and 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium 2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate on infarct size

	Infarct size, % of control
Control	100.0 ±5.9
Meldonium dihydrate 20 mg/kg	117.9 ±7.9
Gamma-butyrobetaine 20 mg/kg	87.6 ±11.4
3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium 2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate 20 mg/kg	60.6 ±6.7*,#,$

Table 4

Effects of Meldonium dihydrate, gamma-butyrobetaine and 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium dihydrogen phosphate on infarct size

	Infarct size, % of control
Control	100.0 ±5.9
Meldonium dihydrate 20 mg/kg	117.9 ±7.9
Gamma-butyrobetaine 20 mg/kg	87.6 ±11.4
3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium dihydrogen phosphate 20 mg/kg	56.1 ±4.4$^{*,\#,\$}$

Table 5

Effects of Meldonium dihydrate, gamma-butyrobetaine and 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium 3-carboxypropanoate on infarct size

	Infarct size, % of control
Control	100.0 ±5.9
Meldonium dihydrate 20 mg/kg	117.9 ±7.9
Gamma-butyrobetaine 20 mg/kg	87.6 ±11.4
3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium 3-carboxypropanoate 20 mg/kg	62.9 ±4.7$^{*,\#,\$}$

Each values in mentioned Tables from 1-5 represents the mean ± s.e.m. of 9-10 animals.

*$p<0.05$ compared with control group; $^{\#}p<0.05$ compared with Gamma-butyrobetaine group, $^{\$}p<0.05$ compared with Meldonium dihydrate group

As it is presented in *Tables 1-5*, Meldonium dihydrate treatment at a dose of 20 mg/kg had no therapeutical effect; gamma-butyrobetaine has decreased infarct size by 12.4 %.

3-Carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium 2-(acetyloxy)benzoate at dose of 20 mg/kg decreased infarction size by 38.4 %.

Carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium (2*E*)-3-carboxyacrylate at dose of 20 mg/kg decreased infarction size by 53.5 %.

3-Carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium 2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate at dose of 20 mg/kg decreased infarction size by 39.4 %.

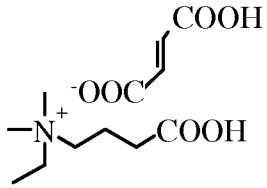
3-Carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium dihydrogen phosphate at dose of 20 mg/kg decreased infarction size by 43.9 %.

3-Carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium 3-carboxypropanoate at dose of 20 mg/kg decreased infarction size by 37.1 %.

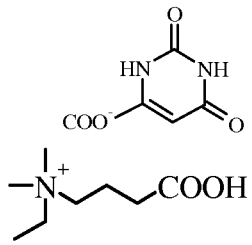
AMENDED CLAIMS

received by the International Bureau on 22 October 2012 (22.10.2012)

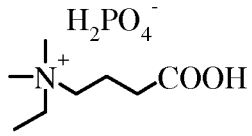
1. 3-Carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium (2*E*)-3-carboxyacrylate



2. 3-Carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium 2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate



3. 3-Carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium dihydrogen phosphate



4. A process for preparing 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salt comprising:
   a. adding *N,N*-dimethylethylamine to ethyl 4-bromobutanoate in appropriate solvent to obtain 4-ethoxy-*N*-ethyl-*N,N*-dimethyl-4-oxo-1-butanaminium bromide;
   b. passing 4-ethoxy-*N*-ethyl-*N,N*-dimethyl-4-oxo-1-butanaminium bromide through ion exchange resin column to obtain 4-[ethyl(dimethyl)ammonio] butanoate;
   c. adding acid selected from the group, consisting of fumaric acid, orotic acid and phosphoric acid in appropriate solvent to obtain the corresponding 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salt.
5. A process according to claim 4, wherein in step a) the appropriate solvent is acetonitrile or acetone.
6. 3-Carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salt, selected from the group consisting of 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium (2*E*)-3-carboxyacrylate, 2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate and dihydrogenphosphate for use as a medicament.

7. The use of 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium salt, selected from the group consisting of 3-carboxy-*N*-ethyl-*N,N*-dimethylpropan-1-aminium, (2*E*)-3-carboxyacrylate and 2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate and dihydrogenphosphate for treatment of cardiovascular diseases.
8. The use according to claim 7, wherein the cardiovascular disease is ischemic heart disease.
9. The use according to claim 8, wherein wherein the ischemic heart disease is myocardial infarction.