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Synthesis of chroman analogues of lipoic acid and evaluation of their activity against reperfusion arrhythmias

Maria Koufaki,[a,*] Anastasia Detsi,[a] Elissavet Theodorou,[a] Christina Kiziridi,[a] Theodora Calogeropoulou,[a] Athanasios Vassilopoulos,[b] Angeliki P. Kourounakis,[c] Eleni Rekka,[d] Panos N. Kourounakis,[d] Catherine Gaitanaki[b] and Panagiota Papazafiri[b]

[a]*Institute of Organic and Pharmaceutical Chemistry, National Hellenic Research Foundation, 48 Vasileos Constantinou Avenue, 116 35 Athens, Greece*
[b]*Department of Animal & Human Physiology, School of Biology, University of Athens, Panepistimiopolis, 15784 Athens, Greece*
[c]*Department of Pharmaceutical Chemistry, School of Pharmacy, University of Athens, Panepistimiopolis, 15771 Athens, Greece*
[d]*Department of Pharmaceutical Chemistry, School of Pharmacy, Aristotelian University of Thessaloniki, 54006 Thessaloniki, Greece*

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Abstract—Novel hybrids of lipoic acid and trolox connected through triamine spacers as well as analogues in which the lipoic acid was attached at different positions of the chroman moiety of vitamin E through an amide bond, were synthesized and exhibited strong inhibition of the microsomal lipid peroxidation. Moreover, the new molecules, at 1 μM concentration, reduced reperfusion arrhythmias and MDA content on isolated rat heart preparations, with the 2- and 5-subtituted chromans possessing the better cardioprotective activity.

1. Introduction

Coronary artery occlusion can result in acute myocardial infarction, which remain a major biomedical problem in developed countries. The establishment of blood flow to the myocardium, by procedures such as thrombolysis, angioplasty, and coronary bypass surgery, reduces the mortality of ischemic tissues. However, these therapies do not protect the heart from the damage caused by the reactive oxygen species (ROS) produced upon the readmission of oxygenated blood into the ischemic myocardium (reperfusion).[1] There is evidence that oxygen free radicals react with the phospholipid components of the myocardium affecting selective permeability of cell membranes and resulting in the development of life threatening ventricular arrhythmias and/or fibrillations.

A variety of antioxidant therapeutic approaches against myocardial reperfusion injury have been developed, which include administration of antioxidant enzymes or antioxidant vitamins.[2] Since lipid peroxidation is an important element of ischemia–reperfusion injury, chain breaking antioxidants such as vitamin E could protect the myocardiun from ROS produced during reperfusion.[3] However, vitamin E, showed limited efficacy, which may be attributed to its highly lipophilic properties making it only slowly available to cardiomyocytes, the principal target of oxidative injury.[4]

Current antioxidant strategies for emergency reperfusion therapy involve small molecules that can act as ROS scavengers but can also gain access to the intracellular compartment. Up to date, attempts have been made to develop analogues of the antioxidant vitamins with improved pharmacological profiles.[4,5]

Lipoic acid is a 1,2-dithiolane analogue, effective against a variety of ROS, which is used as a dietary supplement.[6,7] Recent studies[8] compared the cardioprotective effects of the reduced form of lipoic acid, DHLA, and vitamin E during the reoxygenation of hypoxic hearts. The results showed that the mechanism of action of these two antioxidants is different and thus, a combined treatment of lipoic acid and vitamin E could result in improved mitochondrial function. Other studies showed

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* Corresponding author. Tel.: +30-210-7273818; fax: +30-210-7273831; e-mail: mkoufa@eie.gr

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that dietary supplementation of vitamin E and α-lipoic acid decreased lipid peroxidation during in vivo ischemia–reperfusion in young adult rats. However, the combination did not influence cardiac performance.[9]

We previously reported[10] the synthesis of a series of compounds that contain lipoic acid and trolox connected through spacers. All analogues tested were strong inhibitors of lipid peroxidation, while two of them totally suppressed reperfusion arrhythmias at 5 μM concentration.

As a continuation of our efforts in the field of novel antioxidants, against lipid peroxidation and arrhythmias associated with ischemia–reperfusion injury, we synthesized new compounds, which contain lipoic acid and the analogue of vitamin E, trolox connected through triamine spacers as well as analogues in which the lipoic acid moiety was attached at different positions of the benzopyran ring of vitamin E through an amide bond.

The new compounds were tested against lipid peroxidation in rat liver microsomal membranes induced by ferrous ions and ascorbate.

The cardioprotective effect of the novel compounds was evaluated using reperfusion arrhythmias as end-point. Thus, the new molecules were tested, at 1 μM concentration, on isolated heart preparations using the Krebs perfused Langerdorff model.

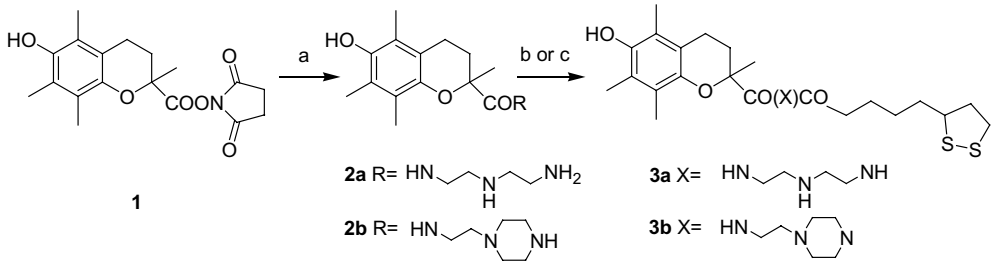
2. Chemistry

The synthesis of the novel compounds is shown in Schemes 1 and 2. The diaminoamides **2a** and **2b** were prepared from *N*-hydroxysuccinimide-activated trolox (**1**) and diethylenetriamine or 1-(2-aminoethyl)piperazine. The synthesis of the final diamides **3a** and **3b** involves the reaction of the aminoamides **2a** and **2b** with *N*-hydroxysuccinimide-activated lipoic acid or lipoic acid chloride in THF/H_2O, respectively.[10] Amine **4** was prepared following the procedure described by Hellberg et al.[11] with minor modifications. Thus, activation of trolox with 1,1′-carbonyldiimidazole (CDI) and subsequent coupling to benzylamine afforded the corresponding amide. The benzylamide was reduced, using boron dimethyl sulfide, to the protected amine, which was debenzylated using 10% Pd/C and ammoniun formate to afford amine **4**. Reaction of **4** with *N*-hydroxysuccinimide-activated lipoic acid gave the amide **5**. For the synthesis of the amide analogue at position 4 of the chroman ring, chromanone **6** was used as starting material. Conversion to oxime **7** followed by reduction using $TiCl_4$ and $NaBH_4$ in DME gave the amine **8**, which was in turn coupled with lipoic acid chloride in THF/H_2O to afford **9**. Amine **10**, which was prepared according to our previously described methodology,[12] was reacted with lipoic acid chloride in the presence of triethylamine to afford **11**, which was deprotected using $BF_3(SMe)_2$[13] to give amide **12**. The chromanyl-5-methylamide analogue **19** was synthesized from aldehyde **13**,[12] which was reduced to the benzylic alcohol **14** by $NaBH_3CN$ in acetic acid. Reduction using $NaBH_4$ did not give satisfactory yield. Alcohol **14** was converted to bromide **15**, using PBr_3 in CH_2Cl_2 and then to azide **16** using NaN_3. Reduction of **16** with PPh_3 in THF/H_2O,[14] gave the corresponding benzylamine **17**, which was coupled with *N*-hydroxysuccinimide-activated lipoic acid, to afford amide **18** and upon demethylation the analogue **19**.

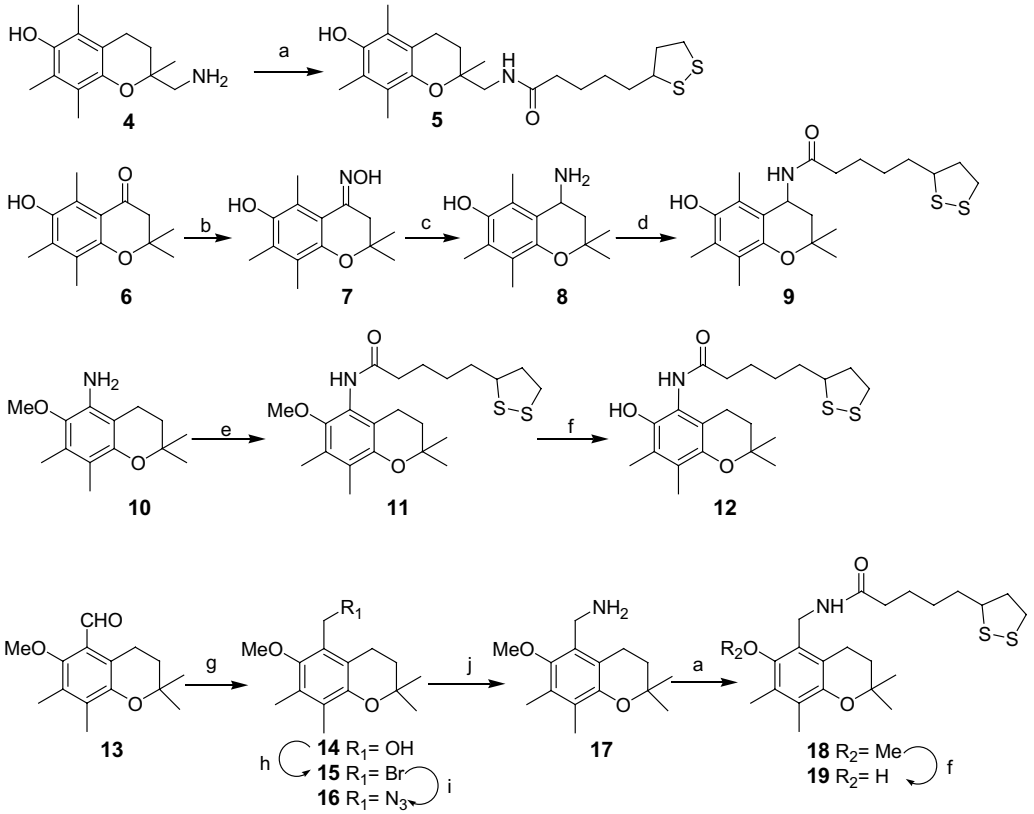
3. Results and discussion

The activity of the new analogues against in vitro peroxidation of rat hepatic microsomal membrane lipids[15–17] is expressed as the concentration of compound that inhibited lipid peroxidation by 50% after 45 min of incubation (IC_{50} values) and is shown in Table 1. All the final compounds are stronger inhibitors than trolox. Amides at position 2, compounds **3a**, **3b**, and **5**, are the most active in this assay.

The new molecules were evaluated for their activity against reperfusion arrhythmias, on isolated heart preparations using the Krebs perfused Langerdorff model[18] (Table 2). Arrhythmia scores were calculated for the first 10 min of reperfusion. The arrhythmic score of controls was 13.7 ± 3.6 and was mainly due to premature beats. Among the compounds tested, **3a**, **3b**, **5**, **12**, and **19** almost totally suppressed reperfusion arrhythmias while compound **9** and trolox/lipoic acid mixture reduced the arrhythmia score by 63.5% and 53%, respectively. It should also be noted that the observed arrhythmias in the presence of the new compounds were due to premature beats and were not accompanied by ventricular fibrillations.



Scheme 1. Reagents and conditions: (a) diethylenetriamine or 1-(2-aminoethyl)piperazine; (b) *N*-hydroxysuccinimide-activated lipoic acid; (c) lipoic acid chloride, $NaHCO_3$, THF, H_2O.



Scheme 2. Reagents and conditions: (a) *N*-hydroxysuccinimide-activated lipoic acid; (b) $NH_2OH{\cdot}HCl$, pyridine; (c) $TiCl_4$, $NaBH_4$, DME; (d) lipoic acid chloride, $NaHCO_3$, THF, H_2O; (e) lipoic acid chloride, Et_3N, THF, (f) $BF_3{\cdot}S(Me)_2$, CH_2Cl_2; (g) $NaBH_3CN$, AcOH, acetonitrile; (h) PBr_3, CH_2Cl_2; (i) NaN_3, DMF; (j) PPh_3, THF, H_2O.

Table 1. Inhibition of in vitro peroxidation (LP) of rat hepatic microsomal membrane lipids

Compound	LP IC_{50} (μM)
Lipoic	>1000
Trolox	24.8 ± 1.0
3a	1.50 ± 0.08
3b	0.95 ± 0.05
5	0.38 ± 0.03
9	8.40 ± 0.20
12	14.0 ± 0.4
19	3.9 ± 0.3

Results are expressed as mean ± SEM, $n = 3$.

Table 2. Antiarrhythmic and antioxidant activity in hearts perfused in vitro

Compound, 1 μM	Premature beats, %	MDA
None	13.7 ± 3.6	230 ± 32
3a	0.53 ± 0.2[a]	121 ± 14[a]
3b	0.95 ± 0.05[a]	154 ± 23[b]
5	0.85 ± 0.15[a]	162 ± 8[b]
9	5.2 ± 0.05[b]	192 ± 17
12	0.95 ± 0.05[a]	184 ± 7
19	2.1 ± 0.7[a]	112± 16[a]
Lipoic acid + trolox	6.45 ± 1.3[b]	165 ± 33

Antiarrhythmic activity is presented as incidence of premature beats: % of total heart beats during the first 10 min of reperfusion, for each treatment. Antioxidant activity is expressed as malondialdehyde content (nmol/g wet tissue), at the end of reperfusion.

[a] $p < 0.01$ versus control.

[b] $p < 0.05$ versus control; $n = 3$–4.

In general, we observe a correlation between the activity against lipid peroxidation and the activity of the compounds against reperfusion arrhythmias.

The antioxidant capacity of the new analogues in isolated hearts is expressed as malondialdehyde (MDA) content at the end of reperfusion.[19] As shown in Table 2, the MDA content in hearts perfused in the absence of test compounds is 230 ± 30 nmol/g of tissue. MDA content in hearts nonsubjected to ischemia/reperfusion is 147 ± 24. All new compounds were tested at a concentration of 1 μM. The analogues **9** and **12** were unable to reduce the total lipid peroxidation level of the myocardium while, analogues **3a** and **19** induced a statistically significant decrease of MDA content ($p < 0.01$). Compounds **3b** and **5** showed a moderate activity against lipid peroxidation. When the isolated hearts were perfused simultaneously with trolox and lipoic acid, at a concentration of 1 μM, the MDA content was reduced by 43% ($p < 0.05$). However, we cannot deduce any clear trend between MDA reduction and suppression of reperfusion arrhythmias for the compounds tested. It is possible that our compounds affect pathways of ischemia–reperfusion injury, which are not reflected on the MDA produced.

Trolox–lipoic acid diamides **3a** and **3b**, having triamines as spacers, are strong inhibitors of the peroxidation of

rat hepatic microsomal membrane lipids as those with diamines, reported previously by our group,[10] but they exhibit better cardioprotective activity.

Among the analogues **5**, **9**, **12**, and **19** in which lipoic acid is attached to the chroman skeleton at position 2, 4, and 5, respectively, through an amide bond, compound **5**, is the strongest inhibitor of in vitro lipid peroxidation and is equipotent with **3a** or **3b** in reducing reperfusion arrhythmias and MDA content. The constrained benzylic analogue **9** is less potent than the benzylic analogue **19** against peroxidation of microsomal membrane lipids, against reperfusion arrhythmias as well as in reducing the MDA content. Compounds **12** and **19**, which bear the amide functionality at position 5 are equipotent against reperfusion arrhythmias but benzylic amide **19** is better antioxidant than **12**.

4. Conclusions

In conclusion, we have synthesized a series of new benzopyran analogues of lipoic acid, which are more effective in suppressing reperfusion arrhythmias than the combination of trolox and lipoic acid, with 2 and 5 substituted chromans possessing the better cardioprotective activity. Their effect against reperfusion arrhythmias is related to their strong antioxidant activity against lipid peroxidation. These compounds probably protect phospholipid components of the myocardium, maintaining selective permeability of cell membranes and thus avoiding the breakdown of transmembrane ionic gradients and development of life threatening ventricular arrhythmias and/or fibrillations.

5. Experimental-chemistry

NMR spectra were recorded on a Bruker AC 300 spectrometer operating at 300 MHz for ^{1}H and 75.43 MHz for ^{13}C. ^{1}H NMR spectra are reported in units of δ with $CHCl_3$ resonance at 7.26 ppm used as the chemical shift reference. ^{13}C NMR spectra are reported in units of δ relative to $CDCl_3$ at 77.00 ppm. Silica gel plates (Merck F254) were used for thin layer chromatography. Chromatographic purification was performed with silica gel (200–400 mesh). Elemental analyses were carried out on a Perkin–Elmer CHN analyzer. Trolox and lipoic acid are racemic.

5.1. Preparation of *N*-hydroxysuccinimide-activated trolox (1)

To a mixture of trolox (500 mg, 2.0 mmol) and *N*-hydroxysuccinimide (NHS) (230.2 mg, 2.0 mmol), in 8 mL anhydrous 1,4-dioxane, *N*,*N*′-dicyclohexyl carbodiimide (DCC) (412 mg, 2.0 mmol) was added at 0 °C. After stirring at ambient temperature overnight, the mixture was cooled at 10 °C and the precipitate was filtered. The filtrate was evaporated in vacuo to yield 712.0 mg of **1**, which was used further without purification.

5.2. *N*-[2-[(2-Aminoethyl)amino]ethyl]-6-hydroxy-2,5,7,8-tetramethyl-2-chromanecarboxamide (2a)

To a solution of diethylenetriamine (445.9 mg, 4.3 mmol) in 10 mL CH_2Cl_2, was added dropwise a solution of **1** (150 mg, 0.43 mmol) in 5 mL CH_2Cl_2, at 0 °C and the mixture was stirred at ambient temperature for 18 h. CH_2Cl_2 (30 mL) and saturated aqueous Na_2CO_3 were then added. The organic layer was washed with 5% solution of NaCl, dried over Na_2SO_4, and the solvent was evaporated in vacuo. The crude product was purified by column chromatography (CH_2Cl_2–CH_3OH, 9:1). Yield: 130.5 mg, 91%, yellow oil. ^{1}H NMR δ 6.76 (br s, 1H, –CON*H*–), 3.09–3.01 (m, 2H), 2.55–2.37 (m, 9H), 2.10 (s, 6H, Ar–CH_3), 2.02 (s, 3H), 1.81–1.74 (m, 1H), 1.46 (s, 3H, –CH_3).

5.3. 6-Hydroxy-2,5,7,8-tetramethyl-*N*-(2-piperazinoethyl)-2-chromanecarboxamide (2b)

Compound **4** was prepared according to the procedure described above from trolox-*N*-hydroxysuccinimide (100 mg, 0.29 mmol) and 1-(2-aminoethyl)piperazine (372 mg, 2.9 mmol). The crude product was purified by column chromatography (CH_2Cl_2–CH_3OH, 8:2). Yield: 80 mg, 76.4%, yellow oil. ^{1}H NMR δ 7.01 (br s, 1H), 3.40–3.15 (m, 2H), 2.80–2.20 (m, 13H), 2.16 (s, 3H), 2.15 (s, 3H), 2.05 (s, 3H), 1.90–1.80 (m, 1H), 1.51 (s, 3H).

5.4. *N*-{2-[(2-{[5-(1,2-Dithiolan-3-yl)pentanoyl]-amino}ethyl)amino]ethyl}-6-hydroxy-2,5,7,8-tetramethyl-2-chromanecarboxamide (3a)

To a solution of aminoamide **2a** (100 mg, 0.3 mmol) in 10 mL CH_2Cl_2 were added 96 mg (0.3 mmol) of *N*-hydroxysuccinimide-activated lipoic acid in 5 mL CH_2Cl_2 at 0 °C. After stirring at ambient temperature for 24 h, 30 mL CH_2Cl_2 and saturated aqueous Na_2CO_3 were added. The organic layer was washed with saturated aqueous NaCl, dried with Na_2SO_4, and the solvent was evaporated in vacuo. The crude product was purified by column chromatography (CH_2Cl_2–MeOH, 9:1). Yield: 51 mg, 31%, yellow oil. ^{1}H NMR δ 6.59 (br s, 1H), 5.92 (br s, 1H), 3.58–3.46 (m, 2H), 3.20–3.01 (m, 6H), 2.65–2.48 (m, 8H), 2.18 (s, 6H), 2.08 (s, 3H), 2.18–2.10 (m, 3H), 1.93–1.42 (m, 9H), 1.54 (s, 3H). ^{13}C NMR δ 174.7, 173.3, 145.6, 144.4, 122.9, 121.7, 120.5, 117.9, 78.4, 56.3, 48.4, 40.2, 38.4, 36.3, 34.6, 29.6, 28.9, 25.2, 24.8, 22.3, 21.0, 12.2, 11.9, 11.5. Anal Calcd for $C_{26}H_{41}N_3O_4S_2$: C, 59.62; H, 7.89; N, 8.02. Found: C, 59.87; H, 8.12; N, 8.00.

5.5. *N*-(2-{4[5-(1,2-Dithiolan-3-yl)pentanoyl]piperazino}-ethyl)-6-hydroxy-2,5,7,8-tetramethyl-2-chromanecarboxamide (3b)

To a mixture of THF (3 mL) and H_2O (2 mL) were added 110 mg (0.3 mmol) of aminoamide **2b**, 135 mg (0.6 mmol) lipoic acid chloride, and 100 mg (1.2 mmol) of $NaHCO_3$. After stirring at ambient temperature for 2 h, H_2O was added and the mixture was extracted with ethyl acetate. The organic layer was washed with satu-

rated aqueous NaCl, dried with Na_2SO_4, and the solvent was evaporated in vacuo. The crude product was purified by column chromatography (CH_2Cl_2–MeOH, 9:1). Yield: 89.0 mg, 44.0%, white solid, mp 82–84 °C. ^{1}H NMR δ 6.93 (br s, 1H), 3.65–3.50 (m, 1H), 3.50–3.00 (m, 8H), 2.70–1.50 (m, 20H), 2.17 (s, 3H), 2.14 (s, 3H), 2.06 (s, 3H), 1.57 (s, 3H). ^{13}C NMR δ 174.5, 173.4, 145.7, 144.2, 122.2, 121.6, 119.9, 117.7, 78.0, 56.3, 52.8, 52.3, 45.4, 41.4, 40.2, 38.4, 35.4, 34.9, 29.5, 29.0, 24.9, 24.9, 24.7, 20.5, 12.4, 12.3, 11.5. Anal. Calcd for $C_{28}H_{43}N_3O_4S_2$: C, 61.17; H, 7.88; N, 7.64. Found: C, 61.05; H, 7.98; N, 8.03.

5.6. 5-(1,2-Dithiolan-3-yl)-*N*-[(6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydro-2*H*-chromen-2-yl)methyl]pentanamide (5)

4 (170 mg, 0.73 mmol), prepared by slight modification of the method described in the literature[11] and *N*-hydroxysuccinimide-activated lipoic acid (233 mg, 0.73 mmol) were treated as described above for the synthesis of compound **3a**. The crude product was purified by column chromatography (pet. ether–acetone, 7:3). Yield: 302.1 mg, 98.0%, yellow foam. ^{1}H NMR δ 5.80 (br s, 1H), 4.53 (s, 1H), 3.54–3.37 (m, 3H, –C*H*–S, –CH_2–NH), 3.15–2.95 (m, 2H, CH_2–S–S), 2.73–2.50 (m, 2H), 2.48–2.10 (m, 3H), 2.15 (s, 3H, Ar–CH_3), 2.10 (s, 6H, 2Ar–CH_3), 1.98–1.30 (m, 9H), 1.15 (s, 3H, –CH_3). ^{13}C NMR δ 172.9, 145.2, 144.3, 122.4, 121.4, 118.8, 117.1, 74.3, 56.4, 47.3, 40.2, 38.4, 36.6, 34.6, 28.9, 28.8, 25.4, 21.4, 20.3, 12.2, 11.9, 11.3. Anal Calcd for $C_{22}H_{33}NO_3S_2$: C, 62.37; H, 7.85; N, 3.31. Found: C, 62.61; H, 7.84; N, 3.16.

5.7. 6-Hydroxy-2,2,5,7,8-pentamethyl-2,3-dihydro-4*H*-chromen-4-one (6)

To a solution of 2,4,5-trimethyl-3,6-dihydroxyacetophenone (2.9 g, 15 mmol) in 15 mL absolute ethanol was added acetone (1 mL, 15 mmol), pyrrolidine (4 mL, 45 mmol), and powdered 3 Å molecular sieves (2.5 g). The resulting mixture was heated at 50–60 °C overnight. The reaction mixture was poured to ice, 10 N HCl was added, and the mixture was extracted with ether. The organic layer was extracted with saturated aqueous NaCl and was dried with anhydrous Na_2SO_4. The solvent was evaporated in vacuo and the crude product was purified by flash column chromatography (pet. ether–ethyl acetate, 8:2). ^{1}H NMR δ 11.90 (s, 1H), 2.65 (s, 2H), 2.16 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 1.41 (s, 6H); ^{13}C NMR δ 198.2, 156.9, 154.2, 147.6, 115.3, 114.5, 104.9, 77.9, 47.9, 26.7, 16.9, 11.2, 10.5.

5.8. 6-Hydroxy-2,2,5,7,8-pentamethyl-2,3-dihydro-4*H*-chromen-4-one oxime (7)

To a solution of **6** (210 mg, 0.9 mmol) in dry pyridine (5 mL) was added hydroxylamine hydrochloride (890 mg, 12.8 mmol) and the resulting mixture was stirred at 70 °C overnight. After cooling the reaction mixture to room temperature the pyridine was evaporated in vacuo and the residue was taken up with ethyl acetate. The organic layer was extracted with water and saturated aqueous NaCl and was dried with anhydrous Na_2SO_4. The solvent was evaporated in vacuo and the crude product was used without further purification for the next step. Yield: 0.224 g, 100%. ^{1}H NMR δ 10.97 (s, 1H), 7.50 (br s, 1H), 2.88 (s, 2H), 2.18 (s, 3H), 2.14 (s, 3H), 2.07 (s, 3H), 1.37 (s, 6H); ^{13}C NMR δ 155.7, 152.8, 140.1, 124.5, 115.3, 109.8, 100.8, 74.2, 33.7, 26.9, 16.5, 11.5, 11.3.

5.9. 4-Amino-2,2,5,7,8-pentamethyl-6-chromanol (8)

To a solution of $TiCl_4$ (0.17 mL, 1.53 mmol) in 1,2-dimethoxyethane (2 mL) at 0 °C was added $NaBH_4$ (116 mg, 3.06 mmol). The mixture was stirred at 0 °C for 10 min and subsequently a solution of **7** (125 mg, 0.51 mmol) in 2 mL 1,2-dimethoxyethane was added dropwise. The mixture was stirred at ambient temperature overnight. The reaction mixture was cooled to 0 °C and water was added. The mixture became basic with the addition of 28% aqueous ammonia and was extracted with CH_2Cl_2. The organic layer was extracted with saturated aqueous NaCl and was dried with anhydrous Na_2SO_4 and the solvent was evaporated in vacuo. The product was used as such for the next step. Yield: 74 mg, 63%. ^{1}H NMR δ 4.16–4.10 (m, 1H), 2.14 (s, 3H), 2.11 (s, 3H), 2.04 (s, 3H), 2.22–1.99 (m, 2H), 1.38 (s, 3H), 1.19 (s, 3H).

5.10. 5-(1,2-Dithiolan-3-yl)-*N*-(6-hydroxy-2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-chromen-4-yl)pentanamide (9)

Compound **8** (30 mg, 0.13 mmol) was treated as described for the preparation of **3b**. The product was purified by flash column chromatography (pet. ether–acetone, 7:3). Yield: 25 mg, 46.3%, yellow oil. ^{1}H NMR δ 7.55–7.51 (m, 1H), 5.94–5.92 (m, 1H), 5.25–5.21 (m, 1H), 3.58–3.52 (m, 1H), 3.14–3.10 (m, 2H), 2.45–2.42 (m, 1H), 2.20–2.15 (m, 2H), 2.14 (s, 3H), 2.12 (s, 3H), 2.05 (s, 3H), 1.92–1.42 (m, 9H), 1.30 (s, 3H), 1.25 (s, 3H). Anal Calcd for $C_{22}H_{33}NO_3S_2$: C, 62.37; H, 7.85; N, 3.31. Found: C, 62.08; H, 7.73; N, 3.47.

5.11. 5-(1,2-Dithiolan-3-yl)-*N*-(6-methoxy-2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-chromen-5-yl)pentanamide (11)

To a solution of 6-methoxy-2,2,7,8-tetramethyl-3,4-dihydro-2*H*-chromen-5-ylamine **10** (60 mg, 0.26 mmol) in 5 mL THF were added lipoic acid chloride (117 mg, 0.52 mmol) and 0.4 mL triethylamine at 0 °C. After been stirred at ambient temperature for 12 h the mixture was poured into H_2O and extracted with AcOEt. The organic layer was successively washed with saturated aqueous $NaHCO_3$, saturated aqueous NaCl and dried. The product was purified by flash column chromatography (pet. ether–acetone, 8:2). Yield: 100 mg, 90%, yellow oil. ^{1}H NMR δ 7.07 (br s, 1H), 3.59 (s, 3H), 3.59-3.50 (m, 1H), 3.20–3.05 (m, 2H), 2.65–2.53 (m, 2H), 2.20–2.35 (m, 3H), 2.15 (s, 3H), 2.07 (s, 3H), 1.94–1.89 (m, 1H), 1.80–1.68 (m, 6H), 1.60–1.50 (m, 2H), 1.30 (s, 6H).

5.12. 5-(1,2-Dithiolan-3-yl)-*N*-(6-hydroxy-2,2,5,7,8-pentamethyl-3,4-dihydro-2*H*-chromen-5-yl)pentanamide (12)

To a solution of 80 mg (0.18 mmol) **11** in 8 mL CH_2Cl_2 were added 0.19 mL (1.8 mmol) $BF_3{\cdot}S(Me)_2$ at 0 °C. After stirring at ambient temperature for 12 h, the solvent was evaporated under argon and the residue was taken up in ethyl acetate and H_2O. The organic layer was washed with saturated aqueous NaCl, dried, and the solvent was evaporated. The residue was purified by column chromatography (ethyl acetate–pet. ether, 1:1). Yield: 64 mg, 86.5%, yellow oil. ^{1}H NMR δ 9.80 (br s, 1H), 7.15 (br s, 1H), 3.60–3.50 (m, 1H), 3.15–3.00 (m, 2H), 2.90–2.35 (m, 5H), 2.25 (s, 3H), 2.10 (s, 3H), 2.00–1.56 (m, 9H), 1.35 (s, 6H). Anal Calcd for $C_{21}H_{31}NO_3S_2$: C, 61.58; H, 7.63; N, 3.42. Found: C, 61.61; H, 7.73; N, 3.64.

5.13. (6-Methoxy-2,2,7,8-tetramethyl-3,4-dihydro-2*H*-chromen-5-yl)-methanol (14)

To a solution of 600 mg (2.42 mmol) of aldehyde **13** in 11 mL of acetic acid and 3 mL of acetonitrile at 0 °C, was added 122 mg, (1.94 mmol) sodium cyanoborohydride. After being stirred at ambient temperature for 2 h the mixture was poured into ice, neutralized by adding 2 N NaOH, and extracted with ethyl acetate. The organic layer was washed with saturated aqueous NaCl, dried, and the solvent was evaporated in vacuo. The product was used as such for the next step. Yield: 604 mg, 100%. ^{1}H NMR: δ 4.68 (s, 2H), 3.69 (s, 3H), 2.81 (t, J = 6.6 Hz, 2H), 2.19 (s, 3H), 2.12 (s, 3H), 1.77 (t, J = 6.6 Hz, 2H), 1.30 (s, 6H).

5.14. 5-(Bromomethyl)-2,2,7,8-tetramethyl-3,4-dihydro-2*H*-chromen-6-yl methyl ether (15)

To a solution of alcohol **14** (290 mg, 1.16 mmol) in 6 mL CH_2Cl_2 at 0 °C, was added PBr_3 (0.11 mL, 1.16 mmol) and the mixture was stirred at 0 °C for 30 min and at ambient temperature for 90 min. The reaction mixture was then poured into ice and extracted with CH_2Cl_2. The organic layer was washed with saturated aqueous NaCl, dried with anhydrous Na_2SO_4, and the solvent was evaporated in vacuo. The product was used as such for the next step. Yield: 313 mg, 86%. ^{1}H NMR δ 4.65 (s, 2H), 3.80 (s, 3H), 2.80 (t, J = 6.6 Hz, 2H), 2.18 (s, 3H), 2.10 (s, 3H), 1.82 (t, J = 6.6 Hz, 2H), 1.31 (s. 6H).

5.15. 5-(Azidomethyl)-2,2,7,8-tetramethyl-3,4-dihydro-2*H*-chromen-6-yl methyl ether (16)

A mixture of 200 mg (0.64 mmol) of **15** and 415 mg (6.4 mmol) sodium azide in 6 mL DMF was stirred at 40 °C for 12 h. The mixture was then diluted with ethyl acetate, washed with saturated aqueous NaCl, dried, and evaporated. Yield: 175 mg, 100%. ^{1}H NMR: δ 4.42 (s, 2H), 3.70 (s, 3H), 2.75 (t, J = 6.6 Hz, 2H), 2.20 (s, 3H), 2.11 (s, 3H), 1.80 (t, J = 6.6 Hz, 2H), 1.31 (s. 6H).

5.16. (6-Methoxy-2,2,7,8-tetramethyl-3,4-dihydro-2*H*-chromen-5-yl)-methanamine (17)

To a solution of 175 mg (0.64 mmol) of **16** in 6 mL THF and 0.25 mL H_2O was added 183.5 mg (0.70 mmol) triphenylphosphine and the mixture was stirred at rt for 16 h. The solvents were then evaporated and the residue was purified by column chromatography (ethyl acetate–pet. ether, 9:1 and then MeOH). Yield: 130 mg, 82.3%. ^{1}H NMR: δ 3.81 (s, 2H), 3.69 (s, 3H), 2.78 (t, J = 6.7 Hz, 2H), 2.18 (s, 3H), 2.09 (s, 3H), 1.79 (t, J = 6.7 Hz, 2H), 1.56 (br s, 2H), 1.31 (s. 6H).

5.17. 5-(1,2-Dithiolan-3-yl)-*N*-[(6-methoxy-2,2,7,8-tetramethyl-3,4-dihydro-2*H*-chromen-5-yl)methyl]pentanamide (18)

Prepared from 120 mg (0.48 mmol) of **17** and *N*-hydroxysuccinimide-activated lipoic acid according to the procedure described above for **3a**. Yield: 201 mg, 96.6%, yellow oil. ^{1}H NMR: δ 5.89 (t, J = 4.8 Hz, 1H), 4.37 (d, J = 4.8 Hz, 2H), 3.61 (s, 3H), 3.48–3.43 (m, 1H), 3.11–3.00 (m, 2H), 2.71 (t, J = 6.6 Hz, 2H), 2.45–2.30 (m, 1H), 2.15–2.10 (m, 2H), 2.12 (s, 3H), 2.03 (s, 3H), 1.85–1.30 (m, 9H), 1.22 (s, 6H). ^{13}C NMR: δ 172.2, 150.1, 148.3, 128.1, 125.8, 125.7, 117.4, 73.1, 60.3, 56.3, 40.1, 38.4, 36.3, 34.6, 32.6, 28.8, 26.8, 25.3, 20.9, 20.1, 12.6, 12.0.

5.18. 5-(1,2-Dithiolan-3-yl)-*N*-[(6-hydroxy-2,2,7,8-tetramethyl-3,4-dihydro-2*H*-chromen-5-yl)methyl]pentanamide (19)

Prepared from 80 mg (0.18 mmol) of protected amide **18**. Yield: 60 mg, 78.9%, yellowish foam. ^{1}H NMR: δ 6.46 (t, J = 6.7 Hz, 1H), 4.33 (d, J = 6.7 Hz, 2H), 3.54–3.46 (m, 1H), 3.18–3.01 (m, 2H), 2.66 (t, J = 6.6 Hz, 2H), 2.43–2.35 (m, 1H), 2.25–2.10 (m, 2H), 2.08 (s, 3H), 2.02 (s, 3H), 1.87–1.30 (m, 9H), 1.22 (s, 6H). ^{13}C NMR: δ 175.0, 146.7, 145.0, 126.5, 124.8, 119.2, 115.5, 72.4, 56.3, 40.1, 38.4, 35.9, 34.4, 32.9, 28.7, 26.7, 25.0, 20.7, 12.5, 12.0. Anal Calcd for $C_{22}H_{33}NO_3S_2$: C, 62.37; H, 7.85; N, 3.31. Found: C, 62.49; H, 8.13; N, 3.07.

6. In vitro lipid peroxidation

Hepatic microsomal fraction from untreated female Fischer-344 rats (180–220 g) was prepared as described earlier.[15–17] Various concentrations (1 mM–0.025 μM) of the test compounds dissolved in dimethylsulfoxide (DMSO) were tested. Lipid peroxidation was assessed spectrophotometrically (535 against 600 nm) by the determination of the TBA reactive material.[17] DMSO, was tested and found not to interfere with the assay. Each experiment was performed at least in duplicate.

7. Animals and heart preparations

Male Wistar rats weighing about 300–350 g were housed under controlled light (12L:12D) and temperature with free access to food and water in compliance with the prescriptions for the care and use of laboratory animals.

Rats were anesthetized with pentobarbital (30–40 mg per animal). After intravenous administration of heparin, the chests were opened the hearts were rapidly excised and mounted on a nonrecirculating Langendorff perfusion apparatus. Retrogade perfusion was established at a pressure of 90 cm H_2O with an oxygenated normothermic Krebs–Hensleit bicarbonate (KHB) buffer (25 mmol L^{-1} $NaHCO_3$, 118 mmol L^{-1} NaCl, 2.5 mmol L^{-1} $CaCl_2$, 4.7 mmol L^{-1} KCl, 1.4 mmol L^{-1} $MgSO_4$, 1.2 mmol L^{-1} KH_2PO_4, pH 7.2 at 25 °C) supplemented with 11 mmol L^{-1} glucose and equilibrated with 95% O_2/5% CO_2. The temperature of the hearts and perfusates was maintained at 37 °C by the use of a water-jacketed apparatus. All hearts were equilibrated for 20 min under these conditions. At the end of the equilibration period, hearts were made ischemic for 15 min by perfusing them with the ischemic KHB (KHB with Tris–HCl 10 mM instead of glucose and equilibrated with N_2 before use) followed by 60 min of reperfusion. The antioxidants were present during reperfusion at a final concentration of 1 μM.

8. Evaluation of antiarrhythmic activity

Electrocardiograms were recorded during equilibration, ischemia, and reperfusion. Arrhythmias were scored according to the Lambeth Convention Guidelines.[18] Arrhythmia scores (AS) were calculated for the first 10 min of reperfusion comprising mainly premature beats.

9. Evaluation of antioxidant activity

At the end of the perfusions, hearts were 'freeze-clamped' between aluminum tongs, cooled in liquid N_2 and after the removal of the atria, the ventricles were pulverized under liquid N_2 and powders were stored at −80 °C. A portion of the tissue powder was analyzed for malondialdehyde (MDA) content by using the thiobarbituric acid assay.[19] To prevent auto-oxidation of the samples, homogenization was carried out at 4 °C in nitrogen equilibrated solution in the presence of 0.04% butylated hydroxytoluene, 1.6% ethanol. The values were expressed as nanomoles of TBA reactive substances (MDA equivalent) per gram of tissue. 1,1,3,3-Tetraethoxypropane (0, 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0 nmol) served as external standard. Results are expressed as mean ± SEM. Differences between groups were assessed by Student's unpaired and ANOVA *t*-tests and considered significant when $p < 0.05$.

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