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Bifunctional Agents for Reperfusion Arrhythmias: Novel Hybrid Vitamin E/Class I Antiarrhythmics

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Abstract—We have synthesized a series of hybrid compounds combining the pharmacophoric redox moieties of vitamin E and key features responsible for the antiarrhythmic properties of the class I antiarrhythmics procainamide and lidocaine. Procainamide analogue (**2a**) and lidocaine analogues (**14a**) and (**14b**) are very strong inhibitors of lipid peroxidation. All analogues tested at 100 or 30 μM enhanced the post ischemic recovery without inducing ventricular fibrillations while there was no evidence in our experiments for drug-induced pro-arrhythmia. In addition, they induced a widening of the QRS intervals. Our data suggest that the efficacy of the new compounds in preventing reperfusion arrhythmias could be attributed to their combined effects involving inhibition of free radical mediated damage coupled with antiarrhythmic properties.

Introduction

Coronary artery occlusion results in reduced blood flow that can produce myocardial cell injury and necrosis leading to diminished and consequently fatal cardiac function. These acute myocardial infarctions remain the leading disease in developed countries. In general, treatment of acute myocardial ischemia involves the use of either thrombolytic agents or percutaneous transluminal coronary balloon angioplasty (PTCA), which effectively restore blood flow to the myocardium and reduce overall mortality. 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] It is, thus, postulated 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 fibrillation.

A variety of antioxidant therapeutic approaches against myocardial reperfusion injury have been developed, principal among which is the administration of antioxidant enzymes or alternatively the use of redox vitamins. A number of studies using superoxide dismutase and/or catalase in experimental myocardial ischemia/reperfusion (I/R) models showed great disparity in results, thus, leading to considerable skepticism about the therapeutic potential of antioxidant enzymes for acute myocardial infarction in humans.[2] On the other hand, experimental findings support the hypothesis that lipid peroxidation inhibitors such as vitamin E protect the myocardiun from I/R injury.[3] However, other studies failed to verify a protective effect of antioxidant vitamins.[4] Concerning vitamin E, its limited efficacy may be attributed to its hydrophobic highly lipophilic properties making it only slowly available to cardiomyocytes, the principal target of oxidative injury.[5]

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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.[5–7]

Relatively little is known about the effects of antiarrhythmic drugs on reperfusion arrhythmias. Controlled trials suggested the effectiveness of routine lidocaine prophylaxis in preventing ventricular fibrillation due to acute myocardial infarction.[8,9] However, the use of antiarrhythmic drugs in suppressing reperfusion arrhythmias and preventing sudden cardiac death has demonstrated limited success,[10–12] principally due to the associated increased risk of proarrhythmias and their lack of selectivity for ion channels.

An attractive approach toward the development of new molecules against I/R damage involves the design and synthesis of bifunctional agents acting as *antiarrhythmic antioxidants*. These molecules would be expected to preferentially segregate in the membrane and produce their antiarrhythmic effects and simultaneously assist in the protection of membrane lipids by scavenging free radicals. In this communication, we set out to explore the therapeutic potential of the above concept. The principal features of our design are as follows:

1. Vitamin E is chosen as the prototype reactive oxygen species scavenger. Since the extremely lipid soluble character of tocopherol limits its efficacy in emergency reperfusion therapy, we preserved in our design only the essential molecular features required for antioxidant activity and eliminated some of the hydrophobic moieties.
2. Lidocaine and procainamide are chosen as prototype antiarrhythmic drugs. Thus, our new bifunctional molecules encompass the required 'antiarrhythmic' molecular features of these two molecules.

In our target compounds, the 6-hydroxy benzopyran ring of vitamin E and the diethylamino amide moiety of class I antiarrhythmics, procainamide and lidocaine, were combined in a single molecular entity (Fig. 1).

To achieve favorable partitioning within the myocardial membranes, some of the hydrophobic moieties of the parent compound were curtailed in order to moderate the lipophilic nature of the hybrids. To explore the optimal balance in lipophilicity, analogues in which the phytyl group of Vitamin E was replaced by an alkyl chain of one, six or twelve carbon atoms were synthesized.

At this early stage of exploration, we chose not to address stereochemical considerations associated with the C2 asymmetric center especially since existing information indicates that this is not a factor in the antioxidant activity of Vitamin E analogues.[13]

Chemistry

Compound **2a** was prepared from trolox and *N,N*-diethylethylenediamine in the presence of 1,1′-carbonyldiimidazole (CDI)[13] (Scheme 1). Compounds **2b**,**c** possessing longer alkyl chains at position 2 of the chroman skeleton were synthesized from the corresponding 6-hydroxychroman-2-carbonitriles obtained using methods previously described.[14,15] Alkaline hydrolysis of the nitriles afforded the corresponding acids **1b**,**c** which were subsequently reacted with *N,N*-diethylethylenediamine in the presence of CDI to give the desired 2-alkyl-6-hydroxychroman-2-carboxamides **2b**,**c** (Scheme 1).

Condensation of 2,3-dimethylhydroquinone with the appropriate allylic alcohol **3b**,**c**, in the presence of HCOOH, gave the 2-alkyl-6-hydroxychromans **4b**,**c**[16] (Scheme 2). The allylic alcohols **3b**,**c** were synthesized from methyl vinyl ketone, by 1,2-addition of hexyl lithium or dodecyl lithium, generated by metal halogen exchange with Li from 1-bromohexane and 1-bromo-

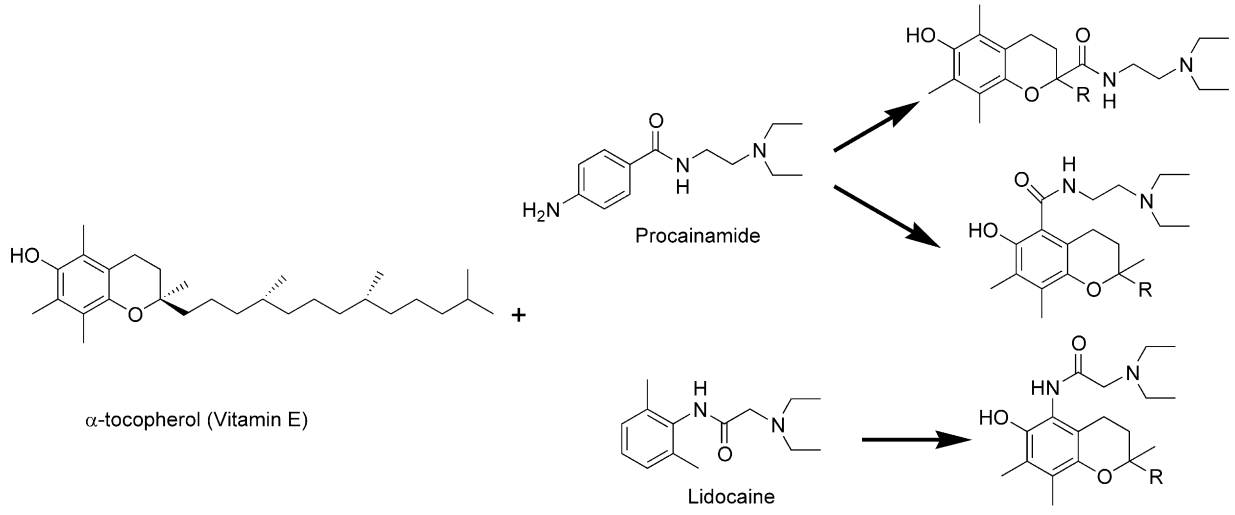
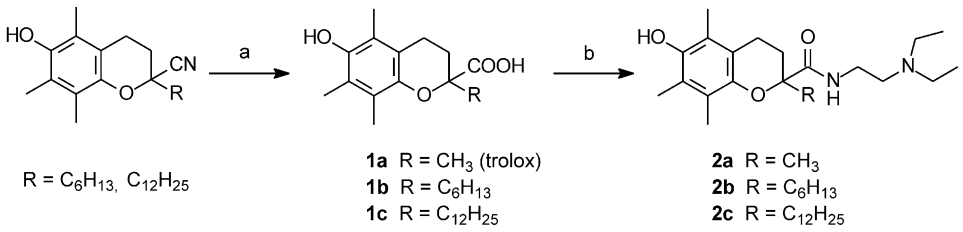
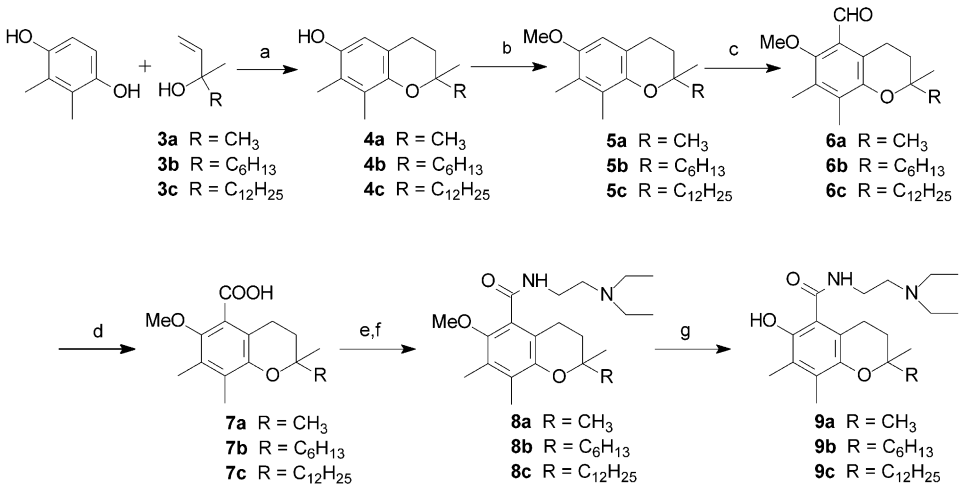


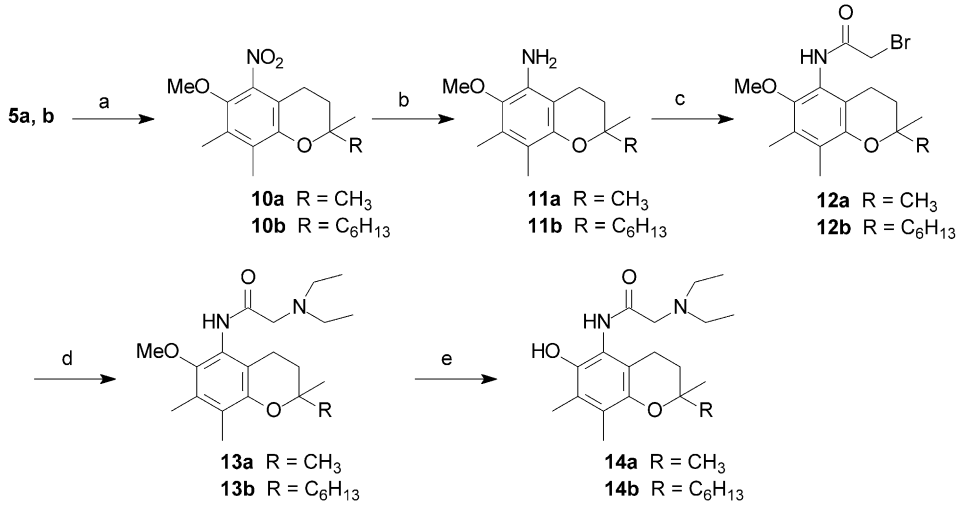
Figure 1. Bifunctional antioxidant/antiarrhythmic hybrids.



Scheme 1. (a) 85% KOH, ethyleneglycol; (b) CDI, *N,N*-diethyl-ethylenediamine, THF.



Scheme 2. (a) HCOOH, THF; (b) $(MeO)_2SO_2$, K_2CO_3, acetone; (c) Cl_2CHOCH_3, $TiCl_4$, CH_2Cl_2; (d) $NaClO_2$, $NaH_2PO_4H_2O$, H_2O_2; (e) $SOCl_2$, benzene; (f) *N,N*-diethyl-ethylenediamine, THF; (g) EtSNa, DMF.



Scheme 3. (a) Acetylnitrate, Ac_2O/AcOH; (b) $NaBH_4$, CuCl, EtOH; (c) bromoacetyl chloride, Et_3N, THF; (d) diethylamine, toluene; (e) EtSNa, DMF.

dodecane, respectively. The 6-hydroxy-2,2,7,8-tetramethylchroman (**4a**) was prepared according to the procedure described in the literature.[17] Protection of the 6-hydroxyl group using dimethylsulfate in the presence of K_2CO_3, afforded in quantitative yields, the corresponding compounds **5a–c** which were further elaborated to analogues **9a–c** and **14a,b**.

Formylation[18] of **5a–c**, using α,α-chloromethyl methyl ether and $TiCl_4$ gave, in quantitative yields, the 5-chromanaldehydes **6a–c** which were in turn oxidized to the corresponding acids **7a–c** by treatment with sodium chlorite-hydrogen peroxide.[19] Acids **7a–c** were then converted to the acid chlorides which upon reaction with *N,N*-diethylethylenediamine afforded the corresponding amides **8a–c**. Deprotection of the methoxy group using sodium ethanethiolate in DMF, gave the desired 6-hydroxy amides **9a–c** (Scheme 2).

Nitration of **5a–c** by acetyl nitrate[20] followed by reduction[21] of the resulting 5-nitrochromans **10a,b** using $NaBH_4$ and CuCl in EtOH, afforded the corresponding

amines **11a**,**b** which were acylated by bromoacetylchloride to give bromoamides **12a**,**b**. Reaction of **12a**,**b** with diethylamine[22] produced aminoamides **13a**,**b**, respectively, which were in turn treated with sodium ethanethiolate in DMF to afford amides **14a**,**b** (Scheme 3).

Results and Discussion

Antioxidant effects

The new analogues were tested against lipid peroxidation induced in rat liver microsomes by ferrous ions and ascorbate. Table 1 shows the effect of the new compounds on lipid peroxidation at a concentration of 10 μM. Compounds **14a** and **14b** were also tested at 5 μM concentrations since they exhibited 100% inhibition at 10 μM. Compound **9b** showed 87.4% inhibition at 50 μM, while compounds **2c** and **9c** inhibited lipid peroxidation by 90% at 500 μM (data not shown).

The differences in antioxidant activity appear to be related to the position of the amide substituent, and the length of the alkyl side chain. Thus, for the amidoamino group of procainamide analogues **2a–c**, **9a–c**, attachment on C2 (compounds **2a–c**) seems to be more favorable for this action than C5 (compounds **9a–c**), possibly due to electronic reasons, since this group exerts the opposite inductive effect compared to that of the methyl group (compounds **2a–c**) which stabilizes the phenoxyradical. Alternatively, the expected hydrogen bond between the 6-hydroxy and the 5-carbonyl groups may prevent the formation of a stable phenoxyradical. This hypothesis is supported by the observation that the lidocaine analogues, **14a–b**, in which the amidoamino functionality is connected to the chroman sceleton, at C5, through the amido nitrogen, exhibit strong antioxidant activity. In this case the 6-hydroxy group may serve as a hydrogen bond acceptor in its interaction with the NH group and, thus, favor the formation of a phenoxyl radical. This is congruent with results from theoretical calculations of substituent effects on the O–H bond strength in phenolic antioxidants related to vitamin E.[23] Among the compounds presented here the lidocaine analogue **14a** is the most active with a 72.4% inhibition of lipid peroxidation at 5 μM. Furthermore, antioxidant activity decreases significantly with increasing alkyl chain length. Thus, compounds **2a**, **9a** and **14a** bearing a methyl group at C2 of the chroman ring are the most active analogues within each series, while compounds **2c** and **9c** with a 12 carbon side chain are inactive at 10 μM. The observed decreased antioxidant activity of these more lipophilic compounds, may be attributed to a less effective access to their antioxidant target, the cell membranes.

Table 1. Inhibitory effects on lipid peroxidation

Compd	% Inhibition at 10 μM	Compd	% Inhibition at 10 μM
2a	95.2	**14a**	100
2b	7.7	**14b**	100
2c	0		% inhibition at 5 μM
9a	38.4	**14a**	72.4
9b	0	**14b**	17.4
9c	0	Trolox	16.3

Antiarrhythmic effects

Evaluation of the antiarrhythmic activity of the new compounds was carried out on isolated rat heart preparations using the non-recirculating Langendorff mode. The compounds were present during the last 5 min of ischemia and during reperfusion at final concentrations of 100, 30 or 5 μM. The results on arrhythmia score represent premature beats during the first 10 min of reperfusion in the presence of the compounds under study (Table 2). We found that at 5 μM all compounds were inactive (data not shown). In contrast, all analogues tested at 100 or 30 μM enhanced the post ischemic recovery without inducing ventricular fibrillations. Additionally, there was no evidence in our experiments for drug-induced pro-arrhytmia even though the concentration of KCl used, could allow for the development of pro-arrhythmic activity.

Among the procainamide analogues **2a–c**, and **9a–c** only the C2-aminoamide **2a** produced a decrease in premature beats (5±2 and 7±3.5 at 100 and 30 μM,

Table 2. Anti-arrhythmic activity: drug effects are presented as incidence of premature beats (% of total heart beats during exactly the same period (10 min) for each treatment in the first 15 min of reperfusion, ±SD)

Compd	30 μM	100 μM
None	12±4	12±4
2a	7±3.5	5±2*
2b	9±3	8±4
2c	11±2	11±3
9a	12±5	10±3
9b	13±2	12±3
9c	10±2.5	8±4
13a	9±3	7±3.5
14a	8±2.1	7±2
14b	6±3*	6±2.5*
Procainamide	6±2.5	5±3*
Lidocaine	5±3	4±3*

*$p < 0.05$ versus control, $n = 4$–6.

Table 3. QRS intervals in milliseconds±SD during reperfusion: Changes in ECG intervals were measured approximately 2 min after the beginning of reperfusion

Compd	QRS intervals[a] 30 μM	QRS intervals[a] 100 μM
None	35±5	35±5
2a	54±8	60±6
2b	45±5	49±7
2c	46±5	50±5
9a	42±3	50±8
9b	44±5	45±6
9c	46±3	54±6
13a	49±5	61±13
14a	63±7*	71±6*
14b	66±6*	78±8*
Procainamide	58±5*	66±10*
Lidocaine	57±4*	70±9*

*$p < 0.05$ versus control (none).

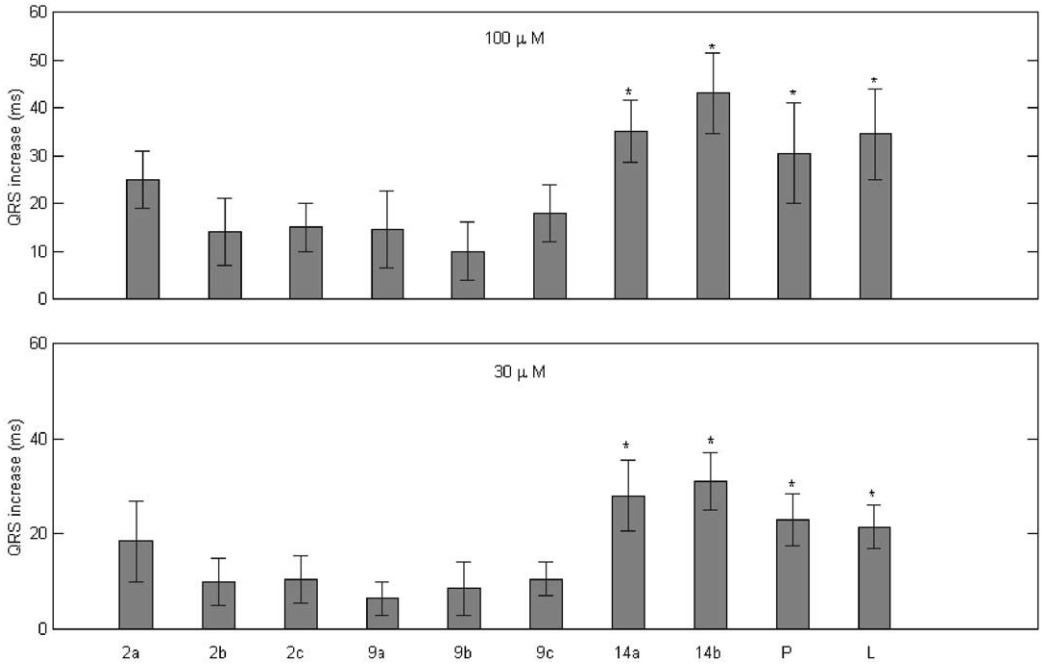


Figure 2. Effects of new compounds on ECG in hearts perfused in vitro. Changes in ECG intervals were measured approximately 2 min after starting reperfusion. Pre-compound values for QRS interval duration in hearts perfused with normal buffer were 36±4 and 34±7 ms, during equilibration and reperfusion respectively. P, procainamide; L, lidocaine; * QRS interval duration in the presence of these compounds was statistically different from control values ($p < 0.05$, $n = 4$–6).

respectively) comparable to procainamide the control drug (5±3 and 6±2.5 at 100 and 30 μM, respectively). Both lidocaine related derivatives **14a** and **14b** caused a decrease in the occurrence of premature beats (**14a:** 7±2 and 8±2.1 at 100 and 30 μM, respectively; **14b:** 6±2.5 and 6±3 at 100 and 30 μM, respectively) only lightly less than lidocaine (4±3 and 5±3 at 100 and 30 μM, respectively). The new compounds induced a widening of the QRS intervals (Table 3, Fig. 2). Analogue **2a** caused an increase in QRS intervals similar to that of procainamide (**2a:** 60±6, 54±8 ms at 100 and 30 μM, respectively; procainamide: 66±10, 58±5 ms at 100 and 30 μM, respectively) while, **14a**,**b** to that of lidocaine at both 30 and 100 μM (**14a**: 70±6, 63±7 ms at 100 and 30 μM, respectively; **14b**: 78±8, 66±6 ms at 100 and 30 μM, respectively; lidocaine: 70±9, 57±4 ms at 100 and 30 μM, respectively). Our results suggest that the above compounds maintain the characteristics of Class I antiarrhythmics at least at the same level as the control drugs procainamide and lidocaine, indicating a moderate to potent Na^+ channel blocking action.

Our data also indicate that compounds exhibiting most potent antioxidant activity also produce pronounced antiarrrhythmic effects although **14b**, is a weaker antioxidant than **14a** is more effective in reducing premature beats and prolonging QRS intervals at both 30 and 100 μM. Additionally, the 6-methoxy aminoamide **13a** was shown to reduce premature beats to the same extent as the corresponding 6-hydroxy derivative **14a**. However, **13a** has its phenolic group masked and is expected to be a much weaker inhibitor of lipid peroxidation than **14a** its phenolic counterpart. Overall, these results suggest that the efficacy of the new compounds in preventing reperfusion arrhythmias has a dual origin and may not be attributed solely to their abilities to inhibit free radical mediated damage but also to their anti-arrhythmic properties.

Conclusions

The concept of antioxidant anti-arrhythmics as a strategy for the treatment of reperfusion arrhythmias was exemplified by the synthesis of hybrids of vitamin E and class I anti-arrhythmics. Compounds **2a**, **14a** and **14b** are very strong inhibitors of lipid peroxidation and are also as effective in inhibiting reperfusion arrhythmias, as the standard anti-arrhythmics procainamide and lidocaine. However, the antioxidant properties of the different compounds do not closely parallel their antiarrhythmic effects **14b** is a weaker antioxidant than **14a** but induces more pronounced anti-arrhythmic effects. The same is the case with **13a** which exhibits anti-arrhythmic activity with only minimal expected antioxidant properties.

Our present study proves the validity of our bifunctional strategy approach for myocardial reperfusion injury therapy. However, remaining to be determined is the proper mix between antioxidant and anti-arrhythmic properties for obtaining optimal suppression of reperfusion arrhythmias with minimal undesirable effects. The novel compounds described here were shown to maintain both class I antiarrhythmic activity and radical scavenging capacity and may, thus, provide superior long term protection of the ischemic myocardium.

Experimental

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 relative to internal $CHCl_3$ at 7.24 ppm. ^{13}C NMR shifts are expressed in units of δ relative to $CDCl_3$ at 77.0 ppm. ^{13}C NMR spectra were proton noise decoupled. All NMR spectra were recorded in $CDCl_3$. Silica gel plates (Merck F254) were used for thin layer chromatography. Chromatographic purification was performed with silica gel (200–400 mesh).

General procedure for the preparation of 3,4-dihydro-6-hydroxy-2-alkyl-5,7,8-trimethyl-2*H*-1-benzopyran-2-carboxylic acids

3,4-Dihydro-2-hexyl-6-hydroxy-5,7,8-trimethyl-2*H*-1-benzopyran-2-carbonitrile or 3,4-dihydro-2-dodecyl-6-hydroxy-5,7,8-trimethyl-2*H*-1-benzopyran-2-carbonitrile (4.65 mmol) were added to a solution of 85% KOH in ethyleneglycol (20 mL) and the mixture was heated at 150 °C overnight. The mixture was then cooled, poured into water and extracted with ether. The ether extracts were discarded and the aqueous solution was acidified with 2 N HCl and extracted with AcOEt. Evaporation of the solvent gave the desired carboxylic acids **1b** and **1c**, respectively.

3,4-Dihydro-2-hexyl-6-hydroxy-5,7,8-trimethyl-2*H*-1-benzopyran-2-carboxylic acid (1b). Yield 1.18 g (79.1%). ^{1}H NMR δ 4.42 (s, 1H), 2.64–2.57 (m, 2H), 2.33–2.28 (m, 1H), 2.17 (s, 6H), 2.11 (s, 3H), 1.9–1.85 (m, 1H), 1.5–1.25 (m, 10H), 0.86 (t, J = 6.3 Hz, 3H); ^{13}C NMR δ: 176.9, 145.7, 144.2, 81.0, 37.0, 31.8, 29.4, 28.8, 23.4, 22.6, 20.4, 14.1, 12.3, 11.9, 11.3; Anal: calcd for $C_{19}H_{28}O_4$: C, 71.22; H, 8.81; found: C, 70.95; H, 9.20.

3,4-Dihydro-2-dodecyl-6-hydroxy-5,7,8-trimethyl-2*H*-1-benzopyran-2-carboxylic acid (1c). Yield 1.39 g (73.8%). ^{1}H NMR δ 2.62–2.59 (m, 2H), 2.40–2.20 (m, 1H), 2.18 (s, 1H), 2.18 (s, 3H), 2.09 (s, 3H), 1.91–1.86 (m, 1H), 1.5–1.24 (m, 22H), 0.88 (t, J = 6.3 Hz, 3H).

General procedure for the preparation of *N* (2-alkyl-3,4-dihydro-6-hydroxy-5,7,8-trimethyl-2*H*-1-benzopyran-2-carbonyl)-*N*′,*N*′-diethyl-ethylenediamines

1,1′-Carbonyldiimidazole (537 mg, 3.3 mmol) was added to a solution of chroman carboxylic acid (3 mmol) in 15 mL THF. After stirring for 1 h at ambient temperature, a solution of *N,N*-diethyl-ethylenediamine (0.26 mL, 3.2 mmol) in 12 mL THF was added dropwise. The mixture was allowed to stir for 24 h at ambient temperature. The solvent was then evaporated in vacuo and the residue was taken up with ethyl acetate and the organic layer was washed with brine and dried (Na_2SO_4). The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH 9:1).

***N*-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-carbonyl)-*N*′,*N*′-diethyl-ethylenediamine (2a).** Yield 0.73 g (70%), white foam. ^{1}H NMR δ 7.13 (bs, 1H), 3.28–3.18 (m, 2H), 2.59–2.31 (m, 9H), 2.18 (s, 3H), 2.17 (s, 3H), 2.16 (s, 3H), 1.89–1.85 (m, 1H), 1.50 (s, 3H), 0.90 (t, J = 7.2 Hz, 6H); ^{13}C NMR δ 174.3, 145.5, 144.3, 121.9, 121.6, 119.1, 117.6, 78.1, 51.3, 46.4, 36.5, 29.4, 24.4, 20.5, 12.2, 11.9, 11.7, 11.3; Anal: calcd for $C_{20}H_{32}N_2O_3$: C, 68.93; H, 9.26; N, 8.04; found: C, 68.84; H, 9.50; N, 8.34.

***N*-(3,4-Dihydro-2-hexyl-6-hydroxy-5,7,8-trimethyl-2*H*-1-benzopyran-2-carbonyl) - *N*′,*N*′ - diethyl - ethylenediamine (2b).** Yield 0.308 g (65.5%), white foam. ^{1}H NMR (δ): 7.10 (bs, 1H), 4.50 (bs, 1H), 3.37–3.19 (m, 2H), 2.61–2.40 (m, 6H), 2.29–2.20 (m, 1H), 2.16 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H), 1.95–1.69 (m, 5H), 1.44–1.23 (m, 8H), 0.95 (t, J = 7 Hz, 6H), 0.84 (t, J = 6.3 Hz, 3H); ^{13}C NMR (δ): 175.3, 146.2, 143.1, 122.5, 121.9, 119.6, 117.2, 79.4, 51.2, 46.9, 37.8, 36.3, 31.4, 29.1, 28.4, 23.1, 22.3, 20.0, 13.8, 12.1, 11.8, 11.7, 11.2. Anal. calcd for $C_{25}H_{42}N_2O_3 \cdot 0.5H_2O$: C, 70.22; H, 10.13; N, 6.55; found: C, 70.28; H, 10.10; N, 6.28.

***N*-(3,4-Dihydro-2-dodecyl-6-hydroxy-5,7,8-trimethyl-2*H*-1-benzopyran-2-carbonyl)-*N*′,*N*′-diethyl-ethylenediamine (2c).** Yield 0.323 g (58.4%), white foam. ^{1}H NMR δ 7.15 (bs, 1H), 4.40 (bs, 1H), 3.36–3.24 (m, 2H), 2.61–2.43 (m, 6H), 2.29–2.22 (m, 1H), 2.17 (s, 3H), 2.13 (s, 3H), 2.05 (s, 3H), 2.01–1.73 (m 5H), 1.44–1.23 (m, 20H), 1.01 (t, J = 7.1 Hz, 6H), 0.87 (t, J = 6.1 Hz, 3H); ^{13}C NMR δ 175.7, 146.1, 143.3, 122.4, 121.9, 119.6, 117,5, 79.7, 50.0, 47.6, 47.4, 36.6, 34.8, 31.8, 29.5, 29.3, 29.2, 28.4, 23.3, 22.5, 20.1, 13.9, 12.4, 11.9, 11.5 Anal. calcd for $C_{31}H_{54}N_2O_3$: C, 74.06; H, 10.83; N, 5.57; found: C, 73.72; H, 10.86; N, 5.97.

General procedure for the preparation of allylic alcohols 3b,c

To Li metal (25.04 mmol, 0.174 g) was added 1 mL of a solution of the appropriate alkylbromide (12.52 mmol) in 8.5 mL diethyl ether and the mixture was stirred until Li became shiny. Subsequently the mixture was cooled to 0 °C and the remaining solution of the alkylbromide was added dropwise over 1 h. The cooling bath was removed and the mixture was stirred at room temperature for 2 h, the unreacted Li was removed and the reaction mixture was cooled to 0 °C and a solution of methyl vinyl ketone (12.52 mmol, 1.95 mL) in diethyl ether (8 mL) was added dropwise and the resulting mixture was stirred at 0 °C until completion of the reaction. The reaction was quenched by addition of saturated NH_4Cl and the aqueous layer was extracted with ethyl acetate. The organic layer was extracted with saturated NaCl solution, was dried with Na_2SO_4 and evaporated in vacuo. The residue was purified by flash column chromatography using petroleum ether 40–60 °C/ethyl acetate 9:1.

3-Methyl-non-1-en-3-ol (3b). 0.60 g (32%), pale yellow liquid. ^{1}H NMR δ 5.85–5.80 (dd, J = 17.2, 10.8 Hz, 1H), 5.13 (d, J = 17.2 Hz, 1H), 4.96 (d, J = 10.8 Hz, 1H), 1.84 (s, 1H), 1.45–1.43 (m, 2H), 1.30–1.18 (m, 7H), 0.82 (t, J = 6.4 Hz, 3H).

3-Methyl-pentadec-1-en-3-ol (3c). Yield 0.74 g (27%), pale yellow liquid. ^{1}H NMR δ 5.90 (dd, J = 17.2, 10.8 Hz, 1H), 5.17 (d, J = 17.2 Hz, 1H), 5.01 (d, J = 10.8 Hz, 1H), 1.84 (s, 1H), 1.51–1.47 (m, 2H), 1.26–1.01 (m, 23H), 0.84 (t, J = 6.4 Hz, 3H); ^{13}C NMR δ 145.3, 111.5, 73.3, 42.4, 31.9, 30.1, 29.7, 29.4, 27.7, 23.9, 22.7, 14.1.

General procedure for the preparation of 2-alkyl-6-hydroxy-2,7,8-trimethylchromans

A solution of 2,3-dimethylhydroquinone (3 g, 21.7 mmol) in 28 mL HCOOH and 4 mL THF, was heated to reflux and the appropriate allyl alcohol (14.8 mmol) was added in three portions. The mixture was refluxed for 3 h, poured into ice and worked up as previously described.[16,17]

The desired 6-hydroxychroman was purified by flash column chromatography (petroleum ether/AcOEt 9:1).

2-Hexyl-6-hydroxy-2,7,8-trimethylchroman (4b). Yield 2.04 g (50%), waxy solid. ^{1}H NMR δ 6.34 (s, 1H), 4.54 (s, 1H), 2.65 (t, J = 6.6 Hz, 2H), 2.12 (s, 3H), 2.10 (s, 3H), 1.70–1.52 (m, 4H), 1.50–1.25 (m, 8H), 1.23 (s, 3H), 0.88 (t, J = 6.5 Hz, 3H).

2-Dodecyl-6-hydroxy-2,7,8-trimethylchroman (4c). Yield: 3.41 g (64%), waxy solid. ^{1}H NMR δ 6.35 (s, 1H), 2.70 (t, J = 6.6 Hz, 2H), 2.09 (s, 3H), 2.02 (s, 3H), 1.68–1.38 (m, 4H), 1.34–1.08 (m, 20H), 1.21 (s, 3H), 0.87 (t, J = 6.5 Hz, 3H). Anal. calcd for $C_{24}H_{40}O_2$: C, 79.94; H, 11.18; found: C, 79.99; H, 11.38.

General procedure for the preparation of 2-alkyl-6-methoxy-2,7,8-trimethylchromans

To a solution of 6-hydroxychroman (7.3 mmol) in 60 mL acetone K_2CO_3 (3.5 g, 25.4 mmol) were added and the mixture was heated to 50 °C. Then dimethylsulfate (2.8 mL, 29.2 mmol) was added and the mixture was refluxed for 8 h. The reaction was quenched by saturated NH_4Cl and extracted with ether. The crude compound was purified by flash column chromatography using petroleum ether/ether 9:1.

6-Methoxy-2,2,7,8-tetramethylchroman (5a). Yield 1.3 g (81.3%), waxy solid. ^{1}H NMR δ 6.43 (s, 1H), 3.76 (s, 3H), 2.74 (t, J = 6.8 Hz, 2H), 2.13 (s, 3H), 2.12 (s, 3H), 1.77 (t, J = 6.8 Hz, 2H), 1.31 (s, 6H). ^{13}C NMR δ 150.7, 145.8, 125.8, 124.2, 117.1, 108.8, 73.3, 56.1, 33.0, 26.9, 23.0, 11.8.

2-Hexyl-6-methoxy-2,7,8-trimethylchroman (5b). Yield 1.9 g (91.7%), waxy solid. ^{1}H NMR δ 6.41 (s, 1H), 3.74 (s, 3H), 2.71 (t, J = 6.5 Hz, 2H), 2.12 (s, 3H), 2.11 (s, 3H), 1.75–1.50 (m, 4H), 1.47–1.27 (m, 8H), 1.23 (s, 3H), 0.87 (t, J = 6.6 Hz, 3H). ^{13}C NMR δ 150.6, 146.0, 125.9, 124.2, 117.4, 108.5, 75.4, 56.1, 39.8, 31.8, 31.4, 29.8, 24.0, 23.6, 22.6, 14.1, 11.8.

2-Dodecyl-6-methoxy-2,7,8-trimethylchroman (5c). Yield 2.46 g (90%). ^{1}H NMR δ 6.41 (s, 1H), 3.74 (s, 3H), 2.70 (t, J = 6.5 Hz, 2H), 2.11 (s, 3H), 2.10 (s, 3H), 1.78–1.54 (m, 4H), 1.48–1.25 (m, 20H), 1.23 (s, 3H), 0.87 (t, J = 6.6 Hz, 3H). Anal. calcd for $C_{25}H_{42}O_2$: C, 80.16; H, 11.30; found: C, 79.81; H, 11.61.

General procedure for the preparation of 2-alkyl-3,4-dihydro-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyran-5-carboxaldehydes

To a solution of the appropriate 2-alkyl-3,4-dihydro-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyran (5.45 mmol) in 4 mL CH_2Cl_2 was added α,α-chloromethyl methyl ether (1.2 mL, 14.5 mmol). The mixture was cooled to 0 °C and a solution of $TiCl_4$ (0.8 mL, 7.8 mmol) in 4 mL CH_2Cl_2 was added drop wise. The resulting mixture was stirred at ambient temperature for 2 h, poured into ice, extracted with CH_2Cl_2 and the organic layer was washed with saturated aqueous $NaHCO_3$, brine and dried (Na_2SO_4).

3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-carboxaldehyde (6a). Yield 1.35 g (100%), gummy solid. ^{1}H NMR δ 10.49 (s, 1H), 3.76 (s, 3H), 3.09 (t, J = 6.5 Hz, 2H), 2.20 (s, 3H), 2.16 (s, 3H), 1.73 (t, J = 6.2 Hz, 2H), 1.29 (s, 6H). Anal. calcd for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12; found: C, 72.42; H, 8.28.

3,4-Dihydro-2-hexyl-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyran-5-carboxaldehyde (6b). Yield 1.56 g (98%), gummy solid. ^{1}H NMR (δ): 10.47 (s, 1H), 3.74 (s, 3H), 3.05 (t, J = 6.4 Hz, 2H), 2.19 (s, 3H), 2.15 (s, 3H), 1.8–1.6 (m, 2H), 1.55–1.51 (m, 2H), 1.36–1.25 (m, 8H), 1.22 (s, 3H), 0.85 (t, J = 6.6 Hz, 3H); ^{13}C NMR (δ): 193.0, 156.5, 148.0, 134.4, 128.8, 124.0, 120.1, 75.6, 63.5, 39.5, 31.8, 30.8, 29.8, 23.8, 23.5, 22.6, 21.1, 14.0, 13.0, 11.9.

3,4-Dihydro-2-dodecyl-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyran-5-carboxaldehyde (6c). Yield 2.19 g (100%), gummy solid. ^{1}H NMR δ 10.48 (s, 1H), 3.75 (s, 3H), 3.06 (t, J = 7 Hz, 2H), 2.20 (s, 3H), 2.16 (s, 3H), 1.76–1.55 (m, 2H), 1.54–1.49 (m, 2H), 1.40–1.25 (m, 20H), 1.23 (s, 3H), 0.86 (t, J = 6.7 Hz, 3H). Anal. calcd for $C_{26}H_{42}O_3$: C, 77.56; H, 10.51; found: C, 77.87; H, 10.82.

General procedure for the preparation of 2-alkyl-3,4-dihydro-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyran-5-carboxylic acids

A solution of $NaClO_2$ 80% (800 mg, 7 mmol) in 7 mL H_2O was added dropwise to a stirred mixture of the appropriate 2-alkyl-3,4-dihydro-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyrancarboxaldehyde (4 mmol), $NaH_2PO_4 \cdot H_2O$ (160 mg, 1.3 mmol), 0.5 mL H_2O_2 35% and 2 mL H_2O and 10 mL acetonitrile at 10 °C. The mixture was stirred at ambient temperature overnight. Subsequently, 50 mg of Na_2SO_3 were added to destroy HOCl and excess H_2O_2 and the aqueous phase was extracted with CH_2Cl_2. The organic phase was washed with 2 N NaOH. Acidification of the aqueous phase (concd HCl) and extraction with CH_2Cl_2 afforded the pure carboxylic acids.

3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-carboxylic acid 7a. Yield 0.760 g (72%), beige gummy solid. ^{1}H NMR δ 11.0 (bs, 1H), 3.77 (s, 3H),

2.93 (t, $J=6.7$ Hz, 2H), 2.19 (s, 3H), 2.13 (s, 3H), 1.76 (t, $J=6.7$ Hz, 2H), 1.31 (s, 6H). Anal. calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63; found: C, 67.97; H, 7.73.

3,4-Dihydro-2-hexyl-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyran-5-carboxylic acid (7b). Yield 1.24 g (93%), beige gummy solid. ^{1}H NMR δ 3.67 (s, 3H), 2.80–2.70 (m, 2H), 2.12 (s, 3H), 2.06 (s, 3H), 1.60–1.40 (m, 4H), 1.4–1.25 (m, 8H), 1.17 (s, 3H), 0.85 (t, $J=6.5$ Hz, 3H).

3,4-Dihydro-2-dodecyl-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyran-5-carboxylic acid (7c). Yield 1.60 g (96%), beige gummy solid. ^{1}H NMR δ 3.76 (s, 3H), 2.95 (t, $J=6.6$ Hz, 2H), 2.19 (s, 3H), 2.13 (s, 3H), 1.74–1.37 (m, 4H), 1.35–1.24 (m, 20H), 1.23 (s, 3H), 0.87 (t, $J=6.2$ Hz, 3H).

General procedure for the preparation of *N*-(2-alkyl-3,4-dihydro-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyran-5-carbonyl)-*N*′,*N*′-diethyl-ethylenediamines

To a solution of the appropriate 2-alkyl-3,4-dihydro-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyran-5-carboxylic acid (1.9 mmol) in 5 mL benzene was added $SOCl_2$ (0.6 mL, 7.5 mmol) and the mixture was refluxed for 2 h. The solvent and the excess thionyl chloride were removed in vacuo. The residue was then diluted with 7 mL THF, *N,N*-diethylethylenediamine (0.17 mL, 2 mmol) was added and the mixture was stirred overnight at ambient temperature. The solvent was then evaporated, the residue was taken up with AcOEt and the organic layer was washed with brine. The crude product was purified by flash column chromatography (CÇ$_2$Cl$_2$/ÌeÏÇ 95:5).

***N*-(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-carbonyl)-*N*′,*N*′-diethyl-ethylenediamine (8a).** Yield 0.51 g (74.5%), light brown viscous oil. ^{1}H NMR δ 6.67 (bs, 1H), 3.67 (s, 3H), 3.62–3.56 (m, 2H), 2.73 (t, $J=6.7$ Hz, 2H), 2.71–2.63 (m, 6H), 2.15 (s, 3H), 2.09 (s, 3H), 1.72 (t, $J=6.7$ Hz, 2H), 1.3 (s, 6H), 1.09 (t, $J=7.2$ Hz, 6H). Anal. calcd for $C_{21}H_{34}N_2O_3\cdot 0.5H_2O$: C, 67.89; H, 9.49; N, 7.54; found: C, 68.02; H, 9.54; N, 7.34.

***N*-(3,4-Dihydro-2-hexyl-6-methoxy 2,7,8-trimethyl-2*H*-1-benzopyran-5-carbonyl)-*N*′,*N*′-diethyl-ethylenediamine (8b).** Yield 0.68 g (82.5%), light brown viscous oil. ^{1}H NMR δ 6.80 (bs, 1H), 3.63 (s, 3H), 3.61–3.57 (m, 2H), 2.81 (t, $J=6$ Hz, 2H), 2.78–2.65 (m, 6H), 2.11 (s, 3H), 2.06 (s, 3H), 1.8–1.6 (m, 2H), 1.6–1.24 (m, 10H), 1.21 (s, 3H), 1.11 (t, $J=7.2$ Hz, 6H), 0.84 (t, $J=6.7$ Hz, 3H); ^{13}C NMR δ 168.3, 148.0, 147.5, 128.5, 127.4, 127.3, 116.1, 75.6, 62.1, 51.6, 46.9, 39.8, 36.4, 31.8, 30.8, 29.7, 24.1, 23.5, 22.5, 20.0, 14.0, 12.3, 12.1, 10.6. Anal. calcd for $C_{26}H_{44}N_2O_3\cdot 1.5H_2O$: C, 67.93; H, 10.30; N, 6.09; found; C, 67.84; H, 10.06; N, 6.43.

***N*-(3,4-dihydro-2-dodecyl-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyran-5-carbonyl)-*N*′,*N*′-diethyl-ethylenediamine (8c).** Yield 0.83 g (85%), light brown viscous oil. ^{1}H NMR δ 6.6 (bs, 1H), 3.67 (s, 3H), 3.63–3.54 (m, 2H), 2.77–2.6 (m, 8H), 2.15 (s, 3H), 2.09 (s, 3H), 1.75–1.65 (m, 2H), 1.60–1.24 (m, 22H), 1.23 (s, 3H), 1.06 (t, $J=7$ Hz, 6H), 0.84 (t, $J=6.2$ Hz, 3H). Anal. calcd for $C_{32}H_{56}N_2O_3$: C, 74.37; H, 10.92; N, 5.42; found: C, 73.96; H, 11.07; N, 5.67.

General procedure for the preparation of of *N*-(2-alkyl-3,4-dihydro-6-hydroxy-2,7,8-trimethyl-2*H*-1-benzopyran-5-carbonyl)-*N*′,*N*′-diethyl-ethylenediamines

To an ice-cooled slurry of NaH (240 mg, 10 mmol) in 5 mL DMF was added EtSH (0.9 mL, 12 mmol) and the mixture was stirred for 15 min at 0 °C. A solution of the appropriate *N*-(2-alkyl-3,4-dihydro-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyran-5-carbonyl)-*N*′,*N*′-diethyl-ethylenediamine (1.24 mmol) in 10 mL DMF was added and the mixture was stirred at 90 °C overnight. The mixture was then poured into H_2O and extracted with AcOEt. The organic layer was washed with brine, dried (Na_2SO_4) and the solvent was removed in vacuo. Purification by column chromatography (CH_2Cl_2/MeOH 95:5) gave the desired compounds.

***N*-(3,4-dihydro-6-hydroxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-carbonyl)-*N*′,*N*′-diethyl-ethylenediamine (9a).** Yield 0.42 g (97%), light brown waxy solid. ^{1}H NMR δ 6.80 (bs, 1H), 3.48 (bs, 2H), 2.82 (t, $J=6.5$ Hz, 2H), 2.64–2.52 (m, 6H), 2.12 (s, 3H), 2.09 (s, 3H), 1.68 (t, $J=6.5$ Hz, 2H), 1.28 (s, 6H), 1.0 (t, $J=7.1$ Hz, 6H); ^{13}C NMR δ 170.0, 149.6, 144.5, 130.0, 124.1, 115.2, 114.0, 72.7, 51.1, 46.1, 36.9, 32.0, 26.8, 22.4, 12.4, 11.9, 11.1. Anal. calcd for $C_{20}H_{32}N_2O_3\cdot H_2O$: C, 65.54; H, 9.35; N, 7.64; found: C, 65.88; H, 9.01; N, 7.74.

***N*-(3,4-Dihydro-2-hexyl-6-hydroxy-2,7,8-trimethyl-2*H*-1-benzopyran-5-carbonyl)-*N*′,*N*′-diethyl-ethylenediamine (9b).** Yield 0.48 g (93%), light brown waxy solid. ^{1}H NMR δ 6.74 (bs, 1H), 3.52 (bs, 2H), 2.84 (t, $J=6$ Hz, 2H), 2.66–2.56 (m, 6H), 2.16 (s, 3H), 2.12 (s, 3H), 1.80–1.60 (m, 2H), 1.60–1.28 (m, 10H), 1.25 (s, 3H), 1.01 (t, $J=7$ Hz, 6H), 0.87 (t, $J=6.2$ Hz, 3H); ^{13}C NMR δ 170.1, 150.1, 144.5, 130.2, 124.2, 114.6, 114.1, 74.8, 51.0, 46.1, 39.8, 36.9, 31.9, 31.3, 29.8, 24.1, 23.6, 22.6, 22.3, 14.1, 12.5, 11.9, 11.3. Anal. calcd for $C_{25}H_{42}N_2O_3\cdot 0.5H_2O$: C, 70.22; H, 10.13; N, 6.55; found: C, 69.92; H, 10.17; N, 6.18.

***N*-(3,4-dihydro-2-dodecyl-6-hydroxy-2,7,8-trimethyl-2*H*-1-benzopyran-5-carbonyl)-*N*′,*N*′-diethyl-ethylenediamine (9c).** Yield 0.60 g (97%), light brown waxy solid. ^{1}H NMR δ 7.1 (bs, 1H), 3.56 (bs, 2H), 2.88 (t, $J=6$ Hz, 2H), 2.69–2.60 (m, 6H), 2.16 (s, 3H), 2.12 (s, 3H), 1.75–1.56 (m, 4H), 1.25 (m, 20H), 1.23 (s, 3H), 1.03 (t, $J=7.1$ Hz, 6H), 0.87 (t, $J=6.3$ Hz, 3H); ^{13}C NMR δ 170.1, 149.9, 144.5, 130.1, 124.2, 114.8, 114.1, 74.7, 51.0, 46.1, 39.8, 36.7, 31.8, 31.3, 29.6, 29.3, 24.0, 23.6, 22.6, 22.2, 14.0, 12.4, 11.9, 10.9. Anal. calcd for $C_{31}H_{54}N_2O_3$: C, 74.06; H, 10.83; N, 5.57; found: C, 74.24; H, 11.09; N, 5.27.

General procedure for the preparation of 2-alkyl-3,4-dihydro-6-methoxy-5-nitro-2,7,8-trimethyl-2*H*-1-benzopyrans

Acetyl nitrate (1.8 mL) (prepared from 0.4 mL HNO_3 70% and 1.4 mL Ac_2O at 0 °C) were added to a solution of the appropriate 2-alkyl-3,4-dihydro-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyran (1.6 mmol) in 2 mL Ac_2O

and 2 mL AcOH at 0 °C. The mixture was stirred at 0 °C for 1 h and then poured into ice-water. The aqueous mixture was extracted with AcOEt and the organic layer was washed with saturated aqueous $NaHCO_3$, brine and dried. The desired compounds were purified by column chromatography (petroleum ether/AcOEt 9:1).

3,4-Dihydro-6-methoxy-5-nitro-2,2,7,8-tetramethyl-2*H*-1-benzopyran (10a). Yield 0.40 g (94.8%), yellow foam. ^{1}H NMR δ 3.75 (s, 3H), 2.65 (t, J = 6.8 Hz, 2H), 2.19 (s, 3H), 2.11 (s, 3H), 1.75 (t, J = 6.8 Hz, 2H), 1.32 (s, 6H) ^{13}C NMR δ 147.9, 143.5, 142.3, 130.0, 128.0, 110.29, 74.3, 62.7, 31.6, 26.9, 26.7, 18.4, 12.6, 12.2 Anal. calcd for $C_{14}H_{19}NO_4$: C, 63.38; H, 7.22; N, 5.28; found: C, 62.99; H, 7.17; N, 5.42.

3,4-dihydro-6-methoxy-5-nitro-2-hexyl-2,7,8-trimethyl-2*H*-1-benzopyran (10b). Yield 0.40 g (74.5%), yellow foam. ^{1}H NMR δ 3.75 (s, 3H), 2.63 (t, J = 6.7 Hz, 2H), 2.19 (s, 3H), 2.11(s, 3H), 1.76–1.36 (m, 4H), 1.36–1.28 (m, 11H), 1.25 (s, 3H), 0.85 (t, J = 6.9 Hz, 3H). Anal. calcd for $C_{19}H_{29}NO_4$: C, 68.03; H, 8.71; N, 4.18; found: C, 67.85; H, 8.73; N, 3.93.

General procedure for the preparation of 2-alkyl-5-amino-3,4-dihydro-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyrans

To a solution of 2-alkyl-3,4-dihydro-6-methoxy-5-nitro-2,7,8-trimethyl-2*H*-1-benzopyran (1.4 mmol) in 15 mL EtOH were sequentially added CuCl (620 mg, 6.3 mmol) and $NaBH_4$ (480 mg, 12.6 mmol) at 0 °C. The mixture was refluxed for 1 h, then cooled to ambient temperature, basified ($NaHCO_3$), filtered and washed with CH_2Cl_2. The filtrate was evaporated and the residue extracted with CH_2Cl_2 and washed with brine. The organic layer was dried and the solvent was removed in vacuo.

5-Amino-3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran (11a). Yield 0.27 g (82.3%), brown oil. ^{1}H NMR δ 3.69 (s, 3H), 3.58 (bs, 2H), 2.49 (t, J = 6.8 Hz, 2H), 2.18 (s, 3H), 2.05 (s, 3H), 1.82 (t, J = 6.8 Hz, 2H), 1.31 (s, 6H); ^{13}C NMR δ 148.0, 138.7, 134.6, 127.6, 114.3, 104.7, 72.8, 59.7, 32.4, 26.7, 18.5, 12.3, 11.1.

5-Amino-2-hexyl-3,4-dihydro-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyran (11b). Yield 0.39 g (91.6%), brown oil. ^{1}H NMR δ 3.67 (s, 3H), 3.55 (bs, 2H), 2.46 (t, J = 6.8 Hz, 2H), 2.15 (s, 3H), 2.03 (s, 3H), 1.90–1.65 (m, 2H), 1.55–1.50 (m, 2H), 1.50–1.27 (m, 8H), 1.22 (s, 3H), 0.87 (t, J = 6.6 Hz, 3H); ^{13}C NMR δ 147.8, 139.0, 134.5, 127.5, 114.4, 105.0, 74.8, 59.4, 39.6, 31.8, 30.8, 29.8, 23.8, 23.6, 22.6, 18.2, 14.1, 12.3, 11.1.

General procedure for the preparation of *N*-(2-alkyl-3,4-dihydro-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyran-5-yl)-bromoacetamides

To a solution of 2-alkyl-5-amino-3,4-dihydro-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyran (1.28 mmol) in 8 mL THF were added 1 mL bromoacetylchloride and triethylamine at 0 °C. After been stirred at ambient temperature for 1 h the mixture was poured into H_2O and extracted with AcOEt. The organic layer was washed with saturated aqueous $NaHCO_3$, brine and dried.

***N*-(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)-bromoacetamide (12a).** Yield 0.43 g (94.9%), brown viscous oil. ^{1}H NMR δ 7.88 (bs, 1H), 4.05 (s, 2H), 3.63 (s, 3H), 2.58 (t, J = 6.7 Hz, 2H), 2.17 (s, 3H), 2.09 (s, 3H), 1.72 (t, J = 6.7 Hz, 2H), 1.31 (s, 6H) ^{13}C NMR δ 164.4, 148.0, 145.5, 128.3, 125.2, 116.5, 73.7, 61.1, 32.3, 29.0, 26.9, 19.4, 12.4, 12.0.

***N*-(3,4-dihydro-2-hexyl-6-methoxy-2,7,8-trimethyl-2H-1-benzopyran-5-yl)-bromoacetamide (12b).** Yield 0.50 g (92%). ^{1}H NMR (δ): 7.90 (bs, 1H), 4.04 (s, 2H), 3.63 (s, 3H), 2.56 (t, J = 6.5 Hz, 2H), 2.17 (s, 3H), 2.09 (s, 3H), 1.80–1.65 (m, 2H), 1.65–1.50 (m, 2H), 1.48–1.27 (m, 8H), 1.23 (s, 3H), 0.86 (t, J = 6.7 Hz, 3H) ^{13}C NMR δ 164.4, 148.3, 145.7, 128.2, 125.2, 124.5, 116.8, 75.7, 61.0, 53.4, 40.0, 31.8, 30.7, 29.8, 29.0, 23.9, 23.6, 22.6, 19.0, 14.1, 12.4, 12.0.

General procedure for the preparation of *N*-(2-alkyl-3,4-dihydro-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyran-5-yl)-(diethylamino)acetamides. To a solution *N*-(2-alkyl-3,4-dihydro-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyran-yl)-bromoacetamide (1.18 mmol) in 7 mL toluene at 0 °C was added diethylamine (0.3 mL, 3 mmol). After stirring for 2 days at ambient temperature the mixture was extracted with 2 N HCl. The aqueous layer was made basic with 2 N NaOH and extracted with CH_2Cl_2. The organic layer was dried and evaporated.

***N*-(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)-(diethylamino) acetamide (13a).** Yield 0.29 g (72%), brown viscous oil. ^{1}H NMR δ 8.97 (bs, 1H), 3.59 (s, 3H), 3.19 (s, 2H), 2.66 (q, J = 7.1 Hz, 4H), 2.59 (t, J = 6.7 Hz, 2H), 2.16 (s, 3H), 2.08 (s, 3H), 1.71 (t, J = 6.7 Hz, 2H), 1.30 (s, 6H), 1.13 (t, J = 7.1 Hz, 6H); ^{13}C NMR δ 170.7, 148.3, 145.8, 128.0, 125.4, 124.3, 116.6, 73.5, 60.9, 57.7, 48.9, 32.5, 26.9, 19.6, 12.4, 12.3, 11.9.

***N*-(3,4-dihydro-2-hexyl-6-methoxy-2,7,8-trimethyl-2*H*-1-benzopyran-5-yl)-(diethylamino)acetamide (13b).** Yield 0.36 g (74.5%). ^{1}H NMR δ 8.96 (bs, 1H), 3.59 (s, 3H), 3.19 (s, 2H), 2.66 (q, J = 7.2 Hz, 4H), 2.57–2.50 (m, 2H), 2.16 (s, 3H), 2.07 (s, 3H), 1.80–1.56 (m, 4H), 1.50–1.27 (m, 8H), 1.23 (s, 3H), 1.12 (t, J = 7.1 Hz, 6H) 0.86 (t, J = 6.5 Hz, 3H); ^{13}C NMR δ 170.7, 149.0, 145.7, 128.0, 125.4, 124.3, 116.8, 75.5, 60.9, 57.6, 48.8, 40.0, 31.8, 30.8, 29.8, 23.9, 23.6, 22.6, 19.2, 14.1, 12.5, 12.4, 11.8.

General procedure for the preparation of (2-alkyl-3,4-dihydro-6-hydroxy-2,2,7,8-trimethyl-2*H*-1-benzopyran-5-yl)-(diethylamino)actamides

The deprotection of the methoxy group was carried out following the general procedure for **9a–c**. The amides were purified by column chromatography (CH_2Cl_2/MeOH 95:5).

***N*-(3,4-Dihydro-6-hydroxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)- (diethylamino)acetamide (14a).** Yield 0.19 g (81%), light brown waxy solid. ^{1}H NMR δ 9.5 (bs, 1H), 8.27 (bs, 1H), 3.23 (s, 2H), 2.69 (q, $J=7.2$ Hz, 4H), 2.54 (t, $J=6.8$ Hz, 2H), 2.20 (s, 3H), 2.09 (s, 3H), 1.79 (t, $J=7$ Hz, 2H), 1.23 (s, 6H), 1.09 (t, $J=7.2$ Hz, 6H); ^{13}C NMR δ 171.2, 145.1, 140.8, 126.2, 124.0, 120.4, 109.3, 72.5, 57.4, 48.6, 32.4, 26.5, 19.4, 12.4, 12.3, 11.8 Anal. calcd for $C_{19}H_{30}N_2O_3$: C, 68.23; H, 9.04; N, 8.38; found: C, 68.51; H, 9.11; N, 8.00.

***N*-(3,4-dihydro-2-hexyl-6-hydroxy-2,7,8-trimethyl-2*H*-1-benzopyran-5-yl)-(diethylamino)acetamide (14b).** Yield 0.12 g (62%), light brown waxy solid. ^{1}H NMR δ 9.5 (bs, 1H), 8.27 (bs, 1H), 3.24 (s, 2H), 2.69 (q, $J=7$ Hz, 4H), 2.53 (t, $J=6$ Hz, 2H), 2.20 (s, 3H), 2.09 (s, 3H), 1.80–1.65 (m, 2H), 1.60–1.26 (m, 10H), 1.21 (s, 3H), 1.09 (t, $J=7$ Hz, 6H), 0.86 (t, $J=6.5$ Hz, 3H); ^{13}C NMR δ 171.2, 145.0, 140.8, 126.2, 124.1, 120.4, 109.5, 73.7, 57.4, 48.6, 39.4, 31.8, 31.0, 29.7, 23.6, 23.5, 22.6, 19.1, 14.0, 12.4, 11.8. Anal. calcd for $C_{24}H_{40}N_2O_3$: C, 71.25; H, 9.97; N, 6.92; found: C, 71.32; H, 9.83; N, 6.55.

In vitro lipid peroxidation

Hepatic microsomal fraction from untreated female Fischer-344 rats (180–220 g) was prepared as described earlier.[24] The incubation mixture contained heat inactivated (90 °C for 90 s) hepatic microsomal fraction, corresponding to 2.5 mg protein/mL (final concentration) or 4 mM fatty acid residues,[25] ascorbic acid (0.2 mM) in Tris–HCl/KCl buffer (50 mM/150 mM, pH 7.4) and various concentrations (5 μM–1 mM) of the test compounds dissolved in dimethylsulphoxide (DMSO). The reaction was started by the addition of a freshly prepared $FeSO_4$ solution (10 μM) and the mixture was incubated at 37 °C for 45 min. Lipid peroxidation was assessed spectrophotometrically (535 against 600 nm) by the determination of the thiobarbituric acid (TBA) reactive material.[26] DMSO, was tested and found not to interfere with the assay. Each experiment was performed at least in duplicate.

Isolated heart preparation

The evaluation of the anti-arrhythmic activity of the compounds was carried out on isolated heart preparations using the non-recirculating Langendorff mode.[11] Adult male (250–300 g) Wistar rats were anesthetized with pentobarbitone (5 mg/100 g body weight) and heparinized intravenously (1000 IU). Hearts were excised and washed in ice cold modified Krebs-Henseleit (KHB) buffer of the following composition in mM: NaCl 118, KCl 4.7, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.4, $CaCl_2$ 2.5, Glucose 11. Hearts were mounted on a Langendorff apparatus and perfused at a pressure 80 cm H_2O with KHB equilibrated with 95% O_2/5% CO_2 for 20–30 min (coronary flow 9 mL/min). The temperature of the perfusate and the heart was maintained at 37 °C by the use of a water-jacketed apparatus. After the end of the equilibration period, the 'ischemic' KHB solution was applied for 15 min (this solution is a KH buffer in which the glucose is substituted by 10 mM Tris/HCl and equilibrated with N_2 before use). The hearts were then reperfused with normal KHB for 30 min. Drugs were present during the last 5 min of ischemia and during reperfusion at the final concentration of 5, 30 and 100 μM. Arrhythmias were scored according to the Lambeth Convention Guidelines.[27] Electrocardiograms were recorded during equilibration, ischemia and reperfusion. Results are expressed as mean ± SD. Differences between groups were assessed by Student's unpaired and ANOVA *t*-tests and considered significant when $p<0.05$.

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