Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 14 (2006) 6666–6678
Bioorganic & Medicinal Chemistry

Synthesis and biological evaluation of benzopyran analogues bearing class III antiarrhythmic pharmacophores

Maria Koufaki,[a,*] Christina Kiziridi,[a] Panagiota Papazafiri,[b] Athanasios Vassilopoulos,[b] Andràs Varró,[c] Zsolt Nagy,[c] Attila Farkas[c] and Alexandros Makriyannis[d]

[a]*Institute of Organic and Pharmaceutical Chemistry, National Hellenic Research Foundation, 48 Vas. Constantinou Avenue, 116 35 Athens, Greece*
[b]*Department of Animal and Human Physiology, School of Biology, University of Athens, Panepistimiopolis, 15784 Athens, Greece*
[c]*Department of Pharmacology and Pharmacotherapy University of Szeged, Szeged Dom ter 12 H-6720, Hungary*
[d]*Northeastern University, Center for Drug Discovery, 360 Huntington Avenue, 116 Mugar Hall, Boston, MA 02115, USA*

Received 13 March 2006; revised 25 May 2006; accepted 31 May 2006
Available online 19 June 2006

Abstract—We have synthesized a series of compounds combining the hydroxy-benzopyran ring of vitamin E with the methylsulfonylaminophenyl group of class III antiarrhythmic drugs, connected through tertiary amine moieties. Evaluation of the antiarrhythmic and antioxidant activity of the new compounds was carried out on isolated rat heart preparations using the non-recirculating Langendorff mode. The new analogues were present, at 10 μM concentration, during ischemia and reperfusion. Selected compounds were further studied by a conventional microelectrode method in order to get insight into their cellular mode of action. The most active compound, *N*-[4-[2-[[2-(3,4-dihydro-6-hydroxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)ethyl] methylamine]ethyl]phenyl]methanesulfonamide (**19a**), reduces premature beats, prolongs QT and QRS intervals during ischemia and reperfusion, and reduces MDA content, leading to a fast recovery of the heart. In addition, it exhibits moderate class III antiarrhythmic action.

1. Introduction

Arrhythmias account for nearly one quarter of all cardiovascular-related deaths. The majority of such deaths is caused by the degeneration of a normal cardiac rhythm into ventricular tachycardia (VT) followed by ventricular fibrillation (VF).[1] The shape of the action potential of the heart cells is strongly controlled by the correct interplay of ion channels.[2] The main ion channels contributing to the action potential are sodium, potassium, and calcium channels. Changes in the ionic mechanism responsible for the generation and the propagation of the normal action potential can cause abnormalities in the electrical activity of the heart.

Although cardiac rhythm disturbances may be the result of a variety of pathophysiological conditions, coronary artery disease which has resulted in prior ischemia is most important. Myocardial ischemia causes profound alterations in normal cardiac electrophysiology and cellular metabolism, precipitating ventricular arrhythmias or fibrillation.[3] 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, the reactive oxygen species (ROS), produced upon the readmission of oxygenated blood into the ischemic myocardium (reperfusion),[4] affect selective permeability of cell membranes, leading to the development of life-threatening ventricular arrhythmias and/or fibrillation.

Moreover, post-operative atrial fibrillation is a common complication after open heart surgery; it increases morbidity, hospital stay, and costs.[5] Pharmacologic strategies and regimens aimed at preventing post-operative atrial fibrillation are necessary to patients undergoing open heart operations. The prevalence of arrhythmia in the population is increasing as more people survive for longer with cardiovascular disease.[6] Since many patients experience a decrease in physical performance as

Keywords: Benzopyran analogue; Antiarrhythmic agents; Ischemia/reperfusion; Cellular electrophysiology.
* Corresponding author. Tel.: +30 210 7273818; fax: +30 210 7273831; e-mail: mkoufa@eie.gr

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.05.065

well as a diminished quality of life, there is still a need for antiarrhythmic drug therapy.

According to the classification of Vaughan Williams (1970), based on electrophysiological actions, the antiarrhythmic drugs can be defined by four classes:[7] class I consist of antiarrhythmic agents that block sodium channels, reducing the maximum increase rate of depolarization (V_{max}). Class II are β-blockers. Class III act through delaying repolarization of cardiac myocytes and thus cause a lengthening of APD (potassium-channel blockers). Class IV block calcium currents in cardiac tissue.

In the case of antiarrhythmic drugs the delicate balance between drug efficacy and unexpected adverse side effects is narrower than in any other class of therapeutic agents. Concerning class I antiarrhythmics, controlled trials suggested the effectiveness of routine lidocaine (class Ib) prophylaxis in preventing ventricular fibrillation due to acute myocardial infarction.[8,9] However, in 1980 CAST (Cardiac Arrhythmia Suppression Trial), with the drugs encainide and flecainide (class Ic), uncovered the inefficacy and even proarrhythmic risk of sodium channel blockers in post-infarction patients. To circumvent the problems with class I antiarrhythmics, pharmacological and clinical research shifted toward the class III agents.[10] Amiodarone (combining class I–IV properties),[11a,11b] d,l-sotalol (class II, III)[11c] and ambasilide (class II, III), azimilide (class I, III, IV) are complex class III compounds, while 'pure' class III agents are d-sotalol, dofetilide, ibutilide.

Sotalol, amiodarone, ibutilide, and dofetilide are moderately effective in patients with chronic atrial fibrillation. However, amiodarone appears to be most efficacious. Moreover, amiodarone and dofetilide are safe in patients who have had a myocardial infarction and those with heart failure. The safety of commercially available d,l-sotalol in these patients is poorly understood. Torsades de pointes is the most serious adverse effect of sotalol and dofetilide. Amiodarone has minimal proarrhythmic risk but has numerous noncardiac toxicities that require frequent monitoring.[12a,12b] Dronedarone a noniodinated benzofuran derivative has been shown to be more effective in vivo than amiodarone in several arrhythmia models, particularly in preventing ischemia- and reperfusion-induced ventricular fibrillation and in reducing mortality. However, further experimental studies and long-term clinical trials are required to provide additional evidence of efficacy and safety of this drug.[12c] Azimilide statistically reduced the incidence of new atrial fibrillation in recent survivors of myocardial infarction at high risk for sudden cardiac death.[13] In addition, class III antiarrhythmic agents are increasingly being used as adjunct therapy to decrease the frequency of ICD discharges in patients with ventricular arrhythmias and implantable cardioverter defibrillators (ICDs).[14] The antiarrhythmic efficacy of most pure class III drugs is compromised by their inherent property to induce excessive lengthening of the action potential and their inability to prolong the action potential when most needed, namely during tachycardia. Overall, an ideal antiarrhythmic agent does not exist, and drug selection should be highly individualized.[15,16]

Thus, it is important to develop therapeutic agents which could improve heart function with minimal side effects. We have previously synthesized[17] a series of hybrid compounds combining the pharmacophoric redox moiety of vitamin E and key features responsible for the antiarrhythmic properties of the class I antiarrhythmics procainamide and lidocaine. Some of these compounds, at concentrations of 30–100 μM, prolonged QRS intervals during reperfusion and enhanced the post-ischemic recovery without inducing ventricular fibrillations. Moreover, there was no evidence in our experiments for drug-induced proarrhythmia.

Based on this experience in antiarrhythmic field, we were interested in continuing our work and to focus our activities on the synthesis of novel cardioprotective compounds with improved efficacy in the treatment of life-threatening arrhythmias. Thus, we synthesized series of molecules that combine the hydroxy-benzopyran ring of vitamin E with the methylsulfonylaminophenyl moiety of class III antiarrhythmic drugs.

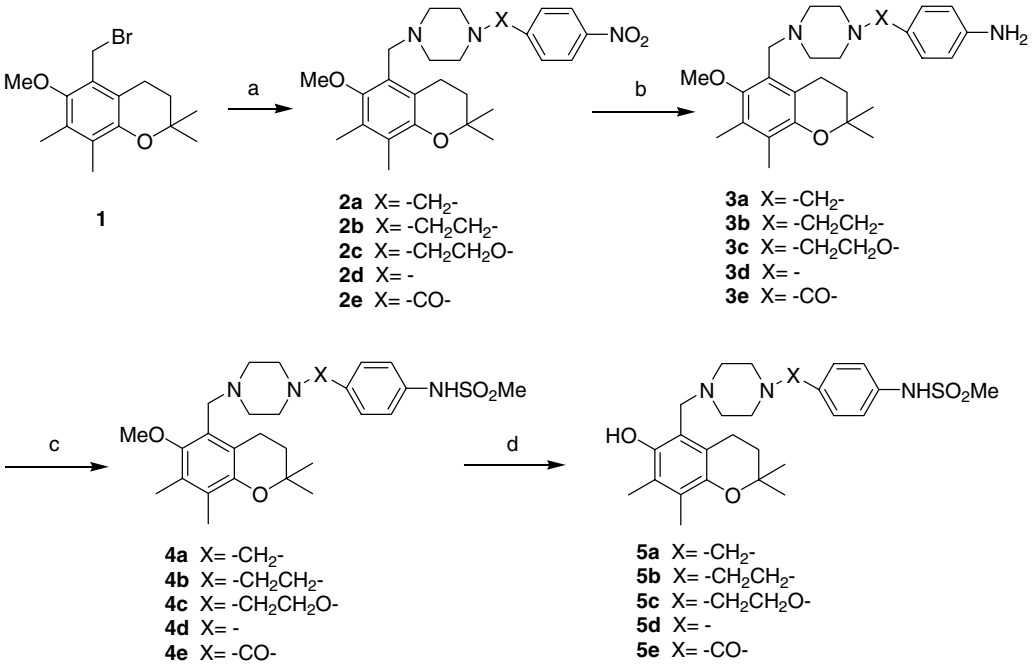
Specifically, the new compounds combine pharmacophores identified for the most active class III antiarrhythmics. Thus, they contain two aromatic rings, one methylsulfonyl amino group and at least one tertiary amine, such as a 1,4-piperazine or methylamine moiety.

Evaluation of the antiarrhythmic and antioxidant activity of the new compounds was carried out on isolated rat heart preparations using the non-recirculating Langendorff mode. The new analogues were present, at 10 μM concentration, during ischemia and reperfusion. Selected compounds were further studied by a conventional microelectrode method in order to get insight into their cellular mode of action.

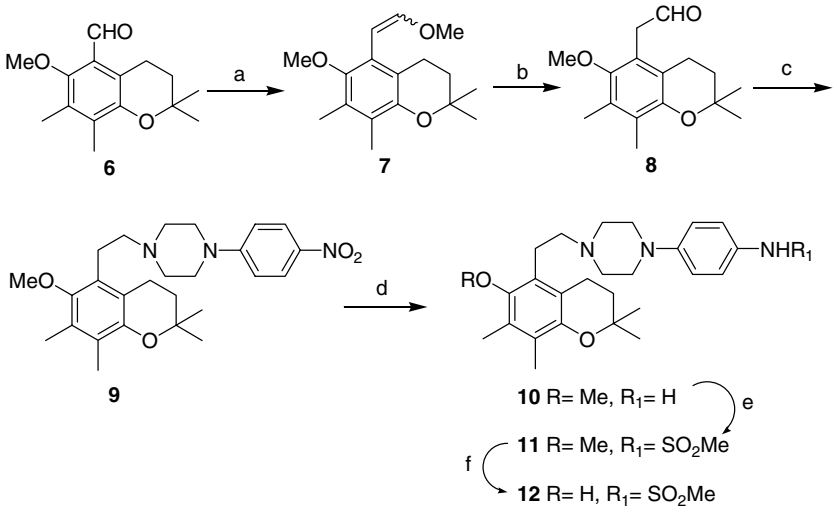
2. Chemistry

The synthesis of the disubstituted piperazine derivatives **5a**–**e** is depicted in Scheme 1. Analogues **2a**–**e** were synthesized by alkylation of the appropriate monosubstituted piperazines (prepared by the appropriate bromides or acid chloride and 8-fold excess of piperazine) with bromide **1**[18] in the presence of K_2CO_3 and TBAI in CH_3CN (compounds **2a**–**c**,**e**) or DMF (compound **2d**). Reduction of the nitro group using $NaBH_4$ and CuCl gave analogues **3a**–**e**, which in turn were converted to the corresponding methanesulfonamides **4a**–**e** using CH_3SO_2Cl in pyridine. Deprotection of the chroman hydroxyl group was achieved using $BF_3{\cdot}S(CH_3)_2$ in CH_2Cl_2, to afford the methanesulfonamides **5a**–**e**.

The 1-[(benzopyran-5-yl)ethyl]piperazine analogue **12** is synthesized as shown in Scheme 2. Wittig reaction of aldehyde **6** with $Ph_3P^+CH_2OCH_3Cl^-$ in the presence of *t*-BuOK gave the enol ether **7** which upon hydrolysis[19] afforded aldehyde **8**. Reductive amination of **8** with 1-(4-nitrophenyl)-piperazine produced the nitro



Scheme 1. Reagents and conditions: (a) 4-substituted piperazine, K_2CO_3, TBAI, anhyd CH_3CN; (b) 1-(4-nitrophenyl)piperazine, K_2CO_3, TBAI, anhyd DMF, 80 °C; (c) $NaBH_4$, CuCl, EtOH, 80 °C; (d) CH_3SO_2Cl, pyridine, anhyd CH_2Cl_2; (e) $BF_3{\cdot}S(CH_3)_2$, anhyd CH_2Cl_2.



Scheme 2. Reagents and conditions: (a) $Ph_3P^+CH_2OCH_3Cl^-$, *t*-BuOK, anhyd THF; (b) *p*-toluenesulfonic acid, dioxane, H_2O; (c) 1-(4-nitrophenyl)piperazine, $NaBH_3CN$, CH_3COOH, CH_3CN; (d) $NaBH_4$, CuCl, EtOH, 80 °C; (e) CH_3SO_2Cl, pyridine, anhyd CH_2Cl_2; (f) $BF_3{\cdot}S(CH_3)_2$, anhyd CH_2Cl_2.

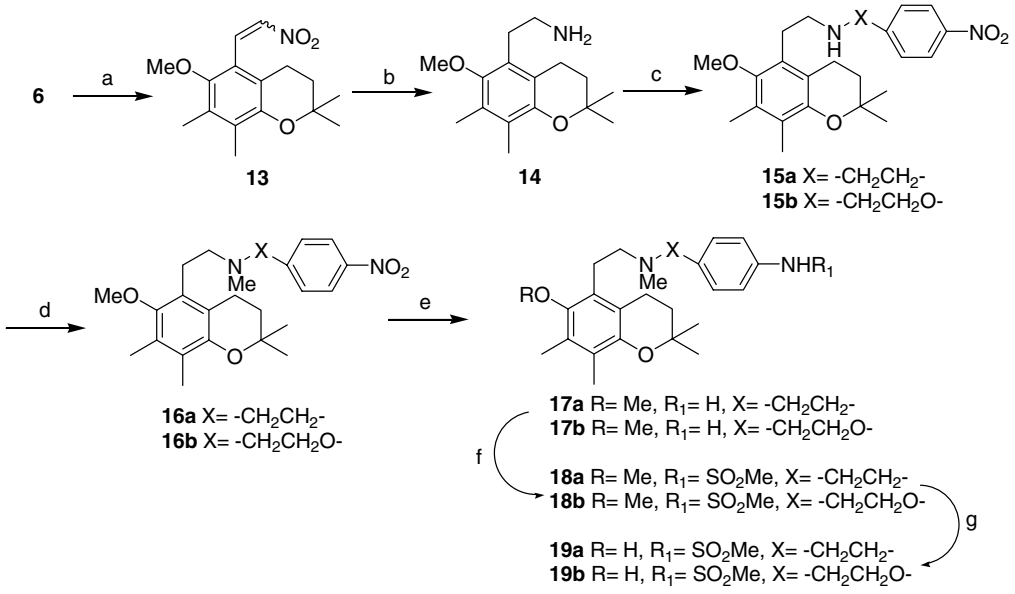
analogue **9** which was converted to methanesulfonamide **12** using the synthetic procedure described above for compounds **5a**–**e**.

The synthesis of methylamino derivatives **19a**,**b** is depicted in Scheme 3. Condensation of aldehyde **6** with CH_3NO_2, in the presence of CH_3COONH_4, followed by reduction using $LiAlH_4$,[20] gave amine **14**, which in turn was alkylated with 4-nitrophenyl or 2-(4-nitro-phenoxy)ethyl bromide to afford amines **15a**,**b**. Methylation of the secondary amines using HCHO 36% and HCOOH[21] afforded nitro compounds **16a**,**b** which were converted to methanesulfonamides **19a**,**b**.

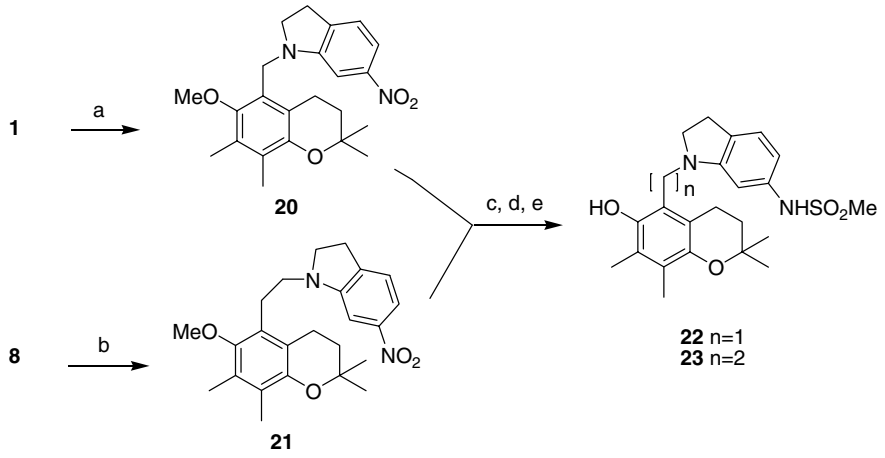
For the synthesis of the constrained derivative **22** (Scheme 4), bromide **1** and 6-nitroindoline were used as starting materials. Analogue **21** was synthesized by reductive alkylation of 6-nitroindoline with aldehyde **8** and then converted to the final compound **23**.

3. Results and discussion

The antiarrhythmic activity of the new compounds, at a concentration of 10 μM, is shown in Table 1 and it is expressed as incidence of premature beats during the first 10 min of reperfusion. The antioxidant activity is



Scheme 3. Reagents and conditions: (a) CH_3NO_2, CH_3COONH_4, 100 °C; (b) $LiAlH_4$, THF; (c) 4-nitrophenethyl or 2-(4-nitrophenoxy)ethylbromide, K_2CO_3, TBAI, anhyd CH_3CN; (d) HCHO 36%, HCOOH, 100 °C; (e) $NaBH_4$, CuCl, EtOH, 80 °C; (f) CH_3SO_2Cl, pyridine, anhyd CH_2Cl_2; (g) $BF_3{\cdot}S(CH_3)_2$, anhyd CH_2Cl_2.



Scheme 4. Reagents and conditions: (a) 6-nitroindoline, K_2CO_3, TBAI, CH_3CN; (b) 6-nitroindoline, $NaBH_3CN$, CH_3COOH, CH_3CN; (c) $NaBH_4$, CuCl, EtOH, 80 °C; (d) CH_3SO_2Cl, pyridine, anhyd CH_2Cl_2; (e) $BF_3{\cdot}S(CH_3)_2$, anhyd CH_2Cl_2.

Table 1. Antiarrhythmic and antioxidant activity, at a concentration of 10 μM, of the new compounds

Compound	Premature beats (%)	MDA (nmol/g)	Remarks
None (control)	3 ± 0.6	134 ± 11	Tachycardia
5a	3 ± 0.28	137 ± 13	Bradycardia
5b	3.15 ± 1.06	141 ± 9	Tachycardia
5c	3.15 ± 1.9	146 ± 25	Bradycardia
5d	8.2 ± 1.8	129 ± 35	Increase of premature beats
5e	2.8 ± 1.04	95 ± 35	Bradycardia
12	5.35 ± 2.1	139 ± 7	Fibrillation
18a	3.9 ± 1.68	105 ± 7	Suppression of tachycardia
19a	1.35 ± 0.58*	76 ± 15**	Fast recovery of the heart
19b	3.05 ± 1.06	110 ± 14	Suppression of tachycardia
22	3.85 ± 1.4	104.5 ± 16	Fast recovery
23	2.11 ± 0.75	83 ± 2.8**	Fast recovery
Amiodarone	5.11 ± 3.5	116 ± 5.6	Fibrillation

n = 3–4.
* $p < 0.05$, versus control.
** $p < 0.01$, versus control.

expressed as MDA content (nmoles/gr of cardiac tissue) at the end of reperfusion.

In the absence of compound (control) tachycardia was observed during reperfusion, while the QT and QRS intervals shorten during ischemia and reperfusion (Table 2).

Compound **5b**, which induces tachycardia upon reperfusion, and compounds **5d** and **12**, which increase the incidence of premature beats, are the less active analogues.

Piperazine analogue **5a** suppresses tachycardia during reperfusion, prolongs QT interval during ischemia and reperfusion, while it affects QRS interval only during ischemia. Compound **5c** suppresses also reperfusion tachycardia, prolongs QT interval during ischemia and reperfusion, and prolongs QRS interval only during reperfusion. Compound **5e** is the only piperazine derivative that reduces premature beats and exhibits antioxidant activity.

Among the methylamino analogues, compound **19b**, does not reduce the premature beats but suppresses reperfusion tachycardia. Although this analogue does not influence the QT interval during ischemia, it causes a significant QT widening during reperfusion and it slightly decreases MDA content. Compound **19a**, bearing ethylene group instead of ethylenoxy group of compound **19b**, and reduces premature beats, prolongs QT and QRS intervals during ischemia and reperfusion, reduces MDA content, leading to a fast recovery of the heart. In addition, analogue **19a** exhibits better antiarrhythmic and antioxidant activity than its methoxy derivative **18a**.

The two constrained analogues **22** and **23** induced fast recovery of the heart during reperfusion. It should be also noted that the presence of compounds **5c**, **19a**,**b**, and **22** facilitated the recovery of QRS and QT intervals to the normal values.

The effect of compounds **5a**–**e** and **19a**,**b** on the action potential parameters was investigated at 5 μM, in rabbit ventricular muscles. The results are summarized in Table 3. The compounds did not change the resting potential (RP), the action potential amplitude (APA), and the maximal rate of depolarization (V_{max}). The observed variations of V_{max} in some experiments most likely reflect inconsistency of the impalement of the microelectrode. These data suggest no major change of the fast inward sodium current (I_{Na}) after the application of the analogues. Compounds **5a**,**b**,**d** did not alter the repolarization process reflected as no change of the 50% and 90% action potential durations (APD_{50} and APD_{90}). This indicates no, or minimal effect of the major repolarizing potassium channels. However, compounds **5c**,**e** and **19a**,**b** prolonged APD by 5–14% which suggests moderate inhibition of the repolarizing potassium current most likely of the rapid delayed rectifier potassium current (I_{Kr} or HERG). The latter effect represents moderate class III antiarrhythmic actions and may need further investigations.

4. Conclusions

Among piperazine derivatives, compounds **5c** and **5e** suppress reperfusion tachycardia, while compound **5e** reduces premature beats and MDA content, combining antiarrhythmic and antioxidant properties. The presence of phenyl piperazine moiety (analogues **5d** and **12**) abolishes antiarrhythmic activity.

Methylamino derivative **19a** and its constrained analogue **23** exhibit antioxidant activity, reduce premature beats, and induce a fast recovery of the heart during reperfusion.

Compounds **5c**, **19a**,**b**, and **22** facilitated the recovery of QRS and QT intervals during reperfusion, to the normal values. Moreover, the cardioprotective compounds **5c**,**e** and **19a**,**b** do not induce excessive lengthening of the action potential, exhibiting moderate class III antiarrhythmic actions. Further studies on animal models as well as on the possible influence on specific potassium channels such as I_{Kr}, I_{Ks}, I_{to}, and I_{K1} should clarify the mecha-

Table 2. QRS and QT intervals (ms) of the analogues **5a**,**e**,**c**, **19a**,**b**, **22**, and **23** during ischemia and reperfusion

Compound	Equilibration		Ischemia		Reperfusion	
	QRS	QT	QRS	QT	QRS	QT
None (control)	39 ± 3.2	105 ± 7.3	28 ± 3.6	61.5 ± 4.3	24.4 ± 2.1	55.4 ± 4.8
5a			36 ± 3.1*	114 ± 9.6***	26.4 ± 2.2	121.3 ± 11.7***
5c			31 ± 2.4	96 ± 7.4**	42.7 ± 3.2***	119.7 ± 12***
5e			29.14 ± 1	97.4 ± 8.4**	41.7 ± 4.3***	129.15 ± 14.6***
19a			38.5 ± 4.2**	89.3 ± 6.8**	30.6 ± 2.7*	107.6 ± 10.3***
19b			21.3 ± 4	63.3 ± 9.3	43.4 ± 1.4***	121.3 ± 5.7***
22			32.5 ± 4.4	108.1 ± 12.5**	33.12 ± 4.7*	106.16 ± 24.7**
23			33.8 ± 2.1	102.8 ± 3.6***	39.44 ± 1.7***	143.44 ± 9.2***
Amiodarone			36.3 ± 3.7**	129.1 ± 18.4***	42.4 ± 2.3***	134.1 ± 17.2***

n = 3-4.
* $p < 0.05$, versus control.
** $p < 0.01$, versus control.
*** $p < 0.001$, versus control.

Table 3. Effect of analogues **5a–e** and **19a,b**, at a concentration of 5 μM, on the action potential parameters in rabbit isolated right ventricular papillary muscle

Compound	Experiments	RP	APA	APD_{50}	APD_{90}	V_{max}
Control	1	−88	122	164	201	201
5a		−88	125	167	201 (0%)	201
Control	2	−89	117	124	165	171
5a		−87	115	133	173 (4.8%)	186
Control	1	−84	110	107	139	186
5b		−85	118	110	140 (0%)	208
Control	2	−93	114	136	169	156
5b		−91	115	136	168 (0%)	163
Control	1	−87	121	167	205	223
5c		−86	122	188	224 (9.3%)	230
Control	2	−77	98	126	175	267
5c		−84	109	159	199 (13.7%)	267
Control	1	−90	104	157	195	171
5d		−88	100	157	189 (−3.1%)	208
Control	2	−89	114	112	161	178
5d		−89	112	137	169 (5%)	216
Control	1	−84	117	127	175	305
5e		−84	117	150	191 (9.1%)	297
Control	2	−93	113	154	200	163
5e		−92	112	167	211 (5.5%)	171
Control	1	−90	113	197	230	201
19a		−91	120	214	246 (6.9%)	230
Control	2	−82	105	118	155	193
19a		−86	106	127	170 (9.7%)	260
Control	1	−86	119	110	150	171
19b		−86	117	117	160 (6.7%)	178
Control	2	−86	117	199	237	334
19b		−83	103	215	255 (7.6%)	201

RP, resting membrane potential.
APA, action potential amplitude.
$APD_{50–90}$ = 50% and 90% action potential duration.
V_{max} = maximal rate of depolarization.

nism and provide additional evidence of efficacy of these compounds.

5. Experimental

5.1. Chemistry

Melting points were determined on a Buchi 510 apparatus and are uncorrected. NMR spectra were recorded on a Bruker AC 300 spectrometer operating at 300 MHz for ^1H and 75.43 MHz for ^{13}C. ^1H NMR spectra are reported in units of δ with $CHCl_3$ resonance at 7.26 ppm used as the chemical shift resonance. ^{13}C NMR spectra are reported in units of δ relative to $CDCl_3$ at 77.00 ppm. $CDCl_3$ was used as solvent. Silica gel plates Macherey-Nagel Sil G-25 UV_{254} were used for thin-layer chromatography. Chromatographic purification was performed with silica gel (200–400 mesh). Mass spectra were recorded on a Varian Saturn 2000 GC–MS instrument in the EI mode. Elemental analyses were carried out on a Perkin-Elmer Series II CHNS/O 2400 analyser.

5.2. General procedure for the synthesis of disubstituted piperazines 2a–e (method A)

To a solution of bromide **1** (0.200 g, 0.64 mmol) in 5 mL anhyd CH_3CN, were added, at 0 °C, K_2CO_3 (0.132 g, 0.96 mmol) and a solution of the appropriate monosubstituted piperazine (0.64 mmol) in 5 mL anhyd CH_3CN. A catalytic amount of TBAI was then added and the mixture was stirred at ambient temperature for 24 h. After completion of the reaction, the solvent was evaporated, the residue was taken up with AcOEt and washed with water. The organic layer was dried and concentrated, and the residue was purified by column chromatography (CH_2Cl_2/CH_3OH 9.5:1.5).

5.2.1. 1-[(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)methyl]-4-[(4-nitrophenyl)methyl]-piperazine (2a). Yield: 85%, yellow viscous oil. ^1H NMR δ: 8.14 (d, 2H, J = 9.1 Hz, Ar*H*), 7.49 (d, 2H, J = 9.1 Hz, Ar*H*), 3.66 (s, 3H, –OCH_3), 3.55 (s, 2H, ArCH_2N–), 3.50 (s, 2H, –NCH_2Ar), 2.86 (t, 2H, J = 6.7 Hz, ArCH_2), 2.51–2.49 (m, 8H, CH_2),

2.18 (s, 3H, ArCH_3), 2.10 (s, 3H, ArCH_3), 1.76 (t, 2H, J = 6.7 Hz, CH_2), 1.31 (s, 6H, CH_3) ^{13}C NMR δ: 150.6, 148.0, 147.0, 146.7, 129.4, 127.6, 125.9, 125.1, 123.4, 119.0, 72.9, 62.1, 61.5, 53.5, 52.8, 32.9, 26.9, 20.1, 12.8, 12.1. MS m/z 453 (M^+1%), 268 (100%), 233, 217. Anal. Calcd for $C_{26}H_{35}N_3O_4$: C, 68.85; H, 7.78; N, 9.26. Found: C, 69.09; H, 7.88; N, 9.59.

5.2.2. 1-[(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)methyl]-4-[2-(4-nitrophenyl)ethyl]-piperazine (2b). Yield: 80%, yellow viscous oil. ^{1}H NMR δ: 8.09 (d, 2H, J = 9.1 Hz), 7.33 (d, 2H, J = 9.1 Hz), 3.63 (s, 3H), 3.47 (s, 3H), 2.84–2.82 (m, 4H), 2.58 (t, 2H, J = 6.7 Hz), 2.50–2.48 (m, 8H), 2.16 (s, 3H), 2.08 (s, 3H), 1.74 (t, 2H, J = 6.7 Hz), 1.28 (s, 6H). ^{13}C NMR δ: 150.6, 148.5, 148.0, 146.4, 129.5, 127.6, 125.9, 125.1, 123.6, 119.1, 72.9, 61.5, 59.4, 53.4, 52.8, 33.5, 32.9, 26.9, 20.1, 12.8, 12.0.

5.2.3. 1-[(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)methyl]-4-[2-(4-nitrophenoxy)ethyl]-piperazine (2c). Yield: 45%, yellow viscous oil. ^{1}H NMR δ: 8.17 (d, 2H, J = 9.1 Hz), 6.92 (d, 2H, J = 9.1 Hz), 4.17 (t, 2H, J = 5.5 Hz), 3.64 (s, 3H), 3.47 (s, 2H), 2.84–2.79 (m, 4H), 2.52–2.50 (m, 8H), 2.17 (m, 3H), 2.09 (s, 3H), 1.75 (t, 2H, J = 6.7 Hz), 1.29 (s, 6H). ^{13}C NMR δ: 163.8, 150.5, 148.0, 141.5, 127.6, 125.8, 125.1, 119.1, 114.5, 114.4, 72.9, 66.9, 65.0, 61.5, 56.8, 54.0, 53.1, 52.7, 32.9, 26.9, 20.1, 12.8, 12.0. Anal. Calcd for $C_{27}H_{37}N_3O_5$: C, 67.06; H, 7.71; N, 8.69. Found: C, 67.12; H, 7.63; N, 8.32.

5.2.4. 1-[(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)methyl]-4-(4-nitrophenyl)piperazine (2d). Bromide **1** and 1-(4-nitrophenyl)piperazine (0.100 g, 0.48 mmol), in anhyd DMF at 80 °C, were treated according to method A. Yield: 0.154 g (73%), orange solid, mp 126–129 °C. ^{1}H NMR δ: 8.09 (d, 2H, J = 9.1 Hz), 6.78 (d, 2H, J = 9.1 Hz), 3.64 (s, 3H), 3.52 (s, 2H), 3.36–3.34 (m, 4H), 2.87 (t, 2H, J = 6.7 Hz), 2.59–2.57 (m, 4H), 2.19 (s, 3H), 2.10 (s, 3H), 1.76 (t, 2H, J = 6.7 Hz), 1.30 (s, 6H). ^{13}C NMR δ: 154.9, 150.5, 127.8, 125.9, 125.2, 119.0, 112.4, 73.0, 61.6, 52.3, 47.2, 32.8, 26.9, 20.1, 12.8, 12.1. MS m/z: 234 (100%), 219. Anal. Calcd for $C_{25}H_{33}N_3O_4$: C, 68.31; H, 7.57; N, 9.56. Found: C, 67.92; H, 7.62; N, 9.16.

5.2.5. 1-[(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)methyl]-4-(4-nitrobenzoyl)piperazine (2e). Yield: 95%, yellow solid, mp 192–194 °C. ^{1}H NMR δ: 8.20 (d, 2H, J = 8.5 Hz), 7.52 (d, 2H, J = 8.5 Hz), 3.74–3.72 (m, 2H), 3.59 (s, 3H), 3.51 (s, 2H), 3.25–3.23 (m, 2H), 2.81 (t, 2H, J = 6.7 Hz), 2.59–2.57 (m, 2H), 2.39–2.37 (m, 2H), 2.12 (s, 3H), 2.05 (s, 3H), 1.74 (t, 2H, J = 6.7 Hz), 1.25 (s, 6H). ^{13}C NMR δ: 167.8, 150.5, 148.2, 142.2, 128.0, 127.7, 125.6, 125.0, 123.8, 118.9, 73.0, 65.0, 61.5, 52.7, 47.9, 42.5, 32.8, 26.9, 20.0, 12.7, 12.0. MS m/z: 234 (100%), 219. Anal. Calcd for $C_{26}H_{33}N_3O_5$: C, 66.79; H, 7.11; N, 8.99. Found: C, 66.86; H, 7.05; N, 8.67.

5.3. General procedure for the synthesis of 4-substituted anilines (method B)

To a solution of the appropriate disubstituted piperazine (0.35 mmol) in 8 mL abs EtOH, CuCl (1.56 mmol) was added, at 0 °C, followed by $NaBH_4$ (3.18 mmol) and the mixture was refluxed for 2 h. The mixture was then filtered through Celite and washed with CH_2Cl_2. The filtrate was washed with sat aqueous NaCl, dried, and evaporated to dryness.

5.3.1. [4-[4-[(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)methyl]piperazin-1-yl]methyl]aniline (3a). Yield: 95%, yellow viscous oil. ^{1}H NMR δ: 7.09 (d, 2H, J = 8.5 Hz), 6.61 (d, 2H, J = 8.5 Hz), 3.63 (s, 3H), 3.47 (s, 2H), 3.41 (s, 2H), 2.81 (t, 2H, J = 6.7 Hz), 2.49–2.47 (m, 8H), 2.16 (s, 3H), 2.08 (s, 3H), 1.73 (t, 2H, J = 6.7 Hz), 1.28 (s, 6H).

5.3.2. [4-[4-[(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1- benzopyran-5-yl)methyl]piperazin-1-yl]ethyl]aniline (3b). Yield: 88%, yellow viscous oil. ^{1}H NMR δ: 7.05 (d, 2H, J = 9.1 Hz), 6.63 (d, 2H, J = 9.1 Hz), 3.66 (s, 3H), 3.47 (s, 3H), 2.85–2.82 (m, 4H), 2.59 (t, 2H, J = 6.7 Hz), 2.51–2.48 (m, 8H), 2.17 (s, 3H), 2.08 (s, 3H), 1.74 (t, 2H, J = 6.7 Hz), 1.27 (s, 6H).

5.3.3. 4-[2-[4-[(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)methyl]piperazin-1-yl]ethoxy]-aniline (3c). Yield: 80%, yellow viscous oil. ^{1}H NMR δ: 6.71 (d, 2H, J = 8.5 Hz), 6.61 (d, 2H, J = 8.5 Hz), 4.05 (t, 2H, J = 5.5 Hz), 3.64 (s, 3H), 3.47 (s, 2H), 2.84 (t, 2H, J = 6.7 Hz), 2.75 (t, 2H, J = 5.5 Hz), 2.52–2.50 (m, 8H), 2.16 (s, 3H), 2.08 (s, 3H), 1.74 (t, 2H, J = 6.7 Hz), 1.28 (s, 6H).

5.3.4. 4-[4-[(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)methyl]piperazin-1-yl]aniline (3d). Yield: 0.137 g (96%), green viscous oil. ^{1}H NMR δ: 6.78 (d, 2H, J = 8.5 Hz), 6.63 (d, 2H, J = 8.5 Hz), 3.66 (s, 3H), 3.55 (s, 2H), 2.99–2.97 (m, 4H), 2.87 (t, 2H, J = 6.7 Hz), 2.63–2.61 (m, 4H), 2.19 (s, 3H), 2.10 (s, 3H), 1.75 (t, 2H, J = 6.7 Hz), 1.29 (s, 6H).

5.3.5. 4-[[4-[(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)methyl]piperazin-1-yl]carbonyl]aniline (3e). Yield: 78%, yellow viscous oil. ^{1}H NMR δ: 7.23 (d, 2H, J = 8.5 Hz), 6.61 (d, 2H, J = 8.5 Hz), 3.84–3.82 (m, 2H), 3.63 (s, 3H), 3.48–3.47 (m, 2H), 2.84 (t, 2H, J = 6.7 Hz), 2.44–2.42 (m, 4H), 2.16 (s, 3H), 2.08 (s, 3H), 1.75 (t, 2H, J = 6.7 Hz), 1.28 (s, 6H).

5.4. General procedure for the synthesis of 4-substituted phenylmethanesulfonamides (method C)

To a solution of the appropriate aniline (0.5 mmol) in 6 mL CH_2Cl_2 and 2 mL pyridine was added at 0 °C CH_3SO_2Cl (1 mmol) and the mixture was stirred at ambient temperature. After completion of the reaction, AcOEt and sat aqueous solution of NH_4Cl were added. The organic layer was further washed by satd aqueous NaCl, dried, and concentrated in vacuo. The residue

was purified by column chromatography (CH_2Cl_2/CH_3OH 9.5:1.5).

5.4.1. *N*-[4-[[4-[(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)methyl]piperazin-1-yl]methyl]-phenyl]methanesulfonamide (4a). Yield: 55%, white solid, mp 80–82 °C. ^{1}H NMR δ: 7.27 (d, 2H, J = 8.5 Hz), 7.15 (d, 2H, J = 8.5 Hz), 3.64 (s, 3H), 3.47 (s, 2H), 3.42 (s, 2H), 2.98 (s, 3H), 2.83 (t, 2H, J = 6.7 Hz), 2.47–2.38 (m, 8H), 2.16 (s, 3H), 2.08 (s, 3H), 1.74 (t, 2H, J = 6.7 Hz), 1.28 (s, 6H). ^{13}C NMR δ: 150.5, 148.0, 135.5, 130.3, 127.6, 125.9, 125.0, 120.9, 119.1, 72.9, 62.2, 61.5, 53.4, 52.8, 39.2, 32.9, 26.9, 20.1, 12.8, 12.0. MS *m*/*z*: 234 (100%), 219, 179. Anal. Calcd for $C_{27}H_{39}N_3O_4S$: C, 64.64; H, 7.84; N, 8.38. Found: C, 64.36; H, 7.56; N, 8.16.

5.4.2. *N*-[4-[2-[4-[(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl- 2*H*-1-benzopyran-5-yl)methyl]piperazin-1-yl]ethyl]phenyl]methanesulfonamide (4b). Yield 80%, white solid, mp 152–155 °C. ^{1}H NMR δ: 7.15–7.13 (m, 4H), 3.65 (s, 3H), 3.48 (s, 2H), 2.96 (s, 3H, –$NHSO_2CH_3$), 2.84 (t, 2H, J = 6.7 Hz), 2.75–2.70 (m, 2H), 2.53–2.51 (m, 10H,), 2.17 (s, 3H), 2.09 (s, 3H), 1.75 (t, 2H, J = 6.7 Hz), 1.29 (s, 6H). ^{13}C NMR δ: 150.5, 148.0, 137.9, 134.7, 129.8, 127.6, 125.9, 125.1, 121.6, 119.1, 72.9, 61.5, 60.2, 53.4, 52.8, 39.1, 32.9, 26.9, 20.1, 12.8, 12.1. MS *m*/*z*: 461, 234 (100%), 219, 179. Anal. Calcd for $C_{28}H_{41}N_3O_4S$: C, 65.21; H, 8.01; N, 8.15. Found: C, 64.82; H, 8.35; N, 7.73.

5.4.3. *N*-[4-[2-[4-[(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)methyl]piperazin-1-yl]ethoxy]phenyl]methanesulfonamide (4c). Yield: 74%, white viscous oil. ^{1}H NMR δ: 7.17 (d, 2H, J = 8.5 Hz), 6.83 (d, 2H, J = 8.5 Hz), 4.10 (t, 2H, J = 5.5 Hz), 3.62 (s, 3H), 3.47 (s, 2H), 2.91 (s, 3H), 2.83 (t, 2H, J = 6.7 Hz), 2.77 (t, 2H, J = 5.5 Hz), 2.52–2.50 (m, 8H), 2.16 (s, 3H), 2.08 (s, 3H), 1.73 (t, 2H, J = 6.7 Hz), 1.28 (s, 6H). ^{13}C NMR δ: 160.0, 150.7, 129.4, 124.7, 119.1, 115.5, 73.1, 65.9, 65.0, 61.4, 56.8, 53.4, 52.7, 52.1, 38.9, 32.8, 26.9, 20.2, 12.8, 12.1. Anal. Calcd for $C_{28}H_{41}N_3O_5S{\cdot}H_2O$: C, 61.18; H, 7.88; N, 7.64. Found: C, 61.27; H, 7.58; N, 7.53.

5.4.4. *N*-[4-[4-[(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)methyl]piperazin-1-yl]phenyl]-methanesulfonamide (4d). Yield: 82%, white viscous oil. ^{1}H NMR δ: 7.13 (d, 2H, J = 9.1 Hz), 6.85 (d, 2H, J = 9.1 Hz), 3.66 (s, 3H), 3.53 (s, 2H), 3.10–3.08 (m, 4H), 2.91 (s, 3H), 2.87 (t, 2H, J = 6.7 Hz), 2.60–2.58 (m, 4H), 2.19 (s, 3H), 2.10 (s, 3H), 1.77 (t, 2H, J = 6.7 Hz), 1.29 (s, 6H). ^{13}C NMR δ: 150.5, 150.0, 148.1, 127.6, 125.3, 124.5, 119.1, 116.5, 73.0, 61.6, 52.7, 49.2, 38.7, 32.9, 26.9, 20.1, 12.8, 12.1. Anal. Calcd for $C_{26}H_{37}N_3O_4S$: C, 64.04; H, 7.65; N, 8.62. Found: C, 63.75; H, 7.73; N, 8.66.

5.4.5. *N*-[4-[[4-[(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)methyl]piperazin-1-ylcarbonyl]phenyl]methanesulfonamide (4e). Yield: 50%, yellowish solid, mp 194–196 °C. ^{1}H NMR δ: 7.34 (d, 2H, J = 8.5 Hz), 7.21 (d, 2H, J = 8.5 Hz), 3.67–3.65 (m, 2H), 3.62 (s, 3H), 3.49 (s, 2H), 3.33–3.31 (m, 2H) 2.97 (s, 3H), 2.83 (t, 2H, J = 6.7 Hz), 2.52–2.50 (m, 2H), 2.38–2.36 (m, 2H), 2.15 (s, 3H), 2.08 (s, 3H), 1.75 (t, 2H, J = 6.7 Hz), 1.28 (s, 6H). ^{13}C NMR δ: 169.8, 150.6, 148.2, 139.0, 131.4, 128.7, 127.8, 119.6, 73.1, 61.5, 52.9, 52.6, 50.6, 39.4, 32.8, 29.7, 26.9, 20.1, 12.8, 12.1. Anal. Calcd for $C_{27}H_{37}N_3O_5S$: C, 62.89; H, 7.23; N, 8.15. Found: C, 62.65; H, 6.96; N, 8.14.

5.5. General procedure for the synthesis of final phenylmethanesulfonamides (method D)

To a solution of the appropriate phenylmethanesulfonamide (0.1 mmol) in 4 mL anhyd CH_2Cl_2 was added, at 0 °C, $BF_3{\cdot}S(CH_3)_2$ (1 mmol) and the mixture was stirred at ambient temperature for 24 h. The solvent is then evaporated under argon, and AcOEt and water were added. The organic layer was dried and evaporated to dryness. The residue was purified by column chromatography (CH_2Cl_2/CH_3OH 9.5:1.5).

5.5.1. *N*-[4-[[4-[(3,4-Dihydro-6-hydroxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)methyl]piperazin-1-yl]methyl]-phenyl]methanesulfonamide (5a). Yield: 24%, white solid, mp 212–215 °C. ^{1}H NMR δ: 7.30 (d, 2H, J = 8.5 Hz), 7.17 (d, 2H, J = 8.5 Hz), 3.63 (s, 2H), 3.50 (s, 2H), 3.00 (s, 3H), 2.61–2.56 (m, 10H), 2.12 (s, 3H), 2.08 (s, 3H), 1.74 (t, 2H, J = 6.7 Hz), 1.25 (s, 6H). ^{13}C NMR δ: 148.7, 144.4, 130.4, 124.9, 120.8, 115.7, 114.8, 72.2, 61.9, 56.3, 52.7, 39.4, 33.0, 29.7, 26.6, 20.7, 11.8, 11.7. MS *m*/*z*: 487 (M^+), 438, 257, 219 (100%). Anal. Calcd for $C_{26}H_{37}N_3O_4S$: C, 64.04; H, 7.65; N, 8.62. Found: C, 63.88; H, 7.35; N, 8.97.

5.5.2. *N*-[4-[2-[4-[(3,4-Dihydro-6-hydroxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)methyl]piperazin-1-yl]ethyl]phenyl]methanesulfonamide (5b). Yield: 41%, white solid, mp 185–187 °C. ^{1}H NMR δ: 7.24 (d, 2H, J = 8.5 Hz), 7.07 (d, 2H, J = 8.5 Hz), 3.78 (s, 2H), 3.61–3.59 (m, 2H), 3.15–3.13 (m, 2H), 3.06–3.03 (m, 8H), 2.93 (s, 3H), 2.58 (t, 2H, J = 6.7 Hz), 2.08 (s, 3H), 2.06 (s, 3H), 1.72 (t, 2H, J = 6.7 Hz), 1.24 (s, 6H). ^{13}C NMR δ: 147.8, 145.1, 136.7, 132.4, 129.7, 126.1, 121.5, 116.3, 113.9, 72.5, 65.0, 58.0, 55.2, 51.8, 48.9, 39.2, 32.8, 29.4, 26.6, 20.8, 12.0, 11.9. Anal. Calcd for $C_{27}H_{39}N_3O_4S$: C, 64.64; H, 7.84; N, 8.38. Found: C, 64.78; H, 8.10; N, 8.53.

5.5.3. *N*-[4-[2-[4-[(3,4-Dihydro-6-hydroxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)methyl]piperazin-1-yl]ethoxy]phenyl]methanesulfonamide (5c). Yield: 36%, yellowish solid, mp 202–204 °C. ^{1}H NMR δ: 7.17 (d, 2H, J = 9.1 Hz), 6.87 (d, 2H, J = 9.1 Hz), 4.09–4.07–4.05 (m, 2H), 3.65 (s, 2H), 2.93 (s, 3H), 2.89–2.86 (m, 2H), 2.71–2.69 (m, 8H), 2.59 (t, 2H, J = 6.7 Hz), 2.12 (s, 3H), 2.09 (s, 3H), 1.75 (t, 2H, J = 6.7 Hz), 1.26 (s, 6H). ^{13}C NMR δ: 156.5, 148.2, 144.5, 130.0, 125.0, 124.2, 122.5, 115.9, 115.3, 114.8, 72.3, 65.6, 56.8, 56.1, 53.4, 53.2, 51.8, 38.5, 32.9, 26.5, 20.6, 11.8, 11.6. Anal. Calcd for $C_{27}H_{39}N_3O_5S$: C, 62.64; H, 7.59; N, 8.12. Found: C, 63.02; H, 7.97; N, 8.36.

5.5.4. *N*-[4-[4-[(3,4-Dihydro-6-hydroxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)methyl]piperazin-1-yl]phenyl]-methanesulfonamide (5d). Yield: 33%, yellowish viscous oil. ^{1}H NMR δ: 7.16 (d, 2H, J = 8.5 Hz), 6.87 (d, 2H,

J = 8.5 Hz), 3.72 (s, 2H), 3.22–3.20 (m, 4H) 2.93 (s, 3H), 2.70–2.68 (m, 4H), 2.62 (t, 2H, J = 6.7 Hz) 2.14 (s, 3H), 2.10 (s, 3H), 1.77 (t, 2H, J = 6.7 Hz), 1.28 (s, 6H). ^{13}C NMR δ: 150.3, 148.9, 148.0, 126.9, 125.1, 124.0, 119.3, 115.8, 72.8, 62.1, 52.5, 38.8, 32.9, 26.6, 20.8, 11.9, 11.6. Anal. Calcd for $C_{25}H_{35}N_3O_4S$: C, 63.40; H, 7.45; N, 8.87. Found: C, 63.77; H, 7.06; N, 8.51.

5.5.5. *N*-[4-[[4-[(3,4-Dihydro-6-hydroxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)methyl]piperazin-1-yl]carbonyl]phenyl]methanesulfonamide (5e). Yield: 54%, white solid, mp 124–126 °C. ^{1}H NMR δ: 7.34 (d, 2H, J = 8.5 Hz), 7.22 (d, 2H, J = 8.5 Hz), 3.67–3.65 (m, 2H), 3.65 (s, 2H), 3.47–3.45 (m, 2H), 3.00 (s, 3H), 2.61–2.56 (m, 6H), 2.12 (s, 3H), 2.09 (s, 3H), 1.75 (t, 2H, J = 6.7 Hz), 1.28 (s, 6H). ^{13}C NMR δ: 169.6, 148.3, 138.7, 131.6, 128.9, 119.5, 115.8, 114.4, 72.3, 65.0, 56.1, 53.4, 52.3, 39.7, 33.0, 26.6, 20.8, 11.9, 11.7. Anal. Calcd for $C_{26}H_{35}N_3O_5S$: C, 62.25; H, 7.03; N, 8.38. Found: C, 62.27; H, 7.36; N, 8.66.

5.6. (3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5)-vinyl-methyl-ether (7)

To a solution of $Ph_3P^+CH_2OCH_3Cl^-$ (1.35 g, 3.9 mmol) in 8 mL anhyd THF was added, at 0 °C, *t*-BuOK (0.295 g, 2.6 mmol), and the red mixture was stirred at 0 °C for 15 min. A solution of aldehyde **6** (0.320 g, 1.29 mmol) in 8 mL THF was then added and the mixture was stirred at 0 °C for 15 min and at ambient temperature for 24 h. Satd aqueous $NaHCO_3$ was then added followed by extraction with diethyl ether. The organic layer was washed with satd aqueous NaCl, dried and the solvent was evaporated. Purification by column chromatography (pet ether/AcOEt 8:2) afforded a mixture of cis/trans isomers. Yield: 0.330 g (93%), yellow oil. ^{1}H NMR δ (trans isomer): 7.09 (d, 1H, J = 13.4 Hz, –C*H*=CH–OCH$_3$), 5.71 (d, 1H, J = 13.4 Hz, –CH=C*H*–OCH$_3$), 3.72 (s, 3H, –CH=CH–OC*H*$_3$), 3.61 (s, 3H), 2.69 (t, 2H, J = 6.7 Hz), 2.20 (s, 3H), 2.11 (s, 3H), 1.77 (t, 2H, J = 6.7 Hz), 1.31 (s, 6H).

5.7. 3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-acetaldehyde (8)

To a solution of compound **7** (0.330 g, 1.19 mmol) in 25 mL 1,4-dioxane and 13 mL H_2O, a catalytic amount of *p*-toluenesulfonic acid was added and the mixture is refluxed for 24 h. After completion of the reaction, the mixture was extracted with diethyl ether and the organic layer was washed with satd aqueous NaCl, dried, and the solvent was evaporated. Yield: 0.310 g (100%). ^{1}H NMR δ: 10.49 (s, 1H, –C*H*O), 3.76 (s, 3H), 3.59 (s, 2H, ArC*H*$_2$CHO–), 3.09 (t, 2H, J = 6.5 Hz), 2.20 (s, 3H), 2.16 (s, 3H), 1.73 (t, 2H, J = 6.2 Hz), 1.29 (s, 6H).

5.8. 1-[2-(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)ethyl]-4-(4-nitrophenyl)piperazine (9)

To a solution of compound **8** (0.094 g, 0.36 mmol) in 2 mL CH_3COOH and 2 mL CH_3CN, at 0 °C, a solution of 1-(4-nitrophenyl)piperazine (0.049 g, 0.24 mmol) in 2 mL CH_3COOH was added and after stirring for 10 min at 0 °C, $NaBH_3CN$ (0.018 g, 0.29 mmol) was added and the mixture was stirred at ambient temperature for 24 h. After completion of the reaction the mixture was poured into ice and NaOH 2 N was added until pH 6. The mixture was then extracted with AcOEt, the organic layer was washed with satd aqueous NaCl, dried and the solvent evaporated. The residue was purified by column chromatography (AcOEt/pet. ether 8:2) Yield: 0.035 g (25%), orange viscous oil. ^{1}H NMR δ: 8.12 (d, 2H, J = 8.5 Hz), 6.82 (d, 2H, J = 8.5 Hz), 3.67 (s, 3H), 3.49–3.47 (m, 4H), 2.82–2.79 (m, 2H), 2.71–2.52 (m, 8H), 2.17 (s, 3H), 2.07 (s, 3H), 1.76 (t, 2H, J = 6.7 Hz), 1.27 (s, 6H). Anal. Calcd for $C_{26}H_{35}N_3O_4$: C, 68.85; H, 7.78; N, 9.26. Found: C, 69.11; H, 7.87; N, 9.64.

5.9. 4-[4-[2-(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)ethyl]piperazin-1-yl]aniline (10)

This compound was prepared using method B. Yield: 0.028 g (98%), yellow viscous oil. ^{1}H NMR δ: 6.82 (d, 2H, J = 8.5 Hz), 6.61 (d, 2H, J = 8.5 Hz), 3.75–3.73 (m, 2H), 3.67 (s, 3H), 3.12–3.10 (m, 2H), 2.82–2.80 (m, 2H), 2.73–2.51 (m, 8H), 2.17 (s, 3H), 2.07 (s, 3H), 1.76 (t, 2H, J = 6.7 Hz), 1.25 (s, 6H).

5.10. *N*-[4-[4-[2-(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)ethyl]piperazin-1-ylphenyl]methanesulfonamide (11)

This compound was prepared using method C. Yield: 0.029 g (41%) yellowish viscous oil. ^{1}H NMR δ: 7.15 (d, 2H, J = 8.5 Hz), 6.90 (d, 2H, J = 8.5 Hz), 3.67 (s, 3H), 3.26–3.23 (m, 4H), 2.93 (s, 3H), 2.86–2.81 (m, 2H) 2.73–2.68 (m, 6H), 2.56–2.52 (m, 2H), 2.17 (s, 3H), 2.07 (s, 3H), 1.77 (t, 2H, J = 6.7 Hz), 1.29 (s, 6H). ^{13}C NMR δ: 149.9, 148.2, 128.2, 124.6, 124.1, 117.0, 116.7, 72.8, 61.2, 58.4, 53.0, 48.9, 38.9, 32.9, 26.9, 23.9, 20.4, 12.8, 11.9. Anal. Calcd for $C_{27}H_{39}N_3O_4S{\cdot}H_2O$: C, 62.40; H, 7.95; N, 8.09. Found: C, 61.98, H, 7.58; N, 7.73.

5.11. *N*-[4-[4-[2-(3,4-Dihydro-6-hydroxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)ethyl]piperazin-1-yl]phenyl]methanesulfonamide (12)

This compound was prepared using method C. Yield: 0.010 g (40%), yellowish solid, mp 202–205 °C. ^{1}H NMR δ: 7.15 (d, 2H, J = 8.5 Hz), 6.91 (d, 2H, J = 8.5 Hz), 3.28–3.25 (m, 4H), 2.93 (s, 3H), 2.85–2.81 (m, 2H), 2.73–2.68 (m, 6H), 2.56–2.52 (m, 2H), 2.17 (s, 3H), 2.07 (s, 3H), 1.77 (t, 2H, J = 6.7 Hz), 1.28 (s, 6H). ^{13}C NMR δ: 149.2, 148.2, 128.0, 124.6, 123.8, 117.0, 116.4, 72.5, 61.0, 58.4, 48.6, 38.9, 32.9, 26.9, 23.7, 20.4, 12.1, 11.9. Anal. Calcd for $C_{26}H_{37}N_3O_4S$: C, 64.04; H, 7.65; N, 8.62. Found: C, 63.77; H, 7.73; N, 8.66.

5.12. 3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-5-(2-nitroethenyl)-2*H*-1-benzopyran (13)

Aldehyde **6** (0.200 g, 0.8 mmol) was added in a mixture of 3 mL anhyd CH_3NO_2 and cat amount of

CH_3COONH_4. The mixture was stirred at 100 °C for 2 h. The solvent is then evaporated and H_2O and a mixture of diethyl ether/CH_2Cl_2 9:1 were added. The organic layer was washed with H_2O (2× 50 mL), HCl 3 N (2× 25 mL), and satd aqueous NaCl, dried, and the solvent was evaporated. Yield: 0.223 g (96%), yellow solid, mp 101–103 °C. ^{1}H NMR δ: 8.24 (d, 1H, J = 13.4 Hz), 7.95 (d, 1H, J = 13.4 Hz), 3.62 (s, 3H), 2.84 (t, 2H, J = 6.7 Hz), 2.19 (s, 3H), 2.13 (s, 3H), 1.83 (t, 2H, J = 6.7 Hz), 1.31 (s, 6H) MS m/z: 291 (M^+, 100%).

5.13. 2-(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)ethylamine (14)

To a slurry of $LiAlH_4$ (0.087 g, 2.31 mmol) in 20 mL anhyd THF was added dropwise, at 0 °C, a solution of compound **13** (0.223 g, 0.77 mmol) in 20 mL anhyd THF and the mixture was refluxed for 2 h. Some drops of THF/H_2O (1:1) were then added to destroy the excess of $LiAlH_4$. The mixture was diluted in AcOEt and Na_2SO_4 was added. Filtration through Celite and evaporation of the solvent afforded the desired amine. Yield: 0.200 g (98%), yellow oil. ^{1}H NMR δ: 3.62 (s, 3H), 2.83–2.79 (m, 2H), 2.75–2.72 (m, 2H), 2.67 (t, 2H, J = 6.7 Hz), 2.15 (s, 3H), 2.06 (s, 3H), 1.75 (t, 2H, J = 6.7 Hz), 1.31 (s, 6H).

5.14. General procedure for the synthesis of secondary amines 15a,b (method E)

To a solution of 4-nitrophenethyl- or 2-(4-nitrophenoxy)ethylbromide (0.5 mmol) in 5 mL anhyd CH_3CN was added, at 0 °C, K_2CO_3 (0.75 mmol), a solution of amine **14** (0.5 mmol) in 5 mL anhyd CH_3CN, and a catalytic amount of TBAI, and the mixture was stirred at 50 °C for 24 h. The solvent was then evaporated and the residue was extracted with AcOEt and H_2O. The organic layer was washed with satd aqueous NaCl, dried, and evaporated to dryness. The residue was purified by column chromatography (CH_2Cl_2/CH_3OH 9.5:1.5).

5.14.1. *N*-[2-(4-Nitrophenyl)ethyl]-2-(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)ethylamine (15a). Yield: 36%, yellow viscous oil. ^{1}H NMR δ: 8.11 (d, 2H, J = 9.1 Hz), 7.31 (d, 2H, J = 9.1 Hz), 3.62 (s, 3H), 2.93–2.90 (m, 4H), 2.78–2.76 (m, 4H), 2.66 (t, 2H, J = 6.7 Hz), 2.15 (s, 3H), 2.07 (s, 3H), 1.75 (t, 2H, J = 6.7 Hz), 1.27 (s, 6H). ^{13}C NMR δ: 149.7, 148.2, 148.0, 146.5, 129.5, 128.1, 127.4, 124.2, 123.6, 117.0, 72.8, 60.9, 50.3, 49.6, 36.1, 32.8, 26.9, 26.8, 20.4, 12.8, 11.9.

5.14.2. *N*-[2-(4-Nitrophenoxy)ethyl]-2-(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)ethylamine (15b). Yield: 42%, yellowish viscous oil. ^{1}H NMR δ: 8.17 (d, 2H, J = 9.1 Hz), 6.93 (d, 2H, J = 9.1 Hz), 4.16 (t, 2H, J = 4.9 Hz, –CH_2CH_2O–) 3.65 (s, 3H), 3.08 (t, 2 H, J = 4.9 Hz, –CH_2CH_2O–) 2.83 (m, 4H, ArCH_2CH_2NH–), 2.69 (t, 2H, J = 6.7 Hz), 2.16 (s, 3H), 2.07 (s, 3H), 1.76 (t, 2H, J = 6.7 Hz), 1.27 (s, 6H). ^{13}C NMR δ: 163.9, 149.7, 148.2, 141.5, 128.1, 127.3, 125.9, 124.2, 117.0, 114.5, 72.8, 68.3, 65.0, 49.9, 48.2, 32.8, 27.1, 26.9, 20.4, 12.8, 11.9.

5.15. General procedure for the synthesis of methylamines 16a,b (method F)

To 0.2 mmol of the appropriate secondary amine were added at 0 °C HCOOH (1 mL) and HCHO 36% in water (0.05 mL) and the mixture was heated at 100 °C for 2 h. NaOH 3 N was then added until pH 8 and the mixture was extracted with AcOEt. The organic layer was washed with satd aqueous NH_4Cl, sat aqueous NaCl, dried, and evaporated to dryness.

5.15.1. *N*-Methyl-*N*-[2-(4-nitrophenyl)ethyl]-2-(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)ethylamine (16a). Yield: 93%, yellowish viscous oil. ^{1}H NMR δ: 8.13 (d, 2H, J = 8.5 Hz), 7.35 (d, 2H, J = 8.5 Hz), 3.66 (s, 3H), 2.93–2.88 (m, 2H) 2.77–2.71 (m, 4H), 2.67 (t, 2H, J = 6.7 Hz), 2.57–2.52 (m, 2H), 2.43 (s, 3H, –N(CH_3)–) 2.17 (s, 3H), 2.08 (s, 3H), 1.76 (t, 2H, J = 6.7 Hz), 1.28 (s, 6H). ^{13}C NMR δ: 149.7, 148.6, 148.2, 146.4, 129.5, 128.1, 127.7, 124.0, 123.6, 116.8, 72.8, 61.1, 58.5, 57.0, 42.1, 33.7, 32.9, 26.8, 24.3, 20.3, 12.8, 11.9. MS m/z: 246 (100%), 234, 218. Anal. Calcd for $C_{25}H_{34}N_2O_4$: C, 70.39; H, 8.03; N, 6.57. Found: C, 70.82; H, 8.35; N, 6.73.

5.15.2. *N*-Methyl-*N*-[2-(4-nitrophenoxy)ethyl]-2-(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)ethylamine (16b). Yield: 93%, yellowish viscous oil. ^{1}H NMR δ: 8.17 (d, 2H, J = 9.1 Hz), 6.96 (d, 2H, J = 9.1 Hz), 4.17 (t, 2H, J = 5.5 Hz) 3.65 (s, 3H), 2.93 (t, 2H, J = 5.5 Hz) 2.82–2.77 (m, 2H), 2.68 (t, 2H, J = 6.7 Hz), 2.62–2.56 (m, 2H), 2.48 (s, 3H), 2.17 (s, 3H), 2.07 (s, 3H), 1.76 (t, 2H, J = 6.7 Hz), 1.28 (s, 6H). ^{13}C NMR δ: 163.9, 149.7, 148.2, 141.5, 132.0, 128.1, 127.5, 125.8, 124.0, 116.8, 114.5, 72.8, 67.1, 65.0, 61.1, 57.8, 55.7, 50.8, 42.8, 32.9, 26.8, 24.3, 20.3, 12.8, 11.9. Anal. Calcd for $C_{25}H_{34}N_2O_5$: C, 67.85; H, 7.74; N, 6.33. Found: C, 67.92; H, 7.58; N, 6.32.

5.16. 4-[2-[*N*-Methyl-2-(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)ethylamine]ethyl]aniline (17a)

This compound was synthesized using method B. Yield: 85%, yellowish viscous oil. ^{1}H NMR δ: 7.00 (d, 2H, J = 7.9 Hz), 6.62 (d, 2H, J = 7.9 Hz), 3.67 (s, 3H), 2.83–2.68 (m, 8H), 2.66 (t, 2H, J = 6.7 Hz), 2.45 (s, 3H) 2.18 (s, 3H), 2.08 (s, 3H), 1.78 (t, 2H, J = 6.7 Hz), 1.29 (s, 6H).

5.17. 4-[2-[*N*-Methyl-2-(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl- 2*H*-1-benzopyran-5-yl)ethylamine]ethoxy]-aniline (17b)

This compound was synthesized using method B. Yield: 97%, yellowish viscous oil. ^{1}H NMR δ: 6.75 (d, 2H, J = 8.5 Hz), 6.63 (d, 2H, J = 8.5 Hz), 4.06 (t, 2H, J = 5.5 Hz), 3.66 (s, 3H), 2.90–2.62 (m, 8H), 2.49 (s, 3H, –NCH_3), 2.17 (s, 3H), 2.07 (s, 3H), 1.76 (t, 2H, J = 6.7 Hz), 1.28 (s, 6H).

5.18. *N*-[4-[2-[[2-(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)ethyl]methylamine]ethyl]-phenyl]methanesulfonamide (18a)

This compound was synthesized using method C. Yield: 70%, yellowish viscous oil. ^{1}H NMR δ: 7.17–7.15 (m, 4H), 3.66 (s, 3H), 2.97 (s, 3H, –NHSO$_2$CH_3), 2.81–2.55 (m, 10H), 2.45 (s, 3H, –NCH_3), 2.17 (s, 3H), 2.07 (s, 3H), 1.76 (t, 2H, J = 6.7 Hz), 1.27 (s, 6H). ^{13}C NMR δ: 149.7, 148.2, 137.9, 134.6, 129.9, 128.1, 127.6, 124.0, 121.5, 116.9, 72.8, 61.1, 59.0, 56.9, 42.0, 39.2, 32.9, 26.9, 24.1, 20.3, 12.8, 11.9. Anal. Calcd for $C_{26}H_{38}N_2O_4S$: C, 65.79; H, 8.07; N, 5.90. Found: C, 65.92; H, 7.69; N, 6.04.

5.19. *N*-[4-[2-[[2-(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)ethyl]methylamine]ethoxy]-phenyl]methanesulfonamide (18b)

This compound was synthesized using method C. Yield: 66%, yellowish solid, mp 125–127 °C.

^{1}H NMR δ: 7.17 (d, 2H, J = 8.5 Hz), 6.86 (d, 2H, J = 8.5 Hz), 4.07 (t, 2H, J = 5.5 Hz), 3.64 (s, 3H), 2.91 (s, 3H), 2.89–2.85 (m, 2H), 2.81–2.76 (m, 2H), 2.66 (t, 2H, J = 6.7 Hz), 2.60–2.56 (m, 2H), 2.46 (s, 3H) 2.15 (s, 3H), 2.05 (s, 3H), 1.77 (t, 2H, J = 6.7 Hz), 1.26 (s, 6H). ^{13}C NMR δ: 157.3, 149.7, 148.2, 129.1, 128.1, 127.5, 124.7, 124.0, 116.9, 115.5, 72.8, 66.4, 61.1, 57.8, 55.9, 53.4, 42.7, 38.9, 32.9, 26.9, 24.1, 20.3, 12.8, 11.9. MS *m*/*z*: 428, 246 (100%), 231. Anal. Calcd for $C_{26}H_{38}N_2O_5S$: C, 63.65; H, 7.81; N, 5.71. Found: C, 63.88; H, 7.58; N, 5.35.

5.20. *N*-[4-[2-[[2-(3,4-Dihydro-6-hydroxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)ethyl]methylamine]ethyl]-phenyl]methanesulfonamide (19a)

This compound was synthesized using method D. Yield: 53%, yellowish viscous oil. ^{1}H NMR δ: 7.12–7.10 (m, 4H), 2.96 (s, 3H), 2.76–2.67 (m, 8H), 2.61 (t, 2H, J = 6.7 Hz), 2.45 (s, 3H) 2.16 (s, 3H), 2.10 (s, 3H), 1.77 (t, 2H, J = 6.7 Hz), 1.27 (s, 6H). ^{13}C NMR δ: 148.1, 145.0, 137.8, 135.0, 129.9, 128.0, 127.5, 123.9, 121.4, 115.7, 72.2, 60.1, 53.4, 42.2, 39.2, 33.2, 26.7, 24.0, 21.1, 12.5, 12.0. Anal. Calcd for $C_{25}H_{36}N_2O_4S$: C, 65.19; H, 7.88; N, 6.08. Found: C, 64.82; H, 7.73; N, 6.41.

5.21. *N*-[4-[2-[[2-(3,4-Dihydro-6-hydroxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)ethyl]methylamine]ethoxy]-phenyl]methanesulfonamide (19b)

This compound was synthesized using method D. Yield: 37%, yellowish viscous oil. ^{1}H NMR δ: 7.15 (d, 2H, J = 8.5 Hz), 6.79 (d, 2H, J = 8.5 Hz), 4.08 (t, 2H, J = 5.5 Hz), 2.93 (s, 3H), 2.90–2.87 (m, 2H), 2.82–2.78 (m, 4H), 2.59 (t, 2H, J = 6.7 Hz), 2.54 (s, 3H) 2.14 (s, 3H), 2.05 (s, 3H), 1.74 (t, 2H, J = 6.7 Hz), 1.25 (s, 6H). ^{13}C NMR δ: 151.2, 149.7, 148.2, 129.5, 128.0, 127.3, 125.1, 124.0, 116.7, 115.3, 72.8, 66.4, 61.0, 57.8, 55.5, 42.7, 38.9, 32.9, 26.9, 24.3, 20.3, 12.2, 11.9. Anal. Calcd for $C_{25}H_{36}N_3O_5S$: C, 63.00; H, 7.61; N, 5.88. Found: C, 62.79; H, 8.01; N, 5.53.

5.22. 3,4-Dihydro-6-methoxy-5-[(6-nitro-2,3-dihydro-1*H*-indol-1-yl)methyl]-2,2,7,8-tetramethyl-2*H*-1-benzopyran (20)

The synthesis of this compound was carried out following method A. Yield: 93%, orange solid, mp 145–147 °C. ^{1}H NMR δ: 7.52 (d, 1H, J = 7.93 Hz), 7.34 (s, 1H), 7.08 (d, 1H, J = 7.9 Hz), 4.26 (s, 2H), 3.64 (s, 3H), 3.28 (t, 2H, J = 8.6 Hz), 2.91 (t, 2H, J = 8.6 Hz), 2.74 (t, 2H, J = 6.7 Hz), 2.20 (s, 3H), 2.12 (s, 3H), 1.75 (t, 2H, J = 6.7 Hz), 1.29 (s, 6H). ^{13}C NMR δ: 156.2, 153.4, 150.4, 148.6, 148.2, 138.2, 128.2, 126.1, 124.0, 118.2, 113.3, 100.2, 73.1, 61.5, 52.4, 43.6, 38.5, 32.7, 28.2, 26.9, 20.0, 12.9, 12.1. MS *m*/*z*: 396 (M^+), 233, 217. Anal. Calcd for $C_{23}H_{28}N_2O_4$: C, 69.68; H, 7.12; N, 7.07. Found: C, 69.96; H, 6.92; N, 7.32.

5.23. 3,4-Dihydro-6-methoxy-5-[(6-nitro-2,3-dihydro-1*H*-indol-1-yl)ethyl]-2,2,7,8-tetramethyl-2*H*-1-benzopyran (21)

Aldehyde **8** was treated with 6-nitroindoline as described for analogue **9**. Yield: 35%, yellowish viscous oil ^{1}H NMR δ: 7.50 (d, 1H, J = 7.9 Hz), 7.26 (s, 1H), 7.07 (d, 1H, J = 7.9 Hz), 3.72 (s, 3H), 3.62 (t, 2H, J = 8.6 Hz), 3.28 (t, 2H, J = 7.9 Hz), 3.05 (t, 2H, J = 8.6 Hz), 2.82 (t, 2H, J = 7.9 Hz), 2.72 (t, 2H, J = 6.7 Hz), 2.21 (s, 3H), 2.09 (s, 3H), 1.79 (t, 2H, J = 6.7 Hz), 1.29 (s, 6H). ^{13}C NMR δ: 155.8, 152.9, 150.7, 148.0, 136.3, 128.4, 125.1, 122.3, 118.5, 112.3, 101.2, 72.9, 61.0, 53.5, 44.6, 37.2, 33.5, 31.8, 28.4, 25.9, 20.3, 12.6, 11.9.

5.24. *N*-{1-[(3,4-Dihydro-6-hydroxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)methyl]-2,3-dihydro-1*H*-indol-6-yl}methanesulfonamide (22)

The synthesis of this compound was carried out using method D. Yield: 33%, yellowish solid, mp 149–152 °C. ^{1}H NMR δ: 7.09 (d, 1H, J = 7.9 Hz), 6.70 (d, 1H, J = 6.7 Hz), 6.59 (s, 1H), 4.25 (s, 2H), 3.30 (t, 2H, J = 7.9 Hz), 2.94 (m, 5H), 2.66 (t, 2H, J = 6.7 Hz), 2.14 (s, 3H), 2.09 (s, 3H), 1.78 (t, 2H, J = 6.7 Hz), 1.29 (s, 6H). ^{13}C NMR δ: 153.3, 151.0, 149.7, 144.5, 135.6, 124.2, 121.5, 120.0, 118.3, 117.6, 106.2, 100.3, 72.9, 61.5, 53.1, 40.1, 38.5, 31.4, 27.7, 26.9, 20.5, 12.1, 11.8 Anal. Calcd for $C_{23}H_{30}N_2O_4S$: C, 64.16; H, 7.02; N, 6.51. Found: C, 63.88; H, 6.97; N, 6.35.

5.25. *N*-{1-[(3,4-Dihydro-6-hydroxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-yl)ethyl]-2,3-dihydro-1*H*-indol-6-yl}methanesulfonamide (23)

This compound was synthesized using method D. Yield: 29%, yellowish viscous oil. ^{1}H NMR δ: 7.01 (d, 1H, J = 7.9 Hz), 6.52 (d, 1H, J = 6.7 Hz), 6.46 (s, 1H), 3.46 (t, 2H, J = 7.9 Hz), 3.27 (t, 2H, J = 6.7 Hz), 2.93 (m, 7H), 2.70 (t, 2H, J = 6.7 Hz), 2.12 (s, 3H), 2.09 (s, 3H), 1.81 (t, 2H, J = 6.7 Hz), 1.29 (s, 6H). ^{13}C NMR δ: 155.3, 151.0, 148.2, 143.5, 133.1, 124.0, 121.1, 120.3, 118.8, 116.3, 107.5, 101.3, 72.5, 61.3, 52.1, 40.3, 38.5, 31.4, 28.5, 27.1, 26.3, 20.5, 12.4, 11.9 Anal. Calcd for $C_{24}H_{32}N_2O_4S$: C, 64.84; H, 7.25; N, 6.30. Found: C, 64.82; H, 7.41; N, 6.73.

5.26. Biology

5.26.1. Evaluation of the activity of the new compounds against reperfusion arrhythmias. Male Sprague–Dawley 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 non-recirculating Langendorff perfusion apparatus. Retrograde 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} $MgSO_4$, and 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 10 min by perfusing them with the ischemic KHB (KHB with Tris–HCl 10 mM, pH 6.4, instead of glucose and equilibrated with N_2 before use) followed by 15 min of reperfusion. The new compounds were present during ischemia and reperfusion at a final concentration of 10 μM.

5.26.2. Evaluation of antiarrhythmic activity. Electrocardiograms were recorded during equilibration, ischemia, and reperfusion. Arrhythmias were scored according to the Lambeth Convention Guidelines.[22] Arrhythmia scores (AS) were calculated for the first 10 min of reperfusion as the percentage of premature beats.

5.26.3. 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.[23] 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 means ± SEM. Differences between groups were assessed by Student's unpaired and ANOVA *t*-tests and considered significant when $p < 0{,}05$.

5.26.4. Investigation of the new analogues by a conventional microelectrode method. New Zeeland rabbits (1.5–2 kg) were anesthetized by pentabarbital (iv 50 mg/kg). After chest was opened, hearts were immediately rinsed in oxygenated Tyrode's solution containing (in mM): NaCl, 115; KCl 5; $CaCl_2$ 1.2; $MgCl_2$ 1; $NaHCO_3$ 21.4; and glucose 11. The pH of this solution is 7.35–7.45 when gassed with 95% O_2 and 5% CO_2 at 37 °C. The tip of the papillary muscles from the right ventricle was prepared and individually mounted in a tissue chamber (volume ≈ 50 mL).

The preparations were continuously stimulated (by HSE [Hugo Sachs Electronik] Stimulator type 215/II, March-Hugstetten, Germany) at a basic cycle length of 1000 ms using 2 ms long rectangular constant voltage pulses isolated from ground and delivered across bipolar platinum electrodes in contact with the preparation. At least 1 h was allowed for each preparation to equilibrate while they were continuously superfused with Tyrode's solution. Temperature of the superfusate was kept constant at 37 °C.

Transmembrane potentials were recorded using conventional microelectrode techniques. Microelectrodes filled with 3 M KCl and having tip resistances of 5–20 MΩ were connected to the input of a high impedance electrometer (Experimetria Microelectrode Amplifier Type), which was referenced to the ground. The voltage outputs from the amplifier was displayed on a dual beam memory oscilloscope (Tektonix 2230 100 MHz Digital Storage Oscilloscope, Beaverton, OR, USA).

The maximal diastolic potential (MDP) ('resting potential,' RP), action potential amplitude (APA), the first derivative of transmembrane potentials (V_{max}) and action potential duration measured at 50% and 90% repolarization (APD_{50-90}), were obtained using a software developed in the Department of Pharmacology, Univ, of Szeged (HSE-APES). After the control measurements, the compounds were added to the tissue bath at 5 μM concentration and the measurements were prepared after 30–40 min incubation time. When the impalement was lost during measurement, readjustment was attempted. If the difference between the original and the readjusted action potential parameters did not exceed 15%, the experiment was continued, otherwise it was terminated.

Acknowledgments

This work was supported by the Greek General Secretariat for Research and Technology, grant Bilateral Collaboration of Greece–Hungary 736, by the National Institute of Health, DA3801 and by the Hungarian National Research Foundation, OTKA T-048698.

References and notes

1. Pugsley, M. K. *Drug Dev. Res.* **2002**, *55*, 3–16.
2. (a) Ravens, U.; Wettwer, E.; Hála, O. *Cell Calcium* **2004**, *35*, 575–582; (b) Tamargo, J.; Caballero, R.; Gómez, R.; Valenzuela, C.; Delpón, G. *Cardiovasc. Res.* **2004**, *62*, 9–33.
3. Carmeliet, E. *Physiol. Rev.* **1999**, *79*, 917–987.
4. (a) Lefer, D. J.; Granger, N. *Am. J. Med.* **2000**, *109*, 315–323; (b) Vergely, C.; Maupoil, V.; Clermont, G.; Bril, A.; Rochette, L. *Arch. Biochem. Biophys.* **2003**, *420*, 209–216.

5. (a) DiDomenico, R. J.; Massad, M. G. *Ann. Thorac. Surg.* **2005**, *79*, 728–740; (b) Knotzer, H.; Dunser, M. W.; Mayr, A. J.; Hasibeder, W. R. *Curr. Opin. Crit. Care* **2004**, *10*, 330–335.
6. Elming, H.; Brendorp, B.; Pehrson, S.; Pedersen, O. D.; Kober, L.; Torp-Petersen, C. *Expert Opin. Drug Saf.* **2004**, *3*, 559–577.
7. Nattel, S.; Singh, B. N. *Am. J. Cardiol.* **1999**, *84*, 11R–19R.
8. Koster, R. W. *N. Eng. J. Med.* **1985**, *313*, 1105–1110.
9. Li, G.-R.; Ferrier, G. R. *J. Pharmacol. Exp. Ther.* **1991**, *257*, 997–1004.
10. Nair, L. A.; Grant, A. O. *Cardiovasc. Drugs Ther.* **1997**, *11*, 149–167.
11. (a) Rochetaing, A.; Barbe, C.; Kreher, P. *J. Cardiovasc. Pharmacol.* **2001**, *38*, 500–511; (b) Dogrell, S. A.; Hancox, J. C. *Expert Opin. Investig. Drugs* **2004**, *13*, 415–426; (c) Tse, H. F.; Lau, C. P. *J. Am. Coll. Cardiol.* **2002**, *40*, 2150–2155.
12. (a) Brendorp, B.; Pedersen, O.; Torp-Pedersen, C.; Sahebzadah, N.; Kober, L. *Drug Saf.* **2002**, *25*, 847–865; (b) Purerfellner, H. *Curr. Med. Chem. Cardiovasc. Hematol. Agents* **2004**, *2*, 79–91; (c) Kathofer, S.; Thomas, D.; Karle, C. A. *Cardiovasc. Drug Rev.* **2005**, *23*, 217–230.
13. Carlson, M. *Expert Rev. Cardiovasc. Ther.* **2005**, *3*, 387–391.
14. Sager, P. T. *Curr. Opin. Cardiol.* **2000**, *15*, 41–53.
15. Vos, M. A. *J. Cardiovasc. Electrophysiol.* **2001**, *12*, 1034–1036.
16. MacKenzie, I. *Drug Dev. Res.* **2002**, *55*, 73–78.
17. Koufaki, M.; Calogeropoulou, T.; Rekka, E.; Chryselis, M.; Papazafiri, P.; Gaitanaki, C.; Makriyannis, A. *Bioorg. Med. Chem.* **2003**, *11*, 5209–5219.
18. Koufaki, M.; Detsi, A.; Theodorou, E.; Kiziridi, C.; Calogeropoulou, T.; Vassilopoulos, A.; Kourounakis, A.; Rekka, E.; Kourounakis, P.; Gaitanaki, C.; Papazafiri, P. *Bioorg. Med. Chem.* **2004**, *12*, 4835–4841.
19. Novák, J.; Salemink, C. A. *Tetrahedron Lett.* **1981**, *22*, 1063–1064.
20. Monte, A. P.; Waldman, S. R.; Marona-Lewicka, D.; Wainscott, D. B.; Nelson, D. L.; Sanders-Bush, E.; Nichols, D. E. *J. Med. Chem.* **1997**, *40*, 2997–3008.
21. Liu, H.; JI, M.; Luo, X.; Shen, J.; Huang, X.; Hua, W.; Jiang, H.; Chen, K. *J. Med. Chem.* **2002**, *45*, 2953–2969.
22. Walker, M.; Curtis, M.; Hearse, D. *Cardiol. Res.* **1988**, *22*, 447–455.
23. Draper, H. H.; Hardley, M. *Methods Enzymol.* **1990**, *186*, 283–293.