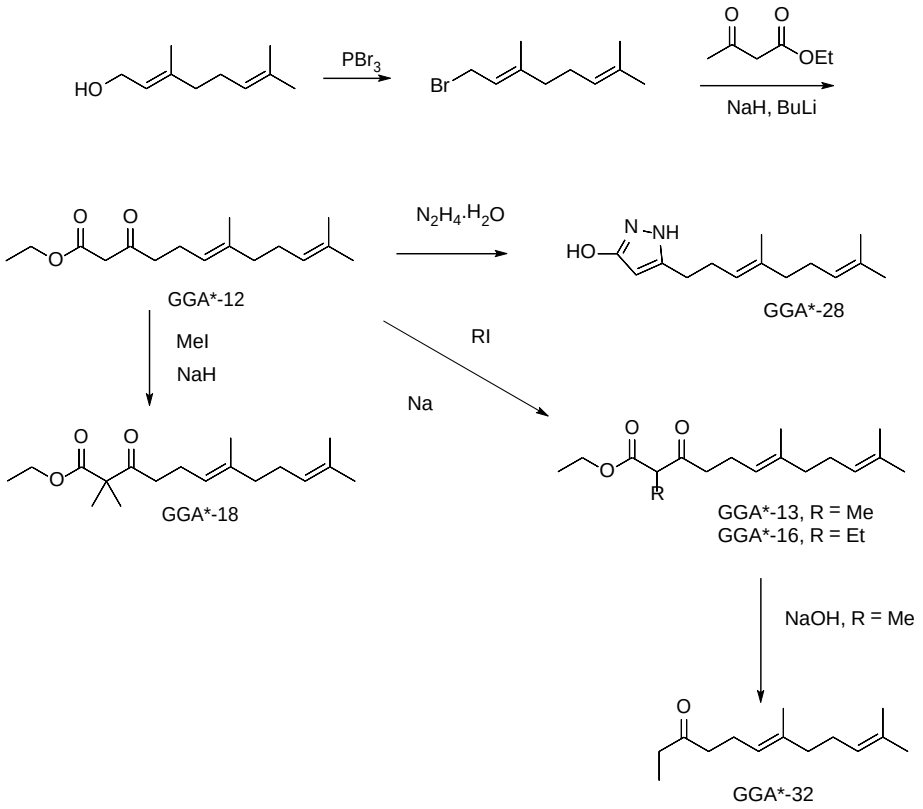
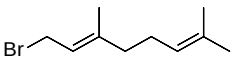
Supplementary materials

Material and methods

Synthesis of GGA-derivatives

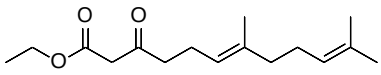


Synthesis of GGA-12, -13, -16, -28 and -32*



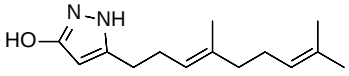
(*E*)-1-bromo-3,7-dimethylocta-2,6-diene. Geraniol (10 g, 11.4 mL, 64.8 mmol, 1.0 eq) was dissolved in DCM (50 mL), under a nitrogen atmosphere. The solution was cooled to -20 °C. A solution of PBr_3 (3.0 mL, 32.4 mmol, 0.5 eq) in DCM (10 mL) was added drop-wise, keeping the temperature < -16 °C. The color changed to green/blue during addition. The reaction mixture was stirred for 3h at ~ -20 °C. Water (50 mL) was added carefully at -40 °C. The water layer was extracted with Et_2O (3 x 50 mL). The

combined organic layers were washed with sat. aq. NH_4Cl. sol. (3 x 50 mL), dried over Na_2SO_4 and concentrated *in vacuo* to provide a brown oil (14.5 g, 66.6 mmol, 100%.)



GGA*-12

(E)-Ethyl 7,11-dimethyl-3-oxododeca-6,10-dienoate (GGA*-12). NaH (60% in oil, 2.63 g, 65.8 mmol, 1.0 eq) was suspended in THF (20 mL), under a nitrogen atmosphere. The solution was cooled to 0 °C with an ice/water bath. Ethylacetoacetate (8.3 mL, 65.8 mmol, 1.0 eq) was added drop-wise in 1 h. During addition a thick suspension was formed which later became a yellow solution. Gas formation was visible and an exothermic reaction was observed. The temperature was kept <10 °C. The reaction mixture was stirred for 10 min at 0 °C. n-BuLi (2.5M in hexanes, 26.3 mL, 65.8 mmol, 1.0 eq) was added drop-wise in 10 min. An very exothermic reaction was observed, during addition the flask was cooled with an ice/MeOH bath. A bright yellow suspension was formed which changed into a yellow solution. The temperature was kept at 0 °C for 10 min. Geranylbromide (ABCR, 9.1 mL, 46.1 mmol, 0.7 eq) was added drop-wise and a suspension was formed. The reaction mixture was warmed to RT and stirred for 1 h. The reaction mixture was poured in sat. aq. NH_4Cl sol. (100 mL) and the water layer was extracted with TBME (3 x 100 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to provide a yellow oil (17.1 g). The crude product was purified by automated column chromatography (eluens, 0 to 100% DCM in heptanes) affording a colorless oil (6.0 g, 22.4 mmol, 49%).

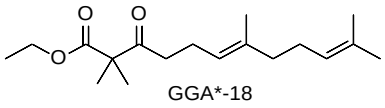


GGA*-28

(E)-5-(4,8-dimethylnona-3,7-dien-1-yl)-1H-pyrazol-3-ol (GGA*-28). GGA*-12 (500 mg, 1.88 mmol, 1.0 eq) was dissolved in EtOH (10 mL), under a nitrogen atmosphere. The solution was cooled to 0 °C with an ice/water bath. Hydrazine (64% water, 0.12 mL, 2.44 mmol, 1.3 eq) was added and the solution was warmed to RT overnight. The solvents were removed *in vacuo* and Et_2O (10 mL) was added to the residue. The solids were isolated by filtration and purified by automated column

chromatography (eluens, 50 to 100% EtOAc in heptanes) yielding a white solid (81 mg, 0.35 mmol, 18%).

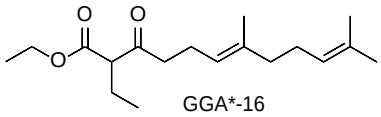
^{1}H-NMR ($CDCl_3$, in ppm): 1.61 (s, 3H), 1.64 (s, 3H), 1.69 (s, 3H), 2.03 (m, 4H), 2.37 (m, 2H), 2.42 (t, 1H), 2.60 (t, 1H), 5.17 (m, 2H), HPLC-MS: $(M+1)^+$ = 235,2



GGA*-18

(E)-ethyl 2,2,7,11-tetrameyhyl-3-oxododeca-6,10-dienoate (GGA*-18). NaH (70 mg, 1.8 mmol, 2.6 eq) was suspended in TFH (6 mL), under a nitrogen atmosphere, and cooled to 0 °C with an ice/water bath. A solution of GGA*-12 (180 mg, 0.68 mmol, 1.0 eq) in THF (20 mL) was added and the solution was allowed to warm to RT and subsequently stirred for 30 min at this temperature. A solution of MeI (101 μL, 1.6 mmol, 2.4 eq) in THF (20 mL) was added and the solution was heated to reflux temperature and stirred overnight. The solvents were removed *in vacuo*. Water (30 mL) was added and the water layer was extracted with TBME (3 x 30 mL) and EtOAc (1 x 30 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by automated column chromatography (eluens 0 to 100% DCM in heptanes) affording a colorless oil (20.1 mg, 0.068 mmol, 10%)

^{1}H-NMR ($CDCl_3$, in ppm): 1.21 (t, 3H), 1.38 (s, 6H), 1.59 (s, 3H), 1.60 (s, 3H), 1.63 (s, 3H), 2.00 (m, 4H), 2.24 (m, 2H), 2.44 (m, 2H), 4.18 (q, 2H), 5.03 (m, 2H), HPLC-MS: $(M+1)^+$ = 295,2

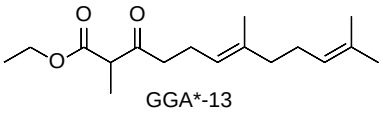


GGA*-16

(E)-Ethyl-2-ethyl-7,11-dimethyl-3-oxododeca-6,10-dienoate (GGA*-16). NaH (60% in oil, 39 mg, 0.98 mmol, 1.3 eq) was suspended in THF (6 mL), under a nitrogen atmosphere, and cooled to 0 °C with an ice/water bath. A solution of GGA*-12 (200 mg, 0.75 mmol, 1.0 eq) in THF (20 mL) was added and the solution was warmed to RT and stirred for 30 min. A solution of iodoethane (72 μL, 0.90 mmol, 1.2 eq) in THF (20 mL) was added and the mixture was heated at reflux temperature overnight and cooled to RT over the weekend. The solvents were removed *in vacuo*. Water (30 mL) was added and the water layer was extracted with TBME (3 x 30 mL). The combined organic layers were dried over

Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by automated column chromatography (eluens 0 to 100% DCM in heptanes) affording a colorless oil (37 mg, 0.13 mmol, 17%).

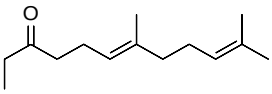
^{1}H-NMR ($CDCl_3$, in ppm): 0.93 (t, 3H), 1.29 (t, 3H), 1.60 (s, 3H), 1.62 (s, 3H), 1.71 (s, 3H), 2.00 (m, 6H), 2.30 (m, 2H), 2.58 (m, 2H), 3.39 (q, 1H), 4.20 (q, 2H), 5.04 (m, 2H), HPLC-MS: $(M+1)^+$ = 295,2



GGA*-13

(E)-Ethyl 2,7,11-trimethyl-3-oxododeca-6,10-dienoate (GGA*-13). NaH (60% in oil, 39 mg, 0.976 mmol, 1.3 eq) was suspended in THF (6 mL), under a nitrogen atmosphere. The solution was cooled to 0 °C with an ice/water bath. A solution of GGA*-12 (200 mg, 0.751 mmol, 1.0 eq) in THF (20 mL) was added and the solution was warmed to room temperature and stirred for 30 min. A solution of MeI (56 µL, 0.90 mmol, 1.2 eq) in THF (20 mL) was added and the reaction mixture was heated at reflux temperature overnight. The THF was removed *in vacuo* and water (25 mL) was added to the residue. The water layer was extracted with TBME (3 x 20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to provide a brown oil (367 mg). The crude product was purified twice by automated column chromatography (1. eluens o to 10% EtOAc in heptanes and 2. eluens 0 to 100% DCM in heptanes) affording a colorless oil (24 mg, 0.086 mmol, 9%).

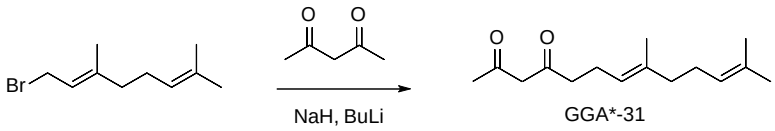
^{1}H-NMR ($CDCl_3$, in ppm): 1.29 (t, 3H), 1,31 (d, 3H), 1.60 (s, 3H), 1.62 (s, 3H), 1.71 (s, 3H), 2.00 (m, 6H), 2.30 (m, 2H), 2.58 (m, 2H), 3.39 (q, 1H), 4.20 (q, 2H), 5.04 (m, 2H), HPLC-MS: $(M+1)^+$ = 281,4



GGA*-32

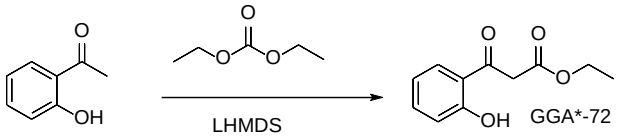
(E)-7,11-dimethyldodeca-6,10-dien-3-one (GGA*-32). GGA*-13 (24 mg, 0.086 mmol, 1.0 eq) was dissolved in EtOH (5 mL). NaOH (1.2 M, 3.6 mL, 4.3 mmol, 50 eq) was added and the solution was heated at 50 °C for 3h. The solution was acidified with acetic acid and concentrated *in vacuo*. Water (20 mL) was added to the residue and the water layer was extracted with Et_2O (3 x 20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by automated column chromatography (eluens 0 to 100% DCM in heptanes) affording a colorless oil (18 mg, 0.09 mmol, quant.).

^{1}H-NMR ($CDCl_3$, in ppm): 1.05 (t, 3H), 1.61 (s, 3H), 1,64 (s, 3H), 1.66 (s, 3H), 2.00 (m, 4H), 2.30 (m, 2H), 2.42 (m, 4H), 5.05 (m, 2H), HPLC-MS: $(M+1)^+$ = 209,3



(E)-8,12-Dimethyltrideca-7,11-diene-2,4-dione (GGA*-31). Sodium hydride (60%, 2 g, ca. 50 mmol) was added under nitrogen atmosphere to THF (125 mL) while stirring. The suspension was cooled to 0 ^{o}C for 10 min and 2,4-pentanedione (5 g, 50 mmol) was added drop-wise over ca. 3 min. A slightly exothermic reaction took place, resulting in some gas evolution and a thick white suspension was obtained. nBuLi (19 mL, 2.5 M in hexanes) was added in ca. 15 sec with a plastic syringe. A slightly yellow, clear solution was obtained. After 20 min geranyl bromide (7.3 g, 33.62 mmol) was added. The resulting suspension was stirred while warming to RT for 1h. The reaction mixture was quenched with sat. aq. NH_4Cl (70 mL). Extraction with TBME (2 x 150 mL), drying of the organic fractions with Na_2SO_4 and concentration under vacuum gave the crude product (9.66 g, >100%). Purification of a small sample by ISCO chromatography gave 63 mg pure (E)-8,12-dimethyltrideca-7,11-diene-2,4-dione (GGA*-31) as an oil.

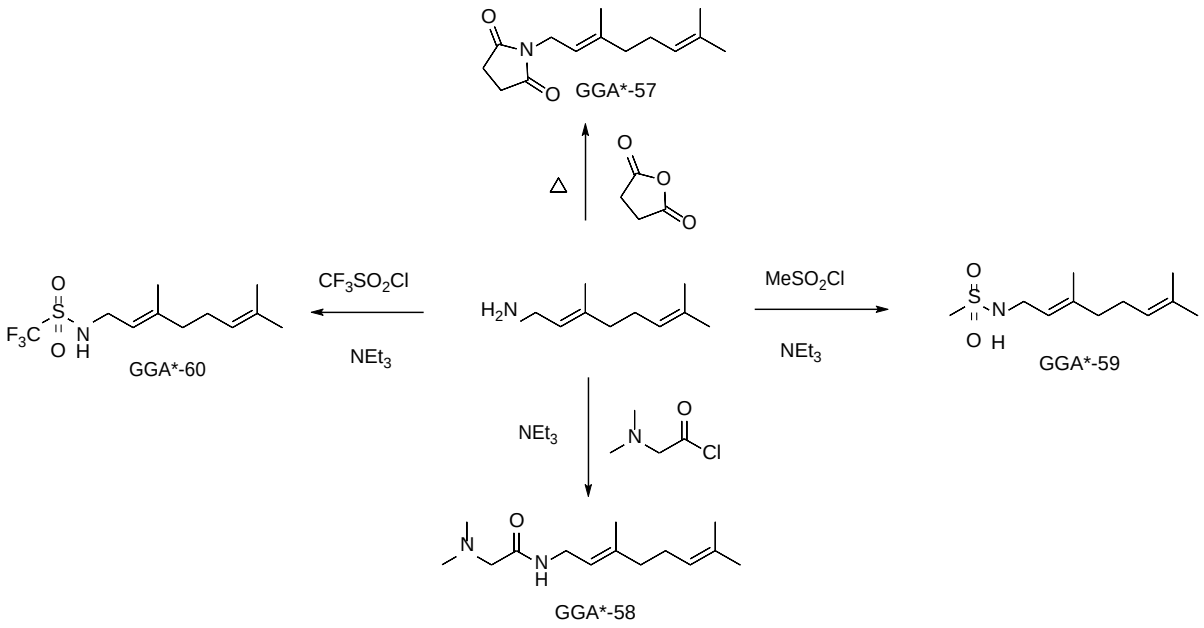
^{1}H-NMR ($CDCl_3$, in ppm): 1.70 (s, 3H), 1.72 (s, 3H), 1.77 (s, 3H), 2.01 (m, 9H), 2.35 (m, 2H), 3.59 (s, 2H), 5.10 (m, 2H), GC-MS: $(M)^+$ = 236,2



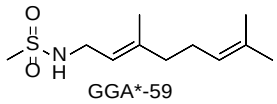
Ethyl 3-(2-hydroxyphenyl)-3-oxopropanoate (GGA*-72). A solution of LHMDS (1M, 220 mL, 220 mmol, 3.0 eq) was under N_2-atmosphere cooled to –78 °C. A solution of 2'-hydroxyacetophenone (8.84 mL, 73.5 mmol, 1.0 eq) in THF (300 mL) was added drop-wise in 30 min. The temperature was maintained at -78 °C for 1 h and 2 h at -10 °C. The solution was cooled again to -78 °C and subsequently a solution of diethylcarbonate (9.8 mL, 80.8 mmol 1.1 eq) in THF (30 mL) was added. The reaction mixture was allowed to warm to RT over the weekend and poured in a mixture of HCl (37%, 50 mL) and ice (1.5 L). The layers were separated and the water layer was extracted with DCM (2 x 500 mL). The combined organic layers were washed with brine (1 x 0.5 L), dried over Na_2SO_4 and concentrated *in vacuo* yielding a yellow oil (16.4 g). The crude product was stirred in DCM and the solids formed

were removed by filtration. The filtrate was purified by automated column chromatography (eluens, 0 to 30% EtOAc in heptanes) affording a colorless oil (11.6 g, 55.6 mmol, 76%).

^{1}H-NMR ($CDCl_3$, in ppm): 1.29 (t, 3H), 4, 01 (s, 2H), 4.23 (q, 2H), 6.95 (t, 1H), 7.02 (d, 1H), 7.58 (m, 1H), 7.70 (d, 1H). HPLC-MS: $(M+1)^+$ = 209,2

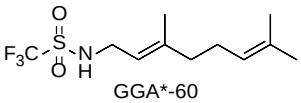


Synthesis of GGA-57, -58, -59 and -60*



(E)-N-(3,7-Dimethylocta-2,6-dien-1-yl)methanesulfonamide (GGA*-59). A solution of geranylamine (306 mg, 2.0 mmol) and triethylamine (360 mg, 3.6 mmol) in dichloromethane (2 mL) was cooled to 0 ^{o}C with an ice bath and methanesulphonyl chloride (229 mg, 2 mmol) was added. After stirring overnight while warming to RT water (10 mL) was added. Extraction with dichloromethane (2 x 10 mL), drying of the combined organic layers with Na_2SO_4 and concentration under vacuum provided crude GGA*-59. Purification by ISCO chromatography afforded (E)-N-(3,7-dimethylocta-2,6-dien-1-yl)methanesulfonamide GGA*-59 (208 mg, 0,90 mmol, 45%)

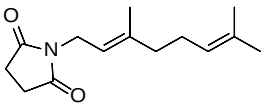
^{1}H-NMR ($CDCl_3$, in ppm): 1.60 (s, 3H), 1.68 (s, 3H), 1.70 (s, 3H), 2.05 (m, 4H), 2.99 (s, 3H), 3.79 (t, 2H), 5.10 (m, 1H), 5.22 (m, 1H). HPLC-MS: $(M+1)^+$ = 231.2



GGA*-60

(E)-N-(3,7-Dimethylocta-2,6-dien-1-yl)-1,1,1-trifluoromethanesulfonamide (GGA*-60). Geranylamine (0.50 g, 3.26 mmol, 1.0 eq) was dissolved in DCM (20 mL) and cooled in a NaCl/ice bath. Triethylamine (0.82 mL, 0.59 g, 5.87 mmol, 1.8 eq) was added and the solution cooled to -13 °C. Trifluoromethanesulfonic acid (0.55 mL, 0.920 g, 1.0 eq) was carefully added dropwise in 15 min, keeping internal temperature below -10 °C, addition was very exothermic. The reaction mixture was stirred between -15 and -11 °C for 30 min, then warmed to 0 °C and stirred at that temperature for 1 hour. Water (40 mL) and DCM were added (10 mL), the layers were mixed and separated, and the aqueous phase was extracted with DCM (2x 20 mL). The organic layers were combined, dried with sodium sulfate, filtered and concentrated *in vacuo* to afford a yellow oil (1.02 g) which was purified by ISCO chromatography (Silica, 10% EtOAc/Heptanes) yielding 223 mg (24%) of GGA*-60

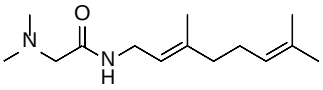
^{1}H-NMR ($CDCl_3$, in ppm): 1.60 (s, 3H), 1.68 (s, 6H), 2.07 (m, 4H), 3.92 (m, 2H), 5.05 (m, 1H), 5.22 (m, 1H). HPLC-MS: $(M-1)^+$ = 284,20



GGA*-57

(E)-1-(3,7-Dimethylocta-2,6-dien-1-yl)pyrrolidine-2,5-dione (GGA*-57). A mixture of geranylamine (300 mg, 2.0 mmol) and succinic anhydride (200 mg, 2 mmol) in dichloromethane (2 mL) was heated with a heating gun for 5 min. The reaction mixture was concentrated *in vacuo*. Purification by ISCO chromatography afforded GGA*-57 (47 mg, 0,20 mmol, 10%).

^{1}H-NMR ($CDCl_3$, in ppm): 1.58 (s, 3H), 1.65 (s, 3H), 1,78 (s, 3H), 2.01 (m, 4H), 2.70 (s, 4H), 4.09 (d, 2H), 5.03 (m, 1H), 5.17 (m, 1H). GC-MS: $(M)^+$ = 235.2



GGA*-58

(E)-2-(Dimethylamino)-N-(3,7-dimethylocta-2,6-dien-1-yl)acetamide (GGA*-58). A solution of geranylamine (311 mg, 2.03 mmol) and triethylamine (360 mg, 3.6 mmol) in dichloromethane (2 mL) was treated with N,N-dimethylaminoacetyl chloride hydrochloride salt (360 mg, 2.3 mmol) at RT. After stirring overnight at RT water (10 mL) was added. Extraction with dichloromethane (2 x 10 mL), drying of the combined organic layers with Na_2SO_4 and concentration under vacuum furnished crude GGA*-58. Purification by ISCO chromatography afforded (E)-2-(dimethylamino)-N-(3,7-dimethylocta-2,6-dien-1-yl)acetamide GGA*-58 (153 mg, 0,64 mmol, 32%).

^{1}H-NMR ($CDCl_3$, in ppm): 1.61 (s, 3H), 1.75 (s, 6H), 2.04 (m, 4H), 2.31 (s, 6H), 2.97 (s, 2H), 3.95 (t, 2H), 5.08 (m, 1H), 5.21 (m, 1H). GC-MS: $(M)^+$ = 238.2

For all compounds LogP was calculated with ChemDraw.

HL-1 mouse atrial cardiomyocytes culture, tachypacing and CaT measurements

In short, the coverslips with HL-1 cardiomyocytes were placed in 4-well rectangular dishes (Nuclon, The Netherlands) and placed into C-Dish100™-Culture Dishes (IonOptix Corporation, MA). Cardiomyocytes were subjected to tachypacing by electrical field stimulation (4,5 Hz with 20-ms pulses) for 8 h via the CPace100™-Culture Pacer (IonOptix Corporation, The Netherlands), since 8 h of tachypacing previously induced significant CaT loss.[46]

To measure CaT, HL-1 cardiomyocytes were incubated for 30 min with the Ca^{2+}-sensitive Fluo-4-AM dye (2 µM) (Invitrogen, The Netherlands), followed by 3 washing steps with Dulbecco's Modified Eagle Medium (DMEM, Gibco). CaT were live recorded in Fluo-4-AM loaded cardiomyocytes in full Claycomb medium (at 1 Hz of stimulation in a temperature (37 °C) controlled system) by exciting them at 488 nm. Light emitted at 500-550 nm was visually recorded with a 40x objective, using a Solamere-Nipkow-Confocal-Live-Cell-Imaging system (based on a Leica DM IRE2 Inverted microscope). Absolute fluorescent signals were recorded and analyzed with ImageJ software (National Institutes of Health, USA). To compare the fluorescent signals between experiments, the fluorescent signal at any given time (F) was divided by the fluorescent signal at rest (F0) (Fcal = F/F0).[47] Mean values from

each experimental condition were based on measurements of at least 10 cardiomyocytes per condition.

GGA and GGA-derivative treatment and heat shock

HL-1 cardiomyocytes, seeded into 6 wells plates, were treated with 10 µM GGA or GGA-derivative or control (DMSO) for 6 h with or without heat shock pre-treatment. In case of heat shock treatment, HL-1 cardiomyocytes were first subjected to a mild heat shock (44 °C) for 10 min, to pre-activate a heat shock response, followed by 10 min recovery at 37 °C before control (DMSO), GGA or GGA-derivative treatment.[34] HL-1 cardiomyocytes were harvested after 6 h for protein or RNA isolation. In case of CaT measurements, HL-1 cardiomyocytes were treated with 10 µM GGA or GGA-derivative or control (DMSO) for 6 h before tachypacing (pre-treatment) or immediately after tachypacing (post-treatment) for 24 h.

To check the HSF1 activation and the effect of a GGA-derivative on HSF1 activation, HL-1 cardiomyocytes were subjected to a mild heat shock (44 °C), recovered for 10 min and treated with DMSO or GGA*-59. Proteins were isolated after 10 min, 30 min, 1 h, 2 h and 6 h recovery periods.

Drosophila tachypacing and drug treatment

Wild-type W1118 *Drosophila* strain was obtained from Genetic Services Inc. (Massachusetts, USA). Flies were maintained on standard Bloomington medium, supplemented with dried yeast powder, at 25 °C. After fertilization, adult flies were removed and 200 µL GGA or GGA-derivative (final concentration 100 µM) diluted in demineralized water or control solution (demineralized water with equal amount of DMSO as compounds) was added to the food of the larvae. Larvae consumed the drug/DMSO-containing food for 2-3 days after which prepupae were collected and placed on 1% agarose gel in PBS. Per condition, at least five prepupae were subjected to tachypacing (5 Hz for 20 min) by the use of a C-Pace100 culture pacer (IonOptix Corp). Before and after tachypacing, the hearts of the prepupae were visualized through a microscope at 10x magnification, movies of the beating heart were made (3x 10 sec) before and after tachypacing with a camera and heart wall contractions were calculated in Hz as previously described in.[6,26,48]

Protein isolation and Western blot analysis

HL-1 cardiomyocytes were lysed on ice with 125 µL SDS sample loading buffer (10% SDS, 50% glycerol, 0.33 M Tris–HCl pH 6.8, 10% β-mercaptoethanol, 0.05% bromophenol blue) and passed through a syringe. After centrifugation, supernatant was collected and boiled for 6 min. Equal amounts of protein (20 µg) were separated on SDS-PAGE 4-20% PreciseTM Protein gels (Thermo Fisher Scientific, USA) and transferred onto nitrocellulose membranes (GE Healthcare, The Netherlands). Membranes were blocked in 5% skim milk for 1 h at room temperature. Overnight incubation at 4 °C with primary antibody (mouse-anti-HSPA1A, Enzo-Lifesciences, USA and Rabbit-anti-HSF1, Cell Signaling Technology, USA) was followed by secondary horseradisch peroxidase-conjugated antibody (goat-anti-mouse or goat-anti-rabbit, Dako Cytomation, Denmark) incubation for 1 h at room temperature. Western blot signals of at least two independent experiments with HL-1 cardiomyocytes were detected by Super Signal (Thermo Scientific, The Netherlands) and quantified by densitometry. The amount of protein was expressed relative to GAPDH.

RNA isolation and PCR analysis

Total RNA was extracted from HL-1 cardiomyocytes by using the NucleoSpin® II RNA isolation kit (Macherey-Nagel, Germany). cDNA was synthesized according to standard methods, using random hexamers, reverse transcriptase (RT), RT buffer, dNTP's and RNAsin (Promega, USA). 1 µg cDNA per reaction in triplicate was used as a template for real-time reverse-transcriptase PCR (qRT-PCR) which was supplemented with SYBRGreen Rox mix (5 µL per sample, Thermo Fisher Scientific, USA), primers (0,3 µL of 10 µM) (see Table 1) and water. mRNA levels were expressed in relative units based on the standard curve (serial dilutions of a pooled cDNA mix) and normalized against GAPDH.

Short interfering RNA HSPB1 knock-down

HL-1 cardiomyocytes were cultured on coverslips coated with 0.02% gelatin in Claycomb medium. After 24 h (80-90% confluency) HL-1 cardiomyocytes were cotransfected with the reporter construct CD8 and siRNA-HSPB1 construct in a ratio of 1:3 (siRNA-HSPB1: from 5′ to 3′; forward, GATCCCC GACCAAGGATGGCGTGGTG TTCAAGAGA CACCACGCCATCCTTGGTC TTTTTA; reverse, AGCTTAAAAA GACCAAGGATGGCGTGGTG TCTCTTGAA CACCACGCCATCCTTGGTC GGG) or as a control for transfection with mock siRNA (forward, GATCCCC GCTGCAAAATCCGATGAGA

TTCAAGAGA TCTCATCGGATTTTGCAGC TTTTTA; reverse, AGCTTAAAAA GCTGCAAAATCCGATGAGA TCTCTTGAA TCTCATCGGATTTTGCAGC GGG) in 1 mL Optimem with 3 µL of lipofectamine2000 per well. 24 h after transfection GGA or GGA-derivatives were added to the medium (final concentration 10 µM), incubated for 8 h and followed by tachypacing or non-pacing for 8 h. After loading the cardiomyocytes with the Fluo-4-AM dye, cardiomyocytes were incubated with anti-CD8 coated beads (11147D, Thermo Fisher). CaT measurements were performed only on viable, CD8 positive cardiomyocytes. For each condition, we measured at least 10 cardiomyocytes.

Figures

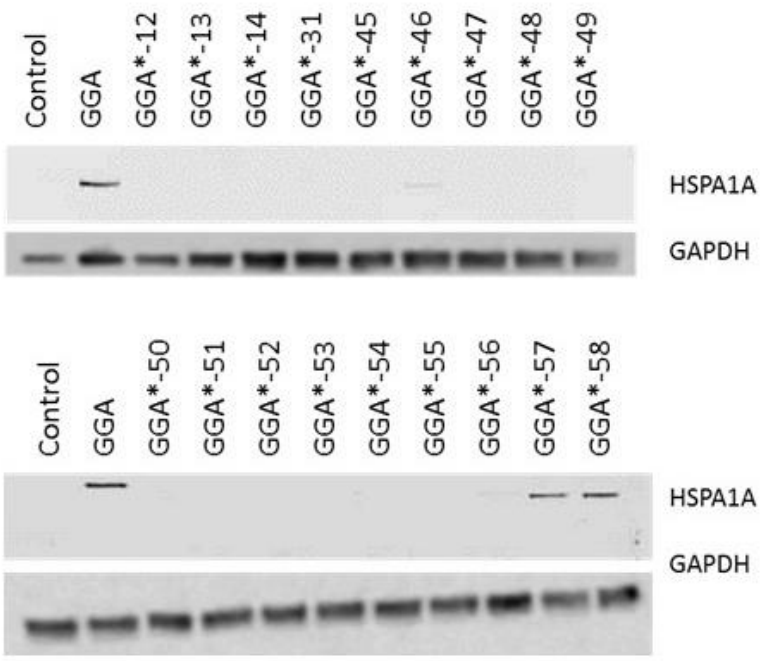


Figure S1. Treatment with GGA and GGA-derivatives gives minor induction of HSPA1A expression in HL-1 cardiomyocytes

HL-1 cardiomyocytes pre-treatment with 10 µM GGA and GGA-derivatives for 6 h reveal minor effect on HSPA1A protein expression.

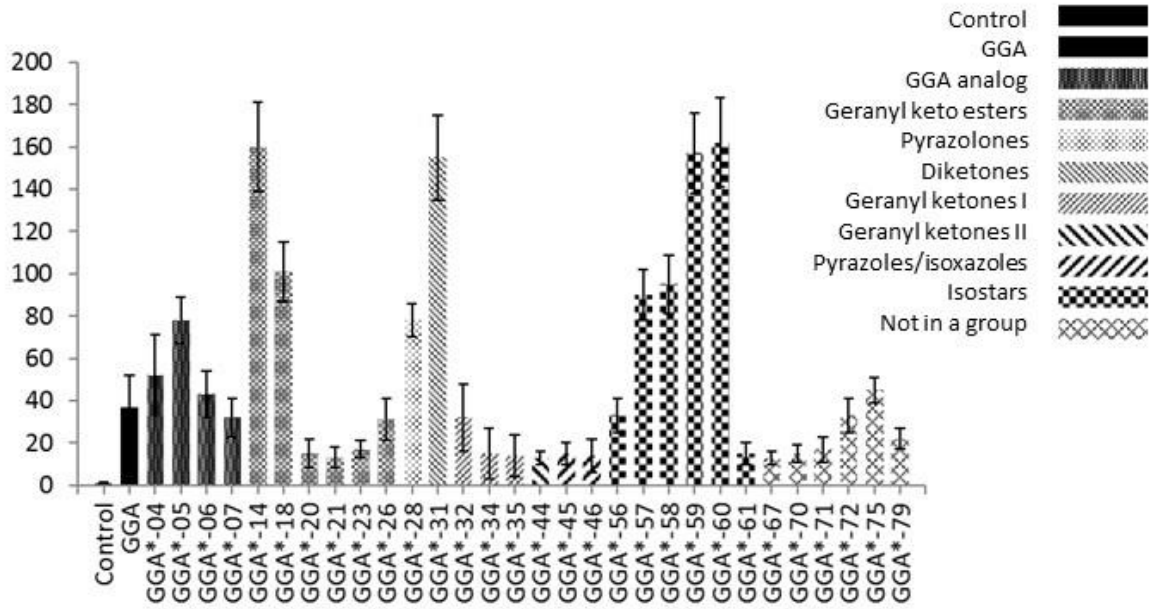


Figure S2. HSPA1A inducing effects of GGA-derivatives cannot be related to groups of same molecular structure.

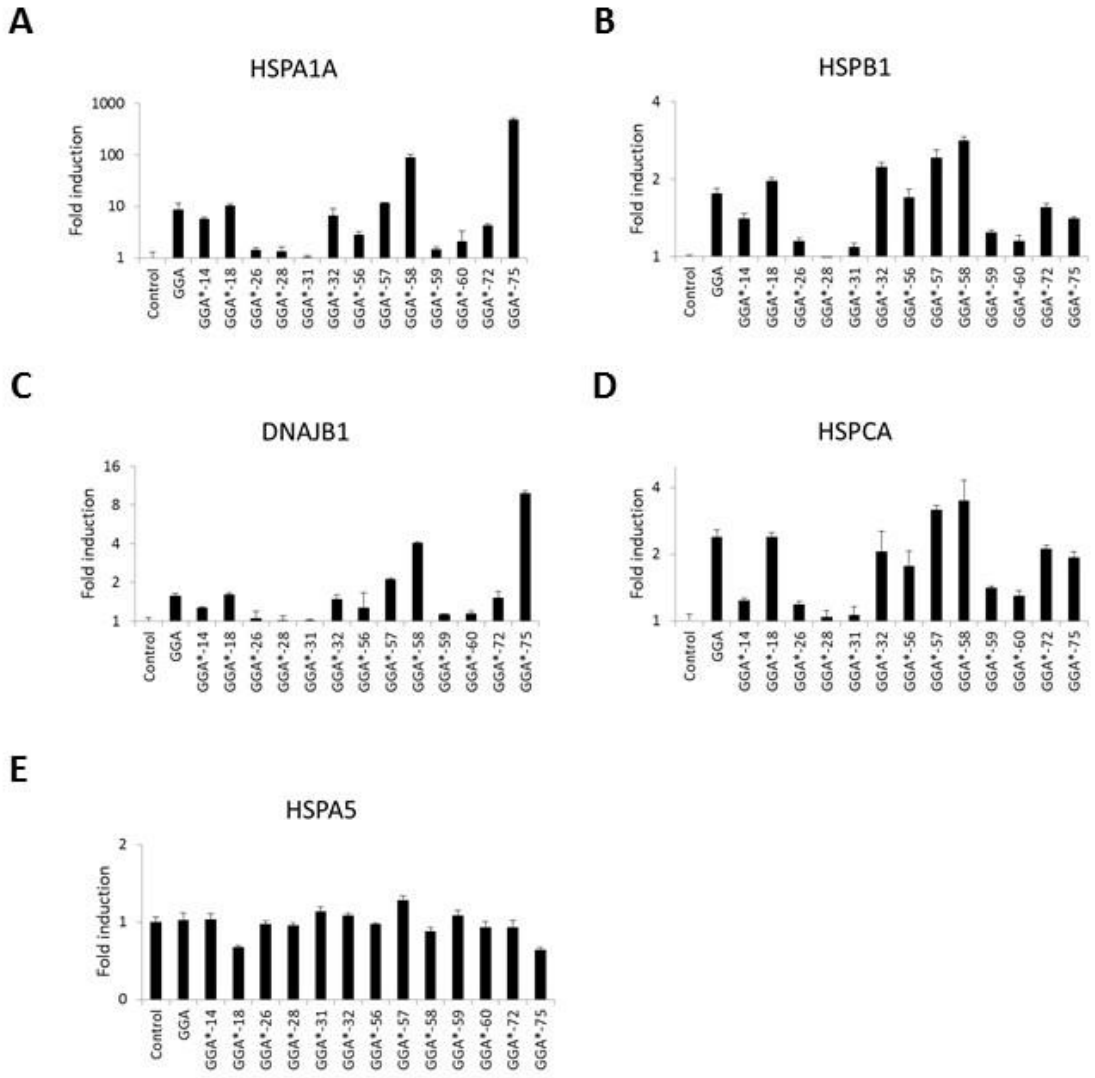


Figure S3 GGA-derivatives induce HSPA1A (**A**), HSPB1 (**B**), DNAJB1 (**C**) and HSPCA (log scale) (**D**), but not HSPA5 (linear scale, not HSF-1 mediated) (**E**) mRNA expression in heat shocked HL-1 cardiomyocytes compared to non-treated heat-shocked control HL-1 cardiomyoyctes.

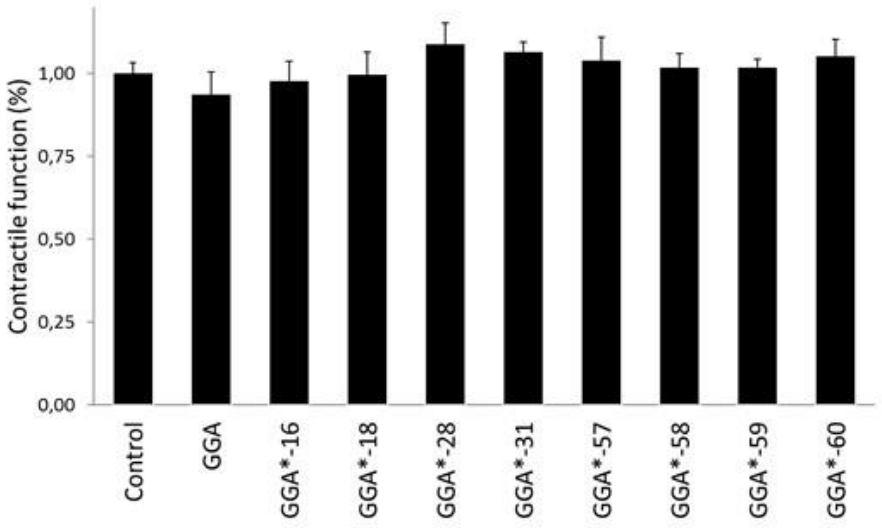


Figure S4 GGA or GGA-derivatives do not affect basal heart rate in *Drosophila* prepupae.

Video S1 Beating heart wall of nonpaced control *Drosophila* prepupa.

Video S2 Beating heart wall of tachypaced control *Drosophila* prepupa

Video S3 Beating heart wall of nonpaced *Drosophila* prepupa pretreated with GGA*-59

Video S4 Beating heart wall of tachypaced *Drosophila* prepupa pretreated with GGA*-59 (same prepupa as S3, but different part of the body to view heart wall)

References

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2. Paredes RM, Etzler JC, Watts LT, Zheng W, Lechleiter JD. Chemical calcium indicators. Methods. 2008;46(3):143–151.
3. den Hoed M, Eijgelsheim M, Esko T, et al. Identification of heart rate-associated loci and their effects on cardiac conduction and rhythm disorders. Nat Genet. 2013;45(6):621–631.