Graphical Abstract

(*E*)- *N'*-[1-(3-Oxo-3*H*-benzo[f]chromen-2-yl)ethylidene benzohydrazide protecting rat heart tissues from isoproterenol toxicity: Evidence from *in vitro* and *in vivo* tests

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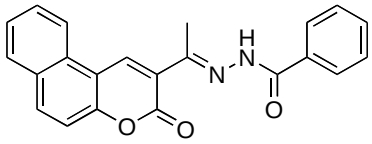

1c: (*E*)- *N'*-[1-(3-oxo-3*H*-benzo[f]chromen-2-yl)ethylidene benzohydrazide

Protection of rat heart tissues by **1c** from isoproterenol toxicity

Title page

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Abstract

The current study was aimed to assess the protective effect of a new molecule **(***E*)-*N'*-(1-(3-oxo-3*H*-benzo[f]chromen-2-yl)ethylidene)benzohydrazide, denoted **1c**, against cardiac remodeling process in isoproterenol (Isop) induced myocardial infarction (MI) in rats. Male *Wistar* rats were randomly divided into four groups, control, Isop (85 mg/kg body weight was injected subcutaneously into rats at an interval of 24 h for 2 days (6^{th} and 7^{th} day) to induce MI and pretreated animals with acenocoumarol (Ace) (150 µg/kg bw) and **1c** (150 µg/kg bw) by oral administration during 7 days and injected with isoproterenol (Isop + Ace) and (Isop + **1c**) groups. Results *in vitro* showed that **1c** is endowed with potent inhibition of angiotensin-converting enzyme (ACE) with an IC_{50} 39.12 µg/ml. The *in vivo* exploration evidenced alteration in the ECG pattern, notable cardiac hypertrophy and increase in plasma level of fibrinogen, troponin-T, CK-MB and LDH, AST and ALT by 171%, 300%, 50%, 64% and 75% respectively with histological myocardium necrosis and cells inflammatory infiltration. However, pre-treatment with **1c** improved the ECG pattern reduced significantly the cardiac dysfunction markers and ameliorated the thrombolytic process by decreasing fibrinogen level as compared to untreated infracted rats. Overall, (*E*)-*N'*-(1-(3-oxo-3*H*-benzo[f]chromen-2-yl)ethylidene)benzohydrazide **1c** could be used as anticoagulant agent to prevent thrombosis in acute myocardial infarction.

Keywords: (*E*)-*N'*-(1-(3-oxo-3*H*-benzo[f]chromen-2-yl)ethylidene)benzohydrazide; isoproterenol; myocardial infarction; ACE; thrombolytic.

1. Introduction

Myocardial infarction (MI) is a frequent cardiovascular disease and the main cause of cardiovascular deaths, which is estimated to reach 23.3 million by 2030 (World Health Organization, 2019). Myocardial infarction is the result of prolonged imbalance between the myocardial oxygen supply and demand of the myocardium leading to myocardial necrosis (Ahsan et al., 2014). Abnormalities in the electrocardiogram (ECG) pattern are detected during infarctions and reflect the severity of cardiac injuries (Nabel et al., 2012). Moreover, MI seriously induces ventricular remodeling process as an adaptive response in which damaged tissue is replaced by collagen. The left ventricle remodeling process is endowed with loss of cardiac contractility and hemodynamic properties and relates to mural hypertrophy (Sutton et al, 2000). Experimentally, the induction of MI was performed through surgical procedures or the administration of sub-acute dose of isoproterenol (Isop) in animal, which offered rapid myocardial damage similar to that seen in acute MI in humans (Mnafgui et al., 2015). Hence, isoproterenol (Fig. 1) is a synthetic catecholamine, and is a non-selective β-agonist (Sahu et al., 2014). Supra maximal administration induces myocardial damage typically in the sub-endocardial layer of the left ventricle and evidences severe myofibrillar degeneration (Shukla et al. 2015). Several previous studies reported that subcutaneous treatment with Isop induced remarkable increase in reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, and hydroxyl radicals and lipid peroxidation (LPO), which further exacerbate the myocardial damage (Ramya et al., 2014).

The design and construction of novel poly-heterocycles and/or poly-functional containing different privileged substructures within a same molecule represent an important strategy in medicinal chemistry (Abdallah et al., 2017; Chekir et al., 2018; Dong et al., 2014). In fact, it is generally proven that the presence of two or more pharmacophores linked and/or fused within a same framework could contribute to provide a significant positive effect on the overall biological efficiency in the resulting poly-functions (Filali et al., 2016). Coumarins are a class of outstanding organic molecules with a wide array of biological activities and therapeutic functions (Jameel et al., 2016), due to their significant applications such as antimicrobial (Kovvuri et al., 2018), antifungal (Elias et al., 2019), antiviral (Conti et al., 2019), anti-tyrosinase (Pintus et al., 2017), anticancer (Chen et al., 2013; Kostova, 2005), anti-thrombotic (Kirsch et al., 2016), anti-inflammatory (Fylaktakidou et al., 2004; Gagliotti Vigil de Mello and Frode, 2018), anticoagulant (Razavi et al., 2013) and anti-oxidant properties (Pérez-Cruz et al., 2018). They are predicted to play a crucial role of the MI management. In particularly, various researches suggest that the presence of functional groups at the 3-position of coumarin skeleton include specific activities (Keri et al., 2015). A similar trend is seen for hydrazones have been shown to be active in several pharmacological tests both *in vitro* and/or *in vivo* such as antiplatelet and antithrombotic activities (Chelucci et al., 2014).

Several hydrazone derivatives synthesized have been shown to promote intense vasodilatation and antihypertensive activity, for instance, 3,4-methylenedioxybenzoyl-2-thienylhydrazone (LASSBio-294) (Braga et al., 2012) and its analogue 2-thienyl-3,4-methylenedioxybenzoylhydrazone (LASSBio-1027) (Leal et al., 2012).

This encouraged us to prepare a new class of hybrid conjugate associating these two pharmacophores within a same molecular target, namely the hydrazone coumarin and study their biological activity. In this context, the present study aims to examine the anti-thrombotic, anti-inflammatory effect and the potential anti-ischemic properties of a new synthetic hydrazone coumarin (*E*)-*N'*-(1-(3-oxo-3*H*-benzo[f]chromen-2-yl)ethylidene)benzohydrazide **1c** on infracted rats.

2. Material and methods

2.1 Chemistry section

General: Thin-layer chromatography (TLC) was accomplished on 0.2-mm pre-coated plates of silica gel 60 F-254 (Merck) with appropriate eluent. Visualization was made with ultraviolet light (254 and 365 nm) or with a fluorescence indicator. Melting points were determined on a Kofler melting point apparatus and were uncorrected. HPLC analysis was performed on a Perkin Elmer chromatographic system equipped with a 200 series pump, a 20 µl automatic loop injector and UV-Visible detector. Analysis was realized on a Eurospher 100-10 C18 column (250 x 4 mm) using MeOH/water as mobile phase with appropriate composition and flow rate. The analytical process is managed by TotalChrom Navigator workstation. Infrared (IR) spectra were registered on a Perkin Elmer spectrum 100 FTIR Spectrometer apparatus using KBr pellets. ^{1}H NMR spectra were recorded on BRUKER AC 400 P (400 MHz) spectrometer and ^{13}C NMR spectra on BRUKER AC 400 P (100 MHz) spectrometer. Chemical shifts are expressed in parts per million downfield from tetramethylsilane TMS as an internal standard. Data are given in the following order: δ value, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; quint, quintuplet; m, multiplet; br, broad), number of protons, coupling constants *J* is given in Hertz. The high-resolution mass spectra (HRMS) were recorded in positive mode using direct Electrospray infusion on a Thermo Fisher Scientific Q-Exactive spectrometer at the "Centre Régional de Mesures Physiques de l'Ouest" ScanMAT platform (CRMPO platform, UMS 2001 CNRS ScanMAT, Rennes, France). Benzoylhydrazine **b**, 2-hydroxy-1-naphthaldehyde **1** purchased from Sigma-Aldrich (Tunisia), piperidine and methyl 3-oxobutanoate **a** purchased from Fluka (Tunisia), ethanol (purchased from LOBA Chemie, Tunisia) were analytical grade and used as received.

*2.1.1 2-Acetyl-3H-benzo[f]chromen-3-one (**1a**).*

In a round bottomed flask (25 ml) equipped with a condenser and magnetic stirring, piperidine (14 mg, 0.17 mmol, 10% mol) was added directly in a stirred (37 g) solution of substituted 2-hydroxy-1-naphthaldehyde **1** (0.336 g, 1.7 mmol) and ethyl 3-oxobutanoate **a** (0.2 g, 1.7 mmol) in 10 ml of ethanol pa. The resulting mixture was stirred at 80°C during 30 min. (monitored by thin layer chromatography on 0.2 mm plates of silica gel 60 F254 Merck using appropriate eluent). After cooling down to room temperature, the insoluble material was collected by filtration in a Buchner funnel (porosity N°4) and the precipitate was washed with cooled (4°C) ethanol (3 x 5 ml). The desired solid was further dried under high vacuum (10^{-3} Torr) and afforded the desired 2-acetyl-3*H*-benzo[f]chromen-3-one **1a** in 87% yield as yellowish powder, which was used later without further purification. Mp = 194-196°C. IR (KBr, cm^{-1}): 1737 (C=O, ArOCO), 1676 (C=O, MeCO), 1624 (C=C). ^{1}H NMR ($CDCl_3$): δ = 9.26 (s, 1H, H_4), 8.32 (d, *J* = 7.8 Hz, 1H, $H_{5'}$), 8.07 (d, *J* = 8.9 Hz,

1H, H_7), 7.90 (d, *J* = 7.8 Hz, 1H, $H_{6'}$), 7.73 (t, *J* = 7.8 Hz, 1H, $H_{5''}$), 7.60 (t, *J* = 7.8 Hz, 1H, $H_{6''}$), 7.44 (d, *J* = 8.9 Hz, 1H, H_8), 2.77 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ = 195.6, 159.4, 156.1 , 143.3, 136.3, 130.2, 129.8, 129.2, 126.6, 122.3, 121.8, 116.5, 112.7, 30.7.

*2.1.2 (E)-N'-(1-(3-Oxo-3H-benzo[f]chromen-2-yl)ethylidene)benzohydrazide (**1c**).*

In a round bottomed flask (25 ml) equipped with a condenser and magnetic stirring, a mixture of 2-acetyl-3*H*-benzo[f]chromen-3-one **1a** (0.3 g, 1.25 mmol)**,** benzoylhydrazine (**b**) (0.17 g, 1.25 mmol) in ethanol pa (10 ml) and a catalytic amount of glacial acetic acid (0.1% mol.) was refluxed during 12 h under vigorous magnetic stirring (37 g). Progress of the reaction mixture was monitored by thin layer chromatography on 0.2 mm plates of silica gel 60 F254 Merck using appropriate eluent. After cooling down to room temperature, the insoluble material was collected by filtration in a Buchner funnel (porosity N°4) and the precipitate was washed with cooled (4°C) ethanol (3 x 5 ml). Purification of the desired compound **1c** was conducted by recrystallization from dry ethanol, then (*E*)-*N'*-(1-(3-oxo-3*H*-benzo[f]chromen-2-yl)ethylidene)benzohydrazide **1c** was further dried under high vacuum (10^{-3} Torr) during 30 min. at room temperature which gave **1c** as pale white powder in 82% yield. Mp: 240-242°C. IR (KBr, cm^{-1}): 1694 (C=O, ArOCO), 1676 (C=O, NHCOPh), 1633 (C=N), 3302 (NH). ^{1}H NMR (DMSO-d_6): δ = 10.93 (br s, 1H, NH), 8.89 (s, 1H, H_4), 8.51 (d, *J* = 7.8 Hz, 1H, $H_{5'}$), 8.20 (d, *J* = 8.8 Hz, 1H, H_7), 7.91 (d, *J* = 7.2 Hz, 2H, H_{11}), 8.04 (d, *J* = 7.8 Hz, 1H, $H_{6'}$), 7.73 (t, *J* = 7.8 Hz, 1H, $H_{5''}$), 7.63-7.52 (m, 5H, H_{12}, H_{13}, H_8, $H_{6''}$), 2.41 (s, 3H, CH_3) ppm. ^{13}C NMR (DMSO-d_6): δ = C_2 (159.6), C_3 (126.3), $C_{3'}$ (153.8), C_4 (137.8), C_{4a} (129.4), C_5 (129.4), $C_{5'}$ (122.6), $C_{5''}$ (130.3), C_6 (113.2), $C_{6'}$ (129.3), $C_{6''}$ (126.6), C_7 (134.2), C_8 (116.8), C_{8a} (153.8), C_9 (159.6), C_{10} (129.0), C_{11} (128.5), C_{12} (128.5), $C_{12'}$ (132.1), C_{13} (128.8) ppm. HRMS (ES^+, MeOH/CH_2Cl_2 9:1), *m/z:* 379.1054 found (calculated for $C_{22}H_{16}N_2O_3Na$ $[M+Na]^+$ requires 379.1053); 395.0788 found (calculated for $C_{22}H_{16}N_2O_3K$ $[M+K]^+$ requires 395.0793).

2.2 Biology section

2.2.1 In vitro ACE inhibitory activity assays

The ACE inhibitory activity was assayed by the method reported in literature (Wu et al., 2002) with captoril as positive control. The total reaction volume of 100 µl was made up of 50 µl of 2.17 mM Hippuryl-*L*-histidyl-*L*-leucine (HHL), 25 µl of 2 mU of ACE, 25 µl tested samples in different concentrations (all prepared with 100mM borate buffer, containing 300 mM NaCl, pH 8.3) or 25 µl borate buffer for control. The mixture of HHL and sample of **1c** or ACE alone was incubated at 37°C in shaking water bath type (HETO, SBD-50, Denmark) with continuous agitation at 30 g for 10 min to achieve thermal equilibrium. The reaction was started by adding ACE to tube containing HHL and samples or borate buffer. The reaction was terminated after 30 min incubation by addition

of 300 µl of HCl (1 M) and the solution filtered through a 0.45 nm nylon syringe filter for reversed-phase (RP)-HPLC analysis. HPLC analysis was performed on a LC-10ATvp system equipped with a diode array detector (Shimadzu, Japan). ZORBAX SB-C18 column (4.6 mm id x150 mm, 5 µm particle size) was used and HA (hippuric acid) and HHL were detected at 228 nm. External standard of HA sample was prepared freshly on a daily basis and used for the qualification of HA. The peak area of HA was recorded and the percentage inhibition of ACE activity was calculated using equation:

$$\mathrm{FI} = \frac{(\mathrm{Absorbance}_{control} - \mathrm{Absorbance}_{test})}{\mathrm{Absorbance}_{control}} \times 100$$

2.2.2 Animals

In the current study, a total of 24 adult male Wistar rats with 12 weeks of age weighing 260 ± 23 g were purchased from SIPHAT (Tunisia). Their standard pellet diet was purchased from the Industrial Society of Rodents' Diet (SNA, Sfax, Tunisia). The experiments were carried out according to the local Institute Ethical Committee Guidelines for the Care and Use of laboratory animals issued by the University of Gafsa, Tunisia. After one weeks period of adaptation in the laboratory, animals were maintained under constant conditions in polypropylene cages on a 12 h light/dark cycle and at a temperature of 22 ± 2°C; humidity: 60 ± 5 % with free access to food and water. Approval for this study was received from the Ethics Committee of the Faculty of Sciences of Gafsa, Tunisia, according to the Medical Ethical Committee for the Care and Use of Laboratory Animals of Pasteur Institute of Tunis (Approval No: LNFP/Pro 152012).

2.2.3 Experimental animals

After acclimatization, animals were assigned into four groups consisting of six rats per group. Treatment was then carried out as follows:

- Group I: served as negative control (Control) rats received standard laboratory diet and drinking saline water *ad libtum*.
- Group II: (Isop) Animals received saline water for 7 days, were subcutaneously injected at the sixth day with isoproterenol (85 mg/kg/day) once per day at an interval of 24 h for 2 continued days for induction of myocardial infarction and served as isoproterenol group (Ghazouani et al., 2019).
- Group III: (Isop + Ace) Animals were pre-co treated with Acenocoumarol (150 µg/kg body weight), by gastric gavage for 7 days and injected with isoproterenol (85 mg/kg body weight) on the sixth and seventh day.

- Group IV: (Isop + **1c**) Animals received **1c** pre-co treatment (150 µg/kg body weight) by gastric gavage for 7 days and were injected with isoproterenol (85 mg/kg body weight) at the sixth day once per day, for 2 consecutive days.

2.2.4 Experimental induction of MI

Isoproterenol (Isop) was dissolved in normal saline and injected to rats (85 mg/kg BW) subcutaneously at an interval of 24 h for 2 consecutive days to induce experimental myocardial infarction (Mnafgui et al., 2015). Animals were killed 48 h after the first dose of isoproterenol.

2.2.5 Electrocardiography

At the end of the experimental period, after 24h of the second dose of isoproterenol, all rats from the four groups included in the present study were anesthetized intraperitoneally with ketamine hydrochloride (100 mg/kg bw). Needle electrodes were then inserted under the skin of the animals in lead II position. ECG recordings were taken using veterinary electrocardiograph (ECG VET 110, Biocare, China).

2.2.6 Sample collection and biochemical analysis in plasma

After recording the electrocardiogram, all the rats were killed by cervical decapitation. Blood was collected and plasma was removed from each sample, stored at - 20°C and used thereafter for various biochemical and enzymatic estimations such CK-MB (Ref. 97217 from Biolabo, France) and (LDH (Ref. 20011), ALT (Ref. 20046), AST (Ref. 20042) are purchased from Biomaghreb, Tunisia) or determination of the lipid profile (total cholesterol and triglycerides) according to the manufacturer's instructions of the commercial reagent kits purchased from Biomaghreb (Tunisia). The levels of plasma cardiac troponin-T were measured using a standard kit by electrochemiluminescence immunoassay (Roche Diagnostics, Switzerland, Ref, 07007302 119). The amount of fibrinogen was assessed using chronometry-based methods and according to the manufacturer's instructions in the commercial reagent kits (Biolabo, France, Ref. 13450). The technique is based on the work of Clauss et al. (1957), validated by Destaing et al. (1960). In the presence of an excess of thrombin, the coagulation time of previously diluted plasma is inversely proportional to the fibrinogen concentration. Immediately after kill, the heart was excised out, washed with saline and fixed in a Bouin solution for 24 h, and then embedded in paraffin. Sections of 5 µm thickness were stained with hematoxylin-eosin. The slides were photographed with an Olympus U-TU1X-2 camera connected to an Olympus CX41 microscope (Tokyo, Japan).

2.2.7 Statistical analysis

All data were expressed as mean ± standard deviation (S.D) of six rats in each group. Statistical differences between the means of the different groups were examined using a one-way analysis of variance (ANOVA) followed by the Fisher test using Statistical Package for Social Sciences (SPSS) Software Package Version 17.0. A P-value < 0.05 was considered as statistically significant.

3. Results

*3.1. Chemistry: Synthesis and structure of (E)-N'-(1-(3-oxo-3H-benzo[f]chromen-2-yl)ethyliden)benzohydrazide (**1c**)*

The synthetic route used for the preparation of the title compound **1c** was achieved only in two steps, as presented in Scheme 1. In the first step, reaction of commercial 2-hydroxy-1-naphthaldehyde **1** with methyl 3-oxobutanoate **a** produced mainly the corresponding 2-acetyl-3*H*-benzo[f]chromen-3-one **1a** in a 87% yield as yellowish powder after purification by simple washing with ethanol pa. This reaction involving a Knoevenagel condensation and a ring closure (Knoevenagel, 1898) was catalyzed by amount of piperidine. For the second step, the 2-acetyl-3*H*-benzo[f]chromen-3-one **1a** derivative was converted into its hydrazone coumarin **1c** in the presence of glacial acetic acid (0.1% mol.) as catalyst. The desired compound **1c** was purified by classical recrystallization from ethanol in 82% yield (71% overall yield) as pale white powder. Purity of compound **1c** was controlled by HPLC analysis using Eurospher 100-10 C18 column (250 x 4 mm, 5 µm spherical particle size) with UV detector at 254 nm using MeOH/water 8:2 v/v as mobile phase (flow rate = 0.5 ml/min., injection = 10 µl). For **1c**, we obtained only one peak, the retention time was measured at 2.86 min. (the corresponding chromatogram was reported in Supplementary Materials).

IR spectra comparison between the starting 2-acetyl-3*H*-benzo[f]chromen-3-one **1a** and the product of condensation **1c** showed the following points: - firstly, the disappearance of the absorption band of **1a** initially located at 1673 cm^{-1} which indicated the participation of the exocyclic ketone group in the nucleophilic addition process; - secondly, the appearance of the characteristic band for the C=N bond at 1678 cm^{-1} and the absorption band at 3300 cm^{-1} can be attributed to the NH vibrator in the desired compound **1c**.

In ^{1}H NMR spectrum of compound **1c** recorded in DMSO-d_6 solution, showed the presence of a broad singlet at d 10.93 ppm for NH proton (I = 1H), a doublet centered at δ 7.91 ppm with a coupling constant J = 7.2 Hz (I = 2H) for H_{11} and a multiplet at 7.63 < δ < 7.52 ppm attributable to the respective protons H-12, H-13, H-8 and H-6". These attributions were based on the study of the 2D-^{1}H-^{13}C (H-H COSY, HSQC and HMBC) spectroscopy and the NOESY spectra. In ^{13}C NMR

spectrum, conversion of 2-acetyl-3*H*-benzo[f]chromen-3-one **1a** into its phenylhydrazone coumarin derivative **1c** was confirmed by disappearance of signal for MeCO moiety in compound **1a** and appearance of a signal at δ 153.86 ppm can be attributed to the imino C=N group in **1c**. The *Z*- or *E*-stereochemistry of final compound **1c** was determined by NOESY experiments. In the NOESY-map, it showed correlation between NH/H_{Me} and it was in agreement for a geometric (*E*)-configuration in this sole isomer for this C=N bond. The steric hindrance of the phenylamino moiety on this C=N bond is certainly the major factor that induces this unique *E*-stereochemistry and was in agreement with results of the literature (Syakaev et al., 2006; Patorski et al., 2013; Ershov et al., 2009; Gonzaga et al., 2016; Glidewell et al., 2004). HRMS of **1c** completed the analytical data and was in accordance with this structure.

3.2 Biological assays

3.2.1 Angiotensin II- Converting Enzyme (ACE) inhibition, in vitro assay

Table 1 data showed that the compound **1c** caused a dose dependent inhibition of angiotensin converting enzyme activity with an IC_{50} 39.12 µg/ml.

3.2.2 ECG patterns of experimental groups

As shown in Fig. 2, control rats evidenced normal ECG pattern with regular sinus rhythm. However, irregular sinus rhythm with significant elevation of ST-segment and disappearance of Pardee wave were observed in ECG of untreated infracted rats as compared to normal animals. While, pretreatment with acenocoumarol (Ace, Fig. 1) did not ameliorate the ST-segment elevation. Moreover, pre-cotreatment with **1c** mitigated the ECG pattern abnormalities of infracted rats and restored the regular sinus rhythm.

*3.2.3 Effect of **1c** on heart weight to body weight ratio of experimental rats*

The effects of **1c** and acenocoumarol (Ace) pre-co treatment on heart weight to body weight ratio at the end of experiment are presented in Table 2. The heart weight to body weight ratio markedly increased ($P < 0.05$) in isoproterenol-induced rats when compared to control group by 28%. Whereas, a significant decrease ($P < 0.05$) in cardiac hypertrophy by 13 % was observed in infracted rats pre-co treated with **1c** as compared to myocardial infarction of untreated rats.

*3.2.4 Effect of **1c** on the levels of cardiac injury marker enzymes in rats*

As shown in Fig. 3**,** remarkable increase ($P < 0.05$) in plasma levels of CK-MB, LDH, troponin-T, AST and ALT by 50%, 26 %, 300%, 64% and 75% respectively in infracted rats as compared to control group. Though, prior administration of **1c** followed by isoproterenol-induced MI in rats has significantly prevented the depletion of these myocardial markers as evidenced by a significant

decrease (P<0.05) in the levels of all the above mentioned biomarkers by 31 %, 6%, 39%, 14 % and 40 % respectively when compared to isoproterenol (Isop) induced infracted rats. Nearly, the same effect was shown by pre-co treatment with acenocoumarol (Ace).

3.2.5 Plasma fibrinogen level in the experimental groups

As shown in Fig. 4**,** our results showed that Isop-induced rats exhibited a significant rise ($P < 0.05$) in plasma fibrinogen by 171 % when compared to control group. However, pre-co treatment with **1c** or Ace significantly reduced the plasma fibrinogen level ($P < 0.05$) by 42 % and 40 %, respectively, in comparison to Isop alone-administered rats.

*3.2.6 Effect of **1c** on lipid profile*

As shown in Table 3, animals treated with Isop showed remarkable increases ($P < 0.05$) in total cholesterol and triglycerides levels by 111% and 21 % respectively as compared to normal control group. However, pre-co treatment either with **1c** or Ace induced significant decrease in these two parameters activity by 35% and 15 % respectively.

3.2.7. Histopathological observations of heart tissues

Normal myocardium architecture was observed in the control group. However, the non-treated infarcted group showed myocardial cells necrosis, separation of cardiac myofibrillar and large inflammatory cells infiltration. While pre-co treatment with **1c** improved the normal myocardial arrangement and transverse striations (Fig. 5).

4. Discussion

Myocardial infarction (MI) is a leading life-menacing manifestation of ischemic heart disease (IHD) causing mortality worldwide (Benjamin et al., 2017). Clinical searches have shown that statins therapy can prevent or ameliorate cardiac injury. Rather, evidence has shown that statins is able to cause various adverse effect (Attalla et al., 2019). Nowadays, the coumarins and its derivatives have being used for the counteracting effect and treatment of cardiac dysfunction (Mnafgui et al., 2019). These bioantioxidants may play key role in help in cardiac remodeling and preventing disease by its anti-inflammatory, anticoagulant, antithrombotic, antioxidant, and hypolipidemic effects (Kontogiorgis et al. 2015; Paracatu et al. 2016; Srikrishna et al. 2018). Hence, based on the aforementioned literature, the present study was designed to investigate, for the first time, the potential preventive capacity of a novel synthetic hydrazone coumarin, (E)-N'-(1-(3-oxo-3H-benzo[f]chromen-2-yl) ethylidene) benzohydrazide "**1c**", against MI induced in experimental male Wistar rats.

In vitro, results showed that the compound 1c caused a dose dependent inhibition of angiotensin converting enzyme activity with an IC_{50} 39.12 µg/ml. Previous studies have reported that ACE

inhibitors were widely used in treatment of myocardial infarction to prevent left ventricular remodeling process (Mnafgui et al., 2016; Daoud et al., 2017). In fact, the inhibition of ACE activity inactivated expression of cytokine transforming growth factor (TGF-β1), a major mediator of the remodeling process and fibrosis tissues (Border et al., 1994; Borghi et al., 2006).

An electrocardiogram (ECG) is a medical test that detects infarction by measuring the electrical activity generated by the heart as it contracts (Daoud et al., 2017). ECG recordings in Isop treated groups revealed ischemic and conduction abnormalities as demonstrated by significant elevation of ST segments and unidentifiable P wave. These conduction abnormalities indicate the presence myocardial necrosis of consequent loss of cell membrane in an injured myocardium as reported by Mnafgui et al., 2016. Pre-treatment with the synthesized hydrazone coumarin **1c** restored normal ECG pattern, suggestive of its cell membrane protective effects.

In this work, all treated groups showed insignificant change in the body weight of the rats, and increase in heart weight to body weight ratio indicating hypertrophy which was significantly improved only by **1c** pre-treatment. The observed effect in relative heart weight could be related to invasion of inflammatory cells and increased fibrosis (Mnafgui et al., 2015) as confirmed by biochemical and histological examinations in the present work.

Elevated cardiac enzymes are well-known markers of myocardial infarction. Isop induced MI promotes rupture of cardiac membrane and subsequent leakage of these enzymes into the plasma (Khalil et al., 2015; Paula et al., 2017). The current findings revealed deterioration in the cardiac function as shown by increased plasma levels of LDH, ALT, AST and CK-MB activities, in accordance with recent studies of Paula et al. 2017 and Khan et al. 2018. Isop treated rats showed also elevated troponin levels, which predict the risk of both cardiac death and subsequent infarction (Saravanan, et al., 2013). Interestingly, **1c** pre-treatment revealed a significant decrease in plasma amounts of all these cardiac markers. The decreased amounts observed in these enzymes were close to these pretreated in with acenocoumarol.

This data indicating that the synthesized antioxidant compound might could prevent rats against isoproterenol toxicity in the heart, by maintaining myocardial membrane integrity and restricting the leakage of cardiac enzymes into the circulation, as signaled by Ghazouani et al., 2019.

Hyperlipidaemia and hypercholesterolaemia has been reported to be the major pathogenesis in the progression and development of the pathogenesis of heart and myocardial infarction (Abbas 2016). In the current study, results showed also that the levels of TC, TGs increased in plasma of treated rats compared to the control group. The increased level of cholesterol in Isop treated rats is due to increased level of LDL-C taken from the blood circulation (Anandan et al., 2007). For TGs, may be due to increased hydrolysis of stored triglyceride via hormone-sensitive lipase activity (Khalil et al., 2015). However, a strong inhibition of the alteration of lipids profile by **1c** was observed in plasma

of treated rats, which may be due to the hypocholesterolaemic and hypolipidemic activities of **1c** (Pari et al., 2014).

Several prospective epidemiological studies and clinical observations have reported a wide range of data regarding the potential role of fibrinogen as a biomarker of cardiovascular disease (Danesh et al., 2005). Indeed, fibrinogen is one of the most common hemostatic factors that contributes to thrombosis and development of atherosclerosis (Machlus et al., 2011). Further, several factors involved in coagulation or thrombosis are associated with acute myocardial infarction and inflammation, including high fibrinogen concentrations by promoting thrombus formation and altering coagulation kinetics (Mohseni et al., 2014).

Results of the current study showed that isoproterenol intoxication induced increase in the amount of this glycoprotein, which could explain in part the observed cardiac damage. However, pretreatment with **1c** lessened the increased level of fibrinogen produced by isoproterenol metabolism thereby inhibited the thrombosis phenomenon, which prevented cardiac damage, and myocardial injury. Similar results were noted by Daoud et al., 2017 and Ghazouani et al., 2019. In fact, **1c** could inhibit the thrombotic process by acting as a vitamin K antagonist (VKA) through preventing carboxylation of glutamic acid residues of vitamin K-dependent coagulation factors II, VII, IX, and X, a crucial step in the coagulation process (Ufer, 2005; Ghazouani et al., 2019; Mnafgui et al., 2019).

Histopathology study of the MI group evidenced focal lesions in the cardiac tissue, separation of cardiac myofibrillar and large inflammatory cells infiltration, which are in accordance with previous searchs (Mnafgui et al., 2016). The preventive effects of **1c** on the abolition of myocardium cells necrosis could be attributed to their antioxidant potential (Attalla et al., 2018). In fact, the composition of this new synthesized hydrazone coumarin containing different privileged substructures within a same molecule (coumarin) and function (hydrazone), represent a good strategy to fight against oxidative stress (Mnafgui et al., 2019).

5. Conclusion

In conclusion, the present study revealed that isoproterenol induced in rats an increased level of plasma cardiac troponin-T, plasmatic cardiac markers and elevated ST-segments, as well as, increased levels of plasma cholesterol and triglyceride. Pre- and co-treatment with **1c** ameliorated cardiac injury, dyslipidemia and elevated ST-segments in isoproterenol induced myocardial infarcted rats. Histopathology of myocardium correlated with these biochemical findings. Thus, the compound named **(***E*)-*N'*-(1-(3-oxo-3*H*-benzo[f]chromen-2-yl) ethylidene) benzohydrazide **1c** could be used as an anticoagulant agent to prevent thrombosis in acute myocardial infarction.

Declaration of interest

The authors of the present work declare no conflict of interest The authors alone are responsible for the content and writing of this article.

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Supplementary Material

The following contents as IR, ^{1}H and ^{13}C NMR, H-H COSY, HSQC, HMBC, NOESY for compounds **1a** and **1c** are available online at https://dx.doi.org/10.1016/j.ejphar.2019.xx.xxx.

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Scheme, Figures and Table legends

Scheme 1: Preparation of (*E*)-*N'*-(1-(3-oxo-3H-benzo[f]chromen-2-yl)ethylidene)benzohydrazide **1c**

Fig 1: Structures of isoproterenol (Isop) and acenocoumarol (Ace)

Fig 2: Effect of **1c** on electrocardiographic (ECG) pattern in normal and experimental rats. Control: Recording of the control rats. Isop: Recordings of the rats injected with isoproterenol (85 mg/kg). Isop + Ace: Recording of rats treated with acenocoumarol (Ace) and isoproterenol (Isop). Isop + **1c**: Recording of rats treated with **1c** and isoproterenol.

Fig 3: Variation of plasma markers of the myocardial infarction in control and treated rats. Values are given as mean ± standard deviation / S.D. for groups of 6 animals each. The values are statistically introduced as follows: * $P < 0.05$ significant difference compared to the control rats; $^{\#}$ $P < 0.05$ significant difference compared to isoproterenol (Isop) groups; $^{@}$ $P < 0.05$ significant difference compared to treated rats with Acenocoumarol (Ace). Fig. 3 (A): The creatine kinase (CK-MB), Fig. 3 (B): the lactate deshydrogenase (LDH) and Fig.3 (C): the troponin (cTn-1), Fig.3 (D): the aspartate aminotransferase (AST), and Fig.3 (E): the alanine aminotransferase (ALT).

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Fig 4: Plasma fibrinogen level variation in control and treated rats. Values are given as mean ± standard deviation/ S.D. for groups of 6 animals each. The values are statistically presented as follows: * $P < 0.05$ significant difference compared to the control rats; $^{\#}$ $P < 0.05$ significant differences compared to isoproterenol groups.

Fig 5: Impact of 1c pre-co-treatment on histology of myocardial tissue (H&E X 400). Control group revealed normal myocardial structure with clear transverse striations. Isop treated group showing myocardial cells necrosis with separation of cardiac myofibrillar and excessive leukocyte infiltration.. Isop+Ace treated group showing moderate injury with few inflammatory cell infiltration. Isop+1c showing normal myocardial arrangement and limited focal neutrophil infiltration in a small area.

⟹ leucocyte infiltration; ★ separation of cardiac myofibrillar; ⟶◆ cells necrosis

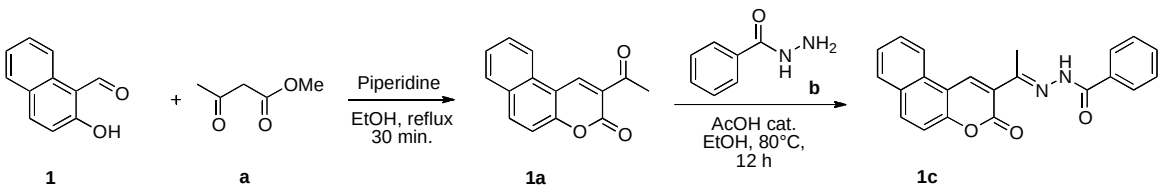
Table 1: Angiotension converting enzyme inhibition activity of compound **1c**

Table 2: Effect of **1c** pre-treatment on heart weight index (HWI) in isoproterenol (Isop) induced myocardial infraction in rats. (HWI) was computed as HWI = heart weight (HW)/body weight (BW) × 100. Values are given as mean ± standard deviation/S.D. for groups of 6 animals each.

Table 3: Effect of **(1c)** on biochemical parameters in plasma of experimental rats. (TC) total cholesterol, (TG) triglyceride.

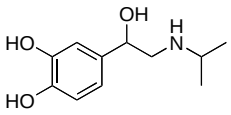
Schemes

Scheme 1.

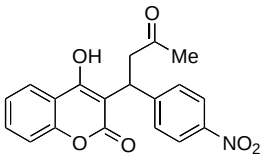


1 **a** **1a** **1c**

Fig.1



Isoproterenol (Isop)



Acenocoumarol (Ace)

Fig. 2

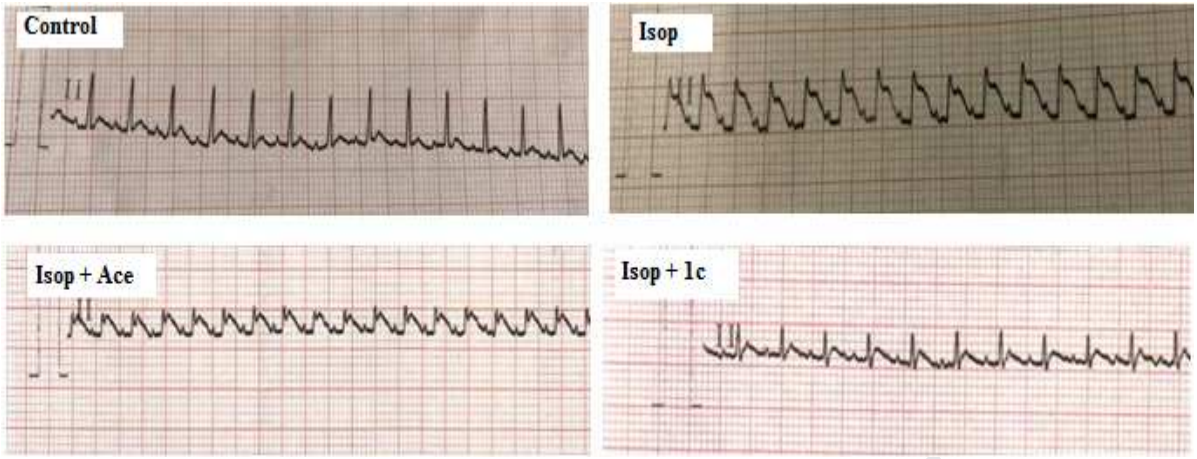


Fig. 3.

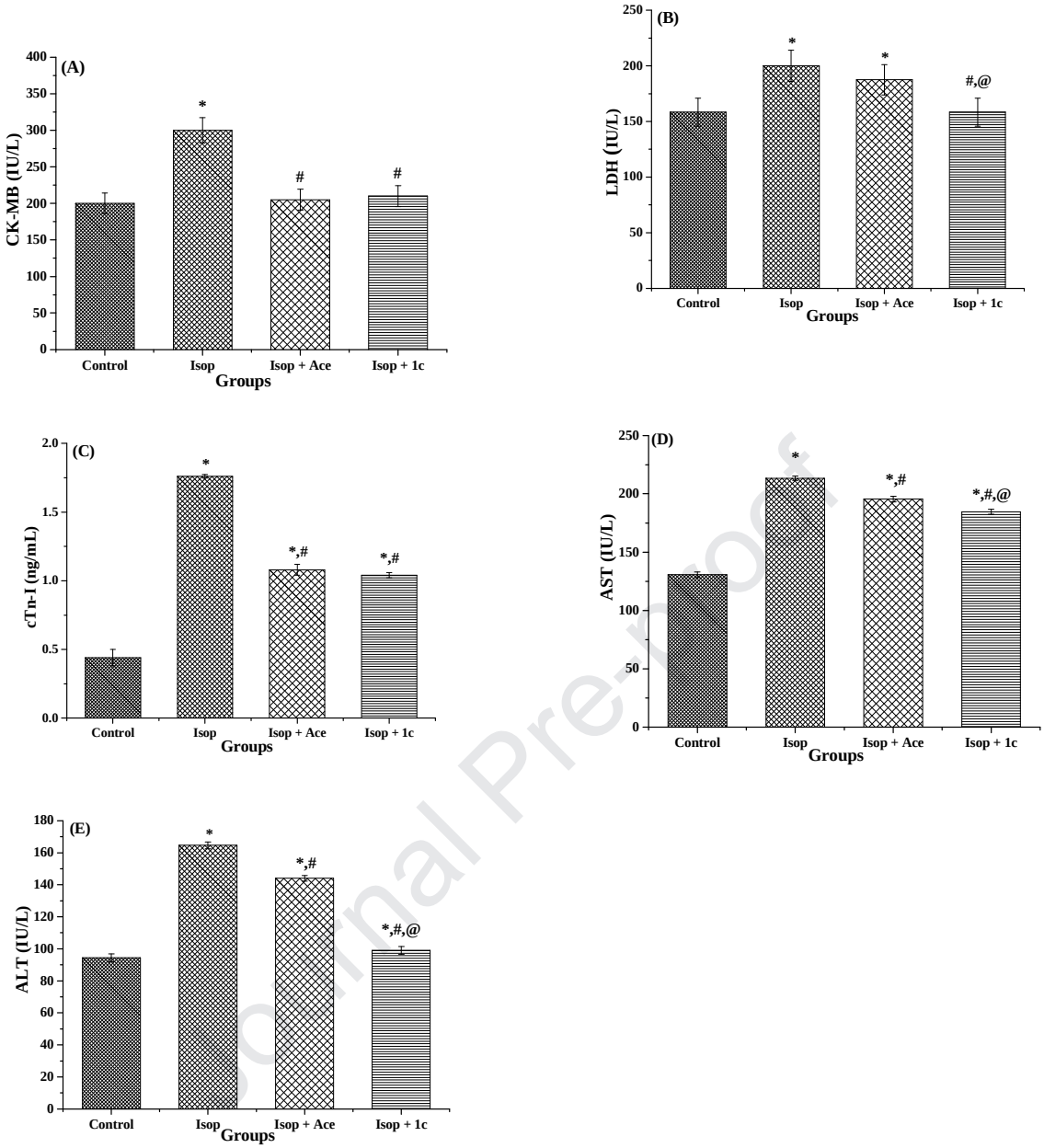


Fig. 4

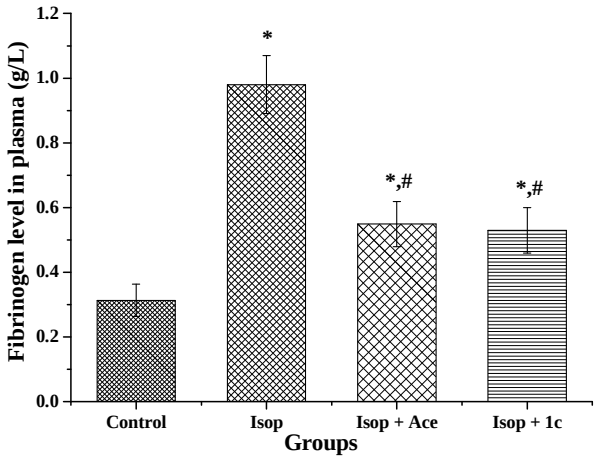
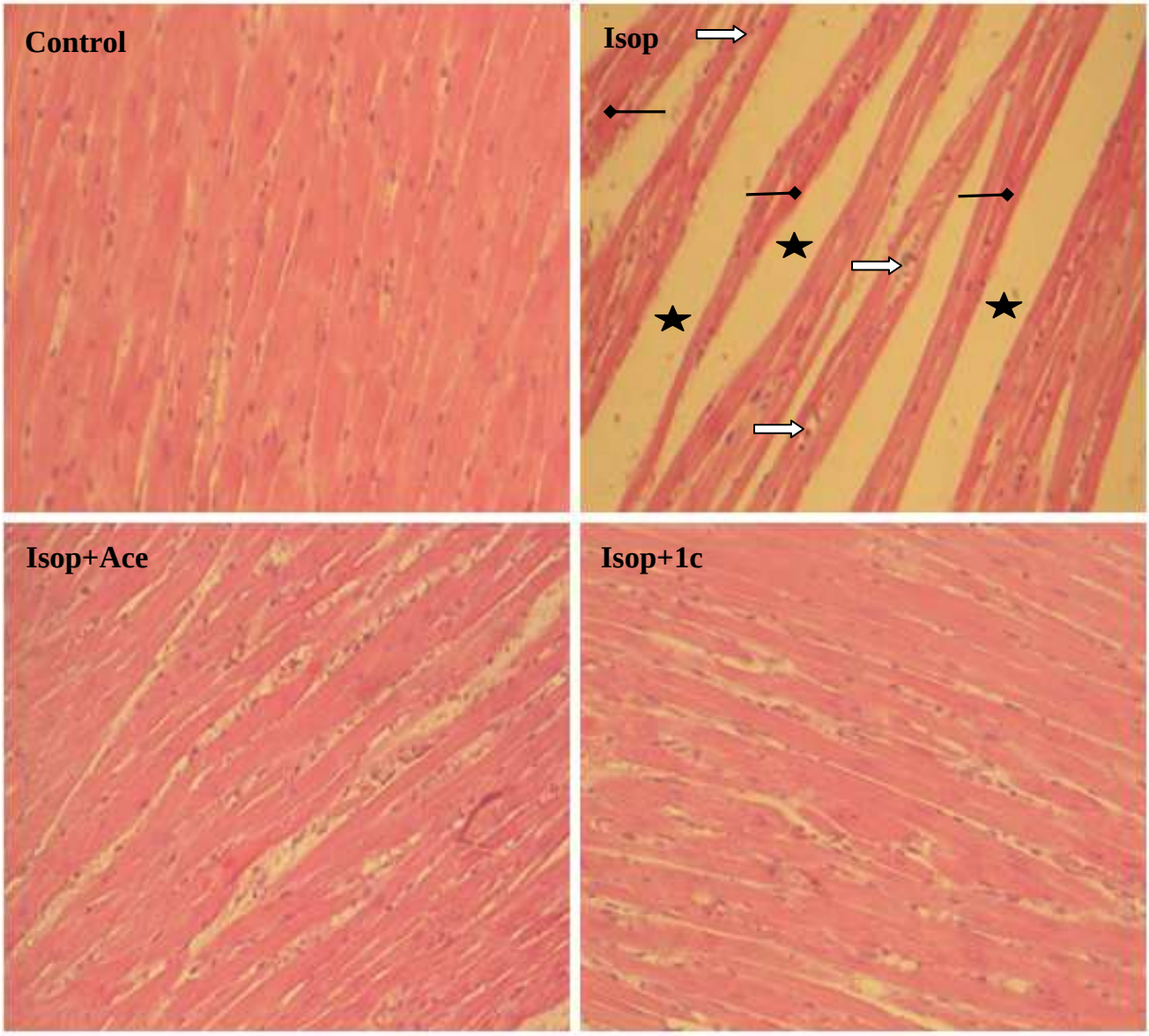


Fig. 5



Tables

Table 1: Angiotension converting enzyme inhibition activity of compound **1c**

Concentration of **1c** (µg/ml)	% Inhibition	IC_{50} (µg/ml)
25	31.33 ± 1.44	
50	63.90 ± 1.89	
75	70.53 ± 1.22	39.12
100	80.55 ± 1.11	
200	81.70 ± 1.08	

The data are expressed in mean ± S.E.M. n = 3 in each tested concentration.

Table 2: Effect of **1c** pre-treatment on heart weight index (HWI) in isoproterenol (Isop) induced myocardial infraction in rats. (HWI) was computed as HWI = heart weight (HW)/body weight (BW) × 100. Values are given as mean ± standard deviation/S.D. for groups of 6 animals each.

Parameters	Control	Isop	Isop + Ace	Isop + **1c**
Initial BW (g)	261.63 ± 20.11	261.33 ± 19.71	260.83 ± 20.14	261.83 ± 19.90
Final BW (g)	272.70 ± 20.71	273.70 ± 21.00	283.60 ± 20.00	280.20 ± 20.90
Heart weight (g)	0.73 ± 0.03	0.95 ± 0.02[a]	0.86 ± 0.02[a, b]	0.80 ± 0.01[a, b, c]
HWI	0.28 ± 0.04	0.35 ± 0.02[a]	0.30 ± 0.01[b]	0.29 ± 0.01[b]

BW: Body Weight (g)

Values are statistically introduced as follows: [a] $P < 0.05$ significant difference compared to control rats, [b] $P < 0.05$ significant difference compared to treated rats with isoprotenol (Isop), [c] $P < 0.05$ significant difference compared to treated rats with acenocoumarol (Ace).

Table 3: Effect of **(1c)** on biochemical parameters in plasma of experimental rats. (TC) total cholesterol, (TG) triglyceride

Parameters	Control	Isop	Isop + Ace	Isop + **1c**
TC (mg/dl)	34.5 ± 1.35	72.8 ± 1.29[a]	46.0 ± 1.45[a,b]	47.0 ± 1.01[a, b]
TG (mg/dl)	74.6 ± 1.00	90.4 ± 1.49[a]	75. 1 ± 1.51[a,b]	76.7 ± 1.20[a, b]

Values are given as mean ± standard deviation / S.D. for groups of 6 animals each. The values are statistically presented as follows: [a] $P < 0.05$ significant difference compared to the control rats; [b] $P < 0.05$ significant difference compared to treated rats with isoprotenol (Isop).

Highlights:

- Synthesis of new (*E*)- *N'*-[1-(3-oxo-3*H*-benzo[f]chromen-2-yl)ethylidene benzohydrazide **1c**
- *In vitro* tests showed potent inhibition of angiotensine I-converting enzyme (ACE)
- *In vivo* assays (on rats), pre-treatment with **1c** improved the ECG pattern
- **1c** reduced the cardiac dysfunction markers and ameliorated the thrombolytic process