ChemInform Abstract: Synthesis and Biological Evaluation of 5-Membered Spiro Heterocycle-Benzopyran Derivatives Against Myocardial Ischemia.

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · MARCH 2011

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2011.01.003 · Source: PubMed

8 AUTHORS, INCLUDING:

Simona Rapposelli
Università di Pisa
75 PUBLICATIONS **852** CITATIONS
SEE PROFILE

Vincenzo Calderone
Università di Pisa
139 PUBLICATIONS **1,779** CITATIONS
SEE PROFILE

Alma Martelli
Università di Pisa
65 PUBLICATIONS **718** CITATIONS
SEE PROFILE

Lara Testai
Università di Pisa
64 PUBLICATIONS **841** CITATIONS
SEE PROFILE

European Journal of Medicinal Chemistry 46 (2011) 966–973

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis and biological evaluation of 5-membered spiro heterocycle-benzopyran derivatives against myocardial ischemia

Simona Rapposelli [a,*], Maria Cristina Breschi [b], Vincenzo Calderone [b,**], Maria Digiacomo [a], Alma Martelli [b], Lara Testai [b], Michael Vanni [a], Aldo Balsamo [a]

[a] Dipartimento di Scienze Farmaceutiche, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy
[b] Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy

ARTICLE INFO

Article history:
Received 20 July 2010
Received in revised form
25 November 2010
Accepted 6 January 2011
Available online 13 January 2011

Keywords:
Mito-K_{ATP} channel openers
Myocardial ischemia
Mitochondrial ATP-sensitive potassium channels
Spiro oxazolidinones
Imino-spirooxazolidindiones
Spiro-substituted benzopyrans

ABSTRACT

The activation of ATP-sensitive potassium channels (K_{ATP}), play a key role in an endogenous "self-defence" mechanism, known as ischemic preconditioning (IPC), which is fundamentally involved in the protection of the heart against the ischemia/reperfusion injury. Presently, it is widely accepted that IPC is mainly (albeit not exclusively) mediated by the activation of K_{ATP} channels expressed in the mitochondrial inner membrane (mito-K_{ATP}) rather than the sarcoplasmatic ones (sarc-K_{ATP}). Consistently, exogenous activation of K_{ATP} channels by pharmacological tools can be viewed as one of the most promising strategies for the therapy of myocardial ischemia. As part of our research program devoted to the synthesis and the evaluation of new cardioprotective agents, we extensively studied several six-membered spiro-heterocycle-benzopyran compounds endowed of a significant anti-ischemic activity. The positive results obtained, prompted us to further explore the influence on the biopharmacological effects, of the spiro-substitution at C4 benzopyran nucleus by replacing the six-membered spirocycle of the most active compounds with 5-membered-one.

The preliminary evaluation of the new compounds on cultured H9c2 cardiomyoblasts exposed to anoxia/reperfusion and on Langendorff-perfused rat hearts submitted to ischemia/reperfusion cycles, showed that some of them can exert a cardioprotective effect. This anti-ischemic activity was antagonized by 5-hydroxydecanoic acid, a selective blocker of mito-K_{ATP} channels, confirming the involvement of this channel in the cardioprotective activity.

1. Introduction

It is presently well-known that brief periods of ischemia are able to protect myocardial tissue from following and more prolonged ischemic events [1]. Such a "self-defence" mechanism, known as ischemic preconditioning (IPC), involves several receptor-dependent and receptor-independent processes. Pharmaceutical agents able to mimic IPC could represent the basis of an innovative rational therapy for myocardial ischemia [2]. It is widely accepted that the activation of cardiac ATP-sensitive potassium channels (K_{ATP}) is probably the most significant among the various signals involved in IPC. K_{ATP} channels have been clearly identified in both sarcolemmal and mitochondrial membranes (sarc- and mito-K_{ATP}, respectively). Recent experimental data indicate that the endogenous activation of mito-K_{ATP} channels play a key role in anti-ischemic myocardial protection [3].

Ischemia is associated with the accumulation of Ca^{++} ions into the mitochondrial matrix, and this is a key step for the irreversible formation of the mitochondrial membrane permeability transition pore (MPTP) during reperfusion [4]. Experimental evidences highlight that an increased influx of K^+ ions into the matrix accounts for a depolarization of the mitochondrial inner membrane, thus reducing the driving force for Ca^{++} uptake into the matrix [5,6]. Therefore, the activation of mito-K_{ATP}-mediated flows of K^+ ions in the ischemic phase might prevent Ca^{++} accumulation in the matrix, blunting the following irreversible opening of MPTP in the reperfusion phase [7] (Fig. 1).

Several well-known non-selective sarc-/mito-K_{ATP}-openers (KCOs), such as cromakalim, bimakalim and diazoxide, elicit strong cardioprotective effects both in *in vitro* and *in vivo* models of myocardial ischemia. Since a non-selective K_{ATP}-activation can

Abbreviations: IPC, Ischemic preconditioning; MPTP, Membrane permeability transition pore; RPP, Rate pressure product.

* Corresponding author. Tel.: +39 050 2219582; fax: +39 050 2219577.
** Corresponding author. Tel.: +39 050 2219589; fax: +39 050 2219609.
E-mail addresses: simona.rapposelli@farm.unipi.it (S. Rapposelli), vincenzo.calderone@farm.unipi.it (V. Calderone).

0223-5234/$ – see front matter © 2011 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2011.01.003

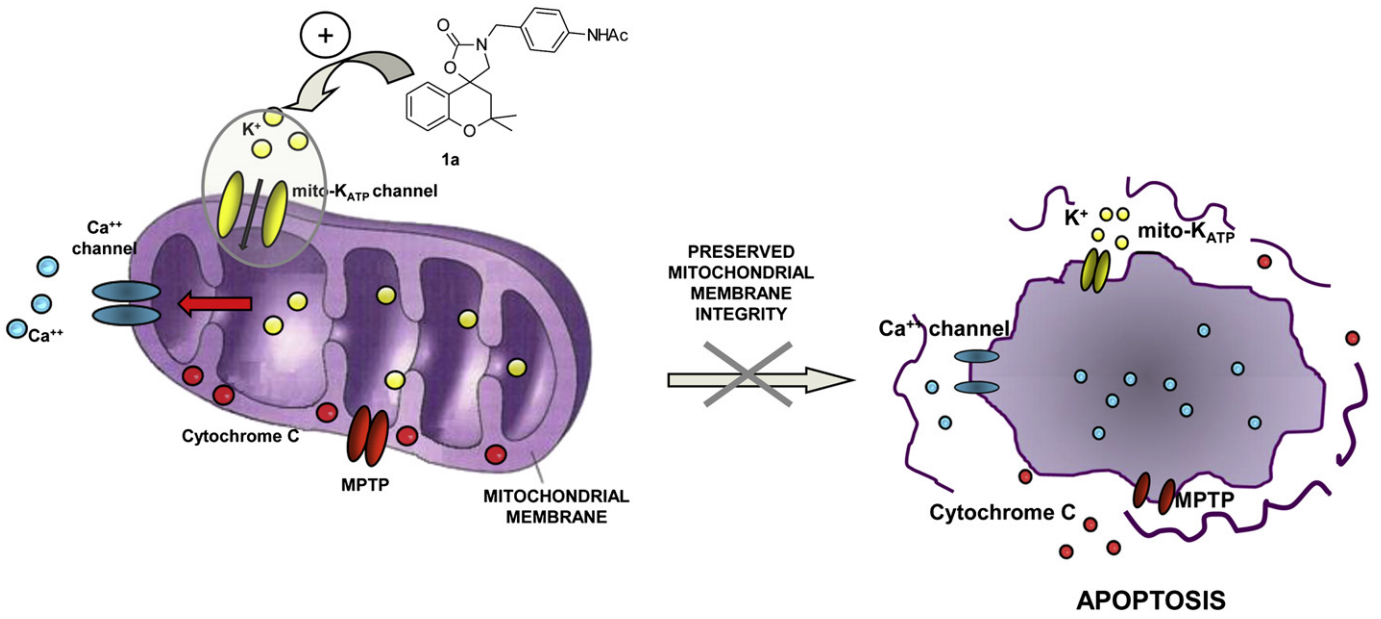


Fig. 1. Schematic and partial description of one of the different mechanisms which link the activation of mito-K_{ATP} channels with the mitochondrial calcium movements and the activation of the membrane permeability transition pore (MPTP). The activation of mito-K_{ATP} channels causes an in-ward flow of potassium ions (yellow circles), a membrane depolarization and thus a reduced driving-force for calcium accumulation into the matrix (blue circles), limiting the formation of MPTP and its opening. This mechanism can, at least in part, reduce the release of mitochondrial pro-apoptotic factors during reperfusion and thus preserve the mitochondrial membrane integrity. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

trigger a plethora of undesired effects, such as hypotension, vasodilatation and hyperglycemia, related to the activation of sarc-K_{ATP} channels expressed in myocardiocytes, vascular smooth muscle cells and pancreatic β-cells [8,9], the possibility to arrange of molecules that selectively activate mito-K_{ATP} channels seem to be a challenging goal in drug discovery. To date, few examples of selective activators of mito-K_{ATP} channel exist. Among them, it may be included the benzopyran derivative BMS-180448, which exhibits anti-ischemic activity and poor vasorelaxing effects, and BMS-191095, which is at least 30-fold more selective than BMS-180448 [10,11] (Fig. 2). Despite the large number of chemical modifications on the benzopyran scaffold, only in a few cases the insertion of C4 in a conformationally restricted nucleus has been reported, although both X-ray and NMR studies on cromakalim and the results obtained with other conformationally restricted 4-spiro-benzopyran derivatives showed to confer a good activity towards K_{ATP} channel [12]. Bearing in mind these informations and the SAR hypothesis suggesting the presence of lipophilic substituents as structural requirements for cardio-selectivity [13], our research targeted on the identification of an original collection of new C4 spiro-substituted benzopyran derivatives variously substituted with additional lipophilic groups on the spirocycle. First, we synthesized a limited number of new constricted benzopyran derivatives in which the C4 carbon is inserted in a spiromorphone or a spiro morfoline ring (SMBs) (Fig. 2) [14,15]. Some derivatives showed cardioprotective activity on Langendorff-perfused rat hearts submitted to ischemia–reperfusion cycle (I/R), with modest effects on vascular smooth muscle. Among them, the compound **A**(4′-(N-(4-acetamidobenzyl))-2,2-dimethyl-2,3-dihydro-5′H-spiro[chromene-4,2′-[1,4]oxazinan-5′-one) (Fig. 3), showed

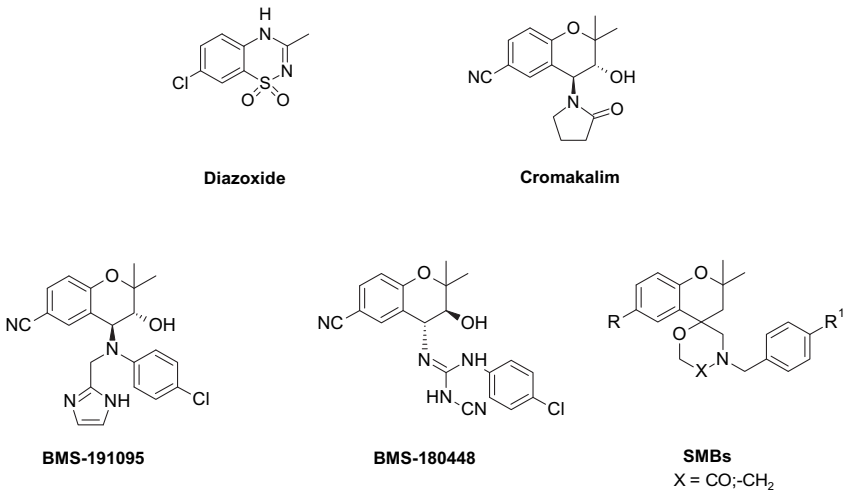
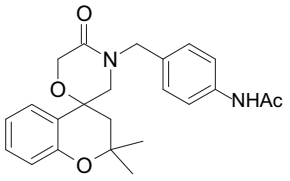
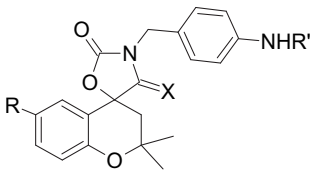


Fig. 2. Chemical structures of non-selective sarc-/mito-K_{ATP}-openers (KCOs), cromakalim and diazoxide and selective activators of mito-K_{ATP} channel BMS-191095, BMS-180448, SMBs.



Compound A



1-4

Compd	X	R	R'
1a	H	H	COMe
1b	H	Br	COMe
2a	H	H	SO_2Me
2b	H	Br	SO_2Me
3a	NH	H	COMe
3b	NH	Br	COMe
4a	NH	H	SO_2Me
4b	NH	Br	SO_2Me

Fig. 3. Chemical structures of compound **A** and the 5-membered spiro-oxazolidinones (**1a,b** and **2a,b**) and the imino-oxazolidin-dione analogues (**3a,b** and **4a,b**).

improved properties in terms of cardio-activity and cardio-selectivity both in *in vitro* and *in vivo* models of myocardial ischemia [16]. Moreover, compound **A** showed a good enantioselectivity which let us to assign the cardioprotective effect to the S-(-)-eutomer [17].

All together these results suggest us to further explore the spiro-heterocycle-benzopyran core as a challenging chemotype for the development of analogues with an improved pharmacological profile. In this context, we retained of interest to evaluate, through a preliminary study, the effects induced on their biopharmacological properties by the constriction of the six-membered spirocycle in the 4-position of compound **A** to 5-membered-ones. For this purpose, were selected as heterocycles the oxazolidinone (as in **1a,b**, **2a,b**) and the imino-oxazolodindione (as in **3a,b**, **4a,b**) rings (Fig. 3). The basic backbone of the new molecules has been substituted with the same groups studied for the SMB derivatives in order to allow the best comparison with the corresponding six-membered analogues and therefore to infer the possible role played on the biopharmacological properties by the spiro-heterocycle.

2. Chemistry

The desired 4-spiro-oxazolidinones **1a,b** and **2a,b** were obtained by first preparing the (4′-aminobenzyl)-4-spiro-oxazolidinones **9a,b**. Briefly, trimethylsilylcyanidrine derivatives **5a,b** [14] were submitted to $LiAlH_4$ reduction affording aminoalcohols **6a,b**. 4-Spiro-oxazolidinones **8a,b** were obtained by cyclization with CDI and subsequent N-alkylation with the 4-nitro-benzylbromide. Reduction of nitro derivatives **8a,b** with hydrazine hydrate in the presence of a catalytic amount of ferric chloride and charcoal gave the corresponding amines **9a,b**. Treatment of **9a,b** with acetic anhydride afforded the corresponding acetamides **1a,b**, while methanesulfonylation of **9a,b** gave compounds **2a,b** (Scheme 1). 4-Spiro-imino-oxazolidindione derivatives **3a,b** and **4a,b** were obtained in a one-pot reaction by treatment of cyanohydrines **10a,b** with carbonyldiimidazole (CDI) and the 4-amino-benzylamine (Scheme 2). The subsequent reaction of **11a,b,** with acetic anhydride and methanesulfonyl chloride afforded the acetamido- (**3a,b**) and methanesulfonamido- (**4a,b**) compounds, respectively.

3. Results and discussion

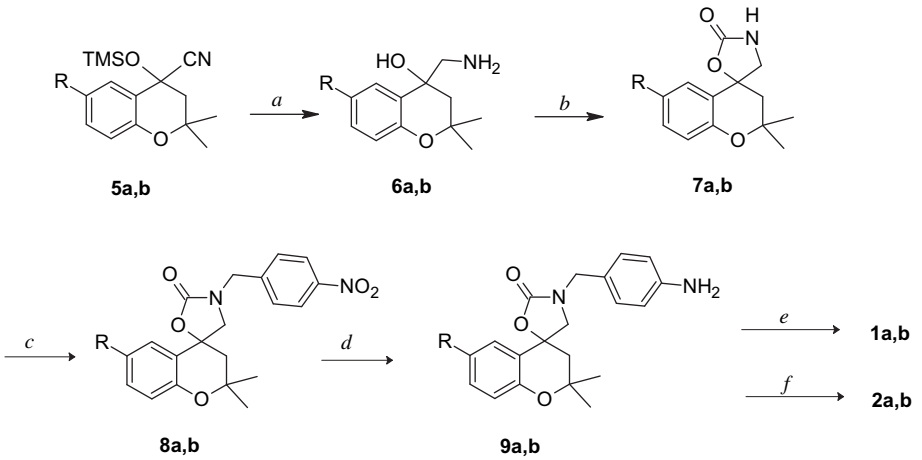
3.1. Biological evaluation

3.1.1. Anti-ischemic activity on H9c2 cells exposed to anoxia/reperfusion cycle

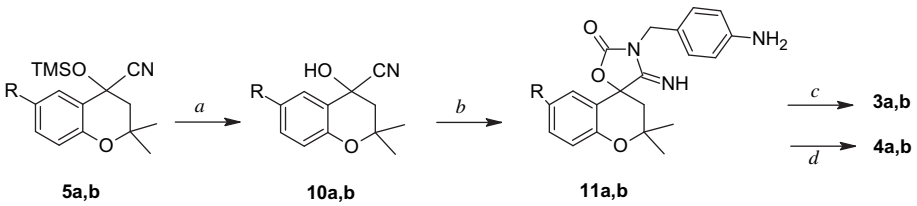
Aiming at evaluating the anti-ischemic activity throughout a reliable screening method we decided to test the newly synthesized compounds as racemic mixture on cultured H9c2 cells exposed to anoxia/reperfusion cycle. In the previous study [16] the results obtained with this experimental protocol were perfectly consistent with the results obtained in isolated rat hearts and with the *in vivo* model of heart infarct. Thus this protocol has been selected as a preliminary screening method to evaluate the synthesized compounds as well as the reference ones (compound **A** and diazoxide).

The anoxia/reperfusion cycle (Fig. 4, Panel A) induced a large degree of cells death in the vehicle-treated H9c2 myocardial cells (cell viability 63 ± 2%). As already observed, the pharmacological treatment with diazoxide, i.e. the reference drug, and spiromorpholone **A** fully protected the cells from the anoxia/reperfusion injury (cell viability 103 ± 6% and 99 ± 3%, respectively).

The spiro-oxazolidinone (**1a**), which represents the ring-contracted analogue of compound **A**, fully prevented the cells damage



Scheme 1. [a]Reagents and conditions. (a) $LiAlH_4$, THF; (b) CDI, THF; (c) 4-nitro-benzylbromide, NaH, DMF; (d) $NH_2NH_2 \cdot H_2O$, $FeCl_3$, MeOH; (e) Ac_2O, acetone; (f) $MeSO_2Cl$, pyridine, dioxane.



Scheme 2. [a]Reagents and conditions. (a) HCl 1N, THF; (b) 4-amino-benzylamine, CDI, CH_2Cl_2; (c) Ac_2O, acetone; (d) $MeSO_2Cl$, pyridine, dioxane.

induced by anoxia/reperfusion causing an almost complete survival of myocardial cells (cell viability 92 ± 3%).

The 6-Br substitution on the benzopyran nucleus (**1b**) decreased the activity. Indeed, compound **1b** exhibited a reduced albeit significant cytoprotective activity against anoxia/reperfusion (cell viability 81 ± 8%).

The replacement of the acetamido group of compound **1a** with a sulphonamido one afforded to compound **2a** which still exhibited cytoprotective activity (cell viability 84 ± 2%), although it was significantly lower than that of **1a**, thus indicating that the acetamido group is a preferable structural requirement with respect to the sulphonamido one.

A deleterious result has been observed with the insertion of the imino-functionality in the spiro-oxazolidinone nucleus as in **3a**, **3b**, **4b**. This kind of functionality caused a detrimental impact on the pharmacological profile and indeed compound **3a,** as well as all the other imino-analogues (**3b**, **4b**), did not show significant anti-ischemic effects (cell viability 68 ± 6%, 76 ± 2% and 78 ± 5, respectively %).

The above results on H9c2 cells showed compounds **1a**, i.e. the most closely related to compound **A**, as the most interesting molecule to subject to further investigations.

In order to unmask the possible involvement of mitochondrial K_{ATP} channel in the anti-ischemic activity observed in cultured cells, the above protocol was also carried out in the presence of 5-hydroxydecanoic acid (5-HD) (100 μM). Such a selective mito-K_{ATP} channel blocker antagonized the cardioprotective effect of both **1a** and the two references drugs (compound **A** and diazoxide) thus confirming that the activation of mito-K_{ATP} plays a preminent role in the pharmacological effect of **1a** (Fig. 4, Panel B).

All together these results show that, within this limited series of compounds, the replacement of spiromorpholone nucleus with the oxazolidinone one does not substantially influence the activity. In addition, the types of substituents in the 6-position on benzopyran ring as well as those on the anchored N-benzyl portion dramatically affect the cardioprotective activity. In particular, the acetoamido substitution on the N-benzyl portion improved the cardioprotective effects with respect to the sulphonamido one. As well as the SMBs derivatives, also for the five-spirosubstituted compounds the 6-Br substitution on benzopyran nucleus negatively affects the activity. Moreover, the presence of the eso-imino group on the spiro nucleus of compounds **3a,b** and **4b** is deleterious.

3.1.2. Anti-ischemic activity on Langendorff-perfused rat hearts model

The potential cardioprotective effects of **1a** were then evaluated in a more complex experimental model of myocardial ischemia–reperfusion cycle, i.e. Langendorff-perfused rat hearts.

As shown in Fig. 5 (Panel A), the vehicle-treated rat hearts showed a dramatic reduction of the post-ischemic functionality. On the contrary the hearts isolated from animals pre-treated with compound **1a** showed an almost full recovery of the post-ischemic inotropic parameter (RPP %) as well as compound **A** and this improved functionality was maintained during the whole reperfusion time.

In agreement with the functional measurement the cardioprotective activity of **1a** was also confirmed by the biochemical parameter. In fact in the reperfusion phase the hearts of rats pre-treated with **1a** and compound **A** (Fig. 5, Panel B) released LDH levels lower than that of vehicle-treated animals.

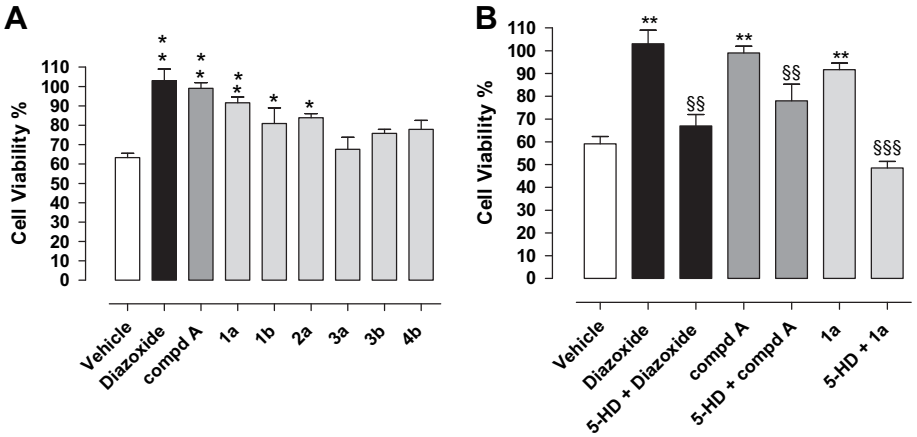


Fig. 4. *Panel A*. The histograms represent the cell viability of H9c2 cells submitted to the different pharmacological treatments (reference drug 100 μM; compounds synthesized 10 μM) and exposed to anoxia/reperfusion. *Panel B*. The histograms represent the cell viability after anoxia/reperfusion in H9c2 cells pre-treated with vehicle, or with diazoxide (100 μM), compound **A** (10 μM), or the selected compound 1a (10 μM), in the absence or in the presence of the mito-K_{ATP}-blocker 5-HD (100 μM). The values are expressed as a percentage of the reference value recorded in reference vehicle-treated cells, which were not submitted to anoxia/reperfusion. The symbol (*) indicates a value of viability significantly different from the vehicle-treated ischemic cells (*$P < 0.05$; **$P < 0.01$). The symbol (§) indicates a statistical difference from the corresponding value obtained in the absence of 5-HD treatment (§§$P < 0.01$; §§§$P < 0.005$).

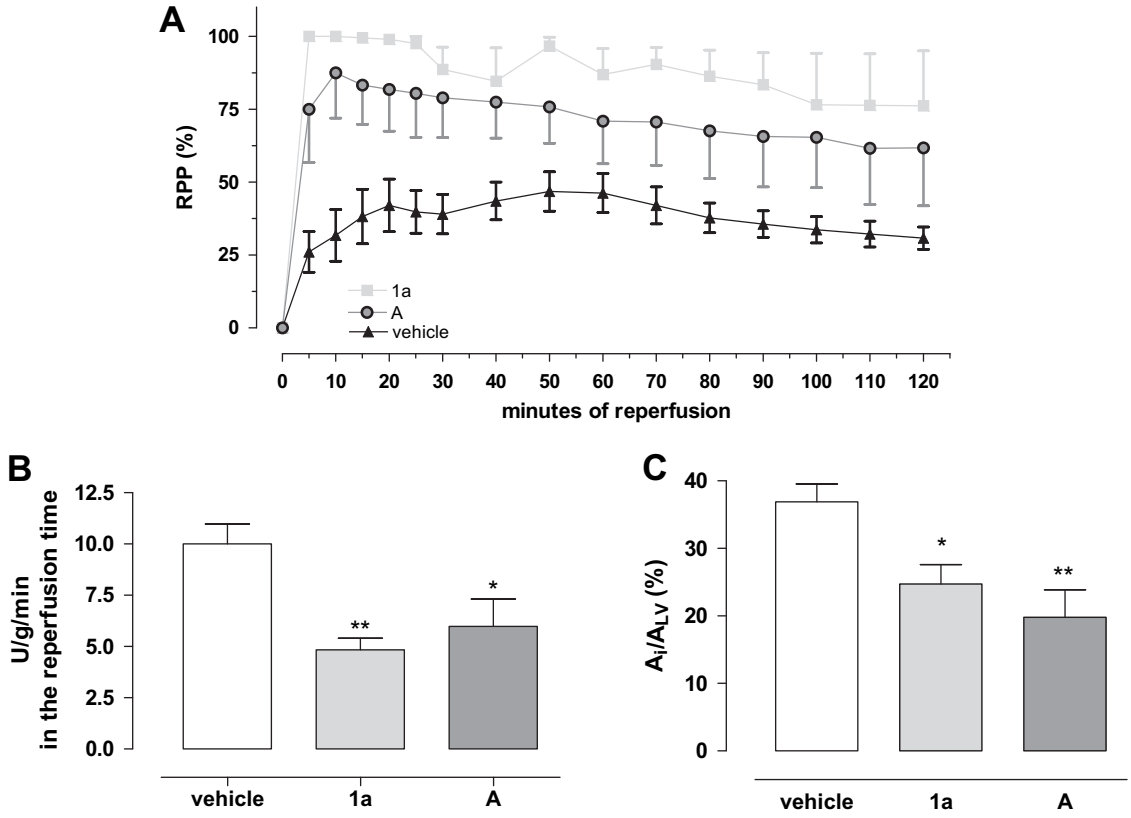


Fig. 5. *Panel A*. The graph represents the post-ischemic functional parameter of RPP recorded during the reperfusion period, expressed as a percentage of the RPP value observed before the ischemia, in Langendorff-perfused hearts of rats pre-treated with vehicle, compound **A** (40 mg/kg) or compound **1a** (40 mg/kg). *Panel B* The histograms represent the influence of the pharmacological treatments (vehicle, compound **A** 40 mg/kg, or compound **1a** 40 mg/kg) on the release of LDH from the Langendorff-perfused hearts during the reperfusion period. The data are expressed as U/min of enzyme and are normalized with the weight of the heart (g). *Panel C*. The histograms represent the parameter of infarct size, expressed as a percentage of the left ventricular area (A_i/A_{LV}%), evaluated in the left ventricles of Langendorff-perfused hearts submitted to ischemia/reperfusion, isolated from animals pre-treated with vehicle, compound **A** (40 mg/kg) or compound **1a** (40 mg/kg). The symbol (*) indicates a statistical difference from the vehicle (*$P < 0.05$; **$P < 0.01$).

Finally, as concerns the morphological analysis, the hearts of vehicle-treated animals submitted to ischemia/reperfusion cycle showed large areas of ischemia injured ventricular tissue, while pre-treatment with **1a** and the reference compound **A** led to a reduction of the injured areas (Fig. 5, Panel C).

4. Conclusion

In conclusion, this study confirmed the great potential of the new class of spiro-heterocycle benzopyrans as innovative anti-ischemic drugs. The structural contraction of the previously synthesized compound **A** to a five-membered spirocycle (**1a**) does not affect the anti-ischemic activity previously found for spiromorpholone derivative, thus indicating that also the spiro-oxazolidinone scaffold represents a valid structural motif to offer cardioprotection. Further pharmacological investigations will be carried out to clarify the mechanism of action, the selectivity for the biological target and the presence/absence of significant side-effects. Moreover the enantiomeric resolution of compound **1a** will be performed, expecting a similar profile of activity for the two enatiomers respect to those of derivative **A**. Within the 4-spiro-oxazolidinone benzopyrans, further structural investigations will be needed to elucidate how the "decorating-substituents" of this scaffold affect the biological activity.

A more detailed study of different 4-spirosubstitution on benzopyran derivatives, may lead us to consider this common molecular scaffold (i.e. 4-spiro-heterocycle-benzopyran) as the pharmacophoric portion in conferring cardioprotection.

5. Experimental

5.1. Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. NMR spectra were obtained with a Varian Gemini 200 MHz spectrometer. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane and referenced from solvent references. Mass spectra were obtained on a Hewlett–Packard 5988 A spectrometer using a direct injection probe and an electron beam energy of 70 eV. The elemental compositions of the compounds agreed to within ±0.4% of the calculated value. Chromatographic separation was performed on silica gel columns by flash (Kieselgel 40, 0.040–0.063 mm; Merck). Reactions were followed by thin-layer chromatography (TLC) on Merck aluminum silica gel (60 F254) sheets that were visualized under a UV lamp. Evaporation was performed in vacuo (rotating evaporator). Sodium sulfate was always used as the drying agent. The trimethylsilyl cyanohydrins **5a,b** were synthesized as previously described [10]. Commercially available chemicals were purchased from Sigma–Aldrich.

5.1.1. 3′-(4-acetamidobenzyl)-2,2-dimethyl-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one (**1a**)

Acetic anhydride (0.10 mL, 0.51 mM) and K_2CO_3 (106 mg, 1.16 mM) were added to a solution of **9a** (172 mg, 0.51 mM) in acetone (5 mL). The reaction mixture was stirred at room temperature for 1 h, then the solvent was evaporated. The residue was dissolved in AcOEt and washed with water and brine. The organic layer was dried, filtered

and concentrated to give a crude product that was purified by flash column chromatography (hexane:AcOEt = 1:1) to obtain the desired amide **1a** (70 mg, 0.18 mM, 36% yield): mp 85–88 °C. ^{1}H NMR ($CDCl_3$) δ: 1.37 (s, 3H, Me); 1.40 (s, 3H, Me); 2.05 (d, 1H, J = 14.5 Hz, CH_2); 2.19 (s, 3H, MeCO); 2.37 (d, 1H, J = 14.5 Hz, CH_2); 3.38 (d, 1H, J = 9.0 Hz, CH_2); 3.65 (d, 1H, J = 9.0 Hz, CH_2); 4.49 (s, 2H, CH_2); 6.77–6.93 (m, 1H, Ar); 7.16–7.32 (m, 6H, Ar); 7.52 (d, 1H, J = 8.2 Hz, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 168.00; 166.50; 154.89; 153.89; 138.21; 135.03; 130.71; 129.45; 120.60; 120.35; 120.13; 119.68; 113.27; 80.40; 74.72; 44.35; 44.24; 30.04; 24.25. Anal. ($C_{22}H_{24}N_2O_4$) C, H, N.

5.1.2. 3′-(4-acetamidobenzyl)-6-bromo-2,2-dimethyl-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one (**1b**)

Compound **1b** was synthesized from **9b** (213 mg, 0.51 mM) following the same procedure described above for the preparation of **1a**. **1b** (119 mg, 0.26 mM, 50% yield): mp 77–80 °C. ^{1}H NMR ($CDCl_3$) δ: 1.36 (s, 3H, Me); 1.37 (s, 3H, Me); 2.02 (d, 1H, J = 14.6 Hz, CH_2); 2.17 (s, 3H, MeCO); 2.31 (d, 1H, J = 14.6 Hz, CH_2); 3.39 (d, 1H, J = 9.2 Hz, CH_2), 3.60 (d, 1H, J = 9.2 Hz, CH_2); 4.41 (d, 1H, J = 14.8 Hz, CH_2); 4.54 (d, 1H, J = 14.8 Hz, CH_2); 6.67 (d, 2H, J = 8.7 Hz, Ar); 7.20–7.29 (m, 3H, Ar); 7.51–7.61 (m, 2H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 168.77; 158.45; 154.64; 136.90; 133.57; 132.44; 130.45; 129.66; ;128.13; 121.42; 114.89; 113.53; 84.51; 75.63; 57.14; 53.78; 52.44; 26.98; 24.60. Anal. ($C_{22}H_{23}BrN_2O_4$) C, H, N.

5.1.3. 3′-(4-methanesulfonamidobenzyl)-2,2-dimethyl-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one (**2a**)

To a solution of **9a** (0.57 g, 1.70 mM) in dry dioxane (18 mL) at 0 °C and under N_2 atmosphere, was added pyridine (1.80 mL, 17.80 mM) and methanesulfonyl chloride (0.17 mL, 2.21 mM). The solution was refluxed for 1 h, then cooled to room temperature and acidified with HCl 1N. Upon dilution with water, the mixture was extracted with AcOEt. The organic phase was dried, filtered, concentrated and purified by trituration with Et_2O to give **2a** (0.67 g, 1.61 mM, 95% yield): mp 120–123 °C. ^{1}H NMR (DMSO) δ: 1.32 (s, 6H, Me); 2.22 (d, 1H, J = 14.8 Hz, CH_2); 2.35 (d, 1H, J = 14.8 Hz, CH_2); 2.99 (s, 3H, SO_2Me); 3.52 (d, 1H, J = 9.4 Hz, CH_2); 3.66 (d, 1H, J = 9.4 Hz, CH_2); 4.42 (s, 2H, CH_2); 6.78 (d, 1H, J = 8.2 Hz, Ar); 6.88–6.96 (m, 1H, Ar); 7.12–7.33 (m, 6H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 158.10; 154.09; 138.03; 131.40; 130.07; 127.43; 123.21; 121.93; 121.81; 118.84; 75.23; 58.55; 48.54; 47.32; 40.12; 29.34; 26.38. Anal. ($C_{21}H_{24}N_2O_5S$) C, H, N.

5.1.4. 3′-(4-methanesulfonamidobenzyl)-6-bromo-2,2-dimethyl-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one (**2b**)

Compound **2b** was synthesized from **9b** (0.71 g, 1.70 mM) following the same procedure described above for the preparation of **2a**. The crude product was purified by crystallization from EtOH to give **2b** (0.56 g, 1.12 mM, 66% yield): mp 165–168 °C. ^{1}H NMR (DMSO) δ: 1.31 (s, 3H, Me); 1.32 (s, 3H, Me); 2.22 (d, 1H, J = 14.9 Hz, CH_2); 2.36 (d, 1H, J = 14.9 Hz, CH_2); 2.99 (s, 3H, SO_2Me); 3.52 (d, 1H, J = 9.5 Hz, CH_2); 3.68 (d, 1H, J = 9.5 Hz, CH_2); 4.41 (s, 2H, CH_2); 6.77 (d, 1H, J = 8.4 Hz, Ar); 7.23 (d, 2H, J = 8.4 Hz, Ar); 7.31 (d, 2H, J = 8.7 Hz, Ar); 7.33–7.41 (m, 2H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 158.45; 153.84; 136.80; 133.77; 130.55; 129.46; 128.93; 126.79; 119.42; 114.44; 113.53; 83.73; 76.55; 57.54; 53.33; 52.80; 42.92; 27.38. Anal. ($C_{21}H_{23}BrN_2O_5S$) C, H, N.

5.1.5. 4′-imino-2,2-dimethyl-3′-[4-acetamidobenzyl]-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one (**3a**)

Compound **3a** was synthesized from **11a** (179 mg, 0.51 mM) following the same procedure described above for the preparation of **1a**. The crude product was purified by flash column chromatography eluting with hexane/AcOEt (1:4) to give **3a** (108 mg, 0.23 mM, 45% yield): mp 190–193 °C. ^{1}H NMR (DMSO) δ: 1.30 (s, 3H, Me); 1.44 (s, 3H, Me); 2.06 (s, 3H, MeCO); 2.35 (d, 1H, J = 15.0 Hz, CH_2); 2.61 (d, 1H, J = 15.0 Hz, CH_2); 4.68 (s, 2H, CH_2); 6.84–6.94 (m, 3H, Ar); 7.26–7.32 (m, 3H, Ar); 7.56 (d, 2H, J = 8.4 Hz, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 168.99; 166.00; 155.09; 154.65; 136.23; 132.48; 128.77; 127.43; 126.32; 121.45; 120.11; 116.80; 112.26; 87.20; 76.76; 47.35; 46.64; 26.94; 24.20. MS (m/z): 393 (M+, 14%); 349 (M+-$COCH_3$, 90%); 334 (M+-NHCOMe, 25%); 106 (100%). Anal. ($C_{22}H_{23}N_3O_4$) C, H, N.

5.1.6. 6-Bromo-4′-imino-2,2-dimetil-3′-[4-acetamidobenzil]-2,3-diidro-2′H-spiro[cromene-4,5′-[1,3]oxazolidin]-2′-one (**3b**)

Compound **3b** was synthesized from **11b** (219 mg, 0.51 mM) following the same procedure described above for the preparation of **1a**. The crude product was purified by flash column chromatography eluting with hexane/AcOEt (3:7) to give **3b** (75 mg, 0.16 mM, 32% yield): mp 110–113 °C. ^{1}H NMR ($CDCl_3$) δ: 1.37 (s, 3H, Me); 1.48 (s, 3H, Me); 2.15–2.24 (m, 5H, CH_2, MeCO); 4.83 (s, 2H, CH_2); 6.75 (d, 1H, J = 8.9 Hz, Ar); 6.86–6.92 (m, 1H, Ar); 7.14–7.18 (m, 1H, Ar); 7.32–7.54 (m, 4H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 168.00; 166.50; 154.89; 153.89; 138.21; 135.03; 130.71; 129.45; 120.60; 120.35; 120.13; 119.68; 113.27; 80.40; 74.72; 44.35; 44.24; 30.04; 24.25. MS (m/z): 471 (M+, 13%); 429 (M+-COMe, 66%). Anal. ($C_{22}H_{22}BrN_3O_4$) C, H, N.

5.1.7. 4′-imino-2,2-dimethyl-3′-[4-methanesulfonamidobenzyl]-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one (**4a**)

Compound **4a** was synthesized from **11a** (0.70 g, 2.00 mM) following the same procedure described above for the preparation of **2a**. The crude product was purified by flash column chromatography eluting with hexane/AcOEt (1:1) to give **4a** (0.20 g, 0.52 mM, 26% yield): mp 87–90 °C. ^{1}H NMR ($CDCl_3$) δ: 1.40 (s, 3H, Me); 1.49 (s, 3H, Me); 2.23 (d, 1H, J = 14.9 Hz, CH_2); 2.35 (d, 1H, J = 14.9 Hz, CH_2); 3.01 (s, 3H, SO_2Me); 4.83 (s, 2H, CH_2); 6.79–6.90 (m, 3H, Ar); 7.20 (d, 2H, J = 8.4 Hz, Ar); 7.24–7.32 (m, 1H, Ar); 7.46 (d, 2H, J = 8.4 Hz, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 166.00; 155.29; 154.65; 135.73; 128.65; 127.77; 126.23; 120.12; 119.51; 116.82; 112.33; 87.46; 76.34; 47.22; 46.59; 42.98; 26.94. MS (m/z): 429 (M+, 12%); 385 (M+-CO_2, 100%); 79 (SO_2Me, 53%). Anal. ($C_{21}H_{23}N_3O_5S$) C, H, N.

5.1.8. 6-Bromo-4′-imino-2,2-dimethyl-3′-[4-methanesulfonamidobenzyl]-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one (**4b**)

Compound **4b** was synthesized from **11b** (0.86 g, 2.00 mM) following the same procedure described above for the preparation of **2a**. The crude product was purified by precipitation from hexane/AcOEt to give **4b** (0.41 g, 0.80 mM, 40% yield): mp 85–88 °C. ^{1}H NMR ($CDCl_3$) δ: 1.38 (s, 3H, Me); 1.50 (s, 3H, Me); 2.14 (d, 1H, J = 15.1 Hz, CH_2); 2.56 (d, 1H, J = 15.1 Hz, CH_2); 3.02 (s, 3H, SO_2Me); 4.89 (s, 2H, CH_2); 6.69–6.81 (m, 3H, Ar); 7.21–7.31 (m, 1H, Ar); 7.32–7.38 (m, 1H, Ar); 7.44–7.53 (m, 2H, Ar) ppm. ^{13}C NMR ($CDCl_3$): δ 166.22; 155.45; 153.89; 135.66; 133.42; 129.55; 127.97; 126.63; 119.72; 119.01; 114.62; 113.43; 86.36; 76.66; 47.55; 46.60; 42.58; 27.00. MS (m/z): 508 (M+, 25%); 465 (M+-CO_2, 16%); 184 (24%), 106 (18%); 79 (SO_2CH_3, 35%). Anal. ($C_{21}H_{22}BrN_3O_5S$) C, H, N.

5.1.9. 2,2-Dimethyl-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one (**7a**)

To a solution of 1,1′-carbonyldiimidazole (1.02 g, 6.33 mM) in THF (11 mL) at 0 °C was added a solution of **6a** (1) (1.30 g, 6.33 mM) in THF (11 mL). The mixture was stirred at room temperature for 1 h, then the solvent was evaporated. The residue was diluted with AcOEt and washed with HCl 1N and aqueous K_2CO_3. The organic layer was dried, filtered and concentrated to give a crude product that was purified by trituration with Et_2O (1.15 g, 4.93 mM, 78% yield): ^{1}H NMR ($CDCl_3$) δ: 1.42 (s, 6H, Me); 2.17 (d, 1H, J = 14.6 Hz, CH_2); 2.46 (d, 1H, J = 14.6 Hz, CH_2); 3.63 (d, 1H, J = 8.8 Hz, CH_2); 3.89 (d, 1H,

J = 8.8 Hz, CH_2); 6.83 (d, 1H, J = 8.2 Hz, Ar); 6.93–7.01 (m, 1H, Ar); 7.22–7.29 (m, 1H, Ar); 7.49 (dd, 1H, J = 7.8, 1.3 Hz, Ar) ppm. Anal. ($C_{13}H_{15}NO_3$) C, H, N.

5.1.10. 6-Bromo-2,2-dimethyl-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one (**7b**)

Compound **7b** was synthesized from **6b** [10] (1.81 g, 6.33 mM) following the same procedure described above for the preparation of **7a**. **7b** (1.66, 5.31 mM, 84% yield): ^{1}H NMR ($CDCl_3$) δ: 1.41 (s, 6H, Me); 2.14 (d, 1H, J = 14.6 Hz, CH_2); 2.42 (d, 1H, J = 14.6 Hz, CH_2); 3.64 (d, 1H, J = 9.0 Hz, CH_2); 3.86 (d, 1H, J = 9.0 Hz, CH_2); 6.72 (d, 1H, J = 8.8 Hz, Ar); 7.33 (dd, 1H, J = 8.8, 2.4 Hz, Ar); 7.58 (d,1H, J = 2.4 Hz, Ar) ppm. Anal. ($C_{13}H_{14}BrNO_3$) C, H, N.

5.1.11. 2,2-Dimethyl-3′-(4-nitrobenzyl)-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one (**8a**)

To a stirred solution of NaH (0.31 g; 13.52 mM, 60% dispersion in mineral oil) in dry DMF (10 mL) and under N_2 atmosphere, was added the compound **7a** (1.05 g; 4.51 mM). After 30 min at room temperature, the reaction mixture was cooled to 0 °C and a solution of 2-nitrobenzylbromide (1.17 g, 5.41 mM) in DMF (2 mL) was added. The mixture was stirred at room temperature for 1 h. Then water was added and the aqueous phase was extracted with AcOEt. The combined organic phases were washed with ice and NaCl, dried, filtered, and concentrated. The residue was purified by flash column chromatography eluting with hexane/AcOEt (1:1) to afford **8a** (0.83 g, 2.25 mM, 50% yield): mp 78–81 °C. ^{1}H NMR ($CDCl_3$) δ: 1.39 (s, 3H, Me); 1.41 (s, 3H, Me); 2.08 (d, 1H, J = 14.6 Hz, CH_2); 2.40 (d, 1H, J = 14.6 Hz, CH_2); 3.42 (d, 1H, J = 8.9 Hz, CH_2); 3.71 (d, 1H, J = 8.9 Hz, CH_2); 4.64 (s, 2H, CH_2); 6.79–6.84 (m, 1H, Ar); 6.88–6.95 (m, 1H, Ar); 7.20–7.30 (m, 1H, Ar); 7.53 (d, 2H, J = 8.6 Hz, Ar); 7.69 (d, 1H, J = 8.8 Hz, Ar); 8.27 (d, 2H, J = 8.6 Hz, Ar) ppm. Anal. ($C_{20}H_{20}N_2O_5$) C, H, N.

5.1.12. 6-Bromo-2,2-dimethyl-3′-(4-nitrobenzyl)-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one (**8b**)

Compound 8**b** was synthesized from **7b** (1.81 g, 6.33 mM) following the same procedure described above for the preparation of **8a**. **8b** (1.13 g, 2.53 mM, 40% yield): ^{1}H NMR ($CDCl_3$) δ: 1.38 (s, 3H, Me); 1.40 (s, 3H, Me); 2.04 (d, 1H, J = 14.6 Hz, CH_2); 2.38 (d, 1H, J = 14.6 Hz, CH_2); 3.44 (d, 1H, J = 9.1 Hz, CH_2); 3.68 (d, 1H, J = 9.1 Hz, CH_2); 4.56 (d, 1H, J = 15.3 Hz, CH_2); 4.71 (d, 1H, J = 15.3 Hz, CH_2); 6.70 (d, 1H, J = 7.7 Hz, Ar); 7.26–7.33 (m, 2H, Ar); 7.55 (d, 2H, J = 8.6 Hz, Ar); 8.29 (d, 2H, J = 8.6 Hz, Ar) ppm. Anal. ($C_{20}H_{19}BrN_2O_5$) C, H, N.

5.1.13. 3′-(4-aminobenzyl)-2,2-dimethyl-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one (**9a**)

To a solution of the compound **8a** (0.37 g, 1.00 mM) in MeOH (10 mL) was added carbon (0.55 g) and a catalytic amount of $FeCl_3$. The mixture was warmed up to 60 °C, and hydrazine monohydrate (0.82 mL, 17 mM) was added dropwise. The reaction mixture was refluxed overnight and then filtered through a celite pad with several methanol washes. The filtrate was concentrated and the crude product was triturated with Et_2O to give **9a** (0.33 g, 0.99 mM, 99% yield): ^{1}H NMR ($CDCl_3$) δ: 1.37 (s, 3H, Me); 1.39 (s, 3H, Me); 2.03 (d, 1H, J = 14.6 Hz, CH_2); 2.34 (d, 1H, J = 14.6 Hz, CH_2); 3.36 (d, 1H, J = 9.2 Hz, CH_2); 3.62 (d, 1H, J = 9.2 Hz, CH_2); 4.96 (d, 1H, J = 14.4 Hz, CH_2); 4.45 (d, 1H, J = 14.4 Hz, CH_2); 6.67 (d, 2H, J = 8.4 Hz, Ar); 6.78 (d, 1H, J = 8.2 Hz, Ar); 6.84–6.92 (m, 1H, Ar); 7.12 (d, 2H, J = 8.4 Hz, Ar); 7.20–7.26 (m, 2H, Ar) ppm. Anal. ($C_{21}H_{22}N_2O_3$) C, H, N.

5.1.14. 3′-(4-aminobenzyl)-6-bromo-2,2-dimethyl-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′one (**9b**)

Compound **9b** was synthesized from **8b** (0.45 g, 1.00 mM) following the same procedure described above for the preparation of **9a**. **9b** (0.29 g, 0.70 mM, 70% yield): ^{1}H NMR ($CDCl_3$) δ: 1.36 (s, 3H, Me); 1.38 (s, 3H, Me); 2.00 (d, 1H, J = 15.4 Hz, CH_2); 2.32 (d, 1H, J = 15.4 Hz, CH_2); 3.36 (d, 1H, J = 9.2 Hz, CH_2); 3.57 (d, 1H, J = 9.2 Hz, CH_2); 4.31 (d, 1H, J = 14.6 Hz, CH_2); 4.51 (d, 1H, J = 14.6 Hz, CH_2); 6.64–6.71 (m, 3H, Ar); 7.13 (d, 2H, J = 8.2 Hz, Ar); 7.26–7.31 (m, 2H, Ar) ppm. Anal. ($C_{21}H_{21}BrN_2O_3$) C, H, N.

5.1.15. 4-Hydroxy-2,2-dimethyl-3,4-dihydro-2H-chromene-4-carbonitrile (**10a**)

An aqueous solution of HCl 1N (6 mL) was added to a solution of **5a** [10] (0.99 g, 3.60 mM) in THF and was refluxed for 1h. Then the solvent was evaporated and the residue was dissolved in AcOEt and washed with water. The organic phase was dried, filtered and concentrated to give **10a** (0.65 g, 3.20 mM, 89% yield): ^{1}H NMR ($CDCl_3$) δ: 1.43 (s, 3H, Me); 1.48 (s, 3H, Me); 2.37 (d, 1H, J = 14.4 Hz, CH_2); 2.49 (d, 1H, J = 14.4 Hz, CH_2); 6.87 (d, 1H, J = 8.4 Hz, Ar); 6.99–7.07 (m, 1H, Ar); 7.20–7.36 (m, 1H, Ar); 7.60 (dd, 1H, J = 7.7; 1.6 Hz, Ar) ppm. Anal. ($C_{12}H_{13}NO_2$) C, H, N.

5.1.16. 6-Bromo-4-hydroxy-2,2-dimethyl-3,4-dihydro-2H-chromene-4-carbonitrile (**10b**)

Compound **10b** was synthesized from **5b** [10] (1.27 g, 3.60 mM) following the same procedure described above for the preparation of **10a**. **10b** (0.98 g, 3.49 mM, 97% yield): ^{1}H NMR ($CDCl_3$) δ: 1.42 (s, 3H, Me); 1.48 (s, 3H, Me); 2.36 (d, 1H, J = 14.5 Hz, CH_2); 2.47 (d, 1H, J = 14.5 Hz, CH_2); 6.76 (d, 1H, J = 8.8 Hz, Ar); 7.39 (dd, 1H, J = 8.8; 2.4 Hz, Ar); 7.73 (d, 1H, J = 2.4 Hz, Ar) ppm. Anal. ($C_{12}H_{12}BrNO_2$) C, H, N.

5.1.17. 4′-imino-2,2-dimethyl-3′-(4-aminobenzyl)-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one (**11a**)

A solution of **10a** (0.59 g, 2.90 mM) in dry CH_2Cl_2 (8.7 mL) was added dropwise to a solution of carbonyldiimidazole (0.51 g, 3.20 mM) in dry CH_2Cl_2 (6.0 mL), at 0 °C under N_2 atmosphere. The resulting mixture was stirred at room temperature for 20 min, and then 4-(aminomethyl)aniline (0.35 g, 2.90 mM) was added. The reaction mixture was stirred for 2 h. Then, the solution was washed with HCl 1N and water. The combined aqueous phases were alkalinized with NaOH 1N and extracted with Et_2O. The organic phase was dried, filtered, concentrated and purified by crystallization from Et_2O/hexane to give **11a** (0.26 g, 0.87 mM, 30% yield): mp 155–160 °C. ^{1}H NMR ($CDCl_3$) δ: 1.39 (s, 3H, Me); 1.48 (s, 3H, Me); 2.19 (d, 1H, J = 15.0 Hz, CH_2); 2.34 (d, 1H, J = 15.0 Hz, CH_2); 4.74 (s, 2H, CH_2); 6.64–6.68 (m, 2H, Ar); 6.79–6.90 (m, 3H, Ar); 7.21–7.34 (m, 3H, Ar) ppm. MS (*m*/*z*): 351 (M+, 18%); 307 (-CO_2, 100%); 121 (24%); 106 (91%). Anal. ($C_{20}H_{21}N_3O_3$) C, H, N.

5.1.18. 6-Bromo-4′-imino-2,2-dimethyl-3′-(4-aminobenzyl)-2,3-dihydro-2′H-spiro[chromene-4,5′-[1,3]oxazolidin]-2′-one (**11b**)

Compound **11b** was synthesized from **10b** (0.82 g, 2.90 mM) following the same procedure described above for the preparation of **11a**. The crude product was purified by flash column chromatography eliting with hexane/AcOEt (6:4) to give **11b** (0.36 g, 0.84 mM, 29% yield): ^{1}H NMR ($CDCl_3$) δ: 1.38 (s, 3H, Me); 1.48 (s, 3H, Me); 2.18 (d, 1H, J = 15.0 Hz, CH_2); 2.35 (d, 1H, J = 15.0 Hz, CH_2); 4.75 (s, 2H, CH_2); 6.68 (d, 2H, J = 8.4 Hz, Ar); 6.74 (d, 1H, J = 8.7 Hz, Ar); 6.86 (d, 1H, J = 2.3 Hz, Ar); 7.26–7.30 (m, 2H, Ar); 7.34 (dd, 1H, J = 2.3; 8.7 Hz, Ar) ppm. Anal. ($C_{20}H_{20}BrN_3O_3$) C, H, N.

5.2. Pharmacology

5.2.1. Material and methods

Male Wistar rats (250–350 g) were housed and cared for in conformity with the Guidelines of the European Community Council Directive 86/609, adopted by Italian law D.L. 116/92, and with the Guide for the Care and Use of Laboratory Animals (NIH n°

85-23, revised 1996). The protocols were approved by the ethical committee of University of Pisa.

5.2.2. Cell culture

H9c2 cells, derived from embryonic rat ventricular myocytes (ATTC, Manassas, VA), were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma–Aldrich) supplemented with 10% fetal bovine serum (FBS, Sigma–Aldrich), 100 units/ml penicillin and 100 μg/ml streptomycin in tissue culture flasks at 37 °C in humidified atmosphere of 5% CO_2. The *in vitro* experimental protocols followed the procedures already described [16].

5.2.3. Langendorff-perfused rat hearts

A group of animals was submitted to an IPC procedure, achieved by 2 cycles of 5′ occlusion/10′ reperfusion, followed by 30′ coronary occlusion and 120′ reperfusion. Each experimental group was composed by at least 6 animals. The experimental protocols pursued to evaluate the activity on myocardial ischemia/reperfusion model (Langendorff-perfused rat hearts) have been previously described [14,15].

Appendix. Supplementary information

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.ejmech.2011.01.003.

References

[1] C.E. Murry, R.B. Jennings, K.A. Reimer, Circulation 74 (1986) 1124–1136.
[2] I. Andreadou, E.K. Iliodromitis, M. Koufaki, D. Farmakis, A. Tsotinis, D.T. Kremastinos, Curr. Med. Chem. 15 (2008) 3204–3213.
[3] K.D. Garlid, A.D.T. Costa, C.L. Quinlan, S.V. Pierre, P. Dos Santos, J. Mol. Cell. Cardiol. 46 (2009) 858–866.
[4] A.P. Halestrap, Biochem. J. 278 (1991) 715–719.
[5] K.D. Garlid, Basic Res. Cardiol. 95 (2000) 275–279.
[6] K.D. Garlid, P. Dos Santos, Z.J. Xie, A.D. Costa, P. Paucek, Biochim. Biophys. Acta 1606 (2003) 1–21.
[7] S.Z. Zhang, Q. Gao, C.M. Cao, I.C. Bruce, Q. Xia, Life Sci. 78 (2006) 738–745.
[8] K.D. Garlid, P. Paucek, V. Yarov-Yarovoy, X. Sun, P.A. Schindler, J. Biol. Chem. 271 (1996) 8796–8799.
[9] P. Lebrun, M.H. Antoine, A. Herchuelz, Life Sci. 51 (1992) 795–806.
[10] K.S. Atwal, G.J. Grover, S.Z. Ahmed, F.N. Ferrara, T.W. Harper, K.S. Kim, P.G. Sleph, S. Dzwonczyk, A.D. Russell, S. Moreland, J.R. McCullough, D.E. Normandin, J. Med. Chem. 36 (1993) 3971–3974.
[11] G.J. Grover, A.J. D'Alonzo, K.D. Garlid, R. Bajgar, N.J. Lodge, P.G. Sleph, R.B. Darbenzio, T.A. Hess, M.A. Smith, P. Paucek, K.S. Atwal, J. Parmacol. Exp. Ther. 297 (2001) 1184–1192.
[12] R.C. Gadwood, B.V. Kamdar, L.A. Dubray, M.L. Wolfe, M.P. Smith, W. Watt, S.A. Mizsak, V.E. Groppi, J. Med. Chem. 36 (1993) 1480–1487.
[13] R. Mannhold, Curr. Top. Med. Chem. 6 (2006) 1031–1047.
[14] M.C. Breschi, V. Calderone, A. Martelli, F. Minutolo, S. Rapposelli, L. Testai, F. Tonelli, A. Balsamo, J. Med. Chem. 49 (2006) 7600–7602.
[15] M.C. Breschi, V. Calderone, M. Digiacomo, M. Manganaro, A. Martelli, F. Minutolo, S. Rapposelli, L. Testai, F. Tonelli, A. Balsamo, J. Med. Chem. 51 (2008) 6945–6954.
[16] V. Calderone, L. Testai, A. Martelli, S. Rapposelli, M. Digiacomo, A. Balsamo, M.C. Breschi, Biochem. Pharmacol. 79 (2010) 39–47.
[17] S. Rapposelli, V. Calderone, R. Cirilli, M. Digiacomo, C. Faggi, F. La Torre, M. Manganaro, A. Martelli, L. Testai, J. Med. Chem. 52 (2009) 1477–1480.