Pharmaceutical Sciences and Drug Design
ISSN: 3062-4428
2025, Volume 5, Page No: 205-216

Available online at: www.galaxypub.co/page/journals

Galaxy Publication

Roflumilast Protects Cardiomyocytes from Doxorubicin-Induced Damage by Modulating Inflammatory Responses and Cellular Aging via SIRT1

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Received: 21 May 2025; **Revised:** 05 October 2025; **Accepted:** 06 October 2025

ABSTRACT

Doxorubicin is widely used for cancer therapy but is limited by its cardiotoxic effects. Traditional approaches, such as lowering the drug dose, aim to reduce cardiac side effects but may compromise anticancer efficacy. This study investigates whether Roflumilast can protect heart cells from Doxorubicin-induced inflammation and premature cellular aging, and explores the role of SIRT1 in this process. H9c2 cardiomyocytes were exposed to 5 µmol/L Doxorubicin to induce cell injury. Cell survival was measured using the MTT assay. Levels of oxidative stress marker 4-HNE were assessed by immunofluorescence. Gene expression of pro- and anti-inflammatory cytokines (IL-6, IL-17, TNF-α, IL-4) and senescence-related markers (p21, PAI-1, SIRT1) were evaluated using qRT-PCR, while Western blot measured protein levels of Gpx4, PAI-1, p21, and SIRT1. Secretion of cytokines and cardiac injury markers (CK-MB, cTnI) was quantified by ELISA. Cellular senescence was examined with SA-β-Gal staining. To determine the involvement of SIRT1, its expression was silenced using siRNA in H9c2 cells. Doxorubicin markedly reduced cardiomyocyte viability and induced oxidative stress, inflammatory cytokine release, and cellular senescence. Treatment with Roflumilast reversed these effects, restoring cell viability, reducing 4-HNE levels, suppressing IL-6 and IL-17 secretion, and decreasing SA-β-Gal staining. Roflumilast also counteracted Doxorubicin-induced increases in p21 and PAI-1 and restored SIRT1 expression. Silencing SIRT1 abolished the protective, anti-senescent effects of Roflumilast, confirming that SIRT1 mediates its cardioprotective action. Roflumilast demonstrates potential as a cardioprotective agent against Doxorubicin-induced damage by limiting inflammation and cellular aging, primarily through SIRT1 activation.

Keywords: Roflumilast, Doxorubicin, SIRT1, Cardiomyocyte Senescence, Inflammation

How to Cite This Article: Abdelrahman M, Osman S. Roflumilast Protects Cardiomyocytes from Doxorubicin-Induced Damage by Modulating Inflammatory Responses and Cellular Aging via SIRT1. Pharm Sci Drug Des. 2025;5:205-16. https://doi.org/10.51847/Dh6x6Hnj1J

Introduction

Recent advances in cancer therapy have improved treatment outcomes; however, the adverse effects of chemotherapy are increasingly recognized as a critical challenge. Among these, chemotherapy-induced cardiomyopathy has emerged as a significant concern, potentially causing long-term cardiac complications in cancer survivors. Clinical studies have demonstrated that chemotherapy can lead to cardiotoxic effects years after treatment, including hypertension, left ventricular dysfunction, and heart failure [1]. The estimated risk of cardiovascular mortality in cancer patients is 2–6 times higher than in the general population [2]. Therefore, treatment planning must carefully consider the potential cardiotoxicity and continuously monitor patient responses [3].

Anthracyclines, a widely used class of chemotherapeutic agents, have been employed to treat various malignancies for decades. Doxorubicin, one of the most commonly used anthracyclines, has been regarded as a potent anticancer agent either alone or in combination with other drugs since the late 1960s [4, 5]. Despite its efficacy, Doxorubicin is associated with both acute and chronic toxicities, including bone marrow suppression, nausea, vomiting, arrhythmias, and chronic heart failure, the latter often resulting from irreversible cardiac damage [6, 7]. Pathological mechanisms underlying Doxorubicin-induced cardiotoxicity involve excessive inflammation,

oxidative stress, and cellular senescence [8, 9]. Notably, senescence plays a key role and is triggered through upregulation of PAI-1 and p21 or downregulation of SIRT1 [10–12]. Understanding these mechanisms is critical for developing effective strategies to mitigate Doxorubicin cardiotoxicity in clinical settings.

Roflumilast, a selective phosphodiesterase-4 (PDE4) inhibitor, prevents the degradation of intracellular cAMP and exhibits anti-inflammatory effects [13, 14]. Approved by the US Food and Drug Administration (FDA) for severe chronic obstructive pulmonary disease (COPD), Roflumilast reduces airway edema, downregulates Mucin 5AC expression in epithelial cells, and suppresses reactive oxygen species (ROS) production in neutrophils, smooth muscle cells, and airway epithelia [15–18]. Previous studies suggest that cardiomyocyte apoptosis in chronic heart failure is triggered by oxidative stress, hypoxia, or ischemia-reperfusion injury [19]. Increasing intracellular cAMP levels has been shown to protect cardiomyocytes from apoptosis and improve survival in acute myocardial infarction models [20]. PDE4 inhibitors, by preventing cAMP hydrolysis, represent promising anti-inflammatory therapeutic options. Notably, Roflumilast has demonstrated protective effects against nitric oxide-induced apoptosis in both H9c2 cells and neonatal rat cardiomyocytes via cAMP–PKA/CREB and Epac/Akt-dependent pathways [21].

The present study aimed to investigate the protective role of Roflumilast on Doxorubicin-induced damage in H9c2 cardiomyocytes and to explore its potential as a therapeutic strategy for managing chemotherapy-induced cardiotoxicity.

Materials and Methods

Cell culture and treatments

H9c2 cardiomyocytes (ATCC, USA) were cultured in DMEM supplemented with 10% fetal bovine serum at 37°C under 5% CO2. Doxorubicin and Roflumilast (Sigma, USA) were used for treatment. Confluent H9c2 cells (90%) were exposed to 5 μM Doxorubicin, either alone or combined with Roflumilast at 1, 2.5, or 5 μM for 24 hours.

Quantitative reverse transcriptase PCR (qRT-PCR)

Total RNA was extracted using an RNA Extraction Kit (Thermo Fisher Scientific, USA) according to manufacturer instructions. RNA concentration was measured with a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). Complementary DNA was synthesized using oligo(dT) primers, and qRT-PCR was performed with SYBR Premix Ex TaqTM on a Bio-Rad CFX96 system (Genscript, China). Relative expression levels of IL-6, IL-17, PAI-1, p21, and SIRT1 were normalized to GAPDH and calculated using the $2^{-\Delta\Delta Ct}$ method. Each experiment was performed in triplicate.

Western blot analysis

Proteins were extracted from H9c2 cells using a Nuclear and Cytoplasmic Protein Extraction Kit (Thermo Fisher Scientific, USA). Protein samples (40 μg) were separated on 12% SDS-PAGE and transferred to PVDF membranes (Millipore, USA). Membranes were blocked with 5% non-fat milk in TBST and incubated overnight at 4°C with primary antibodies against GPX4, PAI-1, p21, SIRT1, and GAPDH (1:1000, Abcam, USA). HRP-conjugated secondary antibody (1:5000, Abcam, USA) was applied for 1 hour at room temperature. Detection was performed using ECL reagents and a chemiluminescent imaging system (Tanon 5200-multi, China). Experiments were repeated three times.

Immunofluorescence

Cells were washed three times with PBS and incubated with primary antibody against 4-HNE (1:1000, Abcam, USA) overnight at 4°C. After washing, cells were incubated with Alexa Fluor488-conjugated secondary antibody (1:200, Abcam, USA) for 30 minutes at room temperature. Nuclei were counterstained with DAPI for 5 minutes, and samples were mounted with 50% glycerol. Fluorescence images were captured using an Olympus microscope (Tokyo, Japan).

Senescence-associated β-galactosidase (SA-β-Gal) staining

Cellular senescence was evaluated using SA-β-Gal staining according to the manufacturer's protocol (Cell Signaling Technology, Boston, USA). Cells were rinsed with PBS and fixed for 30 minutes at room temperature. Following fixation, cells were incubated overnight at 37°C with the SA-β-Gal staining solution. Senescent cells

exhibiting positive staining were imaged under a microscope, and the percentage of stained cells was calculated from five randomly selected fields per sample. Each experiment was repeated three times to ensure reproducibility.

siRNA-mediated knockdown of SIRT1

For gene silencing studies, H9c2 cells were seeded in 6-well plates 24 hours before transfection. SIRT1-specific siRNAs were delivered using Lipofectamine 2000 or X-tremeGENE HP (Invitrogen, Carlsbad, USA) following the manufacturer's instructions. Cells were collected 24–48 hours after transfection for downstream analyses. All transfections were conducted in triplicate.

Evaluation of cardiomyocyte injury

H9c2 cells were exposed to 5 µM Doxorubicin with or without Roflumilast (2.5 or 5 µM) for 24 hours. Release of cardiac injury markers, cTnI and CK-MB, into the culture medium was quantified using ELISA kits (Cusabio Technology LLC; cTnI #CSB-E08594r, CK-MB #CSB-E14403r). Results were normalized to the vehicle-treated group and presented as fold changes.

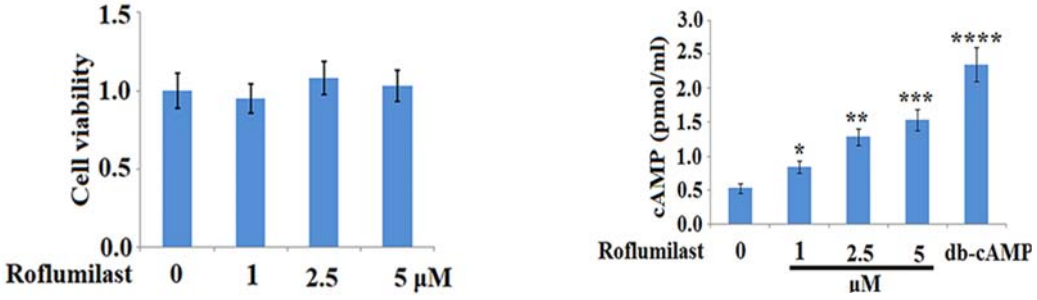
Statistical analysis

Data are expressed as mean ± SD. Statistical differences were assessed using one-way ANOVA followed by Tukey's post hoc test (GraphPad Prism 6.0). A p-value < 0.05 was considered statistically significant.

Results and Discussion

Roflumilast protects H9c2 cells from doxorubicin-induced damage

To determine whether Roflumilast could protect H9c2 cardiomyocytes from Doxorubicin-induced injury, cell viability was measured using the MTT assay. H9c2 cells treated with Roflumilast alone at concentrations of 1, 2.5, or 5 µM did not show any significant alterations in viability compared to untreated controls **(Figure 1a)**. Next, intracellular cAMP levels were measured to explore the mechanistic effects of Roflumilast. Short-term treatment (15 minutes) with Roflumilast led to a dose-dependent increase in cAMP within the cells **(Figure 1b)**, indicating activation of the cAMP signaling pathway as a potential protective mechanism.



a) b)

Figure 1. Roflumilast increases concentrations of cyclic adenosine monophosphate (cAMP) in H9c2 cardiac cells. (a) Cells were treated with Roflumilast (1, 2.5, 5 µM) for 24 hours. Cell viability was measured using MTT assay; (b) Cells were treated with Roflumilast (1, 2.5, 5 µM) for 15 minutes. 200 µM dibutyryl cyclic adenosine monophosphate (db-cAMP) was used as a positive control. Concentrations of cAMP have been measured (*, **, ***, ****P<0.05, 0.01, 0.001, 0.0001 vs vehicle group).

Subsequently, the cell viability and LDH release were determined after the H9c2 cardiac cells were incubated with 5 µmol/L Doxorubicin in the presence or absence of Roflumilast (1, 2.5, 5 µM) for 24 hours. As shown in **Figures 2a and 2b**, the cell viability was significantly inhibited by the introduction of Doxorubicin but was greatly promoted by Roflumilast at the concentrations of 2.5 and 5 µM. LDH release was promoted by Doxorubicin and was suppressed by Roflumilast at concentrations of 2.5 and 5 µM.

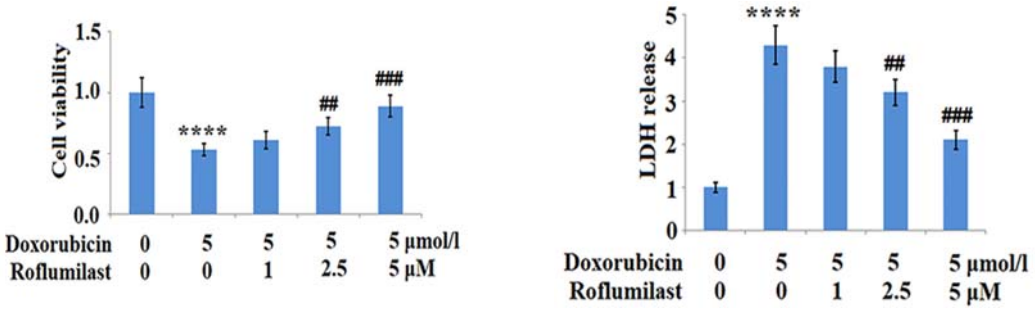


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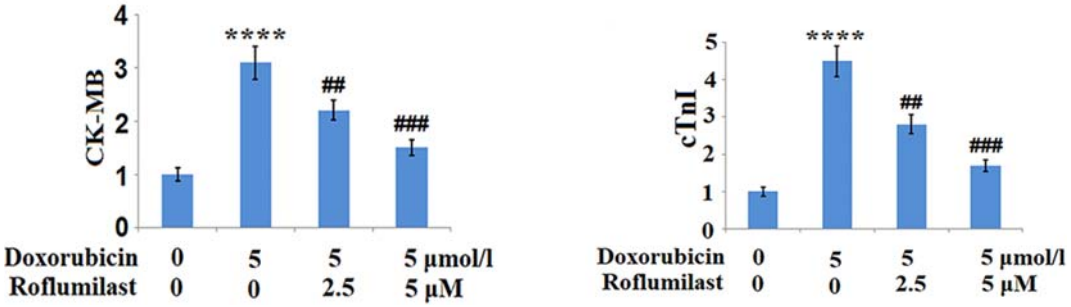
Figure 2. Roflumilast ameliorates Doxorubicin-induced cell death in H9c2 cardiac cells. Cells were treated with 5 µmol/L Doxorubicin in the presence or absence of Roflumilast (1, 2.5, 5 µM) for 24 hours. (a) Cell viability and (b) Lactate dehydrogenase (LDH) release was assayed (****P<0.0001 vs vehicle control; ##, ###P<0.01, 0.001 vs Doxorubicin treatment)

To further examine the effects of Roflumilast against Doxorubicin-induced cell injury in H9c2 cardiac cells, the release of CK-MB and cTnI, two important biomarkers of cardiomyoblast injury, were measured. Results indicate that Doxorubicin treatment significantly increased the release of both CK-MB **(Figure 3a)** and cTnI **(Figure 3b)** but there were remarkably reduced by Roflumilast in a dose-dependent manner.

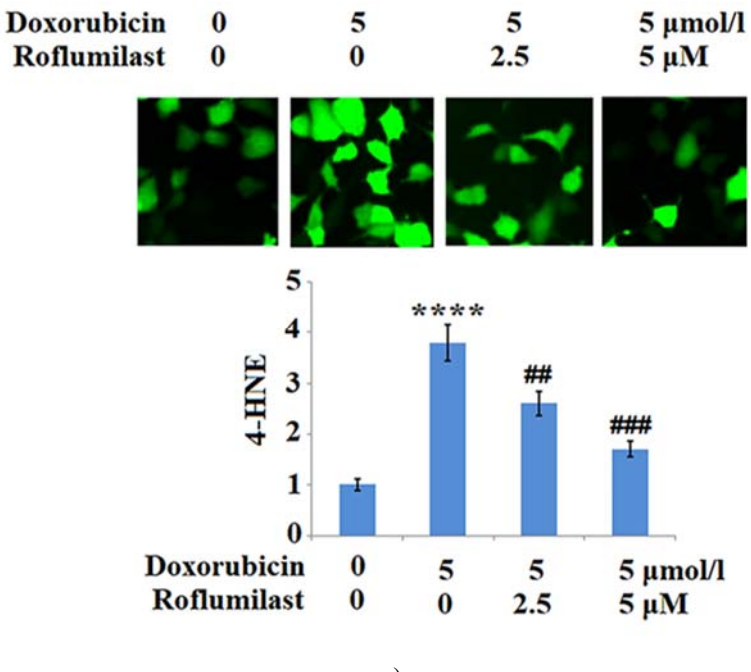


a) b)

Figure 3. Roflumilast reduces the Doxorubicin-induced release of creatine kinase-muscle/brain (CK-MB) and cardiac troponin (cTnI). Cells were treated with 5 µmol/l Doxorubicin in the presence or absence of Roflumilast (2.5, 5 µM) for 24 hours. (a) The release of CK-MB; (b) The release of cTnI (****P<0.0001 vs vehicle control; ##, ###P<0.01, 0.001 vs Doxorubicin treatment).

Roflumilast alleviates doxorubicin-induced oxidative stress

H9c2 cardiac cells were treated with 5 µM Doxorubicin in the presence or absence of Roflumilast (2.5, 5 µM) for 24 hours and the expressions of 4-HNE and GPX4 were evaluated to illustrate the effects of Roflumilast on the activated oxidative stress. As shown in **Figure 4a**, 4-HNE in the H9c2 cardiac cells was upregulated by the introduction of Doxorubicin but was significantly downregulated by Roflumilast. The inhibited expression level of GPX4 by Doxorubicin was significantly rescued by Roflumilast **(Figure 4b)**.



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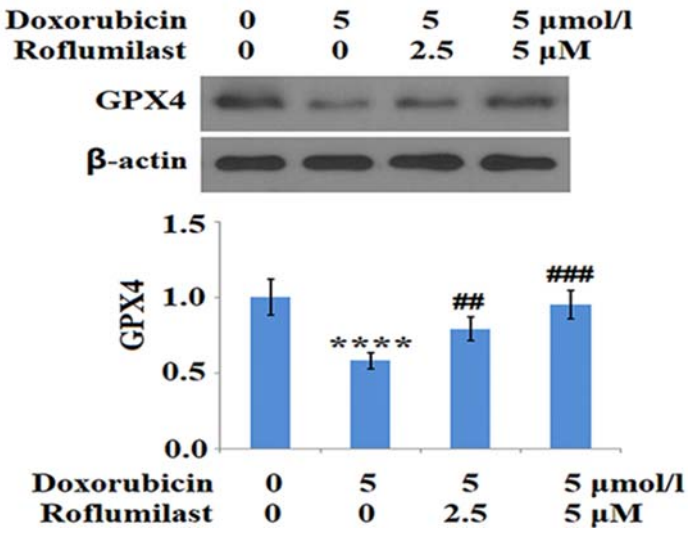

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Figure 4. Roflumilast Mitigates Doxorubicin-Induced Oxidative Stress in H9c2 Cardiomyocytes
H9c2 cells were exposed to 5 µM Doxorubicin with or without Roflumilast (2.5 and 5 µM) for 24 hours. (a) Immunofluorescence detection of 4-Hydroxynonenal (4-HNE) levels; (b) Western blot analysis of glutathione peroxidase 4 (GPX4) expression. Statistical significance: ****P < 0.0001 versus vehicle-treated control; ##, ### P < 0.01, 0.001 versus Doxorubicin-treated cells.

Roflumilast suppresses doxorubicin-induced inflammatory responses

To examine the anti-inflammatory effects of Roflumilast, the levels of pro-inflammatory cytokines IL-6, IL-17, TNF-α, and the anti-inflammatory cytokine IL-4 were measured in H9c2 cardiomyocytes. As illustrated in **Figures 5a–5h**, Doxorubicin treatment led to significant elevations in IL-6, IL-17, and TNF-α, which were markedly reduced following Roflumilast administration. Conversely, the Doxorubicin-induced reduction in IL-4 expression was reversed by Roflumilast in a dose-dependent manner. These findings suggest that Doxorubicin triggers a strong inflammatory response in H9c2 cells, which can be effectively mitigated by Roflumilast treatment.

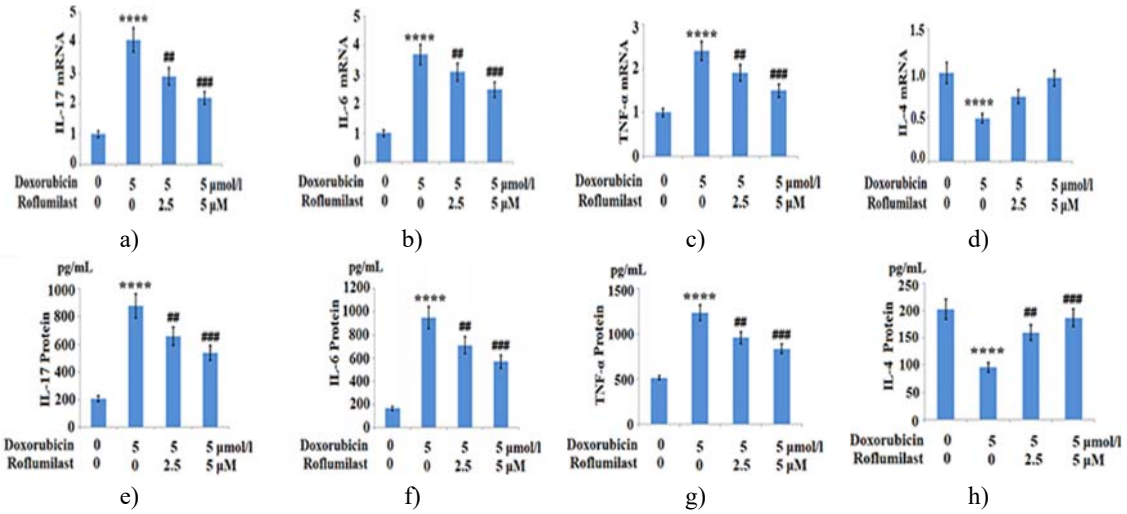


Figure 5. Impact of Roflumilast on Doxorubicin-Induced Cytokine Expression in H9c2 Cardiomyocytes H9c2 cells were exposed to 5 µM Doxorubicin with or without Roflumilast (2.5 and 5 µM) for 24 hours. Panels show: (a) IL-17 mRNA; (b) IL-6 mRNA; (c) TNF-α mRNA; (d) IL-4 mRNA; (e) IL-17 protein; (f) IL-6 protein; (g) TNF-α protein; (h) IL-4 protein. Statistical significance: ****P < 0.0001 versus vehicle control; ##, ### P < 0.01, 0.001 versus Doxorubicin-treated cells.

Roflumilast reduces doxorubicin-induced cellular senescence

Cellular senescence is a key pathological effect caused by Doxorubicin treatment. In this study, H9c2 cardiomyocytes were incubated with 5 µM Doxorubicin in the presence or absence of Roflumilast (2.5 and 5 µM) for 24 hours, and senescence was assessed using SA-β-Gal staining. As depicted in **Figure 6**, Doxorubicin significantly increased SA-β-Gal-positive staining, indicating elevated cellular senescence. Treatment with Roflumilast, however, markedly reduced SA-β-Gal staining in a dose-dependent manner, suggesting that Roflumilast effectively attenuates Doxorubicin-induced senescence in H9c2 cells.

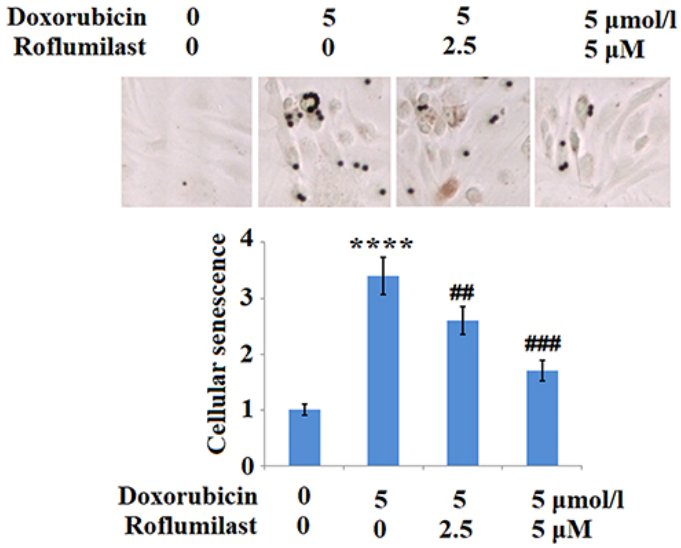
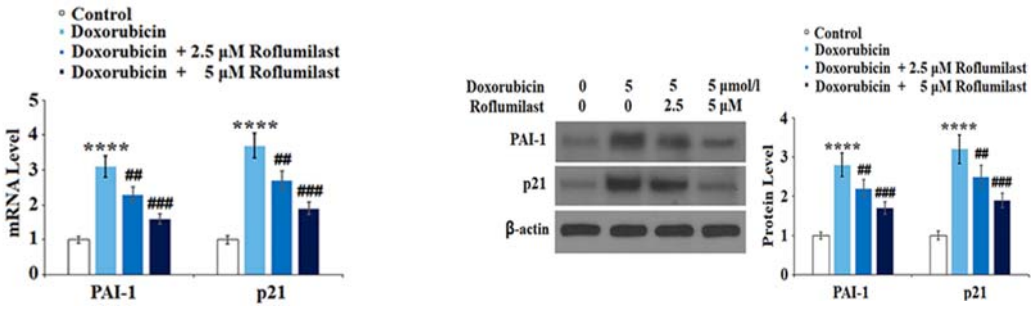


Figure 6. Roflumilast Reduces Senescence in H9c2 Cardiomyocytes Triggered by Doxorubicin.

H9c2 cells exposed to 5 µM Doxorubicin were treated with or without Roflumilast (2.5 or 5 µM) for 24 hours, and cellular aging was assessed using SA-β-Gal staining. Doxorubicin markedly increased the proportion of senescent cells, whereas Roflumilast treatment significantly decreased the number of SA-β-Gal-positive cells in a concentration-dependent manner, indicating its protective effect against drug-induced senescence (****P < 0.0001 vs control; ##, ### P < 0.01, 0.001 vs Doxorubicin alone).

Roflumilast inhibits doxorubicin-induced elevation of p21 and PAI-1

To determine whether Roflumilast could counteract senescence signaling initiated by Doxorubicin, H9c2 cardiomyocytes were treated with 5 µM Doxorubicin in the presence or absence of Roflumilast (2.5 or 5 µM) for 24 hours. Both qRT-PCR and Western blot analyses revealed that Doxorubicin substantially increased the expression of the senescence markers p21 and PAI-1. Co-treatment with Roflumilast markedly suppressed these elevations, demonstrating its potential to mitigate cellular aging pathways triggered by chemotherapeutic stress.



a) b)

Figure 7. Roflumilast Suppresses Doxorubicin-Induced Upregulation of PAI-1 and p21 in H9c2 Cardiomyocytes

H9c2 cells exposed to 5 µM Doxorubicin were treated with or without Roflumilast (2.5 or 5 µM) for 24 hours. Analysis of both mRNA **(Figure 7a)** and protein levels **(Figure 7b)** revealed that Doxorubicin significantly increased the expression of the senescence markers PAI-1 and p21. Co-treatment with Roflumilast effectively reduced these elevations in a concentration-dependent manner (****P < 0.0001 vs control; ##, ### P < 0.01, 0.001 vs Doxorubicin alone), indicating its protective effect against Doxorubicin-induced cellular aging.

Roflumilast restores SIRT1 levels reduced by doxorubicin

The impact of Roflumilast on SIRT1, a protein known to counteract cellular senescence, was examined in H9c2 cells treated with 5 µM Doxorubicin for 24 hours. Both qRT-PCR and Western blot analyses showed that Doxorubicin markedly suppressed SIRT1 expression. However, co-treatment with Roflumilast (2.5 or 5 µM) significantly restored SIRT1 levels, suggesting that Roflumilast may exert its anti-senescent effect through the upregulation of this protective protein.

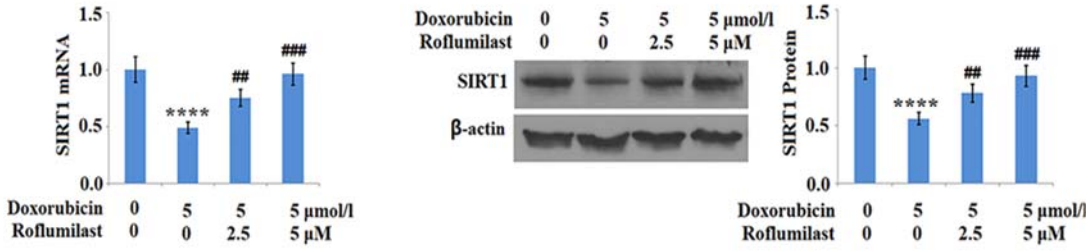


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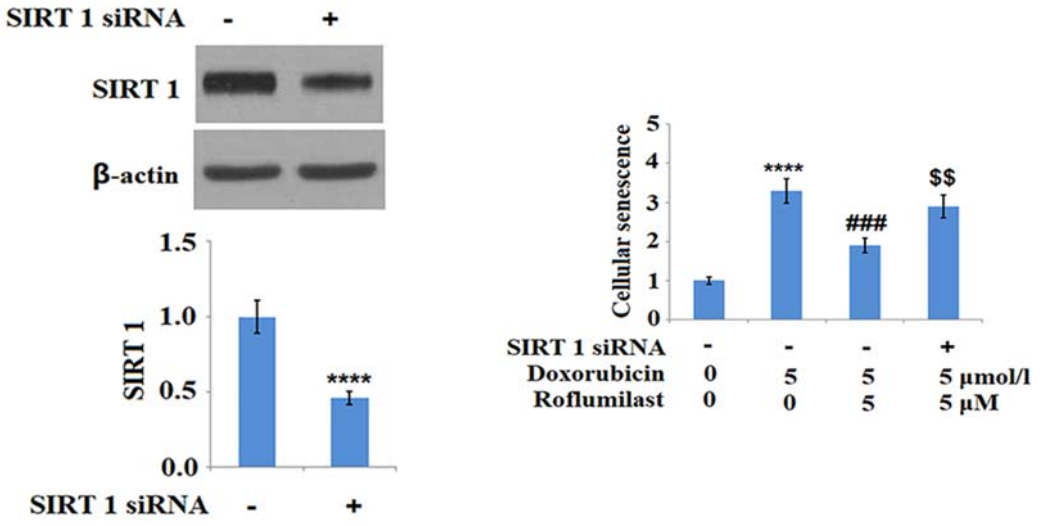
Figure 8. Roflumilast Reverses Doxorubicin-Induced Suppression of SIRT1 in H9c2 Cardiomyocytes

H9c2 cells were exposed to 5 µM Doxorubicin with or without Roflumilast (2.5 or 5 µM) for 24 hours. Analysis of both mRNA **(Figure 8a)** and protein levels **(Figure 8b)** demonstrated that Doxorubicin significantly reduced SIRT1 expression. Administration of Roflumilast counteracted this effect in a concentration-dependent manner (****P < 0.0001 vs control; ##, ### P < 0.01, 0.001 vs Doxorubicin alone), suggesting that SIRT1 plays a crucial role in mediating the protective effect of Roflumilast against cellular senescence.

SIRT1 knockdown negates the anti-senescent effect of roflumilast

To determine whether SIRT1 is required for Roflumilast's anti-senescent action, H9c2 cells were transfected with siRNA to suppress SIRT1 expression. Western blot confirmed efficient knockdown of SIRT1 **(Figure 9a)**. Following treatment with Doxorubicin in the presence or absence of Roflumilast (2.5 or 5 μM) for 24 hours, SA-β-Gal staining revealed that the protective effect of Roflumilast against Doxorubicin-induced cellular senescence was lost **(Figure 9b)**. These results indicate that the ability of Roflumilast to inhibit senescence in H9c2 cardiomyocytes depends on the modulation of SIRT1 expression.



a) b)

Figure 9. SIRT1 Silencing Abrogates Roflumilast's Protective Effect in H9c2 Cardiomyocytes. H9c2 cells were transfected with SIRT1-specific siRNA and subsequently exposed to 5 μM Doxorubicin, in the presence or absence of Roflumilast (2.5 or 5 μM) for 24 hours. (a) Western blot analysis confirmed effective knockdown of SIRT1. (b) Cellular senescence was assessed via SA-β-Gal staining. Silencing SIRT1 abolished the anti-senescent effect of Roflumilast induced by Doxorubicin (****P < 0.0001 vs vehicle control; ###P < 0.001 vs Doxorubicin; $$P < 0.01 vs Doxorubicin + Roflumilast).

Doxorubicin is an anthracycline chemotherapeutic widely employed for the treatment of various malignancies but is restricted clinically due to its cardiotoxic side effects, including dilated cardiomyopathy and chronic heart failure (CHF) [22]. Cardiac cells isolated from patients demonstrate reduced proliferation and activity following Doxorubicin exposure, which is exacerbated by prolonged administration [22]. At present, dose reduction is the most common strategy to mitigate cardiac toxicity; however, it does not prevent or reverse the progression of CHF. Although key mediators of CHF have been identified, the precise molecular mechanisms remain incompletely understood, involving changes in proteins such as PAI-1, p21, and SIRT1 [23].

In this study, an in vitro model of Doxorubicin-induced CHF was established using H9c2 cells. The model was validated through measurements showing reduced cell viability, elevated LDH release, oxidative stress, and inflammatory activation. Additionally, Doxorubicin markedly induced cellular senescence, upregulated CHF-related proteins PAI-1 and p21, and downregulated SIRT1, reflecting molecular alterations associated with CHF. These results highlight the need for therapeutic agents capable of attenuating Doxorubicin-induced cardiotoxicity. Our findings demonstrate that Roflumilast exerts protective effects against these adverse changes in H9c2 cells, suggesting potential benefits for patients receiving Doxorubicin chemotherapy [24, 25].

Mature cardiomyocytes are terminally differentiated, with minimal proliferative capacity. Exposure to chronic stress, oxidative damage, and inflammation can trigger cell death, which contributes to cardiac dysfunction. Beyond cell death, a distinct process termed "stress-induced" or "irritable" senescence has been observed in cardiomyocytes under pathological conditions such as chemotherapy or obesity [26, 27]. Unlike replicative senescence, which arises after a defined number of cell divisions, irritable senescence is triggered by external stimuli and is characterized by flat morphology, increased permeability, upregulation of senescence markers, enhanced SA-β-Gal activity, and telomerase-independent growth arrest [28, 29]. Senescent cells may impair organ function by secreting pro-inflammatory mediators including IL-6, IL-8, IL-17, GM-CSF, MCP-1, and MMP-1

[30]. In our experiments, Doxorubicin induced significant senescence in H9c2 cells, which was alleviated by Roflumilast, supporting its potential role in counteracting CHF-related cellular changes. However, further studies in animal models or clinical settings are needed to fully evaluate Roflumilast's therapeutic potential in Doxorubicin-induced CHF.

P21 is a critical regulator of senescence, promoting mitochondrial dysfunction and ROS production through pathways such as GADD45-MAPK14(p38MAPK)-GRB2-TGFBR2-TGFβ [31]. PAI-1 also contributes to cellular senescence via the TGF-β1/p53 signaling pathway [32]. SIRT1 is a well-established anti-senescent factor with documented effects on PARP1 and NF-κB signaling [33, 34]. In this study, Roflumilast reversed Doxorubicin-induced upregulation of p21 and PAI-1 and restored SIRT1 expression, suggesting that its anti-senescent effect may involve these proteins. Knockdown of SIRT1 abolished Roflumilast's protective effects, confirming that SIRT1 upregulation is essential for its action. A schematic of the proposed molecular mechanism is presented in **Figure 10**.

The sirtuin family (SIRT1-7) plays key roles in cardiometabolic health and aging. Prior research has shown that Doxorubicin exposure reduces SIRT1 in H9c2 cells [35]. Among sirtuins, SIRT1 and SIRT3 are highly expressed in cardiomyocytes and both influence Doxorubicin cardiotoxicity [36], with SIRT1 serving as the dominant regulator of cardiomyocyte injury [37]. Our findings indicate that Roflumilast mitigates Doxorubicin-induced SIRT1 reduction, reinforcing its protective role against drug-induced cardiac dysfunction.

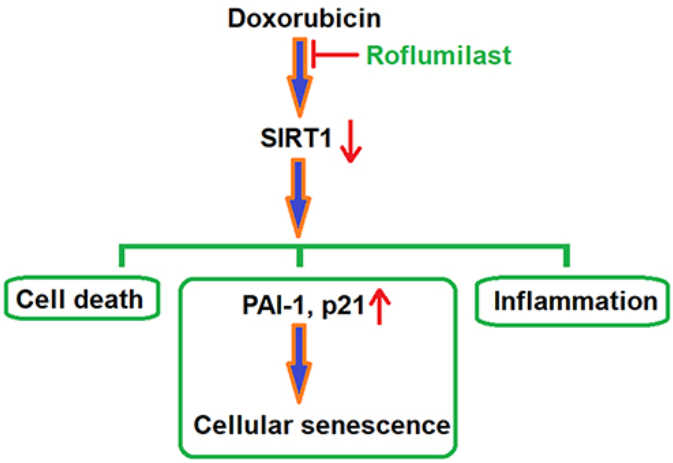


Figure 10. A representative schematic of the molecular mechanism.

Several limitations exist in the current study. The protective effects of Roflumilast against Doxorubicin-induced cardiotoxicity were evaluated using H9c2 cells derived from rat cardiac tissue. While this cell line is widely accepted as a convenient in vitro model for studying Doxorubicin cardiotoxicity [38], it cannot fully replicate the behavior of primary cultured cardiomyocytes. H9c2 cells are undifferentiated myoblasts, and their immortalized, actively dividing nature differs from fully differentiated, spontaneously contracting cardiomyocytes found in vivo [39]. Consequently, the transcriptional and functional responses to Roflumilast in H9c2 cells may not fully represent those in native cardiomyocytes. Future studies should validate these findings using primary rat cardiomyocytes or appropriate in vivo cardiotoxicity models.

Phosphodiesterase (PDE) enzymes are important targets in drug development. In rodents, PDE4 is the predominant isoform, whereas in the human heart, PDE3 is expressed at higher levels. Previous research suggests that PDE4 expression in the human heart may help reduce susceptibility to arrhythmias [40]. Accordingly, Roflumilast is not expected to induce cardiac side effects in humans, provided it is not administered concurrently with PDE3 inhibitors [41].

Conclusion

In summary, our results indicate that the PDE4 inhibitor Roflumilast mitigates Doxorubicin-induced inflammation and cellular senescence in cardiomyocytes, likely via upregulation of SIRT1. Roflumilast shows a protective effect against Doxorubicin-mediated cardiac cell injury in vitro. Further studies are warranted to elucidate the detailed

mechanisms underlying its cardioprotective actions, which may support its potential therapeutic application in chemotherapy-induced cardiotoxicity.

Acknowledgments: This work was supported by the National Natural Science Foundation of China (No.81170102 & No.81441011 & No.81670328), the Fourth Period Project "333" of Jiangsu Province (BRA2012207), and the Natural Science of Jiangsu Province Youth Fund (BK-20141020).

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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