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# Novel Agonists of Adenosine Receptors in Animal Model of Acute Myocardial Infarction

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**Background and Purpose:** Current treatments for acute myocardial infarction (AMI) include pain relief and attempts to improve survival. This study investigated the effects of two new ligands of the adenosine receptor, LASSBio-1027 and LASSBio-1860, on cardiac function in an experimental model of AMI.

**Methods:** AMI was induced in Wistar rats by ligating the anterior descending coronary arteries. Infarcted animals were treated orally with vehicle (DMSO), LASSBio-1027 (30 and 70 µmol/kg), or LASSBio-1860 (70 µmol/kg) for seven days. Hemodynamic parameters were observed using echocardiography, whereas inflammation and fibrosis were detected using histological analysis.

**Results:** MI increased the filling pressure from 23.0 ± 1.6 and 14.0 ± 2.0 to 37.0 ± 3.7 and 33.2 ± 8.0, respectively indicating diastolic dysfunction. However, treatment with LASSBio-1027 (70 µmol/kg) and LASSBio-1860 (70 µmol/kg) reduced this parameter to 23.9 ± 5.4 and 17.1 ± 6.7. An impairment in ejection fraction from 57.1 ± 3.2 to 36.6 ± 2.0% was observed after MI, partially recovered to 47.0 ± 7.4% by LASSBio-1027 and fully restored to 61.8 ± 4.3% after 7 days of treatment with LASSBio-1860. After MI, collagen deposition in LV free wall was increased to 31.4 ± 11.0% and treatment with LASSBio-1027 reduced to 23.4 ± 6.0 and 19.7 ± 8.0% at 30 and 70 µmol/kg, respectively. Similarly, LASSBio-1860 reduced collagen levels to 63.1 ± 2.0%.

**Conclusion:** Fibrosis and inflammatory components of MI reduced following treatment with agonist of adenosine receptor subtype A2A. Cardiac remodeling induced by LASSBio-1027 and LASSBio-1860 may be responsible for the improvement in cardiac function in AMI through the activation of A2A adenosine receptors.

**Keywords:** cardiac remodeling, inflammation, myocardial infarction, adenosine receptors

## Introduction

### Pathophysiology of Acute Myocardial Infarction (AMI)

AMI remains the leading cause of death among patients with cardiovascular disease. AMI results from sustained ischemia and consists of three phases: inflammatory, proliferative, and reparative. Each phase is intricately regulated by various cell types and cytokine levels.[1,2] Notably, cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, and IL-6 not only promote cell recruitment, extracellular matrix (ECM) degradation, and fibroblast activation within the necrotic core, but also adversely affect healthy myocardial regions, contributing to cardiac dysfunction, ventricular remodeling, and heart failure.[3] Recent evidence highlights the pivotal role of cardiac fibroblasts in fibrosis, responding to microenvironmental stimuli and directly acting on the ECM in cardiac tissue.[4] During the healing process, this appears as upregulation in fibrosis-associated genes, promoting ECM synthesis and remodeling.[5] Consequently, persistent stimuli that disrupt the ventricular structure ultimately result in impaired systolic and diastolic functions.[6]

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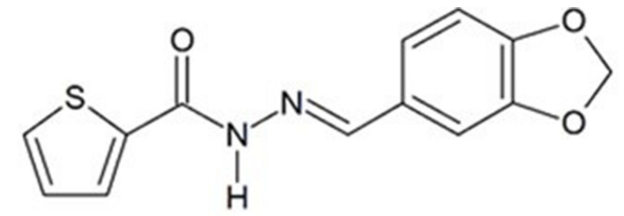
## Role of Adenosine Receptors in AMI

Current guidelines for AMI treatment do not provide pharmacological strategies to control inflammation.[7,8] Therefore, it is important to identify new therapeutic targets that regulate the inflammatory response during the early phase of AMI, thereby improving cardiac performance.

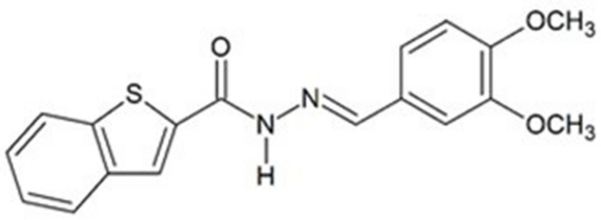
One potential target is the adenosine system, which promotes different physiological or pathological responses in several tissues, including the immune, cardiovascular, and nervous systems.[9] Adenosine activates four subtypes of membrane G protein-coupled receptors, named adenosine receptors (AR): $A_1$-AR, $A_{2A}$-AR, $A_{2B}$-AR, and $A_3$-AR. Activation of $A_1$-AR and $A_3$-AR, coupled to Gi, inhibits adenylate cyclase (AC) activity and reduces cAMP levels, while $A_{2A}$-AR and $A_{2B}$-AR, coupled to Gs, activate AC and increase intracellular cAMP, thereby opening $Ca^{2+}$ channels. $A_1$-AR are ubiquitously expressed in the cardiovascular system, particularly in atria, vascular smooth muscle and coronary artery endothelium, and $A_2$-AR are primarily located in vessels, atrial and ventricular tissues. The main effects of adenosine on the cardiovascular system include changes in heart rate (HR) and blood pressure (BP), but also exerts a protective action by reducing oxygen demand, inflammation, and angiogenesis. Activation of $A_{2A}$-AR promotes fibroblast inhibition, reduction of collagen synthesis, and anti-inflammatory action and vasodilation,[10–14] while $A_3$-AR has anti-inflammatory activity by modulating the NF-κB pathway.[15] Therefore, a reduction in the inflammatory milieu of AMI by activating adenosine receptors could represent an alternative strategy to prevent ventricular remodeling and subsequent HF.

## Compound Development and Mechanism

A novel *N*-acylhydrazone, (*E*)-*N*'-(benzo[*d*][1,3]dioxol-5-ylmethylene)thiophene-2-carbohydrazide (LASSBio-1027), was designed as a retroisoster of (*E*)-*N*'-(thiophen-2-ylmethylene)benzo[*d*][1,3]dioxole-5-carbohydrazide (LASSBio-294),[16] which improves MI-induced cardiac dysfunction in rats.[17] Therefore, the thiophene ring linked to the imine function of LASSBio-294 is bound to the carbonyl of the *N*-acylhydrazonic subunit in LASSBio-1027.[18] LASSBio-1027 was previously described to have affinity for $A_{2A}$-AR and $A_3$-AR and, as a consequence, produces vascular relaxation and reduces blood pressure.[19] Optimization of this compound by opening the dioxolane ring and extension of acyl-bound aromatic system (6π to 10π) generated (*E*)-*N'*-3,4-dimethoxybenzylidenebenzo[*b*]thiophene-2-carbohydrazide, named LASSBio-1860 (Figure 1), which binds the $A_{2A}$-AR and displays increased lipophilicity.[20]




LASSBio-1027




LASSBio-1860

**Figure 1** Chemical structure of LASSBio-1027 and LASSBio-1860.

## Rationale for Study

As modulation of the adenosine system has the potential to prevent MI-induced cardiac remodeling, the present study investigated the effects of a new $A_{2A}$-AR and $A_3$-AR ligand, LASSBio-1027, compared with its analog LASSBio-1860, a ligand for $A_{2A}$-AR, in the early phase of MI in rats.

# Materials and Methods

All protocols were approved by the Ethics Committee on the Use of Animals at Universidade Federal do Rio de Janeiro. Male Wistar rats (180–200 g) were kept under a light/dark cycle of 12 hours at 24 °C, with free access to water and pellet-type feed. Animals were kept in accordance with Brazilian Guide of Production, Maintenance and Utilization of Animals for Teaching or Scientific Research Activities (1st edition, 2016) approved by the National Council for Control of Animal Experimentation. Ketamine and isoflurane were provided by Cristália Produtos Químicos e Farmacêuticos Ltda (Itapira, SP, Brazil), LASSBio-1027 and LASSBio-1860 (Figure 1) were synthetized by Laboratório de Avaliação e Síntese de Substâncias Bioativas (LASSBio®, Universidade Federal do Rio de Janeiro (Rio de Janeiro, Brazil).

## Myocardial Infarction Model

Animals were anesthetized with isoflurane 3% and maintained at 1.5% until the end of the surgical procedure. Myocardial infarction was induced by total occlusion of the anterior descending coronary artery, followed by mechanical ventilation until recovery from anesthesia.

The sham group underwent the same surgical procedure but without coronary occlusion.[17,21] Four hours after surgery, the animals were subjected to echocardiographic analysis (Vevo 770, FUJIFILM Visual Sonic, Inc., Toronto, Ontario, Canada) to confirm MI by observing the absence of left ventricle (LV) wall motility (M-mode short axis image).[17] Animals were divided into Sham and MI groups, and randomly orally treated daily with either vehicle (dimethylsulfoxide, DMSO), LASSBio-1027 (30 µmol/kg or 70 µmol/kg), or LASSBio-1860 (70 µmol/kg) for 7 days. Treatment was initiated immediately after confirmation of MI by echocardiography (4 hours after occlusion). The dose of the substances was initially established based on the results of the prototype substance LASSBio-294 (molecular weight: 274 g/mol), which demonstrated satisfactory results in subchronic treatment after myocardial infarction at a dose of 70 µmol/kg.[17,21,22] The doses of 30 and 70 µmol/kg LASSBio-1027 (molecular weight: 274 g/mol) corresponded to 8.22 and 19.18 mg/kg, respectively, whereas for LASSBio-1860 (molecular weight: 340 g/mol) a dose of 70 µmol/kg was equivalent to 23.8 mg/kg.

## Transthoracic Echocardiography

Cardiac structure and function were evaluated using echocardiography, and images of the short and long parasternal axes were obtained in animals under anesthesia with isoflurane (3%). The structural parameters obtained were LV internal diameter and thickness of the anterior and posterior LV walls during systole and diastole, respectively. Simpson's method was used to determine ejection fraction (EF), cardiac output, and rapid (E) and late (A) transmitral filling velocities, and rapid mitral annular velocity (e') was obtained using pulsed tissue Doppler imaging to calculate the ventricular filling pressure (E/e').[17]

## Left Ventricle Catheterization

LV pressure measurements were obtained after the animals were kept under anesthesia with ketamine (80 mg/kg, i.p.) and xylazine (15 mg/kg, i.p.) for catheter insertion into the right carotid artery, which was connected to a calibrated pressure transducer (MLT884, ADInstruments). After blood pressure stabilization, the catheter was introduced until it reached the LV to record the LV systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), and rates of contraction and relaxation (+dP/dt and −dP/dt). All parameters were recorded (PowerLab, ADInstruments) and stored on a computer for further analysis using LabChart (ADInstruments, version 7.0).[17]

## Histological Analysis

At the end of the protocol, the animals were euthanized, and their hearts were quickly removed and divided into two fragments, to immediately freeze in liquid nitrogen for Western blot analysis or fix in 10% buffered formalin for

histological and immunohistochemical analysis. Fixed tissue was embedded in paraffin and cut into 5 µm sections (Lupe, model MRP 03) and the presence of inflammatory infiltrate was detected using hematoxylin and eosin staining. Interstitial cells were observed in 10 fields on each slide using a digital camera (Canon, USA) connected to an optical microscope (400x, Axiostar plus, Zeiss, Germany).

The fibrotic area in the myocardium was measured using picrosirius red staining observed in 10 fields of the peri-infarcted region located in the free wall of the LV, using an optical microscope (400x, Zeiss AXIOSTAR-plus). Collagen fibers stained red, muscle stained yellow, and nuclei stained black were analyzed using ImageJ software. Using the same histological staining method, the infarction area was determined for the infarcted groups and calculated as the ratio between the measurement of the internal circumference of the LV and the area of free wall fibrosis.

Changes in protein expression at the border of the injured myocardium were detected using immunohistochemical techniques to explore the following proteins involved in inflammatory and proliferative processes and fibroblast activation: α-SMA (Sigma, A2547); TNF-α (Abcam, abI793), MAPK p38 (Abcam, ab1793), c-Fos (AbCam, ab1793), and inducible nitric oxide synthase (iNOS) (AbCam, ab1793). Immunodetection was performed by exposure to goat secondary antibody (Rat Max PO MULTI, Nichirei, Japan) at room temperature in the dark, followed by the addition of the chromogenic substrate diaminobenzidine (DAB; Spring, USA), which produced brown staining. Ten photos of each slide were taken using an optical microscope (400x) for the quantification of α-SMA, TNF-α, and iNOS, and the area corresponding to the immunostaining in each photo was measured using Fiji/ImageJ2 software. Measurement of MAPK p38- and c-Fos-labeled nuclei was observed in relation to the total number of nuclei in each area (1000x).

### Statistical Analysis

Data are expressed as the mean ± SEM. One-way ANOVA was used for comparisons between sham and experimental groups. Differences between the experimental groups were considered statistically significant at $p < 0.05$.

## Results

### LASSBio-1027 and LASSBio-1860 on Cardiac Structure After MI

The structural cardiac parameters of all experimental groups were observed using echocardiography (Figure 2A). MI induced a reduction of LV anterior wall thickness during systole from 2.1 ± 0.4 mm to 0.5 ± 0.3 mm (Figure 2B), and during diastole from 1.2 ± 0.2 mm to 0.4 ± 0.2 mm ($p < 0.05$) (Figure 2C). Treatment with LASSBio-1027 did not alter this parameter but, in contrast, LASSBio-1860 prevented this change, since the LV thickness of the anterior wall during systole was 1.5 ± 0.3 mm and during diastole 1.0 ± 0.1 mm ($p < 0.05$). Consequently, there was a significant increase in the internal diameter from 4.42 ± 0.44 to 6.73 ± 0.88 mm ($p < 0.05$) in systole and from 6.12 ± 0.78 to 8.18 ± 0.99 mm ($p < 0.05$) in diastole while the treatment with LASSBio-1860 recovered to 4.25 ± 0.68 and 6.52 ± 0.44 mm.

### LASSBio-1027 and LASSBio-1860 on Cardiac Function After MI

The filling pressure (E/e') of the Sham groups was 23.0 ± 1.6 and 14.0 ± 2.0, which increased to 37.0 ± 3.7 and 33.2 ± 8.0, respectively, indicating diastolic dysfunction after 1 week of MI induction (Figure 3A and B). However, treatment with LASSBio-1027 (70 µmol/kg) and LASSBio-1860 (70 µmol/kg) reduced this parameter to 23.9 ± 5.4 and 17.1 ± 6.7 (Figure 3A and B).

LV echocardiography during systole demonstrated that MI reduced EF from 57.1 ± 3.2 and 62.3 ± 18.0 to 36.6 ± 2.0 and 46.2 ± 3.8% ($p < 0.05$) (Figure 3C and D). EF increased to 47.0 ± 7.4% after treatment with LASSBio-1027 (70 µmol/kg) (Figure 3C) and returning to 61.8 ± 4.3% after 7 days of treatment with LASSBio-1860 (70 µmol/kg) (Figure 3D).

Sham-operated animals had LVEDP of 3.2 ± 0.9 mmHg, which increased to 18.2 ± 2.4 in the MI group (Figure 4). Improvement in diastolic function was observed after treatment with either LASSBio-1027 or LASSBio-1860. LVEDP reduced to 9.2 ± 1.4 and 6.4 ± 1.6 mmHg at the dose of 30 and 70 µmol/kg of LASSBio-1027 and to 15.5 ± 8.0 mmHg after treatment with LASSBio-1860 (Figure 4).

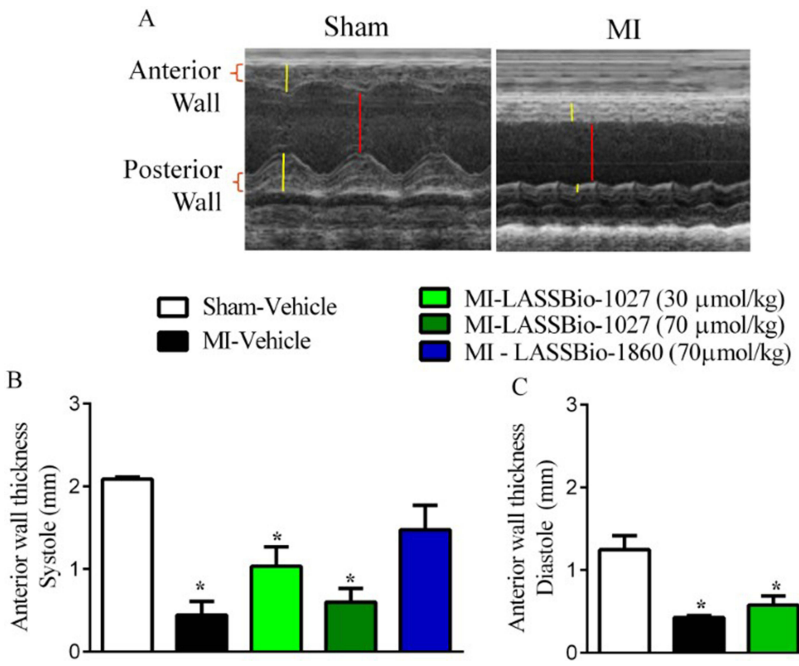



**Figure 2** LASSBio-1027 and LASSBio-1860 on left ventricule (LV) wall thickness and internal diameter obtained through echocardiography in MI-induced animals. (**A**) Representative image of LV in M-Mode of Sham-vehicle and MI-induced. (**B**) Measurement of anterior wall thickness in systole; (**C**) Measurement of anterior wall thickness in diastole from animals treated with either vehicle or LASSBio-1027 or LASSBio-1860. *p<0.05 compared to Sham-vehicle. #p<0.05 compared to MI-vehicle. Data expressed as mean ± SEM. n = 7 animals/group.

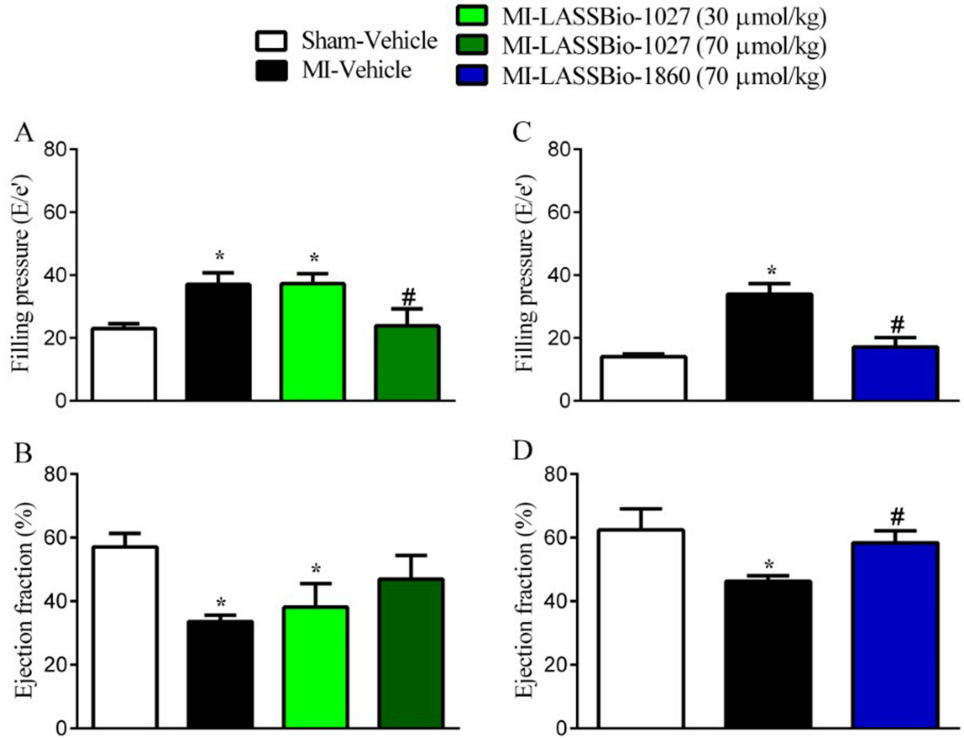



**Figure 3** LASSBio-1027 and LASSBio-1860 on left ventricule (LV) hemodynamics in MI-induced animals. (**A**) LV filling pressure (E/e') after treatement whit vehicle or LASSBio-1027. (**B**) Ejection fraction after treatement whit vehicle or LASSBio-1027. (**C**) LV filling pressure (E/e') after treatement whit vehicle or LASSBio-1860. (**D**) Ejection fraction after treatement whit vehicle or LASSBio-1860. *p<0.05 compared to Sham-vehicle. #p<0.05 compared to MI-vehicle. n = 7 animals/group.

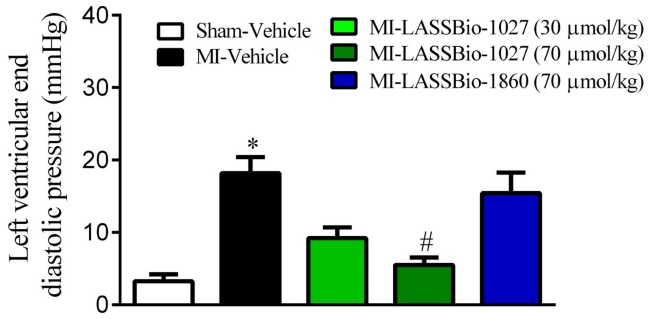



**Figure 4** LASSBio-1027 and LASSBio-1860 on left ventricule (LV) hemodynamics in MI-induced animals. LV end diastolic pressure from animals treated with either vehicle or LASSBio-1027 or LASSBio-1860. *p<0.05 compared to Sham-vehicle. #p<0.05 compared to MI-vehicle. n = 7 animals/group.

## LASSBio-1027 and LASSBio-1860 on Cardiac Remodeling – Inflammation and Fibrosis After MI

To determine cellular infiltration, we used the interstitial cell count, which increased in the MI vehicle group from 92.4 ± 14.0 to 227.5 ± 31.1 cells/µm$^2$ (Figure 5). However, the cellular infiltration was reversed by treatment with LASSBio-1027 and LASSBio-1860. Interstitial cells decreased in the MI-LASSBio-1027 groups at 30 and 70 µmol/kg to 138.5 ± 16.6 and 137.4 ± 12.6 cells/µm$^2$ and in the MI-LASSBio-1860 group (70 µmol/kg) to 104.0 ± 23.0 cells/µm$^2$ (Figure 5B).

Increased collagen deposition was observed in the free wall of the LV after MI (Figure 6A). The percentage of collagen was 3.6 ± 2.2% in the sham group, but increased to 31.4 ± 11.0% in the MI vehicle group (Figure 6B). Treatment with LASSBio-1027 reduced to 23.4 ± 6.0 and 19.7 ± 8.0% at 30 and 70 µmol/kg, respectively. Similarly, LASSBio-1860 reduced collagen levels, and, consequently, cardiac fibrosis.

In infarcted animals, the total ischemic zone was 63.1 ± 2.0%, which was determined through histological analysis using picro sirius-red staining. The infarct size calculated by the ratio between fibrosis area and LV internal diameter was not significantly different in all experimental groups because ischemic area was 64.7 ± 1.5 and 62.8 ± 3.1% after treatment with LASSBio-1027, 30, and 70 µmol/kg, respectively.

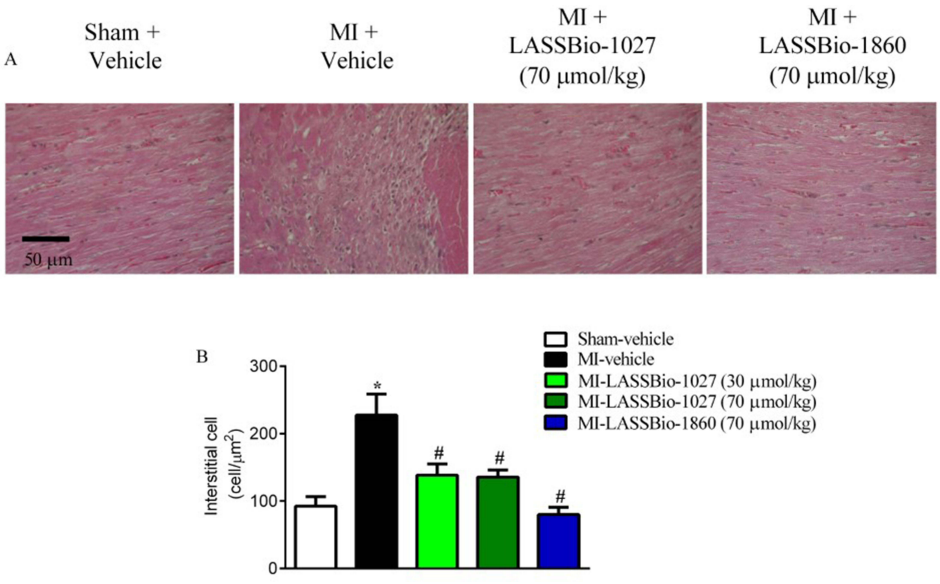



**Figure 5** LASSBio-1027 and LASSBio-1860 on left ventricule (LV) histology in MI-induced animals. (**A**) Representative micrographs of LV sections with hematoxylin-eosin stain. (**B**) Interstitial cell density in MI border zone in LV tissue from animals treated with either vehicle or LASSBio-1027 or LASSBio-1860. *p<0.05 compared to MI-vehicle. #p<0.05 compared to Sham-vehicle. Data are expressed as mean ± SEM. n = 7 animals/group.

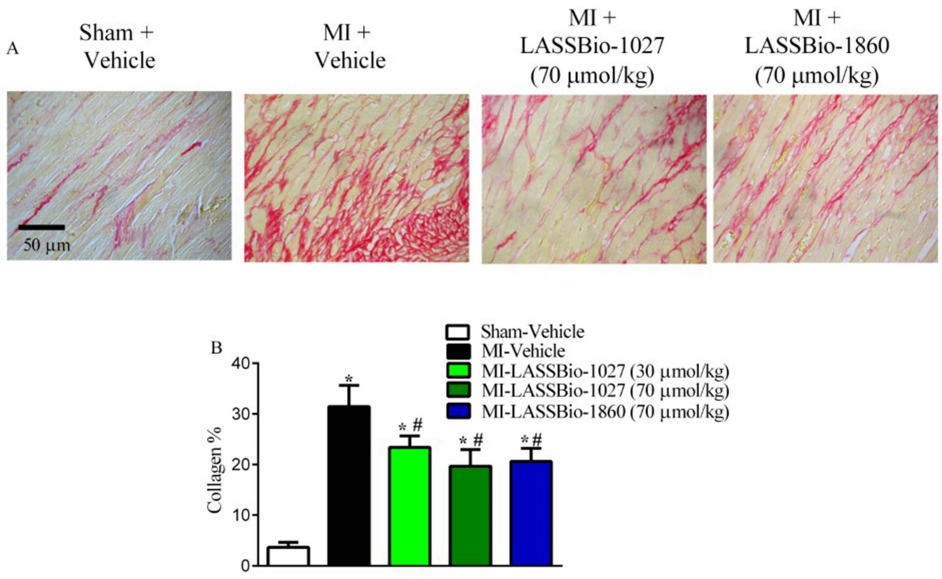



**Figure 6** LASSBio-1027 and LASSBio-1860 on left ventricule (LV) histology in MI-induced animals. (**A**) Representative micrographs of LV sections with picro sirius red stain. (**B**) Collagen deposition in MI border zone in LV tissue from animals treated with either vehicle or LASSBio-1027 or LASSBio-1860. *p<0.05 compared to Sham-vehicle. #p<0.05 compared to MI-vehicle. n = 7 animals/group.

Activation of fibroblasts in myofibroblasts is detected by the observation of α-SMA (Figure 7). Representative micrographs of LV sections for negative control, Sham-vehicle and MI treated with either vehicle, LASSBio-1027 or LASSBio-1860 are shown in Figure 7A. MI increased the α-SMA labelling from 3.2 ± 0.7 to 11.8 ± 2.9% and treatment with LASSBio-1027 reduced to 1.0 ± 2.8 and 1.4 ± 4.9% at 30 and 70 µmol/kg (Figure 7B). Similarly, LASSBio-1860 recovered to 1.5 ± 0.3%.

Experimental MI increased the inflammatory process, as evidenced by the increase in TNF-α and iNOS expression (Figures 8 and 9). Figure 8A shows the effects of LASSBio-1027 and LASSBio-1860 on LV immunohistochemistry for TNF-α stain. TNF-α expression increased from 17.9 ± 5.2 to 57.6 ± 1.9% detected using immunohistochemistry and oral administration of 30 and 70 µmol/kg of LASSBio-1027 reduced expression to 23.0 ± 4.0 and 25.4 ± 4.0%, respectively (Figure 8B). The same profile was observed with LASSBio-1860, reducing expression to 23.3 ± 10.1% (Figure 8B).

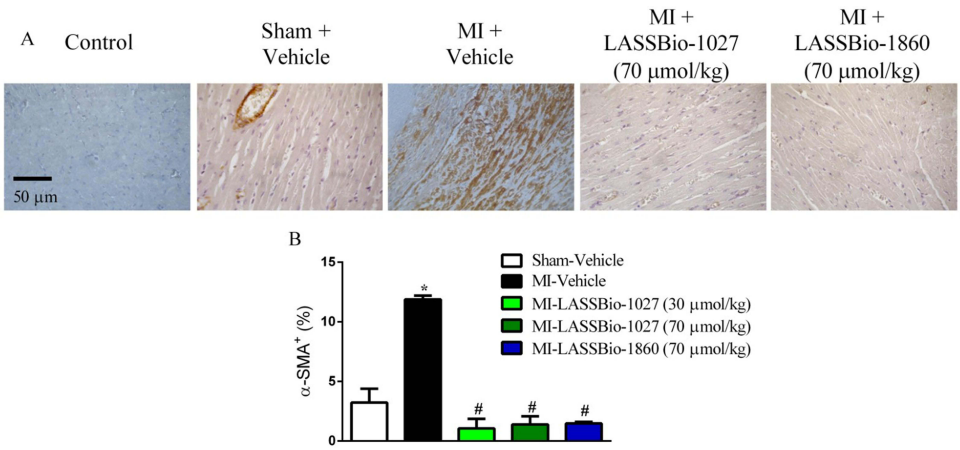



**Figure 7** LASSBio-1027 and LASSBio-1860 on left ventricule (LV) immunohistochemistry in MI-induced animals. (**A**) Representative micrographs of LV sections with immunohistochemistry for smooth muscle α-actin (α-SMA) stain in negative control, Sham-vehicle and MI treated with vehicle, LASSBio-1027 or LASSBio-1860. (**B**) Percentage of stain for α-SMA in MI border zone in LV tissue, from animals treated with either vehicle or LASSBio-1027 or LASSBio-1860. *p<0.05 compared to Sham-vehicle. #p<0.05 compared to MI-vehicle. n = 7 animals/group.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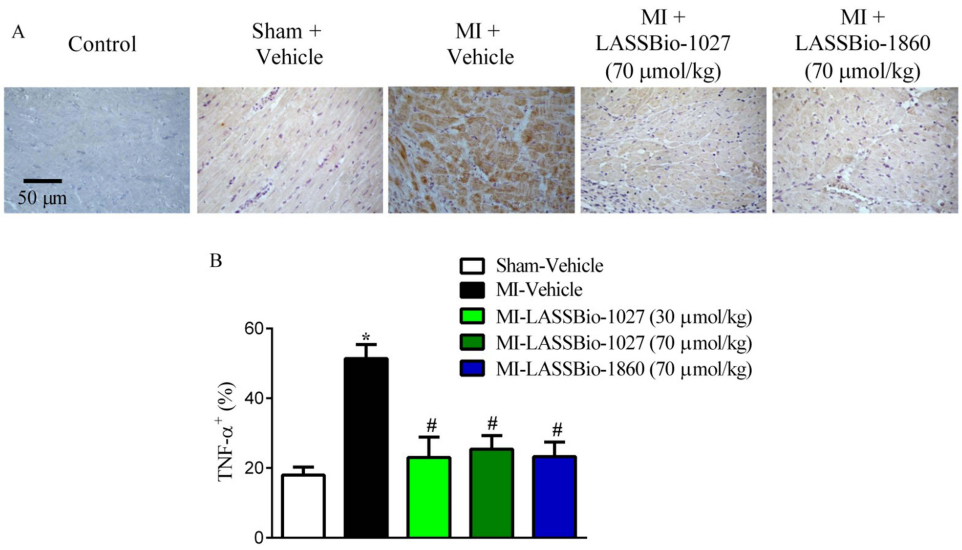


**Figure 8** LASSBio-1027 and LASSBio-1860 on left ventricule (LV) immunohistochemistry in MI-induced animals. (**A**) Representative micrographs of LV sections with immunohistochemistry for TNF-α stain in negative control, Sham-vehicle and MI treated with vehicle, LASSBio-1027 or LASSBio-1860. (**B**) Percentage of stain for TNF-α in MI border zone in LV tissue from animals treated with either vehicle or LASSBio-1027 or LASSBio-1860. *p<0.05 compared to Sham-vehicle. #p<0.05 compared to MI-vehicle. n = 7 animals/group.

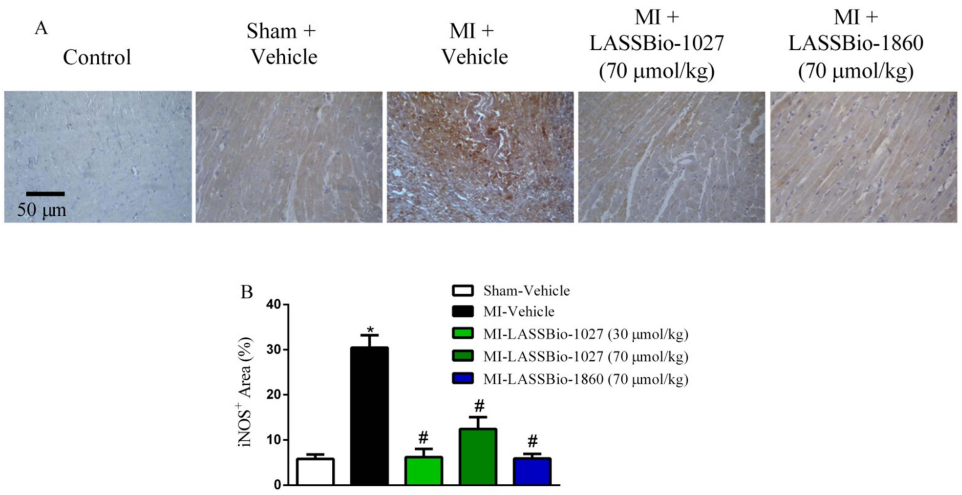


**Figure 9** LASSBio-1027 and LASSBio-1860 on left ventricule (LV) immunohistochemistry in MI-induced animals. (**A**) Representative micrographs of LV sections with immunohistochemistry for inducible nitric oxide synthase (iNOS) stain in negative control, Sham-vehicle and MI treated with vehicle, LASSBio-1027 or LASSBio-1860. (**B**) Percentage of stain for TNF-α in MI border zone in LV tissue from animals treated with either vehicle or LASSBio-1027 or LASSBio-1860. *p<0.05 compared to Sham-vehicle. #p<0.05 compared to MI-vehicle. n = 7 animals/group.

The importance of the inflammatory component in MI was reinforced by the increased iNOS expression (Figure 9). iNOS expression in MI border zone of LV tissue collected from different experimental groups is observed in Figure 9A. Immunohistochemistry detected increased iNOS staining in cardiac tissue from the MI-vehicle group of 30.5 ± 4.0 which was reduced to 6.2 ± 2.0 and 12.5 ± 2.6% after treatment with LASSBio-1027 at 30 and 70 µmol/kg (Figure 9B). Subsequently, the same protocol was repeated with LASSBio-1860, which decreased the expression to 5.9 ± 1.8% (Figure 9B). Increased cell proliferation in the MI was observed through an increase in c-Fos expression. In AMI, c-FOS expression is elevated, corresponding to cardiac hypertrophy. As shown in Figure 10, the expression of c-FOS into nucleus (area labelled in brown) increased from 12.7 ± 2.3 (Sham-vehicle group) to 37.3 ± 4.8 (p<0.05) and the treatment with LASSBio-1027 (70 µmol/kg) and LASSBio-1860 (70 µmol/kg) reduced to 9.6 ± 1.8 and 10.2 ± 1.2%, respectively (Figure 10B).

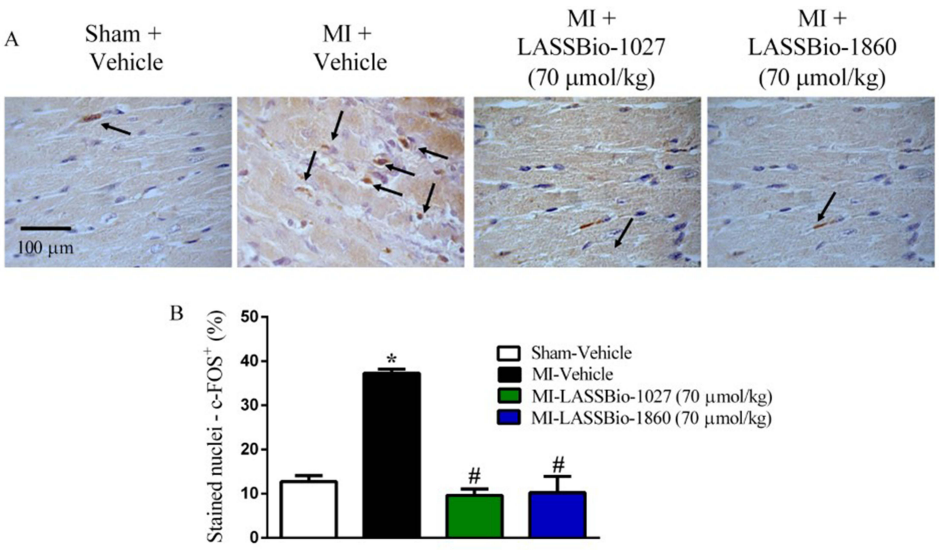


**Figure 10** LASSBio-1027 and LASSBio-1860 on left ventricule (LV) immunohistochemistry in MI-induced animals. (**A**) Representative micrographs of LV sections with immunohistochemistry for c-Fos stain, Sham-vehicle and MI treated with vehicle, LASSBio-1027 or LASSBio-1860; Arrows indicate nuclei stain by anti-c-Fos antibody. (**B**) Percentage of marker nuclei by anti-cFos in relation to the number of total nuclei in MI border zone in LV tissue from animals treated with either vehicle or LASSBio-1027 or LASSBio-1860. *p<0.05 compared to Sham-vehicle. #p<0.05 compared to MI-vehicle. n = 7 animals/group.

## Discussion

Experimental AMI causes cardiac structural and functional changes related to the long-term development of HF. HF is a multifactorial syndrome and symptoms can range from dyspnea during physical activities or rest, fatigue, fluid retention to pulmonary congestion, according to the progression of the disease.[23] In recent years, patients with HF with poor prognosis has increased.[24] The adenosine system has been shown to be a good pharmacological target for several pathologies, including HF due to MI.

Adenosine is an endogenous purine nucleoside that regulates several physiological functions involving the cardiovascular system. Activation of $A_{2A}$-AR promotes improvement in cholesterol homeostasis, platelet aggregation and inflammatory response.[25] $A_{2A}$-AR are involved in NO production, which activates guanylate cyclase and increases intracellular cGMP levels in smooth muscle.[21,22] Activation of $A_{2A}$-AR regulates vascular tone, promoting relaxation of arterial smooth muscles which in turn, decreases vascular resistance facilitating blood flow and also, decreases blood pressure and heart rate.[25]

As activation of $A_{2A}$-AR is involved in blood pressure control, LASSBio-1027 normalizes systolic and diastolic blood pressure in hypertensive but not in normotensive rats, without altering heart rate.[26] Data from infarcted rats reinforce that LASSBio-1027 did not alter blood pressure and heart rate on normotensive animals. Additionally, activation of the adenosine system decreases cellular infiltration, LVEDP, and exercise intolerance, suggesting that it could interfere with cardiac remodeling and progression of HF.[17,27,28]

It has been previously demonstrated that activation of $A_{2A}$-AR increases myocardial contractility due to increased $Ca^{2+}$ reuptake by the sarcoplasmic reticulum through increased SERCA2a expression.[29–31] $A_{2A}$-AR ligands reduce the area of fibrosis and reverse cardiac depression due to experimental MI in rats. Cardioprotection is also mediated by the activation of $A_3$-AR in MI, since $N^6$-(3-iodobenzyl)-adenosine-5'-*N*-methylcarboxamide (IB-MECA) reduces infarct size in ischemic preconditioning conditions.[32] In different animal models of MI, $A_3$-AR plays a role in the heart function through the opening of ATP-sensitive potassium ($K_{ATP}$) channels and iNOS.[33–35]

LASSBio-1027, a ligand of both $A_{2A}$-AR and $A_3$-AR, promotes vasodilation and normalizes blood pressure in spontaneously hypertensive rats.[26] Similarly to LASSBio-1027, the analog LASSBio-1860 shows affinity for the $A_{2A}$-AR,[20] which prompted a comparative investigation of its protective effects of acute MI in rats. In the present work, AMI reduced EF and thickness of the LV anterior wall and increased filling pressure and internal diameter in systole and diastole. Reduced EF consequent to occlusion of the anterior descending coronary artery is associated to a decrease in cardiac contractility due to the ischemic area and death of local cardiomyocytes. MI-induced increased LVEDP is associated with a higher probability of HF,

and increased filling pressure is related to lower survival.[36] Changes are common in patients with AMI and occurs due to impaired ventricular relaxation, leading to an increase in the end-diastolic volume, indicating the establishment of diastolic dysfunction. Treatment with 30 and 70 µmol/kg (8.2 and 19.2 mg/kg) of LASSBio-1027 was effective in preventing cardiac functional and structural changes induced by AMI. Low dose of LASSBio-1027 (30 µmol/kg) did not interfere with altered filling pressure (Figure 3A) or EF (Figure 3B), but slight reduction in LVEDP was observed (Figure 4). However, increased dose of LASSBio-1027 (70 µmol/kg) reverted the diastolic dysfunction because normalized filling pressure (Figure 3A) and LVEDP (Figure 4) and partially recovered the EF (Figure 3B). Since the improvement in cardiac remodeling was better with the high dose, LASSBio-1860 was evaluated at 70 µmol/kg (23.8 mg/kg). LASSBio-1860 binds selectively to the adenosine $A_{2A}$ receptor, unlike LASSBio-1027, which has an affinity for both adenosine $A_{2A}$ and $A_3$ receptors. Reduction in LV dysfunction was observed after treatment with LASSBio-1027 and LASSBi-1860, indicating the involvement of $A_{2A}$-AR and $A_3$-AR in cardioprotection. After tissue injury, the increased intracellular $Ca^{2+}$ due to decreased expression or activity of SERCA2a could worsen due to increased ROS levels.[37,38] As activation of $A_{2A}$-AR is associated with increased cardiac contractile force and increased expression of SERCA2a,[30] it suggests that LASSBio-1027 and LASSBio-1860 improve cardiac dysfunction by activating these receptors.

Inflammation was observed in the cardiac tissue from infarcted animals in the presence of interstitial cells and increased TNF-α and iNOS labeling. The inflammatory process plays an important role in the changes detected in AMI, because TNF-α is responsible for triggering the generation of ROS, which reduces myocardial contractility.[39] Increased TNF-α has been observed in most infarcted patients, which is correlated with intense tissue injury, considerable collagen deposition, and infarct size.[40,41] Both compounds prevented the increased expression of proteins TNF-α, iNOS, and c-Fos, which are involved in inflammation and proliferation induced by MI. Decrease in TNF-α labeling induced by LASSBio-1027 and LASSBio-1860 may be related to the prevention of HF, because of the reduction of the inflammatory process through the activation of $A_{2A}$-AR and $A_3$-AR. Similar results were observed when adenosine was infused in the early phase of MI in rats with improved cardiac function by modulating the activation of NF-κB and TNF-α.[42] With an increase in high levels of NF-κB and TNF-α in infarcted heart,[43,44] there is an increase in the expression of iNOS.[45] Hypoxia in AMI produces iNOS and elevated levels of NO in fibroblasts and endothelial cells, which contribute to contractile dysfunction. The increase of iNOS after MI occurs in the second phase of MI and the elevated NO levels causes damage to cardiomyocytes favoring the development of HF.[45–47] Furthermore, it was demonstrated in a mouse model that treatment with an agonist of $A_{2A}$-AR, decreased iNOS expression with simultaneous improvement in cardiac function, suggesting the involvement of AR.[48] Thus, LASSBio-1027 and LASSBio-1860 can prevent the development of HF by reducing the expression of iNOS in cardiac tissue.

In the acute phase of MI, considered as the inflammatory phase, fibroblasts in the ischemic region of the cardiac tissue transdifferentiate towards a pro-inflammatory phenotype and mediate the recruitment of inflammatory cells. Fibroblasts in the infarction area then convert into myofibroblasts and begin to express contractile proteins, such as α-SMA, and synthesize large amounts of ECM proteins.[4] Thus, MI increases α-SMA in cardiac tissue, indicating the activation of fibroblasts to myofibroblasts and cardiac fibrosis detected by the accumulation of collagen in the myocardium. Increased deposition of ECM contributes to the expansion of fibrosis and to cardiac remodeling with consequent progression to HF.[4,49] Activation of the $A_{2A}$-AR improves cardiac function and suppresses cardiac fibrosis, thus preventing adverse cardiac remodeling by reducing the expression of fibronectin, MMP-9, collagen I and collagen III and inflammatory cytokines.[50] Treatment with LASSBio-1027 or LASSBio-1860 decreased α-SMA labeling, reducing fibrosis and demonstrating to be a potential target for HF prevention. These new agonists of AR, which decrease the production of collagen, could decrease the occurrence of myocardial fibrosis and consequently improve cardiac contractile activity and compliance and prevent HF after AMI.

The presence of an apoptotic process, which indicates the presence of MI-induced proliferation in the heart, was confirmed by increased α-SMA labeling and increased expression of the molecular marker c-Fos. The nuclear protein c-Fos is involved in the proliferative process, and its activation is dependent on its phosphorylation by MAPK p38α.[51] LV hypertrophy is also related to the higher collagen content in the myocardium, as observed by the greater labeling of collagen fibers in the staining of picrosirius red in infarcted animals, contributing to LV dysfunction. Cardiac hypertrophy was reduced by the oral administration of LASSBio-1027 or LASSBio-1860, possibly promoted by AR activation. These findings are in accordance with the demonstration that decreased expression of c-Fos could attenuate cardiac fibrosis and

hypertrophy in neonatal cardiomyocytes of rats and mice.[52] There are no effective drugs in clinical use that modulate the adenosine system and prevent cardiac remodeling induced by MI. Dipyridamole inhibits adenosine uptake and metabolism by increasing coronary vasodilation and blood flow through $A_{2A}$-AR activation. However, since it is a non-selective adenosine receptor agonist, it can promote adverse effects through activation of the adenosine $A_1$ receptor. Acadesine, an adenosine-regulating agent, has not demonstrated good clinical outcomes in revascularization studies. Regadenoson, a selective agonist for $A_{2A}$-AR, could be promising for avoiding cardiac remodeling, however, its high cost limits its clinical use.[53] According to our results, LASSBio-1027 and LASSBio-1860 demonstrated promising effects in preventing MI-induced cardiac remodeling through activation of adenosine receptors.

## Conclusion

New agonists of AR contribute to improving changes in the LV and reverse remodeling and dysfunction, reinforcing the importance of the adenosine system as a potential therapeutic target for the treatment of the early phase of MI.

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## Disclosure

The authors report no conflicts of interest in this work.

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