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Synthesis and vasorelaxant evaluation of novel 7-methoxyl-2,3-disubstituted-quinoxaline derivatives

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ABSTRACT

An array of novel 7-methoxyl-2,3-disubstituted quinoxaline derivatives was designed, synthesized and their potential antihypertensive activities were examined, in an attempt to discover potent small molecules with vasorelaxant effects. The vasoactivities of these compounds on vascular tone, as well as underlying mechanisms were hereby explored. Results showed that five compounds (**7s**, **7t**, **7v**, **7w**, **7γ**) could induce endothelium-independent relaxation in high extracellular K^+- and phenylephrine-precontracted C57 mice aortic rings. These five compounds, unlike other commonly used vasodilators, could slowly but effectively inhibit vasoconstriction.

Cardiovascular diseases (CVDs), including atherosclerosis, myocardial infarction, congenital and rheumatic heart disease, heart failure, coronary syndromes and vascular inflammation, have been among the leading causes of death worldwide. The morbidity and mortality of CVDs have increased due to a rapidly aging population in recent years. Additionally, uncontrolled or inadequately treated hypertension can accelerate atherosclerosis, increasing the risk of myocardial infarction, stroke, heart attack and end-stage kidney failure [1]. Although multiple risk factors, contribute to the pathogenesis of various CVDs, directly or indirectly, systemic arterial hypertension, or persistently high blood pressure, remains the most common modifiable factor, which has been well-established to have strong correlations with the occurrence of all types of CVDs across a wide range of age groups, according to a recent high-resolution survey [2]. Therefore, the importance of effective blood pressure-lowering agents with relatively clear mechanism for treatment of hypertension and also for amelioration of peripheral circulation is urgently emphasized.

In normal circumstances, the dynamic balance of vasoconstriction and vasodilation collaboratively contributes to the maintenance of homeostasis within the vasculature system. Vasodilation typically depends on the vascular endothelium, which is located between circulating blood and vascular smooth muscle, making it a basic bio-target and point of regulating vascular tone. Extensive evidence supports that many vasodilation mediators, *e.g.* nitric oxide (NO) and reactive oxygen species (ROS) from endothelium, are involved in vasodilation *via* endothelium-dependent relaxation of vascular smooth muscle [3]. Endothelium-independent vasodilation is also critical, since structural vascular changes and alterations in vascular smooth muscle cells may mediate endothelium-independent vasodilation, which will be impaired in case of cardiovascular-related pathologies, such as atherosclerosis [4]. Vasoconstriction, on the other hand, is activated by several mediators in vascular smooth muscle cells, including but not limited to, calmodulin, myosin light chain kinase and phosphorylated myosin light chain [5]. Thus, inhibition of vasoconstriction signaling pathways will cause vasodilation.

In an effort to identify bioactive heterocyclic molecules with promising vasorelaxant effects, we noticed many *N*-containing heterocycles, a class of important pharmacophoric platforms in drug development, have been frequently found in an array of natural or synthetic occurring derivatives, such as Fasudil (HA-1077), a clinically available vasodilator for the treatment of cerebral vasospasm which exerts its activity *via* inhibition of RhoA/Rho kinase (ROCK) [6], Lancifoliaine, a new

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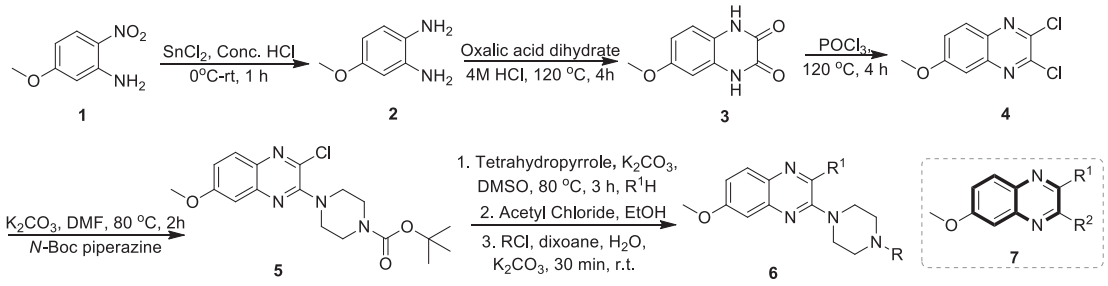


Scheme 1. General synthetic approach for the final products.

bisbenzylisoquinoline that was isolated from the stem bark of *Litsea lancifolia* [7], Curine, a bisbenzylisoquinoline alkaloid [8], Rutaecarpine, an indolopyridoquinazolinone alkaloid [9], and so forth [10–12]. Moreover, efforts from Li's group have revealed several first-in-class *N*-bearing heterocyclic derivatives, exemplified by quinoline derivate (**A**) and its isoelectronic and isostructural quinoxaline analogues (**B**) & (**C**), to be effective vasodilators owing to their noticeable vasorelaxation effects, thereby designating them as good templates for discovering more potent vasodilators [13].

Enlightened by these findings, our newly designed heterocyclic molecules were optimized as follows. The quinoxaline framework of (**C**) was kept unchanged, owing to the fact that the quinoxaline nucleus, acting as a bioisostere of isoquinoline and a well-tolerated structure in humans [14], has been proven to be a privileged platform that can be used as an effective building block for constructing numerous drug-like chemotypes with potent therapeutic applicability for the treatment of different pathophysiological conditions [15–17]. Further chemical diversification was expanded by introducing various substituents at the 2,3-positions of the quinoxaline backbone and by examining their influence to vasorelaxant activities. Meanwhile, 6-nitro group of (**C**) was replaced by an electron-donating methoxyl fragment, due to the potential genotoxic threat of the nitro group [18]. Overall, a novel class of 7-methoxyl quinoxaline derivatives was proposed, their vasorelaxant activities on vascular tone, along with underlying mechanisms of action were also investigated, with the expectation of identifying more promising small molecular blood pressure-lowering agents.

The target 7-methoxyl-2,3-disubstituted quinoxaline derivatives in our synthetic protocol were readily prepared from the commercially available 5-methoxy-2-nitroaniline (**1**), following the processes listed in Scheme 1. Reduction of (**1**) led to 4-methoxybenzene-1,2-diamine (**2**), which was subjected to condensation with oxalic acid under acidic conditions to assemble the quinoxaline nucleus (**3**). This compound was then submitted to chlorination in the presence of $POCl_3$ to furnish the dichlorinated product (**4**), which sequentially underwent the sequences of nucleophilic substitution referenced by literature method with modifications [19,20], deprotection of *N*-Boc group and another nucleophilic substitution to afford the final product (**7a-7γ**) with relatively satisfactory yields. To conveniently elucidate the target compounds, the general molecular formula, molecular weight (MW), melting point (m.p.), yield and calculated log*P* (clog*P*) value of target compounds were shown in gridlines (Table 1).

Next, we proceeded to screen the modulatory effects of these quinoxaline derivatives on vessel tone using the myograph assay [21]. Aortic segments from mice were exposed to α-adrenergic receptor agonist phenylephrine (phe) at 1 μM to induce contraction. Among these compounds, five compounds (**7s**, **7t**, **7v**, **7w**, **7γ**) were observed to relax vascular contraction in the mouse aorta by completely reversing phe-induced pre-contraction, while the remaining compounds had no effects (Fig. 1A and B). For the sake of clarity, only a part of the compounds that had no modulatory effects on vessel tone were shown in Fig. 1A, and the phe SEM results containing the endothelium were presented in Fig. 1B.

The myograph assay was also performed on the mouse aortas to examine whether these five compounds could reverse the vascular contraction elicited by high extracellular K^+. The results showed that they induced dose-dependent relaxation of mouse aortas when pre-contracted with 60 mM KCl at concentrations ranging from 3 to 300 μM (Fig. 2A and B). In addition, deletion of endothelium could not significantly alter the effect of the compounds on the relaxation responses (Fig. 2C and D). These results further supported the vasorelaxant functions of these five compounds (**7s**, **7t**, **7v**, **7w**, **7γ**) exerted by acting on vascular smooth muscle cells.

Further examination of the vasorelaxant effects of the five effective compounds was performed using the myograph method. As exhibited in Fig. 3A-D, phe pre-contracted arteries were statically relaxed by three compounds (**7v**, **7w** and **7γ**) in a dose-dependent (3 ~ 300 μM) and endothelium-independent pattern.

Given that compounds **7v**, **7w** and **7γ** exhibited dose-dependent and endothelium-independent vasorelaxant effects on vessel tone, notably stronger than the others in all the 7-methoxyl-2,3-disubstituted quinoxaline derivatives, we consequently examined whether such vasorelaxation was derived from the activation of eNOS. To this end, these three compounds were pre-treated with a nitric oxide (NO) synthase inhibitor N^{ω}-nitro-l-arginine methyl ester (L-NAME). In the presence of L-NAME, all the compounds decreased contractile responses of vessel tone to Phe or high KCl considerably (Fig. 4A-D), suggesting that compounds **7v**, **7w** and **7γ** induced vasorelaxation without activating the eNOS.

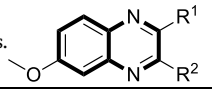
Our present study revealed that in relation to commonly used acute vasodilation drugs, such as acetylcholine (ACH), the 7-methoxyl-2,3-disubstituted quinoxaline derivatives, exemplified by compounds **7v**, **7w** and **7γ**, demonstrated slow, albeit effective vasorelaxant effects on Phe induced pre-contracted mice aortas. In particular, treatment of phe pre-contracted arteries with compound **7γ** at 300 μM for about 40 min led to approximately 72.7% decrease in maximal contractility (Fig. 5A and B), whereas others (**7v**, **7w**) decreased maximal contractility by 50% Fig. 6.

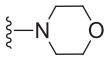
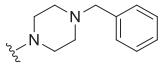
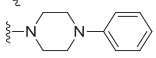
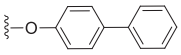
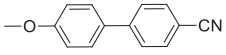
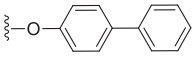
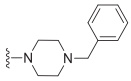
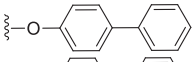
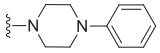
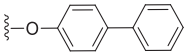
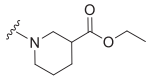
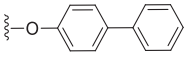
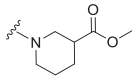
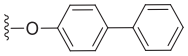
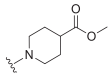
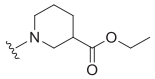
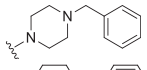
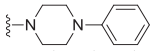
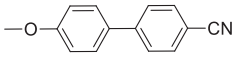
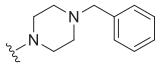
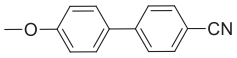
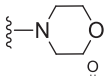
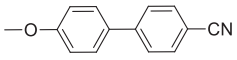
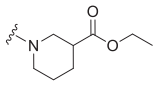
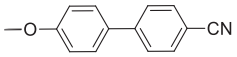
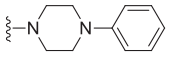
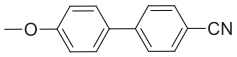
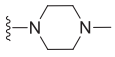
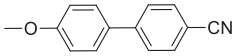
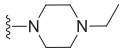
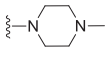
To sum up, the present results suggested that in our newly synthesized 7-methoxyl-2,3-disubstituted quinoxaline derivatives, some compounds, **7v**, **7w** and **7γ** in particular, induced slow but effective vasorelaxation dose-dependent and endothelium-independent effects in high extracellular K^+- and phe-precontracted C57 mice aortic rings. At present stage, although we can't reach a clear SAR conclusion since there were no common structural characteristics among these active derivatives, it is reasonable to conclude that these three quinoxaline derivatives might act as potential leads for further structural modifications and offer therapeutic potential for the treatment of hypertension. Future efforts will concentrate on structural optimizations of these leads to find out the clear structure–activity relationships (SARs) by adding more chemical varieties.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

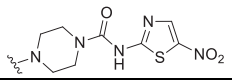
Table 1

The detailed structures and properties of the final compounds. 

Entry	R^1	R^2	mw	m.p.	Yield[a]	clog*P*[b]
7a			314.17	110–111 °C	74.6%	2.888
7b			403.24	115–116 °C	75.2%	5.171
7c			389.22	68–70 °C	71.4%	4.880
7d			397.18	130–132 °C	76.1%	5.678
7e			314.17	110–111 °C	66.5%	6.111
7f			502.24	128–130 °C	82.5%	8.138
7g			488.22	152–153 °C	84.9%	7.847
7h			483.22	117–118 °C	88.2%	7.335
7i			469.20	112–114 °C	78%	6.096
7j			413.17	128–129 °C	75.9%	5.855
7k	NHCN		285.12	212–213 °C	74.2%	1.612
7l	NHCN		341.15	193–194 °C	71.2%	1.853
7m	NHCN		355.16	184–185 °C	84.3%	3.092
7n	NHCN		374.19	204–205 °C	77.3%	3.895
7o	NHCN		360.17	196–197 °C	81.6%	3.604
7p			527.23	152–153 °C	85.5%	7.571
7q			438.17	208–209 °C	90.2%	5.288
7r			508.21	164–166 °C	92.1%	6.768
7s			513.22	180–182 °C	89.2%	7.280
7t			451.20	174–176 °C	88.6%	5.730
7u			465.22	120–122 °C	83.2	6.378
7v			327.21	107–108 °C	77.6%	3.330
7w			341.22	70–72 °C	78.4%	3.978
7x			384.22	105–108 °C	69.1%	4.368
7y			370.20	113–115 °C	82.3%	3.129
7z			426.21	120–121 °C	79.5%	6.297
7α			440.22	130–132 °C	80.6%	6.945
7β			494.20	181–182 °C	57.1%	5.683

(*continued on next page*)

Table 1 (*continued*)

Entry	R^1	R^2	mw	m.p.	Yield[a]	clog*P*[b]
7γ		Cl	449.07	166–168 °C	69.8%	3.131

[a] Final step yield.
[b] clog *P* values of target compounds were predicted *via* ChemBio3D Ultra program.

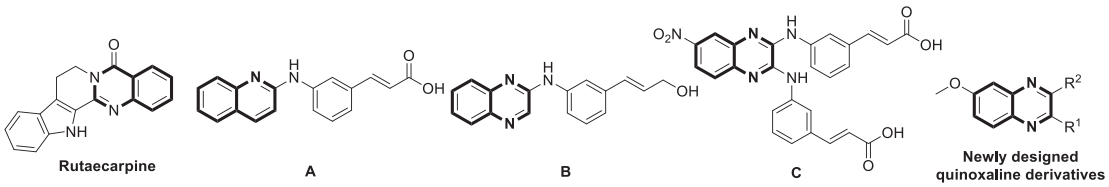


Fig. 1. Our newly proposed 7-methoxyl-2,3-disubstituted quinoxaline derivatives were designed from several *N*-containing natural or synthetic heterocycles that act as potent vasodilators.

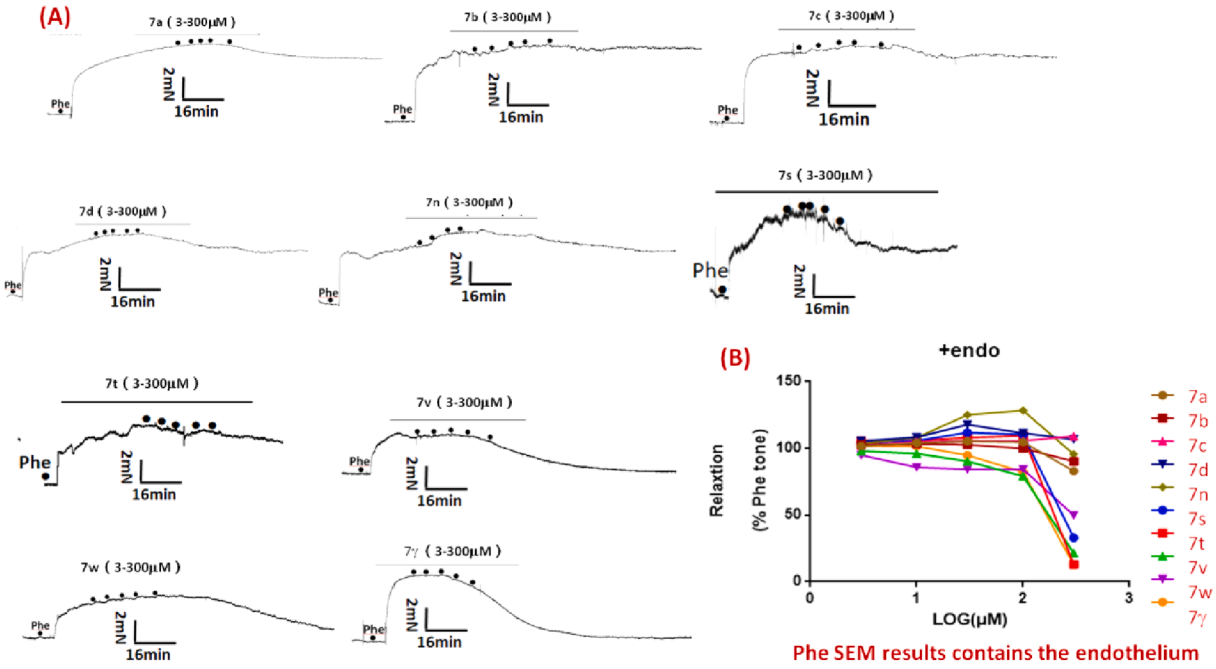


Fig. 2. Modulatory effects of a part of quinoxaline derivatives on vessel tone.

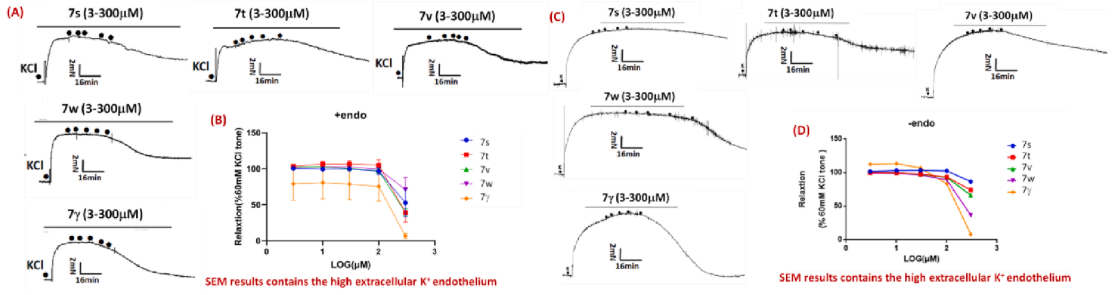


Fig. 3. Vasorelaxant effects of three effective compounds on high K^+ pre-contracted arteries were in dose-dependent and endothelium-independent mode.

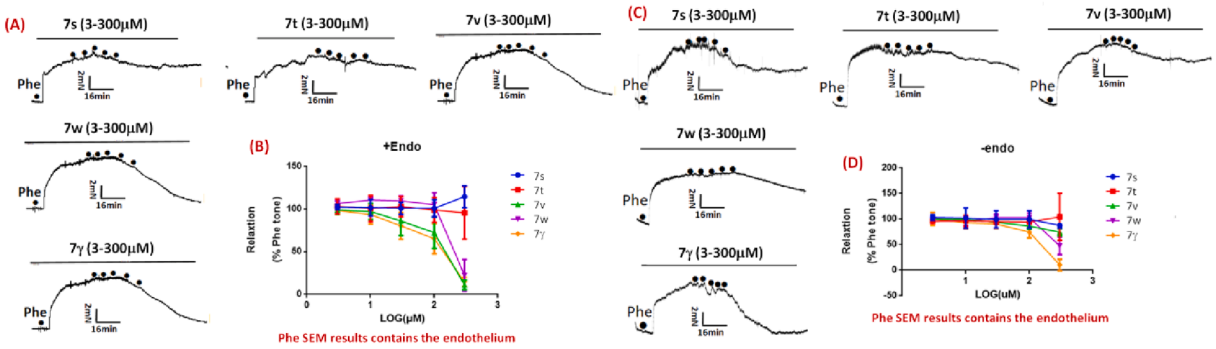


Fig. 4. Vasorelaxant effects of three effective compounds on phe pre-contracted arteries were in dose-dependent and endothelium-independent mode.

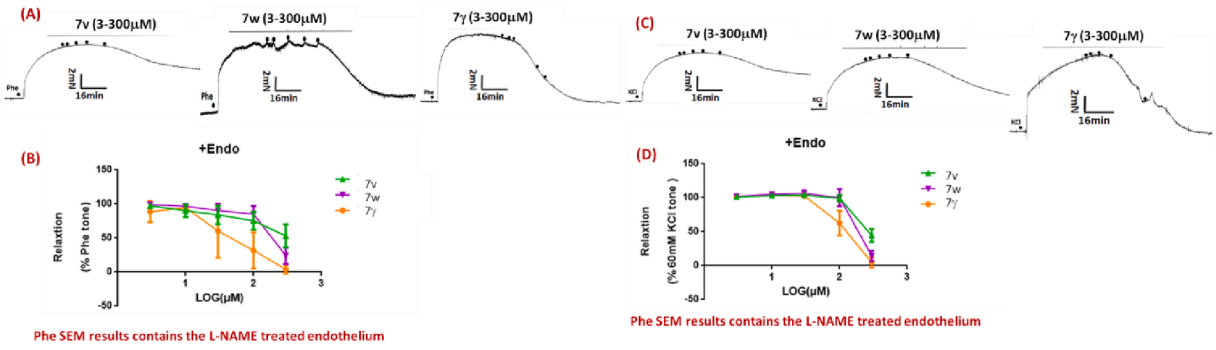


Fig. 5. Pre-treatment with NO synthase inhibitor L-NAME does not affect the activities of compounds **7v**, **7w** and **7γ** on mouse aorta relaxation.

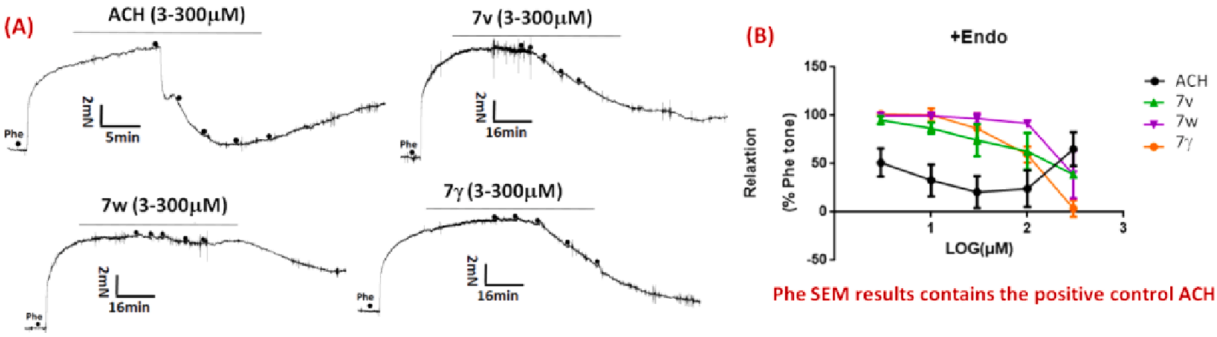


Fig. 6. Compounds **7v**, **7w** and **7γ** demonstrated slow but effective vasorelaxant effects.

the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127785.

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21 Protocol of measurement of vascular tension: Briefly, male C57 adult mice were killed by carbon dioxide suffocation. After euthanasia, the mouse thoracic aortas were quickly dissected free and cut into 2-mm long rings in ice-cold and oxygenated Krebs buffer, which contained in mM: 119 NaCl, 4.7 KCl, 2.5 CaCl2, 1 MgCl2, 25 NaHCO3, 1.2 KH2PO4, and 11 D-glucose. The vascular rings were mounted onto two thin stainless-steel holders in a myograph filled with Krebs solution bubbled with 95% O2 and 5% CO2 at 37 °C. The optimum baseline tension was set at 3 mN. After an equilibration period and 60 mM KCl solution pretreatment (which contained in mM: 63.7 NaCl, 60 KCl, 2.5 CaCl2, 1 MgCl2, 25 NaHCO3, 1.2 KH2PO4, and 11 D-glucose), the rings were exposed to 1 mM phenylephrine to test their contractile responses and subsequently challenged with acetylcholine to certify the integrity of endothelium.