Life Sciences 278 (2021) 119560

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

# Discovery of *trans*-3,4,4′-trihydroxystilbene as new lead vasorelaxant agent for antihypertensive drug development

Yean Chun Loh [a], Sock Ying Chan [a], Chuan Wei Oo [a,*], Mun Fei Yam [b,c,**]

[a] Department of Organic Chemistry, School of Chemical Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia
[b] Department of Pharmacology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia
[c] College of Pharmacy, Fujian University of Traditional Chinese Medicine, 1 Qiuyanng Road, Shangjie, Minhou, Fuzhou 350122, Fujian, China

ARTICLE INFO

*Keywords:*
Antihypertensive
Resveratrol analogue
Stilbenoid
Structure-activity relationship
Vasorelaxation

ABSTRACT

*Aims:* The structure-vasorelaxant activity relationships (SARs) assessment in previous study has found that *trans*-3,4,4′-trihydroxystilbene (344OH) could potentially act as a vasorelaxing agent with demonstration of over 2-fold maximal relaxation ($R_{max}$) compared to its analogue, resveratrol. The present study focuses on the mechanism of actions and pathways employed by 344OH and compared to its analogue to further speculate the SAR of stilbenoids towards vasorelaxation.

*Materials and methods:* The 344OH employed in present study was synthesized based on the protocol in previous study. The vascular responses towards the cumulative addition of 344OH were evaluated using in vitro rat aortic rings assays.

*Key findings:* The $pEC_{50}$ and $R_{max}$ values were found to be 4.33 ± 0.05 and 106 ± 3.99%, respectively. Results showed that the vasorelaxation of 344OH were predominated by G-protein-coupled muscarinic- ($M_3$) and $\beta_2$-adrenergic receptors, followed by $PGI_2$/AC/cAMP- and NO/sGC/cGMP-dependent pathways. It was also identified that 344OH employed voltage-activated- ($K_v$), calcium-activated- ($K_{ca}$) and inwardly-rectifying ($K_{ir}$) potassium channels and act as an antagonist for both VOCC and $IP_3R$ while regulating the action potential in the vasculature.

*Significance:* The different position of hydroxyl substituent located in A-ring of the stilbenoid backbone in 344OH compared to resveratrol resulted in a significant difference in mechanistic actions that lead to 344OH's fast-acting and less time-dependent vasorelaxation behaviour. This has substantially increased the potential of 344OH to be developed as an effective antihypertensive drug in future. Present findings further strengthen our inferences where the SARs study approach should be carried out as the mainstream methodology in future drug development research.

## 1. Introduction

Hypertension is defined by the World Health Organization (WHO) as a persistently elevated pressure within the walls of the blood vessels and is technically diagnosed when the blood pressure is above 140/90 mmHg. It has always been and will always be the cornerstone risk factor

*Abbreviations:* 2-APB, 2-aminoethoxydiphenyl borate; 344OH*trans*, 3,4,4′-trihydroxystilbene; 4-AP, 4-aminopyridine; AC, adenylyl cyclase; ACE2, angiotensin-converting enzyme 2; Ach, acetylcholine; ARASC, Animal Research and Service Centre; $BaCl_2$, barium chloride; $\beta_2$, beta-adrenergic receptor; COX, cyclooxygenase; EGTA, ethylene glycol tetra-acetic acid; eNOS, endothelial nitric oxide synthase; $K_{ca}$, calcium-activated potassium channel; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; $C_{max}$, maximal contraction; GPCRs, G-protein-coupled receptors; IACUC, Institutional Animal Care and Use Committee; $IP_3R$, Inositol triphosphate; $K_{ir}$, Inwardly-rectifying potassium channel; $K_v$, voltage-activated potassium channel; L-NAME, Nω-nitro-L-arginine methyl ester hydrochloride; $M_3$, Muscarinic receptor type 3; MB, methylene blue; NO, nitric oxide; ODQ, 1H-[1,2,4]oxadiazolo[4,3,-*a*]quinoxalin-1-one; $pEC_{50}$, efficient concentration; PE, phenylephrine; $PGI_2$, prostacyclin; RAAS, renin-angiotensin-aldosterone system; $R_{max}$, maximal relaxation; SARs, structure-vasorelaxant activity relationship; SD, Sprague Dawley; sGC, soluble guanylyl cyclase; TEA, tetraethyl ammoniumchloride; VOCC, voltage-operated calcium channel; WHO, World Health Organization.

* Correspondence: C.W. Oo, Department of Organic Chemistry, School of Chemical Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia.
** Correspondence: M.F. Yam, Department of Pharmacology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia.
*E-mail addresses:* oocw@usm.my (C.W. Oo), yammunfei@usm.my (M.F. Yam).

https://doi.org/10.1016/j.lfs.2021.119560
Received 5 January 2021; Received in revised form 8 April 2021; Accepted 22 April 2021
Available online 26 April 2021

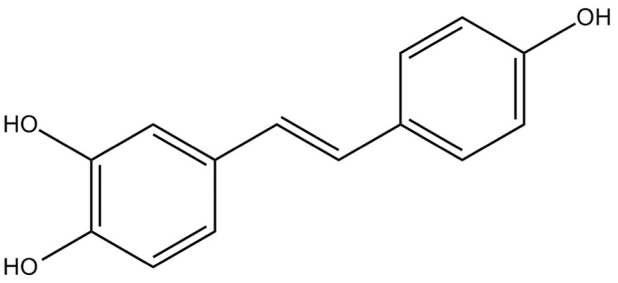


**Fig. 1.** *Trans*-3,4,4′-trihydroxystilbene.

for cardiovascular conditions within our community. Based on the latest reports from WHO, it is postulated that 15% of the population worldwide has hypertension which translates to approximately 1.13 billion people. With this significant number of people who are affected by the condition, it is not surprising that our research community and the healthcare team have always been on a look out for the best practices in management of this condition. However, even with endless resources and manpower motivated in finding the solution for hypertension, there is still gap in its management and the numbers of patients seen to be on the rise. Despite the evolution in antihypertensive medication with close to 200 available choices in the market, it is by far one of the most challenging topics for global scientist as there is not a single agent that is able to be the front cover solution to effectively manage the blood pressure to its optimal levels [1–3]. Therefore, most clinical practice guidelines recommend combination therapies whereby different classes of antihypertensive are introduced to a single patient to execute the blood pressure lowering effects using multiple pathways. It is a better approach as compared to monotherapy management as the employment of different mechanism of actions allow for better elicitation of vasorelaxant effects. However, with the increment in number of drugs that the patients are exposed to, there is a simultaneous increment in potential side effects that our patients will have to manage and live with. Thus, there has yet to be a clear solution where an agent is able to exhibit optimal blood pressure lowering abilities with minimal adverse drug reactions [4,5].

With the recent outbreak of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that resulted in a worldwide pandemic since the end of 2019, it has reignited and instigated the attention of scientists worldwide back to the antihypertensive studies due to increasing evidences pointing the finger towards hypertension as one of the most prevalent co-morbidity in COVID-19 patients [6–10]. Recent findings claimed the angiotensin-converting-enzyme 2 (ACE2) can potentially mediate the entry of SARS-CoV-2 into the host cells [11]. Therefore, employment of renin-angiotensin-aldosterone system (RAAS) inhibitors which includes ACE inhibitors such as lisinopril and angiotensin receptor blockers such as losartan which are most frequently used first line antihypertensive medications currently in the market may lead to higher SARS-CoV-2 infectivity and severe virulence in the ongoing Covid-19 pandemic [12–15]. Besides that, there was another report demonstrating that the replacement of antihypertensive drugs to the patient diagnosed with COVID-19 had eventually lead to lethal outcomes [16].

Looking deeper into the development of antihypertensive agents, the most crucial investigated characteristics would be the vasorelaxant effects of the compound. Thus, all compounds with potential vasorelaxation abilities will be tested in vitro before proceed to in vivo study to understand the potential effects they may exhibit on the blood vessels [3]. There is a rallying team globally that has tested the vasorelaxant effects of hundreds of phytochemicals over the past three decades. However the outcome was not encouraging with only a limited number of these compounds are successfully adapted into current medical practices [17]. Majority of these research and studies were applying conventional research techniques which means exhaustingly searching for undiscovered compounds followed by assessing the potential vasorelaxant activity and their potential pharmacological effects [3]. Seeing that it has not produced fruitful results, we strongly propose an alternative approach that could add value to previous vascular tone-related research findings by investigating the structure-vasorelaxant activity relationships (SARs) of each category of phytochemicals through a series of chemical derivatives synthesis and in vitro screening approaches. With this proposed methodoloy, in our recent studies, we had revealed that the tri-hydroxy structure with *ortho*- occupied position in stilbenoid compounds plays crucial roles to induce stronger vasorelaxant effect and thus producing a new strong vasorelaxant compounds, *trans*-3,4,4′-trihydroxystilbene (344OH) (IUPAC name: (E)-4-(4-hydroxystyryl)benzene-1,2-diol) (Fig. 1) which was successfully synthesized via the Witting reaction [18,19].

This newly synthesized compound demonstrated a very strong vasorelaxant activity upon single concentration (0.08 mg/ml) application with at least 2-fold the maximal relaxation ($R_{max}$) value achieved in comparison with its analogue, resveratrol [18]. According to the efficient concentration ($pEC_{50}$) value reported by Tan et al., resveratrol is considered as the second strongest vasorelaxing agent till date compared to all other studied phytochemicals [3,20]. Thus, we strongly believe and hypothesized that 344OH could be the new lead vasorelaxing agent that demonstrated excellent vasorelaxant activity over the resveratrol. Therefore, our present study was designed to determine the $pEC_{50}$ and $R_{max}$ values upon cumulative addition of 344OH using the “golden tool” of antihypertensive drug development method; isolated thoracic aortic ring assay in vitro followed by thoroughly investigating the key mechanism pathways elicited by 344OH to allow for production of such promising vasorelaxant effect including the endothelium-dependent and independent, enzyme-linked and channel-linked receptors pathways [21].

## 2. Methods

### 2.1. Preparation of chemicals

Acetylcholine chloride (Ach) and phenylephrine hydrochloride (PE) were both purchased from Acros Organics in Belgium. 4-aminopyridine (4-AP) was purchased from Merck, Germany while ethylene glycol tetraacetic acid (EGTA) and methylene blue (MB) were all obtained from Promedipharm Sdn. Bhd., Malaysia. Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME), tetraethyl ammoniumchloride (TEA), barium chloride ($BaCl_2$), propranolol hydrochloride, and atropine were bought from the same supplier - Sigma Aldrich, USA. All these chemicals mentioned were dissolved in distilled water using the micropipette (Eppendorf, Malaysia) to form a solution. On the other hand, materials such as indomethacin, glibenclamide, 2-aminoethoxydiphenyl borate (2-APB), 1H-[1,2,4]oxadiazolo[4,3,-*a*]quinoxalin-1-one (ODQ) that were obtained from Sigma Aldrich, USA and nifedipine that was purchased from Acros Organics, Belgium were dissolved using <1% of Tween 80. Resveratrol was purchased from Fortochem, Hong Kong (code: FTC16040901). Resveratrol and synthesized 344OH were both dissolved in <1% of Tween 80. All the chemicals and materials were prepared upon need for use.

### 2.2. Synthesis of trans-3,4,4′-trihydroxystilbene

The *trans*-3,4,4′-trihydroxystilbene (344OH) was synthesized using the scheme elaborated in our previous study. Briefly, 344OH was synthesized from *trans*-3,4-diethoxy-4′-methoxystilbene via poly *O*-dealkylation. The *trans*-3,4-diethoxy-4′-methoxystilbene (1.50 g, 4.8 mmol) and *N*,*N*-dimethylaniline (30 ml) were stirred at 100 °C. Anhydrous AlCl3 (3.0 equiv.) was slowly added into the mixture and heated at 180 °C for 5 h. Then, the resultant mixture was quenched with ice water followed by the step-wise addition of 37% HCl until the colour of the mixture turned to brownish orange colour. The mixture was then

extracted by using ethyl acetate and then the excess solvent was evaporated off. The crude product was washed with chloroform to yield the desired compound 344Oh as off-white solid. The chemical structures of synthesized 344OH were characterized using FT-IR (PerkinElmer, USA), $^{1}H$ NMR and $^{13}C$ NMR (Bruker, USA) spectroscopy [18].

### 2.3. Preparation of in vitro aortic ring assays and vascular response to cumulative concentration of 344OH and resveratrol

Male Sprague Dawley (SD) rats weighing between 180 and 220 g (age around 8–12 weeks) were obtained from Animal Research and Service Centre (ARASC), Universiti Sains Malaysia and acclimated in the animal transit room for 12 h light-dark cycles with free access to food and water. All the procedures elaborated herein were based on the Guideline of Universiti Sains Malaysia Institutional Animal Care and Use Committee (IACUC, USM) with the following approval: USM/IACUC/2019/(120)(1026). Krebs-Henseleit (Krebs') solution was freshly prepared by dissolving 118.0 mM NaCl, 2.5 mM $CaCl_2$, 11.0 mM D-glucose, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM $KH_2PO_4$ and 25.0 mM $NaHCO_3$ in $dH_2O$ at pH 7.4. The SD rat was executed with overdose inhalation of $CO_2$ for 2 min. Then, the thoracic aorta was excised and placed in the Petri dish containing the Krebs' solution continuously aerated with carbogen (5% $CO_2$ + 95% $O_2$) at 37 °C. The adipose tissue was carefully removed and the aorta was trimmed into 3–4 mm ring segments. The aortic rings were hanged in the tissue bath containing 10 ml of Krebs' solution and aerated with carbogen at 37 °C using two needle hooks with one side connected to force-electricity transducer (GRASS Force-Displacement Transducer FT03C Isometric Measurement, QUINCY, MASS., USA), whereas the second hook was fixed on the L-shaped braces. The aortic rings were left equilibrate for at least 45 min and the Krebs's solution was changed at 15-minute intervals for 3 times. The resting tension was re-adjusted to 1.0 g upon necessary. Once stabilized, the isolation of the rat's aorta and preliminary testing of vascular integrity using the contractile agent (PE) and relaxing agent (Ach) were conducted based on the protocols described in previous studies [18,21,22]. Upon reaching the plateau stage after 30 min of PE pre-contraction, cumulative concentrations of 344OH starting from 5 to 345 μM were instilled into the tissue bath that contains 10 mL of Krebs-Henseleit buffer solution at 20-minute intervals. The cumulative concentration-response curve was sketched and the $pEC_{50}$ and $R_{max}$ values were calculated and assigned as the control study for further comparison with different antagonists pre-treated groups [18,21,23,24]. In addition to that, the vascular responses to the cumulative concentration of resveratrol (5–345 μM) were evaluated and both its $pEC_{50}$ and $R_{max}$ were recorded.

### 2.4. Preliminary evaluation on endothelium- and channel-linked receptors dependencies

The mechanisms of action were investigated based on the protocols designed and executed in previous studies [22,24]. The endothelium dependency of 344OH in eliciting vasorelaxation was preliminary determined by exposure of the ingredient to endothelium-denuded aortic rings. These aortic rings were prepared by manually removing the intima surface of the isolated aorta using a stainless steel rod before mounting them in the tissue bath. The integrity of the endothelium-denuded aortic rings was confirmed with nil vascular response towards the addition of Ach. Then the denuded rings will be pre-contracted with PE and subsequently introduced to a cumulative concentration of 334OH. Besides identification of endothelium dependent pathway of the active ingredient, we investigated the channel-linked receptors dependency by replaced PE with KCl (80 mM) for 30 min pre-contraction before the cumulative addition of 344OH.

### 2.5. Evaluation on the roles of endothelium-dependent relaxing factors (EDRFs)

In EDRFs study, antagonists were added to the tissue bath and responses were identified. The antagonists includes L-NAME (10 μM), ODQ (1 μM) and MB (10 μM) which inhibits endothelial NO synthase (eNOS), soluble guanylyl cyclase (sGC) and cyclic guanosine-5′-monophosphate (cGMP) respectively. Endothelium-intact aortic rings were individually pre-incubated with each of the antagonists listed above for 20 min before PE pre-contraction were introduced [25]. Subsequently 344OH (5-345 μM) was then added to the final cumulative concentration of 345 μM into the tissue bath at 20-min intervals.

### 2.6. Evaluation on the roles of G-protein-coupled receptors (GPCRs)

For this part of the study, endothelium-intact aortic rings were incubated with the antagonist of the cyclooxygenase (COX), indomethacin (10 μM) for 20 min before pre-contracted with PE. On the other hand, $\beta_2$-adrenergic and muscarinic type 3 ($M_3$) receptors were evaluated by adding propranolol (1 μM) and atropine (1 μM) as their respective antagonist individually into the tissue bath for 20 min pre-incubation before PE pre-contraction [26]. 344OH (5-345 μM) was then added into the tissue bath to cumulative concentration of 345 μM at 20-min intervals between each addition. The results were then recorded.

### 2.7. Investigate the roles of potassium and calcium channels

This part of the investigation involves identifying the roles of potassium and calcium channels in exhibiting the vasodilatory effects of 344OH. The ATP-sensitive $K^+$ channels ($K_{ATP}$), calcium-activated $K^+$ channels ($K_{ca}$), inwardly-rectifying $K^+$ channels ($K_{ir}$) and voltage-activated $K^+$ channels ($K_v$) were all evaluated using their respective antagonist - glibenclamide (10 μM), TEA (1 mM), $BaCl_2$ (10 μM) and 4-AP (1 mM). Endothelium-intact aortic rings were pre-incubated with the respective antagonist in individual tissue baths for 20 min before pre-contracted using PE. Subsequently 344OH (5-345 μM) were then added into the tissue bath cumulatively at 20-min intervals. For the study of voltage-operated calcium channels (VOCC), three groups of experiments were conducted including a control, introduction of nifedipine as a positive control and the experimental groups of 344OH. To execute this part of the study, endothelium-intact aortic rings were rinsed with EGTA (0.2 mM) for 10 min in an attempt to remove $Ca^{2+}$ residues. Subsequently they were rinsed with $Ca^{2+}$-free high $K^+$ Krebs' solution twice at 10-minute intervals each. The resting potential of the aortic rings were adjusted to 1.0 g. In the control set, cumulative concentration of $CaCl_2$ (0.01–10 mM) were added into the tissue bath at 3-minute intervals, whereas in the positive control, 0.1, 0.3 and 1 μM of nifedipine were added into the tissue bath for pre-incubation of 20 min before introduction of cumulative $CaCl_2$ concentrations. The experimental sets of tissue baths looked at addition of 344OH at the following concentration - 11, 43.8 and 175.25 μM for 20 min pre-incubation before the addition of cumulative concentration of $CaCl_2$ similar to the control set. The concentration-response curves were then constructed and maximal contractile values ($C_{max}$) were recorded and compared between the three different groups. Besides that, the role of inositol triphosphate receptors ($IP_3R$) were also evaluated using the same protocol as described in VOCC. The only difference from the VOCC protocol was that the PE (1 μM) was applied to induce a transient contraction instead of cumulative addition of $CaCl_2$. About 100 μM of 2-APB and 344OH (11, 43.8 and 175.25 μM) were separately used to pre-incubate with the endothelium-impaired aortic rings for 20 minutes before the addition of PE in both sets of positive control and experimental group, respectively. The $C_{max}$ values were noted and compared to identify its effects [22,27].

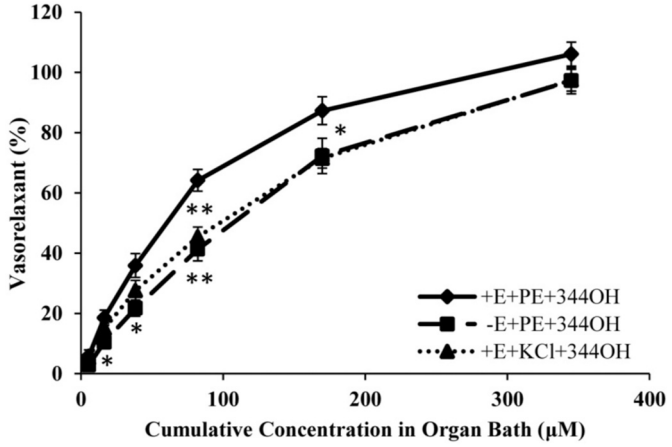


**Fig. 2.** The cumulative concentration-response curves of vasorelaxant effect elicited by *trans*-3,4,4′-trihydroxystilbene (344OH) on PE pre-contracted endothelium-intact (+E), endothelium-denuded (−E), or KCl pre-contracted isolated endothelium-intact rat aortic rings ($n = 8$). The cumulative concentration-response curve of 344OH in endothelium-intact aortic rings was assigned as control. Significance at $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; as compared to the control.

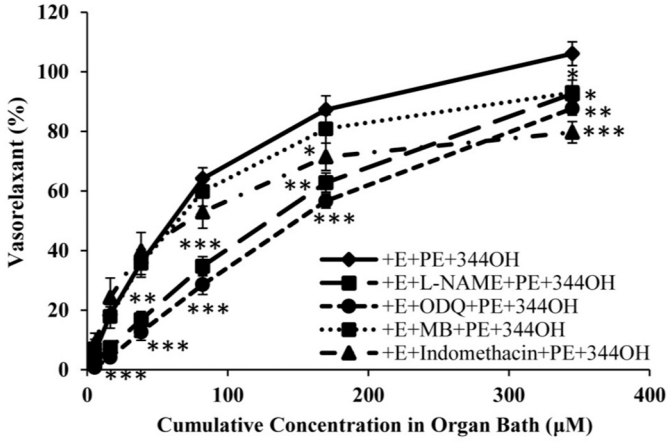


**Fig. 3.** The cumulative concentration-response curves of vasorelaxant effect elicited by *trans*-3,4,4′-trihydroxystilbene (344OH) in the presence of L-NAME, ODQ, MB, or indomethacin on PE pre-contracted isolated endothelium-intact (+E) aortic rings. All antagonists significantly inhibited 344OH's vasorelaxant effects ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $n = 8$).

**Table 1**
The impacts of single and cumulative concentration application of 344OH and resveratrol in PE-primed endothelium-intact aortic rings towards the $R_{max}$ values.

| Compounds | From experiment | | | From equation of cumulative concentration-response curve |
|---|---|---|---|---|
| | Equations of cumulative concentration-response curve | $R_{max}$ values (%) | | |
| | | Single concentration (350 μM) [18] | Cumulative concentration (Final concentration at 345 μM) | Substitution of x = 350, then y =? (Final concentration at 350 μM) |
| 344OH | y = 25.46 ln(x)-46.88 | 89.47 ± 3.88 | 106.10 ± 3.99 | 102.26 |
| Resveratrol [24] | y = 30.03 ln(x)-42.82 | 42.90 ± 1.67 | 122.5 ± 4.99 | 133.09 |

Notes: x: concentration of compound; $R_{max}$: y: maximal vasorelaxation.

### 2.8. Data analysis

All the results obtained from the experiments were expressed as the mean ± standard deviation. All the antagonist pre-treated groups were compared to control using one-way ANOVA followed by Dunnett's post-hoc test using SPSS version 22 software. The entire test were executed to be 2-tailed and the significance level was set as $P < 0.05$.

## 3. Results

### 3.1. Vascular response to 344OH

Referencing the response curve plotted in Fig. 2, it is clearly observed that addition of 344OH at concentration of 5-345 μM resulted in apparent relaxation effect of the endothelium-intact aortic rings in both concentration- and time-dependent manner, with $pEC_{50}$ and $R_{max}$ values of 4.33 ± 0.05 and 106.10 ± 3.99%, respectively (Table 2). The vasorelaxant effects of 344OH was seen to be significantly attenuated in both endothelium-impaired aortic rings and KCl-primed conditions with $pEC_{50}$ values of only 4.11 ± 0.06 and 4.16 ± 0.04, respectively, whereas $R_{max}$ values achieved in both these conditions demonstrated significant reduction of only 97.28 ± 4.34% ($P < 0.01$) and 97.46 ± 3.62% ($P < 0.01$), respectively. It can be observed that both $pEC_{50}$ and $R_{max}$ values obtained upon cumulative addition of resveratrol to the endothelium-intact aortic rings were 4.66 ± 0.03 and 122.50 ± 1.76%, respectively as shown in Table 2. In addition, Table 1 showed the results recorded from previous study where the $R_{max}$ value of 344OH was 2-fold higher than resveratrol upon comparison of only a single concentration (0.08 mg/ml equivalent to 350 μM) addition into the PE-primed endothelium-intact aortic rings [18]. Looking at the terms of cumulative concentration application of 344OH and resveratrol (5–345 μM) from the present study, the $R_{max}$ value of 344OH achieved was slightly lower than the resveratrol despite having achieved maximum vasorelaxation (>100%). The equations obtained from the cumulative concentration-response curve for both the 344OH and resveratrol were shown in Table 1, with

**Table 2**
Significance of $pEC_{50}$ values and comparison of $R_{max}$ values among different pre-treated groups.

| Treatments | $pEC_{50}$ values | $R_{max}$ values (%) |
|---|---|---|
| Preliminary screening | | |
| Control | 4.33 ± 0.05 | 106.10 ± 3.99 |
| KCl | 4.16 ± 0.04 | 97.46 ± 3.62[b] |
| Denuded | 4.11 ± 0.06 | 97.28 ± 4.34[b] |
| EDRFs and NO cascade mechanisms | | |
| L-NAME | 4.01 ± 0.05 | 92.65 ± 2.39[a] |
| ODQ | 3.91 ± 0.04 | 87.69 ± 2.25[a] |
| MB | 4.27 ± 0.07 | 93.04 ± 4.19[c] |
| GPCRs | | |
| Indomethacin | 4.22 ± 0.12 | 79.71 ± 3.60[a] |
| Atropine | 3.49 ± 0.17 | 61.94 ± 5.43[a] |
| Propranolol | 3.56 ± 0.14 | 66.01 ± 5.01[a] |
| Potassium channel-linked receptors | | |
| TEA | 4.05 ± 0.09 | 83.21 ± 4.01[b] |
| Glibenclamide | 4.41 ± 0.09 | 108.91 ± 2.87 |
| 4-AP | 3.76 ± 0.10 | 70.94 ± 4.28[a] |
| $BaCl_2$ | 4.06 ± 0.08 | 88.11 ± 5.47[b] |
| Resveratrol | 4.66 ± 0.03 | 122.50 ± 1.76 |

Notes: $pEC_{50}$: efficient concentration; EDRFs: endothelium-derived relaxing factors; GPCRs: G-protein-coupled receptors; $R_{max}$: maximal vasorelaxation.
[a] Significance $P < 0.001$.
[b] Significance $P < 0.01$.
[c] Significance $P < 0.05$.

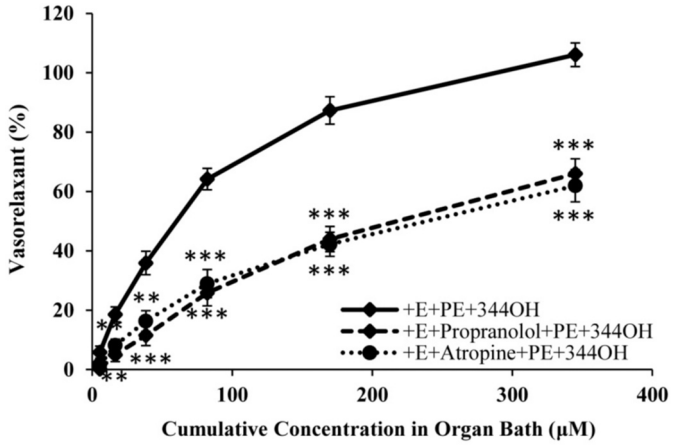


**Fig. 4.** The cumulative concentration-response curves of vasorelaxant effect elicited by *trans*-3,4,4′-trihydroxystilbene (344OH) in the presence of atropine or propranolol on PE pre-contracted isolated endothelium-intact (+E) aortic rings. Significant decrease in 344OH's vasorelaxant effects either in the presence of propranolol or atropine (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; $n = 8$).

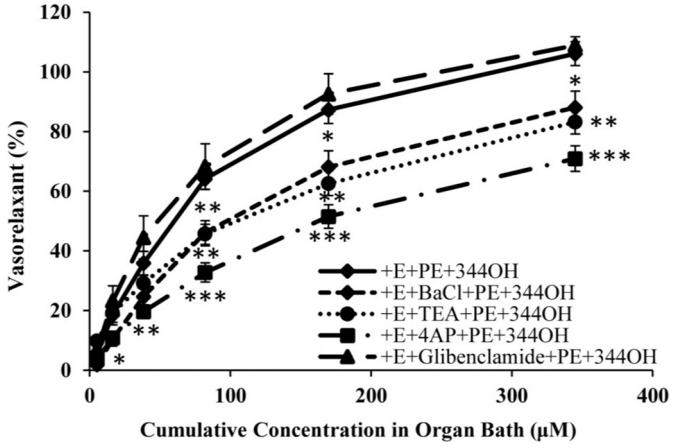


**Fig. 5.** The cumulative concentration-response curves of vasorelaxant effect elicited by *trans*-3,4,4′-trihydroxystilbene (344OH) in the presence of TEA, glibenclamide, 4-AP, or $BaCl_2$ on PE pre-contracted isolated endothelium-intact (+E) aortic rings. All antagonists significantly inhibited 344OH's vasorelaxant effects, except glibenclamide (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; $n = 8$).

substitution of x equal to 350 μM, the $R_{max}$ values achieved were 102.26 and 133.09%, respectively.

### 3.2. Roles of EDRFs in 344OH vasorelaxation

As demonstrated in Fig. 3, the vasorelaxation effects elicited by 344OH were significantly diminished in the presence of L-NAME, ODQ and MB where their $pEC_{50}$ values obtained were reduced significantly to 4.01 ± 0.05, 3.91 ± 0.04 and 4.27 ± 0.07, respectively as compared to the control group. The $R_{max}$ achieved were only at 92.65 ± 2.39% ($P < 0.001$), 87.69 ± 2.25 ($P < 0.001$) and 93.04 ± 4.19 ($P < 0.05$), respectively as shown in Table 2. Thus, it can be concluded that these agents were inhibitory towards the vasorelaxation effects of 344OH and is evident that their inhibitory effects of EDRF antagonists in ascending orders are ODQ > L-NAME > MB.

### 3.3. Roles of GPCRs in 344OH vasorelaxation

Table 2 provides an insight to the presence of non-steroidal anti-inflammatory drug (NSAID), indomethacin towards the effect of 344OH. It

**Table 3**
Comparison of $C_{max}$ values among different treatments on calcium channels mechanism studies.

| Treatments (μM) | $C_{max}$ values (g) |
|---|---|
| VOCC | |
| Control | 0.836 ± 0.060 |
| 0.1 μM Nif | 0.155 ± 0.009[a] |
| 0.3 μM Nif | 0.108 ± 0.008[a] |
| 1 μM Nif | 0.049 ± 0.007[a] |
| 11 μM 344OH | 0.723 ± 0.051 |
| 43.8 μM 344OH | 0.490 ± 0.043[a] |
| 175.25 μM 344OH | 0.255 ± 0.022[a] |
| $IP_3R$ | |
| Control | 0.675 ± 0.010 |
| 100 μM 2-APB | 0.024 ± 0.009[a] |
| 11 μM 344OH | 0.559 ± 0.019[b] |
| 43.8 μM 344OH | 0.453 ± 0.021[a] |
| 175.25 μM 344OH | 0.299 ± 0.015[a] |

Notes: 2-APB: 2-aminoethoxydiphenyl borate; 344OH: *trans*-3,4,4′-trihydroxystilbene; $C_{max}$: maximal contraction; $IP_3R$: inositol triphosphate receptor; Nif: nifedipine; VOCC: voltage-operated calcium channel.
[a] Significance $P < 0.001$.
[b] Significance $P < 0.05$.

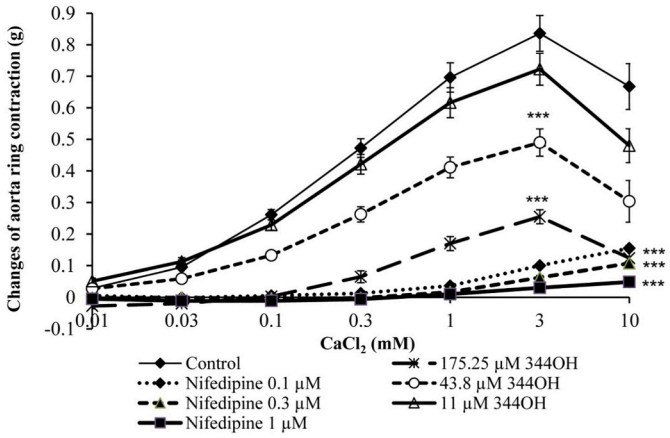


**Fig. 6.** Effect of different concentrations of *trans*-3,4,4′-trihydroxystilbene (344OH) (11, 43.8, 175.25 μM) and nifedipine (0.1, 0.3, 1 μM) on $CaCl_2$-induced contraction in endothelium-intact (+E) isolated aortic rings in $Ca^{2+}$-free Krebs' solution. Both 43.8 and 175.25 μM of 344OH significantly inhibited the $CaCl_2$-induced contraction, but in a lesser extent compared to nifedipine (***$P < 0.001$; $n = 8$).

can be observed that the presence of indomethacin significantly decreased the $R_{max}$ value of 344OH to approximately only a quarter; 79.71 ± 3.60% ($P < 0.001$) compared to the control (Fig. 3), whereas the $pEC_{50}$ value obtained was 4.22 ± 0.12. Besides that, the presence of the propranolol and atropine both drastically reduced the vasorelaxation effect of 344OH by 37% or 41% respectively in endothelium-intact aortic rings (Fig. 4). Hence, the $R_{max}$ values obtained were only 66.01 ± 5.01% ($P < 0.001$) and 61.94 ± 5.43% ($P < 0.001$), while $pEC_{50}$ values were reduced to 3.56 ± 0.14 and 3.49 ± 0.17, respectively (Table 2). From the results of the study it can be seen that the inhibitory effects caused by GPCR antagonists are most significant with atropine followed by propranolol and indomethacin.

### 3.4. Roles of channel-linked receptors in 344OH vasorelaxation

As shown in Fig. 5, the presence of $BaCl_2$, TEA or 4AP had significantly reduced the $R_{max}$ values of 344OH to 88.11 ± 5.47% ($P < 0.01$), 83.21 ± 4.01% ($P < 0.01$) and 70.94 ± 4.28% ($P < 0.001$) respectively.

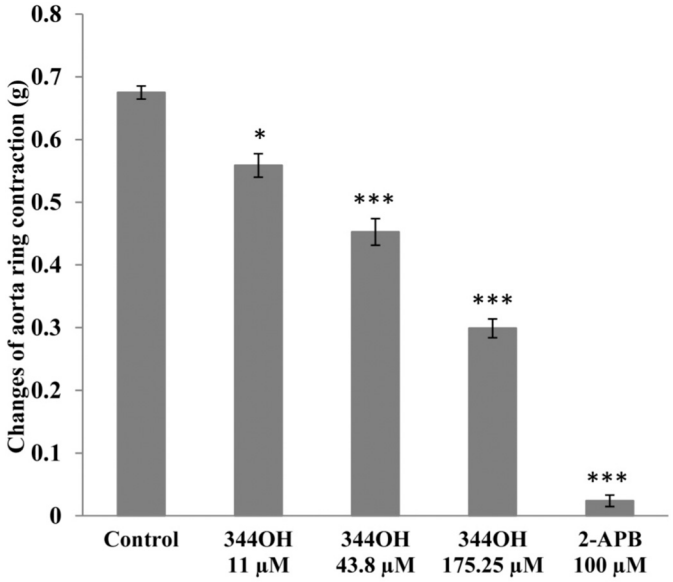


**Fig. 7.** Effects of different concentrations of *trans*-3,4,4′-trihydroxystilbene (344OH) (11, 43.8, 175.25 μM) and 100 μM of 2-APB on PE-induced contraction from intracellular $Ca^{2+}$ release in isolated endothelium-denuded aortic rings in $Ca^{2+}$-free Krebs' solution. Both 344OH and 2-APB were significantly inhibited PE-induced contraction from intracellular $Ca^{2+}$ release through $IP_3R$ (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n = 8).

Whereas the presence of glibenclamide did not affect the 344OH vasorelaxation activity (Table 2). From the observed $pEC_{50}$ values, the significance of antagonizing effects can be arranged as such: 4AP (3.76 ± 0.10) > TEA (4.05 ± 0.09) > $BaCl_2$ (4.06 ± 0.08). Shifting the attention to VOCC evaluation, the $C_{max}$ value of the control set was 0.836 ± 0.060 g as portrayed in Table 3, whereas the presence of L-type calcium channels blocker, nifedipine (0.1, 0.3 and 1 μM) almost completely diminish ($P < 0.001$) the contraction ($C_{max}$ values achieved at 0.155 ± 0.009, 0.108 ± 0.008 and 0.049 ± 0.007 g, respectively) induced by externally applied $CaCl_2$, in a concentration-dependent manner as shown in Fig. 6. The addition of 344OH at concentration of 43.8 and 175.25 μM resulted in significant reduction of $C_{max}$ values to 0.490 ± 0.043 g ($P < 0.001$) and 0.255 ± 0.022 g ($P < 0.001$), respectively. However it was observed that low concentration of 344OH (11 μM) did not produce any worthwhile effects to the aortic rings, Thus, it can be postulated that high concentrations of 344OH (175.25 μM) has the potential to suppress more than one third of the $C_{max}$ value achieved in the control via blocking of VOCC (approximately 69%), but yet unable to reach the drastic effects produced by nifedipine even at its low concentration of 0.1 μM. Furthermore, Fig. 7 showed that the presence of 2-APB drastically diminished the $C_{max}$ value to only 0.024 ± 0.009 g ($P < 0.001$) in comparison with the control of 0.675 ± 0.010 g via inhibition of $IP_3R$. Similarly, the presence of 344OH at 11, 43.8 and 175.25 μM significantly suppressed the contraction of the aorta with $C_{max}$ values achieved at 0.559 ± 0.019 ($P < 0.05$), 0.453 ± 0.021 ($P < 0.001$) and 0.299 ± 0.015 g ($P < 0.001$), respectively (Table 3).

## 4. Discussion

The research world has extensively investigated stilbenoids in the past due to their significant medical and pharmacological effects and abilities. Among the stilbenoids available, resveratrol (*trans*-3,4′,5-trihydroxystilbene) is one of the most largely studied compound [28]. It belongs to the polyphenolic group and is commonly available in plant-based foods especially grapes and berries thus making it abundant in red wine as well. Reveratrol has a proven role in the "French Paradox"- French has lower occurrences of cardiovascular disease due to daily consumption of red wine despite taking in high saturated fats in their regular [28–30]. There has been numerous published studies that reported positive pharmacological effects of resveratrol with some of those articles investigating the underlying mechanism of action that results in the highly antioxidant effect and blood pressure lowering activity of the compound [24,31,32]. With that being said, there are still limited applicability due to its low bioavailability and poor chemical stability and solubility [33–36]. Thus, it still remains as one of the largest concerns towards the potential trend of pharmacological research and we strongly urge towards our suggested SARs research methodology.

It has been identified from our previous study that 344OH (*trans*-3,4,4′-trihydroxystilbene) exhibits excellent vasorelaxant effects towards PE-primed endothelium-intact aortic rings as compared to others synthesized stilbenoid derivatives and the SARs were revealed, but yet the mechanism of actions remain largely unidentified [18]. Based on the results of our study, $R_{max}$ values obtained with a single concentration application of 344OH at 350 μM resulted in 2-fold higher than that of resveratrol. However it showed a significantly lower $R_{max}$ when it's concentration were being added cumulatively despite both compounds producing maximal relaxation of the aorta [24]. This could be a result of fast-acting response of 344OH in eliciting vasorelaxation as compared to resveratrol. With the substitution of the same concentration of 344OH and resveratrol at 350 μM in their respective cumulative concentration-response curve's equation, the $R_{max}$ values of 344OH were seen to have only increased by 16% as compared to a single concentration application which is far lesser than 80% increment shown in resveratrol. This indicates that 344OH required lesser time to induce vasorelaxation hence achieving its maximal relaxation in blood vessel more effectively upon single and low concentration application compared to resveratrol. The underlying mechanisms of actions attributed to 344OH fast-acting and short time-dependent vasorelaxation nature were further investigated.

Fundamentally, the vasorelaxant activity exerted by a compound are mainly attributed to three major categories of vasculature-relaxing receptor pathways; endothelium-dependent receptors (mainly EDRFs), endothelium-independent (mainly GPCRs) and channel-linked receptors pathways [21,23,37]. Previous findings showed that resveratrol mainly employed the EDRFs pathways including $PGI_2$, NO/sGC/cGMP-dependent pathways followed by G-protein-coupled $\beta_2$- and $M_3$-receptors to induce vasorelaxant effects on the aortic rings [24]. However, in present study, the results obtained from the preliminary screening, there were significant reduction of $R_{max}$ values in both KCl-primed endothelium-intact and PE pre-contracted endothelium-impaired aortic rings indicating the involvement of both channel-linked receptors and EDRFs in the vasorelaxation induced by 344OH. At this point, the $R_{max}$ values achieved were fast approaching 100%, suggesting that the major pathway mediating 344OH's vasorelaxant effects caused by endothelium-independent relaxing factors rather than EDRFs, that is opposed to the mechanism employed by resveratrol [24].

Nitric oxide (NO) is best known as the principal compound of EDRFs. Theoretically, NO is produced from the breakdown of L-arginine by the enzymatic reaction of eNOS in the endothelium. Subsequently, NO will diffuse to the adjacent vascular smooth muscle cells (VSMCs) resulting in activation of sGC and its second messenger, cGMP. This subsequently leads to the up-regulation of protein kinase G (PKG) activity and thus led to vessel relaxation [38]. According to the results obtained in the present study, selective antagonist of eNOS, L-NAME was utilized to inhibit the release of NO into the endothelium which caused a significant reduction on 344OH's vasorelaxation ability. This result clearly demonstrated that 344OH promotes the enzymatic reaction of eNOS in the endothelium to up-regulate the NO production in endothelial cells. Next, moving down the NO-signaling cascade, presence of selective blockers, ODQ and MB result in massively reduction of the vasorelaxant activity of 344OH. This portrays that 344OH enhances the synthesis of cGMP from guanosine triphosphate (GTP) through catalyzing of sGC. Subsequently the produced cGMP will binds to the regulatory subunit of PKG and lead to vasorelaxation [39,40]. Overall, from the results it is clear that 344OH

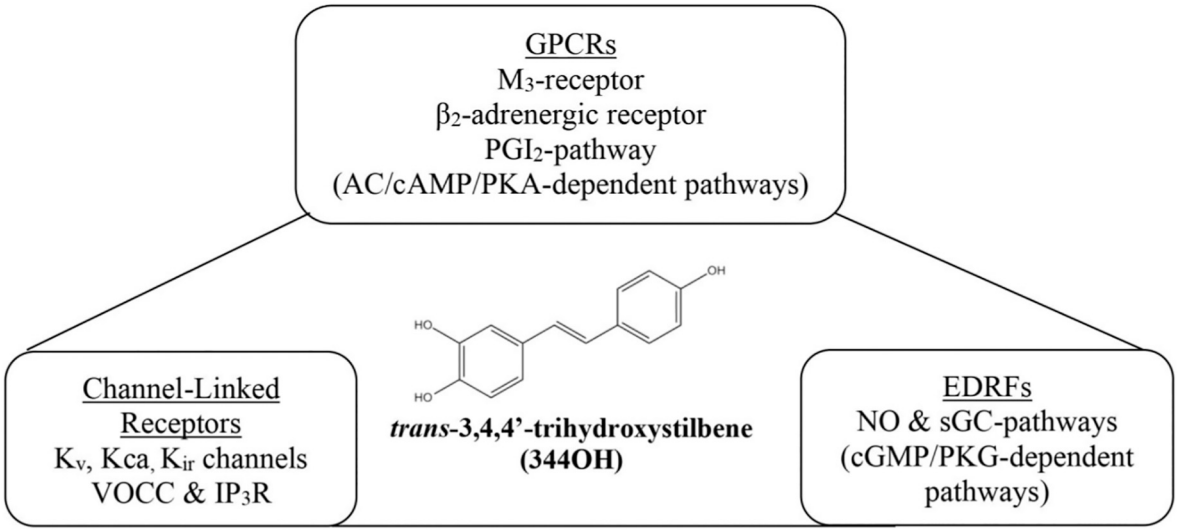


**Fig. 8.** The overview of signaling mechanism pathways employed by 344OH in eliciting vasorelaxant effects.

employed the NO/sGC/cGMP-dependent vasorelaxant pathways with its significance of effect be arranged as sGC > NO > cGMP similar to that of resveratrol.

Prostacyclin ($PGI_2$) is another well-known vasodilator that is released into the endothelial cells from arachidonic acid (AA) via the action of cyclooxygenase (COX) [41]. The effects of $PGI_2$ takes place when it binds to $G_s$-protein-coupled prostacyclin receptors (IP) located in the adjacent VSMCs. Besides the prostacyclin receptors, $\beta_2$-adrenergic receptor is another $G_s$-protein-coupled receptor that is abundantly found in the VSMCs and it is the most frequently studied endothelium-independent relaxing pathways. Upon the binding of the agonists to their respective GPCRs located in VSMCs, adenylyl cyclase (AC) will be activated followed by an elevation of the intracellular cyclic adenosine monophosphate (cAMP) and stimulation of protein kinase A (PKA) action that results in vasorelaxation [39]. According to the results obtained, the $R_{max}$ value of 344OH drastically reduced to 25% in presence of non-selective COX inhibitor, indomethacin and has a drop by 40% in the presence of non-selective β-adrenoreceptor blocker, propranolol. These results demonstrated that both antagonists bound to their respective GPCRs deactivate the AC enzymatic reaction followed by down-regulation in the production of cAMP second messenger and thus causing less PKA activity. This in turn results in the significant reduction of 344OH vasorelaxant effects. In other words, the vasorelaxation induced by 344OH in aortic rings were massively mediated by AC/cAMP/PKA-dependent pathways. Other than the $G_s$-protein-coupled receptors discussed above, we studied the role of the muscarinic receptors which has an important role in vasculature activities. The $M_3$-receptor is known to be greatly prevalent in the endothelial cells of vasculature [42,43]. From the results of this study, the non-selective muscarinic receptor blocker, atropine significantly inhibited the up-regulation of phospholipase C (PLC), thus reducing the production of both the second messengers, inositol triphosphate ($IP_3$) and intracellular calcium in the endothelium, leading to a massive drop of vasodilatory effects elicited by 344OH on the aortic rings. Corresponding to GPCRs, 344OH utilizes a significant portion of the $M_3$-receptor pathway followed by the $\beta_2$-adrenergic receptors, and least the $PGI_2$ pathway.

Other than EDRFs and GPCRs, the vascular tone generation is highly dependent on the action potential that occurs in the VSMCs, regulated by channel-linked receptor pathways which includes both potassium and calcium channels. In theory, we understand that potassium channels are mainly utilized for repolarization and hyperpolarization of membrane potential through efflux of $K^+$ ions from the intracellular to extracellular spaces [21,44]. The four crucial potassium channels were investigated in the present study by application of their selective antagonists and results obtained showed that $K_{ir}$, $K_v$ and $K_{ca}$ were all mediated by 344OH in order to exhibit their vasorelaxant activity. $K_{ATP}$ was the single potassium channel that was excluded from 344OH pathway utilization. The extent to which these potassium channels were employed by 344OH could be arranged in descending order as such: $K_v$ > $K_{ca}$ > $K_{ir}$. Unlike resveratrol which utilized all four potassium channels, it was duly noted that both 344OH and resveratrol produce similar vasorelaxant effects even with the difference in utilization of potassium channel pathways [24]. Moving our focus towards the action potential generation - 344OH repolarizes the membrane potential by activating both $K_v$ and $K_{ca}$ allowing efflux of $K^+$ from the cytosol. When reaching the state of membrane hyperpolarization, 344OH will then cause activation of $K_{ir}$ to hasten the recovery of the membrane potential back to its resting state. It is at this point that the repolarization and hyperpolarization will lead to VSMCs relaxation. The efflux of the $K^+$ will subsequently lead to an increase in $K^+$ concentration within the myoendothelial space, hence enhancing the $K_{ir}$ activation. In the endothelium, the activation of $K_{ir}$ induces endothelium-derived hyperpolarizing factors (EDHFs) that leads to vasorelaxation [45].

For the study of calcium channel, it is theorized that cytosolic $Ca^{2+}$ concentration will increase either by extracellular $Ca^{2+}$ influx through the VOCC or $Ca^{2+}$ released from the sarcoplasmic reticulum (SR) store through the $IP_3R$ which subsequently result in membrane depolarization and vasoconstriction [20,37]. $Ca^{2+}$ is the one of the most important second messengers within our vasculature that plays completely opposite roles in endothelium and VSMCs. In the endothelium, intracellular increment of calcium will trigger NO production however in the VSMCs, calcium binds to calmodulin to form $Ca^{2+}$-calmodulin complexes and stimulate the forward reaction of MLC kinase (MLCK) that leads to vasoconstriction [46,47]. Therefore, in this part of the study endothelium-denuded aortic rings were used to eliminate the possibility of endothelium-induced vasoactivity by calcium as well as removal of all excess calcium residue by introducing the aortic rings in $Ca^{2+}$-free high $K^+$ Krebs' solution which was removed with EGTA. Then, the extracellular $Ca^{2+}$ entry pathway was investigated through the cumulative addition of calcium, whereas the intracellular release of $Ca^{2+}$ was stimulated by application of PE, which will bind to its second messenger, $IP_3$ to $IP_3R$ [48]. From the results obtained, the inhibitory effects of 344OH towards the VOCC were similar to the positive control group, nifedipine in terms of concentration-dependent manner although it did not reach equally excellent abolishment effects. Similar phenomenon was observed in $IP_3R$ mechanistic study, thus implying that 344OH act as a blocker for both VOCC and $IP_3R$ in a concentration-dependent manner. Fig. 8 shows the overall mechanistic actions of 344OH.

## 5. Conclusion

It is clearly elucidated from the results of all comprehensive studies done on major vasculature relaxing pathways that 344OH induces its vasorelaxant effects mainly from utilization of GPCR pathways. The major pathways that attribute to its effects were the $M_3$-receptor and both $\beta_2$-adrenergic receptor and $PGI_2$ via the AC/cAMP/PKA-dependent pathways, followed by EDRFs through the NO/sGC/cGMP-dependent pathway. With respect to the vascular tone, 344OH employed $K_v$, $K_{ca}$ and $K_{ir}$ signaling pathways and acts as a blocker of both the VOCC and $IP_3R$ in regulating the action potential in VSMCs. It is safely concluded that the mechanism of actions employed by 344OH differ from that of its analog, resveratrol due to the positioning of hydroxyl (-OH) substituent in the A-ring of the stilbenoid backbone. It is also due to this key structure that contributes to the fast-acting and less time-dependent vasodilation effects of 344OH as compared to resveratrol thus projecting the potential for this compound to be developed into an effective antihypertensive agent in the near future. Current finding of this study has yet again prove the reliability of our aforementioned proposed research idea whereby synthesizing a new lead compound according to the SAR characteristics will potentially create higher efficacy drugs in treatment of different diseases [3].

## Declaration of competing interest

The authors declare no conflict of interest in current research.

## Acknowledgments

This manuscript is supported by Fundamental Research Grant Scheme – FRGS (203/PKIMIA/6711793) from the Ministry of Education (MOE) of Malaysia. The author would like to extend heartiest thank you to all the contributions made by co-authors.

## References

[1] R. Düsing, Optimizing blood pressure control through the use of fixed combinations, Vasc. Health Risk Manag. 6 (2010) 321.
[2] C. Llorens-Cortes, R.M. Touyz, Evolution of a new class of antihypertensive drugs: targeting the brain renin-angiotensin system, Hypertension 75 (1) (2020) 6–15.
[3] Y.C. Loh, S.Y. Chan, W.Y. Tew, C.W. Oo, M.F. Yam, New flavonoid-based compound synthesis strategy for antihypertensive drug development, Life Sci. (2020) 117512.
[4] D.P. Zipes, P. Libby, R.O. Bonow, D.L. Mann, G.F. Tomaselli, Braunwald's Heart Disease E-Book: A Textbook of Cardiovascular Medicine, Elsevier Health Sciences, 2018.
[5] R.J. Johnson, J. Feehally, J. Floege, M. Tonelli, Comprehensive Clinical Nephrology E-Book, Elsevier Health Sciences, 2018.
[6] T. Chen, D. Wu, H. Chen, W. Yan, D. Yang, G. Chen, K. Ma, D. Xu, H. Yu, H. Wang, T. Wang, W. Guo, J. Chen, C. Ding, X. Zhang, J. Huang, M. Han, S. Li, X. Luo, J. Zhao, Q. Ning, Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study, BMJ 368 (2020) m1091.
[7] K. Ferdinand, T. Batieste, M. Fleurestil, Contemporary and future concepts on hypertension in African Americans: COVID-19 and beyond, J. Natl. Med. Assoc. 112 (3) (2020) 315–323.
[8] B. Li, J. Yang, F. Zhao, L. Zhi, X. Wang, L. Liu, Z. Bi, Y. Zhao, Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China, Clin. Res. Cardiol. 109 (5) (2020) 531–538.
[9] M. Tadic, C. Cuspidi, G. Mancia, R. Dell'Oro, G. Grassi, COVID-19, hypertension and cardiovascular diseases: should we change the therapy? Pharmacol. Res. (2020) 104906.
[10] J. Yang, Y. Zheng, X. Gou, K. Pu, Z. Chen, Q. Guo, R. Ji, H. Wang, Y. Wang, Y. Zhou, Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis, Int. J. Infect. Dis. 94 (2020) 91–95.
[11] H. Cui, W. Feng, Z. Fan, X. Cheng, J. Cheng, M. Fan, The effects of renin-angiotensin system inhibitors (RASI) in Corona Virus Disease (COVID-19) with hypertension: a retrospective, single-center trial, Med. Clin. (Barc.) 155 (7) (2020) 295–298.
[12] J.H. Diaz, Hypothesis: angiotensin-converting enzyme inhibitors and angiotensin receptor blockers may increase the risk of severe COVID-19, J. Travel Med. 27 (3) (2020) 1–2.
[13] M. Esler, D. Esler, Can angiotensin receptor-blocking drugs perhaps be harmful in the COVID-19 pandemic? J. Hypertens. 38 (5) (2020) 781–782.
[14] C.M. Ferrario, J. Jessup, M.C. Chappell, D.B. Averill, K.B. Brosnihan, E.A. Tallant, D.I. Diz, P.E. Gallagher, Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2, Circulation 111 (20) (2005) 2605–2610.
[15] J. Lubel, M. Garg, Renin-angiotensin-aldosterone system inhibitors in Covid-19, N. Engl. J. Med. 382 (2020), e92.
[16] K. Rao, L. Xie, J. Wu, T. Weng, L. Tang, J. Zhou, COVID-19 in a young man with hypertension: a case study of missed opportunities in intensive progression, Intensive Crit. Care Nurs. 60 (2020) 102898.
[17] Y.C. Loh, S.Y. Chan, C.W. Oo, M.F. Yam, Creation of novel antihypertensive agent via structure-activity relationship study on phytochemicals towards vasorelaxant activity, J. Pharmacopunct. 23 (2) (2020) 88–89.
[18] S.Y. Chan, Y.C. Loh, C.W. Oo, M.F. Yam, In vitro study and structure-activity relationship analysis of stilbenoid derivatives as powerful vasorelaxants: discovery of new lead compound, Bioorg. Chem. 104 (2020), 104239.
[19] S.Y. Chan, C.W. Oo, M.F. Yam, Y.C. Loh, Synthesis and Characterization of New Stilbenoid Derivatives as Potential Vasodilators, Paper Presented at the 7th International Conference for Young Chemists (ICYC 2019), 2020, p. 16.
[20] F.J. Luna-Vázquez, C. Ibarra-Alvarado, A. Rojas-Molina, I. Rojas-Molina, M.Á. Zavala-Sánchez, Vasodilator compounds derived from plants and their mechanisms of action, Molecules 18 (5) (2013) 5814–5857.
[21] Y.C. Loh, C.S. Tan, Y.S. Ch'ng, Z.Q. Yeap, C.H. Ng, M.F. Yam, Overview of the microenvironment of vasculature in vascular tone regulation, Int. J. Mol. Sci. 19 (1) (2018) 120.
[22] Y.C. Loh, C.S. Tan, Y.S. Ch'ng, C.H. Ng, Z.Q. Yeap, M.F. Yam, Mechanisms of action of Panax notoginseng ethanolic extract for its vasodilatory effects and partial characterization of vasoactive compounds, Hypertens. Res. 42 (2) (2019) 182–194.
[23] Y.C. Loh, C.S. Tan, Y.S. Ch'ng, M. Ahmad, M.Z. Asmawi, M.F. Yam, Overview of antagonists used for determining the mechanisms of action employed by potential vasodilators with their suggested signaling pathways, Molecules 21 (4) (2016) 495.
[24] C.S. Tan, Y.C. Loh, W.Y. Tew, M.F. Yam, Vasorelaxant effect of 3, 5, 4′-trihydroxy-trans-stilbene (resveratrol) and its underlying mechanism, Inflammopharmacology (2020) 1–7.
[25] B. Yilmaz, C. Usta, Ellagic acid-induced endothelium-dependent and endothelium-independent vasorelaxation in rat thoracic aortic rings and the underlying mechanism, Phytother. Res. 27 (2) (2013) 285–289.
[26] B. Kim, Y. Kwon, S. Lee, K. Lee, I. Ham, H.Y. Choi, Vasorelaxant effects of Angelica decursiva root on isolated rat aortic rings, BMC Complement. Altern. Med. 17 (1) (2017) 474.
[27] M.F. Yam, C.S. Tan, M. Ahmad, R. Shibao, Mechanism of vasorelaxation induced by eupatorin in the rats aortic ring, Eur. J. Pharmacol. 789 (2016) 27–36.
[28] H. Krawczyk, The stilbene derivatives, nucleosides, and nucleosides modified by stilbene derivatives, Bioorg. Chem. 90 (2019), 103073.
[29] B.C. Akinwumi, K.M. Bordun, H.D. Anderson, Biological activities of Stilbenoids, Int. J. Mol. Sci. 19 (3) (2018).
[30] K.L. Gordish, W.H. Beierwaltes, Resveratrol induces acute endothelium-dependent renal vasodilation mediated through nitric oxide and reactive oxygen species scavenging, Am. J. Physiol.-Renal Physiol. 306 (5) (2014) F542–F550.
[31] V.W. Dolinsky, S. Chakrabarti, T.J. Pereira, T. Oka, J. Levasseur, D. Beker, B. N. Zordoky, J.S. Morton, J. Nagendran, G.D. Lopaschuk, S.T. Davidge, J.R. Dyck, Resveratrol prevents hypertension and cardiac hypertrophy in hypertensive rats and mice, Biochim. Biophys, Acta 1832 (10) (2013) 1723–1733.
[32] M. Shen, L. Zhao, R.X. Wu, S.Q. Yue, J.M. Pei, The vasorelaxing effect of resveratrol on abdominal aorta from rats and its underlying mechanisms, Vasc. Pharmacol. 58 (1–2) (2013) 64–70.
[33] U.G. Haider, D. Sorescu, K.K. Griendling, A.M. Vollmar, V.M. Dirsch, Resveratrol suppresses angiotensin II-induced Akt/protein kinase B and p70 S6 kinase phosphorylation and subsequent hypertrophy in rat aortic smooth muscle cells, Mol. Pharmacol. 62 (4) (2002) 772–777.
[34] Q. Liu, C. Kim, Y.H. Jo, S.B. Kim, B.Y. Hwang, M.K. Lee, Synthesis and biological evaluation of resveratrol derivatives as melanogenesis inhibitors, Molecules 20 (9) (2015) 16933–16945.
[35] M. Murias, W. Jager, N. Handler, T. Erker, Z. Horvath, T. Szekeres, H. Nohl, L. Gille, Antioxidant, prooxidant and cytotoxic activity of hydroxylated resveratrol analogues: structure-activity relationship, Biochem. Pharmacol. 69 (6) (2005) 903–912.
[36] W. Nawaz, Z. Zhou, S. Deng, X. Ma, X. Ma, C. Li, X. Shu, Therapeutic versatility of resveratrol derivatives, Nutrients 9 (11) (2017).
[37] M. Rameshrad, H. Babaei, Y. Azarmi, D.F. Fouladia, Rat aorta as a pharmacological tool for in vitro and in vivo studies, Life Sci. 145 (2016) 190–204.
[38] R.F. Furchgott, The role of endothelium in the responses of vascular smooth muscle to drugs, Annu. Rev. Pharmacol. Toxicol. 24 (1984) 175–197.
[39] S.J. Enna, D.B. Bylund, S. Elsevier, XPharm: The Comprehensive Pharmacology Reference. http://www.sciencedirect.com/science/referenceworks/9780080552323, 2008.
[40] M. Feelisch, P. Kotsonis, J. Siebe, B. Clement, H.H. Schmidt, The soluble guanylyl cyclase inhibitor 1H-[1, 2, 4] oxadiazolo [4, 3,-a] quinoxalin-1-one is a nonselective heme protein inhibitor of nitric oxide synthase and other cytochrome P-450 enzymes involved in nitric oxide donor bioactivation, Mol. Pharmacol. 56 (2) (1999) 243–253.
[41] S. Moncada, R. Gryglewski, S. Bunting, J. Vane, An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation, Nature 263 (5579) (1976) 663–665.
[42] L. Brunton, J. Lazo, K. Parker, Goodman & Gilman's the Pharmacological Basis of Therapeutics, Eleventh edition, McGraw-Hill Education, 2005.
[43] L. Walch, C. Brink, X. Norel, The muscarinic receptor subtypes in human blood vessels, Therapie 56 (3) (2001) 223–226.

[44] N.R. Tykocki, E.M. Boerman, W.F. Jackson, Smooth muscle ion channels and regulation of vascular tone in resistance arteries and arterioles, Comprehensive Physiol. 7 (2) (2017) 485–581.
[45] M. Feletou, P. Vanhoutte, EDHF: The Complete Story, CRC Press, 2005.
[46] Y. Gao, K. Kawano, S. Yoshiyama, H. Kawamichi, X. Wang, A. Nakamura, K. Kohama, Myosin light chain kinase stimulates smooth muscle myosin ATPase activity by binding to the myosin heads without phosphorylating the myosin light chain, Biochem. Biophys. Res. Commun. 305 (1) (2003) 16–21.
[47] R.C. Webb, Smooth muscle contraction and relaxation, Adv. Physiol. Educ. 27 (1–4) (2003) 201–206.
[48] W.F. Jackson, Ion channels and vascular tone, Hypertension 35 (1 Pt 2) (2000) 173–178.