Bioorganic & Medicinal Chemistry 19 (2011) 4051–4056

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Vasorelaxant effects of macrocyclic bis(bibenzyls) from liverworts

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ARTICLE INFO

Article history:
Received 28 March 2011
Revised 13 May 2011
Accepted 13 May 2011
Available online 19 May 2011

Keywords:
Bis(bibenzyls)
Vasorelaxant effects
Liverwort
NO
VDC
ROC

ABSTRACT

Vasorelaxant effects of a series of bis(bibenzyls) from liverworts such as *Marchantia polymorpha* and *Marchantia paleacea* on rat aorta demonstrated that they relaxed phenylephrine (PE)-induced contractions, which may be mediated through the increased release of NO from endothelial cells as well as opening of K^+ channels, and inhibition of Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCs) and/or receptor-operated Ca^{2+} channels (ROCs). Structure–activity relationship based on their structures was discussed. The presence of two aromatic rings which can be connected through two atoms bridge spacer may play an important role for vasorelaxant effect.

1. Introduction

The vasodilators are useful for treatment of cerebral vasospasm and hypertension, and for improvement of peripheral circulation. Several endothelium-dependent vasodilators, such as bradykinin, acetylcholine, and histamine, have been reported to elevate Ca^{2+} levels in endothelial cells and activate NO release, leading to vasorelaxation.[1] On the other hand, contractile response in smooth muscle is caused by Ca^{2+} influx through VDCs and/or ROCs.[2] The endothelium-independent vasodilators, such as nicardipine, niphedipine, dirtiazem, and verapamil, have been reported to inhibit VDCs and led to decrease the intracellular Ca^{2+} concentration in smooth muscle, leading to vasorelaxation.[2]

K^+ channels play important roles in the regulation of vascular tone.[3–5] Indeed, the K^+ channels present in blood vessels indirectly influence vascular tension by changing the resting membrane potential. By so doing, not only do they help to maintain the resting membrane potential of vascular smooth muscle, but they also modulate the relaxant/dilator response of blood vessel.[4,5] Many vascularly active agents and drugs induce their vasodilator of vasoconstrictor effects by opening or closing K^+ channels.[6]

During our search for bioactive natural products inducing vasorelaxation from medicinal plants,[7] we found that a series of bis(bibenzyls) from the liverwort remarkably exhibited vasodilation against rat aorta. Liverworts are rich sources of both terpenoids and aromatic compounds with various biological activities.[8] Bis(bibenzyls) are aromatic compounds mainly found in liverworts from genera such as *Riccardia, Marchantia, Plagiochila, Preissia, Reboulia, Monoclea, Ricciocarpos,* and *Blasia*.[8] These compounds are cyclic or acyclic and have one or two diaryl ether or biphenyl bonds possessing a diaryl ether or a biphenyl bonds, and have shown a wide range of antibacterial, antifungual, and 5-lipoxygenase inhibitory activity.[4] Bis(bibenzyls) arise biogenetically from lunularic acid or lunularin, which is widely distributed in leafy and thalloid liverworts.[8]

This paper describes vasorelaxant effects of a series of bis(bibenzyls) from the liverworts on rat aorta as well as mode of action and structure–activity relationship.

2. Materials and methods

A male Wistar rat weighting 260 g was sacrificed by bleeding from carotid arteries under an anesthetization. A section of the thoracic aorta between the aortic arch and the diaphragm was removed and placed in oxygenated, modified Krebs–Henseleit solution (KHS:118.0 mM NaCl, 4.7 mM KCl, 25.0 mM $NaHCO_3$, 1.8 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, and 11.0 mM glucose). The aorta was cleaned of loosely adhering fat and connective tissue and cut into ring preparations 3 mm in length. The tissue was placed in a well-oxygenated (95% O_2, 5% CO_2) bath of 5 mL KHS solution at 37 °C with one end connected to a tissue holder and the other to a force-displacement transducer (Nihon Kohden, TB-611T). The tissue was equilibrated for 60 min under a resting

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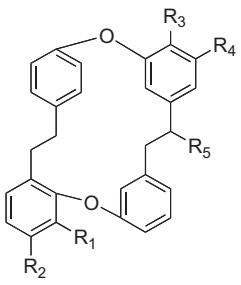
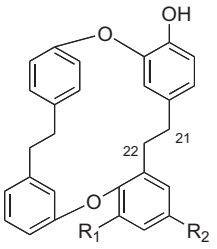
doi:10.1016/j.bmc.2011.05.019

tension of 1.0 g. During this time the KHS in the tissue bath was replaced every 20 min.

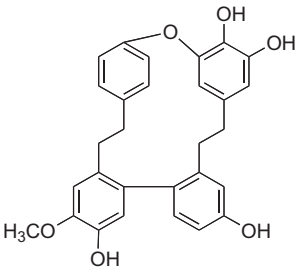


	R_1	R_2	R_3	R_4	R_5
marchantin A (**1**):	OH	H	OH	OH	H
marchantin B (**2**):	OH	OH	OH	OH	H
marchantin C (**3**):	OH	H	OH	H	H
marchantin E (**4**):	OH	H	OH	OH	OMe
marchantin B tetramethyl ether (**5**):	OMe	OMe	OMe	OMe	H
marchantin A trimethyl ether (**6**):	OMe	H	OMe	OMe	H

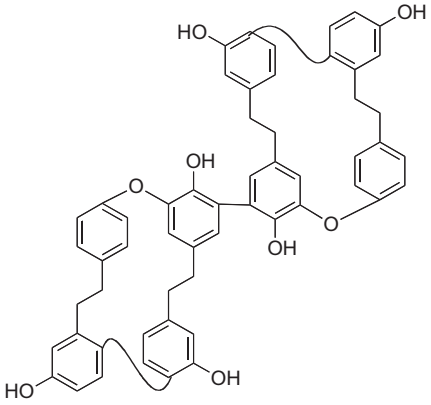


	R_1	R_2
isomarchantin C (**7**):	OH	H
ptychantol A (**8**):	H	OH

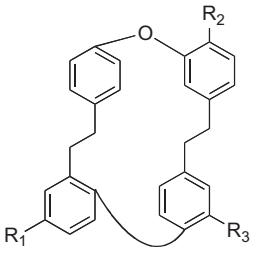
$\Delta^{21,22}$



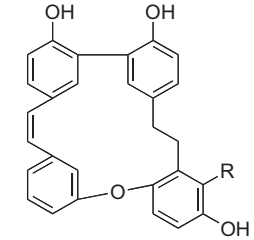
plagiochin A (**9**)



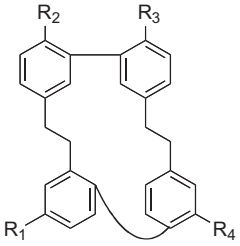
pusilatin B (**10**)



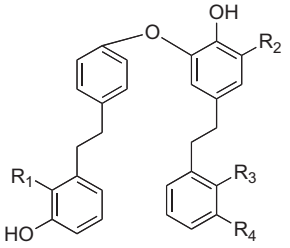
	R_1	R_2	R_3
riccardin A (**11**):	OMe	OH	OH
riccardin C (**12**):	OH	OH	OH
riccardin F (**13**):	OH	OMe	OH



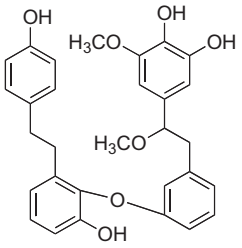
	R
isoplagiochin A (**14**)	H
isoplagiochin B (**15**)	OH



	R_1	R_2	R_3	R_4
isoplagiochin D (**16**):	OH	OH	OH	OH
isoplagiochin D tetramethyl ether (**17**):	OMe	OMe	OMe	OMe



	R_1	R_2	R_3	R_4
perrottetin F (**18**):	H	OH	H	OH
perrottetin E -11'-methyl ether (**20**):	H	H	H	OMe
compound **21**:	OH	H	OH	OH



paleatin A (**19**)

After equilibration, each aortic ring was contracted by treatment with PE (0.3 μM). The presence of functional endothelial cells (EC) was confirmed by demonstrating relaxation to acetylcholine (ACh, 10 μM). And then, aortic ring in which 80% relaxation occurred, were regarded as intact endothelium. Following washout, these rings were contracted once again with the same concentration of PE. When the PE-induced contraction reached a plateau, each sample (30 μM) or ACh (1 nM to 10 μM) was added and evaluated. IC_{50} of ACh was approximately 1 μM in this experimental system.

Data are expressed as means ± SD. Statistical comparisons between time-response curves were made using a one-way analysis of variance (ANOVA), with Bonferroni's correction for multiple comparisons being performed post hoc (P <0.05 being considered significant).

These animal experimental studies were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University and under the supervision of the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Science, Sports Culture, and Technology of Japan.

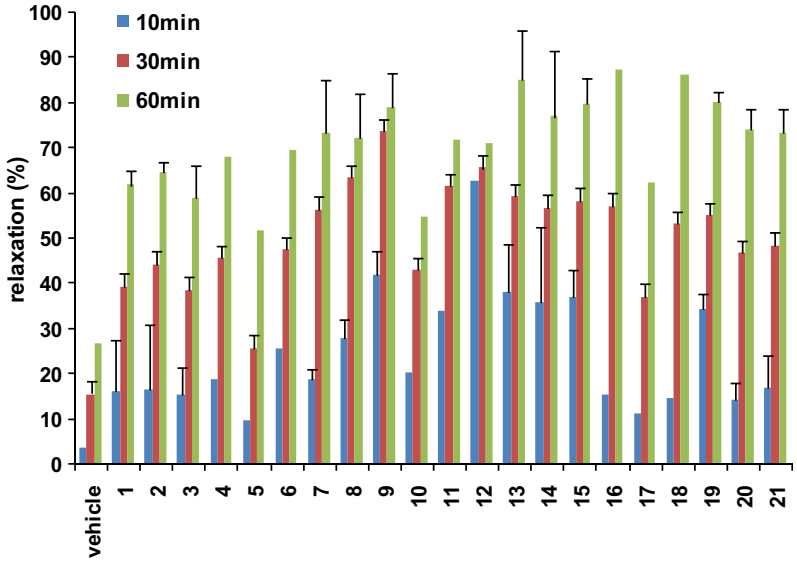


Figure 1. Vasorelaxant effects of a series of bis(bibenzyls) (**1–21**, 30 μM) from the liverworts on the rat aortic rings pre-contracted with PE (0.3 μM). Values are the mean ± SD (n = 3).

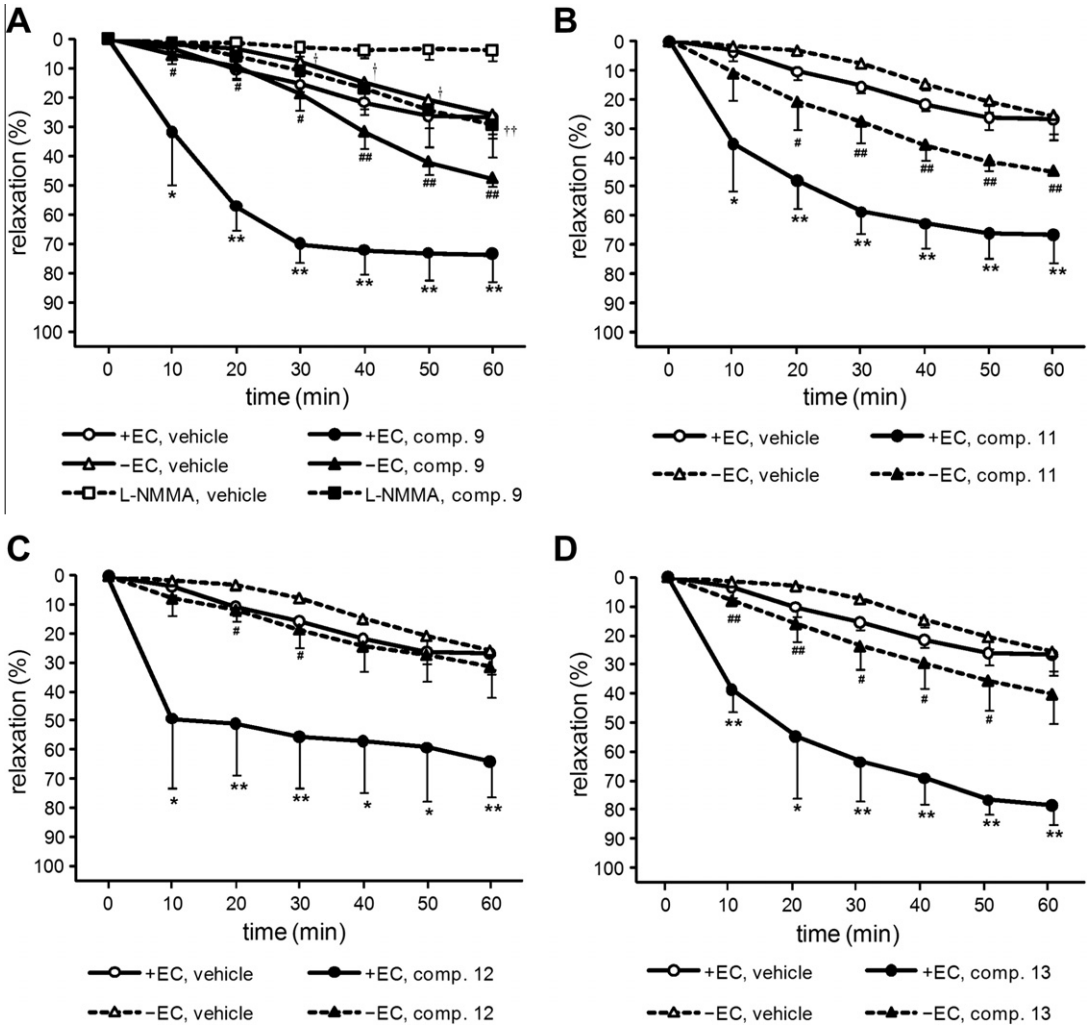


Figure 2. Vasorelaxant effects of **9** (A), **11** (B), **12** (C), and **13** (D) on endothelium-intact (+EC) and endothelium-denuded (−EC) rings cut from rat arteries pre-contracted with PE (0.3 μM). Vasorelaxant effect of **9** (A) on isolated rat aortic rings pre-contracted with PE (0.3 μM) in the presence of L-NMMA (100 μM). Values are the mean ± SD (n = 3) $^{*}P$ <0.05 and $^{**}P$ <0.01, respectively, versus vehicle group. #P <0.05 and ##P <0.01, respectively, versus vehicle group. †P <0.05 and ††P <0.01, respectively, versus vehicle group.

3. Results and discussion

The isolated macrocyclic bis(bibenzyls)[8] could be divided into five types such as **type A** (18-membered ring with two ether linkage; **type A1**: **1**–**6**, **type A2**: **7** and **8**), **type B** (16-membered ring with one ether and one C–C linkage, **type B1**: **9**, **type B2**: **10**), **type C** (18-membered ring with one ether and one C–C linkage, **11**–**13**), **type D** (16-membered ring with a *cis* double bond, one ether and one C–C linkage, **14** and **15**), **type E** (16-membered ring with two C–C linkage, **16** and **17**), and **type F** (acyclic type with one ether linkage, **18**–**21**).

All bis(bibenzyls) were tested for vasorelaxant activity against rat aorta. When PE (0.3 μM) was applied to thoracic aortic rings with endothelium after achieving a maximal response, a series of bis(bibenzyls) was added and showed potent vasorelaxant effects at 30 μM (Fig. 1). The excellent activity could be observed for plagiochin A (**9**), riccardins A, C, and F (**11**–**13**) at early stage within 10–30 min after injection of each sample. Furthermore, vasorelaxant effect was examined by using endothelium-denuded aortic tissues for the selected bis(bibenzyls) such as plagiochin A (**9**), riccardins A, C, and F (**11**–**13**), isoplagiochin B (**15**), isoplagiochin D (**16**), and perrottetin F (**18**), which showed potent activity (Figs. 2 and 3). Interestingly, different mechanism of relaxation on rat aorta was demonstrated depending on a structural type of bis(bibenzyls) between **types A**–**F**.

The potent vasorelaxant effects of **9**, **11**–**13**, **15**, **16**, and **18** were observed in a time-dependent manner as well as a dose-dependent manner at 100, 30, and 10 μM (data not shown) to induce more than 60–80% relaxation of rings pre-contracted with PE (0.3 μM). In endothelium-denuded aortic tissues, **9** and **11**–**13** did not cause vascular relaxation (Fig. 2), whereas bis(bibenzyls) **15**, **16**, and **18** showed relaxation in a time-dependent manner (Fig. 3). Treatment with N^G-monomethyl-L-arginine (L-NMMA, 100 μM),[9] an inhibitor of nitric oxide (NO) synthase, also inhibited plagiochin A (**9**)-induced vasorelaxation (Fig. 2). The vasorelaxant effect of **9** may be mediated through the increased release of NO from endothelial cells. The vasorelaxant effects of **11**–**13** were also attenuated by endothelium removal and their vasorelaxant effects were also attributed to their actions on the endothelial cells to release NO (Fig. 2). The structural feature for these bis(bibenzyls) which induced an endothelium-dependent vasorelaxation on rat aorta was the presence of 18-membered ring with one ether and one C–C linkage. Thus, the subsequent studies were focused on the direct effect of **15**, **16**, and **18** on vascular smooth muscle cells.

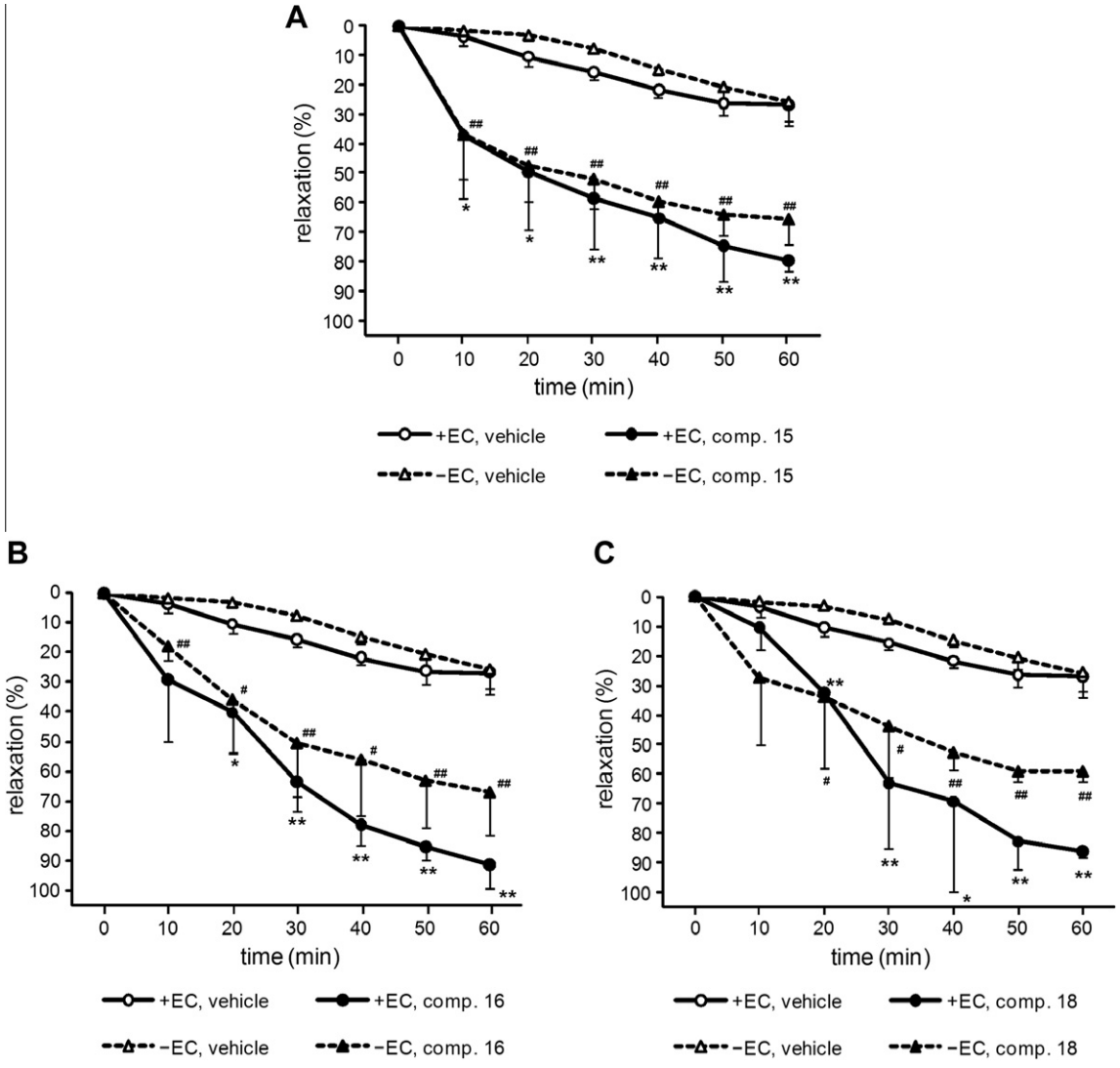


Figure 3. Vasorelaxant effects of **15** (A), **16** (B), and **18** (C) on isolated rat aortic rings pre-contracted with PE (0.3 μM) in presence and absence of endothelium. Values are the mean ± SD (n = 3). $^{*}P$ <0.05 and $^{**}P$ <0.01, respectively, versus vehicle group. #P <0.05 and ##P <0.01, respectively, versus vehicle group.

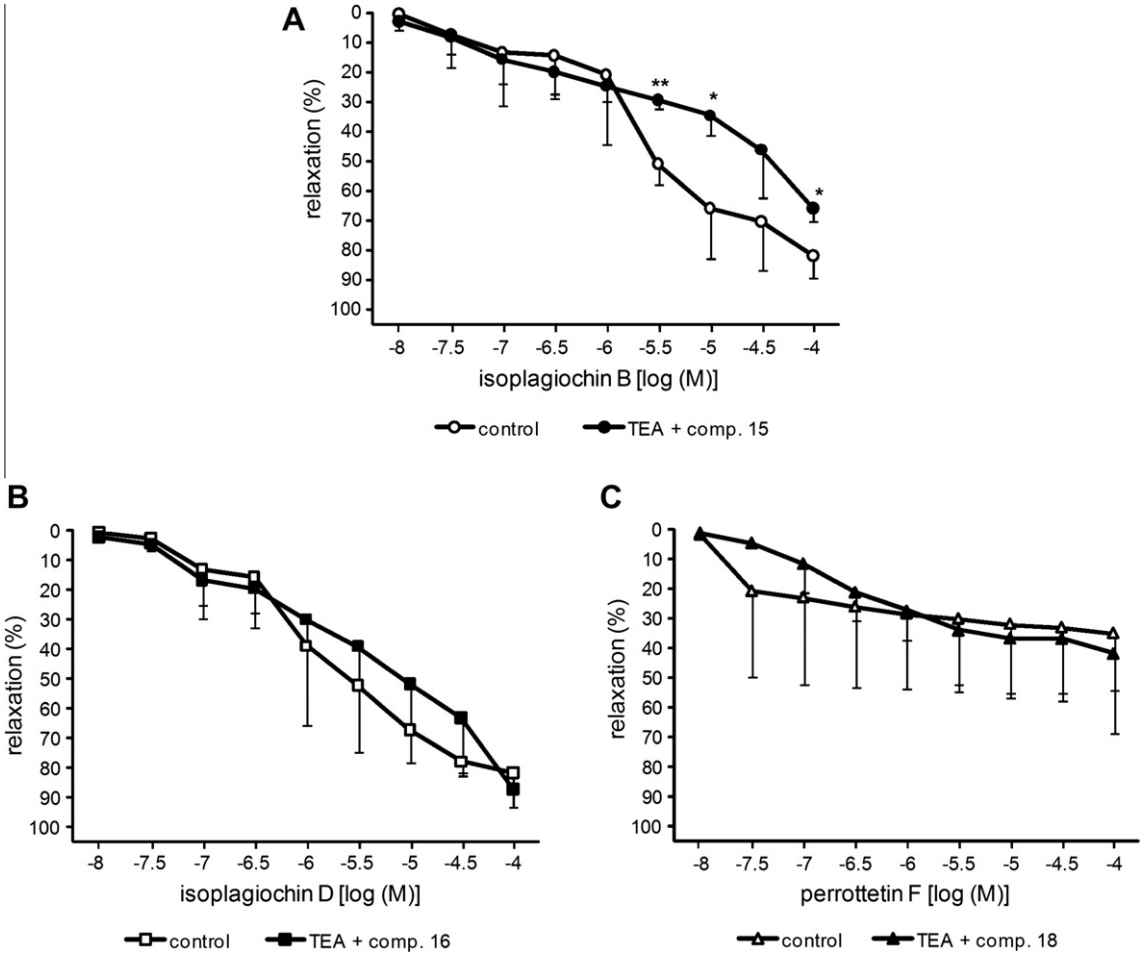


Figure 4. Vasorelaxant effects of **15** (A), **16** (B), and **18** (C) on endothelium-denuded rat aortic rings pre-contracted with PE (0.3 μM) in the presence of TEA (1 mM). Values are the mean ± SD ($n = 3$). $^{*}P < 0.05$ and $^{**}P < 0.01$, respectively, versus control group.

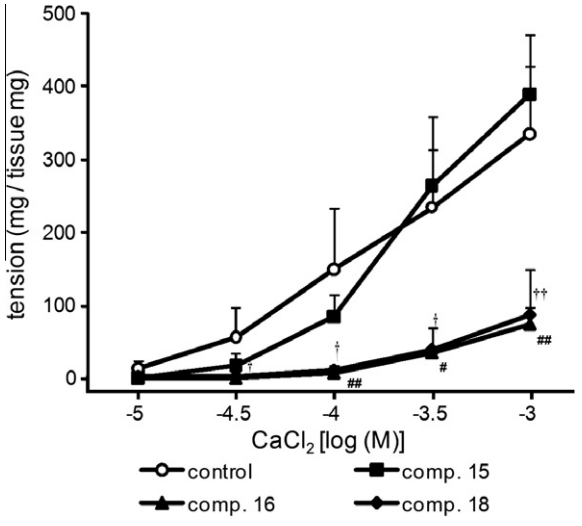


Figure 5. Effects of **15**, **16**, and **18** at 30 μM on the concentration-response curves of $CaCl_2$ in endothelium-denuded rat aortic rings in Ca^{2+}-free K^{+}-rich (60 mM KCl) medium. Values are the mean ± SD ($n = 3$). #$P < 0.05$ and ##$P < 0.01$, respectively, versus control group. †$P < 0.05$ and ††$P < 0.01$, respectively, versus control group.

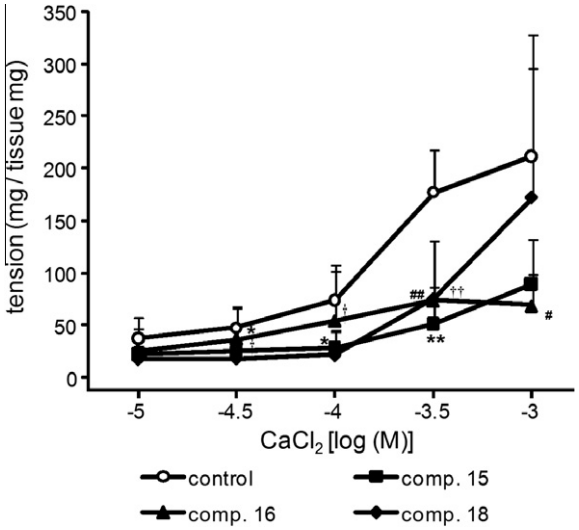


Figure 6. Effects of **15**, **16**, and **18** at 30 μM on the concentration-response curves of $CaCl_2$ in endothelium-denuded rat aortic rings in Ca^{2+}-free medium pre-incubated with PE (1 μM) and nicardipine (1 μM). Values are the mean ± SD ($n = 3$). $^{*}P < 0.05$ and $^{**}P < 0.01$, respectively, versus control group. #$P < 0.05$ and ##$P < 0.01$ respectively, vs control group. †$P < 0.05$ and ††$P < 0.01$, respectively, versus control group.

Bis(bibenzyls) **15**, **16**, and **18** also induced more than 80% relaxation but showed a slow relaxation against PE (0.3 μM)-induced contractions on rat aorta with/without endothelium. In an attempt to elucidate the possible mechanisms involved in the vasorelaxant effects, the effects of **15**, **16**, and **18** were examined with pre-incu-

bation of tetraethylammonium chloride (TEA, 1 mM)[10] as an inhibitor of K^+ channel in PE-contracted endothelium-denuded rings. Incubation of endothelium-denuded rings with TEA slightly shifted the concentration-response curve for isoplagiochin B (**15**) to the right, whereas the curves for isoplagiochin D (**16**) and perrottetin F (**18**) were not affected. These results were shown in Figure 4.

Ca^{2+} can contract aortic rings concentration dependently in Ca^{2+}-free KHS after depolarization with isotonic high K^+ (60 mM) by Ca^{2+} influx via VDCs; this contraction was significantly inhibited by isoplagiochin D (**16**) and perrottetin F (**18**) at 30 μM, whereas isoplagiochin B (**15**) at 30 μM did not inhibit the contraction induced by Ca^{2+} (Fig. 5).

In addition, the PE (1 μM)-induced contractions of the aortic rings in the presence of nicardipine (1 μM) in Ca^{2+}-free KHS occurred in Ca^{2+} (10 μM–1 mM) concentration-dependent manner, presumably due to Ca^{2+} influx via ROCs. Isoplagiochin B (**15**), isoplagiochin D (**16**), and perrottetin F (**18**) showed moderate inhibitions of these contractions at 30 μM, suggesting that **15**, **16**, and **18** exert inhibitory effects on Ca^{2+} influx via ROCs (Fig. 6).

The presence of two aromatic rings which can be connected through two atoms bridge spacer may serve to maintain the backbone conformation as well as vasorelaxant effects. Efforts are currently underway to determine the precise backbone conformation and its relationship to vasorelaxant activity of a series of bis(bibenzyls) such as a series of marchantins as vasorelaxant agents.

In conclusion, some of bis(bibenzyls) from liverworts showed potent vasorelaxant effect on rat aorta. Mode of action for plagiochin A (**9**), riccardins A, C, and F (**11**–**13**) was deduced to be mediated through the increased release of NO from endothelial cells. Vasorelaxant effect of isoplagiochin B (**15**) may be mediated through opening of K^+ channels and Ca^{2+} influx through ROCs. Isoplagiochin D (**16**) and perrottetin F (**18**) may be attributed to integral inhibitory effects such as VDCs and ROCs. As far as we know, this is the first report to demonstrate the vasorelaxant effect of a series of bis(bibenzyls) on rat aortic artery.

Further studies on a series of bis(bibenzyls), such as mechanisms about vasorelaxant effects which may be mediated by NO production from endothelium and/or various K^+ and Ca^{2+} channels, are necessary to develop a novel vasorelaxant agent with controlling vascular tone.

Acknowledgment

This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and a grant from the Open Research Center Project.

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