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Design and Synthesis of Novel Xyloketal Derivatives and Their Vasorelaxing Activities in Rat Thoracic Aorta and Angiogenic Activities in Zebrafish Angiogenesis Screen

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A novel series of xyloketal derivatives (**1**−**21**) were designed and prepared. The majority of the compounds demonstrated vasorelaxation action on 60 mM KCl-induced contractions rat isolated aortic rings in a concentration-dependent manner, and the action is mediated by both endothelium-independent and endothelium-dependent mechanisms. Compounds **9**, **12**, **13**, **14**, **15**, and **19** showed higher vasorelaxation activities comparing with the lead compound **3**. In addition, these derivatives had potential protective action against oxLDL-induced endothelial oxidative injury and enhanced NO production in HUVECs without toxic effects. The NO release was completely inhibited by eNOS inhibitor L-NAME. Furthermore, **3** significantly promoted the angiogenesis in zebrafish in a concentration-dependent manner at 0.1, 1, and 10 μM. Compounds **9**, **12**, **14**, **16**, **20**, and **21** exhibited stronger angiogenic activities than **3**. Therefore, xyloketal derivatives are unique compounds with multiple pharmacological properties and may have potential implications in the treatment of cardiovascular diseases.

Introduction

In recent years, numerous studies have shown that vascular function is compromised in a number of pathological conditions including hypertension and atherosclerosis.[1,2] Hypertension is a chronic cardiovascular disease associated with an altered balance in the proportion or biological action of vasodilator and vasoconstrictor molecules and alterations in vascular tissue.[3] The disturbed balance between nitric oxide (NO[a]) and reactive oxygen species (ROS) plays an important role in vascular abnormality during atherosclerosis and other cardiovascular diseases.[4,5] Decreased NO levels are coupled with impaired endothelium-dependent vasodilation during many cardiovascular diseases.[6] Furthermore, ROS can react with the endogenous vasodilator NO, depleting available NO and leading to vasoconstriction and reduced perfusion.[7−9] Angiogenic activity plays an important role at all stages of development, from early in embryogenesis when it is involved in the growth of new vessels to adulthood when revascularization of tissues is critical for their recovery from ischemic injury.[10−12]

The marine environment is a rich source of novel and unusual secondary metabolites,[13,14] many of which have already shown considerable promise for development as therapeutic agents. Xyloketals (Chart 1), a series of novel natural products isolated from marine mangrove endophytic fungus from the South China Sea,[15−19] have shown strong L-calcium channel blocking activities[16] and inhibitory activities of acetylcholine esterase. Subsequently, we found that (±)-xyloketal B protected against oxidatively modified low density lipoprotein (oxLDL) induced cell injury in vitro without either toxic or proliferative effects. In addition, (±)-xyloketal B concentration-dependently attenuates oxLDL-induced ROS generation, peroxynitrite formation, and decrease of Bcl-2 protein expression and augments NO release.[20] Furthermore, (±)-xyloketal B is able to protect PC12 cells against oxygen glucose deprivation (OGD) induced cell injury; the antioxidative property and protective action on mitochondria may account for its neuroprotective actions.[21] In view of the remarkable structural and biological properties of xyloketals, much interest has currently been in the total synthesis of these natural products. Over the past few years, Wilson et al[22−26] successfully synthesized the racemic and asymmetric xyloketals via two routes: (1) the electrophilic aromatic substitution reactions which were promoted by boron trifluoride diethyl etherate; (2) cycloaddition reaction of an *o*-quinone methide with a dihydrofuran. Krohn et al[27,28] reported the racemic synthesis of demethyl analogues of xyloketal A, B, D, and H and total synthesis of (+)-xyloketal D from enone and phenols by a concise way.

Previous studies of xyloketals have focused on the total synthesis and biological activities in vitro. To our knowledge, no research has been reported on the design of derivatives and

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[a] Abbreviations: NO, nitric oxide; ROS, reactive oxygen species; LDL, low density lipoproteins; HUVECs, human umbilical vein endothelial cells; eNOS, endothelial nitric oxide synthase; OGD, oxygen glucose deprivation; PC12 cells, rat pheochromocytoma; oxLDL, oxidatively modified low density lipoprotein; L-NAME, N(G)-nitro-L-arginine methyl ester; cAMP, 3′,5′-cyclic adenosine monophosphate; DIEA, *N*,*N*-diisopropylethylamine; MTT, 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyltetrazolium bromide; EC_{50}, half-maximal effective concentration; EDRF, endothelium-derived relaxing factor; PGC, primordial germ cells.

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Chart 1. Structures of Xyloketals A, B, C, D, and H

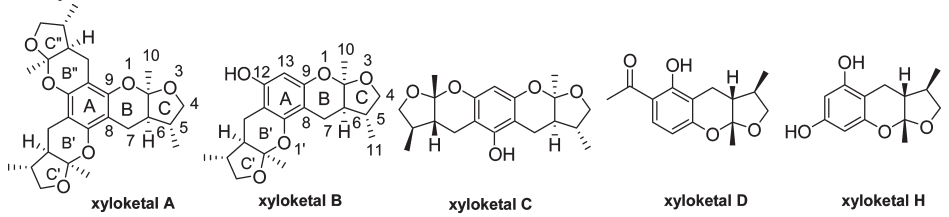
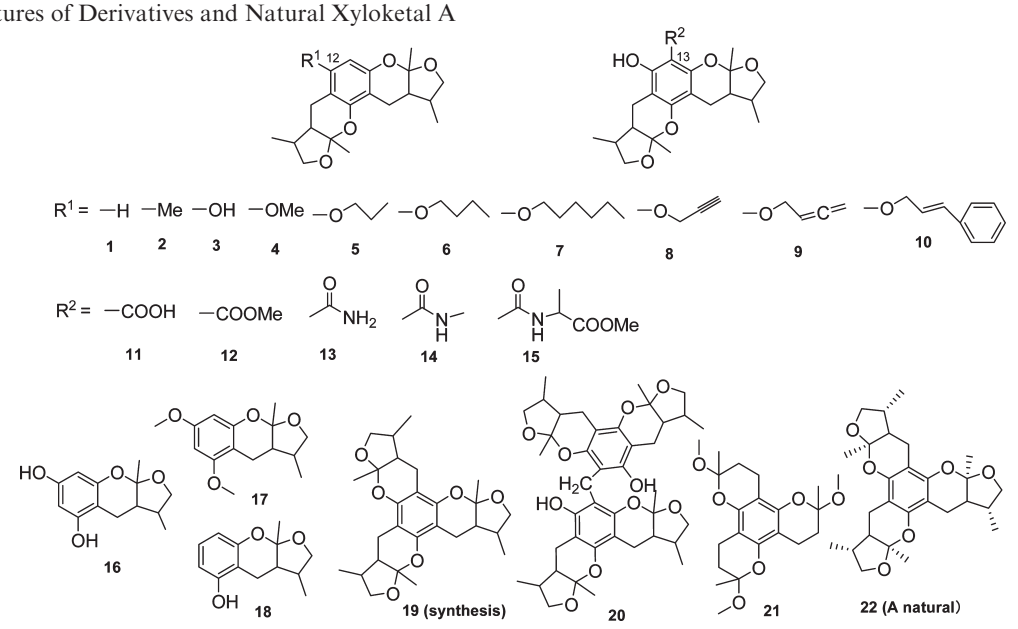
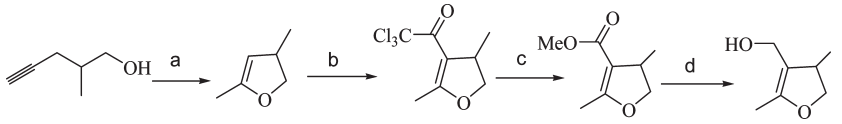


Chart 2. Structures of Derivatives and Natural Xyloketal A



Scheme 1[a]



[a] Reagents and conditions: (a) $NaNH_2$, 170 °C, 12 h; (b) Cl_3CCOCl, CH_2Cl_2, pyridine, 170 °C, 30 min; (c) MeOH, $NaHCO_3$, reflux, 6 h; (d) $LiAlH_4$, Et_2O, 2 h.

structure−activity relationship of these naturally occurring compounds. Therefore, we have keenly become interested in designing and synthesizing novel chemopreventive agents by using xyloketal B as lead compound. Because of the complexity of stereoselective synthesis of xyloketals, it is difficult to provide a great amount of the optically pure samples for biological activity evaluation; we decided to start the studies from racemic xyloketal B. This paper describes the synthesis and biological activities of a new series of xyloketal derivatives, **1**−**21** (Chart 2), bearing a variety of substituents at the 12-position and 13-position of the aromatic ring of (±)-xyloketal B (**3**), as well as different segments of the tetrahydrofuranobenzopyran ring. The aim was to assess the effects of the modifications at these positions on pharmacological activities. The biological activities of **1**−**21** together with natural xyloketal A (**22**) were initially evaluated with a vasorelaxing assay in rat thoracic aorta. The mechanisms underlying vasorelaxing activities were first explored using endothelium-intact aorta rings. The potentially endothelium-independent vasorelaxing activities of compounds were further studied in denuded rings. And then protective actions against oxLDL-induced endothelial oxidative injury and promoting NO production in human umbilical vein endothelial cells (HUVECs) of these derivatives have been investigated. In addition, zebrafish angiogenesis screen was utilized to examine the angiogenic activity of these compounds. Our results demonstrated that these synthetic xyloketal derivatives are promising drug candidates in the treatment of cardiovascular diseases.

Chemistry

The synthetic route of **1**−**21** is shown in Schemes 1−6. The core intermediate 4-dimethyl-3-hydroxymethyl-4,5-dihydrofuran was prepared from 2-methyl-4-pentyn-1-ol. An intramolecular addition reaction gave racemic 2,4-dimethyl-4,5-dihydrofuran in 75% yield. Then substitution by trichloroacetyl chloride afforded 2,4-dimethyl-3-trichloroactyl-4,5-dihydrofuran in 45% yield. Methanolysis and then reduction gave the core intermediate 2,4-dimethyl-3-hydroxymethyl-4,5-dihydrofuran in 97% yield in two steps (Scheme 1).

Compounds **1**−**3** were obtained through a boron trifluoride diethyl etherate promoted electrophilic aromatic substitution reaction between 2,4-dimethyl-3-hydroxymethyl-4,5-dihydrofuran and the corresponding phenol[24−26] (Scheme 2), and **3** is the racemate of natural xyloketal B. Compound **4** was readily

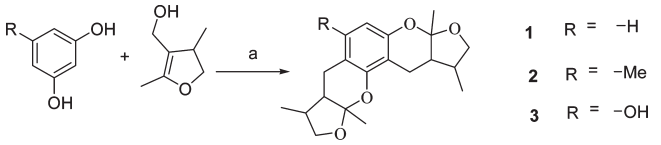
synthesized by a substitution reaction of **3** with Me_2SO_4. The synthesis of monosubstituted xyloketal B derivatives **5**−**10** was carried out by Williamson reaction of various substituted alkyl bromides and **3** in the presence of anhydrous K_2CO_3 in yields of 87.3−94.4% (Scheme 3).

The xyloketal B acid (**11**) was synthesized by electrophilic aromatic substitution reaction shown in Scheme 4. Compound **12** was obtained by methylation of **11** with CH_2N_2 in ether in quantitative yield. **13** and **14** were easily obtained by ammonolysis of **12** in high yield. The amino acid derivative **15** was prepared via coupling of **11** with H-Ala-OMe·HCl in the presence of castor reagent and *N*,*N*-diisopropylethylamine (DIEA) in 89% yield.

Compounds **16** and **18** were also prepared by electrophilic aromatic substitution reaction with phloroglucinol and resorcinol, respectively. Compound **17** was obtained through a substitution reaction of Me_2SO_4 with **16**. Dimer xyloketal B derivative **20** was synthesized from **3** by the Lederer−Manasse reaction in the presence of polyformaldehyde.[16] The tris adduct, racemic chroman **21** was prepared by a Michael-type reaction in excellent 95% yield; methanol was both reagent and solvent.

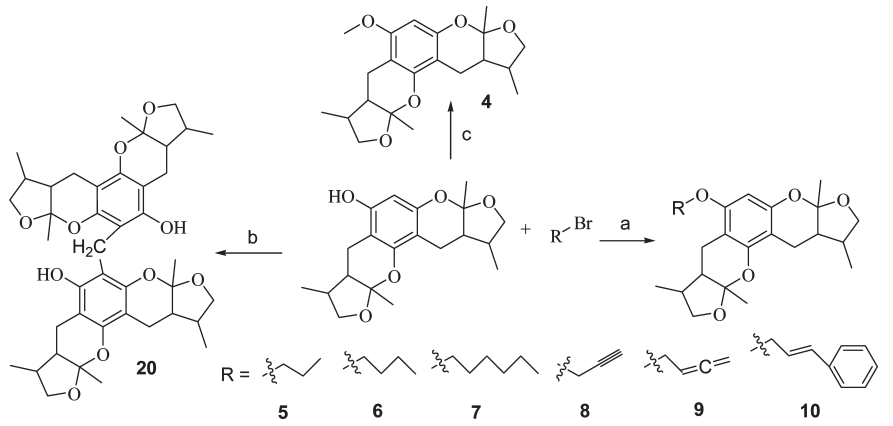
No asymmetric synthesis was employed, and all synthetic compounds were a mixture of stereoisomers. Polarimeter test of optical rotation showed that all the compounds are racemic. The stereochemistry of these xyloketal derivatives was complicated. In principle, the two oxygen-containing pyran and furan rings B and C can be connected in a cis or trans fashion. The methyl group at C-5 or C-5′ could be cis or trans with respect to the stereogenic centers at the junction at C-2 or C-2′ and C-6 or C-6′. Many previous studies[28,29] indicated that in all subsequent condensations leading to xyloketal derivatives, rings B and C were always cis orientated. Therefore, it was possible that two sets of stereoisomers can be formed for **3**. The very close relationship of the isomers was evident in NMR. The hydrogen atom at the 13-position and the carbon atoms of benzene ring showed that the ratio of isomers of **3** is about 1:1. Compounds **4** and **5**−**10** also showed two isomers; the ratio is also about 1:1, which was detected by NMR. For the 13-position xyloketal B derivatives, the very

Scheme 2[a]



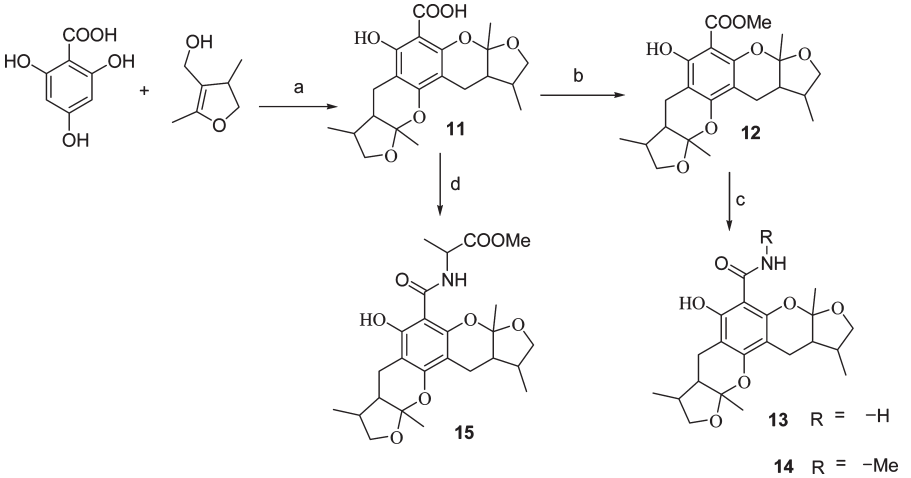
[a] Reagents and conditions: (a) $MgSO_4$, $BF_3 \cdot Et_2O$, Et_2O, 0 °C to room temp.

Scheme 3[a]



[a] Reagents and conditions: (a) K_2CO_3, acetone, room temp; (b) polyformaldehyde, 2 N HCl, room temp; (c) Me_2SO_4, K_2CO_3, acetone, room temp.

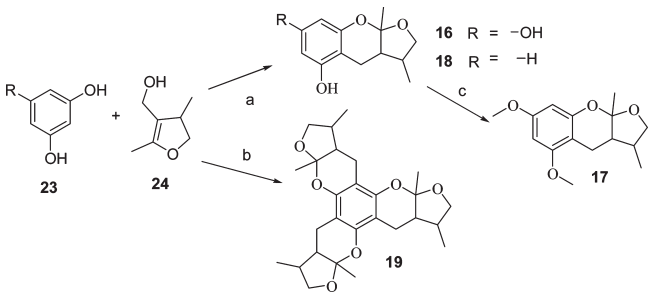
Scheme 4[a]



[a] Reagents and conditions: (a) $MgSO_4$, $BF_3 \cdot Et_2O$, Et_2O, 0 °C to room temp; (b) CH_2N_2, Et_2O, 0 °C; (c) amine, acetone, 50 °C; (d) Bop, Ala-OMe.HCl, DIEA, CH_2Cl_2, room temp.

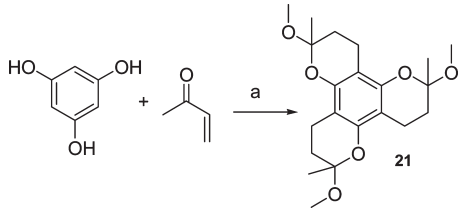
close relationship of the isomers was evident in NMR. The hydrogen atoms of methyl and the carbon atoms at benzene were in a ratio of about 5:4 for compounds **11**−**15**.

Scheme 5[a]



[a] Reagents and conditions: (a) **23**/**24** (1:2), $MgSO_4$, $BF_3 \cdot Et_2O$, Et_2O, 0 °C to room temp; (b) **23**/**24** (1:4), $MgSO_4$, $BF_3 \cdot Et_2O$, Et_2O, 0 °C to room temp; (c) Me_2SO_4, K_2CO_3, acetone, room temp.

Scheme 6[a]



[a] Reagents and conditions: (a) $MgSO_4$, *p*-TsOH, MeOH, 0 °C to room temp.

Vasorelaxation Activity in Rat Thoracic Aorta

Vasorelaxation activities of xyloketal derivatives were evaluated in rat isolated thoracic aorta from which the potency half maximal effective concentration (EC_{50}) were determined. Endothelium intact and denuded rings were pretreated with an isotonic, high potassium physiological salt solution (KPSS, 60 mM KCl) to similar levels, and the concentration−response curves to various xyloketals were generated allowing quantification of the potency and efficacy.

The results (Figure 1, Table 1) showed that most of the examined compounds had moderate to strong vasorelaxing activities. Compounds **9**, **12**, **13**, **14**, **15**, and **19** showed higher vasorelaxing activities, with EC_{50} of 3−12 μM (endothelium-intact) and 10−15 μM (endothelium-denuded), than the lead compound **3** with EC_{50} of 19 μM (endothelium-intact) and 41 μM (endothelium-denuded). Vasorelaxing activities of most compounds were stronger in endothelium intact ring than in denuded rings, suggesting that the vasorelaxing activities of these compounds may be mediated by endothelium-dependent mechanisms and to a lesser degree by endothelium-independent mechanisms.[30−32]

Structure analysis revealed that the methyl, alkynyl, and allenic ether groups substituted at the 12-position of **3**, like **4**, **8**, and **9**, improved the vasodilation activity and that a large functional group at the 12-position was unfavorable. Moreover, dehydroxyl (**1**) and methyl (**2**) xyloketal B reduced vasorelaxation activity. Substituents at the 13-position of **3** were more important than the unsubstituted xyloketal B for vasodilation. **12**−**15** were the preferred compounds in terms of vasorelaxing activities. With the increase of tetrahydrofuranobenzopyran rings in **16**, **3**,

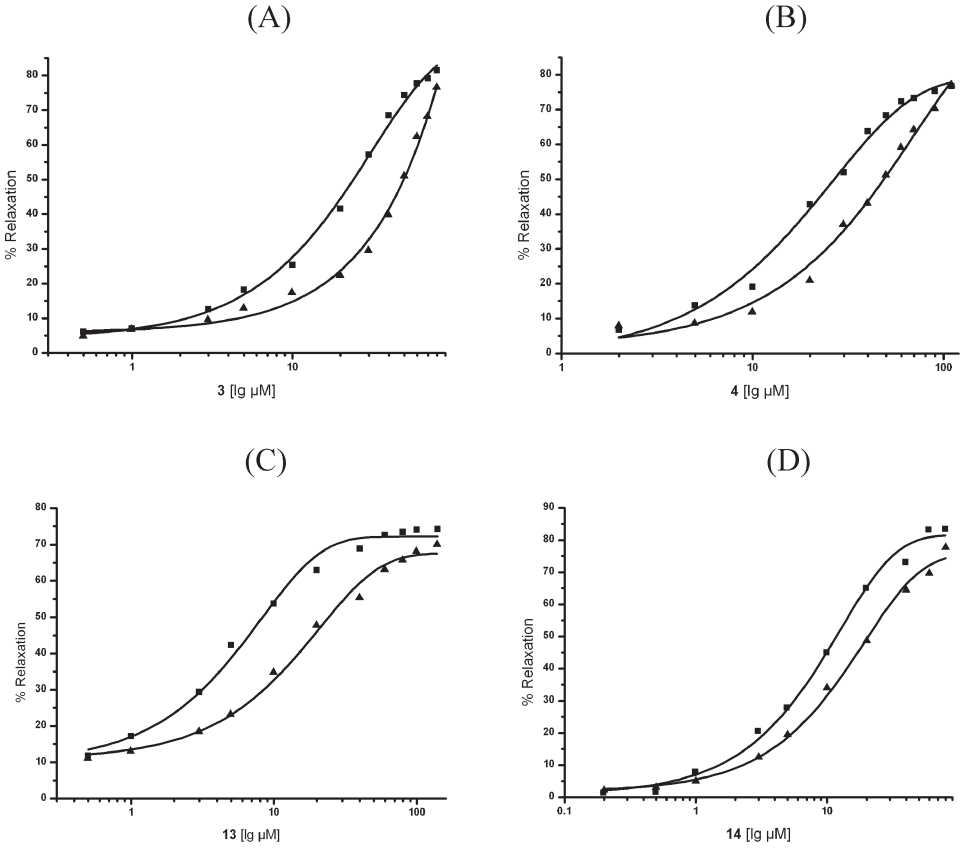
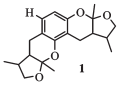
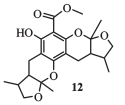
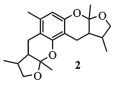
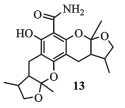
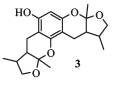
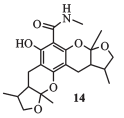
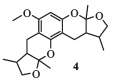
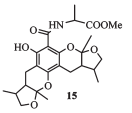
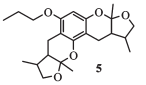
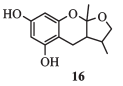
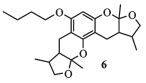
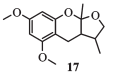
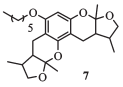
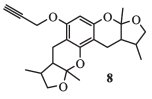
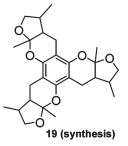
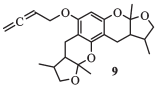
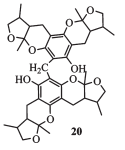
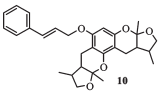
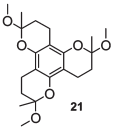
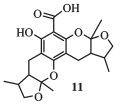
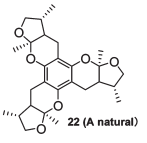


Figure 1. Vascular relaxation effects of (A) **3**, (B) **4**, (C) **13**, and (D) **14** on rat isolated aortic rings precontracted with 60 mM KCl. Data are expressed as ±MSD. Results were obtained from six independent experiments (endothelium-intact vs endothelium-denuded; $p < 0.05$ indicates there are endothelium-dependent activities): (■) endothelium-intact; (▲) endothelium-denuded.

Table 1. Comparison of the EC_{50} to Different Xyloketals in Endothelium-Intact and Endothelium-Denuded Thoracic Aortic Rings from Rats

No.	EC_{50} [a] (μM)		No.	EC_{50} [a] (μM)	
	endothelium-intact	endothelium-denuded		endothelium-intact	endothelium-denuded
 1	55 ± 5	72 ± 6	 12	8 ± 2	14 ± 2
 2	40 ± 3	52 ± 2	 13	3 ± 2*	10 ± 3
 3	19 ± 2*	41 ± 4	 14	9 ± 3*	15 ± 2
 4	17 ± 2*	33 ± 3	 15	5 ± 1*	13 ± 2
 5	43 ± 4	77 ± 5	 16	39 ± 3*	66 ± 5
 6	111 ± 5	> 200	 17	37 ± 3*	80 ± 3
 7	> 200	> 200	 18	128 ± 5	> 200
 8	23 ± 2	22 ± 3	 19 (synthesis)	6 ± 2*	11 ± 2
 9	12 ± 2	11 ± 2	 20	87 ± 4	> 200
 10	64 ± 6	> 200	 21	65 ± 5	118 ± 6
 11	> 200	> 200	 22 (A natural)	12 ± 4*	31 ± 2

[a] Each experiment was independently performed six times and expressed as mean values: (*) vs endothelium-denuded group, $p < 0.05$, has endothelium-dependent activities.

and **19**, their vasorelaxing activities were further enhanced. However, **20** with four tetrahydrofuranobenzopyran rings did not enhance their activities perhaps because of its steric hinerance. Tris adduct chroman **21** reduced the vasorelaxing activity. These data suggested that tetrahydrofuranobenzopyran ring was an important segment for good vasorelaxing

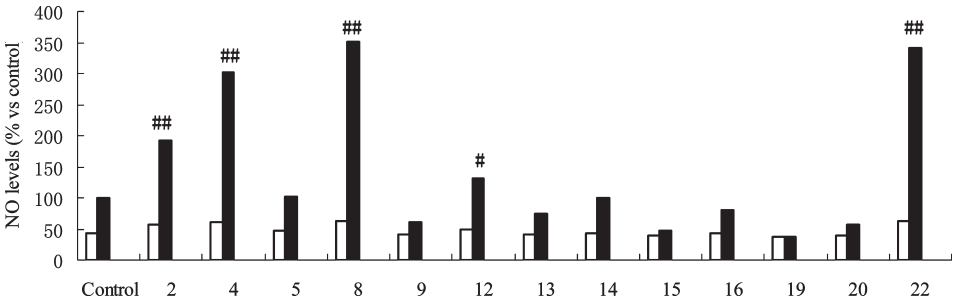


Figure 2. Effects of xyloketals on endothelial NO generation of human umbilical vein endothelial cells (HUVECs). HUVECs were harvested in the presence of 20 μM xyloketals (black bars) or in the presence of 20 μM xyloketals plus 100 μM L-NAME (white bars). The levels of the nitric oxide (NO) derivative nitrite were determined together with the Griess reaction. Values are the mean ± SD (n = 6): (#) vs control group, $p < 0.05$; (##) vs control group, $p < 0.01$.

activities and might be a determinant factor for vasorelaxing activities.

Effect of Xyloketals on NO Release of Endothelial Cells

NO has been shown to be an endothelium-derived relaxing factor (EDRF), and its impairment contributes to cardiovascular disease.[33] Our data demonstrated that most of xyloketals had good vasorelaxing activities and their vasorelaxing action was mainly endothelium dependent. Therefore, we further investigated whether xyloketals affect endothelial NO generation of HUVECs. Generally, HUVECs were preincubated with 20 μM xyloketals for 24 h, and levels of the nitric oxide (NO) derivative nitrite were determined together with the Griess reaction. Compounds **2**, **4**, **8**, and **22** significantly enhanced NO release in HUVECs, as shown in Figure 2. N(G)-nitro-L-arginine methyl ester (L-NAME) (100 μM), an eNOS inhibitor, was used to determine if the increased production of NO came from eNOS. Xyloketal-induced NO release was completely inhibited by L-NAME, suggesting that the NO production induced by xyloketals may be mainly derived from eNOS. Furthers studies will be conducted to reveal the complex mechanisms involved in the vasorelaxing action of xyloketal derivatives.

Cell Viability Assay

Cell viability was assessed by the mitochondrial tetrazolium assay (MTT) in HUVECs. Cells were treated with 200 μM xyloketals or vehicle for 24 h. MTT solution (10 μL/well, 5 μg/mL) was added and processed to examine cell viability. Optical density was read at 570 nm by a Biotek Elx-800 plate reader. At tested concentrations, all the xyloketals showed no significant effects (> 200 μM) on cell viability (data not shown).

Xyloketals Protect Endothelial Cells against oxLDL-Induced Injury

OxLDL-induced endothelial cell injury was an early key event in the pathogenesis of atherosclerosis and many other cardiocerebrovascular diseases.[34] OxLDL-induced apoptosis mainly through oxidative stress.[35,36] To examine the inhibitory effects of xyloketals on oxLDL-induced injury of HUVECs, HUVECs were preincubated with 20 μM xyloketals for 30 min, respectively, followed by native LDL, and 150 μM oxLDL was then added to the medium. After incubation for 24 h, cell viability was determined using MTT reduction assay. As shown in Figure 3, all examined compounds significantly attenuated oxLDL-induced endothelial oxidative injury, both morphological changes and decrease in cellular viability

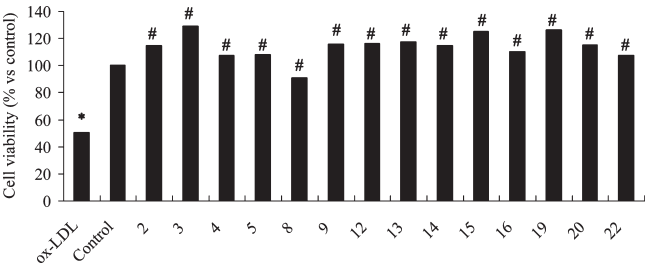


Figure 3. Effects of xyloketals on oxLDL-induced injury of HUVECs. HUVECs were preincubated with 20 μM xyloketals for 30 min, and 150 μM oxLDL was then added to the medium. After incubation for 24 h, cell viability was determined using MTT reduction assay. Values are the mean ± SD (n = 6): (*) vs control group; (#) vs oxLDL treated group, $p < 0.01$.

($p < 0.01$). With an increase of the tetrahydrofuranobenzopyran ring, the antioxidative actions of **16**, **3**, and **19** were slightly improved. With the replacement of a hydroxyl group at the 12-position of **3** with ether or methyl, such as **4**, **5**, **8**, **9**, and **2**, the antioxidative action was slightly decreased relatively to **3**. Therefore, the hydroxyl group at the 12- position of **3** is important but not the necessary functional group for the protective action against OxLDL-induced endothelial oxidative injury.

Angiogenic Activity in Zebrafish Angiogenesis Screen

Many zebrafish organs are remarkably similar to their human counterparts at anatomical, physiological, and molecular levels.[37−39] This is consistent with the fact that most human genes have orthologues in zebrafish.[40] The transparency of zebrafish embryos permits direct visualization of primordial germ cells (PGC) development, a coordinated sequence of events involving cell fate specification, proliferation, and directed migration,[41] and also is used to examine the effect of different compounds on embryonic angiogenesis.[42] Generally, zebrafish embryos were obtained by natural pairwise mating and raised at 28.5 °C in embryo water (0.2 $g \cdot L^{-1}$ of Instant Ocean Salt in distilled water). Compounds were added to embryo water from the shield stage (about 6 h).

Zebrafish embryos incubation with **3** at 0.1, 1, and 10 μM significantly enhanced the angiogenesis in zebrafish embryos in a concentration dependent manner (Figure 4). When zebrafish embryos were incubated with xyloketals at a final concentration of 100 μM for 72 h, the other 12 xyloketals (**2**, **4**, **5**, **8**, **9**, **12**−**16**, **19**, **20**, and **22**) also exhibited angiogenic action in zebrafish embryos. Compounds **9**, **12**, **14**, **16**, **20**, and **21** had stronger angiogenic action compared with **3** ($p < 0.05$, Figure 5). It is

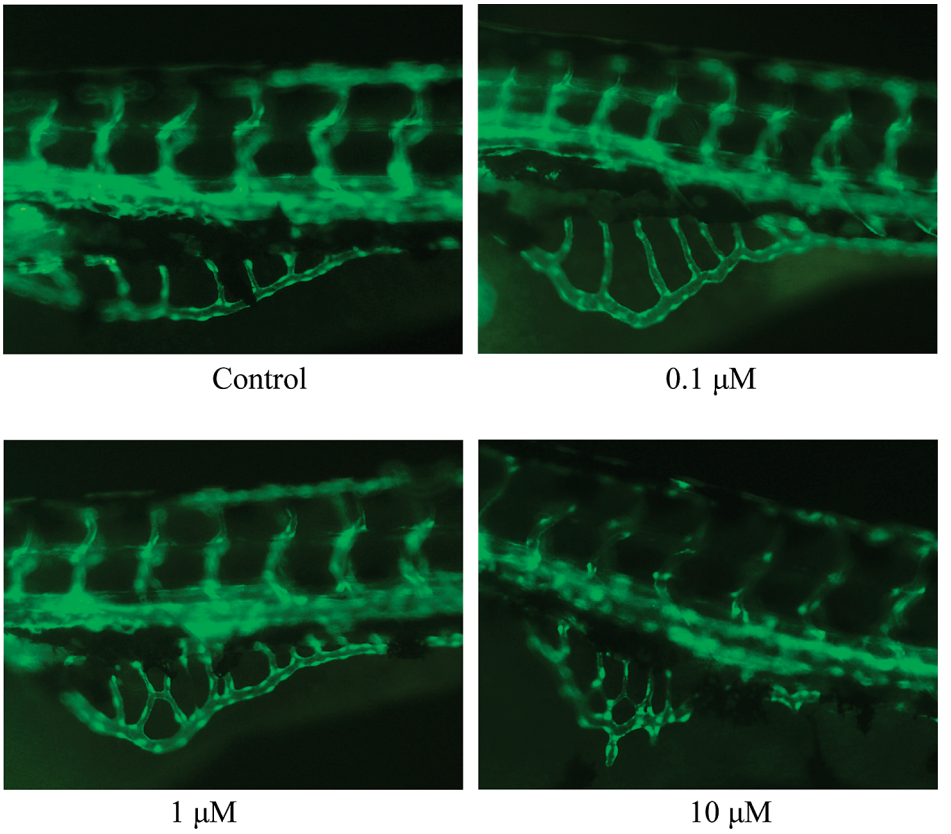


Figure 4. Effect of **3** on angiogenesis in zebrafish. Zebrafish embryos were incubated with **3** at 0.1, 1, and 10 μM for 72 h. Results were obtained from three independent experiments ($p < 0.05$).

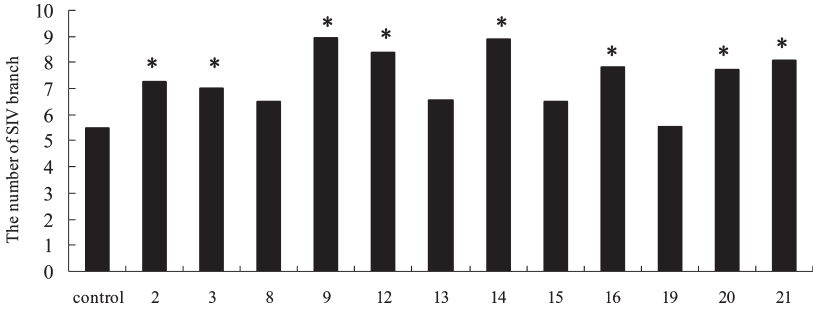


Figure 5. Effect of xyloletals on angiogenesis in zebrafish. Zebrafish embryos were incubated with xyloketals at a final concentration 100 μM for 72 h. Data are expressed as the mean ± SD. Results were obtained from three independent experiments (vs control group, $p < 0.05$).

noted that there was a discrepancy in the effective dose range between vasorelaxation and zebrafish assays. This discrepancy may be due to the different techniques used in these two assays. Given that zebrafish angiogenesis screen is an in vivo assay and the permeability of zebrafish embryos is relatively low, the efficacy of compounds on angiogenesis must be tested in a high-dose range. Alternatively, the dose difference may be due to the different mechanisms underlying the effect of xyloketal derivatives on vasorelaxation and angiogenesis. Angiogenesis is a very complex process. In addition to the vasodilation of preexisting capillaries, angiogenesis includes the proliferation, migration, differentiation, and changes in vascular permeability. Nevertheless, our data suggest that xyloketal derivatives are powerful vasoactive compounds and have an angiogenic effect at high doses.

Conclusion

A novel series of xyloketal derivatives were successfully synthesized. These compounds can relax blood vessels, enhance angiogenesis, promote endothelial cell NO production, and attenuate oxLDL-induced oxidative stress. Interestingly, the vasorelaxing action of these derivatives is mediated by both endothelium-independent and endothelium-dependent mechanisms. Furthermore, eNOS-derived NO plays a critical role in endothelium-dependent vasodilatation of xyloketal derivatives. Therefore, natural xyloketals and their derivatives will be the promising compounds for further evaluation in the treatment of cardiovascular diseases. The efforts aiming at exploiting further biological activities and their action mechanism of xyloketal derivatives are actively continuing in our laboratories.

Experimental Section

Chemistry. All reagents and solvents were of commercial quality and used without further purification. ^{1}H and ^{13}C NMR data were recorded on a Varian Inova 400 MB NMR spectrometer operating at 400 and 125 MHz for ^{1}H and ^{13}C,

respectively. All chemical shifts are in ppm (δ) with respect to tetramethylsilane (TMS) as internal standard, and coupling constants (J) are in Hz. Mass spectra were obtained on DSQ (low resolution mass spectrometer) and MAT95XP (high resolution mass spectrometer) instruments. Melting points were determined on an X-4 micromelting point apparatus and were uncorrected. The purities (> 95%) of all target compounds were checked by HPLC using a LC-2010c equipped with UV detector. Samples were injected on a Merck Purospher STAR RP-18e 125 cm × 4.6 mm (5 μm) column equipped with a Merck Lichrocart precolumn (Merck, Germany). Analyses were run according to A, B, or C solvent system (see below). The traces were recorded at 220 nm, and the column temperature is 40 °C. A table summarizing the purity and spectra of target compounds are given in Supporting Information. Solvent system A consists of the following: binary system with 0.1% (v/v) aqueous formic acid and methanol 15:75 (v/v), flow rate of 0.6 mL/min. Solvent system B consists of the following: binary system with 0.1% (v/v) aqueous formic acid and methanol 15:75 (v/v), flow rate of 0.8 mL/min. Solvent system C consists of the following: binary system with 0.1% (v/v) aqueous formic acid and acetonitrile 20:80 (v/v), flow rate of 0.8 mL/min.

NMR data are given in Supporting Information.

4-Dimethyl-3-hydroxymethyl-4,5-dihydrofuran. The 2-methyl-4-pentyn-1-ol (5.0 g, 0.051 mol) and sodium amide (0.28 g, 0.007 mol) were heated at 170 °C for 12 h. Direct distillation of the reaction mixture at atmospheric pressure afforded the corresponding dihydrofuran as a colorless oil (3.75 g, 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.038 (d, J = 6.6 Hz, 3H), 1.772 (m, 3H), 3.008 (m, 1H), 3.831 (t, J = 7.5 Hz, 1H), 4.382 (t, J = 9.0 Hz, 1H), 4.546 (s, 1H).

2,4-Dimethyl-3-trichloroactyl-4,5-dihydrofuran. To a solution of the above dihydrofuran (3.0 g, 0.031 mol) and pyridine (4.9 g, 0.062 mol) in 10 mL of dichloromethane at 170 °C was added trichloroacetyl chloride (10.2 g, 0.056 mol) dropwise. The reaction mixture was stirred for 30 min, and then a saturated aqueous solution of sodium bicarbonate (10 mL) was added. The resultant mixture was diluted with dichloromethane (20 mL), washed with water (2 × 10 mL), dried over anhydrous sodium sulfate, and concentrated in vacuo. Flash chromatography afforded the title compound (3.4 g, 45% yield) as a pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.295 (d, J = 6.6 Hz, 3H), 2.299 (s, 3H), 3.582 (m, 1H), 4.192 (d, J = 9.0 Hz, 1H), 4.472 (d, J = 8.7 Hz, 1H).

2,4-Dimethyl-3-hydroxymethyl-4,5-dihydrofuran. To a solution of 2,4-dimethyl-3-trichloroactyl-4,5-dihydrofuran (1.0 g, 4.16 mmol) in methanol was added 25 mL of sodium bicarbonate (0.13 g, 1.58 mmol), and the resultant mixture was heated at reflux for 6 h. The mixture was cooled to room temperature. Then a saturated aqueous solution of sodium bicarbonate (10 mL) was added. The resultant mixture was diluted with ether (100 mL), washed with water (2 × 10 mL), dried over anhydrous sodium sulfate, and concentrated in vacuo to afforded the title compound (0.63 g, 97% yield) as a pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.81 (d, J = 6.0 Hz, 3H), 2.183 (s, 3H), 3.220 (m, 1H), 3.711 (s, 3H), 3.990 (m, 1H), 4.444 (t, J = 9.0 Hz, 1H).

A solution of the above methyl ester (1.49 g, 9.6 mmol) in ether (5 mL) was added to a suspension of lithium aluminum hydride (1.15 g, 30 mmol) in ether (30 mL) at 0 °C. The resultant mixture was allowed to warm to room temperature over 2 h, and then aqueous solution of sodium hydroxide (2 M) and an additional quantity of water were then added. The resultant mixture was filtered and washed with ether (3 × 50 mL), and the combined filtrates were concentrated in vacuo to afford the unstable alcohol as a pale-yellow oil (1.18 g, 96% yield). This material was immediately diluted with ether and used for the next reaction.

Dehydroxy Xyloketal B (1). To a stirred mixture of resorcin (62 mg, 0.56 mmol), 2,4-dimethyl-3-hydroxymethyl-4,5-dihydrofuran (215 mg, 1.68 mmol), and anhydrous magnesium sulfate (140 mg) in ether,[24−26] boron trifluoride diethyl etherate (79.2 mg, 0.56 mmol) was added at 0 °C. The mixture was allowed to warm to room temperature. The resultant mixture was stirred for 24 h at room temperature. The reaction mixture was then diluted with 100 mL of ethyl acetate, washed with a saturated aqueous solution of ammonium chloride 1 × 20 mL, brine 1 × 20 mL, dried over anhydrous magnesium sulfate, and concentrated in vacuo. Purification by flash chromatograghy using petroleum ether and ethyl acetate as the eluant afforded the title compound (155.6 mg, 84.2%) as a white solid; mp 134−135 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.732 (s, 1H), 6.281(d, J = 5.6 Hz, 1H), 4.103 (t, J = 8.0 Hz, 2H), 3.444 (t, J = 8.0 Hz, 2H), 2.908 (d, J = 6.0 Hz, 1H), 2.875 (d, J = 6.0 Hz, 1H), 2.658 (d, J = 8.0 Hz, 1H), 2.620(d, J = 8.0 Hz, 1H), 2.081 (m, 2H), 1.857 (m, 2H), 1.489 (s, 3H), 1.477 (s, 3H), 1.016(d, J = 7.0 Hz, 3H), 1.005(d, J = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 152.46, 152.39, 128.96, 111.47, 111.29, 107.58, 107.43, 104.70, 73.75, 48.60, 48.46, 34.94, 29.54, 23.93, 23.87, 23.42, 23.18, 15.94. HREIMS m/z 330.1825 (calculated for $C_{20}H_{26}O_4$, 330.1826).

Methyl Xyloketal B (2). The title compound was obtained from 5-methylresorcinol and 2,4-dimethyl-3-hydroxymethyl-4,5-dihydrofuran with a procedure similar to that for compound **1** as a white solid. Yield, 89.2%; mp 156−157 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.247 (s, 1H), 4.168 (t, J = 8.5 Hz, 2H), 3.400 (t, J = 8.5 Hz, 2H), 2.735 (d, J = 8.0 Hz, 4H), 2.131 (m, 2H), 2.124 (s, 3H), 1.910 (m, 2H), 1.488 (s, 3H), 1.486 (s, 3H), 1.055(d, J = 7.0 Hz, 3H), 1.051(d, J = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$): 152.34, 152.30, 135.08, 110.04, 109.97, 106.68, 106.60, 103.18, 73.86, 48.35, 35.50, 35.45, 22.74, 22.65, 22.41, 16.04, 16.02, 14.73. HREIMS m/z 344.1981 (calculated for $C_{21}H_{28}O_4$, 344.1982).

(±)-Xyloketal B (3). The title compound was obtained from phloroglucinol and 2,4-dimethyl-3-hydroxymethyl-4,5-dihydrofuran with a procedure similar to that for compound **1**. Yield, 93%; white solid; mp 201−203 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.210 (s, 1H), 4.203 (t, J = 8.0 Hz, 1H), 4.152 (t, J = 8.0 Hz, 1H), 3.546 (t, J = 8.0 Hz, 1H), 3.484 (t, J = 8.0 Hz, 1H), 2.881 (dt, J = 1.2, 5.6 Hz, 1H), 2.838 (dt, J = 1.2, 5.6 Hz, 1H), 2.722 (m, 1H), 2.602 (dt, J = 5.6, 17.2 Hz, 1H), 2.151 (m, 2H), 1.922 (m, 2H), 1.541 (s, 3H), 1.519 (s, 3H), 1.072 (d, J = 6.8 Hz, 3H), 1.027 (d, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 153.49, 151.99, 151.62, 107.93, 107.67, 98.61, 98.58, 96.08, 73.97, 73.87, 47.98, 47.55, 35.50, 35.37, 23.45, 23.07, 18.69, 16.09, 15.78. HREIMS m/z 346.1776 (calculated for $C_{20}H_{26}O_5$, 346.1775).

Xyloketal B Methyl Ether (4). To a stirred solution of K_2CO_3 (64 mg, 0.46 mmol) and (80 mg, 0.23 mmol) xyloketal B (**3**) in acetone (5 mL), Me_2SO_4 (58 mg, 0.46 equiv) was added. The resulting reaction mixture was stirred for 24 h and then poured into water. The aqueous layer was extracted with Et_2O, 3 × 20 mL. The combined organic extracts were washed with saturated brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. Purification by flash chromatograghy using petroleum ether and ethyl acetate as the eluant afforded the title compound (60.7 mg, 73.2% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.210 (s, 1H), 4.168 (m, 2H), 3.750 (s, 3H), 3.503 (m, 2H), 2.872 (m, 2H), 2.658 (t, J = 6.4 Hz, 2H), 2.122 (m, 2H), 1.888 (m, 2H), 1.508 (s, 3H), 1.497 (s, 3H), 1.061 (d, J = 6.8 Hz, 3H), 1.054 (d, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 156.85, 152.16, 151.58, 107.49, 107.30, 99.27, 99.16, 91.85, 73.98, 55.22, 47.58, 47.44, 35.33, 29.63, 22.91, 22.68, 18.67, 18.61, 16.04, 15.88. HREIMS m/z 360.1934 (calculated for $C_{21}H_{28}O_5$, 360.1932).

Xyloketal B Propyl Ether (5). The title compound was obtained from **3** and bromopropane with a procedure similar to that for compound **4**. Yield, 87.3%; white solid; mp 67−68 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.992 (s, 1H), 4.175 (apparent t, J = 8.4 Hz, 2H), 3.846 (m, 2H), 3.400 (apparent t, J = 8.4 Hz, 2H), 2.839 (dd, J = 17.4 1.2 Hz, 1H), 2.828 (dd, J = 17.4 1.2 Hz, 1H), 2.646 (m, 2H), 2.125 (m, 2H), 1.883 (m, 2H), 1.781 (m, 2H), 1.502 (s, 3H), 1.497 (s, 3H), 1.061 (d, J = 6.4 Hz, 3H), 1.050 (d,

J = 6.4 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 156.27, 152.06, 151.42, 107.43, 107.14, 99.49, 98.98, 92.70, 74.05, 69.32, 47.66, 47.53, 35.38, 29.69, 22.97, 22.84, 22.59, 18.77, 18.63, 16.09, 15.76, 10.65. HREIMS m/z 388.2245 (calculated for $C_{23}H_{32}O_5$, 388.2244).

Xyloketal B Butyl Ether (6). The title compound was obtained from **3** and 1-bromobutane with a procedure similar to that for compound **4**. Yield, 91.1%; white solid; mp 124−125 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.992 (s, 1H), 4.171 (apparent t, J = 8.4 Hz, 2H), 3.882 (t, J = 6.4 Hz, 2H), 3.519 (t, J = 8.4 Hz, 2H), 2.822 (m, 2H), 2.638 (m, 2H), 2.119 (m, 2H), 1.879 (m, 2H), 1.749 (m, 2H), 1.499 (s, 3H), 1.484 (s, 3H), 1.252 (m, 2H), 1.058 (d, J = 6.4 Hz, 3H), 1.029 (d, J = 6.4 Hz, 3H), 0.968 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 156.37, 152.07, 151.43, 107.54, 107.32, 99.48, 98.97, 92.64, 74.04, 67.57, 47.65, 47.53, 35.37, 31.32, 29.68, 22.97, 22.74, 19.34, 18.72, 18.56, 16.09, 15.93, 13.86. HREIMS m/z 402.2396 (calculated for $C_{24}H_{34}O_5$, 402.2397).

Xyloketal B Hexyl Ether (7). The title compound was obtained from **3** and 1-bromohexane with a procedure similar to that for compound **4**. Yield, 89.4%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.985 (s, 1H), 4.163 (q, J = 8.5 Hz, 1H), 4.154 (q, J = 8.5 Hz, 1H), 3.872 (t, J = 6.5 Hz, 2H), 3.507 (q, J = 8.5 Hz, 1H), 3.502 (q, J = 8.5 Hz, 1H), 2.818 (apparent t, J = 8.0 Hz, 2H), 2.632 (m, 2H), 2.100 (m, 2H), 1.869 (m, 2H), 1.754 (m, 2H), 1.491 (s, 3H), 1.485 (s, 3H), 1.446 (m, 2H), 1.327 (m, 4H), 1.052 (d, J = 6.0 Hz, 3H), 1.023 (d, J = 6.0 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 156.37, 152.07, 151.43, 107.54, 107.32, 99.48, 98.9, 92.64, 74.04, 67.57, 47.65, 47.53, 35.37, 31.32, 29.68, 22.97, 22.74, 19.37, 18.72, 18.56, 16.09, 15.93, 13.86. HREIMS m/z 430.2713 (calculated for $C_{26}H_{38}O_5$, 430.2714).

Xyloketal B Propargyl Ether (8). The title compound was obtained from **3** and propargyl bromide with a procedure similar to that for compound **4**. Yield, 93.4%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.110 (s, 1H), 4.619(d, J = 2.5 Hz, 2H), 4.160 (t, J = 8.0 Hz, 2H), 3.510 (t, J = 8.0 Hz, 2H), 2.831 (m, 2H), 2.650 (m, 2H), 2.511 (t, J = 2.5 Hz, 1H), 2.121 (m, 2H), 1.886 (m, 2H),1.400 (s, 3H), 1.489 (s, 3H), 1.050 (d, J = 7.0 Hz, 3H), 1.037 (d, J = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 155.02, 152.14, 151.78, 107.65, 107.23, 100.18, 99.92, 93.28, 78.74, 75.28, 74.00, 56.01, 47.59, 47.49, 35.45, 35.35, 22.97, 22.73, 18.72, 18.56, 16.08, 15.86. HREIMS m/z 384.1929 (calculated for $C_{23}H_{28}O_5$, 384.1931).

Xyloketal B Allenic Ether (9). The title compound was obtained from **3** and 4-bromobuta-1,2-diene with a procedure similar to that for compound **4**. Yield, 87.8%; light-yellow solid; mp 38−39 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.011 (s, 1H), 5.383 (tt, J = 6.8 Hz, 1H), 4.856 (dt, J = 6.4, 2.4 Hz, 2H), 4.488 (dt, J = 6.8, 2.4 Hz, 2H), 4.181 (apparent t, J = 8.0 Hz, 2H), 3.508 (m, 2H), 2.837 (dd, J = 17.6, 5.6 Hz, 2H), 2.645 (m, 2H), 2.112 (m, 2H), 1.883 (m, 2H), 1.400 (s, 3H), 1.492 (s, 3H), 1.062 (d, J = 6.8 Hz, 3H), 1.036 (d, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 209.17, 155.62, 152.11, 151.68, 107.55, 107.32, 99.81, 99.52, 93.19, 87.24, 76.46, 73.98, 65.72, 47.62, 47.53, 35.45, 35.36, 22.95, 22.71, 18.80, 18.63, 15.98, 15.74. HREIMS m/z 398.2087 (calculated for $C_{24}H_{30}O_5$, 398.2088).

Xyloketal B Cinnamyl Ether (10). The title compound was obtained from **3** and cinnamyl bromide with a procedure similar to that for compound **4**. Yield, 94.4%; light-yellow solid; mp 57−58 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.414 (d, J = 7.2 Hz, 2H), 7.321 (m, 2H), 7.247 (m, 2H), 6.715 (d, J = 16.0 Hz, 1H), 6.408 (dt, J = 16.0, 5.6 Hz, 1H), 6.065 (s, 1H), 4.615 (dd, J = 5.6, 0.8 Hz, 2H), 4.179 (apparent t, J = 8.4 Hz, 2H), 3.533 (apparent t, J = 8.4 Hz, 2H), 2.858 (m, 2H), 2.677 (m, 2H), 2.130 (m, 2H), 1.899 (m, 2H), 1.508 (s, 3H), 1.504 (s, 3H), 1.063 (d, J = 6.4 Hz, 3H), 1.034 (d, J = 6.4 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 155.85, 152.10, 151.50, 136.45, 132.53, 128.49, 127.75, 126.48, 124.68, 107.41, 107.31, 99.60, 99.39, 92.67, 73.98, 68.51, 47.55, 47.45, 35.31, 22.92, 22.68, 18.84, 18.52, 15.98, 15.71. HREIMS m/z 462.2403 (calculated for $C_{29}H_{34}O_5$, 462.2401).

Xyloketal B Acid (11). The title compound was obtained from 2,4,6-trihydroxybenzoic acid and 2,4-dimethyl-3-hydroxymethyl-4,5-dihydrofuran with a procedure similar to that for compound **1**. Yield, 84.2%; white solid; mp 159−160 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 11.462 (s, 1H), 4.124(m, 2H), 3.511 (t, J = 8.5 Hz, 2H), 2.827 (m, 2H), 2.600 (m, 2H), 2.028 (m, 2H), 1.922 (m, 2H),1.533 (s, 3H), 1.470 (s, 3H), 1.025 (s, 6H). ^{13}C NMR ($CDCl_3$) δ: 171.08, 161.37, 156.59, 151.19, 111.40, 108.66, 99.68, 98.09, 93.92, 74.54, 74.07, 47.41, 46.89, 35.08, 34.95, 22.81, 22.50, 18.14, 17.96, 15.62, 15.51. HREIMS m/z 390.1672 (calculated for $C_{21}H_{26}O_7$, 390.1673).

Xyloketal B Methyl Formate (12). Potassium hydroxide (215 mg, 3.83 mmol) was dissolved in 6 mL of EtOH. This solution was treated with Diazald (164 mg, 0.765 mmol). The diazomethane generated at 0 °C was blown via a stream of nitrogen into a flask containing **11** (90 mg, 0.26 mmol) in Et_2O at 0 °C. Nitrogen was continually blown into the flask until the yellow color in both flasks disappeared. The ether solution was concentrated to give **12** (76.9 mg, 100% yield) as a light-yellow solid; mp 47−48 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.159 (m, 2H), 3.901 (s, 3H), 3.553 (t, J = 8.5 Hz, 1H), 3.480 (q, J = 8.5 Hz, 1H), 2.904 (dd, J = 17.0, 3.5 Hz, 1H), 2.081 (t, J = 15.5 Hz, 1H), 2.631 (m, 2H), 2.066 (m, 2H), 1.909 (m, 2H),1.524 (s, 3H), 1.514 (s, 3H), 1.079 (d, J = 7.0 Hz, 3H), 1.047 (d, J = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 172.03, 160.74, 156.15, 153.49, 108.42, 108.32, 99.28, 98.22, 96.12, 74.12, 73.86, 51.93, 47.54, 47.20, 35.87, 35.13, 23.10, 22.75, 18.88, 18.28, 15.96, 15.82. HREIMS m/z 404.1815 (calculated for $C_{22}H_{28}O_7$, 404.1814).

Xyloketal B Formamide (13). A mixture of compound **12** (105 mg, 0.26 mmol) and 5 mL of ammonia was stirred at 50 °C for 6 h and then poured into water. The aqueous layer was extracted with Et_2O (3 × 20 mL). The combined organic extracts were washed with saturated brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. Purification by flash chromatograghy using petroleum ether and ethyl acetate as the eluant afforded the title compound (88.3 mg, 87.3%) as a light-yellow solid; mp 152−153 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.878 (s, 1H), 4.163 (apparent q, J = 8.4 Hz, 2H), 3.556 (t, J = 8.4 Hz, 2H), 2.865 (m, 2H), 2.637 (m, 2H), 2.099 (m, 2H), 1.938 (m, 2H), 1.556 (s, 3H), 1.509 (s, 3H), 1.069 (d, J = 6.0 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ: 172.519, 161.804, 155.060, 151.387, 109.508, 108.217, 99.182, 97.708, 95.962, 74.319, 74.188, 47.399, 47.179, 35.446, 35.202, 23.091, 22.821, 18.585, 18.318, 16.052, 15.933. HREIMS m/z 389.1834 (calculated for $C_{21}H_{27}O_6N_1$, 389.1833).

Preparation of Compound 14. The title compound was obtained from **12** and methylamine with a procedure similar to that for compound **13**. Yield, 90.8%; light-yellow solid; mp 53−54 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.168 (t, J = 8.0 Hz, 2H), 3.547 (t, J = 8.0 Hz, 2H), 2.948 (q, J = 4.8 Hz, 3H), 2.860 (m, 2H), 2.629 (m, 2H), 2.085 (m, 2H), 1.935 (m, 3H), 1.536 (s, 3H), 1.509 (s, 3H), 1.074 (d, J = 6.0 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ: 170.914, 161.186, 154.376, 150.838, 109.226, 108.135, 99.273, 97.367, 96.552, 74.310, 74.094, 47.450, 47.186, 35.529, 35.188, 26.066, 23.142, 22.842, 18.643, 18.357, 16.023, 15.903. HREIMS m/z 403.1987 (calculated for $C_{22}H_{29}O_6N_1$, 403.1989).

Preparation of Compound 15. The xyloketal acid **11** (90 mg, 0.26 mmol) and HCl salt Ala-OMe (61.4 mg, 0.39 mmol) were dissolved in 5 mL of CH_2Cl_2. This solution was treated with BOP (174.9 mg, 0.39 mmol) and DIEA (107.7 μL, 6 mmol). After being stirred at room temperature for 12 h, the reaction mixture was then diluted with 100 mL of ethyl acetate. The resultant solution was washed with a saturated aqueous solution of ammonium chloride, brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. Purification by flash chromatograghy using petroleum ether and ethyl acetate as the eluant afforded the title compound (113.1 mg, 91.6% yield) as a yellow solid; mp 42−43 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.696 (dt, J = 1.6, 7.2 Hz, 1H), 4.168 (apparent q, J = 8.4 Hz, 2H), 3.779 (s, 3H), 3.546 (apparent t, J = 8.4 Hz, 2H), 2.902 (apparent d, J = 17.0 Hz, 1H), 2.850 (apparent d, J = 17.0 Hz,

1H), 2.683 (apparent dd, J = 6.0, 17.0 Hz, 1H), 2.602 (apparent dd, J = 6.0, 17.0 Hz, 1H), 2.090 (m, 2H), 1.954 (m, 2H), 1.601 (d, J=8.4 Hz, 3H), 1.523 (s, 3H), 1.514(s, 3H), 1.088 (d, J = 6.4 Hz, 3H), 1.062 (d, J = 6.4 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 173.27, 170.00, 161.59, 154.95, 151.75, 110.20, 108.47, 99.66, 98.33, 96.86, 74.63, 74.46, 52.70, 48.45, 48.13, 47.63, 36.14, 35.61, 23.68, 23.12, 19.06, 18.81, 18.73, 16.41, 16.26. HREIMS m/z 475.2202 (calculated for $C_{25}H_{33}O_8N_1$, 475.2201).

(±)-Xyloketal H (16). To a stirred mixture of phloroglucinol (70 mg, 0.56 mmol), 2,4-dimethyl-3-hydroxymethyl-4,5-dihydrofuran (143 mg, 1.12 mmol), and anhydrous magnesium sulfate (140 mg) in ether,[20,21] boron trifluoride diethyl etherate (52.3 mg, 0.37 mmol) was added at 0 °C and allowed to warm to room temperature. The resultant mixture was stirred for 24 h at room temperature. The reaction mixture was then diluted with 100 mL of ethyl acetate, washed with a saturated aqueous solution of ammonium chloride (1 × 20 mL), brine (1 × 20 mL), dried over anhydrous magnesium sulfate, and concentrated in vacuo. Purification by flash chromatograghy using petroleum ether and ethyl acetate as the eluant afforded xyloketal B (99.2 mg, 51.2%) as a white solid and xyloketal H (**16**) (57.7 mg, 43.7% yield) as a yellow solid; mp 112−113 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.992 (s, 2H), 4.161 (t, J = 8.0 Hz, 1H), 3.519 (t, J = 8.8 Hz, 1H), 2.770 (d, J = 17.2 Hz, 1H), 2.614 (dd, J = 17.2, 6.4 Hz, 1H), 2.094 (m, 1H), 1.908 (m, 1H), 1.503 (s, 3H), 1.026 (d, J = 6.4 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 163.61, 159.64, 155.04, 107.38, 99.35, 93.41, 91.36, 74.14, 47.58, 35.11, 22.52, 18.36, 15.77. HREIMS m/z 236.1028 (calculated for $C_{13}H_{16}O_4$, 236.1029).

Xyloketal H Dimethyl Ester (17). The title compound was obtained from **16** and Me_2SO_4 with a procedure similar to that for compound **4** as a yellow solid; mp 69−70 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.044 (s, 1H), 6.039 (s, 1H), 4.184 (t, J = 8.0 Hz, 1H), 3.785 (s, 3H), 3.734 (s, 3H), 3.516 (t, J = 8.8 Hz, 1H), 2.808 (dd, J = 17.6, 1.2 Hz, 1H), 2.623 (dd, J = 17.2, 6.4 Hz, 1H), 2.117 (m, 1H), 1.888 (ddd, J = 10.8, 6.8, 1.2 Hz, 1H), 1.499 (s, 3H), 1.052 (d, J = 6.4 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 159.61, 158.64, 154.04, 107.28, 99.45, 93.51, 91.32, 74.04, 55.33, 55.18, 47.51, 35.23, 22.58, 18.39, 15.89. HREIMS m/z 264.1353 (calculated for $C_{15}H_{20}O_4$, 264.1356).

Dehydroxy Xyloketal H (18). The title compound was obtained from resorcin with a procedure similar to that for compound **16** as a white solid. Yield, 74.2%; mp 123−124 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.914 (d, J = 8.4 Hz, 1H), 6.476 (d, J = 2.4 Hz, 1H), 6.406 (dd, J = 8.4, 2.4 Hz, 1H), 4.143 (t, J = 8.0 Hz, 1H), 3.486 (t, J = 8.4 Hz, 1H), 2.924 (ddd, J = 8.4, 5.6, 1.2 Hz, 1H), 2.672 (dd, J = 8.4, 1.6 Hz, 1H), 2.108 (m, 1H), 1.941 (ddd, J = 10.8, 5.6, 1.6 Hz, 1H), 1.567 (s, 3H), 1.037 (d, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 155.72, 153.77, 129.72, 111.24, 108.33, 108.25, 103.98, 73.91, 48.82, 35.08, 23.96, 23.81, 15.89. HREIMS m/z 220.1095 (calculated for $C_{13}H_{16}O_3$, 220.1094).

(±)-Xyloketal A (19). The title compound was obtained from phloroglucinol (100 mg, 0.79 mmol) and 2,4-dimethyl-3-hydroxymethyl-4,5-dihydrofuran (406 mg, 3.17 mmol) with a procedure similar to that for compound **1**. Yield, 84.2%; white solid; mp 139−140 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.171 (t, J = 8.4 Hz, 3H), 3.528 (t, J = 8.4 Hz, 3H), 2.865 (d, J = 17.6 Hz, 3H), 2.642 (dd, J = 17.6, 6.8 Hz, 3H), 2.132 (m, 3H), 1.893 (m, 3H), 1.511 (s, 9H), 1.071 (d, J = 6.8 Hz, 9H). ^{13}C NMR ($CDCl_3$) δ: 149.73, 107.28, 98.91, 74.03, 47.52, 35.49, 22.93, 18.89, 16.01. HREIMS m/z 456.2511 (calculated for $C_{27}H_{36}O_6$, 456.2510).

Preparation of Compound 20. To a solution of (50 mg, 0.145 mmol) xyloketal B (**3**) in THF was added 35 mg of polyformaldehyde and 6 d of 2 N HCl. The mixture was stirred for 24 h at room temperature and then neutralized by addition of K_2CO_3 solution, The resultant solution was washed with a saturated aqueous solution of ammonium chloride, brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. Purification by flash chromatograghy using petroleum ether and ethyl acetate as the eluant afforded the title compound (42 mg, 82.0% yield) as a white solid; mp 121−122 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 4.282 (t, J = 8.4 Hz, 2H), 4.168 (t, J = 8.4 Hz, 2H), 3.742 (s, 2H), 3.591 (t, J = 8.4 Hz, 2H), 3.534 (t, J = 8.4 Hz, 2H), 2.871 (d, J = 17.2 Hz, 2H), 2.829 (d, J = 17.2 Hz, 2H), 2.801 (dd, J = 17.6, 6.4 Hz, 2H), 2.626 (dd, J = 17.6, 6.4 Hz, 2H), 2.112 (m, 2H), 2.033 (m, 2H), 1.926 (dd, J = 17.6, 6.4 Hz, 2H), 1.878 (dd, J = 17.6, 6.4 Hz, 2H), 1.605 (s, 6H), 1.591 (s, 6H), 1.067 (d, J = 6.4 Hz, 6H) 1.053 (d, J = 6.4 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ: 152.49, 150.13, 148.02, 109.17, 107.42, 106.04, 99.97, 97.91, 74.43, 73.88, 47.44, 47.32, 35.19, 35.03, 22.78, 22.19, 19.10, 18.77, 17.12, 15.83, 15.78. HREIMS m/z 704.3554 (calculated for $C_{41}H_{52}O_{10}$, 704.3552).

Preparation of Compound 21. To a suspension of phloroglucinol (1.0 g, 7.94 mmol), methyl vinyl ketone (2.2 g, 31.75 mmol), anhydrous magnesium sulfate (2.0 g), and methanol (30 mL) at 0 °C was added p-TsOH (2.56 g, 15.88 mmol). The resultant mixture was allowed to warm to room temperature and was stirred for 8 h. The reaction mixture was then diluted with ethyl acetate. The resultant solution was washed with a saturated aqueous solution of ammonium chloride, brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. Purification by flash chromatograghy using petroleum ether and ethyl acetate as the eluant afforded the title compound **21** (2.85 g, 95%) as a white solid; mp 134−135 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 3.234 (s, 9H), 2.641 (m, 6H), 2.044 (m, 3H), 1.745 (m, 3H), 1.520 (s, 9H). ^{13}C NMR ($CDCl_3$) δ: 147.99, 147.90, 147.82, 102.70, 102.64, 102.42, 98.12, 98.01, 97.96, 48.99, 48.91, 31.41, 31.34, 23.16, 15.69, 15.66, 15.50. HREIMS m/z 378.2035 (calculated for $C_{21}H_{30}O_6$, 378.2037).

Pharmacological Assays. Isolation of Rat Thoracic Aortic Rings. Male Sprague−Dawley rats (200−250 g) were euthanized by inhalation of 80% CO_2/20% O_2 and subsequent cervical dislocation. The chests were opened, and the thoracic aortae were isolated. After removal of the superficial connective tissue, the aorta was cut into ring segments, 2−3 mm in width, which were then used for relaxation assays in vitro.

Relaxation by Xyloketals. Aortic rings were mounted in standard 10 mL organ baths containing Krebs' bicarbonate solution. The bath medium was maintained at 37 °C with a pH of 7.4 and continuously bubbled with carbogen (5% CO_2 in O_2). When required, the endothelium was mechanically removed by gently rubbing the lumen with the tips of fine forceps. Aortic rings were equilibrated for 60 min at a resting tension of 1 g. The rings were precontracted with an isotonic, high-potassium physiological salt solution (Krebs' potassium salt solution, KPSS, 60 mM KCl) to achieve the maximum tension. After a washout of 30 min, the integrity of the endothelium was tested. Rings were precontracted with phenylephrine (PE, 1 μM). Only rings showing greater than 80% relaxation in response to acetylcholine (Ach, 1 μM) were accepted as endothelium-intact, and lack of responsiveness to Ach was confirmed as endothelium-denuded. Following testing of endothelial integrity, rings were repeatedly washed and re-equilibrated to the resting tension.

(1) Effect on KCl-Induced Precontraction. Isotonic, high potassium physiological salt solution (KPSS, 60 mM KCl) was then used to establish a stable active force in the range of 40−60% of KPSS (60 mM KCl) induced contraction. Cumulative concentration−response curves were conducted for all xyloketals (0.5−200 μM) or vehicle (2% DMSO, 98% KPSS). Only one concentration−response curve was obtained from each thoracic aortic ring.

(2) Effect of the Removal of Endothelium on the Responses to Xyloketals. In order to examine the role of endothelium in the vasorelaxation caused by xyloketals, cumulative response curves were determined in the absence and the presence of endothelium for KPSS (25 mM for endothelium denuded rings or 30 mM KCl for endothelium intact rings) precontracted vessels.

Cell Culture. The HUVECs cell line was from China Center for Typical Culture Collection (CCTCC, Wuhan, China). The cells were cultured in medium 199 containing 20% fetal calf serum, penicillin (100 U/mL), streptomycin (100 U/mL), and

heparin (50 U/mL), supplemented with L-glutamine (2 mM), sodium pyruvate (1 mM), and endothelial cell growth factor (β-ECGF, 5 ng/mL), at 37 °C in 5% CO_2 on 0.1% gelatin-coated culture flasks.

Cell Viability Assay. Cell viability was assessed by the mitochondrial tetrazolium assay (MTT) in HUVECs. Confluent cells were treated with various concentrations of xyloketals or vehicle for 48 h. MTT solution (10 μL/well) was added and processed to examine cell viability. Optical density was read at 570 nm by Biotek Elx-800 plate reader. Cells alone were used as a control group, and the cell viability of the control group was taken as 100%. At tested concentrations, xyloketals showed no significant effects on cell viability in both cell types. To exclude possible toxicity from solvent, various concentrations of DMSO were incubated with each cell type, respectively; no toxicity was detected at the concentrations used in the present study.

Effects of Xyloketals on oxLDL-Induced Cell Injury. The effects of xyloketals on oxLDL-induced cell injury in HUVECs were visualized by the mitochondrial tetrazolium assay (MTT). Briefly, cells were pretreated for 30 min with 20 μM xyloketals, followed by vehide or oxLDL (150 μg/mL) incubation for 24 h. MTT solution (10 μL/well) was added, and optical density was read at 570 nm by a Biotek Elx-800 plate reader after 4 h.

Nitric Oxide Generation Detection. Levels of the nitric oxide (NO) derivative nitrite were determined together with the Griess reaction.[43] The nitrite detection kit was used according to instructions provided by the manufacturer. The samples were assayed in triplicate, and a standard curve using $NaNO_2$ was generated for each experiment for quantification. Then 100 μL of medium or standard $NaNO_2$ was mixed with 100 μL of Griess reagent in a 96-well plate. After 15 min, optical density was read in a microplate reader at 540 nm.

Embryo Collection and Drug Treatment. Zebrafish embryos were used to examine the effect of different compounds on embryonic angiogenesis. These compounds were added to embryo water from the shield stage (about 6 h). Zebrafish embryos were generated by natural pairwise mating and raised at 28.5 °C in embryo water (0.2 g/L of Instant Ocean Salt in distilled water). Embryos were maintained in embryo water (0.2 g/L of Instant Ocean Salt in distilled water) at 27 °C, and the embryos were sorted for viability and developmental stage (shield stage) at 6 h. Three embryos were placed into each well of a 96-well plate containing 100 μL of embryo water with or without drug. The embryo water was replenished daily with compounds at 24, 48, and 72 h. The embryos were examined daily for viability, gross morphological abnormalities, and blood vessel development using an inverted Olympus DP70 epifluorescence microscope (Olympus, Tokyo, Japan). Because the fish embryo receives nourishment from an attached yolk ball for the duration of the experiment, no additional maintenance was required during the duration of the experiments. At 72 h, the embryos were anesthetized using 0.05% 2-phenoxyethanol in embryo water, and each embryo was examined for the presence of ectopic vessels in the subintestinal vessel plexus (SIV).

Statistics. Data are expressed as the mean ± SEM. ANOVA with posthoc tests was used for analyzing the value between vehicle- and xyloketal-treated group. Values of $P < 0.05$ were considered significant.

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Supporting Information Available: NMR data and spectra; HPLC data and chromatograms. This material is available free of charge via the Internet at http://pubs.acs.org.

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