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Phenylsulfonylfuroxan NO-donor phenols: Synthesis and multifunctional activities evaluation

Yundong Xie [a], Yaping Yang [a], Sen Li [b], Yanhong Xu [a], Wenfang Lu [a], Zizhang Chen [a], Guangde Yang [a], Yiping Li [a], Yongxiao Cao [b], Xiaoli Bian [a,*]

[a] College of Pharmacy, Xi'an Jiaotong University, No. 76 Yanta West Road, Xi'an, Shaanxi Province 710061, People's Republic of China
[b] School of Basic Medical Science, Xi'an Jiaotong University, No. 76 Yanta West Road, Xi'an, Shaanxi Province 710061, People's Republic of China

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ABSTRACT

Phenylsulfonyfuroxan nitric oxide (NO)-donor phenols were designed, synthesized and evaluated. The compounds were designed through a symbiotic approach using selected phenols and phenylsulfonylfuroxan NO-donor. The antioxidant activities of the hybrid compounds **T_2–T_6** showed to be good *in vivo*. Compounds **T_4** and **T_6** revealed excellent yeast α-glucosidase inhibitory activity and *anti*-glycosylation activity. All of the compounds exhibited strong NO releasing activity and significant *anti*-platelet aggregation activity. The inhibition of platelet aggregation was more than 50% at low concentration (1.5 μM) and 95% at higher concentration (15 μM and 150 μM). The vasodilatation experiment demonstrated that the six compounds under test exhibited definite vasodilation activity (pIC_{50} ranged from 5.698 to 6.383), especially compound **T_6** (pIC_{50} was 6.383) which was similar to sodium nitroprusside (pIC_{50} was 6.786). Both anticoagulant and vasodilatation effects were correlated with their NO releasing activities. These hybrid phenylsulfonyfuroxan-based NO-donor phenols offer a multifunctional prodrug design concept for the development of therapeutic or preventive agents for metabolic syndrome.

1. Introduction

Metabolic syndrome (MetS) is a cluster of risky factors, including raised blood pressure, dyslipidemia (raised triglycerides and lowered high-density lipoprotein cholesterol), raised fasting glucose, and central obesity, for the development of cardiovascular disease (CVD), renal and liver diseases and type 2 diabetes mellitus (T2DM).[1] Now MetS has become a major public health and clinical problem, and its morbidity and mortality are increasing worldwidely.[2] MetS leads to an increased risk of T2DM and CVD, in the forms of coronary or peripheral atherosclerosis and heart failure.[3] Increasing evidence suggests that MetS is associated with increased oxidative stress.[4,5] The raised oxidative stress, which in turn leads to endothelial dysfunction, is clearly related to increased cardiovascular morbidity and mortality.[6,7]

As we all know, long sustained hyperglycemia is the characteristic of diabetic patients, which results in an activation of oxidative stress with an overproduction of reactive oxygen species (ROS). This activation in turn produces a cascade of deleterious metabolic events such as increased formation of advanced glycation end-products (AGEs), enhanced polyol activity, activation of nuclear factor κB (NF-κB) and protein kinase C, and increased hexosamine pathway flux. It is now well established that hyperglycemia during postprandial period results in exaggerated and accelerated glycation. As a consequence, both postprandial hyperglycemia and AGEs are identified as the fundamental cause of pathology of diabetic complications.

In the pathological mechanism of atherosclerotic plaque, there is an abnormal production of superoxide anion by the endothelium.[8] Hydrogen peroxide is formed from this radical, under the action of the superoxide dismutase, and a source of the very toxic hydroxyl radical. Low density lipoproteins (LDL) are subject to oxidative modifications under the action of this radical.[8] In endothelial dysfunction, the excess superoxide anions induce alterations in the nitric oxide signaling system.[9] It is reported that superoxide anion traps NO to generate peroxynitrite. That in turn can afford two very reactive and toxic radicals: the hydroxyl radical and the nitrogen dioxide radical. Therefore, the lack of NO has been recognized as a key feature in endothelial dysfunction. Moreover, studies showed that oxidative stress contributes to the etiology of hypertension in animals and humans. Hypertensive patients have impaired endogenous and exogenous antioxidant defense mechanisms, increased plasma oxidative stress, and an

* Corresponding author.
E-mail address: xieshijiaoda405@stu.xjtu.edu.cn (X. Bian).

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exaggerated oxidative stress response to various stimuli.[10–12] There is also some evidence that in an atherosclerotic vessel the production of NO by the endothelial cells could be decreased. NO acts as a vascular endothelial protection factor, displaying numerous biological effects such as mediating vascular dilatation, acting as an anti-inflammatory, scavenging superoxide anions, and preventing platelet aggregation.[13–17] On these bases, ameliorating oxidative stress and attempting to increase NO concentrations or to improve responses to NO stimulation have attracted great interest for the pharmaceutical treatment of MetS.

At present, the best treatment for MetS is prevention through dietary modulation.[1] However, too often we find that they are hard to carry out. Therefore, we have designed and synthesized a series of compounds in which appropriate phenylsulfonyfuroxan NO-donor was linked to different natural antioxidants such as cinnamic acid, p-hydroxycinnamic acid, ferulic acid, caffeic acid, 3,4-dihydroxybenzoic acid and 3, 4-dihdroxyphenylacetic acid. The natural antioxidant molecules have antioxidant activity and α-glucosidase inhibition activity.[18] It has been reported that antioxidants and radical scavengers prevent the formation of advanced glycation end-products (AGEs).[19] Researches have revealed that compounds with combined antioxidant and anti-glycosylation properties are more effective in treating dysglycemia.[20]

These hybrid compounds are examples of multi-target drugs that single chemical entities are able to simultaneously modulate more than one target. Now, it is interesting to use this kind of drugs for the treatment of complex diseases such as MetS, T2DM, and CVD. Here we report the conclusive results of a study on the capacities of antioxidant, inhibiting yeast α-glucosidase, anti-glycosylation, anti-platelet aggregation, and vasodilator properties of a series of phenylsulfonyfuroxan NO-donor phenols. These target compounds were formally obtained by containing natural antioxidant molecules, characterized by extensively modulated antioxidant properties, with appropriate NO-donor moiety (Chart 1). The NO-donor moiety that we used was phenylsulfonyfuroxan NO-donor moiety, which shows extensively modulated *in vitro* NO-dependent anti-platelet aggregation and vasodilator properties.[21]

2. Results and discussion

2.1. Chemistry

The intermediate compound **M1** was prepared according to the procedure reported in Scheme 1. The intermediate 4-(2-hydroxyethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**M1**) was synthesized starting from the thiophenal (**1**) that was transformed into the 2-(phenylthio) acetic acid (**2**) by sodium carbonate in 95% ethanol solution. The action on **2** of hydrogen peroxide in acetic acid to get 2-(phenylsulfonyl) acetic acid (**3**), then reacted with nitric acid to give the 3,4-bis(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**4**), which was transformed, in THF solution, in the presence of glycol and 25% sodium hydroxide, into the final intermediate compound **M1**.

A generalized synthetic approach to the $\mathbf{T_1}$ was showed in Scheme 2. Intermediate reacted with cinnamic acid (**5**) in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) and 4-N,N-dimethylaminopyridine (DMAP) in N,N-dimethylformamide (DMF) solution to give $\mathbf{T_1}$.

The synthesis of $\mathbf{T_2}$ and $\mathbf{T_3}$ were completed as depicted in Scheme 3. The hydroxy group of the p-hydroxycinnamic (**6**) and ferulic acid (**7**) were benzyl-protected using benzyl bromide in

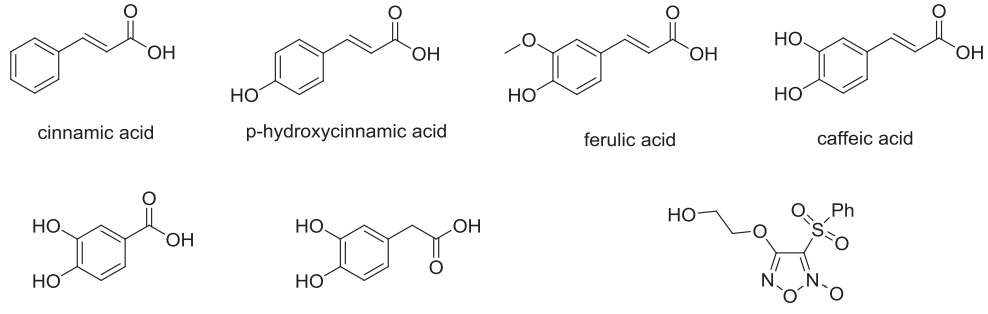
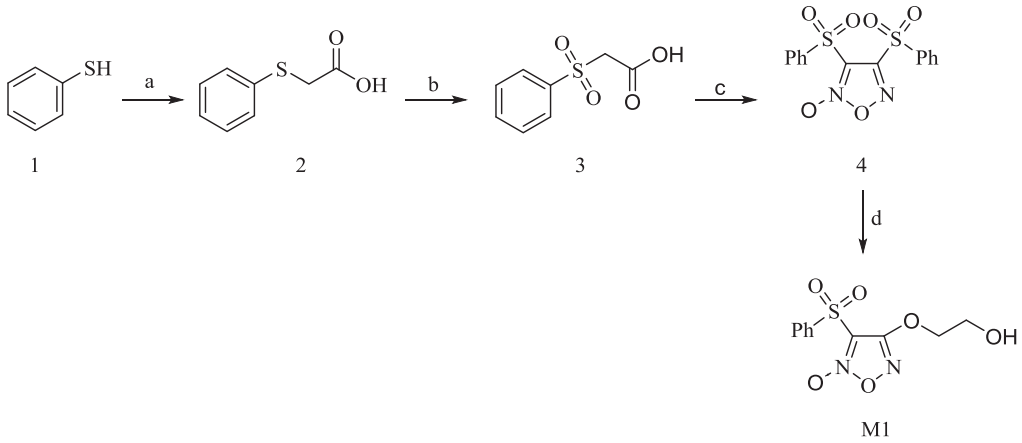
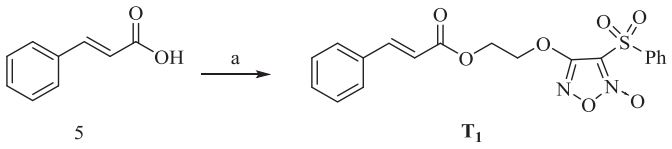


Chart 1.



Scheme 1. Synthesis route of intermediate **M1**. Reagents and conditions: (a) $ClCH_2COOH$, Na_2CO_3, 95% EtOH; (b) H_2O_2, AcOH; (c) HNO_3; (d) $HOCH_2CH_2OH$, 25%NaOH, THF.



Scheme 2. Synthesis route of compounds T_1. Reagents and conditions: (a) **M1**, DMAP, EDCI, DMF, r. t.

acetone solution in the presence of potassium carbonate to give the corresponding derivatives **8** and **9**. Ester hydrolysis of these products using 6 M sodium hydroxide in THF solution was under 60 °C, then HCl acid gave the related acid **10** and **11**. The final protected products **12** and **13** were obtained by reacting with intermediate **M1** in the presence of EDCI and DMAP in DMF. Deprotection of these products using titanium tetrachloride ($TiCl_4$) in dichloromethane (CH_2Cl_2) solution gave the target compounds T_2 and T_3.

The target compounds T_4–T_6 were prepared via the synthetic route showed in Scheme 4. The hydroxy groups of the caffeic acid (**14**), 3,4-dihydroxybenzoic acid (**15**) and 3, 4-dihdroxyphenylacetic acid (**16**) were diphenylmethylene-protected using dichlorodiphenylmethane in methylbenzene under 110 °C to give the corresponding derivatives **17**, **18** and **19**. The final protected products **20**, **21** and **22** were obtained by the action of intermediate **M1** on the EDCI and DMAP in DMF solution. Deprotection of these products using acetic acid-water (4:1) solution under 90 °C afforded target compounds T_4, T_5 and T_6.

2.2. Biological activity

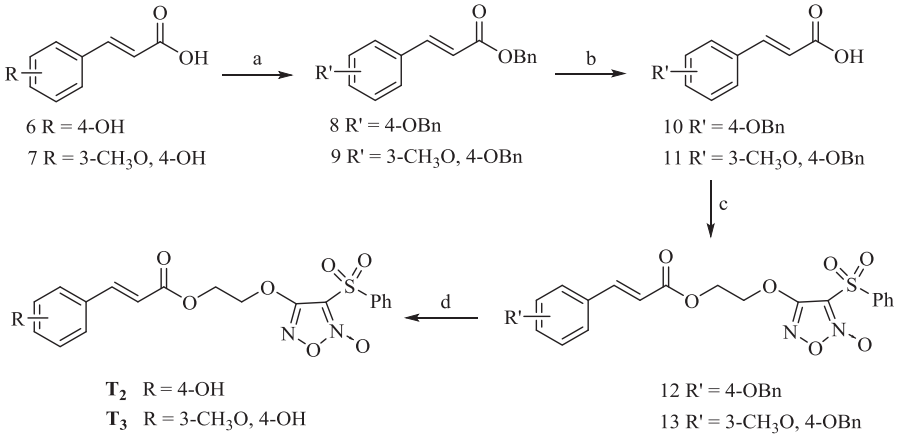
2.2.1. Antioxidant properties in vitro

The antioxidant activities of target compounds were determined by scavenging radicals (DPPH· and OH·) and inhibiting lipid peroxidation. The results are reported in Table 1, which showed that all of compounds have the same orders in scavenging radicals and inhibiting lipid peroxidation. Phenolic hydroxyl is the favorable structure for antioxidation, especially the catechol. T_4–T_6

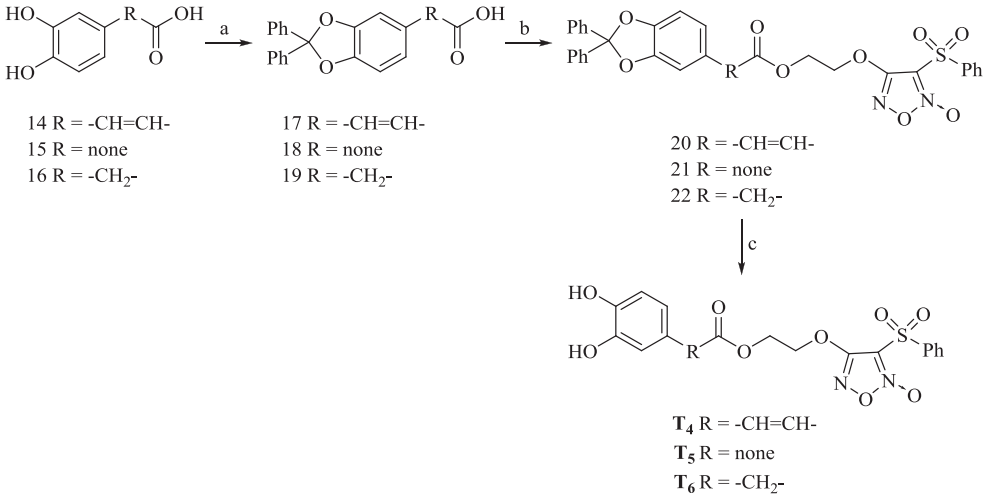
Table 1
Antioxidant activities of target compounds T_1–T_6 *in vitro*.[a]

Compds	DPPH· (mM)[a]	OH· (mM)[a]	anti-lipid peroxidation (μM)[a]
T_1	>10	>10	>100
T_2	1.385 ± 0.009	6.235 ± 0.186	41.421 ± 2.947
T_3	0.181 ± 0.010	0.669 ± 0.043	5.132 ± 0.492
T_4	0.042 ± 0.011	0.219 ± 0.069	0.221 ± 0.047
T_5	0.060 ± 0.006	0.275 ± 0.040	0.713 ± 0.060
T_6	0.016 ± 0.005	0.236 ± 0.037	0.361 ± 0.053
VitC	0.163 ± 0.010	0.895 ± 0.042	15.350 ± 0.165

Data were expressed as Mean ± SE.
[a] The results summarized are the mean values of n = 3 for IC_{50} values.



Scheme 3. Synthesis route of compounds T_2 and T_3. Reagents and conditions: (a) BnBr, K_2CO_3, acetone; (b) 6 M NaOH, THF, 60 °C, HCl; (c) **M1**, EDCI, DMAP, DMF, r. t.; (d) $TiCl_4$, CH_2Cl_2.



Scheme 4. Synthesis route of compounds T_4–T_6. Reagents and conditions: (a) Ph_2CCl_2, PhMe, 110 °C; (b) **M1**, EDCI, DMAP, DMF, r. t.; (c) AcOH: H_2O = 4:1, 90 °C.

containing catechol have a similar antioxidant capacity, which were much better than both **T_2** and **T_3** containing one phenolic hydroxyl. Comparing **T_2** and **T_3**, we found that the *ortho*-methoxy of **T_3** can enhance the antioxidant activity of phenolic hydroxyl. These can indicate that the more the number of phenolic hydroxyl groups, the stronger the antioxidant capacity.

2.2.2. Yeast α-glucosidase inhibitory activity

The activity of the synthesized compounds was evaluated *in vitro* against yeast α-glucosidase from *Saccharomyces cerevisiae*. The results are expressed as the inhibitor concentration required to achieve 50% inhibition of α-glucosidase activity (IC_{50}) and reported in Table 2. The IC_{50} data demonstrates that the synthesized compounds contained phenolic hydroxyl group inhibited α-glucosidase with IC_{50} values ranging from 0.132 mM to 0.279 mM, suggesting that the phenolic hydroxyl group of target compounds had significant effects on the inhibitory potencies and the catechol derivatives **T_4**, **T_5** and **T_6** were more potent against yeast α-glucosidase than acarbose (IC_{50} = 0.232 mM), with IC_{50} values of 0.187, 0.132 and 0.147 mM, respectively. Phenolic hydroxyl is the favorable structure for inhibition of α-glucosidase, especially the catechol. The results of our present study support the previous researches that polyphenols inhibit α-glucosidase *in vitro*.[22]

2.2.3. Anti-glycosylation property

In this experiment, bovine serum albumin (BSA)-methylglyoxal (MGO) system was used to simulate the glycation reactions of the body.[23] The effects of target compounds **T1–T6** on AGEs formation are represented in Table 2. Compounds **T3–T6** showed higher inhibition of AGEs formation than, with IC_{50} values of 0.985, 0.158, 0.464, and 0.390 mM, respectively. **T_1** and **T_2** did not show anti-glycosylation activity. Free radicals have been showed to participate in the formation of AGEs. Antioxidants and radical scavengers inhibit these processes.[24] It has been reported that naturally occurring polyphenols play a good role in preventing AGEs formation.[25] The results of our present study support these viewpoints. Moreover, reports indicated that compounds with combined antiglycation and antioxidant properties are more effective in protecting against glycation reactions in the early and late stages of AGEs formation.[20]

2.2.4. Nitric oxide (NO) releasing activity in vitro

The levels of NO produced by **T_1–T_6** were determined by Griess assay.[26] As showed in Fig. 1, all of the six target compounds exhibited excellent NO releasing ability. The active catechol compounds **T_4–T_6** released considerable amount of NO (45.01, 50.20, and 49.29 μM, respectively). Likewise, the less active compounds **T_1–T_3** under the same conditions released slight lower levels of NO (40.01, 35.72, and 39.20 μM, respectively). The findings of our study indicate that the NO release of phenylsulfonyfuroxan-phenols hybrids may be associated with phenolic hydroxyl, which

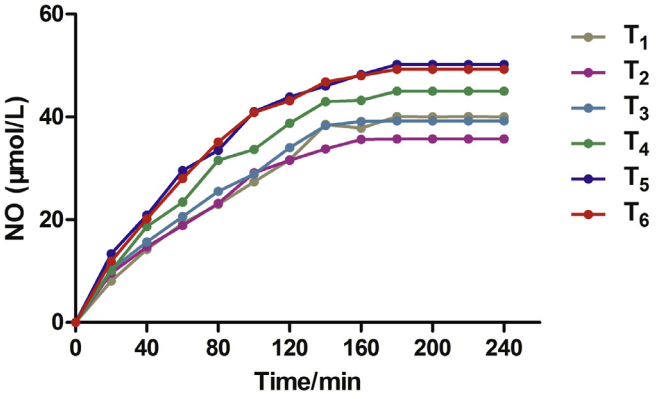


Figure 1. The levels of NO released by target compounds were determined by Griess assay *in vitro*. NO data are expressed as Mean ± SE (n = 5) in μM at each time point.

can weakly alter the NO releasing of phenylsulfonyfuroxan. Since all of the compounds have good NO releasing activity, we further study the anti-platelet aggregation and vasorelaxant properties *in vitro*.

2.2.5. Anti-platelet aggregation activity

The *in vitro* inhibitory effects of the target compounds **T_1–T_6** on adenosine diphosphate (ADP)-induced platelet aggregation in human platelet rich plasma (PRP) were assayed using Born's turbidimetric method.[26] As showed in Table 3, all of the target compounds showed more stronger inhibitory effects than aspirin at the same concentrations. When at concentrations 15 μM and 150 μM, all of inhibitory rate of the target compounds were over 95%. Furthermore, at lower concentrations 1.5 μM, **T_1**(56.44%), **T_2**(58.14%), **T_3**(65.53%), **T_4**(73.54%), **T_5**(60.34%) and **T_6**(62.50%) displayed significant inhibitory effects, especially compound **T_4**, which were superior to aspirin (5.96%), the clinically used. Notably, **T_1–T_6** were almost tenfold more potent than aspirin. The anti-platelet aggregation activity of synthetic compounds were related to their NO releasing ability. It indicated that NO releasing ability of these compounds may be positively correlated to their platelet aggregation inhibitory activity.

2.2.6. Vasorelaxant ability

To study the vasorelaxant effects of furoxan nitric oxide donors, 60 mM potassium chloride (KCl) Krebs solution was added to the baths to induce contraction of the artery ring segments. After the sustained tension was obtained, cumulative concentration of the donor **T_1–T_6** (10^{-10}–$10^{-3.5}$ M) were applied to the arteries,

Table 2
The effects of target compounds **T_1–T_6** on α-glucosidase inhibitory and anti-glycosylation activity.

Compds	Yeast α-glucosidase (μM)[a]	Anti-glycosylation (mM)[b]
T_1	>1500	>10
T_2	278.54 ± 4.75	>10
T_3	263.47 ± 5.33	0.985 ± 0.047
T_4	187.34 ± 4.24	0.158 ± 0.021
T_5	132.46 ± 4.58	0.464 ± 0.041
T_6	147.38 ± 3.08	0.390 ± 0.044
Acar	232.41 ± 4.88	–
AG	–	1.510 ± 0.057

Data were expressed as Mean ± SE.
[a] The results summarized are the mean values of n = 3 for IC_{50} values.
[b] The results summarized are the mean values of n = 5 for IC_{50} values.

Table 3
Inhibitory effects of target compounds **T_1–T_6** on platelet aggregation induced by ADP *in vitro*.

Compds	Inhibition rate (%)		
	150 μM	15 μM	1.5 μM
T_1	96.12 ± 1.20***	95.23 ± 1.25***	56.44 ± 1.27***
T_2	98.10 ± 0.75***	95.59 ± 1.20***	58.14 ± 1.05***
T_3	97.08 ± 0.85***	95.07 ± 0.80***	65.53 ± 0.88***
T_4	98.02 ± 0.71***	95.83 ± 0.85***	73.54 ± 0.87***
T_5	97.82 ± 0.84***	96.94 ± 0.98***	60.34 ± 1.02***
T_6	98.01 ± 0.66***	97.73 ± 0.81***	62.50 ± 1.07***
Aspirin	28.11 ± 1.24	12.44 ± 1.16	5.96 ± 0.79

PRP and different concentrations of tested compounds (150 μM, 15 μM, and 1.5 μM) were preincubated at 37 °C for 5 min followed by the addition of ADP (5 μL). Data were expressed as Mean ± SE of each group (n = 6) and analyzed by one-way analysis of variance (ANOVA) test.
*** $P < 0.001$ vs Aspirin.

Table 4
Inhibitory effects of target compounds **T1**–**T6** on artery contraction induced by KCl 60 mM.

Compds	n	R_{max}[a] (%)	pIC_{50}
DMSO	8	13.0 ± 1.5	–
$\mathbf{T_1}$	7	96.5 ± 2.1**	5.875 ± 0.069
$\mathbf{T_2}$	7	93.5 ± 1.4**	6.171 ± 0.041
$\mathbf{T_3}$	8	97.8 ± 1.6**	5.872 ± 0.081
$\mathbf{T_4}$	6	98.1 ± 3.2**	5.698 ± 0.116
$\mathbf{T_5}$	8	101.5 ± 4.1**	5.796 ± 0.086
$\mathbf{T_6}$	7	102.6 ± 1.9**	6.383 ± 0.065
Sodium nitroprusside	8	89.0 ± 5.1**	6.786 ± 0.119

Data were expressed as Mean ± SE, n = the number of artery ring segments, and analyzed by one-way analysis of variance (ANOVA) test. $^{**}P < 0.01$ vs DMSO.
[a] R_{max}: maximal vasodilative ratio.

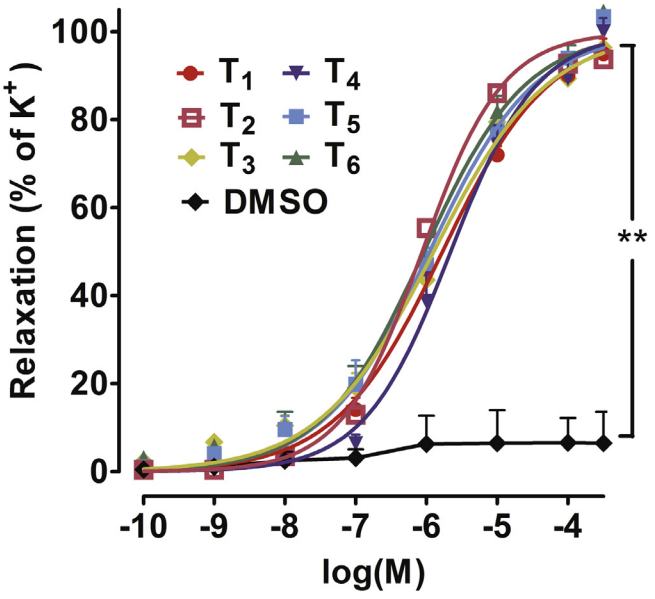


Figure 2. The vasorelaxant effects of target compounds $\mathbf{T_1}$–$\mathbf{T_6}$ on contraction induced by KCl 60 mM of rat mesenteric arteries. Mean ± SE, n = 6–8, $^{**}P < 0.01$ vs DMSO.

respectively. Meanwhile, effect of DMSO with the same volumes used to dissolve the compounds was also evaluated.[27] As depicted in Table 4 and Fig. 2, $\mathbf{T_1}$–$\mathbf{T_6}$ (10^{-10}–$10^{-3.5}$ M) induced concentration-dependent relaxations on the mesenteric arteries pre-constricted by KCl 60 mM, respectively. Relaxant effects of $\mathbf{T_2}$ (pIC_{50} = 6.171) and $\mathbf{T_6}$ (pIC_{50} = 6.383) became significant at approximately 10^{-7} M, Which were both close to SNP (pIC_{50} = 6.786), while $\mathbf{T_1}$ (pIC_{50} = 5.875), $\mathbf{T_3}$ (pIC_{50} = 5.872), $\mathbf{T_4}$ (pIC_{50} = 5.698) and $\mathbf{T_5}$ (pIC_{50} = 5.796) at approximately 10^{-6} M.

3. Conclusion

In summary, in the present study a multi-target-directed approach led us to design and synthesize six phenylsulfonyfuroxan-phenols hybrids, which are characterized by a unique multimodal profile. The antioxidant, yeast α-glucosidase inhibition, anti-glycosylation, and anti-platelet aggregation as well as vasodilator properties, showed that: (i) catechol $\mathbf{T_4}$–$\mathbf{T_6}$ had strong antioxidant capacities measured by scavenging radicals (DPPH· and OH·) and inhibiting lipid peroxidation, and significant yeast α-glucosidase inhibition as well as anti-glycosylation; (ii) polyphenols, having anti-diabetic and free radical scavenging ability, were beneficial; (iii) antioxidants and radical scavengers inhibited AGEs formation; (iv) NO releasing ability of these compounds may be positively correlated to their platelet aggregation inhibitory activity; (v) $\mathbf{T_6}$ had obvious vasodilation capacity which closes to SNP; (vi) NO has an important impact on the antiplatelet effects and vasodilation property. Altogether, the collective results strongly suggest that all these properties could be conducive to the amelioration of VCD and T2DM by attenuating oxidative stress, raising NO levels and decreasing postprandial hyperglycemia as well as inhibiting AGEs formation. The consumingly efficient and moderately balanced activities of $\mathbf{T_4}$–$\mathbf{T_6}$ provide them optimized multifunctional molecules operational in the treatment of metabolic syndrome disorders.

4. Experimental sections

4.1. Chemistry

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. The progress of the reactions was monitored by TLC using Qingdao Haiyang Chemical Co. Ltd, HG/T2354-92 Silica Gel GF254. Purifications of compounds were made by flash column chromatography using Qingdao Haiyang Chemical Co. Ltd, silica gel (100–200 mesh). Melting points were recorded using Tech. Instrument Co. Ltd, XT-4 melting point apparatus and were uncorrected. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded with a Bruker AV400 spectrometer,-chemical shifts were recorded in ppm, tetra-methylsilane was used as the internal standard and coupling constants (*J*) were given in Hertz. Mass spectra were obtained on Shimadzu HPLC-MS-QP2010 instrument.

*4.1.1. 2-(Phenylthio)acetic acid (**2**), 2-(phenylsulfonyl)acetic acid (**3**), 3,4-bis(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**4**) and 4-(2-hydroxyethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**M1**)*

Compounds **2** (Mp: 60–62 °C), **3** (Mp: 110–112 °C), **4** (Mp: 154–156 °C) and **M1** (mp 112–114 °C) were prepared according to the literature procedure.[28]

4.1.2. 4-(2-(Cinnamoyloxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (T_1)

Compound **5** (0.19 g, 1.28 mmol) in DMF solution was mixed with **M1** (0.32 g, 1.07 mmol), EDCI (0.37 g, 1.92 mmol), DMAP (0.02 g, 0.128 mmol). The mixture was stirred at room temperature for 8–12 h. The crude product was purified by column chromatography (petroleum ether-ethyl acetate, 6:1) to yield $\mathbf{T_1}$ as white solid (0.2 g, 43.48%). Mp: 65–67 °C. EI-MS (*m*/*z*): 416.1 (M^+). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.08–7.95 (m, 2H, Ar′-H), 7.84 (t, 1H, *J* = 7.5 Hz, Ar′-H), 7.76(dd, 2H, J_1 = 6.5 Hz, J_2 = 3.0 Hz, Ar′-H), 7.72 (d, 1H, *J* = 15.9 Hz, ArCH=CHCOO–), 7.68 (dd, 2H, J_1 = 5.8 Hz, J_2 = 1.8 Hz, Ar-H), 7.52–7.41 (m, 3H, Ar-H), 6.69 (d, 1H, ArCH=CHCOO–), 4.75–4.66 (m, 2H, –COOCH_2CH_2O–), 4.59–4.50 (m, 2H, –COOCH_2CH_2O–) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.89, 159.63, 146.12, 138.07, 136.98, 134.77, 131.58, 130.85, 129.89, 129.35, 129.21, 118.38, 111.41, 70.30, 62.41.

4.1.3. (E)-4-(2-((3-(4-Hydroxyphenyl)acryloyl)oxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (T_2)

Compound **10** was prepared according to the literature procedure (Mp: 109–110 °C).[29] Compound **10** (0.64 g, 2.5 mmol) in DMF solution was mixed with **M1**(0.60 g, 2.10 mmol), EDCI (0.573 g, 3.00 mmol), DMAP (0.03 g, 0.25 mmol). The mixture was stirred at room temperature for 8–12 h. The crude product was purified by column chromatography (petroleum ether-ethyl acetate, 4:1) to yield **12** as light green oil (0.66 g, 50.19%). To the solu-

tion of compound **12** (0.66 g, 1.30 mmol) in dry CH_2Cl_2 was added $TiCl_4$ (290 μL, 2.60 mmol). The mixture was stirred at room temperature under the atmosphere of N_2. The crude product was purified by column chromatography (chloroform-methanol, 60:1) to yield **T_2** as light green solid (0.32 g, 58.18%). Mp: 151–153 °C. EI-MS (*m*/*z*): 432.1 (M^+). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (s, 1H, Ar-OH), 8.01 (d, 2H, *J* = 7.3 Hz, Ar′-H), 7.85 (t, 1H, *J* = 7.5 Hz, Ar′-H), 7.69 (t, 2H, *J* = 7.9 Hz, Ar′-H), 7.61(d, 1H, *J* = 15.9 Hz, Ar-CH = CH–), 7.60–7.50 (m, 2H, Ar-H), 6.82 (d, 2H, *J* = 8.6 Hz, Ar-H), 6.43 (d, 1H, *J* = 15.9 Hz, Ar-CH = CH–), 4.75–4.62 (m, 2H, –COOCH$_2$-CH$_2$O–), 4.56–4.42 (m, 2H, –COOCH$_2$CH$_2$O–) ppm, ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.84, 160.53, 159.19, 145.92, 137.68, 136.50, 130.90, 130.38, 128.78, 125.43, 116.33, 113.97, 110.94, 70.06, 61.68.

4.1.4. (E)-4-(2-((3-(4-Hydroxy-3-methoxyphenyl)acryloyl)oxy) ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**T_3**)

Compound **T_3** was prepared following the procedure described above. Mp: 130–132 °C. EI-MS (*m*/*z*): 462.1 (M^+). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.68 (s, 1H, Ar-OH), 8.01 (dd, 2H, J_1 = 8.4 Hz, J_2 = 1.1 Hz, Ar′-H), 7.85 (t, 1H, *J* = 7.5 Hz, Ar′-H), 7.73–7.66 (m, 2H, Ar′-H), 7.60 (d, 1H, *J* = 15.9 Hz, Ar-CH = CH–), 7.37 (d, 1H, *J* = 1.8 Hz, Ar-H), 7.15 (dd, 1H, J_1 = 8.2 Hz, J_2 = 1.8 Hz, Ar-H), 6.82 (d, 1H, *J* = 8.2 Hz, Ar-H), 6.54 (d, 1H, *J* = 15.9 Hz, Ar-CH = CH–), 4.74–4.63 (m, 2H, –COOCH$_2$CH$_2$O–), 4.56–4.47 (m, 2H, –COOCH$_2$CH$_2$O–), 3.83 (s, 3H, Ar-OCH$_3$) ppm, ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.90, 159.22, 150.04, 148.46, 146.27, 137.66, 136.53, 130.41, 128.78, 125.94, 123.83, 116.03, 114.27, 111.66, 110.97, 69.98, 61.71, 56.16.

4.1.5. (E)-4-(2-((3-(3,4-Dihydroxyphenyl)acryloyl)oxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (T_4)

Compound **17** was prepared according to the literature procedure.[30] Compound **17** (0.72 g, 2.10 mmol) in DMF solution was mixed with **M1**(0.50 g, 1.75 mmol), EDCI (0.60 g, 2.52 mmol), DMAP (0.025 g, 0.21 mmol). The mixture was stirred at room temperature for 8–12 h. The crude product was purified by column chromatography (petroleum ether-ethyl acetate, 4:1) to yield **20** as light yellow oil (0.62 g, 48.43%). Compound **20** (0.62 g, 1.01 mmol) was dissolved in acetic acid-water (24 mL:6 mL). The mixture was stirred at room temperature under the atmosphere of N_2. The crude product was purified by column chromatography (chloroform-methanol, 90:1) to yield **T_4** as white solid (0.23 g, 51.11%). Mp: 154–156 °C. EI-MS (*m*/*z*): 448.1 (M^+). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.64 (s, 1H, Ar-OH), 9.17 (s, 1H, Ar-OH), 8.05–7.97 (m, 2H, Ar′-H) 7.85 (t, 1H, *J* = 7.5 Hz, Ar′-H), 7.70 (t, 2H, *J* = 7.9 Hz, Ar′-H), 7.53 (d, 1H, *J* = 15.9 Hz, Ar-CH = CHCOO–), 7.08 (d, 1H, *J* = 2.0 Hz, Ar-H), 7.04 (dd, 1H, J_1 = 8.1 Hz, J_2 = 2.0 Hz, Ar-H), 6.79 (d, 1H, *J* = 8.1 Hz, Ar-H), 6.30 (d, 1H, *J* = 15.9 Hz, Ar-CH = CHCOO–), 4.72–4.64 (m, 2H, COOCH$_2$CH$_2$–), 4.54–4.47 (m, 2H, COOCH$_2$CH$_2$O–) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.78, 159.20, 149.09, 146.32, 146.10, 137.68, 136.55, 130.42, 128.78, 125.85, 122.01, 116.26, 115.38, 113.77, 110.96, 69.95, 61.65.

4.1.6. 4-(2-((3,4-Dihydroxybenzoyl)oxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**T_5**)

Compound **T_5** was prepared following the procedure described above. Mp: 145–147 °C. EI-MS (*m*/*z*): 422.0 (M^+). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.91 (s, 1H, Ar-OH), 9.42 (s, 1H, Ar-OH), 7.96 (dd, 2H, J_1 = 8.5 Hz, J_2 = 1.1 Hz, Ar′-H), 7.82 (t, 1H, *J* = 7.5 Hz, Ar′-H), 7.61 (t, 2H, *J* = 7.9 Hz, Ar′-H), 7.40 (d, 1H, *J* = 2.1 Hz, Ar-H), 7.33 (dd, 1H, J_1 = 8.3 Hz, J_2 = 2.1 Hz, Ar-H), 6.84 (d, 1H, *J* = 8.3 Hz, Ar-H), 4.73 (d, 2H, *J* = 4.5 Hz, COOCH$_2$CH$_2$O), 4.58 (d, 2H, *J* = 4.3 Hz, COOCH$_2$CH$_2$O) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.94, 159.22, 151.19, 145.62, 137.70, 136.47, 130.32, 128.65, 122.53, 120.62, 116.96, 115.80, 110.91, 69.95, 61.99.

4.1.7. 4-(2-(2-(3,4-Dihydroxyphenyl)acetoxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**T_6**)

Compound **T_6** was prepared following the procedure described above. Mp: 125–127 °C. EI-MS (*m*/*z*): 436.1 (M^+). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.85 (s, 2H, Ar-OH), 8.01 (dd, 2H, J_1 = 8.5 Hz, J_2 = 1.1 Hz, Ar′-H), 7.90 (t, 1H, *J* = 7.5 Hz, Ar′-H), 7.73 (t, 2H, *J* = 7.9 Hz, Ar′-H), 6.67 (d, 1H, *J* = 2.1 Hz, Ar-H), 6.65 (d, 1H, *J* = 8.0 Hz, Ar-H), 6.50 (dd, 1H, J_1 = 8.3 Hz, J_2 = 2.1 Hz, Ar-H), 4.64–4.59 (m, 2H, COOCH$_2$CH$_2$O), 4.42–4.38 (m, 2H, COOCH$_2$CH$_2$O), 3.50 (s, 2H, ArCH$_2$COO–) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.93, 159.14, 145.57, 144.75, 137.63, 136.56, 130.41, 128.82, 125.14, 120.52, 117.23, 115.91, 110.93, 79.60, 69.78, 62.04.

4.2. Biological evaluation

4.2.1. Antioxidant activity

The antioxidant activity of the newly synthetic compounds were evaluated towards scavenging radicals (DPPH· and OH·) and inhibiting lipid peroxidation.

4.2.1.1. DPPH radical scavenging activity. DPPH· is based on the nitrogen atom as the center of the structure and can be existed stably in organic reagents.[31] The absorption of the DPPH· solution after addition of the antioxygen is reduced at the wavelength of 517 nm. An aliquot (0.5 mL) of 0.08 g/mL DPPH**.** solution was combined with 0.5 mL anhydrous alcohol, pH 5.5 and 0.1 M acetate sodium acetate buffer solution and 0.5 mL sample (antioxygen or anhydrous alcohol reagent). After addition of each component, the solution should be mixed well, then placed at r. t. for 30 min in the dark. The absorption is monitored at 517 nm and the results are expressed as follows:

$$\text{DPPH radical scavenging activity } (\%) = [(\text{Ao} - \text{A1})/\text{Ao})] \times 100$$

wherein Ao is the absorbance of the solvent control reaction and A1 is the absorbance of varying concentrations of samples. Each absorbance was corrected by using blank solution. All tests were performed as parallel for three times.

4.2.1.2. Hydroxyl radical scavenging activity. The assay is based on the reduction of ferrous ions while the color of solution change.[32] Hydroxyl radicals were generated in the Fe^{2+}-Phenanthroline-H_2O_2 system (Fenton reaction). The reaction mixture contained 2.1 mM phenanthroline, 50 mM KH_2PO_4-NaOH buffer (pH 7.4), 2.1 mM freshly prepared $FeSO_4$, 0.03% H_2O_2 (V/V), 100 mM ascorbic acid, solvent, and varying concentrations of tested target compounds. The 10 mL tubes covering reaction solutions were incubated for 60 min at 37 °C. After incubation, the absorbance was measured at 536 nm against an appropriate blank group (without target compounds replaced by an equal amount of distilled water). Hydroxyl radical scavenging percentage was evaluated by comparing the test and blank solutions and the results are calculated as follows:

$$\text{Hydroxyl radical scavenging activity } (\%) = [1 - (A_{s0} - A_{s1})/(A_{b0} - A_{b1})] \times 100.$$

wherein A_{s0} is the absorbance of the sample reaction which not contain the H_2O_2 solution and A_{s1} is the absorbance of the sample reaction which contains the H_2O_2 solution and A_{b0} is the absorbance of the control reaction which not contain the H_2O_2 solution and A_{s1}

is the absorbance of the control reaction which contains the H_2O_2 solution. All absorbance against an appropriate blank mixture (without samples) and all tests were performed as parallel for three times.

4.2.1.3. Anti-lipid peroxidation activity[33]. About 0.10 mL of sample, 0.20 mL of yolk suspension, 0.20 mL of $FeSO_4$ solution (25 mM) and 1.50 mL of 0.20 M sodium phosphate buffer (Na_2HPO_4-NaH_2PO_4, pH 7.45) were mixed well and incubated in water bath at 37 °C for 30 min. After incubation, 1.00 mL of 20% trichloroacetic acid (TCA) was added and centrifuged at 3500*g* for 15 min. Then TBA (1.00 mL, 0.80%) was added and incubated at 100 °C for 15 min and cooled to r. t.. The absorbance was then measured at 532 nm. All tests were performed triply. The results were expressed as follows:

$$\text{Anti-lipid peroxidation activity } (\%) = (A_0 - A_S)/A_0 \times 100.$$

Wherein A_0 is the absorbance of the control reaction and A_S is the absorbance of the sample reaction.

4.2.1.4. Inhibitory activity towards yeast α-glucosidase. The inhibitory activity of target compounds towards yeast α-glucosidase was determined as described in the literature[14] with a slight modification. Briefly, α-glucosidase (from *Saccharomyces cerevisiae*, Sigma Aldrich, USA) was dissolved in phosphate buffer (pH 6.86) at a final concentration of 10 U/mL. The enzyme solution (10 μL) preincubated with the target products (50 μL) at varying concentrations in phosphate buffer (pH 6.86) at 37 °C for 15 min. The reaction was started by the addition of 20 μL 4-nitrophenyl-α-d-glucopyranoside (p-NPG, final concentration 5.3 mM) and stopped after 15 min with 50 μL of 0.5 M Na_2CO_3. The amount of released 4-nitrophenol from p-NPG was measured as the absorbance at 405 nm. In each set of experiments the assay was performed in triplicate and at least two times. The increased absorbance was compared with that of the control containing 50 μL of phosphate buffer in place of the test solution. IC_{50} values were measured graphically by a plot of percent activity versus log of the test compound concentration.

4.2.1.5. Anti-glycosylation activity. Bovine serum albumin (BSA)-methylglyoxal (MGO) system was used to simulate the glycation reactions of the body.[23] A fluorometric spectrophotometry was used to determine the AGEs. For this, taken 2.0 mL BSA into 2.0 mL MGO and mixed well, then added varying concentrations of samples and 2.0 mL PBS. After that the reaction tubes were incubated at 55 °C for 40 h. All tests were carried out in quintic and averaged. The results were expressed as following formula:

$$\text{Inhibition } (\%) = 1 - F_{sample}/F_{blank} \times 100.$$

Where F_{sample} was the fluorescence intensity of the sample, F_{blank} was the fluorescence intensity of the blank control.

4.2.1.6. Anti-platelet aggregation activity[26]. Blood samples were withdrawn from six volunteers and mixed with 3.8% sodium citrate solution (9:1, v/v), followed by centrifuging at 810*g* for 8 min at room temperature. After the resulting platelet-rich plasma (PRP) supernatant was collected, the residue was centrifuged at 3000*g* for another 10 min at room temperature to obtain platelet-poor plasma (PPP). The PRP was adjusted with PPP in order to obtain platelet counts of 400–450 × 10^9 Pl/L. Platelet aggregation was determined by Born's turbidimetric method using a four-channel aggregometer (LBY-NJ4 Platelet-Aggregometer, Beijing, China) within 3 h after blood collection. Briefly, PRP (280 μL) was pre-incubated with vehicle, positive control or different concentrations of individual compounds (10 μL) for 5 min at 37 °C, followed by the addition of 60 μM of Adenosine 5′-diphosphate sodium salt (ADP, Sigma Aldrich, USA) to induce the platelet aggregation. The maximum aggregation rate (MAR) was recorded within 5 min at 37 °C. The inhibition rate of the tested compounds on platelet aggregation was calculated with the following formula:

$$\text{Inhibition rate } (\%) = (100\% - \text{MAR of tested compound})/\text{MAR of vehicle}$$

4.2.1.7. Vasorelaxant ability[34]. Rats were sacrificed by CO_2 asphyxiation. The superior mesenteric arteries of rats were gently removed and immersed in cold Krebs solution (mM): NaCl, 119; KCl, 4.6; $MgCl_2 \cdot 6H_2O$ 1.2; $CaCl_2$, 1.5; $NaH_2PO_4 \cdot 2H_2O$, 1.2; $NaHCO_3$, 15; glucose 5.6, pH 7.4. The arteries, dissected free of adhering tissue under a microscope, were cut into 2 mm-long ring segments. Then, a sensitive myograph (610 M, Myo Technology A/S, Danmark) was used for recording the isometric tension of the artery ring segments. The artery ring segments were threaded onto two 40-μm-diameter stainless steel wires and mounted into the baths filled with 5 mL Krebs solution, which were continuously aerated with 95% O_2 + 5% CO_2. A normalization procedure was applied to obtain an optimal initial tension for the ring segments. After equilibration for 1 h at the initial tension, the contractile capacity of each artery ring segment was tested by exposure to KCl 60 mM solution. After the sustained tension was obtained, the compounds (10^{-10}–$10^{-3.5}$ M) were added to the baths to induce relaxation, respectively. When two reproducible contraction were achieved, the arteries were used for the further experiments.

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