Combination of Paclitaxel and Nitric Oxide as a Novel Treatment for the Reduction of Restenosis

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Received August 26, 2003

The combination of a nitric oxide (NO) donor and a paclitaxel−NO donor conjugate coated on a vascular stent was tested in a rabbit iliac artery model of stenosis as a potential therapy for restenosis. Paclitaxel was conjugated with a NO donor at the 7-position to give compound **7**. An adamantane-based NO donor **14** was synthesized and combined with **7** to provide a burst of NO in the first few critical hours following injury to the vessel wall. Both **7** and **14** demonstrated antiproliferative activity (IC_{50} = 20 nM and 15 μM, respectively) and antiplatelet activity (IC_{50} = 10 and 1 μM, respectively). Stents were coated with a layer of a polymer containing test compounds. The total amount of NO eluted from the stents after a 6 h implantation in the rabbit iliac artery was 35%, 95%, and 69% of the original content for the stents coated with **7**, **14**, and the combination of **7** and **14**, respectively. The antistenotic activity of **7** and **14** was determined in a 28-day rabbit model with two control groups (uncoated stents and polymer-coated stents) and two study groups (paclitaxel-coated stents and stents coated with the combination of **7** and **14**). Polymer-coated stents caused inflammation and increased stenosis by 39% when compared to the uncoated stents. The stents coated with **7** plus **14** were as good as the uncoated stents, 41% better than the polymer-coated stents and 34% better than the paclitaxel-coated stents. These data indicate a beneficial effect of adding NO to an antiproliferative agent (paclitaxel) and suggest a potential therapeutic combination for the treatment of stenotic vessel disease.

Introduction

Blood flow through an obstructed coronary artery can be effectively restored by percutaneous transluminal coronary angioplasty (PTCA). Statistically, 30−40% of patients undergoing PTCA require additional surgical intervention[1] because of a combination of factors including elastic recoil, thrombosis, vessel remodeling, local tissue inflammation, and neointima formation.[2] The incidence of elastic recoil has been reduced by the postangioplasty deployment of vascular stents, which reduce the need for follow-up surgery by some 30%.[3] However, even with stent implantation, restenosis is still a significant medical problem after PTCA.

Nitric oxide (NO), the endothelium-derived relaxing factor, is responsible for regulating vascular tone, blood pressure, smooth muscle proliferation, and platelet aggregation.[4] Controlled delivery of NO to a damaged vessel has been postulated as a promising way of preventing restenosis.[5] The antineoplastic agent paclitaxel has diverse mechanisms of action including microtubule stabilization, interruption of cell mitosis, retardation of cell migration, and immunomodulation.[6] Results of a recent phase III clinical study demonstrate that paclitaxel-coated stents significantly reduce the occurrence of restenosis.[7] Paclitaxel-coated stents have been approved for clinical use in Europe and is expect to reach the U.S. market in 2004.

Because of the complementary biological activities of paclitaxel and NO, the antistenotic profile of paclitaxel may be enhanced by adjunctive NO. In this study, we set out to determine if vascular stents coated with a combination of paclitaxel−NO donor conjugate **7** and an adamantane-based NO donor **14** would be more efficacious than paclitaxel alone in a 28-day rabbit iliac artery model of stenosis. Compounds containing the nitrosothiol functional group were chosen as the NO source. Since the literature[8] indicated that the antiproliferative effects of paclitaxel were least affected by modification at the 7-OH, this position was chosen as the site of derivatization with a NO donor. An ester group was chosen as an appropriate linking functionality because, in the unlikely event that the C7 derivative would be a much less potent antiproliferative agent, hydrolysis of the ester bond would produce paclitaxel itself. Since there is a large difference between the potency of paclitaxel as an antiproliferative agent ($IC_{50} \approx 1$ nM) and the potency of nitrosothiols on cell proliferation and platelets (IC_{50} typically in micromolar), we anticipated that the NO content of **7** was likely to be too low to see a beneficial NO effect from this molecule alone. Envisioning that there might be a requirement to increase the NO load on the stent and realizing that there would be an upper limit of drug that could be physically embedded into the coating matrix, we elected to augment the load with a small-molecule nitrosothiol. From our library of nitrosothiol-containing compounds, adamantane derivatives had the most ap-

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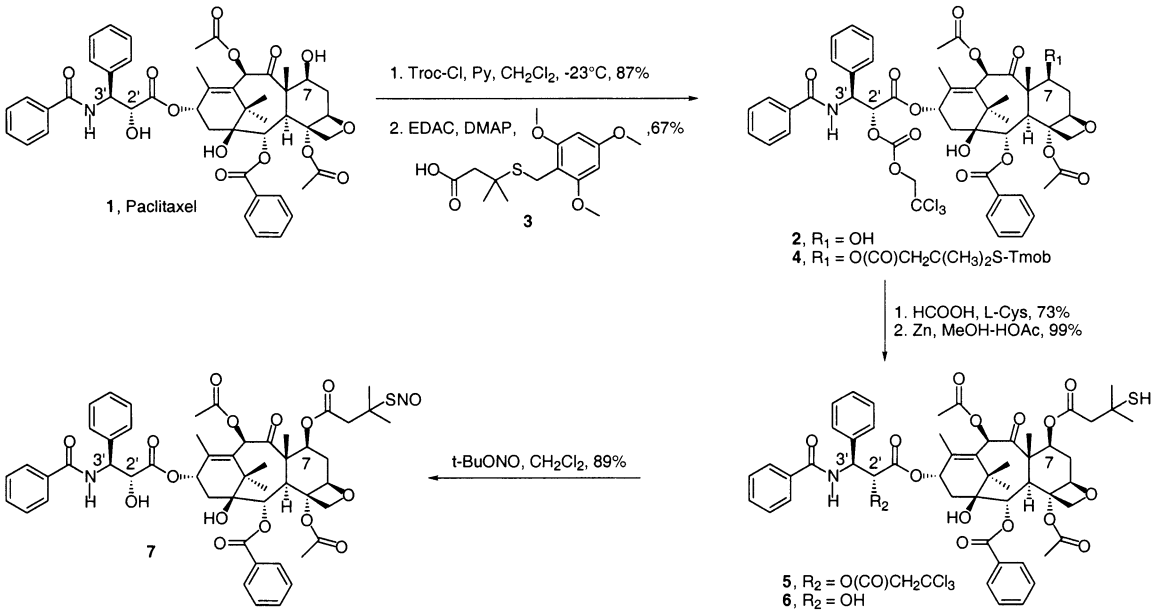
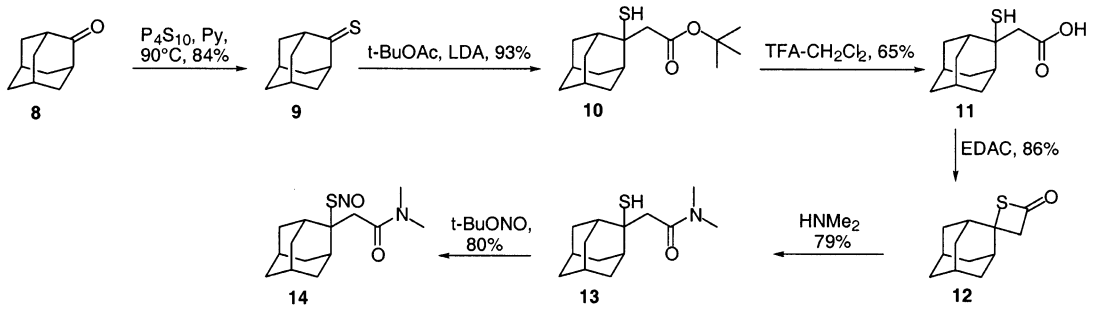
10.1021/jm0304111 CCC: $27.50
Published on Web 03/26/2004

Scheme 1



Scheme 2



propriate combination of nitrosothiol stability and hydrophobicity. For these reasons, the adamantane nitrosothiol **14** was utilized. In earlier studies, we found that low molecular weight nitrosothiols tended to elute much more rapidly from stent coatings. Thus, **7** and **14** were used both to maximize the total NO donor load and to regulate the NO release kinetics from the stent coating. We anticipated that elution of **14** would provide a rapid burst of NO from the vessel wall immediately after injury while that **7** would be released more slowly. This was felt to be an attractive profile, since previous work[9] has shown that the effects of NO are likely to be most beneficial in the early phases of the injury-healing process, whereas a potent antiproliferative agent would be the most effective during the later vessel remodeling phase.

Chemistry

Our previous research had identified many low molecular weight nitrosothiol compounds containing various functional groups, allowing for their covalent conjugation to other molecules to yield NO-containing derivatives. Since paclitaxel has three hydroxyl groups, it seemed most appropriate to consider using a thiol-containing carboxylic acid to incorporate the NO donor. As mentioned above, since the 7-OH position of paclitaxel could be modified without adversely affecting its antiproliferative activity, this position was chosen as the site of conjugation with the NO donor. In order for this to occur successfully, the 2′-OH requires prior protection. Among the reported 2′-protected paclitaxel derivatives,[10] we chose 2′-(2,2,2-trichloroethoxycarbonyl)-protected paclitaxel for its appropriate stability profile and the mild conditions required for protecting group removal.

Reaction of paclitaxel with 2,2,2-trichloroethylchloroformate was conducted as reported[10] on a multigram scale to provide compound **2** (Scheme 1). The 7-OH of compound **2** was then coupled with the acid **3** under standard conditions, using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) to give compound **4**. The subsequent removal of the 2,4,6-trimethoxybenzyl (Tmob) group with L-cysteine in trifluoroacetic acid, conditions previously developed by us for the efficient and convenient removal of this group,[11] caused cleavage of the oxetane. Since paclitaxel survives exposure to formic acid,[10] removal of the Tmob group with L-cysteine in formic acid was attempted with compound **4** and found to work very well to afford **5** in 73% yield (Scheme 1). Subsequent zinc dust and acetic acid treatment provided compound **6** in 99% yield. The thiol in compound **6** was nitrosylated using *tert*-butyl nitrite to give the target paclitaxel−NO donor conjugate **7**.

The adamantane-based nitrosothiol **14** was prepared as shown in Scheme 2. Adamantane-2-thione, **9**, was prepared from adaman-2-one, **8**, and phosphorus pentasulfide (Scheme 2) according to a known procedure.[12] *tert*-Butyl acetate was converted to its enolate with LDA

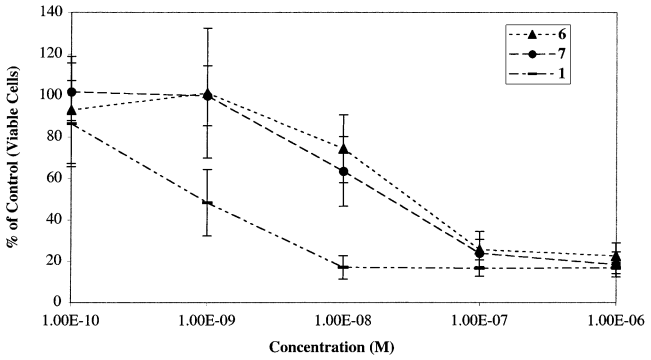


Figure 1. Inhibition of hCASMC proliferation.

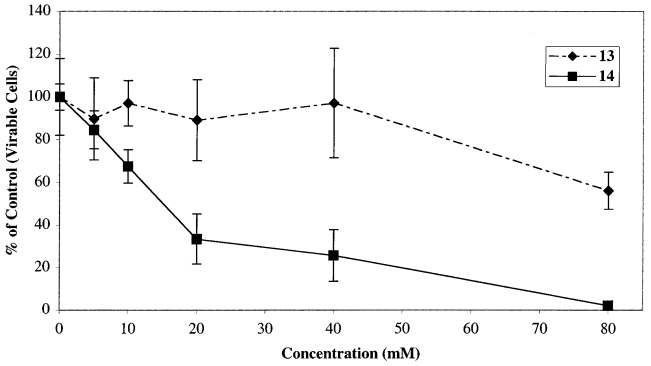


Figure 2. Inhibition of hCASMC proliferation.

and then reacted with the thione **9** to give the tertiary thiol **10** in 93% yield. The ester group was cleaved with trifluoroacetic acid to give the easily isolated crystalline thiol containing acid **11**. Compound **11** was converted to the thiolactone **12** with EDC in 86% yield, and compound **12** was reacted with dimethylamine to give compound **13** in 79% yield. The thiol of **13** was nitrosylated using *tert*-butyl nitrite to give the nitrosothiol **14** in 80% yield.

In Vitro Antiproliferative and Antiplatelet Activities

Compounds **7** and **14** were first tested in vitro for their antiproliferative effects on cultured human coronary artery smooth muscle cells (hCASMC) as well as for their antiplatelet activities in rabbit platelet rich plasma (PRP). As an antiproliferative agent, the paclitaxel−NO donor conjugate **7** ($IC_{50} \approx 20$ nM) was about 20-fold less potent than paclitaxel ($IC_{50} \approx 1$ nM, Figure 1). The antiproliferative potency of the thiol **6** was about the same as the nitrosothiol **7**, indicating that the antiproliferative activity of **7** is due to the action of paclitaxel.

The antiproliferative IC_{50} of **14** was about 15 μM (Figure 2), a value typical of many nitrosothiols. Since the thiol **13** had no significant antiproliferative effect up to 80 μM, we concluded that the antiproliferative activity of the nitrosothiol **14** can be attributed to the NO-donor functionality.

As a platelet antiaggregatory agent, the paclitaxel−NO donor conjugate **7** has an IC_{50} of about 10 μM, whereas the adamantane-based NO donor **14** is approximately 10-fold more potent (Figure 3). That the precursor thiols **6** and **13** are both inactive at the maximum tested concentration indicates that the antiplatelet

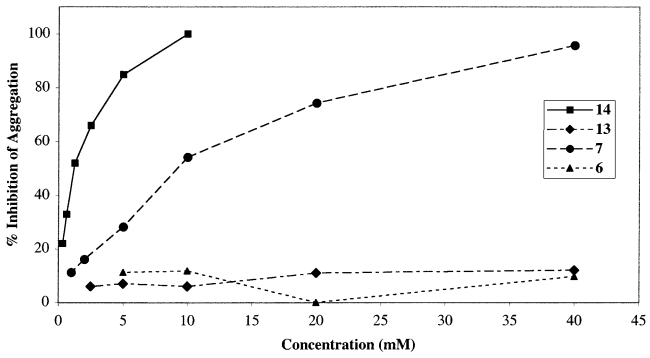


Figure 3. Inhibition of rabbit platelet aggregation in PRP.

Table 1. NO Content of Coated Stents before and after Electron-Beam Radiation

stent	sterilization	mercury displaceable NO[a]
7 coated	before	349 ± 29
	after	316 ± 11
14 coated	before	436 ± 3
	after	440 ± 25
7 + **14** coated	before	733 ± 15
	after	746 ± 89

[a] Values are expressed as the mean ± SD in nmol/stent.

activities of **7** and **14** are attributable to the nitrosothiol functionality.

Stent Coating and Sterilization

Stainless steel stents were spray-coated with polymethylacrylate-based polymer alone or a combination of the polymer and the test compounds in 1-pentanol containing 1% total solids. The polymer contains zwitterionic groups intended to mimic the zwitterionic structure of naturally occurring phospholipids and long hydrocarbon chains intended to mimic the lipid tails of phospholipids. The stents were dried at room temperature under vacuum overnight and examined by light microscopy for coating uniformity. The coating integrity was checked by balloon expanding stents from each treatment group and then examining them by light microscopy for signs of flaking or cracking. The weights of stents before and after balloon expansion were also compared and found not to differ as a result of the procedure. All stents utilized in vivo were sterilized by electron-beam radiation.

The NO content of the stents was determined by dissolving the stent coating and analyzing the solution by ozone chemiluminesence.[13] The results in Table 1 demonstrate that there is no statistically significant loss of nitrosothiol content following sterilization by electron-beam radiation.

In Vivo NO Release Profile

The complex etiology of restenosis involves several sequential events: elastic recoil, thrombosis, vessel wall remodeling, and neointima formation. Therefore, an attractive profile for NO release might be an initial burst of NO to reduce platelet deposition at the injury site followed by a sustained release to decrease smooth muscle cell proliferation. To determine the in vivo NO release profile, coated stents were implanted into rabbit iliac arteries and harvested after 6 h. The coating was then extracted from the stents, and the extract was analyzed for the total nitrosothiol content by ozone

Table 2. In Vivo NO Release Profile

stent (elution time, h)	mercury displaceable NO[a]	remaining NO, %
7 ($t = 0$)	369 ± 8	
7 ($t = 6$)	214 ± 54	65
14 ($t = 0$)	417 ± 43	
14 ($t = 6$)	18 ± 4	5
7 + **14** ($t = 0$)	627 ± 36	
7 + **14** ($t = 6$)	209 ± 13	31

[a] Values are expressed as the mean ± SD in nmol/stent.

Table 3. Stenosis in Rabbit Iliac Arteries

stents	% stenosis[a]	difference from bare stents,[b] %	difference from polymer-coated stents,[b] %	net effect of NO over paclitaxel-coated stents,[b] %
uncoated[c]	18.4 ± 1.7			
polymer[c]	25.5 ± 4.4	39[f]		
paclitaxel[d]	22.6 ± 3.6	23[g]	−11[i]	
7 + **14**[e]	15.0 ± 5.1	−18[h]	−41[f]	−34[f]

[a] Values are expressed as the mean ± SD. [b] Statistic analysis is done using the *t*-test. [c] 10 rabbits per group. [d] 11 rabbits per group. [e] 12 rabbits per group. [f] $P < 0.001$. [g] $P < 0.01$. [h] $P = 0.053$. [i] $P = 0.101$.

chemiluminesence. After 6 h, stents coated with compound **7** retained 65% of their nitrosothiol content, whereas stents coated with compound **14** retained only 5% of their original nitrosothiol load (Table 2). Stents coated with **7** and **14** (1:1.4, mol/mol) retained 31% of their NO content.

In Vivo Stenosis Experiment in Rabbits

Stents were implanted in the iliac arteries of New Zeland White rabbits (1 stent/rabbit) under fluoroscopic guidance with angiography to confirm the position of the deployed stent. The animals were sacrificed 28 days after implantation, and the stented arteries were collected and fixed in formalin prior to histologic analysis. Both stent implantation and histopathological evaluation were performed in a blinded fashion. In this study, bare metal stents and stents coated with polymer alone served as controls. The drug-coated stent groups included a group coated only with paclitaxel and a group coated with a combination of compounds **7** and **14**. From this experimental design, any difference between these two drug-coated stent groups would be indicative of a net NO effect on stenosis.

Bare stents ($n = 10$), polymer-coated stents (744 ± 34 μg of polymer/stent; $n = 10$), paclitaxel-coated stents (381 ± 30 nmol of paclitaxel in 651 ± 52 μg of polymer/stent; $n = 11$), and stents coated with compounds **7** and **14** (352 ± 7 nmol of **7** and 501 ± 11 nmol of **14** in 987 ± 21 μg of polymer/stent; $n = 12$) were used in this study. The results of the morphometric analysis of the stent sections from each group expressed as percent stenosis are summarized in Table 3. The detailed morphometry for this analysis is provided in the Supporting Information.

Histopathologic examination of the polymer-alone coated stents showed significant inflammation in the tissue surrounding the stent, and this in turn is thought to have caused the increased stenosis (+39%) observed in this group relative to the uncoated stents. The paclitaxel-coated stents were able to moderate the increased stenosis caused by the polymer, but the paclitaxel group still showed an overall increase in stenosis of 23% over the uncoated stents. Stents coated with compounds **7** and **14** showed an 18% reduction in stenosis that is almost significant ($P = 0.053$) compared to bare stents.

Since the polymer coating caused inflammation and increased stenosis, we felt it would be reasonable to assay the efficacy of the tested compounds by comparison with the group coated with polymer alone. By use of this comparison, the group coated with compounds **7** and **14** showed a 41% reduction in stenosis over the group coated with polymer alone. The difference between the group coated with polymer alone and the group coated with paclitaxel was not statistically significant.

When comparing two drug-coated stent groups for a net effect of NO, the 34% less stenosis in the group coated with compounds **7** and **14** over the group coated with paclitaxel demonstrated that in this study there was a beneficial reduction of stenosis attributable to the actions of the NO donor.

Conclusions

In this study, novel *S*-nitrosothiol derivatives of paclitaxel and an adamantane amide have been synthesized and shown to exert both antiproliferative and antiplatelet activities. Data from our in vivo rabbit study indicate that a combination of an NO donor and an antiproliferative agent might be effective in the prevention of restenosis. A polymer with an improved biocompatibility profile is needed for future studies to confirm this hypothesis.

Experimental Section

General Methods. All reagents and solvents were obtained from commercial sources and used without further purification. Flash chromatography was performed on silica gel (Merck, 230−400 mesh). ^{1}H and ^{13}C NMR were recorded on a Brucker AMX-300 instrument. Low-resolution mass spectra were recorded on a Perkin-Elmer API-150EX spectrometer with atmospheric pressure turbo ion spray. Elemental analyses were done at Robertson Microlit Laboratories, Madison, NJ.

2′-(2,2,2-Trichloroethoxycarbonyl)paclitaxel (2). To paclitaxel (**1**, 1.3777 g, 1.6134 mmol) under argon was added dichloromethane (26 mL) at room temperature. The solution was cooled to −23 ± 3 °C (internal temperature), and pyridine (2.8 mL) was added. 2,2,2-Trichloroethylchloroformate (225 μL, 346 mg, 1.63 mmol) was slowly added. Stirring was maintained at −23 ± 3 °C for 45 min, and then more 2,2,2-trichloroethylchloroformate (200 μL, 308 mg, 1.45 mmol) was added. Stirring at −23 ± 3 °C was continued for an additional 45 min. The reaction solution was diluted with dichloromethane, washed with water and brine, dried (sodium sulfate), concentrated, and purified by chromatography (silica gel, ethyl acetate/hexane 1:3, then ethyl acetate/hexane 2:3) to give **2** (1.4988 g, 1.4561 mmol, 90%): mp 164−173 °C; ^{1}H NMR ($CDCl_3$−D_2O) δ 8.16−8.14 (m, 2 H), 7.77−7.74 (m, 2 H), 7.64−7.59 (m, 1 H), 7.54−7.49 (m, 3 H), 7.42−7.38 (m, 7 H), 6.93 (d, $J = 9.3$ Hz, 1 H), 6.29 (s, 1 H), 6.29 (t, $J = 9.1$ Hz, 1 H), 6.05 (dd, $J = 2.6$ and 9.3 Hz, 1 H), 5.69 (d, $J = 7.0$ Hz, 1 H), 5.44 (d, $J = 2.8$ Hz, 1 H), 4.97 (d, $J = 8.2$ Hz, 1 H), 4.78 (AB q, $J = 11.9$ Hz, $\Delta\nu_{AB} = 17.8$ Hz, 2 H), 4.43 (dd, $J = 6.6$ and 10.8 Hz, 1 H), 4.31 (d, $J = 8.4$ Hz, 1 H), 4.20 (d, $J = 8.4$ Hz, 1 H), 3.82 (d, $J = 7.0$ Hz, 1 H), 2.55 (m, 1 H), 2.47 (s, 3 H), 2.41 (m, 1 H), 2.22 (s, 3 H), 2.22 (m, 1 H), 1.91 (s, 3 H), 1.91 (m, 1 H), 1.68 (s, 3 H), 1.24 (s, 3 H), 1.14 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 203.7, 171.2, 169.9, 167.3, 167.2, 167.0, 153.3, 142.3, 136.4, 133.6, 133.4, 133.0, 132.1, 130.2, 129.2, 128.7, 127.2, 126.6, 93.9, 84.4, 81.1, 79.1, 77.6, 77.2, 76.4, 75.5, 75.1, 72.4, 72.1, 58.5, 52.7, 45.6, 43.2,

35.5, 29.7, 26.8, 22.7, 22.1, 20.1, 14.7, 9.6; LRMS (APIMS) *m/z* 1028 (MH^+), 1046 ($M + NH_4^+$).

3-Methyl-3-(2,4,6-trimethoxyphenylmethylthio)butyric Acid (3). To a solution of 3-mercapto-3-methylbutyric acid[14] (4.6 g, 34 mmol) in dichloromethane (250 mL) under nitrogen and cooled over ice/salt to 5 °C (internal temperature) was added trifluoroacetic acid (82 g, 0.72 mol). To this was then added dropwise a solution of 2,4,6-trimethoxybenzyl alcohol[15] (6.45 g, 32 mmol) in dichloromethane (150 mL) such that the reaction temperature did not rise above 5 °C. After the addition was complete, the mixture was stirred for an additional 5 min at 5 °C and the volatiles were removed in vacuo. The residue was partitioned between diethyl ether and water, and the organic phase was dried over anhydrous sodium sulfate and filtered, and the volatile material was removed in vacuo. The residue was treated with activated charcoal and recrystallized from diethyl ether/hexane to give the title compound **3** (7 g, 70%): mp 103−105 °C; ^{1}H NMR ($CDCl_3$) δ 10.5 (broad s, 1 H), 6.12 (s, 2 H), 3.80−3.85 (m, 11 H), 2.74 (s, 2 H), 1.47 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 173.9, 160.6, 158.6, 105.6, 90.5, 55.7, 55.3, 45.9, 43.6, 28.4, 21.0. Anal. ($C_{15}H_{22}O_5S$) C, H.

2′-(2,2,2-Trichloroethoxycarbonyl)-7-[3-methyl-3-(2,4,6-trimethoxyphenylmethylsulfanyl)butyryl]paclitaxel (4). To compound **2** (2.3804 g, 2.3126 mmol) in dichloromethane (50 mL) was added **3** (2.4110 g, 7.6686 mmol), 4-(dimethylamino)pyridine (509.7 mg, 4.172 mmol), and then 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (1.4583 g, 7.6072 mmol) at room temperature. The reaction mixture was stirred overnight and washed with water, saturated sodium bicarbonate, 0.2 M citric acid, saturated sodium bicarbonate, and brine. The organic phase was dried (sodium sulfate), concentrated, and purified by chromatography twice (silica gel, ethyl acetate/hexane 1:3, and silica gel, methanol/dichloromethane 1:99) to give the title compound **4** (2.0752 g, 1.5653 mmol, 67%): mp 129−138 °C; ^{1}H NMR ($CDCl_3$−D_2O) δ 8.15−8.12 (m, 2 H), 7.77−7.74 (m, 2 H), 7.61 (m, 1 H), 7.54−7.49 (m, 3 H), 7.42−7.38 (m, 7 H), 6.94 (d, J = 9.3 Hz, 1 H), 6.28 (s, 1 H), 6.26 (t, J = 9.3 Hz, 1 H), 6.10 (s, 2 H), 6.05 (dd, J = 2.6 and 9.3 Hz, 1 H), 5.68 (m, 2 H), 5.56 (d, J = 2.8 Hz, 1 H), 4.97 (d, J = 8.2 Hz, 1 H), 4.78 (AB q, J = 11.9 Hz, $\Delta\nu_{AB}$ = 17.8 Hz, 2 H), 4.34 (d, J = 8.4 Hz, 1 H), 4.29 (d, J = 8.4 Hz, 1 H), 3.98 (d, J = 6.8 Hz, 1 H), 3.82 (s, 6 H), 3.79 (s, 2 H) 3.78 (s, 3 H), 2.71 (s, 2 H), 2.67 (m, 1 H), 2.47 (s, 3 H), 2.42 (m, 1 H), 2.22 (m, 1 H), 2.14 (s, 3 H), 2.00 (s, 3 H), 1.92 (m, 1 H), 1.83 (s, 3 H), 1.47 (s, 3 H), 1.41 (s, 3 H), 1.21 (s, 3 H), 1.17 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 201.9, 170.0, 169.6, 168.6, 167.4, 167.0, 166.9, 160.2, 158.6, 153.2, 140.9, 136.4, 133.7, 133.5, 132.7, 132.0, 130.2, 129.2, 129.1, 128.7, 128.6, 127.1, 126.6, 107.7, 93.9, 90.7, 84.1, 81.0, 78.6, 77.5, 77.2, 76.3, 75.2, 74.6, 72.3, 71.4, 56.0, 55.7, 55.3, 52.8, 47.0, 46.6, 43.8, 43.3, 35.3, 33.2, 28.7, 28.0, 26.5, 22.7, 21.2, 20.8, 20.6, 14.5, 10.9; LRMS (APIMS) *m/z* 1341 ($M + NH_4^+$).

2′-(2,2,2-Trichloroethoxycarbonyl)-7-(3-methyl-3-sulfanylbutyryl)paclitaxel (5). L-Cysteine (7.21 g, 59.5 mmol) was dissolved in formic acid (100 mL). Compound **4** (1.6357 g, 1.2338 mmol) in dichloromethane (100 mL) was added at room temperature to give a colorless solution. The reaction mixture was stirred at room temperature for 40 min, concentrated to dryness, treated with ethyl acetate, and washed with saturated sodium bicarbonate three times and brine twice. The organic phase was dried (sodium sulfate), concentrated, and purified by chromatography (silica gel, ethyl acetate/hexane 1:4, then ethyl acetate/hexane 1:3) to give the title compound **5** (1.3099 g, 1.1435 mmol, 73%): mp 157−164 °C; ^{1}H NMR ($CDCl_3$−D_2O) δ 8.15−8.11 (m, 2 H), 7.77−7.74 (m, 2 H), 7.62 (m, 1 H), 7.54−7.48 (m, 3 H), 7.44−7.35 (m, 7 H), 6.94 (d, J = 9.3 Hz, 1 H), 6.26 (s, 1 H), 6.26 (t, J = 9.3 Hz, 1 H), 6.05 (dd, J = 2.7 and 9.3 Hz, 1 H), 5.69 (d, J = 6.9 Hz, 1 H), 5.63 (dd, J = 7.0 and 11.3 Hz, 1 H), 5.55 (d, J = 2.8 Hz, 1 H), 4.96 (d, J = 8.3 Hz, 1 H), 4.78 (AB q, J = 11.9 Hz, $\Delta\nu_{AB}$ = 17.8 Hz, 2 H), 4.33 (d, J = 8.4 Hz, 1 H), 4.19 (d, J = 8.4 Hz, 1 H), 3.97 (d, J = 6.8 Hz, 1 H), 2.64 (m, 3 H), 2.48 (s, 3 H), 2.42 (m, 1 H), 2.24 (m, 1 H), 2.15 (s, 3 H), 1.99 (s, 3 H), 1.89 (m, 1 H), 1.82 (s, 3 H), 1.49 (s, 3 H), 1.45 (s, 3 H), 1.21 (s, 3 H), 1.17 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 201.9, 169.7, 169.6, 168.7, 167.4, 167.2, 166.9, 153.2, 140.9, 136.4, 133.7, 133.5, 132.7, 132.0, 130.2 129.2, 129.1, 128.7, 128.6, 127.1, 126.6, 93.8, 83.9, 80.9, 78.6, 77.5, 77.2, 76.3, 75.2, 74.5, 72.3, 71.6, 56.0, 52.7, 50.2, 46.9, 43.3, 41.7, 35.3, 33.4, 32.9, 32.3, 26.4, 22.6, 21.2, 20.7, 14.4, 10.9; LRMS (APIMS) *m/z* 1161 ($M + NH_4^+$). Anal. ($C_{55}H_{60}NO_{17}SCl_3$) C, H, N.

7-(3-Methyl-3-sulfanylbutyryl)paclitaxel (6). To compound **5** (1.2353 g, 1.0784 mmol) in methanol/acetic acid (9:1, 80 mL) was added zinc dust (4.5349 g, 69.352 mmol). The reaction suspension was stirred at room temperature for 20 min. The zinc dust was removed by filtration. The filtrate was concentrated to dryness, treated with dichloromethane, and washed with water and brine. The organic phase was dried (sodium sulfate), concentrated, and purified by chromatography (silica gel, ethyl acetate/hexane 1:3, then ethyl acetate/hexane 8:17) to give the title compound **6** (1.0353 g, 1.0672 mmol, 99%): mp 161−166 °C; ^{1}H NMR ($CDCl_3$) δ 8.13−8.10 (m, 2 H), 7.78−7.75 (m, 2 H), 7.62 (m, 1 H), 7.53−7.48 (m, 3 H), 7.44−7.34 (m, 7 H), 7.07 (d, J = 8.9 Hz, 1 H), 6.21 (s, 1 H), 618 (t, J = 9.3 Hz, 1 H), 5.81 (dd, J = 2.1 and 9.0 Hz, 1 H), 5.67 (d, J = 6.8 Hz, 1 H), 5.59 (dd, J = 7.2 and 10.0 Hz, 1 H), 4.95 (d, J = 8.7 Hz, 1 H), 4.79 (d, J = 2.5 Hz, 1 H), 4.32 (d, J = 8.4 Hz, 1 H), 4.19 (d, J = 8.4 Hz, 1 H), 3.93 (d, J = 6.8 Hz, 1 H), 3.62 (broad s, 1 H), 2.64 (m, 3 H), 2.38 (s, 3 H), 2.34 (m, 2 H), 2.16 (s, 3 H), 1.89 (m, 1 H), 1.85 (s, 3 H), 1.83 (s, 3 H), 1.76 (broad s, 1 H), 1.67 (broad s, 1 H), 1.49 (s, 3 H), 1.45 (s, 3 H), 1.20 (s, 3 H), 1.17 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 201.8, 172.4, 170.4, 169.9, 168.8, 166.9, 140.4, 138.0, 133.8, 133.7, 133.0, 131.9, 130.2, 129.1, 129.0, 128.7, 128.3, 127.1, 127.0, 83.9, 81.1, 78.5, 77.2, 75.3, 74.3, 73.3, 72.2, 71.7, 56.1, 54.9, 50.3, 47.1, 43.2, 41.8, 35.6, 33.5, 33.0, 32.4, 26.6, 22.5, 20.8, 14.7, 10.9; LRMS (APIMS) *m/z* 970 (MH^+), 987 ($M + NH_4^+$), 992 ($M + Na^+$). Anal. ($C_{52}H_{59}NO_{15}S$) C, H, N.

7-(3-Methyl-3-nitrosothiobutyryl)paclitaxel (7). To compound **6** (659.2 mg, 0.6795 mmol) in dichloromethane (8.5 mL) was added *tert*-butyl nitrite (120 μL, 104 mg, 1.01 mmol) at room temperature. The reaction mixture was stirred at room temperature for 15 min, concentrated to dryness, and dissolved in dichloromethane. The dichloromethane solution was washed with water and brine. The organic phase was dried (sodium sulfate), concentrated, dried in a vacuum, and purified by chromatography (silica gel, ethyl acetate/hexane 1:3, then ethyl acetate/hexane 1:2) to give the title compound **7** (602.5 mg, 0.6030 mmol, 89%) as a green solid: mp 165−170 °C (melt to a brown oil, losing green color between 120 and 130 °C); ^{1}H NMR ($CDCl_3$−D_2O) δ 8.11−8.08 (m, 2 H), 7.76−7.74 (m, 2 H), 7.61 (m, 1 H), 7.52−7.46 (m, 3 H), 7.42−7.31 (m, 7 H), 7.12 (d, J = 8.9 Hz, 1 H), 6.19 (s, 1 H), 616 (t, J = 8.8 Hz, 1 H), 5.79 (dd, J = 2.0 and 8.7 Hz, 1 H), 5.65 (d, J = 6.8 Hz, 1 H), 5.57 (dd, J = 7.2 and 10.3 Hz, 1 H), 4.91 (d, J = 8.7 Hz, 1 H), 4.78 (d, J = 2.3 Hz, 1 H), 4.30 (d, J = 8.4 Hz, 1 H), 4.17 (d, J = 8.4 Hz, 1 H), 3.90 (d, J = 6.7 Hz, 1 H), 3.25 (s, 2 H), 2.52 (m, 1 H), 2.36 (s, 3 H), 2.31 (m, 2 H), 2.16 (s, 3 H), 1.99 (s, 3 H), 1.95 (s, 3 H), 1.88 (m, 1 H), 1.82 (s, 3 H), 1.77 (s, 3 H), 1.19 (s, 3 H), 1.15 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 201.7, 172.3, 170.4, 169.2, 168.9, 167.0, 166.8, 140.4, 138.0, 133.7, 133.6, 132.9, 131.9, 130.1, 129.0, 128.9, 128.7, 128.3, 127.04, 127.02, 83.8, 81.0, 78.5, 76.4, 75.2, 74.2, 73.2, 72.1, 71.9, 56.0, 54.9, 53.6, 47.1, 47.0, 43.2, 35.5, 33.3, 29.5, 28.6, 26.5, 22.5, 20.71, 20.76, 14.6, 10.8; LRMS (APIMS) *m/z* 999 (MH^+), 1016 ($M + NH_4^+$), 1021 ($M + Na^+$). Anal. ($C_{52}H_{58}N_2O_{16}S$) C, H, N.

Adamantane-2-thione (9). 2-Adamantanone **8** (48.46 g, 322.6 mmol) in pyridine (300 mL) was heated to 90 °C, and phosphorus pentasulfide (17.84 g, 40.13 mmol) was added. The reaction mixture was stirred at 90 °C for 2 h, and heating was turned off. The reaction mixture was set at room temperature to cool slowly. After sitting at room temperature overnight, a gum phase and a solution phase were formed. The pyridine solution phase was decanted and concentrated to dryness. The residual semisolid was treated with hexane (400 mL) to give an orange solution containing a light-brown suspension. The suspension was removed by filtration. The filtrate was concentrated to dryness to give an orange solid (50.36 g) after

drying in a vacuum. This product was purified by chromatography (silica gel, hexane) to give the title compound **9** (45.15 g, 271.5 mmol, 84%): ^{1}H NMR ($CDCl_3$) δ 3.43 (s, 2 H), 2.1−1.9 (m, 12 H); ^{13}C NMR ($CDCl_3$) δ 222.4, 57.5, 41.1, 36.5, 27.4.

***tert*-Butyl 2-(2-Sulfanyladamantan-2-yl)acetate (10).** To *tert*-butyl acetate (25 mL, 21.6 g, 186 mmol) in dry THF (400 mL) at −78 °C was added lithium diisopropylamide monotetrahydrofuran (1.5 M solution in cyclohexane, 100 mL, 150 mmol) under nitrogen. The mixture was stirred at −78 °C for 40 min. Compound **9** (21.88 g, 131.57 mmol) in THF (400 mL) was added. The cold bath was removed, and the reaction was stirred at room temperature for 2 h. The reaction mixture was diluted with dichloromethane, and 2 M HCl (75 mL) was added. The organic phase was separated, washed with brine (4 × 40 mL), dried (magnesium sulfate), filtered, and concentrated. The crude product was purified by column chromatography (silica gel, ethyl acetate/hexane 1:19) to give the title compound (34.67 g, 122.7 mmol, 93%): mp 56−62 °C; ^{1}H NMR ($CDCl_3$) δ 2.87 (s, 2 H), 2.47 (m, 2 H), 2.38 (s, 1 H), 2.11 (m, 2 H), 1.98 (s, 2 H), 1.96 (m, 2 H), 1.84−1.62 96 (m, 6 H), 1.47 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 170.7, 80.7, 54.0, 47.2, 38.9, 38.1, 33.9, 33.2, 28.1, 27.4, 26.8; LRMS (APIMS) *m/e* 283 (MH^+). Anal. ($C_{16}H_{26}O_2S$) C, H.

2-(2-Sulfanyladamantan-2-yl)acetic Acid (11). To compound **10** (10.76 g, 38.1 mmol) in dichloromethane (15 mL) was added trifluoroacetic acid (15 mL). The reaction mixture was stirred at room temperature for 40 min and concentrated to dryness. The residue was treated with dichloromethane and concentrated to dryness three times. The residual solid was triturated with dichloromethane (20 mL). The solid was collected by filtration, washed with a small amount of dichloromethane, and dried in a vacuum to give the title compound (5.6447 g, 24.94 mmol, 65%): mp 178−180 °C; ^{1}H NMR ($CDCl_3$) δ 9.5 (broad, 1 H), 3.04 (s, 2 H), 2.49 (m, 2 H), 2.25 (s, 1 H), 2.1−2.0 (m, 4 H), 1.9 (m, 2 H), 1.7−1.6 (m, 6 H); ^{13}C NMR ($CDCl_3$) δ 177.7, 53.4, 46.3, 38.9, 37.8, 33.8, 33.2, 27.4, 26.8; LRMS (APIMS) *m/e* 225 ($M - H^-$). Anal. ($C_{12}H_{18}O_2S$) C, H.

Spiro[adamantane-2,4′-thietane]-12-one (12). To a solid mixture of compound **11** and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (444.8 mg, 2.320 mmol) was added dichloromethane (10 mL). The reaction solution was stirred at room temperature for 1 h and diluted with dichloromethane and washed with 0.1 M HCl and brine. The organic phase was dried (magnesium sulfate), filtered, concentrated, and purified by chromatography (silica gel, ethyl acetate/hexane 1:3, then 1:1) to give the title compound **12** (0.41 g, 1.97 mmol, 86%): mp 77−78 °C; ^{1}H NMR ($CDCl_3$) δ 3.61 (s, 2 H), 2.20 (m, 2 H), 1.95−1.78 (m, 12 H); ^{13}C NMR ($CDCl_3$) δ 191.8, 63.4, 54.9, 39.9, 36.5, 35.6, 33.7, 26.6, 25.8; LRMS (APIMS) *m/z* 209 (MH^+), 226 ($M + NH_4^+$). Anal. ($C_{12}H_{16}OS$) C, H.

***N*,*N*-Dimethyl-2-(2-sulfanyladamantan-2-yl)acetamide (13).** To compound **12** (1.35 g, 6.48 mmol) in dichloromethane (15 mL) at room temperature was added dimethylamine (2.0 M in methanol, 5.5 mL, 11 mmol). The reaction solution was stirred at room temperature for 40 min and concentrated to dryness. The crude product was purified by chromatography (silica gel, dichloromethane) to give the title compound **13** (1.30 g, 5.13 mmol, 79%): mp 69−71 °C; ^{1}H NMR ($CDCl_3$) δ 3.09 (s, 2 H), 3.00 (s, 3 H), 2.97 (s, 3 H), 2.57−2.53 (m, 3 H), 2.16 (m, 2 H), 2.11−2.07 (m, 2 H), 1.86 (m, 2 H), 1.76−1.70 (m, 4 H), 1.65−1.60 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 171.2, 54.6, 42.8, 39.2, 38.0, 37.8, 35.4, 33.8, 33.4, 27.7, 27.0; LRMS (APIMS) *m/z* 254 (MH^+), 507 ($2M + H^+$), 524 ($2M + NH_4^+$), 529 ($2M + Na^+$). Anal. ($C_{14}H_{23}NOS$) C, H, N.

***N*,*N*-Dimethyl-2-[2-(nitrosothio)adamantan-2-yl]acetamide (14).** To compound **13** (449.7 mg, 1.774 mmol) in dichloromethane (5 mL) was added *tert*-butyl nitrite (430 μL, 373 mg, 3.62 mmol) at room temperature. The reaction solution was stirred at room temperature for 20 min, concentrated to dryness, and then treated with dichloromethane and water. The organic phase was separated, dried (magnesium sulfate), filtered, and concentrated. The crude product was purified by chromatography (silica gel, dichloromethane) to give the title compound **14** (398.9 mg, 1.412 mmol, 80%): mp 62−64 °C; ^{1}H NMR ($CDCl_3$) δ 3.73 (s, 2 H), 3.02 (s, 2 H), 2.82 (s, 6 H), 2.45−2.41 (m, 2 H), 2.13−2.08 (m, 3 H), 1.96−1.92 (m, 3 H), 1.86 (m, 2 H), 1.77−1.70 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 170.1, 67.4, 39.4, 38.9, 37.8, 35.6, 35.3, 33.9, 33.4, 27.32, 27.28; LRMS (APIMS) *m/z* 283 (MH^+), 565 ($2M + H^+$), 582 ($2M + NH_4^+$). Anal. ($C_{14}H_{22}N_2O_2S$) C, H, N.

In Vitro Platelet Aggregation Assay. A New Zealand white rabbit was premedicated with acepromazine (0.1−0.2 mg/kg) and anesthetized with a ketamine/xylazine mixture (40 and 5 mg/kg, respectively). Blood was drawn from the right common carotid artery with a 5F introducer sheath. After allowance of a few milliliters of blood to spill away, 10 volumes of blood were collected into a sterile syringe containing 1 volume of 3.8% trisodium citrate, pH 6.5. The blood was mixed by careful inversion a few times, then gently transferred to a 15 mL polypropylene centrifuge tube prior to centrifugation at 180*g* for 15 min at 21 °C. The turbid platelet-rich plasma (PRP) was collected and used for the aggregation assay. The PRP was first preincubated with each test compound (dissolved at 500× in ethanol) for 2 min at 37 °C prior to induction of aggregation with ADP (5−10 μM). Aggregation is measured in a Chrono-Log Lumi-Ionized Calcium Aggregometer, model 560-Ca, Chrono-Log Corp., Havertown, PA. The percent inhibition of aggregation was calculated relative to the maximum aggregation amplitude.

In Vitro Human Coronary Artery Smooth Muscle Cell (hCASMC) Antiproliferation Assay. hCASMC samples were seeded at 3×10^4 cells in 2 mL of the commercial growth medium SmGM2 (Clonetics Corp., San Diego, CA) per well of a 24-well tissue culture plate. The test compounds (dissolved at 1000× in ethanol) were added at the indicated concentrations to the cells ($n = 4$ per data point) after cell attachment (3−4 h after seeding). Control cultures received an equal volume of ethanol. Three days (68−72 h) later, cultures were trypsinized, and viable cells were counted as Trypan Blue negative cells with a hemacytometer (results are the mean ± SD).

In Vivo NO Release. New Zealand White rabbits on a normal diet were premedicated with acepromazine (0.1−0.2 mg/kg) and anesthetized with ketamine (40−50 mg/kg). The femoral artery was isolated with airtight ligatures, and access was obtained via the superficial femoral artery. Stents were deployed as follows. By use of fluoroscopic guidance, a steerable guide wire was inserted into a 5F sheath, bypassing the heart, and was placed into the iliac artery lumen. A 3.0 balloon with the stent was advanced over the guidewire and into the iliac artery. The balloon was inflated to 8 atm for 30 s and deflated by negative pressure held for 30 s. Angiography confirmed the position of the deployed stent. Six hours later, the animal was euthanized with 120 mg/kg of intravenous sodium pentobarbital. The stented iliac artery was collected, and the stent was recovered from the arteries for NO analysis.

In Vivo Stenosis Experiment. New Zealand White rabbits on a normal diet were premedicated with acepromazine (0.1−0.2 mg/kg) and anesthetized with ketamine (40−50 mg/kg). The femoral artery was isolated with airtight ligatures, and access was obtained via the superficial femoral artery. Stents were deployed as detailed above. Postintervention drug treatment included aspirin (90 mg, po), heparin (1000 units), and gentamycin (2−4 mg/kg, im). Following recovery, the animal was held for 28 days, at which time the animal was euthanized with 120 mg/kg of intravenous sodium pentobarbital. The vessel was fixed with buffered formalin, and the stented iliac artery was collected. The harvested artery was sectioned for histological analysis with computer imaging. Both stent implantation and histopathological evaluation were done in blinded fashion.

Supporting Information Available: Morphometic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0304111