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New Thioderivatives of Gossypol and Gossypolone, as Prodrugs of Cytotoxic Agents

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Abstract—New dithiane or dithiolane derivatives of gossypol and gossypolone were synthesized with dithiolethane or dithiolpropane in the presence of BF_3/Et_2O. These thioderivatives exhibited low toxicity on KB cells (human epidermoid carcinoma cells of the mouth). They react easily with electrophiles in aprotic solvents to regenerate gossypolone or to form dehydrogossypoldithianes and dehydrogossypoldithiolanes, which display higher toxicity on KB cells. In addition, the low toxicity of gossypol thioderivatives was reversed by nitric oxide donors in physiological media. These experiments suggest that gossypol and gossypolone dithianes and dithiolanes can be used as prodrugs that target tumor cells surrounded by high concentrations of nitric oxide.

Introduction

Gossypol is a natural product of the cotton plant that has been studied as a potential male contraceptive. In addition to its antifertility properties, gossypol exhibits other interesting biological effects,[1] and it may be useful as an anti-neoplastic drug.[2] However, the concentration of gossypol needed to effect a specific drug response relative to its overall toxicity may preclude its direct use as a therapeutic agent. Consequently, there is interest in synthesizing and testing derivatives and analogues of gossypol for their activity and toxicity.

The activity of gossypol has been often attributed to the presence of the aldehyde groups,[3] which are capable of reacting with nucleophiles. Of particular interest is gossypol's ability to bind to the ε-amino groups of lysine in peptides or proteins[4] and the likelihood that these complexes interfere with cellular function. Several derivatives of the aldehyde group have been synthesized, including periacylated gossylic nitriles,[5] gossylic iminolactones,[6] and a series of gossypol Schiff's bases[7] (Fig. 1). These derivatives were found to exhibit a varying degree of activity relative to gossypol. In most cases, the compounds were found to have less toxicity and less biological activity. Also important for maintaining activity is the retention of the 6, 6′, 7, and 7′ hydroxyl groups. These observations were recently confirmed with gossypol, gossypolone, gossypol Schiff's bases, gossypolone Schiff's bases, methylated gossypol, and gossypolol (reduced gossypol).[8]

In this work, we report on the formation of dithiane and dithiolane aldehyde derivatives of gossypol and gossypolone. We also determined the relative toxicity of these compounds against KB cells (human epidermoid carcinoma cells of the mouth). As expected, the chemistry was readily reversed in the presence of electophiles,[9] and we tested the possibility that these masking groups could be deprotected with NO in physiological media. The results suggest that this class of analogues can be used as less-toxic prodrugs that become activated near tumor cells surrounded by high concentrations of NO.[10–14]

Results and Discussion

Chemistry

The potential for forming stable aldehyde derivatives of gossypol is limited because the aldehydes groups inter-

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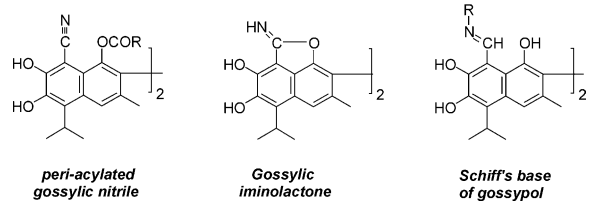

Figure 1.

act with the 1 and 1′ hydroxyl groups to form lactol in neutral or basic conditions. However, dithiane derivatives of aldehydes (or ketones) are usually formed in acidic conditions by the acid-catalyzed condensation of thiols. These aldehyde modifications have not yet been reported for gossypol. To form these derivatives, gossypol was treated with dithioethane and dithiopropane in the presence of various acid catalysts, such as concentrated aqueous HCl, ZnI_2, $TiCl_4$, $SiCl_4$, and BF_3/Et_2O and the reaction was monitored by HPLC.

The formation of the derivatives was highly dependent on the catalyst. With concentrated aqueous HCl or ZnI_2/dioxane, the reaction did not proceed. With $TiCl_4$ and $SiCl_4$ the reaction led to tarring, probably due to the complexation with the gossypol phenolic hydroxyl groups. Only BF_3/Et_2O proved to be an efficient catalyst for these reactions. We obtained dithiolanes and dithiane derivatives of gossypol (**1**, **2**) and gossypolone (**3**, **4**) in yields $>60\%$ (Fig. 2).

Under typical acid or base conditions, dithiolanes and dithianes are very stable, which has led to their synthetic use as carbonyl protecting groups or as synthons. For gossypol and gossypolone however, these products cannot be used as intermediates without prior protection of the phenolic hydroxyl groups, which are easily oxidized. In an attempt to form protected thioderivatives, 7, 7′, 8, 8′-tetramethylgossypol and hexamethylgossypol were treated with dithioethane and dithiopropane in the presence of BF_3/Et_2O. However, instead of forming the expected dithiolanes or dithianes, we obtained 8,8′ bridged thioacetals (**5**–**7**) in yields ranging from 20 to 60% (Fig. 3).

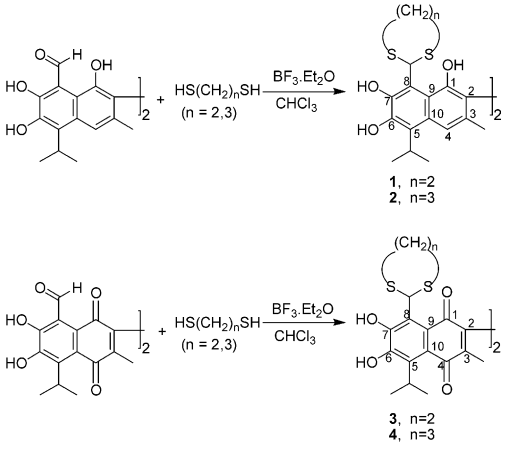

Figure 2.

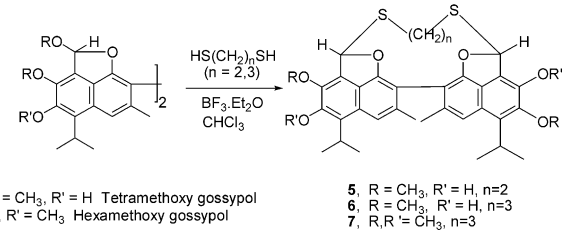

Figure 3.

The bridging reaction appeared to proceed by a simple displacement of the lactol ethers by the electron-rich sulphur atoms (Fig. 4a). The length of an extended unrestrained dithioethane molecule is just long enough to cover the distance between the acetal carbon atoms if the naphthalene rings are slightly distorted from perpendicular. Consequently, this is the smallest dithio-bridge possible. In comparison, gossypol binaphthalene bridges have also been formed by condensation reactions with diamines (Fig. 4b).[15] These reactions proceed via the aldehyde tautomer with aliphatic bridging chains greater than or equal to four carbon atoms.[15] The shorter thio-based bridges are possible because of the longer C–S bond lengths compared to the C–N and C=N bond lengths and because the distance between the acetal carbon atoms of the lactols is shorter than the distance between the carbonyl carbon atoms of the aldehydes.

Because it is well established that (−)-gossypol posseses greater antitumor activity than (+)-gossypol,[16,17] the potential of these bridges to stabilize the gossypol enantiomers is of particular interest. Research on these thio-bridged gossypol derivatives is continuing

In the presence of strong electrophiles, such as Ag^+,[18] or NO^+,[19] aldehyde groups can be recovered from dithianes. Reaction of **4** with catalytic amounts of $NO^+.BF_4^-$ produced the expected gossypolone (Fig. 5a). Reaction of **2** at the same conditions resulted in a new compound, dehydrogossypoldithiane (**8**). The structure of **8** was determined by HPLC-electron spray ionization mass spectroscopy, which indicated the loss of four hydrogen atoms ($m/z=694$). The ^1H and ^{13}C NMR spectra were consistent with the coexistence of two isomers **8a** and **8b** (Fig. 5b), **8a** being the more abundant form ($\delta_{c=o}=187.8$ ppm). The unexpected reactivity was attributed to the presence of the 1 and 1′ phenolic hydroxyl groups in **2**, which can interact intramolecularly with the enones at the 8 and 8′ positions. The same product was formed when the dithiane derivative of gossypol was exposed to NO in DMF with Fe^{3+}.

Biological studies

The toxicity of the synthesized derivatives (**1**–**8**) on KB cells is reported in Table 1. The results confirm that masking the aldehyde groups decreases gossypol and gossypolone toxicity (100-fold for **2** compared to gossypol and 10-fold for **4** compared to gossypolone). The methylated gossypol derivatives were also less toxic.

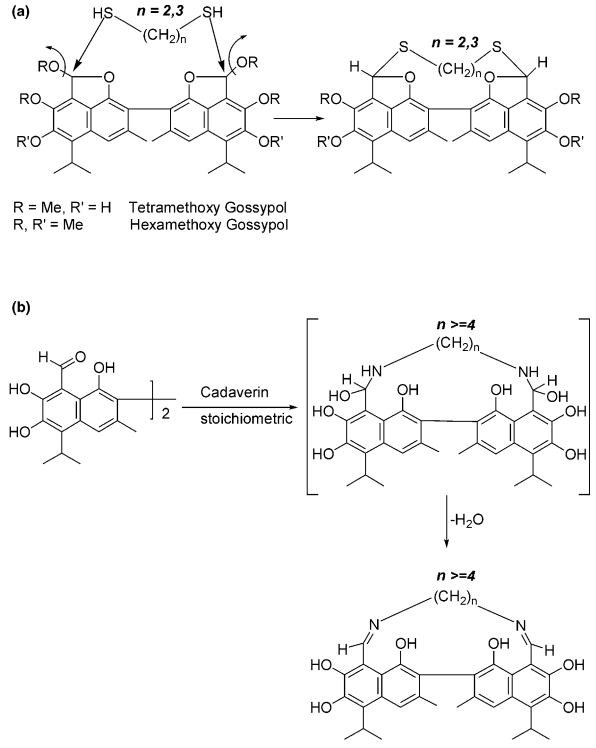

Figure 4.

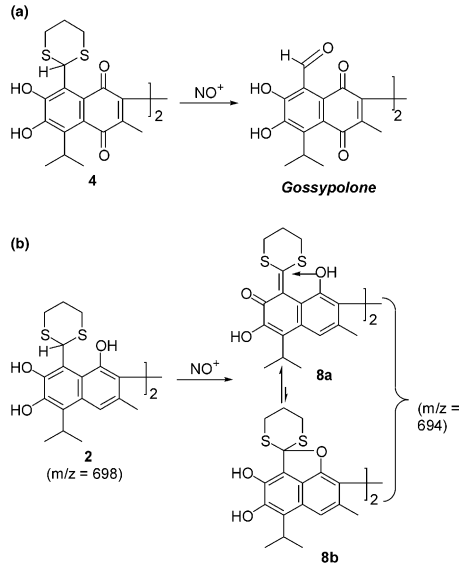

Figure 5.

Cytotoxicities of gossypol thioderivatives in the presence of NO

The dithianes and dithiolanes were found to react readily with a nitronium donor in organic solvent. When the reaction mixture was poured into water, gossypolone dithiane was deprotected to give gossypolone, and gossypol dithiane was rearranged to give **8**, which has a toxicity on KB cells similar to gossypol (Table 1). For both classes of compounds, the toxicity of the thioderivatives was significantly less than for the original compounds and the chemistry resulting from the exposure to nitric oxide essentially restored the toxicity.

Table 1. Toxicity of the thioderivatives on KB cells

Compound	IC_{50}
Gossypol	*5.7 10^{-6}M*
1	4.5 10^{-4}M
2	1.2 10^{-4}M
Gossypolone	*2.4 10^{-6}M*
3	2.0 10^{-5}M
4	3.5 10^{-5}M
Tetramethoxy Gossypol	*> 25 10^{-6}M*
5	Atoxic
6	Atoxic
Hexamethoxy Gossypol	*> 25 10^{-6}M*
7	Atoxic
8	5.5 10^{-6}M

Table 2. Toxicity of **2** in the presence of DPTA NONOate

Concentration of **2**	Without DPTA NONOate	With DPTA NONOate		
		10^{-4} M	5 10^{-5} M	10^{-5} M
5 10^{-5} M	29%	59%	22%	11%
10^{-5} M	0%	41%	11%	0%

In biological media, nitronium ions can be formed from NO binding to the heme of cytochromes. This may serve as a potential source of nitronium ions to initiate the formation of nitrosothiols.

$$NO + Fe^{3+} \rightarrow [NO\text{-}Fe^{3+}] \rightarrow [NO^{+}] + Fe^{2+}$$
$$NO^{+} + R\text{-}S^{-} \rightarrow RS\text{-}NO$$

The low toxicity of the gossypol thioderivatives prompted us to examine their toxicity in physiological media in the presence of a NO donor. The experiment was performed by incubating **2** on a KB cell culture system with dipropylenetriamine nonoate (DPTA NONOate). We observed that the presence of DPTA NONOate (at concentrations that were not toxic to KB cells) significantly increased the toxicity of **2** (Table 2). The result suggests that at physiological conditions NO could release the sulphur moieties from dithiolane and dithiane modified compounds to increase the toxicity of the underlying drugs. More experimentation is needed to confirm the feasibility of this approach for use in drug therapy.

Conclusion

The toxicity of gossypol derivatives has often been attributed to the presence of the aldehyde groups, which can potentially bind or cross-link enzymes and nucleic acids. To further study the influence of the aldehyde moieties of gossypol on activity, several thioderivatives of gossypol were prepared, characterized, and tested for toxicity on KB human tumor cells. The results support

the general hypothesis of the role of the gossypol aldehydes on cell toxicity.

In addition, because NO is generated in high concentration by activated macrophages as part of the immune response against bacteria, viruses, and tumor cells, it is particularly abundent near solid tumors, which are highly vascularized, and it plays a predominant role in angiogenesis and vascular permeability. Thus, high local concentrations of NO could be used as a deprotection reagent (i.e., a chemical trigger) to activate the toxicity of therapeutic agents. The results suggest a new approach to targeted drug therapy.

Experimental

Reagents and solvents were purchased from Fluka Chemie AG. Gossypol (as gossypol-acetic acid), gossypol methyl ethers, and gossypolone were obtained as previously described.[8] DPTA NONOate was from Cayman Chemical Co., Boron trifluoride diethyl ether complex 99% was from Lancaster Co. Mass spectra were determined at the Service de Spectrometrie de Masse de l'Institut de Chimie des Substances Naturelles on AEI MS50 or Navigator-Thermoquest spectrometers. NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on Bruker AM300 or AM400 spectrometers. The structures of **1**–**8** were checked by HMQC and HMBC 2-D NMR.

Analytical-scale HPLC work was performed with a SpectraSYSTEM P1000XR pumping system and a reverse-phase Alltima C-18 column (4.6 mm i.d.×250 mm) with isocratic elution at a flow rate of 1 mL/min. Two mobile phases were used: (I) 10% A and 90% B and (II) 20% A and 80% B, where A was 95:5 (v/v) water/CH_3CN acidified with TFA to a final concentration of 0.1% and B was CH_3CN also acidified with TFA to a final concentration of 0.1%. Compounds were detected with a Waters Corp. Model 996 photo-diode array detector operated from 254 to 400 nm. Preparative work was performed with a Waters Corp. 515 HPLC pumping unit, reverse-phase PrepPak C-18 cartridge column (10 μm particles with 125 Å pores; 25 mm i.d.×100 mm) with isocratic elution and a flow rate of 5 mL/min. The mobile phase was 20:80 (v/v) CH_3CN/water. A Waters Corp. Model 2487 dual wavelength UV detector operated at 254 nm was used for detection.

General procedure for the preparation of 1,3 dithiane or 1,2-dithiolane dervivatives of gossypol and gossypolone and bridged thioacetal of gossypol methyl ethers 1–7. A 0.1–1.0 M solution of either gossypol, gossypolone, or a methylether derivative of gossypol in chloroform was combined with an equimolar amount of either 1,2-dithiolethane or 1,3-dithiolpropane at room temperature. For reaction with gossypol or gossypolone, the solution was kept at room temperature for 1 h before cooling to −20 °C. For reactions with the methyl ether gossypol derivatives, the solutions were cooled on an ice bath immediately after mixing the components. A 0.1 equiv of BF_3-diethylether complex was added dropwise to the cooled solutions. For **1** and **2**, the solutions were warmed to room temperature and allowed to stand for up to 15 h resulting in precipitation of the product. The precipitate was filtered off, washed with Et_2O, and vacuum dried. For **3** and **4**, the progress of the reaction was monitored by HPLC. Upon completion, the solutions were washed successively with a 5% solution of $NaHCO_3$, water, and brine, dried over Na_2SO_4, and the solvent evaporated. The products were precipitated from Et_2O/hexane as green solids. Compounds **5**–**7** were obtained as white solids by following the procedure for **1** and **2**.

8,8′-Bis-[1,3]dithiolan-2-yl-5,5′-diisopropyl-3,3′-dimethyl-[2,2′]binaphthalenyl-1,6,7,1′,6′,7′-hexaol (1). Yield 67%, yellow solid, HPLC: k′(II): 9,17; ESI-MS: *m*/*z* 669 ($M-H^-$); ^1H NMR (400 MHz, DMSO) δ 1.54 (d, 12H, 2HC-(CH_3)$_2$), 2.00 (s, 6H, 2 Ar-CH_3), 3.31, 3.65 (m, 8H, 2 C–S–CH_2–CH_2–S), 3.94 (m, 2H, 2 HC–(CH_3)$_2$), 7.51 (s, 2H, 2 Ar–H), 8.13 (s, 2H, 2 S–HC–S), 7.96, 8.29, 8.63 (s, br, 6H, 6 OH at 1,1′,6,6′,7,7′ positions); ^{13}C NMR (100 MHz, DMSO) δ 20.4 (Ar–CH_3), 20.6 (HC–(CH_3)$_2$), 26.2 (HC–(CH_3)$_2$), 39.7 (C–S–CH_2–CH_2–S), 50.6 (S–HC–S); 111.7 (C-8), 115.4 (C-4), 117.4 and 117.6 (C-2 and C-9), 125.7 (C-5), 129.2 (C-10), 132.3 (C-3), 143.9 (C-6), 146.0 (C-7), 150.7 (C-1).

8,8′-Bis-[1,3]dithian-2-yl-5,5′-diisopropyl-3,3′ dimethyl-[2,2′]binaphthalenyl-1,6,7,1′,6′,7′-hexaol (2). Yield 93%. HPLC: k′(II): 10,25; ESI-MS: *m*/*z* 721 (M + Na); ^1H NMR (400 MHz, DMSO) δ 1.47 (d, 12H, 2HC–(CH_3)$_2$), 1.96 (s, 6H, 2 Ar–CH_3), 1.74, 2.15 (m, 4H, 2 C–S–CH_2–CH_2–CH_2–S), 2.93 (m, 8H, 2 C-S–CH_2–CH_2–CH_2–S), 3.87 (m, 2H, 2 HC–(CH_3)$_2$), 7.55 (s, 2H, 2 Ar-H), 7.83 (s, 2H, 2 S–HC–S), 7.77, 8.25, 8.63 (s, br, 6H, 6 OH at 1,1′,6,6′, 7,7′ positions); ^{13}C NMR (100 MHz, DMSO) δ 20.4 (Ar–CH_3), 20.5 (HC–(CH_3)$_2$), 26.2 (HC–(CH_3)$_2$), 25.0 (C–S–CH_2–CH_2–CH_2-S), 31.6 (C–S–CH_2–CH_2–CH_2-S), 45.7 (S–HC–S), 111.4 (C-8), 115.6, 115.7 (C-4 and C-9), 117.9 (C-2), 125.6 (C-5), 129.4 (C-10), 132.4 (C-3), 143.7 (C-6), 146.7 (C-7), 150.7 (C-1).

8,8′-bis-[1,3]dithiolan-2-yl-6,7,6′,7′-tetrahydroxy-5,5′-diisopropyl-3,3′-dimethyl-[2,2′]binaphthalenyl-1,4,1′,4′-tetraone (3). Yield 65%. HPLC: k′(II): 4,70; ESI-MS: *m*/*z* 697 ($M-H^-$). ^1H NMR (300 MHz, $CDCl_3$) δ 1.43 (dd, 12H, 2HC–(CH_3)$_2$), 1.99 (s, 6H, 2 Ar–CH_3), 3.40, 3.58 (m, 8H, 2 C–S–CH_2–CH_2–S), 4.03 (m, 2H, 2 HC–(CH_3)$_2$), 7.15 (s, 2H, 2 S–HC–S), 6.63, 8.89 (s, br, 4H, 4 OH at 6,6′,7,7′ positions); ^{13}C NMR (75MHz, $CDCl_3$) δ 14.4 (Ar–CH_3), 19.4, 19,6 (HC–(CH_3)$_2$), 28.2 (HC–(CH_3)$_2$), 39.8, 40.0 (C–S–CH_2–CH_2–S), 50.1 (S–HC–S), 119.9 (C-8), 125.5 (C-9), 128.8 (C-10), 136.8 (C-5), 139.4 (C-2), 145.8 (C-3), 147.2 (C-7), 149.7 (C-6), 185.4 (C-1), 187.6 (C-4).

8,8′-bis-[1,3]dithian-2-yl-6,7,6′,7′-tetrahydroxy-5,5′-diisopropyl-3,3′-dimethyl-[2,2′]binaphthalenyl-1,4,1′,4′-tetraone (4). Yield 89%. HPLC: k′(II): 5,97; ESI MS: *m*/*z* 749 (M + Na); ^1H NMR (400 MHz, $CDCl_3$) δ 1.43 (dd, 12H, 2HC–(CH_3)$_2$), 1.99 (s, 6H, 2 Ar–CH_3), 1.87, 2.15 (m, 4H, 2 C–S–CH_2–CH_2–CH_2–S), 2.85, 3.10 (m, 8H, 2

C–S–CH_2–CH_2–CH_2–S), 4.02 (m, 2H, 2 HC–$(CH_3)_2$), 7.23 (s, 2H, 2 S–HC–S), 6.57, 8.18 (s, 4H, 4 OH at 6,6′,7,7′ positions); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5 (Ar–CH_3), 19.9, 20.0 (HC–$(CH_3)_2$), 28.2 (HC–$(CH_3)_2$), 24.8 (S–CH_2–CH_2–CH_2–S), 31.2 (S–CH_2–CH_2–CH_2–S), 43.1 (S–HC–S), 121.5 (C-8), 123.4 (C-9), 128.8 (C-10), 136.8 (C-5), 139.4 (C-2), 145.9 (C-3), 147.0 (C-7), 149.5 (C-6), 185.3 (C-1), 187.8 (C-4).

5,5′-Diisopropyl-2,2′-dithioethane-4,4′-dimethoxy-7,7′-dimethyl-2H,2′H-[8,8′] bi[naphtho [1,8- bc]furanyl]-3,3′-diol (5). Yield 24.2%. HPLC: k′(I): 3.82; EI–MS: *m*/*z* 605 (MH^+); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (d, 12H, 2HC–$(CH_3)_2$), 2.30 (s, 6H, 2 Ar–CH_3); 2.61, 2.95 (d, 4H, C–S–CH_2–CH_2–S), 3.66 (m, 2H, 2 HC–$(CH_3)_2$), 4.10 (s, 6H, 2 OCH_3 at 7,7′ positions) 6.18 (s, 2H, 2 OH at 6,6′ positions), 6.98 (s, 2H, 2 O–HC–S), 7.30 (s, 2H, 2 Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.7 (HC–$(CH_3)_2$, Ar–CH_3), 26.9 (HC-$(CH_3)_2$), 30.7 (C–S–CH_2–CH_2–S), 59.3 (OCH_3 at 7,7′ positions), 90.2 (O-HC–S), 109.4 (C-2), 114.2 (C-4), 118.2 (C-8), 121.2 (C-9), 125.8, 126.1 (C-5, C-10), 137.2 (C-3), 139.8 (C-7), 146.4 (C-6), 156.5 (C-1).

5,5′-Diisopropyl-2,2′-dithiopropane-4,4′-dimethoxy-7,7′-dimethyl-2H,2′H-[8,8′] bi[naphtho [1,8-bc]furanyl]-3,3′-diol (6). Yield 60.3%. HPLC: k′(I): 4,09; ESI-MS: 619 (MH+); ^{1}H NMR (300 MHz, DMSO) δ 1.43 (d, 12H, 2HC–$(CH_3)_2$), 2.14 (s, 6H, 2 Ar–CH_3), 1.67 (m, 4H, S–CH_2–CH_2–CH_2–S), 2.98, 3.02 (m, 4H, S–CH_2–CH_2–CH_2–S), 3.72 (m, 2H, 2 HC–$(CH_3)_2$), 4.09 (s, 6H, 2 OCH_3 at 7,7′ positions), 7.31 (s, 2H, 2 Ar–H), 7.39 (s, 2H, 2 O–HC–S), 8,60 (s, 2H, 2 OH at 6,6′ positions); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.3 (Ar–CH_3), 20.5HC–$(CH_3)_2$, 25.9 (HC–$(CH_3)_2$), 27.0 (S–CH_2–CH_2–CH_2–S), 27.6 (S–CH_2–CH_2–CH_2-S), 58.2 (OCH_3 at 7,7′ positions), 88.6 (O-HC-S), 108.9 (C-2), 113.4 (C-4), 118.1 (C-8), 120.6 (C-9), 125.1, 125.2 (C-5, C-10), 136.2 (C-3), 140.5 (C-7), 147.1 (C-6), 155.1 (C-1).

5,5′ - Diisopropyl - 2,2′ - dithiopropane - 3,3′,4,4′ - tetramethoxy-7,7′-dimethyl-2H,2′H-[8,8′] bi[naphtho [1,8- bc]furanyl] (7). Yield 27.3%. HPLC: k′(I): 10.71; ESI-MS: *m*/*z*, 647 (MH^+); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (d, 12H, 2HC–$(CH_3)_2$), 2.24 (s, 6H, 2 Ar–CH_3), 1.76 (m, 2H, C–S–CH_2–CH_2–CH_2–S), 3.04, 3.34 (m, 4H, C–S–CH_2–CH_2–CH_2–S), 3.82 (m, 2H, 2 HC–$(CH_3)_2$), 3.87 (s, 6H, 2 OCH_3 at 6,6′positions), 4.13 (s, 6H, 2 OCH_3 at 7,7′ positions), 7.12 (s, 2H, 2 O–HC–S), 7.44 (s, 2H, 2 Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.9 (Ar–CH_3), 22.0 (HC–$(CH_3)_2$), 27.1 (HC–$(CH_3)_2$, S-CH_2–CH_2–CH_2–S), 27.5 (S–CH_2–CH_2–CH_2–S), 61.4 (OCH_3 at 6,6′ positions), 59.2 (OCH_3 at 7,7′ positions), 89.5 (O–HC–S), 111.0 (C-2), 115.7 (C-4), 121.4 (C-8), 124.0 (C-9), 125.9 (C-10), 136.0 (C-5), 137.1 (C-3), 145.2 (C-7), 151.0 (C-6), 155.5 (C-1).

Reaction of 1,3 dithiane gossypol (2) and 1,3 dithiane gossypolone (4) with nitrosonium tetrafluoroborate (NO^+, BF_4^-). One hundred and fifty microliters of a 50-mg/mL solution of $NO^+BF_4^-$ in CH_2Cl_2 and 50 μL of DMF were added to 15 mL of a DMF solution containing 150 mg of **2**. After standing 24 h at ambient temperature, the reaction, which was monitored by HPLC, gave compound **8** in a 68% yield. The product was recovered by preparative HPLC. The corresponding reaction with **4** gave gossypolone in a 40% yield after standing 4 days.

8′-Dimercaptomethylene-8-[1,3]dithian-2-ylidene-1,6,1′,6′-tetrahydroxy - 5,5′ - diisopropyl - 3,3′ - dimethyl - 8H,8′H - [2,2′]binaphthalenyl-7,7′-dione (8a) (see Fig. 5). Yield 14%. HPLC: k′(II): 3,19; ESI-MS: *m*/*z* 694 (M); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (d, 12H, 2HC–$(CH_3)_2$), 1.99 (s, 6H, 2 Ar–CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.3–20.7 (Ar–CH_3), 21.3–21.4 (HC–$(CH_3)_2$), 28.1–28.2 (HC–$(CH_3)_2$), 27.0 (C–S–CH_2–CH_2–CH_2–S), 29.6–30.0 (C–S–CH_2–CH_2–CH_2–S), 41.9, 65.1 (S–HC–S), 107.9–149.9, 173.0 and 187.8 (naphthalene nucleus).

Reaction of 1,3 dithiane gossypol 2 with NO in DMF. A saturated solution of NO in DMF was prepared by bubbling NO gas in DMF previously degassed with argon. The NO concentration was determined by adding 2,2′-azino-bis(2-ethylbenzthiazoline-6-sulfonic acid)-(ABTS) with detection by UV–vis spectroscopy at 660 and 750 nm, corresponding to the absorption of a stable cation radical $ABTS^{\cdot+}$.[20] One hundred microliters of a NO/DMF solution (0.53 mmol/L) was poured into 500 μL of DMF containing 10 mg of **2** at room temperature. The reaction mixture was monitored by HPLC and, after 20 min, **2** displayed no transformation. The same experiment performed in the presence of 50 μL of 1% (w/w) aqueous $FeCl_3$ led to the immediate formation of **8**.

Biological studies

KB cells were originally obtained from the American Type Culture Collection. The cell line was grown in minimal essential media with Earle's salt solution (purchased from Seromed) containing 10% fetal calf serum, 2 mM L-glutamine, 60 μg/mL penicillin G, 60 μg/mL streptomycin sulfate, and 40 μg/mL gentamycin. Cells were grown as monolayers in Nunc 24-well plastic plates (~25,000 cells were seeded per well in 1 mL of media).

For toxicity assays, the compounds of interest were dissolved in DMSO and were serially diluted with the same solvent. Immediately after plating the cells, the test compounds were added to the cultures (<10 μL) such that the final concentration of DMSO was <1%. Control cultures received an equal dilution with DMSO. The cultures were incubated at 37 °C in a 95:5 (v/v) air/CO_2 humidified incubator. After incubating for 3 days, cell viability was determined by adding 100 μL of a 0.02% solution of neutral-red vital dye in the growth media to each well and incubating the plate for an additional 8-16 h. The wells were then washed with phosphate buffered saline, and the cells were lysed with a 1% solution of sodium lauryl-dodecyl sulfate. Extracted dye was measured at 540 nm with a Uniskam-II microplate reader (Labosystems, Life Science International).

Most of the compounds were tested at concentrations between 0.5 and 10 μM. Each concentration was added

to duplicate wells on the same plate. The percentage of cell death was plotted against the compound concentration, and IC_{50} values were determined from the plots.

To study the effect of DPTA NONOate on the toxicity of **2**, KB cells were first grown as given above except that the incubation period was reduced to 24 h. Compound **2** and DPTA NONOate were added simultaneously to the cultures. Control samples were also run in the same assays with no compound addition, with only **2** added, and with only DPTA NONOate added. After incubating the cells at 37 °C for 48 h, cell destruction was determined by the neutral-red viability test described above.

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