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Synthesis, Characterization, Mechanism of Decomposition, and Antiproliferative Activity of a Class of PEGylated Benzopolysulfanes Structurally Similar to the Natural Product Varacin

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Abstract

Benzopolysulfanes, 4-CH₃(OCH₂CH₂)₃NHC(O)-C₆H₄-1,2-S_x (x = 3–7, and 9) were synthesized with a PEG group attached through an amide bond and examined for water solubility, antitumor activity, and propensity to equilibrate and desulfurate. LCMS and HPLC data show the PEG pentasulfane ring structure predominates, and the tri-, tetra-, hexa-, hepta-, and nonasulfanes were present at very low concentrations. The presence of the PEG group improved water solubility by 50-fold compared to the unsubstituted benzopolysulfanes, C₆H₄S_x (x = 3, 5, and 7), based on intrinsic solubility measurements. Polysulfur linkages in the PEG compounds decomposed in the presence of ethanethiol and hydroxide ion. The PEG pentathiepin desulfurated rapidly and an S₃ transfer reaction was observed in the presence of norbornene, no S₂ transfer reaction was observed with 2,3-dimethylbutadiene. The antitumor activities of the PEG-substituted benzopolysulfane mixtures were analyzed against four human tumor cell lines PC3 (prostate), DU145 (prostate), MDA-MB-231 (breast), and Jurkat (T-cell leukemia). The PEG conjugated polysulfanes had IC₅₀ values 1.2–5.8 times lower than the parent “unsubstituted” benzopolysulfanes. Complete cell killing was observed for the PEG polysulfanes with 4 μM for PC3 and DU145 cells, and with 12 μM for MDA-MB-231 cells. The results suggest that solubilization of the polysulfur linkage is a key parameter to the success of these compounds as drug leads.

Introduction

Tunicates or their associated microorganisms produce benzopolysulfanes, such as varacin (**1**), lissoclinotoxin A (**2**), and *N,N*-dimethyl-5-(methylthio)varacin (**3**) (Scheme 1).^{1–6} Unnatural benzopolysulfanes have also been synthesized, e.g., 6-(2-aminoethyl)benzopentathiepin (**4**)⁷ and the parent benzopentathiepin (**5B**)^{8,9} (alphabetical

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Supporting Information Available. Spectroscopic data for compounds **6A–F** and **8–17**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

labels will given to some polysulfanes, as will be elaborated on below). Benzopolysulfanes represent an attractive target yet understudied class of compounds for antitumor drug discovery. High nanomolar and low micromolar antiproliferative IC₅₀ values have been reported for benzopolysulfanes.^{1–7,10} It was suggested that the bioactivity of varacin derives from DNA damage because of an observed difference in toxicity toward the CHO cell line EM9 (chlorodeoxyuridine sensitive) compared to BR1.¹

Benzopolysulfanes have not been studied widely in the context of drug discovery due to the instability of the polysulfur ring. Even determining the number of sulfur atoms in *o*-benzopolysulfanes has not been a trivial task.^{11–13} MS fragmentation patterns can be difficult to interpret; for example, the structure of the natural product lissoclinotoxin A was assigned first as a trisulfane³ and later revised as a pentasulfane.¹¹ In 2007, the synthesis and purification of parent *o*-C₆H₄S₅ (**5B**) revealed an equilibration involving elemental sulfur, S₈ (Scheme 2).¹² A facile equilibration took place between the pentasulfane and the tri-, and heptasulfanes (*o*-C₆H₄S₃ and *o*-C₆H₄S₇): the ratio of **5A**:**B**:**C** was 49:45:6 in CH₂Cl₂ over 1–3 days. In **5**, the labels A, B, and C correspond to compounds with 3, 5, and 7 sulfur atoms, respectively. Analysis of GC/MS retention times revealed that **5A** and **5B** differed by 12.3 min, while the retention times of **5B** and **5C** differed by 12.6 min (Table 1).

Because natural benzopolysulfanes are in short supply and their stability is difficult to assess, we synthesized benzopolysulfanes with a short-chain PEG, in which the benzene ring and PEG group replaced the naturally occurring dopamine core **1–3**. The aims of the present work were to determine (1) whether benzopolysulfanes could be synthesized with a PEG side group, (2) whether the pentasulfur species predominates, (3) whether the polysulfur linkage(s) are unstable to medium effects, (4) whether the PEGylated benzopolysulfanes decompose at different rates and transfer sulfur to norbornene and butadiene traps, (5) the extent the PEG group enhances water solubility, (6) whether the polysulfur ring is essential for bioactivity, and (7) whether enhanced benzopolysulfane water solubility is correlated with an enhanced pharmacological activity against human tumor cells. We synthesized a mixture of PEG-benzopolysulfane conjugates 4-CH₃(OCH₂CH₂)₃NHC(O)-C₆H₄-1,2-S_x (x = 3–7 and 9) **6A–F** and explored their stability, and activity in a variety of tumor cell lines [PC3 (prostate), DU145 (prostate), MDA-MB-231 (breast), and Jurkat (T-cell leukemia)]. In **6**, the labels A–E and F correspond to compounds with 3–7 and 9 sulfur atoms, respectively. The octasulfane species was not detected.

Results and Discussion

Synthesis and Characterization

Pentasulfane **6C** was synthesized as the major constituent of a mixture of polysulfanes 4-CH₃(OCH₂CH₂)₃NHC(O)-C₆H₄-1,2-S_x (x = 3–7 and 9) in 8 steps and 1.5% overall yield. A procedure developed by Liénard et al.¹⁴ was used for the conversion of 3,4-dihydroxybenzoic acid (**7**) to 3,4-disulfuranylbenzoic acid (**11**) (steps i–iv, Scheme 3). Dithiastannole-5-carboxylate anion **12** was generated under basic conditions by the reaction of dimethyltin chloride with **11** using a modified procedure by Sato et al.¹⁵ Stannole **12** reacted with *p*-nitrophenol, DCC, and DMAP to yield 4-nitrophenyl ester stannole **13** in 72% yield. Stannole **13** reacted with disulfur dichloride giving 4-nitrophenyl ester benzopentasulfane (**14**) in 27% yield. Amino-terminated poly(ethylene glycol) (**15**) was prepared in 2 steps by the method of Dombi et al.¹⁶ and PEGylated to benzopentasulfane **14** at the 7-position of the benzene ring. It is possible that other cyclic polysulfanes related to benzopentasulfane **14** were formed in ~1–10% yields, but this was not determined. The resulting mixture was purified by column chromatography to afford **6C** in 93% purity. In the ¹H NMR, ¹³C NMR, COSY, HMBC, and HSQC spectra, the benzene and PEG portions of the structure were confirmed (Supporting Information). For example, in COSY NMR, a

strong 3J correlation was found between C9-H and C8-H, and between N-H and C13-H, and a weak 4J -W correlation found between C8-H and C6-H (Scheme 4). The HMBC and HSQC NMR data further bolstered the structural assignment of the non-sulfur portion of **6C**. LC/MS data indicated that **6C** contained 5 sulfur atoms (HRMS calcd for $C_{14}H_{19}NO_4S_5$ = 424.9918, found 424.9926).

Lability of the Pentasulfur Ring

Polysulfanes are challenging structures to study because of their instability. Few reports on benzopolysulfanes describe the distribution of the polysulfanes, or their equilibration, and often incorrectly assume the pentathiepin to be the sole compound present.¹² We found that over a 12–24 hour period **6C** equilibrates S_8 and forms low concentrations of structurally related polysulfanes in aqueous methanol at room temperature (Scheme 5). Table 2 lists the polysulfane masses and retention times to compare the number of sulfur atoms in the compounds. The LCMC spectra of polysulfanes **6A–F** contained peaks spaced by ~ 0.7 min per additional sulfur atom (Figure 1). For example, a minor amount of a sulfur-rich compound was assigned as nonasulfane **6F** (HRMS calcd for $C_{14}H_{19}NO_4S_9$ = 552.8800, found 552.8788). The ratio of **6A:B:C:D:E:F** was 1.5:1.5:93:0.3:0.1:0.1 by HPLC (methanol/water), and there was $\sim 2\%$ uncharacterized material and $\sim 1.5\%$ of elemental sulfur. The moderate solubility of the elemental sulfur in methanol/water suggested it to be a residue or colloid particles of the orthorhombic cyclo- S_8 form, but neither the monoclinic S_8 ring form (usually found at $\sim 95^\circ C$), nor the polymeric, oligomeric, or amorphous forms (insoluble materials).^{17–19} The overlaid-ion extracted LCMS of **6** in Figure 1 displayed a series of polysulfanes, analogous to that seen for parent **5** by SIM-GCMS.¹² Preference for odd-membered ring compounds was observed experimentally for the parent system o - $C_6H_4S_x$ ($x = 3, 5, 7$) in CH_2Cl_2 .¹² Gas-phase DFT calculations showed the odd-membered o - $C_6H_4S_x$ rings to be conformationally stable with gauche adjacent lone-pair electron interactions, whereas eclipsing lone-pair electron interactions occurred in the even-numbered cases.¹³ It may be noted that Nakayama et al. also observed odd-membered ring products (namely, the trithiolane and penathiepan) in the reaction of elemental sulfur with a benzobarrelene compound and an acenaphthylene compound.^{20,21} However, the preference for odd-membered polysulfur rings does not apply to the PEG benzopolysulfanes, which is likely because the equilibrium is at an early stage and has not been reached in aqueous methanol, even after 24 h. The effect of solvent or HPLC conditions on the equilibrium of **6A–F** and S_8 was investigated next.

Influence of Solvent on Equilibration of Polysulfanes 6A–F

To better understand the equilibration between **6A–F** and S_8 , the HPLC elution methods were modified. When eluting at 1:1 methanol:water, the ratio of **6A:B:C:D:E:F**: S_8 was 2.2:1.8:93.5:0.4:0.3:0.2:1.6 (Method 1, Table 3). Increasing the methanol concentration in water produces more pronounced polysulfane equilibration. When eluting at 85:15 methanol:water, the ratio of **6A:B:C:D:E:F**: S_8 was 5.3:5.7:79.0:3.4:1.2:0.1:5.3 (Method 5, Table 3). The HPLC measurements do not ensure that thermodynamic conditions have been reached. Equilibrium at a new condition is established in 1–3 days in CH_2Cl_2 ,¹² and probably much longer in methanol/water. Our results are similar with observations of 7-methylbenzopolysulfane equilibrations enhanced in polar solvents with higher solvation compared to nonpolar solvents.²² Elemental S_8 also involves equilibration with S_6 and S_7 , which was more pronounced in polar than nonpolar organic solvents,²³ and sulfur-reactive solvents such as pyridine or other amines which dissolve elemental sulfur readily.¹⁸ The enhanced equilibration in methanol compared to methanol/water is likely a result of the increase in the solubility of S_8 , which is pivotal to the reversible addition reactions leading to the ring compounds **6A–F**.

Desulfuration of 6A-F in the Presence of Nucleophiles and Trapping Agents

The PEGylated benzopolysulfanes **6A-F** decomposed in the presence of NaOH or ethanethiol (Table 4), which led to an increase in elemental S₈ and uncharacterized products, such as oligomers and/or polymers of desulfurated PEGylated benzopolysulfanes. The reaction of hydroxide ion with benzopolysulfanes **6A-F** was comparatively slower than with ethanethiol and could be monitored by HPLC. As can be seen in Table 4, the decomposition rate of pentasulfane **6C** was rapid while the concentrations of the other benzopolysulfanes were barely reduced. Determining whether the concentration of tetrasulfane **6B** increased or decreased was not possible because it eluted along with an unidentified compound (X in Table 4) and the two peaks could not be de-convoluted. The data suggested that pentasulfane **6C** desulfurated faster compared to other polysulfanes, which led us to trapping studies to analyze the sulfur-transfer reaction with butadiene and norbornene traps.

The reaction of benzopolysulfanes **6A-F** with ethanethiol and norbornene (**16**) led to the formation of norbornenetriethiolane (**17**) and desulfurated or polymerized PEGylated benzopolysulfanes (Scheme 6). Unlike **6C**, the concentrations of **6A**, **6B**, and **6D-F** did not change significantly over the course of the trapping experiment. Thus, we propose that **6C** is the most reactive polysulfane and responsible for the S₃-transfer to norbornene. Interestingly, the decomposition of benzopolysulfanes **6A-F** did not show an S₂ transfer reaction. The sulfuration of 2,3-dimethylbutadiene (**18**) by **6A-F** with ethanethiol did not yield the disulfide product **19**. A possible mechanism for the desulfuration of pentathiepin **6C** is shown in Scheme 7, which begins with an equatorial attack of ethanethiolate ion to the sulfur atom (S1) adjoining the aryl ring. A previous DFT study showed that attack of the HS[−] nucleophile at the S1 position of a pentathiepin was preferred to S2.²⁴ The resulting open-chain polysulfide anion (**20**) has the potential for thiozone (S₃) elimination driven by the delocalization of the negative charge in the remaining carbon-sulfur fragment (**21**)^{24,25} followed by thiozonation of norbornene. *Ab initio* calculations predict the open C_{2v} zwitterionic form²⁶ of thiozone S₃ to be energetically preferred to the cyclic D_{3h} form.^{27,28}

Intrinsic Solubilities

Elemental S₈ suffers from poor solubility.^{19,29,30} We investigated the intrinsic solubilities of elemental S₈, **5A-C**, and **6A-F** (Table 5). Aliquots of methanol or water were added to 1–3 mg sample quantities and the solutions were vortexed (2 min) and stirred (10–15 min) at room temperature. As expected, the presence of the PEG group at the 7- position of **6A-F** significantly increased their solubilities. By comparison to **6A-F**, elemental sulfur S₈ and **5A-C** had ~50-fold lower solubility. Our aqueous solubility measurements are consistent with previous values for elemental sulfur S₈ reported in the literature, namely, the solubility of S₈ in water was reported to be 0.4 µg/mL.²⁹ The solubility of S₈ in ethanol was reported to be 0.51 mg/mL.³⁰

Antiproliferative Activities of Polysulfanes

We examined the effects of 1,2-benzenedithiol, *o*-benzopolysulfanes **5A-C**, and PEGylated benzopolysulfanes **6A-F** on the proliferation of several cancer cell lines. Their bioactivities are shown in Table 6 and Figure 3. 1,2-Benzenedithiol was nontoxic; it stimulated the growth of PC3 cells, and had a minimal effect on the proliferation of MDA-MB-231 cells. However, at concentrations of 10 µM and above, it inhibited the proliferation of DU145 cells by ~40%. In a MTS assay (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium in the presence of phenazine methosulfate),³¹ 1,2-benzenedithiol was also not effective in inhibiting Jurkat cell growth (IC₅₀ of 60 µM). The requirement of the polysulfur ring was also observed by Molinski et al.⁵ in the natural lissoclinotoxin A system when judged against the corresponding benzenedithiol compound.

Parent polysulfanes **5A-C** had a moderate antiproliferative effect on MDA-MB-231 cells, inhibiting the proliferation with an IC_{50} value of 28 μM . PC3 cell proliferation was inhibited with an IC_{50} of 11 μM with complete cell killing at 20 μM . DU145 and Jurkat cells were sensitive to parent polysulfanes **5A-C**; 6 μM killed 100% of the DU145 cells, and the IC_{50} was 0.5 μM for Jurkat cells. PEGylated benzopolysulfanes **6A-F** were the most potent of the three compound classes for the four cell lines examined, and they were toxic for all four cell lines. Complete cell killing was observed for **6A-F** with 4 μM for PC3 and DU145 cells, and with 12 μM for MDA-MB-231 cells. The PEG compounds **6A-F** were cytotoxic in Jurkat cells with an IC_{50} of 0.4 μM .

The data in Table 6 indicate that the polysulfur ring is significant in the enhancement of antiproliferative activity. The data also demonstrate that the PEG group leads to an increase in the antiproliferative effect. The PEGylated benzopolysulfanes **6A-F** have higher water solubility compared to the parent polysulfanes **5A-C**, and produced the highest growth inhibition. It was previously reported that lissoclinotoxin **A 2** was cytotoxic against L1210 leukemia cells (IC_{50} of 3.1 μM) and *N,N*-dimethylvaracin **3** was cytotoxic against MDA-MB-231 cells (IC_{50} of 3.6 μM). Interestingly, varacin **1** showed greater cytotoxicity in HCT-116 colon cancer cells (IC_{90} of 0.15 μM).

Potential of Benzopolysulfanes as Drug Candidates

Even though high nanomolar and low micromolar antiproliferative IC_{50} values for benzopolysulfanes were mentioned in the Introduction^{1-7,10} and the above results for **6A-F** appear promising, *in vivo* stability and deliverability studies are needed to evaluate benzopolysulfane lability to glutathione, protein thiols, etc. Like some other drugs, benzopolysulfanes are reductively activated. Our *in vitro* studies showed that ethanethiol-induced desulfuration of **6C** in aqueous methanol took ~2 min, but in benzene took 4 h, suggesting greater thiol ionization/nucleophilicity increases the polysulfane lability. Thus, hydrophobic or mild acidic conditions which maintain the less reactive thiol form will preserve **6C**, but in the presence of thiolate ion **6C** decomposes rapidly. In the absence of thiol and thiolate ion, the solvent effect is in the opposite direction, benzopolysulfane equilibria in organic solvents such as CH_2Cl_2 was more facile than in methanol or methanol/water, driven by solvation of elemental sulfur and the benzopolysulfanes (*vide supra*). Lastly, although a DNA cleaving study of 7-methylbenzopentathiepin suggested a metal- and oxygen-dependent Fenton pathway,^{32,33} more work is needed to evaluate a potential anaerobic S_3 -transfer pathway that may underlie the antitumor activity.

Conclusion

The following conclusions can be made (1) the synthesis of benzopolysulfanes **6A-F** was accomplished with attached PEG groups of 160 Da molecular weight via an amide linkage in 1.5% overall yield. (2) Pentathiepin **6C** was the main product, but even after its purification, benzopolysulfanes **6A**, **6B**, **6D-F** formed in very low concentrations. (3) The pentasulfur linkage of **6C** was sensitive to the solvent composition in the HPLC experiments. Methanol rich elution conditions reduced the ratio of **6C** relative to the other polysulfanes. (4) Thiol-initiated reactions of **6A-F** led to an S_3 -transfer to norbornene, but no S_2 -transfer was observed to 2,3-dimethylbutadiene. (5) While the cause of the poor solubility of benzopolysulfanes is the polysulfur linkage itself, a key issue in the present study was the enhanced water solubility the PEG group provided, which did not reduce the prevalence of the pentasulfur ring species. (6) The results confirm the requirement of the polysulfur ring for low micromolar antiproliferative IC_{50} values, 1,2-benzenedithiol showed little or no antiproliferative activity. (7) The PEG polysulfanes **6A-F** were more water soluble and more active against four cancer cell lines than the parent polysulfanes **5A-C**.

suggesting that enhanced solubilization of benzopolysulfanes holds promise for advancing these compounds as drug candidates.

Experimental Section

3,4-Dihydroxybenzoic acid, *N,N*-dimethylthiocarbamoyl chloride, DABCO, sodium hydroxide, potassium hydroxide, ethanethiol, acetanilide, formic acid, ammonium formate, dimethyltin chloride, *p*-nitrophenol, DCC, S₂Cl₂, triethylene glycol monomethyl ether, phthalimide, triphenylphosphine, DIAD, hydrazine monohydrate, elemental sulfur (S₈), norbornene, 2,3-dimethylbutadiene, DMAP, sodium sulfate (anhydrous), magnesium sulfate (anhydrous), NaCl, DMF, Na₂CO₃, NaHCO₃, THF, CHCl₃, CH₂Cl₂, methanol, ethanol, ethyl acetate, hydrochloric acid (12 M), diphenyl ether, benzene, acetone-*d*₆, CDCl₃, CD₃CN, CD₃OD, and hexanes were used as received without further purification. Purification of product mixtures was carried out by column chromatography using silica gel with 40–60 Å particle size. TLC was carried out using silica gel 60F 254 TLC-plates. Proton NMR data were acquired at 400 MHz and ¹³C NMR data were acquired at 100.6 MHz. HRMS, GCMS, HPLC, and melting point data were collected.

HPLC Instrumentation and Analysis

The HPLC instrument was equipped with an autosampler and diode array detector. The C18 column was 150 mm × 3.9 mm in size. The flow rate was 1 mL/min, and the injection volume was 50 µL. The mobile phase consisted of methanol and water. Compounds were detected by UV at 254 nm at room temperature.

LCMS Instrumentation and Analysis

The LCMS system consisted of a high-resolution TOF mass spectrometer attached to an HPLC equipped with an autosampler, diode array detector, and binary pump. The chromatography was conducted with a 2.1 × 30 mm SB-C18 3.5 µm column using water containing 0.1% formic acid and 5 mM ammonium formate (solvent A) and Methanol containing 0.1% formic acid and 5 mM ammonium formate (solvent B) at a flow rate 0.5 mL/min. The gradient program was as follows 15–85% B (0–13 min) 85% B (2 min) 85–15% B (1 min). The mass spectra were collected over a range of 100–1600 *m/z*. The reference masses used were purine with (M+H⁺) ion at 121.05087 *m/z* and HP-922 with ion at 922.00980 *m/z*. They were infused into the spray chamber using a calibrant delivery system.

GCMS Instrumentation and Analysis

GCMS samples were ionized using the EI auto mode. The capillary column was a VF-5ms 30 m × 0.25 mm (ID DF=0.25). The solvent delay was set to 3 min. Temperature program was as follows; 80 °C (0–5 min), 80–250 °C (5–22 min, at a rate 10 °C/min) and 250 °C (22–28 min). Total run was 28 min. The instrument parameters were set as follows: Injector temperature 200 °C, column flow rate 1 mL/min. Data were collected with the instrument set to mass range 40–650 *m/z*.

Equilibration and Solubility Determinations

To determine the extent of polysulfane equilibration, a 6.4 mg sample of dry **6A-F** was dissolved in 3 mL methanol or methanol/water mixtures by stirring for 2 min at room temperature. Then 0.2 mL was placed in a vial and diluted to 1 mL with methanol. The solution was stirred for 1 min, in which the concentration of each sample was 0.42 mg/mL. Finally, 50-µL of the solution was injected into the HPLC auto-sampler and quantitated by the absorption signal at 254 nm. The solubilities of elemental S₈, **5A-C**, or **6A-F** were determined by adding 10–50 µL aliquots of methanol or water to 1–3 mg of the compounds.

The solutions were vortexed for 2 min, and then stirred 10–15 min at room temperature. Polysulfane decompositions were also conducted in the presence of sodium hydroxide. To a solution of polysulfanes **6A-F** (2.5 mM) and internal standard, acetanilide, (0.25 mM) was added NaOH (0.625, 1.25, 1.875 or 2.5 mM) in 0.1 mL methanol. After certain periods of time, the reaction was analyzed by HPLC by injecting 15 μ L of the sample.

Sulfur Transfer and Trapping Studies

Trapping studies were carried out in 0.2 mL benzene solution. To solution of PEGylated benzopenthasulfanes **6A-F** (2.3 mM) and norbornene or 2,3-dimethylbutadiene (2.3 mM) was added ethanethiol (2.3 mM). The reaction mixture was vortexed for two minutes and analyzed in GCMS by injecting 1 μ L of the sample. Both scan and single ion mode (SIM) analyses were conducted on the sample.

Cell Proliferation Assays

The cell lines were grown from frozen stocks originally obtained from the American Type Culture Collection. The prostate cancer cell lines PC3 and DU145 cells were grown in F12K and DMEM, respectively. MDA-MB-231 breast cancer cells were grown in DMEM. All the media were supplemented with 10% FBS and penicillin/streptomycin. Cell proliferation was assessed by a cell proliferation assay as previously described.³⁴ Briefly, equal numbers of cells were distributed in 96-well plates and the cells were incubated at 37 °C in a 5% CO₂ incubator until they were in log phase growth. The medium was removed, and replaced with a growth medium containing the sulfur-containing compounds (0–30 μ M). After 48 h incubation, the media was removed and the plates were frozen at –80 °C. The assay was performed by warming the plates to rt and followed by the addition of the reagent to the wells. The fluorescence was measured with a plate reader (excitation/emission, 485/530 nm).

The cytotoxicity assay was conducted as follows: Cells were cultured in RPMI-1640 medium with 10% fetal bovine serum and penicillin-streptomycin (100 U/mL and 100 μ g/mL, respectively) and maintained in a 37 °C humidified 5% CO₂ incubator. On the day before the drug treatment, cells were plated onto each well of the 96-well plate at 2,000 cells/well (200 μ L of the medium per well). After 24 h, cells were treated with different concentrations of the sulfur-containing compounds and incubated for 72 h. After the incubation, cell growth was evaluated using a 96-titer solution cell proliferation assay. UV absorption (490 nm) of each well was quantified with a microplate reader.

N-(2-(2-(2-Methoxyethoxy)ethoxy)ethyl)benzo[d][1,2,3]trithiole-5-carboxamide (**6A**)

HPLC (150 mm $\times$ 3.9 mm C18 column, 90% acetonitrile in water, flow rate 1 mL/min): t_R = 20.3 min. LCMS: t_R = 4.6 min; HRMS (+ESI) calcd for C₁₄H₁₉NO₄S₃ = 361.0476, found 361.0478.

N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[e][1,2,3,4]tetrathiine-6-carboxamide (**6B**)

HPLC (150 mm $\times$ 3.9 mm C18 column, 90% acetonitrile in water, flow rate 1 mL/min): t_R = 22.6 min. LCMS: t_R = 5.6 min; HRMS (+ESI) calcd for C₁₄H₁₉NO₄S₄ = 393.0197, found 393.0193.

N-(2-(2-(2-Methoxyethoxy)ethoxy)ethyl)benzo[f][1,2,3,4,5]pentathiepine-7-carboxamide (**6C**)

Yield 9.6 mg (53%). A solution of 3,6,9-trioxadecylamine **15** (13.8 mg, 0.0847 mmol) in 1.5 mL of THF was added to a solution of 4-nitrophenyl benzo[f][1,2,3,4,5]pentathiepine-7-carboxylate **14** (17 mg, 0.0423 mmol) in 2.5 mL of THF. The reaction mixture was stirred under argon atmosphere overnight. The solvent was evaporated and the residue was

dissolved in 30 mL of CH₂Cl₂. The organic layer was washed with saturated aqueous NaHCO₃ solution (3 × 30 mL), 1 M HCl (3 × 30 mL), and water (3 × 30 mL). The organic solvent was evaporated and the crude product was chromatographed (CHCl₃/MeOH, 10:1) to yield 9.6 mg of **6C** (93% purity), *R*_f = 0.57, ¹H NMR (CHCl₃, 400 MHz): δ 8.30 (d, *J* = 1.9 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.78 (dd, *J* = 7.90, 1.9 Hz, 1H), 7.18 (br s, 1H), 3.66 (m, 10H), 3.54 (m, 2H), 3.32 (s, 3H); ¹³C NMR (CHCl₃, 100 MHz): δ 162.4, 155.2, 150.3, 145.8, 1145.0, 137.3, 136.3, 131.5, 130.2, 125.4, 122.5; HPLC (150 mm × 3.9 mm C18 column, 90% acetonitrile in water, flow rate 1 mL/min): *t*_R = 28.7 min. LCMS: *t*_R = 6.5 min; HRMS (+ESI) calcd for C₁₄H₁₉NO₄S₅ = 424.9918, found 424.9926.

N-(2-(2-(2-methoxyethoxy)ethoxy)ethyl)benzo[g][1,2,3,4,5,6]hexathiocine-8-carboxamide (6D)

HPLC (150 mm × 3.9 mm C18 column, acetonitrile/water 1:9 to 9:1 over 53 min, flow rate at 1 mL/min): *t*_R = 30.0 min. LCMS: *t*_R = 7.1 min; HRMS (+ESI) calcd for C₁₄H₁₉NO₄S₆ = 456.9638, found 456.9636.

N-(2-(2-(2-Methoxyethoxy)ethoxy)ethyl)benzo[h][1,2,3,4,5,6,7]heptathionine-9-carboxamide (6E)

HPLC (150 mm × 3.9 mm C18 column, acetonitrile/water 1:9 to 9:1 over 53 min, flow rate at 1 mL/min): *t*_R = 36.0 min. LCMS: *t*_R = 7.8 min; HRMS (+ESI) calcd for C₁₄H₁₉NO₄S₇ = 488.9359, found 488.9354.

N-(2-(2-(2-Methoxyethoxy)ethoxy)ethyl)benzo[j][1,2,3,4,5,6,7,8,9]nonathiacyclo-undecine-11-carboxamide (6F)

HPLC (150 mm × 3.9 mm C18 column, acetonitrile/water 1:9 to 9:1 over 53 min, flow rate 1 mL/min): *t*_R = 40.8 min. LCMS: *t*_R = 9.2 min; HRMS (+ESI) calcd for C₁₄H₁₉NO₄S₉ = 552.8800, found 552.8788. The identification of peak **6F** at 9.2 min in the spectrum is complicated because the former is a shoulder in the chromatogram of **3A**. Peak **6F** has been assigned based on the extracted ion chromatogram feature of the software.

Methyl 3,4-dihydroxybenzoate (8)

Yield 1.018 g (76%). 3,4-Dihydroxybenzoic acid **7** (1.0 g, 6.49 mmol) was dissolved in 40 mL MeOH. A catalytic amount of concentrated HCl (5 drops) was added to the methanol solution, which was then refluxed overnight at 80 °C. Water (300 mL) was added, and the mixture extracted with EtOAc/hexane (60:40) and dried over anhydrous MgSO₄. The solvent was removed affording a light brown solid (mp 137–139 °C). ¹H NMR (acetone-*d*₆) δ 3.81 (s, 3H), 6.90 (d, *J* = 8.3 Hz, 1H), 7.45 (dd, *J* = 8.3, *J* = 2.0 Hz, 1H), 7.50 (d, *J* = 2.0 Hz, 1H), 8.47 (br s, 2H); ¹³C NMR (acetone-*d*₆) δ 51.9, 115.8, 117.2, 123.0, 123.4, 145.6, 150.8, 167.1; HRMS (+ESI) calcd for C₈H₈O₄ = 168.0423, found 168.0423.

Methyl 3,4-bis[(dimethylamino carbothioyl)oxy]benzoate (9)

Yield 0.546 g (74 %). Methyl-3,4-dihydroxybenzoate **8** (738 mg, 4.39 mmol), DABCO (17.6 mmol), and *N,N*-dimethylthiocarbamoyl chloride (2.17 g, 17.6 mmol) were stirred in DMF (10 mL) for 30 min. The white solid that formed was dissolved in 50 mL of water. The product was extracted with EtOAc (3 × 30 mL), and dried over anhydrous MgSO₄. The solvent was removed affording a yellow-green oil. Benzoate **9** was obtained by flash chromatography (EtOAc/hexanes, 2:3); *R*_f = 0.4 or via recrystallization in 95% EtOH as white crystals (mp 110–112 °C). ¹H NMR (CDCl₃) δ 8.01 (dd, *J* = 8.4, *J* = 2.0 Hz, 1H), 7.85 (d, *J* = 2.0, 1H), 7.25 (d, *J* = 8.4, 1H), 3.90 (s, 3H), 3.43 (s, 3H), 3.30 (s, 6H); ¹³C NMR (CDCl₃) δ 186.6, 186.1, 165.6, 149.4, 145.6, 128.7, 128.1, 125.9, 124.4, 52.3, 43.4, 43.3, 38.9, 38.8; HRMS (+ESI) calcd for C₁₄H₁₈O₄S₂N₂ calcd 342.0708, found 342.0708;

(Lit.:¹⁴ ¹H NMR (CDCl₃) δ 8.00 (dd, *J* = 8.5, *J* = 2.0 Hz, 1H), 7.86 (d, *J* = 2.0 Hz, 1H), 7.25 (d, *J* = 8.5 Hz, 1H), 3.90 (s, 3H), 3.43, 3.42, 3.30, 3.29, 3.30 (4 × br s, 4 × 3H); ¹³C NMR (CDCl₃) δ 186.4, 186.0, 165.6, 149.3, 145.5, 128.7, 128.2, 125.9, 124.4, 52.3, 43.4, 43.3, 38.9).

Methyl 2-oxo-1,3-benzodithiole-5-carboxylate (10)

Yield 0.079 g (40%). Methyl 3,4-bis([dimethylamino-carbothioyl]oxy)benzoate **9** (300 mg, 0.876 mmol) was heated at 240 °C in 10 mL of diphenyl ether for 30 min. The reaction mixture was purified by flash chromatography using a gradient of EtOAc/hexane (2:98 to 40:60) to afford 79 mg (40%) of **10** as a light brown solid. *R*_f = 0.75 (EtOAc/hexane 1:2), mp 140–142°C. ¹H NMR (CDCl₃) δ 3.95 (s, 3H), 7.57 (d, *J* = 8.5, 1H), 7.98 (dd, *J* = 8.5, *J* = 2.0 Hz, 1H), 8.18 (d, *J* = 2.0 Hz, 1H); ¹³C NMR (CDCl₃): δ 52.5, 122.9, 124.1, 128.2, 129.2, 133.0, 138.0, 165.7, 188.8; MS (EI): *m/z* = 226 (M), 198 (M-CO), 167 (M-CO₂Me) and 139 (M-CO₂Me - CO); Molecular peak (C₉H₆O₃S₂, M=226) isotopic ratio (calcd %, found %): [M+1] 227 (11.5, 11.4), [M+2] 228 (9.0, 9.8), and [M+3] 229 (1.2, 1.5); HRMS (+ESI) calcd for C₉H₆O₃S₂ = 225.9758, found 225.9757. (Lit.:¹⁴ (CDCl₃) ¹H NMR δ 3.95 (s, 3H), 7.57 (d, *J* = 8.5 Hz, 1H), 7.98 (dd, *J* = 8.5, *J* = 2.0 Hz, 1H), 8.18 (d, *J* = 2.0 Hz, 1H); ¹³C NMR (CDCl₃) δ 52.6, 122.9, 124.1, 127.8, 129.1, 133.0, 137.9, 165.7, 189.0).

3,4-Disulfanylbenzoic acid (11)

Yield 0.52 mg (82%). An aqueous solution of NaOH (1 N, 3 mL) was added to **10** (79 mg, 0.35 mmol). The resulting mixture was heated at 70 °C under nitrogen atmosphere for 4 h. The reaction mixture was cooled to room temperature and acidified with HCl (1 N). The white precipitate was collected by filtration, washed several times with H₂O and dried overnight to afford **11**. m.p. 218–220 °C. ¹H NMR (CD₃CN): δ 9.50 (br s, 1H), 7.98 (d, *J* = 1.8 Hz, 1H), 7.66 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.45 (d, *J* = 8.1 Hz, 1H), 4.34 (br s, 2H). (CD₃OD): δ 8.07 (d, *J* = 1.8 Hz, 1H), 7.73 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.52 (d, *J* = 8.1 Hz, 1H), ¹³C NMR (CD₃OD): δ 168.9, 140.4, 132.9, 131.5, 130.5, 129.5, 128.4 ppm. HRMS (-ESI): calcd for C₇H₆O₂S₂ = 185.9809, found 185.9811. (Lit.:¹⁴ (CD₃OD): δ 8.03 (d, *J* = 2 Hz, 1H), 7.70 (dd, *J* = 8, 2 Hz, 1H), 7.47 (d, *J* = 8 Hz, 1H), 4.05 (s, 1H), 3.67 (s, 1H). ¹³C NMR (CD₃OD) δ 167.9, 139.4, 131.9, 130.4, 129.5, 128.5, 127.4 ppm.

2,2-Dimethylbenzo[d][1,3,2]dithiastannole-5-carboxylic acid (12)

Compound **12** was synthesized using a modified literature procedure by Lee et al.³⁵ To an ethanolic solution (10 mL) of **11** (116 mg, 0.623 mmol) was added an ethanolic solution of NaOH (5 mL, 0.2 M). After the mixture was stirred at rt for 15 min, an aqueous solution (5 mL) of dimethyltin chloride (273 mg, 1.24 mmol) was added. The solution was stirred for 1.5 h, acidified with 1N HCl and extracted with CH₂Cl₂ (30 mL × 3). The resultant organic layers were combined and washed with water and brine solution. The organic layer was dried over anhydrous MgSO₄ and the solvent was removed affording 155 mg (75%) of **12**. ¹H NMR (CD₃CN) 7.95 (d, *J* = 1.6 Hz, 1H), 7.45 (d, *J* = 8.1 Hz, 1H), 7.40 (dd, *J* = 8.1, 1.6 Hz, 1H), 0.96 (s, 6H); ¹³C NMR (CDCl₃) δ 166.5, 146.5, 139.9, 129.7, 129.0, 125.1, 124.1 4.76 ppm; HRMS (+ESI) calcd for C₉H₁₀O₂S₂Sn = 325.9170, found 325.9171.

4-Nitrophenyl 2,2-dimethylbenzo[d][1,3,2]dithiastannole-5-carboxylate (13)

Yield 149 mg (72%). Compound **12** (155 mg, 0.465 mmol) was dissolved in dry CH₂Cl₂ (5 mL). To this solution was added a catalytic amount of DMAP, with stirring for 5 min. DCC (113 mg, 0.51 mmol) was added. After the solution was stirred for 10 min, *p*-nitrophenol (138 mg, 0.93 mmol) was added, and the solution was stirred overnight and 30 mL of CH₂Cl₂ was added. The white precipitate of urea was removed by filtration. The organic layer was washed with concentrated citric acid solution, saturated aqueous NaHCO₃

solution, water, and the organic layer was dried over anhydrous MgSO_4 . The solvent was removed affording crude compound **13**. The crude mixture was purified by column chromatography using EtOAc/hexanes (2:3) to afford **10**; $R_f = 0.54$, mp 198–199 °C; ^1H NMR (CDCl_3) δ 8.33 (d, $J = 8.3$ Hz, 2H), 8.30 (d, $J = 1.6$ Hz, 1H), 7.67 (dd, $J = 8.1, 1.6$ Hz, 1H), 7.59 (d, $J = 8.1$ Hz, 1H), 7.39 (d, $J = 8.3$ Hz, 2H), 1.09 (s, 6H); ^{13}C NMR (CDCl_3) δ 163.6, 155.9, 147.4, 145.4, 139.7, 131.2, 130.0, 125.6, 125.2, 124.4, 122.6, 3.2 ppm; HRMS (-ESI) calcd for $\text{C}_{15}\text{H}_{13}\text{NO}_4\text{S}_2\text{Sn} = 446.9334$, found 446.9324.

4-Nitrophenyl benzo[f][1,2,3,4,5]pentathiepine-7-carboxylate (**14**)

Yield 9.5 mg (27%). Compound **14** was synthesized using a modification of the procedure of Sato et al.¹⁵ To a solution of **10** (40 mg, 0.088 mmol, in 7 mL of dry CH_2Cl_2) at 0 °C was added dropwise a solution of S_2Cl_2 (23.8 mg 0.176 mmol in 3 mL of dry CH_2Cl_2) at 0 °C. The reaction mixture was allowed to reach rt and stirred for 24 hr. After 15 mL of CH_2Cl_2 was added, the reaction mixture was washed with water (20 mL $\times$ 3). The organic layer was dried with anhydrous MgSO_4 and evaporated. The product was purified by column chromatography using EtOAc/hexanes (1:4) to afford **14**; $R_f = 0.44$ (CHCl_3 /hexanes, 1:2) ^1H NMR (CHCl_3) δ 8.66 (d, $J = 1.5$ Hz, 1H), 8.36 (d, $J = 8.1$ Hz, 2H), 8.13 (dd, $J = 8.1, 1.5$ Hz, 1H), 8.02 (d, $J = 8.1$ Hz, 1H), 7.42 (d, $J = 8.3$ Hz, 2H); ^{13}C NMR (CDCl_3) δ 162.4, 155.2, 150.3, 145.8, 1145.0, 137.3, 136.3, 131.5, 130.2, 125.4, 122.5.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Ion extracted $[M+H^+]$ LCMS spectra of compounds **6A-F**. Chromatograms were normalized to the largest peak **6C**. The “counts” on the Y-axis are equal to the number of ions detected.

Figure 2. HPLC chromatogram of polysulfanes; peaks include, 1, **6A**; 2, **6B**; 3, **6C**; 4, **6D**; 5, **6E**; 6, **6F**. Elemental sulfur S₈ elutes in the 11–13 min range. Peaks marked with an asterisk belong to unidentified substances.

Figure 3.

Effect of 1,2-benzenedithiol, *o*-benzopolysulfanes **5A-C**, and PEGylated benzopolysulfanes **6A-F** on the growth of human cancer cell lines. Prostate cancer cell lines DU145 and PC3 cells, breast cancer cell line MDA-MB-231 and Jurkat cells, a leukemia cell line, were grown in 96-well plates. When the cells were in exponential growth they were incubated with different concentrations of 1,2-benzenedithiol, **5A-C**, or **6A-F** for 48 h (DU145, PC3, MDA-MB-231) or 72 h (Jurkat). The increase in cell numbers relative to controls were determined by the CyQuant assay (A-C) and by the MTS assay (D) as described in the Experimental Section. The values were expressed as the increase in cell numbers relative to controls without any compound. Data are the mean $\pm$ SE ($n = 6-8$).

Scheme 1.
Natural and Unnatural Benzopolysulfanes

Scheme 2.

Equilibration Between Tri-, Penta-, and Heptasulfanes

Scheme 3^a Synthesis of PEG Conjugated Benzopentasulfane 6C

^a Reagents and conditions: (i) HCl (cat.), MeOH, reflux 80°C, 7 h; (ii) Me₂NC(=S)Cl, DABCO, DMF, RT, 30 min; (iii) Ph₂O, 230 °C, 40 min; (iv) (a) aqueous NaOH, under N₂, 70°C, 4 h, (b) 1N HCl; (v) (a) Me₂SnCl₂, KOH, EtOH, water, (b) 1N HCl; (vi) *p*-nitrophenol, DCC, DMAP, CH₂Cl₂, 1 d; (vii) S₂Cl₂, CH₂Cl₂, 0 °C, 30 min, then warmed to RT, 24 h; (viii) H₂N-(CH₂O)₃-CH₃ **15** (Scheme 4), THF, RT, 12 h.

Scheme 4.
COSY NMR Couplings of Benzopentasilfane 6C

Scheme 5.

Equilibration of Pentasulfane 6C with the Minor Amounts of Tri-, Tetra-, Hexa-, Hepta- and Nonasulfanes in Methanol/Water (1:1 vol/vol)

Scheme 6.

PEGylated Benzopolysulfanes 6A-F as Sulfur-Transfer Reagents to Norbornene but not to 2,3-Dimethylbutadiene

Scheme 7.
Potential Mechanism for the Triatomic Sulfur-Transfer of Benzopentasulfane 6C

Table 1GC/MS Detection of the Parent Benzopolysulfanes 5A-C^a

Compound	No. of S atoms	MS t_R (min) ^b	Molecular Weights	
			calcd	found ^c
<i>o</i> -C ₆ H ₄ S ₃ 5A	3	14.2	172	172
<i>o</i> -C ₆ H ₄ S ₅ 5B	5	26.5	236	236
<i>o</i> -C ₆ H ₄ S ₇ 5C	7	39.1	300	300

^aRef. 12. See also Ref. 13.^bGCMS retention time.^cLow-resolution GCMS. Support for the GC/MS peak assignments came from spectroscopic comparisons of **5A-C**, which were independently synthesized and examined shortly after their purification, i.e., before equilibration was pronounced, which took 1–3 days.

Table 2

Mass Spectrometry Data for PEGylated Benzopolysulfanes 6A-F

Compound	Formula	Retention time ^a (min)	Calculated mass	Experimental mass ^b [(M+H ⁺)-H ⁺] ^b	Error (ppm)
6A	C ₁₄ H ₁₉ NO ₄ S ₃	4.6	361.0476	361.0478	0.62
6B	C ₁₄ H ₁₉ NO ₄ S ₄	5.6	393.0197	393.0193	-1.00
6C	C ₁₄ H ₁₉ NO ₄ S ₅	6.5	424.9918	424.9926	1.99
6D	C ₁₄ H ₁₉ NO ₄ S ₆	7.1	456.9638	456.9636	-0.60
6E	C ₁₄ H ₁₉ NO ₄ S ₇	7.8	488.9359	488.9354	-1.08
6F	C ₁₄ H ₁₉ NO ₄ S ₉	9.2	552.8800	552.8787	-2.34

^aLCMS retention time. Chromatography was performed on a SB-C18 3.5 3m column using water containing 0.1% formic acid and 5 mM ammonium formate (solvent A) and methanol containing 0.1% formic acid and 5 mM ammonium formate (solvent B) at a flow rate 0.5 mL/min. The gradient program was as follows 15–85% B (0–13 min) 85% B (13–15 min) 85–15% B (1 min).

^bThe experimental exact mass is calculated by the subtraction of a proton (H⁺; 1.00728 Da) to the measured *m/z* value the [M+H⁺] ions for the molecular formulas of interest.

Table 3

Polysulfane Distribution as a Function of HPLC Solvent Elution Condition^a

Method ^b	Ratio of MeOH-H ₂ O (V/V)	Polysulfanes and Elemental Sulfur							
		6A	6B	6C	6D	6E	6F	S ₈	
1	50–50	2.2	1.8	93.5	0.4	0.3	0.2	1.6	
2	60–40	2.5	2.1	94.7	-	0.6	-	-	
3	75–25	4.3	3.2	84.6	1.5	0.9	0.5	3.4	
4	80–20	4.7	4.8	83.6	1.0	0.3	0.4	5.1	
5	85–15	5.3	5.7	79.0	3.4	1.2	0.1	5.3	

^a HPLC analysis at 254 nm at room temperature with a flow rate of 1 mL/min in water-methanol mixtures.

^b HPLC methods:

Method 1 consisted of a gradient of methanol from 10% to 90% over 53 min and maintained for 2 min before reverting to 10% methanol over 1 min. Method 2 consisted of 60% methanol run isocratically over 91 min. Method 3 consisted of a gradient of methanol from 60% to 95% over 30 min, which was maintained for 10 min before reverting to 15% methanol over 5 min. Method 4 consisted of a gradient of methanol from 15% to 95% over 30 min, which was maintained for 15 min before reverting to 15% methanol over 3 min. Method 5 consisted of a gradient of methanol from 15% to 95% over 15 min, which was maintained for 15 min before reverting to 15% methanol over 2 min. Method 6 consisted of water (0.1% formic acid and 5 mM ammonium formate in water) and methanol (0.1% formic acid and 5 mM ammonium formate in MeOH) with a gradient of methanol from 15% to 85% over 13 min, which was maintained for 2 min before reverting to 15% methanol over 1 min.

Table 4

Decomposition of PEGylated Benzopolysulfanes 6A-F as a Function of Hydroxide Ion Equivalents Added^a

Hydroxide ion equivalents	Internal standard	Polysulfanes and elemental sulfur distribution ^b						
		6A	6B+X ^c	6C	6D	6E	6F	S ₈
0.25	100	10.9	11.2	362.4	2.3	3.0	2.1	12.3
0.5	100	13.2	19.6	289.2	2.1	2.8	3.7	23.1
0.75	100	14.2	38.1	222.9	1.8	4.7	3.8	26.7
1.0	100	14.2	64.9	163.9	1.5	1.6	3.1	32.4
1.0 ^d	100	14.9	85.5	113.6	0.7	2.2	-	3.1

^a Reaction of benzopolysulfanes **6A-F** (2.5 mM) and hydroxide ion in the presence of internal standard acetanilide (0.25 mM) in methanol/water (99.9:0.1 vol/vol).

^b Polysulfane ratios were determined by HPLC monitoring at 254 nm at room temperature with the flow rate 1 mL/min using method 4 (Table 3). HPLC analysis was carried out after 1 hr of equilibration between benzopolysulfanes **6A-F** and hydroxide ion.

^c X is unidentified compound that was eluted with **6B**.

^d Analysis was done after 24 hrs.

Table 5

Experimental Solubility Values

Reagent	Solvent	
	Methanol (mg/mL)	Water (μ g/mL)
Elemental sulfur, S ₈	0.33 $\pm$ 0.03	0.14 $\pm$ 0.02
<i>o</i> -Benzopolysulfanes 5A-C (97%)	0.37 $\pm$ 0.02	0.18 $\pm$ 0.02
PEGylated benzopolysulfanes 6A-F (93%)	19.00 $\pm$ 0.05	8.70 $\pm$ 0.25

^a Equilibrium time of 24 h was used in the solubility study.

^b Measurements were conducted three times and the solubility value was averaged.

Table 6

Inhibition of Cancer Cell Growth by 1,2-Benzenedithiol, *o*-Benzopolysulfanes 5A-C, and PEGylated Benzopolysulfanes 6A-F

Compound	IC ₅₀ μM ^a			
	PC-3	DU145	MDA-MB-231	Jurkat
1,2-Benzenedithiol	No effect	>30	>30	60
<i>o</i> -Benzopolysulfanes 5A-C (97% purity)	10.5 (20) ^b	4.9 (6) ^b	27	0.5
PEGylated benzopolysulfanes 6A-F (~98% total purity)	1.8 (4) ^b	3 (4) ^b	5.5 (12) ^b	0.4

^aIC₅₀ values were obtained for 24 h incubations with Jurkat cells and 48 h for PC3, DU145 and MDA-MB-231 cells.

^bConcentrations that resulted in 100% cell kill are shown in parentheses. Experiments were conducted in 96 well plates with 8 replicates for each concentration (0–30 μM) of the indicated compounds.