Design, Synthesis, and Biological Evaluation of (*E*)-Styrylbenzylsulfones as Novel Anticancer Agents

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Cell cycle progression is regulated by cyclins and cyclin-dependent kinases, which are formed at specific stages of the cell cycle and regulate the G1/S and G2/M phase transitions, employing a series of "checkpoints" governed by phosphorylation of their substrates. Tumor development is associated with the loss of these checkpoint controls, and this provides an approach for the development of therapeutic agents that can specifically target tumor cells. Here, we describe the synthesis and SAR of a novel group of cytotoxic molecules that selectively induce growth arrest of normal cells in the G1 phase while inducing a mitotic arrest of tumor cells resulting in selective killing of tumor cell populations with little or no effect on normal cell viability. The broad spectrum of antitumor activity in vitro and xenograft models, lack of in vivo toxicity, and drug resistance suggest potential for use of these agents in cancer therapy.

Introduction

Cancer is now believed to result from unlimited growth of a given cell, which is often due to a block in the ability of cells to undergo differentiation and/or apoptosis. Most of our understanding of how cells grow and divide comes from the study of cells grown in vitro. The cell cycle is typically divided into four phases: G1, S, G2, and M. The periods associated with DNA synthesis (S phase) and mitosis (M phase) are separated by gaps called G1 and G2.[1–6] The past 2 decades have seen a series of discoveries that have provided us with a better understanding of the complexity of the control mechanisms, which ensure ordered progression of cell cycle. It is becoming apparent that the order and timing of the cell cycle are critical for accurate transmission of genetic information, and consequently, a number of biochemical pathways have evolved to ensure that initiation of a particular cell cycle event is dependent on the accurate completion of the others. These biochemical pathways have been termed "checkpoints".[1–6]

Most normal cells, unless they have received a stimulus to proliferate or differentiate, remain in a resting state, termed G_o. However, when the organism requires additional cells, extracellular stimuli induce the cells to enter the G_1 phase of the cell cycle and become committed to cell division. It is at a late point in the G_1 phase of the cell cycle that a potentially dividing cell reaches the "restriction point", a time at which the cell must determine whether the conditions are suitable for continued proliferation.[4,7,8] Provided that conditions are conducive to proliferation, the cell proceeds past this checkpoint. An absolute prerequisite for cell growth is the duplication of its genetic material, which occurs during the S phase. Once the DNA has been replicated, the cell "ascertains" whether this process has been correctly executed during the second checkpoint during G_2, and provided that it has, the cell divides during mitosis, or M phase.[8,9] The ordered growth process seen in normal cells is a result of regulatory control mechanisms that restrain cell cycle machinery. The genetic changes seen in a malignant cell are primarily aimed at overriding this negative regulation and result in the loss of one or both of the intrinsic checkpoints that are normally used by their normal counterparts. While some of the oncogenes, such as *ras*, force progression through G_1, other genes such as Rb, which are termed tumor suppressor genes, function as "gatekeepers" of these restriction points.[10,11] Cancer is characterized by a loss of one or more tumor suppressor genes, which enables a malignant cell to ignore all of the safeguards that are aimed at preventing unwanted cell division.

An important rule associated with cell cycle progression (for both normal and tumor cells) is the fact that once a cell crosses the "restriction point" (which is the G1/S boundary), it has to either divide into two daughter cells or die[4] because of the fact that most eukaryotic cells can exist in S, G2, and M phases of the cell cycle for only a limited time. Most chemotherapeutic agents, such as paclitaxel, that are currently used in cancer therapy function by blocking cell cycle progression at a point beyond the G_1/S boundary (M phase in the case of paclitaxel), resulting in the death of the tumor cell.[12] A major problem with many of the current drugs is their inability to discriminate between normal and tumor cells. As a result, normal cells undergoing active cell division also become blocked at the mitotic phase of the cell cycle and enter programmed cell death pathways, the effects of which are often manifested as the toxic side effects seen in patients treated by these drugs. A second problem appears to be the development of resistance to many of the chemotherapeutic agents often due to overexpression of drug transporters. Our quest was to design new chemical entities that exhibit reduced toxicity in normal cells and are not recognized by drug transporters that are overexpressed in drug-resistant tumor cells. In this communication, we describe the synthesis of a group of styrylbenzylsulfones that induce apoptotic death of a wide variety of human tumor cell lines at subnanomolar concentrations while exhibiting relatively low toxicity to normal human cells. More importantly, these compounds were found to be active against a wide variety of

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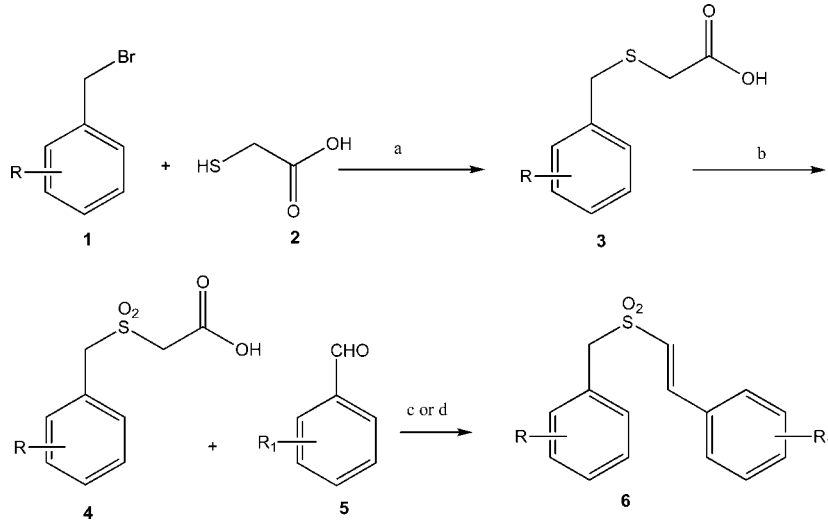
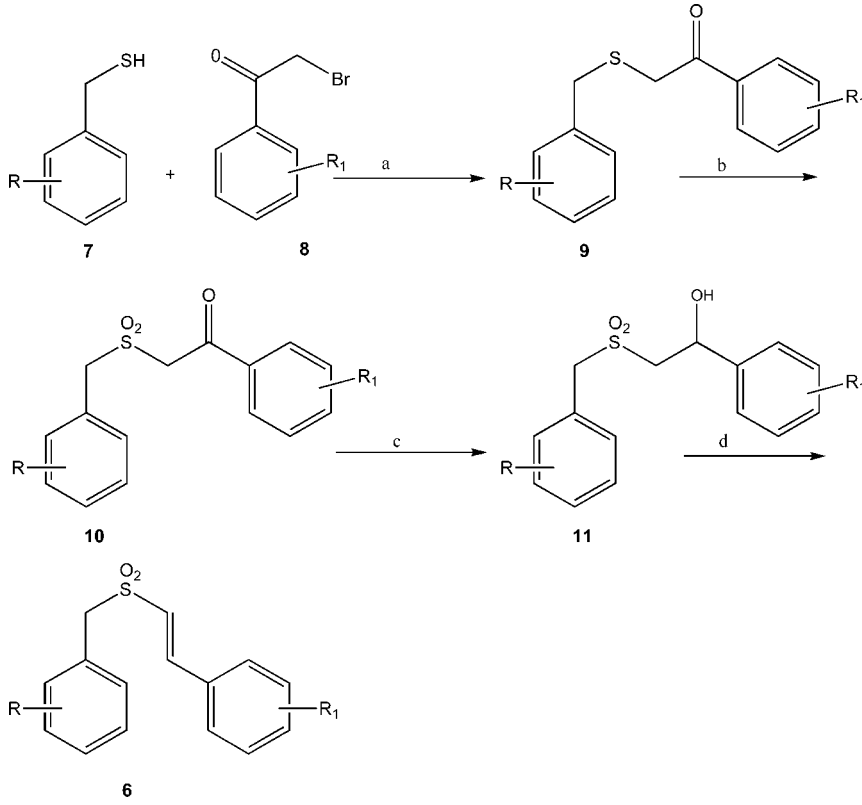
Scheme 1. Synthesis of (*E*)-Styrylbenzylsulfones[a]



[a] Reagents and conditions: (a) NaOH, MeOH, 4 h, room temp, 94%; (b) 30% H_2O_2, CH_3COOH, 18 h, room temp, 92%; (c) piperidine, benzoic acid, toluene, 120 °C, 2–4 h, 48%; (d) CH_3COOH, benzylamine, 118 °C, 2–8 h, 66%.

Scheme 2. Alternative Method for the Synthesis of (*E*)-Styrylbenzylsulfones[a]



[a] Reagents and conditions: (a) NaOH, MeOH, 4 h, room temp, 93%; (b) 30% H_2O_2, CH_3COOH, 24 h, room temp, 94%; (c) $NaBH_4$, EtOH, 30 min, 0 °C, 88%; (d) *p*-toluenesulfonic acid, C_6H_6, 3 h, 80 °C, 65%.

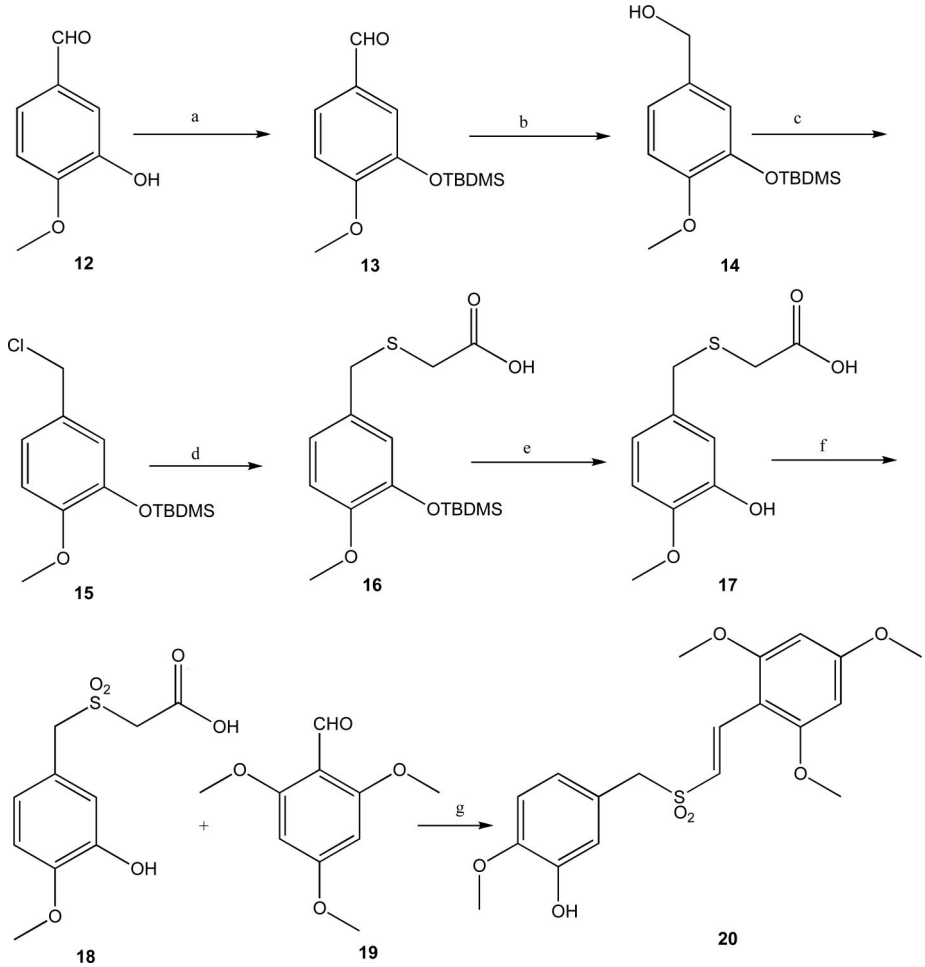
human tumor cell lines that are resistant to the activity of many of the cytotoxic agents.

Chemistry

The synthetic routes for the preparation of substituted styrylbenzylsulfones are shown in Schemes 1 and 2. In Scheme 1 substituted benzyl bromides, **1**, were reacted with thioglycollic acid **2** in the presence of mild or strong bases to obtain compound **3**. Complete oxidation of **3**, with hydrogen peroxide in the presence of glacial acetic acid, gave **4**.[13] Knoevenagel condensation of **4** with aromatic aldehydes **5** in toluene in the presence of catalytic amounts of piperidine and benzoic acid yielded styrylbenzylsulfones, **6**.[14] Alternately, the condensation between **4** and **5** was also carried out in glacial acetic acid in the presence of a catalytic amount of benzylamine to obtain **6**.[13]

Monosubstituted styrylbenzylsulfones were also prepared as shown in Scheme 2. Benzyl mercaptans with desired substituents, **7**, were reacted with α-bromo substituted acetophenones, **8**, in absolute alcohol in the presence of a base to obtain **9**.

Scheme 3. Synthesis of (*E*)-2,4,6-(Trimethoxystyryl)-3-hydroxy-4-methoxybenzylsulfone[a]



[a] Reagents and conditions: (a) TBDMS−THF, *N*,*N*-diisopropylethylamine, DMF, room temp, 12–16 h, 100%; (b) $NaBH_4$, MeOH, 0 °C, 30 min, 73%; (c) $SOCl_2$, C_6H_6, 0 °C, 2 h, 70%; (d) $HSCH_2COOH$, NaOH, MeOH, 3 h, room temp, 75%; (e) TBAF−THF, THF, room temp, 2 h, 50%; (f) 30% H_2O_2, CH_3COOH, 18 h, room temp, 60%; (g) piperidine, benzoic acid, toluene, 120 °C, 2–4 h, 63%.

Oxidation of **9** with hydrogen peroxide in glacial acetic acid gave **10**.[13] Reduction of the carbonyl group in **10** with sodium borohydride in ethanol gave corresponding alcohol **11**.[14] Elimination of water from **11** in refluxing solution of *p*-toluenesulfonic acid in benzene afforded **6**.[15]

Schemes 3 and 4 depict the method used for the preparation of 2,4,6-trimethoxystyryl-3-hydroxy-4-methoxybenzylsulfone **20** and its water-soluble disodium phosphate salt **29** (Scheme 5).

The synthesis of compound **20** is outlined in Scheme 3. TBDMS protected isovanillin **13** was reduced to alcohol **14** with sodium borohydride. The benzyl alcohol **14** was converted to benzyl chloride **15** with $SOCl_2$ and was subsequently converted to **16** by condensing with thioglycollic acid. Deprotection of TBDMS with tetrabutylammonium fluoride and subsequent oxidation of **17** with hydrogen peroxide provided **18**. Condensation of **18** with aldehyde **19** provided styrylbenzylsulfone **20**.

Alternatively, **20** was also prepared as shown in Scheme 4. Isovanillin **12** was reacted with 4- toluenesulfonyl chloride in the presence of pyridine to obtain compound **21**.[16] Reduction of **21** with sodium borohydride gave **22**. Treatment of **22** with thionyl chloride resulted in **23**, which on condensation with thioglycollic acid yielded benzylthioacetic acid **24**. Oxidation of **24** with hydrogen peroxide gave corresponding sulfonylacetic acid **25**. Knoevenagel type condensation of **25** with 2,4,6-trimethoxybenzaldehyde **19** in the presence of a base produced unsaturated sulfone **26**. Removal of tosyl group by treating **26** with sodium hydroxide gave the styrylbenzylsulfone **20**.

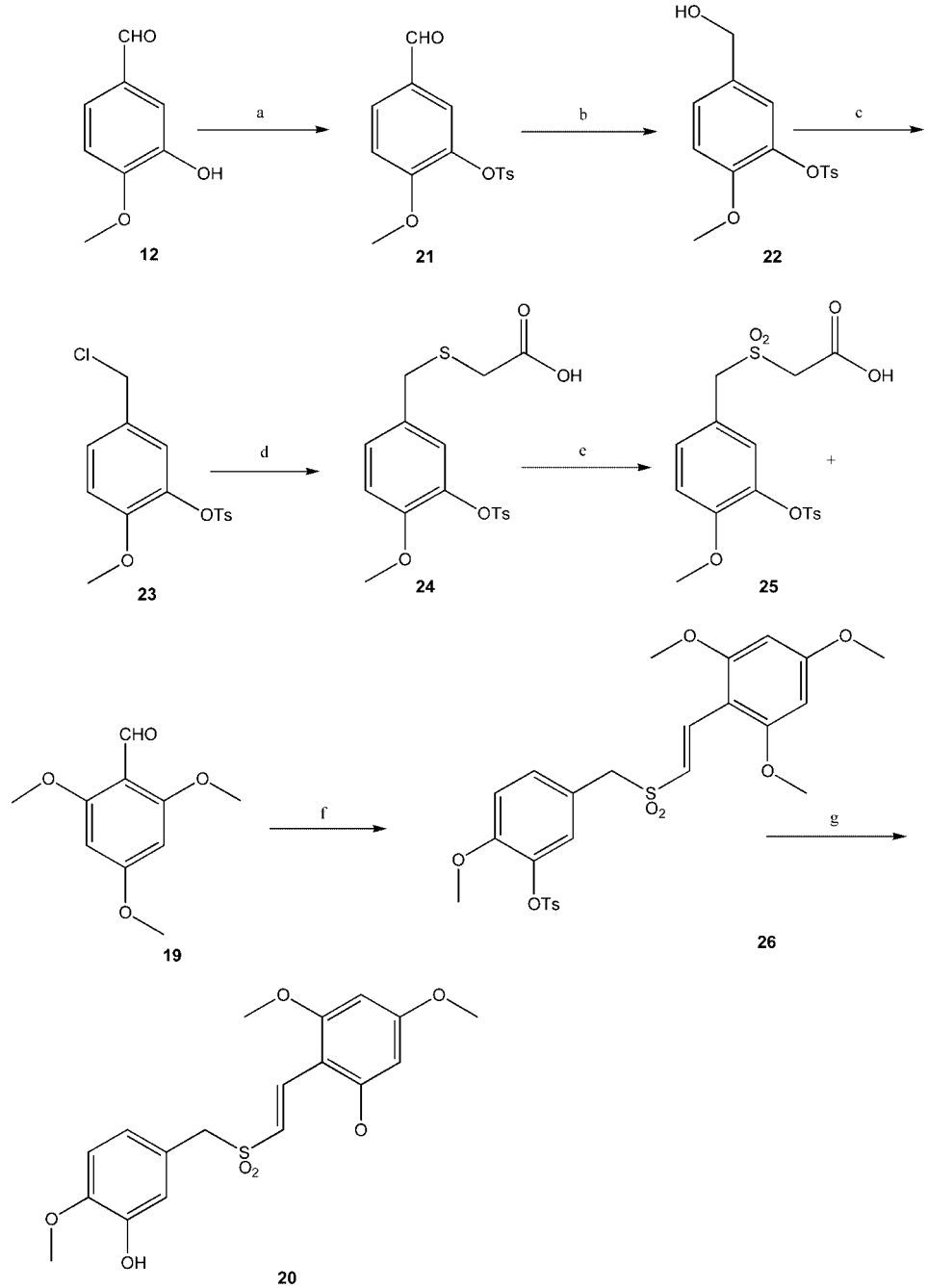
To enhance the bioavailability and water solubility of (*E*)-2,4,6-trimethoxystyryl-3-hydroxy-4-methoxybenzylsulfone **20**, its sodium phosphate prodrug was synthesized (Scheme 5). The prodrug was synthesized in three steps starting from styrylbenzylsulfone **20**. Phosphorylation of the phenolic group in **20** employing dibenzyl phosphite under basic conditions gave *O*-dibenzyl phosphate **27**. Cleavage of benzyl groups with bromotrimethylsilane in acetonitrile produced 3-*O*-phosphate **28**.[18] Treatment of the phosphate **28** with sodium hydroxide in anhydrous ethylene glycol dimethyl ether yielded disodium *O*-phosphate **29**.

Structure–Activity Relationships (SAR)[a]

Following the synthesis of this group of compounds, their in vitro cytotoxicity was assessed using four different human tumor cell lines derived from human breast (BT20), prostate (DU145), lung (H157), and colorectal (DLD1) cancers. The results of this study are presented in Table 1. These studies show that the cytotoxic activity of the styrylbenzylsulfones is completely dependent on the nature and position of the substituents on the two aromatic rings. In a majority of the compounds described here, we have kept a methoxy group constant at the fourth

[a] Abbreviations: SAR, structure–activity relationship; MDR, multidrug resistance; HFL, human lung fibroblasts; PARP, poly(ADP-ribose) polymerase-1; HUVEC, human vascular endothelial cells; NBT, nitroblue tetrazolium.

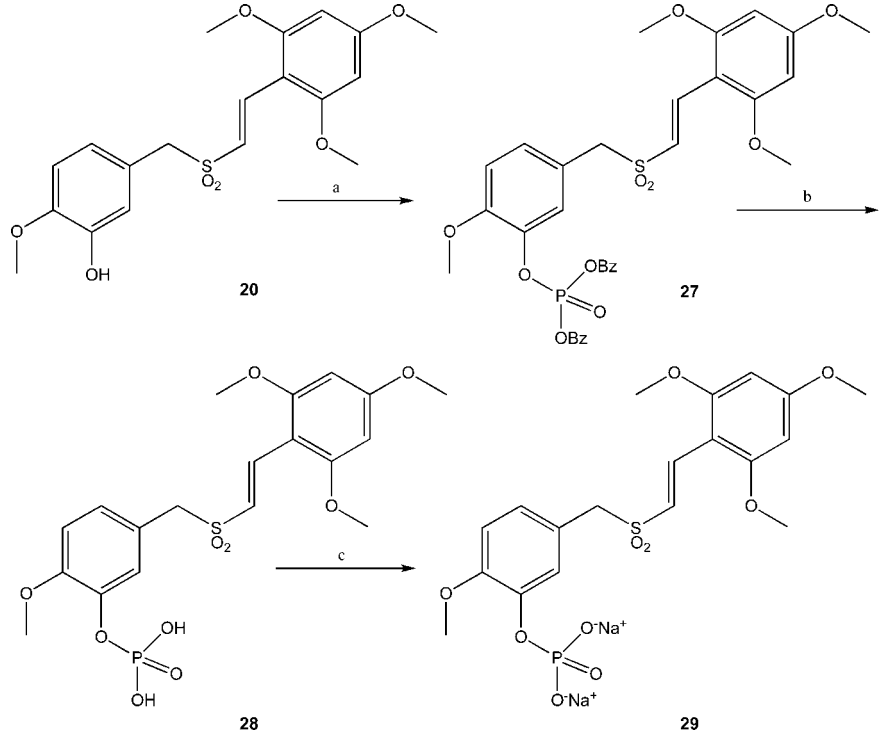
Scheme 4. Alternative Method for the Synthesis of (*E*)-2,4,6-(Trimethoxystyryl)-3-hydroxy-4-methoxybenzylsulfone[a]



[a] Reagents and conditions: (a) *p*-toluenesulfonyl chloride, pyridine, 70–80 °C, 2 h, 98%; (b) $NaBH_4$, MeOH, 20 °C, 30 min, 97%; (c) $SOCl_2$, C_6H_6, 15–20 °C, 2 h, 90%; (d) $HSCH_2COOH$, NaOH, MeOH, 65 °C, 5 h, 93%; (e) 30% H_2O_2, CH_3COOH, room temp, 18 h, 80%: (f) piperidine, benzoic acid, C_6H_6, 80 °C, 4–5 h, 65%; (g) 20% NaOH, MeOH, 65 °C, 3–4 h, 95%.

position on the aromatic ring of the benzyl moiety. A moderate cytotoxic activity was seen when a fluorine atom was present at the 4-position (**6b**) on the styryl aromatic ring. Changing the fluorine atom at the 4-positon with a chlorine (**6c**), a nitro (**6d**), a methoxy (**6a**), or an amino (**6e**) group gradually decreased the activity of the molecules. When the position of the methoxy group is changed from the 4- to the 2-position (**6f**) on the styryl aromatic ring, the molecule partially recovered the lost activity. The introduction of a chlorine atom in the 2-position (**6g**) in **6b** retained cytotoxic activity, whereas a methoxy group in the same position (**6i**) resulted in the loss of activity. Dimethyl substitutions on the styryl aromatic ring with a methoxy group at the 4-position on the benzyl aromatic ring (**6h**, **6k**, and **6l**) resulted in the molecules possessing a low level of cytotoxicity. Whereas the results are quite surprising for the molecules that are disubstituted with methoxy groups (**6j**, **6m**, **6n**, **6o**, and **6p**) on the styryl aromatic ring, the results obtained in cytotoxicity assays using these compounds (**6j**, **6m**, **6n**, **6o**, and **6p**) clearly show that the methoxy group, when present at the 2,6-positions (**6m**), enhances the activity of the molecule by greater than 40-fold when compared to other disubstituted methoxysulfones (**6j**, **6n**, **6o**, and **6p**). Because the introduction of two methoxy groups on the styryl aromatic ring enhanced the biological activity, we have synthesized some trimethoxystyryl analogues to determine if this further enhances their cytotoxic properties. Analysis of these compounds (**6q**, **6r**, **6s**, and **6t**) in the cell-killing assays showed that 4-methoxybenzyl-2,4,6-trimethoxystyrylsulfone (**6s**) is 20-fold more active than **6m**, whereas the other trisubstituted

Scheme 5. Method for the Synthesis of (*E*)-2,4,6-(Trimethoxystyryl)-3-*O*-phosphate Disodium 4-Methoxybenzylsulfone[a]



[a] Reagents and conditions: (a) CH_3CN, triethylamine, CBr_4, dibenzyl phosphite, KH_2PO4, room temp, 2 h, 73%; (b) CH_3CN, bromotrimethyl silane, sodium thiosulfate, room temp, 45 min, 44%; (c) $NaOH/H_2O$, ethyleneglycoldimethyl ether, room temp, 3 h, 98%.

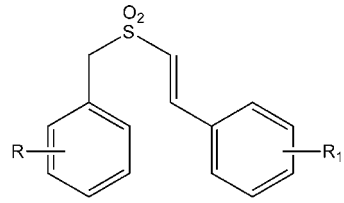
styrylsulfones (**6q**, **6r**, and **6t**) were totally inactive at the highest concentration (20 μM) tested. These results show that when the 2, 4, and 6 positions on the styryl aromatic ring are occupied by methoxy groups, the molecules attain optimum biological activity. To validate whether methyl groups at those positions can replace the methoxy groups and retain the activity, we have prepared 2,4,6-trimethylstyrylsulfone (**6w**), which was found to be inactive in cell-killing assays. To further assess the significance of the methoxy group on the 4-position of styryl ring in **6m**, we replaced the methoxy group at that site with a hydroxy (**6u**) or a fluoro (**6v**) substituent. Both of these replacements resulted in either a reduced level or a total loss of activity. Once the methoxy subtituents are fixed at the 2, 4, and 6 positions of the styryl ring, to further enhance the activity of the molecule, we then determined the effect of other substituents on the benzyl aromatic ring. Replacing the methoxy group at the 4-position on the benzylic aromatic ring of **6s** by chloro (**6ae**), nitro (**6af**), cyano (**6ag**), carboxy (**6ah**), and hydroxy (**6ai**) resulted in molecules that substantially lost activity. These results show that the methoxy group is indispensable at the 4-position of the benzyl aromatic ring of **6s** with respect to its biological activity. To analyze the effect of the additional substituents on the benzyl aromatic ring, we have synthesized a number of analogues containing 4-methoxy-3-halo, nitro, cyano, carboxy, methoxy (data not shown), hydroxy (**6aa**), 3,4,5-trimethoxy (**6ac**), and 2,3,4-trimethoxy (**6ad**) benzylsulfones. Cytotoxicity analyses of these analogues on four cancer cell lines showed that the compound with the hydroxy substituent at position 3 (**6aa**) exhibited the best activity in the entire series. This compound, **6aa**, is almost 8- to 10-fold more active than **6s** in all four cell lines. Further, the introduction of a hydroxy group at the third position not only enhanced the potency of the molecule but also created a method to generate a water-soluble analogue (**6ab**), which is critical for intravenous administration of the compound. The conversion of the hydroxyl group in **6aa** to a disodium phosphate **6ab** derivative did not alter the potency of the molecule.

Biological Results and Discussion

In Vitro Antitumor Effects of 6s and 6aa Compounds. We next tested the activity of two of the most active compounds listed in Table 1 against 94 different human tumor cell lines, and surprisingly, they were found to induce apoptosis of all of these cell lines with very similar GI_{50} values (selected data shown in Table 2). Some of these compounds (such as **6s**, **6aa**) were also tested by the National Cancer Institute through its Developmental Therapeutics Program (DTP) against their panel of 60 human cancer cell lines.[21] Their results showed that these compounds exhibited broad-spectrum activity and inhibited the growth of all of the tested cell lines, including drug-resistant cell lines, at nanomolar concentrations. Notably, the GI_{50} and LC_{50} values for many of these cell lines were similar, indicating that they induced apoptosis in these cells. Statistical comparison (using the NCI algorithm COMPARE) revealed that these drugs are mitotic blockers of tumor cells.

6s and 6aa Compounds Are Highly Active against Drug-Resistant Tumor Cell Lines. Development of resistance to classical chemotherapeutic agents is widely observed in patients who have not responded or have relapsed after first round therapy and is the primary cause of treatment failure. In the initial screening experiments, we observed that both our cell panel and the NCI panel included several cell lines that are multidrug resistant[19,20] but were highly sensitive to the proapoptotic effects of this series of compounds. To further investigate the activity of these compounds against MDR positive tumor types, we determined the IC_{50} values of **6s** and **6aa** using two classical MDR positive cell lines. The results shown in Figure 1A show a 96 h dose response of the uterine sarcoma cell line MES-SA and the multidrug resistant subline MES-SA/DX5[19]

Table 1. In Vitro Cytotoxicity of Styrylbenzylsulfones



			$IC_{50}(\mu M)$			
compd	R	R_1	BT20	DU145	H157	DLD1
6a	4-OMe	4-OMe	>20	>20	>20	>20
6b	4-OMe	4-F	1.5	1.25	2.5	2.0
6c	4-OMe	4-Cl	2.0	2.5	3.5	3.0
6d	4-OMe	4-NO_2	5.0	7.5	7.5	5.0
6e	4-OMe	4-NH_2	>20	>20	>20	>20
6f	4-OMe	2-OMe	5.0	3.5	5.0	7.5
6g	4-OMe	2-Cl,4-F	2.5	2.0	2.5	4
6h	4-OMe	2,4-$(CH_3)_2$	15	12	15	15
6i	4-OMe	2-OMe,4-F	15	10	20	15
6j	4-OMe	3,4-$(OMe)_2$	>20	>20	>20	>20
6k	4-OMe	3,5-$(CH_3)_2$	>20	>20	>20	>20
6l	4-OMe	2,6-$(CH_3)_2$	>20	>20	>20	>20
6m	4-OMe	2,6-$(OMe)_2$	0.40	0.25	0.70	0.70
6n	4-OMe	2,4-$(OMe)_2$	>20	>20	>20	>20
6o	4-OMe	2,5-$(OMe)_2$	>20	>20	>20	>20
6p	4-OMe	3,5-$(OMe)_2$	>20	>20	>20	>20
6q	4-OMe	2,4,5-$(OMe)_3$	>20	>20	>20	>20
6r	4-OMe	2,3,4-$(OMe)_3$	>20	>20	>20	>20
6s	4-OMe	2,4,6-$(OMe)_3$	0.020	0.025	0.030	0.040
6t	4-OMe	3,4,5-$(OMe)_3$	>20	>20	>20	>20
6u	4-OMe	2,6-$(OMe)_2$,4-OH	>20	>20	>20	>20
6v	4-OMe	2,6-$(OMe)_2$,4-F	0.75	0.5	0.5	0.75
6w	4-OMe	2,4,6-$(CH_3)_3$	>20	>20	>20	>20
6x	4-OCF_3	2,4,6-$(OMe)_3$	3	4	6	4
6y	4-OMe,3-OH	3,4,5-$(OMe)_3$	7.5	5	10	10
6z	4-OMe,3-OH	2,6-$(OMe)_2$,4-OH	0.25	0.5	0.5	0.5
6aa	4-OMe,3-OH	2,4,6-$(OMe)_3$	0.010	0.003	0.004	0.003
6ab	4-OMe,3-OPO_3Na_2	2,4,6-$(OMe)_3$	0.005	0.0025	0.007	0.007
6ac	3,4,5-$(OMe)_3$	2,4,6-$(OMe)_3$	>20	>20	>20	>20
6ad	2,3,4-$(OMe)_3$	2,4,6-$(OMe)_3$	15	12.5	15	15
6ae	4-Cl	2,4,6-$(OMe)_3$	10	7.5	15	15
6af	4-NO_2	2,4,6-$(OMe)_3$	15	15	20	15
6ag	4-CN	2,4,6-$(OMe)_3$	12.5	7.5	15	20
6ah	4-COOH	2,4,6-$(OMe)_3$	15	10	17.5	15
6ai	4-OH	2,4,6-$(OMe)_3$	15	7.5	20	20

treated with **6aa**. This cell line has been shown to express high levels of P-glycoprotein and is resistant to a number of drugs including doxorubicin, paclitaxel, vincristine, vinblastine, etoposide, mitoxantrone, dactinomycin, and daunorubucin. The activity of our compounds was then compared to the activity of paclitaxel (MDR sensitive drug). Our results show that the parental cell line was very sensitive to paclitaxel ($IC_{50} = 4$ nM), but the MDR positive subline was greater than 100-fold resistant ($IC_{50} = 750$ nM). When the two cell lines were treated with **6aa**, both the parental and the MDR positive cell lines were equally sensitive to the cell killing activity of the compound. We also investigated as to whether atypical multidrug resistant cells are sensitive to **6aa**. For these studies, we employed the parental leukemic cell line CEM and its MDR subline CEM/C2.[20] CEM/C2 was selected and subcloned for resistance to camptothecin and has cross-resistance to etoposide, dactinimycin, bleomycin, mitoxantrone, doxorubicin, and daunorubicin. Our results show that the campothecin-resistant subline, CEM/C2, was highly sensitive to the styrylbenzylsulfone series of compounds, suggesting that these compounds do not share any cross-resistance to classical MDR and atypical MDR cell lines (Table 2).

Effect of 6aa and 6ab on Soft Agar Colony formation. We next tested the antitumorigenic activity of **6aa** and **6ab** in soft agar. For soft agar assays, we used three different cell lines, BT20, DU145, and MIA-PaCa-2, representing breast, prostate, and pancreatic cancers, respectively. In all cases we observed complete inhibition of the growth of tumor cells in a dose-dependent manner for the individual compounds (Figure 1B shows data for MIA-PaCa-2). In these assays, paclitaxel was used as a positive control, which showed a slightly lower potency than **6aa** and **6ab** (Figure 1B).

Effects of 6s and 6aa on Cell Cycle Progression of Normal and Tumor Cells. We next examined the effect of these compounds on normal and tumor cell cycle progression using FACS analysis. Figure 2A shows the effect of **6aa** on the cell cycle progression of human vascular endothelial cells (HUVEC) and DU145 (prostate cancer) cells. The results of this study show that the addition of the **6aa** to HUVEC cells resulted in a block of their cell cycle progression in the G_1 phase, causing growth arrest without a loss of viability. On the other hand, tumor cells treated with this compound gradually accumulated in the G2/M phase of the cell cycle and appeared to be unable to exit from this phase, leading to the activation of

Table 2. Tumor Cell Killing Concentrations (IC_{50}, μM) of **6s** and **6aa**

cell line	tumor type	6s	6aa
T47D	breast (ER+)	0.025	0.006
MCF-7	breast (ER+)	0.003	0.004
DU145	prostate (AR−)	0.025	0.007
PC-3	prostate (AR+)	0.03	0.008
OV-CAR-3	ovarian	0.008	0.006
Sk-OV-3	ovarian	ND	0.006
MIA-PaCa2	pancreatic	0.008	0.004
U87	glioblastoma	0.04	0.007
H157	NSCLC	0.03	0.007
A549	NSCLC	0.02	0.01
H187	SCLC	0.015	0.007
N417	SCLC	0.008	0.005
AGS	gastric	0.02	0.007
RF1	gastric	0.008	0.006
RF48	gastric	0.01	0.005
COLO-205	colorectal	0.015	0.009
DLD-1	colorectal	0.04	0.008
HCT-116	colorectal	ND	0.009
HCT-15	colorectal	0.02	0.008
SW480	colorectal	ND	0.007
SK-MEL-28	melanoma	0.04	0.007
CEM	leukemic	0.03	0.009
K562	CML	ND	0.004
MOLT-4	T-lymphoblastic: all	0.009	0.005
Namalwa	Burkitt's lymphoma (B-cell)	0.015	0.006
Daudi	Burkitt's lymphoma (B-cell)	0.008	0.007
Raji	Burkitt's lymphoma (B-cell)	0.009	0.004
MES-SA	sarcoma	0.01	0.006
MESSA/DX5[a]	resistant sarcoma	0.01	0.005
CEM	leukemic	0.03	0.01
CEM/C2[a]	resistant leukemic	0.01	0.01
2008	ovarian	ND	0.005
2008/17/4[a]	resistant ovarian	ND	0.004

[a] These cell lines constitute multidrug-resistant cell lines and show up-regulation of MDR and in the case of CEM/C2, additional mutations in the Topo-2 gene.[19,20]

apoptotic pathways as judged by PARP [poly(ADP-ribose) polymerase-1] cleavage,[22] which is a marker for caspase activation (Figure 2B). No PARP cleavage was observed in HUVEC cells following similar treatment with **6aa** compounds (Figure 2B).

In Vivo Antitumor Effects of 6s and 6aa of Compounds. In order to determine in vivo efficacy, we utilized the nude mouse model system. A highly aggressive human estrogen negative breast carcinoma cell line (BT20) was xenografted into athymic nude mice. The animals were treated with either 50 mg/kg **6s** using a Q4D schedule or 25 mg/kg using a Q2D schedule. The animals were treated when the tumors were approximately 70 mm^3 in size. Figure 3A shows that intraperitonial (ip) injections of **6s** using either of the two schedules were able to inhibit the growth of the tumors. The vehicle control treated tumors, on average, increased in volume over the 22 day period by 5-fold (62−335.5 mm^3), while the Q2D **6s** treated tumors increased in volume by only 2.5-fold (67.5−165 mm^3). The Q4D **6s** treated tumors also had significant but slightly less tumor growth inhibition, whereby these tumors increased in average volume by only 2.9-fold (74−217 mm^3). **6s** was well tolerated at these doses, as determined by body weights and physical observations. These studies show that **6s** is efficacious against human tumor xenografts while showing no signs of toxicity at the schedules tested under this study.

Because **6s** is poorly water-soluble, we synthesized **6ab**,[18] which was highly water-soluble and allowed intravenous administration. To test the effects of **6ab** in vivo, we used two groups of mice. One group received the vehicle alone, while the second group received the compound by intravenous (iv) injection into the tail vein (Figure **3B**). The tumor size was then measured on alternative days, and the total length of the experiment was 21 days. The results presented in Figure 3B show that **6ab** readily inhibited tumor growth in this xenograft model system. Of the eight mice included in each group, 100% of the control mice (placebo administered) showed a doubling or tripling of the total tumor volume. On the other hand, the majority of the mice administered with **6s** or the phosphate salt of **6aa** showed growth arrest or a gradual reduction in their tumor volume, suggesting that these compounds, with proper formulation, can be valuable anticancer therapeutics.

In Vivo Toxicity Studies in Mice. To assess the in vivo toxicity profile, 100 mg/kg **6aa** was intravenously administered into mice and its effect on the in vitro hematopoietic colony formation of bone marrow cells was determined at 12, 24, and 48 h intervals following the injection of the drug. These studies (Figure 4) show that there was no reduction in total, myeloid, or lymphoid colony formation. Single-dose and repeat-dose (28 daily injections) toxicology studies and detailed biochemical and cellular analysis of one of the water-soluble analogues (**6ab**) revealed that unlike most other cytotoxic agents, this drug did not cause hematotoxicity (no myleosuppression), liver damage, or detectable neurotoxicity in these animals. High dose-dependent drug levels were sustained in circulation, suggesting that therapeutic levels could be achieved without overt toxicity.

Acute and Repeat Toxicity Studies in Rats. We have carried out single-dose and repeat-dose (28 daily injections) toxicology studies with one water-soluble form of this series (**6ab**) to assess the safety of intravenously administered drug in rats. Detailed biochemical and cellular analysis revealed that this drug did not cause hematotoxicity (no myleosuppression), liver damage, or any detectable neurotoxicity in these animal studies. High dose-dependent drug levels were sustained in circulation, suggesting that therapeutic levels could be achieved without overt toxicity.

Conclusion

In this communication, we describe the synthesis of a group of styrylbenzylsulfones that induce apoptotic death of a wide variety of human tumor cell lines at subnanomolar concentrations while exhibiting relatively low toxicity to normal human cells. Our studies show that the cytotoxic activity of the styrylbenzylsulfones is completely dependent on the nature and position of the substituents on the two aromatic rings. In a majority of the compounds described here, we have kept a methoxy group constant at the fourth position on the aromatic ring of the benzyl moiety. These structure–function studies show that when the 2, 4, and 6 positions on the styryl aromatic ring are occupied by the methoxy groups, the molecules attain optimum biological activity (**6s**). This activity could be further enhanced by the introduction of a hydroxyl group at the third position of the benzylic ring (**6aa** and **6ab**). Biological evaluation of the activity of these compounds shows that these compounds are highly active against a wide variety of human tumor cell lines including those that are resistant to the activity of many of the currently used chemotherapeutic agents.

The low toxicity profile, both in vitro and in vivo, and their potent tumor inhibitory activity as seen in soft agar and nude mouse xenograft assays point to the potential value of these compounds as safe therapies for cancer, lacking many of the side effects normally associated with current chemotherapeutic agents. Recent studies with **6s**, **6aa**, and **6ab** show that these compounds altered the growth and cell cycle status of mantle cell lymphoma cell lines and potently inhibited the expression of several important proteins, including cyclin-dependent kinase

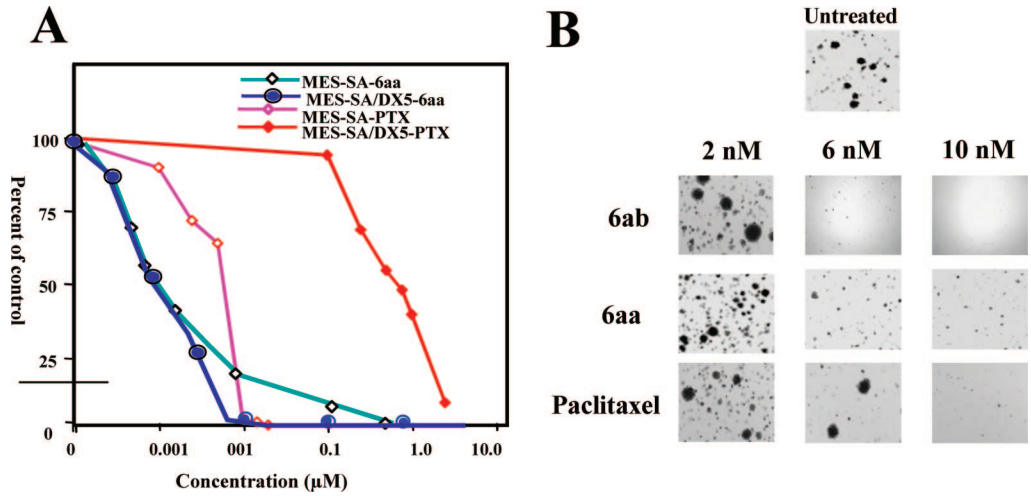


Figure 1. Antitumor effects of **6aa**. (A) **6aa** inhibits the growth of parental (MES-SA) and paclitaxel-resistant (MES-SA/DX5) cell lines with equal efficiency. The parental uterine sarcoma cells and the MDR positive (MES-SA/DX5) cells were plated into six-well dishes and treated with various concentrations of **6aa** and paclitaxel for 96 h. The number of viable cells from duplicate plates was determined by trypan blue exclusion. (B) Shown are results from soft agar assays. MIA-PaCa-2 cells (1.0×10^5) were plated in soft agar containing various concentrations of each compound in triplicate. After 3 weeks of growth, the plates were stained for 48 h using 0.05% nitroblue tetrazolium solution. Representative plates were photographed using an Olympus stereoscope mounted with a Sony digital camera system (DKC5000, Sony Inc.).

4, p53, mouse double minute 2 (MDM2), and cyclin D.[23] Since **6s**, **6aa**, and **6ab** are highly effective in various combinations with conventional chemotherapy,[23] the lack of overt hematotoxicity of these compounds may be beneficial for testing novel combinations for advanced cancers, including tumors resistant to conventional chemotherapy. In addition, their safety profile seen with normal hematopoietic cells suggests that these compounds have a potential use in in vitro purging of tumor cells from patient bone marrow for use in autologous bone marrow transplantation. Clinical and preclinical studies currently underway will reveal the best way to utilize these compounds in cancer therapy.

Experimental Section

Chemistry. General Methods. All reagents and solvents were obtained from commercial suppliers and used without further purification unless otherwise stated. Solvents were dried using standard procedures, and reactions requiring anhydrous conditions were performed under N_2 atmosphere. Reactions were monitored by thin layer chromatography (TLC) on precoated silica gel F254 plates (Sigma-Aldrich) with a UV indicator. Column chromatography was performed with Merck 70–230 mesh silica gel 60 Å. Yields were of purified product and were not optimized. Melting points were determined using an Electrothermal Mel-Temp 3.0 micromelting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were obtained with a Bruker AM 300 and 400 MHz spectrometer. The chemical shifts are reported in parts per million (δ) downfield using tetramethylsilane (Me_4Si) as internal standard and $CDCl_3$ as the solvent except where indicated. Spin multiplicities are given as s (singlet), d (doublet), br s (broad singlet), m (multiplet), and q (quartet). Coupling constants (J values) were measured in hertz (Hz). Combustion analyses were performed with a Perkin-Elmer 2402 series II CHNS/O analyzer by Quantitative Technologies Inc. (White House, NJ). All the compounds gave satisfactory combustion analysis results (C, H, N within 0.4% of calculated values). Benzylsulfonylacetic acids were prepared according to the procedure reported in the literature.[13]

General Procedure for the Preparation of (*E*)-Styrylbenzylsulfones (6). Method A (Scheme 1). A mixture of benzylsulfonyl acetic acid **4** (10 mmol), araldehyde **5** (10 mmol), acetic acid (10 mL), and a catalytic amount of benzylamine (150 μL) was refluxed for about 2–8 h. After completion of the reaction (TLC monitoring, $CHCl_3$ on silica gel plate), with the contents cooled to room temperature, the precipitated product was filtered and washed with 2-propanol. If solid was not formed, the reaction mixture was diluted with ether and washed with saturated $NaHCO_3$, dilute hydrochloric acid, and water. The organic layer was dried over sodium sulfate, filtered, and concentrated under vacuum to obtain the desired crude product **6**. The crude product was recrystallized in 2-propanol to yield an analytically pure sample of **6**. The following (*E*)-styrylbenzylsulfones **6** were prepared using the above procedure.

(*E*)-4-Methoxystyryl-4-methoxybenzylsulfone (6a). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 4-methoxybenzaldehyde following the procedure as described in method A. Yield, 51%; white solid, mp 150–152 °C. ^{1}H NMR: δ 3.65 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 4.07 (s, 2H, CH_2), 6.36 (d, 1H, J = 14.4 Hz, =CH), 6.71−6.76 (m, 4H, Ar−H), 7.09–7.22 (m, 5H, Ar−H + vinylic). Anal. ($C_{17}H_{18}O_4S$) C, H.

(*E*)-4-Fluorostyryl-4-methoxybenzylsulfone (6b). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 4-fluorobenzaldehyde following the procedure as described in method A. Yield, 60%; white solid, mp 148–149 °C. ^{1}H NMR: δ 3.81 (s, 3H, OCH_3), 4.25 (s, 2H, CH_2), 6.61 (d, 1H, J = 14.0 Hz, =CH), 6.91–7.45 (m, 9H, Ar−H + vinylic). Anal. ($C_{16}H_{15}FO_3S$) C, H.

(*E*)-4-Chlorostyryl-4-methoxybenzylsulfone (6c). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 4-chlorobenzaldehyde following the procedure as described in method A. Yield, 66%; white solid, mp 176–177 °C. ^{1}H NMR: δ 3. 56 (s, 3H, OCH_3), 4.00 (s, 2H, CH_2), 6.40 (d, 1H, J = 16.0 Hz, =CH), 6.63–7.14 (m, 9H, Ar−H + vinylic). Anal. ($C_{16}H_{15}ClO_3S$) C, H.

(*E*)-4-Nitrostyryl-4-methoxybenzylsulfone (6d). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 4-nitrobenzaldehyde following the procedure as described in method A. Yield, 56%; light-yellow solid, mp 179–181 °C. ^{1}H NMR: δ 3.76 (s, 3H, OCH_3), 4.24 (s, 2H, CH_2), 6.78 (d, 1H, J = 15.6 Hz, =CH), 6.84 (m, 2H, Ar−H), 7.20–7.24 (m, 2H, Ar−H), 7.41 (d, 1H, J = 15.6 Hz, CH=), 7.52 (d, 2H, J = 13.3 Hz, Ar−H), 8.19 (d, 2H, J = 11.1 Hz, Ar−H). Anal. ($C_{16}H_{15}NO_5S$) C, H, N.

(*E*)-4-Aminostyryl-4-methoxybenzylsulfone (6e). In a 100 mL round-bottomed flask fitted with condenser, 5% Pd/C (0.073 g) was taken, and 20 mL of ethanol was added slowly. Compound **6d** (0.5 g, 1.4 mmol) and hydrazine hydrate (1.24 g, 38.7 mmol) were added to the contents of the flask, and the mixture was refluxed for 6 h. Completion of the reaction was monitored by TLC ($CHCl_3$ on silica

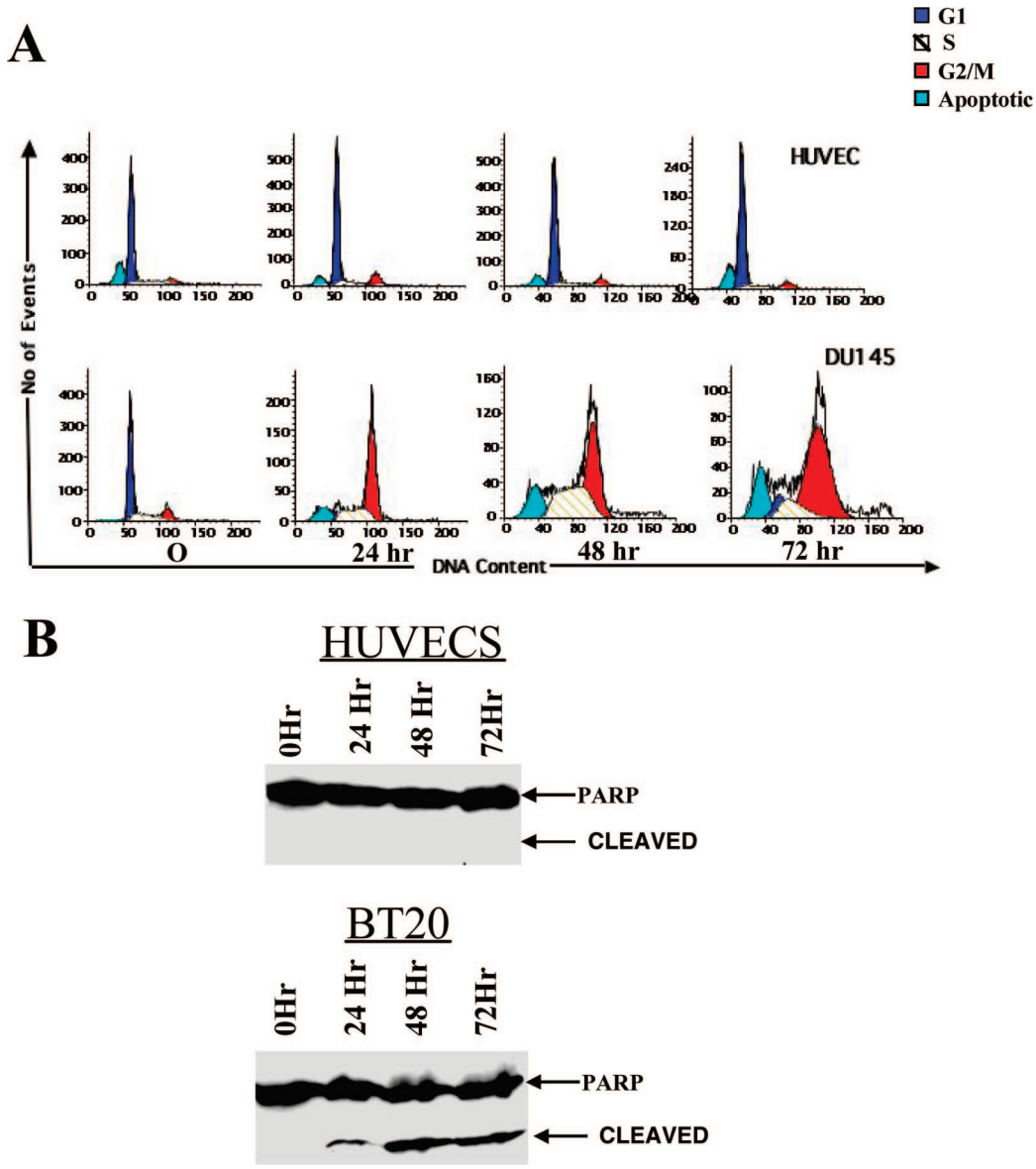


Figure 2. Preferential tumor cell killing activity of **6aa**. (A) Cell cycle analyses. Normal (HUVEC) and tumor cells (DU145) were treated with 20 nM **6aa** and incubated in medium containing 10% fetal bovine serum. At 24 h intervals, the cells were fixed, stained with propidium iodide, and analyzed for their DNA content by flow cytometry. (B) Induction of apoptosis in normal (HUVEC) and tumor cells (DU145) was assessed by Western blot analysis of cell lysates treated with **6aa** for 24, 48, and 72 h. The Western blots were probed with anti-PARP antibodies to assess the cleavage of the protein.

gel plate). The reaction mixture was filtered through Celite and concentrated to 50% volume. The concentrated mixture was poured onto crushed ice, and the solid formed was filtered, washed with cooled water, and dried to get the desired product **6e**. Yield, 52%; light-yellow solid, mp 164–168 °C. ^{1}H NMR: δ 3. 80 (s, 3H, OCH_3), 4.22 (s, 2H, CH_2), 4.04 (br s, 2H, NH_2), 6.42 (d, 1H, J =15.6 Hz, =CH), 6.60–7.31 (m, 9H, Ar−H + vinylic). Anal. ($C_{16}H_{17}NO_3S$) C, H, N.

(*E*)-2-Methoxystyryl-4-methoxybenzylsulfone (6f). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 2-methoxybenzaldehyde following the procedure as described in method A. Yield, 53%; white solid, mp 115–117 °C. ^{1}H NMR: δ 3.75 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 4.18 (s, 2H, CH_2), 6.82–7.27 (m, 9H, Ar−H + vinylic), 7.56 (d, 1H, J = 15.6 Hz, CH=). Anal. ($C_{17}H_{18}O_4S$) C, H.

(*E*)-2-Chloro-4-fluorostyryl-4-methoxybenzylsulfone (6g). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 2-chloro-4-fluorobenzaldehyde following the procedure as described in method A. Yield, 56%; white solid, mp 154–155 °C. ^{1}H NMR: δ 3.75 (s, 3H, OCH_3), 4.22 (s, 2H, CH_2), 6.62 (d, 1H, J = 15.6 Hz, =CH), 6.83–7.41 (m, 7H, Ar−H), 7.68 (d, 1H, J = 15.6 Hz). Anal. ($C_{16}H_{14}ClFO_3S$) C, H.

(*E*)-2,4-Dimethylstyryl-4-methoxybenzylsulfone (6h). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 2,4-dimethylbenzaldehyde following the procedure as described in method A. Yield, 55%; white solid, mp 126–128 °C. ^{1}H NMR: δ 2.21 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 3.74 (s, 3H, OCH_3), 4.19 (s, 2H, CH_2), 6.49 (d, 1H, J = 15.4 Hz, =CH), 6.83–7.24 (m, 7H, Ar−H), 7.55 (d, 1H, J = 15.4 Hz). Anal. ($C_{18}H_{20}O_3S$) C, H.

(*E*)-4-Fluoro-3-methoxystyryl-4-methoxybenzylsulfone (6i). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 4-fluoro-3-methoxybenzaldehyde following the procedure as described in method A. Yield, 55%; white solid, mp 105–107 °C. ^{1}H NMR: δ 3.69 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 4.20 (s, 2H, CH_2), 6.55 (d, 1H, J = 15.5 Hz, =CH), 6.82–7.31 (m, 8H, Ar−H + vinylic). Anal. ($C_{17}H_{17}FO_4S$) C, H.

(*E*)-3,4-Dimethoxystyryl-4-methoxybenzylsulfone (6j). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 3,4-dimethoxybenzaldehyde following the procedure as described in method A. Yield, 54%; white solid, mp 160–161 °C.

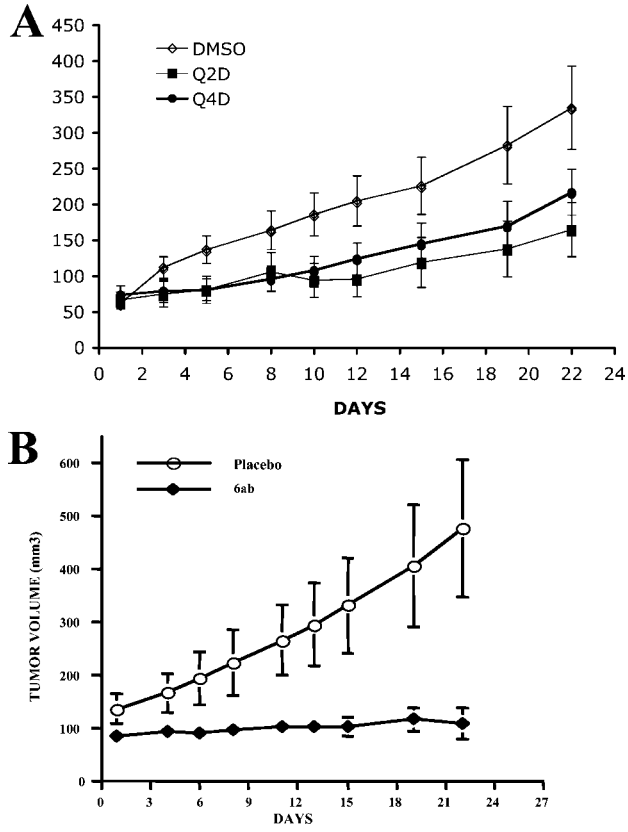


Figure 3. In vivo antitumor effects of **6s** and **6ab**. Female athymic (NCr-nu/nu) mice were injected subcutaneously with (0.5–1) × 10^7 ER-negative human breast tumor cells (BT-20) in 0.2 mL of PBS and the tumors allowed to grow to a size of 100–150 mm^3 in about 14 days. The mice were then paired such that the pairs harbored equal sized tumors, which were then used to test the therapeutic effects of **6s** and **6ab**. (A) Of the pairs, the animals were treated with either 50 mg/kg **6s** following a Q4D schedule or 25 mg/kg using a Q2D schedule or vehicle (DMSO) control. The tumor size was then measured on alternate days in two dimensions and the volume determined using the following equation: $V = (L(S^2))\pi/6$, where L is the longer and S is the shorter of the two measurements. (B) **6ab** was dissolved in PBS and was administered intravenously (50 mg/kg) through the tail vein on every alternate day. Tumor measurements were done as in Experimental Section.

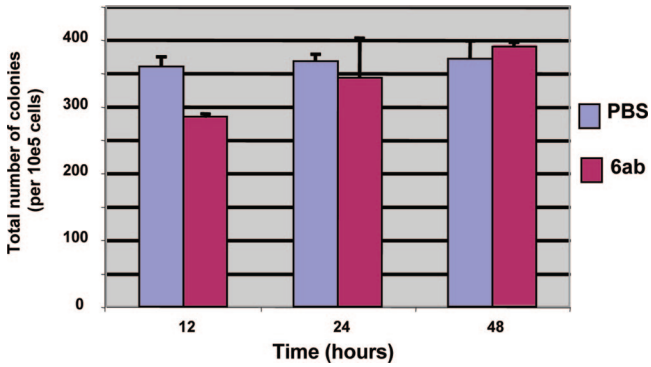


Figure 4. Bone marrow toxicity profile of **6ab**. To assess the toxicity of **6ab**, the phosphate salt of the drug was injected into mice (100 mg/kg) and bone marrow harvested from femur and tibia after 12, 24, and 36 h following the injection of the drug. The bone marrow cells were cultured in methylcellulose medium supplemented with a mixture of stem cell factor, GM-CSF, IL-3, and erythropoietin for 1 week, and colony forming units were determined.

^{1}H NMR: δ 3.23 (s, 3H, OCH_3), 3.55 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 4.37 (s, 2H, CH_2), 6.71 (d, 1H, J = 15.4 Hz, =CH), 6.64–7.05 (m, 7H, Ar−H), 7.55 (d, 1H, J = 15.4 Hz). Anal. ($C_{18}H_{20}O_5S$) C, H.

(*E*)-3,5-Dimethylstyryl-4-methoxybenzylsulfone (6k). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 3,5-dimethylbenzaldehyde following the procedure as described in method A. Yield, 58%; white solid, mp 127–130 °C. ^{1}H NMR: δ 2.08 (s, 6H, $2XCH_3$), 3.57 (s, 3H, OCH_3), 3.99 (s, 2H, CH_2), 6.40 (d, 1H, J = 15.5 Hz, =CH), 6.83–7.24 (m, 7H, Ar−H), 7.11 (d, 1H, J = 15.5 Hz). Anal. ($C_{18}H_{20}O_3S$) C, H.

(*E*)-2,6-Dimethylstyryl-4-methoxybenzylsulfone (6l). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 2,6-dimethylbenzaldehyde following the procedure as described in method A. Yield, 53%; white solid, mp 99–101 °C. ^{1}H NMR: δ 2.15 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 4.13 (s, 2H, CH_2), 6.41 (d, 1H, J = 15.4 Hz, =CH), 6.83–7.35 (m, 7H, Ar−H), 7.55 (d, 1H, J = 15.4 Hz). Anal. ($C_{18}H_{20}O_3S$) C, H.

(*E*)-2,6-Dimethoxystyryl-4-methoxybenzylsulfone (6m). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 2,6-dimethoxybenzaldehyde following the procedure as described in method A. Yield, 51%; white solid, mp 136–138 °C. ^{1}H NMR: δ 3.81(s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 4.23 (s, 2H, CH_2), 7.19 (d, 1H, J = 15.4 Hz, =CH), 6.64–7.05 (m, 7H, Ar−H), 7.90 (d, 1H, J = 15.4 Hz). ^{13}C NMR: δ 160.6, 160.3, 135.9, 132.9.9, 132.6, 126.4, 120.9, 114.5, 110.7, 104.0, 61.7, 56.2, 55.7. Anal. ($C_{18}H_{20}O_5S$) C, H.

(*E*)-2,4-Dimethoxystyryl-4-methoxybenzylsulfone (6n). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 2,4-dimethoxybenzaldehyde following the procedure as described in method A. Yield, 59%; white solid, mp 161–162 °C. ^{1}H NMR: δ 3.73 (s, 3H, OCH_3), 3.77 (s, 6H, 2 × OCH_3), 4.14 (s, 2H, CH_2), 6.71 (d, 1H, J = 15.5 Hz, =CH), 6.37−7.23 (m, 7H, Ar−H), 7.42 (d, 1H, J = 15.5 Hz). Anal. ($C_{18}H_{20}O_5S$) C, H.

(*E*)-2,5-Dimethoxystyryl-4-methoxybenzylsulfone (6o). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 2,5-dimethoxybenzaldehyde following the procedure as described in method A. Yield, 54%; white solid, mp 105–107 °C. ^{1}H NMR: δ 3.71 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 4.18 (s, 2H, CH_2), 6.78–7.26 (m, 8H, Ar−H + vinylic), 7.52 (d, 1H, J = 15.6 Hz). Anal. ($C_{18}H_{20}O_5S$) C, H.

(*E*)-3,5-Dimethoxystyryl-4-methoxybenzylsulfone (6p). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 3,5-dimethoxybenzaldehyde following the procedure as described in method A. Yield, 62%; white solid, mp 119–121 °C. ^{1}H NMR: δ 3.70 (s, 6H, 2 × OCH_3), 3.71 (s, 3H, OCH_3), 4.15 (s, 2H, CH_2), 6.55 (d, 1H, J = 15.5 Hz, =CH), 6.42–7.20 (m, 7H, Ar−H), 7.23 (d, 1H, J = 15.5 Hz). Anal. ($C_{18}H_{20}O_5S$) C, H.

(*E*)-2,4,5-Trimethoxystyryl-4-methoxybenzylsulfone (6q). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 2,4,5-trimethoxybenzaldehyde following the procedure as described in method A. Yield, 66%; white solid, mp 146–148 °C. ^{1}H NMR: δ 3.73 (s, 3H, OCH_3), 3.78 (s, 6H, 2 × OCH_3), 3.81 (s, 3H, OCH_3), 4.20 (s, 2H, CH_2), 6.86 (m, 4H, Ar−H), 7.00 (d, 1H, J = 15.6 Hz, =CH), 7.31 (d, 2H, J = 8.9 Hz), 7.61 (d, 1H, J = 15.6 Hz, CH=). Anal. ($C_{19}H_{22}O_6S$) C, H.

(*E*)-2,3,4-Trimethoxystyryl-4-methoxybenzylsulfone (6r). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 2,3,4-trimethoxybenzaldehyde following the procedure as described in method A. Yield, 54%; white solid, mp 154–156 °C. ^{1}H NMR: δ 3.75 (s, 3H, OCH_3), 3.79 (s, 6H, 2 × OCH_3), 3.83 (s, 3H, OCH_3), 4.18 (s, 2H, CH_2), 6.76 (d, 2H, J = 8.9 Hz, Ar−H), 6.86 (d, 2H, J = 9.0 Hz, Ar−H), 6.99 (d, 1H, J = 15.6 Hz, =CH), 7.31 (d, 2H, J = 8.9 Hz), 7.61 (d, 1H, J = 15.6 Hz, CH=). Anal. ($C_{19}H_{22}O_6S$) C, H.

(*E*)-2,4,6-Trimethoxystyryl-4-methoxybenzylsulfone (6s). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 2,4,6-trimethoxybenzaldehyde following the procedure as described in method A. Yield, 34%; white solid, mp 143–145 °C. ^{1}H NMR: δ 3.80 (s, 3H, OCH_3), 3.82 (s, 6H, 2 × OCH_3), 3.85 (s, 3H, OCH_3), 4.20 (s, 2H, CH_2), 6.08 (s, 2H, Ar−H), 6.88 (d, 2H, J = 9.2 Hz, Ar−H), 7.00 (d, 1H, J = 15.6 Hz, =CH), 7.31 (d, 2H, J = 8.8 Hz), 7.81 (d, 1H, J = 15.6 Hz, CH=). ^{13}C NMR: δ 164.2,

161.8, 160.2, 135.9, 132.6, 123.0, 121.1, 114.4, 104.2, 90.9, 61.8, 56.1, 55.8, 55.6. Anal. ($C_{19}H_{22}O_6S$) C, H.

(*E*)-3,4,5-Trimethoxystyryl-4-methoxybenzylsulfone (6t). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 3,4,5-trimethoxybenzaldehyde following the procedure as described in method A. Yield, 54%; white solid, mp 138–141 °C. ^{1}H NMR: δ 3.76 (s, 3H, OCH_3), 3.80 (s, 6H, 2 × OCH_3), 3.83 (s, 3H, OCH_3), 4.16 (s, 2H, CH_2), 6.78 (d, 2H, J = 9.1 Hz, Ar−H), 6.91 (d, 2H, J = 8.9 Hz, Ar−H), 7.04 (d, 1H, J = 15.6 Hz, =CH), 7.37 (d, 2H, J = 8.8 Hz), 7.79 (d, 1H, J = 15.6 Hz, CH=). Anal. ($C_{19}H_{22}O_6S$) C, H.

(*E*)-2,6-Dimethoxy-4-hydroxystyryl-4-methoxybenzylsulfone (6u). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 2,6-dimethoxy-4-hydroxybenzaldehyde following the procedure as described in method A. Yield, 58%; white solid, mp 134–136 °C. ^{1}H NMR: δ 3.47 (s, 6H, 2 × OCH_3), 3.55 (s, 3H, OCH_3), 3.98 (s, 2H, CH_2), 5.77 (s, 2H, Ar−H), 6.63 (d, 2H, J = 8.5 Hz, Ar−H), 6.73 (d, 1H, J = 15.6 Hz, =CH), 7.05 (d, 2H, J = 8.5 Hz), 7.55 (d, 1H, J = 15.6 Hz, CH=). Anal. ($C_{18}H_{20}O_6S$) C, H.

Preparation of 4-Fluoro-2,6-dimethoxybenzaldehyde. Phosphorus oxychloride (1.8 mL, 19.3 mmol) was added slowly to a well-stirred mixture of 1-fluoro-3,5-dimethoxybenzene (2.6 mL, 19.25 mmol) and *N*,*N*-dimethylformamide (2.5 mL, 20 mmol) while the temperature was kept below −5 °C. Stirring was continued at room temperature for 1.5 h and at 60 °C for another 2 h. Reaction completion was monitored by TLC. The reaction mixture was cooled and hydrolyzed with ice–water (60 mL). The resulting suspension was neutralized by addition of 5 N NaOH and extracted with ethyl acetate (2 × 30 mL). The aqueous phase was adjusted to pH 10 by 5 N NaOH and re-extracted with ethyl acetate (2 × 30 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution (30 mL) and brine (30 mL) and dried over anhydrous sodium sulfate. The dried solution was concentrated to get the crude product, which on purification by column chromatography afforded a colorless pure product. Yield, 75%; white solid, mp 79–81 °C. ^{1}H NMR: δ 3.85 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 6.25 (S, 2H, Ar−H), 10.24 (s, 1H, CHO). Anal. Calcd for $C_{18}H_{19}FO_5S$: C, 58.70; H, 4.92. Found: C, 58.64; H, 4.91.

(*E*)-2,6-Dimethoxy-4-fluorostyryl-4-methoxybenzylsulfone (6v). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 2,6-dimethoxy-4-fluorobenzaldehyde following the procedure as described in method A. Yield, 55%; white solid, mp 146–148 °C. ^{1}H NMR: δ 3.47 (s, 6H, 2 × OCH_3), 3.55 (s, 3H, OCH_3), 3.98 (s, 2H, CH_2), 5.77 (s, 2H, Ar−H), 6.63 (d, 2H, J = 8.5 Hz, Ar−H), 6.73 (d, 1H, J = 15.6 Hz, =CH), 7.05 (d, 2H, J = 8.5 Hz), 7.55 (d, 1H, J = 15.6 Hz, CH=). Anal. ($C_{18}H_{19}FO_5S$) C, H.

(*E*)-2,4,6-Trimethylstyryl-4-methoxybenzylsulfone (6w). The title compound was obtained from 4-methoxybenzylsulfonylacetic acid and 2,4,6-trimethylbenzaldehyde following the procedure as described in method A. Yield, 51%; white solid, mp 97–99 °C. ^{1}H NMR: δ 2.16 (s, 3H,CH_3), 2.28 (s, 6H, 2 × CH_3), 3.76 (s, 3H, OCH_3), 4.13 (s, 2H, CH_2), 6.08 (s, 2H, Ar−H), 6.42 (d, 1H, J = 15.4 Hz, =CH), 6.82 (m, 4H, Ar−H), 7.56 (d, 1H, J = 15.4 Hz, CH=). Anal. ($C_{19}H_{22}O_3S$) C, H.

(*E*)-2,4,6-Trimethoxystyryl-4-trifluoromethoxybenzylsulfone (6x). The title compound was obtained from 4-trifluoromethoxybenzylsulfonylacetic acid and 2,4,6-trimethoxybenzaldehyde following the procedure as described in method A. Yield, 52%; white solid, mp 133–135 °C. ^{1}H NMR: δ 3.82 (s, 6H, 2 × OCH_3), 3.87 (s, 3H, OCH_3), 4.26 (s, 2H, CH_2), 6.10 (s, 2H, Ar−H), 6.99 (d, 1H, J = 15.6 Hz, =CH), 7.20–7.48 (m, 4H, Ar−H), 7.78 (d, 1H, J = 15.6 Hz, CH=). Anal. ($C_{19}H_{19}F_3O_6S$) C, H.

(*E*)-3,4,5-Trimethoxystyryl-3-hydroxy-4-methoxybenzylsulfone (6y). The title compound was obtained from 3-hydroxy-4-methoxybenzylsulfonylacetic acid and 3,4,5-trimethoxybenzaldehyde following the procedure as described in method A. Yield, 60%; white solid, mp 118–120 °C. ^{1}H NMR: δ 3.83 (s, 6H, 2 × OCH_3), 3.85 (s, 3H. OCH_3), 3.89 (s, 3H. OCH_3), 4.16 (s, 2H, CH_2), 5.60 (s, 1H, OH), 6.09 (s, 2H, Ar−H), 6.82–6.96 (m, 3H, Ar−H), 7.05 (d, 1H, J = 15.6 Hz, =CH), 7.85 (d, 1H, J = 15.6 Hz, CH=). Anal. ($C_{19}H_{22}O_7S$) C, H.

(*E*)-2,6-Dimethoxy-4-hydroxystyryl-3-hydroxy-4-methoxybenzylsulfone (6z). The title compound was obtained from 3-hydroxy-4-methoxybenzylsulfonylacetic acid and 2,6-dimethoxy-4-hydroxybenzaldehyde following the procedure as described in method A. Yield, 54%; white solid, mp 123–125 °C. ^{1}H NMR: δ 3.77 (s, 6H, 2 × OCH_3), 3.81 (s, 3H, OCH_3), 4.28 (s, 2H, CH_2), 6.10 (s, 2H, Ar−H), 6.71–6.92 (m, 3H, Ar−H), 7.00 (d, 1H, J = 15.5 Hz, =CH), 7.59 (d, 1H, J = 15.5 Hz, CH=). Anal. ($C_{18}H_{20}O_7S$) C, H.

(*E*)-2,4,6-Trimethoxystyryl-3-hydroxy-4-methoxybenzylsulfone (6aa). The synthesis of the title compound was described in the preparation of **20** (Scheme 3). Yield, 63%; white solid, mp 125–127 °C. ^{1}H NMR: δ 3.83 (s, 6H, 2 × OCH_3), 3.85 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 4.16 (s, 2H, CH_2), 5.60 (s, 1H, OH), 6.09 (s, 2H, Ar−H), 6.82–6.96 (m, 3H, Ar−H), 7.05 (d, 1H, J =15.6 Hz, =CH), 7.85 (d, 1H, J = 15.6 Hz, CH=). ^{13}C NMR: δ 164.1, 161.9, 147.3, 145.9, 135.7, 123.4, 123.1, 122.2, 117.5, 111.1, 105.5, 104.4, 90.9, 62.0, 56.3, 56.1, 55.8. Anal. ($C_{19}H_{22}O_7S$) C, H.

(*E*)-2,4,6-Trimethoxystyryl-3^1-*O*-phosphate Disodium 4-Methoxybenzylsulfone (6ab). The synthesis of the title compounds was described in the preparation of **29** (Scheme 5). Yield, 98%; white solid, mp 152–154 °C. ^{1}H NMR (D_2O): δ 3.68 (s, 6H, 2 × OCH_3), 3.71 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.35 (s, 2H, CH_2), 5.92 (s, 2H, Ar−H), 6.91 (s, 2H, Ar−H), 6.97 (d, 1H, J = 15.6 Hz, =CH), 7.39 (s, 1H, Ar−H), 7.43 (d, 1H, J = 15.6 Hz, CH=). ^{13}C NMR (D_2O): δ 164.4, 161.7, 151.1, 143.8, 136.8, 125.9, 123.4, 120.6, 120.2, 113.1, 103.4, 91.1, 61.0, 56.4, 56.2, 55.9. Anal. ($C_{19}H_{21}O_{10}Na_2PS$) C, H.

(*E*)-2,4,6-Trimethoxystyryl-3,4,5-trimethoxybenzylsulfone (6ac). The title compound was obtained from 3,4,5-trimethoxybenzylsulfonylacetic acid and 2,4,6-trimethoxybenzaldehyde following the procedure as described in method A. Yield, 53%; white solid, mp 151–153 °C. ^{1}H NMR: δ 3.81(s, 6H, 2 × OCH_3), 3.83 (s, 6H, 2 × OCH_3), 3.84 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.19 (s, 2H, CH_2), 6.10 (s, 2H, ArH), 6.60 (s, 2H, ArH), 7.03 (d, 1H, J = 15.6 Hz, =CH), 7.83 (d, 1H, J = 15.6 Hz, CH=). Anal. ($C_{21}H_{26}O_8S$) C, H.

(*E*)-2,4,6-Trimethoxystyryl-2,3,4-trimethoxybenzylsulfone (6ad). The title compound was obtained from 2,3,4-trimethoxybenzylsulfonylacetic acid and 2,4,6-trimethoxybenzaldehyde following the procedure as described in method A. Yield, 52%; white solid, mp 94–96 °C. ^{1}H NMR: δ 3.77, 3.83, 3.84, 3.86, 3.90 (s, 6 × 3H, OCH_3), 4.32 (s, 2H, CH_2), 6.08 (s, 2H, aromatic), 6.67 (d, J = 8.4 Hz, 1H, aromatic), 7.11 (d, J = 15.6 Hz, 1H, =CH), 7.16 (d, J = 8.4 Hz, 1H, aromatic), 7.79 (d, J = 15.6 Hz, 1H, CH=). Anal. ($C_{21}H_{26}O_8S$) C, H.

(*E*)-2,4,6-Trimethoxystyryl-4-chlorobenzylsulfone (6ae). The title compound was obtained from 4-chlorobenzylsulfonylacetic acid and 2,4,6-trimethoxybenzaldehyde following the procedure as described in method A. Yield, 60%; white solid, mp 181–184 °C. ^{1}H NMR: δ 3.83 (s, 2 × 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.22 (s, 2H, CH_2), 6.09 (s, 2H, Ar−H), 6.99 (d,1H, J = 15.5 Hz, =CH), 7.29 (s, 4H, Ar−H), 7.76 (d, 1H, J = 15.5 Hz, CH=). Anal. ($C_{18}H_{19}ClO_5S$) C, H.

(*E*)-2,4,6-Trimethoxystyryl-4-nitrobenzylsulfone (6af). The title compound was obtained from 4-nitrobenzylsulfonylacetic acid and 2,4,6-trimethoxybenzaldehyde following the procedure as described in method A. Yield, 60%; light-yellow solid, mp 179–184 °C. ^{1}H NMR: δ 3.83 (s, 2 × 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.35 (s, 2H, CH_2), 6.09 (s, 2H, Ar−H), 7.01 (d, 1H, J = 15.5 Hz, =CH), 7.57 (d, 2H, J = 9.0 Hz, Ar−H), 7.76 (d, J = 15.5 Hz, 1H, CH=), 8.21 (d, 2H, J = 9.0 Hz, Ar−H). Anal. ($C_{18}H_{19}NO_7S$) C, H, N.

(*E*)-2,4,6-Trimethoxystyryl-4-cyanobenzylsulfone (6ag). The title compound was obtained from 4-cyanobenzylsulfonylacetic acid and 2,4,6-trimethoxybenzaldehyde following the procedure as described in method A. Yield, 58%; white solid, mp 140–142 °C. ^{1}H NMR: δ 3.81 (s, 3H, 2 × OCH_3), 3.87 (s, 3H, OCH_3), 4.21 (s, 2H, CH_2), 6.00 (s, 2H, Ar−H), 6.78 (d, 2H, J = 8.5 Hz, Ar−H), 7.07 (d, 1H, J = 15.6 Hz, =CH), 7.29 (d, 2H, J = 8.5 Hz, Ar−H), 7.86 (d, 1H, J = 15.6 Hz, CH=). Anal. ($C_{19}H_{19}NO_5S$) C, H, N.

(*E*)-2,4,6-Trimethoxystyryl-4-carboxybenzylsulfone (6ah). The title compound was obtained from 4-carboxybenzylsulfonylacetic acid and 2,4,6-trimethoxybenzaldehyde following the procedure as described in method A. Yield, 60%; white solid, mp 143–145 °C. ^{1}H NMR: δ 3.83 (s, 6H, 2 × OCH_3), 3.88 (s, 3H, OCH_3), 4.11 (s, 2H, CH_2), 6.01 (s, 2H, Ar−H), 6.68 (d, 2H, J = 8.5 Hz, Ar−H), 6.97 (d, 1H, J = 15.6 Hz, =CH), 7.13 (d, 2H, J = 8.5 Hz, Ar−H), 7.76 (d, 1H, J = 15.6 Hz, CH=). Anal. ($C_{19}H_{20}O_7S$) C, H.

(*E*)-2,4,6-Trimethoxystyryl-4-hydroxybenzylsulfone (6ai). The title compound was obtained from 4-*tert*-butoxybenzylsulfonylacetic acid and 2,4,6-trimethoxybenzaldehyde following the method A procedure. During the condensation the protective *tert*-butoxy group cleaved to the hydroxy group. Yield, 52%; white solid, mp 141–143 °C. ^{1}H NMR: δ 3.73 (s, 6H, 2 × OCH_3), 3.77 (s, 3H, OCH_3), 4.11 (s, 2H, CH_2), 4.36 (s, 1H, OH), 6.01 (s, 2H, Ar−H), 6.68 (d, 2H, J = 8.5 Hz, Ar−H), 6.97 (d, 1H, J = 15.6 Hz, =CH), 7.13 (d, 2H, J = 8.5 Hz, Ar−H), 7.76 (d, 1H, J = 15.6 Hz, CH=). Anal. ($C_{15}H_{20}O_6S$) C, H.

Method B. See Scheme 2.

Preparation of Phenacylbenzylsulfones (10). General Procedure. To a cooled solution of sodium hydroxide (100 mmol) in absolute methanol (50 mL), taken in a 250 mL round-bottomed flask, benzylthiol **7** (100 mmol) was added slowly through a dropping funnel, and the reaction mixture was stirred for 5 min. An appropriate phenacyl bromide **8** (100 mmol) was added in portions to the contents of the flask and stirred for 3–4 h. After completion of the reaction, the contents of the flask were poured onto crushed ice and the compound formed was washed with ice-cold water and dried to get phenacylbenzyl sulfide **9**.

The above crude phenacylbenzyl sulfide **9** (50 mmol) in glacial acetic acid (100 mL) was taken in a 250 mL round-botomed flask, and 30% hydrogen peroxide (60 mL) was added in portions at frequent intervals. Then the reaction mixture was kept at room temperature for 24 h. The solid, if any formed, was separated by filtration, and the filtrate was poured onto crushed ice. The compound separated was filtered, washed with water, dried, and added to the first crop, if any. The total product on recrystallization from methanol afforded pure phenacylbenzylsulfones (**10**).

4-Methoxyphenacyl-4-methoxybenzyl sulfone (10a). The title compound was obtained from 4-methoxybenzylthiol and 4-methoxyphenacyl bromide followed by oxidation of the resultant compound. Yield, 80%; white solid, mp 128–130 °C. ^{1}H NMR: δ 3.78(s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.60 (s, 2H, CH_2), 4.89 (s, 2H, CH_2), 6.98 (d, 2H, J = 8.7 Hz, Ar−H), 7.09 (d, 2H, J = 8.9 Hz, Ar−H), 7.36 (d, 2H, J = 8.7 Hz, Ar−H), 8.04 (d, 2H, J = 8.9 Hz, Ar−H). Anal. Calcd for $C_{17}H_{18}O_5S$: C, 61.06; H, 5.42. Found: C, 61.09; H, 5.40.

4-Chlorophenacyl-4-methoxybenzylsulfone (10b). The title compound was obtained from 4-methoxybenzylthiol and 4-chlorophenacyl bromide followed by oxidation of the resultant compound. Yield, 82%; white solid, mp 141–143 °C. ^{1}H NMR: δ 3.89 (s, 3H, OCH_3), 4.50 (s, 2H, CH_2), 4.79 (s, 2H, CH_2), 7.06 (d, 2H, J = 8.3 Hz, Ar−H), 7.47 (d, 2H, J = 8.4 Hz, Ar−H), 7.60 (dd, 4H, J = 8.2, 9.4 Hz, Ar−H). Anal. Calcd for $C_{16}H_{15}ClO_4S$: C, 56.72; H, 4.46. Found: C, 56.69; H, 4.42.

Preparation of β-Hydroxybenzylsulfones (11). General Procedure. To an ethanolic solution (20 mL) of phenacylbenzylsulfone **10** (10 mmol) maintained at 0 °C was added $NaBH_4$ (10 mmol) slowly under N_2 atmosphere. The reaction mixture was maintained at 0 °C for 0.5 h. After completion of the reaction, monitored by TLC, the contents were poured onto crushed ice. The solid was filtered, washed with water, and dried under vacuum to yield **11**.

2-(4-Methoxybenzylsulfonyl)-1-(4-methoxyphenyl)ethanol (11a). The title compound was obtained by the reduction of **10a** with sodium borohydride. Yield, 70%; white solid, mp 112–114 °C. ^{1}H NMR: δ 3.43 (dd, 2H, J = 9.9 and 4.6 Hz), 3.76 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.47 (dd, 2H, J =15.7 and 13.6 Hz), 5.09 (m, 1H, CHOH), 6.00 (d, 1H, J = 4.2 Hz, OH), 6.93 (d, 2H, J = 8.6 Hz, Ar−H), 6.99 (d, 2H, J = 8.6 Hz, Ar−H), 7.35 (t, 4H, J = 8.8 Hz, Ar−H). Anal. Calcd for $C_{17}H_{20}O_5S$: C, 60.69; H, 5.99. Found: C, 60.73; H, 5.97.

1-(4-Chlorophenyl)-2-(4-methoxybenzylsulfonyl)ethanol (11b). The title compound was obtained by the reduction of **10b** with sodium borohydride. Yield, 78%; white solid, mp 130–132 °C. ^{1}H NMR: δ 3.63 (dd, 2H, J =10.1 and 4.4 Hz), 3.93 (s, 3H, OCH_3), 4.75 (dd, 2H, J = 13.5 and 10.6 Hz), 5.26 (d, 1H, J = 8.3 Hz, CHOH), 6.17 (br s, 1H, OH), 7.10 (d, 2H, J = 8.3 Hz, Ar−H), 7.51(d, 2H, J = 8.3 Hz, Ar−H), 7.64 (dd, 4H, J = 9.4 and 8.2 Hz, Ar−H). Anal. Calcd for $C_{16}H_{17}ClO_4S$: C, 56.39; H, 5.03. Found: C, 56.34; H, 4.99.

Preparation of (*E*)-Styrylbenzylsulfones (6). *p*-Toluenesulfonic acid (1 mmol) was added in one portion to a mixture of β-hydroxybenzylsulfone **11** (5 mmol) in anhydrous benzene (25 mL) at room temperature and under N_2 atmosphere. The temperature was raised to 80 °C, and the mixture was refluxed for 3 h using a Dean–Stark water separator. After completion of the reaction monitored by TLC, the reaction mixture was concentrated under reduced pressure and then quenched by the addition of water (25 mL). The aqueous layer was neutralized with a saturated aqueous solution of sodium hydrogen carbonate and extracted with dichloromethane (3 × 25 mL). The combined organic extracts were washed with brine (2 × 25 mL), dried over Na_2SO_4, and filtered, and the solvent was evaporated under reduced pressure to afford crude product, which on recrystallization in 2-propanol afforded the desired product **6** in excellent yield.

(*E*)-4-Methoxystyryl-4-methoxybenzylsulfone (6a). The title compound was obtained by dehydration of **11a** as described in the above procedure. Yield, 65%; white solid, mp 151–153 °C. Analytical data are same as for **6a** obtained by method A.

(*E*)-4-Chlorostyryl-4-methoxybenzylsulfone (6c). The title compound was obtained by dehydration of **11b** as described in the above procedure. Yield, 69%; white solid, mp 175–177 °C. Analytical data is same as for **6c** obtained by method A.

Synthesis of (*E*)-2,4,6-Trimethoxystyryl-3-hydroxy-4-methoxybenzylsulfone, 6aa. See Scheme 3.

Preparation of 3-[(*tert*-Butyldimethylsilyl)oxy]-4-methoxybenzaldehyde (13). To a cooled solution of 3-hydroxy-4-methoxybenzaldehyde **12** (10.0 g, 65.7 mmol) in dry *N*,*N*-dimethylformamide (75 mL) was added diisopropylethylamine (16.99 g, 131.4 mmol). Before the addition of 1.0 M solution of *tert*-butyldimethylsilyl chloride in tetrahydrofuran (11.89 g or 78.9 mL, 78.85 mmol), the mixture was stirred under nitrogen for 10 min. After complete addition over 30 min, the reaction mixture was left overnight (12–16 h). Reaction completion was checked by TLC (chloroform on silica gel plate). Water was added to the reaction mixture, extracted with dichloromethane, and the organic layer was washed with a saturated sodium bicarbonate solution and water and dried. Removal of solvent in vacuo yielded as an oil, which was subjected to filter column chromatography (eluant, chloroform) to afford a yellow viscous oil **13** (100%). ^{1}H NMR: δ 0.21 (s, 6H, 2 × CH_3), 1.01 (s, 9H, 3 × CH_3), 3.91 (s, 3H, OCH_3), 6.94 (d, 1H, J = 8.5 Hz, 5H), 7.36 (d, 1H, J = 2 Hz, 2H), 7.47 (dd, 1H, J = 8.5, 2 Hz, 6H), 9.89 (s, 1H, CHO). Anal. Calcd for $C_{14}H_{22}O_3Si$: C, 63.12; H, 8.32. Found: C, 63.09; H, 8.30.

Preparation of 3-[(*tert*-Butyldimethylsilyl)oxy]-4-methoxybenzyl Alcohol (14). To a cooled solution of 3-[(*tert*-butyldimethylsilyl)oxy]-4-methoxybenzaldehyde **13** (17.5 g, 65.7 mmol) in methanol (100 mL) under nitrogen, sodium borohydride (2.98 g, 78.8 mmol) was added. The mixture was stirred at room temperature for 30 min. After the reaction was complete as indicated by TLC (chloroform on silica gel plate), ice was added to the reaction mixture and extracted with ethyl acetate. The organic layer was separated, washed with water, and dried. Removal of solvent in vacuo yielded a yellow oil **14** (73.5%). ^{1}H NMR: δ 0.18 (s, 6H, 2 × CH_3), 1.00 (s, 9H, 3 × CH_3), 1.90 (br s, 1H, OH), 3.82 (s, 3H, OCH_3), 4.56 (s, 2H, CH_2OH), 6.94 (br s, 3H, Ar−H). Anal. Calcd for $C_{14}H_{24}O_3Si$: C, 62.64; H, 9.01. Found: C, 62.59; H, 9.03.

Preparation of 3-[(*tert*-Butyldimethylsilyl)oxy]-4-methoxybenzyl Chloride (15). To a cooled solution of 3-[(*tert*-butyldimethylsilyl)oxy]-4-methoxybenzyl alcohol **14** (9.5 g, 35.4 mmol) in benzene (50 mL) under nitrogen, thionyl chloride (6.32 g or 3.87 mL, 53.1 mmol) dissolved in benzene (5 mL) was added over 10

min, and the temperature was maintained at 0 °C for 2 h. The completion of reaction was checked by TLC (chloroform on silica gel plate). Ice was added to the reaction mixture, and the solution was extracted with ethyl acetate. The organic layer was washed with saturated bicarbonate solution and water and dried over anhydrous sodium sulfate. Removal of the solvent in vacuo afforded **15**. Yield, 98.5%; yellow oil. ^{1}H NMR: δ 0.16 (s, 6H, 2 × CH_3), 1.00 (s, 9H, 3 × CH_3), 3.80 (s, 3H, OCH_3), 4.44 (s, 2H, CH_2), 6.70–7.01 (m, 3H, Ar−H). Anal. Calcd for $C_{14}H_{23}ClO_2Si$: C, 58.62; H, 8.08. Found: C, 58.69; H, 8.03.

Preparation of 3-[(*tert*-Butyldimethylsilyl)oxy]-4-methoxybenzylthioacetic Acid (16). To a solution of sodium hydroxide (2.79 g, 69.7mmol) in methanol (30 mL) was added mercaptoacetic acid (3.21 g or 2.42 mL, 34.9 mmol) slowly and stirred under nitrogen for 10 min. 3-[(*tert*-Butyldimethylsilyl)oxy]-4-methoxybenzyl chloride **15** (10.0 g, 34.9 mmol) was added slowly to the reaction mixture and stirred at room temperature for 3 h. Reaction completion was checked by TLC (chloroform on silica gel plate). The reaction mixture was then poured onto ice and neutralized with concentrated HCl. The resulting material was extracted with ethyl acetate. The ethyl acetate solution was washed with water and dried over anhydrous sodium sulfate. Removal of the solvent in vacuo afforded **16**. Yield, 75%; white solid, mp 57–59 °C. ^{1}H NMR: δ 0.18 (s, 6H, 2 × CH_3), 1.02 (s, 9H, 3 × CH_3), 3.34 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 4.04 (s, 2H, CH_2), 6.80–7.01 (m, 3H, Ar−H). Anal. Calcd for $C_{16}H_{26}O_4SiS$: C, 56.10; H, 7.65. Found: C, 56.08; H, 7.61.

Preparation of 3-Hydroxy-4-methoxybenzylthioacetic Acid (17). To a cooled solution of 3-[(*tert*-butyldimethylsilyl)oxy]-4-methoxybenzylthioacetic acid **16** (8.75 g, 25.5 mmol) in tetrahydrofuran (40 mL) was added 1.0 M solution of tetra-*n*-butylammonium fluoride in tetrahydrofuran (6.68 g or 25.54 mL, 25.5 mmol) slowly and stirred under nitrogen for 2 h at room temperature. The progress of the reaction was monitored by TLC (9:1 chloroform/methanol on silica gel plate). Water was added to the reaction mixture and extracted with ethyl acetate. The organic layer was washed with water and dried. Removal of the solvent in vacuo yielded a semisolid that was subjected to column chromatography (initial with chloroform and finally with ethyl acetate) to afford the pure product **17**. Yield, 50%; white solid, mp 128–130 °C. ^{1}H NMR: δ 3.34 (s, 2H, CH_2), 3.84 (s, 3H, OCH_3), 4.04 (s, 2H, CH_2), 6.80–7.01 (m, 3H, Ar−H). Anal. Calcd for $C_{10}H_{12}O_4S$: C, 52.62, H, 5.30. Found: C, 52.58, H, 5.35.

Preparation of 3-Hydroxy-4-methoxybenzylsulfonylacetic Acid (18). To a solution of 3-hydroxy-4-methoxybenzylthioacetic acid **17** (2.9 g, 12.7 mmol) in glacial acetic acid (15 mL) was added 6 mL of 30% hydrogen peroxide and stirred overnight (18 h). The completion of the reaction was determined by TLC. The mixture was then poured onto ice–water and extracted with ethyl acetate. The organic layer was washed with water and dried. Removal of the solvent in vacuo afforded pure product **18**. Yield, 60%; white solid, mp 164–165 °C. ^{1}H NMR: δ 3.84 (s, 3H, OCH_3), 4.04 (s, 2H, CH_2), 4.29 (s, 2H, CH_2), 6.85–7.11 (m, 3H, Ar−H). Anal. Calcd for $C_{10}H_{12}O_6S$: C, 46.15; H, 4.65. Found: C, 46.21; H, 4.69.

Preparation of (*E*)-2,4, 6-Trimethoxystyryl-3-hydroxy-4-methoxybenzylsulfones (20, 6aa). A mixture of 3-hydroxy-4-methoxybenzylsulfonylacetic acid **18** (1.9 g, 7.3 mmol), 2,4,6-trimethoxybenzaldehyde **19** (1.58 g, 8.0 mmol), benzoic acid (0.134 g, 1.1 mmol), and piperidine (0.081 g, 0.95mmol) in toluene (50 mL) was refluxed for 2–3 h with continuous removal of water using a Dean–Stark water separator. Reaction completion was determined by TLC (9:1 chloroform/methanol on silica gel plate). The reaction mixture was then cooled to room temperature, and water was added and extracted with ethyl acetate. The organic layer was washed with saturated sodium bicarbonate solution, dilute hydrochloric acid, and water and dried. Removal of the solvent in vacuo yielded a crude product, which on recrystalization from 2-propanol resulted in pure product **20/6aa**.

Alternative Method for the Synthesis of (*E*)-2,4,6-Trimethoxystyryl-3-hydroxy-4-methoxybenzylsulfone 6aa (Scheme 4). Preparation of 3-[(*p*-Toluenesulfonyl)oxy]-4-methoxybenzaldehyde (21). A mixture of 3-hydroxy-4-methoxybenzaldehyde **12** (5.0 g, 32 mmol) and *p*-toluenesulfonyl chloride (10.0 g, 52.5 mmol) was dissolved in pyridine (12.5 mL, 154 mmol). The reaction mixture was stirred for 5 min and maintained at 70–80 °C. The clear reaction mixture became turbid and became a slurry. The stirring was continued for 2 h at 70–80 °C. The reaction completion was determined by TLC, and the contents of the flask were cooled to room temperature and poured onto cold water. The white crystalline solid formed was filtered, washed successively with 5 mL of 1:1 HCl/H_2O, 5 mL of 5% NaOH solution, and water till the filtrate was free from pyridine, and dried to constant weight to get the desired product **21**. Yield, 98%; white solid, mp 148–151 °C. ^{1}H NMR: δ 2.40 (s, 3H, CH_3), 3.71 (S, 3H, OCH_3), 6.91–7.83 (m, 7H, Ar−H), 9.83 (s, 1H, CHO). Anal. Calcd for $C_{15}H_{14}O_5S$: C, 58.81; H, 4.61. Found: C, 58.79; H, 4.59.

Preparation of 3-[(*p*-Toluenesulfonyl)oxy]-4-methoxybenzyl Alcohol (22). To a cooled solution of 3-[(*p*-toluenesulfonyl)oxy]-4-methoxybenzaldehyde **21** (9.0 g, 29 mmol) in methanol (25 mL) was added sodium borohydride (0.55 g, 14.5 mmol) in methanol (2.5 mL) over a period of 5–10 min, maintaining the temperature around 15–20 °C. The reaction mixture was maintained at that temperature for a further 30 min, and TLC was used to check for completion of the reaction. Water was added to the reaction mixture, and the solid formed was filtered, washed with water, and dried to afford **22**. Yield, 97%; white solid, mp 88–90 °C. ^{1}H NMR: δ 2.41 (s, 3H, CH_3), 3.59 (s, 3H, OCH_3), 4.57 (s, 2H, CH_2), 6.80–7.79 (m, 7H, Ar−H). Anal. Calcd for $C_{15}H_{16}O_5S$: C, 58.43; H, 5.23. Found: C, 58.49; H, 5.19.

Preparation of 3-[(*p*-Toluenesulfonyl)oxy]-4-methoxybenzyl Chloride (23). To a cooled solution of 3-[(*p*-toluenesulfonyl)oxy]-4-methoxybenzyl alcohol **22** (8.0 g, 26 mmol) in benzene (25 mL) was added thionyl chloride (1.9 mL, 26 mmol) slowly over 5–10 min, maintaining the temperature around 15–20 °C. The reaction was maintained at those conditions for 2 h, and TLC was used to check for the completion. The flask was connected to a high vacuum through a trap containing formic acid under mild heating to remove excess thionyl chloride. The slurry was formed after complete removal of thionyl chloride and benzene, filtered, washed with hexane, and dried to afford **23**. Yield, 90%; white solid, mp 102–105 °C. ^{1}H NMR: δ 2.43 (s, 3H, CH_3), 3.61 (s, 3H, OCH_3), 4.50 (s, 2H, CH_2), 6.80–7.79 (m, 7H, Ar−H). Anal. Calcd for $C_{15}H_{15}ClO_4S$: C, 55.13; H, 4.63. Found: C, 53.20; H, 4.59.

Preparation of 3-[(*p*-Toluenesulfonyl)oxy]-4-methoxybenzylthioacetic Acid (24). To a solution of sodium hydroxide (1.72 g, 42.9 mmol) in methanol (30 mL) was added mercaptoacetic acid (1.5 mL, 21.5 mmol) in portions and stirred under nitrogen atmosphere for 10 min. 3-[(*p*-toluenesulfonyl)oxy]-4-methoxybenzyl chloride **23** (7.0 g, 21.5 mmol) was then added slowly to the reaction mixture and stirred at reflux temperature for 5 h. The reaction mixture was then poured onto ice containing concentrated HCl. The white crystalline solid was filtered, washed with water, and dried to get the desired product **24**. Yield, 93%; white solid, mp 116- 120 °C. ^{1}H NMR: δ 2.41 (s, 3H, CH_3), 3.03 (s, 2H, SCH_2), 3.61 (s, 3H, OCH_3), 3.79 (s, 2H, CH_2S), 6.80–7.81 (m, 7H, Ar−H). Anal. Calcd for $C_{17}H_{18}O_6S_2$: C, 53.39; H, 4.74. Found: C, 53.33; H, 4.71.

Preparation of 3-[(*p*-Toluenesulfonyl)oxy]-4-methoxybenzylsulfonylacetic Acid (25). To a solution of 3-[(*p*-toluenesulfonyl)oxy]-4-methoxybenzylthioacetic acid **24** (7.0 g, 18.4 mmol) in glacial acetic acid (35 mL) was added 21 mL of 30% hydrogen peroxide and stirred overnight (18 h). The reaction mixture was poured onto ice–water, and the solid that separated was filtered, washed with cold water, and dried to get pure **25**. Yield, 80%; white solid, mp 142–146 °C. ^{1}H NMR: δ 2.50 (s, 3H, CH_3), 3.61 (s, 3H, OCH_3), 4.03 (s, 2H, SCH_2), 4.60 (s, 2H, CH_2S), 7.09–7.74 (m, 7H, Ar−H), 13.4 (br s, 1H, OH). Anal. Calcd for $C_{17}H_{18}O_8S_2$: C, 49.27; H, 4.38. Found: C, 49.22; H, 4.34.

Preparation of (*E*)-2,4,6-Trimethoxystyryl-3-[(*p*-toluenesulfonyl)oxy]-4-methoxybenzylsulfone (26). A mixture of 3-[(*p*-toluenesulfonyl)oxy]-4-methoxybenzylsulfonylacetic acid **25** (6.0 g, 14.5 mmol), 2,4,6-trimethoxybenzaldehyde **19** (2.85 g, 14.5 mmol),

benzoic acid (0.27 g, 2.2 mmol), and piperidine (0.19 mL, 1.9 mmol) in benzene (50 mL) was refluxed for 4–5 h with continuous removal of water using a Dean–Stark water separator. The reaction completion was checked by TLC (9:1 chloroform/methanol on silica gel plate). The reaction mixture was then cooled to room temperature, and the crystalline solid formed was filtered, washed with cold benzene, and dried to get the desired product **26**. Yield, 65%; white solid, mp 159–166 °C. ^{1}H NMR: δ 2.42 (s, 3H, CH_3), 3.61(s, 3H. OCH_3), 3.81 (s, 3H, 3 × OCH_3), 4.21 (s, 2H, CH_2), 6.06 (s, 2H, Ar−H), 6.80–7.74 (m, 7H, Ar−H), 7.01 (d, 1H, J =15.5 Hz, =CH), 7.83 (d, 1H, J = 15.5 Hz, CH=). Anal. Calcd for $C_{26}H_{28}O_9S_2$: C, 56.90; H, 5.14. Found: C, 56.83; H, 5.11.

Preparation of (*E*)-2,4,6-Trimethoxystyryl-3-hydroxy-4-methoxybenzylsulfone (6aa). A mixture of (*E*)-2,4,6-trimethoxystyryl-3-[(*p*-toluenesulfonyl)oxy]-4-methoxybenzylsulfones **26** (5.0 g, 9.1 mmol), 50 mL (20%) of sodium hydroxide solution, and methanol (50 mL) was taken in a round bottomed flask and refluxed until the reaction mixture was clear without any turbidity (3–4 h). The progress of the reaction was monitored by TLC. The reaction mixture was cooled to room temperature and neutralized with cold dilute HCl solution. The precipitate that separated after neutralization was filtered, washed with water, and dried to get the crude product, which on recrystallization from 2-propanol resulted in an analytically pure sample of **6aa**.Yield, 95%; white solid, mp 124–127 °C.

Synthesis of (*E*)-2,4,6-Trimethoxystyryl-3-*O*-phosphate Disodium 4-Methoxybenzylsulfone (6ab) (Scheme 5). Preparation of (*E*)-2,4,6-Trimethoxystyryl-3-*O*-bis(benzyl)phosphoryl-4-methoxybenzyl Sulfone (27). To a stirred solution of (*E*)-2,4,6-trimethoxystyryl-3-hydroxy-4-methoxybenzylsulfone **6aa** (3.8 g, 9.6 mmol) in acetonitrile (48 mL) under nitrogen atmosphere was added carbon tetrabromide (3.88 g, 11.72 mmol) and triethylamine (1.46 g, 14.4 mmol), and stirring was continued for 10 min. Dibenzyl phosphite (3.20 g, 11.6 mmol) dissolved in acetonitrile (32 mL) was added to the reaction mixture slowly. After the addition, the reaction mixture was stirred for 2 h and TLC was used to check for completion of the reaction. The phosphorylation was terminated by dropwise addition of potassium dihydrogen phosphate (20 mL, 0.5 M) to the reaction mixture over 10 min. The solution was then extracted with ethyl acetate (3 × 60 mL). The organic extracts were combined and washed with water, dried, and concentrated in vacuo. The thick liquid obtained after concentration was purified on silica column using chloroform/methanol with increasing polarity. The purified product was concentrated in vacuo to afford pure dibenzyl ester **27**. Yield, 73%; semisolid. ^{1}H NMR: δ 3.68 (s, 6H, 2 × OCH_3), 3.71(s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 4.07 (s, 2H, CH_2), 4.96–5.04 (m, 4H, OCH_2), 5.98 (s, 2H, Ar−H), 6.60–7.42 (m, 14H, Ar−H + vinylic), 7.71 (d, 1H, J = 15.6 Hz, CH=). Anal. Calcd for $C_{33}H_{35}O_{10}PS$: C, 60.54; H, 5.39. Found: C, 60.48; H, 5.44.

Preparation of (*E*)-2,4,6-Trimethoxystyryl-3-*O*-phosphoryl-4-methoxybenzylsulfone (28). To a stirred solution of the above dibenzyl ester **27** (4.36 g, 6.7 mmol) in anhydrous dichloromethane (40 mL) under nitrogen at 0 °C was added bromotrimethylsilane (2.14 g, 14.1 mmol). The stirring was continued for 45 min at the same temperature, and TLC was used to check for completion of the reaction. Sodium thiosulfate (1%, 50 mL) was added to the reaction mixture, and stirring was continued for an additional 5 min. The separated aqueous phase was extracted with ethyl acetate (3 × 25 mL). The organic extracts were concentrated in vacuo to afford the crude phosphoric acid **28**, which was purified on a silica column using chloroform/methanol with increasing polarity. The purified product was concentrated in vacuo to afford pure acid **28**. Yield, 44.3%; white solid, mp 202–205 °C. ^{1}H NMR (DMSO-d_6): δ 3.78(s, 6H, 2 × OCH_3), 3.85(s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.34 (s, 2H, CH_2), 6.30 (s, 2H, Ar−H), 7.02 (m, 2H, Ar−H), 7.12 (d, 1H, J = 15.6 Hz, =CH), 7.32 (s, 2H, OH), 7.52 (s, 1H,Ar−H), 7.61 (d, 1H, J = 15.6 Hz, CH=). Anal. Calcd for $C_{19}H_{23}O_{10}PS$: C, 48.10; H, 4.89. Found: C, 48.14; H, 4.92.

Preparation of (*E*)-2,4,6-Trimethoxystyryl-3^1-*O*-phosphate Disodium 4-Methoxybenzylsulfone (6ab). To a stirred solution of the above phosphoric acid **28** (1.35 g, 2.85 mmol) in ethylene glycol dimethyl ether (125 mL) was added 2 N sodium hydroxide (0.27 g dissolved in 13.66 mL of H_2O, 6.8 mmol) and stirred for 3 h. The solid formed was filtered, washed with acetone (2 × 25 mL), and dried under vacuum to get the product **6ab**. Yield, 98%; white solid, mp 152–154 °C. ^{1}H NMR (D_2O): δ 3.68 (s, 6H, 2 × OCH_3), 3.71 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.35 (s, 2H, CH_2), 5.92 (s, 2H, Ar−H), 6.91 (s, 2H, Ar−H), 6.97 (d, 1H, J = 15.6 Hz, =CH), 7.39 (s, 1H, Ar−H), 7.43 (d, 1H, J = 15.6 Hz, CH=). ^{13}C NMR (D_2O): δ 164.4, 161.7, 151.1, 143.8, 136.8, 125.9, 123.4, 120.6, 120.2, 113.1, 103.4, 91.1, 61.0, 56.4, 56.2, 55.9. Anal. ($C_{19}H_{21}O_{10}Na_2PS$) C, H.

Biology. Tissue Culture and Reagents. Paclitaxel was purchased from Sigma. Cell lines were purchased from ATCC. Cell lines were routinely grown in DMEM or RPM1 (CellGro) supplemented with 10% fetal bovine serum (Atlas) and 1 unit/mL penicillin−streptomycin (Gibco).

Cytotoxicity Assay. We have tested a number of tumor cell lines using a dose response end point assay system. The cells were grown in either DMEM or RPMI supplemented with 10% fetal bovine serum and 1 unit/mL penicillin−streptomycin solution. The tumor cells were plated into six-well dishes at a cell density of 1.0×10^5 cells/mL/well, and compounds were added 24 h later at various concentrations. Cell counts were determined from duplicate wells after 96 h of treatment. The total number of viable cells was determined by trypan blue exclusion.

Soft Agar Assay. The soft agar plates were prepared as described by Cosenza et al.[24] Briefly, Noble bottom agar (0.8%) was plated onto 60 mm tissue culture plates. Exponentially growing MIA-PaCa-2 cells (1.0×10^5) were mixed with growth medium with various concentrations of each compound and mixed with Noble agar to a final concentration of 0.4%. Each concentration was plated in triplicate. The top agar was allowed to solidify, and the plates were then incubated at 5% CO_2 at 37 °C for 3 weeks. The plates were then stained with 0.05% nitroblue tetrazolium (NBT) solution, and representative plates were photographed using an Olympus stereoscope mounted with a Sony digital camera system (DKC5000, Sony Inc.).

Flow Cytometry. Human prostate tumor cells, DU145 cells, and normal diploid human lung fibroblasts, HFL-1 cells, were grown in DMEM (Cellgro) supplemented with 10% fetal bovine serum and 1 unit/mL penicillin−streptomycin. The cells were plated onto 100 mm^2 dishes at a cell density of 1.0×10^6 cells/dish, and 24 h later, they were treated with 2.5 μM of the compound. The cells were harvested 24, 48, and 72 h after treatment. The cells were removed from the plate by trypsin digestion and combined with the nonattached cells found in the medium. The cell pellets were washed in phosphate buffered saline (PBS) and fixed in ice cold 70% ethanol for at least 24 h. The fixed cells were then washed with room temperature PBS and stained with propidium iodide (50 μg/mL) and RNase A (0.5 mg) for 30 min at 37 °C. The stained cells were then analyzed on a Becton-Dickinson (BD) (FACScan) flow cytometer and the data analyzed by cell cycle analysis software (Modfit, BD).

PARP Western. BT20 cells were plated at a density of 3.0×10^6 cells per 150 mm^2 plate and treated 24 h later with either DMSO or **6aa**. The cells were collected at the indicated time points, and cell pellets were frozen. The frozen cell pellets were lysed in 1% NP40/PBS lysis buffer containing protease inhibitors. Equal amounts of total cellular protein were then resolved on a 10% SDS−polyacrylamide gel. The gels were transferred onto nitrocellulose paper (S/S), hybridized with anti-PARP antibodies (BD) and developed using ECL (Perkin-Elmer, MA) solution.

Nude Mouse Assay. Female athymic (NCR-nu/nu, Taconic) nude mice were injected with (0.5–1.0) × 10^7 BT20 cells subcutaneously in the hind leg using a 1 mL tuberculin syringe equipped with a 271/2 gauge needle. Approximately 14 days later, mice were paired (N = 8) and injected with **6ab** or phosphate buffered saline as the vehicle control. The intravenous injections were performed in the mouse tail vein using a 1 mL tuberculin syringe equipped with a 30 gauge needle. The animals were injected following a Q_2D ×3 schedule. Tumor measurements (two dimen-

sions) were done three times per week using traceable digital vernier calipers (Fisher). Tumor volume was calculated using the following equation: $V = (L(S^2)\pi/6$, where L is the longer and S is the shorter of the two dimensions. Body weight was determined during each measurement. The animals were observed for signs of toxicity. The time of tumor volume doubling was calculated and the $T - C$ value (difference in the average times post-treatment for tumors of the treated groups to attain a doubling in volume compared to the average of the control group) was determined. We did not observe body weight loss of more than 10% in any group nor were there any animal deaths. All studies were performed under the guidelines of Temple University IACUC.

Bone Marrow Harvest and Colony Formation Assay. Bone marrow was harvested from femur and tibia of CD-1 mice injected with 200 μL of PBS or **6ab** [10 mg/mL:100 mg/kg dose] at 12, 24, or 48 h before the sacrifice. The bone marrow cells were cultured in methylcellulose medium supplemented with 50 ng/mL rmStem cell factor, 10 ng/mL rmIL-3, 10 ng/mL rh IL-6, 200 μg/mL human transfeffin, and 3 units/mL rhErythropoietin (Stem Cell Technologies, Vancouver, British Columbia, Canada). Cultures were seeded in duplicate using 35 mm plastic Petri dishes, and colony forming units were determined after 1 week.

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

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