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Discovery of a Clinical Stage Multi Kinase Inhibitor Sodium (E)-2-{2-Methoxy-5-[(2', 4', 6'-trimethoxystyrylsulfonyl)methyl]phenylamino}acetate (ON 01910.Na): Synthesis, Structure Activity Relationship and Biological Activity

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Abstract

Cyclin D proteins are elevated in many cancer cells and targeted deletion of Cyclin D1 gene in the mammary tissues protects mice from breast cancer. Accordingly, there is an increasing awareness of this novel non-enzymatic target for cancer therapeutics. We have developed novel, non-alkylating styryl benzyl sulfones that induce cell death in wide variety of cancer cells without affecting the proliferation and survival of normal cells. The development of derivatized Styryl Benzyl Sulfones followed logically from a tumor cell cytotoxicity screen performed in our laboratory that did not have an *a priori* target profile. Modifications of some of the precursor molecules led to lead optimization with regard to tumor cell cytotoxicity. In this report we describe the synthesis and structure-activity relationships of novel, non-alkylating (E) styryl benzyl sulfones, and the development of the novel anti-cancer agent sodium (E)-2-{2-methoxy-5-[(2',4',6'-trimethoxystyrylsulfonyl)methyl]phenylamino}-acetate (ON 01910.Na), which is in Phase III trials for myelodysplastic syndromes (MDS) associated with aberrant expression of cyclin D proteins.

Introduction

In a recent paper,¹ we described the synthesis of a group of styryl benzyl sulfones that induce apoptotic death of a wide variety of human tumor cell lines at nanomolar

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concentrations while exhibiting relatively low toxicity to normal human cells. Our studies showed that the cytotoxic activity of styryl benzyl sulfones is completely dependent on the nature and position of the substituents on the two aromatic rings. Structure–function studies showed that position of functional groups on the styryl aromatic ring play a critical role in determining the biochemical and biological activity of these molecules. Biological evaluation of the activity of these compounds showed 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 pointed to the potential value of these compounds as safe therapies for cancer, lacking many of the side effects normally associated with current chemotherapeutic agents. Many of the compounds described in this study were found to act as allosteric inhibitors of serine/threonine and tyrosine kinases providing a rationale for further expansion of this chemotype for applications related to cancer therapy.

The present report describes the synthesis and structure-activity relationships of novel, non-alkylating (E)-styryl benzyl sulfones, and the development of the novel anti-cancer agent sodium (E)-2-{2-methoxy-5-[(2', 4', 6'-trimethoxystyrylsulfonyl)methyl]phenylamino}acetate (ON 01910.Na, **28**)². The development of derivatized styryl benzyl sulfones followed logically from a tumor cell cytotoxicity screen performed in our laboratory. Precursors of **28** were identified specifically based upon their ability to target cancer cells while leaving non-malignant cell cultures virtually unaffected. Modifications of some of the precursor molecules led to lead optimization with regard to tumor cell cytotoxicity. Structure-activity studies confirmed that the nature, number, and position of substituents on the two aromatic rings of the parent molecule are the determining factor in the tumor cell cytotoxicity of these compounds.

In previous studies in our laboratory, **28** displayed desirable pharmacokinetic and pharmacodynamic properties, and was able to reduce tumor size and increase survival in mice carrying tumor cell xenografts.² **28** Received orphan drug status for the myelodysplastic syndrome, a heterogeneous hematopoietic stem cell disorder that affects cell proliferation, differentiation, and function. MDS is characterized by dyspoiesis, hyperproliferative bone marrow, and peripheral blood cytopenias involving one or more lineages.^{3–5} Majority of untreated patients with high risk MDS die from progressive bone marrow failure within one year due to hemorrhage and/or infection. *In vitro* studies with **28** showed that incubation of human leukemic cells with this compound results in the inhibition of PI3K/AKT pathway, down regulation of cyclin D1, induction of NOXA and BIM and activation of JNK pathway.⁶ Treatment of MDS patients with **28** results in a dramatic reduction of cytogenetically abnormal cells with a minimal inhibition of normal hematopoiesis. This drug is currently in Phase III clinical trials.

Chemistry

Multiple synthetic routes for synthesis of the (E)-styryl benzyl sulfone scaffold were explored. The initial method (Method A) involved the synthesis of substituted (E)-styryl benzyl sulfones by the reaction of benzyl bromides (**2**) with mercaptoacetic acid in presence

of a strong base, sodium hydroxide in methanol, to obtain benzylthioacetic acids (**5**) in quantitative yields (Scheme 1). Oxidation of **5** with 30% hydrogen peroxide (H_2O_2) in glacial acetic acid afforded benzylsulfonylacetic acids **6**.⁷ Knoevenagel condensation of **6** with aromatic aldehydes **7** either in benzylamine/acetic acid⁷ or piperidine/benzoic acid⁸ in toluene, afforded (E)-styryl benzyl sulfones **8** in good yields.

Some of the substituted benzyl bromides **2** that are not commercially available were synthesized as shown in the scheme 2. Substituted toluenes **1** were brominated with N-bromosuccinimide (NBS) in presence of a catalytic amount of benzoyl peroxide in carbon tetrachloride (CCl_4) to obtain **2**.⁹ Some of these nitro substituted benzyl bromides **2** were also made starting from substituted nitro benzaldehydes **3** which were reduced with sodium borohydride (NaBH_4)¹⁰ and on subsequent bromination of the resulting alcohol **4** with phosphorous tribromide (PBr_3) (scheme 2).¹¹

In method B, 3-Nitro-4-methoxybenzyl mercaptan (**9**) was treated with 4-methoxyphenacyl bromide (**10**) to obtain 4-methoxyphenacyl-3-nitro-4-methoxybenzyl sulfide **11** which on oxidation with 30% H_2O_2 gave 4-methoxyphenacyl-3-nitro-4-methoxybenzyl sulfone **13**.⁷

Sequential reduction of **13** with sodium borohydride¹ and subsequent dehydration with p-toluenesulfonic acid (p-TSA) afforded the desired **8** in moderate yields (Scheme 3).¹² Method A is superior to method B for the synthesis of **8** as it involves steps with relatively higher yields. The 3-nitro-4-methoxy benzyl mercaptan **9** was in turn synthesized from **2a** as outlined in Scheme 4. **2a** on treatment with thiourea in water gave an intermediate isothiuronium salt (**12**) which on reduction with ammonia yielded **9** in low to moderate yields.¹³

To see the effect of *trans* sulfide and sulfoxide on the biological activity of these (E)-styryl benzyl sulfones, we made (Z) (**16**) and (E) (**17**) 4-methoxy-3-nitrobenzyl-2',4',6'-trimethoxystyryl sulfides by the reaction of 3-nitro-4-methoxybenzyl mercaptan (**9**) and 2,4,6-trimethoxyphenyl acetylene (**15**) in the presence of triethylborane-hexane (Et_3B) in benzene (Scheme 5).¹⁴ In this reaction, (Z) to (E) ratio of 40:60 resulting in mainly *trans* isomers. **17** on controlled oxidation with 1,1,1,3,3,3-hexafluoro-2-propanol and 30% H_2O_2 at room temperature resulted in sulfoxide **20**,¹⁵ which on reduction with sodium hydrosulfite in acetone-water mixture at 50 °C afforded the corresponding amine **21**.⁹

The sulfoxide **20** was oxidized to sulfone **22** with 30% hydrogen peroxide in glacial acetic acid⁷ which was later reduced with sodium hydrosulfite to corresponding sulfone **23**.⁹ The nitro sulfide **17** was also converted to corresponding amino sulfide **24** which on further oxidation with m-chloroperoxybenzoic acid (m-CPBA) afforded sulfone **23**. The acetylene **15** was synthesized starting from 2,4,6-trimethoxybenzaldehyde **18** and tetrabromomethane (CBr_4) in the presence of triphenylphosphine (Ph_3P) in dichloromethane.¹⁶ The resulting 2',2'-dibromovinyl-1,3,5-trimethoxybenzene **19** on treatment with n-BuLi in THF at -78 °C gave **15**¹⁶ in high yields. The synthesis of nitro (E)-styryl benzyl sulfoxide **20** and amino (E)-styryl benzyl sulfoxide **21** was also achieved from 4-methoxy-3-nitrobenzylthioacetic acid **5a** by oxidation to sulfoxide **25**¹⁷ and then condensation with 2,4,6-

trimethoxybenzaldehyde **18** in the presence of benzylamine and acetic acid (Scheme 7).⁷ The resulted **20** was reduced to **21** as described earlier in Scheme 5.

To enhance the solubility and bioavailability of these (E)-styryl benzyl sulfones, several 3-amino substituted esters and acids were made from (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-aminobenzylsulfone (**8p**) and (E)-3',4',5'-trimethoxystyryl-4-methoxy-3-aminobenzylsulfone (**8q**) (Scheme 8). **8p** and **8q** were treated with different α -bromo esters in the presence of mild base sodium acetate in ethanol for 48 h to give amine esters (**26**) which on subsequent hydrolysis with sodium hydroxide in ethanol afforded the corresponding acids **27**.

The lead compound of our investigation, **28**, was synthesized from **8p** by its reaction with methyl 2-bromo acetate in the presence of a mild base sodium acetate in methanol for 4–6 h to give amino substituted methyl ester **26a** which on subsequent hydrolysis with sodium hydroxide in aqueous ethanol and dichloromethane followed by methyl ethyl ketone washing afforded crystalline **28** in high yields with 2 molecules of water of hydration as determined by Karl-Fisher analysis (scheme 9).

Structure-Activity Relationships (SAR)

After the synthesis of these compounds, their in vitro cytotoxicity was assessed using two different human tumor cell lines derived from human prostate (DU145) and leukemic (K562) cancers. The results of the study are presented in Table 1. These studies reveal that the cytotoxicity of the (E)-styryl benzyl sulfones is totally dependent on the nature and position of the substituents present on the two aromatic rings. For the purpose of structure activity relationship, we have selected a few compounds from a library of two thousand (E)-styryl benzyl sulfones synthesized from our laboratory. In most of the selected compounds described here, we have kept a methoxy group at the fourth position and an amino group at the third position of benzylsulfonyl aromatic ring and one or more methoxy groups on styryl aromatic ring at different positions. A moderate cytotoxicity in tumor cells was seen in a compound when an amino and methoxy groups are at 3rd and 4th position of the benzyl ring and a methoxy group at the 4th position of the styryl ring (**8d**). By introduction of second methoxy group on the styryl ring of **8d**, the cytotoxicity of the resulting compounds (**8g**) (**8h**) and (**8i**) was further enhanced by several folds. Whereas the results are quite surprising for the molecules that are disubstituted with methoxy groups on the styryl aromatic ring compared to **8d**, the results obtained in cytotoxicity assays using these compounds (**8g**, **8h**, **8i**, **8j** and **8k**) clearly shows that the methoxy group, when present at 2, 6 positions (**8i**), enhances the activity of the molecule by greater than 10 fold when compared to other disubstituted methoxy groups (**8g** and **8h**). It is also clear from the cytotoxicity profile of the compounds **8j** and **8k** that when amino and methoxy groups are placed at other than 3rd and 4th position, the compounds exhibit decreased cell killing activity: 15 to 500 fold. Because the introduction of two methoxy groups on the styryl aromatic ring enhanced the biological activity, we have synthesized some trimethoxystyryl analogs to determine if this further enhances their cytotoxic properties. Analysis of these compounds (**8n**, **8o**, **8p**, **8q**, and **8r**) in cell-killing assays showed that (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-aminobenzylsulfone (**8p**) exhibited best activity in the entire series. This compound, **8p**, is

almost 11 folds more active than **8i** in both cell lines. These results also show that when 2, 4 and 6 positions of the styryl ring are occupied by methoxy groups (**8p**) the molecule attains optimum biological activity compared to other trimethoxy substituted styryl sulfones (**8n**, **8o**, **8q** and **8r**). To validate the critical requirement of the methoxy group at 4th position of 2', 4', 6'-trimethoxystyryl moiety, we have replaced the methoxy group at 4th position by a hydroxy (**8t**) and a carboxypropoxy group (**8u**). Both the replacements in **8p** caused severe loss of cytotoxicity in the molecules indicating the indispensability of a methoxy group at 4th position of the ring. Further replacing the methoxy group with halogen atoms or changing the position of the amino group in benzyl aromatic ring resulted in molecules (**8w**, **8x**, **8y** and **8aa**) that suffered substantial loss of cytotoxicity. Also the compounds formed by replacing all three methoxy groups of the styryl ring by fluorine atoms (**8ab** and **8ac**) in **8p** have reduced level of cytotoxicity. The SAR analysis clearly shows that all the compounds with nitro substitutions on the benzylic ring (**8a**, **8c**, **8e**, **8f**, **8l**, **8m**, **8s**, **8v** and **8z**) are far less active than the corresponding amino compounds indicating that the amino group in that position is critical for the interaction of the compounds with their target and activity.

Once we established that 3-amino-4-methoxy groups on benzyl aromatic ring and 2, 4, 6-trimethoxy groups on styryl ring produced a compound (**8p**) with best cytotoxic activity, we then analyzed the role of sulfone in the activity of the molecule (Table 2). To understand the oxidative state of sulfur in the molecule, we have made a sulfide (**24**) and sulfoxide (**21**) analogs of **8p**. Both sulfide (**24**) and sulfoxide (**21**) analogs are 10 fold less active than sulfone (**8p**) indicating complete oxidative state of sulfur in the compound is required for optimum activity.

The compound **8p**, which has a high potency, has very low solubility in aqueous buffers and solutions. In order to enhance its water solubility and bioavailability the amino group in third position was converted to amino acids (**27a–27l**). From Table 3, it is clear that all 3-substituted amino acids (**27a–27l**) are more active than the corresponding esters (**26a–26l**). As the 3-glycine substituted compound **27a** has superior activity over other molecules (**27b–27l**), and as it doesn't have a chiral center at glycine carbon, we have made a water-soluble sodium analogue of it (**28**) to carry out our preclinical and clinical studies. Compound **28** has 28mg/ml solubility in water and other aqueous buffers which makes it suitable for intravenous, intraperitoneal and oral administration of the compound.

Biological Results and Discussion

In vitro anti-tumor effects of **8p** and **28** compounds

We next tested the activity of two of the most active compounds (**8p** and **28**) listed in Tables 1–3 against 94 different human tumor cell lines and surprisingly, they were found to induce apoptosis of all of these cell lines with very similar GI₅₀ values (selected data shown in Table 4). These compounds (such as **8p**, **28**) were also tested by the National Cancer Institute, USA, through its Developmental Therapeutics Program (DTP) against their panel of 60 human cancer cell-lines.¹⁸ 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.. The finding that such a large number of tumor cell lines are sensitive to these compounds suggests that the target is essential for

the proliferation and survival of cancer cells. Statistical comparison (using the NCI algorithm COMPARE) revealed that these drugs are mitotic blockers of tumor cells. This statistical observation was further substantiated by flow cytometry analysis shown in Figure 2.

8p and 28 compounds are highly active against drug resistant tumor cell lines

Development of multidrug resistance (MDR) to classical chemotherapeutic agents is a clinical problem Oncologists face as patients fail first round treatment or become resistant during or following recurrent tumor growth. The MDR phenotype is caused by the overexpression of ATP-binding cassette (ABC) transporters divided into seven subfamily members. The overexpression of various members of this family enables the tumor cells to actively pump out a wide range of amphipathic drugs such that the intracellular concentrations are no longer cytotoxic. To further investigate the activity of these compounds against MDR positive tumor types, we determined the IC₅₀ values of **8p** and **28** using two classical MDR positive cell lines (Table 5). The results shown in Figure 1A show a 96 h dose response of the uterine sarcoma cell line MES-SA and the multidrug resistant sub line MES-SA/DX5¹⁹ treated with **28** compared to a dose response against paclitaxel a known substrate for P-glycoprotein. This cell line has been shown to express high levels of P-glycoprotein (ABCB1) and is resistant to a number of hydrophobic compounds including doxorubicin, paclitaxel, vincristine, vinblastine, etoposide, mitoxantrone, dactinomycin, and daunorubicin. Our results show that the parental cell line was very sensitive to Paclitaxel (IC₅₀ 4 nM) but the MDR positive sub line was greater than 100 fold resistant (IC₅₀ 750 nM). When the two cell lines were treated with **28**, both the parental and the MDR positive cell lines were equally sensitive to the cell killing activity of the compound with an IC₅₀ value of 0.1 μM (Table 5). A second MDR cell line, derived from an ovarian tumor, was also tested. Once again, both the parental and the resistant cell lines were equally sensitive to compound number **28** (Table 5). We also investigated as to whether atypical multidrug resistant cell are sensitive to **28**. For these studies, we employed the parental leukemic cell line CEM and its MDR sub line CEM/C2 (Figure 1B).²⁰ CEM/C2 was selected and sub cloned for resistance to camptothecin and has cross resistance to etoposide, dactinomycin, bleomycin, mitoxantrone, doxorubicin, and daunorubicin. As shown in figure 1B and Table 5, compound **28** was active against both the parental and the camptothecin resistant sub clone, with the CEM/C2 clone being 2 fold more sensitive than the parental. These studies clearly demonstrate that this chemotype will not be a substrate for ABC transport proteins and therefore be a suitable treatment option for tumors expressing both classical and atypical MDR resistant markers.

Effects of 28 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 show the effect of **28** on the cell cycle progression of normal diploid human fetal lung (HFL-1) and DU145 (prostate cancer) cells (Figure 2B). The results of this study show that the addition of the **28** to DU145 cells resulted in a block of their cell cycle progression in G2/M phase of the cell cycle in a dose dependent manner. In addition, treatment of the tumor cells resulted in an accumulation of cells containing subG1 content of DNA which is an indication of cell death. On the other

hand, treatment of normal diploid fibroblasts cells (HFL-1) with **28** resulted in a block of their cell cycle progression in the G₁ and G₂/M phases of the cell cycle, without the corresponding induction of cell death. We further investigated the selective nature of cell killing by running studies aimed at studying the activation of the apoptotic pathway. Tumor and normal cell lines were then treated with **28** and activation of apoptotic pathways, as judged by PARP [Poly(ADP-ribose) polymerase-1] cleavage,²¹ is shown in (Figure 3). Treatment of **28** selectively induced PARP cleavage in the tumor cell line while there was no PARP cleavage in the treated normal cell line. Since it is well known that PARP cleavage is the result of activation of caspase-3 we further investigated the activation of the apoptotic pathway by looking at cellular viability together with the activity of caspases 3/7. A dual viability and caspase activation assay was performed in A549 cells treated with **28** which showed the concentrations at which **28** activated the apoptotic machinery to induce a cytotoxic effect (Figure 4). This data shows that treatment with 0.25 μ M induced loss of viability with the concomitant induction of caspase 3/7 within 24 h of tumor cell treatment. Taken together, this data shows that **28** selectively induces G₂/M cell cycle block with the induction of apoptosis in tumor cells.

In vivo anti-tumor effect of **28**

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 200 mg/kg of **28** using a Q2D $\times$ 20 schedules. The animals were treated when the tumors were approximately 70 mm³ in size. Figure 5A shows that an intraperitoneal (IP) injection of **28** was able to significantly inhibit the growth of the tumors. The tumors of vehicle treated mice, on average, increased in volume over the 22 day period by 5 fold (70 mm³–480 mm³), while the Q2D **28** treated tumors increased in volume by only 2.5 fold (70 mm³–180 mm³). **28** were well tolerated at these doses as determined by body weights and physical observations (Figure 5B). These studies show that **28** is efficacious against human tumor xenografts while showing no signs of toxicity at the schedules tested under this study.

Conclusion

In this communication, we describe the synthesis of a group of (E)-styryl benzyl sulfones which induce apoptotic death of a wide variety of human tumor cell lines at sub nanomolar concentrations while exhibiting relatively low toxicity to normal human cells. Our studies show that the cytotoxic activity of the (E)-styryl benzyl sulfones is completely dependent on the nature and position of the substituents on the two aromatic rings. These structure function studies show that a molecule with a benzyl moiety having 3-amino, 4-methoxy groups and a styryl ring with methoxy groups at 2, 4 and 6-positions showed optimum biological activity (**8p**). Biological evaluation of the activity of these compounds show 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 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 **28** show that this compound altered the growth and cell cycle status of mantle cell lymphoma cell lines and potently inhibited the expression of several important proteins, including cyclin D1, p-mTOR, and PI3-K.⁶ Since **28** is highly effective in various combinations with conventional chemotherapy,⁶ the lack of overt hematotoxicity of this compound is beneficial for testing novel combinations for advanced cancers, including tumors resistant to conventional chemotherapy. In addition, its safety profile seen with normal hematopoietic cells suggest 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 studies in MDS (Phase III) and pancreatic (Phase II/III) patients currently underway will reveal the best way to utilize this compound in cancer therapy.

Experimental Section

Chemistry: General Experimental Procedures

All reactions requiring anhydrous conditions were run under an atmosphere of dry nitrogen and solvents were dried using standard procedures. Reagents and solvents were obtained in the highest available purity and used without further purification unless indicated. Reactions were monitored by thin layer chromatography (TLC) on preloaded silica gel 60 glass-backed plates with F²⁵⁴ plates as the indicator (Sigma – Aldrich), developed using mobile phases of varying compositions of ethyl acetate/hexane, methanol/chloroform and the spots were visualized by a UV indicator. Column chromatography was performed with Merck 70 – 320 mesh silica gel. Melting points were determined using an Electro thermal MEL – Temp 3.0 micro melting point apparatus with a FLUKA 51 K/J electronic thermometer and are uncorrected. Nuclear magnetic resonance spectra for proton (¹H NMR) were recorded on a Bruker Avance III (300 MHz), Varian INOVA (400 MHz) and GE (500 MHz) spectrometer. ¹³C NMR spectra (75 MHz) were obtained on a Bruker Avance III 300 MHz spectrometer. The chemical shift values are expressed in ppm (parts per million) relative to tetramethylsilane as an internal standard: s, singlet; d, doublet; dd, doublet of a doublet; t, triplet; m, multiplet; br s, broad singlet. Coupling constants (J values) were measured in hertz (Hz). Compounds purity was determined by elemental analyses (0.4%) or LC/MS analysis and was confirmed to be > 95% for all compounds. All LC/MS data were gathered on an Agilent 1200 LC with Agilent 6410 triple quadrupole mass spectrometer detectors. The compound solution was infused into the electron spray ionization source operating in positive and negative mode.

General Procedure for the Preparation of benzyl bromides (2). Method A (Scheme 2)

To a well stirred solution of toluene **1** (40 mmol) in carbon tetrachloride (150 mL) were added benzoyl peroxide (4.0 mmol), and N-bromosuccinimide (48 mmol). The reaction mixture was heated at reflux for 18 h. After completion of the reaction (TLC monitoring, hexane/ethyl acetate, 9:1 on silica gel plate), the contents cooled to room temperature, water was added, and the product was isolated by extraction with dichloromethane. The organic

phase was washed with water, brine, dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum to obtain the desired crude product **2**. The pure compound **2** was obtained following purification by silica gel flash column chromatography (hexane/ethyl acetate, 9:1). The following benzyl bromides **2** were prepared using the above procedure.

4-Methoxy-3-nitrobenzyl bromide (2a)—Radical benzylic bromination of 4-methyl-2-nitroanisole yielded the corresponding 4-methoxy-3-nitrobenzyl bromide. The yield of this reaction was 68%, giving light yellow solid with a melting point 106–108 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.97 (s, 3H, OCH₃), 4.47 (s, 2H, CH₂), 7.08 (d, J = 8.7 Hz, 1H, Ar-H), 7.58 (dd, J = 8.7, 2.1 Hz, 1H, Ar-H), 7.89 (d, J = 2.4 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 245.9688; found 245.9682. Anal. (C₈H₈BrNO₃) C, H, N.

4-Methoxy-2-nitrobenzyl bromide (2b)—Radical benzylic bromination of 4-methyl-3-nitroanisole yielded the corresponding 4-methoxy-2-nitrobenzyl bromide. The yield of this reaction was 65%, giving yellow solid with a melting point 60–62 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.89 (s, 3H, OCH₃), 4.81 (s, 2H, CH₂), 7.14 (dd, J = 8.4, 2.7 Hz, 1H, Ar-H), 7.48 (d, J = 8.7 Hz, 1H, Ar-H), 7.56 (d, J = 2.7 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 245.9688; found 245.9681. Anal. (C₈H₈BrNO₃) C, H, N.

4-Bromo-3-nitrobenzyl bromide (2c)—Radical benzylic bromination of 4-bromo-3-nitrotoluene yielded the corresponding 4-bromo-3-nitrobenzyl bromide. The yield of this reaction was 78%, giving yellow solid with a melting point 62–63 °C. ¹H NMR (CDCl₃, 500 MHz): δ 4.45 (s, 2H, CH₂), 7.47 (dd, J = 8.4, 2.4 Hz, 1H, Ar-H), 7.73 (d, J = 8.4 Hz, 1H, Ar-H), 7.89 (d, J = 2.0 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 295.8667; found 295.8662. Anal. (C₇H₅Br₂NO₂) C, H, N.

4-Chloro-3-nitrobenzyl bromide (2d)—Radical benzylic bromination of 4-chloro-3-nitrotoluene yielded the corresponding 4-chloro-3-nitrobenzyl bromide. The yield of this reaction was 87%, giving pale yellow liquid with a boiling point 115–120 °C (0.25 mm Hg). ¹H NMR (CDCl₃, 300 MHz): δ 4.60 (s, 2H, CH₂), 7.42 (dd, J = 8.4, 2.4 Hz, 1H, Ar-H), 7.68 (d, J = 8.4 Hz, 1H, Ar-H), 7.86 (d, J = 2.4 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 249.9192; found 249.9186. Anal. (C₇H₅BrClNO₂) C, H, N.

4-Chloro-2-nitrobenzyl bromide (2e)—Radical benzylic bromination of 4-chloro-2-nitrotoluene yielded the corresponding 4-chloro-2-nitrobenzyl bromide. The yield of this reaction was 69%, giving pale yellow solid with a melting point 40–42 °C. ¹H NMR (CDCl₃, 500MHz): δ 4.80 (s, 2H, CH₂), 7.54 (d, J = 8.3 Hz, 1H, Ar-H), 7.61 (dd, J = 8.3, 2.1 Hz, Ar-H), 8.07 (d, J = 2.1Hz, Ar-H). HRMS: m/z calcd [M + H] 249.9192; found 249.9189. Anal. (C₇H₅BrClNO₂) C, H, N.

4-Fluoro-3-nitrobenzyl bromide (2f)—Radical benzylic bromination of 4-fluoro-3-nitrotoluene yielded the corresponding 4-fluoro-3-nitrobenzyl bromide. The yield of this reaction was 84%, giving yellow solid with a melting point 50–52 °C. ¹H NMR (CDCl₃, 300 MHz): δ 4.49 (s, 2H, CH₂), 7.32(dd, J = 8.7, 1.8 Hz, 1H, Ar-H), 7.66–7.71 (m, 1H, Ar-H), 8.11 (dd, J = 6.9, 2.4 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 233.9488; found 233.9481. Anal. (C₇H₅BrFNO₂) C, H, N.

General Procedure for the Preparation of benzyl alcohols (4). Method B (Scheme 2)

Sodium borohydride (20 mmol) was added in small portions to an ice-cold solution of aldehyde **3** (20 mmol) in dry methanol (100 mL) with stirring. The reaction mixture was left at 0–5 °C for 1 h. After completion of the reaction (TLC monitoring, hexane/ethyl acetate, 9:1 on silica gel plate), the solvent was evaporated, then the chloroform (100 mL) was added to the residue obtained. The organic layer was washed with 5% sodium bicarbonate (50 mL) and water (75 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum to obtain the desired crude product **4**. The pure compound **4** was obtained after recrystallization from benzene/hexane. The following benzyl alcohols **4** were prepared using the above procedure.

4-Methoxy-3-nitrobenzyl alcohol (4a)—Reduction of 4-methoxy-3-nitrobenzaldehyde yielded the corresponding 4-methoxy-3-nitrobenzyl alcohol. The yield of this reaction was 78%, giving yellow solid with a melting point 69–70 °C. ¹H NMR (CDCl₃, 300 MHz): δ 2.65 (br s, 1H, OH), 3.96 (s, 3H, OCH₃), 4.64 (s, 2H, CH₂), 7.12 (d, J = 8.4 Hz, 1H, Ar-H), 7.61 (dd, J = 8.4, 2.1 Hz, 1H, Ar-H), 7.84 (d, J = 2.1 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 184.0532; found 184.0522. Anal. (C₈H₉NO₄) C, H, N.

4-Methoxy-2-nitrobenzyl alcohol (4b)—Reduction of 4-methoxy-2-nitrobenzaldehyde yielded the corresponding 4-methoxy-2-nitrobenzyl alcohol. The yield of this reaction was 73%, giving colorless needles with a melting point 80–82 °C. ¹H NMR (CDCl₃, 400 MHz): δ 2.53 (t, J = 6.8 Hz, 1H, OH), 3.88 (s, 3H, OCH₃), 4.86 (d, J = 6.7 Hz, 2H, CH₂), 7.19 (dd, J = 8.6, 2.7 Hz, 1H, Ar-H), 7.58 (d, J = 8.6 Hz, 1H, Ar-H), 7.60 (d, J = 2.7 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 184.0532; found 184.0523. Anal. (C₈H₉NO₄) C, H, N.

4-Bromo-3-nitrobenzyl alcohol (4c)—Reduction of 4-bromo-3-nitrobenzaldehyde yielded the corresponding 4-bromo-3-nitrobenzyl alcohol. The yield of this reaction was 89%, giving yellow solid with a melting point 60–62 °C. ¹H NMR (CDCl₃, 300 MHz): δ 2.61 (br s, 1H, OH), 4.71 (s, 2H, CH₂), 7.38 (d, J = 8.5 Hz, 1H, Ar-H), 7.67 (d, J = 8.3 Hz, 1H, Ar-H), 7.81 (s, 1H, Ar-H). HRMS: m/z calcd [M + H] 231.9531; found 231.9533. Anal. (C₇H₆BrNO₃) C, H, N.

4-Chloro-3-nitrobenzyl alcohol (4d)—Reduction of 4-chloro-3-nitrobenzaldehyde yielded the corresponding 4-chloro-3-nitrobenzyl alcohol. The yield of this reaction was 81%, giving yellow solid with a melting point 63–65 °C. ¹H NMR (CDCl₃, 300 MHz): δ 2.63 (br s, 1H, OH), 4.49 (s, 2H, CH₂), 7.48 (dd, J = 8.0, 2.0 Hz, 1H, Ar-H), 7.53 (d, J = 8.0 Hz, 1H, Ar-H), 7.85 (d, J = 2.0 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 188.0036; found 188.0031. Anal. (C₇H₆ClNO₃) C, H, N.

4-Chloro-2-nitrobenzyl alcohol (4e)—Reduction of 4-chloro-2-nitrobenzaldehyde yielded the corresponding 4-chloro-2-nitrobenzyl alcohol. The yield of this reaction was 78%, giving pale yellow solid with a melting point 89–91 °C. ¹H NMR (CDCl₃, 500MHz): δ 2.91 (br s, 1H, OH), 4.92 (s, 2H, CH₂), 7.47 (d, J = 8.7 Hz, 1H, Ar-H), 7.72 (dd, J = 8.7, 2.4 Hz, Ar-H), 8.09 (d, J = 2.1 Hz, Ar-H). HRMS: m/z calcd [M + H] 188.0036; found 188.0029. Anal. (C₇H₆ClNO₃) C, H, N.

4-Fluoro-3-nitrobenzyl alcohol (4f)—Reduction of 4-fluoro-3-nitrobenzaldehyde yielded the corresponding 4-fluoro-3-nitrobenzyl alcohol. The yield of this reaction was 91%, giving colorless solid with a melting point 42–44 °C. ¹H NMR (CDCl₃, 300 MHz): δ 2.69 (br s, 1H, OH), 4.76 (s, 2H, CH₂), 7.28 (dd, J = 10.7, 8.6 Hz, 1H, Ar-H), 7.61–7.66 (m, 1H, Ar-H), 8.06 (dd, J = 10.7, 8.6 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 172.0732; found 172.0713. Anal. (C₇H₆FNO₃) C, H, N.

6-Methoxy-3-nitrobenzyl alcohol (4g)—Reduction of 6-methoxy-3-nitrobenzaldehyde yielded the corresponding 6-methoxy-3-nitrobenzyl alcohol. The yield of this reaction was 77%, giving white solid with a melting point 123–125 °C. ¹H NMR (CDCl₃, 300 MHz): δ 2.31 (br s, 1H, OH), 3.97 (s, 3H, OCH₃), 4.73 (d, J = 4.1 Hz, 2H, CH₂), 6.93 (d, J = 9.0 Hz, 1H, Ar-H), 8.19 (dd, J = 9.0, 2.8 Hz, 1H, Ar-H), 8.26 (d, J = 2.8 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 184.0532; found 184.0521. Anal. (C₈H₉NO₄) C, H, N.

General Procedure for the Preparation of benzyl bromides (2). Method B (Scheme 2)

Phosphorous tribromide (24 mmol) was added to a stirred solution of alcohol **4** (20 mmol) and toluene (40 mL) at 40 °C. The solution was heated to 100 °C for 30 min, and after completion of the reaction (TLC monitoring, hexane/ethyl acetate, 9:1 on silica gel plate), the contents cooled to ambient temperature. The liquid was decanted and washed with water (2 × 50 mL) and brine (50 mL). The combined aqueous washes were extracted with ether (2 × 75 mL), and the combined organic fractions were dried and evaporated to give crude residue. The residue was dissolved in ether (100 mL) and washed with water (2 × 50 mL) and brine (50 mL). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to get the crude product **2**, which on silica gel flash chromatography (hexane/ethyl acetate, 9:1) resulted in pure benzyl bromide **2**. The following benzyl bromides **2** were prepared using the above procedure.

4-Methoxy-3-nitrobenzyl bromide (2a)—Bromination of the alcohol **4a** with phosphorous tribromide yielded the corresponding **2a**. The yield of this reaction was 52%. The analytical data are in accord with above method A procedure.

4-Methoxy-2-nitrobenzyl bromide (2b)—Bromination of the alcohol **4b** with phosphorous tribromide yielded the corresponding **2b**. The yield of this reaction was 58%. The analytical data are in accord with above method A procedure.

4-Bromo-3-nitrobenzyl bromide (2c)—Bromination of the alcohol **4c** with phosphorous tribromide yielded the corresponding **2c**. The yield of this reaction was 62%. The analytical data are in accord with above method A procedure.

4-Chloro-3-nitrobenzyl bromide (2d)—Bromination of the alcohol **4d** with phosphorous tribromide yielded the corresponding **2d**. The yield of this reaction was 59%. The analytical data are in accord with above method A procedure.

4-Chloro-2-nitrobenzyl bromide (2e)—Bromination of the alcohol **4e** with phosphorous tribromide yielded the corresponding **2e**. The yield of this reaction was 63%. The analytical data are in accord with above method A procedure.

4-Fluoro-3-nitrobenzyl bromide (2f)—Bromination of the alcohol **4f** with phosphorous tribromide yielded the corresponding **2f**. The yield of this reaction was 54%. The analytical data are in accord with above method A procedure.

6-Methoxy-3-nitrobenzyl bromide (2g)—Bromination of the 6-methoxy-3-nitrobenzyl alcohol **4g** with phosphorous tribromide yielded the corresponding 6-methoxy-3-nitrobenzyl bromide **2g**. The yield of this reaction was 55%, giving white solid with a melting point 76–78 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.97 (s, 3H, OCH₃), 4.73 (s, 2H, CH₂), 6.93 (d, J = 9.0 Hz, 1H, Ar-H), 8.19 (dd, J = 9.0, 2.8 Hz, 1H, Ar-H), 8.26 (d, J = 2.8 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 245.9688; found 245.9674. Anal. (C₈H₈BrNO₃) C, H, N.

General Procedure for the Preparation of benzylthioacetic acids (5). Method A (Scheme 1)

The following benzylthioacetic acids were prepared according to the procedure reported in the literature.⁷

4-Methoxy-3-nitrobenzylthioacetic acid (5a)—Condensation of 4-methoxy-3-nitrobenzyl bromide **2a** with mercaptoacetic acid yielded the corresponding 4-methoxy-3-nitrobenzylthio-acetic acid. The yield of this reaction was 96%, giving pale yellow solid with a melting point 129–133 °C. ¹H NMR (DMSO-d₆, 300 MHz): δ 3.12 (s, 2H, -SCH₂), 3.82 (s, 2H, CH₂S), 3.90 (s, 3H, OCH₃), 7.32 (d, J = 8.4 Hz, 1H, Ar-H), 7.59 (dd, J = 8.7, 2.4 Hz, 1H, Ar-H), 7.82 (d, J = 2.1 Hz, 1H, Ar-H), 12.60 (br s, 1H, COOH). HRMS: m/z calcd [M - H] 256.0358; found 256.0346. Anal. (C₁₀H₁₁NO₅S) C, H, N.

4-Methoxy-2-nitrobenzylthioacetic acid (5b)—Condensation of 4-methoxy-2-nitrobenzyl bromide **2b** with mercaptoacetic acid yielded the corresponding 4-methoxy-2-nitrobenzylthioacetic acid. The yield of this reaction was 86%, giving light yellow solid with a melting point 86–88 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.05 (s, 2H, -SCH₂), 3.81 (s, 3H, OCH₃), 4.11 (s, 2H, CH₂S), 7.04 (dd, J = 8.4, 2.7 Hz, 1H, Ar-H), 7.33 (d, J = 8.7 Hz, 1H, Ar-H), 7.49 (d, J = 2.7 Hz, 1H, Ar-H), 12.41 (br s, 1H, COOH). HRMS: m/z calcd [M - H] 256.0358; found 256.0349. Anal. (C₁₀H₁₁NO₅S) C, H, N.

4-Bromo-3-nitrobenzylthioacetic acid (5c)—Condensation of 4-bromo-3-nitrobenzyl bromide **2c** with mercaptoacetic acid yielded the corresponding 4-bromo-3-nitrobenzylthioacetic acid. The yield of this reaction was 92%, giving yellow solid with a melting point 133–135 °C. ¹H NMR (DMSO-d₆, 300 MHz): δ 3.15 (s, 2H, -SCH₂), 4.05 (s, 2H, CH₂S), 7.51 (d, J = 8.1, Hz, 1H, Ar-H), 7.91 (dd, J = 8.4, 2.1 Hz, 1H, Ar-H), 8.21 (d, J = 1.8 Hz, 1H, Ar-H), 12.60 (br s, 1H, COOH). HRMS: m/z calcd [M - H] 303.9357; found 303.9348. Anal. (C₉H₈BrNO₄S) C, H, N.

4-Chloro-3-nitrobenzylthioacetic acid (5d)—Condensation of 4-chloro-3-nitrobenzyl bromide **2d** with mercaptoacetic acid yielded the corresponding 4-chloro-3-

nitrobenzylthioacetic acid. The yield of this reaction was 90%, giving yellow solid 110–114 °C. ¹H NMR (DMSO-d₆, 300 MHz): δ 3.15 (s, 2H, -SCH₂), 3.86 (s, 2H, CH₂S), 7.42 (d, J = 8.1 Hz, 1H, Ar-H), 7.69 (dd, J = 8.4, 2.1 Hz, 1H, Ar-H), 7.92 (d, J = 1.8 Hz, 1H, Ar-H), 12.70 (br s, 1H, COOH). HRMS: m/z calcd [M - H] 259.9863; found 259.9856. Anal. (C₉H₈ClNO₄S) C, H, N.

4-Chloro-2-nitrobenzylthioacetic acid (5e)—Condensation of 4-chloro-2-nitrobenzyl bromide **2e** with mercaptoacetic acid yielded the corresponding 4-chloro-2-nitrobenzylthioacetic acid. The yield of this reaction was 92%, giving yellow solid with a melting point 103–105 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.13 (s, 2H, -SCH₂), 4.22 (s, 2H, CH₂S), 7.47 (d, J = 8.1 Hz, 1H, Ar-H), 7.57 (dd, J = 8.1, 2.1 Hz, 1H, Ar-H), 8.04 (d, J = 2.1 Hz, 1H, Ar-H), 12.73 (br s, 1H, COOH). HRMS: m/z calcd [M - H] 259.9863; found 259.9851. Anal. (C₉H₈ClNO₄S) C, H, N.

4-Fluoro-3-nitrobenzylthioacetic acid (5f)—Condensation of 4-fluoro-2-nitrobenzyl bromide **2f** with mercaptoacetic acid yielded the corresponding 4-fluoro-3-nitrobenzylthioacetic acid. The yield of this reaction was 88%, giving pale yellow solid with a melting point 74–76 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.12 (s, 2H, -SCH₂), 3.92 (s, 2H, CH₂S), 7.28 (dd, J = 8.4, 2.1 Hz, 1H, Ar-H), 7.61–7.68 (m, 1H, Ar-H), 8.07 (dd, J = 6.9, 2.4 Hz, 1H, Ar-H), 12.61 (br s, 1H, COOH). HRMS: m/z calcd [M - H] 244.0158; found 244.0143. Anal. (C₉H₈FNO₄S) C, H, N.

6-Methoxy-3-nitrobenzylthioacetic acid (5g)—Condensation of 6-methoxy-3-nitrobenzyl bromide **2g** with mercaptoacetic acid yielded the corresponding 6-methoxy-3-nitrobenzylthioacetic acid. The yield of this reaction was 86%, giving pale yellow solid with a melting point 76–78 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.08 (s, 2H, -SCH₂), 3.74 (s, 2H, CH₂S), 3.98 (s, 3H, OCH₃), 7.29 (d, J = 9.0 Hz, 1H, Ar-H), 8.21 (m, 1H, Ar-H), 8.30 (d, J = 3.0 Hz, 1H, Ar-H). HRMS: m/z calcd [M - H] 256.0358; found 256.0349. Anal. (C₁₀H₁₁NO₅S) C, H, N.

General Procedure for the Preparation of benzylsulfonylacetic acids (6). Method A (Scheme 1)

The following benzylsulfonylacetic acids were prepared according to the procedure reported in the literature.⁷

4-Methoxy-3-nitrobenzylsulfonylacetic acid (6a)—Oxidation of 4-methoxy-3-nitrobenzylthioacetic acid **5a** with 30% hydrogen peroxide yielded the corresponding 4-methoxy-3-nitrobenzylsulfonylacetic acid. The yield of this reaction was 51%, giving yellow solid with a melting point 137–139 °C. ¹H NMR (DMSO-d₆, 500 MHz): δ 3.79 (s, 2H, SCH₂), 3.86 (s, 3H, OCH₃), 4.48 (s, 2H, CH₂S), 7.08 (d, J = 9.0 Hz, 1H, Ar-H), 7.59 (dd, J = 8.5, 2.5 Hz, 1H, Ar-H), 7.86 (d, J = 2.5 Hz, 1H, Ar-H), 13.42 (br s, 1H, COOH). HRMS: m/z calcd [M - H] 288.0256; found 288.0251. Anal. (C₁₀H₁₁NO₇S) C, H, N.

4-Methoxy-2-nitrobenzylsulfonylacetic acid (6b)—Oxidation of 4-methoxy-2-nitrobenzylthioacetic acid **5b** with 30% hydrogen peroxide yielded the corresponding 4-

methoxy-2-nitrobenzylsulfonylacetic acid. The yield of this reaction was 63%, giving pale yellow solid with a melting point 158–161 °C. ¹H NMR (DMSO-d₆, 300 MHz): δ 3.87 (s, 3H, OCH₃), 4.27 (s, 2H, -SCH₂), 5.02 (s, 2H, CH₂S), 7.36 (dd, J = 8.4, 2.7 Hz, 1H, Ar-H), 7.53 (d, J = 8.7 Hz, 1H, Ar-H), 7.57 (d, J = 2.4 Hz, 1H, Ar-H), 13.53 (br s, 1H, COOH). HRMS: m/z calcd [M - H] 288.0256; found 288.0244. Anal. (C₁₀H₁₁NO₇S) C, H, N.

4-Bromo-3-nitrobenzylsulfonylacetic acid (6c)—Oxidation of 4-bromo-3-nitrobenzylthioacetic acid **5c** with 30% hydrogen peroxide yielded the corresponding 4-bromo-3-nitrobenzylsulfonylacetic acid. The yield of this reaction was 62%, giving a yellow solid with a melting point 172–174 °C. ¹H NMR (DMSO-d₆, 400 MHz): δ 4.30 (s, 2H, -SCH₂), 4.81 (s, 2H, CH₂S), 7.65 (dd, J = 8.4, 2.1 Hz, 1H, Ar-H), 8.01 (d, J = 8.4 Hz, 1H, Ar-H), 8.08 (d, J = 1.8 Hz, 1H, Ar-H), 13.58 (br s, 1H, COOH). HRMS: m/z calcd [M - H] 335.9256; found 335.9252. Anal. (C₉H₈BrNO₆S) C, H, N.

4-Chloro-3-nitrobenzylsulfonylacetic acid (6d)—Oxidation of 4-chloro-3-nitrobenzylthioacetic acid **5d** with 30% hydrogen peroxide yielded the corresponding 4-chloro-3-nitrobenzylsulfonylacetic acid. The yield of this reaction was 68%, giving yellow solid with a melting point 161–163 °C. ¹H NMR (DMSO-d₆, 300 MHz): δ 4.27 (s, 2H, -SCH₂), 4.77 (s, 2H, CH₂S), 7.61 (dd, J = 8.4, 2.1 Hz, 1H, Ar-H), 7.98 (d, J = 8.4 Hz, 1H, Ar-H), 8.04 (d, J = 1.8 Hz, 1H, Ar-H), 13.60 (br s, 1H, COOH). HRMS: m/z calcd [M - H] 291.9761; found 291.9755. Anal. (C₉H₈ClNO₆S) C, H, N.

4-Chloro-2-nitrobenzylsulfonylacetic acid (6e)—Oxidation of 2-(4-chloro-2-nitrobenzylthioacetic acid **5e** with 30% hydrogen peroxide yielded the corresponding 2-(4-chloro-2-nitrobenzylsulfonylacetic acid. The yield of this reaction was 64%, giving yellow solid with a melting point 113–115 °C. ¹H NMR (DMSO-d₆, 300 MHz): δ 4.36 (s, 2H, -SCH₂), 5.00 (s, 2H, CH₂S), 7.65 (dd, J = 8.4, 3.0 Hz, 1H, Ar-H), 7.80 (d, J = 8.4 Hz, 1H, Ar-H), 8.18 (d, J = 1.8 Hz, 1H, Ar-H), 13.70 (br s, 1H, COOH). HRMS: m/z calcd [M - H] 291.9761; found 291.9753. Anal. (C₉H₈ClNO₆S) C, H, N.

4-Fluoro-3-nitrobenzylsulfonylacetic acid (6f)—Oxidation of 4-fluoro-3-nitrobenzylthioacetic acid **5f** with 30% hydrogen peroxide yielded the corresponding 4-fluoro-3-nitrobenzylsulfonylacetic acid. The yield of this reaction was 69%, giving yellow solid with a melting point 120–122 °C. ¹H NMR (DMSO-d₆, 300 MHz): δ 4.24 (s, 2H, -SCH₂), 4.75 (s, 2H, CH₂S), 7.64 (dd, J = 8.4, 2.7 Hz, 1H, Ar-H), 7.79–7.84 (m, 1H, Ar-H), 8.20 (dd, J = 8.4, 2.1 Hz, 1H, Ar-H), 13.40 (br s, 1H, COOH). HRMS: m/z calcd [M - H] 276.0056; found 276.0046. Anal. (C₉H₈FNO₆S) C, H, N.

6-Methoxy-3-nitrobenzylsulfonylacetic acid (6g)—Oxidation of 6-methoxy-3-nitrobenzylthioacetic acid **5g** with 30% hydrogen peroxide yielded the corresponding 6-methoxy-3-nitrobenzylsulfonylacetic acid. The yield of this reaction was 67%, giving pale yellow solid with a melting point 166–168 °C. ¹H NMR (DMSO-d₆, 300 MHz): δ 3.93 (s, 3H, OCH₃), 4.28 (s, 2H, -SCH₂), 4.79 (s, 2H, CH₂S), 7.30 (d, J = 9.0 Hz, 1H, Ar-H), 8.26–8.28 (m, 1H, Ar-H), 8.31 (d, J = 3.0 Hz, 1H, Ar-H), 13.40 (br s, 1H, COOH). HRMS: m/z calcd [M - H] 288.0256; found 288.0248. Anal. (C₁₀H₁₁NO₇S) C, H, N.

General Procedure for the Preparation of (E)-Styryl benzyl sulfone (**8**). Method A (Scheme 1)

A mixture of benzylsulfonylacetic acid **6** (10 mmol), araldehyde **7** (10 mmol), glacial acetic acid (15 mL), and a catalytic amount of benzylamine (200 μ L) was refluxed for about 2–8 h. After completion of the reaction (TLC monitoring, chloroform 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 successively with saturated sodium bicarbonate, dilute hydrochloric acid, and water. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum to obtain the desired crude product **8**. The crude product was recrystallized in 2-propanol to yield an analytically pure sample of **8**. The following (E)-Styryl benzyl sulfones **8** were prepared using the above procedure.

(E)-2'-Methoxystyryl-4-methoxy-3-nitrobenzylsulfone (8a)—The title compound was obtained from 4-methoxy-3-nitrobenzylsulfonylacetic acid **6a** and 2-methoxybenzaldehyde following the procedure as described in method A. Yield, 56%; yellow solid, mp 169–171 °C. ^1H NMR (CDCl_3 , 300 MHz): δ 3.89 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 4.27 (s, 2H, CH_2), 6.92–6.99 (m, 2H, Ar-H), 7.01 (d, J = 15.6 Hz, 1H, =CH), 7.12 (d, J = 8.7 Hz, 1H, Ar-H), 7.35–7.45 (m, 2H, Ar-H), 7.62 (d, J = 15.6 Hz, 1H, CH=), 7.63 (dd, J = 8.7, 2.4 Hz, 1H, Ar-H), 7.87 (d, J = 2.4 Hz, 1H, Ar-H). HRMS: m/z calcd $[\text{M} + \text{H}]$ 364.0777; found 364.0780. Anal. ($\text{C}_{17}\text{H}_{17}\text{NO}_6\text{S}$) C, H, N.

(E)-2'-Methoxystyryl-4-methoxy-3-aminobenzylsulfone (8b)—(E)-2'-methoxystyryl-4-methoxy-3-nitrobenzylsulfone **8a** (900 mg, 2.5 mmol) was dissolved in acetone:water (40:20 mL) and heated to 50 °C. After 30 min sodium hydrosulfite (8.79 g, 50.0 mmol) was added slowly and maintained temperature at 50 °C for further 30 min. After completion of reaction (TLC monitoring, chloroform on silica gel plate), the contents cooled to room temperature, water was added, and the product was isolated by extraction with ethyl acetate. The organic phase was washed with water (3 X 100 mL), brine (50 mL), dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum to obtain the desired crude product **8b**. The pure compound **8b** was obtained following purification by silica gel flash column chromatography (chloroform). Yield, 51%; white solid, mp 140–142 °C. ^1H NMR (CDCl_3 , 300 MHz): δ 3.78 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 4.15 (s, 2H, CH_2), 6.95–7.02 (m, 2H, Ar-H), 6.98 (d, J = 15.6 Hz, 1H, =CH), 7.12 (d, J = 8.7 Hz, 1H, Ar-H), 7.37–7.45 (m, 2H, Ar-H), 7.62 (d, J = 15.6 Hz, 1H, CH=), 7.63 (dd, J = 8.7, 2.4 Hz, 1H, Ar-H), 7.87 (d, J = 2.4 Hz, 1H, Ar-H). HRMS: m/z calcd $[\text{M} + \text{H}]$ 334.1035; found 334.1066. Anal. ($\text{C}_{17}\text{H}_{19}\text{NO}_4\text{S}$) C, H, N.

(E)-4'-Methoxystyryl-4-methoxy-3-nitrobenzylsulfone (8c)—The title compound was obtained from 4'-methoxy-3-nitrobenzylsulfonylacetic acid **6a** and 4-methoxybenzaldehyde following the procedure as described in method A. Yield, 58%; yellow solid, mp 172–174 °C. ^1H NMR (CDCl_3 , 500 MHz): δ 3.88 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.27 (s, 2H, CH_2), 6.93–6.98 (m, 2H, Ar-H), 6.95 (d, J = 15.5 Hz, 1H, =CH), 7.12 (d, J = 9.0 Hz, 1H, Ar-H), 7.36 (dd, J = 7.5, 1.5 Hz, 1H, Ar-H), 7.42–7.49 (m, 2H, Ar-

H), 7.61 (d, J = 15.5 Hz, 1H, CH=), 7.86 (d, J = 2.0 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 364.0777; found 364.0765. Anal. (C₁₇H₁₇NO₆S) C, H, N.

(E)-4'-Methoxystyryl-4-methoxy-3-aminobenzylsulfone (8d)—The title compound was obtained by the reduction of (E)-4-methoxystyryl-4-methoxy-3-nitrobenzylsulfone **8c** following the procedure as described in compound **8b**. Yield, 49%; yellow solid, mp 152–154 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.89 (s, 3H, OCH₃), 3.99 (s, 3H, OCH₃), 4.27 (s, 2H, CH₂), 6.92–6.99 (m, 2H, Ar-H), 7.01 (d, J = 15.6 Hz, 1H, =CH), 7.12 (d, J = 8.7 Hz, 1H, Ar-H), 7.35–7.45 (m, 2H, Ar-H), 7.62 (d, J = 15.6 Hz, 1H, CH=), 7.63 (dd, J = 8.7, 2.4 Hz, 1H, Ar-H), 7.87 (d, J = 2.4 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 334.1035; found 334.1068. Anal. (C₁₇H₁₉NO₄S) C, H, N.

(E)-2',6'-Dimethoxystyryl-4-methoxy-3-nitrobenzylsulfone (8e)—The title compound was obtained from 4-methoxy-3-nitrobenzylsulfonylacetic acid **6a** and 2,6-dimethoxybenzaldehyde following the procedure as described in method A. Yield, 53%; yellow solid, mp 188–190 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.78 (s, 6H, 2 X OCH₃), 3.90 (s, 3H, OCH₃), 4.17 (s, 2H, CH₂), 6.48 (d, J = 8.7 Hz, 2H, Ar-H), 7.03 (d, J = 8.7 Hz, 1H, Ar-H), 7.13 (d, J = 15.6 Hz, 1H, =CH), 7.26 (t, J = 8.4 Hz, 1H, Ar-H), 7.56 (dd, J = 8.7, 2.4 Hz, 1H, Ar-H), 7.82 (d, J = 2.4 Hz, 1H, Ar-H), 7.84 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 394.0882; found 394.0889. Anal. (C₁₈H₁₉NO₇S) C, H, N.

(E)-2',6'-Dimethoxystyryl-4-methoxy-2-nitrobenzylsulfone (8f)—The title compound was obtained from 4-methoxy-2-nitrobenzylsulfonylacetic acid **6b** and 2,6-dimethoxybenzaldehyde following the procedure as described in method A. Yield, 52%; yellow solid, mp 176–178 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.78 (s, 6H, 2 X OCH₃), 3.80 (s, 3H, OCH₃), 4.74 (s, 2H, CH₂), 6.47 (d, J = 8.7 Hz, 2H, Ar-H), 7.09 (dd, J = 8.4, 2.7 Hz, 1H, Ar-H), 7.16 (d, J = 15.6 Hz, 1H, =CH), 7.25 (t, J = 8.4 Hz, 1H, Ar-H), 7.45 (m, 2H, Ar-H), 7.72 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 394.0882; found 394.0869. Anal. (C₁₈H₁₉NO₇S) C, H, N.

(E)-2',4'-Dimethoxystyryl-4-methoxy-3-aminobenzylsulfone(8g)

Step1: (E)-2',4'-Dimethoxy-styryl-4-methoxy-3-nitrobenzylsulfone: The condensation of 4-methoxy-3-nitrobenzylsulfonylacetic acid **6a** with 2,4-dimethoxybenzaldehyde following the procedure as described in method A resulted in the desired product (E)-2',4'-dimethoxystyryl-4-methoxy-3-nitrobenzylsulfone. Yield, 50%; yellow solid, mp 148–150 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.78 (s, 6H, 2 X OCH₃), 3.90 (s, 3H, OCH₃), 4.17 (s, 2H, CH₂), 6.48 (d, J = 8.7 Hz, 2H, Ar-H), 7.03 (d, J = 8.7 Hz, 1H, Ar-H), 7.13 (d, J = 15.6 Hz, 1H, =CH), 7.26 (t, J = 8.4 Hz, 1H, Ar-H), 7.56 (dd, J = 8.7, 2.4 Hz, 1H, Ar-H), 7.84 (d, J = 2.4 Hz, 1H, Ar-H), 7.82 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 394.0882; found 394.0877. Anal. Calcd. for C₁₈H₁₉NO₇S: C, 54.95%; H, 4.87%; N, 3.56%. Found: C, 55.06%; H, 4.82%; N, 3.43%.

Step 2: (E)-2',4'-Dimethoxystyryl-4-methoxy-3-aminobenzylsulfone (8g): The title compound was obtained by the reduction of (E)-2',4'-dimethoxystyryl-4-methoxy-3-nitrobenzylsulfone following the procedure as described in compound **8b**. Yield, 47%;

yellow solid, mp 136–138 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.86 (s, 6H, 2 X OCH₃), 3.98 (s, 3H, OCH₃), 4.25 (s, 2H, CH₂), 6.46 (d, J = 2.4 Hz, 1H, Ar-H), 6.85 (d, J = 15.3 Hz, 1H, =CH), 7.12 (d, J = 8.7 Hz, 1H, Ar-H), 7.29 (d, J = 8.7 Hz, 1H, Ar-H), 7.51 (d, J = 15.3 Hz, 1H, CH=). 7.63 (dd, J = 8.7, 2.4 Hz, 1H, Ar-H), 7.86 (d, J = 2.4 Hz, 1H, Ar-H). HRMS: m/z [M + H] 364.1140; found 364.1129. Anal. (C₁₈H₂₁NO₅S) C, H, N.

(E)-2',5'-Dimethoxystyryl-4-methoxy-3-aminobenzylsulfone (8h)

Step1: (E)-2',5'-Dimethoxystyryl-4-methoxy-3-nitrobenzylsulfone: The condensation of 4-methoxy-3-nitrobenzylsulfonylacetic acid **6a** with 2,5-dimethoxybenzaldehyde following the procedure as described in method A resulted in the desired product (E)-2',5'-dimethoxystyryl-4-methoxy-3-nitrobenzylsulfone. Yield, 52%; yellow solid, mp 174–175 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.80 (s, 6H, 2 X OCH₃), 3.94 (s, 3H, OCH₃), 4.19 (s, 2H, CH₂), 6.54 (d, J = 8.7 Hz, 2H, Ar-H), 7.13 (d, J = 8.7 Hz, 1H, Ar-H), 7.17 (d, J = 15.6 Hz, 1H, =CH), 7.30 (t, J = 8.4 Hz, 1H, Ar-H), 7.59 (dd, J = 8.7, 2.4 Hz, 1H, Ar-H), 7.78 (d, J = 2.4 Hz, 1H, Ar-H), 7.82 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 394.0882; found 394.0881. Anal. Calcd. for C₁₈H₁₉NO₇S: C, 54.95%; H, 4.87%; N, 3.56%. Found: C, 54.86%; H, 4.75%; N, 3.47%.

Step 2: (E)-2',5'-Dimethoxystyryl-4-methoxy-3-aminobenzylsulfone (8h): The title compound was obtained by the reduction of (E)-2',5'-dimethoxystyryl-4-methoxy-3-nitrobenzylsulfone following the procedure as described in compound **8b**. Yield, 49%; yellow solid, mp 130–132 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.78 (s, 3H, OCH₃), 3.83 (s, 3H, OCH₃), 3.85 (s, 3H, OCH₃), 4.18 (s, 2H, CH₂), 6.68–6.77 (m, 3H, Ar-H), 6.86 (d, J = 9.0 Hz, 2H, Ar-H), 6.91 (d, J = 15.6 Hz, 1H, =CH), 6.93–7.02 (m, 1H, Ar-H), 7.64 (d, J = 15.9 Hz, 1H, CH=). HRMS: m/z [M + H] 364.1140; found 364.1131. Anal. (C₁₈H₂₁NO₅S) C, H, N.

(E)-2',6'-Dimethoxystyryl-4-methoxy-3-aminobenzylsulfone (8i)—The title compound was obtained by the reduction of (E)-2',6'-Dimethoxystyryl-4-methoxy-3-nitrobenzylsulfone **8e** following the procedure as described in compound **8b**. Yield, 48%; pale yellow solid, mp 106–108 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.83 (s, 3H, OCH₃), 3.86 (s, 6H, 2 X OCH₃), 4.16 (s, 2H, CH₂), 6.52–6.59 (m, 2H, Ar-H), 6.71–6.79 (m, 2H, Ar-H), 7.17 (t, J = 8.1 Hz, 1H, Ar-H), 7.23 (d, J = 15.9 Hz, 1H, =CH), 7.32 (t, J = 8.4 Hz, 1H, Ar-H), 7.94 (d, J = 15.9 Hz, 1H, CH=). HRMS: m/z [M + H] 364.1140; found 364.1131. Anal. (C₁₈H₂₁NO₅S) C, H, N.

(E)-2',6'-Dimethoxystyryl-4-methoxy-2-aminobenzylsulfone (8j)—The title compound was obtained by the reduction of (E)-2',6'-Dimethoxystyryl-4-methoxy-2-nitrobenzylsulfone **8f** following the procedure as described in compound **8b**. Yield, 49%; yellow solid, mp 160–164 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.70 (s, 3H, OCH₃), 3.79 (s, 6H, 2 X OCH₃), 4.19 (s, 2H, CH₂), 6.24–6.29 (m, 2H, Ar-H), 6.49 (d, J = 8.4 Hz, 2H, Ar-H), 6.91 (d, J = 8.1 Hz, 1H, Ar-H), 7.23 (d, J = 15.9 Hz, 1H, =CH), 7.29 (t, J = 6.3 Hz, 1H, Ar-H), 7.94 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z [M + H] 364.1140; found 364.1151. Anal. (C₁₈H₂₁NO₅S) C, H, N.

(E)-2',6'-Dimethoxystyryl-6-methoxy-3-aminobenzylsulfone (8k)

Step1: (E)-2',6'-Dimethoxystyryl-6-methoxy-3-nitrobenzylsulfone: The condensation of 6-methoxy-3-nitrobenzylsulfonylacetic acid **6g** with 2,6-dimethoxybenzaldehyde following the procedure as described in method A resulted in the desired product (E)-2',6'-dimethoxystyryl-6-methoxy-3-nitrobenzylsulfone. Yield, 55%; pale yellow solid, mp 189–191 °C. ¹H NMR (CDCl₃, 500 MHz): δ 3.85 (s, 6H, 2 X OCH₃), 3.88 (s, 3H, OCH₃), 4.44 (s, 2H, CH₂), 6.55 (d, J = 8.5 Hz, 2H, Ar-H), 6.93 (d, J = 9.0 Hz, 1H, Ar-H), 7.27 (d, J = 16.0 Hz, 1H, =CH), 7.33 (t, J = 8.5 Hz, 1H, Ar-H), 7.79 (d, J = 16.0 Hz, 1H, CH=), 8.21 (dd, J = 9.0, 3.0 Hz, 1H, Ar-H), 8.31 (d, J = 3.0 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 394.0882; found 394.0891. Anal. Calcd. for C₁₈H₁₉NO₇S: C, 54.95%; H, 4.87%; N, 3.56%. Found: C, 55.10%; H, 4.92%; N, 3.63%.

Step 2: (E)-2',6'-Dimethoxystyryl-6-methoxy-3-aminobenzylsulfone (8k): The title compound was obtained by the reduction of (E)-2',6'-dimethoxystyryl-6-methoxy-3-nitrobenzylsulfone following the procedure as described in compound **8b**. Yield, 47%; light yellow solid, mp 172–174 °C. ¹H NMR (CDCl₃, 500 MHz): δ 3.63 (s, 3H, OCH₃), 3.84 (s, 6H, 2 X OCH₃), 4.33 (s, 2H, CH₂), 6.53 (d, J = 8.5 Hz, 2H, Ar-H), 6.93 (d, J = 9.0 Hz, 1H, Ar-H), 7.27 (d, J = 16.0 Hz, 1H, =CH), 7.33 (t, J = 8.5 Hz, 1H, Ar-H), 7.85 (d, J = 16.0 Hz, 1H, CH=), 8.21 (dd, J = 9.0, 3.0 Hz, 1H, Ar-H), 8.31 (d, J = 3.0 Hz, 1H, Ar-H). HRMS: m/z [M + H] 364.1140; found 364.1134. Anal. (C₁₈H₂₁NO₅S) C, H, N.

(E)-2',4',6'-Trimethoxystyryl-4-methoxy-3-nitrobenzylsulfone (8l)—The title compound was obtained from 4-methoxy-3-nitrobenzylsulfonylacetic acid **6a** and 2,4,6-trimethoxybenzaldehyde following the procedure as described in method A. Yield, 56%; yellow solid, mp 184–186 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.84 (s, 6H, 2 X OCH₃), 3.86 (s, 3H, OCH₃), 3.98 (s, 3H, OCH₃), 4.23 (s, 2H, CH₂), 6.09 (s, 2H, Ar-H), 7.03 (d, J = 15.6 Hz, 1H, =CH), 7.10 (d, J = 8.7 Hz, 1H, Ar-H), 7.63 (dd, J = 8.7, 2.4 Hz, 1H, Ar-H), 7.80 (d, J = 15.6 Hz, 1H, CH=), 7.85 (d, J = 2.1 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 424.0988; found 424.0982. Anal. (C₁₉H₂₁NO₈S) C, H, N.

(E)-2',4',6'-Trimethoxystyryl-4-methoxy-2-nitrobenzylsulfone (8m)—The title compound was obtained from 4-methoxy-2-nitrobenzylsulfonylacetic acid **6b** and 2,4,6-trimethoxybenzaldehyde following the procedure as described in method A. Yield, 54%; yellow solid, mp 158–160 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.84 (s, 6H, 2 X OCH₃), 3.85 (s, 3H, OCH₃), 3.87 (s, 3H, OCH₃), 4.80 (s, 2H, CH₂), 6.10 (s, 2H, Ar-H), 7.05 (d, J = 15.6 Hz, 1H, =CH), 7.16 (dd, J = 8.7, 2.7 Hz, 1H, Ar-H), 7.50–7.53 (m, 2H, Ar-H), 7.70 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 424.0988; found 424.0972. Anal. (C₁₉H₂₁NO₈S) C, H, N.

(E)-2',4',6'-Trimethoxystyryl-4-methoxy-2-aminobenzylsulfone (8n)—The title compound was obtained by the reduction of (E)-2',4',6'-trimethoxystyryl-4-methoxy-2-nitrobenzylsulfone **8m** following the procedure as described in compound **8b**. Yield, 50%; pale yellow solid, mp 147–149 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.78 (s, 3H, OCH₃), 3.85 (s, 6H, 2 X OCH₃), 3.86 (s, 3H, OCH₃), 4.25 (s, 2H, CH₂), 6.11 (s, 2H, Ar-H), 6.32–6.36 (m, 2H, Ar-H), 6.98 (d, J = 8.1 Hz, 1H, Ar-H), 7.13 (d, J = 15.6 Hz, 1H, =CH), 7.93 (d, J =

15.6 Hz, 1H, CH=). HRMS: m/z calcd $[M + H]$ 394.1246; found 394.1261. Anal. ($C_{19}H_{23}NO_6S$) C, H, N.

(E)-2',4',5'-Trimethoxystyryl-4-methoxy-3-aminobenzylsulfone (8o)

Step 1: (E)-2',4',5'-Trimethoxystyryl-4-methoxy-3-nitrobenzylsulfone: The condensation of 4-methoxy-3-nitrobenzylsulfonylacetic acid **6a** with 2,4,5-trimethoxybenzaldehyde following the procedure as described in method A resulted in the desired product (E)-2',4',5'-trimethoxystyryl-4-methoxy-3-nitrobenzylsulfone. Yield, 56%; pale yellow solid, mp 200–202 °C. 1H NMR ($CDCl_3$, 300 MHz): δ 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 4.26 (s, 2H, CH_2), 6.49 (s, 1H, Ar-H), 6.79 (d, J = 15.6 Hz, 1H, =CH) 6.84 (s, 1H, Ar-H), 7.14 (d, J = 8.7 Hz, 1H, Ar-H), 7.56 (d, J = 15.6 Hz, 1H, CH=), 7.63 (dd, J = 8.7, 2.1 Hz, 1H, Ar-H), 7.86 (d, J = 2.1 Hz, 1H, Ar-H). HRMS: m/z calcd $[M + H]$ 424.0988; found 424.1001. Anal. Calcd. for $C_{19}H_{21}NO_8S$: C, 53.89%; H, 5.00%; N, 3.31%. Found: C, 53.76%; H, 4.91%; N, 3.25%.

Step 2: (E)-2',4',5'-Trimethoxystyryl-4-methoxy-3-aminobenzylsulfone (8o): The title compound was obtained by the reduction of (E)-2',4',5'-trimethoxystyryl-4-methoxy-3-nitrobenzylsulfone following the procedure as described in compound **8b**. Yield, 48%; pale yellow solid, mp 112–114 °C. 1H NMR ($CDCl_3$, 300 MHz): δ 3.84 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 4.17 (s, 2H, CH_2), 6.50 (s, 1H, Ar-H), 6.69 (d, J = 2.1 Hz, 1H, Ar-H), 6.73 (dd, J = 8.4, 2.1 Hz, 2H, Ar-H), 6.77 (d, J = 2.1 Hz, 1H, Ar-H), 6.81 (d, J = 15.0 Hz, 1H, =CH), 7.61 (d, J = 15.6 Hz, 1H, =CH). HRMS: m/z calcd $[M + H]$ 394.1246; found 394.1268. Anal. ($C_{19}H_{23}NO_6S$) C, H, N.

(E)-2',4',6'-Trimethoxystyryl-4-methoxy-3-aminobenzylsulfone (8p)—The title compound was obtained by the reduction of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-nitrobenzylsulfone **8l** following the procedure as described in compound **8b**. Yield, 48%; light yellow solid, mp 146–148 °C. 1H NMR ($CDCl_3$, 400 MHz): δ 3.77 (s, 3H, OCH_3), 3.84 (s, 6H, 2 X OCH_3), 3.85 (s, 3H, OCH_3), 4.24 (s, 2H, CH_2), 4.33 (br s, 2H, NH_2), 6.10 (s, 2H, Ar-H), 6.31–6.35 (m, 2H, Ar-H), 6.97 (d, J = 8.3 Hz, 1H, Ar-H), 7.12 (d, J = 15.6 Hz, 1H, =CH), 7.93 (d, J = 15.6 Hz, 1H, CH=). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 163.7, 161.4, 147.6, 136.4, 135.1, 122.8, 121.1, 121.0, 117.2, 110.2, 103.8, 90.3, 61.7, 55.7, 55.5, 55.4. HRMS: m/z calcd $[M + H]$ 394.1246; found 394.1246. Anal. ($C_{19}H_{23}NO_6S$) C, H, N.

(E)-3',4',5'-Trimethoxystyryl-4-methoxy-3-aminobenzylsulfone (8q)

Step 1: (E)-3',4',5'-Trimethoxystyryl-4-methoxy-3-nitrobenzylsulfone: The condensation of 4-methoxy-3-nitrobenzylsulfonylacetic acid **6a** with 3,4,5-trimethoxybenzaldehyde following the procedure as described in method A resulted in the desired product (E)-3',4',5'-trimethoxystyryl-4-methoxy-3-nitrobenzylsulfone. Yield, 52%; pale yellow solid, mp 170–172 °C. 1H NMR ($CDCl_3$, 300 MHz): δ 3.88 (s, 6H, 2 X OCH_3), 3.90 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 4.29 (s, 2H, CH_2), 6.63 (d, J = 15.3 Hz, 1H, =CH), 6.68 (s, 2H, Ar-H), 7.13 (d, J = 8.7 Hz, 1H, Ar-H), 7.39 (d, J = 15.3 Hz, 1H, CH=), 7.62 (dd, J = 8.7, 2.4 Hz, 1H, Ar-H), 7.86 (d, J = 2.4 Hz, 1H, Ar-H). HRMS: m/z calcd $[M + H]$ 424.0988; found 424.0998. Anal. Calcd. for $C_{19}H_{21}NO_8S$: C, 53.89%; H, 5.00%; N, 3.31%. Found: C, 53.71%; H, 4.93%; N, 3.37%.

Step 2: (E)-3',4',5'-Trimethoxystyryl-4-methoxy-3-aminobenzylsulfone (8q): The title compound was obtained by the reduction of (E)-3',4',5'-trimethoxystyryl-4-methoxy-3-nitrobenzylsulfone following the procedure as described in compound **8b**. Yield, 48%; light orange solid, mp 108–110 °C. ¹H NMR (CDCl₃, 400 MHz): δ 3.77 (s, 3H, OCH₃), 3.79 (s, 6H, 2 X OCH₃), 3.82 (s, 3H, OCH₃), 4.11 (s, 2H, CH₂), 6.55 (d, J = 15.4 Hz, 1H, =CH), 6.58 (s, 2H, Ar-H), 6.61 (dd, J = 8.2, 2.1 Hz, 1H, Ar-H), 6.67 (d, J = 8.2 Hz, 1H, Ar-H), 6.70 (d, J = 2.1 Hz, 1H, Ar-H), 7.29 (d, J = 15.4 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 394.1246; found 394.1246. Anal. (C₁₉H₂₃NO₆S) C, H, N.

(E)-2',4',6'-Trimethoxystyryl-6-methoxy-3-aminobenzylsulfone (8r)

Step 1: (E)-2',4',6'-Trimethoxystyryl-6-methoxy-3-nitrobenzylsulfone: The condensation of 6-methoxy-3-nitrobenzylsulfonylacetic acid **6g** with 2,4,6-trimethoxybenzaldehyde following the procedure as described in method A resulted in the desired product (E)-2',4',6'-trimethoxystyryl-6-methoxy-3-nitrobenzylsulfone. Yield, 55%; yellow solid, mp 144–146 °C. ¹H NMR (CDCl₃, 500 MHz): δ 3.83 (s, 6H, 2 X OCH₃), 3.85 (s, 3H, OCH₃), 3.87 (s, 3H, OCH₃), 4.42 (s, 2H, CH₂), 6.08 (s, 2H, Ar-H), 6.92 (d, J = 9.0 Hz, 1H, Ar-H), 7.09 (d, J = 15.5 Hz, 1H, =CH), 7.69 (d, J = 16.0 Hz, 1H, CH=), 8.11 (dd, J = 9.0, 2.5 Hz, 1H, Ar-H), 8.30 (d, J = 2.5 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 424.0988; found 424.0998. Anal. Calcd. for C₁₉H₂₁NO₈S: C, 53.89%; H, 5.00%; N, 3.31%. Found: C, 53.76%; H, 4.94%; N, 3.37%.

Step 2: (E)-2',4',6'-Trimethoxystyryl-6-methoxy-3-aminobenzylsulfone (8r): The title compound was obtained by the reduction of (E)-2',4',6'-trimethoxystyryl-6-methoxy-3-nitrobenzylsulfone following the procedure as described in compound **8b**. Yield, 49%; orange solid, mp 124–128 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.57 (s, 3H, OCH₃), 3.76 (s, 6H, 2 X OCH₃), 3.77 (s, 3H, OCH₃), 4.25 (s, 2H, CH₂), 6.01 (s, 2H, Ar-H), 6.59 (dd, J = 8.2, 2.1 Hz, 2H, Ar-H), 6.77 (d, J = 8.2 Hz, 1H, Ar-H), 7.02 (d, J = 15.9 Hz, 1H, =CH), 7.69 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 394.1246; found 394.1266. Anal. (C₁₉H₂₃NO₆S) C, H, N.

(E)-2',6'-Dimethoxy-4'-hydroxystyryl-4-methoxy-3-nitrobenzylsulfone (8s)—A mixture of 4-methoxy-3-nitrobenzylsulfonylacetic acid **6a** (2.05 g, 5 mmol), 2,6-dimethoxy-4-hydroxybenzaldehyde (1.02 g, 5.5 mmol), benzoic acid (92 mg, 0.75 mmol), and piperidine (55 mg, 0.65 mmol) in toluene (50 mL) was refluxed for 2–4 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 solvent was evaporated and to the residue water was added and extracted with ethylacetate. The organic phase was washed with saturated sodium bicarbonate solution, dilute hydrochloric acid, water and dried over anhydrous sodium sulfate. The organic phase filtered, evaporated the solvent under vacuo yielded a crude product **8s**. The pure compound **8s** was obtained following purification by silica gel flash column chromatography (chloroform). Yield, 48%; light yellow solid, mp 222–224 °C. ¹H NMR (DMSO-d₆, 300 MHz): δ 3.77 (s, 6H, 2 X OCH₃), 3.91 (s, 3H, OCH₃), 4.51 (s, 2H, CH₂), 5.79 (s, 1H, OH), 6.11 (s, 2H, Ar-H), 6.99 (d, J = 15.6 Hz, 1H, =CH), 7.37 (d, J = 9.0 Hz, 1H, Ar-H), 7.48 (d, J = 15.6 Hz, 1H, CH=),

7.62 (dd, $J = 8.7, 2.4$ Hz, 1H, Ar-H), 7.86 (d, $J = 2.1$ Hz, 1H, Ar-H). HRMS: m/z calcd $[M + H]$ 410.08; found 410.09. Anal. ($C_{18}H_{19}NO_8S$) C, H, N.

(E)-2',6'-Dimethoxy-4'-hydroxystyryl-4-methoxy-3-aminobenzylsulfone (8t)—

The title compound was obtained by the reduction of (E)-2',6'-dimethoxy-4'-hydroxystyryl-4-methoxy-3-nitrobenzylsulfone **8s** following the procedure as described in compound **8b**. Yield, 45%; light yellow solid, mp 132–134 °C. 1H NMR ($CDCl_3$, 400 MHz): δ 3.67 (s, 6H, 2 X OCH_3), 3.76 (s, 3H, OCH_3), 4.07 (s, 2H, CH_2), 5.75 (bs, 1H, OH), 5.95 (s, 2H, Ar-H), 6.65 – 6.69 (m, 3H, Ar-H), 6.92 (d, $J = 15.6$ Hz, 1H, =CH), 7.73 (d, $J = 15.6$ Hz, 1H, CH=). HRMS: m/z calcd $[M + H]$ 380.1090; found 380.0184. Anal. ($C_{18}H_{21}NO_6S$) C, H, N.

(E)-2',6'-Dimethoxy-4'-phenoxybutanoic acidstyryl-4-methoxy-3-aminobenzylsulfone (8u)

Step 1: (E)-2',6'-dimethoxy-4'-phenoxybutanoic acidstyryl-4-methoxy-3-nitrobenzylsulfone:

The condensation of 4-methoxy-3-nitrobenzylsulfonylacetic acid **6a** with 4-(4-formyl-3,5-dimethoxyphenoxy) butyric acid following the procedure as described in method A resulted in the desired product (E)-2',6'-dimethoxy-4'-phenoxybutanoic acidstyryl-4-methoxy-3-nitrobenzylsulfone. Yield, 56%; pale yellow solid, mp 198–200 °C. 1H NMR ($CDCl_3$, 400 MHz): δ 2.11–2.18 (m, 2H, CH_2), 2.61 (t, $J = 7.1$ Hz, 2H, CH_2), 3.83 (s, 6H, 2 X OCH_3), 3.97 (s, 3H, OCH_3), 4.08 (t, $J = 6.1$ Hz, 2H, CH_2), 4.23 (s, 2H, CH_2), 6.09 (s, 2H, Ar-H), 7.02 (d, $J = 15.6$ Hz, 1H, =CH), 7.11 (d, $J = 8.7$ Hz, 1H, Ar-H), 7.63 (dd, $J = 8.7, 2.3$ Hz, 1H, Ar-H), 7.78 (d, $J = 15.6$ Hz, 1H, CH=), 7.84 (d, $J = 2.2$ Hz, 1H, Ar-H). HRMS: m/z calcd $[M + H]$ 496.1199; found 496.1984. Anal. Calcd. for $C_{22}H_{25}NO_{10}S$: C, 53.33%; H, 5.09%; N, 2.83%. Found: C, 53.42%; H, 5.01%; N, 2.87%.

Step 2: (E)-2',6'-Dimethoxy-4'-phenoxybutanoic acidstyryl-4-methoxy-3-aminobenzylsulfone (8u):

The title compound was obtained by the reduction of (E)-2',6'-dimethoxy-4'-phenoxybutanoic acidstyryl-4-methoxy-3-nitrobenzylsulfone following the procedure as described in compound **8b**. Yield, 48%; light yellow solid, mp 176–178 °C. 1H NMR ($CDCl_3$, 400 MHz): δ 2.04–2.10 (m, 2H, CH_2), 2.53 (t, $J = 7.1$ Hz, 2H, CH_2), 3.75 (s, 6H, 2 X OCH_3), 3.77 (s, 3H, OCH_3), 4.00 (t, $J = 6.0$ Hz, 2H, CH_2), 4.06 (s, 2H, CH_2), 6.02 (s, 2H, Ar-H), 6.66–6.70 (m, 3H, Ar-H), 6.99 (d, $J = 15.6$ Hz, 1H, =CH), 7.77 (d, $J = 15.6$ Hz, 1H, CH=). HRMS: m/z calcd $[M + H]$ 466.1457; found 466.1443. Anal. ($C_{22}H_{27}NO_8S$) C, H, N.

(E)-2',4',6'-Trimethoxystyryl-4-bromo-3-nitrobenzylsulfone (8v)—

The title compound was obtained from 4-bromo-3-nitrobenzylsulfonylacetic acid **6c** and 2, 4, 6-trimethoxybenzaldehyde following the procedure as described in method A. Yield, 56%; yellow solid, mp 186–188 °C. 1H NMR ($CDCl_3$, 300 MHz): δ 3.84 (s, 6H, 2 X OCH_3), 3.86 (s, 3H, OCH_3), 4.73 (s, 2H, CH_2), 6.10 (s, 2H, Ar-H), 7.03 (d, $J = 15.9$ Hz, 1H, =CH), 7.10 (d, $J = 8.7$ Hz, 1H, Ar-H), 7.63 (dd, $J = 8.7, 2.4$ Hz, 1H, Ar-H), 7.80 (d, $J = 15.6$ Hz, 1H, CH=), 7.85 (d, $J = 2.1$ Hz, 1H, Ar-H). HRMS: m/z calcd $[M + H]$ 471.9987; found 471.9972. Anal. ($C_{18}H_{18}BrNO_7S$) C, H, N.

(E)-2',4',6'-Trimethoxystyryl-4-bromo-3-aminobenzylsulfone (8w)—The title compound was obtained by the reduction of (E)-2',4',6'-trimethoxystyryl-4-bromo-3-nitrobenzylsulfone **8v** following the procedure as described in compound **8b**. Yield, 49%; white solid, mp 162–164 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.80 (s, 6H, 2 X OCH₃), 3.84 (s, 3H, OCH₃), 4.63 (s, 2H, CH₂), 6.01 (s, 2H, Ar-H), 6.70 – 6.77 (m, 2H, Ar-H), 7.06 (d, J = 15.9 Hz, 1H, =CH), 7.27 (s, 1H, Ar-H), 7.85 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 442.0174; found 442.0189. Anal. (C₁₈H₂₀BrNO₅S) C, H, N.

(E)-2',4',6'-Trimethoxystyryl-4-chloro-3-aminobenzylsulfone (8x)

Step 1: (E)-2',4',6'-Trimethoxystyryl-4-chloro-3-nitrobenzylsulfone: The condensation of 4-chloro-3-nitrobenzylsulfonylacetic acid **6d** with 2,4,6-trimethoxybenzaldehyde following the procedure as described in method A resulted in the desired product (E)-2',4',6'-trimethoxystyryl-4-chloro-3-nitrobenzylsulfone. Yield, 51%; pale yellow solid, mp 173–174 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.76 (s, 6H, 2 X OCH₃), 3.78 (s, 3H, OCH₃), 4.06 (s, 2H, CH₂), 6.02 (s, 2H, Ar-H), 6.59 (dd, J = 8.1, 2.1 Hz, 1H, Ar-H), 6.77 (d, J = 2.1 Hz, 1H, Ar-H), 7.00 (d, J = 15.6 Hz, 1H, =CH), 7.13 (d, J = 8.1 Hz, Ar-H), 7.75 (d, J = 15.9 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 428.0489; found 428.0476. Anal. Calcd. for C₁₈H₁₈ClNO₇S: C, 50.53%; H, 4.24%; N, 3.27%. Found: C, 50.72%; H, 4.29%; N, 3.24%.

Step 2: (E)-2',4',6'-Trimethoxystyryl-4-chloro-3-aminobenzylsulfone (8x): The title compound was obtained by the reduction of (E)-2',4',6'-trimethoxystyryl-4-chloro-3-nitrobenzylsulfone following the procedure as described in compound **8b**. Yield, 50%; pale yellow solid, mp 138–140 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.76 (s, 6H, 2 X OCH₃), 3.78 (s, 3H, OCH₃), 4.00 (br s, 2H, NH₂), 4.06 (s, 2H, CH₂), 6.02 (s, 2H, Ar-H), 6.59 (dd, J = 8.1, 2.1 Hz, 1H, Ar-H), 6.77 (d, J = 2.1 Hz, 1H, Ar-H), 6.95 (d, J = 15.6 Hz, 1H, =CH), 7.13 (d, J = 8.1 Hz, Ar-H), 7.75 (d, J = 15.9 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 398.0842; found 398.0848. Anal. (C₁₈H₂₀ClNO₅S) C, H, N.

(E)-2',4',6'-Trimethoxystyryl-4-chloro-2-aminobenzylsulfone (8y)

Step 1: (E)-2',4',6'-Trimethoxystyryl-4-chloro-2-nitrobenzylsulfone: The condensation of 4-chloro-2-nitrobenzylsulfonylacetic acid **6e** with 2,4,6-trimethoxybenzaldehyde following the procedure as described in method A resulted in the desired product (E)-2',4',6'-trimethoxystyryl-4-chloro-2-nitrobenzylsulfone. Yield, 54%; pale yellow solid, mp 206–208 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.79 (s, 6H, 2 X OCH₃), 3.84 (s, 3H, OCH₃), 4.27 (s, 2H, CH₂), 6.10 (s, 2H, Ar-H), 6.59 (dd, J = 8.1, 2.1 Hz, 1H, Ar-H), 6.84 (d, J = 2.1 Hz, 1H, Ar-H), 7.10 (d, J = 15.6 Hz, 1H, =CH), 7.21 (d, J = 8.1 Hz, Ar-H), 7.82 (d, J = 15.9 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 428.0489; found 428.0469. Anal. Calcd. for C₁₈H₁₈ClNO₇S: C, 50.53%; H, 4.24%; N, 3.27%. Found: C, 50.45%; H, 4.20%; N, 3.22%.

Step 2: (E)-2',4',6'-Trimethoxystyryl-4-chloro-2-aminobenzylsulfone (8y): The title compound was obtained by the reduction of (E)-2',4',6'-trimethoxystyryl-4-chloro-2-nitrobenzylsulfone following the procedure as described in compound **8b**. Yield, 48%; yellow solid, mp 184–186 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.86 (s, 6H, 2 X OCH₃), 3.88 (s, 3H, OCH₃), 4.27 (s, 2H, CH₂), 4.50 (br s, 2H, NH₂), 6.12 (s, 2H, Ar-H), 6.74 (dd, J = 8.1, 2.1 Hz, 1H, Ar-H), 6.79 (d, J = 2.1 Hz, 1H, Ar-H), 6.99 (d, J = 8.1 Hz, Ar-H), 7.11 (d, J =

15.9 Hz, 1H, =CH), 7.92 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 398.0842; found 398.0836. Anal. (C₁₈H₂₀ClNO₅S) C, H, N.

(E)-2',4',6'-Trimethoxystyryl-4-fluoro-3-nitrobenzylsulfone (8z)—The title compound was obtained from 4-fluoro-3-nitrobenzylsulfonylacetic acid **6f** and 2,4,6-trimethoxybenzaldehyde following the procedure as described in method A. Yield, 53%; light yellow solid, mp 161–163 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.85 (s, 6H, 2 X OCH₃), 3.87 (s, 3H, OCH₃), 4.28 (s, 2H, CH₂), 6.10 (s, 2H, Ar-H), 7.02 (d, J = 15.6 Hz, 1H, =CH), 7.31 (dd, J = 8.7, 1.8 Hz, 1H, Ar-H), 7.69–7.74 (m, 1H, Ar-H), 7.75 (d, J = 15.3 Hz, 1H, CH=), 8.07 (dd, J = 6.9, 2.1 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 412.0791; found 412.0774. Anal. (C₁₈H₁₈FNO₇S) C, H, N.

(E)-2',4',6'-Trimethoxystyryl-4-fluoro-3-aminobenzylsulfone (8aa)—The title compound was obtained by the reduction of (E)-2',4',6'-trimethoxystyryl-4-fluoro-3-nitrobenzylsulfone **8z** following the procedure as described in compound **8b**. Yield, 46%; pale yellow solid, mp 129–131 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.76 (s, 6H, 2 X OCH₃), 3.78 (s, 3H, OCH₃), 4.09 (s, 2H, CH₂), 6.02 (s, 2H, Ar-H), 6.57–6.62 (m, 1H, Ar-H), 6.80 (dd, J = 8.4, 2.1 Hz, 1H, Ar-H), 6.85 (dd, J = 8.4, 2.7 Hz, 1H, Ar-H), 6.96 (d, J = 15.9 Hz, 1H, =CH), 7.27 (s, 1H, Ar-H), 7.76 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 382.1087; found 382.1105. Anal. (C₁₈H₂₀FNO₅S) C, H, N.

(E)-2',4',6'-Trifluorostyryl-4-methoxy-3-aminobenzylsulfone (8ab)

Step 1: (E)-2',4',6'-Trifluorostyryl-4-methoxy-3-nitrobenzylsulfone: The condensation of 4-methoxy-3-nitrobenzylsulfonylacetic acid **6a** with 2,4,6-trifluorobenzaldehyde following the procedure as described in method A resulted in the desired product (E)-2',4',6'-trifluorostyryl-4-methoxy-3-nitrobenzylsulfone. Yield, 52%; yellow solid, mp 141–143 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.89 (s, 3H, OCH₃), 4.28 (s, 2H, CH₂), 6.20 (s, 2H, Ar-H), 6.80–6.90 (m, 2H, Ar-H), 6.97–7.05 (m, 1H, Ar-H), 7.10 (d, J = 15.9 Hz, 1H, =CH), 7.62 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 388.0388; found 388.0379. Anal. Calcd. for C₁₆H₁₂F₃NO₅S: C, 49.61%; H, 3.12%; N, 3.62%. Found: C, 49.75%; H, 3.17%; N, 3.68%.

Step 2: (E)-2',4',6'-Trifluorostyryl-4-methoxy-3-aminobenzylsulfone (8ab): The title compound was obtained by the reduction of (E)-2',4',6'-trifluorostyryl-4-methoxy-3-nitrobenzylsulfone following the procedure as described in compound **8b**. Yield, 48%; light yellow solid, mp 110–112 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.86 (s, 3H, OCH₃), 4.18 (s, 2H, CH₂), 6.67–6.83 (m, 5H, Ar-H), 7.01 (d, J = 16.2 Hz, 1H, =CH), 7.50 (d, J = 15.9 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 358.0646; found 358.0637. Anal. (C₁₆H₁₄F₃NO₃S) C, H, N.

(E)-2',4',5'-Trifluorostyryl-4-methoxy-3-aminobenzylsulfone (8ac)

Step 1: (E)-2',4',5'-Trifluorostyryl-4-methoxy-3-nitrobenzylsulfone: The condensation of 4-methoxy-3-nitrobenzylsulfonylacetic acid **6a** with 2,4,5-trifluorobenzaldehyde following the procedure as described in method A resulted in the desired product (E)-2',4',5'-trifluorostyryl-4-methoxy-3-nitrobenzylsulfone. Yield, 51%; pale yellow solid, mp 161–163

°C. ¹H NMR (CDCl₃, 300 MHz): δ 3.91 (s, 3H, OCH₃), 4.19 (s, 2H, CH₂), 6.67 (dd, J = 8.1, 2.1 Hz, 1H, Ar-H), 6.74–6.77 (m, 2H, Ar-H), 6.80 (d, J = 15.6 Hz, 1H, =CH), 6.97–7.05 (m, 1H, Ar-H), 7.17–7.24 (m, 1H, Ar-H), 7.43 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 388.0388; found 388.0394. Anal. Calcd. for C₁₆H₁₂F₃NO₅S: C, 49.61%; H, 3.12%; N, 3.62%. Found: C, 49.50%; H, 3.16%; N, 3.69%.

Step 2: (E)-2',4',5'-Trifluorostyryl-4-methoxy-3-aminobenzylsulfone (8ac): The title compound was obtained by the reduction of (E)-2',4',5'-trifluorostyryl-4-methoxy-3-nitrobenzylsulfone following the procedure as described in compound **8b**. Yield, 46%; white solid, mp 132–134 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.91 (s, 3H, OCH₃), 4.19 (s, 2H, CH₂), 6.67 (dd, J = 8.1, 2.1 Hz, 1H, Ar-H), 6.74–6.77 (m, 2H, Ar-H), 6.80 (d, J = 15.6 Hz, 1H, =CH), 6.97–7.05 (m, 1H, Ar-H), 7.17–7.24 (m, 1H, Ar-H), 7.43 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 358.0646; found 358.0627. Anal. (C₁₆H₁₄F₃NO₃S) C, H, N.

Preparation of 3-Nitro-4-methoxybenzyl mercaptan (9). (Scheme 4)

Step1: 3-Nitro-4-methoxybenzylisothiuronium salt (12)—A solution of 3-nitro-4-methoxybenzyl bromide **2a** (10.0 g, 41 mmol) and thiourea (10.0 g, 131 mmol) in 50 mL water was heated under reflux for 2 h. The reaction mixture was cooled, stirred at room temperature for 2 h and filtered the solid. The resulted dried product used in next step without further purification. The yield of this reaction was 90%, giving a white solid with a melting point 174–176 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.91 (s, 3H, OCH₃), 4.51 (s, 2H, CH₂), 7.39 (d, J = 8.7 Hz, 1H, Ar-H), 7.72 (dd, J = 8.7, 2.4 Hz, 1H, Ar-H), 7.98 (d, J = 2.1 Hz, 1H, Ar-H), 9.12 (br s, 4H, 2 X NH₂). HRMS: m/z calcd [M + H] 323.9939; found 323.9929. Anal. (C₉H₁₄ BrN₃O₃S) C, H, N.

Step 2: 3-Nitro-4-methoxybenzyl mercaptan (9)—The isothiuronium salt **12** was decomposed by boiling several times with concentrated ammonia and hexane (100 mL, 15:85). Concentration of the combined hexane extracts provided crude **9**. The pure compound **9** was obtained on silica gel flash column chromatography (hexane/ethyl acetate, 4:1). The yield of this reaction was 55%, giving a yellow solid with a melting point 48–49 °C. ¹H NMR (CDCl₃, 300 MHz): δ 1.74 (t, J = 7.5 Hz, SH), 3.67 (d, J = 7.5 Hz, 2H, CH₂), 3.89 (s, 3H, OCH₃), 6.98 (d, J = 8.4 Hz, 1H, Ar-H), 7.45 (dd, J = 8.4, 2.1 Hz, 1H, Ar-H), 7.76 (d, J = 2.4 Hz, 1H, Ar-H). HRMS: m/z calcd [M - H] 198.0303; found 198.0320. Anal. (C₈H₉NO₃S) C, H, N.

General Procedure for the Preparation of (E)-Styryl benzyl sulfone (8). Method B (Scheme 3). Preparation of 4'-Methoxyphenacyl 3-nitro-4-methoxybenzyl sulfide (11)

To a cooled solution of sodium hydroxide (100 mmol) in absolute methanol (50 mL), taken in a 250 mL round-bottomed flask, 3-nitro-4-methoxybenzyl mercaptan **9** (100 mmol) was added slowly and the reaction mixture was stirred for 5 minutes. An appropriate 4-methoxyphenacyl bromide **10** (100 mmol) was added in portions to the contents of the flask and stirred for 2 h. After completion of the reaction (TLC, monitoring, hexane/ethylacetate, 8:2 on silica gel plate), the contents of the flask were poured into crushed ice and the compound formed was washed with ice-cold water and dried to get 4'-methoxyphenacyl-3-

nitro-4-methoxybenzyl sulfide **11**. The yield of this reaction was 95%, giving a yellow solid with a melting point 87–89 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.55 (s, 2H, CH₂), 3.67 (s, 2H, CH₂), 3.81 (s, 3H, OCH₃), 3.88 (s, 3H, OCH₃), 6.87 (d, J = 9.0 Hz, 2H, Ar-H), 6.96 (d, J = 8.4 Hz, 2H, Ar-H), 7.49 (dd, J = 8.4, 2.1 Hz, 1H, Ar-H), 7.79 (d, J = 2.4 Hz, 1H, Ar-H), 7.85 (d, J = 8.7 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 348.0827; found 348.0811. Anal. (C₁₇H₁₇NO₅S) C, H, N.

Preparation of 4'-Methoxyphenacyl-3-nitro-4-methoxybenzyl sulfone (**13**)

The crude 4'-methoxyphenacyl-3-nitro-4-methoxybenzyl sulfide **11** (50 mmol) in glacial acetic acid (100 mL) was taken in a 250 mL round-bottomed 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 4'-Methoxyphenacyl-3-nitro-4-methoxybenzyl sulfone (**13**). Yield, 76%; white solid, mp 172–174 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.83 (s, 3H, OCH₃), 3.91 (s, 3H, OCH₃), 4.31 (s, 2H, CH₂), 4.45 (s, 2H, CH₂), 6.87 (d, J = 9.0 Hz, 2H, Ar-H), 7.06 (d, J = 8.7 Hz, 2H, Ar-H), 7.67 (dd, J = 8.7, 2.1 Hz, 1H, Ar-H), 7.90 (d, J = 9.0 Hz, 1H, Ar-H), 7.98 (d, J = 2.1 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 380.0726; found 380.0709. Anal. (C₁₇H₁₇NO₇S) C, H, N.

Preparation of 2-(3-Nitro-4-methoxybenzylsulfonyl)-1-(4'-methoxyphenyl)ethanol (**14**)

To anhydrous tetrahydrofuran (THF) solution (20 mL) of 4-Methoxyphenacyl benzyl sulfone **13** (10 mmol) maintained at 0 °C, was added NaBH₄ (10 mmol) slowly under N₂ atmosphere. The reaction mixture was maintained at 0 °C for 1 h. After completion of the reaction monitored by TLC (hexane/ethylacetate, 8:2 on silica gel plate), the contents were poured on to crushed ice. The solid that separated out was filtered, washed with water and dried under vacuum to yield **14**. Yield, 56%; white solid, mp 152–154 °C. ¹H NMR (DMSO-d₆, 300 MHz): δ 3.43 (dd, J = 9.9, 4.6 Hz, 2H, CH-CH₂), 3.73 (s, 3H, OCH₃), 3.94 (s, 3H, OCH₃), 4.58 (dd, J = 15.7, 13.6 Hz, 2H, SO₂CH₂), 5.05 (m, 1H, CHOH), 6.02 (d, J = 4.2 Hz, 1H, CH), 6.90 (d, J = 8.6 Hz, 2H, Ar-H), 7.32 (d, J = 8.6 Hz, 2H, Ar-H), 7.42 (d, J = 9.0 Hz, 1H, Ar-H), 7.69 (dd, J = 8.7, 1.8 Hz, 1H, Ar-H), 7.93 (d, J = 1.8 Hz, 1H, Ar-H). HRMS: m/z calcd [M+H] 382.0882; found 382.0916. Anal. (C₁₇H₁₉NO₇S) C, H, N.

Preparation of (E)-4'-Methoxystyryl-3-nitro-4-methoxybenzyl sulfone (**8c**)

p-Toluenesulfonic acid (1 mmol) was added in one portion to a mixture of 2-(3-Nitro-4-methoxybenzylsulfonyl)-1-(4'-methoxyphenyl)ethanol **14** (5 mmol) in anhydrous toluene (25 mL) at room temperature and under N₂ atmosphere. The temperature was raised to 120 °C, and the mixture was refluxed for 2–4 h using Dean-Stark water separator. After completion of the reaction monitored by TLC (chloroform, on silica gel plate), 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 Na₂SO₄, filtered and the

solvent was evaporated under reduced pressure to afford crude product, which on recrystallization in 2-propanol afforded the desired product **8c**. Yield: 65%; white solid, 184–186 °C. Analytical data is same as **8c** obtained by method A.

General Procedure for the Preparation of 2,4,6-trimethoxyphenyl acetylene (**15**, Scheme 6)

Step 1: 2-(2',2'-dibromovinyl)-1,3,5-trimethoxybenzene (19**)**—To a solution of 2,4,6-trimethoxybenzaldehyde **18** (5.0 g, 25.5 mmol), and triphenyl phosphine (13.37 g, 51.0 mmol) in anhydrous dichloromethane (60 mL) was added a solution of tetrabromomethane (9.8 g, 30.0 mmol) in dichloromethane (10 mL), keeping the temperature kept below 5 °C. The reaction mixture was stirred for additional 30 min. After completion of the reaction monitored by TLC (hexane/ethylacetate, 9:1 on silica gel plate), the reaction mixture was filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (hexane/ethylacetate, 9:1), resulted pure **19**. The yield of this reaction was 72%, giving a white solid with a melting point 128–130 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.82 (s, 6H, 2 X OCH₃), 3.83 (s, 3H, OCH₃), 6.12 (s, 2H, Ar-H), 7.19 (s, 1H, CH=). HRMS: m/z calcd [M +H] 352.9133; found 352.9112. Anal. (C₁₁H₁₂Br₂O₃) C, H.

Step 2: 2,4,6-Trimethoxyphenyl acetylene (15**)**—A solution of 2-(2',2'-dibromovinyl)-1,3,5-trimethoxybenzene **19** (7.42 g, 21.3 mmol) in dry tetrahydrofuran (135 mL) was cooled to –78 °C, and slowly added n-butyllithium (18.0 mL, 45.0 mmol). The mixture was stirred for further 15 min at –78 °C. After completion of the reaction monitored by TLC (hexane/ethylacetate, 9:1 on silica gel plate), water added (60 mL) and extracted with ethyl acetate (2 × 100 mL). The organic phase was washed with water, dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo* and the residue was purified by flash column chromatography (hexane/ethyl acetate, 9:1) resulted pure **15**. The yield of this reaction was 67%, giving a white solid with a melting point 119–122 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.43 (s, 1H, CH), 3.77 (s, 3H, OCH₃), 3.82 (s, 6H, 2 X OCH₃), 6.04 (s, 2H, Ar-H). HRMS: m/z calcd [M +H] 193.0786; found 193.0764. Anal. (C₁₁H₁₂O₃) C, H.

Synthesis of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-nitrobenzyl sulfide (**17**) and (Z)-2',4',6'-trimethoxystyryl-4-methoxy-3-nitrobenzyl sulfide (**16**). (Scheme 5)

A hexane solution of triethyl borane (10.5 mL, 10.1 mmol) was added to a solution of 2,4,6-trimethoxyphenyl acetylene **15** (5.0 g, 10.1 mmol) and 4-methoxy-3-nitrobenzyl thiol **9a** (2.49g, 12.12 mmol) in benzene (100 mL) at 25 °C under nitrogen atmosphere. The resulting mixture was stirred for 2 h at 25 °C. After completion of the reaction monitored by TLC (hexane/ethylacetate, 9:1 on silica gel plate), the reaction mixture was quenched with 1 molar ammonium chloride solution and extracted with ethyl acetate. The organic phase was washed with water, dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo* and the residue was purified by column chromatography (hexane/ethyl acetate, 9:1 and with gradual increasing in polarity) resulted as a stereo isomeric mixture (E/Z = 60/40).

(E)-2',4',6'-Trimethoxystyryl-4-methoxy-3-nitrobenzyl sulfide (17**)**—The yield of this reaction was 60%, giving a white solid with a melting point 121–123 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.80 (s, 6H, 2 X OCH₃), 3.81 (s, 3H, OCH₃), 3.93 (s, 2H, CH₂S), 3.96

(s, 3H, OCH₃), 6.10 (s, 2H, Ar-H), 6.83 (d, J = 15.6 Hz, 1H, CH=), 7.00 (d, J = 15.6 Hz, 1H, =CH), 7.05 (d, J = 8.7 Hz, 1H, Ar-H), 7.59 (dd, J = 8.7, 2.4 Hz, 1H, Ar-H), 7.80 (d, J = 2.4 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 392.1090; found 392.1084. Anal. (C₁₉H₂₁NO₆S) C, H, N.

(Z)-2',4',6'-Trimethoxystyryl-4-methoxy-3-nitrobenzyl sulfide (16)—The yield of this reaction was 40%, giving a white solid with a melting point 102–104 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.75 (s, 12H, 4 X OCH₃), 3.86 (s, 2H, CH₂S), 6.06 (s, 2H, Ar-H), 6.14 (d, J = 10.2 Hz, 1H, CH=), 6.49 (d, J = 10.5 Hz, 1H, =CH), 6.94 (d, J = 8.4 Hz, 1H, Ar-H), 7.43 (dd, J = 8.7, 2.1 Hz, 1H, Ar-H), 7.75 (d, J = 1.8 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 392.1090; found 392.1084. Anal. (C₁₉H₂₁NO₆S) C, H, N.

Synthesis of (E)-2',4',6'-Trimethoxystyryl-4-methoxy-3-aminobenzyl sulfide (24)

The title compound was obtained by the reduction of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-nitrobenzylsulfide **17** following the procedure as described in compound **8b**. Yield, 43%; pale yellow solid, mp 106–108 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.73 (s, 6H, 2 X OCH₃), 3.76 (s, 6H, 2 X OCH₃), 3.80 (s, 2H, CH₂S), 6.03 (s, 2H, Ar-H), 6.63–6.71 (m, 2H, Ar-H), 6.74 (d, J = 15.6 Hz, 1H, CH=), 7.01 (d, J = 15.9 Hz, 1H, =CH), 7.30 (s, 1H, Ar-H). HRMS: m/z calcd [M + H] 362.1348; found 362.1339. Anal. (C₁₉H₂₃NO₄S) C, H, N.

Synthesis of (E)-2',4',6'-Trimethoxystyryl-4-methoxy-3-nitrobenzyl sulfoxide (20)

A aqueous 30% hydrogen peroxide (0.56 mL, 5.2 mmol) was added to a stirred solution of (E)-2',4',6'-Trimethoxystyryl-4-methoxy-3-nitrobenzyl sulfide **17** (1.0 g, 2.6 mmol) in 1,1,1,3,3,3-hexafluoro-2-propanol (10 mL) at 25 °C. The reaction was monitored by TLC. After the complete disappearance of the sulfide (2 h), the excess hydrogen peroxide was quenched with saturated sodium sulfite (Na₂SO₃) solution (5.0 mL) and the fluoruous organic phase containing the sulfoxide was separated. After removal of the solvent under vacuo, sulfoxide **20** was obtained as a semisolid. Flash column chromatography (chloroform) gave pure **20**. The yield of this reaction was 91%, giving a white solid with a melting point 148–150 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.75 (s, 6H, 2 X OCH₃), 3.77 (s, 3H, OCH₃), 3.89 (s, 3H, OCH₃), 3.96–4.00 (d, J = Hz, 2H, CH₂S), 6.01 (s, 2H, Ar-H), 6.96 (d, J = 15.9 Hz, 1H, CH=), 7.02 (d, J = 8.7 Hz, 1H, Ar-H), 7.36 (d, J = 15.6 Hz, 1H, =CH), 7.46 (dd, J = 8.7, 2.1 Hz, 1H, Ar-H), 7.71 (d, J = 2.1 Hz, 1H, Ar-H). HRMS: m/z calcd [M + H] 408.1039; found 408.1022. Anal. (C₁₉H₂₁NO₇S) C, H, N.

Synthesis of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-aminobenzyl sulfoxide (21)

The title compound was obtained by the reduction of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-nitrobenzylsulfoxide **20** following the procedure as described in compound **8b**. Yield, 46%; pale yellow solid, mp 131–133 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.77 (br s, 12H, 4 X OCH₃), 3.81 (br s, 2H, NH₂), 3.92 (d, J = 12.6 Hz, 2H, CH₂S), 6.04 (s, 2H, Ar-H), 6.60–6.69 (m, 3H, Ar-H), 7.08 (d, J = 15.6 Hz, 1H, CH=), 7.43 (d, J = 15.6 Hz, 1H, =CH). HRMS: m/z calcd [M + H] 378.1297; found 378.1280. Anal. (C₁₉H₂₃NO₅S) C, H, N.

Synthesis of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-nitrobenzyl sulfone (22 & 8l)

The title compound was obtained by the oxidation of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-nitrobenzyl sulfoxide **20** according to the procedure reported in the literature.⁷ The yield of this reaction was 62%; yellow solid, mp 184–186 °C. The analytical data are in accord with **8l**.

Synthesis of (E)-2',4',6'-Trimethoxystyryl-4-methoxy-3-aminobenzyl sulfone (23 & 8p)

Method A—The title compound was obtained by the reduction of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-nitrobenzyl sulfone **22** following the procedure as described in compound **8b**. The yield of this reaction was 52%; light yellow solid, mp 146–148 °C. The analytical data are in accord with **8p**.

Method B—The title compound was obtained by the oxidation of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-aminobenzyl sulfide **24**. To a solution of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-aminobenzyl sulfide **24** (903 mg, 2.5 mmol) in anhydrous dichloromethane (20 mL) at 0 °C was added m-chloroperoxybenzoic acid (1.29 g, 7.5 mmol) slowly under nitrogen atmosphere and the resulting mixture was stirred at 0 °C to room temperature for 3 h. After completion of the reaction (TLC monitoring, chloroform on silica gel plate), 10% sodium hydrogen carbonate solution (25 mL) was added slowly and stirred for 10 min and separated the aqueous layer. The organic layer washed with water (2 X 25 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (chloroform) to obtain the title compound **24**. The yield of this reaction was 64%; light yellow solid, mp 146–148 °C. The analytical data are in accord with **8p**.

Synthesis of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-aminobenzyl sulfoxide (21, Scheme 7)

Step 1: 4-Methoxy-3-nitrobenzyl sulfinylacetic acid (25)—A vigorously stirred solution of sodium hydroxide (0.58 g, 14.5 mmol) and deionized water (30 mL) was treated with 4-methoxy-3-nitrobenzylthioacetic acid **5a** (3.30 g, 12.1 mmol). The resulting suspension was stirred at ambient temperature for 10 min. To this was added sodium bicarbonate (8.00 g, 95 mmol) and acetone (10 mL) and cooled to 0 °C. The oxone® solution (4.85 g in 20 mL of 4×10^{-4} M EDTA) was added over 10 min keeping the reaction below 5 °C. The suspension was stirred for 5 min and immediately quenched at 2 °C with sodium bisulfate (3 g in 6 mL of deionized water) and stirred for 15 min. Ethyl acetate (75 mL) was added and the solution was acidified with 6N (aq) HCl (18 mL). The aqueous phase was isolated, treated with sodium chloride (15.0 g), and re-extracted with ethyl acetate (75 mL). The organic layers were combined and washed with deionized water (50 mL), washed with brine (30 mL), dried over anhydrous sodium sulfate, filtered and concentrated under vacuo. The crystals formed were dried under vacuum to afford pure **25**. The yield of this reaction was 89%, giving a white solid with a melting point 122–124 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.42 (s, 2H, -SCH₂), 3.90 (s, 3H, OCH₃), 4.02 (s, 2H, CH₂S), 7.42 (d, J = 8.4 Hz, 1H, Ar-H), 7.69 (dd, J = 8.7, 2.4 Hz, 1H, Ar-H), 7.92 (d, J = 2.1 Hz, 1H, Ar-H), 12.90 (br s, 1H, COOH). HRMS: m/z calcd [M - H] 272.0307; found

272.0300. Anal.Calcd. for C₁₀H₁₁NO₆S: C, 43.95%; H, 4.06%; N, 5.13%. Found: C, 44.06%; H, 4.09%; N, 5.17%.

Step 2: (E)-2',4',6'-Trimethoxystyryl-4-methoxy-3-nitrobenzyl sulfoxide (20)—

The title compound was obtained from 4-methoxy-3-nitrobenzylsulfinylacetic acid **25** and 2,4,6-trimethoxybenzaldehyde following the procedure as described in method A and scheme 1. The yield of this reaction was 48%. The analytical data are in accord with **20** as reported in scheme 5.

Step 3: (E)-2',4',6'-Trimethoxystyryl-4-methoxy-3-aminobenzyl sulfoxide (21)

—The title compound was obtained by the reduction of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-nitro-benzylsulfoxide **20** following the procedure as described in compound **8b**. The yield of this reaction was 42%. The analytical data are in accord with **21** as reported in scheme 5.

General Procedure for the Preparation of Amino esters (26, Scheme 8)

Sodium acetate (32.8 g, 400 mmol) was dissolved in ethanol (200 mL). Methyl 2-bromoacetate (61.1 g, 400 mmol) was added to the above solution and refluxed for 10 min. To the cooled reaction mixture compound (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-aminobenzylsulfone **8p** or (E)-3',4',5'-trimethoxystyryl-4-methoxy-3-aminobenzylsulfone **8q** (39.35 g, 100 mmol) was added and then refluxed for 48 h. After completion of the reaction monitored by TLC (chloroform/methanol, 9:1 on silica gel plate), the reaction mixture was concentrated under vacuum and poured into ice water. The solid formed was filtered, washed with water and dried under vacuum. The crude product on purification from ethanol resulted analytical pure product **26**. The following amino esters were prepared using the above procedure.

(E)-Methyl 2-(2-methoxy-5-((2',4',6'-

trimethoxystyrylsulfonyl)methyl)phenylamino)-acetate (26a)—Sodium acetate (3.28 g, 39 mmol) was dissolved in methanol (20 mL). Methyl 2-bromoacetate (6.11 g, 40 mmol) was added to the above solution and refluxed for 10 min. To the cooled reaction mixture compound (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-aminobenzylsulfone **8p** (3.95 g, 10 mmol) was added and then reflux for 4–6 h. The reaction mixture was concentrated under vacuum and poured into ice water. The formed precipitate was filtered, washed with water and dried under vacuum. The crude product on recrystallization from ethanol resulted pure product **26a**. Yield, 70%; white solid, mp 150–152 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.69 (s, 3H, OCH₃), 3.75 (s, 6H, 2 X OCH₃), 3.78 (s, 3H, OCH₃), 3.79 (s, 3H, OCH₃), 3.81 (d, J = 5.4 Hz, 2H, CH₂), 4.09 (s, 2H, CH₂), 4.74 (t, J = 5.4 Hz, 1H, NH), 6.01 (s, 2H, Ar-H), 6.41 (d, J = 1.8 Hz, 1H, Ar-H), 6.61–6.68 (m, 2H, Ar-H), 6.97 (d, J = 15.6 Hz, 1H, =CH), 7.73 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 466.1457; found 466.1446. Anal. (C₂₂H₂₇NO₈S) C, H, N.

(E)-Methyl 2-(2-methoxy-5-((3',4',5'-

trimethoxystyrylsulfonyl)methyl)phenylamino)-acetate (26b)—The title compound was obtained by the alkylation of (E)-3',4',5'-trimethoxystyryl-4-methoxy-3-

aminobenzylsulfone **8q** with methyl 2-bromoacetate following the procedure as described in compound **26**. Yield, 63%; light brown solid, mp 82–84 °C. ¹H NMR (CDCl₃, 400 MHz): δ 3.67 (s, 3H, OCH₃), 3.79 (s, 3H, OCH₃), 3.81 (s, 6H, 2 X OCH₃), 3.82 (s, 3H, OCH₃), 3.86 (d, J = 5.4 Hz, 2H, CH₂), 4.14 (s, 2H, CH₂), 4.53 (t, J = 5.4 Hz, 1H, NH), 6.52 (d, J = 15.5 Hz, 1H, =CH), 6.57 (s, 2H, Ar-H), 6.62 (dd, J = 8.2, 2.1 Hz, 1H, Ar-H), 6.67 (d, J = 8.2 Hz, 1H, Ar-H), 6.70 (d, J = 2.1 Hz, 1H, Ar-H), 7.24 (d, J = 15.4 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 466.1457; found 466.1465. Anal. (C₂₂H₂₇NO₈S) C, H, N.

(E)-Ethyl 3-(2-methoxy-5-((2',4',6'-

trimethoxystyrylsulfonyl)methyl)phenylamino)propanoate (26c)—The title compound was obtained by the alkylation of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-aminobenzylsulfone **8p** with ethyl 3-bromopropionate following the procedure as described in compound **26**. Yield, 59%; light green solid, mp 72–74 °C. ¹H NMR (CDCl₃, 300 MHz): δ 1.26 (t, J = 7.2 Hz, 3H, CH₃), 2.58 (t, J = 6.3 Hz, 2H, CH₂), 3.41 (t, J = 6.6 Hz, 2H, CH₂), 3.82 (s, 9H, 3 X OCH₃), 3.84 (s, 3H, OCH₃), 4.04–4.10 (m, 2H, OCH₂), 4.17 (s, 2H, CH₂), 5.06 (t, J = 5.4 Hz, 1H, NH), 6.09 (s, 2H, Ar-H), 6.61–6.64 (m, 1H, Ar-H), 6.66–6.71 (m, 2H, Ar-H), 7.06 (d, J = 15.6 Hz, 1H, =CH), 7.84 (d, J = 15.9 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 480.1614; found 480.1647. Anal. (C₂₃H₂₉NO₈S) C, H, N.

(E)-Methyl 2-(2-methoxy-5-((2',4',6'-

trimethoxystyrylsulfonyl)methyl)phenylamino)-propanoate (26d)—The title compound was obtained by the alkylation of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-aminobenzylsulfone **8p** with methyl 2-bromopropionate following the procedure as described in compound **26**. Yield, 60%; white solid, mp 178–180 °C. ¹H NMR (CDCl₃, 400 MHz): δ 1.24 (d, J = 6.9 Hz, 3H, CH₃), 3.50 (s, 3H, OCH₃), 3.61 (s, 6H, 2 X OCH₃), 3.63 (s, 3H, OCH₃), 3.64 (s, 3H, OCH₃), 3.90–3.92 (m, 1H, CH), 3.94 (s, 2H, CH₂), 4.52 (br s, 1H, NH), 5.88 (s, 2H, Ar-H), 6.32 (d, J = 1.7 Hz, 1H, Ar-H), 6.47 (dd, J = 8.1, 1.7 Hz, 1H, Ar-H), 6.52 (d, J = 8.1 Hz, 1H, Ar-H), 6.81 (d, J = 15.6 Hz, 1H, =CH), 7.59 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 480.1579; found 480.1568. Anal. (C₂₃H₂₉NO₈S) C, H, N.

(E)-Methyl 2'',2''-difluoro-2(2-methoxy-5-((2',4',6'-

trimethoxystyrylsulfonyl)methyl)-phenylamino)acetate (26e)—The title compound was obtained by the alkylation of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-aminobenzylsulfone **8p** with methyl 2-chloro-2,2-difluoroacetate following the procedure as described in compound **26**. Yield, 57%; white solid, mp 186–188 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.74 (s, 3H, OCH₃), 3.83 (s, 6H, 2 X OCH₃), 3.85 (s, 3H, OCH₃), 3.87 (s, 3H, OCH₃), 4.22 (s, 2H, CH₂), 6.09 (s, 2H, Ar-H), 6.86 (d, J = 8.4 Hz, 1H, Ar-H), 7.09 (d, J = 15.6 Hz, 1H, =CH), 7.11 (dd, J = 8.4, 2.1 Hz, 1H, Ar-H), 7.19 (s, 1H, Ar-H), 7.84 (d, J = 15.6 Hz, 1H, CH=), 8.08 (brs, 1H, NH). HRMS: m/z calcd [M + H] 502.1269; found 502.1284. Anal. (C₂₂H₂₅F₂NO₈S) C, H, N.

(E)-Methyl 3'',3'',3''-trifluoro-2-(2-methoxy-5-((2',4',6'-

trimethoxystyrylsulfonyl)-methyl)phenylamino)propanoate (26f)—The title compound was obtained by the alkylation of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-aminobenzylsulfone **8p** with methyl 2-bromo-3,3,3-trifluoropropanoate following the

procedure as described in compound **26**. Yield, 56%; white solid, mp 192–194 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.49 (s, 3H, OCH₃), 3.51 (s, 6H, 2 X OCH₃), 3.55 (s, 3H, OCH₃), 3.57 (s, 3H, OCH₃), 3.95–4.16 (m, 1H, CH), 4.22 (s, 2H, CH₂), 4.85 (t, J = 9.0 Hz, 1H, NH), 5.92 (s, 2H, Ar-H), 6.23 (dd, J = 8.4, 2.1 Hz, 1H, Ar-H), 6.40 (d, J = 8.1 Hz, 1H, Ar-H), 6.53 (d, J = 2.1 Hz, 1H, Ar-H), 6.80 (d, J = 15.6 Hz, 1H, =CH), 7.55 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 534.1345; found 534.1339. Anal. (C₂₃H₂₆F₃NO₈S) C, H, N.

(E)-Methyl 2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino)-2-methylpropanoate (26g)—The title compound was obtained by the alkylation of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-aminobenzylsulfone **8p** with methyl 2-bromo-2-methylpropanoate following the procedure as described in compound **26**. Yield, 60%; white solid, mp 170–172 °C. ¹H NMR (CDCl₃, 500 MHz): δ 1.52 (s, 6H, 2 X CH₃), 3.68 (s, 3H, OCH₃), 3.81 (s, 6H, 2 X OCH₃), 3.82 (s, 3H, OCH₃), 3.83 (s, 3H, OCH₃), 4.11 (s, 2H, CH₂), 6.06 (s, 2H, Ar-H), 6.41 (s, 1H, Ar-H), 6.69–6.71 (m, 2H, Ar-H), 7.02 (d, J = 15.5 Hz, 1H, =CH), 7.80 (d, J = 15.5 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 494.1770; found 494.1761. Anal. (C₂₄H₃₁NO₈S) C, H, N.

(E)-Methyl 2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino)-2-phenylacetate (26h)—The title compound was obtained by the alkylation of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-aminobenzylsulfone **8p** with methyl α-bromophenylacetate following the procedure as described in compound **26**. Yield, 63%; white solid, mp 94–96 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.71 (s, 3H, OCH₃), 3.83 (s, 6H, 2 X OCH₃), 3.86 (s, 3H, OCH₃), 3.88 (s, 3H, OCH₃), 4.06 (s, 2H, CH₂), 4.99 (d, J = 6.0 Hz, 1H, CH), 5.43 (d, J = 6.3 Hz, 1H, NH), 6.10 (s, 2H, Ar-H), 6.36 (d, J = 1.8 Hz, 1H, Ar-H), 6.67 (dd, J = 8.1, 1.8 Hz, 1H, Ar-H), 6.73 (d, J = 8.1 Hz, 1H, Ar-H), 6.99 (d, J = 15.6 Hz, 1H, =CH), 7.29–7.35 (m, 3H, Ar-H), 7.42 (dd, J = 8.1, 1.8 Hz, 2H, Ar-H), 7.77 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 542.1770; found 542.1778. Anal. (C₂₈H₃₁NO₈S) C, H, N.

(E)-Methyl 2-(4''-fluorophenyl)-2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino)acetate (26i)—The title compound was obtained by the alkylation of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-aminobenzylsulfone **8p** with methyl 2-bromo-2-(4-fluorophenyl) acetate following the procedure as described in compound **26**. Yield, 60%; white solid, mp 152–154 °C. ¹H NMR (CDCl₃, 500 MHz): δ 3.72 (s, 3H, OCH₃), 3.82 (s, 6H, 2 X OCH₃), 3.86 (s, 3H, OCH₃), 3.88 (s, 3H, OCH₃), 4.05 (s, 2H, CH₂), 4.98 (d, J = 2.5 Hz, 1H, CH), 6.10 (s, 2H, Ar-H), 6.33 (d, J = 2.0 Hz, 1H, Ar-H), 6.69 (dd, J = 8.5, 2.0 Hz, 1H, Ar-H), 6.73 (d, J = 8.5 Hz, 1H, Ar-H), 6.96–6.99 (m, 2H, Ar-H), 7.00 (d, J = 15.5 Hz, 1H, =CH), 7.37–7.40 (m, 2H, Ar-H), 7.76 (d, J = 16.0 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 560.1676; found 560.1656. Anal. (C₂₈H₃₀FO₈S) C, H, N.

(E)-Methyl 2-(4''-chlorophenyl)-2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino)acetate (26j)—The title compound was obtained by the alkylation of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-aminobenzylsulfone **8p** with methyl 2-bromo-2-(4-chlorophenyl)acetate following the

procedure as described in compound **26**. Yield, 64%; white solid, mp 154–156 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.72 (s, 3H, OCH₃), 3.83 (s, 6H, 2 X OCH₃), 3.86 (s, 3H, OCH₃), 3.88 (s, 3H, OCH₃), 4.05 (s, 2H, CH₂), 4.96 (s, 1H, CH), 6.11 (s, 2H, Ar-H), 6.31 (s, 1H, Ar-H), 6.67–6.75 (m, 2H, Ar-H), 6.99 (d, J = 15.6 Hz, 1H, =CH), 7.25–7.28 (m, 2H, Ar-H), 7.54 (d, J = 8.4 Hz, 2H, Ar-H), 7.76 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 576.1381; found 576.1367. Anal. (C₂₈H₃₀ClNO₈S) C, H, N.

(E)-Methyl 2-(4''-bromophenyl)-2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)-methyl)phenylamino)acetate (26k)—The title compound was obtained by the alkylation of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-aminobenzylsulfone **8p** with methyl 2-bromo-2-(4-bromophenyl)acetate following the procedure as described in compound **26**. Yield, 62%; white solid, mp 150–152 °C. ¹H NMR (CDCl₃, 400 MHz): δ 3.48 (s, 3H, OCH₃), 3.59 (s, 6H, 2 X OCH₃), 3.62 (s, 3H, OCH₃), 3.65 (s, 3H, OCH₃), 3.81 (s, 2H, CH₂), 4.71 (s, 1H, CH), 5.87 (s, 2H, Ar-H), 6.07 (d, J = 1.8 Hz, 1H, Ar-H), 6.44 (dd, J = 8.1, 1.8 Hz, 1H, Ar-H), 6.50 (d, J = 8.1 Hz, 1H, Ar-H), 6.75 (d, J = 15.6 Hz, 1H, =CH), 7.05–7.08 (m, 2H, Ar-H), 7.16–7.19 (m, 2H, Ar-H), 7.53 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 620.0876; found 620.0859. Anal. (C₂₈H₃₀BrNO₈S) C, H, N.

(E)-Methyl 2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino)-2-(4''-methoxyphenyl)acetate (26l)—The title compound was obtained by the alkylation of (E)-2',4',6'-trimethoxystyryl-4-methoxy-3-aminobenzylsulfone **8p** with methyl-2-bromo-2-(4-methoxyphenyl)acetate following the procedure as described in compound **26**. Yield, 61%; white solid, mp 182–184 °C. ¹H NMR (CDCl₃, 500 MHz): δ 3.70 (s, 3H, OCH₃), 3.78 (s, 3H, OCH₃), 3.83 (s, 6H, 2 X OCH₃), 3.85 (s, 3H, OCH₃), 3.87 (s, 3H, OCH₃), 4.06 (s, 2H, CH₂), 4.91 (s, 1H, CH), 6.10 (s, 2H, Ar-H), 6.37 (d, J = 1.5 Hz, 1H, Ar-H), 6.68 (dd, J = 8.1, 1.5 Hz, 1H, Ar-H), 6.73 (d, J = 8.1 Hz, 1H, Ar-H), 6.84 (d, J = 9.0 Hz, 2H, Ar-H), 7.00 (d, J = 15.5 Hz, 1H, =CH), 7.33 (d, J = 8.0 Hz, 2H, Ar-H), 7.77 (d, J = 16.0 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 572.1876; found 572.1896. Anal. (C₂₉H₃₃NO₉S) C, H, N.

General Procedure for the Preparation of Amino acids (**27**, Scheme 8)

To a solution of amine ester **26** (46.5g, 100 mmol) in ethanol (200 mL), 20% aqueous sodium hydroxide solution (200 mL) was added. The reaction mixture was refluxed for 2.5 h. After completion of the reaction (TLC, monitoring, chloroform/methanol, 9:1 on silica gel plate), the solvent removed under vacuum and the remainder was acidified by acetic acid to pH 4. The solid that formed was filtered and dried to get the crude amino acid **27** which on crystallization from acetone (2 X 25 mL) resulted in analytically pure amino acid **27** as white crystals.

(E)-2-(2-Methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino)acetic acid (27a)—The title compound was obtained by the hydrolysis of (E)-methyl 2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino)acetate **26a** following the procedure as described in compound **27**. Yield, 50%; pale yellow solid, mp 172–174 °C. ¹H NMR

(DMSO- d_6 , 300 MHz): δ 3.78 (s, 2H, NH-CH₂), 3.86 (s, 3H, OCH₃), 3.91 (s, 3H, OCH₃), 3.92 (s, 6H, 2X OCH₃), 4.33 (s, 2H, CH₂), 6.35 (s, 2H, Ar-H), 6.48 (d, J = 1.8 Hz, 1H, Ar-H), 6.65 (dd, J = 8.1, 1.8 Hz, 1H, Ar-H), 6.86 (d, J = 8.1 Hz, 1H, Ar-H), 7.17 (d, J = 15.9 Hz, 1H, =CH), 7.62 (d, J = 15.6 Hz, 1H, CH=). ¹³C NMR (DMSO- d_6 , 75 MHz): δ 172.1, 163.5, 160.8, 146.3, 136.9, 132.7, 123.6, 121.3, 119.2, 111.9, 109.3, 102.5, 90.6, 60.3, 55.9, 55.5, 55.4, 44.4, 30.6. HRMS: m/z calcd [M - H] 450.1301; found 450.1311. Anal. (C₂₁H₂₅NO₈S) C, H, N.

(E)-2-(2-Methoxy-5-((3',4',5'-

trimethoxystyrylsulfonyl)methyl)phenylamino)acetic acid (27b)—The title compound was obtained by the hydrolysis of (E)-methyl 2-(2-methoxy-5-((3',4',5'-trimethoxystyrylsulfonyl)methyl)phenylamino)acetate **26b** following the procedure as described in compound **27**. Yield, 55%; light yellow solid, mp 112–114 °C. ¹H NMR (CDCl₃, 400 MHz): δ 3.78 (s, 3H, OCH₃), 3.79 (s, 6H, 2 X OCH₃), 3.81 (s, 3H, OCH₃), 3.87 (s, 2H, CH₂), 4.15 (s, 2H, CH₂), 6.44 (d, J = 1.8 Hz, 1H, Ar-H), 6.55 (d, J = 15.4 Hz, 1H, =CH), 6.59 (s, 2H, Ar-H), 6.62–6.66 (m, 1H, Ar-H), 6.69 (d, J = 8.2 Hz, 1H, Ar-H), 7.26 (d, J = 15.5 Hz, 1H, CH=). HRMS: m/z calcd [M - H] 450.1301; found 450.1314. Anal. (C₂₁H₂₅NO₈S) C, H, N.

(E)-3-(2-Methoxy-5-((2',4',6'-

trimethoxystyrylsulfonyl)methyl)phenylamino)propanoic acid (27c)—The title compound was obtained by the hydrolysis of (E)-ethyl 3-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino)propanoate **26c** following the procedure as described in compound **27**. Yield, 51%; white solid, mp 130–132 °C. ¹H NMR (CDCl₃, 300 MHz): δ 2.56 (t, J = 6.3 Hz, 2H, CH₂), 3.34 (t, J = 6.3 Hz, 2H, CH₂), 3.75 (s, 6H, 2 X OCH₃), 3.76 (s, 3H, OCH₃), 3.77 (s, 3H, OCH₃), 4.11 (s, 2H, CH₂), 6.01 (s, 2H, Ar-H), 6.57 (s, 1H, Ar-H), 6.64–6.71 (m, 2H, Ar-H), 6.99 (d, J = 15.9 Hz, 1H, =CH), 7.76 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M - H] 464.1457; found 464.1471. Anal. (C₂₂H₂₇NO₈S) C, H, N.

(E)-2-(2-Methoxy-5-((2',4',6'-

trimethoxystyrylsulfonyl)methyl)phenylamino)propanoic acid (27d)—The title compound was obtained by the hydrolysis of (E)-methyl 2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino)propanoate **26d** following the procedure as described in compound **27**. Yield, 57%; light yellow solid, mp 116–118 °C. ¹H NMR (DMSO- d_6 , 400 MHz): δ 1.07 (d, J = 6.6 Hz, 3H, CH₃), 3.57 (s, 3H, OCH₃), 3.60 (s, 3H, OCH₃), 3.64 (s, 6H, 2 X OCH₃), 3.79–3.83 (m, 1H, CH), 4.06 (s, 2H, CH₂), 6.08 (s, 2H, Ar-H), 6.26 (s, 1H, Ar-H), 6.35 (d, J = 7.5 Hz, 1H, Ar-H), 6.58 (d, J = 8.1 Hz, 1H, Ar-H), 6.90 (d, J = 15.6 Hz, 1H, =CH), 7.35 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M - H] 464.1457; found 464.1446. Anal. (C₂₂H₂₇NO₈S) C, H, N.

(E)-2'',2''-Difluoro-2-(2-methoxy-5-((2',4',6'-

trimethoxystyrylsulfonyl)methyl)phenylamino)acetic acid (27e)—The title compound was obtained by the hydrolysis of (E)-methyl 2'',2''-difluoro-2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino)-acetate **26e** following the procedure as

described in compound **27**. Yield, 59%; white solid, mp 196–198 °C. ¹H NMR (CDCl₃, 300 MHz): δ 3.85 (s, 6H, 2 X OCH₃), 3.87 (s, 3H, OCH₃), 3.89 (s, 3H, OCH₃), 4.12 (s, 2H, CH₂), 6.12 (s, 2H, Ar-H), 6.58 (d, J = 8.1 Hz, 1H, Ar-H), 6.89 (d, J = 8.4 Hz, 1H, Ar-H), 7.06 (dd, J = 8.4, 2.1 Hz, 1H, Ar-H), 7.29 (d, J = 15.6 Hz, 1H, =CH), 7.84 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M - H] 486.1112; found 486.1101. Anal. (C₂₁H₂₃F₂NO₈S) C, H, N.

(E)-3'',3'',3''-Trifluoro-2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)-phenylamino)propanoic acid (27f)—The title compound was obtained by the hydrolysis of (E)-methyl 3'',3'',3''-trifluoro-2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino) propanoate **26f** following the procedure as described in compound **27**. Yield, 53%; white solid, mp 180–182 °C. ¹H NMR (DMSO-d₆, 300 MHz): δ 3.78 (s, 3H, OCH₃), 3.83 (s, 6H, 2 X OCH₃), 3.87 (s, 3H, OCH₃), 4.05 (d, J = 6.3 Hz, CH), 4.12 (s, 2H, CH₂), 6.28 (s, 2H, Ar-H), 6.61 (d, J = 8.4 Hz, 1H, Ar-H), 6.76 (dd, J = 8.4, 2.1 Hz, 1H, Ar-H), 7.01–7.09 (m, 1H, Ar-H), 7.10 (d, J = 15.6 Hz, 1H, =CH), 7.57 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M - H] 518.1175; found 518.1186. Anal. (C₂₂H₂₄F₃NO₈S) C, H, N.

(E)-2-(2-Methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino)-2-methylpropanoic acid (27g)—The title compound was obtained by the hydrolysis of (E)-methyl 2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino)-2-methylpropanoate **26g** following the procedure as described in compound **27**. Yield, 61%; white solid, mp 142–144 °C. ¹H NMR (CDCl₃, 500 MHz): δ 1.48 (s, 6H, 2 X CH₃), 3.83 (s, 6H, 2 X OCH₃), 3.84 (s, 3H, OCH₃), 3.85 (s, 3H, OCH₃), 4.14 (s, 2H, CH₂), 6.07 (s, 2H, Ar-H), 6.52 (s, 1H, Ar-H), 6.79 (d, J = 8.0 Hz, 1H, Ar-H), 6.85 (d, J = 8.1 Hz, 1H, Ar-H), 7.05 (d, J = 16.0 Hz, 1H, =CH), 7.78 (d, J = 15.5 Hz, 1H, CH=). HRMS: m/z calcd [M - H] 478.1614; found 478.1605. Anal. (C₂₃H₂₉NO₈S) C, H, N.

(E)-2-(2-Methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino)-2-phenylacetic acid (27h)—The title compound was obtained by the hydrolysis of (E)-methyl 2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino)-2-phenylacetate **26h** following the procedure as described in compound **27**. Yield, 60%; white solid, mp 124–126 °C. ¹H NMR (CDCl₃, 400 MHz): δ 3.75 (s, 6H, 2 X OCH₃), 3.77 (s, 3H, OCH₃), 3.79 (s, 3H, OCH₃), 4.00 (s, 2H, CH₂), 4.86 (s, 1H, CH), 6.03 (s, 2H, Ar-H), 6.32 (d, J = 1.4 Hz, 1H, Ar-H), 6.66–6.69 (m, 2H, Ar-H), 6.94 (d, J = 15.6 Hz, 1H, =CH), 7.22–7.26 (m, 3H, Ar-H), 7.32–7.34 (m, 2H, Ar-H), 7.69 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M - H] 526.1614; found 526.1604. Anal. (C₂₇H₂₉NO₈S) C, H, N.

(E)-2-(4''-Fluorophenyl)-2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)-phenylamino)acetic acid (27i)—The title compound was obtained by the hydrolysis of (E)-methyl 2-(4''-fluorophenyl)-2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino) acetate **26i** following the procedure as described in compound **27**. Yield, 60%; light yellow solid, mp 82–84 °C. ¹H NMR (DMSO-d₆, 300 MHz): δ 3.79 (s, 3H, OCH₃), 3.83 (s, 3H, OCH₃), 3.84 (s, 3H, OCH₃), 3.86 (s, 3H, OCH₃), 4.14 (s, 2H, CH₂), 4.42 (s, 1H, CH), 6.29 (s, 2H, Ar-H), 6.33 (d,

J = 2.0 Hz, 1H, Ar-H), 6.69 (dd, J = 8.5, 2.0 Hz, 1H, Ar-H), 6.73 (d, J = 8.5 Hz, 1H, Ar-H), 6.96–6.99 (m, 2H, Ar-H), 7.00 (d, J = 15.5 Hz, 1H, =CH), 7.37–7.40 (m, 2H, Ar-H), 7.76 (d, J = 16.0 Hz, 1H, CH=). HRMS: m/z calcd [M - H] 544.1520; found 544.1434. Anal. (C₂₇H₂₈FNO₈S) C, H, N.

(E)-2-(4''-Chlorophenyl)-2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)-phenylamino)acetic acid (27j)—The title compound was obtained by the hydrolysis of (E)-methyl 2-(4''-chlorophenyl)-2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino) acetate **26j** following the procedure as described in compound **27**. Yield, 60%; white solid, mp 172–174 °C. ¹H NMR (CDCl₃, 400 MHz): δ 3.55 (s, 6H, 2 X OCH₃), 3.57 (s, 3H, OCH₃), 3.60 (s, 3H, OCH₃), 3.80 (s, 2H, CH₂), 4.69(s, 1H, CH), 5.83 (s, 2H, Ar-H), 6.06 (d, J = 1.8 Hz, 1H, Ar-H), 6.44 (dd, J = 8.1, 1.8 Hz, 1H, Ar-H), 6.47 (d, J = 8.1 Hz, 1H, Ar-H), 6.73 (d, J = 15.6 Hz, 1H, =CH), 7.00 (d, J = 7.8 Hz, 2H, Ar-H), 7.08 (d, J = 8.5 Hz, 2H, Ar-H), 7.49 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M - H] 560.1224; found 560.1212. Anal. (C₂₇H₂₈ClNO₈S) C, H, N.

(E)-2-(4''-Bromophenyl)-2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)-phenylamino)acetic acid (27k)—The title compound was obtained by the hydrolysis of (E)-methyl 2-(4''-bromophenyl)-2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino) acetate **26k** following the procedure as described in compound **27**. Yield, 60%; white solid, mp 178–179 °C. ¹H NMR (DMSO-d₆, 400 MHz): δ 3.93 (s, 6H, 2 X OCH₃), 3.94 (s, 3H, OCH₃), 3.96 (s, 3H, OCH₃), 4.25 (s, 2H, CH₂), 4.96(s, 1H, CH), 6.39 (s, 2H, Ar-H), 6.41 (d, J = 1.8 Hz, 1H, Ar-H), 6.69 (dd, J = 8.1, 1.8 Hz, 1H, Ar-H), 6.91 (d, J = 8.1 Hz, 1H, Ar-H), 7.14 (d, J = 15.6 Hz, 1H, =CH), 7.39 (d, J = 8.4 Hz, 2H, Ar-H), 7.57 (d, J = 8.4 Hz, 2H, Ar-H), 7.61 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M - H] 604.0719; found 604.0731. Anal. (C₂₇H₂₈BrNO₈S) C, H, N.

(E)-2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino)-2-(4''-methoxyphenyl)acetic acid (27l)—The title compound was obtained by the hydrolysis of (E)-(4''-Methoxyphenyl)-{2-methoxy-5-[2-(2',4',6'-trimethoxyphenyl)ethenesulfonylmethyl]-phenylamino}acetic acid methyl ester **26l** following the procedure as described in compound **27**. Yield, 60%; white solid, mp 174–175 °C. ¹H NMR (DMSO-d₆, 300 MHz): δ 3.72 (s, 3H, OCH₃), 3.84 (s, 3H, OCH₃), 3.86 (s, 6H, 2 X OCH₃), 3.87 (s, 3H, OCH₃), 4.16 (s, 2H, CH₂), 4.80(s, 1H, CH), 6.31 (s, 2H, Ar-H), 6.37 (d, J = 1.5 Hz, 1H, Ar-H), 6.62 (dd, J = 8.1, 1.5 Hz, 1H, Ar-H), 6.71 (d, J = 8.1 Hz, 1H, Ar-H), 6.82 (d, J = 9.0 Hz, 2H, Ar-H), 7.08 (d, J = 15.6 Hz, 1H, =CH), 7.27 (d, J = 8.7 Hz, 2H, Ar-H), 7.54 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M - H] 556.1720; found 556.1731. Anal. (C₂₈H₃₁NO₉S) C, H, N.

Sodium (E)-2-(2-Methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenylamino)-acetate (ESTYBON[®]) (ON 01910.Na) (28)—The title compound was obtained by the direct hydrolysis of (E)-methyl-2-(2-methoxy-5-((2',4',6'-trimethoxystyrylsulfonyl)methyl)phenyl amino)acetate

26a. To a solution of sodium hydroxide (3.95 g, 99 mmol) in water (11.5 mL) at 20 °C was added ethanol (40 mL), (E)-methyl 2-(2-methoxy-5-((2',4',6'-trimethoxystyryl sulfonyl)methyl)phenylamino)acetate **26a** and dichloromethane (200 mL). The resulting mixture was stirred at room temperature for 3–4 h. After completion of the reaction monitored by TLC (chloroform/methanol, 9:1 on silica gel plate), charcoal was added and stirred for 30 min. The reaction mixture was filtered through celite and washed with ethanol (2 X 20 mL). The combined filtrate was distilled at 50 °C until most of the solvent removed. Methyl Ethyl Ketone (70 mL) was added to the residue and distill of the methyl ethyl ketone at 50 °C. To the residue water (10 mL) was added and the resulting mixture was heated to 70 °C and maintained for 30 min. The reaction mixture cooled to room temperature and stirred for 2 h at room temperature. The solid formed filtered, washed with methyl ethyl ketone (2 X 20 mL) and dried to get the title compound **28**. Yield, 80%; white solid, mp 174–178 °C. ¹H NMR (D₂O, 300 MHz): δ 3.46 (s, 2H, NH-CH₂), 3.63 (s, 6H, 2 X OCH₃), 3.72 (s, 3H, OCH₃), 3.75 (s, 3H, OCH₃), 4.23 (s, 2H, CH₂), 5.89 (s, 2H, Ar-H), 6.44 (d, J = 1.5 Hz, 1H, Ar-H), 6.59 (dd, J = 8.4, 1.5 Hz, 1H, Ar-H), 6.72 (d, J = 8.4 Hz, 1H, Ar-H), 6.91 (d, J = 15.6 Hz, 1H, =CH), 7.39 (d, J = 15.6 Hz, 1H, CH=). HRMS: m/z calcd [M + H] 510.1365; found 510.1378. Anal. (C₂₁H₂₄NNaO₈S. 2H₂O) C, H, N.

Biology. Tissue Culture and Reagents—Paclitaxel was purchased from Sigma. Cell lines were purchased from ATCC. Cell lines were routinely grown in DMEM or RPMI (CellGro) supplemented with 10% fetal bovine serum (Cell Generation Co) 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 6 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.

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 mm² 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 h after treatment. The cells were removed from the plate by trypsin digestion and combined with the non-attached 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 mg/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—DU145 and HFL-1 cells were plated at a density of 3.0×10^6 cells per 150 mm² plate and treated 24 h later with either DMSO or **28**. The cells were collected 48 h

treatment 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 was 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.

Cellular Viability and Caspase 3/7 Activity—Exponentially growing A549 cells were seeded in a white walled 96-well plate at a density of 3,600 cells/well in 100 μ L of DMEM containing 10% FBS and 1% Pen/Strep. Cells were then allowed to adhere overnight at 37 °C in an incubator. The next day, cells were treated with varying concentrations of **28** or DMSO and then returned to the incubator. 24 h later, plates were removed from the incubator and 20 μ L of CellTiter-Blue® Reagent (Promega Cat # G8080) was added individually to each well following manufacturer's instructions. Plates were slowly shaken for 0.5 min and then returned to the incubator. After 3 h, fluorescence was read using a Glomax 96-well plate reader. Next, 120 μ L of Caspase Glo® 3/7 Reagent (Promega Cat # G8090) was added to each well per manufacturer's instructions. Plates were slowly shaken for 0.5 min and allowed to develop at room temperature for 2 h. At the end of this period, luminescence was read using a Glomax 96-well plate reader.

Nude mouse assay—Female athymic (NCR-nu/nu, Taconic) nude mice were injected with $0.5\text{--}1.0 \times 10^7$ 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=9) and injected with 200 mg/Kg **28** 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 Q2D X 20 schedule. Tumor measurements (two dimensions) were done three times per week using traceable digital vernier calipers (Fisher). Tumor volume was calculated using the following equation: $V = (L \times S^2) / 6$, 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

MDS myelodysplastic syndromes

AML	acute myeloid leukemia
PI3K	Phosphoinositide 3-kinase
BIM	Bcl-2-interacting modulator of cell death
FDA	food and drug Administration
DNA	deoxyribonucleic acid
CDKs	cyclin dependent kinases
JNK	Janus kinase
p-mTOR	mammalian target of rapamycin

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Figure 1.

28 is active against multidrug resistant cells (MDR). (A) The MDR-1 positive MES-SA/DX5 clone as well as an atypical MDR resistant to topoisomerase inhibitors CEM/C2 (B) as well as their respective sensitive parental controls were plated into six-well dishes and treated with increasing concentrations of **28** or a representative chemotherapeutic agent, paclitaxel (PTX) or camptothecin (CPT), respectively for 96 h. The number of viable cells from duplicate plates was determined by trypan blue exclusion. As expected, the parentals are sensitive to both **28** and the chemotherapeutic agent, but while the resistant clones are over 500 fold resistant to PTX or CPT, they are remaining sensitive to the cytotoxic action of **28**.

Figure 2.

28 selectively induces mitotic G2/M arrest and apoptosis in cancer cells. (A) Normal diploid fibroblasts HFL-1 cells or (B) human prostate cells (DU145) were treated with increasing concentrations of **28** and incubated in medium containing 10% fetal bovine serum for 24 h and the cells were fixed, stained with propidium iodide and then subjected to FACS analysis and analyzed for their DNA content. **28** induces a mitotic arrest and induction of a subG1 population (blue) indicative of apoptosis in the cancer cell line while only inducing a small mitotic block in the normal cells.

Figure 3.

DU145 and HFL-1 (normal human fibroblasts) cells were treated with increasing concentrations of **28** or DMSO (Vehicle) for 48 h. Cells were harvested and total protein was resolved by 10% SDS-PAGE, western blotted and hybridized to anti-PARP (Cell Signaling # 542) anti body. The blot was then washed and treated with anti-rabbit secondary labeled with infrared dye (700) and scanned using the Odyssey scanner (LiCor).

Figure 4.

Cellular viability together with the activity of caspases 3/7 were assayed concomitantly in A549 cells treated with **28** for 24 h (n=3). Viability was measured after addition of CellTiter-Blue® reagent (Promega Corporation). Caspase 3/7 activity was measured after addition of Caspase-Glo® 3/7 reagent (Promega Corporation). Fluorescence and luminescence were plotted together to reveal the concentrations at which **28** activated the apoptotic machinery to induce a cytotoxic effect.

Figure 5.

Human breast cancer cells (BT20) were injected subcutaneously into female nude mice. The mice were grouped and then treated with 200 mg/kg **28** by intraperitoneal injections Q2D x 20 formulated in phosphate buffered saline (PBS) or placebo (PBS) alone. Tumor volumes (A) and body weights (B) were determined and the average ($\pm$ SEM) for each group (N=9) were determined and plotted. **28** significantly inhibited the in vivo growth of tumors.

Placebo tumors doubled in 6 days while it took over 18 days for the **28** treated tumors to double in size. There was no sign of toxicity or body weight loss.

Method A

Scheme 1. Synthesis of (E)-styryl benzyl sulfones from benzylsulfonylacetic acids^a

^a Reagents and conditions: (a) HSCH₂COOH, MeOH, NaOH, rt, 3 h; (b) 30% H₂O₂, AcOH, rt, 24 h; (c) benzylamine, AcOH, reflux, 2–8 h; (d) piperidine, benzoic acid, toluene, 2–4 h.

R=4-OCH₃,3-NO₂

R=4-OCH₃,2-NO₂

R=4-Br,3-NO₂

R=4-Cl,3-NO₂

R=4-Cl,2-NO₂

R=4-F,3-NO₂

R=6-OCH₃,3-NO₂

Scheme 2.

Synthesis of benzyl bromides^a

^a Reagents and conditions: (a) NBS, benzoyl peroxide, CCl₄, 18 h; (b) NaBH₄, MeOH, 0 °C–5 °C, 1 h; (c) PBr₃, Toluene, 100 °C, 30 min.

Method B

Scheme 3. Synthesis of (E)-styryl benzyl sulfones from phenacyl benzyl sulfones^a^a Reagents and conditions: (a) NaOH, MeOH, rt, 2 h; (b) 30% H₂O₂, AcOH, rt, 24 h; (c) NaBH₄, tetrahydrofuran, 0 °C, 1 h; (d) *p*-toluenesulfonic acid, toluene, 2–4 h.

Scheme 4. Synthesis of 4-methoxy-3-nitrobenzylmercaptan^a^a Reagents and conditions: (a) Thiourea, H₂O, reflux, 2 h; (b) Ammonia, hexane, reflux, 30 min.

Scheme 5.

Synthesis of (Z) and (E)-styryl benzyl sulfides from 4-methoxy-3-nitro benzylmercaptan and 2,4,6-trimethoxyphenyl acetylene and (E)-styryl benzyl sulfoxide and sulfone^a

^a Reagents and conditions: (a) Et₃B-hexane, benzene, 25 °C, 2 h; (b) 1,1,1,3,3,3-hexafluoro-2-propanol, 30% H₂O₂, 25 °C, 2 h; (c) Acetone: water (2:1), sodium hydrosulfite, 50 °C, 30 min; (d) 30% H₂O₂, AcOH, rt, 24 h; (e) *m*-CPBA, CH₂Cl₂, 0 °C - rt, 3 h.

Scheme 6. Synthesis of 2,4,6-trimethoxyphenyl acetylene^a

^a Reagents and conditions: (a) Triphenylphosphine, tetrabromomethane, CH_2Cl_2 , 5°C , 30 min; (b) $n\text{-BuLi}$, dry THF, -78°C , 15 min.

Scheme 7.

Synthesis of (E)-styryl 4-methoxy-3-nitrobenzyl sulfoxide from 4-methoxy-3-nitro benzyl sulfoxideacetic acid^a

^a Reagents and conditions: (a) NaOH/deionized H₂O, NaHCO₃, Acetone, Oxone solution, sodium bisulfite, 6N HCl; (b) benzylamine, AcOH, reflux, 2–8 h; (c) Acetone: water (2:1), sodium hydrosulfite, 50 °C, 30 min.

Scheme 8.

Synthesis of amine esters and acids of 4-methoxy-3-amino styryl benzyl sulfones (**8p** and **8q**)

Scheme 9.
Synthesis of **28**

Table 1*In vitro* cytotoxicity of (E)-styryl benzyl sulfones **8**

Compd	R	R ₁	IC ₅₀ (μM)	
			DU145	K562
8a	3-NO ₂ , 4-CH ₃ O	2-CH ₃ O	20	20
8b	3-NH ₂ , 4-CH ₃ O	2-CH ₃ O	7.5	7.5
8c	3-NO ₂ , 4-CH ₃ O	4-CH ₃ O	25	25
8d	3-NH ₂ , 4-CH ₃ O	4-CH ₃ O	2.5	2.5
8e	3-NO ₂ , 4-CH ₃ O	2, 6-(CH ₃ O) ₂	20	20
8f	2-NO ₂ , 4-CH ₃ O	2, 6-(CH ₃ O) ₂	50	50
8g	3-NH ₂ , 4-CH ₃ O	2, 4-(CH ₃ O) ₂	0.5	0.5
8h	3-NH ₂ , 4-CH ₃ O	2, 5-(CH ₃ O) ₂	0.75	0.75
8i	3-NH ₂ , 4-CH ₃ O	2, 6-(CH ₃ O) ₂	0.05	0.05
8j	2-NH ₂ , 4-CH ₃ O	2, 6-(CH ₃ O) ₂	0.75	0.75
8k	3-NH ₂ , 6-CH ₃ O	2, 6-(CH ₃ O) ₂	25	25
8l	3-NO ₂ , 4-CH ₃ O	2, 4, 6-(CH ₃ O) ₃	2.5	2.5
8m	2-NO ₂ , 4-CH ₃ O	2, 4, 6-(CH ₃ O) ₃	20	20
8n	2-NH ₂ , 4-CH ₃ O	2, 4, 6-(CH ₃ O) ₃	0.025	0.025
8o	3-NH ₂ , 4-CH ₃ O	2, 4, 5-(CH ₃ O) ₃	0.25	0.25
8p	3-NH ₂ , 4-CH ₃ O	2, 4, 6-(CH ₃ O) ₃	0.004	0.003
8q	3-NH ₂ , 4-CH ₃ O	3, 4, 5-(CH ₃ O) ₃	3.0	0.25
8r	3-NH ₂ , 6-CH ₃ O	2, 4, 6-(CH ₃ O) ₃	7.5	7.5
8s	3-NO ₂ , 4-CH ₃ O	2, 6-(CH ₃ O) ₂ , 4-OH	5.0	5.0
8t	3-NH ₂ , 4-CH ₃ O	2, 6-(CH ₃ O) ₂ , 4-OH	0.2	0.3
8u	3-NH ₂ , 4-CH ₃ O	2, 6-(CH ₃ O) ₂ , 4-O(CH ₂) ₃ COOH	25	20
8v	3-NO ₂ , 4-Br	2, 4, 6-(CH ₃ O) ₃	25	25
8w	3-NH ₂ , 4-Br	2, 4, 6-(CH ₃ O) ₃	0.75	0.75
8x	3-NH ₂ , 4-Cl	2, 4, 6-(CH ₃ O) ₃	0.01	0.01

Compd	R	R ₁	IC ₅₀ (μM)	
			DU145	K562
8y	2-NH ₂ , 4-Cl	2, 4, 6-(CH ₃ O) ₃	2.5	2.5
8z	3-NO ₂ , 4-F	2, 4, 6-(CH ₃ O) ₃	25	25
8aa	3-NH ₂ , 4-F	2, 4, 6-(CH ₃ O) ₃	2.5	2.5
8ab	3-NH ₂ , 4-CH ₃ O	2, 4, 6-F ₃	2.0	0.8
8ac	3-NH ₂ , 4-CH ₃ O	2, 4, 5-F ₃	5.0	15

Table 2

In vitro cytotoxicity of (E)-styryl benzyl sulfides (**24**) and sulfoxides (**21**)

Compd	R	R ₁	n	IC ₅₀ (μM)	
				DUI45	K562
17	3-NO ₂ , 4-OCH ₃	2, 4, 6-(OCH ₃) ₃	0	5.0	5.0
20	3-NO ₂ , 4-OCH ₃	2, 4, 6-(OCH ₃) ₃	1	15	15
21	3-NH ₂ , 4-OCH ₃	2, 4, 6-(OCH ₃) ₃	1	0.04	0.02
24	3-NH ₂ , 4-OCH ₃	2, 4, 6-(OCH ₃) ₃	0	0.05	0.03

Table 3*In vitro* cytotoxicity of 3-amino substituted esters **26** and acids **27** and sodium salt **28**

Compd	R ₁	R ₂	IC ₅₀ (μM)	
			DU145	K562
26a	2, 4, 6-(CH ₃ O) ₃	CH ₂	0.1	0.1
26b	3, 4, 5-(CH ₃ O) ₃	CH ₂	75	30
26c	2, 4, 6-(CH ₃ O) ₃	CH ₂ CH ₂	1.5	1.0
26d	2, 4, 6-(CH ₃ O) ₃	CH(CH ₃)	0.75	0.2
26e	2, 4, 6-(CH ₃ O) ₃	CF ₂	0.25	0.25
26f	2, 4, 6-(CH ₃ O) ₃	CH(CF ₃)	0.25	0.25
26g	2, 4, 6-(CH ₃ O) ₃	C(CH ₃) ₂	2.5	0.80
26h	2, 4, 6-(CH ₃ O) ₃	CH(C ₆ H ₅)	0.075	0.03
26i	2, 4, 6-(CH ₃ O) ₃	CH(4-FC ₆ H ₄)	0.3	0.8
26j	2, 4, 6-(CH ₃ O) ₃	CH(4-ClC ₆ H ₄)	0.4	0.2
26k	2, 4, 6-(CH ₃ O) ₃	CH(4-BrC ₆ H ₄)	0.3	0.2
26l	2, 4, 6-(CH ₃ O) ₃	CH(4-MeOC ₆ H ₄)	0.25	0.15
27a	2, 4, 6-(CH ₃ O) ₃	CH ₂	0.075	0.0075
27b	3, 4, 5-(CH ₃ O) ₃	CH ₂	5.0	3.0
27c	2, 4, 6-(CH ₃ O) ₃	CH ₂ CH ₂	5.0	5.0
27d	2, 4, 6-(CH ₃ O) ₃	CH(CH ₃)	0.025	0.015
27e	2, 4, 6-(CH ₃ O) ₃	CF ₂	0.15	0.15
27f	2, 4, 6-(CH ₃ O) ₃	CH(CF ₃)	0.02	0.075
27g	2, 4, 6-(CH ₃ O) ₃	C(CH ₃) ₂	0.15	0.0075
27h	2, 4, 6-(CH ₃ O) ₃	CH(C ₆ H ₅)	0.02	0.015
27i	2, 4, 6-(CH ₃ O) ₃	CH(4-FC ₆ H ₄)	0.15	0.08
27j	2, 4, 6-(CH ₃ O) ₃	CH(4-ClC ₆ H ₄)	0.4	0.25
27k	2, 4, 6-(CH ₃ O) ₃	CH(4-BrC ₆ H ₄)	0.3	0.2
27l	2, 4, 6-(CH ₃ O) ₃	CH(4-OMeC ₆ H ₄)	0.1	0.015
28	ON 01910 Na		0.1	0.015

Table 4Evaluation of **8p** and **28** against a panel of Human Tumor Cell lines and normal fibroblasts.

Cell Line	Tumor Type	IC ₅₀ (μM)	
		8p	28
BT20	Breast (ER−)	0.03	0.08
T47D	Breast (ER+)	0.003	0.17
MCF-7	Breast (ER+)	0.001	0.075
SK-BR-3	Breast (ER−)	0.003	0.075
BT474	Breast (ER+)	0.002	0.05
MDA-MB-231	Breast (Triple Neg)	ND	0.025
MDA-MB-157	Breast (Triple Neg)	ND	0.075
Hcc70	Breast	ND	0.075
HCC1428	Breast	ND	0.06
DU145	Prostate (AR−)	0.005	0.1
PC-3	Prostate (AR+)	0.006	0.15
OV-CAR-3	Ovarian	0.003	0.075
MIA-Paca2	Pancreatic	0.003	0.05
BxPC-3	Pancreatic	ND	0.075
PANC-1	Pancreatic	ND	0.04
U87	Glioblastoma	0.003	0.08
H157	NSCLC	0.004	0.08
A549	NSCLC	0.003	0.09
H1975	NSCLC	0.003	0.09
H187	SCLC	0.004	0.08
N417	SCLC	0.003	0.08
AGS	Gastric	0.003	0.08
RF1	Gastric	0.002	0.05
RF48	Gastric	0.001	0.05
HELA	Cervical	ND	0.1
COLO-205	Colo-rectal	0.005	0.15
DLD-1	Colo-rectal	0.005	0.15
HCT-116	Colo-rectal	0.003	0.075
HCT-15	Colo-rectal	0.003	0.09
COLO-320	Colo-rectal	0.003	0.06
SW480	Colo-rectal	0.005	0.06
RPMI-7951	Melanoma	ND	0.025
WM983A	Melanoma	ND	0.04
WM3211	Melanoma	ND	0.075
WM1341D	Melanoma	ND	0.1

Cell Line	Tumor Type	IC ₅₀ (μM)	
		8p	28
WM278	Melanoma	ND	0.15
WM239A	Melanoma	ND	0.075
WM-793	Melanoma	ND	0.075
451-LU	Melanoma	ND	0.025
DND-1A	Melanoma	ND	0.075
K562	CML	0.0025	0.015
MOLT-4	T-Lymphoblastic: ALL	0.004	0.04
Z138C	Mantle Cell Lymphoma	0.003	0.075
GRANTA-519	Mantle Cell Lymphoma	0.003	0.075
Bel-7402	Hepatoma	ND	0.1
KB	Nasopharyngeal	ND	0.07
HELA	Cervical	ND	0.1
U937	Lymphoma	ND	0.01
LP-1	Multiple Myeloma	0.003	0.03
U266	Multiple Myeloma	0.003	0.025
OPM-2	Multiple Myeloma	0.003	0.015
RPMI-8266	Multiple Myeloma	0.003	0.01
HL-60	AML M3	ND	0.02
KG1a	AML M1	ND	0.03
HEL	AML M6	ND	0.05
Daudi	Burkitt's Lymphoma (B-cell)	0.003	0.15
Raji	Burkitt's Lymphoma (B-cell)	0.002	0.075
Namalwa	Burkitt's Lymphoma (B-cell)	0.005	0.075
Fibroblasts	PS-41	ND	>100
Endothelial	Hu-VEC	ND	>100

Table 5Evaluation of **8p** and **28** against a panel of Multidrug-resistance Human Tumor Cell lines

Cell Line	Tumor Type	IC ₅₀ (μM)	
		8p	28
MES-SA	Sarcoma	0.004	0.1
MES-SA/DX5 ^a	Resistant Sarcoma	0.004	0.1
CEM	Leukemic	0.01	0.1
CEM/C2 ^a	Resistant Leukemic	0.01	0.05
2008	Ovarian	0.003	0.15
2008/17/4	Resistant Ovarian	0.003	0.1

^aThese cell lines constitute multidrug-resistance cell lines and show up-regulation of MDR and in the case of CEM/C2, additional mutations in the Topo-2 gene.^{19,20}