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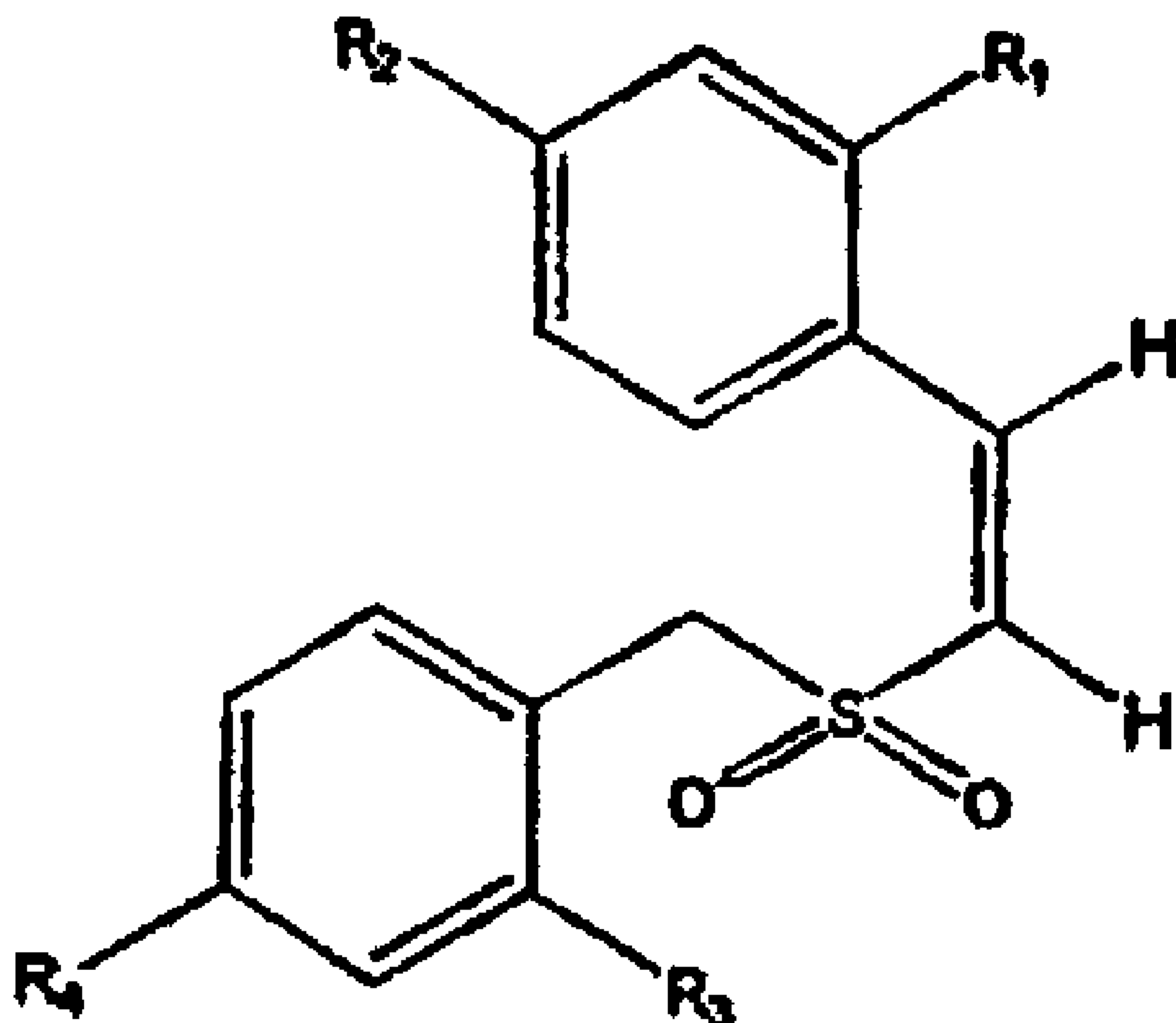
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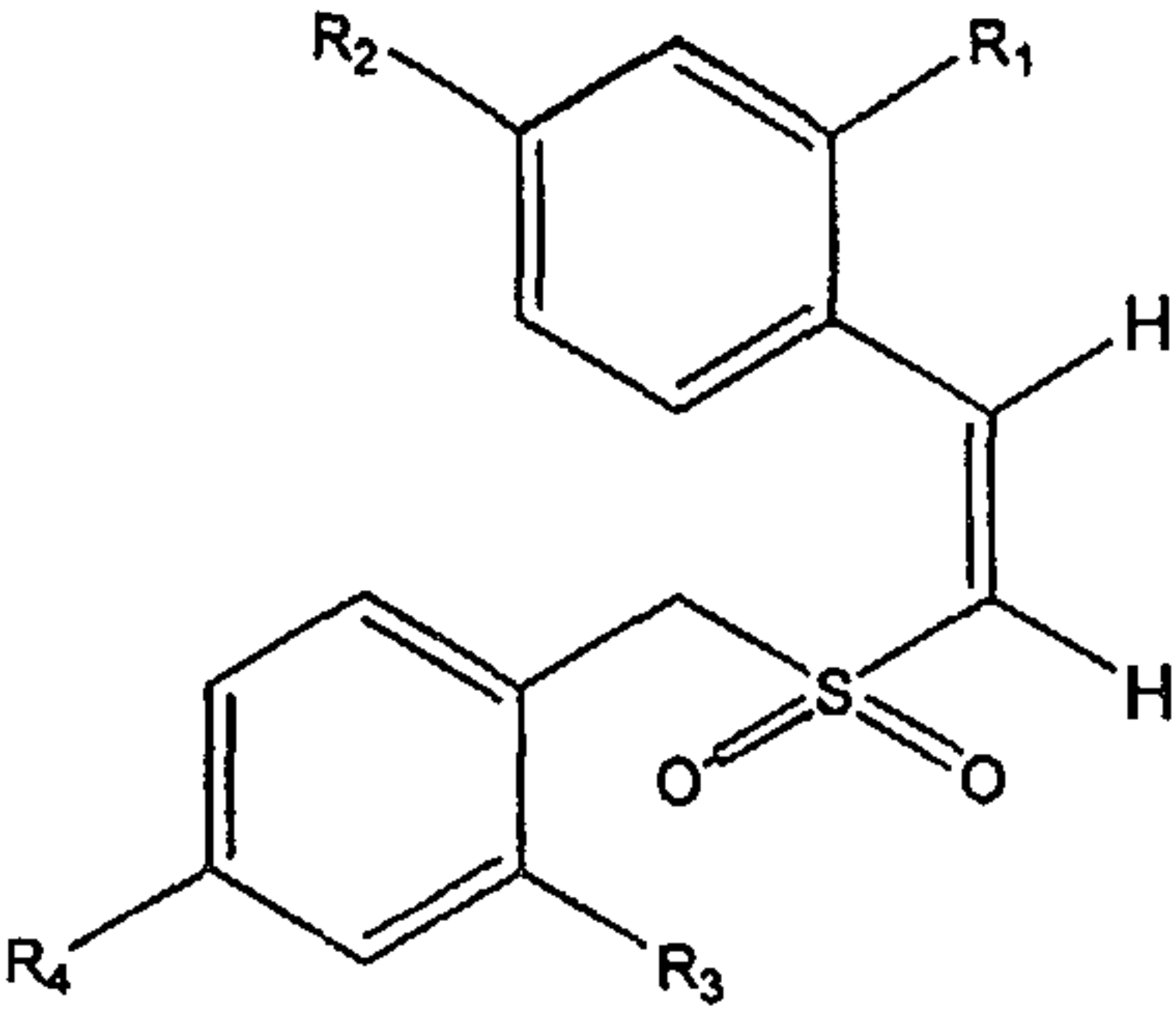
(57) Abrégé/Abstract:

(Z)-styryl benzylsulfones of formula (I) are useful as anticancer agents: wherein R_1 is selected from the group consisting of hydrogen, chloro and nitro; R_2 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, chloro, bromo, iodo and fluoro; and R_3 and R_4 are independently selected from the group consisting of hydrogen, lower alkyl, nitro, chloro, bromo, iodo and fluoro; provided that at least one of R_1 or R_2 is hydrogen. The corresponding (Z)-styryl benzylsulfides are useful as intermediates in the preparation of the biologically active (Z)-styryl benzyl sulfones.



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(54) Title: Z-STYRYL SULFONE ANTICANCER AGENTS (57) Abstract <p>(Z)-styryl benzylsulfones of formula (I) are useful as anticancer agents: wherein R₁ is selected from the group consisting of hydrogen, chloro and nitro; R₂ is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, chloro, bromo, iodo and fluoro; and R₃ and R₄ are independently selected from the group consisting of hydrogen, lower alkyl, nitro, chloro, bromo, iodo and fluoro; provided that at least one of R₁ or R₂ is hydrogen. The corresponding (Z)-styryl benzylsulfides are useful as intermediates in the preparation of the biologically active (Z)-styryl benzyl sulfones.</p> <div style="text-align: center;">  <p style="margin-left: 750px;">(I)</p> </div>		

Z- STYRYL SULFONE ANTICANCER AGENTS

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Field of the Invention

The invention relates to compositions and methods for the treatment of cancer.

10

Background of the Invention

Extracellular signals received at transmembrane receptors are relayed into the cells by the signal transduction pathways (Pelech *et al.*, *Science* **257**:1335 (1992)) which have been implicated in a wide array of physiological processes such as induction of cell proliferation, differentiation or apoptosis (Davis *et al.*, *J. Biol. Chem.* **268**:14553 (1993)).
The Mitogen Activated Protein Kinase (MAPK) cascade is a major signaling system by which cells transduce extracellular cues into intracellular responses (Nishida *et al.*, *Trends Biochem. Sci.* **18**:128 (1993); Blumer *et al.*, *Trends Biochem. Sci.* **19**:236 (1994)). Many steps of this cascade are conserved, and homologous for MAP kinases have been discovered in different species.

In mammalian cells, the Extracellular-Signal-Regulated Kinases (ERKs), ERK-1 and ERK-2 are the archetypal and best-studied members of the MAPK family, which all have the unique feature of being activated by phosphorylation on threonine and tyrosine residues by an upstream dual specificity kinase (Posada *et al.*, *Science* **255**:212 (1992); Biggs III *et al.*, *Proc. Natl. Acad. Sci. USA* **89**:6295 (1992); Garner *et al.*, *Genes Dev.* **6**:1280 (1992)).

Recent studies have identified an additional subgroup of MAPKs, known as c-Jun NH2-terminal kinases 1 and 2 (JNK-1 and JNK-2), that have different substrate specificities and are regulated by different stimuli (Hibi *et al.*, *Genes Dev.* **7**:2135 (1993)). JNKs are members of the class of stress-activated protein kinases (SPKs). JNKs have been shown to be activated by treatment of cells with UV radiation, pro-inflammatory cytokines and environmental stress (Derijard *et al.*, *Cell* **1025** (1994)). The activated JNK binds to the amino terminus of the c-Jun protein and increases the protein's transcriptional activity by phosphorylating it at ser63 and ser73 (Adler *et al.*, *Proc. Natl. Acad. Sci. USA* **89**:5341 (1992); Kwok *et al.*, *Nature* **370**:223 (1994)).

Analysis of the deduced primary sequence of the JNKs indicates that they are distantly related to ERKs (Davis, *Trends Biochem. Sci.* **19**:470 (1994)). Both ERKs and JNKs are phosphorylated on Tyr and Thr in response to external stimuli resulting in their activation (Davis, *Trends Biochem. Sci.* **19**:470 (1994)). The phosphorylation (Thr and Tyr) sites, which play a critical role in their activation are conserved between ERKs and JNKs (Davis, *Trends Biochem. Sci.* **19**:470 (1994)). However, these sites of phosphorylation are located within distinct dual phosphorylation motifs: Thr-Pro-Tyr (JNK) and Thr-Glu-Tyr (ERK). Phosphorylation of MAPKs and JNKs by an external signal often involves the activation of protein tyrosine kinases (PTKs) (Gille *et al.*, *Nature* **358**:414 (1992)), which constitute a large family of proteins encompassing several growth factor receptors and other signal transducing molecules.

Protein tyrosine kinases are enzymes which catalyze a well defined chemical reaction: the phosphorylation of a tyrosine residue (Hunter *et al.*, *Annu Rev Biochem* **54**:897 (1985)). Receptor tyrosine kinases in particular are attractive targets for drug design since blockers for the substrate domain of these kinases is likely to yield an effective and selective antiproliferative agent. The potential use of protein tyrosine kinase blockers as antiproliferative agents was recognized as early as 1981, when quercetin was suggested as a PTK blocker (Graziani *et al.*, *Eur. J. Biochem.* **135**:583-589 (1983)).

The best understood MAPK pathway involves extracellular signal-regulated kinases which constitute the Ras/Raf/MEK/ERK kinase cascade (Boudewijn *et al.*, *Trends Biochem. Sci.* **20**, 18 (1995)). Once this pathway is activated by different stimuli, MAPK phosphorylates a variety of proteins including several transcription factors which translocate into the nucleus and activate gene transcription. Negative regulation of this pathway could arrest the cascade of these events.

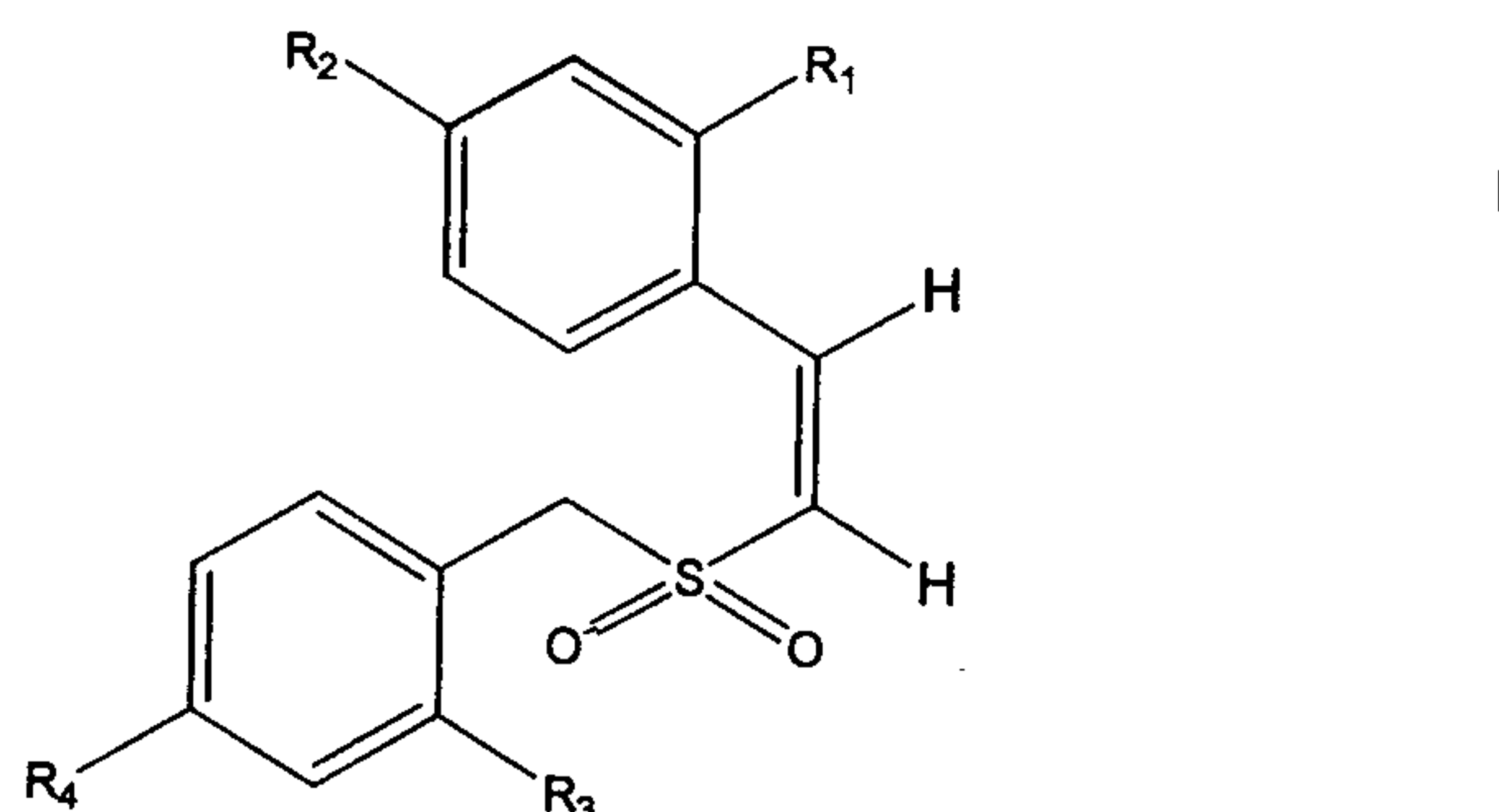
What are needed are new anticancer chemotherapeutic agents which target receptor tyrosine kinases and which arrest the Ras/Raf/MEK/ERK kinase cascade. Oncoproteins in general, and signal transducing proteins in particular, are likely to be more selective targets for chemotherapy because they represent a subclass of proteins whose activities are essential for cell proliferation, and because their activities are greatly amplified in proliferative diseases.

Summary of the Invention

It is an object of the invention to provide compounds, compositions and methods for the treatment of cancer and other proliferative diseases. The biologically active compounds are in the form of (Z)-styryl benzylsulfones.

It is a further object of the invention to provide intermediates useful for the preparation of compounds having anticancer activity. The intermediates comprise (Z)-styryl benzylsulfides.

The present invention provides for pharmaceutical compositions comprising a pharmaceutically acceptable carrier and one or more compounds of the formula I



wherein

5 R_1 is selected from the group consisting of hydrogen, chloro and nitro;

R_2 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, chloro, bromo, iodo and fluoro; and

10 R_3 and R_4 are independently selected from the group consisting of hydrogen, lower alkyl, nitro, chloro, bromo, iodo and fluoro; provided at least one of R_1 or R_2 is hydrogen.

15 According to one embodiment of such compositions, R_2 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, chloro, bromo and fluoro; and R_3 and R_4 are independently selected from the group consisting of hydrogen, lower alkyl, nitro, chloro, bromo and fluoro. According to another embodiment, at least one of R_2 , R_3 and R_4 is iodo.

20 According to one preferred embodiment of the invention, pharmaceutical compositions of compounds of formula I are provided wherein R_1 is hydrogen. More preferably, R_1 and R_3 are hydrogen, and R_2 and R_4 are independently selected from the group consisting of chloro, fluoro, iodo and bromo, most preferably selected from chloro, bromo and fluoro.

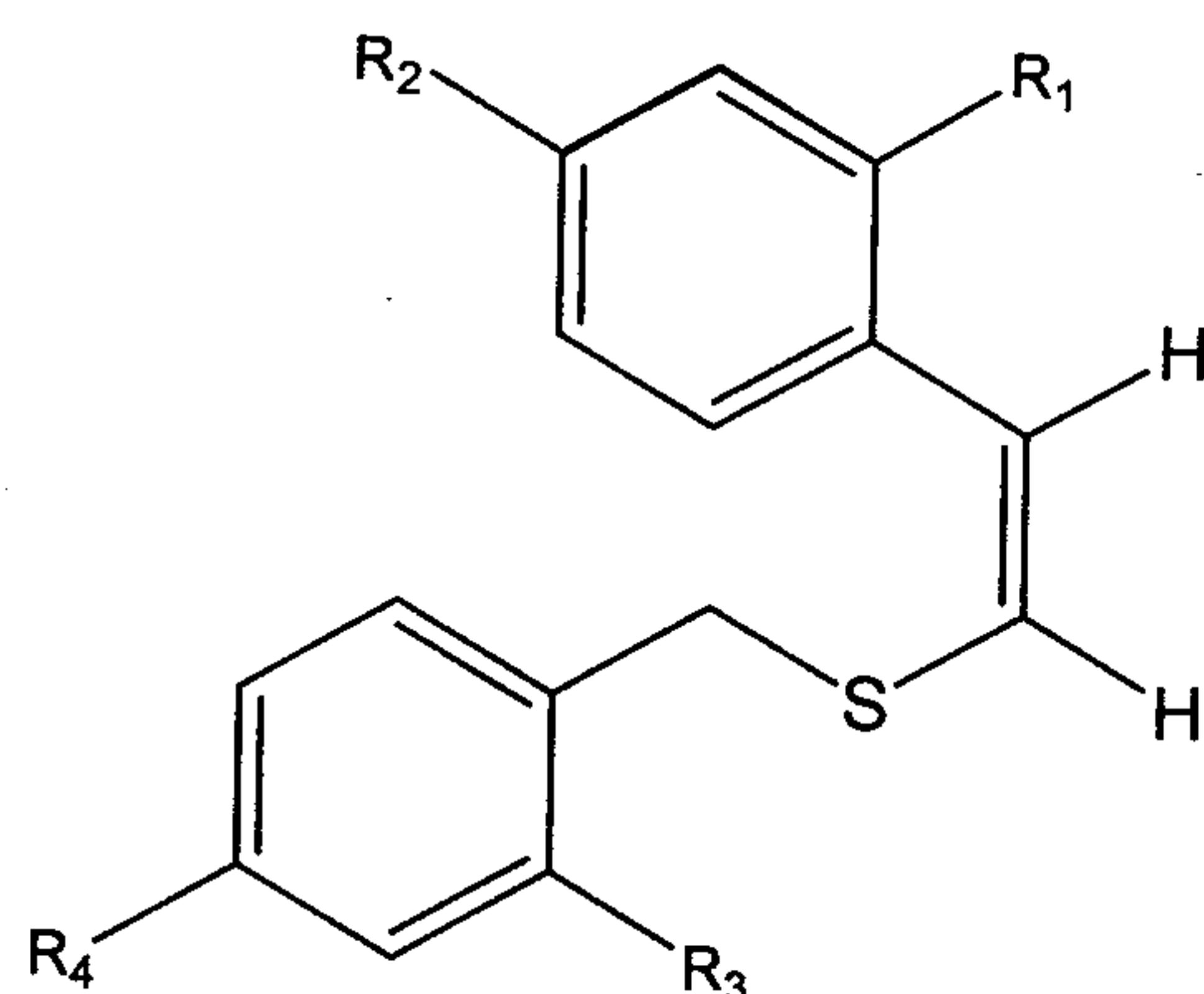
According to another embodiment of the invention, novel compounds of formula I are provided where R_1 , R_2 , R_3 and R_4 are defined as above, provided:

- (a) at least one of R_1 or R_2 is hydrogen;
- 5 (b) R_1 and R_2 may not both be hydrogen when:
 - (i) R_3 and R_4 are both hydrogen,
 - (ii) R_3 is chloro and R_4 is hydrogen, or
 - (iii) R_4 is chloro and R_3 is hydrogen; and
- (c) when R_1 is hydrogen and R_2 is methyl:
 - 10 (i) both R_3 and R_4 may not be hydrogen,
 - (ii) R_3 may not be chloro when R_4 is hydrogen, and
 - (iii) R_4 may not be chloro when R_3 is hydrogen.

According to one embodiment of novel compounds, R_2 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, chloro, bromo and fluoro; and R_3 and R_4 are independently selected from the group consisting of hydrogen, lower alkyl, nitro, chloro, bromo and fluoro. According to another embodiment, at least one of R_2 , R_3 and R_4 is iodo.

Preferably, R_1 is hydrogen in the novel compounds of the invention. More preferably, R_1 and R_3 are hydrogen, and R_2 and R_4 are independently selected from the group consisting of chloro, fluoro, iodo and bromo, most preferably selected from chloro, bromo and fluoro.

According to another embodiment of the invention, novel (Z)-styryl benzylsulfides are provided which are useful as intermediates in the preparation of the biologically active (Z)-styryl benzylsulfones. The (Z)-styryl benzylsulfides have the formula:



wherein:

R_1 is selected from the group consisting of hydrogen, chloro and nitro;

5 R_2 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, chloro, bromo, iodo and fluoro, provided that at least one of R_1 or R_2 is hydrogen;

R_3 and R_4 are independently selected from the group consisting of hydrogen, lower alkyl, nitro, chloro, bromo, iodo and fluoro; provided:

- 10 (a) at least one of R_1 or R_2 is hydrogen;
- (b) R_1 and R_2 may not both be hydrogen when:
- (i) R_3 and R_4 are both hydrogen,
 - (ii) R_3 is chloro and R_4 is hydrogen, or
 - (iii) R_4 is chloro and R_3 is hydrogen; and
- 15 (c) when R_1 is hydrogen and R_2 is methyl:
- (i) both R_3 and R_4 may not be hydrogen,
 - (ii) R_3 may not be chloro when R_4 is hydrogen, and
 - (iii) R_4 may not be chloro when R_3 is hydrogen.

20 According to one embodiment of the aforesaid intermediates, R_2 is selected from the group consisting of hydrogen, lower alkyl, lower alkoxy, chloro, bromo and fluoro; and R_3 and R_4 are independently selected from the group consisting of hydrogen, lower alkyl, nitro, chloro, bromo and

fluoro. According to another embodiment, at least one of R_2 , R_3 and R_4 is iodo.

Preferably, R_1 is hydrogen in the aforementioned intermediates. More preferably, R_1 and R_3 are hydrogen, and R_2 and R_4 are
5 independently selected from the group consisting of chloro, fluoro, iodo and bromo, most preferably selected from chloro, bromo and fluoro.

Where R_2 , R_3 and/or R_4 is halogen, the halogen is preferably selected from the group consisting of chloro, bromo and fluoro.

By "lower alkyl" is meant straight or branched chain alkyl
10 containing from one to six carbon atoms. The preferred alkyl group is methyl. By "lower alkoxy" is meant straight or branched chain alkoxy containing from one to six carbon atoms. The preferred alkoxy group is methoxy.

According to another embodiment of the invention, a method
15 of treating an individual for cancer or other proliferative disorder is provided, comprising administering to said individual an effective amount of the aforesaid pharmaceutical composition.

In another embodiment, a method of inhibiting growth of tumor cells in an individual afflicted with cancer is provided, comprising
20 administering to said individual an effective amount of the aforesaid pharmaceutical composition.

In another embodiment, a method of inducing apoptosis of cancer cells, more preferably tumor cells, in an individual afflicted with cancer is provided, comprising administering to said individual an effective
25 amount of the aforesaid pharmaceutical composition.

Detailed Description of the Invention

According to the present invention, certain (Z)-styryl sulfone derivatives selectively kill various tumor cell types without killing normal cells. Without wishing to be bound by any theory, it is believed that the
30 compounds affect the MAPK signal transduction pathway, thereby affecting

tumor cell growth and viability. This cell growth inhibition is associated with regulation of the ERK and JNK types of MAPK.

5 The compounds of the invention have been shown to inhibit the proliferation of various tumor cells by inducing cell death. The compounds are effective against a broad range of tumor types, including but not limited to the following: breast, prostate, ovarian, lung, brain (i.e, glioma) and renal. The compounds are also effective against leukemic cells. The compounds do not kill normal cells in concentrations at which tumor cells are killed.

10 Treatment of this broad range of tumor cells with the styryl sulfone compounds of the invention leads to inhibition of cell proliferation and induction of apoptotic cell death. In breast tumors, the effect is observed for estrogen receptor (ER) positive as well as estrogen receptor negative cells.

15 The compounds are also useful in the treatment of non-cancer proliferative disorders, including but not limited to the following: hemangiomas in new born, secondary progressive multiple sclerosis, chronic progressive myelodegenerative disease, neurofibromatosis, ganglioneuromatosis, keloid formation, Pagets Disease of the bone, fibrocystic disease of the breast, Peronies and Duputren's fibrosis, restenosis and cirrhosis.

20 Tumor cells treated with the compounds of the invention accumulate in the G2/M phase of the cell cycle. As the cells exit the G2/M phase, they appear to undergo apoptosis. Treatment of normal cells with the styryl sulfones does not result in apoptosis.

25 Both cells treated with the styryl sulfone compounds of the invention and untreated cells exhibit similar levels of intracellular ERK-2, but the biochemical activity of ERK-2, as judged by its ability to phosphorylate the substrate myelin basic protein (MBP), is considerably diminished in drug-treated cell compared to untreated cells. Without wishing to be bound by any theory, these results suggest that the styryl

30

sulfones of the present invention block the phosphorylating capacity of ERK-2.

5 The styryl sulfones of the present invention enhance the ability of JNK to phosphorylate c-Jun protein compared to mock-treated cells. Without wishing to be bound by any theory, this result suggests that the styryl sulfones may be acting like pro-inflammatory cytokines or UV light, activating the JNK pathway, which in turn may switch on genes responsible for cell growth inhibition and apoptosis.

10 Synthesis of (Z)- Styryl Sulfones

The compounds of the present invention were prepared by synthetic methods yielding pure compounds in the (Z)-isomeric configuration. Thus, the nucleophilic addition of the appropriate thiols to substituted phenylacetylene with subsequent oxidation of the resulting sulfide by hydrogen peroxide yields the Z- styryl sulfone. The procedure is generally described by Reddy et al., *Sulfur Letters* 13:83 (1991), the entire disclosure of which is incorporated herein as a reference.

20 The compounds are named according to the Cahn-Ingold-Prelog system, the IUPAC 1974 Recommendations, Section E: stereochemistry, in *Nomenclature of Organic Chemistry*, Pergamon, Elmsford, NY, 1979 (the "Blue Book").

25 In the first step of the synthesis, the sodium salt of benzyl mercaptan or the appropriate substituted benzyl mercaptan is allowed to react with phenylacetylene or the appropriate substituted phenylacetylene forming the pure Z- isomer of the corresponding styryl benzylsulfide in good yield.

In the second step of the synthesis, the (Z)-styryl benzylsulfide intermediate is oxidized to the corresponding sulfone in the pure Z- isomeric form by treatment with hydrogen peroxide.

General Procedure

A. Synthesis of intermediate sulfides

To a refluxing methanolic solution of substituted or unsubstituted sodium benzylthiolate prepared from 460 mg (0.02g atom) of (i) sodium, (ii) substituted or unsubstituted benzyl mercaptan (0.02 mol) and (iii) 80 ml of absolute methanol, is added freshly distilled substituted or unsubstituted phenylacetylene. The mixture is refluxed for 20 hours, cooled and then poured on crushed ice. The crude product is filtered, dried and recrystallized from methanol or aqueous methanol to yield a pure (Z)-styryl benzylsulfide.

B. Synthesis of sulfone

An ice cold solution of a (Z)-styryl benzylsulfide (3.0g) in 30 ml of glacial acetic acid is treated with 7.5 ml of 30% hydrogen peroxide. The reaction mixture is refluxed for 1 hour and then poured on crushed ice. The separated solid is filtered, dried, and recrystallized from 2-propanol to yield the pure (Z)-styryl benzylsulfone. The purity of the compounds is ascertained by thin layer chromatography and geometrical configuration is assigned by analysis of infrared and nuclear magnetic resonance spectral data.

Therapeutic Administration

The styryl sulfones of the invention may be administered in the form of a pharmaceutical composition, in combination with a pharmaceutically acceptable carrier. The active ingredient in such formulations may comprise from 0.1 to 99.99 weight percent. By "pharmaceutically acceptable carrier" is meant any carrier, diluent or excipient which is compatible with the other ingredients of the formulation and to deleterious to the recipient.

The compounds of the invention may be administered to individuals (mammals, including animals and humans) afflicted with breast

or prostate cancer. The compounds may be administered by any route, including oral and parenteral administration. Parenteral administration includes, for example, intravenous, intramuscular, intraarterial, intraperitoneal, intranasal, rectal, or subcutaneous administration. The
5 active agent is preferably administered with a pharmaceutically acceptable carrier selected on the basis of the selected route of administration and standard pharmaceutical practice.

The active agent may be formulated into dosage forms according to standard practices in the field of pharmaceutical preparations.
10 See Gennaro Alphonso, ed., *Remington's Pharmaceutical Sciences*, 18th Ed., (1990) Mack Publishing Co., Easton, PA. Suitable dosage forms may comprise, for example, tablets, capsules, solutions, parenteral solutions, troches, suppositories, or suspensions.

For parenteral administration, the active agent may be mixed
15 with a suitable carrier or diluent such as water, an oil, saline solution, aqueous dextrose (glucose) and related sugar solutions, or a glycol such as propylene glycol or polyethylene glycol. Solutions for parenteral administration preferably contain a water soluble salt of the active agent. Stabilizing agents, antioxidizing agents and preservatives may also be
20 added. Suitable antioxidizing agents include sulfite, ascorbic acid, citric acid and its salts, and sodium EDTA. Suitable preservatives include benzalkonium chloride, methyl- or propyl-paraben, and chlorbutanol.

For oral administration, the active agent may be combined with one or more solid inactive ingredients for the preparation of tablets,
25 capsules, or other suitable oral dosage forms. For example, the active agent may be combined with carboxymethylcellulose calcium, magnesium stearate, mannitol and starch, and then formed into tablets by conventional tableting methods.

The specific dose of compound according to the invention to
30 obtain therapeutic benefit will, of course, be determined by the particular circumstances of the individual patient including, the size, weight, age and sex of the patient, the nature and stage of the disease, the aggressiveness

of the disease, and the route of administration. For example, a daily dosage of from about 0.05 to about 50 mg/kg/day may be utilized. Higher or lower doses are also contemplated.

The practice of the invention is illustrated by the following
5 non-limiting examples.

Example 1

Z-styryl benzylsulfone

A solution of phenylacetylene (0.02 mol) and benzyl mercaptan (0.02 mol) and metallic sodium (0.02g atom) was subjected to
10 the General Procedure, part A, to form Z-styryl benzylsulfide. The title compound was obtained in 65% yield by oxidation of the sulfide according to the General Procedure, part B. ¹HNMR (CDCl₃) δ4.50 (2H, s), 6.65 (1H, d, J_{H,H} = 11.2), 7.18-7.74 (10H aromatic + 1H ethylenic).

Example 2

Z-styryl 4-chlorobenzylsulfone

A solution of phenylacetylene (0.02 mol) and 4-chlorobenzyl mercaptan (0.02 mol) and metallic sodium (0.02g atom) was subjected to
the General Procedure to form Z-styryl 4-chlorobenzylsulfide. The title
compound was obtained in 72% yield following oxidation. ¹HNMR (CDCl₃)
20 δ4.56 (2H, s), 6.68 (1H, d, J_{H,H} = 11.8), 7.20-7.64 (9H aromatic + 1H ethylenic).

Example 3

Z-styryl 2-chlorobenzylsulfone

A solution of phenylacetylene (0.02 mol) and 2-chlorobenzyl
25 mercaptan (0.02 mol) and metallic sodium (0.02g atom) was subjected to the General Procedure to form Z-styryl 2-chlorobenzylsulfide. The title compound was obtained in 68% yield following oxidation. ¹HNMR (CDCl₃) δ4.50 (2H, s), 6.65 (1H, d, J_{H,H} = 12.0), 7.18-7.74 (9H aromatic + 1H ethylenic).

Example 4**Z-styryl 4-fluorobenzylsulfone**

5 A solution of phenylacetylene (0.02 mol) and 4-fluorobenzyl mercaptan (0.02 mol) and metallic sodium (0.02g atom) was subjected to the General Procedure to form Z-styryl 4-fluorobenzylsulfide. The title compound was obtained in 70% yield following oxidation. ¹HNMR (CDCl₃) δ4.58 (2H, s), 6.62 (1H, d, J_{H,H} = 11.86), 7.18-7.60 (9H aromatic + 1H ethylenic).

Example 5**10 Z-4-chlorostyryl benzylsulfone**

A solution of 4-chlorophenylacetylene (0.02 mol) and benzyl mercaptan (0.02 mol) and metallic sodium (0.02g atom) was subjected to the General Procedure to form Z-4-chlorostyryl benzylsulfide. The title compound was obtained in 74% yield following oxidation. ¹HNMR (CDCl₃)
15 δ4.55 (2H, s), 6.66 (1H, d, J_{H,H} = 12.12), 7.16-7.65 (9H aromatic + 1H ethylenic).

Example 6**Z-4-chlorostyryl 4-chlorobenzylsulfone**

20 A solution of 4-chlorophenylacetylene (0.02 mol) and 4-chlorobenzyl mercaptan (0.02 mol) and metallic sodium (0.02g atom) was subjected to the General Procedure to form Z-4-chlorostyryl 4-chlorobenzylsulfide. The title compound was obtained in 76% yield following oxidation. ¹HNMR (CDCl₃) δ4.62 (2H, s), 6.68 (1H, d, J_{H,H} = 11.92), 7.18-7.60 (8H aromatic + 1H ethylenic).

25

Example 7**Z-4-chlorostyryl 2-chlorobenzylsulfone**

A solution of 4-chlorophenylacetylene (0.02 mol) and 2-chlorobenzyl mercaptan (0.02 mol) and metallic sodium (0.02g atom) was subjected to the General Procedure to form Z-4-chlorostyryl 2-

chlorobenzylsulfide. The title compound was obtained in 73% yield following oxidation. ^1H NMR (CDCl_3) δ 4.56 (2H, s), 6.70 (1H, d, $J_{\text{H,H}} = 12.05$), 7.18-7.64 (8H aromatic + 1H ethylenic).

Example 8

5 **Z-4-chlorostyryl 4-fluorobenzylsulfone**

A solution of 4-chlorophenylacetylene (0.02 mol) and 4-fluorobenzyl mercaptan (0.02 mol) and metallic sodium (0.02g atom) was subjected to the General Procedure to form Z-4-chlorostyryl 4-fluorobenzylsulfide. The title compound was obtained in 82% yield
10 following oxidation. ^1H NMR (CDCl_3) δ 4.60 (2H, s), 6.70 (1H, d, $J_{\text{H,H}} = 11.78$), 7.18-7.60 (8H aromatic + 1H ethylenic).

Example 9

Z-4-fluorostyryl benzylsulfone

A solution of 4-fluorophenylacetylene (0.02 mol) and benzyl
15 mercaptan (0.02 mol) and metallic sodium (0.02g atom) was subjected to the General Procedure to form Z-4-fluorostyryl benzylsulfide. The title compound was obtained in 76% yield following oxidation. ^1H NMR (CDCl_3) δ 4.54 (2H, s), 6.68 (1H, d, $J_{\text{H,H}} = 11.94$), 7.12-7.58 (9H aromatic + 1H ethylenic).

20

Example 10

Z-4-fluorostyryl 4-chlorobenzylsulfone

A solution of 4-fluorophenylacetylene (0.02 mol) and 4-chlorobenzyl mercaptan (0.02 mol) and metallic sodium (0.02g atom) was subjected to the General Procedure to form Z-4-fluorostyryl 4-chlorobenzylsulfide. The title compound was obtained in 82% yield
25 following oxidation. ^1H NMR (CDCl_3) δ 4.60 (2H, s), 6.68 (1H, d, $J_{\text{H,H}} = 11.84$), 7.18-7.60 (8H aromatic + 1H ethylenic).

Example 11**Z-4-fluorostyryl 2-chlorobenzylsulfone**

A solution of 4-fluorophenylacetylene (0.02 mol) and 2-chlorobenzyl mercaptan (0.02 mol) and metallic sodium (0.02g atom) was subjected to the General Procedure to form Z-4-fluorostyryl 2-chlorobenzylsulfide. The title compound was obtained in 74% yield following oxidation. ¹HNMR (CDCl₃) δ4.55 (2H, s), 6.66 (1H, d, J_{H,H} = 11.94), 7.20-7.65 (8H aromatic + 1H ethylenic).

Example 12**Z-4-fluorostyryl 4-fluorobenzylsulfone**

A solution of 4-fluorophenylacetylene (0.02 mol) and 4-fluorobenzyl mercaptan (0.02 mol) and metallic sodium (0.02g atom) was subjected to the General Procedure to form Z-4-fluorostyryl 4-fluorobenzylsulfide. The title compound was obtained in 78% yield following oxidation. ¹HNMR (CDCl₃) δ4.60 (2H, s), 6.65 (1H, d, J_{H,H} = 11.83), 7.20-7.65 (8H aromatic + 1H ethylenic).

Example 13**Z-4-bromostyryl benzylsulfone**

A solution of 4-bromophenylacetylene (0.02 mol) and benzyl mercaptan (0.02 mol) and metallic sodium (0.02g atom) was subjected to the General Procedure to form Z-4-bromostyryl benzylsulfide. The title compound was obtained in 80% yield following oxidation. ¹HNMR (CDCl₃) δ4.52 (2H, s), 6.80 (1H, d, J_{H,H} = 11.98), 7.18-7.59 (9H aromatic + 1H ethylenic).

Example 14**Z-4-bromostyryl 4-chlorobenzylsulfone**

A solution of 4-bromophenylacetylene (0.02 mol) and 4-chlorobenzyl mercaptan (0.02 mol) and metallic sodium (0.02g atom) was subjected to the General Procedure to form Z-4-bromostyryl 4-

chlorobenzylsulfide. The title compound was obtained in 87% yield following oxidation. ^1H NMR (CDCl_3) δ 4.58 (2H, s), 6.72 (1H, d, $J_{\text{H,H}} = 12.08$), 7.15-7.68 (8H aromatic + 1H ethylenic).

Example 15

5 **Z-4-bromostyryl 2-chlorobenzylsulfone**

A solution of 4-bromophenylacetylene (0.02 mol) and 2-chlorobenzyl mercaptan (0.02 mol) and metallic sodium (0.02g atom) was subjected to the General Procedure to form Z-4-bromostyryl 2-chlorobenzylsulfide. The title compound was obtained in 84% yield
10 following oxidation. ^1H NMR (CDCl_3) δ 4.57 (2H, s), 6.70 (1H, d, $J_{\text{H,H}} = 11.58$), 7.18-7.58 (8H aromatic + 1H ethylenic).

Example 16

Z-4-bromostyryl 4-fluorobenzylsulfone

A solution of 4-bromophenylacetylene (0.02 mol) and 4-fluorobenzyl mercaptan (0.02 mol) and metallic sodium (0.02g atom) was
15 subjected to the General Procedure to form Z-4-bromostyryl 4-fluorobenzylsulfide. The title compound was obtained in 78% yield following oxidation. ^1H NMR (CDCl_3) δ 4.58 (2H, s), 6.65 (1H, d, $J_{\text{H,H}} = 11.78$), 7.22-7.67 (8H aromatic + 1H ethylenic).

20

Example 17

Z-4-methylstyryl benzylsulfone

A solution of 4-methylphenylacetylene (0.02 mol) and benzyl mercaptan (0.02 mol) and metallic sodium (0.02g atom) was subjected to the General Procedure to form Z-4-methylstyryl benzylsulfide. The title
25 compound was obtained in 70% yield following oxidation. ^1H NMR (CDCl_3) δ 2.48 (3H, s), 4.60 (2H, s), 6.68 (1H, d, $J_{\text{H,H}} = 11.94$), 7.20-7.65 (9H aromatic + 1H ethylenic).

Example 18**Z-4-methylstyryl 4-chlorobenzylsulfone**

A solution of 4-methylphenylacetylene (0.02 mol) and 4-chlorobenzyl mercaptan (0.02 mol) and metallic sodium (0.02g atom) was subjected to the General Procedure to form Z-4-methylstyryl 4-chlorobenzylsulfide. The title compound was obtained in 74% yield following oxidation. ¹HNMR (CDCl₃) δ2.46 (3H, s), 4.64 (2H, s), 6.75 (1H, d, J_{H,H} = 12.21), 7.18-7.57 (9H aromatic + 1H ethylenic).

Example 19**Z-4-methylstyryl 2-chlorobenzylsulfone**

A solution of 4-methylphenylacetylene (0.02 mol) and 2-chlorobenzyl mercaptan (0.02 mol) and metallic sodium (0.02g atom) was subjected to the General Procedure to form Z-4-methylstyryl 2-chlorobenzylsulfide. The title compound was obtained in 76% yield following oxidation. ¹HNMR (CDCl₃) δ2.50 (3H, s), 4.58 (2H, s), 6.80 (1H, d, J_{H,H} = 11.88), 7.20-7.63 (9H aromatic + 1H ethylenic).

Example 20**Z-4-methylstyryl 4-fluorobenzylsulfone**

A solution of 4-methylphenylacetylene (0.02 mol) and 4-fluorobenzyl mercaptan (0.02 mol) and metallic sodium (0.02g atom) was subjected to the General Procedure to form Z-4-methylstyryl 4-fluorobenzylsulfide. The title compound was obtained in 69% yield following oxidation. ¹HNMR (CDCl₃) δ2.46 (3H, s), 4.62 (2H, s), 6.78 (1H, d, J_{H,H} = 11.98), 7.18-7.59 (9H aromatic + 1H ethylenic).

Example 21**Z-4-fluorostyryl 4-iodobenzylsulfone**

A solution of 4-fluorophenylacetylene (0.02 mol) and 4-iodobenzyl mercaptan (0.02 mol) and metallic sodium (0.02g atom) is subjected to the General Procedure to form Z-4-fluorostyryl 4-iodobenzylsulfide. The title compound is obtained following oxidation.

Example 22

Effect of Z-Styryl Sulfones on Breast, Prostate and Ovarian Tumor Cell Lines

A. Cells.

5 The effect of the Z-styryl sulfones on normal fibroblasts and on tumor cells of breast, prostate and ovarian origin was examined utilizing the following cell lines: breast tumor cell lines: MCF-7, BT-20 and 435; prostate tumor cell lines LnCaP and DU-145; and ovarian tumor cell lines OVCAR and SKOV3. NIH/3T3 and HFL cells, which are normal murine
10 and human fibroblasts, respectively, were also tested. LnCap is an androgen-dependent prostate tumor cell line. MCF-7 is an estrogen-responsive breast tumor cell line, while BT-20 and 435 are estrogen-unresponsive breast tumor cell lines. MCF-7, BT-20 and 435 were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal
15 bovine serum supplemented with penicillin and streptomycin. LnCaP and Du145 were cultured in RPMI with 10% fetal bovine serum containing penicillin and streptomycin. NIH3T3 and HFL cells were grown in DMEM containing 10% calf serum supplemented with penicillin and streptomycin. All cell cultures were maintained at 37°C in a humidified atmosphere of 5%
20 CO₂.

B. Treatment with Z-Styryl Sulfones and Viability Assay

Cells were treated with test compound at 2.5 mM concentration and cell viability was determined after 72 hours by the Trypan blue exclusion method. The results are set forth in Table 1.

25 Activity for each compound is reported as a range of cell induced death (% Death) with the lowest activity in the range of 10-20% and the highest being above 75%. For each compound tested, the activity was found to be in the same range for the three cell types.

30 Two of the twenty compounds tested (Examples 8 and 14) had kill rates of over 75%; three compounds (Examples 6, 10, and 16) had rates of 60-70%.

The five compounds exhibiting the highest activity contained halogen in the 4-position in Formula I.

Normal cells HFL and NIH 3T3 were treated with the same compounds in Table 1 under the same conditions of concentration and
5 time. The normal cells were not killed.

Table 1
Effect of (Z)- styryl benzyl sulfones on tumor cells

Ex.	R ₁	R ₂	R ₃	R ₄	Tumor cell type		
					Breast	Prostate	Ovarian
5	1	H	H	H	-	-	-
	2	H	H	Cl	+	+	+
	3	H	H	Cl	+	+	+
	4	H	H	F	+	+	+
	5	H	Cl	H	+	+	+
10	6	H	Cl	Cl	+++	+++	+++
	7	H	Cl	Cl	+	+	+
	8	H	Cl	F	++++	++++	++++
	9	H	F	H	+	+	+
	10	H	F	Cl	+++	+++	+++
15	11	H	F	Cl	+	+	+
	12	H	F	H	++	++	++
	13	H	Br	H	+	+	+
	14	H	Br	Cl	++++	++++	++++
	15	H	Br	Cl	+	+	+
20	16	H	Br	F	+++	+++	+++
	17	H	CH ₃	H	+	+	+
	18	H	CH ₃	Cl	+	+	+
	19	H	CH ₃	Cl	+	+	+
	20	H	CH ₃	F	+	+	+

25

The activity of the compounds at 2.5mM after 72 hours.
Breast cell lines : MCF-7, BT-20, 435
Prostate cell lines: LnCaP, DU-145
Ovarian cell lines : OVCAR, SKOV3

10-20% Death = -
20-25% = +
40-50% = ++
60-70% = +++
above 75% = ++++

Example 23**Effect of Z-Styryl Sulfones on Lung, Renal and Brain Tumor Cell Lines**

The procedure of Example 22 was followed for certain of the (Z)-benzylsulfones, substituting the following cancer cell lines: lung, N417 and H157; renal, CAKI-1 and CAKI-2; glioma, U87 and SW1088. The results are set forth in Table 2.

Table 2**Effect of (Z)- styryl benzyl sulfones on tumor cells**

Ex.	R ₁	R ₂	R ₃	R ₄	Tumor cell type		
					Lung	Renal	Glioma
5	H	Cl	H	H	+	+	+
6	H	Cl	H	Cl	+++	+++	+++
7	H	Cl	Cl	Cl	+	+	+
8	H	Cl	H	F	++++	++++	++++
10	H	F	H	Cl	+++	+++	+++
11	H	F	Cl	Cl	+	+	+
12	H	F	H	F	++	++	++
14	H	Br	H	Cl	++++	++++	++++
15	H	Br	Cl	Cl	+	+	+
16	H	Br	H	F	+++	+++	+++
18	H	CH ₃	H	Cl	+	+	+
20	H	CH ₃	H	F	+	+	+

The activity of the compounds at 2.5mM after 72 hours.

Lung cell lines: N417, H157

Renal cell lines: CAKI-1, CAKI-2

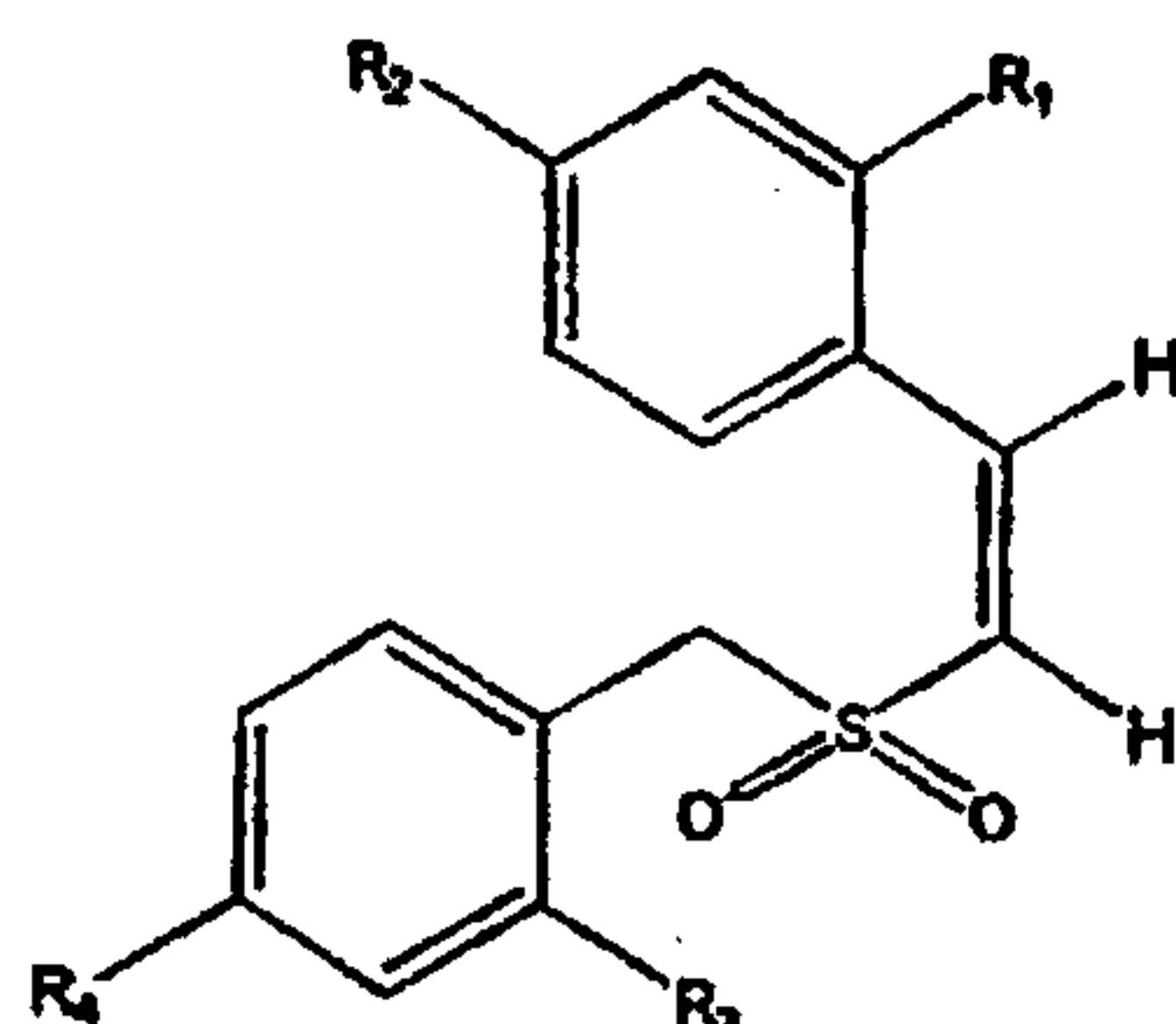
Glioma cell lines: U87, SW1088

10-20% Death	=	-
20-25%	=	+
40-50%	=	++
60-70%	=	+++
above 75%	=	++++

The present invention may be embodied in other specific forms without departing from the spirit or essential attributes thereof and, accordingly, reference should be made to the appended claims, rather than to the foregoing specification, as indication the scope of the invention.

CLAIMS

1. A compound of the formula:



wherein:

R_1 is hydrogen, chloro or nitro;

R_2 is hydrogen, straight or branched chain alkyl containing from one to six carbon atoms, straight or branched chain alkoxy containing from one to six carbon atoms, chloro, bromo, iodo or fluoro;

R_3 and R_4 are independently hydrogen, straight or branched chain alkyl containing from one to six carbon atoms, nitro, chloro, bromo, iodo or fluoro;

provided:

(a) at least one of R_1 or R_2 is hydrogen;

(b) R_1 and R_2 are not both hydrogen when:

(i) R_3 and R_4 are both hydrogen,

(ii) R_3 is chloro and R_4 is hydrogen, or

(iii) R_4 is chloro and R_3 is hydrogen; and

(c) when R_1 is hydrogen and R_2 is methyl:

(i) both R_3 and R_4 are not hydrogen,

(ii) R_3 is not chloro when R_4 is hydrogen, and

(iii) R_4 is not chloro when R_3 is hydrogen.

2. A compound according to claim 1, wherein:

R_2 is hydrogen, straight or branched chain alkyl containing from one to six carbon atoms, lower alkoxy, chloro, bromo or fluoro; and

R_3 and R_4 are independently hydrogen, straight or branched chain alkyl containing from one to six carbon atoms, nitro, chloro, bromo or fluoro.

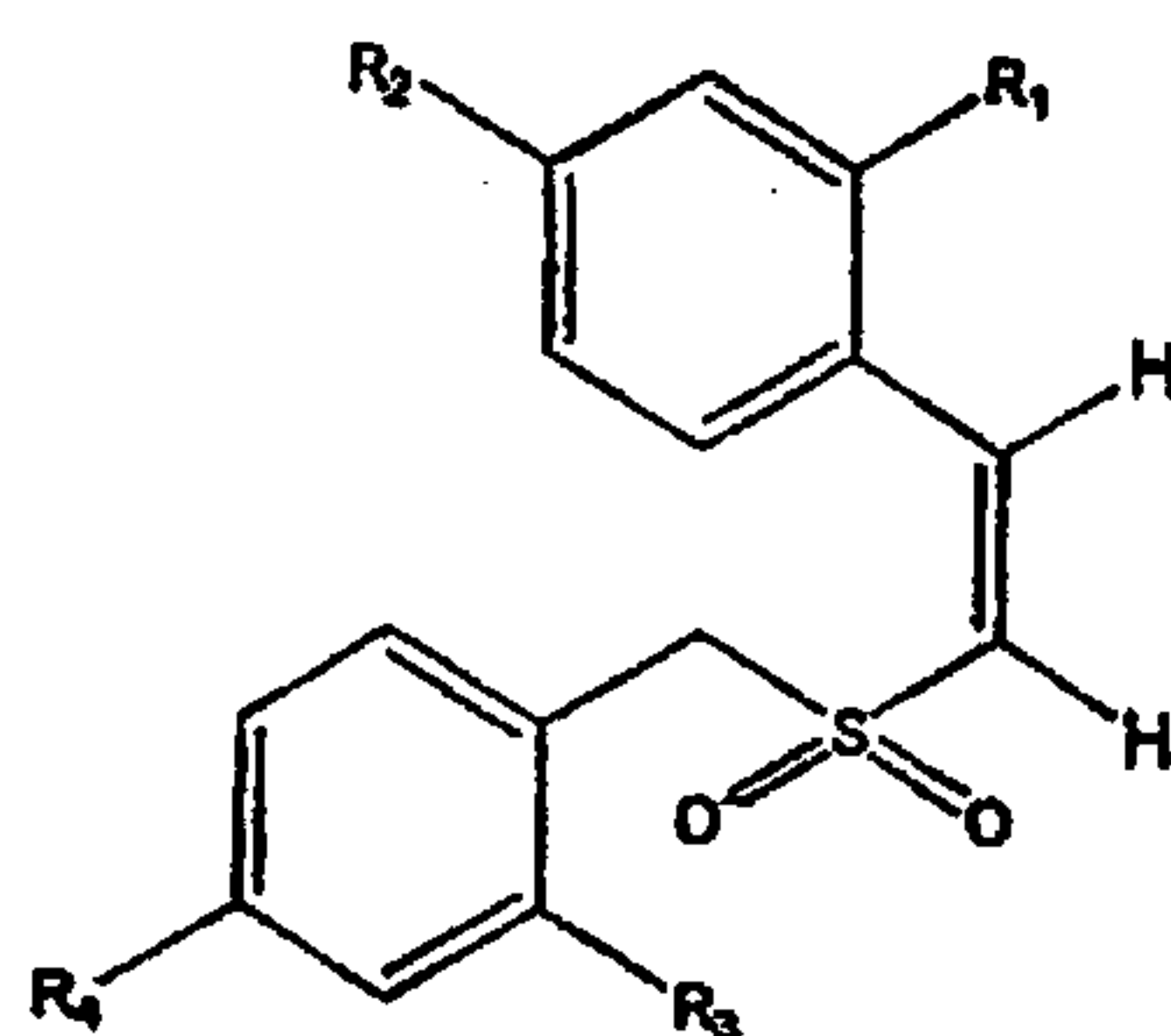
3. A compound according to claim 1, wherein at least one of R_2 , R_3 and R_4 is iodo.
4. A compound according to claim 1, wherein R_1 is hydrogen.
5. A compound according to claim 4, wherein:
 R_3 is hydrogen; and
 R_2 and R_4 are independently chloro, fluoro, iodo or bromo.
6. A compound according to claim 5, wherein the compound is Z-4-fluorostyryl 4-iodobenzylsulfone.
7. A compound according to claim 5, wherein R_2 and R_4 are independently chloro, fluoro or bromo.
8. A compound according to claim 7, wherein the compound is Z-4-chlorostyryl 4-chlorobenzylsulfone.
9. A compound according to claim 7, wherein the compound is Z-4-chlorostyryl 4-fluorobenzylsulfone.
10. A compound according to claim 7, wherein the compound is Z-4-fluorostyryl 4-chlorobenzylsulfone.

11. A compound according to claim 7, wherein the compound is Z-4-fluorostyryl 4-fluorobenzylsulphone.

12. A compound according to claim 7, wherein the compound is Z-4-bromostyryl 4-chlorobenzylsulphone.

13. A compound according to claim 7, wherein the compound is Z-4-bromostyryl 4-fluorobenzylsulphone.

14. A pharmaceutical composition comprising a pharmaceutically acceptable carrier, and a compound of the Formula:



wherein

R_1 is hydrogen, chloro or nitro;

R_2 is hydrogen, straight or branched chain alkyl containing from one to six carbon atoms, straight or branched chain alkoxy containing from one to six carbon atoms, chloro, bromo, iodo or fluoro; and

R_3 and R_4 are independently hydrogen, straight or branched chain alkyl containing one to six carbon atoms, nitro, chloro, bromo, iodo or fluoro;

provided at least one of R_1 and R_2 is hydrogen.

15. A pharmaceutical composition according to claim 14, wherein:
 R_1 is hydrogen, chloro or nitro;

R_2 is hydrogen, straight or branched chain alkyl containing from one to six carbon atoms, straight or branched chain alkoxy containing from one to six carbon atoms, chloro, bromo or fluoro; and

R_3 and R_4 are independently hydrogen, straight or branched chain alkyl containing from one to six carbon atoms, nitro, chloro, bromo or fluoro.

16. A pharmaceutical composition according to claim 14, wherein at least one of R_2 , R_3 and R_4 is iodo.
17. A pharmaceutical composition according to claim 15, wherein:
 - (a) R_1 and R_2 are not both hydrogen when:
 - (i) R_3 and R_4 are both hydrogen,
 - (ii) R_3 is chloro and R_4 is hydrogen, or
 - (iii) R_4 is chloro and R_3 is hydrogen; and
 - (b) when R_1 is hydrogen and R_2 is methyl:
 - (i) both R_3 and R_4 are not hydrogen,
 - (ii) R_3 is not chloro when R_4 is hydrogen, and
 - (iii) R_4 is not chloro when R_3 is hydrogen.
18. A pharmaceutical composition according to claim 14, wherein R_1 is hydrogen.
19. A pharmaceutical composition according to claim 18, wherein:

R_3 is hydrogen; and

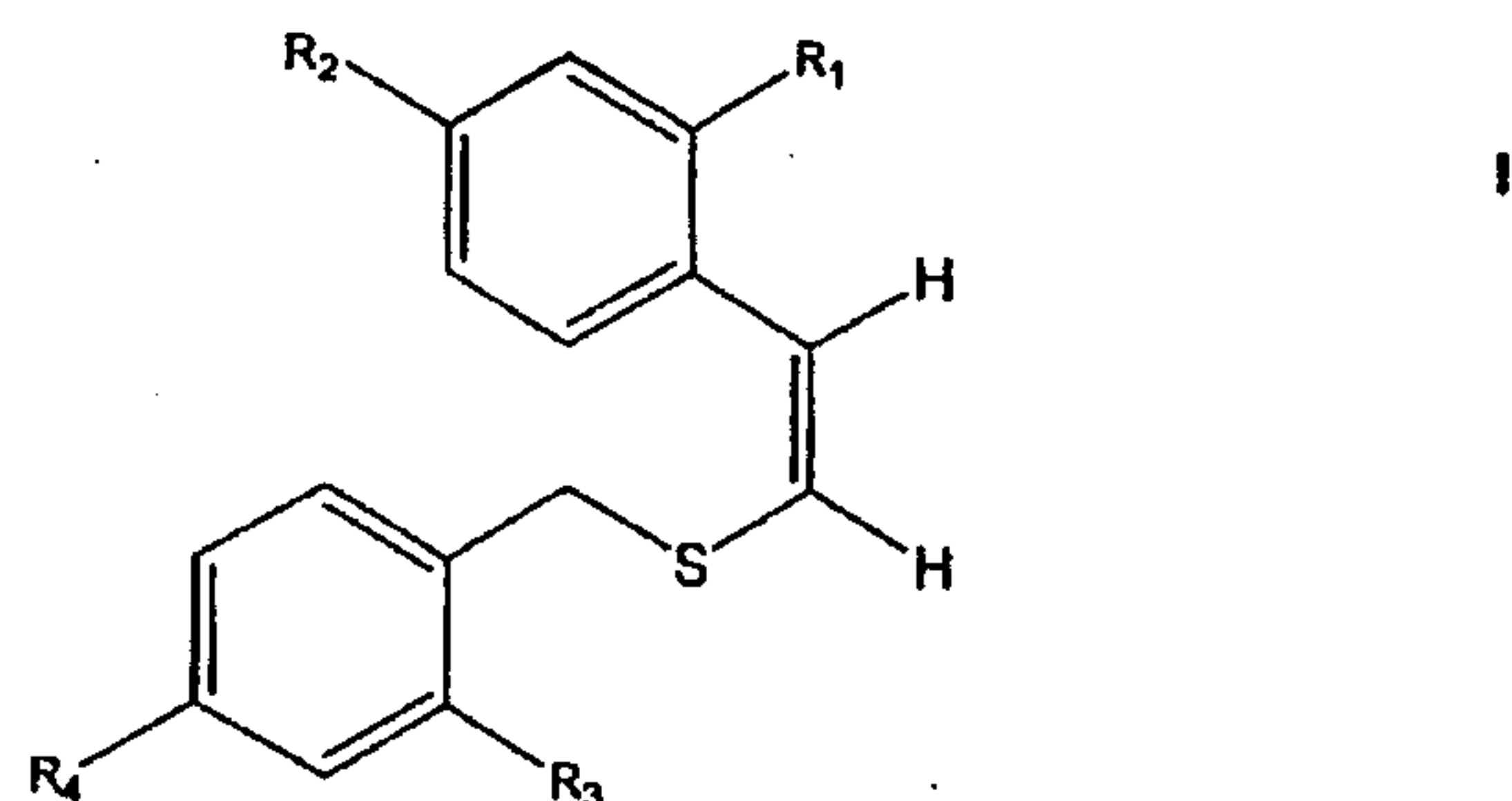
R_2 and R_4 are independently chloro, fluoro, iodo or bromo.
20. A pharmaceutical composition according to claim 19, wherein the compound is Z-4-fluorostyryl 4-iodobenzylsulphone.

21. A pharmaceutical composition according to claim 19, wherein R_2 and R_4 are independently chloro, fluoro or bromo.
22. A pharmaceutical composition according to claim 19, wherein the compound is Z-4-chlorostyryl 4-chlorobenzylsulphone.
23. A pharmaceutical composition according to claim 19, wherein the compound is Z-4-chlorostyryl 4-fluorobenzylsulphone.
24. A pharmaceutical composition according to claim 19, wherein the compound is Z-4-fluorostyryl 4-chlorobenzylsulphone.
25. A pharmaceutical composition according to claim 19, wherein the compound is Z-4-fluorostyryl 4-fluorobenzylsulphone.
26. A pharmaceutical composition according to claim 19, wherein the compound is Z-4-bromostyryl 4-chlorobenzylsulphone.
27. A pharmaceutical composition according to claim 19, wherein the compound is Z-4-bromostyryl 4-fluorobenzylsulphone.
28. The use of a composition according to claim 14, for treating an individual for a proliferative disorder.
29. The use according to claim 28, wherein the proliferative disorder is cancer.
30. The use according to claim 29, wherein the cancer is ovarian, breast, prostate, lung, renal or brain cancer, or the cancer is leukemia.

31. The use of a pharmaceutical composition according to claim 14, for inducing apoptosis of tumour cells in an individual afflicted with cancer.

32. The use according to claim 31, wherein the cancer is ovarian, breast, prostate, lung, renal or brain tumour, or the cancer is leukemia.

33. A compound of the formula



wherein

R_1 is hydrogen, chloro or nitro;

R_2 is hydrogen, straight or branched chain alkyl containing from one to six carbon atoms, straight or branched chain alkoxy containing from one to six carbon atoms, chloro, bromo, iodo or fluoro;

R_3 and R_4 are independently hydrogen, straight or branched chain alkyl containing from one to six carbon atoms, nitro, chloro, bromo, iodo or fluoro;

provided

(a) at least one of R_1 or R_2 is hydrogen;

(b) R_1 and R_2 are not both hydrogen when:

(i) R_3 and R_4 are both hydrogen,

(ii) R_3 is chloro and R_4 is hydrogen, or

(iii) R_4 is chloro and R_3 is hydrogen; and

(c) when R_1 is hydrogen and R_2 is methyl:

(i) both R_3 and R_4 are not hydrogen,

(ii) R_3 is not chloro when R_4 is hydrogen, and

(iii) R_4 is not chloro when R_3 is hydrogen.

34. A compound according to claim 33, wherein:

R_2 is hydrogen, straight or branched chain alkyl containing from one to six carbon atoms, straight or branched chain alkoxy containing from one to six carbon atoms, chloro, bromo or fluoro; and

R_3 and R_4 are independently hydrogen, straight or branched chain alkyl containing from one to six carbon atoms, nitro, chloro, bromo or fluoro.

35. A compound according to claim 33, wherein at least one of R_2 , R_3 and R_4 is iodo.

36. A compound according to claim 33, claim 34 or claim 35, wherein R_1 is hydrogen.

