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TITLE: COMPOSITION AND METHOD FOR TREATING CANCER

Fig. 1

Abstract: The present invention provides a compound for treating cancer including an isolated form of dibenzyl trisulfide (DTS) provided in an effective amount to act as an agent against human diseases, including various forms of cancer. The present invention also provides a compound for treating cancer including DTS isolated from Petiveria alliacea L. (guinea hen weed) for providing an effective, potent anti-proliferation and/or cytotoxic activity on a wide range of cancer cell lines. The present invention further provides DTS derivatives (e.g., DTS-albumin complexes) in effective dosage for providing a potent anti-proliferation and/or cytotoxic activity on a wide range of cancer cell lines. Additionally, the present invention provides methods of isolating and/or providing the DTS and/or its derivatives in an effective amount for providing a potent anti-proliferation and/or cytotoxic activity on cancer cell lines.
COMPOSITION AND METHOD FOR TREATING CANCER

Cross-reference to Prior Applications

[0001] This application claims the benefit of the following applications: U.S. Provisional Patent Application Serial No. 60/919,601, filed March 24, 2007 and U.S. Patent Application Serial No. 11/804,514, filed May 18, 2007 which are incorporated herein by reference in their entirety to the extent permitted by law.

U.S. Government Support

[0002] N/A

Background of the Invention

Area of the Art

[0003] The present invention relates generally to the treatment of various disease states with Dibenzyl Trisulfide (DTS). Further, the present invention relates to the use of DTS for cancer therapeutic treatment.

Description of the Background Art

[0004] The urgent need to find effective and safe therapeutic agents to treat cancers and auto-immune diseases such as Type 1 diabetes, lupus erythematosus, rheumatoid arthritis and multiple sclerosis has been one of the greatest challenges for the pharmaceutical companies (1). Recent studies have revealed that the top 150 propriety drugs used in the western hemisphere, 57 % contained at least one major active compound derived from natural sources (2). However, one of the major foci of the therapeutic industry is to find small molecules which regulate the biochemistry of disease cells via signal transduction modes of action (3), DTS is one such molecule (see Figure 1). Dibenzyl trisulfide was first coded as DBTS when its insecticidal/repellent activities were discovered (4) and re-coded as DTS when its therapeutic potential was found (5). The signal transduction pathways regulate cell biological processes e.g., gene expression, differentiation, cell division and apoptosis generated from interaction/binding of molecules to cell membrane receptors. One of the most intensely investigated therapeutic signal transduction pathway is that which regulates the process of apoptosis or programmed cell death. The apoptotic signal transduction cascade has been implicated in several cancers such as Hodgkin's lymphoma and in Alzheimer's disease, Parkinson's disease, acquired immune deficiency syndrome, transplant rejection as well as autoimmune disorder such as diabetes (6).
[0005] The art in this area includes U.S. Published Patent Application No. 2005/0261321, "Substituted organosulfur compounds and methods of using thereof." This application generally describes using DTS to treat cancer. It also generally mentions DTS formulated with bovine serum albumin, without any cytotoxic evaluation, summarizing Rosner et al. (7). Lastly the application describes using various R groups attached to a carbon molecule connecting the trisulfide to a benzyl group.

[0006] US Patent No. 6,555,712 as relevant to the possible use of DTS and its substituted cousins as therapeutic agents. The patent, entitled "Process for the preparation of diorganotrisulfide," provides a general method for synthesizing various diorganotrisulfides, including DTS and its substituted cousins.

[0007] An article entitled "Synthesis and anti anti-tumor evaluation of new trisulfide derivatives," was written by H. An, J. Zhu, X. Wang, and X. Xu and was published in Bioorganic & Medicinal Chemistry Letters, Volume 16, Issue 18, pages 4826-4829 (September 2006). The article reported information on the anti-tumor activities of DTS and several related species (involving substitution of at least one benzyl ring from the base DTS molecule).

[0008] However, none of the above formulations or methods of using DTS created an enhanced cytotoxic effect against cancer cells; thus there remains a need for such a molecule to more affectively treat cancer, among other ailments. The present invention is provided to solve the problems discussed above and other problems. A full discussion of the features and advantages of the present invention is deferred to the following detailed description, which proceeds with reference to the accompanying drawings.

Summary of the Invention

[0009] A first aspect of the present invention is to provide dibenzyl trisulfide (DTS), particularly as a naturally-occurring therapeutic agent against human diseases, including various forms of cancer. Another aspect of the present invention is to provide DTS isolated from Petiveria alliacea L. (guinea hen weed) for providing an effective, potent anti-proliferation and/or cytotoxic activity on a wide range of cancer cell lines. Yet another aspect of the present invention includes providing DTS derivatives in effective dosage for providing a potent anti-proliferation and/or cytotoxic activity on a wide range of cancer cell lines. Still another aspect of the present invention includes methods of isolating and/or providing the DTS and/or its derivatives in an effective amount for providing a potent anti-proliferation and/or cytotoxic activity on cancer cell lines.

[0010] These and other aspects of the present invention will become apparent to those skilled in the art after a reading of the following description of the preferred embodiment.
Description of the Figures

[0011] Figure 1 is a graphical representation of a DTS molecule according to the present invention.

Detailed Description of the Invention

[0012] In the following description, like reference characters designate like or corresponding parts throughout the several views. Also in the following description, it is to be understood that such terms as "forward," "rearward," "front," "back," "right," "left," "upwardly," "downwardly," and the like are words of convenience and are not to be construed as limiting terms. The description below is for the purpose of describing a preferred embodiment of the invention and is not intended to limit the invention thereto.

[0013] Also, throughout this document, numbers in parenthesis (e.g. "(31)") represent published references which can be found and correlated to the numbers at the end of this document. When used, a number in parenthesis represents that the correlating reference supports at least partially, but not necessarily entirely, the assertion in the sentence or the clause immediately preceding the number.

[0014] The present invention provides a compound for treating cancer including an isolated form of DTS provided in an effective amount to act as an agent against human diseases, including various forms of cancer. The present invention also provides a compound for treating cancer including DTS isolated from Petiveria alliacea L. (guinea hen weed) for providing an effective, potent anti-proliferation and/or cytotoxic activity on a wide range of cancer cell lines. The present invention further provides DTS derivatives in effective dosage for providing a potent anti-proliferation and/or cytotoxic activity on a wide range of cancer cell lines. Additionally, the present invention provides methods of isolating and/or providing the DTS and/or its derivatives in an effective amount for providing a potent anti-proliferation and/or cytotoxic activity on cancer cell lines.

[0015] Dibenzyl trisulfide has the molecular structure shown in Figure 1. The present invention contemplates that DTS is a polysulfide mitogen with a wide range of therapeutic implication in the field of medicine and biochemistry. The molecule signals via the MAPkinase (erK1 / erK 2) pathway.

[0016] The present invention contemplates that DTS in general causes hyper-phosphorylation which can lead to many beneficial effects, including its affect on Cadherin 5, which can lead to enhanced anti-cancer activity. Upregulation of Cadherin-5 through hyper-phosphorylation regulates the gap junction which is likely to reduce metastasis of cancer.
DTS can be extracted from a plant (*Petiveria alliacea*); it can also be synthesized in the laboratory. DTS has anticancer activity and acts to stimulate the production of stem cells from bone marrow and thymus with little toxic effect. DTS has been shown to inhibit growth of numerous cancer cells, including SH-SY5Y neuroblastoma, MCF-7 Mammary Carcinoma, IPC-melanoma, TE-671 Sarcoma, A549 lung cancer, leukemia, and lymphoma. The inhibition mechanism is understood presently to be through microtubule disruption and induced apoptosis. DTS also alters the immunogenicity of antigens through binding with the tyrosine residues in the antigens. It activates stem cells leading to the production of blood and bone marrow cells, with an increased level of phagocytes.

The anti-proliferation/cytotoxic activity of DTS was first discovered by Rosner’s group (7) using the human SH-SY5Y neuroblastoma cells, while the cell differentiation effects on HL-60 promyelocytic cells was reported (8). Subsequently, the IC50 values in μM concentrations (data in parentheses) were reported for DTS on several human cancer cell lines using different bioassay techniques (9,10); SH-SY5Y neuroblastoma (0.43 μM) (9), MCF-7 mammary carcinoma (2.24 μM and 6.6 μM) (9;10); IPC-melanoma (2.90 μM) (9); A549 small cell lung cancer (15.85 μM) (9); A637 primary bladder carcinoma (18.84 μM) (9); Jurkat leukemia (0.35 μM) (10); ovarian A2780 and OVCAR4 (0.40 μM and 1.4 μM, respectively (10); fibrosarcoma HT1080 (1.9 μM) (10); non-small cell lung cancer H460 (5.1 μM) (10); breast M231 (2.4 μM) (10); adenocarcinoma HeLa (2.5 μM) (10). In addition, it was reported that 0.56 μM of DTS gave 44.67 % anti-proliferation/cytotoxic activity on the human TE-671 sarcoma cell line (9). The cytotoxic action of DTS on the human SH-SY5Y neuroblastoma cell lines was enhanced by complexing DTS to Bovine Serum Albumin by 70 fold *in vitro* (11;12).

The present invention, using DTS-albumin and more specifically DTS-BSA complexes, provides enhancement in cytotoxicity up to about 2000 fold over DTS alone, as shown from experiments outlined below. Based on modes of action studies DTS could be useful against prostate cancers for the following reasons; DTS was found to enhance the binding of mitogen-activated protein kinases phosphatase 1 (MKP-1) to its substrate 3-O-methyl-fluorescein phosphate cyclohexyl ammonium salt (OMFP) *in vitro* (12). MKP-1 is a dephosphorylator of various MAPkinases including extracellular regulated kinases 1 and 2 (erk1,2) (13). It is known that in prostate cancers the phosphorylation of erk 1/2 are dramatically increased, up to 1600 % have been reported (14). Thus, if DTS enhanced the activity of the dephosphorylator MKP-1 it could be implicated in the treatment of prostate cancers, as a possible down regulator of the phosphorylation on erk1,2. In addition, MKP-1 is now recognized as a potential therapeutic target in cancer
chemotherapy (15). IPC-melanoma cells exposed to 1.0 µM of DTS undergo nuclear fragmentation to produce micronuclei, indicating mitotic catastrophe (9). The fragmentation of nucleus in cells exposed to cytotoxic agents is one of the diagnostic features of apoptosis. DTS has a strong binding affinity for albumin and RBCs, thus it may have implications for the treatment of cancers in the central nervous system. These findings indicate that DTS could be instrumental in cancer chemotherapy.

[0021] DTS can be derived several different ways. Preferably, according to the present invention, it is chemically synthesized. Alternatively, it can be isolated from Petiveria alliacea L (the guinea hen weed). Preferably, according to the present invention, DTS is bound to an albumin (e.g. bovine serum albumin, BSA), and is synthesized as follows to create the DTS-BSA complex:

1. Prepare a 0.05 % (w/v) stock solution of DTS in methanol, i.e. 5.0 mg of DTS in 10 ml of methanol.
2. Prepare a 0.2 % stock solution of Bovine Serum Albumin (BSA) in Tris acetate buffer pH 6.75, i.e. 2.0 mg BSA in 1.0 ml of the Tris buffer.
3. Combine the DTS solution from step 1 with the BSA solution from step 2 in a ratio of 1:10 (DTS:BSA v/v) e.g., 50 µ_ DTS to 500 µ_ BSA, and incubate for 12 hours at 7 °C in a low temperature refrigerator. NOTE: methanol will not distort the molecular configuration of albumins at concentrations less than 30% (v/v).

[0022] A summary of the results from an experiment involving the above synthesis method were as follows:

1. BSA in Tris acetate buffer at 1.0 mg/mL gave -2.72% anti-proliferation activity (i.e., it was a slight stimulator for the growth of the cells).
2. Tris acetate buffer pH 6.75 (10 µL/mL) gave 0.4 % anti-proliferation activity. DTS at 50 - 0.5 ng/mL + BSA (1.0 mg/mL) gave an average of 92.1 1 % anti-proliferation activity, at 0.05 ng/mL DTS + BSA anti-proliferation activity was lost.
3. DTS at 78 ng/mL (No BSA) gave 38.22 % anti-proliferation activity.
4. DTS at 39 ng/mL (No BSA) gave 1.68% anti-proliferation activity.

[0023] Below in Table 1 are the tabulated results from the above experiment:

Table 1: Anti-proliferation/cytotoxic Activity of DTS and BSA Complex

<table>
<thead>
<tr>
<th>Samples</th>
<th>Percentage anti-proliferation activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTS at 50 – 0.5 ng/mL+ BSA</td>
<td>90.00 to 92.11%</td>
</tr>
<tr>
<td>DTS at 1.25 µg/mL (No BSA)</td>
<td>93.00 %</td>
</tr>
<tr>
<td>DTS at 78 ng/mL (No BSA)</td>
<td>38.22 %</td>
</tr>
<tr>
<td>DTS at 39 ng/mL (No BSA)</td>
<td>1.68 %</td>
</tr>
<tr>
<td>BSA at 1.0 mg/mL</td>
<td>-2.72 %</td>
</tr>
<tr>
<td>BSA in Tris-acetate buffer</td>
<td>(slightly stimulatory)</td>
</tr>
<tr>
<td>Tris acetate buffer alone</td>
<td>0.4 %</td>
</tr>
</tbody>
</table>

[0024] From Table 1, the activity of DTS without BSA at 1.25 µg/mL (1250 ng/mL) is not significantly different from the anti-proliferation activity of DTS with BSA at 0.5 ng/mL. Thus, the enhancement in anti-proliferation activity is 1250 divided by 0.5, which equals 2500 fold.

[0025] Synthesizing DTS-BSA using the above steps causes DTS-BSA to have an unexpected and surprising enhancement in cytotoxic activity toward cancer cells. Doing such enhances the cytotoxic activity of DTS between 100 to 2500 fold on human SH-SY5Y neuroblastoma cells in vitro; as seen from the results from. The present invention contemplates that this enhanced activity is due to the specific bonding configuration and sites between DTS and albumin (e.g., Bovine Serum Albumin).

[0026] According to the preferred embodiment of the present invention, the steps described above create a specific DTS-BSA molecule that results in the enhanced cytotoxic activity mentioned above. In the DTS-BSA complex of the present invention, the hydroxyl of tyrosine amino acid in the BSA binds to the central sulfur atom of the trisulfide bridge in DTS (11). Generally DTS binds to an aromatic region of BSA (7). This region is rich in tyrosine residue and also has phenylalanine which is also an aromatic amino acid. The present invention's preferred synthesis steps described above result in DTS being bound to BSA at the hydroxyl of tyrosine in the BSA. Likewise the present invention's preferred synthesis steps result in the BSA being bound to DTS at the central sulfur atom of the trisulfide bridge in DTS.
According to the present invention, spectrophotometric absorbance binding experiments on pure tyrosine and phenylalanine were conducted and found that DTS binds only to tyrosine. Here is an outline of these experiments:

1. A plot of Absorbance (nm) with time (min.) of the interaction with tyrosine and DTS gives a normal distribution (Gaussian) with the highest optimum at room temperature.

2. As temperature is increased there is a lowering of the optimum on the graph until a straight line is created.

3. These experiments suggest that slight increases in temperature will break the hydrophobic bonds in the interaction between DTS and tyrosine.

4. Phenylalanine, which is an aromatic amino acid, gives a straight line at room temperature with DTS, while DTS and tyrosine give a Gaussian distribution; the height of the curve is a function of the ratio of the DTS used.

5. Thus, the absence of a Gaussian distribution with DTS and phenylalanine and the presence of the Gaussian distribution with DTS and tyrosine indicate that when DTS-BSA is synthesized according to the present invention, the DTS binds only to tyrosine on BSA.

Additionally, tyrosine is a signal transduction activation amino acid, while phenylalanine is not. Thus, the present invention contemplates that the MAP kinase signaling is emerging from the interaction of the DTS with the tyrosine residue in the receptors.

Further, the present invention contemplates that DTS will bind to any proteins or peptides that contained tyrosine (e.g., human serum albumin or bovine serum albumin) or peptides (e.g. pokeweed anti-viral proteins (PAPS), and monoclonal antibodies for immunotherapeutic applications (thus producing new drugs). Thus, the present invention contemplates that DTS binds hydrophobically to the tyrosine residues of peptides or proteins and may produce new drugs (because DTS binds to pure tyrosine amino acid). Any peptide or protein containing tyrosine DTS should interact or bind to these molecules leading to the formulation of other drugs. Also, DTS binds readily to red blood cells (RBCs) without lysis. Thus, DTS can cross the blood-brain barrier by being bound to a carrier protein (e.g., albumin) or on RBCs making it ideal for treating tumors and other diseases of the central nervous system.

The present invention provides an anti-cancer treatment method that includes, but is not limited to, the following steps. First, an organosulfur composition having dibenzyl
DTS is provided that is formulated to be bound to an albumin. Second, the DTS bound to the albumin is formulated to have an increased cytotoxic activity toward cancer cells on the order of about at least 100 times greater than the cytotoxic activity of DTS without albumin. Third, the DTS-albumin is administered to a patient suffering from cancer (and having cancer cells) in a therapeutically effective amount.

[0031] Preferably the albumin is BSA, thereby creating a DTS-BSA formulation. Alternatively, the albumin could be human serum albumin (HSA), or other albumins. When bound to BSA, the present invention provides increased cytotoxic activity toward cancer cells on the order of between about 100 times and about 2500 times greater than the cytotoxic activity of DTS without BSA.

[0032] The DTS-albumin formulation of the present invention can be administered to a patient in a variety of different ways. The administration includes intravenous administration, oral administration, topical administration, parenteral administration, and targeted administration to the cancer cells (e.g. direct injection into a cancerous mass or by molecular selectivity for markers on cancer cells, etc.). Preferably, the administration is intravenous.

[0033] The DTS-albumin formulation of the present invention can also be administered to a patient with a formulation dosage that is predetermined based upon data specific to that patient. For example, weight, metabolic factors, genetic factors, etc. can contribute to varying predetermined dosage amounts that are used to prescribe the specific predetermined dosage to the patient. Preferably the formulation dosage is between about 0.023 mg/kg and about 2.3 mg/kg body weight. The doses were calculated based on a DTS dose of 11 mg/kg body weight which stimulated the immune system in mice which was then co-related to a 240 ng/mL the effective in vitro dose. The DTS-BSA complex was active at 0.5 ng/mL in vitro dose, a ratio of about 480. Thus, 11 divided by 480 is about 0.023 mg/Kg body weight (minimum). Similarly, the maximum is about 2.3 mg/Kg body weight. If we assumed that the maximum weigh of a human to be treated with the DTS-albumin complex is 70 Kg, then the upper limit would be 0.023 X 70 = 1.61 mg/70 kg body weight.

[0034] The present invention further contemplates that DTS may be used to treat other disease states from the same mechanism of up-regulating ERK-1 and ERK-2 pathways and Cadherin 5 to lead to memory enhancement and treatment of diabetic retinopathy, respectively. For instance, the hyper-phosphorylation of ERK 1 and ERK 2 by DTS leads to the release of acetylcholine at the synaptic junction of brain cells which is fundamental to memory enhancement. Also, DTS may stimulate stem cells (including granulocytes)
from bone marrow. DTS additionally may increase thymus mass which leads to the production of more cytotoxic lymphocytes. The following subsections outline the mechanisms of action for the above and other disease states.


[0036] Data obtained from Human Mixed Lymphocytes Responses and CD3 dependent activation revealed that DTS down regulates Th-1 cytokines (11), to which the pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), Interleukin-6 (IL-6), IL-1 (b) and IL-8 belong. At the same time the Th-2 cytokines such as IL-4 were up-regulated. The preferential up-regulation of the IL-4 group of cytokines is an important observation since they are known to regulate the reticuloendothelial system (bone marrow functions) via the MAPkinase signaling pathway. DTS caused an increased in the production of granulocytes and erythrocytes (5; 11), suggesting that it has an effect on bone marrow activity, possible on stem cells. Elevation in the levels of pro-inflammatory cytokines is associated with the onset of auto-immune diseases such as type-1 diabetes, rheumatoid arthritis, multiple sclerosis and lupus erythematosus (16; 17).

[0037] DTS caused an increase in thymic weight in old mice (5; 11), histological analyses of the thymic sections revealed that there was a proliferation of cells in the cortical region infiltrating the medulla (11). It is recognized that mitogenesis (MAPKinase/p21ras) signaling pathway activated from the immuno-receptor tyrosine based activating motifs (ITAMS) within the CD3-TCR receptor complex is critical for positive/negative thymic cell production (18). Cancer patients’ ability to generate T-lymphocytes is inversely related to their age, suggesting an indirect contribution from thymic involution (19). Similarly, the recovery of CD4+ cells is inversely related to age and was enhanced in patients with an enlarge thymus after chemotherapy (19). The functional status of the thymus is regulated by an immuno-endocrine-neurological input emerging from the hypothalamus-pituitary axis (19). The above finding is not surprising because of the fact that there is an interconnectivity of the immune, hormonal and nervous systems via the Zn++ dependent activating Sigma receptor which is mediated via a selective up-regulation of Th-2 cytokines e.g., IL-4 and IL-10 in conjunction with thymulin. Thus, potent cytotoxic effect of DTS on melanoma cells which carry Sigma receptors supports the fact that DTS could be a Sigma receptor agent. Presently, the Sigma receptor group of therapeutic drugs is being critically evaluated for treating/managing various forms of human leukocyte antigen-DR (HLA-DR) immune system dysfunction diseases such as diabetes, osteoarthritis, acquired immune deficiency syndrome (AIDS), asthma and cancers. In addition, the loss of a functional thymus or its absence is common among individuals with immuno compromised congenital diseases e.g., DiGeorge Syndrome (DGS), Chromosomal
Breaking Syndromes (CBSs). The thymus is one of the sites producing cytotoxic lymphocytes (CTLs) which produced the serine protease enzyme granzyme B, a potent inducer of apoptosis in cancer cells and should be assessed. Thus, the thymic enlargement effect of DTS is worthy of detailed investigations.

[0038] The manipulation of various homing factors e.g., L-selectin-mAb LM1-3 and addressin-mAdCAM-1 and integrins such as alpha4beta7 as therapeutic targets on Peyer's patches dendritic cells is a rapidly expanding field in drug development against intestinal bowel diseases (IBD) e.g. colitis, colorectal carcinoma and Crohn's disease (20;21). DTS caused an enlargement in Peyer's patches possible by an activation of cell proliferation. Thus, based on this finding we hereby proposed that the effect of DTS on the Peyer's patches should be elucidated with the hope of developing drugs against IBD.

[0039] Implication of the up-regulation of Cadherin-5.

[0040] DTS upregulates the expression of cadherin-5 in human SH-SY5YTRK-A neuroblastoma cells pre-treated with nerve growth factor (NGF) (11). Cadherin-5 is one of the important factors responsible for the stabilization of tight junctions. The loss of cadherin-5 mediated adhesion has been known to play an important role in the transition of epithelial tumors from a benign to an invasive state (22) and also is important in diabetic retinopathy. Thus, beside the direct toxic effect that DTS has on various forms of cancer cells, it also has the ability to inhibit their metastasis via the up-regulation of cadherin-5.

[0041] Implication of the inhibitory effect of DTS on glycation.

[0042] From 1D 1H NMR studies DTS interacts with BSA at two main sites: (a) at 1.6 - 3.4 ppm (multiplet) and (b) at 6.8 ppm (11). The first site could be attributed to an interaction up on the redox sensitive epsilon-lysine envelope or on threonine. The second interaction signal is associated with the tyrosine envelope of the BSA (11). DTS was found to inhibit the binding of glucose to BSA. From the above findings the following hypothesis could be made; that DTS is interacting with the epsilon-lysine residue in the BSA which suggests that the Amadori rearrangement binding interaction was blocked. The Amadori interaction (glycation) between proteins and glucose is central to the generation of free radicals and destruction of proteins in the body and is the cause of various degenerative diseases such as arterial stiffening, cataracts and neurological impairment (23). Therefore, it would appear that DTS could be developed as an anti-glycation molecule to halt the rapid progression of some degenerative diseases associated with free radicals and stress. It is also interesting to note that DTS is able to
protect BSA from denaturation by 50.83% at 500 ng/mL (0.5 µg/mL) at pH 6.0 (11). This is a feature of non-steroidal anti-inflammatory drugs (24; 25).

[0043] The implication of DTS/growth factor induced hyper-phosphorylation of MAPKinase (erk 1 and erk 2).

[0044] DTS hyper-phosphorylates MAPKinase (erk 1 and erk 2) signaling induced by growth factors e.g., basic fibroblast growth factor (bFGF) by 30% at 1.0 µM and nerve growth factor (NGF) at 0.5 µM (7; 11). The MAPKinase pathway is required for both long-term recognition memory and is associated with hyper-phosphorylation of erk 1 and erk 2 in different sub-region of the entorhinal cortex-hippocampal circuitry (26). In addition, the medial division of the medial geniculate nucleus and adjacent posterior intralaminar nucleus (MGm/PIN) cells that project to the lateral nucleus of the amygdale (LA) contribute to memory formation via erk 1 and erk 2 mediated transcriptions (27). It is also interesting to note that P. alliacea, from which DTS was isolated is used by the Amerindians of Latin America to improve memory (28).

[0045] Implication of DTS induced cell-cell attraction possible through a polarization effect on ankyrins.

[0046] The present invention contemplates that DTS to be effective against many disorders beyond cancer. Erythrocytes (red blood cells, RBCs) separated from white blood cells (WBCs) interact with DTS in buffer induced cell-cell attraction with morphological changes without lysis at concentrations higher than those effective on cancer cells (11; 32). Spectrins are responsible for maintaining the morphological integrity of RBCs. Since DTS interacts with the tyrosyl residues on albumins e.g., BSA (7; 11), the present invention contemplates that the attraction induced by DTS among the RBCs could be on a polarized tyrosine rich domain such as ankyrins (32) and not a classical agglutination effect. 1D 1H NMR analyses revealed that the interaction between DTS and RBCs gave a similar aromatic signal to that observed for the DTS-BSA interaction (11), which suggest that tyrosine is also involved. Ankyrins are tyrosine rich protein domains on the surface of red blood cells (RBCs). Also, ankyrins are present on various mammalian cell types including the membranes of nervous tissues, more specifically at the nodes of Ranvier. Thus, from these observations the present invention contemplates that DTS may activate ankyrin domains located on neurons which are capable of inducing attraction and growth in these cells by causing their redistribution to the plasma membrane with spectrin involvement (29). The above mentioned phenomenon involves the phosphorylation on tyrosine residues in the ankyrins (33), which is one of the central binding modes of DTS leading to the activation of the MAPKinase pathway (7; 11). MAPKinase phosphorylation
emerging from tyrosine residue situated on axons is one of the processes implicated in
the regeneration/growth and possible repair of damaged neurons (34).

[0047] Therefore, the present invention contemplates that DTS should be able to
increase the inter-surface connectivities between cells which have tyrosine residues in
their ankyrins binding domains. Thus, in an infant where neuronal inter-connectivity is
rapidly developing is exposed to DTS the molecule may enhanced these cell-cell inter-
connections; this may lead to enhancing the memory in the infant or child.

[0048] In addition, ankyrins are now found to be important in several demyelinating
human diseases including Multiple Sclerosis. The functions of ankyrins are now been
heavily examined on glial cells node formation. Further, DTS could be important in the
activation of dendritic cells, based on its ability to increase Peyer's patches and thymic
masses, which is a rapidly expanding field in cancer chemotherapy.

[0049] **DTS as a pharmaceutical prototype.**

[0050] Unlike the complex nature of some of the most promising anti-cancer drugs such
as Taxol® and vinblastine; DTS is a simple molecule. Thus, several derivatives of DTS
were produced at low cost, some with higher cytotoxic activities (10; 30). In addition, DTS
was transformed using 2-mercaptoethanol in methanol to methyl benzyl sulfinic
anhydride, a molecule with potent anti-microbial and agrochemical activities (31).

[0051] **Toxicity.**

[0052] DTS did not have any effect on the sensitive process of protein biosynthesis in
Starfish embryos (11). In addition, it seems to have some degree of selectivity to
pathological cells, since it was found not to be toxic to the human fibroblast (HOFA) cell
line over seven days(9). In addition, concentration of up to 30 mg/kg body weigh was not
toxic to mice. DTS seems to activate the bone marrow at 10 mg/kg body weight since
granulocytes differential count was increased by 50 % (5).

[0053] Certain modifications and improvements will occur to those skilled in the art upon
a reading of the foregoing description. By way of example, albumin may be substituted
with other tyrosine containing peptides or proteins to serve as carrier molecules. The
above mentioned examples are provided to serve the purpose of clarifying the aspects of
the invention and it will be apparent to one skilled in the art that they do not serve to limit
the scope of the invention. All modifications and improvements have been deleted herein
for the sake of conciseness and readability but are properly within the scope of the
following claims which are thus to be understood to include what is specifically illustrated
and described above, what is conceptually equivalent, what can be obviously substituted
and also what essentially incorporates the essential idea of the invention. Those skilled in the art will appreciate that various adaptations and modifications of the just-described preferred embodiment can be configured without departing from the scope of the invention.

[0054] References


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What is claimed is:

1. A method for providing an anti-cancer treatment comprising the steps of:
   - providing an organosulfur composition having dibenzyl trisulfide (DTS) formulated to be bound to an albumin, wherein the DTS bound to the albumin is formulated to have an increased cytotoxic activity toward cancer cells on the order of between at least about 100 times and 2500 times greater than the cytotoxic activity of DTS without albumin; and
   - administering the formulation in a therapeutically effective amount to a patient having the cancer cells.

2. The method of claim 1, wherein the albumin is bovine serum albumin (BSA).

3. The method of claim 2, wherein the DTS bound to the BSA is formulated to have an increased cytotoxic activity toward cancer cells on the order of about at least 100 times greater than the cytotoxic activity of DTS without BSA.

4. The method of claim 2, wherein the DTS bound to the BSA is formulated to have an increased cytotoxic activity toward cancer cells on the order of between about 100 times and 2500 times greater than the cytotoxic activity of DTS without BSA.

5. The method of claim 1, wherein the step of administering includes intravenous administration to the patient.

6. The method of claim 1, wherein the step of administering includes topical administration to the patient.

7. The method of claim 1, wherein the step of administering includes oral administration.

8. The method of claim 1, wherein the step of administering includes targeted administration to the cancer cells.
9. The method of claim 1, wherein the formulation dosage for administration is predetermined based upon data specific to each patient.

10. The method of claim 2, wherein the formulation dosage is between about 0.023 mg/kg and about 2.3 mg/kg body weight.

11. A composition for treating cancer patients comprising: an organosulfur composition comprising dibenzyl thsulfide (DTS) formulated to be bound to an albumin, wherein the DTS bound to the albumin is formulated to have an increased cytotoxic activity toward cancer cells on the order of between at least about 100 times and 2500 times greater than the cytotoxic activity of DTS without albumin and wherein the composition is formulated in a therapeutically effective amount for a patient having the cancer cells.

12. The composition of claim 11, wherein the albumin is bovine serum albumin (BSA).

13. The composition of claim 12, wherein the DTS bound to the BSA is formulated to have an increased cytotoxic activity toward cancer cells on the order of about at least 100 times greater than the cytotoxic activity of DTS without BSA.

14. The composition of claim 12, wherein the DTS bound to the BSA is formulated to have an increased cytotoxic activity toward cancer cells on the order of between about 100 times and 2500 times greater than the cytotoxic activity of DTS without BSA.

15. The composition of claim 11, wherein the formulation is functionally active in an intravenous administration to the patient.

16. The composition of claim 11, wherein the formulation is functionally active in a targeted administration to the cancer cells.

17. The composition of claim 11, wherein the formulation dosage for administration is predetermined based upon data specific to each patient.

18. The composition of claim 11, wherein the formulation dosage is between about 0.023 mg/kg and about 2.3 mg/kg body weight.
19. A method of binding dibenzyl trisulfide (DTS) to an albumin comprising the steps of:
   a. preparing an about 0.05 % (w/v) stock solution of DTS in methanol;
   b. preparing an about 0.2 % stock solution of albumin in tris acetate buffer with a pH of about 6.75;
   c. combining the DTS solution with the albumin solution at a ratio of about 1 : 10 (DTS : albumin; v/v); and
   d. incubating the combined DTS and albumin solution for a specified period of time at about 7 degrees Celsius.

20. The method of claim 19, wherein the albumin is selected from the group consisting of bovine serum albumin, human serum albumin, and combinations thereof.
Fig. 1
A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/105(2006.01)i, A61K 38/38(2006.01)i, C07K 14/765(2006.01)i, A61P 35/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 8 as above

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

- Korean Utility models and applications for Utility models since 1975
- Japanese Utility models and applications for Utility models since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKIPASS(KIPO internal), STN(MedLine), "Keyword dibenzyl trisulfide, cancer and albumin"

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
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<td>Y</td>
<td>ROSNER, H et al &quot;Disassembly of microtubules and inhibition of neurotite outgrowth, neuroblastoma cell proliferation, and MAP kinase tyrosine dephosphorylation by dibenzyl trisulfide &quot; Biochim Biophys Acta 22 Aug 2001, Vol 1540, No 2, pp 166-177 See pages 167, 169 and fig 4</td>
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<td>A</td>
<td>US 2005/0261321 A1 ( XU et al ) 24 Nov 2005 See column 1 and claims 10-24</td>
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<td>A</td>
<td>WILLIAMS, LAD et al &quot;A sulfonic anhydride derivative from dibenzyl trisulfide with agro-chemical activities &quot; Chemosphere June 2003, Vol 51, No 8, pp 701-706 See page 702</td>
<td>11-20</td>
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* Special categories of cited documents

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

18 JUNE 2008 (18 06 2008)

Date of mailing of the international search report

18 JUNE 2008

Name and mailing address of the ISA/KR

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Form PCT/ISA/210 (second sheet) (April 2007)
### INTERNATIONAL SEARCH REPORT

<table>
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<tr>
<th>Box No. II</th>
<th>Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)</th>
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| 1 | [37] Claims Nos 1-10 because they relate to subject matter not required to be searched by this Authority, namely  
Claims 1-10 pertain to methods for treatment of the human or animal body by therapy/diagnostic methods, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39 (iv) of the Regulation under the PCT, to search |
| 2 | □ Claims Nos because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically |
| 3 | □ Claims Nos because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 64(a) |

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<td>□ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims</td>
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<td>□ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee</td>
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<td>4</td>
<td>□ No required additional search fees were timely paid by the applicant Consequently, this international search report is restricted to the invention first mentioned in the claims, it is covered by claims Nos</td>
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**Remark on Protest**

- □ The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee  
- □ The additional search fees were accompanied by the applicant’s protest but the applicable protest fee was not paid within the time limit specified in the invitation  
- □ No protest accompanied the payment of additional search fees
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