(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
13 September 2007 (13.09.2007)

PCT

(10) International Publication Number
WO 2007/103273 A2

(51) International Patent Classification:
A61K 31/41 (2006.01)

(21) International Application Number:
PCT/US2007/005530

(22) International Filing Date: 2 March 2007 (02.03.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/778,876 3 March 2006 (03.03.2006) US

(71) Applicant *(for all designated States except US)*: **TRUSTEES OF BOSTON UNIVERSITY** [US/US]; One Sherborn Street, Boston, MA 02215 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **SCHAUS, Scott, E.** [US/US]; 43 St. Germain Street, Apt.3, Boston, MA 02115 (US). **EASTWOOD, Erin, L.** [US/US]; 9 Winthrop Street, Charlestown, MA 02120 (US).

(74) Agent: **BAKER, C., Hunter**; Choate, Hall & Stewart LLP, Two International Place, Boston, MA 02110 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2007/103273 A2

(54) Title: THIOREDOXIN AND THIOREDOXIN REDUCTASE INHIBITORS

(57) Abstract: The present invention relates to sulfone derivatives and to their use as modulators of the thioredoxin/thioredoxin reductase redox system, including for the treatment and/or prevention of pathophysiological conditions mediated by thioredoxin/thioredoxin reductase, such as cancer, HIV/ AIDS, Alzheimer's disease, rheumatoid arthritis, and skin disorders. Also provided are pharmaceutical compositions comprising the inventive sulfones.

Thioredoxin and Thioredoxin Reductase Inhibitors

Related Applications

[1] The present application claims priority from Provisional Application U.S.S.N. 60/778,876 filed on March 3, 2006 and entitled "Thioredoxin and Thioredoxin Reductase Inhibitors". The Provisional Application is incorporated herein by reference in its entirety.

Government Support

[2] Some of the work described herein was funded by the National Institutes of Health (Grant No. P50 GM67041) and National Science Foundation (Grant No. CHE-0349206). The United States government may have certain rights in the invention.

Background of the Invention

[3] A critical step in drug development is the optimization of therapeutic efficacy and the minimization of undesirable side-effects on a candidate drug. Ideally, drug optimization is carried out using knowledge of the drug's mode of action, *i.e.*, the molecular targets that mediate its therapeutic effects and side effects. For many drug candidates, however, the targets are unknown or difficult to identify among the thousands of gene products in a typical genome.

[4] DNA microarray technology enables the simultaneous observation of all genes with a transcriptional response to a compound treatment, and thus provides an opportunity to efficiently identify a compound's targets (M. Schena *et al.*, Science, 1995, 270: 467-479; M Schena *et al.*, Proc. Natl. Acad. Sci. USA, 1996, 93: 10614-10619; D. Schalon *et al.*, Genome Res., 1996, 6: 639-645; D.J. Lockhart *et al.*, Nature Biotechnol., 1996, 14: 1675-1680; J. DeRisi *et al.*, Nature Genet., 1996, 14: 457-460; R.A Heller *et al.*, Proc. Natl. Acad. Sci. USA, 1997, 94: 2150-2155; J.L. DeRisi *et al.*, Science, 1997, 278: 680-686; D.A. Lashkari *et al.*, Proc. Natl. Acad. Sci. USA, 1997, 94: 13057-13062; L. Wodicka *et al.*, Nature Biotechnol., 1997, 15: 1359-1367; R.J. Cho *et al.*, Mol. Cell, 1998, 2: 65-73; N.S. Gray *et al.*, Science, 1998, 281: 533-538; A.D. Cristillo and B.E. Bierer, J. Biol. Chem., 2002, 277: 4465-4476). However, whole genome expression

profiles do not distinguish the genes targeted by a compound from the secondary-response genes.

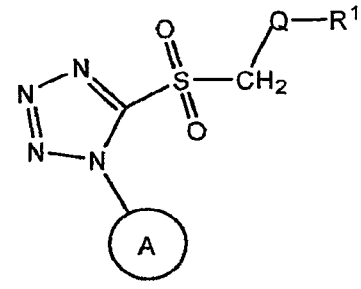
[5] Methods have been developed that allow identification of secondary pathways altered by a drug and detection of drug effects mediated through unintended targets. These methods include, for example, association analysis techniques (T.R. Hughes *et al.*, Cell, 2000, 102: 109–126; M.J. Marton *et al.*, Nat. Med., 1998, 4: 1293–1301; U.S. Pat. No. 5,965,352), haploinsufficiency profiling (G. Giaever. *et al.,* Nature Genetics, 1999, 21: 278–283; G. Giaever *et al.*, Proc. Natl. Acad. Sci. USA, 2004, 101: 793–798; P.Y. Lum *et al.*, Cell, 2004, 116: 121–137), and chemical-genetic interaction mapping (A.B. Parsons *et al.,* Nature Biotechnology, 2004, 22: 62–69). However, these methods require generation of libraries of genetic mutants or fitness-based assays of drug response.

Summary of the Invention

[6] The present Applicants have used a new model-based approach which accurately distinguishes a compound's targets from the secondary responders. Furthermore, this new approach does not require libraries of genetic mutants or fitness-based assays of drug response in contrast to existing methods. More specifically, the new method uses an integrated computational-experimental approach for computing the likelihood that gene products and associated pathways are targets of a compound. This is achieved by filtering the mRNA expression profile of compound-exposed cells using a reverse-engineered model of the cell's gene regulatory network. When applied to a set of 515 whole-genome yeast expression profiles resulting from a variety of treatments (compounds, knockouts, and induced expression), the method correctly enriches for the known targets and associated pathways in the majority of compounds examined.

[7] This new approach was applied to 4-(1-phenyl-1H-tetrazole-5-ylsulfonyl) butanenitrile (PTSB), a novel growth inhibitory compound with a previously unknown mode of action, and identified the thioredoxin/thioredoxin reductase system as its target. Accordingly, the present invention relates to PTSB and PTSB derivatives and their use to inhibit the thioredoxin/thioredoxin reductase system, including in the treatment of diseases mediated by thioredoxin/thioredoxin reductase, such as cancer, HIV/AIDS, Alzheimer's disease, rheumatoid arthritis, and skin disorders.

[8] . In one aspect, the present invention provides compounds with the following structure:



I

or a pharmaceutically acceptable salt or derivative thereof, wherein:

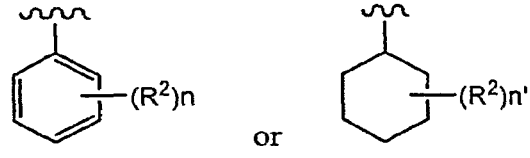
R^1 is hydrogen, -C≡N, or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain, wherein 0–2 methylene units of Q are independently replaced by –O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-;

each R is independently hydrogen or an optionally substituted aliphatic group; and

Ring A is an optionally substituted 3-8 membered bivalent, saturated, partially unsaturated, or aryl monocyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8–10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[9] In certain embodiments, the Ring A is selected from:



or

wherein each wavy line indicates the point of attachment to the tetrazole ring of structure **I**, and wherein:

n is 0 to 4;

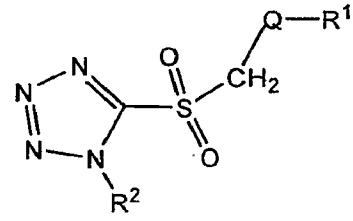
n' is 0 to 10;

each R^2 is independently halogen, R^3, -C≡N, OR^3, SR^3, $N(R^3)_2$, $C(O)R^3$, $C(O)OR^3$, $NR^3C(O)R^3$, $C(O)NR^3$, SO_2R^3, $NR^3SO_2R^3$, $SO_2N(R^3)_2$; and

each R^3 is independently hydrogen or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[10] In some embodiments, R^3 is $-CX_3$, $-CHX_2$, and $-CH_2X$, wherein X is chloro, fluoro, bromo or iodo. In some embodiments, R^1 is -C≡N. In some embodiments, Q is $-CH_2-$ or $-CH_2-CH_2-$.

[11] The present invention also provides compounds with the following structure:



II

or a pharmaceutically acceptable salt or derivative thereof, wherein:

R^1 is hydrogen, -C≡N, or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

R^1 is hydrogen, or an optionally substituted group selected from a C_{1-6} aliphatic group;

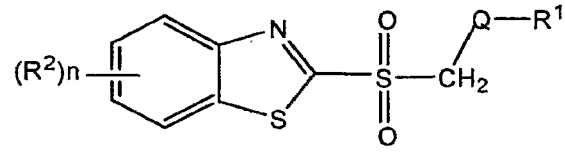
Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain, wherein 0–2 methylene units of Q are independently

replaced by –O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, -SO_2-, -$NRSO_2$-, -SO_2NR-, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-; and

each R is independently hydrogen or an optionally substituted aliphatic group.

[12] In some embodiments, R^1 is -C≡N. In some embodiments,Q is –CH_2- or -CH_2-CH_2-.

[13] The present invention also provides compounds with the following structure:



III

or a pharmaceutically acceptable salt or derivative thereof, wherein:

R^1 is hydrogen, -C≡N, or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain, wherein 0–2 methylene units of Q are independently replaced by –O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, -SO_2-, -$NRSO_2$-, -SO_2NR-, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-;

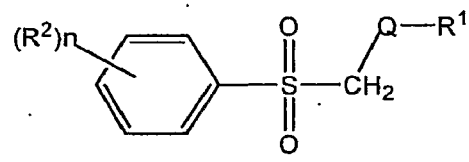
each R is independently hydrogen or an optionally substituted aliphatic group;

each R^2 is independently halogen, R^3, OR^3, SR^3, $N(R^3)_2$, $C(O)R^3$, $C(O)OR^3$, $NR^3C(O)R^3$, $C(O)NR^3$, SO_2R^3, $NR^3SO_2R^3$, $SO_2N(R^3)_2$; and

each R^3 is independently hydrogen or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[14] In some embodiments, R^1 is -C≡N. In some embodiments,Q is –CH_2- or -CH_2-CH_2-.

[15] The present invention also provides compounds with the following structure:



IV

or a pharmaceutically acceptable salt or derivative thereof, wherein:

R^1 is hydrogen, -C≡N, or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain, wherein 0–2 methylene units of Q are independently replaced by –O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-;

each R is independently hydrogen or an optionally substituted aliphatic group;

each R^2 is independently halogen, R^3, OR^3, SR^3, $N(R^3)_2$, $C(O)R^3$, $C(O)OR^3$, $NR^3C(O)R^3$, $C(O)NR^3$, SO_2R^3, $NR^3SO_2R^3$, $SO_2N(R^3)_2$; and

each R^3 is independently hydrogen or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[16] In some embodiments, R^1 is -C≡N. In some embodiments,Q is $-CH_2-$ or $-CH_2-CH_2-$.

[17] The present invention also provides compounds with structure 1, structure 3, structure 4, structure 5, structure 6, structure 9, structure 11, structure 12, structure 13, structure 14, structure 15, structure 33, structure 35, structure 36, structure 37, structure 38, structure 39, structure 40, structure 42, structure 43, structure 44, structure 45, structure 46, structure 47, structure 48, structure 71, structure 97, structure 99, structure

100, structure 101, structure 113, structure 114, structure 116, structure 117, structure 118, and structure 119, as presented in Table 4.

[18] In another aspect, the present invention provides for the use of PTSB or PTSB derivatives, including the compounds disclosed above, for the manufacture of a medicament for use in the treatment of a disease or condition mediated by thioredoxin/thioredoxin reductase and/or a disease or condition associated with oxidative stress. Such diseases and conditions include, for example, cancer, HIV infection/AIDS, Alzheimer's disease, Parkinson's disease, skin diseases such as psoriasis, and cardiovascular diseases, Amyotrophic lateral sclerosis (ALS, sometimes called Lou Gehrig's disease), respiratory distress syndrome, muscular dystrophy, cataractogenesis, progeria, Werner's syndrome, atherosclerosis, diabetes, essential hypertension, cystic fibrosis, ulcerative colitis, and chronic inflammatory disorders such as asthma, chronic obstructive pulmonary disease (COPD), and rheumatoid arthritis.

[19] In another aspect, the present invention provides pharmaceutical compositions comprising an effective amount of a compound of the invention and at least one physiologically acceptable carrier or excipient. A pharmaceutical composition of the present invention can further comprise at least one additional therapeutic agent.

[20] In certain embodiment, the therapeutic agent is a member from the group consisting of chemotherapeutic agents, drugs used in the treatment of Alzheimer's disease, drugs used in the treatment of Parkinson's disease, drugs used in the treatment of HIV infection or AIDS, drugs used in the treatment of psoriasis, drugs used in the treatment of cardiovascular diseases, drugs used in the treatment of rheumatoid arthritis, and combinations thereof.

[21] In another aspect, the present invention provides methods of inhibiting thioredoxin/thioredoxin reductase in a subject or a biological system by administering to the subject or contacting the biological system with an effective amount of a compound of the invention.

[22] In still another aspect, the present invention provides methods of regulating cell proliferation, cell cycle progression, and/or apoptosis in a subject or a biological system by administering to the subject or contacting the biological system with an effective amount of a compound of the invention.

[23] In these methods, the biological system may be a cell, a biological fluid, a biological tissue or an animal.

[24] In yet another aspect, the present invention provides methods of treating a subject suffering from or susceptible to a disease or condition mediated by thioredoxin/thioredoxin reductase. These methods of treatment comprise administering to the subject an effective amount of a compound or pharmaceutical composition of the invention.

[25] In certain embodiments, the disease mediated by thioredoxin/thioredoxin reductase is a member of the group consisting of cancer or cancerous condition, Alzheimer's disease, Parkinson's disease, HIV infection or AIDS, psoriasis, cardiovascular disease, and rheumatoid arthritis.

[26] In certain embodiments, the disease mediated by thioredoxin/thioredoxin reductase is a cancer or cancerous condition that is a member of the group consisting of tumors of the brain and central nervous system, head and/or neck cancer, breast tumors, tumors of the circulatory system, lymphomas, leukemias, Hodgkin's disease, tumors of the excretory system, tumors of the gastrointestinal tract, tumors of the liver, tumors of the digestive organs, tumors of the oral cavity, tumors of the reproductive system, tumors of the respiratory tract, and tumors of the skeletal system, tumors of the skin.

[27] In certain embodiments, the disease mediated by thioredoxin/thioredoxin reductase is a cancer or cancerous condition that is a member of the group consisting of lung cancer, colorectal cancer, cervical cancer, hepatic cancer, and pancreatic cancer.

[28] In the methods of treatment, the compound or pharmaceutical composition of the invention may be administered in combination with another therapeutic agent and/or a therapeutic procedure.

Brief Description of the Drawing

[29] **Figure 1** is a scheme showing an overview of one embodiment of the MNI method. In phase I, a set of treatments, including knockouts, compounds, overexpressions, and/or RNAi, is applied to an organism. Cells or tissues are sampled, and mRNA is collected. The abundance changes of all mRNA species in the organism are measured. The data are used by the MNI algorithm to infer a model of the regulatory influences between genes in the organism (blue-filled circles indicate genes; arrows indicate regulatory influences). In phase II, a test treatment, such as a drug, is applied to the cells and expression changes of all mRNA species are measured. The expression data are then filtered using the network model to distinguish the targets of the test treatment (red-filled circles) from secondary responders.

[30] **Figure 2** shows the structure of the network model. **Fig. 2(A)** is a scheme of the network model showing the regulatory influences (arrows) between transcripts as influence functions for each gene (blue nodes). During phase I, the MNI algorithm identifies the subset of transcripts (the input RNA concentrations) that influence the rate of transcription (the output transcription rate) of each other transcript. The algorithm also learns the coefficients of the interaction function that relates the inputs to outputs. **Fig. 2(B)** is a colored matrix showing a portion of the yeast gene-network model identified by the MNI algorithm. Gene expression profiles are first reduced to a lower-dimensional set of *metagenes* and a network model is learned for the metagenes. The metagenes represent characteristic expression profiles which can be combined to approximate the expression profile of each transcript in the cell. Each pixel in the matrix represents a positive influence (red), negative influence (blue), or no influence (white) of the metagenes on each other. The metagene model, which can be transformed to describe regulatory influences between true genes, is used in phase II of the algorithm to distinguish compound targets from secondary responders.

[31] **Figure 3** is a set of two graphs that help predict targets of itraconazole. **Fig. 3(A)** shows the mRNA changes in 6194 yeast genes following treatment with itraconazole. Changes are plotted as the z-score, x/σ_x, where x is the log(expression ratio) and σ_x is the standard error on the log expression ratio. **Fig. 3(B)** shows the targets of itraconazole predicted by the MNI algorithm using the expression changes in panel **(A)**. Higher MNI scores indicated higher likelihood that the gene is a target. ERG11, a known

target of itraconazole, is the second most likely target identified with the MNI algorithm. Those genes ranked in the top 50 by MNI and annotated with the top ranked GO process, "steroid metabolism" (in addition to ERG11), are shown in orange. The remaining genes ranked in the top 50 by MNI are shown in green. Genes ranked in the top 50 by z-score of mRNA expression change are shown in purple.

[32] **Figure 4(A)** shows the chemical structure of PTSB. **Figure 4(B)** is a scheme that illustrates the DNA microarray construction and microarray sample preparation for PTSB experiments (see Example Section).

[33] **Figure 5** is a scheme presenting the thioredoxin/thioredoxin reductase redox system.

[34] **Figure 6** presents the results of a thioredoxin/thioredoxin reductase activity assay. **Fig. 6(A)** shows the NADPH-dependent reduction of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) by thioredoxin and thioredoxin reductase. **Fig. 6(B)** is a graph showing the reduction of DTNB monitored in the presence of A: 0 μM, B: 1 μM, C: 5 μM and D: 50 μM PTSB.

[35] **Figure 7** shows the chemical structures of known inhibitors of the thioredoxin/thioredoxin reductase system.

[36] **Table 1** presents the results of the MNI approach identifying targets of genetic perturbations. Results of association methods are provided for comparison.

[37] **Table 2** shows the pathways and associated genes targeted by drug compounds.

[38] **Table 3** shows the pathways and associated genes targeted by drug compounds that were not in the original compendium data.

[39] **Figure 8** shows the structure of PTSB (compound **1**) and two derivatives.

[40] **Figure 9** shows diversification points of PTSB.

[41] **Figure 10** shows an example of a plan used for the development of a library of PTSB derivatives.

[42] **Figure 11** shows an example of chemical synthesis used for the preparation of sulfonyl-tetrazole derivatives.

[43] **Table 4** presents the structures and chemical names of 36 compounds in a library of PTSB derivatives.

[44] **Table 5** presents results of a cancer cell growth inhibition screen. Data obtained for each compound of a library are reported as % growth inhibition.

Definitions

[45] Throughout the specification, several terms are employed that are defined in the following paragraphs.

[46] The term "***aliphatic***" or "***aliphatic group***", as used herein, denotes a hydrocarbon moiety that may be straight-chain (*i.e.*, unbranched), branched, or cyclic (including fused, bridging, and spiro-fused polycyclic) and may be completely saturated or may contain one or more units of unsaturation, but which is not aromatic. Unless otherwise specified, aliphatic groups contain 1–20 carbon atoms. In some embodiments, aliphatic groups contain 1–10 carbon atoms. In other embodiments, aliphatic groups contain 1–8 carbon atoms. In still other embodiments, aliphatic groups contain 1–6 carbon atoms, and in yet other embodiments aliphatic groups contain 1–4 carbon atoms. Suitable aliphatic groups include, but are not limited to, linear or branched, alkyl, alkenyl, and alkynyl groups, and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl.

[47] The term "***aryl***", used alone or as part of a larger moiety as in "aralkyl", "aralkoxy", or "aryloxyalkyl", refers to monocyclic, bicyclic, and tricyclic ring systems having a total of five to fourteen ring members, wherein at least one ring in the system is aromatic and wherein each ring in the system contains three to seven ring members. The term "aryl" may be used interchangeably with the term "aryl ring".

[48] In general, an "***effective amount***" of a biologically and/or pharmaceutically active agent is an amount sufficient to achieve a desired biological and/or pharmacological effect when delivered to a cell or organism according to a selected administration form, route, and/or schedule. As will be appreciated by those of ordinary skill in this art, the absolute amount of a particular agent that is effective may vary depending on such factors as the desired biological endpoint, the agent to be delivered, the target tissue, etc. Those of ordinary skill in the art will further understand that an

"effective amount" may be administered in a single dose, or may be achieved by administration of multiple doses. A desired effect may include, for example, one or more of: delaying or preventing the onset of a disease, disorder or condition; slowing down or stopping the progression, aggravation or deterioration of the condition or symptoms of the condition; bringing about ameliorations of the condition or its symptoms; and curing the condition. When a composition of the present invention comprises an inventive compound and other therapeutic agents, the amount of any individual agent required in the composition may be different from the amount required of that agent to achieve its therapeutic effect when administered alone. In some cases, synergies between or among therapeutic agents used in a composition may reduce amounts required; in other cases, inhibitory interactions may increase amounts required. Thus, in general, effective amounts of a combination of agents may utilize different absolute amounts of the agents than what constitute effective amounts of the agents individually.

[49] The terms "***halo***" and "***halogen***", as used herein, refer to an atom selected from fluorine, chlorine, bromine and iodine.

[50] The term "***haloalkyl***" denotes an alkyl group having one, two, or three halogen atoms attached thereto and is exemplified by such groups as chloromethyl, bromoethyl, trifluoromethyl, and the like.

[51] The term "***heteroatom***" means one or more of oxygen, sulfur, nitrogen, phosphorus, or silicon. This includes any oxidized form of nitrogen, sulfur, phosphorus, or silicon; the quaternized form of any basic nitrogen, or a substitutable nitrogen of a heterocyclic ring including =N– as in 3,4-dihydro-2*H*-pyrrolyl, –NH– as in pyrrolidinyl, or =N($R^{\dagger}$)– as in N-substituted pyrrolidinyl.

[52] The term "***in combination***", as used herein with respect to administration of first and second agents, means administration performed such that (i) a dose of the second agent is administered before more than 90% of the most recently administered dose of the first agent has been metabolized to an inactive form or excreted from the body; or (ii) doses of the first and second agents are administered within 48 hours of each; or (iii) the agents are administered during overlapping time periods; or (iv) any combination of the foregoing. The agents may, but need not, be administered together as components of a single composition. The agents may be administered individually at substantially the

same time (by which is meant within less than 10 minutes of one another). The agents may be administered individually within a short time of one another (by which is meant less than 3 hours, sometimes less than 1 hour, apart). The agents may, but need not, be administered by the same route of administration.

[53] The term "***individual***" and "***subject***" are used herein interchangeably. They refer to a higher vertebrate, preferably a human or another mammal (*e.g.*, a mouse, rat, rabbit, monkey, dog, cat, pig, cow, horse, and the like) that may or may not have a disease state or condition mediated by the thioredoxin/thioredoxin reductase redox system.

[54] A "***pharmaceutically acceptable derivative***" of a particular chemical compound include, but is not limited to, pharmaceutically acceptable salts, esters, salts of such esters, or any other adduct or derivative which upon administration to a subject in need is capable of providing, directly or indirectly, a compound as otherwise described herein, or a metabolite or residue thereof. Thus, pharmaceutically acceptable derivatives can include salts, prodrugs, and/or metabolites of relevant compounds. The phrase "pharmaceutically acceptable derivative" may also encompass quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or oil-soluble or dispersable products may be obtained by such quaternization.

[55] As used herein, the term "***pharmaceutically acceptable salt***" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and which are commensurate with a reasonable benefit/risk ratio. A "pharmaceutically acceptable salt" means any non-toxic salt or salt of an ester of a compound of this invention that, upon administration to a recipient, is capable of providing, either directly or indirectly, a compound of this invention or an active metabolite or residue thereof. As used herein, the term "active metabolite or residue thereof" means that a metabolite or residue thereof acts as a thioredoxin or thioredoxin reductase inhibitor.

[56] A wide variety of appropriate pharmaceutically acceptable salts are well known in the art. For example, S.M. Berge *et al.* describe pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 1977, 66: 1-19, incorporated herein by

reference. Pharmaceutically acceptable salts of the compounds of this invention include those derived from suitable inorganic and organic acids and bases.

[57] Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like.

[58] Examples of pharmaceutically acceptable salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and $N^+(C_{1-4}$ alkyl$)_4$ salts. Representative pharmaceutically acceptable alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations, for example formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate and aryl sulfonate.

[59] As used herein, the term "***physiologically acceptable carrier or excipient***" refers to a carrier medium or excipient which does not interfere with the effectiveness of the biological activity of the active ingredients and which is not excessively toxic to the host at the concentrations at which it is administered. The term includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic agents, absorption delaying agents, and the like. The use of such media and agents for the formulation of pharmaceutically active substances is well-known in the art (see, for example, "*Remington's Pharmaceutical Sciences*", E.W. Martin, 18th Ed., 1990, Mack Publishing Co.: Easton, PA, which is incorporated herein by reference in its entirety).

[60] . As described herein, compounds of the invention may contain "***optionally substituted***" moieties. In general, the term "***substituted***", whether preceded by the term "optionally" or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. Combinations of substituents envisioned by this invention are preferably those that result in the formation of stable or chemically feasible compounds. The term "stable", as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein.

[61] Suitable monovalent substituents on a substitutable carbon atom of an "optionally substituted" group are independently halogen; $-(CH_2)_{0-4}R^\circ$; $-(CH_2)_{0-4}OR^\circ$; $-O-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}CH(OR^\circ)_2$; $-(CH_2)_{0-4}SR^\circ$; $-(CH_2)_{0-4}Ph$, which may be substituted with R°; $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ which may be substituted with R°; -CH=CHPh, which may be substituted with R°; $-NO_2$; -CN; $-N_3$; $-(CH_2)_{0-4}N(R^\circ)_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)R^\circ$; $-N(R^\circ)C(S)R^\circ$; $-(CH_2)_{0-4}N(R^\circ)C(O)NR^\circ_2$; $-N(R^\circ)C(S)NR^\circ_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)OR^\circ$; $-N(R^\circ)N(R^\circ)C(O)R^\circ$; $-N(R^\circ)N(R^\circ)C(O)NR^\circ_2$; $-N(R^\circ)N(R^\circ)C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)R^\circ$; $-C(S)R^\circ$; $-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)SR^\circ$; $-(CH_2)_{0-4}C(O)OSiR^\circ_3$; $-(CH_2)_{0-4}OC(O)R^\circ$; $-OC(O)(CH_2)_{0-4}SR-$, $-SC(S)SR^\circ$; $-(CH_2)_{0-4}SC(O)R^\circ$; $-(CH_2)_{0-4}C(O)NR^\circ_2$; $-C(S)NR^\circ_2$; $-C(S)SR^\circ$; $-SC(S)SR^\circ$, $-(CH_2)_{0-4}OC(O)NR^\circ_2$; $-C(O)N(OR^\circ)R^\circ$; $-C(O)C(O)R^\circ$; $-C(O)CH_2C(O)R^\circ$; $-C(NOR^\circ)R^\circ$; $-(CH_2)_{0-4}SSR^\circ$; $-(CH_2)_{0-4}S(O)_2R^\circ$; $-(CH_2)_{0-4}S(O)_2OR^\circ$; $-(CH_2)_{0-4}OS(O)_2R^\circ$; $-S(O)_2NR^\circ_2$; $-(CH_2)_{0-4}S(O)R^\circ$; $-N(R^\circ)S(O)_2NR^\circ_2$; $-N(R^\circ)S(O)_2R^\circ$; $-N(OR^\circ)R^\circ$; $-C(NH)NR^\circ_2$; $-P(O)_2R^\circ$; $-P(O)R^\circ_2$; $-OP(O)R^\circ_2$; $-OP(O)(OR^\circ)_2$; SiR°_3; $-(C_{1-4}$ straight or branched alkylene)O-$N(R^\circ)_2$; or $-(C_{1-4}$ straight or branched alkylene)C(O)O-$N(R^\circ)_2$, wherein each R° may be substituted as defined below and is independently hydrogen, C_{1-6} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5-6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or,

notwithstanding the definition above, two independent occurrences of R°, taken together with their intervening atom(s), form a 3–12-membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, which may be substituted as defined below.

[62] Suitable monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), are independently halogen, $-(CH_2)_{0-2}R^{\bullet}$, -(haloR$^{\bullet}$), $-(CH_2)_{0-2}OH$, $-(CH_2)_{0-2}OR^{\bullet}$, $-(CH_2)_{0-2}CH(OR^{\bullet})_2$; -O(haloR$^{\bullet}$), -CN, $-N_3$, $-(CH_2)_{0-2}C(O)R^{\bullet}$, $-(CH_2)_{0-2}C(O)OH$, $-(CH_2)_{0-2}C(O)OR^{\bullet}$, $-(CH_2)_{0-2}SR^{\bullet}$, $-(CH_2)_{0-2}SH$, $-(CH_2)_{0-2}NH_2$, $-(CH_2)_{0-2}NHR^{\bullet}$, $-(CH_2)_{0-2}NR^{\bullet}{}_2$, $-NO_2$, $-SiR^{\bullet}{}_3$, $-OSiR^{\bullet}{}_3$, -C(O)SR$^{\bullet}$, -(C_{1-4} straight or branched alkylene)C(O)OR$^{\bullet}$, or -SSR$^{\bullet}$ wherein each R$^{\bullet}$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently selected from C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of R° include =O and =S.

[63] Suitable divalent substituents on a saturated carbon atom of an "optionally substituted" group include the following: =O, =S, $=NNR^{*}{}_2$, =NNHC(O)R*, =NNHC(O)OR*, $=NNHS(O)_2R^{*}$, =NR*, =NOR*, $-O(C(R^{*}{}_2))_{2-3}O-$, or $-S(C(R^{*}{}_2))_{2-3}S-$, wherein each independent occurrence of R* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 5–6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents that are bound to vicinal substitutable carbons of an "optionally substituted" group include: $-O(CR^{*}{}_2)_{2-3}O-$, wherein each independent occurrence of R* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[64] Suitable substituents on the aliphatic group of R* include halogen, -R$^{\bullet}$, -(haloR$^{\bullet}$), -OH, -OR$^{\bullet}$, -O(haloR$^{\bullet}$), -CN, -C(O)OH, -C(O)OR$^{\bullet}$, $-NH_2$, -NHR$^{\bullet}$, $-NR^{\bullet}{}_2$, or $-NO_2$, wherein each R$^{\bullet}$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or

a 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[65] Suitable substituents on a substitutable nitrogen of an "optionally substituted" group include $-R^{\dagger}$, $-NR^{\dagger}_2$, $-C(O)R^{\dagger}$, $-C(O)OR^{\dagger}$, $-C(O)C(O)R^{\dagger}$, $-C(O)CH_2C(O)R^{\dagger}$, $-S(O)_2R^{\dagger}$, $-S(O)_2NR^{\dagger}_2$, $-C(S)NR^{\dagger}_2$, $-C(NH)NR^{\dagger}_2$, or $-N(R^{\dagger})S(O)_2R^{\dagger}$; wherein each $R^{\dagger}$ is independently hydrogen, C_{1-6} aliphatic which may be substituted as defined below, unsubstituted -OPh, or an unsubstituted 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of $R^{\dagger}$, taken together with their intervening atom(s) form an unsubstituted 3–12-membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[66] Suitable substituents on the aliphatic group of $R^{\dagger}$ are independently halogen, $-R^{\bullet}$, -(haloR$^{\bullet}$), -OH, $-OR^{\bullet}$, -O(haloR$^{\bullet}$), -CN, -C(O)OH, $-C(O)OR^{\bullet}$, $-NH_2$, $-NHR^{\bullet}$, $-NR^{\bullet}_2$, or $-NO_2$, wherein each $R^{\bullet}$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[67] As used herein, the term "***treatment***" refers to the provision of any type of medical or surgical management to a subject. Treatment can include, but is not limited to, administering a pharmaceutical composition to a subject. Treatment is typically undertaken in an effort to alter the course of a disease, disorder, or undesirable condition in a manner beneficial to the subject. The effect of treatment can generally include reversing, alleviating, reducing severity of, delaying the onset of, inhibiting the progression of, and/or reducing the likelihood of occurrence or reoccurrence of the disease, disorder, or condition to which such term applies, or one or more symptoms or manifestations of such disease, disorder or condition. A compound or composition of the present invention can be administered to a subject who has developed a disease or condition mediated by the thioredoxin/thioredoxin reductase redox system or is at increased risk of developing such a disease or condition. A compound or composition of this invention can be administered prophylactically, *i.e.*, before development of any

symptom or manifestation of a condition, or it can be administered after initiation of the disease or condition, for a therapeutic action.

[68] As used herein, the term "***unit dosage form***" refers to a physically discrete unit suited as unitary dosage for the subject to be treated; each unit containing a predetermined quantity of active agent(s) calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[69] The term "***unsaturated***", as used herein, means that a moiety has one or more units of unsaturation.

Detailed Description of Certain Preferred Embodiments

[70] As mentioned above, the present invention is directed to sulfones, derivatives of PTSB that have biological activity, *e.g.*, thioredoxin/thioredoxin reductase inhibitory activity. The thioredoxin/thioredoxin reductase system was identified as the target of PTSB using a new integrated computational-experimental approach which accurately distinguishes a compound's targets from secondary responders. The inventive sulfones were designed based on structure activity-relationship studies performed with the intent of developing molecules more active than PTSB.

I – Development and Applications of Mode of MNI

[71] The present Applicants have used a new method for drug discovery called Mode of action by Network Identification (MNI) (see U.S. Pat. Appln. No. 2006-0293873; U.S. Pat. Appln. No. 2007-0016390; and International Appln. No. WO/2003/077062). Figure 1 presents an embodiment of such a method. MNI first reverse-engineers a network model of regulatory interactions in the organism of interest using a training data set of whole-genome expression profiles.

[72] The model is then used to analyze the expression profile of compound-treated cells to determine the pathways and genes targeted by the compound. The reverse-engineered model is a directed graph relating the concentrations of transcripts to each other. An edge in the graph means that the activity of one gene product influences the transcription of another gene (Figure 2). Multiple genes may influence the activity of each other gene; these influences are integrated in the model as a weighted sum of the transcript concentrations (Figure 2). Because the model is learned from transcription data

only, regulatory influences between genes may be mediated through protein or metabolite species that are not explicitly represented.

[73] The algorithm assumes that training profiles are obtained in steady state following a variety of treatments, including compounds, RNAi, and gene-specific mutations (Figure 1). The ability to use varied treatment types in the training data is important to advance over earlier model estimation techniques (T.S Gardner *et al.*, Science, 2003, 301: 102-105; J. Tegner *et al.*, Proc. Natl. Acad. Sci. USA, 2003, 100: 5944-5949; M.K.S. Yeung *et al.*, Proc. Natl. Acad. Sci. USA, 2002, 99: 6163-6168), which required knowledge of the gene targets of each training perturbation. This improved flexibility may enable application of the MNI approach to higher model organisms, where gene-specific perturbations are more difficult to implement. To infer a network model without requiring gene-specific perturbations, the algorithm employs an iterative procedure: it first predicts the targets of the treatment using an assumed network model, and then uses those predicted targets to estimate a better model. The procedure continues until convergence criteria are met. This approach is analogous to the Expectation Maximization (EM) algorithm (A. Dempster *et al.*, J. Royal Statistical Society: Series B, 1977, 39: 1-38) commonly used to learn Bayesian networks.

[74] Once the regulatory model is learned, it is applied to the expression profile of a test compound to predict its target. The model acts as a filter, in essence, checking the expression level of each gene in the cell (relative to the level of all other genes in the cell) for consistency with regulatory influences embodied in the learned regulatory model. The genes are then ranked by a z-statistic that measures this level of consistency. The highest-ranked genes are those whose expression is most inconsistent with the mode, and this inconsistency is attributed to the external influence of the compound on those genes.

[75] This new model-based approach was tested by combining two publicly available, whole-genome yeast expression data sets: a compendium of 300 profiles of gene deletions, titratable promoter insertions, and drug compound treatments from Hughes *et al.* (Cell, 2000, 102: 109-126), and a recent set of 215 titratable promoter insertions in essential genes from Mnaimneh *et al.* (Cell, 2004, 118: 31-44). For each treatment/perturbation, a single profile was obtained from yeast cells grown to steady state following the perturbation. A log-transformed expression ratio was computed for

each gene in each profile relative to untreated, wild-type yeast strains. The algorithm was blinded to any information regarding the gene targets of the treatments and mutations.

[76] ***Prediction of Gene Targets of Promoter Insertions.*** The performance of the MNI algorithm was evaluated by testing its ability to predict the gene targets of the 11 promoter insertions of the Hughes compendium (see Table 1 and Example 1). For the 11 mutant profiles tested, the algorithm ranked the targeted gene as the most likely affected gene in 8 out of 11 cases.

[77] ***Prediction of Gene Targets of Drug Compounds.*** The MNI algorithm was then applied to identify probable targets of drug compounds. Unlike promoter insertions which directly influence transcription, compounds predominantly affect protein activity and only indirectly influence transcription. As a result, the algorithm is more likely to identify genes in the same pathway as the affected protein rather than the target itself, *e.g.*, transcriptionally regulated genes downstream of the target protein. On the other hand, when transcriptional feedback regulation is present in the pathway containing the targeted gene, it is likely that the algorithm will also assign a high rank to the targeted gene product. Thus, in analyzing the MNI prediction for compound treatments, are considered as targets both the pathways that are significantly over-represented among the highly-ranked genes and the highly-ranked genes within those pathways. Pathways are identified as significantly over-represented GO processes among the highly ranked genes.

[78] The MNI algorithm was used to identify probable targets of 15 compounds (see Example 1), 13 of which were drawn from the Hughes compendium (Cell, 2000, 102: 109-126) and two from other studies (M. Ueda *et al.*, FEMS Microbiol. Lett., 2003, 219: 93-98). Of the 15 compounds examined, 9 have previously determined targets, while the targets of the other 6 compounds are unknown. For most compounds, the MNI algorithm was successful in correctly identifying the target pathway with the highest significance. Moreover, within a significant pathway, the algorithm typically ranked the target gene product higher than other genes in the pathway. The MNI algorithm's ability to rank both genes and pathways suggests that the most probable targets of novel compounds can be identified as those that act within the most significant over-represented GO processes (pathways) and are highly ranked within those processes. The resulting

small list of probable targets can then be validated for interaction with the compound *via* direct biochemical assays.

II –Thioredoxin / Thioredoxin Reductase Inhibitors

Identification of PTSB's Targets

[79] The method described above was applied to a novel tetrazole-containing compound, 4-(1-phenyl-1H-tetrazole-5-ylsulfonyl)butanenitrile (herein also called PTSB). PTSB (**1**, Figure 8) has been used as an intermediate in the total synthesis of amphidinilide B (B.M. Cid and G. Pattenden, Tetrahedron Letters, 2000, 41: 7373-7378). PTSB was found to exhibit ten times the activity of similar compounds (**2** and **3**) in inhibiting cell growth of A549 human cell lung carcinoma cells in a cell-based cytotoxicity assay of a diverse collection of synthetic compounds from the CMLD-BU (A549, IC_{50} of 5 μM).

[80] The changes in steady-state gene expression in *Saccharomyces cerevisiae* were determined upon treatment with PTSB using oligonucleotide arrays. The MNI algorithm and the reverse-engineered network model described herein were used to obtain a ranking of the most likely targets of PTSB. The most highly over-represented GO process among the top 50 most likely perturbed genes was found to be the "cell redox homeostasis" annotation ($P < 2.2 \times 10^{-3}$). Two genes with that annotation were ranked in the top 50: thioredoxin reductase (*trr1*, rank = 32) and thioredoxin (*trx2*, rank = 36). The results obtained with the MNI algorithm were confirmed and validated (see Example 2) by demonstrating that PTSB efficiently inhibits the thioredoxin/thioredoxin reductase system using a biochemical assay (A. Holmgren and P. Reichard, Eur. J. Biochem., 1967, 2: 187-196).

[81] Thioredoxin is over-expressed in a number of disease states, including cancer. Inhibitors of the thioredoxin/thioredoxin reductase (Trx/TrxR) system are currently under investigation in the treatment of cancer and other diseases. Examples of known inhibitors of the Trx/TrxR system are shown on Figure 7, including PX-12 (T.J. McDonnell, and S.J. Korsemeyer, Nature, 1991, 349: 254-256), NSC 131233 (M.D. Bootman *et al.*, Biochem. Biophys. Res., 190, 166: 1334-1339), BCNU (K.U. Schallretuer *et al.*, Biochim. Biophys. Acta, 1990, 1054: 14-20), Auronofin (S. Gromer *et al.*, J. Biol. Chem., 1998, 273: 20096-20101), and Palmarumycin CP1 (P. Wipf *et al.*, Biomol. Chem., 2004,

2: 1651-1658). Many of the known Trx/TrxR inhibitors have been shown to bind irreversibly to the active site of the TrxR through a disulfide exchange reaction. The structure of PTSB suggests a novel, different mechanism of inhibition.

Thioredoxin/Thioredoxin Reductase System

[82] Thioredoxins are a class of small 12-kDa redox proteins known to be present in almost all eukaryotic and prokaryotic organisms. They are characterized by a highly conserved active site that contains two cysteine residues which are reduced from the oxidized form by NADPH and the flavoenzyme thioredoxin reductase (a 112-130 kDa, selenium-dependent dimeric protein). Mammalian thioredoxin has a variety of biological activities. It was originally studied for its ability to act as hydrogen donor for ribonucleotide reductase, the enzyme that synthesizes deoxyribonucleoside triphosphates for DNA synthesis. It can also serve as a cofactor for methionine sulfoxide reductase, catalyze reduction of protein disulfide bonds and participate in folding of proteins. Thioredoxin can also modulate the DNA binding activity of receptors (AhR receptor) and of some transcription factors (*e.g.*, TFIIIC, NF-κB, AP-1) as well as protein stability (*e.g.*, stability of HIF-1α protein, a component of the hypoxia-inducible factor 1). Furthermore, thioredoxin can protect cells against TNF-induced cytotoxicity, oxidative stress and is able to reduce H_2O_2 and scavenge free radicals.

[83] Pathophysiological effects of thioredoxin/thioredoxin reductase are indicated by over-expression of thioredoxin in human tumors such as lung, colorectal and cervical cancers and leukemia. Secreted thioredoxin has been found to stimulate cancer cell growth and decrease sensitivity to induced apoptosis (G. Powis *et al.*, Chem.-Biol. Interactions, 1998, 111-112: 23-24). *In vivo* data also underline the potential involvement of thioredoxin reductase in the pathogenesis of rheumatoid arthritis. Significantly increased levels of thioredoxin and thioredoxin reductase were found in synovial fluid and tissue, but not in blood plasma, of patients suffering from rheumatoid arthritis. Furthermore, the synovial thioredoxin levels were found to correlate with the local severity of inflammation (M.M. Maurice *et al.*, Arthritis Rheum., 1999, 42: 2430-2439). The thioredoxin/thioredoxin reductase redox system has also been intensely studied in HIV infections. Alterations in seleno(protein) concentrations and increased plasma levels of thioredoxin correlating with the stage of the disease have been observed with HIV-infected patients. It has also been suggested that a decrease in the antioxidant

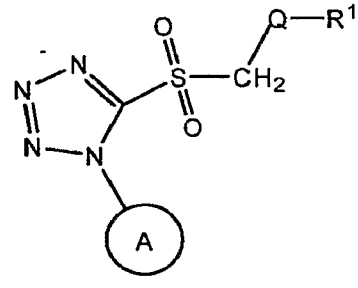
thioredoxin/thioredoxin reductase system may contribute to the increased oxidative stress and subsequent neurodegeneration observed in the brain of patients with Alzheimer's disease (M. Lovell *et al.*, Free Radic. Biol. Med., 2000, 28: 418-427). The thioredoxin/thioredoxin reductase system has also been suggested to play an important role in cellular defense against oxidative stress in cardiovascular diseases. The thioredoxin/thioredoxin reductase system is therefore an important target for therapeutic intervention.

Inventive Thioredoxin/Thioredoxin Reductase Inhibitors

[84] The skeleton of PTSB, which was found to be a thioredoxin/thioredoxin reductase inhibitor by the present Applicants, provides several areas for chemical diversification. An initial structure-activity relationship study has indicated that the sulfone functionality and the nitrile functionality play a role in activity. A library of 36 compounds (presented in Table 4) was designed and developed to include one or both of these functionalities and incorporate some diversification into the structure. Some of these compounds were found to be biologically active in a cancer cell growth inhibition assay (see Example 3 and Table 5).

[85] Accordingly, the present invention provides small molecules that can inhibit the thioredoxin/thioredoxin reductase system. Generally, the inventive Trx/TrxR inhibitors provided herein comprise one or more of a sulfonyl group, a tetrazole group, and a nitrile group.

[86] In some embodiments of the present invention, Trx/TrxR inhibitors have the structure of formula I:



I

or a pharmaceutically acceptable salt or derivative thereof, wherein:

R^1 is hydrogen, -C≡N, or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3-8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8-10 membered saturated, partially unsaturated, or aryl ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain, wherein 0-2 methylene units of Q are independently replaced by -O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-;

each R is independently hydrogen or an optionally substituted aliphatic group; and

Ring A is an optionally substituted 3-8 membered bivalent, saturated, partially unsaturated, or aryl monocyclic ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[87] As defined generally above, the R^1 group of formula **I** is hydrogen, -C≡N, or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[88] In certain embodiments, the R^1 group of formula **I** is a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In other embodiments, the R^1 group of formula **I** is a monocyclic 5-6 membered aryl ring having 0-2 nitrogen atoms, wherein R^1 is optionally substituted with 1 to 3 substituents independently selected from halogen, -C≡N, $-CH_2-C\equiv N$, $-(CH_2)_{0-4}R°$, $-(CH_2)_{0-4}OR°$, $-(CH_2)_{0-4}SR°$, $-(CH_2)_{0-4}Ph$, optionally substituted with R° or OR°, $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ optionally substituted with R° or OR°, -CH=CHPh, optionally substituted with R° or OR°, $-(CH_2)_{0-4}N(R°)_2$, wherein each R° may be substituted as defined herein and is independently hydrogen, C_{1-6} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

According to one embodiment of the present invention, R^1 is phenyl substituted with 1 to 3 groups independently selected from halogen, $-(CH_2)_{0-4}R°$, and $-(CH_2)_{0-4}OR°$. Such groups include chloro, fluoro, OH, OMe, methyl, ethyl, propyl, cyclopropyl, isopropyl, and the like.

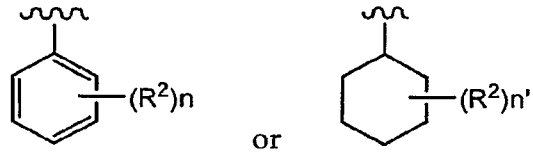
[89] As defined generally above, the Q group of formula **I** is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain, wherein 0–2 methylene units of Q are independently replaced by -O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-. In certain embodiments, Q is a valence bond such that R^1 is directly attached to carbon attached to the sulfur atom. In other embodiments, Q is a bivalent, saturated, and straight C_{1-3} hydrocarbon chain, wherein 0-1 methylene units of Q is replaced by -O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-. In still other embodiments, Q is -O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-. According to another embodiment, Q is $–CH_2-$ or $–CH_2-CH_2-$.

[90] As defined generally above, the Ring A group of formula **I** is an optionally substituted 3-8 membered bivalent, saturated, partially unsaturated, or aryl monocyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8-10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[91] In certain embodiments, Ring A is an optionally substituted 3-8 membered bivalent, saturated, partially unsaturated, or aryl monocyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In other embodiments, Ring A is an optionally substituted 5-6 membered bivalent aryl ring having 0–2 nitrogen atoms. In still other embodiments, Ring A is phenylene optionally substituted with 1 to 4 groups independently selected from halogen, $-(CH_2)_{0-4}R°$, $-(CH_2)_{0-4}OR°$, $-(CH_2)_{0-4}SR°$, $-(CH_2)_{0-4}Ph$, optionally substituted with R° or OR°, $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ optionally substituted with R° or OR°, -CH=CHPh, optionally substituted with R° or OR°, $-(CH_2)_{0-4}N(R°)_2$, wherein each R° may be substituted as defined herein and is

independently hydrogen, C_{1-6} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. According to another embodiment, Ring A is phenylene optionally substituted with 1-2 groups independently selected from halogen, $-(CH_2)_{0-4}R°$, and $-(CH_2)_{0-4}OR°$. Such groups include chloro, fluoro, bromo, iodo, OH, OMe, methyl, ethyl, propyl, cyclopropyl, isopropyl, and the like. In certain embodiments, such groups include $-CX_3$, CHX_2, and $-CH_2X$, wherein X is chloro, fluoro, bromo or iodo.

[92] According to another embodiment, the Ring A group of formula **I** is selected from:



wherein each wavy line indicates the point of attachment to the tetrazole ring, and wherein

n is 0 to 4;

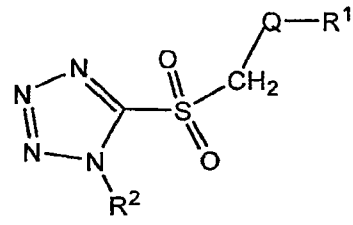
n' is 0 to 10;

each R^2 is independently halogen, $-C{\equiv}N$, R^3, OR^3, SR^3, $N(R^3)_2$, $C(O)R^3$, $C(O)OR^3$, $NR^3C(O)R^3$, $C(O)NR^3$, SO_2R^3, $NR^3SO_2R^3$, $SO_2N(R^3)_2$; and

each R^3 is independently hydrogen or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[93] In certain embodiments, R^3 is a substituted C_{1-6} aliphatic group, such as $-CX_3$, $-CHX_2$, and $-CH_2X$, wherein X is chloro, fluoro, bromo or iodo.

[94] In some embodiments of the present invention, Trx/TrxR inhibitors have the structure of formula **II**:



II

or a pharmaceutically acceptable salt or derivative thereof, wherein:

R^1 is hydrogen, -C≡N, or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

R^2 is hydrogen, or an optionally substituted group selected from a C_{1-6} aliphatic group;

Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain, wherein 0–2 methylene units of Q are independently replaced by –O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-; and

each R is independently hydrogen or an optionally substituted aliphatic group.

[95] As defined generally above, the R^1 group of formula **II** is hydrogen, -C≡N, or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

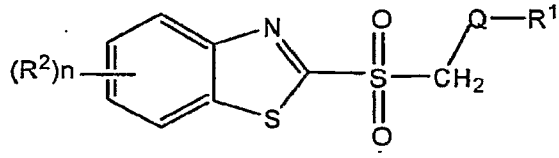
[96] In certain embodiments, the R^1 group of formula **II** is a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In other embodiments, the R^1 group of formula **I** is a monocyclic 5-6 membered aryl ring having 0-2 nitrogen atoms, wherein R^1 is optionally substituted with 1 to 3 substituents independently selected from halogen, -C≡N, $-CH_2-C\equiv N$, $-(CH_2)_{0-4}R°$, $-(CH_2)_{0-4}OR°$, $-(CH_2)_{0-4}SR°$, $-(CH_2)_{0-4}Ph$, optionally substituted with R° or OR°, $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ optionally substituted with R° or OR°, -CH=CHPh, optionally substituted with R° or OR°, $-(CH_2)_{0-4}N(R°)_2$, wherein

each R° may be substituted as defined herein and is independently hydrogen, C_{1-6} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. According to one embodiment of the present invention, R^1 is phenyl substituted with 1 to 3 groups independently selected from halogen, $-(CH_2)_{0-4}R°$, and $-(CH_2)_{0-4}OR°$. Such groups include chloro, fluoro, OH, OMe, methyl, ethyl, propyl, cyclopropyl, isopropyl, and the like.

[97] As defined generally above, the R^2 group of formula **II** hydrogen, or an optionally substituted group selected from a C_{1-6} aliphatic group. In certain embodiments, the R^2 group is a methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, pentyl, and the like.

[98] As defined generally above, the Q group of formula **II** is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain, wherein 0–2 methylene units of Q are independently replaced by -O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-. In certain embodiments, Q is a valence bond such that R^1 is directly attached to carbon attached to the sulfur atom. In other embodiments, Q is a bivalent, saturated, and straight C_{1-3} hydrocarbon chain, wherein 0-1 methylene units of Q is replaced by -O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-. In still other embodiments, Q is -O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-. According to another embodiment, Q is $-CH_2-$ or $-CH_2-CH_2-$.

[99] In some embodiments of the present invention, Trx/TrxR inhibitors have the structure of formula **III**:



III

or a pharmaceutically acceptable salt or derivative thereof, wherein:

R^1 is hydrogen, -C≡N, or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain, wherein 0–2 methylene units of Q are independently replaced by –O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-;

each R is independently hydrogen or an optionally substituted aliphatic group;

each R^2 is independently halogen, R^3, OR^3, SR^3, $N(R^3)_2$, $C(O)R^3$, $C(O)OR^3$, $NR^3C(O)R^3$, $C(O)NR^3$, SO_2R^3, $NR^3SO_2R^3$, $SO_2N(R^3)_2$; and

each R^3 is independently hydrogen or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[100] As defined generally above, the R^1 group of formula **III** is hydrogen, -C≡N, or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

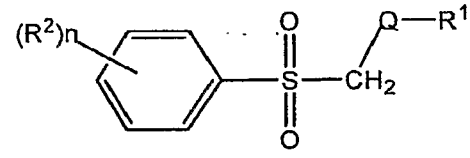
[101] In certain embodiments, the R^1 group of formula **III** is a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In other embodiments, the R^1 group of formula **I** is a monocyclic 5-6 membered aryl ring having 0-2 nitrogen atoms, wherein R^1 is optionally substituted with 1 to 3 substituents independently selected from halogen, -C≡N, $-CH_2-C\equiv N$, $-(CH_2)_{0-4}R°$, $-(CH_2)_{0-4}OR°$, $-(CH_2)_{0-4}SR°$, $-(CH_2)_{0-4}Ph$, optionally substituted with R° or OR°, $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ optionally substituted with R° or OR°, -CH=CHPh, optionally substituted with R° or OR°, $-(CH_2)_{0-4}N(R°)_2$, wherein each R° may be substituted as defined herein and is independently hydrogen, C_{1-6}

aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. According to one embodiment of the present invention, R^1 is phenyl substituted with 1 to 3 groups independently selected from halogen, $-(CH_2)_{0-4}R^\circ$, and $-(CH_2)_{0-4}OR^\circ$. Such groups include chloro, fluoro, OH, OMe, methyl, ethyl, propyl, cyclopropyl, isopropyl, and the like.

[102] As defined generally above, the Q group of formula **III** is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain, wherein 0–2 methylene units of Q are independently replaced by -O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-. In certain embodiments, Q is a valence bond such that R^1 is directly attached to carbon attached to the sulfur atom. In other embodiments, Q is a bivalent, saturated, and straight C_{1-3} hydrocarbon chain, wherein 0-1 methylene units of Q is replaced by -O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-. In still other embodiments, Q is -O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-. According to another embodiment, Q is $-CH_2-$ or $-CH_2-CH_2-$.

[103] As defined generally above, the R^2 group of formula **III** is halogen, R^3, OR^3, SR^3, $N(R^3)_2$, $C(O)R^3$, $C(O)OR^3$, $NR^3C(O)R^3$, $C(O)NR^3$, SO_2R^3, $NR^3SO_2R^3$, or $SO_2N(R^3)_2$, wherein R^3 is independently hydrogen or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In certain embodiments, the R^3 of is a substituted C_{1-6} aliphatic group, such as $-CX_3$, $-CHX_2$, and $-CH_2X$, wherein X is chloro, fluoro, bromo or iodo.

[104] In some embodiments of the present invention, Trx/TrxR inhibitors have the structure of formula **IV**:



IV

or a pharmaceutically acceptable salt or derivative thereof, wherein:

R^1 is hydrogen, -C≡N, or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain, wherein 0–2 methylene units of Q are independently replaced by –O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-;

each R is independently hydrogen or an optionally substituted aliphatic group;

each R^2 is independently halogen, R^3, OR^3, SR^3, $N(R^3)_2$, $C(O)R^3$, $C(O)OR^3$, $NR^3C(O)R^3$, $C(O)NR^3$, SO_2R^3, $NR^3SO_2R^3$, $SO_2N(R^3)_2$; and

each R^3 is independently hydrogen or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[105] As defined generally above, the R^1 group of formula **IV** is hydrogen, -C≡N, or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

[106] In certain embodiments, the R^1 group of formula **IV** is a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. In other embodiments, the R^1 group of formula **I** is a monocyclic 5-6 membered aryl ring having 0-2 nitrogen atoms,

wherein R^1 is optionally substituted with 1 to 3 substituents independently selected from halogen, -C≡N, $-CH_2$-C≡N, $-(CH_2)_{0-4}R°$, $-(CH_2)_{0-4}OR°$, $-(CH_2)_{0-4}SR°$, $-(CH_2)_{0-4}Ph$, optionally substituted with R° or OR°, $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ optionally substituted with R° or OR°, -CH=CHPh, optionally substituted with R° or OR°, $-(CH_2)_{0-4}N(R°)_2$, wherein each R° may be substituted as defined herein and is independently hydrogen, C_{1-6} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5–6-membered saturated, partially unsaturated, or aryl ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. According to one embodiment of the present invention, R^1 is phenyl substituted with 1 to 3 groups independently selected from halogen, $-(CH_2)_{0-4}R°$, and $-(CH_2)_{0-4}OR°$. Such groups include chloro, fluoro, OH, OMe, methyl, ethyl, propyl, cyclopropyl, isopropyl, and the like.

[107] As defined generally above, the Q group of formula **IV** is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain, wherein 0–2 methylene units of Q are independently replaced by -O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2$-, $-NRSO_2$-, $-SO_2NR$-, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-. In certain embodiments, Q is a valence bond such that R^1 is directly attached to carbon attached to the sulfur atom. In other embodiments, Q is a bivalent, saturated, and straight C_{1-3} hydrocarbon chain, wherein 0-1 methylene units of Q is replaced by -O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2$-, $-NRSO_2$-, $-SO_2NR$-, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-. In still other embodiments, Q is -O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2$-, $-NRSO_2$-, $-SO_2NR$-, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-. According to another embodiment, Q is $-CH_2$- or $-CH_2$-CH_2-.

[108] In certain embodiments, a Trx/TrxR inhibitor has a structure selected from the group consisting of structure 1, structure 3, structure 4, structure 5, structure 6, structure 9, structure 11, structure 12, structure 13, structure 14, structure 15, structure 33, structure 35, structure 36, structure 37, structure 38, structure 39, structure 39, structure 43, structure 44, structure 45, structure 46, structure 47, structure 48, structure 71, structure 97, structure 99, structure 100, structure 101, structure 113, structure 114, structure 116, structure 117, structure 118, and structure 119, presented on Table 4.

III – Pharmaceutical Compositions

[109] . . Trx/TrxR inhibitors provided by the present invention may be administered *per se* or in the form of a pharmaceutical composition. Accordingly, the present invention provides pharmaceutical compositions comprising at least one physiologically acceptable carrier or excipient and an effective amount of at least one inventive compound. In some embodiments, the composition further comprises one or more additional therapeutic agents.

[110] Pharmaceutical compositions of the present invention may be administered using any amount and any route of administration effective for achieving the desired effect. The exact amount of pharmaceutical composition to be administered will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the condition, and the like (see below).

Formulation

[111] The optimal pharmaceutical formulation can be varied depending upon the route of administration and desired dosage. Such formulations may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the administered compounds.

[112] The pharmaceutical compositions of the present invention may be formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment.

[113] After formulation with one or more appropriate physiologically acceptable carrier(s) or excipient(s) in a desired dosage, the pharmaceutical compositions of the present invention can be administered to humans or other mammals by any suitable route. Various delivery systems are known and can be used to administer the inventive compositions, including, tablets, capsules, injectable solutions, encapsulation in liposomes, microparticles, microcapsules, etc. Methods of administration include, but are not limited to, dermal, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, pulmonary, epidural, ocular, and oral routes. An inventive composition may be administered by any convenient or otherwise appropriate route, for example, by infusion or bolus injection, by absorption through epithelial or

mucocutaneous linings (*e.g.*, oral, mucosa, rectal and intestinal mucosa, etc) and may be administered together with other biologically active agents. Administration can be systemic or local. For treatment of nasal, bronchial or pulmonary conditions, preferred routes of administration may be oral, nasal, or *via* a bronchial aerosol or nebulizer.

[114] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents, and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a non-toxic parenterally acceptable diluent or solvent, for example, as a solution in 2,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solution or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or di-glycerides. Fatty acids such as oleic acid may also be used in the preparation of injectable formulations. Sterile liquid carriers are useful in sterile liquid from compositions for parenteral administration.

[115] Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use. Liquid pharmaceutical compositions which are sterile solutions or suspensions can be administered by, for example, intravenous, intramuscular, intraperitoneal or subcutaneous injection. Injection may be *via* single push or by gradual infusion (*e.g.*, 30 minute intravenous infusion). Where necessary, the composition may include a local anesthetic to ease pain at the site of injection.

[116] In order to prolong the effect of a drug, it is often desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming micro-encapsuled matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to

. . polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations can also be prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

[117] Liquid dosage forms for oral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, elixirs, and pressurized compositions. In addition to the active ingredients (*i.e.*, an inventive Trx/TrxR inhibitor and, optionally, one or more additional therapeutic agents), the liquid dosage form may contain inert diluents commonly used in the art such as, for example, water or other solvent, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cotton seed, ground nut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, suspending agents, preservatives, sweetening, flavoring, and perfuming agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral administration include water (partially containing additives as above; *e.g.*, cellulose derivatives, such as sodium caboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols such as glycols) and their derivatives, and oils (*e.g.*, fractionated coconut oil and arachis oil)). For pressurized compositions, the liquid carrier can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

[118] Solid dosage forms for oral administration include, for example, capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active ingredient(s) is/are mixed with at least one inert, physiologically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and one or more of: (a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid; (b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia; (c) humectants such as glycerol; (d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (e) solution retarding agents such as paraffin; (f) absorption accelerators such

as quaternary ammonium compounds; (g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate; (h) absorbents such as kaolin and bentonite clay; and (i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. Other excipients suitable for solid formulations include surface modifying agents such as non-ionic and anionic surface modifying agents. Representative examples of surface modifying agents include, but are not limited to, poloxamer 188, benzalkonium chloride, calcium stearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, magnesium aluminum silicate, and triethanolamine. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents. The amount of solid carrier per solid dosage form will vary widely but preferably will be from about 25 mg to about 1 g.

[119] Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition such that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

[120] Pharmaceutical compositions of the present invention may be provided as immediate release formulations or as sustained release formulations. A variety of strategies are known in the art for achieving sustained release, *e.g.*, by prolonging residence time in the stomach (such as through the use of swellable polymers), providing pH or enzyme-sensitive coating, employing bioadhesive coatings that stick to the walls of the stomach or intestine, etc (see, *e.g.*, U.S. Pat. No. 2004-0024018 and references therein.

[121] In certain embodiments, it may be desirable to administer an inventive composition locally to an area in need of treatment. This may be achieved, for example, and not by way of limitation, by local infusion during surgery, topically application, by

injection, by means of a catheter, by means of suppository, or by means of a skin patch or stent or other implant.

[122] For topical administration, the composition is preferably formulated as a gel, an ointment, a lotion, or a cream which can include carriers such as water, glycerol, alcohol, propylene glycol, fatty alcohols, triglycerides, fatty acid esters, or mineral oil. Other topical carriers include liquid petroleum, isopropyl palmitate, polyethylene glycol, ethanol (95%), polyoxyethylenemonolaurate (5%) in water, or sodium lauryl sulfate (5%) in water. Other materials such as antioxidants, humectants, viscosity stabilizers, and similar agents may be added as necessary. Percutaneous penetration enhancers such as Azone may also be included.

[123] In addition, in certain instances, it is expected that the inventive compositions may be disposed within transdermal devices placed upon, in, or under the skin. Such devices include patches, implants, and injections which release the compound onto the skin, by either passive or active release mechanisms. Transdermal administrations include all administrations across the surface of the body and the inner linings of bodily passage including epithelial and mucosal tissues. Such administrations may be carried out using the present compositions in lotions, creams, foams, patches, suspensions, solutions, and suppositories (rectal and vaginal).

[124] Transdermal administration may be accomplished through the use of a transdermal patch containing the active ingredient(s) and a carrier that is non-toxic to the skin, and allows the delivery of the ingredient(s) for systemic absorption into the bloodstream *via* the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient(s) may also be suitable. A variety of occlusive devices may be used to release the active ingredient(s) into the bloodstream such as a semi-permeable membrane covering a reservoir containing the active ingredient(s) with or without a carrier, or a matrix containing the active ingredient.

[125] Suppository formulations may be made from traditional materials, including cocoa butter, with or without the addition of waxes to alter the suppository's melting

point, and glycerin. Water soluble suppository bases, such as polyethylene glycols of various molecular weights, may also be used.

[126] As is understood in the art, in certain embodiments, a pharmaceutically acceptable salt of the Trx/TrxR inhibitor is used in the composition. Pharmaceutically acceptable salts of inventive Trx/TrxR inhibitors (and/or of additional therapeutic agents contained in the pharmaceutical composition) include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acid salts include acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptanoate, glycerophosphate, glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oxalate, palmoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, salicylate, succinate, sulfate, tartrate, thiocyanate, tosylate and undecanoate. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the inventive Trx/TrxR inhibitors (or additional therapeutic agents) and their pharmaceutically acceptable acid addition salts.

[127] Salts derived from appropriate bases include alkali metal (*e.g.*, sodium and potassium), alkaline earth metal (*e.g.*, magnesium), ammonium and $N^+(C_{1-4}$ alkyl$)_4$ salts. This invention also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or oil-soluble or dispersible products may be obtained by such quaternization.

[128] In some embodiments, inventive pharmaceutical compositions include one or more agents intended to protect the active agent(s) against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polyethers, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Certain of the materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions can also be

used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811 and other references listed herein. Liposomes, including targeted liposomes (*e.g.*, antibody targeted liposomes) and pegylated liposomes have been described (C.B. Hansen *et al.*, Biochim. Biophys. Acta, 1995, 1239: 133-144; V.P. Torchilin *et al.*, Biochim. Biophys. Acta, 2001, 1511: 397-411; T. Ishida *et al.*, FEBS Lett., 1999, 460: 129-133).

[129] Materials and methods for producing various formulations are known in the art and may be adapted for practicing the subject invention (see, for example, "*Remington's Pharmaceutical Sciences*", E.W. Martin, 18th Ed., 1990, Mack Publishing Co.: Easton, PA).

Additional Therapeutic Agents

[130] In certain embodiments of the invention, the pharmaceutical compositions further comprise at least one additional therapeutic agent. As will be appreciated by those of ordinary skill in the art, therapeutic agents suitable for use in the inventive compositions include any drug whose administration may be beneficial to the subject receiving a composition of the invention. Thus, therapeutic agents suitable for use in inventive pharmaceutical compositions include, for example, agents that are independently active against the disease or clinical condition mediated by the thioredoxin/thioredoxin reductase system that is to be treated; agents that are active against a disease or condition associated with the disease or clinical condition mediated by the thioredoxin/thioredoxin reductase system; and agents that enhance availability and/or activity of an inventive Trx/TrxR inhibitor (or additional therapeutic agent).

[131] Examples of suitable therapeutic agents include, but are not limited to, is anticancer agents, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), analgesics, antipyretics, sedatives, antianginal agents, antianxiety agents, antidepressants, antipsychotic agents, antiarryhythmics, antihypertensive drugs (*e.g.*, diuretics, beta-adrenergic blocking agents, angiotensin converting enzyme inhibitors, calcium channel-blocking agents, α-adrenoceptor blocking agents, sympatholytics, and vasodilators), antihistamine/antipruritic drugs, immunosuppressants, antimetabolite cytotoxics, neuroprotective agents, T cell inhibitors, antigout agents, anticoagulants, thrombolytic

agents, antifibrinolytic agents, hemorheologic agents, antiplatelet agents, anticonvulsants, agents useful for calcium regulation, antibacterial agents, antifungal agents, antimicrobials, antioxidants, anti-infectives, bronchodilators, hormones, hypoglycemic agents, hypolipidemic agents, proteins, nucleic acids, antibodies, agents useful for erythropoiesis stimulation, antiulcer/antirefluc agents, antinauseants/antiemetics, septic shock agents, Alzheimer's disease agents, Parkinson disease agents, organ transplantation agents, psoriasis agents, angiogenesis inhibitors, anticancer antibiotics, antiproliferative compounds, CD4 binding inhibitors, immunostimulants, photodynamic anticancer agents, photosensitizers, platelet activating factor antagonists, platelet aggregation inhibitors, vitamins, diabetes agents, stroke agents, agents useful for the treatment of neutropenia, agents useful for the treatment of respiratory disorders, agents useful for the treatment of ischemia/reperfusion injury, nitric oxide synthase inhibitors, and the like.

[132] In certain embodiments, the additional therapeutic agent has inhibitory effects on the thioredoxin/thioredoxin reductase system. Clinically applied drugs that have been demonstrated or that are postulated to have effects on thioredoxin reductases include, but are not limited to, carmustine (BCNU) and other nitrosoureas (L.D. Arscott *et al.*, Proc. Natl. Acad. Sci. USA, 1997, 94: 3621-3626; S. Gromer *et al.*, FEBS Lett., 1997, 412: 318-320; K.U. Schallreuter *et al.*, Biochim. Biophys. Acta, 1990, 1054: 14-20); alkylating anticancer agents (A.B. Witte *et al.*, Free Radic. Biol. Med., 2005, 39: 696-703); cisplatin and other platinum-containing anticancer agents (T. Sasada *et al.*, Free Radic. Biol. Med., 1999, 27: 504-514; A.B. Witte *et al.*, Free Radic. Biol. Med., 2005, 39: 696-703); quercetin and myricetin (J. Lu *et al.*, Cancer Res., 2006, 66: 4410-4418); anthracyclines (D. Mustachich and G. Powis, Biochem. J., 2000, 346: 1-8); azelaic acid (S. Gomer *et al.*, Redox Report, 1999, 4: 221-228), auranofin and aurothioglucose (S. Gromer *et al.*, J. Biol. Chem., 1998, 273: 20096-20101), 13-cis-retinoic acid (S. Gomer *et al.*, Redox Report, 1999, 4: 221-228; I. Bruchhaus *et al.*, Arch. Med. Res., 1997, 28: 91-92); and 1-chloro-2,4-dinitrobenzene (D. Mustachich and G. Powis, Biochem. J., 2000, 346: 1-8; S. Gomer *et al.*, Redox Report, 1999, 4: 221-228; L. Zhong *et al.*, J. Biol. Chem., 1998, 273: 8581-8591).

[133] In embodiments where an inventive pharmaceutical composition is to be used in the treatment of Alzheimer's disease, the additional therapeutic agent(s) may be an Alzheimer's disease agents. Examples of Alzheimer's disease agents include, but are not

limited to, ACh release enhancers (*e.g.*, T-588 (benzothiophene derivative)), acetylcholine release stimulants (*e.g.*, DUP-996 and analogues), AMPA agonists (*e.g.*, AMAlex, and Isoxazole compound series), AMPA GluR agonist (*e.g.*, IDRA-21 [7-chloro-3-methyl-3,4-dihydro-2H-1,2,4-benzothiadiazinine]), AMPA GluR antagonists (*e.g.*, S-18986, and related quinolone derivatives), anticholinesterases (*e.g.*, E-2020), Ca-antagonists (*e.g.*, NS-649, spider venom-derived ICM peptides and analogues, and substituted 2-aminoindanes compound series), combined anticholinesterase and muscarinic AChR antagonists (*e.g.*, PD 142676), K-channel blockers (*e.g.*, Trans-R-4-(4-methoxyphenyl-methyl) cyclohexylanine and analogues, and margatoxin-based functional and/or structural analogues), MI muscarinic receptor agonists (*e.g.*, Xanomeline), NMDA antagonists (*e.g.*, certain indole derivatives, and (R-(R1,S 1))-α-(4-hydroxyphenyl)-beta-methyl-4-(phenylmenthyl)-1-piperidinepropanol and analogues), nicotinic AChR agonists (*e.g.*, ABT-418 [isoxazole, 3-meth-5-(1-meth-2-pyrrolidinyl)]), and the like.

[134] In embodiments where an inventive pharmaceutical composition is to be used in the treatment of cancer, the additional therapeutic agent(s) may be a chemotherapeutic agent. A chemotherapeutic agent suitable for use in the present invention may be a synthetic or natural compound, a single molecule, a mixture of different molecules or a complex of different molecules. Chemotherapeutics can belong to any of various classes of molecules including, but not limited to, small molecules, peptides, saccharides, steroids, antibodies (including fragments or variants thereof), fusion proteins, antisense polynucleotides, ribozymes, small interfering RNAs, peptidomimetics, and the like. Suitable chemotherapeutics can also be found in any of the following classes of anti-cancer drugs: alkylating agents, anti-metabolites drugs, anti-mitotic antibiotics, alcoloidal anti-tumor agents, hormones and anti-hormones, interferon, non-steroidal anti-inflammatory drugs, and various other anti-tumor agents such as kinase inhibitors (*e.g.*, inhibitors of Src, BRC/Abl, kdr, aurora-2, glycogen synthase kinase 3 or GSK-3), proteaome inhibitors and NF-κB inhibitors.

[135] Examples of suitable chemotherapeutics are include, but are not limited to, Zyloprim, alemtuzmab, altretamine, amifostine, nastrozole, antibodies against prostate-specific membrane antigen (such as MLN-591, MLN591RL and MLN2704), arsenic trioxide, Avastin™ (bevacizumab), (or other anti-VEGF antibody), bexarotene, bleomycin, busulfan, carboplatin, celecoxib, chlorambucil, cisplatin, cisplatin-epinephrine

gel, cladribine, cytarabine liposomal, daunorubicin liposomal, daunorubicin, daunomycin, dexrazoxane, docetaxel, doxorubicin, Elliott's B Solution, epirubicin, estramustine, etoposide phosphate, etoposide, exemestane, fludarabine, 5-FU, fulvestrant, gemcitabine, gemtuzumab-ozogamicin, goserelin acetate, hydroxyurea, idarubicin, idarubicin, Idamycin, ifosfamide, imatinib mesylate, irinotecan (or other topoisomerase inhibitor, including antibodies such as MLN576 (XR11576)), letrozole, leucovorin, leucovorin levamisole, liposomal daunorubicin, melphalan, L-PAM, mesna, methotrexate, methoxsalen, mitomycin C, mitoxantrone, MLN518 or MLN608 (or other inhibitors of the fit-3 receptor tyrosine kinase, PDFG-R or c-kit), itoxantrone, paclitaxel, Pegademase, pentostatin, porfimer sodium, Rituximab (RITUXAN™), tamoxifen, temozolamide, teniposide, VM-26, topotecan, toremifene, Trastuzumab (Herceptin™ or other anti-Her2 antibody), 2C4 (or other antibody which interferes with HER2-mediated signaling), tretinoin, ATRA, valrubicin, vinorelbine, or pamidronate, zoledronate or another bisphosphonates.

[136] In embodiments where an inventive pharmaceutical composition is to be used in the treatment of HIV infection or AIDS, the additional therapeutic agent(s) may be an anti-HIV or anti-AIDS drug. Examples of such drugs include, but are not limited to, nucleoside reverse transcriptase inhibitors such as AZT (zidovudine, Retrovir®), ddC (zalcitabine, Hivid®), ddI (dideoxyinosine, Videx®), d4T (stavudine, Zerit®), 3TC (lamivudine, Epivir®), abacavir (Ziagen®); nucleotide reverse transcriptase inhibitors such as tenofovir; non-nucleoside reverse transcriptase inhibitors such as nevirapine (Viramune®), efavirenz, and delavirdine (Rescriptor®); protease inhibitors such as saquinavir, ritonavir (Norvir®), indinavir, nelfinavir (Viracept®), amprinavir (Agenerase®), lopinavir and atazanavir; saquinavir (Invirase®), indinavir sulphate (Crixivan®) and fusion inhibitors such as enfuvirtide.

[137] In embodiments where an inventive pharmaceutical composition is to be used in the treatment of cardiovascular diseases, the additional therapeutic agent(s) may be hypertensives, aspirin or statin cholesterol lowering drugs.

[138] Examples of hypertensives include, but are not limited to diuretics, *e.g.* loop diuretics (such as bumetanide, ethacrynic acid, furomeside, and torsemide), thiazide diuretics (such as chlortalidone, epitizide, hydrochlorothiazide and chlorothiazide, and bendroflumethiazide), thiazide-like diuretics (such as indapamide and metolazone) and

potassium-sparing diuretics (such as amiloride and trimaterene); antiadrenergics, *e.g.*, beta-blockers (such as atenolol, metoprolol, nadolol, oxprenolol, pindolol, propanolol, and timolol), alpha-blockers (such as doxazosin, phentolamine, indoramin, phenoxybenzamine, prazosin, terazosin, and tolazoline) and mixed alpha- and beta-blockers (such as bucindolol, carvedilol, and labetalol); calcium-channel blockers, *e.g.*, dihydropyridines (such as amlodipine, felodipine, isradipine, nifedipine, nimodipine, and nitrendipine), and non-hydropyridines (such as diltiazem and verapamil); ACE (angiotensin-converting enzyme) inhibitors, *e.g.*, captopril, enalapril, fosinopril, lisinopril, perindopril, quinopril, ramipril, trandopril, and benzapril; angiotensin II receptor antagonists, *e.g.*, candesartan, irbesartan, losartan, telmisartan, valsartan; aldosterone antagonists, *e.g.*, spironolactone; vasodillators, *e.g.*, sodium nitroprusside; centrally acting adrenergic drugs, *e.g.*, clonidine, guannabenz and methyldopa; and adrenergic neuron blockers, *e.g.*, guanethidine and reserping.

[139] Examples of statin cholesterol lowering drugs include, but are not limited to, atorvastatin (Lipitor® and Torvast®), cerivastatin (Lipobay® and Baycol®), fluvastatin (Lescol®), lovastatin (Mevacor® and Altocor®), mevastatin, pitavastatin (Livalo® and Pitava®), Pravastatin (Pravachol®, Selektine®, Lipostat®), rosuvastatin (Crestor®), simvastatin (Zocor® and Lipex®), and Ezetimine+Simvastatin (Vytorin®).

Pharmaceutical Kits

[140] The present invention also provides pharmaceutical packs of kits comprising one or more containers (*e.g.*, vials, ampoules, test tubes, flasks, or bottles) containing one or more ingredients of the inventive pharmaceutical compositions, for example, allowing for the simultaneous or sequential administration of Trx/TrxR inhibitors and therapeutic agents. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceutical products, which notice reflects approval by the agency of manufacture, use or sale for human administration. Different ingredients may be supplied in solid (*e.g.*, lyophilized) or liquid form. Kits may also include media for the reconstitution of lyophilized ingredients. The individual containers of the kit are preferably maintained in close confinement for commercial use.

IV - Dosage and Administration

[141] A treatment according to the present invention may consist of a single dose or a plurality of doses over a period of time. When an Trx/TrxR inhibitor of the invention is used in combination with at least one other therapeutic agent, the Trx/TrxR inhibitor and therapeutic agent may be administered concurrently or sequentially. For example, the Trx/TrxR inhibitor may be administered prior to or following administration of the therapeutic agent (*e.g.*, one or more hour(s) or one or more day(s) before and/or one or more hour(s) or one or more day(s) after).

[142] Inventive pharmaceutical compositions may be administered according to any desired schedule, typically selected to achieve optimal therapeutic effect. Administration may be one or multiple times daily, weekly (or at some other multiple day interval) or on an intermittent schedule. For example, the Trx/TrxR inhibitor (and optionally additional therapeutic agent) may be administered one or more times per day on a weekly basis for a period of weeks. Alternatively, the Trx/TrxR inhibitor (and optionally additionally therapeutic agent) may be administered daily for a period of days following a period of days without administration, with that cycle repeated a given number of times.

[143] The administration may be carried out in any convenient manner such as by injection (subcutaneous, intravenous, intramuscular, intraperitoneal, or the like) or oral administration.

[144] Depending on the route of administration, effective doses may be calculated according to the body weight, body surface area, or organ size of the subject to be treated. Optimization of the appropriate dosages can readily be made by one skilled in the art in light of pharmacokinetic data observed in human clinical trials. The final dosage regimen will be determined by the attending physician, considering various factors which modify the action of the drugs, *e.g.*, the drug's specific activity, the severity of the damage and the responsiveness of the patient, the age, condition, body weight, sex and diet of the patient, the severity of any present infection, time of administration, the use (or not) of concomitant therapies, and other clinical factors. As studies are conducted using the inventive compounds and compositions, further information will emerge regarding the appropriate dosage levels and duration of treatment.

[145]. In general, pharmaceutical compositions are formulated to contain an amount of active agent(s) (*i.e.*, inventive Trx/TrxR inhibitor and, optionally, one or more therapeutic agents) sufficient to achieve a desired biological or pharmacological effect while minimizing any associated toxicity. Toxicity and therapeutic efficacy of active agents can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ ED_{50}. Agents which exhibit high therapeutic indices are preferred. While agents that exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such agents to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[146] The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such agents lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any agent used in accordance with the present invention, the therapeutically effective amount can typically be estimated initially from cell culture assays. However, it is generally more desirable to establish dosing based on studies in animal models, where amounts required to achieve a circulating plasma concentration range that includes the IC_{50} (*e.g.*, the concentration of the test agent which achieves a half-maximal inhibition of symptoms, half-maximal inhibition of growth or survival of an infectious agent, etc.) can be determined. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

[147] A therapeutically effective amount of an active agent in a pharmaceutical composition typically ranges from about 0.001 to about 100 mg/kg body weight, about 0.01 to about 25 mg/kg body weight, about 0.1 to about 20 mg/kg body weight, about 1 to about 10 mg/kg, about 2 to about 9 mg/kg, about 3 to about 8 mg/kg, about 4 to about 7 mg/kg, or about 5 to about 6 mg/kg body weight. Other exemplary doses include, for example, about 1 μg/kg to about 500 mg/kg, about 100 μg/kg to about 5 mg/, about 1 μg/kg to about 50 μg/kg). In general, smaller doses are typically required for local

administration as contrasted with systemic administration. Furthermore, it is understood by those of ordinary skill in the art that appropriate doses in any particular circumstance depend upon the potency of the Trx/TrxR inhibitor(s) utilized, and may optionally be tailored to the particular recipient, for example, through administration of increasing doses until a preselected desired response is achieved.

[148] As will be appreciated by one skilled in the art, the use of combination of an inventive Trx/TrxR inhibitor and at least one therapeutic agent according to the present invention may allow at least one of the involved agent to be administered at subtherapeutically effective dosages, thereby lessening toxicity associated with the individual agent.

[149] It will also be appreciated that pharmaceutical compositions of the present invention can be employed in combination with additional therapies (*i.e.*, a treatment according to the present invention can be administered concurrently with, prior to, or subsequently to one or more desired therapeutics or medical procedures). The particular combination therapies (therapeutics or procedures) to employ in such a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved.

[150] For example, in embodiments where Trx/TrxR inhibitors are used as anti-cancer agents, methods and compositions can be employed with other procedures including surgery, radiotherapy (*e.g.*, γ-radiation, neuron beam radiotherapy, electron beam radiotherapy, proton therapy, brachytherapy, an systemic radioactive isotopes), endocrine therapy, hyperthermia, and cryotherapy. Alternatively or additionally, methods and compositions of the present invention can be employed together with other agents to attenuate any adverse effects (*e.g.*, antiemetics), and/or with other approved chemotherapeutic drugs, including, but not limited to, alkylating drugs (mechlorethamine, chlorambucil, Cyclophosphamide, Melphalan, Ifosfamide), antimetabolites (Methotrexate), purine antagonists and pyrimidine antagonists (6-Mercaptopurine, 5-Fluorouracil, Cytarabile, Gemcitabine), spindle poisons (Vinblastine, Vincristine, Vinorelbine, Paclitaxel), podophyllotoxins (Etoposide, Irinotecan, Topotecan), antibiotics (Doxorubicin, Bleomycin, Mitomycin), nitrosoureas (Carmustine, Lomustine), inorganic ions (Cisplatin, Carboplatin), enzymes (Asparaginase), and hormones (Tamoxifen, Leuprolide, Flutamide, and Megestrol), to name a few, and/or with a combination of

cytotoxic agents such as CHOPP (cyclophosphamide, doxorubicin, vincristine, prednisone, and procarbazine); CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone); COP (cyclophosphamide, vincristine, and prednisone); CAP-BOP (cyclophosphamide, doxorubicin, procarbazine, bleomycin, vincristine, and prednisone); m-BACOD (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone, and leucovorin), and the like.. For a more comprehensive discussion of updated cancer therapies see, http://www.cancer.gov/, a list of the FDA approved oncology drugs at http://www.fda.gov/cder/cancer/druglistframe.htm, and The Merck Manual, Seventeenth Ed. 1999, the entire contents of which are hereby incorporated by reference.

V - Indications

[151] As already mentioned above, compositions and methods of the present invention can be used to treat any disease, disorder or condition that is mediated by the thioredoxin/thioredoxin reductase system. Examples of such diseases, disorders and conditions include, but are not limited to, cancer, rheumatoid arthritis, HIV infection/AIDS, Alzheimer's disease, skin diseases, and cardiovascular diseases. Compositions and methods can also be used to treat any disease, disorder or condition associated with oxidative stress. Examples of such diseases, disorder and conditions include, but are not limited to, Parkinson's disease and Alzheimer's disease, Amyotrophic lateral sclerosis (ALS, sometimes called Lou Gehrig's disease, Maladie to Charcot or motor neurone disease), Creutzfeldt-Jakob disease, respiratory distress syndrome, muscular dystrophy, cataractogenesis, progeria, Werner's syndrome, atherosclerosis, diabetes, essential hypertension, cystic fibrosis, ulcerative colitis, carcinogenesis, and chronic inflammatory disorders such as asthma, chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, and psoriasis.

[152] In certain embodiments, compositions and methods of the present invention are used in the treatment of cancer (S. Urig and K. Becker, Semin. Cancer Biol., 2006, 16: 452-465; E.S. Arner and A. Holmgren, Semin. Cancer Biol., 2006, 16: 420-426; P. Nguyen *et al.*, Cancer Lett., 2006, 236: 164-174). Inventive compositions and methods can be used to treat primary and/or metastatic cancers, and other cancerous conditions. For example, the inventive compositions and methods can be useful for reducing size of

solid tumors, inhibiting tumor growth or metastasis, treating various lymphatic cancers, and/or prolonging the survival time of mammals (including humans) suffering from these diseases.

[153] Examples of cancers and cancer conditions that can be treated according to the present invention include, but are not limited to, tumors of the brain and central nervous system (*e.g.*, tumors of the meninges, brain, spinal cord, cranial nerves and other parts of the CNS, such as glioblastomas or medulla blastomas); head and/or neck cancer, breast tumors, tumors of the circulatory system (*e.g.*, heart, mediastinum and pleura, and other intrathoracic organs, vascular tumors, and tumor-associated vascular tissue); tumors of the blood and lymphatic system (*e.g.*, Hodgkin's disease, Non-Hodgkin's disease lymphoma, Burkitt's lymphoma, AIDS-related lymphomas, malignant immunoproliferative diseases, multiple myeloma, and malignant plasma cell neoplasms, lymphoid leukemia, myeloid leukemia, acute or chronic lymphocytic leukemia, monocytic leukemia, other leukemias of specific cell type, leukemia of unspecified cell type, unspecified malignant neoplasms of lymphoid, haematopoietic and related tissues, such as diffuse large cell lymphoma, T-cell lymphoma or cutaneous T-cell lymphoma); tumors of the excretory system (*e.g.*, kidney, renal pelvis, ureter, bladder, and other urinary organs); tumors of the gastrointestinal tract (*e.g.*, oesophagus, stomach, small intestine, colon, colorectal, rectosigmoid junction, rectum, anus, and anal canal); tumors involving the liver and intrahepatic bile ducts, gall bladder, and other parts of the biliary tract, pancreas, and other digestive organs; tumors of the oral cavity (*e.g.*, lip, tongue, gum, floor of mouth, palate, parotid gland, salivary glands, tonsil, oropharynx, nasopharynx, puriform sinus, hypopharynx, and other sites of the oral cavity); tumors of the reproductive system (*e.g.*, vulva, vagina, Cervix uteri, uterus, ovary, and other sites associated with female genital organs, placenta, penis, prostate, testis, and other sites associated with male genital organs); tumors of the respiratory tract (*e.g.*, nasal cavity, middle ear, accessory sinuses, larynx, trachea, bronchus and lung, such as small cell lung cancer and non-small cell lung cancer); tumors of the skeletal system (*e.g.*, bone and articular cartilage of limbs, bone articular cartilage and other sites); tumors of the skin (*e.g.*, malignant malonoma of the skin, non-melanoma skin cancer, basal cell carcinoma of skin, squamous cell carcinoma of skin, mesothelioma, Kaposi's sarcoma); and tumors involving other tissues including peripheral nerves and autonomic nervous system,

. . . connective and soft tissue, retroperitoneoum and peritoneum, eye and adnexa, thyroid, adrenal gland, and other endocrine glands and related structures, secondary and unspecified malignant neoplasms of lymph nodes, secondary malignant neoplasm of respiratory and digestive systems and secondary malignant neoplasms of other sites.

[154] Thioredoxin expression has been shown to be increased in a variety of human malignancies including lung, colorectal, cervical, hepatic, and pancreatic cancer (G. Powis *et al.*, Chem-Biol. Interact., 1998, 111-112: 23-34; S.J. Welsh *et al.*, Cancer Res., 2002, 62: 5089-5095; S. Kakolyris *et al.*, Clin. Cancer Res., 2001, 7: 3087-3091; J. Raffel *et al.*, J. Lab. Clin. Med., 2003, 142: 46-51; H.J. Kim *et al.*, Cell Biol. Toxicol., 2003, 19: 285-298; D.T. Lincoln *et al.*, Anticancer Res., 2003, 23: 2425-2434; H. Han *et al.*, Cancer Res., 2002, 62: 2890-2896; D. Hedley *et al.*, Am. J. Pathol., 2004, 164: 557-565; J.H. Choi *et al.*, Anticancer Res., 2002, 22: 3331-3336). Thus, in certain embodiments, inventive compositions and methods are used in the treatment of lung cancer, colorectal cancer, cervical cancer, hepatic cancer, and pancreatic cancer.

[155] In certain embodiments, tumors that can be treated using compositions and methods of the present invention may be refractory to treatment with other chemotherapeutics. The term "refractory", when used herein in reference to a tumor means that the tumor (and/or metastases thereof), upon treatment with at least one chemotherapeutic other than an inventive composition, shows no or only weak anti-proliferative response (*i.e.*, no or only weak inhibition of tumor growth) after the treatment of such an chemotherapeutic agent – that is, a tumor that cannot be treated at all or only with unsatisfying results with other (preferably standard) chemotherapeutics. The present invention, where treatment of refractory tumors and the like is mentioned, is to be understood to encompass not only (i) tumors where one or more chemotherapeutics have already failed during treatment of a patient, but also (ii) tumors that can be shown to be refractory by other means, *e.g.*, biopsy and culture in the presence of chemotherapeutics.

[156] In certain embodiments, compositions and methods of the present invention are used in the treatment of cardiovascular diseases (C.J. World *et al.*, J. Mol. Med., 2006, 84: 997-1003; K. Shioji *et al.*, Antioxidants & Redox Signaling, 2003, 5: 795-802). Cardiovascular diseases are a class of diseases that involve the heart and/or blood vessels (arteries and veins). Cardiovascular diseases include artheriosclerosis, coronary artery disease, heart valve disease, arrhythmia, heart failure, hypertension, orthostatic

hypotension, shock, endocarditis, diseases of the aorta and its branches, disorders of the peripheral vascular system, and congenital heart disease.

[157] It is anticipated that methods and compositions of the present invention will find particular use for other diseases and conditions not mentioned herein.

Examples

[158] The following examples describe some of the preferred modes of making and practicing the present invention. However, it should be understood that these examples are for illustrative purposes only and are not meant to limit the scope of the invention. Furthermore, unless the description in an Example is presented in the past tense, the text, like the rest of the specification, is not intended to suggest that experiments were actually performed or data were actually obtained.

[159] Some of the results presented below have been reported in a scientific publication (D. di Bernardo *et al.*, "Chemogenomic profiling on a genome-wide scale using reverse-engineered gene networks", Nature Biotechnology, March 2005, 23: 377-383), which is incorporated herein by reference in its entirety (including the Supplemental Information section).

Materials and Methods

[160] ***Public Expression Data.*** Two publicly available sets of gene expression profiles (T.R. Hughes *et al.*, Cell, 2000, 102: 109-126; S. Mnaimneh *et al.*, Cell, 2004, 118: 31-44) served as the training data set for the MNI algorithm, with two primary modifications. First, information regarding the identity of compounds used to treat the cells and the identity of the mutated genes in each profile was not provided to the MNI algorithm. Thus the data set was representative of an experimental situation where only treatments with unknown mode of action were applied to the model organism. Second, if the test expression profile (*i.e.*, the profile for which targets were to be identified) was part of the 515 profiles in the compendium, it was removed from the training data set prior to analysis. Note that an additional public data set (M. Ueda *et al.*, FEMS Microbiol. Lett., 2003, 219: 93-98) was used as the source of expression data for one compound, 3-aminotriazole. All expression profiles were pre-processed prior to analysis: missing expression ratios were set to zero, and missing standard errors were estimated.

[161] . ***DNA Microarray Construction for PTSB Experiments.*** A set of 6307 synthesized oligonucleotide 70-mer probes including ten controls was obtained from Operon Technologies, Inc. (Alameda, CA). The plates of DNA were suspended in 3X SSC (0.45 M NaCl, 45 mM sodium citrate, pH 7.0) to make printable aliquots. The DNA solutions were spotted on CMT-GAPS II slides (Corning, NY) using OmniGrid Accent™ (GeneMachines, San Carlos, CA) microarraying robot equipped with a Stealth Printhead (SPH32, Telechem International, Inc., Sunnyvale, CA) containing 16-Stealth Micro Spotting Pins (SMP4, Telechem International, Inc.). Post processing of the slides was accomplished according to published procedures (M.N. Eisen and P.O. Brown, Methods Enzymol., 1999, 303: 179-205).

[162] ***Drug Treatment and Preparation of Microarray Sample.*** An overnight culture of a drug-sensitive strain of *Saccharomyces cerevisiae* was diluted to an OD_{600} of 0.1, treated with 5 μM PTSB and then grown to an OD_{600} of 0.8. Total RNA was isolated from the flash-frozen cultured yeast cells using the acidic phenol method. Poly(A) RNA was isolated using an oligo(dT) resin (Oligotex, Qiagen, Chatsworth, CA). cDNA was synthesized followed by double-strand synthesis. *In vitro* transcription was then used for amplification of antisense RNA (aRNA) (Amino Allyl MessageAMP™ aRNA kit, Ambion). The *in vitro* transcription employed 5-(3'-Amino-allyl)-dUTP for dye conjugation. The control and experimental probes were coupled with Cy3- and Cy5-N-hydroxysuccinamide esters (Amersham Biosciences), respectively, and purified using a MEGAclear™ kit (Ambion). The samples were concentrated and fragmented prior to hybridization. Each experiment was conducted in duplicate.

[163] ***Data Acquisition and Analysis.*** The microarrays were scanned with a GenePix 4000B array scanner (Axon Instruments, Foster City, CA) using GenePix 3.0 software to quantitate the Cy3- and Cy5-fluorescence intensities at each spot and determine the background signal intensities. Signal intensities greater than three standard deviations above the average background were considered for analysis. A scaling factor was calculated using the ratio of the Cy3 average mean signal intensity to the Cy5 average mean signal intensity. The scaling factor was applied to normalize the two channels. The Yeast Protein Database (YDP) and the GeneSpring software package (Silicon Genetics, Redwood City, CA) were used for data analysis.

[164] ***Validation of PTSB Target: Thioredoxin/Thioredoxin Reductase Assay.*** The solution assay of coupled thioredoxin-thioredoxin reductase activity using dithio(bis)nitrobenzoic acid (DTNB) was carried out by the method of Holmgren and Reichard (A. Holmgren and P. Reichard, Eur. J. Biochem., 1967, 2: 187-196), with the following modifications. To an assay mixture of 10 mM Tris, 3.12 mM EDTA (pH 8.0), NADPH and DTNB were added to final concentrations of 0.05 mM and 0.33 mM, respectively. DTNB was prepared prior to the experiment, in ethanol, as a 100 mM stock solution. To this mixture, *E. coli* thioredoxin reductase was added to a concentration of 1 μM as was a variable amount of PTSB (leading to 0, 1, 5, and 50 μM PTSB). The reaction was initiated by the addition of *E. coli* thioredoxin (250 μM, final solution), and monitored by absorption change due to the thiolated anion at 412 nm (at pH = 8.0, $\varepsilon_{412} = 13.6\ mM^{-1}cm^{-1}$). The *E. coli* thioredoxin I protein (*trxA* gene product) is 34% identical to *S. cerevisiae* thioredoxin (TRX2; identified by MNI) and 30-40% identical to human thioredoxins. The *E. coli* thioredoxin reductase protein (*trxB* gene product) is 47% identical to *S. cerevisiae* thioredoxin reductase (TRR1; identified by MNI) and approximately 25% identical to human thioredoxin reductases.

[165] ***MNI Algorithm.*** The algorithm and underlying assumptions are described in detail in the Supplementary Information section of D. di Bernardo *et al.*, Nature Biotechnology, March 2005, 23: 377-383. Briefly, the algorithm operates in two phases. In the first phase (the training phase), a model of regulatory influences in the cell is learned from an $N \times M$ data matrix, X, consisting of measurements of steady-state expression ratios of N genes in M experiments. In prior work (T.S. Gardner *et al.*, Science, 2003, 301: 102-105), it was shown that such a regulatory model can be constructed provided that specific genes are perturbed in each of the M experiments. The gene-specific perturbations enable the construction of an $N \times M$ matrix, P, of external influences on the genes. Regulatory influences are obtained as coefficients in the matrix, A that provide a sparse solution to a linearized steady-state model of the regulatory network: $A(X\text{-}1) = P$.

[166] In the MNI algorithm, a similar strategy is used. However, gene-specific perturbations are assumed to be unavailable. Thus, the matrix P is unknown and the prior approach is inapplicable. To estimate the network model A, with no date on P, the MNI algorithm uses a recursive strategy. The algorithm begins by using a naïve model of the

regulatory structure (*i.e.*, no genes regulate any other genes) to estimate P from the expression data X. The estimate of P is then used, along with X, to determine A *via* principal components regression (T. Hastie *et al.*, "*The Elements of Statistical Learning: Data Mining, Inference, and Prediction*", 2001, Springer: New York). The estimates of A and P are then used to recursively re-estimate one another until the estimates converge. The recursive approach is much like the Expectation Maximization (EM) algorithm (A. Dempster *et al.*, J. Royal Statistical Society: Series B, 1977, 39: 1-38) commonly used to learn Bayesian networks. The estimation of P corresponds to the "E-step", and the estimation of A corresponds to the "M-step".

[167] In past work (T.S. Gardner *et al.*, Science, 2003, 301: 102-105), expression-ratio data were used to compose the data matrix X, thereby allowing the inference of a linearized model of regulatory influences. The MNI algorithm, however, uses log-transformed expression-ratio data in the matrix X. This transformation improves the statistical properties of the data by stabilizing the variances of the expression ratios, and it enables the identification of a log-linear model (J.C. Liao *et al.*, Proc. Natl. Acad. Sci. USA, 2003, 100: 15522-15527) of gene regulation. The log-linear model enables the capture of some non-linear properties of the regulatory network, providing better predictive power.

[168] In the second phase of the algorithm, the A matrix, representing a model of regulatory influences in the cell, is used to estimate the targets of a test compound. The test compound is incorporated in the model as an $N \times 1$ vector, p, of gene-specific influences that result in the log-transformed expression-ratios, x, measured for the compound. The p vector is then calculated directly from the log-linear regulatory model as: $p = Ax$. The significance of each element of the p vector is then calculated as a z-score. Genes are ranked according to the z-score of their corresponding element in the p vector, and the top-ranked genes and pathways are selected as probable targets of the test compound.

Example 1: Chemogenomic profiling on a genome-wide scale using reverse-engineered gene networks

[169] ***Prediction of Gene Targets of Promoter Insertions.*** The performance of the MNI algorithm was evaluated by testing its ability to predict the gene targets of the 11

promoter insertions of the Hughes compendium (see Table 1). For the 11 mutant profiles tested, the algorithm ranked the targeted gene as the most likely affected gene in 8 out of 11 cases. Two of the remaining perturbed genes were correctly ranked in the top 10 of the most likely affected genes (*rho1* and *pma1*). The final perturbed gene, *erg11*, was ranked 42nd, which is a substantial enrichment over its ranking based on the significance of its expression change alone (it was ranked 2820 by z-score of expression change; Table 1). Similarly, for the other promoter insertions, the ranking by expression change identified the affected gene with high significance (ranked among the top 10 out of 6,000 genes) for only 3 of the 11 mutations, which is significantly worse than the MNI algorithm.

[170] The performance of the MNI algorithm was compared to two association analysis approaches: a correlated method (T.R. Hughes *et al.*, Cell, 2000, 102: 109-126; M.J. Marton *et al.*, Nature Med., 1998, 4: 1293-1301) and a linear combination method (U.S. Pat. No. 5,965,352) (see Table 1). The correlation method computes the correlation coefficient between the expression profile of a test compound and each profile in the training data set. The mutant profiles with the greatest similarity to the compound profile are considered the most likely targets. The linear combination approach finds a weighted sum of mutant profiles that best match the profile of the test compound. The strongest-weighted mutants are considered the most likely targets. The primary limitation of these methods is that they can only identify the target of a compound if a mutant strain for that target has been included in the training data set. For 9 of the 11 titratable promoter profiles, no corresponding profile exists.

[171] ***Prediction of Gene Targets of Drug Compounds.*** The MNI algorithm was then used to identify probable targets of 15 compounds, 13 of which were drawn from the Hughes compendium (Cell, 2000, 102: 109-126) and two from other studies (M. Ueda *et al.*, FEMS Microbiol. Lett., 2003, 219: 93-98). Of the 15 compounds examined, 9 have previously determined targets, while the targets of the other 6 compounds are unknown. The pathways and protein targets of the 9 compounds of known mode of action are shown in Table 2 and Table 3. For each of these compounds, the MNI algorithm was used to rank more than 6,000 yeast genes by the likelihood that they are the targets of each drug treatment. The 50 highest ranked genes were then subjected to pathway analysis, using the GO Term Finder tool (www.yeastgenome.org), to identify over-represented GO

biological process annotations. The most significant annotation for each case is reported in Table 2 and Table 3, along with the highly ranked genes in that pathway.

[172] The most over-represented pathways identified among the genes ranked by the MNI algorithm matched the known targeted pathway for 7 of the 9 compounds (Table 2). The four studied compounds that target ergosterol biosynthesis (terbinafine, lovastatin, itraconazole, and dyclonine) affect genes that are enriched for steroid and lipid metabolism, of which ergosterol biosynthesis is a more specific sub-category that also shows significant enrichment. The top pathways identified for each of the four compounds contain a high preponderance of ergosterol biosynthetic enzymes, and the gene encoding the known target protein for each respective compound is ranked near the top for each pathway (Table 2 and Table 3; Figure 3).

[173] In determining the targets of hydroxyurea, a ribonucleotide reductase inhibitor, the algorithm identifies "DNA replication", the primary pathway of hydroxyurea's targets (RNR2 and RNR4), as the second most significant unique annotation. The algorithm identified *rnr4* and *rnr2* as the top ranked genes in that pathway (second and sixth overall, respectively), as well as two other genes encoding proteins in the ribonucleotide reductase complex (*rnr1* and *rnr3*). The highest ranked annotated processes were related to DNA repair, in which the RNR complex plays an important role (A. Chabes *et al.*, Cell, 2003, 112: 391-401); the "heteroduplex formation" genes *rad51* and *rad54* act in double-strand break repair *via* homologous recombination, and are highly ranked by the MNI algorithm. In the case of cycloheximide, the most significant annotation did not match the known pathway, but the MNI algorithm, ranked two genes (*rpl26b* and *rps29a*) in the top 50 that are members of the ribosome complex, which is targeted by the drug.

[174] For 3 of the 9 compounds with known modes of action (tunicamycin, nikkomycin, and 3-aminotriazole), the MNI algorithm did not identify the known target. However, for tunicamycin and 3-aminotriazole, the MNI algorithm did identify the targeted biosynthetic pathways and gene products acting adjacent to the known targeted proteins (ALG7 and HIS3, respectively) in those pathways (Table 2 and Table 3, respectively). The target of tunicamycin, ALG7, is an integral membrane protein of the endoplasmic reticulum (ER) that catalyzes the transfer of N-acetylglucosamine-1-P from UDP-N-acetylglucosamine to dolichol phosphate in the first step of liquid-linked

oligosaccharide synthesis (M. Kaneshisa and S. Goto, Nucleic Acids Res., 2000, 28: 27-30). The MNI algorithm identified several protein-ER targeting proteins (SEC62, SIL1, and SEC59) among the top 50 most likely targets for tunicamycin. The final step in the synthesis of dolichol phosphate, the substrate of ALG7, is catalyzed by SEC59, which is ranked third in the top-ranked pathway by MNI (Table 2). Similarly, a target of 3-aminotriazole, HIS3, catalyzes the sixth step in the synthesis of histidine from 5-phosphoribosyl 1-pyrophosphate (M. Kaneshisa and S. Goto, Nucleic Acids Res., 2000, 28: 27-30). The following (seventh) step in that biosynthetic pathway is catalyzed by HIS5, which is ranked tenth in the top-ranked pathway by MNI (Table 3).

[175] The MNI algorithm requires that the training perturbations influence a diversity of cell functions. If a particular cellular pathway does not show a response in any experiment, then a regulatory model for that pathway cannot be learned and thus no predictions can be made about that pathway. For instance, while in principle it is possible to use expression response profiles from environmental stimuli and stresses with this algorithm, even large data sets (A.P. Gash *et al.*, Mol. Biol. Cell, 2000, 11: 4241-4257) sampling many unique environmental stresses can yield training data with low information content. Thus, the failure to identify the target of nikkomycin may be due to insufficient stimulation of the pathway related to its function.

[176] The predicted pathways and genes for the six compounds with currently unknown targets were also examined. For example, MMS is an alkylating agent that damages DNA; it is not thought to have a direct protein target. However, prior studies have shown that RNR3 deletion strains are most sensitive to MMS treatment (T.R. Hughes *et al.*, Cell, 2000, 102: 109-126), and thus RNR3 is a likely mediator of the effects of MMS. The MNI algorithm ranks *rnr3* as the sixth most likely target of MMS. Interestingly, the most significant pathway among the top ranked genes was "sterol biosynthesis" ($P < 5.0 \times 10^{-5}$), containing the highly ranked genes *erg5*, *cyb5*, *hmg1*, and *mvd1*. Previous studies have shown that disruption of ergosterol biosynthesis leads to MMS sensitivity (C. Bennett *et al.*, Nature Genetics, 2001, 29: 426-434), possibly due to defective mitochondrial mitogenesis, as discussed in the examination of the membrane-associated progesterone receptor family protein and probable sterol synthesis regulator, DAP1 (R.A Hand *et al.*, Eukaryotic Cell, 2003, 2: 306-317).

[177] The performance of the association analysis approaches, and the raw expression change ranking in identifying target genes and pathways for all of the compounds considered were also examined. This comparison showed that the inventive MNI algorithm outperformed these approaches.

[178] Overall, the results presented herein show that for most compounds, the MNI algorithm is successful in correctly identifying the target pathway with the highest significance. Moreover, within a significant pathway, the algorithm typically ranks the target gene product higher than other genes in the pathway. This performance is likely due to the "tournament" strategy used to rank genes. For a particular test compound profile, the algorithm is applied repeatedly to rank genes. In each application of the algorithm, gene profiles are collapsed into a small number of principal components ("metagenes"). The metagenes represent the behavior of a group of similarly expressed genes. Such genes are likely to be involved in the same pathway. Thus, in initial rounds, genes within a pathway may be treated and ranked similarly. In each subsequent application of the algorithm, the 1/3 most highly ranked genes are selected and re-analyzed. Thus, a fewer number of gene profiles are collapsed into the representative metagenes, and the resolution of the predictions is improved. Thus, in later iterations of the algorithm, genes within a pathway can be differentiated.

[179] The MNI algorithm's ability to rank both genes and pathways suggests that the most probable targets of novel compounds can be identified as those that act within the most significant over-represented GO processes (pathways) and are highly ranked within those processes. The resulting small list of probable targets can then be validated for interaction with the compound *via* direct biochemical assays.

Example 2: Identification and Validation of PTSB's Targets

[180] The method described above was used on a novel tetrazole-containing compound, 4-(1-phenyl-1H-tetrazole-5-ylsulfonyl)butanenitrile or PTSB. The changes in steady-state gene expression in *Saccharomyces cerevisiae* were determined upon treatment with PTSB using oligonucleotide arrays. The MNI algorithm and the reverse-engineered network model were used to obtain a ranking of the most likely targets of PTSB. The most highly over-represented GO process among the top 50 most likely perturbed genes was found to be the "cell redox homeostasis" annotation ($P < 2.2 \times 10^{-3}$).

Two genes with that annotation were ranked in the top 50: thioredoxin reductase (*trr1*, rank = 32) and thioredoxin (*trx2*, rank = 36).

[181] To validate the predictions made by the MNI algorithm, a biochemical assay was performed to monitor the NADPH-dependent reduction of DTNB by thioredoxin and thioredoxin reductase (A. Holmgren and P. Reichard, Eur. J. Biochem., 1967, 2: 187-196; see Examples Section). Accumulation of the reduced DTNB product, a thiolate anion, was observed spectroscopically (λ_{max} = 412 nm) in the presence of 0, 1, 5 and 50 μM PTSB (as shown in Figure 6). The results obtained demonstrate that PTSB efficiently inhibits the thioredoxin/thioredoxin reductase system.

Example 3: Design and Development of a PTSB-based Library and Biological Activity Screening

[182] A library was designed to include the sulfone and nitrile functionalities, which, according to initial biological data, are important for activity, and incorporate some diversifications into the structure, as shown on Figure 9. The library plan, detailed on Figure 10, was to synthesize a library of 1-(substituted)-5-mercapto-tetrazoles and other derivatives and screen these compounds for increased biological activity compared to PTSB. Tetrazole-5-thiols were synthesized using commercially available isothiocyanates and sodium azide. The overall yield for the tetrazole syntheses ranged from 50% to 98% after purification. The alkylation reactions were employed using primary bromides and iodides. The reaction proceeded at 50°C in THF in the presence of potassium trimethylsilanolate. Excess halide was scavenged using a thiol derived silica. Alkylation was followed by oxidation to the sulfone using magnesium mono-peroxyphthalate hexahydrate (MMPP). The synthetic plan is detailed on Figure 11.

[183] Thirty-six (36) compounds (presented in Table 4) were synthesized and plated into 96-well format for biological assessment. The library was screened for growth inhibition of cancer cell lines (A549 and HeLa-S3) as well as yeast cells. Results of a cancer cell growth inhibition screen, conducted at 10 μM against small cell lung carcinoma a549, are reported for each compound of the library in Table 5. The nitrile sulfone of Structure 97, or BF-PTSB, was found to be the most active compound of the library. A IC50 of 568 nM was measured for this compound in A549 cells.

[184] To further examine the mechanism of Trx/TrxR inhibition by PTSB (or PTSB derivatives), additional assays have been designed to examine whether the addition of NADPH will release the cells from the effects of PTSB. Docking experiments suggest that PTSB is competing with NADHP for the binding pocket in TrxR. Similar studies with the new library members indicate that several compounds have a higher docking rating in the active site than PTSB, suggesting that they may be better inhibitors of Trx/TrxR.

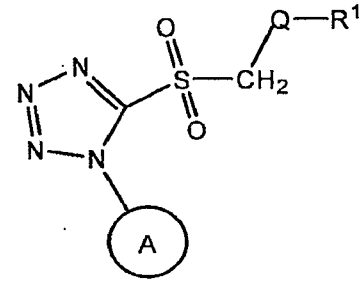
Other Embodiments

[185] Other embodiments of the invention will be apparent to those skilled in the art from a consideration of the specification or practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope of the invention being indicated by the following claims

Claims

What is claimed is:

1. A compound with the following structure:



I

or a pharmaceutically acceptable salt or derivative thereof, wherein:

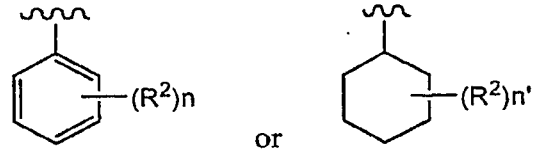
R^1 is hydrogen, -C≡N, or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain, wherein 0–2 methylene units of Q are independently replaced by –O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-;

each R is independently hydrogen or an optionally substituted aliphatic group; and

Ring A is an optionally substituted 3-8 membered bivalent, saturated, partially unsaturated, or aryl monocyclic ring having 0–4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or an optionally substituted 8–10 membered bivalent saturated, partially unsaturated, or aryl bicyclic ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

2. The compound of claim 1, wherein the Ring A is selected from:



wherein each wavy line indicates the point of attachment to the tetrazole ring of structure **I**, and wherein:

n is 0 to 4;

n' is 0 to 10;

each R^2 is independently halogen, R^3, -C≡N, OR^3, SR^3, $N(R^3)_2$, $C(O)R^3$, $C(O)OR^3$, $NR^3C(O)R^3$, $C(O)NR^3$, SO_2R^3, $NR^3SO_2R^3$, $SO_2N(R^3)_2$; and

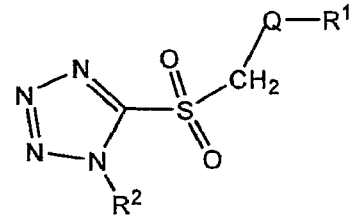
each R^3 is independently hydrogen or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

3. The compound of claim 2, wherein R^3 is $–CX_3$, $-CHX_2$, and $–CH_2X$, wherein X is chloro, fluoro, bromo or iodo.

4. The compound of claim 1, wherein R^1 is -C≡N.

5. The compound of claim 1, wherein Q is $–CH_2-$ or $–CH_2-CH_2-$.

6. A compound with the following structure:



II

or a pharmaceutically acceptable salt or derivative thereof, wherein:

R^1 is hydrogen, -C≡N, or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen,

oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

R^1 is hydrogen, or an optionally substituted group selected from a C_{1-6} aliphatic group;

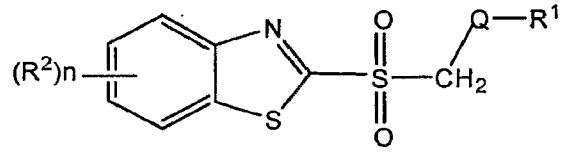
Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain, wherein 0–2 methylene units of Q are independently replaced by –O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-; and

each R is independently hydrogen or an optionally substituted aliphatic group.

7. A compound of claim 6, wherein R^1 is -C≡N.

8. A compound of claim 6, wherein Q is $–CH_2-$ or $–CH_2-CH_2-$.

9. A compound with the following structure:



III

or a pharmaceutically acceptable salt or derivative thereof, wherein:

R^1 is hydrogen, -C≡N, or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain, wherein 0–2 methylene units of Q are independently replaced by –O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-;

each R is independently hydrogen or an optionally substituted aliphatic group;

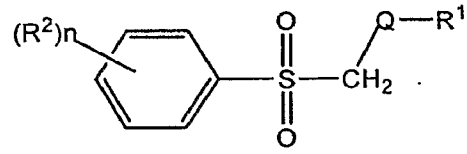
each R^2 is independently halogen, R^3, OR^3, SR^3, $N(R^3)_2$, $C(O)R^3$, $C(O)OR^3$, $NR^3C(O)R^3$, $C(O)NR^3$, SO_2R^3, $NR^3SO_2R^3$, $SO_2N(R^3)_2$; and

each R^3 is independently hydrogen or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

10. The compound of claim 9, wherein R^1 is -C≡N.

11. The compound of claim 9, wherein Q is $–CH_2-$ or $–CH_2-CH_2-$.

12. A compound with the following structure:



IV

or a pharmaceutically acceptable salt or derivative thereof, wherein:

R^1 is hydrogen, -C≡N, or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Q is a valence bond or a bivalent, saturated or unsaturated, straight or branched C_{1-6} hydrocarbon chain, wherein 0–2 methylene units of Q are independently replaced by –O-, -NR-, -S-, -OC(O)-, -C(O)O-, -C(O)-, -SO-, $-SO_2-$, $-NRSO_2-$, $-SO_2NR-$, -NRC(O)-, -C(O)NR-, -OC(O)NR-, or -NRC(O)O-;

each R is independently hydrogen or an optionally substituted aliphatic group;

each R^2 is independently halogen, R^3, OR^3, SR^3, $N(R^3)_2$, $C(O)R^3$, $C(O)OR^3$, $NR^3C(O)R^3$, $C(O)NR^3$, SO_2R^3, $NR^3SO_2R^3$, $SO_2N(R^3)_2$; and

each R^3 is independently hydrogen or an optionally substituted group selected from a C_{1-6} aliphatic group, a monocyclic 3–8 membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or a bicyclic 8–10 membered saturated, partially

unsaturated, or aryl ring having 0–5 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

13. The compound of claim 12, wherein R^1 is -C≡N.

14. The compound of claim 12, wherein Q is $-CH_2-$ or $-CH_2-CH_2-$.

15. A compound with a structure selected from the group consisting of: structure 1, structure 3, structure 4, structure 5, structure 6, structure 9, structure 11, structure 12, structure 13, structure 14, structure 15, structure 33, structure 35, structure 36, structure 37, structure 38, structure 39, structure 40, structure 42, structure 43, structure 44, structure 45, structure 46, structure 47, structure 48, structure 71, structure 97, structure 99, structure 100, structure 101, structure 113, structure 114, structure 116, structure 117, structure 118, and structure 119.

16. A pharmaceutical composition comprising an effective amount of a compound of any one of claims 1-15 and at least one physiologically acceptable carrier or excipient.

17. The pharmaceutical composition of claim 16 further comprising a therapeutic agent.

18. The pharmaceutical composition of claim 17, wherein the therapeutic agent is a member of the group consisting of chemotherapeutic agents, drugs used in the treatment of Alzheimer's disease, drugs used in the treatment of Parkinson's disease, drugs used in the treatment of HIV infection or AIDS, drugs used in the treatment of psoriasis, drugs used in the treatment of cardiovascular diseases, drugs used in the treatment of rheumatoid arthritis, and combinations thereof.

19. The pharmaceutical composition of claim 17, wherein the therapeutic agent comprises a chemotherapeutic agent.

20. A method comprising a step of:

administering an effective amount of the compound of any one of claims 1-15 to a subject in need thereof.

21. The method of claim 20, wherein the subject is a mammal.

22. The method of claim 20, wherein the subject is a human.

23. The method of claim 20, wherein the subject is suffering from or susceptible to a disease mediated by thioredoxin/thioredoxin reductase.

24. The method of claim 23, wherein the disease mediated by thioredoxin/thioredoxin reductase is a member of the group consisting of cancer or cancerous condition, Alzheimer's disease, Parkinson's disease, HIV infection or AIDS, psoriasis, cardiovascular disease, and rheumatoid arthritis.

25. The method of claim 23, wherein the disease mediated by thioredoxin/thioredoxin reductase is a cancer or cancerous condition.

26. The method of claim 25, wherein the cancer or cancerous condition is a member of the group consisting of tumors of the brain and central nervous system, head and/or neck cancer, breast tumors, tumors of the circulatory system, lymphomas, leukemias, Hodgkin's disease, tumors of the excretory system, tumors of the gastrointestinal tract, tumors of the liver, tumors of the digestive organs, tumors of the oral cavity, tumors of the reproductive system, tumors of the respiratory tract, tumors of the skeletal system, and tumors of the skin.

27. The method of claim 23, wherein the cancer or cancerous condition is a member of the group consisting of lung cancer, colorectal cancer, cervical cancer, hepatic cancer, and pancreatic cancer.

28. The method of claim 20 further comprising a step of:
administering an effective amount of at least one additional therapeutic agent to the subject.

29. The method of claim 28, wherein the compound and therapeutic agent are administered substantially simultaneously.

30. The method of claim 28, wherein the compound and therapeutic agent are administered sequentially.

31. The method of claim 28, wherein the additional therapeutic agent is selected from the group consisting of chemotherapeutic agents, drugs used in the treatment of Alzheimer's disease, drugs used in the treatment of Parkinson's disease, drugs used in the treatment of HIV infection or AIDS, drugs used in the treatment of psoriasis, drugs used in the treatment of cardiovascular diseases, drugs used in the treatment of rheumatoid arthritis, and combinations thereof.

32. The method of claim 28, wherein the disease mediated by thioredoxin/thioredoxin reductase is a cancer or cancerous condition, and the at least one additional therapeutic agent is a chemotherapeutic agent.

33. A method comprising a step of:
contacting a biological system with a compound of any one of claims 1-15.

34. The method of claim 33, wherein the biological system is a cell, a biological fluid, a biological tissue or an animal.

35. The method of claim 33, wherein the compound is contacted in an amount effective to inhibit thioredoxin/thioredoxin reductase in the biological system.

36. The method of claim 33, wherein the compound is contacted in an amount effective to regulate cell proliferation, regulate cell cycle progression, and/or regulated apoptosis in the biological system.

37. The method of claim 33, wherein the contacting step is performed *in vivo*.

38. The method of claim 33, wherein the contacting step is performed *ex vivo*.

39. The method of claim 33, wherein the contacting step is performed *in vivo*.

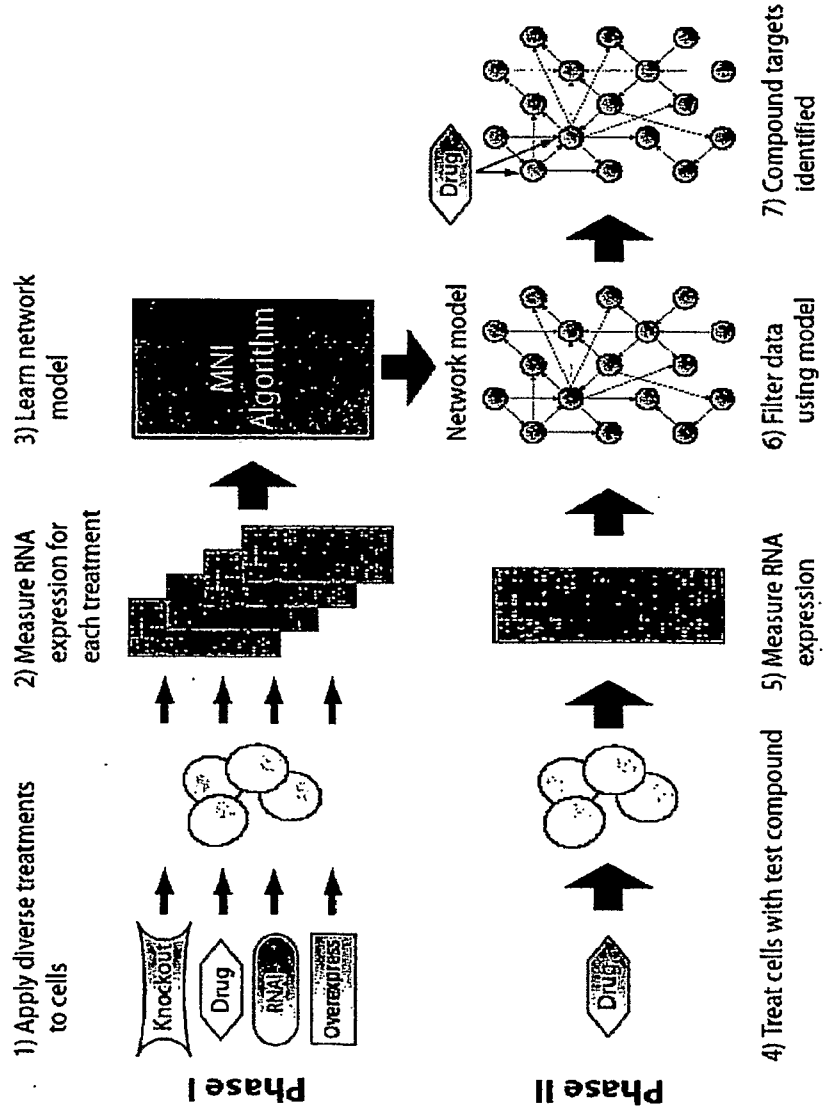

1) Apply diverse treatments to cells
2) Measure RNA expression for each treatment
3) Learn network model
Knockout
Drug
RNAi
Overexpress
MNI Algorithm
Network model
Drug
4) Treat cells with test compound
5) Measure RNA expression
6) Filter data using model
7) Compound targets identified
Phase I
Phase II

Figure 1

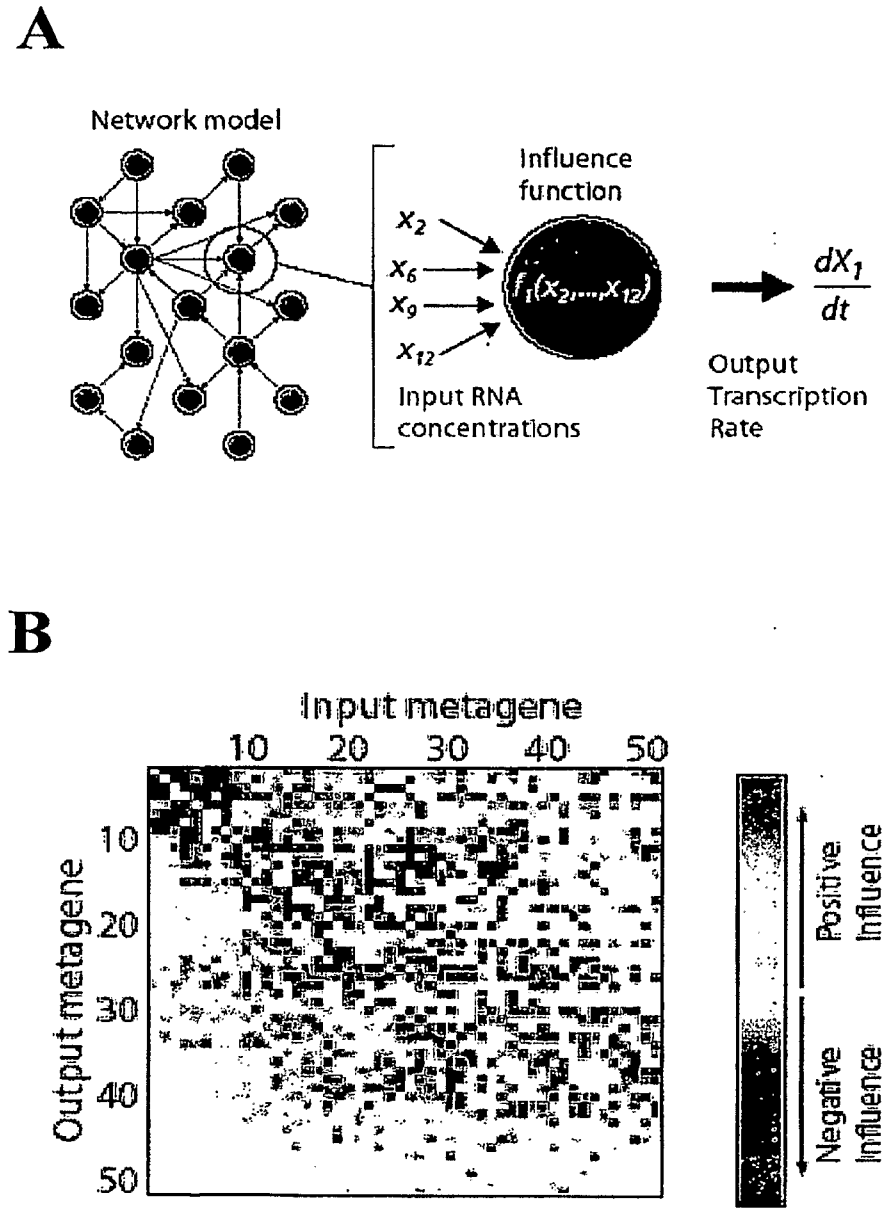

A
Network model
Influence function
x_2
x_6
x_9
x_{12}
$f_1(x_2,\ldots,x_{12})$
$\frac{dX_1}{dt}$
Input RNA concentrations
Output Transcription Rate
B
Input metagene
10
20
30
40
50
Output metagene
10
20
30
40
50
Positive Influence
Negative Influence

Figure 2

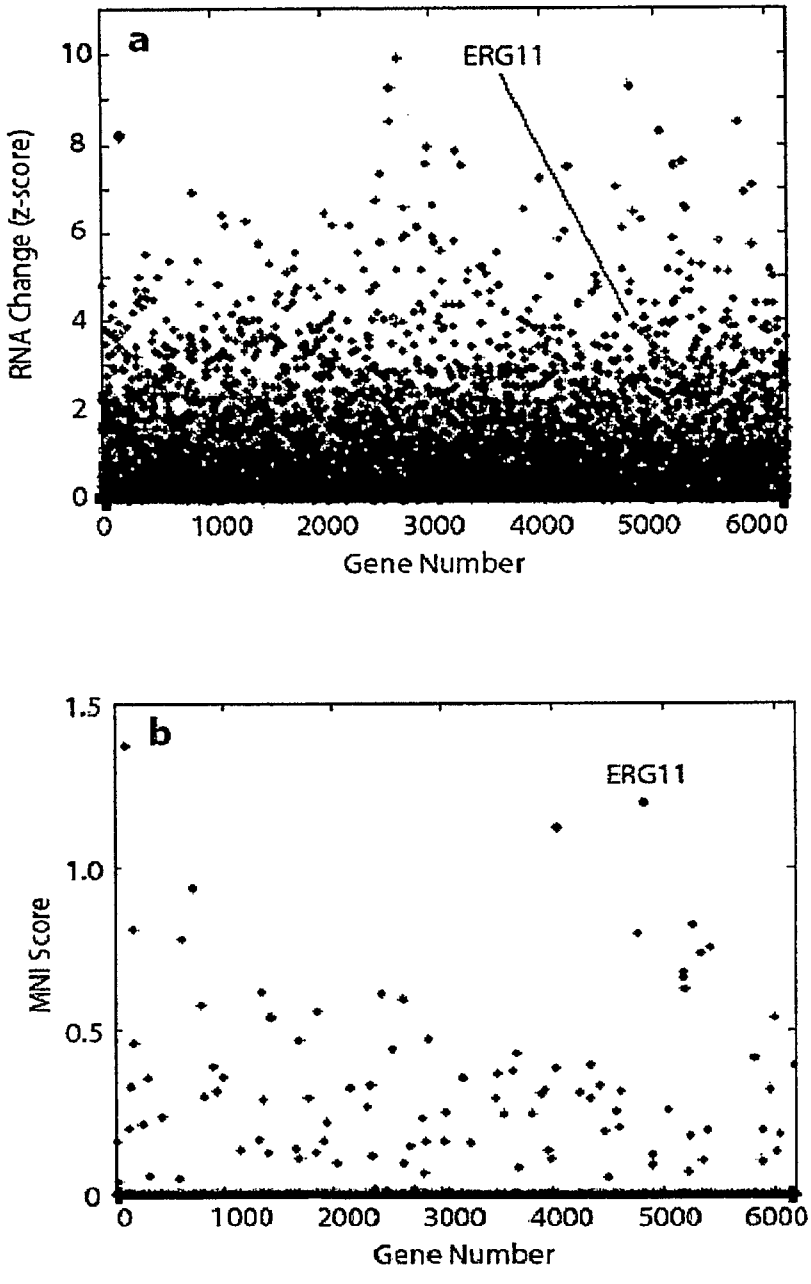
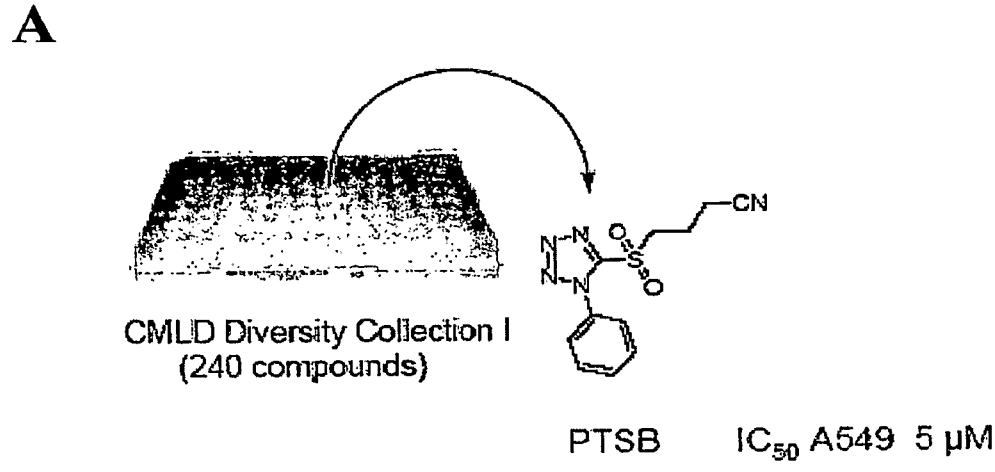

a
ERG11
RNA Change (z-score)
10
8
6
4
2
0
0
1000
2000
3000
4000
5000
6000
Gene Number
b
ERG11
MNI Score
1.5
1.0
0.5
0
0
1000
2000
3000
4000
5000
6000
Gene Number

Figure 3


A
CMLD Diversity Collection I
(240 compounds)
CN
PTSB
IC50 A549 5 μM

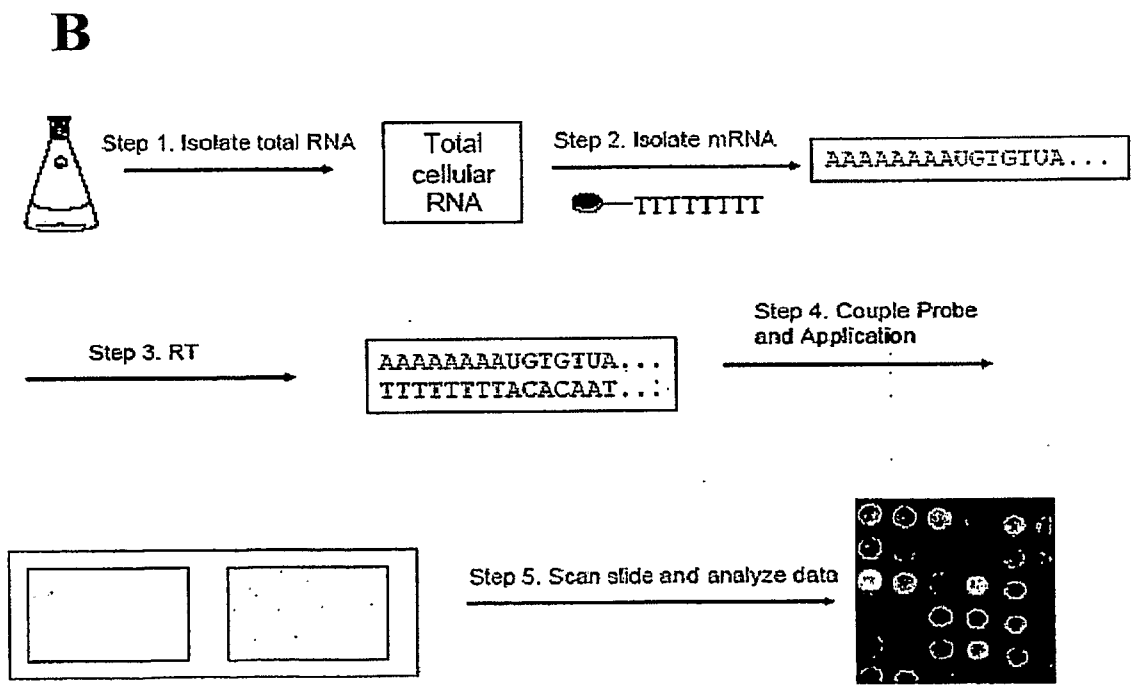

B
Step 1. Isolate total RNA
Total cellular RNA
Step 2. Isolate mRNA
AAAAAAAAUGTGTUA...
TTTTTTTT
Step 3. RT
AAAAAAAAUGTGTUA...
TTTTTTTTACACAAT...
Step 4. Couple Probe and Application
Step 5. Scan slide and analyze data

Figure 4

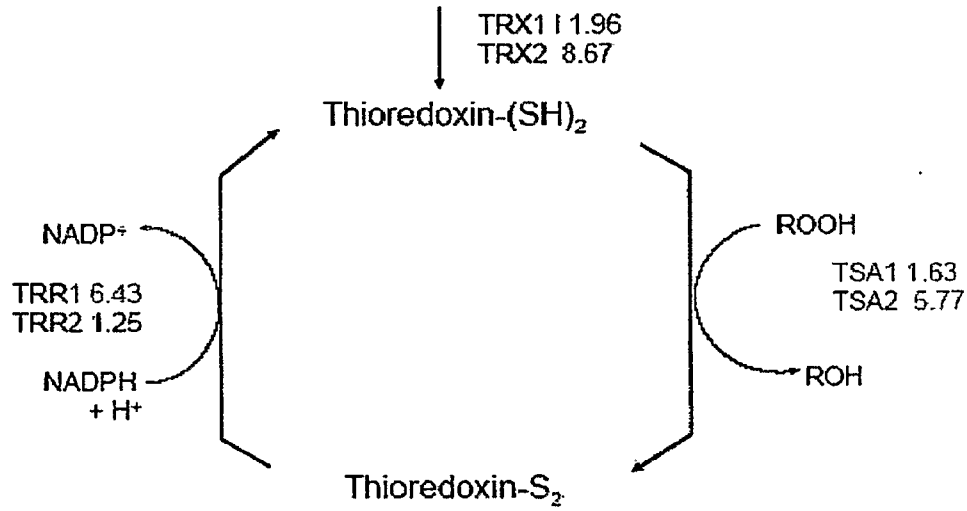
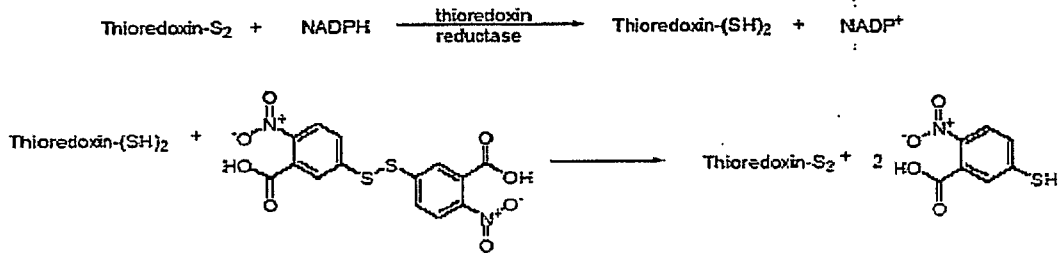

TRX1 1.96
TRX2 8.67
Thioredoxin-(SH)2
NADP+
TRR1 6.43
TRR2 1.25
NADPH
+ H+
ROOH
TSA1 1.63
TSA2 5.77
ROH
Thioredoxin-S2

Figure 5

A


Thioredoxin-S2 + NADPH
thioredoxin reductase
Thioredoxin-(SH)2 + NADP+
Thioredoxin-(SH)2 +
Thioredoxin-S2 + 2

B

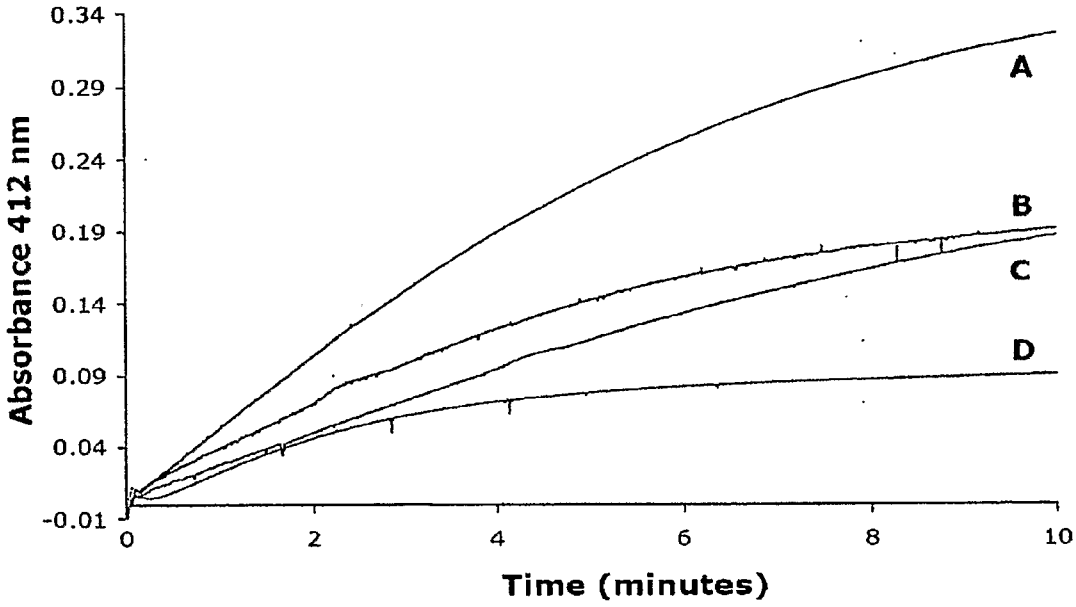
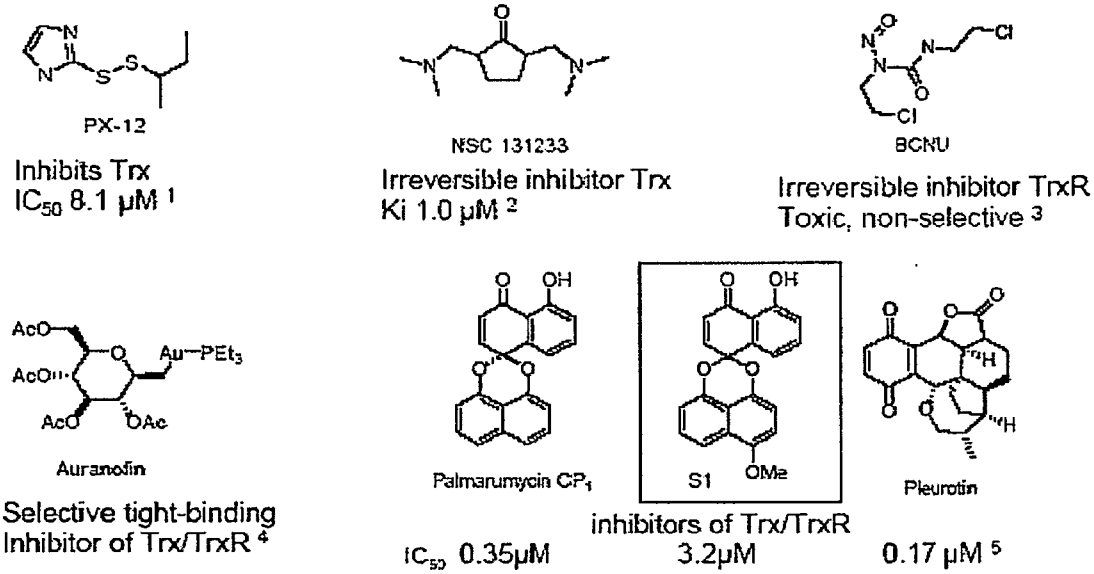

Absorbance 412 nm
0.34
0.29
0.24
0.19
0.14
0.09
0.04
-0.01
0
2
4
6
8
10
Time (minutes)
A
B
C
D

Figure 6



1 McDonnell, T. J.; Korsmeyer, S. J. *Nature*. 1991, *349*, 254-256.
2 Bodman, M. D.; Ray, G. F.; Rick, C. E.; Toes, M.A. *Biochem. Biophy. Res. Commun.* 1990, *166*, 1334-39.
3 Schallreiter, K. U.; Gleason, F.K.; Wood, J. M. *Biochim. Biophys. Acta.*, 1990, *1054*, 14-20.
4 Gromer, S. Arscott, L. D.; Williams Jr., C.H.; Schirmer, R. H.; Becker, K. J. *Biol. Chem.*, 1998, 273, 20096-20101.
5 Wipf, P.; Lynch, S. M.; Birmingham, A.; Tamayo, G.; Jimenez, A.; Campos, N. Powis, G. *Org. Biomol. Chem.* 2004, 2, 1651-1656

Figure 7

Table 1. Results of the MNI approach identifying targets of genetic perturbations. Results of association methods are provided for comparison. Promoter mutants are obtained by replacing the endogenous promoter with a tet-regulatable promoter.

Promoter mutant	Target	rank MNI	rank LC	rank C	rank R
tet-idi1	idi1	1	–	–	1
tet-rho1	rho1	4	–	–	1
tet-yef3	yef3	1	–	–	116
tet-aur1	aur1	1	–	–	14
tet-fks1	fks1	1	89	2	41
tet-kar2	kar2	1	–	–	64
tet-cdc42	cdc42	1	278	22	141
tet-hmg2	hmg2	1	–	–	19
tet-pma1	pma1	6	–	–	22
tet-erg11	erg11	42	–	–	2820
tet-cmd1	cmd1	1	–	–	1

LC: linear combination; C: correlation; R: RNA change (z-score); "–" indicates association analysis methods do not identify target genes that are not themselves perturbed.

Table 2. Pathways and associated genes targeted by drug compounds.

Drug	Known Pathway	Known Target	Significant GO ontology (rank, P-value)	Ranked Pathway Genes (rank)
Terbinafine	ergosterol biosynthesis [a]	ERG1	**steroid metabolism** (1, 10^{-14})	erg7 (4), **erg1 (5)**, erg8 (11), erg26 (13), upc2 (17), erg28 (18), erg11 (20), dap1 (33), hes1 (34), atf2 (36), erg5 (49)
Lovastatin	ergosterol biosynthesis [b]	HMG2, HMG1	**lipid metabolism** (1, 10^{-4})	bst1 (1), erg1 (18), ysr3 (23), **hmg2 (30)**, lcb5 (31), erg13 (36), vrg4 (48)
Itraconazole	ergosterol biosynthesis [c]	ERG11	**steroid metabolism** (1, 10^{-8})	**erg11 (2)**, erg24 (4), erg1 (6), erg25 (13), cyb5 (16), erg27(19), atf2 (23)
Hydroxyurea	DNA replication [d]	RNR2, RNR4	heteroduplex formation (1, 10^{-4})	rad51 (15), rad54 (47)
			DNA replication (2, 10^{-2})	**rnr4 (2)**, **rnr2 (6)**, rnr1 (14), rnr3 (23)
Cycloheximide	protein biosynthesis [e]	ribosome	nuclear mRNA splicing,via spliceosome (1, 10^{-4})	syf1 (3), smd3 (19), hsh49 (42)
			–	**rpl26b (32)**, **rps29a (34)**
Tunicamycin	N-linked glycosylation [f]	ALG7	**protein-ER targeting** (1, 10^{-3})	sec62 (1), sil1 (31), *sec59 (43)
Nikkomycin	cell wall chitin biosynthesis [g]	CHS3	protein amino acid alkylation (1, 10^{-3})	swd2 (3), rmt2 (6)

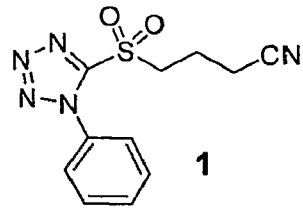
bolded text indicates matches with previously reported targets and pathways for each compound. "–" indicates that the known target pathway is not significantly over-represented among ranked genes, or
that the target pathway or gene is unknown. * SEC59 catalyzes the reaction immediately preceding ALG7 (tunicamycin's target) in the dolichol pathway of N-linked glycosylation.
[a] V. Klobucnikova *et al.*, Biochem. Biophys. Res. Commun., 2003, 309: 666-671; [b] J. Rine *et al.*, Proc. Natl. Acad. Sci. USA, 1983, 80: 6750-6754; [c] G. Daum *et al.*, Yeast, 1998, 14: 1471-1510; [d] D. Rittberg and J.A. Wright, Biochem. Cell Biol., 1989, 67: 352-357; [e] W. Stocklein and W. Piepersberg, Antimicrob. Agents Chemother., 1980, 18: 863-867; [f] G. Barned *et al.*, Mol. Cell Biol., 1984, 4: 2381-2388; [g]J.P. Gaughran *et al.*, J. Bacteriol., 1994, 176: 5857-5860.

Table 2

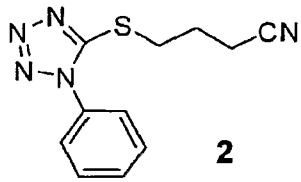
Table 3. Pathways and associated genes targeted by drug compounds not in the original compendium data.

Drug	Known Pathway	Known Target	Significant GO ontology (rank, P-value)	Ranked Pathway Genes (rank)
3-aminotriazole	histidine biosynthesis [(a)]; oxygen and reactive oxygen species metabolism [(b)]	HIS3; CTA1	**organic acid metabolism** (1, 10^{-7})	frm2 (8), bio5 (9), yat2 (10), aro10 (18), aro9 (20), cha1 (21), bio3 (31), arg1 (33), arg4 (37), his5* (42), lys1 (47),sam2 (50)
Dyclonine	ergosterol biosynthesis [(c)]	ERG2	**sterol biosynthesis** (1, 10^{-18})	erg3 (1), erg6 (2), cyb5 (3), **erg2 (4)**, erg11 (6), erg28 (10), erg1 (12), erg5 (13), erg27 (18), mvd1 (23), erg24 (30), erg26 (37)
Novel drug with unknown mode of action				
PTSB	–	–	**cell redox homeostasis** (1, 10^{-3})	**trr1 (32), trx2 (36)**

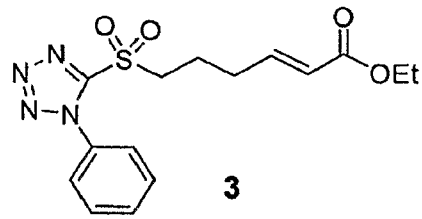
bolded text indicates matches with previously reported targets and pathways for each compound. "–" indicates that the known target pathway is not significantly over-represented among ranked genes, or
that the target pathway or gene is unknown. * HIS5 catalyzes the reaction immediately following HIS3 (3-aminotriazole's target) in the histidine biosynthesis pathway.
[(a)] R.M. Anderson *et al.*, Science, 2003, 302: 2124-2126; [(b)] M. Ueda *et al.*, FEMS Microbiol. Lett., 2003, 219: 93-98; [(c)] T.R. Hughes *et al.*, Cell, 2000, 102: 109-126.



1



2



3

Figure 8

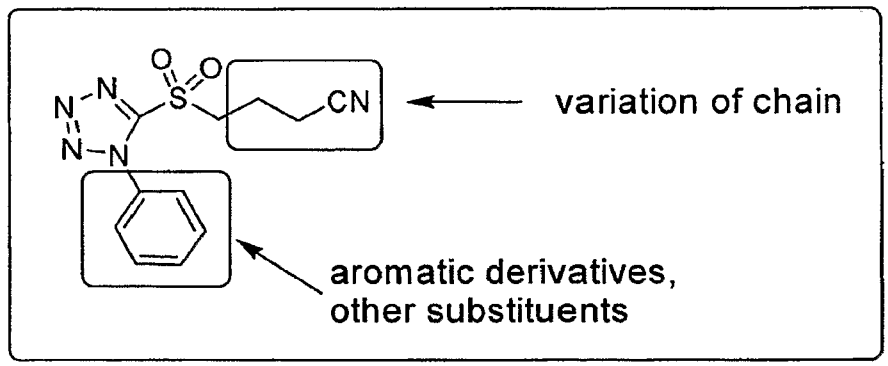

CN
variation of chain
aromatic derivatives,
other substituents

Figure 9

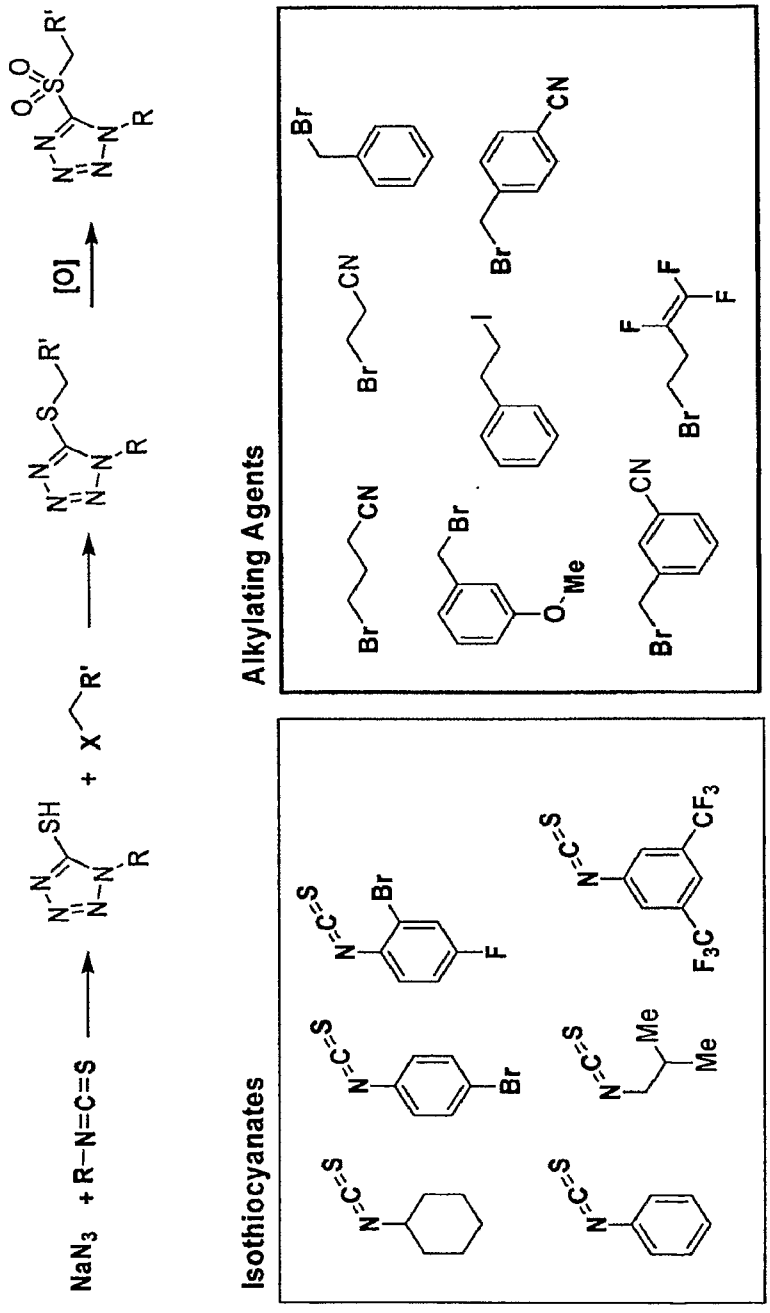


Figure 10

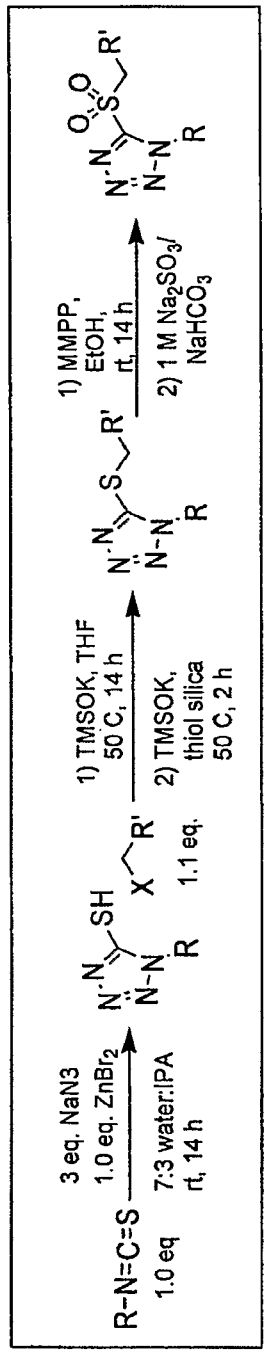
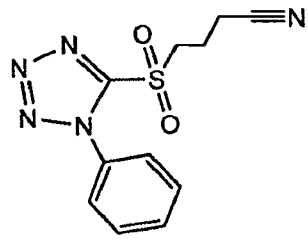
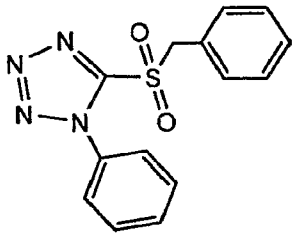
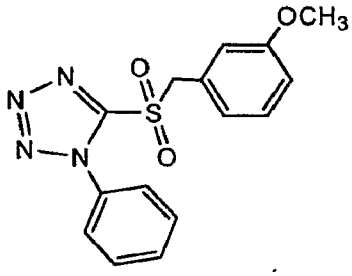
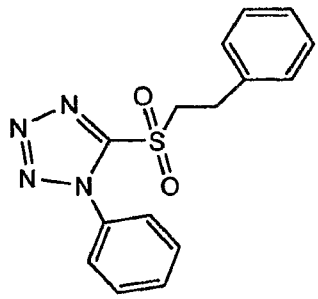
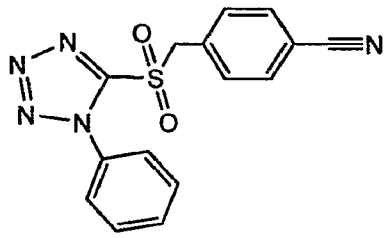

R-N=C=S
1.0 eq
3 eq. NaN3
1.0 eq. ZnBr2
7:3 water:IPA
rt, 14 h
1.1 eq.
1) TMSOK, THF
50 C, 14 h
2) TMSOK,
thiol silica
50 C, 2 h
1) MMPP,
EtOH,
rt, 14 h
2) 1 M Na2SO3/
NaHCO3

Figure 11

FORMULA	STRUCTURE	CHEMICAL NAME
	Structure 1	4-(1-Phenyl-1H-tetrazole-5-sulfonyl)-butyronitrile
	Structure 3	1-Phenyl-5-phenylmethanesulfony -1H-tetrazole
	Structure 4	5-(3-Methoxy-phenylmethane-sulfonyl)-1-phenyl-1H-tetrazole
	Structure 5	1-Phenyl-5-(2-phenyl-ethane-sulfonyl)-1H-tetrazole
	Structure 6	4-(1-Phenyl-1H-tetrazole-5-sulfonyl-methyl)-benzonitrile

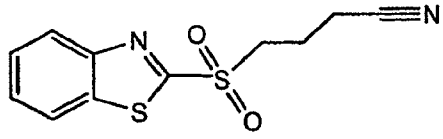
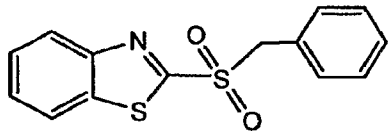
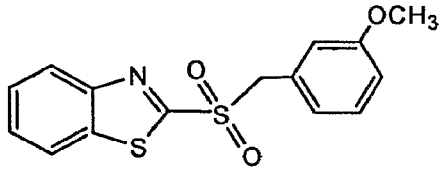
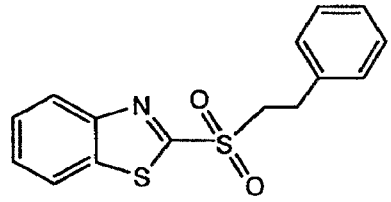
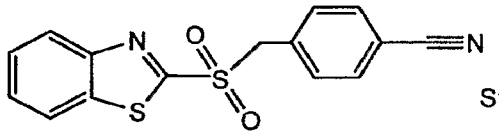
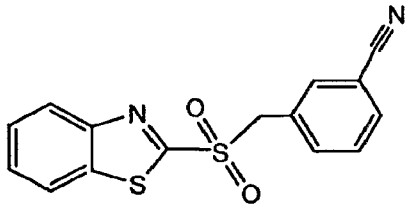
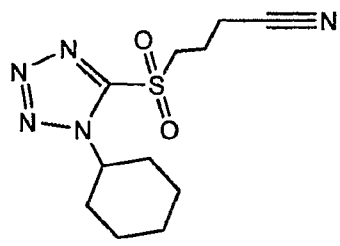
FORMULA	STRUCTURE	CHEMICAL NAME
	Structure 9	4-(Benzothiazole-2-sulfonyl)-butyronitrile
	Structure 11	2-Phenylmethanesulfonyl-benzothiazole
	Structure 12	2-(3-Methoxy-phenylmethane-sulfonyl)-benzothiazole
	Structure 13	2-(2-Phenylethanesulfonyl)-benzothiazole
	Structure 14	4-(Benzothiazole-2-sulfonylmethyl)-benzonitrile
	Structure 15	3-(Benzothiazole-2-sulfonylmethyl)-benzonitrile
	Structure 33	4-(1-Cyclohexyl-1H-tetrazole-5-sulfonyl)-butyronitrile

Table 4 (page 2 of 7)

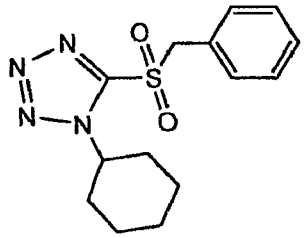
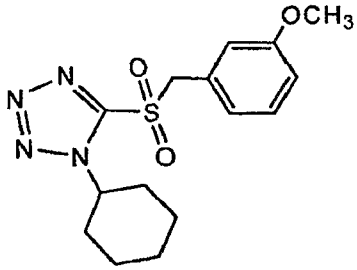
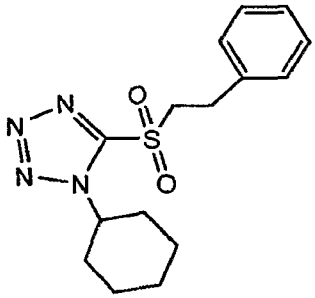
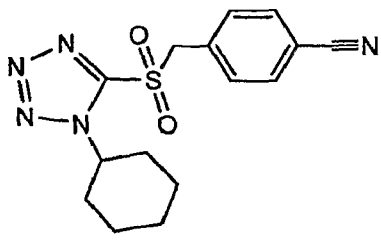
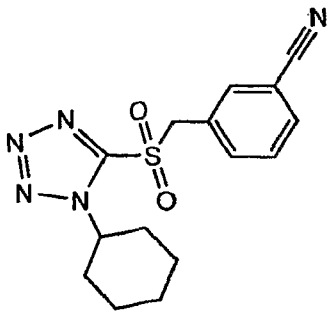
FORMULA	STRUCTURE	CHEMICAL NAME
	Structure 35	1-Cyclohexyl-5-phenylmethane-sulfonyl-1H-tetrazole
	Structure 36	1-Cyclohexyl-5-(3-methoxy-phenyl-methanesulfonyl)-1H-tetrazole
	Structure 37	1-Cyclohexyl-5-(2-phenyl-ethane-sulfonyl)-1H-tetrazole
	Structure 38	4-(1-Cyclohexyl-1H-tetrazole-5-sulfonylmethyl-benzonitrile
	Structure 39	3-(1-Cyclohexyl-1H-tetrazole-5-sulfonylmethyl-benzonitrile

Table 4 (page 3 of 7)

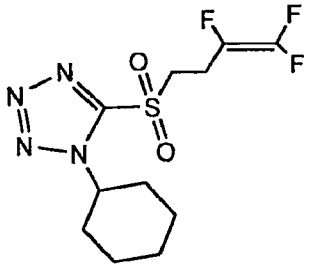
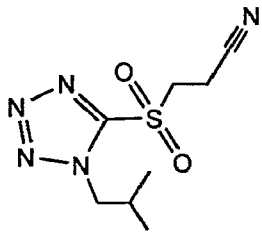
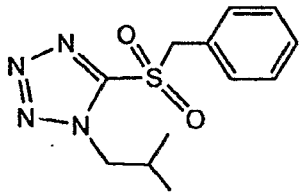
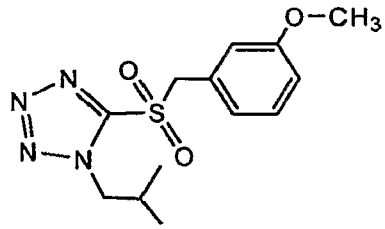
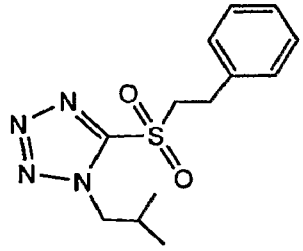
FORMULA	STRUCTURE	CHEMICAL NAME
	Structure 40	1-Cyclohexyl-5-(3,4,4-trifluoro-but-3-ene-1-sulfonyl)-1H-tetrazole
	Structure 42	3-(1-Isobutyl-1H-tetrazole-5-sulfonyl)-propionitrile
	Structure 43	3-Isobutyl-5-phenylmethanesulfonyl-1H-tetrazole
	Structure 44	1-Isobutyl-5-(3-methoxy-phenyl-methanesulfonyl)-1H-tetrazole
	Structure 45	1-Isobutyl-5-(2-phenyl-ethanesulfonyl)-1H-tetrazole

Table 4 (page 4 of 7)

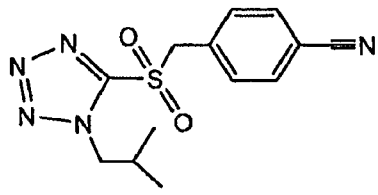
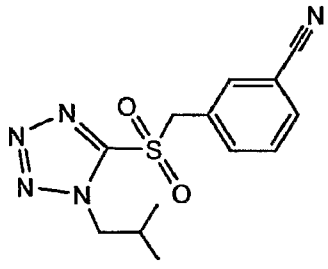
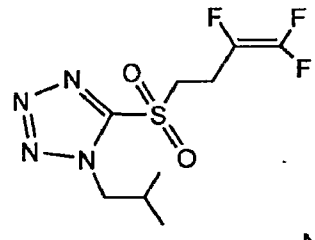
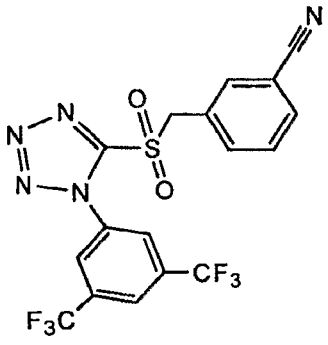
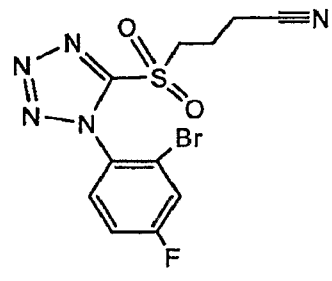
FORMULA	STRUCTURE	CHEMICAL NAME
	Structure 46	4-(1-Isobutyl-1H-tetrazole-5-sulfonyl-methyl)-benzonitrile
	Structure 47	3-(1-Isobutyl-1H-tetrazole-5-sulfonyl-methyl)-benzonitrile
	Structure 48	1-Isobutyl-5-(3,4,4-trifluoro-but-3-ene-1-sulfonyl)-1H-tetrazole
	Structure 71	3-[1-(3,5-Bis-trifluoromethyl-phenyl)-1H-tetrazole-5-sulfonylmethyl]-benzonitrile
	Structure 97	4-[1-(2-Bromo-4-fluoro-phenyl)-1H-tetrazole-5-sulfonyl]-butyronitrile

Table 4 (page 5 of 7)

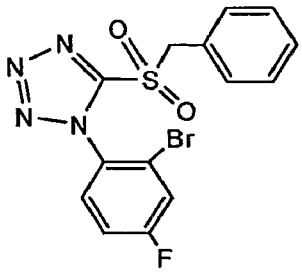
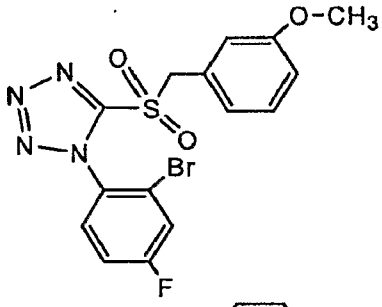
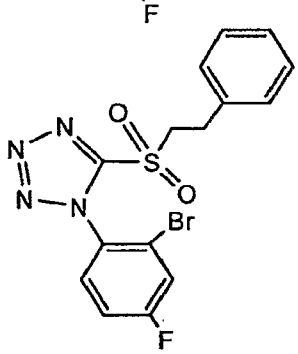
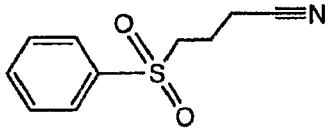
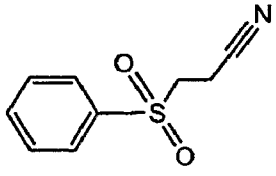
FORMULA	STRUCTURE	CHEMICAL NAME
	Structure 99	1-(2-Bromo-4-fluoro-phenyl)-5-phenyl-methanesulfonyl-1H-tetrazole
	Structure 100	1-(2-Bromo-4-fluoro-phenyl)-5-(3-methoxy-phenylmethanesulfonyl -1H-tetrazole
	Structure 101	1-(2-Bromo-4-fluoro-phenyl)-5-(2-phenyl-ethanesulfonyl)-1H-tetrazole
	Structure 113	4-Benzenesulfonyl-butyronitrile
	Structure 114	3-Benzenesulfonyl-priopionitrile

Table 4 (page 6 of 7)

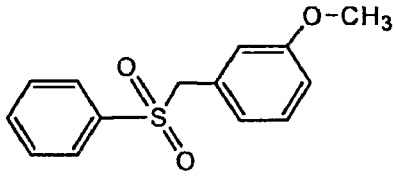
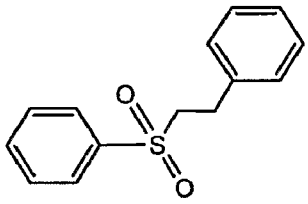
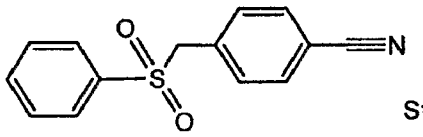
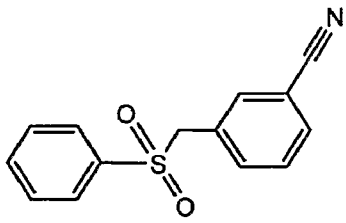
FORMULA	STRUCTURE	CHEMICAL NAME
	Structure 116	1-Benzenesulfonylmethyl-3-methoxy-benzene
	Structure 117	Phenylsulfonyl-ethylbenzene
	Structure 118	4-Benzenesulfonylmethyl-benzonitrile
	Structure 119	3-Benzenesulfonylmethyl-benzonitrile

Table 4 (page 7 of 7)

PTSB derivative	% growth Inhibition
Structure 1	12.7552
Structure 3	-14.31642
Structure 4	16.56685
Structure 5	24.28602
Structure 6	23.61924
Structure 9	20.36009
Structure 11	47.92369
Structure 12	19.08621
Structure 13	-16.69824
Structure 14	-22.28000
Structure 15	25.19884
Structure 33	21.01670
Structure 35	23.75196
Structure 36	22.38237
Structure 37	15.59516
Structure 38	12.85345
Structure 39	-42.01362
Structure 40	-28.96405
Structure 42	11.44704
Structure 43	22.24432
Structure 44	1.91947
Structure 45	9.07087
Structure 46	6.88507
Structure 47	19.13155
Structure 48	-28.88758835
Structure 71	-13.15865885
Structure 97	**89.91925724**
Structure 99	0.397619896
Structure 100	13.30622742
Structure 101	6.663015136
Structure 113	-3.245484499
Structure 114	5.556647571
Structure 116	-19.45098954
Structure 117	-12.93940096
Structure 118	-2.522965655
Structure 119	9.011068593

Table 5