

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
21 September 2006 (21.09.2006)

PCT

(10) International Publication Number
WO 2006/099095 A2

(51) International Patent Classification:
A61K 45/00 (2006.01) **A61P 35/00** (2006.01)

(21) International Application Number:
PCT/US2006/008501

(22) International Filing Date: 8 March 2006 (08.03.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/661,306 11 March 2005 (11.03.2005) US

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(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, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, 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, 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).

Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv))

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.

(54) Title: RIBOSOME INHIBITORS AS INHIBITORS OF TYROSYL-DNA-PHOSPHODIESTERASE

(57) Abstract: Disclosed herein are methods of using ribosome inhibitors that inhibit tyrosyl-DNA-phosphodiesterase (Tdp) activity to enhance an antineoplastic effect of a DNA-damaging therapy. Also provided herein are pharmaceutical compositions that include at least one chemotherapeutic agent and at least one ribosome inhibitor that inhibits Tdp activity.



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***RIBOSOME INHIBITORS AS INHIBITORS OF
TYROSYL-DNA-PHOSPHODIESTERASE***

5 **CROSS-REFERENCE TO RELATED APPLICATION**

 This application claims priority to and benefit of United States Provisional Application 60/661,306 filed March 11, 2005, which is incorporated herein by reference in its entirety.

10 **FIELD OF THE DISCLOSURE**

 This disclosure relates to methods of using a ribosome inhibitor that inhibits tyrosyl-DNA-phosphodiesterase (Tdp) activity to enhance an antineoplastic effect of a DNA-damaging therapy, and related compositions.

15 **BACKGROUND**

 Cancer has long been a leading cause of mortality in the United States. Significant efforts have been and continue to be made to find new approaches for treating this constellation of diseases. Cancerous tumors result when a cell escapes from its normal growth regulatory mechanisms and proliferates in an uncontrolled fashion. Tumor cells can metastasize to secondary sites if treatment of the primary tumor is either not complete or not initiated before substantial progression of the disease. Early diagnosis and effective treatment of cancerous tumors is therefore beneficial for survival.

20 DNA-damaging therapies, such as radiotherapy and certain types of chemotherapy, are the methods of choice for treating subjects with metastatic cancer or subjects with diffuse cancers such as leukemias. However, radiotherapy can cause substantial damage to normal tissue in the treatment field, resulting in scarring and loss of function of the normal tissue, and secondary tumors, especially at higher radiation doses. Chemotherapy can provide a therapeutic benefit in many cancer subjects, but it

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often fails to treat the disease because cancer cells may become resistant to the chemotherapeutic agent. To overcome these limitations additional antineoplastic strategies are needed, such as the use of potentiating agents that restore or amplify the effect of antitumor agents.

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SUMMARY OF THE DISCLOSURE

A method for enhancing an antineoplastic effect of a DNA-damaging therapy has been identified and is described herein. The method includes administering to a subject having a neoplasm a therapeutically effective amount of the DNA-damaging therapy and a ribosome inhibitor that inhibits Tdp activity, wherein the ribosome inhibitor is administered in a sufficient amount to enhance the DNA-damaging therapy. In disclosed examples, the ribosome inhibitor is an aminoglycoside antibiotic and the DNA-damaging therapy is a topoisomerase inhibitor such as a camptothecin. In particular examples, the DNA-damaging therapy is a therapy that induces DNA damage that is at least partially repaired by a Tdp, such as Tdp1.

This disclosure also provides pharmaceutical compositions that include at least one antitumor chemotherapeutic agent and at least one ribosome inhibitor that inhibits Tdp activity, wherein the chemotherapeutic agent and the ribosome inhibitor are present in a therapeutically effective amount for the ribosome inhibitor to enhance an antineoplastic effect of the chemotherapeutic agent. In disclosed examples, the chemotherapeutic agent is a DNA-damaging agent such as a topoisomerase inhibitor. In particular examples, the chemotherapeutic agent is a DNA-damaging agent that induces DNA damage that is at least partially repaired by a Tdp, such as Tdp1.

The foregoing and other features and advantages will become more apparent from the following detailed description of several embodiments, which proceeds with reference to the accompanying figures.

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BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a schematic representation of a Tdp biochemical assay.

FIG. 2A-2B illustrate the effect of pH on Tdp1 activity.

FIG. 3A-3D show the chemical structures (FIG. 3A) of the aminoglycosides
5 neomycin B (Neo), paromomycin I (Par) and lividomycin (Liv) in their fully protonated
cationic states and illustrate the inhibition of Tdp1 activity by Neo, Par and Liv.

FIG. 4A-4B illustrate the kinetics for processing of D14Y by Tdp1 in the
absence or presence of neomycin.

FIG. 5A-5C illustrate that neomycin can inhibit Tdp1 activity both with a
10 duplex and a single-stranded DNA substrate.

FIG. 6A-6B illustrate that Tdp1 inhibition by neomycin is independent of the
order of addition.

FIG. 7A-7D illustrate that inhibition of Tdp1 by neomycin can be overcome by
increasing Tdp1 but not by increasing D14Y.

FIG. 8 illustrates that neomycin inhibition is competitive relative to the D14Y
15 substrate. Lineweaver-Burk plot representation for D14Y processing by Tdp1 in the
absence and presence of neomycin.

FIG. 9 is a schematic drawing, showing the chemical structures of additional
aminoglycoside ribosome inhibitors listed in Table I.

FIG. 10 is a schematic drawing, showing the chemical structures of non-
20 aminoglycoside ribosome inhibitors listed in Table I.

DETAILED DESCRIPTION

25 I. Abbreviations

BCNU:	1,3-bis (2-chloroethyl)-1-nitrosourea
°C:	degrees Celsius
Liv:	lividomycin
min:	minute(s)
30 ml:	milliliter
Neo:	neomycin B

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	PAGE:	polyacrylamide gel electrophoresis
	Par:	paromomycin
	Tdp:	tyrosyl-DNA-phosphodiesterase
	TopI:	topoisomerase I
5	TopII:	topoisomerase II
	µg:	microgram(s)
	µl:	microliter(s)
	µm:	micrometer(s)
10	µM:	micromolar

II. Terms

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes VII*, published by Oxford University Press, 2000 (ISBN 019879276X);
 15 Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Publishers, 1994 (ISBN 0632021829); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by Wiley, John & Sons, Inc., 1995 (ISBN 0471186341); and other similar references.

20 In order to facilitate review of the various embodiments of this disclosure, the following explanations of specific terms are provided:

Animal: Living multi-cellular vertebrate organisms, a category that includes, for example, mammals and birds. The term mammal includes both human and non-
 25 human mammals. Similarly, the term “**subject**” includes both human and veterinary subjects, for example, humans, non-human primates, dogs, cats, horses, and cows.

Antineoplastic: Having antitumor activity, for example inhibiting the development or progression of a tumor, including local tumor growth or recurrence or metastatic spread.

30 **Cancer or malignant neoplasm:** A neoplasm that has undergone characteristic anaplasia with loss of differentiation, increased rate of growth, invasion of surrounding tissue, and which is capable of metastasis.

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Chemotherapeutic agent: Any chemical compound with therapeutic usefulness in the treatment of a disease characterized by abnormal cell growth, such as a neoplasm.

Chemotherapy: The therapeutic use of a chemical compound to inhibit or kill
5 neoplastic cells and shrink or ablate tumors.

DNA-interacting agent: A chemical compound characterized by its ability to interact with DNA. There are several mechanisms by which a compound can interact with DNA, such as alkylation of nucleophilic sites within the DNA double helix. Exemplary DNA alkylating agents include, but are not limited to, cyclophosphamide, chlorambucil, melphalan, BCNU, and platinum derivatives. An additional mechanism
10 by which a compound can interact with DNA is intercalation. Exemplary DNA intercalating agents include, but are not limited to, doxorubicin and mitoxantrone.

DNA-damaging therapy: Any treatment method or chemical compound that induces DNA-damage when applied to a cell. Exemplary treatment methods include, but are not limited to, radiation that induces DNA-damage, such as gamma-irradiation, X-rays, UV-irradiation, microwaves, electronic emissions, and the like. Particular
15 examples of DNA-damaging therapies are such therapies that induce damage that is at least partially reversed or repaired by a Tdp, such as Tdp1.

A variety of chemical compounds, also described by those of skill in the art as
20 “chemotherapeutic agents,” induce DNA-damage. Exemplary chemical compounds include, but are not limited to, radiomimetic agents, such as bleomycin and neocarcinostatin; Topoisomerase I inhibitors, such as camptothecins (for example, topotecan and irinotecan), indolocarbazole derivatives, indenoisoquinolines, and homocamptothecins; Topoisomerase II inhibitors, such as epipodophyllotoxins (for
25 example, etoposide and teniposide), anthracyclines (for example, doxorubicin, idarubicin and epirubicin), ellipticines, and acridines (for example, m-AMSA); and agents that target DNA, such as DNA alkylating agents (cyclophosphamide, chlorambucil, melphalan, BCNU, and platinum derivatives) and ecteinascidin 743.

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Enhancing: Altering an outcome for the improvement of a specific value, such as an increase or a decrease in a particular parameter of an antineoplastic effect of a DNA-damaging therapy. In one embodiment, enhancement refers to at least a 25%, 50%, 100% or greater than 100% increase in a particular parameter. In another
5 embodiment, enhancement refers to at least a 25%, 50%, 100% or greater than 100% decrease in a particular parameter. In one specific, non-limiting example, enhancement of an antineoplastic effect of a DNA-damaging therapy refers to an increase in the ability of the therapy to inhibit or treat a neoplasm, such as at least a 25%, 50%, 100%, or greater than 100% increase in the effectiveness of the DNA-damaging therapy.

10 **Inhibiting or treating:** With respect to disease (such as neoplasm or metastasis), either term includes (i) preventing the disease, for example, causing the clinical symptoms of the disease not to develop in a subject that may be exposed to or predisposed to the disease but does not yet experience or display symptoms of the disease, (ii) restraining the disease, for example, arresting the development of the
15 disease or its clinical symptoms, (iii) ameliorating the disease, for example, delaying onset of the clinical symptoms of the disease in a susceptible subject or a reduction in severity of some or all clinical symptoms of the disease, or (iv) relieving the disease, for example, causing regression of the disease or its clinical symptoms.

Isolated or purified: An "isolated" or "purified" biological component (such
20 as a nucleic acid, peptide or protein) has been substantially separated, produced apart from, or purified away from other biological components in the cell of the organism in which the component naturally occurs, that is, other chromosomal and extrachromosomal DNA and RNA, and proteins. Nucleic acids, peptides and proteins that have been "isolated" thus include nucleic acids and proteins purified by standard
25 purification methods. The term also embraces nucleic acids, peptides and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids or proteins. The term "isolated" or "purified" does not require absolute purity; rather, it is intended as a relative term. Thus, for example, an isolated biological component is one in which the biological component is more enriched than the

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biological component is in its natural environment within a cell. Preferably, a preparation is purified such that the biological component represents at least 50%, such as at least 70%, at least 90%, at least 95%, or greater of the total biological component content of the preparation.

5 **Metastasis:** The process by which malignant cells transfer from one organ or part of the body to a separate organ or part of the body. This term also refers to a growth of malignant cells distant from the site of the primary neoplasm from which the malignant cells arose.

Neoplasm: An abnormal growth of cells or tissue, particularly a new growth of
10 cells or tissue in which the growth is uncontrolled and progressive. A tumor is an example of a neoplasm.

Parenteral: Administered outside of the intestine, for example, not via the alimentary tract. Generally, parenteral formulations are those that will be administered through any possible mode except ingestion. This term especially refers to injections,
15 whether administered intravenously, intrathecally, intramuscularly, intraperitoneally, or subcutaneously, and various surface applications including intranasal, intradermal, and topical application, for example. Parenteral administration is preferred for some chemical compounds to avoid degradation of the chemical compound in the gastrointestinal tract.

20 **Pharmaceutically acceptable carriers:** The pharmaceutically acceptable carriers (vehicles) useful in this disclosure are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of one or more therapeutic compounds or molecules, such as one or more
25 antitumor chemotherapeutic agents and at least one ribosome inhibitor that inhibits Tdp activity.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids

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such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (for example, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition
5 to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Radiomimetic agent: A chemical compound that imitates the damaging effects
10 of radiation on a cell. Exemplary radiomimetic agents include, but are not limited to, bleomycin and neocarcinostatin.

Radiotherapy: The use of radiation from X-rays, gamma rays, neutrons, and other sources to kill neoplastic cells and shrink or ablate tumors. Radiation may come from a source outside the body (for example, external-beam radiation therapy), or it
15 may come from radioactive material placed in the body near neoplastic cells (for example, internal radiation therapy, implant radiation or brachytherapy).

Resistant: Failure of a neoplastic cell to respond or remit following DNA-damaging therapy.

Ribosome inhibitor: A chemical compound characterized by its ability to
20 inhibit protein synthesis by interfering with ribosomal function. Particular examples of ribosome inhibitors are antibiotic ribosome inhibitors that have antibacterial effects. Exemplary ribosome inhibitors include, but are not limited to, aminoglycosides, such as neomycin, paromomycin, lividomycin, amikamicin, apramycin, kanamycin, netilmicin, streptomycin, and tobramycin; aminocyclitols, such as spectinomycin; tetracyclines,
25 such as tetracycline, minocycline and doxycycline; macrolides, such as erythromycin, azithromycin and clarithromycin; lincosamides, such as lincomycin and clindamycin; streptogramins, such as streptogramin A and streptogramin B; oxazolidinones, such as linezolid; hygromycin B; puromycin; and thiostrepton.

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Therapeutically effective amount: A quantity of a specified agent sufficient to achieve a desired effect in a subject being treated with that agent. For example, this may be the amount of a DNA-damaging therapy and a ribosome inhibitor that inhibits Tdp activity necessary to inhibit a neoplasm or metastasis of a malignant cell. Ideally, a therapeutically effective amount of an agent is an amount sufficient to effect the desired result without causing a substantial cytotoxic effect in the subject. The effective amount of an agent useful for preventing or treating a neoplasm or inhibiting metastasis of a malignant cell will be dependent on the subject being treated, the severity of the affliction, and the manner of administration of the therapeutic composition.

A therapeutically effective amount of a DNA-damaging therapy and a ribosome inhibitor that inhibits Tdp activity may be administered in a single dose, or in several doses, for example daily, during a course of treatment. However, the frequency of administration is dependent on the preparation applied, the subject being treated, the severity and type of the affliction, and the manner of administration of the therapy or compound.

Topoisomerase: A reversible nuclease that attaches covalently to a DNA backbone phosphate, thereby breaking a phosphodiester bond in the DNA strand and forming a bond to an active site tyrosine of the enzyme. This reaction is reversible, and the phosphodiester bond re-forms as the enzyme leaves the DNA. One type of topoisomerase, topoisomerase I (TopI) produces a transient single-strand break (or nick). A second type of DNA topoisomerase, topoisomerase II (TopII) forms a covalent linkage to both strands of the DNA helix at the same time, making a transient double-strand break in the helix. DNA topoisomerases help to relieve helical winding and DNA tangling problems, such as are associated with DNA replication and DNA repair following DNA damage.

Topoisomerase inhibitor: A chemical compound characterized by its ability to inhibit an activity of a DNA topoisomerase, such as the religation function of these enzymes. Exemplary topoisomerase inhibitors include, but are not limited to, Topoisomerase I inhibitors, such as camptothecins (for example, topotecan and

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irinotecan), indolocarbazole derivatives, indenoisoquinolines, and homocamptothecins, and Topoisomerase II inhibitors, such as epipodophyllotoxins (for example, etoposide and teniposide), anthracyclines (for example, doxorubicin, idarubicin and epirubicin), ellipticines, and acridines (for example, m-AMSA). Topoisomerase inhibitors have
5 been found to exert an antitumor effect in which proliferation of tumor cells is inhibited.

Tumor: A neoplasm that may be either malignant or non-malignant and includes both solid tumors and non-solid tumors (such as hematologic malignancies).

Tyrosyl-DNA-phosphodiesterase (Tdp): An enzyme capable of hydrolyzing phosphodiester bonds between tyrosine and the 3'-phosphate of DNA, which are
10 typically generated in a transient manner by DNA topoisomerases. Tdp functions to cleave a covalent 3'-tyrosyl phosphodiester bond between a topoisomerase and a polynucleotide. Therefore, binding to a topoisomerase-polynucleotide complex is a "Tdp activity." Another "Tdp activity" is cleavage of a 3'-tyrosyl phosphodiester bond, such as a 3'-tyrosyl bond between a topoisomerase and a polynucleotide. As used
15 herein, the term "**Tdp activity**" is intended to include both binding of Tdp to a topoisomerase-polynucleotide complex and cleavage of a 3'-tyrosyl bond between a topoisomerase and a polynucleotide.

As used herein, the singular terms "a," "an," and "the" include plural referents
20 unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. Also, as used herein, the term "comprises" means "includes." Hence "comprising A or B" means including A, B, or A and B. It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides
25 are approximate, and are provided for description. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification,

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including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

III. Overview of Several Embodiments

5 Provided herein in various embodiments is a method for enhancing an antineoplastic effect of a DNA-damaging therapy. In one embodiment, the method includes administering to a subject having a neoplasm a therapeutically effective amount of the DNA-damaging therapy and a ribosome inhibitor that inhibits Tdp activity, wherein the ribosome inhibitor is administered in a sufficient amount to
10 enhance an antitumor effect (such as an antiproliferative effect) of the DNA-damaging therapy. In a specific, non-limiting example, the neoplasm is resistant to the DNA-damaging therapy, such as reduced or absent responsiveness of the neoplasm to the DNA-damaging therapy which has developed following treatment with the DNA-damaging therapy. In yet another specific example of the method, the therapeutically
15 effective amount of the DNA-damaging therapy in the presence of the ribosome inhibitor is lower than when the DNA-damaging therapy is administered to the subject alone. In still another specific example of the provided method, the neoplasm is a malignant neoplasm and the DNA-damaging therapy is a therapy effective against a malignant neoplasm.

20 In a further specific example of the provided method, the DNA-damaging therapy includes antitumor radiotherapy and/or chemotherapy. Exemplary chemotherapeutic agents include, but are not limited to, radiomimetic agents, TopI inhibitors, TopII inhibitors, and DNA-interacting agents. Exemplary ribosome inhibitors include, but are not limited to, aminoglycosides, tetracyclines, macrolides,
25 lincosamides, streptogramins, oxazolidinones, spectinomycin, thiostrepton, and puromycin. In one embodiment, the ribosome inhibitor is administered prior to administration of the DNA-damaging therapy. In another embodiment, the ribosome inhibitor is administered simultaneously with the administration of the DNA-damaging therapy.

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A method for treating a malignant neoplasm is also described herein. This method includes administering to a subject having a malignant neoplasm a therapeutically effective amount of a TopI inhibitor and a therapeutically effective amount of a ribosome inhibitor that inhibits Tdp activity, thereby treating the malignant neoplasm in the subject. In a specific, non-limiting example, the TopI inhibitor includes a camptothecin and the ribosome inhibitor includes an aminoglycoside.

Pharmaceutical compositions are also disclosed that include a pharmaceutical carrier, at least one chemotherapeutic agent and at least one ribosome inhibitor that inhibits Tdp activity, wherein the chemotherapeutic agent and the ribosome inhibitor are present in a therapeutically effective amount for the ribosome inhibitor to enhance an antineoplastic effect of the chemotherapeutic agent. Representative chemotherapeutic agents include, but are not limited to, radiomimetic agents, TopI inhibitors, TopII inhibitors, and DNA-interacting agents. Representative ribosome inhibitors include, but are not limited to, aminoglycosides, tetracyclines, macrolides, lincosamides, streptogramins, oxazolidinones, spectinomycin, thiostrepton, and puromycin.

Also provided herein is a method for inhibiting Tdp activity in a biological sample with Tdp activity, including contacting the sample with a ribosome inhibitor, thereby inhibiting Tdp activity. Exemplary ribosome inhibitors include, but are not limited to, aminoglycosides, tetracyclines, macrolides, lincosamides, streptogramins, oxazolidinones, spectinomycin, thiostrepton, and puromycin.

IV. Ribosome Inhibitors That Inhibit Tdp Activity

Tdp is an enzyme that hydrolyzes a 3'-tyrosyl phosphodiester, such as a 3'-tyrosyl phosphodiester bond between a topoisomerase and the 3'-end of a DNA molecule to which the topoisomerase is bound. Topoisomerases are cellular enzymes that function by breaking the DNA backbone, allowing DNA to undergo topological change, and then resealing the break. During this process, topoisomerases form a covalent bond with the DNA prior to the resealing step. Under circumstances in which the resealing step fails (for example, in the presence of a topoisomerase inhibitor), the

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active site tyrosine of the enzyme remains covalently linked to the 3'-end of broken DNA. Such topoisomerase-DNA complexes disrupt normal cellular replication, leading to decreased proliferation and resulting in cell death. For example, DNA mismatches, nicked DNA, camptothecin-like drug induced- and topoisomerase-induced mutations
5 have been shown to cause covalent complexes to accumulate in vitro (Yeh *et al.*, *J. Biol. Chem.* 269:15498-504, 1994 and Lanza *et al.*, *J. Biol. Chem.* 271:6978-86, 1996). Tdp is a repair enzyme that inhibits the accumulation of these faulty covalent complexes. Inhibiting Tdp activity thus promotes cell death by increasing the accumulation of faulty covalent complexes, such as topoisomerase-DNA complexes.
10 Therefore, inhibitors of Tdp provide a means of enhancing an antineoplastic effect of a DNA-damaging therapy.

The present disclosure provides methods for inhibiting Tdp activity, including contacting a biological sample with Tdp activity with a ribosome inhibitor that inhibits Tdp activity, or administering a ribosome inhibitor that inhibits Tdp activity to a
15 subject. In some embodiments of the present disclosure, inhibition of Tdp activity enhances an antineoplastic effect of a DNA-damaging therapy. A ribosome inhibitor that inhibits Tdp activity may include an aminoglycoside, an aminocyclitol, a tetracycline, a macrolide, a lincosamide, a streptogramin, an oxazolidinone, hygromycin B, puromycin, and thiostrepton.

20 Aminoglycosides are a clinically important group of bactericidal compounds. The family includes, for example, neomycin, paromomycin, lividomycin, amikamicin, apramycin, kanamycin, netilmicin, streptomycin, and tobramycin. The aminocyclitols, such as spectinomycin, are closely related and have a similar mode of action. Aminoglycosides have a variety of effects within a bacterial cell but principally they
25 inhibit ribosome function by binding to the 30S ribosomal subunit to prevent the formation of an initiation complex with messenger RNA. They also cause misreading of the messenger RNA message, leading to the production of nonsense peptides.

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Tetracyclines are a class of bacteriostatic compounds that inhibit binding of the aminoacyl tRNA to the 30S ribosomal subunit in bacteria. The family includes, for example, tetracycline, minocycline and doxycycline.

Macrolides are a class of bacteriostatic compounds that bind to the 50S
5 ribosomal subunit in bacteria and inhibit either peptidyl transferase activity or translocation of the growing peptide. The family includes, for example, erythromycin, azithromycin and clarithromycin.

Lincosamides are a class of bacteriostatic compounds that bind to the 50S
ribosomal subunit in bacteria and inhibit either peptidyl transferase activity or
10 translocation of the growing peptide. Representative lincosamides include, for example, lincomycin and clindamycin.

Streptogramins are a class of bacteriostatic compounds with varied sites of action within the bacterial ribosome. Representative streptogramins include, for example, streptogramin A and streptogramin B.

15 Oxazolidinones are a class of bacteriostatic compounds that bind to the 50S ribosomal subunit in bacteria and interfere with protein initiation. Linezolid, for example, is a representative oxazolidinone.

Hygromycin B is an aminoglycosidic compound that kills bacteria, fungi and higher eukaryotic cells by inhibiting protein synthesis. Puromycin is a compound that
20 inhibits the growth of bacteria and various animal and insect cells by causing premature chain termination during translation. Thiostrepton is a highly-modified multi-cyclic peptide compound that inhibits protein synthesis in bacteria.

Inhibition of Tdp activity by a ribosome inhibitor can be determined using a variety of methods. For example, the Tdp biochemical assay provided herein in
25 Example 1 can be used to assay inhibition of Tdp activity by ribosome inhibitors. Tdp activity/inhibition can also be assayed using substrates consisting of nucleopeptides or DNA with a 3'-phosphodiester linkage to a tyrosyl, paranitrophenol or coumarin substituent. Tdp activity is followed as the release of the tyrosyl, paranitrophenol or coumarin substituent from the DNA. As understood by those of skill in the art, assay

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methods for determining the ability of a compound to inhibit Tdp activity generally require comparison to a control. An exemplary control is an isolated Tdp preparation or biological sample with Tdp activity that is treated substantially the same as the test preparation or sample exposed to a ribosome inhibitor, except that the control is not exposed to the ribosome inhibitor.

Tyrosyl-DNA-phosphodiesterase 1 that is to be inhibited by a ribosome inhibitor can be found in a subject, or contained in a variety of biological samples. For example, Tdp can be contained in a histologic section of a specimen obtained by biopsy, cells obtained from body fluids or cells that are placed in or adapted to tissue culture.

Tyrosyl-DNA-phosphodiesterase 1 can also be contained in a cell that recombinantly expresses Tdp and in a lysate or fraction of such a cell. Isolated or purified Tdp is removed or separated from at least one component with which it is naturally associated. Therefore, isolated Tdp can be contained in a subcellular fraction or extract prepared from cells containing Tdp, such as a cytoplasmic lysate, a membrane preparation, a nuclear extract, or a crude or purified protein preparation. A sample containing Tdp can be prepared by methods known in the art suitable for the particular format of the detection method. For example, biochemical methods such as precipitation, chromatography and immunoaffinity methods can be used to isolate Tdp from a cell which expresses Tdp endogenously or recombinantly. Procedures for preparing subcellular fractions, such as nuclear fractions and cell lysates, are well known to those of skill in the art, and include, for example, cell disruption followed by separation methods such as gradient centrifugation and biochemical purification methods.

V. Methods of Using Ribosome Inhibitors That Inhibit Tdp Activity

The present disclosure includes methods for enhancing an antineoplastic effect of a DNA-damaging therapy, including administering to a subject having a neoplasm a therapeutically effective amount of the DNA-damaging therapy and a ribosome inhibitor that inhibits Tdp activity. In some methods, a therapeutically effective amount of a DNA-damaging therapy and a ribosome inhibitor that inhibits Tdp activity is

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administered to a subject to inhibit the development of or treat an existing neoplasm of an exposed body surface. Additional methods involve administering to a subject or contacting one or more malignant cells of a subject with a therapeutically effective amount of a DNA-damaging therapy and a ribosome inhibitor that inhibits Tdp activity
5 to inhibit metastasis of the malignant cells. Any living, multicellular, vertebrate organism capable of developing one or more neoplasms is contemplated as a subject for the disclosed methods. Thus, in particular examples, a subject of a disclosed method is a human or veterinary subject.

A therapeutically effective amount of a DNA-damaging therapy and a ribosome
10 inhibitor that inhibits Tdp activity can be used to treat or prevent, or inhibit metastasis from, any neoplasm. Non-limiting examples of neoplasms include tumors of the skin, such as squamous cell carcinoma, basal cell carcinoma, melanoma, skin appendage tumors, papilloma, cutaneous T-cell lymphoma (mycosis fungoides), apocrine carcinoma of the skin, or Merkel cell carcinoma, breast carcinomas, for example,
15 lobular and duct carcinomas and other solid tumors, sarcomas and carcinomas of the lung, such as small cell carcinoma, large cell carcinoma, squamous carcinoma, adenocarcinoma, and mesothelioma of the lung, colorectal adenocarcinoma, stomach carcinoma, prostatic adenocarcinoma, ovarian carcinoma, such as serous cystadenocarcinoma and mucinous cystadenocarcinoma, and ovarian germ cell tumors,
20 testicular carcinomas, germ cell tumors, pancreatic adenocarcinoma, biliary adenocarcinoma, hepatocellular carcinoma, bladder carcinoma, including transitional cell carcinoma, adenocarcinoma and squamous carcinoma, renal cell adenocarcinoma, endometrial carcinomas, including adenocarcinomas and mixed Mullerian tumors (carcinosarcomas), carcinomas of the endocervix, ectocervix and vagina, such as
25 adenocarcinoma and squamous carcinoma, esophageal carcinoma, carcinomas of the nasopharynx and oropharynx, including squamous carcinoma and adenocarcinomas, salivary gland carcinomas, brain and central nervous system tumors, including tumors of glial, neuronal and meningeal origin, tumors of peripheral nerve, soft tissue sarcomas

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and sarcomas of bone and cartilage, and non-solid hematopoietic tumors, such as leukemias.

Non-limiting examples of DNA-damaging therapies include the use of therapeutic doses of DNA-damaging radiation, such as gamma-irradiation, X-rays, UV-irradiation, microwaves, electronic emissions, and the like, and the use of chemotherapeutic agents. Exemplary antitumor chemotherapeutic agents include, but are not limited to, therapeutically effective amounts of radiomimetic agents, such as bleomycin and neocarzinostatin; Topoisomerase I inhibitors, such as camptothecins (for example, topotecan and irinotecan), indolocarbazole derivatives, indenoisoquinolines, and homocamptothecins; Topoisomerase II inhibitors, such as epipodophyllotoxins (for example, etoposide and teniposide), anthracyclines (for example, doxorubicin, idarubicin and epirubicin), ellipticines, and acridines (for example, m-AMSA); and agents that target DNA, such as DNA alkylating agents (cyclophosphamide, chlorambucil, melphalan, BCNU, and platinum derivatives) and ecteinascidin 743.

Exemplary methods involve treating, preventing, or inhibiting metastasis of an neoplasm. Treatment of a neoplasm using a disclosed method can involve, for example, inhibiting the growth of the neoplasm, reducing the size of the neoplasm, inducing apoptosis of the neoplasm, or inhibiting metastasis of the neoplasm. Inhibiting the growth of a neoplasm conveys a wide-range of inhibitory effects that a treatment (for example, a DNA-damaging therapy and a ribosome inhibitor that inhibits Tdp activity) can have on the initiation and growth of a neoplasm, for example, as compared to an untreated (or pre-treatment) neoplasm. Thus, inhibiting the growth of a neoplasm includes situations wherein an incidence of neoplasm is reduced or the normal growth rate of the neoplasm has slowed (for example, the number of neoplastic cells still increases over time, but not as rapidly as in a control neoplastic cell population), equals zero (for example, there is substantially no change in number of neoplastic cells in the population over time; for instance, neoplastic cell growth is approximately equal to cell death or quiescence in the same population), or becomes negative (for example, the number of neoplastic cells decreases over time; for instance, cell death exceeds cell

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growth or quiescence). A reduction in the size of a neoplasm can be determined using any methods or standard known to the ordinarily skilled artisan. In one embodiment, the decrease in one or more physical dimensions of a neoplasm (such as, diameter, volume, length, width, or weight), as compared to corresponding measurement(s) made at an earlier time point (such as pre-treatment or earlier in a course of treatment), can indicate a neoplasm size reduction.

Inhibiting metastasis of a neoplasm (or malignant cells thereof) conveys a wide-range of inhibitory effects that a treatment (for example, a DNA-damaging therapy and a ribosome inhibitor that inhibits Tdp activity) can have on metastasis of such neoplasm (or malignant cells). For example, inhibiting metastasis may be considered relative to an untreated (that is, uninhibited or control) rate of metastasis of a particular malignant cell or population of malignant cells of interest. Thus, inhibiting metastasis includes situations wherein the metastatic rate of a cell or cell population has slowed (that is, the number metastatic cells decreases over time as compared to a control population), or is reduced to near zero (that is, there are substantially no metastatic cells in the population over time).

Toxicity and therapeutic efficacy of a treatment, such as a DNA-damaging therapy and a ribosome inhibitor that inhibits Tdp activity, can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, for example, by determining the MIC₅₀ (the lowest tested concentration that inhibits the growth of the population by at least 50%), LD₅₀ (the dose lethal to 50% of the population) and/or the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it may be expressed, for example, as the ratio LD₅₀/ED₅₀. A combination DNA-damaging therapy and ribosome inhibitor that inhibits Tdp activity that exhibit large therapeutic indices are useful, as are combinations that exhibit toxic side effects. However, in the case of combinations with toxic side effects, it can be helpful to design a delivery system that targets such combinations to the site of affliction to minimize potential damage to normal cells and, thereby, reduce side effects.

VI. *Pharmaceutical Compositions*

This disclosure contemplates administering to a subject a chemotherapeutic agent and a ribosome inhibitor that inhibits Tdp activity as a method for enhancing an antineoplastic effect of the chemotherapeutic agent. Any delivery system or treatment regimen that effectively treats or inhibits the development of a neoplasm (or metastasis) of interest can be used. Accordingly, pharmaceutical compositions comprising at least one chemotherapeutic agent and at least one ribosome inhibitor that inhibits Tdp activity are also described herein.

Formulations for pharmaceutical compositions are well known in the art. For example, *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition, 1975, describes exemplary formulations (and components thereof) suitable for pharmaceutical delivery of a chemotherapeutic agent and a ribosome inhibitor that inhibits Tdp activity. Pharmaceutical compositions comprising at least one chemotherapeutic agent and at least one ribosome inhibitor that inhibits Tdp activity can be formulated for use in human or veterinary medicine. Particular formulations of a disclosed pharmaceutical composition may depend, for example, on the mode of administration (for example, topical, oral or parenteral) and/or on the location of the neoplasm to be treated. In some embodiments, formulations include a pharmaceutically acceptable carrier in addition to at least one chemotherapeutic agent and at least one ribosome inhibitor that inhibits Tdp activity. In other embodiments, other medicinal or pharmaceutical agents, for example, with similar, related or complementary effects on the affliction being treated (such as a neoplasm), can also be included as active ingredients in a pharmaceutical composition.

The pharmaceutical compositions comprising at least one chemotherapeutic agent and at least one ribosome inhibitor that inhibits Tdp activity described herein may be formulated in a variety of ways depending, for example, on the mode of administration and/or on the location and type of neoplasm to be treated. For example, such pharmaceutical compositions may be formulated as a pharmaceutically acceptable

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salt of a disclosed chemotherapeutic agent and/or ribosome inhibitor that inhibits Tdp activity. As another example, parenteral formulations may comprise injectable fluids that are pharmaceutically and physiologically acceptable fluid vehicles, such as water, physiological saline other balanced salt solutions, aqueous dextrose, glycerol or the like.

5 Excipients may include, for example, nonionic solubilizers, such as cremophor, or proteins, such as human serum albumin or plasma preparations. If desired, the pharmaceutical composition to be administered may also contain non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example, sodium acetate or sorbitan monolaurate.

10 The dosage form of the pharmaceutical composition will be determined by the mode of administration chosen. For example, in addition to injectable fluids, topical and oral formulations may be employed. Topical preparations may include eye drops, ointments, sprays and the like. The compositions can be applied onto an exposed body surface using any known or otherwise effective application technique including, but not
15 limited to, the techniques of rubbing, brushing, painting, wiping, and stroking a composition onto the skin.

 When the pharmaceutical composition administered in a cutaneous or topical carrier or diluent, the carrier or diluent may be chosen from any known in the cosmetic or medical arts; for example, any gel cream, lotion, ointment, liquid or non liquid
20 carrier, emulsifier, solvent, liquid diluent or other similar vehicle which does not exert deleterious effect on the skin or other living animal tissue. The carrier or diluent is usually a mixture of several ingredients, including, but not limited to liquid alcohols, liquid glycols, liquid polyalkylene glycols, water, liquid amides, liquid esters, liquid lanolin, lanolin derivatives and similar materials. Alcohols include mono and
25 polyhydric alcohols, including ethanol, glycerol, sorbitol, isopropanol, diethylene glycol, propylene glycol, ethylene glycol, hexylene glycol, mannitol and methoxyethanol. Typical carriers may also include ethers (such as, diethyl and dipropyl ether), methoxypolyoxyethylenes, carbowaxes, polyethyleneglycerols, polyoxyethylenes and sorbitols. In some embodiments, the topical carrier includes both

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water and alcohol in order to maximize the hydrophilic and lipophilic solubility (for instance, a mixture of ethanol or isopropanol with water). One skilled in the art may choose other carriers or diluents to adapt to specific dermatologic needs.

Other methods of administering the pharmaceutical compositions comprising at least one chemotherapeutic agent and at least one ribosome inhibitor that inhibits Tdp activity described herein include parental or enteral routes, such as intrathecal, intradermal, intramuscular, intraperitoneal (ip), intravenous (iv), subcutaneous, intranasal, epidural, and oral routes. For solid compositions, conventional non-toxic solid carriers may include pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art.

The pharmaceutical compositions can be administered by any convenient route, including, for example, infusion or bolus injection, absorption through epithelial or mucocutaneous linings (for example, oral mucosa, rectal and intestinal mucosa, and the like), ophthalmic, nasal, and transdermal, and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce a pharmaceutical composition by any suitable route, including intraventricular and intrathecal injection. Intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir. Pulmonary administration can also be employed (for example, by an inhaler or nebulizer), for instance using a formulation containing an aerosolizing agent.

In a specific embodiment, it may be desirable to administer a pharmaceutical composition locally to an area in need of treatment (for example, to an area of the body with a solid tumor). This can be achieved by, for example, local or regional infusion or perfusion during surgery, topical application, injection, catheter, suppository, or implant (for example, implants formed from porous, non-porous, or gelatinous materials, including membranes, such as sialastic membranes or fibers), and the like. In one embodiment, administration can be by direct injection at the site (or former site) of a neoplasm that is to be treated. In another embodiment, the pharmaceutical composition

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is delivered in a vesicle, such as liposomes (see, for example, Langer, *Science* 249:1527-33, 1990 and Treat *et al.*, in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez Berestein and Fidler (eds.), Liss, N.Y., pp. 353-65, 1989).

In yet another embodiment, the pharmaceutical composition can be delivered in
5 a controlled release system. In one example, a pump can be used (see, *e.g.*, Langer, *Science* 249:1527-33, 1990; Sefton, *Crit. Rev. Biomed. Eng.* 14:201-40, 1987; Buchwald *et al.*, *Surgery* 88:507-16, 1980; Saudek *et al.*, *N. Engl. J. Med.* 321:574-79, 1989). In another example, polymeric materials can be used (see, for example, Levy *et al.*, *Science* 228:190-92, 1985; During *et al.*, *Ann. Neurol.* 25:351-56, 1989; Howard *et al.*, *J. Neurosurg.* 71:105-12, 1989). Other controlled release systems, such as those
10 discussed by Langer (*Science* 249:1527-33, 1990), can also be used.

The ingredients in various embodiments are supplied either separately or mixed together in unit dosage form, for example, in solid, semi-solid and liquid dosage forms such as tablets, pills, powders, liquid solutions, or suspensions, or as a dry lyophilized
15 powder or water free concentrate in a hermetically sealed container such as an ampoule or sachet indicating the quantity of active agent. Where the pharmaceutical composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the pharmaceutical composition is administered by injection, an ampoule of sterile water or
20 saline can be provided so that the ingredients may be mixed prior to administration.

VII. Therapeutically Effective Amounts and Dosage Regimens

Therapeutic treatments can include a therapeutically effective amount of a DNA-damaging therapy and a ribosome inhibitor that inhibits Tdp activity. Ideally, a
25 therapeutically effective amount of an agent is an amount sufficient to effect the desired result (for example, inhibiting a neoplasm or metastasis of a malignant cell), without causing a substantial cytotoxic effect in the subject. The effective amount of an agent useful for preventing or otherwise treating a neoplasm or inhibiting metastasis of a malignant cell will be dependent on the subject being treated, the severity of the

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affliction, and the manner of administration of the therapeutic composition. Effective amounts can be determined by standard clinical techniques.

For example, when administering a pharmaceutical composition comprising at least one chemotherapeutic agent and at least one ribosome inhibitor that inhibits Tdp activity, the precise dose to be employed in the formulation will depend on the route of administration, and should be decided according to the judgment of the health care practitioner and each patient's circumstances. The concentration of an active ingredient (such as a chemotherapeutic agent and a ribosome inhibitor that inhibits Tdp activity) in a topical composition (such as an ointment, cream, gel, or lotion) is typically from about 0.2% to about 1% (by weight relative to the total weight of the topical composition); for example, from about 0.3% to about 0.9%, from about 0.4% to about 0.8%, and from about 0.5% to about 0.7%. Within the ranges, higher concentrations allow a suitable dosage to be achieved while applying the lotion, ointment, gel, or cream in a lesser amount or with less frequency. In other embodiments, a dosage range for non-topical administration (such as oral administration, or intravenous or intraperitoneal injection) of a pharmaceutical composition containing at least one chemotherapeutic agent and at least one ribosome inhibitor that inhibits Tdp activity is from about 0.1 to about 200 mg/kg body weight in single or divided doses; for example from about 1 to about 100 mg/kg, from about 2 to about 50 mg/kg, from about 3 to about 25 mg/kg, or from about 5 to about 10 mg/kg.

Acceptable dosages of the active ingredients (such as a chemotherapeutic agent and a ribosome inhibitor that inhibits Tdp activity) of the pharmaceutical compositions of the present disclosure are, for example, dosages that achieve a target tissue concentration similar to that which produces the desired antiproliferative effect *in vitro*. Acceptable dosages of both chemotherapeutic agents and ribosome inhibitors that inhibit Tdp activity are known in the art. It is anticipated that these known dosages can be used in combination to provide the superior antitumor effects of the present methods.

The pharmaceutical compositions of the present disclosure can be administered at about the same dose throughout a treatment period, in an escalating dose regimen, or

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in a loading-dose regime (for example, in which the loading dose is about two to five times the maintenance dose). In some embodiments, the dose is varied during the course of a treatment based on the condition of the subject being treated, the severity of the neoplasm, the apparent response to the therapy, and/or other factors as judged by one of ordinary skill in the art. In some embodiments long-term treatment with a disclosed pharmaceutical composition is contemplated, for instance in order to prevent reoccurrence of a neoplasm.

The subject matter of the present disclosure is further illustrated by the following non-limiting Examples.

EXAMPLES

Example 1

Inhibition of Tdp1 Activity by Ribosome Inhibitors

This example demonstrates the ability of ribosome inhibitors to inhibit Tdp1 activity.

³²P-radiolabeling of the partially duplex oligonucleotide D14Y (FIG. 1) was at the 5'-terminus of the upper 14mer strand. Tdp1 catalyzes hydrolysis of the 3'-phosphotyrosine bond and converts D14Y to an oligonucleotide product with 3'-phosphate (D14P; FIG. 1). The 3'-phosphate oligonucleotide (D14P) runs slower than the corresponding D14Y in polyacrylamide gel electrophoresis (PAGE).

To examine the kinetic processing of the D14Y substrate (see FIG. 1) by Tdp1 under different pH conditions, 100 µl reaction mixtures containing 0.25 µM D14Y and 10 ng of Tdp1 were incubated at 25°C for the indicated time at pH 6.4 (circle in FIG. 2B), pH 6.8 (triangle in FIG. 2B), pH 7.4 (square in FIG. 2B), or pH 8.0 (diamond in FIG. 2B). Aliquots were taken at 0.5, 1, 3, 8, 20, and 40 min, after addition of a 3-fold excess of loading buffer to stop the reactions. Mixtures were analyzed by denaturing PAGE (FIG. 2A). Substrate (Y) and product (P) are shown in representative gels. FIG 2B is a graph showing densitometric analysis of the gels shown in FIG. 2A.

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To determine the ability of ribosome inhibitors to inhibit Tdp1 activity, reactions were performed in 20 μ l reactions containing 0.025 μ M D14Y, 1 ng Tdp1 and various ribosome inhibitors. Exemplary results using the aminoglycosides Neo, Par and Liv are shown in FIG. 3. Concentrations of Neo, Par and Liv are indicated above the gel picture (FIG. 3B). Reactions were incubated at 25°C for 20 min and stopped by addition of 60 μ l of loading buffer (98% v/v formamide, 1% w/v xylene cyanol, 1% w/v bromophenol blue). Mixtures were analyzed by denaturing (7 M urea) PAGE (FIG. 3B is a representative gel showing Tdp1 inhibition in pH 8.0 reaction buffer). Y: substrate D14Y (Y); P: product D14P (P). The inhibition of Tdp1 activity by Neo, Par and Liv in pH 8.0 reaction buffer (FIG. 3C) and in pH 6.4 reaction buffer (FIG. 3D) was also determined. Neomycin (circle), paromomycin (triangle) and lividomycin (square). The ability of additional ribosome inhibitors to inhibit Tdp1 activity is shown in Table I, which includes a summary of IC₅₀ values for the indicated compounds as Tdp1 inhibitors. Reactions were performed for 20 min at 25°C and pH 8.0.

Table 1 Inhibition of Tdp1 by antibiotics interfering with ribosome function

Chemicals	IC ₅₀ (mM)
<i>Aminoglycoside</i>	
Neomycin	8
Paromomycin	21
Lividomycin A	30
Amikamicin	18
Apramycin	21
Kanamycin	30
Netilmicin	8
Streptomycin	12
Tobramycin	21
<i>Non-aminoglycoside</i>	
Clindamycin 2-phosphate	9.1
Hygromycin B	> 30
Puromycin	18
Spectinomycin	> 30
Thiostrepton	1.8

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Example 2

Inhibition Substrates and Kinetics

This example demonstrates the substrate preference of the exemplary ribosome inhibitor neomycin and the kinetics of Tdp1 inhibition.

5 To examine the kinetics for processing D14Y by Tdp1 in the absence or presence of neomycin, a 100 μ l reaction mixture containing 0.25 μ M D14Y and 10 ng Tdp1 was incubated in pH 8.0 buffer at 25°C in the absence of drug (No Neo), or in the presence of 1 or 2 mM neomycin. Aliquots were taken at 0.2, 0.5, 1, 3, 8, 20 and 40 min. Reaction mixtures were analyzed by denaturing PAGE. Substrate D14Y (Y) and
10 product D14P (P) are shown in a representative gel (FIG. 4A). FIG. 4B is a graph showing densitometric analysis of the gel shown in FIG. 4A. Tdp1 activity was calculated as the percentage of D14Y converted to D14P.

The ability of neomycin to inhibit Tdp1 activity on both a duplex and a single-stranded DNA substrate was also examined. A schematic representation of the partially
15 duplex substrate D14Y and of the single-stranded substrate N14Y is shown in FIG. 5A. Oligonucleotide sequences are the same as in FIG. 1. To examine the ability of neomycin to inhibit Tdp1 activity on both a duplex and a single-stranded DNA substrate, a 100 μ l reaction mixture containing 0.25 μ M D14Y (circle in FIG. 5B) or 0.25 μ M N14Y (triangle in FIG. 5B) and 10 ng Tdp1 was incubated in pH 8.0 buffer at
20 25°C with increasing concentrations of neomycin. FIG. 5B is a graphical representation of Tdp1 inhibition by Neo using D14Y (circle) and N14Y (triangle) as substrates. Tdp1 activity was calculated as the percentage of substrate converted. FIG. 5C is an autoradiograph of a representative gel, showing the differential effect of neomycin on the single- and double-stranded substrate.

25 The effect of the order of addition of neomycin and Tdp1 on Tdp1 inhibition by neomycin was also examined. FIG. 6A is a schematic representation (upper panel) of the three protocols used. Reaction mixtures were analyzed by denaturing PAGE, and a representative gel showing kinetic inhibition of Tdp1 by neomycin in the three different protocols is shown in FIG. 6A (lower panel). All reactions were performed with D14Y

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as substrate and at 25°C; 100 µl reaction mixtures contained 0.25 µM D14Y, 10 ng Tdp1, without neomycin (open circles in FIG. 6B) or 1 mM neomycin (filled symbols in FIG. 6B). Protocol a: Neo, Tdp1 and DNA were added at the same time and reactions were incubated for indicated times (filled triangles in FIG. 6B). Protocol b: Neo and Tdp1 were pre-incubated for 20 min before addition of DNA to start the reactions (filled squares in FIG. 6B). Protocol c: Neo was first incubated with D14Y for 20 min before addition of Tdp1 to start the reactions (filled diamonds in FIG. 6B). FIG. 6B is a graphical representation of the kinetics of Tdp1 inhibition by neomycin in the three protocols shown in FIG. 6A. Aliquots were taken at the indicated times and reactions were stopped with loading buffer. Tdp1 activity was calculated as the percentage of D14Y converted to D14P.

Example 3

Overcoming Inhibition of Tdp1

This example demonstrates that inhibition of Tdp1 by the exemplary ribosome inhibitor neomycin can be overcome by increasing Tdp1.

Overcoming inhibition of Tdp1 by neomycin by increasing Tdp1 was examined, using 20 µl reaction mixtures that contained 0.025 µM D14Y and increasing amounts of Tdp1 (0.5-8 ng) in the absence (open circles in FIG. 7B) or presence of 10 mM neomycin (filled circles in FIG. 7B). Reactions were at 25°C for 20 min and then stopped by adding loading buffer. Inhibition of Tdp1 by neomycin can be overcome by increasing Tdp1 (FIG. 7A). FIG. 7B is a graphical representation of Tdp1 inhibition by neomycin (filled circles) corresponding to the autoradiograph of the representative gel shown in FIG. 7A. Tdp1 activity was calculated as the percentage of D14Y converted to D14P.

Overcoming inhibition of Tdp1 by neomycin by increasing D14Y was examined, using 20 µl reaction mixtures contained 1 ng Tdp1 and increasing amounts of D14Y (0.025-6.4 µM) without (open circles in FIG. 7D) or with 10 mM neomycin (filled circles in FIG. 7D). Reactions were at 25°C for 20 min and then stopped by

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adding loading buffer. Inhibition of Tdp1 by neomycin cannot be overcome by increasing D14Y (FIG. 7C). FIG. 7D is graphical representation of Tdp1 inhibition by neomycin (filled circles) corresponding to the autoradiograph of the representative gel shown in FIG. 7C. Tdp1 activity was calculated as the percentage of D14Y converted to D14P.

To examine the competition of the inhibition, 20 μ l reaction mixtures containing 2 ng Tdp1 and increasing concentrations of D14Y (0.025, 0.1, 0.4, 1.6 and 6.4 μ M) in the absence (circles in FIG. 8) or presence (1 mM, triangles in FIG. 8; 2 mM, diamonds in FIG. 8) of neomycin were prepared. Reactions were incubated at 25°C for 1 min and stopped with loading buffer. Velocity (V) was calculated as pmol D14P produced (FIG. 8).

Example 4

Identification of Ribosome Inhibitors That Inhibit Tdp Activity

This example illustrates a method for identifying additional ribosome inhibitors that inhibit Tdp activity.

Based on the principals and procedures described in the present application, additional ribosome inhibitors that inhibit Tdp activity can be identified. Inhibition of Tdp activity by ribosome inhibitors can be determined using a variety of methods. For example, the Tdp biochemical assay provided herein in Example 1 can be used to assay Tdp activity and the inhibition of Tdp activity by ribosome inhibitors. Tdp activity/inhibition can also be assayed using substrates consisting of nucleopeptides or DNA with a 3'-phosphodiester linkage to a tyrosyl, paranitrophenol or coumarin substituent. Tdp activity is followed as the release of the tyrosyl, paranitrophenol or coumarin substituent from the DNA. As understood by those of skill in the art, assay methods for determining the ability of a compound to inhibit Tdp activity generally require comparison to a control. An exemplary control is an isolated Tdp preparation or biological sample with Tdp activity that is treated substantially the same as the test

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preparation or sample exposed to a ribosome inhibitor, except that the control is not exposed to the ribosome inhibitor.

Toxicity of ribosome inhibitors that inhibit Tdp activity, can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, for example, by determining the MIC₅₀ (the lowest tested concentration that inhibits the growth of the population by at least 50%), LD₅₀ (the dose lethal to 50% of the population) and/or the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it may be expressed, for example, as the ratio LD₅₀/ED₅₀. Ribosome inhibitors that inhibit Tdp activity that exhibit large therapeutic indices are useful, as are those that exhibit toxic side effects. However, in the case of ribosome inhibitors that inhibit Tdp activity with toxic side effects, it can be helpful to design a delivery system that targets such inhibitors to the site of affliction to minimize potential damage to normal cells and, thereby, reduce side effects.

Example 5

Ribosome Inhibitors That Inhibit Tdp Activity Enhance the Antineoplastic Effects of DNA-Damaging Therapies

This example illustrates the ability of ribosome inhibitors that inhibit Tdp activity to enhance the antineoplastic effects of DNA-damaging therapies.

Tdp1 genetic deficiency sensitizes cells to DNA damage induced by DNA-damaging therapies used to treat tumors (for example, TopI inhibitors, TopII inhibitors and ionizing radiation) (El-Khamisy *et al.*, *Nature* 434:108-13, 2005; Barthelmes *et al.*, *J. Biol. Chem.* 279:55618-625, 2004; Zhou *et al.*, *Nucleic Acids Res.* 33:289-97, 2005). Potentiation by Tdp inhibitors can be expected to be greater in cells with cell checkpoint deficiencies (Pouliot *et al.*, *Science* 286:552-55, 1999; Pommier *et al.*, *Mutat. Res.* 532:173-203, 2003). Since tumors are characterized by cell cycle checkpoint deficiencies (Pommier *et al.*, *Mutat. Res.* 532:173-203, 2003), Tdp inhibitors are expected to increase the therapeutic index of DNA-damaging therapies.

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The dosage regimes for combining ribosome inhibitors that inhibit Tdp activity with DNA-damaging therapies can be tailored to a subject's conditions and response in a manner that is conventional for any antitumor therapy, and can be adjusted in response to changes in conditions. Those of ordinary skill in the art will know how to
5 tailor such dosage regimes.

While this disclosure has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiments may be used and it is intended that the disclosure may be
10 practiced otherwise than as specifically described herein. Accordingly, this disclosure includes all modifications encompassed within the spirit and scope of the disclosure as defined by the claims below.

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CLAIMS

We claim:

- 5 1. A method for enhancing an antineoplastic effect of a DNA-damaging therapy, comprising administering to a subject having a neoplasm a therapeutically effective amount of the DNA-damaging therapy and a ribosome inhibitor that inhibits tyrosyl-DNA-phosphodiesterase (Tdp) activity, wherein the ribosome inhibitor is administered in a sufficient amount to enhance the DNA-damaging therapy.
- 10 2. The method of claim 1, wherein the DNA-damaging therapy is a therapy that induces DNA damage that is at least partially repaired by Tdp.
3. The method of claim 1, wherein the neoplasm is resistant to the DNA-
15 damaging therapy.
4. The method of claim 3, wherein the resistance is reduced or absent responsiveness of the neoplasm to the DNA-damaging therapy which has developed following treatment with the DNA-damaging therapy.
- 20 5. The method of claim 1, wherein the therapeutically effective amount of the DNA-damaging therapy in the presence of the ribosome inhibitor is lower than when the DNA-damaging therapy is administered to the subject alone.
- 25 6. The method of claim 1, wherein the neoplasm is a malignant neoplasm, and wherein the DNA-damaging therapy is a therapy effective against a malignant neoplasm.

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7. The method of claim 1, wherein the DNA-damaging therapy comprises radiotherapy or chemotherapy.

8. The method of claim 1, wherein the DNA-damaging therapy is
5 chemotherapy.

9. The method of claim 8, wherein the chemotherapy comprises administration of a radiomimetic agent, a topoisomerase I (TopI) inhibitor, a topoisomerase II (TopII) inhibitor, or a DNA-interacting agent.
10

10. The method of claim 1, wherein the ribosome inhibitor is selected from the group consisting of an aminoglycoside, a tetracycline, a macrolide, a lincosamide, a streptogramin, an oxazolidinone, spectinomycin, thiostrepton, puromycin, and combinations thereof.
15

11. The method of claim 1, wherein the ribosome inhibitor comprises an aminoglycoside.

12. The method of claim 11, wherein the aminoglycoside is neomycin.
20

13. The method of claim 1, wherein the ribosome inhibitor is administered prior to administration of the DNA-damaging therapy.

14. The method of claim 1, wherein the ribosome inhibitor is administered
25 simultaneously with the administration of the DNA-damaging therapy.

15. A method for treating a malignant neoplasm, comprising administering to a subject having a malignant neoplasm a therapeutically effective amount of a topoisomerase I (TopI) inhibitor and a therapeutically effective amount of a ribosome

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inhibitor that inhibits tyrosyl-DNA-phosphodiesterase (Tdp) activity, thereby treating the malignant neoplasm in the subject.

16. The method of claim 15, wherein the TopI inhibitor comprises a
5 camptothecin and the ribosome inhibitor comprises an aminoglycoside.

17. The method of claim 16, wherein the camptothecin comprises topotecan or irinotecan and the aminoglycoside is neomycin.

10 18. A pharmaceutical composition, comprising a chemotherapeutic agent and a ribosome inhibitor that inhibits tyrosyl-DNA-phosphodiesterase (Tdp) activity, wherein the chemotherapeutic agent and the ribosome inhibitor are present in a therapeutically effective amount for the ribosome inhibitor to enhance an antineoplastic effect of the chemotherapeutic agent.

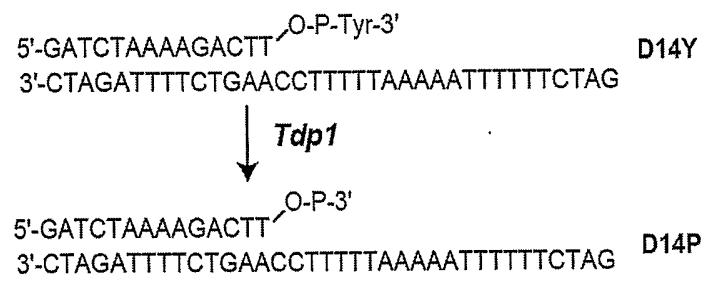
15

19. The pharmaceutical composition of claim 18, wherein the chemotherapeutic agent is a topoisomerase I (TopI) inhibitor and the ribosome inhibitor is an aminoglycoside.

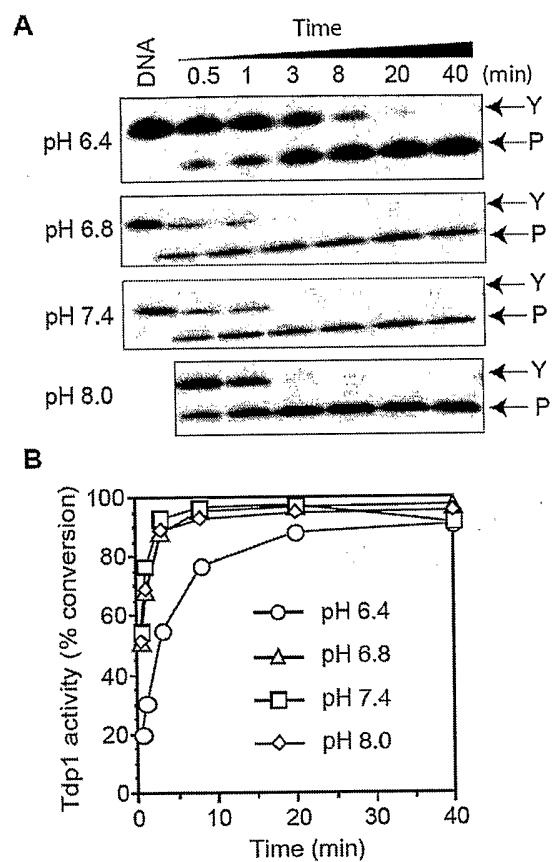
20 20. A method for inhibiting tyrosyl-DNA-phosphodiesterase (Tdp) activity in a biological sample with Tdp activity, comprising contacting the sample with a ribosome inhibitor, thereby inhibiting Tdp activity.

21. The method of claim 20, wherein the ribosome inhibitor is selected from
25 the group consisting of an aminoglycoside, a tetracycline, a macrolide, a lincosamide, a streptogramin, an oxazolidinone, spectinomycin, thiostrepton, puromycin, and combinations thereof.

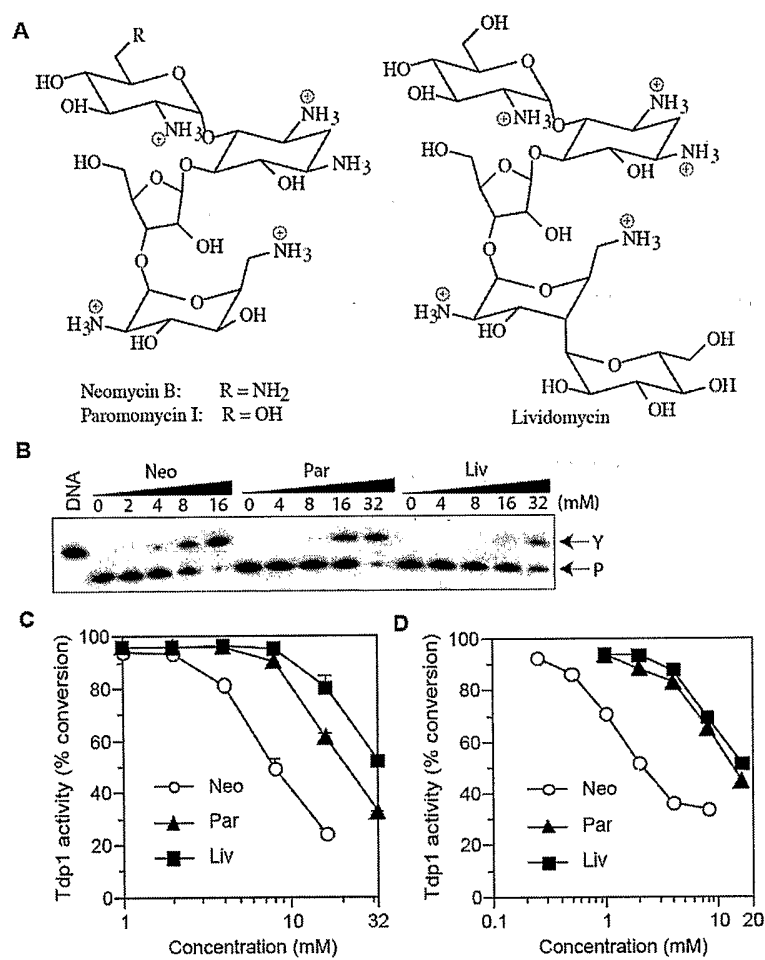
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**Figure 1**

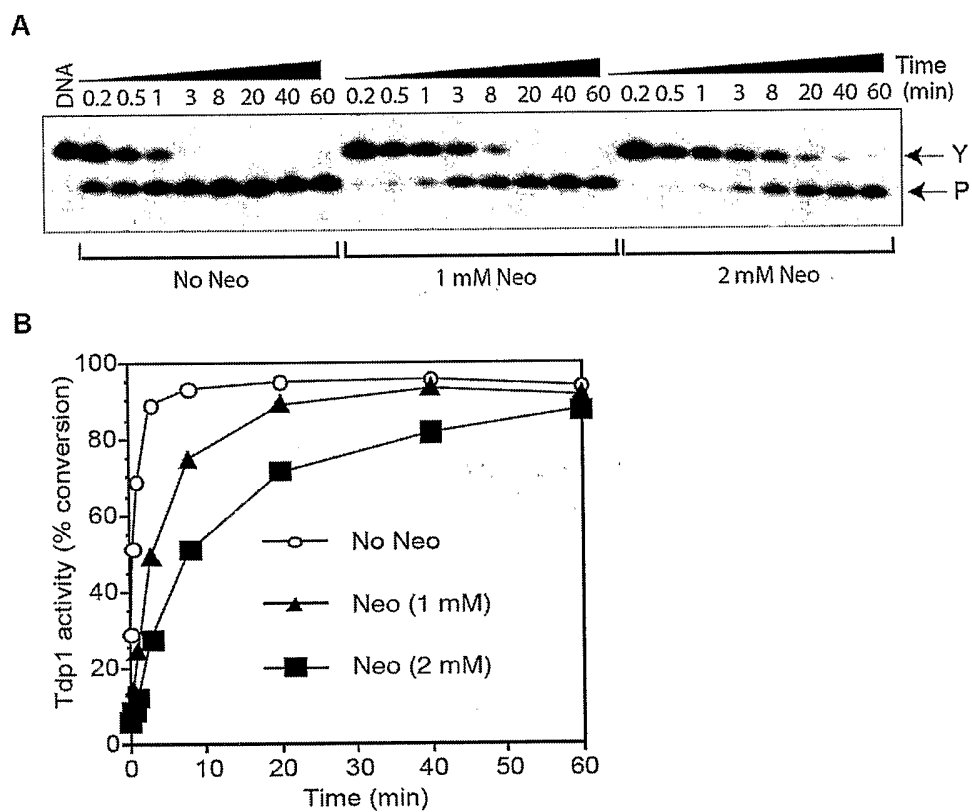
2/10

**Figure 2**

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**Figure 3**

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**Figure 4**

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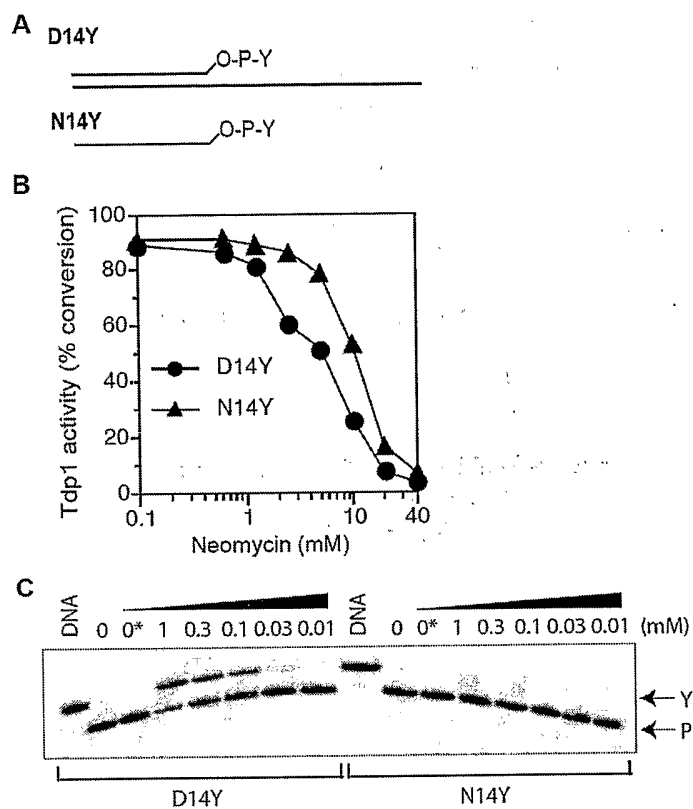


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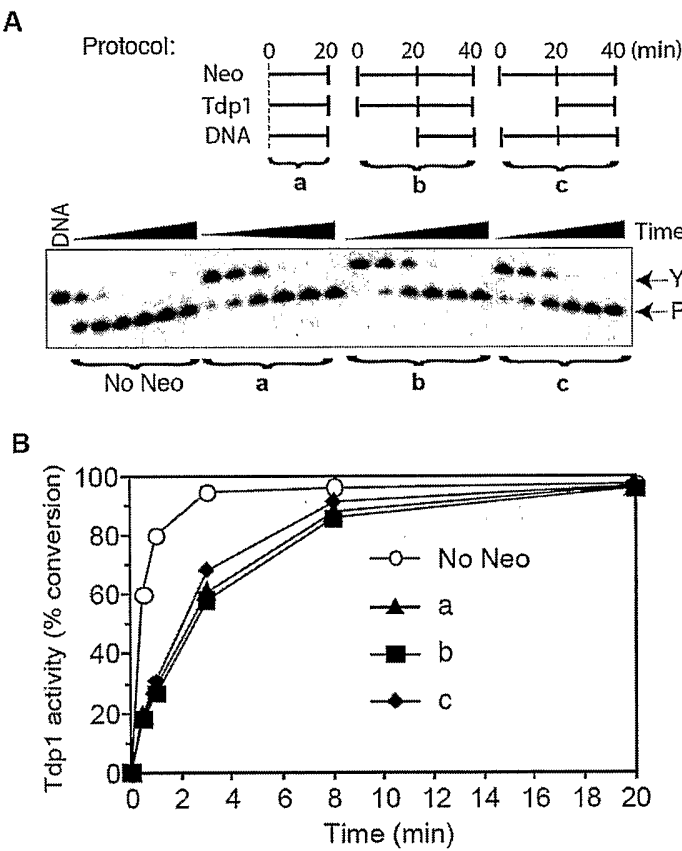


Figure 6

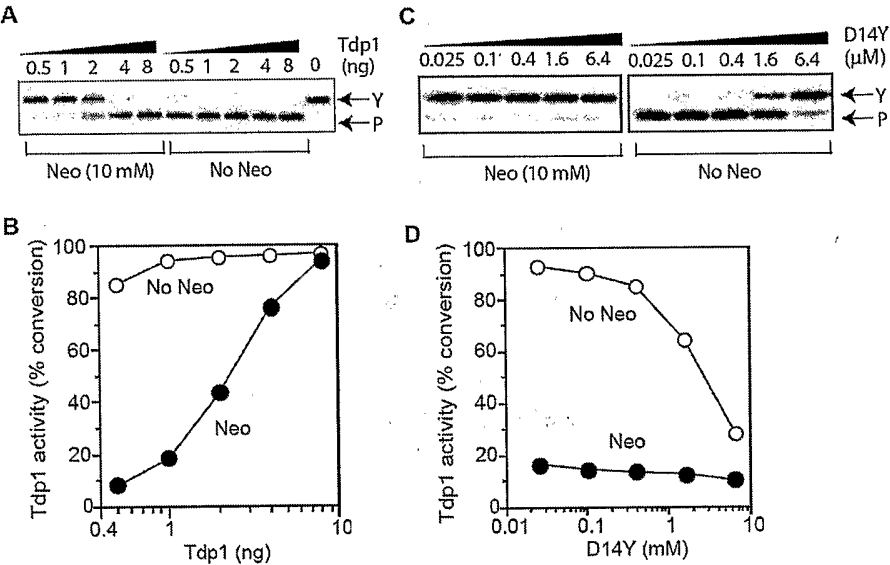
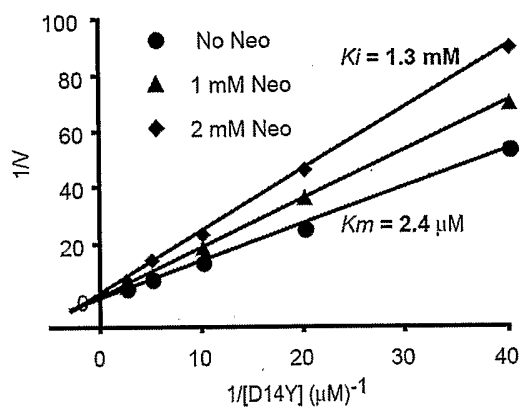


Figure 7

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**Figure 8**

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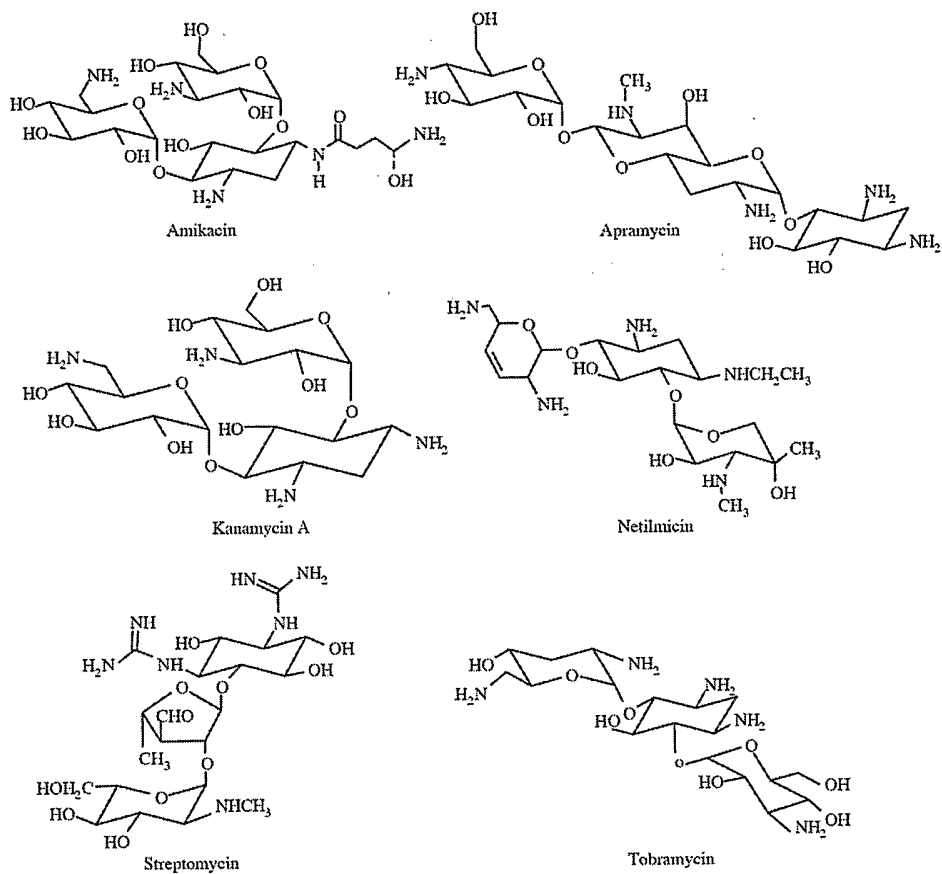
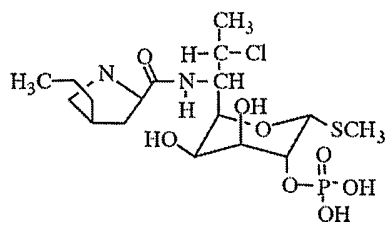
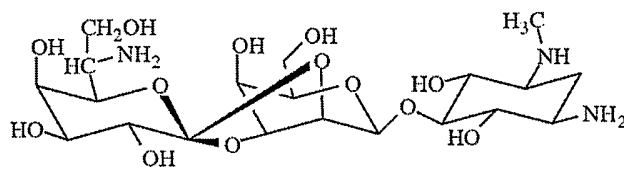


Figure 9

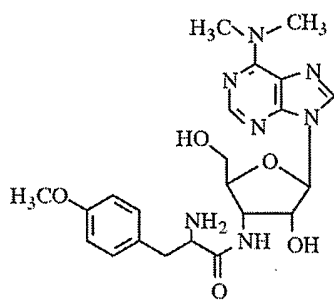
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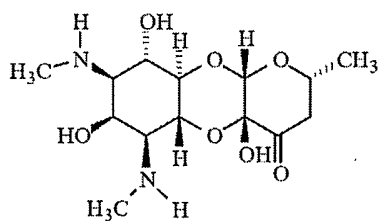
Clindamycin 2-phosphate



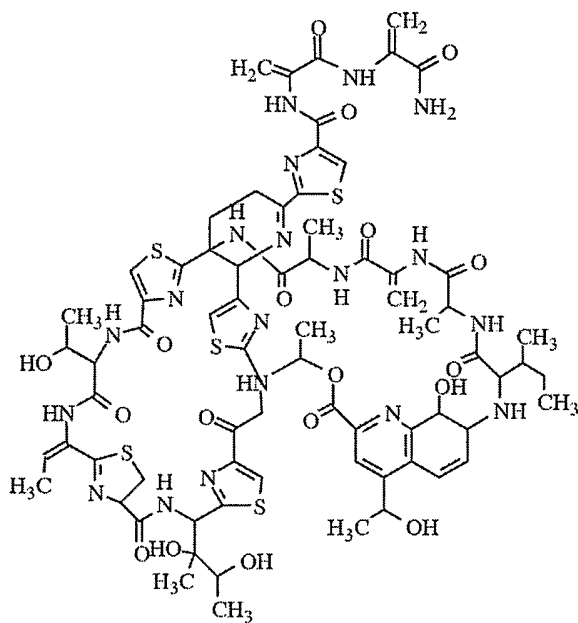
Hygromycin B



Puromycin



Spectinomycin



Thiostrepton

Figure 10