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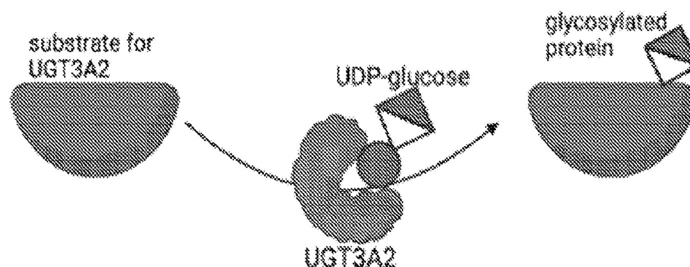
(54) Title: NEOPLASTIC CYTOTOXICITY INDUCED BY GLYCOSYLATION

FIG. 1



(57) Abstract: The disclosure provides agents that sensitize neoplastic cells through glycosylation by a glycosyltransferase. In the mechanism of cytotoxicity discussed herein, compounds become glycosylated resulting in comitant cytotoxicity. Exemplary compounds include: Compound 1 (BRD9645), Compound 2 ((R112), Compound 3 (Tioxolone), Compound 4 (Baf A1).

NEOPLASTIC CYTOTOXICITY INDUCED BY GLYCOSYLATION

CROSS REFERENCE TO RELEATED APPLICATIONS

The present application claims the benefit of and priority to U.S. App. No. 63/521,286, filed June 15, 2023, which is hereby incorporated by reference in its entirety.

5

BACKGROUND

Cancer kills over 550,000 people in the United States and over 8 million people world-wide each year. New agents, including small molecules, molecules that impact tissue-specific growth requirements, and immunomodulatory agents, have been shown to benefit a subset of patients whose cancers have unique genomic mutations or other characteristics. 10 Unfortunately, many cancer patients are still left without effective therapeutic options. Some therapeutic options target specific proteins such as enzymes and enzymes to help confer cytotoxicity.

Glycosyltransferases (GTFs, Gtfs) are enzymes that establish natural glycosidic linkages. They catalyze the transfer of saccharide moieties from an activated nucleotide sugar 15 (also known as the "glycosyl donor") to a nucleophilic glycosyl acceptor molecule, the nucleophile of which can be oxygen-, carbon-,nitrogen-, or sulfur-based. UDP-glycosyltransferases (UGTs) are important conjugation enzymes found in all kingdoms of life, catalyzing a sugar conjugation with small lipophilic compounds and playing a crucial role in detoxification and homeostasis. Glycosyltransferases are not implicated in apoptosis 20 or cytotoxicity.

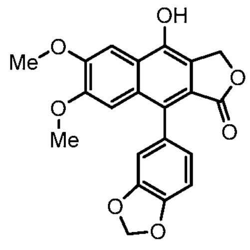
SUMMARY

In accordance with the foregoing objectives and others, the present disclosure provides compounds that are converted into active compounds by the presence of a glycosyltransferase within a cell. The present disclosure is partially based on the discovery 25 that glycosyltransferases are able to glycosylate certain compounds to induce cytotoxicity of neoplastic cells. Glycosyltransferases such as UGT3A2 are often tissue specific, therefore only relegating the active compounds to tissue specific neoplasias that are characterized by expression of the glycosyltransferase. These neoplasia include Ewing sarcoma, rhabdomyosarcoma, and various lymphoid cancers.

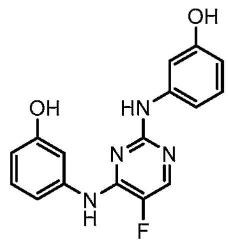
30 Methods are provided for treating a selected subject having a neoplasia, the method comprising administering to the subject a compound that is a substrate for a

glycosyltransferase and forms a glycosylated compound upon interaction with the glycosyltransferase,

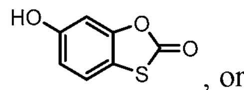
wherein the subject is or was selected by characterizing the neoplasia for the presence of the glycosyltransferase. In some embodiments, the compound comprises an aromatic ring substituted with a hydroxyl or an amide group. In some embodiments, the compound is not doxorubicin or etoposide. For example, the compound may have the structure:



Compound 1
BRD9645

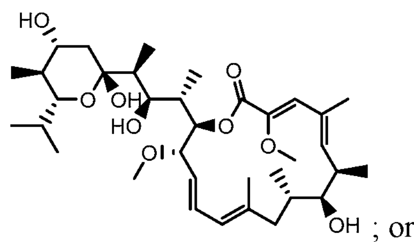


Compound 2
(R112)



, or

Compound 3
(Tioxolone)



; or

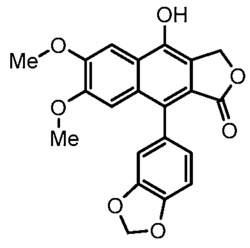
Compound 4
(Baf A1)

a pharmaceutically acceptable salt thereof.

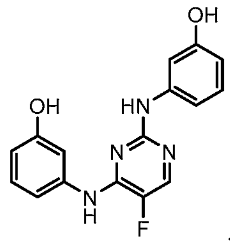
The disclosure also includes pharmaceutical compositions for the treatment of a neoplasia characterized by the presence of the glycosyltransferase;

wherein the compound is a substrate for a glycosyltransferase and forms a glycosylated compound upon interaction with the glycosyltransferase. In some embodiments, the compound comprises an aromatic ring substituted with a hydroxyl or an amide group. In

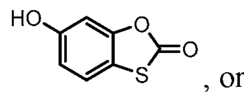
some embodiments, the compound is not doxorubicin or etoposide. For example, the compound may have the structure:



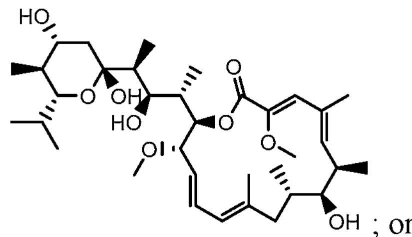
, Compound 1 BRD9645



, Compound 2 (R112)



, or Compound 3 (Tioxolone)

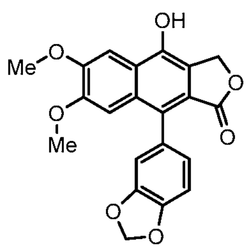


; or Compound 4 (Baf A1)

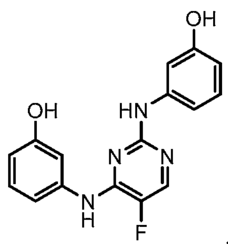
a pharmaceutically acceptable salt thereof.

A method of killing a neoplastic cell are also provided comprising contacting the neoplastic cell with a compound that is a substrate for a glycosyltransferase and forms a glycosylated compound upon interaction with the glycosyltransferase,

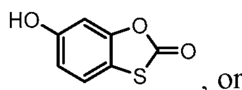
wherein the neoplastic cell is or was selected by characterizing the neoplastic cell for the presence of the glycosyltransferase. In some embodiments, the compound comprises an aromatic ring substituted with a hydroxyl or an amide group. In some embodiments, the compound is not doxorubicin or etoposide. For example, the compound may have the structure:



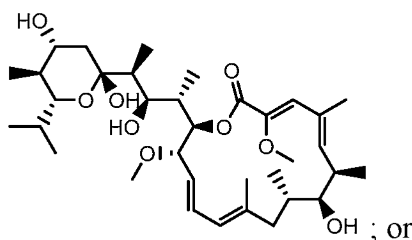
Compound 1
BRD9645



Compound 2
(R112)



Compound 3
(Tioxolone)



Compound 4
(Baf A1)

a pharmaceutically acceptable salt thereof.

The compound or the glycosylated compound may be a substrate for a second protein target expressed by the neoplasia. In some embodiments, the glycosylated compound is a substrate for a second protein target expressed by the neoplasia. In various implementations, the compound or glycosylated compound is a substrate for a second protein target expressed by the neoplasia and the subject is or was selected for characterizing the neoplasia for the presence of both the glycosyltransferase and the second protein target. For example, the second protein target may be an enzyme such as v-ATPase or a H^{+}-ATPase or Carbonic anhydrase. In certain implementations, the enzyme is a kinase.

The UGT gene family is defined by a signature motif in the C-terminal domain where the uridine diphosphate (UDP)-sugar donor binds. Mammals use only 9 sugar nucleotide donors for glycosyltransferases: UDP-glucose, UDP-galactose, UDP-GlcNAc, UDP-GalNAc,

UDP-xylose, UDP-glucuronic acid, GDP-mannose, GDP-fucose, and CMP-sialic acid. The phosphate(s) of these donor molecules are usually coordinated by divalent cations such as manganese, however metal independent enzymes exist. The glycosyltransferase may be a member of the UGT gene family or the SLC35B4 gene (or a protein or polypeptide expressed therefrom). In some embodiments, the glycosyltransferase is a UDP Glycosyltrasferase protein (*e.g.*, UDP glycosyltransferase family 3 member A1, UDP glycosyltransferase family 3 member A2). In particular embodiments, the glycosyltransferase is UDP glycosyltransferase family 3 member A2, UDP-xylose, or UDP N-acetylglucosamine transporter.

These compounds have activity in neoplasias expressing the glycosyltransferase. In various embodiments, the neoplasia is cancer. For example, the cancer may be cancer of the skin, bone, adrenal glands, kidney, spleen, or testicals. In some embodiments, the cancer is Ewing's sarcoma or rhabdomyosarcoma.

Definitions

All terms used herein are intended to have their ordinary meaning in the art unless otherwise provided. All concentrations are in terms of percentage by weight of the specified component relative to the entire weight of the topical composition, unless otherwise defined.

As used herein, "a" or "an" shall mean one or more. As used herein when used in conjunction with the word "comprising," the words "a" or "an" mean one or more than one. As used herein "another" means at least a second or more.

As used herein, all ranges of numeric values include the endpoints and all possible values disclosed between the disclosed values. The exact values of all half-integral numeric values are also contemplated as specifically disclosed and as limits for all subsets of the disclosed range. For example, a range of from 0.1% to 3% specifically discloses a percentage of 0.1%, 1%, 1.5%, 2.0%, 2.5%, and 3%. Additionally, a range of 0.1 to 3% includes subsets of the original range including from 0.5% to 2.5%, from 1% to 3%, or from 0.1% to 2.5%. It will be understood that the sum of all weight % of individual components will not exceed 100%.

Throughout this description, various components may be identified having specific values or parameters, however, these items are provided as exemplary embodiments. Indeed, the exemplary embodiments do not limit the various aspects and concepts of the present

disclosure as many comparable parameters, sizes, ranges, and/or values may be implemented. Unless otherwise specified, the terms "first," "second," and the like, "primary," "secondary," and the like, do not denote any order, quantity, or importance, but rather are used to distinguish one element from another.

5 It will be understood that in the event of any discrepancy between a chemical structure, chemical name, common name (*e.g.*, Baf A3) or other nomenclature, both compounds will be considered to be explicitly disclosed in the application.

By "agent" is meant a small compound, polypeptide or polynucleotide.

By "ameliorate" is meant decrease, suppress, attenuate, diminish, arrest, or stabilize 10 the development or progression of a disease.

By "alteration" is meant a change (increase or decrease) in the expression levels or activity of a gene or polypeptide as detected by standard art known methods such as those described herein. As used herein, an alteration includes a 10% change in expression levels, such as a 25% change, or a 40% change, or a 50% or greater change in expression levels.

15 By "consist essentially" it is meant that the ingredients include only the listed components along with the normal impurities present in commercial materials and with any other additives present at levels which do not affect the operation of the disclosure, for instance at levels less than 5% by weight or less than 1% or even 0.5% by weight.

By "disease" is meant any condition or disorder that damages or interferes with the 20 normal function of a cell, tissue, or organ.

"Detect" refers to identifying the presence, absence or amount of the analyte to be detected.

The term "effective amount" or "therapeutically effective amount" of an agent is meant the amount of an agent (*e.g.*, a compound described herein) required to ameliorate the 25 symptoms of a disease relative to an untreated patient. The effective amount of active compound(s) for therapeutic treatment of a disease varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician will decide the appropriate amount and dosage regimen. Such amount is referred to as an "effective" amount. Agents described herein include compounds that are a 30 substrate for a glycosyltransferase such as Compounds 1-4. In some embodiments, the compounds are administered in an effective amount for the treatment or prophylaxis of a

disease disorder or condition. In another embodiment, in the context of administering an agent that is an anticancer agent, an effective amount of an agent is, for example, an amount sufficient to achieve alleviation or amelioration or prevention or prophylaxis of one or more symptoms or conditions; diminishment of extent of disease, disorder, or condition associated with cancer; stabilized (i.e., not worsening) state of disease, disorder, or condition; preventing spread of disease, disorder, or condition; delay or slowing the progress of the disease, disorder, or condition; amelioration or palliation of the disease, disorder, or condition (*e.g.*, those associated with cancer); and remission (whether partial or total), whether detectable or undetectable, as compared to the response obtained without administration of the agent. In some embodiments, the antiparasitic agent slows the rate of cancer or decreases the neoplastic cells in a host subject or medium.

The term "pharmaceutical composition," as used herein, represents a composition containing a compound described herein formulated with a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition is manufactured or sold with the approval of a governmental regulatory agency as part of a therapeutic regimen for the treatment of disease in a mammal. Pharmaceutical compositions can be formulated, for example, for oral administration in unit dosage form (*e.g.*, a tablet, capsule, caplet, gel cap); for topical administration (*e.g.*, as a cream, gel, lotion, or ointment); for intravenous administration (*e.g.*, as a sterile solution free of particulate emboli and in a solvent system suitable for intravenous use); or in any other formulation described herein (see below).

As used herein, the phrase "pharmaceutically acceptable" generally safe for ingestion or contact with biologic tissues at the levels employed. Pharmaceutically acceptable is used interchangeably with physiologically compatible. It will be understood that the pharmaceutical compositions of the disclosure include nutraceutical compositions (*e.g.*, dietary supplements) unless otherwise specified.

By "reduces" is meant a negative alteration of at least 10%, 25%, 50%, 75%, or 100%.

By "reference" is meant a standard or control condition such as a healthy control cell.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,

16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

As used herein, the terms "treat," treating," "treatment," and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith. It will be appreciated that, although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated.

By "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline. Typical subjects include any animal (*e.g.*, mammals such as mice, rats, rabbits, non-human primates, and humans). A subject in need thereof is typically a subject for whom it is desirable to treat a disease, disorder, or condition as described herein. For example, a subject in need thereof may seek or be in need of treatment, require treatment, be receiving treatment, may be receiving treatment in the future, or a human or animal that is under care by a trained professional for a particular disease, disorder, or condition.

By "substantially identical" is meant a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any nucleic acid sequence encoding a polypeptide described herein). In some embodiments, such a sequence is at least 60%, or at least 80% or at least 85% or at least 90% or at least 95% or even 99% identical at the amino acid level or nucleic acid to the sequence used for comparison.

Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive. Unless specifically stated or obvious from context, as used herein, the terms "a", "an", and "the" are understood to be singular or plural.

Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic of the glycosylation mechanism thought to be responsible for activity of the compounds of the present disclosure. The compound is a substrate for the glycosyltransferase which becomes glycosylated. Without wishing to be bound by theory, this glycosylation is required for sensitivity of the compounds of the present disclosure.

FIG. 2 provides a schematic of the PRISM screen used to identify Compound 1 with selective activity in Ewing sarcoma (left). FIG. 2 also provides an exemplary measurement result from the PRISM screen illustrating the Log2-Fold-Change (LFC) following 625 nM administration of Compound 1 comparing Ewing Sarcoma (EWS) and other characterized cell lines.

FIG. 3A provides the Pearson Correlation of gene expression and compound sensitivity in EWS cells with CRISPR knockout of the indicated gene. FIG. 3B provides the log fold change (LFC) of sensitivity following knockout of genes and corresponding q-values as determined by differential representation methods for CRISPR knockout of genes.

FIG. 4 provides a measurement illustrating the lysotracker+ punctae per cell for several test conditions including administration of Compound 1.

FIG. 5 is a Western Blot of the CRISPR KO clones taken from pooled CRISPR knockout (KO) of UGT3A2. The arrow indicates the two bands associated with UGT3A2 in each clone.

FIGs. 6A-6C provide the concentration of Compound 1 administered and the corresponding number of Lysotracker spots measured (which is correlated with live cells following administration). FIG. 6A provides sensitivity with A673 Cas9 LacZ (which expressed the glycosyltransferase UGT3A2), FIG. 6B provides sensitivity with UGT3A2 KO clone 1 (which did not express the glycosyltransferase UGT3A2), and FIG. 6C provides sensitivity with UG3A2 (which expressed the glycosyltransferase UGT3A2).

FIGs. 7A-7C provide the results of the cell viability assay for Compound 1 (FIG. 7A), Compound 2 (FIG. 7B), Compound 3 (FIG. 7C) as compared to A673 LacZ cells and the UGT3A2 KO clone 1 (which did not express UGT3A2). FIG. 7D compares the Compound 1 with a glycosylated version thereof.

FIGs. 8A-8B provide the results of cell viability assay on RH30 cells as UGT3A KO RH30 cells for Compound 1 (FIG. 8A) and Compound 2 (FIG. 8B).

FIG. 9A shows the mass spectroscopic analysis (measured by area counts) of media and cell lysate at several time points following administration of Compound 1. At each time point, from left to right, the data shows Compound 1 in Media, Compound 1 in Cell Lysate, Glycosylated product in Media, and Glycosylated Product in Cell Lysate. FIG. 9B compares
5 the concentration of glycosylated product measured at several time points in both WT and KO cells.

DETAILED DESCRIPTION

The present disclosure is partially premised on the identification that glycosylation of certain compounds by glycosyltransferases confers cytotoxic activity to certain compounds.
10 These glycosyltransferases are found in many neoplasms including Ewing's sarcoma, rhabdomyosarcoma, and lymphoid cancers.

In particular, compound administration to neoplasms expressing a UDP Glycosyltransferase protein (*e.g.*, UDP glycosyltransferase family 3 member A1, UDP glycosyltransferase family 3 member A2 (UGT3A2)) results in active glycosylated
15 compounds. These glycosyltransferases often act upstream of or within cellular response and are predicted to be integral component of membrane and active in intracellular membrane-bounded organelle. UGT3A2 in particular is biased expression in skin (RPKM 4.5), bone marrow (RPKM 1.8), adrenal glands, kidney, spleen, and the testis.

A "UGT3A2 polypeptide" is typically a protein or fragment thereof associated with
20 the UDP-glucuronosyltransferase 3A2 protein which is in glycosyltransferase family 1 and related proteins with GTB topology, one of two protein topologies observed for nucleotide-sugar dependent glycosyltransferases. The UGT3A2 polypeptide may haveat least 85% amino acid sequence identity to the sequence provided at NCBI Ref No. XP_011512290 which provides the isoform X1. An exemplary UGT3A2 *Homo sapiens* amino acid sequence
25 is

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  1 magqrvlllv gfllpgvlls eaakiltist vggshyllmd rvsqilqdhg hnvtmlnhkr
 61 gpfmpglsds pasasrypri lylqkycqfg lkdfkkeeks yqviswlape dhqrefkksf
121 dffleetlgg rgkfenllnv leylalqcsh flnrkdimds lknenfdmvi vetfdycpfl
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361 shwpkdvhla anvkivdwlp qsdllahpsi rlfvthggqn simeaiqhgv pmvgiplfgd
421 qpenmvrvea kkfgvsiqlk klkaetlalk mkqimedkry ksaavaasvi lrshplsptq
481 rlvgwidhvl qtggathlkp yvfqqpwheq ylldvfvfll gltlgtlwlc gkllgmavww
541 lrgarkvket
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30
35

By "UGT3A2 polynucleotide" is meant any nucleic acid molecule encoding a UGT3A2 polypeptide or fragment thereof. An exemplary UGT3A2 nucleic acid sequence is provided at NCBI Ref: XM_011513988

```
   1 agtgcctttg gcgcactgag gtgcacaggg tcccttagcc gggcgcaggg cgcgcagccc
  61 aggctgagat ccgcggcttc cgtagaagtg agcatggctg ggcagcgagt gcttcttcta
 121 gtgggcttcc ttctccctgg ggtcctgctc tcagaggctg ccaaaatcct gacaatatct
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1561 ctccagacag gggcgcgac gcacctcaag ccctatgtct ttcagcagcc ctggcatgag
1621 cagtacctgc tcgacgtttt tgtgtttctg ctggggctca ctctggggac tctatggctt
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1741 acataaggcc aggtgcagcc ttggcggggt ctgtttggtg ggcgatgtca ccatttctag
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1861 cttgaagaac aggaaaaatg gccaaaaatc atcctttcca cttgctaatt ttgctacaaa
1921 ttcatcctta ctagctcctg cctgctagca gaattctttc cagtcctctt gtcctccttt
1981 gtttgccatc agcaagggct atgctgtgat tctgtctctg agtgacttgg accactgacc
2041 ctcagatttc cagccttaaa atccaccttc cttctcatgc gcctctccga atcacaccct
2101 gactcttcca gcctccatgt ccagacctag tcagcctctc tcactcctgc ccctactatc
2161 tatcatggaa taacatccaa gaaagacacc ttgcatattc tttcagtttc tgttttgttc
2221 tcccacatat tctcttcaat gctcaggaag cctgccctgt gcttgagagt tcagggccgg
2281 acacaggctc acaggtctcc acattgggtc cctgtctctg gtgcccacag tgagctcctt
2341 cttggctgag caggcttgga gactgtaggt ttccagattt cctgaaaaat aaaagtttac
2401 agcgttatct ctccccaacc tca
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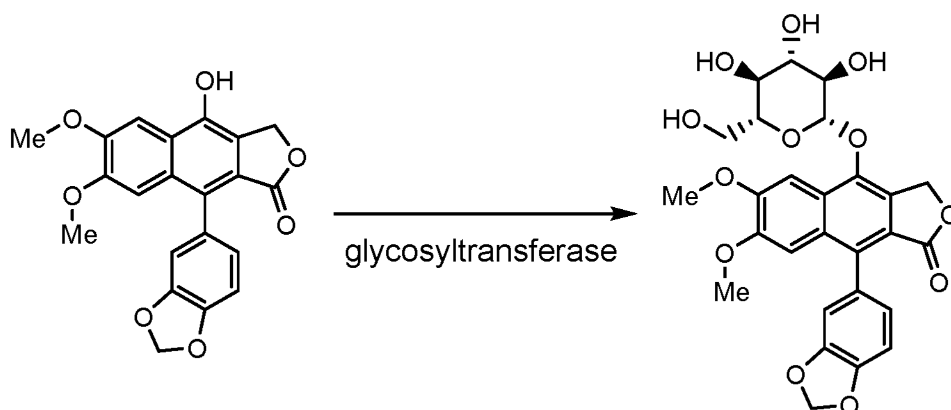
The glycosyltransferase may be a UDP glucuronosyltransferase, a β-1,3-glucuronyltransferase, a β-4-glycosyltransferase, a β-3-glycosyltransferases, a glycosyltransferases group 1 domain protein, a glycosyltransferase family 2 protein, a glycosyltransferasefamily 6 protein, a dolichyl D-mannosylphosphate dependent mannosyltransferases, an exostosin glycosyltransferase family protein, a polypeptide N-acetyl galactosaminyltransferase, a fucosyltransferase, a mannosyl-glycoprotein-N-acetylglucosaminyltransferase, a glycosyltransferase family 8 protein, a glycogen

phosphorylase, a sialyltransferase, a collagen beta(1-O)galactosyltransferase, a UDP-glucose glycoproteinglucosyltransferase, a glucosaminyl (N-acetyl)transferases/xylosyltransferases, an α-1,4-glycosyltransferase, a glycosyltransferase family 90STT3 protein, an oligosaccharyltransferase catalytic subunit, an O-linked N-acetylglucosaminyltransferases, an α-1,3-glucosyltransferases, an α-1,2-glucosyltransferases, a UDP-N-acetylglucosaminyltransferase subunit, a UDP-glucose ceramideglucosyltransferase. In particular embodiments, the glycosyltransferase is a UDP glucouronosyltransferase such asUDP glucuronosyltransferase family 1 member A complex locus (UGT1A), UDP glucuronosyltransferase family 1 member A1 (UGT1A1), UDP glucuronosyltransferase family 1 member A2, pseudogene (UGT1A2P), UDP glucuronosyltransferase family 1 member A3 (UGT1A3), UDP glucuronosyltransferase family 1 member A4 (UGT1A4), UDP glucuronosyltransferase family 1 member A5 (UGT1A5), UDP glucuronosyltransferase family 1 member A6 (UGT1A6), UDP glucuronosyltransferase family 1 member A7 (UGT1A7), UDP glucuronosyltransferase family 1 member A8 (UGT1A8), UDP glucuronosyltransferase family 1 member A9 (UGT1A9), UDP glucuronosyltransferase family 1 member A10 (UGT1A10), UDP glucuronosyltransferase family 1 member A11, pseudogene (UGT1A11P), UDP glucuronosyltransferase family 1 member A12, pseudogene (UGT1A12P), UDP glucuronosyltransferase family 1 member A13, pseudogene (UGT1A13P), UDP glucuronosyltransferase family 2 member A1 complex locus (UGT2A1), UDP glucuronosyltransferase family 2 member A2 (UGT2A2), UDP glucuronosyltransferase family 2 member A3 (UGT2A3), UDP glucuronosyltransferase family 2 member B4 (UGT2B4), UDP glucuronosyltransferase family 2 member B7 (UGT2B7), UDP glucuronosyltransferase family 2 member B10 (UGT2B10), UDP glucuronosyltransferase family 2 member B11 (UGT2B11), UDP glucuronosyltransferase family 2 member B15 (UGT2B15), UDP glucuronosyltransferase family 2 member B17 (UGT2B17), UDP glucuronosyltransferase family 2 member B24, pseudogene (UGT2B24P), UDP glucuronosyltransferase family 2 member B25, pseudogene (UGT2B25P), UDP glucuronosyltransferase family 2 member B26, pseudogene (UGT2B26P), UDP glucuronosyltransferase family 2 member B27, pseudogene (UGT2B27P), UDP glucuronosyltransferase family 2 member B28 (UGT2B28), UDP glucuronosyltransferase family 2 member B29, pseudogene (UGT2B29P), UDP glycosyltransferase family 3 member A1 (UGT3A1), UDP glycosyltransferase family 3 member A2 (UGT3A2), or UDP glycosyltransferase 8 (UGT8). In particular embodiments, the glycosyltransferase is a

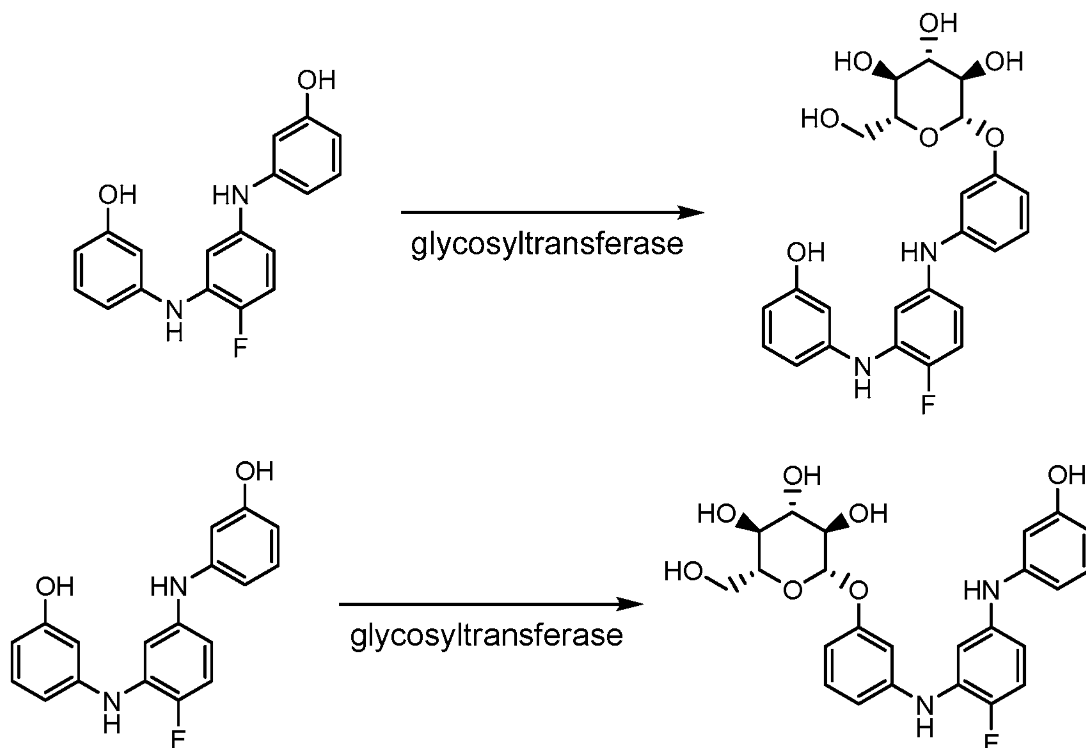
member of UDP glycosyltransferase family 3 such as UDP glycosyltransferase family 3 member A1 (UGT3A1) or UDP glycosyltransferase family 3 member A2 (UGT3A2).

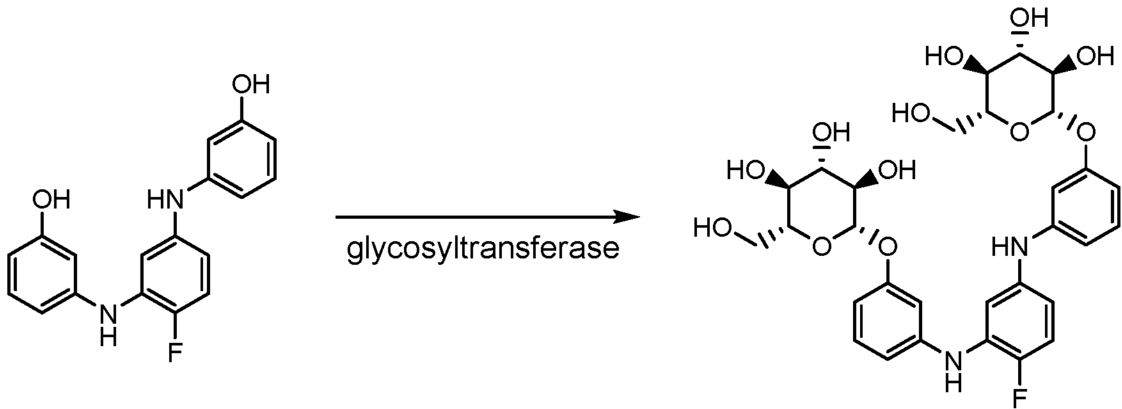
Compound Activity

As shown herein, the compounds of the present disclosure provide activity to
5 neoplasias expressing glycosyltransferases following administration. Without wishing to be bound by theory, the glycosyltransferase glycosylates the compound thereby conferring activity. For example, the glycosyltransferase may convert Compound 1 in the following manner:

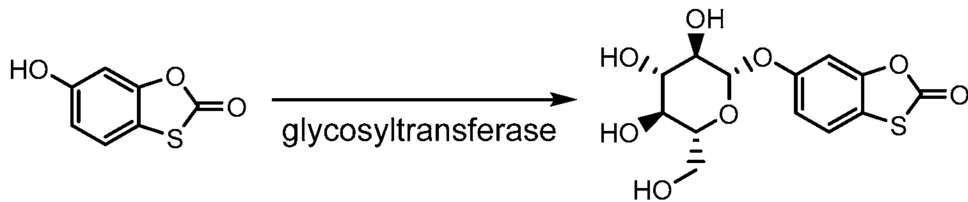


10 The glycosyltransferase may convert Compound 2 (R112) in any of the following manners:

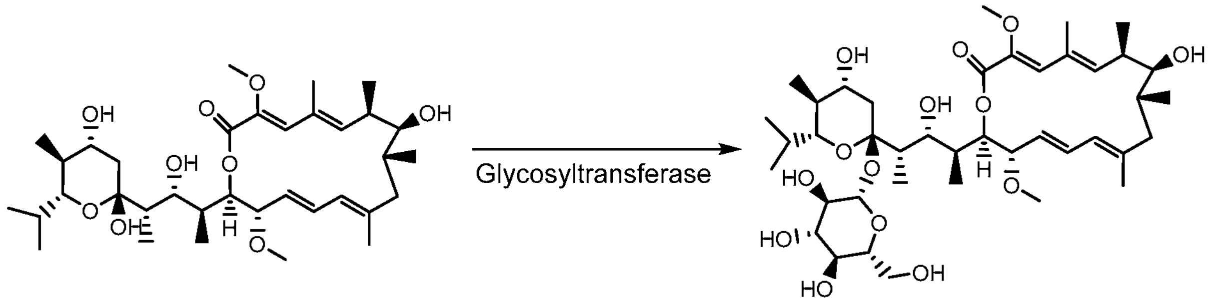




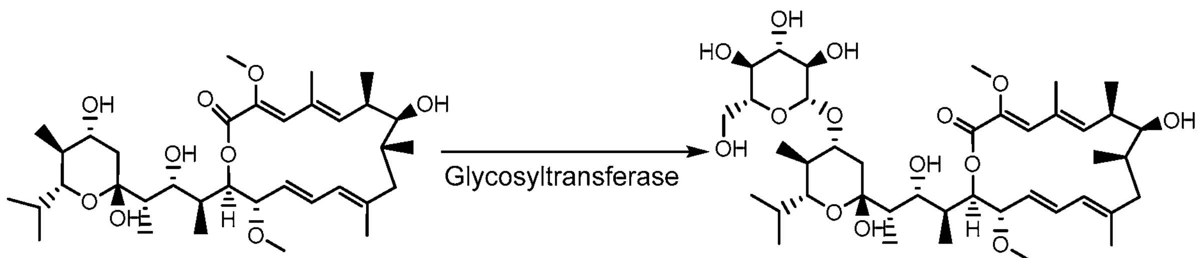
The glycosyltransferase may convert Compound 3 (tioxolone) as follows:

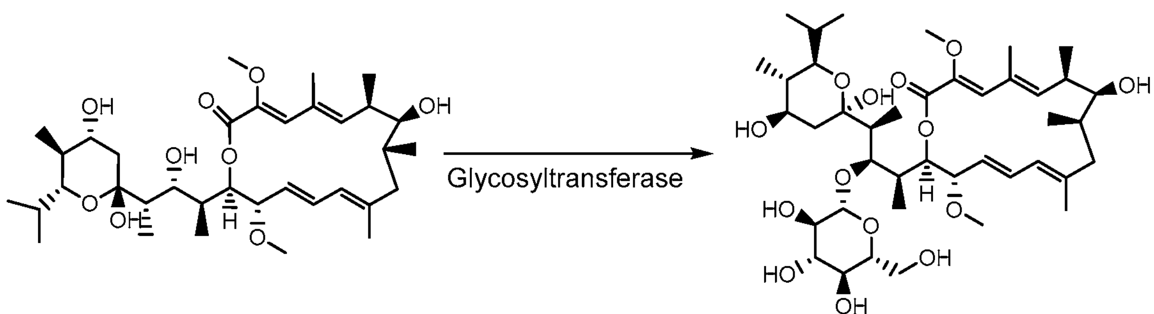


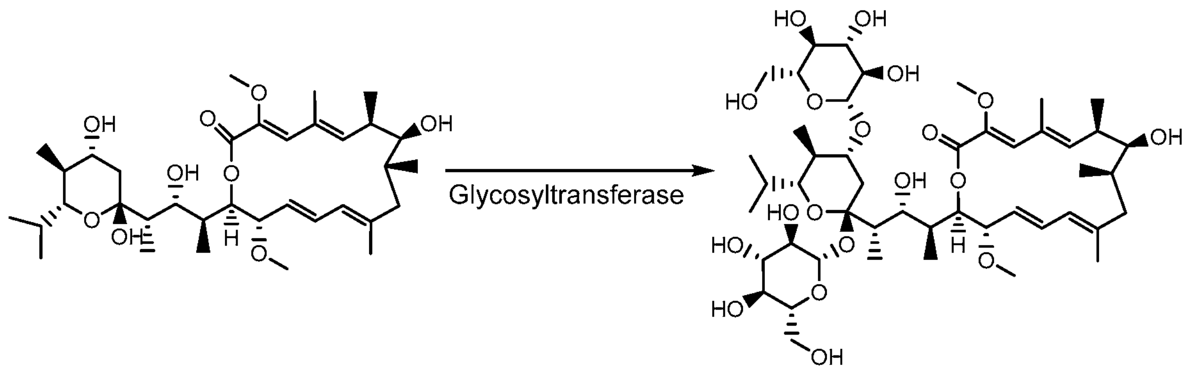
The glycosyltransferase may convert Compound 4 (Bafilomycin A1 or Baf A1) as follows:

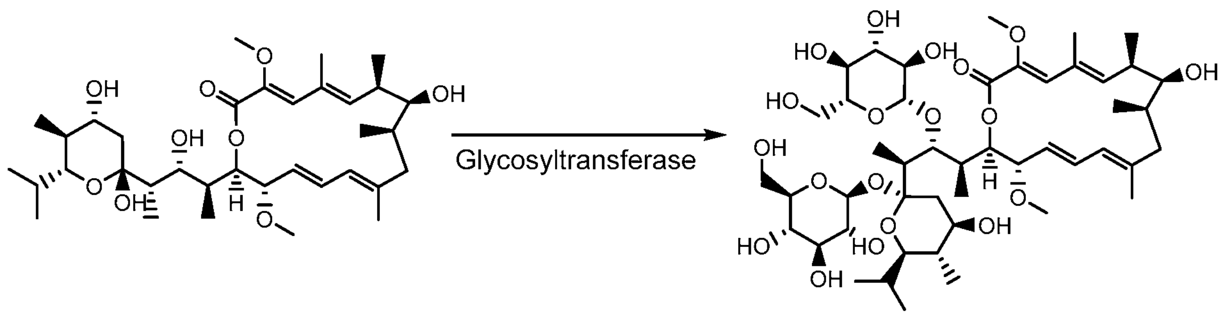


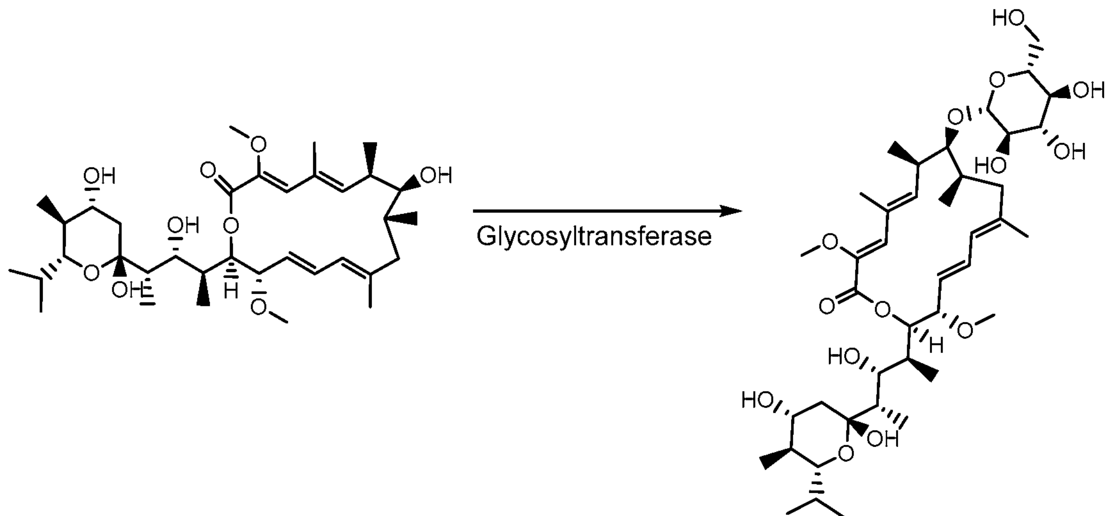
5

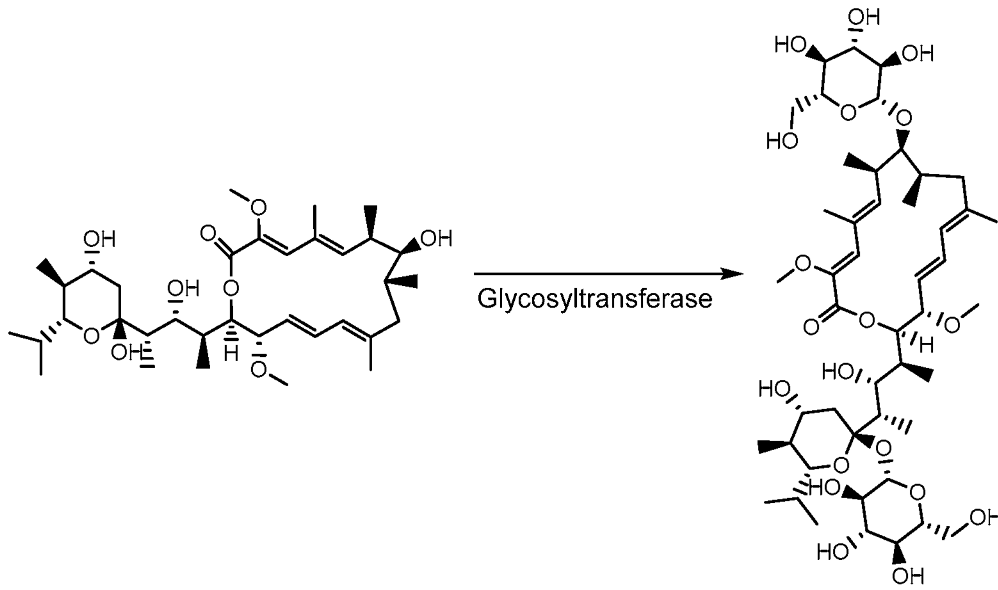


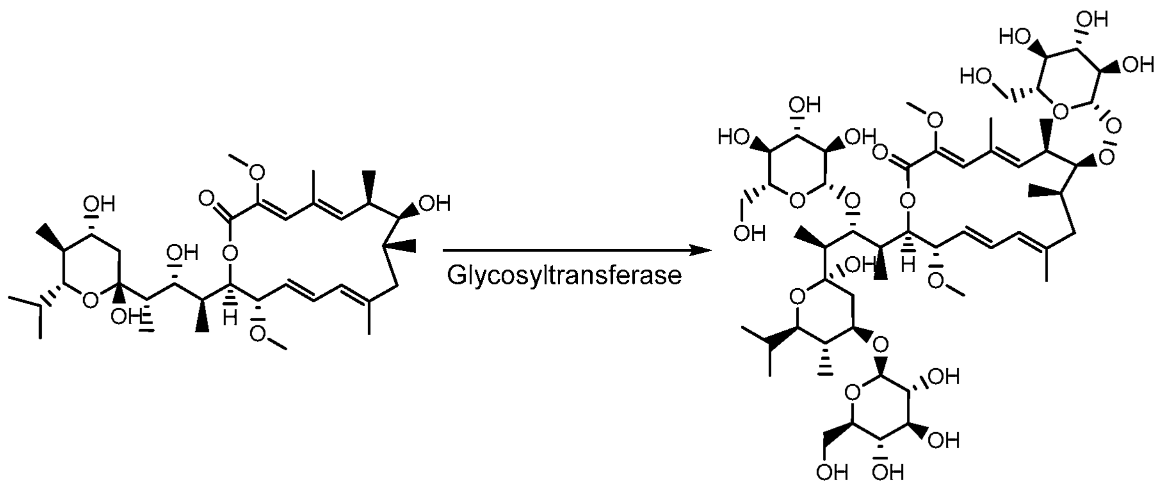


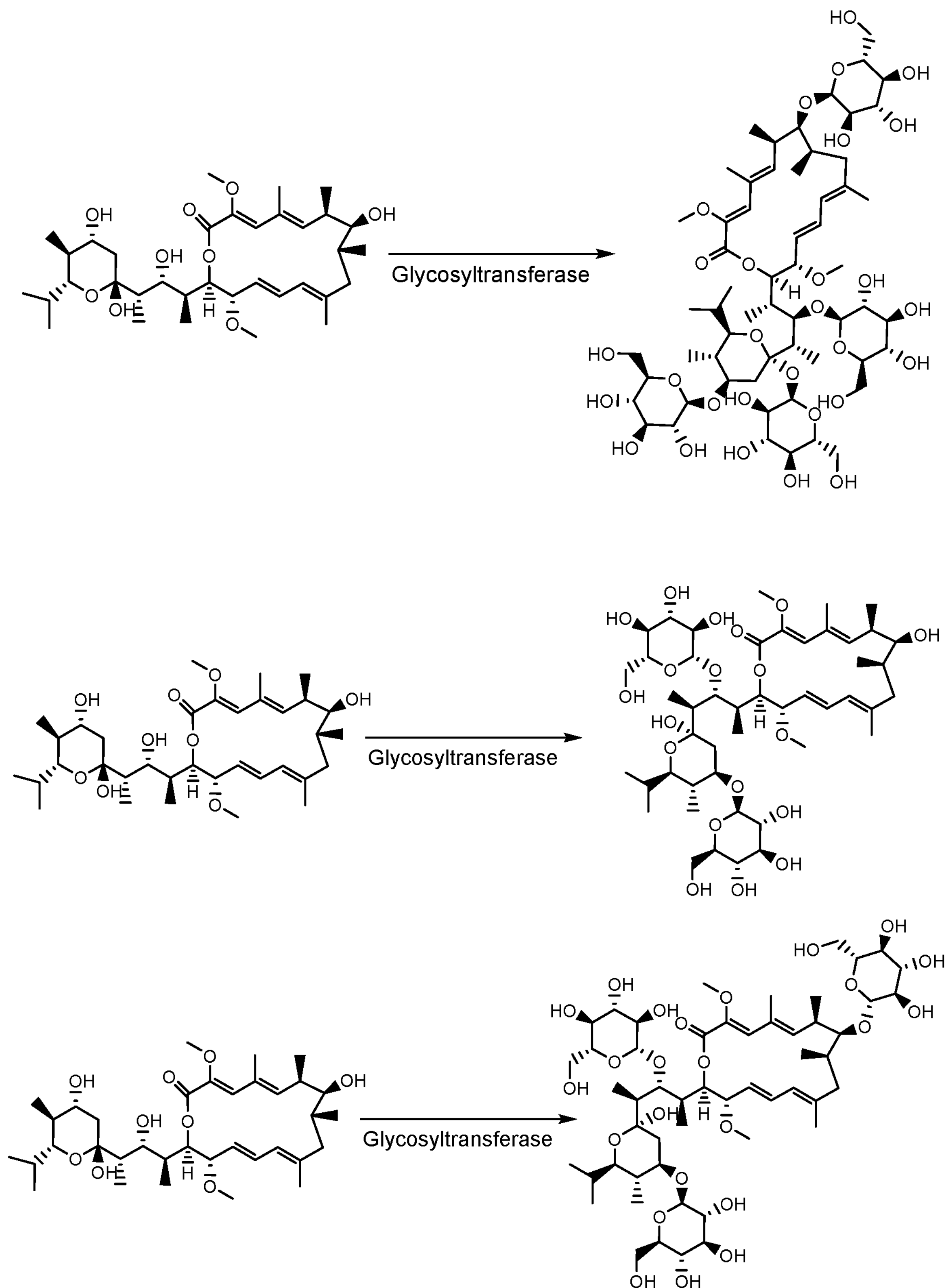

Glycosyltransferase


Glycosyltransferase


Glycosyltransferase


Glycosyltransferase


Glycosyltransferase



The compounds may have an EC_{50} (*e.g.*, as measured by a viability assay) of less than (or 0.1 nM to) 10 µM or less than 1 µM. The glycosylated mechanism is shown in the schematic provided in FIG. 1. The glycosylated compound may then interact with a different protein (*e.g.*, an enzyme, a kinase, v-ATPase, H^+-ATPase, Carbonic anhydrase).

Compounds provided herein can have one or more asymmetric carbon atoms and can exist in the form of optically pure enantiomers, mixtures of enantiomers such as racemates, optically pure diastereoisomers, mixtures of diastereoisomers, diastereoisomeric racemates or mixtures of diastereoisomeric racemates. The optically active forms can be obtained for example by resolution of the racemates, by asymmetric synthesis or asymmetric chromatography (chromatography with a chiral adsorbent or eluant). That is, certain of the disclosed compounds may exist in various stereoisomeric forms including stereoisomers, enantiomers, diastereomers, or racemates (*i.e.*, the compound exists as a mixture containing two enantiomers and does not rotate polarized light). Enantiomers of a compound can be prepared, for example, by separating an enantiomer from a racemate using one or more well-known techniques and methods, such as chiral chromatography and separation methods based thereon.

The compound provided herein may also be present as geometric isomer which differ in the orientation of substituent atoms (*e.g.*, to a carbon-carbon double bond, to a cycloalkyl ring, to a bridged bicyclic system). Atoms (other than H) on each side of a carbon-carbon double bond may be in an E (substituents are on opposite sides of the carbon- carbon double bond) or Z (substituents are oriented on the same side) configuration. "R," "S," "S*," "R*," "E," "Z," "cis," and "trans," indicate configurations relative to the core molecule and may be used to indicate the geometric configuration of the presently disclosed compounds. Certain of the disclosed compounds may exist in atropisomeric forms. Atropisomers are stereoisomers resulting from hindered rotation about single bonds where the steric strain barrier to rotation is high enough to allow for the isolation of the conformers.

The compounds disclosed herein may be prepared as individual isomers by either isomer-specific synthesis or resolved from an isomeric mixture. Conventional resolution techniques include forming the salt of a free base of each isomer of an isomeric pair using an optically active acid (followed by fractional crystallization and regeneration of the free base), forming the salt of the acid form of each isomer of an isomeric pair using an optically active amine (followed by fractional crystallization and regeneration of the free acid), forming an ester or amide of each of the isomers of an isomeric pair using an optically pure acid, amine or alcohol (followed by chromatographic separation and removal of the chiral auxiliary), or resolving an isomeric mixture of either a starting material or a final product using various well known chromatographic methods. When the stereochemistry of a disclosed compound is named or depicted by structure, the named or depicted stereoisomer may be typically more

than 50% (*e.g.*, at least 55%, 60%, 70%, 80%, 90%, 99%, or 99.9%) by weight (or mole fraction) relative to the other stereoisomers. When a single enantiomer is named or depicted by structure, the depicted or named enantiomer is more than 50% (*e.g.*, at least 55%, 60%, 70%, 80%, 90%, 99%, or 99.9%) by weight (or mole fraction) optically pure. When a single diastereomer is named or depicted by structure, the depicted or named diastereomer is more than 50% (*e.g.*, at least 55%, 60%, 70%, 80%, 90%, 99%, or 99.9%) by weight (or mole fraction) pure. Percent optical purity is the ratio of the weight of the enantiomer or over the weight of the enantiomer plus the weight of its optical isomer. Diastereomeric purity by weight is the ratio of the weight of one diastereomer or over the weight of all the diastereomers. Percent purity by mole fraction is the ratio of the moles of the enantiomer or over the moles of the enantiomer plus the moles of its optical isomer. Similarly, percent purity by moles fraction is the ratio of the moles of the diastereomer or over the moles of the diastereomer plus the moles of its isomer. When a disclosed compound is named or depicted by structure without indicating the stereochemistry, and the compound has at least one chiral center, it is to be understood that the name or structure encompasses either enantiomer of the compound free from the corresponding optical isomer, a racemic mixture of the compound or mixtures enriched in one enantiomer relative to its corresponding optical isomer. When a disclosed compound is named or depicted by structure without indicating the stereochemistry and has two or more chiral centers, it is to be understood that the name or structure encompasses a diastereomer free of other diastereomers, a number of diastereomers free from other diastereomeric pairs, mixtures of diastereomers, mixtures of diastereomeric pairs, mixtures of diastereomers in which one diastereomer is enriched relative to the other diastereomer(s) or mixtures of diastereomers in which one or more diastereomer is enriched relative to the other diastereomers. The disclosure embraces all of these forms.

Solvates of the compounds described herein may form the aggregate of the compound or an ion of the compound with one or more solvents. Such solvents may not interfere with the biological activity of the solute. Examples of suitable solvents include, but are not limited to, water, MeOH, EtOH, and AcOH. Solvates wherein water is the solvent molecule are typically referred to as hydrates. Hydrates include compositions containing stoichiometric amounts of water, as well as compositions containing variable amounts of water.

The compounds described herein may be present as a pharmaceutically acceptable salt. Typically, salts are composed of a related number of cations and anions (at least one of

which is formed from the compounds described herein) coupled together (*e.g.*, the pairs may be bonded ionically) such that the salt is electrically neutral. Pharmaceutically acceptable salts may retain or have similar activity to the parent compound (*e.g.*, an ED_{50} within 10%) and have a toxicity profile within a range that affords utility in pharmaceutical compositions. 5 For example, pharmaceutically acceptable salts may be suitable for use in contact with the tissues of humans and animals without undue toxicity, irritation, allergic response and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are described in: Berge et al., *J. Pharmaceutical Sciences* 66:1-19, 1977 and in *Pharmaceutical Salts: Properties, Selection, and Use*, (Eds. P.H. Stahl and C.G. Wermuth), Wiley-VCH, 10 2008. Salts may be prepared from pharmaceutically acceptable non-toxic acids and bases including inorganic and organic acids and bases. Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, dichloroacetate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glutamate, 15 glycerophosphate, hemisulfate, heptonate, hexanoate, hippurate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, isethionate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, mandelate, methanesulfonate, mucate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pantothenate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, 20 succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, and valerate salts. Representative basic salts include alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, and magnesium, aluminum salts, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, 25 triethylamine, caffeine, and ethylamine.

Pharmaceutically acceptable acid addition salts of the disclosure can be formed by the reaction of a compound of the disclosure with an equimolar or excess amount of acid. Alternatively, hemi-salts can be formed by the reaction of a compound of the disclosure with the desired acid in a 2:1 ratio, compound to acid. The reactants are generally combined in a 30 mutual solvent such as diethyl ether, tetrahydrofuran, methanol, ethanol, *iso*-propanol, benzene, or the like. The salts normally precipitate out of solution within, *e.g.*, one hour to ten days and can be isolated by filtration or other conventional methods.

It will be understood that in the event of any inconsistency between a chemical name and formula, both compounds with the indicated chemical name and compounds with the indicated chemical structure will be considered as embraced by the disclosure.

The compounds of the present disclosure include the compounds themselves, as well as their salts and their prodrugs, if applicable. A salt, for example, can be formed between an anion and a positively charged substituent (*e.g.*, amino) on a compound described herein. Suitable anions include chloride, bromide, iodide, sulfate, nitrate, phosphate, citrate, methanesulfonate, trifluoroacetate, and acetate. Likewise, a salt can also be formed between a cation and a negatively charged substituent (*e.g.*, carboxylate) on a compound described herein. Suitable cations include sodium ion, potassium ion, magnesium ion, calcium ion, and an ammonium cation such as tetramethylammonium ion. Examples of prodrugs include C_{1-6} alkyl esters of carboxylic acid groups, which, upon administration to a subject, are capable of providing active compounds that may be glycosylated.

Pharmaceutical Compositions

The compounds described herein (*e.g.*, compounds that are a substrate for a glycosyltransferase such as Compounds 1-4) are useful for the treatment and prophylaxis of neoplasia in a subject in need thereof. The compounds described herein may also be compounds for use in the preparation of a medicament for the treatment of neoplasia in a subject in need thereof. These neoplasias may be characterized for the presence of the glycosyltransferase.

Pharmaceutical dosage forms are provided as well, which may comprise a compound of the present disclosure (*e.g.*, compounds that are a substrate for a glycosyltransferase such as Compounds 1-4) and one or more pharmaceutically acceptable carriers, diluents, or excipients.

Unit dosage forms, also referred to as unitary dosage forms, often denote those forms of medication supplied in a manner that does not require further weighing or measuring to provide the dosage (*e.g.*, tablet, capsule, pellet, caplet). The compositions of the present disclosure may be present as unit dosage forms. For example, a unit dosage form may refer to a physically discrete unit suitable as a unitary dosage for human subjects and other species, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with any suitable pharmaceutical excipient or excipients. Exemplary, non-limiting unit dosage forms include a tablet (*e.g.*, a chewable

tablet), caplet, capsule (*e.g.*, a hard capsule or a soft capsule), lozenge, film, strip, and gel cap. In certain embodiments, the compounds described herein, including crystallized forms, polymorphs, and solvates thereof, may be present in a unit dosage form.

Useful pharmaceutical carriers, excipients, and diluents for the preparation of the compositions hereof, can be solids, liquids, or gases. These include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The pharmaceutically acceptable carrier or excipient does not destroy the pharmacological activity of the disclosed compound and is nontoxic when administered in doses sufficient to deliver a therapeutic amount of the compound. Thus, the compositions can take the form of tablets, pills, capsules, suppositories, powders, enterically coated or other protected formulations (*e.g.*, binding on ion-exchange resins or packaging in lipid-protein vesicles), sustained release formulations, solutions, suspensions, elixirs, and aerosols. The carrier can be selected from the various oils including those of petroleum, animal, vegetable or synthetic origin, *e.g.*, peanut oil, soybean oil, mineral oil, and sesame oil. Water, saline, aqueous dextrose, and glycols are examples of liquid carriers, particularly (when isotonic with the blood) for injectable solutions. For example, formulations for intravenous administration comprise sterile aqueous solutions of the active ingredient(s) which are prepared by dissolving solid active ingredient(s) in water to produce an aqueous solution and rendering the solution sterile. Suitable pharmaceutical excipients include starch, cellulose, chitosan, talc, glucose, lactose, gelatin, malt, rice, flour, chalk, silica, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, and ethanol. The compositions may be subjected to conventional pharmaceutical additives such as preservatives, stabilizing agents, wetting or emulsifying agents, salts for adjusting osmotic pressure, and buffers. Suitable pharmaceutical carriers and their formulation are described in Remington's Pharmaceutical Sciences by E. W. Martin. Such compositions will, in any event, contain an effective amount of the active compound together with a suitable carrier so as to prepare the proper dosage form for administration to the recipient.

Non-limiting examples of pharmaceutically acceptable carriers and excipients include sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and

soybean oil; glycols, such as polyethylene glycol and propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate; coloring agents; releasing agents; coating agents; sweetening, flavoring and perfuming agents; preservatives; antioxidants; ion exchangers; alumina; aluminum stearate; lecithin; self-emulsifying drug delivery systems (SEDDS) such as d-atocopherol polyethyleneglycol 1000 succinate; surfactants used in pharmaceutical dosage forms such as Tweens or other similar polymeric delivery matrices; serum proteins such as human serum albumin; glycine; sorbic acid; potassium sorbate; partial glyceride mixtures of saturated vegetable fatty acids; water, salts or electrolytes such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, and zinc salts; colloidal silica; magnesium trisilicate; polyvinyl pyrrolidone; cellulose-based substances; polyacrylates; waxes; and polyethylene-polyoxypropylene-block polymers. Cyclodextrins such as α-, β-, and γ-cyclodextrin, or chemically modified derivatives such as hydroxyalkylcyclodextrins, including 2-and 3-hydroxypropyl-cyclodextrins, or other solubilized derivatives can also be used to enhance delivery of the compounds described herein.

In various embodiments, the compositions of the disclosure are formulated in pellets or tablets for an oral administration. According to this type of formulation, they comprise lactose monohydrate, cellulose microcrystalline, crospovidone/povidone, aroma, compressible sugar and magnesium stearate as excipients. When the compositions are in the form of pellets or tablets, they are for instance 1 mg, 2 mg, or 4 mg pellets or tablets. Such pellets or tablets are divisible so that they can be cut to suit the posology according to the disclosure in one or two daily takes. In a further embodiment, the compositions of the disclosure are formulated in injectable solutions or suspensions for a parenteral administration. The injectable compositions are produced by mixing therapeutically efficient quantity of torasemide with a pH regulator, a buffer agent, a suspension agent, a solubilization agent, a stabilizer, a tonicity agent and/or a preservative, and by transformation of the mixture into an intravenous, sub-cutaneous, intramuscular injection or perfusion according to a conventional method. Possibly, the injectable compositions may be lyophilized according to a conventional method. Examples of suspension agents include methylcellulose, polysorbate 80, hydroxyethylcellulose, xanthan gum, sodic

carboxymethylcellulose and polyethoxylated sorbitan monolaurate. Examples of solubilization agent include polyoxy ethylene- solidified castor oil, polysorbate 80, nicotinamide, polyethoxylated sorbitan monolaurate, macrogol and ethyl ester of caste oil fatty acid. Moreover, the stabilizer includes sodium sulfite, sodium metalsulfite and ether, while the preservative includes methyl p-hydroxybenzoate, ethyl p- hydroxybenzoate, sorbic acid, phenol, cresol and chlorocresol. An example of tonicity agent is mannitol. When preparing injectable suspensions or solutions, it is desirable to make sure that they are blood isotonic.

In some embodiments, the pharmaceutical composition further comprises a viscosity enhancing agent. In some embodiments, the viscosity enhancing agent includes methylcellulose, hydroxyethylcellulose, hydroxypropylmethylcellulose and smart hydrogel. In some embodiments, the viscosity enhancing agent is hydroxyethylcellulose. In some embodiments, the pharmaceutical composition comprises 0.01-1.0% (w/v) viscosity enhancing agent. In other embodiments, the intranasal pharmaceutical composition comprises 0.05% (w/v) hydroxyethylcellulose.

In some embodiments, the pH of the pharmaceutical composition is from 4.0 to 7.5. In other embodiments, the pH of the pharmaceutical composition is from 4.0 to 6.5. In another embodiment the pharmaceutical composition has a pH of from 5.5 to 6.5. In further embodiments, the pharmaceutical composition has a pH of from 6.0 to 6.5. In various implementations, the pH of said aqueous solution or liquid formulation is from pH 3 to pH 7, from pH 3 to pH 6, from pH 4 to pH 6, or from pH 5 to pH 6. These pH ranges may be achieved through the incorporation of one or more pH modifying agents, buffers, and the like. In some embodiments, a pH modifier such as acetic acid, is present in a final concentration of at least 0.001% or at least 0.01% or between 0.01%-0.2% by weight of the composition.

In terms of their form, compositions of this disclosure may include solutions, emulsions (including microemulsions), suspensions, creams, lotions, gels, powders, or other typical solid or liquid compositions used for application to skin and other tissues where the compositions may be used. Such compositions may contain: additional antimicrobials, moisturizers and hydration agents, penetration agents, preservatives, emulsifiers, natural or synthetic oils, solvents, surfactants, detergents, gelling agents, emollients, antioxidants, fragrances, fillers, thickeners, waxes, odor absorbers, dyestuffs, coloring agents, powders,

viscosity-controlling agents and water, and optionally including anesthetics, anti-itch actives, botanical extracts, conditioning agents, darkening or lightening agents, glitter, humectants, mica, minerals, polyphenols, silicones or derivatives thereof, sunblocks, vitamins, and phytomedicinals. In certain embodiments, the composition of the disclosure is formulated with the above ingredients so as to be stable for a long period of time, as may be beneficial where continual or long-term treatment is intended.

Methods for Neoplasia Treatment and Prophylaxis

Typically, the treatment of a disease, disorder, or condition (*e.g.*, the conditions described herein such as those associated with infection) is an approach for obtaining beneficial or desired results, such as clinical results. Beneficial or desired results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions; diminishment of extent of disease, disorder, or condition; stabilized (i.e., not worsening) state of disease, disorder, or condition; preventing spread of disease, disorder, or condition; delay or slowing the progress of the disease, disorder, or condition; amelioration or palliation of the disease, disorder, or condition; and remission (whether partial or total), whether detectable or undetectable. A disease, disorder, or condition may be palliated which includes that the extent and/or undesirable clinical manifestations of the disease, disorder, or condition are lessened and/or time course of the progression is slowed or lengthened, as compared to the extent or time course in the absence of treatment.

The expression of any biomarker (*e.g.*, a glycosyltransferase such as UGT3A, a second protein target such as an ezyme including kinases or v-ATPases) in the neoplasia may be detected by a method selected from the group consisting of immunoblotting, mass spectrometry, immunoprecipitation quantitative PCR, Northern Blot, microarray, enzyme-linked immunosorbent assay (ELISA), in situ hybridization, and combinations thereof. In certain implementations, expression of a biomarker may be determined by comparison to a reference such as a healthy control cell or a baseline measurement. In certain implementations, expression of a biomarker may be determined by comparison to the reference and the cell is considered to express the biomarker if there is a small difference (*e.g.*, the neoplasia copy number is within 10% of the copy number of the reference, the neoplasia copy number is within 5% of the reference) between expression in the cancer cell and the reference. Genomics may be used to determine expression and relative expression levels. For example, the cell may be considered to not express a biomarker if the number of

copies of the biomarker per cellular genome is less than 1 or less than 2^{-1} or less than 2^{-2} or less than 2^{-3} or less than 2^{-4} or less than 2^{-5}. Conversely, the cell may be considered to express a biomarker if the number of copies of the biomarker per cellular genome is greater than 1 or greater than 2^{-1} or greater than 2^{-2} or greater than 2^{-3} or greater than 2^{-4} or greater than 2^{-5}. In some embodiments, the method involves characterizing the neoplasia as expressing any indicated biomarker (*e.g.*, prior to administration of the compounds of the present disclosure) by identifying the cancer as having increased expression of any indicated biomarker. In some embodiments, the method comprises administering the compounds of the present disclosure. For example, the method may comprise characterizing the neoplasia in a subject in need thereof as expressing a glycosyltransferase and administering the compounds of the present disclosure. In some embodiments, the method does not comprise the characterization step.

Such methods allow for the treatment and/or prevention of hyperproliferative disease, disorders, or conditions caused by the proliferation of cells which may comprise glycosyltransferase. In some embodiments, the cell is a cancer cell. For example, the hyperproliferative disease, disorder, or condition may be selected from bladder, brain, breast, cervical, colorectal, endometrial, esophageal, gallbladder, gastric, glioblastoma, kidney, leukemia (*e.g.*, acute myelogenous leukemia, chronic myelogenous leukemia, chronic lymphocytic leukemia), liver (*e.g.*, hepatocellular carcinoma, intrahepatic cholangiocarcinoma, angiosarcoma, hemangiosarcoma, hepatoblastoma), lung (*e.g.*, non-small cell lung cancer, small cell lung cancer, mesothelioma), melanoma, ovarian, pancreatic, prostate, multiple myeloma, sarcoma (*e.g.*, osteosarcoma, soft-tissue sacrcoma), thyroid, urinary tract, or uterine cancer. In certain implementations the cancer may be a hematopoietic cancer, such as acute lymphoblastic leukemia, acute myelogenous leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, acute monocytic leukemia, Hodgkin's lymphoma, or non-Hodgkin's lymphoma.

The method of prophylaxis or treatment of a neoplasia of a subject in need thereof may comprise administration to the subject a compound (*e.g.*, compounds that are a substrate for a glycosyltransferase such as Compounds 1-4) or composition of the present disclosure. In some embodiments, the neoplasia is associated with a hyperproliferative disease, disorder or condition such as a cancer including acute leukemia (including MLL acute leukemia, MLL partial tandem duplicate acute leukemia, NPM mutated acute leukemia, MOZ acute leukemia, NUP98 acute leukemia, and CALM acute leukemia), chronic lymphocytic leukemia, chronic

myeloid leukemia, myelodysplastic syndrome, polycythemia vera, malignant lymphoma (including B-cell lymphoma), myeloma (including multiple myeloma), brain tumor, cancer of the head and neck, esophageal cancer, thyroid cancer, small cell lung cancer, non-small cell lung cancer, breast cancer, gastric cancer, gallbladder and bile duct cancer, liver cancer, hepatocellular cancer, pancreatic cancer, colon cancer, rectal cancer, anal cancer, chorionepithelioma, endometrial cancer, cervical cancer, ovarian cancer, bladder cancer, urothelial cancer, renal cancer, renal cell cancer, prostate cancer, testicular tumor, testicular germ cell tumor, ovarian germ cell tumor, Wilms' tumor, malignant melanoma, neuroblastoma, osteosarcoma, Ewing's sarcoma, rhabdomyosarcoma, chondrosarcoma, soft tissue sarcoma, or skin cancer. In certain embodimennts, the neoplasia is a neoplasia of the skin, bone, adrenal glands kidney, spleen, or testicals. In particular embodiments, the neoplasia is Ewing's sarcoma or rhabdomyosarcoma. In some embodiments, the neoplasia is a lymphoid cancer such as acute lymphocytic leukemia [ALL], chronic lymphocytic leukemia [CLL], B-cell lymphomas such as diffuse large B-cell lymphoma [DLBCL], follicular lymphoma, Burkitt's lymphoma, mantle cell lymphoma, T-cell lymphomas and leukemias, natural killer [NK] cell lymphomas, Hodgkin's lymphomas, hairy cell leukemia, monoclonal gammopathy of uncertain significance, plasmacytoma, multiple myeloma, or post-transplant lymphoproliferative disorders; the hematological malignancies and related conditions of myeloid lineage are selected from acute myelogenous leukemia [AML], chronic myelogenous leukemia [CML], chronic myelomonocytic leukemia [CM ML], hypereosinophilic syndrome, polycythaemia vera, essential thrombocythaemia primary myelofibrosis, myeloproliferative syndrome, myelodysplastic syndrome, or promyelocytic leukemia.

Methods for preventing the growth of a population of neoplasia in a medium are also provided which may comprise contacting the medium with a compound of the present disclosure (*e.g.*, compounds that are a substrate for a glycosyltransferase such as Compounds 1-4). In some embodiments, the neoplasia is associated with a hyperproliferative disorder such as a cancer including acute leukemia (including MLL acute leukemia, MLL partial tandem duplicate acute leukemia, NPM mutated acute leukemia, MOZ acute leukemia, NUP98 acute leukemia, and CALM acute leukemia), chronic lymphocytic leukemia, chronic myeloid leukemia, myelodysplastic syndrome, polycythemia vera, malignant lymphoma (including B-cell lymphoma), myeloma (including multiple myeloma), brain tumor, cancer of the head and neck, esophageal cancer, thyroid cancer, small cell lung cancer, non-small cell

lung cancer, breast cancer, gastric cancer, gallbladder and bile duct cancer, liver cancer, hepatocellular cancer, pancreatic cancer, colon cancer, rectal cancer, anal cancer, chorionepithelioma, endometrial cancer, cervical cancer, ovarian cancer, bladder cancer, urothelial cancer, renal cancer, renal cell cancer, prostate cancer, testicular tumor, testicular
5 germ cell tumor, ovarian germ cell tumor, Wilms' tumor, malignant melanoma, neuroblastoma, osteosarcoma, Ewing's sarcoma, chondrosarcoma, rhabdomyosarcoma, soft tissue sarcoma, or skin cancer. In certain embodimennts, the neoplasia is a neoplasia of the skin, bone, adrenal glands kidney, spleen, or testicals. In particular embodiments, the neoplasia is Ewing's sarcoma or rhabdomyosarcoma. In some embodiments, the neoplasia is
10 a lymphoid cancer such as acute lymphocytic leukemia [ALL], chronic lymphocytic leukemia [CLL], B-cell lymphomas such as diffuse large B-cell lymphoma [DLBCL], follicular lymphoma, Burkitt's lymphoma, mantle cell lymphoma, T-cell lymphomas and leukemias, natural killer [NK] cell lymphomas, Hodgkin's lymphomas, hairy cell leukemia, monoclonal gammopathy of uncertain significance, plasmacytoma, multiple myeloma, or post-transplant
15 lymphoproliferative disorders; the hematological malignancies and related conditions of myeloid lineage are selected from acute myelogenous leukemia [AML], chronic myelogenous leukemia [CML], chronic myelomonocytic leukemia [CM ML], hypereosinophilic syndrome, polycythaemia vera, essential thrombocythaemia primary myelofibrosis, myeloproliferative syndrome, myelodysplastic syndrome, or promyelocytic
20 leukemia. In certain embodimennts, the neoplasia is a neoplasia of the skin, bone, adrenal glands kidney, spleen, or testicals. In particular embodiments, the neoplasia is Ewing's sarcoma or rhabdomyosarcoma. In some embodiments, the neoplasia is a lymphoid cancer such as acute lymphocytic leukemia [ALL], chronic lymphocytic leukemia [CLL], B-cell lymphomas such as diffuse large B-cell lymphoma [DLBCL], follicular lymphoma, Burkitt's
25 lymphoma, mantle cell lymphoma, T-cell lymphomas and leukemias, natural killer [NK] cell lymphomas, Hodgkin's lymphomas, hairy cell leukemia, monoclonal gammopathy of uncertain significance, plasmacytoma, multiple myeloma, or post-transplant lymphoproliferative disorders; the hematological malignancies and related conditions of myeloid lineage are selected from acute myelogenous leukemia [AML], chronic
30 myelogenous leukemia [CML], chronic myelomonocytic leukemia [CM ML], hypereosinophilic syndrome, polycythaemia vera, essential thrombocythaemia primary myelofibrosis, myeloproliferative syndrome, myelodysplastic syndrome, or promyelocytic leukemia.

In order to treat, prevent, or prevent recurrence of diseases, disorders, or conditions (*e.g.*, compounds that are a substrate for a glycosyltransferase such as Compounds 1-4) as discussed herein, the compounds or compositions of the present disclosure may be administered at least once a day for at least one week. In various embodiments, the composition is administered at least twice a day for at least two days. In certain embodiments, the composition is administered approximately daily, at least daily, twice a week, weekly, or for once a month. In certain embodiments, the composition is administered for several months, such as at least two months, six months, or one year or longer. The compositions may be further suited for long-term use, which may be particularly beneficial for preventing recurring infection, or for preventing infection or conditions in at-risk or susceptible patients, including immune compromised patients. Such long-term use may involve treatment for at least two years, three years, four years, or even five or more years.

The compounds and pharmaceutical compositions can be formulated and employed in combination therapies, that is, the compounds and pharmaceutical compositions can be formulated with or administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder, or they may achieve different effects (*e.g.*, control of any adverse effects).

Examples of other drugs to combine with the compounds described herein include pharmaceuticals for the treatment of neoplasias (*e.g.*, alkylating agents, antimetabolites, antibiotics, platinum complex compounds, camptothecin derivatives, tyrosine kinase inhibitors, serine/threonine kinase inhibitors, phospholipid kinase inhibitors, monoclonal antibodies, interferons, hormones, angiogenic inhibitors, immune checkpoint inhibitors, epigenetics-associated molecular inhibitor, a protein post-translational modification inhibitor, a proteasome inhibitor). In some embodiments, the compounds of the present disclosure may be combined with one or more of a cytostatic agent, cisplatin, doxorubicin, taxotere, taxol, etoposide, irinotecan, camptostar, topotecan, paclitaxel, docetaxel, epothilones, tamoxifen, 5-fluorouracil, methoxtrexate, temozolomide, cyclophosphamide, SCH 66336, R115777, L778,123, BMS 214662, Iressa, Tarceva, antibodies to EGER, Gleevec.TM., intron, ara-C, adriamycin, cytoxan, gemcitabine, Uracil mustard, Chlormethine, Ifosfamide, Melphalan,

Chlorambucil, Pipobroman, Triethylenemelamine, Triethylenethiophosphoramine, Busulfan, Carmustine, Lomustine, Streptozocin, Dacarbazine, Floxuridine, Cytarabine, 6-Mercaptopurine, 6-Thioguanine, Fludarabine phosphate, oxaliplatin, leucovirin, ELOXATIN.TM., Pentostatine, Vinblastine, Vincristine, Vindesine, Bleomycin,
5 Dactinomycin, Daunorubicin, Doxorubicin, Epirubicin, Idarubicin, Mithramycin, Deoxycoformycin, Mitomycin-C, L-Asparaginase, Teniposide 17.alpha.-Ethinylestradiol, Diethylstilbestrol, Testosterone, Prednisone, Fluoxymesterone, Dromostanolone propionate, Testolactone, Megestrolacetate, Methylprednisolone, Methyltestosterone, Prednisolone, Triamcinolone, Chlorotrianisene, Hydroxyprogesterone, Aminoglutethimide, Estramustine,
10 Medroxyprogesteroneacetate, Leuprolide, Flutamide, Toremifene, goserelin, Cisplatin, Carboplatin, Hydroxyurea, Amsacrine, Procarbazine, Mitotane, Mitoxantrone, Levamisole, Navelbene, Anastrazole, Letrazole, Capecitabine, Reloxafine, Droloxafine, Hexamethylmelamine, Avastin, herceptin, Bexxar, Velcade, Zevalin, Trisenox, Xeloda, Vinorelbine, Porfimer, Erbitux, Liposomal, Thiotepa, Altretamine, Melphalan, Trastuzumab,
15 Lerozole, Fulvestrant, Exemestane, Fulvestrant, Ifosfomide, Rituximab, C225, Campath, Clofarabine, cladribine, aphidicolon, rituxan, sunitinib, dasatinib, tezacitabine, Sml1, fludarabine, pentostatin, triapine, didox, trimidox, amidox, 3-AP, and MDL-101,731. Combination methods can involve the use of the two (or more) agents formulated together or separately, as determined to be appropriate. In one example, two or more drugs are
20 formulated together for the simultaneous or near simultaneous administration of the agents.

Kits

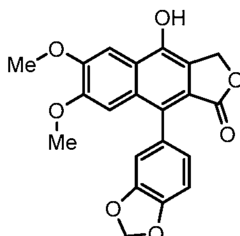
In another aspect, the composition is a contained in a kit, which may contain the compositions of the present disclosure packaged to facilitate dispensing and/or administration of the compositions disclosed herein. The packaging or dispenser may include a bottle, tube,
25 spray bottle, or other dispenser. In certain embodiments, the composition is packaged in a concentrated form, and diluted to a desired concentration upon use by the end user. Typically, in these aspects, the composition may be formulated and packaged in a manner suitable for long-term storage to maintain efficacy of the composition.

30 The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the assay, screening, and therapeutic methods of, and are not intended to limit the scope.

EXAMPLES

Example 1: Genetic Modifier Screen

A gene expression correlation screen (PRISM screen) was performed on Ewing Sarcoma (EWS) A673 cells following administration of Compound 1:



Compound 1
BRD9645

5 CRISPR KO cells were generated of Ewing Sarcoma A673 cells. A schematic of the PRISM Screen is shown in FIG. 2. Briefly, pools of cells are treated for 5 days with compounds, then cells are lysed and mRNA is isolated. The barcode sequences associated with characterized CCLE cell lines are amplified by PCR and detected by a Luminex scanner. The quantity of each barcode remaining after treatment serves as a readout to generate cell line sensitivity 10 signatures for each compound.

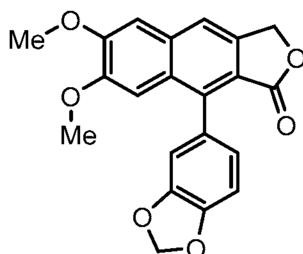
A differential expression analysis FIG. 3A provides the Pearson Correlation coefficients correlating gene expression of EWS specific genes and sensitivity to Compound 1. FIG. 3B correlates the Shrunken log2 fold changes (LFC) for each q-value for each open reading frame (ORF) assigned by differential representation methods. As can be seen, 15 knockout of two genes associated with glycosylation, SLC35B4 and UGT3A2 conferred resistance to Compound 1 in addition to various v-ATPase subunits implicating glycosyltrasferases and V-ATPase in the mechanism of action for Compound 1.

Lysotracker (LTR) Staining was performed on test conditions on several UGT3A2 knockout clones. Briefly, on the day before staining, 35,000 cells were plated in 150 μL in in 20 a 96 well plate. Several test conditions were measured: untreated cells, starvation (treatment with 0.2% by weight fetal bovine serum (FBS)), bafilomycin A1 (Baf A1) treatment and compound 1 treatment at either 400 nM, 2 μM or 10 μM in media. After 5 hours in each test condition, live cells were stained with Lysotracker Red DND-99 and Hoechst for 30 minutes (100 nM Lysotracker Red DND-99, 1:2000 Hoeschst stain by volume). Cells were then 25 washed twice with PBS, fixed for 15 minutes with 4% by volume paraformaldehyde (PFA). Cells were again washed twiced with PBS following fixation and images at 40× on an Opera

Phenix to quantify Lysotracker positive punctae (LTR+) with Columbus analysis. FIG. 4 provides the LTR+ punctae/cell for each test condition. "*" indicates statistical significance ($p<0.05$) as compared to the untreated condition.

A pull down assay of the several UGT3A2 KnockOut (KO) clones following pooled
5 CRISPR KO of UGT3A2. Cas9 LacZ is used as a control. FIG. 5 provides the Western Blot pull down assay of several isolated KO clones following pooled KO of UGT3A2. The arrow marks two bands associated with UGT3A2. As can be seen, clone 1 has lost expression of UGT3A2 while clone 10 does not have UGT3A2 knock out. Similarly, the Cas9 LacZ control also includes expression of UGT3A2.

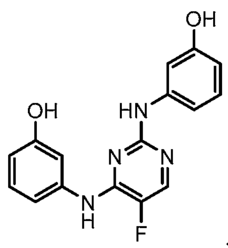
10 The number of spots (correlating with live cells) was measured for the UGT3A2 KO clones. FIGs. 6A-6C correlates the amount of Compound 1 administered to the number of live cells measured. As can be seen, both A673 Cas9 LacZ (FIG. 6A) and Clone 10 (FIG. 6C), both of which express UGT3A2 are sensitive to Compound 1 administration in a dose dependent manner. However, Clone 1 (FIG. 6B), which does not express UGT3A2, lost
15 sensitivity to Compound 1. In FIGs. 6A-6C, sensitivity is compared to 9-(benzo[d][1,3]dioxol-5-yl)-6,7-dimethoxynaphtho[2,3-c]furan-1(3H)-one (Compound 1 (No –OH)), a compound having the structure:



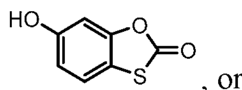
Compound 1 (No –OH)

Compound 1 (No –OH) lacks the hydroxyl at the at the 4 position of the naphtho[2,3-c]furan-1(3H)-one moiety which is the site of UGT3A2 glycosylation in
20 Compound 1.

Cell viability assays were also performed with Compound 2 (R112) and Compound 3 (tioxolone) each having the structure:

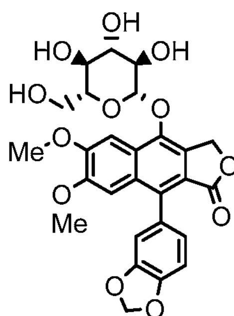


, Compound 2 (R112)



, or Compound 3 (Tioxolone)

As can be seen, like Compound 1, these compounds have moieties capable of glycosylation such as the hydroxyl moiety conjugated to an aromatic ring. FIGs. 7A-7C provide the results of the cell viability assay for Compound 1 (FIG. 7A), Compound 2 (FIG. 7B), Compound 3 (FIG. 7C) as compared to A673 LacZ cells and the UGT3A2 KO clone 1 (which did not express UGT3A2). As can be seen, knockout of UGT3A2 abrogated sensitivity to all compounds which are glycosylated by the glycosyltransferase. FIG. 7D provides the cell viability assay data comparing Compound 1 and the glycosylated version of Compound 1 having the structure:

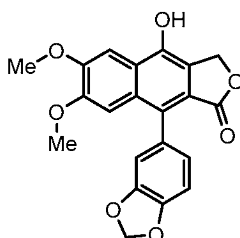


Glycosylated Compound 1

Cell viability assays were also measured RH30 rhabdomyosarcoma cells as well. WT and UGT3A2 knockout ORF cells were measured. FIG. 8A shows the results with Compound 1 administration and FIG. 8B shows the results with Compound 2 (R112) administration. These results further demonstrate the role that UT3GA2 plays in the sensitivity mechanism responsible for cell cytotoxicity. Furthermore, even though Compound 1 and Compound 2 (R112) likely have different targets (v-ATPase and a kinase(s) respectively), there is a common mechanism of modification (glycosylation) by UGT3A2 to produce a cytotoxic compound

Example 2: Detection of Glycosylated Compounds In Lysed Cells

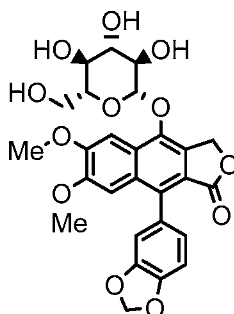
A673 Ewing sarcoma cells were seeded in wells of a 12-well plate (100,000 cells/well). The cells were treated with 10 μM of Compound 1 in dimethyl sulfoxide (DMSO) in media:



Compound 1
BRD9645

5 At each time point (0-72 hours), media were collected and cells with phosphate buffered saline (PBS)and lysed with 500 μL cold acetonitrile: methanol (1:1). The media and cell lysate samples were kept at -80°C until analysis.

To begin analysis, the cell lysate samples were centrifuged at 14,000 rpm for 8 min. 100 μL of the supernatant were collected and diluted with 100 μL of PBS. Each sample was 10 analyzed by high resolution quadrupole time of flight mass spectrometry (QTOF-MS) for the presence of Compound 1 and its glycosylated analog having the structure:



Glycosylated Compound 1

FIG. 9A and 9B provide the measured relative amounts of Compound 1 and Glycosylated Compound 1 found in cell lysate and cell media according to this protocol. FIG. 9B compares WT and KO cells.

15

Other Embodiments

From the foregoing description, it will be apparent that variations and modifications may be made to the disclosure described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

5 The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or subcombination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

All patents and publications mentioned in this specification are herein incorporated by
10 reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference.

CLAIMS

What is claimed is:

1. A method of treating a selected subject having a neoplasia, the method comprising administering to the subject a compound that is a substrate for a glycosyltransferase and forms a glycosylated compound upon interaction with the glycosyltransferase,

wherein the subject is or was selected by characterizing the neoplasia for the presence of the glycosyltransferase.

2. The method of claim 1, wherein the compound comprises an aromatic ring comprising a hydroxyl or an amide group.

3. The method of claim 1 or 2, wherein the compound or the glycosylated compound is a substrate for a second protein target expressed by the neoplasia.

4. The method of claim 1 or 2, wherein the glycosylated compound is a substrate for a second protein target expressed by the neoplasia.

5. The method of claim 1, wherein the compound or glycosylated compound is a substrate for a second protein target expressed by the neoplasia and the subject is or was selected for characterizing the neoplasia for the presence of both the glycosyltransferase and the second protein target.

6. The method according to any one of claims 3-5, wherein the second protein target is an enzyme.

7. The method according to claim 6, wherein the enzyme is a v-ATPase or a H^+-ATPase or Carbonic anhydrase.

8. The method according to claim 6, wherein the enzyme is a kinase.

9. The method according to any one of claims 1-8 wherein the glycosyltransferase is a UDP Glycosyltrasferase protein (*e.g.*, UDP glycosyltransferase family 3 member A1, UDP glycosyltransferase family 3 member A2).

10. The method according to any one of claims 1-8, wherein the glycosyltransferase is UDP glycosyltransferase family 3 member A2.

11. The method according to any one of claims 1-10, wherein the neoplasia is cancer.

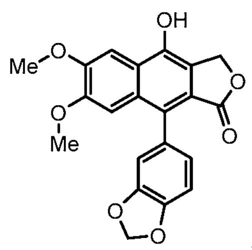
12. The method according to claim 11, wherein the cancer is cancer of the skin, bone,

adrenal glands kidney, spleen, or testicals.

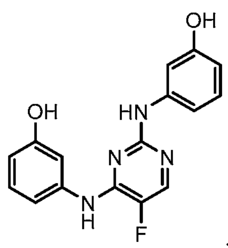
13. The method according to claim 11, wherein the cancer is Ewing's sarcoma or rhabdomyosarcoma.

14. The method according to any one of claims 1-13, wherein the compound is not
5 doxorubicin or etoposide.

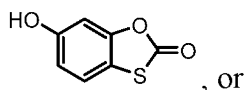
15. The method according to any one of claims 1-14, wherein the compound has the structure:



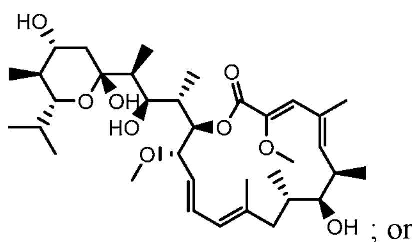
, Compound 1 BRD9645



, Compound 2 (R112)



, or Compound 3 (Tioxolone)



; or Compound 4 (Baf A1)

a pharmaceutically acceptable salt thereof.

16. A composition for the treatment of a neoplasia characterized by the presence of the
10 glycosyltransferase;

wherein the compound is a substrate for a glycosyltransferase and forms a glycosylated compound upon interaction with the glycosyltransferase.

17. The composition of claim 16, wherein the compound comprises an aromatic ring comprising a hydroxyl or an amide group.

5 18. The composition of claim 16 or 17, wherein the compound or the glycosylated compound is a substrate for a second protein target expressed by the neoplasia.

19. The composition of claim 16 or 17, wherein the glycosylated compound is a substrate for a second protein target expressed by the neoplasia.

20. The composition of any one of claims 16-19, wherein the compound or glycosylated
10 compound is a substrate for a second protein target expressed by the neoplasia and the subject is or was selected for characterizing the neoplasia for the presence of both the glycosyltransferase and the second protein target.

21. The composition according to any one of claims 18-20, wherein the second protein target is an enzyme.

15 22. The composition according to claim 21, wherein the enzyme is a v-ATPase or a H^+-ATPase or Carbonic anhydrase.

23. The composition according to claim 21, wherein the enzyme is a kinase.

24. The method according to any one of claims 16-23 wherein the glycosyltransferase is a UDP Glycosyltrasferase protein (*e.g.*, UDP glycosyltransferase family 3 member A1, UDP
20 glycosyltransferase family 3 member A2).

25. The method according to any one of claims 16-24, wherein the glycosyltransferase is UDP glycosyltransferase family 3 member A2.

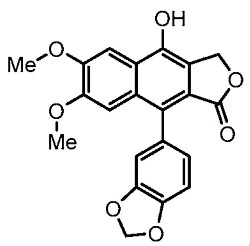
26. The method according to any one of claims 16-25, wherein the neoplasia is cancer.

27. The method according to claim 26, wherein the cancer is cancer of the skin, bone,
25 adrenal glands kidney, spleen, or testicals.

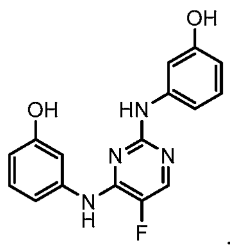
28. The method according to claim 26, wherein the cancer is Ewing's sarcoma or rhabdomyosarcoma.

29. The composition according to any one of claims 16-28, wherein the compound is not doxorubicin or etoposide.

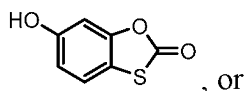
30. The composition according to any one of claims 16-29, wherein said compound has the structure:



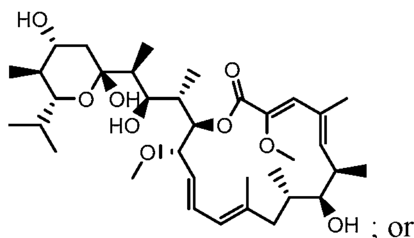
Compound 1
BRD9645



Compound 2
(R112)



Compound 3
(Tioxolone)



Compound 4
(Baf A1)

a pharmaceutically acceptable salt thereof.

31. A method of killing a neoplastic cell comprising contacting the cell with a compound that is a substrate for a glycosyltransferase and forms a glycosylated compound upon interaction with the glycosyltransferase,

wherein the neoplastic cell is or was selected by characterizing the neoplastic cell for the presence of the glycosyltransferase.

32. The method of claim 31, wherein the compound comprises an aromatic ring comprising a hydroxyl or an amide group.

33. The method of claim 31 or 32, wherein the compound or the glycosylated compound

is a substrate for a second protein target expressed by the neoplasia.

34. The method of claim 31 or 32, wherein the glycosylated compound is a substrate for a second protein target expressed by the neoplasia.

35. The method of claim 31, wherein the compound or glycosylated compound is a
5 substrate for a second protein target expressed by the neoplasia and the subject is or was selected for characterizing the neoplasia for the presence of both the glycosyltransferase and the second protein target.

36. The method according to any one of claims 33-35, wherein the second protein target is an enzyme.

10 37. The method according to claim 36, wherein the enzyme is a v-ATPase or a H^+-ATPase or Carbonic anhydrase.

38. The method according to claim 36, wherein the enzyme is a kinase.

39. The method according to any one of claims 31-38 wherein the glycosyltransferase is a UDP Glycosyltrasferase protein (*e.g.*, UDP glycosyltransferase family 3 member A1, UDP
15 glycosyltransferase family 3 member A2).

40. The method according to any one of claims 31-38, wherein the glycosyltransferase is UDP glycosyltransferase family 3 member A2.

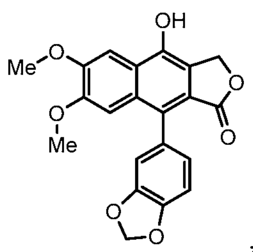
41. The method according to any one of claims 31-40, wherein the neoplasia is cancer.

42. The method according to claim 41, wherein the cancer is cancer of the skin, bone,
20 adrenal glands kidney, spleen, or testicals.

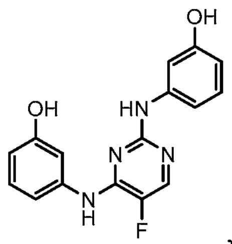
43. The method according to claim 41, wherein the cancer is Ewing's sarcoma or rhabdomyosarcoma.

44. The method according to any one of claims 31-43, wherein the compound is not doxorubicin or etoposide.

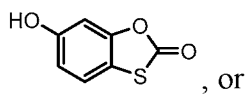
25 45. The method according to any one of claims 31-44, wherein the compound has the structure:



Compound 1
BRD9645

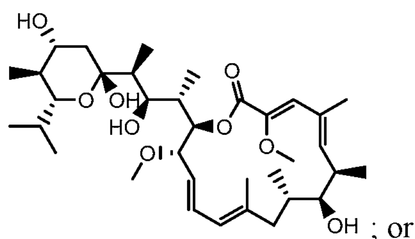


Compound 2
(R112)



, or

Compound 3
(Tioxolone)



; or

Compound 4
(Baf A1)

a pharmaceutically acceptable salt thereof.

46. The method according to any one of claims 31-45, wherein the method is *in vivo*, *ex vivo*, or *in vitro*.

FIG. 1

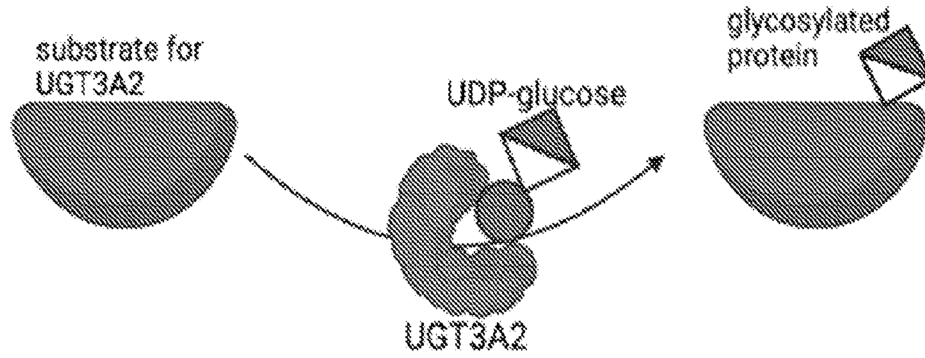


FIG. 2

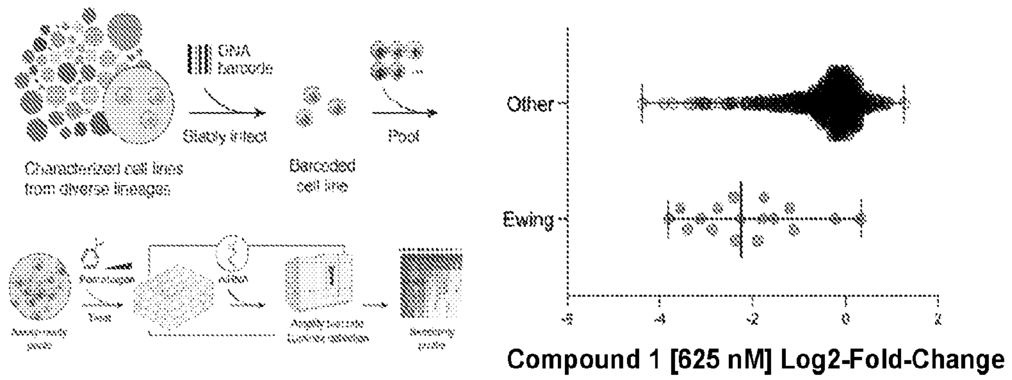


FIG. 3A

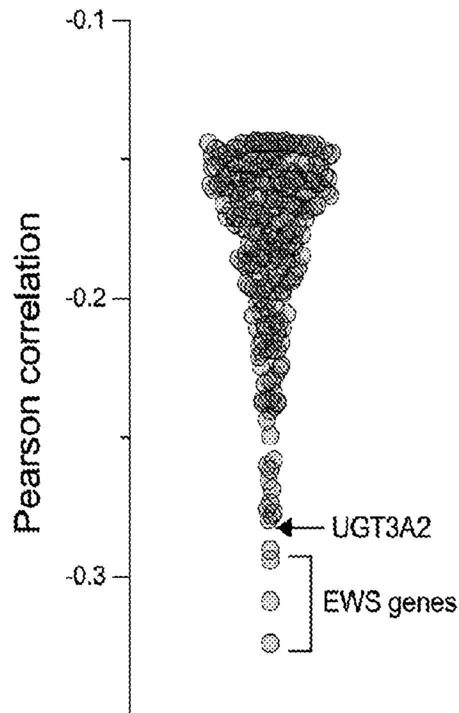


FIG. 3B

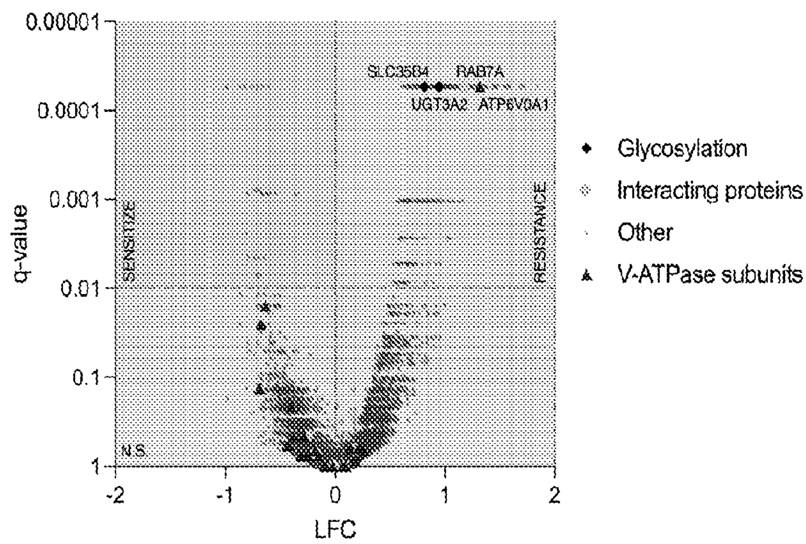


FIG. 4

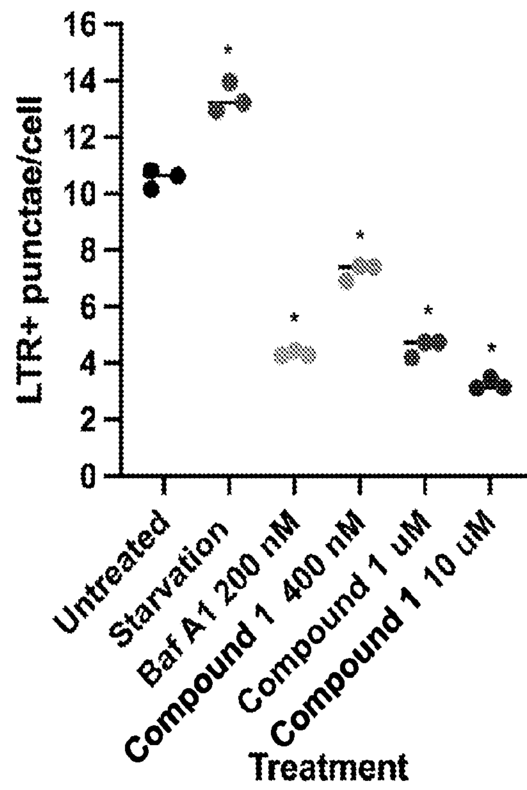


FIG. 5

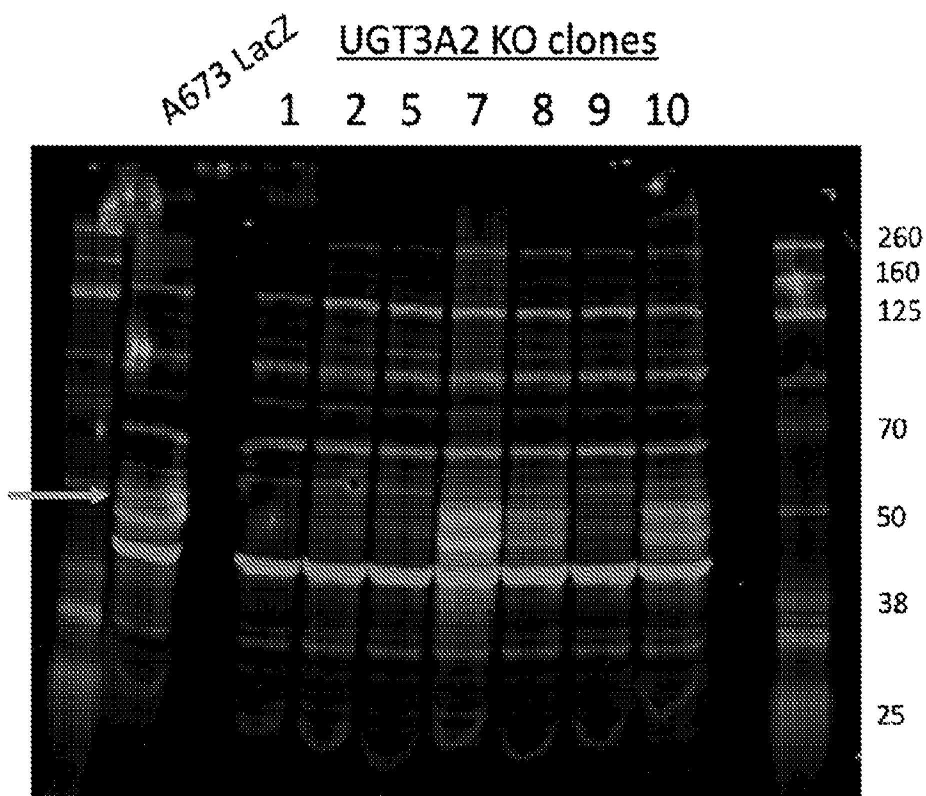


FIG. 6A

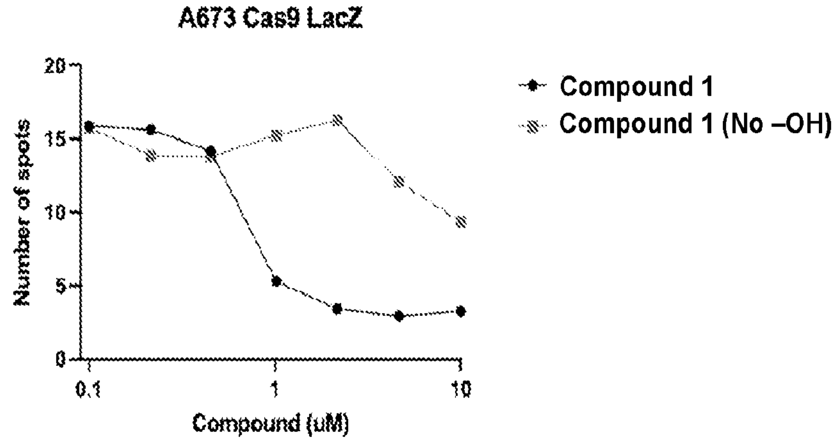


FIG. 6B

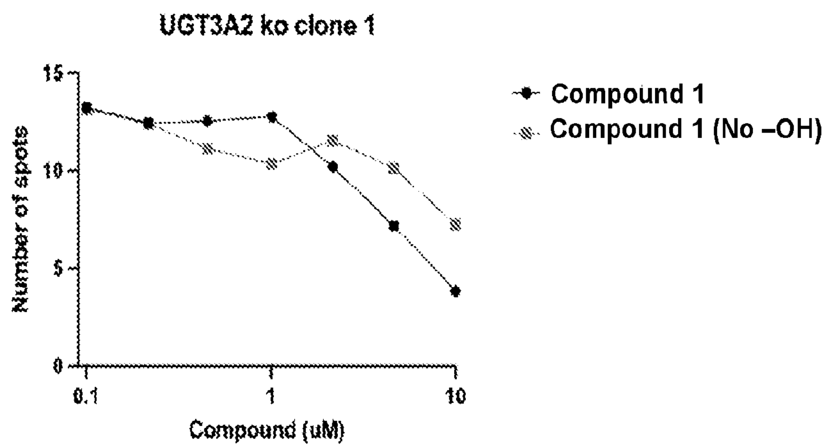


FIG. 6C

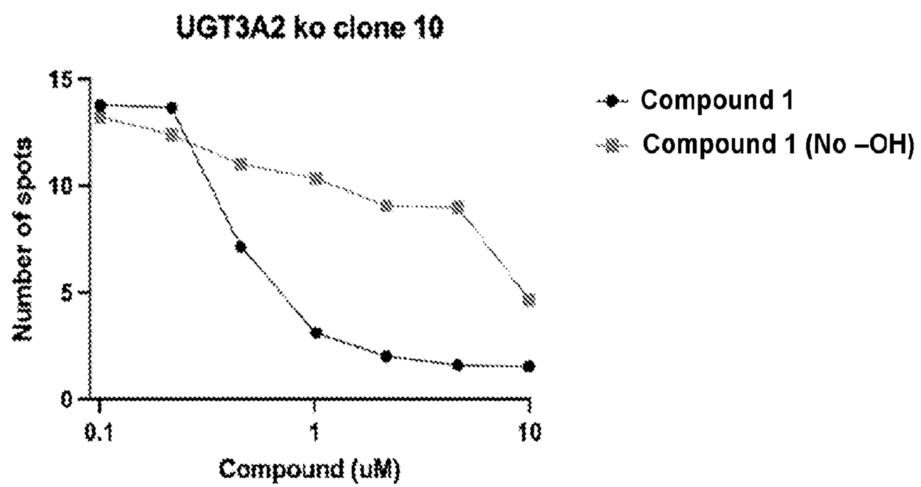
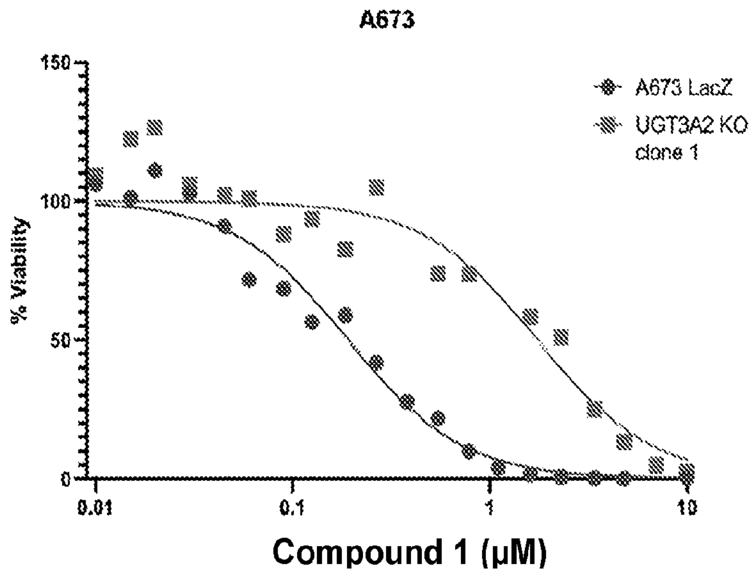


FIG. 7A



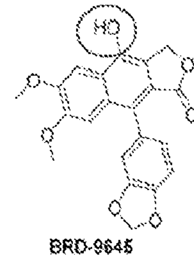
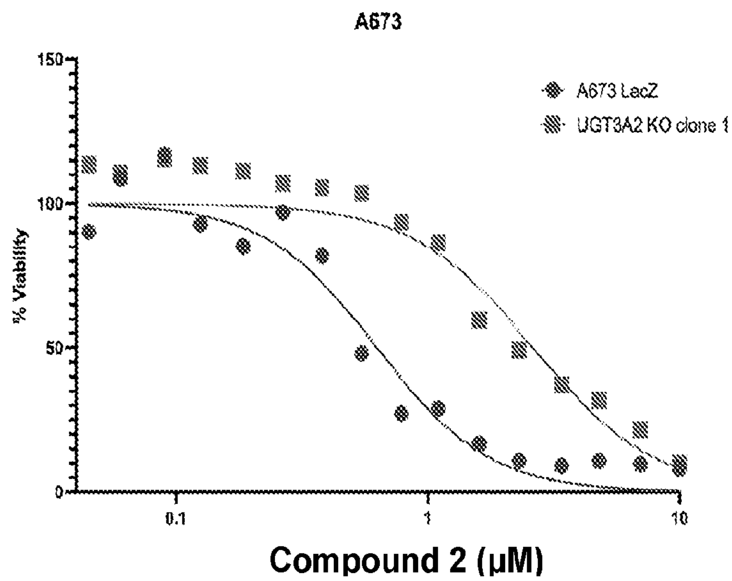


FIG. 7B



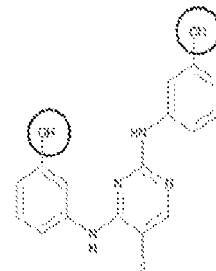


FIG. 7C

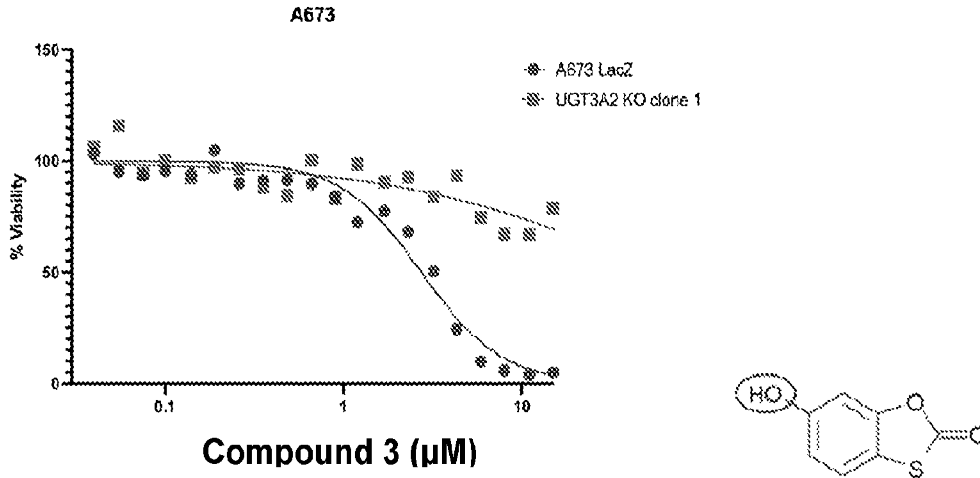


FIG. 7D

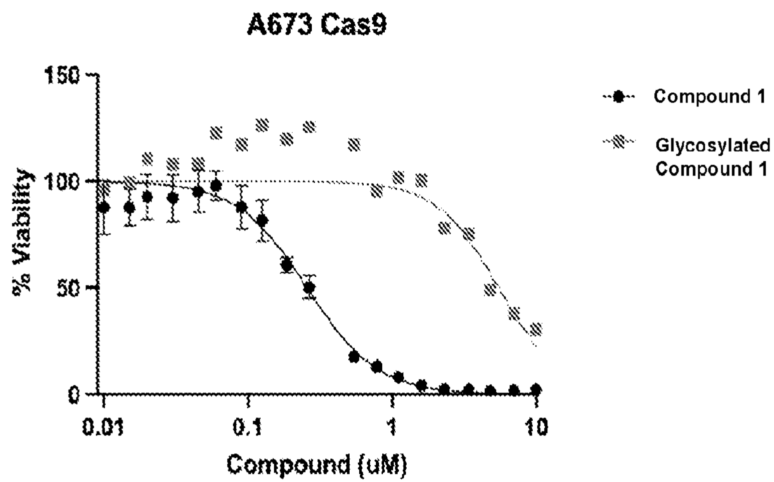


FIG. 8A

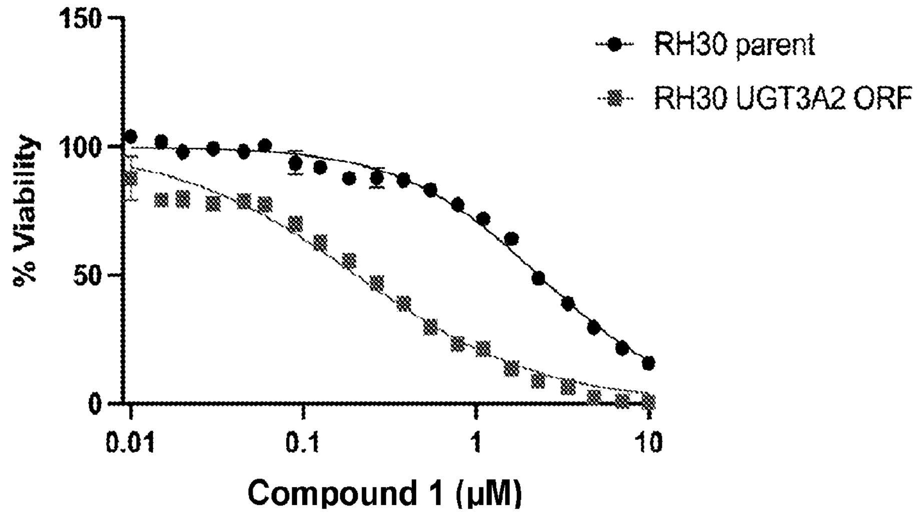


FIG. 8B

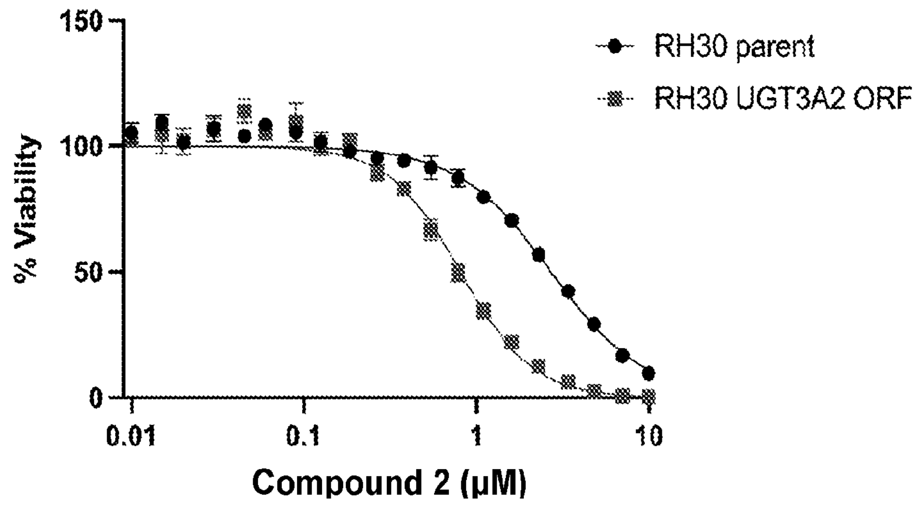


FIG. 9A

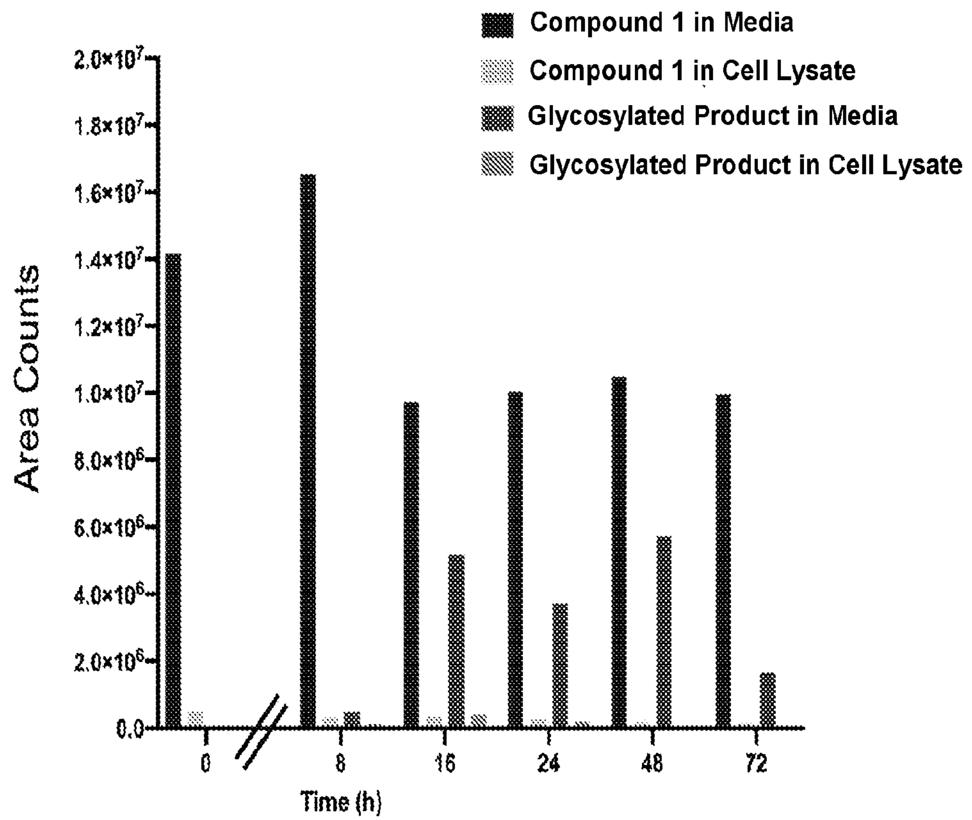
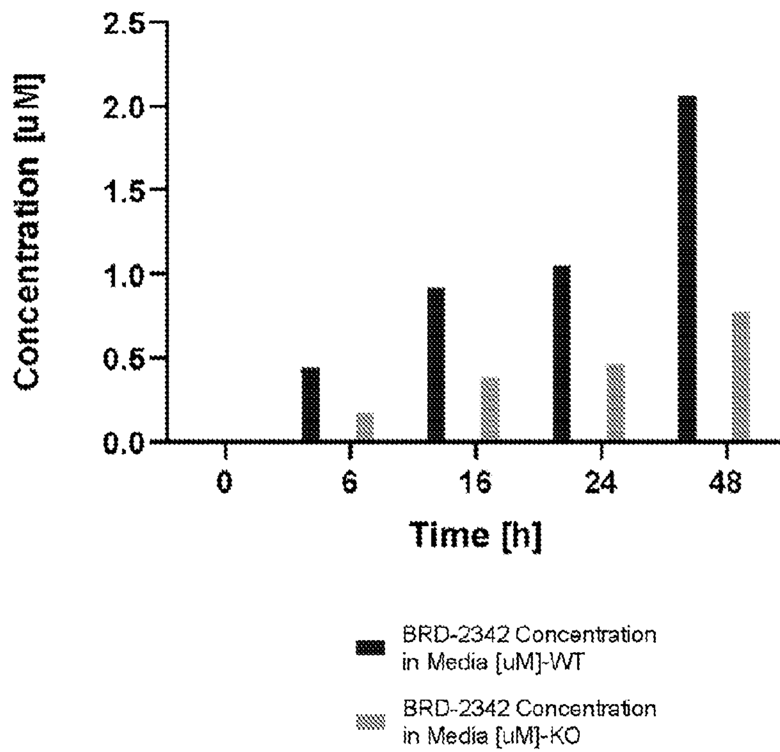


FIG. 9B



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/033347

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61K 31/343*** (2024.01); ***A61P 35/00*** (2024.01); ***C12Q 1/48*** (2024.01); ***G01N 33/68*** (2024.01); *G01N 33/574* (2024.01)
CPC: ***A61K 31/343; A61P 35/00; C12Q 1/48; G01N 33/6893;*** *G01N 33/574*

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2021/0402002 A1 (GLYKOS BIOMEDICAL OY) 30 December 2021 (30.12.2021) entire document	1-5, 16-19, 31-35
Y	US 2017/0233828 A1 (UNIVERSITY OF CAPE TOWN) 17 August 2017 (17.08.2017) entire document	1-5, 16-19, 31-35
A	US 2016/0312292 A1 (CELGENE CORPORATION) 27 October 2016 (27.10.2016) entire document	1-5, 16-19, 31-35
A	AXELSSON et al., Neutralization of pH in the Golgi apparatus causes redistribution of glycosyltransferases and changes in the O-glycosylation of mucins, Glycobiology, Vol. 11, No. 8, September 2001, Pgs. 633–644. [Retrieved on 08 August 2024]. Retrieved from the internet: URL:<https://academic.oup.com/glycob/article/11/8/633/650599>. entire document	1-5, 16-19, 31-35
A	WANG et al. Molecular basis of V-ATPase inhibition by bafilomycin A1, Nat Commun, Vol. 12, No. 1782, 19 March 2021. Pgs. 1-8. [Retrieved on 09 August 2024]. Retrieved from the internet: URL<:https://www.nature.com/articles/s41467-021-22111-5>. entire document	1-5, 16-19, 31-35

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "D" document cited by the applicant in the international application
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 August 2024 (09.08.2024)	**27 August 2024 (27.08.2024)**

Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US **Commissioner for Patents** **P.O. Box 1450, Alexandria, VA 22313-1450**	**MATOS** **TAINA**
Facsimile No. **571-273-8300**	Telephone No. **571-272-4300**

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☐ forming part of the international application as filed.
   b. ☑ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),
      ☑ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/033347

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☑ Claims Nos.: **6-15, 20-30, 36-46**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).