LECTIN-DRUG CONJUGATES

FIELD

The present disclosure relates to protein-drug conjugates, more specifically the disclosure relates to the conjugates of lectin proteins and cytotoxic agents or therapeutic agent, their process of preparation and use in the method of treatment of cancer.

BACKGROUND

Advances in the systemic therapy of cancer, including chemotherapy, hormonal therapy, targeted therapy, and immunotherapy have been responsible for improvements in cancer related mortality. Although such advancements have yet to benefit all cancer types, systemic therapies have led to an improvement in overall survival in both the adjuvant and metastatic setting for many cancers. With the pressure to make therapies available as soon as possible, the side-effects of systemic therapies, in particular long-term side-effects are not very well characterized and understood. Increasingly, a number of cancer types are requiring long-term and even lifelong systemic therapy. Because the incidence of cancer in the world will increase over the next several decades and there will be more people living with cancer, it is important to have an understanding of the potential side-effects of new systemic therapies.

Conventional chemotherapy agents were the first agents in the armamentarium for the war on cancer. Categories today include, but are not limited to, alkylating agents (e.g., cyclophosphamide, temozolomide, cisplatin, oxaliplatin), anti-metabolites (e.g., methotrexate, cytarabine, fluorouracil, capecitabine), anti-tumor antibiotics (e.g., doxorubicin, epirubicin, bleomycin), topoisomerase inhibitors (e.g., etoposide, irinotecan) and microtubule stabilizers (e.g., paclitaxel, docetaxel). Typically, these agents are not tumor specific and frequently their administration results in significant toxic effects to non-cancerous tissues. The success of these agents relies largely upon their differential toxicity for tumor cells which typically have a high mitotic rate and increased dependence on continuous supply of biomolecules for growth, compared to normal non-cancerous tissues. Present and future challenges include identifying the appropriate dosing of cytotoxic agents (and other components of multimodal anti-cancer treatment regimens) to maximize therapeutic efficacy while limiting both acute and long-term side-effects.

Targeted therapy or immunotherapy offers considerable advantages in delivering growth inhibitory or cytotoxic effects in a much more cell-specific manner. Furthermore, these drugs

do not induce the same profile of acute toxicities such as myelosuppression and nausea and vomiting which accompany many of the conventional non-targeted cytotoxic drugs. As described below some of the newer targeted agents have proven highly effective anti-cancer treatments, and have already delivered startling improvements in survival for certain cancer. Examples of targeted therapies include Imatinib Tyrosine Kinase Inhibitor (TKI), and monoclonal antibodies such as trastuzumab, bevacizumab, panitumumab, and cetuximab. Targeted therapy also includes antibody drug conjugates which is a new class of biological drugs wherein the anticancer agent or other therapeutic agent are attached to the antibody with aid of linker. Some of the FDA approved antibody drug conjugates include Gemtuzumabozogamicin, Brentuximab vedotin, Trastuzumab emtansine, inotizimabozogamicin, Moxetumomabpasudotox, Polatuzumabvedotin-piiq, Enfotumabvedotin, Trastuzumab deruxtecan, Sacituzumab govitecan and Belantamabmafodotin.

To date, antibodies have proved to be effective therapeutics. These antibody therapeutics are used in much the same way as injected small-molecule chemotherapeutics.

Radioimmunotherapy provides further examples of the successful use of antibodies in cancer therapeutics.Unfortunately, despite the successful use of radiation-delivery antibody vehicles such as Zevalin and Bexxar, these antibody based drugs have serious shortcomings. For example, both are whole IgG molecules that remain in the circulation for days; they pass through the highly radiation-sensitive bone marrow throughout this period, and bone-marrow toxicity limits the dose of radiation that can be tolerated by patients.

Lectins are proteins or glycoproteins that are an important group of bioactive proteins found in nature. Lectins are used as tools for diagnostic and therapeutic purpose in health care areas. Lectins are also used in the purification of glycoproteins, oligosaccharide analysis and in cell selection processes. Lectins can bind with mono, di or oligosaccharides of the sugar moiety found in polysaccharides, glycoproteins or glycolipids. Lectins are specific to various monosaccharide or glycans and thus have enormous potential in the area of medical assays. Cancer cells are shown to express and/or secrete a variety of glycol conjugates with an aberrant glycan structure. Owing to their sugar specificity towards a particular linkage, lectins have an ability to differentiate changes in glycan structures in cancerous cell from their noncancerous counterparts, leading to their application in cancer diagnosis and treatment.

In the field of protein drug conjugates there are minimal studies which focus on lectin drug conjugates for the treatment of cancer. US9504756, and US6214345, describe antibody drug

conjugates, and lectin is described as an alternative ligand moiety for targeted delivery of cytotoxic drugs for the treatment of cancer.

Indian application 1265/MUM/2004 explicitly discloses a drug or anticancer compound conjugate in which lectin obtained from a fungal culture of*sclerotium rolfsii* (SR, *S. rolfsii*) and *Rhizoctonia bataticola* (RB) is used as carrier or conjugating protein for the transport of anticancer drug to the target cell.

Lectin conjugates, wherein lectinis a carrying agent or target seeking agent, for targeted delivery of cytotoxic drugs and methods of producing the same are disclosed in KR100638706B1, US7015313B2, US7045300B2 and US20060251580A1.

Plant based lectin has been explored for its targeting ability in the field of drug conjugates. Wheat germ agglutinin a plant lectin conjugated to Doxorubicin via acid liable cis-aconityl linker for delivery of prodrug Doxorubicin to the human colonocytes has been studied by Michel writh et al. (Michel writh*et.al*, Pharmaceutical research, Vol 15, NO.7, 1998). Gunjan et al, studied the specific interaction of plant legume lectin such as Peanut agglutinin and concanavalin with Phycocyanin for the application of photodynamic therapy. Peanut agglutinin and concanavalin conjugated with Phycocyanin with the aid of chemical linker such as SPDP and studied for effective delivery of the drug. (Gunjan Pandey et al., Photochemistry and Photobiology, 85: 1126-1133, 2009,). Daichi Kitaguchi et al, studied the binding affinity of rBC2LCN, a lectin isolated from *Burkholderia* that binds cell surface glycans of colorectal cancer for effective delivery of Pseudomonas exotoxin (PE38) for the treatment of cancer. This study also reveals the therapeutic efficacy and toxicity of rBC2LCN-PE38 conjugate in Colo Rectal Cancer mouse xenograft models (Daichi Kitaguchi et al Cancer Science.;111:4548–4557. 2020)

In the case of antibody drug conjugates, only a tiny amount of drug enters the cell, as the number of antigens present on cell surfaces is less and only one antibody interacts with antigen. Hence antibody drug conjugates need repeated dosage in order to achieve good therapeutic effect resulting in exorbitant treatment. Further even though the above references reveal conjugates of lectins, there are no such conjugates which are commercially available as of today. Lectins are highly specific towards glycans present on neoplastic tissues and are potentially better therapeutic ligands than monoclonal antibodies due to their cost-effectiveness, reduced toxicity and reduced side effects. There is a need for improved cancer treatments that are cost effective.

SUMMARY

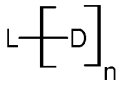
In one embodiment, the present disclosure provides a highly effective product for the treatment of cancer. In one embodiment is provided a recombinant lectin drug conjugate, wherein the recombinant lectin is derived from *Sclerotium rolfsii* lectin (SRL).

Also provided is a method of treatment or prevention of cancer in a subject in need thereof; wherein the method comprises use of therapeutically effective amount of recombinant lectin drug conjugate.

In one embodiment is provided the use of a recombinant lectin drug conjugate as a novel therapeutic agent for the treatment or prevention of cancer, wherein the recombinant lectin is derived from *Sclerotium rolfsii* lectin.

In one embodiment, is provided a composition comprising recombinant lectin drug conjugate for the treatment or prevention of cancer.

According to an aspect of the present disclosure, there is provided a compound of Formula I:



Formula I

or a pharmaceutically acceptable salt thereof, wherein

n is 1 to 9;

L is a recombinant lectin derived from *S. rolfsii*;

D is selected from a cytotoxic agent, therapeutic agent or a compound comprising a cytotoxic agent or therapeutic agent;

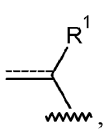
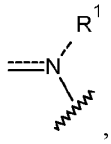
wherein each D is conjugated by L via one of the following;

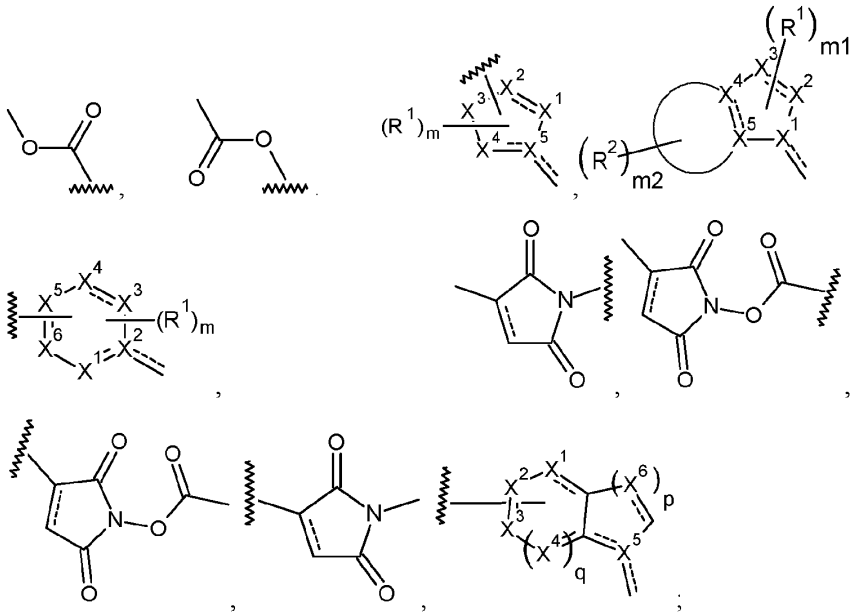
a) a covalent bond, or

b) a group for formula "—A^1-A^2-A^3—";

wherein the other terminal of A^1 and A^3 is connected to L and D, respectively; and wherein A^1 and A^3 is independently selected from:

—CR^1R^2—, —C(O) —, —C(O)NR^1—, —NR^1C(O) —, —NR^1—, —O—, —S—, —S(O) —, —$S(O)_2$—, —R^1C=CR^2—, —C≡C—, —R^1C=CR^2-

R^3C=CR^4—, —R^1C=C=CR^2—, —C(=NR^1)—, , ,

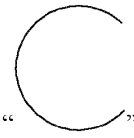


wherein

m, m1 and m2areindependently 0, 1, 2, 3,or 4; p is 1 or 2 and q is 0 or 1;

"wwwww" indicates the point of attachment of A^2 to A^1 or A^3;

"=====" indicates single or double bond;



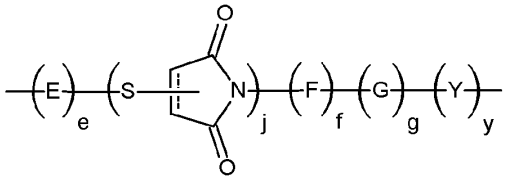
" " indicates a 5 to 10 membered ring including atoms of the ring it is fused to, wherein the 5 to 10 membered ring is an aromatic, non-aromatic, or cycloalkyl ring or a heteroaromatic or heterocycloalkyl ring wherein the heteroaromatic or heterocycloalkyl ring comprise atleast one heteroatom selected from N, O or S;

each of X^1, X^2, X^3, X^4, X^5 and X^6 is independently selected from C, CH, CH_2, N, NH, O or S; and

R^1, R^2, R^3 and R^4 are independently selected from hydrogen, C_1 to C_5 alkyl, $C_2$to C_5 alkenyl, $C_2$to C_5 alkynyl, C_1 to C_5 alkylene, $C_3$to C_{10} cycloalkyl, C_2 to C_9heterocycloalkyl, $C_6$to C_{10} aryl, C_2 to C_9 heteroaryl, $-OR^5$, $-NR^5$ $-SR^5$, halogen selected from fluorine, chlorine, bromine or iodine, and$-C(O)OR^5$, wherein C_1 to C_5 alkyl, $C_2$to C_5 alkenyl, $C_2$to C_5 alkynyl, C_1 to C_5 alkylene, C_3 to C_{10} cycloalkyl, C_2 to C_9heterocycloalkyl, $C_6$to C_{10} aryl, and C_2 to C_9 heteroaryl are unsubstituted or are each further substituted by R^6, and

R^5 and $R^6$are each independently hydrogen, C_1 to C_3 alkyl, OH or halogen;

wherein A^2 is of formula:



wherein

e, j, f, g and yare independently 0 or 1, provided e, f andy are not 0 simultaneously,

S is sulphur atom,

E, F, and Yis a spacer, and

G is an amino acid or apeptide chain comprising 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 amino acids;

and wherein A^1 forms bond with E or S or F and A^3 is connected to Y or G.

According to another aspect of the disclosure, there is provided a compound of Formula II:

L—D

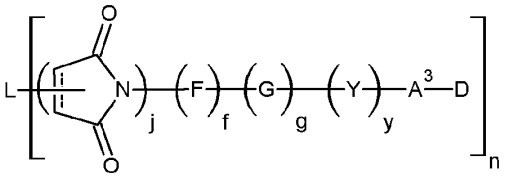
Formula II

or a pharmaceutically acceptable salt thereof, wherein

L is a recombinant lectin derived from *S. rolfsii*;

D is selected from a cytotoxic agent, therapeutic agent or a compound comprising cytotoxic agent or therapeutic agent.

According to yet another aspect of the present disclosure, there is provided a compound of Formula III:

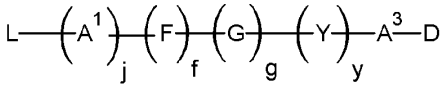


Formula III

or a pharmaceutically acceptable salt thereof, wherein

L, F, G, Y, A^3, D, n, j, f, g, and y are as defined herein before.

According to an aspect of the present disclosure, there is provided a compound of Formula IV:



Formula IV

or a pharmaceutically acceptable salt thereof, wherein

L, A^1, F, G, Y, A^3, D, j, f, g, and y are as defined herein.

According to the preceding aspects, the recombinant lectin derived from *S. rolfsii,* is selected from:

i. lectin protein having amino acid sequence of SEQ ID NO: 1, or

ii. lectin protein having at least 70% sequence identityto the SEQ ID NO: 1.

According to an aspect of the present disclosure, the recombinant lectin derived from *S. rolfsii* is selected from lectin having amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5,SEQ ID NO: 8; or the modified lectin proteins in WO2020044296, which is hereby incorporated by reference.

According to a particular aspect of the present disclosure, the recombinant lectin derived from *S. rolfsii* is selected from lectin having amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 5 or SEQ ID NO. 8.

In an aspect of the present disclosure, D is selected from a DNA binding agent, an anti-microtubule agent, an antimetabolite, an alkylating or alkylating-like antineoplastic agent, a topoisomerase I inhibitor, a topoisomerase II inhibitor, a kinase inhibitor, bortezomib, estramustine, Ixabepilone, everolimus, temsirolimus, neomycin, neamine, a cryptophycin, discodermolide, amanitin, or a pyrrolobenzodiazepine dimer, or a pharmaceutically acceptable salt, polymorph, or solvate of each thereof.

According to any preceding aspect of the present disclosureeach spacer E, F and Y is independently selected from—R^7—, —S—, —NH—,—R^7-NH-R^8—, —R^8-NC(O)-R^7—, —R^8-C(O)N-R^7—,—C(O)—, —C(O)-R^7—, —R^7-C(O)—, —R^8-C(O)-R^7—, —S-R^7—, —R^7-S—,—R^7-S-R^8—, —S(O)—, —S(O)-R^7—, —R^7-S(O)—, —R^7-S(O)-R^8—,—O-R^7—, —R^7-O—, —R^8-O-R^7—, —C(O)O—, —OC(O)-R^7—, —R^7-OC(O)—, — R^8-OC(O)-R^7—, —R^7-OC(O)O—, —OC(O)O-R^7—, —R^7-OC(O)O-R^8—, —NH-R^7-NH—C(O)R^8—, —(CH_2)S-NH-R^7—, —

R^7-C(O)N$(CH_2)_{1-3}$O$(CH_2)_{1-3}$C(O)—, —R^7-NC(O)$(CH_2)_{1-3}$O$(CH_2)_{1-3}$C(O)—,—Si(R^7R^8)—,polyalkyleneglycol optionally attached through oxygen to—R^7—, —S—, —NH—, —R^7-N-R^8—, —R^8-NC(O)-R^7—, —R^8-C(O)N-R^7—, —C(O)—, —C(O)-R^7—, —R^7-C(O)—, —R^8-C(O)-R^7—, —S-R^7—, —R^7-S—, —R^7-S-R^8—, —S(O)—, —S(O)-R^7—, —R^7-S(O)—, —R^7-S(O)-R^8—, —O-R^7—, —R^7-O—, —R^8-O-R^7—, —C(O)O—, —OC(O)-R^7—, —R^7-OC(O)—, — R^8-OC(O)-R^7—, —R^7-OC(O)O—, —OC(O)O-R^7—, —R^7-OC(O)O-R^8—, —NH-R^7-NH—C(O)R^8—, —(CH_2)S-NH-R^7—, —R^7-C(O)N$(CH_2)_{1-3}$O$(CH_2)_{1-3}$C(O)—, —R^7-NC(O)$(CH_2)_{1-3}$O$(CH_2)_{1-3}$C(O)—, —Si(R^7R^8)—;

wherein $R^7$and R^8 are independently selected fromhydrogen,—C_1-C_{10} alkylene, —C_1-C_{10}alkenylene,C_5 to C_{10}saturated or unsaturated cycloalkylene,—C_2-C_{10} alkenylene-C_5 to C_{10} saturated or unsaturated cycloalkylene,$C_6$to C_{10} arylene, —C_2-C_{10} alkenylene-C_6 to C_{10} arylene, heteroarylene ring comprising atleast one heteroatom selected from N, O and S, —C_2-C_{10} alkenylene-C_2 to C_9 heteroarylene; and wherein the —C_1-C_{10} alkylene, —C_2-C_{10}alkenylene, C_5 to C_{10} saturated or unsaturated cycloalkylene,—C_2-C_{10} alkenylene-C_5 to C_{10} saturated or unsaturated cycloalkylene,$C_6$to C_{10} arylene,—C_2-C_{10} alkenylene-C_6 to C_{10} arylene, C_2 to C_9 heteroarylene and —C_2-C_{10} alkenylene-C_2 to C_9 heteroarylene is optionally further substituted with -OH, -C(O), -SH, -NH_2, halogen, C_1 to C_5 alkyl, -NO_2, or -CN;

wherein polyalkylene glycol is a homopolymer or copolymer of alkylene with each unit of alkylene having C_1 to C_8 carbon atoms and total number of alkylene units ranging from 1 to 20; and wherein when polyalkylene glycol is E the -CH_2- end is attached to $A^1$and when polyalkylene glycol is F the -CH_2-end is attached to —R^7—, —S—, —NH—, —R^7-N-R^8—, —R^8-NC(O)-R^7—, —R^8-C(O)N-R^7—, —C(O)—, —C(O)-R^7—, —R^7-C(O)—, —R^8-C(O)-R^7—, —S-R^7—, —R^7-S—, —R^7-S-R^8—, —S(O)—, —S(O)-R^7—, —R^7-S(O)—, —R^7-S(O)-R^8—, —O-R^7—, —R^7-O—, —R^8-O-R^7—, —C(O)O—, —OC(O)-R^7—, —R^7-OC(O)—, — R^8-OC(O)-R^7—, —R^7-OC(O)O—, —OC(O)O-R^7—, —R^7-OC(O)O-R^8—, —NH-R^7-NH—C(O)R^8— or —(CH_2)S-NH-R^7—, —R^7-C(O)N$(CH_2)_{1-3}$O$(CH_2)_{1-3}$C(O)— or —R^7-NC(O)$(CH_2)_{1-3}$O$(CH_2)_{1-3}$C(O)—.

According to the preceding aspect of the present disclosure, Y andA^3 independently or in combination comprise a self immolative groups.

In an aspect of the present disclosure, there is provided a method of treatment or prevention of cancer using compound of Formula I or Formula II or Formula III or Formula IV.

In another aspect of the present disclosure, there is provided a pharmaceutical composition comprising compound of Formula I or Formula II or Formula III or Formula IV.

In yet another aspect of the present disclosure, there is provided a compound of Formula I, Formula II, Formula III or Formula IV or a composition comprising compound of Formula I, Formula II, Formula III or Formula IV for use in the preparation of medicament for the treatment or prevention of cancer.

DEFINITIONS:

The term "alkyl" by itself or as part of another term refers to a substituted or unsubstituted straight chain or branched, saturated or unsaturated hydrocarbon having the indicated number of carbon atoms (e.g., "—C_1-C_8 alkyl" or "—C_1-C_{10}" alkyl refer to an alkyl group having from 1 to 8 or 1 to 10 carbon atoms, respectively). When the number of carbon atoms is not indicated, the alkyl group has from 1 to 8 carbon atoms. Representative straight chain "—C_1-C_8 alkyl" groups include, but are not limited to, -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, -n-hexyl, -n-heptyl and -n-octyl; while branched —C_1-C_8 alkyls include, but are not limited to, -isopropyl, -sec-butyl, -isobutyl, -tert-butyl, -isopentyl, and -2-methylbutyl; unsaturated —C_2-C_8 alkyls include, but are not limited to, -vinyl, -allyl, -1-butenyl, -2-butenyl, -isobutylenyl, -1-pentenyl, -2-pentenyl, -3-methyl-1-butenyl, -2-methyl-2-butenyl, -2,3-dimethyl-2-butenyl, -1-hexyl, 2-hexyl, -3-hexyl, -acetylenyl, -propynyl, -1-butynyl, -2-butynyl, -1-pentynyl, -2-pentynyl and -3-methyl-1 butynyl. In some embodiments, an alkyl group is unsubstituted. An alkyl group can be substituted with one or more groups. In some aspects, an alkyl group is saturated.

The terms "alkenyl" and "alkynyl" refer to straight and branched carbon chains having specified carbon atoms or from about 2 to about 20 carbon atoms (and all combinations and sub-combinations of ranges and specific numbers of carbon atoms therein), with from about 2 to about 8 carbon atoms being preferred. An alkenyl chain has at least one double bond in the chain and an alkynyl chain has at least one triple bond in the chain. Examples of alkenyl groups include, but are not limited to, ethylene or vinyl, allyl, -1-butenyl, -2-butenyl, -isobutylenyl, -1-pentenyl, -2-pentenyl, -3-methyl-1-butenyl, -2-methyl-2-butenyl, and -2,3-dimethyl-2-butenyl. Examples of alkynyl groups include, but are not limited to, acetylenic, propargyl, acetylenyl, propynyl, -1-butynyl, -2-butynyl, -1-pentynyl, -2-pentynyl, and -3-methyl-1-butynyl.

Alkenyl and alkynyl groups, whether alone or as part of another group, can be optionally substituted with one or more groups, preferably 1 to 3 groups (and any additional substituents selected from halogen), including but not limited to, -halogen, —O—(C_1-C_8 alkyl), —O—(C_2-C_8 alkenyl), —O—(C_2-C_8 alkynyl), -aryl, —C(O)R′, —OC(O)R′, —C(O)OR′, —

$C(O)NH_2$, —C(O)NHR′, —$C(O)N(R')_2$, —NHC(O)R′, —SR′, —SO_3R', —$S(O)_2R'$, —S(O)R′, —OH, =O, —N_3, —NH_2, —NH(R′), —$N(R')_2$ and —CN, where each R′ is independently selected from —H, —C_1-C_8 alkyl, —C_2-C_8 alkylenyl, —C_2-C_8 alkynyl, or -aryl and wherein said —O—(C_1-C_8 alkyl), —O—(C_2-C_8 alkenyl), —O—(C_2-C_8 alkynyl), -aryl, —C_1-C_8 alkyl, —C_2-C_8 alkenyl, and —C_2-C_8 alkynyl groups can be optionally further substituted with one or more substituents including, but not limited to, —C_1-C_8 alkyl, —C_2-C_8 alkenyl, —C_2-C_8 alkynyl, -halogen, —O—(C_1-C_8 alkyl), —O—(C_2-C_8 alkenyl), —O—(C_2-C_8 alkynyl), -aryl, —C(O)R″, —OC(O)R″, —C(O)OR″, —$C(O)NH_2$, —C(O)NHR″, —$C(O)N(R'')_2$, —NHC(O)R″, —SR″, —SO_3R'', —$S(O)_2R''$, —S(O)R″, —OH, —N_3, —NH(R″), —$N(R'')_2$ and —CN, where each R″ is independently selected from —H, —C_1-C_8 alkyl, —C_2-C_8 alkenyl, —C_2-C_8 alkynyl, or -aryl.

In further embodiments, the term “alkylene” refers to a saturated branched or straight chain hydrocarbon radical having specified number of carbon atoms or from about 1 to about 20 carbon atoms (and all combinations and sub-combinations of ranges and specific numbers of carbon atoms therein), with from about 1 to about 8 carbon atoms being preferred and having two monovalent radical centres derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkane. Typical alkylenes include, but are not limited to, methylene, ethylene, propylene, butylene, pentylene, hexylene, heptylene, ocytylene, nonylene, decalene, and the like. Alkylene groups, whether alone or as part of another group, can be optionally substituted with one or more groups, preferably 1 to 3 groups (and any additional substituents selected from halogen), including, but not limited to, -halogen, —O—(C_1-C_8 alkyl), —O—(C_2-C_8 alkenyl), —O—(C_2-C_8 alkynyl), -aryl, —C(O)R′, —OC(O)R′, —C(O)OR′, —$C(O)NH_2$, —C(O)NHR′, —$C(O)N(R')_2$, —NHC(O)R′, —SR′, —SO_3R', —$S(O)_2R'$, —S(O)R′, —OH, =O, —N_3, —NH_2, —NH(R′), —$N(R')_2$ and —CN, where each R′ is independently selected from —H, —C_1-C_8 alkyl, —C_2-C_8 alkenyl, —C_2-C_8 alkynyl, or -aryl and wherein said —O—(C_1-C_8 alkyl), —O—(C_2-C_8 alkenyl), —O—(C_2-C_8 alkynyl), -aryl, —C_1-C_8 alkyl, —C_2-C_8 alkenyl, and —C_2-C_8 alkynyl groups can be further optionally substituted with one or more substituents including, but not limited to, —C_1-C_8 alkyl, —C_2-C_8 alkenyl, —C_2-C_8 alkynyl, -halogen, —O—(C_1-C_8 alkyl), —O—(C_2-C_8 alkenyl), —O—(C_2-C_8 alkynyl), -aryl, —C(O)R″, —OC(O)R″, —C(O)OR″, —$C(O)NH_2$, —C(O)NHR″, —$C(O)N(R'')_2$, —NHC(O)R″, —SR″, —SO_3R'', —$S(O)_2R''$, —S(O)R″, —OH, —N_3, —NH_2, —NH(R″), —$N(R'')_2$ and —CN, where each R″ is independently selected from —H, —C_1-C_8 alkyl, —C_2-C_8 alkenyl, —C_2-C_8 alkynyl, or -aryl.

In further embodiments "aryl," by itself or as part of another term, means a substituted or unsubstituted monovalent carbocyclic aromatic hydrocarbon radical of said number of carbon atoms or 6-20 carbon atoms, which is derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system.

The term "arylene," is an aryl group as defined above that has two substitution and can be in the ortho, meta, or para configurations based on relative position of two substitutions, wherein substitutions and 1 and 2 position is said to be ortho substitution, substitution at 1 and 3 position is meta and substitution at 1 and 4 position is para.

In further embodiments a "C_2-C_9 heterocycle," by itself or as part of another term, refers to a monovalent substituted or unsubstituted aromatic or non-aromatic monocyclic or bicyclic ring system having from 2 to 9 carbon atoms (also referred to as ring members) and one to four heteroatom ring members independently selected from N, O or S, and derived by removal of one hydrogen atom from a ring atom of a parent ring system. One or more N, C or S atoms in the heterocycle can be oxidized. The ring that includes the heteroatom can be aromatic or nonaromatic. Unless otherwise noted, the heterocycle is attached to its pendant group at any heteroatom or carbon atom that results in a stable structure. Representative examples of a heterocycle include, but are not limited to, pyrrolidinyl, azetidinyl, piperidinyl, morpholinyl, tetrahydrofuranyl, tetrahydropyranyl, benzofuranyl, benzothiophene, indolyl, benzopyrazolyl, pyrrolyl, thiophenyl (thiophene), furanyl, thiazolyl, imidazolyl, pyrazolyl, pyrimidinyl, pyridinyl, pyrazinyl, pyridazinyl, isothiazolyl, and isoxazolyl.

In further embodiments a "C_3-C_{10} cycloalkyl or carbocycle," by itself or as part of another term, is a 5-, 6-, 7-, 8-, 9- or 10-membered monovalent, substituted or unsubstituted, saturated or unsaturated non-aromatic monocyclic or bicyclic carbocyclic ring derived by the removal of one hydrogen atom from a ring atom of a parent ring system. Representative cycloalkyls include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclopentadienyl, cyclohexyl, cyclohexenyl, 1,3-cyclohexadienyl, 1,4-cyclohexadienyl, cycloheptyl, 1,3-cycloheptadienyl, 1,3,5-cycloheptatrienyl, cyclooctyl, and cyclooctadienyl.

In further embodiments a carbocyclo by itself or as part of another term, refers to a carbocycle group defined above wherein another of the carbocycle groups' hydrogen atoms is replaced with a bond (i.e., it is divalent).

"Substituted alkyl" and "substituted aryl" mean alkyl and aryl, respectively, in which one or more hydrogen atoms are each independently replaced with a substituent. Typical substituents include, but are not limited to, —X, —R, —O_{31}, —OR, —SR, —S−, —NR_2, —NR_3, =NR, —CX_3, —CN, —OCN, —SCN, —N=C=O, —NCS, —NO, —NO2, =N2, —

N3, —NRC(=O)R, —C(=O)R, —C(=O)NR2, —SO_3 −, —SO_3H, —$S(=O)_2R$, —$OS(=O)_2OR$, —S(=O)2NR, —S(=O)R, —$OP(=O)(OR)_2$, —$P(=O)(OR)_2$, —PO^{-}_3, —PO_3H_2, —C(=O)R, —C(=O)X, —C(=S)R, —CO_2R, —CO_2 −, —C(=S)OR, —C(=O)SR, —C(=S)SR, —$C(=O)NR_2$, —$C(=S)NR_2$, or —$C(=NR)NR_2$, where each X is independently a halogen: —F, —Cl, —Br, or —I; and each R is independently —H, —C_1-C_{20} alkyl, —C_6-C_{20} aryl, —C_3-C_{14} heterocycle, a protecting group or a prodrug moiety. Alkylene, carbocycle, carbocyclo, arylene, heteroalkyl, β-alanine, aminoalkanoic acid, aminoalkynoic acid, aminoalkanedioic acid, aminobenzoic acid, amino-heterocyclo-alkanoic acid, heterocyclo-carboxylic acid, citrulline, statine, diaminoalkanoic acid, and derivatives thereof.

The term “protein” as used herein refers to a polymer of amino acid residues.

The term “lectin” or “lectin protein” as used herein refers to a carbohydrate-binding protein of *sclerotium rolfsii*(a soil borne pathogenic fungus of Indian origin),having National Center for Biotechnology Information (NCBI) Accession Number 2OFC_A.

The term “target cell” as used herein refers to a cell bearing receptors for lectins as defined aforementioned.

The term “amino acid”as used herein refers to naturally occurring and synthetic amino acids, as well as amino acid analogues and amino acid mimetics that have a function that is similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code and include the proteinogenic amino acids. Naturally occurring amino acids also include those modified after translation in cells. Synthetic amino acids include non-canonical amino acids such as selenocysteine and pyrrolysine. Typically, synthetic amino acids are not proteinogenic amino acids.

The term “recombinant” means a nucleic acid or a polypeptide has been artificially or synthetically (i.e., non-naturally) altered by human intervention. The alteration can be performed on the material within, or removed from, its natural environment or state. For example, a “recombinant nucleic acid” is one that is made by recombining nucleic acids, e.g., during cloning, DNA shuffling or other well-known molecular biological procedures. A “recombinant DNA molecule” is comprised of segments of DNA joined together by means of such molecular biological techniques. The term “recombinant protein” or “recombinant polypeptide” as used herein refers to a protein molecule which is expressed using a recombinant DNA technology.

The term “linker”, “linker unit”, or “link” as used herein refers to a chemical moiety comprising a covalent bond or a chain of atoms that covalently attaches a protein to a drug

moiety. It is a bi-functional chemical moiety that covalently links the "L" and "D" moieties. The linker aids in maintaining the stability of the recombinant lectin drug conjugate throughout the plasma, by retaining its intactness till it reaches target site. The linkers are also labile and susceptible to the external stimuli of the tumour microenvironment, thereby cleaving/reducing the drug from the recombinant lectin protein drug conjugate, at the target site. The linkers are broadly classified into cleavable and non-cleavable linkers. Cleavable linker may be disulphide containing linkers that are cleavable through disulphide exchange or proteases; acid-labile linkers that are cleavable at acidic pH, and linkers that are cleavable by enzymes such as hydrolases, peptidases, esterases, and glucoronidases.

The term "therapeutic agent" or therapeutically effective agent as used interchangeably here, means an agent administered to a subject for alleviating or curing one or more symptoms of the disease or disorder.

Cytotoxic agentrefers to a substance that inhibits cells or inhibits the function of cells and/or causes destruction of cells. The term is intended to include chemotherapeutic agents, toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including synthetic analogues and derivatives thereof.

The term "conjugate" as used herein refers to the products of the present disclosure. For the purpose of present disclosure, the conjugate may comprise a cytotoxic agent attached to the recombinant lectin protein with the aid of alinker or itmay a comprisecytotoxic agent directly attached to recombinant lectin protein. The structure of the conjugate and the bond between the cytotoxic agent and recombinant lectin protein is an aspect of the present disclosure and is as described herein.

A "spacer", when present, extends the framework of the linker unit to provide more distance between the lectin protein and the cytotoxic agent. The spacer can also act to alter the physiochemical properties of the final products of the present disclosure depending on components of the spacer.

The term"compound comprising a cytotoxic agent" or "therapeutic agent" refers to a compound that comprises a linker or part of a linker along with a cytotoxic agent or therapeutic agent. Examples of compounds comprising a cytotoxic agent are given below.

The term "derivative (s) may refer to one or more of salts (e.g. pharmaceutically acceptable salts), esters, ethers, polymorphs, metabolites, pure form, particle size, isomers, mixtures of isomers, complexes, combinations of the compound(s).

The term "inhibit" or "inhibition of" means to a reduce by a measurable amount, or to prevent entirely.

The term "therapeutically effective amount" refers to an amount of a cytotoxic agent or therapeutic agenteffective to treat a disease or disorder in a mammal. In the case of cancer, the therapeutically effective amount of the cytotoxic agent or therapeutic agent may reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. To the extent the cytotoxic agent may inhibit growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic.

The term "cytotoxic activity" refers to a cell-killing, a cytostatic or an anti-proliferative effect of a compound or an intracellular metabolite of the compound. Cytotoxic activity may be expressed as IC_{50} value, which is the concentration in molar or mass per unit volume at which half the cells survive. The methods of determining IC_{50} value are known to the person skilled in the art. For example, IC_{50} value can be determined using standard MTS-PMS Cytotoxicity assay.

The terms "cancer" and "cancerous" refer to or describe the physiological condition or disorder in mammals that is typically characterized by unregulated cell growth. A "tumor" comprises one or more cancerous cells.

The terms "treat" or "treatment," unless otherwise indicated by context, refer to therapeutic treatment and prophylactic measures to prevent relapse, wherein the object is to inhibit or slow down (lessen) an undesired physiological change or disorder, such as the development or spread of cancer. For purposes of this disclosure, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder.

As used herein "preventing" or "prevention" refers to a reduction in the likelihood that a subject will develop the disease, relative to an untreated subject.

As used herein, the term "Stability unit" refers to a compound that promotes the stability of the conjugate, e.g., by increasing systemic retention of the Ligand when administered to a patient. A Stability unit can also increase the water solubility of the conjugate. An exemplary Stability unit is polyethylene glycol.

The term "excipient" as used herein refers to inactive or usually inert substances that are added to the formulation which do not affect the therapeutic action of the active ingredient, but serves as the vehicle or medium for the active ingredient. It may be used to provide a desired consistency, to improve stability, and/or to adjust osmolality of the composition. For the purpose of this disclosure excipients used are, but not limited to, protein stabilizing agents, buffers, polymers, solubilizers, Cryoprotectants, diluents or mixture thereof.

The term "pharmaceutically acceptable salt" of a given compound refers to salts that retain the biological effectiveness and properties of the given compound and which are not biologically or otherwise undesirable. "Pharmaceutically acceptable salts" or "physiologically acceptable salts" include, for example, salts with inorganic acids, and salts with an organic acid. In addition, if the compounds described herein are obtained as an acid addition salt, the free base can be obtained by basifying a solution of the acid salt. Conversely, if the product is a free base, an addition salt, particularly a pharmaceutically acceptable addition salt, may be produced by dissolving the free base in a suitable organic solvent and treating the solution with an acid, in accordance with conventional procedures for preparing acid addition salts from base compounds. Those skilled in the art will recognize various synthetic methodologies that may be used to prepare nontoxic pharmaceutically acceptable addition salts. Pharmaceutically acceptable acid addition salts may be prepared from inorganic or organic acids. Salts derived from inorganic acids include, e.g., hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include, e.g., acetic acid, propionic acid, gluconic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluene-sulfonic acid, salicylic acid, and the like. Likewise, pharmaceutically acceptable base addition salts can be prepared from inorganic or organic bases. Salts derived from inorganic bases include, by way of example only, sodium, potassium, lithium, aluminum, ammonium, calcium, and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary, and tertiary amines, such as alkyl amines (i.e., NH_2(alkyl)), dialkyl amines (i.e., HN(alkyl)$_2$), trialkyl amines (i.e., N(alkyl)$_3$), substituted alkyl amines (i.e., NH_2(substituted alkyl)), di(substituted alkyl) amines

(i.e., HN(substituted alkyl)$_2$), tri(substituted alkyl) amines (i.e., N(substituted alkyl)$_3$), alkenyl amines (i.e., NH_2(alkenyl)), dialkenyl amines (i.e., HN(alkenyl)$_2$), trialkenyl amines (i.e., N(alkenyl)$_3$), substituted alkenyl amines (i.e., NH_2(substituted alkenyl)), di(substituted alkenyl) amines (i.e., HN(substituted alkenyl)$_2$), tri(substituted alkenyl) amines (i.e., N(substituted alkenyl)$_3$, mono-, di- or tri- cycloalkyl amines (i.e., NH_2(cycloalkyl), HN(cycloalkyl)$_2$, N(cycloalkyl)$_3$), mono-, di- or tri- arylamines (i.e., NH_2(aryl), HN(aryl)$_2$, N(aryl)$_3$), or mixed amines, etc. Specific examples of suitable amines include, by way of example only, isopropylamine, trimethyl amine, diethyl amine, tri(iso-propyl) amine, tri(n-propyl) amine, ethanolamine, 2-dimethylaminoethanol, piperazine, piperidine, morpholine, N-ethylpiperidine, and the like.

The recombinant lectin protein conjugate of the present disclosure may be provided in a pharmaceutically acceptable form, such as a liquid (e.g in an aqueous solution or suspension, or as an oil based solution or suspension.), a solid (e.g a capsule or tablet), a lyophilized powder, a spray, cream, lotion or gel, vesicular drug delivery systems such as, but not limited to, bilosomes, liposomes, niosomes, transferosome, ethosomes, sphingosomes, pharmacosomes, multilamellar vesicles, microsphere and the like.

The terms "homology" or "homologous" as used herein refer to two or more referenced entities that share at least partial identity over a given region or portion. Areas, regions or domains of homology or identity refer to a portion of two or more referenced entities that share homology or are the same. Thus, where two sequences are identical over one or more sequence regions, they share identity in these regions. Substantial homology refers to a molecule that is structurally or functionally conserved such that it has or is predicted to have at least partial structure or function of one or more of the structures or functions (e.g., a biological function or activity) of the reference molecule, or a relevant/corresponding region or portion of the reference molecule to which it shares homology.

In one embodiment, the percentage "homology" between two sequences is determined using the BLASTP algorithm with default parameters (Altschul et al. Nucleic Acids Res. 1997 Sep 1;25(17):3389-402). In particular, the BLAST algorithm can be accessed on the internet using the URL: https://blast.ncbi.nlm.nih.gov/Blast.cgi. In an alternative embodiment, for global sequence alignments, percentage homology between two sequences is determined using the EMBOSS Needle algorithm using default parameters. In particular, the EMBOSS Needle algorithm can be accessed on the internet using the URL: https://www.ebi.ac.uk/Tools/psa/emboss_needle/.

The term "sequence identity" as used herein refers to the occurrence of exactly the same nucleotide or amino acid in the same position in aligned sequences.Unless otherwise indicated, the term "homology" is used interchangeably with the term "sequence identity" or "identity" in the present specification.

The terms "Polyalkylene glycol" or "polyoxyalkylene glycol" are used interchangeably and mean polymers of alkylene oxide. The polymer may be a homopolymer comprising same alkylene oxide, for example polymer comprising all units of ethylene oxide is homopolymer know as polyethylene glycol. The polymer may also be a copolymer comprising different alkylene oxide, for example a polymer comprising three units of ethylene oxide and three units of propylene oxide is known as copolymer. Polyalkylene glycol can be prepared by the processes known to the skilled person or can be procured commercially.

As used herein, the term "subject" refers to an animal that is the object of treatment, observation or experiment. "Animal" includes cold- and warm-blooded vertebrates and invertebrates such as fish, shellfish, reptiles and, in particular, mammals. "Mammal" includes, without limitation, mice, rats, rabbits, guinea pigs, dogs, cats, sheep, goats, cows, horses, primates, such as monkeys, chimpanzees, and apes, and, humans.

Polymorphism is the occurrence of different crystalline forms of a single compound. The term "polymorph" as used herein mean the solids sharing the same molecular formula, yet each having distinct physical properties such as different solubility profiles and/or different melting point temperatures. The polymorph of a compound can be distinguished by X-ray diffraction spectroscopy or by other methods such as, infrared spectrometry.

The term "self-immolative" can also be referred to as self-eliminating group and are chemical linkers that undergo a sequence of self-immolative reactions typically upon cleavage of one or more trigger units. Such self immolative chemical linkers are preferably based on a multifunctional unit which can be linked to both the chemical moiety and to two or more trigger units or other chemical linkers and can further be subjected to electronic cascade self-elimination. A self-immolative group can be selected as per understanding and knowledge of the skilled person or from the references including US patent application 20060269480 or "Self-immolative linkers in polymeric delivery systems" (Blencowe et al., Polymer Chemistry 2, 773-790, 2011).

When one or more molecules of solvent are trapped in the crystal of the compound, the compound is said to be solvated. The term'solvate' may be defined as a compound formed

by solvation. The solvates may be prepared by using solvents known to the skilled person. Non-limiting examples of solvents are water, alcohols such as methanol, ethanol, n-propanol, isopropanol, n-butanol, isobutanol, and t-butanol, glycols or polyalkylene glycols (e.g., polyethylene glycol, polypropylene glycol), acetic acid, formic acid, acetone, dichloromethane and/or mixture thereof. The best-known and preferred solvent is typically water, and solvate compounds formed by solvation with water are termed hydrates.

SEQUENCE REPRESENTATION:

SEQ ID NO:1: represents the native *S. rolfsii* lectin amino acid sequence(reported as SEQ ID NO:1 in WO2010/095143), has the following sequence:

TYKITVRVYQTNPNAFFHPVEKTVWKYANGGTWTITDDQHVLTMGGSGTSGTLRFH ADNGESFTATFGVHNYKRWCDIVTNLAADETGMVINQQYYSQKNREEARERQLSN YEVKNAKGRNFEIVYTEAEGNDLHANLIIG

SEQ ID NO: 2: represents a variant of the *S. rolfsii* lectin amino acid sequence (reported as Rec-2 in WO2010/095143) has the following sequence:

TYKITVRVYQTNPDAFFHPVEKTVWKYANGGTWTITDDQHVLTMGGSGTSGTLRFH ADNGESFTATFGVHNYKRWCDIVTNLAADETGMVINQQYYSQKNREEARERQLSN YQVKNAKGRNFQIVYTEAEGNDLHANLIIG

SEQ ID NO:3: represents a variant of the *S. rolfsii* lectin amino acid sequence (reported as Rec-3 in WO 2010/095143) has the following sequence:

VYKITVRVYQTNPDAFFHPVEKTVWKYANGGTWSITDDQHVLTMGGSGTSGTLRF HADNGESFTATFGVHNYKRWCDIVTNLAADETGMVINQQYYSQKNREEARERQLS NYQVKNAKGRNFQIVYTEAEGNDLHANLIIG

SEQ ID NO: 4: represents a variant of the *S. rolfsii* lectin amino acid sequence (reported in WO 2014/203261) has the following sequence:

VYKITVRVYQTNPDAFFHPVEKTVWKYADGGTWSITDDQHVLTMGGSGTSGTLRF HADNGESFTATFGVHDYKRWCDIVTDLAADETGMVINQEYYSEKDREEARERQNS NYEVKDAKGRNFEIVYTEAEGNDLHADLIIG

SEQ ID NO: 5: represents a variant of the *S. rolfsii* lectin amino acid sequence has the following sequence:

SYKITVRVYQTNPDAFFHPVEKTVWKYANGGTWTITDDQHVLTMGGSGTSGTLRFH
ADNGESFTATFGVHNYKRWGDIVTNLAADETGMVINQQYYSQKNREEARERQLSN
YQVKNAKGRNFQIVYTEAEGNDLHANLIIGC

SEQ ID NO: 6: represents a Nucleotide sequence encoding a protein of SEQ ID NO. 2

ATGACCTATAAAATTACCGTGCGCGTGTATCAGACCAACCCGGATGCCTTTTTCC
ATCCGGTGGAAAAAACCGTGTGGAAATATGCGAATGGCGGTACCTGGACGATTA
CGGATGATCAGCATGTGCTGACGATGGGTGGTAGCGGTACCAGCGGCACCCTGC
GTTTTCACGCAGATAATGGCGAAAGCTTCACCGCCACCTTTGGTGTGCATAATTA
TAAACGCTGGTGTGATATTGTGACCAACCTGGCAGCGGATGAAACCGGCATGGT
TATTAATCAGCAGTATTATAGTCAGAAAAACCGCGAAGAAGCGCGTGAACGCCA
GCTGAGTAACTATCAGGTGAAAAATGCGAAAGGCCGTAACTTCCAGATTGTTTAT
ACCGAAGCGGAAGGCAATGATCTGCATGCGAACCTGATTATCGGC

SEQ ID NO: 7: represents a Nucleotide sequence encoding SEQ ID NO. 5

AGCTATAAAATTACCGTGCGCGTGTATCAGACCAACCCGGATGCCTTTTTCCATC
CGGTGGAAAAAACCGTGTGGAAATATGCGAATGGCGGTACCTGGACGATTACGG
ATGATCAGCATGTGCTGACGATGGGTGGTAGCGGTACCAGCGGCACCCTGCGTTT
TCACGCAGATAATGGCGAAAGCTTCACCGCCACCTTTGGTGTGCATAATTATAAA
CGCTGGGGCGATATTGTGACCAACCTGGCAGCGGATGAAACCGGCATGGTTATT
AATCAGCAGTATTATAGTCAGAAAAACCGCGAAGAAGCGCGTGAACGCCAGCTG
AGTAACTATCAGGTGAAAAATGCGAAAGGCCGTAACTTCCAGATTGTTTATACC
GAAGCGGAAGGCAATGATCTGCATGCGAACCTGATTATCGGCTGC

SEQ ID NO. 8 represents a variant of the *S. rolfsii* lectin amino acid sequence

SYKITVRVYQTNPDAFFHPVEKTVWKYANGGTWTITDDQHVLTMGGSGTSGTLRFH
ADNGESFTATFGVHNYKRWGDIVTNLAADETGMVINQQYYSQKNREEARERQLSN
YQVKNAKGRNFQIVYTEAEGNDLHANLIIGSC

SEQ ID NO. 9 represents a Nucleotide sequence encoding a protein of SEQ ID NO. 8

AGCTATAAAATTACCGTGCGCGTGTATCAGACCAACCCGGATGCCTTTTTCCATC
CGGTGGAAAAAACCGTGTGGAAATATGCGAATGGCGGTACCTGGACGATTACGG
ATGATCAGCATGTGCTGACGATGGGTGGTAGCGGTACCAGCGGCACCCTGCGTTT
TCACGCAGATAATGGCGAAAGCTTCACCGCCACCTTTGGTGTGCATAATTATAAA
CGCTGGGGCGATATTGTGACCAACCTGGCAGCGGATGAAACCGGCATGGTTATT

AATCAGCAGTATTATAGTCAGAAAAACCGCGAAGAAGCGCGTGAACGCCAGCTG
AGTAACTATCAGGTGAAAAATGCGAAAGGCCGTAACTTCCAGATTGTTTATACC
GAAGCGGAAGGCAATGATCTGCATGCGAACCTGATTATCGGCAGCTGC

DETAILED DESCRIPTION OF THE DRAWINGS

FIG.1: Represents reverse phase Mass spectrum for conjugate of Formula 16. The peak 17406 confirms the formation of conjugate of Formula 16 which is also the mass of the final product Formula 16.

FIG.2: Represents reverse phase Mass spectrum for conjugate of Formula 18. The peak 17586 confirms the formation of conjugate of Formula 18which is also the mass of the final product Formula 18.

FIG.3: Represents LC/MS of Formula C with x = 8. The peak at 16041.5000 is related to L alone, whereas peaks at 16553, 17064.5 and 17576 show the formation of Formula C with DAR 1, 2 and 3, respectively. The average DAR being 1.71.

FIG.4: Represents LC/MS of Formula C with x = 12. The peaks at 16729, 17416.5 and 18104.5 show the formation of Formula C having varying DAR values 1, 2 and 3, respectively with average DAR of 1.76

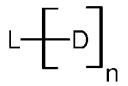
FIG.5: Expression and Solubility analysis of recombinant lectin of SEQ ID NO: 5 in production medium. The figure represents SDS-PAGEanalysis of SEQ ID NO. 5 expression at different times as given below:

Lane	Sample	Band%
1	Standard Protein Marker	NA
2	Seq ID NO: 5 0hr	-
3	Seq ID NO: 5 overnight	56
4	Seq ID NO: 5 overnight pellet	49.6
5	Seq ID NO: 5 supernatant	47.2

FIG.6: Colocalization of Recombinant Lectin having amino acid sequence of SEQ ID NO:2 (Recombinant protein) in different organelles such as mitochondria, golgi bodies, plasma membrane, nuclear envelope, endoplasmic reticulum in 1 cell line.

DETAILED DESCRIPTION

According to a first aspect of the present disclosure, there is provided a compound of Formula I:



Formula I

or a pharmaceutically acceptable salt thereof.

According to an aspect, L is a recombinant lectin derived from *Sclerotium rolfsii* (*S. rolfsii*) lectin. *S. rolfsii*is a soilborne phytopathogenic fungus and *Sclerotium rolfsii* lectin (SRL) is a lectin isolated from *S. rolfsii*. The native SRL has an amino acid sequence of SEQ ID NO: 1.

In an aspect, L is a recombinant lectin having amino acid sequence of SEQ ID NO: 1 or a recombinant lectin having at least 70% sequence identityto SEQ ID NO: 1. In some embodiments the recombinant lectin protein comprises an amino acid sequence having at least 70%, 80%, 90%, 95%, 96%, 97%, 98%or 99% homology to SEQ ID NO: 1.

In an embodiment of the present disclosure, recombinant lectin is a variant of Sclerotium rolfsii Lectin (SRL) represented by SEQ ID NO. 1. In a particular embodiment, the said recombinant lectin retains binding specificity or affinity of the Sclerotium rolfsii Lectin (SRL) represented by SEQ ID NO. 1. The said binding specificity is envisaged to be toward one or more antigens selected from Thomsen-Friedenreich (Galβ1-3GalNAcα-O-Ser/Thr, TF or T) antigen, O-GalNAc Core1 (T antigen), Core2, 'α2,3/6-sialyl Core1' (Sialyl-T antigen), 'α2,6/6-sialyl Core2' and its modified forms such as and altered expression of branched and fucosylated N- and O-glycans, including changes in Lewis antigens (SLex and SLea) which are expressed in more than 90% of human cancers. In addition to alterations in core glycans, each of these carbohydrates can be further modified to generate unique terminal glycan motifs that may also undergo specific changes following neoplastic transformation. For example, highly fucosylated glycans, such as Lewis antigens [Lewisa/b (Lea/b) and Lewisx/y (Lex/y)], can become enriched on the cell surface following neoplastic transformation. Similarly, sialylation, a common terminal glycan modification, can also undergo significant changes during neoplastic progression.

In a more particular embodiment of the present disclosure, variants or modified forms of TF (or T) antigen includes [3OSO3]Galβ1-3GalNAcα; Neu5Acα2-3Galβ1-3 [6OSO3] GalNAcα; Neu5Acα2-3Galβ1-3GalNAcα; GlcNAcβ1-3Galβ1-3GalNAcα; Galβ1-3(Galβ1-4GlcNAcβ1-6) GalNAcα; Galβ1-3(Neu5Acα2-3Galβ1-4GlcNAβ1-6)GalNAc; Galβ1-3(GlcNAcβ1-6)GalNAcα; Galβ1-3(Neu5Acα2-6)GalNAcα; Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GalNAcα; Galβ1-4GlcNAcβ1-6(Galβ1-3) GalNAcα; Galβ1-3GalNAcα (TF antigen); Neu5Acα2-6 (Galβ1-3)GalNAcα; Galβ1-3(Galβ1-4GlcNAcβ1-6)GalNAc; Galβ1-3(GlcNAcβ1-6)GalNAc;

GlcNAcβ1-6 (Galβ1-3) GalNAcα; Galβ1-3 (Neu5Acβ2-6)GalNAcα; Fucα1-2Galβ1-3GalNAcα; Neu5Acβ2-6 (Galβ1-3)GalNAcα; GlcNAcβ1-2Galβ1-3GalNAcα.

WO2010/095143 discloses recombinant lectin variants Rec-2 and Rec-3, which are derived from the native SRL sequence by the substitution of 3 or 5 amino acids respectively. The crystal structure of these variants has been reported (Peppa et al., Molecules. 2015 Jun 12;20(6):10848-65). Rec-2 and Rec-3 is mentioned as SEQ ID NO: 2 and SEQ ID NO: 3, respectively hereinafter. WO 2014/203261 discloses a recombinant lectin variant (mentioned as SEQ ID NO: 4 hereinafter) derived from the native SRL sequence by the substitution of 12 amino acids.WO2020/044296 discloses several variants of SRL with at least one amino acid modification selected from one or more of the following:

a) at least one amino acid modification in a carbohydrate binding site selected from position 27, 28, 47, 48, 70, 71, 72, 77, 78, 80, 101, 105, 112 or 114;
b) at least one amino acid modification in the N-terminus;
c) at least one amino acid modification at position 76; or
d) at least one amino acid modification at position 44 and 89.

WO 2020/044296 further discloses SRL variants with amino acid modifications, wherein the modification refers to addition of at least 1, 2, 3, 4 or 5 amino acids at a particular position in an amino acid sequence.

WO 2020/044296 is incorporated in the present application by way of reference in its entirety. Amino acid sequences from SEQ ID NO: ULLB-0005/001 to SEQ ID NO: ULLB-0005/047of WO 2020/044296 are embodied as an aspect of the present disclosure and are not repeated for the sake of brevity. In a further embodiment, SEQ ID NO: 5 disclosed in present disclosure is a variant of SEQ ID NO: 1 with modification at 1st position T1S; 76th position C76G and with addition of cysteine at C terminal end i.e. 142ndposition.

In a further embodiment, SEQ ID NO. 8 disclosed in the present disclosure is a variant of Seq ID No. 5 with an insertion of amino acid Serine at 142 position between Glycine (141 position) and Cysteine (C terminal end).

SEQ ID NO: 2, 3, 4, 5 and 8have a homology of 97.9%, 96.5%, 91.5%, 96.45% and 96.45% to SEQ ID NO:1 respectively (as determined using EMBOSS Needle).

According to an aspect the lectin proteins of the present disclosureare prepared by recombinant technology. The term “recombinant” means a nucleic acid or a polypeptide has been artificially or synthetically (i.e., non-naturally) altered by human intervention. The

alteration can be performed on the material within, or removed from, its natural environment or state. For example, a “recombinant nucleic acid” is one that is made by recombining nucleic acids, e.g., during cloning, DNA shuffling or other well-known molecular biological procedures. A “recombinant DNA molecule” is comprised of segments of DNA joined together by means of such molecular biological techniques. The term “recombinant protein” or “recombinant polypeptide” as used herein refers to a protein molecule which is expressed using a recombinant DNA technology.

In some embodiments the lectin protein of the present disclosure may be prepared by the processes disclosed in WO 2010/095143, WO 2014/203261, WO 2020/044296 or applicant’s previously filed process patent WO2020074977 or any other method known to the skilled person.

The term "recombinant protein" is intended here to cover any pharmaceutically acceptable salt, solvate, hydrate, prodrug, or any other compound which,is capable of providing (directly or indirectly) the compound as described herein. The preparation of salts, solvates, hydrates, and prodrugs can be carried out by methods known in the art. According to a specific aspect of the present disclosure, L is a recombinant protein selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 8. According to a very particular aspect, L is a recombinant protein selected from SEQ ID NO: 2 or SEQ ID NO: 5 or SEQ ID NO: 8.

In an aspect of the present disclosure, D in Formula I is a cytotoxic agent, therapeutic agent, diagnostic agent or a compound comprising cytotoxic agent or therapeutic agent.According to an aspect, D may be selected from a DNA binding agent, an anti-microtubule agent, an antimetabolite, an alkylating or alkylating-like antineoplastic agent, a topoisomerase I inhibitor, a topoisomerase II inhibitor, a kinase inhibitor, bortezomib, estramustine, Ixabepilone, everolimus, temsirolimus, neomycin, neamine, a cryptophycin, discodermolide, amanitin, or a pyrrolobenzodiazepine dimer, or pharmaceutical acceptable salt, polymorph or solvate thereof.

According to an aspect, D is selected from the DNA binding agent such as bleomycin, netropsin or distamycin or their analogues such as lexitropsins, an enediyne, a mitomycin, a duocarmycin. According to an embodiment the bleomycin may be selected from non-limiting examples of bleomycin A_2, bleomycin B_2, liblomycin, bleomycin substituted with butylamino-3-propylamino-3-propylamine (BAPP), Tallysomycin S10b (TLM S10b), or mixtures thereof, an enediyne for example, may be selected from neocarzinostatin, C-1027, golfomycin A, calicheamicins such as calicheamicin gamma 1 or calicheamicin omega 1;

esperamicins such as esperamicin A1, esperamicin A1b, esperamicin A2, esperamicin A3, esperamicin A4, esperamicin B1, esperamicin B2, and esperamicin X; dynemicin or dynemicin A or lidamycin; the mitomycin may be selected from mitomycin A, mitomycin B or mitomycin C; non-limiting examples of duocarmycin are duocarmycin A, duocarmycin B1, duocarmycin B2, duocarmycin C1, duocarmycin C2, duocarmycin D, duocarmycin SA or CC-1065 and its synthetic analogues such as adozelesin, carzelesin or bizelesin; other non-limiting examples of DNA binding agents are dactinomycin, peptide such as echinomycin and other such agents listed in 'anticancer drugs acting via radical species' (by C. A. Lopez et al. medicinal chemistry of anticancer drugs, chapter 4, pages 133 – 195, Dec 2015) or ionic liquids listed in 'synthesis, characterization, dna binding, anticancer, and molecular docking studies of novel imidazolium-based ionic liquids with fluorinated phenylacetamide tethers' (by N Rezki et al. ACS Omega, volume 5, pages 4807 – 4815, 2020).

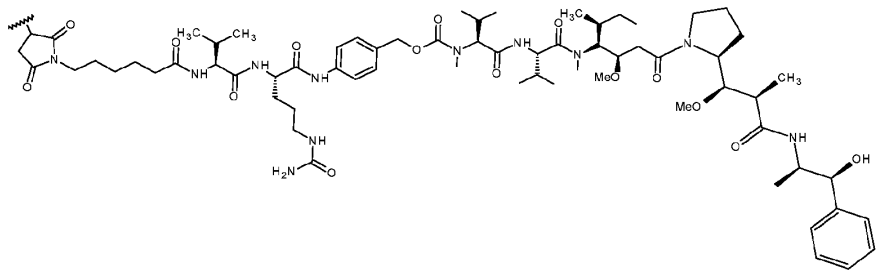
In an embodiment D may be selected from anti-microtubule agent such as an auristatin, a maytansine or its derivatives maytansinoid. These classes of compounds are well known payloads used in the synthesis of antibody-drug conjugates. Non-limiting examples of such class of compounds are dolastatin or its analogues include dolastatin 10, monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF) or PF-063801010 or any other auristatin derivatives listed in 'antibody-drug conjugate payloads, study of auristatin derivatives; (by M. Akaiwa et el. Chemical pharma bulletin, volume 68, page 201-211, 2020),maytansinoidssuch as DM1, DM4 or DM 21. Other non-limiting examples of anti-microtubule agents may be a vinca alkaloid such as vinblastine, vincristine, vindesine or vinorelbine; colchicine or its derivatives, a taxane such as paclitaxel, docetaxel or cabazitaxel, a combretastatin such as combretastatin A-4, combretastatin A-4 phosphate (CA-4P), an epothilone such as epothilones A, epothilones B, epothilones C or epothilones D, eribulin, plinabulin, demecolcine, nocodazole, eleutherobin, rhizoxin, ethidium bromide, or verubulin. In an embodiment D is selected from non-limiting examples of antimetabolite such as capecitabine, hydroxyurea, gemcitabine, methotrexate, mercaptopurine, capecitabine, hydroxyurea, gemcitabine, methotrexate, mercaptopurine, cladribine, pralatrexate, raltitrexed fludarabine, pemetrexed, thioguanine, floxuridine, cytarabine, azacitidine, decitabine, clofarabine, nelarabine, 5-fluorouracil or carmofur; or topoisomerase II inhibitor such as daunorubicin, doxorubicin or its derivatives such as morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin, epirubicin, idarubicin, mitoxantrone, valrubicin, etoposide, teniposide or anthraquinones; or topoisomerase I inhibitor such as Irinotecan, SN-38, govitecan, camptothecin, exatecan (Dxd

1), deruxtecan, topotecan; or alkylating or alkylating-like antineoplastic agent such as a nitrogen mustard such as mustine, cyclophosphamide, chlorambucil, uramustine, melphalan, and bendamustine; ethylenimine derivatives such as aziridine, dimethylenimine, ethyleneimine; or methylenamine derivative or an alkyl sulfonate such as busulfan, improsulfan or piposulfan or a nitrosourea such as arabinopyranosyl-N-methyl-N-nitrosourea, carmustine, chlorozotocin, ethylnitrosourea (ENU), fotemustine, lomustine, nimustine, N-nitroso-N-methylurea (NMU), ranimustine, semustine, streptozocin (streptozotocin); platinum-containing antineoplastic agent such as cisplatin, carboplatin, oxaliplatin, nedaplatin, and lobaplatin; kinase inhibitor such as sorafenib, crizotinib, nilotinib, gefitinib, regorafenib, afatinib, lapatinib, vatalanib, aflibercept, axitinib, ponatinib, sunitinib, pazopanib, bosutinib, dasatinib, erlotinib, imatinib, ruxolitinib, lestaurtinib, AC-430, AC-480, AMG-458, or vandetanib; Pyrrolobenzodiazepine dimer such as tesirine, SG2202, SG3199, talirine, SG2000, SJG-136, SG2285, or SG3376 or pharmaceutical acceptable salt, polymorph or, solvate thereof.

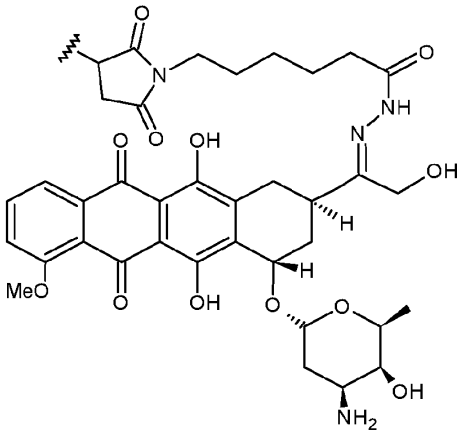
In an embodiment, D may further be selected from non-limiting examples of bortezomib, sutent, letrozole, fulvestrant, leucovorin sirolimus, lonafarnib, a camptothecin (including the synthetic analog topotecan); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); eleutherobin; pancratistatin; spongistatin; chlorambucil, chlornaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, melphalan, prednimustine, trofosfamide, chromophore (chromoprotein enediyne antibiotic chromophores), aclacinomysins, authramycin, azaserine, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, marcellomycin, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, streptonigrin, tubercidin, ubenimex, zinostatin; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; aceglatone; aldophosphamide glycoside; eniluracil; amsacrine; bestrabucil; bisantrene; demecolcine; diaziquone; an epothilone; etoglucid; gallium nitrate; hydroxyurea; razoxane; sizofiran; spirogermanium; triaziquone; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); mitobronitol; mitolactol; pipobroman; arabinoside ("Ara-C") 6-thioguanine; difluoromethylornithine (DMFO); retinoids such as retinoic acid; dacarbazine. D may be used as such or as its salt or derivatives. D may be its most active isomer including stereoisomer or any other isomer having moderate to high cytotoxicity. D may be also

selected from toxins (ricin, Abrin, Pesudomonas Exotoxin PE) or peptides (WT1, GV1001, PAP-114-128, Erbb-2, TCP-1, ANP, F56) or protacs (BETd-260, MZ1ARV-771)having cytotoxic effect that are well known to the skilled person.

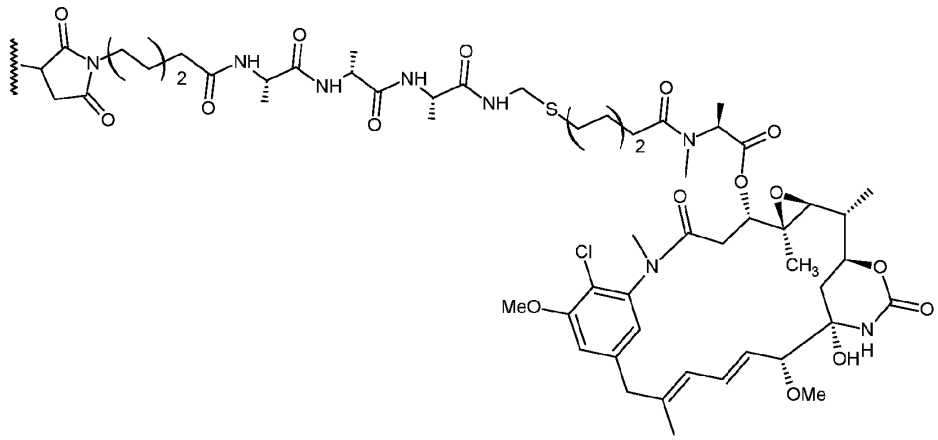
In an embodiment D may be selected from the compounds comprising a cytotoxic agent, therapeutic agent or a diagnosticagent. Such compounds may comprise a linker or part of the linker along with cytotoxic agent or therapeutic agent. Such linker or part of the linker would aid in attachment of D to L. Some of the non-limiting examples of such compounds comprising cytotoxic agents or therapeutic agent are given below, wherein M is maleimide, C is caproyl group, V, C, A, G, F are amino acids valine, citrulline, alanine, glycine, phenylalanine and PAB is para-aminobenzyloxy carbonyl. N-succinimidyl-4-thio butanoate - DM4 is formed when DM4 is reacted with a linker such as N-Succinimidyl-4-(2-pyridyldithio) Butanoate (SPDB), N-succinimidyl 4-(maleimidomethyl)cyclohexanecarboxylate (SMCC).



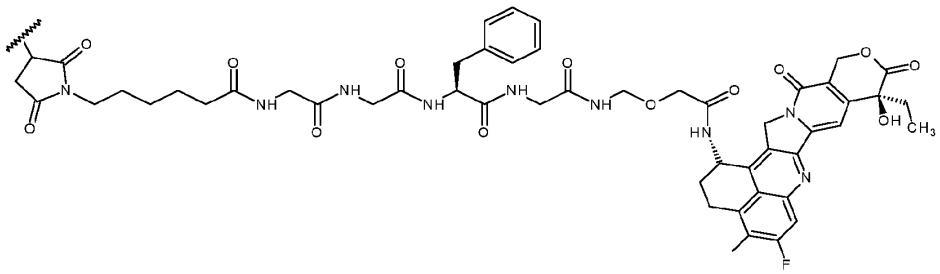
MC-VC-PAB-MMAE;



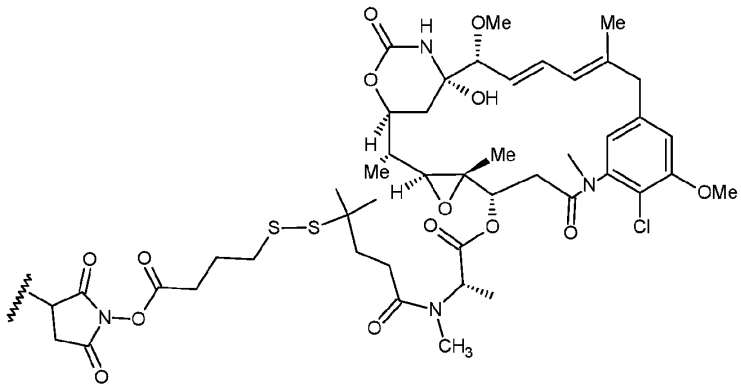
mc-hydrazone-Doxorubicin;



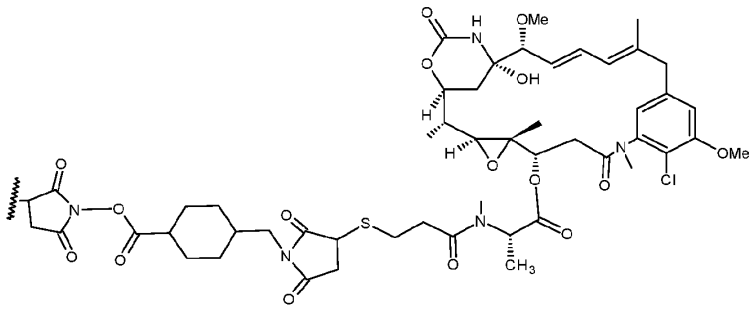
SC-AA'A-DM21-C (wherein A' is D-alanine and A is L-alanine);



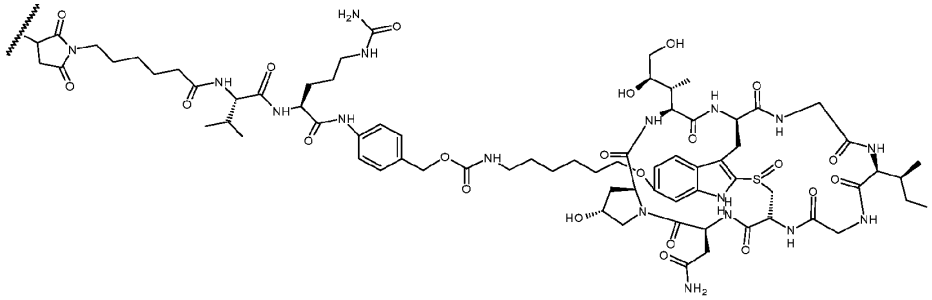
MC-GGFG-NH-CH_2-Exatecan;



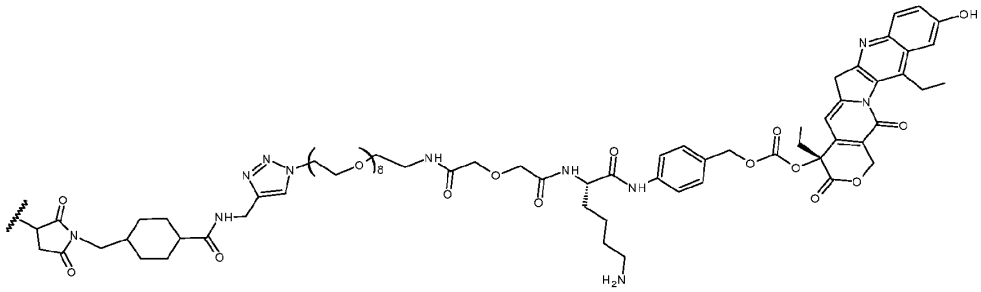
N-succinimidyl-4-thio butanoate -DM4;



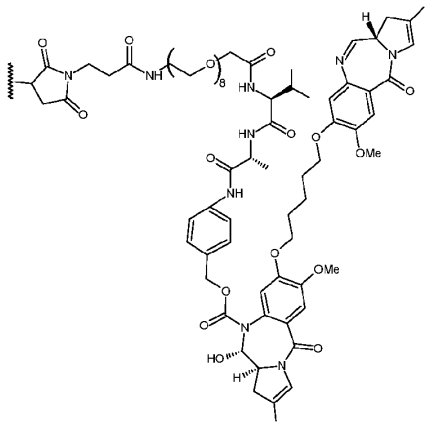
SMCC-DM1;



mc-vc-PAB-C6-α-Amanitin;



Linker- SN38 or Govitecan;



Linker- SG3249 or Tesirine

In a particular embodiment the cytotoxic agents are selected from but not limited to MMAE (monomethyl Auristatin E), Doxorubicin, DM1, DM4, DM21, alpha Amanitin, DXd1, SG3249, SN38, linked to linker selected from but not limited to mc-vc-PAB (melamide Caproic-valine citrulline-Para aminobenzoate), SMCC (Succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, SPDB (N-Succinimidyl-4-(2-pyridyldithio) Butanoate), mc-vc-PAB-C6(melamide Caproic-valine-citrulline-Para aminobenzoate-C6), mc-hydrazone.

According to an aspect of the present disclosure the compound D, may be prepared by any process known in the art. For the purpose ofthe present disclosure D was procured commercially.

The lectin protein, L may be linked to D or to A^1 or to A^2 in the absence of A^1*via* its free amino moiety (-NH- or $-NH_2$ group) of amino acids such as lysine, arginineor its free thiol moiety (-SH group) of amino acid such as cysteine or its free acid (-COOH) group of amino acid such as aspartic acid or its free OH group present in amino acids such as serine. It will be appreciated by the skilled person that all these groups on lectin may form a bond with reactive counterparts on D or A^1 or A^2 in the absence of A^1, thus resulting in more than one unit of D or $-A^1-A^2-A^3-D$ linked to one unit of L.

In an aspect of the present disclosure, one unit of lectin protein, L may be linked to at least one unit of D. In an embodiment one unit of lectin protein may be linked to more than one unit of D. In another embodiment one unit of lectin protein may be linked to at least 2, 3, 4, 5, 6, 7, 8, or 9 units of aD. In antibody drug conjugates such ratio of drug to antibody are called as drug antibody ratio (DAR). In the present disclosure drug to lectin ratio is known as DAR

and in an embodiment, DAR may be in the range from about 1 to about 9, wherein DAR may be integer or a non-integer. For example, DAR value can be 2 or 2.5, which may indicate some of the units of lectin may be linked to more than 2 units of cytotoxic agent or therapeutic agent. In an embodiment of the present disclosure DAR may be preferably in the range from about 1 to about 5.

According to an aspect, the DAR value may be determined by the methods known to the skilled person.Determination of the mass of the product formed could be one of the methods to determine DAR value. It will be appreciated by the person skilled in the art that the mass of the product may be determined by chemical analysis or mass spectrometry or other such methods such as liquid chromatograpy mass spectrometry (LCMS). If the mass of the product calculated (by addition of masses of all the atoms in the product) with varying units of cytotoxic agent or therapeutic agent and the analysed mass indicates the mass of particular product with particular units of cytotoxic agent or therapeutic agent, then that is concluded as DAR. For example the mass spectrometry shows peak at mass value that corresponds to mass of lectin protein with 2 units of cytotoxic agent, then the DAR is 2. As understood by the skilled person, there may be more than one peak on mass spectrum indicating different DAR, in such case DAR is determined by the relative percentage of different drug containing species to give an average value.

In an aspect of the present disclosure the bond "—" between L and D may be a covalent bond or a coordinate covalent bond. As understood by the skilled person a 'covalent bond' is a bond that is formed between two atoms by sharing of the electron pair. A 'coordinate covalent bond' is a covalent bond formed between to atoms, wherein the shared pair of electrons is donated by only one atom. The type of bond formed would depend on both Lectin protein L and D. In a preferred embodiment the bond "—" between L and D is a covalent bond.

According to another aspect, the number of "—" would depend on the DAR value n in Formula I. In some embodiment the value of n for D will be same as value of n for "—"

According to another aspect of the present disclosure the bond "—" between L and D may be a group for formula "—A^1-A^2-A^3— ", wherein the formula"A^1-A^2-A^3" is a linker between L and D and wherein L forms a bond with A^1 and D forms a bond with A^3. The bond between L and A^1 and D and A^3 may be covalent bond or coordinate covalent bond. In preferred embodiments the bond between L and A^1 and D and $A^3$is a covalent bond.

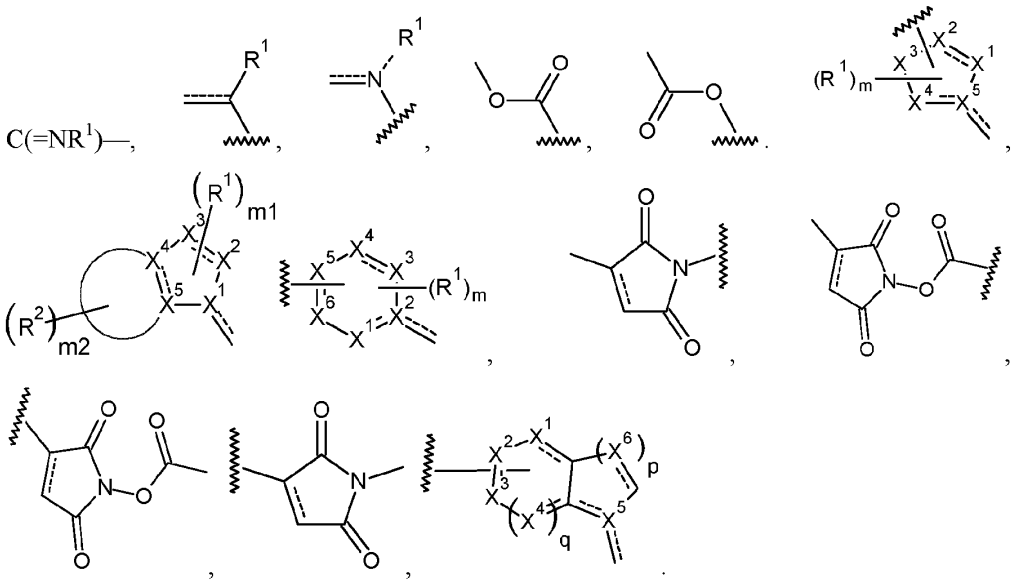
The group for formula "—A^1-A^2-A^3—" is said to be a linker between L and D. It is understood by the skilled person that the linker may be included in the products of the present

disclosurefor several purposes. For example, the linker is added to decrease steric hindrance between the lectin and the agent D or to make the products more stable or soluble or for the safety of the products for therapeutic use so that the products are not disintegrated in the body before reaching their target or combination thereof.

The linker for the present disclosure may be selected or prepared as per the understanding of the skilled person or using the references such as Linkers having crucial role in antibody drug conjugate (Jun Lu et al., Int. J. Mol. Sci 17, 561, 2016,); Current ADC linker chemistry (NareshkumarJain et al., Pharm Res 32: 3526-3540, 2015,); Perspectives about self-Immolative drug delivery systems (Rodrigo et al., Journal of Pharmaceutical Sciences 109, , 3263-3281, 2020); Self hydrolysingmaleimides improve the stability and pharmacological properties of anti-body drug conjugates (Robert et al., Nature Biotechnology, 32, Number 10, 2004).

In an aspect of the present disclosure, A^1 and A^3 is independently selected from:

—CR^1R^2—, —C(O) —, —C(O)NR^1—, —NR^1C(O) —, —NR^1—, —O—, —S—, —S(O) —, —$S(O)_2$—, —$R^1C{=}CR^2$—, —C≡C—, —$R^1C{=}CR^2$-$R^3C{=}CR^4$—, —$R^1C{=}C{=}CR^2$—, —C(=NR^1)—,



According to an embodiment of the present disclosure, A^1 and A^3 may be same or different.

According to another embodiment m, m1 and m2 areindependently 0, 1, 2, 3, or 4, wherein m, m1 or m2 = 0 indicates the substituent R^1 or R^2 is absent and m, m1, or m2 = 1 indicates one R^1 or R^2 present as substituent on the ring and so on for m, m1 or m2 = 2, 3, or 4. It

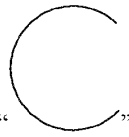
would be appreciated by the skilled person that the value of m, m1, and m2 are independent of each other.

According to yet another embodiment, p is 1 or 2 and q is 0 or 1. When p is 1, the aromatic, cyclic, or non-aromatic ring is 5 membered and when p is 2 the ring is 6 membered. Similarly, when q is 0 the aromatic, cyclic or non-aromatic ring is 5 membered and when its 1, the ring is six membered.

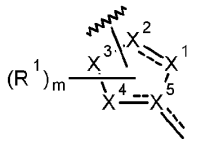
According to an embodiment of the present disclosure"$\sim\sim\sim$" indicates the point of attachment of A^2 to A^1 or A^3. It will be obvious to the skilled person that the other terminal of A^1 and A^3 is connected to L and D respectively.

According to another aspect, "$=$" indicates single or double bond.

It will be appreciated by the skilled person that unless otherwise mentioned the valency of the atom will be satisfied by addition or removal of the hydrogen on or from atom.

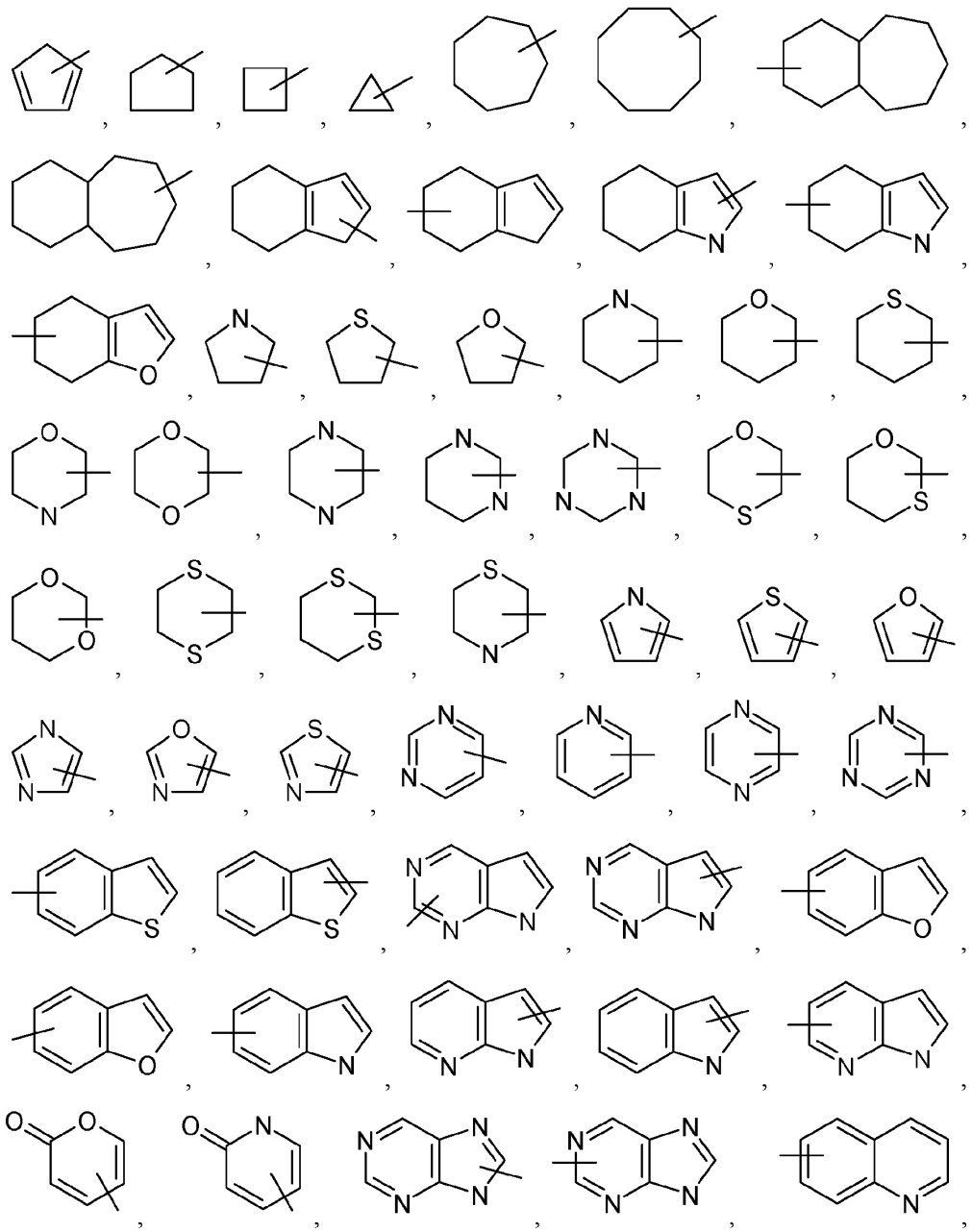
"" indicates a 5 to 10 membered ring including atoms of the ring it is fused to, wherein the 5 to 10 membered ring is an aromatic, non-aromatic, or cycloalkyl ring or a heteroaromatic or heterocycloalkyl ring wherein the heteroaromatic or heterocycloalkyl rings comprise atleast one heteroatom selected from N, O or S .

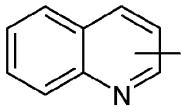
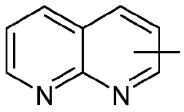
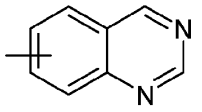
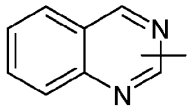
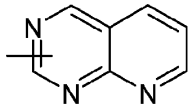
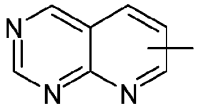
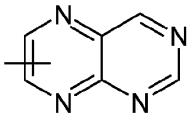
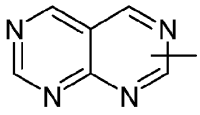
The atoms are depicted as X^1, X^2, X^3, X^4, X^5 and X^6 are independently selected from C, CH, CH_2, N, NH, O or S. It would be appreciated by the skilled person that all the Xs could be same or different and the number of hydrogen on the X will be adjusted as per its valency and the number of bonds it forms.



For example, in a compound of formula, among 5 Xs, 2 Xs may be N or NH and 3 may be C, CH or CH_2. The skilled person is well aware of such interpretations and would understand and consider such variables as an embodiment of the present disclosure.

In A^1 and $A^3$the cyclic, heterocyclic, aromatic or heteroaromatic compounds can be selected as per the knowledge and understanding of the skilled person. Some of the examples of such compounds are phenyl, benzyl, toluene, naphthyl, cyclohexyl,

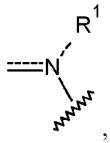
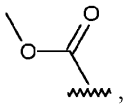
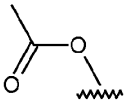
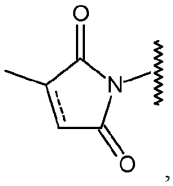
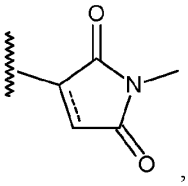


, , , ,
, , , ,

It would be appreciated by the skilled person that the bond shown in the above structure is the bond between D and the structure. The bond with D may be with any carbon or heteroatom if the above structures. The valency of the Carbon and heteroatom would be satisfied by the hydrogen on the same.

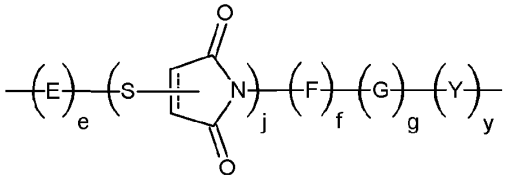
In an aspect of the present disclosure, R^1, R^2, R^3 and R^4 in A^1 and A^3 are independently selected from hydrogen, C_1 to C_5 alkyl, C_2 to C_5 alkenyl, C_2 to C_5 alkynyl, C_1 to C_5 alkylene, C_3 to C_{10} cycloalkyl, C_6 to C_{10} aryl, C_2 to C_9 heteroaryl, $-OR^5$, $-NR^5$ $-SR^5$, halogen selected from fluorine, chlorine, bromine or iodine, $-C(O)OR^5$, wherein C_1 to C_5 alkyl, C_2 to C_5 alkenyl, C_2 to C_5 alkynyl, C_1 to C_5 alkylene, C_3 to C_{10} cycloalkyl, C_2 to C_9 heterocycloalkyl, C_6 to C_{10} aryl, C_2 to C_9 heteroaryl is further substituted by R^6, and R^5 and R^6 is hydrogen, C_1 to C_3 alkyl, OH, or halogen.

According to one aspect, A^1 and A^3 may be selected from

—CR^1R^2—, —C(O) —, —NR^1—, —S—, , , ,
, , wherein R^1, R^2, " wwwww " and " ===== " are same as defined above.

According to another more preferred aspect, A^1 may be —C(O) —.

According to an aspect of the present disclosure, A^2 in group "—A^1-A^2-A^3—" is of the formula:



In an aspect, e, j, f, g and y is independently either 0 or 1. It would be appreciated by the skilled person that whenever e, j, f, g and y are 0, the corresponding groups in A^2 are absent. According to a specific aspect of the present disclosure'e', 'f' and 'y' are not 0 simultaneously, and therefore the groups E, F and Y cannot be absent in a single product of formed according to this aspect. A^1 in the present disclosure forms a bond with E or S or F and A^3 is connected to Y or G.

According to an aspect, S in A^2 is a sulphur atom and E, F and Y is a spacer. The spacer when present, extends the framework of the Linker unit to provide more distance between the lectin protein and the agent D. The spacer can also act to alter the physiochemical properties of the final products of the present disclosure depending on components of the spacer. The spacer may be added in order to increase the solubility of the product and may comprise one or multiple solubility-enhancing groups such as ionic groups or water-soluble polymers. Water-soluble polymers typically include any segment or polymer that is soluble in water at room temperature and includes poly(ethylene) glycol groups as well as other polymers such as polyethyleneimines.

In an aspect of the present disclosure, each spacer E, F and Y is independently selected from —R^7—, —S—, —NH—, —R^7-N-R^8—, —R^8-NC(O)-R^7—, —R^8-C(O)N-R^7—, —C(O)—, —C(O)-R^7—, —R^7-C(O)—, —R^8-C(O)-R^7—, —S-R^7—, —R^7-S—, —R^7-S-R^8—, —S(O)—, —S(O)-R^7—, —R^7-S(O)—, —R^7-S(O)-R^8—, —O-R^7—, —R^7-O—, —R^8-O-R^7—, —C(O)O—, —OC(O)-R^7—, —R^7-OC(O)—, — R^8-OC(O)-R^7—, —R^7-OC(O)O—, —OC(O)O-R^7—, —R^7-OC(O)O-R^8—, —NH-R^7-NH—C(O)R^8—, —(CH_2)S-NH-R^7—, —R^7-C(O)N$(CH_2)_{1\text{-}3}$O$(CH_2)_{1\text{-}3}$C(O)—, —R^7-NC(O)$(CH_2)_{1\text{-}3}$O$(CH_2)_{1\text{-}3}$C(O)—, —Si(R^7R^8)—, polyalkylene glycol optionally attached through oxygen to —R^7—, —S—, —NH—, —R^7-N-R^8—, —R^8-NC(O)-R^7—, —R^8-C(O)N-R^7—, —C(O)—, —C(O)-R^7—, —R^7-C(O)—, —R^8-C(O)-R^7—, —S-R^7—, —R^7-S—, —R^7-S-R^8—, —S(O)—, —S(O)-R^7—, —R^7-S(O)—, —R^7-S(O)-R^8—, —O-R^7—, —R^7-O—, —R^8-O-R^7—, —C(O)O—, —OC(O)-R^7—, —R^7-OC(O)—, — R^8-OC(O)-R^7—, —R^7-OC(O)O—, —OC(O)O-R^7—, —R^7-OC(O)O-R^8—, —NH-R^7-NH—C(O)R^8—, —(CH_2)S-NH-R^7—, —R^7-C(O)N$(CH_2)_{1\text{-}3}$O$(CH_2)_{1\text{-}3}$C(O)—, —R^7-NC(O)$(CH_2)_{1\text{-}3}$O$(CH_2)_{1\text{-}3}$C(O)—, —Si(R^7R^8)—.

According to one preferred embodiment, E may be selected from —R^7-N-R^8—, —R^8-NC(O)-R^7—, —R^8-C(O)N-R^7—, —C(O)-R^7—, —R^7-C(O)—, —R^8-C(O)-R^7—, polyalkylene glycol optionally attached through oxygen to —R^7-N-R^8—, —R^8-NC(O)-R^7—, —R^8-C(O)N-R^7—, —C(O)-R^7—, —R^7-C(O)—, —R^8-C(O)-R^7—.

In an embodiment, R^7andR^8 in E, F or Yis independently selected from hydrogen, —C_1-C_{10} alkylene, —C_1-C_{10}alkenylene, C_5 to C_{10} saturated or unsaturated cycloalkylene, —C_1-C_{10} alkenylene-C_5 to C_{10} saturated or unsaturated cycloalkylene,, C_5 to C_{10} arylene,—C_1-C_{10} alkenylene-C_6 to C_{10} arylene, C_2 to C_9 heteroarylene, —C_1-C_{10} alkenylene-C_2 to C_9 heteroarylene; and wherein —C_1-C_{10} alkylene, —C_1-C_{10}alkenylene, C_5 to C_{10} saturated or unsaturated cycloalkylene,—C_1-C_{10} alkenylene-C_5 to C_{10} saturated or unsaturated cycloalkylene, C_5 to C_{10} arylene,—C_1-C_{10} alkenylene-C_6 to C_{10} arylene, C_2 to C_9 heteroarylene and —C_1-C_{10} alkenylene-C_2 to C_9 heteroarylene is optionally further substituted with -OH, -C(O), -SH, -NH_2 halogen, C_1 to C_5 alkyl, -NO_2, -CN. The definitions and meaning of terms alkylene, alkenylene, cycloalkylene, heteroarylene are known to the skilled person or can be understood as defined hereinbefore.

In another embodiment, polyalkylene glycol in E, F or Ymay be a homopolymer or copolymer of alkylene with each unit of alkylene having C_1 to C_8 carbon atoms and total number of alkylene units ranging from 1 to 20. In a preferred embodiment each unit of alkylene may range from $C_2$to C_5 and the alkylene units may be in the range from 1 to 15.

According to an aspect when polyalkylene glycol is E the -CH_2- end is attached to A^1 and when polyalkylene glycol is F the -CH_2- end is attached to —R^7—, —S—, —NH—, —R^7-N-R^8—, —R^8-NC(O)-R^7—, —R^8-C(O)N-R^7—, —C(O)—, —C(O)-R^7—, —R^7-C(O)—, —R^8-C(O)-R^7—, —S-R^7—, —R^7-S—, —R^7-S-R^8—, —S(O)—, —S(O)-R^7—, —R^7-S(O)—, —R^7-S(O)-R^8—, —O-R^7—, —R^7-O—, —R^8-O-R^7—, —C(O)O—, —OC(O)-R^7—, —R^7-OC(O)—, — R^8-OC(O)-R^7—, —R^7-OC(O)O—, —OC(O)O-R^7—, —R^7-OC(O)O-R^8—, —NH-R^7-NH—C(O)R^8— or —(CH_2)S-NH-R^7—, —R^7-C(O)N$(CH_2)_{1\text{-}3}$O$(CH_2)_{1\text{-}3}$C(O)— or —R^7-NC(O)$(CH_2)_{1\text{-}3}$O$(CH_2)_{1\text{-}3}$C(O)—.

In some aspects of the present disclosure, A^1 and E or A^3 and Y may combine to form a single unit. In such a situation it might be difficult to specify and differentiate between A^1 and E or A^3 and Y. Such situations are well within the understanding and ambit of the knowledge of the skilled person.

In some aspects of the present disclosure the spacer unit F and Y may comprise a cleavable unit. In another aspect F and Y may be linked by a cleavable unit such as G. The cleavable

unit is capable of forming a cleavable bond in the linker. Such bond may be formed with spacer Y or with A^3. In an embodiment in the absence of Y or A^3, a cleavable bond is formed directly with agent D.

Reactive groups for forming cleavable bonds can include, for example, sulfhydryl groups to form disulfide bonds, aldehyde, ketone, or hydrazine groups to form hydrazone bonds, carboxylic or amino groups to form peptide bonds, and carboxylic or hydroxy groups to form ester bonds. The nature of the cleavable unit can vary widely. For example, cleavable linkers include disulfide containing linkers that are cleavable through disulfide exchange, acid-labile linkers that are cleavable at acidic pH, and linkers that are cleavable by enzymes such as hydrolases, peptidases, esterases, and glucoronidases. The cleavable unit can comprise one or multiple cleavage sites.

In some embodiments of the present disclosure, the cleavable unit is G. G may comprise one amino acid or one or more sequences of amino acids. In a particular embodiment G is a peptide chain comprising 2, 3, 4, 5, 6, 7, 8, 9, 10. 11 or 12 amino acids. Thus, G may be a monopeptide, a dipeptide, tripeptide, tetrapeptide, pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide, decapeptide, undecapeptide or dodecapeptide unit. Each amino acid can be natural or unnatural and/or a D- or L-isomer provided of course that there is a cleavable bond. In some embodiments, the G may comprise only natural amino acids. In some aspects, the G may comprise 1 to 12 amino acids in contiguous sequence.

In some embodiments, each amino acid is independently selected from the group consisting of alanine, arginine, aspartic acid, asparagine, histidine, glycine, glutamic acid, glutamine, phenylalanine, lysine, leucine, serine, tyrosine, threonine, isoleucine, proline, tryptophan, valine, cysteine, methionine, selenocysteine, ornithine, penicillamine, β-alanine, aminoalkanoic acid, aminoalkynoic acid, aminoalkanedioic acid, aminobenzoic acid, amino-heterocyclo-alkanoic acid, heterocyclo-carboxylic acid, citrulline, statine, diaminoalkanoic acid, and derivatives thereof.

In another embodiment, each amino acid is independently selected from the group consisting of the following L-(natural) amino acids: alanine, arginine, aspartic acid, asparagine, histidine, glycine, glutamic acid, glutamine, phenylalanine, lysine, leucine, serine, tyrosine, threonine, isoleucine, tryptophan, and valine.

In another embodiment, each amino acid is independently selected from the group consisting of the following D-isomers of these natural amino acids: alanine, arginine, aspartic acid, asparagine, histidine, glycine, glutamic acid, glutamine, phenylalanine, lysine, leucine, serine, tyrosine, threonine, isoleucine, tryptophan, and valine.

In certain embodiments, G can comprise only natural amino acids. In other embodiments, G can comprise only non-natural amino acids. In some embodiments, G can comprise a natural amino acid linked to a non-natural amino acid. In some embodiments, G can comprise a natural amino acid linked to a D-isomer of a natural amino acid.

In some embodimentsY and A^3independently or in combination may comprise multiple self-immolative or non-self immolative groups. In this context, the term "self-immolative group" refers to a bifunctional chemical moiety that is capable of covalently linking together two spaced chemical moieties into a normally stable tripartite molecule. It will spontaneously separate from the second chemical moiety if its bond to the first moiety is cleaved.

In some embodiment of the present disclosure, E and F may be a non-cleavable unit, which is capable of linking a cytotoxic agent or a therapeutic agent to a recombinant lectin protein in a generally stable and covalent manner and is substantially resistant to acid-induced cleavage, light-induced cleavage, peptidase- or esterase-induced cleavage, and disulfide bond cleavage. Cytotoxic agent or therapeutic agent is released from the products of present disclosure containing non-cleavable unit via alternative mechanisms, such as proteolytic ligand degradation.

In some embodiment of the present disclosure, non-cleavable unit may comprise a maleimidomoiety or a moiety selected from but not limited to N-succinimidyl 4-(maleimidomethyl)cyclohexanecarboxylate (SMCC), N-succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxy-(6-amidocaproate), which is a "long chain" analog of SMCC (LC-SMCC), κ-maleimidoundecanoic acid N-succinimidyl ester (KMUA), γ-maleimidobutyric acid N-succinimidyl ester (GMBS), c-maleimidocaproic acid N-hydroxysuccinimide ester (EMCS), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), N-(.alpha.-maleimidoacetoxy)-succinimide ester [AMAS], succinhnidyl-6-(β-maleimidopropionamido)hexanoate (SMPH), N-succinimidyl 4-(p-maleimidophenyl)-butyrate (SMPB), and N-(p-maleimidophenyl)isocyanate (PMPI), N-succinimidyl-4-(iodoacetyl)-aminobenzoate (STAB), N-succinimidyl iodoacetate (SIA), N-succinimidyl bromoacetate (SBA) and N-succinimidyl 3-(bromoacetamido)propionate (SBAP).

In a second aspect of present disclosure, there is provided a compound of formula II:

L-D

Formula II

or a pharmaceutically acceptable salt thereof, wherein

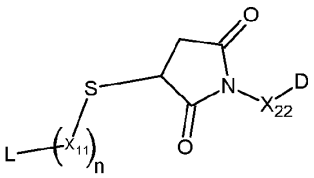
L and D are as defined hereinbefore.

According to a specific aspect of the present disclosure, L is a recombinant protein selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4 and SEQ ID NO: 5. According to a more particular aspect, L is a recombinant protein having amino acid sequence of SEQ ID NO: 5.

According to an aspect the bond "—" between L and D is a covalent bond or co-ordinate covalent bond. According to another aspect the bond "-" between L and D is a group for formula "—A^1-A^2-A^3— ", wherein A^1, A^2 and A^3 are as defined above.

According to an aspect, DAR value for Formula II is 1. Thus, each unit of Formula II comprise one unit of lectin protein and one unit of cytotoxic agent or therapeutic agent.

In one embodiment, a compound of Formula II is;



Formula II

wherein,

L is a recombinant lectin derived from *S. rolfsii*;

D is a Drug or a therapeutic agent.

S is a Sulphur atom;

X_{11} is a group comprising:

a. a group selected from an amine reactive group or sulfhydryl-reactive group;
b. optionally a hydrophilic polymer;
c. a stretcher, wherein S of Formula III is attached to the stretcher;

X_{22} is a group comprising:

a. amine-reactive group, sulfhydryl-reactive group, hydroxyl reactive group or carbonyl reactive group
b. optionally a peptide having 2 to 10 amino acids sequence
c. optionally a hydrophilic polymer;
d. a stretcher;

wherein the amine reactive group, sulfhydryl-reactive group, hydroxyl reactive group, carbonyl reactive group hydrophilic polymer and peptide is as claimed in any of the preceding claims; and

wherein stretcher comprises one or more than one groups selected from R^3, -NR^3R^4, -$NHR^3C(O)R^4$-, -NH-$R^3C(O)$-NHR^4, -NH-(CH_2CH_2O)S-, -NH-(CH_2CH_2O)S-CH_2-, -

NH-(CH_2CH_2NH)S-(CH_2)S-NH-(CH_2CH_2NH)S-(CH_2)S-NH-C(O)-(CH_2)S-NH-(C_3-C_8carbocyclo)-, -NH-(aryl-)-, -NH-(C_3-C_8heterocyclo-)-, -R^3C(O)-, -O(CO)R^3-, -O(CO)R^3NH-, -O(CO)R^3SR^4-, -O(CO)R^3-SR^4CO-NR^3R^4-, C_3-C_8carbocyclo-, -C_3-C_8 carbocyclo-CO-NHR^3-, -C_3-C_8 carbocyclo-CO-N$HR^3$$C_3$-$C_8$heterocyclo-, -OCO($C_3$-$C_8$carbocyclo)-, -OCO($C_3$-$C_8$carbocyclo)-$R^3$-, -OCO($C_3$-$C_8$carbocyclo)-$R^3$-CO-N$R^3R^4$.

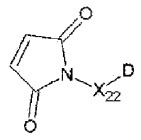
R^3 andR^4 is selected from hydrogen, C_1-C_{10} alkyl, C_1-C_{10} alkelene, C_1-C_{10} alkylene, C_6-C_{10} aryl, C_3-C_8 heteroaryl, C_3-C_8carbocyclyl,

wherein alkyl, alkelene and alkylene is straight or branched chain, and

wherein C_1-C_{10} alkyl, C_1-C_{10} alkelene, C_1-C_{10} alkylene, C_6-C_{10} aryl, C_3-C_8 heteroaryl, C_3-C_8carbocyclyl is further substituted by R^6, wherein R^6 is Oxo.

In one of the embodiments, the compound of formula II is prepared as below steps:

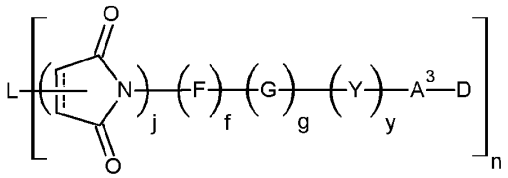
a. reaction of recombinant lectin protein (L) with precursor of linker X_{11}-S-A at room temperature for 60 minutes to produce L-X_{11}-S-A, A is a good leaving group;
b. reaction of L-X_{11}-S-A as prepared in step a) with compound of below formula IIIA to form recombinant lectin Drug conjugate of Formula II



Formula IIIA;

c. purification of compound of Formula II.

In a third aspect of the present disclosure, there is provided a compound of Formula III:



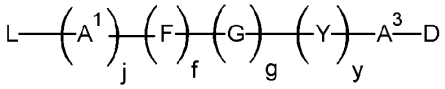
Formula III

or a pharmaceutically acceptable salt thereof, wherein

L, F, G, Y, A^3, D, n, j, f, g and y are as defined herein before.

According to an aspect, the lectin protein L is directly linked to succinimide or maleimide group *via* its free amino, thiol, acid or hydroxy group. More preferably L is linked to succinimide or maleimide group *via*Sulphur of thiol group present in cysteine.

According to a fourth aspect of the present disclosure there is provided a compound of Formula IV:



Formula IV

or a pharmaceutically acceptable salt thereof, wherein

L, A^1, F, G, Y, A^3, D, j, f, g and y are as defined herein before.

According to an aspect, A^1 in Formula IV may be present when j is 1 or absent when j is 0. L is directly linked to F in the absence of A^1. As defined above j, f, g and y can be 0 or 1. In an embodiment s, f and y cannot be 0 in a single compound of Formula IV. In another embodiment j and f cannot be 0 together and f and y cannot be 0 together. In the absence of Y, A^3 may be directly linked to G or F and in the absence of F, A^1 may be directly linked to G or Y.

In some aspects, compounds of Formula I, II, III, IV have a DAR of about 1 to about 2. In some aspects, compounds of Formula I, II, III, IV have a DAR of about 1 to about 1.5. In some aspects, compounds of Formula I, II, III, IV have a DAR of about 1. In some aspects, compounds of Formula I, II, III, IV have a DAR of about 2.

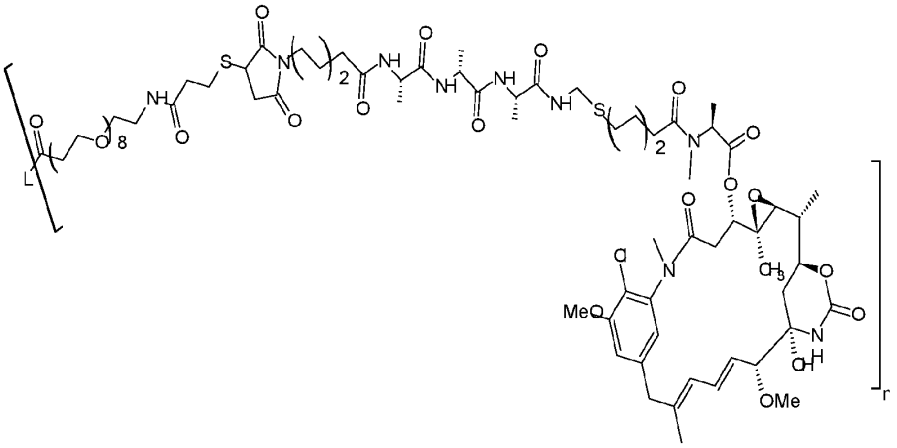
Compounds of this disclosure may have one or more asymmetric centres. Unless otherwise indicated, all chiral (enantiomeric and diastereomeric) and racemic forms of compounds of the present disclosure are included in the present disclosure. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds, and all such stable isomers are contemplated in the present disclosure. Cis and trans geometric isomers of the compounds of the present disclosure are described and may be isolated as a mixture of isomers or as separated isomeric forms. The present compounds can be isolated in optically active or racemic forms.

The compounds of this disclosure include all conformational isomers (e.g., cis and trans isomers). The compounds of the present disclosure have asymmetric centres and therefore exist in different enantiomeric and diastereomeric forms. This disclosure relates to the preparation and use of all optical isomers and stereoisomers of the compounds of the present disclosure and to all pharmaceutical compositions and methods of treatment that may employ

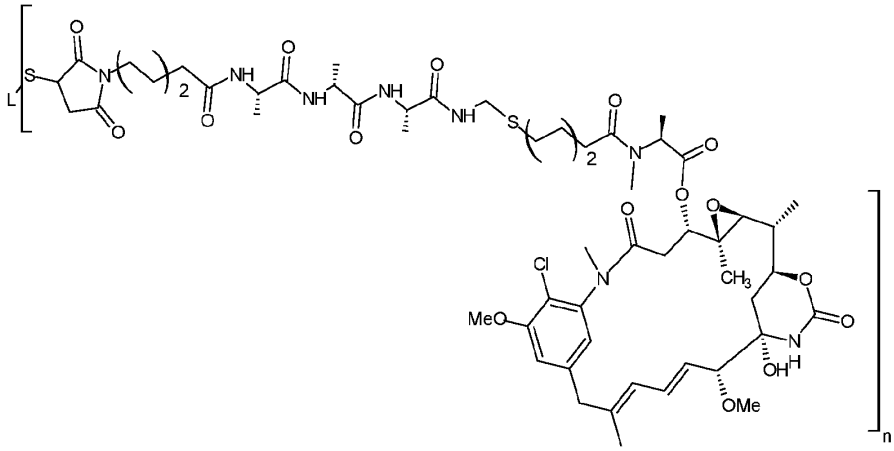
or contain such isomers. In this regard, the disclosure includes both the E and Z configurations. The compounds of present disclosure may also exist as tautomer. This disclosure includes all such tautomers.

In one embodiment of the present disclosure, the use of compound as represented in formula I-IV for the treatment or prevention or cure of cancer or tumor.

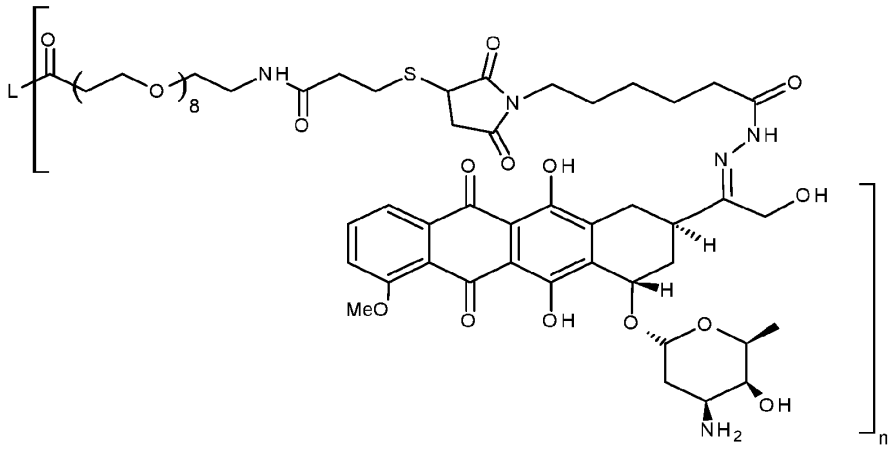
Exemplarily, some of the compounds of present disclosure are compound of Formula 1 to Formula 43.



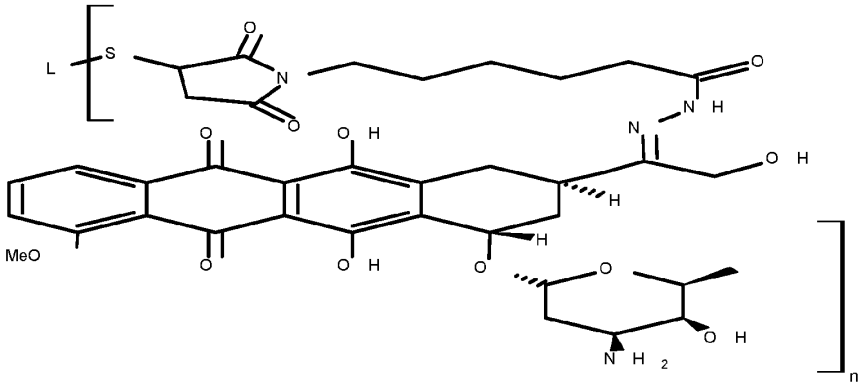
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Formula 2;

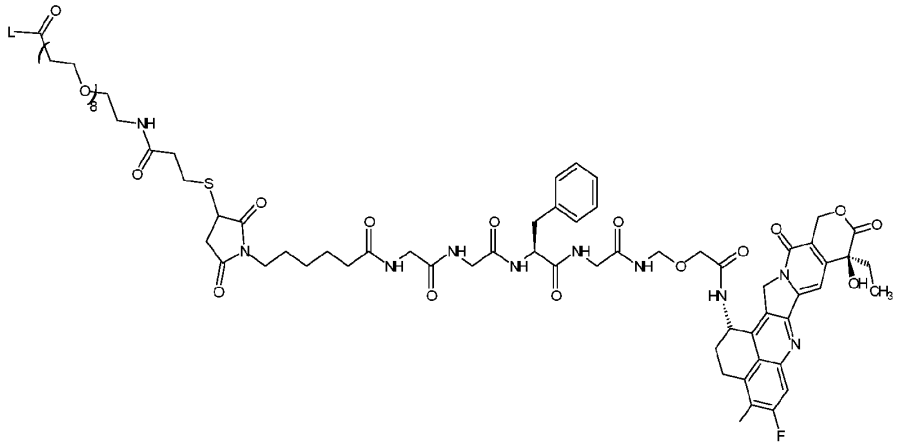


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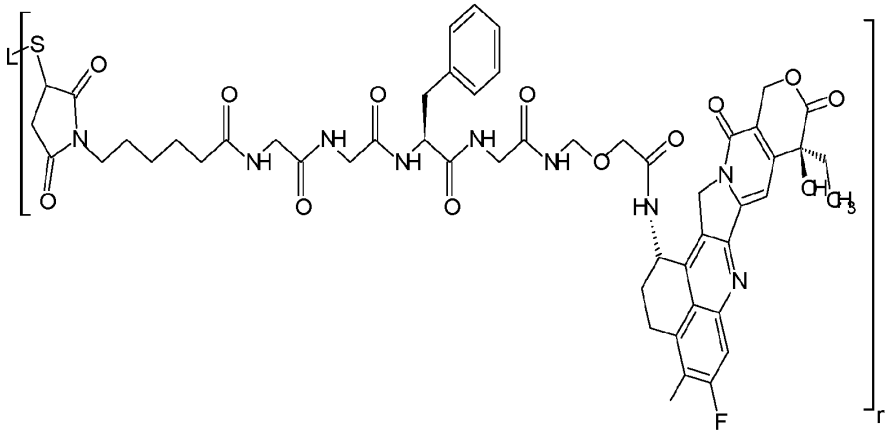


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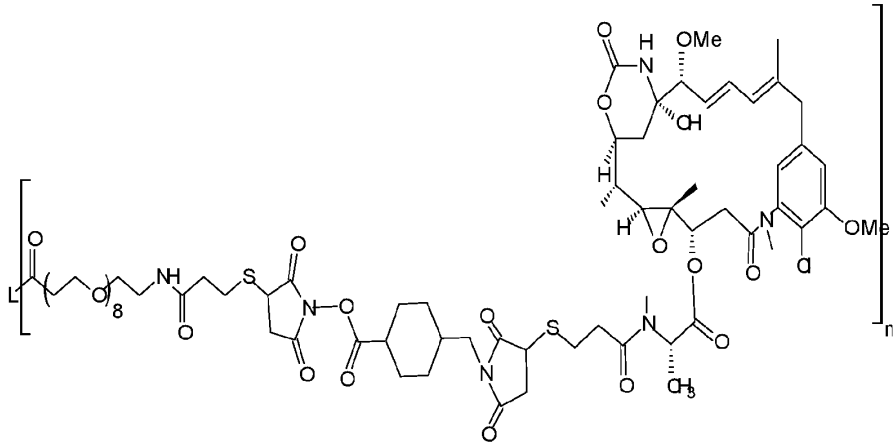
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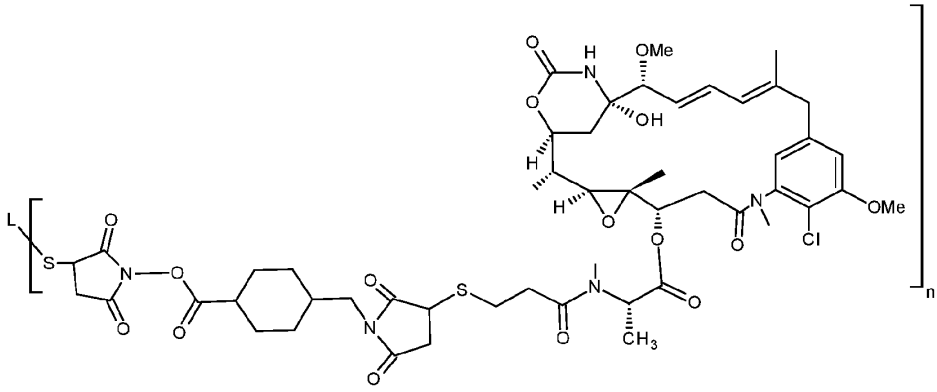
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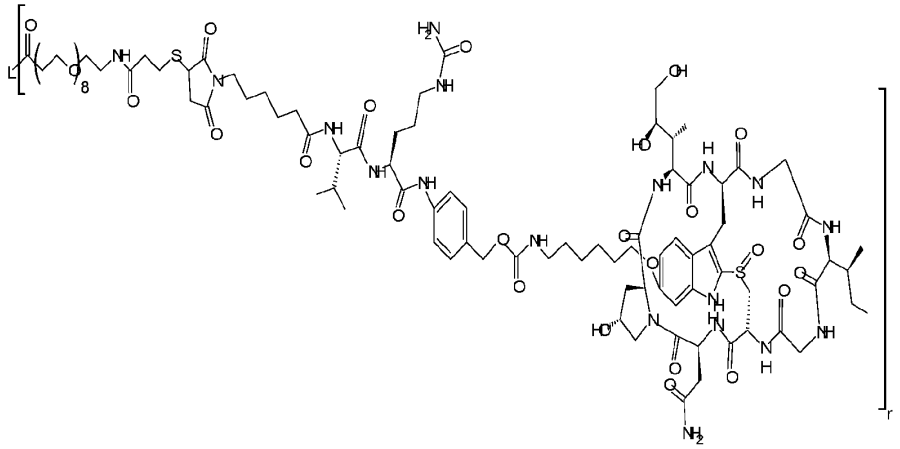
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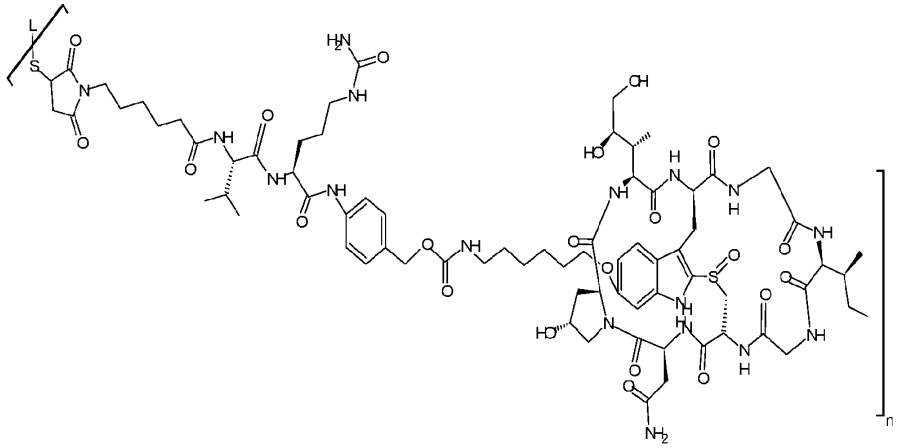
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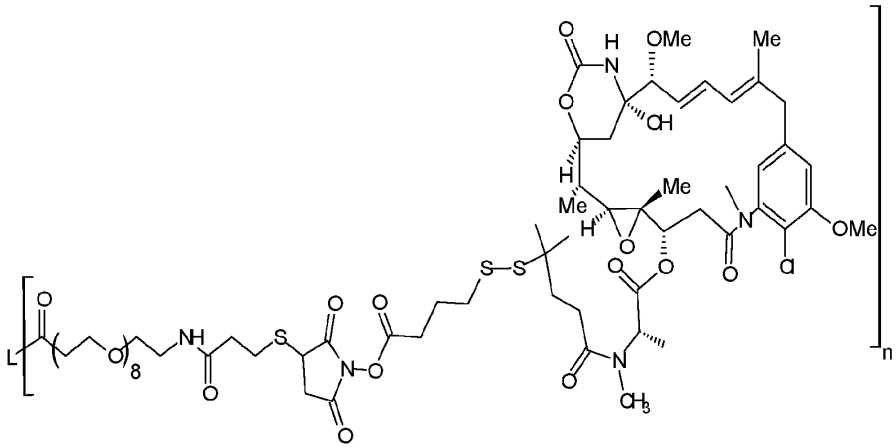
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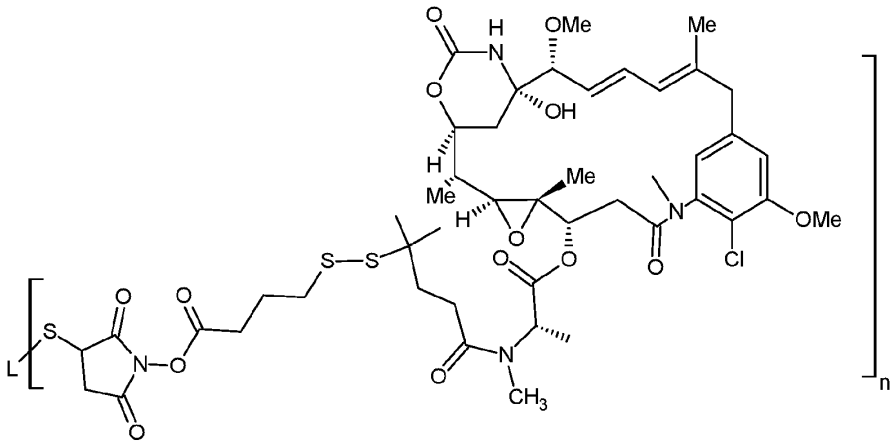
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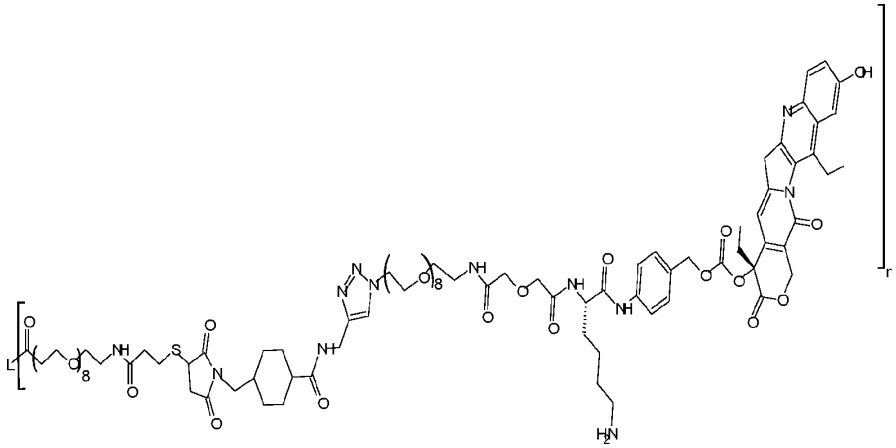
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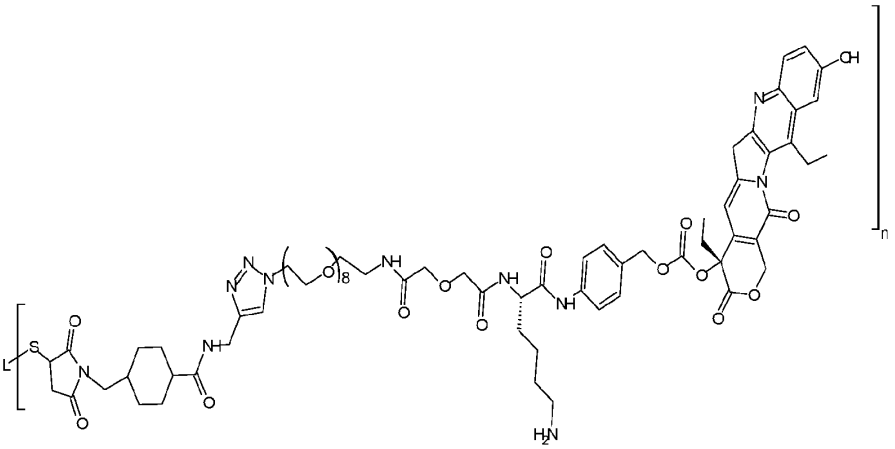
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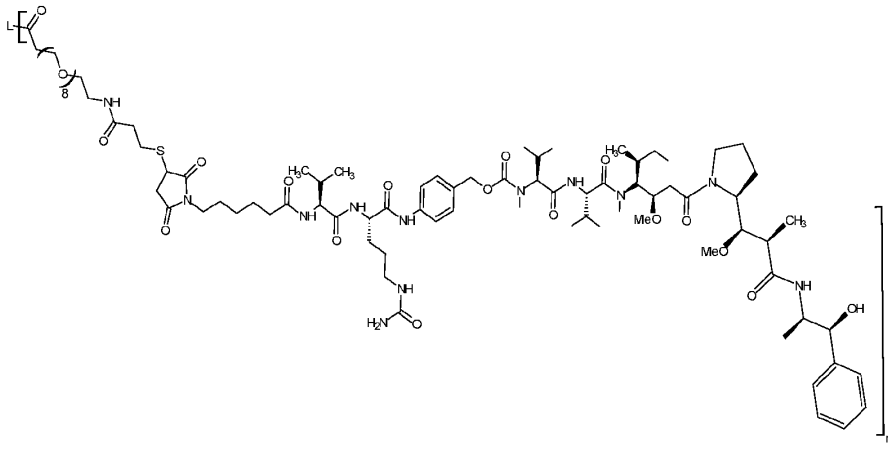
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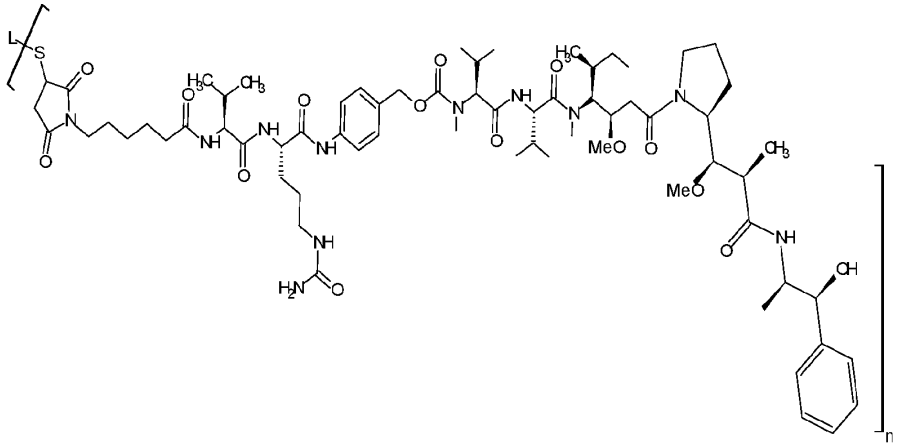
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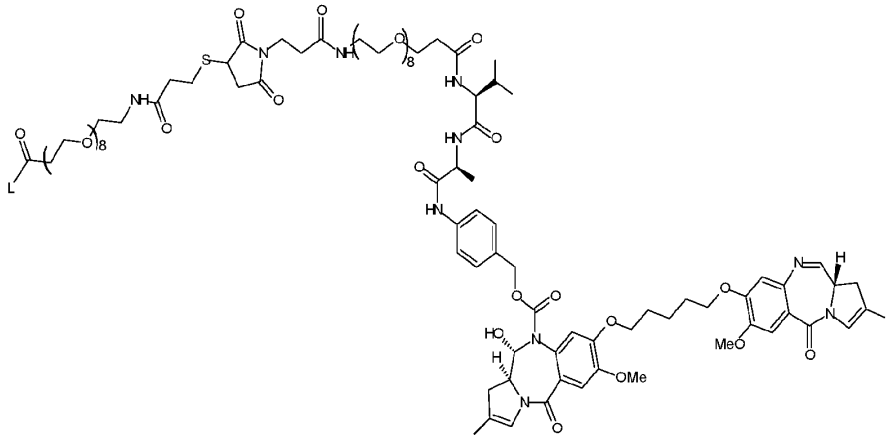
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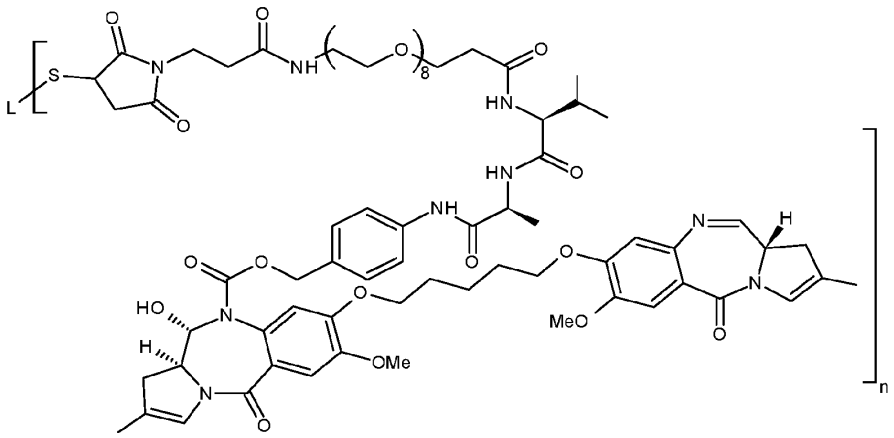
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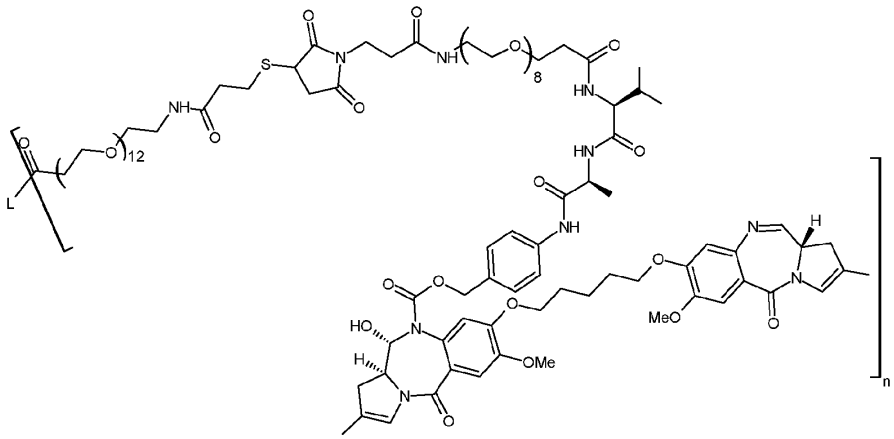
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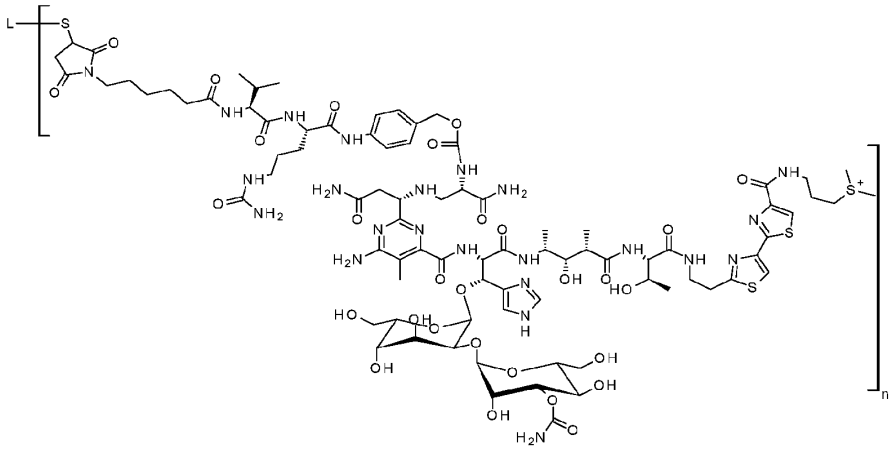
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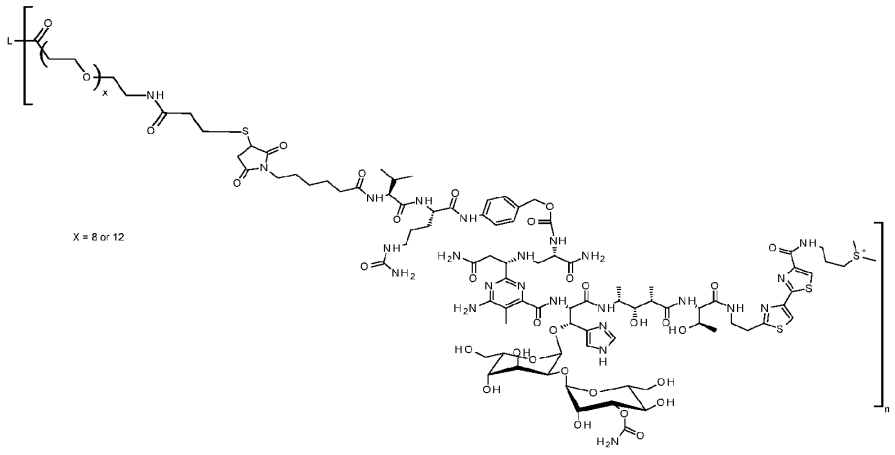
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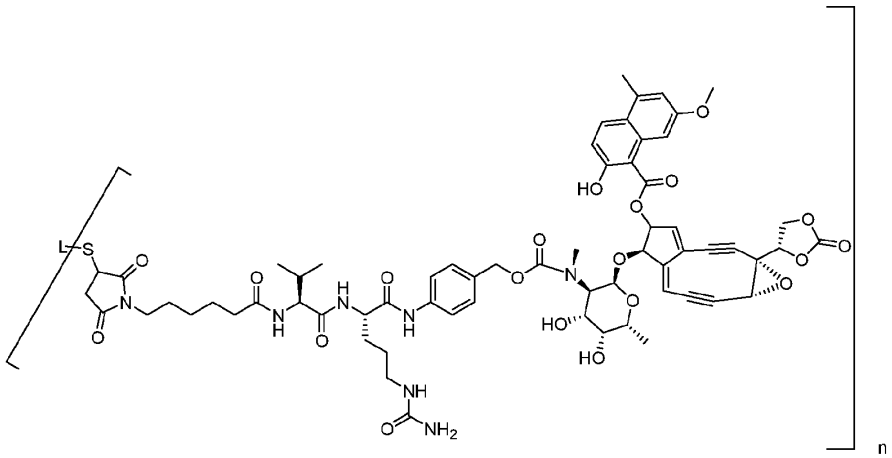
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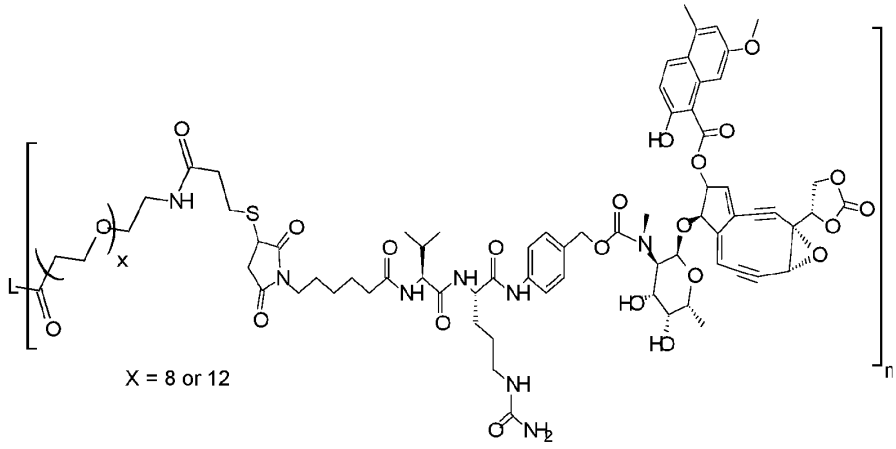
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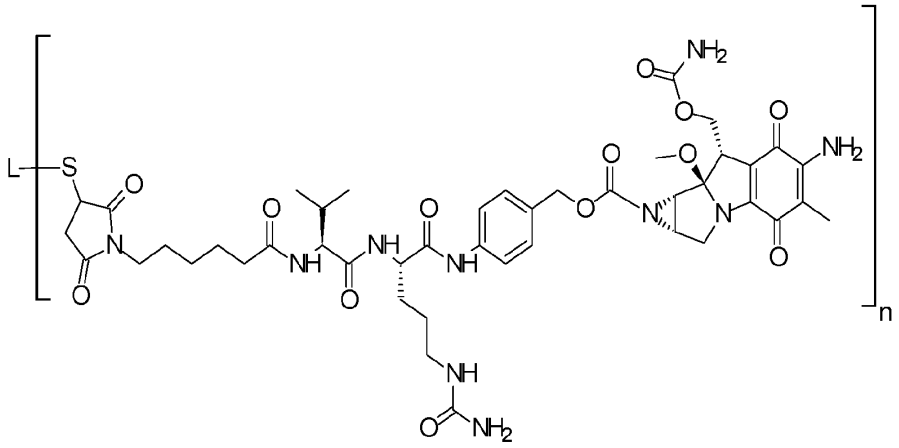


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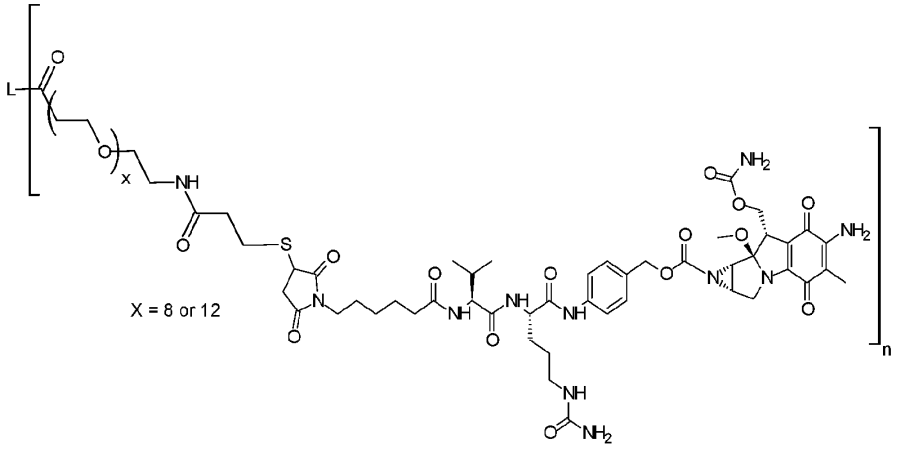


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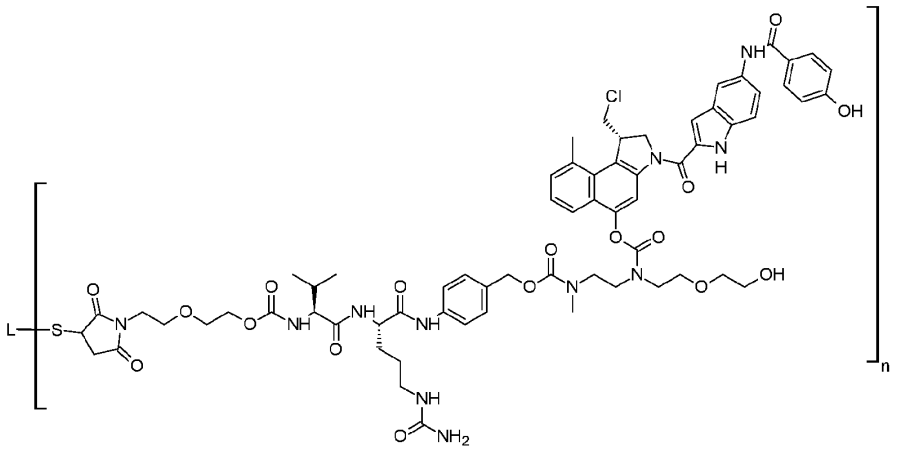
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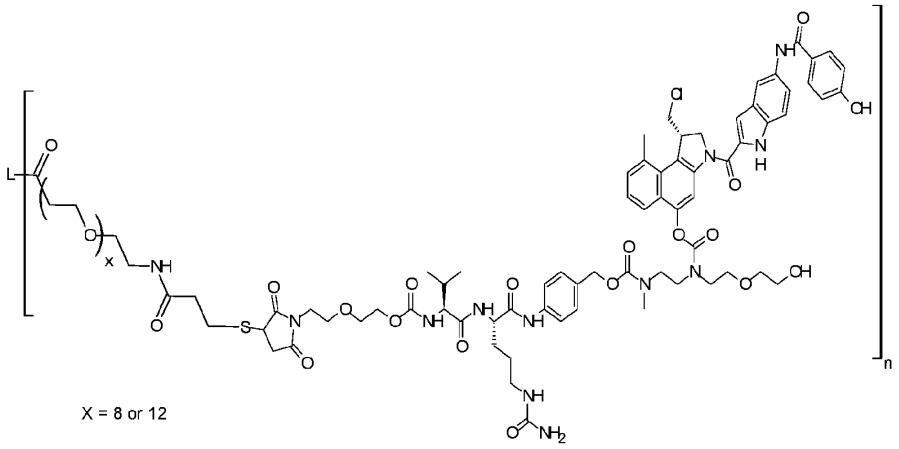
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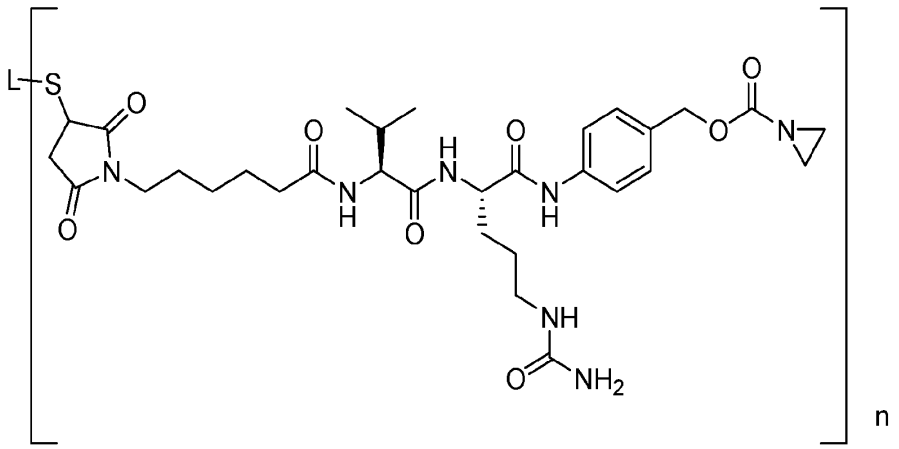
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Formula 26;

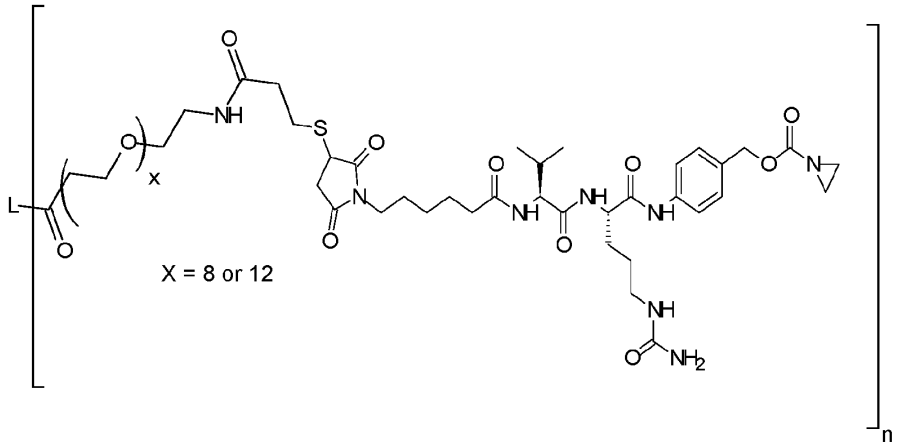


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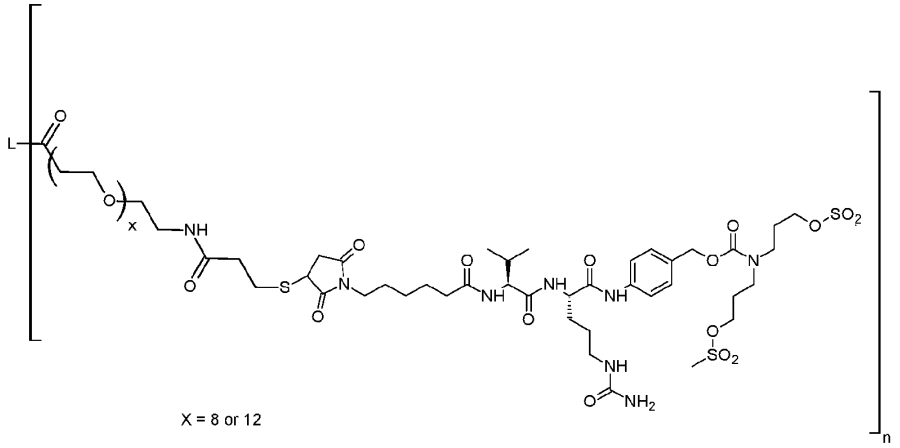


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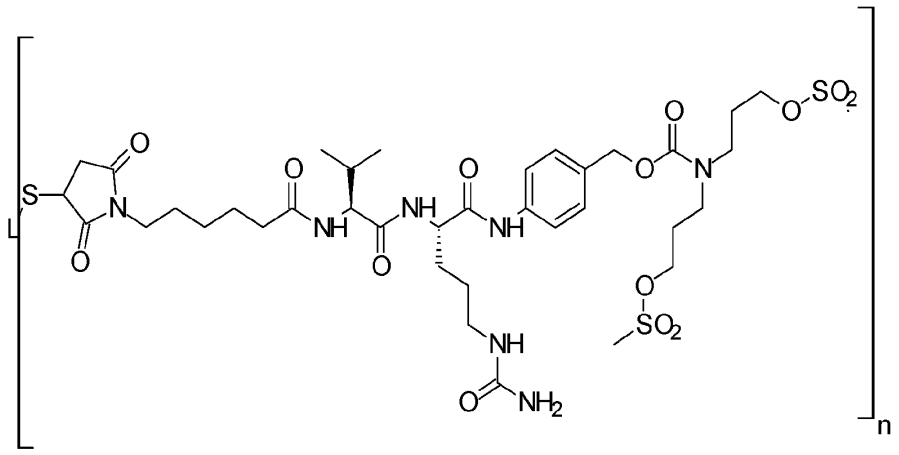
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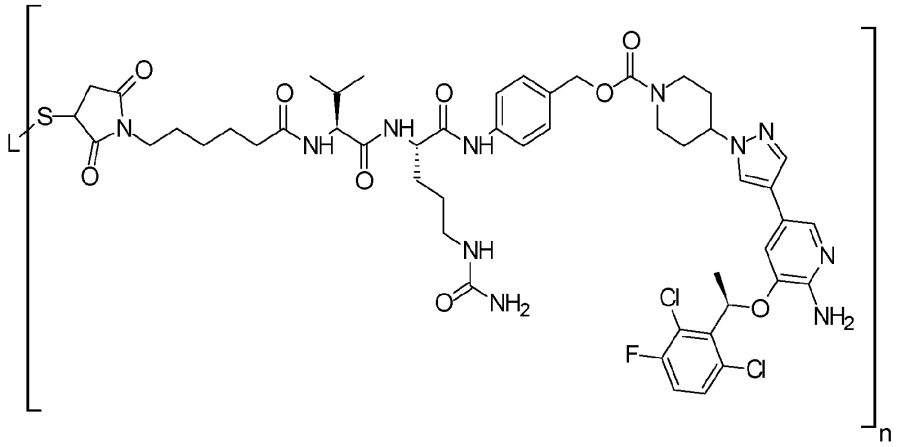
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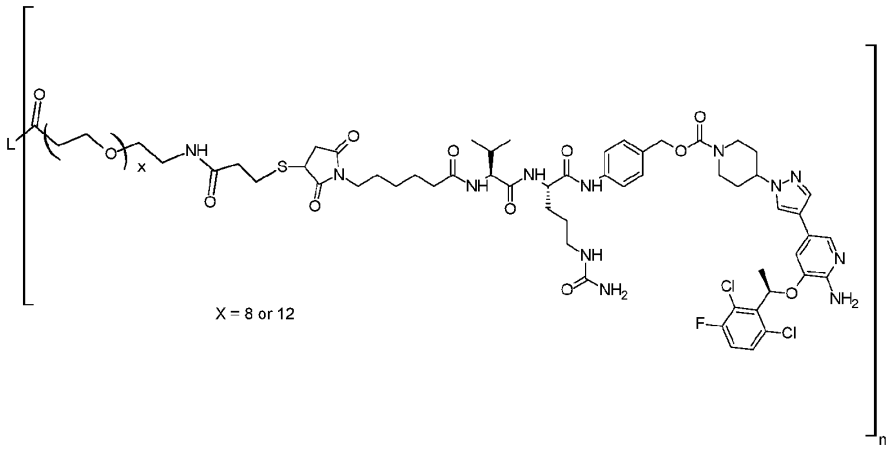
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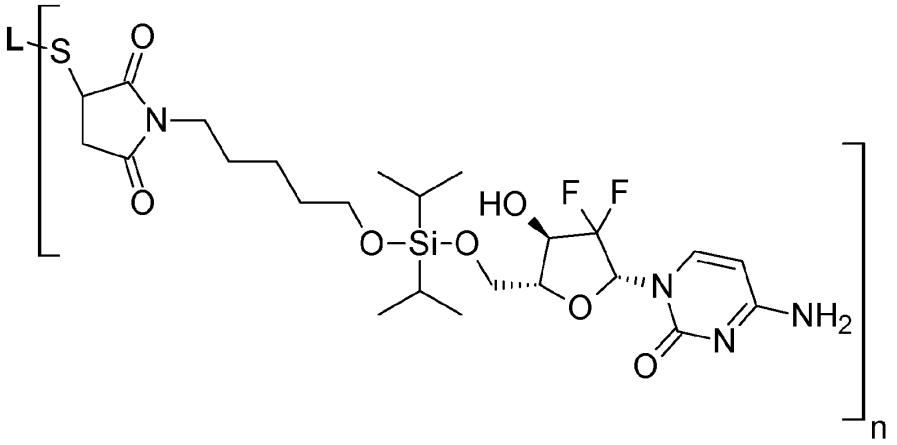
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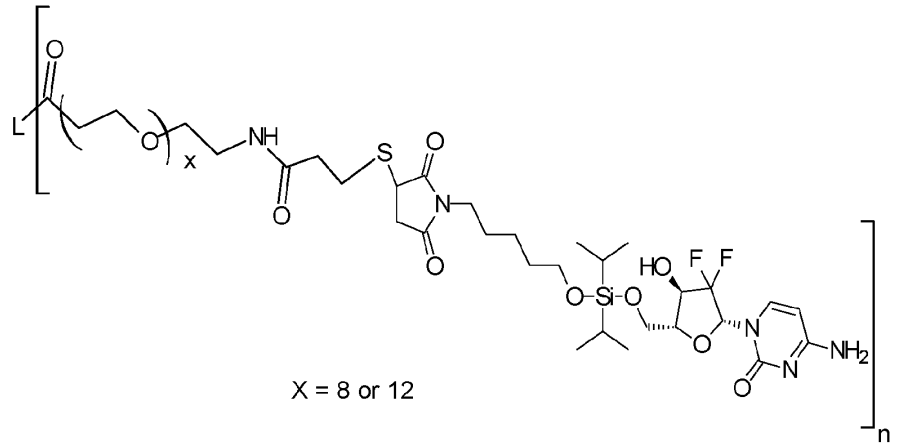
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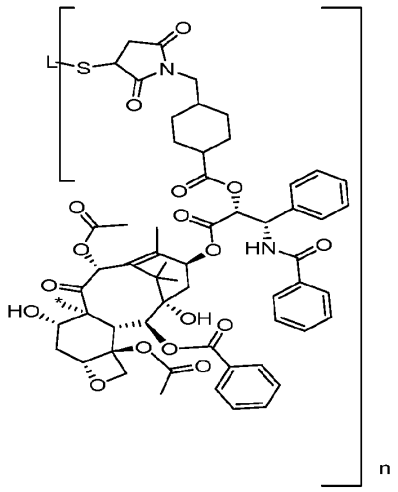
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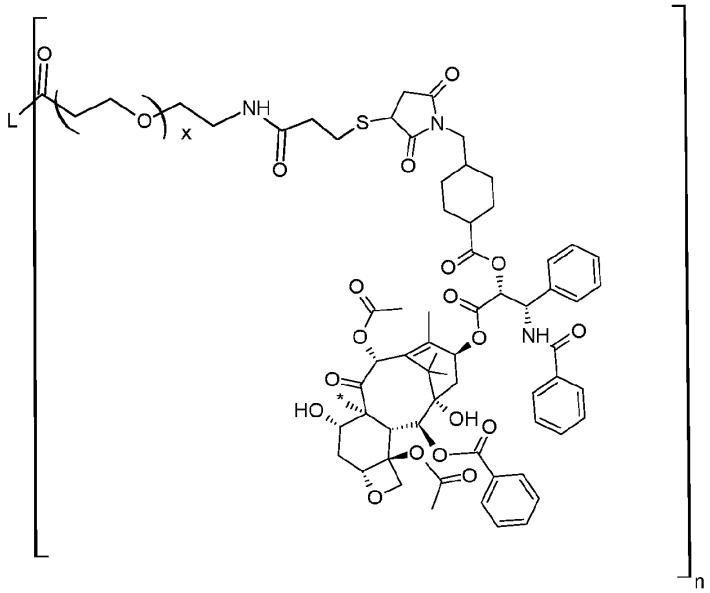
Formula 34;



Formula 35;

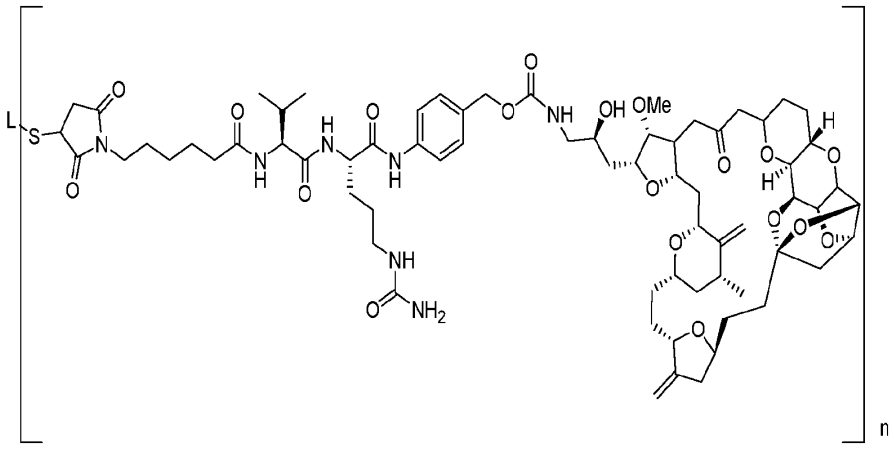


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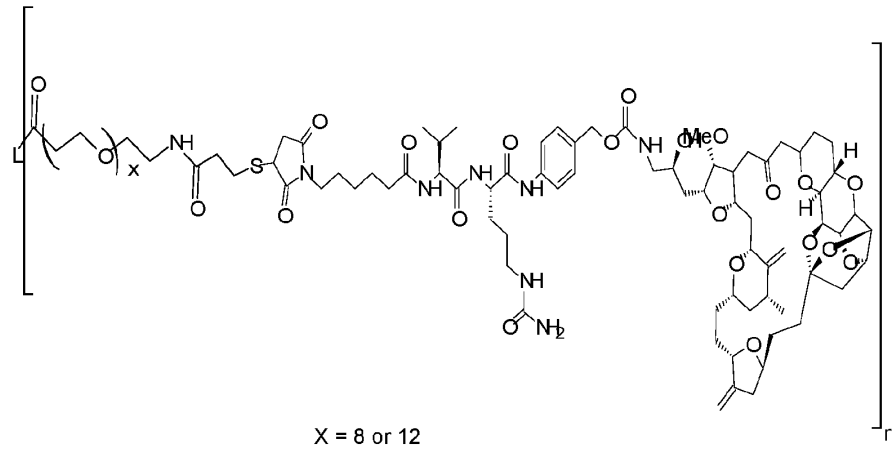


X = 8 or 12

Formula 37;

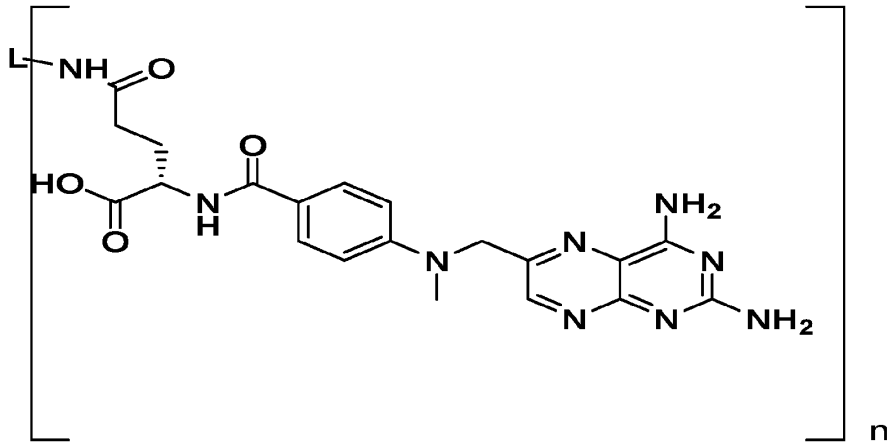


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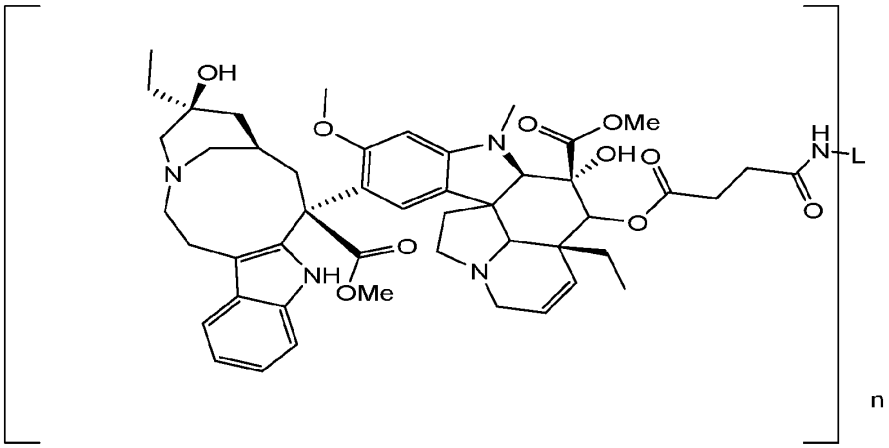


X = 8 or 12

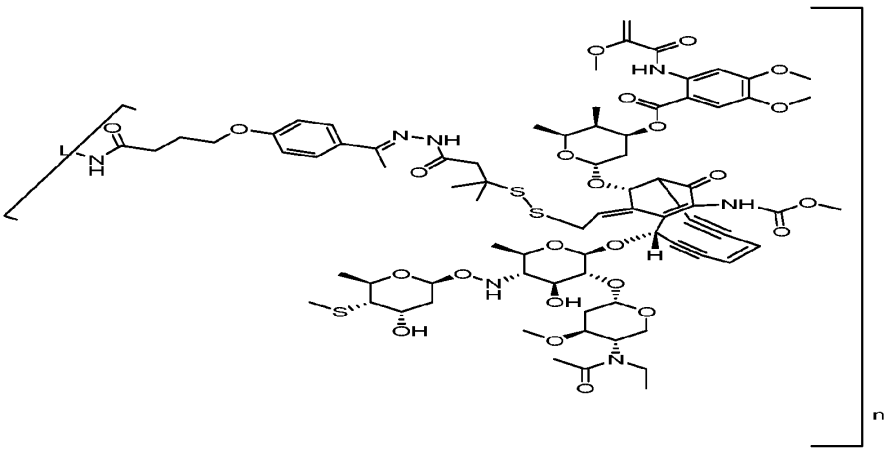
Formula 39;



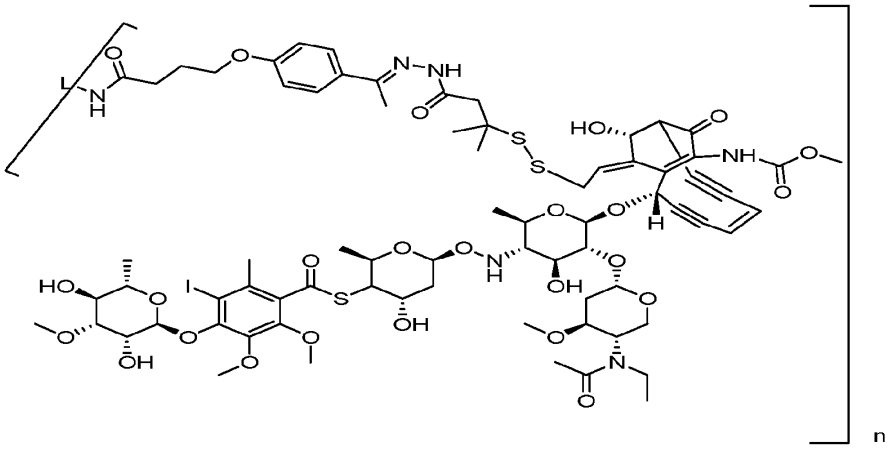
Formula 40;



Formula 41;



Formula 42;



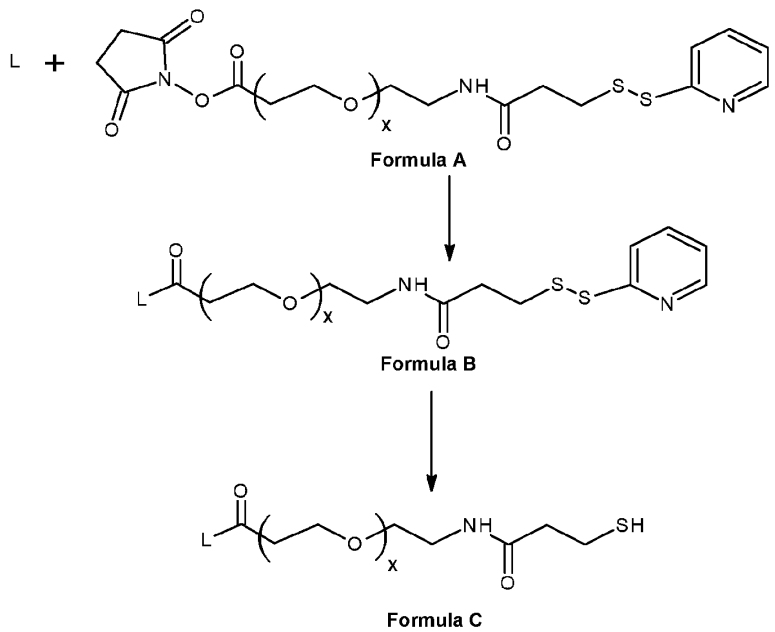
Formula 43;

The inventive aspect of the present disclosure lies in the recombinant lectin conjugates of Formula I, II, III and IV. Though the linker,recombinant lectins and drugs used in the present disclosure are well known, the mere conjugation of the recombinant lectin either to drug directly or with aid of a random linker resulted in protein drug conjugate devoid of either solubility or of desired therapeutic effect. The conjugates described herein have been designed to configure and position several components present in the Formula I, II, III, IV in such a way as to overcome not only one but both the solubility and therapeutic efficacy issues during the course of development. Surprisingly, the protein drug conjugate described herein also resulted in better stability along with improved solubility and therapeutic efficacy as compared to drug or lectin protein individually. Such designing, configuring and positioning of the several components that are part of the compounds of Formula I, II, III, or IV to form the protein drug conjugate with better efficacy, stability and solubility requires inventive step, which has not been taught in or motivated by the teachings of references cited above. Also, there is no teaching in the said references about selection of any specific lectin or drug or the linker which when conjugated would give compounds of the present invention and the desired therapeutic effects.

In an embodiment, the compounds of the present disclosure can be prepared by the methods known to the skilled person or bymethods listed in 'Methods to design and synthesize antibody-drug conjugates (ADCs). International journal of molecular sciences' (by Yao H et al. volume 17, issue 2, page 194, 2016 or in 'Preparation and characterization of antibody-drug conjugates acting on HER2-positive cancer cells' (by Chiang Z-C et al, PLoS ONE, volume 15, issue 9, e02398132020). Exemplarily, the compounds can be prepared by

reactingthe lectin protein with the agent D using coupling reactions in presence of metal catalyst or by heating at feasible temperature or under pressure. As will be appreciated by the skilled person, the reaction of protein needs to be carried out under controlled conditions so that the protein or product comprising protein is not destabilized and denatured.

In an embodiment, the compounds of the present disclosure may be prepared by optionally reacting lectin protein with a moiety which is a part of a linker that may easily form a bond with the D or its pre-cursor or other part of the linker that is linked to D. Such reactions may be carried out at pH between 7 to 9 or sometimes higher or lower, in phosphate or carbonate-bicarbonate, or 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) or borate buffer or tris or glycine buffer. Such reactions may also be carried out at pH below 6 in buffers such as 4-morpholinoethanesulfonic acid (MES) or a carbodiimide reaction buffer. In an example, lectin protein may react with maleimide group at pH between 5 to 8, specifically in the absence of compounds or buffers containing thiol groups. In one particular embodiment of the present disclosure, the recombinant lectin for the preparation of compounds of present disclosure may be mixed with compound of Formula A at room temperature. The resulting compound of Formula B when subjected to reduction, gives compound of Formula C (Scheme I, wherein L is recombinant lectin selected from SEQ ID NO:2 or SEQ ID NO:5 and X ranges from 0-20).



Scheme I

The lectin protein may be sometimes modified in certain un-conventional ways known to the skilled person. For example, addition of a non-natural amino acid to the protein and modifying it using Pictet-Spengler reaction or hydrazine-Pictet-Spengler reaction or other such variants or by using copper catalysed click reaction or variable click reaction such as click reactions in the absence of copper, or in presence or absence of any metal catalyst.

In an embodiment, the lectin protein or modified lectin proteinisfurther reacted with cytotoxic agent or therapeutic agent or its precursor or other part of the linker that is linked to cytotoxic agent or therapeutic agent under conditions known to aperson skilled in the art. In an example, such reaction may be carried out by reductive amination, or amidation, or thiol maleimide reaction. Such reactions may be carried out in buffers at a pH in the range from about 5 to about 9.

According to an embodiment, the final compound may be purified using known methods such as chemical purification or chromatographic purification for example, size exclusion chromatography (SEC) or hydrophobic interaction chromatography (HIC) or otherhigh-performance liquid chromatographic techniques. The intermediates and final products may becharacterized by methods such as Mass spectrometry, nuclear magnetic resonance (NMR) or any other method known to the skilled person. The quantitative distribution of cytotoxic agentor therapeutic agent to lectin protein can also be determined by electrophoresis among other methods described herein before.

According to an embodiment of the present disclosure, the compounds of present disclosure are highly pure. The term 'highly pure' may be construed as compound comprising at least 80% of single compound of present disclosure or at least 85% or at least 90% of compound of present disclosure. The word 'single compound' meaning there is present either of compound of Formula I, or II or III or IV, of which only single entity is present as single isomer or a single stereoisomer or polymorph or the solvate.

In an embodiment, the single compound means themixture of compounds comprising different DAR values with same L, D and the linker. For example, compound of formula 15 with DAR values 1, 2 or 3 may be present as mixture and are considered as single compound for the purpose of the present disclosure. The compound of the present disclosure is said to be highly pure, if it comprises at least 80% of the mixture of products of same formula with different DAR values.

In another embodiment, a single compound is a compound of present disclosure with one DAR value. The compound is said to be highly pure if it comprises at least 80% of the compound with one DAR value and less than 20% of the compound comprises compound with other DAR values. For example, compound of Formula 15 is said to be highly pure if it comprises at least 80% of compound with DAR value 1 and the compound of Formula 15 with DAR 2 is present in less than 20%. Such compounds with same formula and different DAR values may be separated using methods known to the skilled person. For example, such compounds may be separated using size exclusion chromatography.

It would be appreciated by the skilled person that the purity of the compounds of the present disclosure is both in the solution form and the separated form. The compounds of the present disclosure may form aggregates in the solution. The pure compound of the present disclosure in solution comprises at least 80% of aggregates of compounds of single formula.

In fifth aspect of the present disclosure, there is provided a composition comprising compound of Formula I or Formula II or Formula III or Formula IV. The composition of the present disclosure may be formulated as an aqueous liquid or solid. In some embodiment the composition may be liquid, suspension, powder, sterile powder or lyophilized for solution formulation. Lyophilized formulation may be reconstituted with Water for Injection (WFI) and/or any suitable pharmaceutically acceptable diluent or mixture thereof to get required concentration as known by the skilled person. The composition is suitable for single dose or multiple doses. A person skilled in the art knows that the type of dosing is dependent on various factors, such as body height and weight, body surface area, age, gender, or general health of the patient, and on the preparation to be administered in particular, the duration and type of administration, and on other medications that may be administered in parallel.

The compositions of the present disclosure may be administered to an individual in a suitable dosage. The administration can take place locally, enterally, or parenterally, for example, intravenously, intraperitoneally, subcutaneously, intramuscularly, locally, intranasally, intrabronchially or intradermally, or via a catheter at a point in an artery. In a particular embodiment, the compositions of present disclosures may be administered parenterally.

According to some embodiments, the effective concentration of compound of Formula I or Formula II or Formula III or Formula IV is in the range from 0.05 µg/mL to 100 µg/mL. In a particular embodiment, the effective concentration is in the range from 0.05 µg/mL to 50

μg/mL. According to some embodiments, the effective concentration of the cytotoxic drug or therapeutic agent is in the range from 0.001 nM to 1 μM.

In an aspect, the composition of the present disclosure may further comprise pharmaceutically acceptable excipients which may be selected from a buffer, a stabilizer, a polymers, a solubilizer, cryoprotectants, lyoprotectants, bulking agents, diluents emulsifiers and preservatives.

According to an aspect, the excipients may include polymer such as polyethylene glycols (PEGs), dextran, hydroxyl ethyl starch (HETA) or combination thereof; and a protein such as human serum albumin or Gelatin or combination thereof.

According to another aspect the stabilizer may be selected from surfactants, detergents, amino acids, pharmaceutically acceptable salt of amino acid, carbohydrates or sugar stabilizers, amines, polyols or combination thereof.

The liquid formulation of the present invention may preferably comprise buffer. The buffer for example may be selected from phosphate buffer such as sodium monohydrogen phosphate dehydrate, sodium dihydrogen phosphate dehydrate, phosphate buffer such as dibasic potassium phosphate, monobasic potassium phosphate citrate buffer, acetate buffer, TRIS-sodium chloride buffer, or histidine. The concentration of the buffer may range from 1 mM to 300 mM. As understood by the skilled person, pH of the formulation may be regulated between 5 and 9.

According to a specific aspect of the present disclosure, there is provided a composition comprising compound of formula I, formula II, formula III or formula IV and TRIS-sodium chloride buffer.

The composition of the present disclosure may be formulated using the standard methods known to the skilled person or by the methods disclosed in "Protein formulation and Delivery"by E. J. McNally *et* al, or in "Rational Design of Stable Protein Formulations Theory and Practice" by J. F. Carpenter *et* al.

In one embodiment, the compounds of present disclosure has application in the prevention of growth or metastasis of tumor, treatment or to cure cancer. According to some embodiments, the compound of Formula I or Formula II or Formula III or Formula IVmay be used for prevention or treatment of adenocarcinoma, Squamous cell carcinoma, Transitional cell carcinoma, Basal cell carcinoma, Sarcomas, Lymphomas, epithelial cell carcinomas or non-epithelial cell carcinomas. According to the particular embodiment, therapeutically effective

compound of Formula I or Formula II or Formula III or Formula IVof the present disclosure may be used for prevention or treatment of pancreatic cancer, abdominal cancer, liver cancer, prostate cancer, oral cancer, colon cancer, ovary cancer, bladder cancer, kidney cancer, stomach cancer, breast cancer, bone marrow cancer, melanoma, leukemia or central nervous system cancer in a subject.

In sixth aspect of the present disclosure, there is provided a method of treatment or prevention of cancer using compound of Formula I or Formula II or Formula III or Formula IV. The method of treatment or prevention may comprise administering a therapeutically effective amount of the said compound to the subject. In some embodiments, the compound of present disclosure is administered at a dose of from 0.01 to 1000 mg/kg, from 0.05 to 100 mg/kg or from 0,1 to 50 mg/kg. It will be within the capabilities of the skilled person to determine an amount of compound of Formula I or Formula II or Formula III or Formula IV to be administered according to the nature of the condition being treated and the subject.

In one embodiment of the present disclosure the compounds of present disclosure such as compounds of Formula 15 to Formula 19 were subjected to cytotoxicity assay against the bladder cancer T24 cell lines by determining the IC_{50} values. The compound of Formula 15 showed IC_{50} value of 41.13 nM, whereas compound of Formula 17 showed IC_{50} value of 4.216 nM, Formula 16 showed 325.4 nM, compound of Formula 18 showed IC_{50} of 19.93 nM and compound of Formula 19 showed IC_{50} valued of 70.64 nM. Similarly, in ancytotoxicity assay against the ovarian cancer PA-1 cell lines compound of formula 15 showed IC_{50} value of 40.9 nM, Formula 16 showed IC_{50} value of 17.7 nM, compound of formula 17 showed 2.61 nM, Formula 18 showed 1.04 nM and compound of formula 19 showed 5.22 nM.

MMAE individually indicated IC_{50} value of 3.23nM and mc-vc-PAB-MMAE, indicated 646.9 nM whereas Tesirine indicated 13.42 nM against PA-1 cell line. On the other end MMAE individually indicated IC50 value of 0.24 nM whereas Tesirine indicated 144.8 nM against T24 cell line.

Abbreviations:

DMA	Dimethylacetamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid

DO	Dissolved Oxygen
DTPA	Diethylenetriamine pentaacetate
DTT	Dithiothreitol
E. coli	Escherichia coli
EDTA	Ethylenediamine tetraacetic acid
EMEM	Eagle's Minimum Essential Medium
FBS	FBS
FDA	Food and Drug Administration
HCl	Hydrochloric acid
HIC	Hydrophobic interaction chromatography
HPLC	High performance Liquid Chromatography
IgG	Immunoglobulin G
IPTG	Isopropyl ß-D-1-thiogalactopyranoside
K_2HPO_4	Dipotassium hydrogen phosphate
KDa	Kilo Dalton
LCMS	Liquid Chromatography Mass spectrometry
$MgSO_4.7H_2O$	Magnesium sulfate heptahydrate
MTS- PMS	MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt and PMS an electron coupling reagent (phenazine methosulfate).
Na_2HPO_4	Disodium phosphate
NaCl	Sodium chloride
NEM	N-Ethylmaleimide
$(NH4)_2SO_4$	Ammonium sulfate
OD	Optical Density
PBS	Phosphate Buffer Saline
SEC	Size exclusion Chromatography
TBS	Tris-Buffered Saline
TFF	Tangential Flow Filtration
v/v	Volume/volume
vvm	Volume of liquid per minute

w/v	weight/volume
PDC	Protein Drug Conjugate

EXAMPLES

Various aspects of the disclosure are further described by way of below listed examples. The examples are illustrious, and the skilled person is well aware and informed about the obvious variations of them. The examples therefore do not limit the scope of this disclosure in any manner.

Example 1: Clone and Manufacturing Process of Recombinant Lection having amino acid sequence of SEQ ID NO:2

The nucleotide sequence (SEQ ID NO: 6) coding for the recombinant lectin having amino acid sequence of SEQ ID NO:2 cloned into pET27b vector (sourced from Novagen) using *Nde*I and *Bam*HI to generate pET27b-Lec construct. Further the pET27b-Lec construct was transformed into propagation host *E. coli* Top10 competent cells. Plasmid was isolated form transformed cells and insert integrity was confirmed by restriction digestion analysis and the gene sequence is confirmed by DNA sequencing. Verified pET27b-Lec plasmid was further transformed into *E. coli* BL21 DE3 (Gold) expression host. Transformed clones were tested for recombinant protein expression and the clone showing high expression is verified by DNA sequencing and further used for cell bank preparation. Characterized cell bank is used in fermentation process.

For fermentation, seed culture was prepared by inoculating the culture from glycerol stock in medium containing 2 % Hiveg Luria broth, 0.75 % Na_2HPO_4, 0.5% dextrose and kanamycin (20 µg/ml). The culture was grown at 30±2°C and 110 rpm for 16 hours. Approximately 300 ml culture was inoculated into 2.3 L of production medium containing. 1% yeast extract (w/v), 1.2% Dextrose (w/v), 0.3% KH_2PO_4 (w/v), 1.25% K_2HPO_4 (w/v), 0.5% $(NH_4)_2SO_4$ (w/v), 0.05% NaCl (w/v), 0.1% $MgSO_4.7H_2O$ (w/v), 0.1% (v/v) trace metal solution, kanamycin (20µ/ml). Fermentation was carried out in 5L fermenter (BIOSTAT B, Sartorius Stedim) with 1-2 vvm aeration, dissolved oxygen was maintained at 50%-60% and pH maintained was maintained at 6.6-7.0 with alkali. Initial growth was carried out at a temperature of 37°C. Temperature was gradually decreased and maintained at 22°C during the induction phase. Feeding of Carbon (glycerol) and Nitrogen source (yeast extract) was initiated after 5 log hours at predetermined feeding rates, maintaining C:N ratio in the range of 4:1. Culture was induced at cell density of ~45 (OD600) with 1 mM IPTG. Fermentation

was continued till 24 hrs and culture broth was harvested by centrifugation at 9000 rpm for 15 minutes. The wet weight of cell mass obtained from fermentation broth was approximately 350 g.

The cell pellet was suspended in lysis buffer (25 mM tris, 1 mM EDTA, pH 8.5) in a ratio of 1:10 (w/v) and stirred for at least 2 hr on an overhead stirrer to form a homogenous suspension. The suspension was lysed by high pressure homogenization at ~18,000 psi. The cell lysate was centrifuged at 9,000 rpm for 15 min at 15°C. The resultant supernatant was retained and processed for further clarification by treating with at least 0.025% polyethyleimine by stirring for 15-30 mins. Treated cell lysate was clarified using a 0.1µm hollow fiber of 3600 cm2 area pre equilibrated with 25 mM Tris buffer containing 1 mM EDTA, pH 8.0±0.5. Transmembrane pressure of 5 -10 psi was maintained throughout the clarification process.

The recombinant lectin was purified using four step chromatography. In the first step cell lysate was loaded on Cellufine Max Q-r resin pre equilibrated with 25 mM Tris buffer containing 1 mM EDTA, pH 8.0±0.5 and washed with the equilibration buffer containing 1-3 g/L NaCl. Protein with 77.4% purity was eluted with 25 mM Tris buffer containing 1 mM EDTA, 11-15 g/L NaCl, pH 8±0.5. In second step the pH of the column 1 elute was adjusted to 4.5 with acetic acid followed by ammonium sulphate precipitation. After centrifugation clear supernatant was processed on the HIC resin (Cellufine Max Butyl) equilibrated with 25 mM sodium acetate buffer containing 1 mM EDTA, 0.5 - 2M ammonium sulphate, pH 4.5. After washing with equilibration buffer, the protein with purity of 90.3% was eluted with 25 mM Sodium acetate, 1 mM EDTA, 15 g/L ammonium sulphate, pH 4.5. In third step the protein is purified by cation exchange chromatography, where the column 2 elute was diluted to approximately 20 mS/cm conductivity with purified water and loaded onto SP Sepharose FF resin pre equilibrated with 25 mM Sodium acetate buffer containing 1mM EDTA, pH 4.5. After washing with equilibration buffer, the protein with purity of 93.0% was eluted by a step gradient of 70% using elution buffer containing 25mM Sodium acetate, 1 mM EDTA, 0.5 M NaCl, pH 4.5. In fourth step the column 3 elute was buffer exchanged with 25mM Tris buffer, pH 8.0 using 3 KDa membrane and processed onto Source 30Q anion exchange resin, pre equilibrated with 25 mM Tris buffer, pH 8.0. After washing the elution was carried out by linear gradient using elution buffer containing 25 mM Tris, 0.5 M NaCl, pH 8.0 for 15 column volumes. The fractions having purity of >99.0% by HPLC analysis were pooled and total of 9.0 grams of protein was obtained as measured by optical density at 280nm

considering 1OD at 280nm equals 1mg. The final elute fractions were buffer exchanged against TBS Buffer (50 mM Tris buffer, 150 mM NaCl, pH 7.8).

Example 2: Clone and Manufacturing Process of Recombinant Lection having amino acid sequence of SEQ ID NO: 5

The nucleotide sequence (SEQ ID NO: 7) coding for the recombinant lectin having amino acid sequence of SEQ ID NO: 5 cloned into pET27b vector using *Nde*I and *Bam*HI to generate pET27b construct. Further the pET27b construct was transformed into propagation host *E. coli* Top10 competent cells. Plasmid was isolated form transformed cells and insert integrity was confirmed by restriction digestion analysis and the gene sequence is confirmed by DNA sequencing. Verified pET27b plasmid was further transformed into *E. coli* BL21 DE3 (Gold) expression host. Transformed clones were tested for recombinant protein expression and the clone showing high expression is verified by DNA sequencing and further used for cell bank preparation. Characterized cell bank is used in fermentation process.

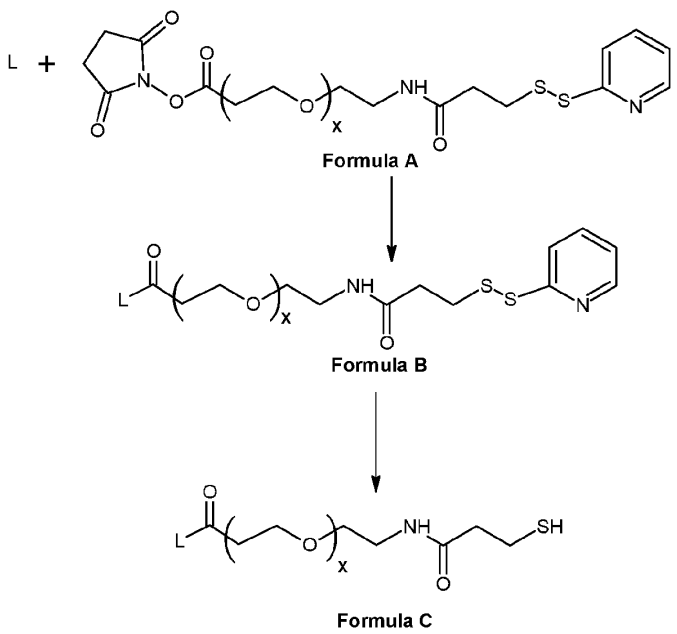
For fermentation seed culture was prepared by inoculating the culture from glycerol stock in medium containing yeast extract (10 g/L), potassium dihydrogen phosphate (3 g/L), di–potassium hydrogen phosphate (12.54 g/L), ammonium sulphate (5 g/L), sodium chloride (0.5 g/L), dextrose (12g/L), magnesium sulphate heptahydrate (1 g/L), kanamycin sulphate (20 mg/L) and trace metal solution (1 ml/L). The fermentation was initiated at 30°C and later the temperature was gradually decreased to 18°C. DO level was maintained at 60% and pH was maintained at 6.80±0.05 with alkali solution except during carbon source shifting. Fed batch fermentation process was used with glycerol (50%) and yeast extract (40%) as carbon and nitrogen source respectively as feed medium. Induction was done with 0.15 mM IPTG at OD600nm around 60. Total batch run time was 48 to 72 hours and maximum OD600 achieved was in the range of 107 after 52 log hours. Expression of the recombinant protein having amino acid sequence of SEQ ID NO 5 was around 6.3 g/L. Fermentation broth was harvested from the fermenter. It is then centrifuged at 9000 rpm, for 10-15 minutes at 10°C to obtain compact cell pellet. Pellet obtained is then suspended in 1:10 ratio (w/v) of lysis buffer containing 25 mM Tris HCl, 1 mM EDTA, pH 8.5 and suspension is stirred for 1-2 hours using mechanical stirrer maintaining a temperature of 10±4°C. Lysis is carried out using high pressure homogenizer at 1100±200 bar by giving 1-2 passes. The cell lysis efficiency of >90-95% is obtained (determined by OD600) and lysate collect at low temperature.

The cell lysate was clarified using 0.1µ TFF and permeate was collected. In order to recover maximum quantity of protein, the retentate was washed 4-6 times with 25 mM Tris-HCl

buffer, 1mM EDTA, pH 8.0 in step mode till protein absorbance at 280 nm came down to less than 4-6. The solution was later clarified using 0.1µ hollow fiber filter with Trans Membrane Pressure of 2-10 psi. The washes that contained the protein were pooled with the main permeate. Temperature was maintained below 25°C during entire clarification process.

The recombinant lectin of SEQ ID NO:5 was purified using three step chromatography. In first step cell lysate was loaded on Cellufine Max Q-r resin pre equilibrated with 25 mM Tris buffer containing 1 mM EDTA, pH 8.0±0.5 and washed with the 25 mM Tris HCl, 1mM EDTA, 50mM NaCl at pH 8.0. Protein was eluted with 25 mM Tris HCl, 1mM EDTA, 200mM NaCl at pH 8.0. Entire peak was collected as a single fraction. In second step the recombinant lectin of SEQ ID NO:5 eluted from first step was bound to the CM Sepharose column pre equilibrated with the equilibration buffer at a binding capacity of 20mg/ml. Post complete loading, column was washed with the equilibration buffer25mM Sodium Acetate, 5mM β- Mercaptoethanol, pH 5.1 to remove the unbound protein. Elution was carried out giving a linear gradient from 0-50% 25mM Sodium Acetate, 1.0M NaCl, 5mM β-Mercaptoethanol, pH 5.1 for 15 column volumes followed by a wash of 100% buffer 25mM sodium scetate, 1.0M NaCl, 5mM β- Mercaptoethanol, pH 5.1. Elute obtained from CM Sepharose column then subjected to pH adjustment to 8.0 with Tris buffer and buffer exchanged against 25mM Tris, 5mM Beta mercaptoethanol, pH 8.0 to get conductivity to less than 2.0mS/cm using 5KDa membrane. Then recombinant lectin of Seq ID: 5 was loaded in third column Source 30Q pre-equilibrated with equilibration buffer: 25 mM Tris, 5mM Beta mercaptoethanol, pH 8.0. The recombinant protein of Seq ID No:5 was then eluted using elution buffer of 25 mM Tris, 5mM Beta mercaptoethanol, 500mM NaCl, pH 8.0.The recombinant protein of Seq ID No: 5 with purity in excess of 95% was obtained.

Example 3: General preparation of Compound of Formula C



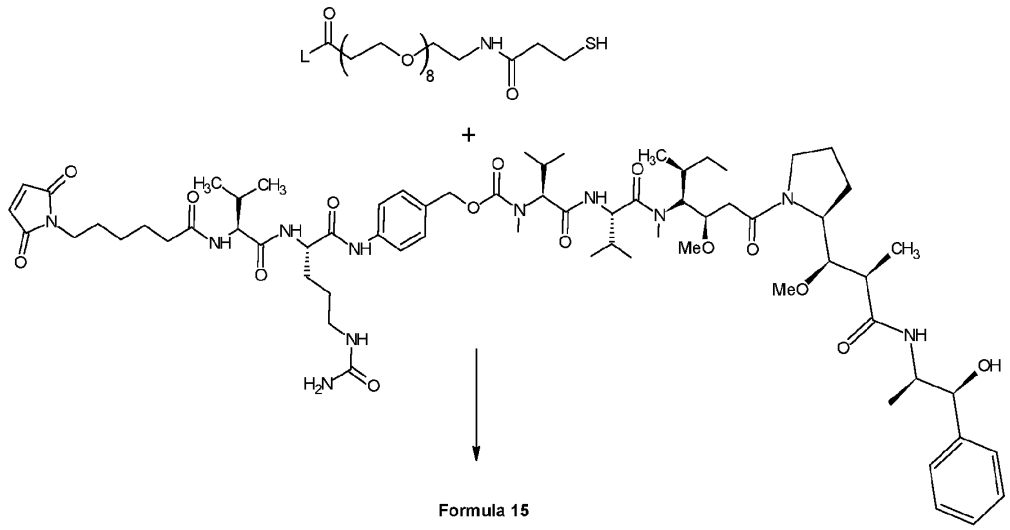
Wherein

L- Recombinant lectin of SEQ ID NO: 2

X- 8 or 12

A solution of recombinant lectin of SEQ ID NO: 2 12.2 mg/L in 1X PBS was diluted with a 1:1 volume of 100 mM sodium phosphate dibasic, 150 mM NaCl and 10 mMDTPA, pH 8.0. To this was added 2.4 equivalents (with respect to SEQ ID NO: 2) of commercially available Formula A (10 mM DMSO)purchased from Quanta Biodesign Limited. The reaction was incubated with gentle mixing for 2 hours and the degree of conjugation was determined by LCMS. If Drug to protein ratio (DAR) was less than 2, another equivalent of 10 mM Formula A was added and incubated another hour at room temperature. After incubation and confirmation of formation of Formula B with DAR of more than 2 by LCMS analysis, DTT was added to the reaction mixture to achieve a final concentration of 50 mM and incubated at 37 °C for 30 minutes in order to obtain a compound of Formula C, which was further purified using Superdex 75 SEC column in 1x PBS, 10 mM EDTA to generate purified compound of Formula C with which gave a final concentration of 4.8 mg/mL in 1x PBS, 10 mM EDTA.

Example 4: Preparation of Conjugate of Formula 15

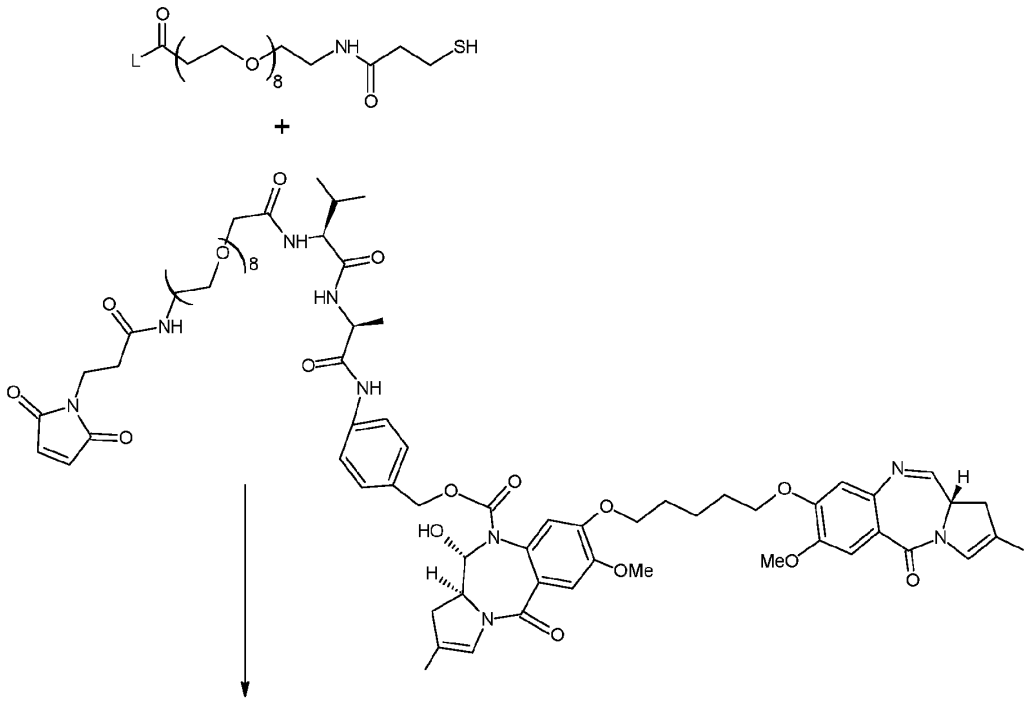


Formula 15

L- Recombinant Lectin of SEQ ID NO 2

45 mg of a solution (4.8 mg/mL) of Compound of formula C in 1X PBS and 10 mM EDTA was further diluted with to final concentration 2.5 mg/ml by 1X PBS with 10 mM EDTA, DMSO and 1.5 eq of 10 mM MC-VC-PAB-MMAE in DMSO containing 6% of cyclodextrin that resulted in a final DMSO concentration of 35% (v/v). The solution was stirred at room temperature for 60 min followed by capping with excess 10 eq NEM. The drug to protein ratio (DAR) of the conjugate Formula 15 was determined by LCMS analysis. Further the conjugate of Formula 15 was subjected to SEC using GE10-300 Superdex 75 column) with buffer exchange to 50 mM Tris, 150 mM NaCl and pH was adjusted to 8.0 using tris buffered saline TBS. Post SEC purification, DAR value of conjugate of Formula 15 was determined with the aid of LCMS. The DAR value was determined to be 1.09.

Example 5: Preparation of Conjugate of Formula 17

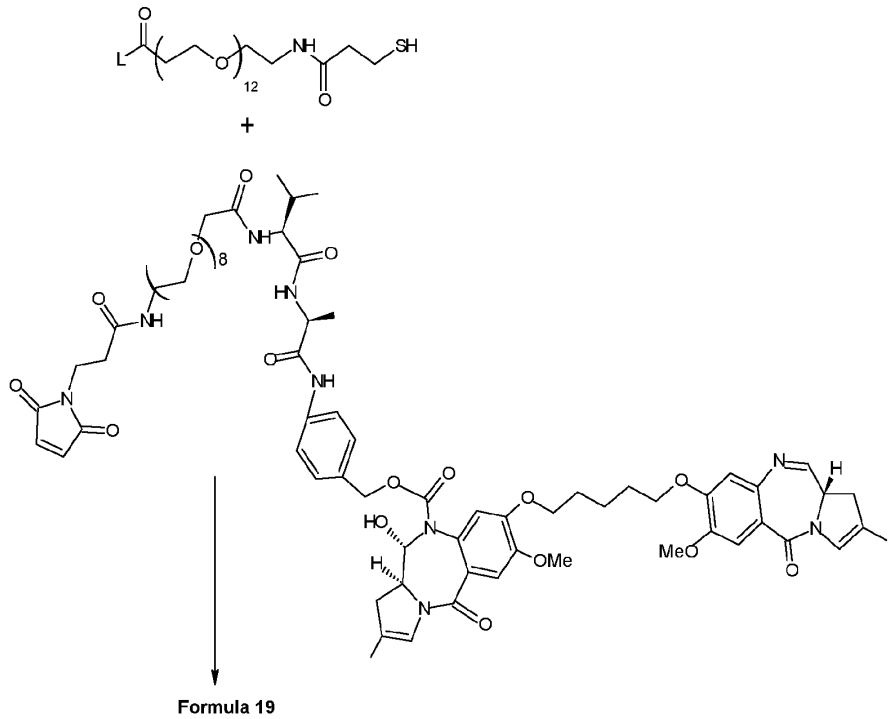


Formula 17

L- Recombinant Lectin of SEQ ID NO 2

34 mg of a solution (4.8 mg/mL) of Formula C in 1X PBS and 10 mM EDTA was further diluted with to final concentration 2.5 mg/ml by 1X PBS with 10 mM EDTA, DMSO and 2.5 eq of 10 mM Tesirine in DMSO that resulted in a final DMSO concentration of 35% (V/V). The solution was stirred at room temperature for 60 min followed by capping with excess 10 eq NEM. The drug to protein ratio DAR of conjugate Formula 17 formedwas determined using LCMS analysis. The final product of Conjugate of Formula 17 was subjected to SEC using GE10-300 Superdex 75 column with buffer exchange to 50 mM Tris, 150 mM NaCl and pH was adjusted to 8.0 using TBS. Post SEC purification DAR of Conjugate of Formula 17was determined using reverse phase liquid chromatography and was found to be1.25.

Example 6: Preparation of Conjugate of Formula 19

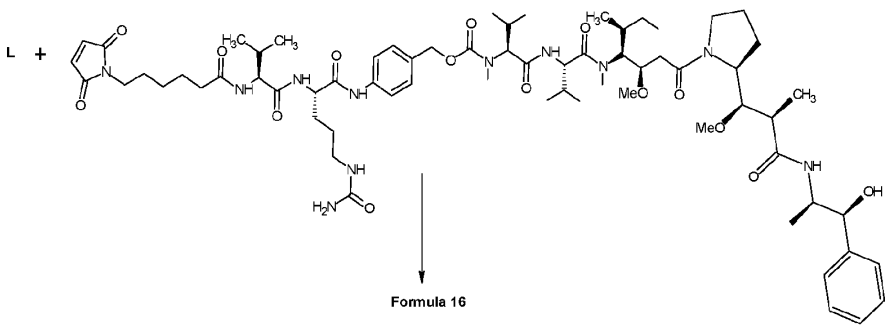


Formula 19

L- Recombinant Lectin of SEQ ID NO 2

10.3 mg of Formula C and 10 mM EDTA was further diluted with to final concentration 2.5 mg/ml by 1X PBS with 10 mM EDTA, DMSO and 2.5 eq of 10 mM Tesirine in DMSO that resulted in a final DMSO concentration of 35% (v/v). The solution was stirred at room temperature for 60 min followed by capping with excess 10 eq NEM. Then the drug to protein ratio DAR of conjugate of Formula 19 was determined by LCMS analysis. The Conjugate of Formula 19 was subjected to SEC using GE10-300 Superdex 75 column with buffer exchange to 50mM Tris, 150 mM NaCl and pH was adjusted to 8.0 using TBS. Post SEC purification DAR value of conjugate of Formula 19 was determined to be 1.32.

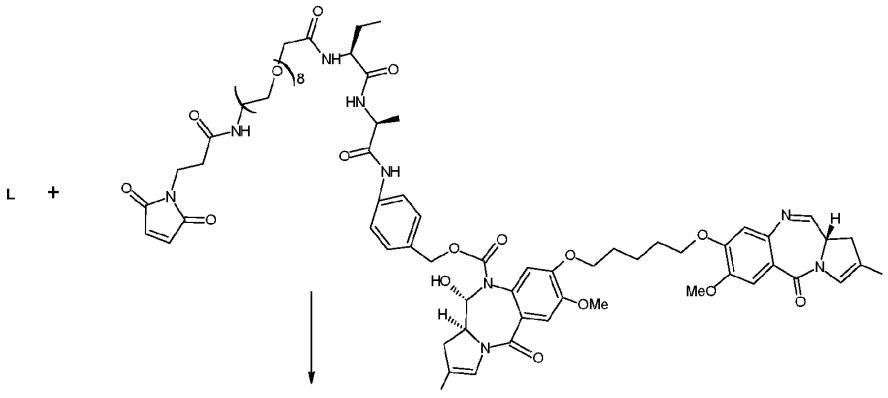
Example 7. Preparation of Conjugate of Formula 16 wherein the L is linked via a cystine (SH) of to the linker drug complex and the linkage is directly to the protein Cysteine SH.



L- Recombinant Lectin of SEQ ID NO 5

30 mg of recombinant lectin of Seq ID No 5 in 1X PBS (7.0 mg/ml) was further diluted with to final concentration 2.5 mg/ml by 1X PBS with 10 mM EDTA, DMA and 3.0 eq of 10 mM MC-Val-Cit-PAB-MMAE in DMA that resulted in a final DMA concentration of 20% (V/V). After 2 hours the reaction was incomplete and another 3.0 eq of MC-Val-Cit-PAB-MMAE was added and incubated for 2 h. Then the conjugate of Formula 16 was subjected to LCMS analysis in-order to determine the DAR value. The product formed was subjected to size exclusion chromatography (SEC) using GE10-300 Superdex 75 column with buffer exchange to 50mM Tris, 150 mM NaCl and pH was adjusted to 8.0 using TBS. Post SEC purification DAR value of conjugate of Formula 16 was determined to be 1.0.

Example 8. Preparation of Conjugate of Formula 18



L- Recombinant Lectin of SEQ ID NO 5

5 mg of recombinant lectin of Seq ID No 5 in 1X PBS (7.0 mg/ml) was further diluted to final concentration 2.5 mg/ml by 1X PBS with 10 mM EDTA, DMA and 3.0 eq of 10 mM Tesirine in DMA that resulted in a final DMA concentration of 20% (v/v). After 2 hours the reaction was incomplete and another 3.0 eq of Tesirine was added and incubated for 2 h.

Then the conjugate Formula 18 was subjected to LCMS analysis in-order to determine the DAR value. The product formed was subjected to SEC using GE10-300 Superdex 75 column with buffer exchange to 50mM Tris, 150 mM NaCl and pH was adjusted to 8.0 using TBS. Post SEC purification DAR value of conjugate of Formula 18 was determined to be 1.05.

Example 9: Bioassay - Cytotoxicity effect of Conjugate of Formula 15, 17 and 19 against ovarian cancer cell line (PA-1) and human urinary Bladder cancer cell line (T-24).

T-24 Cell line were cultured in McCoy's 5a Medium Modified supplemented with 10 % FBS and the PA-1 Cell line was cultured in EMEM Media supplemented with 10% FBS. The assay was performed by plating 5,000 cells/well and incubated at 37 °C overnight. Recombinant lectin protein of SEQ ID NO: 2 conjugates of formula 15, 17 and 19 were made in respective media and diluted 10x starting from 10 micromolar concentration for 11 points of concentration. Each concentration was added in triplicate and media-only wells used as a control. After incubation for 72 h cell titer glow (Promega) reagent (50 µL) was added to each well and the plates shaken for 5 mins and the luminescence was recorded. Percent viability was determined by dividing luminescence of the treated well by the non-treated well and multiplying by 100. Data was analysed using non-linear regression with a logarithmic scale.

Table 1: Cytotoxicity effect of Conjugate of Formula 15, 17 and 19 against ovarian cancer cell line (PA-1) and human urinary Bladder cancer cell line (T-24)

compound	IC_{50} values	
	PA-1 Cell line	**T-24 cell line**
Formula 15	40.9 nM	41.13 nM
Formula 17	2.61 nM	4.216 nM
Formula 19	5.225 nM	70.64 nM
SEQ ID No:2	20.3 µg/ml	10.4 µg/ml

Conclusion:

SEQ ID No. 2 does not exhibit major cytotoxic effects below 10uM concentration in both cell lines. IC50 values are in the rage of 15-25 uM for SEQ ID No. 2, whereas for Formula 15, 17 and 19; IC50 values are in nanomolesi.e., conjugates displayed potent activity against PA-1 cell lines and T-24 cell lines.

Example 10: Bioassay - Cytotoxicity effect Conjugate of Formula 16 and 18 against ovarian cancer cell line (PA-1) and human urinary Bladder cancer cell line (T-24).

5,000 cells/well were plated and incubated at 37°C overnight. Conjugates/vehicle control dilutions were made in respective media and added to cells. Volume added: 50uL to each well. Starting treatment concentration was 1 uM for Conjugate of formula 16 and 18, and control SEQ ID NO: 5 alone and 10% for vehicle control (1X PBS with 20% DMA) and then diluted 10-fold down for a total of 11 treatment dilutions. Each concentration was analysed in triplicates. Media-only wells was used as a control to calculate percent viability. Cell titer glow reagent (volume: 50uL) was added to each well, the plate was shaken for 5 mins and the luminescence was recorded. Drug Treatment Time: 72 hours. Percent viability was calculated by dividing the luminescence signal obtained for each treated well by the untreated well (media-only control) and multiplying by 100.

Data was next transformed using X= Log (x) and then analysed with nonlinear regression (curve fit), Dose Response inhibition – log (inhibitor) vs response (3 parameters) using PRISM software to determine the IC50 value.

Table 2: Cytotoxicity effect of Conjugate of Formula 16 and 18 against ovarian cancer cell line (PA-1) and human urinary Bladder cancer cell line (T-24):

Compound	**IC50 values**	
	PA-1 Cell line	**T-24 cell line**
Formula 16	17.7 nM	325.4 nM
Formula 18	1.04 nM	19.93 nM
SEQ ID NO:5	16µg/ml	-

Conclusion:

SEQ ID No. 5 does not exhibit major cytotoxic effects below 10uM concentration in both cell lines.
Seq ID NO. 5 IC50 values are in the rage of 15-25 uM; whereas for Formula 16 and 18, IC50 values are in nanomolesi.e., conjugates displayed potent activity against PA-1 cell lines and T-24 cell lines.

Example 11 : Glycan binding study and co-localization study

a. **Glycan binding studywas performed to understand the presence and specificity of the Lectin having SEQ ID NO 2 towards glycans expressed on cancer cells.**

Lectin of Seq ID No 2was assayed with following glycan arrays for identifying specific binding motifs:

- O-Glycan array containing 94 glycans; and
- N-Glycan array containing 100 glycans

Observations:

- The said Lectin having Seq ID No 2 showed specific binding to N-glycans containing GlcNac at the non-reducing end; estimated Kd with terminal N-glycan GlcNAc motifs were between 0.238 to 0.460 Kd (ug/ml).
- Lectin having Seq ID No 2 showed strong and broad binding profile to O-glycans, specifically to T-antigen and its extended core structures. Main binding motifs found were O-GalNAc Core1 (T antigen) ; Extended Core1 structures; Core2; α2,3/6-sialyl Core1; and α2,6/6-sialyl Core2. Estimated Kd for T antigen is 0.319, Sialyl T antigen is 0.670 to 1.756 and for core 2 is 0.212 Kd (ug/ml).

b. Colocalization study of SEQ ID NO 2

Study was performed to analyze the localization of SEQ ID NO 2 (test item) in cell organelles using Confocal microscopy.

Test Item provided as aqueous solution was tagged with FITC label at Bioklone Biotech Pvt Ltd. Resultant concentration of FITC tagged test Item was 1mg/mL.

The stock solution of Test Item was diluted in Serum Free Medium (SFM) at single concentration- 100µg/mL.

Cells were plated on a glass coverslip at a density of $0.1x10^6$cells per well. After complete adhesion of the cells on the coverslip, cells were treated with 100µg/mL of FITC- Recombinant Lectin having amino acid sequence of SEQ ID NO: 2 for 2 hours at 37°C.

Following the incubation cells were washed with PBS (3 washes) and fixed using 4% paraformaldehyde for 10 min at RT. After fixation, cells were permeabilized using 0.5% IGEPAL for 10 min at RT (Permeabilization was not done for cells that were stained with Pan-Cadherin). Post permeabilization, cells were blocked using 5% normal goat serum for 1 hour at RT. Post blocking, cells were treated with organelle specific markers for 1.5 hours at RT followed by secondary antibody for 2 hours at RT.

Following antibodies were used for different organelles:

Table 3: Antibodies used for organelles

Organelle	Organelle Specific marker	Dilution	Secondary antibody	Dilution
Mitochondria	MTCO2	1: 50	Texas red Goat Anti-mouse IgG	1:500
Endoplasmic reticulum	Calnexin	1: 50	Anti-mouse Texas red	1:500
Golgi bodies	GM130	1: 50	Anti-mouse Texas red	1:500
Nuclear Envelope	Lamin	1: 50	Anti-mouse Texas red	1:500
Plasma Membrane	Pan Cadherin	1: 50	Anti-rabbit alexa fluor red	1:500

Post incubation, cells were washed using PBST (3 washes) and PBS (3 washes) and stained with DAPI (nucleus stain) for 3 min at RT.

Cells were washed (3 washes) and mounted on a glass slide using Prolong Glass antifade mountant. Imaging was done using Confocal fluorescence microscope (Make: Olympus FV1000) at AIRF, JNU. Images were captured using software FluoView.

To determine the co-localization of Recombinant Lectin having amino acid sequence of SEQ ID NO:2 (Recombinant Protein), bladder cancer cells (T24) were incubated with Recombinant Lectin having amino acid sequence of SEQ ID NO:2 labelled with FITC tag for 2 h. This was followed by staining with organelle specific markers-MTCO2 (Mitochondria), Calnexin (Endoplasmic reticulum), GM130 (Golgi bodies), Pan Cadherin (Plasma membrane) and Lamin A/C (Nuclear Membrane). Cells were imaged using confocal microscope.

Results demonstrated that Recombinant Lectin having amino acid sequence of SEQ ID NO:2 (Recombinant Protein) (Recombinant Protein) colocalized in Mitochondria, Endoplasmic reticulum, Golgi bodies and Plasma membrane (red fluorescence). However, no localization was observed in the nuclear envelope.[Refer Figure 6].

Example 12: Cytotoxic effect of conjugates were evaluated in a panel of cancer cell lines and a normal cell line by MTT assay

Cells were treated with Test Items at various concentrations (0.0001µM – 10 µM) for 48 h. The cells corresponding to Positive Control group were treated with Doxorubicin at a concentration ranging from 0.1µM-100 µM. The Untreated cells were used as Control. After 48h of incubation, the effect of Test Items on cytotoxicity of cells was determined by MTT assay, the plates were taken out and 20 µl of 5 mg/ml of MTT 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide solution was added to all the wells. Cells were incubated for 3h at 37°C. The supernatant was aspirated. 150 µl of DMSO was added to each well to dissolve formazan crystals. The absorbance of each well was then read at 540 nm using Synergy HT micro plate reader.

The % Cytotoxicity was calculated using the formula:

% Cytotoxicity = [(R-X)/R] *100

Where, X = Absorbance of cells treated with Test Item; R = Absorbance of Control cells (Untreated cells maintained in growth medium only).

The IC50 value was calculated as the concentration leading to 50% inhibition of cell growth with respect to untreated cells using Graph pad prism version 4.01.

Selectivity Index was calculated using the formula:

SI = IC50 in Normal cell / IC50 in cancer cell.

Activity scores of the Selective Index values were categorized as:

- Not selective (SI value ≤ 1)
- Moderately selective (1 < SI value < 5)
- Selective (SI value ≥ 5)

Results obtained were as follows:

Table 4a- CYTOTOXIC EFFECT OF SEQ ID No. 2, MMAE AND FORMULA 15 (DRUG CONJUGATE) AFTER 48HOURS OF TREATMENT

Cell Lines	Doxorubicin (µm) Positive control (µm)		SEQ ID No. 2 (µm)		MMAE (µm)		FORMULA 15 (DRUG CONJUGATE) (µm)	
	Concentration	Percent cytotoxicity wrt control	Concentration	Percent cytotoxicity wrt control	Concentration	Percent cytotoxicity wrt control	Concentration	Percent cytotoxicity wrt control
MG-63 Bone	0.1	35.3	0.0001	27	0.0001	81.7	0.0001	33.2
	1	60.7	0.001	38.7	0.001	85.5	0.001	16.7

	10	82	0.01	50.4	0.01	86.2	0.01	58.5
	50	85.5	0.1	59.1	0.1	87.4	0.1	77.1
	100	75.5	1	72.3	1	85.3	1	82.5
	-	-	10	74	10	82.3	10	85.6
PA-1 Ovary	0.1	19.6	0.0001	0.9	0.0001	13.4	0.0001	25.3
	1	62.6	0.001	-5.6	0.001	57.5	0.001	20.7
	10	73.2	0.01	7.4	0.01	50	0.01	49.3
	50	79	0.1	4.5	0.1	57.5	0.1	45.9
	100	71.5	1	51	1	61.7	1	50.3
	-	-	10	72.2	10	58.3	10	73.9
KB Cervix	0.1	28.7	0.0001	-1.5	0.0001	90.5	0.0001	-7
	1	53.1	0.001	-2.6	0.001	95.8	0.001	-10.8
	10	95.7	0.01	-3.9	0.01	96.1	0.01	9.4
	50	91.7	0.1	20.4	0.1	96.3	0.1	48.2
	100	84.2	1	51.2	1	96.2	1	84.3
	-	-	10	54.9	10	96.3	10	90.4
T24 Bladder	0.1	21	0.0001	9.7	0.0001	51.8	0.0001	11.8
	1	34.8	0.001	11.1	0.001	51.1	0.001	6.3
	10	72.8	0.01	9.1	0.01	46.1	0.01	5.5
	50	66.6	0.1	10.5	0.1	44.4	0.1	41.6
	100	52.7	1	43.1	1	44.4	1	47.9
	-	-	10	68.7	10	53.2	10	55
MDA-MB-231 Breast	0.1	3.4	0.0001	13.7	0.0001	11.3	0.0001	9.1
	1	68	0.001	15.1	0.001	13.3	0.001	6.9
	10	74.3	0.01	16.2	0.01	28.9	0.01	19.4
	50	71.3	0.1	28.1	0.1	23.8	0.1	-3.4
	100	58	1	48.5	1	16.8	1	53.1
	-	-	10	66.4	10	26.4	10	57.7
U251 MG Brain	0.1	-13.5	0.0001	-9.9	0.0001	77.8	0.0001	-14.8
	1	41.7	0.001	-13.2	0.001	52.5	0.001	-16.8
	10	57.9	0.01	-14.5	0.01	58.6	0.01	15.2
	50	71	0.1	-5.3	0.1	63.1	0.1	14
	100	65.6	1	15.9	1	64.7	1	28.4
	-	-	10	62.5	10	71	10	72.6
HT29 Colon	0.1	3.2	0.0001	14	0.0001	44.1	0.0001	5.4
	1	1.7	0.001	16.6	0.001	44.6	0.001	2.4
	10	32.7	0.01	22.5	0.01	50.6	0.01	37.2
	50	61.9	0.1	24.7	0.1	50.4	0.1	52.8
	100	59.8	1	35.4	1	51.9	1	63.9
	-	-	10	54.1	10	55.9	10	68.2
AGS Stomach	0.1	15.6	0.0001	7.8	0.0001	30.9	0.0001	1.6
	1	72.4	0.001	8.5	0.001	31.7	0.001	39.2
	10	74.5	0.01	15.9	0.01	32.6	0.01	42.4

	50	74.8	0.1	37	0.1	48.2	0.1	49.2
	100	79	1	49.9	1	48.4	1	56.8
	-	-	10	57.2	10	54.4	10	67.6
PANC-1 Pancreatic	0.1	-3.4	0.0001	9.5	0.0001	38.1	0.0001	50.2
	1	18.6	0.001	17	0.001	41	0.001	53.1
	10	67.3	0.01	20.5	0.01	64.1	0.01	56.2
	50	71.6	0.1	22.5	0.1	67.7	0.1	65.5
	100	70.1	1	34.4	1	77.1	1	70.6
	-	-	10	36.4	10	69.7	10	72.5
A549 Lungs	0.1	3.3	0.0001	-0.5	0.0001	27.9	0.0001	-8.8
	1	10.2	0.001	-15.5	0.001	39.3	0.001	-8.9
	10	54.2	0.01	-1.1	0.01	43.5	0.01	12.8
	50	75.4	0.1	-6.9	0.1	58.8	0.1	39.1
	100	76.2	1	31.9	1	83.6	1	60.2
	-	-	10	48.2	10	75.8	10	76.7
PBMC Normal	0.1	5.3	0.0001	-7.4	0.0001	-2.7	0.0001	-7.6
	1	29.3	0.001	19.9	0.001	29.8	0.001	24.7
	10	37.4	0.01	24	0.01	34.3	0.01	28.1
	50	40.5	0.1	44.9	0.1	34.1	0.1	40.7
	100	37.1	1	49.5	1	26.6	1	57.2
	-	-	10	43.4	10	32.5	10	53.7

Conclusion:
The compound of FORMULA 15 (DRUG CONJUGATE) showed cytotoxicity towards panel of cell-lines tested.

Table 4b- CYTOTOXIC EFFECT OF TESIRINE AND FORMULA 17 (DRUG CONJUGATES) AFTER 48 HOURS OF TREATMENT

Cell Lines	TESIRINE (µm)		FORMULA 17 (DRUG CONJUGATES) 2A (µm)		FORMULA 17 (DRUG CONJUGATES)2B (µm)		FORMULA 17 (DRUG CONJUGATES)2C (µm)	
	Concentration	Percent cytotoxicity wrtcontrol	Concentration	Percent cytotoxicity wrtcontrol	Concentration	Percent cytotoxicity wrtcontrol	Concentration	Percent cytotoxicity wrtcontrol
MG-63 Bone	0.0001	46.6	0.0001	56.8	0.0001	28.6	0.0001	42.7
	0.001	69.4	0.001	50.5	0.001	33.3	0.001	45.9
	0.01	81.2	0.01	53.4	0.01	31.3	0.01	28
	0.1	79.9	0.1	88.8	0.1	72.4	0.1	46.1
	1	82.3	1	91.7	1	89.6	1	90.7
	10	81.8	10	91.6	10	86	10	90.4
PA-1	0.0001	53.1	0.0001	26.1	0.0001	10.2	0.0001	17.8

Ovary	0.001	66.8	0.001	36.4	0.001	18.4	0.001	25.2
	0.01	79.1	0.01	75.1	0.01	56.2	0.01	61.2
	0.1	79.9	0.1	80	0.1	80.3	0.1	80.6
	1	78.1	1	79.9	1	78.9	1	79.6
	10	80.5	10	80.7	10	80.2	10	80.3
KB Cervix	0.0001	-9.5	0.0001	15.3	0.0001	15.7	0.0001	-2
	0.001	1	0.001	27.1	0.001	17.6	0.001	11.5
	0.01	76.2	0.01	57.6	0.01	36.5	0.01	30.1
	0.1	93.8	0.1	95.5	0.1	91.2	0.1	90.2
	1	89.1	1	95.7	1	95.8	1	95.9
	10	92.7	10	95.9	10	94.2	10	93
T24 Bladder	0.0001	57.5	0.0001	21.6	0.0001	21.8	0.0001	24.3
	0.001	60.6	0.001	28.7	0.001	26.7	0.001	22.4
	0.01	57.5	0.01	33.7	0.01	26.3	0.01	23.3
	0.1	61.4	0.1	51	0.1	44.8	0.1	40.2
	1	62.7	1	57.7	1	55.4	1	59.6
	10	80.4	10	59.4	10	66.2	10	74.4
MDA-MB-231 Breast	0.0001	45.9	0.0001	14.5	0.0001	58.5	0.0001	65.4
	0.001	41.9	0.001	-0.6	0.001	62.9	0.001	67.8
	0.01	58.9	0.01	1.7	0.01	62.8	0.01	63.8
	0.1	68.6	0.1	44.7	0.1	68.3	0.1	69.8
	1	69.2	1	55.3	1	75.4	1	74.3
	10	80.7	10	77.6	10	73.7	10	81.8
U251 MG Brain	0.0001	-10.3	0.0001	-1	0.0001	10.2	0.0001	11.5
	0.001	26.5	0.001	16.4	0.001	9.7	0.001	6.8
	0.01	23.1	0.01	75.5	0.01	18.1	0.01	18.5
	0.1	19.2	0.1	62.6	0.1	77.9	0.1	77.4
	1	27.2	1	54.7	1	65.8	1	67.1
	10	60.5	10	70.3	10	77.3	10	78.6
HT29 Colon	0.0001	37.4	0.0001	17.5	0.0001	5.7	0.0001	12.8
	0.001	44.5	0.001	20.9	0.001	6.9	0.001	11.6
	0.01	47.7	0.01	22.9	0.01	15.8	0.01	10.7
	0.1	48.3	0.1	30.1	0.1	15.6	0.1	26.2
	1	49	1	53.2	1	39.2	1	43.5
	10	80.7	10	63.9	10	59.5	10	68.6
AGS Stomach	0.0001	66.4	0.0001	7.6	0.0001	27.7	0.0001	22.6
	0.001	77.5	0.001	3.2	0.001	25.3	0.001	19.9
	0.01	77.8	0.01	59.2	0.01	28.1	0.01	38.3
	0.1	81.3	0.1	69.6	0.1	73.3	0.1	71.7
	1	81.3	1	72.1	1	75	1	75.1
	10	81.6	10	78.8	10	71.6	10	78.4
PANC-1	0.0001	46.6	0.0001	13.5	0.0001	-2	0.0001	9.3
	0.001	51.1	0.001	11.4	0.001	24	0.001	6.5

Pancreatic	0.01	58.3	0.01	17.1	0.01	18.1	0.01	6.4
	0.1	55	0.1	35.7	0.1	21.6	0.1	18.7
	1	59.2	1	67.8	1	13.2	1	14.6
	10	71	10	77.5	10	58.9	10	68.6
A549 Lungs	0.0001	13.7	0.0001	-6.3	0.0001	-10.3	0.0001	-3.8
	0.001	15.2	0.001	-3.1	0.001	-15.4	0.001	-19.1
	0.01	34.4	0.01	12	0.01	-2	0.01	1
	0.1	45.2	0.1	84.3	0.1	11.6	0.1	9.1
	1	53.3	1	75.2	1	38.4	1	68.6
	10	66.7	10	79.7	10	78.5	10	70.8
PBMC Normal	0.0001	16.9	0.0001	-4.4	0.0001	9.1	0.0001	29.1
	0.001	36.5	0.001	22.9	0.001	14	0.001	25.5
	0.01	40.3	0.01	18.8	0.01	30.2	0.01	18.2
	0.1	38.7	0.1	45.9	0.1	34.4	0.1	33.1
	1	38.2	1	42.7	1	68.8	1	65.9
	10	55.3	10	65.7	10	68.5	10	70.4

Conclusion:
The compound of Formula 17 showed cytotoxicity towards panel of cell-lines tested.

TABLE 5: IC50 VALUES OF Formula 15 AND Formula 17(RECOMBINANT- PROTEIN DRUG CONJUGATES) AFTER 48H OF TREATMENT

	Cell Lines	Doxorubicin PC (µm)	SEQ ID NO. 2 (µm)	MMAE(µm)	Formula 15 Conjugate (µm)	TESIRINE (µm)	Formula 17 Conjugate 2A (µm)	Formula 17 Conjugate 2B (µm)	Formula 17 Conjugate 2C (µm)
IC50 Values	**MG-63 Bone**	0.4	0.0127	0.0001	0.0067	0.00003	0.00021	0.012	0.009
	PA-1 Ovary	1	1.535	0.03011	0.1422	5.99E-06	0.002	0.011	0.007
	KB Cervix	0.6	0.88	0.0001	0.12	0.006	0.005	0.014	0.019
	T24 Bladder	5.5	2.13	0.00001	2.19	0.002	0.27	0.43	0.34
	MDA-MB-231 Breast	2.1	1.35	>10	1.58	0.0014	0.87	7.7DE-07	7.96E-09
	U251 MG Brain	8.1	5.95	0.0002	2.82	6.82	0.04	0.07	0.07

	HT29 Colon	35.3	9.39	0.05	0.18	0.02	1.09	3.87	1.63
	AGS Stomach	4.3	1.72	1.54	0.14	4.33E-10	0.03	0.03	0.02
	PANC-1 Pancreatic	8.2	>10	0.0015	0.001	0.0007	0.31	1	0.44
	A549 Lungs	11	10	0.01	0.45	0.39	0.04	1.83	0.78
	PBMC Normal	>100	>10	>10	0.94	5.09	0.96	0.21	0.49
Conclusion: PDC conjugates showed superior cytotoxic activity in all cancer types tested compared to Seq. ID. 2. The IC 50 values PDCs are very low in cancer cells when compared to normal cells.									

TABLE 6: SELECTIVITY INDEX OF Formula 15 AND Formula 17 (RECOMBINANT-PROTEIN DRUG CONJUGATES) AFTER 48H OF TREATMENTWRT PBMCs

	Cell Lines	SEQ ID NO. 2(µm)	MMAE (µm)	Formula 15 Conjugate 1A (µm)	TESIRINE (µm)	Formula 17 Conjugate 2A (µm)	Formula 17 Conjugate 2B (µm)	Formula 17 Conjugate 2C (µm)
Selectivity wrt PBMC	MG-63 Bone	786.78	100000	140.3	169666.7	4541.2	18.26	56.4
	PA-1 Ovary	6.51	332.1	6.6	849182.5	449.9	19.14	73.2
	KB Cervix	11.33	100000	7.9	844.8	193	14.89	26.1
	T24 Bladder	4.69	1062837	0.4	2412.3	3.6	0.49	1.4
	MDA-MB-231 Breast	7.39	1	0.6	3707.2	1.1	272762.7	61573259.6
	U251 MG Brain	1.68	49019.6	0.3	0.7	23.4	2.93	7.1
	HT29 Colon	1.07	208.4	5.1	236.1	0.9	0.05	0.3
	AGS Stomach	5.81	1	6.7	1.18E+10	37.7	7.04	20.7

	PANC-1 Pancreatic	1	6662.2	987.8	7829.6	3.1	0.21	1.1
	A549 Lungs	1	887.3	2.1	13	26.8	0.11	0.6

Selectivity Index (SI) is interpreted as
SI Value <1 = Not selective; 1<SI Value <5 = Moderately selective; SI Value >5 = Selective;

Conclusion:

PDC conjugates showed high selectivity index in cancer types tested compared to normal cells.

Example 13:

In vivo anti-tumor potential of compound of Formula 16 **was evaluated using T24 human bladder carcinoma xenograft model**

In vivo anti-tumor potential of compound of Formula 16 was evaluated using T24 human bladder carcinoma xenograft model in mice by assessing biological endpoints such as tumor growth and tumor growth inhibition.

Thirty-five healthy female nude mice bearing T24 tumor with volume of ~100mm3 were selected, randomized and allocated into five groups (G1-G5, n=7 animals per group). Body weight of each mouse was recorded on the day of randomization and daily till the end of the study. The tumor size was measured weekly twice from the day of test item/reference drug/vehicle dosing till the end of the experiment. Tumor size was measured by digital caliper (MITUTOYO, Japan) by measuring length and width. Body weight and tumor volume data was statistically analysed using two-way ANOVA (Bonferroni Test). Statistical analysis was conducted using PRISM software, version 5.01.p values of <0.05 were considered as statistically significant. Percentage survival proportions were analyzed by "Kaplan Meier" method.

RESULTS:

Table 7:

Group and treatment	Percent body weight change Day 22	Tumor volume Day 22 (cu.mm)	Percent TGI Day 22	Percent T/C Day 22	Tumor growth delay (days)	Percent survival

G1, Vehicle control (5mL/kg)	5.67	1052.0	0.00	100.00	NA	100
G2, Cisplatin (5mg/kg), Weekly once	6.3	622.6	40.82	59.18	10.5	100
G3, Formula 16(0.5mg/kg), Alternate days	4.73	540.6	48.62	51.38	7.5	100
G4, Formula 16(1mg/kg),once in three days	1.53	705.2	32.96	67.04	6	100
G5, Formula 16 (2mg/kg), once in six days	5.84	859.8	18.27	81.73	3	100
Abbreviation TGI: Tumor Growth Inhibition T/C: Test/Control						

Conclusion:

The biological end points such as mean tumor volume, tumor growth inhibition (TGI) and % Test/Control (5T/C) showed that the treatment of Formula 16at the given doses and regimen showed reduction in tumor volume in T24 xenograft model without body weight loss. Statistically significant anti-tumor activity was observed at the doses of 0.5mg/kg, alternate days ($p<0.001$; TGI:48.62%) on Day 22 when compared to vehicle control group G1. Hence it can be concluded based on the study conducted that the Protein drug conjugate Formula 16showed invivo anti-tumor efficacy in human urinary bladder carcinoma xenograft model.

Example 14: Histopathological Investigation of Tumour Tissue

Effect of compound of Formula 16 was evaluated using T24 human bladder carcinoma xenograft model.

Table 8:

Group and treatment	Apoptosis	Grade	
G1, Vehicle control (5mL/kg)	Mild	3	Moderately Differentiated
G2, Cisplatin (5mg/kg), Weekly once	Mild	2	Moderately Differentiated
G3, Formula 16(0.5mg/kg), Alternate days	Moderate	2	Moderately Differentiated
G4, Formula 16(1mg/kg),once in three days	Moderate	2	Moderately Differentiated
G5, Formula 16 (2mg/kg), once in six days	Marked	1	Well Differentiated
Note: Low grade indicates a better apoptosis. Grade 1 (Well differentiated) - <25% cells anaplastic; Grade 2 (Moderately Differentiated) - <25-50% cells anaplastic; Grade 3 (Moderately Differentiated) - <50-75% cells anaplastic; Grade 4 (Poorly Differentiated) - >75% cells anaplastic			

Conclusion:

Treatment Group (G3, G4, G5) showed

- increased apoptosis, angiogenesis, low mitotic figures; and
- lower grade as compared to vehicle group indicated anti tumour effect of compound of Formula 16 in T24 human bladder carcinoma xenograft model.

Example 15: Effect of compound of Formula 16on Immune system

Effect of compound of Formula 16 was evaluated using T24 human bladder carcinoma xenograft model.

Results:

Table 9:

Effect of compound of Formula 16 on Immune System						
Group and Treatment	Th1 cytokines		Th2 cytokines		Immunoglobulins	
	IFN gamma (pg/ML)	IL-2 (pg/ml)	IL-10 (pg/ml)	IL-4 (pg/ml)	IgG (ng/mL)	IgM (ng/mL)
G1, Vehicle Control, 5mL/kg	50.4	148.4	BLQ	68.7	54.6	39.5
G2, Cisplatin, 5mg/kg, i.p. (q7dX2)	80.8	259.3	BLQ	60	54	18
G3, Formula 16 0.5 mg/kg, i.v. (q2dX7)	54.8	182.5	652.9	63.4	56.7	24.6
G4, Formula 16 1mg/kg, i.v. (q3dX5)	104.4	229.5	219.1	61.1	59.1	29.5
G5,Formula 16 2mg/kg, (q6dX3)	73.3	220.8	331.7	NA	55.6	26.5

Conclusion:

- Th1 cytokines were found to be increased demonstrating immune cell activation for NK cell mediated suppression in tumour growth.
- Cisplatin group showed reduction in IgM indicating immuno suppression.
- Treatment group (G3, G4, G5) showed moderately altered level of IgM.

Example 16: Evaluation of Acute toxicity of compound of Formula 16 in BALB/c mice

Twenty-five healthy female BALB/c mice were used in the study. Animals were acclimatized for five days. After completion of acclimatization, animals were randomized based on the body weight and grouped into five groups (G1-G5), comprising five animals in each group. Animals from G1, G2, G3, G4 and G5 received Formula 16 intravenously once at the dose of 0.5 mg/kg, 2.5 mg/kg, 5 mg/kg, 7.5 mg/kg and 10 mg/kg respectively at a dose volume of 5 mL/kg. Animals were observed for clinical sign of toxicity and mortality once daily up to 14 days. Body weight was measured on day 1, 2, 3, 7 and 14. On day 15, animals were necropsied for gross pathological investigation of vital organs was carried out.

No clinical signs of toxicity were observed in G1 and G2 animals. In G3 group, 3 animals showed mild symptoms (Lethargy) whereas 2 animals showed severe symptoms (Lethargy, Tremor, Convulsion) however animals recovered after ~15-20 minutes post dosing. In G4 group, 1 animal died immediately post dosing and necropsied for gross pathology, reddish

coloration of all lobes of lungs was found. Whereas 4 animals showed severe symptoms (Lethargy, Tremor, Convulsion, Gasping) however animals recovered later. In G5 group, all animals died immediately post dosing and necropsied for gross pathology, reddish coloration of all lobes of lungs was observed. All the surviving animals from groups G1-G4 were necropsied on day 15 and no visible changes were found during the gross pathological examination of vital organs.

Based on the study, single i.v.dose.of compound of Formula 16 at concentrations 0.5 mg/kg, 2.5 mg/kg, 5 mg/kg was found to be well tolerated and safe.

We Claim:

1. A protein drug conjugate comprising:
   a) a lectin; and
   b) a drug;
   wherein lectin is conjugated to drug;
   wherein lectin is a recombinant lectin having amino acid sequence of SEQ ID NO: 1; or the lectin having at least 70%identity to SEQ ID NO: 1.
2. The protein drug conjugate as claimed in claim 1, wherein thelectin has binding affinity towards one or more antigen selected from the group comprising of Thomsen-Friedenreich antigen (O-GalNAc Core1 antigen), and its extended core structures (Core2, α2,3/6-sialyl Core1 (Sialyl-T antigen), α2,6/6-sialyl Core2), and its modified forms.
3. The protein drug conjugate as claimed in claim 1, wherein the lectin having at least 70% identity to SEQ ID NO: 1is selected from a group consisting of SEQ ID NO: 2, 3,4, 5and8.
4. The protein drug conjugate as claimed in claim 1, wherein lectin is conjugated to drugby a covalent bond or a coordinate covalent bond.
5. The protein drug conjugate as claimed in claim 1, wherein lectin is attached to drug via free amino moiety (-NH- or $-NH_2$ group) or free thiol moiety (-SH group) or free acid (-COOH group)or free hydroxyl (OH group) available on amino acid.
6. The protein drug conjugate as claimed in claim 1, wherein the lectin is bound to the drug by a covalent bond or a coordinate covalent bond, via linker.
7. The protein drug conjugate as claimed in claim 1, wherein lectin is used for delivery of drug to the target cell.
8. The protein drug conjugate as claimed in claim 1, wherein drug is selected from a group comprising of therapeutic agent, cytotoxic agent, anti-cancer agent, diagnostic agent, and combinations thereof.
9. The protein drug conjugate as claimed in claim 1, wherein the drug is selected from a group comprising of monomethyl auristatin E (MMAE), Tesirine, DM1, DM4, DM 21, Doxorubicin, Govitecan, Dxd 1, and Amantin.
10. The protein drug conjugate as claimed in claim 1, wherein the lectin is conjugated to drug selected from MC-VC-PAB-MMAE, mc-hydrazone-Doxorubicin, DM21-C,

DXd(1), SMCC-DM1, Govitecan (Linker- SN38), SPDB-DM4, mc-vc-PAB-C6-α-Amanitin, MC-GGFG-NH-CH2-Exatecan, Tesirine (Linker- SG3249).

11. The protein drug conjugate as claimed in claim 1, wherein the conjugate is used in treatment or prevention of cancer.

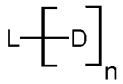
12. A pharmaceutical composition comprising:

a) a protein drug conjugate as claimed in claim 1; and

b) one or more pharmaceutically acceptable excipient(s).

13. The pharmaceutical composition as claimed in claim 12, wherein the said composition is used in treatment or prevention of cancer.

14. A protein drug conjugate of Formula I;



Formula I

or a pharmaceutically acceptable salt thereof; wherein

L is lectin;

D is drug;

"— " between L and D is a bond; and

n is 1 to 9.

15. The protein drug conjugate as claimed in claim 14, wherein lectin is a recombinant lectin having amino acid sequence of SEQ ID NO: 1; or the lectin having at least 70% identity to SEQ ID NO: 1.

16. The protein drug conjugate as claimed in claim 14, wherein drug is selected from a group comprising of therapeutic agent, cytotoxic agent, anti-cancer agent, diagnostic agent, and combinations thereof.

17. The protein drug conjugate as claimed in claim 16, wherein the cytotoxic or therapeutic agent is selected from a group comprising of monomethyl auristatin E (MMAE), Tesirine, DM1, DM4, DM 21, Doxorubicin, Govitecan, Dxd 1, and Amantin.

18. The protein drug conjugate as claimed in claim 14, wherein drug is selected from an DNA binding agent, an anti-microtubule agent, an antimetabolite, an alkylating or alkylating-like antineoplastic agent, a topoisomerase I inhibitor, a topoisomerase II inhibitor, a kinase inhibitor, bortezomib, estramustine, Ixabepilone, everolimus,

temsirolimus, neomycin, neamine, a cryptophycin, discodermolide, amanitin, or a pyrrolobenzodiazepine dimer, or a pharmaceutically acceptable salt, polymorph, and solvate thereof.

19. The protein drug conjugate as claimed in claim 18, wherein the DNA binding agent is selected from bleomycin, netropsin, distamycin or their analogues including lexitropsins, enediyne,mitomycin, and duocarmycin.

20. The protein drug conjugate as claimed in claim 18, wherein anti-microtubule agent is selected from auristatin, maytansine, and maytansinoid.

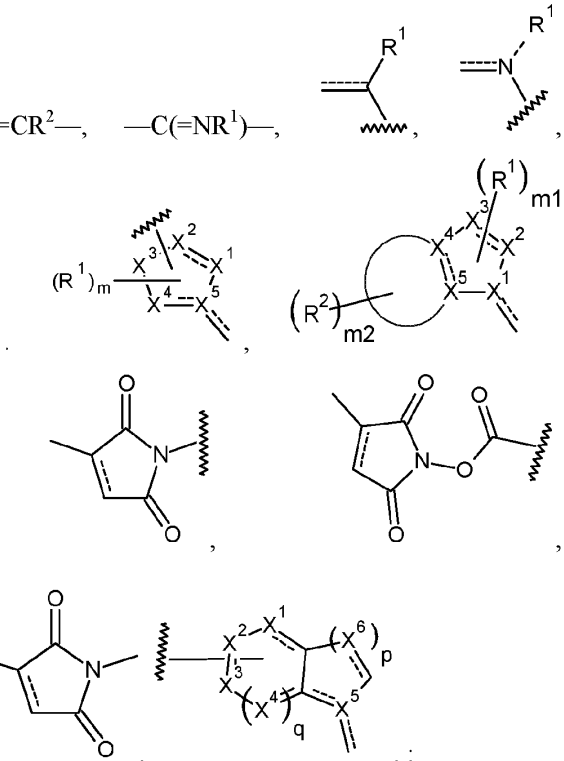
21. The protein drug conjugate as claimed in claim 14, wherein each drug is conjugated to L via:

a) a covalent bond, or

b) a group of formula "—A^1-A^2-A^3—";

wherein the other terminal of A^1 and A^3 is connected to L and D respectively; and wherein A^1 and A^3 is independently selected from:

—CR^1R^2—, —C(O) —, —C(O)NR^1—, —NR^1C(O) —, —NR^1—, —O—, —S—, —S(O) —, —$S(O)_2$—, —R^1C=CR^2—, —C≡C—, —R^1C=CR^2-R^3C=CR^4—, —R^1C=C=CR^2—, —C(=NR^1)—,

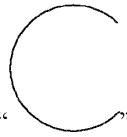


wherein

m, m1 and m2 are independently 0, 1, 2, 3, or 4; p is 1 or 2 and q is 0 or 1;

"wwwww" indicates the point of attachment of A^2 to A^1 or A^3;

"=====" indicates single or double bond;



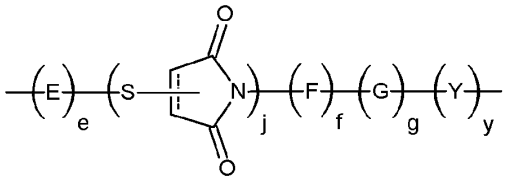
" " indicates a 5 to 10 membered ring including atoms of the ring it is fused to, wherein the 5 to 10 membered ring is an aromatic, non-aromatic, or cycloalkyl ring or a heteroaromatic or heterocycloalkyl ring wherein the heteroaromatic or heterocycloalkyl ring comprise at least one heteroatom selected from N, O or S ;

each of X^1, X^2, X^3, X^4, X^5 and X^6 is independently selected from C, CH, CH_2, N, NH, O or S; and

R^1, R^2, R^3 and R^4 are independently selected from hydrogen, C_1 to C_5 alkyl, C_2 to C_5 alkenyl, C_2 to C_5 alkynyl, C_1 to C_5 alkylene, C_3 to C_{10} cycloalkyl, C_2 to C_9heterocycloalkyl, C_6 to C_{10} aryl, C_2 to C_9 heteroaryl, $-OR^5$, $-NR^5$ $-SR^5$, halogen selected from fluorine, chlorine, bromine or iodine, and $-C(O)OR^5$, wherein C_1 to C_5 alkyl, C_2 to C_5 alkenyl, C_2 to C_5 alkynyl, C_1 to C_5 alkylene, C_3 to C_{10} cycloalkyl, C_2 to C_9heterocycloalkyl, C_6 to C_{10} aryl, and C_2 to C_9 heteroaryl are unsubstituted or are each further substituted by R^6, and

R^5 and R^6 are each independently hydrogen, C_1 to C_3 alkyl, OH or halogen;

wherein A^2 is of formula:



wherein

e, j, f, g and y are independently 0 or 1, provided e, f and y are not 0 simultaneously,

S is sulphur atom,

E, F, and Y is a spacer, and

G is an amino acid or apeptide chain comprising 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 amino acids;

and wherein A^1 forms bond with E or S or F and A^3 is connected to Y or G.

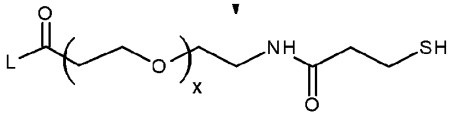
22. The protein drug conjugate as claimed in 21, wherein each spacer E, F and Y is independently selected from —R^7—, —S—, —NH—, —R^7-N-R^8—, —R^8-NC(O)-R^7—, —R^8-C(O)N-R^7—, —C(O)—, —C(O)-R^7—, —R^7-C(O)—, —R^8-C(O)-R^7—, —S-R^7—, —R^7-S—, —R^7-S-R^8—, —S(O)—, —S(O)-R^7—, —R^7-S(O)—, —R^7-S(O)-R^8—, —O-R^7—, —R^7-O—, —R^8-O-R^7—, —C(O)O—, —OC(O)-R^7—, —R^7-OC(O)—, — R^8-OC(O)-R^7—, —R^7-OC(O)O—, —OC(O)O-R^7—, —R^7-OC(O)O-R^8—, —NH-R^7-NH—C(O)R^8—, —(CH2)S-NH-R^7—, —R^7-C(O)N(CH2)1-3O(CH2)1-3C(O)—, —R^7-NC(O)(CH2)1-3O(CH2)1-3C(O)—, —Si(R^7R^8)—, polyalkylene glycol optionally attached through oxygen to —R^7—, —S—, —NH—, —R^7-N-R^8—, —R^8-NC(O)-R^7—, —R^8-C(O)N-R^7—, —C(O)—, —C(O)-R^7—, —R^7-C(O)—, —R^8-C(O)-R^7—, —S-R^7—, —R^7-S—, —R^7-S-R^8—, —S(O)—, —S(O)-R^7—, —R^7-S(O)—, —R^7-S(O)-R^8—, —O-R^7—, —R^7-O—, —R^8-O-R^7—, —C(O)O—, —OC(O)-R^7—, —R^7-OC(O)—, — R^8-OC(O)-R^7—, —R^7-OC(O)O—, —OC(O)O-R^7—, —R^7-OC(O)O-R^8—, —NH-R^7-NH—C(O)R^8—, —(CH2)S-NH-R^7—, —R^7-C(O)N(CH2)1-3O(CH2)1-3C(O)—, —R^7-NC(O)(CH2)1-3O(CH2)1-3C(O)—, —Si(R^7R^8)—;

wherein R^7 and R^8 are independently selected from hydrogen, —C1-C10 alkylene, —C1-C10 alkenylene, C5 to C10 saturated or unsaturated cycloalkylene, —C2-C10 alkenylene-C5 to C10 saturated or unsaturated cycloalkylene, C6 to C10 arylene, —C2-C10 alkenylene-C6 to C10 arylene C2 to C9 heteroarylene, —C2-C10 alkenylene-C2 to C9 heteroarylene; and wherein the —C1-C10 alkylene, —C2-C10 alkenylene, C5 to C10 saturated or unsaturated cycloalkylene, —C2-C10 alkenylene-C5 to C10 saturated or unsaturated cycloalkylene, C6 to C10 arylene, —C2-C10 alkenylene-C6 to C10 arylene, C2 to C9 heteroarylene and —C2-C10 alkenylene-C2 to C9 heteroarylene is optionally further substituted with -OH, -C(O), -SH, -NH2, halogen, C1 to C5 alkyl, -NO2, or -CN;

wherein polyalkylene glycol is a homopolymer or copolymer of alkylene with each unit of alkylene having C1 to C8 carbon atoms and total number of alkylene units ranging from 1 to 20; and wherein when polyalkyleneglycol is E the -CH2- end is attached to A1 and when polyalkylene glycol is F the -CH2- end is attached to —R^7—, —S—, —NH—, —R^7-N-R^8—, —R^8-NC(O)-R^7—, —R^8-C(O)N-R^7—, —C(O)—, —C(O)-R^7—, —R^7-C(O)—, —R^8-C(O)-R^7—, —S-R^7—, —R^7-S—, —R^7-S-R^8—, —S(O)—, —S(O)-R^7—, —R^7-S(O)—, —R^7-S(O)-R^8—, —O-R^7—, —R^7-O—, —R^8-O-R^7—, —C(O)O—, —OC(O)-R^7—, —R^7-OC(O)—, — R^8-OC(O)-R^7—, —R^7-

OC(O)O—, —OC(O)O-R^7—, —R^7-OC(O)O-R^8—, —NH-R^7-NH—C(O)R^8— or —(CH2)S-NH-R^7—, —R^7-C(O)N(CH2)1-3O(CH2)1-3C(O)— or —R^7-NC(O)(CH2)1-3O(CH2)1-3C(O)—.

23. A compound of Formula C represented as



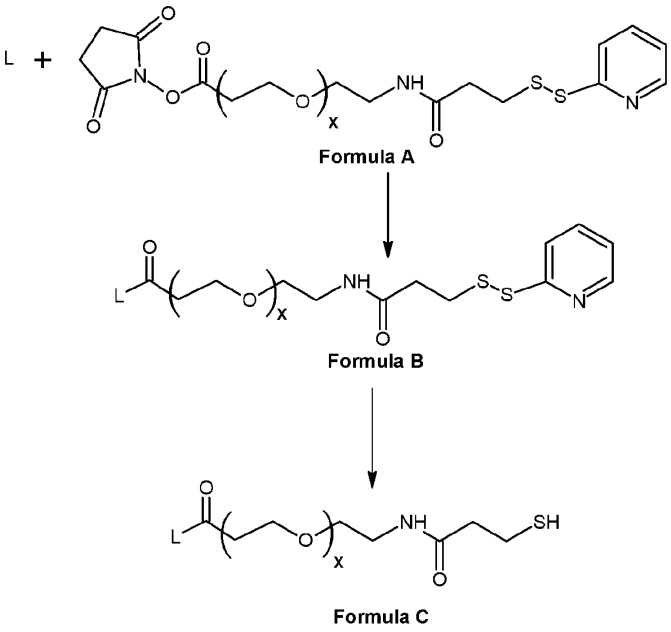
Formula C

wherein

x is 8 or 12; and

L is a lectin having amino acid sequence of SEQ ID NO: 1; or the lectin having at least 70% identity to SEQ ID NO: 1.

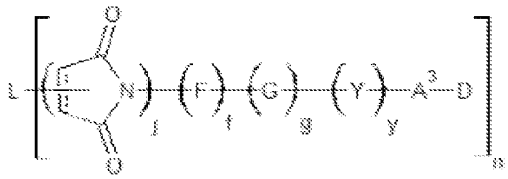
24. The compound of Formula C as claimed in claim 23, wherein the compound of formula C is prepared by process comprising :



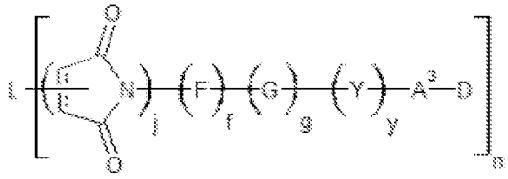
25. The protein drug conjugate as claimed in claim 14, whereincompound of is selected from Formula II, Formula III, and Formula IV:

L—D

Formula II;



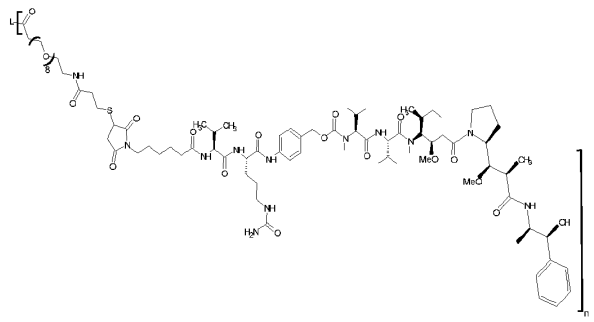
Formula III;



Formula IV

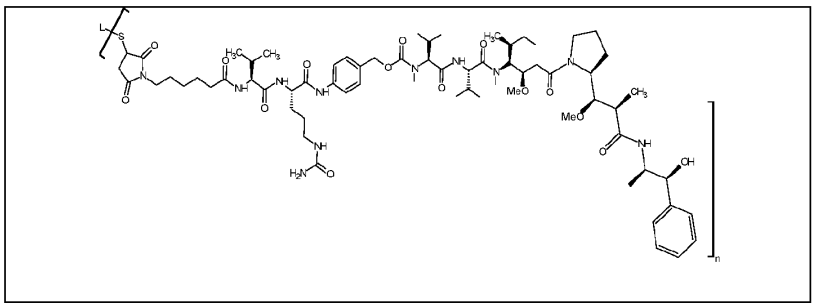
or a pharmaceutically acceptable salt thereof, wherein L, F, G, Y, A^1, A^3, D, n, j, f, g, and y are as defined herein before.

26. A protein drug conjugate, selected from the group comprising of Formula 15, 16, 17, 18 and 19;

Formula 15


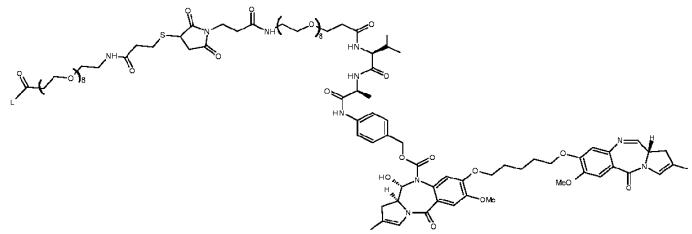
;

Formula 16



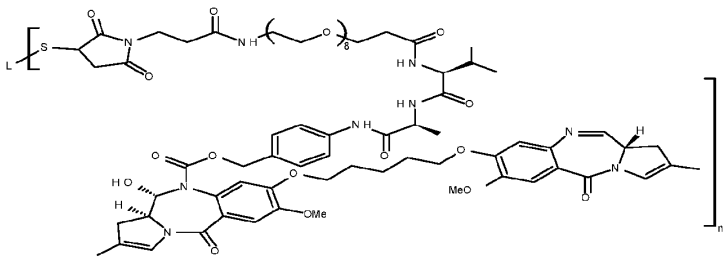
;

Formula 17



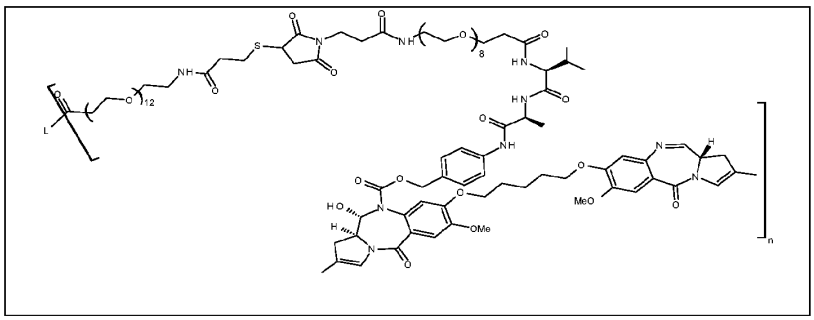
.
;

Formula 18



5 ;

Formula 19



;

or a pharmaceutically acceptable salt or derivatives thereof;

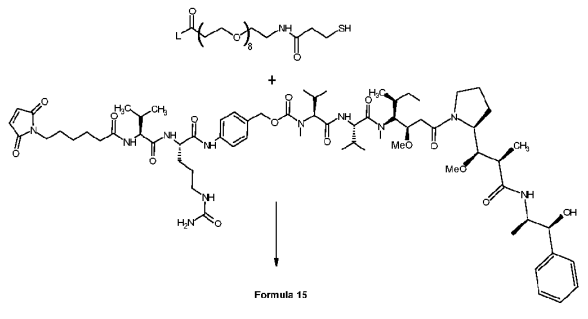
L is a lectin having amino acid sequence of SEQ ID NO: 1; or the lectin having at least 70% identity to SEQ ID NO: 1.

27. The protein drug conjugate as claimed in claim 25, wherein

conjugate of Formula 15, 17 and19 comprises of Lectin of Seq ID No. 2 conjugated to drug via an intermediate compound represented by Formula C; and

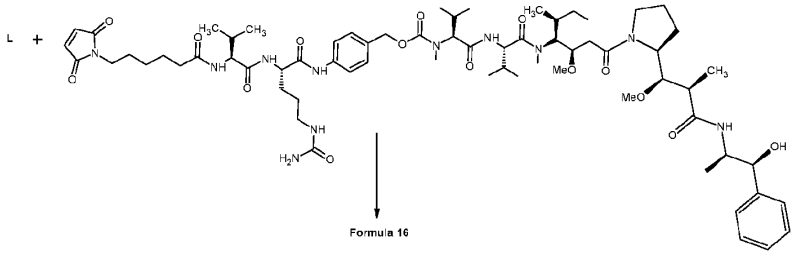
conjugate of Formula 16 & 18 comprises of Lectin of Seq ID No. 5 conjugated to drug via an Cysteine (SH bond) at carboxy terminal of Lectin of Seq ID No. 5.

28. The protein drug conjugate as claimed in claim 25, wherein

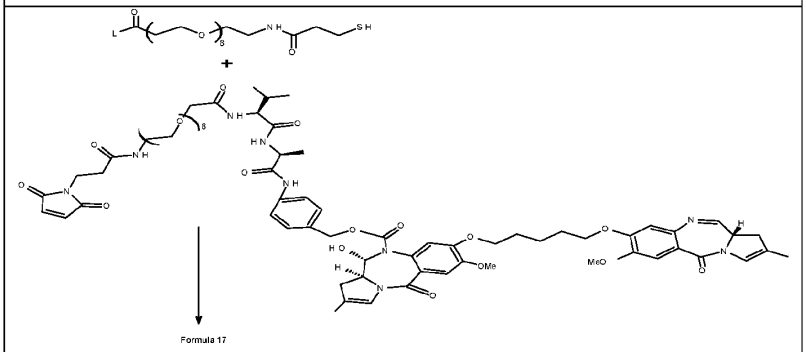
the process for preparation of Formula 15 comprises:
 Formula 15

,

the process for preparation of Formula 16 comprises:

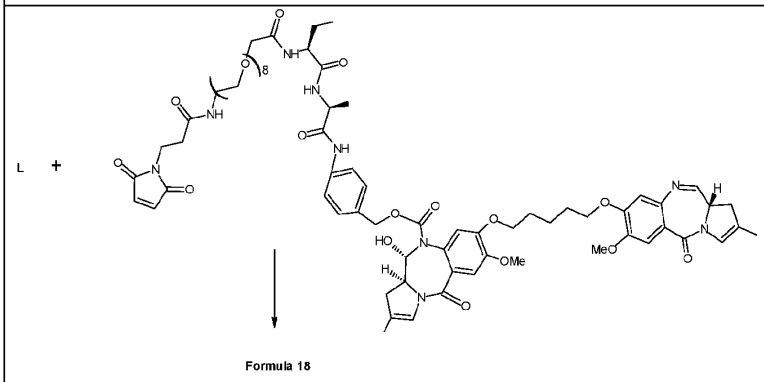


the process for preparation of Formula 17 comprises:



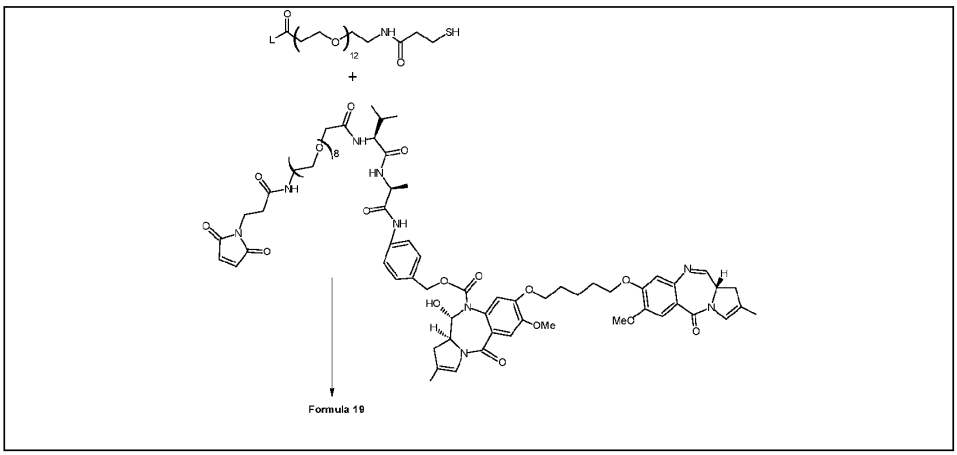
,

the process for preparation of Formula 18 comprises:



,

the process for preparation of Formula 19 comprises:


L
O
12
NH
SH
+
8
HN
HO
H
OMe
MeO
Formula 19