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(54) TAGGABLE HETEROAROMATIC DRUGS AND CONJUGATES THEREOF

MARKIERUNGSGIEBELHETEROAROMATISCHE ARZNEIMITTEL UND KONJUGATE DAVON

MÉDICAMENTS HÉTÉROAROMATIQUES ÉTIQUETABLES ET LEURS CONJUGUÉS

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Description

TECHNICAL FIELD

[0001] The present invention provides heteroarene-based drug derivatives having a "clickable" ketone group, as well as conjugates of said drug derivatives with targeting moieties capable of binding to extracellular antigens and pharmaceutical compositions thereof.

[0002] **Abbreviations: ACN,** acetonitrile; **AcOH,** acetic acid; **Ac_2O,** acetic anhydride; **$CHCl_3$,** chloroform; **CPT,** camptothecin; **DBTL,** dibutyltin dilaurate; **DCM,** dichloromethane; **DMAP,** dimethylaminopyridine; **DMF,** dimethylformamide; **DMSO,** dimethylsulfoxide; **Et_2O,** diethyl ether; **EtOAc,** ethylacetate; **EtOH,** ethanol; **FBS,** fetal bovine serum; **FR,** folate receptor; **Hex,** *n*-hexane; **HBTU,** (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate); **HMPA,** hexamethylphosphoramide **[?]; HMTA,** hexamethylenetetra amine; **HPLC,** high pressure liquid chromatography; **LHMDS,** lithium hexamethyldisilazide; **MeOH,** methanol; **MTX,** methotrexate; **PBS,** phosphate buffered saline; **PEG,** polyethylene glycol; **PTSA,** *p*-toluenesulfonic acid; **RPMI,** Roswell Park Memorial Institute; **TBHP,** tertbutylhydroperoxide; **TFA,** trifluoroacetic acid; **THF,** tetrahidrofuran; **TLC,** thin layer chromatography; **TMZ,** temozolomide; **TsOH,** *p*-toluenesulfonic acid.

BACKGROUND ART

[0003] Bioconjugation is an essential tool in chemical biology for attaining controlled release of a small molecule with medicinal activity. It is typically implemented through chemoselective modification of native functional groups present on the target molecule (Zhou *et al.,* 2013a). For example, amines can be chemoselectively derivatized through amide linkages, azides or acetylenes through the "click" reaction (Kolb *et al.,* 2001) and carbonyl groups through the oxime ligation (Ulrich *et al.,* 2014). Although many medicinal agents contain traditional "taggable" functional groups, some compounds present the challenge of not having any apparent chemical handles. As a further layer of complexity, some molecules require an orthogonal handle for bioconjugation.

[0004] A general C-H functionalization method for the tagging of heteroarenes, more particularly heteroarene-based drugs, has been recently been introduced by the group of Baran (Gui *et al.,* 2014; Zhou *et al.,* 2013b; Fujiwara *et al.,* 2012; Bruckl *et al.,* 2012; Ji *et al.,* 2011). According to that method, an azide-containing sulfinate reagent, more specifically sodium (difluoroalkylazido)sulfinate, allows the appendage of azidoalkyl chain onto a heteroaromatic, and the synthesized azide-linked drug is then attached to a monoclonal antibody, via a succinimide-containing linker, by reacting with a dibenzoazocan-4-yne (*referred to in the publication as dibenzylazacyclooctyne*)-containing monoclonal antibody in a copper-free azide-alkyne cycloaddition, so as to obtain a drug-antibody conjugate.

SUMMARY OF INVENTION

[0005] The present invention relates to a new chemical linker strategy for construction of photo-labile or acid-sensitive conjugates, e.g., folate conjugates, of heteroarene-based drugs or bioactive reagents including those that *a priori* have neither limited or no tagging option, and demonstrates the efficacy and chemical properties of such conjugates. The main advantage of this methodology is the ability to introduce a "clickable" ketone group into the heteroarene-based drug molecule while preserving its biological activity. Upon hydrolysis under slightly acidic conditions, i.e., physiological conditions, the drug conjugate releases the functionalized drug in a wide-ranging release rates.

[0006] In one aspect, the present invention provides a compound of the formula I:

I $Y\text{-}CF_2\text{-}(CR_1R_2)_n\text{-}X\text{-}(CR_1R_2)_m\text{-}R_1$

wherein

Y is a drug or a bioactive reagent, comprising a heteroaromatic ring and linked via a carbon atom of said heteroaromatic ring;

X is carbonyl, or cyclic ketal substituted with 1 to 4 groups each independently is phenyl or naphtyl substituted ortho to the carbon of attachment with $-NO_2$, and optionally further substituted at any position other than ortho to the carbon of attachment with one or more groups each independently selected from $-O-(C_1-C_8)$, $-(C_1-C_8)$alkyl, $-N(R')_2$, or halogen, wherein R' each independently is $-(C_1-C_8)$alkyl or H;

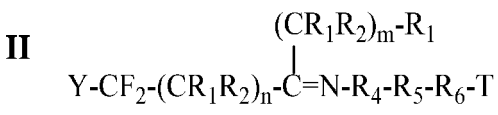
R_1 and R_2 each independently is H, halogen, $-OR_3$, $-CO-R_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, $-CN$, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CO-N(R_3)_2$, $-(C_1-C_8)$alkyl, $-(C_2-C_8)$alkenyl, $-(C_2-C_8)$alkynyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl, or 4-12-membered heterocyclyl;

R_3 is H, $-(C_1-C_{18})$alkyl, $-(C_2-C_{18})$alkenyl, or $-(C_2-C_{18})$alkynyl; and

n and m each independently is an integer of 1-8,

or a pharmaceutically acceptable salt thereof.

[0007] In another aspect, the present invention provides a conjugate of the formula II:



wherein

Y is a drug or a bioactive reagent, comprising a heteroaromatic ring and linked via a carbon atom of said heteroarimatic ring;

R_1 and R_2 each independently is H, halogen, $-OR_3$, $-CO-R_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, -CN, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CO-N(R_3)_2$, $-(C_1-C_8)$alkyl, $-(C_2-C_8)$alkenyl, $-(C_2-C_8)$alkynyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl, or 4-12-membered heterocyclyl;

R_3 is H, $-(C_1-C_{18})$alkyl, $-(C_2-C_8)$alkenyl, or $-(C_2-C_{18})$alkynyl;

R_4 is absent, or is selected from $-NH-(CH_2)_p-$, $-NH-CO-(CH_2)_p-$, $-NH-CO-NH-(CH_2)_p-$, $-NH-CO-O-(CH_2)_p-$, $-O-(CH_2)_p-$, $-O-CO-(CH_2)_p-$, $-O-CO-NH-(CH_2)_p-$, or $-O-CO-O-(CH_2)_p-$;

R_5 is a polymer-, protein-, peptide-, or carbohydrate moiety;

R_6 is H, $-(CH_2)_y-OH$, $-(CH_2)_y-SH$, $-(CH_2)_y-NH_2$, $-(CH_2)_y-COOH$, $-(CH_2)_y-SO_3H$, or a divalent radical selected from $-(CH_2)_y-O-$, $-(CH_2)_y-S-$, $-(CH_2)_y-NH-$, $-(CH_2)_y-COO-$ or $-(CH_2)_y-SO_3-$;

n and m each independently is an integer of 1-8;

p and y each independently is an integer of 0-8; and

T is absent, or is a targeting moiety capable of binding to an extracellular antigen and linked via a functional group thereof, provided that when T is absent R_6 is not a divalent radical, and when T is a targeting moiety R_6 is a divalent radical,

or a pharmaceutically acceptable salt thereof.

[0008] In one particular such aspect, the present invention provides a conjugate of the formula II wherein R_6 is H, $-(CH_2)_y-OH$, $-(CH_2)_y-SH$, $-(CH_2)_y-NH_2$, $-(CH_2)_y-COOH$, or-$(CH_2)_y-SO_3H$, wherein y is an integer of 0-8, and T is absent; and in another particular such aspect, the present invention provides a conjugate of the formula II wherein R_6 is a divalent radical, and T is a targeting moiety capable of binding to an extracellular antigen.

[0009] In a further aspect, the present invention provides a pharmaceutical composition comprising either a compound of the formula I as defined above wherein X is not carbonyl; or a conjugate of the formula II as defined above, or a pharmaceutically acceptable salt thereof, herein also referred to as the ***active agent,*** and a pharmaceutically acceptable carrier.

[0010] The active agent as referred to herein can be used for prevention, treatment of management of various diseases, disorders or indications treatable by administration of the drug or a bioactive reagent Y composing said compound or conjugate.

[0011] Also disclosed herein is a method for treatment of a cancer in an individual in need thereof, comprising administering to said individual a therapeutically effective amount of a conjugate of the formula II as defined above, or a pharmaceutically acceptable salt thereof, wherein Y is an anticancer drug or an antineoplastic drug, and said targeting moiety is capable of binding to an extracellular antigen present on the cells of said cancer.

[0012] In still another aspect, the present invention relates to a conjugate of the formula II as defined above, or a pharmaceutically acceptable salt thereof, wherein Y is an anticancer drug or an antineoplastic drug, and said targeting moiety is capable of binding to an extracellular antigen present on the cells of said cancer, for use in the treatment of cancer.

BRIEF DESCRIPTION OF DRAWINGS

[0013]

Fig. 1 illustrates schematically the bioconjugation approach disclosed herein, as well as the bioconjugate hydrolysis releasing the drug-derivative.

Figs. 2A-2C show tumor cell growth inhibition assays of CPT **(2A),** TMZ **(2B),** and bosutinib **(2C)** *vs*. their ketone analogues.

Fig. 3 shows CPT and CPT-ketone **2a** cell viability assay on six different cell lines.

Fig. 4 shows bosutinib and bosutinib-ketone **4a** cell viability assay on different cell lines.
Figs. 5A-5B show the release of CPT-ketone **2a** with and without irradiation of prodrug **6 (5A);** and U-87 human primary glioblastoma cell growth inhibition assay of CPT-ketone **2a,** and prodrug **6** before and after irradiation **(5B).**
Fig. 6 shows the hydrolysis rate of CPT-ketone-semicarbazone-PEG-FA **7** as a function of time after incubation in buffer at pH 4.8, 6.0, 6.5, 7.0, and 7.4, at 37°C.
Figs. 7A-7B show measurement of binding affinity of folate-conjugate **7** with FR receptors using high expressed-FR KB cells **(7A);** and cytotoxicity assay for CPT-folic acid conjugate **7** using HiFR KB cells **(7B).**

DETAILED DESCRIPTION

[0014] According to the C-H functionalization method developed by the group of Baran and discussed above, an azide-containing sulfinate reagent allows the addition of azidoalkyl chain onto a heteroarene-based drug, and the azide-linked drug synthesized is then attached to a monoclonal antibody, via a linker, by reacting with a dibenzoazocan-4-yne-containing monoclonal antibody in a copper-free azide-alkyne cycloaddition, so as to obtain a drug-antibody conjugate. Baran does not show the hydrolysis of the drug conjugate obtained as a function of pH and incubation time; however, it may be postulated that upon hydrolysis under physiological conditions, either the drug-difluoroalkylene-azide or the drug-difluoroalkylene-8,9-dihydro-1H-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocine is released. As currently known, such an azide-linked drug loses most, i.e., up to 99%, of its biological activity compared with the underivatized (native) drug.

[0015] It has now been found, in accordance with the present invention, that heteroarene-based drugs, i.e., drugs comprising a heteroaromatic ring, which lack an appropriate functional group for linker chemistry, can be functionalized using an alkylating sulfinate reagent bearing a protected ketone functional group, recently developed by the present inventors, more particularly sodium 1,1-difluoro-4-(2-methyl-1,3-dioxolan-2-yl)butane-1-sulfinate via a one-step synthesis. As surprisingly found, a heteroarene-based drug derivative prepared using said alkylating sulfinate reagent preserves the biological activity of the underivatized drug.

[0016] The introduction of a "clickable" ketone group into the heteroarene-based drug molecule allows for the bioconjugation of the drug molecule via an acid labile hydrazone linkage or through a photo-labile ketal, for controlled-release applications. When a stable linkage is required, the oxime ligation could also be used to link between the ketone-tagged moiety and an amine-oxy derivative. Indeed, as further shown herein, drug derivatives as described above can be bioconjugated through a linker such as polyethylene glycol (PEG) or a pseudoPEG, having suitable functional groups, to a targeting moiety capable of binding to an extracellular antigen, e.g., an antibody, sugar, lectin, hormone, peptidomimetic, or folic acid as exemplified herein, as schematically illustrated in **Scheme 1.**

[0017] Upon hydrolysis under slightly acidic (pH 4.8-6.0) conditions that might mimic endosomal and liposomal environments, the drug conjugate releases the functionalized drug in a wide-ranging release rates. The bioconjugation approach disclosed herein, as well as the bioconjugate hydrolysis releasing the drug-derivative, is schematically illustrated in **Fig. 1.**

[0018] In one aspect, the present invention thus provides a compound, also referred to herein as "drug derivative", of the formula I:

I $Y\text{-}CF_2\text{-}(CR_1R_2)_n\text{-}X\text{-}(CR_1R_2)_m\text{-}R_1$

wherein

Y is a drug or a bioactive reagent, comprising a heteroaromatic ring and linked via a carbon atom of said heteroaromatic ring;
X is carbonyl, or cyclic ketal substituted with 1 to 4 groups each independently is phenyl or naphtyl substituted ortho to the carbon of attachment with $-NO_2$, and optionally further substituted at any position other than ortho to the carbon of attachment with one or more groups each independently selected from $-O-(C_1-C_8)$, $-(C_1-C_8)$alkyl, $-N(R')_2$, or halogen, wherein R' each independently is $-(C_1-C_8)$alkyl or H;
R_1 and R_2 each independently is H, halogen, $-OR_3$, $-CO-R_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, $-CN$, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CO-N(R_3)_2$, $-(C_1-C_8)$alkyl, $-(C_2-C_8)$alkenyl, $-(C_2-C_8)$alkynyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl, or 4-12-membered heterocyclyl;
R_3 is H, $-(C_1-C_{18})$alkyl, $-(C_2-C_{18})$alkenyl, or $-(C_2-C_{18})$alkynyl; and
n and m each independently is an integer of 1-8,

or a pharmaceutically acceptable salt thereof.

[0019] The term "halogen" as used herein refers to a halogen and includes fluoro, chloro, bromo, and iodo, and it is preferably fluoro or chloro.

[0020] The term "alkyl" as used herein typically means a linear or branched saturated hydrocarbon radical having 1-18

carbon atoms and includes, e.g., methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, sec-butyl, isobutyl, tert-butyl, *n*-pentyl, isoamyl, 2,2-dimethylpropyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, *n*-decyl, *n*-undecyl, *n*-dodecyl, *n*-tridecyl, *n*-tetradecyl, *n*-pentadecyl, *n*-hexadecyl, *n*-heptadecyl, *n*-octadecyl, and the like. Preferred are (C_1-C_8)alkyl groups, more preferably (C_1-C_4)alkyl groups, most preferably methyl, ethyl or propyl. The terms "alkenyl" and "alkynyl" typically mean linear and branched hydrocarbon radicals having 2-18 carbon atoms and 1 double or triple bond, respectively, and include ethenyl, propenyl, 3-butenyl, 2-ethenylbutyl, 1- and 2-pentenyl, 1-, 2- and 3-hexenyl, 1-, 2-, 3- and 4-heptenyl, 1-, 2-, 3- and 4-octenyl, 1-, 2-, 3- and 4-nonenyl, 1-, 2-, 3-, 4- and 5-decenyl, and the like, and propynyl, 2-butynyl, 1- and 2-pentynyl, 1-, 2- and 3-hexynyl, 1-, 2-, 3- and 4-heptynyl, 1-, 2-, 3- and 4-octynyl, 1-, 2-, 3- and 4-nonynyl, 1-, 2-, 3-, 4- and 5-decynyl, and the like. C_2-C_6 alkenyl and alkynyl radicals are preferred, more preferably C_2-C_4 alkenyl and C_2-C_4 alkynyl. The alkyl, alkenyl and alkynyl defined herein may optionally be substituted with one or more groups each independently selected from -OR',-COR', -COOR', -OCOOR', -OCON(R')$_2$, -CN, -NO_2, -SR', -(C_1-C_8)alkyl, -N(R')$_2$,-CON(R')$_2$, -SO_2R', -SO_2NHR', or -S(=O)R', wherein R' is H or unsubstituted (C_1-C_8)alkyl.

[0021] The term "alkylene" as used herein means typically means a divalent straight or branched hydrocarbon radical having 1-8 carbon atoms and includes, e.g., methylene, ethylene, propylene, butylene, 2-methylpropylene, pentylene, 2-methylbutylene, hexylene, 2-methylpentylene, 3-methylpentylene, 2,3-dimethylbutylene, heptylene, octylene and the like. Preferred are (C_3-C_8)alkylene, more preferably (C_3-C_6)alkylene.

[0022] The term "cycloalkyl" as used herein means a cyclic or bicyclic hydrocarbyl group having 3-10 carbon atoms such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, adamantyl, bicyclo[3.2.1]octyl, bicyclo[2.2.1]heptyl, and the like. Preferred are (C_5-C_{10})cycloalkyls, more preferably (C_5-C_7)cycloalkyls. The cycloalkyl defined herein may optionally be substituted with one or more groups each independently selected from -OR, -COR', -COOR', -OCOOR', -OCON(R')$_2$, -CN, -NO_2, -SR', -(C_1-C_8)alkyl, -N(R')$_2$, -CON(R')$_2$, -SO_2R', -SO_2NHR', or -S(=O)R', wherein R' is H or unsubstituted (C_1-C_8)alkyl.

[0023] The term "aryl" denotes an aromatic carbocyclic group having 6-14 carbon atoms consisting of a single ring or multiple rings either condensed or linked by a covalent bond such as, but not limited to, phenyl, naphthyl, phenanthryl, and biphenyl. The aryl defined herein may optionally be substituted with one or more groups each independently selected from -OR', -COR', -COOR', -OCOOR', -OCON(R')$_2$, -CN, -NO_2, -SR', -(C_1-C_8)alkyl,-N(R')$_2$, -CON(R')$_2$, -SO_2R', -SO_2NHR', or -S(=O)R', wherein R is H or unsubstituted (C_1-C_8)alkyl.

[0024] The term "heterocyclic ring" denotes a mono- or poly-cyclic non-aromatic ring of 4-12 atoms containing at least one carbon atom and one to three heteroatoms selected from sulfur, oxygen or nitrogen, which may be saturated or unsaturated, i.e., containing at least one unsaturated bond. Preferred are 5- or 6-membered heterocyclic rings. The term "heterocyclyl" as used herein refers to any univalent radical derived from a heterocyclic ring as defined herein by removal of hydrogen from any ring atom. Examples of such radicals include, without limitation, piperidino, 4-morpholinyl, or pyrrolidinyl. The heterocyclyl defined herein may optionally be substituted, at any position of the ring, with one or more groups each independently selected from -OR, -COR', -COOR', -OCOOR',-OCON(R')$_2$, -CN, -NO_2, -SR', -(C_1-C_8)alkyl, -N(R')$_2$, -CON(R')$_2$, -SO_2R', -SO_2NHR', or-S(=O)R', wherein R' is H or unsubstituted (C_1-C_8)alkyl.

[0025] In certain embodiments, the present invention provides a compound of the formula I, wherein R_1 and R_2 each independently is H, halogen, -OR_3, -CO-R_3, -CO-OR_3,-OCO-OR_3, -OCO-N(R_3)$_2$, -CN, -NO_2, -SR_3, -N(R_3)$_2$, -CO-N(R_3)$_2$, -(C_1-C_4)alkyl, -(C_2-C_4)alkenyl, -(C_2-C_4)alkynyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl, or 4-12-membered heterocyclyl, wherein R_3 is H, -(C_1-C_4)alkyl, -(C_2-C_4)alkenyl, or -(C_2-C_4)alkynyl. Preferred such embodiments are those wherein R_3 is H, methyl, ethyl or propyl, more preferably H.

[0026] In certain embodiments, the present invention provides a compound of the formula I, wherein n is 3, 4 or 5, preferably 3; or m is 1, 2 or 3, preferably 1.

[0027] In certain embodiments, the present invention provides a compound of the formula I, wherein R_1 and R_2 each independently is H, halogen, -OR_3, -CO-R_3, -CO-OR_3,-OCO-OR_3, -OCO-N(R_3)$_2$, -CN, -NO_2, -SR_3, -N(R_3)$_2$, -CO-N(R_3)$_2$, -(C_1-C_4)alkyl, -(C_2-C_4)alkenyl, -(C_2-C_4)alkynyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl, or 4-12-membered heterocyclyl, wherein R_3 is H, -(C_1-C_4)alkyl, -(C_2-C_4)alkenyl, or -(C_2-C_4)alkynyl, preferably H, methyl, ethyl or propyl; n is 3, 4 or 5, preferably 3; and m is 1, 2 or 3, preferably 1. Particular such embodiments are those wherein R_1 and R_2 each independently is H, halogen, -OH, -COH, -COOH, -OCOOH, -OCO-NH_2, -CN, -NO_2, -SH, -NH_2, -CO-NH_2, -(C_1-C_4)alkyl, -(C_2-C_4)alkenyl, -(C_2-C_4)alkynyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl, or 4-12-membered heterocyclyl; n is 3; and m is 1. In more particular such embodiments, R_1 and R_2 are H.

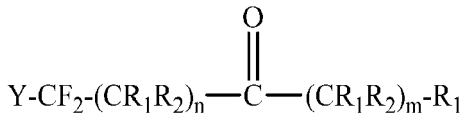
[0028] In certain embodiments, the present invention provides a compound of the formula I, wherein the drug or bioactive reagent Y is selected from anticancer drugs, antineoplastic drugs, antifungal drugs, antibacterial drugs, antiviral drugs, cardiac drugs, neurological drugs, psychoactive drugs such as caffeine, drugs of abuse, i.e., drugs taken for nonmedicinal reasons (usually for mind-altering effects), alkaloids, antibiotics, bioactive peptides, steroids, steroid hormones, peptide (e.g., polypeptide) hormones, interferons, interleukins, narcotics, nucleic acids, pesticides, or prostaglandins.

[0029] In particular such embodiments, the drug or bioactive reagent Y is an anticancer drug such as a chemotherapeutic drug, e.g., camptothecin or a derivative thereof such as 10-hydroxycamptothecin or any other camptothecin

substituted at the 7-, 9- or 10-position (as described in Basil and Moro, Expert Opin Ther Pat., 2009, 19(5), 555-574), bosutinib, or methotrexate formerly known as amethopterin; or an antineoplastic drug such as an alkylating antineoplastic agent, e.g., temozolomide, uramustine or bendamustine.

[0030] In certain embodiments, the present invention provides a compound of the formula I as defined in any one of the embodiments above, wherein X is carbonyl, i.e., a compound of the formula Ia:

Ia

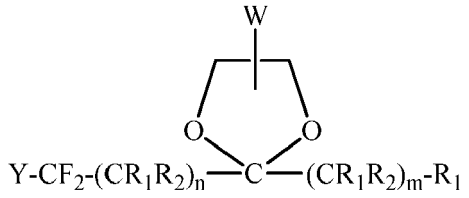


$$Y\text{-}CF_2\text{-}(CR_1R_2)_n\text{-}C(=O)\text{-}(CR_1R_2)_m\text{-}R_1$$

[0031] Particular compounds of the formula Ia are those wherein R_1 and R_2 each independently is H, halogen, -OH, -COH, -COOH, -OCOOH, -OCO-NH_2, -CN, -NO_2, -SH, -NH_2, -CO-NH_2, -(C_1-C_4)alkyl, -(C_2-C_4)alkenyl, -(C_2-C_4)alkynyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl, or 4-12-membered heterocyclyl, preferably H; n is 3; and m is 1. Specific such compounds exemplified herein are those wherein R_1 and R_2 are H; n is 3; m is 1; and the drug moiety Y is CPT, TMZ, bosutinib or MTX, herein identified compounds **2a, 3a, 4a,** and **5a,** respectively.

[0032] In certain embodiments, the present invention provides a compound of the formula I as defined in any one of the embodiments above, wherein X is cyclic ketal substituted as defined above, i.e., a compound of the formula Ib. As shown herein, such compounds are, in fact, prodrugs for the corresponding compounds wherein X is carbonyl, and are converted to said corresponding compounds upon exposure to an irradiation, e.g., UV irradiation as exemplified herein, or a visible or near infra-red irradiation.

Ib



$$Y\text{-}CF_2\text{-}(CR_1R_2)_n\text{-}C(\text{-}O\text{-}W\text{-}O\text{-})\text{-}(CR_1R_2)_m\text{-}R_1$$

W = 1-4 groups each being phenyl or naphtyl substituted as defined above

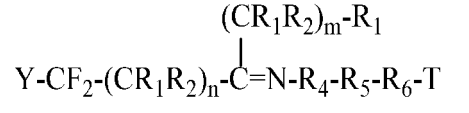
[0033] In particular such embodiments, X is cyclic ketal substituted with 1 or 2 groups each independently is phenyl substituted ortho to the carbon of attachment with -NO_2, -CN, or -COO-(C_1-C_4)alkyl, preferably -NO_2, and optionally further substituted at any position other than ortho to the carbon of attachment with one or more groups each independently selected from -O-(C_1-C_4), preferably -O-(C_1-C_2), more preferably -OCH_3. In more particular such embodiments, X is cyclic ketal substituted with 4,5-dimethoxy,2-nitrophenyl group, or cyclic ketal substituted with two 4,5-dimethoxy,2-nitrophenyl groups wherein each one of said groups is linked to a different carbon atom of the ketal.

[0034] Particular such compounds are those wherein X is cyclic ketal substituted with 4,5-dimethoxy,2-nitrophenyl group; R_1 and R_2 each independently is H, halogen, -OH,-COH, -COOH, -OCOOH, -OCO-NH_2, -CN, -NO_2, -SH, -NH_2, -CO-NH_2, -(C_1-C_4)alkyl,-(C_2-C_4)alkenyl, -(C_2-C_4)alkynyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl, or 4-12-membered heterocyclyl, preferably H; n is 3; and m is 1. Specific such compounds are those wherein R_1 and R_2 are H; n is 3; m is 1; and the drug moiety Y is CPT (herein identified compound 6), TMZ, bosutinib, or MTX.

[0035] The drug derivatives of the formula I wherein X is carbonyl may be synthesized according to any technology or procedure known in the art, e.g., via a one-step synthesis using the alkylating sulfinate reagent sodium 1,1-difluoro-4-(2-methyl-1,3-dioxolan-2-yl)butane-1-sulfinate, as described in the Examples section hereinafter with respect to, e.g., CPT, TMZ, bosutinib and MTX. Conversion of these drug derivatives to their corresponding prodrugs, wherein X is cyclic ketal substituted as defined above, can be carried out, e.g., by reacting said drug derivatives with ethylene glycol substituted at one or both of its carbon atoms with, e.g., 4,5-dimethoxy,2-nitrophenyl group, as described in the Examples section with respect to such a CPT prodrug herein identified compound 6.

[0036] In another aspect, the present invention provides a conjugate of the formula II:

II



$$Y\text{-}CF_2\text{-}(CR_1R_2)_n\text{-}C(\text{-}(CR_1R_2)_m\text{-}R_1)\text{=}N\text{-}R_4\text{-}R_5\text{-}R_6\text{-}T$$

wherein

Y is a drug or a bioactive reagent, comprising a heteroaromatic ring and linked via a carbon atom of said heteroarimatic ring;

R_1 and R_2 each independently is H, halogen, $-OR_3$, $-CO-R_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, -CN, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CO-N(R_3)_2$, $-(C_1-C_8)$alkyl, $-(C_2-C_8)$alkenyl, $-(C_2-C_8)$alkynyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl, or 4-12-membered heterocyclyl;

R_3 is H, $-(C_1-C_{18})$alkyl, $-(C_2-C_{18})$alkenyl, or $-(C_2-C_{18})$alkynyl;

R_4 is absent, or is selected from $-NH-(CH_2)_p-$, $-NH-CO-(CH_2)_p-$, $-NH-CO-NH-(CH_2)_p-$, $-NH-CO-O-(CH_2)_p-$, $-O-(CH_2)_p-$, $-O-CO-(CH_2)_p-$, $-O-CO-NH-(CH_2)_p-$, or $-O-CO-O-(CH_2)_p-$;

R_5 is a polymer-, protein-, peptide-, or carbohydrate moiety;

R_6 is H, $-(CH_2)_y-OH$, $-(CH_2)_y-SH$, $-(CH_2)_y-NH_2$, $-(CH_2)_y-COOH$, $-(CH_2)_y-SO_3H$, or a divalent radical selected from $-(CH_2)_y-O-$, $-(CH_2)_y-S-$, $-(CH_2)_y-NH-$, $-(CH_2)_y-COO-$ or $-(CH_2)_y-SO_3-$;

n and m each independently is an integer of 1-8;

p and y each independently is an integer of 0-8; and

T is absent, or is a targeting moiety capable of binding to an extracellular antigen and linked via a functional group thereof, provided that when T is absent R_6 is not a divalent radical, and when T is a targeting moiety R_6 is a divalent radical,

or a pharmaceutically acceptable salt thereof.

[0037] In certain embodiments, the present invention provides a conjugate of the formula II, wherein R_1 and R_2 each independently is H, halogen, $-OR_3$, $-CO-R_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, -CN, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CO-N(R_3)_2$, $-(C_1-C_4)$alkyl, $-(C_2-C_4)$alkenyl, $-(C_2-C_4)$alkynyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl, or 4-12-membered heterocyclyl, wherein R_3 is H, $-(C_1-C_4)$alkyl, $-(C_2-C_4)$alkenyl, or $-(C_2-C_4)$alkynyl. Preferred such embodiments are those wherein R_3 is H, methyl, ethyl or propyl, more preferably H.

[0038] In certain embodiments, the present invention provides a conjugate of the formula II, wherein n is 3, 4 or 5, preferably 3; or m is 1, 2 or 3, preferably 1.

[0039] In certain embodiments, the present invention provides a conjugate of the formula II, wherein R_4 is $-NH-CO-(CH_2)_p-$ or $-NH-CO-NH-(CH_2)_p-$, wherein p is an integer of 0-8.

[0040] In certain embodiments, the present invention provides a conjugate of the formula II, wherein R_5 is a polymer moiety, more specifically a biocompatible and biodegradable water-soluble polymer moiety. Such a polymer may be selected from a linear or branched PEG or copolymers thereof; a pseudo PEG interrupted by at least one group, each independently preferably selected from -NH-CO-, -CO-NH-, or (C_3-C_8)alkylene interrupted by at least two groups each independently selected from -NH-CO- or -CO-NH-; poly(lactic acid) or copolymers thereof; polyesters selected from polylactide (PLA), polyglycolide (PGA), polycaprolactone (PCL) or copolymers thereof; or polyamides based on polymethacrylamide or copolymers thereof.

[0041] The term "pseudo PEG" as used herein refers to a hydrophilic chain having great structural similarities with the PEG chain, which differs via the presence of one or more, e.g., one, two, three, four or more, groups such as ester (-CO-O-, -O-CO-), amide (-CO-NH-, -NH-CO-), carbamate (-O-CO-NH-, -NH-CO-O-), urea (-NH-CO-NH-), and (C_3-C_8)alkylene interrupted by at least two groups each independently selected from ester, amide, carbamate and urea, within it. According to the present invention, the "pseudo PEG" may in fact consist of either a sole PEG chain interrupted as defined above, or two or more separate PEG chains wherein each couple of those PEG chains are linked to each other via a group such as those listed above.

[0042] In particular such embodiments, R_5 is a polymer moiety, and said polymer is a linear or branched PEG, or a pseudo PEG interrupted by at least one group each independently selected from -NH-CO-, -CO-NH-, or (C_3-C_8)alkylene interrupted by at least two groups each independently selected from -NH-CO- or -CO-NH-, preferably wherein said PEG or pseudo PEG has a molecular weight of 150-20000 Da (PEG/pseudo PEG_{150} to PEG/pseudo $PEG_{20,000}$), more preferably 500-2000 Da (PEG/pseudo PEG_{500}-PEG/pseudo PEG_{2000}) or 500-1000 Da (PEG/pseudo PEG_{500}-PEG/pseudo PEG_{1000}), e.g., a pseudo PEG having the structure $(-CH_2-CH_2-O)_3-(CH_2)_3-NH-CO-(CH_2)_2-CO-NH-(CH_2)_3-(O-CH_2-CH_2-)_3$ as exemplified herein, i.e., a pseudo PEG consisting of two separate PEG chains linked via an interrupted (C_8)alkylene chain of the formula $-(CH_2)_3-NH-CO-(CH_2)_2-CO-NH-(CH_2)_3-$.

[0043] In certain embodiments, the present invention provides a conjugate of the formula II, wherein R_5 is a protein moiety, and said protein is albumin such as human serum albumin (HSA), a modified albumin such as a cationized bovine serum albumin (CBSA) or a cationized human serum albumin (CHSA), or a protein containing globin-like domains having long half-life in circulation such as hemoglobin A or S.

[0044] In certain embodiments, the present invention provides a conjugate of the formula II, wherein R_5 is a peptide moiety, e.g., an oligopeptide or polypeptide.

[0045] The term "peptide" as used herein refers to a chain of amino acid monomers (residues) linked by peptide bonds,

i.e., the covalent bond formed when a carboxyl group of one amino acid reacts with an amino group of another. Such peptides include oligopeptides and polypeptides, as well as peptides consisting of more than 50 amino acid monomers that are, in fact, proteins of low or medium molecular weight.

[0046] In certain embodiments, the peptide is a dipeptide, tripeptide or tetrapeptide consisting of 2, 3 or 4 amino acid residues, respectively, or consists of 5-50, 5-40, 5-30, 5-20, 5-15, or 5-10 amino acid residues and configured as a linear peptide, cyclic peptide, bicyclic peptide or a combination thereof. In more particular such embodiments, said peptide is a polypeptide consisting of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acid residues. All such peptides may consist of residues of both natural and non-natural amino acids.

[0047] The term "amino acid" as used herein refers to an organic compound comprising both amine and carboxylic acid functional groups, which may be either a natural or non-natural amino acid. The twenty two natural amino acids are aspartic acid (Asp), tyrosine (Tyr), leucine (Leu), tryptophan (Trp), arginine (Arg), valine (Val), glutamic acid (Glu), methionine (Met), phenylalanine (Phe), serine (Ser), alanine (Ala), glutamine (Gln), glycine (Gly), proline (Pro), threonine (Thr), asparagine (Asn), lysine (Lys), histidine (His), isoleucine (Ile), cysteine (Cys), selenocysteine (Sec), and pyrrolysine (Pyl). Non-limiting examples of non-natural amino acids include diaminopropionic acid (Dap), diaminobutyric acid (Dab), ornithine (Orn), aminoadipic acid, β-alanine, 1-naphthylalanine, 3-(1-naphthyl)alanine, 3-(2-naphthyl)alanine, γ-aminobutiric acid (GABA), 3-(aminomethyl) benzoic acid, *p*-ethynyl-phenylalanine, *p*-propargly-oxy-phenylalanine, *m*-ethynyl-phenylalanine, *p*-bromophenylalanine, *p*-iodophenylalanine, *p*-azidophenylalanine, *p*-acetylphenylalanine, azidonorleucine, 6-ethynyl-tryptophan, 5-ethynyl-tryptophan, 3-(6-chloroindolyl)alanine, 3-(6-bromoindolyl)alanine, 3-(5-bromoindolyl)alanine, azidohomoalanine, *p*-chlorophenylalanine, α-aminocaprylic acid, O-methyl-L-tyrosine, N-acetylgalactosamine-α-threonine, and N-acetylgalactosamine-α-serine.

[0048] In certain embodiments, the present invention provides a conjugate of the formula II, wherein R_5 is a carbohydrate moiety.

[0049] The term "carbohydrate" refers to a molecule containing carbon, hydrogen and oxygen atoms. The carbohydrate can be cyclic or linear, saturated or unsaturated and substituted or unsubstituted. Preferably, the carbohydrate residue comprises one or more saccharide residues. The phrase "saccharide residue" as used herein encompasses any residue of a sugar moiety, including monosaccharides, oligosaccharides and polysaccharides. Alternatively, the saccharide can be a saccharide derivative such as, but not limited to, glucosides, ethers, esters, acids and amino saccharides. Monosaccharides consist of a single sugar molecule which cannot be further decomposed by hydrolysis. Examples of monosaccharides include, without limitation, pentoses such as, but not limited to, arabinose, xylose and ribose. Oligosaccharides are chains composed of saccharide units. As commonly defined in the art and herein, oligosaccarides are composed of up to nine saccharide units. Examples of oligosaccharides include, without limitation, disaccharides such as, but not limited to, sucrose, maltose, lactose and cellobiose; trisaccharides such as, but not limited to, mannotriose, raffinose and melezitose; and tetrasaccharides such as amylopectin, Syalyl Lewis X (SiaLex) and the like. The term "polysaccharide" refers to a compound composed of at least 10 saccharide units and up to hundreds and even thousands of monosaccharide units per molecule, which are held together by glycoside bonds and range in their molecular weights from around 5,000 and up to millions of Daltons. Non-limiting examples of common polysaccharides include starch, glycogen, cellulose, gum arabic, agar and chitin.

[0050] In certain embodiments, the present invention provides a conjugate of the formula II, wherein the drug or bioactive reagent Y is selected from anticancer drugs, antineoplastic drugs, antifungal drugs, antibacterial drugs, antiviral drugs, cardiac drugs, neurological drugs, psychoactive drugs such as caffeine, drugs of abuse, alkaloids, antibiotics, bioactive peptides, steroids, steroid hormones, peptide hormones, interferons, interleukins, narcotics, nucleic acids, pesticides, or prostaglandins.

[0051] In particular such embodiments, the drug or bioactive reagent Y is an anticancer drug such as a chemotherapeutic drug, e.g., camptothecin or a derivative thereof such as 10-hydroxycamptothecin or any other camptothecin substituted at the 7-, 9- or 10-position, bosutinib, or methotrexate; an antineoplastic drug such as an alkylating antineoplastic agent, e.g., temozolomide, uramustine or bendamustine; or an antimetabolite such as methotrexate or 5-fluorouracil.

[0052] In certain embodiments, the present invention provides a conjugate of the formula II, wherein the targeting moiety is a protein, peptide, polypeptide, glycoprotein, lipoprotein, lipid, phospholipid, oligonucleotide or a mimic thereof, steroid, hormone, lymphokine, growth factor, albumin, cytokine, enzyme, coenzyme, vitamin, cofactor, human antigen, hapten, receptor protein, antibody or a fragment thereof, a substance used or modified such that it functions as a targeting moiety, or a combination thereof. Preferred such embodiments are those wherein the targeting moiety is a vitamin such as vitamin B9 (folic acid).

[0053] According to the present invention, the targeting moiety optionally composing the conjugate of the formula II is capable of binding to an extracellular antigen. Such an extracellular antigen may be any antigen presented on the membrane of a mammalian cell, more particularly a human cell. Particular such extracellular antigens are cancer antigens, i.e., tumor-specific antigens (TSA, which are present only on tumor cells) or tumor-associated antigens (TAA, which are present on some tumor cells and also some normal cells) such as, without being limited to, epidermal growth factor

receptor (EGFR; ErbB-1; HER1 in humans), human epidermal growth factor receptor 2 (HER2), or prostate specific membrane antigen (PSMA).

[0054] In certain embodiments, the present invention provides a conjugate of the formula II as defined in any one of the embodiments above, wherein R_1 and R_2 each independently is H, halogen, $-OR_3$, $-CO-R_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, -CN, $-NO_2$, $-SR_3$,$-N(R_3)_2$, $-CO-N(R_3)_2$, $-(C_1-C_4)$alkyl, $-(C_2-C_4)$alkenyl, $-(C_2-C_4)$alkynyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl, or 4-12-membered heterocyclyl, wherein R_3 is H, $-(C_1-C_4)$alkyl, $-(C_2-C_4)$alkenyl, or $-(C_2-C_4)$alkynyl, preferably H, methyl, ethyl or propyl; n is 3, 4 or 5, preferably 3; m is 1, 2 or 3, preferably 1; R_4 is $-NH-CO-(CH_2)_p$ or $-NH-CO-NH-(CH_2)_p$; and R_5 is a PEG moiety or a pseudo PEG interrupted by at least one group each independently selected from -NH-CO-, -CO-NH-, or (C_3-C_8)alkylene interrupted by at least two groups each independently selected from -NH-CO- or -CO-NH-, preferably wherein said PEG or pseudo PEG has a molecular weight of 150-20000, more preferably 500-2000 or 500-1000. Particular such embodiments are those wherein R_1 and R_2 each independently is H, halogen, -OH, -COH, -COOH, -OCOOH, $-OCO-NH_2$, -CN, $-NO_2$, -SH, $-NH_2$, $-CO-NH_2$, $-(C_1-C_4)$alkyl, $-(C_2-C_4)$alkenyl, $-(C_2-C_4)$alkynyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl, or 4-12-membered heterocyclyl; n is 3; and m is 1. In more particular such embodiments, R_1 and R_2 are H; and/or R_5 is a PEG moiety or a pseudo PEG moiety having the structure $(-CH_2-CH_2-O)_3-(CH_2)_3-NH-CO-(CH_2)_2-CO-NH-(CH_2)_3-(O-CH_2-CH_2-)_3$, i.e., a pseudo PEG consisting of two separate PEG chains linked via an interrupted (C_8)alkylene chain of the formula $-(CH_2)_3-NH-CO-(CH_2)_2-CO-NH-(CH_2)_3-$.

[0055] In certain particular such embodiments, R_6 is a divalent radical and T is a targeting moiety capable of binding to an extracellular antigen. More particular such embodiments are those wherein R_6 is $(CH_2)_y-NH-$, preferably wherein y is 1 or 2; and said targeting moiety is folic acid linked via a carboxylic group thereof, i.e., through its alpha (α)- or gamma (γ)- carboxyl group but preferably through its γ-carboxyl group. More specific such embodiments are those wherein (i) R_4 is $-NH-CO-NH-CH_2-$; R_5 is a pseudo PEG moiety having the structure $(-CH_2-CH_2-O)_3-(CH_2)_3-NH-CO-(CH_2)_2-CO-NH-(CH_2)_3-(O-CH_2-CH_2-)_3$; R_6 is $-(CH_2)-NH-$; and Y is camptothecin or a derivative thereof such as 10-hydroxycamptothecin, temozolomide, bosutinib, or methotrexate; (ii) R_4 is $-NH-CO-CH_2-$; R_5 is a pseudo PEG moiety having the structure $(-CH_2-CH_2-O)_3-(CH_2)_3-NH-CO-(CH_2)_2-CO-NH-(CH_2)_3-(O-CH_2-CH_2-)_3$; R_6 is $-(CH_2)-NH-$; and Y is camptothecin or a derivative thereof such as 10-hydroxycamptothecin, temozolomide, bosutinib, or methotrexate; or (iii) R_4 is -NH-CO-; R_5 is a PEG moiety having the structure $(-CH_2-CH_2-O)_3-$; R_6 is $-(CH_2)_2-NH-$; and Y is camptothecin or a derivative thereof such as 10-hydroxycamptothecin, temozolomide, bosutinib, or methotrexate.

[0056] In other particular such embodiments, R_6 is H, $-(CH_2)_y-OH$, $-(CH_2)_y-SH$, $-(CH_2)_y-NH_2$, $-(CH_2)_y-COOH$ or $-(CH_2)_y-SO_3H$, preferably $-(CH_2)_y-SH$, $-(CH_2)_y-NH_2$, $-(CH_2)_y-COOH$, and T is absent. More particular such embodiments are those wherein y is 1, 2 or 3.

[0057] As shown in the Example section herein, the conjugate of the formula II can be prepared, e.g., by condensing a drug derivative of the formula I with a targeting moiety linked to a linker having a terminal semicarbazide (-NH-CO-NH-NH_2) group, under acidic conditions, thus reacting said semicarbazide group with the ketone group of said drug derivative, and purifying the conjugate obtained, e.g., by HPLC. PEG- and pseudo PEG-based linker having a terminal semicarbazide group can be prepared according to any technology or procedure known in the art, e.g., as described with respect to the pseudo PEG-based linker exemplified herein, having the structure $(-CH_2-CH_2-O)_3-(CH_2)_3-NH-CO-(CH_2)_2-CO-NH-(CH_2)_3-(O-CH_2-CH_2-)_3$. The conjugate obtained by this process can thus be represented as a drug derivative-linker-targeting moiety triconjugate, wherein said linker is in fact represented by the sequence $-R_4-R_5-R_6-$ in the formula II, wherein R_4 is the $-NH-CO-NH-(CH_2)_p-$ of the semicarbazide group forming an acid labile hydrazone linkage with the drug derivative of the formula I, and R_6 comprises a functional group through which said linker is linked to the targeting moiety. As stated above, when a stable linkage between the targeting moiety and the linker is required, the oxime ligation could also be used to link between the ketone group of the drug derivative and an amine-oxy derivative.

[0058] In a further aspect, the present invention provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an active agent as referred to herein, i.e., either a compound of the formula I as defined in any one of the embodiments above wherein X is not carbonyl, i.e., a prodrug of the formula Ib; or a conjugate of the formula II as defined in any one of the embodiments above, or a pharmaceutically acceptable salt thereof.

[0059] The pharmaceutical compositions of the present invention can be provided in a variety of formulations, e.g., in a pharmaceutically acceptable form and/or in a salt form, as well as in a variety of dosages.

[0060] In one embodiment, the pharmaceutical composition of the present invention comprises a non-toxic pharmaceutically acceptable salt of a compound of the formula Ib; or a conjugate of the general formula II. Suitable pharmaceutically acceptable salts include acid addition salts such as, without being limited to, the mesylate salt, the maleate salt, the fumarate salt, the tartrate salt, the hydrochloride salt, the hydrobromide salt, the esylate salt, the *p*-toluenesulfonate salt, the benzenesulfonate salt, the benzoate salt, the acetate salt, the phosphate salt, the sulfate salt, the citrate salt, the carbonate salt, and the succinate salt. Additional pharmaceutically acceptable salts include salts of ammonium (NH_4^+) or an organic cation derived from an amine of the formula R_4N^+, wherein each one of the Rs independently is selected from H, C_1-C_{22}, preferably C_1-C_6 alkyl, such as methyl, ethyl, propyl, isopropyl, *n*-butyl, sec-butyl, isobutyl, tert-butyl, *n*-pentyl, 2,2-dimethylpropyl, *n*-hexyl, and the like, phenyl, or heteroaryl such as pyridyl, imidazolyl, pyrimidinyl, and the like, or two of the Rs together with the nitrogen atom to which they are attached form a 3-7 membered ring

optionally containing a further heteroatom selected from N, S and O, such as pyrrolydine, piperidine and morpholine. Furthermore, where the compounds of the formula Ib or conjugates of the formula II carry an acidic moiety, suitable pharmaceutically acceptable salts thereof may include metal salts such as alkali metal salts, e.g., lithium, sodium or potassium salts, and alkaline earth metal salts, e.g., calcium or magnesium salts.

[0061] Further pharmaceutically acceptable salts include salts of a cationic lipid or a mixture of cationic lipids. Cationic lipids are often mixed with neutral lipids prior to use as delivery agents. Neutral lipids include, but are not limited to, lecithins; phosphatidylethanolamine; diacyl phosphatidylethanolamines such as dioleoyl phosphatidylethanolamine, dipalmitoyl phosphatidylethanolamine, palmitoyloleoyl phosphatidylethanolamine and distearoyl phosphatidylethanolamine; phosphatidylcholine; diacyl phosphatidylcholines such as dioleoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, palmitoyloleoyl phosphatidylcholine and distearoyl phosphatidylcholine; phosphatidylglycerol; diacyl phosphatidylglycerols such as dioleoyl phosphatidylglycerol, dipalmitoyl phosphatidylglycerol and distearoyl phosphatidylglycerol; phosphatidylserine; diacyl phosphatidylserines such as dioleoyl- or dipalmitoyl phosphatidylserine; and diphosphatidylglycerols; fatty acid esters; glycerol esters; sphingolipids; cardiolipin; cerebrosides; ceramides; and mixtures thereof. Neutral lipids also include cholesterol and other 3β hydroxy-sterols.

[0062] Examples of cationic lipid compounds include, without being limited to, Lipofectin® (Life Technologies, Burlington, Ontario) (1:1 (*w*/*w*) formulation of the cationic lipid N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride and dioleoylphosphatidyl-ethanolamine); Lipofectamine™ (Life Technologies, Burlington, Ontario) (3:1 (*w*/*w*) formulation of polycationic lipid 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanamin-iumtrifluoroacetate and dioleoylphosphatidyl-ethanolamine), Lipofectamine Plus (Life Technologies, Burlington, Ontario) (Lipofectamine and Plus reagent), Lipofectamine 2000 (Life Technologies, Burlington, Ontario) (Cationic lipid), Effectene (Qiagen, Mississauga, Ontario) (Non liposomal lipid formulation), Metafectene (Biontex, Munich, Germany) (Polycationic lipid), Eu-fectins (Promega Biosciences, San Luis Obispo, Calif.) (ethanolic cationic lipids numbers 1 through 12: $C_{52}H_{106}N_6O_4 \cdot 4CF_3CO_2H$, $C_{88}H_{178}N_8O_4S_2 \cdot 4CF_3CO_2H$, $C_{40}H_{84}NO_3P \cdot CF_3CO_2H$, $C_{50}H_{103}N_7O_3 \cdot 4CF_3CO_2H$, $C_{55}H_{116}N_8O_2 \cdot 6CF_3CO_2H$, $C_{49}H_{102}N_6O_3 \cdot 4CF_3CO_2H$, $C_{44}H_{89}N_5O_3 \cdot 2CF_3CO_2H$, $C_{100}H_{206}N_{12}O_4S_2 \cdot 8CF_3CO_2H$, $C_{162}H_{330}N_{22}O_9 \cdot 13CF_3CO_2H$, $C_{43}H_{88}N_4O_2 \cdot 2CF_3CO_2H$, $C_{43}H_{88}N_4O_3 \cdot 2CF_3CO_2H$, $C_{41}H_{78}NO_8P$); Cytofectene (Bio-Rad, Hercules, Calif.) (mixture of a cationic lipid and a neutral lipid), GenePORTER® (Gene Therapy Systems, San Diego, Calif.) (formulation of a neutral lipid (Dope) and a cationic lipid) and FuGENE 6 (Roche Molecular Biochemicals, Indianapolis, Ind.) (Multi-component lipid based non-liposomal reagent).

[0063] The pharmaceutically acceptable salts of the present invention may be formed by conventional means, e.g., by reacting a free base form of the active agent with one or more equivalents of the appropriate acid in a solvent or medium in which the salt is insoluble, or in a solvent such as water which is removed *in vacuo* or by freeze drying, or by exchanging the anion/cation of an existing salt for another anion/cation on a suitable ion exchange resin.

[0064] The pharmaceutical compositions provided by the present invention may be prepared by conventional techniques, e.g., as described in Remington: The Science and Practice of Pharmacy, 19th Ed., 1995. The compositions can be prepared, e.g., by uniformly and intimately bringing the active agent into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product into the desired formulation. The compositions may be in liquid, solid or semisolid form and may further include pharmaceutically acceptable fillers, carriers, diluents or adjuvants, and other inert ingredients and excipients. In one embodiment, the pharmaceutical composition of the present invention is formulated as nanoparticles.

[0065] The compositions can be formulated for any suitable route of administration, but they are preferably formulated for parenteral, e.g., intravenous, intraarterial, intramuscular, intraperitoneal, intrathecal, intrapleural, intratracheal, subcutaneous, or topical administration, as well as for inhalation. The dosage will depend on the state of the patient, i.e., individual treated, and will be determined as deemed appropriate by the practitioner.

[0066] The pharmaceutical composition of the invention may be in the form of a sterile injectable aqueous or oleagenous suspension, which may be formulated according to the known art using suitable dispersing, wetting or suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent. Acceptable vehicles and solvents that may be employed include, without limiting, water, Ringer's solution, PEG, 2-hydroxypropyl-β-cyclodextrin (HPCD), Tween-80, and isotonic sodium chloride solution.

[0067] Pharmaceutical compositions according to the present invention, when formulated for inhalation, may be administered utilizing any suitable device known in the art, such as metered dose inhalers, liquid nebulizers, dry powder inhalers, sprayers, thermal vaporizers, electrohydrodynamic aerosolizers, and the like.

[0068] Pharmaceutical compositions according to the present invention, when formulated for administration route other than parenteral administration, may be in a form suitable for oral use, e.g., as tablets, troches, lozenges, aqueous, or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs.

[0069] Pharmaceutical compositions intended for oral administration may be formulated so as to inhibit the release of the active agent in the stomach, i.e., delay the release of the active agent until at least a portion of the dosage form has traversed the stomach, in order to avoid the acidity of the gastric contents from hydrolyzing the active agent. Particular such compositions are those wherein the active agent is coated by a pH-dependent enteric-coating polymer. Examples

of pH-dependent enteric-coating polymer include, without being limited to, Eudragit® S (poly(methacrylicacid, methylmethacrylate), 1:2), Eudragit® L 55 (poly (methacrylicacid, ethylacrylate), 1:1), Kollicoat® (poly(methacrylicacid, ethylacrylate), 1:1), hydroxypropyl methylcellulose phthalate (HPMCP), alginates, carboxymethylcellulose, and combinations thereof. The pH-dependent enteric-coating polymer may be present in the composition in an amount from about 10% to about 95% by weight of the entire composition.

[0070] Pharmaceutical compositions intended for oral administration may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and may further comprise one or more agents selected from sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients, which are suitable for the manufacture of tablets. These excipients may be, e.g., inert diluents such as calcium carbonate, sodium carbonate, lactose, calcium phosphate, or sodium phosphate; granulating and disintegrating agents, e.g., corn starch or alginic acid; binding agents, e.g., starch, gelatin or acacia; and lubricating agents, e.g., magnesium stearate, stearic acid, or talc. The tablets may be either uncoated or coated utilizing known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated using the techniques described in the US Patent Nos. 4,256,108, 4,166,452 and 4,265,874 to form osmotic therapeutic tablets for control release. The pharmaceutical composition of the invention may also be in the form of oil-in-water emulsion.

[0071] The pharmaceutical compositions of the invention may be formulated for controlled release, i.e., extended- or sustained-release, of the active agent. Such compositions may be formulated as controlled-release matrix, e.g., as controlled-release matrix tablets in which the release of a soluble active agent is controlled by having the active diffuse through a gel formed after the swelling of a hydrophilic polymer brought into contact with dissolving liquid (*in vitro*) or gastro-intestinal fluid (*in vivo*). Many polymers have been described as capable of forming such gel, e.g., derivatives of cellulose, in particular the cellulose ethers such as hydroxypropyl cellulose, hydroxymethyl cellulose, methylcellulose or methyl hydroxypropyl cellulose, and among the different commercial grades of these ethers are those showing fairly high viscosity. In other configurations, the compositions comprise the active agent formulated for controlled release in microencapsulated dosage form, in which small droplets of the active agent are surrounded by a coating or a membrane to form particles in the range of a few micrometers to a few millimeters.

[0072] Another contemplated formulation is depot systems, based on biodegradable polymers, wherein as the polymer degrades, the active ingredient is slowly released. The most common class of biodegradable polymers is the hydrolytically labile polyesters prepared from lactic acid, glycolic acid, or combinations of these two molecules. Polymers prepared from these individual monomers include poly (D,L-lactide) (PLA), poly (glycolide) (PGA), and the copolymer poly (D,L-lactide-co-glycolide) (PLG).

[0073] The active agent as referred to herein, as well as the pharmaceutical composition thereof, can be used for prevention, treatment of management of various diseases, disorders or indications treatable by administration of the drug or a bioactive reagent Y composing said active agent.

[0074] Further disclosed herein is a method for treatment of a cancer in an individual in need thereof, comprising administering to said individual a therapeutically effective amount of a conjugate of the formula II as defined in any one of the embodiments above, or a pharmaceutically acceptable salt thereof, wherein Y is an anticancer drug, e.g., a chemotherapeutic drug such as camptothecin or a derivative thereof, bosutinib or methotrexate, or an antineoplastic drug, e.g., an alkylating antineoplastic agent such as temozolomide, uramustine or bendamustine; and said targeting moiety is capable of binding to an extracellular antigen present on the cells of said cancer. In a disclosure, the cancer treated by this method is characterized by folate receptor overexpressing cells, and said targeting moiety is folic acid linked via a carboxylic group thereof, preferably through its gamma-carboxyl group. In another disclosure, said cancer is carcinoma.

[0075] In still another aspect, the present invention relates to a conjugate of the formula II as defined in any one of the embodiments above, or a pharmaceutically acceptable salt thereof, wherein Y is an anticancer drug or an antineoplastic drug, and said targeting moiety is capable of binding to an extracellular antigen present on the cells of said cancer, for use in the treatment of a cancer. In a particular embodiment, the conjugate is used in the treatment of a cancer characterized by folate receptor overexpressing cells, e.g., a carcinoma, wherein said targeting moiety is folic acid linked via a carboxylic group thereof, preferably through its gamma-carboxyl group.

[0076] The invention will now be illustrated by the following non-limiting Examples.

EXAMPLES

Synthesis schemes and experimental procedures

Materials and Methods

[0077] All reactions requiring anhydrous conditions were performed under argon atmosphere. All reactions were carried out at room temperature unless stated otherwise. Chemicals and solvents were either standard analytical grade (A.R. grade) or purified by standard techniques. TLC: silica gel plates Merck 60 F_{254}, compounds were visualized by irradiation with UV light. Flash chromatography: silica gel Merck 60 (particle size 0.040-0.063 mm), eluent given in parentheses. HPLC: C18 5μ, 250×4.6mm, eluent given in parentheses. Preparative HPLC: C18 5μ, 250×21mm, eluent given in parentheses. ^{1}H-NMR and ^{13}C-NMR spectra were measured using Bruker Avance operated at 400MHz as mentioned. All general reagents, including salts and solvents, were purchased from Sigma-Aldrich. Absorption and fluorescence spectra were recorded on Spectramax-M2 fluorescent spectrometer using quartz cuvettes or quartz 96-wells plate reader.

Compound 1c

[0078] Mercaptopyridine was reacted with bromodifluoromethyl diethylphosphonate under basic conditions. The non-isolated thio-pyridine product was oxidized using sodium periodate to afford the sulfonyl-pyridine derivative **1a**.

[0079] Pentaiodoacetal **1b** was prepared first by replacement reaction of 5-chloro-2-pentanone with sodium iodide in acetone. The carbonyl group of 5-iodo-2-pentanone was protected with ethylene glycol catalyzed by PTSA.

[0080] As depicted in **Scheme 2,** to a flask equipped with a magnetic stirrer, HMPA (4 mL) was added under N_2 atmosphere to a solution of 2-(difluoromethylsulfonyl)-pyridine (1.5 g, 7.8 mmol, 1.0 equiv.) in THF (50 mL). The reaction mixture was cooled to -98°C (CH_3OH/liquid nitrogen bath), then 2-methyl-2-(3-iodo)-propyl-1,3-dioxolane (3.15 mL, 0.0194, 2.5 equiv.) was added. A THF solution of LHMDS (2.72 mL, 3.5 equiv.) was added dropwise over 35 minute. After 30 minutes, the reaction mixture was quenched with saturated aqueous NH_4Cl solution (2 mL) at -98°C. The cold bath was removed and distilled water (1 mL) was added. The mixture was extracted with EtOAc. The combined organic solution was treated with saturated aqueous NaCl to remove HMPA and dried over Na_2SO_4. The solvent was removed under reduced pressure to obtain a crude product which was purified by flash column chromatography (EtOAc/Hex) to give a pure product **1c** (1.67 g, 67% yield) as a light yellow viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.90-8.84 (m, 1H), 8.17 (dd, J = 7.9, 0.7 Hz, 1H), 8.04 (td, J = 7.8, 1.6 Hz, 1H), 7.67 (ddd, J = 7.7, 4.7, 1.0 Hz, 1H), 4.01-3.85 (m, 2H), 2.58-2.31 (m, 2H), 1.81-1.71 (m, 4H), 1.32 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.65, 151.45, 139.21, 129.55, 128.55, 125.70 (t), 109.80, 65.10, 38.58, 30.78(t), 24.25, 16.07. MS (ESI+) m/z: 322.

Ketal sulfinate 1

[0081] As depicted in **Scheme 2,** sodium ethanethiol (1.2 g, 14.3 mmol, 3 equiv) was dissolved in THF (60 mL) at 0°C under argon atmosphere and stirred at 0°C for 5 minutes. THF (30 mL) solution of **1c** (1.5 g, 4.7 mmol, 1.0 equiv.) was added. The flask was sealed with a cap and further wrapped with parafilm. The mixture was stirred at 0°C for 2 h, then at room temperature for 10 h. The solvent was removed under vacuum, and the residue was purified by column chromatography (MeOH/DCM) to give ketal sulfinate **1** (4.1 g, 93% yield). ^{1}H NMR (400 MHz, DMSO-d^6): δ 3.88-3.79 (m, 2H), 1.90-1.70 (m, 2H), 1.63-1.54 (m, 2H), 1.46 (m, 2H), 1.22 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 134.04, 131.23, 128.42, 112.76, 67.55, 41.75, 30.20, 29.99, 29.78, 26.03, 18.63. MS (ESI-) m/z: 243.

CPT-ketone (compound 2a)

[0082] To a solution of CPT (**2,** 150 mg, 0.43 mmol, 1.0 equiv.), ketal sulfinate **1** (343 mg, 1.29 mmol, 3.0 equiv.) and $ZnCl_2$ (123 mg, 0.64 mmol, 1.5 equiv.) in DCM (2.5 mL) and H_2O (1 mL) was added PTSA (54 mg, 0.43 mmol, 1.0 equiv.). The reaction mixture was cooled in ice bath and TBHP (70% solution in water, 0.214 mL, 5.0 equiv.) was added dropwise with vigorous stirring. The stirring was continued at this temperature for 5 minutes. The reaction was warmed to room temperature and monitored by TLC until completion. After 24 h, a second addition of $ZnCl_2$ (123 mg, 0.64 mmol, 1.5 equiv.), ketal kulfinate **1** (343 mg, 1.29 mmole, 3.0 equiv.) and TBHP (0.214 mL 5.0 equiv.) was performed to drive the reaction further. Upon consumption of the starting material, the reaction was partitioned between DCM and saturated aqueous $NaHCO_3$ (2.0 mL). The organic layer was separated, and the aqueous layer was extracted with DCM (3×2.0 mL). The combined organic solution was dried over Na_2SO_4, concentrated in vacuum and purified by column chromatography to give product **2a** (140 mg, 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.27 (dd, J = 16.1, 8.5 Hz, 2H), 7.87 (t, J = 7.6 Hz, 1H), 7.79-7.65 (m, 2H), 5.46 (d, J = 3.1 Hz, 2H), 2.56 (td, J = 7.1, 3.6 Hz, 2H), 2.51-2.38 (m, 2H), 2.13 (s,3H), 1.99-1.78 (m, 4H), 1.07 (t, J = 7.4 Hz, 3H). MS (TOF-ESI): m/z 483.3 $[M+H]^+$, 481.4 $[M-H]^-$. ^{13}C NMR (101 MHz, $CDCl_3$)

δ 208.01, 174.62, 158.13, 153.28, 150.80, 150.66, 146.25, 137.89, 131.68, 131.31, 129.62, 126.67, 125.41, 124.60, 119.87, 98.75, 73.45, 67.10, 52.04, 42.86, 38.69, 32.34, 30.70, 30.42, 16.91, 8.54. MS (ESI+) m/z: 483, 405 [M+Na].

10-hydroxy CPT-ketone

[0083] Using the synthetic procedure described above for CPT-ketone, an analogue of 10-hydroxy CPT-ketone, a compound having a similarity to the SN-38 anti-cancer drug, can be prepared.

TMZ-ketone (compound 3a)

[0084] To a solution of TMZ (**3,** 25 mg, 0.13 mmol, 1.0 equiv.), ketal sulfinate **1** (120 mg, 0.45 mmol, 3.5 equiv.) and $ZnCl_2$ (31 mg, 0.22 mmol, 1.75 equiv.) in DMSO (1 mL) was added TFA (0.02 mL, 0.19 mmol, 1.5 equiv.). The reaction mixture was cooled in ice bath and TBHP (70% solution in water, 0.071 mL, 5.5 equiv.) was added dropwise with vigorous stirring. The stirring was continued at this temperature for 5 minutes. The reaction was warmed to 50°C and monitored by HPLC. The reaction stopped after 1h and purified by preperative HPLC to give product **3a** (7 mg, 28% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (s, 1H), 7.26 (s, 1H), 6.28 (s, 1H), 4.07 (s, 3H), 2.68-2.52 (m, 4H), 2.16 (s, 3H), 1.98-1.84 (m, 2H). MS (ESI+) m/z: 329, 351 [M+Na].

Bosutinib-ketone (compound 4a)

[0085] To a solution of bosutinib (**4,** 20 mg, 0.04 mmol, 1.0 equiv.), Ketal Sulfinate **1** (30 mg, 0.11 mmol, 3 equiv.) and $ZnCl_2$ (11 mg, 0.05 mmol, 1.5 equiv.) in DMSO:H_2O (0.2:0.2 mL) was added TFA (12 μL, 0.15 mmol, 4 equiv.). The reaction mixture was cooled in ice bath and TBHP (70% solution in water, 0.019 mL, 5 equiv.) was added dropwise with vigorous stirring. The stirring was continued at this temperature for 5 minutes. The reaction was warmed to 50°C and monitored by HPLC. After 24 h, a second addition of $ZnCl_2$ (11 mg, 0.056 mmol, 1.5 equiv.), ketal sulfinate **1** (30 mg, 0.11 mmole, 3.0 equiv.) and TBHP (0.019 mL 5.0 equiv.) was performed to drive the reaction further. The reaction stopped after 24 h and purified by preparative HPLC to give product **4a** (17 mg, 73% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.16 (d, J = 7.9 Hz, 1H), 7.70 (d, J = 8.8 Hz, 2H), 6.71 (d, J = 8.9 Hz, 2H), 5.01 (s, 1H), 4.32 (s, 1H), 3.19 (s, 2H), 2.37-2.28 (m, 3H), 2.12-1.99 (m, 4H), 1.89 (s, 1H), 1.73-1.66 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 208.68, 154.42, 153.30, 152.64, 150.51, 150.40, 145.39, 137.50, 130.74, 121.38, 118.84, 117.81, 117.62, 115.00, 114.79, 110.21, 106.11, 102.10, 91.39, 66.17, 56.66, 55.91, 54.76, 50.78, 50.09, 49.88, 49.67, 49.45, 49.24, 48.99, 43.22, 42.82, 34.87, 29.95, 24.18, 16.42. MS (ESI+) m/z: 665, 687 [M+Na].

MTX-ketone (compound 5a)

[0086] To a solution of MTX (**5,** 30 mg, 0.06 mmol, 1.0 equiv.), ketal sulfinate **1** (105 mg, 0.42 mmol, 6 equiv.) and $ZnCl_2$ (38 mg, 0.19 mmol, 3 equiv.) in DMSO (1 mL) was added PTSA (27 mg, 0.19 mmol, 3 equiv.). The reaction mixture was cooled in ice bath and TBHP (70% solution in water, 0.066 mL, 10 equiv.) was added dropwise with vigorous stirring. The stirring was continued at this temperature for 5 minutes. The reaction was warmed to 50°C and monitored by HPLC. The reaction stopped after 24 h and purified by preparative HPLC to give product **5a** (13 mg, 35% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.16 (d, J = 7.9 Hz, 1H), 7.70 (d, J = 8.8 Hz, 2H), 6.71 (d, J = 8.9 Hz, 2H), 5.01 (s, 1H), 4.32 (s, 1H), 3.19 (s, 2H), 2.37-2.28 (m, 3H), 2.12-1.99 (m, 4H), 1.89 (s, 1H), 1.73-1.66 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 209.11, 174.97, 163.12, 152.59, 132.87, 132.71, 130.69, 129.99, 129.32, 128.06, 123.00, 122.09, 117.32, 112.18, 54.22, 52.89, 42.85, 31.58, 31.20, 30.94, 27.14, 17.36, 16.90. MS (ESI+) m/z: 589.

Compound 9

[0087] As depicted in **Scheme 3,** a solution of benzylchloroformate (1.3 mL, 9 mmol) in DCM (20 mL) was added over 1.5 h to a solution of compound **8** (2 gr, 9 mmol) and triethyl amine (3.8 ml, 27 mmol) in DCM (40 mL) cooled at 0°C. The solution was stirred for 2 h at 0°C, then warmed to room temperature. After completion the crude was concentrated by evaporation and the product was purified by column chromatography (Hex:EtOAc) to give product **9** (1.3 gr, 40% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21-7.11 (m, 5H), 4.89 (s, 2H), 3.46-3.31 (m, 12H), 3.07 (dd, J = 12.6, 6.3 Hz, 2H), 2.94 (t, J = 6.5 Hz, 2H), 1.84-1.76 (m, 1H), 1.64-1.56 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.58, 137.35, 129.06, 128.58, 128.47, 70.78, 70.54, 70.40, 69.63, 66.97, 50.32, 39.36, 39.16, 30.06, 27.39. MS (ESI+) m/z: 255.

Compound 10

[0088] As depicted in **Scheme 3,** compound **9** (100 mg, 0.28 mmol), DMAP (70 mg, 0.6 mmol) and succinic anhydride

(28 mg, 0.28 mmol) were dissolved into 4 mL of DCM. The solution was stirred for 2 h. After completion, the mixture was extracted with 1 M HCl, the organic layer dried over Na_2SO_4, and concentrated to give product **10** (96 mg, 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28-7.13 (m, 1H), 6.98 (s, 1H), 5.79 (s, 1H), 4.92 (s, 1H), 3.52-3.27 (m, 3H), 3.17-3.08 (m, 1H), 2.52-2.41 (m, 1H), 2.38-2.30 (m, 1H), 1.60 (dt, J = 12.6, 6.2 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.87, 173.23, 157.42, 137.41, 129.09, 128.61, 128.38, 70.97, 70.63, 70.56, 70.03, 69.92, 67.01, 39.54, 38.29, 31.35, 30.39, 30.05, 29.44. MS (ESI-) m/z: 425. MS (ESI+) m/z: 247.

Compound 11

[0089] As depicted in **Scheme 4,** in one flask, triphosgene (288 mg, 0.5 mmol, 1.2 equiv.) was dissolved in toluene (16 mL). In a separate flask, tert-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (200 mg, 0.4 mmol, 1 equiv.) was dissolved in toluene (12 mL) and the resulted solution was added dropwise within 10 minutes to the mixture in the first flask at reflux. The reaction mixture was kept at reflux for 30 minutes. The toluene was removed under reduced pressure to obtain the corresponding crude isocyanate intermediate product which was dissolved in a solution of toluene (10 mL), DBTL (3 drops) and tert-butyl carbazate (104 mL, 0.4 mmol, 1 equiv.), then the mixture was heated to reflux for 30 minutes and monitored by TLC (Hex:EtOAc). The toluene was removed under vacuum and the crude product was purified by column chromatography (Hex:EtOAc) to give product **11** (200 mg, 50% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10-7.19 (m, 5H), 7.12 (s, 1H), 6.59 (s, 1H), 6.19 (s, 1H), 5.54 (s, 1H), 5.07 (s, 2H), 3.79-3.42 (m, 12H), 3.42-3.16 (m, 4H), 1.85-1.64 (m, 4H), 1.44 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.76, 157.36, 156.79, 137.50, 129.17, 128.83, 128.70, 81.97, 71.32, 71.11, 70.88, 70.73, 70.63, 70.06, 67.16, 39.74, 39.49, 30.11, 29.65, 28.95.

Compound 12

[0090] As depicted in **Scheme 4,** a mixture of compound **11** (100 mg, 0.19 mmol), 5% Pd/C (cat.), and methanol was stirred under a balloon of hydrogen at ambient pressure at room temperature overnight. The reaction mixture was then filtered through a short plug of Celite and the filtrate was concentrated in vacuo to give product **12** (68 mg, 93 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.53 (m, 14H), 3.22 (m, 2H), 3.04 (m, 1H), 1.92 (s, 2H), 1.61 (m, , 4H), 1.36 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.20, 157.36, 81.66, 71.03, 70.55, 70.20, 70.01, 42.11, 41.72, 39.83, 38.46, 30.09, 28.96, 27.92. MS (ESI+) m/z: 379, 401 [M+Na].

Compound 13

[0091] As depicted in **Scheme 4,** compound **12** (50 mg, 0.13 mmol), DMAP (33 mg, 0.26 mmol) and succinic anhydride (14 mg, 0.13 mmol) were dissolved into 3 mL of DCM. The solution was stirred for 2 h. After completion, the mixture was extracted with 1 M HCl, the organic layer dried over Na_2SO_4, and concentrated to give product **13** (46 mg, 70% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.55-3.37 (m, 12H), 3.15 (m, 2H), 2.63-2.41 (m, 4H), 2.32 (m, 2H), 1.72-1.61 (m, 4H), 1.33 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.12, 173.48, 160.94, 156.80, 82.01, 72.20, 71.09, 70.87, 70.04, 40.53, 37.40, 32.12, 31.20, 30.41, 30.08, 29.97, 29.12, 29.03. MS (ESI+) m/z: 451.

Compound 14

[0092] As depicted in **Scheme 5,** DMAP (68 mg, 0.55 mmol, 3 equiv.) and compound **10** (70 mg, 0.18 mmol, 1 equiv.) were dissolved in DCM (2 mL). HBTU (210 mg, 0.55 mmol, 3 equiv.) was added and the reaction mixture was stirred in room temperature for 10 minutes, then compound **13** (84 mg, 0.19 mmol, 1 equiv.) was added, and the reaction was monitored by TLC (Hex:EtOAc). After completion the product was purified by coloumn chromatography (Hex:EtOAc), to give product **14** (97 mg, 82% yield).

Compound 15

[0093] As depicted in **Scheme 5,** a mixture of compound **14** (97 mg, 0.0.151 mmol), 5% Pd/C (cat.), and methanol was stirred under a balloon of hydrogen at ambient pressure at room temperature overnight. The reaction mixture was then filtered through a short plug of Celite and the filtrate was concentrated in vacuo and directly reacted.

[0094] DMAP (25 mg, 0.21 mmol, 8 equiv.) and folic acid (50 mg, 0.105 mmol, 4 equiv.) were dissolved in DMSO (4 mL). HBTU (20 mg, 0.052 mmol, 2 equiv.) was added and the reaction mixture was stirred in room temperature for 10 minutes, then compound **14a** (13 mg, 0.026 mmol, 1 equiv.) was added, the reaction progress was monitored by HPLC (ACN/H_2O). After completion the product was purified by preparative HPLC (ACN/H_2O) to give product **15** (24 mg, 60% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.68 (s, 1H), 7.64 (d, J = 6.5 Hz, 2H), 6.63 (d, J = 8.7 Hz, 2H), 4.52 (s, 2H), 4.26 (d, J = 9.6 Hz, 1H), 3.61 - 3.17 (m, 22H), 3.06 (dd, J = 22.9, 6.1 Hz, 10H), 2.26-1.98 (m, 8H), 1.58 (dd, J = 16.1, 9.8 Hz,

8H), 1.37 (s, 9H). ^{13}C NMR (101 MHz, DMSO) δ 175.06, 175.06, 172.42, 172.42, 167.42, 159.59, 154.11, 154.11, 151.82, 149.35, 149.35, 130.17, 130.17, 129.16, 129.16, 112.40, 112.40, 80.11, 70.92, 70.92, 70.70, 70.70, 69.45, 69.22, 69.22, 53.39, 46.98, 38.03, 37.78, 36.95, 36.95, 33.18, 32.49, 32.02, 32.02, 31.16, 30.85, 30.53, 30.53, 29.25, 29.25, 27.70. MS (ESI+) m/z: 1223, 1245 [M+Na].

Compound 7

[0095] As depicted in **Scheme 6,** compound **15** was dissolved in a mixture of 2 ml DCM and 2 ml TFA, and stirred for 1h. After completion the product was concentrated in vacuum and directly reacted in the next step.

[0096] CPT-ketone **2a** (20 mg, 0.0414 mmol, 1 equiv.) was dissolved in methanol (5 mL). TFA (5 drops) and compound **15a** (82 mg, 0.083 mmol, 2 equiv.) were added and the mixture was stirred for 2 hour at 40°C. The reaction progress was monitored by HPLC (ACN/H_2O). After completion, the crude was purified by preparative HPLC (ACN/H_2O) to give **7** (40 mg, 75% yield). ^{1}H NMR (400 MHz, DMSO) δ 8.90 (s, 1H), 8.61 (d, J = 2.2 Hz, 1H), 8.22-8.26 (m, 3H), 8.01-7.61 (m, 8H), 7.10-6.85 (m, 2H), 6.59 (m, 3H), 5.53-5.15 (m, 3H), 4.87 (s, 1H), 4.46 (d, J = 4.4 Hz, 2H), 4.31 (m, 1H), 3.50 (m, 22H), 3.16-2.93 (m, 10H), 2.51 (m, 2H), 1.57 (m, 8H), 0.88 (t, J = 7.1 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 173.69, 172.41, 157.77, 157.41, 153.76, 151.83, 151.19, 149.99, 149.71, 145.85, 142.15, 132.77, 131.59, 131.05, 130.23, 129.92, 129.72, 129.15, 128.86, 127.60, 125.91, 124.40, 122.96, 120.26, 113.55, 112.42, 97.90, 73.57, 70.90, 70.68, 69.70, 69.21, 66.43, 51.44, 47.09, 38.33, 37.82, 36.93, 35.67, 33.34, 32.04, 31.43, 31.19, 30.51, 25.20, 19.49, 16.82, 9.97, 8.95. MS (ESI+) m/z: 1689, 1711 [M+Na].

Compound 7b

[0097] As depicted in **Scheme 7,** compound **13** was dissolved in a mixture of 2 ml DCM and 2ml TFA, and mixed for 1h. After completion the product **13a** was concentrated in vacuum and directly reacted in the next step.

[0098] CPT-ketone **2a** (10 mg, 0.02 mmol, 1 equiv.) was dissolved in methanol (5 mL). TFA (5 drops) and compound **13a** (21 mg, 0.06 mmol, 3 equiv.) were added and the mixture was stirred for 2 hour at 40°C. The reaction progress was monitored by HPLC (ACN/H_2O). After completion, the crude was purified by preparative HPLC (ACN/H_2O) to give product **7b** (8 mg, 50 % yield). ^{1}H NMR (400 MHz, DMSO) δ 8.89 (s, 1H), 8.24 (dd, J = 18.5, 8.4 Hz, 2H), 7.95-7.87 (m, 1H), 7.78-7.67 (m, 2H), 6.54 (t, J = 5.8 Hz, 1H), 5.27 (d, J = 12.4 Hz, 2H), 3.72-3.35 (m, 16H), 3.12 (d, J = 6.3 Hz, 2H), 2.57 (s, 4H), 2.23 (t, J = 7.3 Hz, 2H), 2.10 (m, 1H), 2.02 (m, 1H), 1.89-1.53 (m, 10H), 0.85 (t, J = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 178.93, 174.79, 171.67, 162.02, 157.40, 154.92, 150.04, 149.70, 141.95, 136.93, 131.46, 129.50, 128.52, 125.86, 124.15, 102.24, 100.54, 95.33, 84.25, 81.26, 70.90, 70.72, 69.70, 69.34, 59.27, 56.59, 52.43, 38.36, 37.81, 36.94, 36.74, 32.65, 31.19, 30.68, 30.28, 29.98, 29.16, 28.54, 19.52, 16.76, 10.26, 9.60. MS (ESI+) m/z: 943.

Compound 6

[0099] As depicted in **Scheme 8,** compound **16** (20 mg, 0.082 mole, 2 eq.) and CPT-ketone **2a** (10 mg, 0.041 mole, 1 eq.) were dissolved in toluene and catalytic amount of PTSA was added. The reaction was heated to 110°C and stirred for 5 h. The reaction progress was monitored by HPLC (ACN/H_2O). After completion, the crude was purified by preparative HPLC (ACN/H_2O) to give product **6** (15 mg, 75 % yield).

[0100] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40-8.14 (m, 2H), 7.85 (d, J = 1.4 Hz, 1H), 7.69 (m, 3H), 7.38-7.29 (m, 1H), 5.73 (m, 1H), 5.58-5.20 (m, 3H), 5.03-4.87 (m, 1H), 4.71-4.53 (m, 1H), 3.95 (m, 6H), 2.61-2.45 (m, 2H), 2.31-2.15 (m, 2H), 1.96-1.78 (m, 2H), 1.71-1.57 (m, 2H), 1.41 (s, 3H), 1.10-1.01 (t, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.60, 158.12, 154.56, 153.33, 150.68, 148.71, 146.22, 139.89, 132.92, 131.76, 131.32, 130.76, 130.47, 129.61, 125.46, 124.71, 119.92, 114.80, 111.75, 111.22, 110.29, 108.94, 108.75, 98.84, 75.92, 74.66, 73.45, 71.98, 71.06, 67.08, 65.11, 57.15, 57.02, 52.05, 42.86, 39.37, 38.41, 36.66, 35.59, 34.56, 32.65, 32.33, 32.16, 31.36, 30.90, 30.42, 30.25, 30.06, 29.96, 27.94, 26.24, 25.13, 23.66, 23.42, 19.85, 17.47, 14.86, 8.54, 1.75.

Buffer Preparation Protocols:

[0101] Preparation of acetate buffers. The buffer solutions were prepared by addition of acetic acid solution (pH 4, 284.4 mL; pH 4.8, 87.2 mL; pH 5, 73.4 mL; pH 6 52.4 mL) and sodium hydroxide solution (1M, 50 mL) in 500 mL volumetric flask. The mixture was diluted with distilled water to a final volume of 500 mL.

[0102] Preparation of PBS buffers. 1M NaH_2PO_4 solution was prepared by dissolving 13.8 g of NaH_2PO_4•H_2O in distilled H_2O to make a final volume of 100 mL. 1M Na_2HPO_4 solution was prepared by dissolving 14.2 g of Na_2HPO_4 in distilled H_2O to make a final volume of 100 mL. 1M NaH_2PO_4 (pH 7.0, 42.3 mL; pH 7.4, 22.6 mL) and 1M Na_2HPO_4 (pH 7.0, 57.7 mL; pH 7.4 mL) were mixed and diluted to 1L with H_2O.

[0103] Hydrolysis of compound **7** in buffer: pH 4.8, pH 6.0, pH 6.5, pH 7.0 and pH 7.4. A stock solution of **7** (10 mM)

was prepared by dissolving 17.95 mg of the compound in 1 mL DMSO. A sample of 25 μL from the stock solution was added to 75 μL DMSO, and the resulting solution was added slowly while stirring to 840 μL of the appropriate buffer (pH 4.8, 6.0, 6.5, 7.0, or 7.4). Samples were taken at different times and analyzed by HPLC. The hydrolysis percentage of the carrier was calculated based on the HPLC integration ratio of the starting material and the corresponding hydrolytic products.

[^{3}H]-Folic acid competition assay

[0104] KB HiFR cells (nasopharyngeal epidermal carcinoma cells overexpressing the folate receptor) were growth in folate depleted RPMI + 10% FBS for 15 days in order to overexpress the folate receptor. KB cells were growth in regular RPMI + 10% FBS. KB or KB HiFR cells were seeded in 24 wells/plate, 1×10^6 cells/well in 500 μL of folate depleted RPMI without FBS. The cells were treated as follow: 3 wells: 10 μL/wel of RMPI without FBS; 3 wells: 10 μL/well of cold folic acid (100 μM, final concentration in each well 2 μM); 3 wells: 10 μL/well of compound **7** ([FA]=100 μM, final concentration in each well 2 μM); 3 wells: 10 μL /well of compound **7b** ([FA]=100 μM, final concentration in each well 2 μM); 3 wells: 10 μL /well of CPT ([FA]=100 μM, final concentration in each well 2 μM). The cells were incubated for 3 hours at 37°C, 5% CO_2. After 3 h of incubation 10 μL of [^{3}H]-FA (5 μCi/mL, 10 μM, final concentration in each well 0.2 μM) were added to each well. After 3 h of incubation the medium was collected (100 mL were kept and added to 3 mL of scintillation fluid for counting, as reference), the cells harvested with trypsin, added to the medium and the suspension was centrifuged. The supernatant was discarded and the pellet washed 3 times with 3 mL of cold PBS. After the last wash, each sample was treated overnight with 500 μL of 0.5N NaOH, followed by neutralization with 0.5N HCl. After 30 minutes, 600 μL of the suspension was added to 3 mL of scintillation fluid and the amount of radioactivity was determined by liquid scintillation counting.

Proliferation assay

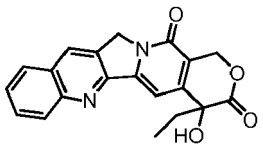
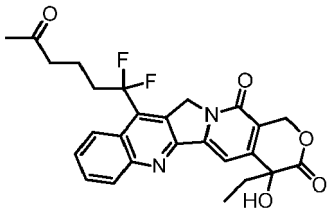
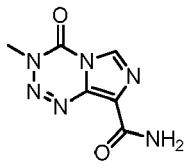
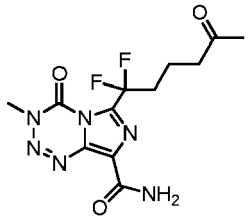
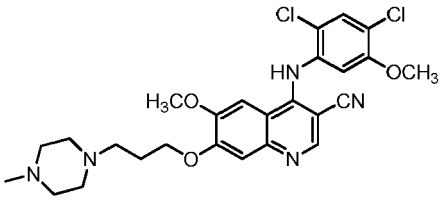
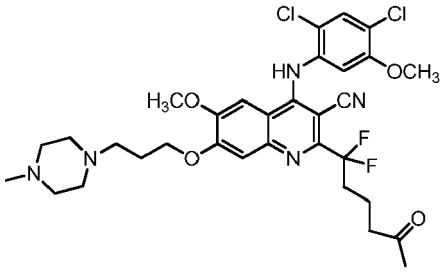
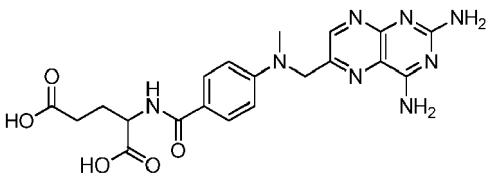
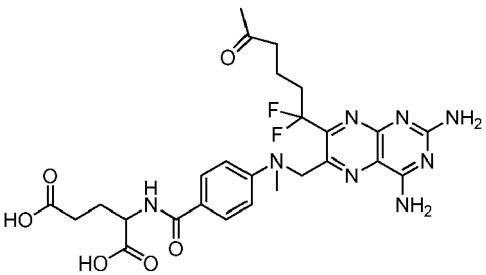
[0105] KB HiFR were seeded in 96 well/plates, 2×10^3 cell/100 μL in folate depleted RPMI + 10% FBS. After 24 h incubation at 37°C, 5% CO_2, 100 μL of serial dilutions (200-0.00002 μM CPT equivalent concentrations) of CPT and CPT-derivatives were added to the wells (final concentration 100-0.00001 μM). In the short exposure (pulse and chase) experiment the treatments were removed after 10 min incubation at 37°C, 5% CO_2, the cells were washed once with PBS and incubated with fresh folate depleted RPMI + 10% FBS for 72h. In the long exposure experiment, the cells were incubated for 72 h with the treatment. After 72 h incubation, 30 μL of mg/mL solution of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) in PBS were added to each well, and incubated for 5h. After incubation the medium was removed and the cells lysed in 200 μL DMSO and kept under gentle stirring protected from light for 20 min. Absorbance was measured at 560 nm and survival plotted as percentage of untreated control.

Example 1.

[0106] Sodium difluoroalkyl ketal sulfinate **1** is a ketone-protected reagent, capable of reacting with a heteroarene C-H bond under oxidative acidic conditions to produce the corresponding ketone derivative **I (Scheme 1).** Deprotection of the ketal should occur *in situ* under the hydrolytic acidic conditions of the reaction to afford the ketone functional group. The heteroarene-ketone analogue can be readily masked through an acid-labile hydrazone linkage (Patil *et al.,* 2012; Yang *et al.,* 2007) **(III)** or a photo-labile ketal protecting group (Gravel *et al.,* 1983) **(II).**

[0107] The synthesis of ketal sulfinate **1** was achieved in an analogous manner to that recently described for other sulfinate salts **(Scheme 2).** Thus, alkylation of ketal **1b** by pyridine derivative **1a** generated sulphone **1c.** The latter was reacted with sodium mercaptoethanol to afford sulfinate salt **1.** This synthesis could be easily performed on multi-gram scale to produce sulfinate **1** with good yields. Heteroarene difluoroalkylation by sulfinate salt **1** to produce the corresponding ketone derivative was initially established on caffeine as a test substrate. Caffeine successfully reacted with sulfinate **1** through direct functionalization of its C-H bond to afford ketone derivative **Id** in 87% yield.

Table 1. Difluoroalkylation by sulfinate salt **1** of CPT, TMZ, bosutinib and MTX

Heteroarene drug	Heteroarne-ketone derivative	Isolated yield
 **2**	 **2a**	78%
 **3**	 **3a**	28%
 **4**	 **4a**	67%
 **5**	 **5a**	35%

[0108] Next, we sought to evaluate the functionalization-capability of sulfinate salt **1** on CPT **(2),** TMZ **(3),** bosutinib **(4)** and MTX **(5),** all heteroarene known antineoplastic drugs. CPT is a topoisomerase inhibitor with limited options for bioconjugation through its tertiary hydroxyl group. TMZ is a cytotoxic alkylating agent, which is considered as an untaggable compound due to the absence of suitable functional group. Likewise, bosutinib, an approved tyrosine kinase inhibitor based drug, has no functional group available for conjugation. MTX, an antifolate-based chemotherapeutic drug, is another heteroarene with limited bioconjugation options **(Table 1).** The four heteroarene drugs were successfully functionalized by sulfinate salt **1** to afford the corresponding ketone analogues in moderate to good yields. For CPT, bosutinib and MTX, sulfinate salt **1** was able to selectively functionalize the heteroarenes at the most electron-deficient C-H bond.

[0109] In order to find out whether the new derivatives retain their potency, the cytotoxicity of the CPT-, TMZ- and bosutinib-ketone analogues was evaluated in comparison to that of the parent drugs. **Fig. 2** shows representative cell-growth inhibition plots for each drug and its ketone analogue, wherein the applied cell lines and the calculated IC_{50}s are presented in **Table 2.**

Table 2. IC_{50}s values calculated from cell-growth inhibition assays for CPT, TMZ and bosutinib, and their ketone analogues

Drugs	IC_{50}	Cell line
CPT	7 nm	U-87
CPT-ketone	10 nm	U-87
TMZ	36 μm	U-251
TMZ-ketone	35 μm	U-251
Bosutinib	17 μm	SF-295
Bosutinib-ketone	17 μm	SF-295

[0110] Remarkably, for three of the four drugs, tumor cell-growth inhibition assays showed almost identical cytotoxicity (IC_{50} values) for the ketone-derivatives and their native drugs. Functionalization of CPT, TMZ and bosutinib using sulfinate salt **1,** resulted in ketone analogues that retained their cytotoxic activity. However, the ketone analogue of MTX completely lost its cytotoxicity. The assays were repeated with several tumorous cell-lines and the obtained results were similar **(Figs. 3-4).** These results indicate that it is possible for certain biologically relevant heteroarenes to maintain their original activity, after C-H functionalization by sulfinate salt **1,** at an appropriate position.

Example 2.

[0111] To determine the usefulness of the drug analogues produced harboring a ketone for controlled release and bioconjugation, CPT-ketone **2a** was selected for further evaluation. As presented in **Scheme 1,** a ketone functional group can be masked either through an acid-labile hydrazone linkage or with a photo-labile ketal protecting group. CPT-ketone **2a** was masked with a photo-labile protecting group (Gravel *et al.,* 1983; Klan *et al.,* 2013) to produce ketal derivative **6 (Fig. 5).** This derivative can be considered as prodrug form of CPT-ketone **2a.** Irradiation of prodrug **6** with UV light over 2 hours under physiological conditions resulted in release of CPT-ketone **2a (Fig. 5A).** No release was observed without irradiation. The cytotoxicity of prodrug **6** was then evaluated in a standard cell-growth inhibition assay before and after irradiation with UV light **(Fig. 5B).** As expected, derivative **6** before irradiation exhibited typical prodrug behavior, with an IC_{50} value of 350nM. Prodrug **6** after irradiation has showed significantly higher cytotoxicity with an IC_{50} value of 10nM, similarly to that obtained for CPT-ketone **2a** (IC_{50}=5nM). These results demonstrate how the newly installed ketone of the heteroarene analogues can be used to obtain prodrugs with a photo-labile controlled-release pathway. In this example, the prodrug was activated with UV light; however, there are analogues protecting groups that can be removed through a visible or near infrared light (Lu *et al.,* 2003). To our knowledge, this is the first demonstration of a prodrug photo-activation, which is based on unmasking of a ketone group (Klan *et al.,* 2013).

[0112] Next, we sought to evaluate the acid-labile hydrazone linkage, for controlled-release and bioconjugation between CPT-ketone **2a** and a targeting vehicle. Hydrolysis via an acid-labile linkage is a useful controlled-release mechanism for cell-penetrating vehicles such as folic acid (FA) (Zhao *et al.,* 2008) conjugate **(Scheme 9).** Thus, CPT-ketone **2a** was reacted with a semicarbazide derivative of folic acid **(7a),** via a semicarbazone linkage, to generate CPT-folic acid conjugate **7** with an acid-labile controlled-release mechanism (see Synthesis schemes and experimental procedures). A PEG linker derivative was used as a spacer to connect between the folic acid and CPT-ketone **2a.**

Example 3.

[0113] To validate the release of CPT-ketone **2a** from the folate conjugate **7,** we monitored the hydrolysis of the semicarbazone linkage under various pHs (physiological conditions - pH, 7.4; early endosome stage - pH, 6.5 and 6.0; and late endosome stages - pH, 4.8). The kinetic plots of CPT-ketone **2a** release are presented in **Fig. 6.** The semicarbazone-base conjugate exhibited excellent selective hydrolysis under acidic conditions. Fast release kinetics of CPT-ketone **2a** was observed under acidic pHs, while no release at all was observed over 24 hr. at physiological pH. Importantly, at relatively mild acid conditions (early-stage endosome pH, 6.5), release of CPT-ketone **2a** (about 50%) still effectively took place (Yang *et al.,* 2007).

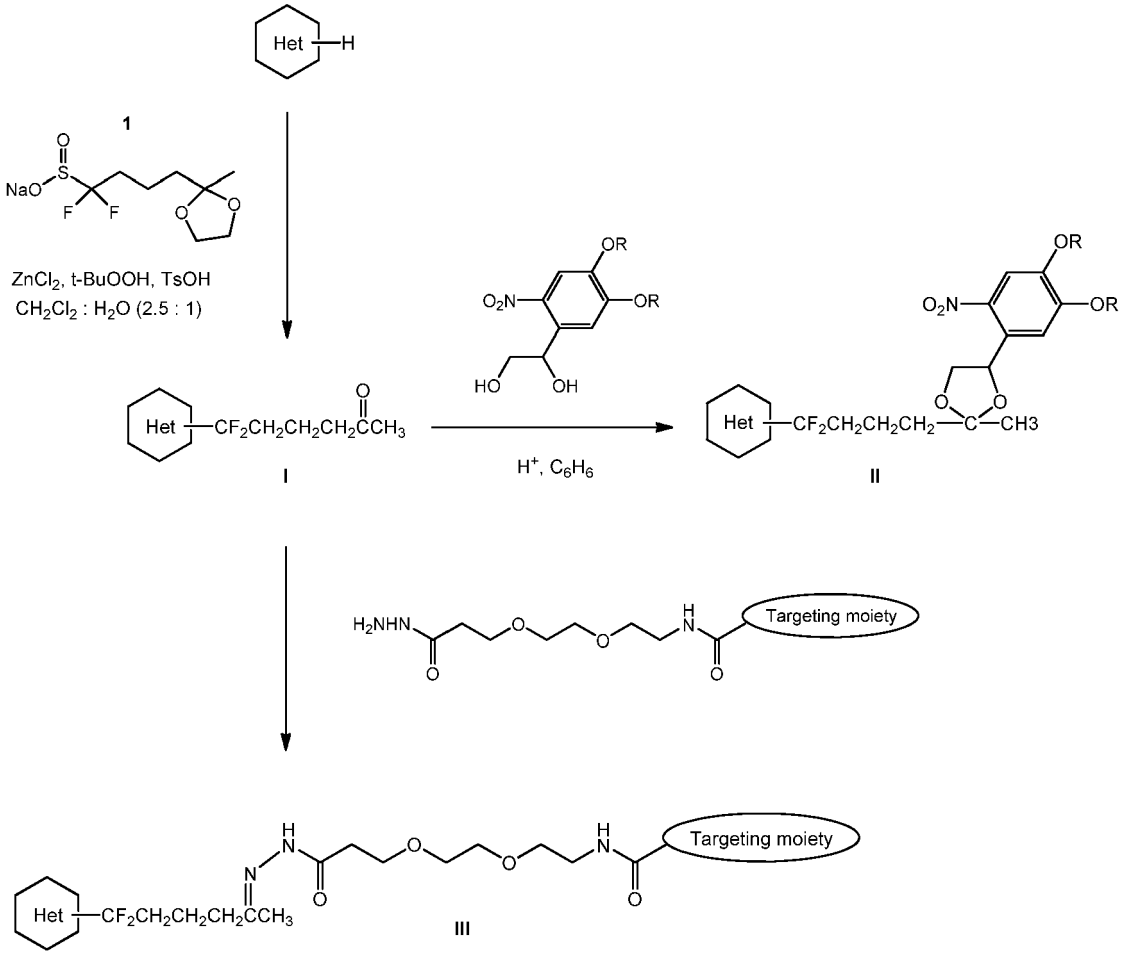
[0114] The binding affinity of folate-conjugate **7** with FR receptors was evaluated with highly expressed-FR KB cells (HiFR-KB). CPT-PEG derivative **7b,** which lacks the folic acid was used as a control **(Fig. 7A).** The obtained measurements showed 82% binding of conjugate **7** to FR receptors in comparison to that of free folic acid. Cytotoxicity evaluation of CPT-folic acid conjugate **7** on HiFR KB cells results with relatively similar IC_{50} values for CPT-ketone **2a** and its folic

acid conjugate **7 (Fig. 7B).** CPT-PEG derivative **7b** showed IC_{50} value of 15-fold higher in comparison to that of conjugate **7.** These results support the cell-penetrating ability of the folic acid-CPT conjugate and *in vitro* CPT-ketone controlled-release through the acid-labile semicarbazone linkage.

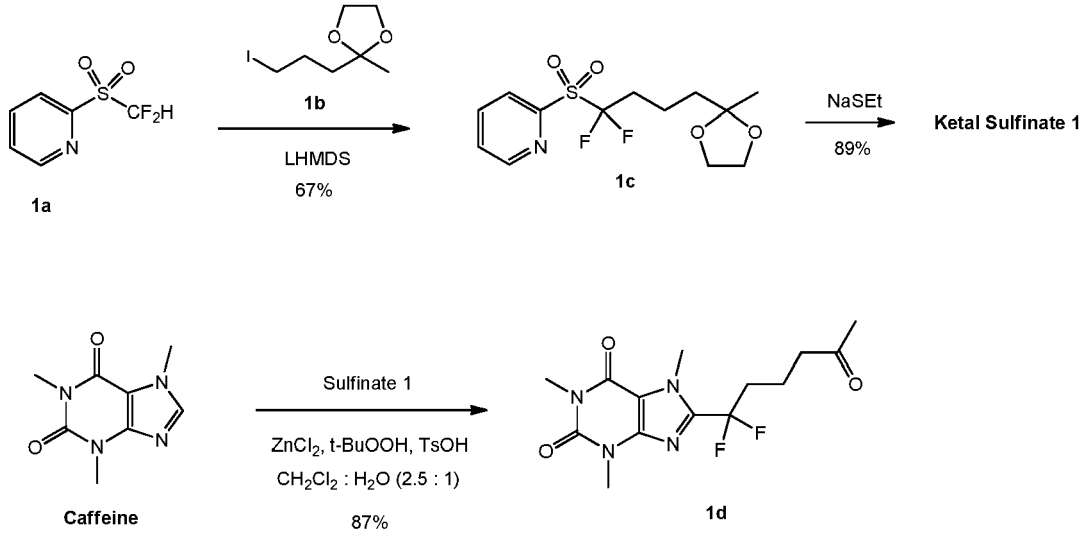
APPENDIX

[0115]

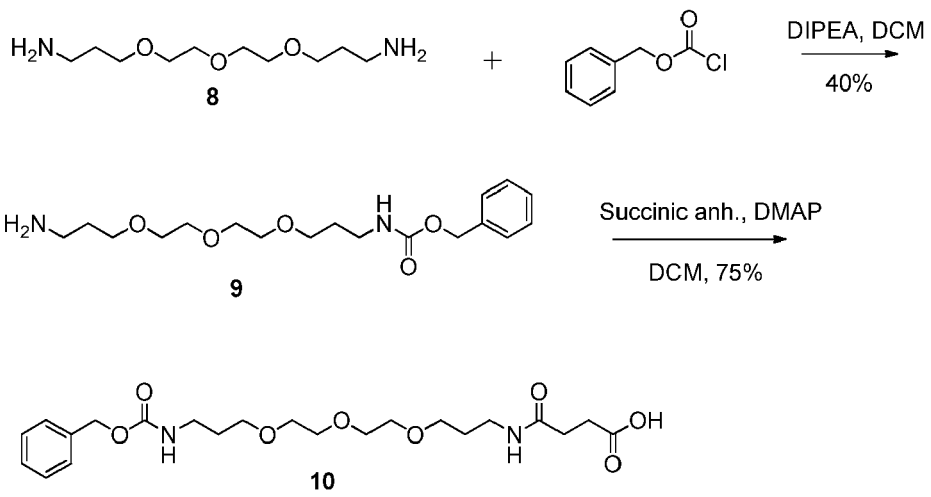
Scheme 1. Employing of sodium ketal sulfinate **1** for tagging a heteroarene C-H inert bond with a ketone functional group and its bioconjugation through an acid-labile hydrazone linkage or a photo-labile ketal group for controlled-release applications



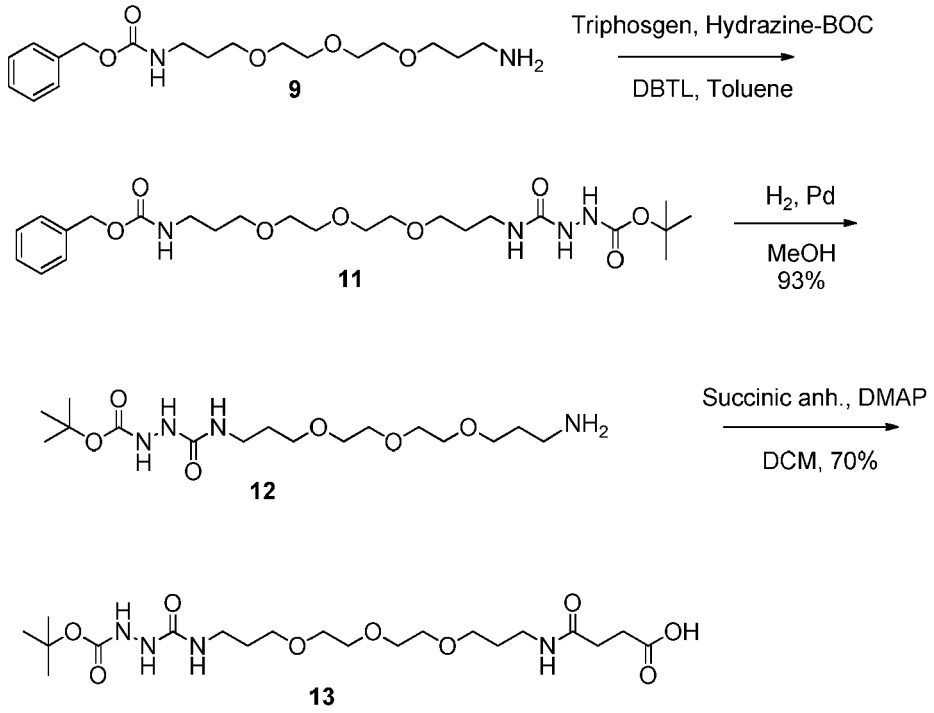
Scheme 2. Chemical synthesis of sodium ketal sulfinate **1**



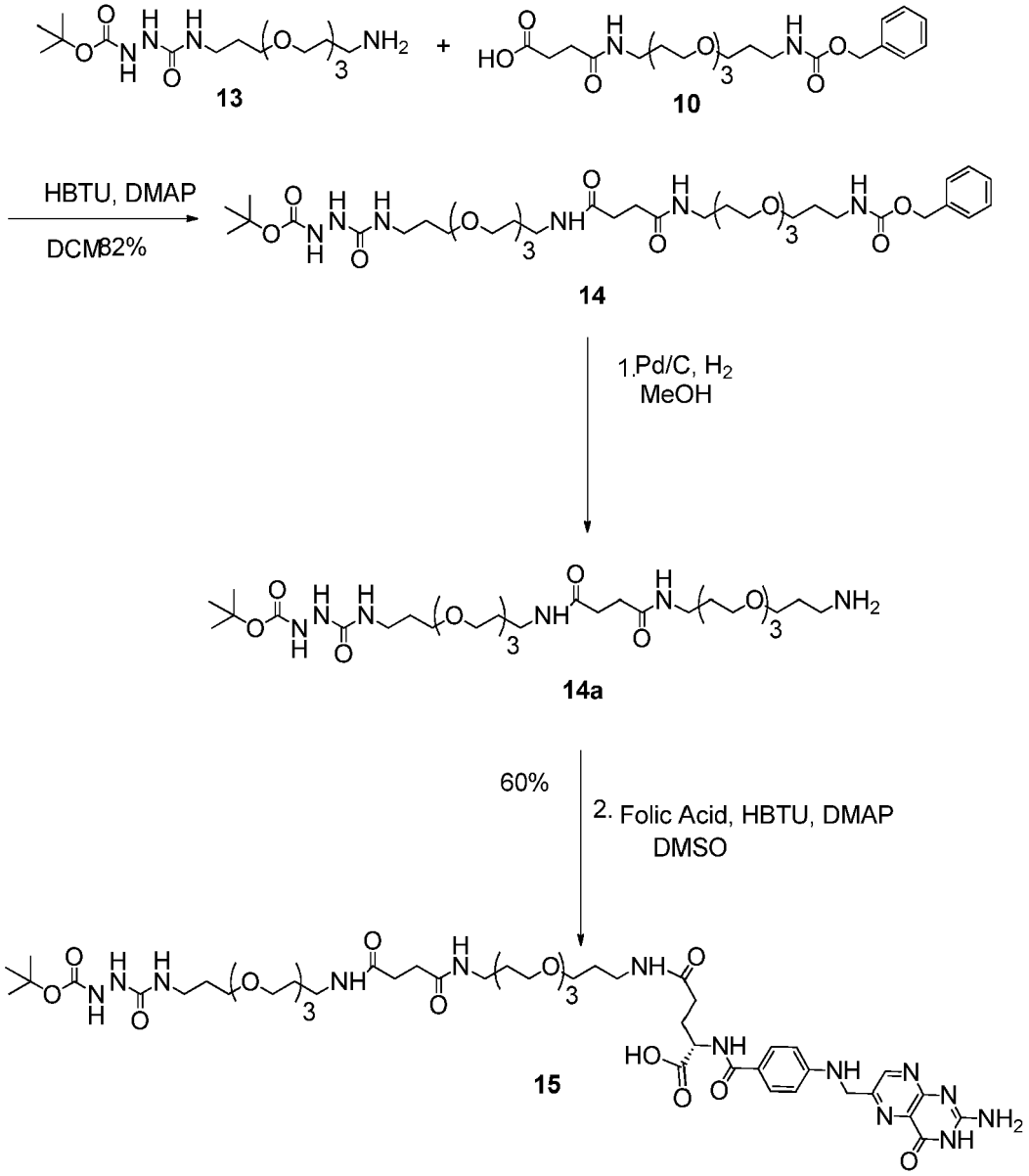
Scheme 3. Chemical synthesis of compound **10**



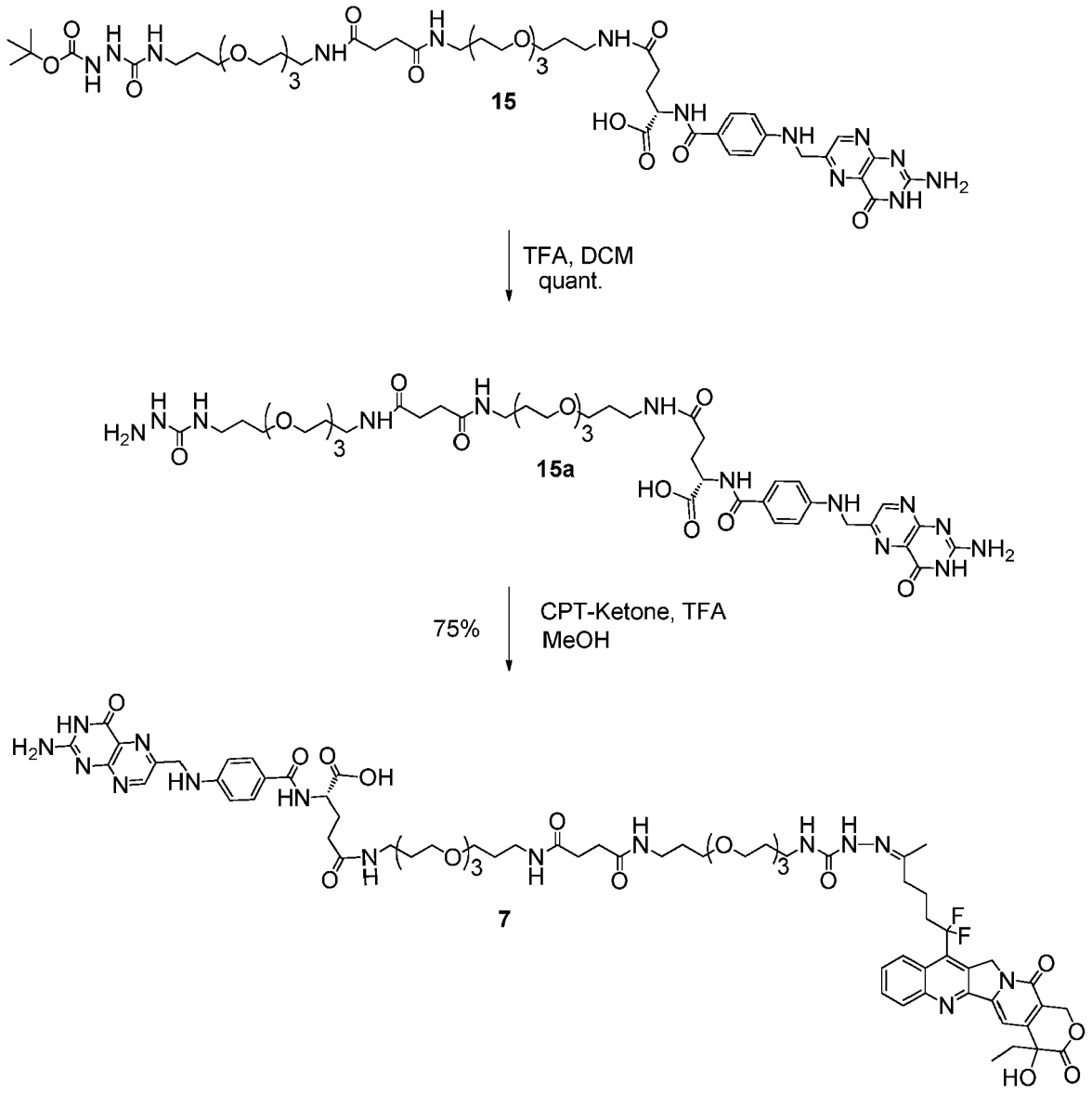
Scheme 4. Chemical synthesis of compound **13**



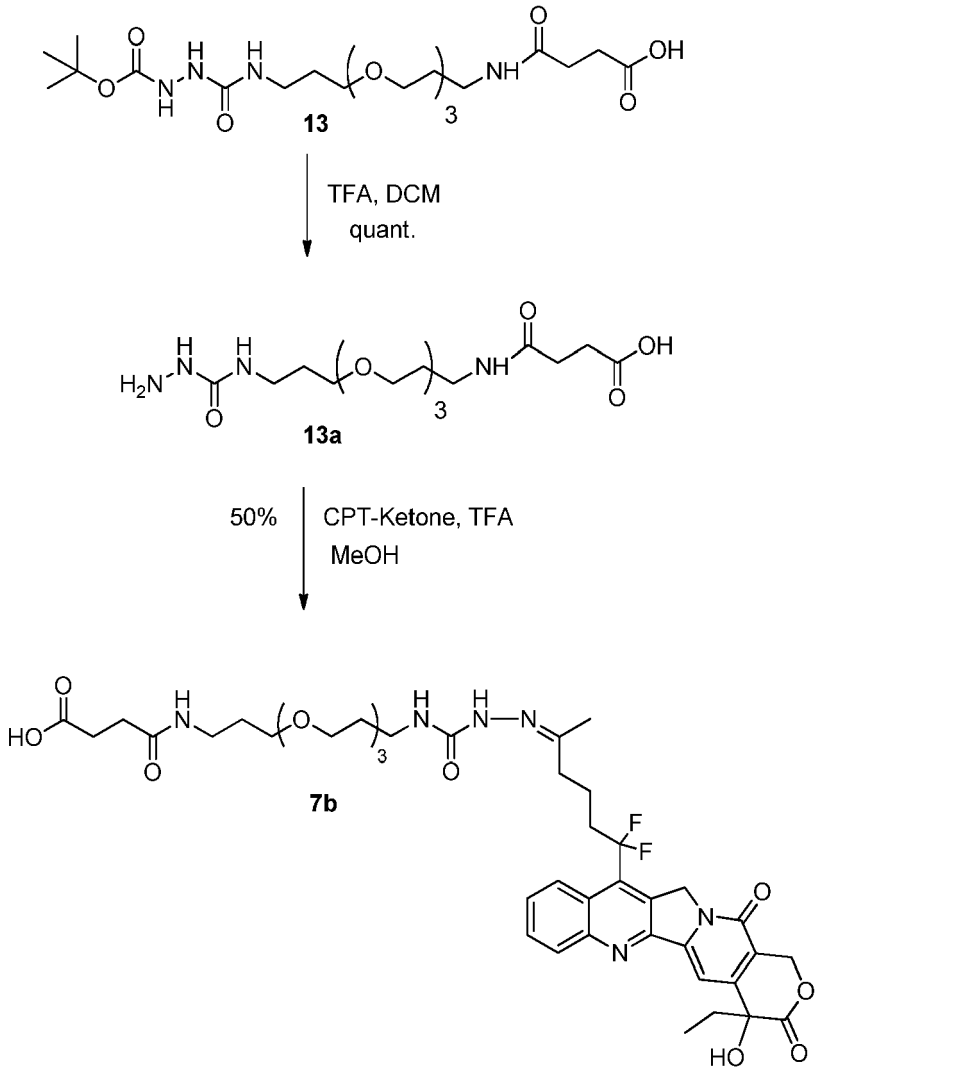
Scheme 5. Chemical synthesis of compound **15**



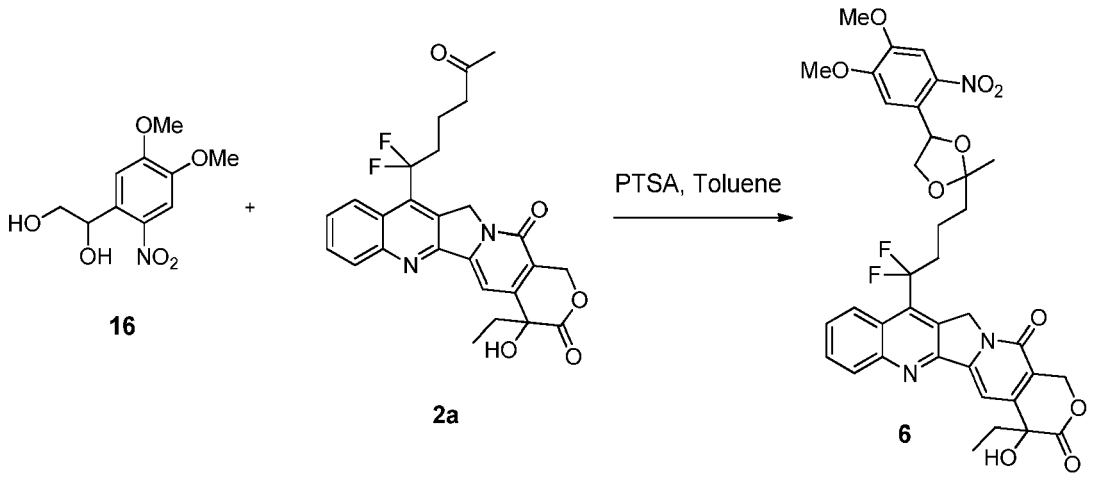
Scheme 6. Chemical synthesis of compound **7**



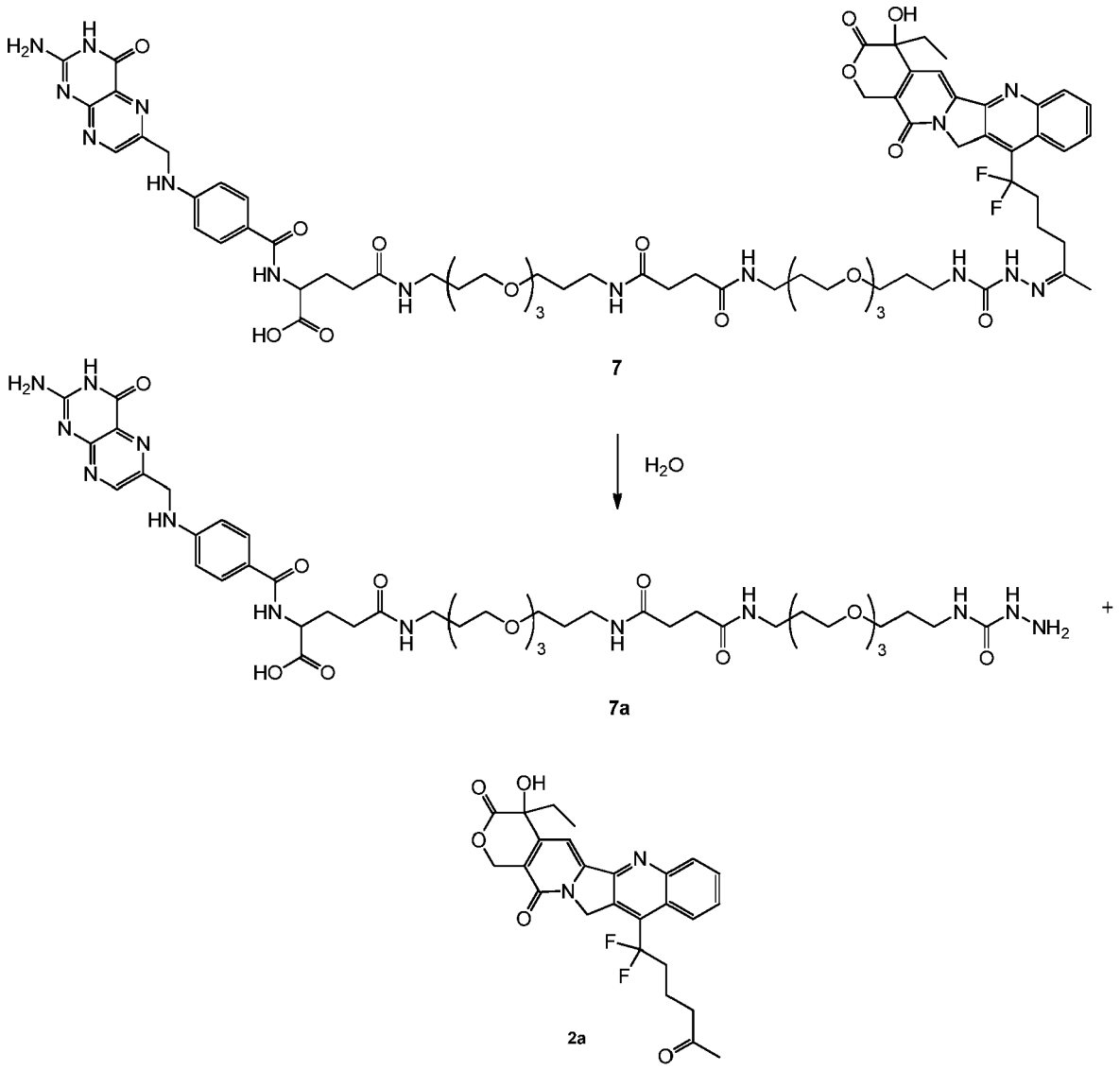
Scheme 7. Chemical synthesis of compound **7b**



Scheme 8. Chemical synthesis of compound **6**



Scheme 9. Release of CPT-ketone **2a** from a folic acid conjugate via acid-labile controlled-release mechanism of a semicarbazone linkage.



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Claims

1. A compound of the formula I:

I $Y\text{-}CF_2\text{-}(CR_1R_2)_n\text{-}X\text{-}(CR_1R_2)_m\text{-}R_1$

wherein

Y is a drug or a bioactive reagent, comprising a heteroaromatic ring and linked via a carbon atom of said heteroaromatic ring;
X is carbonyl, or cyclic ketal substituted with 1 to 4 groups each independently is phenyl or naphtyl substituted ortho to the carbon of attachment with $-NO_2$, and optionally further substituted at any position other than ortho to the carbon of attachment with one or more groups each independently selected from $-O-(C_1\text{-}C_8)$, $-(C_1\text{-}C_8)$alkyl, $-N(R')_2$, or halogen, wherein R' each independently is $-(C_1\text{-}C_8)$alkyl or H;
R_1 and R_2 each independently is H, halogen, $-OR_3$, $-CO\text{-}R_3$, $-CO\text{-}OR_3$, $-OCO\text{-}OR_3$, $-OCO\text{-}N(R_3)_2$, -CN, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CO\text{-}N(R_3)_2$, $-(C_1\text{-}C_8)$alkyl, $-(C_2\text{-}C_8)$alkenyl, $-(C_2\text{-}C_8)$alkynyl, $(C_3\text{-}C_{10})$cycloalkyl, $(C_6\text{-}C_{14})$aryl, or 4-12-membered heterocyclyl;
R_3 is H, $-(C_1\text{-}C_{18})$alkyl, $-(C_2\text{-}C_{18})$alkenyl, or $-(C_2\text{-}C_{18})$alkynyl; and
n and m each independently is an integer of 1-8,

or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein:

(i) R_1 and R_2 each independently is H, halogen, $-OR_3$, $-CO\text{-}R_3$, $-CO\text{-}OR_3$,$-OCO\text{-}OR_3$, $-OCO\text{-}N(R_3)_2$, -CN, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CO\text{-}N(R_3)_2$, $-(C_1\text{-}C_4)$alkyl, $-(C_2\text{-}C_4)$alkenyl, $-(C_2\text{-}C_4)$alkynyl, $(C_3\text{-}C_{10})$cycloalkyl, $(C_6\text{-}C_{14})$aryl, or 4-12-membered heterocyclyl, wherein R_3 is H, methyl, ethyl or propyl;
(ii) n is 3, 4, or 5; or
(iii) m is 1, 2, or 3.

3. The compound of claim 1, wherein R_1 and R_2 each independently is H, halogen, $-OR_3$, $-CO\text{-}R_3$, $-CO\text{-}OR_3$, $-OCO\text{-}OR_3$, $-OCO\text{-}N(R_3)_2$, -CN, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CO\text{-}N(R_3)_2$, $-(C_1\text{-}C_4)$alkyl, $-(C_2\text{-}C_4)$alkenyl, $-(C_2\text{-}C_4)$alkynyl, $(C_3\text{-}C_{10})$cycloalkyl, $(C_6\text{-}C_{14})$aryl, or 4-12-membered heterocyclyl, wherein R_3 is H, methyl, ethyl or propyl; n is 3, 4, or 5; and m is 1, 2, or 3, preferably wherein R_1 and R_2 are each H.

4. The compound of claim 1, wherein Y is an anticancer drug, antineoplastic drug, antifungal drug, antibacterial drug, antiviral drug, cardiac drug, neurological drug, psychoactive drug, drug of abuse, alkaloid, antibiotic, bioactive peptide, steroid, steroid hormone, peptide hormone, interferon, interleukin, narcotic, nucleic acid, pesticide, or prostaglandin.

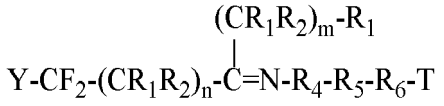
5. The compound of claim 4, wherein said anticancer drug is a chemotherapeutic drug such as camptothecin or a derivative thereof, bosutinib, or methotrexate; or said antineoplastic drug is an alkylating antineoplastic agent such as temozolomide, uramustine or bendamustine.

6. The compound of any one of claims 1 to 5, wherein X is (i) carbonyl; or (ii) cyclic ketal substituted with 1 or 2 phenyl

groups each independently substituted ortho to the carbon of attachment with $-NO_2$, and optionally further substituted at any position other than ortho to the carbon of attachment with one or more groups each independently selected from $-O-(C_1-C_8)$.

7. The compound of claim 6, wherein X is cyclic ketal substituted with 4,5-dimethoxy,2-nitrophenyl group.

8. A conjugate of the formula II:



wherein

Y is a drug or a bioactive reagent, comprising a heteroaromatic ring and linked via a carbon atom of said heteroarimatic ring;
R_1 and R_2 each independently is H, halogen, $-OR_3$, $-CO-R_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, $-CN$, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CO-N(R_3)_2$, $-(C_1-C_8)$alkyl, $-(C_2-C_8)$alkenyl, $-(C_2-C_8)$alkynyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl, or 4-12-membered heterocyclyl;
R_3 is H, $-(C_1-C_{18})$alkyl, $-(C_2-C_{18})$alkenyl, or $-(C_2-C_{18})$alkynyl;
R_4 is absent, or is selected from $-NH-(CH_2)_p-$, $-NH-CO-(CH_2)_p-$, $-NH-CO-NH-(CH_2)_p-$, $-NH-CO-O-(CH_2)_p-$, $-O-(CH_2)_p-$, $-O-CO-(CH_2)_p-$, $-O-CO-NH-(CH_2)_p-$, or $-O-CO-O-(CH_2)_p-$;
R_5 is a polymer-, protein-, peptide-, or carbohydrate moiety;
R_6 is H, $-(CH_2)_y-OH$, $-(CH_2)_y-SH$, $-(CH_2)_y-NH_2$, $-(CH_2)_y-COOH$, $-(CH_2)_y-SO_3H$, or a divalent radical selected from $-(CH_2)_y-O-$, $-(CH_2)_y-S-$, $-(CH_2)_y-NH-$, $-(CH_2)_y-COO-$ or $-(CH_2)_y-SO_3-$;
n and m each independently is an integer of 1-8;
p and y each independently is an integer of 0-8; and
T is absent, or is a targeting moiety capable of binding to an extracellular antigen and linked via a functional group thereof, provided that when T is absent R_6 is not a divalent radical, and when T is a targeting moiety R_6 is a divalent radical,

or a pharmaceutically acceptable salt thereof.

9. The conjugate of claim 8, wherein:

(i) R_1 and R_2 each independently is H, halogen, $-OR_3$, $-CO-R_3$, $-CO-OR_3$,$-OCO-OR_3$, $-OCO-N(R_3)_2$, $-CN$, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CO-N(R_3)_2$, $-(C_1-C_4)$alkyl, $-(C_2-C_4)$alkenyl, $-(C_2-C_4)$alkynyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl, or 4-12-membered heterocyclyl, wherein R_3 is H, methyl, ethyl or propyl;
(ii) n is 3, 4, or 5;
(iii) m is 1, 2, or 3;
(iv) R_4 is $-NH-CO-(CH_2)_p-$ or $-NH-CO-NH-(CH_2)_p-$;
(v) R_5 is a polymer moiety, and said polymer is linear or branched polyethylene glycol (PEG) or copolymers thereof; a pseudo PEG interrupted by at least one group each independently selected from -NH-CO-, -CO-NH-, or (C_3-C_8)alkylene interrupted by at least two groups each independently selected from -NH-CO- or -CO-NH-, such as $(-CH_2-CH_2-O)_3-(CH_2)_3-NH-CO-(CH_2)_2-CO-NH-(CH_2)_3-(O-CH_2-CH_2-)_3$; poly(lactic acid) or copolymers thereof; polyesters selected from polylactide (PLA), polyglycolide (PGA), polycaprolactone (PCL) or copolymers thereof; or polyamides based on polymethacrylamide or copolymers thereof;
(vi) R_5 is a protein moiety, and said protein is albumin, a modified albumin, or a protein containing globin-like domains having long half-life in circulation;
(vii) R_5 is a peptide moiety;
(viii) R_5 is a carbohydrate moiety;
(ix) Y is an anticancer drug, antineoplastic drug, antifungal drug, antibacterial drug, antiviral drug, cardiac drug, neurological drug, psychoactive drug, drug of abuse, alkaloid, antibiotic, bioactive peptide, steroid, steroid hormone, peptide hormone, interferon, interleukin, narcotic, nucleic acid, pesticide, or prostaglandin; or
(x) said targeting moiety is a protein, peptide, polypeptide, glycoprotein, lipoprotein, lipid, phospholipid, oligonucleotide or a mimic thereof, steroid, hormone, lymphokine, growth factor, albumin, cytokine, enzyme, coenzyme, vitamin, cofactor, human antigen, hapten, receptor protein, antibody or a fragment thereof, a substance

used or modified such that it functions as a targeting moiety, or a combination thereof, preferably a vitamin such as vitamin B9 (folic acid).

10. The conjugate of claim 9, wherein said anticancer drug is a chemotherapeutic drug such as camptothecin or a derivative thereof, bosutinib, or methotrexate; or said antineoplastic drug is an alkylating antineoplastic agent such as temozolomide, uramustine or bendamustine.

11. The conjugate of any one of claims 8 to 10, wherein R_1 and R_2 each independently is H, halogen, $-OR_3$, $-CO-R_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, -CN, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CO-N(R_3)_2$, $-(C_1-C_4)$alkyl, $-(C_2-C_4)$alkenyl, $-(C_2-C_4)$alkynyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl, or 4-12-membered heterocyclyl, wherein R_3 is H, methyl, ethyl or propyl; n is 3, 4, or 5; m is 1, 2, or 3; R_4 is $-NH-CO-(CH_2)_p-$ or $-NH-CO-NH-(CH_2)_p-$; and R_5 is a PEG moiety or a pseudo PEG moiety interrupted by at least one group each independently selected from -NH-CO-, -CO-NH-, or (C_3-C_8)alkylene interrupted by at least two groups each independently selected from -NH-CO- or -CO-NH-.

12. The conjugate of claim 11, wherein R_1 and R_2 are H; n is 3; m is 1; and R_5 is a PEG moiety or a pseudo PEG moiety having the structure $(-CH_2-CH_2-O)_3-(CH_2)_3-NH-CO-(CH_2)_2-CO-NH-(CH_2)_3-(O-CH_2-CH_2-)_3$.

13. The conjugate of claim 12, wherein (i) R_6 is a divalent radical; or (ii) R_6 is H,$-(CH_2)_y-OH$, $-(CH_2)_y-SH$, $-(CH_2)_y-NH_2$, $-(CH_2)_y-COOH$ or $-(CH_2)_y-SO_3H$, preferably-$(CH_2)_y-SH$, $-(CH_2)_y-NH_2$, or $-(CH_2)_y-COOH$, preferably wherein y is 1, 2 or 3; and T is absent.

14. The conjugate of claim 13, wherein R_6 is $-(CH_2)_y-NH-$, preferably wherein y is 1 or 2; and said targeting moiety is folic acid linked via a carboxylic group thereof, preferably through its gamma-carboxyl group.

15. The conjugate of claim 14, wherein (i) R_4 is $-NH-CO-NH-CH_2-$; R_5 is a pseudo PEG moiety having the structure $(-CH_2-CH_2-O)_3-(CH_2)_3-NH-CO-(CH_2)_2-CO-NH-(CH_2)_3-(O-CH_2-CH_2-)_3$; R_6 is $-(CH_2)-NH-$; and Y is camptothecin, 10-hydroxycamptothecin, temozolomide, bosutinib, or methotrexate; (ii) R_4 is $-NH-CO-CH_2-$; R_5 is a pseudo PEG moiety having the structure $(-CH_2-CH_2-O)_3-(CH_2)_3-NH-CO-(CH_2)_2-CO-NH-(CH_2)_3-(O-CH_2-CH_2-)_3$; R_6 is $-(CH_2)-NH-$; and Y is camptothecin, 10-hydroxycamptothecin, temozolomide, bosutinib, or methotrexate; or (iii) R_4 is -NH-CO-; R_5 is a PEG moiety having the structure $(-CH_2-CH_2-O)_3-$; R_6 is $-(CH_2)_2-NH-$; and Y is camptothecin, 10-hydroxycamptothecin, temozolomide, bosutinib, or methotrexate.

16. A pharmaceutical composition comprising a compound of any one of claims 1 to 7 wherein X is not carbonyl; or a conjugate of any one of claims 8 to 15, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

17. A conjugate of any one of claims 8 to 15, or a pharmaceutically acceptable salt thereof, wherein Y is an anticancer drug or an antineoplastic drug, and T is a targeting moiety capable of binding to an extracellular antigen present on the cells of said cancer, for use in the treatment of a cancer.

Patentansprüche

1. Verbindung der Formel I :

I $\quad Y-CF_2-(CR_1R_2)_n-X-(CR_1R_2)_m-R_1$

worin

Y ein Wirkstoff oder ein bioaktives Reagenz ist, umfassend einen heteroaromatischen Ring und verbunden über ein Kohlenstoffatom des heteroaromatischen Rings;
X Carbonyl oder ein cyclisches Ketal ist, das mit 1 bis 4 Gruppen substituiert ist, die jeweils unabhängig Phenyl oder Naphtyl sind, substituiert mit $-NO_2$ in ortho-Position zu dem Verbindungskohlenstoffatom, und gegebenenfalls ferner substituiert an irgendeiner anderen Position als in ortho-Position zu dem Verbindungskohlenstoffatom mit einer oder mehreren Gruppen jeweils unabhängig ausgewählt aus $-O-(C_1-C_8)$, $-(C_1-C_8)$alkyl, $-N(R')_2$ oder Halogen, wobei R' jeweils unabhängig $-(C_1-C_8)$alkyl oder H ist;
R_1 und R_2 jeweils unabhängig H, Halogen, $-OR_3$, $-CO-R_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, -CN, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CO-N(R_3)_2$, $-(C_1-C_8)$alkyl,

$-(C_2-C_8)$alkenyl, $-(C_2-C_8)$alkinyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl oder eine 4-bis 12-gliedrige Heterocyclylgruppe sind;
R_3 H, $-(C_1-C_{18})$alkyl, $-(C_2-C_{18})$alkenyl oder $-(C_2-C_{18})$alkinyl ist; und
n und m jeweils unabhängig eine ganze Zahl von 1-8 sind,

oder ein pharmazeutisch annehmbares Salz davon.

2. Verbindung nach Anspruch 1, worin:

(i) R_1 und R_2 jeweils unabhängig H, Halogen, $-OR_3$, $-CO-R_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, -CN, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CO-N(R_3)_2$, $-(C_1-C_4)$alkyl, $-(C_2-C_4)$alkenyl, $-(C_2-C_4)$alkinyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl oder eine 4- bis 12-gliedrige Heterocyclylgruppe sind, wobei R_3 H, Methyl, Ethyl oder Propyl ist;
(ii) n 3, 4 oder 5 ist; oder
(iii) m 1, 2 oder 3 ist.

3. Verbindung nach Anspruch 1, worin R_1 und R_2 jeweils unabhängig H, Halogen, $-OR_3$, $-CO-R_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, -CN, $-NO_2$, $-SR_3$,
-N(R3)2, $-CO-N(R_3)_2$, $-(C_1-C_4)$alkyl, $-(C_2-C_4)$alkenyl, $-(C_2-C_4)$alkinyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl oder eine 4-bis 12-gliedrige Heterocyclylgruppe sind, wobei R_3 H, Methyl, Ethyl oder Propyl ist; n 3, 4 oder 5 ist; und m 1, 2 oder 3 ist, wobei bevorzugt R_1 und R_2 jeweils H sind.

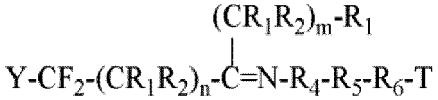
4. Verbindung nach Anspruch 1, wobei Y ein Antikrebswirkstoff, ein antineoplastischer Wirkstoff, ein antimykotischer Wirkstoff, ein antibakterieller Wirkstoff, ein antiviraler Wirkstoff, ein Herzmedikament, ein neurologischer Wirkstoff, ein psychoaktiver Wirkstoff, ein Missbrauchswirkstoff, ein Alkaloid, ein Antibiotikum, ein bioaktives Peptid, ein Steroid, ein Steroidhormon, ein Peptidhormon, ein Interferon, ein Interleukin, ein Narkotikum, eine Nukleinsäure, ein Pestizid oder ein Prostaglandin ist.

5. Verbindung nach Anspruch 4, wobei der Antikrebswirkstoff ein Chemotherapeutikum wie zum Beispiel Camptothecin oder ein Derivat davon, Bosutinib oder Methotrexat ist; oder wobei der antineoplastische Wirkstoff ein alkylierender antineoplastischer Wirkstoff wie zum Beispiel Temozolomid, Uramustin oder Bendamustin ist.

6. Verbindung nach einem der Ansprüche 1 bis 5, wobei X (i) Carbonyl; oder (ii) ein cyclisches Ketal ist, das mit 1 oder 2 Phenylgruppen substituiert ist, die jeweils unabhängig in ortho-Position zu dem Verbindungskohlenstoffatom mit $-NO_2$ substituiert sind, und gegebenenfalls ferner an irgendeiner anderen Position als in ortho-Position zu dem Verbindungskohlenstoffatom mit einer oder mehreren Gruppen substituiert sind, die jeweils unabhängig ausgewählt sind aus $-O-(C_1-C_8)$.

7. Verbindung nach Anspruch 6, wobei X ein cyclisches Ketal ist, das mit einer 4,5-Dimethoxy,2-nitrophenylgruppe substituiert ist.

8. Konjugat der Formel II:



worin:

Y ein Wirkstoff oder ein bioaktives Reagenz ist, umfassend einen heteroaromatischen Ring und verbunden über ein Kohlenstoffatom des heteroaromatischen Rings;
R_1 und R_2 jeweils unabhängig H, Halogen, $-OR_3$, $-CO-R_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, -CN, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CO-N(R_3)_2$, $-(C_1-C_8)$alkyl,
$-(C_2-C_8)$alkenyl, $-(C_2-C_8)$alkinyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl oder eine 4-bis 12-gliedrige Heterocyclylgruppe sind;
R_3 H, $-(C_1-C_{18})$alkyl, $-(C_2-C_{18})$alkenyl oder $-(C_2-C_{18})$alkinyl ist;
R_4 abwesend ist oder ausgewählt ist aus $-NH-(CH_2)_p-$, $-NH-CO-(CH_2)_p-$, $-NH-CO-NH-(CH_2)_p-$, $-NH-CO-O-(CH_2)_p-$, $-O-(CH_2)_p-$, $-O-CO-(CH_2)_p-$, $-O-CO-NH-(CH_2)_p-$ oder $-O-CO-O-(CH_2)_p-$;

R_5 ein Polymer-, Protein-, Peptid-, oder Kohlenhydratrest ist;
R_6 H, $-(CH_2)_y-OH$, $-(CH_2)_y-SH$, $-(CH_2)_y-NH_2$, $-(CH_2)_y-COOH$, $-(CH_2)_y-SO_3H$ oder ein zweiwertiger Rest ausgewählt aus $-(CH_2)_y-O-$, $-(CH_2)_y-S-$, $-(CH_2)_y-NH-$, $-(CH_2)_y-COO-$ oder $-(CH_2)_y-SO_3-$ ist;
n und m jeweils unabhängig eine ganze Zahl von 1-8 sind;
p und y jeweils unabhängig eine ganze Zahl von 0-8 sind; und
T abwesend ist oder eine Zieleinheit ist, die imstande ist, an ein extrazelluläres Antigen zu binden und gebunden ist mittels einer funktionellen Gruppe davon, unter der Voraussetzung, dass wenn T abwesend ist, R_6 keine zweiwerte Gruppe ist und wenn T eine Zieleinheit ist, R_6 eine zweiwertige Gruppe ist,

oder ein pharmazeutisch annehmbares Salz davon.

9. Konjugat nach Anspruch 8, wobei:

(i) R_1 und R_2 jeweils unabhängig H, Halogen, $-OR_3$, $-CO-R_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, -CN, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CO-N(R_3)_2$, $-(C_1-C_4)$alkyl,
$-(C_2-C_4)$alkenyl, $-(C_2-C_4)$alkinyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl oder eine 4- bis 12-gliedrige Heterocyclylgruppe sind, wobei R_3 H, Methyl, Ethyl oder Propyl ist;
(ii) n 3, 4 oder 5 ist;
(iii) m 1, 2 oder 3 ist;
(iv) R_4 $-NH-CO-(CH_2)_p-$ oder $-NH-CO-NH-(CH_2)_p-$ ist;
(v) R_5 eine Polymergruppe ist und das Polymer lineares oder verzweigtes Polyethylenglycol (PEG) oder Copolymere davon; ein Pseudo-PEG, das durch mindestens eine Gruppe unterbrochen ist, die jeweils unabhängig ausgewählt ist aus -NH-CO-, -CO-NH- oder (C_3-C_8)alkylen, das durch mindestens zwei Gruppen unterbrochen ist, die jeweils unabhängig ausgewählt sind aus -NH-CO- oder -CO-NH-, wie zum Beispiel $(-CH_2-CH_2-O-)_3-(CH_2)_3-NH-CO-(CH_2)_2-CO-NH-(CH_2)_3-(O-CH_2-CH_2-)_3$; Polymilchsäure oder Copolymere davon; Polyester ausgewählt aus Polylactid (PLA), Polyglycolid (PGA), Polycaprolacton (PLC) oder Copolymere davon; oder Polyamide basierend auf Polymethacrylamid oder Copolymere davon, ist;
(vi) R_5 eine Proteingruppe ist und das Protein Albumin, ein modifiziertes Albumin oder eine proteinhaltige globinähnliche Domäne mit einer langen Halbwertszeit im Umlauf, ist;
(vii) R_5 eine Peptidgruppe ist;
(viii) R_5 eine Kohlenhydratgruppe ist;
(ix) Y ein Antikrebswirkstoff, ein antineoplastischer Wirkstoff, ein antimykotischer Wirkstoff, ein antibakterieller Wirkstoff, ein antiviraler Wirkstoff, ein Herzmedikament, ein neurologischer Wirkstoff, ein psychoaktiver Wirkstoff, ein Missbrauchswirkstoff, ein Alkaloid, ein Antibiotikum, ein bioaktives Peptid, ein Steroid, ein Steroidhormon, ein Peptidhormon, ein Interferon, ein Interleukin, ein Narkotikum, eine Nukleinsäure, ein Pestizid oder ein Prostaglandin ist; oder
(x) die Zieleinheit ein Protein, Peptid, Polypeptid, Glykoprotein, Lipoprotein, Lipid, Phospholipid, Oligonukleotid oder ein Mimetikum davon, Steroid, Hormon, Lymphokin, Wachstumsfaktor, Albumin, Cytokin, Enzym, Coenzym, Vitamin, Cofaktor, menschliches Antigen, Hapten, Rezeptorprotein, Antikörper oder ein Fragment davon, eine Substanz, die so verwendet oder modifiziert ist, dass sie als Zieleinheit fungiert, oder eine Kombination davon, bevorzugt ein Vitamin wie zum Beispiel Vitamin B9 (Folsäure), ist.

10. Konjugat nach Anspruch 9, wobei der Antikrebswirkstoff ein chemotherapeutischer Wirkstoff wie zum Beispiel Camptothecin oder ein Derivat davon, Bosutinib oder Methotrexat ist; oder der antineoplastische Wirkstoff ein alkylierendes antineoplastisches Mittel wie zum Beispiel Temozolomid, Uramustin oder Bendamustin ist.

11. Konjugat nach einem der Ansprüche 8 bis 10, wobei R_1 und R_2 jeweils unabhängig voneinander H, Halogen, $-OR_3$, $-CO-R_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, -CN, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CO-N(R_3)_2$, -(C1-C4)-alkyl, -(C2-C4)-alkenyl, $-(C_2-C_4)$-alkinyl, (C_3-C_{10})cycloalkyl, (C_6-C_{14})aryl oder eine 4-bis 12-gliedrige Heterocyclylgruppe sind, wobei R_3 H, Methyl, Ethyl oder Propyl ist; n 3, 4 oder 5 ist; m 1, 2 oder 3 ist; R_4 $-NH-CO-(CH_2)_p-$ oder $-NH-CO-NH-(CH_2)_p-$ ist; und R_5 eine PEG-Einheit oder eine Pseudo-PEG-Einheit unterbrochen durch mindestens eine Gruppe, die jeweils unabhängig ausgewählt ist aus -NH-CO-, -CO-NH- oder (C_3-C_8)alkylen, das durch mindestens zwei Gruppen unterbrochen ist, die jeweils unabhängig ausgewählt sind aus -NH-CO- oder-CO-NH-.

12. Konjugat nach Anspruch 11, worin R_1 und R_2 H sind; n 3 ist; m 1 ist; und R_5 eine PEG-Einheit oder eine Pseudo-PEG-Einheit mit der Struktur $(-CH_2-CH_2-O)_3-(CH_2)_3-NH-CO-(CH_2)_2-CO-NH-(CH_2)_3-(O-CH_2-CH_2-)_3$ ist.

13. Konjugat nach Anspruch 12, worin (i) R_6 ein zweiwertiger Rest ist; oder (ii) R_6 H, $-(CH_2)_y-OH$, $-(CH_2)_y-SH$,

$-(CH_2)_y-NH_2$, $-(CH_2)_y-COOH$ oder $-(CH_2)_y-SO_3H$ ist, vorzugsweise $-(CH_2)_y-SH$, $-(CH_2)_y-NH_2$ oder $-(CH_2)_y-COOH$, vorzugsweise worin y 1, 2 oder 3 ist; und T fehlt.

14. Konjugat nach Anspruch 13, worin R_6 $-(CH_2)_y-NH-$ ist, bevorzugt worin y 1 oder 2 ist; und die Zieleinheit Folsäure ist, die über eine Carboxylgruppe davon, vorzugsweise über ihre gamma-Carboxylgruppe, gebunden ist.

15. Konjugat nach Anspruch 14, wobei (i) R_4 $-NH-CO-NH-CH_2-$ ist; R_5 eine Pseudo-PEG-Einheit mit der Struktur $(-CH_2-CH_2-O)_3-(CH_2)_3-NH-CO-(CH_2)_2-CO-NH-(CH_2)_3-(O-CH_2-CH_2-)_3$ ist; R_6 $-(CH_2)-NH-$ ist; und Y Camptothecin, 10-Hydroxycamptothecin, Temozolomid, Bosutinib oder Methotrexat ist; (ii) R_4 $-NH-CO-CH_2$ ist; R_5 eine Pseudo-PEG-Einheit mit der Struktur $(-CH_2-CH_2-O)_3-(CH_2)_3-NH-CO-(CH_2)_2-CO-NH-(CH_2)_3-(O-CH_2-CH_2-)_3$ ist; R_6 $-(CH_2)-NH-$ ist; und Y Camptothecin, 10-Hydroxycamptothecin, Temozolomid, Bosutinib oder Methotrexat ist; oder (iii) R_4 $-NH-CO-$ ist; R_5 eine PEG-Einheit mit der Struktur $(-CH_2-CH_2-O)_3-$ ist; R_6 $-(CH_2)_2-NH-$ ist; und Y Camptothecin, 10-Hydroxycamptothecin, Temozolomid, Bosutinib oder Methotrexat ist.

16. Pharmazeutische Zusammensetzung, umfassend eine Verbindung nach einem der Ansprüche 1 bis 7, worin X nicht Carbonyl ist; oder ein Konjugat nach einem der Ansprüche 8 bis 15 oder ein pharmazeutisch annehmbares Salz davon und ein pharmazeutisch annehmbarer Träger.

17. Konjugat nach einem der Ansprüche 8 bis 15 oder ein pharmazeutisch annehmbares Salz davon, wobei Y ein Antikrebswirkstoff oder ein antineoplastischer Wirkstoff ist und T eine Zielgruppe ist, die in der Lage ist, an ein extrazelluläres Antigen zu binden, das auf den Zellen des Krebses vorhanden ist, zur Verwendung bei der Behandlung von Krebs.

Revendications

1. Composé de formule I :

$$Y-CF_2-(CR_1R_2)_n-X-(CR_1R_1)_m-R_1 \qquad (I)$$

dans laquelle

Y est un médicament ou un réactif bioactif, comprenant un cycle hétéroaromatique, et lié via un atome de carbone dudit cycle hétéroaromatique ;
X est un carbonyle, ou un cétal cyclique substitué par 1 à 4 groupes qui sont chacun indépendamment phényle ou naphtyle substitué en ortho par rapport au carbone de rattachement par $-NO_2$, et éventuellement encore substitué en n'importe quelle position autre qu'en ortho par rapport au carbone de rattachement par un ou plusieurs groupes, chacun indépendamment choisi parmi $-O-(C_1-C_8)$, -alkyle en C_1 à C_8, $-N(R')_2$, ou halogène, où chaque R' est indépendamment -alkyle en C_1 à C_8 ou H ;
chacun de R_1 et R_2 est indépendamment H, halogène, $-OR_3$, $-CO-R_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, $-CN$, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CO-N(R_3)_2$, - alkyle en C_1 à C_8, -alcényle en C_2 à C_8, -alcynyle en C_2 à C_8, cycloalkyle en C_3 à C_{10}, aryle en C_6 à C_{14}, ou hétérocyclyle à 4 à 12 chaînons ;
R_3 est H, -alkyle en C_1 à C_{18}, -alcényle en C_2 à C_{18}, ou -alcynyle en C_2 à C_{18} ; et
chacun de n et m est indépendamment un entier de 1 à 8,

ou un sel pharmaceutiquement acceptable de celui-ci.

2. Composé selon la revendication 1, dans lequel :

(i) chacun de R_1 et R_2 est indépendamment H, un halogène, $-OR_3$, $-COR_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, $-CN$, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CON(R_3)_2$, -alkyle en C_1 à C_4, -alcényle en C_2 à C_4, -alcynyle en C_2 à C_4, cycloalkyle en C_3 à C_{10}, aryle en C_6 à C_{14}, ou hétérocyclyle à 4 à 12 chaînons, où R_3 est H, méthyle, éthyle ou propyle ;
(ii) n vaut 3, 4 ou 5 ; ou
(iii) m vaut 1, 2 ou 3.

3. Composé selon la revendication 1, dans lequel chacun de R_1 et R_2 est indépendamment H, un halogène, $-OR_3$, $-CO-R_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, $-CN$, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CO-N(R_3)_2$, -alkyle en C_1 à C_4, - alcényle en C_2 à C_4, -alcynyle en C_2 à C_4, cycloalkyle en C_3 à C_{10}, aryle en C_6 à C_{14}, ou hétérocyclyle à 4 à 12 chaînons,

où R_3 est H, méthyle, éthyle ou propyle ; n vaut 3, 4 ou 5 ; et m vaut 1, 2 ou 3, de préférence dans lequel chacun de R_1 et R_2 est H.

4. Composé selon la revendication 1, dans lequel Y est un médicament anticancéreux, un médicament antinéoplasique, un médicament antifongique, un médicament antibactérien, un médicament antiviral, un médicament cardiaque, un médicament neurologique, un médicament psychoactif, une drogue addictive, un alcaloïde, un antibiotique, un peptide bioactif, un stéroïde, une hormone stéroïdienne, une hormone peptidique, un interféron, une interleukine, un narcotique, un acide nucléique, un pesticide, ou une prostaglandine.

5. Composé selon la revendication 4, dans lequel ledit médicament anticancéreux est un médicament chimiothérapeutique tel que la camptothécine ou un dérivé de celle-ci, le bosutinib, ou le méthotrexate ; ou ledit médicament antinéoplasique est un agent antinéoplasique d'alkylation tel que le témozolomide, l'uramustine ou la bendamustine.

6. Composé selon l'une quelconque des revendications 1 à 5, dans lequel X est (i) carbonyle ; ou (ii) un cétal cyclique substitué par 1 ou 2 groupes phényle, chacun indépendamment substitué en ortho par rapport au carbone de rattachement par $-NO_2$, et éventuellement encore substitué en n'importe quelle position autre qu'ortho par rapport au carbone de rattachement par un ou plusieurs groupes, chacun indépendamment choisi parmi $-O-(C_1-C_8)$.

7. Composé selon la revendication 6, dans lequel X est un cétal cyclique substitué par un groupe 4,5-diméthoxy,2-nitrophényle.

8. Conjugué de formule II :

$$\begin{array}{l} (CR_1R_2)_m–R_1 \\ \quad | \\ Y–CF_2–(CR_1R_2)_n–C=N–R_4–R_5–R_6–T \end{array} \qquad (II)$$

dans laquelle

Y est un médicament ou un réactif bioactif, comprenant un cycle hétéroaromatique, et lié via un atome de carbone dudit cycle hétéroaromatique ;
chacun de R_1 et R_2 est indépendamment H, un halogène, $-OR_3$, $-COR_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, $-CN$, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CON(R_3)_2$, -alkyle en C_1 à C_8, -alcényle en C_2 à C_8, -alcynyle en C_2 à C_8, cycloalkyle en C_3 à C_{10}, aryle en C_6 à C_{14}, ou hétérocyclyle à 4 à 12 chaînons ;
R_3 est H, -alkyle en C_1 à C_{18}, -alcényle en C_2 à C_{18}, ou -alcynyle en C_2 à C_{18} ;
R_4 est absent ou est choisi parmi $-NH-(CH_2)_p-$, $-NH-CO-(CH_2)_p-$, $-NH-CO-NH-(CH_2)_p-$, $-NH-CO-O-(CH_2)_p-$, $-O-(CH_2)_p-$, $-O-CO-(CH_2)_p-$, $-O-CO-NH-(CH_2)_p-$, ou $-O-CO-O-(CH_2)_p-$;
R_5 est un fragment de polymère, protéine, peptide, ou hydrate de carbone ;
R_6 est H, $-(CH_2)_y-OH$, $-(CH_2)_y-SH$, $-(CH_2)_y-NH_2$, $-(CH_2)_y-COOH$, $-(CH_2)_y-SO_3H$, ou un radical divalent choisi parmi $-(CH_2)_y-O-$, $-(CH_2)_y-S-$, $-(CH_2)_y-NH-$, $-(CH_2)_y-COO-$ ou $-(CH_2)_y-SO_3-$;
chacun de n et m est indépendamment un entier de 1 à 8 ;
chacun de p et y est indépendamment un entier de 0 à 8 ; et
T est absent ou est un fragment de ciblage capable de se lier à un antigène extracellulaire, et est lié via un groupe fonctionnel de celui-ci, sous réserve que, lorsque T est absent, R_6 ne soit pas un radical divalent, et lorsque T est un fragment de ciblage, R_6 soit un radical divalent,

ou un sel pharmaceutiquement acceptable de celui-ci.

9. Conjugué selon la revendication 8, dans lequel :

(i) chacun de R_1 et R_2 est indépendamment H, un halogène, $-OR_3$, $-COR_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, $-CN$, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CON(R_3)_2$, -alkyle en C_1 à C_4, -alcényle en C_2 à C_4, -alcynyle en C_2 à C_4, cycloalkyle en C_3 à C_{10}, aryle en C_6 à C_{14}, ou hétérocyclyle à 4 à 12 chaînons, où R_3 est H, méthyle, éthyle ou propyle ;
(ii) n vaut 3, 4 ou 5 ;
(iii) m vaut 1, 2 ou 3 ;
(iv) R_4 est $-NH-CO-(CH_2)_p-$ ou $-NH-CO-NH-(CH_2)_p-$;

(v) R_5 est un fragment de polymère, et ledit polymère est un polyéthylèneglycol (PEG) linéaire ou ramifié ou ses copolymères ; un pseudo-PEG interrompu par au moins un groupe, chacun indépendamment choisi parmi -NH-CO-, -CO-NH-, ou alkylène en C_3 à C_8 interrompu par au moins deux groupes, chacun indépendamment choisi parmi -NH-CO- ou -CO-NH-, tel que $(-CH_2-CH_2-O)_3-(CH_2)_3-NH-CO-(CH_2)_2-CO-NH-(CH_2)_3-(O-CH_2-CH_2-)_3$; le poly(acide lactique) ou ses copolymères ; les polyesters choisis parmi le polylactide (PLA), le polyglycolide (PGA), la polycaprolactone (PCL) et leurs copolymères ; ou les polyamides à base de polyméthacrylamide ou leurs copolymères ;
(vi) R_5 est un fragment de protéine, et ladite protéine est l'albumine, une albumine modifiée, ou une protéine contenant des domaines de type globine ayant une longue demi-vie en circulation ;
(vii) R_5 est un fragment de peptide ;
(viii) R_5 est un fragment d'hydrate de carbone ;
(ix) Y est un médicament anticancéreux, un médicament antinéoplasique, un médicament antifongique, un médicament antibactérien, un médicament antiviral, un médicament cardiaque, un médicament neurologique, un médicament psychoactif, une drogue addictive, un alcaloïde, un antibiotique, un peptide bioactif, un stéroïde, une hormone stéroïdienne, une hormone peptidique, un interféron, une interleukine, un narcotique, un acide nucléique, un pesticide, ou une prostaglandine ; ou
(x) ledit fragment de ciblage est une protéine, un peptide, un polypeptide, une glycoprotéine, une lipoprotéine, un lipide, un phospholipide, un oligonucléotide ou un imitateur de celui-ci, un stéroïde, une hormone, une lymphokine, un facteur de croissance, l'albumine, une cytokine, une enzyme, une coenzyme, une vitamine, un cofacteur, un antigène humain, un haptène, une protéine de récepteur, un anticorps ou un fragment de celui-ci, une substance utilisée ou modifiée de façon à fonctionner comme un fragment de ciblage, ou une combinaison de ceux-ci, de préférence une vitamine telle que la vitamine B9 (acide folique).

10. Conjugué selon la revendication 9, dans lequel ledit médicament anticancéreux est un médicament chimiothérapeutique tel que la camptothécine ou un dérivé de celle-ci, le bosutinib, ou le méthotrexate ; ou ledit médicament antinéoplasique est un agent antinéoplasique d'alkylation tel que le témozolomide, l'uramustine ou la bendamustine.

11. Conjugué selon l'une quelconque des revendications 8 à 10, dans lequel chacun de R_1 et R_2 est indépendamment H, un halogène, $-OR_3$, $-CO-R_3$, $-CO-OR_3$, $-OCO-OR_3$, $-OCO-N(R_3)_2$, -CN, $-NO_2$, $-SR_3$, $-N(R_3)_2$, $-CO-N(R_3)_2$, - alkyle en C_1 à C_4, -alcényle en C_2 à C_4, -alcynyle en C_2 à C_4, cycloalkyle en C_3 à C_{10}, aryle en C_6 à C_{14}, ou hétérocyclyle à 4 à 12 chaînons, où R_3 est H, méthyle, éthyle ou propyle ; n vaut 3, 4 ou 5 ; m vaut 1, 2 ou 3 ; R_4 est $-NH-CO-(CH_2)_p-$ou $-NH-CO-NH-(CH_2)_p-$; et R_5 est un fragment PEG ou un fragment pseudo-PEG interrompu par au moins un groupe, chacun indépendamment choisi parmi -NH-CO-, -CO-NH-, ou alkylène en C_3 à C_8 interrompu par au moins deux groupes, chacun indépendamment choisi parmi -NH-CO- ou -CO-NH-.

12. Conjugué selon la revendication 11, dans lequel R_1 et R_2 sont H ; n vaut 3 ; m vaut 1 ; et R_5 est un fragment PEG ou un fragment pseudo-PEG de structure $(-CH_2-CH_2-O)_3-(CH_2)_3-NH-CO-(CH_2)_2-CO-NH-(CH_2)_3-(O-CH_2-CH_2-)_3$.

13. Conjugué selon la revendication 12, dans lequel (i) R_6 est un radical divalent ; ou (ii) R_6 est H, $-(CH_2)_y-OH$, $-(CH_2)_y-SH$, $-(CH_2)_y-NH_2$, $-(CH_2)_y-COOH$ ou $-(CH_2)_y-SO_3H$, de préférence $-(CH_2)_y-SH$, $-(CH_2)_y-NH_2$ ou $-(CH_2)_y-COOH$, de préférence où y vaut 1, 2 ou 3 ; et T est absent.

14. Conjugué selon la revendication 13, dans lequel R_6 est $-(CH_2)_y-NH-$, de préférence où y vaut 1 ou 2 ; et ledit fragment de ciblage est l'acide folique lié via un groupe carboxylique de celui-ci, de préférence par l'intermédiaire de son groupe gamma-carboxyle.

15. Conjugué selon la revendication 14, dans lequel (i) R_4 est $-NH-CO-NH-CH_2-$; R_5 est un fragment pseudo-PEG de structure $(-CH_2-CH_2-O)_3-(CH_2)_3-NH-CO-(CH_2)_2-CO-NH-(CH_2)_3-(O-CH_2-CH_2-)_3$; R_6 est $-(CH_2)-NH$; et Y est la camptothécine, la 10-hydroxycamptothécine, le témozolomide, le bosutinib, ou le méthotrexate ; (ii) R_4 est $-NH-CO-CH_2-$; R_5 est un fragment pseudo-PEG de structure $(-CH_2-CH_2-O)_3-(CH_2)_3-NH-CO-(CH_2)_2-CO-NH-(CH_2)_3-(O-CH_2-CH_2-)_3$; R_6 est $-(CH_2)-NH-$; et Y est la camptothécine, la 10-hydroxycamptothécine, le témozolomide, le bosutinib, ou le méthotrexate ; ou (iii) R_4 est -NH-CO- ; R_5 est un fragment PEG de structure $(-CH_2-CH_2-O)_3-$; R_6 est $-(CH_2)-NH$; et Y est la camptothécine, la 10-hydroxycamptothécine, le témozolomide, le bosutinib, ou le méthotrexate.

16. Composition pharmaceutique comprenant un composé de l'une quelconque des revendications 1 à 7 dans lequel X n'est pas carbonyle ; ou un conjugué de l'une quelconque des revendications 8 à 15, ou un sel pharmaceutiquement acceptable de celui-ci, et un véhicule pharmaceutiquement acceptable.

17. Conjugué selon l'une quelconque des revendications 8 à 15, ou un sel pharmaceutiquement acceptable de celui-ci, dans lequel Y est un médicament anticancéreux ou un médicament antinéoplasique, et T est un fragment de ciblage capable de se lier à un antigène extracellulaire présent sur les cellules dudit cancer, pour une utilisation dans le traitement d'un cancer.

Fig. 1

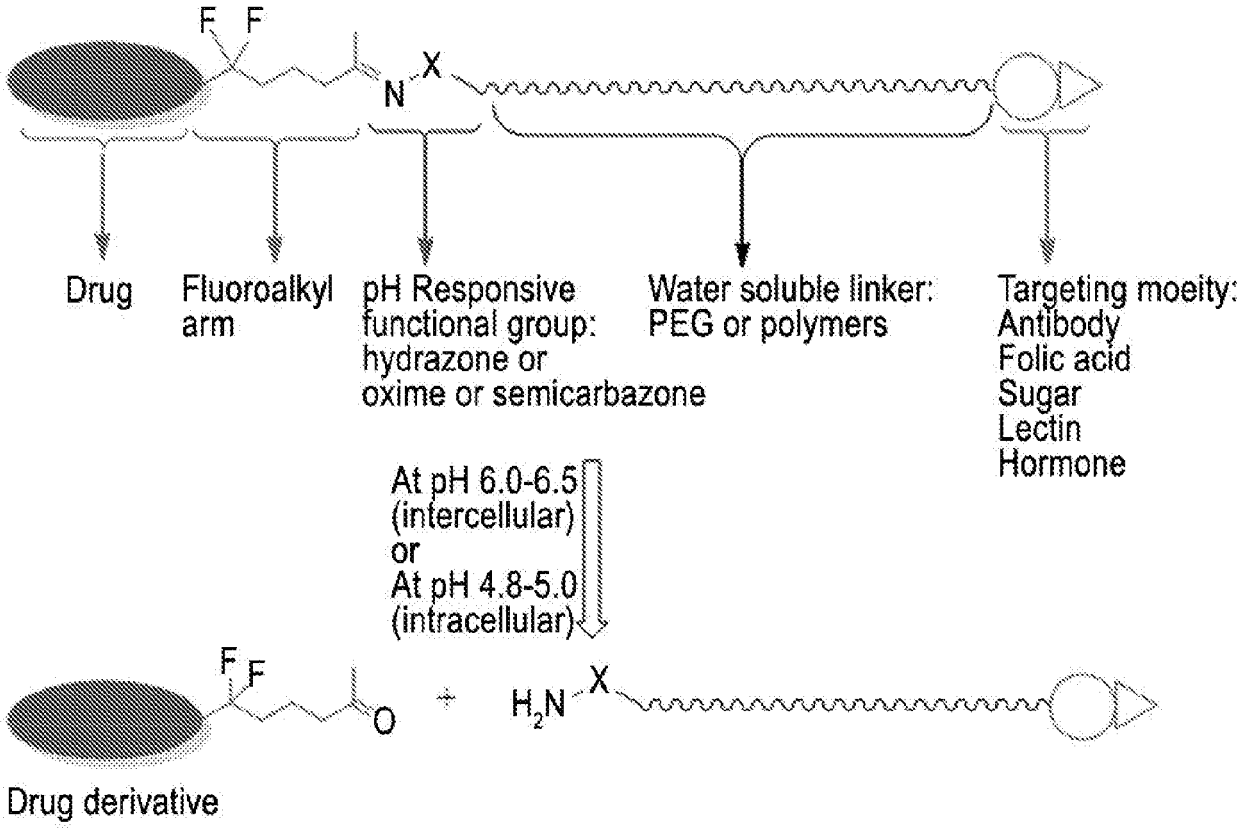

F F
X
N
Drug
Fluoroalkyl arm
pH Responsive functional group: hydrazone or oxime or semicarbazone
Water soluble linker: PEG or polymers
Targeting moeity: Antibody Folic acid Sugar Lectin Hormone
At pH 6.0-6.5 (intercellular) or At pH 4.8-5.0 (intracellular)
F F
O
+
H_2N
X
Drug derivative

Fig. 2A

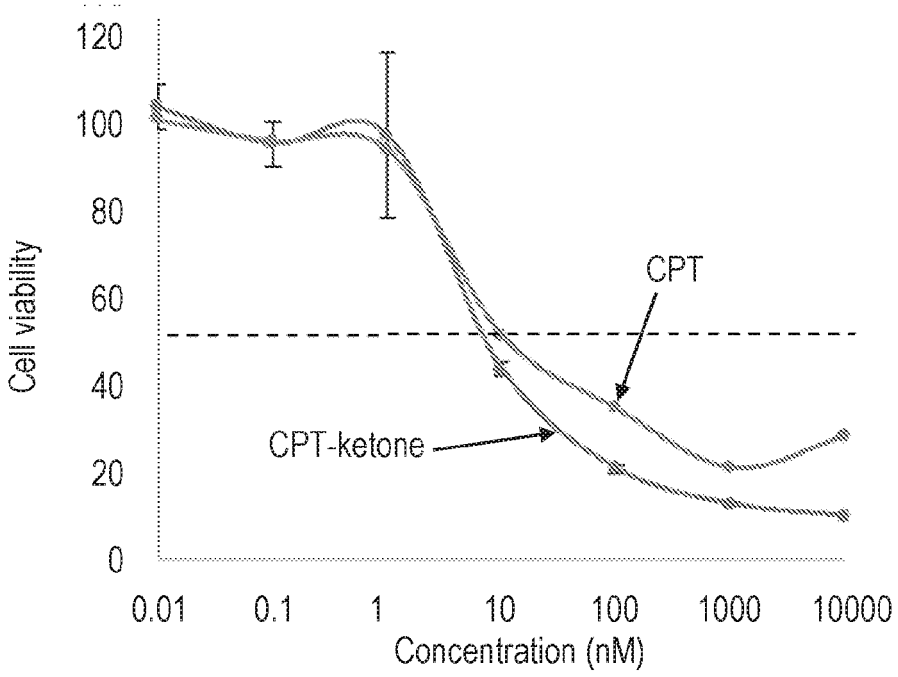

120
100
80
60
40
20
0
Cell viability
CPT
CPT-ketone
0.01
0.1
1
10
100
1000
10000
Concentration (nM)

Fig. 2B

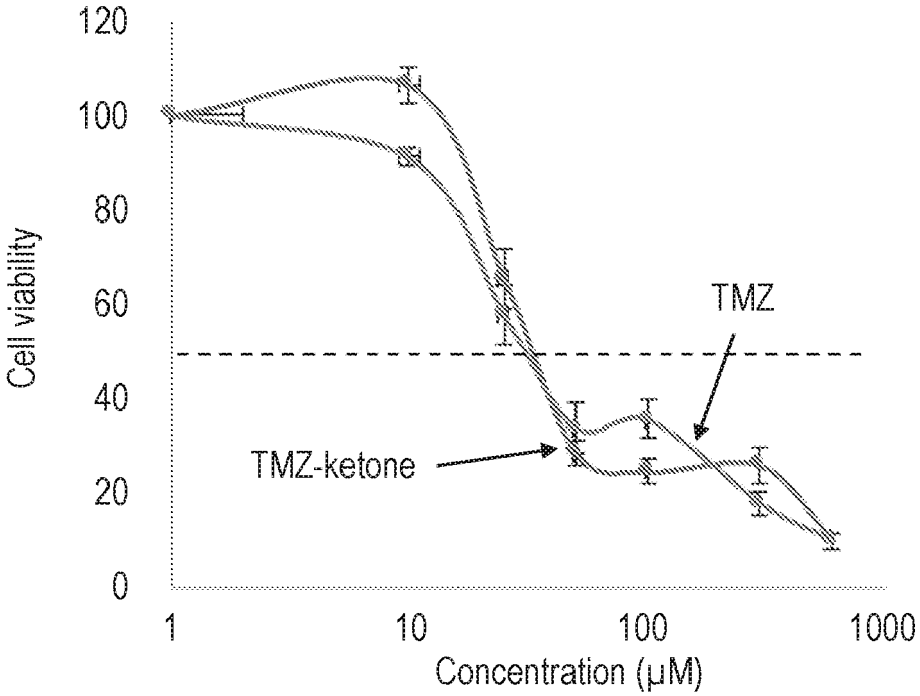

120
100
80
60
40
20
0
Cell viability
TMZ
TMZ-ketone
1
10
100
1000
Concentration (μM)

Fig. 2C

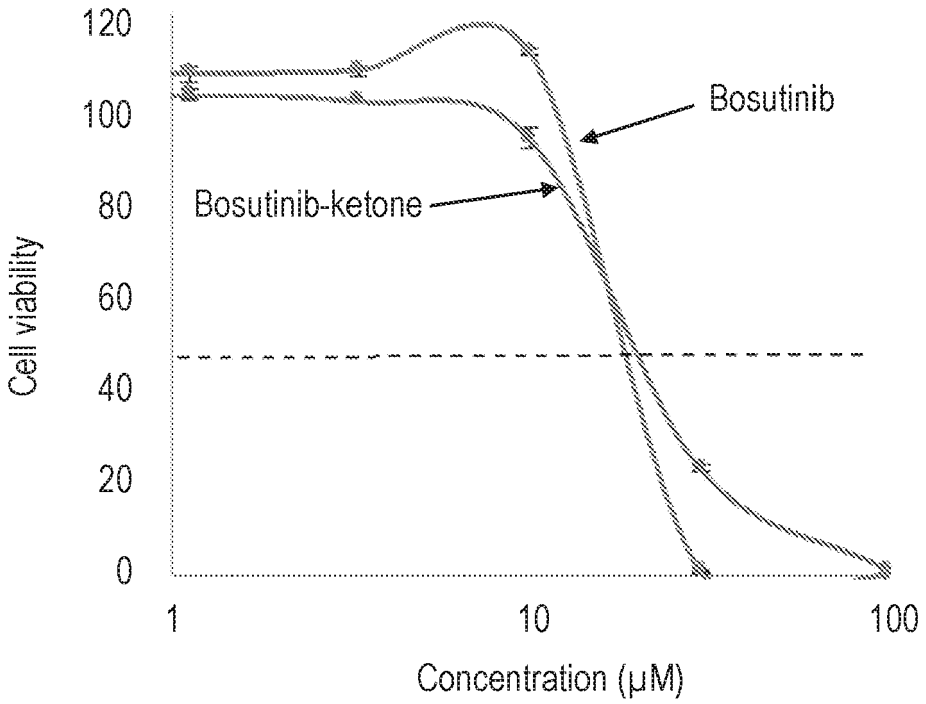

120
100
80
60
40
20
0
Cell viability
Bosutinib
Bosutinib-ketone
1
10
100
Concentration (μM)

Fig. 3

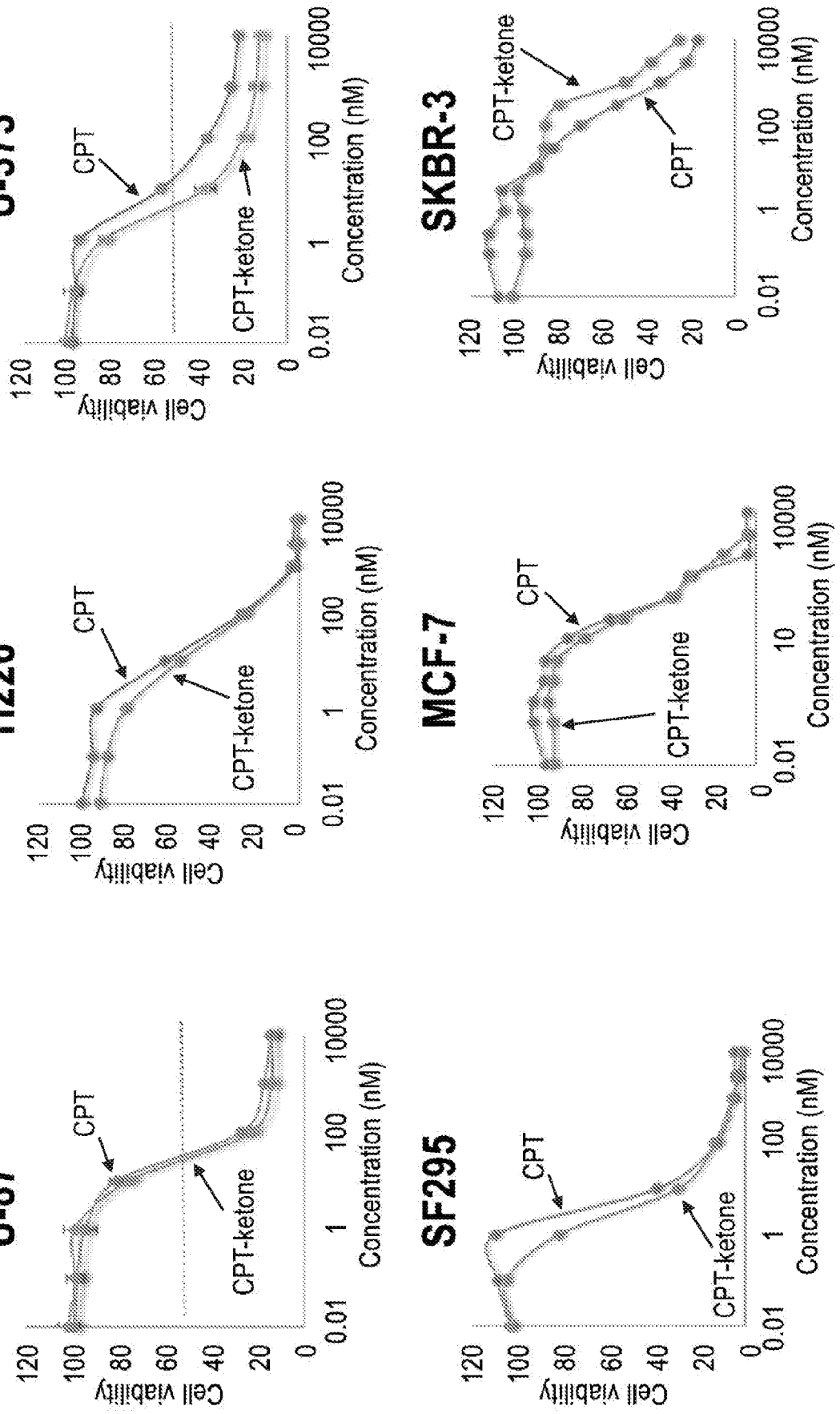

U-87
H226
U-373
SF295
MCF-7
SKBR-3
CPT
CPT-ketone
Cell viability
Concentration (nM)
0
20
40
60
80
100
120
0.01
1
100
10000
10

Fig. 4

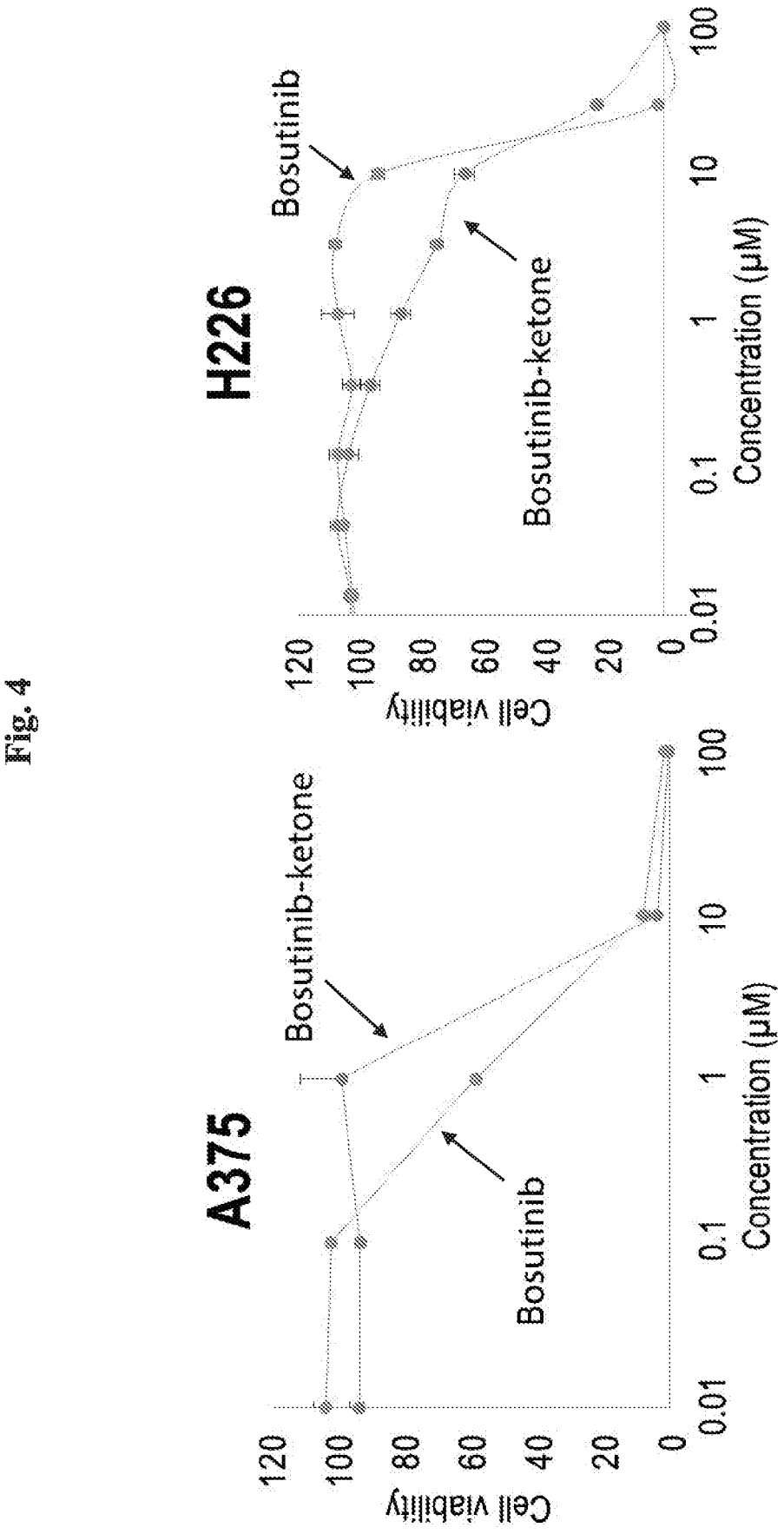

A375
H226
Cell viability
Concentration (μM)
Bosutinib
Bosutinib-ketone
0
20
40
60
80
100
120
0.01
0.1
1
10
100

Fig. 5A

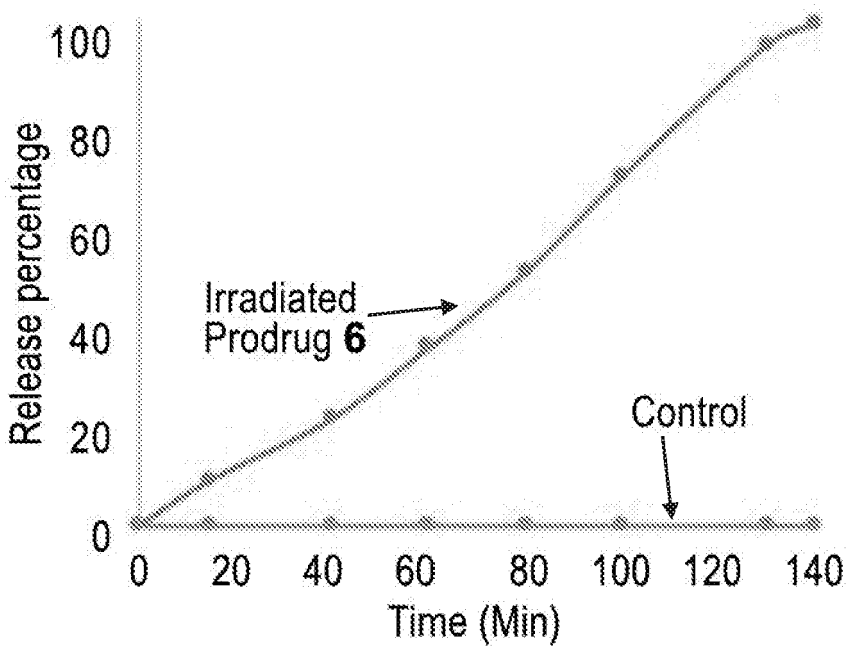

100
80
60
40
20
0
Release percentage
Irradiated
Prodrug 6
Control
0
20
40
60
80
100
120
140
Time (Min)

Fig. 5B

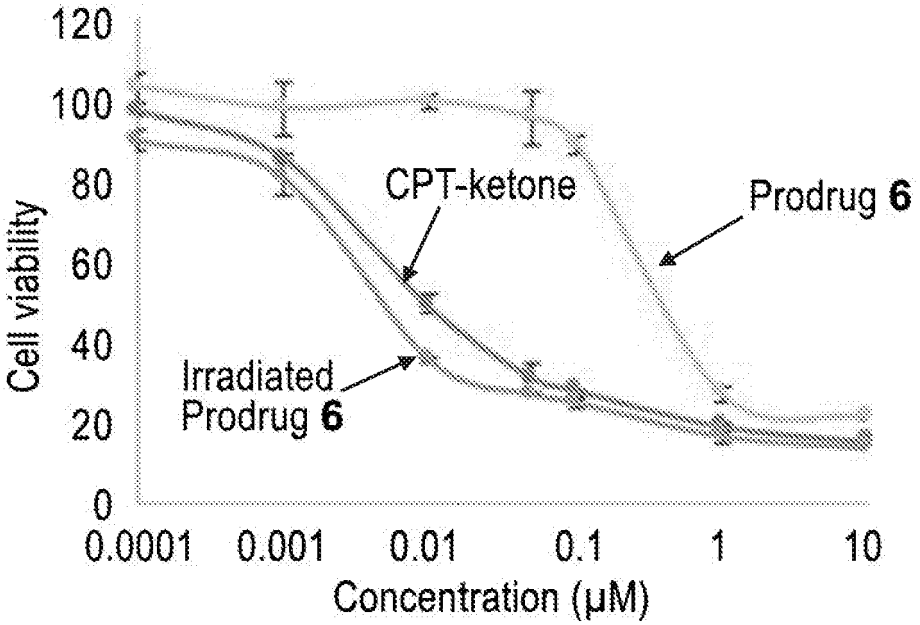

120
100
80
60
40
20
0
Cell viability
CPT-ketone
Prodrug 6
Irradiated
Prodrug 6
0.0001
0.001
0.01
0.1
1
10
Concentration (μM)

Fig. 6

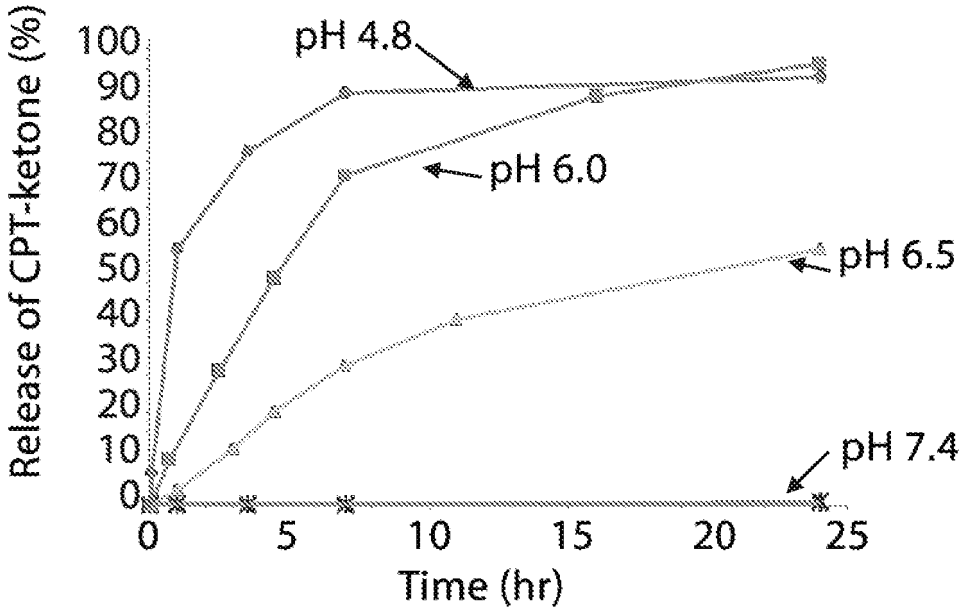

Release of CPT-ketone (%)
100
90
80
70
60
50
40
30
20
10
0
pH 4.8
pH 6.0
pH 6.5
pH 7.4
0
5
10
15
20
25
Time (hr)

Fig. 7A

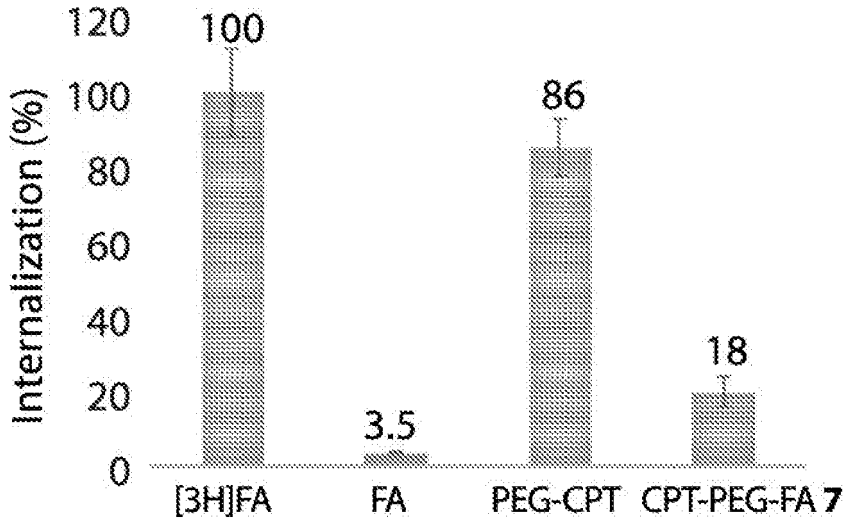
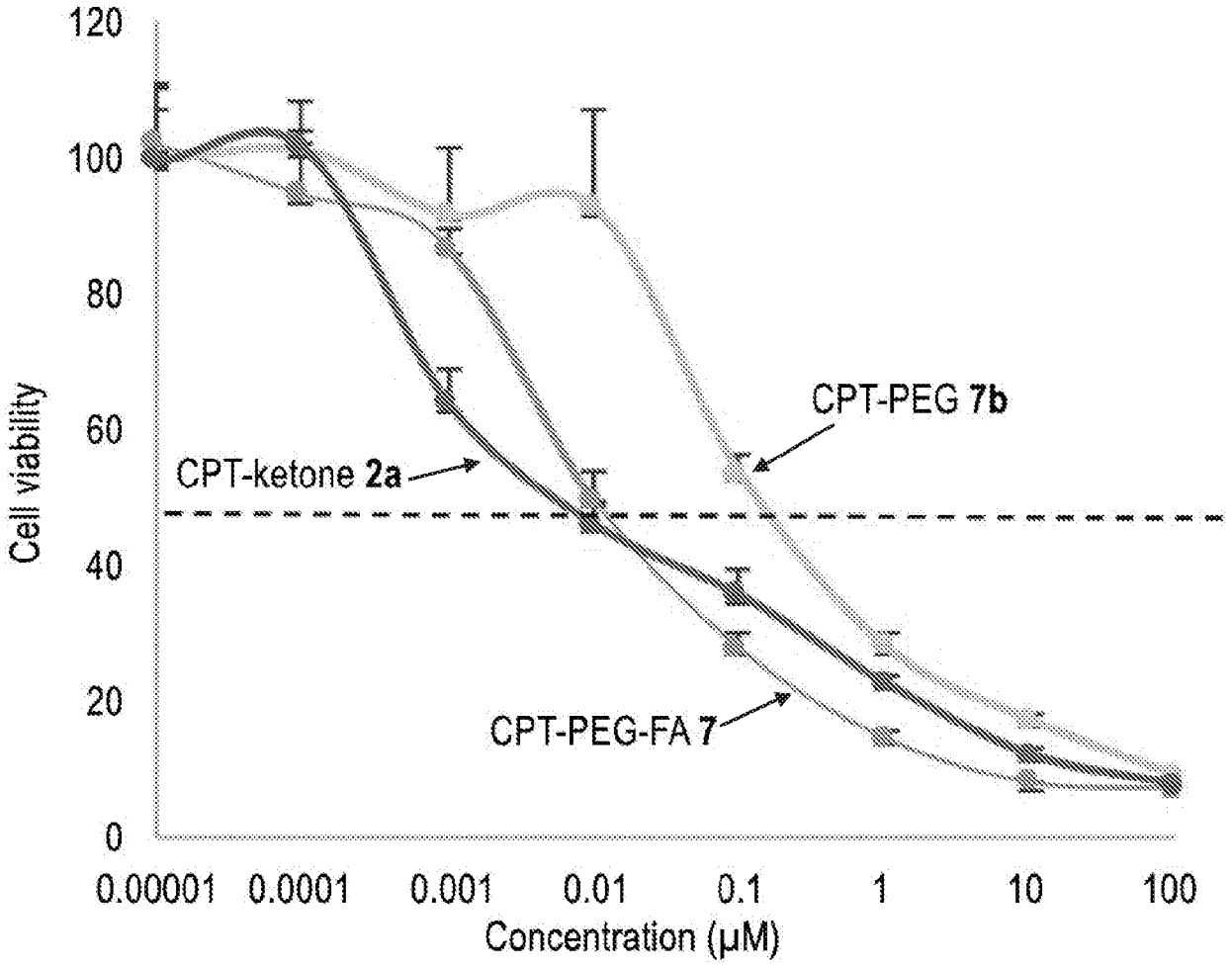

Internalization (%)
120
100
80
60
40
20
0
100
3.5
86
18
[3H]FA
FA
PEG-CPT
CPT-PEG-FA 7

Fig. 7B

Compound	IC_{50}(nM)
CPT-ketone **2a**	5
CPT-PEG-FA **7**	10
CPT-PEG **7b**	150



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