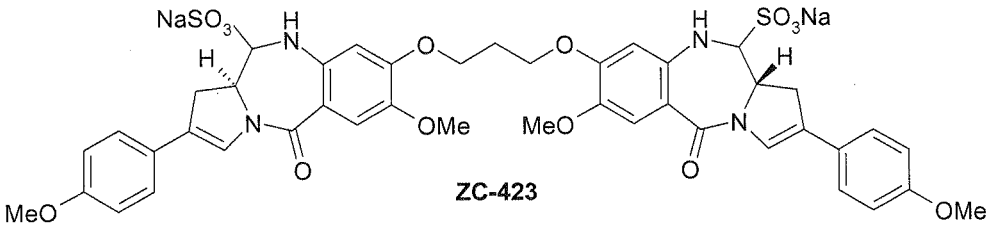


ZC-423

These compounds have been shown to be highly useful cytotoxic agents (Howard, P.W., *et al.*, *Bioorg. Med. Chem.* (2009), 19 (22), 6463-6466, doi: 10.1016/j.bmcl.2009.09.012).

Due to the manner in which these highly potent compounds act in cross-linking DNA, these molecules have been made symmetrically. This provides for straightforward synthesis, either by constructing the PBD moieties simultaneously having already formed the dimer linkage, or by reacting already constructed PBD moieties with the dimer linking group.

WO 2010/043880 discloses unsymmetrical dimeric PBD compound bearing aryl groups in the C2 position of each monomer, where one of these groups bears a substituent designed to provide an anchor for linking the compound to another moiety. Co-pending International application PCT/US2011/032664, filed 15 April 2011, published as WO 2011/130613, discloses the inclusion of these compounds in targeted conjugates. Co-pending International application PCT/US2011/032668, filed 15 April 2011, published as WO 2011/130616, discloses unsymmetrical dimeric PBD compound bearing an aryl group in the C2 position of one monomer bearing a substituent designed to provide an anchor for linking the compound to another moiety, the other monomer bearing a non-aromatic group in the C2 position. The inclusion of these compounds in targeted conjugates is also disclosed.

Disclosure of the invention

Summary

The present inventors have developed further unsymmetrical dimeric PBD compounds for inclusion in targeted conjugates, eliminating the need for an aryl group bearing an anchor. The C2 group bearing a substituent designed to provide an anchor for linking the compound to another moiety in the present ~~invention~~ disclosure is a propargyl group.

In a first aspect, t~~T~~he present ~~invention comprises~~ disclosure provides a compound with the formula I:

selected from C_{1-4} alkyl amido and C_{1-4} alkyl ester; or, when one of R^{26a} and R^{26b} is H, the other is selected from nitrile and a C_{1-4} alkyl ester;

R^6 and R^9 are independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', nitro, Me_3Sn and halo;

where R and R' are independently selected from optionally substituted C_{1-12} alkyl, C_{3-20} heterocyclyl and C_{5-20} aryl groups;

R^7 is selected from H, R, OH, OR, SH, SR, NH_2, NHR, NHRR', nitro, Me_3Sn and halo;

either:

(a) R^{10} is H, and R^{11} is OH, OR^A, where R^A is C_{1-4} alkyl;

(b) R^{10} and R^{11} form a nitrogen-carbon double bond between the nitrogen and carbon atoms to which they are bound; or

(c) R^{10} is H and R^{11} is SO_zM, where z is 2 or 3 and M is a monovalent pharmaceutically acceptable cation;

R'' is a C_{3-12} alkylene group, which chain may be interrupted by one or more heteroatoms, e.g. O, S, NR^{N2} (where R^{N2} is H or C_{1-4} alkyl), and/or aromatic rings, e.g. benzene or pyridine;

Y and Y' are selected from O, S, or NH;

$R^{6'}$, $R^{7'}$, $R^{9'}$ are selected from the same groups as R^6, R^7 and R^9 respectively and $R^{10'}$ and $R^{11'}$ are the same as R^{10} and R^{11}, wherein if R^{11} and $R^{11'}$ are SO_zM, M may represent a divalent pharmaceutically acceptable cation.

A second aspect of the present ~~invention~~ disclosure provides the use of a compound of the first aspect of the ~~invention~~ present disclosure in the manufacture of a medicament for treating a proliferative disease. The second aspect also provides a compound of the first aspect of the ~~invention~~ present disclosure for use in the treatment of a proliferative disease.

One of ordinary skill in the art is readily able to determine whether or not a candidate conjugate treats a proliferative condition for any particular cell type. For example, assays which may conveniently be used to assess the activity offered by a particular compound are described in the examples below.

A third aspect of the present ~~invention comprises~~ disclosure provides a compound of formula **II**:

selected from C_{1-4} alkyl amido and C_{1-4} alkyl ester; or, when one of R^{26a} and R^{26b} is H, the other is selected from nitrile and a C_{1-4} alkyl ester;

R^6 and R^9 are independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', nitro, Me_3Sn and halo;

where R and R' are independently selected from optionally substituted C_{1-12} alkyl, C_{3-20} heterocyclyl and C_{5-20} aryl groups;

R^7 is selected from H, R, OH, OR, SH, SR, NH_2, NHR, NHRR', nitro, Me_3Sn and halo;

either:

(a) R^{10} is carbamate nitrogen protecting group, and R^{11} is O-$Prot^O$, wherein $Prot^O$ is an oxygen protecting group;

(b) R^{10} is a hemi-aminal nitrogen protecting group and R^{11} is an oxo group;

R'' is a C_{3-12} alkylene group, which chain may be interrupted by one or more heteroatoms, e.g. O, S, NR^{N2} (where R^{N2} is H or C_{1-4} alkyl), and/or aromatic rings, e.g. benzene or pyridine;

Y and Y' are selected from O, S, or NH;

$R^{6'}$, $R^{7'}$, $R^{9'}$ are selected from the same groups as R^6, R^7 and R^9 respectively and $R^{10'}$ and $R^{11'}$ are the same as R^{10} and R^{11}.

An ~~fourth aspect~~ embodiment of the present ~~invention~~ disclosure comprises a method of making a compound of formula I from a compound of formula II by deprotection of the imine bond.

The unsymmetrical dimeric PBD compounds of the present ~~invention~~ disclosure are made by different strategies to those previously employed in making symmetrical dimeric PBD compounds. In particular, the present inventors have developed a method which involves adding each each C2 substituent to a symmetrical PBD dimer core in separate method steps. Accordingly, an ~~fifth aspect~~ embodiment of the present ~~invention~~ disclosure provides a method of making a compound of the first or third aspect of the ~~invention~~present disclosure, comprising at least one of the method steps set out below.

In an ~~sixth aspect~~embodiment, the present ~~invention~~ disclosure relates to Conjugates comprising dimers of PBDs linked to a targeting agent, wherein the PBD is a dimer of formula I (supra).

In some embodiments, the Conjugates have the following formula III:

$$L - (LU-D)_p \qquad (III)$$

wherein L is a Ligand unit (i.e., a targeting agent), LU is a Linker unit and D is a Drug unit that is a PBD dimer (see below). The subscript p is an integer of from 1 to 20. Accordingly, the Conjugates comprise a Ligand unit covalently linked to at least one Drug unit by a Linker unit. In an aspect, there is provided a Conjugate having formula III: L - $(LU-D)_p$ (III), wherein L is a Ligand unit, LU is a Linker unit, p is 1 to 20; and D is a Drug unit which is a PBD dimer as disclosed herein, wherein LU is connected to D via the Q substituent of R^2. The Ligand unit, described more fully below, is a targeting agent that binds to a target moiety. The Ligand unit can, for example, specifically bind to a cell component (a Cell Binding Agent) or to other target molecules of interest. Accordingly, the present ~~invention~~ disclosure also provides methods for the treatment of, for example, various cancers and autoimmune disease. In an aspect, there is provided the use of a Conjugate as disclosed herein, in the manufacture of a medicament for treating a proliferative disease or an autoimmune disease. These methods encompass the use of the Conjugates wherein the Ligand unit is a targeting agent that specifically binds to a target molecule. The Ligand unit can be, for example, a protein, polypeptide or peptide, such as an antibody, an antigen-binding fragment of an antibody, or other binding agent, such as an Fc fusion protein.

The PBD dimer D is of formula I, except that Q is selected from the group comprising: O, S, NR^N, wherein R^N is selected from the group comprising H and C_{1-2} alkyl.

Definitions

Pharmaceutically acceptable cations

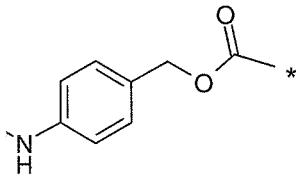
Examples of pharmaceutically acceptable monovalent and divalent cations are discussed in Berge, *et al.*, *J. Pharm. Sci.*, **66**, 1-19 (1977), which is incorporated herein by reference.

The pharmaceutically acceptable cation may be inorganic or organic.

Examples of pharmaceutically acceptable monovalent inorganic cations include, but are not limited to, alkali metal ions such as Na^+ and K^+. Examples of pharmaceutically acceptable divalent inorganic cations include, but are not limited to, alkaline earth cations such as Ca^{2+} and Mg^{2+}. Examples of pharmaceutically acceptable organic cations include, but are not limited to, ammonium ion (i.e. NH_4^+) and substituted ammonium ions (e.g. NH_3R^+, $NH_2R_2^+$, NHR_3^+, NR_4^+). Examples of some suitable substituted ammonium ions are those derived from: ethylamine, diethylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary ammonium ion is $N(CH_3)_4^+$.

where the asterisk indicates the point of attachment to the Drug unit, the wavy line indicates the point of attachment to the L^1, Y is -N(H)-, -O-, -C(=O)N(H)- or -C(=O)O-, and n is 0 to 3.

83. The Conjugate of claim 82, wherein L^2 is:



84. The use of a Conjugate of according to any one of claims 71 to 83, in the manufacture of a medicament for treating a proliferative disease or an autoimmune disease.

85. The ~~use of a~~ Conjugate according to any one of the claims 71 to 83 for use in treating a proliferative disease or an autoimmune disease.

~~86. A method of treating a mammal having a proliferative disease or an autoimmune disease, comprising administering an effective amount of the Conjugate of any one of claims 71 to 83.~~