consequences for the therapeutic window and the safety of the amatoxin conjugates for therapeutic approaches.

[008] Patent application WO 2012/041504 describes conjugates of an amatoxin with a binding molecule, which use a urea moiety as linker to the binding molecule. Such linkage could be shown to be significantly more stable than an ester linkage.

[009] Thus, significant progress has already been made in the development of amatoxin-based conjugates for therapeutic uses. However, the present inventors have found that constructs based on α- and β-amatoxin were not fully stable under stress conditions in plasma and resulted in a substantial degree of cross-linked products (see Figures 2 to 4). However, the stability of the conjugates comprising a highly toxic amatoxin is of utmost importance for the envisaged use as a therapeutic molecule for administration to human beings.

OBJECT OF THE INVENTION

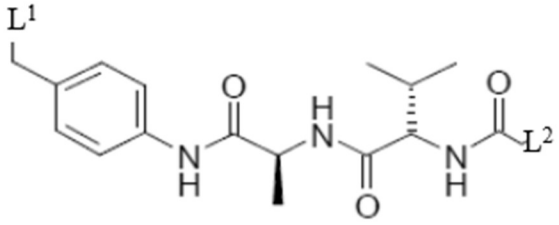
[0010] Thus, there was still a great need for amatoxin variants with an improved stability. The solution to this problem, i.e. the identification of certain modifications to the backbone of eight amino acid residues forming the basic amatoxin structure was neither provided nor suggested by the prior art.

SUMMARY OF THE INVENTION

[0011] The present disclosure is based on the unexpected observation that a variant form of amatoxins with (i) an amino acid 4 with a 6'-deoxy position; and (ii) an amino acid 8 with an S-deoxy position, shows an increased stability under stress conditions and an improved therapeutic index.

[0012] Thus, in one aspect the present disclosure refers a conjugate comprising (a) an amatoxin comprising (i) an amino acid 4 with a 6'-deoxy position;

and (ii) an amino acid 8 with an S-deoxy position; (b) a target-binding moiety; and (c) a linker linking said amatoxin and said target-binding moiety, wherein said linker is an enzymatically cleavable linker and/or a linker comprising the structure



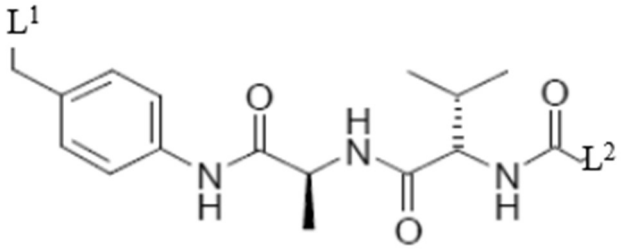
L^1-L*-L^2

wherein L^1 is a part of the linker that connects L* to the amatoxin, and L^2 is a part of the linker that connects L* to the target-binding moiety. In one example, there is described a conjugate comprising (a) an amatoxin comprising (i) an amino acid 4 with a 6'-deoxy position; and (ii) an amino acid 8 with an S-deoxy position; (b) a target-binding moiety; and (c) optionally a linker linking said amatoxin and said target-binding moiety. In particular, the optional linker of (c) is present and is a cleavable linker.

[0013] In a second aspect, the present disclosure refers to a pharmaceutical composition comprising the conjugate of the present invention.

[0014] In a third aspect, the present disclosure refers to use of a conjugate as disclosed herein in the manufacture of a medicament for treating cancer in a patient, particularly wherein the cancer is selected from the group consisting of breast cancer, pancreatic cancer, cholangiocarcinoma, colorectal cancer, lung cancer, prostate cancer, ovarian cancer, prostate cancer, stomach cancer, kidney cancer, malignant melanoma, leukemia, and malignant lymphoma.

[0015] In a fourth aspect, the present disclosure refers a construct comprising (a) an amatoxin comprising (i) an amino acid 4 with a 6'-deoxy position; and (ii) an amino acid 8 with an S-deoxy position; and (b) a linker moiety carrying a reactive group for linking said amatoxin to a target-binding moiety, wherein said linker moiety is an enzymatically cleavable linker moiety and/or a linker



L^1-L*-L^2

moiety comprising the structure

wherein L^1 is a part of the linker moiety that connects L* to the amatoxin, and L^2 is a part of the linker moiety that comprises the reactive group. In one example, there is disclosed a construct comprising (a) an amatoxin comprising (i) an amino acid 4 with a 6'-deoxy position; and (ii) an amino acid 8 with an S-deoxy position; and (c) a linker moiety, particularly a linker that is cleavable, carrying a reactive group for linking said amatoxin to a target-binding moiety.

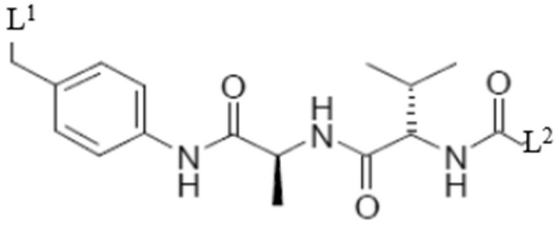
BRIEF DESCRIPTION OF THE DRAWING

[0016] **Fig. 1** shows the structural formulae of different amatoxins. The numbers in bold type (1 to 8) designate the standard numbering of the eight amino acids forming the amatoxin. The standard designations of the atoms in amino acids 1, 3 and 4 are also shown (Greek letters α to γ, Greek letters α to δ, and numbers from 1' to 7', respectively).

[0017] **Fig. 2** shows the results of a stress testing experiment in an anti-amanitin Western blot. A trastuzumab-amanitin conjugate (Her-30.0643; lysine conjugation via 6`-OH; stable linker) was incubated for 5 days at 37°C in PBS, pH 7.4, which led to extensive inter- and intrachain cross-linking; cross-linking of antibody chains could be prevented by addition of free cysteine.

CLAIMS

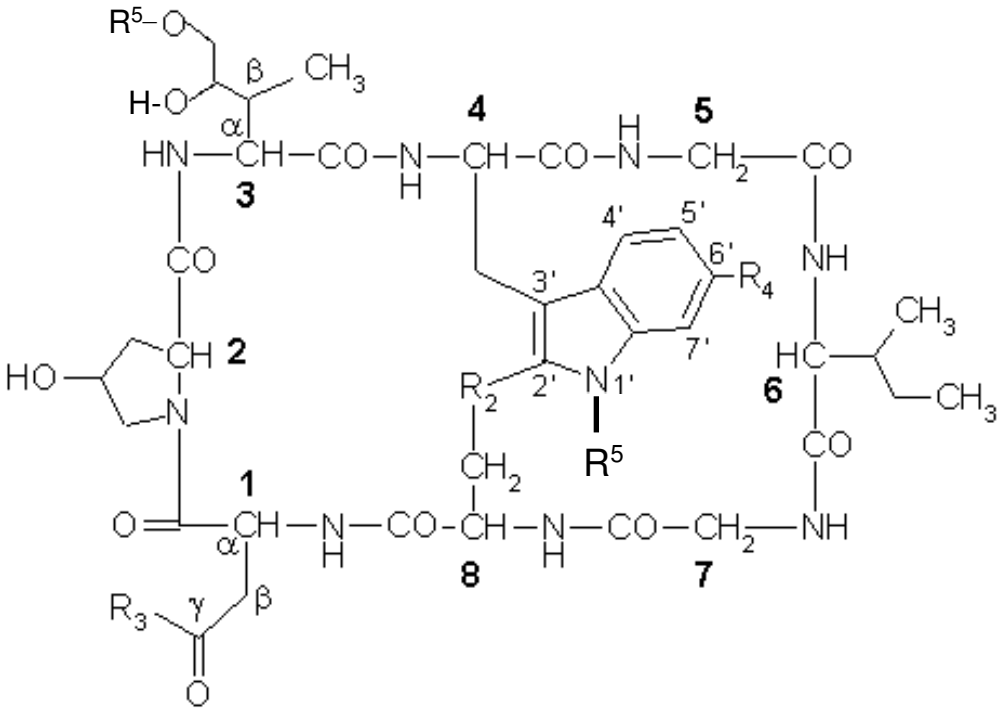
1. A conjugate comprising (a) an amatoxin comprising (i) an amino acid 4 with a 6'-deoxy position; and (ii) an amino acid 8 with an S-deoxy position; (b) a target-binding moiety; and (c) a linker linking said amatoxin and said target-binding moiety, wherein said linker is an enzymatically cleavable linker and/or a linker comprising the structure



L^1-L*-L^2

wherein L^1 is a part of the linker that connects L* to the amatoxin, and L^2 is a part of the linker that connects L* to the target-binding moiety.

2. The conjugate of claim 1 having structure **I**



I

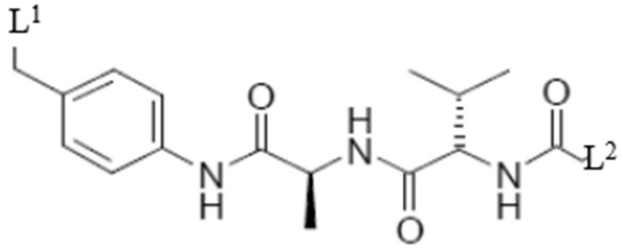
wherein:

R^2 is S;

R^3 is selected from -NHR^5, -NH-OR^5, and -OR^5;

R^4 is H; and

wherein one of R^5 is -L_n-X, wherein L is a linker, which is an enzymatically cleavable linker and/or a linker comprising the structure



L^1-L*-L^2

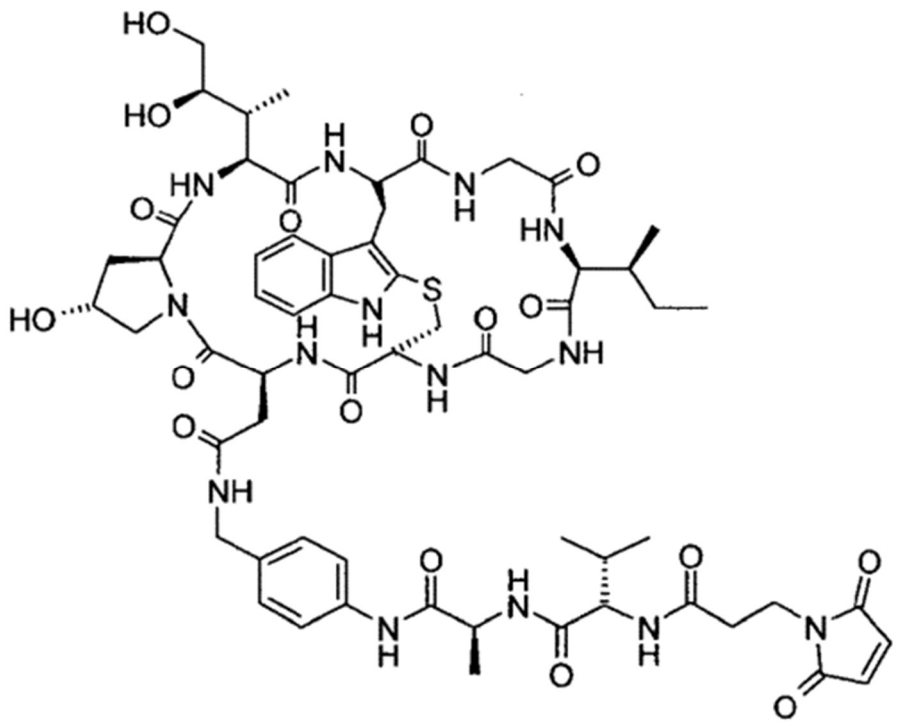
wherein L^1 is a part of the linker that connects L* to the amatoxin, and L^2 is a part of the linker that connects L* to the target-binding moiety; n is selected from 0 and 1, and X is a target-binding moiety, and wherein the remaining R^5 are H.

3. The conjugate of claim 2, wherein R^3 is selected from -NH-L_n-X, -NH-O-L_n-X, and -O-L_n-X.

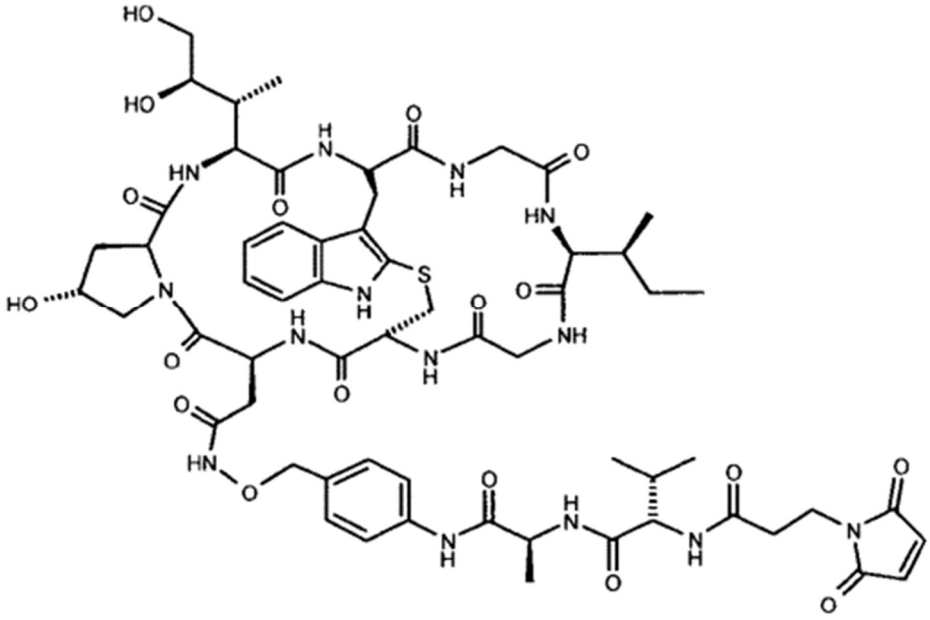
4. The conjugate of any one of claims 1 to 3, wherein said enzymatically cleavable linker comprises a dipeptide selected from the group consisting of: Phe-Lys, Val-Lys, Phe-Ala, Val-Ala, Phe-Cit, and Val-Cit.

5. The conjugate of claim 4, wherein said enzymatically cleavable linker further comprises a p-aminobenzyl (PAB) spacer between said dipeptide and said amatoxin.

6. The conjugate of any one of claims 1 to 5, wherein said target-binding moiety is attached to a construct selected from constructs HDP 30.2115 and HDP 30.2179



HDP 30.2115

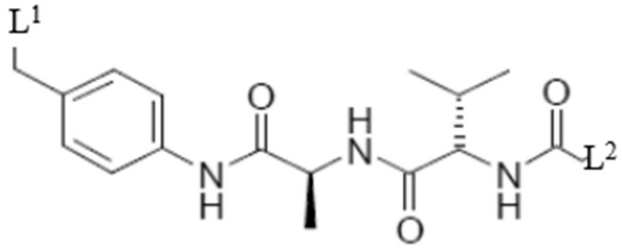


HDP 30.2179.

7. A pharmaceutical composition comprising the conjugate of any one of claims 1 to 6.

8. Use of a conjugate according to any one of claims 1 to 6 in the manufacture of a medicament for treating cancer in a patient, particularly wherein the cancer is selected from the group consisting of breast cancer, pancreatic cancer, cholangiocarcinoma, colorectal cancer, lung cancer, prostate cancer, ovarian cancer, stomach cancer, kidney cancer, malignant melanoma, leukemia, and malignant lymphoma.

9. A construct comprising (a) an amatoxin comprising (i) an amino acid 4 with a 6'-deoxy position; and (ii) an amino acid 8 with an S-deoxy position; and (b) a linker moiety carrying a reactive group for linking said amatoxin to a target-binding moiety, wherein said linker moiety is an enzymatically cleavable linker moiety and/or a linker moiety comprising the structure



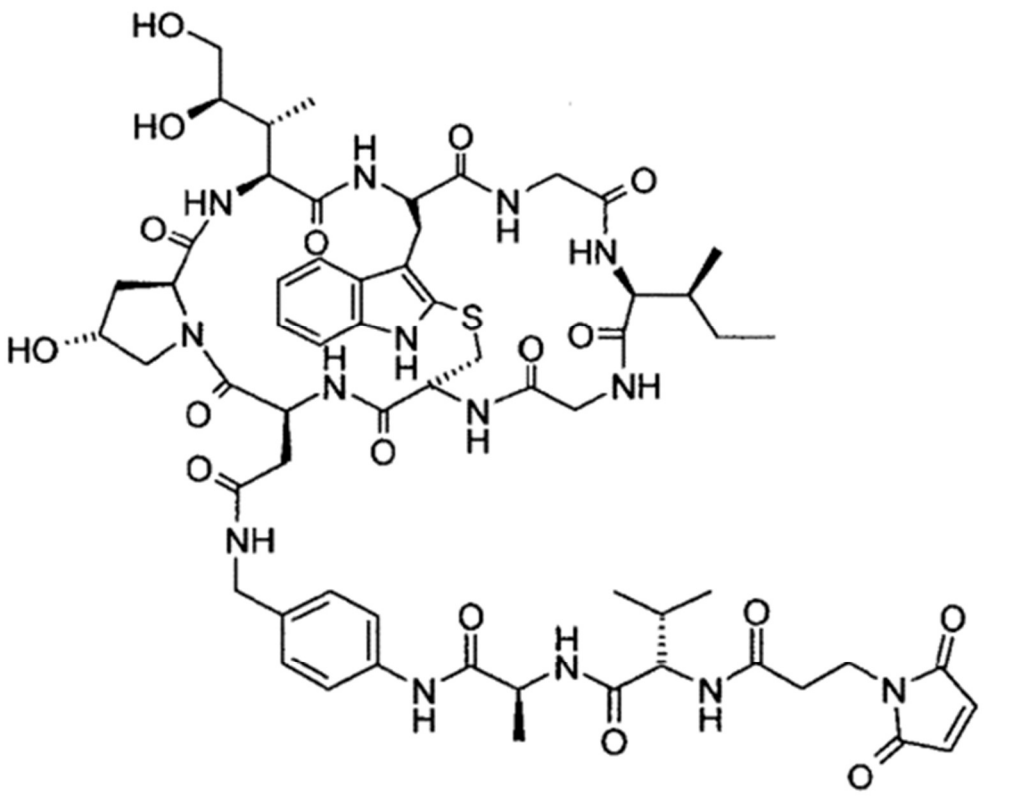
L^1-L*-L^2

wherein L^1 is a part of the linker moiety that connects L* to the amatoxin, and L^2 is a part of the linker moiety that comprises the reactive group.

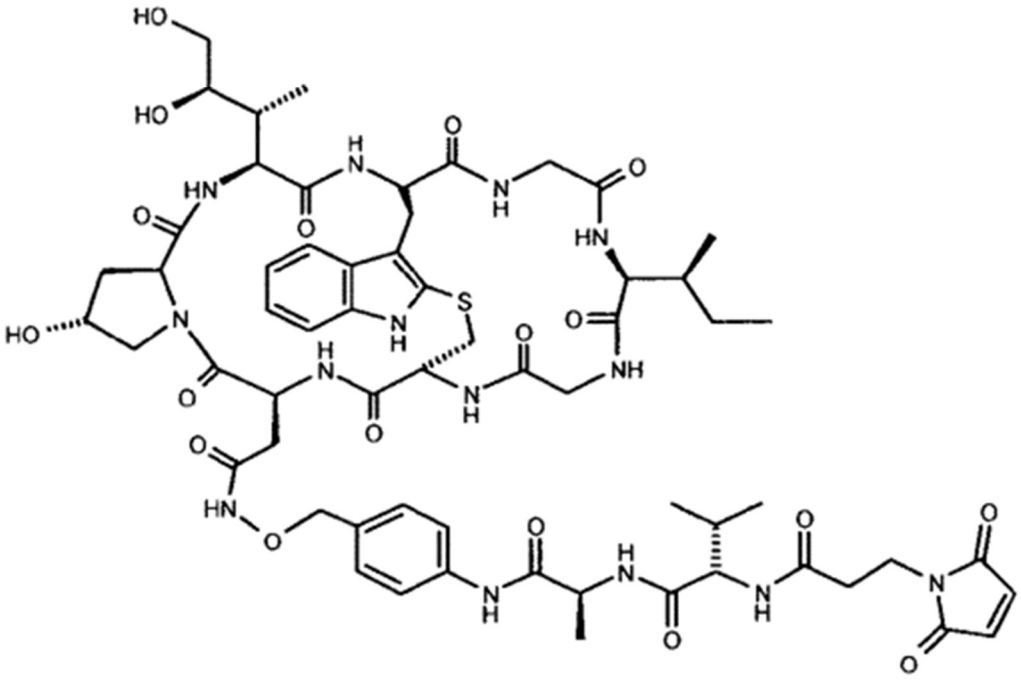
10. The construct of claim 9, wherein said reactive group is a thiol-reactive group.

11. The construct of claim 10, wherein said thiol-reactive group is selected from bromoacetamide, iodoacetamide, maleimide, a maleimide having a leaving group in position 3, a 1,2-dihydropyridazine-3,6-dione having a leaving group in position 4, methylsulfonyl benzothiazole, methylsulfonyl phenyltetrazole, methylsulfonyl phenyloxadiazole, a 3-arylpropionitrile, 5-nitro-pyridin-2-yl-disulfide, a maleimide having two leaving groups in positions 3 and 4, a 1,2-dihydropyridazine-3,6-dione having two leaving groups in positions 4 and 5.

12. The construct of claim 11, which is selected from constructs HDP 30.2115 and HDP 30.2179



HDP 30.2115



HDP 30.2179.