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

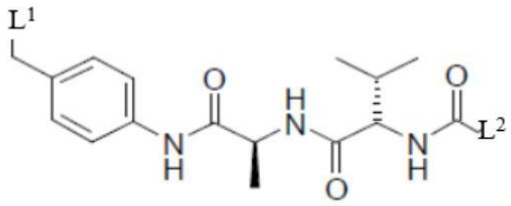
[0010] Thus, there was still a great need for amatoxin variants with an improved stability. The solution to this problem, i.e. the identification of certain modifications to the backbone of eight amino acid residues forming the basic amatoxin structure was neither provided nor suggested by the prior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

SUMMARY

[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.

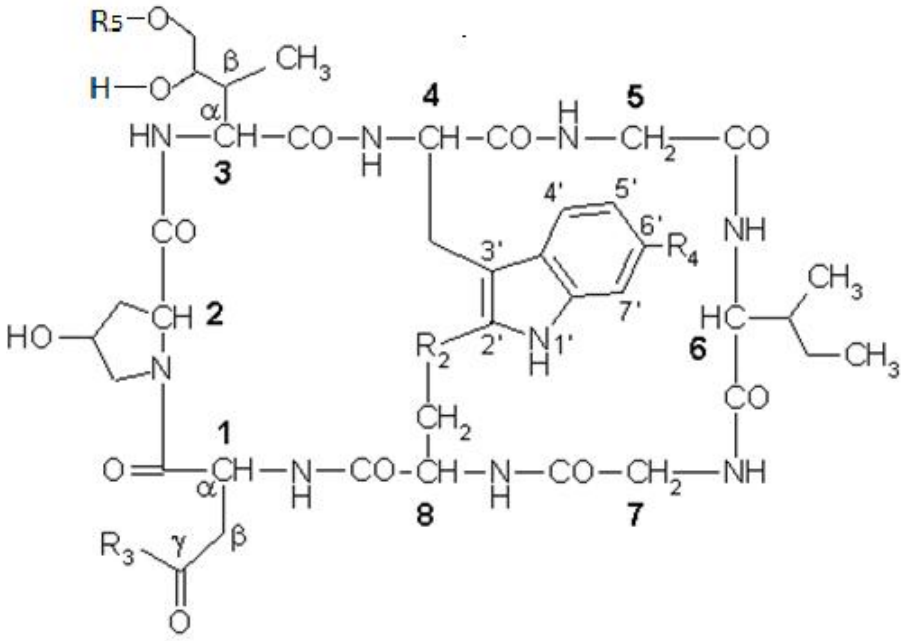
[0011a] In an aspect, there is provided a construct comprising (a) an amatoxin comprising (i) an amino acid 4 with a 6'-deoxy position; and (ii) an amino acid 8 with

an S-deoxy position; and (c) a cleavable linker moiety carrying a reactive group Y for linking said amatoxin to a target-binding moiety, wherein said target-binding moiety is an antibody or antigen-binding fragment thereof, and wherein said cleavable linker is an enzymatically cleavable 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 said reactive group Y, and wherein the construct has the structure II



II

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-Y, wherein L is said cleavable linker, and Y is a reactive group for linking said construct to a target-binding moiety.

[0012] Thus, in one example the present disclosure relates to 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 example, the present disclosure relates to a pharmaceutical composition comprising the conjugate of the present invention.

[0014] In a third example, the present disclosure relates to a conjugate of the present invention for use in the treatment of 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 example, the present disclosure relates to 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.

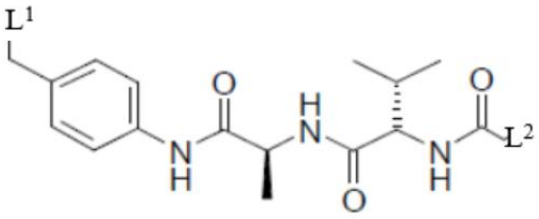
BRIEF DESCRIPTION OF THE DRAWING

[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.

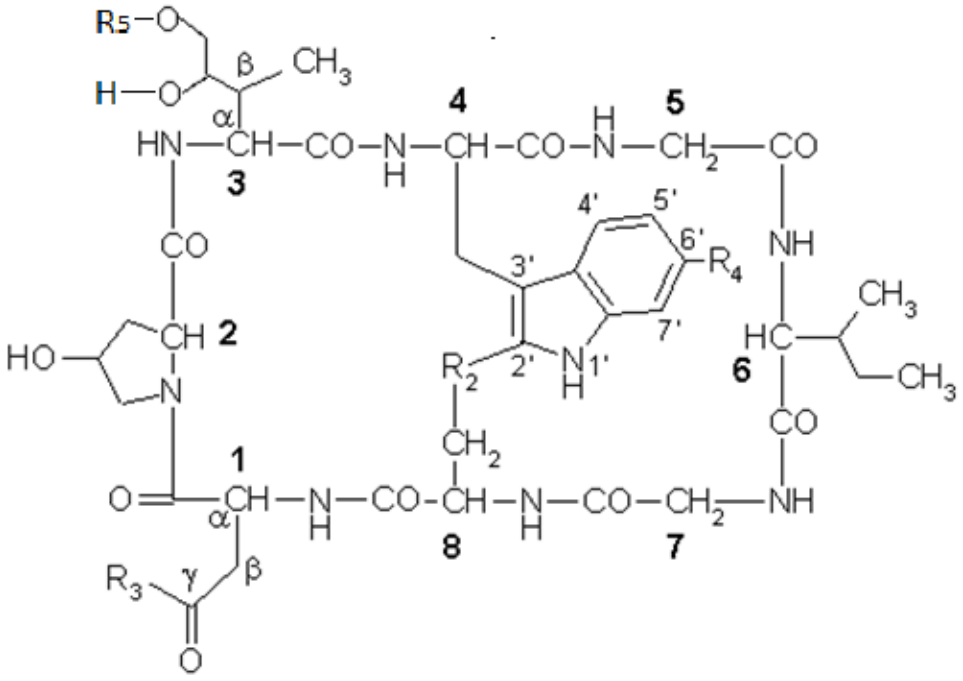
CLAIMS

1. A 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 cleavable linker moiety carrying a reactive group Y for linking said amatoxin to a target-binding moiety, wherein said target-binding moiety is an antibody or antigen-binding fragment thereof, and wherein said cleavable linker is an enzymatically cleavable 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 said reactive group Y, and wherein the construct has the structure II



II

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-Y, wherein L is said cleavable linker, and Y is a reactive group for linking said construct to a target-binding moiety.