comprising elements such as amide, ester, ether, thioether, disulfide, urea, thiourea, hydrocarbon moieties and the like, but no such constructs were actually shown, and no more details, such as attachment sites on the Amatoxins, were provided.

[005] It is known that amatoxins are relatively non-toxic when coupled to large biomolecule carriers, such as antibody molecules, and that they exert their cytotoxic activity only after the biomolecule carrier is cleaved off. In light of the toxicity of amatoxins, particularly for liver cells, it is of outmost importance that amatoxin conjugates for targeted tumour therapy remain highly stable after administration in plasma, and that the release of the amatoxin occurs after internalization in the target cells. In this context, minor improvements of the conjugate stability may have drastic consequences for the therapeutic window and the safety of the amatoxin conjugates for therapeutic approaches.

OBJECT OF THE INVENTION

[006] Thus, there was a high need in the prior art for target-binding moiety amatoxin conjugates that are stable in plasma, so that harmful side effects to non-target cells are minimized.

SUMMARY OF THE INVENTION

[006a] In one aspect, there is provided a conjugate comprising a target-binding moiety linked via a linker L to an amatoxin, wherein the linker L is connected to the amatoxin via

(i) the γ C-atom of amatoxin amino acid 1, particularly via an amide linkage;

(ii) an oxygen atom bound to the δ C-atom of amatoxin amino acid 3, particularly via an ester linkage, an ether linkage or a urethane linkage; or

(iii) the 6' C-atom of amatoxin amino acid 4, particularly via an oxygen atom bound to the 6' C-atom of amatoxin amino acid 4;

in each case wherein the linker L is connected to the target-binding moiety via a urea moiety.

[006b] In one aspect, there is provided a pharmaceutical composition comprising the conjugate as defined above and further comprising one or more pharmaceutically acceptable diluents, carriers, excipients, fillers, binders, lubricants, glidants, disintegrants, adsorbents; and/or preservatives.

[006c] In one aspect, there is provided an amatoxin-conjugation molecule comprising a linker L connected to an amatoxin via

(i) the γ C-atom of amatoxin amino acid 1, particularly via an amide linkage;

(ii) an oxygen atom bound to the δ C-atom of amatoxin amino acid 3, particularly via an ester linkage, an ether linkage or a urethane linkage; or

(iii) the 6' C-atom of amatoxin amino acid 4, particularly via an oxygen atom bound to the 6' C-atom of amatoxin amino acid 4;

in each case wherein the linker L comprises a carbamic acid derivative -NH-C(O)-X, wherein X is a leaving group that can be replaced by a primary amine of a target-binding moiety.

[006d] In one aspect, there is provided a method for synthesizing a conjugate as defined above, comprising the step of reacting an amatoxin-conjugation molecule as defined above with a target-binding moiety comprising a primary amino group.

[007] In one~~a first~~ aspect the present invention relates to a conjugate comprising a target-binding moiety linked via a linker L to an amatoxin, wherein the linker L is connected to the amatoxin via

(i) the γ C-atom of amatoxin amino acid 1, particularly via an amide linkage;

(ii) an oxygen atom bound to the δ C-atom of amatoxin amino acid 3, particularly via an ester linkage, an ether linkage or a urethane linkage; or

(iii) the 6' C-atom of amatoxin amino acid 4, particularly via an oxygen atom bound to the 6' C-atom of amatoxin amino acid 4;

in each case wherein the linker L is connected to the target-binding moiety via a urea moiety.

CLAIMS

1. A conjugate comprising a target-binding moiety linked via a linker L to an amatoxin, wherein the linker L is connected to the amatoxin via
   (i) the γ C-atom of amatoxin amino acid 1, particularly via an amide linkage;
   (ii) an oxygen atom bound to the δ C-atom of amatoxin amino acid 3, particularly via an ester linkage, an ether linkage or a urethane linkage; or
   (iii) the 6' C-atom of amatoxin amino acid 4, particularly via an oxygen atom bound to the 6' C-atom of amatoxin amino acid 4;
   in each case wherein the linker L is connected to the target-binding moiety via a urea moiety.

2. The conjugate of claim 1, wherein the conjugate has a structure selected from one of the following structures:
   (i) amatoxin-γC(O)-NH-L-NH-C(O)-NH-target-binding moiety;
   (ii) amatoxin-δC-O-C(O)-L-NH-C(O)-NH-target-binding moiety;
   (iii) amatoxin-δC-O-L-NH-C(O)-NH-target-binding moiety;
   (iv) amatoxin-δC-O-C(O)-NH-L-NH-C(O)-NH-target-binding moiety; and
   (v) amatoxin-6'C-O-L-NH-C(O)-NH-target-binding moiety.

3. The conjugate of claim 1 or 2, wherein the target-binding moiety is connected to the linker L via an amino group present in the target-binding moiety, wherein said amino group forms part of said urea moiety.

4. The conjugate of any one of claims 1 to 3, wherein the amatoxin is selected from α-amanitin, β-amanitin, γ-amanitin, ε-amanitin, amanin, amaninamide, amanullin, or amanullinic acid, or from salts or analogues thereof.

5. The conjugate of any one of claims 1 to 4, wherein the linker L is an alkylene, heteroalkylene, alkenylene, heteroalkenylene, alkynylene, heteroalkynylene, cycloalkylene, heterocycloalkylene, arylene, heteroarylene, aralkylene, or a heteroaralkylene group, optionally substituted.

6. The conjugate of any one of claims 1 to 5, wherein the linker L comprises a moiety selected from one of the following moieties: a disulfide, an ether, an amine, an ester, a carboxamide, a urethane, and a urea moiety.

7. The conjugate of any one of claims 1 to 6 wherein the target-binding moiety specifically binds to an epitope that is present on a tumour cell, particularly wherein the target-binding moiety specifically binds to an epitope of epithelial cell adhesion molecule (EpCAM).

8. The conjugate of any one of claims 1 to 7, wherein the target-binding moiety is selected from the group consisting of:
   (i) antibody or antigen-binding fragment thereof;
   (ii) antibody-like protein; and
   (iii) nucleic acid aptamer.

9. The conjugate of claim 8, wherein the antibody or the antigen-binding fragment thereof is selected from a diabody, a tetrabody, a nanobody, a chimeric antibody, a deimmunized antibody, a humanized antibody or a human antibody.

10. The target-binding moiety toxin conjugate of claim 8 or 9, wherein the antigen binding fragment is selected from the group consisting of Fab, $F(ab')_2$, Fd, Fv, single-chain Fv, and disulfide-linked Fvs (dsFv).

11. The conjugate of any one of claims 1 to 10 for use as a medicament.

12. The conjugate of any one of claims 1 to 11 for the treatment of cancer in a patient, wherein the cancer is selected from the group consisting of pancreatic cancer, cholangiocarcinoma, breast cancer, colorectal cancer, lung cancer, prostate cancer, ovarian cancer, stomach cancer, kidney cancer, malignant melanoma, leukemia, and malignant lymphoma.

13. Pharmaceutical composition comprising the conjugate according to any one of claims 1 to 10 and further comprising one or more pharmaceutically acceptable diluents, carriers, excipients, fillers, binders, lubricants, glidants, disintegrants, adsorbents; and/or preservatives.

14. An amatoxin-conjugation molecule comprising a linker L connected to an amatoxin via

(i) the γ C-atom of amatoxin amino acid 1, particularly via an amide linkage;

(ii) an oxygen atom bound to the δ C-atom of amatoxin amino acid 3, particularly via an ester linkage, an ether linkage or a urethane linkage; or

(iii) the 6' C-atom of amatoxin amino acid 4, particularly via an oxygen atom bound to the 6' C-atom of amatoxin amino acid 4;

in each case wherein the linker L comprises a carbamic acid derivative -NH-C(O)-X, wherein X is a leaving group that can be replaced by a primary amine of a target-binding moiety.

15. The amatoxin-conjugation molecule claim 14, wherein X is selected from: -^{t}butyloxy, -succinimidyloxy, -1-O-succinimidyloxy-3-sulfonate (-Sulfo-NHS), -O-(4-nitrophenyloxy), -O-(3-nitrophenyloxy), -O-(2,4-dinitrophenyloxy), -O-(2,4-dichloro-6-nitrophenyloxy), -pentafluorophenyloxy, -pentachlorophenyloxy, -O-(2,4,5-trichlorophenyloxy), -O-(3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine-3-yl), -O-(endo-1-hydroxy-5-norbornene-2,3-dicarboximide-1-yl), -1-phthalimidoyloxy, -1-benzotriazolyloxy, -1-(7-aza-benzotriazolyl)oxy,), and -N-imidazolyl.

16. A method for synthesizing a conjugate of any one of claims 1 to 12, comprising the step of reacting an amatoxin-conjugation molecule of claim 14 or 15 with a target-binding moiety comprising a primary amino group.

Claims

1. A conjugate comprising a target-binding moiety linked via a linker L to an amatoxin, wherein the linker L is connected to the amatoxin via

   (i) the γ C-atom of amatoxin amino acid 1, particularly via an amide linkage;
   (ii) an oxygen atom bound to the δ C-atom of amatoxin amino acid 3, particularly via an ester linkage, an ether linkage or a urethane linkage; or
   (iii) the 6' C-atom of amatoxin amino acid 4, particularly via an oxygen atom bound to the 6' C-atom of amatoxin amino acid 4;

   in each case wherein the linker L is connected to the target-binding moiety via a urea moiety.

2. The conjugate of claim 1, wherein the conjugate has a structure selected from one of the following structures:

   (i) amatoxin-γC(O)-NH-L-NH-C(O)-NH-target-binding moiety;
   (ii) amatoxin-δC-O-C(O)-L-NH-C(O)-NH-target-binding moiety;
   (iii) amatoxin-δC-O-L-NH-C(O)-NH-target-binding moiety;
   (iv) amatoxin-δC-O-C(O)-NH-L-NH-C(O)-NH-target-binding moiety; and
   (v) amatoxin-6'C-O-L-NH-C(O)-NH-target-binding moiety.

3. The conjugate of claim 1 or 2, wherein the target-binding moiety is connected to the linker L via an amino group present in the target-binding moiety, wherein said amino group forms part of said urea moiety.

4. The conjugate of any one of claims 1 to 3, wherein the amatoxin is selected from α-amanitin, β-amanitin, γ-amanitin, ε-amanitin, amanin, amaninamide, amanullin, or amanullinic acid, or from salts or analogues thereof.

5. The conjugate of any one of claims 1 to 4, wherein the linker L is an alkylene, heteroalkylene, alkenylene, heteroalkenylene, alkynylene, heteroalkynylene, cycloalkylene, heterocycloalkylene, arylene, heteroarylene, aralkylene, or a heteroaralkylene group, optionally substituted.

6. The conjugate of any one of claims 1 to 5, wherein the linker L comprises a moiety selected from one of the following moieties: a disulfide, an ether, an amine, an ester, a carboxamide, a urethane, and a urea moiety.

7. The conjugate of any one of claims 1 to 6 wherein the target-binding moiety specifically binds to an epitope that is present on a tumour cell, particularly wherein the target-binding moiety specifically binds to an epitope of epithelial cell adhesion molecule (EpCAM).

8. The conjugate of any one of claims 1 to 7, wherein the target-binding moiety is selected from the group consisting of:

   (i) antibody or antigen-binding fragment thereof;
   (ii) antibody-like protein; and
   (iii) nucleic acid aptamer.

9. The conjugate of claim 8, wherein the antibody or the antigen-binding fragment thereof is selected from a diabody, a tetrabody, a nanobody, a chimeric antibody, a deimmunized antibody, a humanized antibody or a human antibody.

10. The target-binding moiety toxin conjugate of claim 8 or 9, wherein the antigen binding fragment is selected from the group consisting of Fab, $F(ab')_2$, Fd, Fv, single-chain Fv, and disulfide-linked Fvs (dsFv).

11. The conjugate of any one of claims 1 to 10 for use as a medicament.

12. The conjugate of any one of claims 1 to 11 for the treatment of cancer in a patient, wherein the cancer is selected from the group consisting of pancreatic cancer, cholangiocarcinoma, breast cancer, colorectal cancer, lung cancer, prostate cancer, ovarian cancer, stomach cancer, kidney cancer, malignant melanoma, leukemia, and malignant lymphoma.

13. Pharmaceutical composition comprising the conjugate according to any one of claims 1 to 10 and further comprising one or more pharmaceutically acceptable diluents, carriers, excipients, fillers, binders, lubricants, glidants, disintegrants, adsorbents; and/or preservatives.

14. An amatoxin-conjugation molecule comprising a linker L connected to an amatoxin via

(i) the γ C-atom of amatoxine amino acid 1, particularly via an amide linkage;
(ii) an oxygen atom bound to the 6 C-atom of amatoxin amino acid 3, particularly via an ester linkage, an ether linkage or a urethane linkage; or
(iii) the 6' C-atom of amatoxin amino acid 4, particularly via an oxygen atom bound to the 6' C-atom of amatoxin amino acid 4;

in each case wherein the linker L comprises a carbamic acid derivative -NH-C(O)-X, wherein X is a leaving group that can be replaced by a primary amine of a target-binding moiety.

15. The amatoxin-conjugation molecule claim 14, wherein X is selected from: -^{t}butyloxy, -succinimidyloxy, -1-O-succinimidyloxy-3-sulfonate (-Sulfo-NHS), -O-(4-nitrophenyloxy), -O-(3-nitrophenyloxy), -O-(2,4-dinitrophenyloxy), -O-(2,4-dichloro-6-nitrophenyloxy), -pentafluorophenyloxy, -pentachlorophenyloxy, -O-(2,4,5-trichlorophenyloxy), -O-(3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine-3-yl), -O-(endo-1-hydroxy-5-norbornene-2,3-dicarboximide-1-yl), -1-phthalimidoyloxy, -1-benzotriazolyloxy, -1-(7-aza-benzotriazolyl)oxy,), and -N-imidazolyl.

16. A method for synthesizing a conjugate of any one of claims 1 to 12, comprising the step of reacting an amatoxin-conjugation molecule of claim 14 or 15 with a target-binding moiety comprising a primary amino group.