[0004] Different research teams have previously tethered polypeptides with cysteine residues to a synthetic molecular structure (Kemp and McNamara (1985), J. Org. Chem; Timmerman *et al*. (2005), ChemBioChem). Meloen and co-workers had used tris(bromomethyl)benzene and related molecules for rapid and quantitative cyclisation of multiple peptide loops onto synthetic scaffolds for structural mimicry of protein surfaces (Timmerman *et al*. (2005), ChemBioChem). Methods for the generation of candidate drug compounds wherein said compounds are generated by linking cysteine containing polypeptides to a molecular scaffold as for example TATA (1,1',1''-(1,3,5-triazinane-1,3,5-triyl)triprop-2-en-1-one, Heinis *et al.* Angew Chem, Int Ed. 2014; 53:1602–1606).

[0005] Phage display-based combinatorial approaches have been developed to generate and screen large libraries of bicyclic peptides to targets of interest (Heinis *et al*. (2009), Nat Chem Biol *5* (7), 502-7 and WO 2009/098450). Briefly, combinatorial libraries of linear peptides containing three cysteine residues and two regions of six random amino acids (Cys-$(Xaa)_6$-Cys-$(Xaa)_6$-Cys) were displayed on phage and cyclised by covalently linking the cysteine side chains to a small molecule scaffold.

SUMMARY

[0005a] In an aspect of the present disclosure, there is provided a use of a Bicycle toxin conjugate BT5528, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, in the manufacture of a medicament for preventing or treating a disease, disorder, or condition characterised by overexpression of EphA2 in a patient, wherein the Bicycle toxin conjugate BT5528 is to be administered to the patient to provide a tumor MMAE concentration of about 20 pmol/gram or more.

[0005b] In another aspect of the present disclosure, there is provided a Bicycle toxin conjugate, which is BT5528 as shown herein, or a pharmaceutically acceptable salt thereof.

[0005c] In a further aspect of the present disclosure, there is provided a pharmaceutical composition comprising Bicycle toxin conjugate BT5528, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier, adjuvant, or vehicle.

[0005d] In another aspect of the present disclosure, there is provided a Bicycle toxin conjugate, which is BCY10188 as shown herein, or a pharmaceutically acceptable salt thereof.

[0005e] In a further aspect of the present disclosure, there is provided a pharmaceutical composition comprising Bicycle toxin conjugate BCY10188, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier, adjuvant, or vehicle.

[0005f] In another aspect of the present disclosure, there is provided a Bicycle toxin conjugate BCY10188, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, for use in preventing or treating a disease, disorder, or condition characterised by overexpression of EphA2 in a patient.

[0005g] In a further aspect of the present disclosure, there is provided a use of a Bicycle toxin conjugate BCY10188, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, in the manufacture of a medicament for preventing or treating a disease, disorder, or condition characterised by overexpression of EphA2 in a patient.

[0006] It had been found that Bicycle toxin conjugates BT5528 and BCY10188, and pharmaceutically acceptable salts and pharmaceutical compositions thereof, are effective in treating diseases, disorders, or conditions characterised by overexpression of EphA2, for example, cancers.

[0007] In one example, the present disclosure provides Bicycle toxin conjugate BT5528, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof.

[0008] In one example, the present disclosure provides Bicycle toxin conjugate BCY10188, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof.

[0009] In one example, the present disclosure provides a method of preventing or treating a disease, disorder, or condition characterised by overexpression of EphA2 in a patient, comprising administering to the patient BT5528, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof.

[0010] In one example, the present disclosure provides a method of preventing or treating a disease, disorder, or condition characterised by overexpression of EphA2 in a patient, comprising

CLAIMS

1. Use of a Bicycle toxin conjugate BT5528, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, in the manufacture of a medicament for preventing or treating a disease, disorder, or condition characterised by overexpression of EphA2 in a patient, wherein the Bicycle toxin conjugate BT5528 is to be administered to the patient to provide a tumor MMAE concentration of about 20 pmol/gram or more.

2. The use of claim 1, wherein Bicycle toxin conjugate BT5528, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, is to be administered to a patient to provide a tumor MMAE concentration of about 50 pmol/gram or more.

3. The use of claim 1 or 2, wherein Bicycle toxin conjugate BT5528, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, is to be administered to a patient to provide a system exposure of BT5528 and/or MMAE for about 2 hours or less.

4. The use of claim 3, wherein the system exposure of BT5528 is measured by the time when the concentration of BT5528 in plasma is about 2% or more of the maximum concentration of BT5528 in plasma.

5. The use of claim 3 or 4, wherein the system exposure of BT5528 is measured by the time when the concentration of BT5528 in plasma is about 15 pmol/gram or more.

6. The use of claim 3, wherein the system exposure of MMAE is measured by the time when the concentration of MMAE in plasma is about 10% or more of the maximum concentration of MMAE in plasma.

7. The use of claim 3 or 6, wherein the system exposure of MMAE is measured by the time when the concentration of MMAE in plasma is about 2 pmol/gram or more.

8. The use of any one of the preceding claims, wherein Bicycle toxin conjugate BT5528, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, is to be administered to a patient to induce mitotic arrest in tumor within about 24 hours.

9. The use of claim 8, wherein mitotic arrest in tumor is induced when there is about 15% or more pHH3+ nuclei in tumor.

10. The use of any one of the preceding claims, wherein Bicycle toxin conjugate BT5528, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, is to be administered to a patient to induce measurable tumor regression by day 4 post dosing.

11. The use of any one of the preceding claims, wherein Bicycle toxin conjugate BT5528, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, is to be administered to a patient at about 0.3 mg/m^2 to about 9 mg/m^2 each dose.

12. The use of any one of the preceding claims, wherein Bicycle toxin conjugate BT5528, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, is to be administered to a patient by an intravenous bolus injection.

13. The use of any one of claims 1-11, wherein Bicycle toxin conjugate BT5528, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, is to be administered to a patient by an intravenous infusion.

14. The use of claim 13, wherein the intravenous infusion is an about 1 hour infusion.

15. The use of any one of claims 1-11, wherein Bicycle toxin conjugate BT5528, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, is to be administered to a patient by a subcutaneous infusion.

16. The use of claim 15, wherein the subcutaneous infusion is an about 24 hour infusion.

17. The use of any one of the preceding claims, wherein Bicycle toxin conjugate BT5528, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, is to be administered to a patient two or more times, with at least 48 hours in between two consecutive administrations.

18. The use of any one of the preceding claims, wherein Bicycle toxin conjugate BT5528, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, is to be administered to a patient two or more times, with about one week in between two consecutive administrations.

19. A Bicycle toxin conjugate, which is BT5528 as shown herein, or a pharmaceutically acceptable salt thereof.

20. A pharmaceutical composition comprising Bicycle toxin conjugate BT5528, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier, adjuvant, or vehicle.

21. A Bicycle toxin conjugate, which is BCY10188 as shown herein, or a pharmaceutically acceptable salt thereof.

22. A pharmaceutical composition comprising Bicycle toxin conjugate BCY10188, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier, adjuvant, or vehicle.

23. A Bicycle toxin conjugate BCY10188, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, for use in preventing or treating a disease, disorder, or condition characterised by overexpression of EphA2 in a patient.

24. Use of a Bicycle toxin conjugate BCY10188, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, in the manufacture of a medicament for preventing or treating a disease, disorder, or condition characterised by overexpression of EphA2 in a patient.

25. The Bicycle toxin conjugate BCY10188 for use of claim 23, or the use of claim 24, wherein Bicycle toxin conjugate BCY10188, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, is to be administered to a patient at about 0.3 mg/m^2 to about 9 mg/m^2 each dose.

26. The Bicycle toxin conjugate BCY10188 for use, or the use of any one of claims 23 to 25, wherein Bicycle toxin conjugate BCY10188, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, is to be administered to a patient by an intravenous bolus injection.

27. The use of any one of claims 1-18, or the Bicycle toxin conjugate BCY10188 for use, or the use of any one of claims 23-26, wherein one or more other therapeutic agents are to be administered.

28. The use of any one of claims 1-18, or the Bicycle toxin conjugate BCY10188 for use, or the use of any one of claims 23-27, wherein the disease, disorder, or condition characterised by overexpression of EphA2 is a cancer.

29. The use, or the Bicycle toxin conjugate BCY10188 for use of claim 28, wherein the cancer is selected from prostate cancer, lung cancer (such as non-small cell lung carcinomas (NSCLC)), breast cancer (such as triple negative breast cancer), gastric cancer, ovarian cancer, oesophageal cancer, multiple myeloma, pancreatic cancer, and fibrosarcoma.