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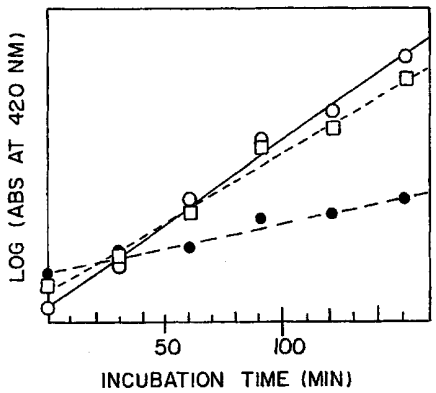
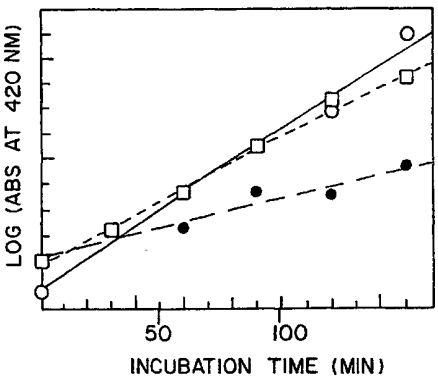
(54) Title: COMPOSITIONS AND METHODS FOR TARGETED ENZYMATIC RELEASE OF CELL REGULATORY COMPOUNDS





(57) Abstract: Novel pro-drugs and methods for their use to alter the growth and biological characteristics of living cells, tissues, or whole organisms are described. The methods allow for selective activation of the pro-drugs at or near transformant host cells expressing a gene for an enzyme that activates the pro-drugs. Pro-drugs according to a preferred embodiment of the invention are conjugates of a bioactive compound and a chemical group that is capable of being cleaved from the bioactive compound by action of an enzyme. Methods according to this invention include: (a) introducing into targeted cells a gene encoding an enzyme and (b) administering a pro-drug, wherein the enzyme releases the pro-drug from conjugation. In a preferred embodiment of the invention, the gene encoding the enzyme is a marker gene.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

AMENDED CLAIMS

[received by the International Bureau on 9 April 2001 (09.04.01);
original claims 1, 11, 12, and 28 amended; original claims 2, 19, 30 and 42 cancelled;
remaining claims unchanged (6 pages)]

1. A method of targeted delivery of a bioactive compound comprising:

(a) introducing into targeted cells a gene encoding an enzyme, wherein said enzyme does not naturally occur in the targeted cells in an amount effective to release the bioactive compound from conjugation;

(b) administering an inactive conjugate of the bioactive compound, said inactive conjugate having structure BLOCK-X-DRUG;

wherein the enzyme releases the bioactive compound from conjugation, said released bioactive compound having structure H-X-DRUG.

3. The method of claim 1 wherein the enzyme is an hydrolitic enzyme.

4. The method of claim 1 wherein the enzyme is a marker gene.

5. The method of claim 1 wherein the enzyme is an active component of *lacZ* β-galactosidase from the bacteria *Escherichia coli.*

6. The method of claim 1 wherein the enzyme is an active component of *GUS* β -glucuronidase from the bacteria *Escherichia coli.*

7. The method of claim 1 wherein the enzyme is an active component of firefly luciferase from *Photinus pyralis.*

8. The method of claim 1 wherein the enzyme is an active component of firefly luciferase from *Renilla reniformis.*

9. The method of claim 1 wherein the enzyme is an active component of β-lactamase from the bacteria *Escherichia coli.*

10. The method of claim 1 wherein the enzyme is an active component of alkaline phosphatase.

11. The method of claim 1 wherein the bioactive compound is selected from the group consisting of cycloheximide, dexamethasone, 4'-hydroxymethyl-trimethylpsoralen, chloramphenicol, 5-fluorouridine, tetracycline, doxorubicin, resveratrol, phorbol octanoate acetate, dioctanoyl glycerol, 4-hydroxyphenylretinamide, mitoxantrone, and thymidine.

12. A method of hindering cell growth comprising:

(a) introducing into targeted cells a gene encoding an enzyme; and

(b) administering in an amount effect to hinder growth of the targeted cells an inactive conjugate of a bioactive compound, said inactive conjugate having structure BLOCK-X-DRUG;

wherein the enzyme does not naturally occur in the targeted cells in an amount effective to release the bioactive compound from conjugation, and the enzyme releases the bioactive compound from conjugation, said released bioactive compound having structure H-X-DRUG.

13. The method of claim 12 wherein the targeted cells are the cancer cells.

14. The method of claim 13 wherein the targeted cells are proximal to the cancer cells.

15. The method of claim 13 wherein the drug initiates a host immune response to the cancer cells.

16. The method of claim 13 wherein the cancer cells are present in a mammal.

17. The method of claim 12 wherein the cells are plant cells.

18. The method of claim 12 where in the cells are bacterial cells.

20. The method of claim 12 wherein the enzyme is an hydrolitic enzyme.

21. The method of claim12 wherein the enzyme is a marker gene.

22. The method of claim 12 wherein the enzyme is an active component of *lacZ* β-galactosidase from the bacteria *Escherichia coli.*

23. The method of claim 12 wherein the enzyme is an active component of *GUS* β -glucuronidase from the bacteria *Escherichia coli.*

24. The method of claim 12 wherein the enzyme is an active component of firefly luciferase from *Photinus pyralis.*

25. The method of claim 12 wherein the enzyme is an active component of firefly luciferase from *Renilla reniformis.*

26. The method of claim 12 wherein the enzyme is an active component of β-lactamase from the bacteria *Escherichia coli.*

27. The method of claim 12 wherein the enzyme is an active component of alkaline phosphatase.

28. A method of enhancing cell growth comprising:

(a) introducing into the cells a gene encoding an enzyme; and

(b) administering a conjugate of a bioactive compound in an amount effective to enhance cell growth an inactive conjugate of a bioactive compound having structure BLOCK-X-DRUG;

wherein the bioactive compound has structure H-X-DRUG, and enhances cell growth and the enzyme does not naturally occur in the cells in an amount effective to release the bioactive compound from conjugation, and the enzyme releases the bioactive compound from conjugation.

29. The method of claim 28 wherein enhancing cell growth increases the production of a protein in a cell culture.

31. The method of claim 28 wherein the enzyme is an hydrolitic enzyme.

32. The method of claim 28 wherein the enzyme is a marker gene.

33. The method of claim 28 wherein the enzyme is an active component of *lacZ* β-galactosidase from the bacteria *Escherichia coli.*

34. The method of claim 28 wherein the enzyme is an active component of *GUS* β-glucuronidase from the bacteria *Escherichia coli.*

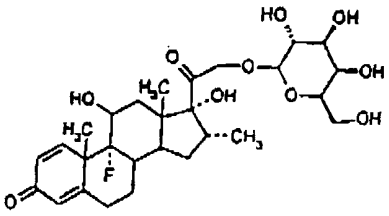
35. The method of claim 28 wherein the enzyme is an active component of firefly luciferase from *Photinus pyralis.*

36. The method of claim 28 wherein the enzyme is an active component of firefly luciferase from *Renilla reniformis.*

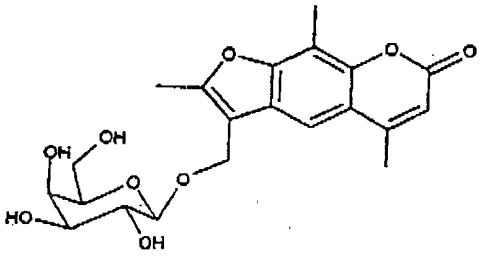
37. The method of claim 28 wherein the enzyme is an active component of β - lactamase from the bacteria *Escherichia coli.*

38. The method of claim 28 wherein the enzyme is an active component of alkaline phosphatase.

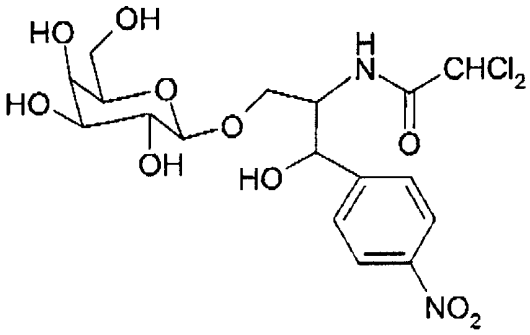
39. An inactive conjugate of a bioactive compound having the formula



40. An inactive conjugate of a bioactive compound having the formula



41. An inactive conjugate of a bioactive compound having the formula



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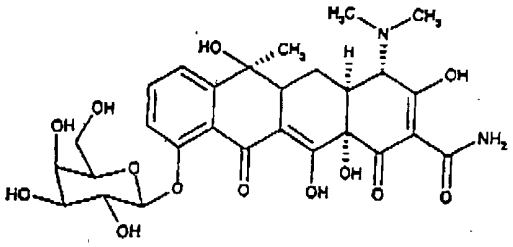

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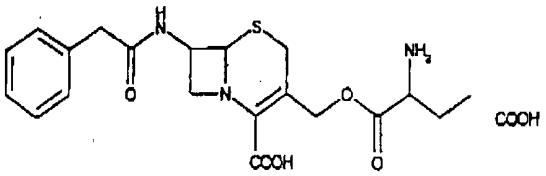

45. An inactive conjugate of a bioactive compound having the formula