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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.

(54) Title: METHODS FOR ALTERING POLYKETIDE SYNTHASE GENES

(57) Abstract: Ketoreductase (KR) domains of modular polyketide synthase (PKS) enzymes can be inactivated by one or more point mutations in the domain. Replacement or insertion of a KR domain can be used to introduce a cis or trans double bond into a polyketide by appropriate selection or inactivation of the type of KR domain that codes for a particular stereochemical configuration of a hydroxyl moiety. Inactivation of a DH domain can be used to produce a polyketide having a hydroxyl moiety with a desired stereochemical configuration.



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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/25087

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 9/00; C12P 19/62

US CL : 435/183, 76

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/183, 76

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAPLUS - search terms: polyketide synthase, modular, ketoreductase, KR

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KAO et al. Alcohol stereochemistry in polyketide backbones is controlled by the b-ketoreductase domains of modular polyketide synthases. J. Am. Chem. Soc. February 1998, Vol. 120, pages 2478-2479, especially page 2479, column-bridging paragraph.	9, 11, 14
X	HOLZBAUR et al. Molecular basis of Celmer's rules: the role of the ketosynthase domain in epimerisation and demonstration that ketoreductase domains can have altered product specificity with unnatural substrates. Chemistry & Biology. April 2001, Vol. 8, pages 329-340, entire document.	9, 12, 13
A,P	OSTERGAARD et al. Stereochemistry of catalysis by the ketoreductase activity in the first extension module of the erythromycin polyketide synthase. Biochemistry. 29 January 2002, Vol. 41, pages 2719-2726.	9-14
A	KAO et al. Gain of function mutagenesis of the erythromycin polyketide synthase. 2. Engineered biosynthesis of an eight-membered ring tetraketide lactone. J. Am. Chem. Soc. 19 November 1997. Vol. 119, No. 46, pages 11339-11340.	9-14

☒ Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

02 September 2003 (02.09.2003)

Date of mailing of the international search report

23 SEP 2003

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/25087

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐
☐

- The additional search fees were accompanied by the applicant's protest.
No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-8, drawn to methods of inactivating a ketoreductase domain of a modular polyketide synthase.

Group II, claim(s) 9-14, drawn to methods of altering a modular polyketide synthase to introduce a cis double bond into the polyketide produced.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the technical features of Group I, namely the method steps, are not found in the methods of Group II, which perform different steps to produce different products. The only feature in common is the use of polyketide synthases, a feature which is well known in the art. Thus, Groups I and II cannot share a special technical feature.

INTERNATIONAL SEARCH REPORT

PCT/US02/25087

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HOLZBAUR et al. Molecular basis of Celmer's rules: the role of two ketoreductase domains in the control of chirality by the erythromycin modular polyketide synthase. Chem. & Biol. 04 March 1999, Vol. 6, No. 4, pages 189-195, entire document.	9-14
Y	US 5,672,491 A (KHOSLA et al) 30 September 1997 (30.09.1997), entire document.	9,10
T,E	REID et al. A Model of Structure and Catalysis of Ketoreductase Domains in Modular Polyketide Synthases. Biochemistry. 2003, Vol. 42, pages 72-79.	1-14