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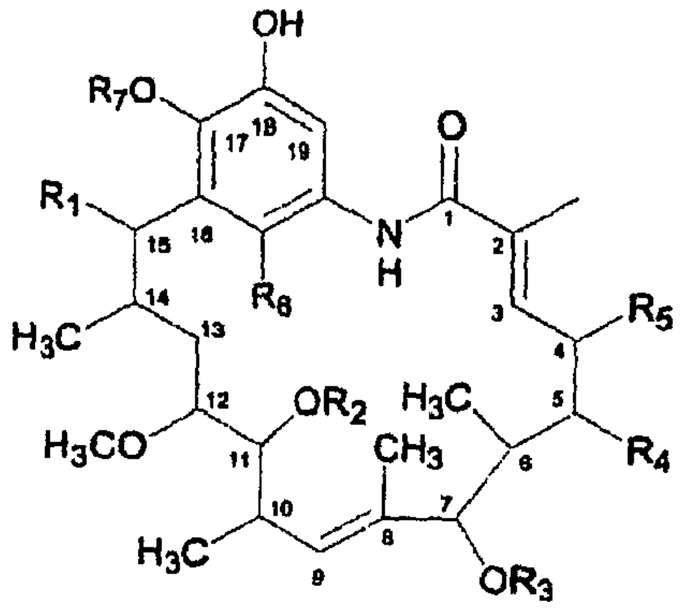
(54) Titre : DERIVES DE 17-OXYMACBECIN ET LEUR UTILISATION DANS LE TRAITEMENT DU CANCER ET/OU DES MALIGNITES TOUCHANT LES LYMPHOCYTES B

(54) Title: 17-OXYMACBECIN DERIVATIVES AND THEIR USE IN THE TREATMENT OF CANCER AND/OR B-CELL MALIGNANCIES



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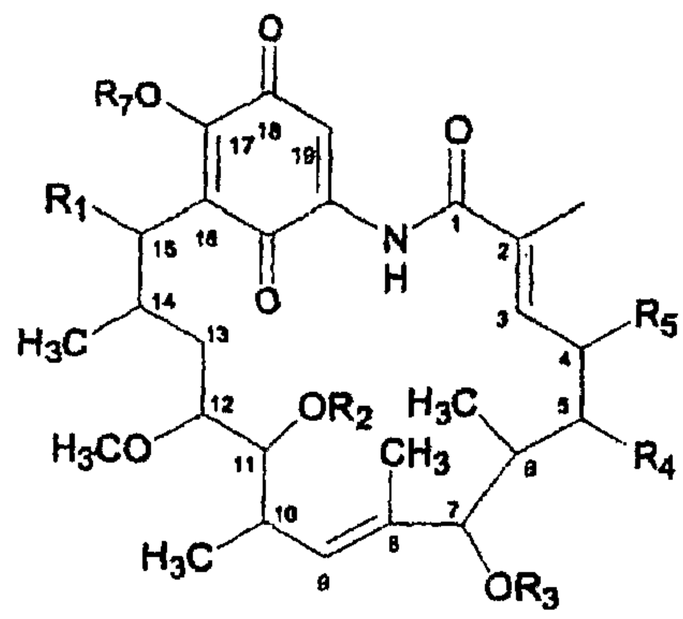

(IB)

(57) Abrégé/Abstract:
The present invention relates to 17-oxymacbecin analogues according to the formula (IA) or (IB) below, or a pharmaceutically acceptable salt thereof, wherein: R_1 represents H, OH or OCH_3; R_2 represents H or CH_3 R_3 represents H or $CONH_2$ R_4 and R_5

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(13) A1

(57) Abrégé(suite)/Abstract(continued):
either both represent H or together they represent a bond (i.e. C4 to C5 is a double bond); and R_6 represents H or OH; and R_7 represents H or CH_3. that are useful, e.g. in the treatment of cancer, B-cell malignancies, malaria, fungal infection, diseases of the central nervous system and neurodegenerative diseases, diseases dependent on angiogenesis, autoimmune diseases and/or as a prophylactic pretreatment for cancer. The present invention also provides methods for the production of these compounds and their use in medicine, in particular in the treatment and / or prophylaxis of cancer or B-cell malignancies.

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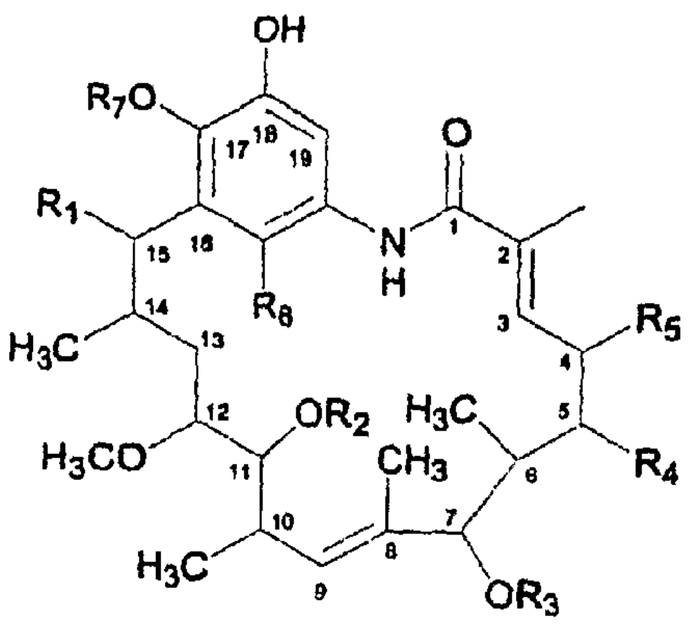

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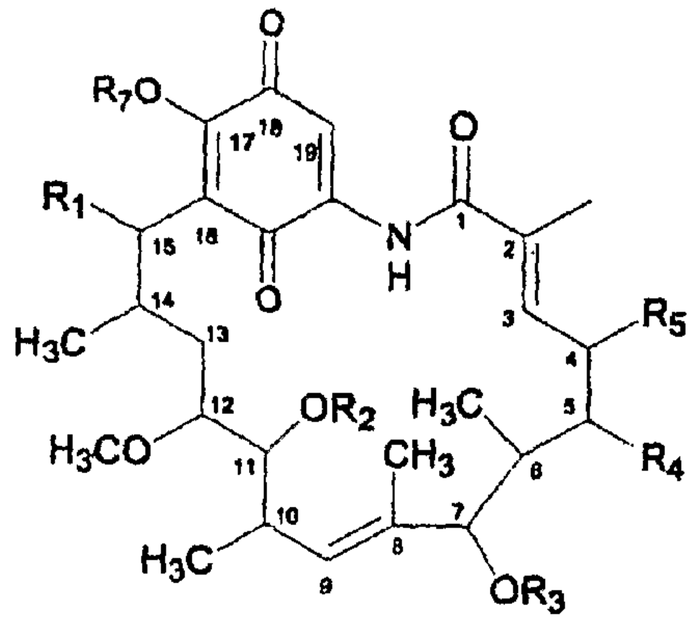

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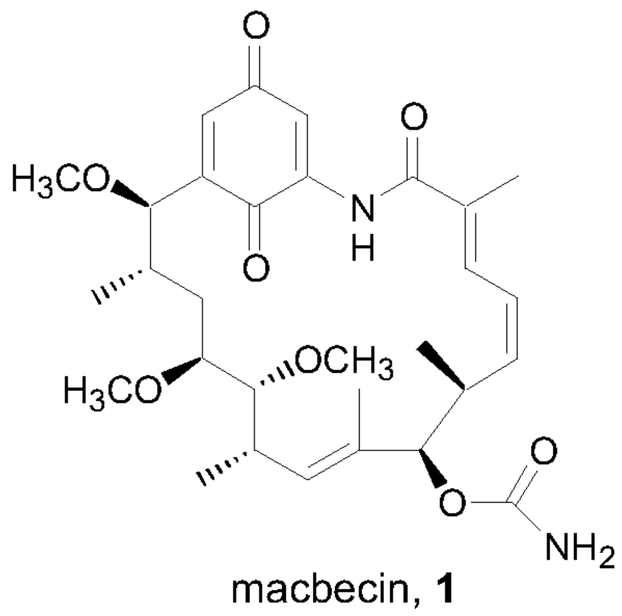
17-OXYMACBECIN DERIVATIVES AND THEIR USE IN THE TREATMENT OF CANCER AND/OR B-CELL MALIGNANCIES

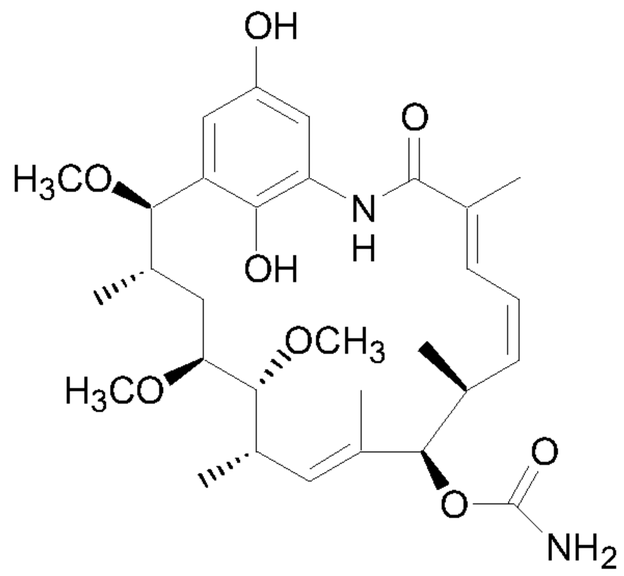
Background of the invention

The 90 kDa heat shock protein (Hsp90) is an abundant molecular chaperone involved in the folding and assembly of proteins, many of which are involved in signal transduction pathways (for reviews see Neckers, 2002; Sreedhar *et al.*, 2004a; Wegele *et al.*, 2004 and references therein). So far nearly 50 of these so-called client proteins have been identified and include steroid receptors, non-receptor tyrosine kinases e.g. *src* family, cyclin-dependent kinases e.g. cdk4 and cdk6, the cystic transmembrane regulator, nitric oxide synthase and others (Donzé and Picard, 1999; McLaughlin *et al.*, 2002; Chiosis *et al.*, 2004; Wegele *et al.*, 2004; http://www.picard.ch/downloads/Hsp90interactors.pdf). Furthermore, Hsp90 plays a key role in stress response and protection of the cell against the effects of mutation (Bagatell and Whitesell, 2004; Chiosis *et al.*, 2004). The function of Hsp90 is complicated and it involves the formation of dynamic multi-enzyme complexes (Bohen, 1998; Liu *et al.*, 1999; Young *et al.*, 2001; Takahashi *et al.*, 2003; Sreedhar *et al.*, 2004; Wegele *et al.*, 2004). Hsp90 is a target for inhibitors (Fang *et al.*, 1998; Liu *et al.*, 1999; Blagosklonny, 2002; Neckers, 2003; Takahashi *et al.*, 2003; Beliakoff and Whitesell, 2004; Wegele *et al.*, 2004) resulting in degradation of client proteins, cell cycle dysregulation and apoptosis. More recently, Hsp90 has been identified as an important extracellular mediator for tumour invasion (Eustace *et al.*, 2004). Hsp90 was identified as a new major therapeutic target for cancer therapy which is mirrored in the intense and detailed research about Hsp90 function (Blagosklonny *et al.*, 1996; Neckers, 2002; Workman and Kaye, 2002; Beliakoff and Whitesell, 2004; Harris *et al.*, 2004; Jez *et al.*, 2003; Lee *et al.*, 2004) and the development of high-throughput screening assays (Carreras *et al.*, 2003; Rowlands *et al.*, 2004). Hsp90 inhibitors include compound classes such as ansamycins, macrolides, purines, pyrazoles, coumarin antibiotics and others (for review see Bagatell and Whitesell, 2004; Chiosis *et al.*, 2004 and references therein).

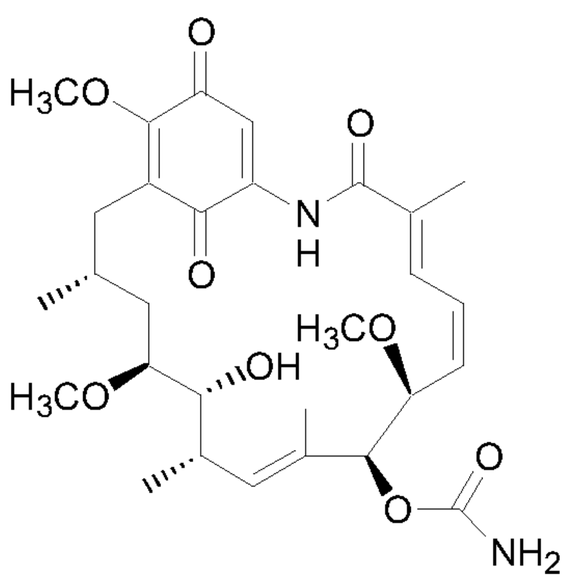
The benzenoid ansamycins are a broad class of chemical structures characterised by an aliphatic ring of varying length joined either side of an aromatic ring structure. Naturally occurring ansamycins include: macbecin and 18,21-dihydromacbecin (also known as macbecin I and macbecin II respectively) (**1** & **2**; Tanida *et al.*, 1980), geldanamycin (**3**; DeBoer *et al.*, 1970; DeBoer and Dietz, 1976; WO 03/106653 and references therein), and the herbimycin family (**4**; **5**, **6**, Omura *et al*., 1979, Iwai *et al.*, 1980 and Shibata *et al,* 1986a, WO 03/106653 and references therein).



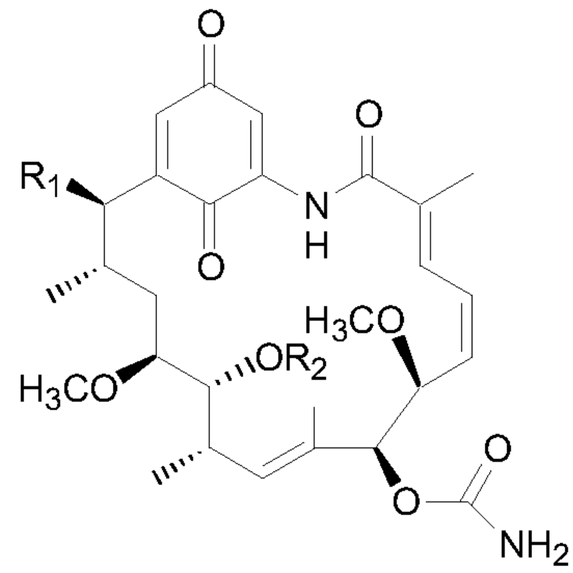
macbecin, **1**



18,21-dihydromacbecin (macbecin II), **2**



geldanamycin, **3**



herbimycin A, **4** R_1=OCH_3, R_2=CH_3
herbimycin B, **5** R_1=H, R_2=H
herbimycin C, **6** R_1=OCH_3, R_2=H

Ansamycins were originally identified for their antibacterial and antiviral activity, however, recently their potential utility as anticancer agents has become of greater interest (Beliakoff and Whitesell, 2004). Many Hsp90 inhibitors are currently being assessed in clinical trials (Csermely and Soti, 2003; Workman, 2003). In particular, geldanamycin has nanomolar potency and apparent specificity for aberrant protein kinase dependent tumour cells (Chiosis *et al.*, 2003; Workman, 2003).

It has been shown that treatment with Hsp90 inhibitors enhances the induction of tumour cell death by radiation and increased cell killing abilities (e.g. breast cancer, chronic myeloid leukaemia and non-small cell lung cancer) by combination of Hsp90 inhibitors with cytotoxic agents has also been demonstrated (Neckers, 2002; Beliakoff and Whitesell, 2004). The potential for anti-angiogenic activity is also of interest: the Hsp90 client protein HIF-1α plays a key role in the progression of solid tumours (Hur *et al.*, 2002; Workman and Kaye, 2002; Kaur *et al.*, 2004).

Hsp90 inhibitors also function as immunosuppressants and are involved in the complement-induced lysis of several types of tumour cells after Hsp90 inhibition (Sreedhar *et al.*, 2004). Treatment with Hsp90 inhibitors can also result in induced superoxide production (Sreedhar *et al.*, 2004a) associated with immune cell-mediated lysis (Sreedhar *et al.*, 2004). The use of Hsp90 inhibitors as potential anti-malaria drugs has also been discussed (Kumar *et*

al., 2003). Furthermore, it has been shown that geldanamycin interferes with the formation of complex glycosylated mammalian prion protein PrP^c (Winklhofer *et al.*, 2003).

As described above, ansamycins are of interest as potential anticancer and anti-B-cell malignancy compounds, however the currently available ansamycins exhibit poor pharmacological or pharmaceutical properties, for example they show poor water solubility, poor metabolic stability, poor bioavailability or poor formulation ability (Goetz *et al.*, 2003; Workman 2003; Chiosis 2004). Both herbimycin A and geldanamycin were identified as poor candidates for clinical trials due to their strong hepatotoxicity (review Workman, 2003) and geldanamycin was withdrawn from Phase I clinical trials due to hepatotoxicity (Supko *et al.*, 1995; WO 03/106653).

Geldanamycin was isolated from culture filtrates of *Streptomyces hygroscopicus* and shows strong activity *in vitro* against protozoa and weak activity against bacteria and fungi. In 1994 the association of geldanamycin with Hsp90 was shown (Whitesell *et al.*, 1994). The biosynthetic gene cluster for geldanamycin was cloned and sequenced (Allen and Ritchie, 1994; Rascher *et al.*, 2003; WO 03/106653). The DNA sequence is available under the NCBI accession number AY179507. The isolation of genetically engineered geldanamycin producer strains derived from *S. hygroscopicus* subsp. *duamyceticus* JCM4427 and the isolation of 4,5-dihydro-7-*O*-descarbamoyl-7-hydroxygeldanamycin and 4,5-dihydro-7-*O*-descarbamoyl-7-hydroxy-17-*O*-demethylgeldanamycin were described recently (Hong *et al.*, 2004). By feeding geldanamycin to the herbimycin producing strain *Streptomyces hygroscopicus* AM-3672 the compounds 15-hydroxygeldanamycin, the tricyclic geldanamycin analogue KOSN-1633 and methyl-geldanamycinate were isolated (Hu *et al.*, 2004). The two compounds 17-formyl-17-demethoxy-18-*O*-21-*O*-dihydrogeldanamycin and 17-hydroxymethyl-17-demethoxygeldanamycin were isolated from *S. hygroscopicus* K279-78. *S. hygroscopicus* K279-78 is *S. hygroscopicus* NRRL 3602 containing cosmid pKOS279-78 which has a 44 kbp insert which contains various genes from the herbimycin producing strain *Streptomyces hygroscopicus* AM-3672 (Hu *et al.*, 2004). Substitutions of acyltransferase domains have been made in four of the modules of the polyketide synthase of the geldanamycin biosynthetic cluster (Patel *et al.*, 2004). AT substitutions were carried out in modules 1, 4 and 5 leading to the fully processed analogues 14-desmethyl-geldanamycin, 8-desmethyl-geldanamycin and 6-desmethoxy-geldanamycin and the not fully processed 4,5-dihydro-6-desmethoxy-geldanamycin. Substitution of the module 7 acyltransferase (AT) domain lead to production of three 2-desmethyl compounds, KOSN1619, KOSN1558 and KOSN1559, one of which (KOSN1559), a 2-demethyl-4,5-dihydro-17-demethoxy-21-deoxy derivative of geldanamycin, binds to Hsp90 with a 4-fold greater binding affinity than geldanamycin and an 8-fold greater binding affinity than 17-AAG. However this is not reflected in an improvement in the IC_{50} measurement using SKBr3. Another analogue, a novel nonbenzoquinoid geldanamycin,

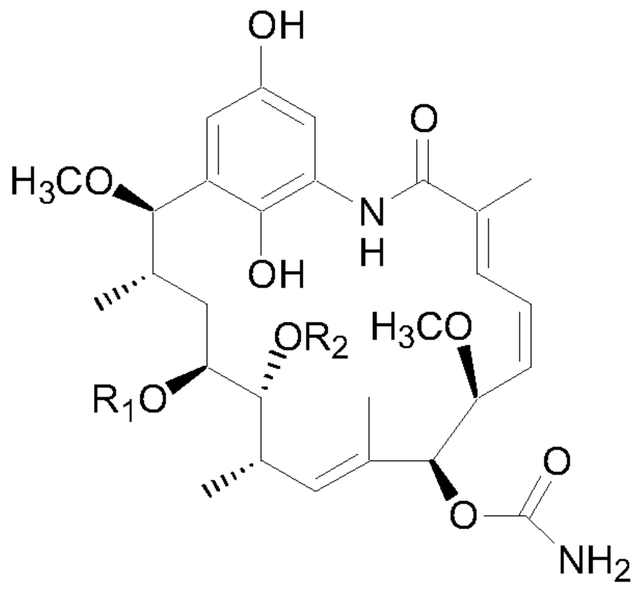
designated KOS-1806 has a monophenolic structure (Rascher *et al.*, 2005). No activity data was given for KOS-1806.

In 1979 the ansamycin antibiotic herbimycin A was isolated from the fermentation broth of *Streptomyces hygroscopicus* strain No. AM-3672 and named according to its potent herbicidal activity. The antitumour activity was established by using cells of a rat kidney line infected with a temperature sensitive mutant of Rous sarcoma virus (RSV) for screening for drugs that reverted the transformed morphology of the these cells (for review see Uehara, 2003). Herbimycin A was postulated as acting primarily through the binding to Hsp90 chaperone proteins but the direct binding to the conserved cysteine residues and subsequent inactivation of kinases was also discussed (Uehara, 2003).

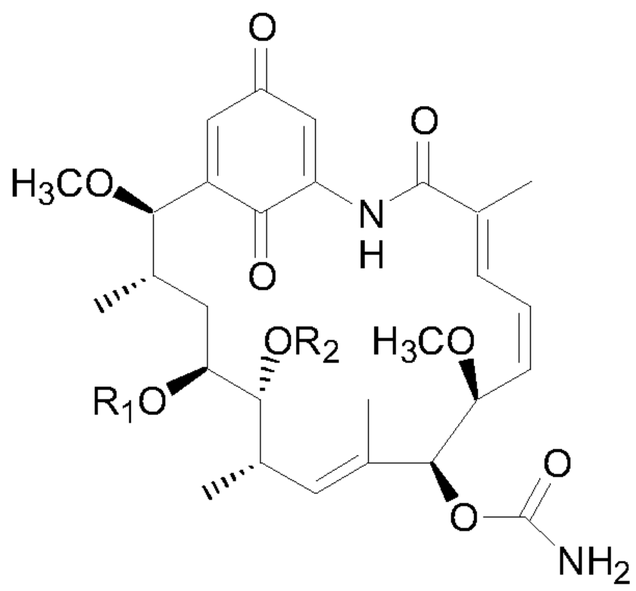
Chemical derivatives have been isolated and compounds with altered substituents at C19 of the benzoquinone nucleus and halogenated compounds in the ansa chain showed less toxicity and higher antitumour activities than herbimycin A (Omura *et al.*, 1984; Shibata *et al.,* 1986b). The sequence of the herbimycin biosynthetic gene cluster was identified in WO 03/106653 and in a recent paper (Rascher *et al.*, 2005).

The ansamycin compounds macbecin (**1**) and 18,21-dihydromacbecin (**2**) (C-14919E-1 and C-14919E-1), identified by their antifungal and antiprotozoal activity, were isolated from the culture supernatants of *Nocardia* sp No. C-14919 (*Actinosynnema pretiosum subsp pretiosum* ATCC 31280) (Tanida *et al.*, 1980; Muroi *et al.*, 1980; Muroi *et al.*, 1981; US 4,315,989 and US 4,187,292). 18,21-Dihydromacbecin is characterized by containing the dihydroquinone form of the nucleus. Both macbecin and 18,21-dihydromacbecin were shown to possess similar antibacterial and antitumour activities against cancer cell lines such as the murine leukaemia P388 cell line (Ono *et al.*, 1982). Reverse transcriptase and terminal deoxynucleotidyl transferase activities were not inhibited by macbecin (Ono *et al.*, 1982). The Hsp90 inhibitory function of macbecin has been reported in the literature (Bohen, 1998; Liu *et al.*, 1999). The conversion of macbecin and 18,21-dihydromacbecin after adding to a microbial culture broth into a compound with a hydroxy group instead of a methoxy group at a certain position or positions is described in patents US 4,421,687 and US 4,512,975.

During a screen of a large variety of soil microorganisms, the compounds TAN-420A to E were identified from producer strains belonging to the genus *Streptomyces* (**7-11,** EP 0 110 710).



TAN-420A, **7** R_1=H, R_2=H
TAN-420C, **9** R_1=H, R_2=CH_3
TAN-420E, **11** R_1=CH_3, R_2=CH_3

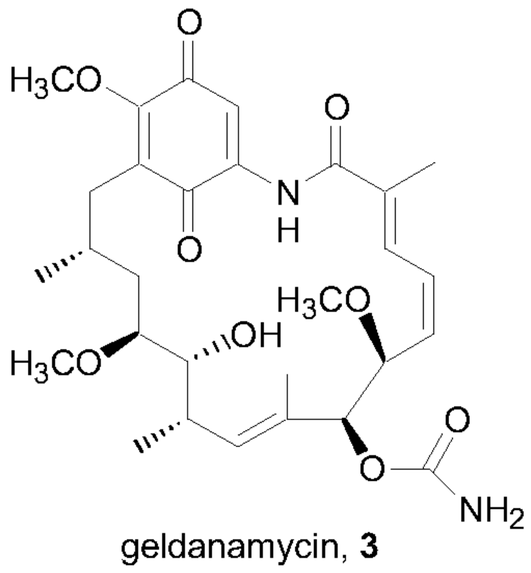


TAN-420B, **8** R_1=H, R_2=H
TAN-420D, **10** R_1=H, R_2=CH_3

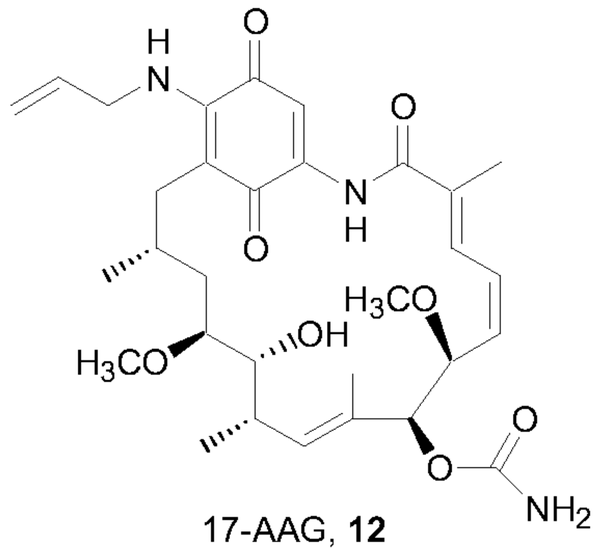
In 2000, the isolation of the geldanamycin related, non-benzoquinone ansamycin metabolite reblastin from cell cultures of *Streptomyces* sp. S6699 and its potential therapeutic value in the treatment of rheumatoid arthritis was described (Stead *et al.*, 2000).

A further Hsp90 inhibitor, distinct from the chemically unrelated benzoquinone ansamycins is Radicicol (monorden) which was originally discovered for its antifungal activity from the fungus *Monosporium bonorden* (for review see Uehara, 2003) and the structure was found to be identical to the 14-membered macrolide isolated from *Nectria radicicola*. In addition to its antifungal, antibacterial, anti-protozoan and cytotoxic activity it was subsequently identified as an inhibitor of Hsp90 chaperone proteins (for review see Uehara, 2003; Schulte *et al.*, 1999). The anti-angiogenic activity of radicicol (Hur *et al.*, 2002) and semi-synthetic derivates thereof (Kurebayashi *et al.*, 2001) has also been described.

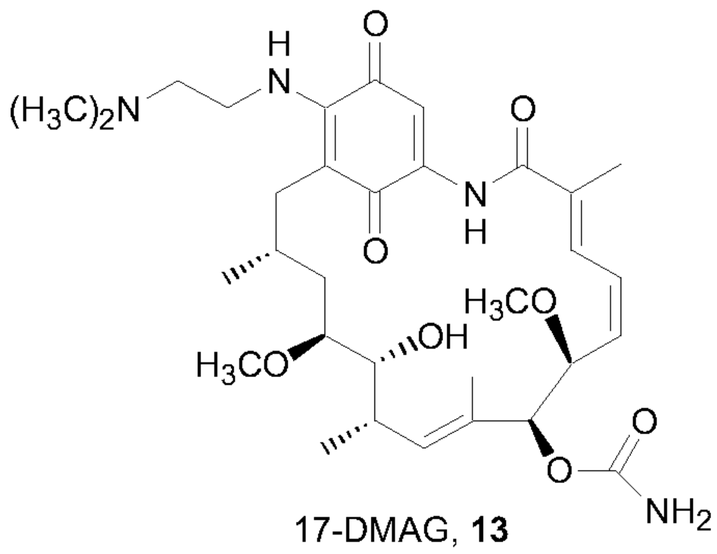
Recent interest has focussed on 17-amino derivatives of geldanamycin as a new generation of ansamycin anticancer compounds (Bagatell and Whitesell, 2004), for example 17-(allylamino)-17-desmethoxy geldanamycin (17-AAG, **12**) (Hostein *et al.*, 2001; Neckers, 2002; Nimmanapalli *et al.*, 2003; Vasilevskaya *et al.*, 2003; Smith-Jones *et al.*, 2004) and 17-desmethoxy-17-N,N-dimethylaminoethylamino-geldanamycin (17-DMAG, **13**) (Egorin *et al.*, 2002; Jez *et al.*, 2003). More recently geldanamycin was derivatised on the 17-position to create 17-geldanamycin amides, carbamates, ureas and 17-arylgeldanamycin (Le Brazidec *et al.*, 2003). A library of over sixty 17-alkylamino-17-demethoxygeldanamycin analogues has been reported and tested for their affinity for Hsp90 and water solubility (Tian *et al.*, 2004). A further approach to reduce the toxicity of geldanamycin is the selective targeting and delivering of an active geldanamycin compound into malignant cells by conjugation to a tumour-targeting monoclonal antibody (Mandler *et al.*, 2000).



geldanamycin, **3**



17-AAG, **12**



17-DMAG, **13**

Whilst many of these derivatives exhibit reduced hepatotoxicity they still have only limited water solubility. For example 17-AAG requires the use of a solubilising carrier (e.g. Cremophore®, DMSO-egg lecithin), which itself may result in side-effects in some patients (Hu *et al.,* 2004).

Most of the ansamycin class of Hsp90 inhibitors bear the common structural moiety: the benzoquinone which is a Michael acceptor that can readily form covalent bonds with nucleophiles such as proteins, glutathione, etc. The benzoquinone moiety also undergoes redox equilibrium with dihydroquinone, during which oxygen radicals are formed, which give rise to further unspecific toxicity (Dikalov *et al.,* 2002). For example treatment with geldanamycin can result in induced superoxide production (Sreedhar *et al.*, 2004a).

Therefore, there remains a need to identify novel ansamycin derivatives which may have utility in the treatment of cancer and / or B-cell malignancies, preferably such ansamycins have improved water solubility, an improved pharmacological profile and/or reduced side-effect profile for administration. The present invention discloses novel ansamycin analogues generated by genetic engineering of the parent producer strain. In particular the present invention discloses novel 17-oxymacbecin analogues which generally have improved pharmaceutical properties compared with the presently available ansamycins; in particular they are expected show improvements in respect of one or more of the following properties: activity against different cancer sub-types, toxicity, water solubility, metabolic stability, bioavailability and formulation ability. Preferably the 17-oxymacbecin analogues show improved water solubility and/or bioavailability.

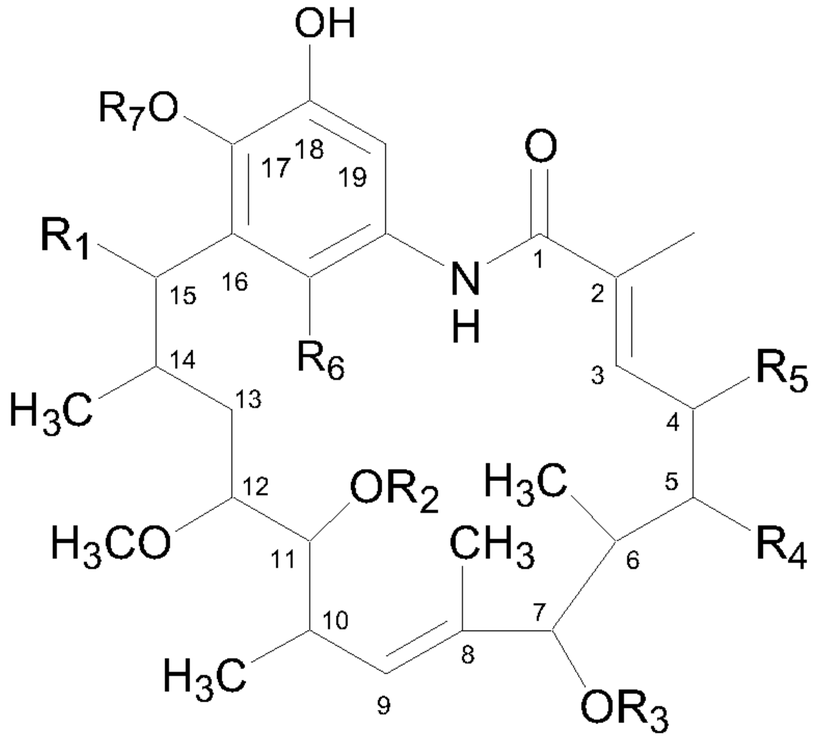
Summary of the invention

The present invention provides novel 17-oxymacbecin analogues which have either a hydroxy or a methoxy group at position C17, methods for the preparation of these compounds, and methods for the use of these compounds in medicine or as intermediates in the production of further compounds.

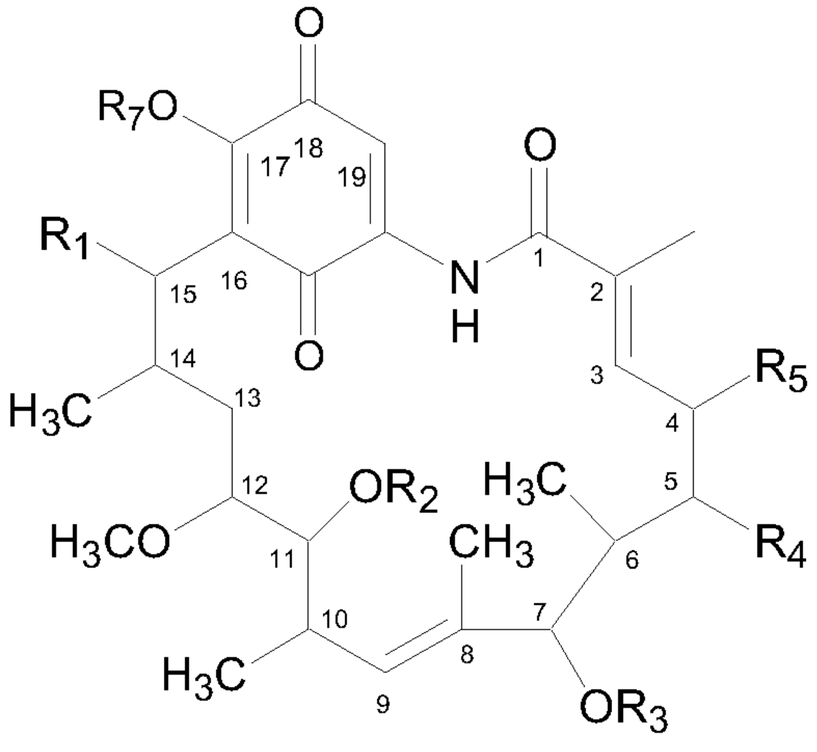
Therefore, in a first aspect the present invention provides analogues of macbecin which have a hydroxy or a methoxy group at position C17, the macbecin analogues may either have a

benzoquinone (i.e. they are macbecin I analogues) or have a dihydroquinone moiety (i.e., they are 18,21-dihydromacbecin or macbecin II analogues).

In a more specific aspect the present invention provides **17-oxymacbecin analogues** according to the formula (IA) or (IB) below, or a pharmaceutically acceptable salt thereof:



(IA)



(IB)

wherein:

R_1 represents H, OH or OCH_3;

R_2 represents H or CH_3

R_3 represents H or $CONH_2$

R_4 and R_5 either both represent H or together they represent a bond (i.e. C4 to C5 is a double bond); and

R_6 represents H or OH; and

R_7 represents H or CH_3.

The above macbecin analogues according to Formula (IA) or (IB) are also referred to herein as “compounds of the invention”, such terms are used interchangeably herein. Compounds of formula (IA) and (IB) are referred to collectively in the foregoing as compounds of formula (I).

The above structure shows a representative tautomer and the invention embraces all tautomers of the compounds of formula (I) for example keto compounds where enol compounds are illustrated and vice versa.

The invention embraces all stereoisomers of the compounds defined by structure (I) as shown above.

In a further aspect, the present invention provides macbecin analogues such as compounds of formula (I) or a pharmaceutically acceptable salt thereof, for use as a pharmaceutical.

Definitions

The articles "**a**" and "**an**" are used herein to refer to one or to more than one (i.e. at least one) of the grammatical objects of the article. By way of example "an analogue" means one analogue or more than one analogue.

As used herein the term "**analogue(s)**" refers to chemical compounds that are structurally similar to another but which differ slightly in composition (as in the replacement of one atom by another or in the presence or absence of a particular functional group).

As used herein, the term "**homologue(s)**" refers a homologue of a gene or of a protein encoded by a gene disclosed herein from either an alternative macbecin biosynthetic cluster from a different macbecin producing strain or a homologue from an alternative ansamycin biosynthetic gene cluster e.g. from geldanamycin, herbimycin or reblastatin. Such homologue(s) encode a protein that performs the same function of can itself perform the same function as said gene or protein in the synthesis of macbecin or a related ansamycin polyketide. Preferably, such homologue(s) have at least 40% sequence identity, preferably at least 60%, at least 70%, at least 80%, at least 90% or at least 95% sequence identity to the sequence of the particular gene disclosed herein (see in particular Table 3, SEQ ID NO: 11 which is a sequence of all the genes in the macbecin biosynthetic gene cluster, from which the sequences of particular genes may be deduced and Figure 6A and 6B, SEQ ID NOs: 20 and 21 which show the nucleic acid and encoded amino acid sequences of *gdmL*). Percentage identity may be calculated using any program known to a person of skill in the art such as BLASTn or BLASTp, available on the NCBI website.

As used herein, the term "**cancer**" refers to a benign or malignant new growth of cells in skin or in body organs, for example but without limitation, breast, prostate, lung, kidney, pancreas, brain, stomach or bowel. A cancer tends to infiltrate into adjacent tissue and spread (metastasise) to distant organs, for example to bone, liver, lung or the brain. As used herein the term cancer includes both metastatic tumour cell types, such as but not limited to, melanoma, lymphoma, leukaemia, fibrosarcoma, rhabdomyosarcoma, and mastocytoma and types of tissue carcinoma, such as but not limited to, colorectal cancer, prostate cancer, small cell lung cancer and non-small cell lung cancer, breast cancer, pancreatic cancer, bladder cancer, renal cancer, gastric cancer, gliobastoma, primary liver cancer and ovarian cancer.

As used herein the term "**B-cell malignancies**" includes a group of disorders that include chronic lymphocytic leukaemia (CLL), multiple myeloma, and non-Hodgkin's lymphoma (NHL). They are neoplastic diseases of the blood and blood forming organs. They cause bone marrow and immune system dysfunction, which renders the host highly susceptible to infection and bleeding.

As used herein, the term "**bioavailability**" refers to the degree to which or rate at which a drug or other substance is absorbed or becomes available at the site of biological activity after

administration. This property is dependent upon a number of factors including the solubility of the compound, rate of absorption in the gut, the extent of protein binding and metabolism etc. Various tests for bioavailability that would be familiar to a person of skill in the art are for example described in Egorin *et al.* (2002).

The term "**water solubility**" as used in this application refers to solubility in aqueous media, e.g. phosphate buffered saline (PBS) at pH 7.3. An exemplary water solubility assay is given in the Examples below

As used herein the term "**post-PKS genes(s)**" refers to the genes required for post-polyketide synthase modifications of the polyketide, for example but without limitation monooxygenases, *O*-methyltransferases and carbamoyltransferases. This term also specifically encompasses the genes required for the addition of the oxygen to position C17, e.g. *gdmL* and homologues thereof. Particularly, the term "**macbecin post-PKS gene(s)**" refers to those modifying genes in the macbecin PKS gene cluster, i.e. *mbcM, mbcN, mbcP, mbcMT1, mbcMT2* and *mbcP450.*

The pharmaceutically acceptable salts of compounds of the invention such as the compounds of formula (I) include conventional salts formed from pharmaceutically acceptable inorganic or organic acids or bases as well as quaternary ammonium acid addition salts. More specific examples of suitable acid salts include hydrochloric, hydrobromic, sulfuric, phosphoric, nitric, perchloric, fumaric, acetic, propionic, succinic, glycolic, formic, lactic, maleic, tartaric, citric, palmoic, malonic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, fumaric, toluenesulfonic, methanesulfonic, naphthalene-2-sulfonic, benzenesulfonic hydroxynaphthoic, hydroiodic, malic, steroic, tannic and the like. Other acids such as oxalic, while not in themselves pharmaceutically acceptable, may be useful in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable salts. More specific examples of suitable basic salts include sodium, lithium, potassium, magnesium, aluminium, calcium, zinc, N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, N-methylglucamine and procaine salts. References hereinafter to a compound according to the invention include both compounds of formula (I) and their pharmaceutically acceptable salts.

As used herein the terms "18,21-dihydromacbecin" and "macbecin II" (the dihydroquinone form of macbecin) are used interchangeably.

Brief Description of the Drawings

Figure 1: Representation of the biosynthesis of macbecin showing the first putative enzyme free intermediate, pre-macbecin and the post-PKS processing to macbecin. The list of PKS processing steps in the figure in not intended to

represent the order of events. The following abbreviations are used for particular genes in the cluster: AL0 - AHBA loading domain; ACP – Acyl Carrier Protein; KS - ß–ketosynthase; AT - acyl transferase; DH - dehydratase; ER - enoyl reductase; KR - β-ketoreductase.

Figure 2: Depiction of the sites of post-PKS processing of pre-macbecin to give macbecin.

Figure 3: Diagrammatic representation of the generation of an *Actinosynnema pretiosum* strain in which the *mbcP, mbcP450, mbcMT1 and mbcMT2* genes have been deleted in frame.

Figure 4: Sequence of the amplified PCR product 1+2a (SEQ ID NO: 14)

Figure 5: Sequence of the amplified PCR product 3b+4 (SEQ ID NO: 17)

Figure 6: A – nucleic acid sequence of the PCR product containing *gdmL*

B – amino acid sequence of GdmL

Description of the Invention

The present invention provides 17-oxymacbecin analogues, as set out above, methods for the preparation of these compounds, methods for the use of these compounds in medicine and the use of these compounds as intermediates or templates for further semi-synthetic derivatisation or derivatisation by biotransformation methods.

Suitably the 17-oxymacbecin analogues have a structure according to Formula IA.

Suitably the 17-oxymacbecin analogues have a structure according to Formula IB.

Suitably R_3 represents $CONH_2$

Suitably R_6 represents OH. Alternatively R_6 represents H.

Suitably R_7 represents H.

In a specific embodiment, the 17-oxymacbecin analogues have a structure according to Formula (IA), wherein R_1 represents H, R_2 represents H, R_3 represents $CONH_2$, R_4 and R_5 each represent H, R_6 represents OH and R_7 represents H.

In a specific embodiment, the 17-oxymacbecin analogues have a structure according to Formula (IB), wherein R_1 represents H, R_2 represents H, R_3 represents $CONH_2$, R_4 and R_5 each represent H, and R_7 represents H

In a specific embodiment, the 17-oxymacbecin analogues have a structure according to Formula (IA), wherein R_1 represents H, R_2 represents H, R_3 represents $CONH_2$, R_4 and R_5 each represent H, R_6 represents OH and R_7 represents CH_3.

In a specific embodiment, the 17-oxymacbecin analogues have a structure according to Formula (IB), wherein R_1 represents H, R_2 represents H, R_3 represents $CONH_2$, R_4 and R_5 each represent H, and R_7 represents CH_3.

In a specific embodiment, the 17-oxymacbecin analogues have a structure according to

Formula (IA), wherein R_1 represents H, R_2 represents H, R_3 represents $CONH_2$, R_4 and R_5 each represent H, R_6 represents H and R_7 represents H.

In a specific embodiment, the 17-oxymacbecin analogues have a structure according to Formula (IA), wherein R_1 represents H, R_2 represents H, R_3 represents $CONH_2$, R_4 and R_5 each represent H, R_6 represents H and R_7 represents CH_3.

The preferred stereochemistry of the non-hydrogen sidechains to the ansa ring is as shown for macbecin in Figures 1 and 2 (that is to say the preferred stereochemistry follows that of macbecin).

The compounds of the invention where R_6 represents OH, may be isolated from the fermentation broth in their benzoquinone form or in their dihydroquinone form. It is well-known in the art that benzoquinones can be chemically converted to dihydroquinones (reduction) and *vice versa* (oxidation), therefore these forms may be readily interconverted using methods well-known to a person of skill in the art. For example, but without limitation, if the benzoquinone form is isolated then it may be converted to the corresponding dihydroquinones. As an example (but not by way of limitation) this may be achieved in organic media with a source of hydride, such as but not limited to, $LiAlH_4$ or $SnCl_2$-HCl. Alternatively this transformation may be mediated by dissolving the benzoquinone form of the compound of the invention in organic media and then washing with an aqueous solution of a reducing agent, such as, but not limited to, sodium hydrosulfite ($Na_2S_2O_4$ or sodium thionite). Preferably, this transformation is carried out by dissolving the compound of the invention in ethyl acetate and mixing this solution vigorously with an aqueous solution of sodium hydrosulfite (Muroi *et al.*, 1980). The resultant organic solution can then be washed with water, dried and the solvent removed under reduced pressure to yield an almost quantitative amount of the 18,21-dihydro form of the compound of the invention.

In order to oxidise a dihydroquinone to a quinone several routes are available, including, but not limited to the following: the dihydroquinone form of the compound of the invention is dissolved in an organic solvent such as ethyl acetate and then this solution is vigorously mixed with an aqueous solution of iron (III) chloride ($FeCl_3$). The organic solution can then be washed with water, dried and the organic solvent removed under reduced pressure to yield an almost quantitative amount of the benzoquinone form of the macbecin compound.

The present invention also provides a pharmaceutical composition comprising a 17-oxymacbecin analogue, or a pharmaceutically acceptable salt thereof, together with a pharmaceutically acceptable carrier.

The present invention also provides for the use of a 17-oxymacbecin analogue as a substrate for further modification either by biotransformation or by synthetic chemistry.

In one aspect the present invention provides for the use of a 17-oxymacbecin analogue in the manufacture of a medicament. In a further embodiment the present invention provides for the

use of a 17-oxymacbecin analogue in the manufacture of a medicament for the treatment of cancer and/or B-cell malignancies. In a further embodiment the present invention provides for the use of a 17-oxymacbecin analogue in the manufacture of a medicament for the treatment of malaria, fungal infection, diseases of the central nervous system, diseases dependent on angiogenesis, autoimmune diseases and/or as a prophylactic pre-treatment for cancer.

In another aspect the present invention provides for the use of a 17-oxymacbecin analogue in medicine. In a further embodiment the present invention provides for the use of a 17-oxymacbecin analogue in the treatment of cancer and/or B-cell malignancies. In a further embodiment the present invention provides for the use of a 17-oxymacbecin analogue in the manufacture of a medicament for the treatment of malaria, fungal infection, diseases of the central nervous system and neurodegenerative diseases, diseases dependent on angiogenesis, autoimmune diseases and/or as a prophylactic pre-treatment for cancer.

In a further embodiment the present invention provides a method of treatment of cancer and/or B-cell malignancies, said method comprising administering to a patient in need thereof a therapeutically effective amount of a 17-oxymacbecin analogue. In a further embodiment the present invention provides a method of treatment of malaria, fungal infection, diseases of the central nervous system and neurodegenerative diseases, diseases dependent on angiogenesis, autoimmune diseases and/or a prophylactic pre-treatment for cancer, said method comprising administering to a patient in need thereof a therapeutically effective amount of a 17-oxymacbecin analogue.

As noted above, compounds of the invention may be expected to be useful in the treatment of cancer and/or B-cell malignancies. Compounds of the invention may also be effective in the treatment of other indications for example, but not limited to malaria, fungal infection, diseases of the central nervous system and neurodegenerative diseases, diseases dependent on angiogenesis, autoimmune diseases such as rheumatoid arthritis and/or as a prophylactic pre-treatment for cancer.

Diseases of the central nervous system and neurodegenerative diseases include, but are not limited to, Alzheimer's disease, Parkinson's disease, Huntington's disease, prion diseases, spinal and bulbar muscular atrophy (SBMA) and amyotrophic lateral sclerosis (ALS).

Diseases dependent on angiogenesis include, but are not limited to, age-related macular degeneration, diabetic retinopathy and various other ophthalmic disorders, atherosclerosis and rheumatoid arthritis.

Autoimmune diseases include, but are not limited to, rheumatoid arthritis, multiple sclerosis, type I diabetes, systemic lupus erythematosus and psoriasis.

"Patient" embraces human and other animal (especially mammalian) subjects, preferably human subjects. Accordingly the methods and uses of the 17-oxymacbecin analogues of the invention are of use in human and veterinary medicine, preferably human medicine.

The aforementioned compounds of the invention or a formulation thereof may be administered by any conventional method for example but without limitation they may be administered parenterally (including intravenous administration), orally, topically (including buccal, sublingual or transdermal), via a medical device (e.g. a stent), by inhalation, or via injection (subcutaneous or intramuscular). The treatment may consist of a single dose or a plurality of doses over a period of time.

Whilst it is possible for a compound of the invention to be administered alone, it is preferable to present it as a pharmaceutical formulation, together with one or more acceptable carriers. Thus there is provided a pharmaceutical composition comprising a compound of the invention together with one or more pharmaceutically acceptable diluents or carriers. The diluents(s) or carrier(s) must be "acceptable" in the sense of being compatible with the compound of the invention and not deleterious to the recipients thereof. Examples of suitable carriers are described in more detail below.

The compounds of the invention may be administered alone or in combination with other therapeutic agents. Co-administration of two (or more) agents may allow for significantly lower doses of each to be used, thereby reducing the side effects seen. It might also allow resensitisation of a disease, such as cancer, to the effects of a prior therapy to which the disease has become resistant. There is also provided a pharmaceutical composition comprising a compound of the invention and a further therapeutic agent together with one or more pharmaceutically acceptable diluents or carriers.

In a further aspect, the present invention provides for the use of a compound of the invention in combination therapy with a second agent e.g. a second agent for the treatment of cancer or B-cell malignancies such as a cytotoxic or cytostatic agent..

In one embodiment, a compound of the invention is co-administered with another therapeutic agent e.g. a therapeutic agent such as a cytotoxic or cytostatic agent for the treatment of cancer or B-cell malignancies. Exemplary further agents include cytotoxic agents such as alkylating agents and mitotic inhibitors (including topoisomerase II inhibitors and tubulin inhibitors). Other exemplary further agents include DNA binders, antimetabolites and cytostatic agents such as protein kinase inhibitors and tyrosine kinase receptor blockers. Suitable agents include, but are not limited to, methotrexate, leukovorin, prenisone, bleomycin, cyclophosphamide, 5-fluorouracil, paclitaxel, docetaxel, vincristine, vinblastine, vinorelbine, doxorubicin (adriamycin), tamoxifen, toremifene, megestrol acetate, anastrozole, goserelin, anti-HER2 monoclonal antibody (e.g. trastuzumab, trade name Herceptin™), capecitabine, raloxifene hydrochloride, EGFR inhibitors (e.g. gefitinib, trade name Iressa ®, erlotinib, trade name Tarceva™, cetuximab, trade name Erbitux™), VEGF inhibitors (e.g. bevacizumab, trade name Avastin™), proteasome inhibitors (e.g. bortezomib, trade name Velcade™). Further suitable agents include, but are not limited to, conventional chemotherapeutics such as

cisplatin, cytarabine, cyclohexylchloroethylnitrosurea, gemcitabine, Ifosfamid, leucovorin, mitomycin, mitoxantone, oxaliplatin, taxanes including taxol and vindesine; hormonal therapies ; monoclonal antibody therapies such as cetuximab (anti-EGFR); protein kinase inhibitors such as dasatinib, lapatinib; histone deacetylase (HDAC) inhibitors such as vorinostat; angiogenesis inhibitors such as sunitinib, sorafenib, lenalidomide; and mTOR inhibitors such as temsirolimus. A further suitable agent is imatinib, trade name Glivec ® . Additionally, a compound of the invention may be administered in combination with other therapies including, but not limited to, radiotherapy or surgery.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient (compound of the invention) with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

The compounds of the invention will normally be administered orally or by any parenteral route, in the form of a pharmaceutical formulation comprising the active ingredient, optionally in the form of a non-toxic organic, or inorganic, acid, or base, addition salt, in a pharmaceutically acceptable dosage form. Depending upon the disorder and patient to be treated, as well as the route of administration, the compositions may be administered at varying doses.

For example, the compounds of the invention can be administered orally, buccally or sublingually in the form of tablets, capsules, ovules, elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate-, delayed- or controlled-release applications.

Such tablets may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate and glycine, disintegrants such as starch (preferably corn, potato or tapioca starch), sodium starch glycollate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethylcellulose (HPMC), hydroxy-propylcellulose (HPC), sucrose, gelatine and acacia. Additionally, lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate and talc may be included.

Solid compositions of a similar type may also be employed as fillers in gelatine capsules. Preferred excipients in this regard include lactose, starch, a cellulose, milk sugar or high molecular weight polyethylene glycols. For aqueous suspensions and/or elixirs, the compounds of the invention may be combined with various sweetening or flavouring agents, colouring matter or dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol and glycerine, and combinations thereof.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. povidone, gelatine, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (e.g. sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethylcellulose in varying proportions to provide desired release profile.

Formulations in accordance with the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatine and glycerine, or sucrose and acacia; and mouth-washes comprising the active ingredient in a suitable liquid carrier.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

Pharmaceutical compositions adapted for topical administration may be formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, impregnated dressings, sprays, aerosols or oils, transdermal devices, dusting powders, and the like. These compositions may be prepared via conventional methods containing the active agent. Thus, they may also comprise compatible conventional carriers and additives, such as preservatives, solvents to assist drug penetration, emollient in creams or ointments and ethanol or oleyl alcohol for lotions. Such carriers may be present as from about 1% up to about 98% of the composition. More usually they will form up to about 80% of the composition. As an illustration only, a cream or ointment is prepared by mixing sufficient quantities of hydrophilic material and water, containing from about 5-10% by weight of the compound, in sufficient quantities to produce a cream or ointment having the desired consistency.

Pharmaceutical compositions adapted for transdermal administration may be presented as discrete patches intended to remain in intimate contact with the epidermis of the recipient for a

prolonged period of time. For example, the active agent may be delivered from the patch by iontophoresis.

For applications to external tissues, for example the mouth and skin, the compositions are preferably applied as a topical ointment or cream. When formulated in an ointment, the active agent may be employed with either a paraffinic or a water-miscible ointment base.

Alternatively, the active agent may be formulated in a cream with an oil-in-water cream base or a water-in-oil base.

For parenteral administration, fluid unit dosage forms are prepared utilizing the active ingredient and a sterile vehicle, for example but without limitation water, alcohols, polyols, glycerine and vegetable oils, water being preferred. The active ingredient, depending on the vehicle and concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the active ingredient can be dissolved in water for injection and filter sterilised before filling into a suitable vial or ampoule and sealing.

Advantageously, agents such as local anaesthetics, preservatives and buffering agents can be dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum. The dry lyophilized powder is then sealed in the vial and an accompanying vial of water for injection may be supplied to reconstitute the liquid prior to use.

Parenteral suspensions are prepared in substantially the same manner as solutions, except that the active ingredient is suspended in the vehicle instead of being dissolved and sterilization cannot be accomplished by filtration. The active ingredient can be sterilised by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the active ingredient.

The compounds of the invention may also be administered using medical devices known in the art. For example, in one embodiment, a pharmaceutical composition of the invention can be administered with a needleless hypodermic injection device, such as the devices disclosed in U.S. 5,399,163; U.S. 5,383,851; U.S. 5,312,335; U.S. 5,064,413; U.S. 4,941,880; U.S. 4,790,824; or U.S. 4,596,556. Examples of well-known implants and modules useful in the present invention include : US 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; US 4,486,194, which discloses a therapeutic device for administering medicaments through the skin; US 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; US 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; US 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and US 4,475,196, which discloses an osmotic drug delivery system. Many other such implants, delivery systems, and modules are known to those skilled in the art.

The dosage to be administered of a compound of the invention will vary according to the particular compound, the disease involved, the subject, and the nature and severity of the disease and the physical condition of the subject, and the selected route of administration. The appropriate dosage can be readily determined by a person skilled in the art.

The compositions may contain from 0.1% by weight, preferably from 5-60%, more preferably from 10-30% by weight, of a compound of invention, depending on the method of administration.

It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of a compound of the invention will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the age and condition of the particular subject being treated, and that a physician will ultimately determine appropriate dosages to be used. This dosage may be repeated as often as appropriate. If side effects develop the amount and/or frequency of the dosage can be altered or reduced, in accordance with normal clinical practice.

In a further aspect the present invention provides methods for the production of 17-oxymacbecin analogues.

Macbecin can be considered to be biosynthesised in two stages. In the first stage the core-PKS genes assemble the macrolide core by the repeated assembly of 2-carbon units which are then cyclised to form the first enzyme-free intermediate “pre-macbecin”, see Figure 1. In the second stage a series of “post-PKS” tailoring enzymes (e.g. P450 oxygenases, methyltransferases, FAD-dependent oxygenases and a carbamoyltransferase) act to add the various additional groups to the pre-macbecin template resulting in the final parent compound structure, see Figure 2. The 17-oxymacbecin analogues of the invention may be biosynthesised in a similar manner.

This biosynthetic production may be exploited by genetic engineering of suitable producer strains to result in the production of novel compounds. In particular, the present invention provides a method of producing 17-oxymacbecin analogues said method comprising:

a) providing a first host strain that produces macbecin or an analogue thereof when cultured under appropriate conditions

b) inserting one or more post-PKS genes capable of oxidising the C17 position of macbecin,

c) culturing said modified host strain under suitable conditions for the production of novel compounds; and

d) optionally isolating the compounds produced.

In step (a) by “macbecin or an analogue thereof” is meant macbecin or those analogues of macbecin that are embraced by the definition of R_1.

In step (b) the inserted post-PKS gene(s) is preferably *gdmL,* or a homologue therof

The method may additionally comprise the following step:

e) deleting or inactivating one or more macbecin post-PKS genes, or homologues thereof, said step usually occurring prior to step c) and may occur prior to step b).

In step e), deleting or inactivating one or more post-PKS genes, will suitably be done selectively.

Alternative methods additionally comprise the step of

f) reintroducing one or more of the deleted post-PKS genes, said step usually occurring prior to step c; and/or

g) introducing post-PKS genes from other PKS clusters, said step usually occurring prior to step c).

In a further embodiment, step e) comprises inactivating one or more post-PKS genes, or a homologue thereof, by integration of DNA into the gene(s) such that functional protein is not produced. In an alternative embodiment, step e) comprises making a targeted deletion of one or more post-PKS genes, or a homologue thereof. In a further embodiment one or more post-PKS genes, or a homologue thereof, are inactivated by site-directed mutagenesis. In a further embodiment the host strain of step a) is subjected to mutagenesis and a modified strain is selected in which one or more of the post-PKS enzymes, or a homologue thereof, is not functional. The present invention also encompasses mutations of the regulators controlling the expression of one or more post-PKS genes, or a homologue thereof, a person of skill in the art will appreciate that deletion or inactivation of a regulator may have the same outcome as deletion or inactivation of the gene.

In a further embodiment the strain of step e) is complemented with one or more of the genes that have been deleted or inactivated, or a homologue thereof.

In a further embodiment the strain of step e) is complemented with one or more post-PKS genes from a different PKS cluster for example but not limited to a gene expressing a protein capable of transferring a methyl group onto the hydroxy at C17.

In a particular embodiment of the present invention, a method of selectively inserting a post PKS gene comprises:

(i) isolating the gene responsible for C17-hydroxylation by PCR amplification using genomic DNA as a template, where the genomic DNA is of a strain that itself produces a related suitably hydroxylated molecule, for example isolating the *gdmL* gene from a geldanamycin producer either by using specific primers based on the published sequence of *gdmL* or degenerate primers based on the published sequence of *gdmL* if the template is a *gdmL* gene or homogloue of *gdmL* for which the sequence is not available.

(ii) Cloning this gene into a suitable vector for transfer into the host cell, that will be maintained in the cell and will allow expression of the *gdmL* gene or

homologue thereof to produce a functional C17-hydroxylase. For example, but not limited to, cloning of the *Streptomyces hygroscopicus* NRRL 3602 *gdmL* gene to place it under the *actI* promoter in a vector also containing the *actII*-ORF4 activator to facilitate expression of *gdmL*. The vector used in example 2 also contains the *oriT* for conjugal transfer, a phiBT1 attachment site and an apramycin resistance marker.

(iii) Transformation of the host cell with this vector for example by conjugation.

One skilled in the art will readily accept that maintenance of a piece of DNA in a host cell can be achieved by a number of standard methods. In a preferred embodiment the promoter and *gdmL* or a homologue thereof may be introduced into the chromosomal phage attachment site of the *Streptomyces* phage phiBT1 (Gregory *et al*., 2003) as described in example 2. One skilled in the art will appreciate that expression of the target gene is not limited to introducing the vector at this phage attachment site, or indeed to the use of an attachment site. Therefore, the expression vector can be introduced into other phage attachment sites such as the attachment site for *Streptomyces* phage phiC31 for example by using a derivative of pSET152 (Bierman *et al*., 1992). Such integration may similarly be performed using other available integration functions including but not limited to: vectors based on pSAM2 integrase (*e.g.* in pPM927 (Smovkina *et al*., 1990)), R4 integrase (*e.g.* in pAT98 (Matsuura *et al*., 1996)), VWB integrase (*e.g.* in pKT02 (Van Mellaert *et al*., 1998)), and L5 integrase (e.g. Lee *et al.,* 1991). One skilled in the art will recognise that there are many Actinomycete phages which may be expected to contain integration functions that could be transferred to a delivery vector along with a suitable promoter to generate further systems that can be used to introduce genes into *A. pretiosum*. Indeed many phages have been identified from Actinomycetes and integration functions could be obtained from those and utilised in a similar way. As more phages are characterised one would expect there to be further available integrases that could be ued similarly. In some cases this may need alteration of the host strain by addition of the specific *attB* site for the integrase to enable high efficiency integration. Introduction of *gdmL* or a homologue thereof under an appropriate promoter can also be effected by, without limitation, homologous recombination into a neutral position in the chromosome, homologous recombination into a non-neutral position in the chromosome (for example to disrupt a chosen gene). Self-replicating vectors can also be used for example, but not limited to, vectors containing the *Streptomyces* origin of replication from pSG5 (e.g. pKC1139 Bierman *et al*., 1992), pIJ101 (e.g. pIJ487, Kieser *et al*., 2000) and SCP2* (*e.g.* pIJ698, Kieser *et al*., 2000).

One skilled in the art will also readily accept that there are many promoters that can be used for production of GdmL or a homologue thereof, for example one could use a promoter from a secondary metabolite biosynthetic cluster such as the *gdmL* promoter, the actI or actIII promoters which are generally used along with their cognate activator actII-ORF4 (Rowe et al.,

1998) as in example 2, promoters responding to stress such as the promoter for resistance to pristinamycin (Blanc *et al*., 1995) and the erythromycin resistance gene ermE promoter, P_{ermE} (Bibb et al., 1985) and the mutated version, P_{ermE*}.

In a particular embodiment of the present invention, a method of selectively deleting or inactivating a post PKS gene comprises:

(i) designing degenerate oligos based on homologue(s) of the gene of interest (e.g. from the geldanamycin PKS biosynthetic cluster and/or from the herbimycin biosynthetic cluster) and isolating the internal fragment of the gene of interest (or a homologue thereof) from a suitable macbecin producing strain for example by using these primers in a PCR reaction,

(ii) integrating a plasmid containing this fragment into either the same, or a different macbecin producing strain followed by homologous recombination, which results in the disruption of the targeted gene (or a homologue thereof),

(iii) culturing the strain thus produced under conditions suitable for the production of the macbecin analogues.

In a specific embodiment, the macbecin-producing strain in step (i) is *Actinosynnema mirum (A. mirum).* In a further specific embodiment the macbecin-producing strain in step (ii) is *Actinosynnema pretiosum (A. pretiosum)*

A person of skill in the art will appreciate that an equivalent strain may be achieved using alternative methods to that described above, e.g.:

- Degenerate oligos may be used to amplify the gene of interest from one of a number of macbecin producing strains for example, but not limited to *A. pretiosum,* or *A. mirum*
- Different degenerate oligos may be designed which will successfully amplify an appropriate region of the target gene of a macbecin producer, or a homologue thereof.
- The sequence of the target gene of the *A. pretiosum* strain may be used to generate the oligos which may be specific to the target gene of *A. pretiosum* and then the internal fragment may be amplified from any macbecin producing strain e.g *A. pretiosum* or *A. mirum*.
- The sequence of the target gene of the *A. pretiosum* strain may be used along with the sequence of homologous genes to generate the degenerate oligos and then the internal fragment may be amplified from any macbecin producing strain e.g *A. pretiosum* or *A. mirum*.

Figure 2 shows the activity of the post-PKS genes in the macbecin biosynthetic cluster. A person of skill in the art would thus be able to identify which additional post-PKS genes would need to be deleted or inactivated in order to arrive at a strain that will produce the compound(s) of interest.

It may be observed in these systems that when a strain is generated in which an additional post-PKS gene has been inserted and optionally in which one or more of the post-PKS genes, or a homologue thereof, does not function as a result of one of the methods described including inactivation or deletion, and optionally further post-PKS genes have been re-inserted, that more than one macbecin analogue may be produced. There are a number of possible reasons for this which will be appreciated by those skilled in the art. For example there may be a preferred order of post-PKS steps and removing a single activity leads to all subsequent steps being carried out on substrates that are not natural to the enzymes involved. This can lead to intermediates building up in the culture broth due to a lowered efficiency towards the novel substrates presented to the post-PKS enzymes, or to shunt products which are no longer substrates for the remaining enzymes possibly because the order of steps has been altered. Aternatively there may be effects on the expression of some genes in the biosynthetic pathway.

A person of skill in the art will appreciate that the ratio of compounds observed in a mixture can be manipulated by using variations in the growth conditions.

When a mixture of compounds is observed these can be readily separated using standard techniques some of which are described in the following examples.

17-oxymacbecin analogues may be screened by a number of methods, as described herein, and in the circumstance where a single compound shows a favourable profile a strain can be engineered to make this compound preferably. In the unusual circumstance when this is not possible, an intermediate can be generated which is then biotransformed to produce the desired compound.

The present invention provides novel macbecin analogues generated by the selected insertion of one or more post-PKS genes capable of oxidising the 17 position of macbecin, optionally in combination with the deletion or inactivation of one or more post-PKS genes from the macbecin PKS gene cluster. In particular, the present invention relates to novel 17-oxymacbecin analogues produced by the insertion of *gdmL* or a homologue thereof optionally combined with the selected deletion or inactivation of one or more post-PKS genes, or a homologue thereof, from the macbecin PKS gene cluster. In a specific embodiment, one or more post-PKS genes selected from the group consisting of: *mbcP, mbcM, mbcN, mbcP450, mbcMT1* and *mbcMT2* are additionally deleted or inactivated in the host strain. In a further embodiment, two or more of the post-PKS genes selected from the group consisting of *mbcP, mbcM, mbcN, mbcP450, mbcMT1* and *mbcMT2* are additionally deleted or inactivated. In a further embodiment, three or more of the post-PKS genes selected from the group consisting of *mbcP, mbcM, mbcN, mbcP450, mbcMT1* and *mbcMT2* are additionally deleted or inactivated. In a further embodiment, four or more of the post-PKS genes selected from the group consisting of *mbcP, mbcM, mbcN, mbcP450, mbcMT1* and *mbcMT2* are additionally deleted or inactivated. In a further

embodiment, five or more of the post-PKS genes selected from the group consisting of *mbcP, mbcM, mbcN, mbcP450, mbcMT1* and *mbcMT2* are additionally deleted or inactivated.

In a specific embodiment *mbcP, mbcP450, mbcMT1* and *mbcMT2* have been deleted and *gdmL* has been introduced (eg at a phage attachment site) and expressed from a promoter to yield 4,5-dihydro-11-*O*-desmethyl-15-desmethoxy-17-hydroxymacbecin.

In a specific embodiment *mbcM* has been deleted and *gdmL* has been introduced (eg at a phage attachment site) and expressed from a promoter to yield 4,5-dihydro-11-*O*-desmethyl-15-desmethoxy-17-hydroxy-21-desoxymacbecin.

In a specific embodiment *mbcM* has been deleted and *gdmL* has been introduced (eg at a phage attachment site) and expressed from a promoter to yield 4,5-dihydro-11-*O*-desmethyl-15-O-desmethyl-17-hydroxy-21-desoxymacbecin.

In a specific embodiment *mbcM, mbcP, mbcP450, mbcMT1* and *mbcMT2* have been deleted and *gdmL* is introduced (eg at a phage attachment site) and expressed from a promoter to yield 4,5-dihydro-11-*O*-desmethyl-15-desmethoxy-17-methoxy-21-desoxymacbecin.

In a specific embodiment *mbcM, mbcP, mbcP450, mbcMT1* and *mbcMT2* has been deleted and *gdmL* has been introduced (eg at a phage attachment site) and expressed from a promoter to yield 4,5-dihydro-11-*O*-desmethyl-15-O-desmethyl-17-methoxy-21-desoxymacbecin.

A person of skill in the art will appreciate that a gene does not need to be completely deleted for it to be rendered non-functional, consequentially the term “deleted or inactivated” as used herein encompasses any method by which a gene is rendered non-functional including but not limited to: deletion of the gene in its entirety, deletion of part of the gene, inactivation by insertion into the target gene, site-directed mutagenesis which results in the gene either not being expressed or being expressed in an inactive form, mutagenesis of the host strain which results in the gene either not being expressed or being expressed in an inactive form (e.g. by radiation or exposure to mutagenic chemicals, protoplast fusion or transposon mutagenesis). Alternatively the function of an active gene can be impaired chemically with inhibitors, for example metapyrone (alternative name 2-methyl-1,2-di(3-pyridyl-1-propanone), EP 0 627 009) and ancymidol are inhibitors of oxygenases and these compounds can be added to the production medium to generate analogues. Additionally, sinefungin is a methyl transferase inhibitor that can be used similarly but for the inhibition of methyl transferase activity *in vivo* (McCammon and Parks 1981).

In an alternative embodiment, in a strain in which one or more post-PKS genes capable of oxidising the 17 position has been inserted, all of the post-PKS genes may be deleted or inactivated and then one or more of the genes, may then be reintroduced by complementation (e.g. at an attachment site, on a self-replicating plasmid or by insertion into a homologous

region of the chromosome). Therefore, in a particular embodiment the present invention relates to methods for the generation of 17-oxyhydromacbecin analogues, said method comprising:

a) providing a first host strain that produces macbecin when cultured under appropriate conditions

b) selectively inserting one or more post-PKS genes capable of oxidising the C17 position of macbecin,

c) selectively deleting or inactivating all the post-PKS genes,

d) culturing said modified host strain under suitable conditions for the production of novel compounds; and

e) optionally isolating the compounds produced.

Preferably in step b) the post-PKS gene is *gdmL* or a homologue thereof,

In an alternative embodiment, one or more of the macbecin post-PKS genes that are deleted or inactivated in step c) are reintroduced. In a further embodiment, one or more of the post-PKS genes selected from the group consisting of *mbcP*, *mbcM, mbcN, mbcP450, mbcMT1* and *mbcMT2* are reintroduced. In a further embodiment, two or more of the post-PKS genes selected from the group consisting of *mbcP*, *mbcM, mbcN, mbcP450, mbcMT1* and *mbcMT2* are reintroduced. In a further embodiment, three or more of the post-PKS genes selected from the group consisting of *mbcP*, *mbcM, mbcN, mbcP450, mbcMT1* and *mbcMT2* are reintroduced. In a further embodiment, four or more of the post-PKS genes selected from the group consisting of *mbcP*, *mbcM, mbcN, mbcP450, mbcMT1* and *mbcMT2* are reintroduced. In a further embodiment, five or more of the post-PKS genes selected from the group consisting of *mbcP*, *mbcM, mbcN, mbcP450, mbcMT1* and *mbcMT2* are reintroduced. In a further alternative embodiment, *mbcP*, *mbcM, mbcN, mbcP450, mbcMT1* and *mbcMT2* are reintroduced.

Additionally, it will be apparent to a person of skill in the art that in a strain in which one or more post-PKS genes capable of oxidising the C17 position, has been inserted wherein at least one of said post-PKS genes is *gdmL* or a homologue thereof, a subset of the macbecin post-PKS genes could be deleted or inactivated and a smaller subset of said post-PKS genes could be reintroduced to arrive at a strain producing 17-oxymacbecin analogues.

A person of skill in the art will appreciate that there are a number of ways to generate a strain that contains the biosynthetic gene cluster for macbecin which additionally expresses one or more post-PKS genes capable of oxidising the C17 position, wherein at least one of said post-PKS genes is *gdmL* or a homologue thereof.

It is well known to those skilled in the art that polyketide gene clusters may be expressed in heterologous hosts (Pfeifer and Khosla, 2001). Accordingly, the present invention includes the transfer of the macbecin biosynthetic gene cluster with *gdmL*, or a homologue thereof, with or without resistance and regulatory genes, either otherwise complete or containing additional deletions, into a heterologous host. Alternatively, the macbecin biosynthetic gene cluster could

be transferred to a strain which naturally contains *gdmL* or a homologue thereof. Methods and vectors for the transfer as defined above of such large pieces of DNA are well known in the art (Rawlings, 2001; Staunton and Weissman, 2001) or are provided herein in the methods disclosed. In this context a preferred host cell strain is a prokaryote, more preferably an actinomycete or *Escherichia coli*, still more preferably include, but are not limited to *Actinosynnema mirum (A. mirum)*, *Actinosynnema pretiosum subsp. pretiosum (A. pretiosum)*, *S. hygroscopicus*, *S. hygroscopicus sp.*, *S. hygroscopicus var. ascomyceticus*, *Streptomyces tsukubaensis*, *Streptomyces coelicolor*, *Streptomyces lividans*, *Saccharopolyspora erythraea*, *Streptomyces fradiae, Streptomyces avermitilis*, *Streptomyces cinnamonensis, Streptomyces rimosus, Streptomyces albus, Streptomyces griseofuscus, Streptomyces longisporoflavus, Streptomyces venezuelae, Streptomyces albus, Micromonospora sp., Micromonospora griseorubida, Amycolatopsis mediterranei* or *Actinoplanes sp. N902-109.* Further examples include *Streptomyces hygroscopicus subsp. geldanus and Streptomyces violaceusniger.*

In one embodiment the entire biosynthetic cluster is transferred, with *gdmL* or a homologue thereof. In an alternative embodiment the entire PKS is transferred without any of the associated macbecin post-PKS genes, but with *gdmL* or a homologue thereof. Optionally this can be carried out step-wise. Optionally some of the post-PKS genes can be introduced appropriately. Optionally additional genes from other clusters such as the geldanamycin or herbimycin pathways can be introduced appropriately.

In a further embodiment the entire macbecin biosynthetic cluster with *gdmL* or a homologue thereof is transferred and then manipulated according to the description herein.

In an alternative aspect of the invention, the 17-oxymacbecin analogue of the present invention may be further processed by biotransformation with an appropriate strain. The appropriate strain either being an available wild type strain for example, but without limitation *Actinosynnema mirum*, *Actinosynnema pretiosum subsp. pretiosum*, *S. hygroscopicus*, *S. hygroscopicus sp..* Alternatively, an appropriate strain may be a engineered to allow biotransformation with particular post-PKS enzymes for example, but without limitation, those encoded by *mbcM, mbcN, mbcP450, mbcMT1, mbcMT2* (as defined herein)*, gdmN, gdmM, gdmP,* (Rascher *et al.,* 2003) the geldanamycin O-methyl transferase, *hbmN*, *hbmL*, *hbmP*, (Rascher *et al.,* 2005) herbimycin *O*-methyl transferases and further herbimycin mono-oxygenases, *asm7*, *asm10*, *asm11*, *asm12*, *asm19* and *asm21* (Cassady *et al.,* 2004, Spiteller *et al.,* 2003). Where genes have yet to be identified or the sequences are not in the public domain it is routine to those skilled in the art to acquire such sequences by standard methods. For example the sequence of the gene encoding the geldanamycin O-methyl transferase is not in the public domain, but one skilled in the art could generate a probe, either a heterologous probe using a similar O-methyl transferase, or a homologous probe by designing degenerate primers from available homologous genes and amplifying a DNA fragment from the producing

organism, which can then be used to carry out Southern blots on a geldanamycin producing strain and thus acquire this gene to generate biotransformation systems. Similarly, the published sequence of the herbimycin cluster appears not to have one of the P450 monooxygenases that is required for the final structure. One skilled in the art could generate a probe, either a heterologous probe using a similar P450, or a homolgous probe can be isolated by designing degenerate primers using sequences of available homologous genes and amplifying a DNA fragment from the producing organism, which can then be used to carry out Southern blots on a herbimycin producing strain and thus acquire this gene to generate biotransformation systems.

In an alternative embodiment a C17-*O*-methyl transferase is co-expressed with *gdmL* or a homologue thereof to produce C17 methoxy macbecin analogues.The *O*-methyl transferase may be isolated from a geldanamycin producing strain using degenerate primers as described above.

In a particular embodiment the strain may have had one or more of its native polyketide clusters deleted, either entirely or in part, or otherwise inactivated, so as to prevent the production of the polyketide produced by said native polyketide cluster. Said engineered strain may be selected from the group including, for example but without limitation, *Actinosynnema mirum*, *Actinosynnema pretiosum subsp. pretiosum*, *S. hygroscopicus*, *S. hygroscopicus sp.*, *S. hygroscopicus var. ascomyceticus*, *Streptomyces tsukubaensis*, *Streptomyces coelicolor*, *Streptomyces lividans*, *Saccharopolyspora erythraea*, *Streptomyces fradiae, Streptomyces avermitilis*, *Streptomyces cinnamonensis, Streptomyces rimosus, Streptomyces albus, Streptomyces griseofuscus, Streptomyces longisporoflavus, Streptomyces venezuelae, Micromonospora sp., Micromonospora griseorubida, Amycolatopsis mediterranei* or *Actinoplanes sp. N902-109.* Further possible strains include *Streptomyces hygroscopicus subsp. geldanus and Streptomyces violaceusniger.*

In a further aspect the present invention provides host strains which naturally produce macbecin or analogue therof, in which the *gdmL* gene, or a homologue thereof, has been inserted such that it thereby produces 17-oxymacbecin or an analogue thereof (e.g. a 17-oxymacbecin analogue as defined by compounds of formula (I)) and their use in the production of 17-oxymacbecin or analogues thereof.

Therefore, in one embodiment the present invention provides a genetically engineered strain which naturally produces macbecin in its unaltered state, said strain having one or more post-PKS genes capable of oxidising the C17 position inserted, wherein at least one of said post-PKS genes is *gdmL* or a homologue thereof, and optionally one or more post-PKS genes from the macbecin PKS gene cluster deleted.

The invention embraces all products of the inventive processes described herein.

Although the process for preparation of the 17-oxymacbecin analogues of the invention as described above is substantially or entirely biosynthetic, it is not ruled out to produce or interconvert 17-oxymacbecin analogues of the invention by a process which comprises standard synthetic chemical methods.

In order to allow for the genetic manipulation of the macbecin PKS gene cluster, first the gene cluster was sequenced from *Actinosynnema pretiosum subsp. pretiosum* however, a person of skill in the art will appreciate that there are alternative strains which produce macbecin, for example but without limitation *Actinosynnema mirum*. The macbecin biosynthetic gene cluster from these strains may be sequenced as described herein for *Actinosynnema pretiosum subsp. pretiosum*, and the information used to generate equivalent strains.

Further aspects of the invention include:

-An engineered strain based on a macbecin producing strain in which a gene encoding an activity capable of oxidising macbecin at the 17-position, eg *gdmL* has been introduced. Optionally further post-PKS genes for example *mbcP*, *mbcP450*, *mbcMT1* and *mbcMT2,* may be deleted or inactivated, and optionally some or all of these may be reintroduced, and/or optionally one or more post-PKS genes from heterologous clusters may be introduced. These steps may be carried out in any order. Suitably the macbecin producing strain is *A. pretiosum* or *A. mirum*.

-A process for producing a 17-oxymacbecin analogue which comprises culturing an aforementioned strain. The strains will be cultured in suitable media known to a skilled person and provided with suitable feed materials eg appropriate starter acids.

-Such a process further comprising the step of isolating 17-oxymacbecin or an analogue thereof. Isolation may be performed by conventional means eg chromatography (eg HPLC).

-Use of such an engineered strain in the preparation of a 17-oxymacbecin analogue.

Compounds of the invention are advantageous in that they may be expected to have one or more of the following properties: good activity against one or more different cancer sub-types compared with the parent compound; good toxicological profile such as good hepatotoxicity profile, good nephrotoxicity, good cardiac safety; good water solubility; good metabolic stability; good formulation ability; good bioavailability; good pharmacokinetic or pharmacodynamic properties such as tight binding to Hsp90, fast on-rate of binding to Hsp90 and/or good brain pharmacokinetics; good cell uptake; and low binding to erythrocytes.

EXAMPLES

General Methods

Fermentation of cultures

Conditions used for growing the bacterial strains *Actinosynnema pretiosum* subsp. *pretiosum* ATCC 31280 (US 4,315,989) and *Actinosynnema mirum* DSM 43827 (KCC A-0225, Watanabe *et al*., 1982) were described in the patents US 4,315,989 and US 4,187,292.

Methods used herein were adapted from these patents and are as follows for culturing of broths in tubes or flasks in shaking incubators, variations to the published protocols are indicated in the examples. Strains were grown on ISP2 agar (Medium 3, Shirling, E.B. and Gottlieb, D., 1966) at 28 °C for 2-3 days and used to inoculate seed medium (Medium 1, see below adapted from US 4,315,989 and US 4,187,292). The inoculated seed medium was then incubated with shaking between 200 and 300 rpm with a 5 or 2.5 cm throw at 28 °C for 48 h. For production of macbecin, 18,21-dihydromacbecin and macbecin analogues such as 17-oxymacbecins the fermentation medium (Medium 2, see below and US 4,315,989 and US 4,187,292) was inoculated with 2.5% - 10% of the seed culture and incubated with shaking between 200 and 300 rpm with a 5 or 2.5 cm throw initially at 28 °C for 24 h followed by 26 °C for four to six days. The culture was then harvested for extraction.

Media

Medium 1 - Seed Medium

In 1 L of distilled water

Glucose	20g
Soluble potato starch (Sigma)	30g
Spray dried corn steep liquor (Roquette Freres)	10g
'Nutrisoy' toasted soy flour (Archer Daniels Midland)	10g
Peptone from milk solids (Sigma)	5g
NaCl	3g
$CaCO_3$	5g
Adjust pH with NaOH	7.0

Sterilisation was performed by autoclaving at 121°C for 20 minutes.

Apramycin was added when appropriate after autoclaving to give a final concentration of 50 mg/L.

Medium 2 - Fermentation Medium

In 1 L of distilled water

Glycerol	50g
Spray dried corn steep liquor (Roquette Freres)	10g
'Bacto' yeast extract (Difco)	20g
KH_2PO_4	20g
$MgCl_2.6H_2O$	5g
$CaCO_3$	1g
Adjust pH with NaOH	6.5

Sterilisation was performed by autoclaving at 121°C for 20 minutes.

Medium 3 – ISP2 Medium

In 1 L of distilled water

Malt extract	10g
Yeast extract	4g
Dextrose	4g
Agar	15g
Adjust pH with NaOH	7.3

Sterilisation was performed by autoclaving at 121°C for 20 minutes.

Medium 4 - MAM

In 1 L of distilled water

Wheat starch	10g
Corn steep solids	2.5g
Yeast extract	3g
$CaCO_3$	3g
Iron sulphate	0.3g
Agar	20g

Sterilisation was performed by autoclaving at 121°C for 20 minutes.

Extraction of culture broths for LCMS analysis

Culture broth (1 mL) and ethyl acetate (1 mL) was added and mixed for 15-30 min followed by centrifugation for 10 min. 0.5 mL of the organic layer was collected, evaporated to dryness and then re-dissolved in 0.25 mL of methanol, or 0.23mL of methanol + 0.02mL of a 1% $FeCl_3$ solution.

LCMS analysis procedure

LCMS may be performed using an Agilent HP1100 HPLC system in combination with a Bruker Daltonics Esquire 3000+ electrospray mass spectrometer operating in positive and/or negative ion mode. Chromatography may be achieved over a Phenomenex Hyperclone column (C_{18} BDS, 3u, 150 x 4.6 mm) eluting at a flow rate of 1 mL/min using the following gradient elution process; T=0, 10%B; T=2, 10%B; T=20, 100%B; T=22, 100%B; T=22.05, 10%B; T=25, 10%B. Mobile phase A = water + 0.1% formic acid; mobile phase B = acetonitrile + 0.1% formic acid. UV spectra may be recorded between 190 and 400 nm, with extracted chromatograms taken at 210, 254 and 276 nm. Mass spectra may be recorded between 100 and 1500 amu.

NMR structure elucidation methods

NMR spectra may be recorded on a Bruker Advance 500 spectrometer at 298 K operating at 500 MHz and 125 MHz for ^{1}H and ^{13}C respectively. Standard Bruker pulse sequences may be used to acquire ^{1}H-^{1}H COSY, APT, HMBC and HMQC spectra. NMR

spectra may be referenced to the residual proton or standard carbon resonances of the solvents in which they were run.

Assessment of compound purity

Purified compounds may be analysed using the LCMS method described above. Purity may be assessed by MS and at multiple wavelengths (210, 254 & 276 nm). All compounds may be >95% pure at all wavelengths. Purity may be finally confirmed by inspection of the ^{1}H and ^{13}C NMR spectra.

Assessment of water solubility

Water solubility may be tested as follows: A 10 mM stock solution of the 17-oxymacbecin analogue is prepared in 100% DMSO at room temperature. Triplicate 0.01 mL aliquots are made up to 0.5 mL with either 0.1 M PBS, pH 7.3 solution or 100% DMSO in amber vials. The resulting 0.2 mM solutions are shaken in the dark, at room temperature on an IKA® vibrax VXR shaker for 6 h, followed by transfer of the resulting solutions or suspensions into 2 mL Eppendorf tubes and centrifugation for 30 min at 13200 rpm. Aliquots of the supernatant fluid are then analysed by LCMS as described above.
Compounds are quantified by peak area measurement at 258 nm. All analyses are performed in triplicate and the solubility of the 17-oxymacbecin compounds calculated by comparing PBS solutions with 0.2 mM in DMSO (with an assumed solubility of 100 % in DMSO).

In vitro *bioassay for anticancer activity*

In vitro evaluation of compounds for anticancer activity in a panel of human tumour cell lines in a monolayer proliferation assay may be carried out at the Oncotest Testing Facility, Institute for Experimental Oncology, Oncotest GmbH, Freiburg. The characteristics of the selected cell lines are summarised in Table 1.

Table 1 – Test cell lines

#	Cell line	Characteristics
1	CNXF 498NL	CNS
2	CXF HT29	Colon
3	LXF 1121L	Lung, large cell ca
4	MCF-7	Breast, NCI standard
5	MEXF 394NL	Melanoma
6	DU145	Prostate - PTEN positive

The Oncotest cell lines are established from human tumor xenografts as described by Roth *et al.,* (1999). The origin of the donor xenografts was described by Fiebig *et al.*, (1999). Other cell lines are either obtained from the NCI (DU145, MCF-7) or purchased from DSMZ, Braunschweig, Germany.

All cell lines, unless otherwise specified, were grown at 37 °C in a humidified atmosphere (95 % air, 5 % CO_2) in a 'ready-mix' medium containing RPMI 1640 medium, 10 % fetal calf serum, and 0.1 mg/mL gentamicin (PAA, Cölbe, Germany).

A modified propidium iodide assay may be used to assess the effects of the test compound(s) on the growth of human tumour cell lines (Dengler *et al.*, (1995)).

Briefly, cells are harvested from exponential phase cultures by trypsinization, counted and plated in 96 well flat-bottomed microtitre plates at a cell density dependent on the cell line (5 - 10.000 viable cells/well). After 24 h recovery to allow the cells to resume exponential growth, 0.010 mL of culture medium (6 control wells per plate) or culture medium containing macbecin are added to the wells. Each concentration is plated in triplicate. Compounds are applied in two concentrations (1 µg/mL and 10 µg/mL). Following 4 days of continuous exposure, cell culture medium with or without test compound is replaced by 0.2 mL of an aqueous propidium iodide (PI) solution (7 mg/L). To measure the proportion of living cells, cells are permeabilized by freezing the plates. After thawing the plates, fluorescence is measured using the Cytofluor 4000 microplate reader (excitation 530 nm, emission 620 nm), giving a direct relationship to the total number of viable cells.

Growth inhibition is expressed as treated/control x 100 (% T/C).

Example 1 – Sequencing of the Macbecin PKS gene cluster

Genomic DNA was isolated from *Actinosynnema pretiosum* (ATCC 31280) and *Actinosynnema mirum* (DSM 43827, ATCC 29888) using standard protocols described in Kieser *et al.*, (2000) DNA sequencing was carried out by the sequencing facility of the Biochemistry Department, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW using standard procedures.

Primers BIOSG104 5'-GGTCTAGAGGTCAGTGCCCCCGCGTACCGTCGT-3' (SEQ ID NO: 1) AND BIOSG105 5'-GGCATATGCTTGTGCTCGGGCTCAAC-3' (SEQ ID NO: 2) were employed to amplify the carbamoyltransferase-encoding gene *gdmN* from the geldanamycin biosynthetic gene cluster of *Streptomyces hygroscopicus* NRRL 3602 (Accession number of sequence: AY179507) using standard techniques. Southern blot experiments were carried out using the DIG Reagents and Kits for Non-Radioactive Nucleic Acid Labelling and Detection according to the manufacturers' instructions (Roche). The DIG-labeled *gdmN* DNA fragment was used as a heterologous probe. Using the *gdmN* generated probe and genomic DNA isolated from *A. pretiosum* 2112 an approximately 8 kb *Eco*RI fragment was identified in

Southern Blot analysis. The fragment was cloned into Litmus 28 applying standard procedures and transformants were identified by colony hybridization. The clone p3 was isolated and the approximately 7.7 kb insert was sequenced. DNA isolated from clone p3 was digested with *Eco*RI and *Eco*RI/*Sac*I and the bands at around 7.7 kb and at about 1.2 kb were isolated, respectively. Labelling reactions were carried out according to the manufacturers' protocols. Cosmid libraries of the two strains named above were created using the vector SuperCos 1 and the Gigapack III XL packaging kit (Stratagene) according to the manufacturers' instructions. These two libraries were screened using standard protocols and as a probe, the DIG-labelled fragments of the 7.7 kb *Eco*RI fragment derived from clone p3 were used. Cosmid 52 was identified from the cosmid library of *A. pretiosum* and submitted for sequencing to the sequencing facility of the Biochemistry Department of the University of Cambridge. Similarly, cosmid 43 and cosmid 46 were identified from the cosmid library of *A. mirum*. All three cosmids contain the 7.7 kb *Eco*RI fragment as shown by Southern Blot analysis.

An around 0.7 kbp fragment of the PKS region of cosmid 43 was amplified using primers BIOSG124 5'-CCCGCCCGCGCGAGCGGCGCGTGGCCGCCCGAGGGC-3' (SEQ ID NO: 3) and BIOSG125 5'- GCGTCCTCGCGCAGCCACGCCACCAGCAGCTCCAGC-3' (SEQ ID NO: 4) applying standard protocols, cloned and used as a probe for screening the *A. pretiosum* cosmid library for overlapping clones. The sequence information of cosmid 52 was also used to create probes derived from DNA fragments amplified by primers BIOSG130 5'-CCAACCCCGCCGCGTCCCCGGCCGCGCCGAACACG-3' (SEQ ID NO: 5) and BIOSG131 5'-GTCGTCGGCTACGGGCCGGTGGGGCAGCTGCTGT-5' (SEQ ID NO: 6) as well as BIOSG132 5'- GTCGGTGGACTGCCCTGCGCCTGATCGCCCTGCGC-3' (SEQ ID NO: 7) and BIOSG133 5'- GGCCGGTGGTGCTGCCCGAGGACGGGGAGCTGCGG-3' (SEQ ID NO: 8) which were used for screening the cosmid library of *A. pretiosum*. Cosmids 311 and 352 were isolated and cosmid 352 was sent for sequencing. Cosmid 352 contains an overlap of approximately 2.7 kb with cosmid 52. To screen for further cosmids, an approximately 0.6 kb PCR fragment was amplified using primers BIOSG136 5'-CACCGCTCGCGGGGGTGGCGCGGCGCACGACGTGG CTGC-3' (SEQ ID NO: 9) and BIOSG 137 5'- CCTCCTCGGACAGCGCGATCAGCGCCGCGC ACAGCGAG-3' (SEQ ID NO: 10) and cosmid 311 as template applying standard protocols. The cosmid library of *A. pretiosum* was screened and cosmid 410 was isolated. It overlaps approximately 17 kb with cosmid 352 and was sent for sequencing. The sequence of the three overlapping cosmids (cosmid 52, cosmid 352 and cosmid 410) was assembled. The sequenced region spans about 100 kbp and 23 open reading frames were identified potentially constituting the macbecin biosynthetic gene cluster, (SEQ ID NO: 11). The location of each of the open reading frames within SEQ ID NO: 11 is shown in Table 3

Table 2 – Summary of the cosmids

Cosmid	Strain
Cosmid 43	*Actinosynnema mirum* ATCC 29888
Cosmid 46	*Actinosynnema mirum* ATCC 29888
Cosmid 52	*Actinosynnema pretiosum* ATCC 31280
Cosmid 311	*Actinosynnema pretiosum* ATCC 31280
Cosmid 352	*Actinosynnema pretiosum* ATCC 31280
Cosmid 410	*Actinosynnema pretiosum* ATCC 31280

Table 3 – location of each of the open reading frames within SEQ ID NO: 11

Nucleotide position in SEQ ID NO: 11	Gene Name	Function of the encoded protein
14925-17909*	*mbcRII*	transcriptional regulator
18025-19074c	*mbcO*	aminohydroquinate synthase
19263-20066c*	*mbc?*	unknown, AHBA biosynthesis
20330-40657	*mbcAI*	PKS
40654-50859	*mbcAII*	PKS
50867-62491*	*mbcAIII*	PKS
62500-63276*	*mbcF*	amide synthase
63281-64852*	*mbcM*	C21 monooxygenase
64899-65696c*	*PH*	phosphatase
65693-66853c*	*OX*	oxidoreductase
66891-68057c*	*Ahs*	AHBA synthase
68301-68732*	*Adh*	ADHQ dehydratase
68690-69661c*	*AHk*	AHBA kinase
70185-72194c*	*mbcN*	carbamoyltransferase
72248-73339c	*mbcH*	methoxymalonyl ACP pathway
73336-74493c	*mbcI*	methoxymalonyl ACP pathway
74490-74765c	*mbcJ*	methoxymalonyl ACP pathway
74762-75628c*	*mbcK*	methoxymalonyl ACP pathway
75881-76537	*mbcG*	methoxymalonyl ACP pathway
76534-77802*	*mbcP*	C4,5 monooxygenase
77831-79054*	*mbcP450*	P450
79119-79934*	*mbcMT1*	*O*-methyltransferase
79931-80716*	*mbcMT2*	*O*-methyltransferase

[Note 1: c indicates that the gene is encoded by the complement DNA strand; Note 2: it is sometimes the case that more than one potential candidate start codon can been identified. One skilled in the art will recognise this and be able to identify alternative possible start codons. We have indicated those genes which have more than one possible start codon with a '*' symbol. Throughout we have indicated what we believe to be the start codon, however, a person of skill in the art will appreciate that it may be possible to generate active protein using an alternative start codon.]

Example 2 Production of 4,5-dihydro-11-*O*-desmethyl-15-desmethoxy-17-hydroxy-macbecin.

An *Actinosynnema pretiosum* strain was generated in which the *mbcP, mbcP450, mbcMT1 and mbcMT2* genes had been deleted in frame, in this strain *gdmL* was additionally expressed to produce of 4,5-dihydro-11-*O*-desmethyl-15-desmethoxy-17-hydroxy-macbecin.

2.1 Cloning of DNA homologous to the downstream flanking region of *mbcMT2*

Oligos ls4del1 (SEQ ID NO: 12) and ls4del2a (SEQ ID NO: 13) were used to amplify a 1595 bp region of DNA from *Actinosynnema pretiosum* (ATCC 31280) in a standard PCR reaction using cosmid 52 (from example 1) as the template and Pfu DNA polymerase. A 5' extension was designed in oligo ls4del2a to introduce an *Avr*II site to aid cloning of the amplified fragment (Figure 3). The amplified PCR product (1+2a, Figure 4 SEQ ID NO: 14) encoded 196 bp of the 3' end of *mbcMT2* and a further 1393 bp of downstream homology. This 1595 bp fragment was cloned into pUC19 that had been linearised with *Sma*I, resulting in plasmid pLSS1+2a.

ls4del1 (SEQ ID NO: 12)

5' – GGTCACTGGCCGAAGCGCACGGTGTCATGG – 3'

ls4del2a (SEQ ID NO: 13)

5' – CTAGGCGACTACCCCGCACTACTACACCGAGCAGG – 3'

2.2 Cloning of DNA homologous to the upstream flanking region of *mbcM.*

Oligos ls4del3b (SEQ ID NO: 15) and ls4del4 (SEQ ID NO: 16) were used to amplify a 1541 bp region of DNA from *Actinosynnema pretiosum* (ATCC 31280) in a standard PCR reaction using cosmid 52 (from example 1) as the template and Pfu DNA polymerase. A 5' extension was designed in oligo ls4del3b to introduce an *Avr*II site to aid cloning of the amplified fragment (Figure 3). The amplified PCR product (3b+4, Figure 5, SEQ ID NO: 17) encoded 95 bp of the 5' end of *mbcP* and a further 1440 bp of upstream homology. This 1541 bp fragment was cloned into pUC19 that had been linearised with *Sma*I, resulting in plasmid pLSS3b+4.

ls4del3b (SEQ ID NO: 15)

5' – CCTAGGAACGGGTAGGCGGGCAGGTCGGTG – 3'

ls4del4 (SEQ ID NO: 16)

5' – GTGTGCGGGCCAGCTCGCCCAGCACGCCCAC – 3'

The products 1+2a and 3b+4 were cloned into pUC19 to utilise the *Hin*dIII and *Bam*HI sites in the pUC19 polylinker for the next cloning step.

The 1621 bp *Avr*II/*Hin*dIII fragment from pLSS1+2a and the 1543 bp *Avr*II/*Bam*HI fragment from pLSS3b+4 were cloned into the 3556 bp *Hin*dIII/*Bam*HI fragment of pKC1132 to make pLSS315. pLSS315 therefore contained a *Hin*dIII/*Bam*HI fragment encoding DNA homologous to the flanking regions of the desired four ORF deletion region fused at an *Avr*II site (Figure 3).

2.3 Transformation of *Actinosynnema pretiosum subsp. pretiosum*

Escherichia coli ET12567, harbouring the plasmid pUZ8002 was transformed with pLSS315 by electroporation to generate the *E. coli* donor strain for conjugation. This strain was used to transform *Actinosynnema pretiosum subsp. pretiosum* by vegetative conjugation (Matsushima *et al*, 1994) Exconjugants were plated on MAM medium (1% wheat starch, 0.25% corn steep solids, 0.3% yeast extract, 0.3% calcium carbonate, 0.03% iron sulphate, 2% agar) and incubated at 28°C. Plates were overlayed after 24 h with 50 mg/L apramycin and 25 mg/L nalidixic acid. As pLSS315 is unable to replicate in *Actinosynnema pretiosum subsp. pretiosum*, apramycin resistant colonies were anticipated to be transformants that contained plasmid integrated into the chromosome by homologous recombination via the plasmid borne regions of homology.

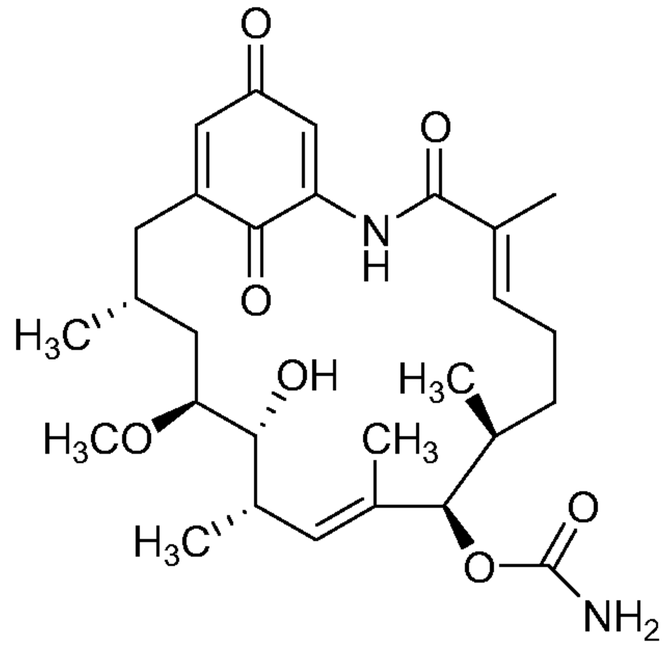
2.4 Screening for secondary crosses

Six macbecin producing exconjugates were selected for further analysis. Genomic DNA was isolated from the six exconjugants and digested and analysed by Southern Blot. The blot showed that in five out of the six isolates integration had occurred in the RHS region of homology and in one of the six isolates homologous integration had occurred in the LHS region. One strain resulting from homologous integration in the LHS region (BIOT-3829; *Actinosynnema pretiosum*:pLSS315#9) and two strains resulting from homologous integration in the RHS region (BIOT-3826; *Actinosynnema pretiosum*:pLSS315#3 and BIOT-3830; *Actinosynnema pretiosum*:pLSS315#12) were chosen for subculturing to screen for secondary crosses.

Strains were patched onto MAM media (supplemented with 50 mg/L apramycin) and grown at 28 °C for four days. A 1 cm^2 section of each patch was used to inoculate 7 mL of ISP2

(0.4% yeast extract, 1% malt extract, 0.4% dextrose, not supplemented with antibiotic) in a 50 mL falcon tube. Cultures were grown for 2-3 days then subcultured (5% inoculum) into 7 mL of ISP2 in a 50 mL falcon tube. After 4-5 rounds of subculturing the cultures were sonicated, serially diluted, plated on MAM media and incubated at 28 °C for four days. Single colonies were then patched in duplicate onto MAM media containing apramycin and onto MAM media containing no antibiotic and the plates were incubated at 28 °C for four days. Patches that grew on the no antibiotic plate but did not grow on the apramycin plate were re-patched onto +/- apramycin plates to confirm that they had lost the antibiotic marker. The desired mutant strains have a deletion of 3892 bp of the macbecin cluster containing the genes *mbcP*, *mbcP450*, *mbcMT1* and *mbcMT2*. One colony originating from *Actinosynnema pretiosum*:pLSS315#12 that contains the correct deletion was designated BIOT-3852.

The fermentation broth from this strain was extracted and analysed as described in General Methods. LCMS analysis showed that no macbecin was produced but a single, more polar, major component **14** with retention time of 15.0 min and m/z = 515.5 $[M-H]^-$, 539.5 $[M+Na]^+$ was observed. This was indistinguishable by LCMS and NMR (after isolation) with the compound 4,5-dihydro-11-O-desmethyl-15-desmethoxymacbecin produced elsewhere.



14

2.5 Isolation of plasmid Lit28gdmL

Oligos BioSG110 (SEQ ID NO: 18) and BioSG111 (SEQ ID NO: 19) were used to amplify a 1512 bp region of DNA from the geldanamycin biosynthetic gene cluster of *Streptomyces hygroscopicus* NRRL 3602 (Accession number of sequence: AY179507) using standard techniques. (SEQ ID NO: 20; Figure 6A, the amino acid sequence of gdmL is also shown, Figure 6B, SEQ ID NO: 21). The *Xba*I and *Nde*I restriction sites introduced at the end of the primers are underlined. The amplified PCR product was cloned into vector Litmus28 previously linearised with *Eco*RV using standard techniques. Plasmid Lit28gdmL was isolated and confirmed by DNA sequence analysis.

BioSG110 (SEQ ID NO: 19):

5'-GGCATATGTTGACGGAGAGCACGACCGAGGTCGTTG-3'

BioSG111 (SEQ ID NO: 18):

5'-GGTCTAGAGGTCAGGGCACCCTCGCGAGGTCGCCGG-3'

2.6 Isolation of plasmid pGP9*gdmL*

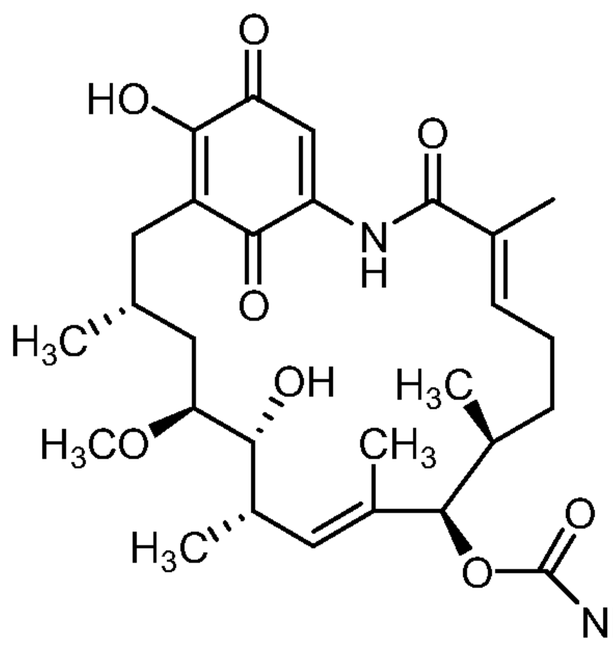
Plasmid Lit28gdmL was digested with *Nde*I/*Xba*I and the about 1.5 kb insert DNA fragment was isolated and cloned into *Nde*I/*Xba*I treated vector pGP9. Plasmid pGP9gdmL was isolated using standard techniques. The construct was confirmed by restriction digest analysis.

2.7 Complementation of BIOT-3852 with pGP9gdmL

Conjugation experiments with BIOT-3852 using plasmid pGP9gdmL were carried out as follows. *Escherichia coli* ET12567, harbouring the plasmid pUZ8002 was used to transform pGP9gdmL by electroporation to generate the *E. coli* donor strain for conjugation. This strain was used for conjugation experiments in combination with BIOT-3852 (Matsushima *et al*, 1994). Exconjugants were plated on Medium 4 (MAM medium) and incubated at 28°C. Plates were overlayed after 24 h with 50 mg/L apramycin and 25 mg/L nalidixic acid.

Transformants were patched into MAM plates (medium 4) containing 50 mg/L apramycin and 25 mg/L nalidixic acid . A 6 mm circular plug from each patch was used to inoculate individual 50 mL falcon tubes containing 10 mL seed medium (adapted from medium 1 - 2% glucose, 3% soluble starch, 0.5% corn steep solids, 1% soybean flour, 0.5% peptone, 0.3% sodium chloride, 0.5% calcium carbonate) supplemented with 50 mg/L apramycin. These seed cultures were incubated for 2 days at 28°C, 200 rpm with a 2 inch throw. These were then used to inoculate (0.5 mL into 10 mL) production medium (medium 2 - 5% glycerol, 1% corn steep solids, 2% yeast extract, 2% potassium dihydrogen phosphate, 0.5% magnesium choride, 0.1% calcium carbonate) and were grown at 28°C for 24 hours and then at 26°C for a further 6 days.

The extraction of fermentation broth and subsequent LCMS analysis was performed as described in General Methods. In one such extract, in addition to the production of **14**, the production of small amount of a new compound (**15**) was also observed which eluted with a retention time of 13.4 minutes. This displayed characteristic ions with m/*z* = 531.4 $[M-H]^-$ and 555.4 $[M+Na]^+$ which are consistent with **15** being the compound 4,5-dihydro-11-*O*-desmethyl-15-desmethoxy-17-hydroxymacbecin.



All references including patent and patent applications referred to in this application are incorporated herein by reference to the fullest extent possible.

Throughout the specification and the claims which follow, unless the context requires otherwise, the word 'comprise', and variations such as 'comprises' and 'comprising', will be understood to imply the inclusion of a stated integer or step or group of integers but not to the exclusion of any other integer or step or group of integers or steps.

References

Allen, I. W. and Ritchie, D.A. (1994) Cloning and analysis of DNA sequences from *Streptomyces hygroscopicus* encoding geldanamycin biosynthesis. Mol. Gen. Genet. 243: 593-599.

Bagatell, R. and Whitesell, L. (2004) Altered Hsp90 function in cancer: A unique therapeutic opportunity. Molecular Cancer Therapeutics 3: 1021-1030.

Beliakoff, J. and Whitesell, L. (2004) Hsp90: an emerging target for breast cancer therapy. Anti-Cancer Drugs 15:651-662.

Bibb, M. J., G. R. Janssen, et al. (1985). "Cloning and analysis of the promoter region of the erythromycin resistance gene (*ermE*) of *Streptomyces erythraeus*." Gene **38**(1-3): 215-26.

Bierman, M., Logan, R., O'Brien, K., Seno, ET., Nagaraja Rao, R. and Schoner, BE. (1992) "Plasmid cloning vectors for the conjugal transfer of DNA from *Escherichia coli* to *Streptomyces spp*." Gene 116: 43-49.

Blagosklonny, M.V. (2002) Hsp-90-associated oncoproteins: multiple targets of geldanamycin and its analogues. Leukemia 16:455-462.

Blagosklonny, M.V., Toretsky, J., Bohen, S. and Neckers, L. (1996) Mutant conformation of p53 translated *in vitro* or *in vivo* requires functional HSP90. Proc. Natl. Acad. Sci. USA 93:8379-8383.

Blanc, V.; Salah-Bey, K.; Folcher, M.; Thompson, C. J. *Mol. Microbiol.* **1995**, *17*, 989-999.

Bohen, S.P. (1998) Genetic and biochemical analysis of p23 and ansamycin antibiotics in the function of Hsp90-dependent signaling proteins. Mol Cell Biol 18:3330-3339.

Carreras, C.W., Schirmer, A., Zhong, Z. and Santi D.V. (2003) Filter binding assay for the geldanamycin-heat shock protein 90 interaction. Analytical Biochemistry 317:40-46.

Cassady, J.M., Chan, K.K., Floss, H.G. and Leistner E. (2004) Recent developments in the maytansinoid antitumour agents. Chem. Pharm. Bull. 52(1) 1-26.

Chiosis, G., Huezo, H., Rosen, N., Mimnaugh, E., Whitesell, J. and Neckers, L. (2003) 17AAG: Low target binding affinity and potent cell activity – finding an explanation. Molecular Cancer Therapeutics 2:123-129.

Chiosis, G., Vilenchik, M., Kim, J. and Solit, D. (2004) Hsp90: the vulnerable chaperone. Drug Discovery Today 9:881-888.

Csermely, P. and Soti, C. (2003) Inhibition of Hsp90 as a special way to inhibit protein kinases. Cell.Mol.Biol.Lett. 8:514-515.

DeBoer, C and Dietz, A. (1976) The description and antibiotic production of *Streptomyces hygroscopicus* var. *geldanus*. J. Antibiot. 29:1182-1188.

DeBoer, C., Meulman, P.A., Wnuk, R.J., and Peterson, D.H. (1970) Geldanamycin, a new antibiotic. J. Antibiot. 23:442-447.

Dengler W.A., Schulte J., Berger D.P., Mertelsmann R. and Fiebig HH. (1995) Development of a propidium iodide fluorescence assay for proliferation and cytotoxicity assay. *Anti-Cancer Drugs*, 6:522-532.

Dikalov, s., Landmesser, U., Harrison, DG., 2002, Geldanamycin Leads to Superoxide Formation by Enzymatic and Non-enzymatic Redox Cycling, The Journal of Biological Chemistry, 277(28), pp25480-25485

Donzé O. and Picard, D. (1999) Hsp90 binds and regulates the ligand-inducible α subunit of eukaryotic translation initiation factor kinase Gcn2. Mol Cell Biol 19:8422-8432.

Egorin MJ, Lagattuta TF, Hamburger DR, Covey JM, White KD, Musser SM, Eiseman JL. (2002) “Pharmacokinetics, tissue distribution, and metabolism of 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (NSC 707545) in CD2F1 mice and Fischer 344 rats. “ Cancer Chemother Pharmacol, 49(1), pp7-19.

Eustace, B.K., Sakurai, T., Stewart, J.K., *et al.* (2004) Functional proteomic screens reveal an essential extracellular role for hsp90α in cancer cell invasiveness. Nature Cell Biology 6:507-514.

Fang, Y., Fliss, A.E., Rao, J. and Caplan A.J. (1998) *SBA1* encodes a yeast Hsp90 cochaperone that is homologous to vertebrate p23 proteins. Mol Cell Biol 18:3727-3734.

Fiebig H.H., Dengler W.A. and Roth T. Human tumor xenografts: Predictivity, characterization, and discovery of new anticancer agents. In: Fiebig HH, Burger AM (eds). Relevance of Tumor Models for Anticancer Drug Development. *Contrib. Oncol*. 1999, 54: 29 - 50.

Goetz, M.P., Toft, D.O., Ames, M.M. and Ehrlich, C. (2003) The Hsp90 chaperone complex as a novel target for cancer therapy. Annals of Oncology 14:1169-1176.

Gregory, M.A., Till R, and Smith M.C.M. (2003) Integration site for *Streptomyces* phage ΦBT1 and the development of site-specific integrating vectors. *Journal of Bacteriology* **185**: 5320-5323.

Harris, S.F., Shiau A.K. and Agard D.A. (2004) The crystal structure of the carboxy-terminal dimerization domain of *htpG*, the *Escherichia coli* Hsp90, reveals a potential substrate binging site. Structure 12: 1087-1097.

Hong, Y.-S., Lee, D., Kim, W., Jeong, J.-K. *et al*. (2004) Inactivation of the carbamoyltransferase gene refines post-polyketide synthase modification steps in the biosynthesis of the antitumor agent geldanamycin. J. Am. Chem. Soc. 126:11142-11143.

Hostein, I., Robertson, D., DiStefano, F., Workman, P. and Clarke, P.A. (2001) Inhibition of signal transduction by the Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin results in cytostasis and apoptosis. Cancer Research 61:4003-4009.

Hu, Z., Liu, Y., Tian, Z.-Q., Ma, W., Starks, C.M. *et al*. (2004) Isolation and characterization of novel geldanamycin analogues. J. Antibiot. 57:421-428.

Hur, E., Kim, H.-H., Choi, S.M., *et al.* (2002) Reduction of hypoxia-induced transcription through the repression of hypoxia-inducible factor-1α/aryl hydrocarbon receptor nuclear translocator DNA binding by the 90-kDa heat-shock protein inhibitor radicicol. Molecular Pharmacology 62:975-982.

Iwai Y, Nakagawa, A., Sadakane, N., Omura, S., Oiwa, H., Matsumoto, S., Takahashi, M., Ikai, T., Ochiai, Y. (1980) Herbimycin B, a new benzoquinoid ansamycin with anti-TMV and herbicidal activities. The Journal of Antibiotics, 33(10), pp1114-1119.

Jez, J.M., Chen, J. C.-H., Rastelli, G., Stroud, R.M. and Santi, D.V. (2003) Crystal structure and molecular modeling of 17-DMAG in complex with human Hsp90. Chemistry and Biology 10:361-368.

Kaur, G., Belotti, D, Burger, A.M., Fisher-Nielson, K., Borsotti, P. *et al.* (2004) Antiangiogenic properties of 17-(Dimethylaminoethylamino)-17-Demethoxygeldanamycin: an orally bioavailable heat shock protein 90 modulator. Clinical Cancer Research 10:4813-4821.

Kieser, T., Bibb, M.J., Buttner, M.J., Chater, K.F., and Hopwood, D.A. (2000) Practical Streptomyces Genetics, John Innes Foundation, Norwich

Kumar, R., Musiyenko, A. and Barik S. (2003) The heat shock protein 90 of Plasmodium falciparum and antimalarial activity of its inhibitor, geldanamycin. J Malar 2:30.

Kurebayashi, J., Otsuke, T., Kurosumi, M., Soga, S., Akinaga, S. and Sonoo, H. (2001) A radicicol derivative, KF58333, inhibits expression of hypoxia-inducible factor-1α and vascular endothelial growth factor, angiogenesis and growth of human breast cancer xenografts. Jpn. J. Cancer Res. 92:1342-1351.

Le Brazidec, J.-Y., Kamal, A., Busch, D., Thao, L., Zhang, L. *et al*. (2003) Synthesis and biological evaluation of a new class of geldanamycin derivatives as potent inhibitors of Hsp90. J. Med. Chem. 47: 3865-3873.

Lee MH, Pascopella L, Jacobs WR Jr, Hatfull GF. (1991), Site-specific integration of mycobacteriophage L5: integration-proficient vectors for Mycobacterium smegmatis, Mycobacterium tuberculosis, and bacille Calmette-Guerin. *Proc Natl Acad Sci U S A.*; **88**:3111-5.

Lee, Y.-S., Marcu, M.G. and Neckers, L. (2004) Quantum chemical calculations and mutational analysis suggest heat shock protein 90 catalyzes trans-cis isomeration of geldanamycin. Chem. Biol. 11:991-998.

Liu, X.-D., Morano, K.A. and Thiele D.J. (1999); The yeast Hsp110 family member, Sse1, is an Hsp90 cochaperone. J Biol Chem 274:26654-26660.

Mandler, R., Wu, C., Sausville, E.A., Roettinger, A.J., Newman, D.J., Ho, D.K., King, R., Yang, D., Lippman, M.E., Landolfi, N.F., Dadachova, E., Brechbiel, M.W. and Waldman, T.A. (2000) Immunoconjugates of geldanamycin and anti-HER2 monoclonal antibodies: antiproliferative activity on human breast carcinoma cell lines. Journal of the National Cancer Institute 92:1573-1581.

Matsushima, P., M. C. Broughton, *et al.* (1994). Conjugal transfer of cosmid DNA from *Escherichia coli* to *Saccharopolyspora spinosa*: effects of chromosomal insertions on macrolide A83543 production. Gene 146(1): 39-45.

Matsuura, M., Noguchi, T., Yamaguchi, D., Aida, T., Asayama, M., Takahashi, H. and Shirai, M. (1996). The sre gene (ORF469) encodes a site-specific recombinase responsible for integration of the R4 phage genome. *J Bact.* 178(11):3374-3376.

McLaughlin S. H., Smith, H.W. and Jackson S.E. (2002) Stimulation of the weak ATPase activity of human Hsp90 by a client protein. J. Mol. Biol. 315: 787-798.

McCammon, M. T. and L. W. Parks (1981). Inhibition of sterol transmethylation by S-adenosylhomocysteine analogs. J Bacteriol **145**(1): 106-12.

Muroi M, Izawa M, Kosai Y, Asai M. (1981) "The structures of macbecin I and II" Tetrahedron, 37, pp1123-1130.

Muroi, M., Izawa M., Kosai, Y., and Asai, M. (1980) Macbecins I and II, New Antitumor antibiotics. II. Isolation and characterization. J Antibiotics 33:205-212.

Neckers, L (2003) Development of small molecule Hsp90 inhibitors: utilizing both forward and reverse chemical genomics for drug identification. Current Medicinal Chemistry 9:733-739.

Neckers, L. (2002) Hsp90 inhibitors as novel cancer chemotherapeutic agents. Trends in Molecular Medicine 8:S55-S61.

Nimmanapalli, R., O'Bryan, E., Kuhn, D., Yamaguchi, H., Wang, H.-G. and Bhalla, K.N. (2003) Regulation of 17-AAG-induced apoptosis: role of Bcl-2, Bcl-x_L, and Bax downstream of 17-AAG-mediated down-regulation of Akt, Raf-1, and Src kinases. Neoplasia 102:269-275.

Omura, S., Iwai, Y., Takahashi, Y., Sadakane, N., Nakagawa, A., Oiwa, H., Hasegawa, Y., Ikai, T., (1979), Herbimycin, a new antibiotic produced by a strain of *Streptomyces*. The Journal of Antibiotics, 32(4), pp255-261.

Omura, S., Miyano, K., Nakagawa, A., Sano, H., Komiyama, K., Umezawa, I., Shibata, K, Satsumabayashi, S., (1984), "Chemical modification and antitumor activity of Herbimycin A. 8,9-epoxide, 7,9-carbamate, and 17 or 19-amino derivatives". The Journal of Antibiotics, 37(10), pp1264-1267.

Ono, Y., Kozai, Y. and Ootsu, K. (1982) Antitumor and cytocidal activities of a newly isolated benzenoid ansamycin, Macbecin I. Gann. 73:938-44.

Patel, K., M. Piagentini, Rascher, A., Tian, Z. Q., Buchanan, G. O., Regentin, R., Hu, Z., Hutchinson, C. R. And McDaniel, R. (2004). "Engineered biosynthesis of geldanamycin analogs for hsp90 inhibition." Chem Biol 11(12): 1625-33.

Pfeifer, B. A. and C. Khosla (2001). "Biosynthesis of polyketides in heterologous hosts." Microbiology and Molecular Biology Reviews 65(1): 106-118.

Rascher, A., Hu, Z., Viswanathan, N., Schirmer, A. *et al.* (2003) Cloning and characterization of a gene cluster for geldanamycin production in *Streptomyces hygroscopicus* NRRL 3602. FEMS Microbiology Letters 218:223-230.

Rascher, A., Z. Hu, Buchanan, G. O., Reid, R. and Hutchinson, C. R. (2005). Insights into the biosynthesis of the benzoquinone ansamycins geldanamycin and herbimycin, obtained by gene sequencing and disruption. Appl Environ Microbiol 71(8): 4862-71.

Rawlings, B. J. (2001). "Type I polyketide biosynthesis in bacteria (Part B)." Natural Product Reports 18(3): 231-281.

Roth T., Burger A.M., Dengler W., Willmann H. and Fiebig H.H. Human tumor cell lines demonstrating the characteristics of patient tumors as useful models for anticancer drug screening. In: Fiebig HH, Burger AM (eds). Relevance of Tumor Models for Anticancer Drug Development. *Contrib. Oncol*. 1999, 54: 145 - 156.

Rowe, C. J.; Cortés, J.; Gaisser, S.; Staunton, J.; Leadlay, P. F. *Gene* **1998**, *216*, 215-223

Rowlands, M.G., Newbatt, Y.M., Prodromou, C., Pearl, L.H., Workman, P. and Aherne, W. (2004) High-throughput screening assay for inhibitors of heat-shock protein 90 ATPase activity. Analytical Biochemistry 327:176-183

Schulte, T.W., Akinaga, S., Murakata, T., Agatsuma, T. *et al*. (1999) Interaction of radicicol with members of the heat shock protein 90 family of molecular chaperones. Molecular Endocrinology 13:1435-1488.

Shibata, K., Satsumabayashi, S., Nakagawa, A., Omura, S. (1986a) The structure and cytocidal activity of herbimycin C. The Journal of Antibiotics, 39(11), pp1630-1633.

Shibata, K., Satsumabayashi, S., Sano, H., Komiyama, K., Nakagawa, A., Omura, S. (1986b) Chemical modification of Herbimycin A: synthesis and *in vivo* antitumor activities of halogenated and other related derivatives of herbimycin A. The Journal of Antibiotics, 39(3), pp415-423.

Shirling, E.B. and Gottlieb, D. (1966) International Journal of Systematic Bacteriology 16:313-340

Smith-Jones, P.M., Solit, D.B., Akhurst, T., Afroze, F., Rosen, N. and Larson, S.M. (2004) Imaging the pharmacodynamics of HER2 degradation in response to Hsp90 inhibitors. Nature Biotechnology 22:701-706.

Smovkina, T., Mazodier, P., Boccard, F., Thompson, C.J. and Guerineau, M. (1990) Construction of a series of pSAM2-based integrative vectors for use in actinomycetes. *Gene* 94: 53-59.

Spiteller, P., Bai, L., Shang, G., Carroll, B.J., Yu, T.-W. and Floss, H. G. (2003). The post-polyketide synthase modification steps in the biosynthesis of the antitumor agent ansamitocin by Actinosynnema pretiosum. J Am Chem Soc 125(47): 14236-7

Sreedhar A.S., Nardai, G. and Csermely, P. (2004) Enhancement of complement-induced cell lysis: a novel mechanism for the anticancer effects of Hsp90 inhibitors. Immunology letters 92:157-161.

Sreedhar, A.S., Söti, C. and Csermely, P. (2004a) Inhibition of Hsp90: a new strategy for inhibiting protein kinases. Biochimica Biophysica Acta 1697:233-242.

Staunton, J. and K. J. Weissman (2001). "Polyketide biosynthesis: a millennium review." Natural Product Reports 18(4): 380-416.

Stead, P., Latif, S., Blackaby, A.P. *et al*. (2000) Discovery of novel ansamycins possessing potent inhibitory activity in a cell-based oncostatin M signalling assay. J Antibiotics 53:657-663.

Supko, J.G., Hickman, R.L., Grever, M.R. and Malspeis, L (1995) Preclinical pharmacologic evaluation of geldanamycin as an antitumor agent. Cancer Chemother. Pharmacol. 36:305-315.

Takahashi, A., Casais, C., Ichimura K. and Shirasu, K. (2003) HSP90 interacts with RAR1 and SGT1 and is essential for RPS2-mediated disease resistance in *Arabidopsis*. Proc. Natl. Acad. Sci. USA 20:11777-11782.

Tanida, S., Hasegawa, T. and Higashide E. (1980) Macbecins I and II, New Antitumor antibiotics. I. Producing organism, fermentation and antimicrobial activities. J Antibiotics 33:199-204.

Tian, Z.-Q., Liu, Y., Zhang, D., Wang, Z. *et al.* (2004) Synthesis and biological activities of novel 17-aminogeldanamycin derivatives. Bioorganic and Medicinal Chemistry 12:5317-5329.

Uehara, Y. (2003) Natural product origins of Hsp90 inhibitors. Current Cancer Drug Targets 3:325-330.

Van Mellaert, L., Mei, L., Lammertyn, E., Schacht, S., and Anné, J. (1998) Site-specific integration of bacteriophage VWB genome into *Streptomyces venezuelae* and construction of a

VWB-based integrative vector. *Microbiology* 144:3351-3358.

Vasilevskaya, I.A., Rakitina, T.V. and O'Dwyer, P.J. (2003) Geldanamycin and its 17-Allylamino-17-Demethoxy analogue antagonize the action of cisplatin in human colon adenocarcinoma cells: differential caspase activation as a basis of interaction. Cancer Research 63: 3241-3246.

Watanabe, K., Okuda, T., Yokose, K., Furumai, T. and Maruyama, H.H. (1982) Actinosynnema mirum, a new producer of nocardicin antibiotics. J. Antibiot. 3:321-324.

Wegele, H., Müller, L. and Buchner, J. (2004) Hsp70 and Hsp90-a relay team for protein folding. Rev Physiol Biochem Pharmacol 151:1-44.

Wenzel, S.C., Gross, F, Zhang, Y., Fu, J., Stewart, A.F. and Müller, R (2005) Heterologous expression of a myxobacterial natural products assembly line in Pseudomonads via Red/ET recombineering. Chemistry & Biology 12: 249-356.

Whitesell, L., Mimnaugh, E.G., De Costa, B., Myers, C.E. and Neckers, L.M. (1994) Inhibition of heat shock protein HSP90-pp60$^{v\text{-}src}$ heteroprotein complex formation by benzoquinone ansamycins: Essential role for stress proteins in oncogenic transformation. Proc. Natl. Acad. Sci. USA 91: 8324-8328.

Winklhofer, K.F., Heller, U., Reintjes, A. and Tatzelt J. (2003) Inhibition of complex glycosylation increases the formation of PrP^{sc}. Traffic 4:313-322.

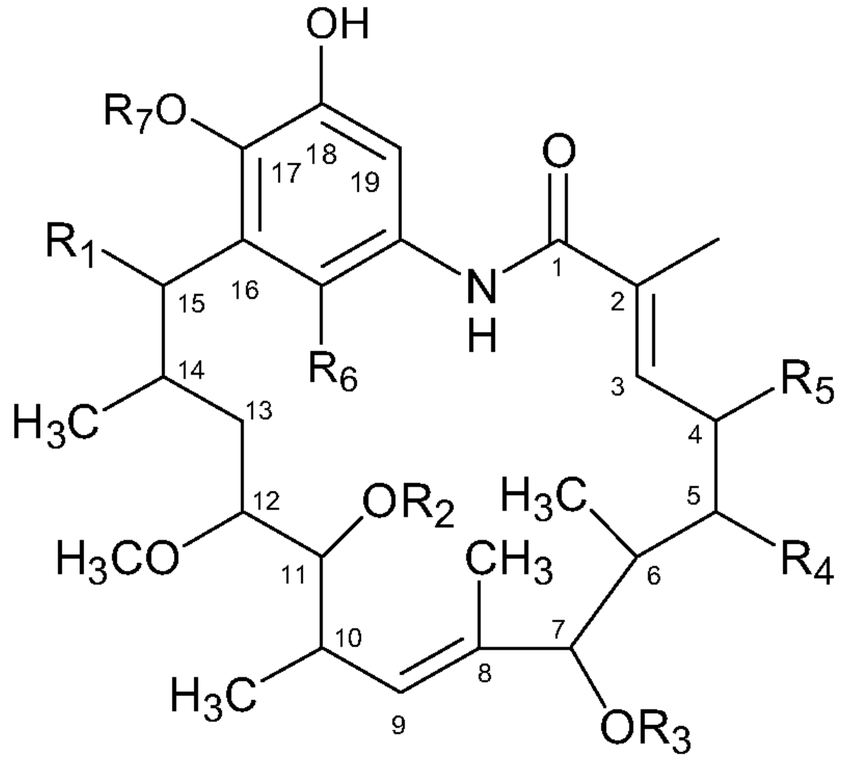
Workman P. (2003) Auditing the pharmacological accounts for Hsp90 molecular chaperone inhibitors: unfolding the relationship between pharmacokinetics and pharmacodynamics. Molecular Cancer Therapeutics 2:131-138.

Workman, P. and Kaye, S.B. (2002) Translating basic cancer research into new cancer therapeutics. Trends in Molecular Medicine 8:S1-S9.

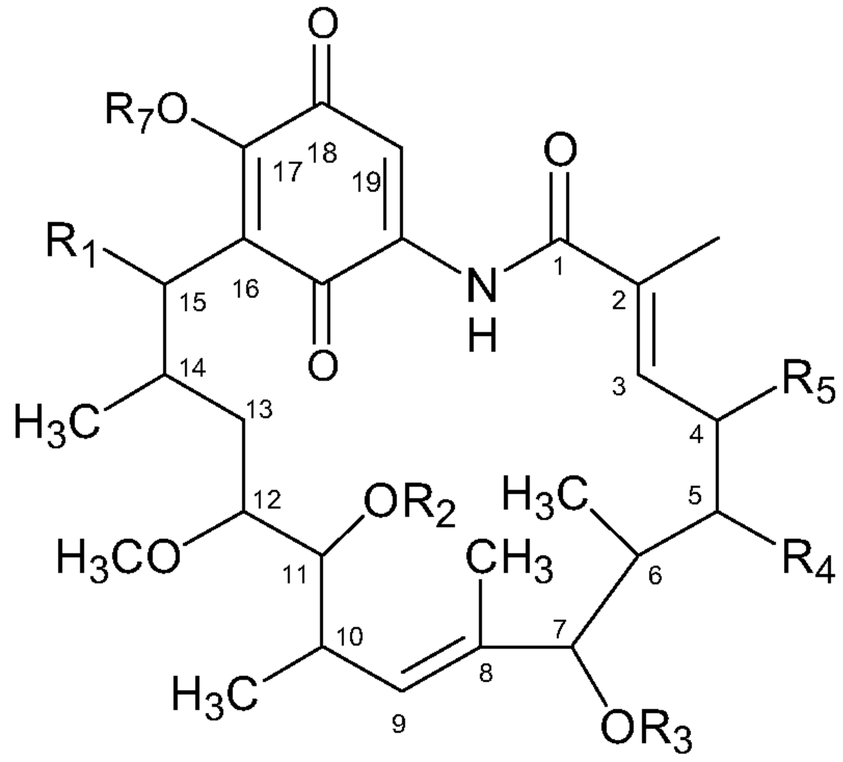
Young, J.C.; Moarefi, I. and Hartl, U. (2001) Hsp90: a specialized but essential protein folding tool. J. Cell. Biol. 154:267-273.

Claims

1. A 17-oxymacbecin analogue according to the formula (IA) or (IB) below, or a pharmaceutically acceptable salt thereof:



(IA)



(IB)

wherein:

R_1 represents H, OH or OCH_3;

R_2 represents H or CH_3

R_3 represents H or $CONH_2$

R_4 and R_5 either both represent H or together they represent a bond (i.e. C4 to C5 is a double bond); and

R_6 represents H or OH; and

R_7 represents H or CH_3.

2. A compound according to claim 1, wherein the 17-oxymacbecin analogue is according to formula (IA)

3. A compound according to claim 1, wherein the 17-oxymacbecin analogue is according to formula (IB)

4. A compound according to any one of claims 1 to 3 wherein R_3 represents $CONH_2$.

5. A compound according to any one of claims 1 to 4 wherein R_6 represents OH.

6. A compound according to any one of claims 1 to 4 wherein R_6 represents H

7. A compound according to any one of claims 1 to 6 wherein R_7 represents H

8. A compound according to claim 1 wherein the 17-oxymacbecin analogue has a structure according to Formula (IA), R_1 represents H, R_2 represents H, R_3 represents $CONH_2$, R_4 and R_5 each represent H, R_6 represents OH and R_7 represents H

9. A compound according to claim 1 wherein the 17-oxymacbecin analogue has a structure according to Formula (IB), R_1 represents H, R_2 represents H, R_3 represents $CONH_2$, R_4 and R_5 each represent H, and R_7 represents H.

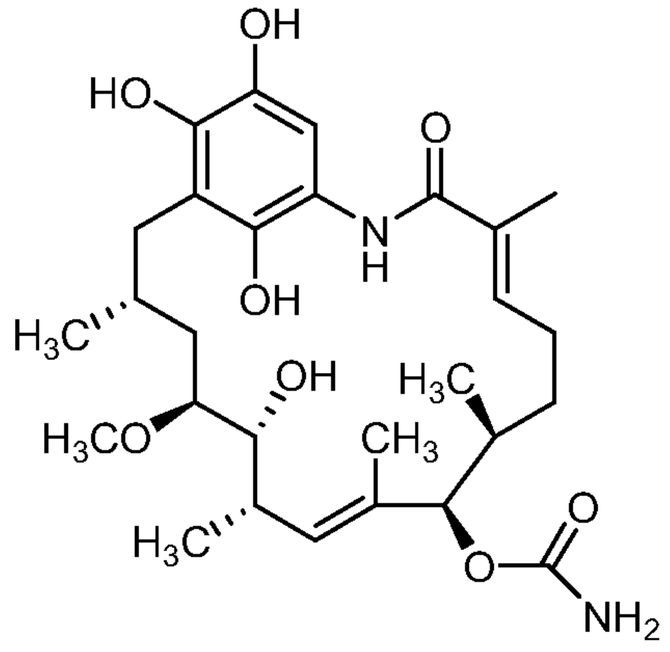
10. A compound according to claim 1 wherein the 17-oxymacbecin analogue has a structure according to Formula (IA), wherein R_1 represents H, R_2 represents H, R_3 represents $CONH_2$, R_4 and R_5 each represent H, R_6 represents OH and R_7 represents CH_3.

11. A compound according to claim 1 wherein the 17-oxymacbecin analogue has a structure according to Formula (IB), wherein R_1 represents H, R_2 represents H, R_3 represents $CONH_2$, R_4 and R_5 each represent H, and R_7 represents CH_3.

12. A compound according to claim 1 wherein the 17-oxymacbecin analogue has a structure according to Formula (IA), wherein R_1 represents H, R_2 represents H, R_3 represents $CONH_2$, R_4 and R_5 each represent H, R_6 represents H and R_7 represents H.

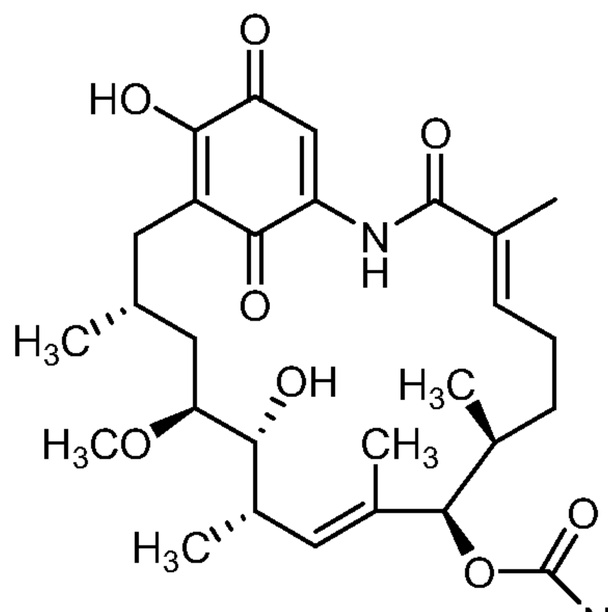
13. A compound according to claim 1 wherein the 17-oxymacbecin analogue has a structure according to Formula (IA), wherein R_1 represents H, R_2 represents H, R_3 represents $CONH_2$, R_4 and R_5 each represent H, R_6 represents H and R_7 represents CH_3.

14. A compound according to claim 1 which is



or a pharmaceutically acceptable salt thereof

15. A compound according to claim 1 which is



or a pharmaceutically acceptable salt thereof

16. A pharmaceutical composition comprising a 17-oxymacbecin analogue according to any one of claims 1 to 15, together with one or more pharmaceutically acceptable diluents or carriers.

17. A 17-oxymacbecin analogue according to any one of claims 1 to 15 for use as a medicament.

18. The use of a 17-oxymacbecin analogue according to any one of claims 1 to 15 in the manufacture of a medicament for the treatment of cancer, B-cell malignancies, malaria, fungal infection, diseases of the central nervous system and neurodegenerative diseases, diseases dependent on angiogenesis, autoimmune diseases and/or as a prophylactic pretreatment for cancer.

19. A 17-oxymacbecin analogue according to any one of claims 1 to 15 for use as a medicament for the treatment of cancer, B-cell malignancies, malaria, fungal infection, diseases of the central nervous system and neurodegenerative diseases, diseases dependent on angiogenesis, autoimmune diseases and/or as a prophylactic pretreatment for cancer..

20. A method of treatment of cancer, B-cell malignancies, malaria, fungal infection, diseases of the central nervous system and neurodegenerative diseases, diseases dependent on angiogenesis, autoimmune diseases and/or as a prophylactic pretreatment for cancer which comprises administering to a patient in need thereof an effective amount of a 17-oxymacbecin analogue according to any one of claims 1 to 15.

21. A 17-oxymacbecin analogue, composition, use or method according to any one of claims 1 to 20, wherein the 17-oxymacbecin analogue or a pharmaceutically acceptable salt thereof is administered in combination with another treatment.

22. A 17-oxymacbecin analogue, composition, use or method according to claim 21 where the other treatment is selected from the group consisting of: methotrexate, leukovorin, prenisone, bleomycin, cyclophosphamide, 5-fluorouracil, paclitaxel, docetaxel, vincristine, vinblastine, vinorelbine, doxorubicin, tamoxifen, toremifene, megestrol acetate, anastrozole, goserelin, anti-HER2 monoclonal antibody, capecitabine, raloxifene hydrochloride, EGFR inhibitors, VEGF inhibitors, proteasome inhibitors, radiotherapy and surgery.

23 ~~22~~. A 17-oxymacbecin analogue, composition, use or method according to claim 21 where the other treatment is selected from the group consisting of conventional chemotherapeutics such as cisplatin, cytarabine, cyclohexylchloroethylnitrosurea, gemcitabine, Ifosfamid, leucovorin, mitomycin, mitoxantone, oxaliplatin; taxanes including taxol and vindesine; hormonal therapies; monoclonal antibody therapies such as cetuximab (anti-EGFR); protein kinase inhibitors such as dasatinib and lapatinib; histone deacetylase (HDAC) inhibitors such as vorinostat; angiogenesis inhibitors such as sunitinib, sorafenib, lenalidomide; mTOR inhibitors such as temsirolimus; and imatinib.

24. A method for the production of a 17-oxymacbecin analogue according to any one of claims 1 to 15, said method comprising:

a) providing a first host strain that produces macbecin or an analogue thereof when cultured under appropriate conditions

b) inserting one or more post-PKS genes not usually associated with the macbecin PKS gene cluster, wherein at least one of said post-PKS genes is *gdmL,* or a homologue therof

c) culturing said modified host strain under suitable conditions for the production of novel compounds; and

d) optionally isolating the compounds produced.

25. A method according to claim 24 which additionally comprises the step of

e) deleting or inactivating one or more macbecin post-PKS genes, or homologues thereof, said step usually occurring prior to step c).

26. A method according to claim 25 which additionally comprises the step of

f) reintroducing one or more of the deleted post-PKS genes, said step usually occurring prior to step c).

27. A method according to claims 24 to 26 which additionally comprises the step of

g) introducing post-PKS genes from other PKS clusters, said step usually occurring prior to step c).

28. A genetically engineered host strain which naturally produces macbecin in its unaltered state, said strain having one or more post-PKS genes not naturally associated with the macbecin PKS gene cluster, wherein at least one of said post-PKS genes is *gdmL* or a homologue thereof inserted

29. The host strain of claim 28 in which one or more post-PKS genes from the macbecin PKS gene cluster have additionally been deleted.

30. The host strain of claim 29 in which one or more of the deleted post-PKS genes have been re-introduced.

31. The host strain of any one of claims 28 to 30 in which one or more post-PKS genes from heterologous PKS clusters have been re-introduced

32. The host strain of claim 29 in which *mbcP*, *mbcP450*, *mbcMT1* and *mbcMT2* have been deleted, and *gdmL* has been introduced.

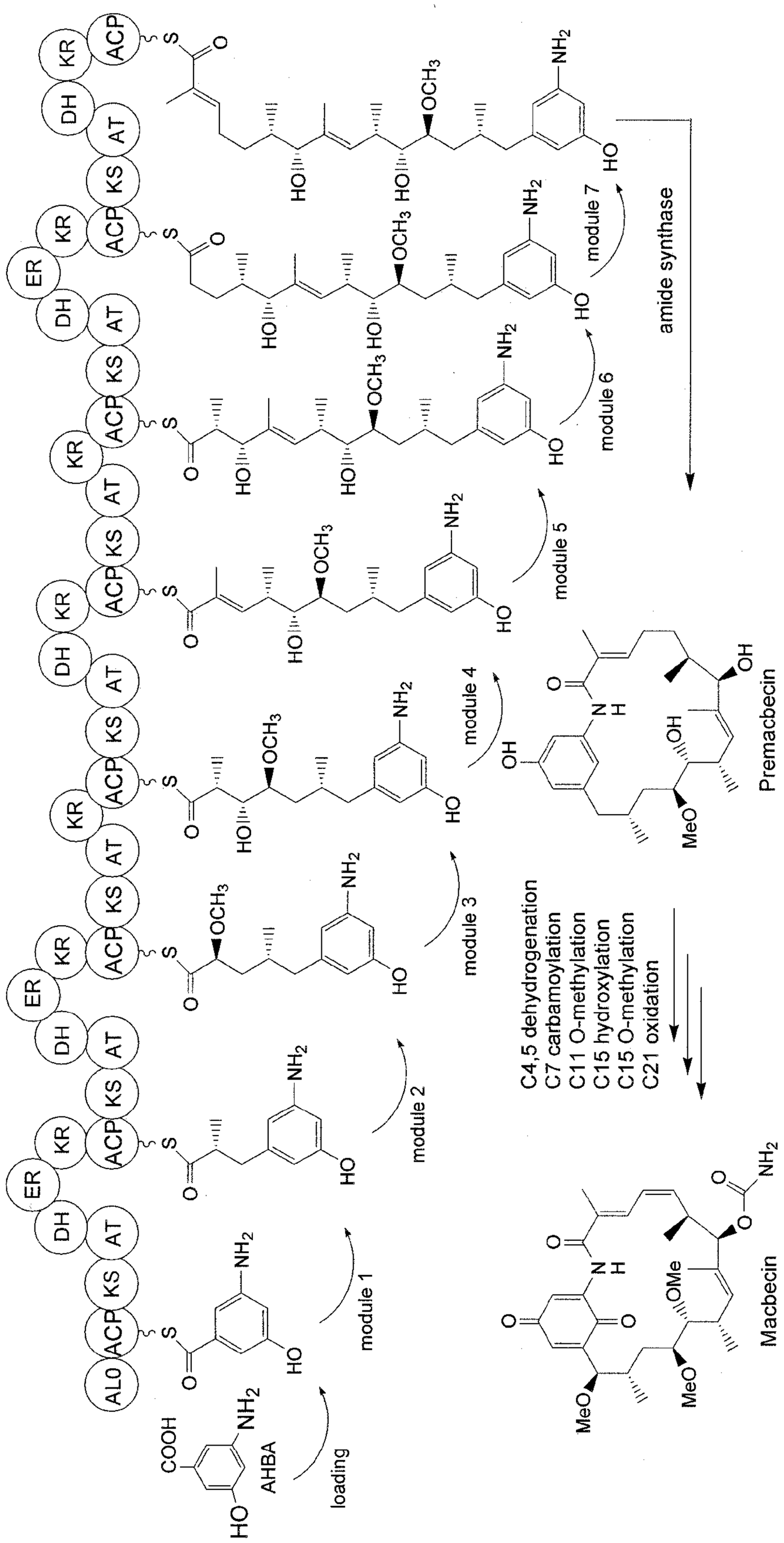
33. A host strain according to any one of claims 28 to 32 which is *A. pretiosum* or *A. mirum*.

34. A process for producing 17-oxymacbecin or an analogue thereof which comprises culturing a strain according to any one of claims 28 to 33.

35. A process according to claim 34 further comprising the step of isolating 17-oxymacbecin or an analogue thereof.

36. Use of a host strain according to claims 28 to 33 for the production of 17-oxymacbecin or analogues thereof.

Figure 1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module 5
module 6
module 7
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C4,5 dehydrogenation
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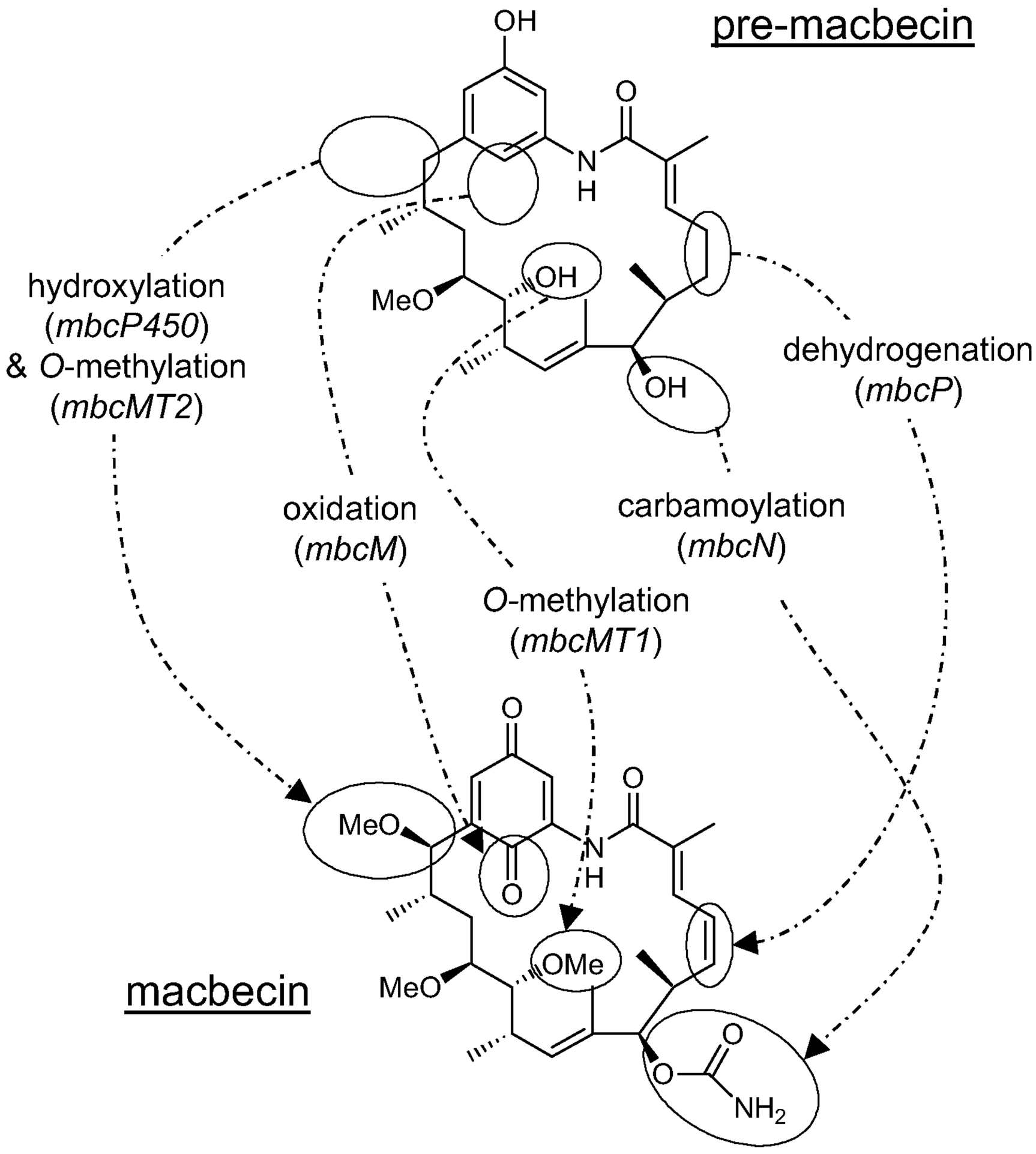
C15 O-methylation
C21 oxidation
Premacbecin
Macbecin

Figure 2


pre-macbecin
hydroxylation
(*mbcP450*)
& *O*-methylation
(*mbcMT2*)
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dehydrogenation
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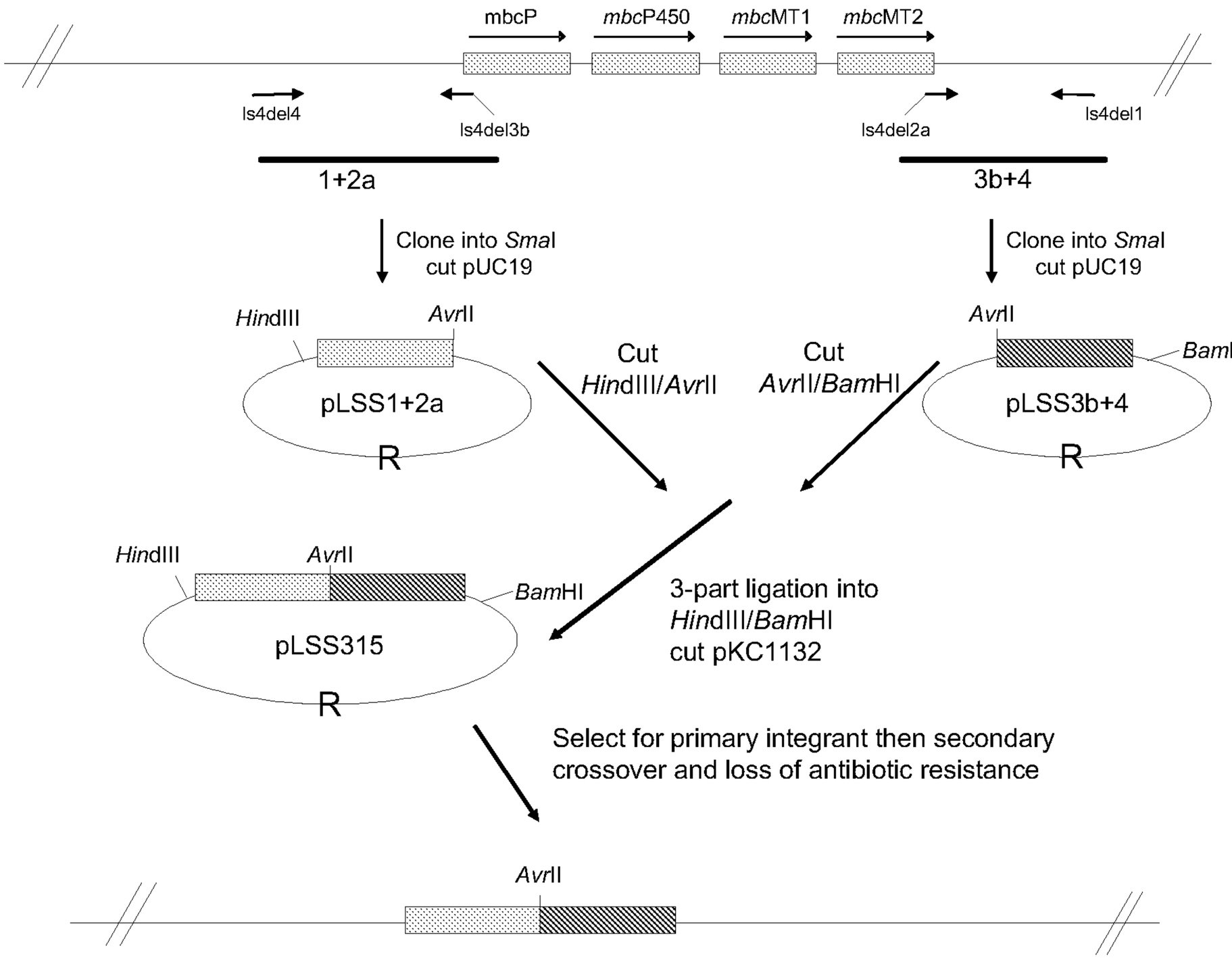
macbecin

Figure 3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1+2a
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Clone into SmaI cut pUC19
Clone into SmaI cut pUC19
HindIII
AvrII
pLSS1+2a
R
Cut HindIII/AvrII
Cut AvrII/BamHI
AvrII
BamHI
pLSS3b+4
R
HindIII
AvrII
BamHI
pLSS315
R
3-part ligation into HindIII/BamHI cut pKC1132
Select for primary integrant then secondary crossover and loss of antibiotic resistance
AvrII

Figure 4

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Figure 5

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1501 GAGGACGTGG CCACCGACCT GCCCGCCTAC CCGTTCCTAG G
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Figure 6A

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1021 TCGCGGCCCA GGTCCTGCAT CACACCTCGG CGCAACGCGT CCTGGCGATT TCGAACCCGA
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1141 ACCGCCATCT CGCGGGGCTG ATGTCCGGCC TCTCGCTGCG CTACGACCTG CCCGGCGATC
1201 ACCCGCTCAC CGGAGAGCGC ATCCCGGACG CCGATCTGGT GACCGAAACC GGCACCACCC
1261 GGCTGTCGAC GCTCTTCGGC TCCGGACACG CCGTCCTGCT CGACCTGGCC GGAGCCGTCC
1321 CGGCCGACCT CCCGCTCCCG CCACGAGTCG ACCTCGTCCG CGCCACATGC GCCGACGACA
1381 TGGGCGCCGC CGCCCTGCTC ATCCGTCCCG ACGGCTATGT CTGCTGGGCT ACGGACACCT
1441 CCGCCGCCTG CGGCGACACC CTGCTGGCCG CGCTCACCGG CGACCTCGCG AGGGTGCCCT
1501 GACCTCTAGA CC
```

Figure 6B

```
   1    MLTESTTEVV VAGAGATGLM LAYELALAGV ETLVLEKLPQ RIQQVKGGTI
  51    QPRTAELLES RGLLEPMLRR AIARDPVGGS FGALPVPLDC APWRTEHPFP
 101    IGIPQWEIEE VLEERATAAG ARVLRGTAVS GVAPDDDGVV VTADGLRARA
 151    HYLVACDGGH STVRKLLGLP FPGRAGTHPA VLADIRLSAV SSLVPRQMGL
 201    MSTMTRHARG YWSMLVPLGG DRYRFTFGHA DQADTARDTP VTHEEIAAAL
 251    QAVYGPETTL GAVDNSSRFS DATRQLEHYR TGRVLFAGDA AHIHPPLGAQ
 301    GLNLGVQDAL NLGWKLAAVL QDRAPNGLLD SYHAERHPVA AQVLHHTSAQ
 351    RVLAISNPSE DVAALRDIFT DLLRLPDTNR HLAGLMSGLS LRYDLPGDHP
 401    LTGERIPDAD LVTETGTTRL STLFGSGHAV LLDLAGAVPA DLPLPPRVDL
 451    VRATCADDMG AAALLIRPDG YVCWATDTSA ACGDTLLAAL TGDLARVP*
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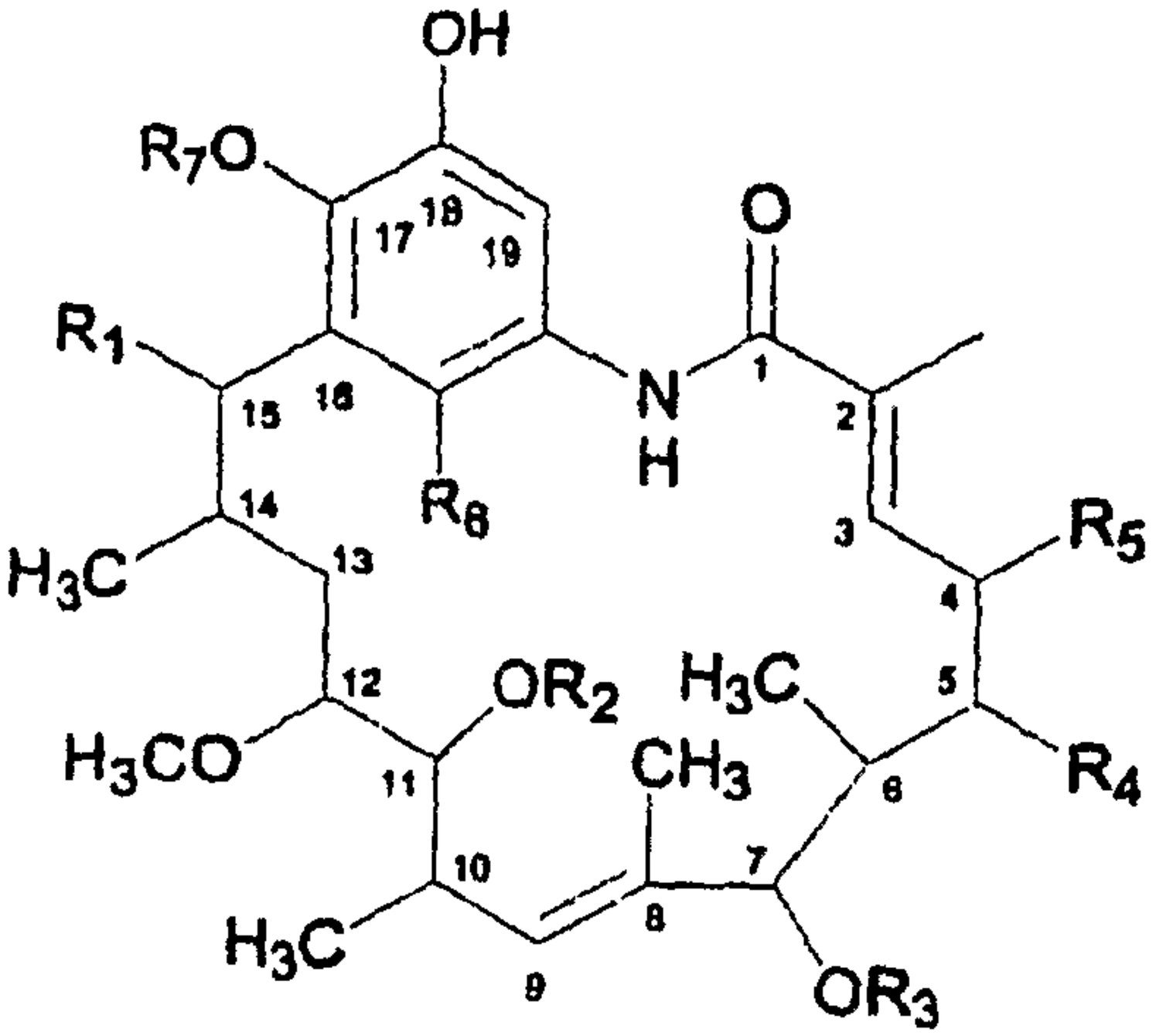
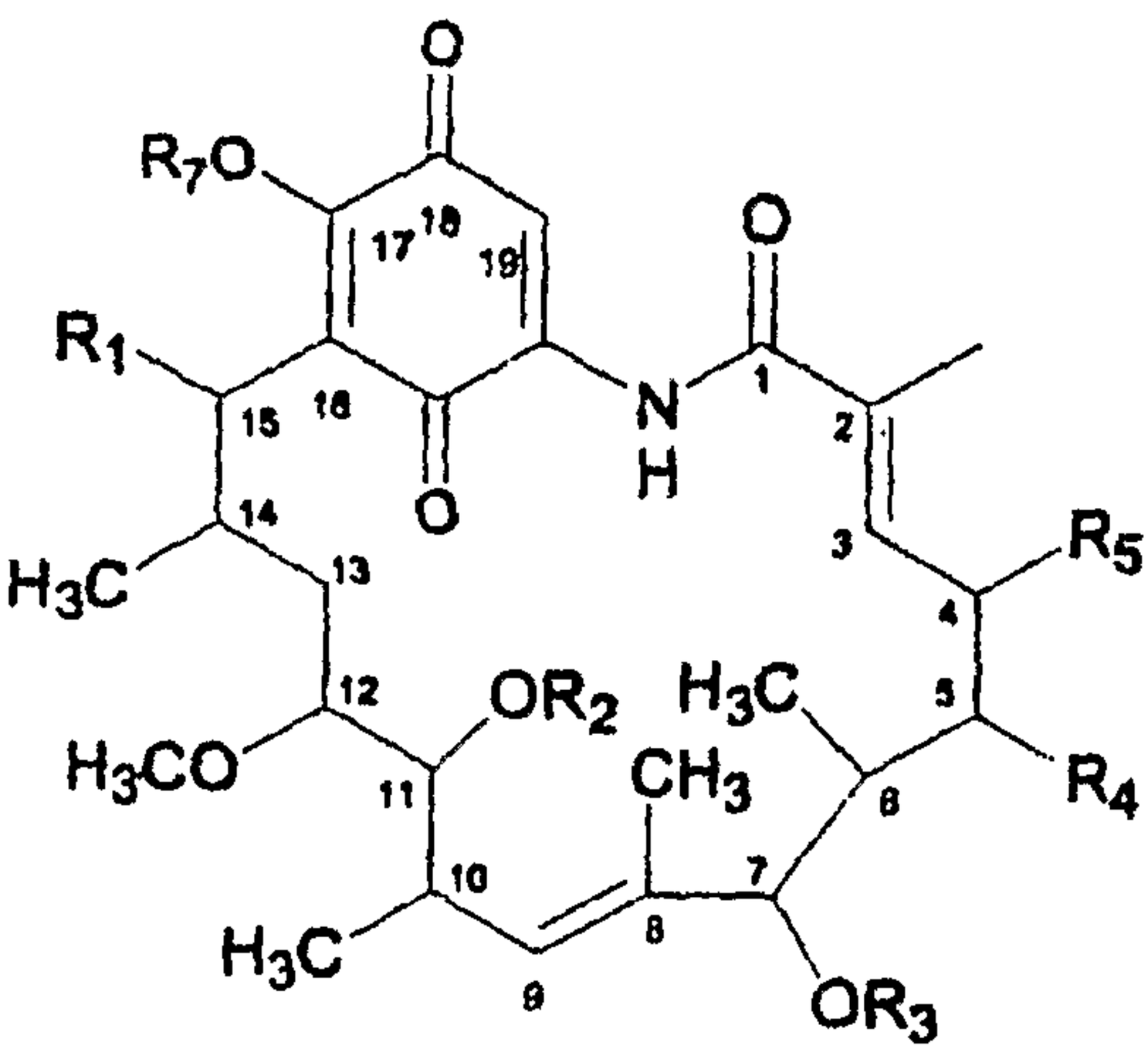

OH
R7O
17
18
19
O
R1
15
16
N
H
1
2
14
R6
3
R5
H3C
13
4
12
OR2
H3C
5
H3CO
11
CH3
6
R4
10
7
H3C
8
9
OR3

(IA)


O
R7O
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R1
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16
N
H
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2
14
O
3
R5
H3C
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4
12
OR2
H3C
5
H3CO
11
CH3
6
R4
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7
H3C
8
9
OR3

(IB)