Chemistry and Biology of Landomycins, an Expanding Family of Polyketide Natural Products

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Abstract: This review covers existing literature (from 1990 to 2008) on landomycins (**LS**), a family of glycosylated angucyclines, with an emphasis on the bioactivity scope of landomycin (**La**)-like structures accessible *via* biocombinatorial manipulations. Some **LS** display strong antitumor activity and have inspired several chemical studies focused mainly on their unusual deoxysugar chains. A decade of genetic studies on **La**-producing bacteria has provided many novel molecules with altered structure and activity. A complex nonlinear correlation between the length of the carbohydrate tail of **LS** and their antitumor activity has also been revealed. It implies that simpler **LS** than the largest member of the family, **LaA**, are still potential drug leads. Combinatorial biosynthesis appears to be a powerful tool to search the chemical space around the **La** scaffold.

Key Words: Landomycins, angucyclines, cancer, structure-activity relationship studies, combinatorial biosynthesis, *Streptomyces*.

INTRODUCTION

Cancer is a leading cause of disease-related mortality around the world. According to the World Health Organization, between 2005 and 2015, 84 million people will die of cancer without intervention. Along with other medical approaches, chemotherapy of malignant tumors is an important component of most modern strategies of treatment of this devastating disease. Currently, the inventory of anticancer drugs is extensive; however, the search for novel molecules cannot stop. The lack of efficient chemotherapies for many types of cancer as well as the relentless development of multidrug resistance by cancer cells [1] are two main factors that define the need for novel anticancer compounds.

Natural products have proven to be an invaluable source of antitumor bioactivity. It is of special interest to discover structurally novel families of natural compounds that display strong anticancer activity, since they might fight multidrug resistant cancers and/or operate *via* a novel mechanism (thus establishing a new drug target and inspiring new rounds of research). Here we focus on a novel family of polyketide natural products, the landomycins (**LS**; Fig. (**1**)), which have attracted the attention of the scientific community interested in novel bioactive molecules, unexploited drug targets and unusual biosynthetic processes [2]. Here, we will review what is known about the biological properties of **LS**, how novel **LS** can be generated, and what has to be done to move these interesting compounds closer to practical use.

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LANDOMYCINS – OCCURRENCE IN NATURE AND STRUCTURAL CHARACTERISTICS

LS fall into the angucycline group of antibiotics, along with numerous other aromatic (polycyclic) polyketides [3-5]. The most salient feature of angucyclines is their uniquely shaped benz[a]anthracene tetracyclic framework with an angularly condensed ring A (Fig. (**1**)). The "curved" aglycon distinguishes angucyclines from other aromatic polyketides and is at least partially responsible for the wider range of bioactivities of angucyclines compared to, for instance, anthracyclines and tetracyclines [4]. To date, only two **La**-producing bacteria have been described, *Streptomyces cyanogenus* S136 (DSM5087; accumulates **LaA** (**1**), **B** (**2**), **C** (**3**) and **D** (**5**)) [6] and *Streptomyces globisporus* 1912 (produces **LaD** (**5**) and **E** (**4**)) [7, 8]. All naturally occurring **LS** share the same aglycon (landomycinone (**8**)); however, depending on culture and extraction conditions, 5,6-anhydroderivatives of compounds **1-3** and **5** can be recovered in large quantities from *S. cyanogenus* [6]. Natural **LS** differ in the structure of the carbohydrate moiety, a linear glycosidic chain containing only di- and trideoxysugars (β-D-olivose and α-L-rhodinose;in **LaC** (**3**) amicetose is also thought to be present [6]). **LaA** (**1**), the biggest member of the family, contains a hexasaccharide chain comprised of two repeating trisaccharides: α-L-rhodinose-(1-3)-β-D-olivose-(1-4)-β-D-olivose. From both chemical and biosynthetic points of view **LaD** (**5**), **E** (**4**), and **B** (**2**) can be regarded as intermediates to **LaA** (**1**), while **LaC** (**3**) seems to be a shunt product. The carbohydrate chains of **La** are connected to the aglycon phenolglycosidically. Phenol glycosides are rare among natural metabolites and the few angucyclines that contain this moiety have much shorter glycosidic chains than **LaA** (**1**). The naphtarazine chromophore-containing aglycon as well as the aromatic A ring (Fig. (**1**)) separate **LS** structurally from other

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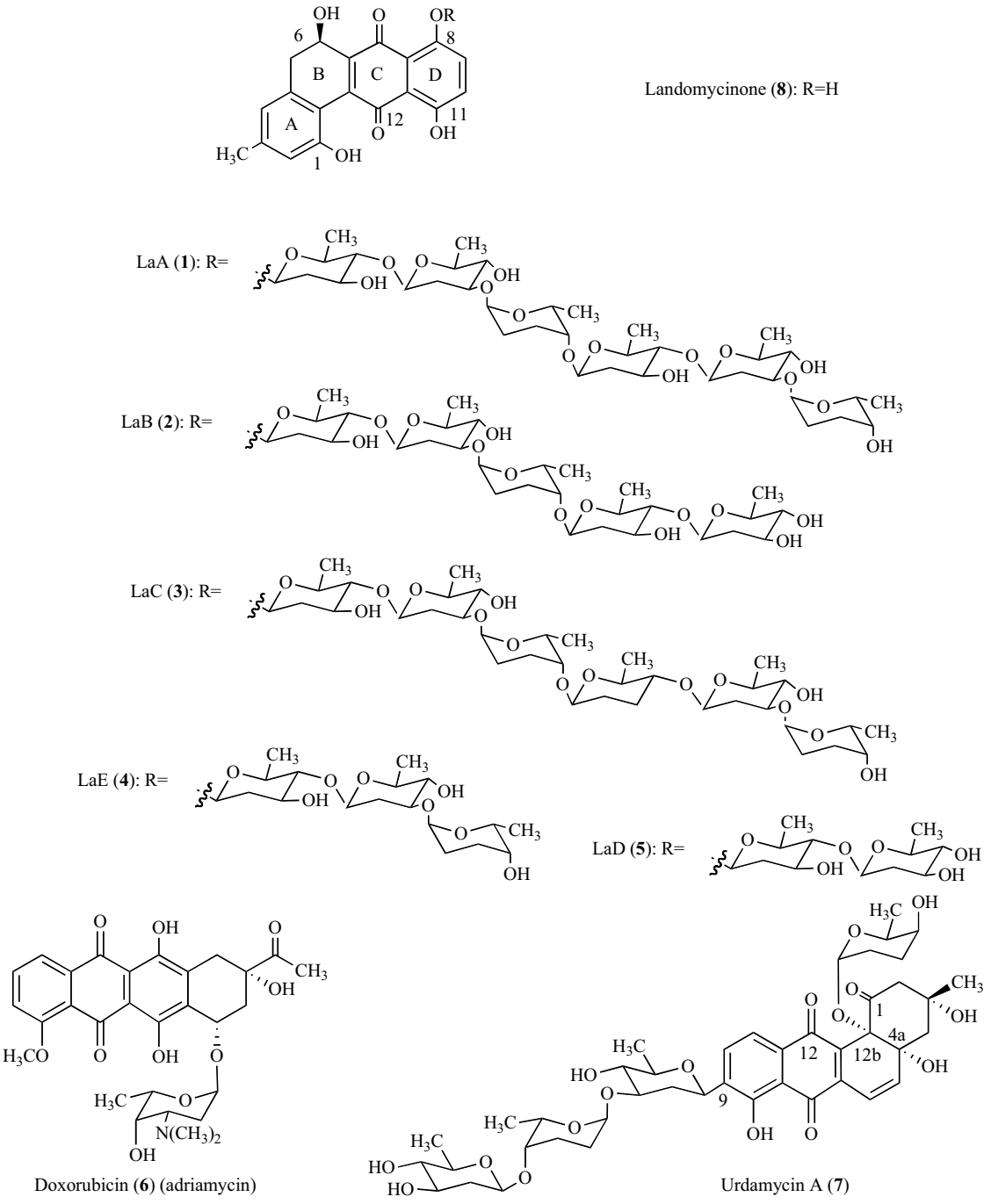

Fig. (1). Structures of naturally occurring **LS**. Structures of angucycline urdamycin A (**7**) and anthracycline doxorubicin (**6**) are shown for comparison. The carbon atom numbering of **La** and urdamycin aglyca is taken from [3].

angucyclines and have prompted further investigations into their biosynthesis and activity.

BIOLOGICAL ACTIVITIES OF THE LANDOMYCINS A AND E

Antibacterial Activity

LS display weak antibacterial activity, mainly against various Streptomycetes and other Gram-positive bacteria [9-12]. Although the mechanisms of their antibacterial action are unknown, it has been shown that **LS** with shorter glycosidic chains (**LaE** (**4**), **LaD** (**5**)) are more potent antibiotics than **LaA** (**1**). Researchers speculate that the long deoxysugar chain of **LaA** (**1**) decreases its diffusion into prokaryotic cells [9].

Molecular Targets in the Mammalian Cells

Initial *in vitro* experiments showed that **LaA** (**1**) interferes with DNA synthesis [13]. However, in contrast to many clinically useful drugs of a similar structure, like the anthracyclines and chromomycins, **LS** do not bind directly to DNA

[14-16]. Therefore, it was proposed that **LaA**-mediated inhibition of DNA replication must be caused by its (**1**) interference with DNA synthesis/repair enzymes. Flow cytometry experiments showed that **LaA** (**1**) specifically blocked cell cycle progression from G_1 phase to S phase (DNA synthesis) [13]. Structurally unrelated anticancer drugs (e.g. bleomycin, mitomycin C, and neocarzinostatin) possess a similar pattern of cell cycle inhibition. However, it remains unknown whether **LaA** (**1**) inhibits cell cycle *via* similar mechanisms. It should be noted that the above mentioned classes of the G_1/S transition inhibitors are also strong DNA intercalators, while **LS** are not, suggesting that they might operate *via* different molecular mechanisms. **LaE**-induced apoptosis in target cells was accompanied by the inhibition of DNA synthesis (shown by a decrease in [^{3}H]-thymidine incorporation into DNA) without pronounced changes in cell cycling and independent of p53 status [15]. It has also been shown that DNA topoisomerase I is not a direct molecular target of the **LaE** (**4**) [17], in agreement with the prediction that landomycinone (**8**) is not planar molecule [3] and can not intercalate DNA. The absence of visible changes in the cell cycling of **LaE**-treated cells points to differences in the mechanisms of its action compared with anthracycline antibiotics, which in most cases cause cell block in G2/M phase, probably due to the inhibition of DNA topoisomerase I and/or II [18-20]. In some tumor cells, **LaE (4)** dose-dependently increased pre-G1 apoptotic DNA fraction [21].

A set of experiments showed that **LaE** (**4**) strongly impairs mitochondria. Mitochondria damage decreases the intracellular pool of ATP and affects the oxidation-reduction processes in this organelle, which may lead to the generation of reactive oxygen species (**ROS**). Negative effects of **ROS** are often blocked by N-acetylcysteine (**NAC**), a known radical scavenger. **NAC** only partially blocked cytotoxic action of the adriamycin, while it efficiently protected **LaE**-treated cells [15]. Such an effect of **NAC** towards **LaE** (**4**) action resembled that towards typical **ROS**-inducing antibiotic bleomycin [22]. Thus, **LaE** (**4**) causes mitochondria damage that is accompanied by both intensive **ROS** generation (impairing other cellular activities) and a cascade of proapoptotic caspases (7 and 3), intracellular proteases important for cellular growth and viability [15] (Fig. (**2**)).

Anticancer Potential

LaA (**1**) and **LaE** (**4**) are model compounds for studies on the anticancer activites of the **LS**. *In vitro* studies show that at low micromolar concentrations **LaA** (**1**) is more active than other clinically used anticancer drugs (e.g. bleomycin, cisplatin, doxorubicin, mitomycin, etoposide, carmustin, vinblastin and paclitaxel). **LaA** (**1**) inhibits tumor colony formation in a dose-dependent manner [23]. Subsequent *in vivo* evaluation revealed that **LaA** (**1**) is highly cytotoxic which precludes its further development as a drug. With the ultimate aim of generating improved **LS**, researchers have focused on gaining insight into mode of action of **LaA** (**1**) and **LaE** (**4**), and defining the relationship between the glycoside chain and antitumor activity.

The intuitive (yet unsubstantiated) idea that all **LS** exert their anticancer action *via* a similar mechanism(s) was popular in early literature [4]. However, recent studies on **LaE** (**4**) have shown that this may not be true. **LaE** (**4**) exerted an anticancer action in the Guerin's carcinoma in rats [24], and, in micromolar concentrations, it dose-dependently inhibited growth and induced apoptosis in various cancer cells *in vitro* [15, 25]. Its cytotoxicity profile is comparable to that of the adriamycin [15, 26]. We have tested **LaE** (**4**) action towards almost 30 cell lines of animal and human tumors of different tissue origin. Some of those results are presented in the Table **1**. It should be noted that several cell lines differed considerably in their resistance to specific anticancer drugs, such as vincristine, cisplatin, mitoxanthrone, and adriamycin. The $IC_{50}$of the **LaE** (**4**) cytotoxic action ranges from 0.37 ug/ml (MDA-468 line of human breast tumor) to 15.04 ug/ml (CCL-64) line of mink lung normal epithelium).

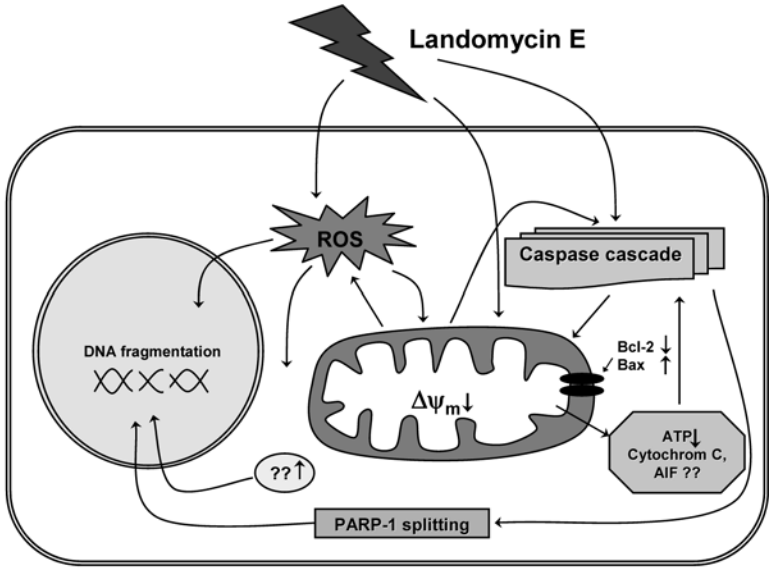

Fig. (2). Summary of the proposed mechanisms of action of **LaE** (**4**).

Table 1. Cytotoxic Action of the LaE (4) Towards Mammalian Cells of Different Origin and Malignancy

Cell line	IC_{50}, ug/ml	IC_{50}, M x 10^{-6}	Origin
MDA-468	0.37	0.52	Human breast adenocarcinoma
MDA-MB-231	0.76	1.06	Human breast adenocarcinoma
MCF-7	1.28	1.79	Human breast adenocarcinoma
T47D	6.68	9.35	Human breast adenocarcinoma
SW-480	3.30	4.6	Human colon carcinoma
HT-29	6.00	8.4	Human colon carcinoma
HL-60	1.87	2.62	Human promyelocytic leukemia
HL-60/adr	4.06	5.68	Human promyelocytic leukemia (MRP1)
HL-60/vinc	3.85	5.39	Human promyelocytic leukemia (P-gp)
A549	2.5	3.5	Human non-small cell lung cancer
KB/S	5.00	7.0	Human epidermal carcinoma-derived cell
KB-3-1	4,3	6.0	Human epidermal carcinoma-derived cell
J774.2	2.68	4.0	Murine macrophage-like cell
L929	2.68	3.75	Murine transformed fibroblasts
CCL-64	15.04	21.0	Normal Mink lung epithelial cells
NIH 3T3	2.04	2.85	Normal Mouse embryonic fibroblasts
L1210	1.75	2.45	Mouse leukemia

Rapid development of the multidrug resistance (**MDR**) in tumor cells has significantly decreased the clinical efficacy of the anthracyclins in cancer chemotherapy. Such resistance is usually caused by the over-expression of plasma membrane ABC transporting systems including P-gp, MRP1 and BCRP which are the products of a large family of **MDR** genes [1, 27, 28]. Our studies (illustrated by Fig. (**3**)) show that, in contrast to other anticancer drugs, (e.g. adriamycin, daunomycin, mitoxantrone), **LaE** (**4**) is a challenging substrate for P-gp and MRP1 and is not transported at all by the BCRP system [15, 29].

Thus, the ability of the **LaE** (**4**) to kill tumor cells possessing various forms of resistance to anticancer drugs is among the most valuable features of this molecule. Further investigation of **LaE** (**4**) interaction with mammalian ABC transporters should uncover the structural peculiarities of the compound which help it escape active export from the target tumor cells. Hence, **LaE** (**4**) seems to be a promising antineoplastic drug in the case of development of drug resistance in tumor cells. It is also possible that ATP-dependent **MDR** transporters could simply be "disarmed" in the **LaE**-treated cells due to the loss of an energy source (*vide supra*). Although the carbohydrate moieties of the **LS** were intensively studied and shown to be significant for the anticancer potential of these molecules [30], a detailed side-by-side evaluation of **LaA** (**1**), **LaE** (**4**) and landomycinone (**8**) has not been completed. Therefore, despite the significance of the **LS** as a new tool to study cancer biology, more extensive investigations are needed to draw definite conclusions on real role of **LS** as novel anticancer drugs.

CHEMICAL SYNTHESES AROUND LANDOMYCIN SCAFFOLD

The total synthesis of any of the **LS** has not yet been reported. The aglycon moiety of **LS** generally resembles that of other angucyclines. Several approaches towards synthesis of angucyclinones were developed and they are summarized in an excellent review by Krohn and Rohr [4]. Hence, we shall not focus on this topic. Since the structural and biological uniqueness of the **LS** were initially defined by their glycan moieties, chemists have developed several synthetic routes to hexa- and trisaccharide chains of the **LS**, as well as to their building blocks, trideoxysugar α-L-rhodinose and dideoxysugar β-D-olivose. The syntheses developed by groups of Sulikowski, Kirschning, Roush and Wang begin with an asymmetric carbohydrate starting material [31-34]. Sulikowski and Guo synthesized fully acetylated β-*p*-methoxyphenol hexasaccharide in a total of 33 steps and an overall yield of <0.01% starting from L- and D-rhamnal. In this synthesis L-rhodinosyl tetrazoles and D-olivosyl phosphites were used as donors to build the corresponding α-L-rhodinoside and β-D-olivoside linkages, respectively. The

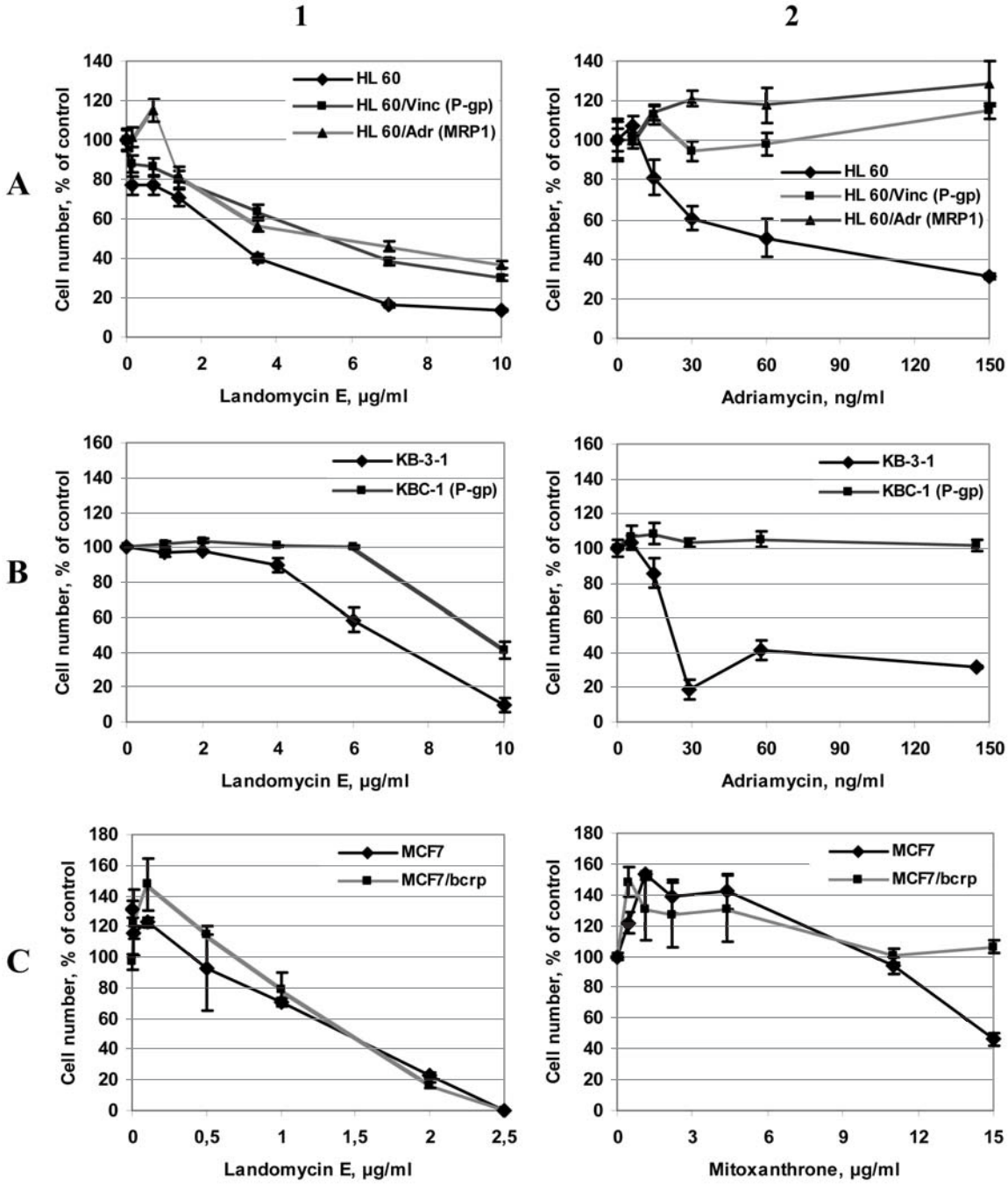

Fig. (3). Comparison of antiproliferative action of **LaE** (**4**) with adriamycin (or mithoxantrone) towards human tumor cell sub-lines differing in their drug resistance. **(A)** dose-dependent action (72 hrs) of **LaE** (1) or adriamycin (2) towards wild type of human promyelocytic leukemia cells (HL60), adriamycin-resistant sub-line cells (HL60/Adr) (overexpression of MRP1); and vincristine-resistant sub-line (HL60/Vinc) cells (overexpression of P-gp); **(B)** dose-dependent action (72 hrs) of **LaE** (1) or adriamycin (2) towards wild type (KB3-1 line) and adriamycin-resistant subline (KBC-1) cells (overexpression of P-gp); **(C)** dose-dependent action (72 hrs) of **LaE** (1) or mitoxanthrone (2) towards to wild type (MCF-7 line) and mitoxanthrone resistant sub-line (MCF-7/BCRP) cells (overexpression of BCRP).

stereoselectivities for the synthesis of the latter linkages were moderate [31]. The Roush and Bennett synthetic route to the hexasaccharide gycal consisted of 35 steps and was achieved in 0.6% overall yield starting from (S)-lactate and triacetyl D-glucal [33]. Taking advantage of the use of 2-deoxy-2-iodo-glucopyranosyl trichloroacetimidate donors for the construction of the required 2-deoxy-β-glycoside linkages, they achieved the synthesis in a highly stereoselective manner. In addition, L-rhodinosyl acetates were successfully employed for the synthesis of the α-L-rhodinoside linkages. In a previous report on the synthesis of an A-B-C trisaccharide derivative by Kirschning, 2,6-dideoxy-2-iodo-glucopyranosyl acetates were employed as donors for the stereoselective synthesis of the β-D-olivoside linkages, albeit in lower yields [32].

Yu and Wang reported the synthesis of the β-*p*-methoxyphenol hexadeoxysaccharide fragment of **LaA** (**1**) in a total of 33 steps and 0.5% overall yield starting from D-mannose and D-xylose [34] and using phenyl 2,3-O-thionocarbonyl-1-thioglycosides as 2-deoxy-β-glycoside precursors. The most recent synthetic endeavor, reported by Zhou and O'Doherty, has led the preparation of a trisaccharide portion of **LaE** (**4**) from achiral starting material. This synthesis relied on regioselective Mitsunobu reaction and resulted in 4.5% overall yield after 22 steps, the best outcome so far reported [35]. The biological properties of the synthesized glycans have not been tested.

Current **La**-centered synthetic approaches show a great improvement over initial ones in terms of yield and efficiency and have now reached the point where the generation of modified carbohydrate moieties is feasible. For example, the generation of **LS** with 7 or more sugars in a single linear chain could be a "stringent test" for synthetic chemistry. It also would help understand whether generation of **LS** with longer/modified carbohydrate tails is a viable approach towards clinically useful molecules.

GENETIC ENGINEERING OF NOVEL LANDOMYCINS, THEIR STRUCTURE-ACTIVITY RELATIONSHIP STUDIES

Cloning of the **LaA** (**1**) biosynthetic (*lan*) genes from *S. cyanogenus* S136 [36] followed by description of the **LaE** (**4**) biosynthetic (*lnd*) genes from *S. globisporus* 1912 [37] has created a new dimension in studies of the **LS**. As usual in actinomycetes, both the *lan* and *lnd* genes form clusters (Fig. (**4**)). These clusters are highly similar at both the level of cluster organization as well as DNA sequence, which could be explained by their recent evolutionary divergence. All homologous *lan* and *lnd* genes studied so far control the same respective steps of **La** biosynthesis (Fig. (**4**)). Still, several notable differences exist between the *lan* and *lnd* clusters. The *lnd* cluster in *S. globisporus* has no *lanGT3* and *lanZ2* homologues, and this genetic difference accounts for accumulation of **LaE** (**4**) as the final **La** in this strain [38]. Also, the *lan* and *lnd* clusters differ in the number and location of genes involved in the regulation of **La** biosynthesis; the reason(s) for that are not clear [39, 40]. The biosynthetic and regulatory pathways governing **La** biosynthesis are well

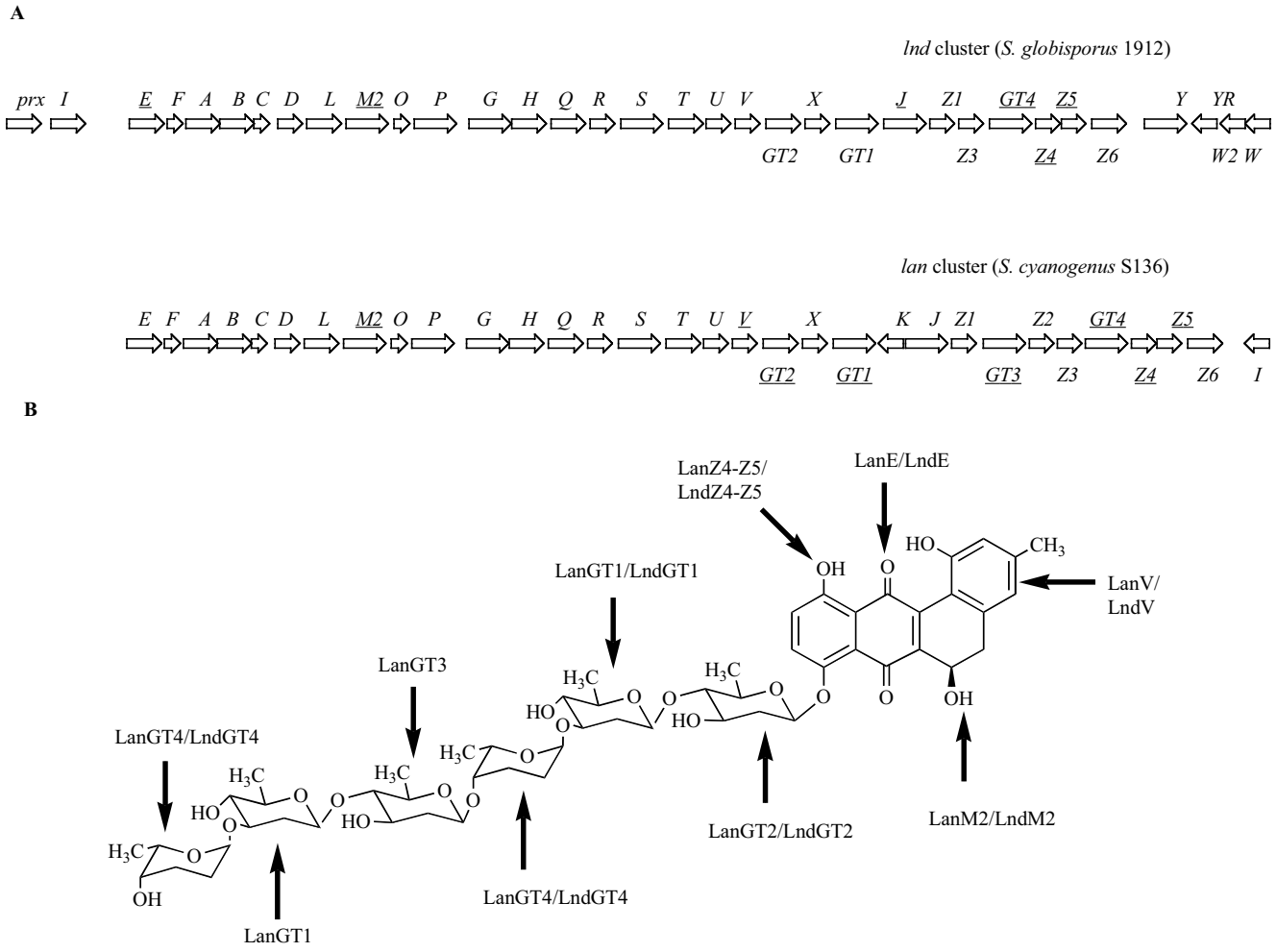

Fig. (4). Landomycin biosynthesis. A. Organization of gene clusters involved in **La** biosynthesis in *S. globisporus* and *S. cyanogenus*. Genes (e.g. their literal symbols) useful for generation of novel molecules are underlined. B. Structure of **LaA** (**1**) and Lan/Lnd enzymes responsible for the introduction of its different functionalities (see text for gene functions).

established and have been summarized in several papers and reviews [5, 41-44].

To generate novel molecules, genes for the biosynthesis of a certain natural product can be expressed in bacteria which make structurally similar compounds (so called "heterologous expression"). Alternatively, the biosynthetic genes can be either knocked out or overexpressed in the wild type producer. The success of these "mix-and-match" experiments hinges on the availability of a pool of genes encoding enzymes with somewhat relaxed substrate specificity [45]. All of the aforementioned approaches as well as combinations thereof have been used to generate novel **La**-like compounds.

Streptomyces fradiae Tu2717, the producer of the urdamycin complex (major product – urdamycin A (**7**), see Fig. (**1**)) [46] turned out to be an excellent host for the expression of *lan* genes which has led to the identification of many novel molecules (Fig. (**5**)). Introduction of the entire *lan* cluster into the wild type Tu2717 strain resulted in the production of 5,6-anhydro-LaA (**9**) instead of **LaA** (**1**). The failure to obtain final product **1** was due to suboptimal fermentation and/or extraction conditions; indeed, as it was mentioned above, 5,6-anhydrolandomycins are isolated from wild type **La** producer *S. cyanogenus* [6]. The production of several novel angucyclines has been achieved through combinatorial expression of the glycosyltransferase genes *lanGT1* or *lanGT1* plus *lanGT4* in *S. fradiae* mutant A-x which makes urdamycinone B (C –olivosylated aglycon of urdamycin A (**7**), Fig. (**1**)) [47]. Particularly, *lanGT1* overexpression in A-x resulted in the accumulation of ladamycins A2 (**11**) and B2 (**13**). Coexpression of *lanGT1* and *lanGT4* in A-x led to the production of ladamycins A3 (**10**), B3 (**12**) and D3 (**14**). The latter compound is an interesting example of non-enzymatic conversion of novel urdamycin A-type into urdamycin D-type molecules through the addition of transaminated tryptophan. Also, the production of linearly assembled tetracyclic compound lourdamycin was observed, pro-bably a result of photochemical rearrangement of some urdamycin metabolite. These experiments showed for the first time that LanGT1 and LanGT4 control the transfer of D-olivose and L-rhodinose, respectively. LanGT4 appeared to be an especially promiscuous enzyme able to rhodinosylate various acceptor substrates. This notion was reinforced through overexpression of *lanGT4* in the wild type urdamycin A producer, which resulted in production two new urdamycins U (**16**) and V (**17**) [48]. Both urdamycins possess tetrasaccharide chains featuring L-rhodinose as the terminal sugar. Thus, LanGT4 can transfer L-rhodinose to angucyclines having different aglyca and carbohydrate moieties of varying lengths. Besides glycosyltransferase genes, an aromatase-ketoreductase gene *lanV* was overexpressed in the *S. fradiae* mutant that accumulates urdamycin B (urdamycin A (**7**) lacking 12b and 4a hydroxyls; see Fig. (**1**)) as a final metabolite) The resulting strain accumulated novel compound 9-*C*-olivosyltetrangulol (**15**) [49].

These results show the potential of the **La** biosynthetic genes for generation of novel angucyclines through heterologous expression. Genes for post-PKS reactions (glycosylation, oxidation-reduction of polyketide backbone) seem to be the most promising ones in this regard. To date, there are no studies on the biological activities of the novel compounds isolated from recombinant *S. fradiae* strains.

The development of gene disruption procedures in *S. globisporus* [50] and *S. cyanogenus* [51] deciphered the sequence of reactions leading to **LaA** (**1**) and generated many "unnatural natural" **LS** depicted in Fig. (**6**). For instance, *S. globisporus* strain GT4.1 impaired in expression of *lndGT4lndZ4lndZ5* genes produces three novel landomycins H (**18**), F (**19**) and G (**20**) (mono-, di- and triglycoside, respectively) [52]. Unlike all naturally occurring **La**, these compounds lack the C11-hydroxyl group. The anticancer activity of **LaA** (**1**), **E** (**4**) and **D** (**5**) and the engineered C11-deoxylandomycins against lung (H460) and breast (MCF-7) cancer cell lines were tested using the sulforhodamine B assay. It has been shown that in "LaA-LaE-LaD" row the antitumor activity generally decreases, supporting the idea about the essential role of a long glycoside chain in bioactivity. Nonetheless, **LaH** (**18**) (1 sugar) is more active than **LaF** (**19**) (2 sugars). With regard to C11-hydroxyl group, **LaF** (**19**) is roughly threefold less active against MCF-7 cell line than its 11-hydroxy counterpart, **LaD** (**5**). It can be concluded, that the C11 hydroxyl improves the antitumor activity of the **LS**. A definite conclusion cannot be drawn without comparative analysis of the C11-deoxy analogs of **LaA** (**1**), **LaB** (**2**) and landomycinone (**8**). An attempt to engineer a *S. cyanogenus* strain producing novel C11-deoxylandomycin derivatives was undertaken. However, the created mutant accumulated two unexpected landomycins M (**21**) and O (**22**) [53]. **LaM** (**21**) and **LaO** (**22**) possess pentasaccharide and disaccharide chains, respectively, and both lack C11 and C6 hydroxyl groups. Hence, although in *S. globisporus* damaged expression of *lndGT4-Z5* genes leads to accumulation of C11-deoxy-**La**, in *S. cyanogenus* the same mutant genotype behaves differently and has a negative effect on both C6-hydroxylation and glycosylation (*e.g.*, production of the hexasaccharide **La** was not observed). The reasons for this are unclear and additional experiments (optimization of culture conditions as well as overexpression of additional **La** oxygenase and glycosyltransferase genes) might help generate the desired C11-deoxy-**LS**. The antitumor activity of **LaM** (**21**) and **O** (**22**) were diminished in comparison to **LaA** (**1**), especially in case of MCF-7 cell line. There was no significant difference between activities of **LaM** (**21**) and **O** (**22**). Interestingly, **LaF** (**19**) (2 sugars, C11-deoxy) is almost twice as active against MCF-7 cell line in comparison to **LaO** (**22**) (2 sugars, C11- and C6-deoxy). Therefore, it seems that both C11- and C6-hydroxyl groups are important for antitumor activities of **LS**, at least in context of certain tumor cell lines.

Recently, the overexpression of olivosyltransferase gene *lanGT3* in wild type **LaA** (**1**) producer *S. cyanogenus* has been reported. The recombinant strain accumulated two novel compounds, **LaI** (**23**) (monoglycoside) and **LaJ** (**24**) (tetraglycoside) [30]. Their isolation allowed for a comprehensive study on the relationship between the length of saccharide chain of the **LS** and their antiproliferative action. Particularly, landomycinone (**8**) and **LS** having the same aglycon and saccharide chains of all possible lengths where subjected to an analysis of their activity against NCI-H460, MCF-7 and LL/2 cell lines (human lung, human breast and

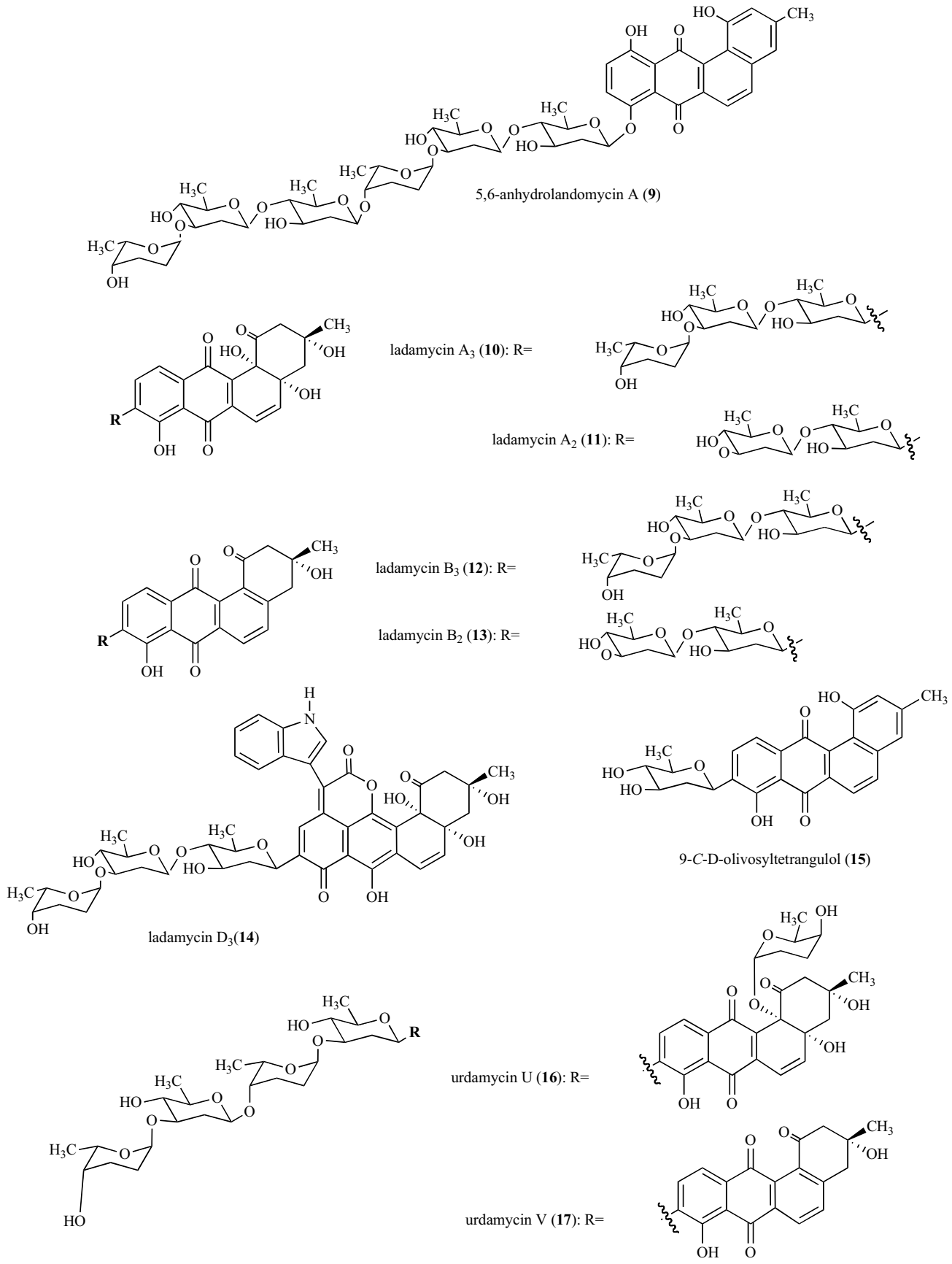

Fig. (5). Novel angucyclines generated through expression of *lan* genes in urdamycin producer *Streptomyces fradiae* Tu2717 or its various *urd*–deficient mutants.

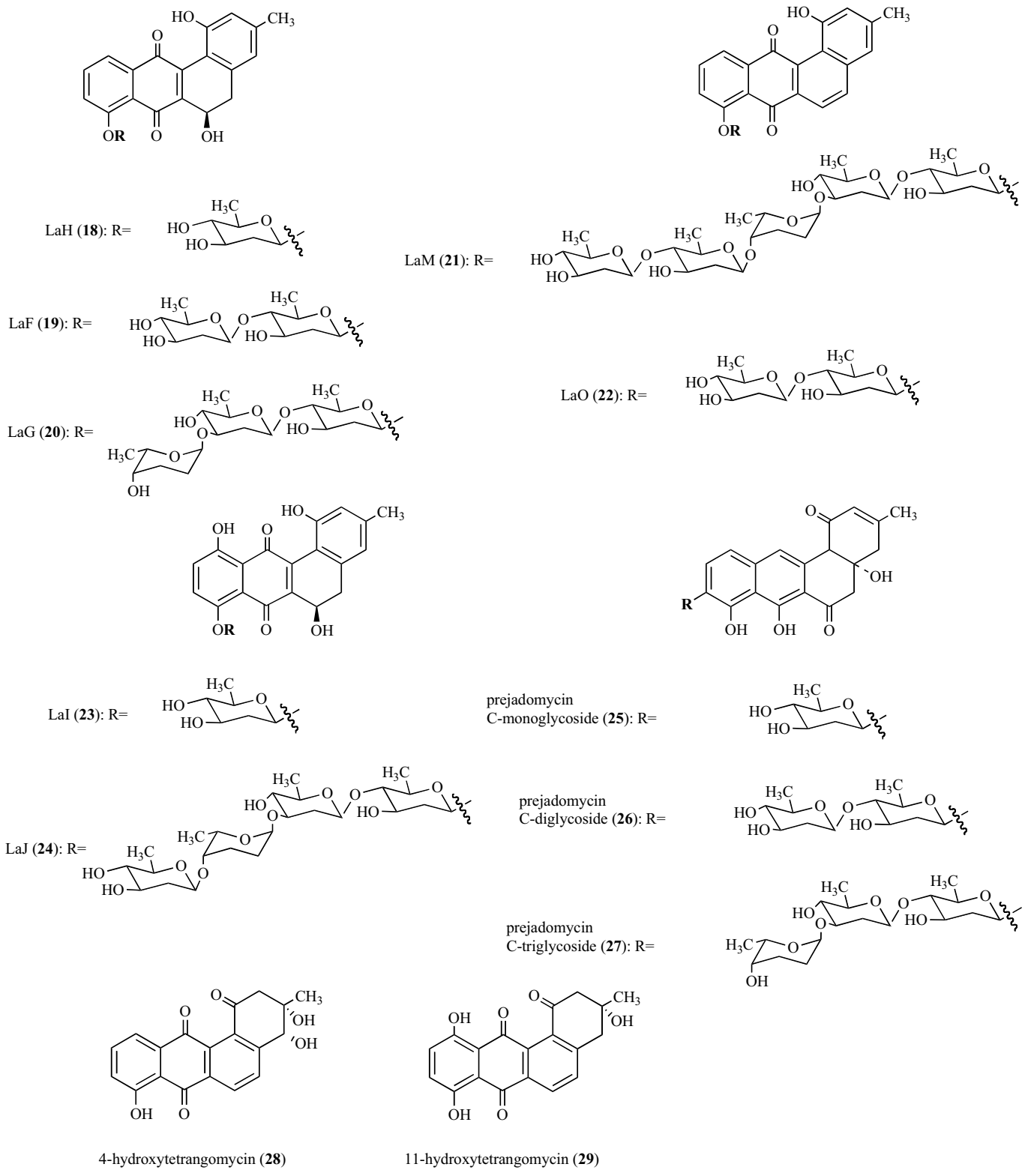

Fig. (6). Novel **LS** obtained through genetic manipulations of *S. globisporus* 1912 and *S. cyanogenus* S136.

murine Lewis lung cancers, respectively). **LS** are generally the most active against LL/2. Although **LaA** (**1**) (the longest sugar chain) was generally the most active compound, the antitumor activities of the **LS** do not increase linearly with an increase in sugar chain length. For example, **LaE** (**4**) and **LaA** (**1**) are equally potent inhibitors of LL/2, however the former is the least active against MCF-7. Also, landomycinone (**8**) turned out to be quite active against all studied cell lines. The researchers suggested that mechanisms of action of **LS** and landomycinone (**8**) could be different. Moreover,

this study and previous ones give us reason to believe that even different **LS** could exert activity against mammalian cells in different ways. Of practical interest, the strong activity and smaller size of **LaE** (**4**), as well as its higher stability (with regard to loss of C6-hydroxyl) makes it an attractive model for the development of **La**-based therapies and further biocombinatorial efforts. This potential is supported by two recent reports elucidating the early steps of landomycinone (**8**) biosynthesis in *S. globisporus*. In the first report, the disruption of oxygenase-reductase gene *lndM2* led to the generation of two unexpected aglyca (**28** and **29** Fig. (**5**)) [54]. Another study examined the effect of deletion of the dioxygenase gene *lndE* in concert with the expression of C-olivosyltransferase gene *urdGT2* in the resulting mutant. This caused the accumulation of novel prejadomycin C-glycosides [44]. Biological activities of these compounds were not tested. Overexpression of *urdGT2* in *lanGT2*-deficient *S. cyanogenus* strain led to accumulation of 9-*C*-olivosyltetrangulol (**15**) [55]. The production of other as-yet-unidentified **LS** was also reported in several papers [12, 56]. It is likely that more extensive "mixing" of angucycline biosynthetic genes can lead to even greater structural diversity. Indeed, our current research shows that overexpression of various post-PKS tailoring genes from simocyclinone, urdamycin and oviedomycin pathways in *S. globisporus* mutants results in production of as-yet-unknown metabolites (V. Fedorenko, A. Kobylyanskyy, unpublished).

CONCLUDING REMARKS

Given the facts that the angucycline group of antibiotics is the largest group of aromatic polyketides and that many angucyclines display anticancer activity [4], it is astonishing that only the **LS** have been extensively studied. Discouraging initial *in vivo* results and misleading assumptions about their modes of action discouraged early research; we argue that more careful investigation of the angucyclines may lead to new insightful results, as our research on **LS** did. Early biological studies on the biggest member of **La** family, **LaA** (**1**), pointed to its exceeding cytotoxicity, barring its further development. Recent investigations on smaller representatives of the family show that their activity is comparable to that of **LaA** (**1**), although their mechanisms of action may be different. Up-to-date, **LaE** (**4**) has been the most intensively studied angucycline antibiotic regarding its molecular targets in the mammalian cells, and its capability to suppress tumor cells resistant to various anticancer drugs widely used in clinics, was demonstrated. C11 and C6 hydroxyl groups of the aglycon are important for antitumor activity, while the relationship between the length of **La** glycoside chain and activity cannot be easily explained. Whatever the fate of **LS** as potential drugs will be, it is apparent that these compounds may operate in mammalian cells *via* novel pathway(s). Hence, their exploration could lead to novel therapies and insights into cancer biology. Identification of the molecular target(s) of **LS** would be the single most important breakthrough in the field of **La** research. Biological studies on a wider set of **LS** were recently initiated and they will be indispensable to reach this milestone. A great number of structurally interesting **La**-like molecules have been generated mostly *via* combinatorial biosynthesis. Their studies will help uncover more details concerning the importance of certain functional groups for the antitumor activity of **LS**. With the exception of amicetose-containing **LaC** (**3**) (Fig. (**1**)), currently all known **LS** carry glycoside chains composed of only L-rhodinose and/or D-olivose. It would be interesting to test **LS** carrying, for example, amino- or monodeoxysugars present in many potent anticancer drugs. Both chemical and biocombinatorial efforts could be combined to generate such molecules. We believe that advances in understanding the molecular biology of **La** action in mammals, coupled with the power of chemical and genetic engineering of **La** derivatives, will soon provide practical answers to the needs of cancer chemotherapy.

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ABBREVIATIONS

La = Landomycin

LS = Landomycins

MDR = multidrug resistance

NAC = N-acetylcysteine

ROS = Reactive oxygen species

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