Nucleoside derived antibiotics to fight microbial drug-resistance: New Utilities for an Established Class of Drugs?[#]

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[#]This work is dedicated to the memory of Prof. Chris McGuigan, a great colleague and scientist, invaluable source of inspiration.

Abstract

Novel antibiotics are urgently needed to combat the rise of infections due to drug-resistant microorganisms. Numerous natural nucleosides and their synthetically modified analogues have been reported to have moderate to good antibiotic activity against different bacterial and fungal strains. Nucleoside-based compounds target several crucial processes of bacterial and fungal cells such as nucleoside metabolism and cell wall, nucleic acid and protein biosynthesis. Nucleoside analogues have also been shown to target many other bacterial and fungal cellular processes though these are not well characterised and may therefore represent opportunities to discover new drugs with unique mechanisms of action. In this perspective we demonstrate that nucleoside analogues, cornerstones of anticancer and antiviral treatments, also have great potential to be repurposed as antibiotics so that an old drug can learn new tricks.

Introduction

Microbe is an umbrella term describing microscopic living organisms from six major groups: bacteria, viruses, fungi, protozoa, algae and archea.[1] Some of these microbes are essential for the health and survival of humans, animals and plants, whereas others

are pathogenic. Since the 19th century, it has been known that microbes can be the cause of serious infectious diseases. Antibiotics are routinely used to treat bacterial infections, and some of these drugs are also effective against fungi and protozoa. However, the efficacy and ease of access of antibiotics has led to the development of drug-resistance.[2] Bacteria and fungi possess an incredible ability to adapt in response to antibiotics, they are able to rapidly evolve mechanisms that interfere with an antimicrobial's mode of action. Acquired antibiotic-resistance can arise by a variety of mechanisms, primarily: enzymatic inactivation of the antibiotic, inhibition of its cellular uptake or increase of its efflux from the cell, and/or alteration of the target site such that the antibiotic cannot recognize it. These adaptations often result from the spontaneous mutation of a microbial gene and selection in the presence of the antibiotic. Most, but not all, antibiotic resistance genes are encoded by plasmids leading to heritable resistance.[3,4, 5] Examples of resistant microbial strains can be found for every class of antibiotic, regardless of their mechanism of action.

Antibiotic resistance is a natural phenomenon that has been enhanced by antibiotic misuse and by the increasing incidence of immunosuppression, meaning there are more individuals who are susceptible to infection. Failure to take antibiotics correctly or strictly when necessary has been proven to have drug resistance as consequence. Farm animals fed with antibiotics also became reservoirs for antibiotic-resistant bacteria that can be easily transmitted to humans. Once resistance has emerged, subsequent dissemination of the resistant strain is easy and can be facilitated by crowded living conditions and poor hygiene standards.

The rise of antibiotic resistance and a concurrent reduction of the investment of the pharmaceutical companies in the development of new antibiotics, pose a serious threat to the entire world population.[4] A 2014 Word Health Organization's report

revealed that antimicrobial resistance is spreading across the world and is threatening the effective prevention and treatment of common infections caused by bacteria, parasites, viruses and fungi.[5] It has been estimated that if no concerted effort to discover and develop new antibiotics is made by all countries, by 2050 the number of deaths per year due to antibiotic-resistant infections will reach 10 million, with an associated cost to the global economy of $1 trillion.[6]

According to a recent report,[4] third-generation cephalosporins, which are the treatment of last resort for gonorrhoea, now appear to be failing, leading to untreatable gonococcal infections. Resistance to fluoroquinolones, which are one of the most commonly used antibacterial drugs for the oral treatment of *Escherichia coli* infections of the urinary tract, is now widely reported. Common intestinal bacterial infections, usually treated with carbapenem antibiotics, are now life-threatening due to the resistance developed by these bacteria. Other examples of important drug resistant bacteria in humans are methicillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile*, *Salmonella, Chlamydia*, and *Mycobacterium tuberculosis*.

Drug-resistant fungi also cause both acute and chronic infections associated with high morbidity and mortality. Invasive *Candida* infections are the fourth leading cause of hospital-acquired blood infections, and they are associated with a high mortality. Besides *Candida*, antifungal resistance occurs in other fungi such as *Aspergillus* and *Cryptococcus neoformans,* which are major opportunistic fungal pathogens in immunocompromised individuals e.g. HIV-infected patients and those receiving immunosuppressive treatment for cancer, organ transplantation, and other serious medical conditions.

A coordinated set of strategies is needed to fight the global threat of antimicrobial resistance. Improving infection, prevention and control practice together with the

optimization of prescribing practice are critical steps that need to be taken. Professional education and training are essential to optimize the consistency in prescription. Better access to, and an improved use of surveillance data, could be used to drive reductions in antimicrobial prescribing rates and total antibiotic consumption. It would certainly be necessary to improve the public engagement and to strengthen international collaboration. Further to these actions, one of the most important measures to tackle antibiotic resistance and to prevent its spread worldwide, is promotion of research and development of novel antimicrobial agents. Unfortunately, only a few classes of antibacterial drugs have been identified in the last 50 years of research.[7] As such, the identification of new therapeutic targets and the development of novel drugs, with the ability to subvert resistance are urgently needed.

Nucleosides and nucleotides are essential molecules for the life processes of all living organisms, including microbes, playing pivotal roles in most fundamental cellular metabolic pathways. For this reason, synthetic analogues of nucleosides have been extensively investigated as therapeutics and have become mainstays of antiviral and anticancer treatment.[8] Naturally occurring nucleosides isolated from bacteria and fungi are well known for their diverse biological activities.[9-16] However, the antibacterial and antifungal potential of nucleoside analogues has not been fully exploited yet; hence, recognition of these important biological properties could therapeutic potential of this class of compounds as antibiotics.

New antimicrobial drugs, potentially with new mechanisms of action, are needed to address the growing problem of resistance. After a brief introduction highlighting the structure of bacteria and fungi and their potential therapeutic targets, we will discuss the current knowledge of nucleoside and nucleotide analogues possessing antimicrobial activity. This perspective will be limited to the potential of this class of

compounds for the treatment of bacterial and fungal infections, focusing on the new therapeutic utilities for these drugs. An account on the state of the art of anti-mycobacterial nucleoside and nucleotide analogues for the treatment of tuberculosis has been recently published,[17] therefore no specific attention will be dedicated to this microorganism. This Perspective covers the efforts directed toward the identification of natural and modified nucleoside analogues active as antibiotics, up to early 2016. This overview is followed by a discussion of the importance of nucleosides as antibacterial and antifungal drugs and will, hopefully, provide valuable information to aid the development of novel nucleoside analogues against new therapeutic targets.

Bacteria: Structure and drug targets

Bacteria are prokaryotic cells that, unlike eukaryotic cells, do not have a well-defined nucleus surrounded by a nuclear envelope (Figure 1). Bacteria are approximately ten times smaller than eukaryotic cells, with sizes ranging between 0.2 and 700 µm in diameter. They have five key structural components: a nucleoid and plasmids containing the genome and any supplementary genetic information; ribosomes which are critical for protein synthesis; the cell membrane and cell wall form protective cell barriers, with the latter protecting the cells against osmotic stress and physical damage,.[18] Additional bacterial cell features include the flagella, responsible for propulsion/movement, inclusion bodies, responsible for the mineral storage of the cell, and pili, which are used for the attachment to the host and for bacterial conjugation. (Figure 1). Bacteria are broadly classified into two types: Gram-positive and Gram-negative. Gram-positive bacteria have a thick cell wall, which is made up of many layers of peptidoglycan. Running perpendicular to the peptidoglycan sheets is a group of anionic glycopolymers called teichoic acids. Gram-negative bacteria

have comparatively thin cell walls, composed of only a few layers of peptidoglycans and surrounded by an outer membrane containing lipopolysaccharides (LPS).[18]

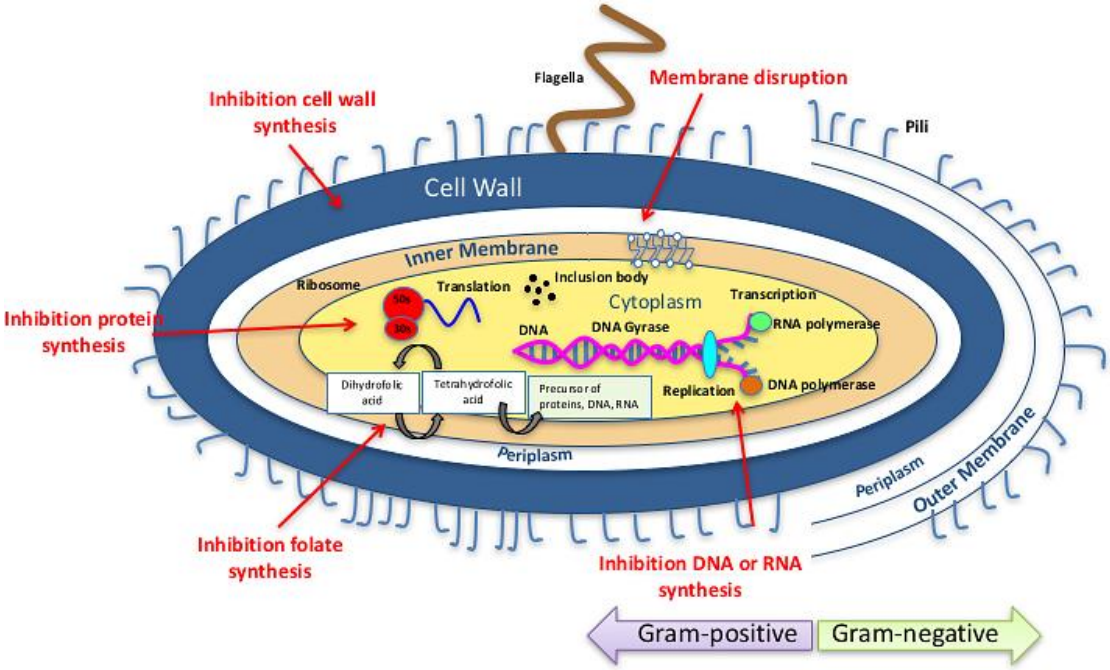


Figure 1. General structure and inhibition targets of bacteria's cell.

Key targets for the treatment of bacterial infections are the various enzymes involved in the synthesis of the cell wall, DNA, proteins and phospholipid membrane, along with enzymes such as DNA gyrase and RNA polymerase, crucial for bacterial replication.[19]

The bacterial cell wall is an important antibiotic target. Cell wall peptidoglycan consists of parallel strands of a polysaccharide composed of *N*acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) in β-(1,4)-linkage (Figure 2). The lactyl side chain of MurNAc continues in a pentapeptide of structure L-Ala-D-Glu-X-D-Ala-D-Ala, of which X is *L*-Lys in Gram-positive and meso-diaminopimelic acid (DAP) in Gram-negative bacteria.[20] The cross-linked structure of peptidoglycan confers mechanical strength and shape to the cell and provides a barrier to withstand internal osmotic pressure. Nevertheless, it has sufficient plasticity to allow cell growth and division. Its fast turnover makes peptidoglycan an attractive target for

antibiotics.[21] Synthesis of peptidoglycan is a three-stage process that occurs in the cytoplasm, as well as in the inner and outer part of the plasma membrane. Peptidoglycan synthesis involves several different enzymes, most of which represent attractive targets for development of antibacterial therapeutics (Figure 2).[22]

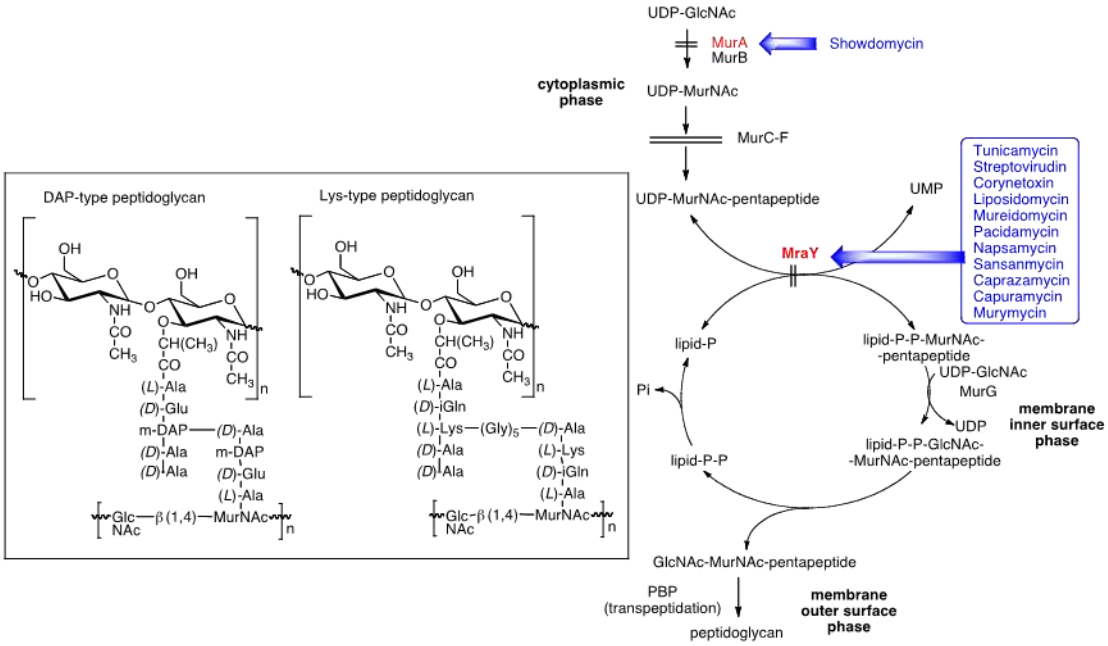


Figure 2. Peptidoglycan structure and biosynthesis.

Fungi: Structure and drug targets

Fungi are eukaryotic unicellular or multicellular microorganisms. Therefore they have a well-defined nucleus as well as membrane-bound organelles.[23, 24] The biggest difference between fungal and mammalian cells is the presence, in fungi, of an outer cell wall. The fungal cell wall is dynamic system that provides the cell with mechanical strength and protection (Figure 3).[24] Other significant differences can be found in the membrane portion, which in fungi contains ergosterol and in mammals contains cholesterol.[24] The biosynthetic pathway for ergosterol is a specific branch of the mevalonate pathway which, being fungi-specific, represents an attractive target for antifungal therapy.[23] Additional therapeutic targets could include regulators of fungal DNA biosynthesis or cell division e.g. mitotic spindles.

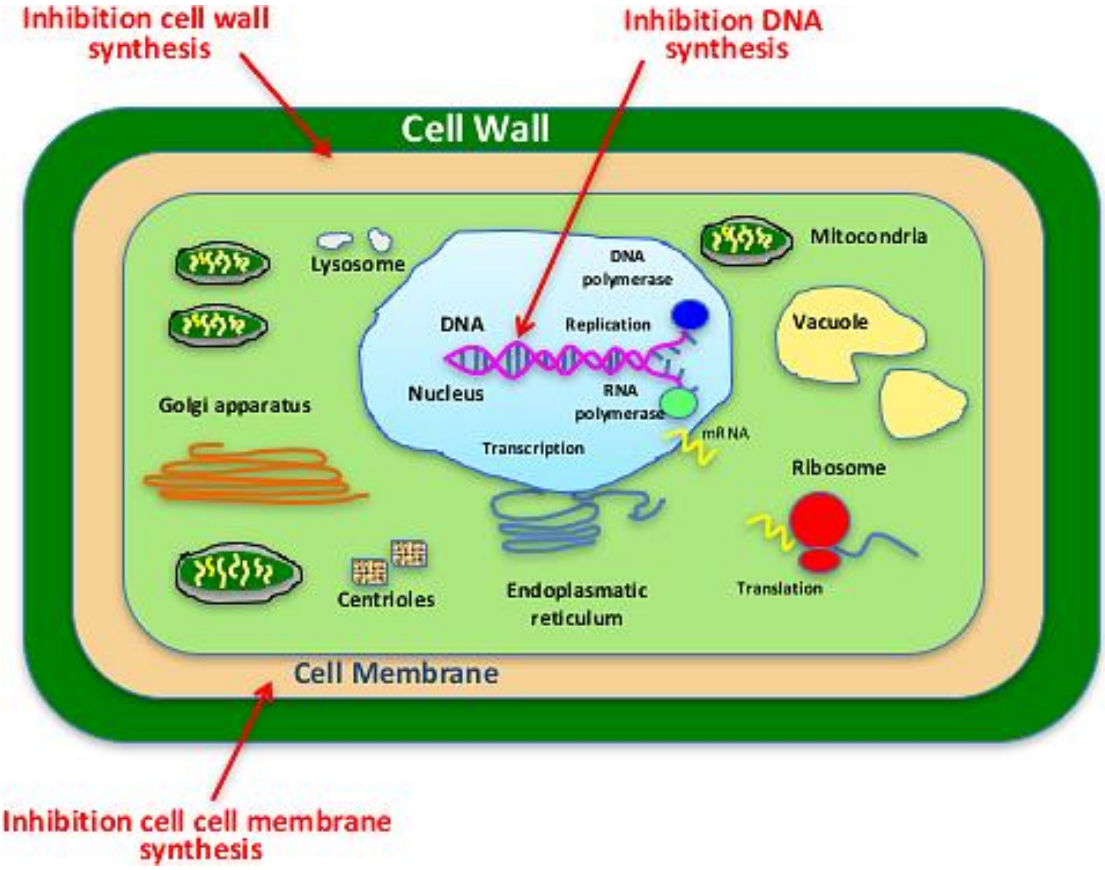


Figure 3. General structure and inhibition targets of the fungal cell.

The fungal cell wall is comprised of three main building blocks: chitin (a polymer of *N*-acetylglucosamine); glucans, (glucose polymers that serve as cross-link between chitin polymers and give rigidity to the cell wall) and glycoproteins.[25] The formation and remodelling of the fungi cell wall involves numerous biosynthetic pathways and the concerted actions of hundreds of fungal proteins.

The chitin and glucan components are synthesized on the plasma membrane and are extruded into the cell wall space during their synthesis (Figure 4). The synthesis of chitin is mediated by chitin synthase, an integral membrane enzyme that catalyses transfer of *N*-acetylglucosamine from uridine diphosphate (UDP)-*N*-acetylglucosamine to a growing chitin chain.

Polymers of glucan are generated by the plasma-membrane associated glucan synthases. Within each glucan chain (approximately 1500 glucose units) some of the glucose residues become sites at which additional glucans are attached to generate branched chains that are in turn cross-linked together and to the chitin.

Glycoprotein synthesis begins in the endoplasmic reticulum with co-translational addition of *N*-linked oligosaccharides. In the Golgi apparatus, the glycosyltransferase further modifies proteins by addition of sugars to generate *O*-linked and *N*-linked oligosaccharides. In addition, some glycoproteins are further functionalized with a glycophosphadityl inositol (GPI) anchor. These glycoproteins are then secreted into the cell wall space where they are covalently combined with sugars units of chitin or glucans, via their *N*-, *O*-, and GPI sugar units. Enzymes such as glycosyl hydrolases and glycosyl transferases assist this process.

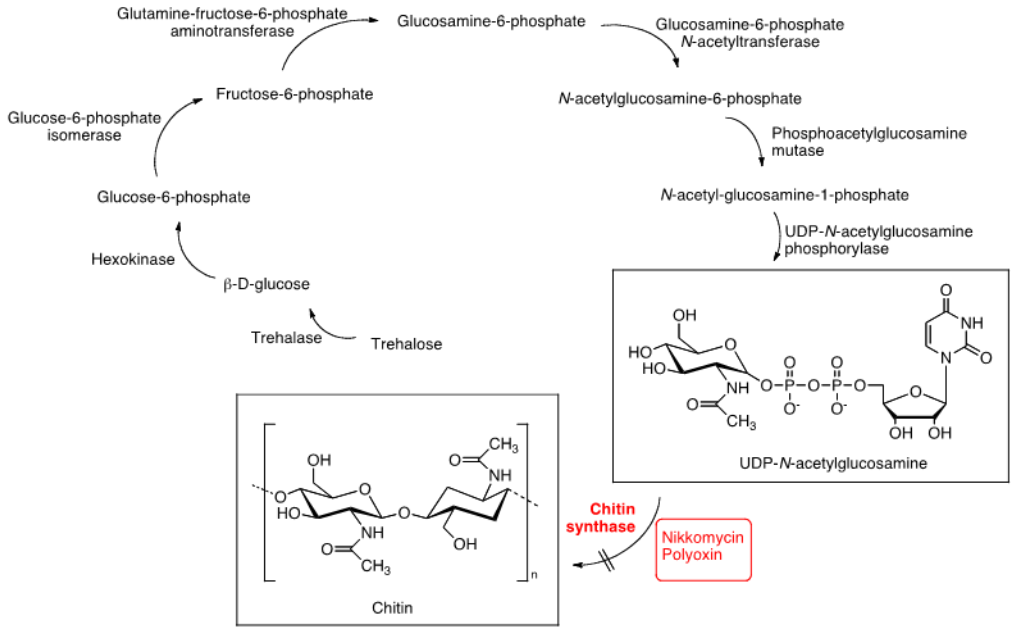


Figure 4. Chitin structure and biosynthesis.

Chitin synthesis is the target of a range of antifungal agents, including nucleosides.[26] The inhibition of glucan synthesis has also been extensively pursued as a means of disrupting wall formation and preventing fungal growth.[27]

Both glycosylation and GPI anchor addition have also been shown to be very important processes in the cell wall biosynthesis. As such, disruption of these pathways is predicted to have significant effects on the structure and function of the fungal cell wall.

Nucleoside analogues as cell wall inhibitors

Since they are not present in mammal cells, the cell wall of bacteria and fungi represents an important target for different classes of antibiotics and this target still possess a great potential for the development of selective therapeutics.

Numerous natural products with nucleosidic structures have been isolated and found to be inhibitors of the cell wall biosynthesis.[12, 14] Synthetic analogues have been further produced in order to expand the bacterial and fungal spectrum of activity and to improve bioavailability. The following sections provide an overview of several classes of nucleoside analogues whose antibacterial activity was found to depend on the inhibition of cell wall biosynthesis.

Nucleoside analogues as inhibitors of peptidoglycan biosynthesis

Important classes of antibacterial nucleoside analogues have been harvested from natural sources and found to inhibit peptidoglycan synthesis, by targeting the MraY enzyme. Among them, the tunicamycins **1a-l**, originally isolated from *S. lysosuperficus* and *S. chartreusis* in 1971, mimic the bis-substrate intermediate of *N*-acetyl-D-hexosamine-1-phosphate translocase or MraY.[28] This class of antibiotics consists of five structural units: uracil (**A**), ribose (**B**), galactosamine (**C**), *N*-

acetylglucosamine (**D**), and a fatty acid (**R**) (Figure 5).[29] The **B** and **C** portions of tunicamycin structure constitute an undecose moiety named tunicamine.[30] Because of their structure, these nucleoside antibiotics target both the bacterial MraY and the eukaryotic GlcNAc-1-P transferase. Targeting MraY inhibits synthesis of precursors for components of bacterial cell wall and inhibiting GlcNAc-1-P transferase prevents *N*-glycosylation of eukaryotic proteins.[29, 31, 32] The antibacterial effect of tunicamycins is directed primarily against Gram-positive bacteria, especially those of the *Bacillus* genus (MICs = 0.1–20 μg mL^{-1}).[33]

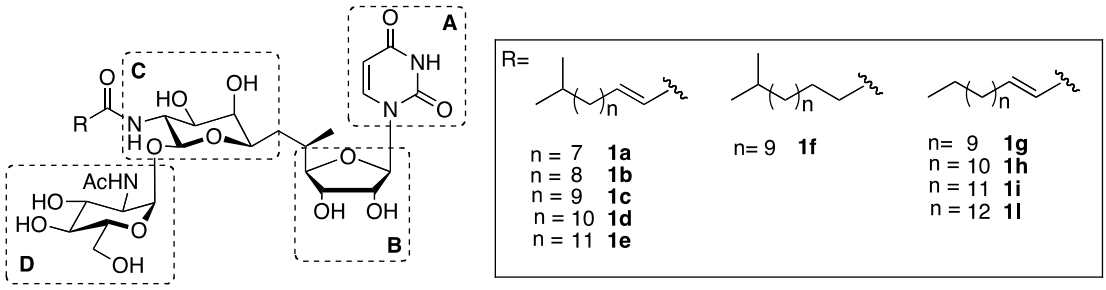


Figure 5. Structures of tunicamycins **1a-l**.

Several years after the discovery of tunicamycin, another class of natural antibiotics named Streptovirudins **2a-j** were isolated as a complex mixture consisting of ten closely related components, from fermentation of *Streptomyces sp.* These compounds were found to be structurally similar to tunicamycins and in fact, streptovirudins **2i** and **2j** were later identified as tunicamycins **1a** and **1b** (Figure 6). The main difference between these two classes of natural products lies in the nucleobase (**X**): uracil in tunicamycins and mainly dihydrouracil in streptovirudins (Figure 6).

The streptovirudin complex, which is inactive against Gram-negative bacteria, does exhibit antibiotic activity against Gram-positive bacteria, with MIC values between 0.31 and 10 μg/mL against different strains of *Bacilli.* Moderate activity was also observed against *Mycobacteria sp.,* with the activity of streptovirudin **2d** as low as

MIC = 3.1 μg/mL against *M. smegmatis*.[34] In acute toxicity studies in mice, the streptovirudin complex was moderately tolerated if administered orally, but more toxic when administered subcutaneously, intravenously or intraperitoneally.

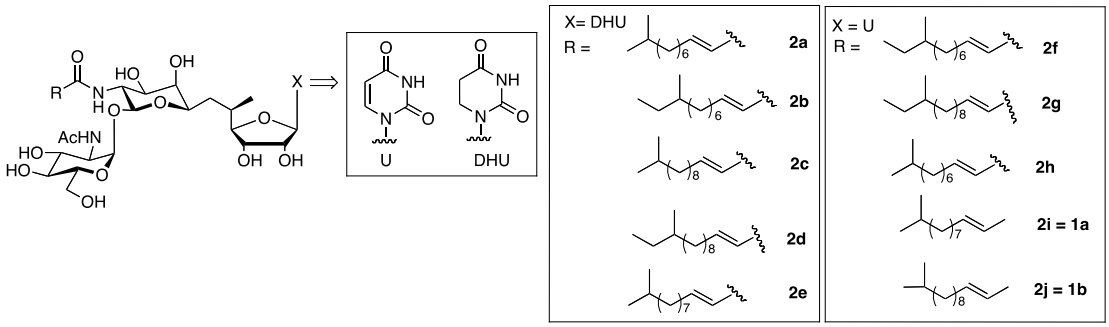


Figure 6. Structures of streptovirudins **2a**-**j**.

Another series of natural nucleoside analogues, structurally related to tunicamycin and streptovirudins, are corynetoxins **3a-n**, isolated from *Corynebacterium sp*.[35] The corynetoxins differ from tunicamycins and streptovirudins because they contain longer fatty-acid residues (**R**), β-hydroxy alkyl chains in addition to the common β-unsaturated and saturated series (Figure 7). The antibacterial activity of these analogues was found to be similar to tunicamycins, with MIC values ranging between 0.1 and 0.8 μg/mL against different *Bacillus* strains.[38] Similarly to tunicamycins, evidence of toxicity was found for this class of compounds, with LD_{50} values of 137 μg/kg on nursling rats (the LD_{50} of tunicamycin is 132 μg/kg in the same assay).[35] The toxicity derives from the inhibition of the mammals protein glycosylation enzyme GlcNAc-1-P transferase.

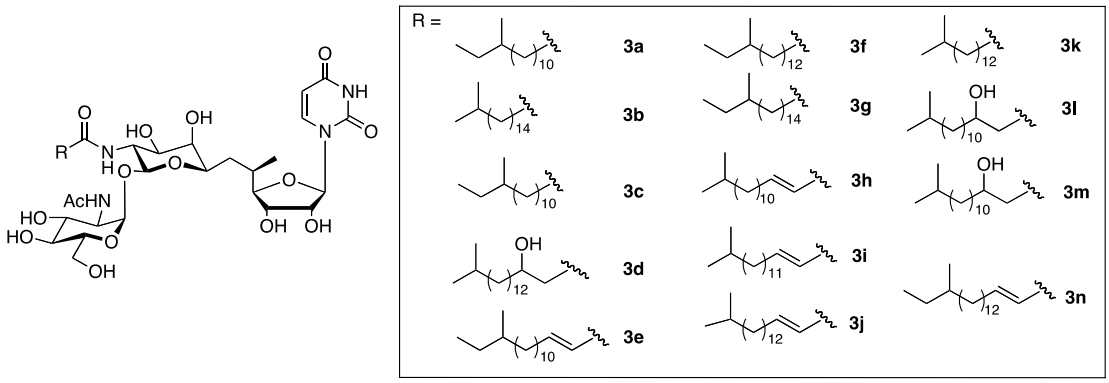


Figure 7. Structures of corynetoxins **3a-n**.

The off-target inhibition of mammalian glycoprotein biosynthesis, precluded the use of translocase I (MraY), tunicamycin, streptovirudins and corynetoxins as therapeutic antibiotics, thus stimulating the search for more selective agents. Subsequent research led to the discovery of two new classes of antibiotics named liposidomycins and mureidomycins, which are able to inhibit the bacterial translocase I without being toxic in mice.

Liposidomycins are characterised by a complex structure containing 5'-substituted uridine (**A**), 5-amino-5-deoxyribose-2-sulfate (**B**), perhydro-1,4-diazepine (**C**), and fatty acyl moieties ($\mathbf{R_1}$) (Figure 8).[36, 37] Despite the structural similarities to tunicamycins, liposidomycins are specific MraY inhibitors, and show no *in vivo* toxicity up to a concentration of 500 mg/kg.[38] Although liposidomycins have potent MraY inhibitory activity *in vitro* (IC_{50} = 0.03 μg/ml), their antimicrobial activity in whole cell assay was lower than expected, potentially due to poor permeability through the bacterial cell membrane.[39] Figure 8 shows the structures of the natural liposidomycin **4a-j** containing the sulfate and 3-methylglutaric acid moieties, which were the first to be isolated from the culture broth of *S. griseosporeus*.[39] More hydrophobic analogues, lacking either the sulfate group **5a,c**, the methylglutaryl moiety **6a**-**j** or both the two groups **7a,c** were isolated later in the fermentation broth of the same microorganism by the change of medium components (Figure 8).[40-42] The

order of peptidoglycan inhibition was found to be **4a-j** > **5a,c** > **7a,c** > **6a-j**, suggesting that the 3-methyl-glutaryl moiety plays a critical part in the MraY inhibition. Compounds **6a-j** and **7a,c** had similar *in vitro* activity compared to **4a-j** and **5a,c** but better *in vivo* properties, due to their higher lipophilicity and consequent improved membrane permeability.[43] Among all the analogues, liposidomycin **6c** was one of the most active against *M. avium* complex (MIC = 1.2-12.5 μg/ml).

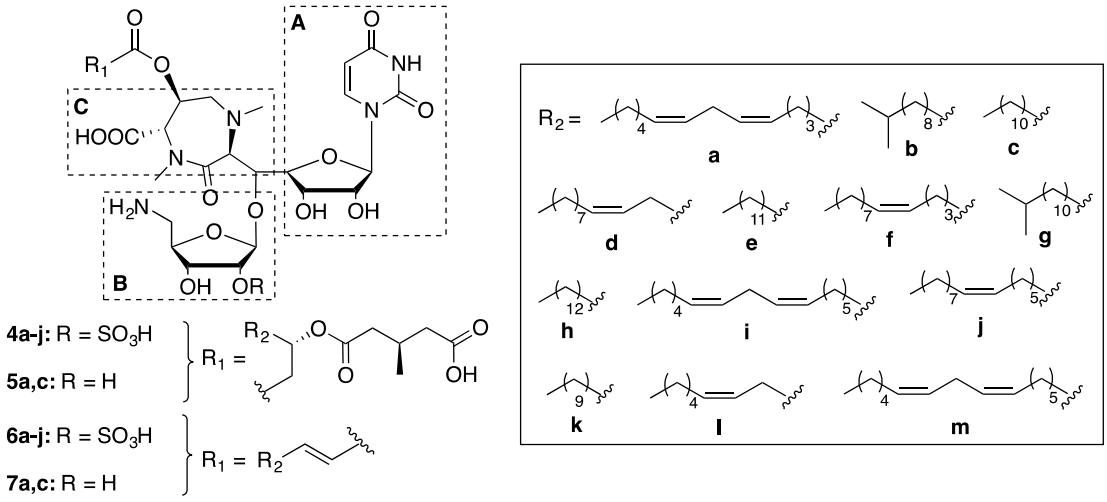


Figure 8. Structures of natural **4a**-**j** and synthetically modified liposidomycins **5a**,**c**, **6a**-**j**; **7a**, **c**.

In order to elucidate the pharmacophore of this family of antibiotics, less complex liposidomycins analogues were synthesised and tested as MraY inhibitors (Figure 9).[44, 45] In this study it was demonstrated the lipophilic fatty acid chain (R_1) was essential for activity as its removal generated a 200-fold less active analogue **8** (Figure 9). Both the 5''-amino group and the uracil moiety were found critical for the inhibition of the bacterial translocase. Modification of the uracil nucleobase via reduction of its double bond and methylation of the imide group led to compounds lacking of any inhibitory activity in translocase assay. Compound **9a,** a simplified molecule based only on the liposidomycin core structure, was found to be a moderate

MraY inhibitor (IC_{50} = 50 μM), while compounds **9b** and **9c**, higher homologues of **9a** containing an additional stereocentre at the 5' position, showed different MraY inhibitory activities. In particular, only the (*S*)-isomer **9c** showed significant activity against MraY with an IC_{50} value of 5 μM (Figure 9).[44, 45]

A compound with a hydroxyl group at the 5" position was found totally inactive in translocase inhibitory assay. Among a series of compounds with different amines at 5" position, compounds **10a-d** (Figure 9) were endowed with MraY inhibitory activity, proving that the primary amine can by replaced by isosters (i.e., secondary amines, amidine or guanidine). The activities were nevertheless low, with IC_{50} ranging from 25 to 50 μM against MraY.[44]

In order to further define the active pharmacophore, additional analogues were synthesised by modification of the hydroxyl group patterns **11a-d** (Figure 9).[46] According to these investigations, only the 3''-hydroxyl group ($\mathbf{R_3}$) was found to be crucial for the inhibition of MraY. Instead, the absence of the 2''-hydroxyl ($\mathbf{R_2}$) or both the 2'- and 3'-hydroxyls (**R** and $\mathbf{R_1}$) resulted in a decrease in activity. The exclusive removal of the 2'-hydroxyl group (**R**) had little impact on the activity while the absence of the 3'- hydroxyl ($\mathbf{R_1}$) gave rise to an inhibitor, which was five times more potent than the parent compound **10a**. In particular, compounds **11a-c** had anti-MraY activities with IC_{50} ranging from 10 to 80 μM, while compound **11d** was completely inactive.[46] From these investigations it was clear that the structural features that appear to be important for high inhibitory activity against MraY include the absence of hydroxyl at the 3'-position, a 5'*S* chiral center, a primary amine group at the 5''-position, and an unmodified uracil moiety. Replacement of the complex diazepanone ring with simple lipophilic substituents was also shown to improve activity **12a-e** (Figure 9).[47] The longer the chains the more powerful the compounds

were found to be in whole cell assays. In fact, antibacterial activity was detected only for compounds with alkyl chain with n = 9 to 13 methylene units, reaching MIC values against *S. aureus* as low as 2.5 μg/mL.[43] On the other hand, the *in vitro* inhibitory activity against MraY was very similar for all compounds with different alkyl chain lengths, with IC_{50} values ranged between 0.33 and 0.90 μM.[47] This may suggest that length and lipophilicity of the hydrocarbon chain are key features necessary for the activity in whole cell assay.

Simplified gem-difluorinated liposidomycin analogues were also evaluated as MraY inhibitors. However, only compound **13** showed moderate activity, with 29 % inhibition of MraY at a concentration of 11.4 mM (Figure 9).[48]

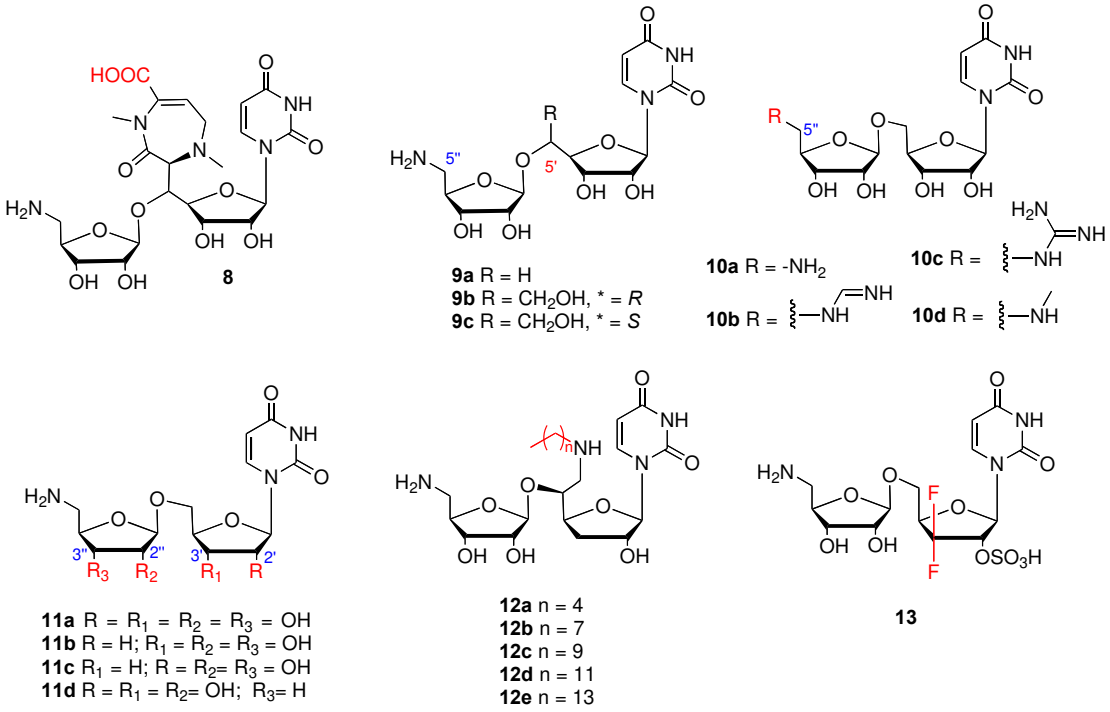


Figure 9. Structure of simplified liposidomycin analogues **8**; **9a-c**; **10a-d**; **11a-d**; **12a-e**.

Mureydomycins **14a-d**, as liposidomycins, belong to the class of uridyl-peptide antibiotics. These compounds have a uridyl moiety (**A**) linked to a peptide chain (**C**) via an enamidic bond (**B**) (Figure 10).[49] Isolated from *S. flavidoviridens* in 1989, these

analogues were found to be potent *in vitro* especially against *Pseudomonas aeruginosa* (MICs = 1.5-12.5 μg/mL). When evaluated in an infected mice model, they also showed ED_{50} doses ranging between 50 and > 100 mg/kg).[50] Analogue **14c** (Mureidomycin A) showed generally the most potent antibacterial activity against β-lactam-resistant strains of *P. aeruginosa* (MICs = 0.1-1.56 μg/mL) together with remarkable *in vivo* activity against *P. aeruginosa* infected mice.[51] Compound **14c** was also found to inhibit selective bacterial translocase MraY without any significant effect on the mammalian glycotransferases.[52, 53]

Mureydomycins **14e** and **14f** (Figure 10) were only later identified and showed lower anti-pseudomonas activity when compared to **14a**.[54]

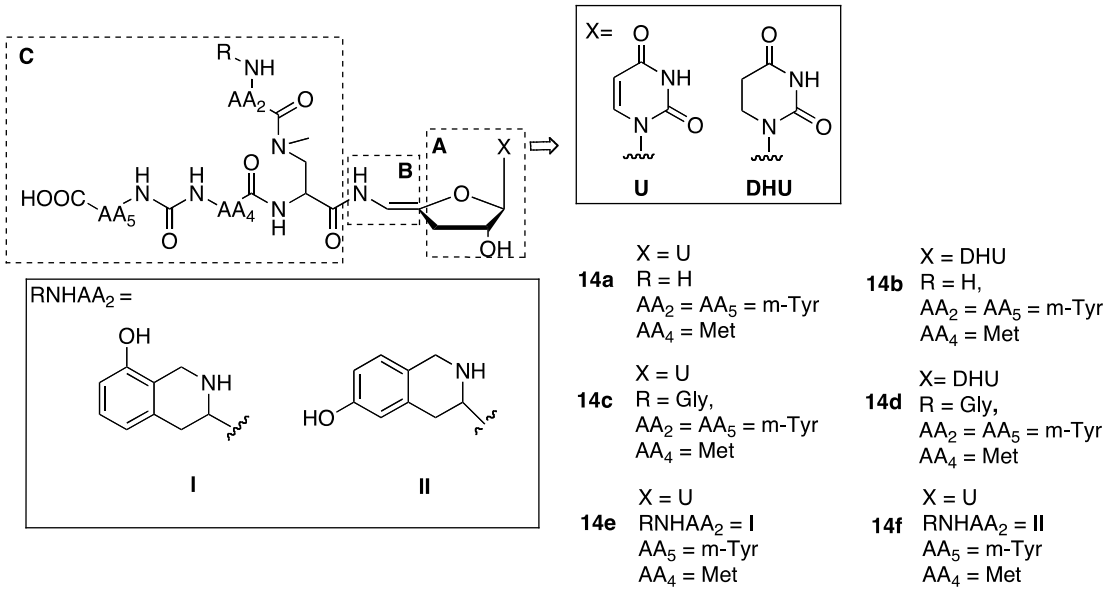


Figure 10. Structures of mureidomycins **14a-f**.

A structure-function study on mureidomycins[55] aimed to examine the role of the enamide linkage in the MraY binging and revealed that the peculiar 4',5'-enamidic bond (**B**; highlighted in the blue square; Figure 10), is not the only key structural feature responsible for the MraY enzyme binding. In support of this, compound **15** (Figure 11), synthesised as a simplified mureydomycin analogue, indeed showed lack

of enzyme inhibition.[55] Different studies showed the greater importance of the *N*-terminal in the peptide chain for MraY inhibition, due most probably to its crucial interaction with several of the enzyme's residues.[56] To confirm this, a series of 5'-uridinyl dipeptide analogues of mureidomycin A in which the diaminobutyric acid unit is replaced with a β-alanine or *N*–methyl-β-alanine were prepared and evaluated for their MraY inhibition and antibacterial activity. The simplified analogue 5'-*O*-(L-ala-*N*-methyl-β-alanyl)uridine (**16**, Figure 11), was found to be an inhibitor of MraY (97 % inhibition at 2.3 mM), and also active against *P. putida* at 100 μg/mL concentration. The activity of such compound was found to be competitive with Mg^{2+} ions suggesting that the primary amino terminus may be able to bind the enzyme in place of the Mg^{2+} cofactor.[57] Analogues without either enamide, or diaminobutyric acid or *N*-methyl functionalities were found to be inactive.[58] Compounds bearing a reduced enamidic portion and different combinations of amino acids in the peptidic region were found to inhibit *M. tuberculosis* growth. The most active compounds **17a-d** (MIC_{90} = 12.5 μg/mL) are reported in Figure 11.[59]

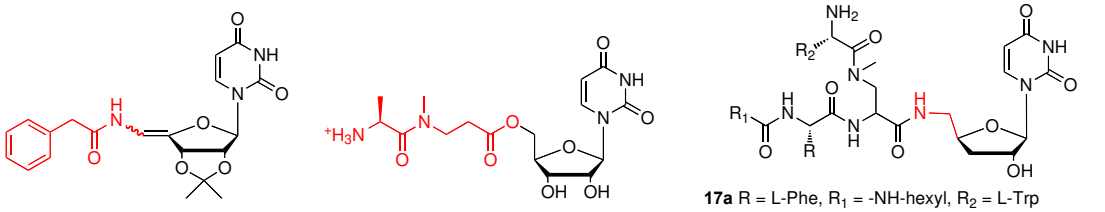
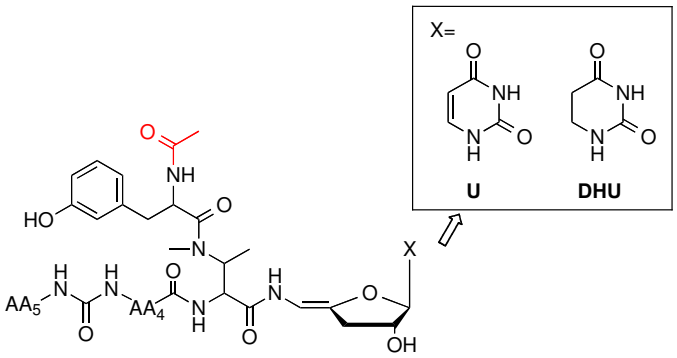


Figure 11. Structures of simplified mureidomycin analogues **15**; **16**; **17a-d**.

Recently, *N*-acetylated mureidomycin analogues **18a-h**, were identified[60] and were revealed to be potent growth inhibitors of *P. aeruginosa* (Figure 12).



18a AA_4 = L-Met, AA_5 = L-*m*-Tyr, X = U
18b AA_4 = L-Met, AA_5 = L-*m*-Tyr, X = DHU
18c AA_4 = L-Met^{SO}, AA_5 = L-*m*-Tyr, X = U
18d AA_4 = L-Met, AA_5 = L-Phe, X = DHU
18e AA_4 = L-Met, AA_5 = L-Phe, X = U
18f: AA_4 = L-Leu-L-Ile, AA_5 = L-*m*-Tyr, X = U
18g AA_4 = L-Leu-L-Ile, AA_5 = L-Phe, X = U
18h AA_4 = L-Leu-L-Ile, AA_5 = L-Phe, X = DHU

Figure 12. Structures of *N*-acetyl mureidomycin analogues **18a-h**.

One additional series of uridyl-peptide antibiotics is represented by pacidamycins **19a-g**, which were originally isolated from *S. coeruleorubidus*.[61-63] Their common scaffold contains a central *N*-β-methyl-2*S*,3*S*-diaminoburytic acid (**A**) moiety, which is α-amino-capped by a ureido dipeptide (**B**), β-amino-capped by a single amino acid or a dipeptide (**C**), and carboxy-linked to a 3′-deoxy-4′,5′- enaminouridine (**D**).

These compounds showed no activity against Gram-positive bacteria but were found to have potent *in vitro* efficacy against *P. aeruginosa* (MICs = 4-16 μg/mL).[63] Unfortunately, these analogues did not show *in vivo* activity in mice. Three additional analogues **19h-j** were later on isolated and had similar *in vitro* activity to the previous analogues against *P. aeruginosa* (for **19h** MIC = 32 μg/mL).[64-66]

The enamide group of pacidamycins can be hydrogenated, with the retention of antibiotic activity, such as in the case of dihydropacidamycin D (**20a**) (Figure 13) with only a minor decrease in the activity (MIC = 64 μg/mL) against *P. aeruginosa*.[67] However, hydrogenation of both the enamidic bond and the nucleobase generated the totally inactive tetrahydropacidamycin D (**20b**) (Figure 13).[67]

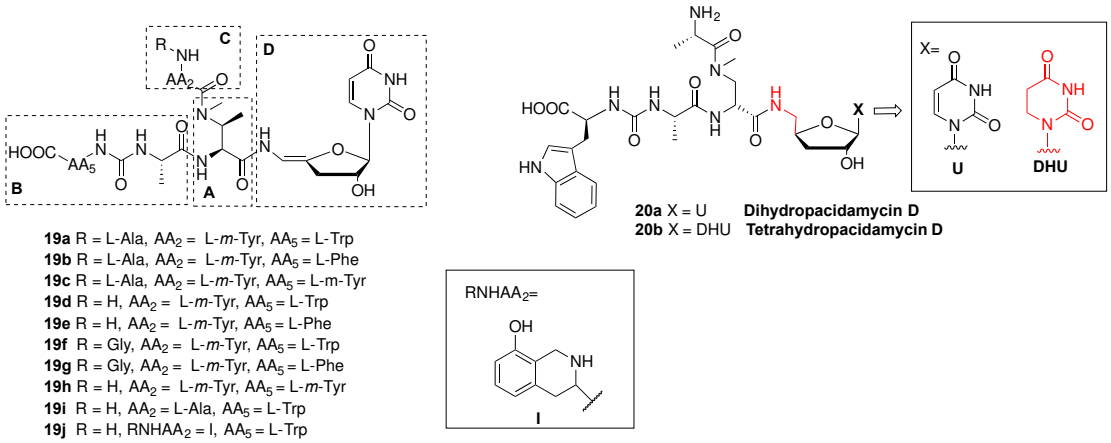


Figure 13. Structures of pacidamycins **19a-j**, dihydropacidamycin D (**20a**) and tetrahydropacidamycin D (**20b**).

Modifications in the peptidic portion of **20a** were introduced. The resulting analogues showed either retention (MIC = 16 μg/mL for **21a-d**) or improvement of the antibacterial activity (MIC = 4 and 8 μg/mL respectively for **21e** and **21f**) against *P. aeruginosa* (Figure 14).

Introduction of different nucleobases in the scaffold of pacidamycin caused a drop in activity, confirming the requirement for an uracil base for antibacterial activity of this class of analogues.[68] In a later study, the culture broth of *S. coeruleorubidus* was fed with halogenated phenylalanine analogues, leading to the biosynthesis of differently halogenated pacidamycin analogues **22a** and **22b**.[66] These compounds were bio-assayed against *P. aeruginosa* and *E. coli* and the activity was compared to the closest structural analogue pacidamycin **19h**. The chlorinated analogue **22a** exhibited a two-fold decrease in activity (MIC = 64 μg/mL) whilst the fluorinated derivative **22b** was totally inactive (Figure 14). Both compounds were inactive against *E. coli*.[66]

In 2013 Okamoto *et al*. carried out the total synthesis of **19i** (Figure 13) and tested it along with the 3'-hydroxy analogue **23** (Figure 14) in a whole cell assay against *P. aeruginosa* and in an enzymatic assay as MraY inhibitor.[69] Compound **23** was found slightly less active against MraY (IC_{50} = 42 nM) than the natural analogue **19i** (IC_{50}

=22 nM). However, the activity in whole cell assays on different strains of *P. aeruginosa* was improved (MIC = 8-32 µg/mL compared to MIC = 16-64 µg/mL for **19i**).[69]

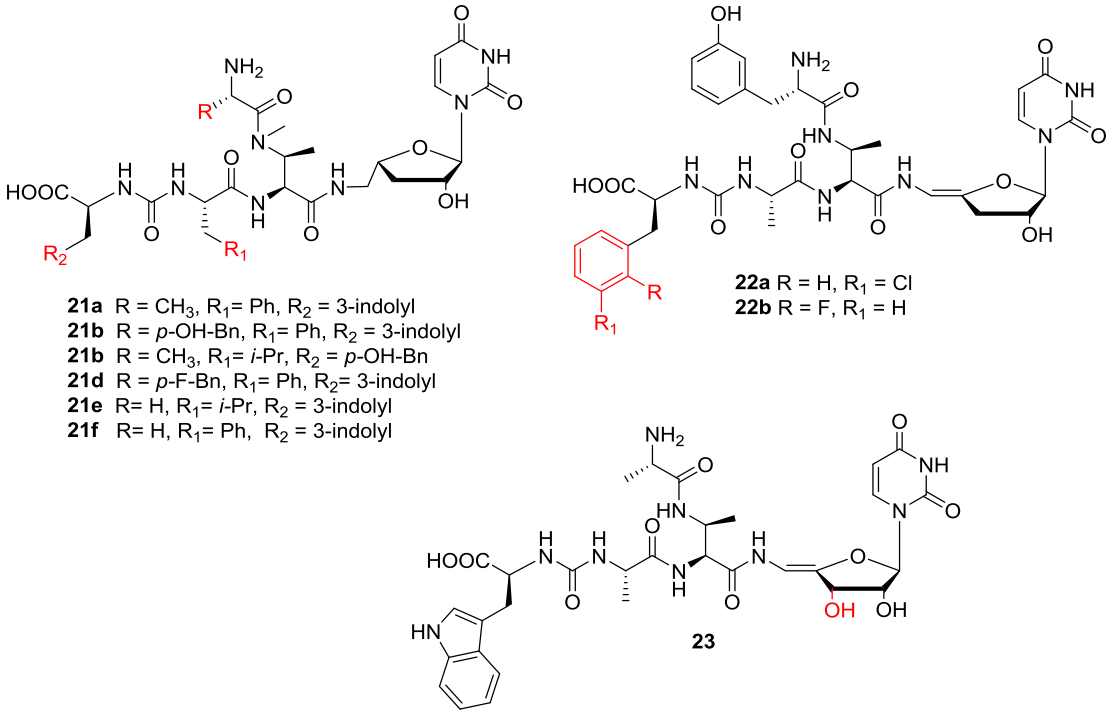


Figure 14. Structures of pacidamycin analogues **22a-f**; **23a-b**; **24**.

In 1994 during the course of a screening program for antibiotics with specific activity against *Pseudomonas sp.* another series of uridyl-peptide antibiotics named napsamycins **24a-d** were isolated from different strains of *Streptomyces* (Figure 15).[70] The general structure of these compounds contains a *N*-methyl diaminobutyric acid (**A**), a methionine (**B**) an ureido group (**C**), and two non-proteinogenic aromatic amino acid residues (**D**) in a peptide backbone that is linked to a 5'-amino-3'-deoxyuridine or 5'-amino-3'-deoxydihydrouridine by an enamide bond (**E**). This class of antibiotic showed anti-pseudomonas activity similar to mureidomycins and pacidamycins (MIC = 6.25-50 µg/ml).[70]

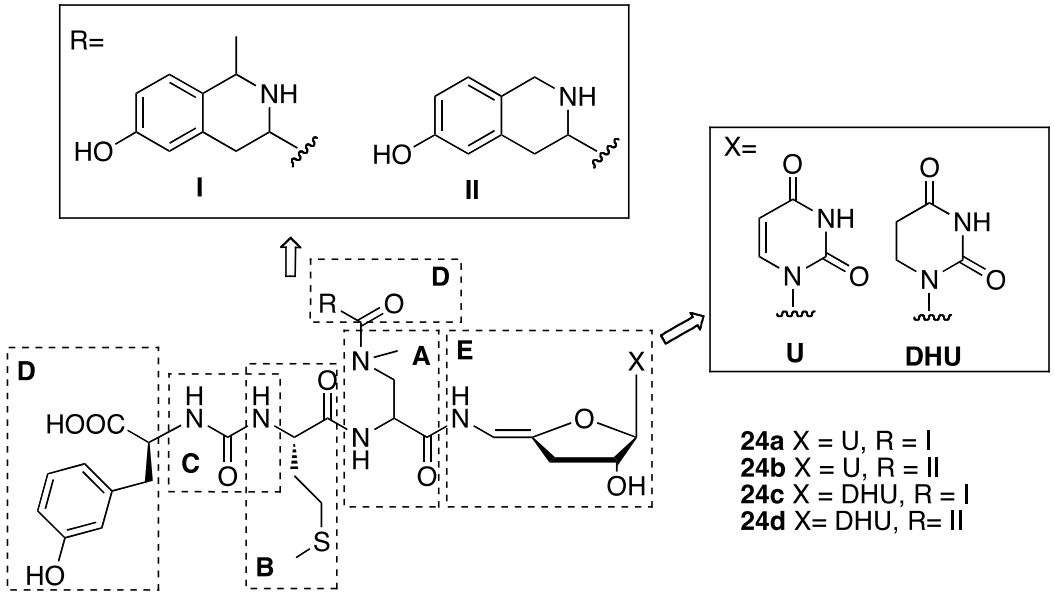


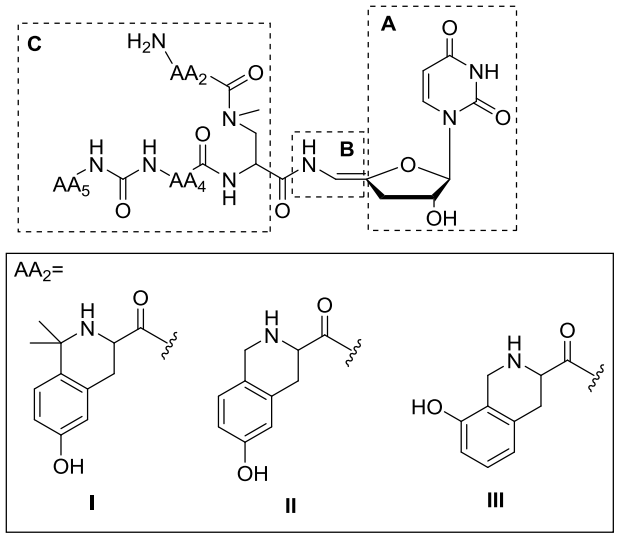
Figure 15. Structures of Napsamycins **24a-d**.

Within the series of uridyl-peptide compounds sansanmycins **25a-n**, are the most recently discovered sansamycin antibiotics (Figure 16). Structurally very similar to mureidomycin, pacidamycin and napsamycin, sansamycin antibiotics consist of a 3'-deoxy uridyl moiety (**A**) linked to a peptide chain (**C**) *via* an enamidic bond (**B**). The difference from the other uridyl-peptide compounds lies in the nature of the amino acids of the peptidic chain. Compound **25a**, first isolated from *Streptomyces sp.* in 2007, showed inhibitory activity against *M. tuberculosis* and *P. aeruginosa* with MIC values respectively of 10 and 12.5 μg/mL.[71] Other analogues bearing diverse amino acids in the peptidic region were later reported **25b-f**.[72, 73] Although, these new compounds were less active than **25a**, compounds **25b** and **25c** showed similar activity against *P. aeruginosa* and *M. tuberculosis* (MICs ranging from 8 to 20 μg/mL).

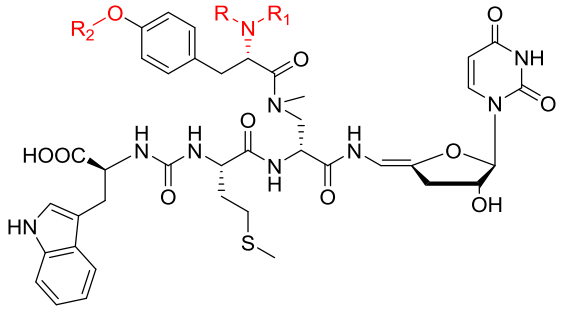
In 2011, novel synthetic sansamycin derivatives **25o,p** were prepared from **25a** by *N*-alkylation of the primary amino group and the O-alkylation of the tyrosine residue.[74] When evaluated as anti-tuberculosis agents (Figure 16), derivatives **25o** and **25p**

retained activity with MIC values of 16 and 8 μg/mL respectively when compared to the parent compound **25a** (MIC value of 16 μg/mL). Compound **25p** retained its activity (MIC value of 8 μg/mL) when tested against *M. tuberculosis* strains resistant to rifampicin and isoniazide. In the same test, **25a** had antibacterial activities with MIC values of 16 and 32 μg/mL against rifampicin and isoniazide-resistant strains respectively.

In 2014, novel natural sansanmycin analogues were identified **25g-n** (Figure 16).[75] Compound **25j** was the most potent analogue, with eight-fold improved anti-tuberculosis activity compared to the parent **25a** (MIC = 2 μg/mL) and potent anti-pseudomonas activity (MIC = 8 μg/mL).



25a AA_2 = L-*m*-Tyr, AA_4 = L-Met, AA_5 = L-Trp
25b AA_2 = L-*m*-Tyr, AA_4 = L-Leu, AA_5 = Trp
24c AA_2 = L-*m*-Tyr, AA_4 = L-Met^{SO}, AA_5 = L-Trp
25d (19d) AA_2= AA_4 = L-Met^{SO}, AA_5= L-*m*-Tyr
25e AA_2 = I, AA_4 = L-Met, AA_5 = Trp
25f AA_2 = I, AA_4 = L-Leu, AA_5 = L-Trp
25g AA_2 = *m*-Tyr, AA_4 = L-Phe, AA_5 = L-Trp
25h AA_2 = *m*-Tyr, AA_4 = L-Phe, AA_5 = L-Trp
25i AA_2 = II, AA_4 = L-Met, AA_5 = L-Tyr
25j AA_2 = III, AA_4 = L-Met^{SO}, AA_5 = L-Tyr
25k AA_2 = II, AA_4 = L-Phe, AA_5 = L-Trp
25l AA_2 = III, AA_4 = L-Phe, AA_5 = L-Trp
25m AA_2 = II, AA_4 = L-Met, AA_5 = L-Trp
25n AA_2 = L-*m*-Tyr, AA_4 = L-Met, AA_5 = L-Tyr



25o R = CH_3, R_1 = CH_3, R_2 = R_4 = H
25p R = $(CH_3)_2CH$, R_1 = R_2 = R_4 = H

Figure 16. Structures of natural **25a-n** and *N*-alkylated synthetic sansanmycin analogues **25o**,**p**.

Caprazamycins **26a-g**, specific inhibitors of MraY, are another series of compounds belonging to the family of lipouridyl antibiotics (Figure 17). First reported in 2003 following isolation from culture broth of *Streptomyces sp.* MK730-62F, these compounds have similar structures to lipidomycins, from which they differ just for the lack of the 2''-sulfate group of the aminoribose (**B**), and for the presence of a seven member diazepanone skeleton (**C**) together with a permethylated *L*-rhamnose (**E**) β-glycosidically linked to a 3-methylglutaryl moiety (**D**) (Figure 17).[76-78]

These analogues are mainly active against acid-fast bacteria, showing excellent *in vitro* anti-mycobacterial activity against drug-susceptible and multidrug resistant *M. tuberculosis* strains (MIC = 3.13 μg/mL) without significant toxicity in mice.[79]

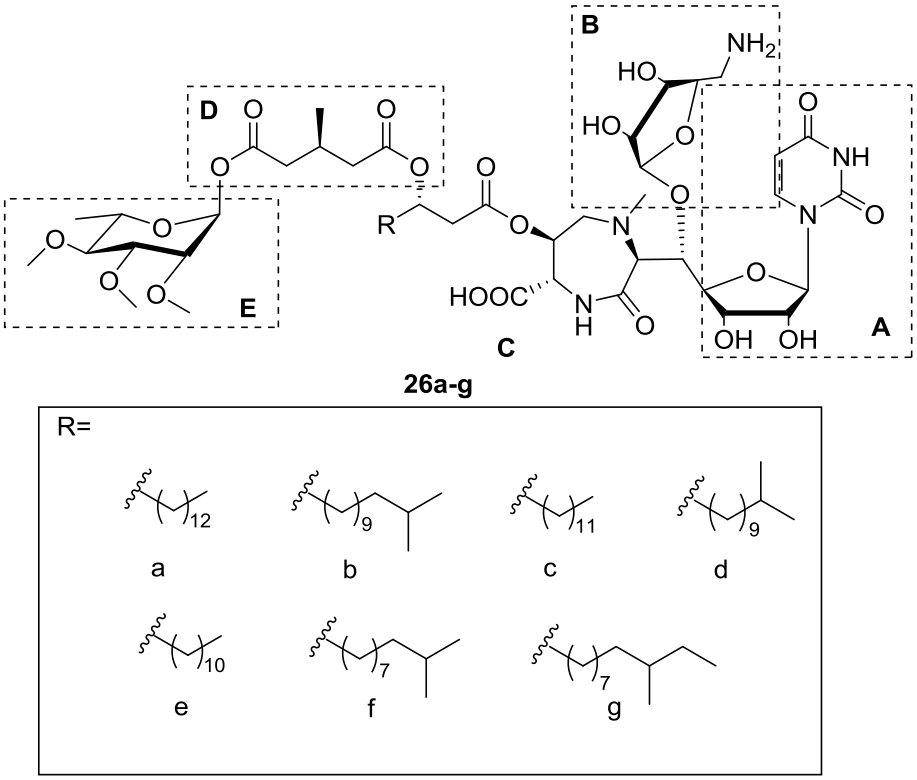


Figure 17. Structures of natural caprazamycins analogues **26a-g**.

Caprazol (**27a**), the common structural motif present in all caprazamycins (Figure 18), has been isolated as minor component in the same fermentation culture of caprazamycins **26a-g.** It can be also prepared synthetically allowing access to several

analogues. [80, 81] Hirano *et al.* tested **27a** along with compounds **27b** and **27c**, in which the OH at the 3''' position is linked to a palmitoyl chain (Figure 18). [79] The caprazol analogue **27a** showed no activity against either *M. tuberculosis* or *S. aureus*. On the other hand, the *N*-methylated **27b** and the *N*-demethylated **27c** palmitoyl derivatives showed good activity against *S. aureus* (MIC = 1.56-6.25 µg/mL) and moderate activity against *Enterococcus faecalis* (MIC = 12.5 µg/mL). The methyl group was found to be important for the activity against *M. tuberculosis*. Compound **27b** was 4-fold more active than **27c** (MICs 6.25 µg/mL and 25 µg/mL respectively). The same research team prepared different caprazamycin analogues lacking either the uridine moiety (**A**), or the aminoribose (**B**). [79] A third compound **28** bearing an acyclic caprazole group was also synthesised (Figure 18).[82] These analogues were tested as anti-mycobacterial agents, and only the acyclic compound **28** retained antibacterial activity against *M. tuberculosis* (MIC = 6.25 µg/mL).

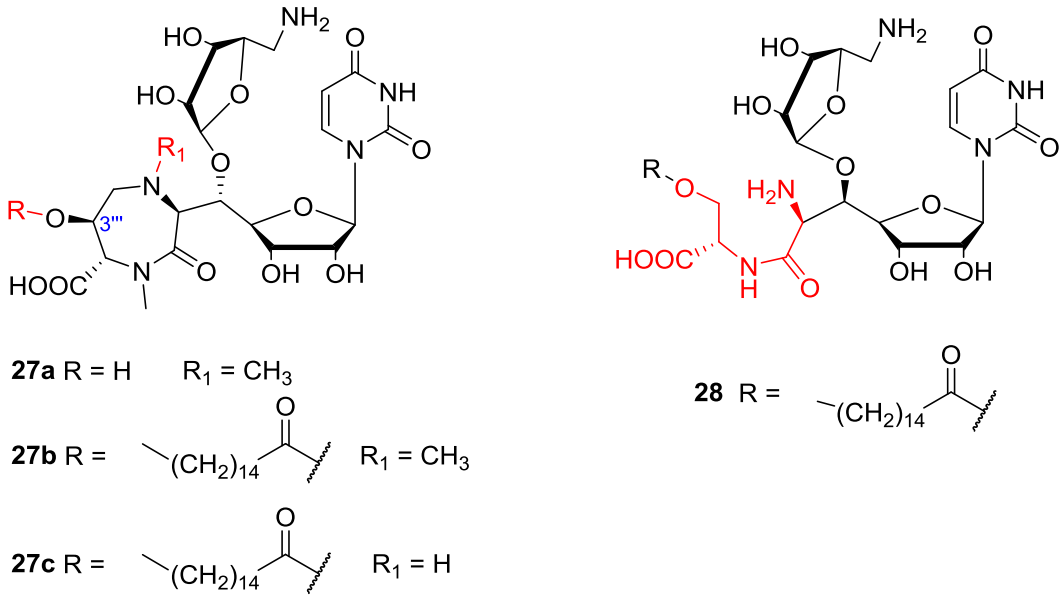


Figure 18. Structures of synthetic caprazamycins analogues **27a-c**; **28**.

Other caprazamycin analogues **29a-f** were synthesised in 2010 by Ii *et al*.[83] In these novel molecules the diazepanone ring was removed and lipophilic chains with amide or urea linkages were attached to either the C-7' or N-6 atom. The acyclic analogues **29a-f** exhibited relatively moderate MraY inhibitory effects: compounds **29a,b** (IC_{50} =18 and 46 μM) were generally more active than **29c-f** (IC_{50} =36- 256 μM). However, when **29a-f** were evaluated against a range of bacterial strains including *S. aureus*, *E. faecalis* and *E. facium* their antibacterial activities were largely reduced compared to the parent natural products, indicating that the diazepanone moiety was not essential for, but contributory to antibacterial activity. In particular, the *C*-amide derivatives **29a,b** exhibited weak antibacterial activity against drug-susceptible and drug-resistant strains *of S. aureus* (IC_{50} = 64 μM) and *Enterococci sp.* (IC_{50} = 16-64 μM). On the other hand, analogues **29c-f**, demonstrated total loss of antibacterial activity. The reduced biological activity observed with compounds **29a-f** was attributed to their excessive flexibility when compared to the natural caprazamycin. Therefore, the same authors decided to restrict the conformation of the acyclic analogues by linking the nitrogen atom at the 6**'**-position and the oxygen atom at the 5'-position of either **29a** or **29c** (Figure 19).[83] As a result oxazolidone-containing compounds, lacking the aminoribose and the diazepanone ring were generated. Among these compounds, **30a-m** showed antibacterial activity with MIC values ranging from 8 to 32 μg/mL against the bacterial resistant strains *S. aureus*, *E. faecalis* and *E. faecium* (Figure 19). Surprisingly, these analogues were only weak MraY inhibitors with IC_{50} of 920-1200 μM, suggesting a different mechanism of action.

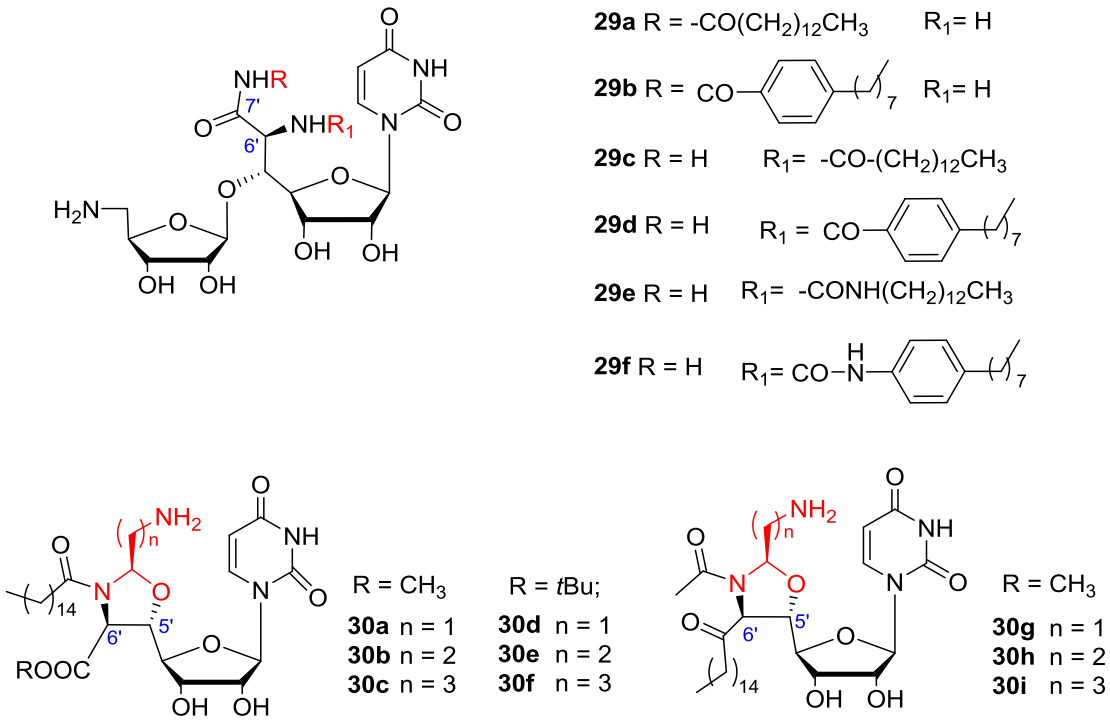


Figure 19. Structures of synthetic caprazamycin analogues **29a,b**; **30a-i**; **31a,b**; **32a,b**.

Acidic treatment of a mixture of natural caprazamycins **26a-g** afforded another simplified caprazamycin analogue **31a** lacking of the permethylated (*L*)-rhamnose (**E**) β-glycosidically linked to a 3-methylglutaryl moiety (**D**). This analogue was named caprazene and represents the core structure of caprazamycins (Figure 20).[77] Although **31a** was found to be devoid of any antibacterial activity, its chemically modified structures were potent anti-mycobacterial agents (Figure 20).[84] Most of these analogues have low MIC values down to 0.05 μg/mL.[84] One of the most active derivatives was **31b** (CPZEN-45, Figure 20), which surprisingly showed an antibacterial spectrum different from that of the natural caprazamycin**.** In particular, the spectrum of **31b** was found to be narrower and it was especially effective against slowly growing mycobacteria, implying that **31b** has a different mode of action from caprazamycins. In further study, decaprenyl-phosphate–GlcNAc-1-phosphate transferase (TagO), an enzyme involved in the biosynthesis of teichoic acid, was

identified as the primary target of **31b** in *B. subtilis.*[85]The same molecule targets the WecA (the orthologue of TagO) in *M. tuberculosis.*

32a (A-90289 A) and **32b** (A-90289 B) (Figure 20) are nucleoside analogues closely related to caprazamycin, which were recently isolated from the culture broth of *Streptomyces sp.* SANK 60405.[86-88] These compounds are characterised by the presence of a sulfate group on 2'-position of the uridine moiety, and were reported to inhibit MraY enzyme at concentrations around 30 ng/mL. In whole cell assay MIC values of **32a** against different strains of *Streptococcus*, *Enterococcus* and *Staphylococcus* ranged between 4 and 16 µg/mL.[86]

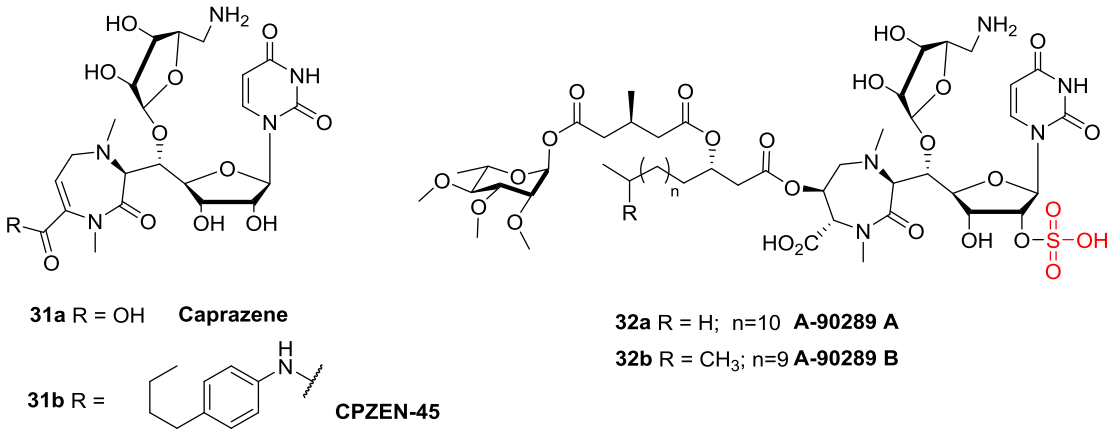


Figure 20. Structures of semi-synthetic **31a,b** and natural caprazamycin analogues **32a,b**.

The search for translocase I inhibitors, led to the discovery of another novel nucleoside antibiotic **33** (A-94964) isolated from the culture broth of the strain *Streptomyces sp.* SANK 60404 (Figure 21).[89, 90] This compound has a unique structure with an extended uronic acid (**A**) with eight-carbon unit. The oxygen atoms at 6' and 7' position of this acid, are connected to a pendant ribose unit (**B**) and to a phosphate bridge (**C**) respectively. The other arm of the phosphate group is then connected to a *N*-acyl glucosamine moieties (**D**). Despite the substantial structural changes, **33** was found to inhibit bacterial MraY with an IC_{50} value of 1.1 µg/m. It

showed antimicrobial activities against *S. aureus* and *E. faecalis* with MIC values of 100 and 50 μg/mL respectively, without any cytotoxicity against mammalian cell lines.[89]

More recently, during an active discovery program focused on inhibitors of bacterial translocase, researchers at Daiichi-Sankyo Co. Ltd in Tokyo reported additional liposidomycin and caprazamycin analogues **34a** (A-97065) and **34b** (A-97065OPO_3H) and muraminocin **35**, which were isolated from culture broths of *Streptomyces sp.* SANK60704 and *Streptospotangium sp.* SANK60501 respectively (Figure 21).[91, 92] These antibiotics, which differ only at the acyl group (**E**), showed similar antibacterial activity to liposidomycin and caprazamycins suggesting that there is no characteristic structure–activity relationship in the fatty acid side chain. Compound **34b** with a phosphate group was found 40-times less potent than the dephosphorylated analogue **34a**.

In 2015, using a gene-targeting approach, Van Lanen and co-workers discovered novel nucleoside-derived antibiotics. In particular, the authors were able to isolate and characterize only one of the species detected in actinomycete (Sphaerisporangium sp. SANK 60911) culture broth. This new compound, named Sphaerimycin A, has a structure (determined by mass spectrometry and NMR) closely related to those of muraymycin with which it shares the uridine moiety. Sphaerimycin A is capable to potently inhibit translocase I (IC_{50} between 12 and 65 ng/mL), displaying also promising antibacterial activity against different strands of Gram-positive bacteria. On the contrary it was found totally inactive against Gram-negative bacteria probably as a results of a pronounced efflux.[93] (Figure 21)

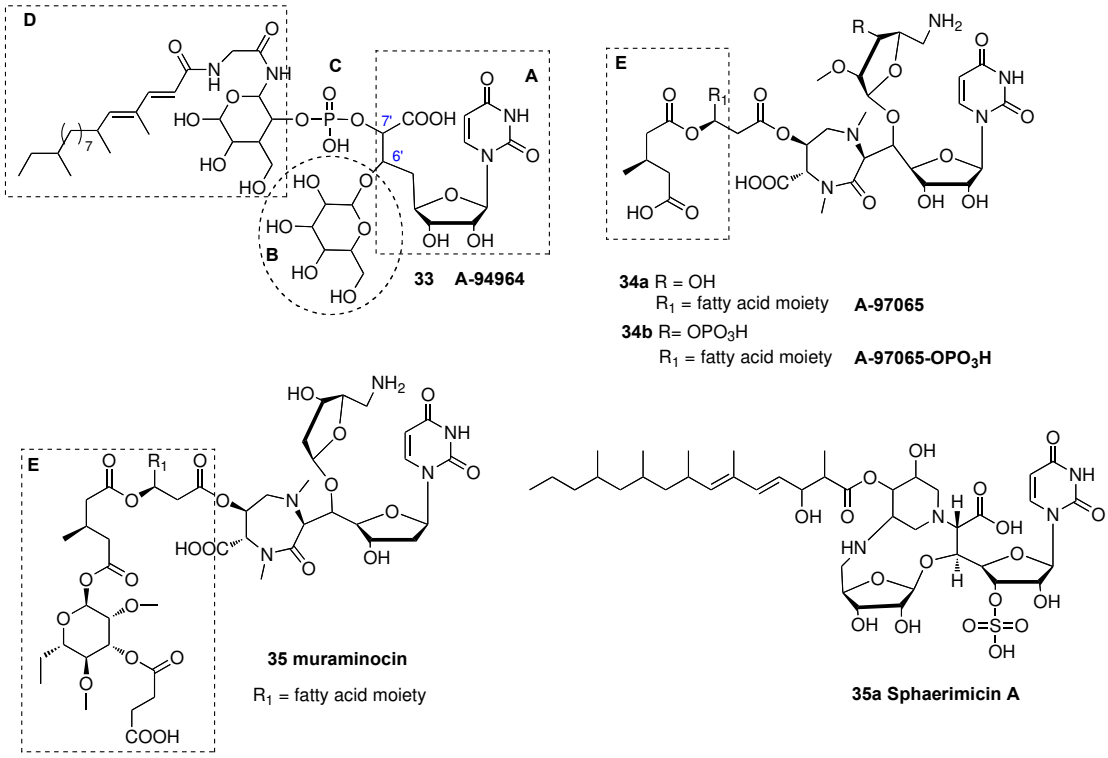


Figure 21. Structures of analogues **33**, **34a-b** and muraminocin **35**.

Another important class of natural nucleoside analogues are capuramycins, which present in their structures a uridine-5'-carboxamide (**A**), an unsaturated *α-D*-mannopyranuronate (**B**) and a *L*-α-amino-ε-caprolactam moiety (**C**) (Figure 22). The first member of this class is the uracil nucleoside analogue **36a**, isolated in 1986 from the culture filtrate of *S. griseus* (Figure 22).[94-97] Compound **36a** proved to have selective anti-mycobacterial activities with MIC values of 3 and 12.5 μg/mL against *M. smegmatis* and *S. pneumoniae* respectively. The antibacterial activity was linked to the inhibitory activity of MraY (K_I = 10 ng/mL).

In 2003 two capuramycin derivatives, **36b** (A-500359A), the methylated version of **36a**, and **36c** (A-500359E), lacking instead of the caprolactam moiety, were isolated from *S. griseus* culture (Figure 22).[98, 99] Compound **36b** was found to have MraY inhibitory (IC_{50} = 0.017 μM) and antibacterial activity against *M. smegmatis* (MIC =

6.25 μg/mL) similar to capuramycins **36a**. On the contrary, although derivative **36c** was still inhibiting MraY (IC_{50}=0.027μM), it was devoid of any antibacterial activity. This result was attributed the hydrophilic nature of this compound, which could potentially prevent it from crossing the cell wall and reaching the target enzyme, translocase I. Therefore, a number of synthetic derivatives with a variety of substituents in place of the diazepan-2-one moiety, were prepared.[100] The discrepancy in activities of these analogues seemed to be linked to the difference in cell permeability. These new capuramycin analogues **36d-g** were synthesized from A-500359E replacing the methyl ester with a variety of amides (Figure 22). When tested in whole cell assay the most potent compound was the phenyl amide **36h** (MIC = 6.25 μg/mL). The other analogues **36d-g** had activities with MIC values ranging between 12.5 and 50 μg/mL.

Compounds **37a-g** were also prepared by acylation of the 2'-hydroxyl of the uridine unit together with the acylation of one or both the two hydroxyl groups on the *a-D*-mannopyranuronate.[101] These modifications caused an increase in cell permeability and also of the antibacterial activity. Many analogues were very potent against different *Mycobacteria* strains. The most active analogues were compounds **37a-d**, with MIC values lower than 0.063 μg/mL against *M. avium*, *M. intracellulare* and *M. kansasii*. This impressive activity was suggested to be dependent on the excellent lipophilicity of these compounds, which enabled them to penetrate the cell membrane of *Mycobacteria* more effectively. It has also been suggested that these compounds may work as prodrugs, being converted into the parent compound **36b**, within the bacterial cell.

The same research group modified the scaffold of **36b** by replacing the diazepam-2-one moiety with diverse aryl groups.[100] Among several aryl derivatives, compound **38**

(Figure 22) showed MICs between 0.5 and 2 μg/mL against different mycobacterial strains. Compounds **37a** (SQ641) and **38** along with **36b** were tested *in vivo* in a murine lung model of tuberculosis.[102] These compounds were administered intranasally at concentrations of 0.1 or 1 mg per mouse per day for 12 days. The mycobacterial load in the lungs was significantly lower in all treated groups when compared to that of the untreated controls, demonstrating the strong potential of capuramycin analogues as anti-tuberculosis agents. The *in vitro* screening of compound **37a** was also extended to a broader spectrum of *Mycobacteria sp*. [103, 104] In these studies 37a demonstrated exceptional anti-mycobacterial activity both alone and in combination with other approved anti-tuberculosis agents. Despite the remarkable *in vitro* potency against *M. tuberculosis*, **37a** had low solubility in water. Hence, studies were addressed at developing new delivery vehicles and routes of delivery, which led to significant improvements in *in vivo* activity.[105] It is also noteworthy that further acylation of two hydroxyl groups on the sugar moiety of **37a** with amino undecanoic acid such as in derivatives **37e-g** expanded the *in vitro* activity of this compound against Gram-negative and Gram-positive bacteria (*Staphylococci* and *Bacilli*), and also improved the activity against *Mycobacteria sp*.[106] Recently, **37a** demonstrated efficacy in the treatment of *C. difficile* in a murine model of infection.[107]

Capuramycin **36a** and its analogs **36b-e**, **37a-g**, **38** are strong translocase I inhibitors that are capable of killing only replicating mycobacteria under aerobic conditions. However, during a screening of new capuramycin analogs against replicating and dormant *M. tuberculosis* strains, it was found that the 2'-methylated capuramycin analog **39** (UT-01320) was able to kill both replicating and non-replicating bacteria.[108] Interestingly, this compound did not exhibit MraY enzyme inhibitory

activity even at high concentrations, but it was instead responsible for the inhibition of bacterial RNA polymerases with IC_{50} values between 100 and 150 nM. In addition, this derivative had a synergistic effect with MraY inhibitor **37a**, significantly improving the *in vitro* anti-mycobacterial activity.[108] Although the exact mechanism of action of **39** is still under investigation, this analogue contains a new pharmacophore that inhibits the polymerization of bacterial RNA at low concentrations in a dose–dependent manner and for this reason deserves further attention.

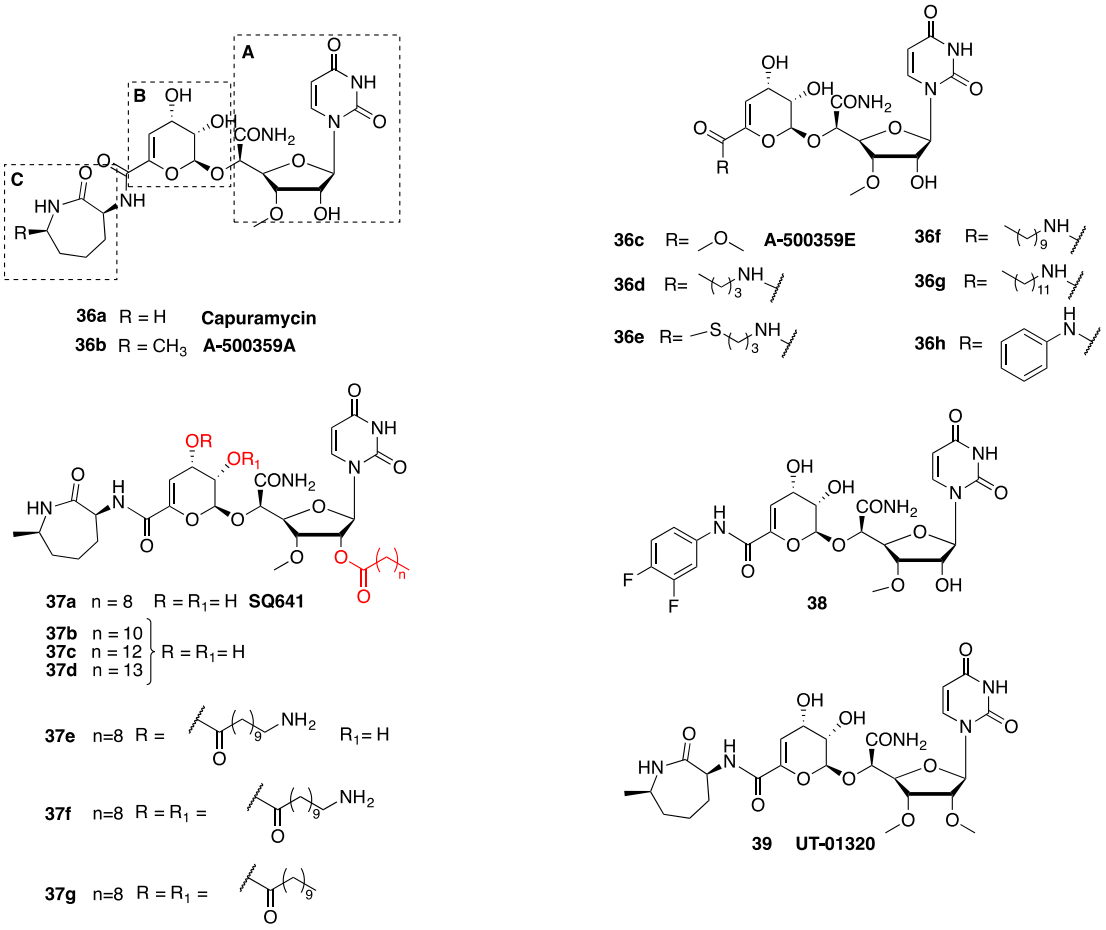


Figure 22. Structured of capuramycin analogues **36a-h**; **37a-g**; **39**.

Antibiotic analogues similar to **36b** were discovered in the culture broth of *S. griseus* SANK 60196.[109-111] The most active compounds were **40a** (A-500359M-1) and **40b**

(A-500359M-2) (Figure 23). These analogues inhibit MraY with IC_{50} values of 0.010 μM and 0.058 μM, respectively. Compound **40a** showed activity similar to **36b** against *Mycobacterium sp,* whereas **40b** showed a broader antibacterial spectrum.[110]

A new derivative of capuramycin **41** (A-102395) bearing a benzene ring with a substituted chain instead of the aminocaprolactam moiety, was isolated from the culture broth of *Amycolatopsis sp.* SANK 60206 (Figure 23).[112]

Although being a potent inhibitor of MraY (IC_{50} = 11 nM), this analogue did not have any antimicrobial activity against various bacterial strains tested. This was suggested to be due to poor permeability through the bacterial membrane.

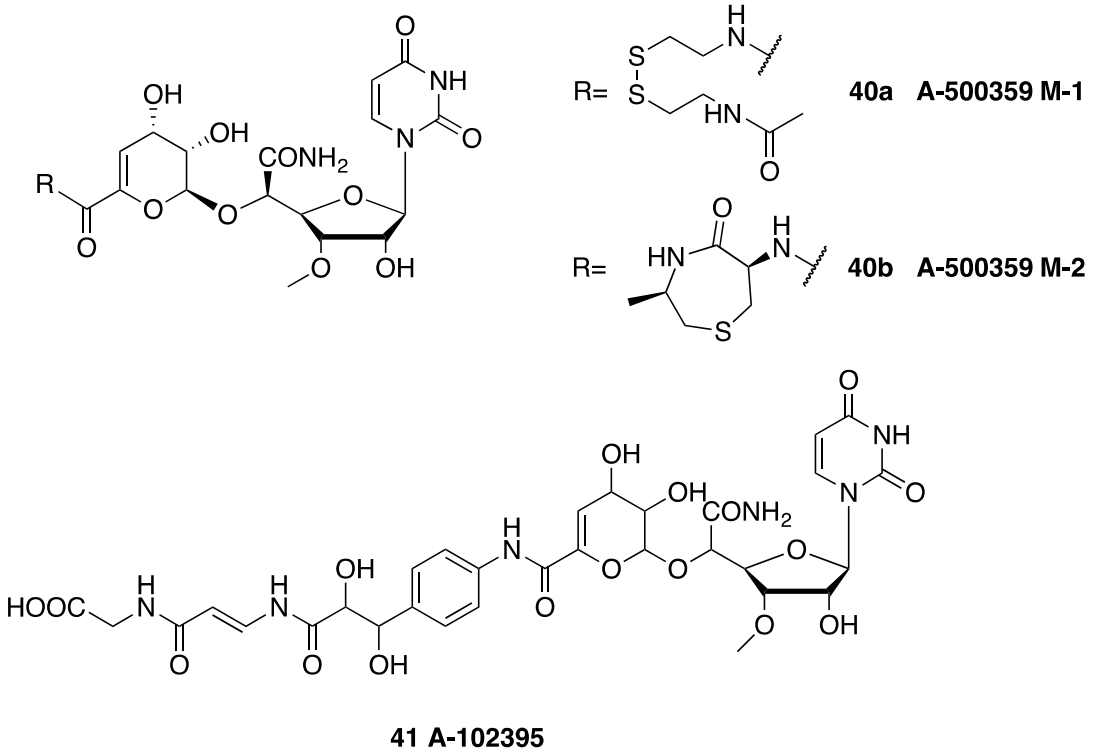


Figure 23. Structures of alternative capuramycin analogues **40a, 40b** and **41**.

In 2002 scientist at Wyeth in New York isolated a new family of antibiotics called muraymycins as complex of nineteen compounds from *Streptomyces sp*.[113] The core structure of these compounds consists of the uronic acid (**A**) connected to a 2-

methoxy-5-amino-5-deoxy-ribofuranoside (**B**) and to a 3-aminopropyl residue (**C**) that is juxtaposed to a hydroxy-leucyl residue (**D**). An unusual feature of the molecule is the presence of a hexahydro-2-imino-4-pyrimidylglycyl moiety (**E**) and a cyclic guanidino amino acid residue. The muraymycins terminate in a basic amino acid-urea-amino acid motif (**F**) effectively reversing the directionality of the peptide chain. Members of the muraymycin family consist of a common peptide-appended glycosylated uronic acid core and differ primarily in the fatty acid (**R**) and the terminal amino sugar (**B**). Compound **42a** (Muraymycin A1) was one of the most active members of the series, showing *in vitro* antibacterial activity with MIC values ranging from 2 to 16 μg/ml against *Staphylococci*, 16 to >64 μg/ml against *Enterococci* and 8 to >64 μg/ml against Gram-negative bacteria.[113] Potent antibacterial activity for **42a** was also evident against a mutant strain of *E. coli* (MIC < 0.03 μg/ml), which has a reduced lipid bilayer, hence suggesting that membrane permeability plays a key role in the *in vivo* activity. Compound **42a** also demonstrated efficacy in *S. aureus* infected mice (ED_{50} 1.1 mg/ kg).[113] The presence and the structure of the fatty acid (**R**) was found to influence activity. Compound **42b**, lacking of the **R** chain, although was showing a good enzyme inhibitory activity and no apparent toxicity did not demonstrate a good antimicrobial activity. This result suggested that the lipophilic 13-hydroxyguanidino lauroyl group (**R**), positioned on the hydroxyl group of the hydroxy leucyl moiety, may be responsible for transporting the compound to the target enzyme in the membrane, and at the same time may also be responsible for its toxicity.

In order to improve the activity and the therapeutic index, scientist at Wyeth investigated semi-synthetic modification of **42b**, having lipophilic groups onto the primary amino group of the amino ribose moiety (structures not shown) and/or a

secondary amino group at the 15-position of muraymycin (Figure 24).[114] Among these analogues compounds **43a** and **43b,** have shown similar inhibitory activity against MraY compared to the parent compound (IC_{50} = 6.25 μg/mL).

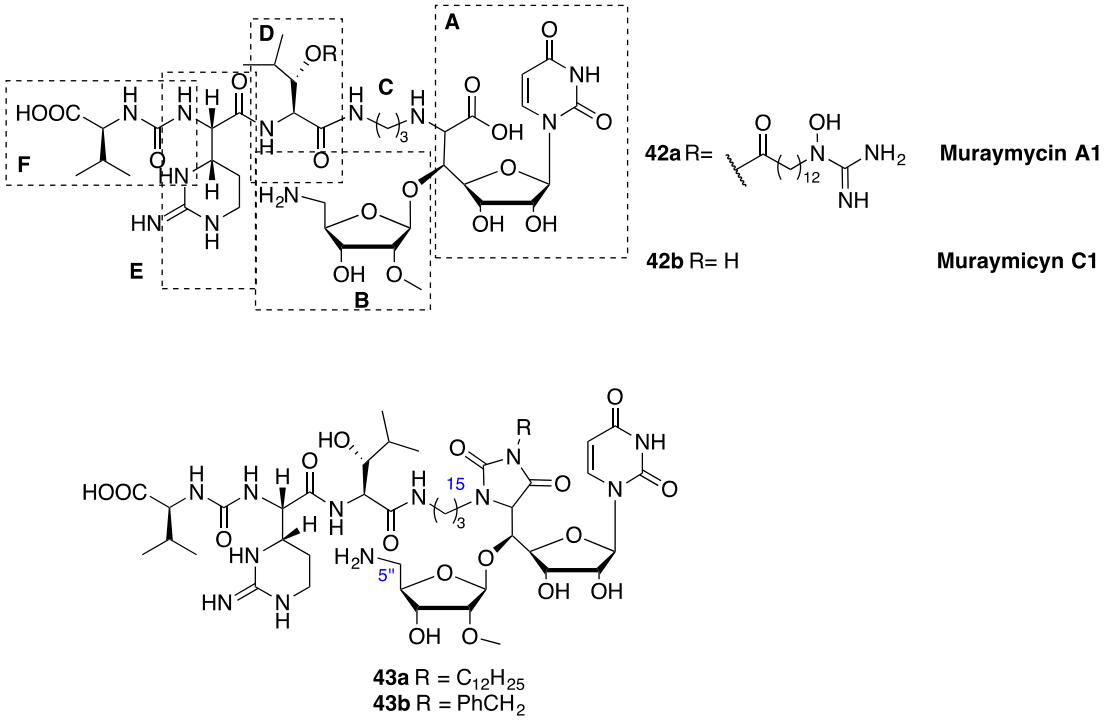


Figure 24. Structures of natural **42a** and **42b** and synthetic muraymycin analogues **43a** and **43b**.

Other synthetic analogues of the muraymycins have also been investigated.[115] Among these compounds the cyclic guanidine amino acid (**E**), the 5'-amino ribose sugar moiety (**B**) and the lipophilic side chain (**R**) were removed. Surprisingly, compounds with a fully protected uridyl moiety **44a-d** (Figure 25), showed improved antibacterial activity. Among truncated muraymycins compound **44a** had activities with MIC values ranging from 4 to 16 μg/mL against *Staphylococci* and from 4 to 8 μg/mL against *Enterococci,* whereas compound **44b** showed MIC values ranging from 1 to 2 μg/mL against the same microorganisms (Figure 25).[115]

Analogues of compounds **44a** and **44b** with 5'-epimeric hydroxyl group (compounds **44c** and **44d**, Figure 25), showed similar activities on Gram-positive bacteria.[116]

Muraymycin analogues with a lipophilic substituent **45b**-**g** (Figure 25) were synthesized and displayed good activity against a range of Gram-positive bacterial pathogens including methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *E. faecium,* without exhibiting significant cytotoxicity against human hepatoceller liver carcinoma (HepG2) cells (IC_{50} > 100 μg/mL).[117] The most active compound was **45b** with MIC values ranging from 2 to 4 μg/mL and from 0.25 to 4 μg/mL against *Staphylococci* and *Enterococci* respectively. The long lipophilic side chain in compound **45b** is able to influence the antibacterial activity by modulating the membrane permeability without jeopardizing the affinity for the target enzyme MraY (IC_{50} = 0.74 μM). Interestingly, analogues with the **"**natural**"** stereochemistry are in general slightly more potent than those with **"**unnatural**"** stereochemistry, although **45b** endowed with unnatural stereochemistry proved to be superior to **45e**. The same research team investigated the mechanism of action of the most promising analogues **45b** and **45e**, and found that they act as inhibitors of MraY with competitive inhibition towards the nucleotide substrate and non-competitive inhibition towards the lipid substrate.[118]

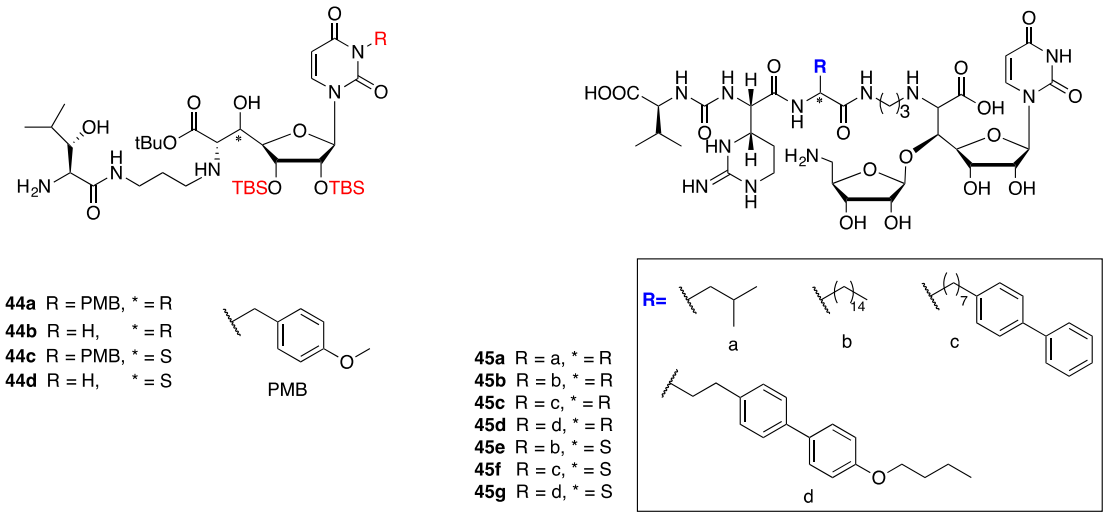


Figure 25. Active truncated muraymycin analogues **44a-d; 45a-g**.

Further investigations were carried out on the scaffold of the lipophilic analogue **45b** (Figure 25), on which the urea-peptide moiety was modified. These analogues **46a-g** (Figure 26) retained good inhibitory activity against resistant *Staphylococci* and *Enterococci* strains (MICs = 1-8 μg/mL).[118]

Other derivatives having a lactam-fused isoxazolidine core showed moderate antimicrobial activity **47a-g**, (Figure 26), with MIC values between 8 and 32 μg/mL against *Staphylococci* and *Enterococci*.[119]

In order to expand the antibacterial spectrum of muraymycins toward *P. aeruginosa*, (a common nosocomial pathogen intrinsically resistant to a variety of drugs currently used in the clinic), a systematic structure−activity relationship study was carried out.[120] From these investigations it was discovered that both the lipophilic side chain and the guanidinium groups are functional groups important for the anti-pseudomonas activity. The most active compounds were **48a** and **48b** (Figure 26) with MIC values ranging from 4 to 16 μg/mL against different strains of *P. aeruginosa*. Some insights into the mechanism of action of these analogues and other MraY inhibitors were published in 2014.[121]

Muraymycin derivatives lacking of the aminoribose unit were also synthesised. [122] The 5'-defunctionalized compound **49** was found to be ideal in terms of stability and chemical accessibility (Figure 26). This analogue showed good inhibitory properties towards the bacterial target protein MraY (IC_{50} = 1.7 μM). Analogue **49** also demonstrated sufficient pharmacokinetic stability and no cytotoxicity against human cells (up to 100 μM), thus making it a promising lead for antibacterial drug development. Studies regarding the mechanism of action of muraymycins were carried out: the highly unusual ω-guanidinylated fatty acid moiety was proposed to mediate membrane penetration, thus enabling the interaction of muraymycins with their intracellular target MraY.[123]

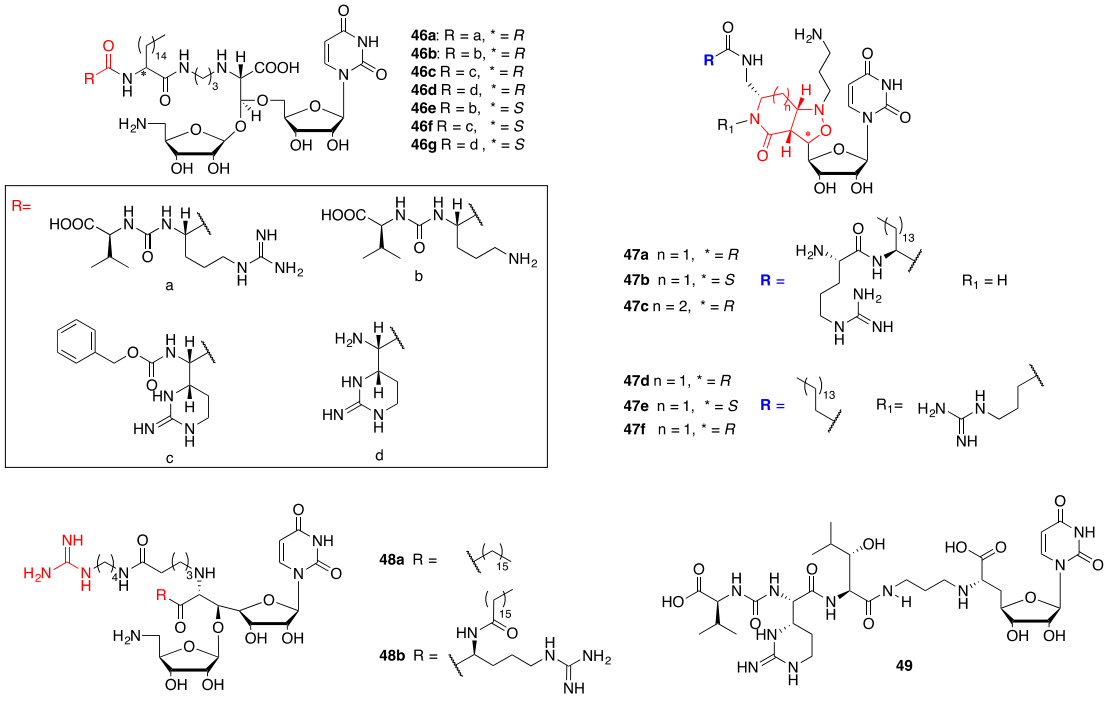


Figure 26. Structures of synthetic muraymycin analogues **46a-g**; **47a-f**; **48a-b**; **49.**

In 2011, Walsh reviewed the biosynthetic pathways for peptidyl nucleosides antibiotics with particular focus on the chemical logic and enzymatic machinery for uridine transformation and coupling to peptides.[124]

Recently Calvet-Vitale and Gravier-Pellettier reported the synthesis and biological evaluation of novel MraY inhibitors. These compounds presented a simplified structure still based on the aminoribosyl-*O*-uridine scaffold, commonly shared between liposidomycin, caprazamycins and other natural MuraY inhibitors.[125, 126]The first series of compounds contained a triazole ring directly attached via carbon-carbon bond to the 5' position of the nucleoside, whereas the second series presented an extra methylene between the nucleoside and the heteroaromatic ring, which was linked via its N- or C atoms to the aminoribosyl-O-uridine. The latest compounds were designed to be more flexible thanks to the extra methylene group. The biological activity of both series was evaluated on the MraY transferase activity as well as on their antimicrobial effects against some common bacteria. Out of thirty compounds tested, eighteen compounds had MraY inhibition with IC_{50} ranging from 15 to 150 μM. In general compounds lacking the "extra" methylene unit were found to be less active that the "methylene spaced" analogues. Among the compounds of the second series the *C*- connected were found slightly superior to the *N*-linked analogues. The best MraYb inhibitory activities (15-100 μM) were observed with compounds **51a-d** and **52a-d**, having a hydrophobic chain on the triazole unit. Promising antibacterial activity against MRSA was also observed for compounds **50c** and **51c** (MIC = 8 μg/mL) (Figure 27). Generally, the hydrophobic character of the chain and the flexible nature of the triazole unit were the key structural motifs to maintain the enzyme inhibition activity. A molecular modeling study was performed to rationalize the observed structure–activity relationships (SAR), and this allowed the authors to correlate the activity of the most potent compounds with their interaction with Leu191 of MraYAA.

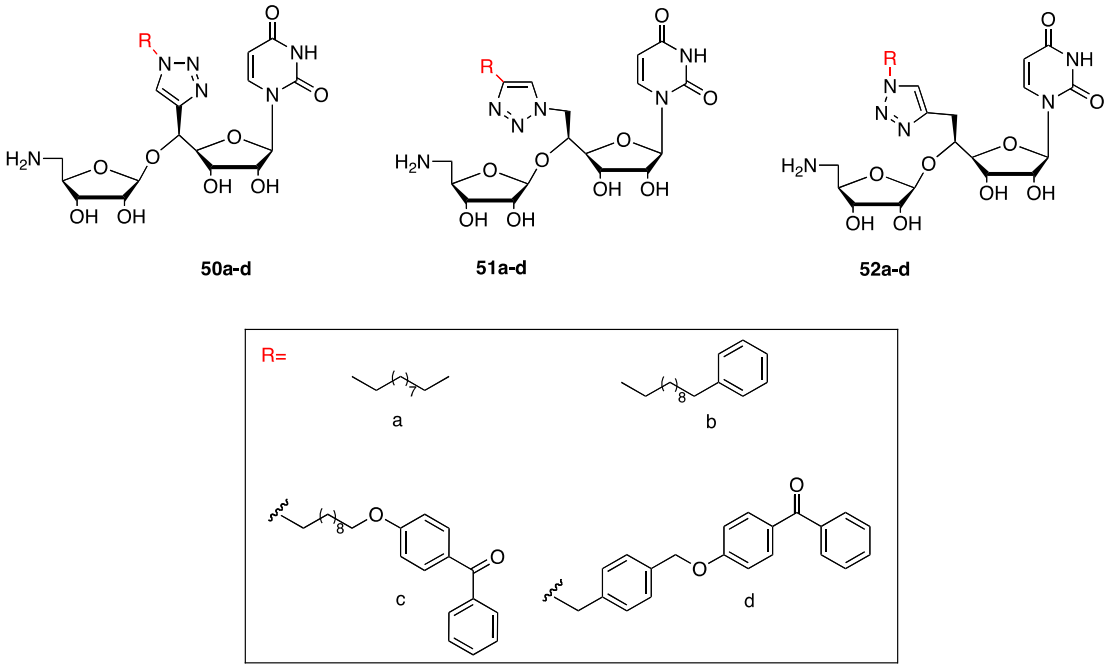
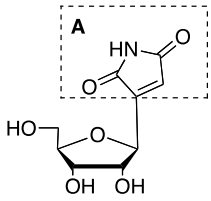


Figure 27. Structures of triazole derivatized aminoribosyl-O-uridine antibiotics **50a-d**, **51a-d** and **52a-d.**

Another particularly important target in peptidoglycan synthesis is the enzyme MurA and this has also been targeted by nucleoside analogues. Showdomycin (**51**, Figure 28) isolated from *S. showdoensis*, is a potent *C*-glycosyl nucleoside antibiotic, which is active against both Gram-positive and Gram-negative bacteria and is especially active against *S. hemolyticus and S. pyogenes*.[127] It displays a high structural similarity to uridine and pseudo-uridine, with the exception that the bases are replaced by an electrophilic maleimide moiety (**A**).[128] It was therefore speculated that nature designed this nucleoside analogue may have evolved as a suicide inhibitor that interferes with the uridine metabolism. Recent studies indicate that the antibiotic effect of showdomycin **51** against *S. aureus* may be due to the inhibition of various essential enzymes, such as MurA, which is required for cell wall biosynthesis.[129]



Showdomycin

51

Figure 28. Structure of Showdomycin (**51**).

Chitin synthase inhibitors

The first chitin synthase (CS) inhibitors were isolated in 1960s from *S. Cacaoi var asoensis* as a result of a screening program for antibiotics active against phytopathogenic fungi.[130] Structurally, they are uridine-based nucleoside-peptide antibiotic constituted by a pyrimidine base (**A**), and a ribose sugar (**B**) to which an oligopeptide moiety (**C**) is attached.[131] These compounds were called polyoxin B, D, L and C (**52a-c**) and were found to be especially useful as agriculture fungicides. (Figure 29) Later on, in 1976, from the fermentation broth of *S. tendae* a new class of antibiotic structurally related to polyoxin were isolated and named Nikkomycin X (**53a**) and Nikkomycin Z (**53b**) (Figure 29).[132, 133]

As CS inhibitors these compounds exhibited a wide spectrum of activities against fungi, whilst they are harmless to plants and human since they do not have the target enzyme. The ability of nikkomycins and polyoxins to block the CS is due to their structural similarity to the natural substrate of this enzyme, the uridine-diphosphate-*N*-acetylglucosamine (UDP-GlcNAc).[134] In particular, polyoxin B (**52a**) was reported to inhibit CS with an IC_{50} value of 0.8 μg/mL, whereas **53a** and **53b** inhibits CS with IC_{50} values of 0.1 and 0.3 μg/mL respectively. In whole cell assay against two wild type strains of *C. albicans* MIC values for **52b** were ranging from 40-20 mg/mL. In the same assay MIC values for **53a** and **53b** were in the range of 5-2.5μg/mL and 10-20 μg/mL respectively.[135]

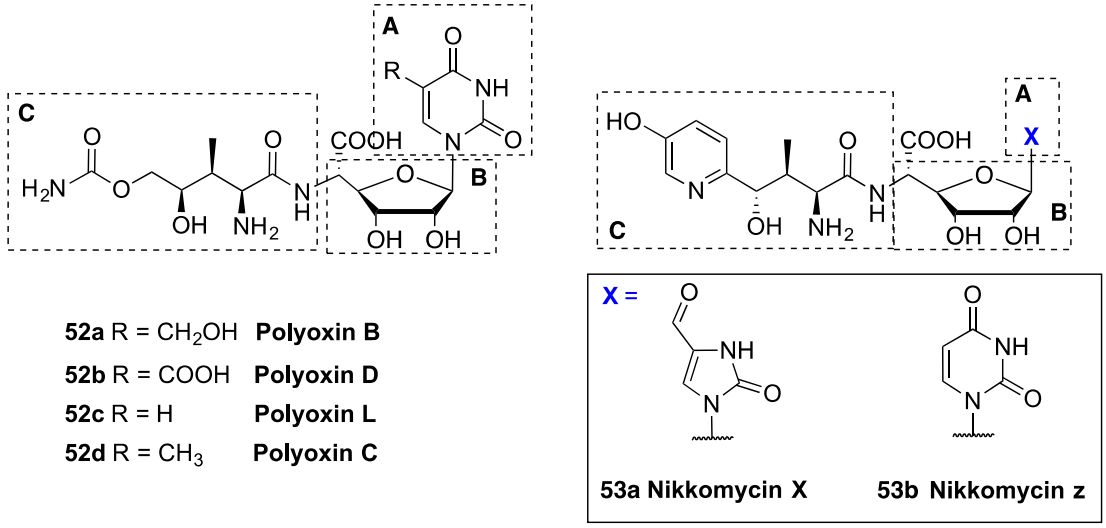


Figure 29. Example of structures of polyoxins **52a-c** and nikkomycins **53a-b**.

A common characteristic of antifungals targeting cell wall formation is the fact that although these molecules often show excellent *in vitro* enzyme inhibition their success is not reflected in *in vivo* applications. This *in vivo* failure is thought to be due to poor intracellular uptake, different susceptibility of fungal species, and variable responses in animal models.[136]

These facts prompted diverse structure-activity-relationship studies in order to identify more bioavailable antifungal agents. Polyoxins and nikkomycins enter the fungal cell via a dipeptide transport pathway. [137] Considerable research has been conducted to replace the amino terminal peptide moiety of polyoxins and nikkomycins producing analogues with better ability to penetrate the cell wall.[133] To prepare these derivatives (Figure 30), compound **54** (uracil polyoxin C) the common precursor for both nikkomycins and polyoxins was used. It can be obtained either by degradation of polyoxins[138] or via multistep synthesis, although this results in low yields.[139]

To implement the uptake of peptidyl nucleoside Naider and colleagues prepared and evaluated several polyoxin and nikkomycin analogues for their antifungal activity and

CS inhibition, in which the terminal amino acid was replaced with a di- or tripeptide residue.[140] The dipeptidyl compounds, were designed to behave as double-targeted drugs: being themselves active and releasing a toxic amino acid e.g. oxalysine and *m*-fluorophenylalanine. Unfortunately the dipetidyl compounds proved to be inactive against *C. albicans* due to either resistance to the pathogen peptidases or to the poor recognition by the peptide transport system. Among all the tripeptide derivatives, designed to behave as prodrugs, only **55a** transported into the pathogen and hydrolysed to the active compound **53a**. However, neither **55a** (MIC 83-166 μg/mL) nor any of the other compounds in the series were more effective than nikkomycin X when tested *in vitro* against *C. albicans*. The low effectiveness of **55a** was suggested to be due to its low affinity for the dipeptide transport system, indicating that the structural specificity for such transporter is quite complex.

In search for more potent CS inhibitors the Obi group synthesised a series of simplified nikkomycin derivatives having a variety of substituents at the terminal amino acid moiety.[141, 142] Among twenty derivatives the authors were able to show that the analogue **55b** lacking of the β–methyl and γ–hydroxyl groups and bearing a phenanthrene moiety (Figure 30), was a slightly more potent CS inhibitor (IC_{50} = 0.31 μg/mL) than **53b** (IC_{50} = 0.39 μg/mL). It is interesting to note that two chiral centres in **53b** can be removed without losing any potency in the inhibition of the enzyme, and with beneficial simplification of the synthetic method.[142]

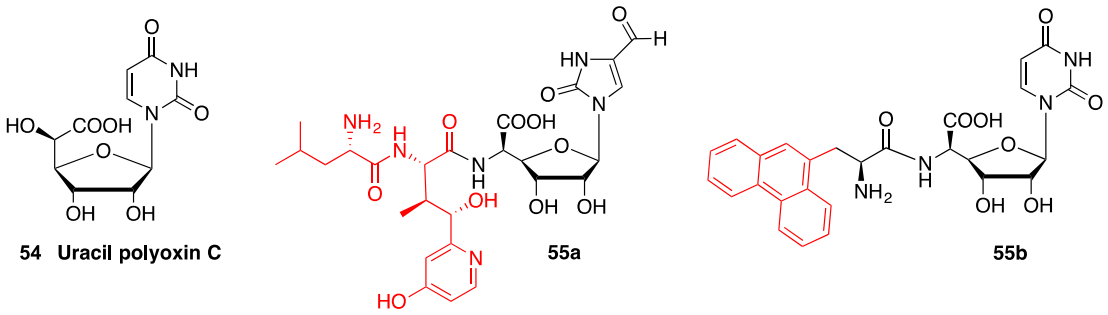


Figure 30. Polyoxin C (**54**), tripeptide nikkomycin X analogue (**55a**) and nikkomycin Z analogue (**55b**).

In 2003, novel heteroaryl nucleosides derivatives (as model CS inhibitors) appeared in the literature.[143] The intent of the authors was to modify the UDP-GlcNAc by replacing the glucopyranosyl moiety (**A**) with a heteroaromatic ring (in red) and the pyrophosphate moiety (**B**) with either malonic or tartaric acid scaffolds as well as a carbohydrate linkage (in blue). Compounds with these three structural motifs (**56a-f**) were designed to mimic the six membered ring pyrophosphate-Mg^{2+} complex (in purple) that is formed in the active site of the enzyme (Figure 31).[144]

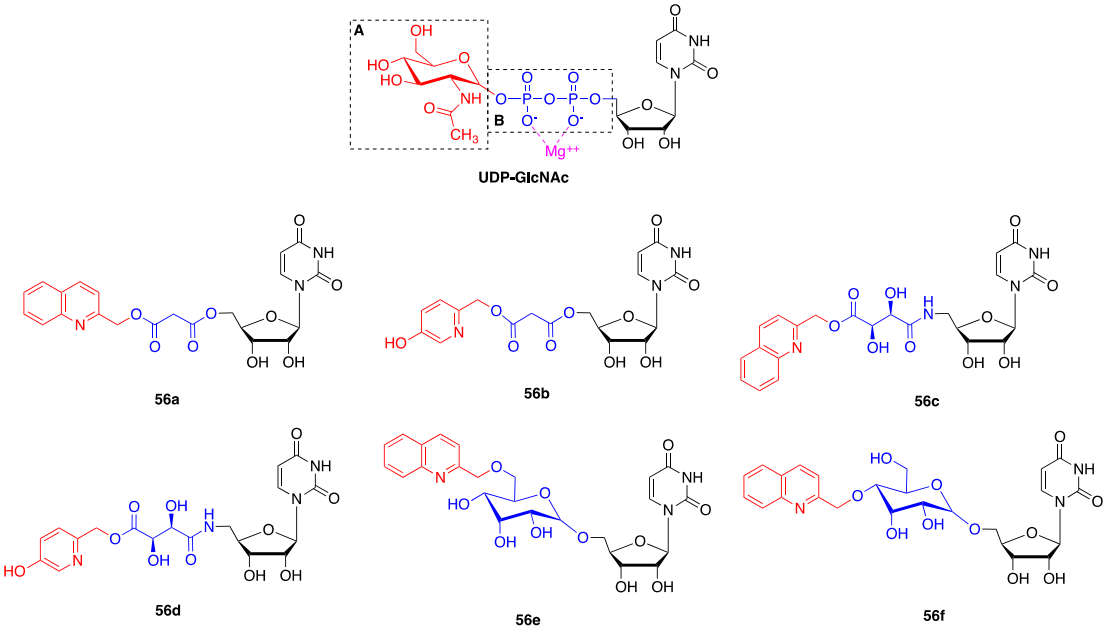


Figure 31. Hetaryl-nucleoside derivatives as CS inhibitors **56a-f**.

All compounds displayed weak CS inhibition with IC_{50} values ranging from 0.8 to 11 mM. Among these structures only compound **56e** showed 80 % growth inhibition of *C. neoformans* and 30 % inhibition of *C. albicans, S. cerevisiae* and *A. fumigatus* at a concentration of 256 µg/mL.

The poor activity of these compounds can be explained by the fact that the mode of action of CS was not completely understood at the time the experiments were conducted. Crucially, it was not known that CS could bind two oppositely orientated GlcNAc units. This CS property may explain why attempts to inhibit the enzyme's catalytic activity with a single UDPGlcNAc analogue, are mostly inefficient. In order to perform this kind of polymerization, it has been postulated that the enzyme should have at least two active sites in the right spatial conformation and at the right distance. However, this theory has been opposed by some authors, based on experimental evidence gathered from proteins sequence analysis, and crystal structure of single-sugar glycosyl transferases.[145] In 2003, an excellent paper reviewing the CS as an antifungal target was also published.[146]

In 2004 the Finney group presented the first chemical evaluation of a two-active site mechanism for CS and provided evidence that supports this mechanism. Dimeric nucleoside analogues **57a-d** with different ethylene glycol spacer lengths (**A**),[147] were synthetized along with the monomeric control **58a** (Figure 32). The basis of this design was that if two active sites are close together, a dimeric substrate with the appropriate spacer length should be able to simultaneously bind and inhibit both sites, hence achieving a more potent antifungal activity. Among all derivatives, those with shorter spacer **57a** (n = 1) and **57b** (n = 2) exhibited the highest inhibition of CS at 1 mM concentration (32 and 45 % respectively). On the other hand, the monomeric control **58a** and compounds with longer spacers **57d-e** showed poor activity at the

same concentrations. At 2 mM concentration **58b** did not lead to a more potent inhibition of CS, which demonstrates that the greater inhibition of the dimers **57a** and **57b** is not simply due to a "double concentration" of the uridine moiety.

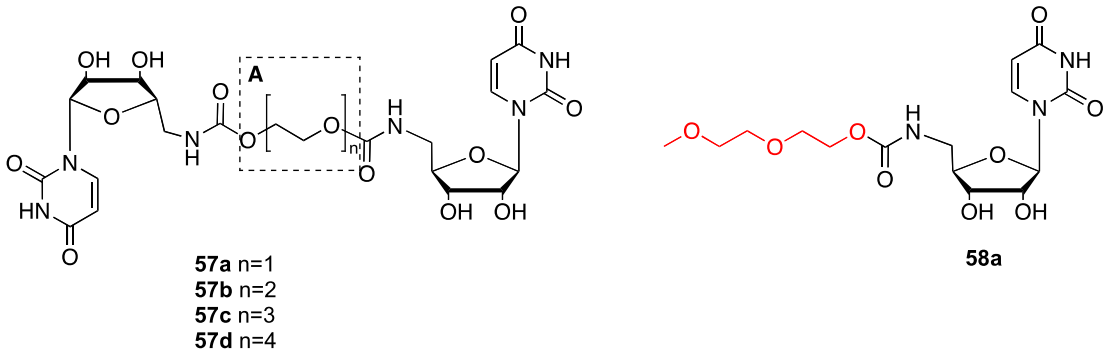


Figure 32. Dimeric nucleosides **57a-d** and monomeric nucleoside **58a** as probes for the CS mechanism.

Dose response analyses showed an IC_{50} value of 2.2 mM for **57a**, 1.1 mM for **57b** and 11.8 mM for **58a**. The authors also proved that the inhibition by the dimer **57a** (n = 1) was competitive. The results combined were supportive for the two-active site model for the CS. Although the CS inhibition by **57b** was an order of magnitude more potent than the corresponding monomer **58a**, **57b** was still a much weaker CS inhibitor than polyoxins and nikkomycins. Thus, with the aim to identify more potent CS inhibitors, a second generation of dimeric compounds was investigated by the same group.[148] These new analogues **59a-c** and their corresponding monomeric compounds **60a** and **60b** displayed as spacer tartaric acid amides (**A**), which could potentially mimic the pyrophosphate group (Figure 33). Monomers **60a** and **60b** showed greater inhibition (12 % and 20 % respectively at 1 mM concentration) when compared with their analogue having the ethylene glycol as spacer **58a** (6 % at 1 mM concentration). In the dimeric series **59a-c**, as in the ethylene glycol series, the increase in the hydrocarbon spacer length corresponded to a diminished inhibitory activity of the molecules with compounds **59a** (n = 1) and **59b** (n = 2) showing 5 and 10 %

inhibition respectively at 1mM. Compounds **59b** (n = 3) and **59c** (n = 4) were totally inactive at the same concentration. In the same studies along with these compounds a short dimer **61** and its monomeric control **62** were also prepared and evaluated as CS inhibitors.[148] The most active compound was compound **61** with 35 % enzyme inhibition at 1mM. Monomer control **62** had reduced activity when compared with compound **60b**, suggesting that the methyl ether is detrimental for the inhibitory activity (Figure 33).

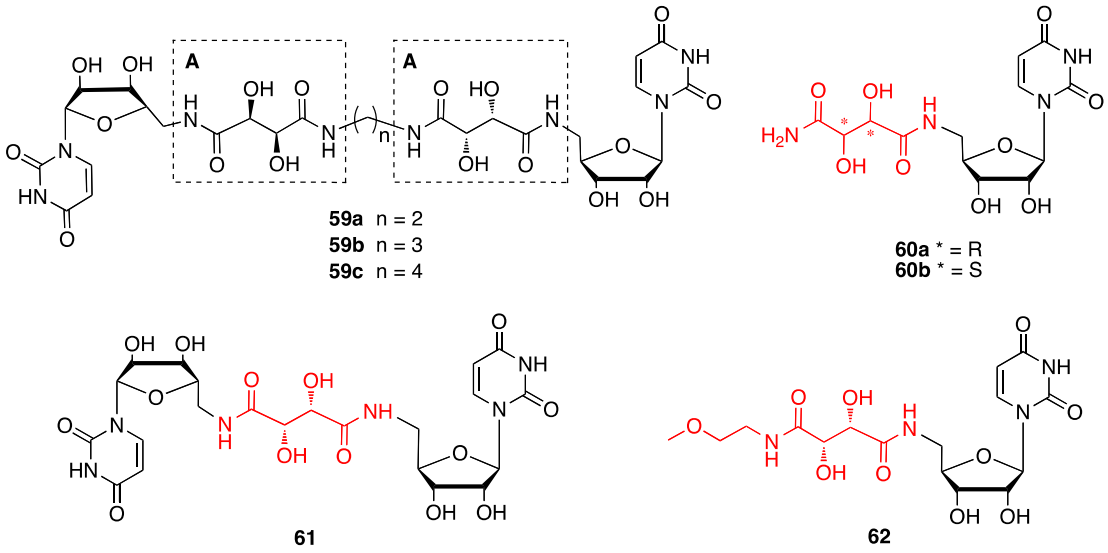


Figure 33. Tartaric amide derivatives **59a-c, 60a,b, 61, 62.**

These results supported the "double active site" theory and suggest that the inter-uridine distance (optimal distance of 12-14 Å) is the primary factor for the inhibitory activity of these compounds. The data also appear to demonstrate that the nature of the spacer between the two-nucleoside units (as pyrophosphate mimic) does not significantly alter enzyme inhibition. [148]

As a part of a research program directed to the identification of novel peptidyl antibiotics, based on nikkomycin scaffold, Dutta and colleagues investigated the effect of the carbohydrate ring size on their biological activity.[149] It was found that nikkomycin analogue **63** with an increased ring size, such as a pyranose ring (**A**), led

to an improvement in activity against different human pathogenic fungi (Figure 34). In particular, compound **63** was found to be more potent against *Coccidioides immitis* (MIC = 0.03 μg/mL) than **53b** (MIC = 0.25 μg/mL), and was equipotent against *C. neoformans* (MIC = 0.03 μg/mL).[144]

Encouraged by this result and by Naider investigations,[140] Dutta's group decided to further transform compound **63,** introducing as consecutive key modification a di- and tri-peptide chain into its scaffold.[150] The intent was to increase the permeability through the fungal cell of this derivative (**63**), by introducing lipophilic amino acids such as leucine, methionine, phenylalanine and tyrosine with the aim of making **63a** better substrate for the fungal peptidyl transport system.

The antifungal activity of the double modified compounds **64a-d** (Figure 34) was evaluated against several fungal species.[149] In general the activity profile of these novel analogues was found to be very similar to that of compound **63**. None of the newly designed compounds were active against *Candida* and *Aspergillus* strains, whereas all the compounds were more potent (MIC = 0.03 μg/mL) than Nikkomycin Z (MIC = 0.25 μg/mL) when tested against *Cryptococcus* and *Coccidiosis* fungal strains.

Cryptococcus and *Coccidioides* are responsible for serious human infections of lungs, brain and bones, so the results obtained with compounds **64a-d** suggested significant therapeutic potential. The findings were particularly impressive if compared to the current standard treatment amphotericin B whose MIC was 0.25 μg/mL. Unfortunately, *in vivo* results for these derivatives have not yet been reported.

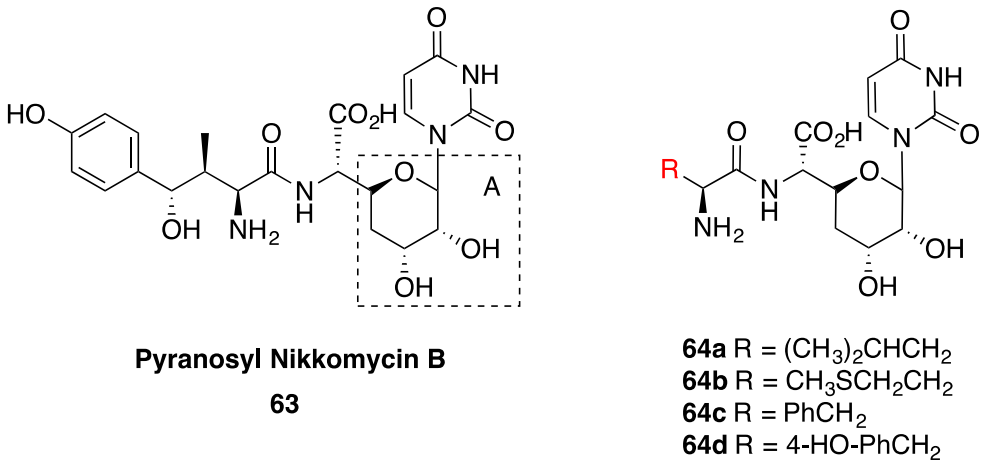


Figure 34. Structures of peptidyl-pyranosyl nucleoside analogues **63** and **64a-d**.

With the aim of increasing the amount of nikkomycins obtained from biological sources (*S. ansochromogens*), Tan and co-workers[151] used recombinant technology to manipulate the *S. ansochromogens* nikkomycins biosynthetic gene cluster by introducing a modified plasmid (pNIK) into the bacteria. This resulted in a 4-fold (880 mg/L against 220 mg/L in non modified strain) increased production of **53a** and 1.8-fold (210 vs. 120 mg/L) increased synthesis of **53b**. The same authors then generated two hybrid antibiotics, via a combinatorial biosynthetic approach. The gene cluster responsible for the synthesis of the dipeptide moiety of polyoxins was inserted into a mutant strain of *S. ansochromogens* (ΔsaN) that responsible for the formation of the nucleoside moiety of **53b**.[152] From the culture broth of this recombinant strain, two new hybrid antibiotics named polynik N (**65**) and polyoxins A (**66**) were isolated (Figure 35). These novel compounds contained the nucleoside core (**A**) from **53a** functionalized with the dipeptide chain (**B**) from **65** and with its 3''-dehydro analogue (**C**) for compound **66** (Figure 35).

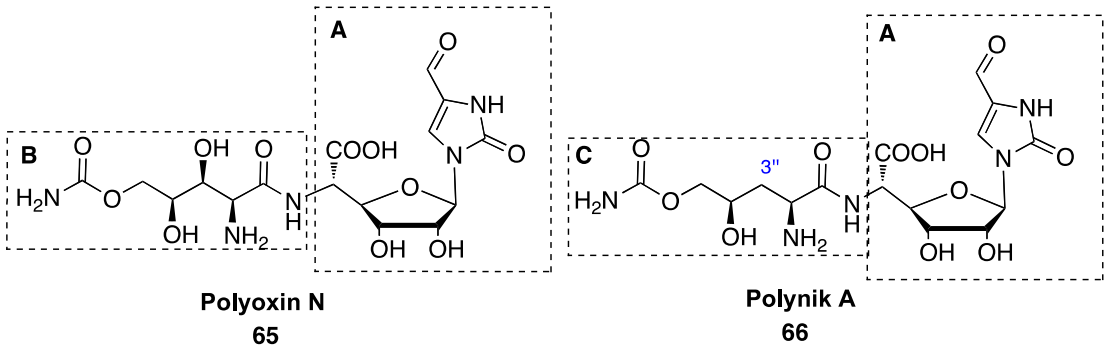


Figure 35. Stucture sof polynik A (**65**) and polyoxin N (**66**).

65 was a naturally existing nucleoside that had been previously isolated and identified, however, **66** was a novel compound, whose structure was unreported. With the nucleoside moiety from **53a**, the antifungal activities of these two hybrids (MIC = 2-10 µg/ml) against various microorganisms were generally higher than those of **52a** (MIC = 10-50 µg/ml). Their activity was lower than those of **53a** (MIC = 0.5-10 µg/ml), though when combined with the peptidyl moiety from polyoxin, **65** and **66** demonstrated greater stability than **53a**.[152] Compared to **52a**, which had weak inhibitory activity against the human pathogen *C. albicans* (MIC = 50 µg/ml), both **65** and **66** showed strong inhibitory activity (MIC= 6 µg/ml for both compounds), similar to that of **53a** (MIC = 2 µg/ml).[152]

These results clearly demonstrated the potential of generating new peptidyl nucleosides antibiotics with improved properties by simple genetic manipulation of an appropriate bacterial strain.

The same group expanded the diversity of polyoxins structures by a combinatorial biosynthetic strategy: transferring the gene responsible for the synthesis of polyoxins in *S. cacaoi,* via pPOL plasmid, into the chromosome of the ΔsanN-mutated strain of *S. ansochromogens.* From the mutated bacteria culture media, two novel antibiotics

named polyoxin P (**67**) and polyoxin O (**68)** (Figure 36) were obtained along with several other known polyoxins.[153]

Characterization of the structure of **67** and **68** by high-resolution mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy indicated that the **67** is characterized by a thymidine portion (**A**) and a dehydroxyl-carbamoylpolyoxamic acid as peptidyl moiety (**B**). Compound **68** instead, contains 5-aminohexuronic acid with N-glycosically bound thymine (**C**) and polyoximic acid (**D**) as the nucleoside and the peptidyl moieties respectively.

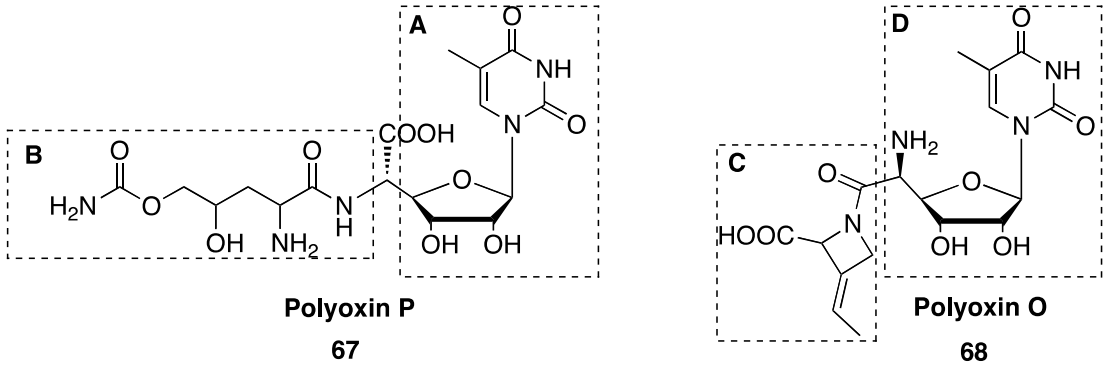


Figure 36. Structure of novel Polyoxin P (**67)** and Polyoxin O (**68**).

Of the two newly produced compounds, **67** was endowed with the best antifungal activity while **68** showed a very weak activity thus indicating that components containing different peptidyl moieties posses different biological activity.

Following the same approach, mutations of industrial *S. aureochromogenes* YB172 (a polyoxins producer) were accomplished by introducing a modified nikkomycin gene cluster from *S. tendae*, resulting in the production of four polyoxin–nikkomycins hybrids: the already known polyoxin **66**, (Figure 35) and the novel nikkoxin B–D (**69-71**).[154] The structures of these hybrid antibiotics, elucidated by high-resolution MS and NMR analysis, are reported in Figure 37. Compounds **66** and **71** were significantly more potent against some human or plant fungal pathogens compared to their parent structures (Table 1).

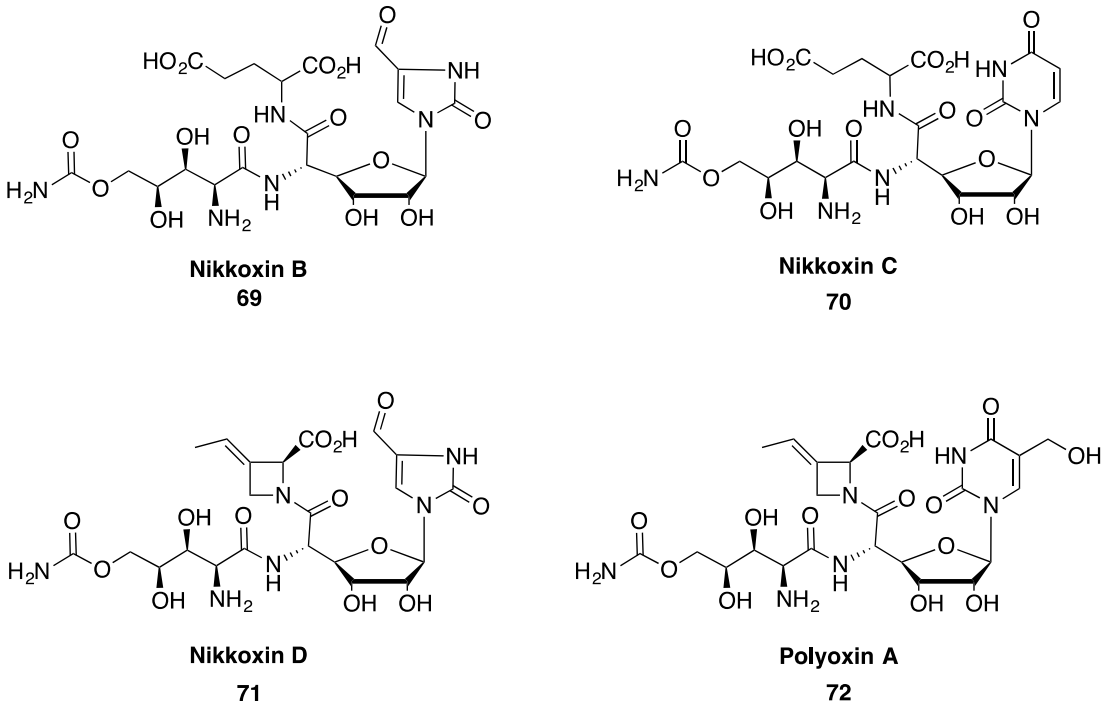


Figure 37. Structures of polyoxin–nikkomycins hybrids **69-72**.

	Natural antibiotic			Hybrid antibiotic*				
Fungal	Nikk Z	Nikk X	PolA	PolB	PolN	Nikox B	Nikox C	Nikox D
Strain	**53a**	**53b**	**72**	**52a**	**66**	**69**	**70**	**71**
C. albicans	16	8	> 256	256	16	> 256	> 256	> 256
T. cutaneum	64	64	64	> 256	256	2	32	0.5

Table 1 MIC (µg/mL) for natural (**3a-b, 72** and hybrid Pol-Nikk antibiotics **66, 69-71;** * Pol: polyoxins; Nikk: nikkomycins; Nikox: nikkoxin hybrids**.**

Tan's group recently synthesised two additional novel nikkomycins structures by feeding mutated *S. ansochromogens* ΔsanL with nicotinic acid.[155] The structures of these two novel nikkomycin analogues named as nikkomycin Pz (**72**) and nikkomycin Px (**73**) were identified by high-resolution MS and NMR (Figure 38). In particular, compound **72** was found to have a nucleoside moiety (**A**) identical to that of **53a**, but with a 4-(3'-pyridinyl)-homothreonine as peptidyl moiety (**B**). For **73**, the same

analyses confirmed the presence of an identical peptidyl moiety (**B**) as in nikkomycin Pz (**72**) but with the 4-formyl-4-imidazoline-2-one as nucleobase (**C**).

Against *C. albicans* and *Achnanthes longipes,* compounds **72** and **73** showed antifungal activities comparable to nikkomycins **53a** and **53b**. These results indicated that the incorporation of the nicotinic acid has no effect on the biological activity of these drugs. However, **72** and **73** were found to have an increased stability under different temperature ranges as well as at different pHs, reinforcing the idea that the peptidyl moiety is able to influence the stability of these classes of compounds.

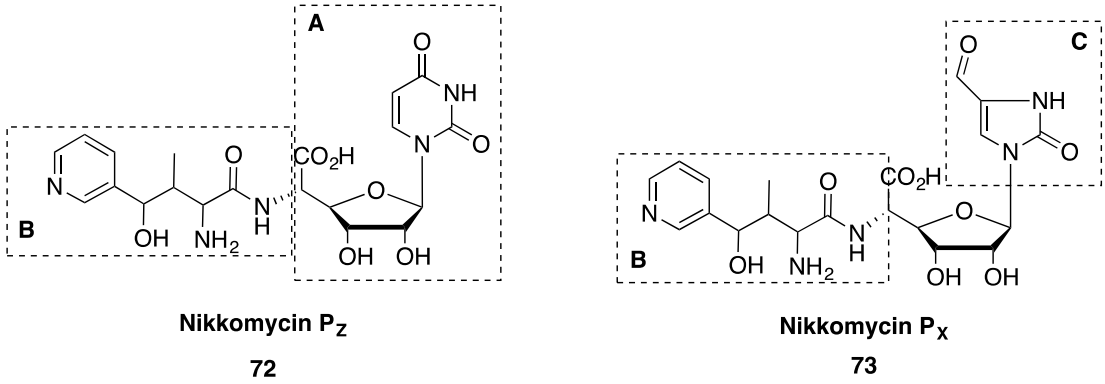


Figure 38. Structures of Nikkomycin Px (**72**) and Nikkomycin Pz (**73**).

The search for novel antibiotics led to the replacement of the peptide bond of nikkomycin with a triazole moiety.[156] The 1,2,3-triazole unit acts as surrogate of the peptide bond, both in terms of atom placement and electronic properties.

Two sets of novel 1,2,3-triazolyl linked uridine derivatives were prepared with either an aryl ester or ether as substituent on the 1,2,3-triazole ring. Several of the synthesised structures were found to be active against *C. albicans* and C. *neoformans* (MICs = 8-64 μg/mL). Compound **74a** (Figure 39) was the most potent against *C. neoformans* with a MIC value of 8 μg/mL, whereas compound **74b** and **74c** were the most potent compounds against *C. albicans* (MIC = 24 μg/mL) and *F. oxysporum* (MIC = 32 μg/mL) respectively. A very interesting review of the synthesis and the

subsequent biological evaluation of both polyoxins and nikkomycins has been also published.[157]

Wandzik and co-workers designed, and evaluated as antifungal agents, a series of 5'-uridine nucleoside analogues **75a-c**.These analogues contained a keto-enolic motif[158] able to mimic the phosphate unit, that is involved in the essential Mg^{2+} coordination in the active site of CS and other glycosyl transferases (Figure 39). Unfortunately, none of these compounds showed activity against *C. albicans* or *A. fumigatus* up to 1,000 µg/mL concentration.

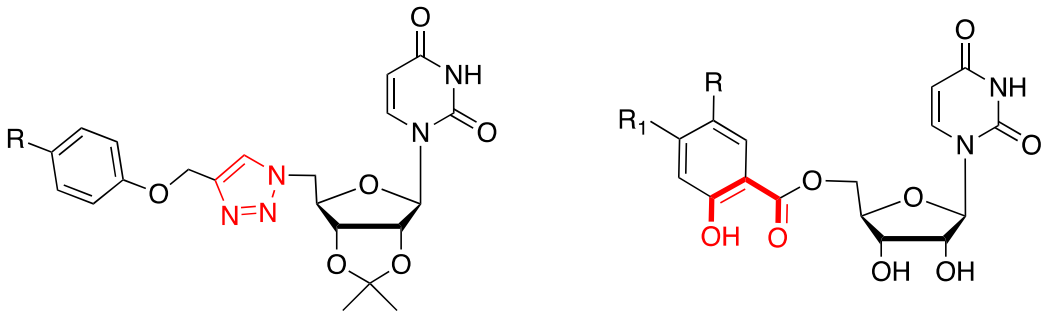


Figure 39. Structures of triazole derivatives **74a-c**, and 5'-uridine nucleoside analogues **75a-c**.

Some other natural peptydil nucleosides, such as ezomycin[159-161] ampipurimycin[162-166] and miharamycins,[167-170] also exhibit potent antifungal activities against a broad range of fungi species. The exact modes of action of these compounds have yet to be defined however, the synthetic "medicinal" chemical studies make these nucleosides potentially attractive new antifungal agents, worthy of further investigation. In this regard, the total synthesis of complex nucleosides antibiotics has been reviewed by Knapp.[171]

Nucleoside antibiotic that interfere with nucleotide metabolism.

Nucleotide biosynthesis is crucial for all living organisms and therefore also for the growth of bacteria. The metabolic requirements for the nucleotides in bacteria can be met either by a salvage pathway from low molecular weight precursors or by *de novo* synthesis.

The salvage pathway of both purines and pyrimidines represents an energy-efficient way to produce the building blocks of DNA and RNA, but depends on the availability of nutrients. During bacterial proliferation in the blood stream, it was shown that *de novo* biosynthesis of pyrimidine and purine nucleosides is crucial for the growth of several virulent *E. coli* strains and for *S. enterica* and *B. antracis.* These findings were ascribed to the limited availability of the nucleosides in the blood.[172] Enzymes involved either in the *de novo* or salvage pathways are critical for the survival and proliferation of pathogenic bacteria and are therefore potential targets for the treatment of these infections.

Phosphoribosyltransferase inhibitors

Phosphoribosyl transferases (PRTs) are enzymes involved in the purine salvage pathway and are essential for the reproduction and survival of different pathogens. Within this family, hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and xanthine-guanine phosphoribosyltransferase (XGPRT) were identified as interesting targets for the development of new anti-bacterial agents.[173, 174]

HGPRT catalyses the Mg^{2+}-dependent reversible transfers of the 5-phosphorybosyl group from 5-phosphoribose-1-pyrophosphate (PRPP) to the N^9 position of 6-oxopurines (H and G), to form inosine monophosphate (IMP) and guanosine monophosphate (GMP) respectively, with concomitant release of inorganic

pyrophosphate (PPi) (Figure 40). XGPRT catalyses the same transfer to xanthine, guanine and (to a lesser extent) hypoxanthine to form XMP (xanthosine monophosphate), GMP, and IMP, respectively.

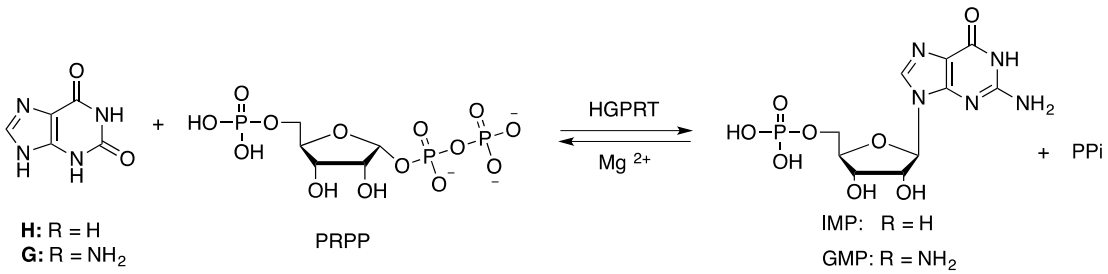
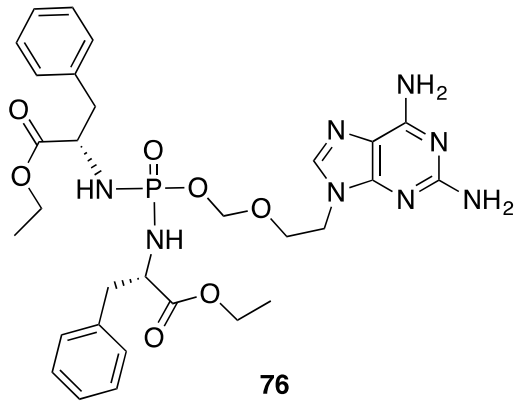


Figure 40. Enzymatic reaction catalyzed by hypoxanthine-guanine phosphorybosyl transferase. H = hypoxanthine, G = guanine, PRPP = 5-phosphoribose-1-pyrophosphate, HGPRT = hypoxanthine-guanine phosphoribosyltransferase, IMP = inosine monophosphate, GMP = guanosine monophosphate, PPi = inorganic pyrophosphate.

Mammalian cells have two different pathways for the synthesis of the essential 6-oxopurine nucleosides monophosphates: the *de novo* (from small molecules) and salvage (of purine bases) pathways. However, clinically important bacteria such as e.g. *M. tuberculosis* and *H. pylori* do not possess the necessary enzymes for the *de novo* synthesis and rely exclusively on the transport of preformed purine bases from the host cell.[175, 176] These differences in metabolism between infectious agents and their host cells can be exploited in the design of drugs aimed at fighting these pathogens. As these organisms have only one pathway for the synthesis of the purine nucleoside mono- phosphates needed for DNA/RNA production, they depend on the activity of their 6-oxopurine PRTases for both reproduction and survival. Acyclic nucleoside phosphonates containing a second phosphonate group have been identified as potent inhibitors of 6-oxopurine phosphoribosyl transferases.[177] Keough *et al.* reported a number of novel acyclic nucleoside phosphonates inhibitors of XGPRT and HGPRT from *E. coli* with K_i values as low as 10 nM.[178] These acyclic nucleoside

phosphonates were also inhibitors of mycobacterial HGPRT, with K_i values as low as 0.69 μM.[179] Prodrugs of these compounds were shown to arrest the growth of a virulent strain of *M. tuberculosis* in cell-based assays with MIC values as low as 4.5 μM, without significant cytoxicity in mammalian cells (CC_{50} > 300 μM). The structure of the most potent compound in the series (**76**) (IC_{50} = 5 μM against *M. tuberculosis*) is depicted in Figure 41.



76

Figure 41. Structure of HGPRT inhibitor **76**.

Guanosine monophosphate synthase inhibitors

GMP synthase is an enzyme that converts XMP to GMP in the presence of adenosine triphosphate (ATP), glutamine and Mg^{2+} (Figure 42). GMP synthase has significantly diverged across prokaryotes and eukaryotes supporting its consideration as an antifungal target.[180]

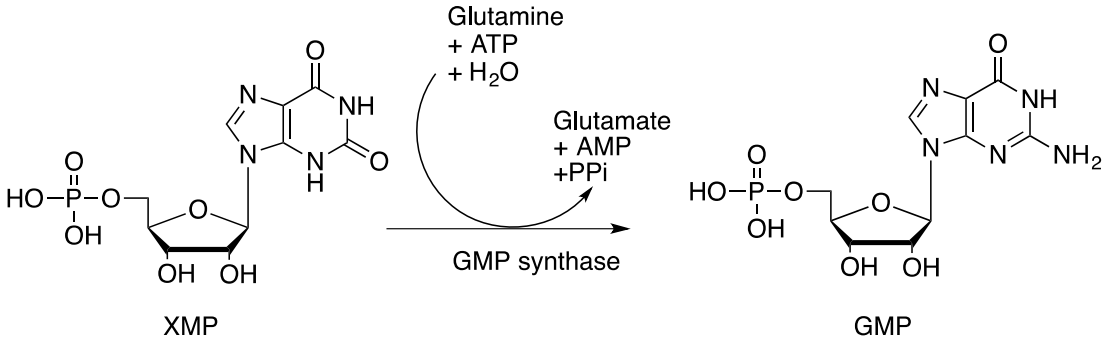


Figure 42. Enzymatic reaction catalysed by guanosine monophosphate synthase. XMP = xanthosine monophosphate; ATP = adenosine triphosphate; AMP = adenosine monophosphate; GMP = guanosine monophosphate; PPi = inorganic pyrophosphate.

Oxanosine (**77**) (Figure 43) is a guanosine analogue, isolated from the culture filtrate of a strain of *Streptomyces*.[181] Compound **77** showed weak growth inhibition of *E. coli* K-12 (MIC 12.5 μg/ml). This bacteriostatic activity was due to the inhibition of GMP synthase, and was antagonised by guanine, guanosine, and guanylic acid.[182]

Psicofuranine (**78**) (Figure 43) is an adenosine analogue, first described in 1959 when it was isolated from culture media of *S. hygroscopicus var. decoyinine*.[183] Since its discovery, the structural similarity to the endogenous nucleoside has been linked to an antimetabolite mode of action. Compound **78** has been shown to inhibit the amination of XMP to GMP catalysed by GMP synthetase in *E coli* and guanine, guanosine and GMP could reverse growth inhibition in psicofuranine treated *S. aureus*.[184]

Mizoribine (**79a**, Figure 43) is a nucleoside analogue bearing an imidazole nucleobase, isolated from the culture medium of the mould *E. brefeldianum* M-2166.[185] Bredinin (**79b**), the monophosphate form of **79a** (Figure 43), exerts antibacterial activity through selective inhibition of GMP (K_i= 100 μM) and IMP synthetases (K_i = 0.1 μM), resulting in the complete inhibition of guanine nucleotide synthesis and arrest of DNA synthesis in the S phase of cell division. Although this compound has been found to have weak antimicrobial activity against *C. albicans*, it has proven

ineffective against experimental candidiasis, probably due to lack of membrane penetration. This compound is mostly used as an immunosuppressive agent due to the inhibition of the lymphocyte proliferation.[186]

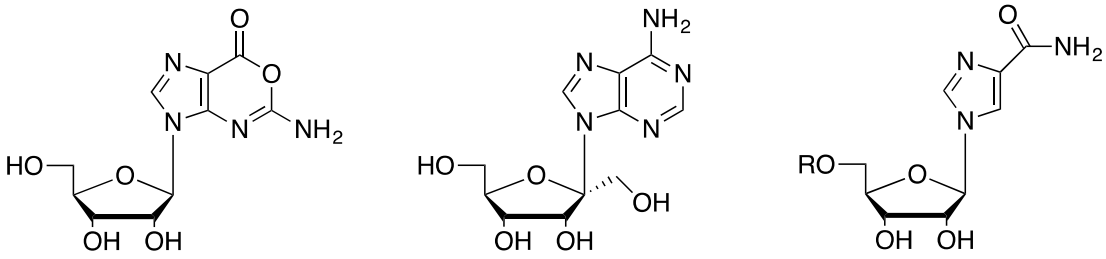


Figure 43. Structures of GMP synthase inhibitors **77-78** and **79a,b**.

Inosine-5-monophosphate dehydrogenase inhibitors

Inosine-5′-monophosphate dehydrogenase (IMPDH) is a purine biosynthetic enzyme that catalyzes the nicotinamide adenine dinucleotide (NAD^+)-dependent oxidation of IMP to XMP, the first and rate-determining step in the *de novo* biosynthesis of guanine nucleotides from IMP (Figure 44). Bacterial IMPDH enzymes show biochemical and kinetic characteristics that are different from the mammalian enzymes, suggesting IMPDH may be an attractive target for the development of antimicrobial agents. [187]

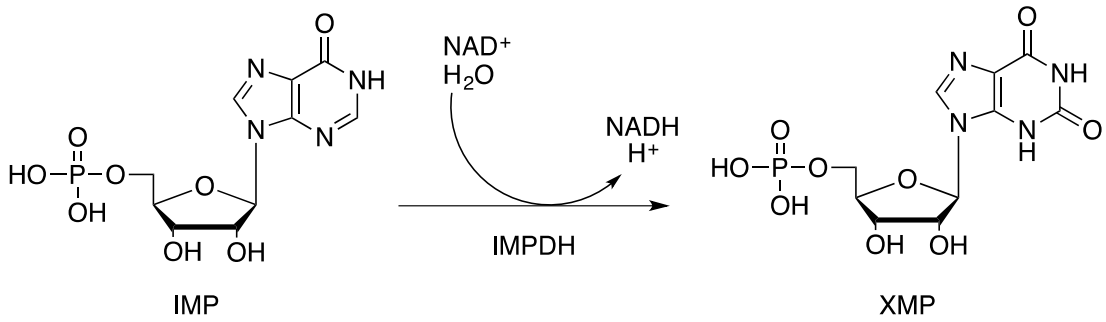
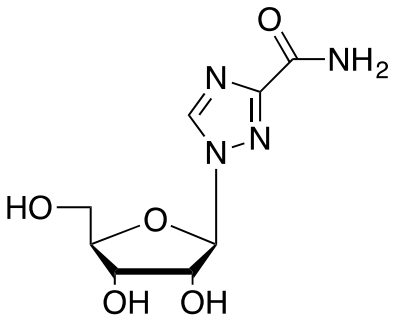


Figure 44. Enzymatic reaction catalyzed by inosine monophosphate dehydrogenase. IMP = inosine monophosphate; XMP = xanthosine monophosphate; IMPDH = inosine monophosphate dehydrogenase; NAD = nicotinamide adenine dinucleotide.

IMPDH is a regulator of the intracellular guanine nucleotide pool, and is therefore important for DNA and RNA synthesis, signal transduction, energy transfer and glycoprotein synthesis, as well as other processes involved in cellular proliferation.

Ribavirin (**80**, Figure 45) discovered in 1972 by Witkowski and coworkers,[188] is a guanosine analogue mainly used as an antiviral agent. However, **80** has also demonstrated antibacterial activity against *P. areuginosa* (MIC = 3 μg/mL). Compound **80** exerts its antibacterial activity as 5'-monophosphate by inhibiting the cellular inosine monophosphate dehydrogenase, thereby depleting intracellular pools of GTP.[189]



Ribavirin
80

Figure 45. Structure of ribavirin (**80**).

Pertussis adenylate cyclase toxin (ACT)

Pertussis adenylate cyclase toxin (ACT) is a bacterial enzyme considered to be an important virulence factor for *B. pertussis* infection.[190] After penetration into the human macrophages ACT is activated by endogenous calmodulin and catalyses the conversion of ATP into cyclic AMP (cAMP). This causes disruption of host cell

functions and facilitates an effective bacterial invasion and colonization of the host (Figure 46).

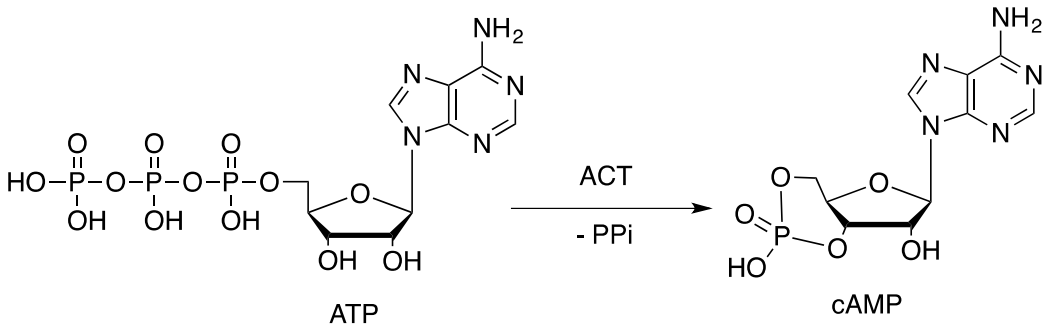
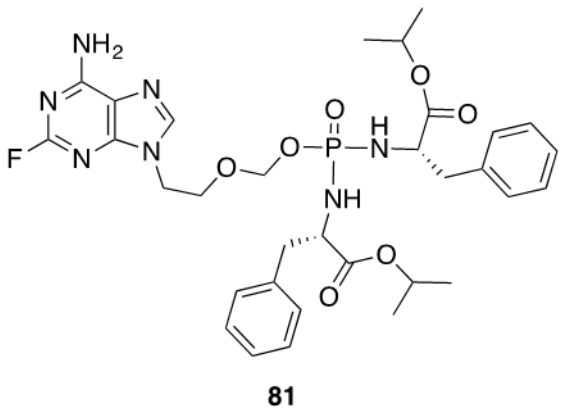


Figure 46. Enzymatic reaction catalysed by Pertussis adenylate cyclase toxin. ATP = adenosine triphosphate; ACT = adenylate cyclase toxin; cAMP = cyclic adenosine monophosphate; PPi = inorganic pyrophosphate.

Novel C^2-modified analogues of 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA, adefovir) (structure not disclosed), in the form of their bis-amidate prodrugs, were found to inhibit the activity of ACT.[191] They are capable of crossing the macrophage cell membrane and then release PMEA, which is subsequently phosphorylated to active PMEA diphosphate, which is in turn able to inhibit the activity of ACT. The most active compound of the series was found to be the 2-fluoro PMEA bis-amidate prodrug **81** (IC_{50}= 0.145 μM) (Figure 47).



81

Figure 47. Structure of 2-fluoro PMEA derivative **81**.

Nucleoside antibiotic that inhibit nucleic acid synthesis

Nucleoside analogues used in the treatment of viral infections and cancer conditions have also demonstrated efficacy in inhibiting the synthesis of bacterial nucleic acids. It has been shown that these nucleoside drugs could be efficiently converted to their active triphosphate form by bacterial deoxynucleoside kinases (dNKs). Such nucleoside analogues may therefore have great potential as antibiotics for the treatment of bacterial infections, in particular by multi-drug resistant strains.

Studies on several FDA approved nucleoside analogue drugs, screened for their potential bactericidal activity against Gram-positive and Gram-negative bacteria, indicated 3'-azido-3'-deoxythymidine (**82**, AZT; zidovudine), 5-fluoro-2'-deoxyruridine (**83**, FdUrd) and 2',2'-difluoro-2'-deoxycytidine (**84**, gemcitabine) as the most active nucleosides (Figure 48).[192, 193] In particular, **84** was found very active against Gram-positive bacteria (e.g. MIC values against *S. aureus* and *S. pyogenes* lower than 0.002 μg/mL and 0.2 μg/mL, respectively) but inactive against Gram-negative bacteria, which were instead susceptible to **82** (MIC values against *E. coli* were between 10 μM to 31.6 μM). Conversely, none of the Gram-positive bacteria was susceptible to AZT. **83** was found active towards both Gram-positive and Gram-negative bacteria; however, the lowest minimal inhibitory concentrations were in the range from 0.003 μM to 1 μM and from 1 μM to 10 μM respectively for the Gram-positive and the Gram-negative strains. These results demonstrate a wide variation in kinase substrate preferences suggesting that the different nucleoside analogues may possess species-specific activity (Figure 48).

To investigate the potential *in vivo* efficacy of **84**, this compound was evaluated in an *in vivo* mouse model of *S. pyogenes* infection. **84** was able to protect the treated

animal from bacterial spread and systemic infection, demonstrating that nucleoside can indeed be used as antibiotics.[192]

More lipophilic AZT derivatives have been developed with the aim of improving its antiviral and bactericidal activity. Among them, the 5' leucine AZT prodrug **85** (Figure 48) was reported to be the most effective antibacterial agent with a MIC value of 0.125 μg/mL against *Klebsiella pneumonaiae*.[194]

Among other nucleosides affecting the nucleic acid synthesis, Sangivamycin (**86**), (Figure 48) isolated from *S. rimosus* had poor antibacterial and antifungal activities.[195] The triphosphate form can inhibit bacterial and fungal nucleic acid synthesis and can also compete with ATP in various reactions, acting as inhibitor of protein kinases.[196]

Blue-green algae have proven to be an excellent source of bioactive compounds. Moore and co-workers isolated, from different *Scytonemataceae* strains, two nucleosides with antifungal activity: tubercidin (**87**) and toyocamycin (**88**) (Figure 48).[197] Compound **87** is responsible for the antifungal activity of these algae and for the antifungal activity of extracts from *Scytonema saleyeriense var. indica.*[198] On the other hand, most of the activity of the extract from *Plectonema radiosum* and *Tolypothrix distorta* was due to the tubercidin 5'-α-D-glucopyranose derivative **89**.[198] Compound **87** serves as a substrate for numerous enzymes involved in the anabolism of adenosine, as demonstrated by its incorporation into RNA and DNA, and by the formation of nicotinamide-deaza-adenine dinucleotide. Moreover, this analogue proved to be a weak inhibitor of adenosine phosphorylase, interfering with the phosphorylation of adenosine and AMP.[199]

Compound **88** (Figure 48) and toyocamycin 5'-α-D-glucopyranose **90**, the minor and major constituent in *Tolypothrix tenuis* extract, are responsible for the cytotoxic and

fungicidal properties of this extract. It was suggested that their mechanism of action is linked to the blocking of the ribosomal RNA synthesis.

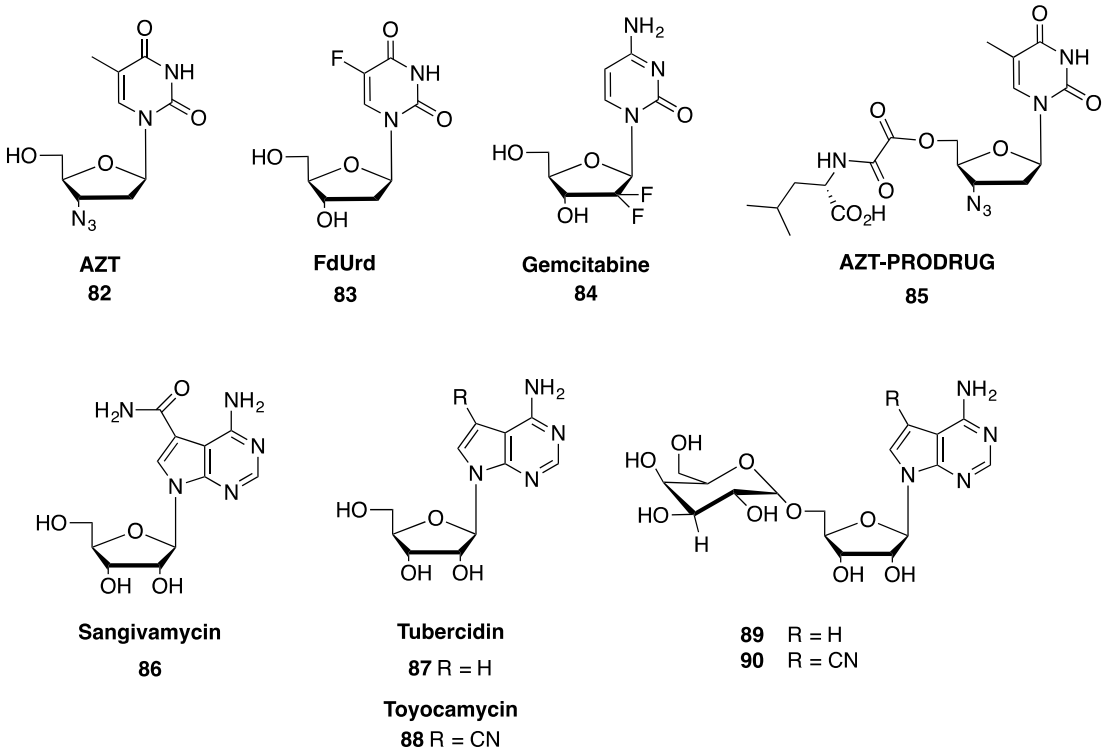


Figure 48: Structures of nucleic acid inhibitors **82**-**84**, AZT prodrug **85**, Sangivamycin (**86**), Tubercidin (**87**) and toyocamycin (**88**) and their glucose derivatives (**89-90**).

Protein synthesis inhibitors

Ribosomal peptidyl center inhibitors

Mildiomycin (**91**, Figure 49) is a nucleoside antibiotic isolated from the culture filtrate of *S. rimofaciens* B-9889 that has shown strong activity against powdery mildews on various plants, and remarkably low toxicity in mammals and fishes.[200-202] The mode of action of this antibiotic was linked to the inhibition of protein synthesis and to a lesser extent to the DNA synthesis.[202] Since it does not affect RNA production, the slight inhibition of DNA synthesis observed with this compound can be explained by the effect observed on translation. Studies on the mode of action of

this nucleoside showed that with **91** present, the binding of the natural substrates to the ribosome was unaffected, whereas inhibition of the peptide-bond formation was observed. This finding suggested that the target of Mildiomycin action is the peptidyl-transferase center located on the larger ribosomal subunit.[203]

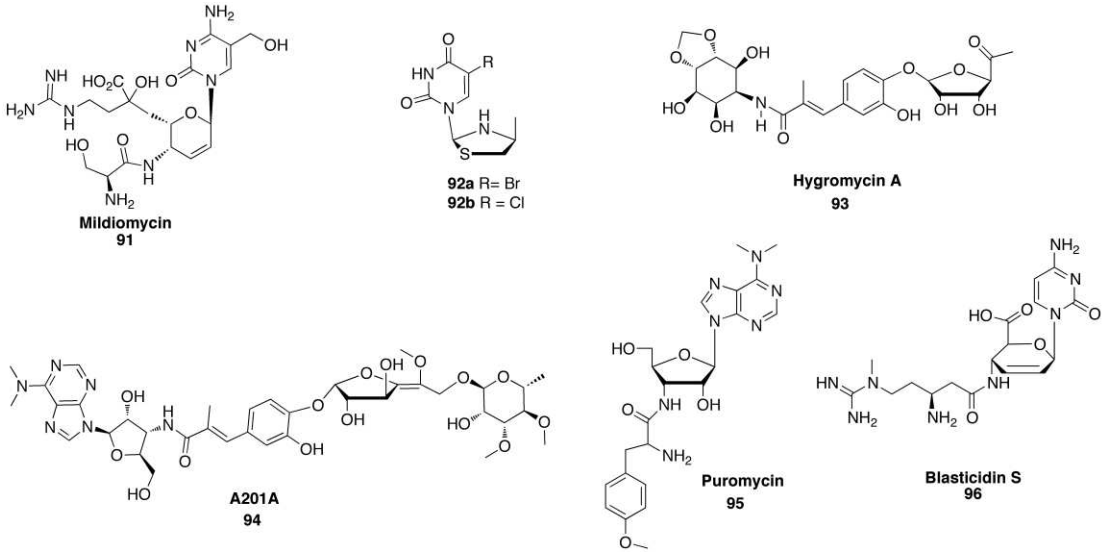


Figure 49. Structures of ribosomal peptidyl center inhibitors: Mildiomycin (**91**), 1,3-Thiazolidines **92a-b**, Hygromycin A (**93**), A201A (**94**), Puromycin (**95**), Blasticidin S (**96**).

1,3-Thiazolidines pyrimidine nucleosides are a class of antimicrobial agents reported by Sriharsha *et al.,* with activity against a broad spectrum of Gram-positive pathogens including *Staphylococci*, *Streptococci* and *Enterococci*.[204] Among these derivatives, compounds bearing bromine and chlorine at the 5 position on the uracil ring **92a,c** (Figure 49) showed increased activity against all tested bacteria, including Bacitracin-resistant bacteria. The mode of action of these compounds implies inhibition of the protein synthesis in actively growing bacteria by binding to the bacterial 50S ribosomal subunit and inhibition of formation of the 70S ribosomal initiation complex, eventually blocking bacterial protein synthesis.

Hygromycin A (**93**) and **94** (A201A) (Figure 49) are antibiotics, which have been found to inhibit bacterial protein synthesis. Compound **93**, first discovered in 1953 as a metabolite produced by *S. hygroscopicus,* has a broad activity mainly against Gram-positive bacteria.[205] **94** is an aminoacyl-nucleoside antibiotic that was first isolated from *S. capreolus* NRRL 3817.[206] It is active against Gram-positive aerobic and anaerobic bacteria and most Gram-negative anaerobic species.

Both antibiotics inhibit translation by binding the peptidyl transferase center on the larger ribosomal subunit, sterically occluding the A site. Despite not impeding the binding of *t*RNA, they prevent its full accommodation in the peptidyl transferase center, thereby inhibiting the peptide bond formation.[207]

Puromycin (**95**, Figure 49) is an amino-nucleoside antibiotic derived from the *S. alboniger*, that causes premature chain termination during translation.[208] **95** contains an amide linkage at the 3' position, which resembles the 3' end of the aminoacylated *t*RNA. The amide moiety makes the molecule much more resistant to hydrolysis when compared to the normal ester linkage of *t*RNA. As such, when puromycin enters the A site of the ribosome, it is transferred to the RNA growing chain, causing the formation of a puromycin-RNA chain and premature chain release. Several analogues of puromycin bearing modifications at the sugar and nucleobase levels were prepared, but their antibacterial activities were lower than, or in the same range as, **95**.[209-212]

Blasticidin S (**96**) is a nucleoside analogue consisting of a cytosine linked to a dideoxyhexose and bonded to a modified arginine (Figure 49).[213-215] This analogue was isolated from the broth of *S. griseochromogenes* in an effort to identify natural non-mercurial fungicides. Compound **96** has been used on a large scale to control rice blast by foliar application against *Pyricularia oryzae*, a virulent fungus that was the cause of a serious disease in Asia. Analogue **96** strongly inhibited the production of

aflatoxin by *A. flavus* without impairing fungal growth, and was found to exert its inhibitory activity by interfering with ribosomal protein synthesis in host cells. Unlike other peptidyl transferase inhibitors that bind to the A site of the large ribosomal subunit, such as the clinically used chloramphenicol, linezolid, lincomycin and clindamycin, , Blasticidin S occupies the P site, where it induces conformational changes of the P-site tRNA.

Aminoacyl *t*RNA synthetase inhibitors

Aminoacyl-*t*RNA synthetases (aaRSs) play an indispensable role in protein synthesis. These proteins aminoacylate *t*RNAs with cognate amino acids in a two-stage process whereby the amino acid is first activated with adenosine monophosphate (AMP) and then esterified at the 2'- or 3'-hydroxyl groups of the 3'-end of respective *t*RNA (Figure 50). Taking advantage of the significant differences between prokaryotic and eukaryotic aaRSs, selective inhibition of bacterial aaRSs enzymes can be used as drug target.[216]

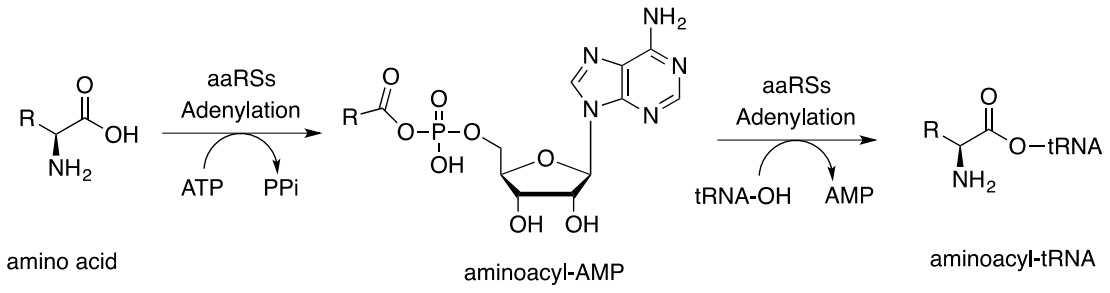


Figure 50. Aminoacyl-*t*RNA synthetases mechanism of action. AA = amino acid; ATP = adenosine triphosphate; PPi =inorganic pyrophosphate; amino-acyl-AMP = 5'-O-aminoacyl adenosine monophosphate; AMP = adenosine monophosphate.

Ascamycin (**97a**) and dealanylascamycin (**97b**) isolated in 1982 from *Streptomyces sp*. culture,[217] are natural nucleoside antibiotics capable of inhibiting the aaRSs. These analogues bear an unusual 5′-*O*-sulfonamide moiety (**A**), which is linked to an adenosine nucleoside (**B**) (Figure 51). **97a** is active against few microorganisms, such

as *Xanthomonas*. **97b** on the other hand shows broad-spectrum inhibitory activity against various Gram-positive and -negative bacteria and eukaryotic *Trypanosoma,* but unfortunately it is also toxic to mice. [218]

Natural compounds **97a** and **97b**, along with amino-acyl adenylate (amino-acyl AMP), an intermediate in the enzymatic reaction, have been used as a starting prototype to design novel aaRS inhibitors. The amino-acyl AMP is a mixed anhydride, which is bound more tightly to the enzyme than amino acid and ATP (the two natural substrates). Thus, structural analogues of this mixed anhydride and of the natural substrates have been designed to study their interactions with the corresponding enzymes. Several diadenosine-5'-tetraphosphate phosphonate analogues have been synthesised and proved to be moderate inhibitors of alanyl, lysyl and phenyl alanyl *t*RNA synthetases from *E Coli*.[219] Ester analogues prepared by Desjardins *et al.* were also weak inhibitors of glutamyl tRNA synthetase.[220]

In order to elucidate the substrate specificity of alanyl-*t*RNA synthetase, Ueda *et al.* reported the synthesis and molecular conformation study, by X-ray single crystal analysis, of 5'-*O*-[*N*-(*L*-alanyl)sulfamoyl]adenosine (**98**), an analogue of **97a** (Figure 51).[221] This research provided useful insights for analysing the mechanism of specific amino acylation. Several other derivatives were successively reported, such as two non-hydrolyzable prolyl adenylate analogues **99a** (*L*-PSA) and **99b** (*D*-PSA) (Figure 51).[222] These compounds were structurally related to the natural antibiotic **97a** and the synthetic derivative **98** reported by Ueda[221] (Figure 51). **99a** was the most potent inhibitor of both human and *E. coli* prolyl-*t*RNA synthetases with K_i in the subnanomolar to low-nanomolar range (K_i= 0.6 nM for human proline synthetase and K_i= 4.3 nM for *E. coli* prolyn synthetase). **99b** was found to be less potent (K_i= 85 nM for human proline synthetase and K_i = 490nM for *E. coli* proline synthetase).

Since both compounds showed activity against the human isoform of the enzyme, they were potentially toxic and therefore unsuitable for clinical antibiotic use. [222]

Cusak's group reported the crystal structures of seryl-*t*RNA synthetase from *Thermus thermophiles,* in complex with two different analogues of seryl adenylate: 5'-*O*-[*N*-(*L*-seryl)-sulfamoyl]adenosine (**100a**) and seryl-hydroxamate-adenosine monophosphate (**100b**) **(**Figure 51).[223] These studies revealed the region of the enzyme involved in the interactions and showed that, apart from the specific recognition of the serine side chain, the interactions were similar to the other amino- acyl synthetases. The inhibition constant of **100a** for the *E. coli* enzyme was found to be in the nanomolar range.[223]

Molecular modelling and synthetic studies on tyrosinyl adenylate analogues[224] have been carried out to probe the interactions seen by Brick *et al*.[225] in the active site of tyrosyl *t*RNA synthetase from *B. stearothermophilus.* These studies also aimed to find new inhibitors of tyrosyl *t*RNA synthetase. Three specific areas of the adenylate interactions were examined: the phosphate moiety, the role of the ribose hydroxyl groups, and the specificity of the adenine binding. To this end a variety of inhibitors of tyrosyl tRNA synthetase with alterations in these three part of tyrosinyl adenylate were synthesized. While it has been found that the level of inhibition of this enzyme can be maintained by replacement of the phosphate with the sulfamate moiety, other variants were shown to lead to substantially decreased potency. Among different derivatives, compounds **101a,b** were found to have an inhibitory activity for tyrosyl *t*RNA synthetases from both *B. stearothermophilus* and *S. aureus* with IC_{50} values in the micromolar and sub-micromolar ranges respectively (for **101a** K_i = 0.011-0.0063 μM; for **101b** K_i = 0.026-0.0093 μM) (Figure 51). Glutaminyl adenylate analogues were also synthesized as inhibitors of glutaminyl-*t*RNA synthetase.[226] Compound **102**

was found to be a competitive inhibitor of glutaminyl-*t*RNA synthetase from *E. coli* (K_i=0.28 μM) (Figure 51).

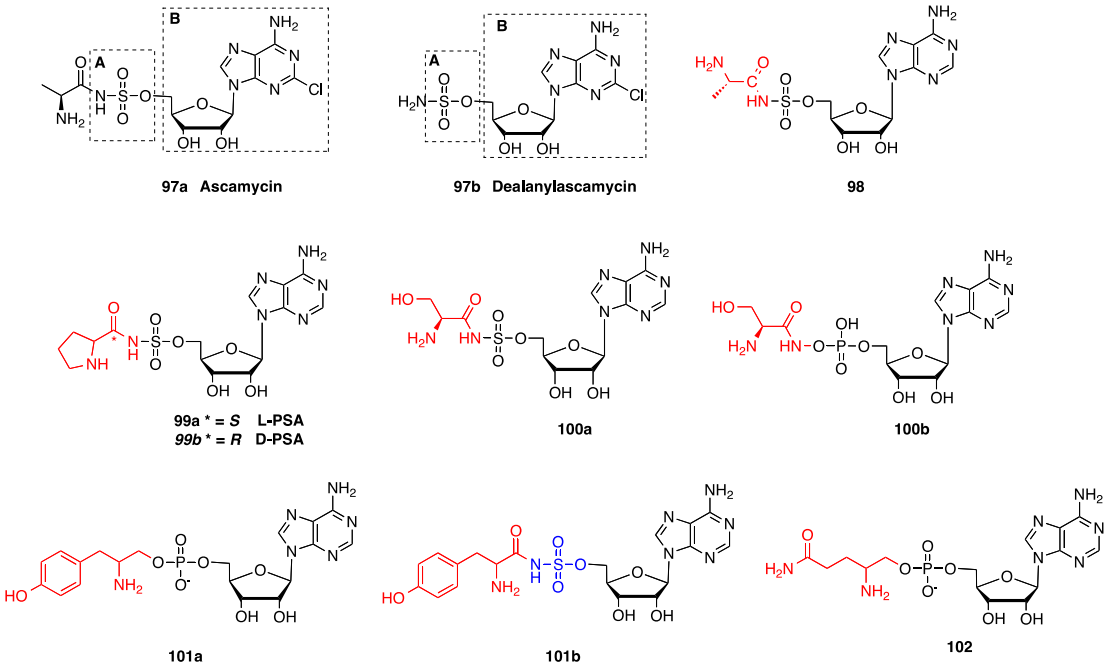


Figure 51. Structures of natural nucleoside antibiotics Ascamycin (**97a**) and Dealanylascamycin (**97b**) and synthetic aatRNAs inhibitors **98-102.**

Subsequently, ester, amide, *N*-alkyl hydroxamate and *O*-alkyl hydroxamate methionyl adenylate analogues **103a-d** were also examined (Figure 52).[227] Their relative activities as inhibitors of methionyl adenylate synthetase from *E. coli* were as follows: **103a** (ester) > **103c** (N-hydroxamate) > **103b** (amide) > **103d** (O-hydroxamate). Among these compounds the ester analogue **103a** was found to be the most potent with K_i = 10.9 μM. Antibacterial activities of methionyl adenylate **103a-d** were examined against different bacterial species. The extent of their activities was proportionally correlated to the extent of their enzyme inhibition, with **103a** showing a strong growth inhibition of *E. coli* (MIC = 0.5 μg/mL) and moderate inhibition of *B. cereus* (MIC =32 μg/mL).

Extensive SARs of ester and hydroxamate analogues of methionyl and isoleucyl adenylate were carried out by introducing a linker between the nucleobase and the

ribose. Adenine and adenine heterocyclic surrogates were linked to ribose by one or two carbon units. Among the tested analogues, compound **104** was found the most potent inhibitors of methionyl *t*RNA synthetase (IC_{50}= 3.6 μM) from *E. coli* (Figure 52).[228]

In 2003, a series of sulfamate surrogates of methionyl and isoleucyl adenylate were investigated as methionyl and isoleucyl *t*RNA synthase inhibitors, by modifications of both the sulfamate linker and adenine moieties.[229] Among this series, the 2-iodo analogue **105** emerged as the most potent inhibitor of isoleucyl *t*RNA synthase from *E. coli* (IC_{50} = 0.014 μM) (Figure 52). Docking studies with the X-ray structure of the isoleucyl *t*RNA synthase from *T. Thermophilus* indicated that a significant hydrophobic interaction between the 2-substituent and the adenine-binding site was responsible for the high potency of this compound. No selectivity toward different species aaRSs was reported/discussed.

Several modifications have been attempted in order to improve the inhibitory activity and selectivity of these aminoacyl-sulfamoyl adenosine analogues. Among them 2'-deoxy, 3'-deoxy, and 2',3'-dideoxyribosyl surrogates of isoleucyl and methionyl sulfamate adenylates have been investigated to identify the pharmacophoric importance of the ribose group for the inhibition of *E. coli* methionyl-*t*RNA and isoleucyl-*t*RNA (IRS) synthetases. Unfortunately, replacement of the ribofuranose with 2'- or 3'-deoxy or 2',3'-dideoxy sugars yielded less potent aaRS inhibitors.[230]

Cubist Pharmaceuticals reported the first examples of selective bacterial aaRSs inhibitors.[216] The structures of the two most interesting compounds **106a** (CB168) and **106b** (CB432) are depicted in Figure 52. Although still based upon aminoacyl sulfamoyl adenosine scaffold, these analogues contain an aryl-tetrazole in place of the adenine moiety, connected via a two-carbon linker to a ribose ring. These analogues

exhibited good inhibitory activity against isoleucyl *t*RNA synthetase from various bacteria species but poor inhibition of the human homologue. **106a** was found to inhibit E coli synthases with a K_i value of 1.3 μg/mL while inhibiting the corresponding human enzyme with a K_i value of 3000 μg/mL. **106b** showed moderate activity against a broad range of bacteria including *S. aureus*, *S. epidermidis*, *S. saprophyticus*, *S. pneumoniae S. pyogenes*, *E. faecium*, *E. faecalis*, *B. subtilis*, *Haemophilus influenzae*, *K. pneumoniae*, and *M. catarrhalis*, with MIC values ranging between 2 and 100 μg/mL. To determine the usefulness of **106b** in a model therapeutic application, this compound was tested in an *in vivo* murine model of *S. pyogenes* infections.[216] Although the compound was able to increase the animal survival, the dose required for its efficacy was too high to be useful for human applications. This high dosage was attributed to low bioavailability due to low cell permeability and strong binding to serum albumin of **106b**.

In order to address the selectivity issue of the sulfamoylated adenosines, Gadakh *et al.* further investigated the pharmacophoric importance of the adenine base.[231] Several isoleucyl-sulfamoyl nucleoside analogues were synthesized, bearing uracil, cytosine, hypoxanthine, guanine, 1,3-dideaza-adenine (benzimidazole) and 4-nitro-benzimidazole as the heterocyclic base. From *in vitro* evaluation, the uracil derivative **106c** showed the best inhibitory activity (Figure 52). Unfortunately, only weak growth inhibitory properties were found for **106c** due to its low cellular uptake. However, similar results were obtained with the hexapeptidyl (formal-MRTGNA-OH) conjugate of **106c** prepared in the effort to promote its bacterial uptake.

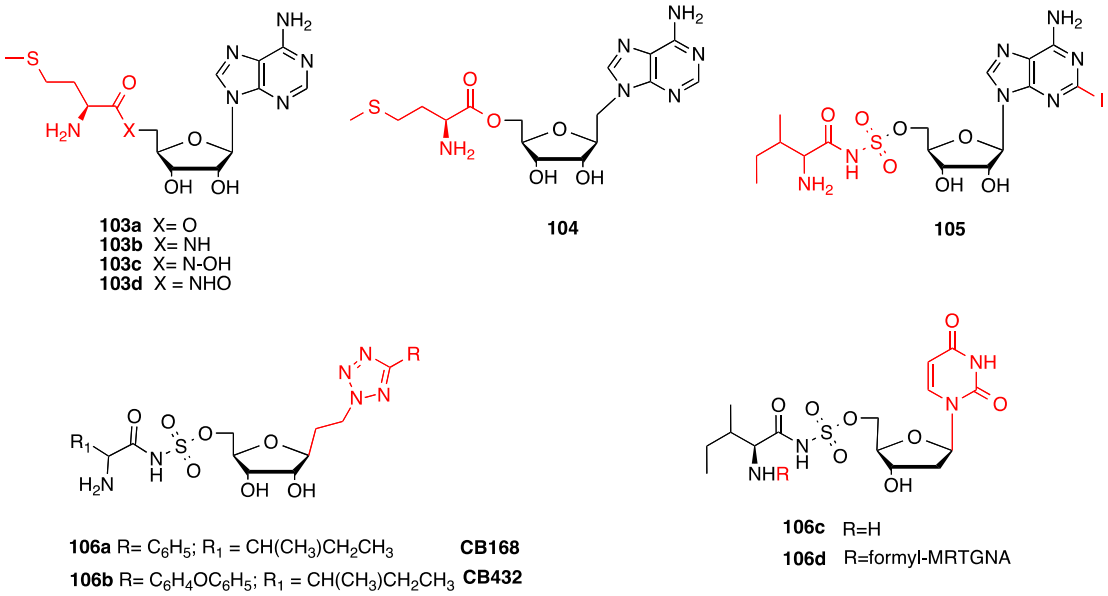


Figure 52. Structures of synthetic aatRNAs inhibitors **103-106.**

To overcome the poor permeation of aminoacyl-adenylates into bacterial cells, the active component that mimic aminoacyl-adenylate intermediates can be conjugated with a specialized module that facilitates its active transport into bacterial cell. The transport module can be subsequently removed, releasing the inhibitor inside the cell.[232] Examples of naturally occurring aminoacyl-adenylate-based antibiotics that employ this strategy include the aspartyl-*t*RNA synthetase inhibitor microcin C (**107a**) the seryl-*t*RNA synthetase inhibitor Albomycin (**108**) and the leucyl-*t*RNA synthetase inhibitor Agrocin 84 (**109**) (Figure 53).

107a is a natural compound produced by *Enterobacteriaceae*, consisting of an adenosine group (**A**) linked to a heptapeptide (**B**) through a *C*-terminal P-N bond (**C**). A propylamine group (**D**) additionally modifies the phosphoroamidate group of the nucleotide part of **107a**.[233, 234] *In vivo*, **107a** appears to target translation with the peptide part responsible for the transport of the antibiotic into the cell, whereas the nucleotide part is involved in the translation inhibition. Once inside the cell, this compound is metabolised by a peptide deformylase and an aminopeptidase enzyme,

which liberate a non-hydrolyzable analogue of aspartyl adenylate **107b**. This compound is able to inhibit the aspartyl *t*RNA synthase in *E. coli*, but is also active against the human isoform.[235] Acquisition of resistance via acetylation of the processed form of the antibiotic and *in vivo* instability of the peptide chain, are serious disadvantages for this drug.[236]

108a is another natural aaRS inhibitor, which belongs to the class of siderophore drug conjugates (Figure 53). It is composed of an iron carrier linked to an antibiotic moiety. The iron carrier is a tripeptide composed of N^5-hydroxy-(*L*)-ornithines (**A**), that mimicks the siderophore ferrichrome. The antibiotic moiety is a 4'-thio-6'-amino heptonic acid bearing a modified cytosine attached to the 1'-position (**B**). The siderophore allows albomycin to be taken up by a ferrichrome transport mechanism, which is expressed in many bacteria. Once inside the cell, the compound is processed with the release of the active moiety **108b**, which resembles seryl adenylate and therefore inhibits seryl *t*RNA synthase.[237] This analogue is particularly effective against Gram-negative bacteria such as *Enterobacteriaceae* and few Gram-positive bacteria like *S. pneumoniae*, *B. subtilis* and *S. aureus*.

109 is a disubstituted analogue of adenosine produced by *Agrobacterium radiobacter* K84, a non-pathogenic member of the genus isolated in Australia (Figure 53). The nucleoside core, which is essential for toxicity, contains 3**'**-deoxyarabinose (**A**) instead of the common ribose unit. The methyl-substituted pentanamide (**B**) at the *5'*-position is also required for activity. Although the glucofuranose (**C**) at the N^6 position does not confer toxicity it is required for transport inside the bacterium and therefore is critical to functionality. Both groups are linked to the nucleoside core by phosphoroamidate bonds (D). **109** is an aaRS inhibitor, active only against certain

strains of the phytopathogenic agrobacteria such as *A. tumefaciens* and *A. rhizogenes*.[238]

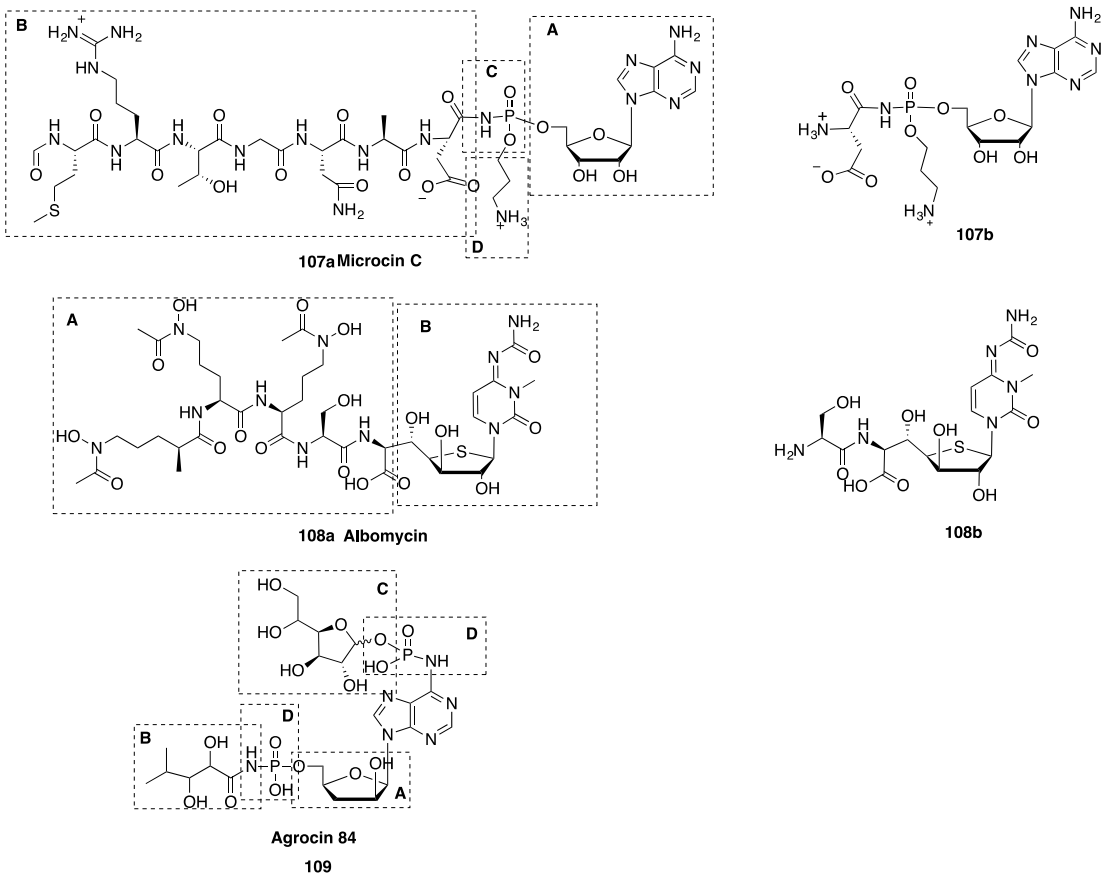


Figure 53. Structures of Microcin C (**107a**), processed Microcin C (**107b**), Albomycin (**108**), processed form of Albomycin (**108b**) and Agrocin 84 (**109**).

In search for bacterial aaRSs inhibitors endowed with *in vivo* activity, Vondenhoff and co-workers reported a series of microcin C **110a-c** and albomycin analogues **111a-b** with aryl tetrazole substituents as nucleobase isosters (Figure 54).[239]

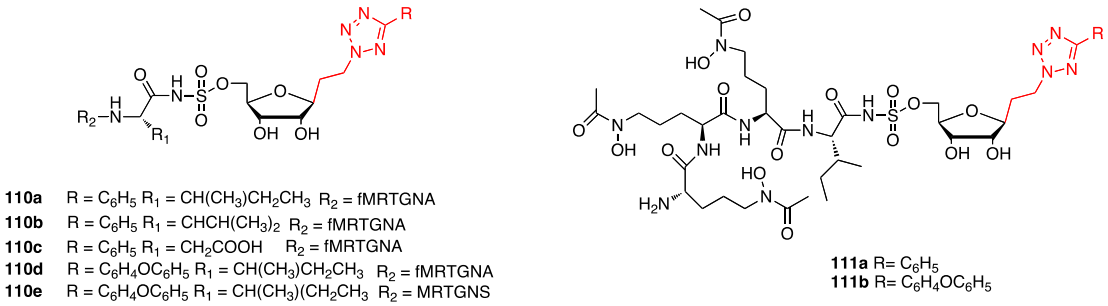
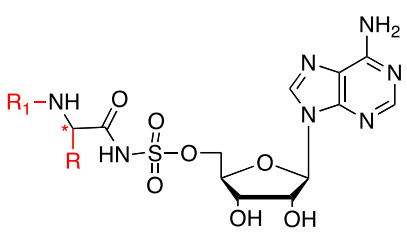
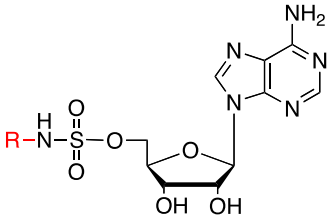


Figure 54. Structures of aryl tetrazole microcin C **110a-e** and albomycin conjugates **111a-b**.

Unfortunately, no antibacterial activity was observed with these new analogues in whole-cell assay. These compounds showed strong activity against isoleucyl *t*RNA synthase in *E. coli* cell extracts. The activity was dependent on processing by cellular peptidases. Since all the compounds were efficiently metabolized, the absence of antibacterial activity can be attributed only to the lack of cellular uptake. These results strongly suggest that not only the siderophore but also the nucleoside portion of these analogues are fundamental for the transport inside the bacterial cell. Using the same strategy, Gadakh *et al.* reported a series of sulfamoylated nucleoside analogues bearing different nucleobases, coupled with a hexapeptide in the effort to improve their uptake.[231] However, no significant activity was found for these derivatives. On the basis of these results, Vonderhoff *et al.* turned their attention to another class of microcin C analogues with a preserved nucleoside core but with the siderophore portion modified with *N*-methyl or D-amino acids (Figure 55).[240] The authors believed that these modifications would potentially help to escape the natural inactivation mechanism of acetylation of the amino group and/or prevent the *in vivo* instability of the peptide chain. A series of *N*-methyl and D-aminoacyl, sulfamoyl adenosine analogues **112a-d**, **113a-e** were prepared and investigated as potential aaRSs inhibitors. Unfortunately, all *N*-methylated aaSAs proved to be inactive in

whole-cell assays. In addition, these analogues were ineffective as *t*RNA aminoacylation inhibitors in cell extracts. The same result was obtained when the amino acid residues in positions 2 to 6 had a D-configuration, indicating that despite the better stability, the metabolism by peptidase enzymes was significant, thus preventing the release of the active moiety and consequent inhibition of aatRNAs. Compounds bearing a D-amino acid in position 1 of the peptidyl chain, were able to inhibit aspartyl-*t*RNA synthetase. However, this effect was short-lived due to the inactivation by acetylation.





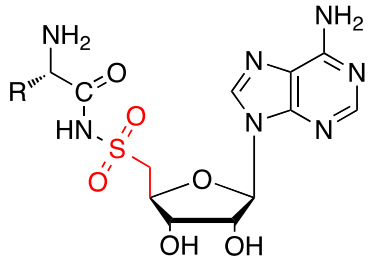
112a R = $CH_2CH(CH_3)_2$ R_1 = CH_3 * = L
112b R= H R_1= CH_3
112c R= CH_2CO_2H (Daa) R_1=H * = D
112d R= CH_2CO_2H (Laa) R_1=H * = L

113a R = Formyl-(L)M-(D)R-(D)T-G-(D)N-(D)A-(L)D-
113b R = Formyl-(L)M-(D)R-(D)T-G-(D)N-(L)A-(L)D-
113c R = Formyl-(L)M-(D)R-(D)T-G-(D)N-(L)S-(L)D-
113d R = Formyl-(L)M-(D)R-(D)T-G-(D)N-(L)S-(L)I-
113e R = Formyl-(L)M-(L)R-(L)T-G-(L)N-(L)A-(D)D-

Figure 55. structures of derivatives **112a-d** and derivatives **113a-e.**

Gadack *et al.* reported a further attempt to improve the stability of aminoacyl sulfamoyl adenosine by synthetizing derivatives lacking of the 5' oxygen **114a-f** (Figure 56).[241] The rationale was that the lack of the oxygen would improve the stability of the resulting aminoacyl sulfamate analogues while making the C-5' less electrophilic and prone attackby N^3 adenine, thus avoiding the formation of a cyclic degradation product. Unfortunately, no inhibitory activity was observed for these derivatives either *in vitro* or in whole cell assays, except for derivative **114f**. This loss of activity has been explained by a concomitant loss of affinity for the target due to the oxygen deletion. Computer simulations showed that the active compound **114f**

was the only one capable of binding the aspartyl *t*RNA synthetase similarly to the natural substrate.



114a R= $CH(CH_3)CH_2CH_3$
114b R= $CH_2CH(CH_3)_2$
114c R = $CH_2C_6H_4$-*p*-OH
114d R= H
114e R= CH_2OH
114f R= CH_2COOH

Figure 56. Synthesis of 5'-aminoacyl-5'-deoxy-nucleoside sulfonamides **114a-f.**

S-adenosylhomocysteine nucleotidase inhibitors

5'-methylthioadenosine/S-adenosylhomocysteine (MTA/SAH) nucleosidase is a bacterial dual-substrate specific enzyme that catalyses the breakdown of *S*-adenosylhomocysteine (SAH) to S-rybosylhomocysteine (SRH) and adenosine (A). It is also responsible for the conversion of methylthioadenosine (MTA) in adenine and 5-thioribose (Figure 57).[242] MTA/SAH nucleosidase plays an important role in biological polyamine biosynthesis and transmethylation reactions. The inhibition of this enzyme causes a feedback inhibition of transmethylation, resulting in the formation of uncapped *m*RNA, which is less efficiently translated. MTA nucleosidase is not found in humans and has been shown to be essential in certain bacteria.[243]

In the mammalian salvage pathway, MTA phosphorylase is the enzyme responsible for the breakdown of MTA to adenine and 5-thioribose 1-phosphate. A careful comparison between the *E. coli* MTA/SAH nucleosidase and the mammalian phosphorylase revealed sequence and structure diversity in the active site region. This suggested the possibility to achieve substrate specificity[244, 245] and these differences have been exploited in the design of inhibitors specific for bacterial nucleosidase.

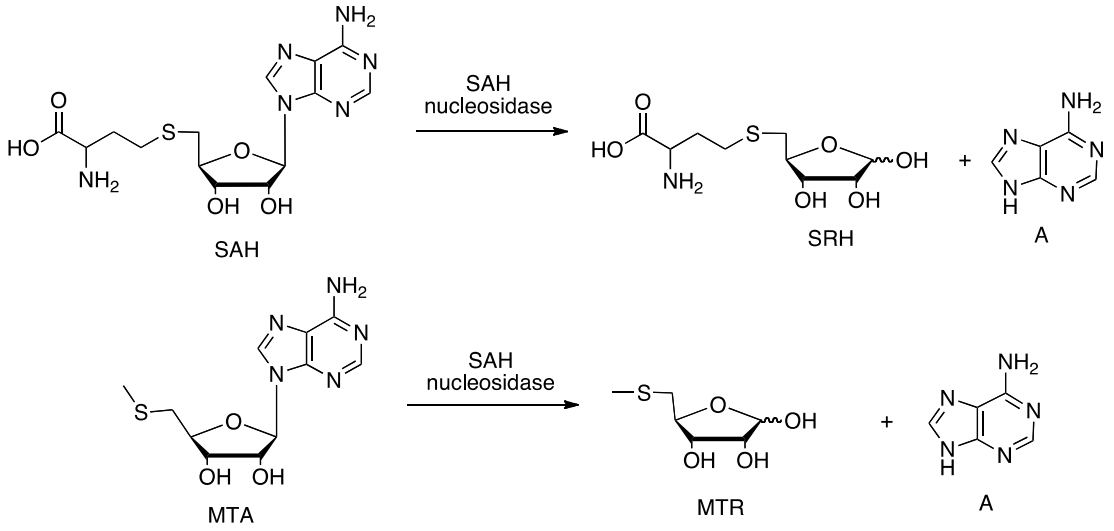


Figure 57. MTA/SAH nucleosidases mechanism of action. SAH = *S*-adenosylhomocysteine; SRH = *S*-ribosylhomocysteine; A = adenine; MTA = 5' methylthioadenosine = MTR =5'-methylthioribose.

Neplanocin A (**115**) and Aristeromycin (**116**) are two naturally occurring carbocyclic nucleosides exhibiting activity against SAH nucleosidase (Figure 58).

115 is a carbocyclic analogue of adenosine in which the ribose moiety has been replaced by a cyclopentene ring (Figure 58). This analogue was originally isolated from *Ampuriella regularis* and has been reported to have growth inhibitory activity against certain fungi.[246] Since this analogue was shown to be a strong inhibitor of purified calf-liver SAH,[247] it was speculated that a possible mode of action for its antifungal activity could be the inhibition of bacterial SAH hydrolase. **116** (Figure 58) is a carbocyclic analogue of adenosine in which the ribose moiety has been replaced by a cyclopentane ring (Figure 58). **116** displays strong activity against some phytopathogenic bacteria and fungi, such as *X. oryzae* and *P. oryzae*. However, no inhibitory activity was reported against yeast and other pathogenic fungi and bacteria.[248]Unfortunately, the close resemblance of **115** and **116** to adenosine renders them susceptible to recognition and phosphorylation by cellular kinases, resulting in

significant cytotoxicity. Strategic removal of the 4'-hydroxymethyl group of **115** and **116** to afford the 4',5'-saturated and 4',5'-unsaturated derivatives has proven to successfully address this limitation, as both families were potent inhibitors of SAHase.[249, 250] Interestingly, neither series of compounds were substrates for adenosine kinase nor adenosine deaminase, thus did not exhibit the toxicity associated with **115** and **116**. Among these derivatives, **117** was found to inhibit SAH with a K_i value of 5 μM. However, no antibacterial activity was reported for any of the compounds.

A number of different SAH analogues with structural similarity with the naturally occurring nucleoside, *S*-adenosylhomocysteine were reported by Srivstava *et al.*[251] These derivatives, when evaluated for their antibacterial and antifungal activities, exhibited good to moderate activity against Gram-positive bacteria (group D *Streptococcus)* and some human pathogenic fungi like *A. fumigatus*, *P. marneffei*, *C. albicans*, *C. neoformans* and *Mucor sp* with MICs ranging from 0.19 to 0.75 μM for the most active compounds **118a-d** (Figure 58).

5'-Methylthioadenosine analogues have been described as inhibitors of MTA/SAH nucleosidase. Among them, **119a** (MTDIA), and **119b** have been reported to be femtomolar inhibitors of *E. coli* MTA nucleotidase (Figure 58).[252, 253] Unfortunately, no activities in whole cell assay have been reported so far.

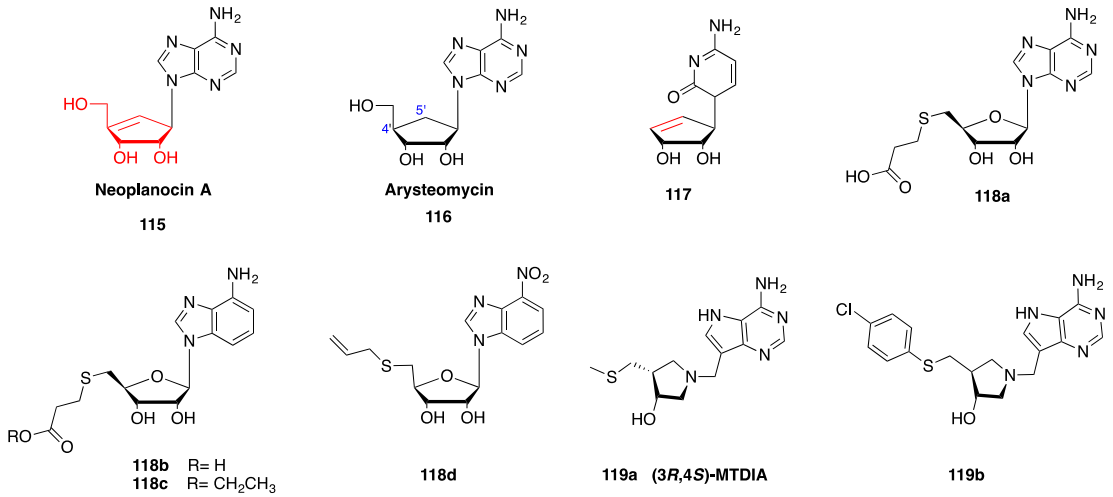


Figure 58. Structures of natural antibiotic Neplanocin A (**115**) and Aristeromycin (**116**) and synthetic MTA/SAH nucleotidase inhibitors **117**; **118a-d**; **119a,b**.

Bacterial cell division

FtsZ is an essential bacterial cytoskeletal protein, and represents the prokaryotic counterpart of tubulin. [254] *In vivo* polymerization of FtsZ is a GTP-induced process that leads to the formation of a macromolecular structure termed the Z ring.[255] This is a key event in bacterial cell division. Agents interfering with FtsZ polymerization potently inhibit septum formation and cell division, and may be developed into a promising class of novel antibiotics with low potential for cross resistance.[256] FtsZ shows only weak sequence identity to the eukaryotic tubulin, although it has a similar three-dimensional structure and conserved longitudinal protofilament contacts.[224] Modified GTP analogues **120a-k** bearing small substituents in C-8 position on the guanine ring were found to inhibit FtsZ (Figure 59).[257]

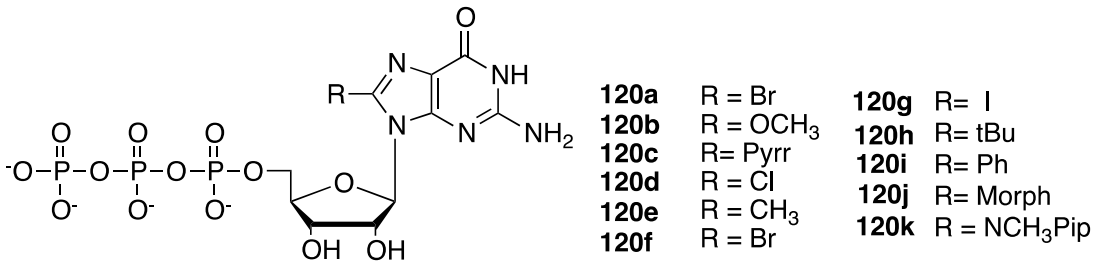
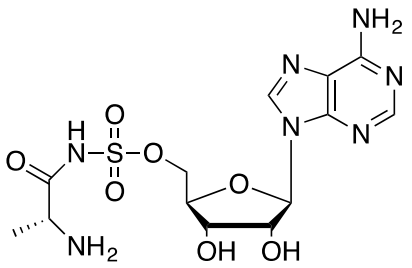


Figure 59. Structures of FtsZ inhibitors (**120a-k**).

They exert their action by binding the enzyme, with the C-8 substituents capable of obstructing the association of a second FtsZ subunit. Compounds with small C-8 substituent did not inhibit tubulin polymerisation, a prerequisite for suitable selective and non-cytotoxic agents. Although these analogues do not have a druglike structure, they may represent a starting point for the further development of more bioavailable FtsZ inhibitors.

Teichoic acid biosynthesis

Lipoteichoic acids (LTAs) and wall teichoic acids (WTAs) are cell wall polymers that play fundamental roles in Gram-positive bacterial physiology and pathogenesis, and have both been proposed as novel antibacterial targets.[258] LTAs and WTAs play critical roles in bacterial viability *in vitro*.[259] LTA (*D*)-alanylation is a step in teichoic acid biosynthesis that crucially has no counterpart in the human body and can be inhibited by (5'-*O*-[*N*-(*D*-alanyl)-sylfamoyl]adenosine (**121**) (Figure 60) (K_i = 232 nM).[260]



121

Figure 60. Structure of 5'-*O*-[*N*-(*D*-alanyl)-sulfamoyl]adenosine (**121**).

Whole cell studies were carried out inhibiting LTA (*D*)-alanylation in wild type *B. subtilis* using this analogue in combination with vancomycin at a concentration of respectively 1 mM and 0.4 nM. No growth was observed after 30 h treatment. In the presence of vancomycin alone, the wild type recovers its growth after 12 h incubation and, after 30 h, reached an attenuance comparable to that of untreated wild type cells.[260]

Conclusion and future direction

The development of antimicrobial resistance is a natural phenomenon and represents a serious and growing problem in the treatment of bacterial infections. The slowdown in development of new antibacterial drugs has coincided with an alarming increase in the number of resistant, multi-resistant, and even totally resistant bacterial infections. At the same time, there has also been an increase in the incidence of fungal infections, which has been also linked to the emergence of antifungal drug resistance. The rise in the occurrence of drug resistant infections has intensified the need for the development of a new generation of antibacterial and antifungal agents. The inappropriate use of antimicrobial drugs, including in animal husbandry, favoured the emergence and selection of resistant strains. This, in combination with poor infection prevention and control practices, contributes to further emergence and spread of

antimicrobial resistance. It is alarming that in countries where antibiotics can be bought without a prescription, emergence and spread of resistance is worse in comparison with countries were this practice is under strict control. Therefore, in our vision, the most urgent point to focus on is the education of the world population. Surveillance data from hospitals and farm veterinary must be extensively used for this purpose with the intent to achieve a better control of antibiotic dosage and use. This is because the pace at which the research for novel antibiotic proceeds is inadequate to cope with the spread of bacterial resistance. At the same time, scientists from academia and industry, must work together to develop new antibiotics.

Alongside the human health concerns it is important to note that when infections become resistant to first-line drugs, more expensive therapies must be used. A longer duration of illness and treatment increases health care costs as well as the economic burden on families and societies.

We have shown here that nucleoside analogues have a great potential as antibiotic agents, and are capable of targeting essential cellular processes. Antibiotic nucleoside analogue targets include: cell wall growth (MrY and CS inhibitors); nucleic acid synthesis; translation (aatRNA synthase and ribosomal peptidyl center inhibitors) and nucleotide metabolism of bacterial and fungal pathogens (phosphoribosyltransferase, guanosine monophosphate synthase, inosine-5-monophosphate dehydrogenase inhibitors). We have also discussed how nucleoside analogues can affect new or poorly characterized targets. Such targets include enzymes involved in a range of processes such as: cell wall biosynthesis (Mur A); in the later lipid-linked steps encompassing both LTA and WTA synthesis (dtA); in microbial cell division (FtsZ) and methionine salvage pathway (SAH/MTA nucleosidase). Other processes such as cell signalling in bacteria mediated by the rei protein,[261, 262] and protein

glycosylation[263] have also been recently identified as potential new targets for nucleoside-based antibiotic agents. These processes, constitute a rich source of crucial targets for antibacterial chemotherapy. Since most of these pathways are unique to bacteria further investigations of the mechanisms involved could help to identify new drug targets. Several groups are already exploiting some of these targets e.g. NovoBiotic Pharmaceuticals, which discovered teixobactin, a new Gram-positive bacteria-targeting antibiotic.[7] This new cell wall inhibitor (lipid III) is able to kill pathogens such as MRSA and *M. tuberculosis* without detectable resistance. Scientists at Merck have also recently identified specific inhibitors of either early or late stage of WTA synthesis.[264] Some of these new compounds do not have intrinsic bioactivity but rather demonstrate potent bactericidal synergy in combination with broad-spectrum β-lactam antibiotics against diverse clinical isolates of MRSA. The combination has also demonstrated robust efficacy in a murine infection model of MRSA, proving that drug combination can be a valuable approach to tackle antibiotic resistance. It is important to mention that another recently developed approach involved generating compounds that inhibit bacterial virulence and biofilm formation, by controlling the quorum sensing.[265] This alternative approach does not involve the killing of the pathogen and therefore does not put on it a pressure to develop resistance.

We believe that structure-function-based studies on novel and underutilized targets, essential to bacterial and fungal viability, could help the optimization of the already known nucleoside antibiotics portfolio and aid the identification of new antibacterial and antifungal agents. The discovery of new nucleoside-based molecules could originate from the exploitation of techniques such as genome mining,[266] analysis of DNA sequence and semi-synthesis,[267] along with the investigation of metabolites

from microorganisms from unusual habitats, such as marine microorganisms and uncultured terrestrial microorganisms.[268, 269] Moreover, mutasynthesis[270] and combinatorial biosynthesis[271] can be employed to generate new variants or hybrid nucleoside antibiotics.

Alongside these strategies is the chemical modification of existing structures via classic organic synthesis. Although more time consuming, and generally more challenging, this strategy can be more flexible in terms of novelty of the structural diversification. From the above discussion, it is clear that the majority of the nucleoside antibiotics, both natural and man-made, have the problem of being poorly available to their biological target because their intrinsic low permeability through the cellular membrane. It is our opinion that synthetic efforts should be directed toward the identification of suitable structural motif capable of increasing the cell-permeability of the new structures without compromising the biological activity. To overcome the poor permeation of aminoacyl-adenylates into bacterial cells, the active component can be conjugated with a specialized module that facilitates its active transport into bacterial cell. In this regard, prodrug approaches, currently successfully used in antiviral and anticancer nucleosides, can be a key answer to the poor permeability problem. For example, our research group hasshown that the pronucleotide (proTide) methodology could be used for the development of active molcules against *M. tuberculosis* and reveals the importance of improving lipophilicity to efficiently pass the mycobacteria wall barrier.[272] Prodrug approaches in combination with a detailed understanding of the biosynthetic pathways of nucleoside-based natural products can be effectively implemented with the use of advanced molecular modelling tools and computer aided drug design.

As far as fungi are concerned, nucleoside analogues capable of interfering with the

cell wall synthesis suffer from the same problem as their counterpart in bacteria: penetration of the fungal cell. The majority of chitin synthase inhibitors are peptide-nucleoside structure that relies on peptide transporter to access the cell. Modification of the peptide part with more lipophilic amino acids (natural and non-natural) could be an effective strategy to improve their *in vivo* antifungal activity. More lipophilic, fluorinated amino acids for example could be structural motifs that may have some success in this respect. It is our opinion that the chemists have at their disposal several potentially effective approaches that have never been used in the antibacterial/antifungal research. For example, the large amount of sugar units in bacterial and fungal cells can be used as target for boron functionalized compounds.[273] Molecules functionalized with boron can have great affinity for the sugar's hydroxyl group. This can be exploited by establishing selective interactions with the critical enzymes (such as chitin synthase): thereby stopping the formation of chitin or glycoproteins important for the cell wall. Research in this field should be pursued with great efforts especially in light of the great progress that boron chemistry has made in recent years. In terms of future drug discovery strategies, it is our view that it will be important to continue to pursue approaches that are likely to minimise, or delay, resistance to a specific molecule by targeting diverse biologically relevant pathways in the bacterial/fungal cell simultaneously.

In conclusion, we strongly believe that nucleoside and nucleotide analogues will play a key role in the quest for more effective antibiotics. Furthermore, there are several bacterial and fungal targets such as bacterial cell division protein (FtsZ) and teichoic acids biosynthesis for which very few nucleosides have been explored as potential therapeutics. We predict that expansion of this promising field of research would lead to the implementation of an effective new arsenal against bacterial and fungal

resistance.

Acknowledgment

We would like to thank Mr Calvin Smith and Dr Jennifer Wymant for their kind help in proofreading the manuscript.

Authors Biography

Fabrizio Pertusati received is MSc in Chemistry in 1999 from the University of Turin (Italy) and his Ph.D. in Asymmetric Organic Chemistry in 2005 at the Cardiff University (UK). After postdoctoral experiences at Emory University with Professor Fredric Menger (surfactant chemistry) and in the laboratories of Nobel Laureate, Professor George Olah at the Loker Hydrocarbon Institute (fluorine and boron chemistry), he is now a Life Science Research Network Wales post-doctoral fellow at the School of Pharmacy and Pharmaceutical Sciences at Cardiff University. His current research involves the development of a diastereoselective synthesis of phosphoroamidate prodrugs of nucleoside analogues. His research interests include the discovery of novel antiviral, anticancer, and neurodegenerative diseases-related agents based on rational drug design.

Michaela Serpi graduated in Medicinal Chemistry in 1998 at the University of Cagliari (Italy). She received her Ph.D. in Medicinal Chemistry in 2005 from the University of Perugia (Italy), with a work on constrained analogues of L-AP4, as ligands at the metabotropic glutamate receptors. After her Ph.D., she joined the group of Professor Chris McGuigan at Cardiff University (UK). She worked from January 2008 to January 2011 as a Post Doctoral Fellow funded by NIH at the University of Southern California in the group of Professor Charles E. Mckenna. Her research

interests include the design, synthesis, and biological evaluation of nucleoside analogues as antitumoral, antinfective, and antibacterial agents. She is currently a Senior Scientist in the School of Pharmacy and Pharmaceutical Sciences of Cardiff University.

Valentina Ferrari graduated in Pharmacy in 2012 at the University of Rome (La Sapienza, Italy) working on the synthesis and biological evaluation of benzotriazoles derivatives as inhibitors of cannabinoids catabolism enzymes. In 2012 she joined the group of Chris McGuigan as Ph.D. student working on the synthesis of phosphoroamidate and phosphonoamidate prodrugs of various nucleosides as anticancer and antiviral therapeutics. She received her PhD in Medicinal Chemistry in March 2016 and she is now working as research assistant in the School of Pharmacy and Pharmaceutical Sciences of Cardiff University.

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Abbreviation:

MRSA	Methicillin-resistant *Staphylococcus aureus*
LPS	Lipopolysaccharides
GlcNAc	*N*acetylglucosamine
MurNAc	*N*-acetylmuramic acid
DAP	Meso-diaminopimelic acid
UDP	Uridine diphosphate
GPI	Glycophosphadityl inositol
CS	Chitin synthase

MIC	Minimum inhibitory concentration
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
PRTs	Phosphoribosyl transferases
HGPRT	Hypoxanthine-guanine phosphoribosyltransferase
XGPRT	Xanthine-guanine phosphoribosyltransferase
PRPP	5-phosphorybosyl group from 5-phosphoribose-1-pyrophosphate
IMP	Inosine monophosphate
GMP	Guanosine monophosphate
PPi	Inorganic pyrophosphate
XMP	Xanthosine monophosphate
G	Guanine
H	Hypoanthine
ATP	Adenosine triphosphate;
AMP	Adenosine monophosphate;
IMPDH	Inosine-5′-monophosphate dehydrogenase
NAD	Nicotinamide adenine dinucleotide
ACT	Pertussis adenylate cyclase toxin
cAMP	cyclic adenosine monophosphate
PMEA	9-[2-(Phosphonomethoxy)ethyl]adenine
dNKs	Deoxynucleoside kinases
aaRSs	Aminoacyl-*t*RNA synthetases
MTA	5'-Methylthioadenosine
SAH	*S*-Adenosylhomocysteine
SRH	*S*-Ribosylhomocysteine
A	Adenine
MTR	5'-Methylthioribose
LTAs	Lipoteichoic acids
WTAs	Wall teichoic acids

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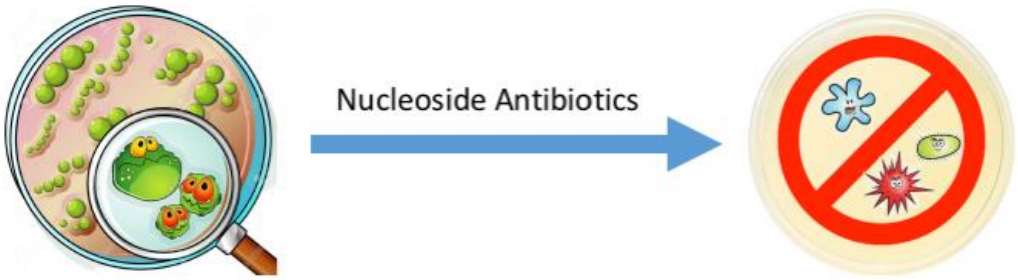
Nucleoside Antibiotics