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Natural products — The future scaffolds for novel antibiotics?

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ABSTRACT

Natural products have played a pivotal role in antibiotic drug discovery with most antibacterial drugs being derived from a natural product or natural product lead. However, the rapid onset of resistance to most antibacterial drugs diminishes their effectiveness considerably and necessitates a constant supply of new antibiotics for effective treatment of infections. The natural product templates of actinonin, pleuromutilin, ramoplanin and tiacumicin B, which are compounds undergoing clinical evaluation, represent templates not found in currently marketed antibacterial drugs. In addition, the new templates present in the recently discovered lead antibacterials arylomycin, GE23077, mannopeptimycin, muraymycin/caprazamycin, nocathiacin and ECO-0501, are discussed. Despite extensive efforts to identify antibiotic leads from molecular targets, only the peptide deformylase inhibitor LBM-415 is currently in clinical trials. It is proposed that new antibacterial assays which combine cell-based screening with molecular targets could offer better prospects for lead discovery.

1. Introduction

The introduction of the sulphonamide antibiotics in the 1930s and penicillin in the 1940s revolutionised medicinal practice by dramatically decreasing the fatality rates associated with bacterial infections [1–3]. These discoveries led to a concerted search for new antibacterial drugs during the following 30 years and resulted in the discovery of most of the antibacterial drug classes known today, many of which were derived from natural product leads (Table 1) [1,4,5]. Given this success, it is surprising to note that only three new antibacterial classes, the topical antibiotic mupirocin in 1985, the oxazolidinone linezolid in 2000 and the lipopeptide daptomycin in 2003, have entered the market since 1970. Over the past 20 years, there has been a 56% decline in the number of antibiotics approved annually by the Food and Drug Administration (FDA) and over the last decade, only 22 new antibacterial drugs have been launched (Table 2) [6–9]. The 12 natural product-derived drugs belong to five different structure classes (β-lactam, streptogramin, macrolide, tetracycline and daptomycin), while the 10 synthetic drugs launched belong to only two antibacterial classes, with the quinolone class accounting for nine of these drugs.

The prevalence of natural product-derived antibacterial drugs may be due to the evolution of secondary metabolites as biologically active chemicals that conferred selectional advantages to the producing organisms. Natural products also are likely to have evolved to penetrate cell membranes and interact with specific protein targets [10]. In addition, natural products have an element of structural complexity which is required for the inhibition of many antibacterial protein targets. Relevant reviews on the role of natural products in modern drug discovery [11,12], natural product-derived compounds in clinical trials [13] and compounds in antibacterial clinical trials have been published recently [14–17].

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Table 1 – Antibiotic class with approximate year of clinical introduction, lead derivation, example of drug and mechanism of action

Antibiotic class	Introduction	Derivation	Example	Mechanism
Sulphonamide	1935	Synthetic	Sulfapyridine	Antifolate
β-Lactam	1941	NP-derived	Penicillin	Bacterial cell wall
Bacterial peptide	1942	NP-derived	Bacitracin	Bacterial cell wall
			Polymixin	Bacterial cell membrane
Aminoglycoside	1944	NP-derived	Streptomycin	Protein synthesis
Cephalosporin	1945	NP-derived	Cephalosporin	Bacterial cell wall
Nitrofuran	1947	Synthetic	Nitrofurantoin	Various
Hexamine	1947	Synthetic	Methenamine mandelate	Release of formaldehyde
Chloramphenicol	1949	NP-derived	Chloramphenicol	Protein synthesis
Tetracycline	1950	NP-derived	Chlortetracycline	Protein synthesis
Isoniazid	1951	Synthetic[a]	Isoniazid	Fatty acid biosynthesis
Viomycin	1951	NP-derived	Viomycin	Protein synthesis
Macrolide	1952	NP-derived	Erythromycin	Protein synthesis
Lincosamide	1952	NP-derived	Lincomycin	Protein synthesis
Streptogramin	1952	NP-derived	Virginiamycin	Protein synthesis
Cycloserine	1955	NP-derived	Cycloserine	Bacterial cell wall
Glycopeptide	1956	NP-derived	Vancomycin	Bacterial cell wall
Novobiocin	1956	NP-derived	Novobiocin	DNA synthesis
Ansamycin	1957	NP-derived	Rifamycin	RNA synthesis
Nitroimidazole	1959	Synthetic	Tinidazole	DNA synthesis
Ethambutol	1962	Synthetic	Ethambutol	Bacterial cell wall
Quinolone	1962	Synthetic	Nalidixic acid	DNA synthesis
Fusidane	1963	NP-derived	Fusidic acid	Protein synthesis
Diaminopyrimidine	1968	Synthetic	Trimethoprim	Antifolate
phosphonate	1969	NP-derived	Fosfomycin	Bacterial cell wall
Pseudomonic acid	1985	NP-derived	Mupirocin	Protein synthesis
Oxazolidinone	2000	Synthetic	Linezolid	Protein synthesis
Lipopeptides	2003	NP-derived	Daptomycin	Bacterial cell membrane

[a] Isoniazid is based on the structure of nicotinamide (vitamin B_2).

The rapid onset of resistance to most antibacterial drugs has diminished their effectiveness and, as a consequence, a continual search for novel antibacterials needs to be undertaken to replenish antibacterial drug pipeline [18,19]. However, despite the clear need for new antibacterial drugs with novel mechanisms of action, many pharmaceutical companies have chosen to reduce or completely cease their antimicrobial R&D efforts [18–25].

Table 2 – Antibacterial drugs launched since 1995 by year with reference to their structure class and derivation

Year	Generic name (trade name)	Class	Classification
1995	Cefozopran (Firstcin®)	β-Lactam – cephalosporin	NP-derived
1997	Cefcapene pivoxil (Flomox®)	β-Lactam – cephalosporin	NP-derived
1997	Faropenem (Farom®)	β-Lactam – penem	NP-derived
1997	Flurithromycin (Ritro®)	Macrolide – erythromycin	NP-derived
1998	Cefoselis (Wincef®)	β-Lactam – cephalosporin	NP-derived
1998	Trovafloxacin (Trovan®)	Quinolone	Synthetic
1999	Dalfopristin and quinupristin (Synercid®)	Streptogramin	NP-derived
1999	Gatifloxacin (Tequin®)	Quinolone	Synthetic
1999	Moxifloxacin (Avelox®)	Quinolone	Synthetic
2000	Linezolid (Zyvox®)	Oxazolidinone	Synthetic
2001	Ertapenem (Invanz™)	β-Lactam – carbapenem	NP-derived
2001	Telithromycin (Ketek®)	Macrolide – erythromycin	NP-derived
2002	Biapenem (Omegacin®)	β-Lactam – carbapenem	NP-derived
2002	Balofloxacin (Q-Roxin®)	Quinolone	Synthetic
2002	Pazufloxacin (Pasi®, Pazucross®)	Quinolone	Synthetic
2002	Prulifloxacin (Sword®)	Quinolone	Synthetic
2002	Voriconazole (Vfend®)	Quinolone	Synthetic
2003	Daptomycin (Cubicin™)	Daptomycin	NP
2004	Gemifloxacin (Factive®)	Quinolone	Synthetic
2004	Fosfluconazole (Prodif®)	Quinolone	Synthetic
2005	Doripenem (Finbax®)	β-Lactam – carbapenem	NP-derived
2005	Tigecycline (Tygacil™)	Tetracycline	NP-derived

This review focuses on new natural product antibacterial templates of compounds currently in clinical trials and selected examples of compounds undergoing preclinical evaluation. In addition, the future prospects of natural product-derived compounds in antibacterial research are discussed.

2. Antibacterial compounds in clinical development

14 of the 19 candidates (Table 3, Figs. 1 and 2) undergoing antibacterial clinical evaluation are derivatives of known drugs: one rifamycin derivative **3** of the ansamycin class, seven β-lactams (cephalosporins **4**, **5**, **6**, carbapenems **7**, **8** and **9** and penem **10**), two vancomycin-type glycopeptide derivatives **11** and **12**, two erythromycin-type macrolide derivatives **13** and **14**, one streptogramin mixture **18** and **19** and one tetracycline derivative **20**. The remaining five, details of which are discussed below, are of interest because they contain antibacterial templates not previously found in drugs marketed for human use.

Bacterial peptide deformylase (PDF) is responsible for removing the *N*-formyl group from the N-terminal methionine following translation, contains three highly conserved catalytic domains and is a metallo hydrolase [26,27]. PDF is an essential gene for bacterial survival and does not share close homology with any mammalian equivalent [28]. In 2000, workers from Vicuron reported that actinonin **2**, a known *Streptomyces*-derived antibiotic, was a potent inhibitor of PDF [29]. Actinonin **2** was identified by searching for natural products that possess a hydroxamate metal chelating group and methionine-like structure after several synthetic transition and/or substrate analogue-based inhibitors were found to be inactive in whole cell assays [29]. Applying a combinatorial chemistry approach to the lead optimisation of actinonin **2** led to the identification of several promising compounds, such as VRC3375 [30,31], VRC4307 [32] and LBM-415 (NVP-PDF-713) **1** [33–35]. LBM-415 **1** is currently in Phase I clinical evaluation by Novartis in collaboration with Vicuron. British Biotech (now Vernalis) also identified a related PDF inhibitor BB-3497 independently by the screening of a chemical library of potential metalloenzyme inhibitors [36]. Resistance to PDF inhibitors has been observed through mutation of the deformylase enzyme [37,38]. Recent papers have reported that LBM-415 **1** has excellent antibacterial activity and a low level of bacterial mutation rate [34,35] but is susceptible to efflux from *Haemophilus influenzae* [39].

Pleuromutilin **16** is a fungal metabolite discovered in the 1950s that exerts its antimicrobial activity by binding to the 50S bacterial ribosome [40]. GlaxoSmithKline have been evaluating a pleuromutilin derivative, retapamulin (SB-275833) **15**, in Phase III clinical trials as a topical antibiotic for skin infections and expect to file a new drug application (NDA) by the end of 2005 [41–43]. Another pleuromutilin derivative (code 565154) is in Phase I clinical trials as an oral antibiotic [40]. Although there are no pleuromutilin **16** derivatives in human clinical use, two semi-synthetic derivatives tiamulin and valnemulin, are widely used as antibiotics for the treatment of swine diseases.

Ramoplanin is a lipopeptide antibiotic complex isolated from *Actinoplanes* sp. ATCC33076, which consists of factors A1, A2 and A3 that have similar antibacterial profiles [44,45]. The major component, factor A2 **17**, is in clinical trials and is known as ''ramoplanin''. In preclinical studies, ramoplanin **17** displayed excellent activity against methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE) and *Clostridium difficile* [46]. Oscient Pharmaceuticals is evaluating ramoplanin **17** for the treatment of *C. difficile*-associated diarrhoea (CDAD) in Phase II trials and was granted fast track status for this use by the FDA in February 2004 [47]. Oscient have also evaluated ramoplanin **17** for the treatment

Table 3 – Natural product-derived compounds in antibacterial clinical trials (current 30 July 2005)

Name (synonym)	Class (lead compound)	Development status	Developer
LBM415 (NVP-PDF-713) **1**	New class[a] (actinonin **2**)	Phase I	Novartis
Rifalazil (ABI-1648, KRM-1648) **3**	Ansamycin (rifamycin B)	Phase II	ActivBiotics
Ceftobripole medocaril (BAL-5788) **4**	β-Lactam – cephalosporin	Phase III	Basilea and J&J
PPI-0903 (TAK-599) **5**	β-Lactam – cephalosporin	Phase I	Cerexa
RWJ-442831 **6**	β-Lactam – cephalosporin	Phase I	J&J
CS-023 (R1558) **7**	β-Lactam – carbapenem	Phase II/Phase I	Roche/Sankyo
Tebipenem pivoxil (ME1211) **8**	β-Lactam – carbapenem	Phase II	Meiji Seika Kaisha
ME1036 (CP5609) **9**	β-Lactam – carbapenem	Phase I	Meiji Seika Kaisha
Faropenem daloxate **10**	β-Lactam – penem	Phase III	Replidyne
Dalbavancin **11**	Glycopeptide (A40926)	NDA	Vicuron
Telavancin (TD-6424) **12**	Glycopeptide (vancomycin)	Phase III	Theravance
Cethromycin **13**	Macrolide (erythromycin)	Phase III	Advanced Life Sciences
EP-013420 **14**	Macrolide (erythromycin)	Phase I/Phase I	Enanta/Shionogi
Retapamulin (topical) (SB-275833) **15**	New class (pleuromutilin **16**)	Phase III	GlaxoSmithKline
Pleuromutilin derivative (565154)	New class (pleuromutilin **16**)	Phase I	GlaxoSmithKline
Ramoplanin **17**	New class (ramoplanin)	Phase II	Oscient
NXL103 (XRP2868)–RPR132552A **18** and RPR202698 **19**	Streptogramin	Phase I	Novexel
PTK 0796 **20**	Tetracycline	Phase I	Paratek
Tiacumicin B (PAR-101, OPT-80) **21**	New class (tiacumicin)	Phase II	Par

[a] Class not previously found in antibacterial drugs marketed for human use.

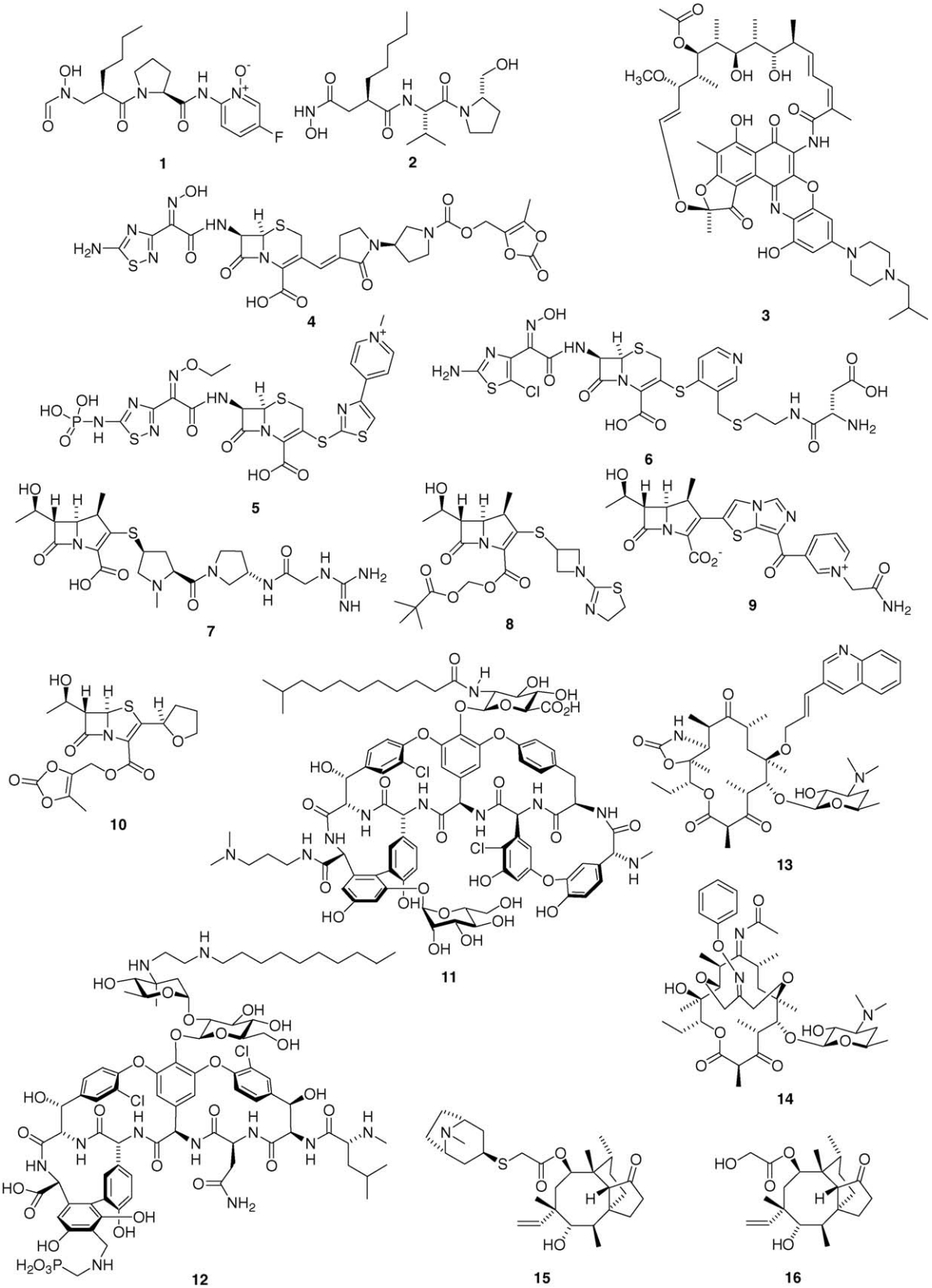

Fig. 1 – Chemical structures of compounds 1–16 in antibacterial clinical trials. Part 1.

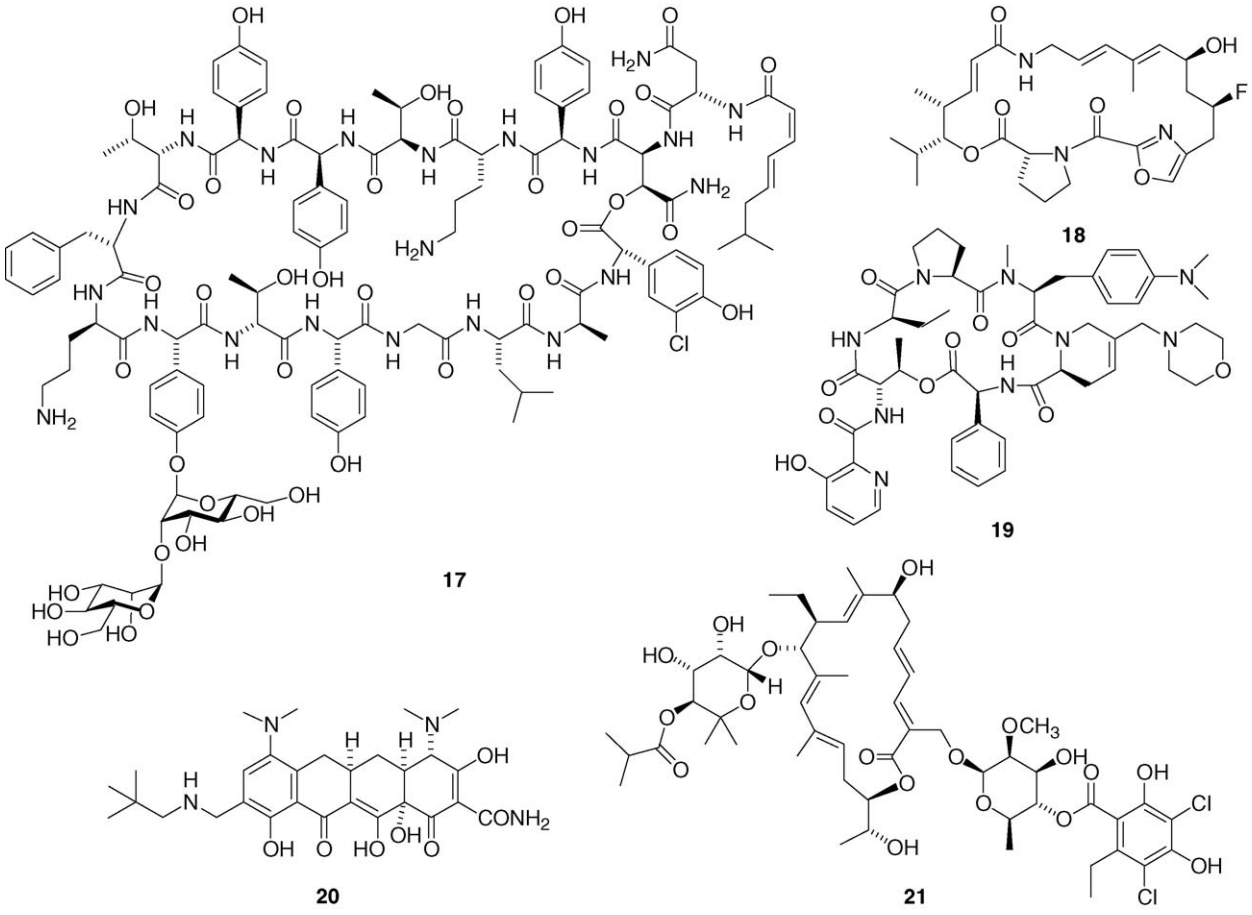

Fig. 2 – Chemical structures of compounds 16–21 in antibacterial clinical trials. Part 2.

of VRE, but no clinical trials are currently in progress. Ramoplanin **17** is thought to exert its antibacterial activity by binding to the peptidoglycan intermediate Lipid II (C_{35}–MurNAc–peptide–GlcNAc) and disrupting bacterial cell wall synthesis [45]. The lipid side chain has been shown to be necessary for antibacterial activity, but not important for Lipid II binding.

Tiacumicin B (PAR-101, OPT-80, lipiarmycin A3 and clostomicin B1) **21** is the major component of the tiacumicin antibiotic complex produced by *Dactylosporangium aurantiacum* spp. *hamdenensis* NRRL 18085 [48–51]. Tiacumicin B **21** exerts its antibacterial activity through inhibition of RNA synthesis [52], possesses broad-spectrum Gram-positive antibacterial activity and is especially active against various *Clostridium* species [53–56]. Tiacumicin B **21** is being evaluated in Phase II clinical trials by Par Pharmaceuticals for the treatment of CDAD and fast track status has been granted by the FDA for this indication [57,58].

3. New antibacterial templates

Drugs which contain new antibacterial templates with novel mechanisms of action should have advantages over known antibacterials in the fight against multi-drug resistant bacteria and the emergence of new pathogens. The following compounds, arylomycin **22**, GE23077 **23**, mannopeptimycin **24**, muraymycin **25**, caprazamycin **26**, nocathiacin **27** and ECO-0501 **28,** potentially represent such new classes of antibacterial agents (Fig. 3).

The arylomycin antibiotic complex, which showed activity against Gram-positive bacteria, was first reported from *Streptomyces* sp. Tü 6075 in 2002 [59,60]. In 2004, Paetzel et al. reported the X-ray crystal structure of arylomycin A_2 **22** in a complex with *Escherichia coli* Type I signal peptidase (SPase) Δ2–75, which established the mechanism of action of these antibiotics [61]. SPase is a membrane-bound serine endopeptidase that catalyzes the cleavage of the amino-terminal signal peptide from secretory and membrane proteins [62]. SPase I is considered an attractive antibacterial target because it is essential for bacterial viability and growth. Shortly after Paetzel's paper was published, workers at Lilly confirmed that related compounds to the arylomycins were competitive inhibitors of SPase I with K_i values of 50–158 nM, but only displayed moderate activity against a panel of Gram-positive and -negative bacteria [63]. However, they were able to demonstrate that these compounds blocked protein secretion and noted that the arylomycins may represent an important new lead for the development of a novel class of broad-spectrum antibiotics [63].

GE23077 is a mixture of four major cyclic heptapeptide factors A1, A2 **23**, B1 and B2, which were isolated from

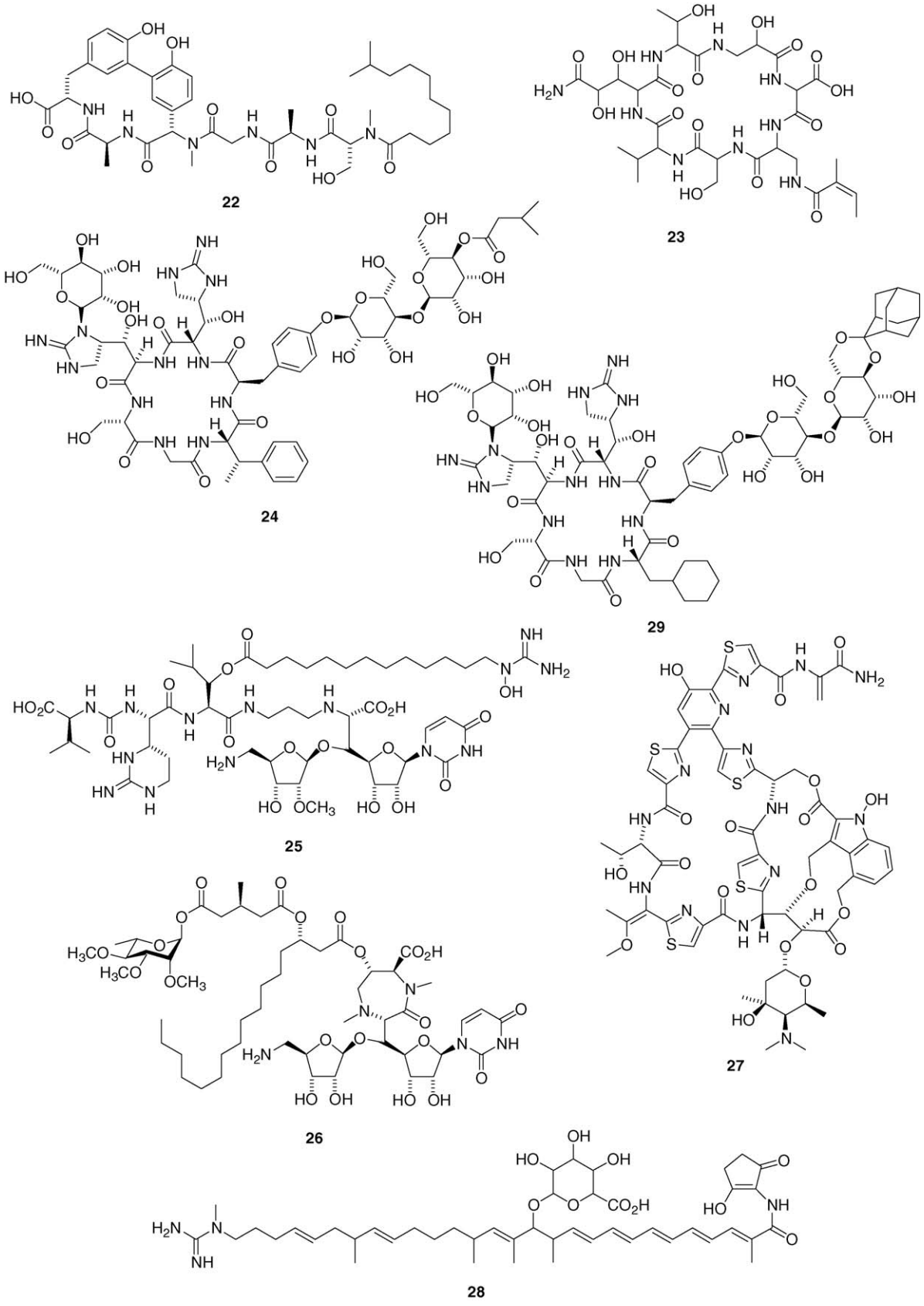

Fig. 3 – Chemical structures of new antibacterial templates.

Actinomadura sp. [64–66]. The GE23077 complex was identified while screening for inhibitors of DNA-directed RNA polymerase (RNAP), which has a central role in DNA transcription and makes it an essential enzyme in bacterial cells. RNAP is the target of the rifampicin group of antibiotics and the clinical candidate tiacumicin B **21**. The factors A and B are closely related and differ only by the presence of an amide with 2-methyl-2-butenoic acid (factor A) and 3-methyl butanoic acid (factor B) and epimers of α-amino-malonic acid unit. Although all the factors display potent inhibitory activity against *Escherichia coli* and *Bacillus subtilis* RNAPs (IC_{50} 0.02 μg/ml), their antimicrobial activity is weak and restricted to only a few pathogens such as *Moraxella catarrhalis*, *Neisseria gonorrhoeae* and *Mycobacterium smegmatis*. It was suggested that the weak whole cell activity was due to poor penetration across the bacterial membrane or, to a lesser extent, because of bacterial efflux. Results from a medicinal chemistry programme that was aimed to improve bacterial membrane penetration and antibacterial activity of GE23077 factors has been reported recently [67].

Workers from Wyeth have isolated a series of related antibiotics, mannopeptimycins α to ε, from *Streptomyces hygroscopicus* LL-AC98, which have activity against MRSA and VRE [68,69]. Interestingly, the AC98 antibiotic complex was discovered in the 1950s at Wyeth and described in 1970 [70], but their structures were elucidated only after a programme was initiated to examine old antibiotics. The mannopeptimycins (e.g. mannopeptimycin ε **24**) are thought to interfere with the late stages of cell wall biosynthesis by inhibition of transglycosylation through binding to Lipid II [68]. The presence and location of the isovaleryl group is important for antibacterial activity and SAR studies have identified semi-synthetic analogues such as AC98-6446 **29** which possess improved antibacterial activity and safety profiles [68,71–73].

The uridyl peptide (or lipo-uridyl) antibiotic class has been shown to inhibit bacterial translocase, an enzyme which catalyzes the transfer of peptidoglycan precursor, phosphoryl-MurNAc pentapeptide, from uridine 5′-monophosphate in the cytosol to the membrane-bound C55-undecapenyl phosphate lipid carrier [74]. Members of this antibacterial class with two adjacent nucleosides attached to the uridyl moiety include the liposidomycins (discovered 1985), FR-900493 (1989) and the newly reported muraymycins (2002) and caprazamycins (2003) [74]. The muraymycins (e.g. muraymycin A1 **25**) were identified by workers at Wyeth from *Streptomyces* sp. and have been reported to have good Gram-positive, but weak Gram-negative antibacterial activity [75,76]. Two reports of synthetic derivatives of muraymycins from Wyeth have been published [77,78]. The caprazamycins were isolated from *Streptomyces* sp. MK730-62F2 and possess activity against acid-fast bacteria including *Mycobacterium tuberculosis* and *M. avium* [79,80]. The caprazamycins (e.g. caprazamycin A **26**) are closely related to the liposidomycins, which also selectively inhibit bacterial translocase I (MraY) and display activity against *Mycobacterium* [74]. Workers at Aventis have described the synthesis of simpler derivatives of liposidomycins, named the riburamycins, which retain biological activity [74,81].

The interest in thiopeptide antibiotics started with the isolation of micrococcin in 1948 and thiostrepton in 1954 and these and related compounds, which belong to five distinct structural classes a–e, have excellent Gram-positive antibacterial activity [82]. Their mechanism of action is through interference with protein synthesis, either by binding to the L11 binding domain on the 23S ribosomal RNA or by binding to elongation factor Tu. Thiostrepton is the most thoroughly studied thiopeptide antibiotic and has an antibacterial profile similar to penicillin, but suffers from rapid resistance and poor aqueous solubility. In 2002, workers at Bristol-Myers Squibb described the isolation of nocathiacin I (BMS-249524, identical to MJ347-81F4-A) **27**, a series e type thiopeptide antibiotic related to nosiheptide, from *Nocardia* sp. ATCC 202099 [82–85]. Nocathiacin I **27** was identified by screening natural product extracts against a multiple drug resistant strain of *Enterococcus faecium* and was found to be more water soluble at low pH compared to other thiopeptide antibiotics. A medicinal chemistry programme was undertaken to identify compounds with improved water solubility while retaining antibacterial activity, but these compounds have not progressed beyond preclinical studies [86–88].

Recently, workers at Ecopia have reported the structure and biological activity of a novel antibiotic ECO-0501 **28**, that was isolated from the vancomycin-producer *Amycolatopsis orientalis* ATCC 43491 [89–91] after genome scanning for novel biosynthetic pathways [92,93]. More details of genome scanning can be found in Ecopia's paper on the isolation of the antifungal ECO-02301 from *Streptomyces aizunensis* NRRL B-11277 [94]. ECO-0501 **28** has shown activity against Gram-positive bacteria, including MRSA and VRE, and was effective in vivo [90]. Although ECO-0501 **28** contains a polyene moiety, it has a good safety profile [90] and is proposed to exert its antibacterial activity through a potentially novel cell membrane and/or cell wall target [91].

4. Future prospects

Despite the past success of antibiotic drug discovery, at least in the industrially developed world, infectious diseases remain the second-leading cause of death worldwide. Bacterial infections cause 17 million deaths globally, particularly in children and the elderly. Of particular concern are the increasing and relentless resistance of nosocomial pathogens such as *Staphylococcus aureus* to mainline antibiotics and the emergence of multi-drug resistant Gram-negative bacteria. The pace of drug resistance has outstripped the discovery of new antimicrobial agents and there is an urgent need for new antibiotic drugs with novel mechanisms of action. The question is about how we tackle the problem more effectively in the future, particularly given the fact that since 1970, only three new classes of antibiotics have been marketed (Table 1).

The paucity of new antibiotic classes in the clinic does not appear to be the result of a lack of trying, at least until recently. Over the past decade, the genomes of more than 140 bacteria have been sequenced and with this effort have come a stream of novel antimicrobial drug targets, many of which have been developed into high throughput screens [95]. Despite the efforts of many companies to identify antibiotic leads for these targets, only one candidate, the PDF inhibitor LBM-415 **1** [26,27,33–35,37–39], appears to be in clinical trials.

Theoretically, there are no reasons why clinically effective inhibitors cannot be found for such targets. The reasons for the lack of success are multifactorial and include:

- Wasted attempts to transform ''hits'' obtained from in vitro target-based high throughput screening into whole cell active agents.
- Reliance on synthetic compound libraries that were compiled with little or no consideration given to essential ''drug-like'' properties (a mistaken focus on quantity and not quality).
- Significantly diminished use of natural products as a source of novel, relevant chemistry for lead optimisation.
- Commercial disincentives for large pharmaceutical companies to invest in antimicrobial R&D.

Whilst conventional whole cell screening for inhibitors of microbial growth is unlikely to reveal new chemical templates very readily, the development of cell-based antimicrobial assays that respond to the inhibition of specific targets offers better prospects [96]. Reporter gene assays for inhibitors of transcription, translation, cell wall and other biosynthetic pathways have been reported. Assays have also been described in which expression of an antisense RNA confers specific sensitivity to compounds targeting the corresponding gene product [97]. This approach enables the development of high throughput cell-based screens for any essential gene, independent of its biochemical function. In addition to targets essential for bacterial cell growth, targeting resistance mechanisms (β-lactamases, bacterial efflux pumps) or essential pathogenesis factors offer attractive options [98].

Exposure of antimicrobial screens to drug-like chemical diversity, including the relatively complex chemical scaffolds and rich functional group display found in natural products, is a key success requirement [19,99,100]. Natural products are a logical starting point for discovering new drugs to treat infectious diseases. Significant advances in compound separation technology and structure elucidation mean that these former bottlenecks in the discovery process no longer exist [11,12]. The major challenge that remains for natural product-based drug discovery is the willingness of medicinal chemists to take up the task of optimising relatively complex, often chiral chemical scaffolds with arrays of diverse functional groups.

Finally, much debate and lines of print have been devoted to how pharmaceutical companies may be encouraged to invest more into antimicrobial R&D activities [18,20–25]. Groups such as the Infectious Diseases Society of America have suggested various incentives that include shortening the approval process for new antibiotics, offering patent extensions, classifying antibiotics as ''orphan drugs'', providing tax credits, limiting liability for adverse effects and offering advanced purchasing commitments by government [18].

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