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FEATURE ARTICLE

# The renewed challenge of antibacterial chemotherapy†

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**We are currently witnessing a dramatic and alarming increase in the incidence of bacterial infections resistant to most common antibiotics. In this article, we examine briefly the background to this situation, and the mechanisms both of antibacterial action and bacterial resistance. Following this introduction is a discussion of the evolution of the various antibacterial classes and of the current approaches being adopted to keep at bay the threat of a return to a pre-antibiotic era.**

Undoubtedly one of the greatest discoveries of modern times was that of penicillin by Sir Alexander Fleming in the 1920s.[1] Prior to the clinical introduction of penicillin in the 1940s bacteria were responsible for many of the world's most lethal diseases such as pneumonia, plague, gas gangrene, wound sepsis and tuberculosis, which has killed more people than any other disease. As a result of the work of Fleming and contemporaries such as Howard Florey and Ernst Chain, few people today consider bacterial infections as being as life threatening as viral infections, heart disease or tumours.

However today, we are approaching a medical crisis which, according to some experts, could see us return to the 'dark age' of the pre-antibiotic era.

There are several reasons for this rapid reversal of fortune in man's battle against infection. The pressures of natural selection are favourable in bacteria due to their rapid rate of multiplication. Thus a colony treated with a particular antibiotic may contain a small number of organisms which have developed a random mutation which renders it less susceptible to the antibiotic. Should these bacteria survive the treatment, for example when the patient fails to complete the prescribed course, they can then multiply, causing an infection less susceptible to the antibiotic class previously used. A second factor is the over-prescription of antibiotics common throughout the world. Many people suffering from viral infections are given, as a matter of routine, antibacterial agents. Similarly, huge quantities of antibiotics are used indiscriminately in meat and dairy farming and crop spraying at doses below those necessary to eradicate bacteria. This is an ideal medium for the selection of resistant strains. Another reason is the increase in the number of immunocompromised patients such as those taking anticancer drugs, immunosuppressants following organ transplants and AIDS patients. These people need to take extended antibacterial regimens as prophylaxis against infection. Pharmaceutical companies also believed for many years that antibacterial agents were no longer a high priority and shifted their resources into other, less well-covered therapeutic areas. Thus, when resistance to the available antibiotics first started to become a problem, there were few novel agents ready to replace them.

However, in the last few years, most of the major pharmaceutical companies have committed themselves to large research programmes aimed at both improving the potency of existing classes and, more notably at discovering novel approaches to antibacterial therapy.

Some of the more common bacterial pathogens and their associated disease states are summarised in Table 1.

Various resistance mechanisms are present in a wide variety of bacteria (Table 2), in many cases because of the transfer of resistance genes between different organisms. Resistant bacteria colonising hospital wards, operating theatres, equipment and even healthcare workers are now a major problem. Only a few years after the introduction of penicillin, *Staphylococcus aureus* strains became resistant due to the production of β-lactamase enzymes which hydrolysed the antibiotic before it had a chance to act.[2] Scientists countered this problem by developing β-lactamase stable agents such as methicillin, but again the bacteria fought back by altering the cell-wall target for β-lactams, the penicillin binding proteins (PBPs), to give methicillin resistant *Staphylococcus aureus* (MRSA). For the life-threatening infections caused by MRSA, the only viable treatment is vancomycin. As will be discussed later, some *Enterobacter* strains, in addition to being unaffected by all other classes of antibiotics, are also resistant to vancomycin (VRE). It has also been shown to be possible to create vancomycin resistant *S. aureus* mutants in the laboratory.[3] If and when this occurs in a hospital man will find himself, for the first time in

**Table 1** Common bacterial pathogens and their associated disease states

| Class | Associated diseases |
|---|---|
| **Gram-positive** | |
| *Staphylococci* | abscesses |
| | endocarditis |
| | meningitis |
| | wound infections |
| *Streptococci* | pneumonia, ear, nose and throat infections |
| *Enterococci* | endocarditis |
| *Clostridium* spp. | gas gangrene |
| | tetanus |
| | colitis |
| *Bacillus* spp. | anthrax |
| | food poisoning |
| **Mycobacteria** | |
| *M. tuberculosis* | tuberculosis |
| *M. leprae* | leprosy |
| *M. avium-intracellulare* | disseminated mycobacterial disease in AIDS patients |
| **Gram-negative** | |
| *Escherichia coli* | food poisoning UTI[a] |
| *Enterobacter* spp. | UTI[a] |
| *Proteus* spp. | UTI[a] |
| *Pseudomonas aeruginosa* | burns infections, cystic fibrosis infections |
| | pneumonia |
| *Neisseria* spp. | meningitis |
| | gonorrhoea |
| *Klebsiella* spp. | pneumonia |
| | UTI[a] |
| *Salmonella* spp. | food poisoning |
| | typhoid fever |
| *Yersinia pestis* | plague |
| *Haemophilus* spp. | meningitis |
| | pneumonia |

[a] UTI = Urinary tract infection.

half a century, unable to treat one of the most problematic pathogens of the pre-antibiotic age.

The rest of this short review is dedicated to an analysis of the various classes of antibiotics currently in either clinical use or undergoing clinical trials and consideration of the problems they face, before taking a look at possible approaches towards the next generation of antibacterial agents which will hopefully arise from the rapid advances in both biology and chemistry we are currently witnessing.

A qualitative impression of the amount of research being carried out on the various antibiotic classes can be gained by examining the number of patents issued each year. A schematic representation of a study of this kind is given in Fig. 1.[4]

## Penicillins

The revolution brought about by Fleming's discovery was based on a very simple β-lactam molecule, penicillin, which acts by irreversibly acylating proteins vital for the construction of the bacterial cell wall. These penicillin binding proteins (PBPs) have no mammalian counterpart, making the β-lactams one of the most selective drug classes known. The evolution of the β-lactam antibiotics has led to the development of several distinct structural classes (Fig. 2). As can be seen, the amount of novel research being carried out on penicillins (penams) is now very limited, and will therefore not be discussed here.[5]

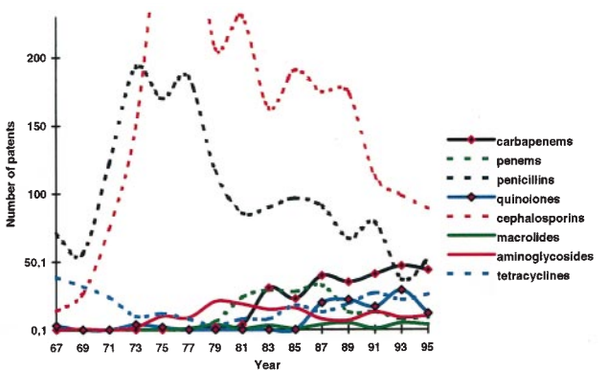



**Fig. 1** The changes in antibiotic research as shown by patent publications

## Cephalosporins

A similar overall picture is apparent also for the cephalosporins (cephems), with increasing structural complexity, heavy patent coverage and the development of resistant strains leading to a reduction in research effort on this class.[6]

What research is still being done on cephalosporins is aimed at trying to obtain activity against specific pathogens. As a way of improving activity against the notoriously impermeable pathogen *Pseudomonas aeruginosa*, some compounds have been made with the aim of exploiting the tonB-dependent iron uptake mechanism by incorporating a catechol or catechol-like group.[7,8] No catechol-bearing β-lactam has yet completed clinical trials but the approach is nevertheless an interesting one.

In an attempt to regain activity against MRSA some cephalosporins bearing bulky aromatic substituted chains on the six-membered ring have been made.[9,10]

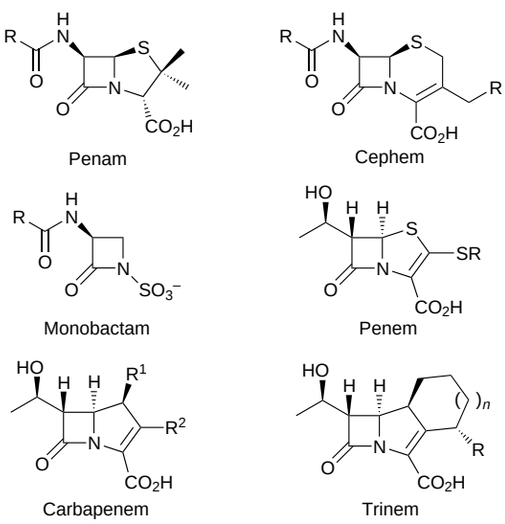


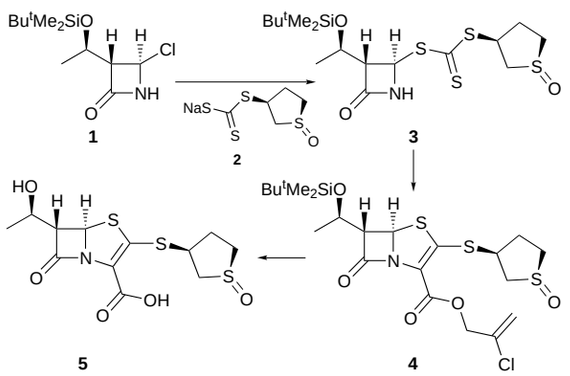

**Fig. 2** Structural classes of β-lactam antibiotics

**Table 2** Antimicrobial resistance by class

| Antimicrobial class | Mode of action | Baterial resistance mechanisms |
|---|---|---|
| β-Lactams | Cell wall biosynthesis inhibition | β-Lactamases<br>Altered PBP targets<br>Altered permeability |
| Glycopeptides | | |
| Fluoroquinolones | DNA gyrase A and topoisomerase IV inhibitors | Alteration of enzyme targets<br>Efflux pumps<br>Altered permeability |
| Aminoglycosides | Protein biosynthesis inhibitors | Enzymatic modification of antibiotic<br>Alteration of ribosome target |
| Chloramphenicol | | Enzymatic modification of antibiotic<br>Altered permeability |
| Macrolides | | Alteration of *23S* rRNA<br>Enzymatic modification of antibiotic |
| Streptogramins | | Alteration of ribosome target<br>Active efflux<br>Enzymatic modification of antibiotic |
| Tetracyclines | | Efflux pumps<br>Aleration of ribosome target<br>Altered permeability<br>Enzymatic modification of antibiotic |
| Sulfonamides<br>Trimethoprim | Folic acid biosynthesis inhibitors | Altered permeability |

## Penems

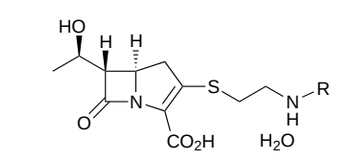
The penems are a purely synthetic class of β-lactam antibacterial agents.[11] It may be said, however, that considering the amount of extremely elegant research invested in the penem class, they have, from the clinical point of view, been decidedly disappointing. The main reasons for this are the difficulty of synthesis on a multi-tonne scale and the advent of the generally more potent carbapenem class. There is still however some interest in the penems, and there are currently a number of penems in clinical development including sulopenem **5** (Scheme 1). The key steps of the synthesis of compound **5** are the introduction of the thioxanthate substituent onto the transient azetinone generated from the 4-chloroazetidinone **1**. The resulting compound **3** is cyclised *via* an oxalimide route to the penem **4** which is then deprotected by standard methods to give sulopenem **5**.



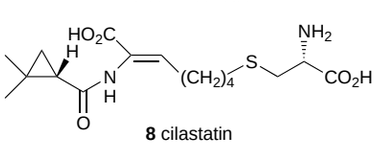
**Scheme 1**

## Carbapenems

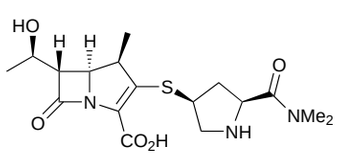
The first carbapenem, thienamicin **6**, was discovered as a natural product of *Streptomyces cattleya* in the late 1970s. Its formamidine derivative imipenem **7** was found to be much more chemically stable, and for a number of years has been an invaluable weapon against serious bacterial infection. Despite its chemical stability (due to the 6-hydroxyethyl side-chain it is also stable to all *conventional* β-lactamases), imipenem is rapidly inactivated in the kidney by mammalian dehydropeptidases (DHP) and is also nephrotoxic. This necessitates its co-administration with cilastatin **8**, which functions both as an inhibitor of DHP and reduces the renal accumulation of imipenem.[12]



**6** R = H
**7** R = CH=NH



**8** cilastatin



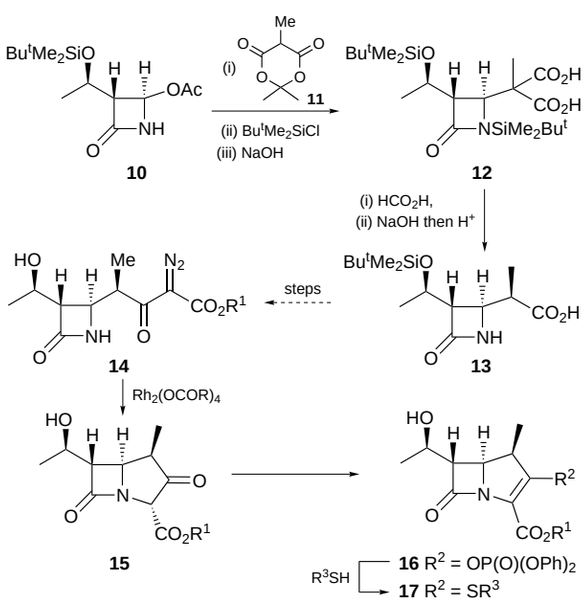
**9** meropenem

Meropenem **9** has, by the introduction of a 1β-methyl substituent, eliminated the necessity of co-administration with cilastatin.[13] This, along with the fact that it is possible to inject the compound as a single large bolus dose prior to surgery (whereas imipenem must be administered more gradually, both to reduce the risk of seizures and to overcome its lower solubility), is considered to be the main advantage of meropenem over imipenem as, in terms of clinical relevance, their antibacterial spectra are both impressive.

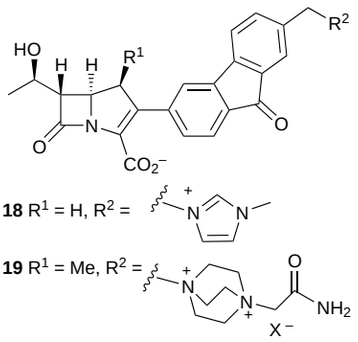
Although carbapenems have their origins as natural products, efficient, large-scale fermentation processes for key intermediates have yet to become commercially viable. Two key intermediates in the synthesis of 1β-methyl carbapenems are the 4-acetoxyazetidinone **10** and the propionic acid derivative **13**, in which all of the stereocentres in the final compounds are defined (Scheme 2).[14] While the need to explore novel syntheses of compound **10** (which is available commercially on a multi-tonne scale) is questionable, the control over the stereochemistry of the methyl group in **13** has led to many elegant approaches. One such route is shown in Scheme 2.[15] After alkylating the 4-acetoxyazetidinone **10** with the Meldrum's acid derivative **11**, *N*-silylation and saponification gave the diacid **12**, which was decarboxylated with catalytic formic acid to give the desired acid **13** in good yield and in a 94 : 6 ratio of β : α isomers. The transformation of acid **13** into a cyclised carbapenem system **17** is also outlined in Scheme 2.[16] Chain elongation to the diazo derivative **14** allowed an intramolecular rhodium octanoate-catalysed carbene insertion to the carbapenam keto ester **15** which was transformed into the enol phosphate **16**, an excellent substrate for a wide range of nucleophiles.

The current challenges in the discovery of novel carbapenems are largely based on broadening the spectrum even further, to include a wider range of problematic pathogens. As in the case of other β-lactams, carbapenems are largely ineffective against MRSA, as they have a low affinity for the PBP2' produced by these strains. The addition of a lipophilic group near the 'tail' of the molecule can increase affinity for PBP2', and workers at Merck have used this approach to synthesise anti-MRSA carbapenems such as L-695,256 **18** and its more soluble analogue L-742,728 **19**[17].

Resistance to carbapenems in *B. fragilis* and *P. aeruginosa* due to novel zinc-mediated metallo β-lactamases is starting to make an appearance, and will almost certainly increase in the coming years.[18] These enzymes are able to recognise the



**Scheme 2**

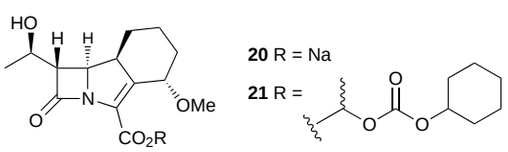



hydroxyethyl side-chain which protects carbapenems from more traditional serine β-lactamases and in order to have stable compounds in the future it will be necessary to either modify the hydroxyethyl side chain (while trying to maintain the affinity for the PBPs) or design a new generation of metallo β-lactamase inhibitors.

A third mechanism of resistance exploited by *P. aeruginosa* is to suppress the synthesis of the D2 porin in the outer membrane through which imipenem and amino acids pass. However, it has been shown that meropenem maintains activity against these resistant strains, indicating that it has an alternative method of reaching its target.[19]

## Trinems

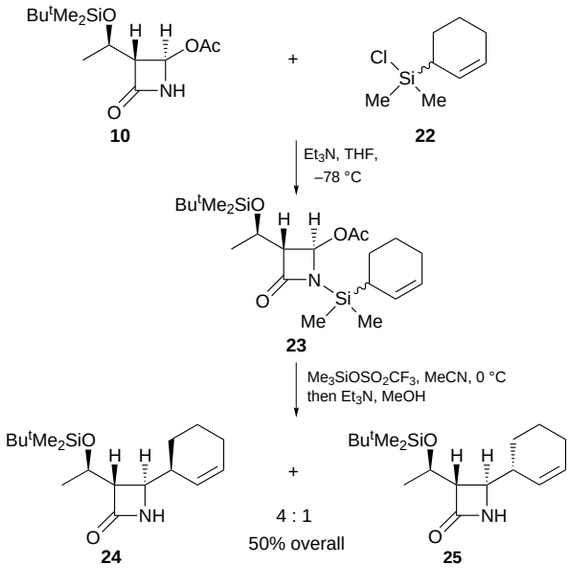
In the past few years there has been an increase in the exploration of novel β-lactam containing ring systems. Despite being structurally and synthetically interesting, many of the compounds generated in these programmes were unable, or unlikely, to exhibit significant antibacterial activity.[20] However, one both synthetically challenging and clinically relevant new class, the trinems, has emerged.[21]




Sanfetrinem **20**, the (4*S*)-methoxytrinem, and its orally bioavailable ester sanfetrinem cilexetil **21** are currently in Phase II clinical trials.[22] Its broad spectrum, which includes anaerobes and penicillin resistant *Streptococcus pneumoniae*, resistance to clinically relevant β-lactamases and stability to the renal dehydropeptidases which inactivate many penems and carbapenems, make it a promising agent for community acquired infections.

The challenge of the large-scale asymmetric synthesis of sanfetrinem has resulted in the recent publication of several quite different approaches. The original large-scale route to this compound, which was used successfully to produce the multikilogram quantity required for the Phase I study, suffered from a low overall yield and high number of steps.[21]

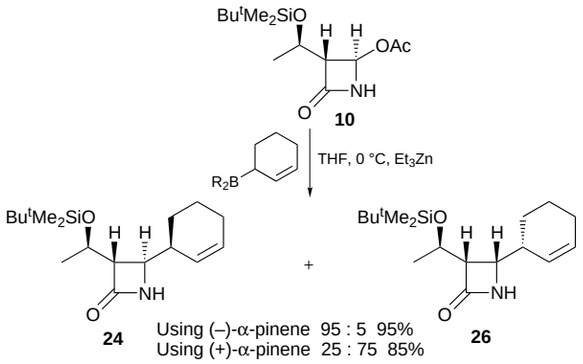
More recent approaches, however, have both reduced the number of steps and improved the overall yields to key intermediates. Two approaches to olefin **24** published recently involve an intramolecular Sakurai reaction with a cyclohexenylsilane[23] (Scheme 3), and dialkylcyclohexenylboranes[24] and appropriate Lewis acids (Scheme 4). An even more direct approach to sanfetrinem is *via* the direct condensation of (2*S*)-methoxycyclohexanone with the 4-acetoxazetidinone **10**. This has been achieved either *via* a Mukaiyama-like condensation (Scheme 5),[25] or more recently *via* zinc[26] or zirconium[27] enolates. A quite different approach to the key methoxy ketone **29** has also been published in a recent total synthesis of sanfetrinem.[28] With this method, the stereocontrol at the 6-position of the cyclohexanone ring is obtained by exploiting



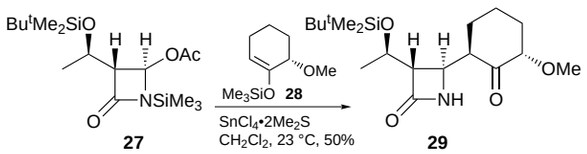

**Scheme 3**

the decarboxylation method originally applied to 1β–methyl carbapenems.[15] More recently still, a route to compound **29** using chelated tin(IV) enolates has been reported (Scheme 6).[29] In this method, optically active 2-methoxycyclohexanone is transformed into its stable tin(IV) chloride chelate **30**, which is then treated with a tertiary amine base, forming the corresponding enolate. This enolate then reacts with the 4-acetoxyazetidinone **10** and additional tin(IV) chloride to give the desired methoxy ketone **29**. This method, which was developed with the aid of low temperature NMR studies, has proved to give both high yields and stereocontrol and appears adaptable to a very large scale.

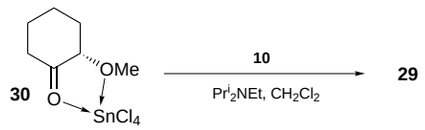
The promising biological activity obtained with sanfetrinem **20**, and the fact that this class presents a considerable synthetic




**Scheme 4**

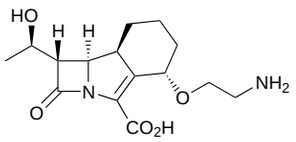



**Scheme 5**




**Scheme 6**

challenge, has encouraged research into other trinem molecules. Among the 4-alkoxy derivatives reported recently,[30] the 4-aminoalkoxy derivative **31** exhibited a promising biological



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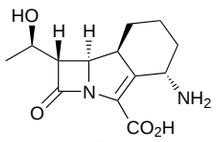
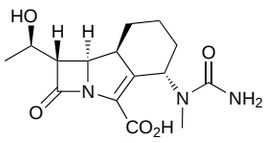


**32**



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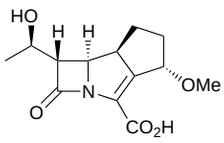

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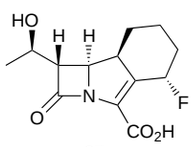

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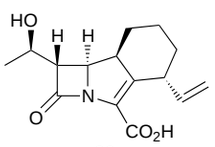


**36**

activity including *P. aeruginosa*. Other trinems reported recently include some 4-amino derivatives such as **32**,[31] 4-ureidotrinems including **33**,[32] the 5-membered ring analogue of sanfetrinem **34**,[33] stereo- and regio-isomers of sanfetrinem,[34] the highly unstable 4-fluorotrinem **35**[35] and a number of 4-alkenyl derivatives such as **36**.[36]

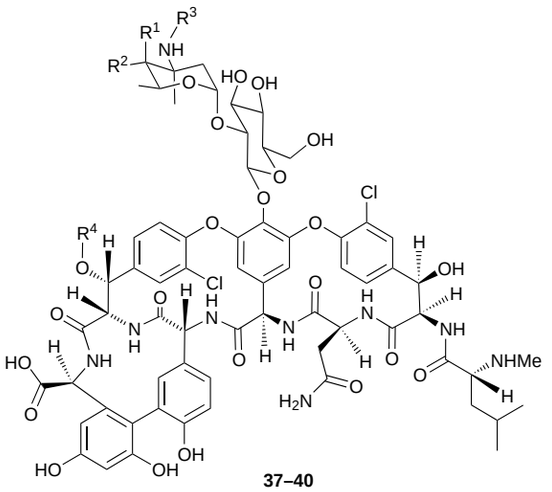
## Glycopeptides

The glycopeptides are a family of antibiotics isolated from a variety of species including actinoplanes and streptomyces.[37] Vancomycin **37** and teicoplanin **41** are the most well-known members of this class and are used widely in the clinic as the drugs of choice in treating resistant Gram-positive species such as (MRSA), coagulase-negative staphylococci and multi-resistant enterococci.

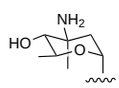
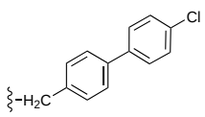
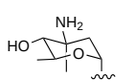
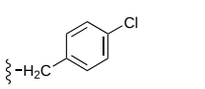
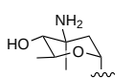
Five of the seven amino acids present are common to all glycopeptides, and their mode of action is to inhibit cell wall synthesis by interfering with a late stage in the assembly of the peptidoglycan. The conformation of the glycopeptides allows them to form a stable complex, by means of a hydrogen bond network, with the terminal dipeptide D-Ala-D-Ala of the peptidoglycan chain. This prevents both transglycosylation (chain elongation) and transpeptidation (cross-linking) with a consequent block of cell growth, subsequently leading to cell death. While the aglycone nucleus is responsible for the complexation of the D-Ala-D-Ala units, the sugar groups stabilise the complex (the modulation effect).

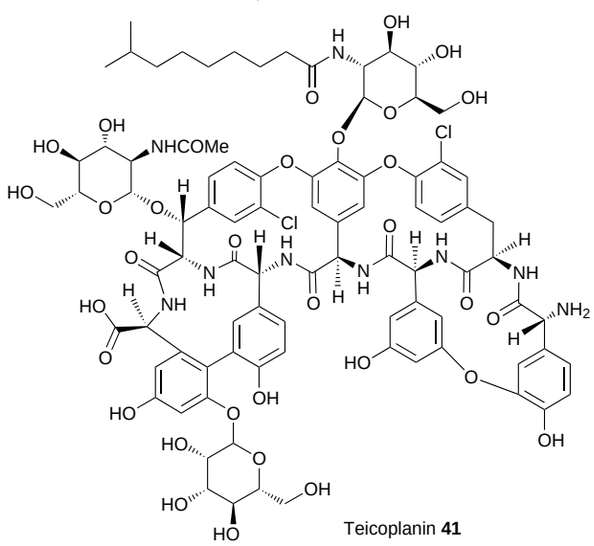
In recent years, a rapid increase in the incidence of infection and colonisation with vancomycin-resistant enterococci (VRE) has been reported from US hospitals, and the possibility of this resistance being transferred to *S. aureus* in the clinic is a cause for major concern. The mechanism of glycopeptide resistance in enterococci is well-understood and involves an alteration of the peptidoglycan precursor in which the terminal D-alanine is replaced with D-lactate, a change that reduces the ability of the antibiotic to bind to its target.[38]

In order to overcome these resistance problems, new semisynthetic glycopeptides have been synthesised. Based on the observation that the introduction of lipophilic chains onto the amino sugar vancosamine of vancomycin enhanced activity against resistant enterococci, several derivatives were prepared *via* reductive alkylation of LY264826 **38**[39] (a derivative of vancomycin). Among these, the *p*-chlorophenylbenzyl derivative LY333328 **39**[40] was identified as the most potent compound against vancomycin resistant enterococci and has undergone preclinical studies. Recent studies on the prototype



**37–40**

| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| vancomycin **37** | OH | H | H | H |
| LY264826 **38** | H | OH | H |  |
| LY333328 **39** | H | OH |  |  |
| LY191145 **40** | H | OH |  |  |

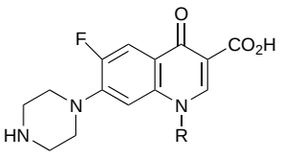


Teicoplanin **41**

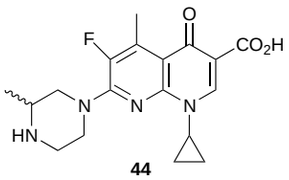
*p*-chlorobenzyl derivative LY191145 **40**[41] have shown it to still have a poor affinity for D-Ala-D-Lac residues. However, the ability of LY191145 **40** to inhibit peptidoglycan biosynthesis may be explained in part by strong dimerization, allowing it to bind to two D-Ala-D-Lac units at the cell surface. Moreover, the hydrophobic side chain confers the ability to bind to bacterial membranes, anchoring the agent at the membrane-associated target site.[38]

## Fluoroquinolones

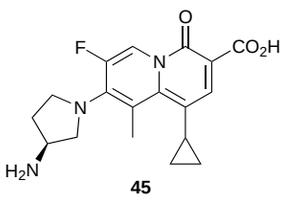
The introduction, in the 1980s of fluoroquinolones such as norfloxacin **42** and ciprofloxacin **43** provided clinicians with a valuable weapon against infections.[42] These compounds are orally active, have a broad-spectrum and favourable pharmacokinetic and safety profiles.



**42** R = Et
**43** R = cyclopropyl



**44**



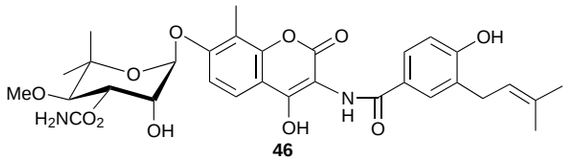
**45**

The primary mode of action of the quinolones is the inhibition of gyrase, a topoisomerase II responsible for the introduction of negative supercoils in bacterial DNA during its replication cycle.[43] Quinolones bind to the gyrase A subunit when it is complexed to the DNA, effectively blocking the supercoiling process. It has been shown that fluoroquinolones are also able to inhibit topoisomerase IV in several species including *S. pneumoniae*, *S. aureus* and *E. coli* and in some cases this may be the principal mode of antibacterial activity.[44]

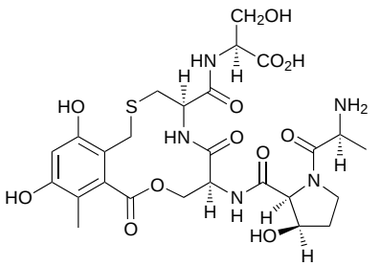
The main challenge of current quinolone research is to improve Gram-positive and anaerobe potency.[45] Two compounds currently addressing these issues are grepafloxacin **44** and the structurally interesting 2-pyridone ABT-719.1 **45**.[46]

## Cyclothialidines

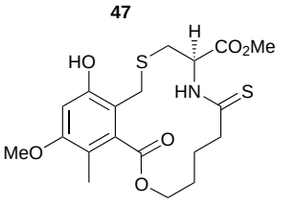
As mentioned previously, quinolones bind to the bacterial gyrase A subunit, thus blocking supercoiling. Gyrase is however a tetrameric protein comprised of two A and two B subunits. The B subunits have ATPase activity, necessary for the completion of the supercoiling cycle. When compared to the effort expended on quinolones, this target has, in the past, been largely overlooked. In the 1960s the coumarin antibiotics, such as novobiocin **46** were used clinically, although their toxicity and resistance development meant that they are now employed very rarely. Recently, however, scientists at both Hoffman-La Roche[47] and GlaxoWellcome discovered a new class of gyrase B inhibitors, the cyclothialidines. Despite having excellent activity against isolated gyrase B, cyclothialidine **47** has little or no activity against bacteria. More recently, scientists at Roche have been able to synthesise analogues, such as compound **48**, which act against a wide range of bacterial species. Several



**46**



**47**

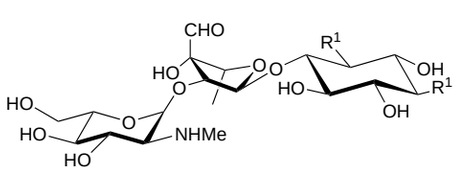


**48**

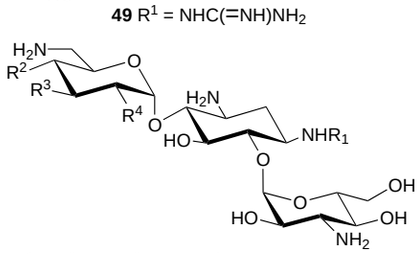
gyrase B protein subunit-inhibitor complexes have also been co-crystallised recently,[48] and it is hoped that the information gained from their X-ray crystal structures may aid the design and synthesis of novel gyrase B inhibitors.

## Aminoglycosides

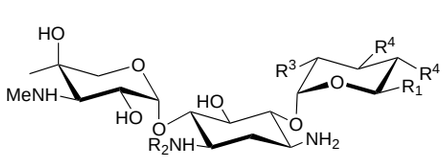
The first aminoglycoside discovered, streptomycin **49** was isolated from *Streptomyces griseus* in 1944. Since then, around ten compounds have found a clinical application, including the natural products gentamicin **50** and tobramycin **52**, and the semisynthetic derivatives amikacin **53**, netilmicin **55** and dibekacin **54**. The aminglycosides are an extremely useful class of antibiotics, especially against serious Gram-negative bacterial infections,[49] and are frequently used in a synergistic combination with β-lactams. The amino groups present in these molecules are essential for biological activity, but may also be the cause of the nephrotoxicity associated with this class. The binding of the aminoglycoside to the ribosome causes misreading of a protein which is then incorporated into membranes, causing the formation of abnormal membrane channels and



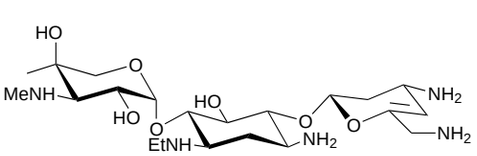
**49** $R^1$ = NHC(=NH)$NH_2$



**52** $R^1 = R^3$ = H, $R^2$ = OH, $R^4 = NH_2$
**53** $R^1$ = (*R*)-COCH(OH)$CH_2CH_2NH_2$, $R^2 = R^3 = R^4$ = OH
**54** $R^1 = R^2 = R^3$ = H, $R^4 = NH_2$



**50** $R^1$ = (*S*)-CH(Me)NHMe, $R^2$=$R^4$=H, $R^3$=$NH_2$
**51** $R^1$ = $CH_2NH_2$, $R^2$ = (*S*)-COCH(OH)$CH_2NH_2$, $R^3 = R^4$ = OH



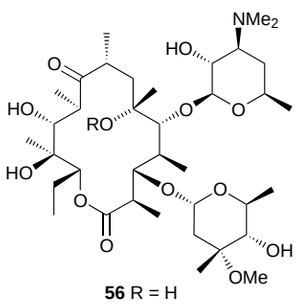
**55**

altered permeability into the bacterial cell. This provokes an autocatalytic increase in aminoglycoside uptake, leading to rapid cell death.

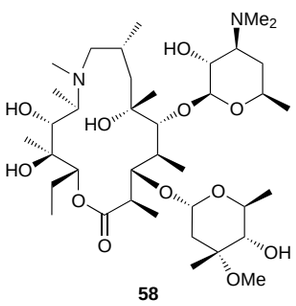
Resistance to aminoglycosides can come about *via* a number of mechanisms. The most significant is the chemical modification of the antibiotic by intracellular enzymes. Low-level resistance may also occur as a result of altered permeability across the cell wall or mutations of the ribosome target. Recently a novel aminoglycoside, isepamicin **51**, has been described as being less susceptible to enzymatic inactivation.[50]

### Macrolides and ketolides

The first macrolide, the 14-membered ring compound erythromycin A **56**, isolated from *Streptomyces erythraeus*, was introduced to clinical use in the early 1950s. Displaying similar activity to penicillins against Gram-positive bacteria, erythromycin A has the advantage of also being active against bacteria having altered outer structures such as *Mycoplasma pneumoniae* and those such as *Legionella* and *Chlamydia* which survive within the host's cells.[51]




**56** R = H




**58**

The macrolides act by inhibiting protein biosynthesis by blocking the peptidyl transferase centre of the *50S* subunit of the bacterial ribosome. Resistance to macrolides occurs either as a result of the enzymatic modification of the antibiotic or modification of targets (mutations of ribosomal proteins or rRNA genes).

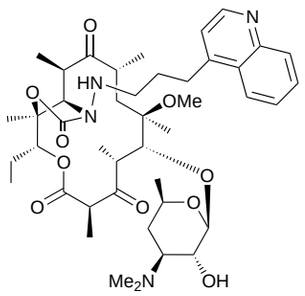
Further research led to the development of semisynthetic macrolides such as dirithromycin, roxithromycin, clarithromycin **57** and azithromycin **58**. These derivatives overcome some of the drawbacks of older agents: poor oral bioavailability as a result of acid instability, gastrointestinal disturbances, poor tissue concentration and short half-life. Azithromycin **58**, a 15-membered azalide antibiotic, is synthesised by a stereospecific Beckmann rearrangement of (9*E*)-erythromycin A oxime, followed by the reduction and reductive *N*-methylation under Eschweiler–Clarke conditions.[52]

Recent research on 14-membered macrolides has led to the discovery of a new distinct class of these macrocyclic lactones, characterized by a 3-keto function instead of the cladinose moiety (hence the name ketolide), well-known for its lability in even weakly acidic media. Currently, the most promising ketolides appear to be RU-66647,[53] RU-64004[54] **59** and TE-802[55] **60**.

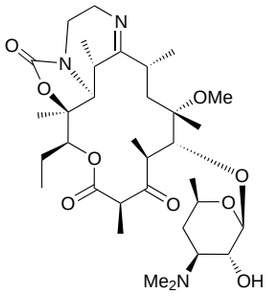
### Streptogramins

Macrolides have the same targets as the lincosamides and streptogramins, and these classes are frequently referred to as the MLS group. The streptogramins are a family of cyclic peptides produced by *Streptomyces* sp.[56] Each member of the class consists of at least two structurally different molecules, distinguished as being of either group A or group B agents. Group A molecules are polyunsaturated macrolactones, whereas group B compounds are cyclic hexadepsipeptides.

Quinupristin **61** and dalfopristin **62** are two injectable semisynthetic streptogramins currently in Phase III clinical trials. The compounds are used in synergistic combination

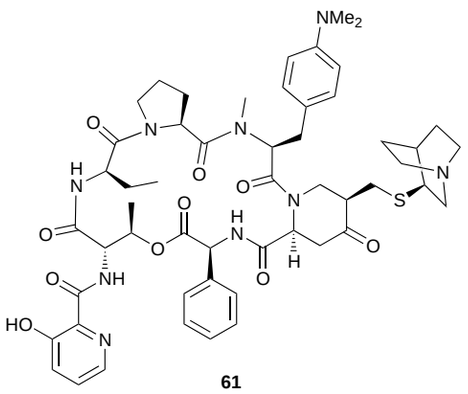



**59**

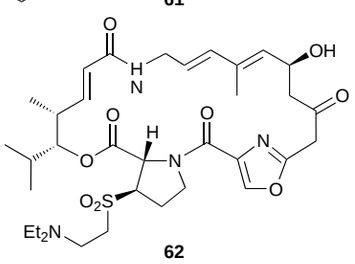



**60**

(RP59500, Synercid®) against Gram-positive bacteria, including multiresistant strains.




**61**




**62**

The synergy observed with the combination of quinopristin **61** and dalfopristin **62** can be explained in the remarkable way they bind to the peptidyl transferase domain of the *50S* ribosomal subunit. This synergy occurs because the attachment of a group A derivative such as dalfopristin **62** to the ribosome results in a conformational change, increasing the affinity for the group B compound quinopristin **61**. The resulting ternary complex is extremely stable and, unlike the bacteriostatic macrolides, streptogramins are bactericidal when used in combination.

More recent research into novel semisynthetic derivatives has allowed the discovery of an orally active streptogramin combination, RP106972, which is currently under investigation.[57]

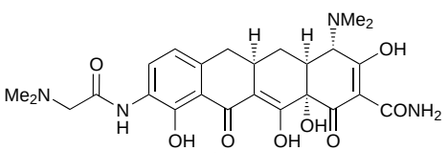
While resistance to single streptogramin components has been documented, it has been suggested that the quinupristin/dalfopristin combination should be less susceptible. However, according to a recent study reported at the 1996 Interscience Conference on Antimicrobial Agents and Chemotherapy (ICCAC) in New Orleans, resistance to the combination has already been seen in vancomycin resistant *Enterococcus faecium*.[58] Whether resistance to streptogramins will become a serious problem will only become apparent as their use in clinical situations becomes more widespread.

### Tetracyclines

Due to their broad spectrum, low toxicity, low incidence of side-effects and low cost, the tetracyclines were used intensively

both in clinical, agricultural and veterinary settings in the half century since their discovery.[59] The tetracyclines are bacteriostatic, and act by blocking the binding of aminoacyl-tRNA to the acceptor site on the *30S* ribosome.

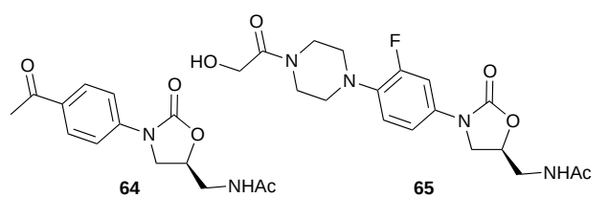
Recently, structurally modified tetracyclines have overcome the severe resistance problems affecting this class. The so-called glycylcyclines maintain potent activity against resistant strains containing ribosomial protection factors (*tetM* and *tetO*) or efflux (*tetA-E*, *-L* and *-K*) determinants.[60] The most promising member of the class is CL 331,928 (DMG-DMDOT) **63** and research into modified tetracyclines is now the subject of new research programmes in several laboratories.



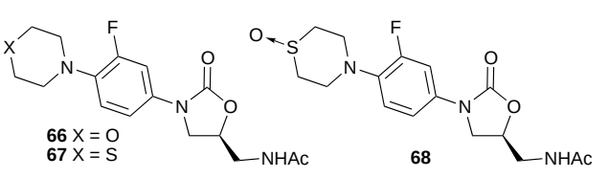
**63**

## Oxazolidinones

The last ten years have seen only one clinically significant novel class of antibiotics emerge from basic research, the oxazolidinones.[61] The pioneering work in this field was carried out at DuPont in the mid-1980s and culminated in the discovery of DUP 721 **64**. However, this molecule was found to exhibit



**64** **65**

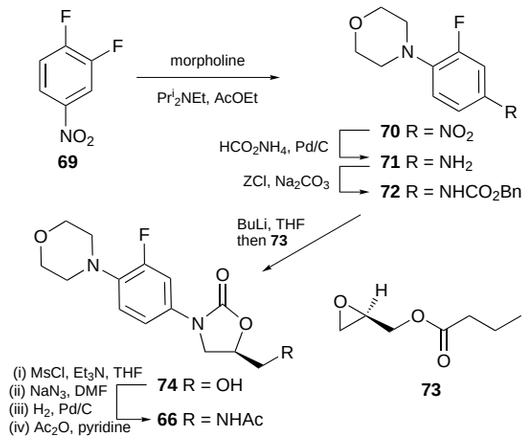


**66** X = O
**67** X = S **68**

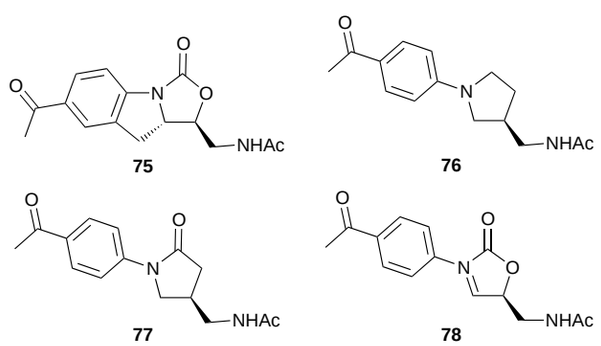
lethal toxicity in *in vivo* tests, and little further work was reported on this class until 1995, when Upjohn published a series of novel compounds. The toxicity problems encountered by DuPont and currently there are two oxazolidinones undergoing clinical trials, U-100592 **65** and U-100766 **66**.[62] Both compounds are active against Gram-positive bacteria, including resistant strains, and offer hope for the treatment of these extremely serious infections in the future. The oxazolidinones are believed to act by inhibiting an early step in bacterial protein synthesis. The versatile asymmetric synthesis of oxazolidinones, exemplified by U-100766 **66**, developed at Upjohn is shown in Scheme 7.

It has also been possible to extend the antibacterial spectrum of the class into mycobacterial species such as *M. tuberculosis* and *M. avium*, the latter being a life-threatening opportunistic infection of immunocompromised patients. Replacing the oxygen atom of the morpholine ring of U-100766 **66** with a sulfur atom gave the potent antimycobacterial oxazolidinone U100480 **67**. It is likely that the *in vivo* activity observed is due, at least in part, to its sulfoxide metabolite U-101603 **68**.

There have been other related but less well documented systems published in the last few years. While Upjohn has recently published *trans*-tricyclic systems such as **75**,[63] other groups have reported oxazolidinone ring-surrogate analogues. In one report, the inactive pyrrolidine **76** and pyrrolidinone **77** were contrasted with the active butenolide **78**.[64] This and related publications have prompted Brickner[62] to define a general structure–activity relationship, summarised in Fig. 3.



**Scheme 7**



**75** **76**

**77** **78**

## The resurgence of tuberculosis

One of the reasons for the interest being shown in the oxazolidinones is their activity against mycobacterial species such as *M. tuberculosis.* Prior to the discovery of the aminoglycoside streptomycin **49** in 1944, there were no effective treatments for tuberculosis (TB). This breakthrough was the start of a new age of chemotherapy for what had been a major killer throughout the world. With the introduction of streptomycin and even more effective therapies, the incidence of tuberculosis, especially in the more developed countries, went into sharp decline, to the point that the disease was no longer perceived to be a serious threat to most people. In recent years, however, this complacent attitude to TB has been seriously challenged. In the majority of the developing countries the number of cases is increasing, and more people died of TB in 1995 than in any other year in history. Even in the industrialised states there is a growing anxiety at the number of new cases being reported. Currently more than one third of the world's population carries *M. tuberculosis*, and in developing countries the disease accounts for over 25% of all preventable deaths.

There are several reasons for this alarming situation. Poverty, resulting in overcrowding and poor hygiene, is undoubtedly a

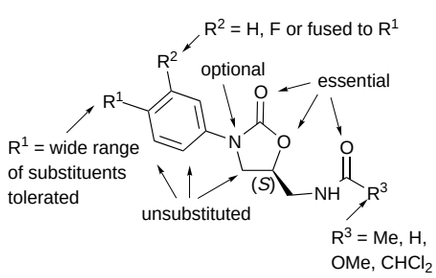
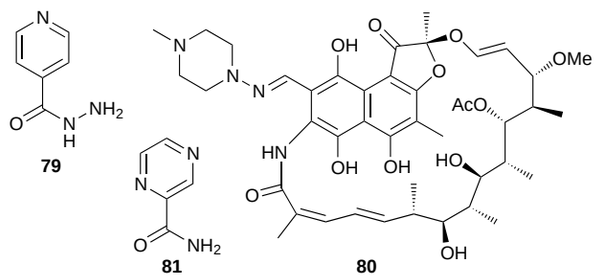


**Fig. 3** General structure–activity relationships

major factor in inner cities. The increase in the number of HIV seropositive people has also created a population less able to combat infection. In Asia, two-thirds of all TB cases are HIV-positive. A third reason is the increase in multidrug-resistant *M. tuberculosis* strains. A typical current therapeutic approach to TB may involve taking isoniazid **79**, rifampin **80** and pyrazinamide **81** for at least six months. As a result, non-



**79** **81** **80**

compliance, *i.e*. patients stopping therapy as soon as an improvement in the symptoms is felt, allows the remaining, more resistant bacteria to survive and spread.

Long-term benefits are unlikely to be gained by modifying existing antimycobacterial agents. The recent increase in research in this field will hopefully lead to the discovery of novel classes of compounds,[65] although the structures of these classes remain, as yet, unclear.

At present, studies are also being carried out to assess the efficacy of existing broad-spectrum antibacterials such as fluoroquinolones.[66] The possibility of using β-lactams in tuberculosis therapy has also been considered.[67] Although *M. tuberculosis* is naturally resistant to this class due to the production of intracellular β-lactamase, the use of elevated doses of newer, β-lactamase-stable agents such as carbapenems has been shown to work *in vitro*.

## Future approaches to antibacterial chemotherapy

The rapid emergence of multidrug resistant bacterial strains and the potential threat they pose to future generations means that the development of novel antibacterials is likely neither to be easy nor to offer a permanent solution to the problem.

Currently, there are two basic strategies for finding novel antibacterials. The first, which has been responsible for the discovery of most novel classes to date, is to screen natural product or corporate compound collections. This strategy provides a compound with demonstrable antibacterial activity, although it may take some time to discover the mode of action of the class, and working with a complex biological system such as a bacterial cell can make the generation of meaningful structure–activity relationships quite problematic, even before taking into account the inter-species diversity encountered.

The second strategy, which has become increasingly popular in recent years, is to identify the essential enzymes of the bacterial cell and to screen compounds as inhibitors of these systems. While this method allows a far greater control over the structure–activity relationship and in many cases facilitates the use of high-throughput screening technology, it involves taking at least one step back from the reality of bacterial infection in that it takes no account of how the compounds are able to permeate the bacterial cell to reach their target, or whether they are rapidly removed from the bacterial cell by efflux or enzymatic inactivation. At present, the number of targets is limited by our knowledge and understanding of gene and protein function. A significant improvement will be brought about by systematic elucidation of complete bacterial genomic sequences.[67] Genes and proteins found to be essential for bacterial cell survival are potential pharmaceutical targets.[68] Thus, using the current state-of-the-art technologies based around the high throughput screening of compound collections, high speed parallel synthesis and natural products will hopefully provide research groups with a number of novel lead classes for further optimisation. In some cases the further optimisation of the classes may be significantly helped by the availability of stuctural information on the target–inhibitor complexes provided by protein crystallography or high field protein NMR and mass spectroscopy.[69]

## Targeting bacterial virulence

An alternative approach currently being examined by several groups is that of modulating the virulence of bacteria.[70,71] For pathogenic bacteria to survive in the hostile environment of another organism they must first be able to attach themselves to host cells and then launch an assault whilst simultaneously debilitating the host immune system. To do this, bacteria produce products called virulence factors, which include attachment factors, toxins and lytic enzymes. Targeting these pathogenesis processes may give protection against a wide range of bacterial species, and result in an infection site-specific as opposed to microorganism specific therapy.

## Conclusions

Owing to the growing threat of infection due to multi-drug resistant bacteria we are already witnessing an increased effort not only aimed at finding more potent examples of existing agents but also, more encouragingly, a serious commitment to discover new classes of antibiotics. These new classes will hopefully evolve through the exploitation of the rapidly expanding number of known targets, the use of the latest techniques for combinatorial chemistry and high throughput screening, and by studying the processes used by the bacteria to establish infection.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## Footnote and References

† This ChemComm is also available in expanded format *via* the World Wide Web: http://chemistry.rsc.org/rsc/cccenha.htm


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