Novelties in the Field of Anti-Infective Compounds in 1999

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In 1999 the number of new compounds reported in the anti-infective field decreased significantly in comparison with previous years, especially for antifungals. The reported new compounds are mainly directed against *Staphylococcus aureus* isolates resistant to methicillin. Few derivatives were reported in the field of anti-infectives for gram-negative bacteria. At the moment, we are in a period of discovery as we await novel compounds that could issue from new engineering.

Despite strong advances, the process of drug discovery is still an arduous task. The main concerns remain gram-positive infections due to resistant strains. The spread of *Streptococcus pneumoniae* strains resistant to penicillin G and erythromycin A and of *Streptococcus pyogenes* resistant to erythromycin A are of concern around the world, with variable but increasing rates. Methicillin-resistant *Staphylococcus aureus* (MRSA) or coagulase-negative staphylococci are still clinical problems, and many compounds have been synthesized or extracted by fermentation from microorganisms and sponges. Compounds targeting MRSA are numerous and belong to many different families of antibacterial compounds, including cephalosporins, carbapenems, coumarinic derivatives, oxazolidinones, even-nomycin, peptides, and cyclins.

The novel drugs of 1999 were efflux pump inhibitors, which were new tools designed to overcome efflux mechanism of resistance by restoring in vitro and in vivo activities of fluoroquinolones, tetracycline, and azole antifungals. Two updates on novelties in the field of anti-infectives have been recently published [1, 2]. The aim of this review is to update the knowledge of new compounds that were presented in 1999.

New Fluoroquinolones Derivatives

Many communications and publications have been issued on investigational fluoroquinolones (table 1). Only 3 series of new fluoroquinolones have been published with minimal or extensive in vitro studies, from Procter & Gamble Pharmaceuticals (Mason, OH), Wakunaga Pharma (Hiroshima, Japan), and the University of Cordoba in Argentina.

WQ-3330 and WQ-2942

A new series of N-1 2,4-difluoro 5′ aminophenyl derivatives has been synthesized and the structure-activity relationships among the subgroup of fluoroquinolones reported [3]. Irrespective of the 7-substituents (azetidinyl, amino group), the replacement of the 2′-fluorine with a 3′-methyl on the N-1 phenyl ring decreased in vitro activity against gram-positive and gram-negative bacteria. At the C-8 position, the presence of the 8-methyl group instead of the 8-chlorine group significantly enhanced the photostability of this class of compounds in an aqueous solution under ultraviolet irradiation.

WQ 3330 and WQ 2942 (figure 1) are both more active in vitro against gram-positive cocci than trovafloxacin. The difference in terms of in vitro activity against gram-positive cocci between both compounds is difficult to conclude; their respective activity depends on the strain. WQ 2942 is more active than ciprofloxacin against Enterobacteriaceae and *Pseudomonas aeruginosa*, except against *Serratia marcescens*. However, WQ 3330 seems to be more active than WQ 2942 against MRSA (MIC_{50}, 1.56 μg/mL vs. 12.5 μg/mL), and other tested fluoroquinolones are inactive (trovafloxacin MIC_{50}, 1.25 μg/mL).

In disseminated staphylococcal infections, WQ 3331 (maleic acid salt) of WQ 3330 is more active than WQ 3345 (ethanolamine salt of WQ 2942) against *S. aureus* Smith (median effective dose [ED_{50}], 0.15 vs. 0.60 mg/kg) and more active than trovafloxacin against MRSA W44 (ED_{50}, 17.5 vs. 39.7 mg/kg). WQ 3345 and WQ 3331 are less active than trovafloxacin against *S. pneumoniae* infection (ED_{50}, 10.5, 23.2, and 8.17 mg/kg for WQ 3345, WQ 3331, and trovafloxacin, respectively). WQ 3345 is more active than WQ 3331 in *P. aeruginosa* infections (ED_{50}, 4.23 vs. 72.4 mg/kg), but ciprofloxacin was not tested in this model.
A 5-week-old male CBA/J mouse was challenged by intranasal infection with \textit{S. pneumoniae} and was administered compounds 6, 24, and 48 h by the oral route. After bacterial challenge, the survival rates after treatment with both compounds were 100%. A significant reduction in lung burden was obtained after an oral dose of 50 mg/kg, but in the same magnitude than that recorded with trovafloxacin at 50 mg/kg. The reduction was over 5 \( \log_{10} \text{cfu per lung} \), 3 \( \log_{10} \text{cfu per lung} \), and over 5 \( \log_{10} \text{cfu per lung} \) for WQ 3345, WQ 3331, and trovafloxacin, respectively. The pharmacokinetics of both compounds have been investigated in 5-week-old male ddY mice who received a dose of 10 mg/kg administered orally. The plasma and lung levels were determined by a microbiological assay and \textit{Bacillus subtilis} ATCC 6633 as test organism.

Plasma concentrations and the area under the curve (AUC) were significantly higher than those recorded with levofloxacin, sparfloxacin, and trovafloxacin with WQ 3345, but significantly lower with WQ 3331, in comparison with reference quinolone. In male beagles (27–39 months old), after an oral administration of 20 mg/kg, apparent elimination half-lives were 4.0 and 5.2 h for WQ 3345 and WQ 3331, respectively. Urine elimination of this compound is low: <10\%, in comparison with 36.9\% for levofloxacin.

Coadministration of both compounds with biphenylacetic acid into the cerebral ventricles of ddY mice did not induce convulsions or death, even with a high dose of 80 \( \mu \text{g} \) per head. No phototoxicity in mice after ultraviolet A irradiation (180 \( \mu \text{W/cm}^2 \)) has been recorded at the higher dose of 80 mg/kg.

The original features of these compounds are the trisubstituted N-1 phenyl group, and especially the good in vitro and in vivo activity of the 7-amino derivative WQ 2942 (or WQ 3345) \[3\].

**PGE 926 2932, PGE 417 5997, and PGE 950 9924**

A new series of 6 des-6-F-fluoroquinolones has been synthesized. The first series lead to the development of T-3811 M \[4\]. The aim of the new series was to obtain new quinolones active against multiresistant gram-positive cocci. A structure-activity study with compounds having an N-1-cyclopropylquinoline core has shown that the size of the 8-substituent is important for the anti-gram-positive activity. Various substituents at position 7 demonstrated that the most potent compounds were obtained with 3-aminoethylpyrrolidinyl and 3-aminoazepinyl rings \[5\]. Furthermore, it has been shown that a 6-fluorine instead of a nonsubstituted ring increased the genotoxicity in the in vitro micronucleus assay (CHO cells), whatever the substituent at position 8 (H, Cl, or OCH\(_3\)).

Three compounds were selected for further investigation: PGE 926 2932, PGE 417 5997, and PGE 950 9926 (figure 2). It has been shown that these new des-6-fluorinated quinolones exhibit a less clastogenic effect than do their 6-fluorinated counterparts \[6\]. The acute iv toxicity in male Sprague-Dawley rats

### Table 1. Late phase or recently registered fluoroquinolones under investigation.

<table>
<thead>
<tr>
<th>Generic name</th>
<th>Code number</th>
<th>Manufacturer</th>
<th>Phase of study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gemifloxacin</td>
<td>SB 265805</td>
<td>SmithKline Beecham (Collegeville, PA)</td>
<td>Not yet registered</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>AM 1155</td>
<td>Bristol-Meyers Squibb (Princeton, NJ)</td>
<td>Registered in the US</td>
</tr>
<tr>
<td>Sitafoxacin</td>
<td>Du 6859a</td>
<td>Daichi Pharma (Tokyo, Japan)</td>
<td>Phase III</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>BAY 12-8039</td>
<td>Bayer Pharma (Wuppertal, Germany)</td>
<td>Registered</td>
</tr>
<tr>
<td>(None yet available)</td>
<td>BMS-284756 (T-3811M)</td>
<td>Bristol-Meyers Squibb/Toyama (Toyama, Japan)</td>
<td>Phase II/III</td>
</tr>
</tbody>
</table>

**NOTE.** US, United States.
Burkholderia cepacia pounds. The 3 derivatives could be considered inactive against comparison with ciprofloxacin, trovafloxacin, and other compounds in vitro against Enterobacteriaceae. They exhibit a good activity against Haemophilus influenzae and Moraxella catarrhalis, similarly active or less active (1 test tube dilution) than ciprofloxacin. Among the 3 compounds, 1 is always less active to PGE 417 5997 than the 2 others that show differential activity, depending on the bacterial species. They displayed activity similar to that of trovafloxacin against staphylococci. They are more active against S. pneumoniae isolates than trovafloxacin, irrespective of their susceptibility to penicillin G.

The most active compound is PGE 926 2932 (MIC$_{so}$, 0.015 µg/mL; MIC$_{so}$, 0.03 µg/mL) in comparison with trovafloxacin (MIC$_{so}$, 0.12 µg/mL; MIC$_{so}$, 0.12 µg/mL). PGE 926 2932 is also very active against S. pyogenes (MIC$_{so}$, 0.015 µg/mL; MIC$_{so}$, 0.03 µg/mL) and the viridans group streptococci (MIC$_{so}$, 0.03 µg/mL; MIC$_{so}$, 0.12 µg/mL) in comparison with trovafloxacin (MIC$_{so}$, 0.12 µg/mL; MIC$_{so}$, 0.50 µg/mL). MIC values of PGE 926 2932 remain low against S. pneumoniae with single par C mutations (MIC, 0.06 µg/mL), double par C mutations (MIC, 0.012 µg/mL), or a combination of double par C mutation and gyr A (MIC, 0.5 µg/mL) in comparison with trovafloxacin [9].

The pharmacokinetic profiles have been established for rats (Sprague-Dawley VAF rats, 230–250 g) and dogs (male beagles, 11–15 kg) who received oral and iv doses. The bioavailability of the compound in dogs was high (65%–100%). The highest bioavailability was reached with PGE 950 9924 (7-amino piperidinyl derivative). For PGE 926 2932 and PGE 417 5997, the oral bioavailability (±SD) was 67% ± 32%, and the apparent elimination half-life was 7.0 ± 1.4 h and 5.4 ± 0.5 h, respectively. Bioavailability was low in the rat (4%–9%), with an apparent elimination half-life of 1.5–2.0 h [10].

In conclusion, it has been clearly demonstrated that these new series of des-6-fluoro fluoroquinolones are more active in vitro against gram-positive cocci than trovafloxacin. However, no in vivo data have been provided. These compounds are highly bioavailable in dogs but not in rats, and no data were provided in mice. Like T-3811-M, these new derivatives seem to have a better toxicological profile, which could be a kickoff for new research in the quinolones field.

NSFQ-105 Derivatives

A new series of benzenesulfonylamido 7-piperazinyl quinolones had been synthesized (figure 3). A first series was published in 1994 [11]; the compounds showed good antipneumococcal activity. This series was synthesized with the aim of exploring the role of 7-substituent in ciprofloxacin analogs on S. pneumoniae organisms resistant to fluoroquinolones. All the derivatives differ from ciprofloxacin by the 4 substitution on the 7-piperazinyl ring. Quinolones could be divided into 2 groups for S. pneumoniae: those acting against gyr A (sparfloxacin and gatifloxacin) and those acting as a first step against par C (ciprofloxacin, norfloxacin, levofloxacin, and trovafloxacin).

It was clearly demonstrated that C-7 substitution is important for governing target selection. The piperazinyl ring of ciprofloxacin is substituted with benzosulfonyamide groups. With this substitution, the primary target on S. pneumoniae became gyr A instead of par C. More studies are needed to explore in detail the putative role of the C-7 substitution on S. pneumoniae.
intracellular targets and to explore the possibility of overcoming cross-resistance [12].

Topical Quinolones

Nadoloxacin was introduced in clinical practice for the topical treatment of acnes vulgaris in Japan. A new series of derivatives have been synthesized [13], with one 8-methyl quinolone compound (figure 4) having a 7-substituted pyridine ring and 3-ethoxy group fixed on the 3-carboxylic group. It is claimed that this compound exhibits a good in vitro activity against gram-positive bacteria, and in particular against Propionibacterium acnes. This compound shows 2 additional characteristics: it has a C-C link between the quinoline ring and the pyridine ring, and it is a 6-des-fluoroquinolone.

Antiparasitic Activity of Fluoroquinolones

In the search of new drugs, fluoroquinolones have been tested against malaria [14], Pneumocystis carinii [15], toxoplasmosis [16–18], leishmaniasis [19, 20], and recently trypanosomiasis [21]. The existence was demonstrated of both mitochondrial and nuclear topoisomerases in trypanosomes [22, 23]. Six fluoroquinolones introduced in clinical practice (norfloxacin, enoxacin, ciprofloxacin, pefloxacin, ofloxacin, and fleroxacin) and 4 tetracyclic investigational fluoroquinolones (figure 5) have been investigated for in vitro activity against the bloodstream form of Trypanosoma brucei brucei. All tested compounds had measurable activity, but the tetracyclic analogs were most potent (EC$_{50}$ 1.7–14 μg/mL), and trypanosomes were more susceptible than L1210 leukemia cells. These results, even if these compounds did not seem to be useful in clinical setting, provide evidence that fluoroquinolone inhibition of type II DNA topoisomerases may be a novel approach for the development of new antitypansomal drugs.

Gatifloxacin anti-toxoplasmal activity was investigated [16] in vitro and in vivo. Gatifloxacin inhibited intracellular replication of RH strain tachyzoites in vitro; the MIC after 48 h of exposure was 0.12 μg/mL with human foreskin fibroblast.

In RH-infected mice (outbred Swiss Webster female mice), treatment with gatifloxacin alone initiated 24 h after challenge and for 10 days (400 mg/kg) resulted in significant prolongation of time to death (>3 days); further, there was a 40% survival benefit at this dose. Combination of gatifloxacin and pyrimethamine provided a significant enhancement in survival of RH-infected mice compared with the activity of either drug alone. However, no data have been presented concerning intracerebral infection and the sterilization of mice. The clinical relevance of these data needs to be documented.

A series of 11 trovafloxacin derivatives was evaluated for their in vitro activities against Toxoplasma gondii, and a tentative structure activity relationships was investigated. Different substituents have been fixed on the 7-bicyclic ring, with either a 2',4'-difluorophenyl ring at position 1 or an N-1 cyclopropyl. Only one derivative was a quinoline analog; all others were 1,8 naphtyridone derivatives. One compound also bears a 5-CH$_3$.

It was previously hypothesized that the 7 ring is critical for antitoxoplasmal activity. Substituted quinoline or 1,8 naphtyridone derivatives with a 7-substituted or no piperazinyl or pyrrolidinyl ring are devoid of antitoxoplasmal activities [16]. It was shown that addition of a methyl group at C-5 of the 1,8 naphtyridone ring, or at C-2' of the azabicyclohexane ring or on the C-6' amino group of the bicyclic ring resulted in a 4- to 6-fold increase in activity. Moreover, replacement of the 2',4'-difluorophenyl moiety by cyclopropyl at N-1 of the 1,8 naphtyridone ring decreased antitoxoplasmal activity [17].
Tolerance of Fluoroquinolones

The year 1999 saw safety concerns arise regarding fluoroquinolone tolerance; classical adverse events are phototoxicity, CNS side effects, arthropathies, QTc prolongation, and, more recently, hepatotoxicity with trovafloxacin. In clinical practice, 2 drugs were no longer given or the dosages recommended by the FDA on the label were reduced because of adverse events in cardiovascular area for grepafloxacin and hepatotoxicity with trovafloxacin. Application for clinafloxacin was given up, probably due to the heavy risk of phototoxicity. In phase II/III clinical trials, a 2.4% (37 of 1544) phototoxicity rate was recorded in patients receiving clinafloxacin, with a higher rate recorded after administration by the oral route (29 of 149; 16.1%) than in those receiving clinafloxacin administered by the iv route (15 of 1544; 1%). The rate increased with dose: 200 mg resulted in a 2.1% toxicity rate, and 400 mg, 9.5%. Clinafloxacin-treated patients experienced higher rates of liver-related adverse events, treatment discontinuations due to liver-related adverse events, and elevation of aspartate aminotransferase or alanine aminotransferase than in patients treated with comparative substances (113 of 1544; 7.3%). The hypoglycemia incidence in clinafloxacin-treated patients in clinical trials is 4% [24].

An extensive investigation has been carried out with T-3811 (BMS 284756) to appreciate the occurrence of CNS side effects in animals [25]. Inhibition of GABA receptors by T-3811 and various quinolones was determined by analyzing rat brain synaptic membranes and investigating the convulsion-inducing effect after intracerebroventricular administration of 10 μg of the quinolone tested in combination with 4-biphenyl acetic. Development of clonic convulsion 1 h after dosing was rare, similar to the rate observed with HSR 903 and ofloxacin. Thirty minutes after oral administration of 10 different nonsteroidal anti-inflammatory drugs to mice, the test drug was administered iv. Clonic convulsions were not recorded with T-3811 up to a dose of 60 mg/kg.

Nausea and vomiting are frequently encountered side effects of quinolones. Emetic effect of T-3811 was explored in 6 male dogs with up to 40 mg/kg administered iv; no emetic effect was noted, in comparison with trovafloxacin, for which the effect occurred after administration of 10 mg/kg. The influence of T-3811ME on motive coordination ability (rotared test) and sense of balance (righting reflex test) were evaluated in mice after iv administration of tested quinolones. No dyscoordination was recorded for T-3811ME (60 mg/kg), whereas with alatrovafloxacin, dyscoordination was noted 5 and 30 min after iv administration. There was no disappearance of the righting reflex in mice at doses of 60 mg/kg, whereas it disappeared at 30 mg/kg with trovafloxacin.

Epidemiological Survey

Respiratory Pathogens

Bacterial resistance of pathogens is an increasing concern worldwide, as is the rapid potential decrease of activity of new drugs introduced in clinical practice. Many recent epidemiological surveys were presented during the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy. These studies mainly investigated the profile of resistance of respiratory pathogens: S. pneumoniae, H. influenzae, and, to a lesser extent, M. catarrhalis.

A total of 15,458 fresh clinical isolates of S. pneumoniae, H. influenzae, and M. catarrhalis were collected in 377 hospitals in the United States in 1997–1998. By use of recommended National Committee for Clinical Laboratory Standards (NCCLS) break points, the resistance incidence rates of several isolates were determined. The incidence rate of 5640 S. pneumoniae isolates resistant to penicillin G was 13.7% and 22.5% for intermediate susceptibility strains, 4.1% and 11.2% were resistant or intermediately susceptible to ceftriaxone, 24% were resistant to erythromycin A, and <0.2% were resistant to fluoroquinolones (levofloxacin, moxifloxacin, trovafloxacin, sparflaxin, and grepafloxacin). No resistant isolates of H. influenzae and M. catarrhalis have been isolated for fluoroquinolones;
33.3% of *H. influenzae* isolates were producing β-lactamase (2195 of 6588), and 92.4% (2983 of 3230) of *M. catarrhalis* isolates were producing β-lactamase [26].

During the 1997–1998 cold season, 27 centers located throughout Great Britain (21 centers), North Ireland (2 centers), and Eire (4 centers) collected isolates of *S. pneumoniae* from patients with lower respiratory tract infections. MIC values were determined by use of NCCLS recommendations and recommended NCCLS break points. The resistance rates throughout Great Britain (England, Wales, and Scotland) for *S. pneumoniae* were 9.1% for penicillin G (intermediate plus resistant strains), 5.7% for amoxicillin-clavulanic acid, 5.3% for cefotaxime, 10.7% for clarithromycin, and 0.3% for levofloxacin. The incidence rate of penicillin G resistance is higher in North Ireland and Eire (27.5% and 24.7%, respectively) for amoxicillin-clavulanic acid; 7.1% of *S. pneumoniae* were resistant to clarithromycin, and no resistant strains were resistant to levofloxacin [27].

A centralized, multicenter study with recent clinical isolates from lower respiratory tract infections from 31 sites in Austria, France, Germany, Italy, and Switzerland found the incidence rate of penicillin G *S. pneumoniae* resistance to be 5% for Austria, 45% (26% intermediate and 19% resistant) for France, 6% for Germany, 8% for Italy, and 23% (13% intermediate and 10% resistant) for Switzerland. The incidence rate for erythromycin A was 20.8% for the 31 sites and ranged 0%–0.6% for fluoroquinolones. No resistant strains of *H. influenzae* and *M. catarrhalis* for fluoroquinolones were found [28].

The emergence of resistance in the Province of Quebec, Canada was investigated in 1996–1998 in 26 centers. Pneumococcal isolates were collected from blood cultures or CSF samples, or from samples taken from other sterile sites. Among the 13,545 *S. pneumoniae* isolates collected, the rates of resistance were found for penicillin G (9.8% in 1996, which increased to 13.6% in 1998), ceftriaxone (7.7%), chloramphenicol (2.7%), erythromycin A (8.1%), rifampin (0.1%), ciprofloxacin (20.2%), and levofloxacin (1.9%). No strains resistant to vancomycin were collected [29].

Before the early 1990s, resistance to penicillin G remained uncommon among clinical isolates of *S. pneumoniae* in Germany. A reduced susceptibility to penicillin G increased from 1.8% in 1992 to 5.2% in 1998 and 6.8% in 1999. A more dramatic increase in resistance was observed with erythromycin A (3.6% to 10.5%). In 1999, a 13.4% resistance rate was recorded for tetracycline, and a 0.2% resistance rate was recorded for levofloxacin [30]. A summary of the incidence rate of antibacterial resistance for *S. pneumoniae* is listed in table 2.

### Mutation Prevention Concentration (MPC)

An interesting study has been presented on MPC, which is defined as the concentration (expressed in μg/mL) of antibacterial agents that prevent the emergence of resistant mutants to the compounds tested [31]. MPC testing was performed by agar dilution by use of an inoculum size of the tested organisms of 10⁶ cfu/mL. In comparison, MIC determinations are investigated with 10⁵ cfu/mL of the organisms tested.

Blondeau et al. [32] showed that MPC is irrespective of the susceptibility to penicillin G of 96 *S. pneumoniae* isolates, which were as follows for 90% of samples: 0.5–4 μg/mL for gatifloxacin, 0.5–4 μg/mL for grepafloxacin, 1–4 μg/mL for levofloxacin, ≤0.06–4 μg/mL for moxifloxacin, and 0.25–4 μg/mL for trovafloxacin. This parameter is an addition in the risk assessment of selection of mutants, but this parameter has to be compared with plasma or tissue concentrations.

### Gyr A Mutation in *Bacteroides fragilis*

The role of gyr A mutations in *B. fragilis* has been explored. In vitro with ciprofloxacin as the selector, 2 steps were needed to obtain ciprofloxacin and trovafloxacin-resistant mutants, and 50% of them harbored a gyr A mutation (Ser 83→Phe).
By contrast, with trovafloxacin as a selector, first step mutants were harboring a new gyr A mutation (Asp 82→Asn and Ala 119→Val). These mutations resulted in an 8- to 16-fold decrease in trovafloxacin activity, but only a 4-fold decrease for activity of ciprofloxacin [33].

Pyridone Derivatives

New series of 2-pyridone derivatives have been synthesized; all new compounds have variations at the 8 position and 3 substitution of the 3-pyrrolidinyl moiety. Nine substituted 3-methall new compounds have variations at the 8 position and 3 substituents at position 3 leads to the best substitution. Instead of a carbamoyl amino group of the 7-pyrrolidinyl ring and by the presence of 4-cyclopropyl fixes on this ring (Figure 7B). A-270117 exhibited in vitro activity against gram-positive cocci similar to that of A-170568 and ciprofloxacin against E. coli Juhl and P. aeruginosa BMH 10.

Figure 6. 2-Pyridone derivatives

The important improvement of A-270117 in comparison with A-170568 is the genotoxic profile. In a V-79 cell cytotoxicity assay, the IC₅₀ values of A-270117 and A-170568 were >100 μg/mL and 15.5 μg/mL, respectively, and inhibition of human topoisomerase II, assayed by the concentration (which induces 7% of additional cleavage baseline), were 1000 μg/mL and 34 μg/mL for A-270117 and A-170568, respectively [37].

Coumarin Derivatives

New synthetic inhibitor compounds acting on gyr B subunit of DNA gyrase have been designed [38–40]. The aim was to provide compounds acting against S. aureus isolates resistant to methicillin. The coumarinic moiety has been substituted at different positions. The aim was to enhance the antibacterial activity of coumarinic analogs, but also to overcome hepatic toxicity of novobiocin and to improve physicochemical properties. By use of a 3-dimensional structure resolution, it was shown that substituents at position 3 and 4 of the coumarin moieties are outside of the protein and could be substituted to improve the physicochemical properties. An oxime group at position 3 leads to the best substitution. Instead of a carbamoyl group in novobiocin, an alkoxycarbamate chain improved inhibition of gyrase B, but it also allowed it to obtain analogs with a better safety profile. One compound was chosen for further investigation, RU 79115 [41].

In vitro activity of RU 79115 (Figure 8) was compared to that of vancomycin and eperzolid (an oxazolidinone derivative) against S. aureus, Streptococcus species, and Enterococcus species. RU 79115 is more active than vancomycin and eperzolid against Streptococcus species (geometric MIC, 0.06, 1.15, and 0.6 μg/mL, respectively). The in vitro activity remains unchanged against S. aureus resistant to oxacillin, ofloxacin, or both. RU 79115 is 2 times more active against S. pneumoniae than vancomycin and eperzolid (geometric MIC, 0.12 μg/mL) and showed similar activity against viridans group streptococci (geometric MIC, 0.59 μg/mL) or other streptococci.
(geometric MIC, 0.47 μg/mL). RU 79115 exhibits a higher activity than the 2 comparative compounds against enterococci, whatever the susceptibility of the isolates to vancomycin or teicoplanin (geometric MIC, 0.26 μg/mL).

In disseminated staphylococcal infection, RU 79115 administered by the subcutaneous route was more active than vancomycin, linezolid, and epererezolid. Against streptococci and enterococci, RU 79115 was less active than the oxazolidinone derivatives, but the data are difficult to interpret. RU 79115 was not always administered by the same route (oral or subcutaneous) than oxazolidinone derivatives. RU 79115 does not inhibit the uridine diphosphate glucuronyl transferase, which is responsible for the conjugation of bilirubin on the liver. Furthermore, no antivitamin K activity was recorded after oral administration of 30 or 100 mg/kg RU 7911 [42].

Macrolides and Ketolides

Research in the field of macrolides in 1999 was directed against descladinosyl erythromycin A, mainly ketolide analogs or 3-O-acyl derivatives of erythromycin A. No new compounds in 16-membered ring macrolides have been reported in published articles. Some research on 12-membered ring macrolides was presented.

C11-C12 Carbamate Erythromycin A Derivatives

It was shown that the addition of a C11-C12 carbamate side chain to the erythronolide A ring permitted it to overcome erythromycin A resistance driven by a mef gene. The antibacterial potency of a given C11-C12 carbamate erythromycin A analog is due to the substituent of the carbamate residue; in particular, compounds having 2 amino groups at the terminal of the chain possess a good in vitro activity against S. pneumoniae strains harboring the mef gene. Moreover, the structure of the carbamate side chain may dramatically increase the in vitro activity against S. pneumoniae strains harboring an erm gene [43].

Descladinosyl Erythromycin A Derivatives

C11-C12 carbamate derivatives. A series of 3-O-acyl erythromycin A was synthesized (figure 9). It was clearly shown that in vitro activity against S. pneumoniae isolates harboring a mef gene is independent of the O-acyl residue at position 3 of the erythronolide A ring. However, substituents of the 3-O-acyl chain seem to be important for the in vitro activity against S. pneumoniae isolates harboring an erm gene (e.g., compound FMA 198, with a 3-methylpyridine ring, which exhibited an MIC of 0.39 μg/mL against S. pneumoniae 205 [erm gene]). Increasing the length of the carbamate side chain by adding 2- or 3-methylene significantly enhances in vitro activity of these analogs against S. pneumoniae isolates harboring an erm gene (e.g., compound 31481, with a 3-methylpyridine ring, which exhibited an MIC of 0.39 μg/mL and 0.78 μg/mL, respectively). However, the level of the in vitro activity is strain dependent. By substituting the carbamate side chain with a quinoline ring, one compound, FMA 481 (figure 9), exhibited good in vitro activity against S. pneumoniae 205 (erm gene), but this analog was unable to overcome MLSb constitutive type of resistance in S. aureus [43].

C6-C9, C11-C12 dicarbonate derivatives. A series of C6-C9, C11-C12 dicarbonate 3-O-acyl derivatives has been described [44]. It was first shown that 3-O-methylpyridine deriv-
Methymycin Derivatives

Twelve-membered ring macrolides, such as methymycin or neomethymycin, were not developed as medicinal drugs. An increasing interest in these molecules has been seen recently in order to find new scaffolds within the macrolide family. Many alterations have been made by engineering and new derivatives have been published, but the antibacterial activities of these analogs were not assessed. Methymycin was altered at the sugar level. One derivative having a D-quinovose sugar instead of D-desosamine was published [45], and a methymycin-calicheamicin hybrid having a N-acetyl deoxyhexose instead of the D-

desosamine was obtained from engineering *Streptomyces venezuelae* [46].

Ketolides

Ketolides are semisynthetic derivatives of erythromycin A that are characterized by the lack of L-cladinose at position 3 of the erythronolide A ring and have a 3-keto function [47]. Numerous compounds have been prepared, but 2 compounds are currently under development [2]: telithromycin [48, 49] and ABT-773. Numerous compounds have been presented during the last few years, such as TE 802 and its derivatives [1]. Telithromycin (HMR 3647) is currently in the registration phase, and new data have been presented in pharmacokinetics, especially in lung tissues [50] and in different populations [51–54]. Data on the antibacterial activities of ABT-773 have been presented extensively, as has data on 2 new 2-fluoroketolides, HMR 3562 and HMR 3787.

*ABT-773*. *ABT 773* is a ketolide derivative characterized by a C11-C12 carbamate side chain and a 6-O-substituted chain (figure 11A) [55]. Physicochemical properties are summarized in table 4 [56]. Many studies were devoted to highlight the in vitro and in vivo activities of ABT-773 against *S. pneumoniae* and other respiratory pathogens. MIC\textsubscript{50} and MIC\textsubscript{90} values of ABT-773 against *S. pneumoniae* are 0.001 \(\mu\)g/mL and 0.002 \(\mu\)g/mL, respectively, and against *S. pyogenes* are \(\leq0.002 \mu\)g/mL and 0.002 \(\mu\)g/mL, respectively [57].

Against *S. pneumoniae* that have *mef* or *erm* genes, MIC\textsubscript{50} values for ABT-773 were 0.016 \(\mu\)g/mL and 0.125 \(\mu\)g/mL; ABT-773 MIC\textsubscript{90} values were 0.03 \(\mu\)g/mL and 0.125 \(\mu\)g/mL. However, the range of the in vitro susceptibilities showed that MICs for ABT-773 could reach 2.0 \(\mu\)g/mL and 4.0 \(\mu\)g/mL when *mef* or *erm* genes are harbored by *S. pneumoniae* isolates [58, 59]. ABT-773 showed good in vitro activity against gram-positive cocci and bacilli, *Corynebacterium jeikeium* (MIC\textsubscript{50} 0.03 \(\mu\)g/mL), Lis-

Methymycin Derivatives

Twelve-membered ring macrolides, such as methymycin or neomethymycin, were not developed as medicinal drugs. An increasing interest in these molecules has been seen recently in order to find new scaffolds within the macrolide family. Many alterations have been made by engineering and new derivatives have been published, but the antibacterial activities of these analogs were not assessed. Methymycin was altered at the sugar level. One derivative having a D-quinovose sugar instead of D-desosamine was published [45], and a methymycin-calicheamicin hybrid having a N-acetyl deoxyhexose instead of the D-
teria monocytogenes (MIC$_{50}$, 0.06 μg/mL; MIC$_{90}$, 0.06 μg/mL),
S. aureus (MIC$_{50}$, 0.03 μg/mL; MIC$_{90}$, 0.06 μg/mL), Staphylococcus epidermidis (MIC$_{50}$, 0.03 μg/mL; MIC$_{90}$, 0.06 μg/mL),
Staphylococcus haemolyticus (MIC$_{50}$, 0.03 μg/mL; MIC$_{90}$, >16 μg/mL), Staphylococcus saprophyticus (MIC$_{50}$, 0.06 μg/mL; MIC$_{90}$, 0.06 μg/mL), and viridans group streptococci (MIC$_{90}$ ≤0.008 μg/mL; MIC$_{90}$, 0.06 μg/mL). ABT-773 MIC$_{50}$ values were 0.06 μg/mL against M. catarrhalis, 0.06 μg/mL against Neisseria gonorrhoeae, and 0.016 μg/mL against Neisseria meningitidis. ABT-773 MIC$_{90}$ values were 0.12 μg/mL against M. catarrhalis, 0.06 μg/mL against N. gonorrhoeae, and 0.12 μg/mL against N. meningitidis [59].

ABT-773 is less active than clarithromycin against Helicobacter pylori (MIC$_{50}$, 0.012 μg/mL and MIC$_{90}$, 0.25 μg/mL vs. MIC$_{50}$, 0.015 μg/mL and MIC$_{90}$, 0.03 μg/mL for clarithromycin) [60], and ABT-773 is fully cross-resistant with clarithromycin [57]. In vitro ABT-773 is moderately active against Mycobacterium avium complex (MAC; MIC, 16 μg/mL) in comparison with clarithromycin and is fully cross-resistant against MAC resistant to clarithromycin [57]. ABT-773 displays a good activity against Legionella pneumophila [61].

ABT-773 exhibits antitoxoplasmal activity in vitro and in vivo [62]. ABT-773, like telithromycin, is highly bactericidal against S. pneumoniae strains susceptible to erythromycin A at 2 times the MIC. ABT-773 also exhibits a bactericidal activity against S. pneumoniae isolates resistant to erythromycin A. ABT-773 shows only a bacteriostatic activity against H. influenzae [63].

The mechanism of action of ABT-773 against S. pneumoniae was explored. ABT-773 binds to the 23S ribosomal RNA sites A-2058 and A-2059. ABT-773 binds with unmethylated ribosome 10- to 100-fold higher than erythromycin A. In S. pneumoniae strains containing erm, the estimated affinity with methylated ribosome is ~100-fold lower than with unmethylated ribosomes [64]. ABT-773 does not induce erm resistance at the difference of another ketolide TE 802 [65]. In vivo ABT-773 exhibited similar activity to that of telithromycin against erythromycin A–susceptible S. aureus and S. pneumoniae in mouse protective test and rat pulmonary infection [66].

ABT-773 showed good efficacy in murine pneumococcal lung infections, irrespective of the underlying mechanism of resistance to erythromycin A [67]. In rats, dogs, and monkeys, 4 main metabolites have been demonstrated: M-1 (N-desmethyl ABT-773), M4 (10-hydroxy N-desmethyl ABT-773), M-6 (oxidation of ABT-773), M-3 (10-hydroxyl ABT-773), and M-2 (di-N-desmethyl ABT-773) [68].

HMR 3562 and HMR 3787. HMR 3562 and HMR 3787 are 2-fluoroketolides (figure 11B). The relative importance of position 2 of erythronolide A of ketolides has been studied by the introduction of various electrophiles (halogen atoms or a methyl group) as well as new 2,3-enolether derivatives [69]. Position 2 of ketolides needs to remain tetrahedral and tolerates only very small substituents such as fluorine; planar analogs such as the 2,3-enolether result in a loss of antibacterial activities. It was shown that the 2-chloro and 2-methyl analogs were less active than clarithromycin or the parent ketolide, especially against S. aureus harboring an inducible MLSB resistance. The most active compounds are HMR 3562 and HMR 3787, with a 2-fluorine. HMR 3562 differs from a structural point of view from telithromycin by having a 2-fluorine, and HMR 3787 by having a bicyclic ring on the carbamate side chain. Previous series of 2-fluoroketolides have appeared [70]. A fluorine atom, a chlorine atom, or a bromine atom replaced the C-2 hydrogen. The C-2 bromine and C-2 chlorine analogs were poorly active, and the 2-fluoro TE-802 exhibited only a slight improvement in vivo. Both compounds were tested for their in vitro activities against gram-positive and gram-negative cocci and some gram-negative bacilli, as well as against Mycoplasma pneumoniae, L. pneumophila, Chlamydia species, and Mycobacterium tuberculosis [71].

HMR 3787 seems to be more active against gram-positive cocci than HMR 3562, except against S. pneumoniae isolates resistant to erythromycin A, against which HMR 3562 is 1 test tube dilution more active (MIC$_{50}$, 0.015 μg/mL; MIC$_{90}$, 0.12 μg/mL), and Enterococcus faecium resistant to erythromycin A (MIC$_{50}$, 0.5 μg/mL; MIC$_{90}$, 0.5 μg/mL). Both compounds are active against Corybacterium diphtheriae, L. monocytogenes, N. gonorrhoeae, N. meningitidis, M. catarrhalis, and Bordetella pertussis. Against H. influenzae, MIC$_{50}$ was 1.0 μg/mL and MIC$_{90}$ was 2.0 μg/mL for HMR 3562, and MIC$_{50}$ was 1.0 μg/mL and MIC$_{90}$ was 1.0 μg/mL for HMR 3787. They exhibit a good activity against Helicobacter pylori, L. pneumophila, Chlamydia trachomatis, Chlamydia (chlamydiophila) pneumoniae, M. pneumoniae, and Ureaplasma urealyticum. Only HMR 3787 exhibits a significant activity against Mycoplasma hominis (MIC$_{50}$, 0.5 μg/mL; MIC$_{90}$, 1.0 μg/mL). They are inactive against M. tuberculosis (MIC, ≥64 μg/mL).
In murine disseminated infections, both compounds are more active than clarithromycin against *S. aureus*, *S. pyogenes*, *S. pneumoniae*, and *Streptococcus agalactiae* infections, irrespective of the susceptibility of the bacterial isolates to erythromycin A. The difference in in vivo activity between both compounds is strain dependent [72]. Female Swiss (OF-1) mice (20–23 g) were infected intratracheally with ~10⁷ cfu of *S. pneumoniae* (erythromycin A susceptible and erythromycin A resistant isolates; MIC, >128 µg/mL). Treatment was initiated by the subcutaneous route 18 h after bacterial challenge every 8 h for 3 days. After 50 or 100 mg/kg, the survival rate, when challenged with a *S. pneumoniae*–susceptible strain, was 80%–100%; but with a *S. pneumoniae* strain resistant to erythromycin A, the survival rates were ~90% for HMR 3562 and 45%–60% for HMR 3787. The bacterial lung clearance was higher with HMR 3562 than with HMR 3787 [73].

*Other ketolides.* New 8a-aza-ketolides have been published in patent; they show in vitro activities [74]. In previous publications, it was reported that 9a-aza-ketolides were devoid of any antibacterial activities [75]. Wu [76] described new ketolide derivatives that display a broad antibacterial spectrum, covering both gram-positive and gram-negative bacteria (figure 12).

**Table 4. Physicochemical properties of ABT-773.**

<table>
<thead>
<tr>
<th>Physicochemical property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>766 Da</td>
</tr>
<tr>
<td>pK1 (Isoquinoline)</td>
<td>4.1</td>
</tr>
<tr>
<td>pK2 (Desosamine)</td>
<td>8.6</td>
</tr>
<tr>
<td>log P&lt;sub&gt;app&lt;/sub&gt; (n-octanol/pH 7.4 buffer)</td>
<td>2.6</td>
</tr>
<tr>
<td>log P&lt;sub&gt;app&lt;/sub&gt; (n-octanol/pH 6.8 buffer)</td>
<td>1.7</td>
</tr>
<tr>
<td>pH Maximum stability</td>
<td>3–4</td>
</tr>
<tr>
<td>Solubility pH 4.0</td>
<td>&gt;143 mg/mL</td>
</tr>
<tr>
<td>pH 6.7</td>
<td>3.6 mg/mL</td>
</tr>
<tr>
<td>pH 7.44</td>
<td>0.44 mg/mL</td>
</tr>
<tr>
<td>0.9% saline in water</td>
<td>0.06 mg/mL</td>
</tr>
</tbody>
</table>

**Other New Macrolides**

New data of interest regarding available 14-membered ring macrolides were published on the nonantibiotic effects of clarithromycin in lung carcinoma [77].

**Carbapenems**

Few carbapenems to be delivered by oral and parenteral routes are currently in the preclinical stage or under development. New investigational compounds were shown in 1999,
Figure 12. New ketolide analogs from Pfizer, Inc.

some of them the result of extensive chemical modifications and biological investigations.

**Oral Carbapenems**

*OCA-983.* A series of 1β-methylcarbapenems containing a 2-aminomethylene–substituted tetrahydrofuran-thio side chain was synthesized and reported in 1994 [78]. The aim of this series of 1β-methylcarbapenem analogs was to obtain derivatives with enhanced antibacterial activity against gram-negative bacilli and with enhanced activity when delivered by the oral route, with both mediated through the use of a peptide transport system [79]. A double ester prodrug has been prepared, and it is transported through the phospholipid bilayer [80].

An effort was initiated to produce synthetic peptidic prodrugs of these carbapenem derivatives by addition of amino acids at the aminomethyl group of each selected carbapenem molecule in an attempt to improve oral absorption and efficacy through the di- and tripeptide transport system [81, 82]. The pure diastereoisomer CL 191,121 was extensively esterified with different amino acids: alanine, valine, isoleucine, and phenylalanine.

Efficacy of CL 191,121 and the 4 prodrug analogs were evaluated in a murine (female CD-1 mice, 20 ± 2 g) disseminated infection model induced by Enterobacteriaceae (including strains producing broad and extended spectrum β-lactamases), *S. aureus* Smith, and *S. pneumoniae* isolates susceptible or resistant to penicillin G. The parent compound, CL 191,121, when nonesterified, possesses good in vivo efficacies when administered subcutaneously, but only a modest efficacy after oral administration due to poor enteral absorption. Peptidic derivatives (alanine and valine) were strongly active against all challenging strains, including those containing extended spectrum β-lactamase. These increasing oral efficiencies can be attributed to an increasing level of circulating parent compound in serum [83].

CL 191,121 is less active in vitro than meropenem against Enterobacteriaceae, especially against *Enterobacter* species, *Morganella morganii*, *Proteus mirabilis*, *Providencia* species, and *S. marcescens* (MIC₉₀ >2.0 μg/mL). It is as active as imipenem against gram-positive cocci, including *S. pneumoniae* isolates, irrespective to their susceptibility to penicillin G. CL 191,121 is hydrolyzed by metallo–β-lactamases. It has the same hydrolyzing stability than imipenem by serine β-lactamases but an enhanced stability to Sme-1 and Imi-1 in comparison with imipenem [84, 85].

CL 191,121 esterified with a valine residue, OCA-983 (figure 13A), was selected for further investigation. CL 191,121 is highly stable to human renal dehydropeptidase hydrolysis, with a relative rate of hydrolysis of 4.4 versus 100 for imipenem. However, the relative hydrolysis rate is similar to that of meropenem (4.4 vs. 5.3 for meropenem) and higher than that observed with biapenem (4.4 vs. 1.7 for biapenem). In preliminary oral pharmacokinetic studies (ascending dose) of OCA-983, after administering 100–800 mg oral doses to volunteers, peak plasma concentrations ranged from 0.8 μg/mL (100 mg) to 3.5 μg/mL (800 mg), with an AUC of 2.34 (100 mg) to 13.5 μg/mL (800 mg). Apparent elimination half-life was 1.4 h [84].

*Other oral carbapenems.* New data have been provided on L-084, which is the prodrug of LJC 11,036, by esterification of the 4-carboxylic group of the penem ring with a pivaloyloxy methyl group. In a phase I study, after a single ascending oral dose of 25–200 mg, a linear dose response was observed of 25–150 mg. The mean peak plasma concentrations ranged from 1.27 ± 0.62 μg/mL (25 mg) to 5.93 ± 2.16 μg/mL (200 mg), with an AUC of 0.90 ± 0.12 μg/mL (25 mg) to 7.03 ± 1.14 μg/mL (200 mg). The apparent elimination half-lives were 0.28–0.43 h. Elimination by urine is predominant, with 63%–73% of the administrated doses recovered in urine. A metabolite was identified in urine, which represents 10% of the eliminated compound.
The ester side chain is metabolized in pivalic acid, leading to a net decrease of L-carnithine. It is a well-known adverse effect due to the pivaloyloxyethyl side chain. Usually the metabolism of this side chain gives pivalic acid and formaldehyde; the level of the later metabolite was not given [86]. Series of double ester 2-pyrrolidinyl 1β-methylcarbapenem for oral use have been disclosed [87] (figure 13B).

**Parenteral Carbapenems**

No new parenteral carbapenem has been reported in a published article; new data on compounds already disclosed in previous years have been presented, and information on compounds has been published in patents. A series of new data has been reported for E-1010 (figure 14A), which was referenced as ER-35786. In vitro activity of E-1010 ranged between that of imipenem (for gram-positive cocci) and that of meropenem (for gram-negative bacilli).

**DK-35C.** For another carbapenem, DK-35C (figure 14B), which has been known since 1994 [88], data on its ability to inhibit [3H] muscimol (5 nM) binding to GABA receptors were assayed with crude synaptic membranes prepared from the rat cerebral cortex. The concentrations, which inhibit 50% of the specific binding, were 0.6, 18, 15.4 and 27.6 nM for imipenem, cefazolin, DK-35C, and meropenem, respectively. After intracerebroventricular injections, the doses that induced convulsions in 50% of rats were 57 nmol per rat for imipenem, 96 nmol per rat for cefazolin, 377 nmol per rat for DK-35C, and >3000 nmol per rat for meropenem. In ICR mice, pretreatment with 200–800 mg/kg of tested β-lactams delivered by the iv route, followed by intraperitoneal administration of pentylenetetrazole, induce convulsions at 800 mg/kg with cefazolin and 200 mg/kg with imipenem. At 400 mg/kg, no convulsions were recorded with cefazolin, meropenem, and DK-35C. No data have been provided after a dose of 800 mg/kg for DK-35C (figure 14B). DK-35C possesses a risk of convulsive activities mediated through an interaction with GABA A receptors [89].

**J 111,225.** Previous data have been published on the in vitro and in vivo activities of J 111,225 [1], but no data have been published concerning the correlation between the chemical structure and anti-MRSA activities and epileptogenicity due to the C-5 stereochernistry of the side chain. J 111,225 is characterized by an unusual C-5 stereochemistry of the side chain in comparison with known carbapenems that have a cis-substituted 3-pyrrolidinylthio side chain, such as meropenem. The 4 diastereoisomer analogs of J 111,225 were synthesized to investigate their in vitro activities and their epileptogenicity (figure 15).

When considering the C-3 configuration, the 3-S isomers were significantly more active than the corresponding 3-R isomers. Of the two 3-S isomers, the trans-(5-R) isomer was 4 times more active than the corresponding cis-(5-S) isomers against MRSA and *P. aeruginosa*. In addition, no epileptogenicity was recorded after intracerebroventricular injection (200 μg per rat head) of the 5-trans isomer analog, whereas the 5-cis isomer analog produced severe adverse effect at the same dose [90].

**2-Naphtosultamyl carbapenems.** In 1998 a series describing 2-naphtosultamyl 1β-methylcarbapenem was published. One compound was investigated: L-786,392. This compound is active against MRSA but poorly active against gram-negative bacilli [1]. Among a series of 2-naphtylcarbapenem analogs...
having an appropriate positioning of a cationic moiety, one compound (figure 14C) was found to have enhanced activity against MRSA (MIC range, 0.25–8 μg/mL) and retained some activity against Enterobacteriaceae, but not against P. aeruginosa [91].

New C-2 pyrrolidinylthio substituted carbapenems. A new series of 1β-methylcarbapenems in which the C-2 side chain is very close to that of E-1010 has been synthesized. On the C-2 pyrrolidinylthio group, a 3',4'-disubstituted group at 5' of the pyrrolidine ring was introduced. Among these new analogs, one compound with unsubstituted diol (figure 14D) was the most active compound against gram-positive cocci except E. faecium and retained a good activity against Enterobacteriaceae and P. aeruginosa. In vitro activity of this compound ranged between those of imipenem (gram-positive cocci) and meropenem (gram-negative bacilli). It is less stable to porcine DHP-1 hydrolysis than meropenem (>40% reduction of stability) and possesses a similar pharmacokinetic profile in mice to meropenem. The in vivo protective activities in mice are similar to those of meropenem [92].

Glycylcyclines and Other Tetracycline Derivatives

Glycylcyclines

GAR-936 is the 9-t-butylglycylamido derivative of minocycline. GAR-936 was shown to be able to overcome the 2 main mechanisms responsible for tetracycline resistance: ribosomal protection and active efflux [2]. New data have been provided in 1999 for GAR-936. GAR-936 exhibits a good activity against gram-positive cocci and bacilli, including C. jejuni (MIC_LQ 0.5 μg/mL; MIC_90 2 μg/mL), Lactobacillus species (MIC_LQ 0.06 μg/mL; MIC_90 0.12 μg/mL), Leuconostoc species (MIC_LQ 0.12 μg/mL; MIC_90 0.12 μg/mL), and Pediococcus species (MIC range, 0.03–1.0 μg/mL) [93].

It had been shown that GAR-936 exhibited a good activity against M. pneumoniae susceptible to tetracycline (MIC_LQ 0.12 μg/mL; MIC_90 0.25 μg/mL) and M. hominis isolates susceptible to tetracycline (MIC_LQ 0.25 μg/mL; MIC_90 0.5 μg/mL), but it demonstrated a lower activity than minocycline (MIC_LQ 0.12 μg/mL; MIC_90 0.12 μg/mL). GAR-936 is poorly active against U. urealyticum strains resistant to tetracycline (MIC_LQ 4.0 μg/mL; MIC_90 8.0 μg/mL) [94]. GAR-936 displays activity against gram-positive anaerobic bacteria that is similar or higher than the activity of minocycline.

GAR-936 is less active than minocycline against B. fragilis (MIC_LQ 0.25 μg/mL and MIC_90 0.5 μg/mL, vs. MIC_LQ 0.03 μg/mL and MIC_90 8.0 μg/mL for minocycline), but minocycline activity is distributed as a bimodal population. GAR-936 showed activity similar to minocycline against Porphyromonas species and Prevotella species (MIC_LQ 0.06 μg/mL; MIC_90 0.06 μg/mL) and Fusobacterium species (MIC_LQ 0.01 μg/mL; MIC_90 0.06 μg/mL) [95].

The pharmacokinetics of GAR-936 was investigated in a randomized, double-blind, placebo-controlled, ascending single-dose phase I study in 8 male volunteers gathered in 7 study groups. GAR-936 was administered both as 1 h (12.5–300 mg) and 4 h (200–300 mg) iv infusions. Peak plasma concentration at the end of infusion ranged from 0.11 μg/mL (12.5 mg) to 2.8 μg/mL (300 mg). The mean apparent elimination half-life was 36 h. Less than 15% of the administered dose was eliminated unchanged in urine [96].

GAR-936, like all cyclines, is highly distributed in rat bones (male Sprague-Dawley rats), probably due to calcium chelation and bone adhesion [97].
Other Tetracycline Derivatives

New tetracyclic derivatives with 3 new substituents (figure 16) have been described. One compound within this series exhibits an MIC of 6.25 μg/mL against MRSA. These derivatives show a good activity against gram-positive cocci and gram-negative bacteria, including isolates resistant to cycline [98].

Cephems

The number of new cephems presented is very limited. One new series of oral cephems and one modified cephem in the parenteral class were presented in 1999.

Oral Cephem: LB10827

A new class of cephems bearing 3-pyrimidinyl-substituted sulfonyl, sulfonylmethyl, or sulfonylmethyl-sulfonyl group at the C-3 position of the cephem ring has been synthesized. The targeted bacteria were S. pneumoniae isolates resistant to penicillin G. One compound was selected for further investigation, LB10827 (figure 17A). LB10827 possesses 2,6-diaminopyrimidinyl-4-thiomethylthio at the C-3 position of the cephem ring and bears a hydroxy residue on the oxime group [99]. LB10827 is more active than cefdinir, cefuroxime, cefprozil, and trovafloxacin against S. pneumoniae isolates, irrespective of their susceptibility to penicillin G.

MIC50 and MIC90 values are ≤0.008 μg/mL and 0.016 μg/mL for S. pneumoniae isolates susceptible to penicillin G; 0.13 μg/mL and 0.13 μg/mL for S. pneumoniae isolates intermediately susceptible to penicillin G; and 0.5 μg/mL and 0.5 μg/mL for isolates resistant to penicillin G. In the latter group of microorganisms, trovafloxacin was more active than LB10827 (MIC50 0.25 μg/mL; MIC90 0.25 μg/mL). LB10827 also exhibits a good activity in vitro against H. influenzae isolates that produce or do not produce β-lactamases (MIC50 0.13 μg/mL; MIC90 0.5 μg/mL), and it is 10 times more active than cefuroxime against isolates producing β-lactamases.
roxime. However, no data were provided on its activity against *H. influenzae* that harbor a nonenzymatic mechanism of resistance to ampicillin [100].

The information provided regarding antibacterial activity is difficult to interpret. Data provided with *S. pneumoniae* isolates having a high level of resistance to penicillin G (MIC, 4 µg/mL) showed that LB10827 activity dropped from 0.008 µg/mL to 0.5 µg/mL; and investigation against *H. influenzae* is not well enough documented.

Pneumococcal infection was induced in the lungs of cyclophosphamide-reduced neutropenic rats (Sprague-Dawley rats weighing 80–100 g) by intrabronchial instillation. Tested compounds were administered orally at 18, 26, 42, and 50 h after bacterial challenge with *S. pneumoniae* type III with doses of 2, 10, and 50 mg/kg. About 4 log₁₀ cfu were found per lung, whatever the administered doses were. Higher burden was recorded for coamoxyclav, cefdinir, and trovafloxacin.

In C57 black mice (6 mice weighing 17–19 g), *S. pneumoniae* type III (MIC, 0.008 µg/mL) challenge was obtained after intranasal instillation. Tested agents were administered orally 6 h after bacterial challenge, and then twice daily for 3 days. With LB10827, the survival rate was 100% for a dose of 50, 17, 5.6, and 1.8 mg/kg. The same survival rate was observed with coamoxyclav. With cefdinir, 100% survival rate was recorded after a 50 mg/kg dose and 40% after a 17 mg/kg dose; for lower doses, no survival in mice was recorded [101].

This compound may have a potential for *S. pneumoniae* isolates resistant to penicillin G. However, data are insufficient, with no control rats and with no tests with *S. pneumoniae* resistant to penicillin G with different level of resistance. Other, more convincing studies are warranted.

### Parenterally Administered Cephems

No truly new parenteral cephems were reported in 1999. One compound is currently in the preclinical stage: RWJ-54428 (MC-02,479).

**RWJ-54428 (MC-02,479).** New in vitro data have been provided regarding the efficacy of RWJ-54438 against gram-positive cocci. RWJ-54428 is more active than vancomycin, quinupristin-dalfopristin, and teicoplanin against *S. aureus* strains susceptible (MIC<sub>₉₀</sub>, 0.5 µg/mL; MIC<sub>₅₀</sub>, 0.5 µg/mL) or resistant (MIC<sub>₉₀</sub>, 1 µg/mL; MIC<sub>₅₀</sub>, 2 µg/mL) to methicillin, as well as to coagulase-negative staphylococci susceptible (MIC<sub>₉₀</sub>, 0.25 µg/mL; MIC<sub>₅₀</sub>, 1.0 µg/mL) or resistant (MIC<sub>₉₀</sub>, 1.0 µg/mL; MIC<sub>₅₀</sub>, 4.0 µg/mL) to methicillin. RWJ-54428 is highly active against *S. pneumoniae* susceptible (MIC<sub>₉₀</sub>, ≤0.06 µg/mL; MIC<sub>₅₀</sub>, ≤0.06 µg/mL) or simultaneously susceptible (MIC<sub>₉₀</sub>, 0.12 µg/mL; MIC<sub>₅₀</sub>, 0.25 µg/mL) to penicillin G. However, a decrease in in vivo activity was shown against *S. pneumoniae* isolates resistant to penicillin G (MIC<sub>₉₀</sub>, 0.25 µg/mL; MIC<sub>₅₀</sub>, 0.5 µg/mL), in comparison with MIC against susceptible strains to penicillin G. It is as active as teicoplanin against *Enterococcus faecalis* (MIC<sub>₉₀</sub>, 0.5 µg/mL; MIC<sub>₅₀</sub>, 1.0 µg/mL) but poorly active against *E. faecium* (MIC<sub>₉₀</sub>, 4.0 µg/mL; MIC<sub>₅₀</sub>, 8.0 µg/mL) in comparison with dalfopristin-quinupristin (MIC<sub>₉₀</sub>, 1.0 µg/mL; MIC<sub>₅₀</sub>, 1.0 µg/mL). RWJ-54428 exhibits good antistaphylococcal activity (Lancefield group A, B, and G), with an MIC<sub>₉₀</sub> of ≤0.06 µg/mL and an MIC<sub>₅₀</sub> of ≤0.06 µg/mL [102].

Granuloma pouch models were used for determining the in vivo antistaphylococcal activity of RWJ-54428 in Swiss Webster mice (20–25 g). Mice were infected with 1 x 10<sup>3</sup> cfu of MRSA Col, MRSA 76, or the Michigan glycopeptide-intermediate *Staphylococcus aureus* (GISA) strain HP-5827 in 5% mucin. Immediately after bacterial challenge, mice were treated subcutaneously with tested compounds. MICs of RWJ-54428 against the 3 *S. aureus* isolate strains tested were 2.0 µg/mL.

RWJ-54428 at 20 mg/kg decreased mean bacterial burden by 2.7 log₁₀ cfu/mL in the pouch for MRSA Col. With GISA strains HP-5827, a 3.4 log₁₀ cfu/mL decrease of bacterial burden in the pouch was observed for RWJ-54428, in comparison with 1.8 log₁₀ cfu/mL for vancomycin. A dose-dependent killing was noted for RWJ-54428 [103].

**MC-03,971.** It was recognized that a positively charged group attached through an ortho linkage to the heteroarylthio substituent at C-3 of the cephem nucleus provides good anti-
bacterial activity and also lowers the serum protein binding relative to noncharged analogs. The zwitterionic character of such cephems, with the relatively high lipophilicity needed for anti-MRSA activity, results in insufficient aqueous solubility for iv formulation. By adding a second protonation site, RWJ-54428, it is possible to achieve a better water solubility at low pH. Another method is to append a solubilizing prodrug moiety. A new series of 3-heteroarylthio cephems has been prepared [104] and 1 compound identified for its good in vitro activity and in vivo efficacy against gram-positive cocci: MC-03,971 (figure 17B). In vivo, MC-03,971 ED$_{50}$ were 0.2 mg/kg and 2.5 mg/kg for S. aureus Smith and S. aureus 76 (MRSA), respectively. To enhance water solubility, an N-alanyl prodrug of MC-03,791 was prepared. Alanyl prodrug of the aminothiazolyl ring at C-3 position was evaluated for its bioavailability. Good bioavailability was achieved, as well as a good water solubility [104].

In a new series, the C-3 aminothiazolyl ring was replaced with the more basic aminopyridine heterocyclic and analogs of MC-02,479. MC-02,867 and MC-03,260 (figure 17C) showed higher water solubility (>20 mg/mL at pH 4.5), with good in vitro activity against gram-positive cocci (except against E. faecium) for MC-03,260. In systemic staphylococcal infections due to S. aureus Smith induced in Swiss Webster mice, ED$_{50}$ values were 2.0 and 4.0 mg/kg for MC-02,867 and MC-03,260, respectively [105].

**Inhibitors of β-Lactamases**

β-Lactamase–producing strains of gram-negative bacilli remain a therapeutic problem. Four classes of β-lactamases are of concern: classes A, B, C, and D. Class A β-lactamases are good substrates for clavulanic acid, tazobactam, or sulbactam. However, new classes of β-lactamases (e.g., extended-spectrum β-lactamases) are not overcome by available β-lactams and broad-spectrum β-lactamases are no more inhibited by clavulanic acid. Furthermore, clavulanic acid has recently been shown to be hydrolyzed by specific enzyme. No specific inhibitors are available for metallo-enzymes (class B) and class C enzymes. New compounds were reported in 1999.

**6-Exomethylpenams**

Numerous 6-(substituted methylene) penems have been reported in the literature that are potent inhibitors of cell-free β-lactamases but were ineffective in combination with β-lactams due to their poor ability to penetrate through the outer membrane of gram-negative bacilli. A new series of 6-methyl substituted penems has been prepared; they have a polar group

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**Figure 18.** A, Inhibitor of β-lactamases. B, Inhibitor of β-lactamase J 110441.

**Figure 19.** A, Inhibitors of class A and B β-lactamases. B, Inhibitors of class B β-lactamases.
Rhodamine Derivatives

In the search for non-β-lactam inhibitors of β-lactamases and especially class C enzymes, rhodamine derivatives have been shown to selectively inhibit class C enzymes such as RWJ-157498 (figure 20). The structure-activity relationships were explored, and it was shown that some structures support enzyme inhibition and some other, only antibacterial, activities such as nitrofuran chain [108]. Another series of rhodamine derivatives was shown to inhibit both classes A and C of β-lactamases [109].

Carbapenem J-110,441

Metallo-enzymes are metalloproteins that require zinc ions for their activity. These metallo-enzymes hydrolyze the β-lactam ring by a different mechanism than that of the serine enzymes of classes A, C, and D. Metallo-enzymes described before 1990 are chromosomal-mediated enzymes. In Japan in 1991, a plasmid-encoded metallo-enzyme was described in P. aeruginosa. A plasmid mediated IMP-1 metallo-enzyme was recently described in Enterobacteriaceae in Korea, Singapore, and Europe.

7-Alkylidene Cephalosporins

A series of 7-alkylidene cephems has been reported; they are able to inhibit class A, class C, and, for 2 of them, class B metallo-enzymes. Compounds with an electronegative substitution at position 3 of the cephem nucleus are able to inhibit Enterobacter cloacae P99 enzyme (IC₅₀ 0.01–1.48 μM), and also class A serine β-lactamases (TEM-1) with a wider range of activity (IC₅₀ 0.006–2.52 μM) and PC-1 enzyme with a lower activity (IC₅₀ 0.1–283 μM; figure 19A).

Two compounds with a 3-methylacetyl side chain and with a bromine or chlorine atom at C-7 (figure 19B) exhibit good inhibitory activity against metallo-enzyme, with IC₅₀ values of 82 μM for Bacillus cereus II (Bc II), 3 μM for B. fragilis (Cf A), 0.04 μM for Stenotrophomonas maltophilia (L1), and 12 μM for S. marcescens (IMP-1) [107].
The IMP-1 gene bla\textsuperscript{IMP} is transferable among gram-negative bacilli, and the spread of this gene could be a threat in the near future. Many inhibitors of metallo-enzymes have been described against chromosomal metallo-enzymes. A series of carbapenems active in inhibiting IMP-1 metallo-enzymes was described [110]. By studying the structure inhibitory relationship, it was demonstrated that 1-\(\beta\)-methyl carbapenem having an \(S\)-linked lipophilic side chain significantly inhibited IMP-1 metallo-enzyme (\(\beta\)-lactamase). Some of them are strong inhibitors (IC\textsubscript{50}, <0.1 \(\mu\)M). All these carbapenems are weakly active against \(E.\ coli\) NIH-JC2 and \(P.\ aeruginosa\) but displayed good activity against \(S.\ aureus\) 209P-JC1 (MIC, <0.012 \(\mu\)g/mL). These 1\(\beta\)-methyl carbapenems could be classified into 3 groups according to the C-2 side chain heteroatomic moiety, such as benzothiophene (J-110,441), phenylthiophene ring, \(N,N\)-dialkylthiocarbamoyl with a dicarboxylic moiety, and pyrrolidinyl substituted with aromatic ring (e.g., thiophene) [110].

J-110,441 (figure 18B) was chosen for further investigation. J-110,441 inhibited class B metallo-enzymes such as IMP-1 encoded on the transferable \(bla_{IMP}\) gene (inhibition constant \(K_{i}\), 0.0037 \(\mu\)M), CcrA from \(B.\ fragilis\) (\(K_{i}\), 0.24 \(\mu\)M), L1 from \(S.\ maltophilia\) (\(K_{i}\), 1.02 \(\mu\)M), and \(B.\ cereus\) type II (\(K_{i}\), 0.88 \(\mu\)M). J-110,441 inhibited also TEM-1 enzyme (class A \(K_{i}\), 1.02 \(\mu\)M) and class C serine \(\beta\)-lactamase (\(E.\ cloacae\)), with a \(K_{i}\) of 0.0062 \(\mu\)M.

Inhibition of IMP-1 metallo-\(\beta\)-lactamase is reversible. Combination of imipenem or ceftazidime with J-110,441 shows a synergistic activity on antibacterial activity against IMP-1 producing \(S.\ marcescens\) and derepressed class C \(\beta\)-lactamase of \(E.\ cloacae\). These combinations was not effective against \(P.\ aeruginosa\) harboring the \(bla_{IMP}\) gene, due probably to a poor outer membrane penetration [111].

**Mercaptocarboxylic Inhibitors**

In a screening study to identify metallo-\(\beta\)-lactamase inhibitors, captopril and thiophan, 2 angiotensin-converting enzyme inhibitors were identified as low-potency competitive inhibitors of metallo-\(\beta\)-lactamas. SB 255 666 (figure 21A) was obtained by combining the thiazolidine ring structure of penem ring with the 2-benzoylpropanoyl component present in thiophan. It is one of the most potent broad-spectrum and selective series of metallo-enzyme inhibitors. Against \(B.\ cereus\) type II, \(B.\ fragilis\) (C¢A), and \(S.\ maltophilia\) (L1), \(K_{i}\) were 6.0, 0.43 and 0.8 \(\mu\)M, respectively [112].

The phenylglycine derivative (SB 227 943) was found to be the most active and selective compound, with a \(K_{i}\) of 0.61 \(\mu\)M for IMP-1, 21 \(\mu\)M for \(B.\ cereus\) I, 0.24 \(\mu\)M for \(B.\ fragilis\) (C¢A), and 0.15 \(\mu\)M for \(S.\ maltophilia\). Optimization of thiophene derivatives led to SB 252 619 (figure 21B), which exhibits high affinity for metallo-\(\beta\)-lactamas, with \(K_{i}\) of 0.08 \(\mu\)M for C¢A enzyme, 0.40 \(\mu\)M for L1 enzyme, and 0.09 \(\mu\)M for IMP-1 enzyme. Another derivative that has a phenyl substituted side chain, SB 264 218, also exhibits a high affinity for metallo-\(\beta\)-
lactamases, with $IC_{50}$ values of 0.03 $\mu M$ for Cfi A enzyme, 0.15 $\mu M$ for L1 enzyme, and 0.10 $\mu M$ for IMP-1 [113].

**Oxazolidinones and Derivatives**

Numerous new series of oxazolidinones have been published since 1997 [1, 2]. In 1999 new series of oxazolidinones were prepared.

**Pyrazinoindole and Oxazinoindole Oxazolidinones**

Within a series of pyrazinoindole and oxazinoindole analogs of oxazolidinone, 2 compounds exhibited a higher activity against gram-positive cocci than linezolid, especially the oxazinoindole analog. MIC values were $\leq$0.12–0.25 $\mu g/mL$ for *Staphylococcus* species, *S. pyogenes*, and *S. pneumoniae* (linezolid MICs, 1–2 $\mu g/mL$). An enhanced in vitro activity was shown against *H. influenzae* (MIC, 0.5 $\mu g/mL$). However, the pharmacokinetics after oral absorption are unfavorable (bioavailability $\sim$16%) [114].

**Benzoxazinone and Benzothiazinone Oxazolidinones**

In a series of benzoxazinone and benzothiazinone oxazolidinones, the most active analog exhibited in vitro activity similar to that of linezolid. In female CFW-1 mice, the pharmacokinetic profile after administration of 1 mg/kg iv was better with the selected compound than with linezolid. An apparent elimination half-life of 1.5 h versus 0.5 h for linezolid was recorded [115]. The morpholine ring of linezolid could be modified and antibacterial activities retained [116], especially when the replacement was done with a 3,6-dihydro-4(2H)-thiopyran ring.

**Tetrahydrothiopyranyl Phenylazolidinone Sulfoxides and Sulfones**

A new series of thiopyranyl sulfoxide and sulfone analogs have been synthesized with various side chains replacing the acetamido group. Some analogs in these series exhibited enhanced in vitro activities, including fastidious gram-negative bacilli such as *H. influenzae* and *M. catarrhalis*. Some analogs showed higher water solubility up to 20.2 mg/mL, such as compound B [117] (figure 22).

Biotransformation of PNU-176723 (figure 23), a cis-isomer of the tetrahydro-4(2H)-thiopyranyl phenylazolidinone sulfoxide, to the corresponding trans isomer was investigated in the Sprague-Dawley rat. The cis isomer was biotransformed in trans isomer, and an additional metabolite, a sulfone metabolite, was obtained. It appears that the bioconversion occurs by reduction of the sulfoxide to the sulfide by bacteria in the gastrointestinal tract, followed by the preferential presystemic oxidative metabolism of the sulfoxide to the trans sulfoxide [118].

**Carbon-Carbon Linked 5-Membered Heteroaryl Phenylazolidinone**

With the target of obtaining a new wave of oxazolidinones with enhanced activity against gram-positive cocci and fastid-
ious gram-negative bacilli, the morpholine ring was removed and replaced. One compound with a nitrogen-carbon linked azolylphenyl moiety was selected for further investigation: PNU-172576 [119] (figure 24A). New series of derivatives have been prepared and evaluated in which the nitrogen-carbon bond has been replaced with a carbon-carbon link. The morpholine ring of linezolid was replaced with various 5-membered ring moieties such as pyrazole, thiophene, oxazoles, thiazole, 2,4-oxadiazole, and 2,4-thiadiazole rings.

Two cyanothiophene analogs exhibited good in vitro activity against gram-positive cocci, and one of them displayed good activity against M. catarrhalis (MIC, ≤0.5 μg/mL) but was devoid of antibacterial activity for H. influenzae (MIC, >64 μg/mL). A cyanothiophene analog also exhibited a good activity against gram-positive cocci (MIC, ≤0.12–0.25 μg/mL) and against M. catarrhalis (MIC, 0.25 μg/mL) and H. influenzae (MIC, 2 μg/mL vs. 16 μg/mL for linezolid). A methyloxazole derivative and thiazole analog also displayed good activity against gram-positive bacilli but less activity against fastidious gram-negative bacilli, in comparison with the cyanothiophene analog [120].

A series of thiadiazolyl oxazolidinones was synthesized, and many of them exhibited in vitro activity against gram-positive cocci similar to that of linezolid [121].

Replacement of the Oxazolidinone Ring

Efforts have been focused on the identification of bioisosteric replacements of the oxazolidinone ring. In previous series, the oxazolidinone ring was replaced by dihydrofuran-2-one, pyrrolidin-2-one rings [122].

Isoxazoline ring. In a new series of bioisosteres of oxazolidinone, the isoxazoline ring system was identified for replacement of the oxazolidinone ring. As for oxazolidinone, the absolute configuration at C-5 position is compulsory for antibacterial activities. The (R) isomer is active, whereas the (S) isomer is inactive. Two compounds, PNU-173954 and PNU-171832 (figure 24B), exhibited similar in vivo efficacies in staphylococcal murine disseminated infections [123].

Furanone analogs. In previous series of furanone derivatives, one compound was selected for in vitro evaluation: ZM 302061 (figure 25). A series of new furanone analogs was synthesized. ZM 302061 is more active in vitro against gram-positive cocci than linezolid, especially against staphylococci (MIC, 0.13–0.5 μg/mL). The toxicological assessment was performed on the interaction on bone marrow after a dose of 200 μg/kg. Depending on the side chain, no effects to severe effects were recorded. Some new analogs exhibited equivalent in vivo efficacies in the murine model (Alderley Park mice) than linezolid and are devoid of toxicological effects [124].
**Efflux Pump Inhibitors**

Bacteria resist the poison represented by antibacterial agents in many ways: inactivation, target modification, outer membrane impermeability, and efflux. Efflux mechanisms of resistance are ubiquitous and are found in many bacterial species. This mechanism of resistance was described in gram-positive cocci (e.g., *S. pneumoniae*, *S. pyogenes*, and other streptococci, *S. aureus*, and *Enterococcus* species), and gram-negative bacilli (Enterobacteriaceae and *P. aeruginosa*). Active efflux mechanism of resistance is being recognized as a major cause of resistance for tetracycline (genes, *tet A, B, C, D, F*, and *H*), fluoroquinolones, (e.g., *nor A*), erythromycin A, (genes *mef A, mef E, msr A, msr B*, etc.), and for some β-lactams.

**Inhibitors of Fluoroquinolones**

*S. aureus*. The efflux of fluoroquinolones in *S. aureus* is mainly due to the membrane transporter *Nor A* [125, 126]. The active efflux mediated by *Nor A* can be inhibited by reserpine [127]. Unfortunately, reserpine cannot be used to potentiate the activities of fluoroquinolones because of its neurotoxicity at the concentrations required for *Nor A* inhibition. New chemical structures were selected for their inhibitory activity of *Nor A* (figure 26).

These inhibitors are more potent than reserpine in promoting bactericidal activity of ciprofloxacin against *S. aureus*. They reversed the ciprofloxacin resistance of the *S. aureus* strain and reduced the rate of emergence of ciprofloxacin-resistant mutants of *S. aureus*. These inhibitors are also likely to be active against multidrug transporters of gram-positive bacteria, because the 5 inhibitors also inhibited the *B. subtilis* Bmr multidrug transporter. Furthermore, INF 271 and INF 55 inhibit efflux-mediated fluoroquinolone resistance in *S. pneumoniae* [128].

*P. aeruginosa*. *P. aeruginosa* isolates are characterized by intrinsic resistance to a variety of antibacterial agents. This characteristic has been attributed in part to several efflux systems. Three efflux pumps are involved in *P. aeruginosa* fluoroquinolone resistance: MexAB-OprM, MexCD-OprJ, and MexEF-OprN [129, 130]. Broad-spectrum efflux inhibitors of fluoroquinolones have been identified [131–133]. Prevalence of overexpression of MexAB-OprM was investigated in some countries. For example, in the United States, 16% of 258 *P. aeruginosa* isolates resistant to levofloxacin collected in a surveillance study were harboring only a MexAB-OprM mechanism of resistance [134].

By use of a biological tool, the MPC$_2$ parameter, which is the minimum concentration of an inhibitor to potentiate the MIC of levofloxacin 8-fold, a compound, MC 207,110 (figure
A thigh pseudomonal infection was induced in neutropenic male Swiss-Webster mice. Levofoxacin therapy started 2 h after bacterial challenge and continued for 24 h, in comparison with the combination of levofoxacin 30 mg/kg every 4 h and efflux pump inhibitor. Efflux pump inhibitor was given by the intraperitoneal route alone at doses of 100–350 mg/kg in 2, 3, 6, and 12 divided doses. Addition of efflux pump inhibitor enhanced the rate of bacterial killing by levofoxacin in a time-dependent manner [135].

Other gram-negative bacilli. Bacterial efflux pumps fall into 4 major classes: the resistance-nodulation division family, the major facilitator family, the small multidrug resistance family, and the adenosine triphosphate binding cassette transporter. Resistance-nodulation division pumps confer resistance to various gram-negative bacilli, including *P. aeruginosa*, *E. coli* (Acr AB-ToIC), *H. influenzae* (Acr AB), and *Salmonella typhimurium* (Acr AB).

A combination of levofoxacin and efflux pump inhibitor against Enterobacteriaceae resistant to levofoxacin were synergistic; in addition, efflux pump inhibitor restored the bacterial activity of levofoxacin [133].

**Tetracycline.**

An increase tetracycline resistance within causative agents of bovine respiratory disease, mainly *Pasteurella multocida* and *Pasteurella haemolytica*, led researchers to identify efflux pump inhibitors. Efflux resistance to tetracycline, from *Pasteurella* species (bovine and turkeys), is driven by genes *tet A, tet B, tet C, tet D, tet E*, and *tet H.*

One compound was identified by *tet C* resistance, UK-57,562-01 (figure 27B), which lacks antibacterial activity against *Pasteurella* species (MIC, >25 μg/mL), *E. coli* (MIC, >256 μg/mL).

**Figure 27.** *A,* Efflux pump inhibitors MC 207,110. *B,* Efflux pump inhibitor UK 57,562-01.

**Figure 28.** CHIR 29498
mL), *Streptococcus* species (MIC, >16 μg/mL), and *Staphylococcus* species (MIC, >32 μg/mL). UK-57,562-01 reduced the MIC of tetracycline 4-8-fold against *E. coli* strains harboring Tet A, Tet B, Tet C, and Tet D resistance determinants. In contrast, UK-57,562-01 did not act synergistically with tetracycline against gram-positive cocci and did not inhibit the ubiquitous efflux pump Acr AB in *E. coli* [136].

**Peptide Antibacterials**

Peptide antibacterials are a complex family of antibacterial agents composed of hundreds of compounds. Only a few of them were introduced in clinical practice. Many reviews have been published, gathering the knowledge of this complex class of antibacterials [137, 138]. New data have been provided for Nu-2 [139], bactolysins [140], thrombocytins [141], and cecropins [142]. Only few new compounds were reported in 1999, an update provided for specific characteristics.

**CHIR 29498**

A series of peptides, which are N-substituted glycine trimers with antibacterial activity, have been reported. They were discovered by screening combinatorial chemistry libraries. One trimer peptoid, CHIR 29498, showed some in vitro and in vivo activities (figure 28). CHIR 29498 is rapidly bactericidal. This molecule is likely to interact with the bacterial membrane. CHIR 29498 provided complete protection of female CD-1 mice after staphylococcal (*S. aureus* Smith) challenge and after a single intraperitoneal dose of 10 or 30 mg/kg, administered immediately after bacterial inoculation, or after 2 h [143].

**Analogs of Magainins**

More than 100 peptides representing components of the system of host defense of animals have been discovered, some of them (such as magainins) protegins that have been modified and are under development for human use. New data of a modified magainin, pexiganan (MSI-78), were released. MICs for gram-negative and gram-positive bacteria, as well as for anaerobes, were 4–16 μg/mL. Higher MICs were recorded (64 and 128 μg/mL) for viridans group streptococci and enterococci [144].

**Eremomycin Analogs**

Eremomycin belongs to the glycopeptide family of antibacterial agents. A new series of derivatives has been published [145, 146]. A new series of eremomycin carboxamides was synthesized with the aim to investigate the influence of various substituents on the antibacterial activity against vancomycin-resistant and vancomycin-susceptible gram-positive cocci in comparison with vancomycin and teicoplanin (figure 29). Numerous substitutions have been made on eremomycin or on chloreremomycin on the sugar moieties, and one derivative was selected for clinical development: LY 333328.
In this series, a new site of substitution was chosen: the amide group of eremomycin. Twenty-six carboxamide analogs were prepared. All analogs showed a good antistaphylococcal activities in vitro (MIC, 0.06–0.5 μg/mL). However, the anti-enterococcal activity is variable according to the nature of the substituents. None of them had detectable activity against *E. faecium* strains resistant to vancomycin. One analog with linear lipophilic substituents showed an in vitro activity against vancomycin-resistant enterococci (*vanA, vanB*). Additional alterations were done to enhance the water solubility of these amides analogs by inserting L-lysine or a piperazinyl moiety between decyl and eremomycin parts of the molecule. The most active carboxamide analogs will be considered as leading compounds for further chemical modifications.

**Glycopeptide Derivatives**

An extensive study was carried out to highlight the mechanism of action of modified vancomycin analogs. It is difficult to alter the peptide position of vancomycin. However, vancomycin derivatives having hydrophobic substituents on the vancosamine nitrogen (figure 30, compounds 1 and 2) displayed enhanced activity in comparison with vancomycin against gram-positive cocci strains resistant to vancomycin. By use of modified vancomycin, such as desleucyl vancomycin, or by use of substituted vancosamine sugars, it was shown that these compounds interact with the peptidoglycan by a different way than vancomycin. It seems that their mechanisms of action are independent of peptide binding. They probably interact with the immature peptidoglycan, lipid II, and protein involved in transglycosidation. The fundamental study will open research in glycopeptide chemical modification to obtain new compounds design to overcome vancomycin resistance [147].

**Miscellaneous Peptides**

Two cyclic homopentapeptides, CP 101,680 and CP 163,234, showed a bactericidal activity against animal respiratory pathogens, *Actinobacillus pleuropneumoniae* and *P. haemolytica*.
[148]. Two types of compounds extracted from sponge were published. Psammaplin A, a natural bromotyrosine derivative from an associated form of 2 sponges, *Poecillistra* species and *Jaspis* species, was found to exhibit a good antistaphylococcal activity, including MRSA strains. This compound inhibits DNA synthesis and DNA gyrase activity [149] (Figure 31). Two theonella peptolide-related cyclic depsipeptides have been isolated from a sponge (*Theonella* species). One compound exhibits antistaphylococcal activity (MIC, 8 μg/mL) [150].

Activity of aminopenicillin associated with catechol-containing siderophore (Figure 32) was tested against gram-negative nonfermenting organisms. These compounds displayed good in vitro and in vivo activities against these strains. However, these derivatives have a low safety margin in rats and in mice when administered intravenously [151].

Antimycobacterial Agents

The incidence rate of multidrug-resistant (resistance to 2 major antituberculosis drugs) *M. tuberculosis* varies from one area to another. In western Europe, the incidence rate is <1%, with 2 exceptions: in Berlin, where the incidence rate is ~5%–6%, and in Portugal, where the incidence is 3.7%. In central and eastern Europe, the incidence rate of multidrug-resistant *M. tuberculosis* is higher and has reached 22.1% in Latvia [152]. In other parts of the world, the incidence rate may be very high, but true figures are not always available. Recently some fluoroquinolones, due to their bactericidal activity, were added to the armamentarium of antituberculosis drugs. To increase compliance with antituberculosis treatment regimens, especially in the maintenance phase, a new rifamycin derivative, rifapentine, was granted approval by the US Food and Drug Administration. However, new compounds are needed; a few are in clinical development, such KRMM 1648 (rifalazil), which seems to be in phase II trials. New compounds were screened for in vitro activity against *M. tuberculosis*.

Trifluoperazine

It was shown that there is a correlation between *M. tuberculosis* growth and the presence of calmodulin-like protein, phospholipids, and lipids [153]. Trifluoperazine is a calmodulin antagonist and inhibits the incorporation of phospholipids into the phospholipids bilayers. It was shown that trifluoperazine inhibits the growth of *M. tuberculosis* H37RV. In a
recent study, it was shown that trifluoroperazine, an antipsy-
chotic drug, displayed moderate activity against *M. tuber-
culosis*, even for multiresistant isolates. It could be a companion
drug when a multidrug isolate is involved in this pathological
process [154].

**Isonicotinoyl Hydrazones**

A new series of amino hydrazone analogs, structurally related
to isoniazid, has been described. The pyridylmethyleneamino
derivatives with various substituents on the phenyl ring were
synthesized and investigated for their activity against *M. tu-
berculosis*. All of them were cross-resistant with isoniazid, and
within the tested compounds, there were no analogs with ac-
tivity similar to that of isoniazid (MIC, 0.06 µg/mL vs. analogs
with MICs of >6.25 µg/mL) [155].

**4-Aminobenzoic Acid Hydrazones**

In a series of 4-aminobenzoic acid hydrazid, 1 compound
showed good anti-*M. tuberculosis* activity, with an MIC of 3.13
µg/mL for *M. tuberculosis* H37RV, with the BACTEC 12 B
medium for BACTEC 460 radiometric system. However, this
compound is also very cytotoxic [156] (figure 33).

**Inhibitors of Dihydrofolate Reductase (DHFR)
of Mycobacteria**

It is known that trimethoprim and available benzylpyrimi-
dine derivatives are poor inhibitors of DHFR of *M. tuberculosi-
s* and MAC. The inhibitory activity against DHFR of MAC with
2,4-diamino-5-deazapteridine derivatives was investigated. Various
substituents at position 5, 6, or both of the pteridine moiety
were fixed, and the resulting compounds were tested for their
activities. More than 50% of the 70 analogs inhibited MAC
DHFR with an IC₅₀ of ≤10 nM. MICs for *M. tuberculosis*
varied from 1.28 µg/mL to ≥128 µg/mL [157].

**New Chemical Entities**

**Quinoline-Indole Derivatives**

With the aim of designing compounds active against *S. aureus*
isolates resistant to methicillin, but also to fluoroquinolones
and to vancomycin, a combinatorial library of quinoline indole
Pleuromutilins were prepared by using solid-phase heterocyclic N-oxide chemistry and was screened against a variety of microorganisms. After the identification of a lead structure exhibiting moderate in vitro activity against MRSA, a series of analogs was prepared in order to increase potency and to evaluate the structure-activity relationships of the novel class of antibacterials [158].

Starting from a quinoline ring as a scaffold, it was demonstrated that the 2-quinoline-3-indole ring substituted with a halogen at the C-6 or C-7 position of the quinoline ring, and C-5 of the indole ring gave the most active compounds. The different active compounds were active against MRSA; few of them exhibited a weak activity against vancomycin-resistant *E. faecium*, but they were also active against *S. aureus* resistant to fluoroquinolones due to an active efflux pump. Three compounds were selected for further investigations: SEP-32196, SEP-132617, and SEP-137199 (figure 34).

The most active compound is SEP-137199, which exhibits a good in vitro activity against *S. aureus* (MIC$_{90}$ against MRSA, 0.06 µg/mL), methicillin-resistant (MIC$_{90}$, 0.06 µg/mL), mupirocin-resistant (MIC$_{90}$, 0.06 µg/mL), and fusidic acid–resistant (MIC$_{90}$, 0.03 µg/mL) strains, as well as against *S. epidermidis* (MIC$_{90}$, 0.03 µg/mL) or *S. pyogenes* (MIC$_{90}$, 0.01 µg/mL). SB-268091 is less active than SB-268091, especially against isolates resistant to methillin (MIC$_{90}$, 2.0 µg/mL) or resistant to mupirocin (MIC$_{90}$, 1.0 µg/mL) and against *S. epidermidis* (MIC$_{90}$, 1.0 µg/mL) [160].

Dorsal staphylococcal wound infections were induced in mice, and local treatment began 1 h after challenge. Further doses were administered at 4 and 7 h, and treatment continued thereafter 3 times a day for 3 days. Animals were sacrificed ~17 days post-infection to determine survival rate. SEP-32196 and SEP-132617 showed a better survival rate than vancomycin, while SEP-137199 did not show any beneficial effect. ED$_{50}$ of 12.5 and 15 mg/kg were estimated for SEP-32196 and SEP-132617, respectively. Vancomycin was used as positive control in all studies, and 100% of mice survived after a 10 mg/kg dose of vancomycin administered intraperitoneally [159]. In conclusion, these compounds are novel, but improvement is needed; they are poorly water soluble, highly bound to protein (>95%), and are not as active as vancomycin.

**Pleuromutilins**

Pleuromutilins are natural compounds that are not currently used in human medicine but instead in veterinary medicine. *S. aureus*, coagulase-negative staphylococci, and *S. pyogenes* are the main pathogens involved in skin and skin structure infections. The number of antibacterials used as topical antibiotics are not numerous (mupirocin and fusidic acid), and strains highly resistant to *S. aureus* are not uncommon. New antibiotics for topical use are needed. Two semisynthetic derivatives of pleuromutilins, SB-247386 and SB-268091 (figure 35), were selected for further investigation.

In vitro SB-247386 is particularly active against methicillin-susceptible *S. aureus* (MIC$_{90}$, 0.06 µg/mL), methicillin-resistant (MIC$_{90}$, 0.06 µg/mL), mupirocin-resistant (MIC$_{90}$, 0.06 µg/mL), and fusidic acid–resistant (MIC$_{90}$, 0.03 µg/mL) strains, as well as against *S. epidermidis* (MIC$_{90}$, 0.03 µg/mL) or *S. pyogenes* (MIC$_{90}$, 0.01 µg/mL). SB-247386 is less active than SB-268091, especially against isolates resistant to methillin (MIC$_{90}$, 2.0 µg/mL) or resistant to mupirocin (MIC$_{90}$, 1.0 µg/mL) and against *S. epidermidis* (MIC$_{90}$, 1.0 µg/mL) [160].

Dorsal staphylococcal wound infections were induced in mice, and local treatment began 1 h after challenge. Further doses were administered at 4 and 7 h, and treatment continued thereafter 3 times a day for 3 days. Animals were sacrificed ~17 days post-infection to determine survival rate. SEP-32196 and SEP-132617 showed a better survival rate than vancomycin, while SEP-137199 did not show any beneficial effect. ED$_{50}$ of 12.5 and 15 mg/kg were estimated for SEP-32196 and SEP-132617, respectively. Vancomycin was used as positive control in all studies, and 100% of mice survived after a 10 mg/kg dose of vancomycin administered intraperitoneally [159]. In conclusion, these compounds are novel, but improvement is needed; they are poorly water soluble, highly bound to protein (>95%), and are not as active as vancomycin.
h after therapy ended. The number of viable bacterial cells recovered from the wounds was counted. In control mice, the number of bacterial cells was log10 cfu per wound. Treatment with mupirocin resulted in a 2 log10 reduction in bacterial counts (log10 cfu per wound). A high reduction was obtained with SB-247386 in comparison with control in groups of mice treated with mupirocin: log10 1.8 cfu per wound. No detectable bacteria were recorded in 4 of 7 mice with SB-247386 (1.3 log10 cfu per wound), and 4 of 7 mice showed sterilized wounds.

When infecting mice with S. aureus strain 1080 resistant to mupirocin (MIC, 4.0 µg/mL), mupirocin decreases the wound burden weakly (5.2 ± 1.0 log10 cfu per wound vs. 6.3 ± 0.3 log10 cfu per wound for control animals), but SB 247386 significantly decreased the bacterial burden (2.3 ± 0.4 log10 cfu per wound vs. 6.3 ± 0.3 log10 cfu per wound for control), and bacterial count was under the limit of detection (<1.7 log10 cfu per wound) in 3 of 7 mice [161].

**Spiroisoxazole Derivatives**

A series of spiroisoxazole derivatives has been synthesized with the aim of being active against MRSA and GISA strains.

One compound, KY-9 (figure 36), was investigated. MICs of KY-9 against MRSA and GISA were 3.15–6.25 µg/mL. A synergistic activity was demonstrated in combination with arbekacin. The in vitro activity of KY-9 was not convincing [162].

**Anti-MRSA DHFR Inhibitors**

Elucidation of the mechanism of resistance of staphylococci to trimethoprim and the availability of crystal structures was the starting point for research of DHFR inhibitors against MRSA. Resistance to trimethoprim for S. aureus is originated by a single amino acid substitution (Phe 98→Tyr) in DHFR in S. aureus. This substitution results in the loss of the hydrogen bond between the diamino group at position 4 of trimethoprim and the carbonyl group of leucine 5 [163]. Molecular labeling supported the finding that the resulting loss of binding affinity could be compensated by accurate substitution at position C-3' of trimethoprim to fill a lipophilic pocket in the enzyme. A large series of 2,4-diamino-5-benzylpyrimidines bearing a lipophilic substituent at the position C-3' of the benzene ring was synthesized.

A structure-activity relationship study demonstrated that the diaminopyrimidine backbone is essential, and ≥1 methoxy group is needed in position 5; the best link is 2-propenone. The best residues at position C-3' could be tetrahydroisoquinoline and dihydrophtalazine, which could be substituted at position 1' (R configuration). Substituents at C-3' or C-6' dramatically decreased the antibacterial activity [164]. Two compounds were selected for further investigations: Ro-62-6091 and Ro-64-5781 (figure 37).

Both compounds exhibit low affinity for human DHFR (IC50 >30 µM) but a high affinity for DHFR of S. aureus and S. pneumoniae strains resistant to trimethoprim (IC50 <0.001 and <0.004 µM for S. aureus and S. pneumoniae, respectively). Ro-62-6091 is more active than Ro-64-5781 against S. aureus.
susceptible to trimethoprim (MIC$_{50}$ 0.015 µg/mL and MIC$_{90}$ 0.03 µg/mL vs. MIC$_{50}$ 0.03 µg/mL and MIC$_{90}$ 0.03 µg/mL), but Ro-64-5781 is more active against *S. aureus* resistant to trimethoprim (MIC$_{50}$ 0.125 µg/mL and MIC$_{90}$ 0.5 µg/mL vs. MIC$_{50}$ 0.25 µg/mL and MIC$_{90}$ 0.5 µg/mL). The same figures applied for *S. epidermidis*. Both compounds were highly active against *S. pneumoniae* isolates considered to be susceptible to trimethoprim (MIC$_{50}$ 2 µg/mL; MIC$_{90}$ 4 µg/mL), with MIC$_{50}$ and MIC$_{90}$ values of ≤0.015 and 0.125 µg/mL, and 0.03 and 0.125 µg/mL for Ro-64-5781 and Ro-62-6091, respectively. Both compounds were poorly active against *S. pneumoniae* isolates resistant to trimethoprim (MIC, 128 µg/mL) and resistant to penicillin G; MIC$_{50}$ and MIC$_{90}$ values were 1 and 2 µg/mL, and 4 and 16 µg/mL for Ro-64-5781 and Ro-62-6091, respectively.

The compounds exhibited good in vitro activity against *E. faecalis* and *E. faecium* when isolates were considered as susceptible to trimethoprim (MIC$_{50}$ 0.5 µg/mL; MIC$_{90}$ 2.0 µg/mL) with MIC$_{50}$ and MIC$_{90}$ values of 0.09 and 0.06 µg/mL, and 0.06 and 0.125 µg/mL for Ro-64-5781 and Ro-62-6091, respectively. They were poorly active against *Enterococcus* isolates resistant to trimethoprim (MIC, >128 µg/mL), with MIC$_{50}$ of 16 µg/mL and MIC$_{90}$ of 8 µg/mL, and MIC$_{50}$ >64 µg/mL for Ro-64-5781 and Ro-62-6091, respectively. Both compounds were weakly active against *Enterobacteriaceae*, *P. aeruginosa*, or *S. maltophilia*. They were moderately active against *H. influenzae* susceptible to trimethoprim but inactive against *H. influenzae* isolates resistant to trimethoprim.

Outbred Swiss albino mice were challenged either with *S. aureus* Smith (3 x 10$^6$ cfu per mouse) or *S. aureus* R4 resistant to trimethoprim (MIC, 512 µg/mL; 6 x 10$^6$ cfu per mouse). The test compounds were administered by the iv route 1 and 3 h after bacterial challenge against trimethoprim-resistant *S. aureus*, and by oral and iv routes for *S. aureus* Smith. In the group challenged with *S. aureus* Smith, ED$_{50}$ values were 30 times lower after iv treatment (ED$_{50}$ 0.36 and 1.6 mg/kg for Ro-64-5781 and Ro-62-6091, respectively), in comparison with compounds delivered by the oral route (10.0 and 12.5 mg/kg for both compounds, respectively). In comparison with trimethoprim, ED$_{50}$ values were 5.8 mg/kg and 10.9 mg/kg after delivery by means of the iv and oral routes, respectively.

In *S. aureus* R4, which is trimethoprim resistant, only Ro-64-5781 demonstrated an in vivo activity in disseminated staphylococcal infection, with ED$_{50}$ of 3.6 mg/kg after iv administration; Ro-62-6091 was poorly active (ED$_{50}$ >12.5 mg/kg) [165].

New Bacterial Targets and New Compounds

**Protein Deformylase (PDF)**

In bacteria, protein synthesis is initiated with N-formylmethionine. The nascent synthesized polypeptide is converted to mature protein through sequential removal of N-formyl group and methionine by peptide deformylase and methionine aminopeptidase [166]. The enzyme is lacking in mammalian cells and could be a target for antibacterial agents. It has been shown...
that PDF production is driven by 2 genes in gram-positive cocci (def A, def B, or both) but only one gene seems to drive PDF in E. coli (def A). These genes are essential for bacterial growth [167, 168]. The def A gene in E. coli is under the control of gene tol C, which can regulate PDF expression level with varying concentration of arabinose. The viability of cells is dependent of fmi1 gene product, tRNA-methionyl formyltransferase.

PDF is a metallohydrolase. A screening was initiated of compounds with metal chelating groups. One lead compound VRC483 (actinonin; figure 38) was identified for further investigations. Actinonin exhibits potent inhibitory activity for peptide deformylase, with IC₅₀ of 0.3 nM when using nickel PDF as substrate. Actinonin is a bacteriostatic agent against gram-positive cocci, but the level of activity is low (IC₅₀, >2 µg/mL) for S. epidermidis. MICs of >8 µg/mL were found against other gram-positive cocci; against fastidious gram-negative bacteria, MICs were 0.25-4 µg/mL [169].

The frequency of resistance to VRC483 in S. aureus is 10⁻⁶. The mechanism involved is a mutation in the fmt gene at A108E and G117V, leading to substitution in the formyltransferase protein. VRC483 could be considered as a leading compound, and other series were synthesized in which some analogs showed enhanced antibacterial activities such as VRC3785.

Peptidoglycan Inhibitors

Biarylamide derivatives. A search for new compounds acting on the peptidoglycan synthesis of S. aureus was initiated by screening a variety of compound libraries. Biarylamide side products were identified as the most active compounds within a combinatorial library of ~10,000 semicarbazones. A first series of analogs were synthesized, and 7 compounds exerted bactericidal activity against S. aureus H1 339. Two compounds, PGE 9567567 and PGE 552292, were bactericidal at a concentration inferior of 4 times the MIC (figure 39) but in a time-dependent manner. Some of them lost 50–100-fold in potency when MICs were determined in the presence of 40% bovine serum. These compounds are poorly soluble in water and are highly lipophilic [170]. PGE 9567567 was selected as the first choice for further chemical modification.

By use of combinatorial chemistry techniques, a large set of compounds with various side chains and a mixed set of these side chains or groups was synthesized. One of the main problems was water solubility. Three parameters were used to analyze the biological properties of the compounds issued from this screening: MIC against gram-positive cocci, inhibition of peptidoglycan synthesis of gram-positive cocci, and aqueous solubility. It was shown that increasing the hydrophobicity nature of the end of the molecules increased in vitro activity of analogs. However, increasing the hydrophobicity of the end of the molecule decreased water solubility. Changing the middle of the molecule does not affect the in vitro activity of issuing compounds. To take advantage of these characteristics, modifying groups attached the middle of the molecule may enhance water solubility [171].

In order to increase water solubility, to overcome the loss of potency in the presence of fetal bovine serum, a new series of compounds was synthesized, including 2 ureas, amide, malonamides, semicarbazides, and ureasulfamides (figure 40). All the synthesized compounds were inactive against Enterobacteriaceae (MIC, >300 µg/mL). Many of them showed in vitro activity against gram-positive cocci (geometric mean MIC, 0.9–6.4 µg/mL). All of them were affected by adding 40% fetal bovine serum in media for MIC determination. The loss of activity is very high (~200 times less).

In general, ureasulfamides, semicarbazide analogs, were inactive. Within ureas, biureas, and malonamide and quinazolines analogs, some of them were found to be active against gram-positive cocci. However, improvement in antibacterial activity seems to be accompanied by a significant drop in water solubility [172]. One compound was selected for further investigation, PGE 1393084. It is highly water soluble (829.6 µg/mL), but the affinity for peptidoglycan synthesis is very low (IC₅₀ >300 µM). In disseminated staphylococcal murine (CF-1 male mice) infection, PGE 1393084 after intraperitoneal administration

Table 5. In vitro activity of 6-anilinouracil derivatives.

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC₉₀ µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Staphylococcus aureus OXA-R</td>
<td>16</td>
</tr>
<tr>
<td>S. aureus OXA-S</td>
<td>16</td>
</tr>
<tr>
<td>CNS-OXA-R</td>
<td>32</td>
</tr>
<tr>
<td>CNS-OXA-S</td>
<td>16</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>16</td>
</tr>
<tr>
<td>E. faecium vancomycin-R</td>
<td>16</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>16</td>
</tr>
</tbody>
</table>

NOTE. CNS, coagulate-negative staphylococci; R, resistant; S, susceptible.
tion did significantly reduce bacterial burden at 10–20 mg/kg. However, no reduction of lethality was recorded in pneumococcal disseminated infection, even at a high dose (100 mg/kg) with all the biarylamide analogs tested.

The biarylamide analogs were administrated to rats by the subcutaneous or iv routes or to dogs at a dose of 2 mg/kg. Apparent elimination half-lives (± SD) in rats ranged from 6.3 ± 1.2 h to 337 ± 227 h. The peak plasma concentrations (C\text{max} ± SD) after subcutaneous administrations were low and ranged from 0.1 ± 0.004 to 1.1 ± 0.4 μg/mL, except for 1 compound, for which C\text{max} was 6.7 ± 3.3 μg/mL. The low serum concentration may be due to the large volume of distribution of these compounds (3.3 ± 1.8 to 33.0 ± 27.0 L/kg) [173]. No biarylamide derivatives were selected for further investigation due to their poor water solubility, their moderate in vitro activity against gram-positive cocci, and the lack of in vivo activity.

MurD inhibitors. MurD is an essential enzyme in bacterial cell wall biosynthesis. It is responsible for the addition of D-glutamic acid to the UDPN-acetylmuramoyl-L-alanine substrate in the growing pentapeptide chain of the Park nucleotide precursor. MurD was chosen as an antibacterial target because D-glutamic acid is not found in eukaryotes, and D-glutamic acid is included in all bacterial peptidoglycan cell walls. Series of N-acylated D-glutamic analogs were synthesized, and these analogs exhibited a large range of inhibitory activity (IC\text{50}, 17–400 μg/mL). Indole analogs seem to have a better antibacterial activity. One acyl indole analog was selected for further investigation (IC\text{50}, 17 μg/mL) [174].

Inhibition of Metabolic Pathway

Fumarate reductase. It was shown that lichochalcone A was able to inhibit the activity of fumarate reductase of many bacterial species. Against streptococci, the IC\text{50} values of lichochalcone A on fumarate reductase were 15–64 μM, and against staphylococci, IC\text{50} of lichochalcone A was 20–75 μM. Lichochalcone A inhibited the activity of nicotinamide adenine dinucleotide fumarate reductase of H. pylori, with IC\text{50} of 10 μM [177].

Inhibition of DNA polymerase III. A series of 6-anilino uracil analogs has been synthesized. They are new deoxyguanosine triphosphate (dGTP) analogs that selectively inhibit the replication of the specific DNA polymerase III of gram-positive bacteria. They exhibit their antibacterial activity both glutamic acid analog has an IC\text{50} of 39 μg/mL against MurD. The best derivative, a muramoyl phosphate-phosphinate glutamic acid analog, had an IC\text{50} against MurD of 0.17 μg/mL [175].

Katanosin B and plusbacin A3. Katanosin B and plusbacin A3 are naturally occurring cyclic depsipeptide antibiotics (figure 41) that are active against MRSA and Van A type vancomycin resistant enterococci, with MICs of 0.78 and 3.13 μg/mL for katanosin B and plusbacin A3, respectively.

Katanosin B and plusbacin A3 inhibited the formation of peptidoglycan. They also inhibited the formation of lipid intermediate (glycin addition), with IC\text{50} of 2.2 and 2.3 μg/mL, respectively. They inhibited the formation of nascent peptidoglycan with IC\text{50} of 0.8 and 0.4 μg/mL, respectively. Both compounds did not exert their inhibiting effect through binding to the acyl-D-alanyl-D-alanine terminal part of the lipid intermediate, as vancomycin does, even at high concentrations (800 μg/mL). Both compounds blocked transglycosylolation in a step distinct to that of vancomycin [176].
in vitro and in vivo. Four agents have been explored for their antibacterial activities: hydroxybutyl (6-[3'-ethyl-4'-methylamino]uracil), methoxybutyl (6-[3'-ethyl-4'-methylamino]uracil), hydroxybutyl, and methylbutyl (6-[3'-iodo-4'-methylamino]uracil). The in vitro activities of these few compounds are listed in table 5.

No cross-resistance was shown with fluoroquinolones, rifampin, oxacillin, or vancomycin. MICs were 2–32 μg/mL. It is difficult to provide a statement about these new compounds. Pharmacokinetic study is needed to properly evaluate the future of these compounds [178].

Triazine Derivatives

New leads in triazine derivatives have been selected by screening combinatorial chemistry libraries. The triazine ring is substituted on the C-2 position with an amino group and in C-6 with an amino-substituted side chain. Various side chains were fixed at position C-4 of the triazine ring, and some compounds showed MICs of 4–6 μg/mL and 4–32 μg/mL against _S. aureus_ and _B. subtilis_, respectively [179].

Antibacterial Adherence Compounds

One virulence factor of microorganisms is their ability to express adherence proteins or factors on the cell surface. An extensive review on adherence was recently published [180]. Inhibitor of sortase in gram-positive bacteria was proposed (figure 42) [181].

Miscellaneous

Lincomycin Derivatives

Many lincomycin derivatives have been reported in published articles. Recently, one compound, 7(α)-azido-7-deoxylincomycin, demonstrated a higher in vitro activity than lincomycin but lower than that of clindamycin [182] (figure 43). In a new series of azido derivatives, it was clearly shown that modifi-

![Figure 44. Triazolyl derivative: posaconazole prodrug](image)

![Figure 45. Lipopeptide antifungal A 192 411.29](image)
Table 6. Manufacturers and state of investigation of caspofungin and anidulafungin.

<table>
<thead>
<tr>
<th>Generic name</th>
<th>Code number</th>
<th>Manufacturer</th>
<th>Phase of study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspofungin</td>
<td>MK 0991</td>
<td>Merck</td>
<td>Under registration</td>
</tr>
<tr>
<td>(None yet available) FK 463</td>
<td>Fujisawa Pharm (Ibaraki, Japan)</td>
<td>Phase II/III</td>
<td></td>
</tr>
<tr>
<td>Anidulafungin</td>
<td>LY 303 366</td>
<td>E. Lilly</td>
<td>Phase II</td>
</tr>
<tr>
<td>(None yet available) A 192 411.29</td>
<td>Abbott (Abbott Park, IL)</td>
<td>Preclinical</td>
<td></td>
</tr>
</tbody>
</table>


cations of the azido group at C-7 unfavorably modified the antibacterial activity of the new analogs [183].

Choline Derivatives

An extensive well-done review of choline derivatives [184] highlighted 2 types of mimics: mimics of squalamine and mimics of polymyxin B. A series of cholic acid derivatives was presented and were designed to mimic the polymyxin B activity against gram-positive and gram-negative bacteria. Within these series, some compounds exhibited moderate in vitro activities against both gram-positive and gram-negative bacteria [185].

Antifungals

Only a few new antifungals were presented at the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) held in September 1999 in San Francisco. Three well-done and extensive reviews on new antifungals were issued in 1999 [186–188]. New data on anti- 

Candida activity of a new steryl keto, (3-[3-(4-chlorophenyl)-2-propenoyl]-4-[2-(4-chlorophenyl) vinylene]-1-ethyl-4-piperidinol), has been investigated. Geometric mean MIC for NC 1175 was 1.15 μg/mL (range, 0.25–2.0 μg/mL) in peptone yeast extract glucose broth. NC 1175 exerts a fungicidal activity in a concentration dependent manner. NC 1175 inhibits H+-adenosine triphosphatase of C. neoformans [196].

Phenyl Ethylene Derivatives

One compound, which also displays cytotoxic activity for murine and other animal cell lines and inhibits mammalian tubulin polymerization, was shown to exhibit anti- 

Candida activity in vitro as well as anti- 

C. neoformans activity. The antifungal properties of 1-(3′,4′,5′-trimethoxyphenyl)-2-nitro ethylene is limited due to the cytotoxicity [197].

Cochleates Technology

A new lipid-base carrier for amphotericin B was described [198]. Amphotericin-cochleate showed significant superior activity in murine disseminated 

Candida albicans infection than amphotericin B in reducing kidney and spleen burden [199, 200]. Cochleates are supramolecules formulations based on natural products, phosphatidylserine, and divalent cations. The aim of this formulation is to obtain a gastrointestinal absorption of amphotericin B. In this system, amphotericin B is anchored in the lipid bilayer and is protected from the environmental conditions from degradation.

Inhibitor of Efflux Pump Inhibitors

Multiple mechanisms of resistance are involved in fungal resistance to azole derivatives. Among them are multidrug re-
sistance pumps belonging to different families of transporters. In an intensive screening, efflux pump inhibitors were identified to overcome fluconazole resistance in *C. albicans*. They are active against multiple CDR pumps. They are able to reduce the MIC of fluconazole and posaconazole in *C. albicans*, and they reduced by 8- to 16-fold the MIC of fluconazole or posaconazole for *Candida glabrata* [201]. One compound was identified for further investigations: MC-510,027 (milbemycin α-9; figure 46). Combinations of MC-510,027 with fluconazole, itraconazole, posaconazole, and terbinafine were tested against 9 species of *Candida*. MC-510,027 is devoid of any antifungal activity. MC-510,027 enhances the in vitro activity of azoles and terbinafine against wild-type *C. albicans* and *C. glabrata* and against strains that overexpress adenosine triphosphate–binding cassette transporters (CDR1, CDR2, and Cg CDR1, Cg CDR2) [202]. In vivo, the combination of MC-510,027 and fluconazole was able to potentiate the activity of fluconazole against *C. albicans* strains overexpressing CDR1 and CDR2 without altering the pharmacokinetic behavior of fluconazole [203].

**Bicyclic Guanidines**

By use of a combinatorial technique, 100,000 bicyclic guanidine analogs were generated for activity against *C. albicans*. Some of them showed good activity against *C. albicans* and *C. neoformans* [204].

**Cryptocandin**

A novel lipopeptide antifungal complex (figure 47) has been reported. It is a natural compound obtained from *Cryptosporiopsis cf. quercina*, an endophytic fungus. Cryptocandin shows a good anti-*C. albicans* activity (MIC, 0.03–0.07 μg/mL) and against *Trichophyton mentagrophytes* and *Trichophyton rubrum* [205].

**Antiparasitic Activity**

**Antimalarial Compounds**

*Plasmodium falciparum* resistant to chloroquine as well as to mefloquine, halofantrine, and quinine has spread in endemic areas. New drugs are needed. One combination is currently under clinical development: artemether and lumefantrine (CFP 56697), which seems promising [206] (figure 48). New alkaloids

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**Figure 47. Antifungal: cryptocandin**

**Figure 48. Antimalarial compounds**
have been extracted from the African plant *Strychnos icaja*, and these alkaloids, namely sungucine, usambaresine, and strychnopentamine (figure 49), were investigated for in vitro activity by use of incorporation of [\(^3\)H]-hypoxanthine into 2 *P. falciparum* strains: a chloroquine-sensitive FCA 206 hana and a resistant one, W2 Indochina. All derivatives except sungucine were active against both isolates. Chemically modified sungucine has also been tested, and one derivative, 18-hydroxysungucine, showed a good antimalarial activity. Strychnopentamine and isostrychnopentamine show an unexpectedly high activity against *P. falciparum* ring forms, a stage on which antimalarial compounds have no effect. Probably the mode of action of these alkaloids is original [207] (figure 50).

**Anti*-Pneumocystis carinii* Pneumonia Compounds**

*Pneumocystis carinii* pneumonia is an opportunistic infection commonly encountered in patients with AIDS. Pentamidine remains the drug of choice but is plagued with a high incidence of adverse events. In order to reduce the conformational flexibility of pentamidine (which could support the indiscriminate binding of the compound to nontarget macromolecules), a series of conformationally restricted analogs of pentamidine in which the flexible central bridge has been replaced by trans-cyclopropyl, phenyl, pyridinyl, piperazinyl, or homopiperazinyl groups as conformationally restricted links has been synthesized (figure 50).

In vitro activity of pentamidine analogs was evaluated in cell cultures (human embryonic lung cells). At a concentration of 0.1 \(\mu\)M, 4 compounds out of 10 analogs were more active than pentamidine. One compound, which had a piperazine ring as a central link connecting the benzamidine groups, was the most active and was 15-fold more active than pentamidine. It was also the strongest DNA binder for calf thymus DNA [208].

**Anti-Trypanosomal and Anti-Leishmanial Activity**

Compounds with a new target have been investigated: prenylation inhibitors. Farnesyl transferase attaches farnesyl (15-...
Antitoxoplasmal Compounds: 6-Nitrobenzylthioinosine

A new specific target in Toxoplasma gondii had been reported of the enzyme adenosine kinase. New purine analogs NBMPR (nitrobenzylthioinosine, or 6-[(4-nitrobenzylthio)-9,β-D-ribofuranosyl]purine) were tested for their effect as antitoxoplasmal agents. NBMPR killed T. gondii grown in human fibroblasts in a dose-dependent manner, with an IC₅₀ of 10 μM, without apparent toxicity to host cells [210].

References


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