Drugs of the Future 2009, 34(2): 127-136

CCC: 0377-8282/2009
DOI: 10.1358/dof.2009.34.2.1313642

REVIEW ARTICLE

NEW APPROACHES TO OVERCOMING BACTERIAL RESISTANCE

Y. Fukuda

Discovery Research Laboratories, Kyorin Pharmaceutical Co., Ltd., 2399-1, Nogi, Nogi-machi, Shimotsuga-gun, Tochigi, 329-0114, Japan; e-mail: yasumichi.fukuda@mb.kyorin-pharm.co.jp

CONTENTS

ABSTRACT

The worldwide spread of drug-resistant bacteria is now a critical problem in global healthcare. Hence, the continuous development of effective antibacterial drugs for the treatment of severe infections caused by drug-resistant Gram-positive bacteria is an urgent matter of great importance. This article summarizes our efforts to overcome bacterial resistance through drug discovery and includes other achievements recently reported by other research groups. Approaches can be categorized into three classes: the new approach, the proven approach and the hybrid approach. Our efforts to overcome bacterial resistance have focused on the proven approach, with chemical modifications of mutilin, oxazolidinone and quinolone derivatives. Among them, the mutilin derivative RAM-150 and the oxazolidinone derivative AM-7359 showed highly potent antibacterial activity against methicillin-resistant Staphylococcus aureus (MRSA), penicillin-resistant Streptococcus pneumoniae (PRSP) and vancomycin-resistant enterococci (VRE). AM-1954, a quinolone derivative, showed highly potent antibacterial activity against quinolone/methicillin-resistant S. aureus (QMRSA), quinolone/methicillin-resistant S. pneumoniae (QMRSP) and VRE. These three compounds have been selected as candidates for further evaluation. Other reported compounds representing progress in each category are also described in this paper.

INTRODUCTION

Ever since the "magic bullet" of penicillin was discovered over 60 years ago, physicians have been engaged in a perpetual battle with resistant bacteria. The endeavor to overcome bacterial resistance has always fluctuated between hope and fear. In the last two decades, the uncontrollable spread of drug-resistant bacteria has become a critical problem in global health. The broad and indiscriminate use of antibacterial agents in animals and humans has exerted strong pressure favoring the development of multidrug-resistant bacteria. Of particular importance are severe infections caused by drug-resistant Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP) and vancomycin-resistant *Enterococcus faecalis* (VRE) in hospitals and the community (1-4). Recently, the expansion of Gram-negative drug-resistant bacteria such as β-lactamase-non-producing ampicillin-resistant *Haemophilus influenzae* (BLNAR), extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* or *Escherichia coli* (ESBL) and multidrug-resistant *Pseudomonas aeruginosa* (MDRP) has contributed to this serious situation (5-10). This review summarizes recent efforts in medicinal chemistry to overcome bacterial resistance through drug discovery in terms of three approaches, along with novel compounds representing progress in each approach. These three approaches are the new approach, the proven approach and the hybrid approach.

NEW APPROACH

The new approach typically explores novel antibacterial agents with new modes of action and/or new scaffolds possessing potent antibacterial activity against bacteria with resistance to currently available antibacterial drugs. For about the last decade, bacterial genomics and mechanism studies of antibacterial compounds have revealed many candidates for new drugs with efficacy against resistant bacteria. Selecting good antibacterial mechanisms from the new candidates by high-throughput screening (HTS) is not easy because the outcome of this empirical screening method strongly depends on the quality and quantity of the compound library for HTS. The hit compounds identified from the antibacterial HTS campaigns often include false-positives and nonspecific compounds. In addition, poor chemical tractability and poor drug-like properties of the hits make it hard to overcome the barrier between cell-free activity and whole-cell activity. Even if the initial hits proceed to highly potent compounds, the target mechanism may not result in antibacterial activity or the antibacterial spectrum may be very limited. Overall, it has been reported that the success rate from antibacterial HTS is 4- to 5-fold lower than for targets in other therapeutic areas. In spite of great efforts, genomic approaches have yielded disappointing results (11, 12).

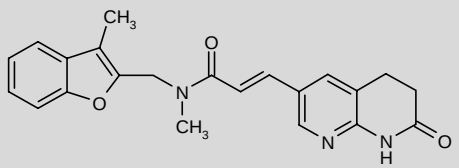
In contrast, efforts to identify new scaffolds with known mechanisms of action have intensified based on both the empirical screening method and structure-based drug design. The most difficult problem in finding new scaffolds for antibacterial drugs is overcoming the barrier between cell-free activity and whole-cell activity. Recently, a group at Merck Research Laboratories reported an elegant solution for identifying antibacterial compounds with new mechanisms of action from among the huge natural product extract library (13). They employed a whole-cell HTS method, a target-based two-plate assay in which each target was differentially expressed by using antisense methodology. The following examples overcame the barrier between cell-free activity and whole-cell activity. **AFN-1252** (Affinium) (14), an enoyl-[acyl-carrier-protein] reductase [NADH] (FabI) inhibitor, showed potent antibacterial activity against MRSA and methicillin-resistant *Staphylococcus epidermidis* (MRSE) and has advanced to phase I clinical trials. **Bederocin** (REP-8839; Replidyne) (15), a methionyl-tRNA synthetase inhibitor, displayed broad-spectrum antibacterial activity against Gram-positive and -negative bacteria; however, the company has discontinued development due to restructuring.

PROVEN APPROACH

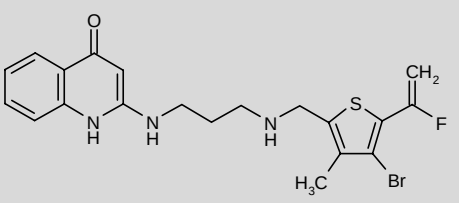
Traditional antibacterial drugs have well-proven clinical efficacy for the treatment of various infections. The proven approach is still the most fruitful strategy for developing new antibacterial drugs for resistant bacteria. Even if the current antibacterial drugs have lost their efficacy against resistant bacteria, effective chemical modification of these compounds is possible to renew their potential against resistant bacteria and extend their antibacterial spectrum. Indeed, many efforts have focused on the chemical modification of proven antibacterial drugs. The following recent examples, including our studies, have succeeded in overcoming bacterial resistance.

β-Lactams and β-lactamase inhibitors

Ceftobiprole medocaril (Basilea Pharmaceutica/Johnson & Johnson) is the first anti-MRSA cephalosporin. Ceftobiprole holds fast track designation in the U.S. for the treatment of complicated skin and skin structure infections (cSSSIs) and for the treatment of hospital-acquired pneumonia and was launched in Canada in 2008 (16). The success of Augmentin® (**amoxicillin/clavulanic acid**; GlaxoSmithKline) (17) led many research groups to develop new combinations of β-lactams with β-lactamase inhibitors. **NXL-104** (Novexel), the first non-β-lactam β-lactamase inhibitor, is one of the most advanced β-lactamase inhibitors, having reached phase II trials in combination with ceftazidime (18).



AFN-1252

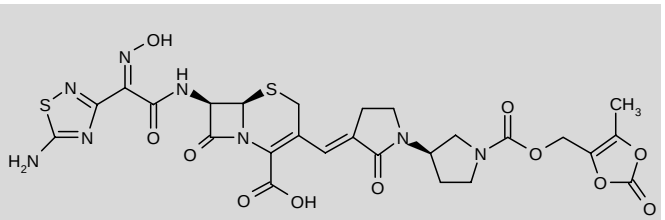


Bederocin

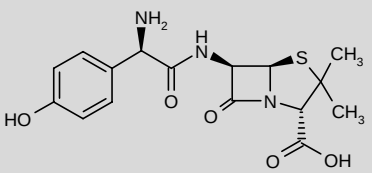
Mutilins

Tiamulin (Novartis), a compound related to the diterpene antibiotic **pleuromutilin**, is a therapeutic agent for veterinary use (19). More recently, the GlaxoSmithKline group reported **SB-264128**, with improved activity against bacteria causing respiratory tract infections, and launched **retapamulin** for the topical treatment of skin infections in 2007 (20, 21).

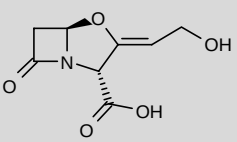
During the course of our initial research to develop novel mutilins, we focused on the chemical modification of the C-12 position of mutilin (22). Our key methodology was regio- and diastereoselective alkylation at the C-12 position of de-ethenylated 4-epimutilin **1**, prepared from pleuromutilin using five steps. Alkylation of **1** with vari-



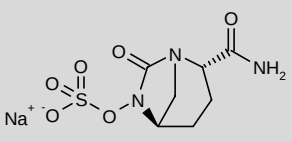
Ceftobiprole medocaril



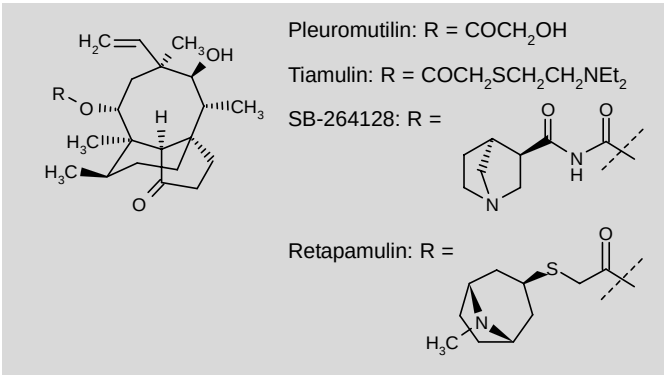
Amoxicillin



Clavulanic acid



NXL-104



ous electrophiles smoothly proceeded to give C-12-alkylated 4-epimutilins in a regio- and diastereoselective manner. The C-12-modified 4-epimutilins **2** were converted to the novel mutilin derivatives **3a-q**, as shown in Scheme 1.

Among the synthesized C-12 alkyl analogues **3a-q**, **RAM-150** (**3a**) (Table I) bearing a propenyl group at the C-12 position exhibited more potent antibacterial activity against methicillin-susceptible *S. aureus* (MSSA), MRSA, penicillin-susceptible *S. pneumoniae* (PSSP), PRSP and VRE than tiamulin and SB-264128. In an MSSA systemic infection model, RAM-150 was more effective compared to pleuromutilin and tiamulin, and equally effective as SB-264128 (23). We have selected RAM-150 as a candidate for further evaluation.

Oxazolidinones

Linezolid (Pfizer) (24), the only oxazolidinone approved for clinical use, represents the first in a new class of antibacterial drugs and is now the last resort for the treatment of Gram-positive infections caused by MRSA or VRE. However, linezolid resistance in Gram-positive bacteria has already been reported (25-30). Linezolid is now in the position of vancomycin, which was also once considered the antibacterial agent of last resort. Therefore, many research groups have attempted to improve the potency and the antibacterial spectrum of linezolid. Currently, two oxazolidinones, **torezolid** (TR-701; Trius Therapeutics, Dong-A) and **radezolid** (Rib-X), are in the clinical stage of development (31, 32).

In our initial research in this area, we identified our lead compound **4** (Scheme 2), showing more potent antibacterial activity against MRSA (MIC = 0.25 μg/mL), quinolone-resistant PRSP (QMRSP; MIC = 0.25 μg/mL) and VRE (MIC = 0.5 μg/mL) compared with linezolid (MIC = 1, 1 and 2 μg/mL, respectively) (33). Our structure–activity relationship (SAR) studies revealed that a cyano group at the C-6 position of the azabicyclo[3.1.0]hex-3-yl ring system greatly contributed to enhancing antibacterial activity. We next focused on the design and the synthesis of novel biaryl oxazolidinones bearing the azabicyclo[3.1.0]hex-6-yl ring system (34). We employed stereoselective cyclopropane ring formation utilizing the 1,2-cyclic sulfate **6** and arylacetonitrile **7** (Scheme 3) to construct a 6-aryl-6-cyanoazabicyclo[3.1.0]hexane ring system. The various azabicyclo[3.1.0]hex-6-yl biaryl oxazolidinones **5a-o** were prepared by way of the Pd-coupling reaction of **8**, as shown in Scheme 3. The synthesized compounds, including **AM-7359** (**5b**) (Table II), showed superior antibacterial activity against MSSA, MRSA, PSSP, PRSP and VRE compared to linezolid. AM-7359 also exhibited highly potent antibacterial activity against resistant clinical isolates such as MRSA and VRE (35). AM-7359 showed greater potency in a mouse thigh infection model caused by linezolid-resistant MRSA (LMRSA) than linezolid (36) and it possesses a significantly lower potential for resistance compared to linezolid (37). We have therefore selected the azabicyclo[3.1.0]hex-6-yl biaryl oxazolidinone AM-7359 as a candidate for further evaluation.

Quinolones

Norfloxacin (Kyorin) (38) opened the door to fluoroquinolones, which have a broad antibacterial spectrum including Gram-positive

Scheme 1.

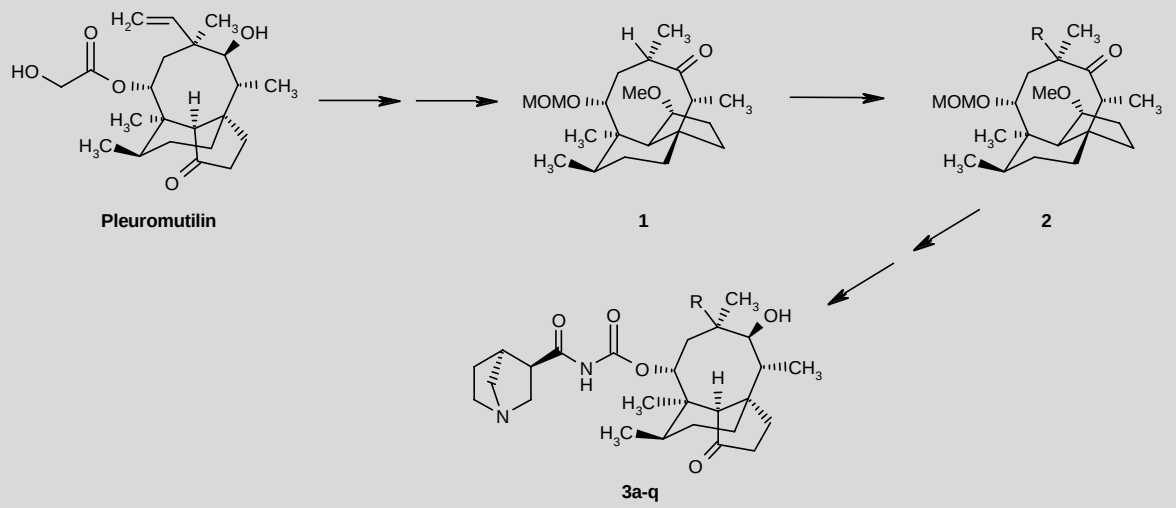
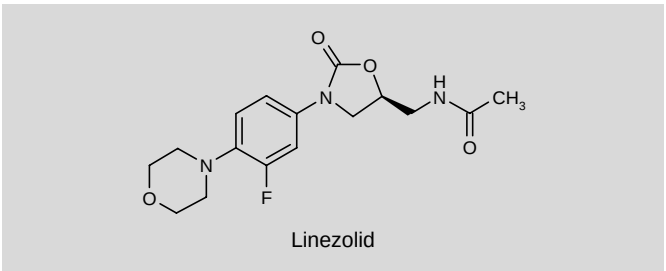


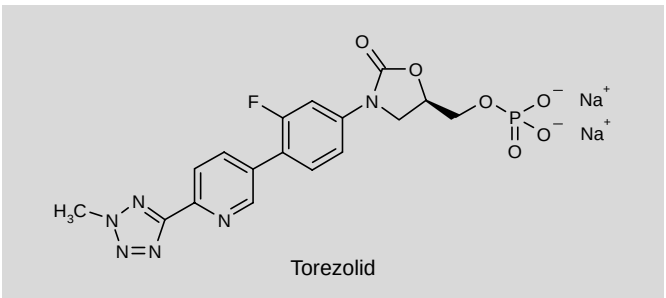
Table I. *Antibacterial activity of mutilin derivatives.*

		MIC (μg/mL)				
Compound	R	MSSA	MRSA	PSSP	PRSP	VRE
3a (RAM-150)	1-propenyl	**0.125**	**0.06**	**0.125**	**0.125**	**0.25**
3b	1-butenyl	0.25	0.25	0.5	0.5	1
3c	1-pentenyl	0.125	0.125	0.25	0.25	0.5
3d	2-Cl-vinyl	0.5	0.5	1	1	2
3e	(*E*)-2-F-vinyl	0.25	0.25	2	0.5	0.5
3f	(*Z*)-2-F-vinyl	0.25	0.25	0.5	0.25	0.5
3g	2-CF_3-vinyl	32	32	> 128	> 128	128
3h	methyl	0.25	0.25	1	2	0.5
3i	fluoromethyl	0.25	0.5	2	2	1
3j	2-fluoroethyl	0.25	0.25	1	0.5	0.5
3k	2,2,2-trifluoroethyl	0.5	0.5	2	2	1
3l	propyl	0.25	0.25	2	2	1
3m	*i*-propyl	0.5	0.5	1	2	1
3n	allyl	0.25	0.25	0.5	0.5	0.5
3o	3-fluoropropyl	2	1	8	8	4
3p	butyl	0.5	0.25	2	2	1
3q	2-methoxyethyl	0.5	0.5	4	8	2
Pleuromutilin		0.5	0.5	16	8	0.5
Tiamulin		0.25	0.25	4	2	0.5
SB-264128		0.25	0.125	1	2	0.5

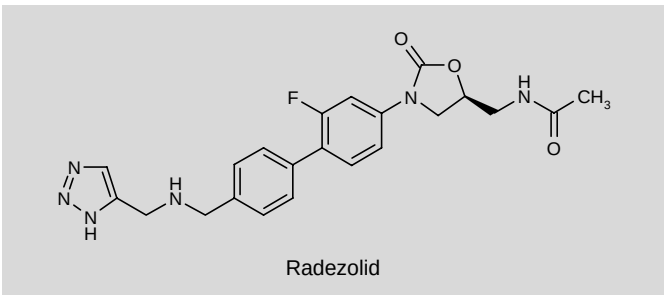
MSSA, methicillin-sensitive *Staphylococcus aureus*; MRSA, methicillin-resistant *S. aureus*; PSSP, penicillin-sensitive *Streptococcus pneumoniae*; PRSP, penicillin-resistant *S. pneumoniae*; VRE, vancomycin-resistant *Enterococcus faecalis*.



Linezolid



Torezolid



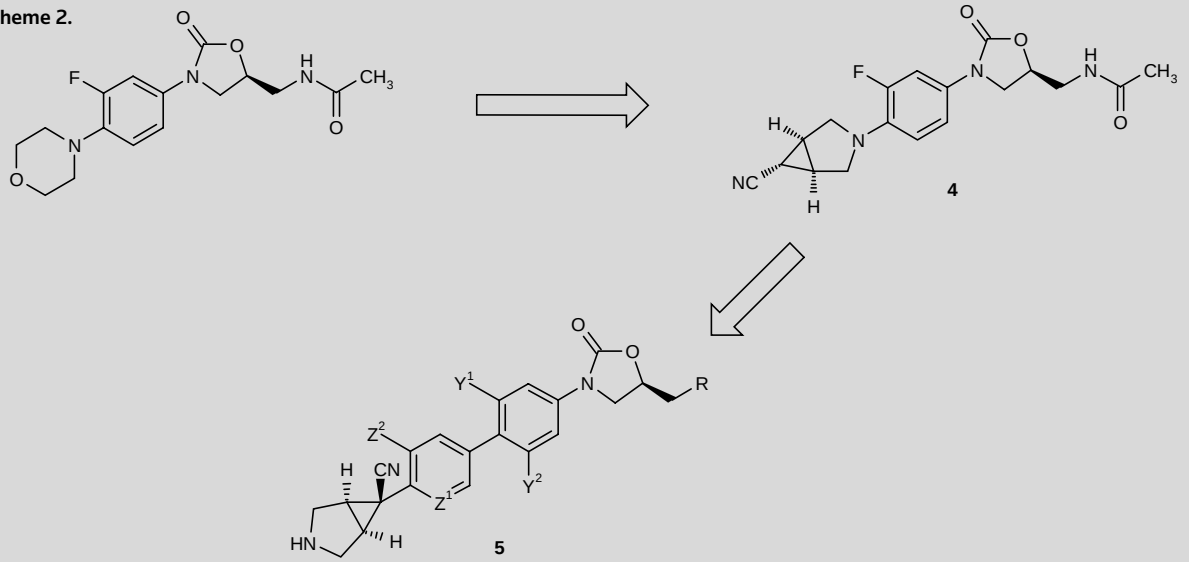
Radezolid

and -negative bacteria. As a consequence, many fluoroquinolones have enjoyed wide clinical use, but some MRSA have acquired quinolone resistance. Recently developed and marketed quinolones include **garenoxacin** (Taisho/Astellas), a nonfluorinated quinolone launched in 2007, and **sitafloxacin** (Daiichi Sankyo), a fluoroquinolone launched in 2008.

Since the launch of **gatifloxacin** (Kyorin) (39), we have continued to develop novel quinolones showing potent activity against multidrug-resistant bacteria including quinolone-resistant bacteria. We first examined a group of 3-ethylaminomethylpyrrolidinyls, including **merafloxacin** (CI-934; Pfizer) (40) and **PD-117558** (Pfizer) (41, 42). Merafloxacin showed enhanced antibacterial activity against Gram-positive bacteria. In contrast, PD-117558 exhibited strong cytotoxicity against mammalian cells.

Although the fluoropyridobenzoxazine **9** was first reported to be effective against *Mycobacterium* spp. (43), we found **9** to have more potent activity against QMRSA and VRE than **clinafloxacin** (Pfizer, Kyorin), the development of which has been discontinued. Thus, we have focused on the design and synthesis of novel fluoropyridobenzoxazine derivatives with highly potent antibacterial activity. The synthesis of (3*R*,4*S*)- and (3*R*,4*R*)-3-cyclopropylaminomethyl-4-methylpyrrolidine (**11a**,**b**) and (3*R*,4*S*)- and (3*R*,4*R*)-3-cyclopropylaminomethyl-4-fluoropyrrolidine (**14a**,**b**) was achieved as shown in Scheme 4. Each pyrrolidine was subsequently subjected to a nucleophilic substitution reaction with the diacetoxyboron chelate of the (3*S*)-methyl- and (3*R*)-fluoromethylpyrido[1,2,3-*de*][1,4]benzoxazinecarboxylic acid derivatives (**15a**,**b**) to give compounds **16a**-**h**. All of the synthesized compounds, including **AM-1954** (**16g**) (Table III), were shown to possess about 8 times higher activity against QMRSA, QMRSP and VRE than clinafloxacin. AM-1954 exhibits

Scheme 2.



Scheme 3.

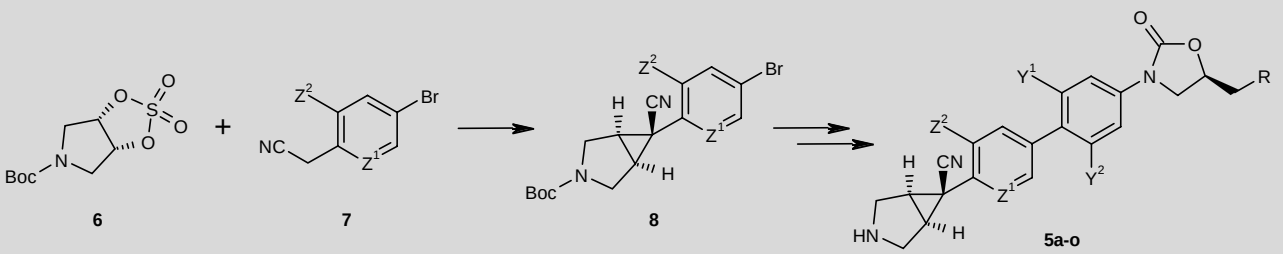


Table II. *Antibacterial activity of oxazolidinone derivatives.*

						MIC (μg/mL)				
Compound	R	Y^1	Y^2	Z^1	Z^2	MSSA	MRSA	PSSP	PRSP	VRE
5a	1,2,3-triazolyl	H	H	N	H	0.125	0.125	0.125	0.125	0.25
5b (AM-7359)	1,2,3-triazolyl	F	H	N	H	**0.06**	**0.06**	**0.06**	**0.06**	**0.125**
5c	1,2,3-triazolyl	F	H	CH	H	0.125	0.06	0.125	0.06	0.25
5d	1,2,3-triazolyl	F	H	CH	F	0.25	0.125	0.25	0.25	0.25
5e	1,2,3-triazolyl	F	H	CF	F	0.5	0.5	1	1	0.25
5f	1,2,3-triazolyl	F	F	N	H	0.125	0.125	0.125	0.125	0.25
5g	NHCOMe	F	H	N	H	0.125	0.125	0.125	0.06	0.25
5h	NHCOEt	F	H	N	H	0.25	0.25	0.25	0.125	0.25
5i	$NHCOCHF_2$	F	H	N	H	0.125	0.125	0.06	0.06	0.25
5j	NHCOcPr	F	H	N	H	0.5	0.25	0.25	0.25	0.5
5k	$NHCO_2Me$	F	H	N	H	0.125	0.125	0.125	0.125	0.125
5l	NHC(=S)OMe	F	H	N	H	0.06	0.03	0.06	0.03	0.06
5m	NHC(=S)Me	F	H	N	H	0.03	0.03	0.03	0.03	0.06
5n	3-isoxazolyl-O	F	H	N	H	0.125	0.125	0.25	0.25	0.5
5o	3-isoxazolyl-NH	F	H	N	H	0.06	0.06	0.125	0.06	0.125
Linezolid						1	1	1	1	2

MSSA, methicillin-sensitive *Staphylococcus aureus*; MRSA, methicillin-resistant *S. aureus*; PSSP, penicillin-sensitive *Streptococcus pneumoniae*; PRSP, penicillin-resistant *S. pneumoniae*; VRE, vancomycin-resistant *Enterococcus faecalis*.

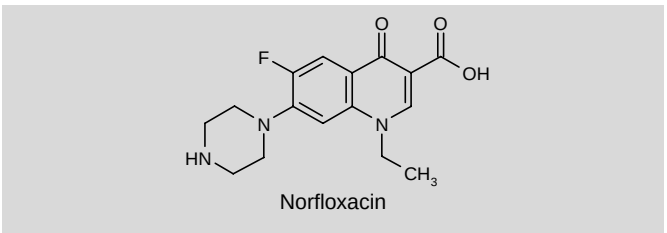
superior in vivo efficacy in systemic infection models caused by MSSA, QMRSA or PSSP. Structure–toxicity relationship studies of AM-1954 revealed that the introduction of a fluorine atom to the C-4 position of the pyrrolidine ring was effective in reducing the single-dose acute toxicity and convulsion-inducing ability, and the introduction of a fluorine atom to the C-3 methyl group of the pyridobenzoxazine ring contributed to complete elimination of phototoxicity (44). We have selected AM-1954 as a candidate for further evaluation.

Other compounds

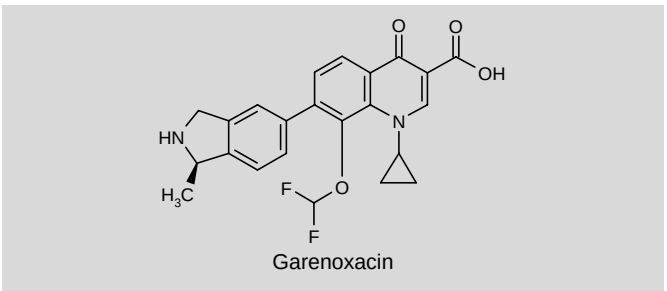
Tigecycline (Wyeth) is the first in a class of glycylcyclines derived from tetracycline and was designed for the treatment of complicated intraabdominal infections and cSSSIs in adults (45). In contrast to tetracyclines, glycylcyclines can overcome tetracycline resistance mediated by acquired efflux pumps and/or ribosomal protection (46). **Iclaprim** (Arpida), a novel trimethoprim derivative which has been submitted for regulatory review, is a dihydrofolate reductase inhibitor that shows potent and extended-spectrum antibacterial activity against various drug-resistant bacteria (47).

HYBRID APPROACH

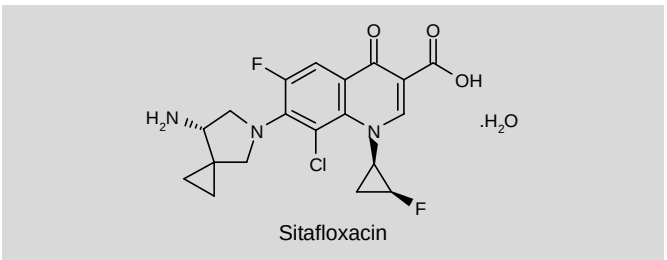
The hybrid approach entails combining several pharmacophores in one molecule with covalent linkages. This approach is considered to be a reasonable strategy for developing new molecules with high potency, extended spectra and low resistance potential. In contrast to drug cocktails, hybrid molecules ensure that the pharmacokinetics, pharmacodynamics and tissue distribution of the pharmacophores are synchronized. **MCB-3837** (Oxaquin®; Biovertis), an oxazolidinone and quinolone hybrid in early clinical development, showed more potent activity against both oxazolidinone- and quinolone-resistant MRSA than respective drug cocktails and possessed a low potential for resistance (48). **CBR-2092** (Cumbre Pharmaceuticals), a 2-pyridone and rifamycin hybrid in phase I clinical evaluation, exhibited more potent activity against both quinolone- and rifamycin-resistant bacteria such as QMRSA than the combination, as well as a lower potential for resistance (49). **TD-**



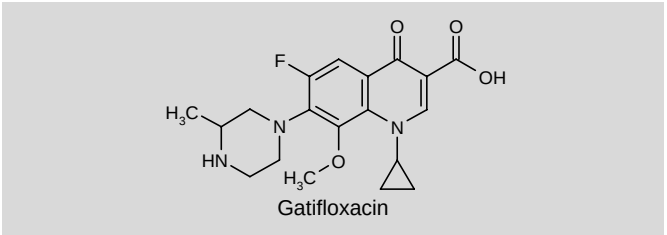
Norfloxacin



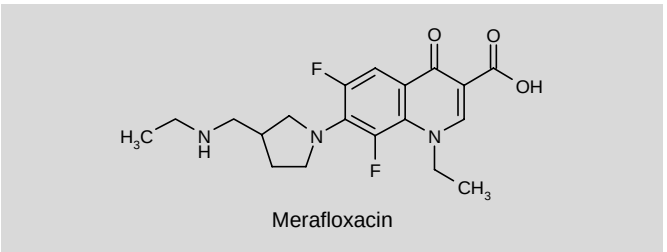
Garenoxacin



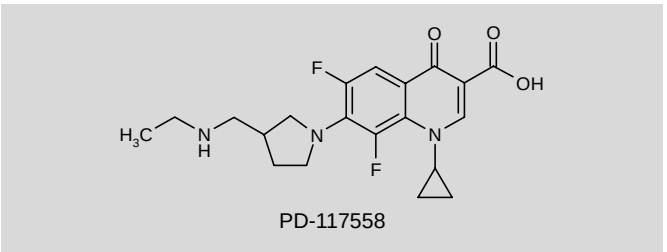
Sitafloxacin



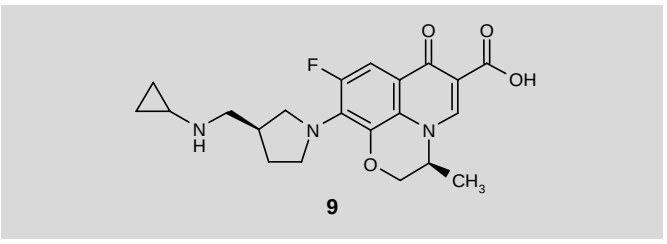
Gatifloxacin



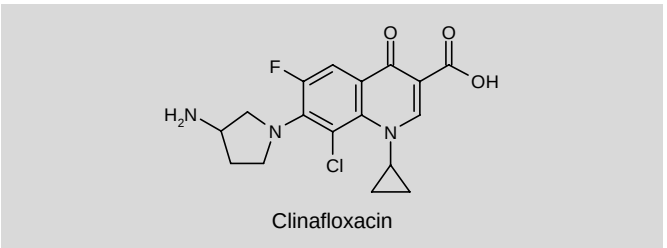
Merafloxacin



PD-117558



9



Clinafloxacin

Scheme 4.

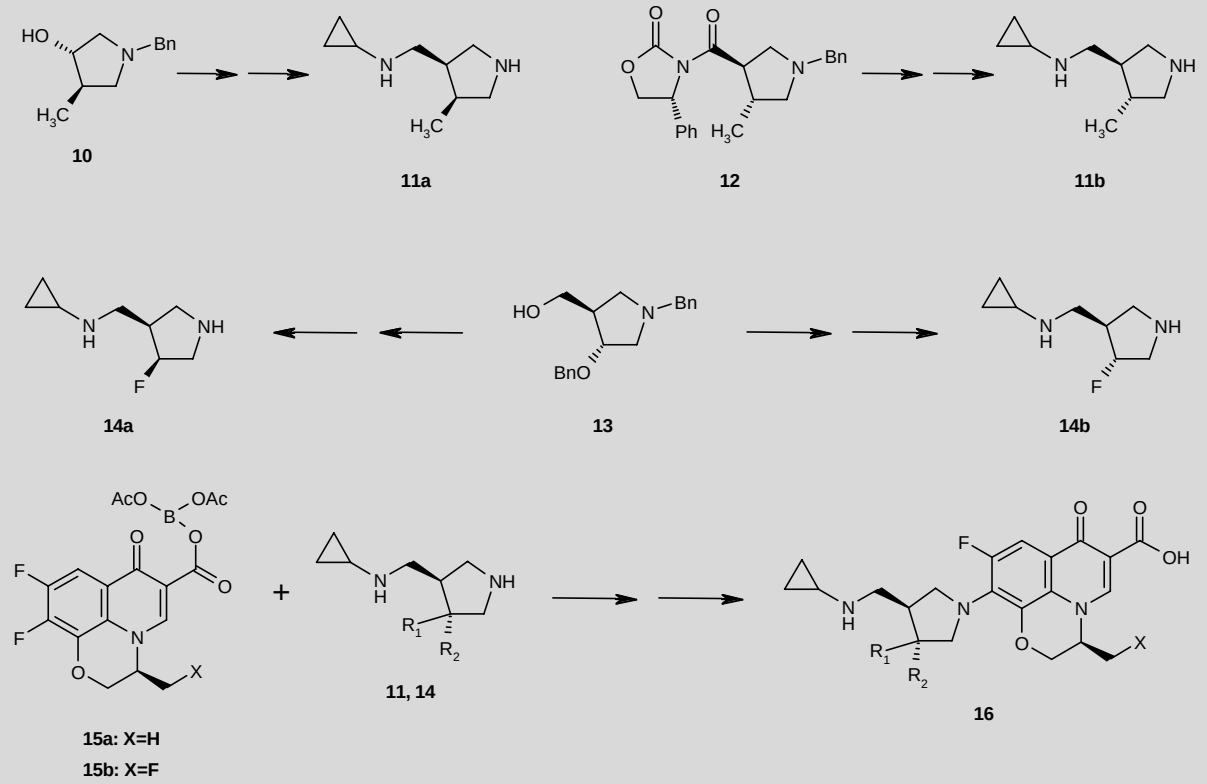


Table III. *Antibacterial activity of quinolone derivatives.*

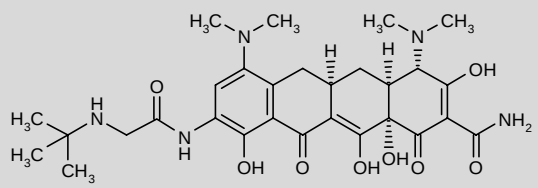
				MIC (μg/mL)				
Compound	X	R_1	R_2	MSSA	QMRSA	PSSP	QMRSP	VRE
16a	H	Me	H	0.008	0.5	0.016	0.125	1
16b	H	H	Me	0.002	2	0.016	0.5	1
16c	H	F	H	0.008	1	0.016	0.125	0.5
16d	H	H	F	0.016	16	0.063	2	4
16e	F	Me	H	0.008	0.5	0.016	0.125	1
16f	F	H	Me	0.008	2	0.031	0.5	1
16g (AM-1954)	F	F	H	0.008	1	0.016	0.25	0.5
16h	F	H	F	0.016	16	0.063	2	4
Clinafloxacin	N	H	F	0.016	8	0.031	0.5	4

MSSA, methicillin-sensitive *Staphylococcus aureus*; QMRSA, quinolone/methicillin-resistant *S. aureus*; PSSP, penicillin-sensitive *Streptococcus pneumoniae*; QPRSP, quinolone/penicillin-resistant *S. pneumoniae*; VRE, vancomycin-resistant *Enterococcus faecalis*.

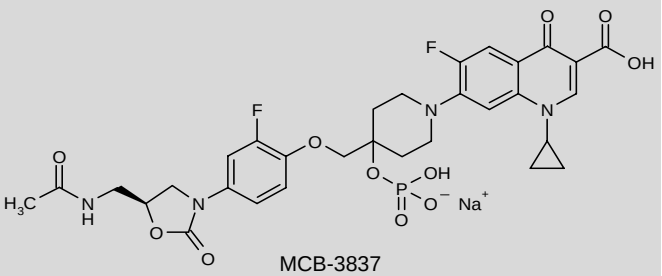
1792 (Theravance), a cephalosporin and vancomycin hybrid, has entered phase II evaluation for cSSSI caused by MRSA (50). It is of interest to note that the hybrids show highly potent antibacterial activity compared to the respective combinations, although the mechanism has not yet been elucidated. It was reported that one possible mechanism for CBR-2092 was the circumvention of intrinsic or mutationally activated efflux systems (49). Mechanism studies of the hybrids may reveal a new strategy for overcoming bacterial resistance. Successful results for these new hybrids possessing particularly potent activity against resistant bacteria will constitute an important new option for the treatment of serious infections caused by resistant bacteria. Our initial research on this approach has identified several novel hybrid compounds, and these studies will be reported in the near future.

CONCLUSIONS

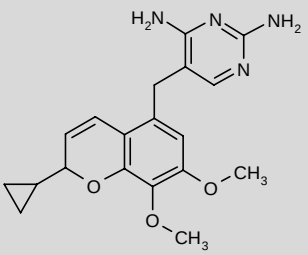
As described above, continuous effort is indispensable for breaking the vicious circle of antibacterial discovery and use and the development of resistant bacteria. The new approach has attractive possibilities for providing new answers to disarming bacterial resistance. The



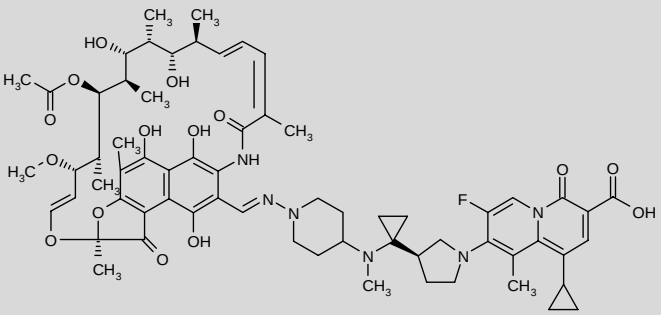
Tigecycline



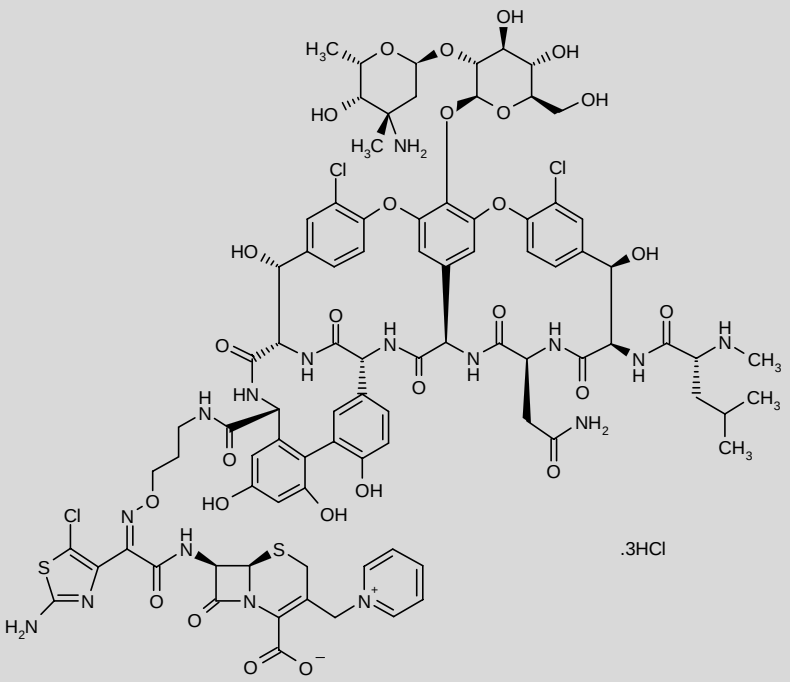
MCB-3837



Iclaprim



CBR-2092



.3HCl

TD-1792

proven approach remains the most reliable methodology to develop new antibacterial drugs. On the basis of this approach, we have identified the mutilin derivative RAM-150, the oxazolidinone derivative AM-7359 and the quinolone derivative AM-1954 as promising candidates. The hybrid approach involving combinations of proven drugs is increasingly being pursued as a method for overcoming resistance.

REFERENCES

1. Pallin, D.J., Egan, D.J., Pelletier, A.J., Espinola, J.A., Hooper, D.C., Camargo, C.A. Jr. *Increased US emergency department visits for skin and soft tissue infections, and changes in antibiotic choices, during the emergence of community-associated methicillin-resistant Staphylococcus aureus.* Ann Emerg Med 2008, 51(3): 291-8.
2. Tong, S.Y., McDonald, M.I., Holt, D.C., Currie, B.J. *Global implications of the emergence of community-associated methicillin-resistant Staphylococcus aureus in indigenous populations.* Clin Infect Dis 2008, 46(12): 1871-8.
3. Izumida, M., Nagai, M., Ohta, A. et al. *Epidemics of drug-resistant bacterial infections observed in infectious disease surveillance in Japan, 2001-2005.* J Epidemiol 2007, 17(Suppl.): S42-7.
4. Deshpande, L.M., Fritsche, T.R., Moet, G.J., Biedenbach, D.J., Jones, R.N. *Antimicrobial resistance and molecular epidemiology of vancomycin-resistant enterococci from North America and Europe: A report from the SENTRY antimicrobial surveillance program.* Diagn Microbiol Infect Dis 2007, 58(2): 163-70.
5. Jansen, W.T., Verel, A., Beitsma, M., Verhoef, J., Milatovic, D. *Longitudinal European surveillance study of antibiotic resistance of Haemophilus influenzae.* J Antimicrob Chemother 2006, 58(4): 873-7.
6. Lavilla, S., González-López, J.J., Sabaté, M. et al. *Prevalence of qnr genes among extended-spectrum beta-lactamase-producing enterobacterial isolates in Barcelona, Spain.* J Antimicrob Chemother 2008, 61(2): 291-5.
7. Cantón, R., Novais, A., Valverde, A., Machado, E., Peixe, L., Baquero, F., Coque, T. M. *Prevalence and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Europe.* Clin Microbiol Infect 2008, 14(Suppl. 1): 144-53.
8. Bush, K. *Extended-spectrum beta-lactamases in North America, 1987-2006.* Clin Microbiol Infect 2008, 14(Suppl. 1): 134-43.
9. Tam, V.H., Gamez, E.A., Weston, J.S. et al. *Outcomes of bacteremia due to Pseudomonas aeruginosa with reduced susceptibility to piperacillin-tazobactam: Implications on the appropriateness of the resistance breakpoint.* Clin Infect Dis 2008, 46(6): 862-7.
10. Nicasio, A.M., Kuti, J.L., Nicolau, D.P. *The current state of multidrug-resistant gram-negative bacilli in North America.* Pharmacotherapy 2008, 28(2): 235-49.
11. Payne, D.J., Gwynn, M.N., Holmes, D.J., Pompliano, D.L. *Drugs for bad bugs; confronting the challenges of antibacterial discovery.* Nat Rev Drug Discovery 2007, 6(1): 29-40.
12. Chan, P.F., Holmes, D.J., Payne, D.J. *Findings the gems using genomic discovery: Antibacterial drug discovery strategies – The successes and the challenges.* Drug Discov Today 2004, 1(4): 519-27.
13. Wang, J., Soisson, S.M., Young, K. et al. *Platensimycin is a selective FabF inhibitor with potent antibiotic properties.* Nature 2006, 441(7091): 358-61.
14. Karlowsky, J.A., Laing, N.M., Baudry, T., Kaplan, N., Vaughan, D., Hoban, D.J., Zhanel, G.G. *In vitro activity of API-1252, a novel FabI inhibitor, against clinical isolates of Staphylococcus aureus and Staphylococcus epidermidis.* Antimicrob Agents Chemother 2007, 51(4): 1580-1.
15. Critchley, I.A., Young, C.L., Stone, K.C., Ochsner, U.A., Guiles, J., Tarasow, T., Janjic, N. *Antibacterial activity of REP8839, a new antibiotic for topical use.* Antimicrob Agents Chemother 2005, 49(10): 4247-52.
16. Noel, G.J., Bush, K., Bagchi, P., Ianus, J., Strauss, R.S. *A randomized, double-blind trial comparing ceftobiprole medocaril with vancomycin plus ceftazidime for the treatment of patients with complicated skin and skin-structure infections.* Clin Infect Dis 2008, 46(5): 647-55.
17. Van Landuyt, H.W., Pyckavet, M., Lambert, A.M. *Comparative activity of BRL 25.000 with amoxycillin against resistant clinical isolates.* J Antimicrob Chemother 1981, 7(1): 65-70.
18. Bassetti, M., Righi, E., Viscoli, C. *Novel beta-lactam antibiotics and inhibitor combinations.* Expert Opin Investig Drugs 2008, 17(3): 285-96.
19. O'Connor, J.J., Baughn, C.O., Pilote, R.R., Alpaugh, W.C., Linkenheimer, W.H., Maplesden, D.C. *Tiamulin in the feed for the prevention of swine dysentery and growth promotion of growing pigs.* J Anim Sci 1979, 49(4): 933-8.
20. Brooks, G., Burgess, W., Colthurst, D. et al. *Pleuromutilins. Part 1. The identification of novel mutilin 14-carbamates.* Bioorg Med Chem 2001, 9(5): 1221-31.
21. Rittenhouse, S., Biswas, S., Broskey, J. et al. *Selection of retapamulin, a novel pleuromutilin for topical use.* Antimicrob Agents Chemother 2006, 50(11): 3882-5.
22. Takadoi, M., Sato, T., Fukuda, Y. *Synthesis of C-20 substituted mutilin.* 234th ACS Natl Meet (Aug 19-23, Boston) 2007, Abst ORGN 166.
23. Takadoi, M., Sato, T., Fukuda, Y. *Synthesis and antibacterial activity of novel C-12 substituted mutilins.* 47th Intersci Conf Antimicrob Agents Chemother (ICAAC) (Sept 17-20, Chicago) 2007, Abst F1-1696.
24. Brickner, S.J., Hutchinson, D.K., Barbachyn, M.R. et al. *Synthesis and antibacterial activity of U-100592 and U-100766, two oxazolidinone antibacterial agents for the potential treatment of multidrug-resistant gram-positive bacterial infections.* J Med Chem 1996, 39(3): 673-9.
25. Tsiodras, S., Gold, H.S., Sakoulas, G. et al. *Linezolid resistance in a clinical isolate of Staphylococcus aureus.* Lancet 2001, 358(9277): 207-8.
26. Auckland, C., Teare, L., Cooke, F. et al. *Linezolid-resistant enterococci: Report of the first isolates in the United Kingdom.* J Antimicrob Chemother 2002, 50(5): 743-6.
27. Jones, R.N., Della-Latta, P., Lee, L.V., Biedenbach, D.J. *Linezolid-resistant Enterococcus faecium isolated from a patient without prior exposure to an oxazolidinone: Report from the SENTRY Antimicrobial Surveillance Program.* Diagn Microbiol Infect Dis 2002, 42(2): 137-9.
28. Herrero, I.A., Issa, N.C., Patel, R. *Nosocomial spread of linezolid-resistant, vancomycin-resistant Enterococcus faecium.* N Engl J Med 2002, 346(11): 867-9.
29. Potoski, B.A., Mangino, J.E., Goff, D.A. *Clinical failures of linezolid and implications for the clinical microbiology laboratory.* Emerging Infect Dis 2002, 8(12): 1519-20.
30. Rahim, S., Pillai, S.K., Gold, H.S., Venkataraman, L., Inglima, K., Press, R.A. *Linezolid-resistant, vancomycin-resistant Enterococcus faecium infection in patients without prior exposure to linezolid.* Clin Infect Dis 2003, 36(11): E146-8.
31. Choi, S., Son, T., Lee, T., Rhee, J. *TR-701 (DA-7218) is significantly more potent than linezolid in skin and soft tissue models of infection.* 47th Intersci Conf Antimicrob Agents Chemother (ICAAC) (Sept 17-20, Chicago) 2007, Abst F1-1691.
32. Lawrence, L., Danese, P., Devito, J., Franceschi, F., Sutcliffe, J. *In vitro activities of the Rx-01 oxazolidinones against hospital and community pathogens.* Antimicrob Agents Chemother 2008, 52(5): 1653-62.
33. Fukuda, Y., Hammond, M.L. (Merck & Co. Inc.; Kyorin Seiyaku KK). *Bicyclic[3.1.0]hexane containing oxazolidinone antibiotic and derivatives thereof.* WO 03027083. Another group independently reported similar

azabicyclo[3.1.0]hexane derivatives, see: Renslo, A.R., Jaishankar, P., Venkatachalam, R., Hackbarth C., Lopez, S., Patel, D.V., Gordeev, M.F. *Conformational constraint in oxazolidinone antibacterials. Synthesis and structure-activity studies of (azabicyclo[3.1.0]hexylphenyl)oxazolidinones.* J Med Chem 2005, 48(15): 5009-24.

34. Komine, T., Kojima, A., Asahina, Y., Saito, T., Takano, H., Shibue, T., Fukuda, Y. *Synthesis and SAR studies of highly potent novel oxazolidinone antibacterials.* J Med Chem 2008, 51(20): 6558-62.

35. Ebisu, H., Abuki, R., Takei, M., Fukuda, H. *Novel oxazolidinones: Antibacterial activities against respiratory pathogens and drug-resistant Gram-positive bacteria.* 46th Intersci Conf Antimicrob Agents Chemother (ICAAC) (Sept 27-30, San Francisco) 2006, Abst F1-966.

36. Gill, C.J., Abruzzo, G.K., Flattery, A.M., Misura, A.S., Bartizal, K., Hickey, E.J. *In vivo efficacy of a novel oxazolidinone compound in two mouse models of infection.* Antimicrob Agents Chemother 2007, 51(9): 3434-6.

37. Overbye, K.M., Mordekhay, D. *AM-7359 — A novel oxazolidinone with low resistance potential and potent activity against drug resistant pathogens.* J Chemother 2007, 19(3): 249-55.

38. Hirai, K., Ito, A., Abe, Y., Suzue, S., Irikura, T., Inoue, M., Mitsuhashi, S. *Comparative activities of AM-715 and pipemidic and nalidixic acids against experimentally induced systemic and urinary tract infections.* Antimicrob Agents Chemother 1981, 19(1): 188-9.

39. Hosaka, M., Yasue, T., Fukuda, H., Tomizawa, H., Aoyama, H., Hirai, K. *In vitro and in vivo antibacterial activities of AM-1155, a new 6-fluoro-8-methoxy quinolone.* Antimicrob Agents Chemother 1992, 36(10): 2108-17.

40. Domagala, J.M., Heifetz, C.L., Mich, T.F., Nichols, J.B. *1-Ethyl-7-[3-[(ethylamino)methyl]-1-pyrrolidinyl]-6,8-difluoro-1,4-dihydro-3-quinolinecarboxylic acid. New quinolone antibacterial with potent Gram-positive activity.* J Med Chem 1986, 29(4): 445-8.

41. Sanchez, J.P., Domagala, J.M., Hagen, S.E., Heifetz, C.L., Hutt, M.P., Nichols, J.B., Trehan, A.K. *Quinolone antibacterial agents. Synthesis and structure—activity relationships of 8-substituted quinolone-3-carboxylic acids and 1,8-naphthyridine-3-carboxylic acids.* J Med Chem 1988, 31(5): 983-91.

42. Domagala, J.M., Heifetz, C.L., Hutt, M.P., Mich, T.F., Nichols, J.B., Solomon, M., Worth, D.F. *1-Substituted 7-[3-[(ethylamino)methyl]-1-pyrrolidinyl]-6,8-difluoro-4-oxo-3-quinolinecarboxylic acids. New quantitative structure–activity relationships at N_1 for the quinolone antibacterials.* J Med Chem 1988, 31(5): 991-1001.

43. Kawakami, K., Namba, K., Tanaka, M., Matsuhashi, N., Sato, K., Takemura, M. *Antimycobacterial activities of novel levofloxacin analogues.* Antimicrob Agents Chemther 2000, 44(8): 2126-9.

44. Asahina, Y., Takei, M., Kimura, T., Fukuda, Y. *Synthesis and antibacterial activity of novel pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid derivatives carrying the 3-cyclopropylaminomethyl-4-substituted-1-pyrrolidinyl group as a C-10 substituent.* J Med Chem 2008, 51(11): 3238-49.

45. Sader, H.S., Mallick, R., Kuznik, A., Fritsche, T.R., Jones, R.N. *Use of in vitro susceptibility and pathogen prevalence data to model the expected clinical success rates of tigecycline and other commonly used antimicrobials for empirical treatment of complicated skin and skin-structure infections.* Int J Antimicrob Agents 2007, 30(6): 514-20.

46. Stein, G.E., Craig, W.A. *Tigecycline: A critical analysis.* Clin Infect Dis 2006, 43(4): 518-24.

47. Kohlhoff, S.A., Sharma, R. *Iclaprim.* Expert Opin Investig Drugs 2007, 16: 1441-8.

48. Gray, C.P., Cappi, M.W., Frimodt-Moller, N. *Efficacy studies of MCB 3837, a dual-action antibiotic, in experimental infections in mice.* 45th Intersci Conf Antimicrob Agents Chemother (ICAAC) (Dec 16-19, Washington, D.C.) 2005, Abst F-513.

49. Du, Q., Doyle, T.B., Duncan, L., Robertson, G.T,. Lynch, A.S. *In vitro microbiology profiling of CBR-2092, a novel rifamycin-quinolone hybrid antibiotic.* 47th Intersci Conf Antimicrob Agents Chemother (ICAAC) (Sept 17-20, Chicago) 2007, Abst F1-2103.

50. Krause, K.M., Difuntorum, S., Blais, J., Turner, S.D., Marquess, D. *TD-1792, a new antibiotic with improved in vitro activity.* 47th Intersci Conf Antimicrob Agents Chemother (ICAAC) (Sept 17-20, Chicago) 2007, Abst E-1626.