RECENT DEVELOPMENTS IN ANTIBACTERIAL ANTIBIOTICS

ACTUALITĂȚI ÎN CERCETAREA DE NOI MOLECULE DE ANTIBIOTICE

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Rezumat. Rezistența bacteriilor la antibioticele autorizate în terapia sau profilaxia infecțiilor este în creștere. Printre măsurile de contracarare a acestui fenomen îngrijorător, în afara utilizării mai raționale a antibioticelor și a optimizării schemelor de tratament, este necesară o intensificare a cercetărilor și investiții mai mari pentru descoperirea de noi molecule de antibiotice, noi inhibitori de betalactamaze, inhibitori ai pompelor de eflux, ai procesului QS, ai formării de biofilm dar și spre identificarea de noi ținte de acțiune.
Metodele utilizate pentru descoperirea de noi antibiotice sunt cele clasice (extracție, biosinteză dirijată, semisinteză, sinteză, urmate de screening antibacterian), chimia organică de sinteză asociată cu studii computerizate de andocare cu ținte specifice, studii QSAR sau identificarea de molecule prototip prin chimie combinatorială.
***Cuvinte cheie**:* rezistența bacteriană, antibiotice, dezvoltare clinică.

Abstract. Bacterial resistance to approved antibiotics (used in therapy and/or as prophylaxis) is increasing. Among the measures to counterwork this alarming phenomenon, beside the more rational use of antibiotics and the optimization of treatment regimens, an intensifying research and greater investments for the discovery of new antibiotic molecules, novel beta-lactamase inhibitors, efflux pump inhibitors, quorum sensing (QS) process and biofilm formation inhibitors, as well as new target identification, are needed.
The methods used for the discovery of new antibiotics are the classical ones (extraction, directed biosynthesis, semi-synthesis, synthesis, followed by antibacterial screening), but also the more modern ones, like synthetic organic chemistry associated with molecular docking studies, QSAR studies or identification of prototype molecules through combinatorial chemistry.
***Keywords**:* bacterial resistance, antibiotics, clinical development.

Introduction

The alarming increase of bacterial resistance to antibiotics over the last few decades became one of the most important concerns of infectious diseases specialists and of medicines regulatory authorities as well. Even if today over 150 antimicrobials are authorized in therapy, mortality due to bacterial infections remains high.

Depending on the level of international alert related to the increasing resistance, pathogenic bacteria are grouped as shown in Table I.

The objectives of the research for new antibiotic molecules

The research of new antibiotics has as main goals:

- the discovery of *new structural profiles*, towards which the bacterial resistance is to be installed as slowly as possible (the discovery of oxazolidinediones' class is an example in this regard). This objective can be achieved by using biosynthesis and extraction methods, chemical semisynthesis or research strategies like *structure-based drug design* through *combinatorial chemistry* associated with HTS rapid testing technique, *computational chemistry*, etc.;
- the discovery of novel bacterial *targets* for the new antibiotic molecules (protease-1, lipid II, peptidyl deformylase, translocase, enoyl-ACP reductase, β-ketoacyl-ACP synthase, leucyl and isoleucyl-tRNA synthetases are examples of recently discovered targets), against which are tested Hit, Leader or Clinical Candidate molecules;
- broadening the existing classes of antibiotics with *new representatives* with optimized pharmacodyna-

Table I. Classification of pathogen bacteria depending onthe international alert level of antibiotic resistance [1-4]

Alert level	Bacteria
Maximum	*Clostridium difficile*, carbapenem-resistant enterobacteriaceae, cephalosporin-(cefixime and ceftriaxone), tetracyclines– and azithromycin-resistant *Neisseria gonorrhoeae*
High	Methicillin-resistant *Staphylococcus aureus* (MRSA), enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs), MDR *Pseudomonas aeruginosa*, MDR *Acinetobacter baumannii*, vancomycin-resistant Enterococcus (VRE), *Salmonella typhi* and *non-typhi*, *Shigella sp*., *Campylobacter jejuni*, *Mycobacterium tuberculosis*
Medium	Vancomycin-resistant *Staphylococcus aureus*, erythromycin-resistant group A Streptococcus, clindamycin-resistant group B Streptococcus

mic, pharmacokinetic, biopharmaceutical and toxicological properties (especially new oxazolidinones, 5th-generation cephalosporins, carbapenems, 4th-generation fluoroquinolones, ketolides, tetracyclines, glycylcyclines, etc.);
- discovering new beta-lactamase inhibitors (avibactam is a recently approved new broad-spectrum beta-lactamase inhibitor);
- the discovery of bacterial efflux pump inhibitors;
- the development of quorum sensing (QS) inhibitors;
- developing agents that inhibit bacterial biofilm formation and biofilm dispersal agents;
- discovering agents that inhibit the adhesion of bacteria to human body cells;
- the development of biological drugs (bacteriophages, vaccines).

If in most therapeutic classes, especially those used in the treatment of diseases of modern societies (cardiovascular diseases, cancer, obesity, depression, diabetes, etc.), there is a significant increase in budgets assigned to clinical development of new bioactive molecules, regarding the research for new antibiotics there has been a regress in the past three decades compared to the 1970-1985 period. For example, from 2000 until now, only two new structural classes of antibiotics were discovered, which, concerning the number of compounds authorized, are still very poorly represented: the oxazolidinones (linezolid as the first representative, was authorized in 2000 and followed by tedizolid only in 2014) and the pleuromutilins (retapamulin, the only class representative, authorized in 2007). Other antibiotics authorized after 2000 are compounds with "mimetic" structures that belong to structural classes of antibiotics that have been known for longer periods of time (ertapenem, telithromycin – 2001; gemifloxacin, daptomycin – 2003; tigecycline, doripenem – 2005; telavancin – 2008, ceftaroline – 2010, fidaxomicin – 2011; ceftobiprole, dalbavancin, oritavancin, tedizolid – 2014). Two combinations of cephalosporins and beta-lactamase inhibitors were also recently approved: ceftolozane/tazobactam – 2014 and ceftazidime/avibactam – 2015, respectively [1].

Among the causes that led to the development and authorization of a small number of antibacterial antibiotics after 2000, there can be listed [4, 5]:
- a *lower profitability* for the pharmaceutical companies that invest in the development of new antibiotics compared to those who develop molecules from classes of therapeutics used to treat chronic diseases with high incidence (cardiovascular diseases, cancer, depression, dyslipidemia, diabetes, osteoporosis, etc.). For example, if we refer only to cancerous diseases, huge investments are directed towards developing agents that modify cell signaling – small molecules and monoclonal antibodies (lots of molecules have been authorized only in the past 10 years) – which are marketed at high prices which ensure a higher profit rate;
- *restricting the prescription* of new antibiotics (according to the purpose for which they were developed) to infections that do not respond to other treatment regimens. The consequence is a relatively low consumption and a lower recovery rate of the costs for these molecules' development;
- phase 3 *clinical study costs* are very high. In addition, the criteria for including subjects into the study are excessively restrictive;
- *the risk of progressive installation of bacterial resistance* remains a real one even after using a new antibiotic and the withdrawal from its clinical use may be possible, due to the gradual reduction of its efficacy.

Despite the difficulties and risks related to the research for new antibacterials, there still are several developers of antibiotic molecules. Among the pharmaceutical companies that have most molecules in their portfolio of clinical studies, Cubist Pharmaceuticals, Actavis, AstraZeneca, Basilea Pharmaceutica, Crestone, Theravance Biopharma, Wockhardt, MicuRx Pharmaceuticals, Polyphor, CrystalGenomics, GlaxoSmithKline, Nabriva Therapeutics, Merck, Paratek Pharmaceuticals, Melinta Therapeutics, Summit, Achaogen, Tetraphase Pharmaceuticals are included.

Among the solutions designed to stimulate research in the field of antibiotics, the following can be listed [6]:
- *better funding* of basic research in universities in order to find new targets and new leader molecules;
- greater investment in applied research through partnerships among governments, private companies and universities;
- simplifying the protocols for clinical trials involving antibiotics;
- extending the period of patent protection (especially for orphan antibiotics).

In USA, the campaign «Bad Bugs Need Drugs» was launched in 2011 with the aim to discover and authorize at least 10 new molecules of antibiotics by 2020. Also, the cooperation between the US and the European Union, through the TATFAR project (Trans-Atlantic Task Force on Antimicrobial Resistance) aims the discovery of 40-50 new leading molecules in the next 10 years [7,8].

The literature from the past few years, in this area of interest is extremely rich in prospecting for new antibacterial molecules, with a structurally different profile from the antibiotics currently available in clinical practice, molecules capable of inhibiting recently discovered bacterial targets and therefore possessing new mechanisms of action. The new molecules belong to extremely varied structural models [3, 9-12].

New antibiotics (or combinations) recently approved or in clinical development phase

Nowadays, there are over 50 new antibiotic molecules in I-III clinical phases. Some of these belong to already-known structural classes (betalactamines, quinolones,

tetracyclines, etc.) and are mainly developed for a more targeted antibacterial activity or for optimized pharmacokinetics, while others belong to new structural models and focus on the bacteria resistant to older antibiotics.

Tables II-VIII present data of the molecules found in clinical development (name, structure, clinical phase and some spectral and therapeutic characteristics) [13,14].

Table II. Betalactamines and combinations

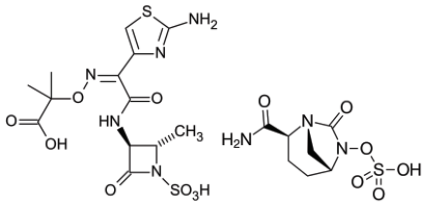
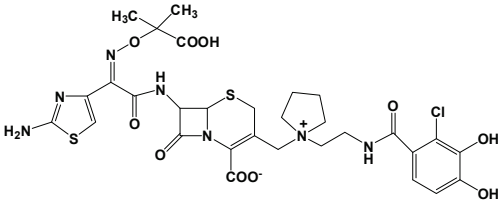
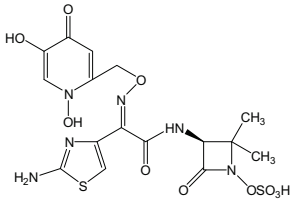
Representatives under development	Characteristics
 Aztreonam+Avibactam	• association of monobactam with non-betalactamic betalactamases inhibitor; • *phase II*: intra-abdominal infections • *phase I*: infections caused by metallo-betalactamases producing enterobacteriaceae [15];
 S-649266	• siderophore-type cephalosporin; • *phase III*: urinary and respiratory infections; • very active against *P. aeruginosa*, *Acinetobacter baumannii* and carbapenem-resistant enterobacteriaceae [16];
 BAL-30072	• siderophore-type sulfactam monobactam, with activity against MDR Gram-negative bacteria; • *phase I*: bronchiectasis and cystic fibrosis; inhalation and intravenous administration [17].

Table III. Tetracyclines [18]

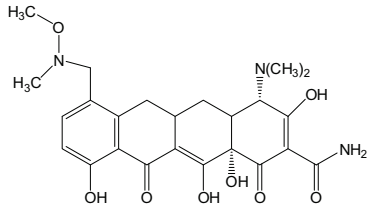
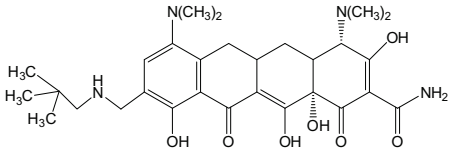
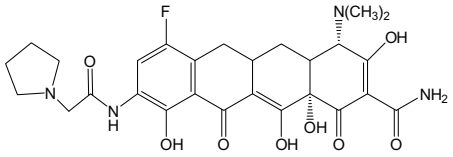
Representatives under development	Characteristics
 Sarecycline	• large spectra against skin bacterial pathogens; • tolerability similar to other authorized tetracyclines; • has an anti-inflammatory effect, as well; • *phase III*: moderate or severe acne; oral administration, in single daily dose;
 Omadacycline	• ultra large spectra, including MRSA; • *phase III*: community-acquired pneumonia; skin and soft tissue; • *phase II*: nosocomial infections; urinary tract infections;
 Eravacycline	• *phase III*: intra-abdominal infections; urinary tract infections; • *phase I*: respiratory tract infections.

Table IV. Oxazolidinones [18]

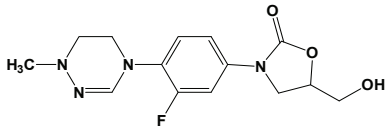
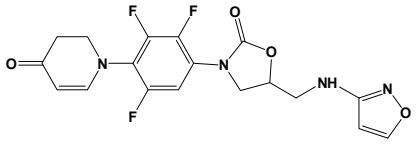
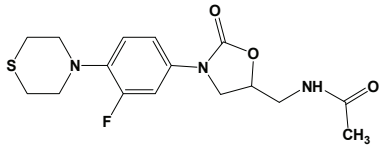
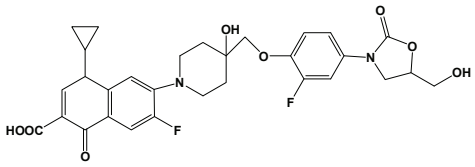
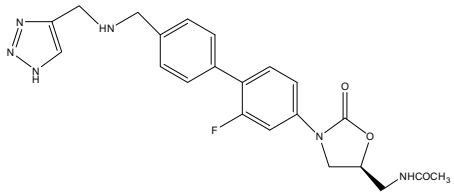
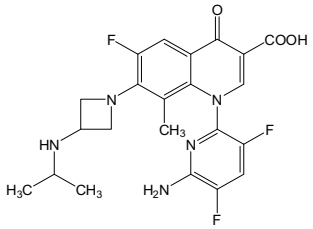
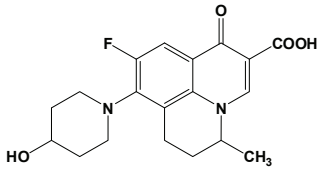
Representatives under development	Characteristics
 LCB01-0371	• *phase I*: Gram-positive infections;
 MRX-I	• *phase II:* skin and soft tissue infections; • reduced myelosuppression;
 Sutezolid	• *phase II*: XDR tuberculosis;
 Cadazolid	• contains the pharmacophores of FQ and oxazolidinone; • *phase III*: *C. difficile* infections
 Radezolid	• *phase II*: complicated staphylococcal infections of the skin and skin tissues (including MRSA) and in community-acquired pneumonia; • safety profile superior to linezolid;

Table V. Quinolone carboxylic acids (quinolones and fluoroquinolones) [18]

Representatives under development or recently approved	Characteristics
 KPI-10	• activity against bacteria currently resistant to other FQ, including *E. coli, N. gonorrhoeae*, MRSA, *Acinetobacter* spp., staphylococci; • excellent pharmacokinetic profile; • oral and parenteral formulation; • *phase I*: bacterial infections;
 Nadifloxacin (WCK 771)	• superior activity against MRSA compared to sparfloxacine and trovafloxacine; • it is not substrate for NorA efflux pumps; • WCK 2349, prodrug of WCK 771, an alanine-based ester; • *marketed*: acne vulgaris;

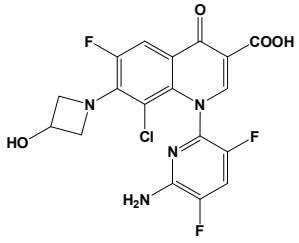
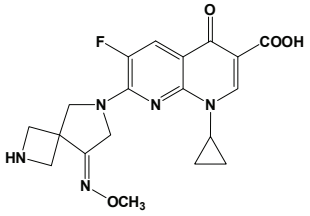
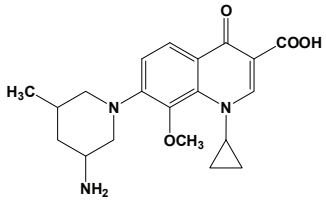
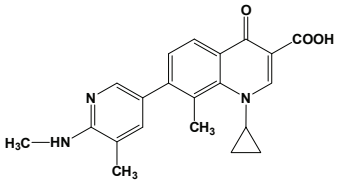
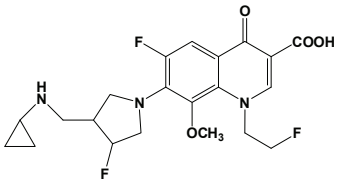
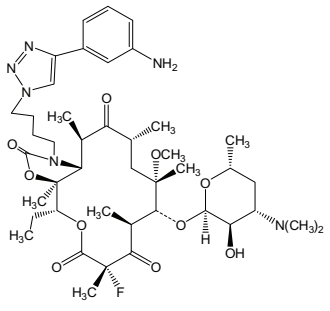
 Delafloxacin	• more active than other fluoroquinolones against MRSA; • unlike other FQ which have a zwitterion-type structure, delafloxacine has an anionic character; • it highly accumulates at cellular level; • *phase III*: community-aquired pneumonia; skin and soft tissue infections; • *phase I*: intra-abdominal infections; nosocomial preumonia; urinary tract infections;
 Zabofloxacin	• *phase III*: respiratory tract infections; • activity against multiresistant Gram-positive bacteria and *N. gonorrhoeae*
 Nemonoxacin	• non-fluorinated quinolone; • very large spectra of activity; • *marketed*: community-acquired pneumonia; • *phase II*: diabetic foot ulcer; • *phase unknown*: skin and soft tissue infections;
 Ozenoxacin	• non-fluorinated quinolone; • active against some Gram-positive bacteria resistant to ordinary quinolones; • *registered*: acne vulgaris; skin and soft tisue infections; • *preregistered*: impetigo;
 Lascufloxacin	• active against respiratory bacterial pathogens; • *phase III*: community-acquired pneumonia; otorhinolariyngological infections; respiratory tract infections.

Table VI. Macrolides (fluoroketolides) and aminoglycosides [18]

Representatives under development or recently approved	Characteristics
 Solithromycin	• carbamate fluoro-ketolide; • stronger interaction with bacterial ribosomes than telithromycin; • *preregistration:*community-acquired pneumonia; • *phase* III: chlamydial infections; gonorrhoea; urethritis; • *phase II:* otorhinolaryngological infections; • *phase I:* anthrax; bacterial infections; tularaemia;

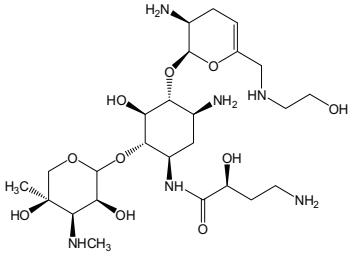
 Plazomicin	• aminoglycoside with large spectra; • *phase III*: bacteraemia; nosocomial pneumonia; pyelonephritis; urinary tract infections.

Table VII. Pleuromutilins [18]

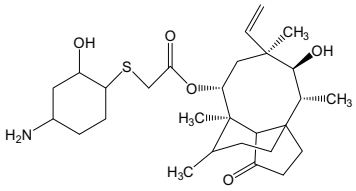
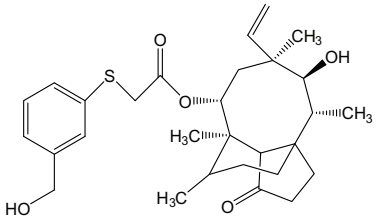
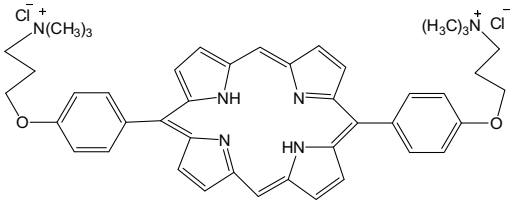
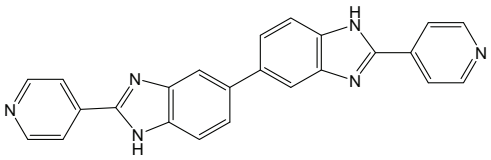
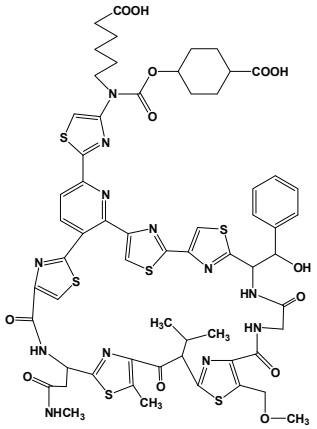
Representatives under development	Characteristics
 Lefamulin (BC-3781)	• oral and i.v. formulation; • active agains MRSA and vancomycin-resistant Enterococcus; • *phase III*: ccommunity-acquired pneumonia; • *phase II*: skin and soft tissue infections;
 BC-7013	• active against some Gram-positive bacteria; • *phase I:* Clostridium infections

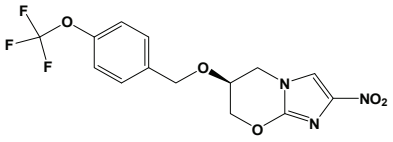
Table VIII. Various structures [18]

Representatives under development or recently approved	Characteristics
 Exeporfinium (XF-73) chloride	• *phase II:* MRSA infections;
NVB-302	• lantibiotic; • *phase I*: *Clostridium difficile* infections
Murepavadin (POL-7080)	• synthetic compound, with peptidomimetic structure; • *phase II:* pseudomonal infections; • *phase I:* Gram-negative infections;
 Ridinilazole (SMT19969)	• nonabsorbable antibiotic; • *phase II*: *C. difficile* infections;



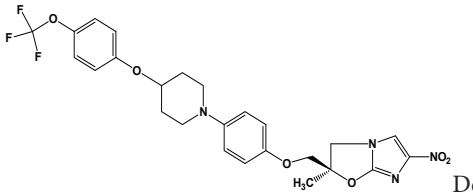
LFF571

- analogue of a natural antibiotic GE2270;
- *phase II*: *C. difficile* infections;



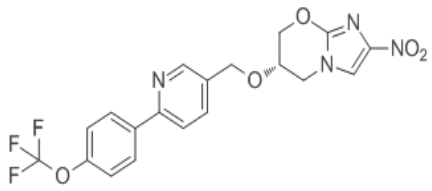
Pretomanid

- bicyclic nitroimidazole;
- inhibits the synthesis of mycolic acid;
- *phase III:* tuberculosis;



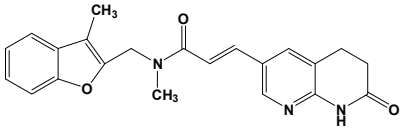
Delamanid

- bicyclic nitroimidazole;
- *marketed:* tuberculosis;



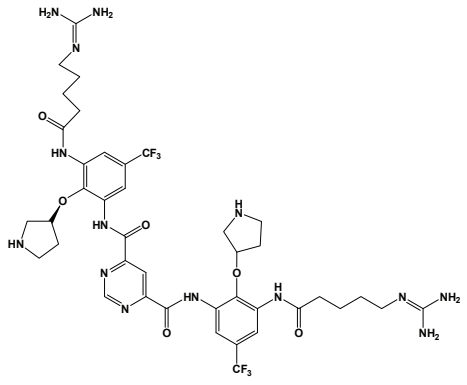
TBA-354

- bicyclic nitroimidazole;
- *phase I:* tuberculosis;



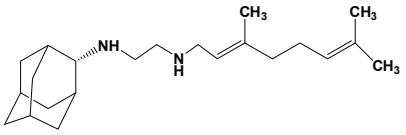
Debio 1542 (AFN 1252)

- *FabI* inhibitor;
- activity against all strains of staphylococci, including MRSA;
- *phase II:* skin and soft tissue infections;
- *phase I:* bacterial infections;



Brilacidin

- class of defensinmimetics;
- mimics the structure of HDP (host defense peptides);
- very active against MRSA;
- *phase II:* skin and soft tissue infections; stomatitis; ulcerative colitis;

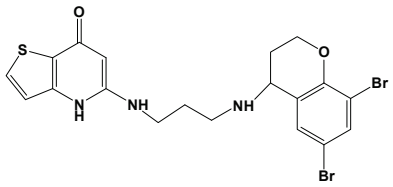


SQ109

- adamantan derivative;
- *phase II/III:* tuberculosis

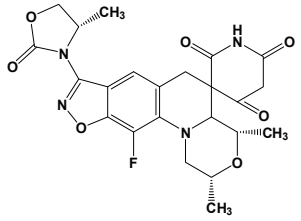
Surotomicin

- cyclic lipopeptide;
- *phase III*: *C. difficile* infections (oral administration);



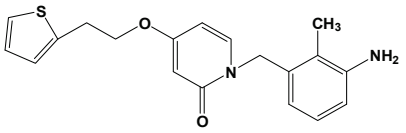
CRS3123

- *methionin-ARNt-synthetase* inhibitor;
- *phase I*: *C. difficile* associated colitis;



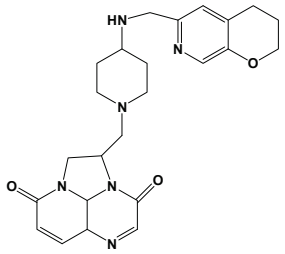
Zolifloxacin (AZD0914)

- *DNA – gyrase* inhibitor;
- *phase I*: gonorrhea;



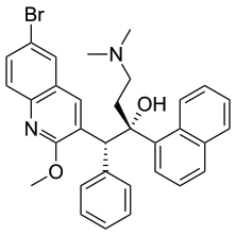
CG400549

- *Enoyl-ACP reductase* inhibitor;
- *phase II:* MRSA infections;
- *phase I:* Vancomycin-resistant *S. aureus* infections;



Gepotidacin (GSK2140944)

- *topoisomerase 2* inhibitor;
- *phase II:* gonorrhoea; skin and soft tissue infections;
- *phase I:* respiratory tract infections;



Bedaquiline

- aryl-quinoline;
- *approved*: MDR tuberculosis.

Conclusions

The discovery of new antibiotic molecules, to be active agaist highly resistant bacteria, must represent a priority of the pharmaceutical research. Currently, approximately 50 new antibiotic molecules, belonging to known antibiotic families or to new structural models, are in clinical development. Most of the promising ones are quinolone carboxylic acid (fluorinated or non-fluorinated molecules), oxazolidinone, tetracycline or pleuromutilin derivatives.

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