Minireview

An Overview of Conventional and Alternative Strategies for Developing New Antibacterial Agents

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Abstract

Although there is an increased need for novel antibacterial compounds, a regress in developing new antibiotic drugs has been registered in the past decades, as a result of economic, medical, social and political factors. The present paper aims to bring together and to discuss the wealth of information in the subject by presenting both traditional and new approaches for antibiotic drug discovery. The route starting from analogues derived from well-known antibiotics seems that will be the one to offer the great part of new antibiotics having the potential to enter the marketplace. The target-based approach, well-validated biomolecular targets and new potential targets emerging from complex resistance mechanisms of bacteria and bacterial virulence, are disscused. Moreover, strategies based on the multiplicity of targets to be addressed by an antibacterial agent in order to limit the problem of antibiotic resistance are highlighted in the present paper. Challenging sources for developing new antibacterial agents, e.g. bacteriophages, non-multiplying and non-culturable bacteria are also considered.

Keywords: Antibiotic, drug discovery, genomics, biomolecular targets, antibiotic resistance, non-culturable bacteria, non-multiplying bacteria

1. Introduction

Antibiotics are drugs critical for treatment of infectious diseases. The terminology *antibiotic* usually refers to derivatives of natural products, while *antibacterial* refers to synthetic molecules. The research of new antibacterial compounds represents a hot topic which implies both academics and economical factors. The programs of fundamental research in the field of antibiotics have been almost exclusively left in charge of pharmaceutical companies. For the past decades, a regress in the development of new antibiotics efficient in the fight against drug resistance has been registered, partly because of a decreased interest of most large pharmaceutical companies in the antibiotics research and development.[1] The spread of resistant bacteria mutants is an inevitable phenomenon, a real public threat which has reached alarming and unprecedented levels. Community- and hospital-acquired infections caused by several resistant pathogens, e.g. methicillin- resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), vancomycin-resistant *Enterococcus* (VRE) and multi-drug resistant *Mycobacterium tuberculosis* (MDR), are of particular interest. The time to the emergence of drug resistance varies largely among organisms and antibiotics. In several cases, resistance to a new antibiotic may arise after four years of its approval by the Food and Drug Administration (FDA).[2]

Antibiotic research efforts have in the last decades provided only analogues of the well-known antibiotics and attempts to develop novel classes of antibiotics with novel mode of action were unsuccessful.[3]

Partly for these reasons, pharmaceutical companies are either abandoning or drastically decreasing the investments in antibiotic research. The dimension of this abandon is suggested by the fact that during 1998–2002 only 3% of new FDA approved drugs were antibacterials and only 6 new antibacterial agents were discovered during 2003–2007.[4]

Despite these problems, there is a serious need for applying innovative solutions and strategies to develop new antibiotics with new mechanisms of action to treat drug-resistant infections. The probable way to move forward should be based on combined efforts coming from

National Institutes of Health, academics, pharmaceutical industry and regulatory authorities.

This review aims to illustrate the progress realized in the field of developing new antibiotics/antibacterials, by considering both conventional and new strategies. Despite the discovery and screening of hundreds of inhibitors of biomolecular targets from bacteria, the process to develop an antibacterial drug candidate is highly complex and other issues, e.g. cell membrane penetration, have to be considered for antibacterial activity of the inhibitors. Many research groups of chemists are involved in the synthesis of potentially more active analogues of some natural moderately antibacterial agents from different microbial sources. Synthetic analogues of lipopeptaibols which are short peptide antibiotics from *Trichoderma* fungi, known as efficient membrane modifiers can be promising tools for the discovery of new antimicrobial agents.[5–6] Another class of potential antimicrobial agents acting as bacterial membrane modifiers are the tetraazamacrocyclic complexes in particular with Ni(II).[7]

A reconsideration of natural products research which has given excellent antibiotic drugs might lead to the discovery of novel encoded molecules as potential drug candidates. This genomic and bioinformatic approach based on the identification and activation of biosynthetic genes clustered in bacterial genomes could deliver new encoded scaffold variants for activity screening.[8]

2. Target-Based Approach for Antibiotic Drug Discovery

The classical strategies of developing antibiotics are based on natural and synthetic compounds targeting logarithmic multiplying bacteria.[9] The route of analogues of well-known antibiotics continues to be successful as shown by some marketed antibiotics that are derivatives of tetracyclines, macrolides and ketolides. Unfortunately, in the last 40 years only two new classes of antibiotics entered the marketplace: oxazolidinones[10] and cyclic lipopeptides.[11–12] Consequently, new approaches are needed for accelerating the antibiotic discovery, e.g. multiple targets approach or alternative sources of new antibiotics (bacteriophages, non-multiplying bacteria and non-culturable bacteria).

The process of antibiotic drug discovery is similar to other therapeutic area, mainly following the target-based approach. This approach aims to identify compounds that interact with a biomolecular target and consequently to develop a structure-based design for the improvement of the activity and the selectivity of these antibacterial compounds. Usually, the aimed targets are proteins and enzymes, but DNA, RNA and ribosomes are also considered.

The next sections will discuss the most representative conventional and novel biomolecular targets, the importance of genomics in the target-based approach, the multiple target approach, and will give representative examples of new and not so new antibiotics active against different single or multiple targets.

2. 1. The Role of Genomics in Identification of Novel Targets of Antibacterial Drugs

Immediately after the elucidation of the first bacterial genome of *Haemophilus influenzae* in 1995,[13] most antibiotic research groups focused on the analysis of new sequence information obtained from bacteria for the identification of new potential targets.[14–15] Genomics proved to be an important tool not only for the identification of new potential targets, but also for the study of pathogenesis[16] and antibiotic resistance phenomenon.[17–18]

The identification and selection of an appropriate target is initiated by the bioinformatics-aided search of open-reading frames (ORFs) conserved across the potential bacterial target organism. The selection of genes and gene products is realized either by applying programs of automated comparison of bacterial genomes[19] or by the analysis of gene expression through DNA microarrays technologies.[20–21] It is known that among the genes of the bacterial genome, 30–50% have apparently unknown functions.[22] The identification of potential targets of antibacterial drugs among these genes is realized by several strategies: (i) structural genomics based on three-dimensional structure determination of the key proteins;[23–25] (ii) motif analysis of a large number of databases (PROSITE) for the search of motifs that might elucidate the gene's biochemical function.[22]

Once a compound with a promising antimicrobial activity and good selectivity has been identified, the evaluation of its mode of action will be realized by applying modern techniques of genomics. Usually, the main route of genomic approach leads to the identification and selection of targets codified by single genes, which are further efficiently analyzed by bioinformatics for the presence of highly conserved sequences across a desired bacterial spectrum, for the existence of homologous in target species and for the selectivity. Unfortunately, the mechanism of bacterial inhibition is not based on competitive binding to a single and unique enzymatic target. Research shows that the best way to discover new efficient antibiotics relies on targeting multiple targets or multiple binding sites at a specific target.[25–26]

Although genomic approach delivered a wealth of molecular targets and led to screening of a sizable number of inhibitors of a specific target by high-throughput screening in enzyme inhibition assays,[27] not all of these molecules showed antibacterial activity and unfortunately no marketed antibiotic resulted from this approach. At this point, the main objective for antibiotic drug dis-

covery is not only the return to the traditional methods not based on a target, but also to continue the genome target-based route, albeit in a different way as initially envisioned. The approach should be designed with a focus on inhibitors of a family of similar molecular targets from a large pathogenic bacterial spectrum. Results have shown that research groups involved in the development of new antibiotics succeeded in identification of inhibitors with performant *in vitro* activity but unfortunately on a low spectrum of bacteria. These molecules were not considered attractive antibacterial agents because of the present empirical therapy which is based mainly on those antibiotics that can be used immediately after the appearance of symptoms and before the antibiogram analysis. For this reason, discovery of new antibiotics with broad spectrum antibacterial activity will remain the essential scope of antibiotic research programs.

2. 2. Conventional and Novel Bacterial Targets

The target-based approach of antibiotic discovery is based on targeting either whole cells of viable multiplying bacteria (intact bacteria), or molecules of bacterial cells, e.g. enzymes (isolated biochemical target). Based on these targets, libraries of natural, recombinant and chemically synthesized compounds (called *hit molecules*) are screened for their binding or biological activity to a defined molecular target. The selected compounds may act by different mechanisms, e.g. inhibition of the *in vitro* catalytic activity of an enzyme, competition to the binding of the natural ligand to its receptor, and agonist/antagonist action at specific receptors. The identified hit molecules are further structurally modified through a multi-step process of synthesis followed by testing the obtained analogues series. From these series, medicinal chemists will select those molecules that have improved chemical characteristics and that may become potential drug candidates (called *lead molecules*). The lead molecules are further optimized by repeated chemical modifications in order to produce antibiotics with optimized properties needed for pre-clinical and clinical trials (Figure 1).

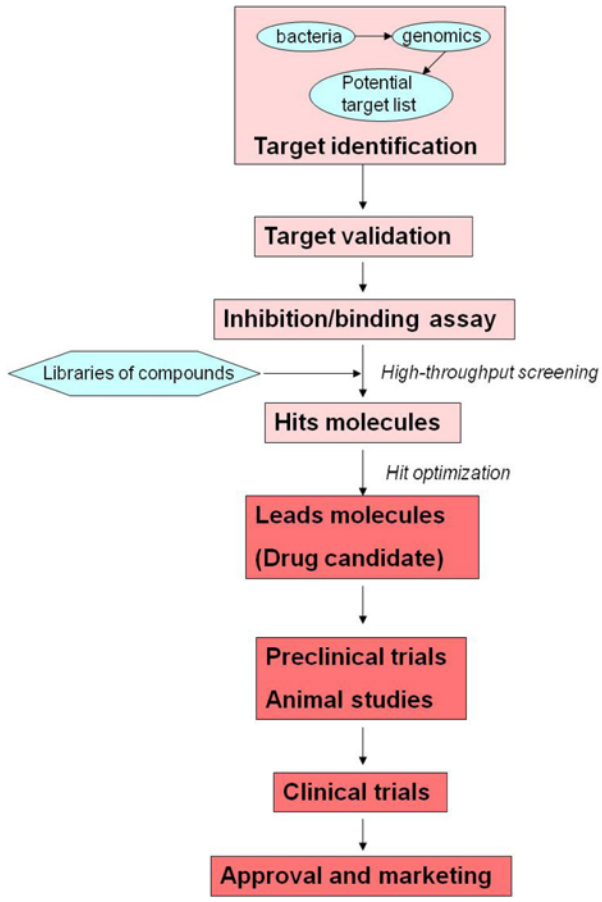

Figure 1. The process of discovery and development of antibiotic drugs.

An ideal target should have the following main characteristics:[28] (i) the bacterial target must not be shared with the human host or should be totally different; (ii) the target must be present in those bacteria that produce an infection for which a treatment is needed; (iii) inhibiting the target should lead to the death of bacteria; (iv) the target should have a well-understood biochemistry.

Majority of the investigated biomolecular targets come from two types of key bacterial cellular processes: (i) genetic processes – DNA replication, transcription in RNA and translation to proteins; (ii) metabolic pathways, e.g. biosynthesis of the cell wall, fatty acids, folic acid, CoA, isoprenoids, and cellular division. These targets are considered conventional. Several different targets of more complex bacterial cell processes, e.g. virulence, gene expression modulation and resistance expression have been studied; along with a modest success in developing new antibiotics. These targets are considered novel.

The present section of the review will briefly cover different conventional and novel targets for various classes of antibiotics and will guide the reader to a great number of publications in the field.

Table 1 shows several types of bacterial cellular processes producing conventional targets of clinically useful antibiotics, while Table 2 includes also new potential targets for novel antibacterials, some of them being under different phases of clinical development.

The key cellular processes in bacteria (replication, transcription and translation of genetic code) which are significantly different from eukaryotic cells offer well-validated targets for drug discovery. Figures 2 and 3 show the chemical structures of several inhibitors that target the prokaryotic replication and translation of the genetic code.

Table 1. Bacterial targets of several therapeutically applied antibiotics and the rate of development of target-related resistance to these antibiotics.

Marketed antibacterials	Type of targeted cellular process in bacteria	Type of enzymatic target	Rate of target-related spontaneous resistance
Quinolones (nalidixic acid)	DNA replication	DNA gyrase (A subunit)	Medium
Fluoroquinolones (levofloxacin, ciprofloxacin)		DNA topoisomerases II and IV (C subunit)	Medium
Coumermycin antibiotics (novobiocin*)		DNA topoisomerases II (B subunit)	Rapid
Ansamycin antibiotics (Rifampin)	Transcription	RNA polymerase (RpoB)	Rapid
-Aminoglycosides (gentamicin, tobramycin, streptomycin), isoleucocyl t-RNA synthetase macrolides (erythromycin, azithromycin), oxazolidinones (linezolid), tetracyclines-Mupirocin	Translation (proteins biosynthesis)	rRNA Aminoacyl-tRNA synthetase	– Slow – Slow for isoleucyl-tRNA synthetase and rapid due to acquisition of the plasmid-mediated *mupA* gene which encodes a second isoleucyl t-RNA synthetase
-β-lactams (penicillins, cephalosporins)	Biosynthesis of cell wall peptidoglycan	Transglycosylases (TG) Transpeptidases (TP)	– Slow – Very slow
-Glycopeptides (vancomycin, bacitracin) Fosfomycin		UDP-*N*-acetylglucosamine Enolpyruvyl transferase (MurA)	Slow
Isoniazid, Triclosan (biocide)	Fatty acid biosynthesis	Enoyl-acyl carrier protein (ACP) reductase (FabI)	Rapid
Sulfonamides (Sulfamethoxazole), benzyldiaminopyrimidines (trimethoprim)	Folic acid biosynthesis	Dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR)	Rapid

*withdrawn from the market

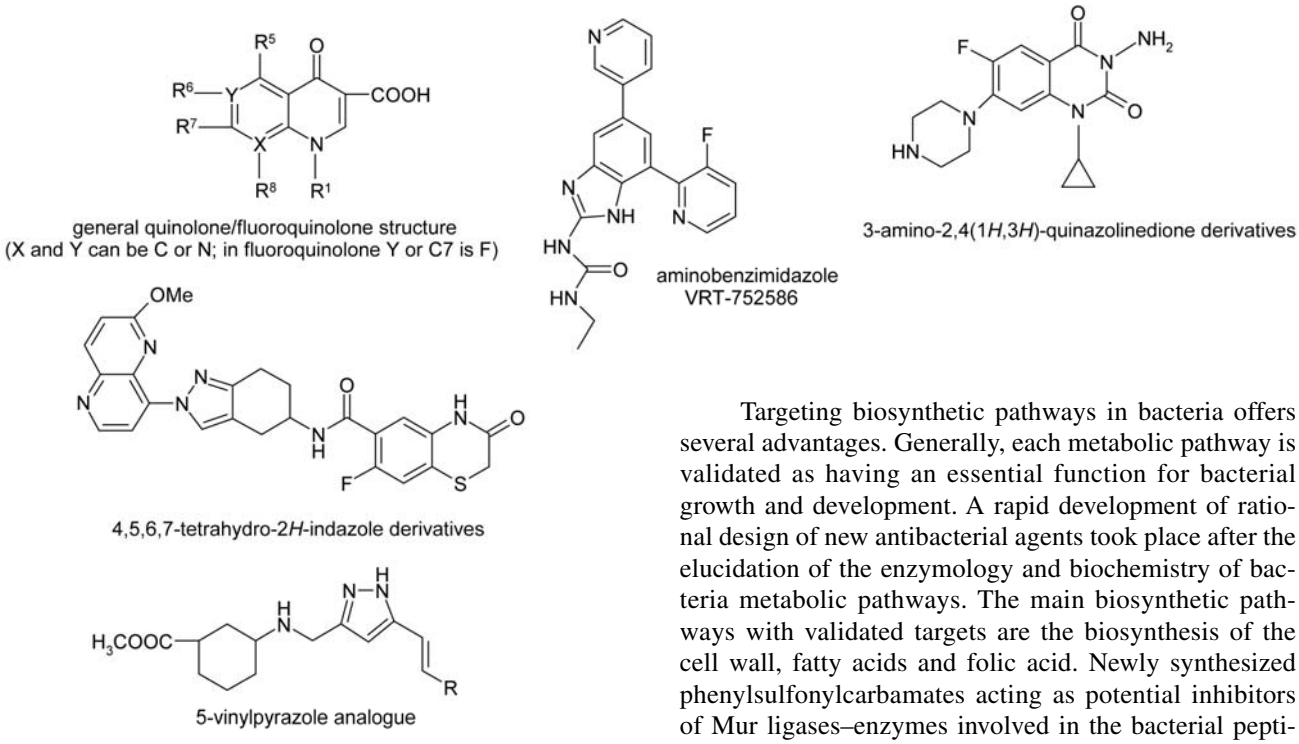

Figure 2. Chemical structure of some important topoisomerase II/IV inhibitors.

Targeting biosynthetic pathways in bacteria offers several advantages. Generally, each metabolic pathway is validated as having an essential function for bacterial growth and development. A rapid development of rational design of new antibacterial agents took place after the elucidation of the enzymology and biochemistry of bacteria metabolic pathways. The main biosynthetic pathways with validated targets are the biosynthesis of the cell wall, fatty acids and folic acid. Newly synthesized phenylsulfonylcarbamates acting as potential inhibitors of Mur ligases–enzymes involved in the bacterial peptidoglycan biosynthesis–are under investigations as potential antibacterial agents.[84] Cell division targets have been

Table 2. New antibacterial compounds targeting both conventional and novel targets from different bacterial cellular processes.

Chemical class	Type of cellular process in bacteria	Target	Ref.
Quinolone and fluoroquinolone analogues	DNA replication	Topoisomerases II and IV	29–41
Quinazolinediones		Topoisomerase II	42–43
Topoisomerase II, B subunit (GyrB)			
		Topoisomerase IV E subunit (ParE)	
Benzimidazoles and benzothiazoles		Pyrazole and indazole analogues	44–49
		Topoisomerase II	50–52
Cyclopeptide microcin J25		RNA polymerase (RpoC)	53–54
Indolmycin, TAK-083	Translation (proteins biosynthesis)	Aminoacyl-tRNA synthetase I	55–57
Chuangxinmycin and analogues			
-Actinonin		Peptide deformylase (PDF)	58–60
-*N*-alkyl urea hydroxamic acid (LBM415)			
-*N*-formyl hydroxylamine-based peptidomimetic (BB-83698)			
Phenyl thiazolyl urea and carbamate derivatives	Biosynthesis of cell wall peptidoglycan	UDP-*N*-acetylglucosamine enolpyruvyl transferase (MurA),	61
Enzyme Lysostaphin		UDP-*N*-acetylglucosamine enolpyruvyl reductase (MurB)	
		Pentaglycine cross-links	62
Cyclic lipopeptide (friulimycin)		complex formation with bactoprenol-phosphate, inhibition of teichoic acid biosynthesis	63
Lipoglycodepsipeptide (Ramoplanin)		UDP-*N*-acetylglucosamine enolpyruvyl transferase (MurA)	
Benzoylaminobenzoic derivatives	Fatty acid biosynthesis	β-ketoacyl-(acyl-carrier-protein (ACP)) synthase III (FabH)	64–66
Thiolactomycin and analogues			
Pseudopeptide pyrrolidinedione antibiotics (moiramide B) and analogues		Acetyl-CoA carboxylase (AccABCD)	67v68
2,3-Epoxy-4-oxo-7,10-dodecadienamide (Cerulenin)		β-ketoacyl-(acyl-carrier-protein (ACP)) synthase I/II (FabF/B)	65
Platensimycin			
N-substituted pantothenamides	CoA biosynthesis	Acyl carrier protein	69
Fosmidomycin	Isoprenoids biosynthesis	1-deoxy-D-xylulose 5-phosphate reducto-isomerase (DXR)	70
Phosphonohydroxamic acids			
Carboxybiphenylindole and indolo[2,3-*a*]quinolizin-7-one derivatives	Cell division	ZipA-FtsZ interaction	71–73
3-(2-indolyl)piperidines and 2-phenyl indoles			
2-carbamoyl pteridine		FtsZ protein	74
Adefovir	Virulence factors	Edema factor (EF)	75–76
Rhodanine derivatives (phenylfuran-2-ylmethylenerhodanineacetic acid derivatives)		Anthrax lethal factor (anthrax LF)	77
Inhibitors produced by *Penicillium spp*. (penicillic acid, patulin).	Quorum-sensing		78
		Transcriptional regulators of *Ps. aeruginosa* QS systems	79
N-acyl homoserine lactones analogues			
Homoserine lactones analogues and natural products from garlic			80–81
Thienopyridine	Two-component signal transduction system (TCST)	Histidine kinases (HK)	82–83
TEP: 3,6-diamino-5-cyano-4-phenylthieno[2,3-*b*]pyridine-2-carboxylic acid (4-bromophenyl)-amide			

less exploited for developing novel antibiotic drugs, despite involving several proteins (FtsA, ZipA, FtsK, FtsQ, FtsL, FtsW, FtsI, FtsN) highly conserved in most bacteria and absent in humans. This is probably due to the difficulties with the inhibition of protein-protein interactions.[85]

Figure 4 shows the chemical structure of several inhibitors targeting different biosynthetic pathways.

New promising strategies for the discovery of antibacterials with new mechanisms of action, that will develop resistance very slowly or not at all, are based on targe-

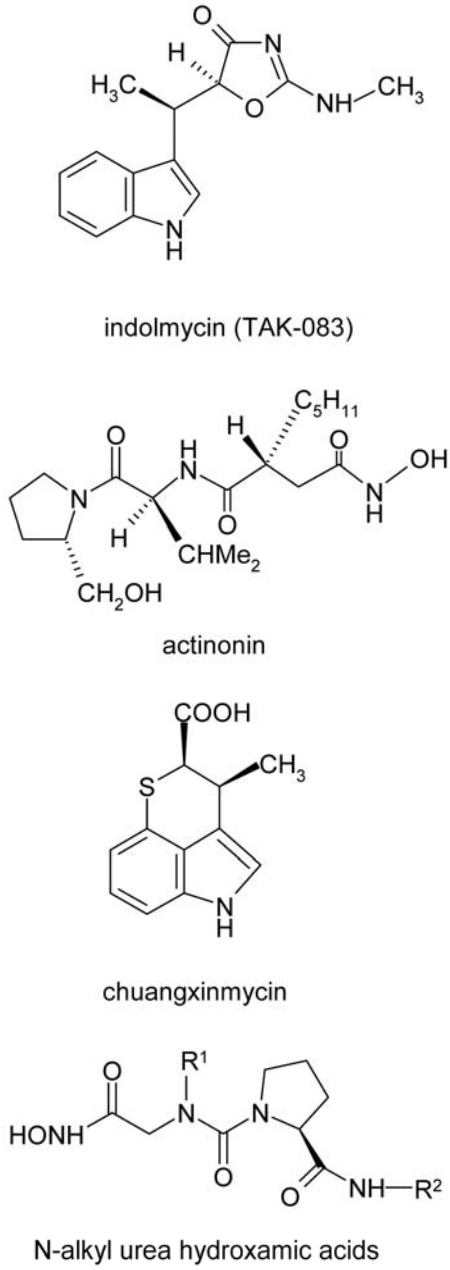

Figure 3. Chemical structure of some important aminoacyl-tRNA synthetase I inhibitors and PDF inhibitors.

ting two essential bacterial processes, e.g. resistance mechanisms and bacterial virulence.

The three main antibiotic resistance mechanisms of bacteria[86] are:

(i) Enzymatic inactivation of the antibiotic; there are few clinically used antibiotics acting as inhibitors of class A β-lactamases, such as clavulanic acid used in combination with amoxicillin or ticarcillin, and sulfone inhibitors (tazobactam and sulbactam). Of particular concern is the increasing number of clinical bacterial isolates which contain genes of class C β-lactamases, for which new inhibitors with improved spectrum activity are needed.[87–89] Few clinical bacterial isolates produce metallo β-lactamases capable of hydrolysing all β-lactams. Research programs provided a new inhibitor–thiomandelic acid, which targets zinc β-lactamase and which can be used efficiently in a combination with carbapenem–the known antibiotic resistant to other classes of β-lactamases.[90]

(ii) Reduction of the intracellular concentration of the antibiotic in bacterial cells by its efflux outside from the cell through bacterial transmembrane efflux pumps (efflux pumps that recognize specific antimicrobials and multidrug efflux pumps);[91] some efflux pumps selectively extrude specific antibiotics, such as tetracyclines and macrolides, and some others, called multidrug resistance (MDR) pumps, recognize varieties of structurally different antibiotics. A novel antibiotic that is not a substrate of the specific efflux pumps but has a broad spectrum activity is tigecycline, that has been recently approved by FDA for clinical trials.[92–93]

(iii) Modification of the biomolecular target by spontaneous mutation of the gene encoding the target, e.g. mutations in RNA polymerase resulting in the resistance to rifamycins and in DNA gyrase resulting in the resistance to quinolones. In other cases, the alteration of the target occurs by the substitution of the target function by an exogenous gene, e.g. acquisition of the *mecA* genes encoding methicillin resistance in *Staphylococcus aureus* and the various *van* genes in enterococci encoding resistance to glycopeptides.

Research efforts are focused on targets of the more complex bacterial cell processes, such as quorum sensing, two-component signal transduction system, DNA methylation and winged-helix transcription factors. Quorum sensing (QS) represents a mode of communication between bacterial colonies in which bacteria regulate sets of genes as a response to an increased population density. When bacteria reach a critical mass, they activate different biological functions, such as secretion of virulence factors, production of biofilms or sporulation of DNA exchange. Gram-positive bacteria that use QS produce signal molecules, *N*-acyl-homoserine lactones (called autoinducers), while Gram-negative bacteria use peptides (called pheromones) in the density-dependent regulation.[94] This efficient communication between bacterial colonies can be disrupted by blocking the genes responsible for QS. Thus, new strategies for developing broad-spectrum QS inhibitors have been considered.[95–96] Several natural and synthetic inhibitors that act by different mechanisms (inhibition of the synthesis of the signal

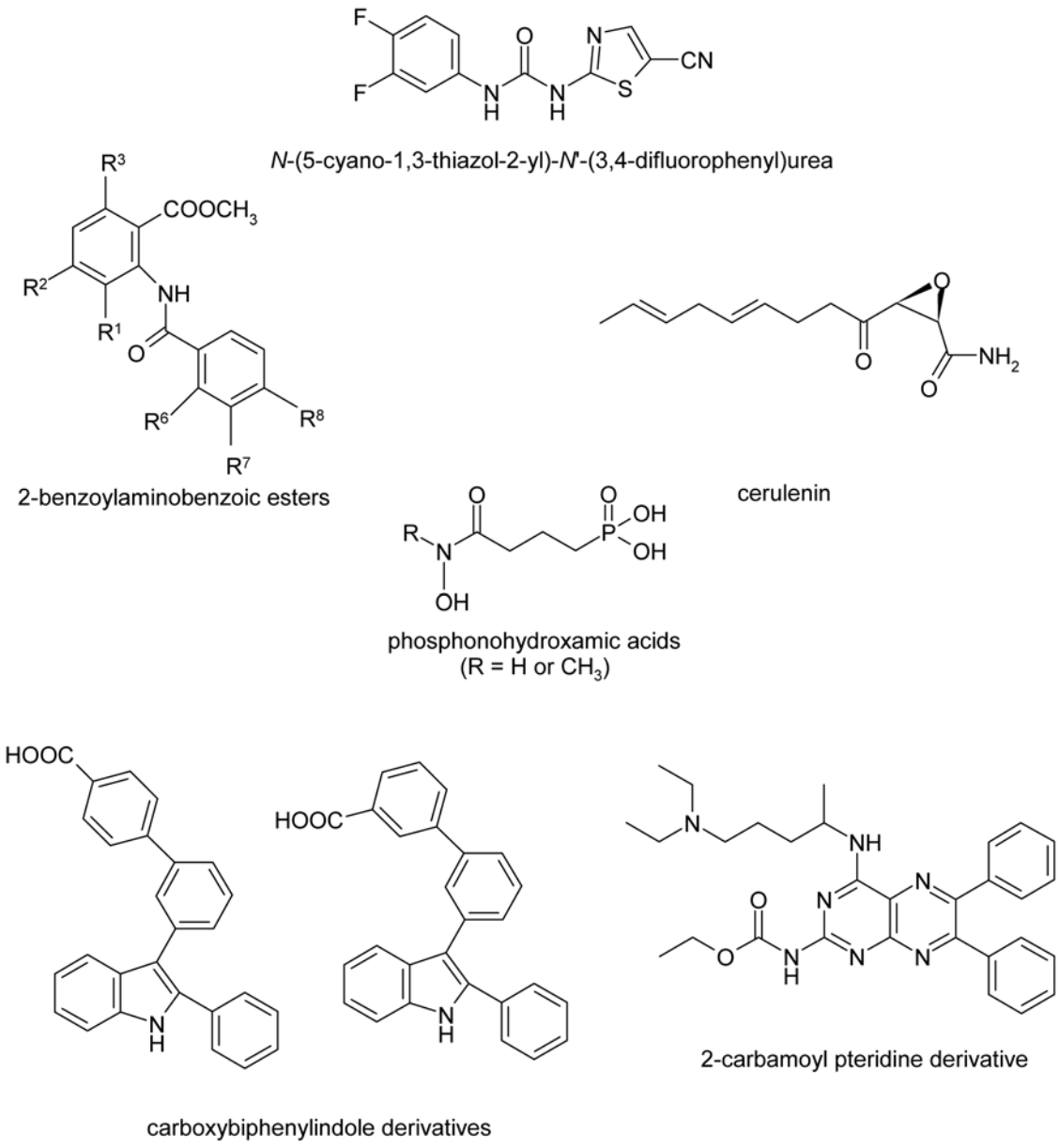

Figure 4. Chemical structure of some important inhibitors which target different bacterial biosynthetic pathways.

molecule, blocking the receptor site of the signal molecule, blocking the synthesis of active dimers essential for binding and expression of target genes) have been proposed.[78–81]

Another important system that bacteria use for gene expression regulation as a result of environmental changes or for virulence factors expression is the two-component signal transduction system (TCST).[97] This system consists of a transmembrane sensor (histidine kinase receptor), which responds to the environmental changes, and a cytoplasmatic response receptor. Histidine kinase was proposed as a novel promising target for developing new antibacterials, e.g. thienopyridine.[82–83]

Figure 5 shows the chemical structure of some new inhibitors targeting the virulence factors, the QS and the TCST.

Recent interest in the discovery of new antibiotics *via* target-based approach has focused attention on some potential new targets: (i) the enzyme DNA adenine methylase (Dam) that is involved in the methylation of DNA;[98] (ii) gene regulators, such as winged-helix transcription factors comprising of small proteins involved in the bacterial efflux pumps (MarR from *Escherichia coli* and MexR from *Pseudomonas aeruginosa*)[99] or family of proteins that regulate the expression of the virulence factors (Sar/Rot from *Staphylococcus aureus*).[100]

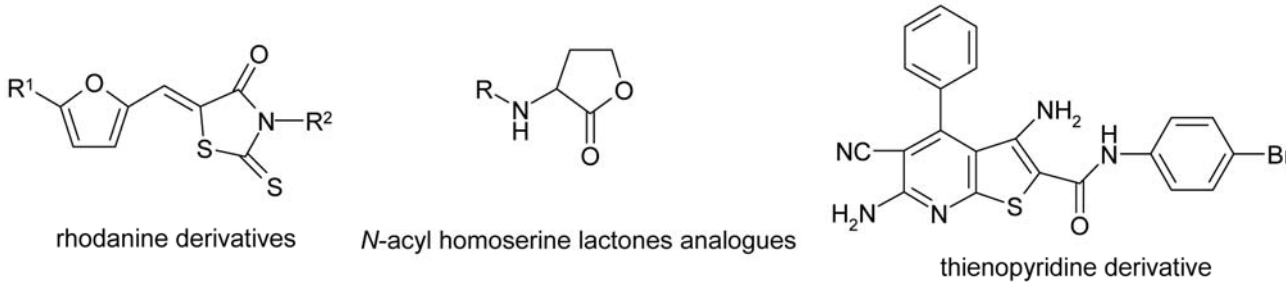

Figure 5. Chemical structure of some important inhibitors targeting the anthrax LF (rhodanine derivatives), transcriptional regulators of QS (*N*-acyl homoserine lactones analogues) and histidine kinases (thienopyridine derivative).

2. 3. Multiple Targets Approach

An increased number of therapeutically useful antibiotics, usually those based on targets of a single gene, are known to generate rapid resistance mutations in the bacterial target gene. These antibiotics are more efficient when used in combination and/or as topical agents.[101–102]

The most important task for developing novel and improved broad-spectrum antibacterial agents through target-based approach remains the focus on several targets in parallel or targets encoded by multiple genes. Thus, considering DNA replication, success can be gained from targeting both topoisomerase IV and DNA gyrase, as do quinolone and fluoroquinolone derivatives,[103–104] or topoisomerase IV, DNA gyrase and rRNA.[105] Regarding the cell wall biosynthesis, attempts have been done to discover inhibitors of both MurA and 5-enolpyruvylshikimate-3-phosphate synthase (AroA).[106] In some cases, two different cellular processes are targeted, such as DNA replication with DNA gyrase as the target, and folic acid biosynthesis with DHPS as the target.[107]

The concept of antibacterial polypharmacology was introduced to define the process by which a drug acts on multiple targets. The following polypharmacologically derived strategies for developing novel antibiotics with limitation of the target-related resistance are described in the literature:[108–109] (i) administration of two antibiotics with different targets (*dual drugs*), e.g. clavulanic acid used in the combination with amoxicillin;[110] (ii) development of next generations of known classes of antibiotics by conferring them new modes of actions or new binding sites; (iii) administration of a single hybrid antibiotic, obtained by binding two pharmacophores with different targets *via* a linker that can be hydrolyzed by the bacterial enzymes in order to release the two pharmacophores which will interact with their specific targets (*dual action drugs* or *hybrids*).[111]

Several pharmaceutical companies adopted the strategy based on implementation of new structural elements in the well-known antibiotics with the purpose to increase antibacterial activity against resistant strains. Representative examples of recently launched antibiotics and antibacterials under clinical development are: (i) two cephalosporins optimized for an increased affinity for the penicillin-binding protein 2a (PBP2a) active site;[112–113] (ii) semisynthetic derivatives of macrolides (ketolides) in which a sugar residue was substituted by a keto group;[114–115] (iii) a new tetracycline (tigecycline) in which a glycil residue was introduced to address a new binding site for the same target (rRNA) that does not induce more bacterial efflux pumps specific for tetracyclines;[116–117] (iv) new antibiotic glycopeptides (telavancin, oritavancin) with new modes of action through the interaction with the bacterial membranes.[118]

The hybrid antibiotic Ro-23-9424 was obtained by binding β-lactams (targeting transpeptidases) to fluoroquinolones (targeting topoisomerases II and IV) *via* an ester bond. Unfortunately, this attempt proved unsuccessful due to low activity, poor solubility, limited metabolic stability or lack of bacterial membrane penetration.[119] The hybrid antibiotic MCB-3837 obtained by linking oxazilidinones (targeting rRNA) to fluoroquinolones (targeting topoisomerases II and IV) *via* stable ether bonds showed improved activity against Gram-positive bacteria, including resistant clinical isolates MRSA, MRSE (methicillin-resistant *Staphylococcus epidermidis*), PRSP, VRE, VISA (vancomycin intermediate *Staphylococcus aureus*), but moderate activity against Gram-negative bacteria.[120–121] The hybrid antibiotic CBR-2092, a stable chiral complex of rifamicyn (targeting RNA polymerase) and fluoroquinolones (targeting topoisomerases II and IV) showed excellent activity against Gram-positive bacteria including rifamicyn-resistant and fluoroquinolones-resistant strains, and a slow rate of resistance appearance due to multiple targets (topoisomerase II, topoisomerase IV and RNA polymerase).[122–123] But this hybrid molecule showed no activity against Gram-negative pathogens. The hybrid antibiotic TD-1792 obtained by direct binding of vancomycin (targeting peptidoglycan synthesis) to cephalosporin (targeting transpeptidases) *via* an amide bond showed an improved activity against Gram-positive bacteria except VRE and no activity against Gram-negative bacteria.[124–125] New hybrid antibacterial agents were obtained by linking one pharmacophore targeting specific enzymes (berberine – an antibacterial alkaloid from *Berberis fremontii*) and a second pharmacophore targeting bacterial efflux pump (2-aryl-5-nitro-1*H*-indoles).[126]

In most cases, attempts to produce hybrid antibiotics showed low or no activity against Gram-negative bacteria, probably due to low bacterial outer membrane penetration as a result of the large molecular mass obtained by linking two different pharmacophores.[127] The future success of such antibiotic research programs may come from the direct linking of the target interaction sites, resulting in a reduced molecular mass of the active molecule.

3. Challenging Sources for Developing Novel Antibiotics

If modern medicine will continue in its actual form (antibiotic overuse), new classes of antibiotics must enter regularly into the marketplace. Even if analogues of the existing classes of antibiotics are useful for a period, new strategies for developing novel antibiotics are needed to manage the increasing resistance of bacteria to antibiotics.

The present section will disscus several promising alternative routes which rely on targeting non-multiplying bacteria, non-culturable bacteria and bacteriophages.

In recent years, antibiotic research efforts have focused on targeting non-multiplying bacteria which are not in the logarithmic phase of multiplication, but in the stationary phase, also called dormant or latent.[128] Clinically, it was suggested that 60% of the infectious diseases caused by bacteria contain a significant part of non-multiplying pathogens.[129] Bacteria in this phase are not easily destroyed by known bacteriostatic antibiotics. Attempts to develop novel antibiotics targeting non-multiplying bacteria have been done, and suggestions were made to their eventual use in combination with antibiotics targeting multiplying bacteria. The main advantages of these novel antibiotics are the reduction of the therapy duration and the potential elimination of resistance. Several problems might arise when using this approach, such as the difficulty to develop new compounds because of the limited number of molecular targets in non-multiplying bacteria, and the existence of many subpopulations of non-multiplying bacteria that require more than one compound to kill them.

Another proposed route for developing novel antibiotics is based on targeting non-culturable bacteria. Viable but non-culturable bacteria (VBNC) is a strategy developed by bacteria to survive the environmental stresses. These bacteria represent a health risk as numerous bacterial pathogens can enter the VBNC state and potentially regrow and return to the infectious state.[130] Today, large fragments of non-culturable bacterial genomes are cloned and expressed using recombinant DNA technology.[131–135] DNA is extracted from non-culturable soil bacteria, inserted into a vector, e.g. artificial bacterial chromosome, which can accept large DNA fragments. The ORFs of these fragments are further expressed in culturable bacteria, e.g. *Streptomyces* spp., which will be consequently screened for antibacterial activity. When considering this route, there are some potential disadvantages: (i) DNA productive fragments may not appear as frequently as to be detected by cloning; (ii) DNA fragments may not contain all the genes necessary for the production of an antibiotic; (iii) the host organism may not correctly express the genes in the DNA fragments.

An alternative route for developing novel antibiotics is using bacteriophages as antibiotics, based on their bactericidal properties.[136–137] The main advantage of this strategy is that bacteriophages present a completely different mechanism of action. Some disadvantages are also encountered with this approach, such as: (i) difficult quality control and standardization; (ii) bacteriophages may become immunogenic and induce synthesis of neutralizing antibodies by a systematic use;[138] (iii) bacterial lysis could induce a toxic shock;[139] (iv) resistance may develop rapidly, so that a combination of phages should be used.[137] The gene products of phages are more promising sources of antibiotics. Thus, the phages' lysins which are hydrolases of the cell wall produced lately in the viral infection cycle, can bind to peptidoglycans leading to the disruption of the cell wall of Gram-positive bacteria.[140–141] The gene products of phages have the advantage of not inducing resistance, neutralizing antibodies or toxic shock. Some research studies reported the efficacy of these products against non-multiplying bacteria and biofilms.[142]

4. Conclusions and Remarks

The alarming increase of multidrug resistant pathogens makes the discovery process of new antibacterial compounds to continue the conventional strategies of targeting known metabolic pathways or bacterial complex processes (replication, transcription and translation of genetic code), which have delivered well validated targets. It seems that the route of analogues of the existing antibiotics will offer the majority of new antibiotics with increased potential to enter the marketplace. At the same time, there is a great need for developing new strategies for antibiotic drugs discovery, as resistance mechanisms of bacteria to some families of antibiotics become more sophisticated. These strategies are based either on new biomolecular targets coming from bacterial cellular processes with still unknown biochemical mechanism, multiplicity of targets, or innovative solutions.

The approaches based on conventional and new targets and the alternative routes based on targeting non-multiplying bacteria, non-culturable bacteria, or bacteriophages discussed here brought together as much information as possibile on the subject and will hopefully help specialists work hand-in-hand in the discovery of novel antibacterials.

Until novel resistance-breaking antibiotics are developed, educational programs based on enhanced hygiene, reduction of misuse and abuse of antibiotics, eradication

of unjustified and inappropiate antibiotic prescriptions and of self-medication should also be considered as important factors to limit the problem of antimicrobial resistance.

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Povzetek

Čeprav obstaja povečana potreba po novih antibakterijskih spojinah, je v zadnjih desetletjih opazno zmanjševanje razvoja registriranih novih antibakterijskih sredstev. To je verjetno posledica ekonomskih, medicinskih, socialnih in političnih vplivov. Prispevek prikazuje in razpravlja o informacijah s tega področja; vključuje tako tradicionalne kot tudi sodobnejše pristope k odkrivanju novih antibiotikov. Za poti, ki izhajajo iz analogov že znanih antibiotikov, se zdi, da imajo največjo možnost, da bodo obrodile antibiotike, ki bi imeli možnost vstopa na tržišče. Obravnavani so tudi pristopi iskanja, ki temeljijo na osnovi tarč, na osnovi ostalih dobro znanih biomolekularnih tarč in tudi na osnovi potencialnih tarč, ki se pojavljajo pri nastopu odpornosti bakterij in tudi pri bakterijski virulenci. Pomembne so tudi strategije, temelječe na več tarčah, ki jih napadejo antibakterijske spojine, saj lahko tako omejimo problem odpornosti na antibakterijske spojine. Omenimo tudi nove izzive pri razvoju antibakterijskih sredstev, kot je npr. uporaba bakteriofagov ter boj proti bakterijam, ki so v nerazmnoževalni fazi, in proti bakterijam, ki jih ni možno gojiti v celičnih kulturah.