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Review

How to cope with the quest for new antibiotics

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ABSTRACT

Since their introduction in therapy, antibiotics have played an essential role in human society, saving millions of lives, allowing safe surgery, organ transplants, cancer therapy. Antibiotics have also helped to elucidate several biological mechanisms and boosted the birth and growth of pharmaceutical companies, generating profits and royalties. The golden era of antibiotics and the scientific and economical drive of big pharma towards these molecules is long gone, but the need for effective antibiotics is increased as their pipelines dwindle and multi-resistant pathogenic strains spread. Here we outline some strategies that could help meet this emergency and list promising new targets.

Antibiotics are, for the vast majority, low molecular weight (<1000 Da) secondary products of microbial, fungal or plant metabolism. Several lines of evidence suggest that these or similar molecules may have been around since the pre-biotic era and may have played an important role as modulators or effectors of the primeval RNA molecules from which "modern" biological structures like the ribosomes and riboswitches have evolved. Indeed, the fact that several antibiotics may have coevolved with RNA is suggested by the fact that they bind select RNA targets and that some of them, like the aminoglycosides, can equally influence ribosomal decoding and inhibit the second step of Group I T4 phage-derived *td* intron splicing, two activities possibly having a common origin in the "RNA World". Furthermore, a large number of antibiotics can be synthesized from amino acids and other compounds which have been detected in meteorites or synthesized in reactions carried out under prebiotic conditions as a result of electric discharge [1,2 and references therein].

Since their discovery and their first therapeutic applications in the second half of the '30s (sulfamides were first used around 1935), antibiotics can be credited with having saved millions of human lives. In addition, the extraordinary contribution given by antibiotics to the progress of science and to the advancement of biotechnology should not be ignored. Studies on the mechanism of action of antibiotics and on both genetics and mechanisms of bacterial resistance to their action have contributed a great deal to the understanding of fundamental biological mechanisms such as DNA duplication, transcription and translation. Genetic characterization of the resistance genes allowed the identification of essential genes such as *gyrA* (*nalA*), *gyrB* (*cou*) and *fus* and gene clusters such as *str* and of their products. The pioneering work of the late Luigi Gorini on streptomycin resistance and dependence and on the *ram* mutations established for the first time the fundamental role played by the ribosome in determining decoding fidelity. Finally, antibiotics have been and are tools of invaluable importance in selection strategies routinely used in genetic engineering.

Nevertheless, after an initial period in which the detection, biological, pharmacological and clinical characterization as well as marketing of new antibiotics have flourished, generating large profits for the pharmaceutical industry, research on new anti-infective agents has slowed down considerably. This has resulted in a long "innovation gap" which extends from 1962, when quinolones and streptogramin were first applied in human therapy, until 2000, when oxazolinidones were introduced [3,4]. During this period no major class of antibiotics has been introduced and, to make matters worse, the decreasing interest and investment in antibiotic research by the big pharma and the consequent decline in antibiotic discovery has been paralleled by the ever more rapid and frightening spread of antibiotic resistance strains [3,4].

The reasons for the disengagement of the big pharma from pursuing antibiotic research are mainly non-scientific and not health-related, but purely economical. The politics of the regulatory authorities, like the US Food and Drug Administration (FDA)

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have also contributed to the problem by failing to approve drugs endowed with non-inferior properties. These issues have been analyzed and discussed in depth in several articles, e.g. [4–6]. Small enterprises (biotech) can carry out competent, efficient and high-quality pre-clinical research. However, since they rely heavily on rather volatile venture capital, the huge costs connected with clinical trials represent an obstacle and, when this is not the case, any delay or drawback occurring during the clinical trials results in economical collapse [4–6]. However, the consequences of this politics pose a formidable threat on all of mankind, not only on the socially and economically less favoured populations of Africa, Asia and Latin America, where lack of prevention and degraded hygienic conditions worsen the effects of the scarcity of therapeutic resources.

The widespread and increasing occurrence of antibiotic resistance should not represent an alibi for discontinuing antibiotic research, but should instead represent a stimulus to pursue antibiotic research with renewed energy and enthusiasm. In fact, alternative approaches to control bacterial infections such as photodynamic therapy of periodontal and skin diseases, low temperature plasma treatments, stimulation of host immune response, use of bacteriophage or bacteriophage components, have so far proven impracticable and/or futile, being sometimes restricted to a rather limited number of specific pathologies.

Indeed, vis-à-vis the worsening of the problems posed by the rise of bacterial resistance, which is considered by the WHO one of the three greatest threats to human health, the IDSA (Infectious Diseases Society of America) has called for a global commitment to the development of 10 new antibacterial drugs by 2020 [7]. Since it would not be possible for both large and small enterprises to escape the logic of the short term profit imposed by the current economical system and therefore taking for granted the persistent lack of interest by the big pharma, in spite of some faint indications of a possible inversion of the current trend [8], only publically -subsidized research or public research *tout-court* could meet the need for new antibiotics.

Once established that new antibiotics are badly needed and therefore that it is necessary to multiply the efforts to discover new molecules or to improve the existing ones, strategies which could offer the best opportunities to reach the goal remain to be discussed, and both positive and negative experiences of the past should help in designing them.

Two very important elements that may have a profound impact on the perspectives of discovering and developing new anti-infectives are the choice of the source of these molecules and the method of screening adopted.

In the golden age of antibiotics discovery (1940s–1960s), whole cell screening of mainly natural compounds led to identification of almost all currently known antibiotic classes. In several cases the natural molecules identified were improved by chemical modifications. However, it soon became clear that the easiest-to-discover anti-infectives had already been identified. Furthermore, the attempts to find antibiotics among natural products encountered a number of difficulties which include the expensive production of high-quality collections, the elaborate and time consuming process of purification and chemical characterization of the active product contained in complex extracts, the difficulties implicit in the large scale production of the molecules of interest, the amenability of the natural drugs to modifications by medicinal chemistry. For these reasons the birth of combinatorial chemistry, favored by the progress of organic synthesis, raised the hope that artificial drugs with potential for therapeutic applications could be easily obtained in a simpler, less expensive way, avoiding the aforementioned difficulties. Thus, about two decades ago (i.e. from late 80's to late 90's) some big pharma such as GW, SKF and Pfizer abandoned the field of natural products in favour of chemical libraries, unlike other companies, such as Merck and Novartis, which had blockbuster natural drugs in their portfolio. However, HTS of chemical libraries turned out to be a flop and, short of matching the high expectations, resulted in a single de novo combinatorial new chemical entity approved by the US Food and Drug Administration (FDA), namely the kinase inhibitor Sorafenib® FDA-approved for renal carcinoma. An interesting account of the various reasons for these failures can be found in the interesting review written by Payne et al. in 2007 [9].

On the other hand, inspection of the long list of antibiotics discovered so far clearly indicates that a large majority of them are either unmodified or modified natural products. Furthermore, natural products still produce the highest hit rate [9]. This is not surprising insofar as natural products represent more diverse chemical classes than traditional synthetic and combinatorial molecules. The natural products derive from common intermediates which had millions of years to evolve into bioactive compounds and are produced by and interact with three dimensional molecules that confer upon them steric complexity [10]. These circumstances alone would be sufficient to indicate that repertoires of natural products were and will remain the best source of new anti-infectives and the old dilemma natural libraries vs. chemical libraries seems to have reached its epilogue [11]. Nevertheless, combinatorial synthesis will probably play an important role in enhancing the diversity of HTS libraries based on scaffolds of proven biological relevance through Diversity-Oriented Synthesis (DOS) or Biology-Oriented Synthesis (BIOS) to produce small compound libraries [12 and references therein].

As to the screening strategy and choice of the target, a new strategy was developed by the mid 90's with the hope of identifying specific, essential and suitable bacterial targets and was largely used by big pharma (e.g. Glaxo-Smith-Kline in Verona). This approach, mainly triggered by the euphoria generated by the promises of genomics, relied on the analysis of the increasing amount of new information obtained from genome sequencing, mainly of pathogenic bacteria, with the aim of targeting the genes products responsible for virulence. In spite of the large material efforts and also as a consequence of a number of strategic mistakes, this approach failed completely, yielding only a few hits. Bacteria proved to bypass easily any attempt to inhibit the selected target. Other likely reasons for the failure were the very short time allotted to the study of each potential target and reliance on chemical libraries instead of repertoires of natural products.

Thus, the current trend remains that of concentrating on fundamental, essential biological targets (e.g. the translational apparatus), possibly focusing on specific steps of these processes representing unexploited or underexploited antibiotic targets. An approach of this kind is expected to yield mainly broad-spectrum antibiotics while the quest for pathogen-specific inhibitors must rely on specific targets of proven relevance.

For what concerns the screening, different strategies have been suggested and/or applied. A traditional way of proceeding is through microbiological primary screenings to identify hits having antimicrobial activity, followed by secondary screenings aimed at obtaining a better characterization of the hits and at the possible selection of lead compounds. This type of approach, in its simplest formulation, although very successful in the past, can be regarded as outdated since it would likely "rediscover" known inhibitors and would also miss a large number of potentially interesting molecules that do not or hardly penetrate the test cells. Thus, improved methods of whole cell screening are necessary. These include "smart screening" using multiple-resistant "superbugs" [13], tests that allow the in vivo measurement of the inhibition of a select, partially silenced target with respect to wild type [14] and genetically modified cells that produce specific responses upon inhibition of a select function [15].

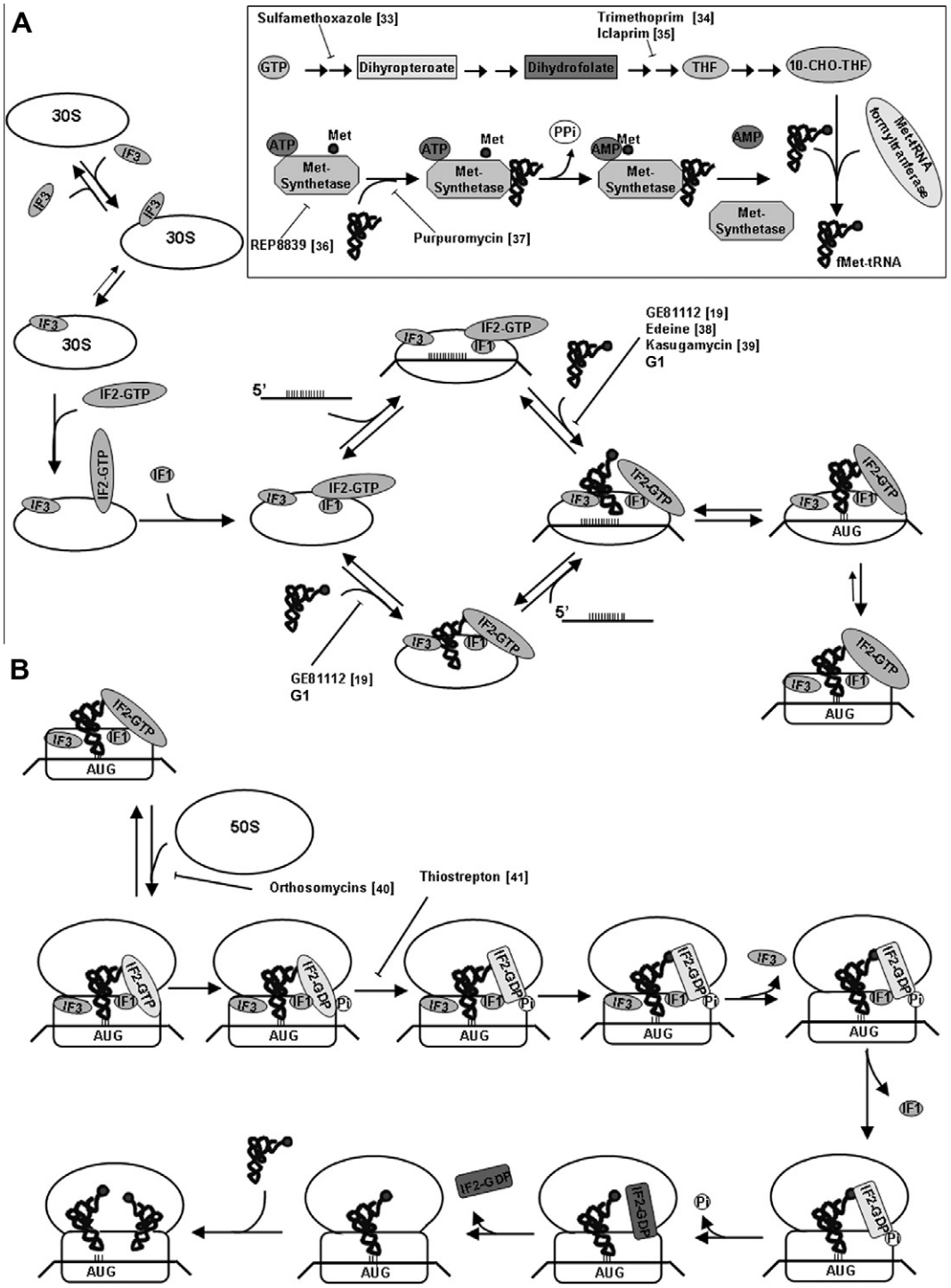

Fig. 1. Translation initiation pathway. (A) 30S initiation complex formation and (B) 70S initiation complex formation. The pathway of the initiator fMet-tRNA formation is presented in the inset. The established/suggested sites of action of the inhibitors are indicated with the corresponding references. (See above-mentioned references for further information.)

When using whole cell screening, it should be considered that large differences in sensitivity may exist among different bacteria. In general, Gram-negative bacteria are much more difficult targets for antibiotics than Gram positives, insofar as their outer membrane is more difficult to be penetrated and because multiple kinds of antibiotics are easily expelled by the efflux pumps which are major non-plasmid-encoded contributors to multidrug resistance. In fact, it has been shown that the screening of an unbiased library produces 10–100 times more hits against *Staphylococcus aureus* than against *Escherichia coli* and even more compared to non-fermenters such as *Pseudomonas aeruginosa*. However, different sensitivity to antibiotics is not restricted to differences between Gram positive and Gram negative bacteria, but is also observed among different species of the same genus or among different strains of the same species, which may have substantial variations in their genome. In this connection, it seems advisable to perform the screening on clinical isolates of interest rather than on model laboratory-tamed strains [16,17].

Another approach, which can complement whole cell screening, is the in vitro screening of select targets. This approach offers the advantage of identifying inhibitors having the desired biological activity, independent from their possible failure to penetrate the cells. If necessary and worthwhile, cell permeability problems can be bypassed by subsequent chemical modifications, e.g. by rendering the inhibitor more hydrophobic or linking it to a cell-permeable molecule such as a polyamine, a sugar or to another, already validated antibiotic. We are aware that approaches of this type have failed in a number of cases, but feel confident that in other cases, knowledge-based modifications could yield the desired result. In this connection it is perhaps worth mentioning the case of the P-site inhibitor GE81112 [18,19] (see also below). This tetrapeptide antibiotic displays a MIC ~0.1 μg/ml in minimal medium but is completely ineffective in rich medium due to competition by other peptides present in the milieu for the peptide pump OPP which is responsible for its entry in both Gram positive and Gram negative cells. Appropriate chemical modifications introduced in a permissive part of the molecule were found to allow the entry of GE81112 bypassing the OPP system and to reduce its MIC in rich medium from ~500 to ~8 μg/ml without altering either nature of the target or mechanism of action in clinical isolates of *P. aeruginosa*, or affecting the encouraging results of the cell toxicity tests (unpublished results). Although economical difficulties have prevented Vicuron Pharmaceuticals from pursuing further these preliminary trials, the outcome of these experiments left the impression that an additional effort along the aforementioned lines could have yielded the desired result of ensuring the efficient entry of the inhibitor into the cell.

Another approach to favour the entry of recalcitrant inhibitors into the cells could be their complexation with cyclodextrin derivatives. Such complexation is expected to improve also the chemical stability of the inhibitors and to increase both their absorption and bioavailability. The choice of the best type of cyclodextrin to be used for antibiotic complexation could be empirically determined in each case depending upon their performance.

Ideally, in vitro screening of select targets should focus on essential cell functions performed by a complex machinery with a well known 3D structure so that rational design and ligand-based design can be applied to improve the performance of the inhibitor. From this point of view ribosomal subunits, RNA polymerase, aminoacyl-tRNA synthetases and riboswitches would appear to represent ideal targets. Along these lines, a target that seemed to be ideal insofar as being essential and bacteria-specific was the metalloenzyme peptide deformylase (PDF) which removes the *N*-formyl group from the N-terminal formyl methionine from bacterial but not from mitochondrial translational products [20 and references therein]. However, to the best of our knowledge the development of PDF inhibitors has been recently discontinued, likely due to the toxicity of these compounds which may interfere with the activity of eukaryotic metalloenzymes. Obviously, the target-directed approach is expected to see only a limited number of inhibitors directed against a narrow window of biological activities; nevertheless, if the chosen target is an unexploited or underexploited function (like, for instance, translation initiation and termination) the potential advantage of this approach is that of minimizing the rediscovery of known antibiotics and identifying instead novel inhibitors, possibly belonging to new classes of molecules for which no bacterial resistance or cross-resistance has developed. These target-based HTS can be combined with microbiological screenings whereby the in vitro-detected hits are "cherry-picked" and subsequently screened for their antibacterial activity using a panel of representative organisms, or may be applied to test hits "cherry-picked" following a microbiological HTS. Both methods could prove successful. Indeed, in our hands upon screening "only" 25,000 natural extracts the first approach identified two new inhibitors, the aforementioned GE81112 [18,19] and GE82832 [21] while following a microbiological screening of 89 000 extracts the second strategy yielded GE107558 [22]. However, the target-related HTS approach can result in rather frustrating results, as experienced by several big (e.g. Glaxo Smith Kline, Pfizer) and small (e.g. Cubist) pharma after large screening campaigns. In spite of this, we do not share the opinion that the target-related screening approach is scientifically worthless and uneconomical, provided that the targets are carefully selected and validated, that the methods of primary screening are further miniaturized and have the characteristics of being simple, specific, robust, sensitive, inexpensive, reproducible and guarantee reliable results, possibly yielding some key information concerning the inhibition mechanism [23,24]. Another condition to avoid the past failures is that the screening must involve libraries of natural or natural-like compounds.

Finding new antibiotics through the aforementioned strategies is not the only way of replenishing the pharmacy shelves with effective anti-infectives. An alternative, commonly used approach, has been that of resorting to medicinal chemistry to modify antibiotics already approved in therapy with the aim of improving the properties and or bypassing the mechanism of resistance or to "revive" inhibitors discovered in the past but never further developed.

Examples of the first approach are the successful chemical modifications introduced into beta-lactams, tetracyclines, aminoglycosides, etc. while an example of the latter approach is exemplified by the recent, successful case of Daptomycin. This drug, discovered in the 80's, was initially neglected and finally approved by the US FDA in 2003 after being developed by Cubist Pharmaceuticals. As pointed out in a recent article a situation similar to that of Daptomycin may apply also to other molecules such as the macrolactone Difimicin, which inhibits RNA transcription and the cyclic lipodecapetide Friulimicin, which inhibits cell wall synthesis [25].

Another successful approach consists in the chemical modification and improvement of select, small molecules through the so-called fragment-based drug discovery (FBDD). As mentioned above, the failure of HTS campaigns was caused, at least in large part by the use of inadequate chemical libraries which had a too limited chemical diversity and physicochemical properties for antibiotic screening. To overcome these limitations and to increase the hit rates in recent years the FBDD approach has been developed. In this method libraries consisting of small (i.e. <300 Da), moderately lipophilic, highly soluble, molecules are screened. Such molecules are more likely to bind to select targets than larger molecules and are prone to be further developed. Biochemical assays, X-ray crystallography, NMR spectroscopy, mass spectrometry, surface plasmon resonance can be used for the detection of active fragments which can be later optimized for potency (increasing the binding affinity usually by structure-guided modifications) and for drug-like properties. The potentiality of FBDD is proven by the entry in clinical trials of several leads active against different targets, especially cancer targets, obtained using this approach. In antibiotic discovery, the FBDD strategy is producing interesting results such as the amino-oxazole inhibiting Gram negative biotin carboxylase [26] and the inhibitors of protein tyrosine phosphatase PtpA or antigen 85C of *Mycobacterium tuberculosis* [27,28].

Another winning approach could be that of rationally re-designing existing antibiotics or designing novel antibiotic scaffolds exploiting the already available or prospective structural data derived from crystallographic studies of essential biological macromolecules and/or macromolecular complexes such as riboswitches and ribosomes in combination with predictive computational chemistry. A successful approach along these lines is that used by Rib-X with Radezolid (RX-1741), an oxazolidinone antibiotic (in phase 2 clinical trial) that exhibits activity against methicil-

lin-resistant *S. aureus* (MRSA) and other Gram-positive organisms, displaying properties superior (wider spectrum and stronger potency) to those of Zyvox® (linezolid). Radezolid was knowledge-base developed exploiting the atomic 3D structure of the 50S ribosomal subunit.

Another promising approach followed by scientists at Rib-X and elsewhere is the generation of chimeric antibiotics. Thus, a chimeric molecule composed of elements of two cyclic peptide antibiotics, the virginiamycin Streptogramin B, a 50S ribosomal subunit inhibitor, and tyrocidine, a compound that destroys the cell membrane was developed. The resulting chimeric antibiotic proved to be able to bypass two of the known resistance mechanisms against Streptogramin B, namely the lyase Vgb-catalyzed hydrolysis and target modification by Erm-dependent 23S rRNA methylation [29]. Other interesting chimerae are the "AU-FQ", hybrid molecules between anilinouracils which inhibit the Gram-positive-specific DNA polymerase pol IIIC and the gyrase inhibitors fluoroquinolones which are at least 15 times more active than the corresponding isolated compounds and proved to be active against both AU- and FQ-resistant Gram-positive bacteria [30].

As mentioned above, widespread antibiotic resistance against all known classes of natural and synthetic compounds is one of

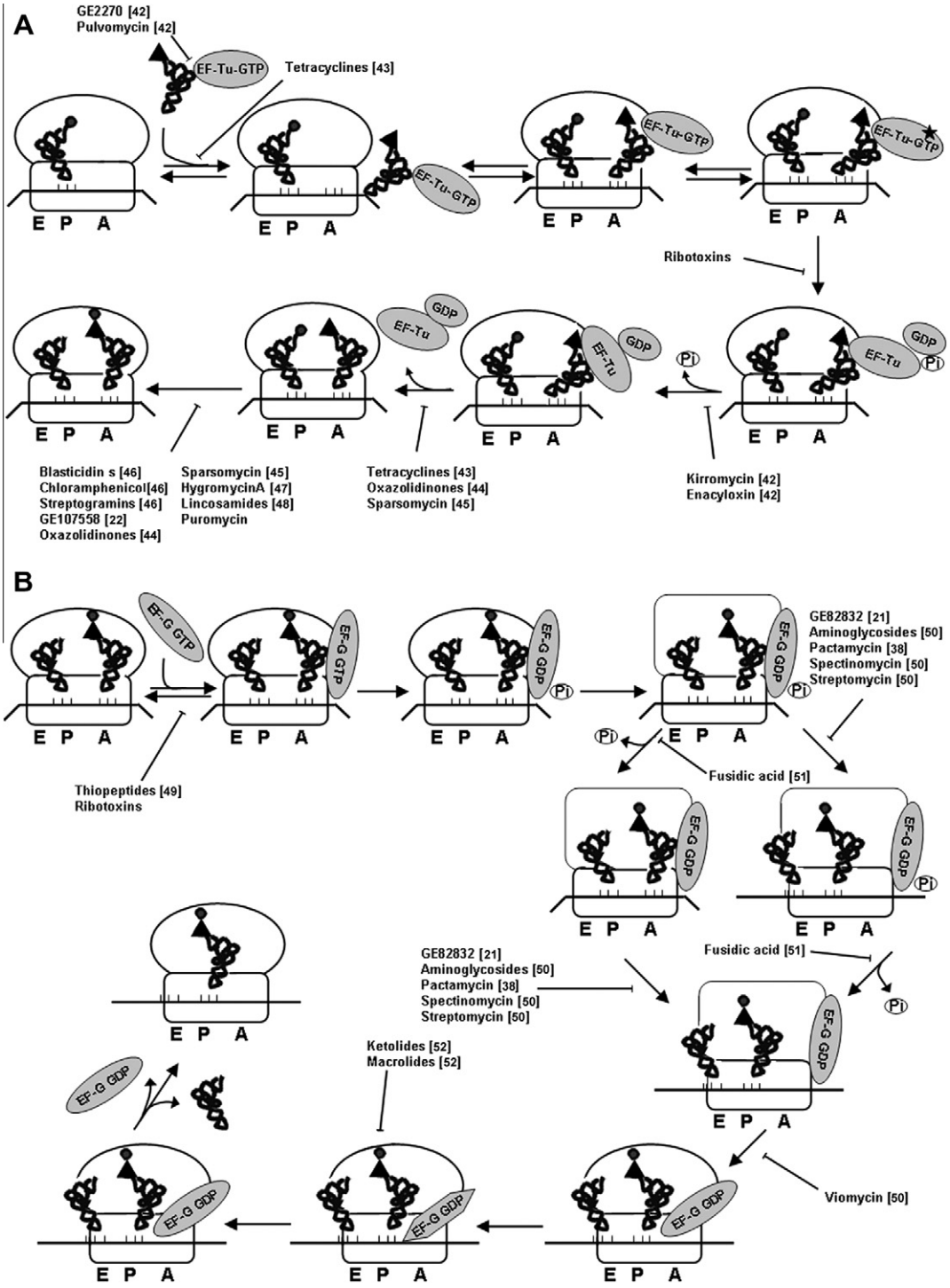

Fig. 2. Translation elongation pathway. (A) Steps involved in the peptide bond formation and (B) EF-G dependent translocation. The established/suggested sites of action of the inhibitors are indicated with the corresponding references. (See above-mentioned references for further information.)

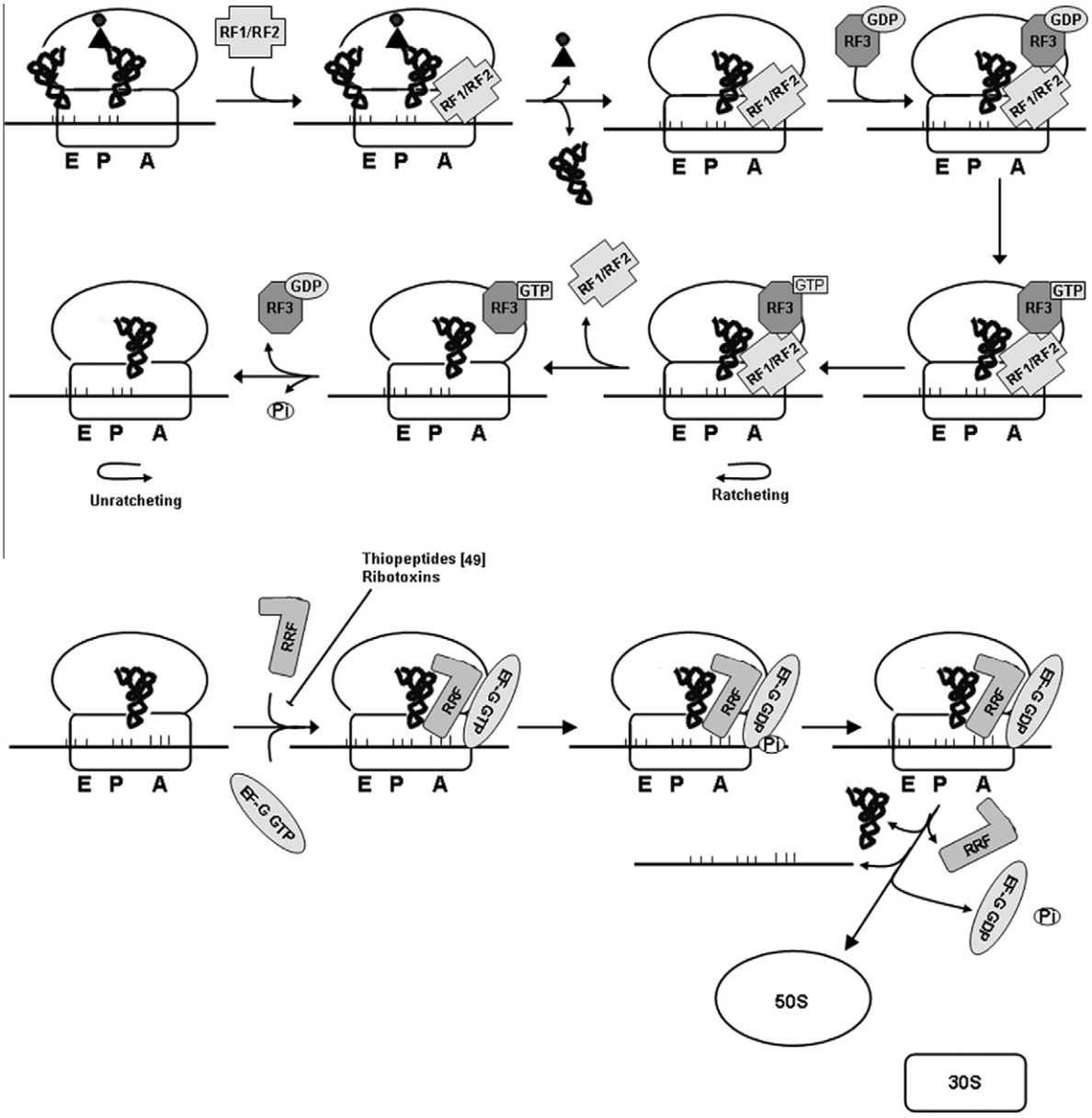

Fig. 3. Translation termination and ribosome recycling pathway. The established/suggested sites of action of the inhibitors are indicated with the corresponding references. (See above-mentioned references for further information.)

the most serious threats to human health. Preventing its occurrence is impossible in light of the high density of resistance genes in the environment, of facile horizontal transfer of these genes and by the adaptive value that they confer upon the bacteria [31]. Aside from using antibiotics more responsibly and trying to exploit the severe loss of fitness that antibiotic resistance may cause [32], the spread and the emergence of resistant bacteria and the consequences of the resistance can be mitigated by the development and use of entirely new antibiotics or of modified versions of antibiotics whose efficacy has been substantially reduced by the emergence of resistance. Some of these approaches have been mentioned above. To cope with vancomycin resistance, one of the most serious threats, several novel second-generation lipoglycopeptides that inhibit bacterial cell wall synthesis have been developed. Among these, the most promising appear to be: (a) Teicoplanin (Targocid), an antibiotic produced by *Actinoplanes teichomyceticus* with an activity spectrum similar to that of vancomycin, being active against Gram-positive bacteria, including MRSA and *Enterococcus faecalis*; (b) Telavancin, a synthetic vancomycin derivative which depolarizes and disrupts bacterial membranes. This antibiotic has been approved by the FDA for treatment of complicated skin and skin-structure infections (cSSSI) caused by susceptible Gram-positive bacteria, including MRSA and methicillin-susceptible (MSSA) strains of *S. aureus*; (c) Dalbavancin (Zeven), initially developed at Biosearch Italia (later Vicuron Pharmaceuticals), which is active against MRSA and a variety of other Gram-positive pathogens; (d) Oritavancin (also known as LY333328) a semi-synthetic glycopeptide with a vancomycin-similar structure which was developed for the treatment of serious Gram-positive infections. Both Dalbavancin and Oritavancin await more convincing results from phase 3 clinical trials before approval.

Antibiotics can be classified according to different criteria such as their economic impact, origin, chemical nature, spectrum of action, therapeutic application or nature of target, the latter being the criterion that we use. By far the main antibiotic target is the

Table 1
Select list of underexploited antibiotic targets and some of their inhibitors.

Targets		Antibiotic	Comments	Ref.
Aminoacyl-tRNA synthetase	Leu-tRNA synthetase (LeuRS)	AN3365	Boron-based new antibiotic; developed by Anacor; new mechanism of action; binds stably LeuRS editing site forming adduct with ribose A76; successfully completed Phase 1 clinical trials; active against AR opportunistic Gr-neg pathogens, including *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *P. vulgaris*, *S. marcescens*, *Providentia* spp, *Enterobacter* spp and *Citrobacter* spp	[a]
	Phe-tRNA synthetase (PheRS)	Benzyl Phenyl Ethers (BPEs)	Inhibits PheRS of *H. influenzae* and *S. pneumoniae*	[56]
	Met-tRNA synthetase (MetRS)	REP8839 (derivative REP3123)	Diaryldyamine; binds at or near Met binding site and inhibits MetRS of MRSA and *S. Pyogenes*; less active on Gr-neg bacteria; not active on mice liver MetRS	[36]
Membranes	LpxC [UDP-3-*O*-(*R*-3 hydroxymyristoyl)-*N*-Ac-glucosamine deacetylase]	CHIR-090	Catalyzes the committed step of lipid A biosynthesis; active against broad range Gr-neg bacteria; may be developed to control *Pseudomonas aeruginosa* infections	[57]
	LptD	POL7080	Induces perturbation of a crucial LPS transport function of LptD in *P. aeruginosa*	[58]
	Membrane integrity	XF-73	Depolarizes and permeabilizes membranes, disrupting multiple cellular process; no cross-resistance in Daptomycin-resistant strains	[59]
	Membrane-bound ATPase (AtpE subunit)	TMC207 (R207910)	Specific against *M. tuberculosis*	[60]
Gyrase DNA topology control	GyrA	GSK299423	Mechanism of inhibition distinct from that of fluoroquinolones and novobiocin; bactericidal against a variety of MDR bacteria, including MRSA and quinolone-resistant pathogens	[61]
	GyrB	QPT-1	Synthetic barbituric acid derivative; mechanism of inhibition distinct from fluoroquinolones and novobiocin; active against a broad spectrum of pathogenic AR bacteria	[62]
Transcription	RNA polymerase (RNAP)	Lipiarmycin (Difimicin, Fidaxomicin, OPT-80, PAR-101)	Developed by Optimer Pharmaceuticals; new class of macrocycles from *Actinoplanes deccanensis*; inhibits open-complex formation preventing ssDNA loading at the active-site cleft of RNAP; bactericidal against *C. difficile*	[63]
	RNA polymerase (RNAP)	Myxopyronin	α-Pyrone produced by *Myxococcus fulvus*; new class of RNAP inhibitors; binds to switch 2 region of RNAP β-subunit, away from rifampicin; possible treatment of tuberculosis	[64]
Fatty acid (FA) synthesis	Fab I (enoyl-ACP reductase) essential for FA biosynthesis	AFN-1252 (API-1252)	Currently in Phase 1 trial; very limited activity spectrum, specific against MSSA and MRSA but not other Gr-pos or Gr-neg bacteria; inhibition may be bypassed in presence of exogenous fatty acids	[65]
	FabF (β-ketoacyl-(acyl-carrier-protein (ACP)) synthase I/II (FabF/B) Involved in cell membranes biosynthesis	Platensimycin	New class natural product of *Streptomyces platensis* containing a pentacyclic motif with a cyclic ether ring; developed by Merck; platensimycin and platencin have a 3-amino-2,4-dihydroxy benzoic acid unit in common and different ketolide portions; blocks enzymes involved in condensation steps in fatty acid biosynthesis; active against Gr-pos, including MRSA, VAR *Enterococci* and linezolid- and macrolide-resistant pathogens. Inhibition may be bypassed in presence of exogenous fatty acids	[66]
	FabF/FabH	Platencin	Differs from platensimycin for presence of a tetracyclic motif and absence of ether ring; targets both FabH and FabF; broad-spectrum Gr-pos bacteria; more active than Platensimycin against VAR *E. faecium* and efflux-negative *E. coli* (*tolC*), less active against *S. pneumoniae*	[67]
	Biotin carboxylase (BC) (subunit of the multisubunit enzyme acetyl-CoA carboxylase)	Pyrido-pyrimidines	Active against Gr-neg pathogens such as *H. influenzae* and *M. catarrhalis*	[68]
Tetrahydrofolate (THF) biosynthesis	ADC synthase/ADC lyase	Abyssomicin C	Complex polyketide-type antibiotic from a *Verrucosispora* strain; inhibits synthesis of *p*-aminobenzoate in THF biosynthetic pathway	[69]
Cell division	Ftsz	PC190723	Small synthetic molecule; inhibits FtsZ and prevents cell division; bactericidal against MRSA and MDRSA; can cure mice infected with a lethal dose of S. aureus	[70]
Quorum sensing dependent virulence	QseC (His kinase sensor of host and bacterial signaling molecules activating virulence cascade)	LED 209	Synthetic benzenesulphonamide derivative; given before infection inhibits virulence, not growth of enterohemorrhagic *E. coli*, *S. typhimurium* and *F. tularensis*	[71]
Protein quality control	ClpP (caseinolytic peptidase P)	Acyldepsipeptides (ADEPs)	Targets catalytic core of peptidase caseinolytic protease P (ClpP) causing a complete functional reprogramming of the Clp–protease complex affecting ClpP-dependent general and regulatory proteolysis; bactericidal activity against MDR Gr-pos bacteria *S. aureus*, *S. pneumoniae* and *Enterobacteriaceae*	[72]
Purine biosynthesis and transport	Guanine riboswitch	PC1	Novel antimicrobial; crystal structure-based rationally designed pyrimidine compound; binds guanine riboswitch in guanine-starved cells; bactericidal against a subgroup of bacterial species including S. aureus and C. difficile	[73]

Abbreviations: Gr-neg = Gram negative; Gr-pos = Gram positive; AR = antibiotic resistant; MDR = multi drug resistant; MRSA = methicillin resistant *Staphylococcus aureus*; MSSA = methicillin sensitive *Staphylococcus aureus*; MDRSA = multi drug resistant *Staphylococcus aureus*; VAR = vancomycin resistant; cSSSI = complicated skin and skin structure infection.

[a] Information available in http://www.anacor.com/pdf/ICAAC2010F1-1637.pdf; http://www.jmilabs.com/data/posters/ICAAC2010/F1-1639.pdf.

translational apparatus, with approximately half of all known inhibitors being directed against its function; here below we present a graphic summary of the sites and/or steps within the initiation (Fig. 1), elongation (Fig. 2) and termination (Fig. 3) pathways of protein synthesis affected by these antibiotics. As seen from these figures, some specific molecular structures or translational steps such as the A-site decoding region of the 30S subunit and the peptidyltransferase center or the adjacent peptide exit tunnel of the 50S subunit, are much more frequently targeted by the inhibitors than others so that at least some of them (e.g. aminoacylation and translation initiation) can be regarded as being underexploited and offer the opportunity of finding new inhibitors for which no resistance has yet been developed. This seems to be the case, for instance for the Class I thiopeptide compounds (i.e. PA–PD series, Thiazomycin and Philipimycin) which target a different region of the ribosome, namely the GTPase-associated region or translation factor binding site, where they interact with both rRNA and ribosomal protein L11 [53–55]. Among these antibiotics are the four inhibitors which are presently under investigation in our laboratory, namely G1 (unpublished results), GE81112 [18,19] (Fig. 1) and GE82832 [21] and GE107558 [22] (Fig. 2).

Aside from translation, a select list of unexploited or underexploited targets is presented in Table 1 along with a non-exhaustive list of antibiotics which interfere with them and are, therefore, in our opinion, of particular interest insofar as they may represent good candidates for the development of new anti-infectives, either as such or upon appropriate structural modifications.

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