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Antibacterial drug discovery—Then, now and the genomics future

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ABSTRACT

Drug discovery research in the area of infectious diseases, in particular that dealing with antibacterial/antibiotic susceptibility and resistance, is in a process of continuing evolution. Steeped in the history of the highly successful intervention with chemotherapeutic agents to treat human infections, the emergence of drug-resistant pathogens worldwide presents a serious unmet medical need, if not a pending catastrophe. Research in both academia and industry over the past 30 years using molecular biology, genetics and more recently – bacterial genomics – has assembled key enabling technologies to increase productivity and success rates in the discovery and development of novel antibacterial agents. However genomics is not limited only to antibacterial target selection but provides the opportunity to further understand key interactions in the use of antibacterial compounds as therapeutic agents (such as resistance emergence, susceptibility, efflux, interactions between compound and pathogen, etc.). Genomics also offers the potential for insights into: bacterial niche adaptation, host susceptibility, treatment regimens, antibiotic resistance, pharmacokinetics (e.g., host metabolism differences), safety and the microbial genesis of chronic diseases (e.g., gastric ulceration).

1. Introduction

The history of antibiotic/antibacterial discovery has been a mix of crude 'live-bug, dead-bug' screening for naturally occurring inhibitors of bacterial growth, selective inhibition of bacterial biochemical targets, ''me-too'' improvements on existing chemical scaffolds primarily found in nature in the 1950s and 1960s, and a decade-long attempt to use sophisticated genomics tools to improve the speed, frequency and quality of antibacterial/antibiotic leads. From the rudimentary tool sets of broad-based screening of natural product extracts and colonies from terrestrial, marine and plant life – to the in silico selection of targets and virtual screening – the industry has sought the same end-products – new chemical entities (NCEs) originating from either natural product screening or compounds from library screening – that can reduce human and animal morbidity and mortality by killing the pathogenic bacteria that infect humans with a minimum of side effects.

To ensure success, the existing 'best' practices in antibacterial drug discovery research must be maintained while incorporating proven new technologies rather than replacing or superceding the former. Thus an optimal genomic-based approach requires the complementary tools of microbiology, biochemistry microbial physiology, genetics and basic molecular biology to discover novel NCEs with genomics complementing the existing approaches that have led to much of the science presented in this special issue of *Biochemical Pharmacology on Antibacterial Drug Discovery*.

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Table 1 – Recently approved antibacterials

Drug name	Brand name	Class	Mechanism-of-action	Company
Tigecycline	Tigacil®	Tetracycline	Bind to 30S ribosomal subunit, block entry of charged tRNAs to the ribosomal A site	Wyeth
Telithromycin	Ketek®	Ketolide	Bind to 23S ribosomal RNA of the 50S subunit, result in premature release of nascent peptide	Aventis
Daptomycin	Cubicin®	Lipopeptide	Alteration of bacterial membrane Potential	Cubist
Gemifloxacin	Factive®	Quinolone	Inhibit DNA gyrase and topoisomerase IV	Oscient
Linezolid	Zyvox®	Oxazolidinone	Bind to 23S ribosomal RNA of the 50S subunit, inhibit translation	Pfizer
Quinupristin/Dalfopristin	Synercid®	Streptogramin	Inhibit peptide chain elongation and peptidyl transferase	Aventis/King Pharmaceuticals
Moxifloxacin	Avelox®	Quinolone	Inhibit DNA gyrase and topoisomerase IV	Bayer
Gatifloxacin	Tequin®	Quinolone	Inhibit DNA gyrase and topoisomerase IV	Bristol-Myers

2. Medical need and commercial opportunity

More than one-third of the world population is likely infected by bacterial pathogens. Two million fatalities occur per year from bacterial infections [1]. New infections caused by drug-resistant strains of *Mycobacterium tuberculosis* range from 0 to 57% (with a median of 10.2%). New infections caused by multi-drug resistant (MDR) forms of *M. tuberculosis* range from 0 to 14.2%, (median 1.1%). Patients undergoing re-treatment harbor MDR strains at a 7% median incidence [2]. Yet the existing pipeline of anti-*M. tuberculosis* agents is weak.

The rapidly evolving resistance development by *Staphylococcus aureus* has the potential to re-create a pre-antibiotic world with mortality rates of 80% for those patients systemically infected [3]. *M. tuberculosis and S. aureus* are two dramatic examples that bacterial pathogens will exist and evolve as long as their host is viable.

Urinary tract infections (UTIs) result in approximately 8 MM annual cases in the United States with an annual cost of US$ 1.6 bn [4,5]. Approximately 20% of these are recurrent UTIs, pointing to an opportunity where genomic information on the genes expressed by the pathogen during the infection could lead to rational targets to both cure the primary disease and prevent recurrence [5]. This special issue details both the unmet medical need and an expansion of the issue of worldwide resistance emergence.

The research that led to the initial golden age of antibiotic drug discovery was stimulated in no small part, by the global conflicts of two World Wars [6]. The current bioterrorist campaigns may thus be anticipated to stimulate the next productive age of antibiotics discovery. Thus the CDC has identified 37 bacterial agents and toxins listed as viable terrorist threats [7]. The theoretical possibility of humans to engineer these agents to enhance pathogen survival, increase lethality and optimize distribution is worrisome and no longer reviewed as remotely possible since the US-based anthrax spore release in 2001. This, along with the isolation of a vancomycin, beta-lactam, macrolide, tetracycline, aminoglycoside and quinolone-resistant strains of *S. aureus* provides an overt message that a continual supply of structurally novel antibacterials with multiple modes of action need to be part of the antibacterial armamentarium. Over the past decade less than a dozen novel antibacterials with significant impact in addressing resistance have emerged, primarily from big pharma (Table 1), but the new wave of agents intent on addressing resistance emergence are mainly from the biotech arena (Table 2).

Table 2 – Biotech antibacterials in clinical development

Drug name/designation	Delivery route/class	Target	Status
Iclaprim (Arpida/Roche)	IV/PO-Diaminopyridine	DHFR inhibitor	Phase III
Faropenem (Replidyne/Suntory)	PO-Penem	Cell wall	Phase III (US)
Tebipenem (Meiji Seika Kaishai/Wyeth)	PO-Carbapenem	Cell wall	Phase II
EP-013420 (Enanta/Shionigi)	PO-Ketolide	Protein synthesis inhibitor	Phase I
PPI-0903M (TAK-599) (Takeda/Peninsula)	IV-Cephalosporin	Membrane disruption	Phase II
MBI 594AN (Migenix)	Topical indolicidin peptide	Membrance disruption	Phase II
Doripenem(J&J/Pennisula Pharma/Shionogi)	IV-Carbapenem	Cell wall	Phase III
RWJ-333441 (Essential Therapeutics/J&J)	IV-Cephalosporin	Cell wall; transpeptidation	Phase I
VRC-4887 (LBM415) (Vicuron/Novartis)	PO-Hydroxamate	Peptide deformylase	Phase I
Ramoplanin (Oscient/Vicuron)	PO-Glycolipo-depsipeptide	Transglycosylation/Lipid II	Phase II/III
Oritavancin (LY333328) (InterMune/Lilly)	IV-Glycopeptide	Cell wall	Phase III
Rifalazil (Activbiotics)	IV/PO-4-Aminobenz-Phase II oxazine		RNA polymerase
BAL-5788 (Ceftobiprole) (J&J/Basilea)	IV-Cephalosporin	Cell wall	Phase III
Dalbavancin (Vicuron/Aventis)	IV-Glycopeptide	Cell wall	Phase III; NDA filed
TD-6424 (Theravance)	IV-Glycopeptide	Cell wall	Phase III
PTK-0796 (Paratek)	Tetracycline	Protein synthesis	Phase I

3. Genomics tools and the potential for change

The genomics paradigm has the promise of providing novel antibacterial targets [8] with the additional possibility of improved safety of drugs based on these targets due to genomic differences between host and pathogen. Genomics also has the potential to identify effective antibacterials against latent microbial forms and can also establish the linkage between persistent microbial forms and chronic disease. For example, is *Mycoplasma* involved in rheumatoid arthritis [9], *Chlamydia* associated with heart disease [10] and are other bacteria involved in the persistent inflammation states that lead to cancer [11]? The 2005 Nobel Prize in medicine was awarded for the discovery that most ulcers were caused by chronic *Helicobacter pylori* infection, a viewpoint that was, in its time, dismissed by scientists in the GI area [12].

Genomics can also detect and characterize pathogen reservoirs and pathogen responses to the environment both before and after, infection [13]. For example, transcriptional arrays can define the response by *S. aureus* to different pHs, enhancing the understanding of consequences of differences in pHs at various staphylococcal infection sites [14]. Similarly, transcription arrays in *S. aureus* biofilms (a model for catheter infection sites [15]) have provided insights into the genes involved in bacterial survival and infection in this infectious niche. A similar study on *E. coli* during UTI [16] has identified enhanced virulence factors and may help define infection-relevant targets for this high morbidity disease. Betts et al. [17] have established and genetically characterized a starvation model for persistent forms of *M. tuberculosis* that are often resistant to the antibiotics that are effective against the rapidly growing pathogen. Proteome, metabolome and transcriptome studies have been used to determine that pulsed treatment of *Streptococcus pneumoniae* with amoxicillin resulted in both autolysin over-expression and increased bactericidal activity only when the dose is near the MIC [18].

4. Scientific approaches to antibiotic discovery—in the beginning

Successful screening strategies for novel antibacterials have followed a paradigm of testing microbial broths for the presence of agents that kill bacteria and that also cured infections in animal models without acting as rodenticides. These screens evolved into searches for NCEs or fermentation broths that globally targeted bacterial cell wall or protein synthesis targets in whole cell assays. The source of active compounds mimicked the successes of the Waksman group in discovering streptomycin and other novel natural product antimicrobials from soil actinomycetes [19]. Actinomycetes dominated the search and discovery of new agents as the consensus became that fungal antimicrobials were often found to be toxins produced in relatively large titers.

Success in the area of synthetic NCEs evolved from the discovery of the sulfonamides and the determination of their mechanism of action in targeting folate biosynthesis and metabolism. A combination of sulfamethoxazole, a *para*-aminobenzoic acid mimic active against the first step in tetrahydrofolate synthesis with trimethoprim, a dihydrofolate reductase inhibitor, together effectively blocked formylation of methionine. N-Formyl methionine activates the tRNA required for prokaryotic protein synthesis. The combination of sulfamethoxazole and trimethoprim is synergistic and less likely to elicit-resistant bacterial strains than either agent used alone [20].

5. Scientific approaches to antibiotic discovery—current

Based upon discrete mechanism of action studies with successful antibacterial agents, the traditional screening paradigm has evolved away from whole cell assays measuring microbial killing to one that relies upon the identification of, and isolation of, antimicrobial targets that can be used in target-based high-throughput screening (HTS) mode [21,22]. This approach has generally not been as successful as anticipated for two reasons: (1) access to the target through the bacterial cell wall/membrane may be limited and (2) pre-existing efflux may limit target accessibility. In general, chemical libraries have been based on Lipinski's 'Rule of 5' [23], these rules having been generated from successful drugs in areas other than infectious disease. Such NCEs were highly effective in vitro against isolated pathogenic targets but lacked MIC efficacy in whole cell pathogen assays [24,25], an indication that the NCEs, cannot access their target. These NCEs may also be substrates for efflux.

Natural product screening was thus de-emphasized since it was not amenable to HTS protocols. Fermentation broths interfered with the efficient operation of in vitro screens by producing agents as mixtures that were considered too difficult to assay and by repeatedly generating uninteresting knowns [26]. On the other hand, the chemical space occupied by natural products selected by nature to be biologically active, particularly against competing microbes, was frequently different from the chemical space occupied by most chemical collections that followed Lipinski's rules [27,28].

The lack of truly novel NCEs emerging from antimicobial drug discovery efforts overlooks two facts: (i) highly effective agents have presumably provided competitive advantages for the microbes that produce them, resulting in the producers becoming more widespread. Therefore, by traditionally screening in a manner where the best agents are on an average the most common, they, not surprisingly, have repeatedly been re-discovered. Ecopia BioSciences has successfully exploited genomics to discover NCEs by analyzing microbial isolates for genetic sequences that predict novel structures and then using this information to find conditions for their production [29]; (ii) microbes often make antimicrobial mixtures [30], containing antibacterials, antifungals, antiparasitics and other microbe-inhibitory agents and toxins, an example of nature indicating that mixtures are more effective than single NCEs and that the pursuit of NCEs active at multiple targets may be a more effective strategy by which to identify novel antibacterials. This is consistent with the hypothesis that resistant strains are less likely to occur when the multiple agents hitting multiple targets are used [31]. Known natural products with unexpected new activities

include the activity of nisin and moenomycin against *E. coli* PBP-1b transpeptidase reinforcing the idea that antibacterials with activity against more than one target can offer a competitive advantage [32]. The majority of known antibiotic classes interact with multiple targets [33], although this was not a selection design/criteria when these agents were initially developed. The use of antibiotics that interact with single targets, e.g., rifampicin (RNA polymerase) often leads to the rapid development of resistance.

Most organisms found in the environment cannot easily be grown [34], nor can all of the NCEs genetically predicted be produced. Transfecting DNA from soil to an industrialized strain may allow these new NCEs to be made [35]. Sequencing of actinomycete strains has revealed the existence of multiple clustered secondary metabolite genes [36,37]. By determining the growth conditions for these cultures using genomic analysis, and then establishing the conditions required to induce expression of their full biosynthetic potential is a necessary approach to natural product drug discovery.

As first generation antibacterial products based on diverse chemical platforms were discovered, the subsequent challenge was to improve stability, pharmacokinetics and the spectrum of efficacy. With the development of resistance to these first generation drugs, there was an additional challenge to identify analogs effective against resistant strains. The results have been outstanding in terms of second and third generation cephalosporins, second generation carbapenems, second generation macrolides, a new generation of cyclines and second generation glycopeptides.

Second generation antibacterial targeting bacteria in a complementary manner by interfering with the *N*-formyl methionine step of protein synthesis are being developed. These are based upon inhibition of peptide deformylase [38] using the natural product, actinonin as a starting point [39]. Inhibition of peptide deformylase not only inhibits peptide deformylation [40], but also enhances the host response by increasing the release of neutrophil activating peptides [41].

HTS screening of chemical libraries for activity against whole cells repetitively selects compounds with detergent-like characteristics, or NCEs that are toxic to mammalian cells, or whose activity is reversed by serum. A notable exception to this are the novel anti-*M. tuberculosis* compounds that were found by screening for inhibition of *M. smegmatis* growth leading to the discovery of 20 potent diarylquinolines, 3 of which have in vivo efficacy [42]. Sequencing of three independently generated laboratory-resistant mutants established that all three had point mutations in *atp*E (coding for part of ATP synthase). The lead compound, R207910 (Fig. 1) inhibits the *Mycobacterium* ATP synthase proton pump. Based on clinical experience this may be the first novel *M. tuberculosis*-specific drug in 40 years.

The need for agents effective against antibiotic-resistant pathogens led many biotech/small pharma companies to re-investigate previously identified NCEs that had failed early tests for various side effect and pharmacokinetic reasons. Among these were daptomycin (Cubist), Telavancin (Theravance) and ramoplanin (Oscient) [43–45].

An unusual natural product success involved the combination of the early generation β-lactams with the β-lactamase inhibitor, clavulanic acid produced by *Streptomyces clavigerus*

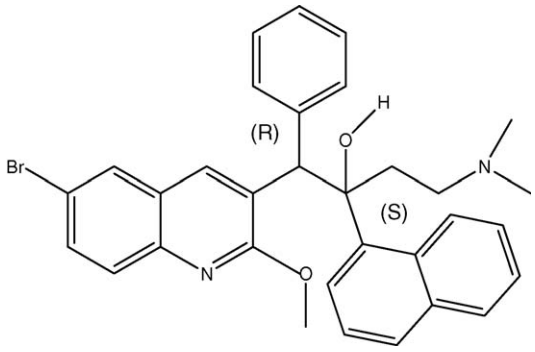

Fig. 1 – Structure of R207910.

[46]. This led to the commercially successful Augmentin®, a combination of amoxicillin and clavulanate providing a therapeutic reprieve for the highly effective and clinically safe early β-lactams.

6. Genomics and the future

A general misperception is that genomics is exclusively focused upon discovering novel targets and building technology platforms to support these efforts [47]. But 'genomics' has a broader utility in antibacterial drug discovery as outlined below:

6.1. *Genomics future—target selection*

Comparison of pathogen gene sequences with human have allowed for the selection and validation of targets including *ileS* and *fabI* as viable targets for antisense technology directed towards the genes resulting in target sensitization to mupirocin and triclosan, respectively [48].

Genomics has also been used to build strains using target or stress promoters linked to reporters for the detection of agents active against the target [49–51]. Antibacterial antisense sequences [52] may be useful as therapeutics, as tools to identify other antibacterials [48] or to determine the MOA of an NCE [53].

The one gene-one target concept while heuristically satisfying can be misleading. Transcription profiling has demonstrated that microbes respond to chemical challenges and ecological signals with global alterations in gene transcription [54,55], presumably resulting in altered sensitivities to the challenging agents, adapting to the ecological challenge. Streptomycin treatment of *Yersinia pestis* increased transcription of 144 genes and decreased transcription of 201 genes [56]. A new antibiotic, furanone induced 92 *Bacillus subtilis* genes and repressed 15 genes [57].

Global genome responses to protein synthesis inhibitors have also been reported [58] that will make the establishment of screening protocols for selected targets more complicated than originally envisioned. As cells are sensitized [59] it would be anticipated that their genomic profile would change in response to the challenge as would the proteomic fingerprint, e.g., *Bacillus subtilis* response to antibiotic action [60]. Global transcription patterns can be a signature for the mode of action for new translation inhibitors [54]. Multi-copy gene

suppression of antibiotic action has recently been used to identify two presumptive dihydrofolate reductase actives and NCEs, the activities of which were diminished when efflux genes were overexpressed [61].

Stress-related transcriptional changes suggest that operon regulators, e.g., *agr* and *sar*A may be responsible in part for virulence expression [62]. The ESX-1 locus may be a more specific virulence target [63]. The dramatic and unexpectedly high levels of transcription increases in the purine salvage pathway by all four translation inhibitors: puromycin, chloramphenicol, tetracycline and erythromycin [54], and the similarly striking elevation of methionine sulfoxide reductase (*msr*A) transcription by the cell wall inhibitors oxacillin, cycloserine and bacillin [64] suggest that genes linked to an appropriate reporter could act as a global indicator of compounds active in each of these target areas.

6.2. Genomics future—additivity, synergy and antagonism

HIV resistance development and *M. tuberculosis* multi-drug-resistant strains have necessarily led to a cocktail approach for drug therapy [65]. As antibiotic resistance spreads to community acquired pathogenic strains, the multi-drug cocktail approach will probably become more necessary unless safe, new antibacterial drugs can be found and applied at a mutant prevention concentration [66].

Acer [67] has suggested that synergistic combinations of antibiotics have generally been developed in response to difficult therapeutic challenges and that the time has come to apply current knowledge of resistance and synergistic mechanisms to develop viable commercial combinations. Two successful synergistic combinations are the β-lactam with a β-lactamase inhibitor that attacks a resistance determinant (β-lactamase) and the newer semi-synthetic natural product combination of the synergistic streptogramins, quinupristin and dalfopristin, introduced in the late 1990s for the treatment of MRSA [31].

Agents that inhibit antibiotic efflux in combination with an otherwise effective agent that is eliminated from the cell may also lead to synergistic combinations [31]. The potential for this type of synergy includes increased sensitivity to antimicrobials in *Pseudomonas* strains mutated to eliminate efflux genes [68]. Extensive work by Microcide and Daiichi have provided proof-of-concept that efflux can be reversed in clinically relevant *P. aeruginosa* strains of bacteria by the addition of an efflux inhibitor [69]. Johnson & Johnson researchers have reported that an agent that targets virulence can be synergistic with ciprofloxacin in a *Pseudomonas* lung infection model [70]. GG918, an inhibitor of tumor drug resistance, can also enhance antibiotic action in *S. aureus* presumably by inhibiting drug efflux [71].

Cationic peptides are widely occurring natural products that function as broad spectrum antimicrobials. These may represent attractive therapeutic agents in part because they are synergistic with traditional antibiotics [72] and also because they can modulate lipopolysaccharide- (LPS) and lipoteichoic acid- (LTA) induced sepsis [73]. Responses to cationic peptides are complex. For example, the cecropin-melittin hybrid peptide, CEMA used in conjunction with LPS-treated macrophages inhibited LPS-induced expression of 36 genes and on its own induced the expression of 35 other genes [74]. Thus in addition to their basic antimicrobial activity, cationic peptides can interfere with LPS binding and modulate the cellular genomic response to LPS. Another group of cationic peptides, the defensins are potent protein kinase inhibitors and would be expected to influence cell signaling cascades [75].

Physicians have empirically combined cell wall antibiotics with other agents in the treatment of difficult infections. Genome-based array technologies may however provide the opportunity to rationally select synergistic combinations based upon an understanding of the bacterial response to an antibiotic challenge. For example, the cell wall stimulon reported by Utaida et al. [55] points to a set of 105 genes up-regulated when the cell is challenged with the cell wall active antibiotics oxacillin, cycloserine or bacillin. Agents that inhibit two key up-regulated genes are one likely place to look for synergistic combinations. In this cell wall stress stimulon case the down-regulation of genes similar to the autolysis genes (*atl* and SA0423) and up-regulation of the autolysis regulators, *fmt* and *lytR* points to an inhibitor of autolysis regulation as a possible synergistic opportunity with cell wall active agents. Similarly, down-regulation of autolysis genes has been noted in *S. aureus* strains that tolerate vancomycin [76]. In the same study it is noteworthy that Utaida et al. [55] observed a marked decrease in transcription of the essential gene infC under conditions of cell wall stress. This may make infC both a reasonable primary antibacterial target and a synergism candidate in combination with cell wall actives that induce the cell wall stress stimulon.

Similar potentials to find logical synergistic target combinations exist we believe in the analysis of pathogens global response to protein synthesis inhibition and DNA synthesis inhibition. The translation inhibitors tested by Ng et al. [54] decreased transcription levels of many of the tRNA synthetase genes and PheS in particular is markedly reduced. A combination of an active targeting PheS with one of the translation inhibitors might reasonably be synergistic. On the other hand, agents that cause marked increases in transcription of known antibiotic targets likely could be antagonistic.

6.3. Genomics future—host susceptibility

Host susceptibility and immune competency represent opportunities for genome-based research. Beyond the global differences in immune competency that impact on treatment outcomes, there are genetic links to specific disease states that are being identified in patients, thus further defining the treatment population. For example, individuals with Nod2 mutations have an increased risk of developing Crohn's disease and they produce a lower level of natural antibacterial peptides [77] creating an opportunity to supply missing or decreased mammalian antibacterials either directly as engineered biologicals or indirectly with agents that stimulate endogenous mammalian cationic peptide production.

6.4. Genomics future—treatment and resistance

Real time PCR can be used to detect antibiotic resistance mediating genes in microbes [78] and to detect rifampin and

isoniazid-resistant mutations in *M. tuberculosis* [79]. Determining, the gene expression profile for persistent bacterial forms, e.g., *M. tuberculosis* [17] should help understand resistance and provide novel targets for pathogenic forms responsible for chronic disease.

Laboratory-induced resistance to fluoroquinolone by *M. tuberculosis* occurs by an increase in the expression of MfpA, a family of proteins that apparently act by mimicking the beta form of DNA [80].

6.5. *Genomics future—safety*

Understanding of the gene transcription and expression patterns of the mammalian response to experimental antibacterial NCEs represents a logical approach to improve the safety margin of such potential drugs. It has been estimated that 25–60% of therapeutic agents have unexpected lower effectiveness because of significant differences in mammalian metabolism or distribution [81]. The ability to predict toxic events due to characterized mammalian responses before clinical trial initiation is critical to make the discovery and development of antibiotics economically viable. Personalized antibiotic dosing may be feasible when mechanisms of action, resistance, or metabolism are understood and the response of a patient can be genomically predicted [82].

6.6. *Genomics future—pharmacokinetics*

The presence of metabolizing, uptake and efflux genes in the bacterial target and the presence of uptake genes in the host may be exploitable in the design of effective agents that are prodrugs or hybrids. Isoniazid, for example, requires metabolism by the target pathogen, *M. tuberculosis*, before it is active [83]. Finding evidence for metabolizing genes in targeted microorganisms may allow the rational synthesis of antimicrobial prodrugs. Similarly, understanding host cell uptake and metabolism may improve pharmacokinetics of therapeutic agents [84].

6.7. *Genomics future—chronic disease linkage*

Studies in the 1950s–1970s linked rheumatoid arthritis (RA) to *Mycoplasma* infection [85]. Symptomatic relief of inflammation associated with tetracycline treatment did little to prove or disprove the hypothesis, due to inadequate methods to measure pathogen load, particularly if the latter are present at low levels as antibiotic-resistant persistent infections. The development of tigecycline (Fig. 2), an antibiotic effective against tetracycline-resistant bacteria [86] together with the development of genomic tools to evaluate the presence of pathogenic DNA [87], may allow for genome-based studies that link improvement in RA disease progression with treatments that may be more effective in hitting persistent forms.

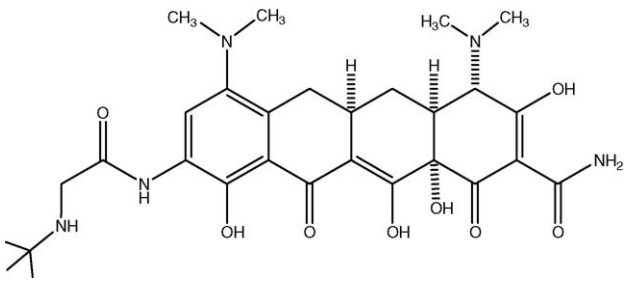

Fig. 2 – Structure of tigecycline.

Similar studies combining agents effective against persistent forms of *Chlamydia* with sensitive genomic-based detection of persistent pathogens could lead to a determination if *Chlamydia* is involved in atherosclerotic disease. Trials of gatifloxacin [88] and azithromycin [89] treatment on advanced heart disease have led to continued ambiguity of the association of microbial pathogens in atherosclerotic disease. Acute coronary syndrome in one case and stable coronary artery disease in the other have left unresolved the microbial question leading to the suggestion that patients with better markers of infection be considered in any future studies [90].

7. Conclusions

A key question is when bacterial genomics will result in new anti-infective drugs it has been argued [8] that: (i) there is no substitute for a detailed understanding of microbial physiology, (ii) target selection must include a determination of what level of inhibition is required to attain clinically effective growth inhibition and/or bactericidal activity and (iii) bacterial genomics can identify novel targets and help develop better assays. Genomics may in the end be a tool that increases understanding of the classical cell wall and protein synthesis targets while improving the ability to identify and develop such agents. But genomics have provided the opportunity for much more impact than mere target identification and the process of drug discovery, and at least six contributions in this *Special Issue* deal with varying aspects of bacterial genomics and the process of drug discovery, providing a comprehensive overview of multiple aspects of bacterial genomics and associated technologies.

Genomics may be viewed as an enabling tool to: (i) identify pathogens responsible for acute and chronic disease; (ii) select antimicrobial targets; (iii) understand drug synergies and antagonism; (iv) improve pharmacokinetics; (v) target the niche of a pathogen and its genomic responses to ecological cues and stress; (vi) understand and avoid resistance.

This overview of the existing uses and potential for genome-based research in antibacterial drug discovery, includes its potential impact beyond acute infections. The events surrounding the emergence of resistance are diverse and for the most part unpredictable, as is the failure of drugs to work in patients that in severe cases can be fatal. For such evens, a better understanding of every aspect of microbial growth, infection, drug intervention and resistance emergence is required, including differences in the genomics/genetics of the host that can be used to define unique targets for effect therapeutic agents. This Special Issue of *Biochemical Pharmacology* containing 26 commissioned articles from leading experts in their fields covering a cross-section of topics dealing with many of these key issues.

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