

Attacking the 'SUPERBUGS'

Less than a hundred years ago, if you developed a microbial infection your immune system couldn't handle, you died. Then came the 'wonder' drugs, antibiotics capable of fighting the infections for you. But times have changed. Now, because of antibiotics, their use and misuse, the rise of the 'Superbugs' is upon us, threatening our once potent arsenal of antibiotics, rendering them useless and impotent against a variety of re-emerging microbes. Now the only 'wonder' comes from your physician, whether an antibiotic prescribed works at all.

Superbugs are growing in number and severity and include methicillin-resistant *Staphylococcus aureus* (MRSA at your local hospital), multidrug-resistant *Mycobacterium tuberculosis* and other species, *Enterococci*, especially those resistant to the drug of last chance, vancomycin (aka Vancomycin Resistant Enterococci, VRE) and lesser publicised but deadly bacteria such as *Acinetobacter* and *Pseudomonas*, microbes for which there is little or no cure. If common bacteria such as *Staphylococcus* become resistant to the antibiotic vancomycin, it could signal the end of the anti-

biotic era, while plagues typical of the last mid-millennium rage across the populace.

Now faced with the danger of returning to the pre-antibiotic era and shortened lifespans, the discovery of new drugs to fight these diseases is gaining ground – albeit slowly. Molecular and structural biologists are just beginning to understand the mechanisms by which microbes can degrade, evade or outwit antibiotics. Medicinal chemists and drug designers have made some headway in understanding the SAR of clinically-used antibiotics to produce newer, more potent antibiotics against microbial pathogens. New microbial

By Dr Mark L. Nelson

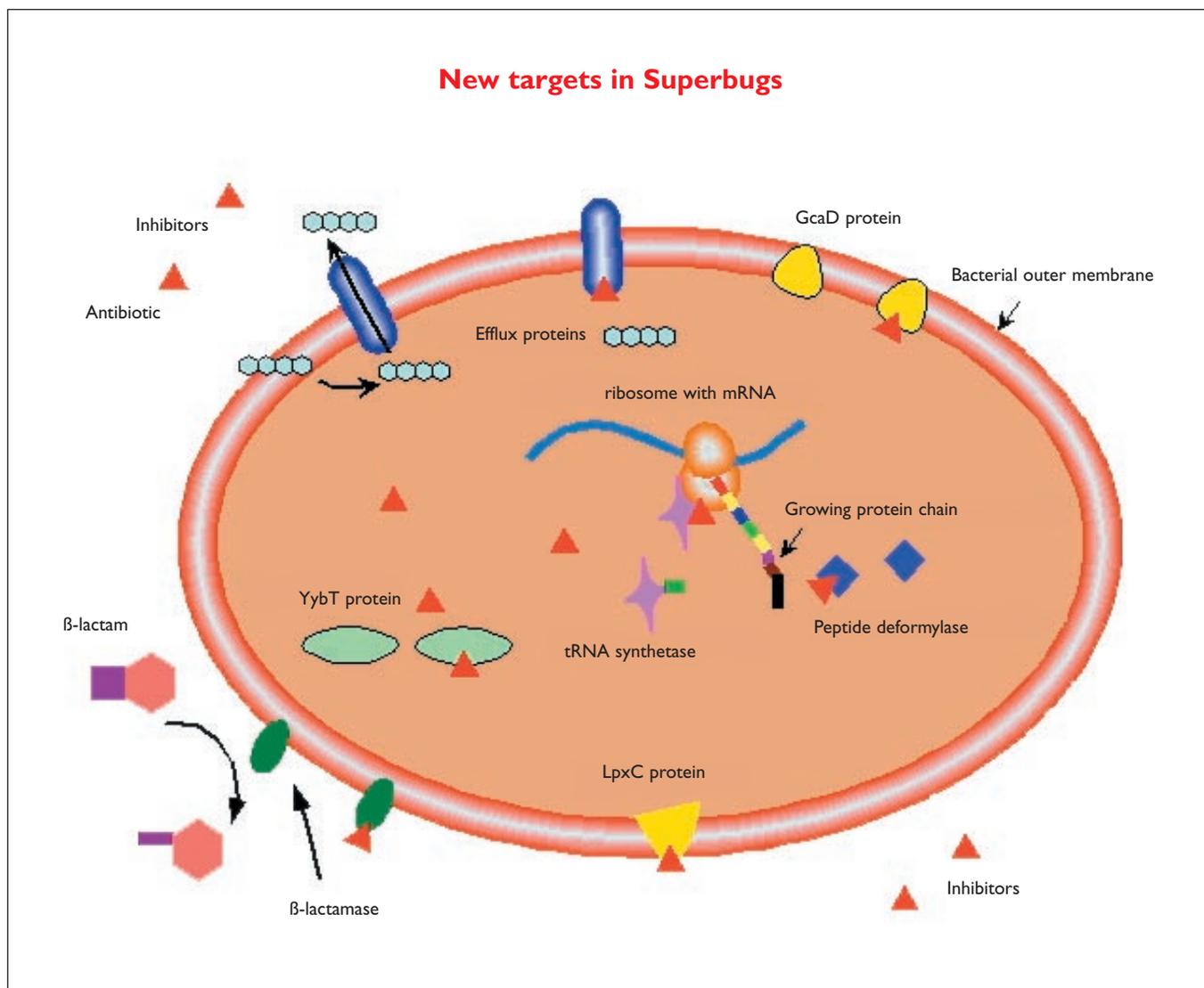


Figure 1 targets are being discovered, through molecular biology, genomics and now are deriving inhibitory molecules capable of attacking the Superbugs. New companies are springing up with better and more ingenious ways of fighting back, with the hopes of delivering the next blockbuster drug, some of which will be presented here. And what are these new microbial targets? And what are the new drugs directed at these targets? Antibacterial targets may be broken down into broad categories: those currently targeted by antibiotics, previously undefined targets prime for molecular inhibition, resistance mechanisms and their modulation, and new paradigms for treatment, such as modulating signalling pathways within bacteria. All of these areas represent the state-of-the-art in antibacterial drug discovery.

Antibiotics *usually* act at specific cellular levels: DNA, RNA and nucleic acid synthesis and metabolism, protein synthesis and modification, and lastly, and the most exploited by antibiotics, inhibitors of cell wall synthesis, membrane integrity and membrane-based cellular pathways. Each level has its own subset of bacterial target proteins while their inhibitors form the basis of our current arsenal of clinically-used antibiotics. But the word *usually* is italicised for a reason. Historically, researchers have sought new antibiotics against these targets. Now we will have to break out of these paradigms in order to fight the attack of the Superbugs.

New drug discovery and synthesis efforts must be applied against new bacterial targets described by genomics and proteomics and can

include: the chemical modification of clinically-used antibiotics ideally producing drugs with higher affinity and/or greater potency against older targets, the discovery of new small molecule scaffolds and inhibitors either through combinatorial or classical synthesis against new bacterial targets, and the discovery of novel natural products against each target and cellular level. In the past, antibiotics that act specifically at the level of peptidoglycan, DNA, RNA or protein synthesis were usually desired, while combinations of drugs that act at two or more mechanisms are now accepted by the medical community. Only a handful of combination drugs targeting different bacterial mechanisms exist, the blockbuster antibiotic mixture of a penicillin derivative and an inhibitor of a penicillin-degrading enzyme being the most popular – GlaxoSmithkline's (GSK) Augmentin currently the most prescribed antibiotic worldwide.

International multi-disciplinary meetings bringing the antibacterial and drug discovery scientists together occur yearly at ICAAC, the Interscience Conference on Antimicrobial Agents and Chemotherapy, where scientists, physicians, academics and industrialists tout their efforts on both the discovery of new antibacterial agents and the description of new, potential drug-able targets in bacteria (Figure 1). The past two years represent a snapshot of current efforts and discoveries while several companies stand out and are reported here.

Microcide Pharmaceuticals, Inc, now Essential Therapeutics (ET), uses genomic techniques to identify essential growth genes in *Staphylococcus aureus* that profile potential *in vitro* and *in vivo* targets, and has compiled a list of 96 genes that are essential for survival. The list of genes presents proteins of all the major metabolic and architectural pathways operative in bacteria and could one day provide target proteins for HTS. One essential gene described is *gcaD*, responsible for producing a protein crucial in cell wall synthesis and now a choice candidate for its drug discovery efforts. ET also reported others inhibitor-based assays for gene products responsible for bacterial survival, screening for inhibitors of the *Staphylococcus* protein YybT. Numerous compounds with preferential activity against *S. aureus* were identified, 12 of which acted against other Gram-positive pathogens possessing the YybT target.

Cubist Pharmaceuticals (CP) and GSK have separately been studying aminoacyl tRNA-syn-

thetases as targets for drug discovery. During protein synthesis, each amino acid is transferred to polypeptide chains by a specific tRNA-synthetase, catalytically growing proteins and bacteria. Potentially, 20 different targets are available for inhibition, which shut down growth and cell viability. 3-D structures are available for more than half of them – further aiding drug discovery and lead optimisation efforts. GSK scientists have been studying inhibitors of tyrosyl-tRNA synthetase with activities in the low micromolar to high nanomolar range, depending on the synthetase studied. Selectivity over human synthetases have been achieved with bacterial inhibitors, while chemical leads have been generated for further SAR determinations.

CP also uses chemoinformatics to discover compounds possessing multiple modes of action in microbes. Using methionyl-tRNA synthetase enzymes and HTS data, heterocyclic substructures were uncovered that block both tRNA synthetase and have whole cell activity, while other chemotypes were found to inhibit both the synthetase and a historic metabolic enzyme target, dihydrofolate reductase. CP's computational methods open the possibility of producing antibiotics with multiple targets and multifaceted modes of action.

Bacterial protein synthesis and its metal-based proteins also serve as targets for inhibition. Polypeptide deformylase (PDF) enzymes help process proteins once made in cells. Inhibiting this reaction stops bacteria from producing growth proteins and eventually cells stop dividing. British Biotech Pharmaceuticals (BBP) has identified numerous metalloenzymes and developed inhibitors that work both *in vitro* and *in vivo* to knock out cellular proliferation. N-formyl hydroxylamines have been found to have activity against major Gram-positive pathogens, including MRSA and antibiotic resistant pneumococci. BBP compounds also had potent activity *in vivo* against *Streptococci* in a once-daily dosing regimen. PDF inhibitors are a promising new class of antibacterial agents.

Versicor also has reported that the compound actonin is active against PDF against a broad spectrum of bacteria with high potency, while combinatorial libraries designed to delineate the active pharmacophore(s) of metalloenzyme binding have been synthesised and actives identified. Versicor also demonstrated an essential gene *lpxC*, whose protein LpxC maintains the permeability barrier of the outer cell wall of *E. coli* and other Gram-negative bacteria. Once inhibited, cells may

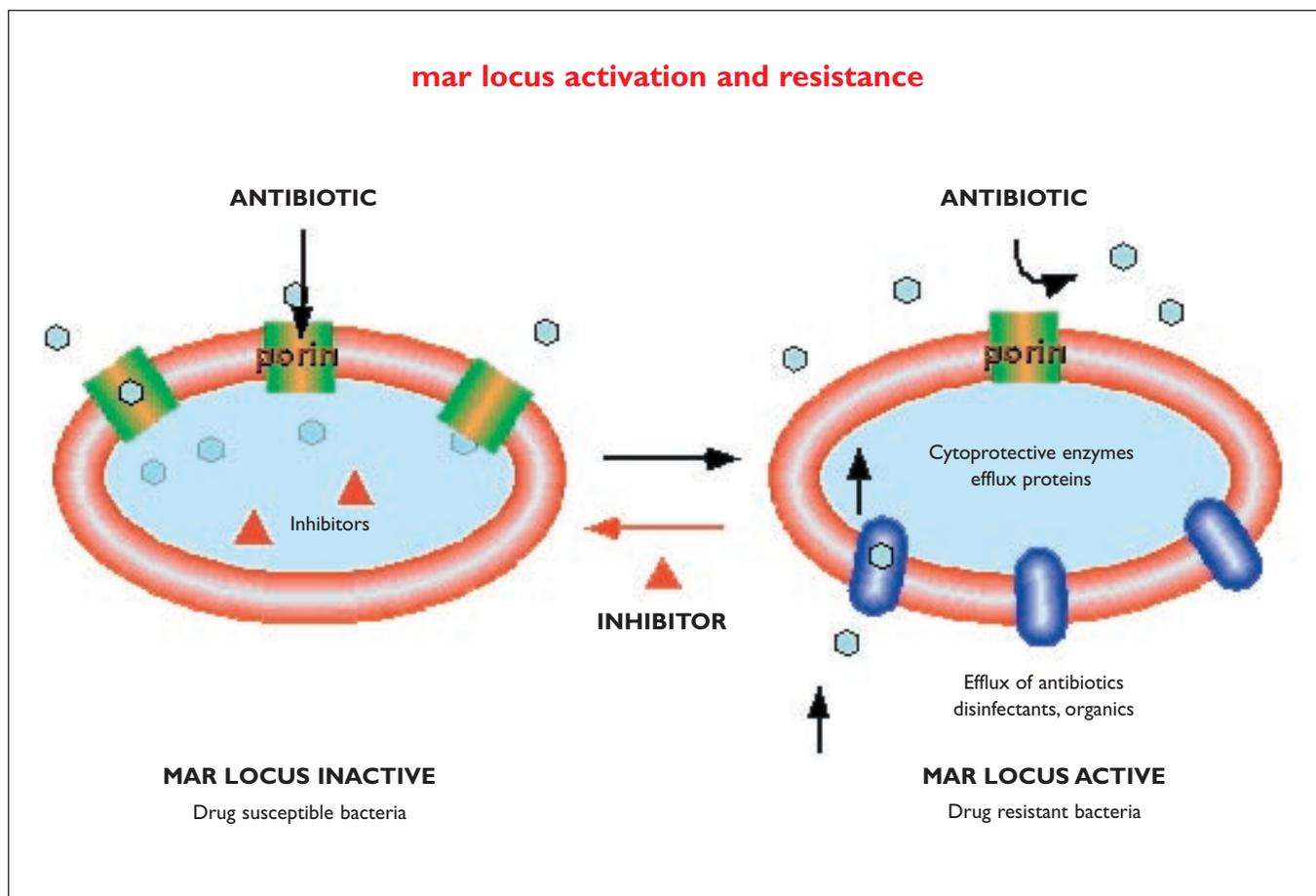


Figure 2 become more permeable to antibiotics, potentially decreasing the amounts needed therapeutically and possibly reviving other, less permeant antibiotics. LpxC awaits further discovery of small molecule inhibitors and proof-of-principle in animal models of infection.

Karo Bio, USA, uses phage display libraries to produce peptide probes that can block essential proteins not targeted by currently used antibiotics. Peptide probes are used as surrogate ligands to outfit HTS assays to discover drugs that inhibit protein function. Karo Bio and GPC Biotech have teamed up to explore TUFs, Targets of Unknown Function. Using sequences of complete microbe genomes, TUFs were identified in parallel and targeted mutually conserved functional sites. Phage display probes combining genomic analysis with biochemical techniques proved fruitful for the development of powerful assays against targets whose biochemical function is unknown.

Novalon Pharmaceuticals has also used peptide probes directed to functional sites on essen-

tial bacterial proteins. Peptides in competitive assays are able to further quantitate inhibition of target protein function. To date, they have identified 35 essential *E. coli* genes and have screened more than 100,000 compounds, delivering hit-rates of >0.2%.

At the annual SMI business conference ‘Superbugs and Superdrugs’ conference held in London in March 2002, industry leaders and scientists gathered and the antibacterial targets keep mounting in number, scope and promise. But it is surprising how small the numbers of actual targets are. Genomic techniques have identified between 2,000 and 3,000 genes in Gram positive pathogens, while Gram negative bacteria, depending on complexity, have between 2,000 and 6,000 genes. Once the genetic differences between man and bacteria are subtracted, around 400 essential genes that can possibly be used to discover new drug targets are found within the latter. Elitra Pharmaceuticals points out that from 2,700 genes in *Staph*, only about 50 genes and protein products are considered as

viable, antibacterial targets by the time genetic filters are applied.

Inhibition of antibacterial resistance mechanisms

Bacteria can evade the most widely prescribed antibiotics in use, the β -lactams, by chemically destroying them (Figure 2). The penicillins, cephalosporins and other antibiotics possessing β -lactam substructures can be hydrolysed by β -lactamase enzymes, which now number over 340 in number, growing every year. Hydrolysis and degradation profiles subdivide the lactamase superfamily of serine proteases or metalloproteases into four subgroups and have been prime targets for inhibition. While the chemistry of β -lactamase inhibition has been developing over the years, only three compounds are used clinically to stop lactamases and are used in combination with β -lactam antibiotics: clavulanic acid (the co-additive in Augmentin), sulbactam and tazobactam. All lactamase inhibitors mimic the structure of the antibiotic, bind to the active site of the lactamase and inhibit further destruction of the β -lactam nucleus. As the β -lactam antibiotics continue to evolve into more complex chemicals and new generation penicillins, cephalosporins and carbapenams arise, β -lactamase inhibitors need to continue to evolve as well to further protect our most widely used antibiotics.

Antibiotic efflux proteins are found in all bacteria, where their main function is to remove antibiotics from the cell, protecting the cellular machinery from chemical assault. Antibiotics and chemical toxins entering into cells are efficiently removed by efflux pumps, ensuring survival. More distressing, bacteria can possess back-up 'pumps', redundant systems used to remove antibiotics. Alternatively, efflux pumps can be thought of as attractive therapeutic targets, where their inhibition can restore activity to antibiotic substrates.

Bacterial efflux pumps can be subdivided into an ever-increasing number of families, based upon their molecular architecture, mechanisms of action, energy requirements and chemical constitution. Each transport family in turn is comprised of multiple pump members typically named after the first compound they were found to remove, ie *acrA* proteins are pumps that remove the dye acridine from the cell. Other potential targets for inhibition include *norA*, an efflux protein capable of removing the antibiotic norfloxacin from *Staphylococcus aureus*, while the bacterium *Pseudomonas aeruginosa*, possesses the Mex family of efflux proteins,

capable of removal multiple structurally-unrelated antibiotics including aminoglycosides, quinolones and even detergents and fatty acids. Since bacterial efflux proteins are found in all pathogens, and are thought responsible for some of the failure of many antibiotics used in medicine, these targets for inhibition are of prime importance.

While efflux pumps have been studied for more than two decades, the number of new compounds found effective in inhibiting them is surprisingly small – only a dozen or so different chemotypes have been described and little or no SAR exists for compounds outside of the tetracycline family of antibiotics. Our laboratory, initially at Tufts University School of Medicine, Boston, was the first to describe SAR for the inhibition of Tet efflux pumps, which remove tetracyclines from bacteria. We developed inhibitors of these pumps, restoring activity to clinically used drugs and essentially reversing antibiotic resistance *in vitro*. As our tetracycline chemistry effort grew we then synthesised novel, potent tetracyclines that inhibited the growth of MRSA *Staphylococcus* and VRE *Enterococcus* bacteria. In 1996, Paratek Pharmaceuticals, Inc was launched from this work, and is devoted to the application of novel chemistries to antibiotics, where our scientists have succeeded in producing a large and structurally-diverse library of novel tetracycline antibiotics to fight antibiotic resistant bacteria both *in vitro* and *in vivo*.

Paratek also is leading the way in the development of new approaches and biologic targets that force change on current drug discovery paradigms to overcome the problem of antibacterial resistance. One unique attack strategy at Paratek is to exploit our discovery of the Multiple Antibiotic Resistance or *mar* operon, a novel and ubiquitous 'master switch' in bacteria that controls a large set of genes that rev up their defence mechanisms to antibiotics. Once *mar* is activated, more than 80 different genes are expressed, some of which control the entry of antibiotics, the efflux of antibiotics and their degradation, while other genes increase the production of cytoprotective enzymes. In pathogens, *mar* increases resistance to key antibiotics such as the β -lactams, fluoroquinolones and even household disinfectants. Paratek has since developed target-based screens to detect drugs paralysing the *mar* locus, decreasing the expression of defensive genes and their products and increasing bacterial susceptibility to a wide variety of antibacterial agents. Paratek is now exploring the use of *mar*

References

- Essential Therapeutics: www.microcide.com
- Cubist Pharmaceuticals: www.cubist.com
- GlaxoSmithKline: www.gsk.com
- British Biotech: www.britbio.co.uk
- Versicor: www.versicor.com
- Karo Bio, USA: www.karobio.com
- GPC Biotech: www.gpc-biotech.com
- Novalon: www.novalon.com
- Elitra Pharmaceuticals: www.elitra.com
- Paratek Pharmaceuticals: www.paratekpharm.com

Further suggested reading

- The challenge of antibiotic resistance, Stuart B. Levy, Scientific American, March 1998, 278, 46-53.
- Multidrug-resistant bacterial infections: the search for new antibiotics, Steven J. Brickner, Chemistry and Industry News, February 17, 1996.
- New targets and strategies for the development of antibacterial agents, Jean-François Desnottes, Trends in Biotechnology, April 1996, 14, 133-140.
- Bacterial resistance mechanisms as therapeutic targets, Ken Coleman, Medha Athalye, Annette Clancy, Mark Davidson, David J. Payne, Caroline R. Perry, Ian Chopra, Journal of Antimicrobial Therapy, 1994, 33, 1091-1116.
- The impact of genomics on novel antibacterial targets, David J. Payne, Nicola G. Wallis, Daniel R. Gentry, Martin Rosenberg, Current Opinion in Drug Discovery and Development, 2000, 3, 177-191.
- Targets, targets everywhere, where are the new antibacterial drugs? Michael J. Pucci, Thomas J. Dougherty, John F. Barrett, Current Opinion in Anti-infective Investigational Drugs, 2000, 2, 123-124.

inhibitors to increase the potency of standard antibiotics and for the production of prophylactic agents for the protection of patients at risk from infection.

Modifying existing antibiotics, improving their spectrum and potency and improving the activity of current antibiotics through disabling bacterial resistance mechanisms are currently under way at Paratek and at other companies as well. Developing drugs to enfeeble bacteria, make them susceptible to antibiotics in current use or to loose pathogenic traits are shifts in current chemotherapy paradigms, and Paratek is well positioned and on target to achieve these goals.

In closing, antibacterial discovery has witnessed the explosion of genomics, proteomics, bioinformatics, high-throughput screening, custom-designed assay formats and truly revolutionary ideas in structural biology. Chemistry has advanced fronts in synthetic techniques, parallel and combinatorial methods and high-throughput synthesis and chemical characterisation. But times are getting rough in antibacterial drug discovery with no sign of subsiding soon. In the last decade, the mood has swung extreme – from elation and unfettered promise to one of dour uncertainty – all the while pushing the science and scientists to their limits.

The biologists look to the chemists for producing compounds they can work with, screen, derive leads from, and eventually develop, but cries of ‘target rich and compound poor’ resound through the halls. The chemists, point likewise, begging for detailed and novel biological bacterial targets worthy of pursuing and chemical and logical interpretation of the mounds of HTS data generated by library and lead optimisation screening. Now, more than ever, we need to think ‘out of the box’ in order to win the attack against Superbugs. It is truly a

grand battle of science, technology and survival, both for bacteria and for mankind. Meanwhile the Superbugs march on. **DDW**

Mark L. Nelson, PhD has a BSc degree in Chemistry and Microbiology from Gannon University, Erie, PA and a doctoral degree in Medicinal Chemistry from Temple University, Philadelphia, PA. Post-doctoral research was conducted at Tufts University School of Medicine, The Center for Adaptation Genetics and Drug Resistance with Stuart B. Levy, MD, Director, studying the SAR of bacterial efflux protein inhibition and the synthesis of novel tetracycline antibiotics. His research with Dr Levy led to the formation of Paratek Pharmaceuticals, Inc in Boston, MA where he is currently the Senior Director of Exploratory Chemistry. He has been a Fulbright Distinguished Lecturer and received the Rho Chi Pharmaceutical Society Kalleis-Lynch Lectureship Award for his research on bacterial resistance mechanisms.

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