



Trip Report for

**“The Challenge of Antibacterial Drug Development: Integrating
Chemistry and Biology”**

San Diego, California

April 23-24, 2008

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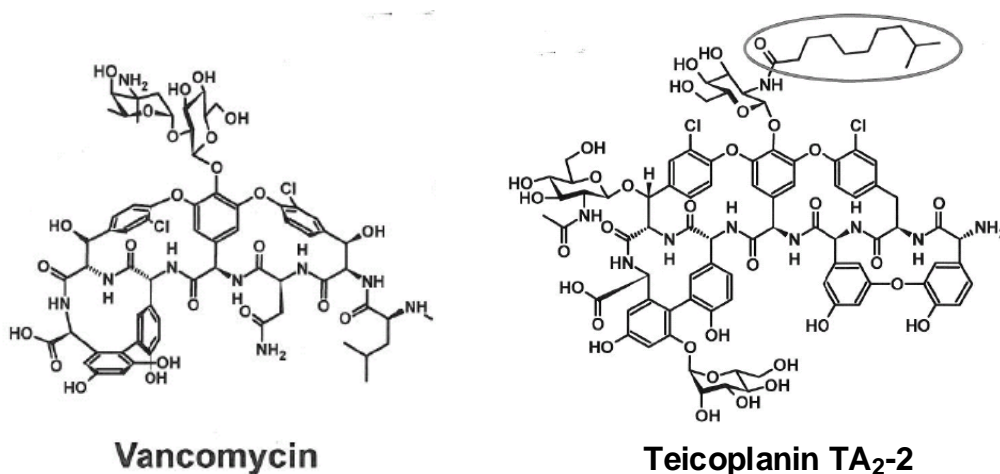
Abstract: *Cambridge Healthtech Institute’s second annual “The Challenge of Antibacterial Drug Development: Integrating Chemistry and Biology” was held in San Diego, California, on April 23-24, 2008. During the two-day conference, topics such as defense strategies, natural product-based screening, approaches by structure, bioinformatics and models, funding and collaboration strategies, beta-lactam antibiotics, identifying novel targets, and the challenge of selecting novel therapeutics were discussed. This report highlights selected material presented at the conference.*

“Oritavancin: The discovery and Development of a Novel Lipoglycopeptide Antibiotic”

Greg Moeck, Targanta Therapeutics Corporation, Cambridge, Massachusetts

The 30-plus year success of vancomycin (Figure 1) and lack of widespread vancomycin resistance, along with the discovery of the teicoplanins has renewed interest by pharmaceutical companies to identify new glycopeptides. Teicoplanin TA₂-2 (Figure 1) is a naturally-occurring fatty acid-substituted glycopeptide complex with greater activity than vancomycin against some isolates, including VanB VRE, with higher degree of protein binding and longer serum half-life than vancomycin.

Figure 1. Structure of Vancomycin and Teicoplanin TA₂-2.



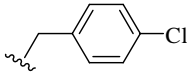
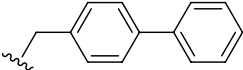
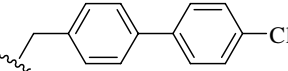
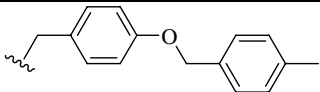
The Eli Lilly strategy for natural products screening to discover novel glycopeptides was to use an anti-vancomycin immunoassay for the identification of glycopeptide-producing cultures. As a result, *Kibdelosporangium aridum* Culture A82846, discovered in Haitian soil, was found to produce a mixture of vancomycin-type glycopeptides including chloroeremomycin. Chloroeremomycin is on average two to ten times more potent than vancomycin which is proposed to be due to the 4-*epi*-vancosamine saccharide moieties ((Table 1 and Figure 2). It was found that chloroeremomycin dimerization and ligand binding were cooperative when interacting with terminal residues of disaccharide pentapeptide (D-Ala-D-Ala) to inhibit bacterial cell wall synthesis.

Table 1. *In vitro* Activity of Chloroeremomycin.

Phenotype	n	MIC ₉₀ (µg/mL)	
		Chloroeremomycin	Vancomycin
MSSA	20	0.25	2
MRSA	40	1	2
VSE	27	1	2
VanA VRE	26	256	2048
VanB VRE	20	128	512

The Eli Lilly vancomycin modification program resulted in the finding that vancosamine *N*-alkylations increased potency and plasma half-life, while in teicoplanin the corresponding *N*-acylation contributed to increased potency and extended plasma half-life relative to vancomycin. Thus, it was discovered that acylation/alkylation at 4-*epi*-vancosamine amine yielded the most active derivatives (Table 2).

Table 2. MIC, Dimerization, and Substrate Binding of Chloroeremomycins.

Compound	Side chain	MIC ₉₀ (μg/mL)	K _{dim} (M ⁻¹)	K _b (D-Ala-D-Ala) (M ⁻¹)
chloroeremomycin	H	0.06	9 x 10 ³	1 x 10 ⁵
LY191145		0.02	1 x 10 ⁴	4 x 10 ⁵
LY307599		0.008	3 x 10 ⁵	6 x 10 ⁵
oritavancin		0.001	6 x 10 ⁵	3 x 10 ⁵
LY377502		0.0007	1 x 10 ⁶	4 x 10 ⁵

The wide range of antimicrobial potency was rationalized by the nature of hydrophobic side chains (linear correlation between logMIC and logK_{dim}), rather than by binding affinity for the soluble ligand (no correlation between logMIC and logK_b). Table 3 shows oritavancin (Figure 1) MIC₉₀ values for Gram positive pathogens.

Table 3. Oritavancin MIC₉₀ values for Gram-positive pathogens.

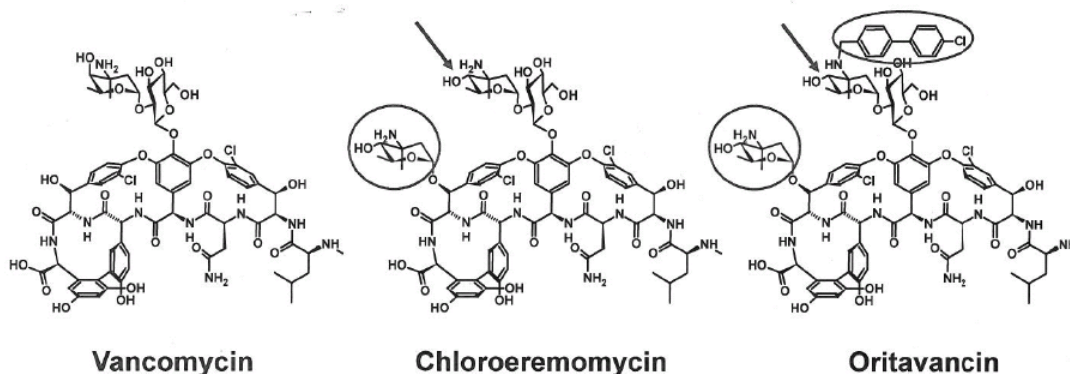
Organism	Phenotype	MIC ₉₀ (μg/mL)
<i>Staphylococcus aureus</i>	Oxacillin S	0.12
	Oxacillin R	0.25
	Daptomycin NS	1
	Linezolid NS	MIC Range: 0.06-0.25
	hVISA	1
	VISA	1
	VRSA	MIC Range: 0.12-0.5
<i>Streptococcus pyogenes</i>	All	0.25
<i>Streptococcus agalactiae</i>	All	0.12
<i>Enterococcus faecalis</i>	Vancomycin S	0.06
	Vancomycin NS	1

Oritavancin showed rapid-kill kinetics against CA-MRSA at predicted free peak concentrations *in vitro*. It is bactericidal within 15 minutes to 6 hours against all isolates of MSSA, MRSA, hVISA and VRSA tested at its free peak level from a single 200 mg dose.

Studies also showed that oritavancin acts by multiple mechanisms: by substrate masking cell wall extension is prevented; by dimerization to provide additional utility; by blocking of transglycosylase enzyme; through disruption of membrane potential. Oritavancin's multiple modes of action reduce the propensity to select for high-level resistance.

Further studies showed oritavancin to be active in multiple animal models. In the rat granuloma pouch model, oritavancin showed single dose efficacy against MSSA, as a single IV dose prevents MSSA regrowth significantly longer than two doses of vancomycin. Oritavancin has further been selected as clinical candidate and Targanta acquired the oritavancin IND in 2006. A Phase II SIMPLIFI complicated skin and skin structure infection (cSSSI) study is underway. Other clinical development programs against diseases such as bacteremia, osteomyelitis, *Clostridium difficile* infection and anthrax infection are also planned.

Figure 2. Structures of vancomycin, chloroeremomycin and oritavancin.



“Bisphosphonated Rifamycin Prodrugs for the Treatment of Osteomyelitis”

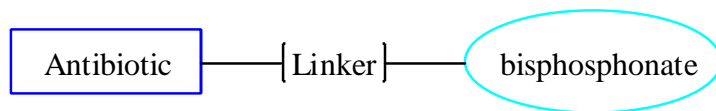
Dario Lehoux, Targanta Therapeutics Corporation, Cambridge, Massachusetts

Chronic bacterial osteomyelitis is a difficult-to-treat bone infection that is essentially caused by gram-positive organisms, primarily *S. aureus*. Currently, surgical debridement and 4 to 6 weeks of post-surgical therapy represent the standard for treatment. Novel, potent antibacterial agents are needed to counteract treatment failures and drug-resistant organisms.

Bisphosphonates (BP) are pyrophosphate analogs with high affinity for calcium in general and bone mineral in particular. A well-established and clinically-used set of compounds in the treatment of osteoporosis, bisphosphonates have been suggested for bone delivery and have been shown to efficiently transport small molecules and proteins to bone.

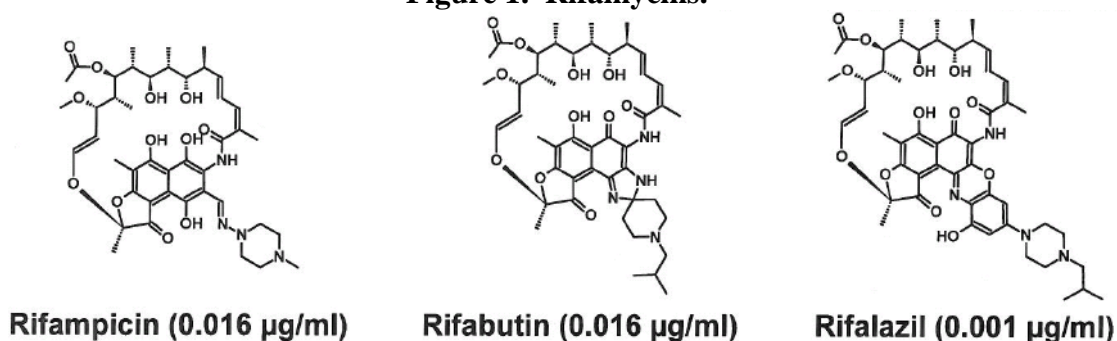
The prodrug strategy is to couple proven antibiotics to bisphosphonates via a cleavable linker, aiming to achieve three goals: efficacy (faster and higher exposure in bone),

convenience (sustained antibiotic release in the bones for infrequent dosing) and safety (lower systemic exposure).



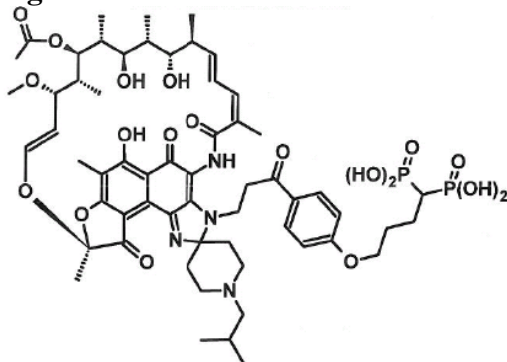
Rifamycins (Figure 1) were selected for coupling to bisphosphonate. Rifamycins are very potent bactericidal antibiotics against *S. aureus* through DNA-dependent RNA-polymerase inhibition in bacteria. They are active against biofilms *in vivo* although emergence of high-level rifamycin resistance is a continuing problem.

Figure 1. Rifamycins.



Rifabutin pharmacodynamics in rat models of chronic osteomyelitis showed that the efficacy of free rifabutin was dose-dependent up to 5 mg/kg/d for 7 days and that rifabutin exposure in tibia is highly correlated with dose. This result supports use of rifabutin in bisphosphonate prodrug design. As a result, bisphosphonated rifabutin prodrug **TT99000647** (Figure 2) was synthesized and was studied *in vitro*. These studies showed that **TT99000647** binds to bone powder (>99% over 1 h) with slow rate of cleavage (0.32% per day). MIC (liberated rifabutin) is 1 µg/mL against *S. aureus*.

Figure 2. Structure of TT99000647.



Prophylactic treatment in a rat osteomyelitis model was used as the primary *in vivo* screening. Antibiotherapy (single dose) was administered for 30 days after which an injection of sclerosing agent and bacteria was administered. After 1 day, a determination

of CFU/g of bone was made. **TT99000647** showed efficacy when administered 5 days prior to infection as it reduced the bacterial load by 3.5 logCFU/g, an improvement when compared to rifabutin.

TT99000647 PK in rabbits compares favorably to PK in rats as shown in Table 1. Long half-life in bone supports infrequent dosing and greater prodrug exposure in rabbit versus rat support the choice of a rabbit model for further efficacy studies. Note that the data in Table 1 represents a slow IV bolus administration of **TT99000647** at 10 mg/kg and that the concentration in the tibia bone (mg/g of bone) was determined between time points of 24 to 168 hours.

Table1. TT99000647 PK in rat and rabbit models.

Parameter	Rat	Rabbit
C _{max}	0.25 ug/g	2 ug/g
AUC _{last}	5,180 ug • h/g	25,900 ug • h/g
T _{1/2} (24h-168h)	159 h	131 h

The efficacy of the rifabutin-bisphosphonate prodrug was evaluated in the rabbit model of chronic *S. aureus* osteomyelitis by injection of the sclerosing agent and 10⁶ CFU MSSA into the tibia. Severity of osteomyelitis was graded by X-ray analyses within 14-15 days, after which animals were randomized and therapy was administered for 28 days in multiple doses. The CFU/g of bone was determined and used to assess resistance in clones from non-sterile bone. Compound **TT99000647** showed efficacy in three separate studies under different dosing schedules in the chronic *S. aureus* osteomyelitis rabbit model, as shown in Table 2. Efficacy of **TT99000647** was found to be superior to the free parent drug (rifabutin) and similar to comparators, albeit with a more convenient dosing regimen.

Table 2. Relative efficacy in rabbit model of chronic *S. aureus* osteomyelitis.

Agent	Dosing Schedule	Total Doses	% Sterile
Rifampin	40 mg/kg qid	112	75
Nafcillin	30 mg/kg qid	112	100
Linezolid	60-70 mg/kg tid	84	71-100
Daptomycin	10 mg/kg bid	56	40
Gatifloxacin	40 mg/kg bid	56	93
Telavancin	30 mg/kg bid	56	80
Vancomycin	30 mg/kg bid	56	80
Rifabutin	30 mg/kg qd	28	73
Rifabutin	30 mg/kg 4xqd + 6xq4d	10	43
TT99000647	50 mg/kg 4xqd + 6xq4d	10	73-100

In summary, **TT99000647** displayed potential as an efficacious and convenient treatment for osteomyelitis. The measured PKs from dose regimen versus efficacy studies confirmed the potential for infrequent use, as it showed marked activity in the “gold standard” rabbit model of chronic osteomyelitis. Further characterization of

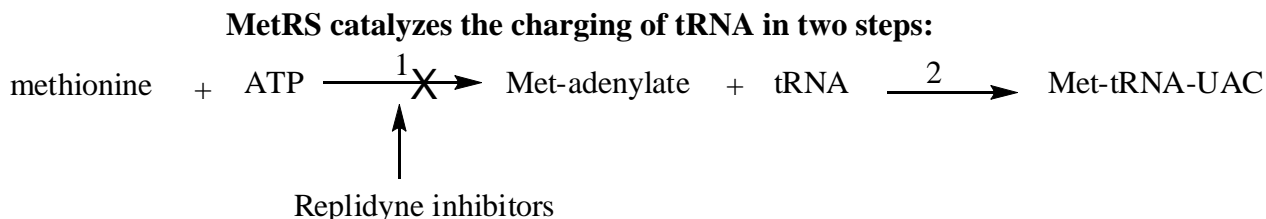
TT99000647 is ongoing as are combination therapies to evaluate the appearance of rifabutin resistant mutants and additional preclinical toxicology studies are underway.

“Identification & Lead Optimization of REP3123: a New Agent for CDAD?”

Joe Guiles, PhD, Replidyne, Inc., Louisville, Colorado

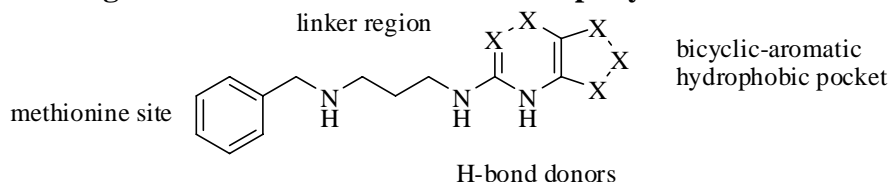
Dr. Guiles presented the discovery of **REP3123**, a new agent being evaluated for the treatment of *clostridium difficile* association disease (CDAD). *C. difficile* is a spore-forming Gram-positive Anaerobe and the leading cause of nosocomially infectious diarrhea and antibiotic-associated GI infection which can lead to colitis, hospitalization and even death. Recurrence occurs in ~20% of patients undergoing the current standard treatments of vancomycin or metronidazole.

To treat effectively the infection by *clostridium difficile*, Replidyne designed a series of inhibitors based on a new mechanism of action which blocks protein synthesis via binding to aminoacyl tRNA synthetase enzymes (MetRS). These enzymes play a key role in protein synthesis and are a family of enzymes that catalyze the attachment of amino acids to their cognate tRNAs. Replidyne inhibitors inhibit protein synthesis by blocking the first step.



The chemical structure of Replidyne inhibitors include three parts: a methionine site, a linker and a bicyclic-aromatic hydrophobic pocket (Figure 1). SAR studies on the left side and on the right side show that the order against *c. difficile* potency on the left side is chroman > thiophene = phenyl while the order on the right side is non-aza-based > aza-based; thienopyridine-based > quinolone-based (Tables 1 and 2, K_i = inhibition constant).

Figure 1. Chemical Structures of Replidyne Inhibitors



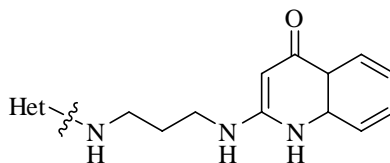


Table 1. Partial SAR on the Left Side.

Compound Number	Heterocycle (Het)	MIC ₉₀ (ug/mL)	K _i (pM)	Compound Number	Heterocycle (Het)	MIC ₉₀ (ug/mL)	K _i (pM)
1		4	260	REP 8839		2	80
2		1	90	4		1	50
3		4	215				

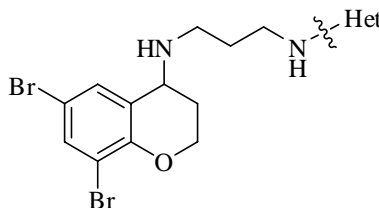
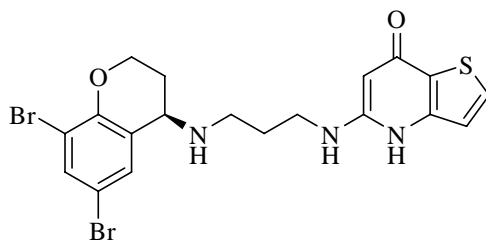


Table 2. Partial SAR on the Right Side.

Compound	Heterocycle (Het)	MIC ₉₀ (ug/mL)	K _i (pM)	Compound	Heterocycle (Het)	MIC ₉₀ (ug/mL)	K _i (pM)
REP 0307		1	50	REP 0308		2	340
REP 0891		4	225	vancomycin	---	0.5	n/a
REP 0897		0.5	70	metroidazole	---	0.25	na

Figure 2. Chemical Structure of REP3123.



REP3123

REP0897 showed good activity against *C. difficile* (0.5 µg/mL) in SAR studies. Between the two enantiomers, only the (*R*)-enantiomer (**REP3123**) was active (Figure 2). Further studies showed that **REP3123** is a highly selective inhibitor of Gram-positive bacterial MetRS (Table 3) and is better against resistance development following serial passages than vancomycin (Table 4).

Table 3. Activity of REP3123 against Gram-positive Bacteria.

Organism	MIC range (µg/mL)
Gram-positive	
<i>C. difficile</i>	0.25-1
<i>S. aureus</i>	0.03-1
Coagulase-negative staphylococci	0.03-0.12
<i>S. pyogenes</i>	0.12-1
<i>E. faecalis</i>	0.008-0.015
<i>E. faeciunt</i>	0.008-0.015
<i>S. pneumoniae</i> (MetRS 1)	0.5-1
<i>S. pneumoniae</i> (MetRS 2)	>8
Gram-negative	
Enterobacteriaceae	>64
Non-fermenting Gram-negative bacilli	>64

Table 4. Comparison of activity of REP3123 and vancomycin against resistance development following serial passages.

Strain	Agent	MIC ₉₀ (ug/mL) (parent)	MIC ₉₀ (ug/mL) (24 passages)
<i>C. difficile</i> ATCC 43255	REP3123	0.25	0.5
	Vancomycin	1	8
<i>C. difficile</i> ATCC 43596	REP3123	0.25	0.5
	Vancomycin	0.5	8
<i>C. difficile</i> MB903	REP3123	0.5	1
	Vancomycin	1	8
<i>C. difficile</i> MB898	REP3123	0.5	0.5
	Vancomycin	1	8

Table 5. Comparison of activity of REP3123, vancomycin and metronidazole against normal gut flora and *C. difficile*.

Organism	MIC ₉₀ range (ug/mL)		
	REP3123	Vancomycin	Metronidazole
<i>Actinomyces spp.</i>	>32	0.5	>32
<i>Bacteroides spp.</i>	>32	>32	1
<i>Bifidobacterium spp.</i>	>32	0.5	>32
<i>Lactobacillus spp.</i>	>32	>32	>32
<i>C. difficile</i>	1	1	0.5

Table 6. Comparison at 0.5 x MIC for REP3123, Vancomycin and Metronidazole

<i>C. difficile</i> strain	properties	% spores at 96 hours		
		REP3123	Vancomycin	Metronidazole
ATCC 43596	Model strain	0	100	100
MB903	<i>tcd</i> variant	1	100	100
RMA 18383	Epidemic strain	0	0	0
RMA 18386	Epidemic strain	0.1	93	0.2

In vivo results showed that **REP3123** is an agent with selective activity against *c. difficile* and has a low propensity for disruption of normal gut flora (Table 5). Comparing to vancomycin and metronidazole, **REP3123** is superior to the leading clinical agents at preventing sporulation as well as in overall survival at the doses and dose schedules evaluated (Table 6). Based on the *in vitro* and *in vivo* results, **REP3123** is a possible clinical agent in treating CDAD.

“Addressing the Bacterial Entry Conundrum”

LL Silver, PhD, LL Silver Consulting, LLC, Springfield, New Jersey

Dr. Silver presented the recent status of bacterial drug development efforts across the industry. With antibacterial resistance on the rise, there is a need to replenish the arsenal of antibacterial drugs to provide physicians with the tools to successfully treat infections in the future. However, after the introduction of streptogramins and quinolones in 1962 and linezolid in 2000, no novel class of antibiotics has been identified and approved for clinical use. Natural products screening has decreased and the quality of chemical libraries has poor antibacterial properties. Enzyme inhibitor hits often have no antibacterial activity largely due to lack of penetration and efflux and single-enzyme inhibitors have high resistance potential.

To address the challenge of antibacterial drug development it is critical to understand physicochemical properties of antibacterial drugs as much as possible from prior efforts, and to apply learned lessons to the discovery of future antibiotics. Novel technologies such as genomics and high-throughput screening must be introduced and applied to improve productivity.

Lipinski's rule represented the first systematic attempt to correlate the physicochemical properties of drugs with the predicted successful matriculation of initial hits and subsequent late-stage leads. It is common to analyze molecular weight (MW), clogP, H-bond donors and H-bond acceptors prior to synthesizing novel candidates. Antibacterial compounds occupy a unique property space that is remarkably different compared to drugs of other therapeutic areas, and have always been considered an exception to these rules primarily because of their higher molecular weight and polarity (Table 1). Sixty four percent of 89 cytoplasm-targeted compounds follow 3 or 4 rules. Average MWs are usually higher for antibacterials, especially the group with Gram-positive only activity. Based on Shea's and Moser's research results, clogP of the reference CMC database and the group showing the Gram-positive activity is similar, but a substantial increase in polarity can be noted for Gram-negative group. This difference is even more striking when comparing clogD_{7.4} values that account for the charged state of molecules at neutral pH. The average value for Gram-negative antibacterials is more than 4 log units lower compared to the CMC data set. Compared to the set of reference compounds, polarity is slightly increased, as demonstrated with an average 36% higher relative PSA and decrease in clogD_{7.4} by almost 2 log units. Compounds with oral bioavailability are in compliance with the general rules established by Lipinski with the exception of macrocyclic compounds (macolides, rifampin, and rifalazil) and fusidic acid that are believed to possess high cellular permeability and carrier-mediated transport mechanism that facilitate oral bioavailability despite large MWs.

Table 1. Comparison of average compound property values of three drug data sets representing general (CMC, excluding antibiotics) and antibacterial drugs.

Class	CMC data set	Antibacterials (only Gram-positive activity)	Antibacterials (Gram-negative activity)
MW	338	813	414
clogP	2.7	2.1	-0.1
clogD _{7.4}	1.6	-0.2	-2.8
PSA(Å ²)	70	243	165
rel PSA(%)	22	30	42
H-donor	1.6	7.1	5.1
H-acceptor	4.9	16.3	9.4

CMC: constant mean curvature; MW: molecular weight; clogD_{7.4}: clogP at pH 7.4; PSA: polar surface area; rel PSA: relative PSA.

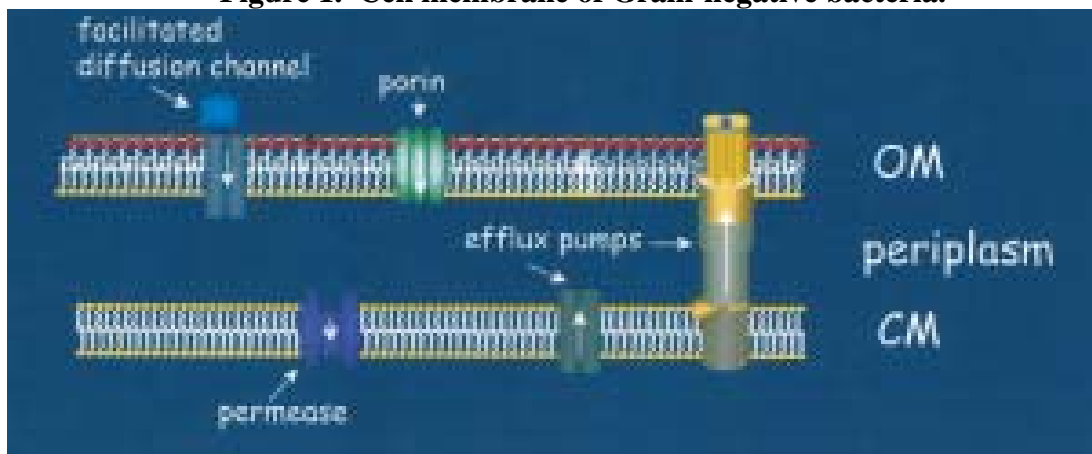
Table 2. Average physicochemical parameters for antibacterial classes.

Class	N	MW	clogP	clogD _{7.4}	PSA(Å ²)	rel PSA(%)	H-donor	H-acceptor
glycopeptides	5	1740	1.3	-1.8	586	37	22.8	37.2
macrolides	8	790	3.5	2.6	189	23	3.6	18
penicillins	14	413	1.4	-2.4	149	39	2.8	8.5
cephems	28	452	0.1	-3.0	210	51	4.1	10.8
penems	6	397	-3.0	-5.8	159	43	4.5	9
sulfa drugs	19	273	0.6	-0.1	112	45	3.1	6.2
fluoroquinolones	24	371	1.3	-0.8	82	25	2.1	6.5
tetracyclines	10	481	-0.7	-3.6	184	40	7.1	10.5
aminoglycosides	12	526	-2.9	-8.1	279	54	14.8	15.4

MW: molecular weight; clogD_{7.4}: clogP at pH 7.4; PSA: polar surface area; rel PSA: relative PSA.

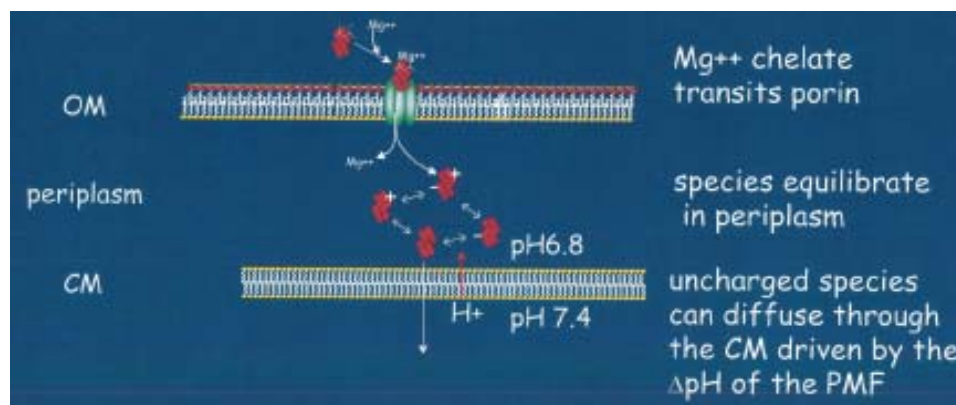
As the diversity of bacterial organisms is large, antibacterial activity is defined in general terms. One key factor for cell activity of drug candidates is their ability to penetrate the cell wall. Comparing to Gram-positive bacteria, Gram-negative bacteria contain an extra outer membrane that serves as an impermeable barrier for many small molecules (Figure 1). Compounds with activity against Gram-negative bacteria must overcome the outer membrane or enter via facilitated diffusion. The penetration of the outer membrane by porins prefers small hydrophilic, charged molecules, but highly charged molecules can not penetrate the cytoplasmic membrane unless actively transported. To fulfill these requirements, zwitterion molecules are beneficial (Figure 2). For instance, tetracycline and some fluoroquinolones are zwitterions that have both charged and uncharged species at physiological pH. The charged molecules can chelate Mg²⁺ and penetrate the outer membrane by porins. After the charged molecules equilibrate in periplasm, the uncharged molecules can diffuse through the cytoplasmic membrane driven by the difference of pH. The process is energy-dependent but not carrier-dependent transport.

Figure 1. Cell membrane of Gram-negative bacteria.



OM: outer membrane; CM: cytoplasmic membrane.

Figure 2. Gram-negative entry.



Efflux pump mechanisms are another barrier for activity as molecules with hydrophobic regions may be effluxed. The additional requirements appear to result in a large MW and an increase in polarity compared to the reference drug set. The increase in polarity is also reflected in the relative polar surface area, hydrogen bond donor, and hydrogen bond acceptor numbers. A large MW and an increase in polarity are partially believed to be driven by the properties of porin proteins that are thought to be major entry gates in Gram-negative bacteria. For example, *P. aeruginosa* has historically been one of the most difficult to treat because of reduced permeability, highly efficient and diverse efflux pumps. With a paucity of novel agents against *P. aeruginosa* under clinical investigation, their MWs are similar to that of the larger class of Gram-negative antibacterials, but the lipophilicity requirement is shifted toward even higher polarity. The $\text{clogD}_{7.4}$ values of the most compounds are below 0 and orally bioavailable compounds with *P. aeruginosa* activity are very narrowly distributed. The characterization of different families of efflux pumps, especially some members of the superfamily (*P. aeruginosa*), revealed the contribution of efflux to the intrinsic drug resistance. These efflux pumps were demonstrated to have wide substrate specificity and often contribute to multi-drug resistance in clinical isolates.

The regions of physicochemical space required to achieve both Gram-negative activity (high polarity to ensure porin permeability) and oral bioavailability (reasonable level of lipophilicity to guarantee lipid membrane permeability) seem to be largely non-overlapping. Fluoroquinolones, however, fulfill both these requirements. The key to meeting both requirements is the capability of molecules to exist in both charged (most zwitterionic) and noncharged form, the former to penetrate porins and the latter to be absorbed in the gut. The mostly zwitterionic fluoroquinolones are the best exemplars of these properties. Essential is the capability of charged and noncharged species to coexist at neutral pH, requiring the pKa values to be close to pH 7.4. For example, the experimental pKa values for ciprofloxacin are 6.15 and 8.66.

The unique physicochemical property space required for antibacterially active compounds must be taken into account during high-throughput screening to identify hits with whole cell activity. Natural products still are a good source to identify novel antibacterial hits and should be increasingly investigated further.