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Review

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A New-Class Antibacterial—Almost. Lessons in Drug Discovery and Development: A Critical Analysis of More than 50 Years of Effort toward ATPase Inhibitors of DNA Gyrase and Topoisomerase IV

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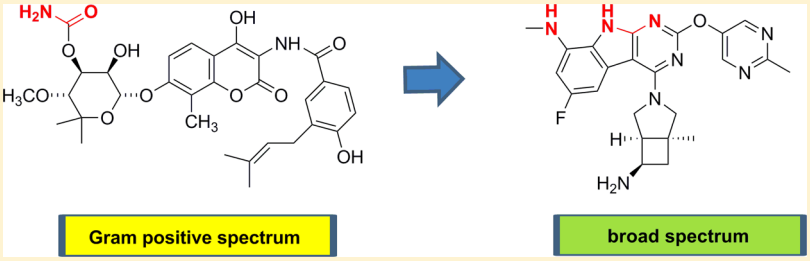

ABSTRACT: The introduction into clinical practice of an ATPase inhibitor of bacterial DNA gyrase and topoisomerase IV (topo IV) would represent a new-class agent for the treatment of resistant bacterial infections. Novobiocin, the only historical member of this class, established the clinical proof of concept for this novel mechanism during the late 1950s, but its use declined rapidly and it was eventually withdrawn from the market. Despite significant and prolonged effort across the biopharmaceutical industry to develop other agents of this class, novobiocin remains the only ATPase inhibitor of gyrase and topo IV ever to progress beyond Phase I. In this review, we analyze the historical attempts to discover and develop agents within this class and highlight factors that might have hindered those efforts. Within the last 15 years, however, our technical understanding of the molecular details of the inhibition of the gyrase and topo IV ATPases, the factors governing resistance development to such inhibitors, and our knowledge of the physical properties required for robust clinical drug candidates have all matured to the point wherein the industry may now address this mechanism of action with greater confidence. The antibacterial spectrum within this class has recently been extended to begin to include serious Gram negative pathogens such as *Pseudomonas aeruginosa, Acinetobacter baumannii*, and *Klebsiella pneumoniae*. In spite of this recent technical progress, adverse economics associated with antibacterial R&D over the last 20 years has diminished industry's ability to commit the resources and perseverance needed to bring new-class agents to launch. Consequently, a number of recent efforts in the ATPase class have been derailed by organizational rather than scientific factors. Nevertheless, within this context we discuss the unique opportunity for the development of ATPase inhibitors of gyrase and topo IV as new-class antibacterial agents with broad spectrum potential.

KEYWORDS: *gyrase, topoisomerase, GyrB, ParE, antibacterial, inhibitor, drug discovery, PK/PD, novobiocin, clorobiocin, coumermycin, BL-C43, RU79115, AZD5099, BTA-C223, VXc-486*

Early in 2011, the following announcement appeared in the Federal Register:

> *"The Food and Drug Administration (FDA) has determined that ALBAMYCIN (novobiocin sodium) capsule, 250 milligrams (mg) was withdrawn from sale for reasons of safety or effectiveness. The Agency will not accept or approve abbreviated new drug applications (ANDAs) for ALBAMYCIN (novobiocin sodium) capsule, 250 mg."*[1]

To many scientists and clinicians working in the area of antibacterial therapy, this announcement must have seemed rather unusual because the antibiotic novobiocin had not been manufactured in the U.S. since 1999 and had not been used therapeutically to treat bacterial infections in humans to any significant extent for many decades. First reported in 1955 and launched shortly thereafter, novobiocin (**1**, Figure 1) was a product of the "golden age" of antibiotics, during which time a great majority of today's antibacterial classes were discovered and put into clinical use.[2,3]

At the time of its introduction, novobiocin was generally viewed as a clinically effective, although imperfect, first-in-class agent. Historically across therapeutic areas, first-in-class agents are typically deficient in some manner and are often later displaced by improved versions of same-class agents, yet novobiocin remained the sole example in its mechanistic class of antibiotics. To this day, no other antibacterial agent has been launched having its novel molecular mechanism of action, namely, the competitive inhibition of the ATPase (adenosine triphosphatase) activities of the GyrB subunit of DNA gyrase and the ParE subunit of topoisomerase IV (topo IV).

For the past several decades, the introduction into clinical practice of novel-mechanism antibacterial agents has been a much sought after yet rarely achieved goal of antibacterial

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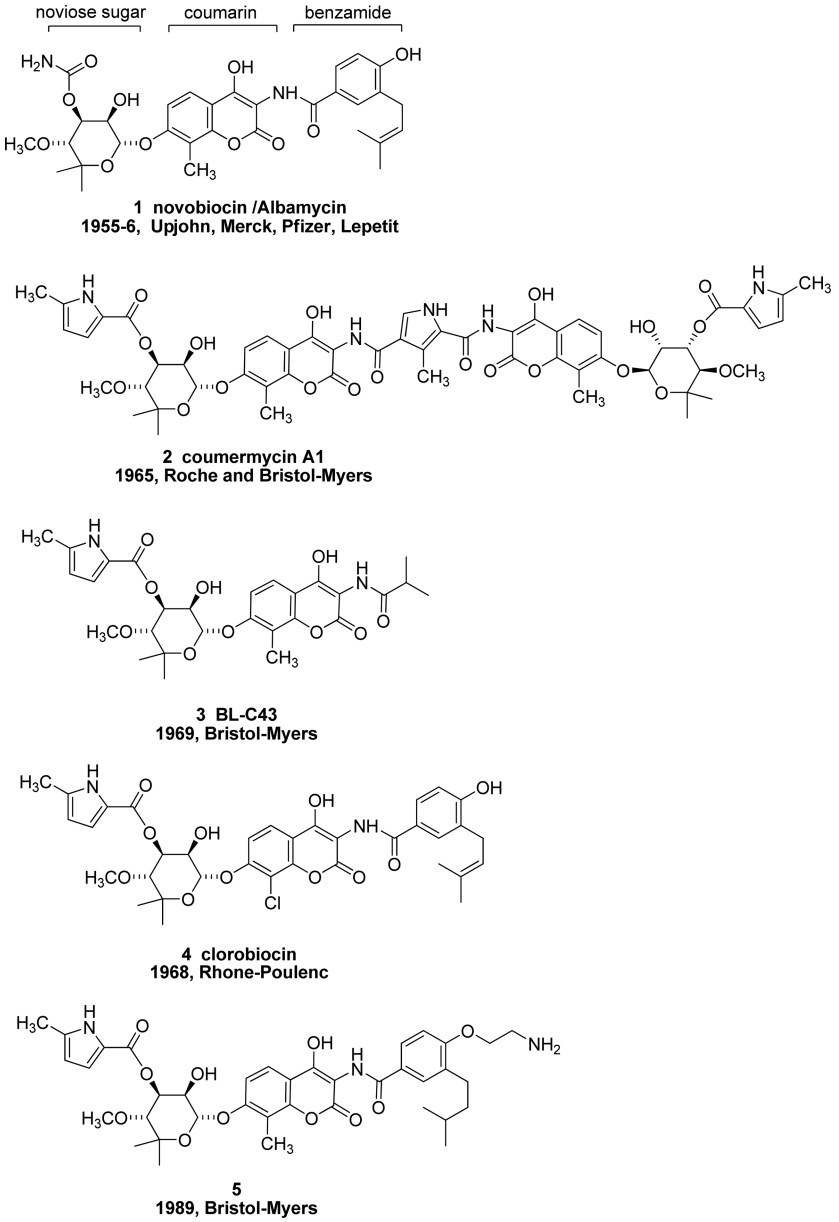

Figure 1. Structures of early coumarin scaffold GyrB/ParE inhibitor antibacterials.

research worldwide. It is broadly accepted that novel mechanism (new-class) agents represent unique and valuable opportunities to achieve significant advances against bacterial resistance because they should not be as susceptible to the pre-existing mechanisms of resistance as are established antibacterial classes, i.e., they should not exhibit cross-resistance.[4,5] This is in contrast to the more common strategy of introducing multiple new members within existing antibacterial classes, a strategy that is typically viewed as an incremental, and thus more temporary and limited, advance against resistance. The latter situation has persisted for most of the antibacterial classes used today including the β-lactams, aminoglycosides, tetracyclines, and quinolones. The introduction into clinical practice today of an ATPase inhibitor of gyrase and topo IV would provide a novel class agent to support our current efforts to combat increasingly resistant bacterial pathogens, including resistant Gram negative pathogens such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*.

In this critical analysis, we examine the historical gyrase/topo IV ATPase inhibitor programs to learn what may have hindered the development and launch of other such antibacterial agents during the last five and a half decades. Clearly, the failure to introduce an improved successor to novobiocin into clinical practice has not been due to lack of effort. Since the mid-1960s, major pharmaceutical companies and, more recently, biotechs have been engaged vigorously in research efforts toward that goal (Chart 1), yet despite seemingly determined and significant efforts by many teams of highly experienced drug R&D professionals over many decades, no antibacterial drug with novobiocin's mechanism has progressed even beyond Phase I. Why?

This narrative-style analysis summarizes individual drug discovery projects along with relevant key scientific discoveries beginning with the discovery of novobiocin and the historical context for which its medical usefulness was established. Over subsequent decades, the other multiple lines of research into same-mechanism agents are examined in their own historical

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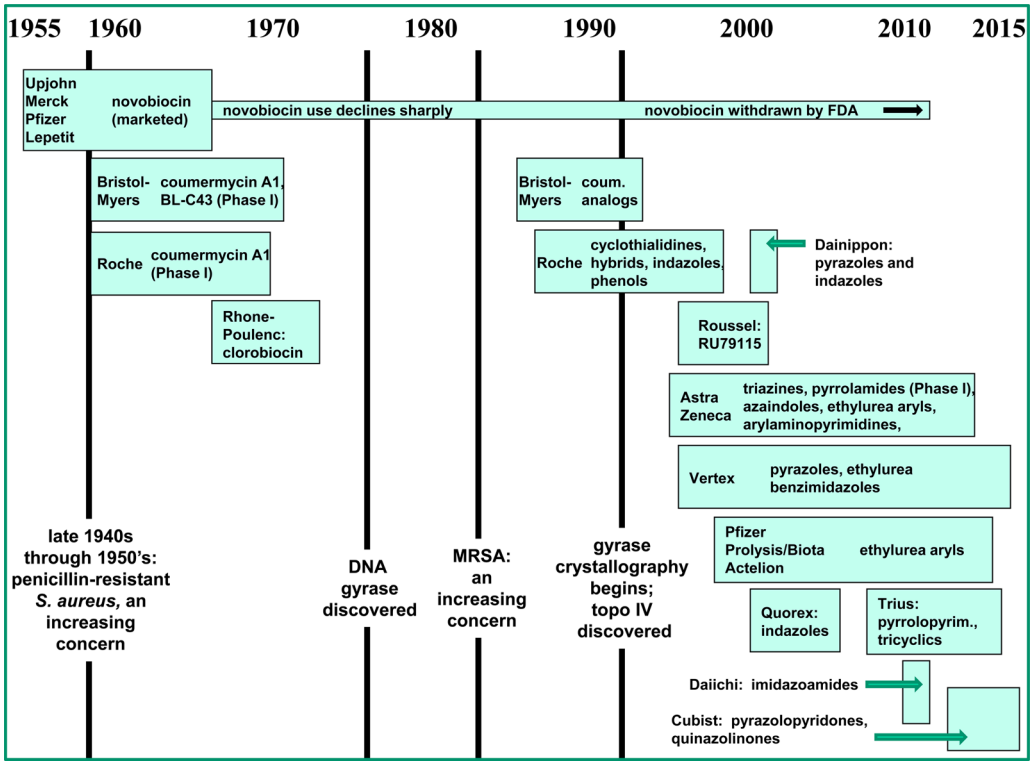
Chart 1. Timeline of GyrB/ParE ATPase Inhibitor Research and Development by Large and Small Biopharmaceutical Companies, along with Key Relevant Clinical and Scientific Events[a]



[a]Compounds or classes that entered human clinical trials are noted. With several exceptions, most project start and termination dates are only approximate because such estimates are most often inferred from dates of the company's patent applications and/or published articles. On occasion, published articles contained specific information on project start and/or termination dates; also a few project leaders were contacted by the authors for this information.

contexts, and as the history of novobiocin itself continued to evolve, they are shown to build on and influence one another. Overall the history can be viewed in three broad phases: (1) a pre-1990s phase, largely empirically driven with natural products being the source of drug discovery projects; (2) the decade of the 1990s wherein X-ray crystallography, the discovery of the second target topo IV, and a growing recognition of the factors governing resistance development and drug permeation through bacterial membranes contributed to an expanded scientific foundation; and (3) drug discovery projects initiated during and after the late 1990s that employed those new scientific foundations and shifted lead-finding methods from natural products to high-throughput screening (HTS) and/or computational techniques. The numerous drug scaffolds identified during this latter period are logically grouped together according to binding mode in the gyrase/topo IV enzymes and more specifically according to the particular molecular motifs bridging to a key aspartic acid and structural water unit residing within the ATP binding pockets of gyrase and topo IV (cf. Figures 7, 10, 12, 16, and 19).

On the basis of this analysis, we argue that the historical lack of success in developing gyrase/topo IV ATPase inhibiting agents lies not in any fundamental flaw in the target or mechanism nor in any of the scientific approaches to the discovery of novel natural product or synthetic scaffolds to engage that particular mechanism. On the contrary, the discovery and launch of agents within this class, especially in light of the considerable insight gained over the last 10−15 years, should now be one of the most scientifically achievable strategies for the introduction of a safe and effective novel mechanism antibacterial agent in the present era. In reviewing the historical attempts to discover and develop agents within this class, we discuss various factors that may have hindered those efforts. With the benefit of accumulated knowledge and experience, we regard those historical technical issues either as quite solvable or avoidable. We conclude this review by providing a broader context for the efforts toward gyrase/topo IV ATPase inhibitors within the evolving history and economics of antibacterial drug discovery and offer some concluding strategic observations that we hope will be useful to leaders of any new-class antibacterial program. We additionally hope that this analysis of the research and development of a specific class of antibacterial drugs might serve as a model for critical analyses of other lines of antibacterial drug discovery in the field or even for drug discovery projects in other therapeutic areas.

1. NOVOBIOCIN, A FIT-FOR-PURPOSE DRUG? HEYDAY AND CRASH (1955−1960s)

During the late 1940s and early 1950s, not only had the usefulness of the sulfonamide class of antibacterials declined drastically because of the emergence of resistant organisms, but penicillin itself was rapidly losing effectiveness against a range of medically important pathogens, most notably against *Staphylococcus aureus*.[6,7] During this time, most major pharmaceutical companies had entered the field of antibacterial R&D and were making important discoveries of new-class agents, several of which could potentially be used as clinically effective antistaphylococcal drugs in place of penicillin. For example

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erythromycin and vancomycin were discovered during the 1950s and entered clinical practice as antistaphylococcal agents, among other indications.

Against this backdrop of intensive antibiotic research and development, novobiocin was discovered and quickly recognized as a potentially important antistaphylococcal replacement for penicillin. Remarkably, this antibiotic was independently discovered through microbial natural products screening within the span of 2 years (1955−1956) by four pharmaceutical companies: Upjohn, Pfizer, Merck, and Lepetit. Each company initially gave it a different name, but the generic name novobiocin was eventually agreed upon and Upjohn commercialized it under the trade name Albamycin.[8−11]

The in vitro antibacterial spectrum of novobiocin is limited to Gram positive pathogens and to a few species of Gram negatives (Table 1, historical data presented).[12,13] In vitro, novobiocin was characterized as being either bactericidal or bacteriostic depending on the pathogen and test conditions used; against *S. aureus* it was described as having a "slow but definite, lethal effect on the cells".[14,15] Therapeutically, novobiocin was used primarily for infections due to penicillin-resistant *S. aureus* or to a lesser extent for pneumococcal pneumonia, especially when penicillin could not be used (for example, in individuals with a severe allergy to penicillin).[14,16−19] In an attempt to broaden the microbiological spectrum, Upjohn also marketed novobiocin as a fixed-dose combination with tetracycline, called Panalba. Fixed-dose combinations of antibacterials were commonly marketed during the 1950s and 1960s, although not without controversy.[20] In particular, one argument against Panalba was that the dose of each of the two ingredients in the combination was too low and would encourage resistance development.[21] Nevertheless, by 1961 it was estimated that annual production of novobiocin in the U.S. alone was about 15 000 kg.[19] When used as monotherapy, the oral dosage of novobiocin was typically 1−2 g per day administered in 2 to 4 divided doses.[12] It was well absorbed, with therapeutically useful concentrations readily achieved in the bloodstream (e.g., average peak serum level of 18.8 μg/mL following 0.5 g oral doses).[22] Although highly protein-bound, its MIC (minimum inhibitory concentration) values in the presence of serum were still sufficiently potent against a number of susceptible species to be therapeutically useful (Table 1, 64-fold increase in MIC for *S. aureus* 209P).[13] The sodium salt of novobiocin is readily soluble in aqueous solutions, and for intravenous administration, a similar daily dose (1 to 2 g) was typically employed.[23]

Table 1. MIC Values and s.c. and Oral ED_{50} Values for Novobiocin (1) and Coumermycin A1 (2)[a]

test organism	novobiocin (1)	coumermycin A1 (2)
	MIC (μg/mL)	
S. aureus 209P	0.05	0.0025
S. aureus 209P + 50% serum	3.2	0.16
S. aureus Smith	0.039	0.0012
S. aureus 52−34 (multiresistant)	0.78	0.0012
S. pneumoniae Type II	0.78	0.78
S. pyogenes Type 3	0.78	0.78
Neisseria sp.	12.5	12.5
E. coli ATCC 9637	50	6.25
S. flexneri	3.12	6.25
K. pneumoniae Type A	3.13	0.78
P. aeruginosa	100	12.5
	ED_{50} (mg/kg)[b]	
S. aureus s.c. administration	3.0	0.13
S. aureus oral administration	5.6	4.3

[a]Data taken from Kawaguchi et al., 1965.[13] [b]Mouse sepsis model.

However, cases of treatment failure were reported because of spontaneous resistance development during therapy with novobiocin.[7,16,17,24] Rash, sometimes severe, was the most commonly reported adverse effect associated with novobiocin use, and occasionally hematological disorders and gastrointestinal intolerance were also seen.[16,18,25,26] Interestingly, however, a small clinical study in the early 1990s that employed novobiocin in the context of methicillin-resistant *S. aureus* (MRSA) carriage found a relatively low incidence of rash (1 out of 45 patients), and the authors proposed that the earlier higher incidences of rash might have resulted from unspecified impurities in early batches of novobiocin that had since been removed.[27] This finding of a low incidence of rash compared to historical rates was further supported by later noninfection clinical studies wherein novobiocin was administered to patients in very high oral doses (3−9 g/day, with plasma concentraions of at least 150 μM sustained for 24 h at a 5.5 g dose) with no serious associated toxicities observed.[28,29]

During the 1960s and early 1970s, penicillinase-stable penicillins (methicillin, oxacillin, etc.) and the first cephalosporins became available, resulting in the decline in use not only of novobiocin but also of many of the other alternative antistaphylococcals. In 1969, a combined panel of the National Academy of Science and National Research Council, which had been systematically reviewing efficacy claims for over 3000 marketed drug products, stated that oral novobiocin should be taken off the market because of the "development of safer and more effective drugs".[21] Although Albamycin remained on the market (albeit with more restrictive claims and side effect warnings), the FDA ordered combination antibiotic Panalba off the U.S. market in 1969.[30] By 1978, one commentator stated that antibacterial therapy with novobiocin "had become more or less obsolete".[19]

2. BRISTOL-MYERS, ROCHE, AND RHONE-POULENC INVESTIGATE NEW NOVOBIOCIN-MECHANSIM DRUGS (1965−1970)

Even as antibacterial therapy with novobiocin was beginning to decline in the 1960s, other companies were eager to explore the development of structurally similar antibiotics. This was still the era when profits from antibiotics were key economic drivers for most pharmaceutical companies.[31,32]

In yet another instance of independent simultaneous antibiotic discovery, both Roche and Bristol-Myers' Japanese research unit reported in 1965 an antibiotic complex called coumermycin, the most potent member of which was the A1 component (**2**, Figure 1), which they had both been investigating since about 1960.[13,33,34] Of particular interest were the very different strategies each company followed toward the development of an antibacterial drug from this common starting point.

Structurally, coumermycin A1 resembles a dimer of novobiocin; a 2-methylpyrrole ester instead of a primary carbamate modifies each noviose sugar, and another pyrrole group asymmetrically links the two coumarin "monomers."

Both Roche and Bristol-Myers quickly recognized potential strengths as well as liabilities of coumermycin A1. Coumermycin A1 demonstrated greater antibacterial potency than novobiocin, including against *S. aureus*, and encompassed roughly the same overall, generally Gram positive spectrum (Table 1). Efficacy

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experiments in mice showed coumermycin A1 to be at least as effective as novobiocin when the compounds were administered by the s.c. or oral routes.[13]

Although well tolerated orally in mice, Roche found coumermycin A1 to be "appreciably toxic" when administered intravenously.[35] Moreover, like novobiocin, coumermycin A1 is highly protein-bound and shows a significant, though not therapeutically unacceptable, loss of microbiological potency in serum (Table 1, 64-fold increase in MIC for *S. aureus* 209P). Roche pushed coumermycin A1 forward into human oral studies but found relatively low oral bioavailability (which could be somewhat improved by formulation with *N*-methylglucamine) as well as gastric irritation.[36−38] Preliminary human efficacy studies using a 1 to 2 g daily dose were reported as satisfactory but additional testing gave "less encouraging results", and further clinical development of coumermycin A1 was terminated by Roche. Both the low oral bioavailability and i.v. tolerability issues associated with coumermycin A1 were ascribed to its extremely poor aqueous solubility.[19,34,39]

Bristol-Myers, after experiencing similar preclinical and clinical issues with coumermycin A1,[19] decided to embark on a semisynthetic analog program. At the outset of this program, they clearly identified four objectives:

(1) improve oral bioavailability,
(2) lower plasma protein binding,
(3) eliminate the dosing irritation liability, and
(4) preserve high activity.[39]

In particular, improving aqueous solubility was seen by the Bristol-Myers team as a means both to enhance the oral bioavailability and to reduce the i.v. dosing irritation liability. The optimization of aqueous solubility for antibacterial agents, then as now, is principally motivated by the desire to allow the formulation of these agents for i.v. administration as well as (where feasible) for oral adminstration. Dosing requirements for i.v. administration of many antibiotics typically require solubilities in the range of ca. 5−20 mg/mL (ca. 10−40 mM), a high bar that can be challenging to achieve. In terms of the optimization of free fraction, it was certainly understood during the 1960s, as it is today, that any significant loss of antibacterial potency due to the binding to plasma proteins ("serum shifted MICs") can be compensated by further optimizing the intrinsic antibacterial potency of the agents. In practice, however, the co-optimization of both properties (along with other properties) is typically undertaken, and a compromise is usually reached.

In their extensive program during the late 1960s, Bristol-Myers prepared more than 160 analogs of the coumermycin A1 "monomer" with variation principally of the right-hand amide group attached to the coumarin core. An optimized analog having an isobutyryl amide group and labeled BL-C43 (**3**, Figure 1) demonstrated all of the desired improvements, whereas at a cost of some in vitro potency compared to that of both coumermycin and novobiocin (Tables 2 and 4). BL-C43 was shown to be bactericidal against *S. aureus*.[39] Encouragingly, efficacy from the oral dosing of BL-C43 (**3**) in several experimental models of infection in mice demonstrated its superiority over both novobiocin and coumermycin A1 (Table 5), which was attributed to its greatly improved free fraction (Table 4) and improved oral absorption. In particular, in humans, although coumermycin A1 gave blood levels of less than 0.1 μg/mL after a single oral dose of 500 mg, an equivalent dose of BL-C43 achieved peak blood levels of over 15 μg/mL in 3 h and the levels were still high after 10 h. The improved pharmacokinetics of BL-C43 seen in animals and man was attributed to both improved solubility and to an unexpected hepatic excretion and reuptake mechanism.[40]

Safety testing in rats and dogs reported that BL-C43 (**3**) caused no gross or microscopic pathological changes at the maximum oral doses administered (100 mg/kg for 36−39 days), so a 14 day tolerance study of healthy human volunteers was initiated. However, after 8−10 days of administration of doses that were only 10−20% of the highest dose used in dog toxicology studies, the majority of the 25 volunteers showed evidence of mild jaundice and/or rash, and one volunteer

Table 2. MIC Values for BL-C43 (3) Compared to Those for Novobiocin (1)

	MIC (μg/mL)	
test organism (number of strains)	novobiocin (**1**)	BL-C43 (**3**)
S. aureus (2)[a]	<0.01	0.5
S. aureus (4)[b]	0.18	0.9
S. pneumoniae (4)	1	3.2
S. pyogenes (4)	0.5	2

[a]Nonpenicillinase producer. [b]Penicillinase producer.

Table 3. MIC Values for Clorobiocin (4) Compared to Those for Novobiocin (1)

	MIC (μg/mL)	
test organism	novobiocin (**1**)	clorobiocin (**4**)
S. aureus ATCC 29213	<0.06	<0.06
S. aureus 42080	0.25	<0.06
S. aureus 80CR5	>32	4
S. pneumoniae 1/1 serotype 6	8	2

Table 4. Protein Binding and Aqueous Solubility for Novobiocin (1), Coumermycin A1 (2), Bl-C43 (3), and Clorobiocin (4)

antibiotic	protein binding; % free (method)	aqueous solubility
novobiocin (**1**)	0.4% (HSA)[a]	freely soluble as sodium salt[c]
coumermycin A 1 (**2**)	1% (95% serum)[b]	insoluble or poorly soluble in any salt form[d]
BL-C43 (**3**)	22% (95% serum)[b]	greatly improved over coumermycin A1[d]
clorobiocin (**4**)	0.01% (HSA)[a]	very soluble as diethanolamine salt[c]

[a]Human serum albumin, Coulson et al.[233] [b]Keil et al.[40] [c]Berger et al.[19] [d]Cron et al.[39]

Table 5. ED_{50} Values for the Oral Administration of Novobiocin (1), Coumermycin A1 (2), BL-C43 (3), and Clorobiocin (4)

	ED_{50} (mg/kg); oral administration; mouse sepsis model			
test organism	novobiocin (**1**)	coumermycin A 1 (**2**)[c]	BL-C 43 (**3**)	clorobiocin (**4**)
S. aureus A9537[a]	26	8	8	ND
S. aureus A9606[b]	38	>80	10	ND
S. aureus[d]	ND[e]	ND	ND	19
S. pneumoniae A9585	800	>200	196	ND
S. pyogenes A9704	>500	>200	180	ND

[a]Nonpenicillinase producer. [b]Penicillinase producer. [c]Toxic at 400 mg/kg. [d]Strain not specified (Berger et al.[19]). [e]ND = no data.

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developed signs of congestive heart failure. The study was stopped, and all volunteers who had adverse events made an uneventful recovery.[34] Because of the clear safety liability, further development of BL-C43 was terminated as was the analog program. There is no indication from the published records that Bristol-Myers contemplated developing a backup to BL-C43. Considering the failure of their animal toxicology panel to predict human tolerability and without any apparent hypotheses to explain the underlying cause(s), it is understandable that no backup program was initiated.

Of interest was an in vitro resistance study for BL-C43, in comparison to novobiocin, erythromycin, and lincomycin.[41] The development of resistance in *S. aureus* A9497 to each of the antibiotics was determined by serially transferring the culture in subinhibitory concentrations of the agents. The changes in the MIC for each drug after the transfers are shown (Table 6). *S. aureus* developed high-level resistance to BL-C43, novobiocin, and erythromycin rapidly, and lincomycin showed a much lower elevation in MIC. These data for novobiocin and erythromycin were consistent with historical clinical experience where high rates of resistance development were seen for both drugs during treatment.[17] On the basis of these in vitro data, it might reasonably be concluded that BL-C43, had its development not been halted by safety issues, may well have shown the same level of resistance-development liabilities as novobiocin in clinical practice.

Table 6. Rates of Resistance Development for *S. aureus* A9497 to BL-C43 (3), Novobiocin (1), and Comparators

	number of transfers on antibiotic-containing plates			
antibiotic	0	4	8	12
	MIC (μg/mL)			
BL-C43 (**3**)	0.63	32	500	1000
novobiocin (**1**)	0.16	8	250	1000
erythromycin	0.16	16	63	>500
lincomycin	0.32	2	32	63

The investigation of clorobiocin (**4**, Figure 1), another coumarin scaffold natural product antibiotic, lagged a few years behind the coumermycin A1 studies of both Roche and Bristol-Myers. First reported by Rhone-Poulenc in 1969,[42] the structure of clorobiocin can be viewed as a hybrid, containing the "monomeric" 2-methylpyrrole-noviose-coumarin functionality of coumermycin but incorporating the right-hand amide functionality of novobiocin. Its antibacterial spectrum is very similar to those of both novobiocin and coumermycin A1 and was generally regarded as more potent in vitro than novobiocin (Table 3). Clorobiocin was more highly protein-bound than novobiocin but had good aqueous solubility as the diethanolamine salt. The efficacy (ED_{50}) of clorobiocin in a mouse model of *S. aureus* infection was reported to be 5.6 mg/kg s.c. and 19 mg/kg p.o. (Table 5, p.o. value compared to reference agents).[19] Little advanced preclinical work was published on clorobiocin, and neither Rhone-Poulenc nor any other sponsor initiated clinical work. We speculate that in light of the contemporary negative experiences from both Roche and Bristol-Myers, as well as the commercial decline of novobiocin itself, Rhone-Poulenc may have been reluctant to develop a closely related analog of its own.

Ironically, starting in 1976, after both the steep decline in novobiocin clinical use and the abandoned efforts of Bristol-Myers, Roche, and Rhone-Poulenc in developing their own coumarin antibiotics, the common mechanism for all these agents (as well as for the quinolone antibacterial class) began to be elucidated. These coumarin-scaffold agents inhibited the ATPase activities of the GyrB and ParE subunits of gyrase and topo IV, respectively (sections 5 and 6 below).

It is worthwhile to mention that contemporary literature, without consulting the original source material, frequently lumps together novobiocin, coumermycin A1, and clorobiocin and describes the coumarin class as "toxic" and having "poor physical properties." In reality, each of those agents had certain individual drawbacks but also certain strengths. For example, novobiocin has excellent aqueous solubility and pharmacokinetics, and its reputation for poor safety seems contradicted by the more recent clinical experience cited above.

3. RISE OF MRSA: NOVOBIOCIN IS BRIEFLY RESURRECTED, AND BRISTOL-MYERS STARTS ANOTHER COUMARIN PROGRAM (1980s)

The emergence of MRSA (methicillin-resistant *S. aureus*) as a clinical concern in the early 1980s prompted Bristol-Myers to reinitiate a program based on semisynthetic analogs of the coumarin class.[43−45] The in vitro susceptibility of clinical MRSA strains to novobiocin, coumermycin A1, and clorobiocin was typically quite good, and as expected because of their different mode of action, there was no cross-resistance to any of the other classes of existing antibacterials. During this period, there were few options for the treatment of MRSA especially by the oral or oral plus i.v. routes. Vancomycin was being used with increasing frequency against MRSA infections, but was an i.v.-only drug. Because novobiocin had already proved to be clinically useful when administered both orally and i.v. against penicillin-susceptible and -resistant staphylococci, the advantage of a dual mode of administration undoubtedly added to the attraction of the coumarin class specifically for the treatment of MRSA.

One aspect of Bristol-Myers' modest medicinal chemistry program, reported during the late 1980s to early 1990s, essentially continued their 1960s strategy by examining additional amide variations on the right-hand side of the methylpyrrole-noviose-coumarin core, this time emphasizing substituents of higher polarity (e.g., charged groups) to ensure high aqueous solubility (e.g., compound **5**, Figure 1). The Bristol-Myers team additionally examined the replacement of the 2-methylpyrrole with several other substituents and also varied the point of attachment of the methylpyrrole to the noviose. Ultimately, however, whereas novel analogs were identified having sub-μg/mL MIC values against MRSA, the potency was inferior to that of the parent coumermycin A1. Bristol-Myers seems to have terminated the program without reporting safety or in vivo animal efficacy data from any of their new analogs. Though it would have been both scientifically useful and technically feasible to generate biochemical data for the inhibition of gyrase, none was reported.

Concurrent with this Bristol-Myers effort, other groups were interested in exploring the usefulness of novobiocin itself against MRSA, for which it was shown to be bactericidal.[46] In 1989, investigators concluded that, on the basis of in vitro susceptibility data, "novobiocin may have a role in contemporary chemotherapy of oxacillin-resistant staphylococcal and other infections".[47] In 1993, a clinical MRSA colonization eradication study concluded that novobiocin plus rifampicin was "an effective and well-tolerated regimen for eradication of MRSA" and, importantly, that the combination controlled the emergence of resistance during therapy.[27] A second 1993 report, however,

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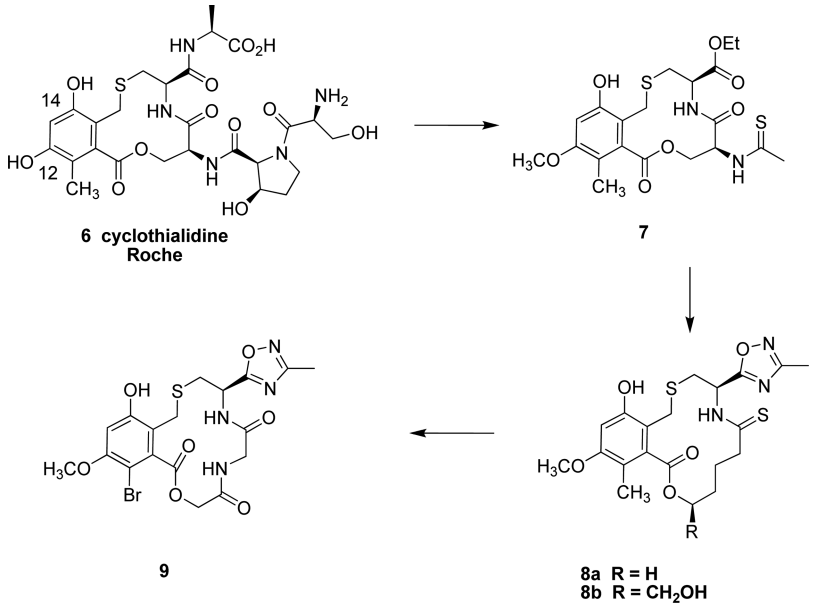

Figure 2. Evolution of Roche's cyclothialidine hit **6**, which possessed only weak MIC values, via analogs **7** and **8**, to an advanced analog **9** having efficacy in an animal model of infection.

Table 7. Biochemical Potencies, MIC Values, and ED_{50} Values for Cyclothialidine (6), Selected Analogs (7−9), and Comparators

enzyme or organism	6	7	8a	8b	9	novobiocin (1)	vancomycin
			Enzyme Potencies MNEC [IC_{50}] (μg/mL)[a]				
E. coli gyrase (supercoiling assay)	0.05 [0.25]	0.1 [0.4]	0.004 [ND][b]	0.005 [ND][b]	0.02 [ND][b]	0.1 [ND][b]	NA[c]
			MIC (μg/mL)				
S. aureus 887* or Smith	>128*	2*	0.06	0.5	1	0.12	1
S. aureus 25923	ND[b]	ND[b]	0.12	0.5	4	0.25	1
S. pyogenes 15	32	8	0.25	0.5	<0.12	1	1
E. faecalis 6	>128	16	0.25	0.25	0.5	4	4
E. coli DC2* or 25922	>128*	64*	>64	>64	>64	>64	>64
			ED_{50} (mg/kg), i.v. Mouse Sepsis Model				
S. aureus Smith	ND[b]	ND[b]	>25	12.5	3	3	1.5

[a]MNEC = maximum noneffective concentration. [b]ND = no data. [c]NA = not applicable.

suggested that novobiocin and rifampicin combinations may not be clinically effective in MRSA infections, primarily because of high serum protein binding of novobiocin.[48] Following this assessment, novobiocin has not been further investigated to any significant extent clinically for the experimental treatment of MRSA infections.

4. ROCHE: CYCLOTHIALIDINES (1987−1997[49])

During 1992−1993, Roche reported using an in vitro *Escherichia coli* DNA supercoiling assay to search for novel natural product gyrase inhibitors.[50−52] Assaying gyrase in this manner would potentially identify inhibitors working through any mode of action on gyrase. After screening 20 000 culture broths, a new lead structure, cyclothialidine (**6**, Figure 2), was disclosed, and Roche quickly elucidated its specific GyrB ATP-site inhibitory mode of action. With parallels to the Bristol-Myers experience, Roche was also once again investigating GyrB ATPase inhibitors two decades after the termination of their own coumermycin A1 clinical program. Significantly, this was the first instance of a noncoumarin inhibitor of the ATPase, an early demonstration of the tolerance of the ATP binding site of gyrase toward very different chemical structures.

Roche clearly considered cyclothialidine to be only a "lead structure" inasmuch as the natural product was extremely weak antimicrobially, inhibiting the growth of only a few Gram positive organisms at high MIC (e.g., the MIC vs *Streptococcus pyogenes* was 32 μg/mL, Table 7). The high MIC values were presumed to be due to poor penetration across the bacterial cytoplasmic membrane, yet intriguingly, the biochemical inhibitory potency of cyclothialidine against *E. coli* gyrase was found to be 2-fold better than that of novobiocin and coumermycin A1 and significantly superior to that of quinolones, including ciprofloxacin (29-fold). In addition cyclothialidine appeared to be "broader spectrum" in its inhibition of gyrases from various pathogens than reference gyrase inhibitors. Cyclothialidine furthermore showed a greater selectivity for bacterial gyrase versus the analogous mammalian Type II topoisomerase (63 000-fold selective) compared to the coumarin antibiotics (6700-fold and 1250-fold for novobiocin and coumermycin A1, respectively) or quinolones such as ciprofloxacin (70-fold).[51,52]

On the basis of these encouraging findings, Roche must have certainly felt a powerful incentive to translate the apparent biochemical superiority and chemical novelty of cyclothialidine into a family of microbiologically active medicines. After all, during the previous two decades, quinolone (GyrA/ParC mechanism) gyrase inhibitors had expanded into a hugely successful antibacterial class. Over the following years, Roche optimistically and repeatedly portrayed cyclothialidine as the

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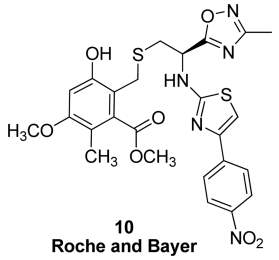
"basis for a new class of antibacterial agents"—a clean break from the older coumarin class agents—as well as mechanistically differentiated from the commercially successful quinolone class.[53,54]

Roche's first medicinal chemistry campaign to improve microbiological potency was described in their original 1992 patent filing, with over 170 analogs of cyclothialidine prepared by total synthesis, a true synthetic tour de force. During these early efforts, there was no crystallographic guidance for medicinal chemists. Nevertheless, critical SAR was quickly discovered, including the essential 14-position phenolic hydroxyl and and the nonessentiality of a major portion of the hydroxyl-ProSer motif on the right-hand side of the molecule. The 12-position hydroxyl could be methylated, affording for the first time analogs with modest potency against *S. aureus*. One such example was Ro46-9288 (**7**, Figure 2), which had MIC_{90} values of 4−8 μg/mL against *S. aureus* and *S. epidermidus*, which were still much less potent than the corresponding MIC_{90} values of <0.25−0.5 μg/mL for novobiocin (Table 7, MIC values). This analog was described as being "partially" bactericidal (less cidal than vancomycin, for example).[51] No in vivo efficacies were reported for these early analogs.

In addition to elucidating the SAR for microbiological activity, Roche monitored the gyrase activity of cyclothialidine analogs using their in vitro *E. coli* supercoiling assay. Conventional IC_{50} values were occasionally reported, but "for practical reasons", Roche most often reported the biochemical activity as maximum non-effective concentration (MNEC) values that were stated to be "3−5 times lower than the IC_{50} values" (Table 7).

Parallel to the extensive SAR studies of cyclothialidine lactone, Roche and Bayer both described acyclic analogs of the cyclothialidine macrolactone scaffold, which interestingly retained similar biochemical and microbiological potency compared to the cyclic analogs, once again demonstrating the tolerance of the GyrB ATP binding site to changes in inhibitor structure.[55,56] Ro 61-6653 (**10**, Figure 3) is a representative



10
Roche and Bayer

Figure 3. Structure of cyclothialidine acyclic analog **10**, which both Roche and Bayer had investigated.

example from this acyclic subclass (IC_{50} and MIC values shown in Table 8). A protein-binding liability in this class was reported for the first time: MIC values shifted at least 64-fold in the presence of 10% horse blood. Therefore, like novobiocin, high protein binding was also apparently an issue with this new natural-product-based series.

Mechanistically, even though both the coumarin-class antibiotics and the cyclothialidines bind to gyase by partially overlapping the ATP binding site of GyrB, they do so with somewhat different sets of interactions. Roche looked at a few spontaneous mutants resistant to cyclothialidines in *S. aureus* and found that most were not cross-resistant to the coumarins.

Table 8. IC_{50} and MIC Values for Acyclic Analog 10 and Effect of Protein Binding by the Addition of 10% Horse Blood

enzyme or organism	**10**	**10** + 10% horse blood
		IC_{50} (μg/mL)
E. coli gyrase (supercoiling assay)	0.1	NA[a]
human DNA Type II topoisomerase	60	NA[a]
		MIC (μg/mL)
S. aureus 133	0.25	16 (64-fold)
S. pyogenes 4851	0.25	16 (64-fold)
E. faecium L4001	0.25	32 (128-fold)
E. coli Neumann	>64	>64

[a]NA = not applicable.

Conversely coumermycin A1 and novobiocin-resistant mutants were still sensitive to cyclothialidines.[57]

In 1996, an X-ray crystal structure of a natural cyclothialidine variant GR122222X (**11**, Figure 4, discovered independently by Glaxo) in complex with the 24 kDa fragment of *E. coli* GyrB was solved.[58,59] Whereas Roche did briefly discuss the crystal structure in several of their subsequent SAR publications, it is unclear how much of their medicinal chemistry effort was truly influenced by it. At the very least, the role of the key 14-phenolic hydroxyl was recognized to be involved in a network of hydrogen bonds with Asp73 and a tightly bound water (Figures 4 and 10).

Reports published during 2003−2004 describe how the cyclothialidine scaffold had evolved further at Roche during the 1990s, with several analogs specifically highlighted, among them compound **8a** (Figure 2), which displayed outstanding gyrase inhibitory as well as antibacterial activity against Gram positive pathogens (Table 7).[54,60] However, it was also appreciated that the hard-won improvements in microbiological potency were not translating into in vivo efficacy because of a combination of factors, i.e., moderate to high protein binding (88−96% bound) as well as glucuronidation of the key 14-hydroxy phenol leading to high clearance. Although aqueous solubility data were not reported for this series, a solubility issue was implied in the published report. As a consequence, a hydroxymethyl group was incorporated into **8b** and related analogs not only to lower the lipophilicity of the scaffold (presumably to lower both the protein binding and mitigate the glucuronidation clearance mechanism) but also to "increase their water solubility". When dosed i.v., analog **8b** demonstrated an ED_{50} of 12.5 mg/kg compared to 3 and 1.5 mg/kg for novobiocin and vancomycin, respectively, in a mouse sepsis model of *S. aureus* infection (Table 7).

Further optimization of the cyclothialidine lactone scaffold provided analogs with excellent antibacterial activities, expressed as MIC_{90} values against panels of contemporary multiresistant Gram positive (*S. aureus*, *Enterococcus faecalis*, *Enterococcus faecium*, and *Streptococcus pneumoniae*) and respiratory Gram negative pathogens (*Haemophilus influenzae* and *Moraxella catarrhalis*).[61] Roche had clearly recognized by this time that optimizing physical properties, e.g., fine tuning lipophilicity, was critical to achieving optimal in vivo efficacy. Its earliest analogs (including cyclothialidine itself) displayed poor MIC values because of high polarity and the consequent inability to penetrate the lipophilic cytoplasmic membrane efficiently, yet the most microbiologically potent compounds were highly lipophilic, leading to high protein binding and high metabolic clearance due to glucuronidation. Hence, Roche tried to optimize within a narrow physical property window. Similar to the Bristol-Myers experience leading to BL-C43 30 years earlier, Roche ultimately

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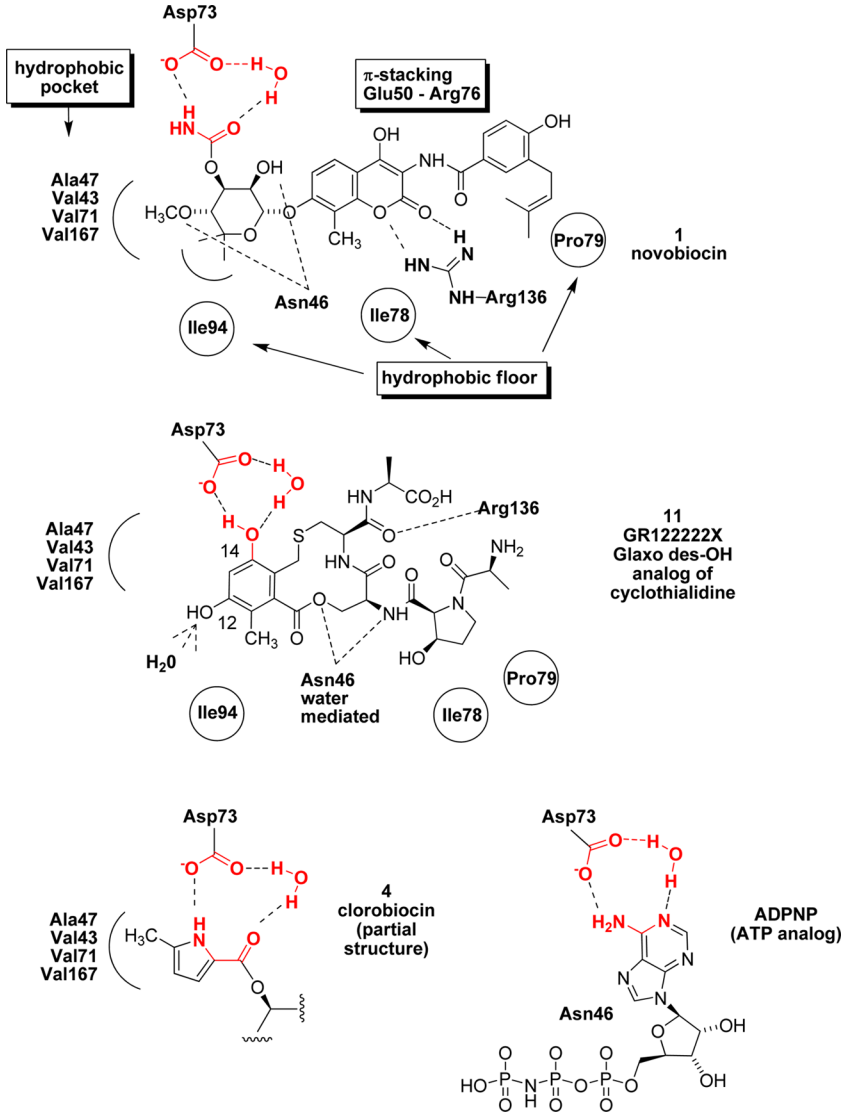

Figure 4. Illustrations of key ligand−enzyme binding interactions of novobiocin, GR12222X, clorobiocin, and ADPNP revealed by X-ray crystal structures using the 24 kDa N-terminal fragment of *E. coli* GyrB. Interactions of the critical Asp73−water motif with residues in each compound are shown in red. The adenine ring of ADPMP engages the Asp73−water motif as illustrated, but the triphosphate analog moiety largely binds in a pocket distinct from the regions that the inhibitor ligands occupy; see also Figure 5B.

found that the optimization of physicochemical properties and exposure came at the expense of some microbiological potency against *S. aureus*. Thus, the somewhat more polar diamide analog **9** (Figure 2), wherein the phenol 12-methyl was additionally replaced by a bromo group, was 16- to 32-fold and 2- to 8-fold less potent microbiologically against *S. aureus* compared to more lipophilic thioamide analogs **8a** and **8b**, respectively, yet it gained a considerable free fraction (resulting in a negligible serum-shifted MIC) along with mitigation of the glucuronidation liability. The net result was that analog **9** achieved a superior ED_{50} of 3 mg/kg when dosed i.v. in an *S. aureus* mouse sepsis model, now comparable to the efficacies of novobiocin and vancomycin (Table 7).

Little safety data was reported by Roche for their optimized series. Although a few optimized analogs were monitored for cytotoxicity in HeLa cell culture, showing variable margins, no safety investigations in animals have been reported.

For historical context, it is worth mentioning that Roche's original gyrase screening campaign 25 years ago was one of the earliest examples of a biochemical, rather than a "whole cell" (phenotypic), screen for antibiotics. It is a testament to the innovation and perseverance of the Roche scientists to have evolved such a microbiologically weak and structurally complex natural product screening hit into simpler molecules with potent MIC values against Gram positive pathogens and with a demonstration of excellent efficacy in *S. aureus* animal models of infection.

5. DISCOVERY OF TOPOISOMERASE IV AND THE CONCEPT OF POTENT DUAL INHIBITION: A KEY CRITERION FOR CONTROLLING RESISTANCE? (1990s ONWARD)

First characterized from *E. coli*, DNA gyrase has been recognized since 1976 as a bacterial target of both the coumarin and quinolone classes.[62−64] Only much later did evidence for the existence of a physiologically relevant "twin" to gyrase, namely, topoisomerase IV (topo IV), begin to accumulate, and by 1990−1992, *E. coli* topo IV had been characterized biochemically.[65,66] Additionally, during the 1990s, gyrase and topo IV from *S. aureus*

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and *S. pneumoniae* were isolated and characterized.[67−71] Investigators also worked out details of the mechanism for these Type II topoisomerases.[72] Briefly, gyrase and topo IV are both ATP-fueled heterotetramers operating by analogous mechanisms. Both enzymes transiently break double-stranded DNA, pass an intact DNA strand through the opening, and then reseal the double-strand nicks. Gyrase is primarily involved in the negative supercoiling of DNA during replication, and topo IV is involved primarily in DNA decatenation. The gyrase tetramer is composed of two subunits of GyrB and two of GyrA $[(GyrB)_2(GyrA)_2]$, and the topo IV tetramer is composed of two subunits of ParE and two of ParC $[(ParE)_2(ParC)_2]$ (Figure 5A). The GyrB and ParE subunits contain the ATP binding site (Figure 5B) and are thus the site of action for the coumarin antibiotics, cyclothialidines, and other more recent GyrB-directed synthetic inhibitors, discussed later. Members of the quinolone class, by contrast, interact with the GyrA and ParC subunits, binding to each in a ternary manner together with the covalently bound (and cleaved) DNA strand.[73,74]

During studies with quinolones in the 1990s, the extent of inhibition of each enzyme (gyrase and topo IV) by individual agents was found to be variable, depending on both the quinolone structure and the pathogen tested. In general, in Gram negative pathogens, gyrase tended to be inhibited more strongly by quinolones, and topo IV tended to be more sensitive in Gram positive pathogens.[75] Eukaryotic Type II topoisomerases exist, but their structures are sufficiently different from the bacterial Type II enzymes that achieving a safe margin of selectivity at the enzyme level, can typically be accomplished.

During the last 15 years, it has been suggested that for any bacterial Type II topoisomerase inhibitor the simultaneous and potent inhibition of both gyrase and topo IV is needed in order to mitigate the development of target-based resistant mutants as measured in vitro or as observed during drug treatment.[70,76−81] This view first grew out of quinolone studies (GyrA/ParC inhibitors) but was then extended to the ATP competitive antibacterial agents (GyrB/ParE inhibitors). For ATP competitive agents, according to this view, the goal is to effect potent inhibition of ATP binding at both gyrase and topo IV. The desirability of inhibiting multiple bacterial targets simultaneously to mitigate spontaneous target-based resistance development is an old concept, with the strategy of combination therapy for treating tuberculosis (TB) being a prime example. More generally, numerous thought leaders in the antibacterial field have stated the benefits of antibacterial "multitargetting" as a means to mitigate target-based resistance, either by the use of a single agent inhibiting two or more critical targets or by the use of a combination of drugs, with each inhibiting a single separate target. In the context of quinolones, David Hooper has remarked that

> *"...for a quinolone congener with equal activity against both topo IV and DNA gyrase, two mutations, one in each target would need to be present simultaneously for the first step in resistance due to an altered target to occur, and thus resistance would be substantially less frequent. This association of a low frequency of resistant mutants (<10^{-10}) and similar drug activities against both gyrase and topo IV has been seen with clinafloxacin in S. pneumoniae and with garenoxacin and gemifloxacin in S. aureus."*[77]

The initial characterization of mutants resistant to coumarin and quinolone antibacterials began in 1976, when bacterial gyrase was first reported. Gellert et al. isolated spontaneous

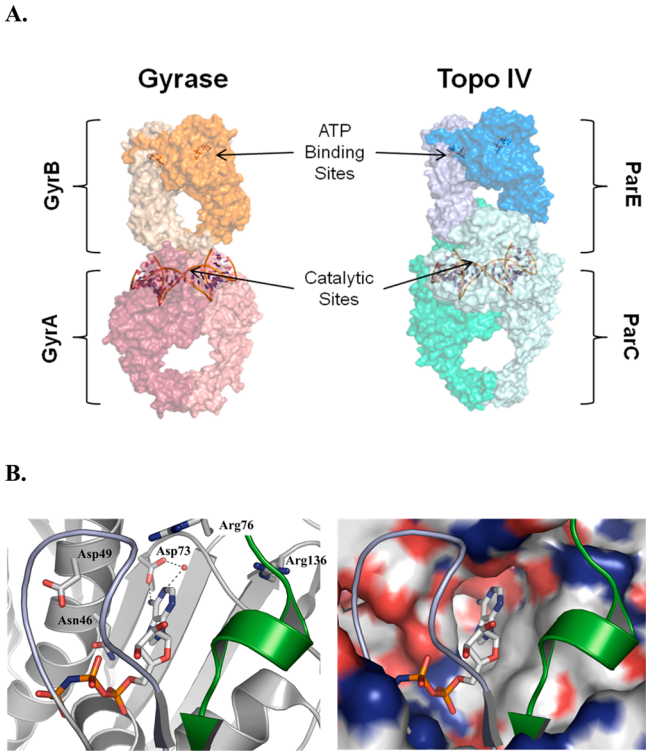

Figure 5. (A) Models for bacterial topoisomerase II tetramers: DNA gyrase (left) and topoisomerase IV (right). The arrows show the relative locations of the ATP binding sites in GyrB and ParE and the DNA cleavage/ligation catalytic sites in GyrA and ParC that are shown to bind the so-called "gateway" segment of DNA. Each monomer within the two tetramers is defined by a different color or shade of color. The models were constructed with *S. pneumoniae* ParC (PDB code 4I3H),[230] *E. coli* ParE (1S16),[162] *C. psychrerythraea* 34H GyrA (3LPX), and *E. coli* GyrB (1EI1)[231] using the X-ray crystal structure of the complete tetramer from *S. cerevisiae* (4GFH)[232] as a template. (B) ATP binding site of *E. coli* GyrB (PDB code 1EI1) in complex with the nonhydrolyzable ATP analog, ADPNP (adenosine 5′-(β,γ-imido)triphosphate). Most of the binding affinity for the nucleotide is derived from interactions between the enzyme and the phosphates. However, the adenine is positioned by a hydrogen-bond network with Asp73 and a bound water (left panel), and there are additional interactions between the base and ribose and the P-loop (or "ATP lid", shown in blue-gray). In the dimer, the N-terminus of the other GyrB subunit (green) packs against the P-loop and interacts with the Arg136, a key residue for binding affinity and selectivity among all known inhibitors of the ATPase. In addition to preventing the binding of ATP, SAR suggests that inhibitor binding disrupts dimer formation by displacing the N-terminus so that the binding pocket relevant to inhibitor design consists of a small hydrophobic pocket (apparent as a gray surface in the right panel, which is colored by atom type) distal to Asp73, an H-bond network with Asp 73 itself, and a largely hydrophobic surface that leads proximally to the basic arginine binding site.

resistant mutants of *E. coli* grown in the presence of novobiocin, although it was reported (without data or further explanation) that the resistance mechanism was due to reduced permeability, as opposed to target mutations. This assessment of the resistance mechanism conflicted with later work (1993) that claimed no evidence of permeability changes in novobiocin-resistant mutants of *E. coli*.[82] Nevertheless, in 1982 Hooper, in an important early study, measured the frequencies of resistance of *E. coli* exposed to a panel of coumarin class inhibitors that included novobiocin, clorobiocin, and coumermycin A1 as well as Bristol-Myers clinical agent BL-C43.[83] In particular, the

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Table 9. Comparative Biochemical Potencies for Novobiocin (1), Clorobiocin (4), and Coumermycin A1 (2) for the Inhibition of *E. coli* and *S. aureus* Gyrase and Topo IV Using Different Assay Formats in Different Laboratories, with Associated Selected MIC Values

enzyme or bacterium	enzyme assay method or bacteria ID	unit	novobiocin (1)	clorobiocin (4)	coumermycin A1 (2)	lab (year of publication)
E. coli gyrase	ATPase	IC_{50} (μg/mL)	0.045			Biota (2013)[91]
E. coli topo IV	ATPase		0.18			
S. aureus	ATCC 29213	MIC (μg/mL)	0.12			
E. coli gyrase	supercoiling	IC_{50} (μg/mL)	0.098			Institute of Microbial Chemistry, Tokyo (2012)[209]
E. coli topo IV	decatenation		>12.5			
S. aureus	Smith	MIC (μg/mL)	0.5		0.008	
	FDA209P		0.025			
	MS16526 MRSA		1			
E. coli gyrase	supercoiling (700 mM K-Glu)	IC_{50} (μM)	0.08	0.03	0.03	Heide (2011)[234]
E. coli topo IV	decatenation (100 mM K-Glu)		10	3	5	
S. aureus gyrase	supercoiling (700 mM K-Glu)		0.01	0.006	0.006	
S. aureus topo IV	decatentation (100 mM K-Glu)		20	10	100	
S. aureus	ATCC 29213	MIC (μg/mL)	0.25	<0.06	<0.06	
E. coli gyrase	supercoiling	IC_{50} (μM)	0.5			Merck (2011)[89]
E. coli topo IV	decatenation		10			
E. coli gyrase	ATPase		0.023			
E. coli topo IV	ATPase		0.45			
S. aureus gyrase	supercoiling		<0.004			
S. aureus topo IV	decatenation		35			
S. aureus	MB 5957	MIC (μg/mL)	0.5		0.008	
	MB2865 MSSA		0.25			
	MB5393 MRSA-COL		0.06			
E. coli gyrase	ATPase	K_i (μM)	0.013			Vertex (2006, 2008)[85,163]
E. coli topo IV	ATPase		0.160			
S. aureus gyrase	ATPase		0.019			
S. aureus topo IV	ATPase		0.900			
S. aureus	54 strains	MIC_{90} (μg/mL)	0.5			
E. coli gyrase	supercoiling	IC_{50} (μg/mL)	0.25	0.15		Hoechst Marion Roussel (2000)[124,125]
S. aureus gyrase	supercoiling		0.5			
S. aureus	011HT3	MIC (μg/mL)	≤0.04	≤0.04		
	011GO64 OfloOxaEry-R		≤0.04			
E. coli gyrase	supercoiling	IC_{50} (μM)	0.49	0.21	0.082−0.14	Hooper (1982)[83]

frequencies of resistance in *E. coli* induced by both novobiocin and BL-C43 ($(2-4) \times 10^{-6}$ and 10^{-6}, respectively) were at a level that, by today's standards, would strongly suggest a risk for clinical resistance development, at least for *E. coli*. The data for *E. coli* are qualitatively consistent with the earlier comparative resistance development studies done with novobiocin and BL-C43 using the serial passage of *S. aureus* (Table 6), both of which showed relatively high rates of spontaneous resistance development.

In 1993, the biochemical inhibition of gyrase and topo IV by novobiocin was measured for the first time: novobiocin inhibited *E. coli* gyrase at nanomolar levels (IC_{50} 6−160 nM), whereas it inhibited topo IV at a much higher concentration (IC_{50} 2.7 μM).[84] Subsequent investigators repeated and confirmed the relatively poor inhibition of *E. coli* topo IV by novobiocin and moreover found that clorobiocin and coumermycin A1 gave similar trends with the *E. coli* enzymes. Starting in the mid-2000s, the extents of inhibition of *S. aureus* gyrase and topo IV by novobiocin, clorobiocin, and coumermycin were measured, with topo IV again showing less sensitivity to inhibition by all three agents.[85,86] In particular, as measured by several investigators for the *S. aureus* enzymes, novobiocin demonstrated gyrase IC_{50} (or K_i) values of between <0.004 and 0.19 μM and topo IV inhibition of between 0.9 and 35 μM. (The factors influencing the large spread of inhibition values for each enzyme are discussed further below.)

Moreover, the frequencies of spontaneous resistance of *S. aureus* grown in the presence of novobiocin at 4-fold the MIC have been determined by several investigators over the past decade and are in the range of 10^{-7} to 10^{-8}.[85,87,88] Although not as high as the frequencies for *E. coli* determined by Hooper in the early 1980s ($(2-4) \times 10^{-6}$), this in vitro level of resistance frequency normally suggests a risk for clinical resistance development. Furthermore, when one considers the exceedingly

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low plasma levels of free (plasma-unbound) novobiocin following the historically recommended dosing protocol in man, it seems apparent that the free concentrations required to inhibit the *S. aureus* targets biochemically, especially topo IV (IC_{50} between 0.9 and 35 μM), cannot be adequately achieved or maintained clinically. In particular, with novobiocin's average peak (total) serum level of 18.8 μg/mL (ca. 30 μM) following administration of the historically recommended 0.5 g oral doses, the peak free (plasma unbound) levels would be 0.075 μg/mL, below the levels for inhibition of topo IV and possibly not at sufficient multiples of the IC_{50} of gyrase to ensure sustained coverage during trough periods. In retrospect, the historical clinical observation of rapid resistance development to novobiocin when used at those doses is consistent with the moderately high in vitro frequency of the spontaneous resistance of *S. aureus* (resulting presumably from potent inhibition at only one target, gyrase) as well as with the likely inability to sustain free drug levels during treatment above the inhibition concentration constants of both biochemical targets for a sufficient length of time.

This analysis suggests that modern GyrB/ParE inhibitors should be designed not only to be highly potent against both targets but also to be sufficiently unbound in plasma to fully manifest the biochemical potency in vivo in order to limit resistance development in addition to driving efficacy. In the authors' experience, agents having greater than 5−10% free plasma levels typically show relatively small serum MIC shifts and a higher propensity to demonstrate in vivo efficacy.

It should be pointed out that the measured values of biochemical inhibition of gyrase and topo IV for any given pathogen are greatly dependent on the assay techniques employed, leading to a high degree of lab-to-lab variation in reported data as illustrated above by the wide ranges reported for the inhibition of *S. aureus* gyrase and topo IV by novobiocin. Moreover some investigators report inhibition data in terms of K_i values whereas others report IC_{50} values (not to mention Roche's custom MNEC inhibition units, see Section 4). The assay format (e.g., gel supercoiling/decatenation versus ATPase format) or differences in concentration of certain assay reagents (e.g., potassium glutamate) can affect the absolute values of the inhibition concentrations by several orders of magnitude. For example, Vertex (2006) using an ATPase assay format reported *S. aureus* gyrase and topo IV K_i values for novobiocin of 0.019 and 0.90 μM, respectively, (a 45-fold difference between the two enzymes), and Merck (2011) reported IC_{50} values of <0.004 and 35 μM in supercoiling (gyrase) and decatenation (topo IV) assays, respectively, a >2000-fold difference (Table 9).[85,89]

In the absence of a single, standard enzyme format or even the rigorous use of a common reference compound such as novobiocin, it is not possible to assess quantitatively what "potent dual inhibition" precisely means. Fortunately, the majority of the published data agree qualitatively at least that novobiocin is a more potent inhibitor of gyrase compared to topo IV (Table 9), yet large differences in the absolute values for inhibition of any given target enzyme, as well as the variations in spread (fold difference) between the two target enzymes, leads to confusion in our mutual understanding of what potent inhibition actually means in practical, quantifiable terms. This uncertainty is potentially problematic because investigators use enzyme inhibition values to guide their medicinal chemistry programs, employing this data as temporary surrogates to forecast in vitro frequencies of spontaneous resistance, which in turn serve as a surrogates to estimate the likelihood and extent of clinical resistance development. Although to date several investigators have associated potent dual target inhibition values with low frequencies of spontaneous resistance in bacteria, the variable data resulting from different enzyme assay methods employed from lab to lab suggests that it is not yet possible to provide a generalizable, quantitative recommendation of the actual extent of potency required at each enzyme in order to achieve adequately low frequencies of resistance (Table 9). Furthermore, there is at the present time no clinical experience beyond novobiocin to set expectations regarding the extent of development of clinical resistance based on laboratory frequencies of resistance for other GyrB/ParE inhibitors. Additional drugs will need to be brought to the clinic to allow the further assessment of any meaningful correlations between in vitro measurements of potent dual inhibition, in vitro frequencies of spontaneous resistance, and the extent of clinical resistance for this class of antibacterials. To complicate matters further, any such correlations based purely on target mutation considerations will likely hold even less well for Gram negative pathogens (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, etc.), which, compared to Gram positives, can more readily develop resistance via nontarget-based mechanisms, especially mechanisms governing drug accumulation (lower permeation and greater efflux) as alluded to earlier in this section.

Finally, it is worth mentioning that the different mechanism of inhibition of gyrase and topo IV displayed by fluoroquinolones compared to ATP site inhibitors leads to some differences in the mode of bacterial cell killing. Whereas fluoroquinolones and GyrB/ParE ATP site inhibitors are both generally regarded as bactericidal, the cidal action of the fluoroquinolones is partially mediated by the toxic action of double strand breaks resulting from the enzyme-DNA-quinolone complexes.[90] Perhaps because of these differences the rate of cell killing by fluoroquinolones has been generally found to be more rapid than that achieved by ATP site inhibitors.[85,91] However, the clinical relevance of bactericidal versus bacteriostatic mechanisms in the treatment of infections is currently controversial;[92,93] therefore, varying degrees of cidality may or may not make a significant difference in the clinical use of these agents.

6. X-RAY CRYSTALLOGRAPHY: A STIMULUS FOR NEW DRUG DISCOVERY EFFORTS (MID-1990s ONWARD)

The X-ray crystal structure of the 43 kDa N-terminal fragment of *E. coli* gyrase B subunit in complex with ADPNP (adenosine 5′-(β,γ-imido)triphosphate), a nonhydrolyzable ATP analog, at 2.5 Å resolution was reported in 1991.[94] This was the first high-resolution structure of the bacterial ATPase domain and revealed that adenine forms a hydrogen bond network with conserved residue Asp73 and a bound water (Figure 4). In 1996, crystal structures were published for novobiocin and GR12222X (**11**), a member of the cyclothialidine (section 4, above) natural product family.[59] In these cases, the complex was with a 24 kDa N-terminal fragment derived from the 43 kDa fragment. Finally in 1997, both the Wigley lab and the Zeneca lab independently solved the structure of clorobiocin with the 24 kDa fragment of GyrB.[95]

The availability of these structures energized GyrB-targeted drug discovery efforts starting in the mid-1990s. As will be seen in the following sections, this new structural information immediately provided confidence to a number of biopharmaceutical companies for the rational redesign of known scaffolds or (more significantly) the pursuit of de novo approaches to lead discovery with the benefit of structure-informed lead optimiza-

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tion. In terms of therapeutic motivation, during the mid to late 1990s there was still the pressing need for new, effective agents against MRSA as well as against increasingly resistant enterococcus pathogens. At that time vancomycin, a parenteral-only drug, was essentially the only reliable agent for MRSA infections, and its effectiveness was waning against some enterococci, especially *E. faecium*. ATP-site GyrB/ParE inhibitors in theory could address those medical needs. The biopharmaceutical companies that entered the GyrB/ParE inhibitor field rapidly set up their own in-house crystallography efforts to facilitate iterative drug design.

The cocrystal structures of ADPNP, novobiocin, clorobiocin, and GR12222X with GyrB (and later ParE) ATPase domain fragments delineated several critical drug−protein interaction themes that were frequently utilized in subsequent drug design efforts over the years. Several of these observed interactions coincided with information derived from prior resistant mutant sequencing studies, thus validating the practical relevance of these drug−enzyme molecular interactions.

The most critical, and seemingly indispensable, interaction is a set of hydrogen bonds provided by Asp73 (*E. coli* gyrase numbering) and an associated water molecule. The adenine of ATP, as assessed through its analog ADPNP, forms a donor/acceptor motif as shown in Figures 4 and 5B. As it happens, the binding sites of ATP and those of all of the reported bacterial GyrB/ParE inhibitors overlap essentially only at this one key site of interaction. Asp73 is so important for anchoring ATP substrate turnover that no resistant mutants have been reported with a change at this amino acid. The remainder of the interactions for GyrB and ParE inhibitors are in a region extending away from the triphosphate binding site of ATP and make use of Arg136, a "hydrophobic floor" consisting of Pro79, Ile78 and Ile94, and a π-stacking "ceiling" formed by Arg76 within a Glu50-Arg76 salt bridge. An important additional hydrophobic pocket revealed by the clorobiocin structure is composed of Val 71, Val43, Val 167, and Ala 47 and is the binding site for the clorobiocin methylpyrrole moiety (Figure 4). The higher enzymatic potency of clorobiocin (and coumermycin A1) has been rationalized by the presence of this additional interaction. The greater microbiological potency of both clorobiocin and coumermycin A1 has been explained by a combination of this greater enzymatic potency along with better membrane permeability. Finally, there are two additional conserved residues (Asn46 and Asp49) that several recent series of inhibitors have targeted (the azaindole scaffold from AstraZeneca and the pyrrolopyrimidine and tricyclic scaffolds from Trius; sections 14 and 16).

From a target drugability standpoint, the binding pocket presents an attractive opportunity for binding small molecules having a balance of polarity and hydrophobicity targeting multiple residues. The ATPase GyrB and ParE domains are highly homologous across species, and in particular, the residues labeled in Figure 4 are largely conserved across pathogenic species and between GyrB and ParE. Thus, the target offers an excellent opportunity for the design and discovery of broad-spectrum antibacterial agents. At the same time, there is low conservation among residues that do not interact directly with the nucleotide in homologous ATPases, particularly in the vicinity of Arg136.

Humans express two Type II topoisomerases, topo IIα and IIβ, which are highly homologous. Despite low sequence homology with bacterial gyrase and topo IV, the human homologues adopt a similar fold.[98] However, the binding pocket is significantly more occluded in human topo II, and the critical Arg136 maps to a Glu (residue 185), making the binding of bacterial topoisomerase inhibitors less favorable. Indeed, in programs at AstraZeneca we routinely saw >3 orders of magnitude selectivity for the bacterial isozymes versus human.

Regarding selectivity versus other ATPases, gyrase is a member of the GHL (gyrase, HSP90, and MutL) subfamily of ATPases that exhibit striking differences from other ATPases[97] and adopt a similar nucleotide binding fold. Here again, important differences in the binding pockets proximal to the adenine binding region make interactions with these related enzymes unlikely. In HSP90, there is an occlusion of the binding pocket, loss of the π-stacking interaction via Arg76, and the mutation of Arg136 to histidine.[98] Similarly, in MutL, among other differences, Arg76 is replaced with a cysteine and Arg136 is replaced with a threonine.[99] Finally, in PMS2, a more recently identified GHL ATPase, there are again large differences, including significant occlusion of the "arginine binding region" by the shift of a helix and the loss of Arg76 to cysteine.[100]

Whereas X-ray crystal structures are useful in guiding design decisions for the optimization of enzyme potency, improvements in antibacterial activity require the co-optimzation of enzyme potency with factors governing cell permeation (see section below), the avoidance of strong plasma protein binding, and other factors governing free plasma concentrations. We have also seen with the Roche cyclothialidine project that the antibacterial potency can be successfully optimized in the absence of enzyme structural information. Nevertheless, with a bounty of newly revealed structural information coupled with the motivation to develop novel antibacterial classes effective against MRSA, it was with renewed energy that the industry approached gyrase drug discovery in the mid-1990s.

7. PROBLEM OF DRUG PENETRATION THROUGH BACTERIAL MEMBRANES: A GROWING AWARENESS YET LARGELY EMPIRICAL SOLUTIONS (1990s ONWARD)

One challenging feature of gyrase and topo IV as drug targets is their location in the cytoplasm, requiring the inhibitors of those enzymes to traverse the hydrophobic cytoplasmic membrane efficiently. For efficacy against Gram negative pathogens, compounds must traverse both the cytoplasmic membrane and the outer membrane, with its opposite physical property requirements and general preference for small, polar compounds.[101,102]

As early as the 1960s, Bristol-Myers observed the effect of compound polarity on *S. aureus* antibacterial activity during their coumarin analog SAR efforts. In particular, increasing the carbon chain length of the right-hand acylamino group gradually improved the antibacterial potency against *S. aureus* up to a maximum of 10 carbons, but further increases in chain length led to a precipitous loss in MIC potency.[103] Bristol-Myers did not specifically link the concept of the degree of polarity (or lipophilicity) to the extent of bacterial cell penetration, however. By contrast, in 1986 when gyrase assays were available, Upjohn reported that both novobiocin and its truncated free amino analog (lacking the lipophilic isopentenyl hydroxybenzoate moiety) inhibited *E. coli* gyrase equally, yet the polar novobiocin fragment was completely inactive microbiologically against *S. aureus*. Upjohn attributed that lack of bacterial activity to a membrane permeation deficiency associated with the more polar novobiocin fragment.[104] By the 1990s, both the Roussel-Uclaf

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coumarin program (described below) and the Roche cyclothialidine program employed the modulation of analog lipophilicity as a specific design strategy to optimize antibacterial potency by facilitating cytoplasmic membrane permeability. Both Roche and AstraZeneca used log *D* measurements to help guide their programs, and both companies concluded, on the basis of simple ratios of enzyme to antibacterial (MIC) potencies in comparison to corresponding log *D* values that for Gram positive bacteria a specific range of low to moderate lipophilicity (log $D \approx 0-3$) was typically optimal for passive cytoplasmic drug permeation.[61,105,106] Complicating any simplistic lipophilicity–MIC correlations, however, are the additional effects that lipophilicity may have on protein binding, aqueous solubility, and enzyme potency itself because of potential interactions of the drug with any of several hydrophobic patches within the GyrB and ParE binding pockets (e.g., the hydrophobic floor).[95]

Recently, substantial progress toward achieving antibacterial activity against important Gram negative pathogens such as *P. aeurginosa*, *A. baumannii*, and *K. pneumoniae* from the GyrB/ParE inhibitor series has been reported, most thoroughly documented by Trius (now Cubist) and described in detail below.[107−109] AstraZeneca and Biota have also recently seen potent MIC values against Gram negative pathogens.[110,111] The scaffolds from these programs are thus able to penetrate both the outer membrane and the cytoplasmic membrane of these pathogens. Expansion of the antibacterial spectrum of GyrB/ParE inhibitors to include many problematic Gram negative pathogens is a highly significant scientific accomplishment in the field. New-class drugs to treat infections caused by such pathogens are critically needed because of the rapidly diminishing therapeutic options for treating life-threatening infections caused by increasingly resistant organisms.

At present, however, beyond imperfect correlations of log *D* and ionic charge with antibacterial potency in Gram positive bacteria, there is disappointingly little further quantifiable and generalizable understanding of those specific factors that facilitate (or hinder) intracellular drug accumulation, especially those special sets of physical property factors that favor simultaneous permeation though both cytoplasmic and outer membranes in Gram negative bacteria and/or the avoidance of bacterial efflux pumps. Nevertheless, it has been suggested, for example, that the zwitterionic fluoroquinolones are able to permeate both membranes of Gram negative bacteria efficiently because of a combination of unique structural features, most important among them being a small size (for facile outer membrane porin transit) and an ability to adjust the polarity by altering ionic charge states within the zwitterionic manifold.[112,113] According to this view, fluoroquinolones may achieve the needed polarity to transit outer membrane porins as well as the needed lipophilicy to transit the cytoplasmic membrane via adjustment of the charged states, although this model may be an oversimplification.[114] The utility of the traditional and widely used informal measure of susceptibility of antibacterial drugs to efflux, i.e., the comparison of the wild-type pathogen MIC to the corresponding efflux-null mutant MIC, has been called into question.[115,116] It is argued that such measures can be misleading in the absence of direct measurements of permeation; for example, a slow rate of permeation could be misinterpreted as a high rate of efflux. Unfortunately, there are still no reliable general assays for the direct, routine measurement of drug permeation through bacterial membranes or for the intracellular accumulation of drug in bacteria, although some recent progress has been made toward such assays.[117,118] Encouragingly, there is ongoing interest and active research to elucidate the molecular influences of drug accumulation (both permeation and efflux) in ways that can be practically applied to new inhibitor design.[113,119,120]

8. ROUSSEL-UCLAF (HOECHST MARION ROUSSEL) RATIONALLY REDESIGNS NOVOBIOCIN: RU79115 (LATE 1990s)

Roussel-Uclaf (later as Hoechst Marion Roussel, HMR), at the Roumainville site near Paris, mounted a determined effort starting in the mid-1990s to develop an antibacterial clinical candidate based on the structure of the original coumarin class.[121−129] It appeared that their effort was partially motivated by the increasing problem of MRSA and partially by the new crystallographic information that could now, for the first time, influence such a medicinal chemistry program. Like Bristol-Myers' earlier efforts, the Roussel team clearly recognized the opportunities for improvement on the coumarin scaffold and announced their vision as the "rational drug design of structurally diverse analogs with improved pharmacological profile and physico-chemical properties". Moreover, differentiated from the two Bristol-Myers coumarin analog efforts, the Roussel group devised a completely synthetic (as opposed to semisynthetic) route to new analogs that allowed maximum design flexibility in ways not previously possible.

After several years of systematic and structure-guided exploration of SAR at many positions around the coumarin scaffold, optimized analog RU79115 (**12**, Figure 6) was reported during 1999−2000.[125,130] The advantage of RU79115 was its potency both in vitro and in vivo against a range of susceptible

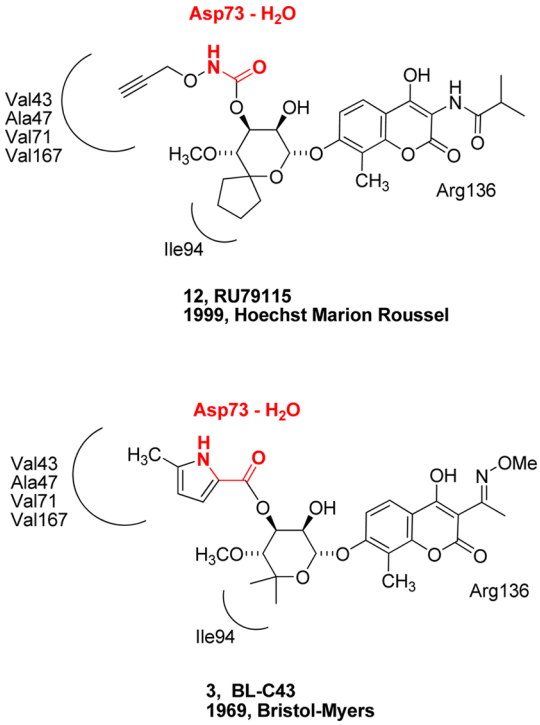

Figure 6. Comparison of **12**, RU79115, Roussel's 1999 structure-guided optimized analog of novobiocin with **3**, BL-C43, and Bristol-Myers optimized analog of coumermycin A1 from 30 years earlier. Several ligand−enzyme interactions are indicated, with Asp73−water interactions shown in red. The binding mode of **3** is inferred on the basis of other inhibitor crystal structures.

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and multiply resistant *S. aureus* strains (Tables 10 and 11). In vitro MIC values against *S. aureus* were comparable to novobiocin and clorobiocin and, not surprisingly, on the basis of structural similarity, there was some cross-resistance with novobiocin as seen with *S. aureus* strain 011HT1. MIC values for RU79115 were somewhat higher than for novobiocin against other Gram positive pathogens such as *S. pyogenes* and *E. faecium*. RU79115 was reported to be bactericidal against both *S. aureus* and *E. faecium*. Favorable pharmacokinetics were shown for RU79115 in mice, with a half-life of 2.5 h and oral bioavailability of 62%; the C_{max} was 5.4 μg/mL following a 10 mg/kg oral dose. Although SAR for the inhibition of *S. aureus* gyrase was developed within the series, neither topo IV inhibition values nor frequencies of spontaneous resistance were reported for RU79115 or its analogs, so it is unclear whether rapid resistance development might be a liability. What is also missing from the published descriptions of RU79115 (as well the majority of other coumarin analogs from the Roussel team) is quantitative assessments of solubility, protein binding (or, alternatively, serum shift MIC measurements), and in vitro or in vivo safety assessments. The absence of reported physicochemical data seems at odds with the original vision statement that set improvements in physical properties as a clear goal. Nevertheless, the low ED_{50} values of orally dosed RU79115 (mouse sepsis model) in comparison to the values for oxazolidinones and vancomycin, and the fact that RU79115 seemed to have been tolerated at the moderate doses used for the efficacy studies, suggest that any physicochemical or safety issues were not severe (Table 11). It is interesting to compare the structure of RU7115

Table 10. IC_{50} and MIC Values for RU79115 (12) Compared to Those for Novobiocin (1) and Clorobiocin (4)

enzyme or organism	RU 79115 (**12**)	novobiocin (**1**)	clorobiocin (**4**)
		IC_{50} (μg/mL)	
S. aureus gyrase (supercoiling assay)	0.2	0.5	0.3
		MIC (μg/mL)	
S. aureus 011HT3[a]	<0.04	<0.04	<0.04
S. aureus 011GO64[b]	<0.04	<0.04	ND[d]
S. aureus 011HT1[c]	1.2	10	0.6
S. pyogenes 02A1UC1	0.15	<0.04	<0.04
E. faecium 02D31P2	0.15	0.6	<0.04

[a]Susceptible strain. [b]Ofloxacin-, oxacillin-, erythromycin-resistant. [c]Novobiocin-resistant. [d]ND = no data.

to Bristol-Myers' clinical analog BL-C43 (**3**) of 30 years earlier (Figure 6). Compared to novobiocin/clorobiocin, both of these advanced analogs have similar highly truncated right-hand sides, and both seem to be able to achieve similar binding interactions within the enzyme. Although comparisons of data separated by 30 years comes with caveats, both compounds demonstrated rather comparable ED_{50} values in *S. aureus* mouse sepsis models (1−7 mg/kg for RU7115 versus 8−10 mg/kg for BL-C43). After the early 2000s, no further information was disclosed on this program, and it appeared to have been terminated.

9. ZENECA'S AMINOTRIAZINE STRUCTURE-BASED EFFORT (LATE 1990s)

Concurrent with the publication of the novobiocin-GyrB crystal structure in 1996, Zeneca was conducting a medicinal chemistry program focused on fully synthetic novobiocin and clorobiocin

Table 11. ED_{50} and MIC Values for RU79115 (12) Compared to Those for Eperezolid (or Linezolid) and Vancomycin

	ED_{50} oral (mg/kg) [MIC μg/mL][a]	ED_{50} s.c. (mg/kg) [MIC μg/mL][a]	
test organism	**12**, RU79115	eperezolid (e) or linezolid (l)	vancomycin
S. aureus HT18	5.6 [<0.04]	20 [0.6] (e)	7.8 [1.2]
S. aureus GR56[b]	1 [0.08]	5 [1.2] (e)	4 [1.2]
S. aureus HT17	2.8 [0.15]	5.8 [1.2] (l)	ND [ND][c]
S. aureus GO3	7.1 [0.3]	8.7 [2.5] (l)	ND [ND][c]
S. pyogenes A1UC1	37.6 [0.15]	22.8 [<0.15] (e)	<1.5 [0.6]
E. faecium D3AP9	44 [0.6]	8.7 [1.2] (e)	>50 [>40]

[a]Mouse sepsis model. [b]Oxacillin-resistant. [c]ND = no data.

analogs constrained by a spiroketal moiety.[131] None of these analogs, however, were biochemically or microbiologically active, which the authors attributed to several nonoptimal enzyme interactions, including poor stacking with Arg76, based on modeling. That series was abandoned. Biochemical and microbiological activity was, however, achieved by Zeneca in a follow-on series of di- and trisubstituted triazines disclosed initially in a 1999 patent application.[132−134] This Zeneca triazine project was noteworthy because it represented the first reported GyrB/ParE inhibitor medicinal chemistry effort not based on natural products. The scaffold was identified from high-throughput screening of the Zeneca corporate compound collection. Both X-ray crystal structure information (for **13**, Figure 8) and protein

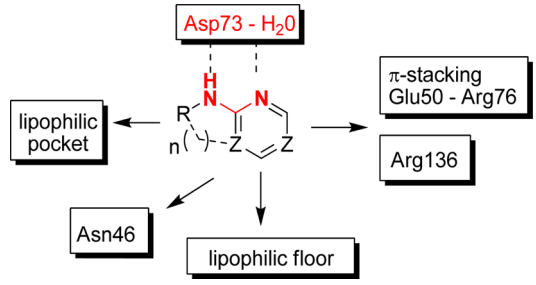

Figure 7. General binding motif for Zeneca's triazines, Roche's hybrid series, AstraZeneca's arylaminopyrimidine, and Trius's pyrrolopyrimidines. R = H, ring carbon, or aryl; n = 0, 1; Z = C, CH or N. Binding motif for ATP but only for Asp73-H20 and Asn46 interactions. The key Asp73−water interaction is shown in red.

NMR structure work (for **14**) confirmed the binding mode within this series (also depicted more generally in Figure 7). Analog **15** inhibited gyrase in a supercoiling assay with an IC_{50} of 0.55 μg/mL, and analog **16** had an MIC range of 2−4 μg/mL against a small panel of susceptible and resistant Gram positive pathogens (Table 12). The Zeneca team formulated a hypothesis correlating cytoplasmic membrane penetration to lipophilicity values (expressed as log *P* or *C* log *P*) for these analogs. Major challenges centered on issues of poor solubility and a potentially undesirable second off-target mechanism.[133]

Although this project was not pursued further at Zeneca, these triazine structures did have some influence on later GyrB/ParE inhibitor projects at other companies, for example, the Roche hybrid effort discussed directly below and, we speculate, aspects of the Trius effort (compare triazine **15** with Trius pyrrolopyr-

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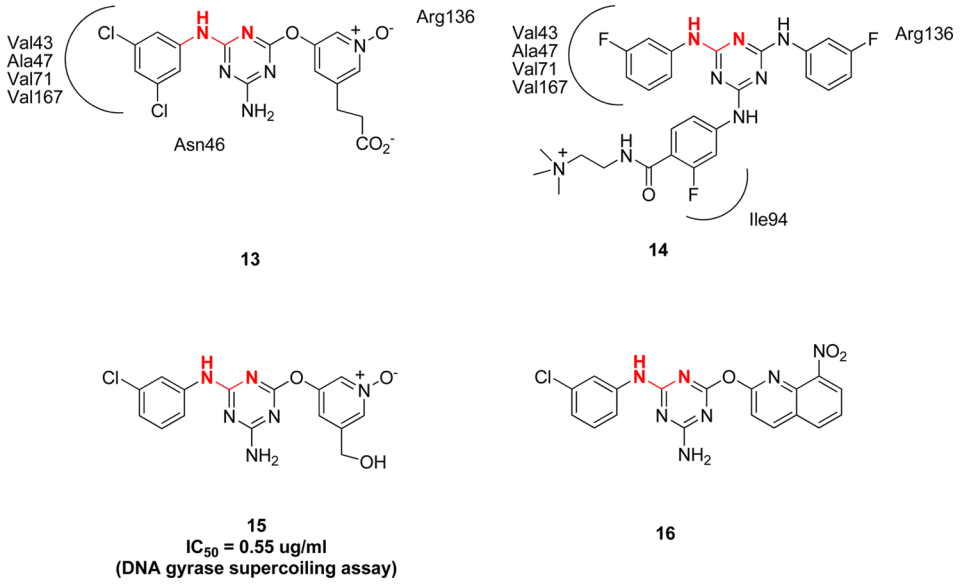

Figure 8. Arylaminotriazine motif efforts at Zeneca. The substructure shown in red interacts with the critical Asp73—water motif in gyrase and topo IV (*E. coli* gyrase numbering) as illustrated in the general binding mode of Figure 7. The aryl groups depicted to the left of the triazine interact with the lipophilic pocket, and the right-hand aryl group interacts with the Arg136/Arg76 motifs. The additional substituted aryl of **14** interacts with Ile94.

Table 12. MIC Values for Zeneca's Triazine 16

test organism	**16** MIC (μg/mL)
S. aureus Oxford	2
S. aureus novobiocin-resistant	2
S. aureus MRQS[a]	4
S. aureus MRQR[b]	2
S. pyogenes C203	4
E. faecalis	4
S. aureus Oxford	2

[a]Methicillin-resistant, quinolone-sensistive. [b]Methicillin-resistant, quinolone-resistant.

imidine **33**, Figure 15). More broadly, this series was the first example of a group of related scaffolds, discussed below, incorporating a 2-amino nitrogen-containing heterocycle motif that hydrogen-bonded with the critical Asp73—water and that allowed suitably designed functionality to project toward other key sites of interaction on the protein (Figure 7).

10. ROCHE'S HYBRID SCAFFOLD: A BLEND OF ATP, NOVOBIOCIN, AND THE ZENECA AMINOTRIAZINE (LATE 1990s)

In parallel with their continuing cyclothialidine efforts during the 1990s, Roche disclosed three additional lines of research exploring ATP competitive GyrB inhibition: a hybrid scaffold program, discussed in this section, and indazole and phenol "needle" (fragment-based) programs, described in sections 11 and 12 below.

In 2000, Roche published a series of hybrid GyrB inhibitors that blended features of ATP, novobiocin, and the Zeneca triazine scaffold (Figure 9).[135] One analog from this series was cocrystallized with a 23 kDa fragment of *S. aureus* DNA GyrB, and the structure was solved by X-ray crystallography. As anticipated, the binding mode demonstrated that the NH_2 group of the core heterocycle formed critical hydrogen bonds to Asp73 and the associated water molecule (Figure 7). The best compound from this limited survey was analog **17** (Figure 9), which showed, at best, comparable MIC values compared to those of novobiocin (Table 13). An important design element in this series discovered by Roche was the recognition that a small lipophilic ethyl group contributed to enzymatic potency. This ethyl group occupied the same lipophilic pocket in GyrB as the propargyl group of RU79115 (**12**) and the pyrrole methyl group of clorobiocin (**4**). Roche provided no physicochemical, efficacy, or safety data for this hybrid series.

11. ROCHE: A PHENOL FRAGMENT-BASED EFFORT (LATE 1990s)

In the same year (2000) that Roche published their hybrid scaffold (Figure 9), they also published an effort called "needle screening", essentially an in-silico structure- and fragment-based approach to identify novel leads for GyrB inhibition.[136] This strategy was different from Roche's other GyrB projects that had focused on natural product starting points (coumarins and cyclothialidine).

One Roche de novo lead discovery effort was based on phenol **18** (Figure 11), a "needle" screening hit.[137] The Roche scientists stated that because of their extensive medicinal chemistry experience with the phenolic cyclothialidine series including knowledge of X-ray structural information, they could reasonably evolve **18** in some productive directions. One optimized analog, **19**, was certainly an improvement over **18** on the basis of both *E. coli* gyrase activity and MIC values for susceptible and resistant *S. aureus* (Table 14). No X-ray data verifying that **19** bound in the expected orientation in GyrB was disclosed, but it is reasonable to assume that these analogs were competitive ATP GyrB inhibitors that bound according to the general motif shown in Figure 10. No physicochemical or other biological data were reported for the series. Because of the phenol moiety, this scaffold might suffer from the same glucuronidation liability and resultant rapid clearance as the early cyclothialidines.

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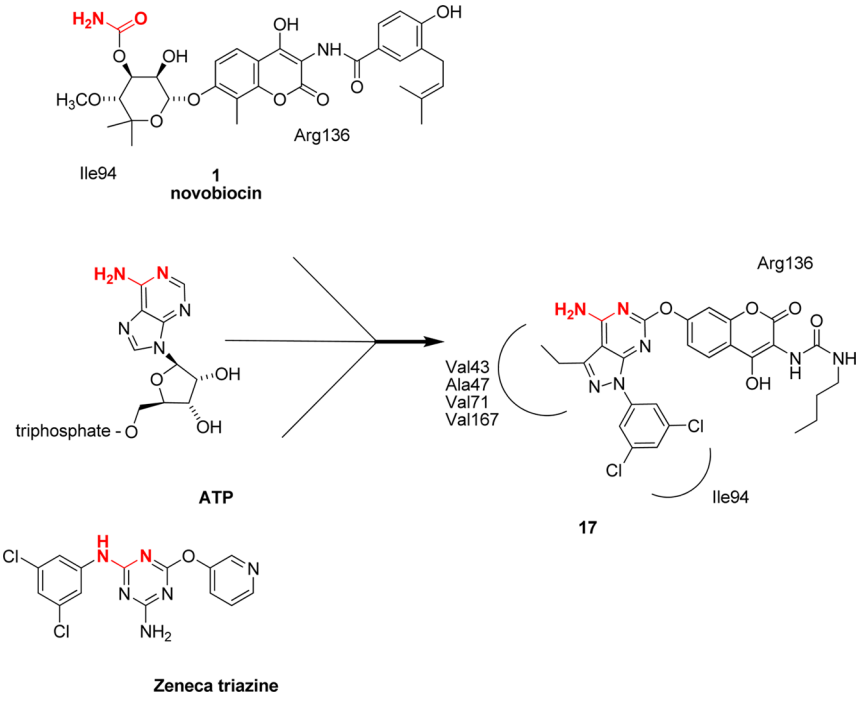

Figure 9. Roche hybrid scaffold **17**. Substructures shown in red interact with the critical Asp73−water motif in gyrase and topo IV (*E. coli* gyrase numbering), as illustrated in Figure 4 and general Figure 7. The ethyl group of **17** interacts with the left-hand hydrophobic pocket.

Table 13. Biochemical Potencies and MIC Values for Roche Hybrid Analog 17 Compared to Those for Novobiocin (1)

enzyme or organism	17	novobiocin (1)
	MNEC (μg/mL)[a]	
E. coli gyrase (supercoiling)	0.5	0.25
	MIC (μg/mL)	
S. aureus ATCC 25923	4	0.25
S. aureus Smith	4	0.25
S. pyogenes b15	2	2
E. faecium van A E23−8	16	8

[a]MNEC = maximum noneffective concentration.

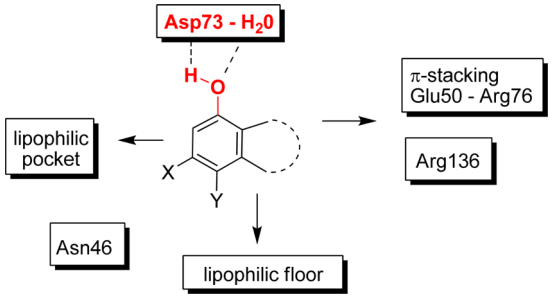

Figure 10. General binding motif for cyclothialidines and acyclic analogs from Roche, Glaxo, and Bayer and for the phenol "needle" optimization program from Roche; X = H, OH, OMe; Y = Me, Br. The key Asp73−water interaction is shown in red.

12. ROCHE AND DAINIPPON: FRAGMENT-BASED INDAZOLE AND PYRAZOLE SCAFFOLDS (1990S−EARLY 2000S)

Roche also identified simple indazole needle **20** (Figure 13) that was evolved, using a structure-guided approach, to analogs **21** and **22**. The binding modes of **20** and **22** were determined by X-ray from a cocrystal with the *S. aureus* GyrB 24 kDa fragment. Indazole ring NH and N of both analogs are shown to interact with Asp73 and a bound water, and for **22**, the benzoic acid substituent interacted with Arg136 and the phenyl interacted with Ile94 on the "hydrophobic floor" of GyrB (general binding mode depicted in Figure 12). Gyrase activity (expressed as MNEC) for analog **21** was 8-fold better than that for novobiocin (Table 15). No physicochemical data or any microbiological data were reported by Roche for this series.

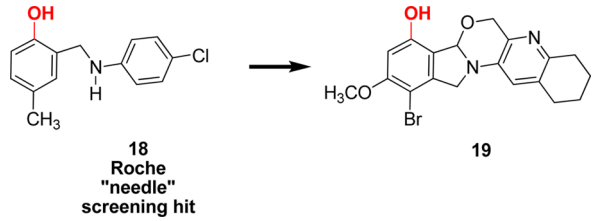

Figure 11. Roche phenol needle optimization effort. The phenol hydroxy shown in red interacts with the critical Asp73−water motif in gyrase and topo IV (*E. coli* gyrase numbering) as illustrated in the general binding mode diagram of Figure 10

Table 14. Biochemical Potencies and MIC Values for Roche Phenol Lead 18 and Optimized Analog 19 Compared to Those for Novobiocin (1)

enzyme or organism	18	19	novobiocin (1)
		MNEC (μg/mL)[a]	
E. coli gyrase (supercoiling assay)	5	0.13	0.25
		MIC (μg/mL)	
S. aureus Smith	16	1	0.25
S. aureus QR-54	16	0.5	0.12
E. faecium van A E23-8	16	>32	8

[a]MNEC = maximum noneffective concentration.

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Table 15. Biochemical Potencies and MIC Values for Roche and Dainippon Indazole (21 and 23) and Pyrazole (24 and 25) Analogs

enzyme or organism	21	23	24	25	novobiocin (1)
			MNEC (μg/mL)[c]		
E. coli gyrase (supercoiling assay)	0.03	ND[d]	ND[d]	ND[d]	0.25
			IC_{50} (μg/mL)[e]		
E. coli gyrase (supercoiling assay)	0.25	4	128	0.5	0.25
E. coli topo IV (decatenation assay)	>128	ND[d]	128	ND[d]	25
human Type II topoisomerase	>400	ND[d]	200	ND[d]	>400
			MIC (μg/mL)[e]		
S. aureus FDA 209P	>128	4	64	8	0.25
S. aureus KMP9[a]	>128	4	64	16	0.25
E. faecium ATCC 29212	>128	8	64	64	2
E. faecium KU 1777[b]	>128	4	64	32	2

[a]Sparfloxacin-, clarithromycin-, and ampicillin-resistant. [b]Sparfloxacin- and vancomycin-resistant. [c]Maximum noneffective concentration; Roche data. [d]ND = no data. [e]Dainippon data.

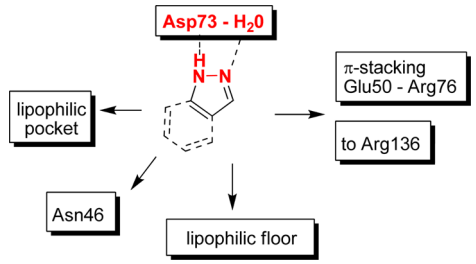

Figure 12. General binding motif for the pyrazole and indazole scaffolds from Roche, Dainippon, Quorex, and Vertex and for the pyrazolopyridone scaffold from Cubist. The key Asp73−water interaction is shown in red.

Dainippon investigated pyrazole **24** (Figure 13), a somewhat related gyrase hit of their own, and studied the Roche indazole series in depth in an attempt to inform and expand their own series.[138,139] Accordingly, Dainippon resynthesized Roche's more advanced analog **21** and prepared several related novel analogs of their own, including indazole **23**, which was specifically highlighted in their paper. As reported by Dainippon, Roche's indazole **21** was equipotent to novobiocin in a gyrase assay and was selective over human topoisomerase II but showed no antibacterial activity, likely because of poor cellular penetration (Table 15). Their own indazole analog **23** lost 16-fold gyrase activity compared to **21** yet showed moderate MIC values of 4−8 μg/mL against susceptible and resistant *S. aureus* and *E. faecium*. We speculate that better physical properties (overall polarity) of Dainippon indazole **23** compared to those of Roche indazole **21** contributed to improvements in microbiological activity despite the decrease in target potency, leading to better penetration through the bacterial cytoplasmic membrane. However, in 10% horse blood, the antibacterial activity of **23** was reported as weak as a result of high protein binding.

Dainippon employed what they learned from the Roche indazole series to evolve their own weak pyrazole hit **24** to the more optimized analog **25** (Figure 13), which bears some structural resemblance to indazole **23**. Even though the gyrase activity for **25** was comparable to that of novobiocin, the antibacterial activity was modest to weak (Table 15). Dainippon did not report the extent of protein binding for their own series. No further work has since been reported by Roche or Dainippon for these related indazole and pyarazole series.

13. VERTEX, QUOREX, AND CUBIST: ADDITIONAL INDAZOLE- AND PYRAZOLE-BASED SCAFFOLDS (2000s)

Three other companies investigated pyrazole- or indazole-based scaffolds, and in each case, those heterocycles were attached to a

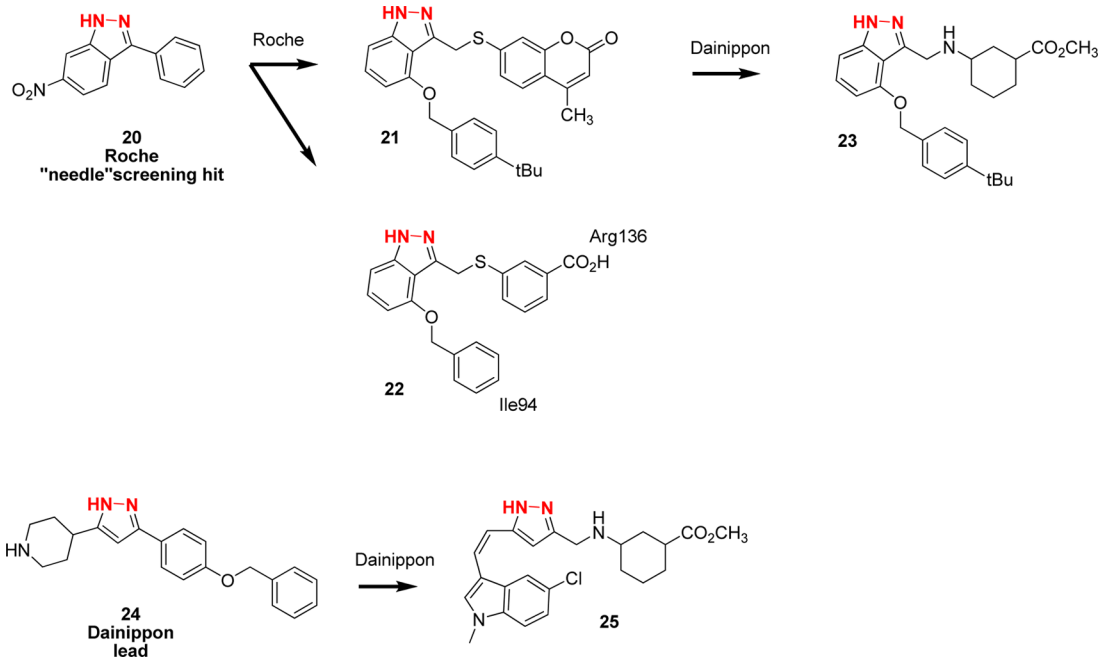

Figure 13. Pyrazole- and indazole-based scaffolds from Roche and Dainippon. Substructures shown in red interact with the critical Asp73−water motif in gyrase and topo IV (*E. coli* gyrase numbering) as illustrated in the general binding mode of Figure 12.

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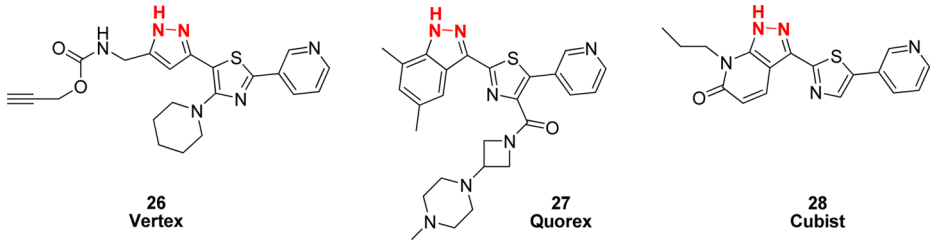

Figure 14. Pyrazole, indazole, and pyrazolopyridone scaffolds from Vertex, Quorex, and Cubist. Substructures shown in red interact with the critical Asp73–water motif in gyrase and topo IV (*E. coli* gyrase numbering) as illustrated in the general binding mode of Figure 12

central thiazole ring. About the same time that Roche was exploring its indazole series, Vertex was working on a pyrazole scaffold (Figure 14).[140−142] Vertex appended to the pyrazole ring small lipophilic groups to interact with the left-hand lipophilic pocket of the enzyme targets. In this regard, Vertex's use of a propargyl group in compound **26** was analogous to Roussel's use of propargyl for their optimized coumarin candidate RU79115, compound **12** (Figure 6). A number of examples from this Vertex pyrazole series had potent GyrB K_i values, although ParE topo IV K_i were not disclosed. Only modest MIC values were achieved for some analogs against *S. aureus* (8−32 μg/mL) and *S. pneumoniae* (1.5−4 μg/mL), and many compounds reportedly suffered from poor solubility and/or chemical instability. Other analogs, although highly potent biochemically (as low as 0.140 μM vs *S. aureus* gyrase and <0.004 μM vs *E. coli* gyrase), were devoid of antibacterial activity against wild-type pathogens for reasons that were not discussed. Vertex apparently deprioritized this series, focusing thereafter on an ethylurea benzimidazole scaffold (section 16).

Quorex Pharmaceuticals developed a series of indazole inhibitors that were disclosed during 2004−2005.[117−223] The detailed data for this series were to be included in posters at the Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) in 2004, but the posters were unexpectedly withdrawn and the only disclosed data were contained in the conference abstracts.[143−148] In those abstracts, the data were associated with compound numbers, but no structures were shown, whereas specific structures, but without associated biological data, were contained in a 2005 patent application. A representative example from the patent is indazole **27** (Figure 14).[149] Quorex's ICAAC abstracts provided data for two compounds, QX-5-2987 and QX-5-3073, that seemed to be similar in their biological profiles. According to the abstracts, crystallographic information was obtained using the N-terminal domain of *E. faecalis* ParE. These crystal structures have not been disclosed, but the binding mode is assumed to be similar to that of the other indazoles and pyrazoles by analogy (Figure 12). Enzyme K_i values and MIC_{90} values for a panel of Gram positive pathogens are shown in Table 16 for QX-5-2987, which was described as bactericidal at concentrations a few fold above the MIC. Compared to the prior pyrazole and indazole efforts of Roche, Daiichi, or Vertex, this series demonstrated higher biochemical and microbiological potency. A "low incidence of resistance" was reported, ascribed to dual gyrase/topo IV inhibition. Oral and i.v. pharmacokinetics were determined in mice, rats, and dogs, and it was stated that the exposure was sufficient to support efficacy. Efficacy in mice was reportedly run in both sepsis and neutropenic thigh models, and ED_{50} values "better than 50 mg/kg" against several pathogens were claimed. The only safety data were in vitro cytotoxicity values in six human cell lines (TC_{50} values reported as >40 μM). Physicochemical

Table 16. K_i and MIC_{90} Values and an ED_{50} Value for Quorex Analog QX-5-2987 (Structure Not Disclosed)

enzyme or species (number of strains)	QX-5-2987
	K_i (nM)
E. faecalis GyrB (ATPase)	1.8
E. faecalis ParE (ATPase)	10
	MIC_{90} (μg/mL)
S. aureus (26)	0.08
S. pyogenes (15)	0.16
S. pneumoniae (18)	0.316
E. faecalis VRE[a] (23)	0.63
	ED_{50}[b]
"for one or more *S. aureus*, *S pneumoniae*, and *E. faecalis*"	<50 mg/kg

[a]Vancomycin-resistant enterococcus. [b]Mouse sepsis model; dosing tid, 1 day; route of administration not specified.

characterization data (solubility, protein binding) were not reported. Quorex was acquired by Pfizer in 2005, and further characterization of the series was carried out there. The compounds fared poorly in animal models of infection at Pfizer because of high clearance caused by rapid glucuronidation of the indazole ring nitrogen. Attempts to solve this issue by modifying the electronics or sterics of the indazole ring with a variety of different substituents were not successful, and the series was terminated.[150]

In a 2013 ICAAC poster, Cubist described a *S. aureus* GyrB NMR-based fragment screening effort that led to a series of pyrazolopyridones, a representative example being compound **28** (Figure 14).[151] An X-ray cocrystal structure was obtained with the 24 kDa fragment of *S. aureus* GyrB that demonstrated the binding mode illustrated in Figure 12. IC_{50} values for *S. aureus* ParE inhibition were 30-fold less potent than for GyrB (Table 17); nevertheless, the spontaneous resistance frequency for **28** was reported to be low (3×10^{-9}) in *S. aureus*. MIC values against a Gram positive panel were low (0.06 to 0.25 μg/mL). The program is in its early stages, and no further characterization data

Table 17. K_i and MIC Values for Cubist Pyrazolopyridone Analog 28

enzyme or organism	**28**
	K_i (nM)
S. aureus GyrB (ATPase assay)	6
S. aureus ParE (ATPase assay)	183
	MIC (μg/mL)
S. aureus 1118 (MRSA)	0.06
S. aureus 1721 (MRSA, ciproR)[a]	0.06
S. pneumoniae ATCC 6303	0.25
E. faecalis ATCC 6569	0.125

[a]Ciprofloxacin-resistant.

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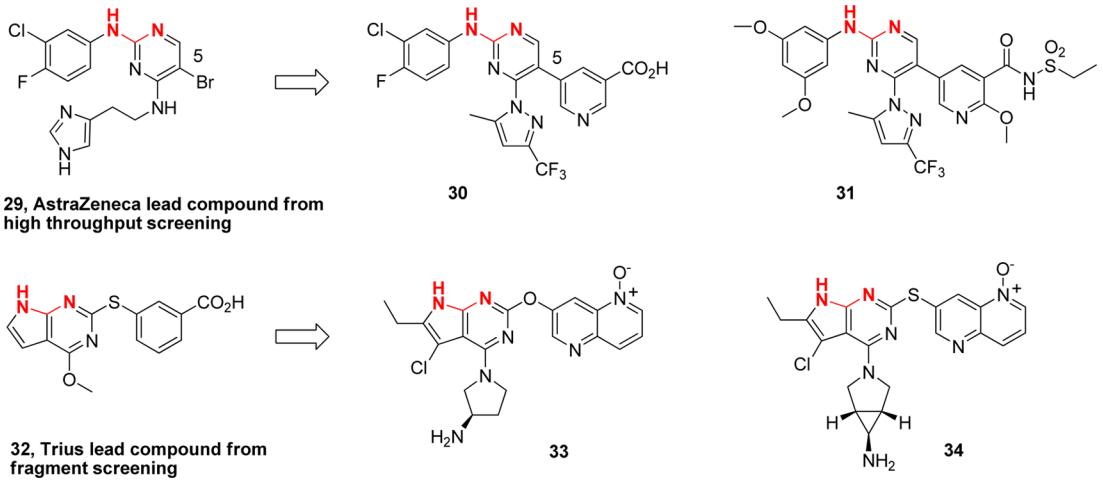

Figure 15. Arylaminopyrimidine scaffold from AstraZeneca and pyrrolopyrimidine scaffold from Trius. Substructures shown in red interact with the critical Asp73—water motif in gyrase and topo IV (*E. coli* gyrase numbering) as illustrated in the general binding mode of Figure 7. The trifluoromethylpyrazole of **30** and **31** interacts with the "lipophilic floor" of the enzymes, and the primary amino group of **33** and **34** interacts with an Asn46 water network.

was disclosed (solubility, protein binding, pharmacokinetics, efficacy, safety).

14. ASTRAZENECA'S ARYLAMINOPYRIMIDINES AND TRIUS'S PYRROLOPYRIMIDINES (2000s)

High-throughput screening by AstraZeneca for the inhibition of *E. coli* ParE ATPase activity identified arylaminopyrimidine **29** (Figure 15) as a potent hit against the enzyme.[152] Potent eukaryotic in vitro cytotoxicity was also observed for **29** because of the cross-inhibition of human kinases, but this was quickly eliminated as a liability by substitution at the pyrimidine 5-position with aryl groups, a strategy guided by crystallographic comparison of the binding pocket of ParE to that of human kinases. X-ray crystal structures in *S. pneumoniae* ParE of lead compound **29** and a number of advanced analogs demonstrated the mode of binding shown in Figure 7.

Compounds **30** and **31** (Figure 15) represent advanced preclinical analogs that displayed low MIC_{90} values against Gram positive pathogens (Table 18) including MRSA and vancomycin-resistant *E. faecium* (VRE). Log *D* values, 0.99 for **30** and 0.60 for **31**, were in the range considered compatible with good cytoplasmic membrane penetration. Potent dual GyrB and ParE enzymatic inhibitory activity was demonstrated routinely for analogs within this series, and spontaneous resistance frequencies in Gram positive pathogens were very low, typically $<10^{-9}$ to $<10^{-10}$ (Table 18). The arylaminopyrimidine series was confirmed to be bactericidal. In an immunocompetent mouse lung model of *S. pneumoniae* infection, an oral 200 mg dose of analog **30** lowered lung CFU (colony-forming unit) counts by almost 2 $\log_{10}$ units after 24 h. Other analogs (not shown[153]) that contained a quinolone or azaquinolone 3-carboxylic acid linked at their 6-positions in place of the pyridine demonstrated MIC_{90} values that were about 10- to 100-fold lower than those for analogs **30** and **31** and had lower human dose estimates compared to the 1.4 g per day dose estimated for **31**. There did not appear to be any serious safety liabilities with the series as judged by in vitro or animal in vivo studies. Disadvantages with this series that were challenging to overcome while retaining target affinity and MIC potency were high protein binding (generally <5% free), poor aqueous solubility, or both. In this respect, the arylaminopyrimidines struggled with some of the same physicochemical issues as the Zeneca triazine series. Ironically, analog **31** as initially prepared demonstrated excellent aqueous solubility (>1000 μM), yet upon i.v. dosing in an animal model, a new low-solubility polymorph crystallized in the i.v. line, halting the experiment. Although phosphate ester prodrugs and nanosuspension formulations were subsequently investigated to mitigate solubility issues within this series,[111] the effort was ultimately terminated. Nevertheless, this series provided an

Table 18. IC_{50}, MIC, MIC_{90}, and Selected Physical Property and Human Dose Values for AstraZeneca Arylaminopyridines 29—31 Compared to Those for Linezolid

enzyme, organism, or property	29	30	31[b]	linezolid
		IC_{50} (μM)		
S. aureus GyrB (ATPase assay)	1	0.002	<0.01	NA[c]
S. pneumoniae ParE (ATPase assay)	17	<0.04	<0.02	NA[c]
	Frequency of Resistance (at an Indicated Multiple of MIC)			
S. aureus ARC516	ND[a]	$<4 \times 10^{-10}$ (2-fold)	10^{-9} (1-fold)	ND[a]
S. pneumonia ARC548	ND[a]	$<10^{-9}$ (2-fold)	$<10^{-10}$ (1-fold)	ND[a]
	MIC (μg/mL)	MIC_{90} (μg/mL) [no. of strains]		
S. aureus MSSA	20	2 [11]	0.5 [40]	2 [40]
S. aureus MRSA	ND[a]	1—2 [4]	0.5 [62]	2 [62]
S. pneumoniae	40	0.06 [11]	0.13 [101]	2 [101]
S. pyogenes	ND[a]	0.12 [10]	0.25 [99]	2 [99]
E. faecalis	ND[a]	0.03 [10]	0.03 [39]	1 [39]
E. faecium	ND[a]	0.25 [10]	0.13 [22]	32 [22]
E. faecium (VRE)	ND[a]	<0.016—0.5 [6]	0.13 [34]	16 [34]
aqueous solubility (μM)	2	612	>1000 (form A)	2700[d]
human PPB (% free)	ND[a]	1.2	2	69[d]
log D	ND[a]	0.99	0.60	1.80[d]
est. human dose (g/day)	ND[a]	ND[a]	1.4	1.2[e]

[a]ND = no data. [b]Data from Eakin and Sherer.[111] [c]NA = not applicable. [d]Values from Benet et al.[235] or from label. [e]Human dose, oral and i.v.; from label.

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opportunity to begin to explore PK/PD (pharmacokinetic/pharmacodynamic) indices and magnitudes for the class of ATP site GyrB/ParE inhibitors. Such measures are important for guiding clinical dose estimates within the class.[154] In particular, efficacy studies in a neutropenic mouse thigh model of *S. aureus* infection using analog **31** dosed i.p. suggested that both unbound drug AUC/MIC and C_{max}/MIC were potentially valid indices, having magnitudes of 40 and 54 (AUC/MIC) and 5.7 and 6 (C_{max}/MIC) for the achievement of stasis (first number in each pair) or 1 log kill (second number in each pair).[155]

In 2009, Trius Therapeutics reported selecting pyrrolopyrimidine **32** (Figure 15) as a starting point for further optimization after exploring a fragment-based crystallographic screen using *E. faecalis* N-terminal GyrB.[107,109] Modifications to **32** during lead optimization included building into the known left-hand lipophilic pocket using chlorine and small alkyl groups, the selection of alternative right-hand groups, and, most importantly, the installation of rigid amino-substituted saturated heterocycles projecting toward the lipophilic floor and Asn46 (Figure 7). Analogs **33** and **34** (Figure 15) are representative of this optimized series. Different from most other GyrB/ParE inhibitor scaffolds, these appended primary or secondary amino groups interact either directly with Asn46 or through an "ordered solvent network [that] has been treated as a conserved structural element during inhibitor optimization". One important consequence of the presence of this basic amino group is the

Table 19. IC_{50} and MIC Values and Serum MIC Shift Effects for Trius's Pyrrolopyrimidine Bicyclics 33 and 34

enzyme or organism	33	34
	IC_{50} (nM)	
E. coli GyrB (ATPase assay)	<0.3	<0.3
E. coli ParE (ATPase assay)	1.7	4.6
	MIC (μg/mL)	
S. aureus ATCC 13709	<0.06	0.13
S. aureus ATCC 13709 + 20% serum	0.13	0.13
S. pneumoniae ATCC 51916	0.25	1
E. coli ATCC 25922	2	4
E. coli ATCC 25922 + 20% serum	4	2
K. pneumoniae ATCC 700603	32	32
A. baumannii ATCC 19606	4	8
P. aeruginosa PAO1	4	16

achievement of MIC values in the range of 2−4 μg/mL against Gram negative pathogens such as *E. coli*, *A. baumannii*, and *P. aeruginosa* and 32 μg/mL against *K. pneumonia* (Table 19). Prior to this report, all GyrB/ParE inhibitors were largely limited to a Gram positive spectrum and a few common respiratory Gram negative pathogens such as *H. influenzae*. To accomplish this unprecedented broadening of the MIC spectrum, Trius cited the potent dual enzyme inhibition and the presence of the basic amine as being important factors. More specifically, the Trius authors cite the ability of their scaffold to encompass populations of both ionized (protonated) and neutral species that may thereby allow permeation both through the nonpolar cytoplasmic membrane and through the polar porin channels of the outer membrane, mimicking, in part, one of the permeation mechanisms proposed for fluoroquinolone antibacterials (see section on permeation, above). The basic amine-containing heterocycles on the Trius scaffolds are in fact largely derived from fluoroquinolone 7-aminoheterocycle substituents. Other structural details of the Trius scaffold, such as the number and location of ring nitrogens in the right-hand heterocycle, appear to be influential for Gram negative potency as well. Noteworthy also is the low serum MIC shift for these analogs (Table 19), suggesting relatively low protein binding. Although no data were provided on in vitro or in vivo safety for this series, it was reported that analogs having certain conservative substitutions off the scaffold result in a loss of selectivity for gyrase and topo IV and acquire alternate mechanisms of action in bacteria.[156]

15. ASTRAZENECA'S FRAGMENT-BASED PYRROLAMIDES AND DAIICHI'S FOLLOW-ON IMIDAZOLAMIDES (2000s)

Following the termination of its triazine scaffold series, Zeneca conducted an NMR-based fragment-screening effort using the 24 kDa N-terminal domain of *S. aureus* GyrB. Building from fragments discovered by this process, prototype pyrrolamide scaffold **35** was designed, showing weak biochemical and microbiological potency (Figure 17).[157] The identification of a

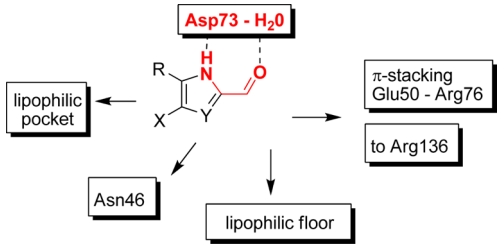

Figure 16. General binding motif of clorobiocin, coumermycin A1, Bristol-Myers' BL-C43, AstraZeneca's pyrrolamides, and Daiichi's imidazolamides. R = Me or Et, X = H or Cl, and Y = N or C−X. The key Asp73−water interaction is shown in red.

methylpyrrole fragment having interactions with the Asp73−water motif in the enzymes defined a certain similarity of this scaffold series with clorobiocin (Figures 16 and 18). One early optimized analog, **36** (AZD1279),[158,159] was advanced to an exploratory IND Phase I study but exhibited suboptimal exposure at low doses in man, believed to be due to rapid anion-transporter-mediated biliary clearance. Preclinical pharmacokinetic data for **36** had not been consistent across several animal species; therefore, it had been difficult to forecast human exposure.[105] Accordingly, analogs were designed for diminished affinity to human bile transporters, and thereafter compounds showed consistently improved clearance across preclinical animal species. The aminothiazole acid on the right-hand side of the scaffold was a preferred motif for interacting with the Arg136, Arg76 enzyme region (Figure 16). Substituents adjacent to the acid group on the thiazole were inserted to decrease the interaction with biliary anion transporters as well as to modulate log *D* and physical properties. Optimized analogs within the pyrrolamide series typically had excellent dual target inhibition, very low frequencies of spontaneous resistance, excellent MIC values against both susceptible and resistant Gram positive pathogens, good solubility, often good animal pharmacokinetics, oral bioavailability, and good efficacy in thigh and lung mouse models of infection. Like many of the other ATP site GyrB/ParE inhibitor series discussed in this review, the pyrrolamide series was shown to be bactericidal. However, plasma protein binding tended to be low to moderate, typically ca. 7% free or less, for compounds having the most potent MIC values.[105]

The in vitro biological and physicochemical property profile for **37** (AZD5099), an advanced analog in this series that was also

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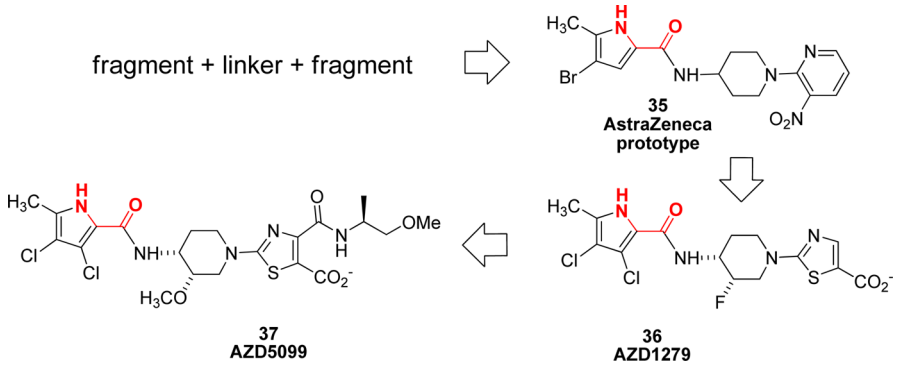

Figure 17. AstraZeneca pyrrolamide optimization program. The functionality shown in red interacts with the critical Asp73−water motif in gyrase and topo IV (*E. coli* gyrase numbering) as illustrated in the X-ray crystal structure diagram in Figure 2 and the general binding mode diagram of Figure 16.

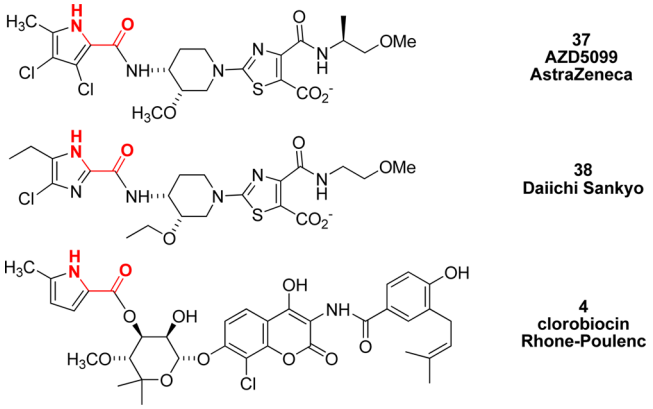

Figure 18. Comparison of AstraZeneca pyrrolamide **37** to Daiichi imidazolamide **38** and both to clorobiocin **4**. Functionality shown in red interact with the critical Asp73−water motif in gyrase and topo IV (*E. coli* gyrase numbering) as illustrated in the general binding mode of Figure 16

taken into the clinic, is shown in Tables 20 and 21.[105] **37** displays excellent enzyme inhibitory potencies, and the frequencies of spontaneous resistance in *S. aureus* and *S. pneumoniae* were below the levels of detection (<9.6×10^{-10} at 4-fold the MIC). MIC and MIC_{90} values were excellent against a panel of Gram positive and fastidious Gram negative pathogens, and the aqueous solubility was encouraging (960 μM). The protein-binding value for **37** (2.5% free) translated into a 22-fold MIC shift for *S. aureus* (from 0.036 to 0.78 μg/mL) in the presence of serum. In a *S. aureus* neutropenic thigh infection mouse model, **37** resulted in a 1.5 $\log_{10}$ reduction in CFU relative to the initial pretreatment inoculum when dosed at 30 mg/kg by intraperitoneal injection. As with the arylaminopyrimidine series, potentially class-relevant preclinical PK/PD studies had been conducted on analog **37**. In particular, detailed studies using the neutropenic mouse thigh model of *S. aureus* infection with analog **37** dosed i.p. suggested that unbound drug AUC/MIC was a valid index, having magnitudes of 105−142 and 137−163 for the achievement of stasis or 1 log kill, respectively.[155] In man, **37** was dosed i.v. up to 500 mg per individual, but further clinical work was discontinued for a combination of factors: (a) high variability in exposure within a small group of healthy volunteers, which eroded confidence that efficacious exposures could be achieved within defined safety margins, and (b) concerns related to mitochondrial changes observed in preclinical safety species.[160] Following this result, the pyrrolamide series was terminated at AstraZeneca.

In 2009, Daiichi Sankyo disclosed an imidazole variation on the AstraZeneca pyrrolamide series in a large patent application; analog **38** represents a typical example from the application (Figure 18).[161] Data were disclosed that showed that analogs from this series, like the pyrrolamides, are potent dual target inhibitors, show low MIC values, and are efficacious in animal models of infection.

16. ETHYLUREAS (VERTEX, PFIZER, EVOTEC/PROLYSIS, BIOTA, ASTRAZENECA, AND ACTELION), AZAINDOLES (ASTRAZENECA), AMINOTRICYCLICS (TRIUS), AND QUINAZOLINONES (CUBIST) (2000s)

Vertex's second inhibitor scaffold after their pyrazole series was a substituted 2-aminobenzimidazole that resulted from a virtual and enzyme-based high-throughput screen. The initial weak hit, an ethyl carbamate derivative (**39**, Figure 20), was optimized, guided by structural information from both *E. coli* GyrB and ParE.[78,85,162,163] Vertex's first patent on the series was published

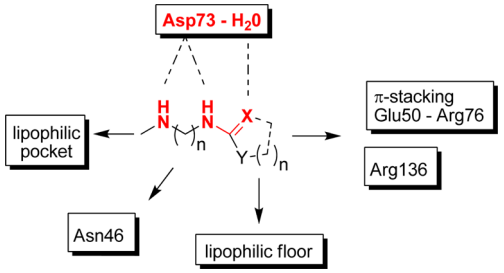

Figure 19. General motif for ethylureas (Vertex, Prolysis, Biota, AstraZeneca, Actelion), azaindoles (AstraZeneca), Trius's tricyclics, and Cubist's quinazolinone scaffolds. n = 1 or 2; X = N or O; Y = NH, N, S, CH. The key Asp73−water interaction is shown in red.

in 2002.[164] Key modifications in the transition from **39** to more advanced analogs included (1) the replacement of the carbamate with an ethylurea, which allowed an additional hydrogen bond donor interaction with Asp73 (Figure 19); (2) improvements in scaffold interactions with the Glu-Arg salt bridge and Arg138; and (3) additional lipophilic interactions on the hydrophobic floor employing an aryl group, typically a 2-pyridyl. Vertex cited the importance of a hydrogen bond between the 2-pyridyl nitrogen and the imidazole NH, thereby restricting the remaining imidazole nitrogen to act as a hydrogen bond acceptor with the structural water associated with Asp73. A second consequence of that internal hydrogen bond was the enforcement of coplainarity

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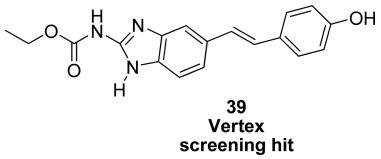

Figure 20. Structure of Vertex screening hit **39**.

between the benzimidazole and the 2-pyridyl, which was deemed to be beneficial for binding. An optimized compound from this design exercise was **40** (Figure 21). The general design concept embodied by an ethylurea moiety flanking a heterocycle was later to have a significant influence on scaffold designs for GyrB/ParE programs run at a number of other companies (Pfizer, Prolysis/Biota, AstraZeneca, and Actelion; Figure 21).

Vertex was an early proponent of potent dual inhibition of the gyrase and topo IV ATPases as a strategy to mitigate target-based resistance development. As discussed previously, this was a concept that had initially gained traction several years earlier in the context of the dual GyrA/ParC targets of quinolones. Compared to novobiocin, compound **40** more potently inhibited both the gyrase and topo IV enzymes of *E. coli* and *S. aureus*, and the spontaneous resistance frequencies of **40** are superior to those of novobiocin when measured at 4- and 8-fold the MIC values (Table 22).

Compound **40** had excellent MIC_{90} values (0.03−0.12 μg/mL) against a range of Gram positive pathogens, a subset of which is shown in Table 22. These values were superior to novobiocin and, for *S. aureus* and enterococcus, far superior to vancomycin. The compounds were also cidal, showing an approximately 1000-fold reduction in bacterial load (*S. aureus*, *E. faecalis* and *S. pneumoniae*) after 24 h following a single dose. However, compound **40** showed a significant (16-fold) shift in the MIC in the presence of 50% serum, indicating that it was highly protein-bound, although the shift is not as severe as the 60-fold MIC shift recorded for novobiocin.[85,163] This analog was

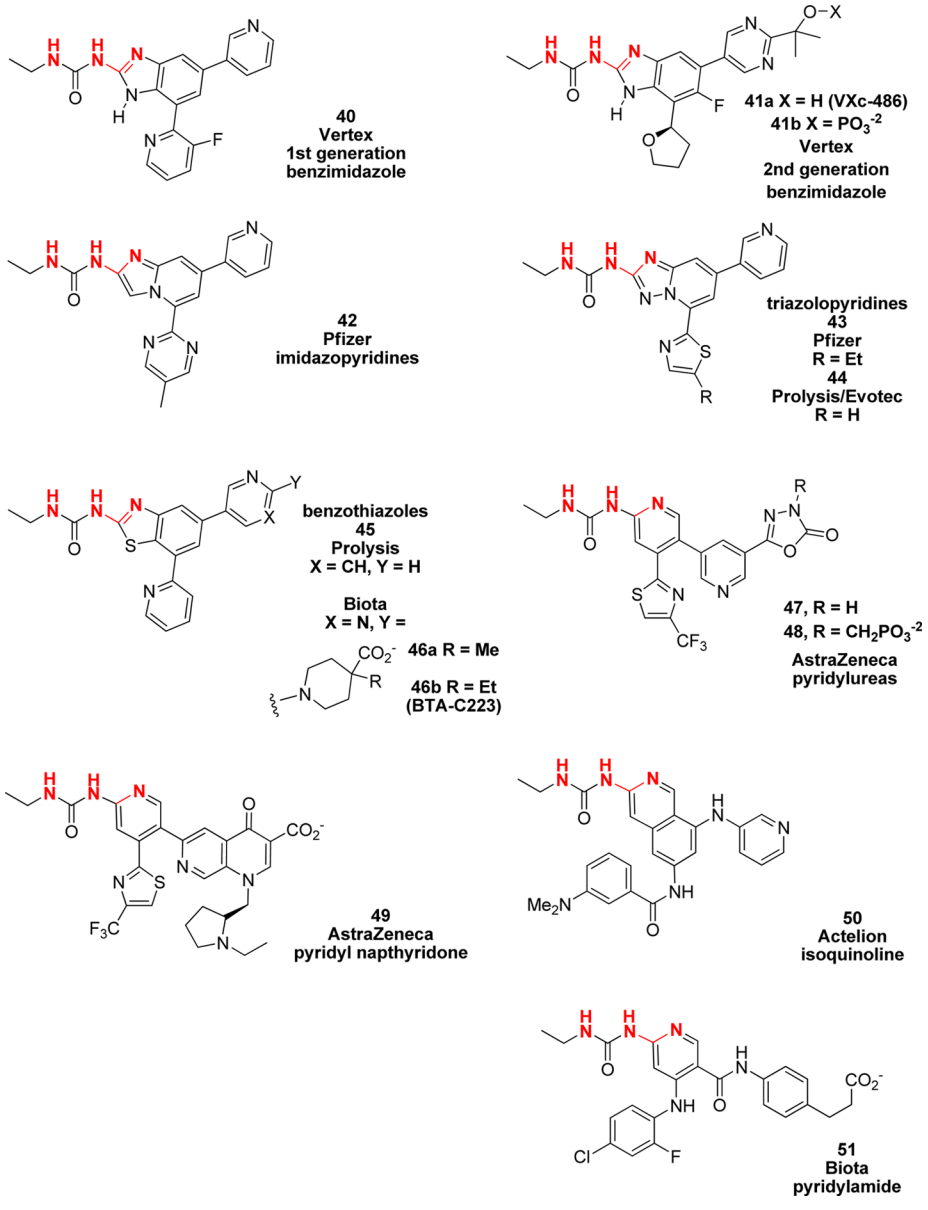

Figure 21. Ethylurea-heteroaryl motif efforts at Vertex, Pfizer, Prolysis/Evotec, Biota, AstraZeneca, and Actelion. The functionality shown in red interacts with the critical Asp73−water motif in gyrase and topo IV (*E. coli* gyrase numbering) as shown in the general binding mode of Figure 19

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Table 20. Data Profile for AstraZeneca Clinical Pyrrolamide 37 (AZD5099)

property, enzyme, or organism	**37** (AZD5099)
	Measured Value for Property
log *D*	1.4
human plasma protein binding	97.50%
aqueous solubility (μM)	960
	IC_{50} (nM)
S. aureus GyrB (ATPase assay)	<10
E. coli ParE (ATPase assay)	72
human topoisomerase II	>50 000
	Frequencies of Resistance (4-fold MIC)
S. aureus (multiple strains)	$<9.6 \times 10^{-10}$
S. pneumoniae (multiple strains)	$<9.6 \times 10^{-10}$
	MIC (μg/mL)
S. aureus MSSA	0.036
S. aureus MSSA + 50% serum	0.78 (22-fold shift)
S. aureus MRQR	0.057
S. pneumoniae 548	0.016
S. pyogenes 538	0.014
H. influenzae 446	0.13
M. catarrhalis	<0.008
E. coli 523	24
E. coli TolC (efflux pump mutant)	0.094

Table 21. MIC_{90} Values for AstraZeneca Clinical Pyrrolamide AZD5099 (37) Compared to Those for Levofloxacin

test species	number of strains (type)	AZD5099 (**37**)	levofloxacin
		MIC_{90} (μg/mL)	
S. aureus	200	0.06	32
	110 (MRSA)	0.06	64
E. faecalis	100	0.015	32
	8 (VR[a])	0.015	64
E. faecium	150	0.06	>128
	50 (VR)	0.06	>128
H. influenzae	200	0.25	0.015
	40 (AR[b])	0.25	0.015

[a]Vancomycin-resistant. [b]Azithromycin-resistant.

efficacious orally in an immunocompetent rat lung infection model (*S. pneumoniae*) and in a neutropenic rat thigh infection model (*S. aureus*) by the i.v. route. As expected, **40** showed no elevation of MIC values against clinical isolates resistant to other antibiotics.

For several years during the mid-2000s, the antibacterial community anticipated the progression of compound **40** into clinical studies, yet this did not happen and it was speculated that poor aqueous solubility, insufficient for i.v. formulation, might be one of the reasons.[165] From a structural perspective, limited aqueous solubility might be associated with the internal hydrogen bond formed between the pyridyl and imidazole. The resulting coplanarity of the urea and pendant pyridine substituents with the benzimidazole core likely raises the lattice energy of the solid state and causes poor dissolution rates. More recently, Vertex discussed protein binding issues with its earlier series, stating that "in general protein binding levels above 95% and serum MIC shifts ranging from 16- to 128-fold were common among these compounds" and negatively impacted the in vivo efficacy.[166] Additionally, Vertex recently disclosed potential safety issues involving a reactive metabolite seen for a close analog of **40** as well as CYP3A4 inhibition that contributed

Table 22. K_i Values, Effect of Human Serum (Protein Binding) on *S. aureus* MIC, and MIC_{90} Values for Vertex Ethylurea Benzimidazole 40 (VRT-752586) Compared to Those for Novobiocin (1) and Vancomycin

enzyme, organism, or species (number of strains)	VRT-752586 (**40**)	novobiocin (**1**)	vancomycin
		K_i (nM)	
E. coli gyrase (ATPase assay)	<4	13	NA[a]
E. coli topo IV (ATPase assay)	23	160	NA[a]
S. aureus gyrase (ATPase assay)	14	19	NA[a]
S. aureus topo IV (ATPase assay)	<6	900	NA[a]
		Frequencies of Resistance	
S. aureus 4X MIC	7.4×10^{-9}	1.8×10^{-8}	ND[b]
S. aureus 8X MIC	$<5.7 \times 10^{-10}$	1.2×10^{-8}	ND[b]
		MIC (μg/mL)	
S. aureus ATCC 29213	0.031	0.125	ND[b]
S. aureus ATCC 29213 + 50% human serum	0.5 (16-fold)	>16 (>128-fold)	ND[b]
		MIC_{90} (μg/mL)	
S. aureus (54)	0.12	0.5	4
S. pyogenes (22)	0.12	4	ND[b]
S. pneumoniae (64)	0.03	2	ND[b]
E. faecalis (35)	0.06	8	16
E. faecium (34)	0.12	2	>32
H. influenzae (36)	>16	<1	ND[b]
M. catarrhalis (23)	0.25	<1	ND[b]

[a]NA = not applicable. [b]ND = no data.

to the decision not to progress into development agents from this earlier series.[167]

The CYP3A4, reactive metabolite, and solubility issues of this earlier series were addressed by Vertex in a recent paper describing a "second generation" series featuring **41a** (Vertex compound number VXc-486) and in a sequence of patent applications featuring the corresponding highly soluble phosphate ester prodrug **41b**.[165,167−170] The metabolism of a compound from the Vertex first generation series occurred on the ethylurea moiety with the formation of the reactive metabolite; replacement of the hydrophobic floor-facing aryl group with a tetrahydrofuran group directed metabolism elsewhere on the scaffold, affording metabolites that were nonreactive. These disclosures as well as recent presentations at the 2014 ICAAC[171−175] provide extensive data on the safety and efficacy of prodrug **41b** and its (bioactive) parent alcohol **41a** in a number of preclinical models and importantly establish wide safety margins in rat, good PK of the prodrug and rapid conversion to parent **41a**, and high oral bioavailability of the parent in cynolmolgus monkeys. In human serum, **41a** has a free fraction of 4%, and in a neutropenic mouse model of *S. aureus* lung infection, prodrug **41b** dosed b.i.d. at 30 mg/kg achieved a >3 $\log_{10}$ reduction in CFU.[174] Analog **41a** and earlier Vertex ethylurea analogs were shown to be bactericidal. Preliminary PK/PD data using an immunocompetent mouse kidney infection model (*S. aureus*) with **41a** dosed orally suggested that unbound drug AUC/MIC was the principal index, although the percent time above MIC (%T > MIC) contributed to a lesser extent. Additional patent applications[176−179] relating to the chemical process and solid form for the parent and phosphate prodrug reinforced the advanced preclinical status of this predominantly Gram positive spectrum compound. Vertex explicitly stated that prodrug **41b** is a development candidate.[167,174]

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Pfizer was the first to scaffold-hop on the basis of the original Vertex ethylurea benzimidazoles, as disclosed in two patent applications: an ethylurea imidazopyridine series, exemplified by **42**, and an ethylurea triazolopyridine series, exemplified by **43** (Figure 21).[180,181] The latter series was also worked on by Prolysis/Evotec (see below). Compound **43** had the most potent antibacterial activity among the triazolopyridines exemplified by Pfizer, with an MIC of 0.06 μg/mL against *Neisseria gonorrheae*. No microbiological data against other pathogens were reported in the application. In Pfizer's hands, the triazolopyridine series had solubility issues that affected its PK performance and efficacy, and the series was not pursued.[182] In the imidazopyridine series, compound **42** had potent IC_{50} values against GyrB and ParE from *S. pneumoniae*, (53 and 250 nM respectively). MIC values

Table 23. IC_{50} and MIC Values for Pfizer's Ethylurea Imidazolopyridine 42

enzyme or organism	**42**	novobiocin (**1**)
	IC_{50} (μM)	
S. pneumoniae GyrB (ATPase assay)	0.053	0.037
S. pneumoniae ParE (ATPase assay)	0.25	2.03
	MIC (μg/mL)	
S. aureus UC76	0.5	0.125
S. aureus MRSA SA-1417	0.5	0.125
S. pyogenes SP1-1	0.5	8
S. pneumoniae SP3	0.125	4
S. pneumoniae FQR SP3765	0.125	4

against a panel of Gram positive pathogens were somewhat weaker than novobiocin versus *S. aureus* but more potent against *S. pyogenes* and *S. pneumoniae* (Table 23). This analog was efficacious in immunocompetent mouse infection models, with PD_{50} values of 24 and 21 mg/kg (oral) in *S. pyogenes* sepsis and *S. pneumoniae* lung models, respectively. Its bioavailability in rats was 99%. Compound **42** was reported as "selected for additional study" according to a 2009 publication,[183] but Pfizer made no additional public disclosures prior to abandoning its entire antibacterial discovery portfolio in 2011.

Investigators at Prolysis pursued the ethylurea motif by designing alternative 5,6-membered ring systems to replace the Vertex benzimidazole, first publishing on a series of ethylurea benzo/pyridylthiazoles, exemplified by **45** (Figure 21).[184] With Evotec, Prolysis also published on the ethylurea triazolopyridine scaffold that Pfizer had also disclosed (Prolysis example **44**).[185] At the time, the triazolopyridine series was selected for further study because of synthesis considerations. However, a comparison of matched molecular pairs between series revealed that triazolopyridines **44** were approximately 100-fold less potent enzymatically than the corresponding benzothiazoles **45**; consequently, only relatively moderate whole-cell activity was observed with **44** (Table 24). In retrospect, the reduced potency could be explained on the molecular level by unfavorable interactions between the two nitrogen lone pairs on adjacent heterocycles, which was anticipated to disrupt the coplanarity, a feature previously noted as beneficial to activity.[163] Although *E. coli* GyrB potencies were moderate, *E. coli* ParE enzyme potencies were much weaker and might not be regarded as truly "dual inhibiting", at least for this pathogen. Nevertheless, the spontaneous resistance frequencies in *S. aureus* of **44** along with two other compounds were reported to be very low, $<1.8 \times 10^{-9}$ (at 2- to 8-fold the MIC), data that Prolysis stated were "consistent with a dual targeting inhibitor series". It is

Table 24. IC_{50} and MIC Values for Prolysis/Evotec's Ethylurea Triazolopyridine 44

enzyme or organism	**44**
	IC_{50} (μM)
E. coli GyrB (ATPase assay)	0.042
E. coli ParE (ATPase assay)	11
	MIC (μg/mL)
S. aureus 601055 MSSA	1
S. aureus Smith ATCC 19636 MSSA	1
S. aureus MRSA ATCC 700698 MRSA	0.5
S. pneumoniae ATCC 49619	1
E. faecalis 1.5604 VRE	1
M. catarrhalis ATCC 25240	0.5
H. influenzae ATCC 49247	>128

conceivable that in *S. aureus* a more balanced and potent dual targeting situation applies, leading to the low resistance frequencies, but *S. aureus* GyrB and ParE enzyme potencies were not reported for these compounds. Compounds from this series were reported to be bactericidal on the basis of time kill curves. The only safety data reported were from a mammalian cytotoxicity assay (HepG2 cells), wherein **44** had an IC_{50} of >64 μg/mL. No protein binding data were reported. Although compounds from the triazolopyridine series (structures not disclosed) were reported to be efficacious when dosed i.p. in a sepsis mouse model of *S. aureus* infection,[186] the triazolopyridine series seems not to have been further pursued at Prolysis.

Ultimately, Prolysis did continue to pursue ethylurea benzothiazole series **45**. In a conference presentation in 2008, they reported data on selected compounds from the series (specific structures not shown) that indicated potent MIC_{90} values (0.03 to 0.06 μg/mL) against a panel of Gram positive pathogens and potent MIC_{90} values against a selection of respiratory tract pathogens.[187] Low spontaneous resistance frequencies against a panel of Gram positive pathogens was reported (e.g., for *S. aureus* $< 6.9 \times 10^{-10}$, compared to 7.7×10^{-9} using novobiocin, both at 8-fold the MIC); however, target potencies against GyrB or ParE were not reported. Efficacy was shown in a mouse sepsis model of *S. aureus* infection (single i.v. dose ED_{50} = 1.73 mg/kg) and in a neutropenic mouse thigh model of *S. aureus* infection using reasonable doses. Protein binding appeared not to be a major issue as judged by the low (2−4-fold) *S. aureus* MIC shifts for many compounds in the presence of 2% bovine serum albumin. Oral bioavailability seemed to be within reach (10−59% in the mouse for four compounds). However, the solubility for this series was low (25−100 μg/mL), which could make it difficult to achieve consistently adequate in vivo exposures by either oral or i.v. dosing.

Prolysis was acquired by Biota in 2009, and Biota continued to focus on the ethylurea benzothiazole series,[91,188] pointing out that initial compounds in the series[184] had good activity but poor solubility. Thus, further work at Biota focused on improving the solubility in this series. This was achieved by the addition of an acidic solubilizing group, with compound **46a** being a representative example (Figure 21 and Table 25). Despite significant improvements in solubility (>800 μg/mL) and the achievement of low in vivo clearance (<1.5 mL/min/kg, rat) and good oral bioavailability (80%, rat), an unfortunate result of this modification was high protein binding (99.3%). For *S. aureus*, the resulting MIC shift in 50% serum was 16-fold (0.12 to 2 μg/mL). Nevertheless, the low spontaneous resistance frequency of **46a**

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Table 25. K_i Values, Effect of Human Serum (Protein Binding) on *S. aureus* MIC, and MIC Values for the Biota Ethylurea Benzthiazole 46a (Me Analog) Compared to Those for Novobiocin (1) and Linezolid

enzyme or organism	46a	novobiocin (1)	linezolid
		IC_{50} (μg/mL)	
S. aureus GyrB (ATPase assay)	0.008	ND[a]	NA[b]
E. coli GyrB (ATPase assay)	0.003	0.045	NA[b]
E. coli ParE (ATPase assay)	0.024	0.18	NA[b]
		Frequencies of Resistance	
S. aureus 4-fold MIC	$<4.5 \times 10^{-10}$	5.8×10^{-8}	$<7.4 \times 10^{-11}$
		MIC (μg/mL)	
S. aureus ATCC 29213	0.12	0.12	2
S. aureus ATCC 29213 + 50% serum	2 (16-fold)	8 (32-fold)	4 (2-fold)
S. aureus MRSA	0.25	ND[a]	1
S. pyogenes ATCC 51339	0.06	1	1
S. pneumoniae ATCC46619	0.008	0.5	1
S. pneumoniae FQR	0.015	ND[a]	1
E. faecalis ATCC29212	0.03	4	2
E. faecium VAN-R	0.06	ND[a]	2
H. influenzae ATCC 49247	2	0.5	4
M. catarrhalis ATCC 25240	0.12	0.25	4

[a]ND = no data. [b]NA = not applicable.

for *S. aureus* (at 4-fold the MIC) seems to be consistent with dual inhibition. (For *S. aureus* only the GyrB IC_{50} is reported, although the *E. coli* target numbers are potent and about equivalent for both targets.) Analog **46a** and other potent members of this series were shown to be bactericidal.

During 2012−2013, Biota described a front runner preclinical candidate, ethyl analog **46b** designated as BTA-C223, that had a low frequency of spontaneous resistance ($<9.4 \times 10^{-11}$ for *S. aureus*), excellent solubility consistent with oral and i.v. dosing, and efficacy in a neutropenic mouse thigh *S. aureus* model and in a mouse lung sepsis model (oral and i.v. dosing for both models)[110,189] Importantly, key safety data were reported: "no observed off-target activity including cardiac channels; well tolerated in MTD and 7 day rodent safety studies". More recently, Biota revealed, however, that **46b** (BTA-C223), like **46a**, was highly protein-bound, showing an MIC shift of 64-fold upon addition of 50% horse serum. (Palmer et al., BMCL 2014) To decrease protein binding, the Biota scientists described a related benzothiazole urea series wherein the right-hand pyrimidine was substituted with a 2-hydroxy-2-propyl group, analogous to recent Vertex compound **41a**. Analogs within this series were also substituted on the lower pyridine with various basic amines to adjust the physical properties further. These analogs maintained low MIC values against *S. aureus* and *S. pyogenes* (typically 0.06 to 0.5 μg/mL) but now without a substantial shift upon addition of 50% serum (typical 1- to 4-fold shift) indicating low protein binding. Good solubility and oral bioavailability were maintained compared to the carboxylate series. One representative compound from this series showed efficacy similar to that of linezolid at a comparable single 30 mg/kg i.v. dose (ca. 3 $\log_{10}$ drop in *S. aureus* CFU count in a mouse thigh model). The analog also showed comparable efficacy in that model by the oral route when dosed at 100 mg/kg.

Intriguingly, Biota described progress (although without revealing exact structures) toward potent Gram negative activity with GyrB/ParE inhibitors having an ethylurea benzothiazole scaffold similar to that for **45** and **46**. Specifically within this series, Biota claimed compounds showing single digit μg/mL MIC values in multiple strains of *A. baumannii*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*.[110]

At AstraZeneca, the ethylurea bis-pyridines, exemplified by **47** (Figure 21), were identified via a fragment-based approach inspired by the Vertex benzimidazole scaffold, swapping a simple pyridine ring for the benzimidazole.[190−192] The incorporation of a second pyridine enhanced the binding affinity via π-stacking with Arg136, and the addition of weakly acidic oxadiazolone further enhanced the potency through a salt bridge with Arg 76. The trifluoromethyl thiazole at the pyridine 4-position interacted with the hydrophobic floor. This series afforded extremely potent inhibitors having subnanomolar IC_{50} values against *S. aureus* GyrB and *S. pneumoniae* ParE. Microbiologially, the series demonstrated very potent activity against Gram positive pathogens along with reasonable pharmacokinetic profiles upon i.v. dosing. Oral bioavailability for this lead series appeared to be low, however, whereas aqueous solubility and the plasma-free fraction for compounds having good whole cell activity also tended to be low. Challenges encountered in simultaneously optimizing for whole cell activity, good free fraction and solubility, and in vitro and in vivo safety resulted in some compromises, with compound **48**, a phosphate prodrug of **47**, emerging as an advanced, though not yet fully optimized, lead compound in this series. Parent **47** has very low spontaneous frequency of resistance in *S. aureus* and excellent activity against a panel of Gram positive and respiratory tract pathogens (Table 26). The free fraction was somewhat low (4.4%), but the exceptional MIC values partially compensated for this liability. Efficacy for **47** was demonstrated in a neutropenic mouse *S. aureus* thigh infection model: a single 300 mg/kg dose i.v. resulted in a 4 $\log_{10}$ reduction in bacterial load relative to the initial pretreatment inoculum. The relatively high dose required was due primarily to suboptimal i.v. clearance for this particular analog (74 mL/min/kg in the mouse). In vitro safety and preclinical in vivo safety data for **47** were encouraging.

Table 26. IC_{50} Values, Frequency of Resistance in *S. aureus*, and MIC_{90} Values for AstraZeneca Ethylurea Pyridine 47

property, enzyme, or organism [number of strains]	47
human plasma protein binding	95.6%
	IC_{50} (μM)
S. aureus GyrB (ATPase assay)	<0.01
S. pneumoniae ParE (ATPase assay)	<0.01
E. coli GyrB (ATPase assay)	<0.01
E. coli ParE (ATPase assay)	0.23
	Frequency of Resistance
S. aureus, at 4-fold MIC	3.0×10^{-10} (16-fold MIC shift)
	MIC_{90} (μg/mL)
S. aureus [11]	0.06
S. pyogenes [10]	0.01
S. pneumoniae [10]	0.01
E. faecalis [10]	<0.01
E. faecium [10]	0.03
H. influenzae [10]	1

Replacing the distal pyridyl of **47** with a naphthyridone led to a potent related series exemplified by **49**, which exhibited superior solubility and free fraction compared to those of the bipyridyl series.[193] The series retained potent dual inhibition and good

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microbiological activity against Gram positive and respiratory tract pathogens. Intriguingly, some members of this class exhibited single digit μg/mL MIC values against Gram negative pathogens such as *E. coli*, *K. pneumoniae*, and *P. aeruginosa*.[111] One example demonstrated a free fraction (human serum albumin) of 57%, a solubility of >700 μM, and an MIC_{90} value of 2 μg/mL against *E. coli*. In a neutropenic mouse thigh *E. coli* infection model, CFU stasis was achieved at a dose of 50−100 mg/kg/day whereas a >1 log_{10} reduction in the CFU count was achieved at a dose of 150 mg/kg/day.

During 2011−2012, Actelion disclosed in two large patent applications a series of inhibitors based on an ethylurea isoquinoline scaffold, with compound **50** being a representative analog (Figure 21).[194,195] The only data disclosed are MIC values versus *S. pneumonia*; **50** was reported with an MIC of <0.031 μg/mL. In 2013, Biota disclosed in a patent application yet another novel series, exemplified by **51**, apparently modeled on the AstraZeneca pyridyl ureas.[196] These compounds display the same overall pharmacophore as previous arylureas and a high degree of sp^2 character, indicating a risk of poor solubility. An acidic group is required for potent (MIC < 1 μg/mL) activity against the full range of pathogens highlighted in the patent, including *S. aureus*, *S. pyogenes*, *E. faecalis*, and *H. influenzae*. A publication in 2014 on this series disclosed moderate serum MIC shifts (4- to 8-fold) for several analogs. No other physical property data or safety or efficacy data were described.[197]

Scaffold designs that are related to this large family of ethylurea heterocycles inasmuch as being encompassed by the general binding motif depicted in Figure 19 are three other series, shown

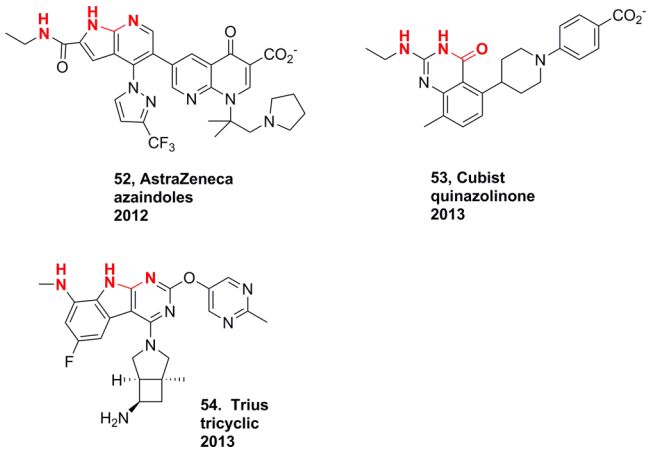

Figure 22. AstraZeneca azaindole, Cubist quinazoline, and Trius tricyclic scaffolds. The functionality shown in red interacts with the critical Asp73−water motif in gyrase and topo IV (*E. coli* gyrase numbering) as illustrated in the general binding mode of Figure 19. The trifluoromethylpyrazole of **52** interacts with the "lipophilic floor" of the enzymes, and the primary amino group of **54** interacts with an Asn46 water network.

in Figure 22, namely AstraZeneca's azaindoles, Trius' tricyclics, and Cubist's quinazolinones described below.

Concurrent with other AstraZeneca GyrB/ParE efforts, the azaindole scaffold exemplified by **52** (Figure 22) was derived from a virtual screen of "kinase-like" cores selected for the presence of a ligand donor−acceptor motif. As was the case with AstraZeneca's ethylurea pyridine scaffold, monocyclic aryl groups were first appended to the azaindole core to interact with the Arg76 and Arg136 enzyme side chains.[106] Evolution to a multicharged naphthyridone acid group afforded compounds with lower protein binding and frequently higher solubility.[198] For example, azaindole **52** had a free fraction of 49% and a solubility of 200 μM. Enzyme potencies for **52** were good and were indicative of a dual targeting inhibitor (Table 27). MIC values for *S. pneumoniae* and susceptible and resistant *S. aureus* were in the range of 0.1 to <0.024 μg/mL, superior to novobiocin (Table 27). In an immunocompetent mouse thigh model of *S. aureus* infection, a 100 mg/kg i.p. dose afforded a 1.5 log_{10} reduction in CFU.[119] To boost the aqueous solubilities in this series further, analogs incorporating a phosphate prodrug moiety (similar to Vertex's recent prodrug **41b**) were prepared. However, the effort on this scaffold was terminated.

Table 27. IC_{50} and MIC Values for AstraZeneca's Azaindole 52 Compared to Those for Novobiocin (1)

enzyme or organism	**52**	novobiocin (**1**)
	IC_{50} (μM)	
S. aureus GyrB (ATPase assay)	0.0005	ND[a]
S. pneumoniae ParE (ATPase assay)	0.0018	ND[a]
	MIC (μM)	
S. aureus MSQS	0.05	0.39
S. aureus MRQR	0.1	0.39
S. pneumoniae	<0.024	0.78

[a]ND = no data.

Cubist reported at ICAAC in 2013 a GyrB/ParE scaffold based on a quinazolinone that seemed to be designed to mimic the aryl ethylureas in a novel way by using a carbonyl group as the Asp73−water hydrogen bond acceptor in place of the standard heteroaryl nitrogen (Figures 19 and 22).[199] An X-ray crystal structure of **53** with the 24 kDa fragment of *S. aureus* GyrB demonstrated the binding mode. This compound showed potent dual GyrB/ParE inhibition along with encouraging MIC values against Gram positive pathogens as well as modest activity (8 μg/mL) against *E. coli* (Table 28). No other data (solubilities, free fraction, frequency of spontaneous resistance, in vitro safety, or any in vivo work) were disclosed for this early program.

Table 28. IC_{50} and MIC Values for Cubist's Quinazolinone 53 Compared to Those for Moxifloxacin

enzyme or organism	**53**	moxifloxacin
	IC_{50} (μM)	
S. aureus GyrB (ATPase assay)	<0.008	ND[a]
S. aureus ParE (ATPase assay)	0.047	ND[a]
E. coli GyrB (ATPase assay)	0.074	ND[a]
	MIC (μg/mL)	
S. aureus 42 MSSA	0.13	ND[a]
S. aureus 1118 MRSA	0.13	ND[a]
S. aureus 1721 MRSA CiproR	0.25	ND[a]
S. pneumoniae	2	0.06
E. faecium	2	0.06
E. faecalis	0.25	>2
E. coli	8	0.01

[a]ND = no data.

Trius followed their earlier bicyclic broad-spectrum scaffold (**33**, **34**; Figure 15) with a related, even more potent tricyclic scaffold, exemplified by **54** (Figure 22).[107] Enzyme potencies for both Gram positive (*E. faecalis*) and Gram negative (*E. coli*) GyrB and ParE were subnanomolar, consistent with a very low

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Table 29. K_i Values, Frequency of Resistance in *E. coli*, and MIC_{90} Values for Trius Tricyclic 54 Compared to Those for Gram Positive Spectrum (Vancomycin) and Broad Spectrum (Ceftazidime and Imipenem) Reference Drugs[a]

enzyme, or organism (number of strains)	54	
	K_i (nM)	
E. faecalis GyrB (ATPase assay)	<0.3	
E. faecalis ParE (ATPase assay)	<0.3	
E. coli GyrB (ATPase assay)	0.3	
E. coli ParE (ATPase assay)	0.9	
	Frequency of Resistance	
E. coli (4-fold MIC)	$<1.9 \times 10^{-11}$	
	MIC_{90}[a] (μg/mL)	
S. aureus (17)	0.008	1 (VAN)
S. pneumoniae (17)	≤0.001	0.25 (VAN)
E. faecalis (10)	≤0.001	>16 (VAN)
H. influenzae (11)	0.25	8 (CFZ)
M. catarrhalis (10)	≤0.008	2 (CFZ)
E. coli (22)	0.5	0.25 (IMP)
K. pneumoniae (31)	1	32 (IMP)
A. baumannii (20)	0.25	64 (IMP)
P. aeruginosa	2	64 (IMP)

[a]VAN = vancomycin; CFZ = ceftazidime, IMP = imipenem.

spontaneous resistance frequency of $<1.9 \times 10^{-11}$ in *E. coli* (Table 29). As with their previous series, the basic amino group in the scaffold was shown by X-ray crystallography to interact with the Asn46 within a structured water network. MIC_{90} values were excellent versus comparators for both Gram positive and Gram negative pathogens, including serious Gram negative pathogens *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae* (Table 29). MIC values were unaffected by 20% mouse serum, indicating low protein binding. Efficacy in a neutropenic mouse thigh model of *E. coli* infection was demonstrated wherein single i.v. doses of 5−15 mg/kg reduced the tissue CFU count by 3 $\log_{10}$ within 24 h, signifying a remarkable level of in vivo potency. Reported safety data for this series, however, was limited to in vitro selectivity against a panel of other ATP-utilizing enzymes. No solubility or pharmacokinetic data has yet been disclosed.

It is interesting to compare one of the earliest structure-influenced scaffolds, the Zeneca triazine, to Trius's very recent tricyclic scaffold, two efforts separated in time by more than 2 decades. Conceptually "removing" one bond in the Trius tricycle opens up a structure that resembles in many respects the overall architecture of the older Zeneca triazine (Figure 23). Yet important details resulting from the Trius design effort, for example, the more rigid scaffold and the precise location of the basic amine, afforded a series that is much more potent and much broader in spectrum than its early predecessor.

17. CRITICAL REFLECTIONS ON PAST AND CURRENT GYRB/PARE PROGRAMS

The 1960s Bristol-Myers' coumarin analog program that identified clinical candidate BL-C43 correctly prioritized the optimization of solubility and the free fraction, accepting diminished MIC potency as a compromise. Iterative mouse efficacy screening additionally revealed an unanticipated pharmacokinetic advantage with BL-C43, namely, a hepatic reuptake mechanism that enhanced blood concentrations. The human tolerability issue that was not predicted by animal safety studies was a problem that researchers of that time could not easily address from a technical perspective. Modern "secondary pharmacology" panels of human receptors and enzymes as well as other standard or custom human-based in vitro tests would be used today to help identify specific issues and guide optimization. Lacking those tools, the Bristol-Myers researchers understandably had limited options and chose to terminate the program. In contrast to the Bristol-Myers program, the concurrent Roche coumermycin A1 development program quickly ran aground and was terminated because of the poor aqueous solubility of the single compound under investigation.

Bristol-Myers 1980s "re-boot" coumarin analog program was initiated to address the rising threat of MRSA during that time. On the basis of the published record, the program seemed limited in scope and therefore appeared to be looking for a quick win. Perhaps shifting priorities at Bristol-Myers contributed to the lack of sustained effort.

By contrast, Roche's second attempt to develop a GyrB/ParE ATPase antibacterial agent demonstrated a significant commitment of resource lasting a decade (1987−1997[49]). The cyclothialidine program, the centerpiece of that effort, demonstrated that a completely novel scaffold having only enzyme potency with no antibacterial activity could be effectively optimized to provide compounds with low MIC values in the absence of initial crystallographic structural guidance. However, Roche did not initially realize that the cyclothialidine scaffold was inherently challenged beyond the usual complexities of co-optimizing the MIC, free fraction, and aqueous solubility: the key phenol "anchor" for enzyme binding also proved to be a key pharmacokinetic liability because of glucuronidation and hence rapid clearance. Nevertheless, with sustained effort Roche did address the glucuronidation liability while co-optimizing the other parameters. A key event in this optimization process was the decision (similar to that made in the Bristol-Myers 1960s effort) to sacrifice substantial microbiological potency in order to

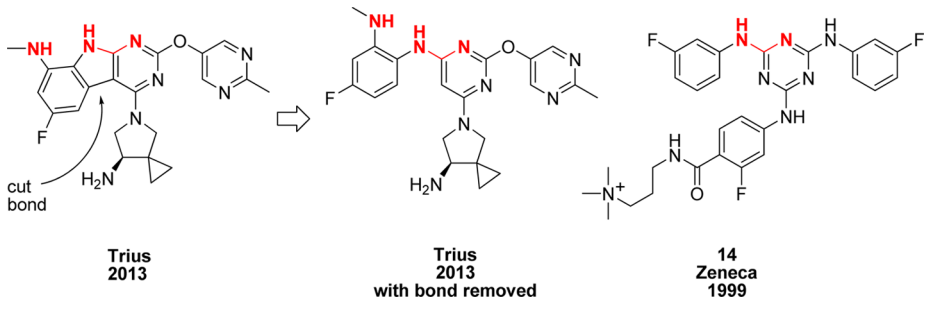

Figure 23. Hypothetical removal of one bond from a Trius tricyclic scaffold example demonstrating the similarity of the resultant pyrimidine core scaffold to one of the older Zeneca triazines.

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achieve superior physicochemical properties, an outcome that translated into superior in vivo efficacy. In our view, it seemed that there was not a significant gap between Roche's most optimized analogs and a potential clinical candidate. Unfortunately, shifting strategic priorities at Roche resulted in the termination of all GyrB/ParE efforts.[49] Roche stopped internal work on antibacterials in 2000, spinning off some antibacterial assets to Basilea, but no projects related to GyrB/ParE inhibition have been reported by Basilea.

Glucuronidation was also a key issue with the indazole series that Pfizer acquired from Quorex. In this case, the glucuronidation issue was not mitigated, and the project was terminated. With the benefit of hindsight, we now clearly recognize several structural motifs (phenols, indazoles, and perhaps pyrazoles) in historical GyrB/ParE projects that represent glucuronidation liabilities. We also recognize many alternative motifs for achieving the key Asp73−water interaction that do not carry a glucuronidation risk. Selecting those motifs appropriately should therefore eliminate the glucuronidation-based clearance liability in the future.

The rational redesign of novobiocin by Roussel-Uclaf that led to the identification of RU79115 was very encouraging from the standpoint of the significant progress made toward a clinical candidate yet discouraging because of the project's seemingly abrupt termination in spite of the continuing medical need at that time for novel class agents to treat MRSA and resistant enterococcus infections. It is not entirely clear whether the program was terminated for scientific or business/portfolio reasons or a combination of the two, but certainly there was a rapid succession of organizational changes that affected momentum.[200] In 1997, Roussel-Uclaf merged with Hoechst, forming Hoechst Marion Roussel (HMR). Then in 1999, the same year that RU79115 was first disclosed at ICAAC, Hoechst Marion Roussel merged with Rhone-Poulenc Rorer, becoming Aventis. In 2004, Aventis was acquired by Sanofi, at which time the infection unit was spun off to become Novexel, a small independent biotech that was then acquired by AstraZeneca in 2006. Neither RU79115 nor any other Roussel GyrB project equity seemed to be part of the assets transferred to AstraZeneca, however.

AstraZeneca, during the course of its numerous programs (triazine, pyrrolamide, arylaminopyrimidine, ethylurea-pyridyl/napthyridone, and azaindole), spent significant time and resources co-optimizing enzyme potency, MIC, free fraction, and solubility for each series in turn. Thousands of compounds were synthesized during lead optimization for these collective programs during a ca. 15 year period. As of 2014, AstraZeneca is the only company to have advanced GyrB/ParE compounds (two pyrrolamides) into the clinic since the Roche and Bristol-Myers programs in the 1960s. The other AstraZeneca program series (aside from the triazines) represented highly optimized preclinical scaffolds with many key scientific issues overcome. Of interest, one cultural factor at AstraZeneca that was influential during many of those years was an emphasis on high numbers of project transitions (such as achieving lead identification or lead optimization status).[201] This metrics-based philosophy artificially encouraged the generation of multiple scaffold series for any given project. In hindsight, we speculate that a valid alternative strategy during those 15 years of GyrB/ParE projects would have been to focus on fewer scaffold series while achieving greater in-depth understanding of the issues preventing progression to or success in the clinic. Conceivably, a more rigorous up-front priortization toward optimizing solubility and the free fraction, similar to the Bristol-Myers' 1960s effort, might have been useful in limiting the resources devoted to scaffolds displaying persistent marginal physical properties. Given that for i.v. dosing, solubilities should be in the range of ca. 5−20 mg/mL (ca. 10−40 mM), attention focused on scaffold series that can deliver high solubility is required to minimize downstream risk. The phosphate (or phosphoramidate) prodrug strategy can successfully be applied to address solubility issues of parent scaffolds for antibacterials, with tedizolid and ceftaroline being two recent commercialized examples, and clindamycin 2-phosphate being an older example.[202,203] As described, AstraZeneca did begin to explore phosphate prodrug strategies for the arylaminopyrimidine and azaindole series, although too late to make a substantial impact prior to the strategic decision to scale back the entire GyrB/ParE effort substantially at AstraZeneca in 2011. Also in retrospect, we believe larger preclinical in vivo data sets may have been beneficial for faster and better-informed decision making. Considering the vast numbers of analogs synthesized, comparatively few animal studies were conducted either for the purpose of continuously benchmarking or capturing serendipity. Moreover, at AstraZeneca the target pathogen profile for the GyrB/ParE programs gradually shifted over time from a Gram-positive-only perspective (with a focus on MRSA and resistant enterococcus) to a focus on broad spectrum, encompassing Gram negatives such as *P. aeruginosa* and *A. baumannii*. This is understandable because the therapeutic need for new Gram positive agents was gradually being satisfied during the 2000s by other agents from new or established classes. This changing focus contributed to decisions to terminate or deprioritize otherwise high-quality series that displayed at that time predominantly Gram positive spectrums. The pyridyl napthyridones (such as **49**) did begin to evolve toward encouragining Gram negative activity but not at a sufficiencly compelling pace to warrant the continuation of this effort in-house.

Vertex by contrast focused primarily on their flagship ethylurea benzimidazole scaffold over the course of 10+ years. On the basis of the published record, it appears that the Vertex GyrB/ParE ATPase effort was significant in the early 2000s and then seemed to pause for several years and was reinitiated about 2010. Scientists at Vertex were early advocates for the concept of potent dual target inhibition within the context of ATPase inhibitors, leading by example with their own series. Their early optimized compounds were exquisitely potent against Gram positive bacteria and had favorably low frequencies of spontaneous resistance, yet marginal physical properties posed a persistent risk. As with AstraZeneca, a stricter policy at program initiation regarding physical property cut-offs may have been useful for Vertex. In any case, Vertex had more recently demonstrated a clear focus on those issues, addressing solubility via a phosphate ester prodrug strategy and a greater appreciation for the importance of the plasma free levels. Seven day rat and monkey safety studies on at least one such recent redesigned analog seemed to indicate acceptable safety margins, and efficacy and exposure data in animal models showed potential for both oral and i.v. dosing. Vertex has clearly positioned this analog for clinical studies; however, it has also recently disclosed that it is closing down its infection therapy area and that **41b** will be developed only if it finds an external partner or buyer.[204] Given today's largely satisfied market for effective Gram positive spectrum agents, that objective may prove difficult to achieve. However, new-class Gram positive spectrum agents having the

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advantage of both oral and i.v. modes of administration could still be attractive.

Biota, preceded by Prolysis/Evotec, also maintained focus over the span of about 8 years on the ethylurea benzothiazole scaffold. That strategy appears to have paid off insofar as credible clinical candidate BTA-C223 has reportedly been identified, as well as a broad spectrum subseries having potent activity against Gram negative pathogens including *P. aeruginosa*. In November 2013, however, Biota announced that because of financial difficulties it has halted all preclinical antibiotic programs and will seek licensing agreements and other collaborative arrangements to advance the development of its candidates. A buyer would need to acquire the assets and make the properly resourced long-term commitment to move not only Gram posive spectrum late-stage equity toward development but also, or perhaps even more important from a medical need perspective, advance the Biota broad spectrum scaffold equity.

Trius's evolution of a novel prototype scaffold into a credible series having an expanded microbiological spectrum encompassing (to varying extents) serious Gram negative pathogens such as *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae* within a relatively short time frame (ca. 2007 to 2011) is an extraordinary accomplishment. Encouragingly, in vitro frequencies of spontaneous resistance (at least in Gram positive pathogens) are very low, and protein binding seems not to be an issue. However, other potential issues such as safety and aqueous solubility are undefined at present. Cubist's early efforts with two different in-house scaffolds seem initially promising, although one of the scaffolds contains a pyrazole that might represent a glucuronidation liability based on the Quorex indazole experience. Trius was acquired by Cubist in 2013, primarily for the oxazolidinone tedizolid that has recently been approved by the FDA. With regard to the now-combined GyrB/ParE equity from both companies but especially the Trius tricyclic scaffold series that shows significant antipseudomonal and antiacinetobacter activity, the challenge for Cubist will be to maintain long-term focus with the application of robust resources to bring that series to the clinic. By do doing, Cubist would add a much-needed new-scaffold, new-mechanism agent to the Gram negative antibacterial pipeline.

As exemplified by the Vertex ethylurea and the AstraZeneca arylaminopyrimidine and pyrrolamide series, there is now an emerging understanding of the PK/PD indices and magnitudes for the class of antibacterial ATP site GyrB/ParE inhibitors. Such an understanding will be important for the optimization of clinical dosing regimens. Free (unbound) drug concentrations (as opposed to total concentrations) are increasingly being employed in these calculations, and this fact reinforces the drug discovery objective for the adequate optimization of free drug levels for any clinical candidate.[205,206]

We would like to comment briefly on the general topic of lead generation for GyrB/ParE projects. As documented in this review, the discovery of tractable chemical starting points having useful levels of enzymatic inhibitory potency toward the ATPase of gyrase and/or topo IV is relatively straightforward. Standard HTS collections have typically provided multiple hits that were quickly progressed to leads and beyond. As described, the inhibitor binding site is tolerant of a wide variety of hydrogen bond accepting/donating pharmacophores capable of productively interacting with the key Asp73—water motif. Other key hydrogen bonding interactions, such as with Arg138, as well as nonpolar interactions with the hydrophobic "floor" and "left-hand pocket" are also readily achieved with a variety of scaffold functionality. This extensive record of quality lead-finding achievements that are amenable to further optimization is somewhat at odds with a 2007 review asserting that standard HTS collections are not diverse enough for productive screening against bacterial targets.[207] At least for the ATP binding site targets of gyrase and topo IV that generalization does not seem to apply. On the basis of our experience, the HTS hit rates seemed comparable to any standard nonbacterial target. Additionally, as we have also seen, natural products have been a historically rich source of GyrB/ParE inhibitor scaffolds, a trend that continues even today.[208,209]

18. GYRB/PARE ATPASE INHIBITORS: CAN A NEW CLASS OF ANTIBACTERIALS BE LAUNCHED IN TODAY'S ECONOMIC CLIMATE?

"Fragile" is an appropriate term to describe the state of antibacterial R&D within the pharmaceutical industry today, and a number of valid explanations have frequently been cited to account for this fragility. Fundamentally, the economics of new antibacterial agents forms the central basis of these explanations, encompassing subtopics such as antibiotic pricing, uncertain regulatory requirements, and the cost of research and development. All of these factors are interrelated and ultimately create the economic calculus that pharmaceutical companies use for strategic resourcing decisions across their portfolios.

In this context, we feel a brief discussion of the cost of discovering and optimizing new-class antibacterial agents might be useful. Over the past decade, it has been said that the "low-hanging fruit" has already been picked, that high-throughput screening of existing corporate compound collections is economically unsustainable, and that new approaches are required. Also frequently discussed is the technical difficulty of converting pure enzyme inhibitors into antibacterially active agents or the elusiveness today of discovering new anti-Gram negative classes.[207] All of these assertions carry some degree of truth when attempting to justify the cost of these discovery, and any subsequent development, efforts in the context of today's returns on antibiotic investment as assessed by net present value (NPV) calculations. Such NPV estimates for new launch antibacterial sales are low compared to returns from agents in many other therapeutic areas, and it understandably follows that companies are reluctant to invest substantial and sustained resources in antibacterial R&D.[210,211] However, to put the low-hanging fruit argument into better perspective, it should be appreciated that preclinical antibacterial research to discover new classes or extend the spectrum of existing classes to encompass Gram negative pathogens such as *P. aeruginosa* has historically, from a technical, time and resource perspective, *always* been highly challenging. What fundamentally differentiates those early decades of antibiotic R&D from the situation we are facing now is the stark difference in antibacterial economics between these two eras.

The cost- and time-intensive early antibiotic discovery efforts were sustained and justified over several decades after World War II by the high profitability of antibiotic sales, initially from purchases of penicillin by the U.S. government and then from sales of other classes of antibiotics directly to the medical and agricultural communities.[32,212,213] The medical sales were driven by the vast unmet need to cure patients infected by a range of bacterial pathogens for which previously there had been no, or few, effective treatment options whatsoever. The agricultural sales were partially driven by the growth-promoting effects of low-dose antibiotics in animals. The entry of the majority of

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American pharmaceutical companies into antibiotic R&D had been strongly encouraged by the significiant economic incentives, largely by investiments in infrastructure and expertise, provided by the U.S. government toward wartime penicillin production. The dominance of antibiotic R&D at most pharmaceutical companies was further sustained in those early decades by the relative sparseness of other scientifically mature, competing research-based therapeutic areas for companies to invest in.[212]

However, even during the most fertile historical decades of natural product screening for antibiotics, attrition rates were staggering by any measure.[32,214−217] The continual rediscovery of existing antibacterials was a significant burden on the workflows throughout that time. Finding effective agents without significant toxicities or agents that were differentiated was also an enormous challenge. A productivity decline in new antibacterial discovery was noted in the early 1960s.[218,219] If one objectively tallies the number of medically significant first-in-class agents discovered and commercialized during the four decades of maximum pharmaceutical participation and resourcing in antibacterial R&D, the productivity for any single company is astonishingly meager. Lilly was probably the most productive in terms of major first-in-class agents: it discovered and commercialized erythromycin and vancomycin during the 1950s and contributed to the technological innovations leading to the semisynthetic cephalosporins, a class that proved to be a significant source of profit for Lilly during the 1970s. Lilly also discovered and partially developed daptomycin, with Cubist completing development and ultimately commercializing it. The companies that collectively became Wyeth (prior to the merger with Pfizer) can be credited with the discovery and commercialization of chlortetracycline and polymyxin B (PMB) as major first-in-class agents, although Wellcome independently discovered PMB at about the same time and commercialized it to a greater extent.[215] Pfizer over its history discovered and commercialized only follow-on in-class agents, among them tetracycline and azithromycin. Purely synthetic new classes were discovered at companies not significantly engaged in natural product screening: Norwich Eaton (nitrofurans), ICI and Sterling (quinolones), Burroughs-Wellcome (trimethoprim), and DuPont (oxazolidinones). During these early decades of intense activity, any single company that both discovered and commercialized a significant new-class antibacterial agent more than once in a 20 year time span exceeded the industry average.

Discovering and launching first-in-class potent Gram negative agents was an even rarer event. In the history of antibacterial R&D only two agents have been discovered and developed possessing therapeutically useful activity against *P. aeruginosa* from the first commercial entry: polymyxin B and thienamycin (commercialized as the chemically stabilized variant imipenem). All other classes of today's antipseudomonal antibacterials (semisynthetic penicillins and cephalosporins; quinolones) took about *20 years* to evolve into variants having potent antipseudomonal activity or required additional natural-product-based screening to discover potent antipseudomonal members of an already known class (aminoglycosides).

The frequently cited disappointment of high-throughput screening of corporate compound libraries against genomically identified bacterial targets reflects expectations that were likely too high.[207] New discovery approaches need time to mature, and breakthrough results may come rarely.[220] Some bacterial targets are better than others from the standpoints of establishing essentiality and being "druggable". In an expensive but necessary learning curve, the resource-intensive HTS exercises during the 1990s and 2000s usefully served to point the field toward certain targets and away from others.

With today's expanded science base encompassing deeper understandings of resistance mechanisms, emerging understandings of the molecular requirements for bacterial cell penetration, and improved crystallographic- and NMR-based structure determination, we have the technical tools to build constructively on the collective efforts of seven decades of historical antibacterial efforts in order to discover and develop new-scaffold, new-mechanism drugs, including those having potent anti-Gram negative activity. With a valid target (or targets) and reasonable chemical lead material, antibacterial programs can succeed from a technical perspective in identifying important new-class clinical candidates that have the potential to reach the market.

Such is especially the case for gyrase/topo IV ATPase inhibitors. Discovering and developing new-class antibacterial agents having this mechanism has multiple immediate advantages over other potential new mechanism agents: (1) the inhibition of two targets simultaneously for the mitigation of target-based resistance; (2) the facile discovery of lead material from corporate compound collections and rapid optimization of those leads into agents having potent antibacterial activity with in vivo efficacy; (3) a bactericidal mechanism; (4) historical clinical validation of the mechanism of action by novobiocin; (5) an emerging understanding of PK/PD indices and magnitudes; (6) the potential for both oral and i.v. administration with a single agent; and finally, from a critical medical need perspective (7) emerging significant broad-spectrum activity including activity against *P. aeruginosa*, *A. baumannii*, and *K. pneumoniae*. As has been amply documented in this review, there has existed for many years now a number of optimized or semioptimized GyrB/ParE inhibitor scaffold series, with several having significant Gram negative potency.

New-class antibacterial drugs, especially for the treatment of Gram negative infections, are urgently needed, yet such agents are essentially absent from the current clinical pipeline.[221] This is a dire situation that nevertheless can be corrected at least from a technical point of view. Crucially, however, the sense of urgency needs to be balanced with focus, patience, and dedicated long-term commitment in the development of such agents. There are no quick wins in the development of novel class antibacterial agents. The required patience and long-term commitment of resources for the successful development of new-class antibacterials have largely been thwarted during the last few decades by the fragile economics of investment in antibacterials. The economic analysis does not permit companies to allocate the substantial levels of resources sustained over the long periods of time required to solve the numerous preclinical and clinical issues that inevitably arise during advancement of new-class agents. Consequently, pharmas both large and small rebalance their portfolios typically at the expense of antibacterials. Those small pharmas that may have the desire to sustain effort in antibacterial R&D can be particularly vulnerable to financial fluctuations.[222,223] With relatively few biopharmaceutical companies currently committed to new-class antibacterial programs (such as GyrB/ParE inhibitors), the loss of even one or two such programs significantly reduces the likelihood of delivery of an agent in that class. Mergers, acquisitions, internal reorganizations, and changes in management adversely affect good scientific programs even in economically robust therapeutic areas such as oncology, most significantly by the loss of the internal champion

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for early clinical or preclinical programs.[224] The negative effects of organizational disruption and project handovers are further magnified for antibacterial programs. Thus, more than any technical or scientific factors, we see the absence of GyrB/ParE new-class antibacterial agents in the clinical pipeline today as a result of these economic forces.

In spite of the perceived gloomy economic picture for antibacterials as a whole, one should keep in mind that two Gram positive spectrum first-in-class agents launched within the last 15 years, linezolid and daptomycin, eventually achieved yearly sales of >$1 billion, notably higher than any in-class antibacterial agents launched during the same period. First-in-class antibacterial agents may have an economic advantage. Other new-class agents, particularly new-mechanism Gram negative agents, could experience a similar economic advantage. Additionally, there has been recent progress toward implementing novel regulatory and economic models governing antibacterials intended to improve the return on investment for future agents that address serious bacterial resistance.[225−229]

Fortunately, with the technical tools and knowledge in hand, the potential of more attractive economic return for future first-in-class agents, and smoother regulatory pathways for antibacterials being implemented, the field may be better prepared now for introducing a GyrB/ParE new-class antibacterial drug than at any time in the past.

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Notes

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ABBREVIATIONS

ADPNP, adenosine 5′-(β,γ-imido)triphosphate
AUC, area under the concentration−time curve (for drug dosed in vivo)
CFU, (bacterial) colony-forming units
C_{max}, peak (in vivo) drug concentration
NPV, net present value
ICAAC, Interscience Conference on Antimicrobial Agents and Chemotherapy
MIC, minimum inhibitory concentration
MIC_{90}, MIC at which 90% of a large panel of same-species (usually clinical) bacterial isolates are susceptible
MRSA, methicillin-resistant *Staphylococcus aureus*
MTD, maximum tolerated dose
PK/PD, pharmacokinetic−pharmacodynamic (relationships)
VRE, vancomycin-resistant enterococcus

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