Chemistry & Biology
Article

Discovery of Kibdelomycin, A Potent New Class of Bacterial Type II Topoisomerase Inhibitor by Chemical-Genetic Profiling in *Staphylococcus aureus*

John W. Phillips,[1,5] Michael A. Goetz,[2] Scott K. Smith,[2] Deborah L. Zink,[2] Jon Polishook,[1] Russell Onishi,[1] Scott Salowe,[1] Judyann Wiltsie,[1] John Allocco,[1] Janet Sigmund,[1] Karen Dorso,[1] Suzy Lee,[1] Stephen Skwish,[1] Mercedes de la Cruz,[3,6] Jesús Martín,[3,6] Francisca Vicente,[3,6] Olga Genilloud,[3,6] Jun Lu,[4] Ronald E. Painter,[1] Katherine Young,[1] Karen Overbye,[1] Robert G.K. Donald,[1] and Sheo B. Singh[1,2,*]
[1]Infectious Diseases
[2]Medicinal Chemistry
Merck Research Laboratories, Merck & Co., PO Box 2000, Rahway, NJ 07065, USA
[3]Centro de Investigación Básica (CIBE), Merck Sharp & Dhome de España, S.A., Madrid, Spain
[4]Global Structural Chemistry, Merck Research Laboratries, West Point, PA 19486, USA
[5]Present address: Allen Institute for Brain Science, Seattle, WA 98103, USA
[6]Present address: Fundación MEDINA, Centro de Excelencia en Investigación de Medicamentos Innovadores en Andalucía, Avda Conocimiento 3, Parque Tecnológico Ciencias de la Salud, 18100 Armilla-Granada, Spain
*Correspondence: Sheo.Singh@merck.com
DOI 10.1016/j.chembiol.2011.06.011

SUMMARY

Bacterial resistance to known therapeutics has led to an urgent need for new chemical classes of antibacterial agents. To address this we have applied a *Staphylococcus aureus* fitness test strategy to natural products screening. Here we report the discovery of kibdelomycin, a novel class of antibiotics produced by a new member of the genus *Kibdelosporangium.* Kibdelomycin exhibits broad-spectrum, gram-positive antibacterial activity and is a potent inhibitor of DNA synthesis. We demonstrate through chemical genetic fitness test profiling and biochemical enzyme assays that kibdelomycin is a structurally new class of bacterial type II topoisomerase inhibitor preferentially inhibiting the ATPase activity of DNA gyrase and topoisomerase IV. Kibdelomycin is thus the first truly novel bacterial type II topoisomerase inhibitor with potent antibacterial activity discovered from natural product sources in more than six decades.

INTRODUCTION

Bacterial strains continue to acquire resistance to clinically used antibiotics with alarming frequency and pose a serious threat to human lives (Klevens et al., 2007). According to a recent report, methicillin-resistant *Staphylococcus aureus* (MRSA) infections alone are responsible for about 18,000 deaths per year in the United States (Klevens et al., 2007). Chemical modifications of the natural product antibiotic lead structures, discovered more than five decades ago, led to incrementally improved antibiotics that continue to serve well and provide the current reservoir of clinical antibiotics (Singh and Barrett, 2006). Regrettably, additional such improvements on existing chemical scaffolds are proving challenging and can be only overcome by discovery of new antibiotic scaffolds with either known or novel modes of action that could be developed as effective treatment options against drug-resistant bacteria. Most antibacterial leads are derived from nature and we believe that nature will continue to serve as a prolific source of new antibacterial leads. However, it is now well documented that one of the biggest challenges in natural products discovery remains the rediscovery of known compounds. The probability of success for the discovery of novel natural product antibiotics can be significantly improved when fundamentally new screening technologies are coupled with new producing sources of natural products. We have made inroads to address both of these problems. First, we have introduced a target-based, whole cell screening approach that uses inducible antisense to down-regulate the expression of individual essential gene targets in *S. aureus*, thereby sensitizing the cells to inhibitors of those targets and enriching the pool of screening hits for inhibitors specific to those targets or pathways at the crude extract stage (Singh et al., 2007). Second, we have expanded our efforts to isolate microorganisms from different geographical regions and habitats (including marine microbes) in combination with improved high-throughput fermentation methods (Bills et al., 2008; Genilloud et al., 2011). The combination of these two approaches resulted in the discovery of platensimycin and platencin by using *fabH*/*fabF* antisense sensitized strains (Jayasuriya et al., 2007; Kodali et al., 2005; Ondeyka et al., 2006; Singh et al., 2006a; Wang et al., 2006, 2007; Young et al., 2006) and a number of other novel natural products by using a *rpsD* sensitized antisense strain (Ondeyka et al., 2007; Singh et al., 2006b, 2008, 2009; Zhang et al., 2009a, 2009b).

The single strain *S. aureus* antisense-based screening approach was recently extended to a genome-wide fitness test assay, its use was validated by mechanistically profiling a diverse set of 59 antibacterials (Donald et al., 2009), and it was applied to the screening of a chemical collection, leading to the discovery of a new class of cell wall inhibitors (Huber et al., 2009). The

S. aureus fitness test assay consists of 245 inducible antisense RNA strains engineered for reduced expression of genes essential for cell growth. Reduced expression of target genes leads to differential growth sensitivity of cells to compounds that either inhibit the targeted gene product or related functions. When combined into pools and grown together for approximately 20 population doublings in the presence of test compounds, these differences in growth lead to specific antisense strains either being depleted or enriched in the treated population. Using multiplex polymerase chain reaction (PCR), capillary electrophoresis and gene fragment analysis to compare the abundances of the strains at the end of the experiment with mock-treated controls generates characteristic antisense-induced strain sensitivity (AISS) profiles that are indicative of a compound's whole-cell mechanism of action (MOA). Compound MOA can either be inferred directly from the AISS profile or by comparison with a database of AISS profiles of known inhibitors (Donald et al., 2009). Both approaches are very useful for the discovery and differentiation of new compounds by not only indicating preliminary mechanistic information but also providing comparative information that is of great value in de-replicating natural products.

In a similar way as the *Candida albicans* fitness test assay was applied for the discoveries of parnafungin (Jiang et al., 2008; Parish et al., 2008) and other novel natural products as antifungal agents (Herath et al., 2009; Ondeyka et al., 2009; Xu et al., 2007), AISS profiling was used in antibacterial discovery to prioritize extracts for natural products chemistry based on whether their activities are likely working through either interesting known or novel MOAs. Those compounds exhibiting a novel AISS profile and likely containing chemical inhibitors not seen before in the assay can be assigned to isolation chemistry. Screening of natural products extracts and AISS profiling led to identification of one such prioritized extract that exhibited an AISS profile with a potentially novel MOA and led to the discovery of coelomycin (Goetz et al., 2010).

Extracts producing AISS profiles that are similar to profiles produced by known drugs with attractive target-based MOAs may contain those known compounds, novel analogs of those compounds, or potentially new structural classes of inhibitors working through established drug targets. Extracts exhibiting a known AISS profile can be analyzed further by high-resolution mass spectrometry and other analytical techniques to either confirm or rule out the presence of known inhibitors before any isolation chemistry steps are undertaken. Continued screening efforts led to the identification of a number of extracts exhibiting known AISS profiles. Here we disclose the discovery of kibdelomycin from *Kibdelosporangium sp.* (MA7385), a new class of natural product bacterial type II topoisomerase inhibitor with potent activity against both DNA gyrase and topoisomerase IV (topoIV). This inhibitor showed broad-spectrum activity against a range of important gram-positive bacteria without cross-resistance to known gyrase inhibitors.

RESULTS

S. aureus Fitness Test Screening of Crude Extracts

We set up a program to systematically screen crude natural product extracts through the *S. aureus* fitness test to discover new antibacterial agents working through target-based MOAs. At the front end of this program, new bacterial and fungal isolates were acquired from around the world, grown under 8–12 different culture conditions, and extracted with acetone, and crude extracts were screened for antibacterial activity against *S. aureus*. Extracts exhibiting *S. aureus* activity were then subjected to the *S. aureus* fitness test for AISS profiling (Donald et al., 2009) for MOA determination and prioritization for natural products chemistry. One such extract derived from a *Kibdelosporangium* strain, isolated from a soil sample collected in a forest in the Central African Republic, generated AISS profiles that were highly correlated to those generated in the *S. aureus* fitness test for the natural product coumarin antibiotic novobiocin (Figure 1). AISS profiles for the coumarin antibiotic novobiocin are characterized by strong depletions in the *parE* and *parC*-antisense (AS) strains (the two subunits of topoIV) and the *gyrB*-AS and *gyrA*-AS strains (the two subunits of DNA gyrase) (Donald et al., 2009). The crude extract shows strong depletions in *parE/C*-AS and *gyrB*-AS and weak depletions of *gyrA*-AS along with subtle differences in other strains (Figure 1).

These fitness test results were predictive of the extract containing a known coumarin antibiotic, a new coumarin analog, or a new structural class working through a similar MOA to novobiocin. Up to this point the program had already identified a number of examples of extracts containing the coumarin antibiotics novobiocin, chlorobiocin, and coumermycin A1 (not shown). Because high-resolution electrospray ionization Fourier transform mass spectral analysis (HRESIFTMS) was unable to identify coumarin antibiotics in the crude extract (data not shown), this extract was prioritized for natural products chemistry to isolate the active component responsible for the coumarin-like AISS profile.

Isolation and Structure Elucidation

The producing organism, *Kibdelosporangium sp.*, MA7385, was grown in a liquid culture for 7 days and broth was extracted with equal volume of acetone. Kibdelomycin was purified from the extract by successive steps of extractions, normal phase (silica gel), and reversed-phase chromatographies. Kibdelomycin (Figure 2) showed a molecular weight of 938 and molecular formula of $C_{44}H_{60}Cl_2N_4O_{14}$ by HRESIFTMS. This formula was supported by the observation of the corresponding number of ^{13}C shifts in the ^{13}C NMR spectrum in methanol-$_{d4}$ (Table 1; see Figure S1 available online) and ^{1}H shifts in the ^{1}H NMR spectrum (Table 1; Figure S2). The structure of kibdelomycin was elucidated by application of two-dimensional NMR techniques (COSY, HMQC, HMBC, and ROESY) in a number of deuterated solvents (methanol-$_{d4}$, pyridine-$_{d5}$, and DMSO-$_{d6}$) but only data in methanol-$_{d4}$ is shown here. The COSY spectrum showed ^{1}H-^{1}H cross peaks that allowed connectivities and establishment of fragments C1-(C20)-C10-C21, C-14-C-17-(C18-C19), C22-C28, C30-C33, C33-C34, and C37-C38. These fragments were connected to establish a planar structure by analysis of HMBC correlations and data is summarized in Table 1. The relative configuration of kibdelomycin was elucidated by ROESY correlations (Table 1). H-4 showed two key ROESY correlations: one with H-10 as a result of a 1,3-diaxial relationship and the other with H-30 as a result of a proximal facial relationship. Further ROESY correlations of H-30 with H-34, H-31$_{ax}$ with H-33 and

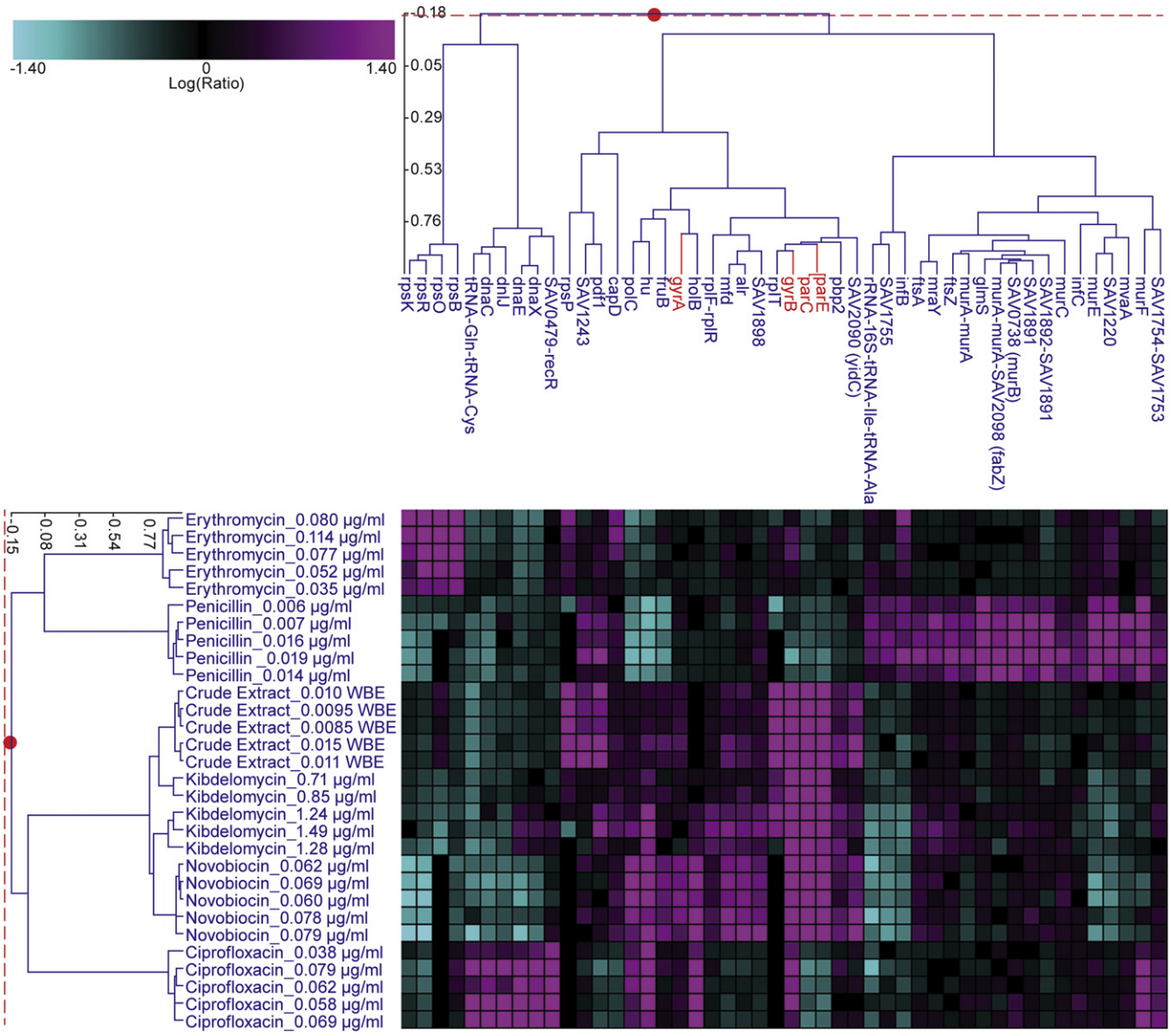

Figure 1. Two-Dimensional Cluster Analysis of *S. aureus* Fitness Test Profiles for the Crude Extract, Kibdelomycin, and Four Compounds of Known Mechanism of Action

Strain depletions are depicted in magenta, strain resistances in cyan. The thresholds for including strains in the cluster analysis are >5-fold depletion, p-value $\leq$ 0.01 in at least 4 of the experiments for any one compound or extract. The compound name is followed by the treatment concentration. Profiles for the Crude Extract and Kibdelomycin cluster tightly with profiles of the antibiotic Novobiocin. The antisense strains targeting the 2 subunits of DNA gyrase (gyrA, gyrB) and DNA topoisomerase IV (parC, parE) are shown in red.

H-37, H-5 with H-9, and H-10 with CH_3-21 helped elucidate the configuration of C1-C10 and C4-C30-C38. The relative configuration of the remainder of the molecule was independently established by ROESY correlations of H-17 with H-22, and H-22 to CH_3-28, H-23, and H-25. The four structural motifs of kibdelomycin were connected with each other with hetero atoms and confirmed by HMBC. The central 4-hydroxy decalin tetramic acid core is tethered on one side to a novel 6-methyl-2-methoxy-3-acetoxy-4-carbamoyl-epiallose residue (Sugar A) by an N-glycosidic linkage to tetramic acid and on the other side to 3-α-aminoethyl-3,6-dideoxy hexopyranose (Sugar B) by an O-glycosidic linkage. The amino group of the amino ethyl substitution of the sugar B forms an amide bond with 3,4-dichloro-5-methyl pyrazole-2-carboxylic acid.

Kibdelomycin Has Broad-Spectrum Gram-Positive Activity

Kibdelomycin exhibited strong antibacterial activity against wild-type *S. aureus* strains and showed a minimum inhibitory concentration (MIC) value of 2 μg/ml (Table 2). It showed strong activity against methicillin-resistant *S. aureus* (MRSA) with a MIC of 0.5 μg/ml. Kibdelomycin inhibited growth of other key

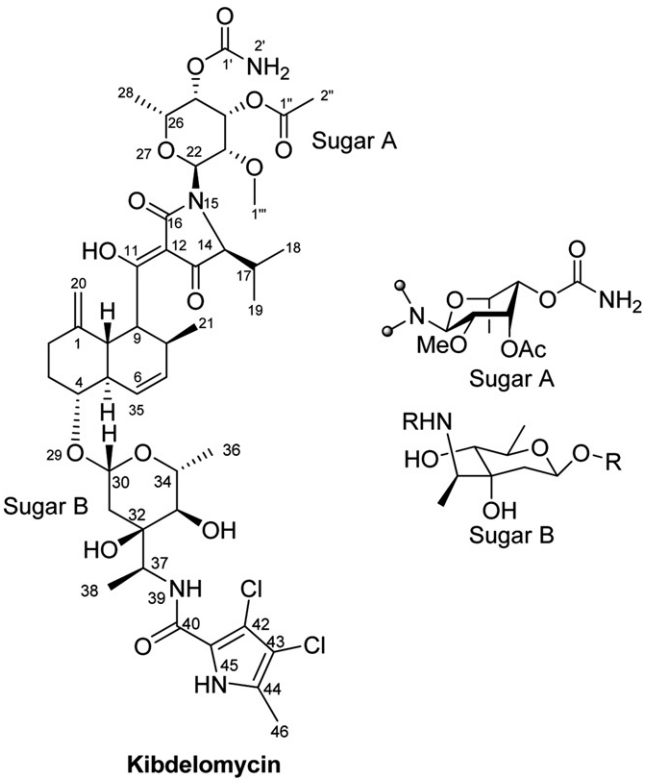

Figure 2. Chemical Structure of Kibdelomycin

gram-positive pathogens, *Streptococcus pneumoniae* and *Enterococcus faecalis*, with MIC values of 1 and 2 μg/ml, respectively. It also inhibited the growth of a gram-negative pathogen, *Haemophilus influenzae*, with a MIC value of 2 μg/ml. It did not exhibit activity against other serious gram-negative wild-type pathogens such as *Escherichia coli* (MIC >64 μg/ml). It did, however, inhibit the growth of a permeable and efflux pump mutant *E. coli* (*envA1*, *tolC*) strain with a MIC value of 32 μg/ml. The activity of kibdelomycin was significantly reduced (MIC 64 μg/ml) when tested in the presence of 50% human serum. Overall, the activity of kibdelomycin was similar to that of novobiocin. Kibdelomycin did not inhibit the growth of *Candida albicans* at 64 μg/ml.

AISS Profiling of Kibdelomycin

Kibdelomycin was subjected to the *S. aureus* fitness test to confirm that it was the active component responsible for the coumarin-like AISS profile of the crude extract. The profile generated by kibdelomycin was highly correlated to the profiles from the crude extract and novobiocin (Figure 1), with strong depletions in the *parE*-AS and *parC*-AS strains (the two subunits of topoIV) and the *gyrB*-AS strain (GyrB subunit of DNA gyrase) along with shared depletions in the *hu*-AS (DNA binding protein), *alr*-AS, and *mfd*-AS strains. Some of the secondary depletions (*mfd*-AS, *alr*-AS, and *hu*-AS) were not as significant for kibdelomycin as they were for novobiocin at all concentrations tested. The most interesting difference between the two AISS profiles was the strong depletions in *gyrA*-AS (GyrA subunit of DNA gyrase) in the novobiocin profiles and a lack of significant *gyrA*-AS depletions for kibdelomycin.

The coumarin antibiotics, novobiocin and coumermycin A1, inhibit the catalytic activity of DNA gyrase and topoIV through inhibition of the ATPase activity of these heterodimeric type II topoisomerases (reviewed: Maxwell and Lawson, 2003; Oblak et al., 2007) leading to inhibition of DNA synthesis and cell death. This differs from the MOA of the fluoroquinolone antibiotics that form a drug-topoisomerase-DNA complex, which traps the covalent intermediate of cleaved DNA to the GyrA or ParC subunit (the cleavable complex), leading to DNA double-strand break-induced cell death (Chen et al., 1996). These mechanistic differences are reflected in the AISS profiles of these classes of compounds (Donald et al., 2009) and suggest that kibdelomycin is likely working through an MOA similar to that of the coumarin antibiotics that involves inhibition of the ATPase activity of DNA gyrase and topoIV (Figure 1).

Kibdelomycin Is a Selective Inhibitor of DNA Synthesis and a Potent Inhibitor of Type II Bacterial Topoisomerases

The macromolecular synthesis assay, in which the inhibition of the intracellular building blocks of macromolecules are measured, provides guidance for understanding the broad MOA of compounds (Wang et al., 2006). In this assay, as predicted by the *S. aureus* fitness test, kibdelomycin inhibited DNA synthesis (IC_{50} 0.03 μg/ml) in *S. aureus* and showed more than 100-fold selectivity over RNA, peptidoglycan, phospholipids, and protein synthesis (Figure 3).

In the cell-free topoisomerase supercoiling and decatenation assays, which measure the inhibitory effect of compounds on the DNA supercoiling activity of the protein complexes of DNA gyrase and the decatenation activity of DNA topoIV, kibdelomycin inhibited *E. coli* gyrase supercoiling and topoIV decatenation activity with IC_{50} values of 60 and 29,000 nM, respectively (Table 3). It showed more potent activity against *S. aureus* gyrase supercoiling (IC_{50} = 9 nM) with a 55-fold reduced potency against *S. aureus* topoIV decatenation activity (IC_{50} = 500 nM) (Table 3). In the catalytic ATPase assays, which measure the inhibition of the holoenzyme ATPase activity, kibdelomycin inhibited *E. coli* DNA gyrase ATPase activity with IC_{50} values of 11 nM and topoIV ATPase activity with an IC_{50} value of 900 nM (Table 3). Similar to novobiocin, kibdelomycin was a more potent inhibitor of *E. coli* DNA gyrase ATPase activity than it was an inhibitor of topoIV ATPase activity.

The gyrase and topoIV activity profiles of kibdelomycin were generally similar to novobiocin but showed significantly better potency against *S. aureus* topoIV decatenation activity. These results support the *S. aureus* fitness test prediction that kibdelomycin works through a mechanism similar to that of the coumarin antibiotic novobiocin that involves inhibition of the ATPase activity of bacterial type II DNA topoisomerases, leading to inhibition of DNA synthesis and cell death.

Kibdelomycin Is Not Cross-Resistant with Gyrase Inhibitors

To test whether kibdelomycin acts at the same site as the coumarin antibiotics, we selected strains resistant to novobiocin or coumermycin A1. Strains MB 5957/A8 and MB 5957/H7 were

selected by fluctuation test (Luria and Delbrück, 1943, Young, 2006) on Miller's Luria agar plates containing 4x the agar dilution MIC of novobiocin and coumermycin A1, respectively.

Kibdelomycin potently inhibited the growth of the wild-type parent *S. aureus* strain with a MIC of 1 μg/ml. The activity of kibdelomycin against MB 5957/A8 (novobiocin resistant) was unaffected (MIC 0.5 μg/ml). Its activity against the *S. aureus* strain harboring a 125-fold resistance to coumermycin A1 (MB 5957/H7) was reduced only four-fold relative to wild-type (Table 4). When tested against a *S. aureus* strain that harbors resistance to the fluoroquinolone antibiotic ciprofloxacin (R35), kibdelomycin's activity was unaffected (MIC 0.5 μg/ml).

The *gyrB* gene was sequenced in strains MB 5957/A8 and MB 5957/H7 and the mutations found were Asp-89-Gly for A8 (novobiocin resistant isolate) and Gln-136-Glu, Ile-175-Thr, and Leu-455-Ile for H7 (coumermycin A1 resistant isolate). The crystal structure of *Thermus thermophilus* GyrB ATPase domain in complex with novobiocin showed strong H-bond interactions of Asp80 (Asp89 in *S. aureus*) with the *p*-hydroxy group of the benzamide unit (Lamour et al., 2002) (Figure S3). The loss of this interaction in the Asp89-Gly mutant (MB5957/A8) would be sufficient to reduce the activity of novobiocin and suggest that this interaction is unlikely to exist for kibdelomycin.

S. aureus Has a Low Frequency of Resistance to Kibdelomycin

The frequency of resistance (FOR) was measured at 2-, 4-, and 8-fold the drug concentrations of the agar plate MIC (Table S1). The FOR was calculated by dividing the total number of spontaneous resistant colonies found on the agar drug plates after 24-hr incubation at 37°C by the total number of colony-forming units (cfu) spread on the drug plates. At 4- and 8-fold concentrations of the plate MIC, the FOR of kibdelomycin was $<5 \times 10^{-10}$ cfu/ml. The FOR of novobiocin and ciprofloxacin was measured simultaneously for comparison and is presented in Table S1. Kibdelomycin displays a FOR similar to that of ciprofloxacin.

DISCUSSION

Kibdelomycin is the first in a new class of natural-product bacterial gyrase inhibitor to be discovered since the cyclothialidines in the early 1990s (Oblak et al., 2007) and it is the first with potent whole-cell antibacterial activity since the discovery of novobiocin and the other coumarin antibiotics in the 1950s (Oblak et al., 2007). The discovery demonstrates the advantages of combining a high-throughput chemical genetic approach with natural products screening for antibacterials. In nearly three years of running the *S. aureus* fitness test natural products screening paradigm, we re-discovered natural-product antibacterials targeting virtually every known major MOA along with dozens of instances of the coumarin antibiotics novobiocin, chlorobiocin, and coumermycin A1 (not shown). Because the AISS profiling of the crude extract identified the likely MOA of the active agent as being inhibition of DNA gyrase, we were able to quickly rule out known inhibitors and confidently pursue chemical isolation/purification of a new type II DNA topoisomerase inhibitor. Although discoveries like that of the anti-fungal parnafungin highlight the power of the fitness test approach for finding compounds with novel MOAs (Jiang et al., 2008; Parish et al., 2008), the discovery of kibdelomycin with the *S. aureus* fitness test demonstrates the other strength of this unbiased screening method, namely the ability to triage rapidly through large numbers of biologically active crude extracts and identify those containing new chemistries against proven therapeutic targets.

Kibdelomycin is a potent and selective inhibitor of DNA synthesis in which it blocks incorporation of ^{14}C-thymidine. The inhibition of DNA synthesis is the result of selective inhibition of the ATPase activity of DNA gyrase and topoIV. Kibdelomycin inhibited the in vitro DNA gyrase and topoIV activities of both *E. coli* and *S. aureus*. The activity profile was similar to that of the coumarin antibiotic novobiocin and not quinolone antibiotics. In general, it showed much better potency against DNA gyrase than DNA topoIV for both *S. aureus*– and *E. coli*–derived enzymes. The inhibition of bacterial DNA synthesis leads to potent antibacterial activity. Although it showed broad-spectrum activity against gram-positive bacteria, its activity against wild-type, gram-negative bacteria was limited to te respiratory tract–infecting bacterium *H. influenzae*. The lack of activity against most wild-type gram-negative bacteria such as *E. coli* was attributed to either poor cell penetration or active efflux, which was validated by its activity against the permeable (*envA1*) and efflux (*tolC*) defective *E. coli* strain. Moreover, it inhibited the in vitro DNA gyrase and topoIV activity of gram-negative bacteria (e.g., *E. coli*), suggesting that wild-type activity against Gram-negative bacteria is achievable by improving cell permeation and/or reducing efflux activity of kibdelomycin analogs. Remarkably, in these initial studies, kibdelomycin did not show cross-resistance to known bacterial type II topoisomerase inhibitors (novobiocin, ciprofloxacin) regardless of whether they interact with GyrB and ParE (coumarins) or GyrA and ParC (quinolones) indicating a unique binding mode of this compound to DNA gyrase and topoIV. Kibdelomycin showed very low frequency of resistance ($<5 \times 10^{-10}$), unlike other known GyrB inhibitors (e. g., novobiocin, 10^{-8}) (Young, 2006), providing additional evidence for binding that is likely significantly different from that of known GyrB inhibitors. Future cross-resistance and mutation studies against a broader spectrum of resistant mutants and pathogenic organisms will be necessary to ultimately explore potential mechanisms of resistance to kibdelomycin.

The structure of kibdelomycin is completely novel compared with the known GyrB and ParE inhibitors novobiocin, chlorobiocin, and coumermycin A_1, which are natural products produced by soil bacteria and were discovered in the 1950s (Oblak et al., 2007). A related compound of unpublished activity, amycolamicin, was recently reported (Tohyama et al., 2010). Novobiocin was one of the first gyrase inhibitors discovered and consists of three domains: a central 8-methyl coumarin domain connected with an amide bond to an isoprenyl hydroxy benzoic acid on one side, and a carbamoyl-methyl-lyxofuranoside (novobiose) on the other. The methyl group of the coumarin and the carbamoyl group of novobiose is substituted by a chloro and a 5-methyl-pyrole-2-carbonyl group in chlorobiocin. The third inhibitor, coumermycin A_1 is more complex and is a dimer consisting of a 3-methyl-pyrrolyl-2,4-dicarbonyl as a central core

Table 1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) Spectral Assignment of Kibdelomycin in CD_3OD

	Kibdelomycin				
Atom #	δ_C	Type	δ_H	HMBC (H→C)	ROESY[b]
1	152.2	C°			
2	35.6	CH_2	2.26, m (eq)	—	
			2.12, m (ax)	C-1, 3	
3	35.3	CH_2	2.25, m (eq)		H-4, 30
			1.26, m (ax)		
4	79.6	CH	3.56, dt, 4, 11	C-5, 6, 30	H-10, 30
5	49.9	CH	1.82, dt, 2.5, 11		H-3ax, 6, 8
6	126.5	CH	5.92, dt, 10, 2	C-4, 5, 8, 10	H-4, 5, 7
7	133.7	CH	5.62, ddd, 10, 4.5, 3		H-8, 21
8	32.1	CH	2.65, m		H-7, 21
9	48.0[a]	CH	4.33, m	C-5, 8, 10, 11, 21	H-5
10	39.5	CH	2.26, m	C-1, 2, 4, 5, 8, 9, 20	H-2ax, 4, 21
11	198.0[a]	C°			
12	105.2[a]	C°			
13	196.6[a]	C°			
14	69.7[a] br	CH	3.52, d, 2.5	C-17, 18, 19, 16	H-17, 18, 19, 22
16	178.1[a]				
17	32.0[a]	CH	2.14, m	C-13, 14, 18, 19	H-17, 18, 19
18	17.8	CH_3	0.97, d, 7	C-14, 17, 19	H-14, 17, 19
19	17.7	CH_3	1.07, d, 7	C-14, 17, 18	H-14, 17, 18
20	106.1	CH_2	4.57, s	C-1, 2, 10	H-2eq
			4.44, s	C-1, 2, 10	
20	—	N			
21	18.9	CH_3	0.80, d, 7	C-7, 8, 9	H-7, 8, 10
22	—	O			
22	77.5[a]	CH	5.02, brd, 9	C-22, 23	H-14(w), 28
23	75.6	CH	4.33, m	C-22, 24, 1‴	H-24, 25
24	69.9	CH	5.88, t, 3	C-22, 23, 25, 26, C-1"	H-23, 25, 1‴
25	69.8	CH	4.91, dd, 6, 3	C-24, 26, 28, C-1′	H-23, 24, 26
26	71.4	CH	4.30, pent, 7	C-22, 25, 28	H-25
28	14.5	CH_3	1.39, d, 7	C-25, 26	H-22, 26
29	—	O			
30	96.8	CH	4.94, dd, 10, 2	C-4, 34	H-4, 3eq, 34
31	38.5	CH_2	1.79, dd, 13.5, 2 (eq)	C-30, 32, 33, 37	H-38
			1.57, dd, 13.5, 10 (ax)	C-30	
32	76.4	C°			
33	74.9	CH	3.17, d, 9	C-32, 34, 36, 37	H-36, 37
34	71.7	CH	3.67, dq, 9, 6	C-30, 33, 36	H-30, 36
36	18.5	CH_3	1.26, d, 6	C-33, 34	H-33, 34
37	52.4	CH	4.37, q, 7	C-31, 32, 33, 38, 40	H-31ax, 33, 38
38	16.2	CH_3	1.24, d, 7	C-32, 37	H-31ax, H-37
39	—	NH			
40	161.7	C°			
41	120.0	C°			
42	112.4	C°			
43	110.6	C°			
44	129.4	C°			
45	—	NH			
46	10.8	CH_3	2.21, s	C-41(w), 42(w), 43, 44	

Table 1. *Continued*

	Kibdelomycin				
Atom #	δ_C	Type	δ_H	HMBC (H→C)	ROESY[b]
1′	158.4	C°			
2′	–	NH_2			
1″	172.0	C°			
2″	21.0	CH_3	2.11, s	C-1″	
1″	57.5	CH_3	3.28, s	C-23	H-23, 24

W, weak; eq, equatorial; ax, axial. C-11 shift suggests a keto form. A multi-keto-enol form is possible, including involvement of imino group. See also Figures S1 and S2.
[a] Broad signals.
[b] Mix 200 and 300 ms.

tethered with two units of novobiocin, one on each side with replacement of carbamoyl group with 5-methyl-2-pyrrolylcarbonyl group.

It has been shown in the co-crystal structure complex of N-terminal domain of *E. coli* gyrase B24-novobiocin (PDB: 1AJ6) and gyrase B24-chlorobiocin (PDB: 1kzn) that the ATP binding site overlaps with novobiocin and chlorobiocin binding sites (Maxwell and Lawson, 2003; Tsai et al., 1997). Because kibdelomycin inhibits gyrase B ATPase activity, it may bind at the gyrase B catalytic site with similar interactions of 3,4-dichloro-5-methyl-pyrrole to chlorobiocin's methyl pyrrole but with additional completely different interactions with the remainder of the molecule.

Further work, including chemical modification, will be necessary to determine whether kibdelomycin can be developed into a therapeutic agent, but the discovery of this unique scaffold from nature will at the very least add valuable insight into new ways to chemically inhibit this extremely important antibacterial drug target. This information may well open the door to designing new synthetic inhibitors and possibly engineering parts of the kibdelomycin biosynthetic pathway to generate additional important new classes of DNA gyrase inhibitors.

SIGNIFICANCE

The emergence of resistant strains of bacteria has led to an urgent need for new antibacterial agents. Burdened by the re-discovery of thousands of known inhibitors what was once one of the most successful paradigms for new antibacterial discovery, empiric screening of crude natural product extracts against pathogenic bacteria has become increasingly difficult in recent years. Here, with the discovery of kibdelomycin, we demonstrate how applying a chemical genetic approach in the pathogenic bacteria *Staphylococcus aureus* for mechanistic profiling of crude natural product extracts can help overcome this obstacle to discover a new chemical scaffold from nature against a clinically important drug target. Using biochemical and microbiological assays we show that kibdelomycin is a potent inhibitor of bacterial type II topoisomerases (DNA gyrase and topoisomerase IV) and that this mechanism leads to inhibition of DNA synthesis and broad-spectrum, gram-positive antibacterial activity.

Kibdelomycin is the first novel class of bacterial gyrase inhibitors with both potent in vitro and whole-cell antibacterial activity to be discovered from natural product sources

Table 2. In Vitro Antimicrobial Activity (Minimum Inhibitory Concentration, μg/ml) of Kibdelomycin and Known DNA Gyrase Inhibitors Novobiocin and Ciprofloxacin

Species	Strain[a]	Kibdelomycin	Novobiocin	Ciprofloxacin
S. aureus (MSSA)	MB 2865	2	0.25	0.12
S. aureus (MSSA)	MB 2865[b]	64	16	0.25
S. aureus (MRSA-COL)	MB5393	0.5	0.06	0.25
S. pneumoniae	CL2883[c]	1	1	2
E. faecalis	CL8516	2	32	2
B. subtilis	MB964	0.12	2	0.06
H. influenzae	MB4572	2	0.12	≤0.015
E. coli	MB2884	>64	>32	≤0.015
E. coli (envA1, tolC)	MB5746	32	2	≤0.015
C. albicans	MY1055[d]	>64	>32	>16

[a] Activities were assayed in cation adjusted Meuller Hinton Broth (CAMHB), except where noted.
[b] CAMHB + 50% human serum.
[c] CAMHB + 2.5% lysed horse blood.
[d] YPD and Sab-Dex media.

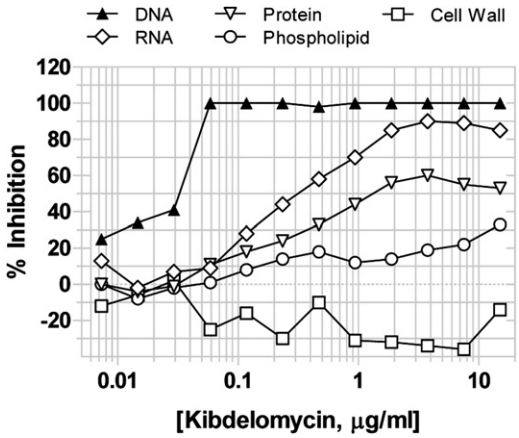

Figure 3. Inhibition of Macromolecular Synthesis of Kibdelomycin
Inhibitory effects across increasing concentrations were determined by measuring incorporation of radiolabeled precursors of either DNA (^{14}C-thymidine), RNA (^{3}H-uridine), phospholipid (2-^{3}H-glycerol), protein (^{3}H-leucine), or cell wall (^{14}C-glycine) in *S. aureus* strain EP167.

since the 1950s. Because kibdelomycin represents a new chemistry, with apparently a new binding mode, against this proven target, it may help circumvent some of the issues of resistance and toxicity of other classes like the coumarin antibiotics. In fact, this new compound does not show cross-resistance with other major classes of DNA gyrase inhibitors and it displays a significantly lower frequency to resistance than the coumarin antibiotic novobiocin. The identification of kibdelomycin demonstrates the power of applying new approaches to natural products discovery. Successes like this have the potential to revitalize interest in natural products-based antibacterial drug discovery.

EXPERIMENTAL PROCEDURES

Producing Organism Isolation and Characterization

The strain was isolated from a soil sample collected in the forest of Pama, a zone of dense, humid vegetation in the plateau of Bangui, in Central African Republic. This soil was suspended in sterile water and pretreated following the method by Hayakawa et al. (Hayakawa et al., 1997) before being plated on selective isolation media and incubated at 28°C for at least 6 weeks. Strain MA7385 (ATCC PTA-10354) was isolated from a NZ-amine–based agar medium containing nalidixic acid (20 ug/ml) and the colony was purified on yeast extract malt extract glucose medium (ISP2) and preserved as frozen agar plugs in 10% glycerol.

Molecular Characterization of the Strain: 16S rDNA Sequencing and Phylogenetic Analysis

The taxonomic position of the strain was determined by phylogenetic analysis of the 16S rDNA sequence with sequences of validated species of the family *Pseudonocardiaceae*. The phylogenetic analysis, based on the Neighbor-Joining method using matrix pairwise comparisons of sequences corrected with Jukes and Cantor algorithm (Jukes and Cantor, 1969; Saitou and Nei, 1987), shows that the strain MA7385 is a new member of the genus *Kibdelosporangium*, a taxonomic position highly supported by the bootstrap analysis (96%) used as a measure of statistical confidence.

Fermentation Procedure

Fermentation of *Kibdelosporangium* sp. (MA7385) was accomplished by inoculating several agar plugs with mycelia into seed broth flasks (50 ml medium in 250 ml baffled flask). The formulation for the seed broth is as follows (g/l, unless specified): soluble starch (20.0), dextrose (10.0), NZ amine type E (5.0)[nitrogen source, mixture of amino acids obtained from casein hydrolysates], beef extract (3.0), peptone (5.0) [another nitrogen source, mixture of amino acids obtained from meat].

Yeast extract (5.0), $CaCO_3$ (1.0) in distilled H_2O (1 l). The pH was adjusted to 7.0 with NaOH before addition of $CaCO_3$. The flasks were incubated at 28°C with 80% relative humidity and shaken on a rotary shaker at 220 rpm. When the seed stage flasks had grown for 3 days, a 1 ml aliquot was used to inoculate each flask of FR23 production medium (50 ml medium in a 250 unbaffled flask). The formulation of FR23 medium consists of (g/l): glucose (5.0), soluble starch (30.0), cane molasses (20.0), and pharmamedia (20.0), in distilled H_2O (1 l). The pH was adjusted to 7.0 with NaOH before sterilization. The flasks were incubated at 28°C with 80% relative humidity on a rotary shaker at 220 rpm for 7 days.

Table 3. Enzyme Inhibitory Activities of Kibdelomycin

Assay	Kibdelomycin (IC_{50}, nM)	Novobiocin (IC_{50}, nM)
E. coli gyrase (Gel)	60	500
E. coli topoIV (Gel)	29,000	10,000
S. aureus gyrase (Gel)	9	<4
S. aureus topoIV (Gel)	500	35,000
E. coli gyrase (ATPase)	11	23
E. coli topoIV (ATPase)	900	450

Purification of Kibdelomycin

A pooled fermentation broth from 240 flasks (12 l) was extracted with 12 l acetone by shaking on a reciprocating shaker for more than 1 hr. The mycelial content was filtered through celite, and the filtrate was concentrated under reduced pressure to remove most of the acetone. The aqueous extract (12 l) was extracted three times with 12 l each of methyl ethyl ketone (MEK). MEK extracts were combined and concentrated under reduced pressure to dryness yielding a gum, which was dissolved in 20 ml of methanol and chromatographed on a 450-cc Sephadex LH 20 column. The column was eluted with methanol, and the appropriate fractions were pooled and concentrated under reduced pressure to dryness. One-third portion of the LH20 fraction was dissolved in minimum volume of methanol and diluted with methylene chloride to a ratio of 90:10, methylene chloride: methanol. This solution was then charged to a 35 ml (10 g) silica gel column and washed with 3–4 column volumes each of 10, 20, and 30% methanol in methylene chloride. The compound of interest eluted in the 10%–20% methanol fraction. This process was repeated twice with the rest of the material, and pooled fractions from the 3 columns were concentrated under reduced pressure to yield a brown gum. This enriched material was dissolved in 10 ml methanol and further fractionated into 5 equal portions on PRP-1 (Hamilton's pH stable high-performance liquid chromatography [HPLC] column, 250 × 21.5 mm) using gradient elution with methanol:0.25M sodium phosphate buffer (pH 7) 60:40 to 80:20 in 40 min at a flow rate of 10 ml/min. Fractions of interest were dried down and repeatedly triturated with methanol to dissolve all but the buffer salts. Another HPLC step followed on Zorbax RX C_8 (250 × 21.5 mm) eluted with a 50-min linear gradient of 50%–100% aqueous methanol. Lyophilization of the fractions containing compound afforded kibdelomycin (60 mg, isolated yield 5 mg/l, titer in the extract = 10 mg/l) from fractions 41–44, as a colorless amorphous powder. Physical and spectral properties of kibdelomycin: $[\alpha]^{23}_{D} = -43.6$ (c, 1.56 MeOH); UV (MeOH) λ_{max} (log ε) = 248 nm (sh) and 276 nm (4.42); IR (ZnSe) ν_{max} = 3417, 2932, 1732, 1611, 1537, 1454, 1376, 1313, 1233, 1159, 1079, 1004, 893, 830, 789, 745 cm^{-1}. HRESIFTMS (*m/z*): observed for M+H = 939.3562, calculated for $C_{44}H_{60}Cl_2N_4O_{14}^{+}H$ = 939.3561.

S. aureus Fitness Test

The *S. aureus* fitness test assay was performed as previously described (Donald et al., 2009). Briefly, 245 strains, each containing a different inducible antisense RNA interference plasmid targeting an essential gene in *S. aureus*

Table 4. In Vitro Antimicrobial Activity of Kibdelomycin against Resistant Strains of Novobiocin, Coumermycin A1, and Ciprofloxacin

Species	Strain[a]	Genotype	Kibdelomycin	Novobiocin	Coumermycin A1	Ciprofloxacin
S. aureus wild-type Parent	MB 5957	Wild-type	1	0.5	0.008	1
S. aureus Novo^R	MB 5957/A8	GyrB (ASP89Gly)	0.5	4	0.004	0.25
S. aureus Cou^R	MB 5957/H7	GyrB (Gln136Glu, Ile175Thr, Leu455Ile)	4	0.25	>1	0.12
S. aureus Cipro^S	MB 5785 (R34)	Parent for R35	1	0.5	0.004	0.5
S. aureus Cipro^R	MB 5786 (R35)	Unknown	0.5	0.25	0.004	>16

See also Figure S3.

[a] Activities were assayed in Miller's Luria broth. MRSA strain R35 is a fluoroquinolone-resistant derivative of strain R34 (Hooper and Wolfson, unpublished data).

RN4220 were pooled into 24 different bins of 6–12 strains each. The bins were then each grown at a different concentration of xylose inducer (ranging from 1.8–55 mM) for ~20 population doublings either in the presence of test compound (acetone extract or purified compound) or as a 2% DMSO mock treatment control. Strain bin growth was performed in 384-well plates (Costar 3680) on a fully automated system using LB medium containing chloramphenicol (34 μg/ml) over 3 cycles of growth ranging from 5–7 hr each for a total time of ~18 hr in a total volume of 50 μl. At the end of the growth period, cells from all 24 bins were pooled for each test treatment or DMSO mock treatment control. Pooled cells were then lysed and subjected to multiplex PCR to amplify specific antisense markers for each strain. Strain specific markers were subsequently identified and peak areas quantitated by DNA fragment analysis on an ABI 3730 genetic analyzer. Peak areas were then normalized and strain depletion ratios were calculated. Statistical significance was determined as a p-value using an error model generated for each individual strain across a discrete set of known standards and unknown test samples selected to represent total coverage of all strains within the array set.

Inhibition of Macromolecular Synthesis

The assay was performed as previously described (Wang et al., 2003; Wang et al., 2006). Briefly, mid-log (A_{600} = 0.5–0.6) *S. aureus* growth was incubated with increasing concentration of inhibitor at 37°C for 20 min with 1 μCi/ml 2-[^{3}H]-glycerol, 1 μCi/ml [^{14}C]-thymidine, 1 μCi/ml [^{3}H]-uridine, 5 μCi/ml 4,5-[^{3}H]-leucine, or [^{14}C]-glycine to measure phospholipids, DNA, RNA, protein, and cell wall synthesis, respectively. Cell wall labeling with [^{14}C]-glycine (*S. aureus*) was performed in the presence of 100 μg/ml chloramphenicol, which prevents protein synthesis and disengages the stringent response. The reaction was stopped by the addition of 10% trichloroacetic acid and the cells were harvested using a glass fiber filter (PerkinElmer Life Sciences, Waltham, MA, 1205-401). The filter was dried and counted with scintillation fluid.

Bacterial DNA Gyrase Supercoiling and Topoisomerase Decatenation Inhibition Assays

DNA gyrase and topoIV assays were performed based on established protocols obtained from the supplier, TopoGEN, Inc. (Port Orange, FL). All of the reactions were stopped by the addition of 10 μl of 3X gel-loading buffer (final concentration: 1.2% SDS, 6 mM EDTA, 10% glycerol, 0.02% bromophenol blue). Twenty μl of this was loaded on a 1% agarose, TAE (40 mM Tris-acetate, 0.01 M EDTA pH 8.3) gel and run for 3–4 hr at 60 V. The gel was stained with 0.5 mg/l ethidium bromide in TAE for 30 min while rocking, then destained for 20 min in deionized water. Fluorescent images were captured on a UV transilluminator imaging system at a wavelength of 300 nm (Epichemi3, UVP Inc. Upland, CA, uvp@uvp.com). The fluorescence intensity of the supercoiled plasmid reaction product, in the case of gyrase, and the decatenation product of topoisomerase were quantitated using ImagQuant software (GE Healthcare, Little Chalfont, UK) and IC_{50} values were determined by non-linear regression analysis in GraphPad Prism (GraphPad Software, Inc., San Diego, CA).

Supercoiling of relaxed plasmid DNA (pBR322 from TopoGEN) was assayed in a reaction volume of 20 μl composed of 35 mM Tris pH 7, 24 mM KCl, 4 mM $MgCl_2$, 2 mM DTT, 1.8 mM spermidine, 6.5% glycerol, 0.1 mg/ml acetylated BSA, 1 mM ATP, and 0.2 μg pBR322 substrate. Compound titrations in DMSO were added and the reactions were initiated with 2 units of *E. coli* gyrase (TopoGEN). The reaction proceeded with shaking on a mini-orbital shaker (Bellco Biotechnology, Vineland, NJ) for 30 min at 37°C. For the *S. aureus* DNA gyrase assay, 5 units of enzyme (TopoGEN) were used in a reaction mix that was supplemented with 0.6 M potassium glutamate.

Decatenation of kinetoplast DNA (TopoGEN) was assayed in a total reaction volume of 20 μl containing 40 mM Tris pH 7.5, 6 mM $MgCl_2$, 10 mM DTT, 100 mM potassium glutamate, 50 μg/ml acetylated BSA, 1 mM ATP, and 0.2 μg kDNA substrate. Compound titrations in DMSO were added and the reactions were initiated with 2 units of *E. coli* topoIV (Topogen) that were incubated with shaking for 30 min at 37°C. For the *S. aureus* enzyme assay, 5 units of *S. aureus* topoIV (TopoGEN) were used in a reaction that was supplemented with 0.6 M potassium glutamate.

ATPase Assays and Data Analysis

The ATPase activity of *E. coli* gyrase and topoIV enzymes was measured with a colorimetric assay for release of inorganic phosphate (Rivetna et al., 1995). *E. coli* gyrase and topoIV holoenzymes, and kinetoplast DNA (kDNA) were obtained from TopoGEN, Inc. The standard gyrase ATPase assay was performed in a 96 well half area, clear, polystyrene microplate with 50 ul of a reaction mix comprised of 100 mM Tris buffer (pH 7.4), 100 mM KCl, 5 mM $MgCl_2$, 200 μM ATP, 15 μg/ml *E. coli* gyrase, and test compound. The standard topoisomerase assay was run under equivalent conditions using 15 μg/ml *E. coli* topoIV in the presence or absence of 19 μg/ml kinetoplast DNA. Background samples contained assay buffer in place of enzymes. Samples were incubated at room temperature for 1 hr before terminating the reaction by the addition of 25 μl 0.016% malachite green/0.4% polyvinyl alcohol and mixing. Color determination was achieved with the addition of 25 μl 0.04M molybdate/2N H_2SO_4 and the samples were again mixed very well. The samples were incubated for 30 min at room temperature and the absorbance at 620 nm was read in a Molecular Devices SpectraMax Plus plate reader (Sunnyvale, CA). Background values were subtracted from all data points, inhibition curves were plotted, and IC_{50} values were calculated with a 4-parameter fit using KaleidaGraph (Synergy Software).

Microbiological Assays

The MIC against each of the strains was determined using Clinical and Laboratory Standards Institute guidelines. The specified medium broth with a 2-serial dilution of compounds was inoculated with 10^5 cfu/ml and was incubated at 37°C for 20 hr. MIC is defined as the lowest concentration of antibiotic that inhibited visible growth.

Agar Dilution MIC, FOR Determination, and Luria-Delbruck Fluctuation Test

Test compounds were incorporated into a solid (LB-agar plate medium). The compounds were diluted in agar at 2-fold serial dilution. A defined number of cells from a late log-phase liquid culture of *S. aureus* grown in LB broth liquid culture were spotted onto the surface of the agar drug medium. The number of cells on the surface of drug agar medium was determined by the cell titer of cells on agar medium without drug. Agar plates with and without drug were incubated for 18 hr at 35°C and then examined for growth. No growth of the test organism on drug-containing plates indicates susceptibility at the

antimicrobial concentration incorporated into the medium and this is referred to as the *agar plate MIC*. The FOR was determined by plating late-log liquid cultures of *S. aureus* onto plates containing 2X, 4X, and 8X the agar plate MIC. The number of cells plated on all plates is determined by the cell titer on the plates without drug.

Luria-Delbruck fluctuation tests were also performed for the control antibiotics novobiocin and coumermycin A1, essentially as described (Young, 2006) on Miller's Luria agar plates containing 4X the agar dilution MIC of novobiocin and coumermycin A1, respectively. Colonies from independent plates were purified by streaking on plates without antibiotic. The resistance was characterized by agar dilution MICs on agar plates containing various antibiotics. MB5957/A8 was chosen for further study because of selective resistance to novobiocin, whereas MB5957/H7 was chosen because of selective resistance to coumermycin A1.

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures and one table and can be found with this article online at doi:10.1016/j.chembiol.2011.06.011.

ACKNOWLEDGMENTS

Authors thank Terry Roemer, David Pompliano, Milton Hammond, David Olsen, Peter Meinke, Lynn Miesel, and Dennis Schmatz for helpful discussions. MRSA strains R34 and R35 were a gift of David C. Hooper, Massachusetts General Hospital, and the late John Wolfson. Some of the authors are employees of Merck & Co., Inc., as stated in the affiliations, and potentially own stock and/or hold stock options in the company.

Received: June 1, 2011
Revised: June 25, 2011
Accepted: June 28, 2011
Published: August 25, 2011

REFERENCES

Bills, G.F., Platas, G., Fillola, A., Jiménez, M.R., Collado, J., Vicente, F., Martín, J., González, A., Bur-Zimmermann, J., Tormo, J.R., and Peláez, F. (2008). Enhancement of antibiotic and secondary metabolite detection from filamentous fungi by growth on nutritional arrays. J. Appl. Microbiol. *104*, 1644–1658.

Chen, C.R., Malik, M., Snyder, M., and Drlica, K. (1996). DNA gyrase and topoisomerase IV on the bacterial chromosome: quinolone-induced DNA cleavage. J. Mol. Biol. *258*, 627–637.

Donald, R.G., Skwish, S., Forsyth, R.A., Anderson, J.W., Zhong, T., Burns, C., Lee, S., Meng, X., LoCastro, L., Jarantow, L.W., et al. (2009). A Staphylococcus aureus fitness test platform for mechanism-based profiling of antibacterial compounds. Chem. Biol. *16*, 826–836.

Genilloud, O., González, I., Salazar, O., Martín, J., Tormo, J.R., and Vicente, F. (2011). Current approaches to exploit actinomycetes as a source of novel natural products. J. Ind. Microbiol. Biotechnol. *38*, 375–389.

Goetz, M.A., Zhang, C., Zink, D.L., Arocho, M., Vicente, F., Bills, G.F., Polishook, J., Dorso, K., Onishi, R., Gill, C., et al. (2010). Coelomycin, a highly substituted 2,6-dioxo-pyrazine fungal metabolite antibacterial agent discovered by Staphylococcus aureus fitness test profiling. J. Antibiot. *63*, 512–518.

Hayakawa, M., Iino, H., Takeuchi, S., and Yamazaki, T. (1997). Application of a method incorporating treatment with chloramine-T for the selective isolation of Streptosporangiaceae from soil. J. Ferment. Bioeng. *84*, 599–602.

Herath, K., Harris, G., Jayasuriya, H., Zink, D., Smith, S., Vicente, F., Bills, G., Collado, J., González, A., Jiang, B., et al. (2009). Isolation, structure and biological activity of phomafungin, a cyclic lipodepsipeptide from a widespread tropical Phoma sp. Bioorg. Med. Chem. *17*, 1361–1369.

Huber, J., Donald, R.G., Lee, S.H., Jarantow, L.W., Salvatore, M.J., Meng, X., Painter, R., Onishi, R.H., Occi, J., Dorso, K., et al. (2009). Chemical genetic identification of peptidoglycan inhibitors potentiating carbapenem activity against methicillin-resistant Staphylococcus aureus. Chem. Biol. *16*, 837–848.

Jayasuriya, H., Herath, K.B., Zhang, C., Zink, D.L., Basilio, A., Genilloud, O., Diez, M.T., Vicente, F., Gonzalez, I., Salazar, O., et al. (2007). Isolation and Structure of Platencin: A Novel FabH and FabF Dual Inhibitor with Potent Broad Spectrum Antibiotic Activity Produced by Streptomyces platensis MA7339. Angew. Chem. Int. Ed. *46*, 4684–4688.

Jiang, B., Xu, D., Allocco, J., Parish, C., Davison, J., Veillette, K., Sillaots, S., Hu, W., Rodriguez-Suarez, R., Trosok, S., et al. (2008). PAP inhibitor with in vivo efficacy identified by Candida albicans genetic profiling of natural products. Chem. Biol. *15*, 363–374.

Jukes, T.H., and Cantor, C. (1969). Evolution.of Protein Molecules. In Mammalian Protein Metabolism, H.N. Munro and J.B. Allison, eds. (New York: Academic Press), pp. 21–132.

Klevens, R.M., Morrison, M.A., Nadle, J., Petit, S., Gershman, K., Ray, S., Harrison, L.H., Lynfield, R., Dumyati, G., Townes, J.M., et al; Active Bacterial Core surveillance (ABCs) MRSA Investigators. (2007). Invasive methicillin-resistant Staphylococcus aureus infections in the United States. JAMA *298*, 1763–1771.

Kodali, S., Galgoci, A., Young, K., Painter, R., Silver, L.L., Herath, K.B., Singh, S.B., Cully, D., Barrett, J.F., Schmatz, D., and Wang, J. (2005). Determination of selectivity and efficacy of fatty acid synthesis inhibitors. J. Biol. Chem. *280*, 1669–1677.

Lamour, V., Hoermann, L., Jeltsch, J.-M., Oudet, P., and Moras, D. (2002). An open conformation of the *Thermus thermophilus* gyrase B ATP-binding domain. J. Biol. Chem. *277*, 18947–18953.

Luria, S.E., and Delbrück, M. (1943). Mutations of bacteria from virus sensitivity to virus resistance. Genetics *28*, 491–511.

Maxwell, A., and Lawson, D.M. (2003). The ATP-binding site of type II topoisomerases as a target for antibacterial drugs. Curr. Top. Med. Chem. *3*, 283–303.

Oblak, M., Kotnik, M., and Solmajer, T. (2007). Discovery and development of ATPase inhibitors of DNA gyrase as antibacterial agents. Curr. Med. Chem. *14*, 2033–2047.

Ondeyka, J.G., Zink, D.L., Young, K., Painter, R., Kodali, S., Galgoci, A., Collado, J., Tormo, J.R., Basilio, A., Vicente, F., et al. (2006). Discovery of bacterial fatty acid synthase inhibitors from a Phoma species as antimicrobial agents using a new antisense-based strategy. J. Nat. Prod. *69*, 377–380.

Ondeyka, J.G., Zink, D., Basilio, A., Vicente, F., Bills, G., Diez, M.T., Motyl, M., Dezeny, G., Byrne, K., and Singh, S.B. (2007). Coniothyrione, a chlorocyclopentandienylbenzopyrone as a bacterial protein synthesis inhibitor discovered by antisense technology. J. Nat. Prod. *70*, 668–670.

Ondeyka, J., Harris, G., Zink, D., Basilio, A., Vicente, F., Bills, G., Platas, G., Collado, J., Gonzáez, A., de la Cruz, M., et al. (2009). Isolation, structure elucidation, and biological activity of virgineone from Lachnum Wirgineum using the genome-wide Candida albicans fitness test. J. Nat. Prod. *72*, 136–141.

Parish, C.A., Smith, S.K., Calati, K., Zink, D., Wilson, K., Roemer, T., Jiang, B., Xu, D., Bills, G., Platas, G., et al. (2008). Isolation and structure elucidation of parnafungins, antifungal natural products that inhibit mRNA polyadenylation. J. Am. Chem. Soc. *130*, 7060–7066.

Rivetna, M.N., Salowe, S.P., Tolman, R.L., and Jones, A.B. (1995). A Medicinal Chemistry Evaluation of the Autoinhibitory Domain of Calcineurin. Bioorg. Med. Chem. Lett. *5*, 1147–1150.

Saitou, N., and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. *4*, 406–425.

Singh, S.B., and Barrett, J.F. (2006). Empirical antibacterial drug discovery—foundation in natural products. Biochem. Pharmacol. *71*, 1006–1015.

Singh, S.B., Jayasuriya, H., Ondeyka, J.G., Herath, K.B., Zhang, C., Zink, D.L., Tsou, N.N., Ball, R.G., Basilio, A., Genilloud, O., et al. (2006a). Isolation, structure, and absolute stereochemistry of platensimycin, a broad spectrum antibiotic discovered using an antisense differential sensitivity strategy. J. Am. Chem. Soc. *128*, 11916-11920 and 15547.

Singh, S.B., Zink, D.L., Huber, J., Genilloud, O., Salazar, O., Diez, M.T., Basilio, A., Vicente, F., and Byrne, K.M. (2006b). Discovery of lucensimycins A and B from Streptomyces lucensis MA7349 using an antisense strategy. Org. Lett. *8*, 5449–5452.

Singh, S.B., Phillips, J.W., and Wang, J. (2007). Highly sensitive target-based whole-cell antibacterial discovery strategy by antisense RNA silencing. Curr. Opin. Drug Discov. Devel. *10*, 160–166.

Singh, S.B., Zink, D.L., Herath, K.B., Salazar, O., and Genilloud, O. (2008). Discovery and antibacterial activity of lucensimycin C from Streptomyces lucensis. Tetrahedron Lett. *49*, 2616–2619.

Singh, S.B., Zink, D.L., Dorso, K., Motyl, M., Salazar, O., Basilio, A., Vicente, F., Byrne, K.M., Ha, S., and Genilloud, O. (2009). Isolation, structure, and antibacterial activities of lucensimycins D-G, discovered from Streptomyces lucensis MA7349 using an antisense strategy. J. Nat. Prod. *72*, 345–352.

Tohyama, S., Takahashi, Y., and Akamatsu, Y. (2010). Biosynthesis of amycolamicin: the biosynthetic origin of a branched alpha-aminoethyl moiety in the unusual sugar amycolose. J. Antibiot. *63*, 147–149.

Tsai, F.T., Singh, O.M., Skarzynski, T., Wonacott, A.J., Weston, S., Tucker, A., Pauptit, R.A., Breeze, A.L., Poyser, J.P., O'Brien, R., et al. (1997). The high-resolution crystal structure of a 24-kDa gyrase B fragment from E. coli complexed with one of the most potent coumarin inhibitors, clorobiocin. Proteins *28*, 41–52.

Wang, J., Galgoci, A., Kodali, S., Herath, K.B., Jayasuriya, H., Dorso, K., Vicente, F., González, A., Cully, D., Bramhill, D., and Singh, S. (2003). Discovery of a small molecule that inhibits cell division by blocking FtsZ, a novel therapeutic target of antibiotics. J. Biol. Chem. *278*, 44424–44428.

Wang, J., Soisson, S.M., Young, K., Shoop, W., Kodali, S., Galgoci, A., Painter, R., Parthasarathy, G., Tang, Y.S., Cummings, R., et al. (2006). Platensimycin is a selective FabF inhibitor with potent antibiotic properties. Nature *441*, 358–361.

Wang, J., Kodali, S., Lee, S.H., Galgoci, A., Painter, R., Dorso, K., Racine, F., Motyl, M., Hernandez, L., Tinney, E., et al. (2007). Discovery of platencin, a dual FabF and FabH inhibitor with in vivo antibiotic properties. Proc. Natl. Acad. Sci. USA *104*, 7612–7616.

Xu, D., Jiang, B., Ketela, T., Lemieux, S., Veillette, K., Martel, N., Davison, J., Sillaots, S., Trosok, S., Bachewich, C., et al. (2007). Genome-wide fitness test and mechanism-of-action studies of inhibitory compounds in Candida albicans. PLoS Pathog. *3*, e92.

Young, K. (2006). Vitro antibacterial resistance selection and quantitation. In Current Protocols in Pharmacology Unit 13A.6, S.J. Enna, M. Williams, J.F. Barret, J.W. Ferkany, T. Kenakin, and R.D. Porsolt, eds. (Hoboken, NJ: John Wiley & Sons, Inc.).

Young, K., Jayasuriya, H., Ondeyka, J.G., Herath, K., Zhang, C., Kodali, S., Galgoci, A., Painter, R., Brown-Driver, V., Yamamoto, R., et al. (2006). Discovery of FabH/FabF inhibitors from natural products. Antimicrob. Agents Chemother. *50*, 519–526.

Zhang, C., Ondeyka, J.G., Zink, D.L., Basilio, A., Vicente, F., Collado, J., Platas, G., Huber, J., Dorso, K., Motyl, M., et al. (2009a). Isolation, structure and antibacterial activity of pleosporone from a pleosporalean ascomycete discovered by using antisense strategy. Bioorg. Med. Chem. *17*, 2162–2166.

Zhang, C., Ondeyka, J.G., Zink, D.L., Basilio, A., Vicente, F., Salazar, O., Genilloud, O., Dorso, K., Motyl, M., Byrne, K., and Singh, S.B. (2009b). Discovery of okilactomycin and congeners from Streptomyces scabrisporus by antisense differential sensitivity assay targeting ribosomal protein S4. J. Antibiot. *62*, 55–61.