A Chiral Benzoquinolizine-2-carboxylic Acid Arginine Salt Active against Vancomycin-Resistant *Staphylococcus aureus*

Noel J. de Souza, Shrikant V. Gupte, Prasad K. Deshpande, Vijaya N. Desai, Satish B. Bhawsar, Ravindra D. Yeole, Milind C. Shukla, Jacob Strahilevitz,† David C. Hooper,† Bülent Bozdogan,‡ Peter C. Appelbaum,‡ Michael R. Jacobs,§ Nitin Shetty, Mahesh V. Patel,* Rasendrakumar Jha, and Habil F. Khorakiwala

Wockhardt Limited, Wockhardt Research Centre, D-4, MIDC, Chikalthana, Aurangabad-431 210, India; Division of Infectious Diseases, Massachusetts General Hospital, 55 Fruit Street, Boston, Massachusetts 02114-2696; Hershey Medical Center, Hershey, Pennsylvania 17033; and Case Western Reserve University, Cleveland, Ohio 44106

Received January 14, 2005

There is an urgent medical need for novel antibacterial agents to treat hospital infections, specially those caused by multidrug-resistant Gram-positive pathogens. The need may also be fulfilled by either exploring antibacterial agents having new mechanism of action or expanding known classes of antibacterial drugs. The paper describes a new chemical entity, compound **21**, derived from hitherto little known "floxacin". The choice of the entity was made from a series of synthesized prodrugs and salts of the active chiral benzoquinolizine carboxylic acid, *S*-(−)-nadifloxacin. The chemistry, physicochemical characteristics, and essential bioprofile of **21** qualifies it for serious consideration as a novel drug entity against hospital infections of multi-drug-resistant *Staphylococcus aureus*, and its progress up to clinical phase I trials in humans is described.

Introduction

On a worldwide basis, nosocomial multidrug-resistant Gram-positive staphylococcal and enterococcal pathogenic isolates are found to display resistance to frontline antimicrobial agents such as methicillin and more recently vancomycin.[1−3] The increasing prevalence of such isolates is disturbing, particularly when taken together with the emergence of vancomycin resistance in enterococci (VRE) and penicillin resistance in pneumococci (PRSP). Three alternatives for the treatment of these life-threatening, hospitalized infections are the recent introductions of the oxazolidinone linezolid, the streptogramins quinupristin−dalfopristin, and the even more recent approval of the lipopeptide daptomycin into the clinical setting. The limitations of these drugs have also been documented in the literature.[4] Additionally, these drugs have a narrow antibacterial spectrum and are potent against only Gram-positive pathogens. Clearly, there is a medical need for an antibacterial agent that is more rapid acting, bactericidal in action, and highly potent with a possible broad antibacterial spectrum and good safety profile for an effective treatment of the above-described multidrug-resistant Gram-positive pathogens. Alternatively, it also needs to be explored if existing known classes of anti-MRSA bacterial agents, for instance, the quinolones, can be expanded to provide novel agents active against vancomycin-intermediate *Staphylococcus aureus* (VISA)/vancomycin-resistant *S. aureus* (VRSA).

During our investigations of bactericidal classes of anti-MRSA agents, the activity profile of nadifloxacin (Figure 1) attracted our attention.[5] *RS*-(±)-nadifloxacin was introduced only for topical use as a liniment against *Propionibacterium acnes*.[6] Chemically, nadifloxacin has a lipophilic tricyclic benzoquinolizine core nucleus, with a 4-hydroxypiperidino moiety at C8 position. This 4-hydroxypiperidine moiety is a singular moiety without distal basic functionality, which is unusual for a side chain of quinolone, as all marketed quinolones bear side

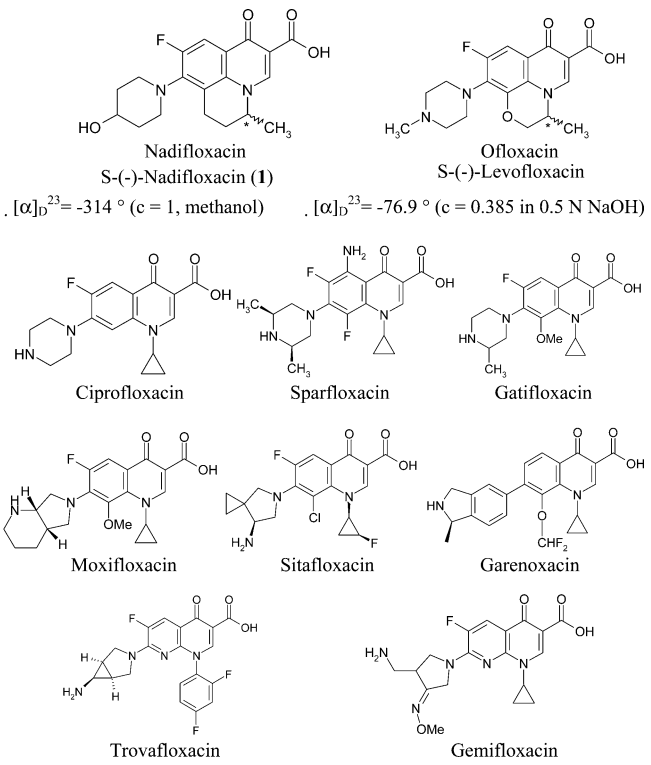


Figure 1. Some quinolones introduced for clinical use.

* To whom correspondence should be addressed. Phone: 91-240-2483854. Fax: 91-240-2489219. E-mail: mpatel@wockhardtin.com.
† Massachusetts General Hospital.
‡ Hershey Medical Center.
§ Case Western Reserve University.

10.1021/jm050035f CCC: $30.25
Published on Web 07/12/2005

Table 1. Distribution Coefficient (log *D*) and Dissociation Constant (pK_a) Data of Marketed Fluoroquinolones

	log *D*				
fluoroquinolone	at pH 2.0	at pH 7.4	pK_{a1}	pK_{a2}	pK_{a3}
nadifloxacin	1.89	1.24	6.8	–	–
trovafloxacin	−0.35	0.42	4.45	8.02	8.13
sparfloxacin	−0.86	−0.02	6.43	9.38	–
gatifloxacin	−1.14	−0.79	5.97	9.82	–
moxifloxacin	−1.33	−0.16	6.2	9.14	–
ciprofloxacin	−1.36	−0.98	6.39	8.68	–
norfloxacin	−1.46	−1	6.54	8.8	–
pefloxacin	−1.58	0.37	6.15	9.97	–
levofloxacin	−2.19	−0.43	6.1	8.12	–

chains with basic functionality, thereby providing two or three pK_a values compared with only one pK_a value for nadifloxacin (Figure 1).

The levorotatory *S*-(−)-nadifloxacin (**1**, Figure 1) is 64−256 times more potent than its *R*-(+)-isomer and approximately twice as active as the racemate against Gram-positive and -negative bacteria.[6] Neither the racemate nor the separate chiral enantiomers are ingredients of an oral or parenteral product. Early in our investigations, we considered the potential of *S*-(−)-nadifloxacin as an anti-MRSA agent.[7] Physicochemical attributes of *S*-(−)-nadifloxacin (**1**), such as a single pK_a value (6.80), high distribution coefficients (log *D*) of 1.89 at pH 2.0 and 1.24 at pH 7.4, and aqueous solubility of 0.06 mg/mL at 28 °C, are unusual among representative fluoroquinolones (Table 1). However, it is envisaged that the less aqueous solubility may be compensated by its high potency coupled with the lipohilicity-driven permeability, which makes *S*-(−)-nadifloaxcin optimizable for an oral dosage form. Alternatively, it is also envisaged that a complete aqueous soluble salt form can be considered for injectable dosage development, acceptable for a systemic use in humans. Various prodrugs and salts of *S*-(−)-nadifloxacin were synthesized and studied for the same purpose.

Herein, we wish to report the choice of the L-arginine salt of *S*-(−)-nadifloxacin (**21**) as an entity for further development as a potential drug candidate. The rationale for its choice and key bioprofile characteristics that led to successful development of **21** up to phase I study in human[8] are described.

Chemistry

S-(−)-Nadifloxacin is *S*-(−)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo-[*i*,*j*] quinolizine-2-carboxylic acid (**1**). Prodrugs and aqueous soluble salts[9] of **1** were synthesized and explored for possible use in parenteral or oral formulations.

Ester prodrugs at the C-2 carboxylic acid were prepared by reacting **1** with an appropriate alcohol or alkyl halide in the presence of a base (**2**−**9**, Table 2). Acyl prodrugs at C-4 hydroxyl of the side chain were prepared by treating **1** with an appropriately protected or unprotected organic acid in the presence of dicyclohexylcarbodiimide (DCC) or with a chloroformate in the presence of a base and subsequent deprotection to provide the carboxylic acid (**10**-**13**, Table 2). The D-glucosyl prodrug **14** was prepared by treating 1-bromo-2,3,4,6-tetra-O-acetyl-β-D-glucopyranose with the methyl ester of **1** in the presence of triethylamine followed by ester saponification and removal of acetyl groups in the presence of lithium hydroxide. The prodrug **15** was prepared by treating **10** with pivaloyloxymethyl chloride in the presence of a base.

The pK_a value of 6.80 of compound **1** permitted salt formation with inorganic bases and basic amino acids. Sodium and potassium salts of **1** were prepared by a conventional method by adding an equimolar quantity of alkali hydroxide to **1** followed by freeze-drying (**16**, **17**, Table 2). A similar procedure then was used for formation of the basic amino acid salt of **1**, that is, treating equimolar quantities of L-arginine and **1** in acetone or aqueous methanol followed by evaporation to dryness yielded two different polymorphs of L-arginine salt (**19**, **20**). The crystalline polymorphic amino acid salts **18** and **21** were prepared by crystallization from the reaction mixture obtained by treating the respective basic amino acid with **1** in an acetone and water mixture.

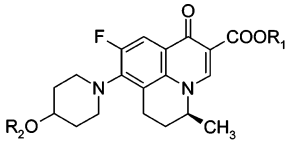
Results and Discussions

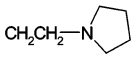
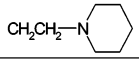
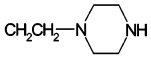
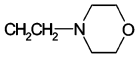
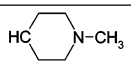
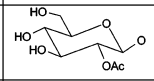
The synthesized prodrugs, initially envisaged for an oral dosage form, were assessed for their stability in simulated gastric fluid. Similarly, salt entities, envisaged for parenteral usage, were studied for aqueous solubility and stability under different relative humidity ranges in order to identify a stable, scalable, polymorphic form. The compounds were also screened for a preliminary assessment of their in vitro and in vivo antibacterial activity (Table 2).

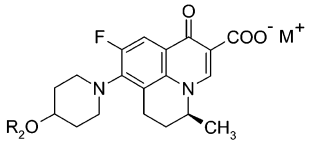
Prodrugs were not considered for further development, as MIC values of prodrugs after esterase action failed to match with the MIC values of original compound **1**, indicating that prodrugs were not completely cleavable by esterase action. Higher ED_{50} values of the prodrugs compared to compound **1** also supported absorption- and/or cleavage-related issues associated with prodrug entities.

The salts merited attention over the prodrugs[10] because of their antibacterial equipotency with **1** and enhanced pH-dependent aqueous solubility**.** Among the alkali metal salts, sodium and potassium salts were not considered, due to their hygroscopic nature. Among the amino acid salts, the L-lysine salt (**18**) and the L-arginine salt (**21**) were further evaluated for pH-dependent aqueous solubility, an essential criterion for injectable dosage formulation. It was observed that aqueous solubility as high as 40 mg/mL at pH 9.2 at 30 °C for compounds **18** and **21** can be acquired by adding additional 1.5 equiv of L-lysine for compound **18** and an additional 0.7 equiv of L-arginine for compound **21**. Thus L-arginine salt was selected, as a lesser quantity of the additional amino acid is required for achieving the required solubility. This solution of **21** is found to be stable over time under the required regulatory temperature and humidity conditions.[11] The L-arginine salt of **1** provided three different polymorphic or pseudopolymorphic forms: the amorphous form **19**, the semicrystalline form **20**, and the crystalline form **21** (Figure 2). The preparation of these polymorphs is solvent dependent. Polymorph **21**, at an ambient temperature of 35 °C and relative humidity of 61%, was found to be a tetrahydrate from the single-crystal X-ray data.[12] On

Table 2. Stability and In Vitro and In Vivo Antibacterial Activity of Prodrugs and Salts of **1**

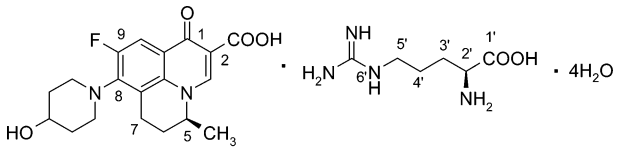


Compound	R1	R2	Stability[a]	MIC[b] (mg/mL) MSSA[c]		ED_{50}[d] (mg/kg) MRSA-32
1	H	H		0.03[e]		*p.o.* 62.3 (46.9-82.6) *s.c.* 24.8 (18.3-33.8)
				Before esterase action	After esterase action	
2	CH_3	H	+	8.0	1.0	>100
3	$CH_2CO_2CH_3$	H	-	8.0	4.0	>150
4	$CH_2CO_2C_2H_5$	H	-	8.0	2.0	>150
5		H	+	1.0	0.06	>150
6		H	+	2.0	0.25	>150
7		H	+	0.5	0.12	>100
8		H	+	0.25	0.1	>100
9		H	+	2.0	1.0	>100
10	H	$C(O)C(CH_3)_3$	+	0.5	0.25	>100
11	H	L-arg	+	1.0	0.1	>100
12	H	L-(NO_2)-arg. TFA	+	0.25	0.06	>75
13	H	L-(NO_2)-arg-L-(NO_2)-arg.TFA	+	0.5	0.12	>75
14	H		-	0.1	0.1	>100
15	$CH_2OC(O)C(CH_3)_3$	$C(O)C(CH_3)_3$	+	>16	>16	>150



Compound	M	R_2	Aqueous Solubility (mg/mL)	Stability[f]	MIC (μg/mL) MSSA	ED_{50}[d] (mg/kg) MRSA-32
16	Na	H	>100	-	0.03	60.0 (54.2-72.5)
17	K	H	>100	-	0.03	>100
18	L-lysine	H	1	+	0.03	>100
19	L-arginine (Amorphous)	H	5	-	0.03	90.3 (74.2-115.8)
20	L-arginine (Semi-crystalline)	H	2	-	0.03	>100
21	L-arginine.$4H_2O$ (Crystalline)	H	1.3	+	0.03	*p.o.* 93.6 (77.7-112.7) *s.c.* 27.8 (18.7 – 41.3)

[a] Stability in simulated gastric fluid up to 8 h. + = >90% intact prodrug. − = <90% intact prodrug. ND = not done. [b] MIC stands for minimum inhibitory concentration: lowest concentration of drug (μg/mL) that inhibits visible growth of the organism. [c] Methicillin-susceptible *S. aureus*. [d] ED_{50} is the amount of drug required to cure 50% of the infected mice by po route. [e] MIC of the parent compound, in the absence of esterase action. [f] Stability at 63 ± 3% RH and 27 °C. +: The substance remains as free flowing powder. −: The substance becomes a hard cake or syrup. ND: not done.



21

Figure 2. Compound **21**.

Table 3. Comparative Venous Toxicity Study of **16** and **21**

	duration of uninterrupted dosing iv (days)		
compound	250 mg/kg	300 mg/kg	NOEL[a] (mg/kg)
16	10	8	<250
21	28	22	250

[a] NOEL = no observed effect level. NOEL is the amount of drug administered to rats at which at 14 days of daily administration no venous blockage or phlebitis is observed.

subjection to heating at 70 °C, under vacuum, polymorph **21** was converted to monohydrate, which, however, reverted to the tetrahydrate form on exposure to humidity levels higher than 22%. X-ray powder diffraction (XRPD) studies of the tetrahydrate and monohydrate forms confirmed this conclusion.[13] The tetrahydrate polymorph is the most stable hydratomorph of the compound, designated **21** (WCK 771) in the text going forward. The crystalline polymorphic form **21** of the L-arginine salt scored over the other polymorphic forms **19** and **20** on the grounds of consistency in its preparation, reproducibility of synthetic batches, stability under ranges of humidity conditions, and pharmaceutical considerations related to provision of thermodynamically stable polymorphic form.[14]

Additionally, the crystallization method used in preparation of compound **21** has the distinct synthetic advantage of using optically impure **1** (chiral purity up to 80%). During crystallization of **21** there is enhancement of the chiral purity of **1**. Thus, as shown by experiment, the optical purity of **1** was enhanced from 88.24% to 97.06% as determined by HPLC during the crystallization of the L-arginine salt from a mixture of acetone and water. This result is due to the differential solubility of the diastereomers formed; this feature is absent in methods used for the preparation of the polymorphs **19** and **20**.

An additional feature for the choice of L-arginine salt **21** as an entity for preclinical development was its superior venous tolerability on multiday uninterrupted dosing to rodents as conveyed by its NOEL value in contrast to that of the alkali metal sodium salt **16** (Table 3). An entity devoid of phlebitis-causing potential is an essential precondition that would permit its progress through an array of preclinical studies required toward an INDA filing stage.

The in vitro antibacterial activity of **21** in comparison to representative clinically used fluoroquinolone drugs is recorded in Table 4. Against the aerobic Gram-positive staphylococci, enterococci, and streptococci, it displayed not only excellent superior potency in contrast to the first-generation ciprofloxacin and levofloxacin, but also higher potency than the third-generation moxifloxacin and gatifloxacin. Against the representative Gram-negative organisms, it was also as equipotent as three of the four comparator drugs against *Klebsiella pneumoniae*. Although generally its spectrum of activity against the Gram-negative organisms was 2−12-fold weaker than the classical/traditionally known ciprofloxacin, its MICs of 0.008−4 μg/mL did not diminish its equality with the other comparator drugs (MICs also of 0.008−4 μg/mL). Against anaerobes,[15] its superiority over ciprofloxacin and equipotency with levofloxacin, sparfloxacin, and moxifloxacin was also evident from its MIC values against *Bacteroides* strain.

The potent antistaphylococcal activity of **21** was further elaborated against a range of methicillin-resistant[16] (MRSA), vancomycin-intermediate-resistant (VISA) and vancomycin-resistant[17] (VRSA) strains (Table 5). Its excellent activity against the VRSA strain makes it currently the first compound in the class of fluoroquinolone antibacterial agents with the highest potency against such a relatively drug-unsusceptible strain. Its overall equipotency with the streptogramin quinupristin−dalfopristin is also to be noted. It is, however, less potent than the lipopeptide daptomycin. Compound **21** was evaluated for in vivo efficacy in lethal mouse infection models employing separately *S. aureus* ATCC 25923, methicillin-resistant *S.* aureus-32 (MRSA-32), and methicillin-resistant *S. aureus*-33 (MRSA-33) as the infectious organisms (Table 6). The in vivo efficacy of **21** is superior to that of levofloxacin and comparable to that of moxifloxacin for both quinolone-sensitive and MRSA-32 strains.

To study the mode of action of **21**, its antistaphylococcal potency was studied. The susceptibility was determined for a wild type *S. aureus* strain and its purified DNA gyrase and topoisomerase enzymes to three compounds: the levorotatory **21**, the dextrorotatory *R*-nadifloxacin, and the comparator drug ciprofloxacin (Table 7).[18] The central event in the interaction between the quinolones and gyrase or topoisomerase IV is formation of a quinolone−enzyme−DNA complex that contains broken DNA. Compound **21** was found to be 2.5−5-fold more potent than the *R*-isomer in DNA cleavage complex (CC) formation and >4-fold more potent in topoisomerase IV CC formation. In comparison with ciprofloxacin, however, **21** was found to be 8-fold more potent in CC formation with gyrase, whereas it was 1−2-fold less potent in topoisomerase IV CC formation. Surprisingly, **21** was itself found to have high potency and almost equipotent affinity both to DNA gyrase and topoisomerase IV, unlike the previously reported data for racemic nadifloxacin showing that the racemate is one of the few fluoroquinolones that in *S. aureus* has DNA gyrase as its primary target.[18] The nearly equipotent affinity of **21** for its two targets explains its propensity to resist selection of resistant mutants.[19] This also supports the dual-target hypothesis, which states that the impact of mutations in either target alone would be relatively low in terms of lowering the antibacterial potency of an equipotently dual-targeting quinolone.[20]

It is also known that membrane protein NorA, encoded by the *norA* gene, mediated efflux resistance is exhibited by fluoroquinolones. We have already demonstrated that compound **21** is found to be least effected by the NorA-mediated efflux in staphylococci.[21]

Table 4. Comparative MICs (μg/mL) of **21** against Representative Staphylococcal, Enterococcal, Streptococcal, Gram-Negative, and Anaerobic Pathogens

	MIC[a] (μg/mL)												
compounds	a	b	c	d	e	f	g	h	i	j	k	l	m
21[b]	0.03	0.015	0.06	0.25	0.12	0.12	0.06	0.12	0.25	0.008	0.12	4	0.5
ciprofloxacin	1.0	0.25	0.5	2	2	2	0.5	2	2	<0.008	<0.008	1	8
levofloxacin	1.0	0.12	0.5	1	1	2	1	1	2	0.008	0.06	4	1
moxifloxacin	0.06	0.06	0.25	0.25	0.25	0.25	0.25	0.12	0.5	0.008	0.06	4	0.5
gatifloxacin	0.1	0.06	0.25	0.25	0.5	0.5	0.25	0.25	0.5	0.008	0.03	4	0.5

[a] MIC: minimum inhibitory concentration. Abbreviations: a, *S. aureus* ATCC 25923; b, *Staphylococcus haemolyticus* ATCC 29970; c, *Staphylococcus saprophyticus* ATCC 15305; d, *Enterococcus faecalis* ATCC 29212; e, *Streptococcus pneumoniae* ATCC 49619; f, *Streptococcus pneumoniae* ATCC 6303; g, *Streptococcus pyogenes* ATCC 19615; h, *Streptococcus agalactiae* ATCC 13813; i, *Streptococcus sanguis* ATCC 10556; j, *K. pneumoniae* ATCC 10031; k, *Escherichia coli* ATCC 25922; l, *Pseudomonas aeruginosa* ATCC 27853; m, *Bacteroides vulgatus* ATCC 29327. [b] MIC values for compound **1** are same that of compound **21**.

Table 5. Comparative MICs (μg/mL) of **21** for Methicillin-Resistant *S. aureus* (MRSA), Vancomycin-Intermediate *S. aureus* (VISA), and Vancomycin-Resistant[17] (VRSA) *S. aureus* Strains

compound	MRSA-32[a]	MRSA-33[b]	MRSA 5023[c]	VISA[d] MU 3	VISA[e] MU 50	*VRSA*[f] Hershey
21	1	1	1	2	1	0.5
levofloxacin	16	8	16	16	8	32
moxifloxacin	2	2	2	4	4	4
vancomycin	1	1	1	8	16	32
quinupristin–dalfopristin	0.5	1	1	1	1	1
daptomycin	0.25	0.25	0.25	0.25	0.25	0.5

[a] Methicillin-resistant *S. aureus*-32. [b] Methicillin-resistant *S. aureus*-33. [c] Methicillin-resistant *S. aureus*-5023. [d] Vancomycin-intermediate *S. aureus* MU 3. [e] Vancomycin-intermediate *S. aureus* MU 50. [f] Vancomyci-resistant *S. aureus* (Hershey[17]).

Table 6. In Vivo Efficacy of **21** against Sensitive and Resistant *S. aureus* Strains in a Systemic Mouse Infection Model

	ED_{50}[a] (mg/kg) (95% confidence limit) by sc route		
compounds	*S. aureus* ATCC 25923	MRSA-32	MRSA-33
21	0.59 (0.37–0.95)	27.8 (18.7–41.3)	38.3 (27.7–53.0)
levofloxacin	5.92 (4.3–6.3)	> 100	> 100
moxifloxacin	0.6 (0.3–1.2)	29.97 (25.3–35.39)	66.22 (56.1–78.0)
vancomycin	6.11 (3.8–9.8)	2.18 (0.6–7.0)	3.7 (1.18–11.7)
linezolid	2.4 (2.1–2.9)	1.14 (0.4–3.1)	3.1 (1.1–4.9)

[a] ED_{50} is the amount of drug required to cure 50% of infected mice by sc route.

The likelihood that a mutant strain will emerge in an individual subject treated with **21** was assessed by determining its mutant prevention concentration (MPC) value (Table 8). MPC has been defined as the lowest concentration that allows no colony growth when more than 10^{10} cells are applied to drug-containing agar plate. It is determined by analysis of the numbers of colonies growing from large populations plated on a series of agar plates containing increasing drug concentrations.[22] MPC/MIC ratios for **21** and moxifloxacin showed that **21** has a 2.5-fold lower selection index[22] than moxifloxacin, in addition to its having a lower MIC value for the strains tested. The mutant selection window for **21** is thus first shifted to lower concentration levels and second is much narrower than for a fluoroquinolone with the ratio of that of moxifloxacin. Compared to gatifloxacin, although the MPC/MIC ratio for gatifloxacin is about the same as that of **21**, the 4-fold higher MIC value of gatifloxacin raises the mutant selection window range to values 4–7-fold higher. At a concentration of 5 μg/mL in plate, **21** did not give rise to resistant mutant colonies of all three MRSA strains tested, whereas both moxifloxacin and gatifloxacin at a comparable concentration failed to suppress emergence of mutants.

The safety aspects by the intravenous (iv) route were assessed for **21**, especially with respect to the class-specific adverse effects known to be associated with the quinolones. The sodium salt **16** was used as a comparator drug. Single dose and repeated dose diverse toxicity studies in Wistar rats and Beagle dogs were conducted, the results of many of which are already reported.[23] The NOAEL (no observed adverse effect level) of **21** for Wistar rats and Beagle dogs was 350 and 50 mg/kg, respectively. **21** was found to be safe with respect to the generally observed class-specific gastrointestinal symptoms and central nervous system disturbances. Even with respect to the serious adverse events such as hepatotoxicity, QT-interval prolongation, phototoxicity, genotoxicty, chondrotoxicity, histopathological changes in organs, and drug interactions which have tended to be quinolone-specific, **21** has acceptable safety. In summary, **21** displayed high intravenous safety and tolerability.

In phase I clinical studies, **21** was safe and well-tolerated following intravenous infusion. In healthy volunteers with 1 h infusion of **21**, up to a dose of 800 mg/volunteer, linearity in C_{max} and AUC was observed throughout the dose range.[24] At a 600 mg iv dose in human volunteers, **21** achieved a serum-unbound C_{max} value of 4 μg/mL with a half-life of 6 h. This level appears to be therapeutically adequate and 8 times higher than the MIC_{90} value of 0.5 μg/mL, with BID dosing, to treat target pathogens such as MRSA, MRSE (methicillin-resistant *Staphylococcus epidermidis*), VISA, and VRSA.

Conclusions

In summary, we have described the preparation of *S*-(−)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*] quinolizine-2-carboxylic acid L-arginine salt **21** as a suitable preclinical developmental entity that has progressed to successful dose-escalation phase I studies in human.

The biological profile makes it worthy for consideration to be developed as a drug candidate for nosocomial infections caused by multidrug-resistant Gram-positive pathogens. The scope of the research supports that L-arginine is optimal for use as a counterion to provide a salt of *S*-nadifloxacin for use in parenteral administration. **21** is a safe, well-tolerated, bactericidal quinolone with efficacy comparable to the best among current clinical quinolones against multidrug-resistant

Table 7. Comparative MICs against *S. aureus* ISP 794 (wild type), *gyrA* Mutant, *grlA* Mutant, and *gyrA*/*grlA* Double Mutant and Drugs-Stimulated Inhibition (IC_{50}) of and DNA Cleavage Efficiencies (CC_{50}) of DNA Gyrase and Topoisomerase-IV

	MIC (μg/mL)[a]				DNA gyrase[b]		topoisomerase IV[b]	
compound	ISP794	gyrA	grlA	*gyrA*/*grlA*	IC_{50}[c]	CC_{50}[d]	IC_{50}[c]	CC_{50}[d]
21	0.016	0.032	0.032	1.0	2.5−5	5.0	2.5−5.0	2.5
R-nadifloxacin	0.250	1.0	1.0	32	10.0	12.5−25.0	>10	>10
ciprofloxacin	0.125	0.125	1.0	16	5.0	30−50	2.5−5	1.25−2.5

[a] Microdilution method,[31] MIC determined after 24 h. [b] Enzyme source: *S. aureus* ISP 794 (wild type). [c] IC_{50} (μg/mL): concentration of drug that inhibits 50% of supercoiling activity, compared to absence of inhibitor. [d] CC_{50} (μg/mL) Concentration of drugs that stimulates 50% of DNA cleavage complex formation, compared to maximum of itself.

Table 8. Comparative MPC (μg/mL) and MPC/MIC of **21** and Frequency of Resistance Emergence for MRSA at 5 μg/mL Concentration

	MRSA-32			MRSA-33			MRSA-5023		
compd	MPC	MPC/MIC	FRE[a]	MPC	MPC/MIC	FRE[a]	MPC	MPC/MIC	FRE[a]
21	2	2	$<6.5 \times 10^{-9}$	2	2	$<3.5 \times 10^{-9}$	2	2	$<4.0 \times 10^{-9}$
moxifloxacin	10	5	4.5×10^{-5}	10	5	2.0×10^{-8}	10	5	3.0×10^{-7}
gatifloxacin	8	2	4.5×10^{-4}	14	3.5	4.0×10^{-5}	8	2	3.5×10^{-6}

[a] FRE stands for frequency of resistance emergence

staphylococccal strains and an ability to cover other aerobic Gram-positive, Gram-negative, and anaerobic pathogens. **21** appears to be an attractive candidate within the quinolone class to provide a new antibacterial agent for use against nosocomial multidrug-resistant Gram-positive infections, in particular MRSA, VISA, and VRSA infections. The possibility continues to be explored for the development of oral formulations of **1**, maybe **21** itself, or sufficiently divergent prodrugs and derivatives of **1**.

Experimental Section

Melting points were obtained manually by capillary method and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on Varian Gemini 2000 (200 MHz) by using tetramethylsilane as an internal reference standard. The mass spectra were recorded on Micromass QuatrroII using the electrospray ionization (ESI) technique. X-ray powder diffraction analysis was performed by using conditions listed below: scan speed 5°/min, sampling time 7 min, scan mode continuous, $2\theta/\theta$ reflection, Cu target (Ni filter) on 40 kV × 40 mA Rigaku D/max 2200 X-ray machine. The differential scanning calorimetric (DSC) analysis was performed on a Mettler Toledo Star system using the heating program from 30 to 300 °C at the rate of 10 °C/min. The infrared (IR) spectra were recorded on FTIR Bruker Vector 22 model. The optical rotation values were recorded on a Rudolph (DIGIPOL DP 1A14) automatic polarimeter at 589 nm wavelength. HPLC measurements were carried out on an HPLC system HP-1100 consisting of a binary solvent delivery system with autoinjector and variable wavelength detector. The chromatography was performed using a 5 μm particle size C18 or Cyano column (YMC Co. Ltd.). Mobile phase used was buffer (0.05% trifluoroacetate or 50 mM ammonium acetate in water) and acetonitrile either in isocratic or gradient mode. The detection was performed at 254 nm. Chiral purity was determined by using reverse phase chromatography consisting of a 5 μm particle size C18 column (YMC Co. Ltd.). The mobile phase used was acetonitrile and phosphate buffer (potassium dihydrogen phosphate and disodium hydrogen phosphate) containing β-cyclodextrin and EDTA in 12:88 ratio. The column temperature was maintained at 50 °C during analysis, with flow rate 2 mL/min. The detection was performed at 290 nm. *S*-(−)-9-Fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic acid was prepared according to the literature procedure.[25]

Free base estimation of sodium, potassium, lysine, and arginine salts was carried out by HPLC by determining free base content against compound **1** as a reference standard. HPLC analysis was performed using a YMC Pack-ODS-AM, 250 × 4.6 mm, 5 μm HPLC column. The mobile phase consisting of a mixture of 0.05% TFA in water and acetonitrile in the ratio of 68:32 was used. The flow rate of the mobile phase was maintained at 1 mL/min. The detection was carried out at 254 nm.

Estimation of the L-lysine and L-arginine content of L-lysine and L-arginine salts was carried out by HPLC. HPLC analysis was performed using a Zorbax SB C-18, 250 × 4.6 mm, 5 μm HPLC column. The mobile phase consisting of a mixture of buffer (0.1 M potassium dihydrogen phosphate and 0.1 M octane sulfonic acid sodium salt in water) and acetonitrile in the ratio of 75:25 was used. The flow rate of the mobile phase was maintained at 1 mL/min. The detection was carried out at 210 nm.

Elemental analysis was performed on Fision EA 1108 CHN S Analyzer.

***S*-(−)-Methyl 9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylate (2).** A mixture of **1** (10 g, 0.027 mol) and K_2CO_3 (7.6 g, 0.055 mol) in *N*,*N*-dimethylacetamide (50 mL) was stirred at 70 °C for 4 h. Methyl iodide (2.6 mL, 0.04 mol) was added and the reaction mixture was stirred overnight. The reaction mixture was cooled to room temperature and the solid was filtered under suction. The filtrate was concentrated under vacuum to dryness and then diluted with water (50 mL). Solid compound separated, which was filtered under suction, dried to give the crude product, and purified using silica gel column chromatography to afford the title compound (4.9 g, 49% yield): mp 142−44 °C; HPLC purity = 99.04%; ^{1}H NMR ($CDCl_3$) δ 1.5 (d, 3H, J = 7.0 Hz), 1.6−1.7 (m, 2H), 1.9−2.2 (m, 5H), 2.7−3.4 (m, 7H), 3.9 (s. 3H), 4.4 (m, 1H), 8.0 (d, 1H, J = 12.0 Hz), 8.5 (s, 1H); MS (ES+) *m/z* 375 (M + H).

(*S*)-(Methoxycarbonyl)methyl 9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylate (3). The title compound was prepared by following the procedure described for compound **2** using methyl bromoacetate: mp 178−80 °C; HPLC purity = 95%; ^{1}H NMR ($CDCl_3$) δ 1.5 (d, 3H, J = 7.0 Hz), 1.6−2.2 (m, 6H), 2.8 (m, 1H), 3.0−3.3 (m, 6H), 3.8 (s, 3H), 3.9 (bs, 1H), 4.4 (m, 1H), 4.9 (s, 2H), 8.0 (d, 1H, J = 12.0 Hz), 8.5 (s, 1H); MS (ES+) *m/z* 433 (M + H).

(*S*)-(Ethoxycarbonyl)ethyl 9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]-quinolizine-2-carboxylate (4). The title compound was prepared by following the procedure described for compound **2** using ethyl bromoacetate: mp 188−90 °C; HPLC purity = 97.4%; ^{1}H NMR ($CDCl_3$) δ 1.3 (t, 3H, J = 7.0 Hz), 1.5 (d, 3H, J = 7.0 Hz), 1.6−1.8 (m, 2H), 2.0−2.2 (m, 4H), 2.7−3.4 (m,

7H), 3.9 (bs, 1H), 4.2 (q, 2H, J = 7.0 Hz), 4.4 (m, 1H), 4.9 (s, 2H), 8.0 (d, 1H, J = 12.0 Hz), 8.5 (s, 1H); MS (ES+) *m/z* 447 (M + H).

(*S*)-2-Pyrrolidin-1-ylethyl 9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylate (5). To a suspension of **1** (3.0 g, 8.33 mmol) in dichloromethane (30 mL) was added triethylamine (7 mL, 50 mmol) and the reaction mixture was stirred at 0 °C. To this mixture was added ethyl chloroformate (5.38 g, 50 mmol). The reaction mixture was stirred at room temperature for 45 min and concentrated under reduced pressure to remove excess of ethyl chloroformate. The residue obtained was dissolved in dichloromethane, and 2-pyrrolidin-1-ylethanol (5.75 g, 50 mmol) was added. The reaction mixture was stirred at room temperature overnight. The solvent was concentrated under vacuum and the residue obtained was purified by column chromatography to give the title compound (1.56 g, 41% yield): mp 220−22 °C; HPLC purity = 98.33%; ^{1}H NMR ($CDCl_3$) δ 1.5 (d, J = 7.0 Hz, 3H), 1.6−1.85 (m, 8H), 1.8−2.2 (m, 5H), 2.6−3.4 (m, 10H), 3.8 (m, 1H), 4.2−4.3 (m, 3H), 8.0 (d, J = 13.0 Hz, 1H), 8.5 (s, 1H); MS (ES+) *m/z* 458 (M + H).

(*S*)-2-Piperidin-1-ylethyl 9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylate (6). The compound was prepared by using 2-piperidin-1-ylethanol in a procedure as described in the previous example: mp 240−42 °C; HPLC purity = 95.34%; ^{1}H NMR ($CDCl_3$) δ 1.4 (d, J = 7.0 Hz), 1.4−1.8 (m, 4H), 1.8−2.4 (m, 8H), 2.5−2.6 (m, 4H), 2.6−2.8 (m, 3H), 2.9−3.4 (m, 5H), 3.8 (m, 1H), 4.4 (m, 3H), 8.0 (d, J = 13.0 Hz, 1H), 8.5 (s, 1H); MS (ES+) *m/z* 472 (M + H).

(*S*)-2-Piperazin-1-ylethyl 9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylate (7). To a suspension of **1** (3.0 g, 8.33 mmol) in dichloromethane (30 mL) at 0 °C was added triethylamine (2.3 mL, 16.66 mmol) followed by isobutyl chloroformate (2.26 g, 16.61 mmol), and the reaction mixture was stirred at for 45 min. Lithium bromide (1.5 g, 16.66 mmol), followed by 1-(*tert*-butoxycarbonyl)-4-(2-hydroxyethyl)piperazine (3.86 g, 16.66 mmol) was added to it and the reaction mixture was allowed to stir at room temperature overnight. The reaction mixture was washed with water (2 × 10 mL), and the dichloromethane layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The residue obtained was purified by column chromatography to yield (*S*)-(2-{4-(*tert*-butoxycarbonyl)piperazin-1-yl}ethyl) 9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylate (1.90 g, 40% yield). This was used as without further purification for the next reaction.

The product obtained in the above reaction was dissolved in dichloromethane (35 mL), and trifluoroacetic acid (5 mL) was added. The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was concentrated under high vacuum to remove the excess of trifluoroacetic acid. The residue obtained was dissolved in dichloromethane and the solution was basified with triethylamine (pH ~ 8). The reaction mixture was washed with water (2 × 10 mL), dried over anhydrous sodium sulfate, and concentrated under vacuum to give title compound (1.23 g, 78% yield): mp 134−36 °C; HPLC purity = 96.94%; ^{1}H NMR ($CDCl_3$, D_2O exchange) δ 1.50 (d, J = 7.0 Hz, 3H), 1.55−2.20 (m, 7H), 2.6 (m, 4H), 2.75−3.20 (m, 10H), 3.8−3.95 (m, 1H), 4.50 (m, 3H), 8.05 (d, J = 13.0 Hz, 1H), 8.5 (s, 1H); MS (ES+) *m/z* 473 (M + H).

(*S*)-2-Morpholin-4-ylethyl 9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylate (8). The compound was prepared by using 2-morpholin-1-ylethanol as in the procedure for compound **5**: mp 160−62 °C; HPLC purity = 95.7%; ^{1}H NMR ($CDCl_3$) δ? 1.5 (d, 3H, J = 7.0 Hz), 1.6−1.8 (m, 2H), 1.9−2.2 (m, 4H), 2.6 (m, 4H), 2.8 (t, 2H, J = 7.0 Hz), 2.8 (m, 1H), 3.0−3.4 (m, 5H), 3.7 (m, 4H), 3.8−3.9 (m, 1H), 4.5 (m, 3H), 8.0 (d, 1H, J = 12.0 Hz), 8.5 (s, 1H); MS (ES+) *m/z* 474 (M + H).

(*S*)-1-Methylpiperidin-4-yl 9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]-quinolizine-2-carboxylate (9). The compound was prepared by using 1-methylpiperidin-4-ol as in the procedure for compound **5**: mp 90−92 °C; HPLC purity = 98.92%; ^{1}H NMR ($CDCl_3$) δ 1.50 (d, 3H, J = 8.3 Hz), 1.60−2.20 (m, 10H), 2.25−2.55 (m, 5H), 2.70−2.90 (m, 3H), 2.95−3.40 (m, 5H), 3.80−4.00 (m, 1H), 4.30−4.50 (m, 1H), 5.00−5.20 (m, 1H), 8.05 (d, 1H, J = 12.5 Hz). 8.40 (s, 1H); MS (ES+) *m/z* 458 (M + H).

***S*-(−)-9-Fluoro-6,7-dihydro-8-(4-pivaloyloxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic Acid (10).** A mixture of **1** (5.0 g, 0.014 mol), triethylamine (2.8 mL, 0.027 mol), and pivaloyl chloride (2.5 mL, 0.020 mol) in dichloromethane (50 mL) was stirred overnight at room temperature. The reaction mixture was washed with water (2 × 10 mL) and the organic layer was concentrated to afford the crude product, which was purified by column chromatography to afford the title compound (2.4 g, 39% yield): mp 208−10 °C; HPLC purity = 99.69%; ^{1}H NMR ($CDCl_3$) δ 1.3 (s, 9H), 1.6 (d, 3H, J = 7.0 Hz), 1.8−2.3 (m, 6H), 2.8−3.5 (m, 6H), 4.6 (m, 1H), 5.0 (m, 1H), 8.1 (d, 1H, J = 12.0 Hz), 8.7 (s, 1H); MS (ES+) *m/z* 445 (M + H).

(2*S*,5*S*)-9-Fluoro-6,7-dihydro-8-{4-(L-argininyloxy)piperidin-1-yl}-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic Acid (11). To a suspension of *N*-Fmoc-L-arginine (0.85 g, 2.08 mmol) in THF (100 mL) was added triethylamine (0.21 mL, 1.52 mmol) at room temperature, and the mixture stirred for 5 min. To the solution was added 2,4,6-trichlorobenzoyl chloride (0.24 mL, 1.52 mmol), and stirring continued at room temperature. After 3 h, DMAP (0.17 g, 1.38 mmol) was added followed by **1** (0.5 g, 1.38 mmol) and stirring continued for 16 h. The solid precipitate was filtered and washed with THF (10 mL). The filtrate was concentrated to dryness under vacuum to afford (2*S*,5*S*)-9-fluoro-6,7-dihydro-8-{4-[*N*-(9-fluorenylmethoxycarbonyl)-L-argininyloxy]piperidin-1-yl}-5-methyl-1-oxo-*1H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic acid (1.5 g) as a yellow solid. The yellow solid was dissolved in THF (10 mL), treated with diethylamine (1.5 mL), and stirred at room temperature for 2 h. The solid precipitate was filtered, washed with THF (3 × 10 mL), and subjected to preparative HPLC to afford the title compound (400 mg, 56% yield): mp 100−105 °C; HPLC purity = 97.9%; ^{1}H NMR (DMSO-d_6) δ 1.4 (d, 1H, J = 7.0 Hz), 1.6−2.1 (m, 12 H), 2.8−3.3 (m, 8H), 4.1 (bs, 1H), 4.9 (bs, 1H), 7.4 (bs, 2H, D_2O exchange), 7.9 (d, 1H, J = 13.0 Hz), 8.0 (bs, 1H, D_2O exchange), 8.5 (bs, 1H, D_2O exchange), 9.0 (s, 1H), 15.3 (bs, 1H, D_2O exchange); MS (ES+) *m/z* 517 (M + H).

(2*S*,5*S*)-9-Fluoro-6,7-dihydro-8-{4-(*N*^{G}-nitro-L-argininyloxy)piperidin-1-yl}-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic Acid Trifluoroacetic Acid Salt (12). A mixture of *tert*-butoxycarbonyl-*N*^{G}-nitro-L-arginine (3.0 g, 9.4 mmol), triethylamine (1.4 mL, 10.3 mmol), and 2,4,6-trichlorobenzoyl chloride (1.65 mL, 10.3 mmol) in tetrahydrofuran was stirred at 0 °C. After stirring for 3 h, **1** (2.3 g, 6.4 mmol) followed by 4-(dimethylamino)pyridine (0.78 g, 6.4 mmol) was added to the reaction mixture, which stirred for 15 h at room temperature. The reaction mixture was diluted with ethyl acetate (250 mL) and the mixture was washed with saturated aqueous solution of ammonium chloride followed by water (20 mL × 2). The organic layer was separated and dried over sodium sulfate and solvent was removed under vacuum to dryness. The residue was stirred with 10% trifluoroacetic acid in dichloromethane (100 mL) at room temperature for 3 h. The reaction mixture was washed with diethyl ether and the solid was filtered. The crude solid was purified using silica gel column chromatography to provide the title compound (2.46 g, 57% yield): mp 222−224 °C; HPLC purity = 95.57%; ^{1}H NMR (CD_3OD) δ 1.40 (d, 3H, J = 7 Hz), 1.45−2.20 (m, 10H), 2.80−3.45 (m, 9H), 4.00−4.20 (bs, 1H), 5.00−5.20 (bs, 1H), 7.75 (d, 1H, J = 12.0 Hz), 8.80 (s, 1H); MS (ES+) *m/z* 562 (M + H).

(2*S*,2′*S*,5*S*)-9-Fluoro-6,7-dihydro-8-{4-(*N*^{G}-nitro-L-argininyl-*N*^{G}-nitro-L-argininyloxy)piperidin-1-yl}-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic Acid Trifluoroacetic Acid Salt (13). A mixture of *tert*-butoxycarbonyl-*N*^{G}-nitro-L-arginine (3.0 g, 9.4 mmol) and triethylamine

(2.1 mL, 13.73 mmol) in tetrahydrofuran (50 mL) and trichlorobenzoyl chloride (1.65 mL, 10.3 mmol) was stirred at 0 °C. After stirring for 3 h, **13** (3.5 g, 5.18 mmol) followed by 4-(dimethylamino)pyridine (0.76 g, 6.2 mmol) was added to the reaction mixture, which stirred for 15 h. The reaction mixture was diluted with ethyl acetate (400 mL) and the mixture was washed with a saturated aqueous solution of ammonium chloride followed by water. The organic layer was separated and dried over sodium sulfate and the solvent was evaporated under vacuum. The residue was stirred with 10% trifluoroacetic acid in dichloromethane (100 mL) at room temperature for 3 h. The reaction mixture was stirred with diethyl ether and the solid was filtered. The crude solid was purified by silica gel column chromatography to provide the title compound (1.72 g, 38% yield): mp 210−212 °C; HPLC purity = 95.57%; ^{1}H NMR (DMSO-d_6) δ 1.40 (d, 3H, J = 7 Hz), 1.45−2.20 (m, 14H), 2.80−3.25 (m, 10H), 4.30−4.40 (m, 1H), 4.7−5.00 (m, 2H), 7.80 (d, 1H, J = 12.0 Hz), 8.80 (s, 1H); MS (ES+) *m/z* 763.4 (M + H).

(5*S*)-9-Fluoro-6,7-dihydro-8-(4-*β*-D-acetylglucopyranosylpiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic Acid (14). To a mixture of 1-bromo-2,3,4,6-tetraacetyl-D-glucose (10 g, 24.3 mmol) and **2** (8.0 g, 21.3 mmol), dissolved in dichloromethane (50 mL) and cooled to 0 °C was added triethylamine (15 mL). After the addition, the reaction was brought to room temperature, allowed to stir for 1 h, and then heated at reflux for 48 h. The solvent was removed under vacuum and the residue was purified by column chromatography to give (5*S*)-methyl 9-fluoro-6,7-dihydro-8-(4-[(*β*-D-tetraacetylglucopyranosyl)oxy]piperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylate (6.2 g, 41% yield) as a solid. ^{1}H NMR ($CDCl_3$, D_2O exchange) δ 1.45 (d, 3H, J = 7.4 Hz), 1.60−2.20 (m, 18H), 2.40−3.40 (m, 6H), 3.80−4.45 (m, 10H), 4.80−5.00 (m, 1H), 5.15−5.25 (m, 1H), 5.75 (d, 1H, J = 5.5 Hz), 8.05 (d, 1H, J = 12.5 Hz), 8.45 (s, 1H), MS (ES+) *m/z* 705 (M + H).

To the above compound (1.0 g, 1.42 mmol), dissolved in a mixture of THF−methanol−water (1:1:1), was added lithium hydroxide (0.5 g, 20.83 mmol), and the mixture stirred at room temperature for 0.5 h. The organic solvent from the reaction mixture was evaporated under reduced pressure, and saturated aqueous ammonium chloride solution was added during which the product precipitated out. The aqueous layer was decanted and the residue was washed with $CHCl_3$ (3 × 5 mL) to obtain the title compound (0.417 g, 52% yield): mp 156−158 °C; HPLC purity = 95.81%; ^{1}H NMR (CD_3OD) δ 1.50 (d, 3H, J = 8.3 Hz), 1.55−2.30 (m, 9H), 2.80−3.40 (br m, 6H), 3.45−4.15 (m, 7H), 4.20−4.30 (m, 1H), 4.30−5.15 (br m, 2H), 5.65 (d, 1H, J = 8.3 Hz), 7.90 (d, 1H, J = 12.5 Hz), 8.75 (s, 1H), MS (ES+) *m/z* 565 (M + H).

***S*-(−)-(Pivaloyloxy)methyl 9-fluoro-6,7-dihydro-8-(4-(pivaloyloxy)piperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*] quinolizine-2-carboxylate (15).** A mixture of **10** (1.0 g, 2.0 mol) and K_2CO_3 (0.62 g, 4.0 mol) in *N*,*N*-dimethylacetamide (5 mL) was stirred at 70 °C. After stirring for 4 h, (pivaloyloxy)methyl chloride (0.5 mL, 3.0 mol) was added, followed by stirring at 70 °C overnight. The reaction mixture was cooled to room temperature and the solid was filtered under suction. The filtrate was concentrated to dryness and then water was added. The solid compound that separated was filtered under suction to give the crude product, which was purified by silica gel column chromatography to afford the title compound (140 mg, 11% yield): mp 192−96 °C; HPLC purity = 99.34%; ^{1}H NMR ($CDCl_3$) δ 1.2 (s, 18H), 1.5 (d, 3H, J = 7.0 Hz), 1.6−2.2 (m, 8H), 2.7−3.4 (m, 5H), 4.4 (m, 1H), 6.0 (s, 2H), 8.0 (d, 1H, J = 12.0 Hz), 8.4 (s, 1H); MS (ES+) *m/z* 559 (M + H).

***S*-(−)-Sodium 9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylate (16).** Aqueous sodium hydroxide solution (1 N, 1.39 mL, 1.39 mmol) was added to the stirred powder of **1** (0.5 g, 1.39 mmol) and diluted with water (10 mL). The resulting solution was stirred for 30 min, passed through a microfilter, and freeze-dried to furnish the title compound (0.54 g, 98% yield) mp 285 °C (dec); HPLC purity = 99%; ^{1}H NMR (D_2O) δ 1.3 (d, 3H, J = 7.0 Hz), 1.4−1.6 (m, 2H), 1.65−2.1 (m, 4H), 2.6−3.2 (m, 6H), 3.71 (m, 1H), 4.5 (m, 1H), 7.6 (d, 1H, J = 13.0 Hz), 8.3 (s, 1H); MS (ES+) *m/z* 383 (M + H). Anal. ($C_{19}H_{20}FN_2O_4Na\cdot1.25H_2O$) C, H, N. HPLC assay of the free base (theoretical free base content) 94.25%, found 93.80%.

***S*-(−)-Potassium 9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*] quinolizine-2-carboxylate (17).** Aqueous potassium hydroxide solution (0.5%, 15.6 mL, 1.39 mmol) was added to the stirred powder of compound **1** (0.5 g, 1.39 mmol). The resulting solution was stirred for 30 min, passed through a microfilter, and freeze-dried to provide the title compound (0.568 g, 99%): mp >300 °C; HPLC purity = 97.3%; ^{1}H NMR (DMSO-d_6) δ 1.3 (d, J = 6.8 Hz, 3H), 1.4−1.7 (m, 2H), 1.7−1.95 (m, 2H), 1.95−2.2 (m, 2H), 2.75−2.98 (m, 2H), 2.98−3.3 (m, 4H), 4.1 (m, 1H), 4.5 (m, 1H), 7.84 (d, J = 12.6 Hz, 1H), 8.3 (s, 1H); MS (ES+) *m/z* 399 (M + H). Anal. ($C_{19}H_{20}FN_2O_4K\cdot2H_2O$) C, H, N. HPLC assay of free base (theoretical free base content) 90.44%, found 91.52%.

***S*-(−)-9-Fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic Acid L-Lysine Salt (18).** To a clear solution of **1** (1.0 g, 2.77 mmol) in methanol (40 mL) was added a solution of L-lysine monohydrate (0.45 g, 2.77 mmol) in water (4 mL) under reflux. The clear solution was stirred for 15 min at reflux. Excess solvent was distilled under vacuum to obtain a powder. The powder was dried at 50−55 °C to furnish the title compound (0.7 g, 99% yield): mp 235−40 °C; HPLC purity = 95.6%; ^{1}H NMR (DMSO-d_6) δ 1.4 (t, 3H, J = 6.8 Hz), 1.5−2.2 (m, 10H), 2.5−2.8 (m, 4H), 2.8−3.4 (m, 6H), 3.7−3.9 (m, 2H), 4.7 (m, 1H), 7.8 (d, 1H, J = 12.6 Hz), 8.8 (s, 1H); MS (ES+) *m/z* 506 (M + H). Anal. ($C_{25}H_{35}FN_4O_6$) C, H, N. HPLC assay of free base (theoretical free base content) 71.15%, found 70.35%. Estimated L-lysine by HPLC (theoretical L-lysine content) 28.85%, found 29.15%.

***S*-(−)-9-Fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic Acid L-Arginine Salt (Amorphous Form) (19).** To a three-necked round-bottom flask fitted on an oil bath and equipped with a mechanical stirrer, a thermometer pocket, and a reflux condenser was charged **1** (100 g, 0.278 mol) followed by acetone (1.25 L). Stirring was started, and the stirred suspension was charged with L-arginine (48.4 g, 0.278 mol) dissolved in distilled water (600 mL). The reaction mixture was stirred at a 40 °C for 30 min to obtain a clear solution. Acetone was removed by distillation. The solid obtained was dried under vacuum at 65−70 °C to furnish title compound (136 g, 92% yield): mp 238−42 °C; ^{1}H NMR (DMSO-d_6) δ 1.4 (d, 3H, J = 7.0 Hz), 1.5−2.2 (m, 8H), 2.8−4.2 (m, 16H), 4.8 (m, 1H), 7.8 (d, 1H, J = 12.8 Hz), 8.8 (s, 1H). MS (ES+) *m/z* 535 (M + H). Anal. ($C_{25}H_{35}FN_6O_6$) C, H, N. HPLC assay of free base (theoretical free base content) 67.41%, found 67.17%. Estimated L-arginine by HPLC (theoretical L-arginine content) 32.59%, found 32.30%.

***S*-(−)-9-Fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic Acid L-Arginine Salt (Semicrystalline Form) (20).** To a suspension of **1** (25 g, 69.44 mmol) in methanol (500 mL) was added L-arginine (12.41 g, 69.44 mmol). To the above solution was added water (30 mL) to obtain a solution clear. The reaction mixture was stirred for 30 min at room temperature. Methanol was removed by distillation and the product was obtained as a white solid. The solid was suspended in hexane (500 mL) and stirred vigorously. The solid was filtered and dried under vacuum at 70 °C to furnish the title compound (14 g, 89% yield): mp 232−34 °C; ^{1}H NMR (DMSO-d_6) δ 1.4 (d, 3H, J = 7.0 Hz), 1.5−2.2 (m, 8H), 2.8−4.2 (m, 16H), 4.8 (m, 1H), 7.8 (d, 1H, J = 13.0 Hz), 8.8 (s, 1H). MS (ES+) *m/z* 535 (M + H). HPLC assay of free base (theoretical free base content) 67.41%, found 67.20%. Estimated L-arginine by HPLC (theoretical L-arginine content) 32.59%, found 33.06%.

***S*-(−)-9-Fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic Acid L-Arginine Salt Tetrahydrate (Crystalline Form) (21).** To a three-necked round-bottom flask fitted on an oil bath and equipped with a mechanical stirrer, a thermometer pocket, and a reflux condenser was charged **1** (100 g, 0.278 mol) followed by acetone (300 mL). Stirring was started and to the stirred suspension was charged powdered L-arginine (48.4 g, 0.278 mol) followed by distilled water (250 mL). The reaction mixture was stirred at a temperature between 50 and 60 °C for 1 h to obtain a clear solution. Activated charcoal (3 g) was added to the solution and the solution was filtered hot. To the filtrate was then added acetone (700 mL) and the reaction mixture was allowed to cool to 30−35 °C. The reaction mixture was stirred for an additional 2 h at this temperature. The crystalline solid was filtered under reduced pressure and the wet cake was washed with acetone (100 mL). The resulting solid was dried under vacuum at 65−70 °C to furnish **21** (137 g, 92% yield): mp 236−240 °C; ^{1}H NMR (DMSO-d_6) δ 1.4 (d, 3H, J = 7.0 Hz), 1.5−2.2 (m, 8H), 2.8−4.2 (m, 16H), 4.8 (m, 1H), 7.8 (d, 1H, J = 13.0 Hz), 8.8 (s, 1H). MS (ES+) *m/z* 535 (M + H). Anal. ($C_{25}H_{35}FN_6O_6 \cdot 4H_2O$) C, H, N. HPLC assay of free base (theoretical free base content) 67.41%, found 67.16%. Estimated L-arginine by HPLC (theoretical L-arginine content) 32.59%, found 32.14%.

Enhancement of the Optical Purity by Preparing the L-Arginine Salt of *S*-(−)-9-Fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H* -benzo[*i*,*j*]-quinolizine-2-carboxylic Acid. A mixture of the two enantiomers of nadifloxacin [88.24% *S*-(−)-isomer and 11.76% (*R*)-9-fluoro-6,7-dihydro-8-(4-hydroxypiperidin-1-yl)-5-methyl-1-oxo-1*H*,5*H*-benzo[*i*,*j*]quinolizine-2-carboxylic acid] (1.5 g, 4.16 mmol) was suspended in an acetone (4.5 mL) and water (3.8 mL) mixture. To the stirred suspension was added L-arginine (0.725 g, 4.16 mmol). The reaction mixture was warmed to temperature between 50 and 60°C to obtain a clear solution. Acetone (11 mL) was added to the reaction mixture. It was cooled to 30−35 °C and stirred for 2 h. The crystalline solid was filtered with suction and the residue was washed with 2 mL of acetone. The resulting solid was dried to furnish 2.05 g (92%) of the title compound. The chiral analyses of the crystals and mother liquor were performed by chromatography on an analytical chiral column. The optical purity of the crystals and mother liquor was found to be 97.06% and 62.37%, respectively. Thus, the optical purity has been enhanced from 88.24% simply by crystallizing the L-arginine salt from a mixture of acetone and water.

Determination of Dissociation Constant (pK_a). The pK_a value[26] was determined by dissolution of the compound in dilute aqueous sodium hydroxide solution and pH metric titration of the solution at 27 °C with 0.1 N hydrochloric acid (Table 1).

Solubility Determinations. Aqueous solubility[27] was measured in double distilled water, after equilibration with excess test substance for 16 h. Typically, a sufficient amount of test substance was added to 3 mL of water to achieve a supersaturated solution. The supersaturated solution was shaken on a mechanical shaker for 16 h at room temperature (27 °C) and then filtered through 0.2 μm nylon syringe filter (Whatman Inc.). Aliquots of filtrate were diluted appropriately and assayed by HPLC (Table 2).

Chemical Stability of Prodrugs in Simulated Gastric Fluid. The stability profiles of prodrugs **2**−**15** were determined in simulated gastric fluid.[28] To 5 mg of prodrug was added 10 mL of simulated gastric fluid and the mixture mixed. At desired time points (initial, 1 h, 3, 5, and 8 h) the solution was analyzed by HPLC (Table 2).

Physical Stability. The physical stability of the compounds **16**−**21** was determined at 63 ± 3% relative humidity and 27 °C by exposing the compound to controlled humidity conditions.[29] About 1 g of the compound was spread on a glass Petri dish, kept in controlled humidity chambers for 24 h, and then observed for change in physical appearance (Table 2).

Determination of Distribution Coefficient (log *D*). Distribution coefficients at pH 2.0 and 7.4 were determined by using the shake flask method.[30] The concentration of test compound in both the octanol and buffer phases was analyzed by HPLC (Table 2).

Determination of Antibacterial Activity. The bacterial strains used for susceptibility studies were either clinical isolates from the Wockhardt culture collection or reference strains from the American Type Culture Collection (ATCC).

In Vitro Susceptibility Test. Minimal inhibitory concentrations (MICs) were determined by standard broth microdilution methods for aerobic bacteria[31] and for anaerobic bacteria.[32] MIC was defined as the lowest drug concentration that prevented visible growth of bacteria (Tables 2, 4, 5, 7, and 8).

Esterase Stability of Prodrugs. Freshly collected rat blood was allowed to coagulate for 1 h and serum was obtained by centrifugation. To 1 mL of serum was added 50 μL of DMSO solution containing 1 mg of drug. The reaction mixture, after shaking, was incubated at 37° C for 4 h and then serially 2-fold diluted in water. In parallel, prodrugs were dissolved in DMSO at a comparative concentration and treated and diluted similarly as serum samples. The MIC of solution was determined against *S. aureus* ATCC 29523 by the NCCLS recommended agar dilution method.[33] The extent of prodrug stability was judged by the fall in MIC values of prodrugs on cleavage by serum esterases due to the formation of parent bioactive compound (Table 2).

In Vivo Efficacy by Oral Route. A group of six Swiss mice consisting of three males and three females, weighing 18−22 g, was infected with 0.5 mL of MRSA-32 cell suspension, prepared from overnight growth, by ip route. The MRSA-32 cell suspension was prepared in sterile 5% hog gastric mucin (Difco). The treatment was given with compounds **1**−**21** suspended in 5% Tween-80 saline and administered at doses of 50, 75, 100, and 150 mg/kg, 1 h and 4 h postinfection, by oral route. Untreated mice served as infection control and showed 100% mortality within 24 h. Treated mice were observed for survival up to 7 days. The results were recorded as ED_{50} and ED_{100} with the help of probit analysis.[33] Each experiment was repeated three times (Table 2).

In Vivo Efficacy by Subsutaneous Route. ED_{50} determinations were conducted as previously described.[34] The methicillin-sensitive bacteria used for the infection were *S. aureus* (MSSA) ATCC 25923. The quinolone-resistant strains were methicillin-resitant *S. aureus* MRSA-32 and MRSA-33. The infecting dose was 2.5×10^8 cfu/animal suspended in 5% sterile hog gastric mucin. Following infection, graded doses of **21** and comparators were administered by the sc route. The ED_{50} values, including 95% confidence limits, were calculated by the probit method from survival rates on day 7 postinfection (Table 6).

Comparative Venous Toxicity Study of 21 and 16. 21 (75 mg) was dissolved in 1 mL of an L-arginine solution of concentration 27 mg/mL. **16** (75 mg) was dissolved in 1 mL of water. Groups of 6-week-old Wistar rats (six male and six females/group) were administered intravenously with either **21** or **16** at doses of 0, 250, and 300 mg/kg/day for 14 consecutive days. The tail vein of each rat was monitored for venous blockage or phlebitis. The NOEL (mg/kg) value was determined (Table 3).

Mutant Prevention Concentration (MPC). The MPC of **21** and moxifloxacin for MRSA-32, MRSA-33, and MRSA-5023 at 2, 4, 8 and 16 times the MIC concentrations was determined by uniformly spreading 200 μL of 10^{10} cfu/mL density cell suspension, obtained from overnight grown broth culture, on five replicate MHA plates for each concentration. Plates were incubated at 37 °C for 48 h and observed for growth. MPC is defined as the minimum concentration of the compound that prevents emergence of mutant colonies of the test organism (Table 8).

Frequency of Selection of Mutants. Overnight cultures of three MRSA strains were concentrated in normal saline before plating on MHA without any antibiotic or containing

21, moxifloxacin, or gatifloxacin at 5 μg/mL concentration. Selection plates were incubated at 37 °C. Frequency of selection of resistant mutants was calculated as the ratio of the number of resistant colonies at 48 h to the number of cells plated (Table 8).

Acknowledgment. The authors thank Mr. Ravindra G. Manwatkar for typing the manuscript and organizing it for Web submission.

Supporting Information Available: XRPD spectra of **19**−**21**. HPLC, HR-MS, and elemental analysis data of assayed compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Centers for Disease Control and Prevention. In The Killer Bug. *Fortune* **2002**, September. The Public Health Research Institute, New Jersey, http://www.phri.org/news/news_inthenews09.asp.

(2) (a) Abbanat, D.; Macielag, M.; Bush, K. Novel Antibacterial Agents for the Treatment of Serious Gram-positive Infections. *Expert Opin. Investig. Drugs* **2003**, *12* (3), 379−99. (b) Hooper, D. C. Fluoroquinolone Resistance among Gram-positive Cocci. *Lancet Infect. Dis.* **2002**, *2*, 530−538. (c) Diekema, D. J.; Jones, R. N. Oxazolidinone Antibiotics. *Lancet* **2001**, 358 (9297), 1975−82. (d) Linden, P. K. Treatment Options for Vancomycin Resistant Enterococcal Infections. *Drugs* **2002**, *62* (3), 425−41. (e) Thornsberry, C.; Sahm, D. F.; Kelly, L. J. Regional Trends in Antimicrobial Resistance Among Clinical Isolates of *Streptococcus pneumoniae. Haemophilus influenzae* and *Moraxella catarrhalis* in the United States: Results from the TRUST Surveillance Program, 1999−2000. *Clin. Infect. Dis.* **2002**, *34* (Suppl 1), S4−S16. (f) Sutcliffe, J. A. Antibacterial Agents: Solutions for the Evolving Problems of Resistance. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4159−4161.

(3) (a) Centers for Disease Control and Prevention. *Staphylococcus aureus* Resistant to Vancomycin-United States. *Morbidity and Mortality Weekly Report* **2002**, *51*, 565−567. (b) Center for Disease Control and Prevention, Public Health Despatch. Vancomycin-Resistant *Staphylococcus aureus*-Pennsylvania. *Morbidity Mortality Weekly Rep.* **2002**, *51*, 902−903.

(4) (a) Hachem, R. Y.; Hicks, K.; Huen, A.; Raad, I. Myelosuppression and Serotonin Syndrome Associated with Concurrent Use of Linezolid and Selective Serotonin Reuptake Inhibitors in Bone Marrow Transplant Recipients. *Clin. Infect. Dis.* **2003**, *37*, e8−11. (b) Hachem, R. Y.; Raad, I. I.; Afif, C.; Husni, R.; Rolston, K. V. Prospective Randomized Pilot Study Comparing Quinupristin/Dalfopristin (QD) vs Linezolid in the Treatment of VRE Infections in Patients with Hematologic Malignancy. *43rd Annual Meeting of American Society of Hematology*, Orlando, FL, Dec 6−17, 2001, Abstract no. 4400.

(5) Ishikawa, H.; Testuyuki, U.; Kano, M.; Nakagawa, K. Pyrroloquinolone and Benzoquinolizine Compounds and Antimicrobial Compositions. US Patent 4,399,134, 1983.

(6) (a) Ishikawa, H.; Tabusa, F.; Miyamoto, H.; Kano, M.; Ueda, H.; Tamaoka, H.; Nakagawa, K. Studies on antibacterial agents. I. Synthesis of substituted 6,7-dihydro-1-oxo-1H,5H-benzo[i,j]-quinolizine-2-carboxylic acids. *Chem. Pharm. Bull*. **1989**, *37*, 2103-2108. (b) Kurokawa, I.; Akamatsu, H.; Nishigima, S.; Asada, Y.; Kawabata, S. Clinical and Bacteriologic Evaluation of OPC-7251 in Patients with Acne: A Double Blind Group Comparison Study vs Cream Base. *J. M. Acad. Dermatol.* **1991**, *25*, 674−81. (c) Morita, S.; Otsubo, K.; Matsubara, J.; Ohtnai, T.; Uchida, M. An Efficient Synthesis of a Key Intermediate towards (S)-(−)-Nadifloxacin. *Tetrahedron: Asymmetry* **1995**, *6* (1), 245−254.

(7) (a) Patel, M. V.; Gupte, S. V.; Sreenivas, K.; Chugh, Y.; Agarwal, S. K.; De Souza, N. J. *S*-(−)-Nadifloxacin: Oral Bioavailbility and Bioefficacy in Mouse Model of Staphylococcal Septicemia. *Abstract of Papers*, *40th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, September 2000;* American Society for Microbiology: Washington, DC, 2000; Poster F-558.

(8) A preliminary version of this work was described in a poster. Deshpande, P. K.; Desai, V. N.; Bhavsar, S. V.; Chaturvedi, N. C.; Ghalsasi, S. A.; Aher, S.; Yeole, R. D.; Pawar, D.; Shukla, M. C.; Patel, M. V.; Gupte, S. V.; De Souza, N. J.; Khorakiwala, H. F. WCK 771−A Chiral Benzoquinolizine-2-carboxylic acid Arginine Salt Active against Vancomycin Intermediate *Staphylococcus aureus* (VISA). *Abstract of Papers*, *43rd Interscience Conference on Antimicrobial Agents and Chemotherapy*, *Chicago*, *September 2003;* American Society for Microbiology: Washington, DC, 2003; Poster F-430.

(9) (a) De Souza, N. J.; Agarwal, S. K.; Patel, M. V.; Bhawsar, S. B.; Beri, R. K.; Yeole, R. D.; Shetty, N.; Khorakiwala, H. F. Chiral Fluoroquinolone Arginine Salt Form. US patent 6,514,986, 2003. (b) De Souza, N. J.; Deshpande, P. K.; Shukla, M. C.; Mukarram S. M. J.; Kulkarni, D. G.; Rahman, A.; Yeole, R. D.; Patel, M. V.; Gupte, S. V. Crystalline Fluoroquinolone Arginine Salt Form. US patent 6,664,267, 2003.

(10) The antibacterial activity of the prodrug is studied after their ester cleavage action.

(11) Patel, M. V. Unpublished in-house data on file.

(12) Patel, M. V. Manuscript under preparation on the single-crystal X-ray data.

(13) In-house XRPD data.

(14) Patel, M. V.; Agarwal, S. K.; Sreenivas, K.; Shetty, N.; Upadhyay, D.; Chaturvedi, N.; Thomas, A.; De Souza, N. J.; Khorakiwala, H. F. Antibacterial Optically Pure Benzoquinolizine Carboxylic acids, Processes, Compositions and Methods of Treatment. US patent 6,514,986, 2003.

(15) Peric, M.; Jacobs, M. R.; Appelbaum, P. C. Antianaerobic Activity of a Novel Fluoroquinolone, WCK 771, Compared to those of Nine Other Agents. *Antimicrob. Agents Chemother.* **2004**, *48*, 3188−3192.

(16) Patel, M. V.; De Souza, N. J.; Gupte, S. V.; Jafri, M. A.; Bhagwat, S. S.; Chugh, Y.; Khorakiwala, H. F.; Jacobs, M. R.; Appelbaum, P. C.; Antistaphylococcal Activity of WCK 771, a Tricyclic Fluoroquinolone, in Animal Infection Models. *Antimicrob. Agents Chemother.* **2004**, *48*, 4754−4761.

(17) (a) Bozdogan, B.; Esel, D.; Whitener, C.; Browne, F. A.; Appelbaum, P. C. Antibacterial Susceptibility of a Vancomycin-Resistant *Staphylococcus aureus* Strain Isolated at the Hershey Medical Center. *J. Antimicrob. Chemother*. **2003**, *52*, 864−868. (b) Bozdogan, B.; Appelbaum, P. C. Bactericidal Activities of New Quinolones WCK 771, WCK 919, and its Two Isomers WCK 1152 and WCK 1153 Against Vancomycin resistant *Staphylococcus aureus* (VRSA). *Abstract of Papers*, *43rd Interscience Conference on Antimicrobial Agents and Chemotherapy*, *Chicago*, *September 2003;* American Society for Microbiology: Washington, DC, 2003, Poster F-438.

(18) (a) Oizumi, N.; Kawabata, S.; Hirao, M.; Watanabe, K.; Okumo, S.; Fujiwara, T.; Kikuchi, M. Relationship Between Mutations in the DNA Gyrase and Topoisomerase IV Genes and Nadifloxacin Resistance in Clinically Isolated Quinolone-Resistant *Staphylococcus aureus. J. Infect. Chemother*. **2001**, *7*, 191−194. (b) Takei, M.; Fukuda, H.; Kishii, R.; Hosaka, M. Target Preference of 15 Quinolones against *Staphylococcus aureus*, Based on Antibacterial Activities and Target Inhibition. *Antimicrob. Agents Chemother*. **2001**, *45*, 3544−3547.

(19) Patel, M. V.; Appelbaum, P. C.; Jacobs, M. R.; Gupte, S. V.; Agarwal, S. K.; Upadhyay, D. J.; Jafri, M. A.; Bhagwat, S. S.; Khorakiwala, H. F.; De Souza, N. J. WCK 771 A−An Investigational Anti-Staphylococcal Fluoroquinolone (FQ) with Strong Bactericidal Activity Against Low and High-Density Cultures. *Abstract of Papers*, *41st Interscience Conference on Antimicrobial Agents and Chemotherapy*, *Chicago*, *September 2001;* American Society for Microbiology: Washington, DC, 2001; Poster F-536.

(20) Hooper, D. C. Mechanisms of Action and Resistance of Older and Newer Fluoroquinolones. *Clin. Infect. Dis*. **2000**, *31*, S24.

(21) (a) Jacobs, M. R.; Bajaksouzian, S.; Windau, A.; Appelbaum, P. C.; Patel, M. V.; Gupte, S. V.; Bhagwat, S. S.; De Souza, N. J.; Khorakiwala, H. F. In Vitro Activity of the New Quinolone WCK 771 against Staphylococci. *Antimicrob. Agents Chemother.* **2004**, *48*, 3338−3342. (b) Gupte, S. V.; Patel, M. V; Agarwal, S. K.; Upadhyay, D. J.; Nair, S. C.; Bhagwat, S. S.; Shetty, N.; Bhawsar, S. B.; De Souza, N. J.; Khorakiwala, H. F. The Impact of NorA Mediated Efflux on In Vitro and In Vivo Anti-Staphylococcal Efficacy of an Investigational Fluoroquinolone (FQ) WCK 771 A and Other FQs. *Abstract of Papers*, *41st Interscience Conference on Antimicrobial Agents and Chemotherapy*, *Chicago*, *September 2001;* American Society for Microbiology: Washington, DC, 2001; Poster F-537.

(22) (a) Zhao X., Drlica K. Resistricting the Selection of Antibiotic Resistant Mutants: A General Strategy Derived from Fluoroquinolone Studies. *Clin. Infect. Dis.*, **2001**, *33* (Suppl 3), S147. (b) Hooper, D. C. Minimising Potential Resistance: The molecular view−A comment on Courvalin and Trieu-Cuot. *Clin. Infect. Dis.* **2001**, *33* (Suppl 3), S157. (c) Drlica, K. The Mutant Selection Window and Antimicrobial Resistance. *J. Antimicrob. Chemother.* **2003**, *52*, 11−17.

(23) Patel, M. V.; Gupte, S. V.; Shetty, N. M.; Chugh, Y.; Pawar, V. D.; Nandanwar, M. B.; Sundar, S. R.; Kansagara, A. J.; Patel, M. I.; Gupta, S. R.; De Souza, N. J.; Khorakiwala, H. F. WCK 771−An Investigational FQ with High Intravenous Tolerability. *Abstract of Papers*, 43rd *Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, September 2003;* American Society for Microbiology: Washington, DC, 2003; Poster F-431.

(24) Maharaj, N.; Jha, R.; Chugh, Y.; Yeole, R.; Patel, M.; De Souza, N. J.; Khorakiwala, H. F.; More, B.; Gogtay, N.; Dalvi, S.; Kshirsagar, N. A Phase I study of escalating single dose of intravenous (IV) WCK 771. *Abstract of Papers*, *44th Interscience Conference on Antimicrobial Agents and Chemotherapy Washington D. C., October 2004*; American Society for Microbiology: Washington, DC, 2003; Poster A-21.
(25) Hashimoto, K.; Okaichi, Y.; Nomi, D.; Miyamoto, H.; Bando, M.; Kido, M.; Fujimura, T. and Minamikawa, J. A Practical Synthesis of (S)-(−)-Nadifloxacin: Novel Acid-Catalyzed Racemisation of Tetrahydroquinaldine Derivative. *Chem. Pharm. Bull*. **1996**, *44* (4), 642−45.
(26) *Analytical Profiles of Drug Substances*; Florey, K., Ed.; Academic Press: New York, 1991; Vol. 20, p 577.
(27) James, I. W. *Pharmaceutical Preformulation: The Physicochemical Properties of Drug Substances*, Ellis Harwood Ltd: Chichester, UK, 1988; p 21.
(28) *United States Pharmacopeia—National Formulary;* United States Pharmacopeia Inc. USP 24 NF 19, 2000; p 2235.
(29) David, R. L. *CRC Handbook of Chemistry and Physics*, 72nd ed.; CRC Press: Boca Raton, FL, 1991−92; pp 15−21.
(30) Sangster, J. *Octanol Water Partition Coefficients*, John Wiley and Sons: Chichester, UK, 1997; pp 57−59.
(31) National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that grow aerobically,* 5th ed. (Approved Standard); NCCLS Document M7-A5; NCCLS: Wayne, PA, 2000.
(32) National Committee for Clinical Laboratory Standards. *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved,* 5th ed (Approved Standard); NCCLS Document M11-A5; NCCLS: Wayne, PA, 2001.
(33) Miller, L. C.; Tainter, M. L. Estimation of the ED50 and its error by means of logarithmic-probit graph paper. *Proc. Soc. Exp. Biol. Med*. **1994**, *57*, 261−264.
(34) Frimodt-Moller, N.; Knudsen, J. D.; Espersen, F. The Mouse Peritonitis/Sepsis Model. In *Handbook of Animal Models of Infection*; Zak, O., Sande, M. A., Eds.; Academic Press: London, 1999; pp 127−136.

JM050035F