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Design, Synthesis, and in vitro Antibacterial Activity of Fluoroquinolone Derivatives Containing a Chiral 3-(Alkoxyimino)-2-(aminomethyl)azetidine Moiety

Kai Lv,[a] Yexin Sun,[a] Lanyin Sun,[a] Zengquan Wei,[a] Huiyuan Guo,[a] Jinwei Wu,[b] and Mingliang Liu*[a]

A series of novel (*R*)/(*S*)-7-(3-alkoxyimino-2-aminomethyl-1-azetidinyl)fluoroquinolone derivatives were synthesized and evaluated for their in vitro antibacterial activity against representative strains. Our results reveal that 12 of the target compounds generally show better activity (MIC: <0.008–0.5 μg mL^{-1}) against the tested Gram-positive strains including MRSA and MRSE than levofloxacin (LVFX, MIC: 0.125–8 μg mL^{-1}). Their activity is similar to that of gemifloxacin (GMFX, MIC: <0.008–4 μg mL^{-1}). However, they are generally less active than the two reference drugs against Gram-negative strains. Moreover, against clinical strains of *S. aureus* including MRSA and *S. epidermidis* including MRSE, the MIC_{50} values (0.06–16 μg mL^{-1}) and MIC_{90} values (0.5–32 μg mL^{-1}) of compounds **16w**, **y**, and **z** are 2–8- and 2–16-fold less than LVFX, respectively, and **16w** (MIC_{90} range: 0.5–4 μg mL^{-1}) was also found to be more active than GMFX (MIC_{90} range: 1–8 μg mL^{-1}).

Introduction

Quinolones have become a major class of antibacterial agents that are used mainly to fight both community-acquired and serious hospital-acquired infections. These antibiotics act by binding two type II bacterial topoisomerases, DNA gyrase (topoisomerase II) and topoisomerase IV.[1,2]

Since the discovery of norfloxacin by Koga et al. in 1980,[3] most research concerning quinolone antibacterials has focused on the basic group at the C7 position, which greatly influences the potency, spectrum, and safety of these compounds. So far, all of the marketed fluoroquinolones, including ciprofloxacin, moxifloxacin, levofloxacin (LVFX), and gemifloxacin (GMFX) (Figure 1) possess a five- or six-membered nitrogen heterocycle at the C7 position.[4,5] There are significantly fewer published reports of azetidinyl-based quinolones than of pyrrolidinyl-, piperazinyl-, and piperidinyl-based analogues.

Recently, a number of quinolone derivatives containing an azetidine moiety, a rather common and stable functional group frequently found in antimicrobial agents such as β-lactams and polyoximic acids,[6,7] were synthesized, some of which were found to have potent in vitro and in vivo antimicrobial activity. It was reported that delafloxacin (ABT-492, RX-3341, Figure 1), bearing a 3-(hydroxy)azetidine moiety, displays excellent activity against Gram-positive pathogens including both methicillin-susceptible *S. aureus* (MSSA) and -resistant *S. aureus* (MRSA),[8] and phase III clinical studies began in April 2008.[9] WQ-3813 (Figure 1), with a C7 3-(isopropylamino)azetidine ring structurally related to delafloxacin, has the potential to be developed into a treatment for respiratory infections in both community and hospital settings[9] owing to its activity against major respiratory pathogens including multidrug- and quinolone-resistant isolates.

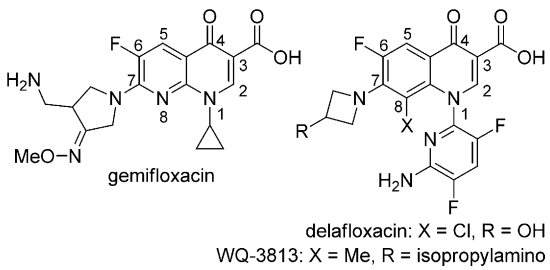

Figure 1. Structures of gemifloxacin, delafloxacin, and WQ-3813.

Fujita et al. reported that the antibacterial activity of 2-aminomethyl-1-azetidinyl-based quinolones is superior to that of the corresponding 3-aminomethyl-1-pyrolidinyl- and piperazin-1-yl-based analogues.[10] In addition, previous work on pyrrolidinyl quinolones emphasized the importance of the oxime functional group with respect to biological activity. For example, GMFX shows much more antibacterial activity than its desmethyloximino analogue.[5]

Inspired by the above research results, we considered the introduction of an alkyloxime moiety to 2-(aminomethyl)azetidine to construct structurally unique compounds, 3-(alkoxyimino)-2-(aminomethyl)azetidines, as side chains at the 7-position of quinolones (Figure 2). Moreover, considering that our side chains have a chiral center at the 2-position of the rings, prep-

[a] *Dr. K. Lv,[+] Y. Sun,[+] L. Sun, Z. Wei, Prof. H. Guo, Prof. M. Liu*
Institute of Medicinal Biotechnology
Chinese Academy of Medical Sciences, 100050 Beijing (China)
E-mail: lmllyx@yahoo.com.cn

[b] *J. Wu*
Zhejiang Starry Pharmaceutical Co. Ltd., 317300 Xianju (China)

[+] *These authors contributed equally to this work.*

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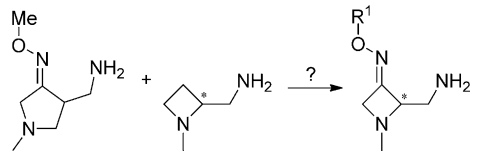


Figure 2. Design of the new azetidine side chain.

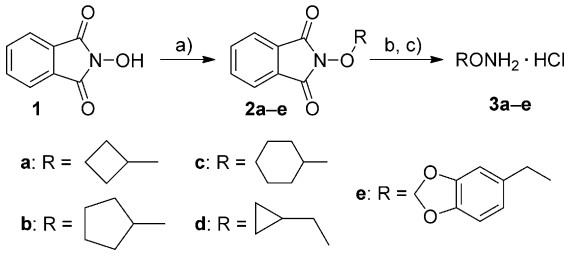
aration of both enantiomers is desirable, based on the concept that biologically active molecules interact with the chiral binding site of the target enzyme, and hence the chirality of a given molecule has a significant impact on its biological activity.[10] Herein we describe the design and synthesis of (*R*)/(*S*)-3-(alkoxyimino)-2-(aminomethyl)azetidine derivatives and a series of novel quinolone compounds containing these cyclic amines at the C7 position. Our primary objective was to optimize the potency of these quinolones against Gram-positive and Gram-negative organisms.

Results and Discussion

Chemistry

Detailed synthetic pathways to new 3-(alkyloximino)-2-(aminomethyl)azetidines (*S*)-**14a**–**h**/(*R*)-**14g**,**h** and novel quinolone derivatives **16a**–**z** are shown below in Schemes 2 and 3, respectively.

Commercially unavailable *O*-alkylhydroxylamines **3a**–**e** were first prepared according to Scheme 1. *N*-Hydroxyphthalimide **1** was treated with bromoalkanes (RBr) in *N*,*N*-dimethylformamide (DMF) in the presence of 18-crown-6 and potassium carbonate at 80 °C, and the resulting condensates **2a**–**e** were subsequently treated with hydrazine hydrate in dichloromethane/methanol to produce the desired compounds **3a**–**e** by following well-established procedures.[11]



Scheme 1. Synthesis of *O*-alkylhydroxylamine hydrochlorides **3a**–**e**. *Reagents and conditions*: a) RBr, 18-crown-6, K_2CO_3, DMF, 80 °C, 6 h, 56–71%; b) $NH_2NH_2{\cdot}H_2O$, CH_2Cl_2, MeOH, RT, 3 h; c) HCl, EtOH, 1 h, RT, 46–61% (for two steps).

Treatment of L/D-serine (*S*)/(*R*)-**4** with di-*tert*-butyl dicarbonate (Boc_2O) in dioxane gave Boc-protected compound (*S*)/(*R*)-**5**,[12] and the hydroxy group of (*S*)/(*R*)-**5** was subsequently protected by reaction with *tert*-butyldiphenylchlorsilane (TBDPSCl) in DMF in the presence of imidazole to yield serine derivative (*S*)/(*R*)-**6**, in an overall yield of 73–74% for the two steps.[13] (*S*)/(*R*)-**6** was treated with isobutyl chloroformate in the presence of *N*-methylmorpholine (NMM) to give a mixed anhydride, which was then treated with diazomethane to afford diazoketone (*S*)/(*R*)-**7** in 68–71% yield. Cyclization of (*S*)/(*R*)-**7** via an intramolecular rhodium-catalyzed N–H insertion reaction in dichloromethane at −78 °C produced the key intermediate (*S*)/(*R*)-**8**.[14,15] Interestingly, we successfully obtained compounds (*S*)/(*R*)-**6** and -**7** with recrystallization methods instead of column chromatographic separation techniques, as in the reported procedures.[13–15]

The azetidine-3-one (*S*)/(*R*)-**8** was smoothly converted into the oximes (*R*)-**9a**–**h**/(*S*)-**9g**,**h** by condensation with the above alkylhydroxylamine derivatives **3a**–**e** obtained as shown in Scheme 1 and commercially available *O*-alkylhydroxylamine hydrochlorides in the presence of pyridine.[16] Removal of the TBDPS protecting group of (*R*)-**9a**–**h**/(*S*)-**9g**,**h** by tetra-*n*-butylammonium fluoride (TBAF) in THF afforded the oxime alcohols (*R*)-**10a**–**h**/(*S*)-**10g**,**h**, which were then converted into the corresponding bromides by nucleophilic substitution with tetrabromomethane in dichloromethane/triphenylphosphine, in 70–80% yield.[17] However, Gabriel reaction of the resulting bromides and phthalimide potassium in DMF met with no success, we were unable to obtain the desired products (*S*)-**11a**–**h**/(*R*)-**11g**,**h**, but instead, 2-methyleneazetidine derivatives via elimination reaction, unexpectedly.

Subsequently, Mitsunobu reaction of (*R*)-**10a**–**h**/(*S*)-**10g**,**h** with phthalimide in diethyl azodicarboxylate (DEAD)/PPh_3/THF was used to furnish condensates (*S*)-**11a**–**h**/(*R*)-**11g**,**h**,[18,19] which, upon hydrazinolysis in ethanol, gave primary amines (*S*)-**12a**–**h**/(*R*)-**12g**,**h**. At this stage, however, we had to protect the amino group with Boc for purification. The bis-Boc protecting groups of (*S*)-**13a**–**h**/(*R*)-**13g**,**h** were then removed by pumping dry hydrogen chloride gas in dichloromethane to afford new azetidine dihydrochlorides (*S*)-**14a**–**h**/(*R*)-**14g**,**h** (Scheme 2).

Finally, the target compounds **16a**–**w** were obtained by coupling the new azetidine derivatives (*S*)-**14a**–**h**/(*R*)-**14g**,**h** with various compounds containing naphthyridone and quinolone cores in the presence of triethylamine. However, for **16s**–**z**, boric chelates **15c**–**f** were required to increase reactivity (Scheme 3).[20] All of the compounds synthesized were thoroughly characterized by analytical techniques.

Because the oxime group is present in the *E* or *Z* configuration, it was necessary to determine the geometries of all the oxime target compounds **16a**–**z**. Unfortunately we were unable to prepare X-ray-quality single crystals of any oxime intermediate or product. Nonetheless, we speculate that the oxime group of the target compounds is present in a mixed configuration of *E* and *Z* isomers due to signal doublings of the azetidine ring of the compounds observed in the ^{1}H NMR spectra. An approximation of the isomer ratio (Table 1) is possible only based on the ^{1}H NMR data of the hydrogen atom linked to the chiral center of the azetidine ring, which are apparently separate for most of the compounds **16a**–**z**. In addition, further separation of compounds **16a**–**z** into *E* and *Z* oxime components by chromatographic techniques is currently in progress.

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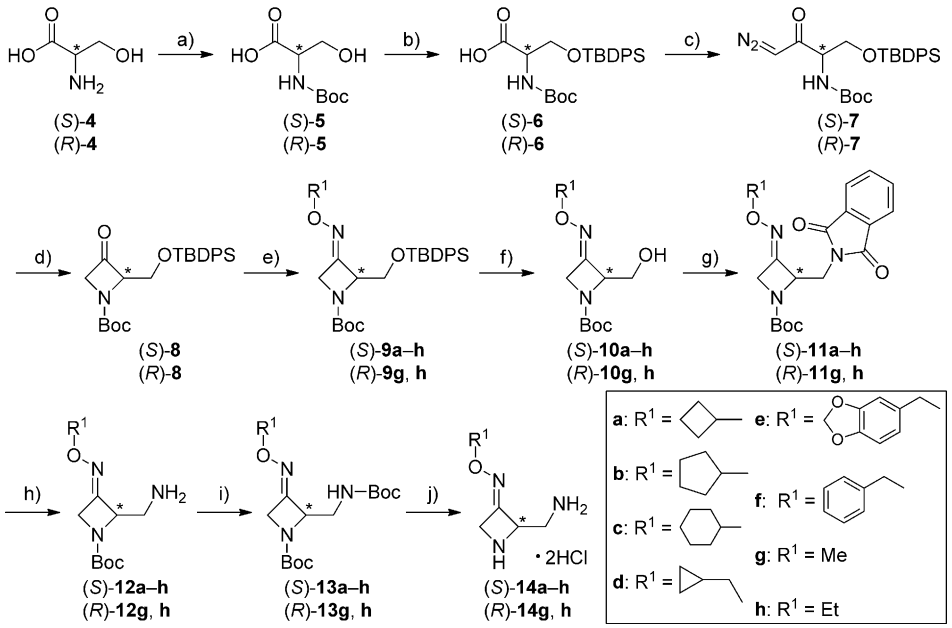
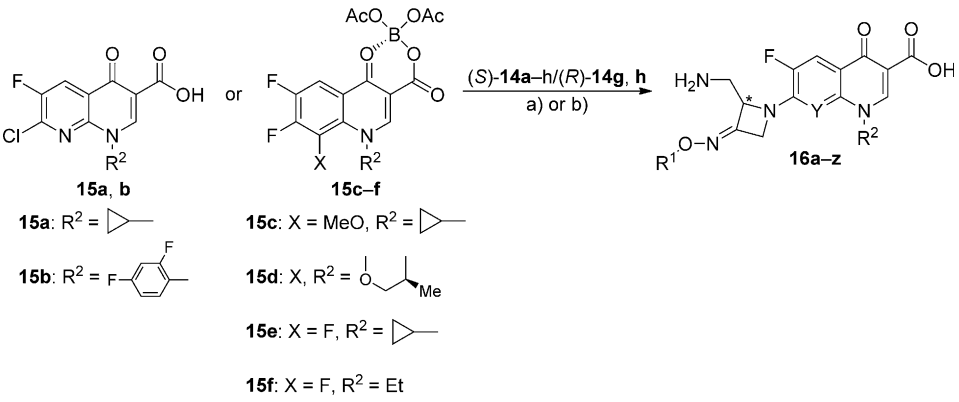


Scheme 2. Synthesis of 3-(alkyloximino)-2-(aminomethyl)azetidines (*S*)-**14a-h**/(*R*)-**14g,h**. *Reagents and conditions*: a) NaOH, $(Boc)_2O$, dioxane, RT, 12 h, then $KHSO_4$; b) TBDPSCl, imidazole, DMF, RT, 7 h, 73–74% (for two steps); c) *i*BuOCOCl, NMM, CH_2N_2, Et_2O, 8 h, RT, 68–71%; d) $Rh_2(OAc)_4$, CH_2Cl_2, −78 °C, 12 h, 46–53%; e) R^1ONH_2·HCl, pyridine, EtOH, 60 °C, 5 h; f) TBAF, THF, RT, 10 h, 41–60% (for two steps); g) phthalimide, Ph_3P, DEAD, THF, RT, 48–71%; h) $NH_2NH_2·H_2O$, EtOH, reflux, 3 h; i) $(Boc)_2O$, MeOH, RT, 12 h, 53–82% (for two steps); j) $HCl_{(g)}$, CH_2Cl_2, 1 h, RT, 47–67%.



Scheme 3. Synthesis of the target compounds **16a–z**. *Reagents and conditions*: a) Et_3N, MeCN, 50 °C, 5 h, 18–35%; b) 1. Et_3N, MeCN, 50 °C, 5 h; 2. 5% NaOH, 50 °C, 12 h, 13–27%. See Table 1 for structures.

Antibacterial activity

The target compounds **16a–z** were evaluated for their in vitro antibacterial activity against representative Gram-positive and Gram-negative strains using standard techniques.[21] Minimum inhibitory concentration (MIC) is defined as the compound concentration required to give complete inhibition of bacterial growth, and MIC values of the synthesized compounds, along with those of the reference drugs LVFX and GMFX for comparison, are listed in Tables 2, 3, and 4. These data suggest that all of the target compounds **16a–z** have considerable activity against the Gram-positive strains (MICs < 0.008–$8\ \mu g\,mL^{-1}$), although they are generally less active than LVFX and GMFX against the Gram-negative strains, with a few exceptions.

Against Gram-positive strains such as MSSA, MRSA, methicillin-susceptible *S. epidermidis* (MSSE), methicillin-resistant *S. epidermidis* (MRSE), and *Streptococcus pneumoniae*, compounds **16a**, **b**, **d**, **g**, **i**, **k**, **l**, **n**, **u**, **w**, **x**, and **z** show strong antibacterial activity (MIC < 0.008–$0.5\ \mu g\,mL^{-1}$), which is generally better than LVFX (MIC 0.125–$8\ \mu g\,mL^{-1}$) and similar to GMFX (MIC < 0.008–$4\ \mu g\,mL^{-1}$). Among them, compounds **16a**, **b**, **i**, **l**, **u**, and **z** (MIC < 0.008–$0.06\ \mu g\,mL^{-1}$) were found to be four- to 1024-fold more active against MSSE and MRSE than the two reference drugs (MIC 0.25–$8\ \mu g\,mL^{-1}$).

Compounds **16w**, **y**, and **z**, with better Gram-positive activity, were chosen for further evaluation of their in vitro activity against the clinical strains of MSSA, MRSA, MSSE, and MRSE. The MIC_{50} and MIC_{90} ranges for these compounds, along with those of LVFX and GMFX for comparison, are listed in Table 4. Against the four species of bacteria tested, the MIC_{50} (0.06–$16\ \mu g\,mL^{-1}$) and MIC_{90} values (0.5–$32\ \mu g\,mL^{-1}$) of **16w**, **y**, and **z** are 2–8- and 2–16-fold less than for LVFX (MIC_{50} 0.25–$32\ \mu g\,mL^{-1}$, MIC_{90} 8–$64\ \mu g\,mL^{-1}$), respectively, and compound **16w** was also found to have superior activity (MIC_{90} 0.5–$4\ \mu g\,mL^{-1}$) to that of GMFX (MIC_{90} 1–$8\ \mu g\,mL^{-1}$).

In the case of Gram-positive strains, the activity imparted to the 1-cyclopropyl-1,8-naphthyridone ring by the alkyl group of the oxime moiety is in the order: cyclobutyl $\geq$ cyclopentyl $\approx$ ethyl $\geq$ cyclopropylmethyl $>$ methyl $\geq$ benzyl $>$ 3′,4′-methylenedioxylbenzyl. However, different results were obtained in comparisons of the activity of the other naphthyridone or quinolone ring systems (Table 2).

Notably, the chirality at C2 of the azetidinyl group of the target compounds influences activity. For example, the *S* enantiomers **16g**, **i**, **q**, and **u** show better activity against Gram-positive strains than the corresponding *R* enantiomers **16h**, **j**, **r**, and **v** (Table 2), and similar results were also obtained in comparisons of the Gram-negative activity (Table 3). This suggests that the *S* enantiomers may fit better into the binding sites of

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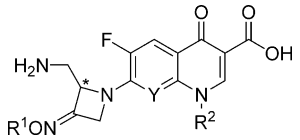
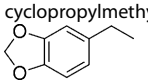
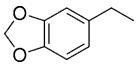
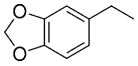
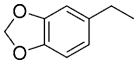
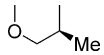
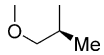
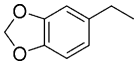
Table 1. Structures and physical data of compounds **16a–z**.



Compd	R^1	R^2	Y	*[a]	Ratio[b]	mp [°C][c]
16a	cyclobutyl	cyclopropyl	N	S	49:51	181–183
16b	cyclopentyl	cyclopropyl	N	S	60:40	157–158
16c	cyclohexyl	cyclopropyl	N	S	85:15	214–216
16d	cyclopropylmethyl	cyclopropyl	N	S	35:65	148–151
16e		cyclopropyl	N	S	45:55	135–137
16f	benzyl	cyclopropyl	N	S	37:63	137–138
16g	methyl	cyclopropyl	N	S	32:68	151–153
16h	methyl	cyclopropyl	N	R	45:55	171–173
16i	ethyl	cyclopropyl	N	S	50:50	170–172
16j	ethyl	cyclopropyl	N	R	20:80	168–170
16k	cyclobutyl	2,4-difluoro-phenyl	N	S	–	148–150
16l	cyclopentyl	2,4-difluoro-phenyl	N	S	50:50	178–180
16m	cyclopropylmethyl	2,4-difluoro-phenyl	N	S	48:52	173–174
16n		2,4-difluoro-phenyl	N	S	20:80	170–172
16o	benzyl	2,4-difluoro-phenyl	N	S	–	174–176
16p	methyl	2,4-difluoro-phenyl	N	S	45:55	193–194
16q	ethyl	2,4-difluoro-phenyl	N	S	–	182–184
16r	ethyl	2,4-difluoro-phenyl	N	R	48:52	183–185
16s		cyclopropyl	C-OMe	S	51:49	120–121
16t	methyl	cyclopropyl	C-OMe	S	51:49	118–120
16u	ethyl	cyclopropyl	C-OMe	S	43:57	108–110
16v	ethyl	cyclopropyl	C-OMe	R	37:63	110–112
16w				S	46:64	154–155
16x	methyl			S	20:80	204–206
16y		cyclopropyl	C-F	S	52:48	145–147
16z	methyl	ethyl	C-F	S	47:53	167–169

[a] Configuration. [b] Isomer ratios based on the ^{1}H NMR spectroscopic data of the hydrogen atom linked to the chiral center of the azetidine ring. [c] Melting points are uncorrected.

the target enzymes, causing more potent enzyme inhibition and hence more potent antibacterial activity.

Conclusions

In summary, a series of (*R*)/(*S*)-7-(3-alkoxyimino-2-aminomethyl-1-azetidinyl)fluoroquinolone derivatives were designed, synthesized, and characterized by ^{1}H NMR, MS, HRMS, and ^{13}C NMR. These derivatives were initially evaluated for their in vitro antibacterial activity; compounds **16w**, **y**, and **z** were then chosen for further evaluation of their activity against clinical isolates. Twelve of the target compounds generally show better activity (MIC $<$0.008–0.5 μg mL^{-1}) against Gram-positive strains including MRSA and MRSE than LVFX (MIC 0.125–8 μg mL^{-1}), and activity similar to GMFX (MIC $<$0.008–4 μg mL^{-1}), although they are generally less active than the two reference drugs against the Gram-negative strains. Against clinical strains of MSSA, MRSA, MSSE, and MRSE, compounds **16w**, **y**, and **z** are more active than LVFX, and **16w** (MIC_{90} 0.5–4 μg mL^{-1}) was also found to be more active than GMFX (MIC_{90} 1–8 μg mL^{-1}).

Experimental Section

Synthesis

Melting points were determined in open capillaries and are uncorrected. ^{1}H NMR (400, 500, or 600 MHz) and ^{13}C NMR (125 or 150 MHz) spectra were recorded at 25 °C on Varian Mercury spectrometers. Chemical shifts (δ) are given in ppm relative to tetramethylsilane or the respective NMR solvent. Electrospray ionization (ESI) mass spectra and high-resolution mass spectra (HRMS) were obtained on a MDSSCIEX Q-Tap mass spectrometer. The reagents were all of analytical grade or chemically pure. TLC was performed on silica gel plates (Merck, ART5554 60 F_{254}).

General procedure for the synthesis of 2a–e: To a stirring solution of 2-(hydroxy)isoindoline-1,3-dione (**1**, 48.94 g, 300 mmol) in DMF (500 mL) was added K_2CO_3 (82.93 g, 600 mmol), alkyl bromides (1200 mmol), and 18-crown-6 (15.85 g, 60 mmol) at room temperature. The reaction mixture was stirred at 80 °C for 6 h. Cooled to room temperature, the mixture was poured into distilled H_2O (1200 mL). The precipitate was filtered, washed with petroleum ether (PE) and dried in vacuo to yield the title compounds **2a–e**.

General procedure for the synthesis of 3a–e: To a stirring solution of **2a–e** (40 mmol) in CH_2Cl_2 (300 mL) and MeOH (30 mL) was added 80% $NH_2NH_2{\cdot}H_2O$ (5.0 mL, 80 mmol) at room temperature. The reaction mixture was stirred at the same temperature for 3 h and then filtered. The filtrate was washed with 10% $NH_{3(aq)}$. The organic layer was dried over anhydrous $MgSO_4$ and then filtered. The filtrate was concentrated under reduced pressure. The residue was dissolved in EtOH (60 mL), concentrated HCl (10 mL) was added, and the mixture was stirred for 1 h at room temperature. The mixture was concentrated under reduced pressure, and the crude product was recrystallized from Et_2O (100 mL) to afford the title compounds **3a–e**.

General procedure for the synthesis of (*S*)/(*R*)-6: To a solution of L/D-serine ((*S*)/(*R*)-**4**, 42.04 g, 400 mmol) in 25% NaOH (180 mL) was added a solution of $(Boc)_2O$ (130.95 g, 600 mmol) in dioxane (250 mL) at 0 °C. The reaction mixture was stirred for 12 h at room temperature, and the dioxane was removed under reduced pressure. The residue was diluted with ice water (1000 mL), adjusted to pH 2–3 with $KHSO_4$, and extracted with EtOAc. The combined extracts were dried over anhydrous $MgSO_4$ and then filtered. The filtrate was concentrated under reduced pressure to afford the crude product (*S*)/(*R*)-**5**, which was used directly in the next step without further purification.

To a solution of the above crude (*S*)/(*R*)-**5** and imidazole (108.93 g, 1600 mmol) in DMF (200 mL) was added dropwise *tert*-butylchlorodiphenylsilane (137.44 g, 500 mmol) over a period of 1 h at room

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Table 2. In vitro antibacterial activity of compounds **16a–z** against Gram-positive strains.

Compd	Strains MIC [μg mL^{-1}]														
	S.a.1[a]	S.a.2[b]	MSSA1[c]	MSSA2[d]	MRSA1[e]	MRSA2[f]	MSSE1[g]	MSSE2[h]	MRSE1[i]	MRSE2[j]	S.p.1[k]	S.p.2[l]	S.p.3[m]	S.p.4[n]	S.p.5[o]
16a	0.06	0.25	0.5	0.125	0.5	0.5	<0.008	<0.008	<0.008	<0.008	0.06	0.03	0.125	0.06	0.03
16b	0.125	0.25	0.25	0.25	0.5	0.5	<0.008	<0.008	<0.008	<0.008	0.5	0.03	0.25	0.5	0.03
16c	0.25	1	1	0.5	1	1	0.5	0.5	0.015	0.015	0.5	0.5	0.5	0.5	0.5
16d	0.06	0.125	0.25	0.25	0.25	0.25	0.25	0.25	<0.008	<0.008	0.25	0.25	0.25	0.25	0.25
16e	0.5	0.5	1	0.5	1	1	1	1	0.125	0.125	1	0.5	0.5	1	1
16f	0.125	0.25	0.25	0.125	0.25	0.25	0.5	0.5	8	8	0.25	0.5	0.125	0.25	0.25
16g	0.25	0.5	0.5	0.25	0.5	0.5	0.5	0.5	0.125	0.125	0.25	0.5	0.25	0.25	0.5
16h	0.06	8	8	4	8	8	8	8	8	8	2	4	4	4	8
16i	0.03	0.5	0.25	0.125	0.5	0.5	<0.008	<0.008	<0.008	<0.008	0.25	0.06	0.125	0.25	0.06
16j	0.015	4	8	4	8	8	0.25	0.25	0.25	0.25	0.5	0.25	4	1	0.25
16k	0.25	0.5	0.5	0.125	0.5	0.5	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
16l	0.125	0.5	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
16m	0.25	0.5	0.25	0.25	0.25	0.25	1	1	1	1	0.25	1	0.25	0.25	1
16n	0.125	0.5	0.25	0.125	0.5	0.5	0.25	0.25	0.5	0.5	0.25	0.25	0.125	0.25	0.25
16o	0.5	1	0.125	0.03	0.125	0.125	1	1	1	1	0.125	0.5	0.03	0.125	1
16p	0.015	0.06	0.25	0.25	0.25	0.25	1	1	1	1	0.125	1	0.25	0.25	1
16q	0.25	0.5	1	0.5	1	1	1	1	<0.008	<0.008	0.5	0.5	0.5	1	1
16r	0.125	0.5	1	0.25	1	1	2	2	2	2	0.5	1	0.25	0.5	2
16s	0.25	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1	1	0.5	0.5	0.5	0.5	0.5
16t	0.25	0.25	0.125	0.06	0.25	0.25	0.25	0.25	4	4	0.125	0.25	0.06	0.25	0.25
16u	0.125	0.25	0.25	0.25	0.25	0.25	0.06	0.06	0.06	0.06	0.25	0.06	0.25	0.25	0.06
16v	2	4	8	8	8	8	2	2	0.125	0.125	4	1	4	4	2
16w	0.015	0.06	0.06	0.06	0.06	0.06	0.5	0.5	0.06	0.06	0.5	0.5	0.06	0.5	0.5
16x	0.06	0.25	0.25	0.125	0.25	0.25	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
16y	0.06	0.125	0.06	0.06	0.25	0.25	0.125	0.125	2	2	0.125	0.125	0.06	0.125	0.125
16z	0.125	0.25	0.06	0.03	0.06	0.06	0.06	0.06	0.03	0.03	0.06	0.06	0.03	0.06	0.06
GMFX[p]	<0.008	0.015	0.03	0.03	0.03	0.03	0.25	0.25	4	4	0.06	0.25	0.03	0.06	0.25
LVFX[q]	0.125	0.25	0.25	0.125	0.25	0.25	0.25	0.25	8	8	0.25	0.25	0.125	0.25	0.25

[a] *S. aureus* ATCC25923. [b] *S. aureus* ATCC43300. [c] Methicillin-sensitive *S. aureus* 12-1. [d] Methicillin-sensitive *S. aureus* 12-4. [e] Methicillin-resistant *S. aureus* 12-3. [f] Methicillin-resistant *S. aureus* 12-15. [g] Methicillin-sensitive *S. epidermidis* 12-2. [h] Methicillin-sensitive *S. epidermidis* 12-22. [i] Methicillin-resistant *S. epidermidis* 12-2. [j] Methicillin-resistant *S. epidermidis* 12-33. [k] *Streptococcus pneumoniae* 12-1. [l] *S. pneumoniae* 12-2. [m] *S. pneumoniae* 12-3. [n] *S. pneumoniae* 12-4. [o] *S. pneumoniae* 12-5. [p] Gemifloxacin. [q] Levofloxacin.

temperature. The reaction mixture was stirred for 7 h at the same temperature, diluted with distilled H_2O (300 mL), and adjusted to pH 2–3 with 3 N citric acid. The mixture was extracted with EtOAc, and the combined extracts were washed with saturated brine, dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure. The crude product was recrystallized from a mixed solvent of EtOAc and PE (1:5, *v*/*v*) to afford the title compound (*S*)/(*R*)-**6**.

General procedure for the synthesis of (*S*)/(*R*)-7: To a stirring solution of (*S*)/(*R*)-**6** (17.72 g, 40 mmol) and isobutyl chloroformate (6.83 g, 50 mmol) in anhydrous THF (100 mL) was added dropwise NMM (5.06 g, 50 mmol) over a period of 10 min at −25 °C. The reaction mixture was stirred for 30 min at the same temperature, and a solution of freshly prepared diazomethane in Et_2O (150 mL, 100 mmol) was added dropwise over a period of 1 h. The mixture was stirred for 8 h at room temperature and concentrated under reduced pressure. The residue was treated with PE (300 mL) at −30 °C and filtered. The precipitate was washed with PE and dried in vacuo to yield the title compound (*S*)/(*R*)-**7**.

General procedure for the synthesis of (*S*)/(*R*)-8: To a solution of (*S*)/(*R*)-**7** (18.68 g, 40 mmol) in anhydrous CH_2Cl_2 was added $Rh_2(OAc)_4$ (0.18 g, 0.4 mmol) at −78 °C. The reaction mixture was stirred for 4 h at the same temperature and overnight at room temperature, then filtered. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (silica gel), eluting with PE and EtOAc (8:1, *v*/*v*) to give the title compound (*S*)/(*R*)-**8**.

General procedure for the synthesis of (*R*)-9a–h/(*S*)-9g–h: To a stirring solution of (*S*)/(*R*)-**8** (21.95 g, 50 mmol) in EtOH was added pyridine and the appropriate *O*-alkylhydroxylamine hydrochloride (60 mmol). The reaction mixture was stirred at 60 °C for 5 h and concentrated under reduced pressure. The residue was treated with EtOAc and washed successively with H_2O and saturated saline, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The crude products (*R*)-**9a–d**, **h** and (*S*)-**9g** were used directly in the next step, whereas (*R*)-**9e–g** and (*S*)-**9h** were purified by column chromatography (silica gel), eluting with PE and EtOAc (10:1, *v*/*v*).

General procedure for the synthesis of (*R*)-**10a–h/**(*S*)-**10g,h**: To a stirring solution of (*R*)-**9a–h**/(*S*)-**9g,h** (40 mmol) in THF (300 mL) was added TBAF (60 mmol, 60 mL 1.0 N solution in THF) at room temperature. The reaction mixture was stirred for 10 h at the same temperature, and concentrated under reduced pressure. The residue was treated with EtOAc, and washed successively with H_2O and saturated saline, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure. The crude products were purified by column chromatography (silica gel), eluting with PE and EtOAc (3:2, *v*/*v*) to afford the title compounds (*R*)-**10a–h**/(*S*)-**10g,h**.

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Table 3. In vitro antibacterial activity of compounds **16a–z** against Gram-negative strains.

Compd	Strains MIC [μg mL^{-1}]														
	P.a.1[a]	P.a.2[b]	P.a.3[c]	P.a.4[d]	P.a.5[e]	E.co.1[f]	E.co.2[g]	E.co.3[h]	E.co.4[i]	E.co.5[j]	E.co.6[k]	K.p.1[l]	K.p.2[m]	K.p.3[n]	K.p.4[o]
16a	8	8	16	16	16	0.125	4	128	128	128	4	0.25	0.25	8	8
16b	4	2	16	16	16	0.25	4	>128	>128	>128	2	0.5	0.5	16	16
16c	16	8	8	8	8	1	16	>128	>128	128	16	4	2	32	32
16d	2	4	4	4	4	0.03	4	128	128	>128	4	0.125	0.125	8	8
16e	8	8	8	8	8	0.5	4	>128	>128	>128	1	8	1	16	16
16f	4	8	16	16	16	0.125	8	>128	>128	>128	8	1	1	16	16
16g	2	2	16	0.5	16	0.03	1	32	128	64	32	0.25	0.25	8	8
16h	32	32	32	32	32	1	16	>128	>128	>128	16	4	4	64	32
16i	4	8	16	16	16	<0.008	1	64	>128	64	8	0.015	0.015	8	8
16j	64	16	8	16	16	1	16	>128	>128	16	32	4	4	64	64
16k	8	16	16	16	16	2	16	>128	>128	>128	8	2	2	32	32
16l	16	8	8	8	8	2	8	>128	>128	>128	>128	4	4	64	>128
16m	8	8	16	16	16	1	8	>128	>128	>128	2	8	2	32	16
16n	8	16	16	16	16	2	16	>128	>128	>128	16	4	4	32	32
16o	16	32	32	64	64	2	16	>128	>128	>128	>128	4	4	64	64
16p	0.5	8	8	16	16	0.015	1	64	>128	0.06	8	0.06	0.06	8	8
16q	4	16	8	16	16	0.5	8	>128	>128	>128	>128	1	1	32	64
16r	8	16	16	16	16	1	8	>128	>128	>128	8	2	2	64	128
16s	4	16	16	16	16	2	16	>128	>128	128	64	4	4	4	32
16t	8	16	16	16	16	0.25	4	64	128	64	16	1	0.5	32	32
16u	8	16	16	16	16	0.5	4	>128	>128	128	2	8	2	8	8
16v	64	8	4	8	16	4	32	>128	128	128	32	8	8	>128	128
16w	1	4	4	4	4	0.125	0.5	64	64	64	0.5	0.5	0.25	4	2
16x	2	4	16	16	16	0.125	1	64	>128	>128	16	1	1	16	32
16y	4	16	8	16	16	0.25	4	128	128	>128	32	1	1	16	16
16z	2	2	16	16	16	0.06	1	64	64	16	16	0.25	0.25	16	16
GMFX[p]	1	8	16	16	16	0.015	0.125	32	16	32	1	0.06	0.06	2	2
LVFX[q]	1	2	8	8	8	0.03	0.5	8	16	16	0.5	0.125	0.125	4	4

[a] *Pseudomonas aeruginosa* ATCC27853. [b] *P. aeruginosa* 12-1. [c] *P. aeruginosa* 12-2. [d] *P. aeruginosa* 12-4. [e] *P. aeruginosa* 12-5. [f] *E. coli* ATCC25922. [g] *E. coli* 12-1. [h] *E. coli* 12-3. [i] Extended-spectrum β-lactamase-producing (ESBL$^+$) *E. coli* 12-1. [j] ESBL$^+$ *E. coli* 12-3. [k] ESBL$^+$ *E. coli* 12-4. [l] *Klebsiella pneumoniae* 12-1. [m] *K. pneumoniae* 12-2. [n] ESBL$^+$ *K. pneumoniae* 12-2. [o] ESBL$^+$ *K. pneumoniae* 12-4. [p] Gemifloxacin. [q] Levofloxacin.

Table 4. Comparative in vitro antibacterial activity of **16w**, **16y**, and **16z** against clinical isolates.

Microorganism	Compd	MIC [μg mL^{-1}]		
		Range	MIC_{50}	MIC_{90}
MSSA[a]	**16w**	0.06–2	0.06	2
	16y	0.06–8	0.125	8
	16z	0.03–16	0.06	16
	GMFX[e]	0.03–4	0.03	4
	LVFX[f]	0.125–32	0.25	32
MRSA[b]	**16w**	0.06–8	2	4
	16y	0.25–16	8	16
	16z	0.06–32	16	32
	GMFX	0.03–8	8	8
	LVFX	0.25–64	32	64
MSSE[c]	**16w**	0.5	0.5	0.5
	16y	0.125–2	0.5	2
	16z	0.06–2	0.125	2
	GMFX	0.06–1	0.25	1
	LVFX	0.25–8	0.5	8
MRSE[d]	**16w**	0.06–0.5	0.5	0.5
	16y	0.06–4	2	4
	16z	0.03–8	2	8
	GMFX	0.015–4	0.5	1
	LVFX	0.125–8	4	8

[a] Methicillin-sensitive *S. aureus*; 10 isolates. [b] Methicillin-resistant *S. aureus*; 10 isolates. [c] Methicillin-sensitive *S. epidermidis*; 10 isolates. [d] Methicillin-resistant *S. epidermidis*; 10 isolates. [e] Gemifloxacin. [f] Levofloxacin.

General procedure for the synthesis of (*S*)-11a–h/(*R*)-11g–h: To a mixture of (*R*)-**10a-h**/(*S*)-**10g,h** (40 mmol), PPh_3 (20.98 g, 80 mmol) and phthalimide (11.76 g, 80 mmol) in anhydrous THF (300 mL) was added dropwise diethyl azodicarboxylate (13.93 g, 80 mmol) at room temperature over a period of 10 min. The reaction mixture was stirred for 12 h at the same temperature and concentrated under reduced pressure. The residue was treated with EtOAc, washed successively with H_2O and saturated saline, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure, and the residue was treated with PE at 0 °C. The precipitate was filtered, and the filtrate was concentrated under reduced pressure. The crude products (*S*)-**11a** and (*R*)-**11g,h** were used directly in the next step, whereas (*S*)-**11b–h** were purified by column chromatography (silica gel), eluting with PE and EtOAc (3:1, *v/v*).

General procedure for the synthesis of (*S*)-13a–h/(*R*)-13g–h: A mixture of (*S*)-**11a–h**/(*R*)-**11g–h** (20 mmol) and 80% $NH_2NH_2{\cdot}H_2O$ (1.88 mL, 30 mmol) in EtOH (150 mL) was heated at reflux and stirred for 3 h at the same temperature. The precipitate was filtered, and the filtrate was concentrated under reduced pressure. The residue was treated with EtOAc, and washed successively with H_2O and saturated saline, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure to give the crude products (*S*)-**12a–h**/(*R*)-**12g–h** as light-yellow oils, which were used directly in the next step.

To a mixture of the above crude (*S*)-**12a–h**/(*R*)-**12g,h** in EtOH (50 mL) was added $(Boc)_2O$ (6.55 g, 30 mmol) at room temperature.

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The reaction mixture was stirred overnight at the same temperature, and concentrated under reduced pressure. The crude product (*S*)-**13a** was used directly in the next step, whereas (*S*)-**13b–h** and (*R*)-**13g,h** were purified by column chromatography (silica gel), eluting with PE and EtOAc (3:1, *v/v*).

General procedure for the synthesis of (*S*)-14a–h/(*R*)-14g,h: To a stirring solution of (*S*)-**13a–h**/(*R*)-**13g,h** (10 mmol) in CH_2Cl_2 (30 mL) was pumped dried $HCl_{(g)}$ for 1 h at 0–5 °C. The reaction mixture was allowed to stir for another 30 min at room temperature, and the precipitate was collected by suction to give the title compounds (*S*)-**14a–h**/(*R*)-**14g,h**.

General procedure for the synthesis of the target compounds 16a–r: To a solution of (*S*)-**14a–h**/(*R*)-**14g,h** (1 mmol) and Et_3N (3 mmol) in anhydrous MeCN (10 mL) was added **15a,b** (0.8 mmol) at room temperature. The reaction mixture was stirred for 5 h at 50 °C, and concentrated under reduced pressure. The residue was dissolved in 20% AcOH (10 mL), stirred for 0.5 h at 50 °C, and filtered. The filtrate was adjusted to pH 6.5–7.5 by 15% NaOH and extracted by CH_2Cl_2. The combined extracts were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel), eluting with CH_2Cl_2 and MeOH (10:1, *v/v*) to afford the title compounds **16a–r**.

General procedure for the synthesis of target compounds 16s–z: To a solution of (*S*)-**14e,g,h**/(*R*)-**14h** (1 mmol) and Et_3N (3 mmol) in MeCN (10 mL) was added **15c–f** (0.8 mmol) at room temperature. The reaction mixture was stirred overnight at 50 °C, and concentrated under reduced pressure. The residue was dissolved in a solution of 5% NaOH (8 mL) and stirred for 1 h at 50 °C. After cooling to room temperature, the mixture was adjusted to pH 7.0–7.5 with 5% AcOH, and extracted with CH_2Cl_2. The combined extracts were concentrated under reduced pressure. The residue was dissolved in 20% AcOH (10 mL), stirred for 0.5 h at 50 °C, and filtered. The filtrate was adjusted to pH 6.5–7.5 by 15% NaOH and extracted by CH_2Cl_2. The combined extracts were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel), eluting with CH_2Cl_2 and MeOH (10:1, *v/v*) to afford the title compounds **16s–z**.

MIC determination

All the target compounds **16a–z** were screened for their in vitro antibacterial activity against representative Gram-positive and Gram-negative strains by using a standard twofold serial dilution method with agar media.[21] Minimum inhibitory concentration (MIC) is defined as the minimum concentration of the compound required to give complete inhibition of bacterial growth after incubation at 35 °C for 18–24 h.

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Keywords: antibiotics · chirality · drug design · medicinal chemistry · quinolones

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[21] MIC values were determined as described by the NCCLS (see: National Committee for Clinical Laboratory Standards, Performance Standards for Antimicrobial Susceptibility Testing: 11th Informational Supplement, Vol. 21, NCCLS, Wayne, PA (USA), 2001, M100–S11). MIC was defined as the lowest concentration of each compound that inhibits visible growth of bacteria after incubation at 35 °C for 18–24 h.

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