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Synthesis and antibacterial activity of 7-(1,2,3,4-tetrahydropyrrolo[1,2-*a*]-pyrazin-7-yl) quinolones

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ABSTRACT

A novel series of 7-(1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazin-7-yl) quinolones has been designed and synthesized in which the heterocyclic side chain is attached to the quinolone core through a carbon–carbon linkage. The antibacterial activity of the compounds was determined against a panel of Gram-positive and Gram-negative pathogens. Compounds **1b** and **1e**, bearing an 8-methoxy group as well as unsubstituted and (3*S*)-methyl substituted 1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazin-7-yl side chains, respectively, demonstrated notable activity against ciprofloxacin-resistant clinical isolates of *Streptococcus pneumoniae*.

Since the discovery of nalidixic acid[1] over four decades ago, the quinolones have evolved into an important class of antibacterial agents.[2] Several of the quinolones, including ciprofloxacin[3] and levofloxacin,[4] are widely used in the clinic to treat multiple bacterial infections. The quinolones act against bacteria by selectively inhibiting the type II topoisomerases DNA gyrase and topoisomerase IV, enzymes that play a critical role in bacterial cell growth and division.[2c] However, extensive clinical use of these agents has led to increasing bacterial resistance to the available quinolones.[2d] Thus, it is a continuing challenge to discover and develop newer quinolone antibacterial agents with activity against resistant organisms, including the respiratory pathogen, *Streptococcus pneumoniae*.

The general chemical structure of the quinolone antibacterial agents consists of a 4-quinolone/naphthyridone-3-carboxylic acid nucleus and a nitrogen-containing side chain, which is attached to the C7 position of the heterocyclic core. While the side chain is typically attached to the quinolone core through a nitrogen atom, there have been a limited number of examples wherein a carbon–carbon linkage was utilized.[5] One of the most noteworthy examples is garenoxacin[6] (Fig. 1), which exhibits a broad spectrum antibacterial profile and is among the newer quinolones to have advanced into late stage clinical trials.

Investigating quinolone analogs with a carbon–carbon linkage between the quinolone core and the C7 side chain is one of the strategies we employed in the search for new and more effective quinolone antibacterial agents. Herein we report the synthesis and antibacterial activity of a series of 7-(1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazin-7-yl) quinolones **1** (Fig. 1).

The general synthetic route to the 7-(1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazin-7-yl) quinolones is outlined in Scheme 1. The key

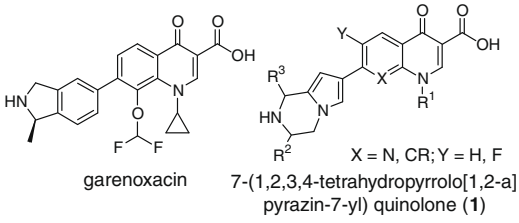
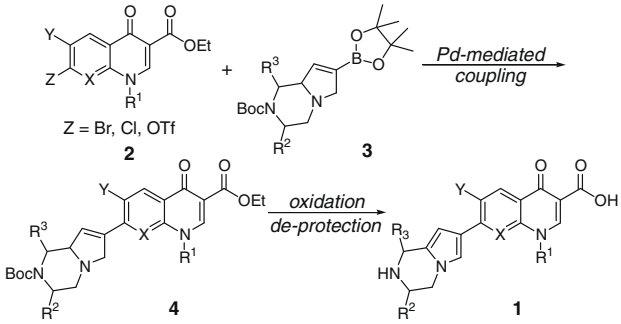


Figure 1.



Scheme 1.

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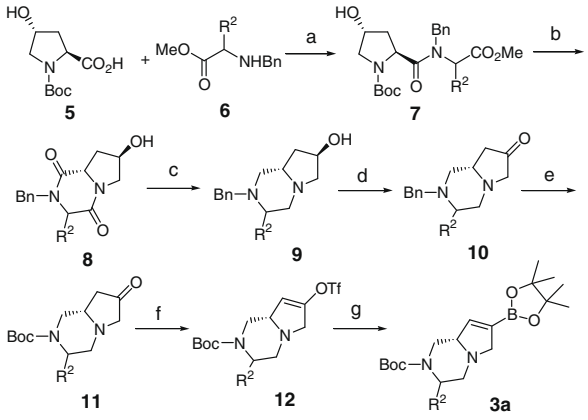
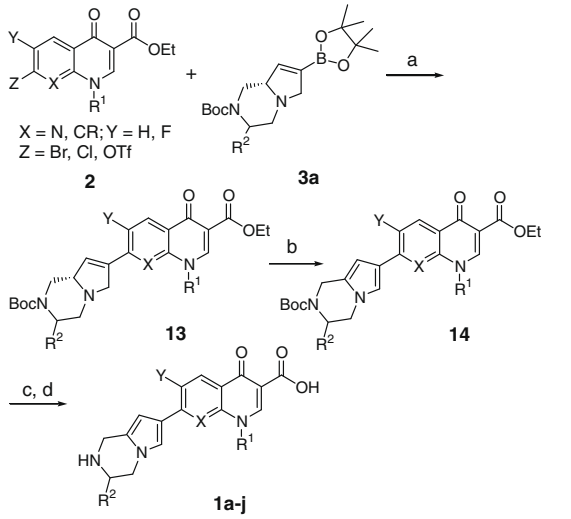


Scheme 2. Reagents and conditions: (a) DCC, CH_2Cl_2; (b) (I) CF_3CO_2H, CH_2Cl_2; (II) aq $NaHCO_3$, THF, rt; (c) $LiAlH_4$, THF, reflux; (d) EDCI, DMSO, PyTFA, CH_2Cl_2; (e) (I) $CH_3CH(Cl)OC(O)Cl$, CH_3CN; (II) aq $NaHCO_3$, THF rt; (III) Boc_2O, THF; (f) $KN(SiMe_3)_2$, $PhNTf_2$, THF, −78 °C to 0 °C; (g) bis(pinacolato)diboron, $Pd(PPh_3)_2Cl_2$, PPh_3, KOPh, toluene, 55 °C.

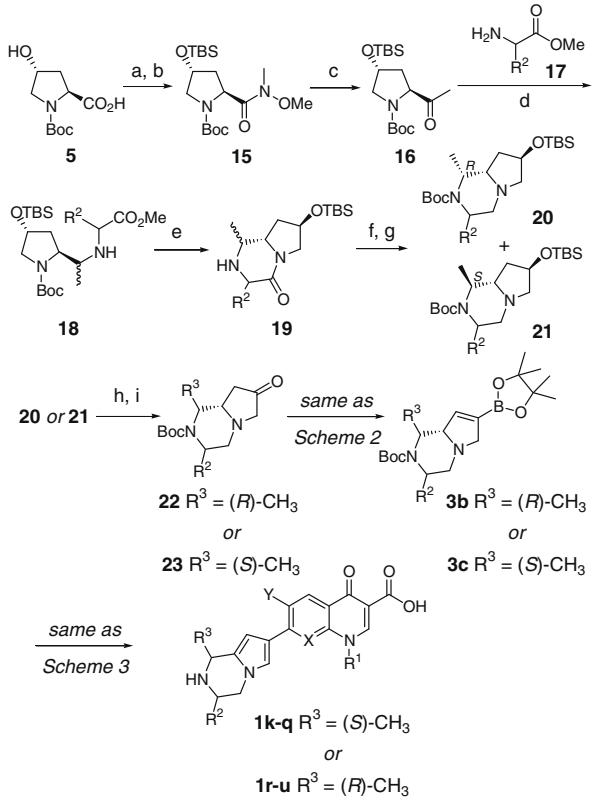


Scheme 3. Reagents and conditions: (a) $Pd(PPh_3)_4$, CsF, THF, reflux; (b) Pd/C, air, MeOH, rt; (c) aq 1 N NaOH, MeOH, THF; (d) 4 N HCl in dioxane, CH_2Cl_2.

step is a palladium-mediated coupling reaction between 7-halo/7-trifloxy-quinolone-3-carboxylic ester **2** and 1,2,3,4,6,8a-hexahydropyrrolo[1,2-*a*]pyrazin-7-yl boronic ester **3** to form the carbon–carbon bond between the quinolone core and the side chain. The resulting 7-(1,2,3,4,6,8a-hexahydropyrrolo[1,2-*a*]pyrazin-7-yl)-quinolone-3-carboxylic ester **4** could then be oxidized and deprotected to give the 7-(1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazin-7-yl) quinolone **1**.

Inspired by the side-chain structure of garenoxacin, we focused primarily on the unsubstituted 1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine side chain and those with small R^2 substituents, such as a methyl group. The synthesis of side-chain intermediate **3a** is illustrated in Scheme 2. Commercially available (4*R*)-hydroxy-*N*-Boc-L-proline **5** was coupled with *N*-benzyl-glycine (R^2 = H) methyl ester or *N*-benzyl-alanine [R^2 = (*S*)-CH_3 or (*R*)-CH_3] methyl ester under standard peptide coupling conditions [*N*,*N*′-dicyclohexylcarbodiimide (DCC), CH_2Cl_2] to give amide **7**. The Boc protecting group of **7** was removed by treatment with trifluoroacetic acid, and the resulting amine was cyclized under basic conditions (aq $NaHCO_3$, THF) to give the bicyclic di-amide compound **8**. Both amide groups of **8** were then reduced by treatment with $LiAlH_4$ to give compound **9**, the hydroxyl of which was then oxidized to give the corresponding ketone **10** using a modified Pfitzner–Moffat procedure [*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI), DMSO, pyridinium trifluoroacetate].[7] The benzyl protecting group of **10** was removed by treatment with 1-chloroethyl chloroformate, and the resulting secondary amine was re-protected with a Boc group to give compound **11**. Compound **11** was reacted with potassium bis(trimethylsilyl)amide at −78 °C, followed by addition of *N*-phenyl-bis(trifluoromethane-sulfonimide) ($PhNTf_2$) to convert the ketone to vinyl triflate **12**. Transforming the vinyl triflate group of **12** to a boronic ester group proved to be a challenging task, mainly due to the ease with which the pyrroline was oxidized to the pyrrole. Gratifyingly, after a number of attempts, the desired boronic ester **3a** was prepared by modifying a procedure reported by Miyaura et al.[8] Vinyl triflate **12** was reacted with bis(pinacolato)diboron under $Pd(PPh_3)_2Cl_2$ catalysis in the presence of potassium phenoxide in toluene at elevated temperature (55 °C) to give vinyl boronic ester **3a**.

The coupling of side-chain intermediate **3a** and quinolone core **2**[9] was catalyzed by $Pd(PPh_3)_4$, in the presence of CsF in refluxing THF to give 7-(1,2,3,4,6,8a-hexahydropyrrolo[1,2-*a*]pyrazin-7-yl) quinolone **13**, which was then subjected to Pd/C catalyzed air-oxidation to yield the corresponding 7-(1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazin-7-yl) quinolone **14**. Hydrolysis under basic conditions (aq NaOH, MeOH, THF) converted the ethyl ester of **14** to the carboxylic acid. Finally, deprotection of the side chain amine under acidic condition (4 N HCl in dioxane, CH_2Cl_2) led to 7-(1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazin-7-yl) quinolones **1a–j** (Scheme 3).

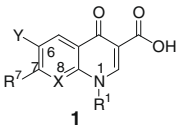


Scheme 4. Reagents and conditions: (a) $CH_3NH(OCH_3)$ HCl, Et_3N, DCC, CH_2Cl_2; (b) TBSCl imidazole, DMF, rt; (c) CH_3MgBr, THF, 0 °C to rt; (d) $NaCNBH_3$, HOAc, MeOH, rt; (e) (I) CF_3CO_2H, CH_2Cl_2; (II) aq $NaHCO_3$, THF; (f) $LiAlH_4$, THF, rt; (g) (I) Boc_2O, THF; (II) chromatography separation; (h) HF (aq 48%), CH_3CN; (i) EDCI, DMSO, PyTFA, CH_2Cl_2.

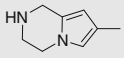
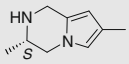
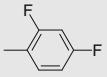
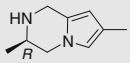
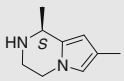
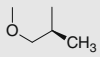
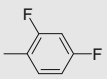
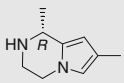
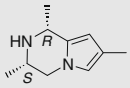
Scheme 4 outlines the synthesis of side-chain intermediates **3b** and **3c**. (4*R*)-Hydroxy-*N*-Boc-L-proline **5** was reacted with *N*,*O*-dim-

ethylhydroxylamine hydrochloride in the presence of Et_3N and *N*,*N*′-dicyclohexylcarbodiimide (DCC) to form the Weinreb amide. The hydroxy group was then protected as a *t*-butyldimethylsilyl ether (TBSCl, imidazole, DMF) to give compound **15**. The Weinreb

Table 1
In vitro antibacterial activity of 7-(1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazin-7-yl) quinolones **1**



1

Compd	Y	R^7	X	R^1	MIC (μg/ml)					
					A	**B**	**C**	**D**	**E**	**F**
Cipro	F		C–H		0.12	1	>16	>16	>16	0.03
1a	H		C–OCHF$_2$		0.06	0.5	1	8	N/A	0.25
1b	F		C–OCH$_3$		0.03	0.25	1	1	1	0.12
1c	F				0.5	2	>16	>16	>16	1
1d	H		C–OCHF$_2$		0.12	1	1	8	1	1
1e	F		C–OCH$_3$		0.03	0.12	0.5	1	0.5	0.25
1f	F		CH		0.06	0.5	2	4	2	0.12
1g	F				0.25	1	4	8	4	0.5
1h	F		N		0.06	0.5	2	8	8	0.25
1i	F		N		0.12	1	16	16	16	2
1j	F		C–OCH$_3$		0.03	0.25	1	2	1	0.5
1k	H		C–OCHF$_2$		0.5	1	4	16	4	2
1l	F		C–OCHF$_2$		0.25	2	1	8	2	2
1m	F		C–OCH$_3$		0.12	0.5	2	4	1	0.5
1n	F		CH		0.25	1	4	16	4	0.5
1o	F				0.25	1	4	8	4	0.5
1p	F		N		0.5	1	8	8	8	1
1q	F		N		1	4	>16	>16	>16	8
1r	H		C–OCHF$_2$		0.5	1	4	16	8	4
1s	F		C–OCH$_3$		0.25	2	8	16	>16	2
1t	F		CH		0.25	1	4	16	8	0.25
1u	F		C–OCH$_3$		0.12	1	2	8	N/A	1

(**A**) *Staphylococcus aureus subsp. aureus* ATCC13709; (**B**) *Streptococcus pneumoniae* ATCC49619; (**C**) *Streptococcus pneumoniae* OC5462; (**D**) *Streptococcus pneumoniae* OC5458; (**E**) *Streptococcus pneumoniae* OC5465; (**F**) *Escherichia coli* ATCC25922. Strains **C**, **D**, and **E** are ciprofloxacin-resistant clinical isolates of *Streptococcus pneumoniae* that contain different representative constellations of amino acid substitutions in the QRDR regions of DNA gyrase and DNA topoisomerase IV.

amide was converted to the methyl ketone by treatment with CH_3MgBr. The resulting ketone **16** was subjected to a reductive amination reaction with glycine methyl ester (R^2 = H) or L-alanine methyl ester [R^2 = (*S*)-CH_3] in the presence of $NaCNBH_3$ to give compound **18** as a mixture of two diastereomers. Removal of the Boc group using trifluoroacetic acid, followed by cyclization under basic conditions (aq $NaHCO_3$, THF) afforded the cyclized amide **19**. The amide group was reduced to a tertiary amine by treatment with $LiAlH_4$. Protecting the secondary amine with a Boc group gave a mixture of two diastereomers **20** and **21**, which could be easily separated by standard chromatography. The TBS protecting group of **20** and **21** was removed by treatment with aq HF in CH_3CN, and the resulting hydroxyl was oxidized to the corresponding ketones **22** and **23**, respectively.

Compounds **22** and **23** were first converted to boronic esters **3b** and **3c**, which were then coupled with the quinolone derivative **2.** The final products **1k–q** and **1r–u** were obtained after oxidation, hydrolysis and de-protection under the same conditions as those described in Scheme 3.

The in vitro antibacterial activity of the 7-(1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazin-7-yl) quinolones **1** was determined against both ciprofloxacin-susceptible and ciprofloxacin-resistant bacteria. Data for representative compounds, along with ciprofloxacin, are presented in Table 1 as the minimum inhibitory concentration (MIC; lowest concentration of compound inhibiting visible growth)[10] against an abbreviated panel of six bacterial strains, including ciprofloxacin-susceptible *Staphylococcus aureus* subsp. *aureus* ATCC13709 (**A**), *S. pneumoniae* ATCC49619 (**B**), and *Escherichia coli* ATCC25922 (**F**), as well as ciprofloxacin-resistant clinical isolates of *S. pneumoniae* OC5462 (**C**), OC5458 (**D**) and OC5465 (**E**).

Compared to ciprofloxacin, the 7-(1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazin-7-yl) quinolones listed in Table 1 showed similar in vitro activity against the ciprofloxacin-susceptible Gram-positive bacteria, *S. aureus subsp. aureus* ATCC13709 (**A**) and *S. pneumoniae* ATCC49619 (**B**), whereas they were generally less active against the ciprofloxacin-susceptible Gram-negative bacteria, *E. coli* ATCC25922 (**F**). In contrast, the majority of the 7-(1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazin-7-yl) quinolones exhibited lower MIC values against all or some of the three ciprofloxacin-resistant *S. pneumoniae* strains **C**, **D** and **E** than ciprofloxacin. The data indicated that substituents at the 1- and 8-positions of the quinolone core as well as the C7 side chain all played crucial roles in determining the antibacterial activity of the corresponding quinolone compounds. Among various 1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine side chains investigated, the combination of a C-8 methoxy group (X = C-OCH_3) and a N-1 cyclopropyl group (R_1 = cyclopropyl) generally led to analogs with better antibacterial activity, as demonstrated by compounds **1b**, **1e**, and **1m**. For methyl substituted 1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine side chains, the position of the methyl group on the tetrahydropyrazine ring was important (**1d**–**1i** vs **1k**, **1m**–**1q**), as well as the stereochemistry (**1e** vs **1j**; **1m** vs **1s**). The (*S*)-configuration of the methyl group seemed to be preferred. Accordingly, incorporation of a second methyl group of the (*R*)-configuration into the tetrahydropyrazine ring did not improve antibacterial activity (**1u** vs **1e**). The unsubstituted 1,2,3,4,-tetrahydropyrrolo[1,2-*a*]pyrazine (**1a**–**1c**) and the (*S*)-methyl substituted 1,2,3,4,-tetrahydropyrrolo[1,2-*a*]pyrazine (**1d**–**1i**) side chains are apparently optimal for this series. In fact, among the 7-(1,2,3,4,-tetrahydropyrrolo[1,2-*a*]pyrazin-7-yl) quinolones evaluated, **1b** and **1e** are the most potent compounds in terms of in vitro antibacterial activity against ciprofloxacin-susceptible and ciprofloxacin-resistant strains.

Table 2
In vivo efficacy of **1e** in the *S. aureus* murine lethal systemic infection model

Compd	ED_{50} (mg/kg)	
	Oral	Subcutaneous
Cipro	10.8	1.4
1e	4.0	<1.25

Compound **1e** was selected for further evaluation in a murine lethal systemic infection model (*S. aureus* subsp. *aureus* ATCC13709),[11] and exhibited more potent in vivo efficacy (oral ED_{50} = 4.0 mg/kg; subcutaneous ED_{50} <1.25 mg/kg) than ciprofloxacin (Table 2).

In summary, we have designed and synthesized a series of novel 7-(1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazin-7-yl) quinolones with a carbon–carbon linkage between the quinolone nucleus and the C7 side chain. The antibacterial activity of these compounds was evaluated against ciprofloxacin-susceptible and ciprofloxacin-resistant bacteria. One of the best analogs of this series (compound **1e**) also demonstrated good in vivo efficacy. Further studies of the antibacterial activity of this series of quinolone compounds will be reported in the future.

Acknowledgment

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11. The in vivo efficacies of compound **1e** and ciprofloxacin were tested in female Swiss-Webster mice infected ip with *Staphylococcus aureus* subsp. *aureus* ATCC13709. Mice were dosed subcutaneously and orally with test compound at 1 h following infection. Mortality was observed over a period of three days. A group of infected mice not treated with drug served as controls.