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METHYLOXIME-SUBSTITUTED AMINOPYRROLIDINE : A NEW SURROGATE FOR 7-BASIC GROUP OF QUINOLONE

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Abstract: Novel fluoroquinolones containing oxime functionalized aminopyrrolidines have been synthesized. They were found to possess potent antibacterial activities against both Gram-negative and Gram-positive organisms, including methicillin resistant *Staphylococcus aureus* (MRSA). Among these compounds, LB20277 (compound **12**) showed the most favorable *in vivo* efficacy and pharmacokinetic profile in animals. Based on these promising results, LB20277 was selected as a candidate for further evaluation.

After the discovery of prototypic norfloxacin, most of the research concerning quinolone antibacterials has been focused on the basic group at the C-7 position. As a results, ciprofloxacin, ofloxacin, lomefloxacin, fleroxacin, and sparfloxacin have been successfully introduced into the market, all of which contain a piperazine derivative at the C-7 position.[2] In 1986, Warner-Lambert reported that this piperazine group has been successfully replaced with 3-(aminomethyl)pyrrolidine.[3] The introduction of this pyrrolidine derivative to the quinolone nucleus resulted in a dramatic improvement of *in vitro* Gram-positive activity compared to piperazinyl analogues. This change, however, also gave rise to undesirable side effects such as cytotoxicity.[4] In order to circumvent these problems, additional attempts have been made to modify the pyrrolidinyl moiety.[5] Nevertheless, so far no quinolone antimicrobial agent possessing a pyrrolidine derivative has been approved on a worldwide basis.[6]

Most of the quinolones currently on the market or under development have only moderate activity against many Gram-positive cocci including *staphylococci* and *streptococci.* This insufficient activity has not only limited their use in infections caused by these organisms, such as respiratory tract infections, but has also been believed to be one of the reasons for the rapidly developing quinolone resistance. Therefore, recent efforts have been directed toward the synthesis of new quinolones that can provide improved Gram-positive antibacterial activity, while retaining the good Gram-negative activity of ciprofloxacin.[7]

In this paper, we wish to describe the design and synthesis of new pyrrolidine derivatives and a series of fluoroquinolone compounds derivatized by these amines at the 7-position. These amines are structurally unprecedented, having an alkyloxime group on the 4-position and an amino substituent on the 3 position of the pyrrolidine ring. We also report herein these new quinolones' excellent antibacterial activity profiles, Structure-Activity Relationship (SAR) and pharmacokinetic data in animals.

The synthesis of novel pyrrolidine derivatives is outlined in **Scheme I.** *N*-BOC-3-pyrroline **1** was reacted with 1.2 equivalents of *m*-chloroperoxybenzoic acid (MCPBA) in chloroform to furnish the epoxide **2** in 82% yield. The epoxide ring of **2** was smoothly opened by sodium azide in the presence of ammonium chloride in refluxing aqueous acetone. The reduction of the azide group of the alcohol **3** was accomplished in

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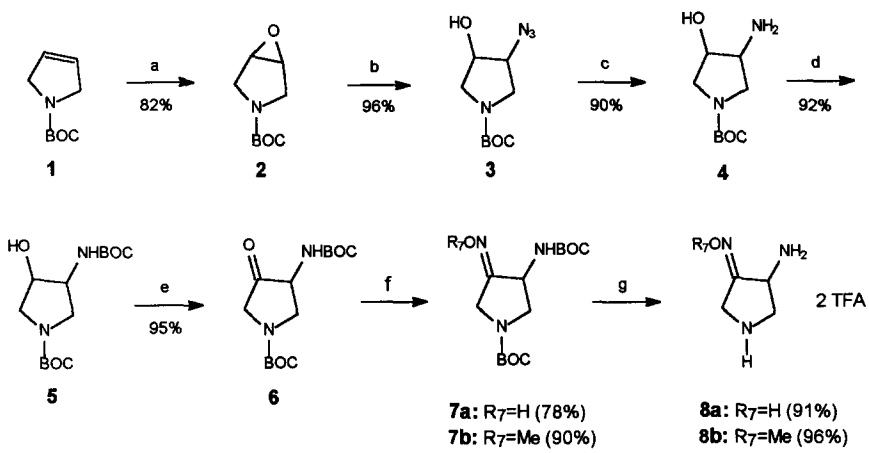
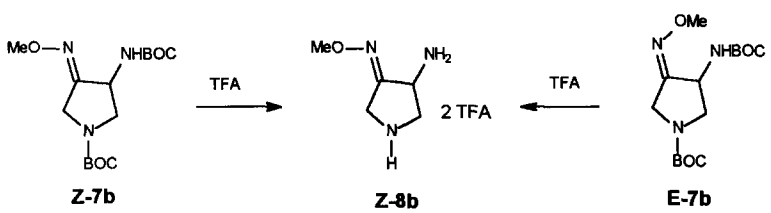
90% yield by treatment of triphenylphosphine and aqueous work up. The resulting amine **4** was subsequently protected by a BOC group using di-*t*-butyl dicarbonate in dioxane-H_2O, which produced the t-BOC protected alcohol **5** in 92% yield. Parikh-Doering oxidant[8] was found to be the best reagent for the oxidation of the alcohol **5**. Thus, the alcohol **5** was cleanly converted to the ketone **6** by treatment with sulfur trioxide-pyridine complex in DMSO in 95% yield. The oxime functional group was introduced onto the ring by coupling **6** with hydroxylamine in EtOH-THF-H_2O to afford the oxime compound **7a**. By reacting the ketone **6** with methoxylamine instead of hydroxylamine, we could also obtain the methyloxime **7b** in 90% yield. Compounds **7a** and **7b**, respectively, were found to be 3:1 mixures of E, Z isomer depending on the oxime geometry. These mixtures were easily separable by silica gel column chromatography, but separation of each isomer at this stage turned out to be unnecessary (*vide infra*).

Scheme I: Synthesis of 3-alkyloxime-4-aminopyrrolidine derivatives



reagents: (a) MCPBA, $CHCl_3$; (b) NaN_3, NH_4Cl, aq.acetone, reflux; (c) PPh_3, THF; (d) $(t\text{-BOC})_2O$, $NaHCO_3$, Dioxane-H_2O; (e) Pyridine-SO_3. Et_3N, DMSO, 5 °C; (f) R_7ONH_2·HCl, $NaHCO_3$, EtOH-THF, 40 °C; (g) Trifluoroacetic acid

Allyl- and 2-fluoroethyl-oxime compounds (compounds **12** and **13**) were synthesized simply by alkylating **7a** with allyl bromide or 2-fluoroethyl bromide (RBr/NaOH/tetra-*n*-butylammonium bromide/CH_2Cl_2-H_2O) in 70-80% yield. Finally, the bis-BOC protective groups of the oxime **7a**, **7b** were removed by trifluoroacetic acid (TFA) to give the new pyrrolidines compounds **8a**, **8b** as white solids. Although *bis*-BOC protected **7a** and **7b** were 3:1 mixtures of geometric isomers of oxime moiety, the diamine salts **8a**

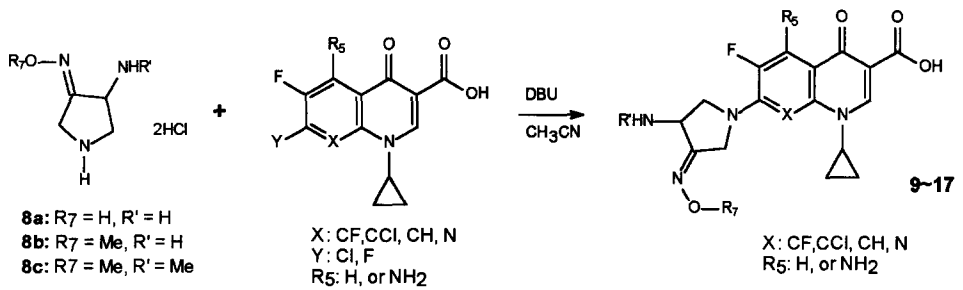


and **8b** were found to be single isomers. This implies rapid E-Z isomerization of the oxime functional group occured during the acidic deprotection. We were able to confirm such facile acid-catalized isomerization by isolating each isomer of Z-**7b** and E-**7b** and treating them separately with TFA. Only Z-**8b** was obtained from Z-**7b** or E-**7b** after TFA treatment.

The thermodynamic stability of the Z isomer over the E isomer could be expected to arise from the unfavorable steric crowding within the E isomer (**E-7b**), whereas **Z-7b** has additional stability originating from the intramolecular hydrogen bonding between the oxime nitrogen and the adjacent amino group. To support this explanation there are several literature precedents of structurally similar cyclic α-amino oximes which exist excusively in a Z form.[9]

The coupling reactions of the new pyrrolidine derivatives with various quinolone and naphthyridone nuclei followed well-established literature procedures (**Scheme II**),[10] using DBU (3 eq) as a base in refluxing acetonitrile. The yields are usually in the range of 45-56 % for quinolones (X= CF, CCl, and CH).[11] In the case of naphthyridines (X= N), the coupling proceeded smoothly at room temperature and the yields are higher (73-85%).

Scheme II : Synthesis of new quinolones

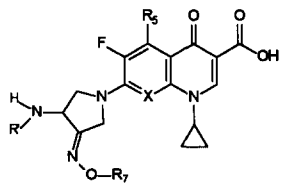


The novel quinolones containing new pyrrolidines thus produced were found to have strong antibacterial activities against not only Gram-negative strains, but also Gram-positive organisms, including methicillin resistant *Staphylococcus aureus* (MRSA) (**Table I**), Most importantly, they showed strong activity against Gram-positive bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis*, with MIC values being mostly close to or lower than 0.016 μg/ml. In particular, they were highly potent against methicillin resistant *Staphylococcus aureus* (MRSA, 4 to 256 fold enhancement compared to ciprofloxacin) and methicillin resistant *Streptococcus epidermidis* (MRSE, 8 to 256 fold enhancement compared to ciprofloxacin), against which ciprofloxacin was virtually ineffective (MIC 64 and 128 μg/ml, respectively).

These remarkable Gram-positive activity profiles are of great significance since in recent years the emergence of resistant bacteria such as MRSA has become a very serious clinical problem. Since *Staphylococci* and *Streptococci* are known to be major pathogenic strains of respiratory tract infections, it is believed the improved potency of these novel quinolones, particularly against *Staphylococci* and *Streptococci*, will supplement the limited therapeutic application of current quinolones to these infections.

The novel quinolones displayed strong activity against Gram-negative strains as well. Their potency was comparable to ciprofloxacin against *E. coli* and *Enterobacter cloacae*. Against other Gram negative strains except *Pseudomonas aeruginosa*, they were almost equipotent to ciprofloxacin. Therefore we believe our

Table I. *In vitro* antimicrobial activities of novel quinolones



compound	9	10	11 LB20277	12	13	14	15	16	17	Cipro-floxacin
	X = CH R_5= H R_7= CH_3 R'= H	X = CCl R_5= H R_7= CH_3 R'= H	X = CF R_5= H R_7= CH_3 R'= H	X = CF R_5= H R_7= C_2H_4F R'= H	X = CF R_5= H R_7= allyl R'= H	X = CF R_5= H R_7= H R'= H	X = CF R_5= NH_2 R_7= CH_3 R'= H	X = N R_5= H R_7= CH_3 R'= H	X = N R_5= H R_7= CH_3 R'= CH_3	X = CH R_5= H piperazine
Strains	Minimum inhibitory concentration (ug/ml)									
S. a. giorgio	0.016	0.016	<=0.008	0.031	0.016	0.063	<=0.008	0.063	0.031	0.25
S. a. 77	0.031	0.016	0.016	0.063	0.031	0.063	0.031	0.063	0.063	0.25
S. a. 241	32	0.5	2	2	2	32	2	8	16	128
S. e. 887E	0.031	0.016	0.016	0.063	0.016	0.13	0.016	0.063	0.063	0.13
S. e. 178	16	0.5	2	2	2	64	2	8	32	128
B. s. ATCC 6633	<=0.008	<=0.008	<=0.008	0.016	<=0.008	0.063	<=0.008	<=0.008	<=0.008	0.031
M.l. ATCC 9341	0.25	0.13	0.13	0.25	0.25	0.25	0.5	1	2	4
E. c. 10536	0.016	0.016	<=0.008	0.031	0.031	0.016	<=0.008	<=0.008	<=0.008	<=0.008
E. c. 3190Y	<=0.008	<=0.008	<=0.008	0.016	<=0.008	<=0.008	<=0.008	<=0.008	<=0.008	<=0.008
E. c. 851E	0.031	0.031	0.031	0.031	0.063	0.031	0.031	0.063	0.063	0.016
*E.c.*TEM5 3739E	0.5	0.25	0.25	0.25	0.5	0.5	0.13	1	1	0.13
P. a. 1012E	1	2	1	2	2	1	1	2	4	0.13
A. c. 15473	0.031	0.031	0.016	0.13	0.031	0.5	0.031	0.063	0.13	0.25
C. d. 2046E	0.031	0.063	0.063	0.13	0.13	0.13	0.063	0.063	0.13	0.016
E. cl. p99	<=0.008	0.016	<=0.008	0.016	0.031	0.031	<=0.008	0.016	<=0.008	<=0.008
K. a. 1076E	0.25	0.25	0.13	0.25	0.25	0.5	0.25	0.25	0. 5	0.13
S. t. 14028	0.13	0.13	0.063	0.13	0.25	0.63	0.063	0.13	0.25	0.031

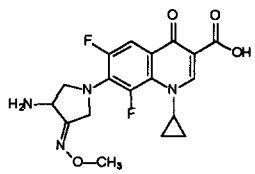
**S.a.: Staphylococcus aureus, S. e.: Staphylococcus epidermis, B. s.: Bacillus subtilis, M. l.: Micrococus luteus, E. c.: Escherichia coli, P. a.: Pseudomonas aeruginosa, A. c.: Acinetobacter calcoaceticus, C. d.: Citrobacter diversus, E. cl.: Enterobacter cloacae, K. a.: Klebsiella aerogenes, S. t.: Salmonella typhimurium*

quinolones are truly strong and broad spectrum antibacterial agents.[12]

The size of the alkyl group of the oxime moiety might be important in determining biological activity. Therefore, we have briefly investigated the structure-activity relationship of the alkyloxime group (**Table I,** compound **11**, **12**, **13**, and **14**). The methyl group seems to be optimal; compound **14** which has an unsubstituted oxime group showed relatively low potency against Gram positive pathogens.

Among the quinolones with strong *in vitro* activities, we selected compound **11** (LB20277) for further

evaluation. Mouse protection tests were used to evaluate the *in vivo* efficacy of LB20277, with the compound being administered orally (**Table II**). The efficacy of this compound was tested against two representative strains. *Staphylococcus aureus* was selected for Gram-positive bacteria, and *Pseudomonas aeruginosa* was chosen for Gram-negative strains.



LB20277

In vivo efficacy is well reflected by *in vitro* inhibitory activity. The ED_{50} of LB20277 against *S. aureus* was 0.87 mg/kg with a 95% confidence limit of 0.47-1.55 mg/kg per day, which was about 8 times stronger than ciprofloxacin (ED_{50} of 7.46 mg/kg). Against the Gram negative strain *P. aeruginosa*, the ED_{50} value of LB20277 (9.69 mg/kg) was higher than that of ciprofloxacin (3.11 mg/kg) as expected.

Table II. *In vivo* Efficacy of LB20277 against Systemic Infection in Mouse.

Infected Bacteria	compound	MIC (μg/ml)	ED_{50} (mg/kg)	95% confidence limit (mg/kg)
S. aureus giorgio	LB20277	<=0.008	0.87	0.47 - 1.55
	Ciprofloxacin	0.13	7.46	3.67 - 17.3
P. aeruginosa 1912E	LB20277	1	9.69	4.24 - 19.5
	Ciprofloxacin	0.13	3.11	1.54 - 6.59

LB20277 demonstrated an excellent pharmacokinetic profile in animals after oral administration (**Table III**). In rats, this compound was extremely well absorbed (AUC= 179.3 ug·h/ml), showed complete bioavailablity (F> 100 %) and had a much longer serum half life ($t_{1/2}$= 5.18 h) than ciprofloxacin (AUC= 2.59 ug·h/ml, F= 44.5 %, $t_{1/2}$= 1.87 h). Also notable is the surprisingly high Cmax (22.75 μg/ml) compared with ciprofloxacin (Cmax= 0.90 μg/ml). Furthermore, in dogs LB20277 also showed complete bioavailability (F> 100 %) and longer serum half-life ($t_{1/2}$= 2.34 h) than ciprofloxacin (F= 71 %, $t_{1/2}$= 1.70 h).

Table III. Pharmacokinetics of LB 20277 in rat* and dog**.

Animal	compound	Route	AUC (μg hr/ml)	Half life (hour)	Cmax (μg/ml)	Tmax (hour)	F (%)
Rat	ciprofloxacin	po	2.59	1.87	0.90	0.11	44.5
	LB20277	po	179.3	5.18	22.75	0.18	>100
Dog	ciprofloxacin	po	4.54	1.70	0.91	1.12	71
	LB20277	po	12.88	2.34	3.44	0.23	>100

SD rat, dose: 20mg/kg ** dose: 4mg/kg

In conclusion, we have designed and synthesized novel quinolone agents derivatized by oxime-substituted aminopyrrolidines. They showed potent antibacterial activities against both Gram-negative and Gram-positive organisms, including methicillin resistant *Staphylococcus aureus* (MRSA). Our development candidate, LB20277, exhibited an excellent *in vivo* efficacy, complete bioavailability, and long half-life. Based on these promising results, LB20277 was advanced to preclinical studies.[14]

References and Notes

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11. Synthesis of 7-(4-amino-3-methoxyiminopyrrolidine-1-yl)-1-cyclo-propyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**11**, LB20277): 1-cyclopropyl-6,7,8-trifluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (0.85g, 3.0 mmole) and 4-amino-pyrrolidin-3-on-*O*-methyloxime bistrifluoroacetate (**8b**) (1.29 g, 3.6 mmol) were dissolved in 10 ml of dry acetonitrile. Then, 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU, 1.37 g, 9.0 mmole) was added and the reaction mixture was refluxed for 1.5 hours. The reaction mixture was cooled to room temperature and diluted with water (10 ml), and extracted with dichloromethane three times (20 ml). The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated *in vacuo* to yield the residue. The residue was purified by preparative HPLC (column: delta pak C18, 30 x 30mm, flow rate: 40 ml/min, eluant: methanol/ water 50/50) to yield 0.57 g (Yield: 48%) of pure final product **11**. ^{1}H NMR (DMSO-d_6, ppm) : δ 8.70 (1H, s), 7.8 (1H, d, J=13.0 Hz), 4.42 (2H, dd, J= 4.7, 13.0 Hz), 4.10 (1H, m), 4.00 (2H, m), 3.83 (3H, s) , 3.52 (1H, m), 3.30 (2H, m), 1.20-1.10 (4H, m). MS (FAB, m/e) : 393 (M+H). *Anal.* Calcd for $C_{18}H_{18}F_2N_4O_4$: C, 55.10; H, 4.62; N, 14.28. Found: C, 55.12; H, 4.60; N, 14.24.
12. There have been two reports where the oxime functionality was introduced into the C-7 amine group of quinolone antibacterials. Abbott and Kaken's scientists independently introduced the oxime function into pyrrolidine ring.[13] Although these quinolones showed enhanced *in vitro* antibacterial activity against Gram-positive strains, they displayed rather weak potency against Gram-negative organisms. In addition, their *in vivo* efficacy was not as good as was anticipated from the *in vitro* results.
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14. LB20277 is currently undergoing preclinical evaluations as a racemic mixture and the asymmetric synthesis of each enantiomer is in progress.