2011 International Conference on Bioscience, Biochemistry and Bioinformatics
IPCBEE vol.5 (2011) © (2011) IACSIT Press, Singapore

Synthesis and Antibacterial Evaluation of Novel Fluoroquinolone Derivatives

Om Prakash Sharma
Department of Pharmacy
Government Polytechnic
Adampur-125052, INDIA
sharmaop1@in.com

Prabodh Chander Sharma
Institute of Pharmaceutical Sciences
Kurukshetra University
Kurukshetra-136119, INDIA
sharma_prabodh@rediffmail.com

Sandeep Jain
Dept. of Pharm. Sciences
Guru Jambheshwar University of Science and Technology
Hisar-125001, INDIA

***Abstract*—A series of six novel 1-ethyl-6-fluoro-7-[4-(1-alkyl-1,4-dihydropyridine-3-carbonyl)-piperazin-1-yl]-4-oxo-1,4-dihydro-quinoline-3-carboxylates has been synthesized and evaluated for antibacterial activity. Norfloxacin was reacted with thionyl chloride, to yield norfloxacin acid chloride which was used immediately in next step by reacting with respective alcohols to furnish the corresponding esters i.e. 1-ethyl-6-fluoro-1,4-dihydro-7-piperazin-1-yl-4-oxoquinoline-3-carboxylates. Nicotinoyl chloride was prepared by adopting reported procedures and was reacted appropriately with previously synthesized esters to yield the amides. Amides were converted into the corresponding quaternary compounds by reacting with suitable alkyl halides. The quaternary compounds were reduced in the inert atmosphere to yield the title compounds. The structures of synthesized compounds were established on the basis of analytical and spectral studies. All the synthesized compounds were evaluated for antibacterial activity against five different strains of bacteria. Compounds exhibited moderate to significant minimum inhibitory concentration.**

Keywords-Fluoroquinolones, norfloxacin, anti-bacterial activity, MIC

I. INTRODUCTION

Infectious diseases caused by bacteria affect millions of people and are leading causes of death worldwide [1,2]. Treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging and increasing number of multidrug resistant microbial pathogens [3]. Considering the antimicrobial resistance phenomenon as one of the greatest challenges in 21st century facing the modern medicine system, discovery of new substances with potential effectiveness against several pathogenic microorganisms becomes highly desirable [4].

Anti-infective chemotherapy is the science of administering chemical agents to treat infectious diseases. Historically, the use of anti-infective agents can be credited with saving more human lives than any other area of medicinal therapy discovered to date [5]. Since their introduction, antimicrobials are one of the most significant weapons in fighting bacterial infections. They have extremely benefited the health related quality of human life [6]. One of the main objectives of organic and medicinal chemistry is the design, synthesis and production of molecules having value as human therapeutic agents [7]. During the past three decades, antimicrobial agents have been introduced at a rate exceeding our ability to integrate them into clinical practice [8].

Development of novel chemotherapeutic agents is an important and challenging task for the medicinal chemists and many research programs are directed towards the design and synthesis of new drugs for their chemotherapeutic usage [9]. Development of new antibiotics has been achieved from derivatives of known antimicrobial agents or by identification of novel agents active against previously unexploited targets [10].

Quinolones and fluoroquinolones are a relatively new class of synthetic antibiotics with potent bactericidal, broad spectrum activity against many clinically important pathogens which are responsible for variety of infections including urinary tract infections (UTI), gastrointestinal infections, respiratory tract infections (RTI), sexually transmitted diseases (STD) and skin infections. They are primarily used against urinary tract infections and are also clinically useful against prostatitis, infections of skin and bones and penicillin resistant sexually transmitted disease. These agents are also employed against bacterial enteric infections, prophylaxis in the immuno compromised neutropenic host. New quinolones provide a valid alternative antibacterial therapy, especially in areas where the prevalence of pencillin resistant and macrolide resistant organisms exist [5].

Since their introduction, fluoroquinolones have become a mainstay in the treatment of serious bacterial infections. These are synthetic antibacterial agents structurally related to nalidixic acid. They depict several favorable properties such as excellent bioavailability, good tissue penetrability and a relatively low incidence of adverse and toxic effects. These drugs are potentially used in the treatment of urinary tract infections and prostatitis. They are also employed against bacterial enteric infections, biliary tract infections, sexually transmitted diseases and prophylaxis in the immuno compromised neutropenic host [8].

On the other hand, despite of much significant advancement in the antimicrobial therapy, many problems remain to be solved for most of the antimicrobial drugs available. It is emphasized that further developments are required in order to combat with the infectious conditions. Hence, discovery of novel antimicrobial agents with better

pharmacological profile is still highly desirable [11]. A potential approach to overcome the resistance problem is to design novel and innovative agents [12]. The development of new antibiotics can be achieved from derivatives of known antimicrobial agents or by identification of novel agents active against previously unexploited targets [13].

Thus, keeping in view the need of novel antibacterial agents with improved antimicrobial profile, and as a part of our ongoing programme on design and discovery of novel pharmacotherapeutic and antimicrobial and agents [14-18], it was also decided to evaluate the newly synthesize compounds for their antibacterial potential.

II. SYNTHETIC CHEMISTRY

The entire chemicals were supplied by E. Merck (Germany) and S.D. Fine Chemicals (India).The melting points of synthesized compounds were determined in open capillary tubes using Veego VMP-1 melting point apparatus, expressed in ºC and are uncorrected. The IR spectra of compounds were recorded on Perkin Elmer Infra Red Spectrophotometer in KBr disc and absorption bands are expressed in cm-1. 1H NMR spectra were recorded on Brucker Aveance 700 MHz NMR Spectrometer (Chemical shift in δ ppm) using TMS as internal standard.

A. Synthesis of Norfloxacin Alkyl Esters and Amides

Syntheses of alkyl esters and amides were carried out by our earlier reported procedure [13].

B. Synthesis of pyridinium halides

Appropriately synthesized amide (0.025 mol) was dissolved in a mixture of acetone (20 mL), triethylamine (10 mL) and ethanol (5 mL). Appropriate quantity of alkyl halide (methyl iodide or ethyl bromide) was added at different time intervals during the 66 to 72 h stirring at room temperature. The solvents were removed under reduced pressure. The product (pyridinium halide) was recrystallized in methanol and yellowish crystals were obtained.

C. Synthesis of Dihydropyridine Derivatives

The quaternary compounds were reduced in a mixture triethyl amine/pyridine/ distilled water and/or ethyl acetate (Figure 4.5). The solution was cooled to about 5 °C. Sodium bicarbonate (2g) and sodium dithionate (1g) were added to the above solution. The reduction was carried out in an atmosphere of nitrogen gas while stirring for 4h to 5h. The organic layer was separated and solvent was removed under reduced pressure. The product (**VIIINa-f**) was recrystallized in methanol and pale yellow crystals were obtained [19,20]. Physical constants of synthesized comounds is presented in Table I.

Spectral characterization

1-*Ethyl-6-fluoro-7-[4-(1-methyl-1,4-dihydropyridine-3-carbonyl)-piperazin-1-yl]-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (VIIINa)*

IR (cm^{-1}, KBr) : 3160 (Ar-C-H), 1648, 1717, 1735, (C=O), 1215 (C-O), 1151 (C-F).

^{1}H NMR, δ ppm (DMSO-d_6) : 14.70 (s, 1H, -COOH); 8.50 (s, 1H, H_2-quinolone); 7.58-7.24 (s, 1H, C_2 dihydropyridine); 7.03-6.85 {m, 2H, aromatic proton (H_5 and H_8-quinolone)}; 5.88-5.74 (d, 1H, C_6 dihydropyridine); 4.72-4.56 {m, 3H, 1H(C_5 dihydropyridine) and 2H (N-CH_2CH_3 quinolone)}; 3.46-3.18 (m, 8H, piperazine); 3.08 (d, 1H, C_4 dihydropyridine); 2.94 (s, 3H, N-CH_3); 1.41 (t, 3H, N-CH_2CH_3 quinolone).

1-Ethyl-7-[4-(1-ethyl-1,4-dihydropyridine-3-carbonyl)-piperazin-1-yl]-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (VIIINb)

IR (cm^{-1}, KBr) : 3152 (Ar-C-H), 1649, 1711, 1737, (C=O), 1215 (C-O), 1156 (C-F).

^{1}H NMR, δ ppm (DMSO-d_6) : 14.68 (s, 1H, -COOH); 8.48 (s, 1H, H_2-quinolone); 7.38-7.24 (s, 1H, C_2 dihydropyridine); 7.18-6.89 {m, 2H, aromatic proton (H_5 and H_8-quinolone)}; 5.86-5.79 (d, 1H, C_6 dihydropyridine); 4.72-4.58 {m, 3H, 1H (C_5 dihydropyridine) and 2H (N-CH_2CH_3 quinolone)}; 3.45-3.12 {m, 10 H, 8H (piperazine) and 2H (N-CH_2CH_3)}; 3.11 (d, 1H, C_4 dihydropyridine); 1.42-1.29 {(t, 6H, 3H (N-CH_2-CH_3) and 3H (N-CH_2CH_3 quinolone)}.

1-Ethyl-6-fluoro-7-[4-(1-methyl-1,4-dihydropyridine-3-carbonyl)-piperazin-1-yl]-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid methyl ester (VIIINc)

IR (cm^{-1}, KBr) : 3157 (Ar-C-H), 1649, 1718, 1737, (C=O), 1226 (C-O), 1154 (C-F).

^{1}H NMR, δ ppm (DMSO-d_6) : 8.39 (s, 1H, H_2-quinolone); 7.32-7.19 (s, 1H, C_2 dihydropyridine); 7.12-6.86 {m, 2H, aromatic proton (H_5 and H_8-quinolone)}; 5.85-5.78 (d, 1H, C_6 dihydropyridine); 4.69-4.53 {m, 3H, 1H (C_5 dihydropyridine) and 2H (N-CH_2CH_3 quinolone)}; 3.61 (s, 3H, CH_3) ; 3.39-3.20 (m, 8H, piperazine); 3.04 (d, 1H, C_4 dihydropyridine); 2.87 (s, 3H, N-CH3); 1.45 (t, 3H, N-CH2CH3 quinolone).

1-Ethyl-7-[4-(1-ethyl-1,4-dihydropyridine-3-carbonyl)-piperazin-1-yl]-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid methyl ester (VIIINd)

IR (cm^{-1}, KBr) : 3153 (Ar-C-H), 1649, 1714, 1739, (C=O), 1222 (C-O), 1153 (C-F).

^{1}H NMR, δ ppm (DMSO-d_6) : 8.38 (s, 1H, H_2-quinolone); 7.28-7.15 (s, 1H, C_2 dihydropyridine); 7.08-6.85 {m, 2H, aromatic proton (H_5 and H_8-quinolone)}; 5.82-5.71 (d, 1H, C_6 dihydropyridine); 4.74-4.58 {m, 3H, 1H (C_5 dihydropyridine) and 2H (N-CH_2CH_3 quinolone)}; 3.64 (s, 3H, CH_3) ; 3.44-3.15 {m, 10 H, 8H(piperazine) and 2H (N-CH_2CH_3)}; 3.07 (d, 1H, C_4 dihydropyridine); 1.50-1.25 {t, 6H, 3H (N-CH_2-CH_3) and 3H (N-CH_2CH_3 quinolone)}.

1-Ethyl-6-fluoro-7-[4-(1-methyl-1,4-dihydropyridine-3-carbonyl)-piperazin-1-yl]-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (VIIINe)

IR (cm^{-1}, KBr) : 3135 (Ar-C-H), 1658, 1714, 1740, (C=O), 1232 (C-O), 1150 (C-F).

^{1}H NMR, δ ppm (DMSO-d_6) : 8.46 (s, 1H, H_2-quinolone); 7.34-7.25 (s, 1H, C_2 dihydropyridine); 7.13-6.84 {m, 2H, aromatic proton (H_5 and H_8-quinolone)}; 5.85-5.76 (d, 1H, C_6 dihydropyridine); 4.72-4.51 {m, 3H, 1H (C_5 dihydropyridine) and 2 H (N-CH_2CH_3 quinolone)}; 4.15 (q, 2H, -CH_2CH_3); 3.42-3.21 (m, 8H, piperazine); 3.04 (d, 1H,

C_4 dihydropyridine); 2.89 (s, 3H, N-CH_3); 1.42-1.26 (t, 6H, -CH_2CH_3 (2 groups)).

1-Ethyl-7-[4-(1-ethyl-1,4-dihydropyridine-3-carbonyl)-piperazin-1-yl]-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (VIIINf)

IR (cm^{-1}, KBr) : 3136 (Ar-C-H), 1662, 1721, 1739, (C=O), 1233 (C-O), 1151 (C-F).

^{1}H NMR, δ ppm (DMSO-d_6) : 8.38 (s, 1H, H_2-quinolone); 7.29-7.18 (s, 1H, C_2 dihydropyridine); 7.10-6.85 {m, 2H, aromatic proton (H_5 and H_8-quinolone)}; 5.84-5.69 (d, 1H, C_6 dihydropyridine); 4.66-4.52 {m, 3H, 1H (C_5 dihydropyridine) and 2H (N-CH_2CH_3 quinolone)}; 4.11 (q, 2H, -CH_2CH_3); 3.45-3.11 {m, 10 H, 8H (piperazine) and 2H (N-CH_2CH_3)}; 3.05 (d, 1H, C_4 dihydropyridine); 1.46-1.24 (t, 9H, CH_2-CH_3 three groups).

III. Antibacterial Activity Assay

The newly synthesized compounds were evaluated for antibacterial activities by minimum inhibitory concentration (MIC) method. Nutrient agar media and King's B media were used for the biological assay as per the following composition: Nutrient agar media (NAM) made up of peptone 5g, beef extract 3g, NaCl 5g, nutrient agar 2% and the final volume of media was adjusted to 1000 mL with double distilled water (pH 7.0). King's B media containing peptone 2%, glycerol 1%, KH2PO4 0.15%, MgSO4 0.15%, agar 2% and the final volume of media was adjusted to 1000 mL with distilled water (pH 7.0). Synthesized compounds were screened for antibacterial activities against two Gram-positive bacteria i.e. *Staphylococcus auerus* (NCDC 110), *Bacillus subtilis* (NCDC 71) and two Gram-negative bacteria i.e. *Escherichia coli* (NCDC 134), *Pseudomonas aeruginosa* (NCDC 105). The bacterial cultures were revived as per the protocol provided by National Collection of Dairy Cultures (NCDC), Karnal, India. *P. aeruginosa* culture was maintained on King's B media while all other cultures were maintained on nutrient agar media. Suspension of each test organism was prepared to evaluate antibacterial activity of the synthetic compounds. All stock cultures were stored at 4°C.

MIC of the synthesized compounds was determined using method described by Kumar *et al* (2009) [21]. A stock solution of 3 mg/mL of each compound was prepared in DMSO and further diluted to get final concentration ranging from 200-0.05 µg/mL. Optical density was measured at 600 nm using UV-visible spectrophotometer. The minimum concentration, where no microbial growth was observed is called as MIC of the compound. The results of antibacterial screening are presented in Table II.

IV. Results And Discussion

In the present investigation, the synthesized compounds (VIIINa-f) were evaluated for their antibacterial activity against Gram-positive and Gram-negative bacterial strains by serial dilution technique and MIC values were determined. All the compounds were screened for their antibacterial activity against three Gram-negative strains (*Escherichia coli*, *Shigella dysentery* and *P. aeruginosa*) and two Gram-positive strains (*Bacillus subtilis* and *Staphylococcus aureus*). Norfloxacin was used as standard drugs for antibacterial activity. When the title compounds (VIIINa-f) were tested against *E. coli*, compounds without substitution at C-3 carboxylic acid group i.e. compounds VIIINa and VIIINb were found most active with MIC of 0.20 µg/mL. All the compounds exhibited good antibacterial activity with MIC ranging from 0.20 µg/mL to 0.30 µg/mL. When screened against another Gram negative bacterial strain *P. aeruginosa*, Compound VIIINa bearing free carboxylic acid group and methyl group at dihydropyridine was most active among norfloxacin derivatives (VIIINa-f) demonstrating good activity (i.e. 20 µg/mL).

All the synthesized norfloxacin derivatives (VIIINa-f) were also screened against these Gram positive bacterial strains. Compound VIIINa with free carboxylic acid group at quinolone ring and and methyl group at dihydropyridine portion was found to be most active against *S. aureus* with MIC of 1.30 µg/mL when tested against standard drug norfloxacin (MIC 1.5 µg/mL). Second most active compounds of series were compound VIIINb and VIIINc (MIC 1.35 µg/mL). Antibacterial screening results against *B. subtilis* depicted that compound VIIINb and VIIINc were most active (MIC 1.35 µg/mL).

V. Conclusion

In conclusion, we have synthesized six new derivatives of fluoroquinolone antibacterial drug norfloxacin and evaluated them for their antimicrobial activities against selected bacterial strains. The compounds have demonstrated moderate to significant activities.

References

[1] World Health Organisation (**1999**). Report on infectious diseases: removing obstacles to healthy development. Geneva, Switzerland: World Health Organization. http://www.who.int/infectious-disease-report/index-rpt99.html (Accessed on July 24, **2010**).

[2] P.C. Sharma, S. Jain, "Synthesis and antibacterial activity of certain novel 1-cyclopropyl-6-flouro-1,4-dihydro-7-4-substituted-piperazin-1-yl-4-oxoquinolin-3-carboxylates." Acta Pharm. Sciencia, 2008, 50,35-40

[3] R. Chawla, U. Sahoo, A. Arora, P.C. Sharma, V. Radhakrishnan, "Microwave assisted synthesis of some novel 2-pyrazoline derivatives as possible antimicrobial agents." Acta Pol. Pharm. Drug Res., 2010, 67, 55-61.

[4] C.G. Almeida, C.G. Diniz, V.L. Silva, M.F. Saraiva, M.L. Hyaric, M.V. Almeida, "Antibacterial activity of lipophillic flouroquinolone derivatives." Med. Chem., 2009, 5, 1-3.

[5] P.C. Sharma, A. Jain. S. Jain, "Flouroquinoline antibacterials: A review on chemistry, microbiology and therapeutic prospects." Acta Pol. Pharm. Drug Res., 2009, 66, 587-604.

[6] R. Chawla, A. Arora, M.K. Parmeswaran, P.C. Sharma, S. Michael, T.K. Ravi, "Synthesis of novel 1,3,4-oxadiazole derivatives as potential antimicrobial agents." Acta Pol. Pharm. Drug Res., 2010, 67, 247-253.

[7] V. Kumar, A. Sharma, P.C. Sharma, "Synthesis of some novel 2,5-disubstituted thiazolidinones from a long chain fatty acid as possible anti-inflammatory and hydrogen peroxide scavenging agents." J. Enz. Inhib. Med. Chem., 2010, in press.: doi 10.3109/14756366.2010.489897.

[8] P.C. Sharma, A. Jain, S. Jain, R. Pahwa, M.S. Yar, "Ciprofloxacin: Review on developments in synthetic, analytical and medicinal aspects." J. Enz. Inhib. Med. Chem., 2010, 25, 577-589.

[9] Aakashdeep, S. Jain, P.C. Sharma, P. Verma, M. Kumar, C.P. Dora, Design and biological evaluation of biphenyl-4-carboxylic acid hydrazide-hydrazone for antimicrobial activity. Acta Pol. Pharm. Drug Res., 2010, 67, 255-259.

[10] P.C. Sharma, A. Jain, S. Jain, In: "Therapeutic Perspestives of flouroquinolone antibacterials: An update." In: Proceedings of 1st Rashtreeya Yuva Vaigyanik Sammelan, NIT, Kurukshetra, Nov. 28-30 Nov, 2008; 234-239.

[11] P.R. Patel, C. Ramalingan, Y.T. Park, "Synthesis and antimicrobial evaluation of guanylsulfonamides." Bioorg. Med. Chem. Lett. 2007, 17, 6610-6614.

[12] R. Kharb, P.C. Sharma, M.S. Yar, "Pharmacological significance of triazole scaffold." *J. Enz. Inhib. Med. Chem..,* in press doi: 10.3109/14756360903524304.

[13] PC. Sharma, S. Jain, "Synthesis and *in vitro* antibacterial activity of some novel N-nicotinyl-1-ethyl-6-flouro-1,4-dihyro-7-piperazin-1-yl-4-oxoquinoline-3-carboxylates." Acta Pol. Pharm. Drug Res., 2008, 65(5), 551-586.

[14] P.C. Sharma, S.V. Sharma, A. Sharma, B Suresh, "3D-QSAR CoMFA study of some heteroarylpyrroles as possible anticandida agents." Ind. J. Pharm. Sci., 2008, 70(2), 154-158.

[15] . P.C. Sharma,; S.V. Sharma,; S. Badami; A. Sharma,; B. Suresh, "Microwave assisted efficient synthesis and anti-fungal evaluation of some N-Phenyl-3-(substituted phenyl) propenamides." Ind. J. Pharm. Educ. Res., 2007, 41(2), 140-145.

[16] P.C. Sharma, S.V. Sharma, S. Jain, S. Singh, B. Suresh, "Synthesis of some new isoxazoline derivatives as possible anti-candida agents." Acta Pol. Pharm. Drug Res., 2009, 66(1), 101-104.

[17] A. Sharma, V. Kumar, S. Jain, P.C. Sharma, "Thiazolidin-4-one and hydrazone derivatives of capric acid as possible anti-inflammatory, analgesic and hydrogen peroxide scavenging agents." J. Enz. Inhib. Med. Chem. In press. DOI 10.3109/14756366.2010.535796.

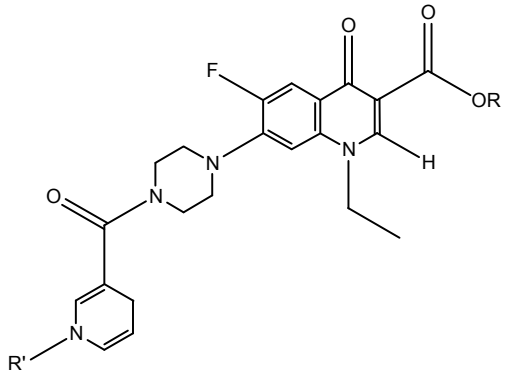
[18] P.C. Sharma, S. Yadav, R. Pahwa, D. Kaushik, S. Jain, "Synthesis and evaluation of novel prodrugs of naproxen." Med Chem Res, in press. DOI: 10.1007/s00044-010-9364-8.

[19] K. Prokai-Tatrai, E. Pop, W. Anderson, J.L. Lin, M.E. Brewster, N. Bodor, "Redox Derivatives of tranylcypromine: synthesis, properties and monoamine oxidase inhibitor activity of some chemical delivery systems." J. Pharm. Sci. 1990; 80: 255-261.

[20] U. Eisher, J. Kuthan, "The chemistry of dihydropyridines." Chem. Rev. 1972, 72, 1-42.

[21] R. Kumar, R.R. Nair, S.S. Dhiman, J. Sharma, O. Prakash. Organoiodine (III)-mediated synthesis of 3-aryl/heteroaryl-5,7-dimethyl-1,2,4-triazolo[4,3-c]pyrimidines as antibacterial agents. Eur. J. Med. Chem. 2009; 44:2254-2260.

TABLE I. PHYSICAL CONSTANTS OF THE SYNTHESIZED COMPOUNDS



Compound	R´	R	M.P. (°C)	Yield (%)	Mol Formula	Mol Wt	Rf value
VIIINa	CH_3	H	141-143	63.6	$C_{23}H_{25}FN_4O_4$	440.47	0.51
VIIINb	C_2H_5	H	164-165	56.5	$C_{24}H_{27}FN_4O_4$	454.49	0.52
VIIINc	CH_3	CH_3	136-137	61.2	$C_{24}H_{27}FN_4O_4$	454.49	0.56
VIIINd	C_2H_5	CH_3	121-122	58.3	$C_{25}H_{29}FN_4O_4$	468.52	0.62
VIIINe	CH_3	C_2H_5	156-157	54.4	$C_{25}H_{29}FN_4O_4$	468.52	0.59
VIIINf	C_2H_5	C_2H_5	158-159	56.6	$C_{26}H_{31}FN_4O_4$	482.55	0.62

TABLE II. MINIMUM INHIBITORY CONCENTRATION IN MG/ML OF COMPOUNDS VIIINA-N AGAINST SELECTED BACTERIAL STRAINS.

Compound No.	Gram negative bacteria			Gram positive bacteria	
	E. coli (NCDC 134)	S. dysentery (NCDC 63)	P. aeruginosa (NCDC 105	B. subtilis (NCDC 71)	S. auerus (NCDC 110)
	MIC (µg/mL)	MIC (µg/mL)	MIC (µg/mL)	MIC (µg/mL)	MIC (µg/mL)
VIIINa	0.20	0.20	0.35	1.40	1.30
VIIINb	0.20	0.25	0.35	1.35	1.35
VIIINc	0.25	0.30	0.40	1.35	1.35
VIIINd	0.30	0.30	0.45	1.45	1.50
VIIINe	0.25	0.35	0.40	1.50	1.60
VIIINf	0.30	0.40	0.45	1.75	1.65
Norfloxacin	0.14	0.12	0.20	1.40	1.50