Synthesis, Characterization and Biological Evaluation of a Series of Levofloxacin Carboxamide Analogues

Najma Sultana, Muhammad Saeed Arayne,*,† Syeda Bushra Shakeb Rizvi,† and Muhammad Ahmed Mesaik‡

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi-75270, Pakistan
*†Department of Chemistry, University of Karachi-75270, Pakistan. *E-mail: msarayne1@gmail.com or msarayne@gmail.com*
‡PCMD, International Centre of Chemical Sciences, University of Karachi, Karachi, Pakistan
Received June 17, 2009, Accepted August 21, 2009

In present work an attempt was made to synthesize various analogues of levofloxacin by introducing new functionality at carboxylic group position *via* nucleophilic substitution reaction. For this purpose the carboxylic group at C-6 was esterified and later subjected to nucleophilic attack at the carbonyl carbon by various aromatic amines. Structure of the analogues was confirmed by different techniques i.e. IR, ^{1}H NMR and mass spectrometry. The antibacterial activity of the derivatives was also assessed and compared with the parent against a series of Gram-positive and Gram-negative bacteria. A synergistic as well as antagonistic behavior was observed in these derivatives. Additionally unlike levofloxacin, the derivatives were also found to modulate oxidative burst response of phagocytes exhibiting moderate to significant inhibitory activity.

Key Words: Levofloxacin, Nucleophilic substitution reaction, Antibacterial activity, Synergistic, Antagonistic

Introduction

Fluoroquinolones have a useful role in the treatment of many bacterial infections.[1,2] The 4-quinolone originates from 1,8-naphthyridine precursor-nalidixic acid, discovered in the early 1960s.[3,4] Later on, quinolones containing fluorine atoms[5,6] were introduced. A broad-spectrum, third-generation fluoroquinolone antibiotic Levofloxacin, (Figure 1) (-)-(*S*)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid produces higher ocular tissue penetration[7] diffuses through the bacterial cell wall and acts by inhibiting DNA gyrase (bacterial topoisomerase II), an enzyme required for DNA replication, RNA transcription, and repair of bacterial DNA. Inhibition of DNA gyrase activity leads to blockage of bacterial cell growth.[8-10]

Much effort has been made in recent years to the synthesis of new quinolones for enhanced antibacterial activity.[11] Structural modifications at all positions of the quinolone nucleus, except the 4-oxo group, have successfully led to the discovery of potent antimicrobial agents.[12]

Most of the quinolone antibacterial research has been focused on the functionality at C-7 position.[13] The structure activity relationship (SAR) reveals that the C-7 substituent is the most adaptable site for chemical change and is an area that determines potency and target preference.[14] During recent years a number of quinolones with substitution on piperazine ring at C-7 position of the basic structure of quinolones were synthesized.[15-17] In earlier studies, the substitution of bulky residue on piperazinyl ring of levofloxacin was carried out with 2-aryl-2-oxoethyl or a 2-aryl-2-oxyiminoethyl moiety.[18] however evidences are found in literature which reports increased antimicrobial profile of the quinolones in the form of carboxylate complexes.[19] Because of lack of data in the literature concerning analogues of levofloxacin at carboxylic group, we are reporting here a third generation broad spectrum azafluoroquinolone antibacterial agent, by introducing new functionality at carboxylic group position. For this purpose the carboxylic group at C-6 was esterified and later subjected to nucleophilic attack at the carbonyl carbon by various aromatic amines.

The present study reports the synthesis (according to figure), spectroscopic analysis (including IR and ^{1}H NMR) and evaluation of biological activities of levofloxacin carboxylic derivatives carrying amino-containing aromatic ring. The mass spectrometry was also performed to establish the structure.

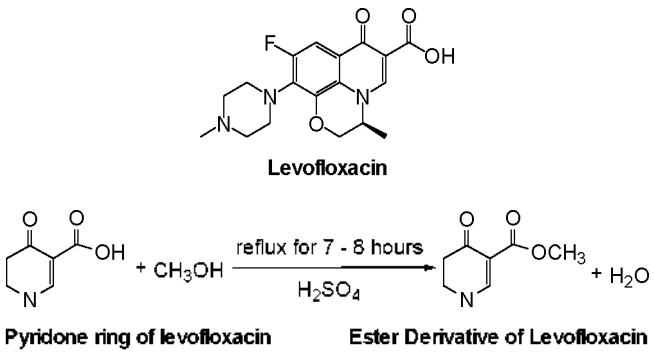

Figure 1

Experimental

Material and equipments. Levofloxacin was gift from Getz Pharmaceutical Laboratories Ltd., Karachi, Pakistan. All the reagents used were of analytical grade. All the glassware was washed with chromic acid followed by a thorough washing with freshly prepared de-ionized water.

Melting points were obtained manually by capillary method. The IR spectra were obtained on shimadzu prestige-21 200 VCE coupled to a PIV-PC and loaded with IR solution version 1.2 software (potassium bromide disks). The absorption peaks were recorded in frequency (cm^{-1}). NMR spectra were recorded on

Bruker FT-NMR 500 MHz with the compounds dissolved in deuterated methanol. Chemical shifts are reported in parts per million (δ) relative to tetra methyl silane as an internal standard. Significant ^{1}H NMR data are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet); and number of proton (s). The mass spectra were recorded on Finnign-MAT212 under electron impact (EI) ionization condition. Thin layer chromatography (TLC) was performed on HSF-254 TLC plate and compounds visualized under UV lamp.

General procedure for preparation of derivatives (a-f). Synthesis of various derivative of levofloxacin was attempted with various aromatic amines i.e., phenylhydrazine, aniline, o-aminophenol, phenylenediamine, 3-aminophenol and α-naphthylamine. Levofloxacin (0.1 moles) was added to the round bottomed flask containing 60 mL of methanol. 1 ~ 2 drops of concentrated sulphuric acid was added to the flask and the reaction was refluxed for about 7 ~ 8 hours. After the consumption of levofloxacin (monitered by TLC) 0.1 molar solutions of aromatic amines (prepared in methanol) were added individually with continuous stirring and the reaction was again refluxed for about 2 ~ 3 hours till completion, indicated by TLC. The volume of the reaction mixture was then reduced by rotary-evaporation. The precipitates were filtrated off, washed with methanol-chloroform (2:8) to give compound.

Spectral data. 9-Fluoro-2,3-dihydro-3-methyl-*N*-phenyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxazine-6-carbohydrazide (a): Yield 32%, m.p. 292 °C (dec.). IR (KBr) ν_{max}: 1669 (C = O) and 3225 (N-H). ^{1}H NMR (MeOD, 300 MHz) δ: 8.51 (s, 1H, 5'aryl H), 6.34 (d, 1H, 8'aryl H, J = 13.1), 3.61; 2.59 (m, 8H, piperznyl H), 2.34 (s, 3H of piperazinyl CH_3), 4.31-4.22 (m, 3H, oxazine H), 3.65 (d, 3H of oxazine ring CH_3, J = 6.1), 7.5-7.9 (s, NH of amide), 7.33 (m, 5H, phenylic H). MS (*m/z*, %): (452.21, 15) M^+. *Anal.* Calcd for $C_{24}H_{26}FN_5O_3$: C, 63.85; H, 5.80; F, 4.21; N, 15.51; Found C, 63.36; H, 5.27; F, 4.07; N, 15.67.

9-Fluoro-2,3-dihydro-3-methyl-*N*-phenyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide (b): Yield 71%, m.p. 301°C (dec.). IR (KBr) ν_{max}: 1641 (C = O) and 3305 (N-H). ^{1}H NMR (MeOD, 300 MHz) δ: 8.54 (s, 1H, 5'aryl H), 6.24 (d, 1H, 8'aryl H, J = 13.5), 3.62; 2.57 (m, 8H, piperznyl H), 2.37 (s, 3H of piperazinyl CH_3), 4.31-4.25 (m, 3H, oxazine H), 3.69 (d, 3H of oxazine ring CH_3, J = 6.7), 7.5-7.9 (s, NH of amide), 7.31 (m, 5H, phenylic H). MS (*m/z*, %): (437.19, 42) M^+. *Anal.* Calcd for $C_{24}H_{25}FN_4O_3$: C, 66.04; H, 5.77; F, 4.35; N, 12.84; Found C, 66.19; H, 5.64; F, 4.25; N, 12.54.

9-Fluoro-2,3-dihydro-3-methyl-*N*-(3-hyroxyphenyl)10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide (c): Yield 52%, m.p. 298 °C (dec). IR (KBr) ν_{max}: 1649 (C = O) and 3064 (N-H). ^{1}H NMR (MeOD, 300 MHz) δ: 8.57 (s, 1H, 5'aryl H), 6.40 (d, 1H, 8'aryl H, J = 13.3), 3.57;2.58 (m, 8H, piperznyl H), 2.34 (s, 3H of piperazinyl CH_3), 4.30-4.27 (m, 3H, oxazine H), 3.65 (d, 3H of oxazine ring CH_3, J = 6.5), 7.5-7.9 (s, NH of amide), 7.33 (m, 5H, phenylic H). MS (*m/z*, %): (453.19, 26) M^+. *Anal.* Calcd for $C_{24}H_{25}FN_4O_4$: C, 63.71; H, 5.57; F, 4.20; N, 12.38; Found C, 63.75; H, 5.71; F, 4.29; N, 12.94.

9-Fluoro-2,3-dihydro-3-methyl-*N*-(2-aminophenyl)10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide (d): Yield 75%, m.p. 280 °C. IR (KBr) ν_{max}:1660 (C = O) and 3150 (N-H). ^{1}H NMR (MeOD, 300 MHz) δ: 8.61 (s, 1H, 5'aryl H), 6.34 (d, 1H, 8'aryl H, J = 13.1), 3.61; 2.60 (m, 8H, piperznyl H), 2.40 (s, 3H of piperazinyl CH_3), 4.29- 4.25 (m, 3H, oxazine H), 3.68 (d, 3H of oxazine ring CH_3, J = 6.1), 7.5-7.9 (s, NH of amide), 7.41 (m, 5H, phenylic H). MS (*m/z*, %): (452.21, 73) M^+. *Anal.* Calcd for $C_{24}H_{26}FN_4O_3$: C, 63.85; H, 5.80; F, 4.21; N, 15.51; Found C, 63.55; H, 5.69; F, 4.10; N, 15.61.

9-Fluoro-2,3-dihydro-3-methyl-*N*-(o'hyroxyphenyl)10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide (e): Yield 72%, m.p. 255 °C. IR (KBr) ν_{max}:1657 (C = O) and 3200 (N-H). ^{1}H NMR δ: 8.62 (s, 1H, 5'aryl H), 6.45 (d, 1H, 8'aryl H, J = 13.1), 3.59;2.57 (m, 8H, piperznyl H), 2.35 (s, 3H of piperazinyl CH_3), 4.31-4.23 (m, 3H, oxazine H), 3.71 (d, 3H of oxazine ring CH_3, J = 6.1), 7.5-7.9 (s, NH of amide), 7.38 (m, 5H, phenylic H). MS (*m/z*, %): (453.19, 26) M^+. *Anal.* Calcd for $C_{24}H_{25}FN_4O_4$: C, 63.71; H, 5.57; F, 4.20; N, 12.38; Found C, 63.77; H, 5.22; F, 4.25; N, 12.54.

9-Fluoro-2,3-dihydro-3-methyl-*N*-(naphthalen-1-yl)10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxamide (f): Yield 65%, m.p. 261 °C. IR (KBr) ν_{max}: 1662 (C = O) and 3157 (N-H). ^{1}H NMR δ: 8.53 (s, 1H, 5'aryl H), 6.35 (d, 1H, 8'aryl H, J = 13.5), 3.63;2.61 (m, 8H, piperznyl H), 2.39 (s, 3H of piperazinyl CH_3), 4.31-4.27 (m, 3H, oxazine H), 3.71 (d, 3H of oxazine ring CH_3, J = 6.1), 7.5-7.9 (s, NH of amide), 7.42 (m, 7*H*, phenylic H). MS (*m/z*, %): (453.19, 26) M^+. *Anal.* Calcd for $C_{28}H_{25}FN_4O_3$: C, 69.12; H, 5.59; F, 3.90; N, 11.52; Found C, 69.84; H, 5.82; F, 3.72; N, 11.59.

Antimicrobial assay. The antimicrobial susceptibility of all the derivatives was tested by the disc diffusion technique developed by Bauer *et al.*[20] For this purpose 50 ppm stock solution of levofloxacin and its derivatives were prepared. The stock solution was diluted to 3 different concentrations i.e. 5, 10 and 20 ppm. Commercially available filter paper discs were soaked in the prepared drug and derivatives solution, dried and applied on the surface of solid culture media (Nutrient Agar), which had been streaked with standardized bacterial inoculums and incubated at 37 °C for 24 h. This method is based on the determination of an inhibited zone proportional to the bacterial susceptibility to the antimicrobial present in the disk.

The results were compared with the parent against 11 different strains of *Gram positive* (*Staphylococcus aureus, Bacillus subtilis, Streptococcus pneumoniae, Corynebacterium hoffmannii*) and *Gram negative* organisms (*Klebsiella pneumoniae, Proteus mirabilis, Shigella flexneri, Escherichia coli, Pseudomonas areuginosa, Citrobacter species*, and *Salmonella typi*).

Phagocyte chemiluminescence. Luminol-enhanced chemiluminescence assay was performed using Helfand *et al.* protocol.[21] Briefly whole blood diluted in modified Hank's solution was incubated with different concentrations of the derivatives (100, 50, 25, 6.25 and 3.1 μg/mL) for 30 min. Zymosan (Sigma Chemical Co., USA) 100 μL (20 mg/mL), followed by 100 uL (7×10^5 M) luminal (Sigma Chemical Co., USA) was added to make a final volume of 0.25 mL. MHS alone was run as a control. Peak chemiluminescence was recorded with a

luminometer (Labsystem Luminoskan RS, Finland). The luminometer was set with repeated scan mode, 50 scans with 30 s intervals and one second point measuring time.

Result and Discussion

FTIR analysis showed broad OH stretching vibration of the carboxylic group of levofloxacin extending from 3600 - 3100 cm^{-1} and strong absorption peak at 1675 - 1707 cm^{-1} due to keto carboxylic group. Our studies revealed that in the spectra of all the derivatives of levofloxacin the absorption intensity of the carboxylic carbonyl group was decreased and shifted to the right near 1650 cm^{-1} which is indicative of the formation of amide. In the spectra of **a**, **b**, **d** and **f** no peak was observed for carboxylic OH absorption and a distinct, strong and un-obscured NH stretch was observed at 3200 cm^{-1} indicating that carboxylic site reacted with the selected amines forming amides. On the other hand, the spectra of **c** and **e** showed sharp NH absorption near 3000 cm^{-1} and also OH stretch at 3600 cm^{-1} as a sharp band due to the presence of free hydroxyl group in the structure of the selected amines.

The spectra of derivatives were similar to the levofloxacin spectrum except the resonance of acidic proton at 11 ppm which was absent in all derivatives spectra, showing the utilization of this moiety in amide formation. Along with the disappearance of carboxylic proton, all derivatives also showed a singlet in the region 7.5 - 7.9 ppm which corresponds to the absorption of sec-amide. The signals for the aliphatic and piperazinyl protons were practically remain same as they were distant from the group modification site of the drug. Further signals corresponding to their respective chemical structure are mentioned in the spectral data.

The electron impact mass spectra (EIMS) of levofloxacin showed M^+ peak at *m/z* 361 which is also the base peak. The fragmentation pattern follows: *m/z* 316 (loss of carboxylic group), *m/z* 262 (loss of piperazinyl ring), *m/z* 218 (loss of both piperazinyl and carboxylic group) and *m/z* 203 (additional loss of methyl group). However all the compounds showed a very low percentage of M^+ peaks owing to their unstable nature.

The fluoroquinolones comprise a major class of antibacterial chemotherapeutic agents, which have a broad spectrum of activity against *Gram positive* and *Gram negative* bacteria. The antibacterial activity generated by fluoroquinolones is caused by the inhibition of two bacterial enzymes: DNA gyrase (a topoiso-

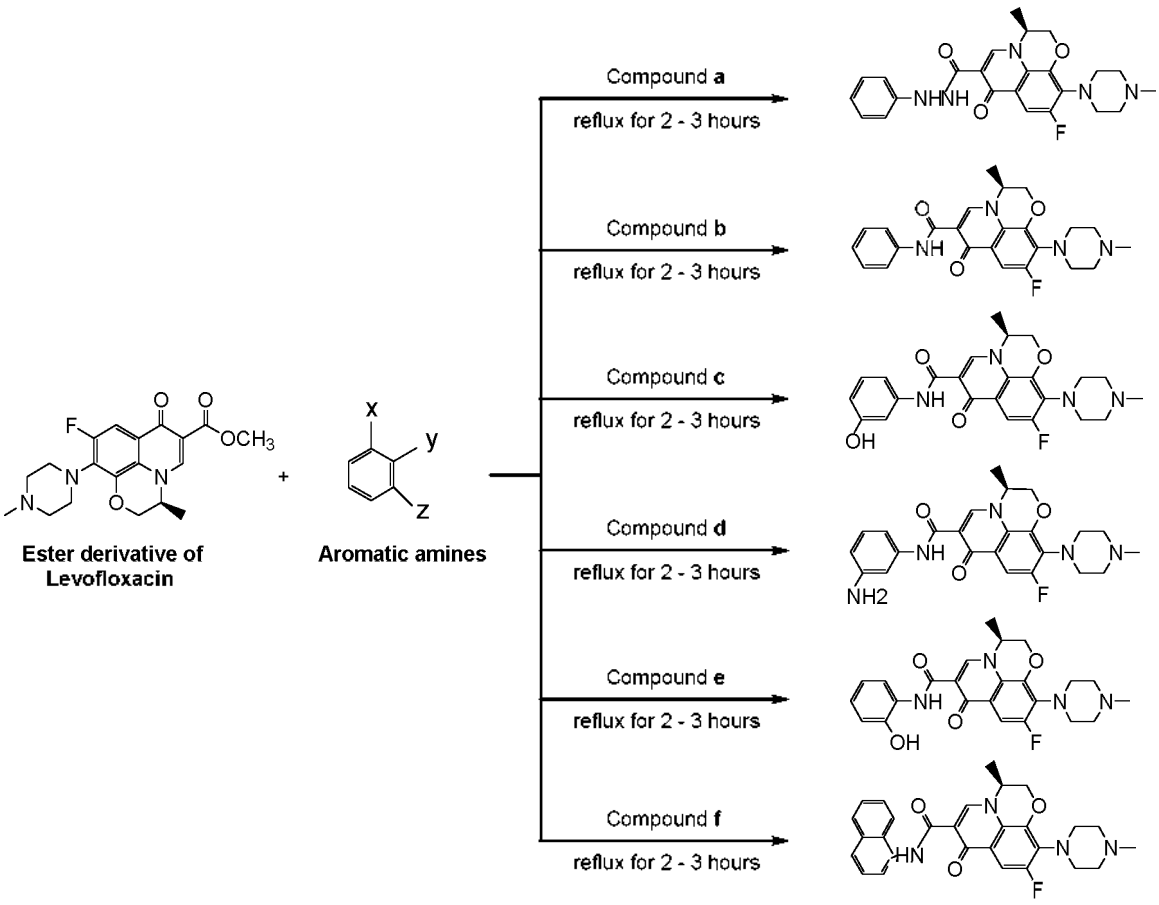

Figure 2. Synthetic Pathway of levofloxacin carboxamide analogues.

Table 1. Zone (mm) of inhibition of Levofloxacin and its derivatives at different concentrations (5, 10, 20 ppm).

S. No.	Microorganisms	**Levofloxacin**			**a**			**b**			**c**			**d**			**e**			**f**		
		5	10	20	5	10	20	5	10	20	5	10	20	5	10	20	5	10	20	5	10	20
		(ppm)			(ppm)			(ppm)			(ppm)			(ppm)			(ppm)			(ppm)		
1	*Staphylococcus aureus*	8	12	18	11	16	25	12	16	27	9	11	18	13	15	28	9	13	19	11	14	25
2	Citrobacter	15	18	21	16	20	22	14	19	20	14	17	20	16	20	22	16	20	21	16	20	22
3	*S. pneumoniae*	13	16	22	15	21	32	12	19	23	12	15	22	14	17	24	15	19	22	14	18	24
4	*Shigella flexneri*	10	12	15	11	13	17	12	17	26	14	15	22	11	13	17	15	18	24	12	14	16
5	*Escherichia coli*	14	16	22	12	15	20	13	15	19	14	15	21	12	15	20	15	18	22	12	15	20
6	*Salmonella typhi*	13	15	17	11	14	15	12	14	16	12	15	18	11	14	15	14	15	19	12	14	16
7	*Pseudomonas aeruginosa*	13	14	14	14	16	18	13	14	16	13	15	16	10	11	12	12	15	16	14	15	16
8	*Bacillus subtilis*	10	12	14	13	17	29	9	13	15	10	13	15	9	11	12	11	13	15	9	11	22
9	*Klebsiella pneumoniae*	12	16	21	13	18	24	11	17	22	11	12	18	13	18	24	10	18	22	13	18	28
10	*Proteus mirabilis*	11	12	14	12	15	18	12	13	14	13	17	24	12	15	18	14	18	26	12	14	16
11	*Corynebacterium hofmannii*	10	10	12	13	16	27	10	12	13	11	11	12	11	15	15	12	14	13	11	13	15

a = Phenyl hydrazine, **b** = Aniline, **c** = 3`amino phenol, **d** = Phenyline diamine, **e** = O`amino phenol, **f** = Alpha naphthylamine.

Table 2. Oxidative burst activity of (a - f) in whole blood phagocytes.

Compound	IC 50 on whole blood (ug/mL)
a	< 5
b	12.3 ± 1.2
c	20 - 25
d	20 - 25
e	< 5
f	13.1 ± 0.1

merase enzyme in bacteria) and topoisomerase IV enzyme.[22-23] The general function of topoisomerases is to facilitate the uncoiling of DNA during DNA replication, as well as to facilitate the recoiling and packaging of DNA once DNA replication has occurred.[24] Targeting DNA gyrase and topoisomerases may be a way to follow in searching for new chemotherapeutic agents against different microorganisms.

When the aryl group is phenyl **b** it was fairly active against *Staphylococcus aureus* and *Shigella flexneri*. Introduction of OH group to aryl amine **c** and **e** showed respectable activity against *Proteus mirabilis* and *Shigella flexneri* respectively. Among these compounds, compound **d** bearing amino group on the phenyl substituent, exhibited the lowest potency against Gram-negative *Pseudomonas areuginosa* and greater potency against Gram-positive *Staphylococcus aureus*. When an aromatic ring was introduced to the phenyl substituent **f** significant enhancements of potency against *Bacillus subtilis*, *Klebsiella pneumoniae* and *Escherichia coli* were achieved. It is also worthwhile to point out that formation of hydrazide **a** at 6 postion exhibited the highest activity against all the *Gram-positive* strains tested, more potent than reference agent. It is proposed that in the terms of structure-activity relationship, the antibacterial activity profile against *Gram-negative* bacteria was modulated and enhanced by the phenyl attachment *via* amide linkage at the 6-position of the levofloxacin molecule.

The overall activity profile of compounds (**a-f**) against microorganisms revealed that there is remarkable difference in zone of inhibition values as compare to parent. In the terms of structure-activity relationship, the antibacterial activity profile against all bacterium was altered by addition of amino group in levofloxacin molecule. The alteration of substitution in amines made marked differences in activity. It seems that expansion of activity is due to better interaction of molecule with target enzymes or for penetration into these bacteria.

Fluoroquinolones have been studied for their immunmodulatory activity. These immunomodulatory effects can be attributed in particular to those flouroquinolones both *in vitro* and *in vivo* with a cyclopropyl-moiety at the position N1 of the quinolone core structure.[25] The immunomodulatory effects of the flouroquinolones are due to their effects on intracellular cyclic AMP and phosphodiesterases, on transcription factors such as NF-kappa B, activitor protein 1 and a triggering effect on the eucaryotic equivalent of bacterial SOS response. All these studies indicate that fluoroquinolones exert immunomodulatory activities in particular in latent or chronic infections.[26]

In order to test the immunomodulatory effect of the drug and its derivatives, we investigated their effect on the oxidative burst activity of whole blood phagocytes. The oxidative burst is an important step in bacterial killing and involves a series of metabolic events that take place when phagocytes are stimulated, resulting in the production of superoxide (O^{-2}), H_2O_2, and other more potent oxidizing radicals [27], which was then quantified by a luminol-enhanced chemiluminescence assay. Results indicate that the zymosan- induced oxidative burst in whole blood phagocytes was inhibited (up to 50% i.e. IC_{50}) by compounds **a** and **e** (< 5 ug/mL) exhibiting very prominent inhibitory activity. While a moderate inhibitory activity was showed by **b** and **f**. Structure activity relationship of certain inflammatory and immunomodulatory agents reveals that unlike levofloxacin, the presence of amino benzene nucleus or its amide analogues with varying substitutions are attributed to their immunomodulatory activity and have any effect on the oxidative burst response.

Conclusion

Development of bacterial resistance has led to the synthesis

of newer and more potent quinolones. As detailed above, ten carboxylic acid derivatives have been designed, synthesized, characterized and evaluated for their biological activities *in vitro* in order to discover potent agents against Gram-positive bacteria and Gram negative bacteria.

It was observed that when an aromatic amino group was introduced to carboxylic side, significant enhancements of potency against organisms were achieved from the levofloxacin nucleus. Moreover some of the derivatives were also found to modulate oxidative burst response of phagocytes. This suggests that they have potential to be anti-inflammatory, as they suppress the production of reactive oxygen species.

References

1. Mascellino, M. T.; Farinelli, S.; Iegri, F.; Iona, E.; De Simone, C. *Drugs Expert. Clin. Res.* **1998**, *24*, 139-151.
2. Hooper, D. C. *Biochim. Biophys. Acta* **1998**, *1400*, 45-61.
3. Lesher, G. Y.; Froelich, E. J.; Gruett, M. D.; Bailey, J. H.; Brundage, R. P. *Journal of Medicinal and Pharmaceutical Chemistry* **1962**, *5*, 1063-1065.
4. Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. *Journal of Medicinal Chemistry* **1980**, *23*(12), 1358-1363.
5. Sanders, C. C. *Rev. Infect Dis.* **1988**, *10*(3), 516-527.
6. Stephen, V. *Advances in Therapy* **2008**, *25*, 979-994.
7. Shen, L. L.; Mitscher, L. A.; Sharma, P. N.; Donnell, T. J.; Chu, D. W. T.; Cooper, C. S.; Rosen, T. *Biochemistry* **1989**, *28*, 3886-3894.
8. Crumplin, G. C.; Smith, J. T. *Antimicrob. Agents Chemother.* **1975**, *8*(3), 251-261.
9. Sorgel, F.; Kinzig, M. *Am. J Med.* **1993**, *94*, 44S-55S.
10. Sato, K.; Hoshino, K.; Tanaka, M.; Hayakawa, L.; Osada, V. *Antimicrob. Agents Chemother.* **1992**, *36*, 1491-1498.
11. Kuo, C. F.; Yeh, L. C.; Jia, Y. S.; Tai, C. W.; Cherng, C. T. *J. Med. Chem.* **2000**, *43*, 3809-3812.
12. Hooper, D. C.; Wolfson, J. S. In *Quinolone Antimicrobial Agents*, 2nd ed.; American Society for Microbiology: Washington, D. C. 1993; p 410-413.
13. Seyyedehsamira, J.; Mohammad, H. M.; Loghman, F.; Saeed, E.; Saeed, R.; Mitra, H.; Farahnaz, P.; Manzarbanoo, E.; Abbas, S.; Alireza, F. *European Journal of Medicinal Chemistry* **2009**, *44*, 1205-1209.
14. Domagala, J. M.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Nichols, J. B.; Solomon, M.; Worth, D. F. *J. Med. Chem.* **1988**, *31*(5), 991-1001.
15. Chen, Y. L.; Fang, K. C.; Sheu, J. Y.; Hsu, S. L.; Tzeng, C. C. *J. Med. Chem.* **2001**, *44*, 2374-2377.
16. Foroumadi, A.; Mansouri, S.; Kiani, Z.; Rahmani, A. *Eur. J. Med. Chem.* **2003**, *38*, 851-854.
17. Xiang, M.; Weicheng, Z.; Reto, B. *Bioorganic & Medicinal Chemistry Letters* **2009**, *19*(3), 986-989.
18. Foroumadi, A.; Emami, S.; Mansouri, S.; Javidnia, A.; Adeli, N. S.; Shirazi, F. H.; Shafiee, A. *European Journal of Medicinal Chemistry* **2007**, *42*(7), 985-992.
19. Anacona, J. R.; Toledo, C. *Transition Metal Chemistry* **2001**, *26*(1), 228-231.
20. Bauer, A. W.; Kirby, W. M. M.; Sherris, J. C.; Truck, M. *Am. J. Clin. Pathol.* **1966**, *45*, 493-496.
21. Helfand, S. L.; Werkmeister, J.; Roder, J. C. *J. Exp. Med.* **1982**, *156*(2), 492-505.
22. Souza, M. V. N. D. *Mini-Rev. Med. Chem.* **2005**, *5*, 1009
23. Anderson, V. E.; Osheroff, N. *Current Pharmaceutical Design* **2001**, *7*, 339-355.
24. Champoux, J. J. *Ann. Rev. Biochem.* **2001**, *70*, 369-413.
25. Dalhoff, A. *Infection* **2005**, *33*, 55-70.
26. Dalhoff, A.; Shalit, I. *The Lancet Infectious Diseases* **2003**, *3*(6), 359-371.
27. Osar, Z.; Samanci, T.; Demirel, G. Y.; Damci, T.; Ilkova, H. *Experimental Diab. Res.* **2004**, *5*, 155-162.