Bioorganic & Medicinal Chemistry Letters, Vol.2, No.7, pp. 643-646, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

DNA - GYRASE INHIBITION AND ANTIBACTERIAL ACTIVITY OF FLUORO-QUINOLONES : INFLUENCE OF THE POSITION OF THE FLUORINE(S).

P. Clairefond *[1], D. Bouzard [1], B. Ledoussal [1], E. Coroneos [1], S. Bazile [2] and N. Moreau [2].

[1] Bristol-Myers-Squibb Pharmaceutical Research Institute, 77422 Marne-La-Vallée Cedex 2, France

[2] CNRS, CERCOA - 2 Rue Henri Dunant, 94320 Thais, France

(*Received* 24 *April* 1992)

Abstract : A series of fluoro-quinolones was evaluated for antibacterial activity and DNA-gyrase inhibitory potency. The relative enhancement of the overall antibacterial activity resulting from C-6 or C-8 fluorine substituent was not accompanied with a major change of DNA-gyrase inhibitory potency.

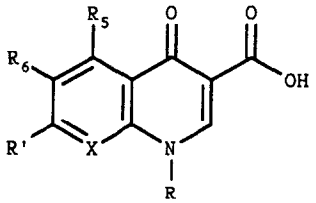
Since the discovery of nalidixic acid by LESHER in 1962[1], the quinolone antibacterials have emerged as a significant class of chemotherapeutic agents. The major structural change in structure-activity of both quinolone and naphtyridone series was the introduction of a fluorine at C-6. This gave rise to compounds which were dramatically more potent than nalidixic acid *in vitro* [2-3]. This modification continues to be a structural feature of all current synthetic analogues and is one of the major factors for the greatly increased activity of all of the current quinolones. However, the precise role of C-6 fluorine substituent has never been clarified.

As a means of gaining additional information on the role displayed by different substitutions of fluorine atom(s) at several positions of quinolones, our group has recently shown that C-6 fluoro atom can be moved to the C-8 position while incurring only minor loss of potency [4].

The primary mechanism of action of quinolones is inhibition of DNA-gyrase activity [5]. The aim of this report was to determine the role displayed by fluorine atom(s) at C-5, C-6 and C-8 of a series of 7-(3(S)aminopyrrolidinyl)-1-cyclopropyl-1,4-dihydro-4-oxo-3-quinoline carboxylic acids at the cellular and molecular level. We compared the antimicrobial activity and inhibitory potency of these compounds against E.Coli DNA-gyrase.

Compounds (Table I) were evaluated for *in vitro* antibacterial activity against a variety of organisms. The minimum inhibitory concentrations (MICs)[6] of these compounds against several Gram-positive and Gram-negative bacteria compared to ciprofloxacin are displayed in Table II.

Table I. STRUCTURE OF COMPOUNDS



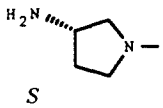
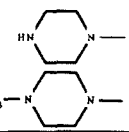
Compounds	X	R5	R6	R	R'
1	CH	H	H		
2	CH	H	F		
3	CF	H	H		
4	CF	F	H		
5	CH	F	H		
Nalidixic acid	N	H	H	Et	Me
Ciprofloxacin	CH	H	F		
Pefloxacin	CH	H	F	Et	

Table II. ANTIBACTERIAL ACTIVITY OF FLUORO-QUINOLONES (MIC, μg/mL)

Bacteria	Strain	CIP §	1	2	3	4	5
S. PNEUMONIAE	9585	0.25	2	0.13	0.5	4	4
E. FAECALIS	9809	0.5	4	0.25	0.25	4	16
S. AUREUS	9606	0.25	0.5	0.06	0.06	1	8
S. EPIDERMIDIS	25783	0.5	0.25	0.03	0.125	0.5	8
E. COLI	20697	0.008	0.03	0.008	0.007	0.06	0.25
K. OXYTOCA	20345	0.06	0.25	0.03	0.06	0.5	1
E. AEROGENES	20985	0.03	0.06	0.016	0.03	0.25	0.5
P. MIRABILIS	9900	0.008	0.13	0.03	0.03	0.13	0.5
M. MORGANII	15153	0.08	0.13	0.016	0.015	0.06	0.25
P. STUARTII	20615	0.03	1	0.03	0.06	0.5	1
S. MARCESCENS	20019	0.03	0.5	0.03	0.03	0.25	0.5
P. AERUGINOSA	9843	0.25	0.5	0.25	0.25	2	4
H. INFLUENZA	21515	0.03	0.08	0.002	0.007	0.06	0.13
P. PERFRINGENS	21284	0.06	N.D.	0.25	0.5	1	N.D.
P. RETTGERI	22424	0.5	8	0.5	1	8	16

§: CIPROFLOXACIN N.D.: Not Determined

Replacement of an hydrogen atom by an halogen atom especially fluorine at C-6 or C-8 resulted in an outstanding enhancement of the overall antibacterial activity. 6-fluoro and 8-fluoro-substituted quinolones showed excellent MICs against Gram-positive and Gram - negative bacteria, and displayed comparable antibacterial activity to ciprofloxacin. Koga *et al.*[7] did the same experiment in another series (1-ethyl-7-piperazinylquinolones) and found, unlike us, the C-8 fluoro compound 10-fold less active *in vitro*. The effect of the fluorine atom seems therefore to be a function of the choice of the N-1 and/or C-7 substituents. Introduction of a fluoro group at C-5 declined markedly the overall antibacterial activity. The 5,8 - difluoro analogue displayed intermediate *in vitro* antibacterial potency. The overall antibacterial activity of the 5,8 - difluoro compound was very similar to that of non - substituted analogue. 5-fluoro substitution effect was therefore counterbalanced by 8-fluoro substitution. Thus, 5-fluoro and 8-fluoro substitutions seem to induce compensating electronic effects.

Quinolones are broad - spectrum antibacterial agents which inhibit bacterial DNA-gyrase activity. The inhibitory activity of these compounds was therefore tested on E.Coli DNA-gyrase [8]. DNA-gyrase was purified from E. Coli HR 560, according to the method of R. Otter and N.R. Cozzarelli [9]. The results are shown in Table III, with MICs against E.Coli HR560 for appropriate comparison.

Table III. IN VITRO INHIBITORY POTENCY OF NEW FLUORO - SUBSTITUTED QUINOLONES ACAINST E. COLI HR 560 DNA - GYRASE (IC_0, IC_{100}, MIC : ug/mL).

COMPOUNDS	DNA - GYRASE		M I C
	IC_0	IC_{100}	
Nalidixic acid	25.0	200	2.5
Ciprofloxacin	0.12	10	0.009
Pefloxacin	0.64	20	0.019
1	0.32	20	0.009
2	0.24	20	0.005
3	0.32	10	0.009
4	0.32	10	0.078
5	0.64	20	0.156

All tested compounds inhibited actively E.Coli DNA-gyrase and displayed an inhibitory potency comparable to that of pefloxacin, and much higher than that of nalidixic acid. Monofluoro- and difluoro-substitutions lacked to modify markedly the inhibitory activity against E.Coli DNA-gyrase supercoiling effect. Even C-5 fluoro-substituted compound was found as potent as pefloxacin at the molecular site of action, while being 10-fold less active at the cellular level.

In summary, optimal antibacterial efficacy elicited through C-6 and C-8 fluorine substitution is not directly connected with the inhibitory activity against E.Coli DNA-gyrase. Transport into the cell and active efflux processes are also involved for some important part in the antibacterial activity. We have shown that the influence induced by fluorine substitution(s) on the antibacterial activity cannot be explained by a direct effect on the enzyme target of quinolones (i.e. DNA-gyrase). Low transport modalities into bacteria and through bacterial membrane, as well as active efflux, might impair the antibacterial efficacy of compounds eliciting a high DNA-gyrase inhibitory activity.

References and Notes :

1. Lesher, G.Y.; Froelich, E.J.; Gruett, M.D.; Bailey, J.H.; Brundage, R.P. *J.Med.Chem.* **1962** , *5* , 1063.

2. Cornett, J.B.; Wentland, M.P. *Annual Report in Medicinal Chemistry* **1986** , *21* , 139.

3. Fernandes, P.B.. *Annual Report in Medicinal Chemistry* **1987** , *22* , 117.

4. Ledoussal, B.; Bouzard, D.; Coroneos, E. *J.Med.Chem.* **1992** , *35* , 198.

5. Gellert, M. *Annu. Rev. Biochem.* **1981** , *50* , 879.

6. The minimal inhibitory concentration (MIC in ug/mL) of all antimicrobial agents was determined by the twofold serial dilution method using Tryptic Soy Browth (Difco). Bacterial inocula contained approximately 10^6 colony-forming units and the bacterial growth was observed after 20-hours incubation at 37°C. MIC endpoints were defined as the lowest browth concentration totally inhibiting organism growth (clear wall) after incubation.

7. Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. *J.Med.Chem.* **1980** , *23* , 1358.

8. a- Preparation of relaxed pBR 322 DNA :
Supercoiled closed pBR 322 plasmid DNA was relaxed using Calf thymus topoisomerase I (BRL), as described by the supplier.

b- Supercoiling assay :
50 ug of relaxed pBR 322 plasmid DNA were supercoiled in standard reaction mixture (20 mM Hepes, KOH (pH 7.6), 25 mM KCl, 4mM dithiothreitol, 6 mM Magnesium acetate, 0.5 mM EDTA, 1.7 mM spermidine, 1.7 mM ATP) with 2mM ATP and one unit of DNA-gyrase to the fully supercoiled form in 30 minutes at 37°C. The reaction was stopped at 0°C by addition of 0.4 mM bromophenol blue in 60% sucrose. Electrophoresis : Samples were loaded onto an 1% agarose gel and run at 72 volts under 312 nm illumination.

c- Inhibition of DNA-gyrase activity :
Inhibition of supercoiling activity was determined by adding variable concentrations of quinolone in the reaction mixtures just before addition of enzyme in each assay.
Increasing series of quinolone were :
0 , 0.04 , 0.08 , 0.12 , 0.16 , 0.24 , 0.32 , 0.48 , 0.64 , 1.0 , 1.3 , 2.6 , 5.2 , 10 , 20 , 40 ug/mL .
Results were expressed as :
- Early inhibition : IC_0, the last concentration without or with very low effect on the enzyme.
- Complete inhibition : IC_{100}, the concentration inactivating totally the enzyme.
All results shown are the mean of at least three separate determinations.

9. Otter, R.; Cozzarelli, N.R. *Methods in Enzymology* **1983** , *100* , 171.
E.Coli DNA-gyrase was purified according to the method of R. Otter and N.R. Cozzarelli, with slight modification : the elution of DNA-gyrase was directly performed with 8M urea.