ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 56 (2001) 37–40

IL FARMACO

Recent advances in the field of quinolones

Makoto Takemura *, Isao Hayakawa

New Product Research Laboratories I, Daiichi Pharmaceutical Company, Ltd., 16-13 Kita-Kasai 1-Chome Edogawa-ku, Tokyo 134-8630, Japan

Keywords: Quinolone; Antibacterial activity; Gram-positive; Gram-negative; Pharmacokinetics; Gyrase; Topoisomerase

1. Introduction

Fluoroquinolones have a broad spectrum of activity against Gram-positive, Gram-negative and mycobacterial organisms as well as anaerobes. The bactericidal effects of quinolones inhibit the function of DNA gyrase and topoisomerase IV [1].

They have excellent oral bioavailability, with good tissue penetration and so their role continues to widen, encompassing infections of the urinary tract, respiratory tract, skin and soft tissues, and sexually transmitted diseases [2]. Despite of these favourable properties, the earlier fluoroquinolones have limited potency against some clinically important organisms, especially Gram-positive pathogens and now the development of resistance of these organisms has become a serious problem. A major recent focus of this class has been the development of quinolones with enhanced activity against Gram-positive bacteria. This paper will review the in vitro activities and important characteristics of several newer agents, which have been prepared by chemical manipulation of the nucleus of the 4-quinolones. The basic molecule has been modified at the N-1 position, with different groups added to the C-5, C-6, C-7, and C-8 positions. These modifications result in changes in the antibacterial activity, pharmacokinetics and toxicological properties of the quinolones.

2. Antibacterial activity

The in vitro activities of several agents, with the exception of Gemifloxacin [3], which will be covered in another paper in this series, against Gram-negative and Gram-positive bacteria are shown in Table 1. The newer quinolones such as Trovafloxacin [4], Moxifloxacin [5], Sitafloxacin [6], and T-3811 [7] have extensive activity against Gram-positive bacteria, particularly against the pneumococcus, staphylococcus and enterococcus species, including resistant strains (Fig. 1).

Among them, Sitafloxacin, which is currently undergoing phase III clinical evaluation in Japan, is one of the most promising newer quinolones with well balanced activities against Gram-negative and Gram-positive bacteria including resistant mutants.

3. Structure–activity relationships for Sitafloxacin

Sitafloxacin has the *cis*-oriented (1*R*,2*S*)-2-fluorocyclopropylamine moiety, which is indispensable for the improvement of toxicological properties and pharmacokinetic profiles (Fig. 2). The in vitro antibacterial activities of Sitafloxacin were almost equal to those of the non-fluorinated analogue (DU-6668). As previously mentioned, the target enzymes of quinolones are bacterial DNA gyrase and topoisomerase IV, which are needed for the replication and transcription of bacterial DNA. DNA gyrase is a member of the type II class of topoisomerases. Mammalian cells also have the same type of enzyme, topoisomerase II. Sitafloxacin was potent in inhibiting DNA gyrase from *Escherichia coli* with similar potency to DU-6668, however it had lower activity against mammalian topoisomerase II than DU-6668, indicating good selectivity for bacterial target enzymes.

In the micronucleus test of these two compounds, Sitafloxacin was negative but the non-fluorinated compound (DU-6668) induced micronuclei in mouse bone marrow cells. Introduction of the fluorine atom to the cyclopropane ring was also effective in reducing the lipophilicity of the molecule. As a result of this effect, the urinary recovery of the unchanged compound of

* Corresponding author.
E-mail address: takemwkr@daiichipharm.co.jp (M. Takemura).

PII: S0014-827X(01)01029-1

Sitafloxacin was at a higher ratio than that of DU-6668, as shown in Table 2. While Sitafloxacin has the spiro-aminopyrrolidine ring at 7 position, the spiro-cyclopropane ring is favourable for reduction of CNS potency, due to quinolone antagonism of γ-amino-butyric acid (GABA) at both pre- and post-synaptic receptors [8], as shown in Table 3.

4. Chemistry

The quinolone derivatives were synthesised in convergent synthesis as outlined in Fig. 3. The trifluorobenzoic acid was transformed into the β-keto ester, which was subsequently converted to its ethoxy-acrylate derivative. Treatment of this ethoxy-acrylate derivative with the amine gave the enamine. Cyclisation of the enamine and hydrolysis gave the 4-oxoquinoline-3-carboxylic acid. The substitution of spiro-aminopyrrolidine at the C-7 position followed by chlorination gave Sitafloxacin. Sitafloxacin has three asymmetric carbon atoms in the molecule.

The most important key intermediate in preparing the component of the N1 position of Sitafloxacin, *cis*-2-fluorocyclopropanecarboxylic acid, was synthesised using transition metal catalysed cyclopropanation

Table 1
Comparative in vitro activities of new quinolones [a]

Organism (number tested)	MIC_{90} values (μg/ml)						
	CPFX	LVFX	TVFX	MFLX	GFLX	T-3811	STFX
Gram-negative aerobes							
E. coli (22)	0.5	0.5	0.5	1	0.5	1	0.06
H. influenzae (21)	0.008	0.015	0.015	0.06	≤0.004	≤0.004	≤0.004
S. marcescens (22)	1	2	2	2	2	8	0.25
K. pneumoniae (25)	0.5	1	1	1	1	2	0.25
P. aeruginosa (24)	0.25	2	2	4	2	4	0.25
Gram-positive aerobes							
Qr-MRSA (25)	>128	64	32	8	16	8	4
Pr-*S. pneumoniae* (18)	8	2	0.25	0.25	0.5	0.12	0.12
S. pyogenes (25)	1	0.5	0.5	0.5	0.5	0.25	0.06
E. faecalis (25)	32	32	16	8	16	2	2
E. faecium (23)	64	128	16	16	32	16	8

[a] MIC_{90}: minimum drug concentration inhibiting growth of 90% of the strain tested; qr: ofloxacin-resistant; MRSA: methicillin-resistant *S. aureus*; pr: penicillin-resistant.

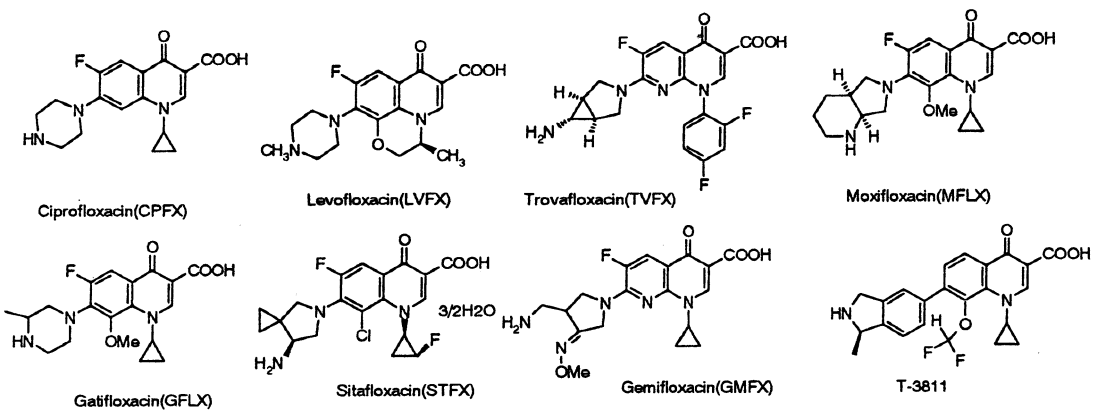

Fig. 1. Structures of quinolone antibacterial agents.

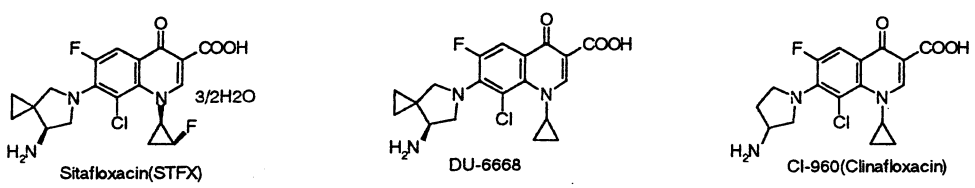

Fig. 2. Chemical structures of Sitafloxacin and related compounds.

Table 2
Influence of fluorine atom on the N_1-cyclopropane ring, MIC (μg/ml)

Organism	STFX	DU-6668	CI-960
E. coli NIHJ	≤0.003	<0.006	0.006
P. aeruginosa 321 21	0.05	0.1	0.1
S. aureus FDA209P	0.013	0.013	0.025
E. faecalis ATCC-19433	0.1	0.1	0.2
MIC (μg/ml) [a] *E. coli* KL-16	0.013	0.013	
IC_{50} (μg/ml) for Gyrase [b]	0.18	0.17	
Topoisomerase II [c]	>1600	434	
Topoisomerase I [c]	>1600	520	
Chromosomal aberration test	−	±	
Micronucleus test	−	+	+
Apparent partition coefficient ($CHCl_3$/0.1 M phosphate buffer, pH 7.4)	3.1	11.1	2.3
Urinary recovery in rats (20 mg/kg p.o.) () +conjugate	22 (23)	7 (14)	24 (29)

[a] Micro broth dilution 10^5 CFU/ml.
[b] Purified from *E. coli* KL-16.
[c] Purified from bovine calf thymus.

reaction. The hydrolysis of the ester and dechlorination gave the mixture(*cis*/*trans*) of 2-fluorocyclopropane–carboxylic acid. The desired *cis*-isomer was separated by fractional distillation. The racemic acid was converted to the chiral acid, which was rearranged into the amine according to the Curtius procedure using diphenyl phosphoryl acid and tert-butanol.

The other component was prepared from diketene, which was converted to the β-ketoamide. After cyclopropanation of active methylene, the ketone was protected, which was converted to chloride and cyclised to the dioxopyrrolidine derivative. The ketone was derived to the oxime, and then reduced to the spiro-aminopyrrolidine. The optical resolution was achieved with L-mandelic acid to give the *S*-enantiomer as shown in Fig. 4. The hydrogenolysis of the benzyl group gave the substituent of the C-7 position.

5. Conclusion

The quinolones described in this paper represent some of the most interesting new members of this class of compounds. Among them, Sitafloxacin is a highly designed compound and it exhibited potent antibacterial activity in vitro and in vivo.

The early clinical studies indicate the potential value of Sitafloxacin in the empirical and directed therapy of serious infection in hospitalised patients.

This will be confirmed in large scale clinical studies.

Table 3
Effect of C_7 spiro-cyclopropane ring on convulsive activity

	STFX	DU-6668	CI-960
Inhibition of GABA receptor binding (%) [Quinolone (10–5 M, 3–4 mg/ml)+BPAA (104 M, 22 mg/ml)]	14	16	70
Incidence of chronic convulsion (%) [Quinolone (100 mg/kg i.v.)+BPAA (400 mg/kg p.o.)]	0	0	100

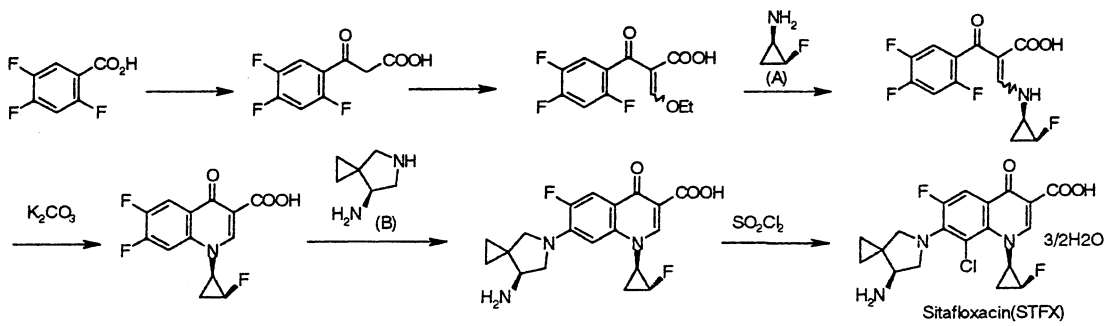

Fig. 3. Synthesis route of Sitafloxacin.

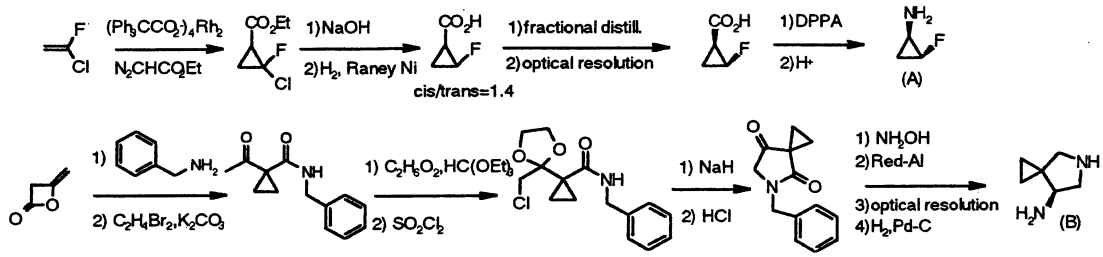

Fig. 4. Synthesis route of the substuents of Sitafloxacin.

References

[1] M. Gellert, K. Mizuuchi, M.H. O'Dea, T. Itoh, J.-I. Tomizawa, Proc. Natl. Acad. Sci. USA 73 (1976) 3872–3876. J. Kato, Y. Nishimura, R. Imamura, H. Niki, S. Hiraga, H. Suzuki, Cell 63 (1990) 393–404.

[2] V.T. Andriole, Drugs 58 (1999) 1–5 and Refs. cited therein.

[3] J.I. Oh, K.S. Paek, M.J. Ahn, M.Y. Kim, C.Y. Hong, I.C. Kim, J.H. Kwak, Antimicrob. Agents Chemother. 40 (1996) 1564–1568.

[4] K.E. Brighty, T.D. Gootz, A. Girard, J.A. Sutcliffe, M.J. Castaldi, D. Girard, M.R. Anderson, R. Borovoy, J. Faiella, S.L. Haskell, T. McKibben, S.A. Miller, 33rd Interscience Conf. On Antimicrobial Agents and Chemotherapy, 1993, Abstract 1509.

[5] A. Julia, B. Balfour, L.R. Wiseman, Drugs 57 (1999) 363–373.

[6] Y. Kimura, S. Atarashi, K. Kawakami, K. Sato, I. Hayakawa, J. Med. Chem. 37 (1994) 3344–3352.

[7] K. Hayashi, Y. Todo, S. Hamamoto, K. Ojima, M. Yamada, T. Kito, M. Takahata, Y. Watanabe, H. Narita, 37th Interscience Conf. On Antimicrobial Agents and Chemotherapy, 1997, Abstract F158.

[8] S. Hori, J. Shimada, A. Saito, T. Migahara, S. Kurioka, M. Matsuda, 25th Interscience Conf. On Antimicrobial Agents and Chemotherapy, 1985, Abstract 396; 26th Interscience Conf. On Antimicrobial Agents and Chemotherapy, 1986, Abstract 438.