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NMR Signal Assignment of a New Quinolone Antibiotic Substance

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Abstract : A new fluoroquinolone (DW-116) with a broad antibacterial spectrum was synthesized by introducing functional fluoropyridyl and methylpyrazine groups on N1, C7 position of quinolone moiety, respectively. ^{1}H and ^{13}C NMR signal assignments and structure were completely elucidated by 2D-NMR methods. Physicochemical properties of products were also investigated. DW-116 is decomposed at 306.9°C and the decomposition starts at around 285°C. The free base form is melt at 280.7°C and started to be decomposed immediately. DW-116 has two kinds of polymorphism which is important in drug action but these two plate and rod types have the same solubility in water. However the solubility is quite different in less or polar solvent. The plate type is more soluble in less polar solvent except in dichloromethane.

INTRODUCTION

In the late 1980s and 1990s, several quinolone agents with good activity against bacterial infections were synthesized and developed, such as sparfloxacin and ciprofloxacin These compounds are absorbed efficiently when taken orally, have long serum elimination half-lives and have a broad range of activities against aerobic pathogens. A new fluoroquinolone {DW-116[1,2], 1-(5-fluoro-2-pyridyl)-6-fluoro-7-(4-methyl-1-piperazinyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid hydrochloride, $C_{20}H_{18}F_2N_4O_3$•HCl, mol.wt 436.84} with a broad antibacterial spectrum[3-5] was synthesized by Dong-Wha pharmaceutical company in Korea and is currently under phase II clinical trials. Interestingly despite the fact that the antibacterial activity of DW-116 toward certain microorganisms *in vitro* was ~27.9 – 55.9 times less effective than that found with

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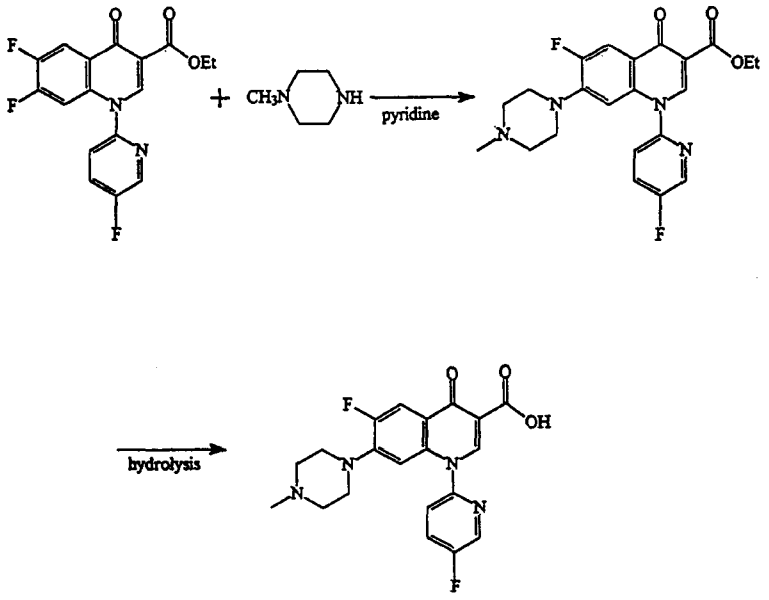
ciprofloxacin, the activity of orally administered DW-116 is comparable or superior to other fluoroquinolones in experiments utilizing infected mice as models.[6] One of the most important things in the developing new drug with good activity is elucidating and understanding the physicochemical properties of the active substances. In this report we describe the physicochemical properties of DW-116 and the structure elucidated by NMR.

EXPERIMENTS

Synthesis of DW-116

Fluoro-2-pyridyl group was introduced on N1 of quinolone moiety to display good antibacterial activities, and further introduction of methylpyrazine was made on C7 position of product by refluxing under pyridine solvent. The final DW116 was obtained by hydrolysis of ethylester form. Enhanced pharmacokinetics and antibacterial activity by substituting N1 position with even bulky group are demonstrated in tosufloxacin and temafloxacin. The procedures of synthetic steps, reaction conditions and yields are described in previous work.[2]



DW-116

Fig. 1. Chemical structure of DW-116 and synthetic routines.

Physicochemical Properties

Melting point of free base form of DW-116 was measured because DW-116 starts to be decomposed at 285°C with wide temperature range. 2g of DW-116 was dissolved in 200mL of water and 0.1N NaOH solution was added in until the precipitation did not take place any more. Melting point was measured by Mettler FP62 after the precipitation was filtered, washed with water and dried at 105°C for 2hrs. DSC(difference scanning colorimeter) experiment was performed with Perkin-Elmer DSC 7. Temperature was raised from 45°C to 350°C with scanning rate 5.0 °C /min.

Solubility test for the two kinds of crystal was carried out in many kinds of solvents such as methanol, ethanol, water, acetic acid, acetone, dichloromethane, chloroform. Enough amounts of two kind crystals were dissolved in each solvent individually so that the solution was saturated. Two types of crystal including plate and rod form are summarized in Table 1.

Table 1. Types of crystal according to the recrystallizing solvents

solvents	type of crystals
water	plate
methanol	rod
methanol + water (50 : 50)	plate
ethanol + water (95 : 5)	rod

The concentration of DW-116 was determined by using the clear supernatant solution after the solution was shaken for 30min was filtered and, if needed, diluted with mobile phase and injected to HPLC. Hygroscopic properties were tested as follows. Dried DW-116 was weighed which was dried at 120°C in vacuum oven and put in under relative humidity 80% condition so that the specimen absorbed water. The specimen was weighed according to elapsed time. EI-MS was measured by BIOTECH VB 2000. MS spectrum of DW-116 shows the peaks, m/z of 400, which is assigned to $[M]^+$(molecule), m/z 356, which is assigned to $[M\text{-}CO2]^+$, m/z 96, which is assigned to 5-fluoro-2- pyridyl group.

Spectroscopic Properties

IR spectrum was obtained by UNICAM Mattson 1000, using KBr pellet. IR spectrum has absorption at 3069, 1728, 1630, 1476, 1400, 1323, 1276, 1230, 561 cm^{-1}, UV absorption was measured with wave range from 200 to 400nm by Philips PU 8730. DW-116 was dissolved in methanol, water, 1N HCl(absorptions are 2830.6 nm, 318.4nm, 330.5 nm in methanol), 1N NaOH(absorptions are 273.1 nm, 324 nm, 333.7 nm in

methanol), buffer(absorptions are 269.6 nm, 324 nm, 333.7 nm in0.2 M KH2PO4, pH 6.5 by 2 N NaOH) and made to 0.0005%(w/v) solution.

NMR experiments were performed by Varian unity 300 (300 MHz) under deuterated trifluoroacetic acid at 22°C. ^{1}H NMR and ^{13}C NMR chemical shifts are reported in ppm with the chemical shift of the residual protons of the solvent used as internal standard. ^{1}H NMR, ^{13}C NMR and DEPT experiment were performed for chemical shift signal assignments. 2D NMR experiments including COSY, HMQC and HMBC experiments were performed in order to determine the molecular structure.

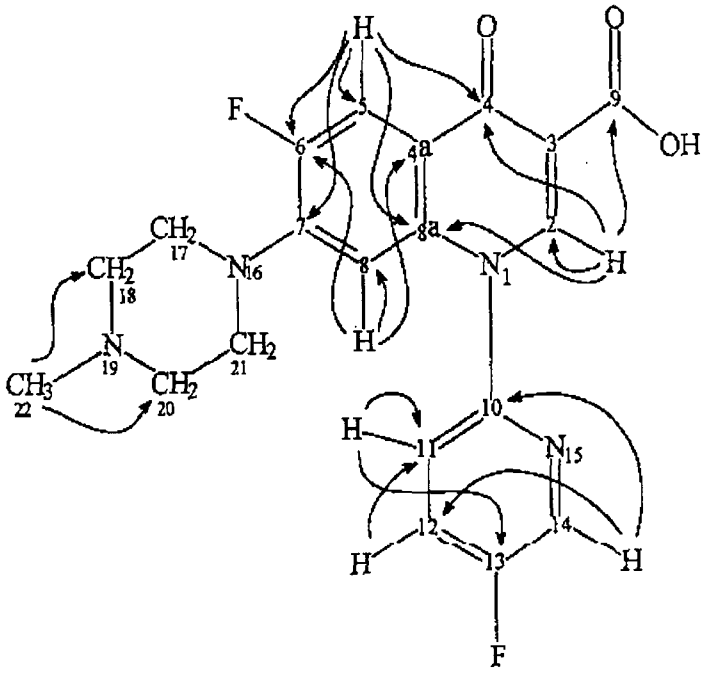


Fig. 2 Chemical structure of DW-116 with numbering scheme and selected ^{1}H-^{13}C J-network connectivities

Table 2. ^{1}H- and ^{13}C-NMR signal assignments for DW-116

Carbon Position	^{1}H- signal (multiplicity)	^{13}C-signal (multiplicity)
1		
2	9.10(S)	150.07
3		108.17
4		176.96
4a		119.81
5	8.23(d)	113.60(d)
6		156.25(d)
7		147.46(d)
8	6.75(d)	107.80
8a		140.52
9		170.76
10		148.75
11	7.83(m)	125.13
12	8.07(m)	129.76(d)
13		162.48(d)
14	8.71(d)	140.69(d)
17	3.55(m)	47.73
18	3.38(m)	54.96
20	3.85(m)	54.96
21	4.01(m)	47.83
22	3.08 (s)	44.68

RESULTS and DISCUSSION

IR spectrum for DW-116 has distinct absorptions at 2700~2250 cm^{-1}, which exhibit N-H stretching for tertiary ammonium and 1728, 1628 cm^{-1}, which exhibit two carbonyl functions and UV spectrum has the maximum absorption peaks at 269.9, 326.3, 333.7 nm in buffer solution(0.2 M KH2PO4, pH 6.5 by 2 N NaOH). UV λ_{max} varies on solvents and pH values. DSC pattern shows that DW-116 is decomposed at 306.9°C and the decomposition starts at around 285°C. The free base form is melt at 280.7°C and started to be decomposed

immediately. X-ray diffractograms show that DW-116 has two kinds of polymorphisms such as plate and rode type, but these two types of DW-116 have the same solubility in water, however, the solubility is quite different in less polar solvent. The plate type is more soluble in less polar solvent except in dichloromethane. DW-116 is sparingly soluble in water, slightly soluble in methanol and acetic acid, very slightly soluble in ethanol, acetone, dichloromethane and chloroform. DW-116 is hygroscopic and absorbs water to 8.3% a day and to 11.4% in 3days elapsed. Complete NMR signal assignments were made and chemical shifts are summarized in table 2. ^{1}H-^{13}C correlation experiments enabled to establish the covalent linkage of product as shown in fig. 2..

Acknowledgement

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