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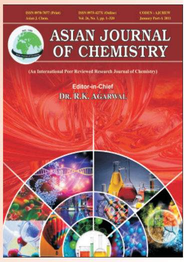

Synthesis of Three Fluoroquinolone Compounds

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Three fluoroquinolone derivatives were respectively synthesized by hydrolysis reactions of (*S*)-9-fluoro-10-cyano-3-methyl-2,3-dihydro-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid ethyl ester (compound **2**), using different hydrolysis conditions: LiOH, KOH and NaOH. Compound **2** was synthesized by a nucleophilic substitution reaction from (*S*)-9,10-difluoro-3-methyl-2,3-dihydro-7-oxo-7*H*-pyridine[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid ethyl ester (compound **1**), using solid sodium cyanide as the source of cyano group. The structures of these three fluoroquinolone compounds have been identified in appropriate methods.

Keywords: Pazufloxacin, Quinolone, Nucleophilic reaction, Hydrolysis.

INTRODUCTION

Pazufloxacin, one of the representative compounds of many fluoroquinolones (**Scheme-I**), was jointly developed by Toyama Chemical Co., Ltd. and Mitsubishi Pharma Corporation in 2002. Because of an intriguing action on Topoisomerase and DNA gyrase collectively, pazufloxacin has an advantage in inhibiting bacterial DNA replication and exerting antibacterial effect[1-7]. Many synthetic procedures about fluoroquinolones were described in the last thirty years[8-11]. The biological activity of fluoroquinolones is highly dependent on their constitutions: R_1 group can help to enhance the antibacterial effect and prolong the time of half-life; R_2 and R_4 groups are related to phototoxicity; and R_3 group is associated with many adverse drug reactions *in vivo*[12-18]. When if the R_3 group of fluoroquinolones is amino cyclopropyl (pazufloxacin), will help enhance the water-solubility and the antibacterial activity of Gram-positive bacteria. Some reported efficient antibacterial drugs, such as gemifloxacin[19] and moxifloxacin[20], both have appropriate modification in R_3 group.

After more than a decade's clinical application, there have been many adverse drug reactions of pazufloxacin. It is now well documented that the slight adverse drug reactions in gastrointestinal tract are related to the basic structure of fluoroquinolone, especially the R_3 analoges (**Scheme-II**)[21].

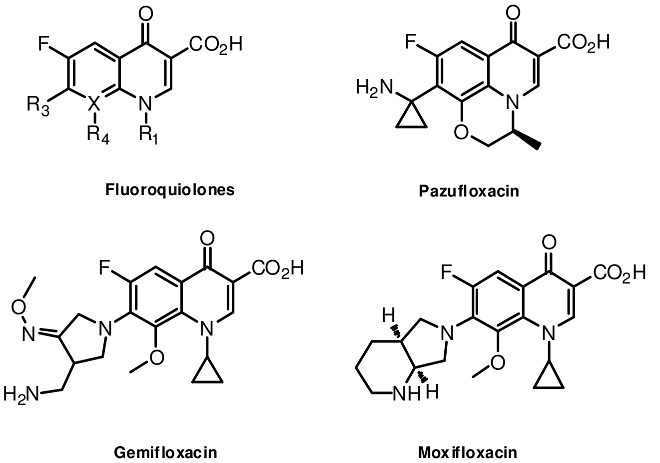
EXPERIMENTAL

Melting points were determined with a SGW X-4 micro melting point apparatus. ^{1}H NMR and ^{13}C NMR spectras were recorded using Bruker AV 500MHz spectrometer. ESI-MS were recorded on Dionex MSOPlus mass spectrometer. High resolution mass spectra were recorded on Finnigan MAT XL95 mass spectrometer. HPLC were recorded on Dionex Ultimate 3000. Optical rotations were obtained on a Perkin-Elmer 241 Autopol polarimeter.

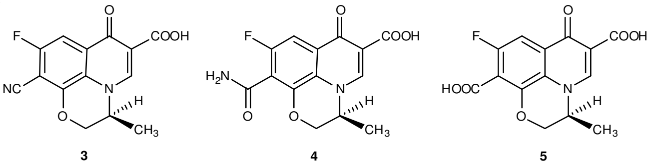
(*S*)-9-Fluoro-10-cyano-3-methyl-2,3-dihydro-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid ethyl ester (2): A mixture of **1** (5 g, 16.2 mmol) and sodium cyanide (1.6 g, 32.4 mmol) was stirred in DMF (50 mL) at 80 °C for 10 h. After cooling to ambient temperature, the precipitate was collected by filtration, washed with H_2O (5 mL) and dried in the vacum (60 °C) to give a pale yellow solid **2** (3.11 g, 9.8 mmol, 60.8 %), purity was determined 99.1 % by HPLC, which was directly used without any further purification. The filtrate was quenched by the aqueous solution of $FeSO_4$.

$[\alpha]_D^{20}$ = - 3.060 (c 1.0, NMP); m.p. 308-313 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.75 (s, 1H), 7.59 (d, J = 9.6 Hz, 1H), 4.95-4.80 (m, 1H), 4.73 (dd, J = 9.8, 6.2 Hz, 1H), 4.58 (d, J = 11.3 Hz, 1H), 4.32-4.18 (m, 2H), 1.51-1.39 (m, 3H), 1.34-1.26 (m, 3H). ESI-MS (*m/z*): 316.9 $(M + 1)^+$. ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.02, 163.58, 151.04, 147.47, 146.62, 130.23, 124.35, 115.60, 113.56, 110.89, 108.10, 69.85, 60.09, 53.87, 17.53, 14.24.

(*S*)-9-Fluoro-10-cyano-3-methyl-2,3-dihydro-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid (3): A magnetically stirred solution containing intermediate **2** (10 g, 31.6 mmol) in N-methyl pyrrolidone (730 mL) was treated



Scheme-I: Structure of fluoroquinolones



Scheme-II: Three possible impurities of pazufloxacin

with saturated aqueous solution of LiOH (60 mL) dropwise at 20-40 °C. The mixture was then stirred at room temperature for 20 min before addition of 2 N HCl (300 mL) and concentrated hydrochloric acid (10 mL). After cooling to ambient temperature, the precipitate was collected by filtration, washed with MeOH (20 mL), crystallization with EtOH-H_2O and dried in vacum (60 °C) to give a pale yellow solid **3** (5 g, 17.34 mmol, 54.9 %), HPLC 97.2 %.

$[\alpha]_D^{20}$ = + 2.558 (c 1.0, NMP); m.p. 240-245 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.90 (s, 1H), 10.10 (s, 1H), 8.25 (s, 1H), 5.02 (d, *J* = 5.8 Hz, 1H), 4.86 - 4.61 (m, 1H), 4.39 (d, *J* = 10.8Hz, 1H), 1.32 (d, *J* = 6.4Hz, 3H). ESI-MS (*m/z*): 288.9 (M + 1)$^+$.

(*S*)-9-Fluoro-10-carbamoyl-3-methyl-2,3-dihydro-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid (4): A solution of intermediate **2** (1 g, 3.16 mmol) in H_2O (20 mL) was heated to 70 °C. Then after the addition of KOH (1.8 g, 16 mmol), stirring was continued for another 3 h at 70 °C. The system was then cooled to room temperature and quenched with 2N HCl until a pH of 5 was reached. The precipitate was collected by filtration, dried in the oven and purified by column chromatography to give a pale yellow solid **4** (0.31 g, 1.01 mmol, 32.1 %), HPLC 97.0 %.

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.10 (s, 1H), 8.30 (s, 1H), 8.01 (s, 1H), 7.71-7.41 (m, 2H), 5.02 (s, 1H), 4.68 (d, *J* = 11.5Hz, 1H), 4.52 (d, *J* = 10.7 Hz, 1H), 1.54 (d, *J* = 6.5 Hz, 3H). ESI-MS (*m/z*): 306.8 (M + 1)$^+$.

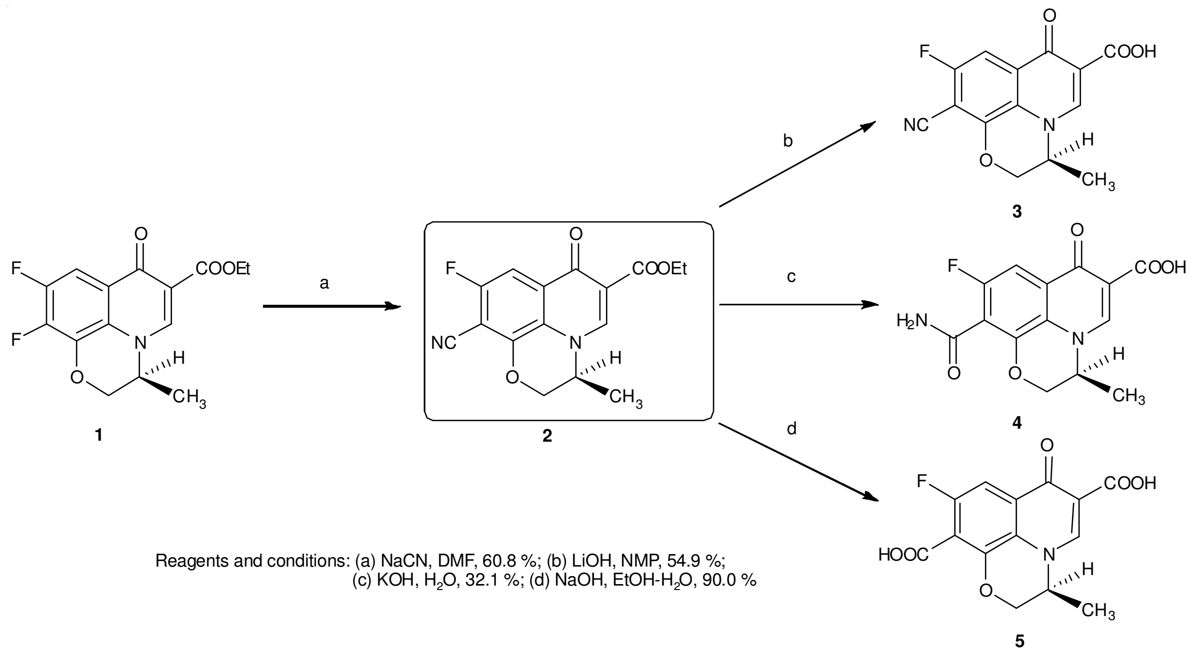
(*S*)-9-Fluoro-10-carboxyl-3-methyl-2,3-dihydro-7-oxo-7*H*-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid (5): A solution of intermediate **2** (1 g, 3.16 mmol) in EtOH (10 mL) was treated with aqueous solution of NaOH (10 mL, 2 mol/L) dropwise at room temperature. Then the system was heated to 100 °C for 5 h or more. After cooling to ambient temperature, the system was quenched by 6N HCl until pH < 1. The precipitate was collected by filtration, washed by water (5 mL), crystallization with EtOH-H_2O and dried in vacum to give a pale solid **5** (0.73 g, 2.37 mmol, 90 %), HPLC 98.9 %.

$[\alpha]_D^{20}$ = - 2.108 (c 1.0, NMP); m.p. 295-298 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.77 (s, 1H), 13.64 (s, 1H), 9.12 (s, 1H), 8.42 (s, 1H), 4.99 (d, *J* = 6.4 Hz, 1H), 4.58 (t, *J* = 24.8 Hz, 1H), 4.42 (dd, *J* = 22.2, 10.9 Hz, 1H), 1.49 (d, *J* = 6.6 Hz, 3H). ESI-MS (*m/z*): 308.3 (M + 1)$^+$.

RESULTS AND DISCUSSION

According to the reported synthetic route of pazufloxacin[22], we provide here a method of synthesizing some possible impurities.

We firstly synthesized compound **2**, which has never been reported before, with intermediate **1** as the starting material.



Reagents and conditions: (a) NaCN, DMF, 60.8 %; (b) LiOH, NMP, 54.9 %; (c) KOH, H_2O, 32.1 %; (d) NaOH, EtOH-H_2O, 90.0 %

Scheme-III: Synthetic procedure of impurities of pazufloxacin

TABLE-1
OPTIMIZATION OF REACTION CONDITIONS OF INTERMEDIATE 2a

Entry	Solvent	Temp. (± 2 °C)	Time (h)	Yield (%)[b]
1	formamide	80	7	47.6
2	DMAC	80	9	59.3
3	DMF	80	10	60.8
4	NMP	80	10	60.2
5	DMF	50	16	50.7
6	DMF	60	13	54.8
7	DMF	70	12	56.3
8	DMF	90	9	59.0
9	DMF	100	7	57.6

[a]Reaction conditions: 2eq solid sodium cyanide, intermediate **1** (16.2 mmol) in 50 mL solvent. [b]The yield was based on the data after recrystallization

We selected 2eq solid sodium cyanide as the source of cyano group after the preliminary examination. Nucleophilic substitution reaction generally requires heating with basic solvent, which is thought would help promote this reaction. The experimental results showed that DMF was better in this reaction (Table-1, entry1-4). We then tested different temperature and found that reaction at 80 ± 2 °C gave intermediate **2** in 60.8 % yield (entry 3, Table-1). It should be noted that higher temperature led to the decrease of intermediate **2** (entry 8-9, Table-1). It might be related to the slight decomposition of the intermediate **2**.

With the key intermediate **2** in hand, we then made some efforts to investigate the hydrolysis reactions of the cyano and eater group. In the reported hydrolysis reactions, cyano group could convert to carbamoyl group, or further carboxyl group, up to the reaction conditions. Accordingly, we chose LiOH, which is a relative moderate base and more selective to ester group in the presence of cyano group. Different solvents, such as THF, MeOH, NMP, were chosen in this selective hydrolysis reaction and the experimental results showed that NMP was the best choice. Briefly, dissolved in NMP, intermediate **2** was converted to product **3** in 54.9 % yield by dropping the saturated aqueous solution of LiOH.

Subsequently, based on the above results, the following reaction for the formation of product **4** required highly selective hydrolysis conditions. The hydrolysis process of cyano group in basic conditions contained two steps, first to amide and then hydrolyzed to acid. To obtain product **4**, we needed to keep the hydrolysis reaction staying at the first step. Then, with KOH and solvent H_2O in a moderate temperature, we got product **4**, isolated in 32.1 % yield.

Different from the former two products, it was not necessary to consider the selectivity in the synthetic process of diacid **5**. Therefore, it was proposed that the reaction should be smoothly carried out in strong basic conditions. Finally, in the presence of NaOH, EtOH-H_2O and high temperature (100 °C), we obtained product **5**, in 90 % crystallization yield.

Conclusion

In summary, an appropriate route is developed to several pazufloxacin impurities in two steps from intermediate **1** through a nucleophilic substitution reaction followed by three different hydrolysis reactions, which could help study the side effects of pazufluxacin. The structures of compounds **2-5** have been identified in appropriate methods. Further research work was under investigation in our laboratory.

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