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Isolation and Characterization of an Impurity Obtained During the Synthesis of the Antibiotic Drug Sparfloxacin

Nirmala MUNIGELA * [1], Moses BABU J. [1], Anjaneyulu YERRAMILLI [3], Peddy VISHWESHWAR [1], Ganesh YADDANAPUDI SESHA SIVA [2], Narayana Murthy VALAVALA [2]

[1] Department of Analytical Research, Discovery Research, Dr. Reddy's Laboratories, Miyapur, Hyderabad 500 049, India.
[2] Process R&D, Custom pharmaceutical synthesis-II, Dr. Reddy's Laboratories Ltd., Jeedimetla, Hyderabad, India.
[3] Jawaharlal Nehru Technological University, Kukatpally, Hyderabad-500072, India.

* Corresponding author. E-mail: nirmalam@drreddys.com (N. Munigela)

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Abstract

During the synthesis of Sparfloxacin, a fluoroquinolone antibiotic drug, an unknown impurity (**SF5-IMP**) was identified in the fifth stage of the synthetic process. The impurity has been isolated from the mother liquor of intermediate **SF5**. The mother liquor was concentrated to dryness added dichloromethane and stirred for 1 h and filtered to generate **SF5-IMP** and the molecular structure was elucidated as 7-amino-1-cyclopropyl-5,6,8-trifluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid by ^{19}F NMR and single crystal X-ray diffraction studies. The structural features of **SF5-IMP** and **SF5** have been discussed here.

Keywords

Sparfloxacin •^{1}H NMR • ^{19}F NMR • Single crystal X-ray diffraction

Introduction

Sparfloxacin, a fluoroquinolone antibiotic sold under the trade name, Zagam and other drugs belonging to the same class are Ofloxacin and Ciprofloxacin. Sparfloxacin has demonstrated better activity in the clinical trials than other quinolones [1] against a broad range of gram-positive, gram-negative [2] and atypical pathogens. These organisms cause

common lower respiratory tract infections and are responsible for severe mortality and morbidity around the world. Sparfloxacin was granted final FDA approval for the treatment of community-acquired pneumonia (CAP) [3] and acute bacterial exacerbations of chronic bronchitis [4] in 1996. In addition, Sparfloxacin showed *in vitro* activity against many penicillin-resistant strains of the gram-positive pathogen, *streptococcus pneumoniae*, as well as multi-drug resistant strains of the gram-negative pathogens *haemophilus influenzae* and *Moraxella catarrhalis*, although clinical efficacy has not yet been established.

In general, the synthesis of Active Pharmaceutical Ingredient (API) constitutes multiple steps. In an effort to synthesize Sparfloxacin, at fifth stage an impurity was isolated in very small amounts (0.15 % by HPLC). The purity of the drug substance is of paramount importance and any impurity should be controlled at 0.1 %. The impurities have to be adequately identified and characterized as per the International Conference on Harmonization (ICH) guidelines [5] and the United States Food and Drugs Administration (US-FDA) guidelines [6]. The structural characterization of this impurity is presented here.

Results and discussion

Chemistry

The synthesis of Sparfloxacin is outlined in Scheme 1. Chlorination of pentafluorobenzoic acid (**SF**) with thionyl chloride gave pentafluorobenzoyl chloride (**SF1)**, which on treatment with diethyl malonate in sulphuric acid gave 3-oxo-3-(pentafluorophenyl)propionic acid ethyl ester (**SF2)**. Treatment of **SF2** with cyclopropyl amine gave the precursor 1-cyclopropyl-5,6,7,8-tetrafluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**SF3**). **SF3** on further treatment with benzyl amine resulted 5-(benzylamino)-1-cyclopropyl-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester (**SF4**) in major quantities and also an impurity (**SF4-IMP**) was found but not isolated. The presence of this impurity was confirmed by LC-MS. Treatment of **SF4** with Conc. HCl gave an intermediate namely, 5-amino-1-cyclopropyl-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**SF5**). This on treatment with 2,6-dimethylpiperazine and *N*-methyl-2-pyrrolidone gave 5-amino-1-cyclopropyl-7-(3,5-dimethyl-piperazin-1-yl)-6,8-difluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (Sparfloxacin).

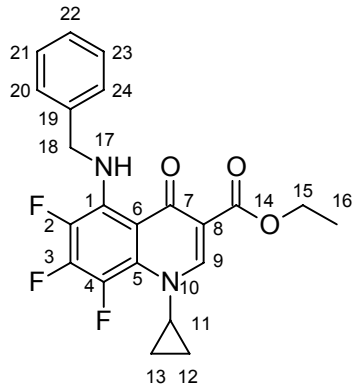
About 0.15% (by HPLC) of unknown impurity was isolated at the fifth stage (Scheme 1). The impurity is labeled as **SF5-IMP**. Isolation and structural characterization of this impurity has been carried out by ^{19}F NMR and single crystal X-ray diffraction. The spectroscopic data (^{1}H NMR, 2D-NMR, ^{19}F NMR and HR-MS) of all the intermediates and impurity were recorded. The structure elucidation of key intermediates **SF3**, **SF4**, **SF5** and **SF5-IMP** are described below.

Structure elucidation of SF3

The HR-MS of **SF3** has shown molecular ion at *m/z* 330.0749 (calculated mass = 330.0753), corresponding to the chemical composition $C_{15}H_{12}NO_3F_4$. The ^{19}F NMR displayed doublet of a doublet at δ -145.99 ppm (J=23,7Hz), broad doublet of a doublet at δ −147.10 ppm (J=23,7Hz), doublet of a triplet at δ −152.138 ppm (J=23,7Hz) and another triplet with δ −163.420 ppm (J=23,7Hz). Based on the three bond fluorine coupling (F-C-C-F; J= 23 Hz) we can deduce that all the four fluorine's are adjacent to one another.

Structure elucidation of SF4

The HR-MS of **SF4** intermediate showed the molecular ion at *m/z* 417.1426 (calculated mass = 417.4126), corresponding to the chemical composition $C_{22}H_{20}N_2F_3O_3$. The expected major component, **SF4** was confirmed with ^{19}F NMR (Table 1, Figure 1; CAS No. 110872-04-3). LC-MS reveals minor quantities of impurity (**SF4-IMP**) with same molecular formula (not isolated). The data indicates that the impurity could be an isomer of **SF4**.



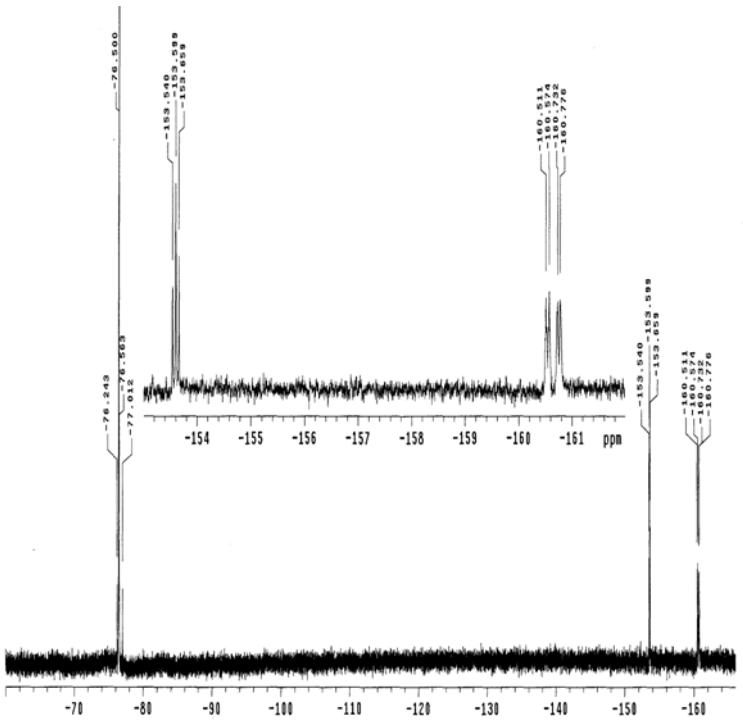


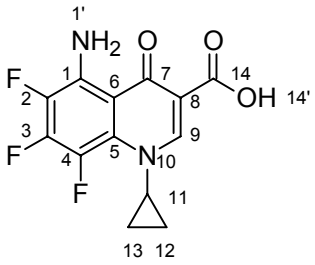
Fig. 1a **Fig. 1b**

Fig. 1a,b. Molecular Structure (left) and ^{19}F NMR spectrum (right) of **SF4**.

Structure elucidation of SF5 and SF5-IMP

To obtain structural insight of **SF5** and **SF5-IMP**, the spectroscopic data was generated. The HR-MS data of **SF5** and **SF5-IMP** displayed the protonated molecular ions at *m/z* 297.0490 (calculated mass = 297.0487), corresponding to the molecular formula $C_{13}H_9N_2F_3O_3$ and at *m/z* 297.0481 (calculated mass = 297.0487), corresponding to the molecular formula $C_{13}H_9N_2F_3O_3$, respectively. Since both **SF5** and **SF5-IMP** have the same mass, the impurity could be an isomer of **SF5.** The ^1H and 2D NMR data revealed that the basic quinolone ring is intact in both **SF5** and **SF5-IMP**. Hence, it is speculated that the position of amino group could be different on the aromatic ring.

To identify the position of the amino group, ^{19}F NMR spectrum of **SF5** (Fig. 2 and Table 1) was recorded. In **SF5**, the fluorine groups appeared as broad doublet at δ -161.92 ppm (J = 24.4 Hz), triplet at δ -152.11ppm (J = 24.4 Hz) and doublet of doublet at δ -162.96 ppm (J = 24.4, 7 Hz). From the splitting pattern and coupling constant values, it can be rationalized that all the three fluorine groups are adjacent to each other thus confirming the structure of **SF5** (Fig. 2). The single crystal X-ray diffraction also supports the molecular structure of **SF5** (Fig. 3).



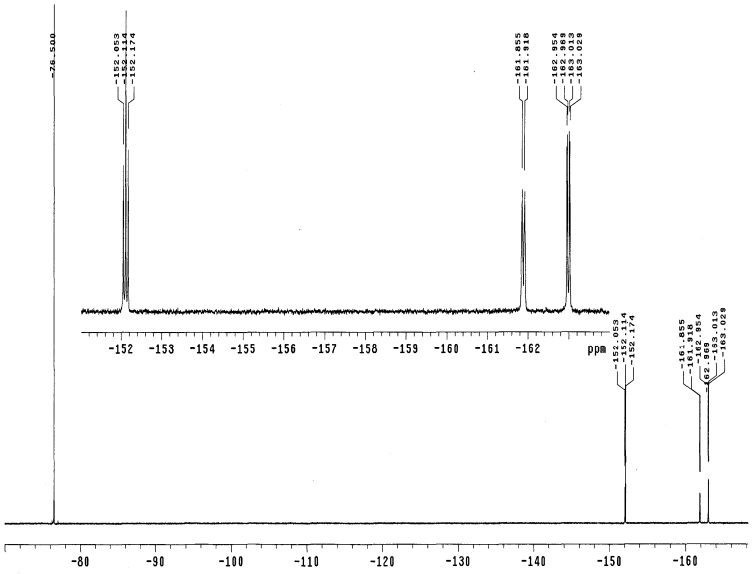


Fig. 2a **Fig. 2b**

Fig. 2a,b. Molecular Structure (left) and ^{19}F NMR spectrum (right) of **SF5**.

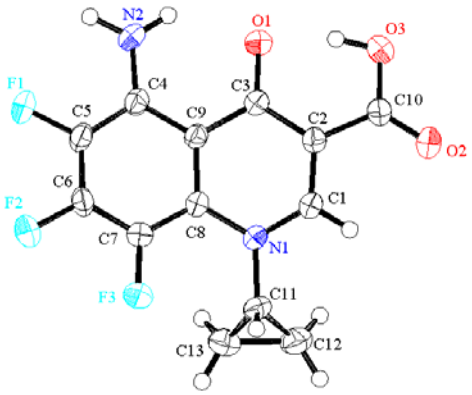
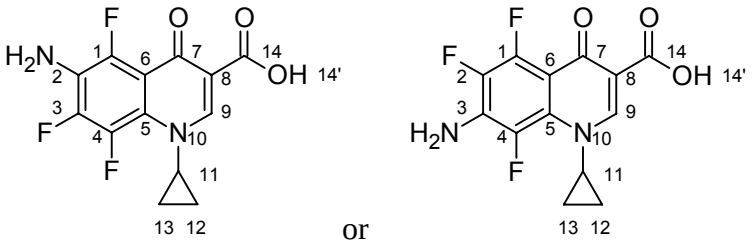


Fig. 3. The ORTEP of **SF5**, showing the atom-labeling scheme, with displacement ellipsoids drawn at the 50 % probability level for non-hydrogen atoms. H atoms are represented by circles of arbitrary size.

The ^{1}H NMR, ^{19}F NMR and ^{13}C NMR assignments of **SF5-IMP** are tabulated (Table 1). The ^{19}F NMR spectrum of **SF5-IMP** (Fig. 4) displayed signals of a broad singlet at δ -145.57ppm, doublet at δ -146.85 ppm (J = 23 Hz) and another doublet at δ -158.81 ppm (J = 23 Hz). This change in splitting pattern indicates that only two fluorine's atoms in **SF5-IMP** are adjacent to each other. The ^{19}F NMR suggests that the amine group could be at position 2 or 3 (Scheme 2). The single crystal X-ray structure of **SF5-IMP** (Fig. 5) confirmed the presence of amino group at position 3 (**II** in Scheme 2). Thus, **SF5-IMP** has been elucidated as 7-amino-1-cyclopropyl-5,6,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (Fig. 4b). The molecular structure of **SF5-IMP** reveals that the impurity has formed during the hydrolysis of the precursor, **SF4-IMP**. Hence, **SF4-IMP** is 7-(benzylamino)-1-cyclopropyl-5,6,8-trifluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (Fig. 6).



Sch. 2. The tentative molecular structures of SF5-IMP by ^{19}F NMR.

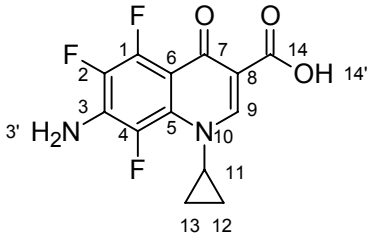


Fig. 4a

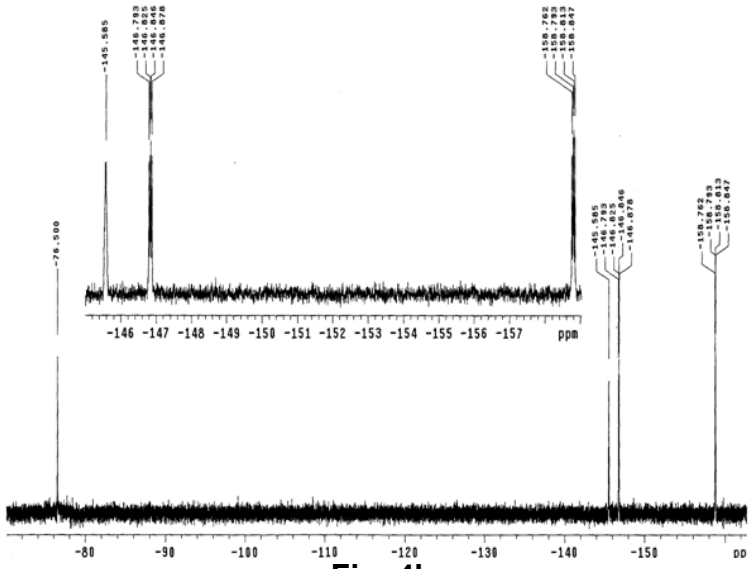


Fig. 4b

Fig. 4a,b. Molecular Structure and ^{19}F NMR Spectrum of **SF5-IMP**.

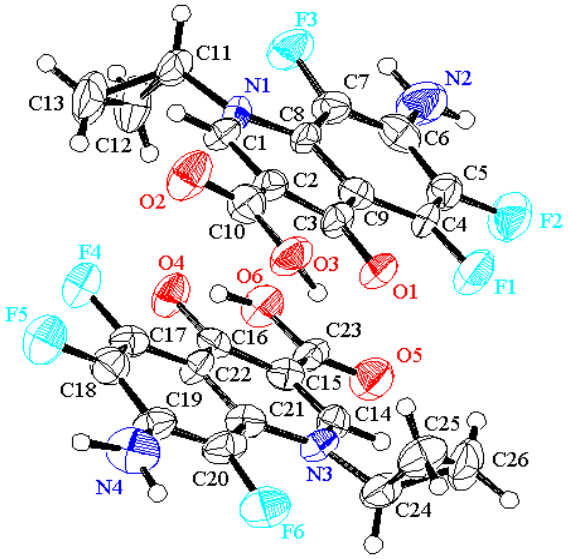


Fig. 5. The ORTEP of **SF5-IMP**, showing the atom-labeling scheme, with displacement ellipsoids drawn at the 50 % probability level for non-hydrogen atoms. H atoms are represented by circles of arbitrary size.

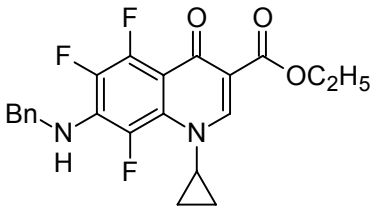


Fig. 6. Molecular Structure of **SF4-IMP**.

Experimental

Materials and methods:

The investigated samples were synthesized at Custom Pharmaceutical Synthesis (CPS)-II of Dr. Reddy's Laboratories Ltd. The solvents used for synthesis were LR grade purchased from the SD fine chemicals and Ranbaxy Laboratories, India.

Syntheses

a) Synthesis of 2,3,4,5,6-Pentafluorobenzoyl chloride (SF1):

To a solution of 2,3,4,5,6-pentafluorobenzoic acid (**SF**) (5.0 g, 23.7 mmol) in ethyl acetate (5 mL), catalytic amount of DMF (0.069 g 0.94 mmol) and thionylchloride (3.434 g, 28.86 mmol) were added at room temperature. The contents were refluxed at 80 °C for 12 hrs. Excess thionylchloride and solvent were removed by distillation and the crude reaction mass of **SF1** was directly used in the next step.

b) Synthesis of Ethyl 3-oxo-3-(pentafluorophenyl)propanoate (SF2):

A Grignard reagent of diethylmalonate was prepared by adding 1,2-dibromoethane (0.305 g, 0.16 mmol), magnesium (Mg) flakes (0.843 g, 34.70 mmol) in 5.70 mL ethanol and diethylmalonate (4.342 g, 16.30 mmol) and refluxed for 1 h and cooled to 25–30 °C. **SF1** (3.0 g, 13.04 mmol) was added to this mixture and refluxed for ca. 2 hrs. The reaction mass was quenched with 2N H_2SO_4 at less than 15 °C, and the solvent removed under reduced pressure to afford the crude product which was directly used in the next step.

c) Synthesis of 1-Cyclopropyl-5,6,7,8-tetrafluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (SF3):

To a solution of triethylorthoformate (2.836 g, 19.13 mmol), **SF2** (5.0 g, 17.72 mmol) and acetic anhydride (2.116 g, 20.73 mmol) were added at 25–30 °C and the contents were warmed to 100–105 °C, stirred under nitrogen atmosphere for one hour the distillate was collected (without vacuum) using a Dean-stork apparatus. This reaction mass was cooled to 70 °C and the acetic anhydride was distilled under vacuum and allowed to cool to about 30 °C. Toluene (25 mL) was added to this reaction mass, cooled to 5 °C. A solution of cyclopropyl amine (0.830 g, 14.5 mmol) in toluene (10 mL) was added and the contents stirred at 5 °C for about 2 hrs. The contents were subsequently warmed to about 25 °C and the mixture was washed with 5 mL of water, 3 mL of 2 % sodium carbonate (15 mL) and 5 mL of water. The organic layer was collected and potassium carbonate (3.778 g, 27.33 mmol) was added and the contents were heated to about 45 °C for 12 hrs. Toluene was distilled under reduced pressure and the solid obtained was washed with about 5 mL of water followed by 2.5 mL of methanol. The yellow colored solid obtained was dried under vacuum.

Tab. 1. ^{1}H, ^{19}F NMR values of **SF5**, **SF4** and **SF5-IMP**.

Position[1]	SF5							SF4							SF5-IMP						
	^{1}H	δ (ppm)	J (Hz)[2]	^{13}C	^{19}F	δ (ppm)	J C-F(Hz)[3]	^{1}H	δ (ppm)	J (Hz)[2]	^{13}C	^{19}F	δ (ppm)	J C-F(Hz)[3]	^{1}H	δ (ppm)	J (Hz)[2]	^{13}C	^{19}F	δ (ppm)	J C-F(Hz)[3]
1	2H	7.71	s	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1F	-158.75	dd
2	-	-	-	-	1F	-161.92	dd (24.4)	-	-	-	-	1F	-160.6	d (17.6)	-	-	-	-	1F	-146.81	dd
3	-	-	-	-	1F	-152.14	(24.4	-	-	-	-	1F	-153.6	t (24.4)	-	-	-	135.65#	-	-	-
3'	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2H	7.02 s	-	-	-	-	-
4	-	-	-	-	1F	-163.02	dd (6, 24.4)	-	-	dd (6, 24.4)	-	1F	-160.7	dd (6, 24.4)	-	-	-	-	1F	-145.59	br.s
5	-	-	-	128.16#	-	-	-	-	-	-	128.16#	-	-	-	-	-	-	128.59#	-	-	-
6	-	-	-	128.16#	-	-	-	-	-	-	128.16#	-	-	-	-	-	-	128.59#	-	-	-
7	-	-	-	164.73#	-	-	-	-	-		174.73#	-	-		-	-	-	166.18#	-	-	-
8	-	-	-	106.23#	-	-	-	-	-		196.30#	-	-	-	-	-	-	107.13#	-	-	-
9	1H	8.57	-	150.21*	-	-	-	1H	8.4		150.21*	-	-	-	1H	8.53	-	150.05*	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	1H	4.04	m (5.2)	40.42*	-	-	-	1H	3.95	-	39.25*	-	-	-	1H	4.06	m	40.05*	-	-	-
12	2H	1.17	d (4.4)	8.45*	-	-	-	2H	1.07	-	8.33	-	-	-	2H	1.16	m	8.24*	-	-	-
13	2H	1.13	d (8.4)	8.45*	-	-	-	2H	1.08	-	8.33*	-	-	-	2H	1.10	m	8.24*	-	-	-
14	-	-	-	179.96*	-	-	-	-	-	-	178.32*	-	-	-	-	-	-	177.09#	-	-	-
14'	1H	14.3	s	-	-	-	-	1H	15.08	-	-	-	-	-	1H	15.08	s	-	-	-	-
15	-	-	-	-	-	-	-	2H	m	4.22	59.95*	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	3H	m	1.26	14.25*	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	1H	s	10.58	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	2H	-	4.62	50.24*	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	142.14#	-	-	-	-	-	-	-	-	-	-
20-24	-	-	-	-	-	-	-	1H	m	~7.32	127.34#	-	-	-		-	-	-	-	-	-

[1] refer figure for numbering. [2] proton coupling constant and multiplicity. * deduced from gHSQC. # deduced from gHMBC. [3] fluorine coupling constant and multiplicity (Hz), s-singlet, d-doublet, dd-doublet of doublet, t-triplet and m-multiplet.

d) Synthesis of 5-(Benzylamino)-1-cyclopropyl-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (SF4):

To a solution of **SF3** (4.0 g, 12.10 mmol) in toluene (20 mL), sodium carbonate (2.008 g, 18.9 mmol) and benzyl amine (1.379 g, 12.8 mmol) were added and the reaction mass was refluxed for 78 hrs. The reaction mixture was cooled to about 25 °C, filtered through celite and the filtrate was washed with 24 mL of water and the solvent distilled to half of the volume and used directly in the next step.

e) Synthesis of 5-Amino-1-cyclopropyl-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (SF5):

To the **SF4** reaction mass, conc. HCl (23.00 mL) was added at about 40 °C and the solution was warmed to 80° C for 7 hrs and cooled to 20 °C. To this about 20 mL of water was added, stirred for 1 hr, filtered the solid, washed with water (40 mL) and dried well.

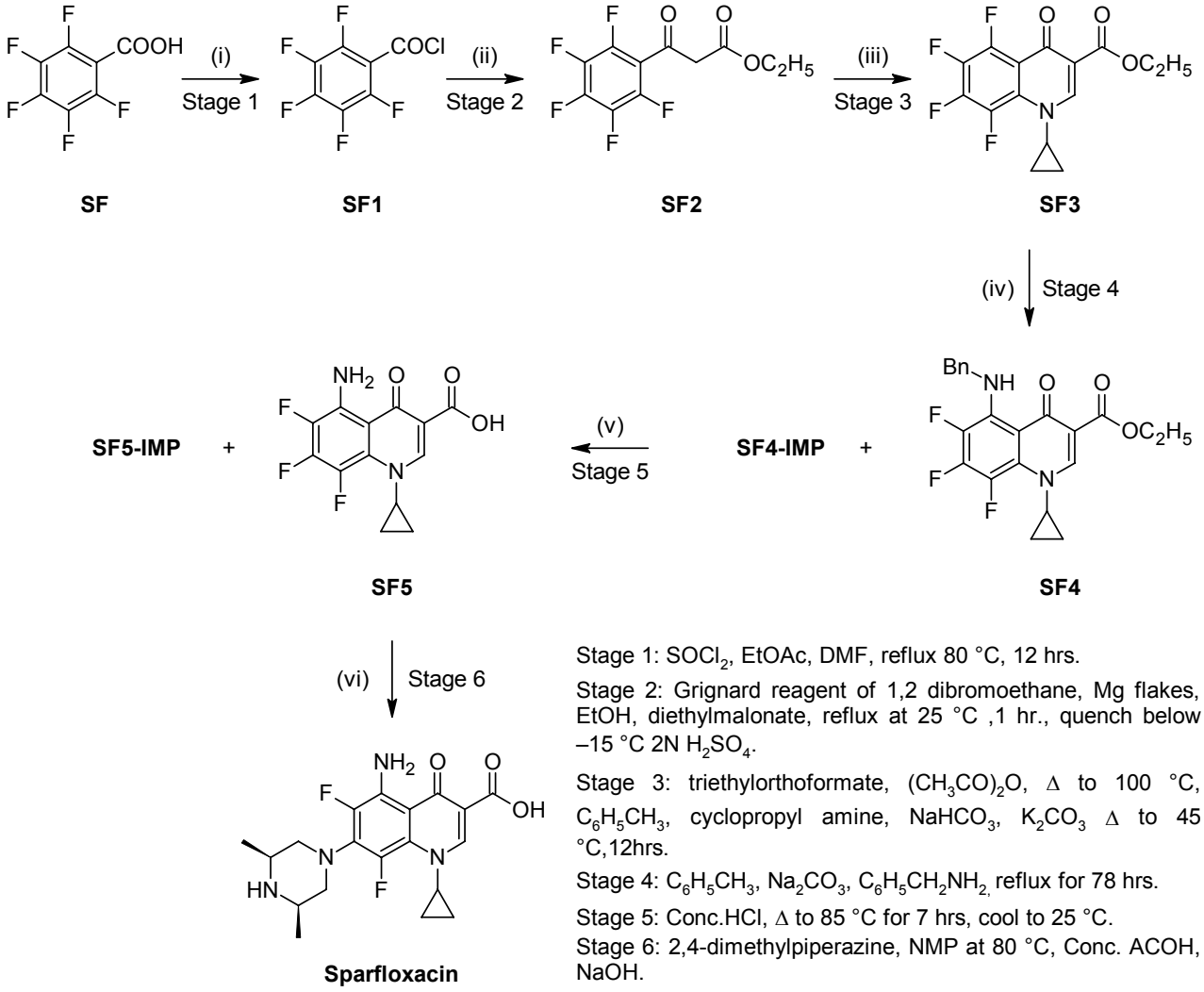
The product consists of **SF5** and **SF5-IMP** (4.0 g, 13.40 mmol). The **SF5** was purified by refluxing with methanol (40 mL) for about 2 hrs and cooled to RT under nitrogen and filtered.

f) Isolation of 7-Amino-1-cyclopropyl-5,6,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (SF5-IMP):

The mother liquor obtained from the above step was concentrated to dryness. Added 2.5 mL of dichloromethane (DCM) and stirred for 1 hr and filtered to obtain **SF5-IMP**. This method was repeated thrice to obtain pure **SF5-IMP** in large quantities.

g) Preparation of 5-Amino-1-cyclopropyl-7-(3,5-dimethylpiperazin-1-yl)-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (Sparfloxacin):

The **SF5** (3.0 g, 10.05 mmol) on treatment with 2,4-dimethylpiperazine (1.16 g, 10.16 mmol) and *N*-methyl-2-pyrrolidone (30 mL) at 80–105 °C gave Sparfloxacin. Purification by acid base treatment using concentrated acetic acid and sodium hydroxide gave pure Sparfloxacin.



Sch. 1. Synthetic scheme of Sparfloxacin.

HPLC

The reagents used for HPLC analysis were Sodium Citrate (Loba chemie), sodium hydroxide (Qualigens 'ExcelaR'), acetonitrile (Merck HPLC grade), methanol (Merck HPLC grade) and milli Q Water.

A Waters Model Alliance 2695 Separations module equipped with a Waters 2996 photo diode array UV detector was used to monitor the reaction by Develosil ODS (150 x 4.6 mm) 5 µm using the mobile phase 0.02M sodium Citrate buffer (pH = 3.0 adjusted with dilute sodium hydroxide), acetonitrile in the ratio of 60:40 at a flow rate of 1.0 mL/min and UV detection at 275 nm. The run time was 35 minutes. The data was recorded using Waters Empower software. All the samples were dissolved in mobile phase, sonicated and filtered with 0.22µ syringe filter and then injected into HPLC.

UPLC-TOF

The UPLC-TOF-MS system consisted of an ACQUITY™ Ultra Performance Liquid Chromatography system and a Micromass LCT Premier XE Mass Spectrometer (High sensitivity orthogonal time-of-flight instrument; Waters, Milford, USA) equipped with a lock mass sprayer, operating in either the positive or negative ion mode. The source temperature was set at 120 °C with a cone gas flow of 10 l/h, a desolvation gas temperature of 250 °C, and a desolvation gas flow of 450 l/h. The capillary voltage was set at 2300V and the cone voltage to 30 V. All analyses were acquired using the lock spray to ensure accuracy and reproducibility; leucine- enkephalin was used as the lock mass. Sample of concentration 0.02 mg/mL in methanol was infused in TOF-MS at a flow rate of 10µL/min. High resolution (W mode, FWHM 10500) positive polarity scan responses were collected from *m/z* 100 to 1000 at a rate of 1.0 s/scan. The dynamic range enhancement (DRE) function was used. The lock spray frequency was set at 5s and data were averaged over 10 scans.

NMR

The ^{1}H, ^{19}F NMR and 2D NMR experiments (gDQCOSY, gHSQC and gHMBC) for intermediate in stage 3 (**SF3**) and intermediates obtained in stage 4 (**SF4)**, stage 5 (**SF5**) and impurity (**SF5-IMP)** shown in (Scheme-1) were performed in dimethyl sulphoxide (DMSO-d_6) solvent using Mercury plus 400MHz FT-NMR spectrometer. The ^{1}H chemical shift values were reported on the δ scale in ppm, relative to TMS (δ = 0.00 ppm) and the chemical shift values were reported relative to chloroform ($CDCl_3$ δ = 77.00 ppm) and DMSO-d_6 (δ = 39.50 ppm) and for the ^{19}F NMR triflouro acetic acid (TFA δ = 76.50 ppm) was used as internal standard.

Single crystal X-ray Diffraction

The X-ray data for the single crystal has been collected on Rigaku AFC-7S diffractometer equipped with Mercury CCD detector using graphite monochromated Mo-K_α radiation (λ = 0.7107 Å). The structure was solved with direct methods (SIR-2004) and refined using least squares procedure (CRYSTALS) using the crystal structure 3.8.1 software.

CCDC 715190 (for **SF5-IMP**) and CCDC 715191 (for **SF5**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

*Single crystals of **SF5**:*

Yellow colored block morphological crystals suitable for single crystal X-ray diffraction were obtained from DMSO by slow evaporation of the solution at ambient conditions. Crystal data: Formula $C_{13}H_9F_3N_2O_3$, M = 298.22, Triclinic, a = 8.339(4) Å, b = 9.010(4) Å, c = 9.470(4) Å, α = 115.192(4)°, β = 90.9041(6)°, γ = 113.736(4), V = 574.2(4) Å^3, T = 298 K, space group $P1$, Z = 2, ρ_{calc} = 1.725 g cm^{-3}, μ(Mo-K$_\alpha$) = 0.155 mm^{-1}, 6340 reflections measured, 2298 unique reflections, 1841 observed reflections [$I > 2.0\sigma(I)$], R_1_obs = 0.043, wR_2_all = 0.044.

*Single crystals of **SF5-IMP**:*

Yellow colored needle morphology crystals suitable for single crystal X-ray diffraction were grown from methanol and acetonitrile 1: 1 (*v*/*v*) mixture by slow evaporation of the solution at ambient conditions. Crystal data: Formula $C_{13}H_9F_3N_2O_3$, M = 298.22, orthorhombic, a = 11.193(12) Å, b = 11.857(11) Å, c = 18.263(18) Å, V = 2424(4) Å^3, T = 298 K, space group $P2_12_12_1$, Z = 8, ρ_{calc} = 1.634 g cm^{-3}, μ(Mo-K$_\alpha$) = 0.147 mm^{-1}, 26601 reflections measured, 3007 unique reflections, 1060 observed reflections [$I > 2.0\sigma(I)$], R_1_obs = 0.052, wR_2_all = 0.101.

Conclusion

Impurities were formed in the fourth (**SF4-IMP**) and fifth stages (**SF5-IMP**) of the Sparfloxacin synthesis. The unknown **SF5-IMP** was isolated and structure was elucidated as 7-Amino-1-cyclopropyl-5,6,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid. The molecular structure of **SF5-IMP** reveals that the impurity has formed during the hydrolysis of the precursor impurity, **SF4-IMP**. Hence, **SF4-IMP** can be inferred as 7-Benzylamino-1cyclopropyl-5,6,7-trifluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester.

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Authors' Statement

Competing Interests

The authors declare no conflict of interest.

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