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Crystal structure and fluorescence property of antofloxacin

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Abstract: Antofloxacin free base is prepared from antofloxacin hydrochloride by removing hydrogen chloride. Its crystal is obtained by slow evaporation of an acetonitrile-methanol mixed solution. Single-crystal X-ray diffraction reveals that the crystallography belongs to a triclinic $P\bar{1}$ space group with cell parameters: $a = 0.663\ 07(13)$ nm, $b = 0.898\ 39(18)$ nm, $c = 1.569\ 0(3)$ nm, $\alpha = 75.12(3)°$, $\beta = 87.92(3)°$, $\gamma = 77.57(3)°$. Antofloxacin shows no fluorescence in solution, but the crystalline state emits strong green light at 510 nm under the excitation of 360 nm, indicating a fluorescence enhancement induced by aggregation. It demonstrates that intermolecular packing and interaction in the crystal lead to the improved fluorescence quantum yield. These results provide important information for the further exploration of the structure-activity relationship of antofloxacin and the development of new drugs.

Key words: antofloxacin; flouroquinolones; crystal structure; fluorescence property

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Quinolone antibacterial agents are among the most attractive drugs in the anti-infective chemotherapy field. They are generally characterized by a broad antimicrobial spectrum, high efficiency and low cardiac toxicity since the advent of Naphthyridine acid in 1962. After nearly 50 years of rapid development, more than 10^5 quinolone compounds have been synthesized and screened for pharmacological activity, which creates a new era of synthetic antibiotics[1–2]. Although the fluorescence properties of quinolones have been reported many times in the literature[3–5], little attention is paid on the correlation between the fluorescence properties and the molecular structure of quinolones, which is important for understanding structure-activity relationships, designing new drugs, and developing new analysis methods[6].

Antofloxacin (see Fig. 1) is the first innovative fluoroquinolone antimicrobial drug developed in China, commonly used as antofloxacin hydrochloride in clinical trials[7]. It is authorized by the Chinese Patent Office on compound, synthesis, antimicrobial drug applications etc. in 2000, and it is approved for marketing by the State Food and Drug Administration (SFDA) in 2009. An HPLC method is established for the assay of antofloxacin hydrochloride which is found to be accurate and convenient for the quality control[8]. A UV spectrophotometry method is also used for the assay of antofloxacin hydrochloride in tablets and is found to be convenient with good reproducibility[9].

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As a new member of the fluoroquinolones, antofloxacin has a long biological half-life, a broad anti-bacterial spectrum, high antibacterial activity, a wide tissue distribution, good absorption and bioavailability, small side effects and other characteristics. Its overall performance is superior to that of similar drugs currently on the market especially for the treatment of infections in the dermal system, the respiratory system and urinary tract[10].

Antofloxacin is likely to become one of the most promising antibacterial drugs on the market. As part of our interests, the crystal structure and its solid-state fluorescence are reported and the correlation between them is analyzed. This research will provide important information for the further exploration of the structure-activity relationship of antofloxacin.

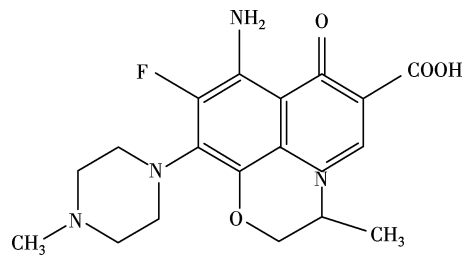

Fig. 1 Chemical structure of antofloxacin

1 Experimental Section

1.1 Chemicals and instruments

Antofloxacin hydrochloride are supplied by Nanjing Jinghua Biological Engineering Co., Ltd. Methanol, acetonitrile, anhydrous ethanol, 95% ethanol and NaOH are analytical reagents. The X-ray data are collected on a Rigaku SCXmini diffractometer (Japan with Manasseh); the absorption spectrum is measured on a V2201 type UV-Vis spectrophotometer (Japan Shimadzu company). The fluorescence spectrum is obtained on an FLS-920 type steady-state/transient fluorescence spectrometer (British Endinburgh Instruments Company).

1.2 Preparation of antofloxacin free base

A sodium hydroxide solution (0.43 g, 2.2 mL) is added dropwise to the solution of antofloxacin hydrochloride (4.13 g, 10.97 mol) in distilled water (65.0 mL) at 55 to 60 ℃ for a period of 15 min. Then the precipitation is filtered, washed with distilled water and 95% ethanol consecutively and then dried in vacuum to afford the antofloxacin free base as a yellow solid.

1.3 Preparation of antofloxacin single crystal

The antofloxacin free base (0.10 g) is dissolved in an acetonitrile solution (20.0 mL) with stirring, and then heated to reflux. A solution of acetonitrile (8.0 mL) and methanol (4.0 mL) is added until the solution is clarified. The

obtained solution is cooled and kept still at room temperature. After 3 d, the large yellow prism crystals of antofloxacin are obtained.

1.4 X-ray crystallographic determination of antofloxacin

The size of a single antofloxacin crystal is 0.20 mm × 0.28 mm × 0.21 mm. The single-crystal X-ray diffraction data of antofloxacin are collected on a Rigaku SCXmini diffractometer with graphite-monochromated Mo-Kα radiation (λ = 0.710 73 nm) by ω-scan technique at 293(2) K. The structures are solved by direct methods with the SHELXS-97 program, and refinements on F^2 are performed with the SHELXL-97 program by full-matrix least-squares techniques with anisotropic thermal parameters. All the non-hydrogen atoms are refined anisotropically, and hydrogen atoms are inserted at their calculated positions and fixed. The methyl H atoms are then constrained to an ideal geometry with C—H of 0.096 nm and $U_{iso,H} = 1.5U_{eq,C}$. All other H atoms are placed in geometrically idealized positions and constrained to ride on their parent atoms with C—H of 0.093 nm and $U_{iso,H} = 1.2U_{eq,C}$.

2 Results and Discussion

2.1 Crystal structure analysis of antofloxacin

The crystallographic data is summarized in Tab. 1. The selected bond length and bond angles are listed in Tab. 2 and Tab. 3, respectively. The molecular view and the crystal packing diagram of antofloxacin are shown in Fig. 2 and Fig. 3, respectively.

In the molecular structure of antofloxacin, the piperazine ring demonstrates a chair type structure with the angles of N1-C20-C22 and C20-C22-N2 being 113.63° and 113.58°, respectively. For the quinolone fragment, atoms F, N3, C17, O2, O3, O4 and C19 are all coplanar with the quinolone plane, while the atoms C24 and C30 show a deviation of 0.069 2 and 0.156 7 nm from the quinolone plane, respectively, and form a distortion angle of 93.23° (C30-C19-N4-C14). The plane of piperazine is nearly perpendicular to that of quinolone with a dihedral angle of 85.50°.

Tab. 1 Summary of crystallographic data for antofloxacin

Parameter	Value
Formula	$C_{18}H_{21}FN_4O_4$
Formula weight	376.39
Crystal system	Triclinic
Space group	$P\bar{1}$
Unit cell dimensions	a = 0.663 07(13) nm, α = 75.12(3)° b = 0.898 39(18) nm, β = 87.92(3)° c = 1.569 0(3) nm, γ = 77.57(3)°
Volume/nm^3	0.882 0(3)
Z	2
F(000)	396
Refinement method	Full-matrix least-squares on F^2
Goodness-of-fit on F^2	2.455
Final R indices [$I > 2\sigma(I)$]	R = 0.204 5, R_w = 0.583 0
R indices (all data)	R = 0.219 5, R_w = 0.590 7

Tab. 2 Selected bond length

Bond	Length/nm	Bond	Length/nm	Bond	Length/nm
O1-C7	0.124 0(8)	F-C12	0.133 7(7)	C5-N4	0.140 3(8)
C6-N2	0.142 5(9)	O2-C17	0.134 2(9)	C11-O4	0.137 7(8)
N4-C14	0.130 5(9)	N4-C19	0.149 0(8)	O3-C17	0.120 7(9)
O4-C24	0.149 1(14)	N2-C22	0.141 7(12)	N2-C27	0.143 0(2)
C19-C24	0.142 9(15)	C19-C30	0.169 0(2)	C20-N1	0.135 6(17)
C20-C22	0.150 0(13)	N1-C29	0.150 6(18)	N1-C23	0.152 0(2)
C23-C27	0.160 0(3)				

Tab. 3 Selected bond angles

Bond	Angle/(°)	Bond	Angle/(°)	Bond	Angle/(°)
C11-O4-C24	108.5(7)	C22-N2-C6	114.9(6)	C22-N2-C27	108.6(15)
C6-N2-C27	115.8(10)	C24-C19-N4	110.0(8)	C24-C19-C30	107.6(10)
N4-C19-C30	105.0(7)	N1-C20-C22	113.7(10)	C20-N1-C29	109.2(12)
C20-N1-C23	106.4(11)	C29-N1-C23	105.5(13)	N2-C22-C20	113.6(8)
N1-C23-C27	100.5(18)	C19-C24-O4	110.4(10)	N2-C27-C23	104(2)

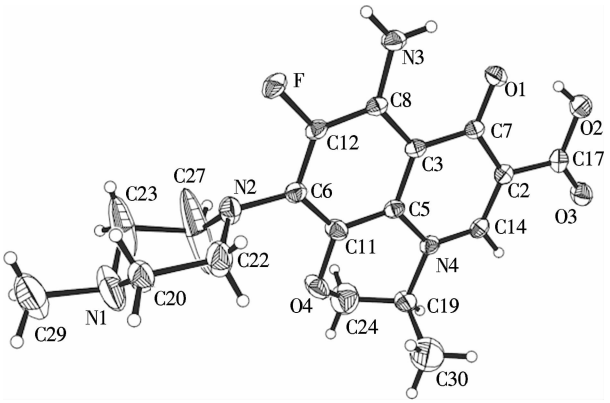

Fig. 2 Molecular structure of antofloxacin with displacement ellipsoids drawn at 30% probability level

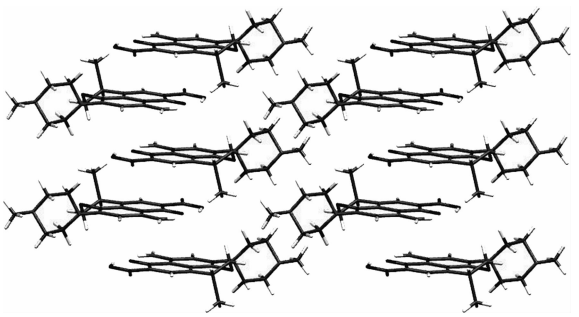

Fig. 3 Crystal packing of antofloxacin along b-axis

In the crystal structure, the intramolecular hydrogen bonds O_2-H_2…O_1 and N_3-H_{3B}…O_1 and the π bond stacking interaction (the adjacent parallel fluoroquinolone rings with the distance of 0.331 1 nm) are observed, which packs the molecules into intricate 3-D structures.

2.2 Fluorescence property of antofloxacin

Plotted in Fig. 4 is the UV-Vis spectrum of the antofloxacin in the ethanol solution. It shows that antofloxacin has absorption peaks at 226.5, 309.5 and 382 nm, respectively, and the strongest absorption peaks are located at 309.5 nm. No fluorescence is found in the solution, but in a solid state a strong green fluorescent light peaking at 510 nm can be found with excitation at 360 nm. Obviously, the fluorescence can be ascribed to the intramolecular π-π^* transition of the antofloxacin, and aggregation-induced fluorescence enhancement effect exists in its crystal.

Antofloxacin has a rigid planar structure with a large delocalized π bond, and has the lowest π-π^* excitation energy. In a crystal state, primary amino is released due to the re-

moval of HCl and forms hydrogen bonds with carboxyl oxygen atoms resulting in enhanced rigidity of the co-planarity, thus reducing the intramolecular non-radiative deactivation probability and improving the quantum efficiency of luminescence[11-12]. Meanwhile, the accumulation of molecules in the crystal mode also plays an enhanced role in their fluorescence.

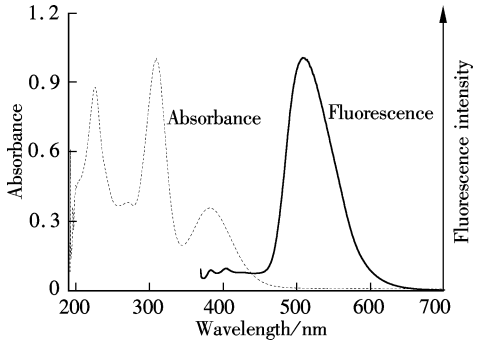

Fig. 4 UV-Vis spectrum of antofloxacin in ethanol solution (10^{-4} mol/L) and fluorescence spectrum in solid state (λ_{ex} = 360 nm)

3 Conclusion

A simple method for the preparation of antofloxacin and its single crystal is developed. The crystallography belongs to triclinic P1 space group. The crystal cell parameters are a = 0.663 07(13) nm, b = 0.898 39(18) nm, c = 1.569 0(3) nm, α = 75.12(3)°, β = 87.92(3)°, γ = 77.57(3)°. The crystalline antofloxacin demonstrates aggregation-induced fluorescence enhancement with an emission peak at 510 nm excited at 360 nm which may be due to the fact of intermolecular packing in the crystal structure. This paper provides important information for further exploration of the structure-activity relationship of antofloxacin and the design and development of new drugs.

References

[1] Hooper D C, Wolfson J S. Mode of action of the quinolone antimicrobial agents: review of recent information [J]. *Rev Infect Dis*, 1989, **11**(Sup 5): 902 - 911.

[2] Chu D T W, Fernandes P B. Recent developments in the field of quinolone antibacterial agents [J]. *Adv Drug Res*, 1991, **21**(1): 39 - 144.

[3] Yao W. Recent advances of fluorescence spectrometric methods for the studies of quinolone drugs[J]. *Journal of Huangshan University*, 2004, **6**(3): 67 - 70. (in Chinese)

[4] Du L M, Jin W J, Dong C, et al. Studies on correlation between protonation and tautomerism and luminescence characteristics of quinolone [J]. *Spectroscopy and Spectral Analysis*, 2001, **21** (4): 518 - 520. (in Chinese)

[5] Qi X J, Wang J X, Liu Z R. Fluorescent spectrometry of balofloxacin [J]. *Chinese Journal of Analytical Chemistry*, 2006, **34**(7): 1047 - 1048. (in Chinese)

[6] Wang Y, Li S W, Wu X M, et al. Study on fluorescence spectra of four quinolones [J]. *Journal of Sichuan Normal University: Natural Science*, 2003, **26**(6): 617 - 619. (in Chinese)

[7] Mei Y J. The novel high efficient and broad-spectrum drug-antofloxacin[J]. *Anhui Medical and Pharmaceutical Journal*, 2010, **14**(2): 229 - 231. (in Chinese)

[8] Hong J W, Hu C Q. Determination of enantiomeric impurity of antofloxacin hydrochloride by high-performance liquid chromatography [J]. *Chinese Journal of Antibiotics*, 2004, **29**(6): 349 - 353. (in Chinese)

[9] Mei Y J, Chen X M, Song C W, et al. Content determination of main components in antofloxacin hydrochloride tablets by ultraviolet spectrophotometry [J]. *Anhui Medical and Pharmaceutical Journal*, 2011, **15**(5): 573 - 574. (in Chinese)

[10] Li X M. Improvement and clinical evaluation of fluoroquinolone[J]. *Evaluation and Analysis of Drug-Use in Hospital of China*, 2003, **3**(2): 83 - 86. (in Chinese)

[11] Wang G P, Cai G Q, Zhu L G. Synthesis and crystal structure of fluoroquinolone complex of copper with two-dimension network [J]. *Chinese Journal of Inorganic Chemistry*, 2000, **16**(6): 987 - 989. (in Chinese)

[12] Yuan R X, Xiong R G, Zhang Z E, et al. Preparation, characterization, and X-ray crystal structure of a one-dimensional calcium-based coordination polymer with strong blue fluorescent emission [J]. *Inorg Chem Commum*, 2001, **4**(8): 430 - 433.

安妥沙星晶体结构及荧光性能

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摘要:以盐酸安妥沙星为原料,通过脱 HCl 反应制备了安妥沙星游离碱,在乙腈-甲醇混合溶剂中培养出单晶,经单晶 X 衍射测得其晶体结构属于三斜晶系,空间群为 $P\bar{1}$,晶胞参数:a = 0.663 07(13) nm,b = 0.898 39(18) nm,c = 1.569 0(3) nm;α = 75.12(3)°,β = 87.92(3)°,γ = 77.57(3)°. 安妥沙星的溶液无荧光,但其晶体在 360 nm 波长光激发下发射较强的绿光,最大发射波长位于 510 nm,存在明显的聚集诱导荧光增强效应,表明晶体内分子间的堆积及其相互作用有效地提高了荧光量子产率. 该研究为进一步探索安妥沙星的构效关系及同类结构新药的设计提供了重要的参考.

关键词:安妥沙星;氟喹诺酮类;晶体结构;荧光性能

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