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Short communication

Isolation, identification and in silico toxicity predictions of two isomers from cefotiam hydrochloride

Ye Tian[a,1], Ya-Nan Wang[b,1], Ying Han[a], Xia Zhang[a], Chang-Qin Hu[a,*]

[a] National Institute for Food and Drug Control, Daxing District, Beijing 102629, People's Republic of China
[b] Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China

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ABSTRACT

Two structural isomers of cefotiam in cefotiam hydrochloride for injection were observed, and the structures of the isomers were determined by mass spectrometry and various 1D and 2D NMR techniques. The thermo-isomerization mechanism of cefotiam was also discussed. Thermo-isomerization occurred not only in cefotiam but also in cephalosporins containing a 1-alkyl-1*H*-tetrazole-5-thiol side chain at C-3. Furthermore, the toxic effects of the two impurities of cefotiam hydrochloride were predicted and it is thought that they could be more toxic than cefotiam. The results reported in this article may be important for quality control and stability studies of this class of drugs.

1. Introduction

Cefotiam (Fig.1) is a second-generation semisynthetic cephalosporin antibiotic with a broad spectrum of activity against Gram-positive and Gram-negative bacteria, which include indole-positive Proteus and clinical isolates of cefazolin-resistant *Escherichia coli* and *Klebsiella pneumonia* [1,2]. Cefotiam hydrochloride is listed in the United States Pharmacopeia 40th Edition and Japanese Pharmacopeia 17th Edition [3,4]. Till date, the impurities from cefotiam hydrochloride have rarely been discussed [5,6]. The *N*-alkyltetrazolthiol ring (Fig. 1) at the C-3 position of cephems is responsible for the antibacterial activity of various drugs based on this class of compounds, such as cefmenoxime, cefamandole, cefoperazone, and cefmetazole [7]. The clinical use of cephalosporins containing an *N*-methyltetrazolthiol ring at C-3 (MTT) is associated with an increased incidence of vitamin K-responsive hypoprothrombinemia (inhibition of vitamin K-epoxide reductase) and a disulfiram-like effect (inhibition of aldehyde dehydrogenase) [8–10].

The impurity profile of a drug material is given in the ICH (International Conference

on Harmonisation) guidelines. For improvement and monitoring of the drug quality as well as the safety and efficacy of drug therapy, impurities exceeding 0.1% in a drug must be identified prior to clinical trials [11]. With the ever-increasing regulatory concerns on the quality and safety of pharmaceuticals, a systematic investigation of the impurities in bulk drug is of paramount importance. In this regard, liquid chromatography coupled with multistage accurate mass spectrometry (LC-MSn) has been widely used to identify the structures of such impurities [12]. However, because of the similarity of the fragmentation patterns, LC/MS alone cannot reveal the final structures of the isomer impurities in the active pharmaceutical ingredient (API); hence, nuclear magnetic resonance (NMR) is employed to obtain more conclusive evidence [13,14]. Herein, two isomer impurities in the API of cefotiam hydrochloride for injection were observed by LC-MSn; in addition, their structures were confirmed by NMR, and the isomerization mechanism was proposed. Furthermore, the toxic effects of the two impurities of cefotiam hydrochloride were predicted.

Abbreviations: ESI, electrospray ionization; DP, declustering potential; EP, entrance potential; CE, collision energy; IS, ion spray; EMS, enhanced MS; EPI, enhanced product ion; MTT, 1-methyl-1*H*-tetrazole-5-thiol; DMMT, 1-(2-dimethylaminoethyl)-1*H*-tetrazol-5-thiol; LC-MSn, liquid chromatography coupled with multistage accurate mass spectrometry; API, active pharmaceutical ingredient; NMR, nuclear magnetic resonance; HSQC, heteronuclear single quantum coherence; COSY, ^{1}H-^{1}H correlation spectroscopy; HMBC, heteronuclear multiple-bond correlation.

* Corresponding author.
E-mail address: hucq@nifdc.org.cn (C.-Q. Hu).
[1] Co first author.

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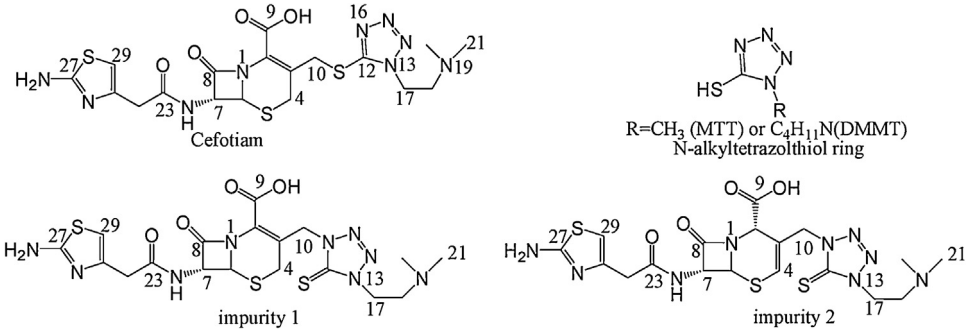

Fig. 1. Chemical structures of Cefotiam; N-alkyltetrazolthiol ring; impurity 1 and impurity 2.

2. Materials and methods

2.1. Chemicals and reagents

Cefotiam hydrochloride RS, Ceftezole hydrochloride RS, and 1-methyl-1*H*-tetrazole-5-thiol (MTT) RS were obtained from the National Institutes for Food and Drug Control (Beijing, China). HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific (Fairlawn, NJ, U.S.). Formic acid (98.0%) was supplied by Sigma-Aldrich Co. Ltd. (St. Louis, MO, US).

All analytical-grade reagents (Na_2HPO_4, KH_2PO_4, and KOH) were obtained from Beijing Chemical Works (Beijing, China). A Milli-Q water purification system (Millipore,Billerica, MA) was used to further purify glass-distilled water.

2.2. Instruments

A P680 HPLC pump, ASI-100 autosampler, PDA-100 diode-array detector (Dionex, Sunnyvale, CA, U.S.), Q-Trap 3200 mass spectrometer (Applied Biosystems, Foster City, CA, U.S.), Bruker Inova-600 NMR spectrometer (Bruker, Zurich, Switzerland), and Mettler Toledo electronic balance (Mettler Toledo, Greifensee, Switzerland) were used for our experiments.

2.3. Mass spectrometry conditions

Tuning and MSn investigation was carried out under the following optimized MS conditions: electrospray ionization (ESI) in positive ionization mode; declustering potential (DP), + 71.0 V; entrance potential (EP), +10.0 V; collision energy (CE), +10.0 V, curtain gas flow, 20.0 L h^{-1}; ion source gas 1, 65.0 L h^{-1}; ion source gas 2, 60.0 L h^{-1}; ion spray voltage (IS), 4500 V, and temperature (TEM), 500.0 °C, with the interface heater on. Enhanced MS (EMS) and enhanced product ion (EPI) spectra were acquired from *m/z* 50 to *m/z* 1200 in 0.1 amu steps with a dwell time of 2.0 s. Analyst software (version 1.5.1) was used for data acquisition and processing.

2.4. Liquid chromatography conditions

A Capcell Pak C18 MGIII (Shiseido,Tokyo, Japan) with dimensions of 250 × 4.6 mm i.d. was used for separation. The column eluent was monitored at a wavelength of 254 nm.

The mobile phase was 800 mL of 0.05 mol disodium hydrogen phosphate with 0.05 mol potassium dihydrogen phosphate (pH 7.2, adjusted by KOH, mobile phase A) and acetonitrile (mobile phase B); the flow rate was 1.0 mL/min. The gradient program is given in Table 1.

Table 1
Gradient program for LC method (analytical).

Time (min)	Mobile phase A (%,v/v)	Mobile phase B (%,v/v)
0	97	3
20	90	10
50	75	25
51	97	3
55	97	3

2.5. NMR measurements

NMR data were acquired using a Bruker-Avance 600 MHz instrument (both for ^1H and ^{13}C) at 25° in DMSO-d_6. The ^1H and ^{13}C chemical shifts are reported on the δ scale (in ppm) relative to TMS ($\delta = 0.00$) and DMSO-d_6 ($\delta = 39.5$ ppm) as the internal standards, respectively.

2.6. High-temperature degradation

Thermal degradation of solid cefotiam hydrochloride for injection was carried out by heating the compound in amber color bottles at the required temperature for the required time.

2.7. Theoretical studies on molecular conformation analysis in aqueous solution

(1) Molecular mechanical computation

The possible conformations were generated and minimized by Discovery Studio 4.0 (Accelrys Inc., San Diego, USA). The detailed density theory method, basis sets, and parameters were the same as those previously reported [15,16]. The conformers with lower energies and characteristic features were chosen as the global minima candidates for quantum chemical optimization.

(2) Quantum mechanical study

Geometry optimization and thermochemistry calculations were performed by the ORCA 3.0.3 program (https://orcaforum.cec.mpg.de/). The detailed density theory method, basis sets, and parameters were the same as those previously reported [15,16].

2.8. Molecular docking

The DS 4.0 software package (Accelrys Software Inc., CA, USA) was used for the docking study of selected targets and ligands. For protein preparation, the three-dimensional (3D) crystallographic structure of hyaluronan synthase 1 (HAS1) was generated through the homology modeling server SWISS-MODEL (https://swissmodel.expasy.org/). Before docking, hydrogen atoms were added to the unoccupied valence of the heavy atoms of the protein. The HAS1 protein was defined as a receptor using DS 4.0. For ligand preparation, the structures of the experimental compounds were downloaded from the PubChem Compound