Original article

Synthesis and in vitro antibacterial activity of Cephradine Benzoate

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Abstract

Aims & Methods: The present study was undertaken to compare the antibacterial activity of a cephradine derivative with that of the parent antibiotic cephradine. Cephradine was converted to its benzoyl derivative by Schotten-Baumann method for the first time. Disc diffusion method was employed to find out the antibacterial activity against *EPEC, ETEC, E. Agg, Salmonella typhi, Salmonella group B, Shigella boydii, Shigella dysenteriae 1, Shigella dysenteriae 2, Shigella flexinariae* and *Shigella sonnei*. Melting point, TLC, HPLC, UV, FTIR and ^{1}H NMR studies were carried out to check the purity and confirm that the derivative was cephradine benzoate. *Results*: The benzoyl derivative showed promising activity against tested bacteria. The results obtained from the study demonstrate that the benzoyl derivative could be a potential antibacterial agent.

Key words: Cephradine benzoate, antibacterial activity, disc diffusion method.

Introduction

The incidence of antibiotic resistance is on the increase in general but is quite high in some sectors like hospitals and childcare centres. It is therefore observed that the low cost antibiotics are not effective for the treatment of frequently seen infections. Therefore the use of newer and more expensive antibiotics are being more employed than before. However, this in turn also leads to resistance towards those drugs[1]. The high cost and development of more effective new antibiotics are the major issues at the moment. Still today, tuberculosis and pneumococcus are prominent examples of once easily treated infections where drug resistance is causing problem[2].

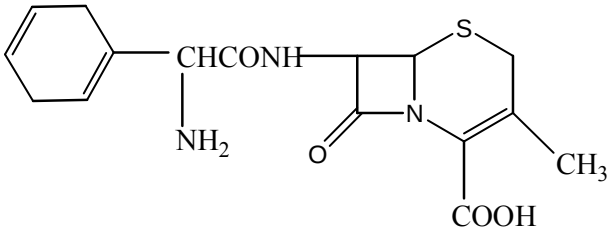

Figure.1: Structure of Cephradine

Cephradine (Fig. 1) is a first generation cephalosporin antibiotic. It has a broad spectrum bactericidal activity against both gram-positive and gram-negative bacteria including penicillinase-producing *staphylococci*[3]. It acts by inhibiting the bacterium's normal growth of cell wall and eventually causes the lysis of bacterial cell[4, 5]. It is indicated for the treatment of uncomplicated urinary tract and upper respiratory tract infections caused by susceptible organisms[6].

In this investigation, cephradine was converted to its benzoyl derivative and subsequently screened against some pathogenic microorganisms for antimicrobial activity.

Materials and Methods

General Experimental Procedure

Cephradine batch No. 010/2007 was collected from Gonoshasthaya Antibiotic Limited, Savar, Dhaka, Bangladesh. The study was carried out with the freshly prepared sample of cephradine. Melting points were determined in open capillary tubes on V scientific (India) melting point apparatus. Analytical thin-layer chromatography (TLC) was performed on precoated silica gel 60F-254 (Merck, Germany) and the spots were visualized under UV light. HPLC was recorded with an HPLC system (Shimadzu Corporation, Japan) consisting of solvent delivery system (LC10ATVP), UV-VIS detector (SPD-10AVP) with manual injector (772Si) and column oven (CTO-10ASVP). UV spectrum was recorded with a Shimadzu visible spectrophotometer (UV-1601PC) and IR spectrum was recorded on a Shimadzu FTIR spectrophotometer (FTIR-8400S). ^{1}H NMR spectrum was recorded on a Bruker DPX-400 spectrophotometer (400 MHz) using

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tetramethylsilane as internal reference at the Dhaka Laboratory of the Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh.

Preparation of Cephradine Benzoate

Benzoyl derivative of cephradine was prepared by following the Schotten-Baumann method[7]. To a solution of cephradine monohydrate, (6.9gm 0.02 mol in 30ml 5% NaOH solution) freshly distilled benzoyl chloride was added drop by drop with constant stirring over a magnetic stirring plate and stirring was continued for 20 minutes. Subsequently the whole content separated out as a pale yellow solid mass. The mass was crystallized from ethanol to give cephradine benzoate (5.2 gm) as pale yellow crystals. (Fig. 2).

Antimicrobial Screening

The antimicrobial activity of the cephradine benzoate was determined by the disc diffusion method[8]. The sample was dissolved in definite volume of methanol and applied to sterile discs at a concentration of 50µg/disc. The blank disc impregnated with methanol was used as control disc. The derivative was tested for antimicrobial activity against a number of gram negative bacteria (Table1). The bacterial strains used for the experiment as pure culture were procured from the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDR'B), Mohakhali. In this investigation cephradine (50µg/disc) was used as reference standard.

Results

The benzoate had m.p. 135-137°C (m.p. of cephradine 176-178°C) and gave one spot with R_f 0.72 in methanol-chloroform (3:7) solvent system, (R_f value of cephradine 0.21). A mobile phase consisting of methanol and distilled water (40:60 v/v) in a reverse C_{18} column was found suitable for elution of cephradine benzoate which gave only one peak (Retention time 7.896 minutes). UV: λ_{max} (methanol) 222.5 and 205nm; IR: ν_{max} (KBr) 3266.22 (ν_{N-H}), 3056.96 (ν_{C-H} aromatic), 1772.46 ($\nu_{C=O}$ of β-lactum), 1688.56 (ν_{CO-NH}), 1631.67 (ν_{CO-NH}, 1528.48 cm^{-1} ($\nu_{C=C}$ aromatic). ^1H NMR (400MHz, $CDCl_3$): δ 8.05 (1H), 7.81 (1H), 7.50 (1H), 7.43 (2H), 7.37 (1H), 7.07 (1H), 6.11 (1H), 5.67 (2H), 5.27 (1H), 5.01 (1H), 3.52 (1H), 3.21 (1H), 2.60 (5H), 2.16 (3H), 2.10 (1H). From the results of antimicrobial activity (Table 1), it is found that the benzoyl derivative showed identical activity to cephradine against *ETEC* having the zone of inhibition 15 mm each. It exhibited almost similar activity against *Salmonella group B* (18mm) and *E. Agg* (12mm). It revealed promising activity against *Shigella flexinariae* (18mm). It moderately inhibited the growth of *Shigella dysenteriae 2.* (10mm), and *Shigella sonnei* (10mm). *EPEC* (8mm) showed least sensitivity towards it. No zone of inhibition was found against *Salmonella typhi, Shigella boydii* and *Shigella dysenteriae 1.*

Discussion

Cephradine was converted to its benzoate by Schotten-Baumann reaction. The benzoyl derivative has not been prepared earlier. The compound showed IR bands at 3266.22, 3056.96, 1772.46, 1688.56 and 1631.67 cm^{-1} which could be attributed to N-H, aromatic C-H, carbonyl stretching of β-

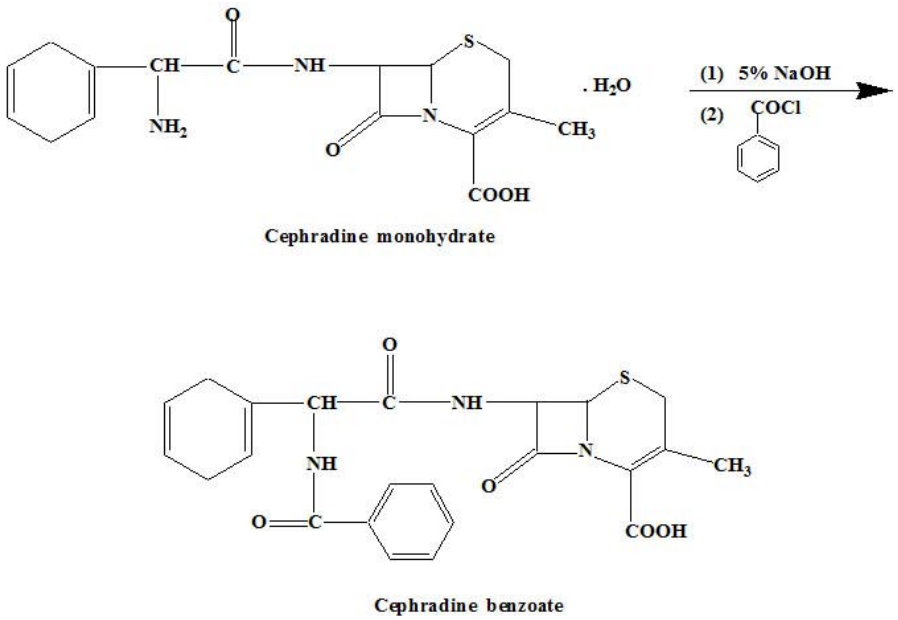

Figure.2: Synthesis of Cephradine benzoate

lactum and two amide group respectively of cephradine benzoate[9]. The ^{1}H NMR (400MHz, $CDCl_3$) spectrum had similar absorptions at δ 7.37 (1H), 7.07 (1H), 6.11 (1H), 5.67 (2H), 5.27 (1H), 5.01 (1H), 3.52 (1H), 3.21 (1H), 2.60 (5H), 2.16 (3H), 2.10 (1H) as in cephradine[10] with additional absorptions at 7.4 (5H) and 7.8 (1H) ppm. The former one can be ascribed to the aromatic protons and the latter to the amide protons of cephradine benzoate respectively. It is thus confirmed that the primary amino group of cephradine was benzoylated.

Antibacterial activity of the benzoate shows lower potency than that of cephradine against most of the tested organisms. However, it shows almost similar potency against *ETEC*, *E. Agg* and *Salmonella group B*. The structural changes by the benzoylation of cephradine obviously lower the polarity as well as basicity. This may explain the observed antibacterial results for cephradine benzoate.

Acknowledgements

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Table 1: Antimicrobial activity of the cephradine benzoate

Test Microorganisms	Conc. in µg/disc	Diameter of zone of inhibition (mm)	
		Cephradine Benzoate	Cephradine
EPEC	50	8	25
ETEC	50	15	15
E. Agg	50	12	15
Salmonella typhi	50	-	23
Salmonella group B	50	18	20
Shigella boydii	50	-	18
Shigella dysenteriae 1.	50	-	24
Shigella dysenteriae 2.	50	10	15
Shigella flexinariae	50	18	23
Shigella sonnei	50	10	24

Conc. represents concentration, µg symbolizes microgram, mm is millimetre and '-'stands for no zone of inhibition.

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