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Synthesis and Stability Studies on a Tripartite Prodrug of Cefuroxime

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ABSTRACT

N-Cbz-tryptophan was attached to cefuroxime at its 4-COOH via L-serine methyl ester to form compound 1. This compound is designed as a tripartite prodrug of cefuroxime for oral use. The compound was stable in pH 6.5 and pH 7.4 phosphate buffer solutions. Sixty percent of this compound remained intact after incubation in a mucosal suspension prepared from rat intestine at 37°C for 2.5 hr. The stability of this compound toward intestinal degradation indicated that it might be feasible as an oral prodrug of cefuroxime. Oral bioavailability in terms of the prodrug and the released parent drug is currently being investigated.

Key words: **cefuroxime, tryptophan, tripartite prodrug, stability.**

INTRODUCTION

Cefuroxime is a widely used β-lactam antibiotic with a broad antibacterial spectrum and resistance to β-lactamase[1], and which is particularly effective on respiratory pathogens[2,3]. Owing to the poor degree of oral absorption, the drug is administered mainly by injection. Even as an injectable drug, frequent administration is necessary due to its short biological half life. Efforts in developing an orally absorbable dosage form for cefuroxime have led to cefuroxime axetil[4-7]. Besides being active for respiratory infection[8], this drug also demonstrated effectiveness in drug combination therapy for the treatment of peptic ulcer where *Helicobacter pylori* is the underlying disease[9,10]. We aimed to develop orally absorbable prodrug derivatives of cefuroxime as alternatives to cefuroxime axetil.

Cefuroxime has a low apparent total volume of distribution (V_d = 11.1-15.8 liters /1.73 m^2). Its low capacity in plasma protein binding (33%), the consequent large portion of the free drug in circulation, and its high hydrophilicity might account for its fast elimination in urine and the short biological half life ($t_{1/2}$ = 1.1 hr)[11]. Based on the fact that cefuroxime has a small tissue volume of distribution, a change of the portion of the free drug in plasma might not effect any change on the total volume of distribution[12,13]. As drug clearance from the body is dependent on the free drug portion in plasma[12, 14], we thought that renal elimination might be delayed as a consequence of increasing the plasma protein bound portion of this drug. We therefore synthesized an ester prodrug 1 of cefuroxime (Fig. 1) with tryptophan

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attached to the molecule for this purpose. The rationale behind the structural design is that (1) as an ester derivative of cefuroxime, this prodrug might be orally absorbed; (2) the plasma bound portion of cefuroxime might be increased by virtue of the attached tryptophan moiety, already known to possess a high degree of plasma protein binding (75%)(15); and (3) tryptophan and the spacer L-serine, which links the cefuroxime and tryptophan moieties, are devoid of toxicity once released in vivo. This report describes the synthesis of this tripartite prodrug 1. Stability of this compound in phosphate buffer solutions, in comparison with a double ester prodrug 2 synthesized previously in this laboratory, was determined(16). As oral administration of the prodrug is desired, stability toward intestinal enzymatic degradation was also examined in a mucosal suspension preparation isolated from rat intestine.

MATERIALS AND METHODS

I. *Materials and Instruments*

Cefuroxime sodium was kindly offered by Standard Chem. and Pharm. Co. Ltd.. Solvents and reagents were commercial products from E. Merck, Aldrich, Wako or Kasai Companies. HPLC grade acetonitrile (CH_3CN) was purchased from Alpus Chemical Company. The MPLC chromatographic system consisted of a Buchi 681 pump, a Borosilicate 3.3cm column, and a ISCO-UA5 Absorbance/ Fluorescence detector. Melting points were determined on a Buchi 510 capillary melting point apparatus and were uncorrected.

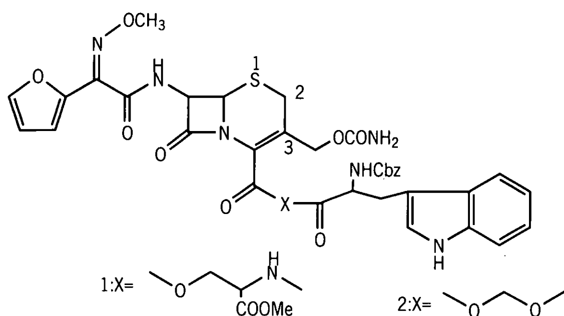


Figure 1. Structures of prodrugs 1 and 2.

Spectral data were obtained from a Perkin-Elmer 1760 FT-IR spectrophotometer, a Jeol JNS-D300 EI or CI mass spectrometer, and a Brucker 200 MHz or 400 MHz NMR spectrophotometer. Elemental analyses were determined with a Perkin-Elmer 240C elemental analyzer. Male Wistar rats weighing 200 - 350 g were used to prepare the intestinal mucosal suspension. The HPLC system contained a reverse-phase Lichrosphere CN column (Merck, 5μm, 250x 4mm) or a Nova-Pak C-18 column (Waters, 4μm, 150x3.9mm), a Waters 600E pump, a U6K injector, a Waters 484 Tunable UV/Vis Absorbance Detector and a Waters 745 Chromatocorder Data Module.

II. *Synthesis*

(I)*Methyl 2-[2-benzyloxyformamido-3-(3-indolyl) -propionyl]amido-3-hydroxylpropionate (3)*

Dicyclohexylcarbodiimide (DCC, 1.22 g, 5.91 mmol) and 1-hydroxy-benzotriazole monohydrate (HOBt, 1.22 g, 5.91 mmol) were added to a CH_2Cl_2 solution (20 ml) containing triethyl amine (0.8 ml, 5.93 mmol), L-serine methyl ester hydrochloride (0.92 g, 5.91 mmol) and N-(Cbz)-tryptophan (2.0 g, 5.91 mmol). The solution was stirred at ice bath temperature for 6 hr. The precipitate was removed by filtration. The filtrate was concentrated and the solid residue was partitioned between EtOAc (100 ml x 3) and water. The combined EtOAc solution was washed subsequently with aqueous $KHSO_4$ solution (5%) and saturated brine, and then dried over $MgSO_4$. EtOAc was evaporated in vacuo and the crude solid residue was recrystallized from toluene to give 1.61 g (58%) of compound 3 as white needles; mp 132-134°C; IR(KBr): 3413, 3378, 3311, 3277, 3052, 2950, 1749, 1734, 1693, 1651, 1640 cm^{-1}; ^1H-NMR (DMSO-d_6): δ 3.05-3.30 (m, 2H , -C$\underline{H}_2$-CH-), 3.30-3.65 (broad 5H, C$\underline{H}_2$-OH & -COOC$\underline{H}_3$), 4.36-4.43 (m, 2H, -C$\underline{H}$-CH_2OH & indole-CH_2-C$\underline{H}$-), 4.94 (m, 3H, -C$\underline{H}_2$-Ph & indole NH), 6.96-7.59 (m, 10H, aromatic H), 7.70 (d, J=8.64, 1H, amide NH), 8.31 (d, J=7.61, 1H, amide NH), 10.77 (s, 1H, -OH) ppm; EIMS m/z

(rel. intensity): 288 (6), 170 (3), 130 (100), 91(29); Anal. for $C_{23}H_{25}N_3O_6$: Calcd. C, 62.86, H, 5.73, N, 9.56; Found C, 63.03, H, 5.79, N, 9.44.

(II)*Preparation of Cefuroxime Free Acid*

Dilute hydrochloric acid (1N) was added dropwise to 100 ml of an ice cold aqueous solution of cefuroxime sodium (8.48 g, 0.02 mol) till the pH of the solution reached 2.0. The precipitate was collected by filtration and was washed with a minimal amount of water. The solid was dissolved in 200 ml of acetone and the solution was dried over $MgSO_4$. Acetone was removed in vacuo and the cefuroxime free acid was obtained as white powder. It was used directly in the following reaction.

(III) *{2'-[2-benzyloxyformamido-3-(3-indolyl) propionyl]amido-2'-methoxy carbonyl} ethyl (6R, 7R)-7-[2-(2-furyl)-2-(methoxyimino) acetamido]ceph-3-em-4-carboxylate (1)*

A mixture of cefuroxime (1.0 g, 2.35 mmol), compound 3 (1.0 g, 2.95 mmol), HOBt (0.40 g, 2.61 mmol) and DCC (0.70 g, 3.53 mmol) in CH_2Cl_2 (20 ml) was stirred in an ice bath temperature for 24 hr. The precipitate was removed by filtration. The filtrate was concentrated in vacuo and the solid residue was partitioned between EtOAc (100 ml) and water (100 ml). The EtOAc solution was washed subsequently with aqueous $KHSO_4$ solution (5%) and saturated brine, and dried over $MgSO_4$. The solvent was evaporated in vacuo. The crude residue was chromatographed three times in MPLC followed by recrystallization from benzene to give 0.61 g (30%) of compound 1 as white needles; mp 124-125°C; IR(KBr): 3359-3060(broad), 1775, 1748, 1717, 1673, 1526 cm^{-1}; ^{1}H-NMR($CDCl_3$): δ 3.10-3.25 (m, 2H, indole-CH_2), 3.46-3.70 (m, 5H, -$COOCH_3$ & C-2 H), 4.08-4.30 (s, 3H, $NOCH_3$), 4.30-4.45 (m, 2H, indole-CH_2-C$\underline{H}$), 4.45-4.50 (m, 2H, CH_2-OCO), 4.70-4.91 (m, 3H, CH_2-OCONH & indole NH), 5.00-5.10 (s, 2H, CH_2-Ph), 5.16 (d, J =7.89 Hz, 1H, C-6 H), 5.80-5.90 (m, 1H, C-7 H), 6.95-7.56 (m, 15H, aromatic H & $OCONH_2$), 8.10 (s, 1H, amide NH), 8.80 (s, 1H, amide NH) ppm; MS m/z (rel. intensity): 288 (10), 209 (3), 170 (8), 152 (3), 130 (100), 124 (10), 91 (32): Anal. for $C_{39}H_{39}N_7O_{13}S$: Calcd. C, 55.43, H, 4.65, N, 11.58; Found C, 55.37, H, 4.56, N, 11.29.

(IV)*Stability in Phosphate Buffer Solutions*

Ten milligrams of each test compound were dissolved in 15 ml of acetonitrile to form stock solutions. One hundred microliters of each stock solution were dissolved in 9.9 ml batches of phosphate buffer solution (pH 6.5 and a pH 7.4). The solutions were incubated in a 37°C water bath and 1 ml of each solution was withdrawn at time intervals from zero to 90 minutes of incubation. Two hundred microliters of each sampled solution were vortexed with 400 microliters of EtOAc and then centrifuged at 15000g rpm for five minutes. Ten microliters of the EtOAc solution were injected into the HPLC for quantitation.

III.*Stability Studies*

(I)*Preparation of Intestinal Mucosa Suspension*

A mucosal suspension was prepared from the intestine of male Wistar rats. After abdominal incision, the intestinal segment between the beginning of the jejunum and the end of the ileum was isolated and everted to expose the interior. The mucosal layer was washed with normal saline and then scraped with glass microscope slides. The scraping was collected, diluted to the ratio of 1:9 (v/v) with ice cold isotonic sucrose solution, and ultrasonicated for 200 seconds. The suspension was centrifuged at 1000g for 5 minutes. The supernatant suspension was stored in an ice bath before being used in the degradation study.

(II)*Degradation Studies in Intestinal Mucosal Suspension*

All the stability studies were conducted immediately after the mucosal suspension was prepared. The stock solution of compound 1 was prepared by diluting 100 μl of a methanolic solution (2.0 mg /2 ml) of 1 with 2.4 ml of an isotonic mannitol buffer (pH 6.5). The L-Gly-L-Phe stock solution was similarly prepared by dissolving the dipeptide directly in isotonic mannitol buffer solu-

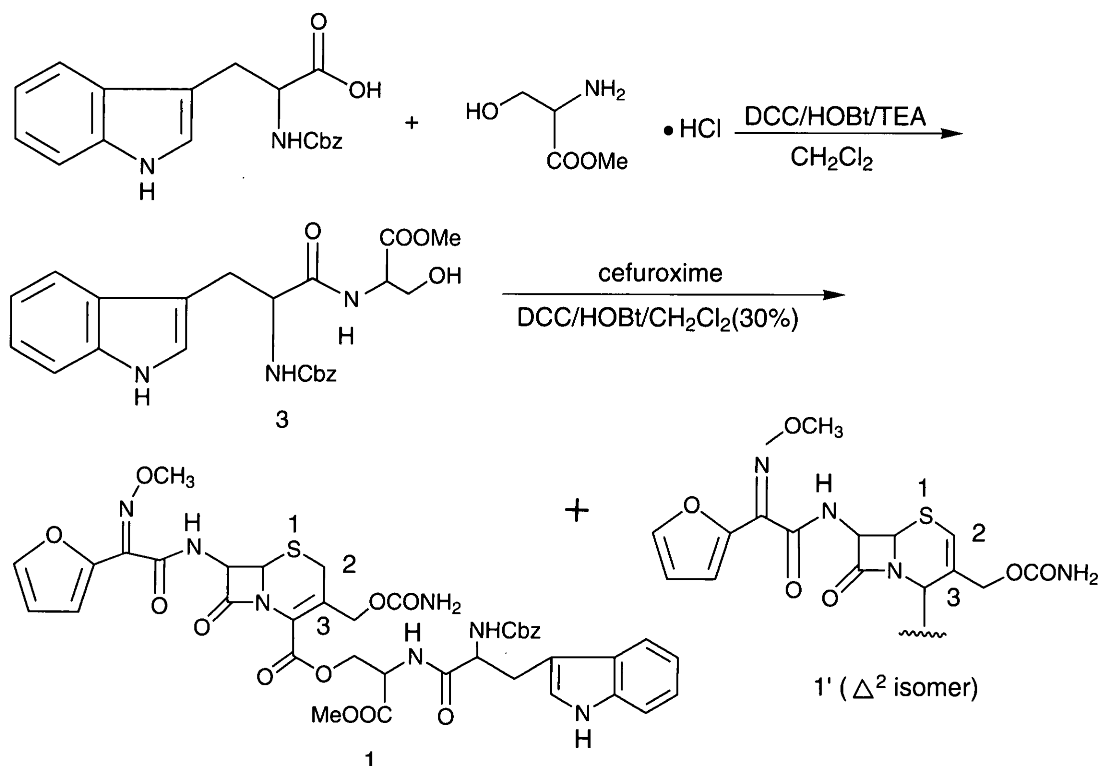
tion (pH 6.5). Two milliliters of each of the stock solutions were mixed with equal volume of the mucosal suspension respectively. The mixtures were incubated in a 37°C water bath and subjected to sampling at time intervals from zero to 1.5 hr. Two hundred microliters of each sampled solution were denatured with 0.8 ml of MeOH, centrifuged at 15000g for 5 minutes and the supernatant weas subjected to HPLC assay.

IV. *Chromatographic Conditions*

HPLC was used to monitor the degradation of compound 1 and L-Gly-L-Phe. The percentage of recovery of each test compound was determined. The assay of compound 1 from phosphate buffer solution was performed on a Lichrospher CN column (5μm, 250x4mm) with CH_3CN : pH 2.5 phosphate buffer = 25 : 75 (v/v) as the mobile phase at a flow rate of 1.0 ml/min. Samples from intestinal mucosal suspension were assayed on a Lichrospher 100 RP-18 column (5μm, 250x4mm) with CH_3CN : pH 2.5 phosphate buffer = 40 : 60 (v/v) as the mobile phase at a flow rate of 1.0 ml/min. L-Gly-L-Phe was analyzed on a Nova-Pak C-18 column (4 μm, 150x3.9mm) with the mobile phase consisting of CH_3CN : citric acid-NH_3 buffer (pH 3) = 25 : 75 (v/v) and 5% of sodium dodecyl sulfate (w/v) at a flow rate of 0.8 ml/min.

RESULTS AND DISCUSSION

N-Cbz-tryptophan was attached to the 4-carboxyl group of cefuroxime via an L- serine methyl ester linkage to form the tripartite prodrug 1. Preparation of this compound is summarized in Scheme 1. Condensation of L-serine methyl ester with N-Cbz-tryptophan in the presence of N,N'-dicyclohexylcarbodiimide (DCC)[17] and 1-hydroxy-benzotriazole (HOBt)[18] afforded the intermediate 3. Coupling of the dipeptide derivative 3 with cefuroxime using DCC as coupling reagent gave compound 1. $\Delta^3 \rightarrow \Delta^2$ isomerization on the cepham ring, commonly reported during preparation of cepham esters,[19,20] was also observed in the reaction. This might account for the poor yield (30%) of product 1. The undesired Δ^2 isomer (1') was removed by column chro-



Scheme 1. Preparation of prodrug 1.

matography. The benzyloxycarbonyl (Cbz) group on tryptophan and the methyl ester on serine were intentionally not detached from compound 1 in order to maintain the necessary lipophilicity for oral absorption.

Compound 1 was stable in pH 6.5 and pH 7.4 phosphate buffer solutions. Only 1.6% and 23% of this compound was degraded respectively after 90 minutes of incubation. This tripartite prodrug was more stable than the double ester prodrug 2 in pH 7.4 phosphate buffer solution (Fig. 2). In compound 1, the tryptophan moiety was attached to the 4-carboxyl group of cefuroxime via the hydroxyl group of L-serine by forming an ester linkage. The spacer, L-serine, in compound 1 was thought to be more of a hindrance to hydrolysis than the methylene linkage in compound 2. Stability of the ester linkage in compound 1, in comparison with that of the double ester linkage made of a methylene moiety in compound 2, may also account for compound 1 being more stable than compound 2.

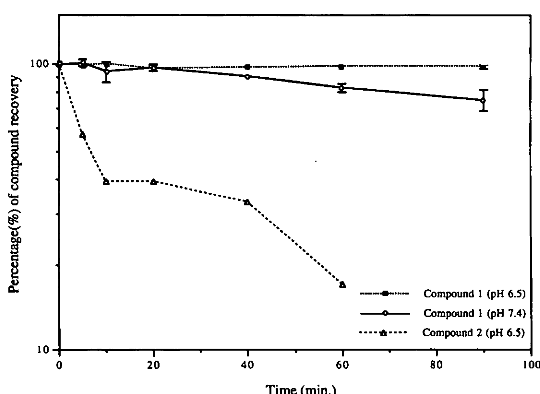


Figure 2. Stability studies of the tripartite compound 1 in pH 6.5 and 7.4 phosphate buffer solution. Each point represents the mean ± S.D. of 3 experiments. Stability of the double ester prodrug 2 of cefuroxime and N(Cbz)-tryptophan in pH 6.5 phosphate buffer solution is presented for comparison.

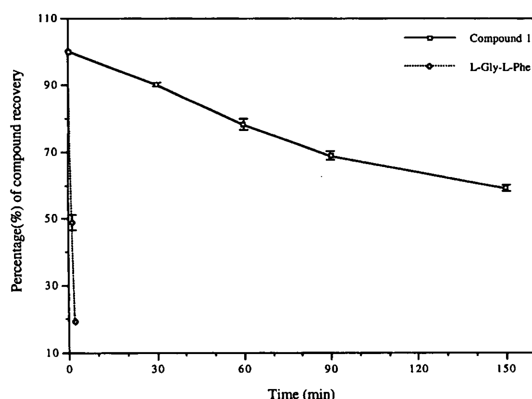


Figure 3. Degradation of compound 1 and dipeptide Gly-Phe in rat intestinal mucosal suspension.

In order to simulate the microclimate of the intestine[21], a pH 6.5 isotonic mucosal suspension was prepared from rat intestine for determining the stability of compound 1. The intestinal enzymatic activity was ensured by monitoring the degradation of dipeptide L-Gly-L-Phe in the suspension. As indicated in Fig. 3, only 19% of L-Gly-L-Phe was recovered after 2 minutes of incubation. However, 60% of compound 1 remained intact after 2.5 hours of incubation. As an ester prodrug of cefuroxime comprising a L-Ser-L-Trp dipeptide moiety, this compound seemed to be more stable than the dipeptide L-Gly-L-Phe.

In conclusion, we have prepared compound 1 which, given its satisfactory stability in the intestine, may be a feasible formulation of an oral prodrug of cefuroxime. Oral bioavailability in terms of both the prodrug and the released parent drug will be determined. Because an increase of the plasma bound fraction of the prodrug is desired, the degree of protein binding of this prodrug in comparison with that of the parent drug will also be pursued.

ACKNOWLEDGEMENT

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含絲胺酸及色胺酸之Cefuroxime先驅藥的合成及安定性研究

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摘　要

Cefuroxime爲臨床上廣爲使用之β-lactam類抗生素，然其缺點爲口服生體可用率低，僅能打針。加上其體內分布體積小、腎臟清除率高，致體內半衰期爲1.1小時。本研究以研發可口服吸收之Cefuroxime先驅藥爲目標，設計合成一含有絲胺酸及色胺酸之三元式先驅藥1。期以先驅藥之脂溶性增加而成爲口服劑型，並藉由色胺酸之高蛋白吸附力而減緩Cefuroxime腎臟清除速率。化合物1之合成係由色胺酸與絲胺酸接合之雙胜肽3藉由絲胺酸雙胜肽了之-OH基接到cefuroxime之4-COOH基形成酯類先驅藥。先驅藥1在pH 6.5及pH 7.4磷酸緩衝溶液中陣搖90分鐘後，分別只有 1.6%及 23%分解。化合物1在以離體大鼠小腸製備之腸膜中培養2.5小時後，仍可偵測到 60%。此化合物在模擬腸液中之安定性將作爲我們進一步測試該藥生體可用率以及評估其作爲cefuroxime口服先驅藥之基礎。

關鍵詞: cefuroxime三元式先驅藥，絲胺酸，色胺酸，合成，腸液中之安定性。