Turk J Chem
29 (2005) , 187 – 191.

Synthesis and Antimicrobial Activities of Some New Flavonyl Pro-drug Esters of Ampicillin

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Received 15.04.2004

A new series of flavonyl pro-drug esters of ampicillin **(Va-b)** was prepared by condensing the appropriate bromomethyl flavone with ampicillin trihydrate potassium salt. Flavone derivatives and ampicillin both have good antibacterial activity. The synthesized compounds were tested for their antifungal and antibacterial activities in vitro. Both of the compounds were active against *Candida krusei* and *Staphylococcus aureus*.

Key Words: Flavone derivatives, Ampicillin, Pro-drug ester, Antimicrobial activities.

Introduction

A flavone ring system is present in many naturally occurring products[1] with diverse pharmacological activities such as antibacterial[2], antifungal[3], antiviral[3−4], antitumor[5], antioxidant[6], spasmolytic and antihepatotoxic[7−8]. It is known that some ampicillin pro-drug esters have antibiotic activity[9−13]. In the light of these results, we aimed to synthesize some flavonyl compounds having an ampicillin side chain and to evaluate their antimicrobial activities.

Experimental

The chemical reagents used in synthesis were purchased from E. Merck (Darmstadt, Germany) and Aldrich (Milwaukee, MI, USA). 3′/4′-Methyl flavone (**Ia-b**)[14] and 3′/4′-bromomethyl flavone (**IIa-b**)[15] were synthesized according to the literature. Melting points were determined with a Büchi SMP-20 melting point apparatus (Büchi, Flawil, Switzerland) and were uncorrected. The IR spectra were recorded on a Jasco FT/IR 420 spectrometer (Jasco Corp., Tokyo, Japan) as potassium bromide disks. ^{1}H NMR spectra were recorded with a Bruker GmbH DPX-400, 400 MHz NMR spectrometer (Bruker, Rheinstetten, Germany)

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using TMS internal standard and $CDCl_3$. All chemical shifts were reported as δ (ppm) values. Mass spectra were recorded on a VG Platform II Micromass spectrometer (Micromass, Manchester, England) (70 eV) with EI (electron ionization) method. Elementary analyses were performed by a Leco CHNS 932 analyzer (Leco, St. Joseph, USA) and satisfactory results (±0.4%) for the calculated values (C, H, N, S) were obtained. All the instrumental analyses were performed by the Instrumental Analysis Laboratory of the Scientific and Technical Research Council of Turkey (TÜBİTAK, Ankara, Turkey).

General procedure for the synthesis of compounds Va-b

*Synthesis of 3′/4′-Bromomethyl flavone (**IIa-b**):* A mixture of N-bromosuccinimide (2.14 g, 0.012 mol) and 3′/4′-methyl flavone **(Ia-b)** (3 g, 0.012 mol) was dissolved in 150 mL of carbon tetrachloride and benzoyl peroxide (0.1 g) was added to this mixture (Scheme 1). Then the reaction mixture was refluxed for 7 h and filtered while still hot. The crude product was crystallized from toluene. [**IIa** m.p.: 136 °C (Lit.: m.p.: 137 °C)[15], **IIb** m.p.: 137 °C (Lit.: m.p.: 139 °C)[15]].

Synthesis of compounds **Va-b**: A suspension of ampicillin trihydrate (**III**) 0.256 g (0.635 mmol) in 3 mL of dimethylformamide was prepared and 0.0635 g (0.635 mmol) of potassium bicarbonate and 0.129 mL (1.27 mmol) benzaldehyde were added at 0 °C. The mixture was stirred at 0 °C for 3.5 h. At the end of this time 0.0635 g (0.635 mmol) of potassium bicarbonate and 0.2 g (0.635 mmol) of 3′ or 4′-bromomethyl flavone (**IIa-b**) were added and stirred at 0 °C for an additional 6 h (Scheme). The mixture was poured onto ice and was extracted with 5 mL (3x) of ethylacetate. After washing the organic phase with 10% sodium chloride and drying on anhydrous sodium sulfate, it was evaporated to dryness in vacuo at 20 °C and a yellow oily residue was obtained. The residue was dissolved in 2.5 mL of acetonitrile. The pH of the solution was adjusted to 2 with 1N HCl and it was stirred for 30 min at 0-5 °C. In order to remove acetonitrile, 6 mL of water was added and the solution was evaporated in vacuo at 20 °C. After washing the aqueous solution with 6 mL (3x) of ethylacetate and saturating with sodium chloride it was extracted with 6 mL (3x) of dichloromethane. The organic phase was dried on anhydrous sodium sulfate and it was held overnight at 5 °C. The residue was filtered and dried.

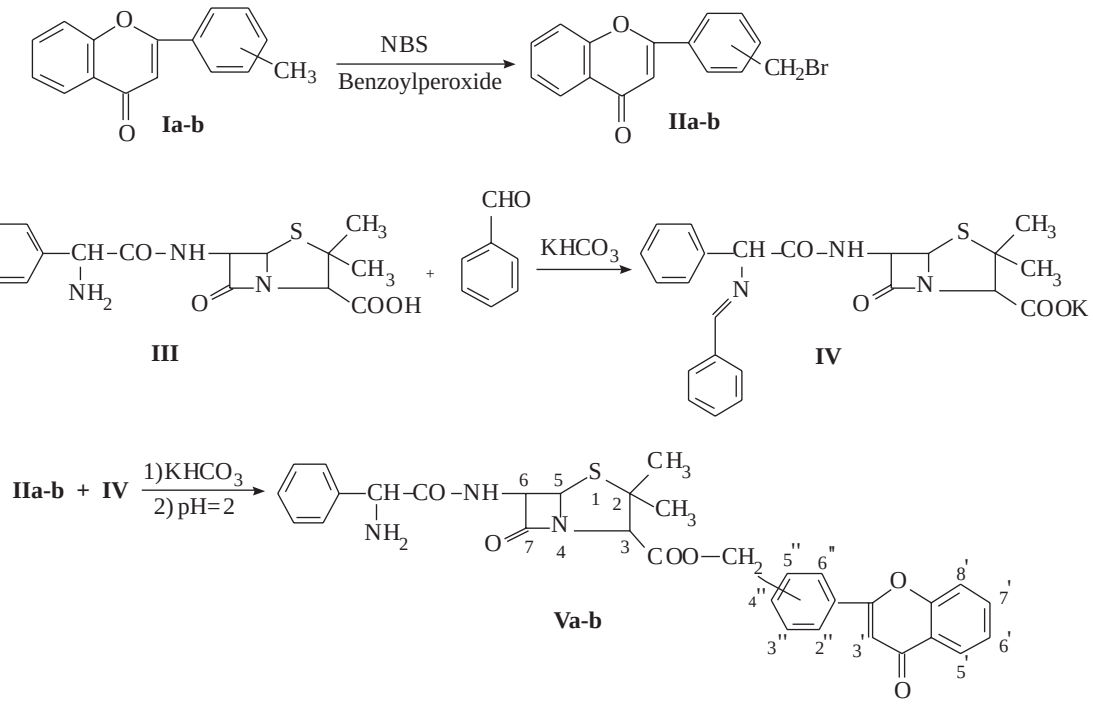
6-(2-Amino-2-phenyl-acetylamino)-3,3-dimethyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid 3-(4-oxo-4H-chromen-2-yl)-benzyl ester (Va)

Yield: 26.5% , m.p.: 139-141 °C. IR (KBr) cm^{-1}: 1628 (C=O, γ pyrone), 1691 (amide CO), 1747 (lactam CO), 1782 (ester CO), ^1H NMR ($CDCl_3$): δ= 1.13 (s, 3H, 2-CH_3), 1.15 (s, 3H, 2-CH_3), 4.29 (s, 1H, 3-H), 5.12 (s, 1H, CH-NH_2), 5.75-5.85 (m, 2H, 5,6-H), 6.74 (s, 2H, Ar-CH_2), 7.19 (s, 1H, 3′-H), 7.29-7.67 (m, 12H, 6′, 7′, 8′, 2″, 4″, 5″, 6″-H and ampicillin phenyl protons), 7.83 (d, 1H, 5′-H), 8.13 (d, 2H, -NH_2), 8.74 (br.s, 1H, CONH). EI MS [m/z (rel. int. %)]: 584 (1.0) [M+1], 281 (100), 236 (3.1), 235 (11.2), 221 (60.0), 194 (7.2), 121 (4.3), 115 (26.2), 101 (6.8), 64 (17.8).

Anal. for $C_{32}H_{29}N_3O_6S$. $3.5H_2O$

Calc. C:59.44, H:5.57, N:6.50, S:4.95

Found C:59.14, H:5.13, N:6.26, S:4.68



Scheme. General synthesis of **Va-b**.

***6-(2-Amino-2-phenyl-acetylamino)-3,3-dimethyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid 4-(4-oxo-4H-chromen-2-yl)-benzyl ester* (Vb)**

Yield: 30.3% , m.p.: 141-142 °C. IR (KBr) cm^{-1}: 1631 (C=O, γ pyrone), 1692 (amide CO), 1747 (lactam CO), 1782 (ester CO), ^{1}H NMR ($CDCl_3$): δ=1.17 (s, 3H, 2-CH_3), 1.19 (s, 3H, 2-CH_3), 4.35 (s, 1H, 3-H), 5.17 (s, 1H, CH-NH_2), 5.75-5.85 (m, 2H, 5,6-H), 6.73 (s, 2H, Ar-CH_2), 7.17 (s, 1H, 3′-H), 7.27-8.10 (m, 13H, $5', 6', 7', 8', 2'', 3'', 5'', 6''$-H and ampicillin phenyl protons), 8.14 (d, 2H, -NH_2), 8.80 (br.s, 1H, CONH). EI MS [m/z (rel. int. %)]: 583 (6.0) [M^+], 584 (2.3) [M+1], 335 (15.9), 236 (7.4), 235 (38.6), 219 (1.8), 194 (4.9), 134 (2.5), 121 (7.1), 115 (31.6), 101 (4.6), 64 (27.4), 29 (100).

Anal. for $C_{32}H_{29}N_3O_6S$. $2.5H_2O$

Calc. C:60.62, H:5.46, N:6.63, S:5.05

Found C:60.79, H:4.84, N:5.82, S:4.74

Antimicrobial activity

Disk diffusion was used for assessing antibacterial activity against *Staphylococcus aureus* ATCC 250 and *Escherichia coli* RSKK 313, and for antifungal activity *Candida krusei* (isolate) was used. Cultures of each bacterium and yeast strain, kept in Mueller-Hinton broth (DIFCO), at 37 °C for 18-24 h and diluted with the same broth to 10^5 cfu/mL, were pipetted into Mueller-Hinton agar plates prepared according to the procedure. Paper disks (8 mm in diameter) embedded into 3000 μg.mL^{-1} compound solution were placed on the surface of the inoculated plates and were placed in an incubator at 37 °C for 18-24 h and were then examined. All the compounds were found to be effective against the tested microorganisms by measuring the diameter of the growth inhibition zone according to Bauer et al.[16].

Results and Discussion

The general method known as the Baker-Venkataraman[14] method was used to prepare 3′- and 4′-methyl flavone **(Ia-b)**. The methyl group of the flavone was converted to bromomethyl with N-bromosuccinimide and a catalytic amount of benzoyl peroxide. Derivatives **Va-b** were synthesized by first reacting ampicillin trihydrate (**III**) with benzaldehyde in the presence of K_2CO_3/DMF. Then compound **IV** was treated with 3′/4′-bromomethyl flavone under the same conditions (Scheme). The structures of the synthesized compounds **(Va-b)** were elucidated by elementary analysis, and ^{1}H-NMR, mass and IR spectral data. All spectral data were in accordance with the assumed structures. The compounds showed strong IR absorption at 1628-1631, 1691-1692, 1747 and 1782 cm^{-1}due to the γ-pyrone, amide, lactam and ester carbonyl stretching bonds, respectively. In the ^{1}H-NMR spectra, characteristic flavone protons were observed between 6.73 and 8.10 δ ppm. Geminal methyl groups of ampicillin resonated at 1.13-1.19 δ ppm. Ampicillin 3-H and 5,6-H were seen at 4.29-4.35 and 5.75-5.85 δ ppm, respectively. $-NH_2$ protons resonated at 8.14 δ ppm as a doublet. In the mass spectra, compound **Vb** has a molecular ion ($M^{+\cdot}$) peak and compounds **Va-b** have M+1 ion peaks.

Compounds **Va-b** were tested for their antimicrobial activity by the agar diffusion method[16] using *C. krusei*, *S. aureus* and *E. coli* and by comparing with ampicillin and miconazole (Table). The resulting inhibition zones against *C. krusei* and *S. aureus* were 15-20 and 26-33 mm, respectively. Both compounds were inactive against *E. coli*. Compound **Vb** showed high activity against *S. aureus* (33 mm) comparable with ampicillin. Compounds **Va-b**, which are designed as pro-drugs, were tested for their antimicrobial activity in vitro. Despite the in vitro conditions, both compounds showed activity against *C. krusei* and *S. aureus*. The in vivo activities will be tested later.

Table. Antimicrobial activities [a] of the compounds **Va-d**.

Compound	*S. aureus*	*C. krusei*	*E. coli*
Va	26	15	*
Vb	33	20	*
Ampicillin	35	-	20
Miconazole	-	30	-

[a]Growth inhibition diameter (mm). *No activity. -:Not tested

Acknowledgment

This work was supported by TÜBİTAK (Project No: SBAG-AYD-243).

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