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Short Note

Ethyl (*S*)-2-Benzamido-5-[(4,6-dimethylpyrimidin -2-yl)amino]pentanoate

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Abstract: Pyrimidines are compounds with a wide range of biological activities, and the synthesis of pyrimidine derivatives—useful in chemical and medicinal applications—is important in medicinal chemistry. This work shows the synthesis under microwave irradiation of the novel compound ethyl (*S*)-2-benzamido-5-[(4,6-dimethylpyrimidin-2-yl)amino]pentanoate (**3**) from (*S*)-*N*-α-benzoyl-L-arginine ethyl ester hydrochloride (**1**) and acetylacetone (**2**). Compound **3** was easily purified, obtained in moderate yield (70%), and fully characterized by UV-Vis, FTIR-ATR spectroscopy, ^{1}H-NMR, ^{13}C-NMR, HRMS, and EI-MS.

Keywords: pyrimidines; L-arginine; microwave irradiation

1. Introduction

Pyrimidines are diazine compounds produced by substituting two nitrogen atoms at positions 1 and 3 of a six-membered ring (1,3-diazine) [1]. The heterocyclic pyrimidine nucleus is the most important within the diazines, and many pyrimidine derivatives are key molecules in alive organisms: DNA, RNA, and numerous natural products such as antibiotics, vitamins, and liposaccharides [2,3]. Furthermore, pyrimidine analogs have shown a range of biological activities, e.g., antibacterial [4,5], antifungal [6], antiparasitic [7], anti-inflammatory [8], antihypertensive [9], antiviral [10], antioxidant [11], herbicide [12], and anticancer [13].

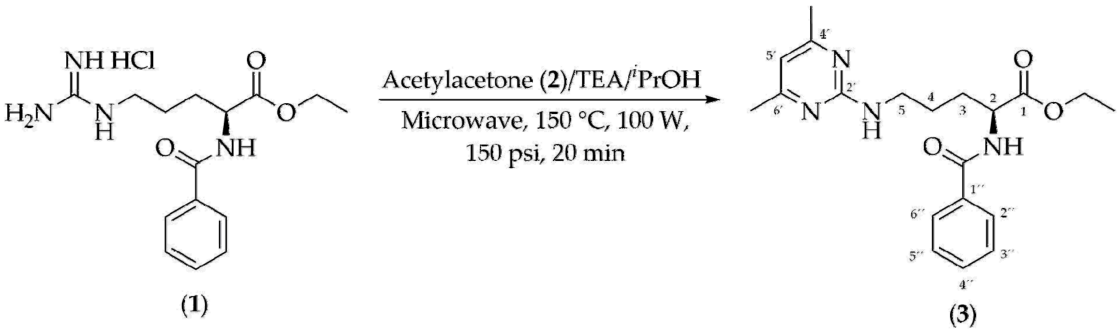
Due to the biological activities of pyrimidines, their synthesis is currently engaged in medicinal chemistry. The principal method of pyrimidine synthesis involves the condensation of β-dicarbonyl and amine compounds [14]. In this sense, α-amino acids show high enantiomeric purity and are versatile building blocks for synthesizing heterocyclic systems [15,16].

L-Arginine is a protein component and precursor of other biomolecules such as nitric oxide, creatine, and urea [17,18]. Arginine has a guanidino group that shows strong basicity, high reactivity, and is critical to protein structure and stability by acting as a bidentate group for hydrogen bonding interactions [19,20]. Thus, L-arginine derivatives are used in the development of drugs such as argatroban [21]. However, the guanidine group is rarely used in such reactions, despite its wide use as an *N*,*N*-binucleophile to synthesize heterocyclic nitrogen systems [22,23].

Therefore, we used the (*S*)-*N*-α-benzoyl-L-arginine ethyl ester hydrochloride (**1**) as a source of amine group to be condensed with acetylacetone (**2**) under microwave irradiation to synthesize the new ethyl (*S*)-2-benzamido-5-[(4,6-dimethylpyrimidin-2-yl)amino]pentanoate.

2. Results

This study shows the synthesis of ethyl (*S*)-2-benzamido-5-[(4,6-dimethylpyrimidin-2-yl)amino] pentanoate using the method reported by Aihua et al. [24] with some modifications. Microwave irradiation of the mixture of (*S*)-*N*-α-benzoyl-L-arginine ethyl ester hydrochloride (**1**), acetylacetone (**2**), and triethylamine (TEA) in isopropanol (^{i}PrOH) produced the novel pyrimidine (**3**) in moderate yield (70%) (Scheme 1). This method was developed to prepare 2,4,6-trisubstituted pyrimidines from chalcones and amidines or guanidine, using TEA as the base, ^{i}PrOH as the solvent, and at 190 °C for 30 min; however, different temperature/time conditions were assayed, obtaining the following yields after column chromatography purification: 69% at 190 °C/30 min, 70% at 150 °C/20 min, and the reaction was incomplete at 150 °C/10 min, as evidenced by the presence of unreacted (*S*)-*N*-α-benzoyl-L-arginine ethyl ester hydrochloride on TLC. Thus, the chosen reaction conditions were 150 °C and 20 min.



Scheme 1. Reaction conditions for the synthesis of ethyl (*S*)-2-benzamido-5-[(4,6-dimethylpyrimidin-2-yl)amino]pentanoate (**3**).

The purified compound **3** showed the expected spectroscopic and spectrometric (UV-Vis, FTIR-ATR, ^{1}H-NMR, ^{13}C-NMR, HRMS, and EI-MS) signals. The specific rotation $[\alpha]_D^{25}$ was + 40 °. Its UV spectrum showed the λ_{max} in methanol at 303 nm (Supplementary Materials, Figure S1). The FTIR-ATR analysis showed the following signals (cm^{-1}): 3289, secondary amide N-H group; 3070, C-H aromatic; 2973, C-H aliphatic; 1741, C=O ester; 1633, C=O amide; 1592, C=C aromatic; and 1043, C-O ester groups (Supplementary Materials, Figure S2). The ^{1}H-NMR signals were at the following δ: 7.80–7.40 for the five benzoyl aromatic hydrogens; doublet at 7.24 with ^{3}J = 4.0 Hz assigned to the hydrogen of the secondary amide; a singlet at 6.26 corresponding to H-5′ in the pyrimidine ring; a broad signal at 5.19 for the hydrogen of the secondary aromatic amine; a multiplet at 4.82 for the asymmetric carbon$_{\alpha}$-hydrogen (H-2) of the arginine residue; a quartet at 4.19 with ^{3}J = 8.0 Hz for the methylene hydrogens of the ethyl group; multiplets at 3.54 and 3.43 for the methylene hydrogens at position H_a-5 and H_b-5; a singlet at 2.22 for the methyl hydrogens in C-4′ and C-6′ of the pyrimidine ring; multiplets at 2.04 and 1.87 for the diastereotopic hydrogens H_a-3 and H_b-3, respectively; a multiplet at 1.71 for the methylene hydrogens at position H-4; and a triplet at 1.26 with ^{3}J = 8.0 Hz for the methyl hydrogens of the ethyl group (Supplementary Materials, Figure S3). The ^{13}C-NMR spectrum showed 16 signals attributable to 20 carbons comprising six quaternary carbons, five methine carbons, four methylene carbons, and two methyl carbons as expected for the molecular formula of **3**. The ^{13}C-NMR δ signals were assigned as follows: 172.8 and 167.5 for the ester and amide carbonyls, respectively; 167.4 and 162.4 correspond to the quaternary carbons C-4′/C-6′and C-2′; 109.9 for the methine carbon C-5′of the pyrimidine ring; four signals for the aromatic system at 134.1 (C-1″), 131.8 (C-4″), 128.6 (C-3″/C-5″), and 127.3 (C-2″/C-6″); 61.6 and 14.3 for the ethyl carbons; 52.9 for the asymmetric carbon$_{\alpha}$ (C-2); three signals for the arginine residue at 40.9 (C-3), 29.5 (C-4), and 26.3 (C5); and 23.9 for the methyl carbons in positions 4′ and 6′ of the pyrimidine ring (Supplementary Materials, Figure S4). The molecular mass and fragmentation pathway of **3** was analyzed by HRMS and EI-MS (Supplementary Materials, Figures S5

and S6). The exact mass/charge (*m*/*z*) of the $[M]^{\bullet+}$ was 370.2038, less than 10 ppm relative error, and corresponds to the formula $C_{20}H_{26}N_4O_3$ (theoretical *m*/*z* 370.2005). The EI-MS spectrum shows the molecular ion $[M]^{\bullet+}$ at *m*/*z* = 370 in relatively good abundance, and its fragments were explained as follows: *m*/*z* = 325, removal of the ethoxy radical from $[M]^{\bullet+}$; *m*/*z* = 248, assigned to the loss of the 4,6-dimethylpyrimidin-2-amine group; the ion at *m*/*z* = 176 can be explained by the loss of the ethoxycarbonyl group from the *m*/*z* = 248 ion; intense fragment at *m*/*z* = 136 (base peak), formation of the 4,6-dimethyl-*N*-methylenepyrimidin-2-aminium cation; and ions at *m*/*z* = 105 and *m*/*z* = 77, expulsion of the benzoyl group from $[M]^{\bullet+}$.

3. Materials and Methods

3.1. General Information

The highest quality available reagents were purchased (Sigma Aldrich, St. Louis, MO, USA) and used without further purification. The reaction progress was monitored by Thin Layer Chromatography (TLC) on aluminum silica gel plates GF_{254} (0.25 mm) and employing different solvents. The reaction was carried out in a Pyrex tube sealed with a silicon septum in a single-mode microwave reactor (Discover-SP model 909150 equipped with an Explorer 12 hybrid model 909505, with 725 W of maximum power, CEM Corp., Matthews, NC, USA) at 100 W initial power and 150 psi pressure. The melting points were determined using a Stuart apparatus model SMP30 (Cole-Parmer, Stone, Staffordshire, UK), and the results were the uncorrected average of three separate experiments. Optical rotation was measured in an MCP-100 Polarimeter (Anton Paar Trading Co. Ltd., Shanghai, China), compound dissolved in reagent grade solvents. UV/Vis spectrum was recorded using a Thermo Scientific/Multiskan GO spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The Fourier transform infrared spectra was recorded on a Cary 660 series FTIR-ATR spectrophotometer (Agilent Technology, Santa Clara, CA, USA). The nuclear magnetic resonance (NMR) spectrum (^{1}H and ^{13}C) was recorded on a JEOL Eclipse 400 (400 MHz) spectrometer (at 400 and 100 MHz) (Bruker Analytische Messtechnik GmbH, Rheinstetten, Germany) with $CDCl_3$ as the solvent and internal standard. Electron ionization mass spectra (EI-MS) and high-resolution mass spectra (HRMS) were obtained on a JEOL MStation MS-700 (JEOL Ltd., Akishima, Tokyo, Japan).

3.2. Synthesis of Ethyl (S)-2-Benzamido-5-[(4,6-dimethylpyrimidin-2-yl)amino]pentanoate

In a 10 mL pressure-rated vial, (*S*)-*N*-α-benzoyl-L-arginine ethyl ester hydrochloride (0.6 mmol, 0.2 g), acetylacetone (0.9 mmol, 0.1 mL), TEA (0.7 mmol, 0.1 mL), and isopropanol (1.0 mL) were mixed. The vial was sealed, stirred, and microwave irradiated at 150 °C for 20 min. Then, the mixture was cooled to 25 °C and added with $CHCl_3$ (3 × 5 mL). The organic layer was recovered and washed twice with saturated aqueous $NaHCO_3$ and twice with H_2O. The organic phase obtained was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The compound was purified by silica gel column chromatography using a mixture of *n*-hexane and ethyl acetate (1:4) as the mobile phase and recrystallized with cold ethanol. In this way, compound **3** was obtained as a yellow solid (0.1300 g, 70%); m.p.: 129–130 °C (cold EtOH); $[\alpha]_D^{25}$ = + 40 (c = 0.33, $CHCl_3$); R_f (*n*-hexane:ethyl acetate 1:2) = 0.5; UV-Vis (MeOH) λ_{max} = 303 nm; ε_{303} = 2007 L mol^{-1} cm^{-1}; IR (FTIR-ATR, cm^{-1}): 3289 (m, N-H), 3070 (m, C-H aromatic), 2973 (m, C-H aliphatic), 1741 (str, C=O ester), 1633 (str, C=O amide), 1592 (str, C=C aromatic), 1043 (str, C-O ester); ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm): 7.81 (d, *J* = 8.0 Hz, 2H), 7.50–7.47 (m, 1H), 7.42–7.39 (m, 2H), 7.24 (d, *J* = 4.0 Hz, 1H), 6.26 (s, 1H), 5.19 (br s, 1H), 4.85–4.80 (m, 1H), 4.19 (q, *J* = 8.0 Hz, 2H), 3.58–3.51 (m, 1H), 3.46–3.39 (m, 1H), 2.22 (s, 6H), 2.07–2.00 (m, 1H), 1.91–1.83 (m, 1H), 1.78–1.64 (m, 2H), 1.26 (t, *J* = 8.0 Hz, 3H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ_C (ppm): 172.8 (C), 167.5 (C), 167.4 (C), 162.4 (C), 134.1 (C), 131.8 (CH), 128.6 (2 × CH), 127.3 (2 × CH), 109.9 (CH), 61.6 (CH_2), 52.9 (CH), 40.9 (CH_2), 29.5 (CH_2), 26.3 (CH_2), 23.9 (2 × CH_3), 14.3 (CH_3). MS (EI$^+$): 370 [M$^+$]. HRMS (EI$^+$): Calculated for $C_{20}H_{26}N_4O_3$: 370.2005; Found: 370.2038.

4. Conclusions

In summary, the reported conditions permitted the easy synthesis and purification in moderate yield (70%) of ethyl (*S*)-2-benzamido-5-[(4,6-dimethylpyrimidin-2-yl)amino]pentanoate (**3**) from (*S*)-*N*-α-benzoyl-L-arginine ethyl ester hydrochloride (**1**) and acetylacetone (**2**). The new pyrimidine compound **3** could be a reagent for the synthesis of novel bioactive pyrimidine derivatives with medicinal applications.

Supplementary Materials: The following spectra of **3** are available online. UV-Vis, FTIR-ATR, ^{1}H and ^{13}C NMR, HRMS, and EI-MS.

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Conflicts of Interest: The authors declare no conflict of interest.

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