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Synthesis, Characterization and Biological Activity of New β-Lactam Analogues Bearing Pyrimidine and N-Acetamido Moieties

SUROR ABDULRAHMAN MAHDI

Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq

Corresponding author: Tel: +964 7722231214; E-mail: surchem_82@yahoo.com

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In this work, a series of new β-lactams were synthesized by Staudinger ketene-imine cyclo-addition reaction after multisteps synthesis. The first step involved preparation of N-acetamido amino acids (3-5) *via* reaction of acetic anhydride with amino acids (alanine, glycine, valine), since acetyl group is common protecting group for amine in organic synthesis. Treatment of compounds **3-5** with thionyl chloride in the second step gave N-acetamido amino acid chlorides (ketenes) (**6-8**) which in turn were treated with Schiff bases (imines) compounds **1** and **2**, which derived from 2-amino pyrimidine and cinnamaldehyde or crotonaldehyde. In the third step to afford a series of the β-lactams (**9-11**) and (**12-14**). The synthesized compounds were identified by FT-IR and some of them by ^{1}H NMR and ^{13}C NMR. The synthesized β-lactams were screened for their antimicrobial activity against *E. coli*, *Pseudomonas aeruginosa* (Gram-negative), *Staphylococcus aureus* (Gram-positive) bacteria respectively and antifungal activity against *Candida albicans*.

Keywords: Amino pyrimidine, β-Lactam, Staudinger addition, Antimicrobial activity.

INTRODUCTION

Pyrimidines exhibit a range of biological activities antibacterial [1], antifungal [2], anticancer [3] and as potential drug candidates for the treatment of prion diseases [4], chronic myeloid leukemia [5]. In the other hand, cinnamaldehyde derivatives possess antibacterial and antioxidant [6], anti-inflammatory [7], antitumor effects [8] and antiviral agents [9].

In another approach, β-lactams have found to be an important chemical entity in the production of novel medical and pharmacological compounds. Besides their structural constituent of β-lactam antibiotics, they have been cholesterol inhibitors [10], anticancer agents [11], anticoagulant drugs [12], antiviral inhibitors [13]. These observations inspire us to synthesize new β-lactams containing pyrimidine and cinnamyl group, which showing good biological activities against three types of bacteria and one type of fungi.

EXPERIMENTAL

Melting points were recorded using GallenKhamp electrothermal melting point apparatus. FT-IR spectra were recorded on SHIMADZU FT-IR 8400 Fourier transform infrared spectrophotometer using KBr disc or thin films. ^{1}H NMR spectra were recorded on Bruker spectro-spin ultra shield magnet 300 MHz instrument using Me_4Si as the internal standard and DMSO-d_6 as a solvent. TLC plates were used with an aluminum backing (0.2 mm, 60 F254), the reactions were monitored by TLC and visualized by the development of the TLC plates. Incubator Heraeus D-63450 (Germany) model was used for incubation samples for biological study.

Synthesis of N-cinnamalidene-2-amino pyrimidine (1): Cinnamaldehyde (3.7 mL, 30 mmol) in (20 mL) dry CH_2Cl_2 was saturated with argon and then was cooled in an ice-water bath while a solution of 2-amino pyrimidine (2.66 g, 28 mmol) in (20 mL) dry CH_2Cl_2 was added dropwise over 15 min. After 30 min anhydrous magnesium sulfate (7.5 g) was added in one portion. The ice-water bath was removed and the reaction mixture was stirred at room temperature for 2 h. The resulting mixture was then filtered and washed with CH_2Cl_2 (2 × 10 mL) and the filtrate is concentrated under reduced pressure by rotary evaporation at room temperature to afford a pale brown powder [13], recrystallized from ethanol:water (1:1) to give a pale yellow powder, yield (87 %).

Synthesis of N-acetamido amino acids (3-5): L-Amino acids (alanine, glycine, valine) (25 mmol) in (15 mL) CH_2Cl_2 and triethylamine (5 mL, 50 mmol) was added at room temperature to give a clear solution. Acetic anhydride (2.5 mL, 25 mmol) was added slowly in ice-bath and the resulting solution was stirred for 24 h at room temperature. The solvent was removed from the mixture under reduced pressure, the solid residue was stirred with 20 mL of cold water, then filtered and washed

with cold water (2 × 5 mL) [13]. Then dried in an air oven and recrystallized from ethanol.

Synthesis of N-acetamido amino chlorides (6-8): A mixture of N-acetamido amino acid (**3-5**) (25 mmol) and thionyl chloride (5.5 mL, 75 mmol) was dissolved in dry CH_2Cl_2 (15 mL). The resulting suspension was stirred at reflux for 18 h, the mixture was cooled to room temperature before removing excess of thionyl chloride under reduced pressure. The residue was suspended in ether (20 mL) and stirred for 1 h at 0 °C [14]. The crystals were filtered, washed with cold ether (5 mL) and dried under vacuum.

Synthesis of N-(2-pyrimidine)-3-(N-acetamido)-4-styryl -2-azetidinones (9-11): To a solution of imine (Schiff base) (**1**) (2 g, 10 mmol) and triethylamine (1.3 mL, 10 mmol) in dry CH_2Cl_2 (20 mL) at -10 °C was added N-acetamido amino acid chlorides (6-8) (15 mmol) in dry (25 mL) CH_2Cl_2 dropwise. After the addition was complete, the solution was stirred at room temperature for 24 h, then the reaction mixture was washed with water (3 × 15 mL). The organic layer was separated and dried Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to give β-lactams (**9-11**) as solids that were recrystallized from ethanol [15].

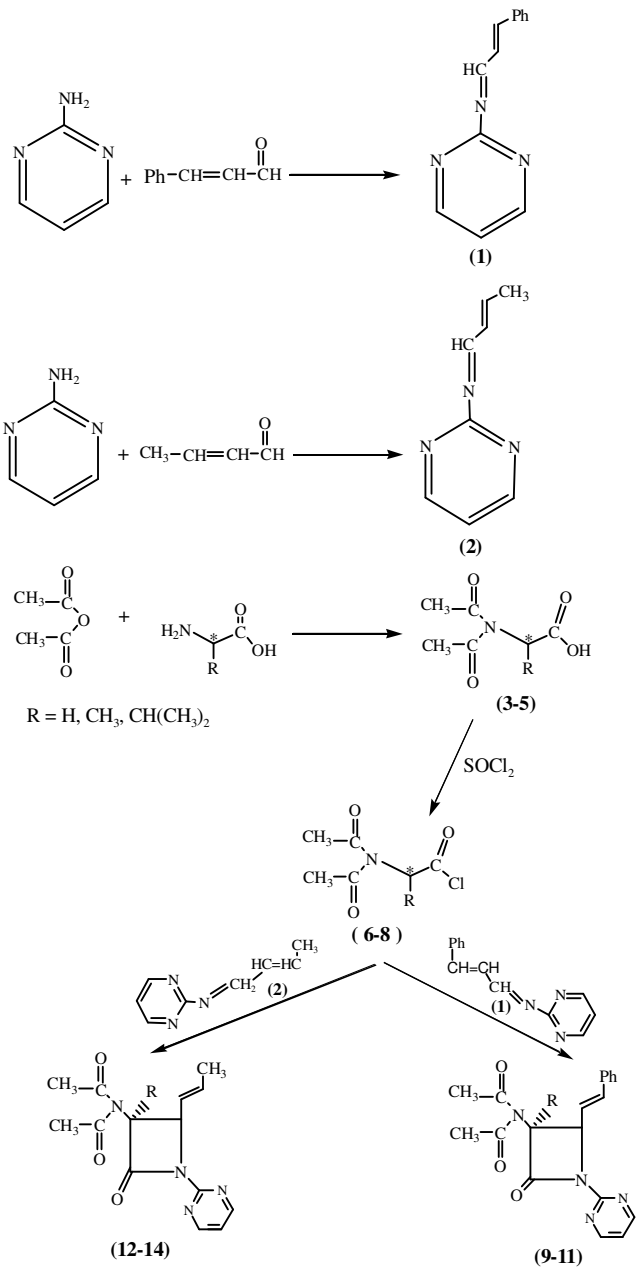
Synthesis of N-crotonylidene-2-amino pyrimidine (2): The titled compound was prepared by following the same procedure used in the preparation of N-cinnamylidene-2-aminopyrimidine except using of crotonaldehyde instead of cinnamaldehyde. The product was purified by recrystallization from ethanol to give compound **2** as off-white precipitate, yield 84 %.

Synthesis of N-(2-pyrimidine)-3-(N-acetamido)-4-propenyl -2-azetidinones (12-14): The titled compounds were prepared by following the same procedure used in the preparation of compounds **9-11** except using of N-crotonalidene-2-aminopyrimidine (**2**) instead of N-cinnamylidene-2-amino pyrimidine (**1**). The final products were purified by recrystallization from ethanol.

Microbiological test: Nutrient agar was added to (1 L) of distilled water in a suitable conical flask with stirring and heating until complete dissolving then the flask was stoppered by cotton and the medium was sterilized in an autoclave for 20 min at 121 °C under pressure at 15 pounds/inch. The medium was placed in Petri dishes about (20 mL) for each one and was left to cool and solidified. The studied bacteria and fungi were placed on the nutrient agar surface using the loop and by streaking processor then the discs saturated with tested compound solutions. The samples were incubated for 24 h at 37 °C [16].

RESULTS AND DISCUSSION

The synthesis route was started with N-acetamido amino acids (**3-5**) which were synthesized from a dehydrative condensation reaction of amino acids (alanine, glycine, valine) with an equimolar quantity of acetic anhydride. These compounds were reacted with thionyl chloride *via* nucleophilic substitution reaction to give N-acetamido amino acid chlorides (**6-8**) as ketenes which subjected to Staudinger cycloaddition reaction with imines (**1**) and (**2**) to give our synthetic goal new β-lactams (**9-11**), (**12-14**) respectively, which play an important role in treatment of tumor cell and antiviral agents, also have a broad spectrum of biological activities. The overall work steps show in **Scheme-I**. The physico-chemical analysis data of synthesized compounds are given in Table-1.

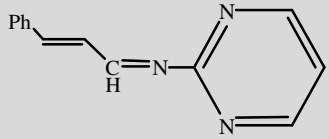
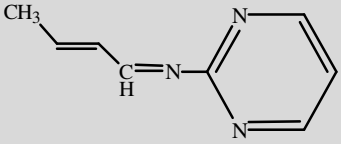
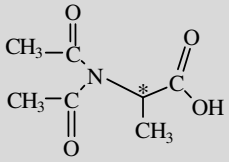
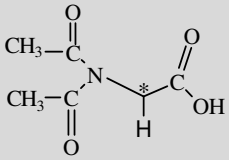
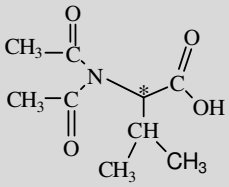
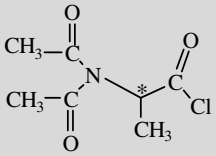
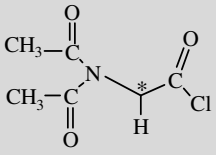
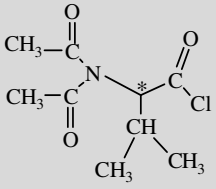
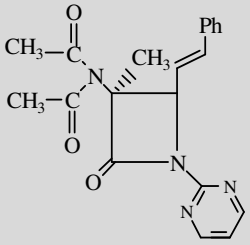
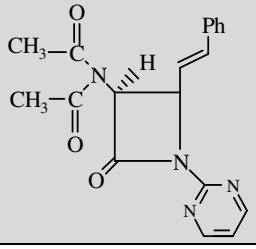


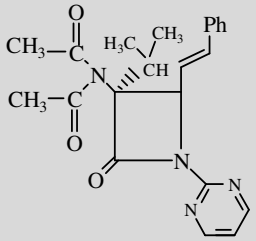
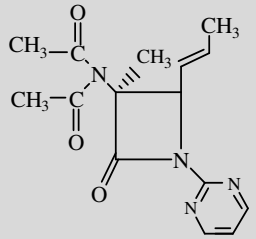
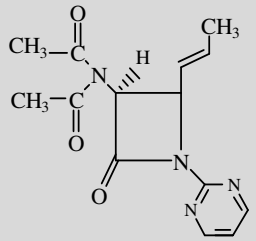
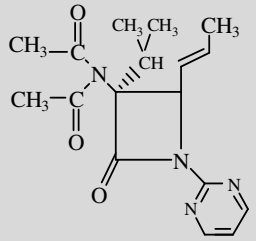
Scheme-I: Synthesis of N-(2-pyrimidine)-3-(N-acetamido)-2-substituted azetidinone (**9-11**) and (**12-14**)

The reaction of 2-amino pyrimidine with cinnamaldehyde and crotonaldehyde afforded compounds **1** and **2**, respectively in high yield.

FT-IR spectrum of compounds **1** and **2** showed absorption bands at 1680 and 1670 cm^{-1}, respectively assigned to ν(C=N) imine and absence of ν(N-H), ^1H NMR spectrum of compound **2** showed singlet signal at δ = 1.7 ppm belong to protons of one methyl group, singlet signal at δ = 5.69 ppm belongs to (CH-N) heteroaromatic, two signal at δ = 6.4 ppm, δ = 6.5 ppm belong to (CH=CH) vinylic and singlet signal at δ = 8.2 ppm belongs to (CH=N) imine proton, ^{13}C NMR spectrum

TABLE-1
PHYSICAL PROPERTIES OF THE SYNTHESIZED COMPOUNDS **1-14**

Compound No.	Structure	Colour	Yield (%)	m.p. (°C)
1		Faint yellow	87	108
2		Off white	84	90
3		Faint brown	75	214 decompose
4		White	73	232-234
5		White	77	263-265
6		Dark brown	88	264 decompose
7		Off white	79	120
8		Off white	81	140 decompose
9		Dark brown	72	120
10		Pale yellow	69	115

Compound No.	Structure	Colour	Yield (%)	m.p. (°C)
11		Pale yellow	67	210 decompose
12		Brown	78	230 decompose
13		Brown	65	100
14		Off white	70	258

showed signal at δ = 17.21 ppm belongs to carbon of methyl group, signal at δ = 59.49 ppm belongs to (C-N), doublet signals at δ = 109.76 ppm and δ = 110.83 ppm belong to (C=C) vinylic, signals at δ = 125.31 ppm and δ = 130.25 ppm belong to (C-C) aromatic carbons and signal at δ = 157.97 ppm belongs to (C=N), that isindicated the formation imine compound **2** [17].

On the other hand, amino acids (alanine, glycine, valine) were condensed with acetic anhydride to afford N-acetamido amino acids (**3-5**) in the first step.

FT-IR spectra of compounds **3-5** showed appearance of several bands, ν(C=O) carboxylic, ν(C=O) imide, ν(C-N) imide at 1739-1716, 1680-1650 and 1352-1334 cm^{-1}, respectively and absence of ν(N-H).

^{1}H NMR spectrum of compound **3** showed signal at δ = 0.9 ppm belong to protons of methyl group, signals at δ = 1.21-1.35 ppm belong to (CH_3-C) acetyl protons, signal at δ = 4.05 ppm belongs to (N-CH-C-OO) proton and signal at δ = 9.52 ppm belongs to (COOH) proton, ^{13}C NMR spectrum of compound **3** showed signal at δ = 8.18-8.51 ppm, belongs to a carbon of methyl, signals at δ = 18.02-22.42 ppm belong to (CH_3-C), a signal at δ = 45.06 ppm belongs to (CH-N), a signal at δ = 168.69 ppm belongs to (-C-N) and a signal at δ = 175.05 ppm belongs to (COOH).

The second step involved a synthesis of N-acetamido amino acid chlorides (**6-8**) *via* reaction of thionyl chloride with compounds **3-5**.

FT-IR spectrum of compounds **6-8** gave absorption bands at 1782-1790 and 808-892 cm^{-1} attributed to ν(C=O) acetyl chloride and ν(C-Cl), respectively, beside this the absence of ν(O-H) at 3346-3415 cm^{-1} indicated success of nucleophilic substitution reaction to afford compound **6-8** as ketenes which subjected to Staudinger cycloaddition reaction by treating with N-cinnamylidene- 2-amino pyrimidine (**1**) or N-crotonylidene-2-aminopyrimidine (**2**) asiminesin the third step to afford N-(2-pyrimidine)-3-(N-acetamido)-4-styryl-2-azetidinones (**9-11**) and N-(2-pyrimidine)-3-(N-acetamido)-4-propenyl-2-azetidi-nones (**12-14**).

The FT-IR spectrum of compounds **9-14** showed the disappearance of band ν(C=N) imine, ν(C-Cl) with appearance bands at 1568-1560 and 1740-1705 cm^{-1} attributed to ν(C-N) β-lactam and ν(C=O) β-lactam, respectively. ^{1}H NMR spectrum of compound **12** showed signal at (δ = 1.75) ppm belongs to (CH_3-C=C) methyl protons, signal at (δ = 3.24) ppm belongs to (N-CH-C=O) proton, signal at δ = 4.65 ppm belongs to (CH-NCO_2-) proton, signal at δ = 6.55 ppm belongs to (CH=CH) proton and multiple signals at 8.21-8.22 ppm belong to (C-H) heteroaromatic protons, ^{13}C NMR showed singlet signal at (δ = 8.34) ppm belongs to (CH_3- C) carbon, signal at (δ = 45.18) ppm belongs to (N-C=O) β-lactam and signal at (δ = 157.97) ppm belongs to (CO-N-CO) carbon [18].

Microbiological test: The prepared β-lactam are screened for their antimicrobial activity against *Staphylococcus aureus*

(Gram-positive) and *Pseudomonas aruginosa*, *E. coli* (Gram-negative) and *Candida albicans*.

They showed different biological activity against these bacteria as showed in Table-2, also antimicrobial activity of the prepared compounds depend on nature of substituents in their molecules thus compounds **9-11**, which are substituted styryl showed high antimicrobial activity against *E. coli* and *Candida albicans* and mild activity against *Staphylococcus aureus*, the compounds **12-14** which are substituted propenyl showed high activity against *E. coli* only, except compound **13** showed high activity against *Candida albicans* too.

TABLE-2
INHIBITION ZONES OF COMPOUNDS **1**, **2** AND **9-14** (mm)

Compd. No.	Gram-negative		Gram-positive	Fungi
	E. coli	*P. aeruginosa*	*S. aureus*	*C. albicans*
1	14	17	N	18
2	11	N	N	12
9	17	N	12	17
10	17	N	11	18
11	13	N	11	15
12	13	N	N	N
13	14	N	N	15
14	15	N	N	N

N = No inhibition, Inhibition zone: 10-12 mm = Mild active, Inhibition zone > 12 mm Highly active.

Compound **1** showed high activity against *E. coli*, *Pseudomonas aeruginosa* and *Candida albicans*, compound **2** showed mild activity against *E. coli* and *Candida albicans*.

Conclusion

The newly synthesized β-lactams were obtained by cyclocondensation of Schiff bases substituted with pyrimidine as (imine) with different N-acetamido amino acid chlorides as (ketene) in good chemical yields. The results showed that β-lactams substituted with styryl group are more active as antibacterial and antifungal toward the tested organism. It is suggested to evaluate their anticancer activities in future.

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