

## Synthesis and Preliminary Antimicrobial Activity of New Schiff Bases of Pyrido [1,2-a] Pyrimidine Derivatives with Certain Amino Acids

Shakir M Alwan<sup>1\*</sup>, Jaafar Abdul-Sahib Al-Kaabi<sup>2</sup>

<sup>1</sup>Pharmaceutical chemistry department, College of Pharmacy, University of Baghdad, Bab Al-Moadham, P.O. Box 14026, Baghdad, Iraq

<sup>2</sup>College of pharmacy, University of Messan, Messan, Iraq

### Abstract

Pyrido [1,2-a] pyrimidine ring structure is one of the most interesting heterocycles in drug design and its derivatives have various potential pharmacological activities. An interesting approach of synthesizing a new series of pyrido-pyrimidine derivatives containing Schiff bases of certain amino acids, as privileged moieties of expected high potential in the field of antibacterial and antitumor agents, were investigated that may provide a synergistic model. The new derivatives 1-6 were synthesized by reacting 3-formyl-2H-pyrido [1, 2-a] pyrimidine-2, 4 (3H)-dione 1b with glycine, alanine, glutamic acid, histidine, tryptophan or leucine in methanol under reflux using glacial acetic acid as catalyst. The chemical structures of the new compounds and their intermediates (1-6, 1a and 1b) were characterized, identified and confirmed by spectral analysis (IR, <sup>1</sup>H-NMR) and elemental microanalysis (CHN) and the results were within the acceptable limits. Disc-diffusion method was used to evaluate the antimicrobial activities of the newly synthesized compounds of interest 1-6, using *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans* and *Escherichia coli*. The synthesized compounds 1-6 showed variable antibacterial activities ranged between good to moderately active, when compared with standards (amoxicillin and ceftriaxone). Compounds 4-6 also showed antifungal activities. However, compounds 5 and 6 are the most potent and have promising results. Compound 6 showed a good activity against all bacterial strains and fungi tested, while compound 5 showed the highest activity against *Pseudomonas aeruginosa*. This approach has afforded the synthesis of new pyrido-pyrimidine derivatives containing Schiff bases of certain amino acids of reasonable and promising antibacterial activities.

**Keywords:** Pyridopyrimidine; Schiff bases; Amino acids

### Introduction

Pyrido [1, 2-a] pyrimidine ring structure is one of the most interesting heterocycles in drug design [1], and compounds containing this moiety have various pharmacological activities [2]. This structural pattern is present in the known psychotropic agents risperidone [3] paliperidone [4], human leukocyte elastase inhibitor (SSR69071) [5], antiallergic agent ramastine [6], and the antioxidants 2-arylpyrido [1, 2-a] pyrimidin-4-ones [7]. Pyrimidines exhibit potential antibacterial [8], antiviral, [9] antitumor [10], anti-HIV [11], antinociceptive [12] activities and are extensively used in neurology, particularly in the treatment of neurodegenerative disorders, such as, Parkinson's disease [13], anti-anxiety disorders [14] and anti-depression cases [15].

Schiff bases have been shown to exhibit a wide range of biological activities including antimicrobial [16], anti-inflammatory and analgesic [17], anti-tubercular [18], antioxidant [19], antiviral and antifungal [20] and anticancer activities [21]. Schiff bases of 2-chloro-3-formyl-4-oxo-4H-pyrido [1, 2-a] pyrimidine with cyclic hydrazides were synthesized and tested for their antihypertensive and MAO-inhibitory activities [16]. The antibacterial and antifungal activities of Schiff bases of amino acids derived from the reaction of 2-hydroxy-1-naphthaldehyde with glycine, alanine, phenylalanine, histidine and tryptophan were reasonably potent [17]. Three new Schiff bases of indole-3-carboxaldehyde with glycine, alanine and valine have indicated better activities against *S. aureus*, *E. coli* and *B. polymyxa* than *C. albicans* [18].

In view of the stated pharmacological properties of the pyrido-pyrimidine derivatives and Schiff bases, a new series of pyrido-pyrimidine derivatives containing Schiff bases of certain amino acids as privileged moieties of expected high potential in the field of antibacterial and antitumor agents were investigated.

### Materials and Methods

#### Chemicals

2H-pyrido [1,2-a] pyrimidine-2,4-(3H) dione, 1a was synthesized

by reacting 2-amino pyridine and diethylmalonate in ethanol at 160-200°C for 4hrs with continuous removal of ethanol by distillation [19] as illustrated in scheme 1. The corresponding aldehyde 1b, 3-formyl-2H-pyrido [1, 2-a] pyrimidine-2,4-(3H) dione, was synthesized by reacting compound 1a with Phosphoryl chloride and N, N-dimethylformamide [20], as shown in scheme 1. The Schiff bases 1-6 were synthesized by reacting 3-formyl-2H-pyrido [1,2-a] pyrimidine-2,4-(3H) dione 1b with either glycine, alanine, leucine, glutamic acid, histidine or tryptophan in methanol in the presence of a catalytic amount of glacial acetic acid (0.5 mL) under reflux [21], as outlined in scheme 1. The amino acids, 2-aminopyridine and diethylmalonate were purchased from Himedia, N,N-dimethylformamide and phosphoryl chloride were obtained from Fluka AG. Petroleum ether (40-60) was from BDH. All other chemicals and solvents were of analar grade.

#### Bacteria

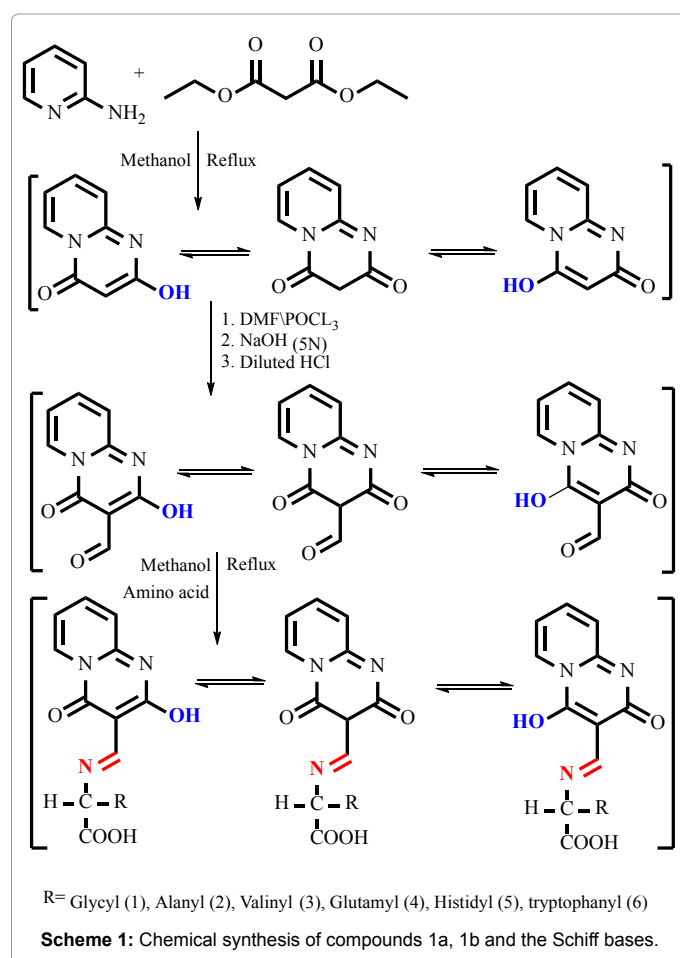
The following pathogenic bacteria and fungi are used to evaluate the antimicrobial activity of the newly synthesized compounds. *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC 27853), *Staphylococcus aureus* (*S. aureus*, ATCC 25923), *Bacillus subtilis* (*B. subtilis*, ATCC 6633), *Candida albicans* (*C. albicans*, isolated from a local hospital and was inoculated on a chocolate agar plate and grown at 37°C for 48 h) and *Escherichia coli* (*E. coli*, ATCC 29522) cultured on Mueller Hinton agar.

**\*Corresponding author:** Shakir M Alwan, Pharmaceutical chemistry department, College of Pharmacy, University of Baghdad, Bab Al-Moadham, P.O. Box 14026, Baghdad, Iraq, Tel: +9647902518888; E-mail: [shakmawales@yahoo.co.uk](mailto:shakmawales@yahoo.co.uk)

**Received** July 08, 2014; **Accepted** August 20, 2014; **Published** August 22, 2014

**Citation:** Alwan SM, Al-Kaabi JAS, Hashim RMM (2014) Synthesis and Preliminary Antimicrobial Activity of New Schiff Bases of Pyrido [1,2-a] Pyrimidine Derivatives with Certain Amino Acids. Med chem 4: 635-639. doi:10.4172/2161-0444.1000206

**Copyright:** © 2014 Alwan SM, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.



## Chemical synthesis

### a) Synthesis of 2H-pyrido [1, 2-a] pyrimidine-2, 4 (3H) dione, 1a

2-aminopyridine (0.106 M, 10 g) and diethyl malonate (0.106 M, 21.22 g) were suspended in ethanol (10 mL) and heated under reflux for 6 hrs in a flask fitted with a still head to extract ethanol continuously. The mixture was then cooled and the obtained precipitate was filtered and washed several times with ethanol and dried in an oven at 50 °C. This product was crystallized from hot water to afford compound 1a [1]. Yield: 85%, white powder, m.p. 298 °C (decomposed). IR spectra ( $\nu$ ,  $\text{cm}^{-1}$ ); 3095 (C-H of alkene), 2904 (assym. C-H of  $-\text{CH}_2-$ ), 2640-2810 (broad enolic OH), 1693 (C=O of COOH), 1653 (C=O of amide) and 1618 (C=N of imine). The  $^1\text{H-NMR}$  spectra (500 MHz, DMSO)  $\delta$ : 3.2 (2H, s,  $\text{C}_3-\text{H}_2$ ), 6.5 (1H, t,  $\text{C}_3-\text{H}$ ), 7.1 (1H, t,  $\text{C}_9-\text{H}$  pyridine), 7.4 (1H, t,  $\text{C}_7-\text{H}$ ), 8.1 (1H, t,  $\text{C}_8-\text{H}$ ), 8.9 (1H, t,  $\text{C}_6-\text{H}$ ), 11.9 (1H, s,  $\text{C}_2-\text{OH}$ ). Addition of  $\text{D}_2\text{O}$  to this compound indicated the disappearance of this proton. The elemental microanalysis (CHN) was recorded for  $\text{C}_8\text{H}_6\text{N}_2\text{O}_2$  (162.0); Calculated; C: 59.26; H: 3.73; N: 17.28; Found; C: 59.6; H: 3.87; N: 16.89.

### b) Synthesis of 3-formyl-2H-pyrido [1,2a] pyrimidine-2, 4-(3H) dione, 1b

Phosphoryl chloride (0.032 M, 3 mL) was added slowly with continuous stirring to the N, N-dimethylformamide (30 mL) incubated in an ice bath. Compound 1a (0.029 M, 4.85 g) was added and the mixture was heated on a water bath at 50°C for 20 min. The mixture was poured slowly into sodium hydroxide (5 N, 50 mL) with vigorous stirring and ice cubes were added as soon as the reaction

became exothermic. The mixture was then acidified to pH 5.5-6.0 by dilute hydrochloric acid and stored in a refrigerator. A precipitate was collected, washed excessively with distilled water and dried in an oven at 50°C. This product was crystallized from N, N-dimethyl formamide to afford compound 1b. The chemical synthesis is represented in scheme (1). Yield: 50%, yellow powder, m.p. 277°C (decomposed). IR spectra ( $\nu$ ,  $\text{cm}^{-1}$ ); 3113 (C-H of alkene), 2773 (C-H of aldehyde), 2640 (broad enolic OH), 1732 (C=O of aldehyde), 1654-1680 (C=O broad of amides) and 1635 (C=N of imine). The  $^1\text{H-NMR}$  spectra (500 MHz, DMSO)  $\delta$ : 4.1 (1H, s,  $\text{C}_3-\text{H}$ ), 6.5 (1H, t,  $\text{C}_3-\text{H}$ ), 7.1 (1H, t,  $\text{C}_9-\text{H}$  pyridine), 7.4 (1H, t,  $\text{C}_7-\text{H}$ ), 8.1 (1H, t,  $\text{C}_8-\text{H}$ ), 8.9 (1H, t,  $\text{C}_6-\text{H}$ ), 10.3 (1H, s,  $\text{C}_2-\text{H}$  aldehyde), 11.9 (1H, s,  $\text{C}_2-\text{OH}$ ). Addition of  $\text{D}_2\text{O}$  to compound 1b displayed no proton at this  $\text{C}_2-\text{OH}$ . The elemental microanalysis (CHN) was recorded for  $\text{C}_9\text{H}_6\text{N}_2\text{O}_3$  (190.2); Calculated; C: 56.85; H: 3.18; N: 14.73; Found; C: 56.91; H: 3.22; N: 14.96.

### c) General procedure for the synthesis of Schiff bases of 3-formyl-2H-pyrido [1,2a] pyrimidine-2, 4-(3H) dione with certain amino acids, 1-6

Schiff bases of 3-formyl-2H-pyrido [1,2-a] pyrimidine-2,4-(3H) dione 1b with certain amino acids were synthesized according to the reported method [18] and as described below.

A mixture of compound 1b (5.2 mM) and the amino acid (5.2 mM) in dry methanol (20 mL) containing a catalytic amount of glacial acetic acid (0.5 mL) were reacted under reflux for 4hrs. The unreacted compound 1b and the amino acid were separated by dissolving in hot water. The product was crystallized from hot ethanol. The chemical syntheses of compounds 1-6 are illustrated on scheme 1.

### d) Synthesis of the Schiff base of 3-formyl-2H-pyrido [1, 2-a] pyrimidine-2, 4-(3H) dione with glycine, 1

2-((2,4-dioxo-3,4-dihydro-2H-pyrido[1,2-a]pyrimidine-3-yl) methylene amino) acetic acid.

Compound 1b (5.2 mM, 1 g) in dry methanol (20 mL) was reacted with glycine (5.2 mM, 0.39 g) suspended in methanol (10 mL) containing glacial acetic acid (0.5 mL) and the mixture was refluxed for 4hrs. The mixture turned to an orange solution, which was cooled in a refrigerator and an orange precipitate was collected, washed thoroughly with hot water to remove unreacted materials (compound 1b and glycine). The orange product was triturated with petroleum ether ( $2 \times 20$  mL) and was dried in an oven at 50°C. Yield: 65%, pink powder, m.p. 220°C (dec.). IR spectra ( $\nu$ ,  $\text{cm}^{-1}$ ); 3097 (C=H, aromatic), 2970, 2920 (C-H), 3000-2800 (keto-enol OH), 1668 (C=O), 1631 (C=N).  $^1\text{H-NMR}$  (500 MHz, DMSO)  $\delta$ : 3.5 (1H, s, enolic  $\text{C}_2-\text{OH}$ ), 4.45 (2H, s,  $-\text{CH}_2-$ ), 6.85 (1H, t,  $\text{C}_9-\text{H}$  pyridine), 7.1 (1H, t,  $\text{C}_8-\text{H}$  pyridine), 7.8 (1H, t,  $\text{C}_7-\text{H}$ ), 8.6 (1H, d,  $\text{C}_6-\text{H}$ ), 8.7 (1H, s,  $\text{N}=\text{CH}-$ ), 10 (1H, s, COOH). The CHN analysis was recorded for  $\text{C}_8\text{H}_6\text{N}_2\text{O}_2$  (247.2); Calculated; C: 59.26; H: 3.73; N: 17.28. Found; C: 59.60, H: 3.86; N: 16.49.

### e) Synthesis of the Schiff base of 3-formyl-2H-pyrido [1, 2-a] pyrimidine-2,4-(3H) dione with alanine, 2

2-((2,4-dioxo-3,4-dihydro-2H-pyrido[1,2-a]pyrimidine-3-yl) methyleneamino) propanoic acid.

Compound 1b (5.2 mM, 1g) in dry methanol (20 mL) was reacted with alanine (5.2 mM, 0.463 g) suspended in methanol (10 mL) and the procedure was continued as previously described. Yield: 47%, orange powder, m.p. 152°C. IR spectra ( $\nu$ ,  $\text{cm}^{-1}$ ); 3096 (C-H), 2970, 2920 (C-H), 3000-2800 (OH), 1674 (C=O), 1618 (C=N).  $^1\text{H-NMR}$  (500 MHz, DMSO)  $\delta$ : 1.5 (3H, d,  $\text{CH}_3$ ), 3.5 (1H, m,  $=\text{N}-\text{CH}-$ ), 4.4 (1H, s,  $\text{C}_2-\text{OH}$ ), 6.9 (1H, t,  $\text{C}_9-\text{H}$ ), 7.7 (1H, d,  $\text{C}_8-\text{H}$ ), 8.1 (1H, t,  $\text{C}_7-\text{H}$ ), 8.5 (1H, d,

C<sub>6</sub>-H), 8.7(1H, s, -CH-N=), 11.2(1H, s, COOH). The CHN analysis was recorded for C<sub>9</sub>H<sub>6</sub>N<sub>2</sub>O<sub>3</sub> (261.2); Calculated; C: 56.86; H: 3.18; N: 14.73. Found: C: 56.91; H: 3.22; N: 14.96.

**f) Synthesis of the Schiff base of 3-formyl-2H-pyrido [1, 2-a] pyrimidine-2,4-(3H) dione with leucine, 3**

*2-((2,4-dioxo-3,4-dihydro-2H-pyrido[1,2-a]pyrimidine-3-yl)methyleneamino)-3-methyl butanoic acid.*

Compound 1b (5.2 mM, 1 g) in dry methanol (20 mL) was reacted with leucine (5.2 mM, 0.608 g) suspended in methanol and was treated as previously described. Yield: 77%, orange powder, m.p. 136-138°C. IR spectra (ν, cm<sup>-1</sup>); 3080, 3040 (C-H), 2955 (C-H), 3000-2800 (OH), 1676 (C=O), 1618 (C=N). <sup>1</sup>H-NMR (500 MHz, DMSO) δ: 0.9 (3H, d, CH<sub>3</sub>), 3.5(1H, d, =N-CH-), 3.6 (1H, s, C<sub>2</sub>-OH), 3.8(6H, m, CH<sub>3</sub>-C-CH<sub>3</sub>), 7.1 (1H, t, C<sub>9</sub>-H), 7.7 (1H, d, C<sub>8</sub>-H), 8.1 (1H, t, C<sub>7</sub>-H), 8.2(1H, d, C<sub>6</sub>-H), 8.4(1H, s, N=CH-), 11.2 (1H, s, COOH). The CHN analysis was recorded for C<sub>15</sub>H<sub>17</sub>N<sub>3</sub>O<sub>4</sub> (303.3); Calculated; C: 59.40; H: 5.65; N: 13.85. Found; C: 58.65; H: 5.95; N: 14.24.

**g) Synthesis of the Schiff base of 3-formyl-2H-pyrido [1, 2-a] pyrimidine-2, 4-(3H) dione with glutamic acid, 4**

*2-((2,4-dioxo-3,4-dihydro-2H-pyrido[1,2-a]pyrimidine-3-yl)methyleneamino)-3-methyl succinic acid.*

Compound 1b (5.2 mM, 1 g) in dry methanol (20 mL) was reacted with glutamic acid (5.2 mM, 0.765 g), as previously described. Yellow product was collected and washed with hot ethanol (3×10 mL) and was dried in an oven at 50°C to afford compound 4. Yield: 41%, yellowish brown, m.p. 212 °C (dec.). IR spectra (ν, cm<sup>-1</sup>); 3078, 3030 (C-H) 3000-2800 (OH), 2935, 2840 (C-H) 1693 (C=O), 1639 (C=O), 1614 (C=N). <sup>1</sup>H-NMR (500 MHz, DMSO) δ: 2.3 (2H, m, -CH<sub>2</sub>-), 2.6 (2H, t, -CH<sub>2</sub>-), 3.6 (1H, s, C<sub>2</sub>-OH), 4.6(2H, s, =N-CH-), 6.9(1H, t, C<sub>9</sub>-H), 7.1(1H, d, C<sub>8</sub>-H), 7.7(1H, t, C<sub>7</sub>-H), 8.5(1H, d, C<sub>6</sub>-H), 8.6(1H, s, N=CH-), 11.2 (1H, s, COOH), 12.4 (1H, s, COOH). The CHN analysis was recorded for C<sub>14</sub>H<sub>13</sub>N<sub>3</sub>O<sub>6</sub> (319.3); Calculated; C: 52.67; H: 4.10; N: 13.16. Found; C: 54.77; H: 3.23; N: 12.91.

**h) Synthesis of the Schiff base of 3-formyl-2H-pyrido [1, 2-a] pyrimidine-2,4-(3H) dione with histidine, 5**

*2-((2,4-dioxo-3,4-dihydro-2H-pyrido[1,2-a]pyrimidine-3-yl)methyleneamino)-3-(1H-imidazol-5-yl) propanoic acid.*

Compound 1b (5.2 mM, 1g) in dry methanol (20 mL) containing glacial acetic acid (0.5 mL) was reacted with histidine (5.2 mM, 0.806 g), as previously described. The Schiff base was collected as a yellow precipitate from the methanolic solution. The precipitate was washed with hot water to remove the unreacted materials. Yield: 68%, yellow powder, m.p. 226°C (dec.). IR spectra (ν, cm<sup>-1</sup>); 3395 (N-H), 3140, 3112 (C-H), 3000-2800 (OH), 2950 (C-H), 1695 (C=O), 1633 (C=N). <sup>1</sup>H-NMR (500 MHz, DMSO) δ: 2.3 (2H, m, -CH - assym. C of histidine), 2.6 (2H, t, -CH<sub>2</sub>-), 3.3 (1H, d, C<sub>3</sub>-H), 3.6 (1H, s, C<sub>2</sub>-OH), 4.6 (1H, s, -CH-N=), 6.9(1H, t, C<sub>9</sub>-H), 7.1(1H, d, C<sub>8</sub>-H), 7.7(1H, t, C<sub>7</sub>-H), 7.3 (1H, s, -CH- imidazole ring), 8.5(1H, d, C<sub>6</sub>-H), 12.4 (1H, s, COOH), 13.2(1H, s, NH imidazole). The CHN analysis was recorded for C<sub>15</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub> (327.3); Calculated; C: 55.05; H: 4.00; N: 21.40. Found; C: 56.47; H: 4.15; N: 21.63.

**i) Synthesis of the Schiff base of 3-formyl-2H-pyrido [1, 2-a] pyrimidine-2,4-(3H) dione with tryptophan, 6**

*2-((2,4-dioxo-3,4-dihydro-2H-pyrido[1,2-a]pyrimidine-3-yl)methyleneamino)-2-(1H-indol-3-yl) acetic acid.*

A similar procedure was conducted to produce compound 6, by

using the followings compound 1b (5.2 mM, 1 g) in dry methanol (20 mL) containing glacial acetic acid (0.5 mL) and tryptophan (5.2 mM, 1.061 g). The Schiff base was collected as a yellow precipitate from the methanolic solution and was washed with hot water to remove unreacted materials. The precipitate was triturated with petroleum ether (2×20 mL) and dried in an oven at 50°C. Yield: 72%, yellow powder, m.p. 205°C (dec.). IR spectra (ν, cm<sup>-1</sup>); 3404 (N-H, indole), 3191, 3136 (C-H), 3000-2800 (OH), 2950, 2835 (C-H), 1724 (C=O), 1658, 1641 (C=N), 1626 (N-H). <sup>1</sup>H-NMR (500 MHz, DMSO) δ: 2.3 (1H, m, -CH- assym. C of tryptophan), 2.6 (2H, t, -CH<sub>2</sub>-), 3.3 (1H, s, C<sub>3</sub>-H), 7.1, 7.4, 8.1 and 8.9 (1H, d, C<sub>9</sub>-H to C<sub>6</sub>-H pyridine), 7.1-7.6 (1H, s, CH aromatic), 10.8 (1H, s, NH indole), 12.4 (1H, s, COOH). The CHN analysis was recorded for C<sub>20</sub>H<sub>16</sub>N<sub>4</sub>O<sub>4</sub> (376.4); Calculated; C: 63.82; H: 4.28; N: 14.89. Found; C: 65.90; H: 4.26; N: 14.66.

## Results

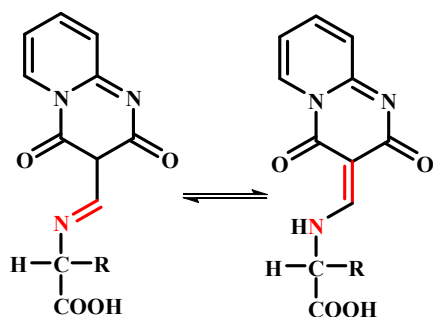
### Spectroscopic characterization of the synthesized compounds

**IR spectra:** The IR spectra of the intermediates and the new derivatives showed the appearance of bands at 3020-3095 cm<sup>-1</sup> for the enolic OH absorbance in all compounds. The bands at 1720 cm<sup>-1</sup> and 1693 cm<sup>-1</sup> are good indication for the carbonyl of the cyclic amides at (C<sub>4</sub>-N<sub>5</sub>) and (N<sub>1</sub>-C<sub>2</sub>) respectively. The characteristic bands (1618-1635 cm<sup>-1</sup>) of imines in compounds 1-6 represent the Schiff bases formed with amino acids. The presence of NH absorption band at 3210-3050 cm<sup>-1</sup> in compounds 1-6 indicated that there is a tautomerism between the imine group and the pyrimidine ring. The IR spectrum of compound 1a showed an absorption band at 1720 cm<sup>-1</sup> and 1693 cm<sup>-1</sup> for the carbonyl of the cyclic amides at (C<sub>4</sub>-N<sub>5</sub>) and (N<sub>1</sub>-C<sub>2</sub>) respectively. A band at 1618 cm<sup>-1</sup> for C=N- stretching of imine group. An absorption band was shown at 3020-3095 cm<sup>-1</sup> for broad enolic OH group. This characteristic band is clear evidence of the keto-enol tautomerism between the carbonyl and OH groups at C<sub>2</sub> and C<sub>3</sub> of the pyrimidine ring. C-H stretching of the corresponding aldehyde 1b appeared at 1392 cm<sup>-1</sup>, while the carbonyl of 1b appeared at 1732 cm<sup>-1</sup>.

**<sup>1</sup>H-NMR spectra:** The <sup>1</sup>H-NMR spectral data of compounds 1a and 1b displayed characteristic protons of the pyridine nucleus of pyridopyrimidine (C<sub>6</sub>-C<sub>9</sub>) and the protons of the two methylene groups at 6.85. 1-6 displayed the characteristic peaks at C<sub>6</sub>-C<sub>9</sub> of the pyridine ring appeared at 8.6, 7.8, 7.1 and 6.85 respectively. The imine protons present in compounds 1-6 was observed and appeared as singlet at δ 8.4-8.7. Proton of OH of the keto-enol form for all compounds appeared at range of δ 3.5-4.4. Addition of D<sub>2</sub>O to compounds 1a and 1b showed disappearance of protons in the <sup>1</sup>H-NMR spectra. This is a further confirmation of the existence of keto-enol form. Protons of COOH for compounds 1-3 and 5-6 appeared weak at 11.2, while the proton of the second carboxyl of glutamic acid in compound 4 also appeared weak at 12.4.

**Elemental microanalysis (CHN):** The elemental microanalyses of the starting materials compounds 1a and 1b and the target compounds 1-6 confirmed their chemical structures and were within the acceptable range.

**Tautomerism phenomenon of the synthesized compounds:** For compounds 1a and 1b, depending on solution, two possible conformations can be described and these are the keto-enol forms, as shown on schemes 1. The chemical structures of 1a is assigned as 4H-pyrido [1, 2-a] pyrimidine-2-hydroxy-4-one or 4H-pyrido [1,2-a] pyrimidine-4-hydroxy-2-one and these were confirmed by spectral analysis. Compounds 1-6 may also undergo further tautomerism leading to few new situations, as shown on schemes 1 and 2. These forms showed the appearance of the imine group and at the same time



**Scheme 2:** Phenomenon of tautomerism of the synthesized Schiff bases.

Compound	Concentration µg/ml	<i>E. coli</i> ATCC 29522	<i>P. aurug.</i> ATCC 27853	<i>S. aureus</i> ATCC 25923	<i>B. Subtilus</i> ATCC 6633	<i>C. Albicans</i>
1	50	+	+	+	-	-
	100	+	+	++	-	+
2	50	+	+	-	-	-
	100	++	+	-	-	+
3	50	-	+	-	-	-
	100	+	++	-	-	-
4	50	++	+	+	+	++
	100	+++	++	-	-	-
5	50	+	++	-	-	+
	100	+++	+++	-	-	++
6	50	++	++	+	-	-
	100	+++	++	++	++	++
Std.1	50	+++	++	++	++	-
	100	+++	+++	+++	+++	-
Std.2	50	++	+++	++	++	-
	100	+++	+++	++	++	-
Std.3	50	-	-	-	-	++
	100	-	-	-	-	+++

**Key to symbols:-** - = no inhibition, + = 14 mm, ++ = 15-17 mm, +++ = more than 18mm.

Std. 1 = Ceftriaxone, Std. 2 = Amoxicillin, Std. 3 = Fluconazole.

**Table 1:** Antimicrobial activity of compounds [1-6].

hydroxyl group and the presence of -NH due to tautomerism with the pyridopyrimidine nucleus. These groupings appeared on the IR spectra of compounds 1-6, which have supported this phenomenon.

## Discussion

The IR spectra of the new derivatives helped to confirm their chemical structures and their tautomeric forms by showing the keto-enol forms. It was reported that such tautomeric forms were observed in the synthesis of compounds 1a and 1b [22]. This situation was also previously observed for synthesis of pyridopyrimidine derivatives [23-26]. For compounds 1-6, there are even further tautomeric forms that are formed between C<sub>2</sub>-C<sub>4</sub> and at C<sub>3</sub> and the imine formed with the amino acids (scheme 2). This tautomerism is governed by the bonds illustrated to include major changes on the molecules and cannot be ruled out. The main differences between these tautomeric forms lie in the intramolecular hydrogen bonding and the relative orientation of the carbonyl groups. Attractive intermolecular interactions occur in this part of the molecule and are responsible for the value of the dihedral angles. These factors have direct impact on the bioactivity of these compounds. This explanation may comply with the observation of the activities of related pyridopyrimidine derivatives [25,26]. The presence of an asymmetric center in the amino acid moiety (scheme

2) adds another factor, which is the existence of isomers and this will definitely affect the bioactivity. The <sup>1</sup>H-NMR spectra of the compounds under study 1-6 revealed that their characteristic peaks experienced noticeable changes. The C<sub>9</sub> proton experienced deshielding due to the only inductive effect of the (=N-C=O) and were recorded downfield as singlet at δ 6.85-7.1. The C<sub>7</sub> proton is experiencing less deshielding effect by virtue of its position and nature of bonding. The C<sub>6</sub> proton is deshielded due to the inductive effect of (-N-C=O) functionality. In all compounds, a very small difference in the chemical shifts of C<sub>6</sub>-C<sub>9</sub> protons was observed, which is probably due to the small inductive electron withdrawing or donating effects of pyridine or pyrimidine moieties. Similarly, a very small difference in the chemical shifts of the imine protons present in compounds 1-6 was observed and appeared as singlet at δ 8.4-8.7.

## Effect of the amino acid moieties

The newly synthesized compounds 1-6 showed reasonable activities against *P. auroginosa*, *B. subtilus*, *E. coli*, *S. aureus* and *C. albicans*. Compounds 1-4 contain two main privileged chemical moieties, Schiff bases with amino acids and pyridopyrimidine, while compounds 5 and 6 contain extra privileged chemical groups and these are the imidazole and indole, respectively. This may be the reason behind the improved antibacterial activities especially against *P. auroginosa*, *C. albicans* and *E. coli* when compared with the standards used. Similar observation was reported with various types of Schiff bases of such amino acids [17,18,27].

## Antimicrobial evaluation

Generally, all the Schiff bases 1-6 showed good to moderate antibacterial activity against the test microbes (Table 1). Compounds 4-6 showed also antifungal activity. Compound 5 showed reasonable activity against *p. aurogenosa* and *C. albican*, while it showed a good activity against *E. coli* and no activity against G (-) bacteria. Compound 4 showed a moderate activity against *E. coli* and a good activity against *Candida*. Compounds 2 and 3 had good activity against *Candida* and a moderate activity against *E. coli*. Compound 1 has a moderate to good activity against all strains, except *B.subtilus*. However, the Schiff bases of the aromatic amino acids, compounds 5-6 showed better antimicrobial activities compared with those of aliphatic amino acids.

## Conclusion

An interesting approach of using two privileged moieties (Schiff bases of amino acids and pyridopyrimidine ring) is successfully accomplished to produce new pyridopyrimidine derivatives. This approach has afforded new derivatives of reasonable and promising antibacterial activities.

## Acknowledgment

The authors are very grateful to University of Baghdad and College of Pharmacy for supporting this research work.

## References

- Zoorob HH, HamamaWS (2002) Chemistry of bicyclic pyridines containing a ring-junction nitrogen. Tetrahedron 58: 6143-6162.
- Katritzky AR, Rogers JW, Witek RM, Nair SK (2011) Synthesis of novel pyrido[2-a]pyrimidinium tetrafluoroborates. ARKIVOC 2: 297-309.
- Khan BU (1997) Brief report: risperidone for severely disturbed behavior and tardive dyskinesia in developmentally disabled adults. J Autism Dev Disord 27: 479-489.
- Jeste DV, Okamoto A, Napolitano J, Kane JM, Martinez RA (2000) Low incidence of persistent tardive dyskinesia in elderly patients with dementia treated with risperidone. Am J Psychiatry 157: 1150-1155.

5. Kapui Z, Varga M, Urban-Szabo K, Mikus E, Szabo T, et al. (2003) Biochemical and pharmacological characterization of 2-(9-(2-piperidinoethoxy)-4-oxo-4H-pyrido[2-a]pyrimidin-2-yl)methyl-4-(1-methylethyl)-6-methoxy-, 2-benzisothiazol-3(2H)-one-, 1-dioxide (SSR69071), a novel orally active elastase inhibitor. *J Pharmacol Exp Ther* 2: 451-459.
6. Awouters F, Vermeire J, Smeyers F, Vermote P, van Beek (1986) Oral antiallergic activity in ascaris hypersensitive dogs: A study of known antihistamines and of the new compounds ramastine (R 57 959) and levocabastine (R 50 547). *Drug Development Research* 8: 95-102.
7. Motta CL, Sartini S, Mugnaini L, Simorini F, Taliani S, et al. (2007) Pyrido[2-a]pyrimidin-4-one derivatives as a novel class of selective aldose reductase inhibitors exhibiting antioxidant activity. *J Med Chem* 4: 4917-4927.
8. Ferrarini PL, Mori C, Armani G, Rossi L (1995) Synthesis of some 4H-pyrido[2-a]pyrimidin-4-ones investigated as antimicrobial agents. *Farmaco* 50: 69-72.
9. Hossain N, Rozenski J, De Clercq E, Herdewijn P (1997) Synthesis and Antiviral Activity of the alpha-Analogues of 5-Anhydrohexitol Nucleosides (5-Anhydro-2,3-dideoxy-D-ribohexitol Nucleosides). *J Org Chem* 62: 2442-2447.
10. Yao P, Zhai X, Liu D, Qi BH, Tan HL, et al. (2010) Synthesis and antiproliferative activity of novel diaryl ureas possessing a 4H-pyrido[2-a]pyrimidin-4-one group. *Arch Pharm (Weinheim)* 343: 17-23.
11. Joseph S, Burke JM (1993) Optimization of an anti-HIV hairpin ribozyme by in vitro selection. *J Biol Chem* 268: 24515-24518.
12. Raghunath BT, Bhausaheb KG, Muddassar AK, Shivaraj PP, Madhukar N (2008) New Approach for the Synthesis of Pyrido[2-a]pyrimidines. *Scholarly Research Exchange* 5-8.
13. Jasinski JP, Butcher RJ, Hakim Al-Arique QN, Yathirajan HS, Narayana B (2009) 3-(2-Chloro-ethyl)-2-methyl-4-oxo-4H-pyrido[2-a]pyrimidin-2,4,6-trinitro-phenolate. *Acta Crystallogr Sect E Struct Rep Online* 65: o2201-2202.
14. Goodacre SC, Street LJ, Hallett DJ, Crawforth JM, Kelly S, et al. (2006) Imidazo[2-a]pyrimidines as functionally selective and orally bioavailable GABA(A)alpha2/alpha3 binding site agonists for the treatment of anxiety disorders. *J Med Chem* 49: 35-38.
15. Bishnoi A, Akst SS (2013) Synthesis, characterization and biological activity of new cyclization products of 3-(4-substituted benzylidene)-2H-pyrido[2-a]pyrimidine-2,4-(3H)-dione. *J Chem Sci* 125: 305-312.
16. George T, Kaul CL, Grewal RS, Tahilramani R (1971) Antihypertensive and monoamine oxidase inhibitory activity of some derivatives of 3-formyl-4-oxo-4H-pyrido (2-a) pyrimidine. *J Med Chem* 14: 913-915.
17. Sakiyan I, Loğoğlu E, Arslan S, Sari N, Sakiyan N (2004) Antimicrobial activities of N-(2-hydroxy-1-naphthalidene)-amino acid (glycine, alanine, phenylalanine, histidine, tryptophane) Schiff bases and their manganese(III) complexes. *BioMetals* 17: 115-120.
18. Nursen Sari Saelais. Antibacterial Activities of Some New Amino Acid-Schiff Bases. *G.U. journal of science*. 2003; 16: 283-288.
19. Gullu M, Razack LA, Utley JHP (1991) Electro-organic Reaction part 35. Efficient Carbon-Oxygen Bond formation in the Anodic Coupling of Pyridopyrimidine Derivatives. *Tetrahedron* 47: 675-684.
20. Eldredge H, Mee CD, Inventors J, Randolph D, Assignee J (1972) Intermediates and the preparation and use thereof. USA patent 3,674,782.
21. Muhammad AA, Karamat MA (2011) Synthesis, Characterization and Biological activity of Schiff bases. *International proceedings on Chemical, biological and environmental engineering*, 10: 1-7.
22. Tschischibabin AE (1924) Formation of bicyclic derivatives of a-aminopyridines. *Ber Dtsch Chem Ges* 57: 1168.
23. Hermecz I, Horvath A, Meszaros Z, De Vos C, Rodriguez L (1984) Nitrogen bridgehead compounds. 44. New antiallergic 4H-pyrido[2-a]pyrimidin-4-ones. 4. *J Med Chem* 27: 1253-1259.
24. Shur M, Israeltam SS (1968) The reaction of amino heterocycles with reactive esters I. Aminopyridines. *J Org Chem* 33: 3015.
25. Hamdouchi C, Ezquerra J, Vega JA, Vaquero JJ, Alvarez-Builla J, et al. (1999) Short synthesis and anti-rhinoviral activity of imidazo[2-a]pyridines: the effect of acyl groups at 3-position. *Bioorg Med Chem Lett* 9: 1391-1394.
26. Rauf A, Liaqat S, Qureshi AM, Yaqub M, Rehman AU, et al. (2012) Synthesis, characterization and urease inhibition of 5-substituted-8-methyl-2H-pyrido [2-a] pyrimidine-2,4-(3H)-diones. *Med Chem Res* 2: 60-74.
27. Evans BE, Rittle KE, Bock MG, DiPardo RM, Freidinger RM, et al. (1988) Methods for drug discovery: development of potent, selective, orally effective cholecystokinin antagonists. *J Med Chem* 31: 2235-2246.