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

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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, 1H-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] pyrimidine-4-ones [7]. Pyrimidines exhibit potential antibacterial [8], antiviral, [9] antitumor [10], anti-HIV [11], antiinociceptive [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 or 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

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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 (v, cm¬¹): 3095 (C-H of alkene), 2904 (C-H of C=C-CH3), 2900 (C-H of CH2), 2870-2810 (C-H of alkene), 1693 (C=O of COOH), 1673 (C=C of amide) and 1618 (C=C of imine); The 1H-NMR spectra (500 MHz, DMSO) δ: 3.2 (2H, s, C 3-CH2), 6.5 (1H, t, C 3-H) 7.1(1H, t, C 9-H pyridine), 7.4 (1H, t, C 7-H), 8.1(1H, t, C 9-H) and 10.3(1H, s, C 1-H pyridine). Addition of D2O to compound 1a displayed no proton at this C 2-OH. The elemental microanalysis (CHN) was recorded for C8H6N2O2 (162.0); Calculated; C: 59.26; H: 3.73; N: 17.28; Found; C: 59.60; H: 3.86; N: 16.49.

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 with water and dried in an oven at 50°C. The mixture was then cooled and the obtained precipitate was filtered and washed few times with ethanol and dried in an oven at 50°C. This product was crystallized from hot water to afford compound 1b. The chemical synthesis is represented in scheme (1). Yield: 50%, yellow powder, m.p. 152°C. IR spectra (v, cm¬¹): 1313 (C-H of alkene), 1773 (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 1H-NMR spectra (500 MHz, DMSO) δ: 4.1 (1H, s, C 2-H) 6.5 (1H, t, C 3-H) 7.1(1H, t, C 9-H pyridine), 7.4 (1H, t, C 7-H), 8.1(1H, t, C 9-H) 8.9 (1H, t, C 6-H),10.3(1H, s, C 1-H pyridine), 11.9 (1H, s, C 2-OH). Addition of D2O to compound 1b displayed no proton at this C 2-OH. The elemental microanalysis (CHN) was recorded for C6H5N2O2 (162.0); Calculated; C: 59.26; H: 3.73; N: 17.28; Found; C: 59.60, H: 3.86; N: 16.49.

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 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 hrs). The mixture turned to a yellow solution, which was cooled in a refrigerator and an orange precipitate was collected, washed thoroughly with distilled water and dried in an oven at 100°C. 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 with water and dried in an oven at 50°C. This product was crystallized from hot water to afford compound 1b. The chemical synthesis is represented in scheme (1). Yield: 65%, pink powder, m.p. 220°C (dec.). IR spectra (v, cm¬¹): 3097 (C-H, aromatic), 2970, 2920 (C-H), 3000-2800 (keto-enol OH), 1668 (C-O), 1631 (C=N). 1H-NMR (500 MHz, DMSO) δ: 3.5 (1H, s, enolic C 2-OH), 4.45 (2H, s, -CH 2 -), 5.2 (1H, t, C 7-H), 6.5 (1H, t, C 3-H) 7.1(1H, t, C 9-H pyridine), 7.4 (1H, t, C 7-H), 8.1(1H, t, C 9-H) and 10.3(1H, s, C 1-H pyridine). Addition of D2O to this compound indicated the disappearance of this proton. The elemental microanalysis (CHN) was recorded for C10H12N2O2 (192.0); Calculated; C: 56.85; H: 3.18; N: 14.73; Found; C: 56.91; H: 3.22; N: 14.96.

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) methylene amino) 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 (v, cm¬¹): 3096 (C-H), 2970, 2920 (C-H), 3000-2800 (OH), 1674 (C=O), 1618 (C=N). 1H-NMR (500 MHz, DMSO) δ: 1.5 (3H, d, CH3), 3.5(1H, m, =N-CH2-), 4.4 (1H, s, C=O-H), 6.9 (1H,t, C 6-H),7.7(1H,d, C 9-H), 8.1(1H,t, C 1-H), 8.5(1H, d,
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: 72%, yellow powder, m.p. 136-138°C. IR spectra (ν, cm-1); 3078, 3030 (C-H), 2950, 2835 (C-H), 1724 (C=O), 1658, 1641 (C=N), 1626 (N-H). The CHN analysis was recorded for C15H13N5O4 (327.3); Calculated; C: 55.05; H: 4.00; N: 21.40. Found; 77%, orange powder, m.p. 136-138°C. 1H-NMR (500 MHz, DMSO) δ: 0.9 (3H, s, CH3), 7.3 (1H, s, -CH- imidazole ring), 8.5 (1H, d, C6-H), 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) diione with glutamic acid, 4**

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

Compound 1b (5.2 mM, 1 g) in dry methanol (20 mL) was reacted with glutamic acid (5.2 mM, 0.608 g) suspended in methanol and was treated 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: 77%, yellowish powder, m.p. 226°C (dec.). IR spectra (ν, cm-1); 3078, 3030 (C-H), 3000-2800 (OH), 2935, 2840 (C-H) 1693 (C=O), 1639 (C=O), 1614 (C=N). 1H-NMR (500 MHz, DMSO) δ: 2.3 (2H, m, -CH2-), 2.6 (2H, t, -CH2-), 3.6 (1H, s, C2-OH), 4.6 (2Hs, -N=CH2), 6.9 (1H, t, C1-H), 7.1 (1Hd, C9-H), 7.7 (1H, t, C9-H), 8.5 (1H, s, -CH-N=), 8.6 (1H, s, C=OH), 11.2 (1H, s, COOH), 12.4 (1H, s, COOH). The CHN analysis was recorded for C19H17N3O4 (376.4); Calculated; C: 65.90; H: 4.26; N: 14.66.

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

2-((2,4-dioxo-3,4-dihydro-2H-pyrido[1,2-a]pyrimidine-3-yl) methylamino)-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 powder, m.p. 205°C (dec.). IR spectra (ν, cm-1); 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). The CHN analysis was recorded for C20H16N4O4 (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⁻¹ for the enolic OH absorbance in all compounds. The bands at 1720 cm⁻¹ and 1693 cm⁻¹ are good indication for the carbonyl of the cyclic amides at (C–N) and (N–C) respectively. 1H-NMR spectra of compound 1a showed an absorption band at 1720 cm⁻¹ and 1693 cm⁻¹ are good indication for the carbonyl of the cyclic amides at (C–N) and (N–C) respectively. 1H-NMR (500 MHz, DMSO) δ: 2.3 (2H, m, -CH2-), 2.6 (2H, t, -CH2-), 3.6 (1H, s, C2-OH), 4.6 (2Hs, -N=CH2), 6.9 (1H, t, C1-H), 7.1 (1Hd, C9-H), 7.7 (1H, t, C9-H), 8.5 (1H, s, -CH-N=), 8.6 (1H, s, C=OH), 11.2 (1H, s, COOH), 12.4 (1H, s, COOH). The CHN analysis was recorded for C19H17N3O4 (376.3); Calculated; C: 65.77; H: 4.32; N: 12.91.

**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 forms showed the appearance of the imine group and at the same time 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 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⁻1); 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). The CHN analysis was recorded for C20H16N4O4 (376.4); Calculated; C: 63.82; H: 4.28; N: 14.89. Found; C: 65.90; H: 4.26; N: 14.66.

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

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

A similar procedure was conducted to produce compound 6, by


Effect of the amino acid moieties

The newly synthesized compounds 1-6 showed reasonable activities against *P. aeruginosa*, *B. subtilis*, *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. aeruginosa*, *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. aerogenosa* 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.

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References


