J. Chem. Sci. Vol. 125, No. 2, March 2013, pp. 305–312.

Synthesis, characterization and biological activity of new cyclization products of 3-(4-substituted benzylidene)-2*H*-pyrido[1,2-*a*]pyrimidine 2,4-(3*H*)-diones

ABHA BISHNOI[a,*], SURUCHI SINGH[a], ANIL K TIWARI[a], KRISHNA SRIVASTAVA[b], RAM RAGHUVIR[c] and CHANDRAKANT M TRIPATHI[c]

[a]Department of Chemistry, University of Lucknow, University Road, Lucknow 226 007, India
[b]Department of Chemistry, Sri Ramswaroop College of Engineering and Management, Faizabad Road, Lucknow 226 001, India
[c]Division of Pharmacology, Central Drug Research Institute, Chattar Manzil Palace, Lucknow 226 001, India
e-mail: dr.abhabishnoi@gmail.com; abhabishnoi5@gmail.com; anilk.tiwari04@gmail.com

MS received 10 March 2012; revised 10 June 2012; accepted 25 July 2012

Abstract. A method is presented for the synthesis of 4-(substituted phenyl)-3-(3-substituted phenyl)4*H*-spiro[isoxazole-5,3′-pyrido[1,2-*a*]pyrimidine]-2′,4′-dione **(3)**, 3-(4-substituted phenyl)-3*H*-isoxazole[3,4-*d*]pyrido[1,2-*a*]pyrimidin-4-(3a*H*)-one **(4)** and 3-(4-substituted phenyl) 3,3a-dihydropyrazolo[3,4-*d*]pyrido[1,2-*a*]pyrimidin-4-(2*H*)-one **(5)** which consists of the conversion of 2*H*-pyrido[1,2-*a*]pyrimidine-2,4(3*H*)-dione **(1)** to chalcones **(2)** and their 1,3-dipolar cycloaddition with appropriate aldoximes to give spiro compounds and heterocyclization using amines to yield isoxazolines and pyrazolines. All the compounds were screened for their antimicrobial and antitubercular activity.

Keywords. Antimicrobial; antitubercular; pyrimidine; spiroisoxazolines; pyrazoline.

1. Introduction

Compounds incorporating heterocyclic ring systems continue to attract considerable interest due to the wide range of biological activities they possess. Amongst them, five-membered heterocyclic compounds occupy a unique place in the realm of natural and synthetic organic chemistry. Five-membered heterocycles like isoxazoline and pyrazoline have found wide applications in the fields of pharmaceutical and agrochemical chemistry.

The value of pyrimidine derivatives is significant among various heterocycles, as they are found to possess antineoplastic,[1–3] antiviral,[4–6] antibiotic,[7] anti-inflammatory,[8] antifungal,[9] antitumour[10,11] and other diverse biological activities.[12–15] Many pyrimidine derivatives are used for the production of thyroid drugs and in the treatment of leukemia also.

In recent years, attention has been increasingly drawn to the synthesis of isoxazolines and pyrazolines as a new source of antibacterial agents. Isoxazoline derivatives have been reported to possess antifungal,[16] antibacterial,[17] antitumour,[18] antiinflammatory,[19] antiviral,[20] analgesic,[21] antidiabetic[22] and anticancer[23] activities. In addition to this, isoxazoline derivatives have played a crucial role in the theoretical development of heterocyclic chemistry and are also used extensively in organic synthesis.

Spiroisoxazolines are heterocyclic nuclei which have stimulated much interest in the field of medicinal and biological chemistry.[24] They display interesting plant growth regulatory,[25] herbicidal[26] and antitumour[27] activities. Antitubercular and antimicrobial activity[28,29] of some spiroisoxazoline derivatives have also been reported recently. The results clearly show that spiroisoxazoline-based compounds have the ability to become drugs of immense use in the above mentioned areas.

Pyrazolopyrimidines and fused heterocycles related to it have also been identified as bioactive molecules. They are known to function as CNS depressants,[30] neuroleptic,[31] antihypertensive,[32] analgesic,[33] antimicrobial[34] and tuberculostatic[35] agents and have also been identified as adenosine receptors.[36]

Encouraged by the diverse biological activities of isoxazoline, pyrazoline, spiroisoxazoline and pyrimidine compounds, in our investigation we found an interesting approach to synthesize these ring systems fused with pyrimidine nucleus.

*For correspondence

2. Experimental

All melting points are uncorrected. IR spectra were recorded on a Perkin-Elmer 1430 spectrophotometer using KBr pellets. Mass spectra were recorded on a Va 70-70H spectrophotometer at 70 eV. ^{1}H and ^{13}C spectra were recorded in $CDCl_3$ or DMSO using TMS as an internal standard on a Bruker F 400 MHz NMR spectrophotometer, respectively. Elemental analyses were carried out on Carlo Erba EA-1108 element analyzer.

2.1 *General procedure for the preparation of 2H-pyrido[1,2-a]pyrimidine-2,4(3H)-dione* ***1***

2-Amino pyridine (2 g, 0.0212 mol) and malonic acid (2.208 g, 0.0211 mol) were refluxed in absolute ethanol (20 mL) under anhydrous conditions for 12 h and excess solvent was then distilled off. Crystalline solid which separated on cooling was collected and recrystallized with methanol. mp 180°C ; IR (KBr)cm^{-1}: 3438 (O–H), 3100 (C–H), 2930 (C–H aliphatic), 1700 (C=O), 1656 (C=N); ^{1}H NMR ($CDCl_3$) δ: 3.29 ($C\underline{H}_2$ protons), 7.28 (t, 1H, $C^{7}\underline{H}$), 7.5 (d, 1H, $C^{9}\underline{H}$), 7.7 (t, 1H, $C^{8}\underline{H}$), 8.82 (d, 1H, $C^{6}\underline{H}$); ^{13}C NMR ($CDCl_3$) δ: 40.20 (CH_2 carbons), 121.7, 124.20, 125.2, 130.3, 149.5, 170.08, 171.1. Anal. Calcd. C: 59.26, H: 3.70, N: 17.28, Found C: 60.00, H: 3.58, N: 17.14%.

2.2 *General procedure for the preparation of 3-(4-substituted benzylidene)-2H-pyrido[1,2-a]pyrimidine-2,4(3H)-dione* ***2a–c***

The desired compounds **2a–c** were prepared via Claisen condensation of **1** with substituted benzaldehydes in the presence of NaOH by known procedure.[37–41]

2.2a *3-Benzylidene-2H-pyrido[1,2-a]pyrimidine-2,4(3H)-dione* ***2a***: Yellowish white crystals, yield 75%, mp 180°C; IR (KBr)cm^{-1}: 3016 (Ar-H), 1680 (C=O), 1634 (C=N); ^{1}H NMR ($CDCl_3$) δ: 6.05–7.09 (m, 5H, Ar-$\underline{H}$), 7.18–7.80 (m, 3H, C^{7-9}), 8.25 (s, 1H, benzylidene), 8.80 (d, 1H, $C^{6}\underline{H}$); ^{13}C NMR ($CDCl_3$) δ: 118.5, 125.6, 127.7, 127.9, 128.4, 128.7, 128.8, 133.2, 134.6, 138.4, 145.2, 148.3, 156.4, 179, 181.3; Anal. Calcd. C: 72.00, H: 4.03, N: 11.20, Found C: 71.89, H: 3.94, N: 11.17%.

2.2b *3-(4-Chlorobenzylidene)-2H-pyrido[1,2-a]pyrimidine-2,4(3H)-dione* ***2b***: Brown crystals, yield 79 %, mp 168°C; IR (KBr)cm^{-1}: 3009 (Ar-H), 1676 (C=O), 1630 (C=N); ^{1}H NMR ($CDCl_3$) δ: 6.15–7.09 (m, 4H, Ar-$\underline{H}$), 7.45–7.97 (m, 3H, C^{7-9}), 8.30 (s, 1H, benzylidene), 8.82 (d, 1H, $C^{6}\underline{H}$); ^{13}C NMR ($CDCl_3$) δ: 117.4, 125.7, 128.2, 128.5, 131.6, 132.9, 133.4, 133.7, 135.1, 138.4, 147.1, 148.9, 157.2, 178.7, 180.7; Anal. Calcd. C: 63.27, H: 3.16, N: 9.84, Found C: 63.45, H: 3.08, N: 9.89%.

2.2c *3-(Benzo[d][1,3]dioxol-5-ylmethylene)-2H-pyrido[1,2-a]pyrimidine-2,4(3H)-dione* ***2c***: Light brown crystals, yield 78%, mp 202°C; Anal. Calcd. C: 65.30, H: 3.40, N: 9.52, Found C: 65.0, H: 3.37, N: 9.49%.

2.3 *General procedure for the preparation of 4-(substituted phenyl)-3-(substituted phenyl)-4H-spiro[isoxazole-5,3′-pyrido[1,2-a]pyrimidine]-2′,4′-dione* ***3a–f***

A mixture of arylidene **2a–c** (0.068 mol) and *para* substituted benzadoximes (0.07 mol) was stirred in chloroform (20 ml) at cold temperature (0–5°C). To this mixture was added 15 ml of sodium hypochlorite (degree = 12 g $NaClO_4$/100 g solution) drop-wise over 20 min keeping the temperature below 5°C. The reaction mixture was then stirred for 4–8 h at room temperature, until TLC indicated complete disappearance of reactants. The chloroform layer was then separated, washed with water and dried over anhydrous sodium sulphate. Excess of chloroform was then distilled off. The compounds **3(a–f)** obtained, were recrystallized from ethanol.

2.3a *3-(3-Nitrophenyl)-4-phenyl-4H-spiro[isoxazole-5,3′-pyrido[1,2-a]pyrimidine]-2′,4′-dione* ***3a***: Pale yellow crystals, yield 66%, mp 167°C; IR (KBr)cm^{-1}: 1710 (C=O), 1533 (C=N), 1310 (C–O); ^{1}H NMR ($CDCl_3$) δ: 5.36 (s, 1H, 4-C$\underline{H}$Ar), 6.05–6.96 (m, 9H, Ar-$\underline{H}$), 7.21–8.05 (m, 3H, protons of pyridine ring), 8.72 (d, 1H, proton adjacent to N-atom); ^{13}C NMR ($CDCl_3$) δ: 33.4 (CH_2 of oxazole), 94.2 (C–O of oxazole), 121, 124.9, 125.1, 125.5, 125.8, 126.3, 127.2, 127.4, 128.5, 128.8, 130.9, 131.3, 134.2, 134.6, 135.7, 140.6, 150.4, 166.4, 175.3, 195.8; Mass: M^{+} 414, 164, 160, 122, 93, 90, 78, 28; Anal. Calcd. C: 66.33, H: 3.52, N: 14.07, Found C: 66.26, H: 3.49, N: 13.96%.

2.3b *3-(4-Chlorophenyl)-4-phenyl-4H-spiro[isoxazole-5,3′-pyrido[1,2-a]pyrimidine]-2′,4′-dione* ***3b***: Light yellow crystals, yield 68%, mp 171°C; IR (KBr)cm^{-1}: 1722 (C=O), 1530 (C–N), 1318 (C–O), 737 (C–Cl); ^{1}H NMR ($CDCl_3$) δ: 5.33 (s, 1H, 4-C$\underline{H}$Ar), 6.12–6.89 (m, 9H, Ar-$\underline{H}$), 6.99–7.94 (m, 3H, protons of pyridine ring), 8.75 (d, 1H, proton adjacent to N-atom);

^{13}C NMR ($CDCl_3$) δ: 32.6, 93.3, 119.5, 125.6, 125.7, 126.2, 127.4, 127.6, 128.2, 128.3, 128.5, 128.7, 128.8, 128.9, 131.8, 135.6, 136.2, 140.9, 151.2, 165.6, 174.8, 192.1; Mass: M^+ 403, 405(M+2), 160, 155, 153, 113, 111, 93, 90, 78, 28; Anal. Calcd. C: 65.43, H: 3.47, N: 10.41, Found C: 65.50, H: 3.51, N: 10.45%.

2.3c *4-(4-Chlorophenyl)-3-(3-nitrophenyl)-4H-spiro[isoxazole-5,3′-pyrido[1,2-a]pyrimidine]-2′,4′-dione* ***3c***: Yellow crystals, yield 66.5%, mp 189°C; IR (KBr) cm^{-1}: 1726 (C=O), 1528 (C–N), 1314 (C–O), 741 (C–Cl); ^{1}H NMR ($CDCl_3$) δ: 5.69 (s, 1H, 4-C<u>H</u>Ar), 6.76–7.18 (m, 8H, Ar-<u>H</u>), 7.26–8.09 (m, 3H, protons of pyridine ring), 8.81 (d, 1H, proton adjacent to N-atom); ^{13}C NMR ($CDCl_3$) δ: 34.2, 93.9, 120.6, 124.8, 125.1, 125.3, 126.2, 128.5, 128.7, 129.2, 129.6, 130.8, 131.4, 131.7, 134.5, 134.6, 135.0, 139.1, 148.5, 164.7, 174.9, 192.6; Mass: M^+ 448, 500 (M+2), 160, 155, 153, 124, 122, 113, 111, 93, 78, 28; Anal. Calcd. C: 61.04, H: 3.00, N: 12.95, Found C: 60.87, H: 2.98, N: 13.02%.

2.3d *3-(4-Chlorophenyl)-4-(4-chlorophenyl)-4H-spiro[isoxazole-5,3′-pyrido[1,2-a]pyrimidine]-2′,4′-dione* ***3d***: Yellowish white crystals, yield 69.2%, mp 185–186°C; IR (KBr)cm^{-1}: 1706 (C=O), 1539 (C=N), 1313 (C–O), 760 (C–Cl); ^{1}H NMR ($CDCl_3$) δ: 5.61 (s, 1H, 4-C<u>H</u>Ar), 6.70–7.09 (m, 8H, Ar-<u>H</u>), 7.55–8.18 (m, 3H, protons of pyridine ring), 8.79 (d, 1H, proton adjacent to N-atom); ^{13}C NMR ($CDCl_3$) δ: 32.1, 93.1, 116.0, 125.3, 125.7, 126.4, 128.5, 128.8, 128.9, 129.4, 129.7, 130.8, 131.4, 131.6, 133.8, 135.1, 135.4, 139.0, 150.2, 165.1, 174.8, 190.2; Mass: M^+ 437, 160, 155, 153, 124, 113, 111, 93, 78, 28; Anal. Calcd. C: 60.27, H: 2.97, N: 9.59, Found C: 60.16, H: 3.0, N: 9.60%.

2.3e *4-(Benzo[d][1,3]dioxol-5-yl)-3-(3-nitrophenyl)-4H-spiro[isoxazole-5,3′-pyrido[1,2-a]pyrimidine]-2′,4′-dione* ***3e***: Shiny white crystals, yield 66%, mp 212°C; IR (KBr)cm^{-1}: 1692 (C=O), 1540 (C=N), 1246 (C–O); ^{1}H NMR ($CDCl_3$) δ: 5.03 (s, 1H, 4-C<u>H</u>Ar), 6.07 (s, 2H, -C<u>H</u>$_2$ of piperonal ring), 6.96–7.44 (m, 7H, Ar-<u>H</u>), 7.69–8.27 (m, 3H, protons of pyridine ring), 8.7 (d, 1H, proton adjacent to N-atom); ^{13}C NMR ($CDCl_3$) δ: 33.7, 94.5, 108.3, 109.2, 117.4, 121.6, 125.3, 125.6, 125.8, 126.2, 130.7, 131.2, 133.8, 134.0, 134.6, 135.3, 146.5, 147.9 (Ar-C), 151.8, 165.1, 178.2, 195.7; Mass: M^+ 458, 164, 160, 134, 122, 93, 78, 28; Anal. Calcd. C: 60.26, H: 3.06, N: 12.23, Found C: 60.18, H: 3.12, N: 12.18%.

2.3f *4-(Benzo[d][1,3]dioxol-5-yl)-3-(3-chlorophenyl)-4H-spiro[isoxazole-5,3′-pyrido[1,2-a]pyrimidine]-2′,4′-dione* ***3f***: White crystals, yield 67%, mp 206°C; IR (KBr)cm^{-1}: 1698 (C=O), 1538 (C=N), 1250 (C–O), 756 (C–Cl); ^{1}H NMR ($CDCl_3$) δ: 5.11 (s, 1H, 4-C<u>H</u>Ar), 6.05 (s, 2H, -C<u>H</u>$_2$ of piperonal ring), 7.05–7.86 (m, 7H, Ar-<u>H</u>), 7.94–8.34 (m, 3H, protons of pyridine ring), 8.70 (d, 1H, proton adjacent to N-atom); ^{13}C NMR ($CDCl_3$) δ: 33.1, 93.9, 100.80, 108.4, 108.9, 116.9, 120.6, 125.1, 125.4, 126.3, 129.3, 130.5, 130.7, 133.9, 134.6, 135.7, 135.8, 145.8, 148.1, 151.5, 164.9, 176.3, 190.5; Mass: M^+ 447, 449 (M+2), 160, 155, 153, 134, 113, 111, 93, 78, 28; Anal. Calcd. C: 61.68, H: 3.13, N: 9.39, Found C: 62.00, H: 3.23, N: 9.42%.

2.4 *General procedure for the preparation of 3-(4-substituted phenyl)-3H-isoxazolo[3,4-d]pyrido[1,2-a]pyrimidin-4(3aH)-one* ***4a–c***

A mixture of compound **2** (0.01 mol) and hydroxylamine hydrochloride (0.01 mol) in 2% ethanolic sodium hydroxide solution (5 ml) was heated to reflux for 6–7 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the solvent was removed under reduced pressure and the residue was added to ice water (15 ml). The resulting solution was neutralized by dilute HCl and extracted with $CHCl_3$ (20 ml). Chloroform layer was dried over anhydrous sodium sulphate, filtered and concentrated to obtain the product. The crude product was recrystallized with appropriate solvent.

2.4a *3-Phenyl-3H-isoxazolo[3,4-d]pyrido[1,2-a]pyrimidin-4(3aH)-one* ***4a***: Light brown crystals, yield 77%, mp >290°C; Recrystallization solvent: Ethanol; IR (KBr) cm^{-1}: 1726 (C=O), 1545 (C=N), 1312 (C–O); ^{1}H NMR ($CDCl_3$) δ: 2.65 (d, 1H, C^3), 4.01 (d, 1H, C^{3a}), 7.22–7.38 (m, 5H, Ar-<u>H</u>), 7.45–8.0 (m, 3H, protons of pyridine ring), 8.24 (d, 1H, proton adjacent to N-atom,); ^{13}C NMR ($CDCl_3$) δ: 40.5, 82.9, 120.6, 125.6, 125.8, 126.8, 127.2, 127.6, 128.3, 128.4, 135.4, 140.7, 150.4, 164.3, 173.2; Mass: M^+ 265, 188, 160, 93, 78, 77; Anal. Calcd. C: 67.92, H: 4.15, N: 15.85, Found C: 68.15, H: 4.06, N: 15.92%.

2.4b *3-(4-Chlorophenyl)-3H-isoxazolo[3,4-d]pyrido[1,2-a]pyrimidin-4(3aH)-one* ***4b***: Brownish white crystals, yield 76.5%, mp 188°C; Recrystallization solvent: Ethanol; IR (KBr) cm^{-1}: 1729 (C=O), 1542 (C=N), 1319 (C–O), 754 (C–Cl); ^{1}H NMR ($CDCl_3$) δ: 2.3 (d, 1H, C^3), 3.75 (d, 1H, C^{3a}), 7.36–7.41 (m, 4H, Ar-<u>H</u>), 7.43–7.50 (m, 3H, protons of pyridine ring), 8.28 (d, 1H, proton adjacent to N-atom); ^{13}C NMR ($CDCl_3$) δ: 40.3, 83.2, 119.7, 125.4, 125.7, 126.3,

126.6, 129.1, 129.4, 133.4, 135.8, 138.6 (Ar-C), 150.3, 164.0, 172.5; Mass: M^+ 299, 301 (M+2), 188, 160, 126, 124, 113, 111, 93, 78; Anal. Calcd. C: 60.10, H: 3.33, N: 14.02, Found C: 59.84, H: 3.30, N: 14.16%.

2.4c *3-(Benzo[d][1,3]dioxol-4-yl)-3H-isoxazolo[3,4-d]pyrido[1,2-a]pyrimidin-4(3aH)-one* ***4c***: Dirty white crystals, yield 71%, mp 168–169°C; Recrystallization solvent: Methanol; IR (KBr)cm^{-1}: 1731 (C=O), 1542 (C=N), 1310 (C–O); ^{1}H NMR ($CDCl_3$) δ: 2.25 (d, 1H, C^3), 3.80 (d, 1H, C^{3a}), 6.12 (s, 2H, O-C$\underline{H}_2$-O-), 7.37 (d, 2H, Ar-$\underline{H}$), 7.43 (d, 1H, Ar-$\underline{H}$), 7.5–8.19 (m, 3H, protons of pyridine ring), 8.56 (d, 1H, proton adjacent to N-atom); ^{13}C NMR ($CDCl_3$) δ: 40.5, 83.5, 101.3, 109.0, 110.1, 116.5, 119.4, 125.4, 125.7, 133.9, 135.2, 146.9, 149.3, 150.8, 164.5, 172.2; Mass: M^+ 309, 188, 160, 121, 93, 78; Anal. Calcd. C: 62.13, H: 3.56, N: 13.59, Found C: 62.18, H: 3.51, N: 13.60%.

2.5 *General procedure for the preparation of 3-(4-substituted phenyl)-3,3a-dihydropyrazolo[3,4-d]pyrido[1,2-a]pyrimidin-4(2H)-one* ***5a–c***

A mixture of compound **2** (0.01 mol) and hydrazine hydrate (0.013 mol) in absolute ethanol (15 ml) was refluxed for 10–12 h. The reaction mixture was concentrated and the crude products thus obtained were then purified by column chromatography using a mixture of *n*-hexane-ethyl acetate (2:1).

2.5a *3-Phenyl-3,3a-dihydropyrazolo[3,4-d]pyrido[1,2-a]pyrimidin-4(2H)-one* ***5a***: Wheatish brown crystals, yield 75%, mp 175°C; IR (KBr) cm^{-1}: 3323 (N–H), 1716 (C=O), 1531 (C=N); ^{1}H NMR ($CDCl_3$) δ: 3.50 (d, 1H, C^3), 4.45 (d, 1H, C^{3a}), 7.06 (s, 1H, -N-$\underline{H}$, D_2O exchangeable), 7.3–7.54 (m, 5H, Ar-$\underline{H}$), 7.57–8.32 (m, 3H, protons of pyridine ring), 8.35 (d, 1H, proton adjacent to N-atom); ^{13}C NMR ($CDCl_3$) δ: 45.8, 53.5, 118.4, 125.3, 125.5, 125.6, 125.9, 126.4, 128.7, 128.9, 135.2, 143.5, 150.7, 156.2, 172.8 (C=O); Mass: M^+ 264, 145, 93, 78, 77, 42; Anal. Calcd. C: 68.18, H: 4.55, N: 21.21, Found C: 67.97, H: 4.52, N: 20.99%.

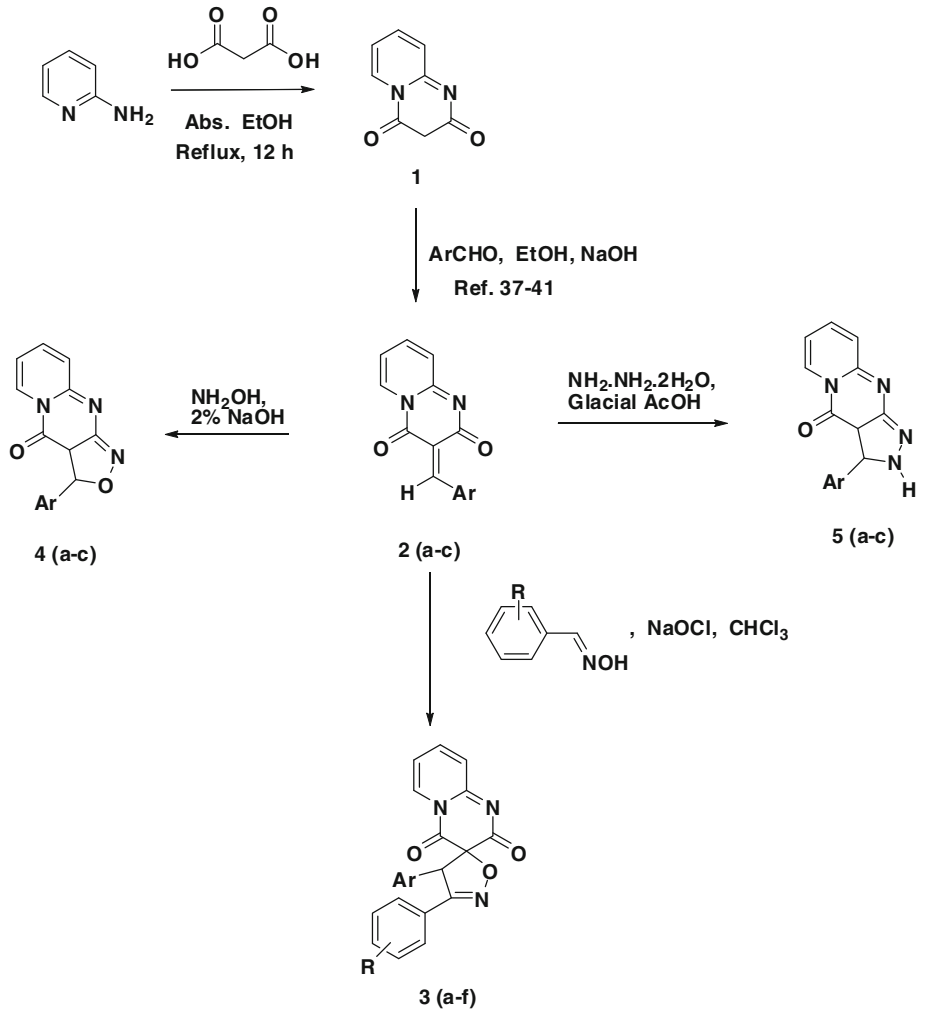
2.5b *3-(4-Chlorophenyl)-3,3a-dihydropyrazolo[3,4-d]pyrido[1,2-a]pyrimidin-4(2H)-one* ***5b***: Muddy brown crystals, yield 72%, mp 193°C; IR (KBr) cm^{-1}: 3286 (N–H), 1712 (C=O), 1527 (C=N), 744 (C–Cl); ^{1}H NMR ($CDCl_3$) δ: 3.54 (d, 1H, C^3), 5.01 (d, 1H, C^{3a}), 7.33–7.40 (m, 4H, Ar-$\underline{H}$), 7.48–8.15 (m, 3H, protons of pyridine ring), 8.42 (d, 1H, proton adjacent to N-atom); ^{13}C NMR ($CDCl_3$) δ: 45.1, 53.1, 116.9, 125.7, 125.9, 127.3, 127.6, 127.8, 128.4, 132.3, 135.1, 141.6, 150.5, 155.7, 172.3 (C=O); Mass: M^+ 298, 300 (M+2), 139, 126, 124, 113, 111, 93, 78, 42; Anal. Calcd. C: 60.30, H: 3.69, N: 18.76, Found C: 60.17, H: 3.72, N: 18.69%.

2.5c *3-(Benzo[d][1,3]dioxol-4-yl)-3,3a-dihydropyrazolo[3,4-d]pyrido[1,2-a]pyrimidin-4(2H)-one* ***5c***: White crystals, yield 76%, mp 179–180°C; IR (KBr) cm^{-1}: 3310 (N–H), 1720 (C=O), 1529 (C=N); ^{1}H NMR ($CDCl_3$) δ: 3.60 (d, 1H, C^3), 5.00 (d, 1H, C^{3a}), 6.09 (s, 2H, O-C$\underline{H}_2$-O-), 7.51 (d, 2H, Ar-$\underline{H}$), 7.64 (d, 1H, Ar-$\underline{H}$), 7.93–8.27 (m, 3H, protons of pyridine ring), 8.68 (d, 1H, proton adjacent to N-atom); ^{13}C NMR ($CDCl_3$) δ: 45.4, 53.4, 101.6, 110.2, 112.4, 116.6, 118.0, 125.3, 125.7, 135.4, 135.9, 145.7, 148.6, 151.2, 155.9, 172.7; Mass: M^+ 308, 187, 145, 121, 93, 78, 70, 42; Anal. Calcd. C: 62.34, H: 3.90, N: 18.18, Found C: 63.14, H: 3.86, N: 18.07%.

2.6 *Chemistry*

The starting material 2*H*-pyrido[1,2-*a*]pyrimidine-2,4(3*H*)dione **1** was prepared via cyclization reaction between 2-amino pyridine and malonic acid in the presence of ethanolic sodium hydroxide. This underwent Claisen condensation with aromatic aldehydes to afford chalcones **2a–c**. The chalcones were reacted successfully with aromatic oximes, hydroxylamine and hydrazine hydrate under different reaction conditions to give cyclized products 4-(substituted phenyl)-3-(3-substituted phenyl)4*H*-spiro[isoxazole-5,3′-pyrido[1,2-*a*]pyrimidine]-2′,4′-dione **3a–f**, 3-(4-substituted phenyl)-3*H*-isoxazole[3,4-*d*]pyrido[1,2-*a*]pyrimidin-4-(3a*H*)-one **4a–c** and 3-(4-substituted phenyl) 3,3a-dihydropyrazolo[3,4-*d*]pyrido[1,2-*a*]pyrimidin-4-(2*H*)-one **5a–c**. In compounds **3a–f**, the oxygen atom of oxime is attached at the most substituted carbon of dipolarophile **2a–c** as generally observed for reaction of nitrones and ethylenic dipolarophiles[42,43] whereas compounds **4a–c** and **5a–c** are formed presumably by way of the hydrazones, followed by cyclization and proton transfer.

The structures of new compounds were assigned on the basis of their analytical and spectral data (IR, ^{1}H, ^{13}C NMR and Mass). The characteristic C=O bands appeared in the 1700–1680 cm^{-1} region in the FT-IR spectra of **1** and **2a–c**. The FT-IR spectra of compounds **3a–f**, **4a–c** and **5a–c** showed the presence of C=N band in the region 1585–1520 cm^{-1}. Compound **1** displayed in its ^{1}H NMR spectra, in addition to ethylenic protons, a doublet at δ 3.29 ppm due to resonance of CH_2 protons. In the benzylidene derivatives **2a–c**, this signal was absent, confirming the condensation had taken place. Regarding compounds **3a–f**, the ^{1}H NMR spectra



Scheme 1. Synthetic route for cyclization products of 3-(4-substituted benzylidene)-2H-pyrido[1,2-a]pyrimidine 2,4-(3H)-diones.

showed the presence of one singlet, H^4 of the isoxazolineic ring at 5.30–5.90 ppm. Compounds **4a–c** and **5a–c** revealed in their ^{1}H NMR spectra, two doublets in the region 2.25–5.01 ppm due to one proton on each 3a–CH and 3–CH, respectively, showing that cyclization had occurred. ^{13}C NMR of **3a–f** showed distinct resonances in agreement with the proposed structure. Spiro carbon resonated at δ 71.93 ppm and C=N carbon at ~162-8 ppm. All the compounds have shown an excellent agreement between calculated and experimentally obtained data for C, H, N analysis (scheme 1).

2.7 *Bioassay*

2.7a *Evaluation of antimicrobial activity*: Compounds **3a–f**, **4a–c** and **5a–c** were evaluated *in vitro* for their antibacterial activity against *Staphylococcus aureus* (ATCC9144), *Bacillus subtilis* (ATCC6633), *Pseudomonas aeruginosa* (ATCC25619), *Klebsiella pneumoniae* and *Escherichia coli* (MTCC739) using ethanol as a solvent by Disc Diffusion method.[44] The activity was compared with ciprofloxacin and gentamycin. The results are presented in table 1. Antifungal activities of these compounds were evaluated against *Candida albicans* (ATCC24433) using ethanol as solvent by the same method.[44] The activity was compared with fluconazole and the results are presented in table 1. The zone of inhibition was measured after 24 h of incubation at 28°C. The zone of inhibition developed, if any, was then measured and recorded. Zone of inhibition for ethanol was done separately and found that there was no activity.

2.7b *Evaluation of antitubercular activity*: All the compounds (**3a–f**, **4a–c** and **5a–c**) have been tested for their antitubercular activity through Microplate Alamar Blue Assay (MABA).[45] The compounds were dissolved in dimethyl sulphoxide (DMSO) to make 5 mg/ml stock solutions. Serial dilutions from stocks were also made in DMSO. Standard anti-TB drugs (isoniazid and

Table 1. Antimicrobial activity of the synthesized compounds.

Compounds	Zone of inhibition (mm) of bacterial (Gram+ and Gram−) strains					Zone of inhibition (mm) of fungal strain
	S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*C. albicans*
3a	10	8	6	6	6	12
3b	11	8	6	7	6	15
3c	9	9	7	7	6	11
3d	12	7	8	7	7	19
3e	18	21	12	9	10	27
3f	24	20	13	9	9	30
4a	10	8	6	9	9	15
4b	11	7	6	8	10	17
4c	9	8	7	11	9	27
5a	8	9	7	7	7	16
5b	10	9	8	9	12	16
5c	10	11	8	8	14	28
Control	6	6	6	6	6	6
Ciprofloxacin/ Gentamycin	20/19		20/17			
Fluconazole						29

Table 2. Antitubercular activity of all the synthesized compounds.

Compounds	%Inhibition@12.5 μg/mL	%Inhibition@6.25 μg/mL	%Inhibition@3.12 μg/mL
3a	4	4	5
3b	2	3	4
3c	9	12	9
3d	27	14	15
3e	28	16	10
3f	23	11	16
4a	0	0	0
4b	0	0	0
4c	2	2	2
5a	0	0	0
5b	0	0	0
5c	0	2	3
Isoniazid	100	100	100

rifampicin) were used as positive control and the vehicle (DMSO) was used as negative control. The percent inhibition at different concentrations is presented in table 2.

3. Results and discussion

3.1 *Antimicrobial activity*

Among the spiro compounds **3a–f**, the one having benzo[*d*] [1,3]dioxole as substituents i.e., **3e** and **3f** showed excellent activity against both bacterial and fungal strains. The zone of inhibition of these substituents was quite considerable against the Gram+ve *S. aureus* (18 and 24 mm), *B. subtilis* (21 and 20 mm) and the fungus *C. albicans* (27 and 30 mm, respectively). It is noteworthy, that these two compounds also inhibited the multiresistant *P. aeruginosa* to some extent which is very encouraging. Rest of the synthesized spiro compounds were moderately active. The results were almost similar for isoxazole **4a–c** and pyrazole derivatives **5a–c** also. The benzo[*d*] [1,3]dioxole substituted compounds **4c** and **5c** were more active than the compounds with other substituents. These compounds showed a marvelous zone of inhibition of 27 and 28 mm against the fungal strain *C. albicans*. The increased activity of benzo[*d*] [1,3]dioxole substitution might be due to its structure leading to a better fit at

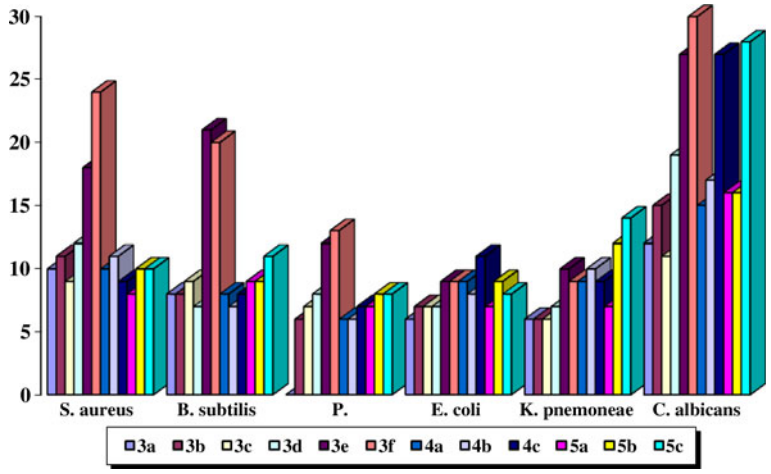


Figure 1. Graphical representation of antimicrobial activity.

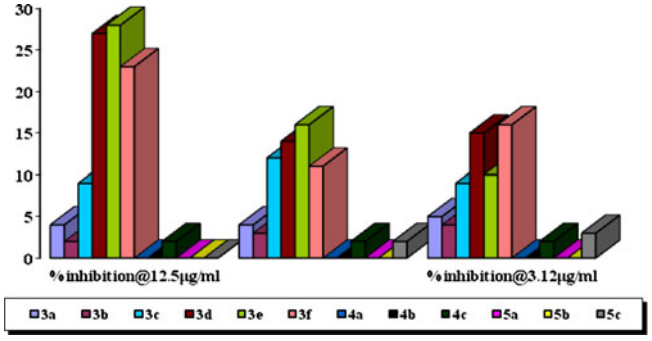


Figure 2. Graphical representation of antitubercular activity.

the receptor site. The Diameter of zone of inhibition is expressed in mm and the results are shown in table 1. The graphical representations are shown in figure 1.

3.2 *Antitubercular activity*

The compounds of the spiro series were somewhat active as they inhibited the mycobacteria to a considerable percentage (23–28%). Compounds **3d**, **3e** and **3f** showed good inhibition at a concentration of 12 µg/mL which was maximum among all the synthesized compounds. It was quite interesting to observe that many compounds viz. **3a**, **3b** and **5c** showed a decreased percentage of inhibition of the mycobacterium when their concentration was increased. The activity of compound **3b** was quite abnormal on increasing its concentration from 3.12 µg/mL to 6.25 µg/mL, the percentage inhibition increased from 9 to 12 but at 12.5 µg/mL the percentage inhibition was again 9 which is a considerable decrease. This may be due to the toxicity of these compounds at a higher concentration or may be due to some other reason. However, it is a matter of further research.

The isoxazole **4a–c** and pyrazole **5a–c** derivatives were found to be more or less inactive. The percentage inhibition at different concentration is expressed in µg/mL and the results are shown in table 2 and, the graphical representations are shown in figure 2.

4. Conclusion

In summary, we tested the new series of heterocycles at several concentrations to evaluate their antimicrobial and antitubercular activity. Our results have shown that among the spiro compounds **3a–f**, the one having benzo[*d*] [1,3]dioxole as a substituent showed excellent activity against both bacterial and fungal strains, however no active antitubercular compound was identified. The active compounds of the series would be promising structural templates for the development of novel and more efficient antimicrobial agents.

Acknowledgements

The authors express their sincere thanks to the Head, Department of Chemistry, Lucknow University, Lucknow, for providing laboratory facilities. They are also thankful to the Director, Central Drug Research Institute, Lucknow for elemental analysis, spectral data and biological activity studies.

References

1. Giller S A, Zhuk R A and Lidak M I U 1967 *Dokl. Akad. Nauk. SSR.* **176** 332
2. Curreri A R, Ansfield R J, McIver F H, Waisman H A and Hedelberger C 1958 *Cancer Res.* **18** 478
3. Chaudhary N K, Montag B J and Heidelberger C 1958 *Cancer Res.* **18** 318

4. Malik V, Singh P and Kumar S 2006 *Tetrahedron* **62** 5944
5. Holý A, Votruba I, Masojídková M, Andrei G, Snoeck R, Naesens L, De Clercq E and Balzarini J 2002 *J. Med. Chem.* **45** 1918
6. Andres P and Marhold A 1996 *J. Fluorine Chem.* **77** 93
7. Reddick J J, Saha S, Lee J, Melnick J S, Perkins J and Begley T P 2001 *Bioorg. Med. Chem. Lett.* **11** 2245
8. Simoni D, Grisolia G, Giannini G, Roberti M, Rondanin R, Piccagli L, Baruchello R, Rossivz M, Romagnoli R, Invidiata F P, Grimaudo S, Jung M K, Hamel E, Gebbia N, Crosta L, Abbadessa V, Di Cristina A, Dusonchet L, Meli M and Tolomeo M 2005 *J. Med. Chem.* **48** 723
9. Gauthier B 1963 *Ann. Pharm. Fr.* **21** 655
10. O' Reilly B C and Atwal K S 1987 *Heterocycles* **26** 1185
11. Overman L E, Rabinowitz M H and Renhowe P A 1995 *J. Am. Chem. Soc.* **117** 2657
12. Ghoneim K M and Youssef R 1986 *J. Indian Chem. Soc.* **53** 914
13. Atwal K S and Moreland S 1991 *Bioorg. Med. Chem. Lett.* **1** 291
14. Godfraind T, Miller R and Wbo M 1986 *Pharmacol. Rev.* **38** 321
15. Ertan M, Balkan A, Sarac S, Uma S, Renaud J F and Rolland Y 1991 *Arch. Pharm.* **324** 135
16. Korgaokar S S, Patil P H, Shab M T and Parekh H H 1996 *Indian J. Pharm. Sci.* **58** 222
17. Cali P, Naerum L, Mukhija S and Hjelmencrantz A 2004 *Bioorg. Med. Chem. Lett.* **14** 5997
18. Getal J 1975 *Antibiot.* **28(1)** 91
19. Rapposelli S, Lapucci A, Minutolo F, Orlandini E, Ortore G, Pinza M and Balsamo A 2004 *Farmaco* **59** 25
20. Sechi M, Sannia L, Carta F, Palomba M, Dallocchio R, Dessi A, Derudas M, Zawahir Z and Neamati N 2005 *Antiviral Chem. Chemother.* **16** 41
21. Carr J B, Durham H G and Hass D K 1977 *J. Med. Chem.* **20** 934
22. Cottineau B, Toto P, Marot C, Pipaud A and Chenault J 2002 *Bioorg. Med. Chem. Lett.* **12** 2105
23. Simoni D, Manfredini S, Tabrizi M A, Bazzanini R, Guarinini M, Ferroni R, Traniello F, Nastruzzi C G and Gambari R 1991 *Top. Mol. Organ. Eng.* **8** 119
24. Faulkner D 2001 *J. Nat. Prod. Rep.* **18** 1
25. Howe R K and Shelton B R 1990 *J. Org. Chem.* **55** 4603
26. Smietana M, Gouverneur V and Mioskowski C 1999 *Tetrahedron Lett.* **40** 1291
27. Najim N, Bathich Y, Zain M M, Hamzah A H and Shaameri Z 2010 *Molecules* **15(12)** 9340
28. Kumar R R, Perumal S, Manju S C, Bhatt P, Yogeeswari P and Sriram D 2009 *Bioorg. Med. Chem. Lett.* **19** 3461
29. (a) Wright A D, King G M and Sticher O 1990 *J. Nat. Prod.* **53** 1615. (b) Abdou I M, Ayman M S and Hussein F Z 2004 *Molecules* **9(3)** 109
30. Filler R 1974 *Chem. Technol.* **4** 752
31. EI-Feky A and Abd-Ei-Samii Z K 1996 *Pharmazie* **51** 540
32. Vijaya Raj K K, Narayan B V, Ashalatha N and Kumari S 2006 *J. Pharmacol. Toxicol.* **1(6)** 559
33. Bazgir A, Khanaposthani M M and Soorki A A 2008 *Bioorg. Med. Chem. Lett.* **18** 5800
34. Moustafa M G, Zeinab H I and Soad A A 2004 *Heterocyclic Chem.* **1** 57
35. Stewart M, Steinig A G, Ma C, Song J P, Kibben B M, Castelhano A L and Lennan S 2004 *J. Biochem. Pharmacol.* **68** 305
36. Cacciarini M, Cordero F M, Faggi C and Goti A 2000 *Molecules* **5** 637
37. Vorlander D and Kunze K 1926 *Ber.* **59** 2078
38. Garland C E and Reid E E 1925 *J. Am. Chem. Soc.* **47** 2333
39. Vorlander D 1925 *Ber.* **58** 118
40. Huitric A C and Kumler W D 1956 *J. Am. Chem. Soc.* **78** 614
41. Pfeiffer P, Angerf O, Backes P, Fitz W, Prahi E, Rheinboldt H and Stoll W 1925 *Ann.* **441** 228
42. Rastelli A, Gandolfi R and Amade M S 2000 *Adv. Quantum Chem.* **36** 151
43. Sahm D F and Washington 1991 *Antibacterial susceptibility tests: dilution methods, Manual of Clinical Microbiology*, 5th ed., (eds) A Balows, W J Hausler Jr., K L Herrmann, H D Isenberg and H J Shadomy, Washington DC, American Society for Microbiology, 1105–1116
44. Collins L A and Franzblau S G 1997 *Antimicrob. Agents Chemother.* **41** 1004
45. Vorländer D and Hobohm K 1886 *Ber.* **29** 1836