NOVEL SYNTHETIC APPROACH OF SOME 3-AMINO-4-OXO-2-SUBSTITUTED BENZOTHIAZOLYL) PYRAZOLO [3,4-B] PYRIDO[1,2-A] PYRIMIDINE DERIVATIVES AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Pyrazolo pyrimido pyrimidine (4a-k) were prepared by the reaction of compound 3-Amino-4-oxo-2-(methylthio) 4H-pyrido [1, 2-a] pyrimidine (3) with hydrazide hydrate, phenyl hydrazide, 2-hydrazino benzothiazole & 6-substitued hydrazine benzothiazole in N,N-dimethyl formamide (DMF) and anhydrous potassium carbonate. All these synthesized compounds were characterized by elemental analysis and spectral data.

Keywords: N,N-dimethyl formamide, Potassium carbonate, Pyrazolo pyrimido pyrimidine.

INTRODUCTION

Interest in the synthesis of pyrimidine derivatives are reported to exhibit antimycobacterial, antitumor, antiviral, anticancer, anti-inflammatory, analgesic, antifolate, antimicrobial, anti-fungal, antiproliferative and antihistaminic activities. They are also effective as antiplatelet agents with analgesic activity and as a new drug for treatment of insomnia anti-AIDS and antinociceptive. Fusion pyrimidines are extensively used in neurology, particularly in the treatment of neurodegenerative disorders such as Parkinson’s disease, antianxiety disorders, and depression. Fusion pyrimidines are selective inhibitors for multidrug resistance (MDR). Cyclization using phosphorus oxychloride or ethanol in sodium ethoxide furnished the pyrido[1,2-a]pyrimidines.

MATERIALS AND METHODS

Melting points were determined by open capillary tubes and were uncorrected. All the reactions monitored by thin layer chromatography, carried out on 0.2 mm silica gel-C plates using iodine vapors for detection. Infrared spectra were recorded in Nujol or as potassium bromide pallets on infrared spectrophotometer, nuclear magnetic resonance spectra were recorded in Nujol or as potassium bromide thin layer chromatography, carried out on 0.2 mm silica and were uncorrected. All the reactions were monitored by open capillary tubes and were uncorrected. Anal. Calcd. For: C, 60.30; H, 3.50; N, 24.80.

General procedure

3-Cyano-4-oxo-2-(methylthio) 4H-pyrido [1, 2-a] pyrimidine (3): A mixture of 2-aminopyridine (1) (0.01 mol) and ethyl di cyano (methylthio) acrylate (2) (0.01 mol) in 15 mL of N, N- dimethyl formamide and anhydrous potassium carbonate (10mg) was refluxed for 5 hours. The reaction mixture was cooled to room temperature and poured in to ice cold water. The separated solid product was filtered, washed with water and recrystallized from a N, N- dimethyl formamide-ethanol mixture to give pure (3).

3-Amino-4-oxo-2-{6'-substituted benzothiazolyl})pyrazolo [3,4-b]pyrido[1,2-a]pyrimidine (4a-k): A mixture of 3 (0.001 mol) and independently with hydrazide hydrate (80 %), phenyl hydrazide, 4-nitro phenyl hydrazine, 2,4-dinitro phenyl hydrazine, 2-hydrazino benzothiazole, 6-methyl 2-hydrazino benzothiazole, 6-methoxy 2-hydrazino benzothiazole, 6-chloro 2-hydrazino benzothiazole, 6-nitro 2-hydrazino benzothiazole, 2,4-dimethyl 2-hydrazino benzothiazole, 6,7-chloro,fluoro 2-hydrazino benzothiazole (0.001mol) in 15 mL of N, N'-dimethyl formamide and anhydrous potassium carbonate (10mg) was refluxed for 6 hours. The reaction mixture was cooled to room temperature and poured in to ice cold water. The separated solid product was filtered, washed with water and recrystallized from a N, N'- dimethyl formamide-ethanol mixture to give pure (4a-k).

3-Cyano4-oxo-2-(methylthio) 4H-pyrido [1, 2-a]pyrimidine (3): Orange powder, yield 78 %, mp 233-238°C (dec.). IR (KBr / cm⁻¹) 1702 (C=O), 2225 (CN); 1H NMR (400 MHz, DMSO-d₆) 2.59 (s, 3H, SCH₃), 7.3-7.4 (d, 2H), 7.8-7.9 (d, 2H), 9.2 (br s, 1H, =NH), EI-MS (m/z: RA %): 217 (M+1, 100%), 215 (35). Anal. Calcld. For: C₉H₇N₂S; C, 60.35; H, 3.55; N, 24.85. Found: C, 60.30; H, 3.50; N, 24.80.

3-Amino-4-oxo-2-(2H) pyrazolo[3,4-b]pyrido [1, 2-a] pyrimidine (4a): Brown powder, yield 75 %, mp 187°C (dec.). IR (KBr/cm⁻¹) 3212cm⁻¹ (NH₂ asym.)1705cm⁻¹ (C=O). 1H NMR (400 MHz, DMSO-d₆) 4.9 (s, 1H), 7.3-7.4 (m, 4H), 5.4 (s, 2H), EI-MS (m/z: RA %); 201 (50%) Anal. Calcld. For C₈H₇N₂O; C, 53.73; H, 3.51; N, 34.81.

3-Amino-4-oxo -2-( phenyl ) pyrazolo [3,4-b] pyrido [1,2-a] pyrimidine (4b): Brown powder, yield 62 %,mp 165°C (dec.). IR (KBr/cm⁻¹) 3221cm⁻¹ (NH₂ asym.) 1700 cm⁻¹ (C=O). 1H NMR (400 MHz, DMSO-d₆) 7.8-7.9 (m, 4H), 7.5 (S 016...
5H), 4.4 (s, 2H). EI-MS (m/z: RA %): 277(M) , 80%, Anal. Calcd. For C_{13}H_{10}N_{2}O: C, 64.97; H, 4.00; N, 25.26.

3-Amino-4-oxo-2-(4'-nitro phenyl) pyrazolo [3,4-b]pyrido [1,2-a] pyrimidine(4c): Brown powder, yield 79%, mp 169°C (dec.). IR (KBr/cm^-1) 3301cm^-1 (NH$_3$ asym.) 1690cm^-1 (C=O). 1H NMR (400 MHz, DMSO-d$_6$) 6.9-7.2 (m, 4H), 6.1 (s, 2H), 7.2 (d 2H), 7.3 (d 2H). EI-MS (m/z: RA %): 323(M+H), 70%, Anal. Calcd. For C$_{13}$H$_{10}$N$_2$O: C, 55.90; H, 3.13; N, 26.08.

3-Amino-4-oxo-2-(2',4'-dinitro phenyl) pyrazolo [3,4-b]pyrido [1,2-a] pyrimidine(4d): Brown powder, yield 68 %, mp 172°C (dec.). IR (KBr/cm^-1) 3300cm^-1 (NH$_3$ asym.) 1685cm^-1 (C=O). 1H NMR (400 MHz, DMSO-d$_6$) 7.2 (d 2H), 7.9 (s 1H), 7.4-7.8 (m, 4H), 6.4 (s, 2H). EI-MS (m/z: RA %): 367 (45) Anal. Calcd. For C$_{13}$H$_{10}$N$_2$O: C, 49.05; H, 2.47; N, 26.70.

3-Amino-4-oxo-2-(2-benzothiazolyl)pyrazolo [3,4-]pyrido [1,2-a] pyrimidine (4f): Brown powder, yield 74 %, mp 192°C (dec.). IR (KBr/cm^-1) 3340cm^-1 (NH$_3$ asym.) 1675cm^-1 (C=O). 1H NMR (400 MHz, DMSO-d$_6$) 7.2 (m 4H), 7.3-7.4 (m, 4H), 5.4 (s, 2H). EI-MS (m/z: RA %): 369(M+H), 50%, Anal. Calcd. For C$_{16}$H$_{14}$N$_4$O: C, 52.11; H, 2.46; N, 22.79.

3-Amino-4-oxo-2-(6'-methyl-2'-benzothiazolyl) pyrazolo [3,4-b]pyrido [1,2-a] pyrimidine(4f): Brown powder, yield 68 %, mp 186°C (dec.). IR (KBr/cm^-1) 3380cm^-1 (NH$_3$ asym.) 1690cm^-1 (C=O). 1H NMR (400 MHz, DMSO-d$_6$) 7.2 (d 2H), 7.4 (s 1H), 7.5-7.8 (m, 4H), 6.8 (s, 2H), 1.9 (s3H). EI-MS (m/z: RA %): 348 (55) Anal. Calcd. For C$_{13}$H$_{10}$N$_2$O: C, 56.61; H, 3.47; N, 24.12; EI-MS (m/z: RA %): 349 (M+H), 80%.

3-Amino-4-oxo-2-(6'-methoxy-2'-benzothiazolyl) pyrazolo [3,4-b]pyrido [1,2-a] pyrimidine (4g): Brown powder, yield 72%, mp 183°C (dec.). IR (KBr/cm^-1) 3340cm^-1 (NH$_3$ asym.) 1689cm^-1 (C=O). 1H NMR (400 MHz, DMSO-d$_6$) 7.1 (d 2H), 6.2 (s1H), 7.5-7.9 (m, 4H), 5.9 (s, 2H), 3.9 (s3H). EI-MS (m/z: RA %): 364 (55%) Anal. Calcd. For C$_{17}$H$_{14}$N$_4$O$_2$: C, 56.04; H, 3.32; N, 23.06.

3-Amino-4-oxo-2-(6'-chloro-2'-benzothiazolyl) pyrazolo [3,4-b]pyrido [1,2-a] pyrimidine(4h): Brown powder, yield 68 %, mp 194°C (dec.). IR (KBr/cm^-1) 3400cm^-1 (NH$_3$ asym.) 1670cm^-1 (C=O). 1H NMR (400 MHz, DMSO-d$_6$) 6.9 (d 2H), 6.4 (s 1H), 7.0-7.1 (m, 4H), 4.9 (s, 2H). EI-MS (m/z: RA %): 368 (55%) Anal. Calcd. For C$_{16}$H$_{14}$N$_5$O: C, 52.11; H, 2.46; N, 22.79.

3-Amino-4-oxo-2-(6'-nitro-2'-benzothiazolyl) pyrazolo [3,4-b]pyrido [1,2-a] pyrimidine(4i): Brown powder, yield 68 %,mp 182°C (dec.). IR (KBr/cm^-1) 3300cm^-1 (NH$_3$ asym.) 1685cm^-1 (C=O). 1H NMR (400 MHz, DMSO-d$_6$) 7.5 (d 2H), 7.9 (s 1H), 7.1-7.2 (m, 4H), 5.4 (s, 2H). EI-MS (m/z: RA %): 379 (55%) Anal. Calcd. For C$_{16}$H$_{14}$N$_7$: C, 50.66; H, 2.39; N, 25.85.

3-Amino-4-oxo-2-(4',6'-dimethyl-2'-benzothiazolyl)pyrazolo [3,4-b]pyrido [1,2-a] pyrimidine (4j): Brown powder, yield 68 %,mp 167°C (dec.). IR (KBr/cm^-1) 3340cm^-1 (NH$_3$ asym.) 1670cm^-1 (C=O). 1H NMR (400 MHz, DMSO-d$_6$) 7.6 (s2H), 7.0-7.4 (m, 4H), 5.8 (s, 2H), 1.9 (s6H). EI-MS (m/z: RA %): 362 (55%) Anal. Calcd. For C$_{19}$H$_{18}$N$_4$: C, 59.65; H, 3.89; N, 23.19.

3-Amino-4-oxo-2-(6',7'-chloro,floro-2'-benzothiazolyl) pyrazolo [3,4-b]pyrido [1,2-a] pyrimidine (4k): Brown powder, yield 68 %,mp 150°C (dec.). IR (KBr/cm^-1) 3340cm^-1 (NH$_3$ asym.) 1698cm^-1 (C=O). 1H NMR (400 MHz, DMSO-d$_6$) 7.5(d 2H), 7.8-8.4 (m, 4H), 5.2 (s, 2H). EI-MS (m/z: RA %): 386 (55%) Anal. Calcd. For C$_{16}$H$_{14}$F$_2$N$_4$: C, 49.68; H, 2.08; N, 21.73.

**Antimicrobial activity**

The synthesized compounds were evaluated for their antibacterial activity against gram-positive species S. aureus and B. subtilis and gram-negative species E. coli and S. typhi by paper disc diffusion method. All the synthesized compounds were dissolved in dimethyl sulphoxide. The synthesized compounds exhibited zone of inhibition of 09-13 mm in diameter whereas standard Streptomycin exhibited zone of inhibition of 18 and 22 mm in diameter against S. aureus and B. subtilis and Penicillin exhibited zone of inhibition of 15 and 16 mm in diameter against E. coli and S. typhi respectively. Amongst the synthesized compounds 4, compound 4g (9, 12, 13, 10 mm) and 4h (11, 11, 10, 12) showed higher zone of inhibition against S. aureus, B. subtilis, E. coli and S. typhi respectively. It seems that the presence of OCH$_3$ & Cl group at 10-position 4a increases antibacterial activity.

**RESULTS AND DISCUSSION**

In the present communication, we have developed new methodology towards the synthesis of 3-Amino -4-oxo-2-(6'-substituted benzothiazolyl) pyrazolo [3,4-b] pyrido [1,2-a] pyrimidine (4) Our method gives single product with high yield. The reaction started with 2-aminopyridines (1) and ethyl dicyano (methylthio) acrylate (2) were refluxed in N,N-dimethyl formamide (DMF) in presence of catalytic amount of anhydrous potassium carbonate to afford (3). **Scheme-1**

The compound (3) possess replaceable active methylthio group at 2- position which is activated by the ring 1- nitrogen atom, electron withdrawing 3-cyano group. Compound (3) was reacting with hydrazine hydrate in presence of N,N-dimethyl formamide (DMF) and catalytic amount of anhydrous potassium carbonate afforded the compound 4a in 78 % yield. the subsequently compound (3) independently heating with phenyl hydrazine, 4-nitro phenyl hydrazine, 2,4-dinitro phenyl hydrazine, 2-hydrazino benzothiazole, 6-methyl 2-hydrazino benzothiazole, 6-methoxy 2-hydrazino benzothiazole, 6-chloro 2-hydrazino benzothiazole, 6-nitro 2-hydrazino benzothiazole,2,4- dimethyl 2-hydrazino benzothiazole, 6,7-chloro,fluoro 2-hydrazino benzothiazole to obtain 3-Amino -4-oxo-2-(6'-substituted benzothiazolyl) pyrazolo [3,4-b] pyrido [1,2-a] pyrimidine derivatives (4a-k) respectively. **Scheme-2**
Scheme 1: Formation of fused pyrido [1, 2-a] pyrimidine.

Scheme 2: Formation of 3-Amino-4-oxo-2-(6’-substituted benzothiazolyl) pyrazolo[3,4-b]pyrido[1,2-a]pyrimidine derivatives.
Table 1: Antibacterial activity of compound (3-4k)

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The structure of these newly synthesized compounds were established on the basis of elemental analysis, IR, PMR and MASS Spectral data, spectral studies of all compounds shows that compounds are stable & do not exhibit any tautomerism.

CONCLUSION

In conclusion, we have described a convenient and practical procedure for the preparation of some novel pyrido[1,2-α] pyrimidine derivatives by the condensation of 2-amino-4,6,7-substitued benzothiazole catalyzed by anhy.K₂CO₃. The milder reaction conditions, simple workup, and good yields are the most significant advantages of this new procedure in synthesis of these potential biologically active compounds. The elemental and spectroscopy analysis of FTIR, 1H- and 13C-NMR were in good agreement with the proposed structure.

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Dr. Sambhaji P. Vartale graduated and post graduated from Dr. B.A.M. University Aurangabad. He awarded “Gold Medal” at M. Sc and qualified SET Exam in appeared. Having 10 year teaching experience to UG and PG level at Yeshwant Mahavidyalaya, Nanded, Maharashtra (NAAC Re-accredited ‘A’ Grade with CGPA 3.31 and ACPE status by UGC). Under his guidance six students awarded M.Phil Degree and presently six students are working for their Ph.D degree. He completed one Minor Research project and ongoing one major research project sanctioned by UGC New Delhi. His area of research is Design synthesis and their pharmacological evaluation. He has published 25 research articles at National and International repute. He is Peer Reviewer of reputed International journal.