American Journal of Advanced Drug Delivery

www.ajadd.co.uk

Original Article

An Efficient One Pot Synthesis of Pyrazolo [3,4-*b*] pyrido[1,2-*a*] pyrimidine and its Antimicrobial Evaluation

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Date of Receipt- 20/04/2013
Date of Revision- 23/04/2013
Date of Acceptance- 04/05/2013

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ABSTRACT

Pyrazolo pyrimido pyrimidine **(4a-j)** were prepared by the reaction of compound 3-cyano-4-imino-7-methyl-2-(methylthio)-*4H*-pyrido [1,2-*a*] pyrimidine **(3)** with hydrazine hydrate, phenyl hydrazine, 2-hydrazino benzothiazole & 6-substuited hydrazine benzothiazole in N,N-dimethyl formamide (DMF) and catalytic amount of anhydrous potassium carbonate. All these newly synthesized compounds were characterized by elemental analysis and spectral data.

Keywords: N,N-dimethyl formamide, potassium carbonate, pyrazolo pyrido pyrimidine & Bis-methylthio methylene malononitrile.

INTRODUCTION

As a part of huge number of therapeutic drugs, the pyrido[1, 2-*a*]pyrimidine have been reported in this paper. These derivatives have diverse biological activities, such as antifungal[1-3], antibacterial [4-7],antiallergic[8] and antiherpes[9]. The ribofuranosides of pyrido[2,3-*d*]pyrimidines have been reported as potential antitumor[10] and anti AIDS agents[11]. The promising multifold exploitations of pyrido[2,3-*d*]pyrimidines. And also pyrazolines exhibiting biological activities like anti-inflammatory [12-14], antidepressant[15], antimicrobial[16-19], antitumor[20-22] drug activity as a stable fragment in biological moieties to synthesize new heterocyclic compounds which is a major topic in current bioorganic synthesis. Environmental synthesis of carbon-heteroatom, carbon-carbon bond formation in organic compounds. Our interest in this area prompted us to synthesize some new pyrido[1,2-*a*]pyrimidines derivatives in antibacterial and antifungal studies have shown promising biological activities.

MATERIALS AND METHODS

Melting points was 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 obtained on brukner advance spectrophotometer 400 MHz mass spectra were recorded on FT-VC-7070 H Mass spectrometer using the EI technique at 70 ev. All the reaction were carried out under ambient atmosphere. Elemental analysis was performed on a Heraeus CHN-O rapid analyzer.

General procedure

3-cyano-4-imino-7-methyl-2-(methylthio)-*4H*-pyrido [1,2-*a*] pyrimidine (3)

A mixture of 2-amino 5-methyl pyridine **(1)** (0.01 mol) and bis (methylthio) methylene malononitrile **(2)** (0.01 mol) in 20 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-j)

A mixture of **3** (0.001 mol) and independently with hydrazine hydrate (80 %), 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, (0.001mol) in 15 mL of N, N'- dimethyl formamide and anhydrous potassium carbonate (10mg) was refluxed for 4-5 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 **(4a-j).**

3-Cyano-4-imino-2-(methylthio)-7-methyl-4H-pyrido [1,2-*a*]pyrimidine (3)

Orange powder, yield 85 %, M.P. 179 °C (dec.). IR (KBr / cm^{-1}) 3342 (=NH), 2212 (CN); ^{1}H NMR (400 MHz,DMSO-d_6, ppm) , δ 2.1 (s , 3H, Ar-CH_3), 2.6 (s, 3H, SCH_3), 5.4-6.4 (m,3H, HC=C), 8.9 (br s, 1H, =NH), EI-MS (m/z: RA %): 230(M^+) , 100% , ^{13}C NMR (300 MHz $CDCl_3$) δ 16,19.5,79,116,120,122, 137,138,150,164,165; Anal.Calcd. M.F. $C_{11}H_{10}N_4S$; C, 57.30; H, 4.38; N, 24.33; Found: C, 57.01; H, 4.03; N, 24.02.

3-Amino-4-imino-7-methyl-2-(H) pyrazolo [3, 4-*b*] pyrido [1, 2-*a*] pyrimidine (4a)

Brown powder, Yield: 78 %, M.P. 187°C (dec.). IR (KBr / cm^{-1}) 3416,3358cm^{-1} (NH_2 asym., sym.), 3210 cm^{-1}(=NH). ^{1}H-NMR: (DMSO-d_6): δ 2.3(s, 3H, Ar-CH_3), 4.0 (broad s, 2H, NH_2, exchangeable with D_2O), δ 5.2-6.5 (m, 3H, CH=CH-), δ 8.9 (s, 1H, =NH exchangeable with D_2O) δ 10.1 (s,1H, NH, exchangeable with D_2O). EI-MS (m/z: RA %): 214(M^{+} +1).Anal. Calcd. For $C_{10}H_{10}N_6$: C, 53.99; H, 4.71; N, 39.23.

3-Amino-4-imino-7-methyl-2-(phenyl) pyrazolo [3,4-*b*] pyrido [1,2-*a*] pyrimidine (4b)

Brown powder, yield 62 %,M.P. 148°C (dec.). IR (KBr / cm^{-1}) 3418, 3322 cm^{-1} (NH_2 asym., sym.), 3211 cm^{-1} (=NH). ^{1}H-NMR: (DMSO-d_6): δ 2.4(s, 3H, Ar-CH_3), 4.2 (broad s, 2H, NH_2, exchangeable with D_2O), δ 5.3-6.7 (m, 8H, Ar-H), δ 9.1 (s, 1H, =NH exchangeable with D_2O) δ 10.3 (s,1H, NH, exchangeable with D_2O). EI-MS (m/z: RA

%): 290 (M), Anal. Calcd. For $C_{16}H_{14}N_6$: C, 66.19; H, 4.86; N, 28.95

3-Amino-4-imino-7-methyl-2-(4'-nitro phenyl) pyrazolo [3,4-b] pyrido [1,2-a] pyrimidine (4c)

Brown powder, Yield 73 %, M.P.164°C (dec.). IR (KBr / cm^{-1}) 3421,3336 cm^{-1} (NH_2 asym., sym.), 3218 cm^{-1} (=NH). ^{1}H-NMR: (DMSO-d_6): δ 2.1(s, 3H, Ar-CH_3), 3.9 (broad s, 2H, NH_2, exchangeable with D_2O), δ 5.6-6.9 (m, 7H, Ar-H), δ 9.2 (s, 1H, =NH exchangeable with D_2O) δ 10.1 (s,1H, NH, exchangeable with D_2O). EI-MS (m/z: RA %): 336 (M+I), Anal. Calcd. For $C_{16}H_{13}N_7O_2$ C, 57.31; H, 3.91; N, 29.24.

3-Amino-4-imino-7-methyl-2-(2',4'-dinitro phenyl) pyrazolo[3,4-b] pyrido [1,2-a] pyrimidine (4d)

Brown powder, Yield 61 %,M.P. 167°C (dec.). IR (KBr / cm^{-1}) 3460, 3318cm^{-1} (NH_2 asym., sym.), 3242 cm^{-1} (=NH). ^{1}H-NMR: (DMSO-d_6): δ 2.2(s, 3H, Ar-CH_3), 4.1 (broad s, 2H, NH_2, exchangeable with D_2O), δ 5.8-7.3 (m, 6H, Ar-H), δ 9.1 (s, 1H, =NH exchangeable with D_2O) δ 10.3 (s,1H, NH, exchangeable with D_2O). EI-MS (m/z: RA %): 380($M^{+\cdot}$)Anal. Calcd. For $C_{16}H_{12}N_8O_4$: C, 50.53; H, 3.18; N, 29.46.

3-Amino-4-imino-7-methyl-2-(2'-benzothiazolyl) pyrazolo [3,4-*b*] pyrido [1,2-*a*]pyrimidine (4e)

Brown powder, Yield 72 %, M.P 173°C (dec.). IR (KBr / cm^{-1}) 3342, 3373cm^{-1} (NH_2 asym., sym.), 3234cm^{-1} (=NH). ^{1}H-NMR: (DMSO-d_6): δ 2.3(s, 3H, Ar-CH_3), 4.3 (broad s, 2H, NH_2, exchangeable with D_2O), δ 5.6-7.1 (m, 7H, Ar-H), δ 9.3 (s, 1H, =NH exchangeable with D_2O) δ 10.1 (s,1H, NH, exchangeable with D_2O). EI-MS (m/z: RA %): 347(M^+), Anal. Calcd. For $C_{17}H_{13}N_7S$: C, 58.77; H, 3.77; N, 28.22.

3-Amino-4-imino-7-methyl-2-(6'-methyl- 2'-benzothiazolyl) pyrazolo [3, 4-b] pyrido [1,2-a] pyrimidine (4f)

Brown powder, Yield 68 %,M.P.176°C (dec.). IR (KBr / cm^{-1}) 3440,3321cm^{-1} (NH_2 asym., sym.),3273 cm^{-1} (=NH). ^{1}H-NMR: (DMSO-d_6): δ 2.4(s, 6H, Ar-CH_3), 3.9 (broad s, 2H, NH_2, exchangeable with D_2O), δ 5.4-6.8 (m, 6H, Ar-H), δ 8.9 (s, 1H, =NH exchangeable with D_2O) δ 10.2 (s,1H, NH, exchangeable with D_2O). EI-MS (m/z: RA %): 361(M^+),Anal. Calcd. For $C_{18}H_{15}N_7S$: C, 59.82; H, 4.18; N, 27.13.

3-Amino-4-imino-7-methyl-2-(6'-methoxy-2'-benzothiazolyl) pyrazolo [3,4-*b*] pyrido [1, 2-*a*] pyrimidine(4g)

Brown powder, Yield72%, M.P.183° C (dec.). IR (KBr/cm^{-1}) 3426,3322 cm^{-1} (NH_2 asym., sym.), 3212cm^{-1} (=NH). ^{1}H NMR: (DMSO-d_6): δ 2.4 (s, 3H, Ar-CH_3), 3.6 (s, 3H, Ar-OCH_3), δ 4.4 (s, 2H, NH_2, exchangeable with D_2O), 6.7-8.3(m, 6H, Ar-H), δ 8.7 (s, 1H, =NH exchangeable with D_2O). EI-MS (m/z: RA %): 377 ($M^{+\cdot}$). Anal. Calcd. For $C_{18}H_{15}N_7OS$: C, 57.28; H, 4.01; N, 25.98.

3-Amino-4-imino-7-methyl-2-(6'-chloro-2'-benzothiazolyl)pyrazolo[3,4-*b*]pyrido[1,2-*a*] pyrimidine (4h)

Brown powder, Yield 69 %,M.P. 180°C (dec.). IR (KBr / cm^{-1}) 3425,3380,cm^{-1} (NH_2 asym., sym.), 3218 cm^{-1} (=NH) . EI-MS (m/z: RA %): 381(M^+) Anal. Calcd. For $C_{17}H_{12}ClN_7S$: C, 53.47; H, 3.17; N, 25.68;

3-Amino-4-imino-7-methyl-2-(6'-nitro-2'-benzothiazolyl) pyrazolo [3,4-*b*] pyrido[1,2-*a*] pyrimidine (4i)

Brown powder, yield 65 %, M.P. Above 300°C (dec.). IR (KBr/cm^{-1}) 3430, 3385,cm^{-1} (NH_2 asym., sym.), 3226cm^{-1} (=NH). EI-MS (m/z: RA %): 379 (55%) Anal. Calcd. For $C_{17}H_{12}N_8O_2S$: C, 52.03; H, 3.08; N, 28.56;

3-Amino-4-imino-7-methyl-2-(4',6'-dimethyl- 2'-benzothiazolyl) pyrazolo [3,4-*b*]pyrido [1,2-*a*] pyrimidine (4 j)

Brown powder, yield 72 %, M.P. 161-62°C (dec.).IR (KBr /cm^{-1}) 3428, 3376,cm^{-1} (NH_2 asym., sym.), 3229cm^{-1} (=NH) . EI-MS (m/z: RA %): 375 (M^+), Anal. Calcd. For $C_{19}H_{17}N_7S$; C, 60.78; H, 4.56; N, 26.11.

RESULTS & 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 (4a-j) Our method gives single product with high yield. The reaction started with 2-amino-5-methyl pyridines (**1**) and bis (methylthio) methylene malononitrile **(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, to obtain 3-Amino-4-oxo-2-(6'-substituted benzothiazolyl) pyrazolo[3,4-*b*]pyrido[1,2-*a*]pyrimidine (4a-j) respectively.**Scheme-2**

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.

Antibacterial activity

The synthesized compounds were evaluated for their antibacterial activity against gram-positive species *S. aureus* and *B. substilis* and gram-negative species *E. coli* and *S. typhi* by paper disc diffusion method [23] . All the synthesized compounds were dissolved in dimethyl sulphoxide. The synthesized compounds exhibited zone of inhibition of 06-16 mm in diameter whereas standard Streptomycin exhibited zone of inhibition of 18 and 22 mm in diameter against *S. aureus* and *B. substilis* 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 **4h** (11, 11, 10, 12 mm) and **4i** (13,16,12,13) showed higher zone of inhibition against *S. aureus, B. substilis, E. coli* and *S. typhi* respectively. It seems that the presence of Cl &NO_2 group at 6- position **4a** increases antibacterial activity. **Table 1**

CONCLUSION

In conclusion, our results demonstrate a simple, mild and efficient method for the synthesis of novel functionalized pyrido [1,2-*a*]pyrimidine derivatives by the condensation of 2-amino-4,6,7-substuited benzothiazole catalyzed by anhy.K_2CO_3. The milder reaction conditions, 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, ^{1}H-NMR were in good agreement with the proposed structure.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. N. V. Kalyankar, Principal, Yeshwant Mahavidyalaya, Nanded, for providing laboratory facilities, To UGC New Delhi for financial assistance under major research project (F.N 39-834/2010 (SR)) and Director, Indian Institute of Chemical Technology, Hyderabad, for providing spectra.

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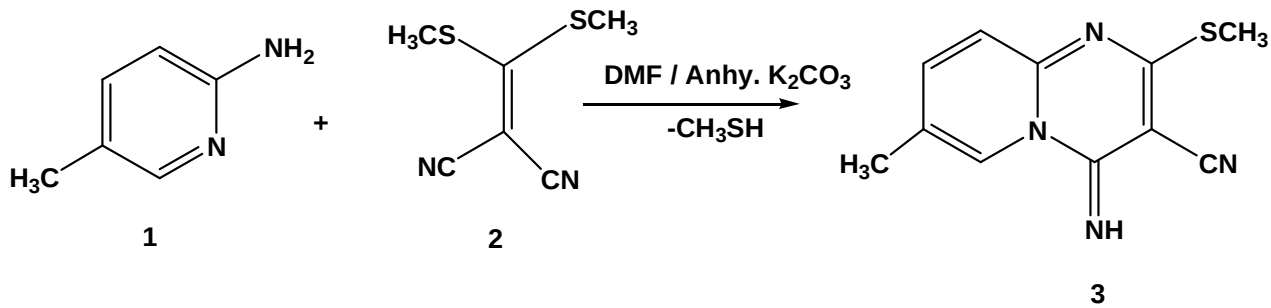
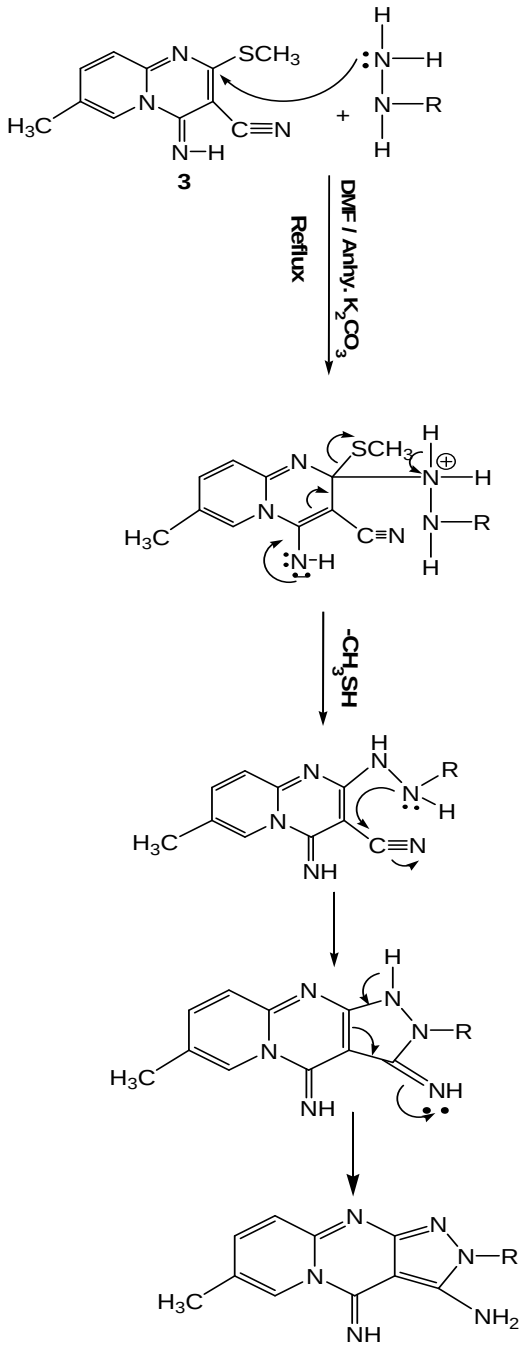
Table 1. Antibacterial activity of compound (3-4j)

Diameter in mm of zone of inhibition at 25 µg/disc				
Comp.	**S.aureus**	**B.substilis**	**E. coli**	**S. typhi**
3	06	08	10	09
4a	08	09	10	07
4b	08	11	09	10
4c	11	14	09	10
4d	12	15	11	12
4e	11	13	06	09
4f	--	08	10	11
4g	10	13	11	12
4h	**11**	**11**	**10**	**12**
4i	**13**	**16**	**12**	**13**
4j	--	08	09	11
Streptomyci	**18**	**22**	---	---
Penicillin	---	---	**15**	**16**



Scheme 1. Formation of fused pyrido [1, 2-*a*] pyrimidine

 ISSN-2321-547X



Comp.No	R
4a	H
4b	
4c	O_2N
4d	O_2N, NO_2
4e	
4f	H_3C
4g	H_3CO
4h	Cl
4i	O_2N
4j	H_3C, CH_3

Scheme 2. formation of fused pyrido [1, 2-*a*] pyrimidine and its substituted derivatives