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AN EFFICIENT SYNTHESIS OF IMINO PYRAZOLO PYRIDO [1,2-*a*]PYRIMIDINES AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

New pyrazolo pyrido pyrimidine derivatives have been synthesized by condensation of 7-bromo-3-cyano-4-imino-2-methylthio-4H-pyrido [1, 2-*a]*pyrimidines (3) with hydrazine and its various derivatives. The chemical structure of the product was proved on the basis of their spectal IR, ^{1}H-NMR, ^{13}C-NMR, Mass and analytical studies. All the newly synthesized compounds have been screened for antimicrobial activity.

KEYWORDS: Bis (methylthio) methylene malononitrile, Pyrazolopyrido pyrimidine, N,N-dimethyl formamide, anhydrous potassium carbonate.

INTRODUCTION

In recent days lots of research work has been done in the synthesis of pyrido pyrimidines because of their wide range of biological activities. After lots of literature survey we came to know that these pyrido pyrimidines are associated with prominent pharmacological activities. These derivatives are found pharmacological potent such as Anticancer,[1] antitumor,[2-4] antibacterial,[5] antiviral,[6] antileishmanial,[7] antineoplastic effects,[8] analgesic activity,[9] antimicrobial,[10-13] anti-inflammatory,[14] antihypertensive,[15] anti-proliferative CDK2 inhibitors[16] Dihydrofolate Reductase Inhibitors.[17] In connection of our interest in pyrido pyrimidines our research group synthesized and evaluated the biological importance of pyrido pyrimidines.[18-19]

Considering all the importance of these pyrido pyrimidine we gain interest to synthesise some new pyrido pyrimidine derivatives. In this paper we report the synthesis of 7-bromo-3-cyano-

4-imino-2-methylthio-4H-pyrido [1,2-*a*] pyrimidines and its hydrazino derivatives which shown promising biological activities.

MATERIALS AND METHODS

Melting point were determined by open capillary tubes and were uncorrected. Progress of reaction was monitored by thin layer chromatography carried out of aluminium silica plates using UV Chember for detection. Infrared spectra were recorded in potassium bromide pallets on an infrared spectrophotometer, nuclear magnetic spectra were obtained on Bruckner advance spectrophotometer; 400MHz mass spectra were recorded on ET-VC-7070H mass spectrophotometer with the use of EI technique at 70ev. All the reactions were carried out under ambient atmosphere. Elemental analysis was performed on Heracus CHN-O rapid analysis.

General peocedure

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

A mixture of 2-amino-5-bromo pyridine (1) (0.01 mole) and bis (methylthio) methylene malononitrile (2) (0.01 mole) in 20 mL N,N-dimethyl formamide and anhydrous potassium carbonate (10mg) was refluxed for 5 hours. The reaction mixture was cooled to room temperature and poured into ice cold water. The separated solid product was filtered, washed with water and recrystallized from alcohol to give pure (3).

3-Amino-7-bromo-4-imino-2-(substituted) 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-chloro- 2-hydrazino benzothiazole, 6-nitro 2-hydrazino benzothiazole, 6-methyl-2-hydrazino benzothiazole, 6-methoxy-2- hydrazino benzothiazole, 4,6-dimethyl 2-hydrazino benzothiazole, (0.001mol) in 15mL 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 N, N'- dimethyl formamide- ethanol mixture to give pure **(4a-j).**

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

Brown powder, yield 85%, m.p. 179 °C (dec.). IR (KBr / cm^{-1}) 3337 cm^{-1} (=NH), 2238 cm^{-1} (CN); ^{1}H NMR (400 MHz,DMSO-*d6*, δ ppm), 2.6 (s, 3H, SCH_3), 5.7-6.6 (m, 3H), 8.1 (s, 1H, =NH), EI-MS (m/z: RA %): 295[M^++1], 100%. ^{13}C NMR (300 MHz $CDCl_3$ δ) 15.1, 80.7, 99.3, 115.5, 120, 134, 137, 150, 164.0, 165.7. Anal.Calcd. M.F. $C_{10}H_7BrN_4S$; C: 40.69, H:2.39, Br:27.07, N:18.98, S:10.86. Found: C:40.35, H:2.28, N:26.85.

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

Brown powder, Yield: 75 %, M.P. 195°C (dec.). IR (KBr / cm^{-1}) 3425, 3345 cm^{-1} (NH_2 asym., sym.), 3242 cm^{-1}(=NH). ^{1}HNMR: (DMSO-*d6*): δ 3.8 (broad s, 2H, NH_2, exchangeable with D_2O), δ 5.1-6.6 (m, 3H, CH=CH-), δ 8.6 (s, 1H, =NH exchangeable with D_2O) δ 9.6 (s,1H, NH, exchangeable with D_2O). EI-MS: m/z = 279 (M^++1) Anal. Calcd. For $C_9H_7BrN_6$: C, 38.20; H, 2.35; N, 30.00.

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

Grey powder, yield 68%, M.P. 160°C (dec.). IR (KBr / cm^{-1}) 3411, 3332 cm^{-1} (NH_2 asym., sym.), 3238 cm^{-1} (=NH). ^{1}HNMR: (DMSO-*d6*): δ 3.8 (broad s, 2H, NH_2, exchangeable with D_2O), δ 5.1-7.5 (m, 8H, Ar-H), δ 9.8 (s, 1H, =NH exchangeable with D_2O). EI-MS (m/z = 355 (M^++1), Anal. Calcd. For $C_{15}H_{11}BrN_6$: C: 50.12; H:3.01; N:23.10.

3-Amino -7-bromo-4- imino -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}) 3409,3321 cm^{-1} (NH_2 asym., sym.), 3230 cm^{-1} (=NH). ^{1}H-NMR: (DMSO-*d6*): δ 4.1 (broad s, 2H, NH_2, exchangeable with D_2O), δ 5.2-8.3 (m, 7H, Ar-H), δ 10.0 (s, 1H, =NH exchangeable with D_2O) d 9.7. EI-MS: m/z = 400 (M^++1), Anal. Calcd. For $C_{15}H_{10}BrN_7O_2$; C: 44.90; H: 2.20; N:24.18.

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

Brown powder, Yield 67 %,M.P. 170°C (dec.). IR (KBr / cm^{-1}) 3455, 3315cm^{-1} (NH_2 asym., sym.), 3238 cm^{-1} (=NH). ^{1}HNMR: (DMSO-*d6*): δ 4.1 (broad s, 2H, NH_2, exchangeable with D_2O), δ 5.1-8.7 (m, 6H, Ar-H), δ 9.5 (s, 1H, =NH exchangeable with D2O). EI-MS: m/z = 445 (M^++1). Anal. Calcd. For $C_{15}H_9BrN_8O_4$: C: 40.10; H: 2.15; N: 25.60.

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

Brown powder, Yield 75 %, M.P 188°C (dec.). IR (KBr / cm^{-1}) 3340, 3380cm^{-1} (NH_2 asym., sym.), 3240cm^{-1} (=NH). ^{1}HNMR: (DMSO-*d6*): δ 4.1 (broad s, 2H, NH_2, exchangeable with D_2O), δ 5.2-8.25 (m, 7H, Ar-H), δ 9.6 (s, 1H, =NH exchangeable with D_2O). EI-MS: m/z = 412 (M^++1), Anal. Calcd. For $C_{16}H_{10}BrN_7S$. C:47.70; H3.35; N:22.90.

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

Brown powder, Yield 69 %,M.P. 180°C (dec.). IR (KBr / cm^{-1}) 3395, 3370 cm^{-1} (NH_2 asym., sym.), 3245 cm^{-1} (=NH). ^{1}H NMR: (DMSO-*d6*): δ 3.7 (s, 2H, NH_2, exchangeable with D_2O), δ 5.3-7.4(m, 6H, Ar- H), δ 8.6 (s, 1H, =NH exchangeable with D_2O). EIMS: m/z = 446 (M^++1). Anal. Calcd. For $C_{16}H_9BrClN_7S$: C: 43.17: H:2.09, N:21.90.

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

Greyish powder, yield 63 %, M.P. Above 295°C (dec.). IR (KBr/cm^{-1}) 3422, 3365,cm^{-1} (NH_2 asym., sym.), 3226cm^{-1} (=NH). ^{1}H NMR: (DMSO-*d6*): δ 3.9 (s, 2H, NH_2, exchangeable with D_2O), 5.2-7.1(m, 6H, Ar- H), δ 8.8 (s, 1H, =NH exchangeable with D_2O). EI-MS: m/z = 457 (M^++1). Anal. Calcd. For $C_{16}H_9BrN_8O_2S$: C:41.85; H:1.85; N:24.65.

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

Brown powder, Yield 68 %,M.P.176°C (dec.). IR (KBr / cm^{-1}) 3430,3315cm^{-1} (NH_2 asym., sym.), 3273 cm^{-1} (=NH). ^{1}H-NMR: (DMSO-*d6*): δ 2.4 (s, 3H, Ar-CH3), δ 3.7 (broad s, 2H, NH_2, exchangeable with D_2O), δ 5.2-7.7 (m, 6H, Ar-H), δ 9.1 (s, 1H, =NH exchangeable with D_2O). EI-MS: m/z = 426 (M^++1). Anal. Calcd. For $C_{17}H_{12}BrN_7S$: C:47.95; H:3.15; N:22.20.

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

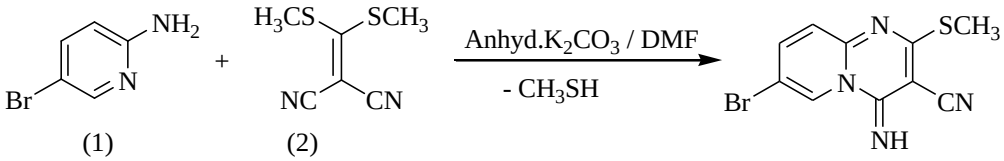
Brown powder, Yield72%, M.P.183° C (dec.). IR (KBr/cm^{-1}) 3430, 3315 cm^{-1} (NH_2 asym., sym.), 3210 cm^{-1} (=NH). ^{1}H NMR: (DMSO-*d6*): 3.7 (s, 3H, Ar-OCH_3), δ 4.1 (s, 2H, NH_2, exchangeable with D_2O), δ 5.2-7.5(m, 6H, Ar- H), δ 8.9 (s, 1H, =NH exchangeable with D_2O). EI-MS: m/z = 442 (M^++1). Anal. Calcd. For $C_{17}H_{12}BrN_7OS$: C: 46.10; H: 2.65; N:22.10.

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

Brown powder, yield 72 %, M.P. 161- 62°C (dec.).IR (KBr /cm^{-1}) 3435, 3365,cm^{-1} (NH_2 asym., sym.), 3220cm^{-1} (=NH) . ^{1}H NMR: (DMSO-*d6*): δ 2.36 (s,3H, Ar-CH_3), δ 2.4 (s,3H,Ar-CH_3) δ 4.2 (s, 2H, NH_2, exchangeable with D_2O), 5.1-7.8 (m, 5H, Ar- H), δ 8.9 (s, 1H, =NH exchangeable with D_2O). EI-MS: m/z = 440 (M^++1) Anal. Calcd. For $C_{18}H_{14}BrN_7S$; C, 49.05; H, 3.25; N, 22.0.

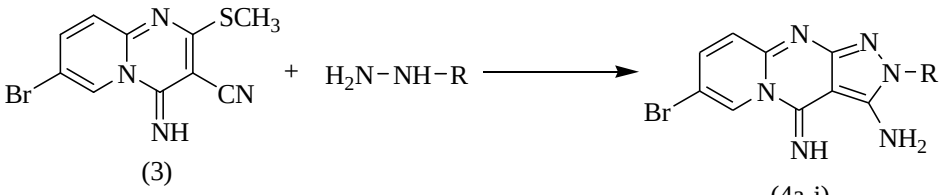
RESULT AND DISCUSSION

In the present investigation, we have reported new efficient method towards the synthesis of 7-bromo-2-cyano-4-imino-2-(sustituted] pyrazolo [3,4-*b*] pyrido [1,2-*a*] pyrimidine [4a-j]. The beauty of our method is to give single product with high yield. The reaction started with 2-amino-5-bromo pyridine (1) and bis (methylthio) methylene malononitrile (2) in N,N-dimethyl formamide in presence of catalytic amount of anhydrous potassium carbonate to afford (3). **Scheme-I**

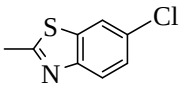
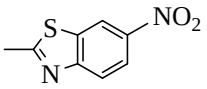
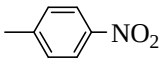
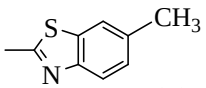
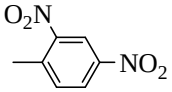
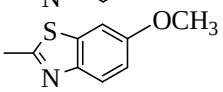
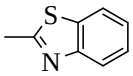
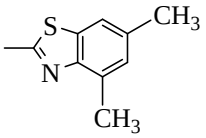


Scheme - I

The compound (3) possess replacable active methylthio group at 2-position which is activated by ring 1- nitrogen atom electron withdrawing 3-cyano group. The susceptibility of 7-bromo-3-cyano-4-imino-2-methylthio-4H-pyrido [1,2-*a*] pyrimidine (3) towards cyclisation with hydrazine hydrate and their different substituted derivatives have been investigated .These reaction results in the formation of 7-bromo-2-cyano-4-imino-2-(sustituted] pyrazolo [3,4-b] pyrido [1,2-*a*] pyrimidine [4a-j].according to these reaction compound (3) reacted with hydrazino hydrate, phenyl hydrazine, 4-nitrophenyl hedrazine, 2,4-dinitrophenyl hydrazine, 2-hydrazino benzothiazole, 6-methyl-2-hydrazino benzothiazole, 6-methoxy-2-hydrozino benzothiazole, 6-chloro-2-hydrozino benzothiazole, 6-nitro-2-hydrozino benzothiazole, 2,4-dimethyl-2-hydrozino benzothiazole to obtain 3-amino-7-bromo-4-imino-2-(6'-substituted) pyrazolo[3,4-*b*]pyrido [1,2-*a*]pyrimidine (4a-j) **scheme-II.**



Scheme - II

Comp. No.	R	Comp. No.	R
4a	−H	4f	
4b	$-C_6H_5$	4g	
4c		4h	
4d		4i	
4e		4j	

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 and do not exhibit any tautomerism.

CONCLUSION

Our results shows a simple and efficient method for the synthesis of novel functionalized pyrido [1,2-*a*] pyrimidine derivatives by condensation of different hydrazino derivatives catalysed by anhydrous K_2CO_3. The milder reaction condition, good yields, are the most significant advantages of this novel synthesis of these biologically potent compounds.

The structure of these newly synthesized derivatives are designated on the basis of elemental data, IR, ^{1}H-NMR, Mass spectral data. The spectral analysis of these compounds are in agreement of the proposed structures.

Antimicrobial activity

In this communication all compounds are synthesized by simple route with mild condition and good yield. Out of these synthesized compounds some compounds shows considerable antimicrobial activity. From the given data it is found that, 4c, 4d, 4f and 4g derivative have significant antibacterial activity. The antimicrobial activity of synthesized compounds are given in following table.

Diameter in mm of zone of inhibition at 25 µg/disc				
Comp. No.	**S. aureus**	**B. substilis**	**E.coli**	**S. typhi**
3	07	09	--	10
4a	08	09	12	15
4b	10	07	09	13
4c	11	13	15	12
4d	13	16	14	12
4e	--	11	12	--
4f	12	15	14	15
4g	18	14	14	15
4h	10	--	13	13
4i	05	06	--	--
4j	05	--	--	06
Streptomycin	**18**	**22**	**--**	**--**
Penicillin	**--**	**--**	**15**	**16**

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