Synthesis and Antimicrobial Activity of Pyrazolo[4,5-*e*] Pyrimido[2,1-*b*] Pyrazines

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Abstract : Based on the potent biological activity of pyrazolo derivatives, we carried out synthesis of 3-amino-4-imino-2-N-(substituted)pyrazolo[4,5-*e*]pyrimido[2,1-*b*] pyrazines (3a-j) have been reported from condensation of 3-cyano-4-imino-2-(methylthio)-4*H*-pyrimido[1,2-*a*]pyrazine (1) and substituted hydrazines and hydrazine benzothiazole (2a-j) using dimethyl formamide as reaction solvent and anhydrous K_2CO_3 as reaction catalyst. These synthesized compounds were further screened for antibacterial activity and cytotoxicity study.

Keywords: Pyrimido pyrazine, substituted hydrazines, anhydrous K_2CO_3.

I. INTRODUCTION

Pyrazolo pyrimidine bearing compounds have great deal of attention due to their biological activity, pyrazolo pyrimidine derivatives shows promising antitumor activity[1], pyrazolo pyrimidine are purine analogue and shows antibacterial activity[2] against gram positive and gram negative bacteria, CNS depressant activity[3], antimicrobial activity[4-5], anti-proliferativeactivity[6], analgesic[7],tuberculostatic activity[8]. Ali Gharib et al.[9] reported catalytic synthesis of pyrazolo[3,4-*d*]pyrimidin-6-thiol by reaction of thiourea, 3-methyl-1-phenyl-5-pyrazolone and various substituted aldehyde in presence of nano zeolite NaX in solvent free condition.

Hichem Ben Jamet et al.[10] reported synthesis of 4-imino-3-methyl-1-phenyl-1*H*,4*H*-pyrazolo[3,4-*d*]pyrimidin-5-ol by refluxing mixture of pyrazole imidates with hydroxyl amine hydrochloride using triethyl amine and ethyl alcohol mixture in 5 ml :20 ml ratio.

J.S. Sandhu et al.[11] reported one pot synthesis of pyrazolo[3,4-*d*]pyrimidines by reaction of 6-hydrazino pyrazole with alkyl/aryl isocyanate using heteroannulation on the double bond of uracil using simple method. In recent time we reported synthesis and antioxidant activity of 3-cyano-4-imino-2-(methylthio)-4*H*-pyrimido[1,2-*a*]pyrazine compounds[12].

Pyrazolo pyrimidines attracted simple interest over the last decades due to their ring system and act as main nucleus in different drugs, by considering these importances of pyrazolo pyrimidine derivatives it was thought worth to synthesize new class of fused heterocyclic compound possessing pyrazolo pyrimido pyrazine which may show positive biological activity. Thus literature survey reveals that pyrazolo pyrimidine derivatives are shows broad range of biological activities which encourage us to synthesise derivatives of these heterocycles.

II. MATERIALS AND METHODS

Open capillary tubes was used for recording melting points of newly synthesized compounds and were uncorrected. FTIR spectrometer was used for the IR spectra of compounds, were as on Brucker advance 300 MHz spectrometer was used for 1H-NMR spectra. FT-VG-7070 Hz mass spectrometer has been utilized for Mass spectra using ESI technique.

General procedure:

Synthesis of 3-amino-4-imino-2-N-(substituted)pyrazolo[4,5-*e*]pyrimido[2,1-*b*] pyrazine (3a).

A mixture of **1** (0.217 g, 0.001 mol) and independently with hydrazine hydrate and its various derivatives (**3a-j**) (0.001 mol) in 15 ml of DMF and anhydrous K_2CO_3 (10 mg) was refluxed for 5-6 hours. The obtained reaction mass was cooled to room temperature and poured into ice cold water having crushed ice (100ml). The separated solid mass was filtered, washed with cold water as well as hot water and recrystallized with absolute ethanol to afford pure compound **(3a-j)**.

3-amino-4-imino-2-N-(*H*)pyrazolo[4,5-*e*]pyrimido[2,1-*b*]pyrazine (3a).

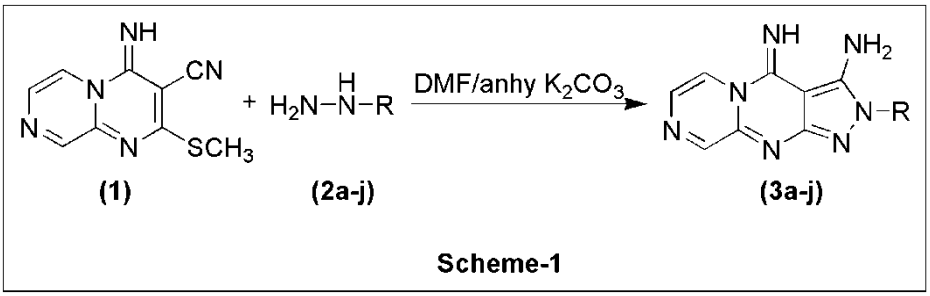
Brown solid, 62% yield, M.P. 232-234°C, IR (KBr) cm^{-1} 3394.48, 3263.33, 3120.61 (NH_2 & =NH stretch), ^{1}H NMR (DMSO d_6, δ ppm) 6.366 (s, 2H, -NH_2), 6.872 (s, 1H, -NH-), 7.235, 7.506-7.554 (s, 3H, Ar-H), 8.586 (s, 1H, =NH), CI-MS (m/z) 201.8 (M +1).

3-amino-4-imino-2-N-(3'-methyl phenyl)pyrazolo[4,5-*e*]pyrimido[2,1-*b*]pyrazine (3e).

Brown solid, 62% yield, M.P. 222-224°C, IR (KBr) cm^{-1} 3359.77, 3282.62, 3155.33 (NH_2 & =NH stretch), ^{1}H NMR (DMSO d_6, δ ppm) 2.502-2.517 (s, 3H, -CH_3), 4.284 (s, 2H, -NH_2), 6.755-7.462 (m, 7H, -Ar-H), 10.245 (s, 1H , =NH), CI-MS (m/z) 291.2 (M^+)

III. RESULTS AND DISCUSSION:

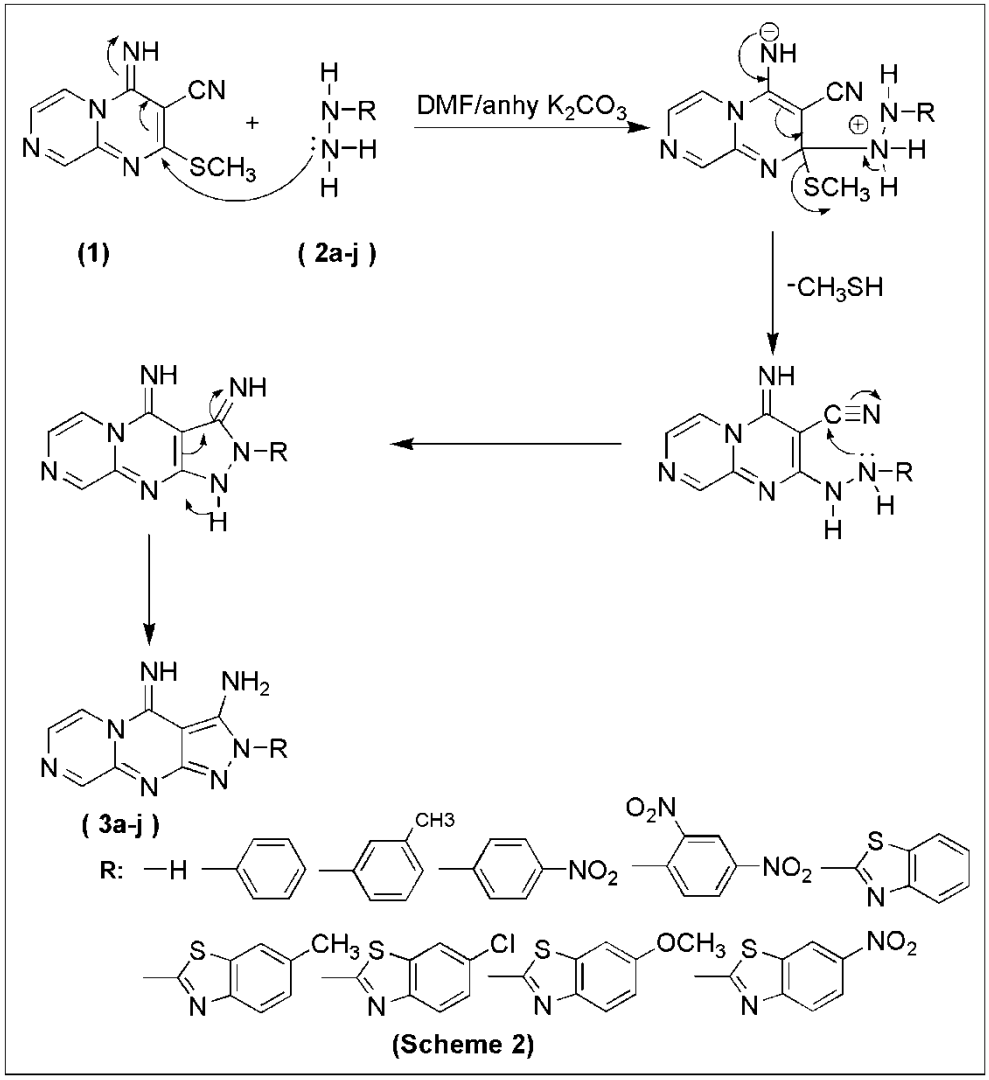
In the present investigation, synthesis of 3-amino-4-imino-2-N-(substituted) pyrazolo[4,5-*e*]pyrimido[2,1-*b*]pyrazine **(3a-j)** have been reported from 3-cyano-4-imino-2-(methylthio)-4*H*-pyrimido[1,2-*a*]pyrazine **(1)** **(Scheme 1).**



Scheme-1

The compound **(1)** has thiomethyl leaving group at 2-position which is activated by pyrimido ring nitrogen atom and electron withdrawing cyano group at adjacent carbon. Hence, the reactivity of the compound **(1)** towards cyclization reaction with hydrazine hydrate and their substituted derivatives **(3a-j)** has been reported. These reactions result into synthesis of 3-amino-4-imino-2-N-(substituted) pyrazolo[4,5-*e*]pyrimido[2,1-*b*]pyrazine **(3a-j)**. Like these reactions, the compound **(1)** independently reacted with hydrazine hydrate (80%) **(2a)**, phenyl hydrazine **(2b)**, 4-nitro phenyl hydrazine **(2c)**, 2,4-dinitro phenyl hydrazine **(2d)**, 3-methyl phenyl hydrazine **(2e)**, 2-hydrazino benzothiazole **(2f)**, 6-methyl-2-hydrazino benzothiazole **(2g)**, 6-methoxy-2-hydrazino benzothiazole **(2h)**, 6-chloro-2-hydrazino benzothiazole **(2i)** and 6-nitro-2- hydrazino benzothiazole **(2j)** in DMF and anhydrous K_2CO_3 to afford, 3-amino -4-imino-2-N-(substituted*)* pyrazolo[4,5-*e*]pyrimido[2,1-*b*]pyrazine (**3a-j**).

Mechanism for the formation of compound **(3a-j)** can be given as follows **(Scheme 2):**



(Scheme 2)

The synthesized compound showed IR absorption bands in the region of **3420-3120** cm^{-1} due to stretching of free -NH_2, -NH, & =NH group. The absence of absorption band of compound (**1**) at **2214.13** cm^{-1} confirm cyano group undergo cyclization to get pyrazole ring.

^{1}H NMR spectra of these synthesized compounds showed absence of singlet peak at **δ2.639** ppm due to -SCH_3 protons shows cyclization took place. The absorption signal appears at **δ4.20-6.78** ppm is due to -NH_2 proton.

The mass spectra reveal that the molecular ion peaks which correspond to molecular mass of concern compounds.

Antibacterial Activity:

Disc diffusion method:

Disc diffusion assay was carried by Kirby-Bauer method. *In vitro* antimicrobial activity was screened by using Mueller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 min and 0.1 % inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 min. The concentrations of compounds were set at 10 µg/disc and were loaded on 5 mm sterile individual discs. The loaded discs were placed on the surface of medium and the compounds were allowed to diffuse for 5 min and the plates were kept for incubation at 37°C for 24 hrs. *Ampicillin* (10 µg/disc) and *Fluconazole* (10 µg/disc) was used as positive control. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Table No. 1: Antimicrobial potential of pyrazolo[4,5-*e*]pyrimido[2,1-*b*]pyrazines

Sr. No.	Compound	Zone of inhibition in mm	
		Antibacterial activity	
		E. coli	***B. subtilis***
1	3a	14	10
2	3b	NA	NA
3	3c	10	NA
4	3d	NA	08
5	3e	10	NA
6	3f	12	06
7	3j	NA	10
8	*Ampicillin*	20	16

(NA-No activity)

The newly synthesized compounds were screened for their antimicrobial activity against gram positive bacteria *B. subtilis*, gram-negative bacteria *E. coli* using *Ampicillin* as standard drug. The antibacterial activity of pyrazolo pyrimido pyrazines is given in table 1.

The compounds **3a**, **3f** exhibit zone of inhibition against the tested bacteria, The compounds like **3b**, **3d**, **3j** were inactive against *E. coli* while **3b**, **3c**, **3e** were not shown zone of inhibition against *B. subtilis*.

IV CONCLUSION:

Simple and efficient synthesis 3-amino-4-imino-2-N-(substituted)pyrazolo[4,5-*e*]pyrimido[2,1-*b*]pyrazines has been presented. Among these synthesized compounds **3a**, **3f** showed antimicrobial activity against *E.Coli*. The result of the present work demonstrate that pyrazolo pyrimido pyrazines are moderate antimicrobial agents and it will attract researchers to design new potent pharmacological pyrazolo pyrimido pyrazines.

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