Heterocyclic Letters
Vol. 13| No.4|691-696|Aug-Oct|2023
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

SYNTHESIS, STRUCTURAL ELUCIDATION AND ANTIBACTERIAL POTENTIAL OF 7,8-DIIMINO PYRAZINO[1,2-*A*]PYRIMIDO[4,5-*D*]PYRIMIDO[2,1-*B*]BENZOTHIAZOLE AND ITS SUBSTITUTED ANALOGS

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ABSTRACT:
The synthesis of 7,8-diimino pyrazino[1,2-*a*]pyrimido[4,5-*d*]pyrimido[2,1-*b*]benzothiazole and its 2/3/5 substituted derivatives have been reported by condensation of 3-cyano-4-imino-2-(methylthio)-4*H*-pyrimido[1,2-*a*]pyrazine with various 2-amino benzothiazole derivatives. To ensure the accuracy of the synthesized compounds' structures, multiple analytical techniques were employed, including Infrared Spectroscopy (IR), Nuclear Magnetic Resonance (NMR), and Mass Spectrometry. Furthermore, the newly synthesized compounds were subjected to an assessment of their antibacterial activity. This evaluation aimed to determine the potential of these compounds in inhibiting bacterial growth, thereby contributing to our understanding of their therapeutic applications.

KEYWORDS:
3-cyano-4-imino-2-(methylthio)-4*H*-pyrimido[1,2-*a*]pyrazine, 2-amino benzothiazoles, N,N-dimethyl formamide, anhydrous potassium carbonate, antibacterial activity.

INTRODUCTION:

Pyrimidine ring system occurs as a principal core skeleton among the drug scaffolds and also play crucial role as an important component of building block of numerous natural products as well as the purine bases of DNA and RNA [i, ii]. Pyrimidine derivatives gained prominence as they exhibit a wide range of biological and medicinal properties such as antitumor [iii, iv], interferon inducer [v], antiviral [vi], anti-hypertensive [vii], hypoglycemic [viii], anticonvulsant[ix], anti-nociceptive [x, xi] and anti-inflammatory [xii] agents.

Pyrimido-benzothiazole is a fascinating heterocyclic compound that has attracted significant attention in the field of medicinal chemistry. It belongs to a class of compounds characterized by a fused pyrimido and benzothiazole ring system, resulting in a unique structure with diverse properties and potential applications. The synthesis of pyrimido-benzothiazole derivatives involves the fusion of pyrimidine and benzothiazole moieties through various synthetic methodologies. This structural fusion imparts distinct chemical and biological properties to the resulting compounds, making them valuable building blocks for the development of novel drugs and bioactive molecules. The presence of the pyrimido-benzothiazole scaffold has been found to exhibit a wide range of biological activities, including antifungal [xiii, xiv], antibacterial [xv], anti-inflammatory [xvi], anthelmintic activity [xvii], antimicrobial activity [xviii]. In view of the reported biological activities of this fused pyrimido benzothiazole heterocycles has fixed the consideration in current era, which may be more effective. Herein we report the synthesis of a library of heterocyclic derivatives of pyrimido pyrazine and benzothiazole moiety are fused to give the pyrazino pyrimido pyrimido benzothiazoles.

EXPERIMENTAL:

All the chemicals used in present works are from analytical grade and used without further purification. 3-cyano-4-imino-2-(methylthio)-4H-pyrimido[1,2-a]pyrazine was prepared by reported method [xix]. Melting points of the products were determined in open capillary tubes on an electro thermal IA 9000 series digital melting point apparatus and were uncorrected. The progress of reactions and the purity of the isolated compounds were monitored by thin layer chromatography on pre-coated sheets of silica-C plates of 0.25 mm thickness (Spectro Chem made). IR spectra were recorded on Shimadzu FT-IR spectrophotometer, 1H NMR spectra were obtained on Bruker advance spectrophotometer 400 MHz in DMSO-d6 using tetramethyl silane as an internal standard. Mass spectra were recorded on FT-VC-7070 H mass spectrometer using the chemical ionization technique at 70 eV.

GENERAL PROCEDURE:

Synthesis of 7,8-diimino pyrazino[1,2-*a*]pyrimido[4,5-*d*]pyrimido[2,1-*b*] benzothiazole and their 2/3/5 substituted derivatives (3a-f).

A mixture of 3-cyano-4-imino-2-(methylthio)-4*H*-pyrimido[1,2-*a*]pyrazine (**1)** (0.217 g, 0.001 mol) and independently with 2-aminobenzothiazole **(2a)**, 2-amino-6-methyl benzothiazole **(2b)**, 2-amino-4,6-dimethylbenzothiazole **(2c)**, 2-amino-6-methoxy benzothiazole **(2d)**, 2-amino-6-chloro benzothiazole **(2e)**, 2-amino-6-nitro benzothiazole **(2f)**, (0.001 mol) in 15 ml of DMF and anhydrous K_2CO_3 (10 mg) was refluxed for 5-6 hours. The reaction mass was cooled to room temperature and then poured into ice cold water crushed ice about 100 ml. The separated solid mass of product was filtered, washed with cold water and recrystallized using absolute ethanol to give pure **(3a-f)** respectively.

7, 8-Diimino pyrazino[1,2-a]pyrimido[4,5-d]pyrimido[2,1-b]benzothiazole (3a):

M.F. $C_{15}H_9N_7S$, Yield: 72%, MP: 272°C. IR spectrum, ν, cm^{-1}: 3421.48, 3348.19, 3220.90 (=NH stretch). ^1H NMR spectrum (δ ppm): 7.436-7.563 (m, 7H, Ar-H), 8.356-8.372 (s, 2H, =NH). Mass spectrum: m/z 320.2 [M+1]

7, 8-Diimino-3-methyl pyrazino[1,2-a]pyrimido[4,5-d]pyrimido[2,1-b] benzothiazole (3b):

M.F. $C_{16}H_{11}N_7S$, Yield: 62%, MP: 248-250°C. IR spectrum, ν, cm^{-1}: 3433.78, 3378.79, 3227.65 (=NH stretch). ^1H NMR spectrum (δ ppm): 2.891-2.931(M,3H, $-CH_3$)7.223-7.314 (m, 6H, Ar-H), 8.477-8.523 (s, 2H, =NH). Mass spectrum: m/z 334.3 [M+1]

7, 8-Diimino-3,5-dimethyl pyrazino[1,2-a]pyrimido[4,5-d]pyrimido[2,1-b]benzothiazole (3c) :

M.F. $C_{17}H_{13}N_7S$, Yield: 69%, MP: 222-224°C. IR spectrum, ν, cm^{-1}: 3407.61, 3341.14, 3213.88 (=NH stretch). ^{1}H NMR spectrum (δ ppm): 2.781-2.894(M,6H,-CH_3), 7.436-7.563 (m, 5H, Ar-H), 8.015-8.124 (s, 2H, =NH). Mass spectrum: m/z 347 [M^+]

7, 8-Diimino-3-methoxy pyrazino[1,2-a]pyrimido[4,5-d]pyrimido[2,1-b]benzothiazole (3d):

M.F. $C_{16}H_{11}N_7OS$, Yield: 71%, MP: 262-264°C. IR spectrum, ν, cm^{-1}: 3432.51, 3339.16, 3225.89 (=NH stretch). ^{1}H NMR spectrum (δ ppm): 3.921(s, 3H, -OCH_3) 7.523-7.841 (m, 6H, Ar-H), 8.110-8.297 (s, 2H, =NH). Mass spectrum: m/z 349 [M^+]

3-Chloro-7,8-diimino pyrazino[1,2-a]pyrimido[4,5-d]pyrimido[2,1-b] benzothiazole (3e):

M.F. $C_{15}H_8ClN_7S$, Yield: 61%, MP: 278-280°C. IR spectrum, ν, cm^{-1}: 3421.48, 3348.19, 3219.85 (=NH stretch). ^{1}H NMR spectrum (δ ppm): 7.351-7.602 (m, 6H, Ar-H), 8.471-8.678 (s, 2H, =NH). Mass spectrum: m/z 353 [M^+]

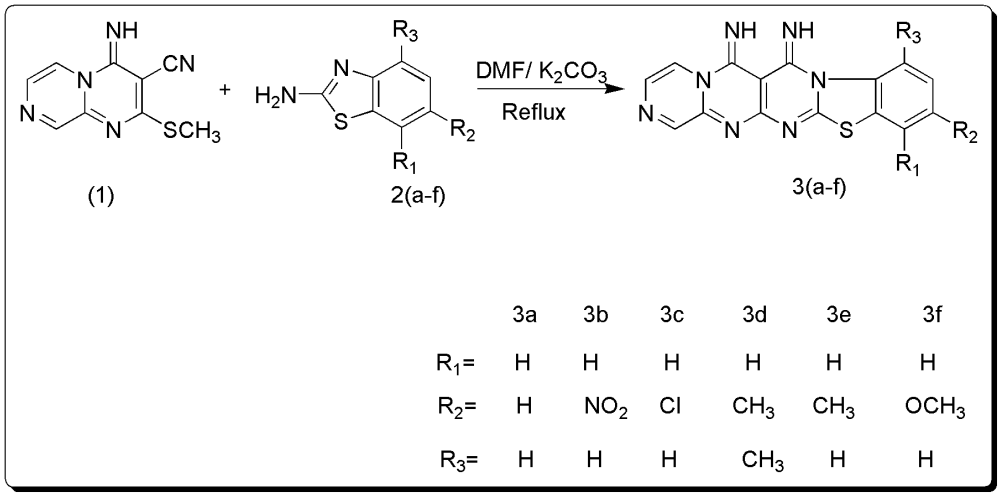
7, 8-Diimino-3-nitro pyrazino[1,2-a]pyrimido[4,5-d]pyrimido[2,1-b]benzothiazole (3f):

M.F. $C_{15}H_8N_8O_2S$, Yield: 75%, MP: 240°C. IR spectrum, ν, cm^{-1}: 3479.34, 3359.77, 3220.90 (=NH stretch). ^{1}H NMR spectrum (δ ppm): 7.436-7.563 (m, 6H, Ar-H), 8.356-8.372 (s, 2H, =NH). Mass spectrum: m/z 364 [M^+]

RESULT AND DISCUSSION:

In present manuscript, we reported the synthesis of 7,8-diimino pyrazino[1,2-*a*]pyrimido[4,5-*d*] pyrimido [2,1-*b*]benzothiazole and its 2/3/5 substituted derivatives **(3a-f)**. The reaction begin with 3-cyano-4-imino-2-(methylthio)-4*H*-pyrimido[1,2-*a*]pyrazine **(1)**. The compound **(1)** was reacted independently with substituted benzothiazoles **(2a-f)** in DMF and anhydrous K_2CO_3 to afford compounds **(3a-f) (Scheme 1)**.

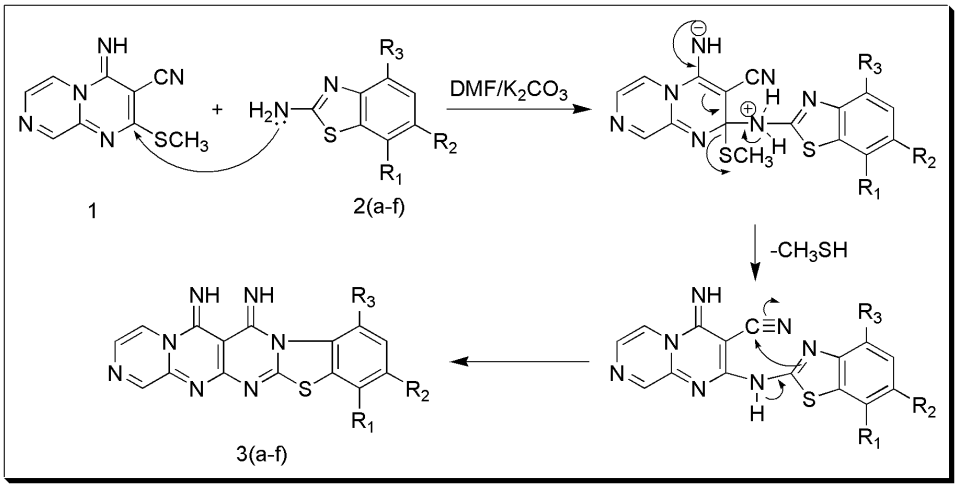
Scheme 1



	3a	3b	3c	3d	3e	3f
R_1=	H	H	H	H	H	H
R_2=	H	NO_2	Cl	CH_3	CH_3	OCH_3
R_3=	H	H	H	CH_3	H	H

The formation of compounds **(3a-f)** begin with nucleophilic attack of amino group of benzothiazoles at thiomethyl flanked carbon of 3-cyano-4-imino-2-(methylthio)-4*H*-pyrimido[1,2-*a*]pyrazine (**1**) resulting in loss of thiomethyl group in the form of thiomethyl alcohol. The obtained secondary amine on intramolecular cyclization with cyano carbon to obtain cyclic product **(3a-f)**. Mechanism for the synthesis of compounds **(3a-f)** can be given as follows **(Scheme 2)**.

Scheme 2



The structures of newly prepared compounds **(3a-f)** were confirmed on the basis of spectral analysis like IR, ^{1}H NMR and Mass spectral technique. The compounds **(3a-f)** showed the absence of CN stretching absorption band in the region **2220-2204** cm^{-1} of IR spectrum which confirm that cyclization took place and exhibit strong absorption bands in the functional group region **3480-3220** cm^{-1} which can be assigned to imino (=NH) stretching. The ^{1}H NMR spectra impart singlet peak at δ **8.960-8.256**, which can be assigned to imino (=NH) proton. Mass spectra exhibit that molecular ion peak which corresponds to its molecular weights of compounds.

ANTIBACTERIAL ACTIVITY:

The antibacterial properties of newly synthesized compounds **3a-3f** were evaluated by the disc diffusion assay using Ampicillin (**10** µg/disc) as the reference compound. 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) 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 diimino pyrazino[1,2-*a*]pyrimido[4,5-*d*] pyrimido[2,1-*b*]benzothiazole and their substituted derivatives.

Sr. No.	Compound	Zone of inhibition in mm	
		Antibacterial activity	
		E. coli	***B. subtilis***
1	3a	12	14
2	3b	17	08
3	3c	14	10
4	3d	18	12
5	3e	11	10

6	3f	10	12
	Ampicillin	20	16

(NA-No activity)

The compounds **3(a-f)** exhibit zone of inhibition against the tested bacteria, some of tested compounds like **3b**, **3d** displayed 17 mm, 18 mm zone of inhibition respectively against *E. coli*, while compounds **3a**, **3d**, **3f** impart 14 mm, 12 mm, 12 mm zone of inhibition respectively against *B. subtilis* as compared with standard *Ampicillin*.

CONCLUSION:

In conclusion, we have reported the synthesis of diverse collections of 7,8-diimino pyrazino[1,2-a]pyrimido[4,5-d]pyrimido[2,1-b]benzothiazole structures through K_2CO_3 catalyzed reactions. This method offers several advantages, including the use of a reusable catalyst, achieving high yields, employing a straightforward procedure, and facilitating the isolation process. Additionally, we conducted an assessment of the antibacterial properties of all the synthesized compounds. Notably, some of these compounds demonstrated significant potential in inhibiting the growth of tested bacteria, specifically *E. coli* and *B. subtilis.*

ACKNOWLEDGEMENT:

The authors duly acknowledge the Principal, Yeshwant Mahavidyalaya, Nanded, for providing laboratory facilities.

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Received on July 1, 2023.