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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF FUSED METHYL DIIMINO PYRIDO PYRIMIDO PYRIMIDO DERIVATIVES

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ABSTRACT

Novel heterocyclic compounds 9-methyl-13,14-di-imino pyrido [1,2-*a*] pyrimido [5,6-*e*] pyrimido[2,3-*b*]benzothiazole and their 1/2/3/4-substuited derivatives **(5a-h)** have been synthesized by condensing 3-cyano-4-imino-2-(methylthio)-7-methyl-4*H*-pyrido [1,2-*a*] pyrimidine **(3)** with 2-amino 1/2/3/4-substituted benzothiazole **(4)** by refluxing with N, N'-dimethyl formamide (DMF) and catalytic amount of anhydrous potassium carbonate.

Key words: Pyrido[1,2-*a*] pyrimidine, N, N'-Dimethyl formamide, Anhydrous potassium carbonate, 2-Amino benzothiazole.

INTRODUCTION

Rising infectious diseases and the growing number of multi-drug resistant microbial pathogens still make the treatment of infectious diseases an important and pressing global problem. Therefore, a substantial research for the discovery for synthesis of new classes of antimicrobial agents is needed.

The synthesis of pyrido [1,2-*a*] pyrimidine and related heterocycles posses a wide application in the field of medicine. Some of them exhibit significant biological and pharmacological activities, such as antifolate activity[1], antibacterial activity[2-5], tyrosine kinase activity[6], antimicrobial activity[7], calcium channel antagonists activity[8,9], anti-inflammatory and analgesic activity[10], antileishmanial activity[11,12], tuber-culostatic activity[13], anticonvulsants activity[14], diuretic and potassium-sparing activity[15], antiaggressive activity[16,17] and antitumor activity[18]. A number of methods have been developed for the

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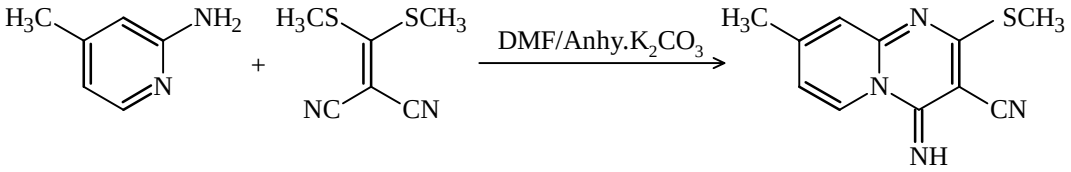
synthesis of pyrido pyrimidine derivatives[19-22], which usually required longer time, complex synthetic pathways and expensive catalyst and often used organic solvent. Hetero fused pyrimidines exhibit promising, anti-AIDS[23] and ntinociceptive[24]. Electron-rich nitrogen heterocycles play an important role in diverse biological activities. Prompted by the varied biological activities of pyrido [1,2-*a*] pyrimidine derivatives, we envisioned our approach towards the synthesis and antimicrobial screening of a novel series of pyrido [1,2-*a*] pyrimidine derivatives.



(1) **(2)** **(3)**

Scheme 1

Table 1

Compd. No.	R_1	R_2	R_3	R_4
5a	H	H	H	H
5b	H	CH_3	H	H
5c	H	OCH_3	H	H
5d	H	Cl	H	H
5e	H	NO_2	H	H
5f	H	CH_3	H	CH_3
5g	Cl	F	H	H
5h	CH_3	CH_3	H	H

Mechanism for the formation of 9-methyl 13,14 di-imino pyrido[1,2-*a*] pyrimido [5,6-*e*] pyrimido [2,3-*b*] benzothiazole

EXPERIMENTAL

Material and methods

All the chemicals used in present study are of analytical grade purchased from Himedia Chemical Co. All the reactions monitored by thin layer chromatography (TLC) on

0.2 mm silica gel-C plates using iodine vapors for detection. Infrared spectra were recorded in nujol or as potassium bromide pellets on infrared spectrophotometer. ^{1}H NMR 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. Melting points of synthesized compounds were determined by a Kofler micro melting point apparatus and were uncorrected. All the reactions were carried out under ambient atmosphere. Elemental analysis was performed on a Heraeus CHN-O rapid analyzer.

General procedure

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

A mixture of 2-amino 5-methyl pyridine **(2)** (0.01 mol) and bis (methylthio) methylene malanonitrile **(1)** (0.01 mol) in 15 mL of N, N'-dimethyl formamide and anhydrous potassium carbonate (10 mg) 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)**.

13,14-Di-imino 9-methyl pyrido [1,2-a] pyrimido [5,6-e] pyrimido [2,3-b] benzothiazole and their 1/2/3/4-substituted derivatives (5a-h)

A mixture of **(3)** (0.001 m mol) and independently with **(4)** 2-amino-6H-benzothiazole, 2-amino 6-methyl benzothiazole, 2-amino 6-methoxy benzothiazole, 2-amino 6-chloro benzothiazole, 2-amino 6-nitro benzothiazole, 2-amino-4,6-dimethyl benzothiazole, 2-amino-7,6-chloro, floro benzothiazole, 2-amino-7,6-dimethyl benzothiazole (0.001 m mol) in 15 mL of N, N'-dimethyl formamide and anhydrous potassium carbonate (10 mg) 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 N, N'-dimethyl formamide- ethanol mixture to give pure **(5a-h)**.

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

Orange powder, yield 63%, mp 179°C (dec.). IR (KBr/ cm^{-1}) 3350 (=NH), 2225 (CN); ^{1}H NMR (400 MHz, DMSO-d_6) d 2.1 (s, 3H, Ar-CH_3), 2.6 (s, 3H, SCH_3), 5.0-6.4 (m, 3H), 9.2 (br s, 1H, = NH). EI-MS (m/z: RA %): 230 (M + I), 100%), 215 (35). Anal. Calcd. M.F. $C_{11}H_{10}N_4S$; C, 57.37; H, 4.38; N, 24.33, Found: C, 57.30; H, 4.38; N, 24.33.

13,14-Diimino 9-methyl pyrido [1,2-a] pyrimido [5,6-e] pyrimido [2,3-b] benzothiazole (5a)

Orange powder, yield 75%, mp 176°C (dec.). IR (KBr/ cm^{-1}) 3342 (=NH), 3248 (=NH) ^{1}H NMR (400 MHz, DMSO- d_6), d 2.3 (s, 3H Ar-CH_3), 7.4-8.7 (m, 7H), 9.2 (br s, 2H,

= NH). EI-MS (m/z: RA %): 333 (M + I) , Anal. Calcd. M.F. $C_{17}H_{12}N_6S$; C, 61.43; H, 3.64; N, 25.28; Found: C, 61.10; H, 3.15; N, 25.02;

13,14-Diimino 3,9-dimethyl pyrido [1,2-a] pyrimido [5,6-e] pyrimido [2,3-b] benzothiazole (5b)

Orange powder, yield 81%, mp 162°C (dec.). IR (KBr/cm^{-1}) 3342 (=NH), 3248 (=NH), ^{1}H NMR (400 MHz, DMSO- d_6) d 2.1 (s, 6H Ar-CH_3), 7.2-8.4 (m, 6H), 9.4 (br s, 2H, = NH) EI-MS (m/z: RA %): 346 (M^+). Anal. Calcd. M.F. $C_{18}H_{14}N_6S$; C, 62.41; H, 4.07; N, 24.26; Found: C, 62.02; H, 3.84; N, 24.01.

13,14-Diimino 9-methyl 3-methoxy pyrido [1,2-a] pyrimido [5,6-e] pyrimido [2,3-b] benzothiazole (5c)

Orange powder, yield 88 %, mp 168°C (dec.). IR (KBr/cm^{-1}) 3349 (=NH), ^{1}H NMR (400 MHz,DMSO- d_6) d 2.3 (s,3H Ar-CH_3), 3.6 (s, 3H Ar-OCH_3), 7.2-8.4 (m, 6H), 9.1 (br s, 2H, =NH) EI-MS (m/z: RA %): 363 (M + I). Anal. Calcd. M.F. $C_{18}H_{14}N_6OS$; C, 59.65; H, 3.89; N, 23.19; Found: C, 59.24; H, 3.52; N, 23.01.

13,14-Diimino 9-methyl 3-chloro pyrido [1,2-a] pyrimido [5,6-e] pyrimido [2,3-b] benzothiazole (5d)

Orange powder, yield 84 %, mp 170 °C (dec.). IR (KBr/cm^{-1}) 3350 (=NH), ^{1}H NMR (400 MHz, DMSO- d_6) d 2.1 (s, 3H Ar-CH_3), 7.5-8.8 (m, 6H), 9.5 (br s, 2H, =NH). EI-MS (m/z: RA %): 366 (M^+). Anal. Calcd. M.F. $C_{17}H_{11}ClN_6S$; C, 55.66; H, 3.02; N, 22.91; Found: C, 55.02; H, 2.86; N, 22.59.

13,14-Diimino 9-methyl 3-Nitro pyrido [1,2-a] pyrimido [5,6-e] pyrimido [2,3-b] benzothiazole (5e)

Orange powder, yield 80 %, mp 162 °C (dec.). IR (KBr / cm^{-1}) 3362 (=NH), ^{1}H NMR (400 MHz,DMSO- d_6) d 1.9 (s,3H Ar-CH_3), 7.2-8.5 (m,6H), 9.1 (br s, 2H, =NH).EI-MS (m/z: RA %): 377(M^+). Anal. Calcd. M.F. $C_{17}H_{11}N_7O_2S$; C, 54.11; H, 2.94; N, 25.98; Found: C, 54.01; H, 2.57; N, 25.62.

13,14-Diimino 1,3,9-trimethyl pyrido [1,2-a] pyrimido [5,6-e] pyrimido [2,3-b] benzothiazole (5f)

Orange powder, yield 79%, mp 170°C (dec.). IR (KBr/cm^{-1}) 3368 (=NH), ^{1}H NMR (400 MHz,DMSO- d_6) d 2.3 (s,9H Ar-CH_3), 7.1-8.4 (m,5H), 9.4 (br s, 2H, =NH). EI-MS (m/z: RA %): 361 (M + I). Anal. Calcd. M.F. $C_{19}H_{16}N_6S$; C, 63.31; H, 4.47; N, 23.32; Found: C, 63.01; H, 4.04; N, 23.16.

13,14-Diimino 9-methyl 4-chloro, 3-fluro pyrido [1,2-a] pyrimido [5,6-e] pyrimido [2,3-b] benzothiazole (5g)

Orange powder, yield 82 %, mp 172°C (dec.). IR (KBr/cm^{-1}) 3354 (=NH), ^{1}H NMR (400 MHz, DMSO- d_6) d 2.3 (s, 3H Ar-CH_3), 7.6-8.8 (m,5H), 9.4 (br s, 2H, =NH). EI-MS (m/z: RA %): 384 (M^+). Anal. Calcd. M.F. $C_{17}H_{10}ClFN_6S$; C, 53.06; H, 2.62; N, 21.84; Found: C, 52.74; H, 2.42; N, 21.48.

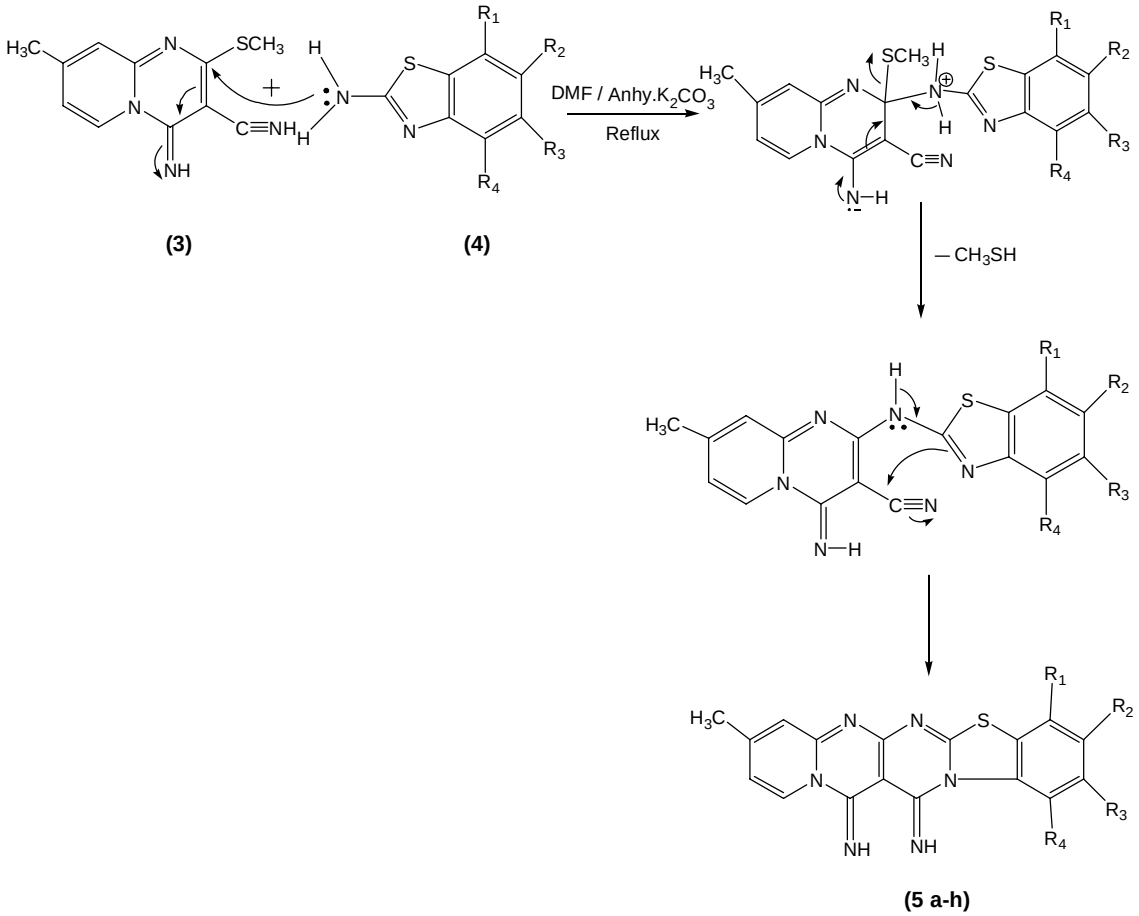
13,14-Diimino 3,4,9-trimethyl pyrido [1,2-a] pyrimido [5,6-e] pyrimido [2,3-b] benzothiazole (5h)

Orange powder, yield 74 %, mp 169°C (dec.). IR (KBr/cm^{-1}) 3363 (=NH), ^{1}H NMR (400 MHz,DMSO- d_6) d 2.1 (s, 9H Ar-CH_3), 7.3-8.6 (m, 5H), 9.1 (br s, 2H, =NH). EI-MS (m/z: RA %): 361 (M + I). Anal. Calcd. M.F. $C_{19}H_{16}N_6S$; C, 63.31; H, 4.47; N, 23.32; Found: C, 63.02; H, 4.07; N, 23.04.

RESULTS AND DISCUSSION

Synthesis of 9-methyl-13,14-diimino pyrido[1,2-*a*]pyrimido [5,6-*e*]pyrimido[2,3-*b*] benzothiazole and their 1/2/3/4-substuited derivatives **(5)** was carried out according to the reported procedure[25]. Our method gives single product with high yield. 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. When equimolar quantities of compound **(3)** was reacted with 2-amino 1/2/3/4 substituted benzothiazole **(4)** in presence of N,N-dimethyl formamide (DMF) and catalytic amount of anhydrous potassium carbonate, it afforded the compound (**5a-h)**, Subsequently, compound **(3)** on independent heating with 2-amino benzothiazole, 2-amino 6-methyl benzothiazole or 2-amino 6-methoxy benzoth-iazole, 2-amino 6-chlorobenzo-thiazole, 2-amino 6-nitro benzothiazole, amino 4,6-dimethyl benzothiazole, 2-amino 7-chloro, 6-floro benzothiazole, 2-amino 6,7-dimethyl benzothiazole to obtain 13,14-diimino pyrido [1,2-*a*] pyrimido [5,6-*e*] pyrimido [2,3-*b*] benzothiazole and their 1/2/3/4-substuited derivatives respectively (**Scheme 2**).

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



Scheme 2

Antibacterial activity

All the newly synthesized compounds were evaluated for their antibacterial activity against species *E. coli, S. typhi, S. aureus* and *B. substilis*, by disc diffusion method[26,27]. These compounds were dissolved in dimethyl sulphoxide. The synthesized compounds exhibited zone of inhibition of 05-12 mm in diameter whereas standard penicillin exhibited zone of inhibition of 15 and 16 mm in diameter against *E. coli* and *S. typhi and* Streptomycin exhibited zone of inhibition of 18 and 22 mm in diameter against *S. aureus* and *B. substilis*, respectively. Amongst the synthesized compounds **(5d)**, **(5e)** and **(5g)** compound possess very good antibacterial activity, due to presence of Cl, NO_2 and F groups on 3 and 4 positions.

Diameter in zone of inhibition (mm)				
Comp.	***E. coli***	***S. typhi***	***S. aureus***	***B. substilis***
5a	09	06	08	12
5b	07	10	11	13
5c	10	09	05	10
5d	**12**	**10**	**13**	**16**
5e	**11**	**12**	**12**	**14**
5f	05	08	09	12
5g	**12**	**11**	**13**	**15**
5h	08	10	09	11
Penicillin	**15**	**16**		
Streptomycin			**18**	**22**

CONCLUSION

In summary, the synthesized pyrido [1,2-*a*] pyrimidine derivatives exhibit promising antibacterial activity. Hence, it has enough scope for further study in developing these as good lead compounds. Moreover, this preliminary study is encouraging to further investigate their broad spectrum pharmacological activities.

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