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Research Article

SYNTHESIS AND ANTIMICROBIAL EVALUATION OF PYRIMIDO PYRIMIDINE DERIVATIVES

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ABSTRACT

Novel heterocyclic pyrimido pyrimidine**3** was prepared by condensing guanidine hydrochloride **1** with ethyl 2-cyano-3,3-bis(methylthio)acrylate**2** in DMF and catalytic amount of anhydrous potassium carbonate. The pyrimido pyrimidine**3** was further condensed with various substituted 2-amino benzothiazoles to gives imino-oxo pyrimido bis benzothiazoles. All newly synthesized substituted imino-oxo pyrimido bis benzothiazoles shows good antibacterial and antifungal activity.

Keywords: 2-Amino benzothiazole; 2-cyano-3,3-bis(methylthio)acrylate; guanidine hydrochloride.

INTRODUCTION

Pyrimidine is a heterocyclic aromatic organic compound containing two nitrogen atoms. It is the biologically important nitrogen-containing molecule called nitrogenous base. Basically pyrimidines are used in our body for the construction of genetic material i.e. deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). In addition pyrimidines also possess antibacterial[1–3], antifungal[4-5], antileishmanial[6], anti-inflammatory[7], analgesic[8], antihypertensive[9], antipyretic[10], antiviral[11], antidiabetic[12], antiallergic[13], antioxidant[14] activities.

In the same waypyrimido pyrimidine also have good biological importance. It also acts as good pharmacophore.Recently some fused heterocyclic compounds containing nitrogen atom showa wide range of pharmacological activities.Pyrimido pyrimidines are annelated to uracils that have considerable interest in recent years[15-16]. Derivatives of pyrimido pyrimidine display potent inhibitory properties regarding tyrosine kinase domain of epidermal growth factor receptor[17]. Pyrimido[4,5-d]pyrimidine fused system represent attractive pharmacological applications such as antitumor[18], antiviral[19], antioxidant[20], antifungal[21] and hepatoprotective activities[22].

Pyrimido pyrimidines have a ring system that can be found marine derived natural products such as crambescidin[23]alkaloid. Various compounds have been found which inhibit platelet aggregation and reduce adhesiveness one of them from which is trimorpholino pyrimido pyrimidine is a synthetic analogue of dipyridamole, this analogue shows powerful inhibitor of platelet aggregation and adhesiveness[24]. The Persantin pyrimido pyrimidine reported to produce coronary vasodilation without an associated increase in cardiac work[25]. Recently our research group reported synthesis of pyrimido thiazine[26-28].

In the literature numerous report have been appeared for synthesis of pyrimido pyrimidine which have force condition, complex synthetic pathway, longer reaction time. Thus new routes for synthesis of these heterocyclic molecule have attracted considerable interest.

The development of pyrimido pyrimidine as a potential host, through a nitrogen atom of pyrimidine ring fused with another biological active nucleus such as 2-amino benzothiazoles.

Keeping this importance in mind herein we report synthesis of heterocycles containing imino, oxo pyrimido pyrimidine which is then fused with various 1/2/3/4-substituted-2-amino benzothiazoles.

MATERIAL AND METHOD

Electrothermal IA 9000 SERIES digital melting point apparatus was used to determine the melting points of synthesized compounds and were uncorrected. All the reactions were monitored by thin layer chromatography, were

carried out on 0.2 mm silica gel-C plates using iodine vapours 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 reactions were carried out under ambient atmosphere. Elemental analysis was performed on a Heraeus CHN-O rapid analyser.

General procedure

4,8-bis(methylsulfanyl)-2,6-dioxo-1,6-dihydro-2*H*-pyrimido[1,2-*a*]pyrimidine-3,7-dicarbonitrile (3)

A mixture of guanidine hydrochloride **1**(0.01mol) and ethyl 2-cyano-3,3-bis (methylthio)acrylate **2** (0.02mol) in 20 ml of DMF and anhydrous potassium carbonate (10mg) was refluxed for 8 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 recrystalized from DMF-ethanol mixture to give pure compound **3**.

Brown powder,Yield 78%,m.p. 220 °C. IR (KBr/cm^{-1}) 3343 (-NH), 2215 (CN), 1661 (CONH-): ^{1}HNMR (400 MHz,DMSO-d_6): δ = 2.6 (s, 6H, SCH_3), 7.8 (s, 1H, NH): EI-MS(m/z: RA%): 305 (M^+). Anal. Calcd for $C_{11}H_7N_5O_2S_2$ C,43.27; H,2.29; N,22.95; found; C,43.22; H,2.23; N,22.89.

Imino-oxo pyrimido pyrimidine bis benzothiazoles (4a-g)

A mixture of **3** (0.001mol) and 2-amino benzothiazole (0.002mol) in 10 ml of DMF and anhydrous potassium carbonate(10mg) was refluxed for 6 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 recrystalized from DMF-ethanol (2:8) mixture to give pure compound **4a.** Similarly compounds**4b-4g** were prepared.

9,20-diimino-8,19-dioxo-18H-benzothiazolyl[2,3-*b*]pyrimido[5,6-*d*] pyrimido[2,3-*b*]pyrimido[5,6-*e*]pyrimido[2,3-*b*]benzothiazole (4a)

Brown powder, Yield 73%, m.p. 246°C. IR (KBr/cm^{-1}) 3367 (=NH), 1658 (CONH-): ^{1}HNMR (400 MHz,DMSO-d_6): 9.0 (s, 2H, =NH), 6.3-6.7 (m, 8H, Ar-H): EI-MS (m/z: RA%): 509 (M^+). Anal. Calcd for $C_{23}H_{11}N_9O_2S_2$ C,54.22; H,2.16; N,24.75; found; C,54.17; H,2.12; N,24.69.

9,20-diimino3,13-dimethyl-8,19-dioxo-18H-benzothiazolyl[2,3-*b*]pyrimido[5,6-*d*] pyrimido[2,3-*b*]pyrimido[5,6-*e*]pyrimido[2,3-*b*]benzothiazole (4b)

Brown powder, Yield 82%, m.p. 275°C. IR (KBr/cm^{-1}) 3354 (=NH), 1665 (CONH-): ^{1}HNMR (400 MHz,DMSO-d_6): δ = 2.3 (s, 6H, Ar-CH_3), 9.2 (s, 2H, =NH), 6.2-6.6 (m, 6H, Ar-H): EI-MS (m/z: RA%): 537 (M^+). Anal. Calcd for $C_{25}H_{15}N_9O_2S_2$ C,55.86; H,2.79; N,23.46; found; C,55.81; H,2.72; N,23.42.

9,20-diimino-3,13-dimethoxy-8,19-dioxo-18H-benzothiazolyl[2,3-*b*]pyrimido[5,6-*d*] pyrimido[2,3-*b*]pyrimido[5,6-*e*]pyrimido[2,3-*b*]benzothiazole (4c)

Brown powder, Yield 71%, m.p. 284°C. IR (KBr/cm^{-1}) 3342 (=NH), 1669 (CONH-): ^{1}HNMR (400 MHz,DMSO-d_6): δ = 3.2 (s, 6H, -OCH_3), 8.9 (s, 2H, =NH), 6.1-6.5 (m, 6H, Ar-H): EI-MS (m/z: RA%): 569 (M^+). Anal. Calcd for $C_{25}H_{15}N_9O_4S_2$ C,52.72; H,2.63; N,22.14; found; C,52.68; H,2.56; N,22.09.

3,13-dibromo-9,20-diimino-8,19-dioxo-18H-benzothiazolyl[2,3-*b*]pyrimido[5,6-*d*] pyrimido[2,3-*b*]pyrimido[5,6-*e*]pyrimido[2,3-*b*]benzothiazole (4d)

yellow powder, Yield 69%, m.p. 262°C. IR (KBr/cm^{-1}) 3347 (=NH), 1672 (CONH-): ^{1}HNMR (400 MHz,DMSO-d_6): δ = 9.4 (s, 2H, =NH), 6.6-7.0 (m, 6H, Ar-H): EI-MS (m/z: RA%): 667 (M^+). Anal. Calcd for $C_{23}H_9N_9O_2S_2Br_2$ C,41.37; H,1.34; N,18.89; found; C,.41.30; H,1.29; N,18.84.

9,20-diimino-1,3,11,13-tetramethyl-8,19-dioxo-18H-benzothiazolyl[2,3-*b*] pyrimido[5,6-*d*]pyrimido[2,3-*b*]pyrimido[5,6-*e*]pyrimido[2,3-*b*]benzothiazole(4e)

Brown powder, Yield 76%, m.p. 298°C. IR (KBr/cm^{-1}) 3350 (=NH), 1678 (CONH-): ^{1}HNMR (400 MHz,DMSO-d_6): δ = 2.2 (s, 12H, -CH_3), 9.0 (s, 2H, =NH), 6.2-6.6 (m, 4H, Ar-H): EI-MS (m/z: RA%): 565 (M^+). Anal. Calcd for $C_{27}H_{19}N_9O_2S_2$ C,57.34; H,3.36; N,22.30; found; C,57.28; H,3.31; N,22.26.

2,3,12,13-tetrachloro-9,20-diimino-8,19-dioxo-18H-benzothiazolyl[2,3-*b*] pyrimido[5,6-*d*]pyrimido[2,3-*b*]pyrimido[5,6-*e*]pyrimido[2,3-*b*]benzothiazole(4f)

Brown powder, Yield 68%, m.p. 266°C. IR (KBr/cm^{-1}) 3320 (=NH), 1660 (CONH-): ^{1}HNMR (400 MHz,DMSO-d_6): δ = 9.2 (s, 2H, =NH), 6.7-7.1 (m, 4H, Ar-H): EI-MS (m/z: RA%): 647 (M^+). Anal. Calcd for $C_{23}H_7N_9O_2S_2Cl_4$

C,41.37; H,1.08; N,19.47; found; C,41.33; H,1.03; N,19.42.

9,20-diimino-2,12-dinitro-8,19-dioxo-18H-benzothiazolyl[2,3-*b*]pyrimido[5,6-*d*] pyrimido[2,3-*b*]pyrimido[5,6-*e*]pyrimido[2,3-*b*]benzothiazole(4g)

Black powder, Yield 83%, m.p. 258°C. IR (KBr/cm^{-1}) 3362 (=NH), 1672 (CONH-): ^{1}HNMR (400 MHz,DMSO-d_6): δ = 9.1 (s, 2H, =NH), 6.9-7.2 (m, 6H, Ar-H): EI-MS (m/z: RA%): 599 (M^+). Anal. Calcd for $C_{23}H_9N_{11}O_6S_2$ C,46.07; H,1.50; N,25.70; found; C,46.03; H,1.45; N,25.64.

RESULT AND DISCUSSION

Synthesis of imino-oxo- pyrimido pyrimidine bis benzothiazoles and their 1/2/3/4 substituted derivatives (4a-g) was carried out by efficient method. Our method gives single product with high yield. Reaction started with guanidine hydrochloride **1** and ethyl 2-cyano-3,3-bis(methyl thio)acrylate **2** were refluxed in DMF in the presence of catalytic amount of anhydrous potassium carbonate to afford **3**.**Scheme-1**.

The compound posseses replaceable active methyl thio group which is activated by nitrogen atom, electron withdrawing cyano group. When compound **3** (1mole) was condensed independently with 2-amino benzothiazole, 2-amino-6-methyl benzothiazole, 2-amino-6-methoxy benzothiazole, 2-amino-6-bromo benzothiazole,2-amino-4,6-dimethyl benzothiazole, 2-amino-5,6-dichloro benzothiazole, 2-amino-5-nitro benzothiazole (2 mole) in the presence DMF and catalytic amount of anhydrous potassium carbonate to obtain imino-oxo- pyrimido pyrimidine bis benzothiazoles (4a-g).

The structure of these compounds were elucided on the basis of elemental analysis,IR,^{1}HNMR,Mass spectral data. Spectral studies of these compound shows that compounds are stable and do not exhibit any tautomerism.

Antimicrobial activity

All newly synthesized compounds were screened for their antibacterial and antifungal activities against *Escherichia coli, Salmonella typhi,Staphylococcus aureus,Bacillus subtilis* and *candida albicus* by paper disk diffusion method. These compounds were dissolved in dimethyl sulphoxide (1mg/ml in DMSO). The incubation period for bacteria was 24 hours at 37°c and for fungi was 4 days at 25±2°c. Activity of compounds was determined by measuring the diameter of zone of inhibition. The newly synthesized compounds show zone of inhibition 5-20 mm in diameter where as standard streptomycin exhibit zone of inhibition 18-22 mm in diameter against *Escherichia coli, Salmonella typhi,Staphylococcus aureus,Bacillus subtilis*. Standard amphotericin B show zone of inhibition 18 mm in diameter. Among all the newly synthesized compounds,**4d** and **4f** showed higher zone of inhibition against*Escherichia coli, Salmonella typhi,Staphylococcus aureus,Bacillus subtilis* and *candida albicus* due to presence of Br and Cl groups.

CONCLUSION

In conclusion, our results demonstrate a simple and efficient method for the synthesis of pyrimido pyrimidine derivatives which shows promising antibacterial and antifungal activities. Hence it has enough scope for further study in developing these as potent compounds. The elemental and spectroscopy analysis were good agreement with the proposed structures.

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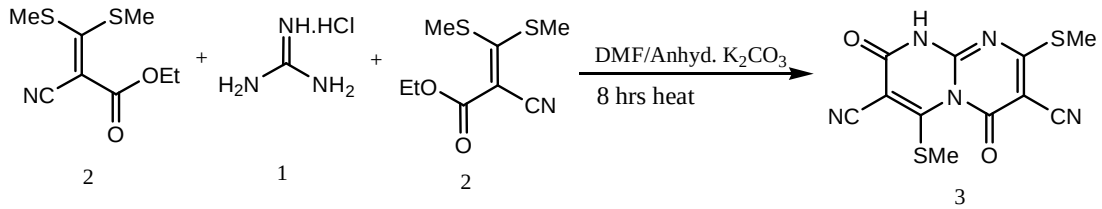
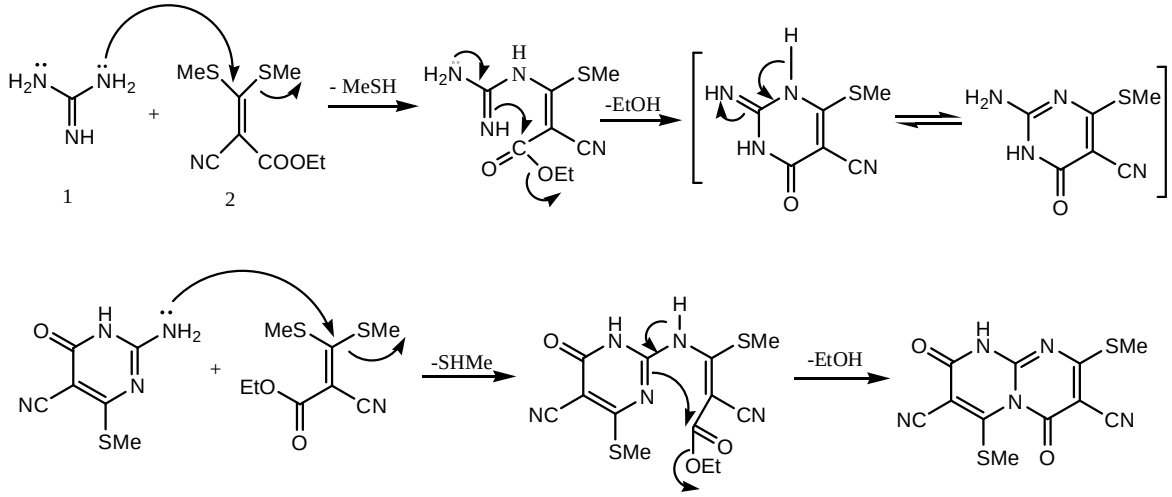
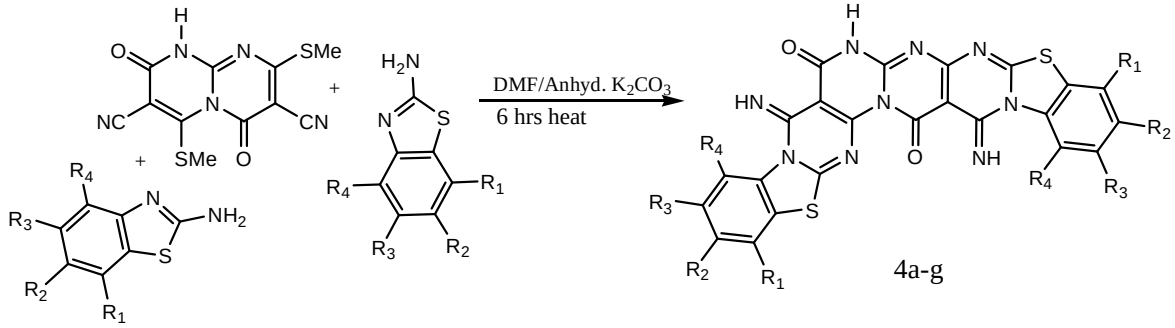


Scheme - 1



Plausible mechanism of formation of compound 3



Scheme-2

Table 1: compound Number and substitution position

Compd. No.	R_1	R_2	R_3	R_4
4a	-H	-H	-H	-H
4b	-H	$-CH_3$	-H	-H
4c	-H	$-OCH_3$	-H	-H
4d	-H	-Br	-H	-H
4e	-H	$-CH_3$	-H	$-CH_3$
4f	-H	-Cl	-Cl	-H
4g	-H	-H	$-NO_2$	-H

Table 2: Antimicrobial activity of compound (4a-g)

Compounds	Bacteria				Fungi
	E.Coli	*S.typhi*	*S.aureus*	*B.subtilis*	*C. Albicus*
4a	++	+	++	++	++
4b	++	++	++	++	++
4c	+	++	++	++	++
4d	**+++**	**++**	**++**	**+++**	**+++**
4e	_	+	++	+	++
4f	**+++**	**+++**	**++**	**+++**	**+++**
4g	+	++	++	++	++
Positive control	+++	+++	+++	+++	+++
	Streptomycin				**Amphoterecin B**

Note: Highly active = +++ = 13-18, Moderate active = ++ = 7-12, Less active = + = 1-6.

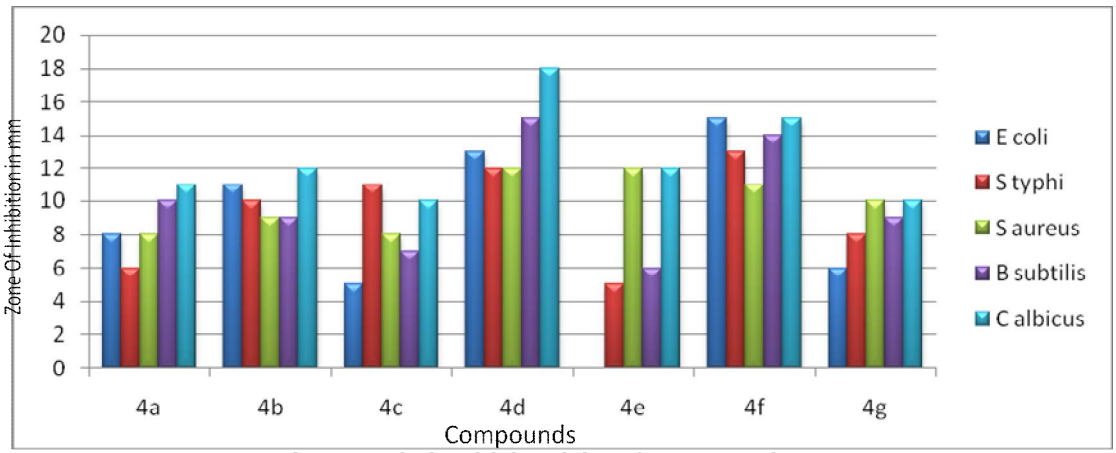


Fig. 1: Antimicrobial activity of compounds 4a-g

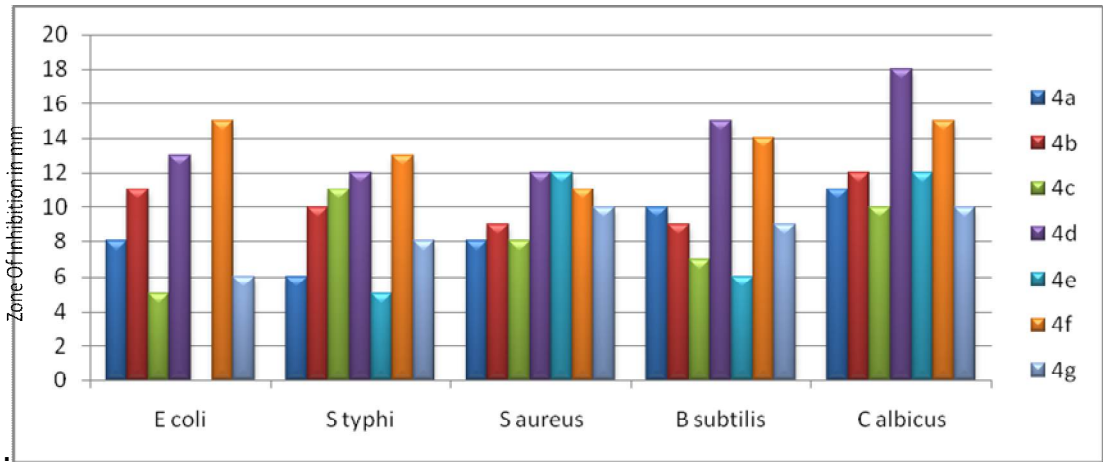


Fig. 2: Comparative Antimicrobial activity of compounds 4a-g

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