Asian Journal of Chemistry Vol. 19, No. 1 (2007), 443–448

Synthesis and Antimicrobial Activities of 1-(Substituted pyrimidino)-substituted thiocarbamides

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1-(4-Hydroxy-6-methyl-pyrimidino)-3-substituted thiocarbamides (**7a–f**) and 1-(4-hydroxy-5-benzyl-6-methyl pyrimidino)-3-substituted thiocarbamides (**8a–f**) have been successfully synthesized by condensing 2-amino-4-hydroxy-6-methyl pyrimidine (**4**) and 2-amino-4-hydroxy-5-benzyl-6-methylpyrimidine (**5**) with various isothiocyanates (**6a–f**) in acetone medium. Compounds (**4**) and (**5**) were obtained by reinvestigating the interaction of guanidine (**1**) with acetoacetic ester (**2**) and ethyl-α-benzyl-β-keto butyrates (**3**) respectively. Antimicrobial activities of these newly synthesized compounds were carried out by MIC method.

Key Words: Guanidine, Acetoacetic ester, Ethyl-α-benzyl-β-keto butyrates, Alkyl/aryl isothiocyanates, Antimicrobial activities.

INTRODUCTION

Guanidine and its derivatives are important organic compounds due to their pharmacological, agricultural and biological activities[1–4]. These compounds have been found as intermediates in the synthesis of various heterocycles[5–10]. Literature survey reveals that thiocarbamidino compounds possess medicinal[11, 12] and agricultural[13–15] applications. Some are used in the treatment of liver disease and also associated with a broad spectrum of biological activities including antituberculosis, anticonvulsant, antiinflammatory, analgesic and antitumour[16–19]. Thus it was interesting to synthesize compounds 1-(4-hydroxy-6-methyl-pyrimidino)-3-substituted thiocarbamides (**7a–f**) and 1-(4-hydroxy-5-benzyl-6-methyl pyrimidino)-3-substituted thiocarbamides (**8a–f**) and to study the antimicrobial activities of these newly synthesized pyrimidinothiocarbamides against *E. coli, S. typhi, S. aureus, P. aeruginosa, B. subtilis* and *C. albicans* pathogens.

EXPERIMENTAL

All the chemicals used were of AnalaR grade. Substituted pyrimidines, *viz.*, **4a–f** and **5a–f**, were synthesized by described method.[20] Substituted isothiocyanates were prepared according to literature method[21]. Melting points of all

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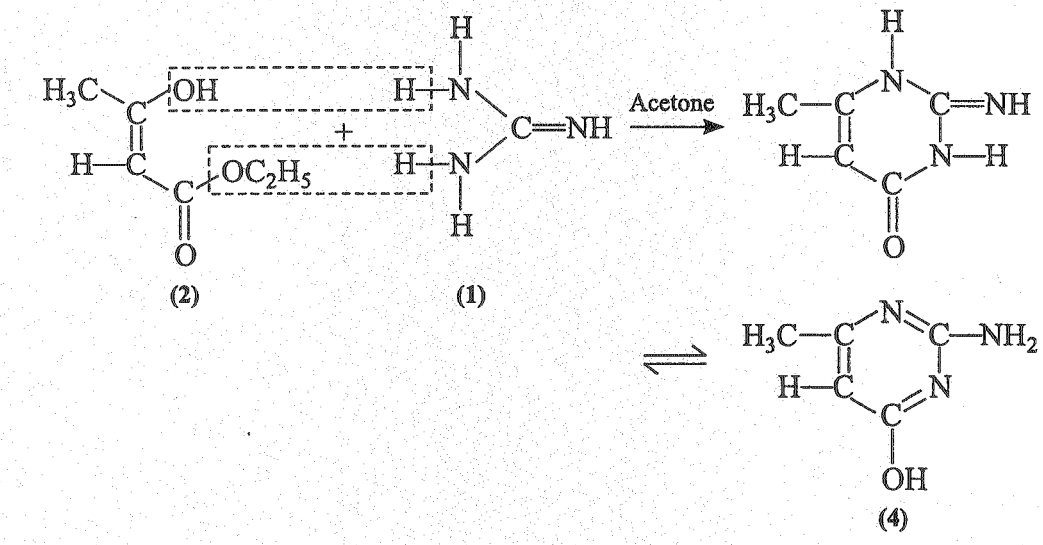
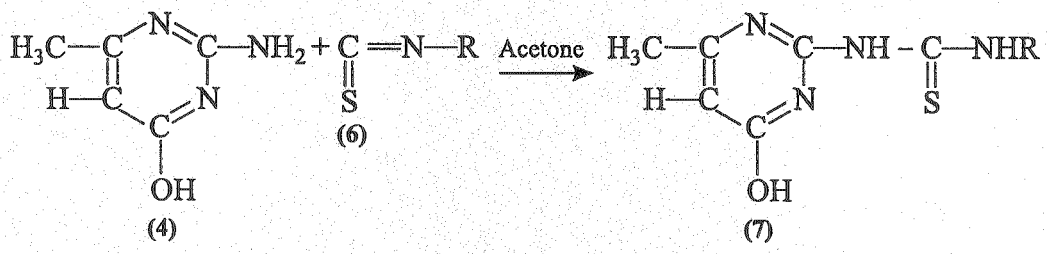
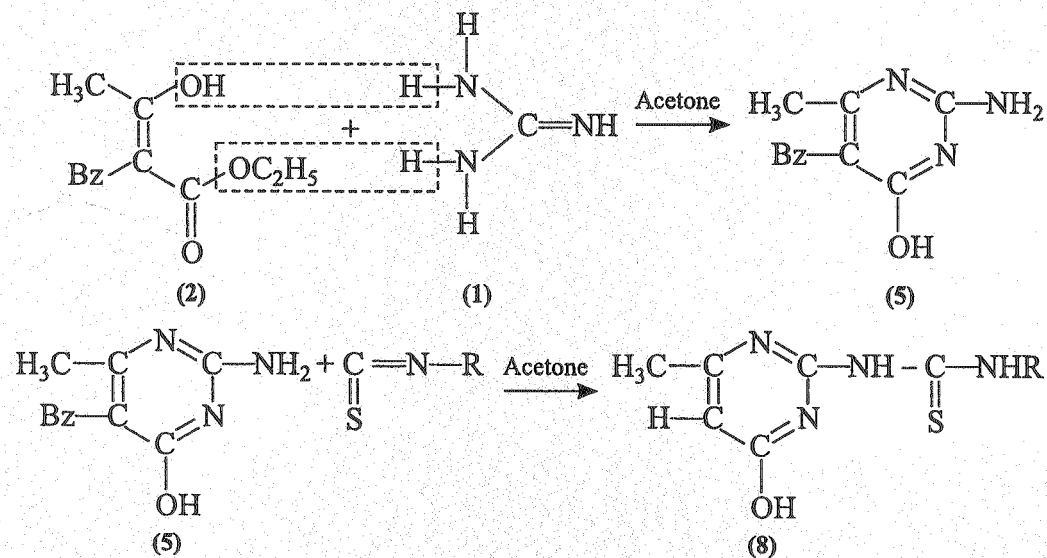
synthesised compounds were determined in open capillary and are uncorrected. IR spectra were recorded on Perkin-Elmer spectrophotometer in the range 4000–400 cm^{-1} in nujol mull as KBr pellets. PMR spectra were recorded with TMS as internal standard using $CDCl_3$ and DMSO-d_6. The purity of the compounds was checked on silica gel-G plate by TLC.





Scheme-I



where R = phenyl, *p*-chlorophenyl, *p*-tolyl, methyl, ethyl, *t*-butyl and Bz = benzyl.

Scheme-II

1-(4-Hydroxy-6-methyl pyrimidino)-3-phenylthiocarbamide (7a): A mixture of 2-amino-4-hydroxy-6-methylpyrimidine (0.05 M) and phenyliso-thiocyanate **(6a)** (0.05 M) was refluxed in acetone (50 mL) on water bath for 8 h; during boiling all the suspended compound **(4)** went into solvent to form clear solution. Brownish coloured crystals were separated out on cooling, filtered and washed with water and finally with ether, m.p. 168°C, yield 74%. The compound was soluble in alcohol, benzene, acetone, dioxane while insoluble in water. It gave picrate (m.p. 171°C). It did not give dye test showing absence of primary aromatic amino group. When aqueous solution of ferric chloride was prepared with **7a** it gave red colouration indicating presence of phenolic group. It was desulphurized by alkaline plumbite solution. The R_f (dioxane) value was found to be 0.32. IR spectra of compound show ν(N—H) 3356 cm^{-1}, ν(CH (Ar)) 3131 cm^{-1}, ν(C = N) 1688 cm^{-1}, ν(>C=NH) 1575 cm^{-1}, ν(C—N) 1294 cm^{-1}, ν(C = S) 1197 cm^{-1}, ν(C—S) 772 cm^{-1}. The PMR spectrum of the compound shows signals due to Ar—OH at δ 9.3008 ppm, signals at δ 8.8343 ppm due to Ar—NH, signals at δ 7.2367–7.6078 ppm due to Ar—H, signals at δ 2.5922 ppm due to Ar—CH_3. Found (Calcd.) (%): C, 54.83 (55.38), H = 3.83 (4.61), N = 20.48 (21.53), S = 11.83 (12.30).

Similarly, other compounds **(7b–f)** were synthesized by the above mentioned method and enlisted in Table-1.

TABLE-1*
PHYSICAL DATA OF THE SYNTHESIZED COMPOUNDS 1-(4-HYDROXY-6-METHYLPYRIMIDINO)-3-SUBSTITUTED THIOCARBAMIDES

Compd. No.	R	Yield (%)	m.p. (°C)	m.w.	m.f.
7a	Phenyl	74	168	260	$C_{12}H_{12}N_4OS$
7b	*p*-Chlorophenyl	82	182	294	$C_{12}H_{11}N_4OSCl$
7c	*p*-Tolyl	69	179	274	$C_{13}H_{14}N_4OS$
7d	Methyl	50	135	198	$C_7H_{10}N_4OS$
7e	Ethyl	57	150	212	$C_8H_{12}N_4OS$
7f	*t*-Butyl	62	127	238	$C_{10}H_{16}N_4OS$

*All compounds gave satisfactory C, H, N and S analysis.

1-(4-Hydroxy-5-benzyl-6-methylpyrimidino)-3-phenylthiocarbamide (8a): A mixture of 2-amino-4-hydroxy-5-benzyl-6-methylpyrimidine **(5)** (0.05 M) and phenylisothiocynates **(6a)** (0.05 M) was refluxed in acetone (50 mL) on a water bath for 8 h; during boiling all the suspended compound **(5)** went into solvent to form clear solution; yellow crystals were separated out on cooling, filtered and washed several times with water and finally with ether, m.p. 192°C, yield 82%. The compound is soluble in acetone, benzene and alcohol. It forms picrate (m.p. 182°C). It gives red colouration with ferric chloride solution indicating presence of phenolic groups. It did not give dye test, showing absence of primary aromatic amino group. It was desulphurized by alkaline plumbite solution. IR spectra of

compound show ν(N—H) 3273 cm^{-1}, ν(CH (Ar)) 3118 cm^{-1}, ν(>C═NH) 1652 cm^{-1}, ν(C═S) 1060 cm^{-1}, ν(C—S) 726 cm^{-1}. The PMR spectra of the compound show signals due to Ar—OH proton at δ 10.7441 ppm, δ 7.2395–7.6424 ppm due to Ar—H, δ 6.2906 ppm due to N—H, δ 4.5838 ppm due to CH_2, δ 2.2214–3.0692 ppm due to Ar—CH_3. Found (Calcd.) (%): C, 62.81 (64.28), H = 3.78% (4.76), N = 15.78 (16.66), S = 8.48 (9.52).

Similarly, other compounds **(8b–f)** were synthesized by the above mentioned method and enlisted in Table-2.

TABLE-2*
PHYSICAL DATA OF THE SYNTHESIzED COMPOUND 1-(4-HYDROXY-5-BENZYL-6-METHYLPYRIMIDINO)-3-SUBSTITUTED THIOCARBAMIDES

Compd. No.	R	Yield (%)	m.p. (°C)	m.w.	m.f.
8a	Phenyl	82	192	336	$C_{18}H_{16}N_4OS$
8b	*p*-Chlorophenyl	79	204	370	$C_{18}H_{15}N_4OSCl$
8c	*p*-Tolyl	65	237	350	$C_{19}H_{18}N_4OS$
8d	Methyl	78	150	274	$C_{13}H_{14}N_4OS$
8e	Ethyl	56	128	288	$C_{14}H_{16}N_4OS$
8f	*t*-Butyl	51	178	314	$C_{16}H_{20}N_4OS$

*All compounds gave satisfactory C, H, N and S analysis.

Antimicrobial activities: The antimicrobial and antifungal activities of all these compounds were studied by MIC process using cup-plate agar diffusion method.

Antibacterial activities: All compounds were screened for their antibacterial activities by using cup-plate agar diffusion method in DMF, using standard Co-trimazine 25 μg/mL against gram positive and gram negative bacteria such as *E. coli, S. typhi, S. aureus, P. aeruginosa* and *B. subtilis.*

Antifungal activities: All compounds were also screened for their antifungal activity by using cup-plate agar diffusion method in DMF using standard griseofulvin (10 μg/mL) against *A. niger* and *C. albicans.*

Cup-plate method: The medium used throughout the experiment was Hi-Media (India make) having the following composition:

Peptone	5 g/L
NaCl	5 g/L
Yeast extract	1.5 g/L
Agar powder	20 g/L
pH	7.4 ± 0.1

The media for antibacterial and antifungal activities were prepared [N-agar for bacterial and Sabouraud's dextrose agar for fungi] by dissolving 28 g of

ingredients in 1 L of distilled water and sterilized in an autoclave at 121°C at 15 lbs/inch pressure for 154 min. The microbes were inoculated into requisite quantity of the medium at temperature 40–50°C and immediately poured the inoculated medium into sterilized petridishes to give a depth of 3–4 mm of uniform thickness. After solidification wells or holes were prepared by well borer. The dimethylformamide solution of the compounds was added in sufficient amount to fill the wells. Then it was kept at room temperature for 4 h as a pre-incubation and then plates of bacteria were inoculated for 18–24 h at 36–38°C and all plates for fungi were inoculated for 48 h at 20–25°C.

After the period of inoculation, zones of inhibition were recorded around the wells. The results are presented in Table-3.

TABLE-3
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF COMPOUNDS

Compd. No.	Antibacterial activity (mm)				Antifungal activity (mm)		
	S. typhi	E. coli	*S. aureus*	*P. aeruginosa*	B. subtilis	*A. niger*	*C. albicans*
7a	1.2	0.8	1.2	0.8	1.2	0.8	0.8
7b	1.6	1.0	1.8	1.2	1.2	1.1	1.1
7c	1.4	1.2	1.4	1.0	1.5	1.2	1.0
7d	0.8	0.2	0.8	0.5	0.8	0.2	0.5
7e	1.0	0.5	0.6	0.5	1.0	0.5	0.5
7f	0.5	0.6	0.4	0.8	1.2	0.6	0.8
8a	1.4	1.1	1.4	1.5	1.8	1.5	1.3
8b	2.1	1.4	2.1	1.3	1.5	1.8	1.6
8c	1.8	1.6	1.8	1.2	2.2	2.0	2.1
8d	1.0	1.8	1.2	0.8	1.0	0.8	0.8
8e	1.6	0.8	0.8	0.5	1.5	0.2	0.6
8f	1.4	0.8	0.8	1.0	1.2	0.5	1.0

All the seven organisms studied are human pathogens. From the results it is clear that all the compounds (except **7a** and **7f**) showed remarkable and considerable antimicrobial activities.

Compounds **7b, 7c, 8a-d** show remarkable antibacterial activity against *E. coli.* Compounds **7a–c, 8a–d** show antibacterial activity against *S. aureus*. Compounds **7b, 7c, 8a–c** and **8f** show antimicrobial activity against *P. aeruginosa.* All the compounds show remarkable antibacterial activity against *B. subtilis* like *S. typhi.*

Compounds **7a, 7b, 8a–c** show remarkable antifungal activity against *A. niger* while **7b, 7c, 8a–c** and **8f** show antifungal activity against *C. albicans.*

From the above data it is clear that these compounds are highly effective against *S. typhi* and *B. subtilis.* So, much more study is required on these

compounds in biochemical and medicinal direction. It is also concluded that these compounds show greater antibacterial activity than antifungal activity.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. D.M. Ambhore, Principal and Prof. J.B. Devhade, Head, Department of Chemistry, Jijamata Mahavidyalaya, Buldana, India, for providing laboratory facilities. The authors are also thankful to Prof. Penshanwar, Shri Shivaji Mahavidyalaya, Chikhali and Dr. Anil Fokmare, Nichol Pharmaceuticals, Indore for screening antimicrobial activity. Thanks are also due to S.A.I.F., C.I.L., Punjab University, Chandigarh for providing IR, NMR spectral and C, H, N and S elemental analyses.

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(*Recieved*: 2 November 2005; *Accepted*: 14 June 2006) AJC-4959