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Synthesis of some new biologically active bis-(thiadiazolotriazines) and bis-(thiadiazolotriazinyl) alkanes

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Abstract

4-Amino-3-mercapto-1,2,4-triazin-5(4*H*)-ones (**1**) were condensed with dicarboxylic acids **2** to yield bis-(4-oxo-4*H*-1,3,4-thiadiazolo[2,3-*c*]-1,2,4-triazin-7-yl)alkanes (**3b**–**d**,**f**–**h**,**j**–**l**,**n**–**p**) and bis-thiadiazolotriazines (**3a**,**e**,**i**,**m**). All the newly synthesised compounds were characterised by analytical, IR, NMR and mass spectral studies. Some of the newly synthesised compounds were screened for their antibacterial and antifungal properties. Among the tested compounds, compound 7,7′-(1,4-butanediyl)-*bis*-(3-*t*-butyl-4-oxo-4*H*-1,3,4-thia-diazolo[2,3-*c*]-1,2,4-triazine (**3p**) exhibited highest degree of antifungal activity.

Keywords: Bis-(thiadiazolotriazinyl)alkanes; Antibacterial activity; Antifungal activity

1. Introduction

In view of the possible pharmacological activity of new purine analogues, a series of 1,3,4-thiadiazolo[2,3-*c*]-1,2,4-triazines were reported in the literature [1]. A number of 1,3,4-thiadiazoles showed antibacterial properties similar to those of well-known sulphonamide drugs [2]. The thiadiazole nucleus which incorporates a N–C–S linkage exhibits a large number of biological activities [3]. Prompted by these findings, and in continuation of our studies on condensed heterocycles [4–12], three 4-amino-6-arylmethyl-3-mercapto-1,2,4-triazin-5(4*H*)-ones (**1a**–**c**) and 4-amino-6-*t*-butyl-3-mercapto-1,2,4-triazin-5(4*H*)-one (**1d**) were prepared and condensed with four dicarboxylic acids to yield new bis-(thiadiazolotriazinyl)alkanes (**3b**–**d**, **f**–**h**, **j**–**l**, **n**–**p**) and bis-thiadiazolotriazines (**3a**, **e**, **i**, **m**). All the newly synthesised compounds were screened for their antibacterial and antifungal activities. The results of such studies are discussed in this paper.

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2. Chemistry

For the present work, three 4-amino-6-arylmethyl-3-mercapto-1,2,4-triazin-5(4*H*)-ones (**1a**–**c**) were prepared by condensing azalactones with thiocarbohydrazide [13]. 4-Amino-6-*t*-butyl-3-mercapto-1,2,4-triazin-5(4*H*)-one (**1d**) was a gift sample from M/s. Rallis India Ltd Bangalore and was prepared as per literature method [14]. The triazinones **1a**–**d** were condensed with dicarboxylic acids **2** in the presence of phosphorus oxychloride to afford bis-(4-oxo-4*H*-1,3,4-thiadiazolo-[2,3-*c*]-1,2,4-triazin-7-yl)alkanes (**3b**–**d**,**f**–**h**, **j**–**l**, **n**–**p**) and bis-(4-oxo-4*H*-1,3,4-thiadiazolo)triazines (**3a**,**e**,**i**,**m**) in rather good yields (Scheme 1). Formation of the title compounds was confirmed by elemental analysis and spectral studies. The characterisation data of compounds **3a**–**p** are given in Table 1.

All the newly synthesised compounds analysed satisfactorily for their nitrogen content. The IR spectrum of 1,2-ethanediyl-*bis*-thiadizolotriazine (**3c**) showed no absorption bands due to the NH_2 and SH groups, thus confirming the bicyclic ring formation. The C=O

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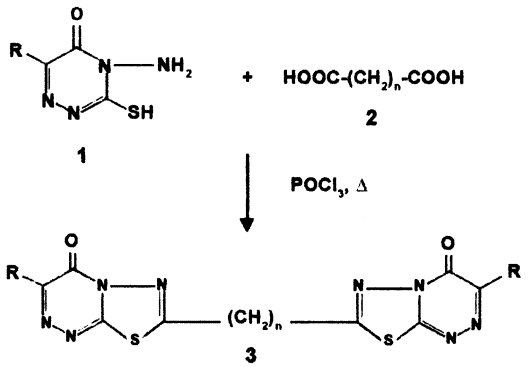


Scheme 1. Condensation of triazinones **1** with dicarboxylic acids **2**. R = benzyl, 4-chlorobenzyl, 3,4-methylenedioxyphenyl, *t*-butyl; $n = 0$, 1, 2, 4.

stretching frequency was seen at 1680 cm^{-1}. The shift in the carbonyl stretching frequency could also be attributed to the formation of the *N*-bridged heterocyclic ring.

The formation of the cyclised products **3** was further supported by recording the ^1H NMR spectra of some selected compounds. The ^1H NMR spectrum of compound **3c** showed a sharp singlet at δ 3.65 integrating for four protons of the alkyl group. The signal for methylene protons of the benzyl moiety was seen as a singlet at δ 4.2 intergrating for four protons. The phenyl protons resonated at δ 7.2 integrating for ten protons.

The ^1H NMR spectrum of compound **3d** showed a broad triplet at δ 1.85 integrating for four protons, which is characteristic of the central CH_2 groups of the alkyl chain while a similar triplet at δ 3.1 integrating for four protons is attributed to the remaining four methylene protons of the alkyl chain. A sharp singlet at δ 4.2 integrating for four protons was observed for the methylene protons of the benzyl moiety. The phenyl protons resonated as a singlet at δ 7.3, integrating for ten protons. The ^1H NMR spectrum of compound **3m** showed a sharp singlet at δ 1.3, integrating for 18 protons of the *t*-butyl group. The disappearance of the signal due to –SH protons at δ 13.8, of the parent triazinones, in the products **3c**, **3d**, and **3h** confirmed the involvement of –SH protons in the condensation reaction.

The mass spectra of the products (**3d**, **e** and **m**) showed molecular ion peaks at m/z 542, 554, 418 which were consistent with their molecular formulae $C_{26}H_{22}N_8O_2S_2$, $C_{22}H_{12}Cl_2N_8O_2S_2$ and $C_{16}H_{18}N_8O_2S_2$, respectively. In all the cases the molecular ion peaks were fairly intense, suggesting the stability of the thiadiazolotriazine ring system. $M+2$ and $M+4$ peaks were seen in the mass spectrum of **3e**, which are indicative of the presence of two chlorine atoms in it. All the three molecular ions underwent fragmentation with the loss of carbon monoxide and produced ions at m/z 514, 526 and 390, respectively. These ions underwent further fragmentation to produce ions at m/z 259, 293 and 225, respectively (Scheme 2). The mass spectrum of **3d** and **3e** showed peaks at m/z 91 and 125, respectively, confirming the presence of corresponding benzyl groups in the condensed products.

3. Experimental

3.1. Chemistry

Melting points were taken in open capillary tubes and are uncorrected. IR spectra in Nujol mull were recorded on a Perkin–Elmer infrared spectrophotometer. ^1H NMR spectra were recorded in DMSO-d_6 on a JEOL GHX400–400 MHz spectrometer using tetramethylsilane as an internal standard. The mass spectra were recorded on a VG Micromass mass spectrometer operating at 70 eV. Purity of the compounds was checked by thin-layer chromatography on silica gel plates using benzene/methanol (9:1) solvent system and iodine as the visualising agent. 4-Amino-6-arylmethyl-3-mercapto-1,2,4-triazin-5(4*H*)-ones (**1a–c**) were prepared according to the method reported by us earlier [13]. 4-Amino-6-*t*-butyl-3-mercapto-1,2,4-triazin-5(4*H*)one (**1d**) was prepared by the method reported by Bonse et al. [14].

3.1.1. Bis-(4-oxo-4H-1,3,4-thiadiazolo[2,3-c]-1,2,4-triazin-7-yl)alkanes and bis-thiadiazolotriazines

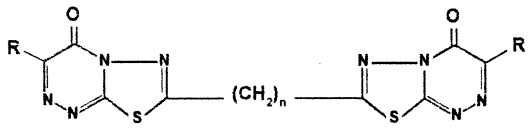
A mixture of triazinone **1a–d** (10 mmol), dicarboxylic acid **2** (5 mmol) and phosphorus oxychloride (10 ml) was refluxed for about 5 h over a water bath. Excess phosphorus oxychloride was removed under reduced pressure. The reaction mixture was cooled and poured onto crushed ice (200 g). The resulting solid was filtered, washed with aqueous (2%) sodium bicarbonate solution and then with water. It was dried and recrystallised from aqueous dimethylformamide. The characterisation data of **3a–p** prepared according to this method are given in Table 1.

3.2. Biological activity

3.2.1. Antibacterial activity

All the newly synthesised compounds were screened for their in vitro antibacterial activity against *Bacillus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* by serial dilution technique [15]. Furacin was used as a standard drug. The results of the screening studies are given in Table 2.

Table 1
Bis-(4-oxo-4H-1,3,4-thiadiazolo[2,3-c]-1,2,4-triazin-7-yl)alkanes **3b–d**, **f–h**, **j–i**, **n–p** and bis-thiadiazolotriazines **3a**, **e**, **i**, **m**



Compound number	R	*n*	% Yield	M.p. (°C)	Molecular formula	% N found (Calcd.)
3a	benzyl	0	70	122–124	$C_{22}H_{14}N_8O_2S_2$	23.09 (23.05)
3b	benzyl	1	70	196–198	$C_{23}H_{16}N_8O_2S_2$	22.48 (22.40)
3c [a]	benzyl	2	72	208–210	$C_{24}H_{18}N_8O_2S_2$	21.84 (21.79)
3d [b]	benzyl	4	70	202–204	$C_{26}H_{22}N_8O_2S_2$	20.59 (20.66)
3e [c]	4-Cl-benzyl	0	71	164–166	$C_{22}H_{12}Cl_2N_8O_2S_2$	20.09 (20.18)
3f	4-Cl-benzyl	1	70	218–220	$C_{23}H_{14}Cl_2N_8O_2S_2$	19.73 (19.68)
3g	4-Cl-benzyl	2	74	204–206	$C_{24}H_{16}Cl_2N_8O_2S_2$	19.26 (19.21)
3h	4-Cl-benzyl	4	68	188–190	$C_{26}H_{20}Cl_2N_8O_2S_2$	18.37 (18.33)
3i	3,4-methylenediozybenzyl	0	68	>300	$C_{24}H_{14}N_8O_6S_2$	19.47 (19.51)
3j	3,4-methylenediozybenzyl	1	74	190–192	$C_{25}H_{16}N_8O_6S_2$	19.11 (19.04)
3k	3,4-methylenediozybenzyl	2	72	104–106	$C_{26}H_{18}N_8O_6S_2$	18.71 (18.66)
3l	3,4-methylenediozybenzyl	4	70	110–112	$C_{28}H_{22}N_8O_6S_2$	17.99 (17.94)
3m [d]	*t*-butyl	0	84	230–232	$C_{16}H_{18}N_8O_2S_2$	26.84 (26.79)
3n	*t*-butyl	1	78	180–182	$C_{17}H_{20}N_8O_2S_2$	25.84 (25.92)
3o	*t*-butyl	2	72	158–160	$C_{18}H_{22}N_8O_2S_2$	25.13 (25.11)
3p	*t*-butyl	4	66	122–124	$C_{20}H_{26}N_8O_2S_2$	23.68 (23.62)

All compounds were analysed satisfactorily for their *N* content.

[a] IR (cm^{-1}): γ(C=O) 1690, γ(C–H def) 1400; γ(C–H, Ar–H def) 740 and 720. ^{1}H NMR (DMSO-d_6): δ 3.65 (s, 4H, alkyl), 4.2 (s, 4H, benzyl protons), 7.2 (s, 10H, aromatic protons).

[b] ^{1}H NMR (DMSO-d_6): δ 1.85 (b.t, 4H, CH_2), 3.1 (b.t, 4H, CH_2), 4.2 (s, 4H, benzyl protons), 7.3 (s, 10H, aromatic protons). MS; *m*/*z* 542 (M^+), 514 $(M-CO)^+$, 259 (A), 96 ($C_6H_5CH_2^+$).

[c] MS; *m*/*z* 554 (M^+), 526 $(M-CO)^+$, 293 (A), 125 (Cl–C_6H_4–CH_2^+).

[d] ^{1}H NMR (DMSO-d_6): δ 1.3 (s, 18H, *t*-butyl group). MS; *m*/*z* 418 (M^+), 390 $(M-CO)^+$, 225 (A).

3.2.2. Antifungal activity

All the title compounds were tested for antifungal activity against *Candida albicans* by serial dilution technique [15] using Sabouraud's agar broth. Fluconazole was used as a standard drug. The results of the screening studies are given in Table 3.

4. Results and discussions

Most of the compounds tested showed higher antibacterial activity than the standard against all the four microorganisms. The test compounds showed better activity against *S. aureus*, *E. coli* and *P. aeruginosa* compared to *Bacillus*. Among the compounds tested for antifungal activity 7,7′-(1,4-butanediyl)-*bis*-(3-*t*-butyl-4-oxo-4*H*-[1,3,4]-thiadiazolo[2,3-*c*]-1,2,4-triazine) (**3p**) showed a high degree of activity. Hence compound **3p** seems to be a promising antifungal agent.

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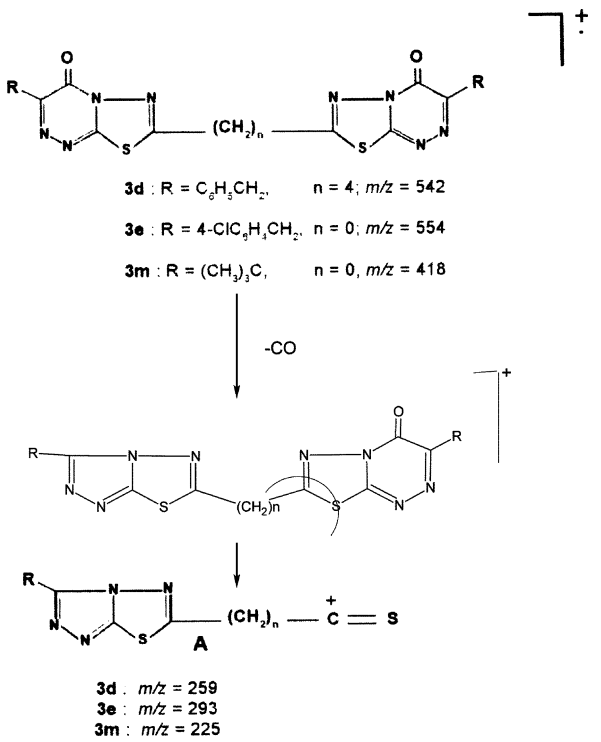

Scheme 2. Mass fragmentation pattern of bis-(thiadiazolotriazinyl)alkanes and bis-thiadiazolotriazines.

Table 2
Antibacterial activities of bis(thiadiazolotriazinyl)alkanes and bis-thiadiazolotriazines (minimum inhibitory concentration (mg ml^{-1})

Comp.	*S. aureus*	*E. coli*	*P. aeruginosa*	*Bacillus*
3a	6	6	3	6
3b	3	3	3	6
3c	3	3	3	6
3d	6	3	6	6
3e	3	3	3	6
3f	3	3	3	6
3g	6	6	6	6
3h	3	3	3	6
3I	3	3	6	6
3j	3	6	8	6
3k	3	3	3	6
3l	3	3	3	6
3m	6	3	3	3
3n	6	3	3	3
3o	8	3	3	6
3p	3	3	3	6
Furacin	12.5	6	12.5	12.5

Table 3
Antifungal activities of bis-(thiadiazolotriazinyl)alkanes and bis-thiadiazolotriazines (minimum inhibitory concentration (μg/ml))

Comp.	*C. albicans*
3a	6
3b	6
3c	6
3d	3
3e	3
3f	6
3g	12.5
3h	3
3i	6
3j	3
3k	3
3l	3
3m	3
3n	3
3o	6
3p	0.75
Fluconazole	6

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