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Studies on bioactive bis-1,3,5-triazinyl dithiocarbamates

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Abstract: The compounds bis(4,6-dichloro/bis[(*p*-methoxyphenyl)amino]-1,3,5--triazin-2-yl)1,2-hydrazine-1,2-dicarbodithioate/1,4-phenylenebis(carbamodithioate)/(1,1'-biphenyl)-4,4'-diylbis(carbamodithioate)/(sulphonyldi-4,1-phenylene)-bis(carbamodithioate/1,2-ethanediylbis(carbamothioate) **4a**–**j** were synthesized by two different methods. In the first method (**A**) for the preparation of **4a**–**e**, 2,4,6-trichloro-1,3,5-triazine **1** was condensed with diammonium 1,2-hydrazine-1,2-dicarbodithioate/1,4-phenylenebis(carbamodithioate)/(1,1'-biphenyl)-4,4'diylbis(carbamodithioate)/(sulphonyl-di-4,1-phenylene)-bis(carbamodithioate)/1,2-ethanediylbis(carbamodithioate) **3a**–**e** to afford **4a**–**e** which undergo reaction with *p*-methoxyaniline to afford **4f**–**j**. In the second method (**B**) of preparation, **1** was condensed with *p*-methoxyaniline to yield **2** followed by the action of **3a**–**e** to yield **4a**–**j**. The structure of the newly synthesized compounds **4a**–**j** was established on the basis of elemental analyses, as well as IR and ^{1}H-NMR spectroscopy. The antimicrobial activities of compounds **4a**–**j** were determined by the cup-plate method against gram-positive bacteria, gram-negative bacteria and fungi. All the synthesized compounds showed significant antimicrobial activity.

*Keywords***:** 1,3,5-triazine, dicarbamodithioates, antimicrobial activity.

INTRODUCTION

Certain dithiocarbamate derivatives have been found to possess a wide range of biological activities, *i.e*., anti-bacterial,[1] tuberculostatic,[2] anti-diuretic,[3] anti-hypertensive,[4] *etc*. *s*-Triazine derivatives also possess biological activities, such as anti-tubercular,[5] antitumor,[6] anti-cancer,[7] sedative,[8] anti-inflammatory[9] and anthelmintic[10] activities. In comparison with a previous publication on 1,3,5-triazinyl dithiocarbamates,[11] in which only one molecule of cyanuric chloride was involved in the reported molecules, a recent literature survey revealed that not a single method for the synthesis of bis(4,6-dichloro-1,3,5-triazin-2-yl) 1,2-hydrazine-1,2--dicarbodithioate **4a**, 1,4-phenylenebis(carbamodithioate) **4b**, (1,1'-biphenyl)-4,4'--diylbis(carbamodithioate) **4c**, (sulfonyldi-4,1-phenylene)bis(carbamodithioate) **4d** and 1,2-ethanediylbis(carbamodithioate) 4e, nor of bis{4,6-bis[(p-methoxyphe-

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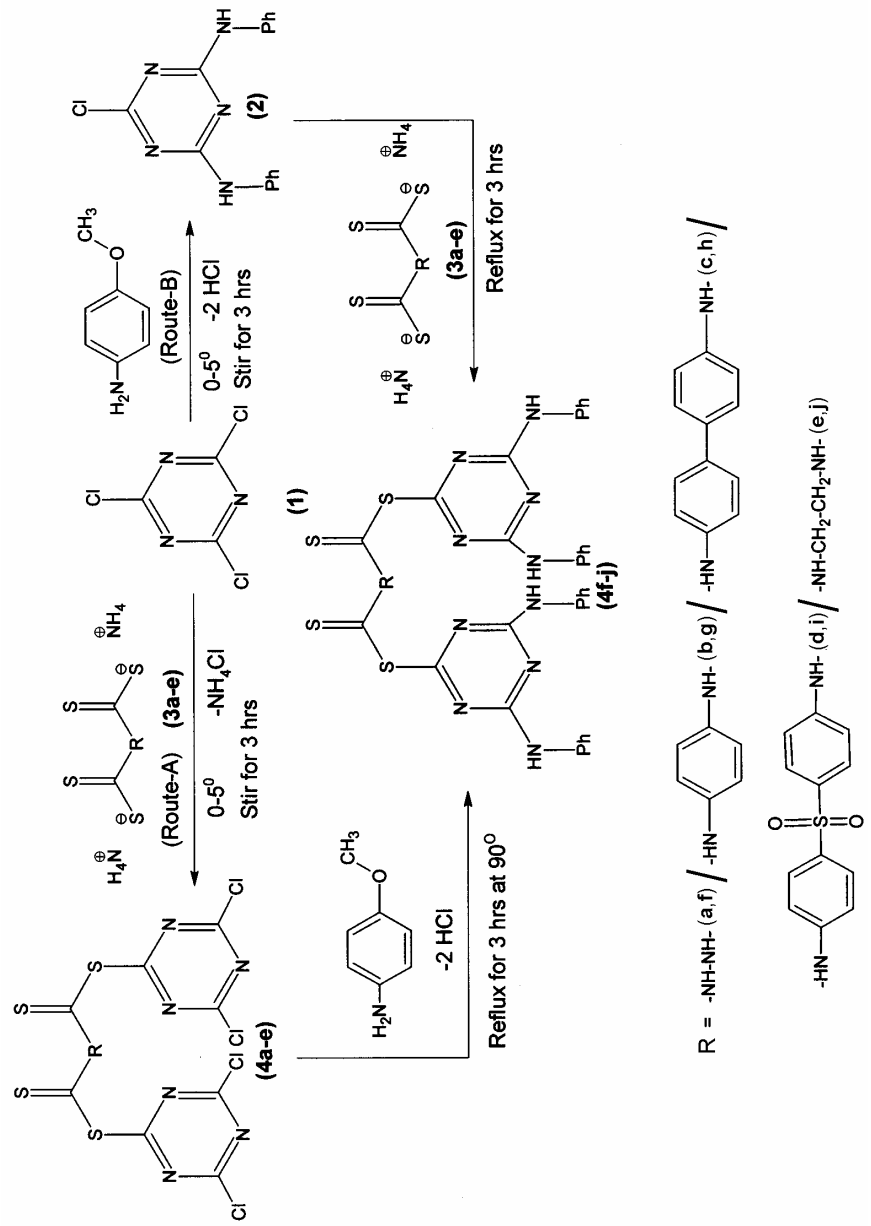
nyl)amino]-1,3,5-triazin-2-yl} esters of the respective acids (**4f–j**) has been reported to date. The present paper reports a mild and facile synthetic method for the condensation of 2,4,6-trichloro-1,3,5-triazine[12] **1** with diammonium 1,2-hydrazine-1,2-dicarbodithioate/1,4-phenylenebis(carbamodithioate)/(1,1'-biphenyl)-4,4'--diylbis(carbamodithioate)/(sulfonyldi-4,1-phenylene)bis(carbamodithioate)/1,2--ethanediylbis(carbamodithioate), followed by reaction with *p*-methoxyaniline. The compounds **4a–j** were evaluated for their biological activities against gram positive and gram negative bacteria (antibacterial activity) and fungi (antifungal activity), as possible potential biological agents. The synthesis of compounds **4a–j** can be achieved in several ways as described in the results and discussion section.

RESULTS AND DISCUSSION

In the first route (**A**), the synthesis of **4a–j** was achieved by the condensation of 1 mole of diammonium 1,2-hydrazine-1,2-dicarbodithioate/1,4-phenylene-bis-(carbamodithioate)/(1,1'-biphenyl)-4,4'-diylbis(carbamodithioate)/(sulfonyldi-4,1--phenylene)bis(carbamodithioate)/1,2-ethanediylbis(carbamodithioate) **3a–e** with 2 moles of 2,4,6-trichloro-1,3,5-triazine **1** at 0 °C in dry acetone for 3 h to afford bis(4,6-dichloro-1,3,5-triazin-2-yl) 1,2-hydrazine-1,2-dicarbodithioate/1,4-phenylene-bis(carbamodithioate)/(1,1'-biphenyl)-4,4'-diylbis(carbamodithioate)/(sulfonyldi--4,1-phenylene)-bis(carbamodithioate)/1,2-ethanediylbis(carbamodithioate) **4a–e** by the removal of 2 moles of ammonium chloride. Subsequently, the action of 4 moles of p-methoxyaniline in dry acetone at 35 °C and 58 °C for 3 h afforded **4f–j** with the removal of 2 moles of hydrochloric acid by maintaining the pH neutral through the addition of a saturated solution of sodium bicarbonate. The compounds **4f–j** were also synthesized by a second route (**B**) involving the chemical reaction between 2 moles of p-methoxyaniline with **1** in dry acetone at 0 °C and 35 °C for 3 h to afford 2,4-bis[(p-methoxyphenyl)amino]-6-chloro-1,3,5-triazine **2** by the removal of 2 moles of hydrochloric acid by maintaining the pH neutral through the addition of a saturated solution of sodium bicarbonate, followed by the action of 0.5 mole of diammonium 1,2-hydrazine-1,2-dicarbodithioate/1,4--phenylenebis(carbamodithioate)/(1,1'-biphenyl)-4,4'-diylbis(carbamodithioate)/(sulfonyldi-4,1-phenylene)bis(carbamodithioate)/1,2-ethanediylbis(carbamodithioate) **3a–e** in dry acetone at 58 °C for 3 h to afford **4f–j**, with the removal of 1 mole of ammonium chloride. A schematic representation of the reaction pathways for the synthesis of compounds **4a–j** are outlined in Scheme 1.

Synthesis of **4a–j** can be achieved by applying both the routes **A** and **B**. A theoretical mechanistic approach for the synthesis of the compounds can be found in a previously published article.[11]

The antimicrobial activity was assayed using the cup-plate agar diffusion method by measuring the zones of inhibition in mm. All the compounds were screened in vitro for their antimicrobial activity against a variety of bacterial strains. Standard drugs such as ampicillin, chloramphenicol, norfloxacin and griseofulvin were used for comparison purposes.


(Route-A) (3a-e) 0-5⁰ -NH4Cl Stir for 3 hrs
(Route-B) 0-5⁰ -2 HCl Stir for 3 hrs
Reflux for 3 hrs
Reflux for 3 hrs at 90⁰
-2 HCl
(1)
(2)
(3a-e)
(4a-e)
(4f-j)
R = -NH-NH- (a,f) / -HN- NH- (b,g) / -HN- NH- (c,h) /
-NH-CH2-CH2-NH- (e,j) / -HN- NH- (d,i) /

Scheme 1.

From the antimicrobial screening of compounds **4a**–**j**, it was observed that **4a**, **4g** and **4h** were more active than the other prepared compounds, but less active in comparison to known standard antibiotics. According to the structural activity relationship of the compounds, when R is an aromatic nucleus, the antimicrobial activity was higher with respect to the aliphatic analogues. In the case of antifungal screening, **4b** and **4i** exhibited more promising activity, whereby **4b** showed a fairly comparable antifungal activity to that of griseofulvin.

EXPERIMENTAL

The melting points of all the synthesized compounds were measured in open glass capillaries and are uncorrected. The yield is presented in percentage. The IR absorption spectra were recorded on a Shimadzu 435-IR spectrophotometer using the KBr pellet method. The ^{1}H-NMR spectra were recorded on a JEOL ^{1}H-NMR spectrophotometer (90 MHz) using TMS as the internal reference. Elemental analyses of the newly synthesized compounds were carried on a Carlo Erba 1108 analyzer and were found to be in the range of the theoretical value. The physical data and antimicrobial activity of the various compounds are presented in Tables I–III. The purity of the compounds was routinely checked by TLC using silica gel G.

Preparation of diammonium 1,2-ethanediylbis(carbamodithioate) ***3e***

A mixture of ethylenediamine (0.01 mol), carbon disulfide (0.02 mol) and ammonium hydroxide (20 ml) was vigorously stirred mechanically at 0 °C for 3 h. The obtained solid was filtered, washed with water and dried. The product was crystallized from ethanol. Yield: 83 %, m.p. 185 °C. IR (KBr) (ν / cm^{-1}): 3364 (N−H str.), 3032 (C−H str. aromatic), 2958 (C−H str. asym.), 1576 (C=S str.), 1554 (N−H str. def), 1300 (C−N str.), 705 (C−S str.); ^{1}H-NMR ($CDCl_3$) (δ / ppm): 7.58 (*s*, 2H, N−H), 2.53 (*s*, 4H, $-CH_2$).

Similarly, the other alkyl/aryl amines were stirred with carbon disulfide and ammonium hydroxide. The physical data of the obtained compounds are reported in Table I.

TABLE I. Physical data of compounds **3a**–**e**

Cpd.	Mol. formula	M.p. / °C	Yield / %	Nitrogen % (Calcd./Found)
3a	$C_2H_{10}N_4S_4$	222	80	25.68/25.65
3b	$C_8H_{14}N_4S_4$	200	82	19.05/19.00
3c	$C_{14}H_{18}N_4S_4$	280	49	15.13/15.02
3d	$C_{14}H_{14}O_2N_4S_5$	143	81	12.90/12.87
3e	$C_4H_{14}N_4S_4$	185	83	22.76/22.70

Route ***A****: Preparation of bis(4,6-dichloro-1,3,5-triazin-2-yl) 1,2-hydrazine-1,2-dicarbodithioate* ***4a***

Diammonium 1,2-hydrazine-1,2-dicarbodithioate **3a** (0.01 mol) dissolved in acetone (15 ml) was added gradually over 3 h to 2,4,6-trichloro-1,3,5-triazine **1** (0.02 mol) suspended in acetone (30 ml) at 0 °C. The obtained solid was filtered, washed with water and dried. The product was crystallized from ethanol. Yield: 72 %, m.p. 310 °C. IR (KBr) (ν / cm^{-1}): 3400 (N−H str.), 1562 (C=S str.), 1547 (N−H str. def), 1311 (C−N str.), 807 (C_3N_3 str. *s*-triazinyl), 726 (C−Cl str.), 660 (C−S str.), 682, 645 (Ar C−H out-of-plane bend); ^{1}H-NMR ($CDCl_3$)(δ / ppm): 6.58 (*s*, 2H, N−H).

Similarly, the other compounds **4b**–**e** were prepared as above. The physical data are reported in Table II.

TABLE II. Physical data of compounds **4a**–**j**

Cpd.	Mol. formula	M.p. / °C	Yield / %	Nitrogen % (Calcd./Found)
4a	$C_8H_2N_8S_4Cl_4$	310	72	23.33/23.31
4b	$C_{14}H_6N_8S_4Cl_4$	302	75	20.14/20.11
4c	$C_{20}H_{10}N_8S_4Cl_4$	307	68	17.72/17.70
4d	$C_{20}H_{10}O_2N_8S_5Cl_4$	310	72	16.09/15.95
4e	$C_{10}H_6N_8S_4Cl_4$	234	65	21.62/21.61
4f	$C_{36}H_{34}O_4N_{12}S_4$	260	68	20.33/20.32
4g	$C_{42}H_{38}O_4N_{12}S_4$	298	71	18.62/18.60
4h	$C_{48}H_{42}O_4N_{12}S_4$	259	72	17.17/17.15
4i	$C_{48}H_{42}O_6N_{12}S_5$	260	68	16.12/15.95
4j	$C_{38}H_{38}O_4N_{12}S_4$	210	65	19.44/19.42

*Preparation of bis{4,6-bis[(*p*-methoxyphenyl)amino]-1,3,5-triazin-2-yl} 1,2-hydrazine-1,2-dicarbodithioate (**4f**)*

A mixture of bis-(4,6-dichloro-1,3,5-triazin-2-yl) 1,2-hydrazine-1,2-dicarbodithioate **4a** (0.01 mol) and 4-methoxyaniline (0.04 mol) in dioxane (50 ml) was stirred for 3 h and then the mixture was refluxed for 3 h at 85–90 °C on a water bath. The content was poured onto crushed ice. The obtained solid was filtered, washed with water and dried. The product was crystallized from ethanol. Yield: 68 %, m.p. 260 °C. IR (KBr) (ν / cm^{-1}): 3404 (N–H str.), 3030 (C−H str. aromatic), 2958 (C−H str. asym.), 2921 (C−H str. CH_2), 2848 (C−H str. CH_3), 1575 (N−H str. def.), 1325 (C−N str.), 1248 (Ar−O−C str.), 818 (C_3N_3 str. *s*-triazinyl), 667 (C−S str.); ^{1}H-NMR ($CDCl_3$) (δ / ppm): 8.67 (*s*, 2H, N−H), 6.62−7.89 (*m*, 16H, Ar−H), 3.79 (*s*, 12H, −OCH_3).

Similarly, the other compounds **4f**–**j** were prepared as above. The physical data are reported in Table II.

*Route **B**: Preparation of 2-chloro-4,6-bis[(*p*-methoxyphenyl)amino]-1,3,5-triazine **2***

A mixture of 2,4,6-trichloro-1,3,5-triazine **1** (0.01 mol) and 4-methoxyaniline (0.02 mol) in dry acetone (50 ml) was stirred at 0 °C for 3 h. The content was poured onto crushed ice. The obtained solid was filtered, washed with water and dried. The product was crystallized from ethanol. Yield: 83 %, m.p. 200 °C. IR (KBr) (ν / cm^{-1}): 3350 (N−H str.), 3050 (C−H str. aromatic), 2970 (C−H str. asym. CH_3), 2850 (C−H str. sym. CH_3), 1580 (C=S str.), 1550 (N−H str. def.), 1310 (C−N str.), 1250 (Ar−O−C str.), 820 (C_3N_3 str. 1,3,5-triazinyl), 705 (C−Cl str.); ^{1}H-NMR ($CDCl_3$) (δ / ppm): 8.87 (*s*, 2H, −N−H), 6.67−7.70 (*m*, 8H, Ar−H), 3.40−4.00 (*s*, 6H, −OCH_3).

*Preparation of bis{4,6-bis[(*p*-methoxyphenyl)amino]-1,3,5-triazin-2-yl]} 1,2-hydrazine-1,2-dicarbodithioate **4f***

2-Chloro-4,6-bis[(*p*-methoxyphenyl)amino]-1,3,5-triazine **2** (0.02 mol) dissolved in dioxane was added to diammonium 1,2-hydrazine-1,2-carbodithioate **3a** (0.01 mol) suspended in dioxane (50 ml) and the mixture was refluxed on a water bath for 3 h. The content was poured onto crushed ice. The obtained solid was filtered, washed with water and dried. The product was crystallized from ethanol. Yield: 68 %, m.p. 260 °C. IR (KBr) (ν / cm^{-1}): 3404 (N−H str.), 3030 (C−H str. aromatic), 2958 (C–H str. asym.), 2921 (C–H str. CH_2), 2848 (C–H str. CH_3), 1325 (C−N str.), 1575 (N−H str. def.), 1248 (Ar−O−C str.), 818 (C_3N_3 str. 1,3,5-triazinyl), 667 (C−S str.); ^{1}H-NMR ($CDCl_3$) (δ / ppm): 8.67 (*s*, 2H, N−H), 6.62–7.89 (*m*, 16H, Ar−H), 3.79 (*s*, 12H, −OCH_3).

Similarly, the other compounds **4g**–**j** were prepared as above. The physical data are reported in Table II.

Antimicrobial activity

Compounds **4a**–**j** were screened for their antibacterial activity against *Bacillus subtilis* (MTCC-441), *Streptococcus pyogenes* (MTCC-442) (gram positive bacteria), *Escherichia coli* (MTCC-443) (gram negative bacteria) and antifungal activity against *Aspergillus niger* (MTCC-282) at a concentration of 50 μg ml^{-1} by the cup-plate method[13] using DMF as the solvent. The zone of inhibition was measured in mm and is presented in Table III.

TABLE III. Antimicrobial activity of compounds **4a**–**j**

Cpd.	Antibacterial Activity			Antifungal Activity
	B. subtilis MTCC-441	*S. pyogenes* MTCC-442	*E. coli* MTCC-443	*A. niger* MTCC-282
4a	14	18	18	16
4b	12	13	14	20
4c	15	15	13	17
4d	14	14	12	13
4e	16	15	16	12
4f	15	14	14	13
4g	18	13	18	15
4h	18	14	19	15
4i	16	18	14	18
4j	16	16	15	11
Ampicillin	22	26	24	–
Chloramphenicol	28	22	19	–
Norfloxacin	19	24	25	–
Griseofulvin	–	–	–	20

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И З В О Д

ИСПИТИВАЊА БИОАКТИВНИХ БИС-1,3,5-ТРИАЗИНИЛ-ДИТИОКАРБАМАТА

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Две различите методе коришћене су за добијање једињења бис{4,6-дихлоро/бис[(*p*-метоксифенил)амино]-1,3,5-триазин-2-ил}-1,2-хидразин-1,2-дикарбодитиоат/1,4-фенилен-бис(карбамодитиоат)/(1,1'-бифенил)-4,4'-диил-бис(карбамодитиоат)/(сулфонил-ди-4,1-фенилен)-бис(карбамодитиоат)/1,2-етан-диил-бис(карбамодитиоат) **4a**–**j**. У првом методу (**A**) за припрему **4a**–**e**, 2,4,6-трихлоро-1,3,5-триазин **1** кондензован је са диамонијум-1,2-хидразин-1,2-дикарбодитиоатом/1,4-фенилен-бис(карбамодитиоатом)/(1,1'-бифенил)-4,4'-диил-бис(карбамодитиоатом)/(сулфонил-ди-4,1-фенилен)-бис(карбамодитиоатом)/1,2-етан-диил-бис(карбамодитиоатом) **3a**–**e** да би се добили **4a**–**e,** који подлежу реакцији са *p*-метоксианилином чиме се добијају **4f**–**j**. У другој методи добијања (**B**), **1** је кондензован са *p*-метоксианилином да би се добио **2** уз накнадно учешће **3a**–**e** да би се добили **4a**–**j**. Структуре новодобијених једињења **4a**–**j** установљене су на основу елементалне анализе, као и IR и ^{1}H-NMR спектро-

скопијама. Антимикробне активности једињења **4a**–**j** на грам-позитивне и грам-негативне бактерије и гљиве одређене су методом бунарчића у агару. Сва добијена једињења показала су знатну антимикробну активност.

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