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Synthesis and radiation stability of novel biologically active sulfur compounds derived from 1,2-bis(4-amino-5-mercapto-s-triazol-3-yl)ethane

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Abstract

Some novel 1,2-bis(s-triazolo[3,4*b*][1,3,4]thiadiazino-3-yl)ethane (**4**–**7**); 1,2-bis(s-triazolo[3,4*b*][1,3,4]thiadiazol-3-yl)ethane (**16a**,**b**) and 1,2-bis(s-triazolo[3,4*b*][1,3,4]thiadiazepino-3-yl)ethane (**17**) were synthesized via reaction of 1,2-bis(4-amino-5-mercapto-s-triazol-3-yl)ethane (**3**) with different reagents. Identification of the new compounds was established by elemental analyses, IR, ^{1}H NMR and mass spectral data. Compounds **12**, **13**, **16b** and **17** were promising antifungal activity. The biologically active compounds **13**, **16b** and **17** were radioresistant retaining their structures unchanged up to 40 k Gy. Radiosterilization of these compounds in the dry state may prove to be applicable. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Triazoles; Triazolothiadiazine; Triazolothiadiazole; Antifungal activity; γ-Rays

1. Introduction

Bisheterocyclic compounds exhibit various biological activities [1,2]. Also triazole derivatives are reported as antifungal [3], antibacterial [4], analgesic and anti-inflammatory activities [5]. In addition some 1,3,4-thiadiazoles and s-triazolo [3,4*b*]-[1,3,4]thiadiazines are reported to have fungicidal [6] and hypotensive activity [7]. Recently, we reported the synthesis of some triazole derivatives as insecticidal [8] and anticancer active compounds [9]. The present investigation describes the synthesis of some bis-sulfur compounds containing triazole rings with the objective of obtaining active antifungal compounds. The application of radiation in pharmaceuticals technology has steadily increased during the past few years [10,11]. In the 1997 edition of the *European pharmacopeia* under 'the methods of preparation of sterile products'; irradiation is one of only three processes that can be used as a terminal sterilization method. Exposure to gamma radiation kills microorganisms with the advantage of having high penetrating power and does not-cause chemical contamination. As our interest is to prepare new heterocyclic compounds with anticipated biological activity, which can be used in the pharmaceutical field, we decided to study the radiation stability of the biologically active synthesized compounds in case of sterilization.

2. Chemistry

This paper describe a facile one step synthesis of bis(4-amino-5-mercapto-s-triazolo-3-y1) derivatives **2** and **3** via reaction between one mole of dicarboxylic acids with two moles of thiocarbohydrazide (**1**) under fusion conditions. This method lead to a higher overall yield and shorter working time in comparison with the reported method [8]. IR spectrum of **2** showed the presence of (NH_2, NH) bands and mass spectrum exhibited a molecular ion peak m/z at 366 (M^+, 1.83%). IR spectrum of compound **3** revealed the presence of (NH_2, NH) while ^{1}H NMR spectrum showed a signal at 3.0 due to the presence of two methylene groups (–CH_2–CH_2–). Mass spectrum of **3** exhibited a molecular ion peak m/z at 258 (M^+ 1.43%).

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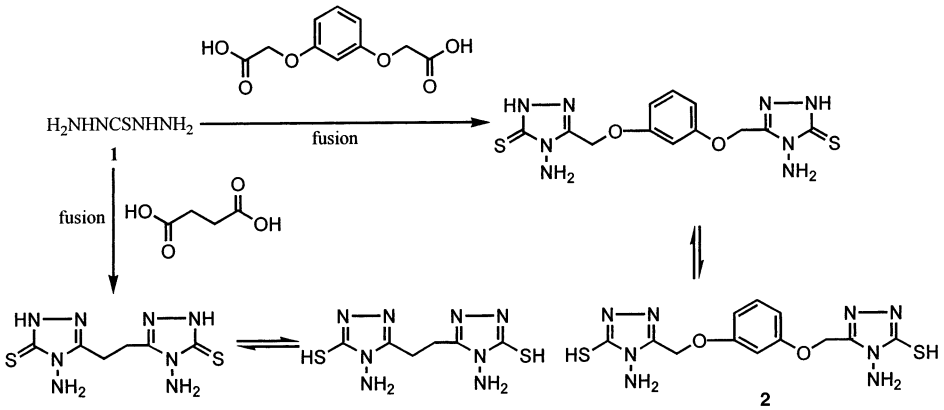
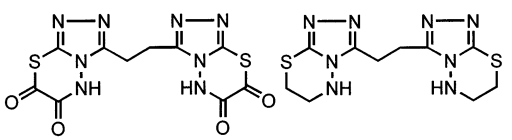


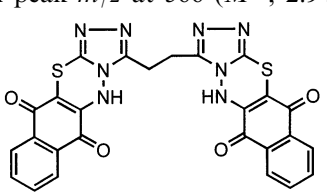
3

The reactivity of compound **3** towards halogenated compounds was discussed. Thus, treatment of compound **3** with oxalyl chloride in dimethylformamide caused cyclization to give the corresponding triazolothiadiazino derivative **4**; its mass spectrum showed a molecular ion peak m/z at 366 ($M^+ + 2$, 4.47%). Also, the triazolothiadiazine derivative **5** was obtained through the interaction of compound **3** with 1,2-dichloroethane in DMF/TEA. IR spectrum of **5** showed the presence of (NH) bands and the mass spectrum exhibited a molecular ion peak m/z at 310 (M^+, 0.98%).



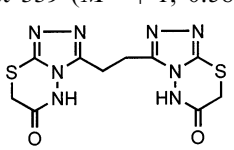
4 5

In addition, interaction of compound **3** with 2,3-dichloronaphthoquinone effected cyclization to furnish thiadiazino derivative **6** through elimination of four moles of HCl. IR spectrum of **6** showed the presence of (C=O) and (NH) bands, while mass spectrum showed a molecular ion peak m/z at 566 (M^+, 2.94%).



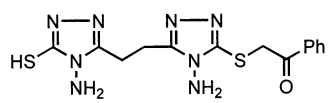
6

Furthermore, interaction of compound **3** with either chloroacetyl chloride or chloroacetic acid yielded the same product which formulated as triazolo[3,4*b*]-thiadiazino derivative **7**. IR spectra exhibited the presence of NH, C=O bands and mass spectrum showed a molecular ion peak m/z at 339 ($M^+ + 1$, 0.58%).



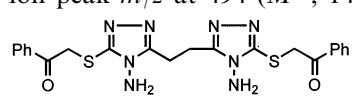
7

Treatment of compound **3** with phenacylbromide in DMF furnished a product with analytical data indicating that only 1 mol of phenacylbromide was consumed with elimination of 1 mol of HBr, which effected the alkylation of the mercapto group. This product was formulated as phenacylthio derivative **8**. IR spectrum showed the presence of NH_2, C=O bands, while mass spectrum showed a molecular ion peak m/z at 376 (M^+, 2.50%).

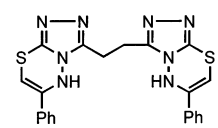


8

Repeating the same reaction in DMF/TEA for a long time with the hope of obtaining the cyclic structure **9b** but instead 2 mol of phenacylbromide were consumed with elimination of 2 mol HBr to give the uncyclized structure **9a**. IR spectrum showed the presence of NH_2, C=O bands. Mass spectrum of **9a** showed a molecular ion peak m/z at 494 (M^+, 14.38%).

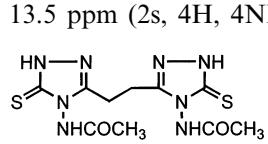


9a



9b

The behavior of compound **3** towards acid derivatives was also discussed. Thus, refluxing compound **3** with acetic anhydride for a short time effected acetylation of the two amino groups to give the acetamido derivative **10**. This reaction was repeated for a long time with the hope of obtaining the thiadiazolo derivative **11** but instead the same acetamido derivative **10** was obtained. IR spectrum showed bands at 3295, 3180 (NH) and 1700 cm^{-1} (C=O). ^1H NMR spectrum revealed signals at 2.2, 2.4 (2s, 6H, $2COCH_3$) and 11.0, 13.5 ppm (2s, 4H, 4NH).



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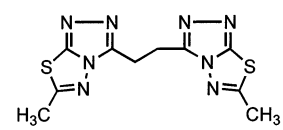
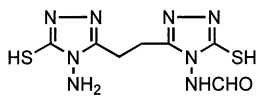


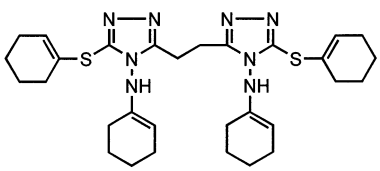
11

When compound **3** was reacted with formic acid, causing formylation for only one amino group to give the *N*-formyl derivative **12**. This was confirmed on the basis of elemental analysis and spectral data. IR spectrum of **12** showed the presence of NH and C=O) bands, while mass spectrum exhibited a molecular ion peak m/z at 286 (M^+, 2.21%).



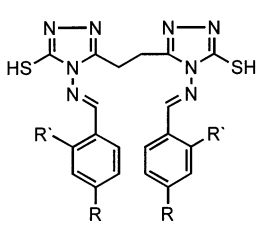
12

This work was extend to cover the reactivity of compound **3** toward carbonyl compounds. Condensation of compound **3** with cyclohexanone gave a product indicated that four moles of cyclohexanone were consumed with the elimination of 4 mol of H_2O. This product was formulated as cyclohexenylamino, derivative **13**, its mass spectrum was compatible with the molecular formula $C_{30}H_{42}N_8S_2$ 578 (M^+, 18.31%).



13

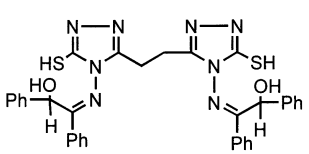
Interaction of compound **3** with aldehydes or bezoin, furnished Schiff's bases **14a**,**b** and **15**, respectively. IR spectra for **14a**,**b** showed the absence of NH_2 bands. Mass spectrum of compound **14a** revealed a molecular ion peak m/z at 466 (M^+, 4.05%). IR spectrum of compound **15** showed the presence of OH bands. Mass spectrum of **15** revealed a molecular ion m/z at 648 ($M^+ + 2$, 0.21%).



14

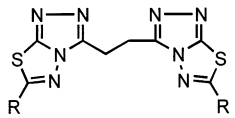
14a; R=H; R`= OH

14b; R= Cl, R`=H



15

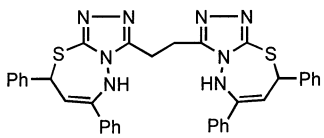
Interaction of compound **3** with ethyl acetoacetate gave the thiadiazolo derivative **16a**. IR spectrum of **16a** showed the presence of CH (aliph.) and C=O bands. Mass spectrum of **16a** revealed a molecular ion peak m/z at 390 (M^+, 12.00%). Also, the reaction of compound **3** with phenyl isothiocyanate yielded thiadiazolo derivative **16b**. IR spectrum of **16b** showed the presence of NH, C=N bands. Mass spectrum of **16b** exhibited a molecular ion peak m/z at 460 (M^+, 0.24%).



16a; R=CH_2COCH_3

16b; R=NHC_6H_5

Finally, the interaction of compound **3** with unsaturated ketone (chalcon) gave a product which was formulated as triazolo[3,4*b*]thiadiazepino derivative **17**. IR spectrum showed the presence of NH and C=N bands. Mass spectrum of compound **17** revealed a molecular ion peak m/z at 636 (M^+, 0.8%).



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3. Experimental

Melting points are uncorrected and were determine on a Stuart melting point apparatus. IR spectra were recorded on a Pye-Unicam SP 3-100 spectrophotometer using the KBr wafer technique. ^{1}H NMR spectra were recorded on a 90 MHz Varian. EM-390 NMR spectrometer in DMSO-d_6 as a solvent, using tetramethylsilane as an internal standard. Mass spectra were run on a HP Model MS-5988. Elemental analyses were determined on a Perkin–Elmer 240 (microanalyses). The samples were irradiated with gamma radiation (^{60}Co) emitted by an Vorsicht Radioaktivitat (Russia), at the National Center for Radiation Research and Technology. A non-irradiated sample (control) was kept as a reference. Powder samples were irradiated at room temperature conditions into polycarbonate vials. UV spectra were recorded using a Ati Unicam-UV–vis Aurora Scan.

3.1. 1,3-Bis[4-amino-5-mercapto-s-triazolo-3-yl]-diacetoxybenzene (2) and 1,2-bis[4-amino-5-mercapto-s-triazolo-3-yl]ethane (3)

3.1.1. General procedure

A mixture of thiocarbohydrazide (**1**) (2.12 g, 0.02 mol) and benzene-1,3-dioxyacetic acid (0.96 g, 0.01 mol) or succinic acid (1.18 g, 0.01 mol) was fused at 180°C in an oil bath for 10 min. After cooling, the reaction mixture was triturated with ethanol and the obtained solid was crystallized from ethanol (Table 1).

2. Yellow crystals from ethanol. IR: ν (cm^{-1}) 3444, 3283, 3177 (NH_2, NH), 2923 (CH aliph.), 1647 (C=N).

MS: m/z 366 (M^+ 1.83%), 63 (100%), 302 (1.83%), 256 (43.28%), 214 (42.97%), 129 (58.76%), 73 (36.97%).

3. Yellow needles from ethanol. IR: ν (cm^{-1}) 3380, 3149 (NH_2, NH), 2939 (CH aliph.), 1640 (C=N). ^1H NMR (DMSO-d_6): δ 3.0 (s, 4H, CH_2–CH_2), 5.5 (s, 4H, $2NH_2$ exchangeable with D_2O), 12.3 (s, 2H 2NH). MS: m/z 258 (M^+ 1.43%), 69 (100%), 214 (7.80%), 185 (5.07%), 160 (7.36%), 149 (29.6%), 109 (14.3%), 81 (53.36%), 73 (24.66%).

*3.2. 1,2-Bis (5,6-dioxo-7H-s-triazolo [3,4b][1,3,4]thiadiazino-3-yl)ethane (**4**)*

A mixture of compound **3** (2.58 g, 0.01 mol), oxalyl chloride (2.52 g, 0.02 mol) and triethylamine (2.02 g, 0.02 mol) in benzene (50 ml) was refluxed for 6 h. The solvent was evaporated under reduced pressure. The precipitated product was collected, washed with water, dried and crystallized from benzene to give shiny yellow needles. IR: ν (cm^{-1}) 3350 (NH), 1700, 1680 (C=O). MS: m/z 366 ($M^+ + 2$; 4.47%), 55 (100%), 313 (4.34%), 256 (7.04%), 185 (10.84%), 137 (7.14%), 97 (36.91%), 83 (48.50%)

*3.3. 1,2-Bis (5,6,7-trihydro-s-triazolo [3,4b][1,3,4] thiadiazin-3-yl)ethane (**5**)*

A mixture of ethylene dichloride (1.98 g, 0.02 mol), compound **3** (2.58 g, 0.01 mol) and triethylamine (2.02 g, 0.02 mol) in ethanol (20 ml) was refluxed for 8 h. After cooling the obtained product was filtered off, washed with sodium bicarbonate solution, dried and crystallized from ethanol to give brown crystals. IR: ν (cm^{-1}) 3296, 3172 (NH), 2917 (CH aliph.), 1624 (C=N). MS: m/z 310 (M^+; 0.98%), 57 (100%) 295 (1.11%), 207 (5.10), 127 (8.94%), 85 (57.20%), 71 (75.32%).

Table 1
Characterization data for newly synthesized compounds

Comp. No.	M.p. (°C)	Yield (%)	Molecular formula (mol. wt)	Analysis required/found (%)			
				C	H	N	S
2	110–112	81	$C_{12}H_{14}N_8O_2S_2$ (366)	39.34 39.10	3.82 3.60	30.60 30.80	17.48 17.60
3	220–222	83	$C_6H_{10}N_8S_2$ (258)	27.90 27.60	3.87 3.70	43.41 43.10	24.80 24.90
4	170–172	62	$C_{10}H_6N_8O_4S_2$ (366)	32.78 32.50	1.64 1.50	30.60 30.90	17.48 17.70
5	110–112	51	$C_{10}H_{14}N_8S_2$ (310)	38.71 38.90	4.51 4.20	36.13 36.30	20.64 20.40
6	>300	74	$C_{26}H_{14}N_8O_4S_2$ (566)	55.12 55.40	2.47 2.60	19.78 19.50	11.30 11.10
7	125–127	75	$C_{10}H_{10}N_8O_2S_2$ (338)	35.50 35.20	2.95 2.60	33.13 33.40	18.93 18.60
8	205–207	70	$C_{14}H_{16}N_8OS_2$ (376)	44.68 44.90	4.25 4.50	29.78 29.40	17.02 16.70
9a	>300	59	$C_{22}H_{22}N_8O_2S_2$ (494)	53.44 53.10	4.45 4.20	22.67 22.40	12.95 12.60
10	>300	66	$C_{10}H_{14}N_8O_2S_2$ (342)	35.08 35.30	4.09 4.20	32.74 32.40	18.71 18.90
12	>300	88	$C_7H_{10}N_8OS_2$ (286)	29.37 29.60	3.49 3.80	39.16 39.40	22.37 22.10
13	210–212	58	$C_{30}H_{42}N_8S_2$ (578)	62.28 62.60	7.26 7.60	19.37 19.60	11.07 11.20
14a	288–290	59	$C_{20}H_{18}N_8O_2S_2$ (466)	51.50 51.20	3.86 3.60	24.03 24.40	13.73 13.50
14b	278–280	61	$C_{20}H_{16}N_8S_2Cl_2$ (503)	47.71 47.50	3.18 3.40	22.26 22.50	12.72 12.40
15	>300	62	$C_{34}H_{30}N_8O_2S_2$ (646)	63.15 63.50	4.64 4.90	17.33 17.10	9.90 9.60
16a	>300	65	$C_{14}H_{14}N_8O_2S_2$ (390)	43.07 43.30	3.59 3.80	28.71 28.50	16.41 16.10
16b	170–172	73	$C_{20}H_{16}N_{10}S_2$ (460)	52.17 52.50	3.47 3.10	30.43 30.60	13.91 13.60
17	270–272	59	$C_{36}H_{26}N_8S_2$ (634)	68.13 67.80	4.10 4.40	17.66 17.90	10.09 10.40

*3.4. 1,2-Bis-4,9-dioxo(naphtho [5,6:2,3][1,3,4]thiadiazino [2,3c]-s-triazolo-3-yl)-ethane (**6**)*

A mixture of compound **3** (2.58 g, 0.01 mol), 2,3-dichloronaphthoquinone (4.54 g, 0.02 mol) and triethylamine (2.02 g, 0.02 mol) in dimethylformamide (20 ml) was refluxed for 5 h. After cooling the obtained product was filtered off, washed with water, dried and crystallized from diluted ethanol as light-yellow needles. IR: ν (cm^{-1}) 3357, 3217 (NH), 1716, 1665 (C=O), 1620 (C=N). MS: m/z 566 (M^+, 2.94%), 57 (100%), 509 (2.24%), 381 (4.44%), 313 (34.18%) 236 (36.82%), 129 (34.42%), 111(39.21%), 71(61.90%).

*3.5. 1,2-Bis(6H-5-oxo-s-triazolo[3,4b][1,3,4]-thiadiazino-3-yl)ethane (**7**)*

Chloroacetyl chloride (2.26 g, 0.02 mol) or chloroacetic acid (1.89 g, 0.02 mol) were added to a stirred solution of compound **3** (2.58 g, 0.01 mol) and triethylamine (2.02 g, 0.02 mol) in dry dioxane (20 ml). The reaction mixture was refluxed for 8 h. The obtained solid was filtered off, washed with water, dried and crystallized from ethanol to give pale-yellow crystals. IR: ν (cm^{-1}) 3410 (NH), 2920 (CH aliph.), 1680 (C=O), 1600 (C=N). MS: m/z 339 ($M^+ + 1$, 0.58%), 63 (100%), 257 (28.42%), 207 (2.17%), 159 (49.88%), 96 (28.41%), 69 (31.24%).

*3.6. 1-[4-Amino-5-phenacylthio-s-triazolo-3-yl]-2-[4′-amino-5-mercapto-s-triazolo-3-yl)ethane (**8**)*

A mixture of equimolecular amounts (0.02 mol) of compound **3** and phenacylbromide in dimethylformamide (20 ml) was heated under reflux for 6 h. The reaction mixture was then cooled, and diluted with water. The obtained solid was crystallized from ethanol to give pale-yellow crystals. IR: ν (cm^{-1}) 3444, 3387, 3189 (NH_2), 2911 (CH aliph.), 1685 (C=O), 1600 (C=N). MS: m/z 376 (M^+, 2.50%), 57 (100%), 368 (17.93%), 256 (7.89%), 207 (9.25%) 137 (13.80%), 97(71.98%), 83 (69.89%), 69 (73.97%).

*3.7. 1,2-Bis[4-amino-5-phenacylthio-s-triazolo-3-yl]ethane (**9a**)*

A mixture of **3** (2.58 g, 0.01 mol), phenacylbromide (3.98 g, 0.02 mol) and triethylamine (2.02 g, 0.02 mol) in dimethylformamide (20 ml) was refluxed for 10 h. It was left to cool, filtered, washed with water, dried and crystallized from dioxane to give pale yellow crystals. IR: ν (cm^{-1}) 3280, 3190 (NH_2), 2920 (CH aliph.), 1690 (C=O), 1610 (C=N). MS: m/z 494 (M^+, 14.38%), 83 (100%), 394 (29.58%), 363 (75.83%), 287 (55.21%), 219 (46.46%), 180 (29.79%), 158 (38.13%), 130 (31.25%), 72 (39.37%), 57 (45.21%).

*3.8. 1,2-Bis[4-acetylamino-5-mercapto-s-triazolo-3-yl]ethane (**10**)*

A solution of **3** (2.58 g, 0.01 mol) in acetic anhydride (20 ml) was heated under reflux for 14 h. After cooling the excess acetic anhydride was removed under reduced pressure. The obtained solid was crystallized from acetic acid to give fluorescent greenish–yellow short needles. IR: ν (cm^{-1}) 3295, 3180 (NH), 2943 (CH aliph.), 1700 (C=O), 1600 (C=N). ^{1}H NMR (DMSO-d_6): δ 2.2, 2.4 (2s, 6H, $2COCH_3$), 2.9 (s, 4H, $–CH_2–CH_2–$), 11.0, 13.5 (2s, 4H, 4NH).

*3.9. 1-[4-Formylamino-5-mercapto-s-triazolo-3-yl]-2-[4′-amino-5-mercapto-s-triazolo-3-yl]ethane (**12**)*

A solution of **3** (2.58 g, 0.01 mol) in formic acid (10 ml) was heated under reflux for 3 h. The solid formed was collected by filtration and crystallized from dioxane to give yellow needles. IR: ν (cm^{-1}) 3279, 3107 (NH_2), NH), 2945 (CH aliph.), 1694 (C=O), 1580 (C=N). MS: m/z 286 (M^+, 2.21%), 55 (100%), 207 (15.91%), 149 (16.41%), 83 (70.60%), 69 (96.48%).

*3.10. 1,2-Bis[4-cyclohexenylamino-5-cyclohexenylthio-s-triazolo-3-yl]ethane (**13**)*

To a solution of **3** (2.58 g, 0.01 mol) in dimethylformamide (10 ml) was added cyclohexanone (1.96 g, 0.02 mol). The reaction mixture was refluxed for 10 h and the obtained solid was crystallized from dioxane to give **13**: IR: ν (cm^{-1}) 3382, 3175 (NH), 2928 (CH aliph.), 1640 (C=N). MS: m/z 578 (M^+, 18.31%), 579 ($M^+ + 1$, 20.97%), 55 (100%, 494 (9.83%), 379 (15.88%), 296 (15.52%), 199 (20.50%), 151 (21.21%), 103 (10.25%), 72 (26.54%).

*3.11. 1,2-Bis[4-(2′-hydroxybenzylidineamino)-5-mercapto-s-triazolo-3-yl]ethane (**14a**) and 1,2-bis[4-(4′-chlorobenzylidineamino)-5-mercapto-s-triazolo-3-yl)ethane (**14b**)*

3.11.1. General procedure

A mixture of **3** (2.58g, 0.01 mol) and the appropriate aldehyde (salicylaldehyde or 4-chlorobenzaldehyde (0.02 mol) in acetic acid (10 ml) was refluxed for 2 h. The solvent was evaporated under reduced pressure. The solid product was collected and crystallized from ethanol.

14a. Yellow crystals from ethanol. IR: ν (cm^{-1}) 3500 (OH), 3100 (CH arom.), 2943 (CH aliph.), 1618 (C=N). MS: m/z 466 (M^+; 4.05%), 91 (100%), 435 (5.80%), 382 (10.64%), 293 (12.27%), 231 (11.60%), 181 (6.59%), 119 (84.23%), 82 (17.74%), 63 (39.36%).

14b. Yellow crystals from ethanol. IR: ν (cm^{-1}) 3100 (CH arom.) 2944 (CH aliph.), 1660 (C=N).

Table 2
Antifungal activity of some newly synthesized compounds (inhibition zones, mm)

Comp. No.	*A. ochraceus Wilhelm* (AUCC-230)	*P. chrysogenum Thom* (AUCC-530)	*A. Flavus Link* (AUCC-164)	*C. albicans (Robin) Berkho* (AUCC-1720)
2	18	20	10	10
3	10	10	10	18
4	20	18	14	16
6	18	10	18	28
7	24	18	30	18
8	18	32	18	16
9a	20	24	16	14
10	20	32	18	18
12	24	36	10	10
13	34	36	34	20
14a	10	24	18	16
14b	20	20	14	10
15	20	18	10	10
16a	20	18	18	18
16b	34	20	36	24
17	18	34	20	36
Mycostatine	36	40	38	40

3.12. Schiff's bases derivative ***15***

Compound **3** (2.58 g, 0.01 mol), benzoin (1.96 g, 0.02 mol) and potassium hydroxide solution (10 ml, 10% solution) in ethanol (30 ml) were refluxed for 6 h. The solvent was evaporated under reduced pressure, the precipitated product was collected, washed with water, dried and crystallized from dioxane to give yellow crystals. IR: ν (cm^{-1}) 3500 (OH), 2926 (CH aliph.), 1630 (C = N). MS: m/z 648 ($M^+ + 2$, 0.21%), 55 (100%), 578 (1.20%), 551 (4.41%), 480 (1.02%), 368 (11.81%), 313 (11.02%), 236 (14.54%), 129 (27.28%), 97 (49.32%), 77 (9.31%).

3.13. 1,2-Bis[5-acetonyl-s-triazolo[3,4b][1,3,4]-thiadiazolo-3-yl]ethane ***(16a)***

A mixture of **3** (2.58 g, 0.01 mol), ethyl acetoacetate (2.60 g, 0.02 mol) and triethylamine (2.02 g, 0.02 mol) in dimethylformamide (20 ml) was refluxed for 12 h. The obtained solid was crystallized from diluted ethanol to give yellow crystals. IR: ν (cm^{-1}) 2922 (CH aliph.), 1727 (C=O), 1627 (C=N). MS: m/z 390 (M^+, 12.00%), 331 (100%), 368 (53.40%), 299 (27.09%), 183 (42.18%), 98 (62.25%), 74 (36.63%).

3.14. 1,2-Bis[5-anilino-s-triazolo[3,4b][1,3,4]-thiadiazolo-3-yl]ethane ***(16b)***

A solution of **3** (2.58 g, 0.01 mol) and phenyl isothiocyanate (2.70 g, 0.02 mol) in dry pyridine (20 ml) was refluxed until the evolution of H_2S has ceased (10 h.) The reaction mixture was poured into ice cold water (100 ml). The product was filtered off, dried and crystallized from dioxane to give light-yellow needles. IR: ν (cm^{-1}) 3264 (NH), 2925 (CH aliph.), 1623 (C=N). MS: m/z 460 (M^+, 0.24%), 93 (100%), 421 (0.71%), 368 (15.54%), 313 (6.89%), 236 (17.08%), 194 (29.30%), 129 (35.59%), 98 (36.92%), 81 (48.87%), 57 (97.94%).

3.15. 1,2-Bis[5,7-diphenyl-s-triazolo[3,4b][1,3,4]-thiadiazepino-3-yl]ethane ***(17)***

A mixture of **3** (2.58 g, 0.01 mol), chalcone (3.92 g, 0.02 mol) and triethylamine (2.02 g, 0.02 mol) in dimethylformamide (20 ml) was refluxed for 10 h. The obtained solid was crystallized from dioxane to give yellow crystals. IR: ν (cm^{-1}) 3160 (NH), 3100 (CH arom.), 2939 (CH aliph.), 1616 (C=N). MS: m/z 636 (M^+; 0.8%), 207 (100%), 579 (0.21%), 429 (0.47%), 368 (3.71%), 281 (2.51%), 131 (34.47%), 77 (66.38%).

4. Antifungal activity

Most of the newly synthesized compounds were screened for their antifungal activity against four species of fungi, namely, *Aspergillus ochraceus Wilhelm* (AUCC-230), *Penicillium chrysogenum Thom* (AUCC-530), *Aspergillus Flavus Link* (AUCC-164) and *Candida albicans (Robin) Berkho* (AUCC-1720), using a cup plate agar diffusion method [12]. The fungi cultures were maintained on Czapek's DOX agar medium.

The tested compounds were dissolved in *N*,*N*-dimethylformamide (DMF) to get a solution of 1 mg/ml concentration. The inhibition zones were measured in (mm) at the end of an incubation period of 48 h at 28°C. Dimethylformamide showed no inhibition zones. Mycostatine was used as a reference to evaluate the potency of the tested compounds. The minimal in-

Table 3
UV spectra of biologically active compounds **13**, **16b** and **17** before and after irradiation

Comp.	Dose (k Gy)	Concentration (mol)	λ_{max}	A (O.D.)
13	0 [a]	1×10^{-4}	280	0.823
	5		280	0.982
	10		280	1.066
	15		280	1.068
	20		280	1.317
	25		280	1.440
	30		280	1.696
	40		280	1.719
16b	0 [a]	1×10^{-4}	283	1.152
	5		283	1.488
	10		283	1.534
	15		283	1.552
	20		283	1.705
	25		283	1.768
	30		283	1.837
	40		283	1.896
17	0 [a]	1×10^{-4}	272	0.896
	5		272	0.904
	10		272	0.953
	15		272	1.002
	20		272	1.108
	25		272	1.173
	30		272	1.353
	40		272	1.827

[a] 0, non-irradiated compound (control); A, optical density (absorbance).

hibitory concentration (MIC) of the active compounds was measured using twofold serial dilution method.

The results are illustrated in Table 2. The antifungal screening of the synthesized compounds showed that the triazole derivatives (**12** and **13**), triazoles containing either thiadiazole **16b** or thiadiazepine **17** were found to be most active compounds. Compound **13** showed high activity against *A. ochraceus*, *P. chrysogenum* and *A. Flavus* (MIC values were 25–50 μg/ml), while compound **12** exhibited high activity against *P. chrysogenum* (MIC < 50 μg/ml). On the other hand, compound **16b** revealed high activity against *A. ochraceus* and *A. flavus* (MIC < 50 μg/ml). Also, compound **17** showed high activity against *P. chrysogenum* and *C. albicans* (MIC < 50 μg/ml). These results indicate that the biologically active compounds **12**, **13**, **16b** and **17** are nearly active as the standard Mycostatine (MIC values were 20–25 μg/ml).

5. Radiation stability of the biologically active compounds

The biologically active compounds **12**, **13**, **16b** and **17** were irradiated in the dry state (dose range 5–40 k Gy) at dose rate 1 k Gy/7 min to investigate their stability of chemical structures in case of radiosterilizations using gamma radiation. Ultraviolet spectra of non-irradiated (control) and irradiated compounds in dimethylformamide (DMF) as solvent are listed in Tables 3 and 4. The results showed that compounds **13**, **16b** and **17** were radioresistant retaining their structure unchanged up to 40 k Gy (the absorbance value above control) (Table 3). Also, thin layer chromatographic analyses for compounds **12**, **13**, **16a** and **17** was conducted before and after irradiation using precoated silica gel G sheet 1B-F and a mixture of 2:1 ethyl acetate–petroleum ether as eluent. Spots were detected by UV lamp at 254 nm. Compounds **13**, **16a** and **17** showed a single distinct spot, before and after irradiation, with the same R_f values of 0.35, 0.81 and 0.26, respectively.

This means that no change occurred in the structure of these compounds, so the radiosterilization of these compounds in the dry form may prove to be applicable.

Compound **12** is sensitive toward gamma radiation because there is reduction in optical densities (Table 4). The reduction percentage was calculated

$$\text{Reduction } \% = \frac{\Delta A}{A_0} \times 100$$

where $\Delta A = A_o - A_i$ and A_o, A_i are the optical densities of the compounds before and after irradiation. Thin layer chromatographic analysis (TLC) of compound **12** before irradiation (control) showed a distinct single

Table 4
UV spectra of biologically active compound **12** before and after irradiation

Comp.	Dose (k Gy)	Concentration (mol)	λ_{max}	A (O.D.)	ΔA	$\Delta A/A_o$ (%)
12	0 [a]	1×10^{-4}	275	1.341	–	–
	5		275	1.227	0.114	8.50
	10		275	1.109	0.232	17.30
	15		275	1.062	0.279	20.80
	20		275	1.041	0.300	22.37
	25		275	0.832	0.509	37.95
	30		275	0.795	0.546	40.71
	40		275	0.673	0.668	49.81

[a] 0, non-irradiated compound (control); A, optical density (absorbance).

before irradiation (control) showed a distinct single spot with R_f value of 0.42, while after irradiation it exhibited a faint single spot having the same R_f, with the appearance of a minor spot or a degraded product at R_f (0.64). This may be attributed to certain degradation, which is confirmed with the UV data (Table 4).

From these results gamma radiation is not the proper method for sterilization of compound **12**.

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