Thiothymine in the synthesis of triazolotriazine and hydrazone compounds: evaluation of antimicrobial activity of some hydrazones

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Background and objectives
Fused triazine derivatives are known to be a very important class of compounds that have significant biological activities. Also, they showed a broad spectrum against leukemic cell lines and cancer cells. Our objective after such a study is, on one hand, to shed some light on the synthesis of triazolotriazine and hydrazone derivatives from the thiothymine compound and, on the other hand, to explore the biological activity of the isolated products from such reactions.
Materials and methods
Reaction of thiothymine **3** and hydrazonoyl chloride **1** in refluxing chloroform in the presence of triethylamine yielded triazolotriazine derivatives **11**. The hydrazino derivative **14** underwent condensation reactions with aldehydes, pyruvic acid, ethyl pyruvate, and ketones to give the corresponding hydrazone derivatives **15** and **16**, respectively. Antimicrobial activities of some newly synthesized compounds were studied using the diffusion plate method.
Results and conclusion
Both antibacterial and antifungal activities of the new synthesized compounds **14**, **15h**, and **16a–d** were studied. Also, the minimum inhibitory concentration values for the highly efficient antibacterial compounds using the most sensitive microorganisms were determined.

Keywords:
antimicrobial activities, cycloaddition reaction, hydrazone, hydrazonoyl halides, thiothymine

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Introduction

Hydrazonoyl halides **1** represent a unique class of compounds which underwent 1,3-base catalyzed elimination reaction to give nitrilimines **2** (Chart 1) [1–7]. The latter are versatile synthetic intermediates especially useful for 1,3-dipolar cycloaddition reaction that have been used in the synthesis of numerous heterocycles [8–13]. In continuation of our study on the chemistry of hydrazonoyl halides **1** [6–13], we report here a synthesis of triazolotriazine and hydrazone derivatives from the thiothymine compound. Evaluation of antimicrobial activity against some microorganisms was investigated.

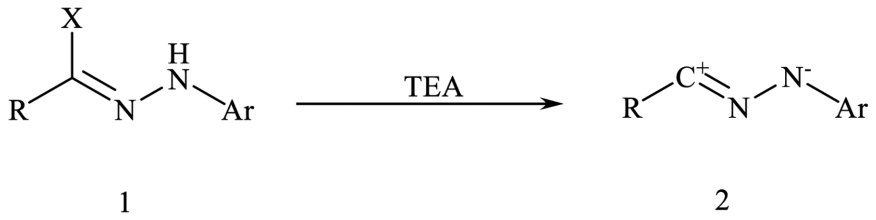
Materials and methods

Melting points were determined on a Stuart melting point apparatus and are uncorrected. The IR spectra were measured as KBr pellets on an FTIR Bruker-Vector 22 spectrophotometer (Manasquan, New Jersey, United States). The ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or $[D_6]$ dimethyl sulfoxide (DMSO) on a Varian Mercury VXR 300 spectrometer (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) using TMS as internal standard. Mass spectra were measured on a Shimadzu GCMS-Q-1000 EX mass spectrometer at 70 eV. The elemental analyses were carried out at the Microanalytical Center, Cairo University, using Automated analyzer CHNS (Vario EL III; Elementar, Hanau, Germany). The hydrazonoyl chloride **1**, 6-methyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one **3**, 6-methyl-3-(methylthio)-1,2,4-triazin-5(4H)-one **13**, and hydrazine derivative **14** were prepared using the reported procedures [12,14–23].

General procedure for the synthesis of 6-methyl-[1,2,4]triazolo[3,4-c][1,2,4]triazine derivatives (11A–l)

To a mixture of hydrazonoyl chloride **1** (6.0 mmol) and 6-methyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one **3** (0.9 g, 6.0 mmol) in chloroform (20 ml), triethylamine (0.6 ml, 6.0 mmol) was added at room temperature. The reaction mixture was refluxed for 6 h and then cooled, the excess chloroform was removed

Chart 1



1 2

1,3-base catalyzed elimination reaction of hydrazonoyl halides.

under reduced pressure and the residue was treated with ethanol (10 ml). The solid that precipitated was collected by filteration and crystallized from a suitable solvent to give compounds **11A–I**. The compounds prepared together with their physical properties are listed below:

*3-Acetyl-6-methyl-1-phenyl-[1,2,4]triazolo[3,4-c][1,2,4] triazin-5(1H)-one (**11Aa**)*

Yellow crystals: mp 242°C (CH_3CN), yield (1.35 g, 80%). IR (KBr): ν=1709 and 1670 (2CO) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ=2.32 (s, 3H, CH_3), 2.71 (s, 3H, CH_3CO) and 7.46–8.06 (m, 5H). ^{13}C NMR (75 MHz, DMSO-d_6): δ=18.1, 27.5, 120.8, 128.2, 129.5, 135.6, 138.7, 148.4, 155.3, 160.4, 185.1. MS, *m/z* (%): 269 (M^+, 5.2), 77 (100). $C_{13}H_{11}N_5O_2$ (269.09): C, 57.99; H, 4.12; N, 26.01. Found: C, 57.83; H, 4.15; N, 26.02.

*3-Acetyl-6-methyl-1-(p-tolyl)-[1,2,4]triazolo[3,4-c][1,2,4] triazin-5(1H)-one (**11Ab**)*

Yellow crystals: mp 232°C (DMF), yield (1.35 g, 76%). IR (KBr): ν=1712 and 1674 (2CO) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ=2.32 (s, 3H, CH_3), 2.40 (s, 3H, $CH_3C_6H_4$), 2.71 (s, 3H, CH_3CO), 7.43 (d, 2H) and 7.90 (d, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ=18.1, 20.6, 27.5, 109.2, 121.0, 129.8, 133.2, 137.8, 148.2, 155.2, 160.5, 185.2. MS, *m/z* (%): 283 (M^+, 50.2), 148 (100). $C_{14}H_{13}N_5O_2$ (283.11): C, 59.36; H, 4.63; N, 24.72. Found: C, 59.20; H, 4.65; N, 24.76.

*3-Acetyl-1-(4-chlorophenyl)-6-methyl-[1,2,4]triazolo[3,4-c] [1,2,4]triaz-in-5(1H)-one (**11Ac**)*

Yellow crystals: mp 264°C (DMF), yield (1.41 g, 74%). IR (KBr): ν=1715 and 1665 (2CO) cm^{-1}. MS, *m/z* (%): 303 (M^+, 11.6), 247 (100). $C_{13}H_{10}ClN_5O_2$ (303.05): C, 51.41; H, 3.32; Cl, 11.67; N, 23.06. Found: C, 51.32; H, 3.36; Cl, 11.69; N, 23.09.

Ethyl 6-methyl-5-oxo-1-phenyl-1,5-dihydro-[1,2,4] triazolo[3,4-c][1,2,4]-triazine-3-carboxylate (**11Ba**)

Yellow crystals: mp 282°C (CH_3CN), yield (1.35 g, 72%). IR (KBr): ν=1741 and 1672 (2CO) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ=1.37–1.41 (t, 3H, CH_3CH_2), 2.33 (s, 3H, CH_3), 4.47–4.54 (q, 2H, CH_2CH_3) and 7.48–8.01 (m, 5H). ^{13}C NMR (75 MHz, DMSO-d_6): δ=13.9, 18.1, 62.8, 121.1, 128.1, 129.4, 134.5, 135.6, 148.4, 154.3, 155.2, 160.5. MS, *m/z* (%): 299 (M^+, 7.4), 91 (100). $C_{14}H_{13}N_5O_3$ (299.10): C, 56.18; H, 4.38; N, 23.40. Found: C, 56.02; H, 4.36; N, 23.47.

*Ethyl 6-methyl-5-oxo-1-(p-tolyl)-1,5-dihydro-[1,2,4]triazolo [3,4-c][1,2,4]triazine-3-carboxylate (**11Bb**)*

White crystals: mp 230°C (DMF), yield (1.35 g, 69%). IR (KBr): ν=1748 (COOEt), 1672 (CO) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ=1.38–1.40 (t, 3H, CH_3CH_2), 2.32 (s, 3H, CH_3), 2.39 (s, 3H, $CH_3C_6H_4$), 4.48–4.52 (q, 2H, CH_2CH_3), 7.40 (d, 2H) and 7.85 (d, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ=13.9, 18.6, 20.6, 62.8, 110.0, 120.6, 129.6, 133.2, 134.3, 137.8, 154.3, 155.2, 160.5. MS, *m/z* (%): 313 (M^+, 14.8), 105 (100). $C_{15}H_{15}N_5O_3$ (313.12): C, 57.50; H, 4.83; N, 22.35. Found: C, 57.42; H, 4.86; N, 22.38.

*Ethyl 1-(4-chlorophenyl)-6-methyl-5-oxo-1,5-dihydro-[1,2,4] triazolo[3,4-c][1,2,4]triazine-3-carboxylate (**11Bc**)*

White crystals: mp 202°C (DMF), yield (1.53 g, 73%). IR (KBr): ν=1751 and 1670 (2CO) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ=1.36–1.41 (t, 3H, CH_3CH_2), 2.33 (s, 3H, CH_3), 4.47–4.53 (q, 2H, CH_2CH_3), 7.69 (d, 2H) and 8.04 (d, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ=13.8, 18.0, 62.9, 122.4, 129.5, 132.3, 134.5, 134.6, 148.3, 154.1, 155.3, 160.3. MS, *m/z* (%): 333 (M^+, 9.9), 334 (M^{+1}, 2.5), 335 (M^{+2}, 3.5), 125 (100). $C_{14}H_{12}ClN_5O_3$ (333.06): C, 50.39; H, 3.62; Cl, 10.62; N, 20.99. Found: C, 50.20; H, 3.65; Cl, 10.64; N, 20.95.

*3-Benzoyl-6-methyl-1-phenyl-[1,2,4]triazolo[3,4-c][1,2,4] triazin-5(1H)-one (**11Da**)*

Yellow crystals: mp 224°C (DMF-Alc), yield (1.65 g, 79%). IR (KBr): ν=1715, 1663 (2CO) cm^{-1}. ^1H NMR

(300 MHz, DMSO-d_6): δ=2.32 (s, 3H, CH_3) and 7.48–8.24 (m, 10H). ^{13}C NMR (75 MHz, DMSO-d_6): δ=18.0, 121.2, 128.1, 128.9, 129.5, 130.5, 134.7, 135.1, 135.7, 138.8, 148.2, 155.2, 160.7, 179.7. MS, *m/z* (%): 331 (M^+, 37.7), 80 (100). $C_{18}H_{13}N_5O_2$ (331.11): C, 65.25; H, 3.95; N, 21.14. Found: C, 65.12; H, 3.96; N, 21.19.

*3-Benzoyl-6-methyl-1-(p-tolyl)-[1,2,4]triazolo[3,4-c][1,2,4]triazin-5-(1H)-one (**11Db**)*
Yellow crystals: mp 234°C (DMF), yield (1.63 g, 75%). IR (KBr): ν=1720, 1660 (2CO) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ=2.31 (s, 3H, CH_3), 2.38 (s, 3H, $CH_3C_6H_4$) and 7.38–8.23 (m, 9H). MS, *m/z* (%): 345 (M^+, 4.6), 105 (100). $C_{19}H_{15}N_5O_2$ (345.12): C, 66.08; H, 4.38; N, 20.28. Found: C, 65.92; H, 4.36; N, 20.29.

*6-Methyl-5-oxo-N-phenyl-1-(p-tolyl)-1,5-dihydro-[1,2,4]triazolo[3,4-c][1,2,4]triazine-3-carboxamide (**11Eb**)*
White crystals: mp 250°C (DMF), yield (1.63 g, 72%). IR (KBr): ν=3296 (NH), 1709 and 1660 (2CO) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ=2.34 (s, 3H, CH_3), 2.39 (s, 3H, $CH_3C_6H_4$), 7.19–7.98 (m, 9H) and 10.94 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ=18.0, 20.6, 120.5, 120.9, 125.0, 128.9, 129.7, 133.4, 136.9, 137.3, 137.5, 147.9, 155.1, 157.1, 160.6. MS, *m/z* (%): 360 (M^+, 42.7), 63 (100). $C_{19}H_{16}N_6O_2$ (360.13): C, 63.33; H, 4.48; N, 23.32. Found: C, 63.20; H, 4.49; N, 23.36.

*6-Methyl-1-phenyl-3-(thiophene-2-carbonyl)-[1,2,4]triazolo[3,4-c][1,2,4]triazin-5(1H)-one (**11Fa**)*
Red crystals: mp 240°C (DMF), yield (1.42 g, 67%). IR (KBr): ν=1670 and 1653 (2CO) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ=2.33 (s, 3H, CH_3) and 7.39–8.40 (m, 8H). MS, *m/z* (%): 337 (M^+, 7.5), 80 (100). $C_{16}H_{11}N_5O_2S$ (337.06): C, 56.97; H, 3.29; N, 20.76; S, 9.50. Found: C, 56.85; H, 3.32; N, 20.78; S, 9.54.

*6-Methyl-3-(thiophene-2-carbonyl)-1-(p-tolyl)-[1,2,4]triazolo[3,4-c][1,2,4]triazin-5(1H)-one (**11Fb**)*
Yellow crystals: mp 236°C (DMF-Alc), yield (1.39 g, 63%). IR (KBr): ν=1673 and 1657 (2CO) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ=2.32 (s, 3H, CH_3), 2.40 (s, 3H, $CH_3C_6H_4$) and 7.39–8.38 (m, 7H). ^{13}C NMR (75 MHz, DMSO-d_6): δ=18.0, 20.6, 120.6, 121.0, 129.4, 129.8, 133.3, 137.8, 138.2, 138.9, 140.1, 146.1, 155.2, 158.6, 160.4. MS, *m/z* (%): 351 (M^+, 68.6), 148 (100). $C_{17}H_{13}N_5O_2S$ (351.08): C, 58.11; H, 3.73; N, 19.93; S, 9.12. Found: C, 58.02; H, 3.78; N, 19.99; S, 9.10.

*3-(Furan-2-carbonyl)-6-methyl-1-phenyl-[1,2,4]triazolo[3,4-c][1,2,4]tri-azin-5(1H)-one (**11Ga**)*
Orange crystals: mp 250°C (DMF), yield (1.23 g, 61%). IR (KBr): ν=1670 and 1661 (2CO) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ=2.32 (s, 3H, CH_3) and 6.90–8.31 (m, 8H). MS, *m/z* (%): 321 (M^+, 66.3), 168 (100). $C_{16}H_{11}N_5O_3$ (321.09): C, 59.81; H, 3.45; N, 21.80. Found: C, 59.73; H, 3.46; N, 21.83.

*6-Methyl-1-phenyl-3-styryl-[1,2,4]triazolo[3,4-c][1,2,4]triazin-5(1H)-one (**11Ha**)*
Yellow crystals: mp 198°C (DMF), yield (1.42 g, 69%). IR (KBr): ν=1670 (CO) cm^{-1}. MS, *m/z* (%): 329 (M^+, 15.8), 287 (100). $C_{19}H_{15}N_5O$ (329.13): C, 69.29; H, 4.59; N, 21.26. Found: C, 69.18; H, 4.56; N, 21.29.

*6-Methyl-1,3-diphenyl-[1,2,4]triazolo[3,4-c][1,2,4]triazin-5(1H)-one (**11Ia**)*
White crystals: mp 228°C (CH_3CN), yield (1.39 g, 73%). IR (KBr): ν=1678 (CO) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ=2.33 (s, 3H, CH_3) and 7.39–8.22 (m, 10H). ^{13}C NMR (75 MHz, DMSO-d_6): δ=18.2, 120.3, 123.6, 127.2, 127.9, 128.9, 129.3, 131.6, 136.2, 154.4, 160.6. MS, *m/z* (%): 303 (M^+, 47.2), 77 (100). $C_{17}H_{13}N_5O$ (303.11): C, 67.32; H, 4.32; N, 23.09. Found: C, 67.26; H, 4.35; N, 23.11.

General procedure for the synthesis of 3-(3-(dimethylamino)acryloyl)-6-methyl-1-aryl-[1,2,4]triazolo[3,4-c][1,2,4]triazin-5(1H)-one (12a–c)
To compounds **11a–c** (30 mmol) in dioxane (50 ml), dimethylformamide dimethyl acetal (DMF-DMA) (4 ml, 30 mmol) was added. The reaction mixture was refluxed for 6 h.The solid product was collected and crystallized from a suitable solvent to afford compounds **12a–c**.

*3-(3-(Dimethylamino)acryloyl)-6-methyl-1-phenyl-[1,2,4]triazolo[3,4-c][1,2,4]triazin-5(1H)-one (**12a**)*
Orange crystals: mp 228°C (DMF), yield (7.0 g, 72%). IR (KBr): ν=1706 and 1666 (2CO) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ=2.48 (s, 3H, CH_3), 3.00 (s, 3H, CH_3N), 3.23 (s, 3H, CH_3N), 5.8 (d, 1H, CH) and 7.26–8.15 (m, 6H, Ph and CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ=18.9, 37.9, 45.8, 89.0, 121.2, 126.3, 128.1, 129.5, 136.2, 147.9, 155.8, 156.6, 161.6, 164.1. MS, *m/z* (%): 324 (M^+, 7.5), 80 (100). $C_{16}H_{16}N_6O_2$ (324.13): C, 59.25; H, 4.97; N, 25.91. Found: C, 59.12; H, 4.99; N, 25.93.

*3-(3-(Dimethylamino)acryloyl)-6-methyl-1-(p-tolyl)-[1,2,4]triazolo[3,4-c][1,2,4]triazin-5(1H)-one (**12b**)*
Orange crystals: mp 240°C (DMF), yield (6.5 g, 64%). IR (KBr): ν=1700 and 1669 (2CO) cm^{-1}. MS, *m/z*

(%): 338 (M^+, 11.8), 80 (100). $C_{17}H_{18}N_6O_2$ (338.15): C, 60.34; H, 5.36; N, 24.84. Found: C, 60.26; H, 5.38; N, 24.89.

*1-(4-Chlorophenyl)-3-(3-(dimethylamino)acryloyl)-6-methyl-[1,2,4]triazolo[3,4-c][1,2,4]tri-azin-5(1H)-one (**12c**)*

Orange crystals: mp 252°C (DMF), yield (6.4 g, 60%). IR (KBr): ν=1698 and 1668 (2CO) cm^{-1}. MS (EI, 70 eV) *m/z* (%): 358 (M^+, 12.1), 359 (M^{+1}, 3.0), 360 (M^{+2}, 4.2) 100 (100). $C_{16}H_{15}ClN_6O_2$ (358.09): C, 53.56; H, 4.21; Cl, 9.88; N, 23.42. Found: C, 53.43; H, 4.25; Cl, 9.85; N, 23.46.

General procedure for the synthesis of 3-(2-arylidenehydrazinyl)-6-methyl-1,2,4-triazin-5(2H)-one (15a–h)

A mixture of 3-hydrazinyl-6-methyl-1,2,4-triazin-5 (2H)-one **14** (0.7 g, 5.0 mmol) and the appropriate aldehyde (5.0 mmol) in ethanol (30 ml) was refluxed for 6 h in the presence of few drops of acetic acid. The reaction mixture was cooled, the precipitate that got separated was collected, and crystallized from the suitable solvent to give the corresponding 3-(2-arylidenehydrazinyl)-6-methyl-1,2,4-triazin-5(2H)-one (**15a–h**).

*6-Methyl-3-(2-(4-methylbenzylidene)hydrazinyl)-1,2,4-triazin-5(2H)-one (**15a**)*

White crystals: mp 292°C (DMF), yield (0.89 g, 74%). IR (KBr): ν=3330 and 3221 (2NH) and 1678 (CO) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ=2.10 (s, 3H, CH_3), 2.33 (s, 3H, $CH_3C_6H_4$), 7.2 (d, 2H), 7.80 (d, 2H), 8.04 (s, 1H, CH), 11.51 (s, 1H, NH) and 12.59 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ=16.9, 21.0, 127.4, 129.2, 131.3, 139.6, 144.8, 147.9, 153.6, 162.3. MS, *m/z* (%): 243 (M^+, 100). $C_{12}H_{13}N_5O$ (243.11): C, 59.25; H, 5.39; N, 28.79. Found: C, 59.16; H, 5.43; N, 28.82.

*3-(2-(2,4-Dichlorobenzylidene)hydrazinyl)-6-methyl-1,2,4-triazin-5(2H)-one (**15b**)*

Yellow crystals: mp 296–298°C (DMF), yield (1.02 g, 69%). IR (KBr): ν=3339 and 3213 (2NH) and 1665 (CO) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ=2.09 (s, 3H, CH_3), 7.38–8.44 (m, 3H, aromatic), 8.47 (s, 1H, CH), 11.81 (s, 1H, NH) and 12.72 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ=16.9, 127.5, 128.9, 130.3, 133.4, 134.8, 139.5, 148.2, 153.5, 162.2, 162.8. MS, *m/z* (%): 297 (M^+, 31.1), 298 (M^{+1}, 7.83), 299 (M^{+2}, 20.0), 123 (100). $C_{11}H_9Cl_2N_5O$ (297.02): C, 44.32; H, 3.04; Cl, 23.78; N, 23.49. Found: C, 44.16; H, 3.08; Cl, 23.80; N, 23.53.

*3-(2-Ethylidenehydrazinyl)-6-methyl-1,2,4-triazin-5(2H)-one (**15c**)*

White crystals: mp 248–250°C (DMF), yield (0.59 g, 71%). IR (KBr): ν=3327 and 3214 (2NH) and 1666 (CO) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ=1.89 (d, 3H, CH_3), 2.13 (s, 3H, CH_3), 7.42 (q, 1H, CH), 11.03 (s, 1H, NH) and 12.36 (s, 1H, NH). MS (EI, 70 eV) *m/z* (%): 167 (M^+, 46.7), 152 (100). $C_6H_9N_5O$ (167.08): C, 43.11; H, 5.43; N, 41.89. Found: C, 43.02; H, 5.46; N, 41.95.

*6-Methyl-3-(2-(2-methylpropylidene)hydrazinyl)-1,2,4-triazin-5(2H)-one (**15d**)*

White crystals: mp 218–220°C (DMF), yield (0.76 g, 79%). IR (KBr): ν=3335 and 3219 (2NH) and 1670 (CO) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ=1.05 (d, 6H, $(CH_3)_2CH$), 2.06 (s, 3H, CH_3), 2.44 (m, 1H, $CH(CH_3)_2$), 7.37 (d, 1H, CH), 11.13 (s, 1H, NH) and 12.27 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ=16.8, 16.9, 19.6, 30.9, 147.8, 153.7, 154.2, 162.9. MS, *m/z* (%): 195 (M^+, 22.1), 80 (100). $C_8H_{13}N_5O$ (195.11): C, 49.22; H, 6.71; N, 35.87. Found: C, 49.13; H, 6.75; N, 35.89.

*3-(2-(Benzo[d][1,3]dioxol-5-ylmethylene)hydrazinyl)-6-methyl-1,2,4-triazin-5(2H)-one (**15e**)*

White crystals: mp 292–294°C (DMF), yield (1.02 g, 75%). IR (KBr): ν=3330 and 3210 (2NH) and 1660 (CO) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ=2.10 (s, 3H, CH_3), 6.07 (s, 2H, CH_2), 6.92–7.95 (m, 3H), 7.98 (s, 1H, CH), 11.50 (s, 1H, NH) and 12.63 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ=16.9, 101.4, 105.5, 108.0, 123.8, 128.6, 147.9, 148.0, 148.9, 153.6, 154.0, 162.2. MS, *m/z* (%): 273 (M^+, 67.1), 134 (100). $C_{12}H_{11}N_5O_3$ (273.09): C, 52.75; H, 4.06; N, 25.63. Found: C, 52.64; H, 4.10; N, 25.68.

*6-Methyl-3-(2-(pyridin-4-ylmethylene)hydrazinyl)-1,2,4-triazin-5(2H)-one (**15f**)*

Yellow crystals: mp 296–298°C (DMSO), yield (0.82 g, 72%). IR (KBr): ν=3329 and 3207 (2NH) and 1663 (CO) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ=2.12 (s, 3H, CH_3), 7.87–8.04 (m, 4H), 8.61 (s, 1H, CH), 11.80 (s, 1H, NH) and 12.79 (s, 1H, NH). MS, *m/z* (%): 230 (M^+, 34.5), 120 (100). $C_{10}H_{10}N_6O$ (230.09): C, 52.17; H, 4.38; N, 36.50. Found: C, 52.00; H, 4.42; N, 36.57.

*6-Methyl-3-(2-(thiophen-2-ylmethylene)hydrazinyl)-1,2,4-triazin-5(2H)-one (**15g**)*

Yellow crystals: mp 286–288°C (DMF), yield (0.81 g, 69%). IR (KBr): ν=3339 and 3210 (2NH) and 1676 (CO) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ=2.09

(s, 3H, CH_3), 7.11–7.68 (m, 3H), 8.27 (s, 1H, CH), 11.67 (s, 1H, NH) and 12.53 (s, 1H, NH). 13C NMR (75 MHz, DMSO-d_6): δ=17.9, 121.3, 129.3, 131.1, 138.9, 148.3, 153.6, 154.1, 160.4. MS, *m/z* (%): 235 (M^+, 61.5), 96 (100). $C_9H_9N_5OS$ (235.05): C, 45.95; H, 3.86; N, 29.77; S, 13.63. Found: C, 45.80; H, 3.90; N, 29.81; S, 13.68.

*3-(2-(1-(1H-Indol-3-yl)ethylidene)hydrazinyl)-6-methyl-1,2,4-triazin-5(2H)-one (**15h**)*

White crystals: mp 270°C (DMF), yield (1.04 g, 74%). IR (KBr): ν=3334, 3280 and 3204 (3NH) and 1664 (CO) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ=2.13 (s, 3H, CH_3), 7.11–7.85 (m, 4H), 8.37 (s, 1H, CH), 8.47 (s, 1H, CH-Indole), 11.20 (s, 1H, NH-Indole), 11.61 (s, 1H, NH) and 12.27 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ=17.0, 111.2, 111.5, 120.3, 122.6, 124.1, 130.6, 137.0, 143.2, 147.7, 153.3, 154.5, 163.0. $C_{13}H_{12}N_6O$ (268.3): C, 58.20; H, 4.51; N, 31.33. Found: C, 58.02; H, 4.46; N, 31.27.

General procedure for the synthesis of 6-methyl-3-(2-(1-aryl-ethyl-idene)hydrazinyl)-1,2,4-triazin-5(2H)-one (16a–h)

A mixture of 3-hydrazinyl-6-methyl-1,2,4-triazin-5 (2H)-one **14** (0.7 g, 5.0 mmol) and the appropriate ketone or pyruvic acid or ethyl pyruvate (5.0 mmol) in absolute ethanol (30 ml) was refluxed for 6 h in the presence of few drops of acetic acid. The reaction mixture in each case was cooled; the precipitate that separated was collected and crystallized from the suitable solvent to give the corresponding hydrazone derivatives **16a–h**.

*6-Methyl-3-(2-(1-phenylethylidene)hydrazinyl)-1,2,4-triazin-5(2H)-one (**16a**)*

White crystals: mp 246°C (dioxane), yield (0.95 g, 79%). IR (KBr): ν=3328 and 3210 (2NH) and 1669 (CO) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ=2.11 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 7.38–8.05 (m, 5H, Ph), 10.98 (s, 1H, NH) and 12.66 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ=13.9, 17.0, 120.6, 126.7, 129.2, 137.5, 147.9, 151.8, 153.7, 160.4. MS, *m/z* (%): 243 (M^+, 12.0), 59 (100). $C_{12}H_{13}N_5O$ (243.27): C, 59.25; H, 5.39; N, 28.79. Found: C, 59.20; H, 5.41; N, 28.76.

*6-Methyl-3-(2-(1-(pyridin-2-yl)ethylidene)hydrazinyl)-1,2,4-triazin-5(2H)-one (**16b**)*

Yellow crystals: mp 248°C (dioxane), yield (0.98 g, 81%). IR (KBr): ν=3324 and 3224 (2NH) and 1679 (CO) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ=2.09 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 7.35–8.56 (m, 4H, pyridine), 11.00 (s, 1H, NH) and 12.69 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ=12.1, 16.9, 121.2, 123.8, 124.5, 136.2, 138.4, 148.2, 151.3, 153.9, 162.6. MS, *m/z* (%): 244 (M^+, 24.9), 78 (100). $C_{11}H_{12}N_6O$ (244.26): C, 54.09; H, 4.95; N, 34.41. Found: C, 54.13; H, 4.90; N, 34.39.

*6-Methyl-3-(2-(1-(pyridin-3-yl)ethylidene)hydrazinyl)-1,2,4-triazin-5(2H)-one (**16c**)*

Yellow crystals: mp 286°C (DMF), yield (0.92 g, 76%). R (KBr): ν=3329 and 32208 (2NH) and 1672 (CO) cm^{-1}. MS, *m/z* (%): 244 (M^+, 34.9), 243 (100). $C_{11}H_{12}N_6O$ (244.26): C, 54.09; H, 4.95; N, 34.41. Found: C, 54.00; H, 4.99; N, 34.35.

*6-Methyl-3-(2-(1-(pyridin-4-yl)ethylidene)hydrazinyl)-1,2,4-triazin-5(2H)-one (**16d**)*

Yellow crystal; mp 268°C (DMF), yield (0.94 g, 78%). IR (KBr): ν=3341 and 3215 (2NH) and 1664 (CO) cm^{-1}. MS, *m/z* (%): 244 (M^+, 10.3), 64 (100). $C_{11}H_{12}N_6O$ (244.26): C, 54.09; H, 4.95; N, 34.41. Found: C, 53.98; H, 4.89; N, 34.44.

*6-Methyl-3-(2-(1-(thiophen-2-yl)ethylidene)hydrazinyl)-1,2,4-triazin-5(2H)-one (**16e**)*

White crystals: mp 260°C (DMF), yield (0.90 g, 73%). IR (KBr): ν=3334 and 3214 (2NH) and 1670 (CO) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ=2.10 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 7.07–7.60 (m, 3H, thienyl), 11.10 (s, 1H, NH) and 12.38 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ=14.9, 16.8, 120.6, 127.5, 128.5, 138.2, 146.6, 152.1, 153.8, 162.3. MS, *m/z* (%): 249 (M^+, 27.71), 59 (100). $C_{10}H_{11}N_5OS$ (249.29): C, 48.18; H, 4.45; N, 28.09; S, 12.86. Found: C, 48.21; H, 4.42; N, 28.07; S, 12.89.

*3-(2-(1-(9H-Fluoren-2-yl)ethylidene)hydrazinyl)-6-methyl-1,2,4-triazin-5(2H)-one (**16f**)*

Yellow crystals: mp 288°C (DMF), yield (1.29 g, 79%). IR (KBr): ν=3338 and 3211 (2NH) and 1677 (CO) cm^{-1}. MS, *m/z* (%): 331 (M^+, 24.1), 205 (100). $C_{19}H_{17}N_5O$ (331.38): C, 68.87; H, 5.10; N, 21.13. Found: C, 68.90; H, 5.15; N, 21.10.

*2-(2-(6-Methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl) hydrazono)propan-oic acid (**16g**)*

White crystals: mp 236–238°C (DMF), yield (0.77 g, 65%). IR (KBr): ν=3578 (OH), 3484 and 3222 (2NH), 1729 and 1660 (2CO) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ=2.08 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 11.87 (s, 1H, NH), 12.33 (s, 1H, NH) and 12.84 (s, 1H, OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ=15.9, 16.8, 146.6, 151.0, 153.4, 164.5, 165.6. MS, *m/z* (%): 211 (M^+, 30.6), 126 (100). $C_7H_9N_5O_3$ (211.20): C,

39.81; H, 4.30; N, 33.16. Found: C, 39.89; H, 4.28; N, 33.13.

*Ethyl 2-(2-(6-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl) hydrazono)-propanoate (**16h**)*

White crystals: mp 198–200°C (DMF), yield (0.77 g, 65%). IR (KBr): ν=3460 and 3214 (2NH), 1718 and 1672 (2CO) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ=1.27–1.31 (t, 3H, CH_3CH_2), 2.05 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 4.18–4.25 (q, 2H, CH_2CH_3), 11.25 (s, 1H, NH) and 12.45 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ=13.0, 13.9, 16.7, 61.0, 145.1, 150.1, 153.5, 161.8, 164.1. MS (EI, 70 eV) *m/z* (%): 239 (M^+, 31.3), 166 (100). $C_9H_{13}N_5O_3$ (239.10): C, 45.19; H, 5.48; N, 29.27. Found: C, 45.02; H, 5.46; N, 29.30.

Biological activity

Antibacterial activity was investigated in-vitro on Gram-positive bacteria *Bacillus subtilis* (ATCC 6051) and *Staphylococcus aureus* (ATCC 12600) and on Gram-negative bacteria *Escherichia coli* (ATCC 1175) and *Pseudomonas aeruginosa* (ATCC 10145). Moreover, antifungal activity against *Aspergillus flavus* (ATCC 15442) and *Candida albicans* (ATCC 26555) was investigated. All these microorganisms were performed at the Microanalytical Center, Cairo University.

The antibacterial and antifungal activity assays were carried out using the diffusion plate method [24,25]. A bottomless cylinder containing a measured quantity (1 ml, mg/ml) of the sample is placed on a plate (9 cm diameter) containing a solid bacterial medium (Kirby–Bauer agar) or fungal medium (Dox's medium), which has been heavily seeded with a spore suspension of the test organism. After incubation at 35±2°C (24 h for bacteria and 5 days for fungi) the diameter of the clear zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism. The solvent used was DMSO and the concentration of the sample used is 10 µl. The references antibiotics Ampicillin and Amphotericin were used as references to evaluate the relative potency of the tested compounds under the same conditions.

Antimicrobial activity evaluation

Antimicrobial activity of the tested compounds was determined using a modified Kirby–Bauer disk diffusion method [24]. Briefly, 100 µl of the test bacteria/fungi was grown in 10 ml of fresh media until they reached a count of ~10^8 cells/ml for bacteria or 10^5 cells/ml for fungi [25]. A measure of 100 µl of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained.

Of the many media available, NCCLS recommends Mueller–Hinton agar or fungal medium (Dox's medium) due to: it results in good batch-to-batch reproducibility. The disk diffusion method for filamentous fungi was tested by using the approved standard method (M38-A) developed by the NCCLS (2002) [26] for evaluating the susceptibilities of filamentous fungi to antifungal agents. The disc diffusion method for yeasts was developed by using the approved standard method (M44-P) by NCCLS (2009) [27].

Minimum inhibitory concentration evaluation

The minimum inhibitory concentration (MIC) values were measured by the broth dilution method [28,29]. A measure of 500 ml of a stock solution (10.24 mg/ml) of each tested compound in DMSO was prepared and then diluted with Mueller–Hinton broth to 1024 µg/ml. The strains were grown briefly at 37°C in Mueller–Hinton media. After 5 h of bacterial growth, the bacterial culture was diluted to obtain a concentration of 5×10^5 cells/ml. Then, 150 µl bacterial suspensions were added to each well of the flat-bottomed 96-well tissue culture plate. Two-fold serial dilutions were carried out from the first well to the tenth well; the final concentrations of the compounds ranged from 1 to 512 µg/ml; and excess media (150 µl) were discarded from the last well. The plates were incubated at 37°C for 24 h in an electro-heating standing temperature cultivator and were read visually. The MIC of the sample showing no turbidity was recorded as the lowest concentration of compound that inhibited bacterial growth completely. Each assay was run in triplicate.

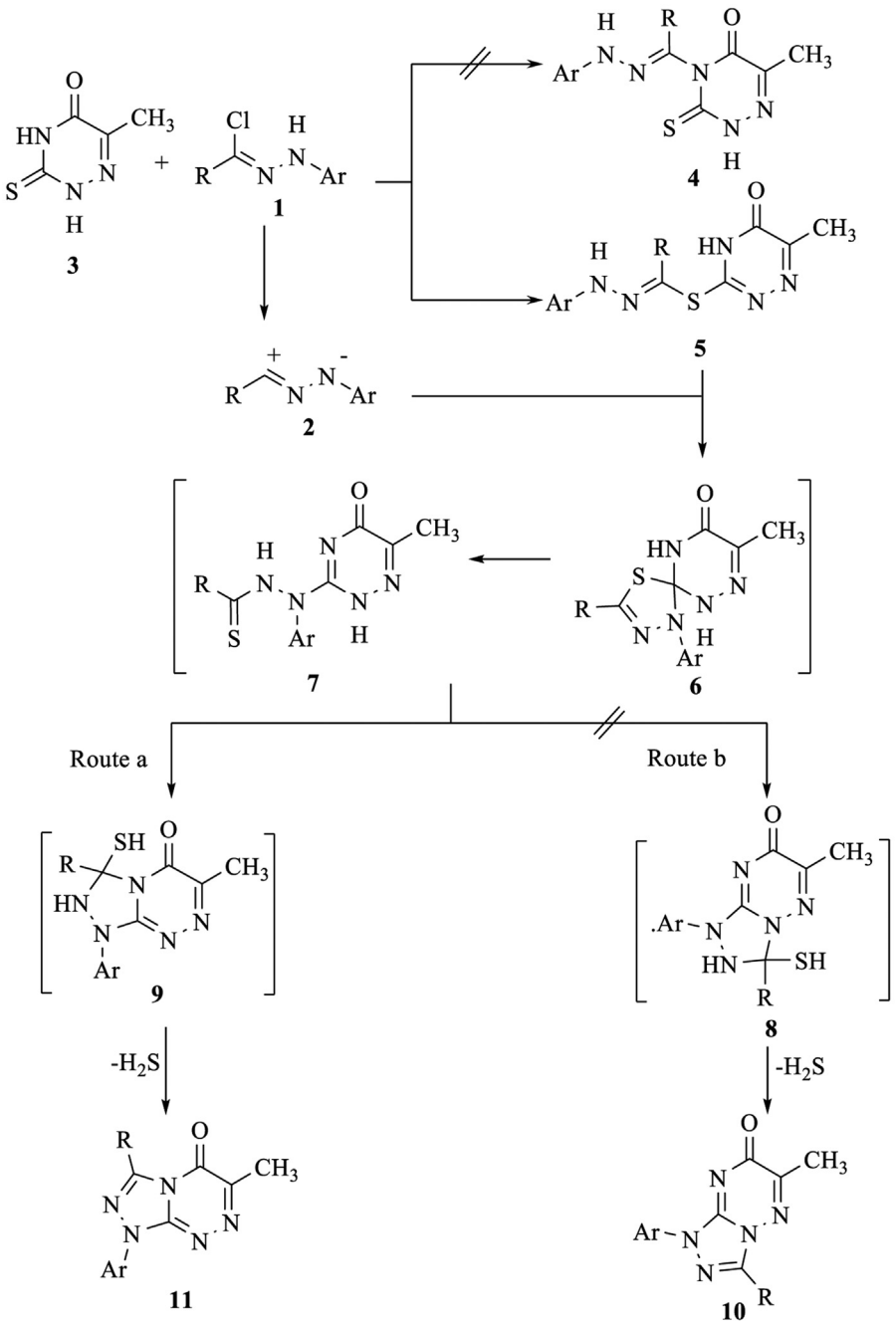
Results and discussion

Chemistry

Reaction of thiothymine **3** [14] with hydrazonoyl chloride **1** in refluxing chloroform in the presence of triethylamine yielded, in each case, a single product. As expected from all our previous results with related compounds [30–32], these products did not contain sulfur. On the basis of this finding, the structures **4–9** were overlooked for the products. Spectral and microanalytical measurements agree with one of the two isomeric forms **10** or **11**. The reaction pathway for obtaining **10** and **11** is believed to proceed by the first production of thiohydrazonate **5** or amidrazone **4**.

Since a structure like **4** is known to be stable [17,32,33], its formation was excluded due to failure of all attempts to its isolation. Moreover, interaction of thiouracils with halogenated reagents affords only S-substituted compounds [8–11]. Upon S→N rearrangement, thiohydrazonates **5** yield the corresponding thiohydrazides **7** via intermediate **6**. The latter can also be produced through 1,3-dipolar cycloaddition of nitrilimines **2** (produced by base-catalyzed dehydrochlorination of the hydrazonoyl chloride **1**) to the thione group C=S. The latter intermediates **7** undergo cyclization followed by elimination of hydrogen sulphide to give the final product **11**. Structural elucidations of the new compounds were confirmed by elementary and spectroscopic analyses (see Experimental). Their structures were assigned **11** rather than the isomeric structure **10** (Scheme 1). For example, the ^{13}C NMR spectrum of **11Aa** showed the

Scheme 1



R: A, CH_3CO; B, C_2H_5OCO; C,CH_3OCO ; D, PhCO; E, PhNHCO; F, 2-Thenoyl; G, 2-Furoyl; H, Cinnamoyl; I, Ph

Ar: a, Ph; b, 4-$CH_3C_6H_4$; c, 4-ClC_6H_4

Synthesis of triazolotriazine derivatives.

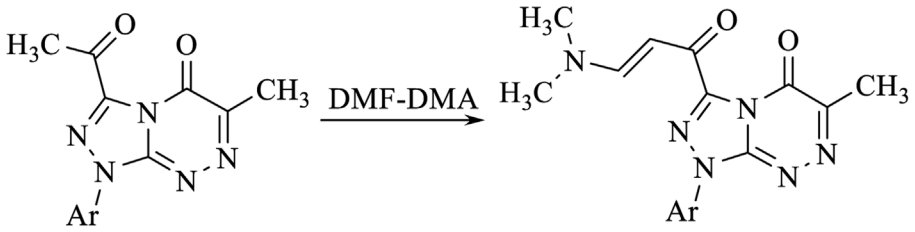
signal for the carbonyl carbon resonance at δ less than 166. This indicates that N (4) is sp^3-hybridized nitrogen atom as in structure **11**. If this nitrogen is sp^2-hybridized as in structure **10**, the signal for the carbonyl carbon will appear near δ 166 [34]. Also, the IR spectrum of **11Aa** showed two carbonyl bands at ν 1709 and 1670 cm^{-1}. Its ^1H NMR spectrum showed two singlet signals at δ 2.32 (3H, CH_3) and 2.71 (3H, CH_3CO), in addition to the multiplet signal at δ 7.46–8.06 (5H) corresponding to aromatic protons (Ph). On the basis of the above data, the structure of **11** is 6-methyl-[1,2,4]triazolo[3,4-*c*][1,2,4]triazine derivatives.

Treatment of compound **11A** with dimethylformamide dimethyl acetal (DMF-DMA) in refluxing dioxane yielded the appropriate enamine **12**. Compatible elementary and spectroscopic analyses were gained for compounds **11A** and **12** (see Experimental) (Scheme 2).

Stirring of **3** with dimethylsulphate in sodium hydroxide solution yielded 6-methyl-3-(methylthio)-1,2,4-triazin-5(4H)-one **13** (Scheme 3) [15]. Treatment of compound **13** with hydrazine hydrate in refluxing ethanol yielded the corresponding hydrazine derivative **14** [15] (Scheme 3). Reaction of hydrazine derivative **14** with the appropriate aldehyde in the refluxing ethanol in the presence of few drops of acetic acid afforded, in each case, one isolated product hydrazone **15** (Scheme 3). The structures of isolated products were identified by their elemental and spectroscopic analyses. For example, the ^1H NMR spectrum of **15a** shows two singlet signals at δ 2.10 (3H, CH_3) and 2.33 (3H, $CH_3C_6H_4$), the pair of doublet of an aromatic protons at δ 7.20 (2H) and 7.80 (2H) in addition to two singlet signals exchangeable protons at δ 11.51 (1H, NH) and 12.59 (1H, NH). Also, its ^{13}C NMR spectrum showed 10 signals.

Next, the treatment of hydrazine **14** with the appropriate ketone, pyruvic acid, or ethyl pyruvate in the refluxing ethanol in the presence of few drops of acetic acid afforded, in each case, one isolated product hydrazone **16** (Scheme 4). The structures of isolated products were identified by their elemental and spectroscopic (IR, ^1H NMR, ^{13}C NMR and MS) analyses (see Experimental).

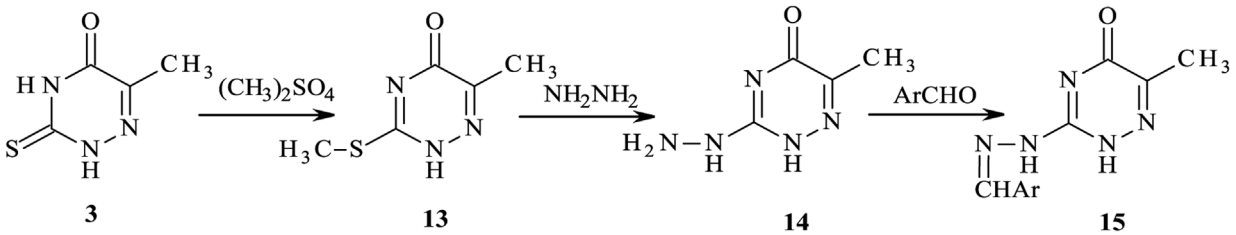
Scheme 2



Ar: a, Ph; b, 4-$CH_3C_6H_4$; c, 4-ClC_6H_4

Synthesis of enaminone derivatives.

Scheme 3



Ar: a, 4-$CH_3C_6H_4$; b, 2,4-$Cl_2C_6H_3$;c, CH_3; d, $(CH_3)_2CH$;
e, Piperonyl; f, 4-Pyridinyl; g, 2-Thienyl; h, 3-Indolyl

Synthesis of hydrazone derivatives.

Antimicrobial screening

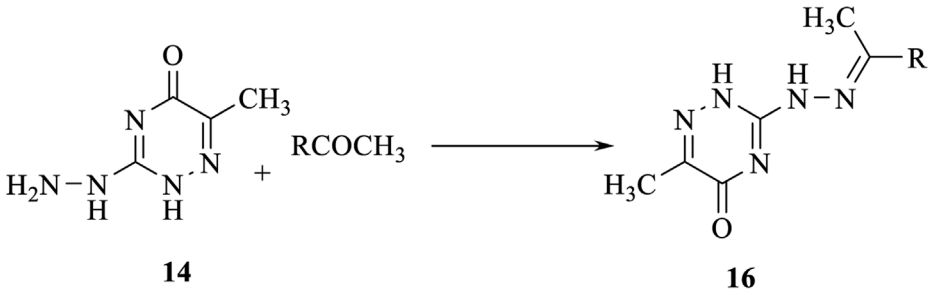
The antibacterial and antifungal activities of new synthesized compounds **14**, **15h**, and **16a–d** were studied by the disk diffusion method. The antibacterial activities were done on the following pathogenic organisms: the Gram-positive bacteria *S. aureus* and *B. subtilis*, the Gram-negative bacteria *E. coli*, and *P. aeruginosa*. Moreover, antifungal activities against *A. flavus* and *C. albicans* were studied. The synthesized compounds were used at a concentration of 20 mg/ml using DMSO as a solvent. Ampicillin 10 µl/disk was used as a standard antibacterial agent and amphotericin B 20 µg/ml as the standard antifungal agent. The results presented in Table 1 suggest that two compounds **14** and **16b** inhibited both bacterial and fungal species; however, the most potent compound that exhibited antibacterial and antifungal activities was **16b**.

Compound **16b** showed high potential in growth inhibition of all tested microorganisms and exceed the activity of standard antibiotics with a relative activity of 200% in *B. subtilis*, 205.5% in *S. aureus*, 154.5% in *E. coli*, 211.7% in *P. aeruginosa*, 280% in *A. flavus*, and 166.6% in *C. albicans*. In-vitro susceptibility tests were performed to evaluate MIC by a broth dilution method [28,35,36]. MIC values were determined for the highly efficient antibacterial compounds using the most sensitive microorganisms. The results illustrated in Table 2 indicated that compound **16b** achieved the lowest MIC values (high efficient derivative) against the sensitive bacterial strain *S. aureus* and fugal strain *A. flavus* with MIC values of 20 and 14 µg/ml, respectively.

Conclusion

In summary, thiothymine **3** was proved to be a useful precursor for the convenient synthesis of a wide variety of triazolotriazine and hydrazone derivatives that have not been reported hitherto.

Scheme 4



R: a, Ph; b, 2-Pyridinyl; c, 3-Pyridinyl; d, 4-Pyridinyl; e, 2-Thienyl; f, Fluorenyl; g, COOH; h, C_2H_5OCO

Condensation reaction of hydrazine.

Table 1 In-vitro antibacterial and antifungal activities of compounds 14, 15h, 16a–d (inhibition zone in mm)a

Compounds	Antimicrobial activity											
	Bacteria								Fungi			
	Gram positive				Gram negative				Filamentous		Unicellular	
	Bacillus subtilis		*Staphylococcus aureus*		*Escherichia coli*		*Pseudomonas aeruginosa*		*Aspergillus flavus*		*Candida albicans*	
	IZ	RA%	IZ	RA%	IZ	RA%	IZ	RA%	IZ	RA%	IZ	RA%
Control: dimethyl sulfoxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0	0	0.0
Standard												
Ampicillin (antibacterial agent)	18	100	18	100	22	100	17	100				
Amphotericin B (antifungal agent)									15	100	18	100
14	16	88.8	17	77.0	15	88.0	15	83.3	0	0.0	0	0.0
15h	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0	0	0.0
16a	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0	0	0.0
16b	36	200.0	37	205.5	34	154.5	36	211.7	42	280.0	30	166.6
16c	10.0	55.5	0.0	0.0	9.0	52.9	0.0	0.0	0	0.0	0	0.0
16d	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0	0	0.0

Amphotericin B, standard antifungal agent; Ampicillin, standard antibacterial agent; RA, relative activity.

Table 2 Minimum inhibitory concentration of compound 16b against the tested bacteria and fungi

Compound	Minimum inhibitory concentration (μg/ml)			
	Bacteria		Fungi	
	Gram positive *Staphylococcus aureus*	Gram negative *Pseudomonas aeruginosa*	Filamentous *Aspergillus flavus*	Unicellular *Candida albicans*
16b	20	26	14	48

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Nil.

Conflicts of interest
There are no conflicts of interest.

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