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Synthesis of new 1-(4-amino-1,2,4-triazol-3-ylmethyl)-pyrimidinedione derivatives under microwave irradiation and their antimicrobial evaluation

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ABSTRACT

The present work represents the reaction of different hydrazides with carbon disulfide in the presence of potassium hydroxide to afford 1-(1,3,4-oxadiazol-5-ylmethyl-2-thio)-pyrimidinedione derivatives. Conventional heating and microwave irradiation (MW) of the 1,2,4-triazoles with hydrazine hydrate afforded the corresponding 4-amino-1H-1,2,4-triazole-5(4H)-thiones. Treatment of the latter compounds either with carbon disulfide in the presence of KOH or with methyl iodide followed by reaction with phthalaldehydic acid under microwave conditions using isopropyl alcohol or dimethylformamide as a solvent afforded the thioxo[1,2,4]triazolo[3,4-b]thiadiazoles and 4-amino-5-(methylthio)-4H-1,2,4-triazoles, respectively. Conventional heating and microwave irradiation (MW) of 4-amino-5-(methylthio)-4H-1,2,4-triazoles with different aromatic aldehyde afforded the corresponding schiff's bases. While reaction of 4-aminotriazoles with phthalaldehydic acid afforded different products depending on the solvent used during the microwave irradiation.

Keywords: Oxadiazole, pyrimidinediones, 1,2,4-triazoles, antimicrobial activity.

INTRODUCTION

The synthesis and screening of compound libraries increased rapidly and became important major objectives in pharmaceutical chemistry leading to new potent leads. Among the five-membered nitrogen heterocycles, 1,2,4-triazoles are associated with a broad spectrum of biological activities. Many 1,2,4-triazoles [1-14] have been reported to possess antibacterial, antifungal, antiviral, anti-inflammatory, anticonvulsant, antidepressant, antitubercular, antihypertensive, analgesic, hypoglycemic, herbicidal, and sedative properties. Our aim in the present work was the synthesis and antimicrobial evaluation of new 2,5-disubstituted 1,2,4-triazoles as well as the attachment of the synthesized derivatives to pyrimidinedione moieties. Much attention has been recently directed in our laboratory to use microwave in organic synthesis [15, 16] as a result of its extensive popularity as a powerful tool for rapid and efficient synthesis of variety of organic compounds. It has not only been used to dramatically accelerate organic reactions, but also to improve the yield. Furthermore, the method saves time, the products were easily isolated, economic, and it is friendly to the environment.

MATERIALS AND METHODS

Synthetic methods, analytical and spectral data

The melting points were measured on a Büchi melting point apparatus and are uncorrected. Dimethylformamide was kept first over alkali and then over P_2O_5. Ethanol was distilled over sodium. IR spectra (KBr) were recorded with a Bruker-Vector22 instrument (Bruker, Bremen, Germany). ^{1}H NMR spectra were recorded with a Varian Gemini spectrometer (300 MHz, DMSO-d_6) with TMS as internal standard. Chemical shifts were reported in δ scale (ppm) relative to TMS as a standard, and the coupling constants (*J* values) are given in Hz. EI-mass spectra were recorded with a HP D5988 A 1000 MHz instrument (Hewlett-Packard, Palo Alto, CA, USA). The progress of the reactions was monitored by TLC using aluminum silica gel plates 60 F_{245}. The microanalyses were performed at the microanalytical unit, Cairo University, Egypt, and were found to agree favourably with the calculated values. Irradiation was carried out in a Teflon closed vessel using a domestic microwave oven (360 Watt). Antiviral activity against HBV was tested at the Liver Institute, Menoufia University, Shebin El-Koam Egypt.

Chemistry

***1-Carbethoxymethyluracils* 2a,b**

Irradiation of a mixture of uracil derivatives **1a,b** (10 mmol) ethyl chloroacetate (1.59 g, 13 mmol) in DMF (5 ml) for 5 min. afforded **2a,b** in 98-99% yield, lit. 85-89% [17].

***1-Acetylhydrazinouracils* 3a,b**

Irradiation of a mixture of **2a,b** (10 mmol), hydrazine hydrate (98%) (2 ml) in isopropyl alcohol (4 ml) for 3 min. gave **3a,b** in a quantitative yield, lit. 90-93% [17].

***1-[(5-Mercapto-1,3,4-oxadiazol-2-yl)methyl]pyrimidine-2,4(1H,3H)-diones* 4a,b**

A mixture of hydrazides **3a,b** (1 mmol), potassium hydroxide (0.056 g, 1 mmol), carbon disulfide (2 ml) in ethanol (10 ml) was irradiated for 3-5 min. The excess of solvent was removed under reduced pressure and the residue was dissolved in water (2 ml) and acidified with 1*N* HCl to give the crude products which were recrystallized from ethanol to afford **4a,b** in 96-99% yields.

1-[(5-Mercapto-1,3,4-oxadiazol-2-yl)methyl]pyrimidine-2,4(1H,3H)-dione (**4a**)

Mp 220-222^{o}C. IR (KBr, cm^{-1}): 2610 (SH). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.45 (s, 2H, CH_2), 6.66 (d, 1H, 5-H, *J* = 5.5 Hz), 8.28 (d, 1H, 6-H, *J* = 5.5 Hz), 11.00 (brs, 1H, NH, D_2O exchangeable), 13.05 (brs, 1H, SH, D_2O exchangeable) ppm. EI-MS: *m/z* 226 [M$^+$]. Anal. Calcd. For $C_7H_6N_4O_3S$; C, 37.17; H, 2.67; N, 24.77. Found: C, 37.10; H, 2.55; N, 24.60.

1-[(5-Mercapto-1,3,4-oxadiazol-2-yl)methyl]-5-methylpyrimidine-2,4(1H,3H)-dione (**4b**)

Mp 244-245^{o}C. IR (KBr, cm^{-1}): 2555 (SH). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.10 (s, 3H, 5-CH_3), 4.43 (s, 2H, CH_2), 8.25 (s, 1H, 6-H), 11.06 (brs, 1H, NH, D_2O exchangeable), 13.11 (brs, 1H, SH, D_2O exchangeable) ppm. EI-MS: *m/z* 240 [M$^+$]. Anal. Calcd. For $C_8H_8N_4O_3S$; C, 40.00; H, 3.36; N, 23.32. Found: C, 39.90; H, 3.33; N, 23.27.

***1-[(4-Amino-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methyl]pyrimidine-2,4(1H,3H)-diones* 5a,b**

Method A. 1,3,4-Oxadiazole-2-thiol derivatives **4a,b** (10 mmol) and hydrazine hydrate (1.5 g, 30 mmol) were dissolved in ethanol (30 ml), and the reaction mixture was refluxed for 6-9 h, then left to cool. The crude products were filtered, dried, and recrystallized from ethanol to give **5a,b** in 70-86% yields.

Method B. 1,3,4-Oxadiazole-2-thiol derivatives **4a,b** (1 mmol) and hydrazine hydrate (0.15 g, 3 mmol) were dissolved in isopropyl alcohol (3 ml), and the mixture was irradiated for 3 min. The crude products were filtered, dried, and recrystallized from ethanol to give **5a,b** in 95-98% yields.

1-[(4-Amino-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methyl]pyrimidine-2,4(1H,3H)-dione (**5a**)

Mp 238-239^{o}C. IR (KBr, cm^{-1}): 3330 (NH_2 + NH), 1670 (C=N), 1275 (C=S). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.52 (s, 2H, CH_2), 5.76 (brs, 2H, NH_2, D_2O exchangeable), 6.60 (d, 1H, 5-H, *J* = 5.5 Hz), 8.22 (d, 1H, 6-H, *J* = 5.5 Hz), 11.09 (brs, 1H, NH, D_2O exchangeable), 13.55 (brs, 1H, NH, D_2O exchangeable) ppm. EI-MS: *m/z* 240 [M$^+$]. Anal. Calcd. For $C_7H_8N_6O_2S$; C, 35.00; H, 3.36; N, 34.98. C, 34.91; H, 3.30; N, 34.88.

1-[(4-Amino-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)methyl]-5-methylpyrimidine-2,4(1H,3H)-dione (**5b**)
Mp 253-254^{o}C. IR (KBr, cm^{-1}): 3310 (NH_2 + NH), 1678 (C=N), 1260 (C=S). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.14 (s, 3H, 5-CH_3), 4.53 (s, 2H, CH_2), 5.78 (brs, 2H, NH_2, D_2O exchangeable), 8.20 (s, 1H, 6-H), 11.04 (brs, 1H, NH, D_2O exchangeable), 13.89 (brs, 1H, NH, D_2O exchangeable) ppm. EI-MS: *m/z* 254 [M^{+}]. Anal. Calcd. For $C_8H_{10}N_6O_2S$; C, 37.79; H, 3.96; N, 33.05. Found: C, 37.60; H, 3.90; N, 32.93.

***1-{6-Thioxo-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-ylmethyl}pyrimidine-2,4(1H,3H)-diones* 6a,b**
Method A. Carbon disulfide (0.152 g, 2 mmol) was added dropwise to a solution of **5a,b** (1 mmol) in ethanol (10 ml) containing aq. KOH (5%, 15 ml) at 0^{o}C. The reaction mixture was refluxed for 8 h, cooled, and poured onto ice-HCl. The obtained solid was filtered off and recrystallized from ethanol to afford **6a,b** in 75-78% yields as pale yellow powders.

Method B. A mixture of **5a,b** (1 mmol), aq. KOH (5%, 15 ml), and ethanol (5 ml) was treated with carbon disulfide (0.152 g, 2 mmol) at 0^{o}C. The reaction mixture was placed in a Teflon closed vessel and irradiated in a domestic microwave oven for 3-5 min. The mixture was cooled and poured onto ice-HCl. The obtained solid was filtered off and recrystallized from ethanol to afford **6a,b** in 96-98% yield as pale yellow powders.

1-{6-Thioxo-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-ylmethyl}pyrimidine-2,4(1H,3H)-dione (**6a**)
Mp 188-189^{o}C. IR (KBr, cm^{-1}): 3135 (NH), 1575 (C=N), 1105 (C=S). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 4.45 (s, 2H, CH_2), 6.58 (d, 1H, 5-H, *J* = 5.5 Hz), 8.20 (d, 1H, 6-H, *J* = 5.5 Hz), 11.02 (brs, 1H, NH, D_2O exchangeable), 14.04 (brs, 1H, NH, D_2O exchangeable) ppm. EI-MS: *m/z* 282 [M^{+}]. Anal. Calcd. For $C_8H_6N_6O_2S_2$; C, 34.04; H, 2.14; N, 29.77. Found: C, 33.92; H, 2.00; N, 29.63.

1-{6-Thioxo-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-ylmethyl}-5-methylpyrimidine-2,4(1H,3H)-dione (**6b**)
Mp 208-210^{o}C. IR (KBr, cm^{-1}): 3135 (NH), 1572 (C=N), 1105 (C=S). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.16 (s ,3H, 5-CH_3), 4.48 (s, 2H, CH_2), 8.21 (s, 1H, 6-H), 11.07 (brs, 1H, NH, D_2O exchangeable), 13.99 (brs, 1H, NH, D_2O exchangeable) ppm. EI-MS: *m/z* 296 [M^{+}]. Anal. Calcd. For $C_9H_8N_6O_2S_2$; ,C 36.48; H, 2.72; N, 28.36. Found: C, 36.33; H, 2.60; N, 28.26.

***1-{[4-Amino-5-(methylthio)-4H-1,2,4-triazol-3-yl]methyl}pyrimidine-2,4(1H,3H)-diones* 7a,b**
A mixture of the thione derivatives **5a,b** (1 mmol), sodium hydroxide (0.04 g, 1 mmol), and methyl iodide (0.17 g, 1.2 mmol) was stirred in water (30 ml) for 5 h. The resulting methylthio derivatives **7a,b** was collecting by filtration, washed with water, dried, and recrystallized from ethanol to give 94-96% yields as white powders.

*1-{[4-Amino-5-(methylthio)-4H-1,2,4-triazol-3-yl]methyl}pyrimidine-2,4(1H,3H)-dion***e** (**7a**)
Mp 166-167^{o}C. IR (KBr, cm^{-1}): 3309 (NH_2), 1628 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.56 (s, 3H, SCH_3), 4.44 (s, 2H, CH_2), 6.16 (brs, 2H, NH_2, D_2O exchangeable), 6.68 (d, 1H, 5-H, *J* = 5.5 Hz), 8.27 (d, 1H, 6-H, *J* = 5.5 Hz), 11.07 (brs, 1H, NH, D_2O exchangeable) ppm. EI-MS: *m/z* 254 [M^{+}]. Anal. Calcd. For $C_8H_{10}N_6O_2S$; C, 37.79; H, 3.96; N, 33.05. Found: C, 37.66; H, 3.88; N, 32.92.

1-{[4-Amino-5-(methylthio)-4H-1,2,4-triazol-3-yl]methyl}-5-methylpyrimidine-2,4(1H,3H)-dione (**7b**)
Mp 178-179^{o}C. IR (KBr, cm^{-1}): 3315, (NH_2), 1626 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.14 (s, 3H, 5-CH_3), 2.58 (s, 3H, SCH_3), 4.46 (s, 2H, CH_2), 6.19 (brs, 2H, NH_2, D_2O exchangeable), 8.25 (s, 1H, 6-H), 11.00 (brs, 1H, NH, D_2O exchangeable) ppm. EI-MS: *m/z* 268 [M^{+}]. Anal. Calcd. For $C_9H_{12}N_6O_2S$; C, 40.29; H, 4.51; N, 31.32. C, 40.20; H, 4.40; N, 31.22.

***(E)-2-{[3-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl]methyl}-5-{[(methylthio)-4H-1,2,4-triazol-4-ylimino]methyl}benzoic acid* 8a,b**
Method A. A mixture of (1 mmol) of 4-amino-5-methylthio-1,2,4-triazoles **7a,b** and 0.15 g (1 mmol) phthalaldehydic acid was dissolved in 10 ml absolute ethanol. Three drops of acetic acid were added and the reaction mixture was refluxed for 7 h, then left to cool. The crude products were filtered, dried, and recrystallized from ethanol to afford **8a,b** in 55-58% yields.

Method B. A mixture of 4-amino-5-methylthio-1,2,4-triazoles **7a,b** (1 mmol) and phthalaldehydic acid (0.15 g, 1 mmol) was dissolved in isopropyl alcohol (4 ml) in a Teflon closed vessel and the mixture was irradiated for 8-10

min. The crude products were filtered, dried, and recrystallized from ethanol to afford **8a,b** in 95-96% yields as white powders.

(E)-2-{[3-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl]methyl}-5-{[(methylthio)-4H-1,2,4-triazol-4-ylimino] methyl} benzoic acid (**8a**)
Mp 180-181^{o}C. IR (KBr, cm^{-1}): 3250-2650 (OH), 1705 (C=O), 1625 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.57 (s, 3H, SCH_3), 4.49 (s, 2H, CH_2), 6.65 (d, 1H, 5-H, *J* = 5.5 Hz), 7.65-7.68 (m, 2H, C_6H_4), 7.78 (d, 1H, C_6H_4, *J* = 6.9 Hz), 7.85 (s, 1H, CH), 7.90-7.95 (m, 1H, C_6H_4), 8.33 (d, 1H, 6-H, *J* = 5.5 Hz), 9.58 (brs, 1H, OH, D_2O exchangeable), 11.11 (brs, 1H, NH, D_2O exchangeable) ppm. EI-MS: *m/z* 386 [M^{+}]. Anal. Calcd. For $C_{16}H_{14}N_6O_4S$; C, 49.74; H, 3.65; N, 21.75. Found: C, 49.66; H, 3.50; N, 21.75.

(E)-2-{[3-(2,4-Dioxo-3,4-dihydro-5-methylpyrimidin-1(2H)-yl]methyl}-5-{[(methylthio)-4H-1,2,4-triazol-4-ylimino] methyl}benzoic acid (**8b**)
Mp 193-194^{o}C. IR (KBr, cm^{-1}): 3250-2650 (OH), 1705 (C=O), 1626 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.18 (s, 3H, 5-CH_3), 2.61 (s, 3H, SCH_3), 4.47 (s, 2H, CH_2), 7.68-7.72 (m, 2H, C_6H_4), 7.77 (d, 1H, C_6H_4, *J* = 6.9 Hz), 7.88 (s, 1H, CH), 7.92-7.95 (m, 1H, C_6H_4), 8.30 (s, 1H, 6-H), 9.61 (brs, 1H, OH, D_2O exchangeable), 11.13 (brs, 1H, NH, D_2O exchangeable) ppm. EI-MS: *m/z* 400 [M^{+}]. Anal. Calcd. For $C_{17}H_{16}N_6O_4S$; C, 50.99; H, 4.03; N, 20.99. Found: C, 50.88; H, 3.90; N, 20.85.

***(E)-2-{[3-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl]methyl}-5-{[thioxo-1H-1,2,4-triazol-4(5H)-ylimino] methyl} benzoic acid* 9a,b**
Method A. A mixture of 4-amino-1,2,4-triazole-5-thiones **5a,b** (1 mmol) and phthalaldehydic acid (0.15 g, 1 mmol) was dissolved in absolute ethanol (10 ml). Three drops of acetic acid were added and the reaction mixture was refluxed for 8 h, then left to cool. The crude products were filtered, dried, and recrystallized from ethanol to afford **9a,b** in 50-53% yields.

Method B. A mixture of 4-amino-1,2,4-triazole-5-thiones **5a,b** (1 mmol) and phthalaldehydic acid (0.15 g, 1 mmol) was dissolved in isopropyl alcohol (4 ml) in a Teflon closed vessel and the mixture was irradiated for 10-12 min. The crude products were filtered, dried, and recrystallized from ethanol to afford **9a,b** in 90-92% yields as pale yellow powders.

(E)-2-{[3-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl]methyl}-5-{[thioxo-1H-1,2,4-triazol-4(5H)-ylimino]methyl} benzoic acid (**9a**)
Mp 185-187^{o}C. IR (KBr, cm^{-1}): 3440-2300 (OH), 3150 (NH), 1708 (C=O), 1629 (C=N), 1255 (C=S). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.54 (s, 2H, CH_2), 6.72 (d, 1H, 5-H, *J* = 5.5 Hz), 7.80-7.85 (m, 3H, C_6H_4), 7.99 (s, 1H, CH), 8.02-8.07 (m, 1H, C_6H_4), 8.30 (d, 1H, 6-H, *J* = 5.5 Hz), 10.55 (brs, 1H, OH, D_2O exchangeable), 11.15 (brs, 1H, NH, D_2O exchangeable), 13.00 (brs, 1H, NH, D_2O exchangeable) ppm. EI-MS: *m/z* 372 [M^{+}]. Anal. Calcd. For $C_{15}H_{12}N_6O_4S$; C, 48.38; H, 3.25; N, 22.57. Found: C, 48.27; H, 3.20; N, 22.50.

(E)-2-{[3-(2,4-Dioxo-3,4-dihydro-5-methylpyrimidin-1(2H)-yl]methyl}-5-{[thioxo-1H-1,2,4-triazol-4(5H)-ylimino] methyl}benzoic acid (**9b**)
Mp 198-200^{o}C. IR (KBr, cm^{-1}): 3447-2251 (OH), 3100 (NH), 1704 (C=O), 1622 (C=N), 1252 (C=S). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.16 (s, 3H, 5-CH_3), 3.53 (s, 2H, CH_2), 7.78-7.83 (m, 3H, C_6H_4), 7.98 (s, 1H, CH), 8.07-8.11 (m, 1H, C_6H_4), 8.29 (s, 1H, 6-H), 10.60 (brs, 1H, OH, D_2O exchangeable), 11.16 (brs, 1H, NH, D_2O exchangeable), 13.30 (brs, 1H, NH, D_2O exchangeable) ppm. EI-MS: *m/z* 386 [M^{+}]. Anal. Calcd. For $C_{16}H_{14}N_6O_4S$; C 49.74; H 3.65; N 21.75. Found: C 49.60; H 3.60; N 21.63.

***1-{[Pyrimidine-2,4(1H,3H)-dione]-3-ylmethyl}[1,2,4]triazolo[4',3':4,5][1,3,4]thiaziazolo-[2,3-a]isoindol-6(10bH)-ones* 10a,b**
A mixture of 4-amino-1,2,4-triazole-5-thiones **5a,b** (1 mmol) and phthalaldehydic acid (0.15 g, 1 mmol) was dissolved in dimethyl formamide (3 ml) in a Teflon closed vessel and the mixture was irradiated for 8-15 min. The crude products were filtered, dried, and recrystallized from ethanol to afford **10a,b** in 90-94% yields.

1-{[Pyrimidine-2,4(1H,3H)-dione]-3-ylmethyl}[1,2,4]triazolo[4',3':4,5][1,3,4]thiaziazolo-[2,3-a]isoindol-6(10bH)-one (**10a**)
Mp 170-171°C. IR (KBr, cm^{-1}): 1720 (C=O), 1650 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 3.28 (s, 2H, CH_2), 4.89 (s, 1H, CH), 6.02 (s, 1H, CH), 6.63 (d, 1H, 5-H, *J* = 5.5 Hz), 7.00 (brs, 1H, NH, D_2O exchangeable), 7.28-7.35 (m, 2H, C_6H_4), 7.77-7.80 (m, 2H, C_6H_4), 8.24 (d, 1H, 6-H, *J* = 5.5 Hz), 11.04 (brs, 1H, NH, D_2O exchangeable) ppm. EI-MS: *m/z* 356 [M^+]. Anal. Calcd. For $C_{15}H_{12}N_6O_3S$; C, 50.56; H, 3.39; N, 23.58. Found: C, 50.45; H, 3.30; N, 23.44.

1-{[5-Methylpyrimidine-2,4(1H,3H)-dione]-3-ylmethyl}[1,2,4]triazolo[4',3':4,5][1,3,4]thiaziazolo-[2,3-a] isoindol-6(10bH)-one (**10b**)
Mp 200-202°C. IR (KBr, cm^{-1}): 1734 (C=O), 1653 (C=N). ^{1}H-NMR (300 MHz, DMSO-d_6): δ 2.15 (s, 3H, 5-CH_3), 3.30 (s, 2H, CH_2), 7.12 (brs, 1H, NH, D_2O exchangeable), 7.25-7.30 (m, 2H, C_6H_4), 7.72-7.78 (m, 2H, C_6H_4), 8.20 (s, 1H, 6-H), 11.17 (brs, 1H, NH, D_2O exchangeable) ppm. EI-MS: *m/z* 370 [M^+]. Anal. Calcd. For $C_{16}H_{14}N_6O_3S$; C, 51.88; H, 3.81; N, 22.69. Found: C, 51.77; H, 3.70; N, 22.53.

Antimicrobial screening
Sample preparation. Each of the test compounds and standards was dissolved in 12.5% DMSO, at concentrations of 500 µg/mL. Further dilutions of the compounds and standards in the test medium were prepared at the required quantities.

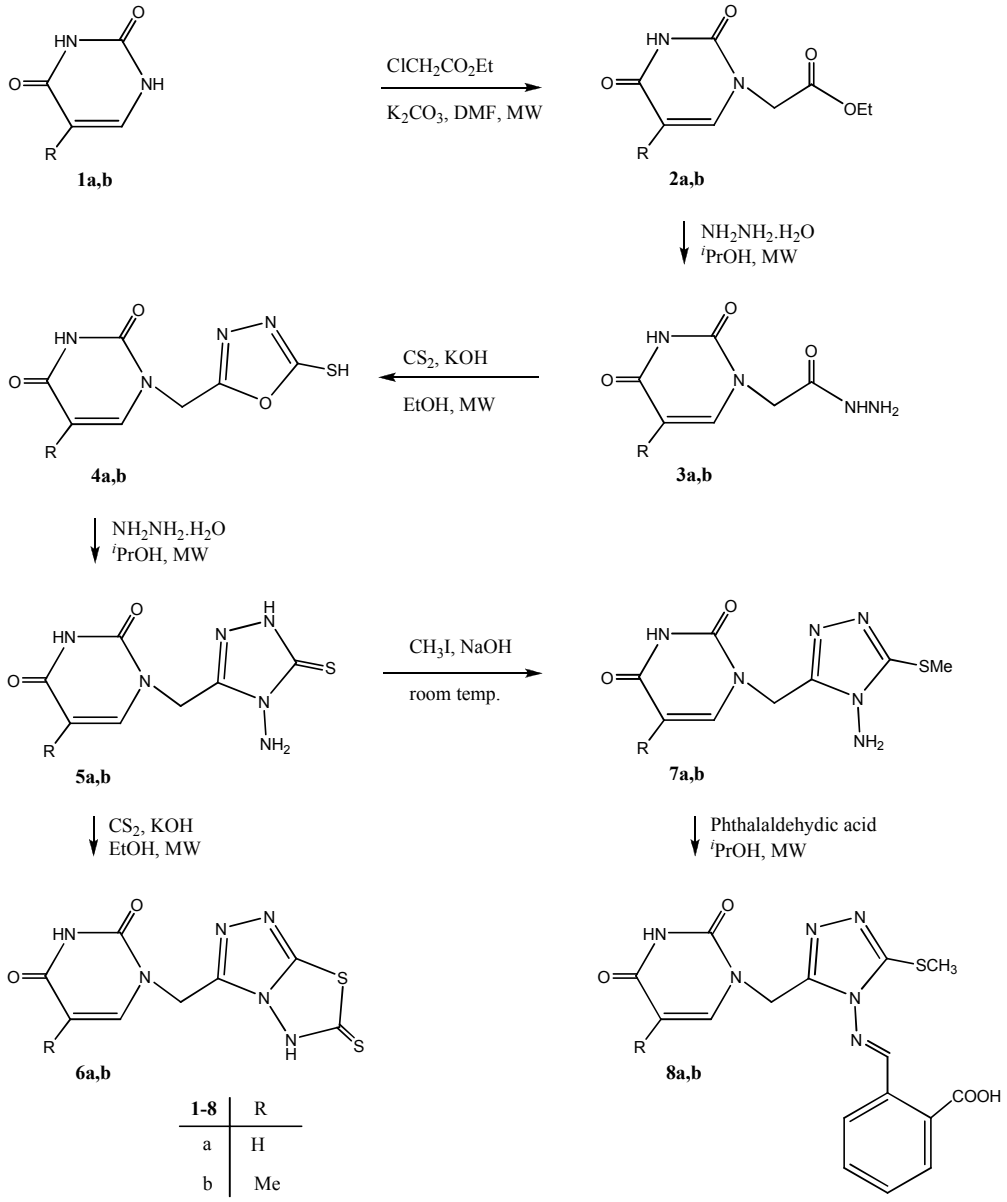
Culture of microorganisms. Bacteria strains were supplied from Botany Department, Faculty of Science, Menoufia University, Shebin El-Koam, Egypt, namely *Bacillus subtilis* (ATCC 6633) (Gram-positive), *Pseudomonas aeruginosa* (ATCC 27853) (Gram-negative), and *Streptomyces* species (Actinomycetes). The bacterial strains were maintained on MHA (Mueller–Hinton agar) medium (Oxoid Chemical Co., UK) for 24 h at 37 °C. The medium was molten on a water bath, inoculated with 0.5 mL of the culture of the specific microorganism and poured into sterile Petri dishes to form a layer of about 3 – 4 mm thickness. The layer was allowed to cool and harden. With the aid of a cork-borer, cups of about 10 mm diameter were produced [18].

Agar diffusion technique. The antibacterial activities of the synthesized compounds were tested against *Bacillus subtilis* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), and *Streptomyces* species (Actinomycetes) using MHA medium (17.5 g casein hydrolysate, 1.5 g soluble starch, 1000 mL beef extract). A stock solution of each synthesized compound (500 µg/mL) in DMSO was prepared and graded quantities of the test compounds were incorporated in specified quantity of sterilized liquid MH medium. Different concentrations of the test compounds in DMF were placed separately in cups in the agar medium. All plates were incubated at 37 ºC overnight. The inhibition zones were measured after 24 h. The minimum inhibitory concentration (MIC) was defined as the intercept of the grave of logarithm concentrations versus diameter of the inhibition zones [19, 20].

RESULTS AND DISCUSSION

The mixture of uracil derivatives **1a,b**, ethyl chloroacetate, dimethylformamide (DMF), and anhydrous potassium carbonate was irradiated in a domestic microwave oven (360 Watt) in a Teflon closed vessel for 5 minutes to afford 1-carbethoxymethyluracils **2a,b** [17] in 98-99% yield. Hydrazinolysis of the ethyl ester group was carried out in isopropyl alcohol under microwave irradiation for 3 minutes, to afford the corresponding hydrazides **3a,b** [17] in a quantitative yield. 1-Acetylhydrazinouracils **3a,b** were treated with CS_2, KOH, and ethanol under MW irradiation for 3 min. to obtain on 1-[(5-mercapto-1,3,4-oxadiazol-2-yl)methyl]pyrimidine-2,4(1*H*,3*H*)-diones **4a,b** in 96-99% yields. Compounds **4a,b** were irradiated for 3-5 minutes with hydrazine hydrate (98%) in isopropyl alcohol in a Teflon closed vessel to afford 1-[(4-amino-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)methyl]pyrimidine-2,4(1*H*,3*H*)-diones **5a,b** in 95-98%. Use of this condition improved the yield and purity of the product, while the yield under classical refluxing conditions was in range 70-86%. (Scheme 1, Table 1). IR spectra of **5a,b** showed a peak in range 3330-3310 cm^{-1} corresponding to the NH and NH_2 groups and a peak in range 1275-1260 cm^{-1} corresponding to C=S group, while no peak in range 2610-2555 cm^{-1} corresponding to the presence of SH group is observed. In addition, the ^{1}H NMR spectra showed a broad peak in range 13.55-13.89 ppm corresponding to NH group rather than SH group, which is in accordance with structure of the thione derivatives **5a,b**. ^{1}H NMR also showed a single peak at range 5.76-5.78 ppm corresponding to the NH_2 group. Both mentioned peaks are exchangeable with D_2O. The structures of **5a,b** were established by IR, ^{1}H NMR, and elemental analyses. Condensation of **5a,b** with CS_2 in ethanol containing KOH in a domestic microwave oven in a Teflon closed vessel

afforded **6a,b** in very good yields (96-98%). Using the conventional method, the condensation required eight hours reflux and gave lower yields (75-78%). IR spectrum of **6a,b** showed a peak at 3135 cm^{-1} corresponding to NH group and a strong peak at 1105 cm^{-1} corresponding to C=S group. Its ^1H NMR spectrum showed the disappearance of the NH_2 and NH peaks respectively, and the appearance of a peak at δ = 13.99-14.04 corresponding to The NH of the cyclic product. Stirring of **5a,b** with methyl iodide and sodium hydroxide for 5 h at room temperature afforded **7a,b** as white powders in 94-96% yields (Scheme 1, Table 1). ^1H NMR spectrum of **7a,b** showed the CH_3 group at δ = 2.56-2.58, while the NH peak was disappeared. Compounds **7a,b** were further allowed to react with phthalaldehydic acid in isopropyl alcohol or dimethylformamide (DMF) in Teflon closed vessel. The reaction was irradiated in a domestic microwave oven (360 Watt) for 8-10 minutes affording the corresponding Schiff′s bases **8a,b** in both cases (Scheme 1, Table 1). Microwave irradiation cause acceleration in the reaction time, and improvement in the yield than via the classical refluxing conditions. IR spectrum of **8a,b** showed a broad peak at 3250-2650 cm^{-1} and a sharp peak at 1705 cm^{-1} confirming the presence of the COOH group. In addition, the ^1H NMR spectrum showed a peak at δ = 9.58-9.61 ppm, exchangeable with D_2O corresponding to the OH group (Scheme 1, Table 1).



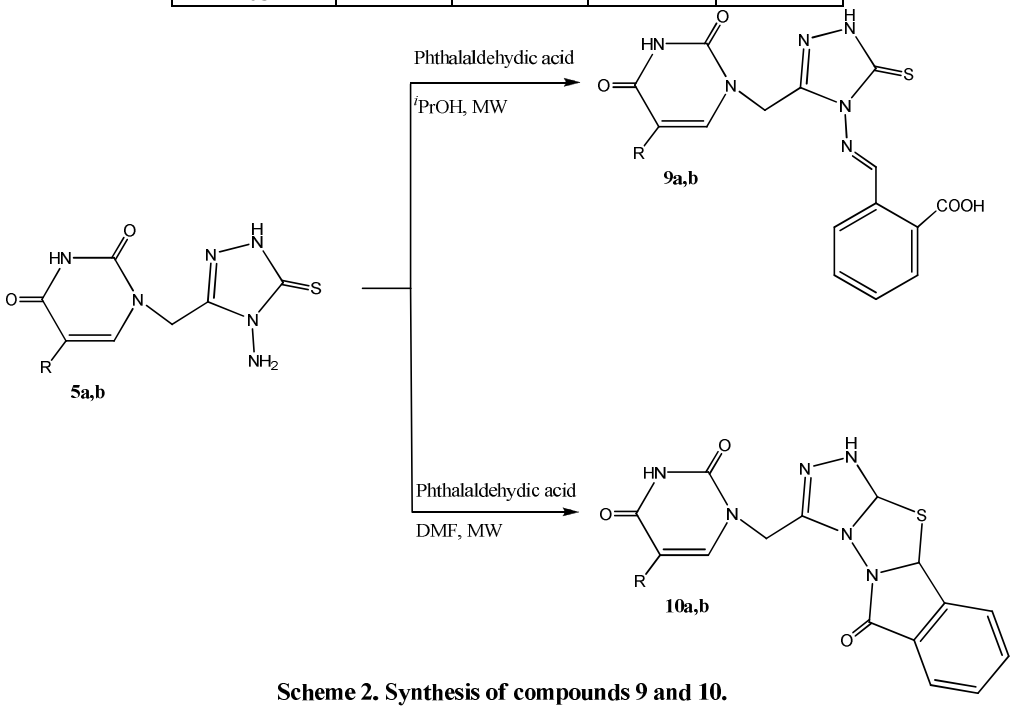
Scheme 1. Synthesis of compounds 2-8.

Following the procedure of Hassan *et al*. [21], condensation of **5a,b** as prototypes with phthalaldehydic acid in 3 ml isopropyl alcohol in a Teflon closed vessel was irradiated in a domestic microwave oven for 10-12 minutes afforded the corresponding Schiff's bases **9a,b** in a better yield than the classical refluxing condition. The IR spectra of **9a,b** gave a peak in the range 3150-3100 cm^{-1} corresponding to the NH group, a broad peak in the range 3440-2300 cm^{-1} corresponding to the OH group and a sharp peak at a range 1710-1715 cm^{-1} corresponding to the C=O group. On the other hand, the ^1H NMR showed a D_2O exchangeable peak in the range 10.55-10.60 ppm corresponding to the OH group. It also showed a peak in the range 13.00-13.30 ppm corresponding to the NH group. The structures of **9a,b** were established by IR, ^1H NMR, and elemental analyses (Scheme 2, Table 1).

The condensation of triazole derivatives **5a,b** with phthalaldehydic acid in 3 ml DMF in a Teflon closed vessel, in a domestic microwave oven (360 Watt) for 8-15 minutes gave the tetracyclic products **10a,b**. The tetracyclic products were obtained in a better yield than the classical refluxing condition. The IR spectra showed one absorption peak at 1720 and 1734 cm^{-1} respectively corresponding to the C=O group (Scheme 2, Table 1).

Table 1. Comparative data of conventional (A) and MW (B) method for the synthesis of compounds 2-10.

Compound No.	Conventional method (A)		Microwave method (B)	
	Time (h)	Yield (%)	Time (min)	Yield (%)
2a	3	85 [ref. 17]	5	98
2b	3	89 [ref. 17]	5	99
3a	4	90 [ref. 17]	3	quantitative
3b	4	93 [ref. 17]	3	quantitative
4a	–	–	3	96
4b	–	–	5	99
5a	6	70	3	95
5b	9	86	3	98
6a	8	75	3	96
6b	8	78	5	98
7a	5	90	–	–
7b	5	94	–	–
8a	7	55	8	95
8b	7	58	10	96
9a	8	50	10	90
9b	8	53	12	92
10a	–	–	8	90
10b	–	–	15	94



Scheme 2. Synthesis of compounds 9 and 10.

The antimicrobial activity of the synthesized compounds was evaluated against three microorganisms; *Bacillus subtilis* (ATCC 6633) (Gram-positive), *Pseudomonas aeruginosa* (ATCC 27853) (Gram-negative), and *Streptomyces* species (Actinomycetes). The values of minimal inhibitory concentrations (MICs) of the tested compounds are presented in Table 2. The results of the antimicrobial activity test revealed that **7a,b** and **8b** showed the highest activity against *B. subtilis* with MIC values of 75 µg/mL followed by compounds **6b**, **9b**, and **10b**. Compound **5a** showed the highest inhibition activity against *P. aeruginosa*, whereas **5a**, **7b**, and **10b** were the most active among the series of tested compounds against *Streptomyces* species with MIC values of 75 µg/mL. The results also revealed that some compounds showed little or no activity against the microorganisms (Table 2).

Table 2. Minimum inhibitory concentration (MIC in µg/mL) of the title compounds. The negative control DMSO showed no activity.

Compd	*Bacillus subtilis* (Gram-positive)	*Pseudomonas aeruginosa* (Gram-negative)	*Streptomyces* species (Actinomycetes)	Compd	*Bacillus subtilis* (Gram-positive)	*Pseudomonas aeruginosa* (Gram-negative)	*Streptomyces* species (Actinomycetes)
Penicillin	31	46	33	Penicillin	31	46	33
3a	250	—[a]	250	**7a**	75	125	100
3b	125	125	500	**7b**	75	100	75
4a	125	500	125	**8a**	250	125	—
4b	—	250	100	**8b**	75	—	125
5a	—	75	75	**9a**	125	500	250
5b	125	125	250	**9b**	100	100	250
6a	125	—	500	**10a**	250	—	125
6b	100	125	—	**10b**	100	250	75

[a] *Totally inactive (MIC > 500 µg/mL).*

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