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Synthesis of some new pyrimidine derivatives and evaluation of their anticancer and antibacterial activities

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Abstract Starting from the reaction of ethyl cyanoacetate with thiourea and the appropriate aldehydes, a series of new pyrimidine derivatives were prepared. Ten selected pyrimidine derivatives were subjected to a screening system for the investigation of their antitumor potency against liver (HEPG2) cell line. The antitumor activity results indicated that most of the selected pyrimidine derivatives showed moderate growth inhibition activity against the tested cell line, but with varying intensities in comparison to the known anticancer drugs: 5-fluorouracil and doxorubicin. Some of the synthesized compounds were also tested for their antimicrobial activity against bacteria as well as fungal isolates.

Keywords Pyrimidine derivatives · Cytotoxic activity · Liver HEPG2 cancer cell lines · Antibacterial activity

Introduction

The pyrimidine moiety with some substitution shows promising antitumor activity, as there are large numbers of pyrimidine-based antimetabolites. An early metabolite prepared was 5-fluorouracil (5-FU) [1]. The biological profiles of this new generation of pyrimidine represent good progress with regard to the older compounds [2]. A pyrimidine derivative followed by 5-thiouracil also exhibits some useful antineoplastic

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activities [3]. Thiouracils are also used as therapeutic agents in anticancer treatment [4], and their nucleoside analogs are found in t-RNA among many prokaryotes [5, 6], and are also used as anticancer and thyroid drugs [7–9]. After the invention of 5-FU as an antimetabolite of uracil [10], it has become one of the most widely used antineoplastic agents. Analogously, some 5-halogenated thiouracils [11] have been synthesized and screened for anticancer activity. It has been reported that the tested compounds have comparable activity to that of uracil [12–14]. From 1961 to 2006, different research laboratories investigated the anticancer activity of some 5-substituted-2-thiouracils and reported that the tested compounds were found to inhibit DNA synthesis [15–18]. We report here the synthesis of novel thiouracil derivatives based on the diverse medicinal uses and biological activities of thiouracil as anticancer drugs [19–24]. Based on these findings, the present work aimed to synthesize a new group of pyrimidine compounds incorporated with different heterocycles as a trial so that the resulting compounds would have better biological activity as antiproliferative agents in the field of liver cancer.

Experimental

Chemistry

All melting points are uncorrected and were taken on electro-thermal capillary melting point apparatus. Infrared spectra were performed on JASCO FT/IR-6100 using KBr discs. ^{1}H-NMR spectra was obtained on Jeol 270 MHz and on Jeol sx 500 MHz spectrometers using TMS as an internal standard. The mass spectra were recorded on a Jeol JMS-AX 500. All reactions were followed and checked by TLC (aluminum sheets) using chloroform–methanol. These (9:1 v/v) eluents and the plates were sprayed with iodine.

General procedure for the preparation of compounds ***1a, b***

A mixture of thiourea (0.1 mol), ethyl cyanoacetate (0.1 mol), and the appropriate aldehydes, namely 4-hydroxybenzaldehyde and 2-hydroxybenzaldehyde, were stirred in sodium ethoxide solution for 48 h, and then the reaction mixtures were poured into ice-water. After acidification with dilute HCl, the precipitate was filtered off, dried under suction, then crystallized from the proper solvent to give compounds **1a**, **b**.

*6-(4-Hydroxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**1a**)*

Crystallized from acetic acid, yellow crystals, m.p. 168 °C, yield 90 %. Analysis: for $C_{11}H_7N_3O_2S$, M. Wt. 245.25, calcd: C, 53.87; H, 2.88; N, 17.13. Found: C, 53.44; H, 2.30; N, 17.02. IR (KBr, cm^{-1}): 1,721 (C=O), 2,228 (–C≡N), 3,018 (NH of thiouracil), and 3,289 (OH). ^{1}H-NMR (DMSO-d_6, δ ppm): 5 (1H, *s*, OH, exchangeable with D_2O), 6.9–7.9 (4H, *m*, aromatic), and 8.1, 13 (2H, *s*, 2NH of thiouracil, exchangeable with D_2O), MS: (m/z) ≈ 245 (10 %).

*6-(2-Hydroxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**1b**)*

Crystallized from dimethylformamide, yellow crystals, m.p. 220 °C, yield 90 %. Analysis: for $C_{11}H_7N_3O_2S$, M. Wt. 245.25, calcd: C, 53.87; H, 2.88; N, 17.13. Found: C, 53.60; H, 2.75; N, 17.01. IR (KBr, cm^{-1}): 1,680 (C=O), 2,225 (–C≡N), 3,300 (NH of thiouracil), and 3,400 (OH). ^{1}H-NMR (DMSO-d_6, δ ppm): 5.4 (1H, *s*, OH, exchangeable with D_2O), 6.8–7.3 (4H, *m*, aromatic), and 8.0, 12 (2H, *s*, 2NH of thiouracil, exchangeable with D_2O). MS: (m/z) ≈ 245 (100 %).

*General procedure for the preparation of compounds **2a, b***

A mixture of **1a**, **b** (0.01 mol) and phosphorus pentachloride (0.01 mol) in phosphorus oxychloride (20 mL) was heated on a steam bath for 3 h and the reaction mixture poured gradually on to crushed ice. The precipitate was filtered off, dried under vacuum, then crystallized from the proper solvent to give compounds **2a**, **b**.

*4-Chloro-6-(4-hydroxyphenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (**2a**)*

Crystallized from dimethylformamide, brown yellow crystals, m.p. 215 °C, yield 90 %. Analysis: for $C_{11}H_6ClN_3OS$, M. Wt. 263.70, calcd: C, 50.10; H, 2.29; N, 15.93. Found: C, 49.85; H, 2.09; N, 15.78. IR (KBr, cm^{-1}): 1,691 (C=N), 2,231 (–C≡N), 3,330 (NH of thiouracil), and 3,400 (OH). ^{1}H-NMR (DMSO-d_6, δ ppm): 5.2 (1H, *s*, OH, exchangeable with D_2O), 6.7–7.4 (4H, *m*, aromatic), and 12 (1H, *s*, NH of thiouracil, exchangeable with D_2O). MS: (m/z) ≈ 263 (0.30 %).

*4-Chloro-6-(2-hydroxyphenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (**2b**)*

Crystallized from acetic acid, brown crystals, m.p. 254 °C, yield 80 %. Analysis: for $C_{11}H_6ClN_3OS$, M. Wt. 263.70, calcd: C, 50.10; H, 2.29; N, 15.93. Found: C, 49.75; H, 2.01; N, 15.15. IR (KBr, cm^{-1}): 1,692 (C=N), 2,226 (–C≡N), 3,200 (NH of thiouracil), and 3,310 (OH). ^{1}H-NMR (DMSO-d_6, δ ppm): 5.6 (1H, *s*, OH, exchangeable with D_2O), 6.9–7.6 (4H, *m*, aromatic), and 12.5 (1H, *s*, NH of thiouracil, exchangeable with D_2O). MS: (m/z) ≈ 263 (7 %).

*General procedure for the preparation of compounds **3a, b***

A mixture of **2a, b** (0.01 mol) and hydrazine hydrate (0.01 mol) in methanol (10 mL) was stirred for 8 h. The precipitate was filtered off, dried under suction then crystallized from the proper solvent to give compounds **3a, b**.

*4-Hydrazinyl-6-(4-hydroxyphenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (**3a**)*

Crystallized from acetic acid, brown crystal, m.p. 245 °C, yield 85 %. Analysis: for $C_{11}H_9N_5OS$, M. Wt. 259.28, calcd: C, 50.95; H, 3.50; N, 27.01. Found: C, 50.55; H,

2.86; N, 26.54. IR (KBr, cm^{-1}): 2,211 (–C≡N), 3,127 (Sec NH), 3,299, 3,400 (Prim NH), and 3,410 (OH). ^{1}H-NMR (DMSO-d_6, δ ppm): 2, 2.2 (3H, *s*, Sec NH, Prim NH, exchangeable with D_2O), 5.3 (1H, *s*, OH, exchangeable with D_2O), 6.6–7.4 (4H, *m*, aromatic), and 13 (1H, *s*, NH of thiouracil, exchangeable with D_2O). MS: m/z ≈ 259 (5 %).

*4-Hydrazinyl-2-thioxo-2,6-dihydropyrimido[5,4-c]quinolin-5(1H)-one (**3b**)*

Crystallized from benzene, yellow crystals, m.p. > 300 °C, yield 80 %. Analysis: for $C_{11}H_9N_5OS$, M. Wt. 259.28, calcd: C, 50.95; H, 3.50; N, 27.01. Found: C, 50.65; H, 2.79; N, 26.74. IR (KBr, cm^{-1}): 1,669 (C=O), 3,281 (Sec NH), 3,310, 3,405 (Prim NH). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.3, 2.4 (3H, *s*, Sec NH, Prim NH, exchangeable with D_2O), 7.1–7.9 (4H, *m*, aromatic), 8 (1H, *s*, Sec NH, exchangeable with D_2O), and 13.4 (1H, *s*, NH of thiouracil, exchangeable with D_2O). MS: $[M + 2]^+$ m/z ≈ 261 (1.89 %).

*General procedure for the preparation of compounds **4a–c***

A mixture of **3a**, **b** and **3c** [25] (0.01 mol) and 2-(bis(methylthio)methylene) malononitrile (0.01 mol) in methanol (20 mL) and triethylamine (1 mL) was refluxed for 8–12 h. The precipitate was filtered off, dried under suction, then crystallized from the proper solvent to give compounds **4a**–**c**.

*4-[5-Amino-4-cyano-3-(methylsulfanyl)-1H-pyrazol-1-yl]-6-(4-hydroxyphenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (**4a**)*

Crystallized from acetic acid, brown crystals, m.p. > 300 °C, yield 65 %. Analysis: for $C_{16}H_{11}N_7OS_2$, M. Wt. 381.43, calcd: C, 50.38; H, 2.91; N, 25.70. Found: C, 50.15; H, 2.36; N, 25.54. IR (KBr, cm^{-1}): 2,220, 2,228 (2–C≡N), 3,290 (NH of thiouracil), 3,330, 3,410 (Prim NH), and 3,420 (OH). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.5 (3H, *s*, SCH_3), 5.1 (1H, *s*, OH, exchangeable with D_2O), 6.6–7.4 (4H, *m*, aromatic), 8 (2H, *s*, Prim NH, exchangeable with D_2O), and 13.4 (1H, *s*, NH of thiouracil, exchangeable with D_2O). MS: m/z ≈ 381 (2 %).

*5-Amino-3-(methylsulfanyl)-1-(5-oxo-2-thioxo-1,2,5,6-tetrahydropyrimido[5,4-c]quinolin-4-yl)-1H-pyrazole-4-carbonitrile (**4b**)*

Crystallized from dimethylformamide, brown crystals, m.p. 266 °C, yield 55 %. Analysis: for $C_{16}H_{11}N_7OS_2$, M. Wt. 381.43, calcd: C, 50.38; H, 2.91; N, 25.70. Found: C, 50.05; H, 2.36; N, 25.14. IR (KBr, cm^{-1}): 1,700 (C=O), 2,228 (–C≡N), 3,281 (Sec NH), 3,330, 3,400 (Prim NH). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.6 (3H, *s*, SCH_3), 7.2–8.2 (4H, *m*, aromatic), 7.9, 8.1 (3H, *s*, Sec NH, Prim NH, exchangeable with D_2O), and 13.5 (1H, *s*, NH of thiouracil, exchangeable with D_2O). MS: m/z ≈ 381 (13 %).

*4-[5-Amino-4-cyano-3-(methylsulfanyl)-1H-pyrazol-1-yl]-6-(2-methoxyphenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (**4c**)*

Crystallized from benzene, orange crystals, m.p. > 300 °C, yield 60 %. Analysis: for $C_{17}H_{13}N_7OS_2$, M. Wt. 395.46, calcd: C, 51.63; H, 3.31; N, 24.79. Found: C, 50.65; H, 2.70; N, 24.34. IR (KBr, cm^{-1}): 2,210, 2,225 (2–C≡N), 3,175 (NH of thiouracil), 3,315, 3,430 (Prim NH). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.8 (3H, *s*, SCH_3), 3.6 (3H, *s*, OCH_3), 6.9–7.9 (4H, *m*, aromatic), 8.1 (2H, *s*, Prim NH, exchangeable with D_2O), and 12.5 (1H, *s*, NH of thiouracil, exchangeable with D_2O). MS: m/z ≈ 395 (9 %).

*General procedure for the preparation of compounds **5a–c***

A mixture of **4a–c** (0.01 mol) and trimethylorthoformate (15 mL) in acetic anhydride (10 mL) was heated under reflux for 2 h. The solid obtained was recrystallized from the proper solvent to give **5a–c**.

*Ethyl{4-cyano-1-[5-cyano-6-(4-hydroxyphenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl]-3-(methylsulfanyl)-1H-pyrazol-5-yl}imidoformate (**5a**)*

Crystallized from benzene, white crystals, m.p. > 300 °C, yield 70 %. Analysis: for $C_{19}H_{15}N_7O_2S_2$, M. Wt. 437.49, calcd: C, 52.16; H, 3.46; N, 22.41. Found: C, 51.05; H, 2.90; N, 22.14. IR (KBr, cm^{-1}): 2,220, 2,228 (2–C≡N), 3,281 (Sec NH), and 3,320 (OH). ^{1}H-NMR (DMSO-d_6, δ ppm): 1.2 (3H, *t*, CH_3), 2.4 (3H, *s*, SCH_3), 3.4 (2H, *q*, CH_2), 5.3 (1H, *s*, OH, exchangeable with D_2O), 6.4–7.7 (4H, *m*, aromatic), 7.5 (1H, *s*, CH), and 13 (1H, *s*, NH of thiouracil, exchangeable with D_2O). MS: m/z ≈ 437 (10 %).

*Ethyl[4-cyano-3-(methylsulfanyl)-1-(5-oxo-2-thioxo-1,2,5,6-tetrahydropyrimido[5,4-c]quinolin-4-yl)-1H-pyrazol-5-yl]imidoformate (**5b**)*

Crystallized from dimethylformamide, brown crystals, m.p. > 300 °C, yield 65 %. Analysis: for $C_{19}H_{15}N_7O_2S_2$, M. Wt. 437.49, calcd: C, 52.16; H, 3.46; N, 22.41. Found: C, 51.75; H, 3.26; N, 21.94. IR (KBr, cm^{-1}): 1,645 (C=O), 2,212 (–C≡N), 3,433 (Sec NH). ^{1}H-NMR (DMSO-d_6, δ ppm): 1.2 (3H, *t*, CH_3), 2.5 (3H, *s*, SCH_3), 3.3 (2H, *q*, CH_2), 7.1–8.2 (4H, *m*, aromatic), 7.1 (1H, *s*, CH), and 8.3, 12.5 (2H, *s*, 2Sec NH, exchangeable with D_2O). MS: m/z ≈ 437 (17 %).

*Ethyl{4-cyano-1-[5-cyano-6-(2-methoxyphenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl]-3-(methylsulfanyl)-1H-pyrazol-5-yl}imidoformate (**5c**)*

Crystallized from benzene, brown crystals, m.p. 184 °C, yield 60 %. Analysis: for $C_{20}H_{17}N_7O_2S_2$, M. Wt. 451.52, calcd: C, 53.20; H, 3.79; N, 21.71. Found: C, 53.05; H, 3.36; N, 21.14. IR (KBr, cm^{-1}): 2,213, 2,228 (2–C≡N), 3,418 (Sec NH). ^{1}H-NMR (DMSO-d_6, δ ppm): 1.1 (3H, *t*, CH_3), 2.4 (3H, *s*, SCH_3), 3.2 (2H, *q*, CH_2), 3.7 (3H, *s*, OCH_3), 6.9–7.2 (4H, *m*, aromatic), 7.3 (1H, *s*, CH), and 12 (1H, *s*, NH of thiouracil, exchangeable with D_2O). MS: m/z ≈ 451 (20 %).

*General procedure for the preparation of compounds **6a–c***

A mixture of **5a–c** (0.01 mol) and hydrazine hydrate (0.01 mol) in methanol (10 mL) was stirred for 8 h. The precipitate was filtered off, dried under suction, then crystallized from the proper solvent to give compounds **6a–c**.

*4-[4-Hydrazinyl-3-(methylsulfanyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl]-6-(4-hydroxyphenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (**6a**)*

Crystallized from benzene, brown crystals, m.p. > 300 °C, yield 55 %. Analysis: for $C_{17}H_{13}N_9OS_2$, M. Wt. 423.47, calcd: C, 48.22; H, 3.09; N, 29.77. Found: C, 47.95; H, 2.66; N, 29.14. IR (KBr, cm^{-1}): 2,215 (–C≡N), 3,281 (Sec NH), 3,330, 3,400 (Prim NH), and 3,300 (OH). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.4 (3H, *s*, SCH_3), 2 (2H, *s*, Prim NH, exchangeable with D_2O), 5.3 (1H, *s*, OH, exchangeable with D_2O), 6.9–7.8 (4H, *m*, aromatic), 8.1 (1H, *s*, CH), and 4.2, 13.2 (2H, *s*, 2 Sec NH, exchangeable with D_2O). MS: m/z ≈ 423 (16 %).

*4-[4-Hydrazinyl-3-(methylsulfanyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl]-2-thioxo-2,6-dihydropyrimido[5,4-c]quinolin-5(1H)-one (**6b**)*

Crystallized from acetic acid, brown crystals, m.p. > 300 °C, yield 70 %. Analysis: for $C_{17}H_{13}N_9OS_2$, M. Wt. 423.47, calcd: C, 48.22; H, 3.09; N, 29.77. Found: C, 47.75; H, 2.88; N, 29.14. IR (KBr, cm^{-1}): 1,670 (C=O), 3,433 (Sec NH), 3,310, 3,400 (Prim NH). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.4 (3H, *s*, SCH_3), 2.1 (2H, *s*, Prim NH, exchangeable with D_2O), 7.1–8.3 (4H, *m*, aromatic), 8.2 (1H, *s*, CH), and 4, 8.1, 13.2 (3H, *s*, 3Sec NH, exchangeable with D_2O). MS: m/z ≈ 423 (75 %).

*4-[4-Hydrazinyl-3-(methylsulfanyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl]-6-(2-methoxyphenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (**6c**)*

Crystallized from benzene, brown crystals, m.p. 262 °C, yield 75 %. Analysis: for $C_{18}H_{15}N_9OS_2$, M. Wt. 437.50, calcd: C, 49.42; H, 3.46; N, 28.81. Found: C, 49.11; H, 2.87; N, 27.24. IR (KBr, cm^{-1}): 2,210 (–C≡N), 3,201 (Sec NH) 3,300, 3,440 (Prim NH). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.6 (3H, *s*, SCH_3), 2.2 (2H, *s*, Prim NH, exchangeable with D_2O), 3.8 (3H, *s*, OCH_3), 6.7–7.2 (4H, *m*, aromatic), 8.2 (1H, *s*, CH), and 4, 12.2 (2H, *s*, 2Sec NH, exchangeable with D_2O). MS: m/z ≈ 437 (50 %).

*General procedure for the preparation of compounds **7a–c***

A mixture of **1a**, **b** and **1c** [25] (0.01 mol) and hydrazine hydrate (0.01 mol) in methanol (10 mL) was heated under refluxed for 5 h. The precipitate was filtered off, dried under suction, then crystallized from the proper solvent to give compounds **7a–c**.

*2-Hydrazinyl-4-(4-hydroxyphenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**7a**)*

Crystallized from dimethylformamide, brown crystal, m.p. 156 °C, yield 80 %. Analysis: for $C_{11}H_9N_5O_2$, M. Wt. 243.22, calcd: C, 54.32; H, 3.73; N, 28.79. Found: C, 53.55; H, 3.16; N, 28.54. IR (KBr, cm^{-1}): 1,700 (C=O), 2,211 (–C≡N), 2,814 (Sec NH), 3,227, 3,312 (Prim NH), and 3,310 (OH). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.2, 2.4 (3H, *s*, Sec NH, Prim NH, exchangeable with D_2O), 5.6 (1H, *s*, OH, exchangeable with D_2O), 6.9–7.8 (4H, *m*, aromatic), and 12.5 (1H, *s*, NH of thiouracil, exchangeable with D_2O). MS: m/z ≈ 243 (6 %).

*2-Hydrazinylpyrimido[5,4-c]quinoline-4,5(3H,6H)-dione (**7b**)*

Crystallized from benzene, yellow crystals, m.p. 219 °C, yield 60 %. Analysis: for $C_{11}H_9N_5O_2$, M. Wt. 243.22, calcd: C, 54.32; H, 3.73; N, 28.79. Found: C, 53.85; H, 3.66; N, 27.94. IR (KBr, cm^{-1}): 1,669, 1,721 (2C=O), 3,334 (Sec NH), 3,270, 3,433 (Prim NH). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.4, 2.5 (3H, *s*, Sec NH, Prim NH, exchangeable with D_2O), 7.2–8.2 (4H, *m*, aromatic), 8.9 (1H, *s*, Sec NH, exchangeable with D_2O), and 12 (1H, *s*, NH of thiouracil, exchangeable with D_2O). MS: $[M + 2]^+$ m/z ≈ 245 (15 %).

*2-Hydrazinyl-4-(2-methoxyphenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**7c**)*

Crystallized from benzene, yellow crystals, m.p. 128 °C, yield 65 %. Analysis: for $C_{12}H_{11}N_5O_2$, M. Wt. 257.24, calcd: C, 56.03; H, 4.31; N, 27.22. Found: C, 55.65; H, 3.86; N, 26.74. IR (KBr, cm^{-1}): 1,721 (C=O), 2,225 (–C≡N), 3,200 (Sec NH), 3,290, 3,335 (Prim NH). ^{1}H-NMR (DMSO-d_6, δ ppm): 2, 2.6 (3H, *s*, Sec NH, Prim NH, exchangeable with D_2O), 3.8 (3H, *s*, OCH_3), 6.9–7.7 (4H, *m*, aromatic), and 13 (1H, *s*, NH of thiouracil, exchangeable with D_2O). MS: m/z ≈ 257 (10 %).

*General procedure for the preparation of compounds **8a–c***

A mixture of **7a–c** (0.01 mol) and 2-(bis(methylthio)methylene)malono nitrile (0.01 mol) in methanol (20 mL) and triethylamine (1 mL) was refluxed for 8–12 h. The precipitate was filtered off, dried under suction, then crystallized from the proper solvent to give compounds **8a–c**.

*2-[5-Amino-4-cyano-3-(methylsulfanyl)-1H-pyrazol-1-yl]-4-(4-hydroxyphenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**8a**)*

Crystallized from acetic acid, brown crystals, m.p. > 300 °C, yield 60 %. Analysis: for $C_{16}H_{11}N_7O_2S$, M. Wt. 365.36, calcd: C, 52.60; H, 3.03; N, 26.84. Found: C, 52.15; H, 2.76; N, 25.94. IR (KBr, cm^{-1}): 1,700 (C=O), 2,220, 2,227 (2–C≡N), 3,190 (NH of thiouracil), 3,230, 3,390 (Prim NH), and 3,400 (OH). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.7 (3H, *s*, SCH_3), 5.3 (1H, *s*, OH, exchangeable with D_2O), 6.6–7.2 (4H, *m*, aromatic), 8.1 (2H, *s*, Prim NH, exchangeable with D_2O), and 12.9 (1H, *s*, NH of thiouracil, exchangeable with D_2O). MS: m/z ≈ 365 (5 %).

*5-Amino-1-(4,5-dioxo-3,4,5,6-tetrahydropyrimido[5,4-c]quinolin-2-yl)-3-(methyl sulfanyl)-1H-pyrazole-4-carbonitrile (**8b**)*

Crystallized from benzene, brown crystals, m.p. 239 °C, yield 65 %. Analysis: for $C_{16}H_{11}N_7O_2S$, M. Wt. 365.36, calcd: C, 52.60; H, 3.03; N, 26.84. Found: C, 52.25; H, 2.86; N, 26.14. IR (KBr, cm^{-1}): 1,645, 1,700 (2C=O), 2,207 (–C ≡ N), 3,169 (Sec NH), 3,281, 3,300 (Prim NH). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.5 (3H, *s*, SCH_3), 7.1–7.9 (4H, *m*, aromatic), 8.2, 8.4 (3H, *s*, Sec NH, Prim NH, exchangeable with D_2O), and 13 (1H, *s*, NH of thiouracil, exchangeable with D_2O). MS: m/z ≈ 365 (29 %).

*2-[5-Amino-4-cyano-3-(methylsulfanyl)-1H-pyrazol-1-yl]-4-(2-methoxyphenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**8c**)*

Crystallized from dimethylformamide, orange crystals, m.p. > 300 °C, yield 60 %. Analysis: for $C_{17}H_{13}N_7O_2S$, M. Wt. 379.39, calcd: C, 53.82; H, 3.45; N, 25.84. Found: C, 53.25; H, 3.16; N, 25.14. IR (KBr, cm^{-1}): 1,715 (C=O), 2,210, 2,220 (2–C ≡ N), 3,270 (NH of thiouracil), 3,335, 3,410 (Prim NH).^{1}H-NMR (DMSO-d_6, δ ppm): 2.4 (3H, *s*, SCH_3), 3.8 (3H, *s*, OCH_3), 6.7–7.8 (4H, *m*, aromatic), 7.7 (2H, s, Prim NH, exchangeable with D_2O), and 13.5 (1H, *s*, NH of thiouracil, exchangeable with D_2O). MS: m/z ≈ 379 (34 %).

*General procedure for the preparation of compounds **9a–c***

A mixture of **8a–c** (0.01 mol) and trimethylorthoformate (15 mL) in acetic anhydride (10 mL) was heated under reflux for 2 h. The solid obtained was recrystallized from the proper solvent to give **9a–c**.

*Ethyl{1-[5-cyano-4-(4-hydroxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl]-4-iso cyano-3-(methylsulfanyl)-1H-pyrazol-5-yl}imidoformate (**9a**)*

Crystallized from benzene, brown crystals, m.p. > 300 °C, yield 70 %. Analysis: for $C_{19}H_{15}N_7O_3S$, M. Wt. 421.43, calcd: C, 54.15; H, 3.59; N, 23.27. Found: C, 53.85; H, 3.36; N, 23.04. IR (KBr, cm^{-1}): 1,669 (C=O), 2,220, 2,229 (2–C ≡ N), 3,190 (Sec NH), and 3,267 (OH). ^{1}H-NMR (DMSO-d_6, δ ppm): 1.1 (3H, *t*, CH_3), 2.6 (3H, *s*, SCH_3), 3.6 (2H, *q*, CH_2), 5.4 (1H, *s*, OH, exchangeable with D_2O), 6.6–7.8 (4H, *m*, aromatic), 7.4 (1H, *s*, CH), and 13.4 (1H, *s*, NH of thiouracil, exchangeable with D_2O). MS: m/z ≈ 421 (19 %).

*Ethyl[1-(4,5-dioxo-3,4,5,6-tetrahydropyrimido[5,4-c]quinolin-2-yl)-4-isocyano-3-(methylsulfanyl)-1H-pyrazol-5-yl]imidoformate (**9b**)*

Crystallized from benzene, brown crystals, m.p. 170 °C, yield 75 %. Analysis: for $C_{19}H_{15}N_7O_3S$, M. Wt. 421.43, calcd: C, 54.15; H, 3.59; N, 23.27. Found: C, 53.75; H, 3.26; N, 22.94. IR (KBr, cm^{-1}): 1,645, 1,742 (2C = O), 2,210 (–C ≡ N), 3,412 (Sec NH). ^{1}H-NMR (DMSO-d_6, δ ppm): 1.3 (3H, *t*, CH_3), 2.5 (3H, *s*, SCH_3), 3.5

(2H, *q*, CH_2), 7.1–8.1 (4H, *m*, aromatic), 7.5 (1H, *s*, CH), and 8.4, 13.5 (2H, *s*, 2Sec NH, exchangeable with D_2O). MS: m/z ≈ 421 (12 %).

*Ethyl{1-[5-cyano-4-(2-methoxyphenyl)-6-oxo-1,6-dihydropyrimidin-2-yl]-4-iso cyano-3-(methylsulfanyl)-1H-pyrazol-5-yl}imidoformate (**9c**)*

Crystallized from acetic acid, brown crystals, m.p. > 300 °C, yield 60 %. Analysis: for $C_{20}H_{17}N_7O_3S$, M. Wt. 435.45, calcd: C, 55.16; H, 3.93; N, 22.52. Found: C, 55.05; H, 3.36; N, 22.14. IR (KBr, cm^{-1}): 1,700 (C=O), 2,211, 2,220 (2–C≡N), 3,330 (Sec NH). ^{1}H-NMR (DMSO-d_6, δ ppm): 1.2 (3H, *t*, CH_3), 2.3 (3H, *s*, SCH_3), 3.1 (2H, *q*, CH_2), 3.5 (3H, *s*, OCH_3), 6.6–7.1 (4H, *m*, aromatic), 7.4 (1H, *s*, CH), and 12.5 (1H, s, NH of thiouracil, exchangeable with D_2O). MS: m/z ≈ 435 (40 %).

*General procedure for the preparation of compounds **10a–c***

A mixture of **9a–c** (0.01 mol) and hydrazine hydrate (0.01 mol) in methanol (10 mL) was stirred for 8 h. The precipitate was filtered off, dried under suction, then crystallized from the proper solvent to give compounds **10a–c**.

*2-[4-Hydrazinyl-3-(methylsulfanyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl]-4-(4-hydroxyphenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**10a**)*

Crystallized from benzene, brown crystals, m.p. 207 °C, yield 80 %. Analysis: for $C_{17}H_{13}N_9O_2S$, M. Wt. 407.40, calcd: C, 50.12; H, 3.22; N, 30.94. Found: C, 49.95; H, 3.06; N, 30.44. IR (KBr, cm^{-1}): 1,700 (C=O), 2,220 (–C≡N), 3,181 (Sec NH), 3,230, 3,332 (Prim NH), and 3,400 (OH). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.6 (3H, *s*, SCH_3), 2.2 (2H, *s*, Prim NH, exchangeable with D_2O), 5.5 (1H, *s*, OH, exchangeable with D_2O), 6.7–7.5 (4H, *m*, aromatic), 8.3 (1H, *s*, CH), and 4.1, 13.5 (2H, *s*, 2Sec NH, exchangeable with D_2O). MS: m/z ≈ 407 (40 %).

*2-[4-Hydrazinyl-3-(methylsulfanyl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-1-yl] pyrimido[5,4-c]quinoline-4,5(3H,6H)-dione (**10b**)*

Crystallized from benzene, brown crystals, m.p. 241 °C, yield 80 %. Analysis: for $C_{17}H_{13}N_9O_2S$, M. Wt. 407.40, calcd: C, 50.12; H, 3.22; N, 30.94. Found: C, 49.75; H, 2.88; N, 30.64. IR (KBr, cm^{-1}): 1,670, 1,710 (2C=O), 3,330 (Sec NH), 3,228, 3,345 (Prim NH). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.5 (3H, *s*, SCH_3), 2 (2H, *s*, Prim NH, exchangeable with D_2O), 7.2–8.2 (4H, *m*, aromatic), 8.4 (1H, *s*, CH), and 4.5, 8.3, 13.5 (3H, *s*, 3Sec NH, exchangeable with D_2O). MS: m/z ≈ 407 (60 %).

*2-[4-Hydrazinyl-3-(methylsulfanyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl]-4-(2-methoxyphenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**10c**)*

Crystallized from benzene, brown crystals, m.p. > 300 °C, yield 75 %. Analysis: for $C_{18}H_{15}N_9O_2S$, M. Wt. 421.43, calcd: C, 51.30; H, 3.59; N, 29.91. Found: C, 50.11; H, 3.36; N, 29.24. IR (KBr, cm^{-1}): 1,660 (C=O), 2,228 (–C≡N), 3,301 (Sec NH) 3,336, 3,422 (Prim NH). ^{1}H-NMR (DMSO-d_6, δ ppm): 2.5 (3H, *s*, SCH_3), 2 (2H, *s*, Prim

NH, exchangeable with D_2O), 3.5 (3H, *s*, OCH_3), 6.8–7.4 (4H, *m*, aromatic), 8 (1H, *s*, CH), and 4.4, 12 (2H, *s*, 2Sec NH, exchangeable with D_2O). MS: m/z $\approx$ 421 (60 %).

Anticancer testing

Measurement of potential cytotoxicity by SRB assay

The selected pyrimidine derivatives **2a**, **1a**, **3c** [25], **7c**, **6b**, **10b**, **9b**, **4c**, **5c**, and **6c** were subjected to a screening system for evaluation of their antitumor activity against liver HEPG2 cancer cell lines in comparison to the known anticancer drugs: 5-FU and doxorubicin (DOX) (Table 1).

Potential cytotoxicity of the selected pyrimidine derivatives was tested using the method of Skehan et al. [26] as follows.

Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the compound(s) to allow attachment of cells to the wall of the plate. Different concentrations of the compound under test (0, 1, 2.5, 5, 10 μg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37 °C and in an atmosphere of 5 % CO_2. Cultures were then fixed with trichloroacetic acid and stained for 30 min with 0.4 % (wt/vol) sulforhodamine B (SRB) dissolved in 1 % acetic acid. Unbound dye was removed by four washes with 1 % acetic acid, and protein-bound dye was extracted with 10 mM unbuffered tris base [tris(hydroxymethyl)aminomethane] for the determination of optical density in a computer-interfaced, 96-well microtiter plate reader. The SRB assay results were linear with the number of cells and with values for cellular protein measured by both the Lowry and Bradford assays at densities ranging from sparse subconfluence to multilayered supraconfluence. The signal-to-noise ratio at 564 nm was approximately 1.5 with 1,000 cells per well. The relation between surviving fraction and drug concentration is plotted to get the survival curve of both cancer cell lines after the specified compound.

Table 1 Effect of some selected newly synthesized compounds on liver carcinoma cell line (HEPG2)

Compound	IC_{50} (μg/mL)
5-Fluorouracil	5
Doxorubicin	3.56
2a	9.6
1a	6.58
3c	8.1
7a	6.85
6b	6.38
10b	9.33
9b	8.26
4c	7.11
5c	3.56
6c	5.23

IC_{50}: dose of the compounds which reduces survival to 50 %

Biochemical Analysis

Animals

Male albino mice weighing 18–20 g were used in the present study. Mice were divided into three main groups as follows:

1-Group (1) Untreated or control group (five mice).
2-Group (2) Divided into two subgroups (five mice for each subgroup) and treated with 5-FU or DOX as reference anticancer drugs.
3-Group (3) Divided into eight subgroups (five mice for each subgroup) and treated with the selected pyrimidine derivatives **2a**, **1a**, **3c** [25], **7c**, **6b**, **10b**, **9b**, **4c**, **5c**, and **6c**.

Treatment

Group (1) Each mouse was given a single intraperitoneal injection of 0.1 mL DMSO.
Group (2) Each mouse was given a single intraperitoneal injection of 0.1 mL containing 12 mg/kg body weight 5-FU or DOX dissolved in sterile water.
Group (3) Each mouse was given a single intraperitoneal injection of 0.1 mL containing 12 mg/kg body weight of the s selected pyrimidine derivatives **2a**, **1a**, **3c** [25], **7c**, **6b**, **10b**, **9b**, **4c**, **5c**, and **6c**, respectively, dissolved in DMSO. Blood was collected after 7 days from all mice groups. The biochemical effects of the selected pyrimidine derivatives **2a**, **1a**, **3c** [25], **7c**, **6b**, **10b**, **9b**, **4c**, **5c**, and **6c** on some liver enzymes such as aspartate and alanine amino transferases (AST and ALT) [27] and alkaline phosphatase (ALP) [28], were done using blood auto analyzer (Olympus AV 400, Japan) (Table 2). Moreover, albumin [29], globulins [30] and creatinine [31], total lipids [32], cholesterol [33], triglycerides [34], and bilirubin [35] in serum of mice were evaluated in comparison to 5-FU and DOX (Tables 3 and 4). Statistical analysis of the results was performed using Chi-square values (SPSS computer program).

Table 2 Biochemical effects of treatment of tested compounds with 5-FU and DOX, on serum ALT, AST, and ALP in mice

Mice groups	Biochemical parameters		
	Alanine amino transferase Mean ± SD ALT (IU/mL)	Aspartate amino transferase Mean ± SD AST (IU/mL)	Alkaline phosphatase Mean ± SD ALP (k.k./dL)
Control	43.5 ± 2.03	108.32 ± 4.19	18.70 ± 1.10
5-FU	51.47 ± 9.02	130.431 ± 8.92	25.485 + 6.03
P<	0.001	0.001	0.001
DOX	59.26 ± 12.03	147.226 ± 16.34	30.317 ± 5.14

Table 2 continued

Mice groups	Biochemical parameters		
	Alanine amino transferase Mean ± SD ALT (IU/mL)	Aspartate amino transferase Mean ± SD AST (IU/mL)	Alkaline phosphatase Mean ± SD ALP (k.k./dL)
P<	0.001	0.001	0.001
2a	80.7 ± 19.09	162.17 ± 34.5	38.58 ± 12.61
P<	0.001	0.001	0.001
1a	38.9 ± 8.9	123.9 ± 11.4	18.83 ± 6.29
P<	n.s.	0.01	n.s.
3c	39.56 ± 6.7	112.54 ± 12.7	19.94 ± 4.35
P<	n.s.	0.01	n.s.
7a	46.21 ± 4.17	107.81 ± 4.25	21.94 ± 3.4
P<	n.s.	n.s.	0.01
6b	53.7 ± 10.08	142.3 ± 29.7	45.42 ± 10.41
P<	0.001	0.001	0.001
10b	81.34 ± 27.3	151.52 ± 45.6	43.7 ± 8.36
P<	0.001	0.001	0.001
9b	33.42 ± 7.05	111.2 ± 11.05	18.32 ± 3.07
P<	n.s.	n.s.	n.s.
4c	60.5 ± 9.7	156.22 ± 20.1	44.26 ± 7.01
P<	0.001	0.001	0.001
5c	68.34 ± 11.9	146.4 ± 28.1	36.9 ± 9.8
P<	0.001	0.001	0.001
6c	50.81 ± 12.01	119 ± 9.56	22.07 ± 3.42
P<	0.01	0.01	0.01

Data are expressed as mean ± SD

$P > 0.05$ insignificant, $P < 0.01$ significant, $P < 0.001$ highly significant, *n.s.* non-significant

Table 3 Biochemical effects of treatment with 5-FU and DOX, and some selected newly synthesized compounds on serum total lipids, cholesterol, triglycerides, and bilirubin in mice

Mice groups	Biochemical parameters			
	Total lipids (mg/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Bilirubin (mg/dL)
Control	323.41 ± 27.1	94.32 ± 13.5	108.7 ± 16.8	0.63 ± 0.04
5-FU	378.2 ± 31.4	105.9 ± 11.7	126.5 ± 19.4	0.75 ± 0.10
P<	0.001	0.001	0.001	0.001
DOX	366.7 ± 6.10	109.3 ± 14.2	137.8 ± 17.10	0.81 ± 0.19
P<	0.001	0.001	0.001	0.001
2a	363.6 ± 29.3	116.4 ± 8.3	98.4 ± 10.6	0.96 ± 0.8
P<	0.001	0.01	n.s.	0.001
1a	329.34 ± 19.7	95.3 ± 9.4	119.7 ± 18.8	0.65 ± 0.9

Table 3 continued

Mice groups	Biochemical parameters			
	Total lipids (mg/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Bilirubin (mg/dL)
P<	n.s.	n.s.	0.01	n.s.
3c	331.63 ± 17.5	96.4 ± 10.5	118.6 ± 19.70	0.68 ± 0.11
P<	n.s.	n.s.	0.01	n.s.
7a	317.4 ± 30.7	93.24 ± 19.53	116.23 ± 20.5	0.51 ± 0.08
P<	n.s.	n.s.	n.s.	0.01
6b	372.8 ± 37.6	110.3 ± 17.8	93.6 ± 9.5	1.08 ± 0.7
P<	0.001	0.01	n.s.	0.001
10b	382.09 ± 23.6	111.76 ± 34.3	172.9 ± 36.3	0.84 ± 0.4
P<	0.001	0.01	0.001	0.01
9b	328.3 ± 13.7	96.8 ± 17.2	112.3 ± 10.6	0.67 ± 0.01
P<	n.s.	n.s.	n.s.	n.s.
4c	374.9 ± 36.6	123.3 ± 26.09	132.8 ± 26.03	0.99 ± 0.07
P<	0.001	0.001	0.001	0.001
5c	326.3 ± 18.7	95.6 ± 14.9	113.7 ± 8.6	0.61 ± 0.04
P<	n.s.	n.s.	n.s.	n.s.
6c	364.19 ± 23.8	105.6 ± 17.4	119.8 ± 19.3	0.76 ± 0.15
P<	0.001	0.01	0.01	0.01

Data are expressed as mean ± SD

$P > 0.05$ insignificant, $P < 0.01$ significant, $P < 0.001$ highly significant, *n.s.* non-significant

Table 4 Biochemical effects of treatment with 5-FU and DOX, on serum albumin, globulin, and creatinine in mice

Mice groups	Biochemical parameters			
	Albumin (mg/dL)	Globulin (mg/dL)	A/G ratio (mg/dL)	Creatinine (mg/dL)
Control	5.63 ± 0.51	4.32 ± 0.9	1.3	0.69 ± 0.03
5-FU	6.49 ± 0.92	5.75 ± 0.8	1.13	0.81 ± 0.06
P<	0.01	0.01	0.01	0.01
DOX	6.37 ± 0.85	5.91 ± 0.63	1.078	0.78 ± 0.04
P<	0.01	0.01	0.01	0.01
2a	7.1 ± 0.3	7.62 ± 0.76	1.003	0.78 ± 0.03
P<	0.01	0.01	0.001	0.01
1a	7.2 ± 0.61	6.62 ± 0.86	1.006	0.8 ± 0.1
P<	0.01	0.01	0.001	0.01
3c	5.97 ± 0.34	4.09 ± 0.63	1.46	0.65 ± 0.09
P<	n.s.	n.s.	n.s.	n.s.
7a	5.92 ± 0.82	5.12 ± 0.9	1.15	0.73 ± 0.04
P<	n.s.	n.s.	n.s.	n.s.

Table 4 continued

Mice groups	Biochemical parameters			
	Albumin (mg/dL)	Globulin (mg/dL)	A/G ratio (mg/dL)	Creatinine (mg/dL)
6b	6.87 ± 0.49	6.86 ± 0.8	1.02	1.7 ± 0.43
P<	0.01	0.01	0.001	0.001
10b	11.43 ± 1.48	8.97 ± 0.9	1.13	0.76 ± 0.25
P<	0.001	0.001	0.01	n.s.
9b	5.62 ± 0.68	4.68 ± 1.06	1.12	0.68 ± 0.08
P<	n.s.	n.s.	n.s.	n.s.
4c	6.47 ± 0.46	6.42 ± 0.7	1.001	0.77 ± 0.05
P<	0.01	0.01	0.001	0.01
5c	5.92 ± 0.81	4.72 ± 0.91	1.15	0.84 ± 0.6
P<	n.s.	n.s.	n.s.	0.01
6c	7.73 ± 0.52	6.25 ± 0.82	1.23	0.84 ± 0.06
P<	0.01	0.01	0.01	0.01

Data are expressed as mean ± SD

$P > 0.05$ insignificant, $P < 0.01$ significant, $P < 0.001$ highly significant, *n.s.* non-significant

Antimicrobial testing

Antimicrobial evaluation of some synthesized compounds

Antimicrobial activities were carried out against highly pathogenic strains; two Gram-positive bacteria (*Listeria monocytogenes, Staphylococcus aureus*), two Gram-negative bacteria (*Salmonella* Typhimurium, *Escherichia coli*) and one mycotic strain (*Candida albicans*) isolated from animal origin. Agar disk diffusion (qualitative method) and minimum inhibitory concentration (MIC) (quantitative method) were used in this study. Fresh cultures were prepared in Muller Hinton Broth for bacterial strains and Sabaroud Dextrose broth for mycotic strains. The inoculated tubes were incubated at 37 and 28 °C for 24 h, respectively. Serial dilutions were carried out for each strain, and dilution matching with 0.5 Mc-Farland was selected for screening of antimicrobial activities. Ciprofloxacin 100 μg/mL and fluconazole 100 μg/mL were used as reference drugs (Oxoid).

Determination of antimicrobial activity by Disk-diffusion method. [36]

Muller Hinton and Sabaroud Dextrose agar plates were prepared. Bacterial (Gram-positive bacteria; *L. monocytogenes* and *S. aureus* and Gram-negative bacteria; *E. coli* and *S*. Typhimurium) and fungal strains (*C. albicans*) matching with 0.5 Mc-Farland was spread onto the surface of the agar plates using sterile cotton swabs. Isolates were isolated from mastitic cow milk and minced meat. For evaluation of antibacterial activities, Whatman no. 1 filter paper disks were saturated with 50 μL of the extract, others were saturated with 50 μL ciprofloxacin (100 μg/mL) and

others 50 μL DMSO as control negative. The same method was used for evaluation of antimycotic activities using fluconazole (100 μg/mL). Disks were placed onto inoculated agar plates and left for 1 h at 25 °C to allow a period of pre-incubation. The plates were re-incubated at 37 and 28 °C for 24 h for bacterial and mycotic strains, respectively. Then, the plates were observed for antimicrobial activities by determining the diameters of the zones of inhibition for each of the samples. Tests were run in triplicate for each strain to avoid any error.

Determination of MIC [37]

The microtiter dilution plate using 96-well sterile microplates is a quantitative method used for evaluation of the antimicrobial activity of testing compounds. Initial concentration of 100 % was used, then twofold serial dilutions of the compounds and reference drugs (ciprofloxacin and fluconazole) were tested. Plates were inoculated with 100 μL of tested strains (0.5 Mc-Farland, about 1×10^8 cells/mL) and incubated at 37–28 °C for 24 h for bacterial and fungal strains, respectively. After incubation, plates were examined visually for bacterial or fungal growth precipitation. The experiment was repeated three times. The lowest concentration that showed complete hindrance of growth was taken as MIC (Table 6).

Results revealed that compound **10b** gives the highest antibacterial activity against all tested strains with a mean zone of inhibition equal to 18.2 mm followed by **5c** (7 mm) then **10a** (7 mm) then **3c** (6.6 mm) then **7b** (6.6 mm) then **2a** (6 mm) then **3b** (5.4 mm) then **6b** (5.2 mm) then **5b** (5.2 mm) then **9b** (5 mm) then **4c** (5 mm) then **6c** (4.8 mm) then **2b** (4.6 mm) then **7a** (4.4 mm) then **4b** (4.2 mm) then **8b** (4.2 mm) and finally **1a** (3.4 mm) as shown in Table 5. Also, compounds **10b** and **3c** showed the highest results in (MIC) test as shown in Table 6.

Table 5 Agar well diffusion method showing antimicrobial activities of testing compounds compared with reference drugs; the results are in mm

Strains samples	*L. monocytogenes* (A)	*S. aureus* (B)	*E. coli* (2)	*S.* Typhimurium (5)	*C. albicans* (6)
2a	18 s	-ve	-ve	12	-ve
1a	17	-ve	-ve	-ve	-ve
3c	12	-ve	-ve	11	10
7a	-ve	-ve	-ve	11	11
6b	15 s	-ve	-ve	11	-ve
10b	14	12	40 s	13	12
9b	13	-ve	-ve	12	-ve
4c	12	-ve	-ve	13	-ve
5c	12	-ve	-ve	13	10
6c	12	-ve	-ve	12	-ve
3b	-ve	-ve	-ve	15	12
7b	10 s	12	-ve	11	-ve
5b	-ve	15	-ve	11	-ve

Table 5 continued

Strains samples	*L. monocytogenes* (A)	*S. aureus* (B)	*E. coli* (2)	*S.* Typhimurium (5)	*C. albicans* (6)
4b	-ve	11	-ve	10	-ve
8b	-ve	10	-ve	11	-ve
2b	-ve	11	-ve	12	-ve
10a	-ve	10	-ve	13	12
Control negative (DMSO)	-ve	-ve	-ve	-ve	-ve
Ciprofloxacin 100 μg/mL	30	40	40	50	–
Fluconazole 100 μg/mL	–	–	–	–	38

Table 6 Minimum inhibitory concentration method showing antimicrobial activities of testing compounds compared with reference drugs; the results are in μg/mL

Strains samples	*L. monocytogenes*	*S. aureus*	*E. coli*	*S.* Typhimurium	*C. albicans*
2a	12.5	-ve	-ve	100	-ve
1a	12.5	-ve	-ve	-ve	-ve
3c	100	-ve	-ve	100	100
7a	-ve	-ve	-ve	100	100
6b	100	-ve	-ve	100	-ve
10b	50	50	50	50	100
9b	50	-ve	-ve	50	-ve
4c	50	-ve	-ve	50	-ve
5c	50	-ve	-ve	50	100
6c	50	-ve	-ve	50	-ve
3b	-ve	-ve	-ve	25	100
7b	-ve	50	-ve	100	-ve
5b	-ve	25	-ve	100	-ve
4b	-ve	100	-ve	100	-ve
8b	-ve	100	-ve	100	-ve
2b	-ve	100	-ve	50	-ve
10a	-ve	100	-ve	50	50
Control negative (DMSO)	-ve	-ve	-ve	-ve	-ve
Ciprofloxacin 100 μg/mL	6.25	1.56	1.56	0.78	–
Fluconazole 100 μg/mL	–	–	–	–	3.125

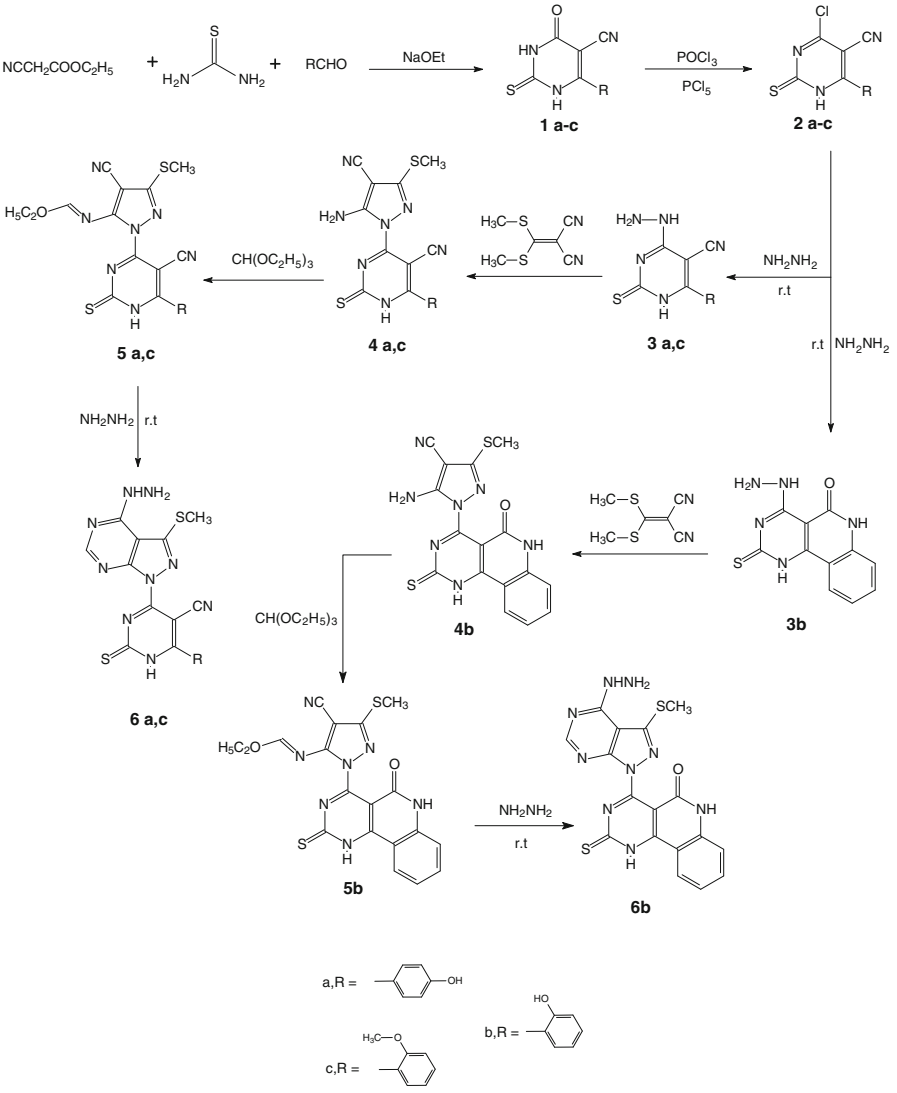
Results and discussion

Chemistry

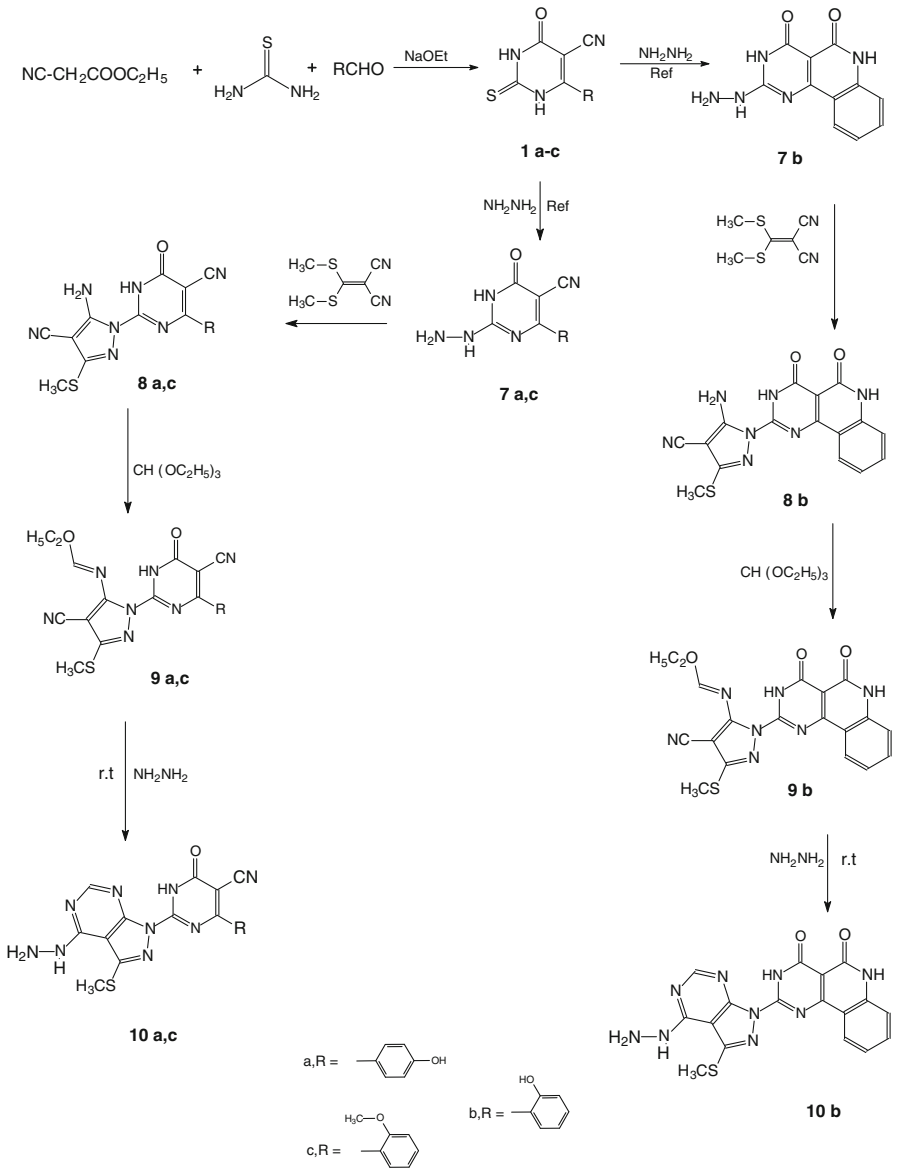
Some new pyrimidine derivatives were synthesized via the reaction of ethyl cyanoacetate with thiourea and the appropriate aldehydes namely 4-hydroxy benzaldehyde, 2-hydroxybenzaldehyde and 2-methoxybenzaldehyde to give **1a**, **b**,

and **1c** [25] (Scheme 1)**.** Compounds **1a**, **b**, and **1c** [25] were chlorinated to give the chloro compounds **2a**, **b**, and **2c** [25] which then reacted with hydrazine hydrate gave compounds **3a**, **b**, and **3c** [25] (Scheme 1)**.**

Compounds **3a**, **b**, and **3c** [25] reacted with 2-(bis(methylthio)methylene)malononitrile to give compounds **4a–c**, respectively, which reacted with trimethylorthoformate to give compounds **5a–c** which then reacted with hydrazine hydrate to give compounds **6a–c** (Scheme 1).



Scheme 1



Scheme 2

Compounds **1a**, **b**, and **1c** [25] were allowed to react with hydrazine hydrate to give compounds **7a–c** (Scheme 2). Compounds **7a–c** reacted with 2-(bis(methylthio) methylene)malononitrile to give compounds **8a–c**, respectively, which reacted with trimethylorthoformate to give compounds **9a–c** which then reacted with hydrazine hydrate to give compounds **10a–c** (Scheme 2).

Anticancer activity

Result

Preliminary screening of the selected pyrimidine derivatives showed that all selected compounds exhibited a moderate to strong growth inhibitory activity on the tested cell line between 1 and 10 μg/mL concentrations in comparison to the known anticancer drugs: 5-FU and DOX. Table 1 indicates the cytotoxic activity of the newly synthesized pyrimidine derivatives **2a**, **1a**, **3c** [25], **7c**, **6b**, **10b**, **9b**, **4c**, **5c**, and **6c** against liver HEPG2 cancer cell lines in comparison to the traditional anticancer drugs: 5-FU and DOX. It can be deduced from the results that compounds **5c** and **6c** were the most active and induced a reasonable growth inhibition in a dose-dependent manner against HEPG2 when compared to 5-FU and DOX (IC_{50} equals 3.56 and 5.23, while 5-FU and DOX were 5 and 3.56 μg/mL).

The effect of antitumor compounds on the biochemical parameters

Data obtained in Table 2 presents the liver enzymatic activities (ALT, AST, and ALP) in serum of control and treated groups of mice. The results showed that the values recorded for AST and ALT were significantly higher ($P < 0.001$) with the 5-FU- and DOX-treated groups of mice than the control. On the other hand, treatment with the new compounds **2a**, **1a**, **3c** [25], **7c**, **6b**, **10b**, **9b**, **4c**, **5c**, and **6c** caused inverse effects, where some values recorded for AST and ALT were non-significant (n.s.) or slightly higher ($P < 0.01$) in comparison to control. Moreover, the recorded data showed that ALP activities were significantly increased ($P < 0.001$) with the treatment of 5-FU and DOX while there were no significant changes in ALP activities upon treatment with some of the new compounds. The data listed in Table 3 demonstrate the comparison between the levels of total lipids, cholesterol, triglycerides, and bilirubin in serum of treated mice and the control group. It can be deduced from the present data that 5-FU and DOX caused a significant increase in the level of these parameters while treatment with the selected compounds showed moderate or no significant changes.

Table 4 represents a comparison between the levels of albumin, globulins, and creatinine in serum of control and treated groups of mice. It is clear from the results in the table that there was a slight increase in the level of albumin and creatinine and globulins in the 5-FU and DOX treated groups of mice while there were moderate or non-significant changes in the other treated groups.

Discussion

Cytotoxic drugs remain the mainstay of cancer chemotherapy and are being administered with novel ways of therapy such as inhibitors of signals [38]. It is therefore important to discover novel cytotoxic agents with spectra of activity and toxicity that differ from those current agents. It is well known that chemotherapy aims to destroy the cancer cells with various types of chemicals. The substances

used are supposed to target mainly the cancer cells, and doses are calculated to minimize the collateral damage to surrounding tissues, which nevertheless occurs [39]. This kind of treatment increases the entropy of the organism, suppresses the immune system, and forms a toxic cell environment which may destroy surrounding healthy cells [40]. So it is important to minimize curing doses to the least amount possible as well as trying to minimize the side effects of these drugs. For this, novel derivatives of pyrimidine possessing a broader spectrum of antitumor activity and fewer toxic side effects than 5-FU and DOX have been sought. The antitumor activities of such compounds were assessed against HEPG2 cancer cell line in comparison to the traditional anticancer drugs: 5-FU and DOX. Regarding the antitumor activity study, some of the selected compounds showed reasonable antitumor activity in comparison to 5-FU and DOX. Moreover, the study of the induced biochemical parameters of the tested compounds in mice showed insignificant differences relative to the control group, which indicates a moderate margin of safety for the selected compounds. Comparable to 5-FU and DOX a dose augmentation of compounds **5c** and **6c**, searching for possible higher potency, seems, consequently, realizable without undesirable implications. Furthermore, the selected compounds have important potential advantages over 5-FU and DOX because of their lower toxicity and their ability to induce lower biochemical parameters. These results are in agreement with Espinosa et al. [41] and Kamalakannan and Venkappayya [42] who have reported novel derivatives of 5-FU possessing a broader spectrum of antitumor activity and fewer toxic side effects than 5-FU.

Conclusion

As a part of our research on the related heterocyclic ring systems, and our attempts to identify new target compounds for future development as antitumor drugs, we previously reported the synthesis and biological of a series of substituted pyrimidine derivatives that have various heterocyclic ring systems. These compounds were selected for further structural modification in an effort to obtain more potent compounds. The goal of this work in making these structural changes was to explore the significance of the spacer on the respective biological activity as having anticancer activity. The anticancer activity data indicate that compounds **5c** and **6c** were the most active and induced a reasonable growth inhibition in a dose-dependent manner against HEPG2 when compared to 5-FU and DOX (IC_{50} equals 3.56 and 5.23, while 5-FU and DOX were 5 and 3.56 μg/mL, respectively).

References

1. P. Callery, P. Gannett, in *Foye's Principles of Medicinal Chemistry*, ed. by D.A. Williams, T.L. Lemke (Lippincott Williams and Wilkins, Philadelphia, 2002), p. 934
2. R. Mishra, I. Tomar, IJPSR **2**(4), 758–771 (2011)

3. O.N. Al Safarjalani, X.J. Zhou, R.H. Ras, J. Shi, R.F. Schinazi, F.N. Naguib, M.H. El Kouni, Cancer Chemother. Pharmacol. **55**, 541–551 (2005)
4. M.S. Masoud, A.A. Ibrahim, E.A. Khalil, A. El-Marghany, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. **67**, 662–668 (2007)
5. A. Odani, H. Kozlowski, J. Swiatek-Kozlowska, J. Brasun, B.P. Operschall, H. Sigel, J. Inorg. Biochem. **101**, 727–735 (2007)
6. C. Vetter, C. Wagner, G.N. Kaluđerović, R. Paschke, D. Steinborn, Inorg. Chim. Acta **362**, 189–195 (2009)
7. M.N. Lipsett, J. Biol. Chem. **240**, 3975 (1965)
8. W. Saenger, *Principles of Nucleic Acid Structures* (Springer, New York, 1984)
9. L. Lapinski, M.J. Nowak, R. Kolos, J.S. Kwiatkowski, J. Leszczynski, Spectrochim. Acta Part A **54**, 685 (1998)
10. C. Heidelberelberger, K.C. Leibman and P.M., Bhargava, Cancer Res. **17**, 39 (1957) C. A. **57**, 16382 c (1957)
11. M.J. Straus, N. Mantel and A. Golden, Cancer Res. **33**, 201(1960), C. A. **63**, 8253 b (1960)
12. M.J. Straus, N. Mantel and A. Golden, *Cancer Res*. P. **126** (1962), C. A. **68**, 32167 a (1962)
13. O.A. Fathalaa, W.A. Zaghary, H.H. Radwan, S.M. Awad, M.S. Mohamed, Arch. Pharm. Res. **25**(3), 258 (2002)
14. P. Langen, *Antimetabolites of Nucleic Acid Metabolism* (Gordon and Breach, New York, 1975)
15. M. Friedland and D.W. Visser, Biochem. Biophys. Act. **51**, 148 (1961), C. A. **70**, 8032 a (1961)
16. G. Vansanten, F. Sorm, E.H. Siman, J. Biol. Chem. **237**, 1271 (1965)
17. R.K. Zahn and U. Hagen, Mol. Pharmac. **1**, 113 (1972), C. A. **90**, 212 a (1972)
18. L. Sui, F. Wang, B.M. Li, Brain Res. **1096**, 53 (2006)
19. O.A. Fathalla, H.H. Radwan, S.M. Awad, M.S. Mohamed, Indian J. Chem. **45B**, 980–985 (2006)
20. A.A. Abu-Hashem, M.M. Youssef, H.A.R. Hussein, J. Chin. Chem. Soc. **58**, 41–48 (2011)
21. S. Prachayasittikul, N. Sornsongkhram, R. Pingaew, S. Techatanachai, S. Ruchirawat, V. Prachayasittikul, Eur. J. Sci. Res. **36**(2), 236–245 (2009)
22. A.Z. Abdel-Hamid, O.A. Fathalaa, J. Pharm. Sci. **34**, 67 (1993)
23. O.A. Fathalaa, H.S.M. Gad, A.S. Maghraby, Arch. Pharm. Res. **23**, 128 (2000)
24. O.A. Fathalla, I.F. Zeid, M.E. Haiba, W.S. El-Serwy, Egypt. Pharm. J. **4**, 593–612 (2005)
25. O.A. Fathalla, I.F. Zeid, M.E. Haiba, A.M. Soliman, ShI Abd-Elmoez, W.S. El-Serwy, World J. Chem. **4**(2), 127–132 (2009)
26. P. Skehan, R. Storeng, D. Scudiero, A. Anne Monks, J. McMahon, D. Vistica, J. Warren, H. Bokesch, S. Kenney, M. Boyd, J. Natl. Cancer Inst. **82**, 1107 (1990)
27. M.H. Abo-Ghalia, A.M. Soliman, Acta Pol. Pharm. Drug Res. **57**, 53 (2000)
28. K. Spencer, C.P. Price, Clin. Chim. Acta **95**, 263 (1979)
29. A. Mays, Lab. Anim. Sci. **19**, 838 (1969)
30. V. Joseph, Vet. Clin. North Am. Exot. Anim. Pract. **2**, 689 (1999)
31. A.M. Soliman, L.M. Faddah, Egypt. J. Bilh. **16**, 8 (1994)
32. A.H. Garde, A.M. Hansen, L.T. Skovgaard, J.M. Christensen, Clin. Chem. **47**, 1877 (2001)
33. E.B. Rietz, G.G. Guilbault, Clin. Chem. **23**, 286 (1977)
34. D.C. Turnell, Ann. Clin. Biochem. **22**, 217 (1985)
35. N.L. Guilbaud, F. Kraus-Berthier, V. Meyer-Losic, C. Malivet, M. Chacun, F. Jan, S. Tillequin, M. Michel, B. Koch, Clin. Cancer Res. **7**, 2573 (2001)
36. S. Bansod, M. Rai, World J. Med. Sci. **3**(2), 81–88 (2008)
37. J.M. Andrews, J. Antimicrob. Chemother. **48**, 5–16 (2001)
38. N.K. Sathish, V.V.S. Rajendra Prasad, N.M. Raghavendra, S.M. Shanta Kumar, Y.C. Mayur, Sci. Pharm. **77**, 19 (2009)
39. S. Pautus, S.W. Yee, M. Jayne, M.P. Coogan, C. Simons, Bioorg. Med. Chem. **14**, 3643 (2006)
40. I. Hayakawa, R. Shioya, T. Agatsuma, H. Furukawa, S. Naruto, Y. Sugano, Bioorg. Med. Chem. Lett. **14**, 4383 (2004)
41. A. Espinosa, J. Marchal, A. Aránega, M. Gallo, S. Aiello, J. Campos, Farmaco **60**, 91 (2005)
42. P. Kamalakannan, D. Venkappayya, J. Inorg. Biochem. **90**, 22 (2002)