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Synthesis of novel *S*-acyl and *S*-alkylpyrimidinone derivatives as potential cytotoxic agents

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Abstract   Two series of 4-phenyl-5-cyanopyrimidin-6-one derivatives bearing various *S*-alkyl or *S*-acyl moieties at position 2 were prepared as cytotoxic agents. All compounds were tested for possible anti-cancer activity on two cell lines (MCF-7 and HCT-116). The MCF-7 cell line was found to be more sensitive than the HCT-116 cell line to the action of the compounds. Compound **8g** was the most potent on the MCF-7 cell line with IC_{50} 18.3 nM/mL, whereas its IC_{50} on the normal cell line (MRC-5) was 64.38 nM/mL, indicating its safety and selectivity towards the MCF-7 cell line. On the other hand, compound **8d** was the most potent compound on the HCT-116 cell line with IC_{50} 23.8 nM/mL. Compound **8g** was screened against five kinases. The compound showed selective inhibitory activity against pim1 kinase with IC_{50} 11.62 μM.

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Graphical Abstract

Synthesis of Novel *S*-acyl and *S*-alkylpyrimidinone Derivatives as Potential cytotoxic Agents

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New *S*-acyl and *S*-alkylpyrimidinones were synthesized as cytotoxic agents. Compound **8g** was the most potent cytotoxic on MCF-7 cell line. Compound **8g** was potent and selective pim1 inhibitor.
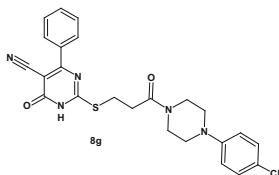
Keywords   Pyrimidine · Antitumor activity · MCF-7 · HCT-116 · pim1

Introduction

Cancer is considered the second leading cause of death worldwide after cardiovascular diseases [1]. In spite of the presence of a large number of chemotherapeutic agents, they cause severe side effects and development of resistance by cancer cells even against drugs developed by targeted therapy such as imitanib [2]. Therefore, there is a continuous need for the development of novel molecules that can overcome cellular resistance.

Since the introduction of 5-flourouracil in the late 1950s as a potent anticancer agent, many uracil and thiouracil derivatives have been reported as cytotoxic agents [3].

In addition, 4-aryl-5-cyanopyrimidinone derivatives were recently reported as cytotoxic agents [4–7]. In most of these derivatives, alkylation of the 2-thio group especially with alkylamino or acetanilide moieties (such as compounds **I–IV**, Fig. 1) was reported to enhance the cytotoxic activity of the resulting pyrimidine derivatives [4–9].

Prompted by these facts, we aimed in this work to prepare 4-phenyl-5-cyanopyrimidin-6-one derivatives bearing various *S*-(substituted amino alkyl) moieties at position 2 (compounds **6a–c** and **8a–g**) to act as cytotoxic agents. Comparable *S*-acyl derivatives (compounds **4a–c**), which were poorly studied in the literature as cytotoxic agents, were also prepared. In both series, different alkyl linkers and different aliphatic and aromatic amines were used to study the effect of these variations on the cytotoxic activity. All compounds were tested for possible anti-cancer activity on two cell lines (MCF-7 and HCT-116). The most active cytotoxic compound (**8g**) was subjected to further studies to explore its mechanism of action.
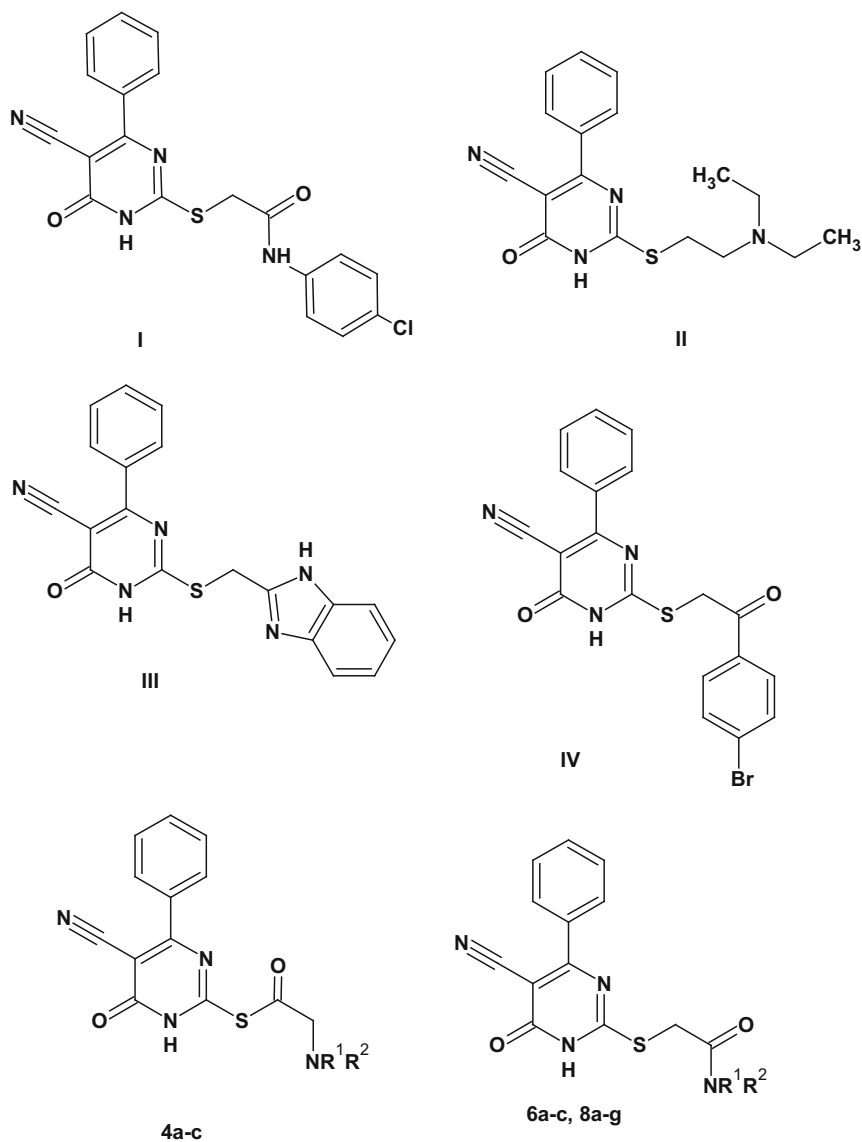
Fig. 1 Structures of 4-phenyl-5-cyanopyrimidin-6-ones with cytotoxic activity

Experimental part

General

All melting points were determined on a Stuart apparatus, and the values given were uncorrected. IR spectra were determined as KBr discs on a Shimadzu IR 435

spectrophotometer, Faculty of Pharmacy, Cairo University, and values were represented in cm^{-1}. ^{1}H-NMR spectra were carried out using a Mercury 300-BB 300 MHz spectrophotometer, Cairo University, Egypt, using TMS as internal standard. Chemical shift values were recorded in ppm on δ scale. ^{13}C-NMR spectra were carried out using a Bruker 100 MHz spectrophotometer, Microanalytical Unit, Faculty of Pharmacy, Cairo University, Egypt, using TMS as internal standard. Chemical shift values were recorded in ppm on δ scale. Mass spectra were recorded on a Hewlett Packard 5988 spectrometer, or Shimadzu QP-2010 plus, Microanalytical Center, Cairo University, Egypt. Elemental analyses were carried out at the Regional Center for Mycology and Biotechnology, Azher University, Egypt. Progress of reactions was monitored by TLC using TLC sheets precoated with UV florescent silica gel Merck 60 F 254, and the sheets were visualized using a UV lamp. All reagents and solvents were purified and dried by standard techniques.

General procedure for the synthesis of S-(5-cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl) 3-chloroalkanethioates (2a–c)

A mixture of compound **1** [10] (0.46 g, 2 mmol), chloroacetyl chloride, 2-chloro-propionyl chloride or 3-choropropionyl chloride (2 mmol), and anhydrous K_2CO_3 (0.28 g, 2 mmol) in dry benzene/dry DMF (20 mL/0.5 mL) was heated under reflux for 10 h. The reaction was cooled and the separated solid was filtered, washed with water (20 mL), dried, and crystallized from DCM.

S-(5-Cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl) chloroethanethioate (**2a**) Yield: 47 %; mp: 218–220 °C; IR (cm^{-1}): 3444 (NH), 2997, 2943 (C–H aliphatic), 2225 (CN), 1716, 1651 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm 3.93 (s, 2H, CH$_2$Cl), 7.47–7.79 (m, 5H, Ar–H), 12.71 (s, 1H, NH, D$_2$O exchangeable); Anal. Calcd. for C$_{13}$H$_8$ClN$_3$O$_2$S: C, 51.07; H, 2.64; N, 13.74. Found: C, 51.16; H, 2.71; N, 13.89.

S-(5-Cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl) 2-chloropropanethioate (**2b**) Yield: 27 %; mp: 282–284 °C; IR (cm^{-1}): 3186 (NH), 2978, 2935 (C–H aliphatic), 2229 (CN), 1732, 1643 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm 1.55–1.57 (d, 3H, CH$_3$CH, J = 7.5 Hz), 4.47–4.55 (q, 1H, CH$_3$CH, J = 7.5 Hz), 7.36–7.97 (m, 5H, Ar–H), 13.10 (s, 1H, NH, D$_2$O exchangeable); Anal. Calcd. for C$_{14}$H$_{10}$ClN$_3$O$_2$S: C, 52.59; H, 3.15; N, 13.14. Found: C, 52.64; H, 3.20; N, 13.28.

S-(5-Cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl) 3-chloropropanethioate (**2c**) Yield: 30 %; mp: 240–242 °C; IR (cm^{-1}): 3400 (NH), 2945, 2926 (C–H aliphatic), 2227 (CN), 1691, 1647 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm 2.72–2.76 (t, 2H, CH$_2$CH$_2$Cl, J = 6.6 Hz), 3.31–3.41 (t, 2H, CH$_2$CH$_2$Cl, J = 6.6 Hz), 7.36–7.95 (m, 5H, Ar–H), 12.51 (s, 1H, NH, D$_2$O exchangeable); ^{13}C NMR (CDCl$_3$) δ ppm 26.2 (CH$_2$CH$_2$Cl), 33.9 (CH$_2$CH$_2$Cl), 116.4 (CN), 93.5, 128.7, 129.0, 132.2, 135.7, 161.6, 166.3 (Ar–C), 167.7 (C=O), 173.2 (SC=O); Anal. Calcd. for C$_{14}$H$_{10}$ClN$_3$O$_2$S: C, 52.59; H, 3.15; N, 13.14. Found: C, 52.65; H, 3.18; N, 13.23.

General procedure for the synthesis of 6-oxo-4-phenyl-2-(substituted amino)-1,6-dihydropyrimidine-5-carbonitriles (3a–e)

A mixture of compound **2a** (0.61 g, 2 mmol), the appropriate secondary aliphatic amine (2 mmol) and triethyl amine (0.28 mL, 2 mmol) in dry benzene/dry DMF (20 mL/0.5 mL) was heated under reflux for 16 h. The reaction was cooled, and the separated solid was filtered, dried, and crystallized from DCM.

6-Oxo-4-phenyl-2-(piperidin-1-yl)-1,6-dihydropyrimidine-5-carbonitrile (**3a**) Yield: 15 %; mp: 238–240 °C; IR (cm^{-1}): 3400 (NH), 2947, 2862 (C–H aliphatic), 2212 (CN), 1626 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm 1.54–1.57 (m, 2H, CH$_2$CH$_2$CH$_2$, J = 4.8 Hz), 1.61–1.62 (m, 4H, CH$_2$CH$_2$N, J = 4.8 Hz), 2.97–2.99 (t, 4H, CH$_2$CH$_2$N, J = 4.8 Hz), 7.44–7.74 (m, 5H, Ar–H), 11.60 (s, 1H, NH, D$_2$O exchangeable); ^{13}C NMR (CDCl$_3$) δ ppm 24.1 (CH$_2$CH$_2$CH$_2$), 25.6 (CH$_2$CH$_2$N), 46.0 (CH$_2$CH$_2$N), 117.8(CN), 128.7, 128.8, 131.4, 137.1 (Ar–C), 170.1 (C=O); MS m/z: 280 [M$^+$, 97.0 %], 251 [100 %], 77 [C$_6$H$_5^+$, 36.4 %]; Anal. Calcd. for C$_{16}$H$_{16}$N$_4$O: C, 68.55; H, 5.75; N, 19.99. Found: C, 68.69; H, 5.73; N, 20.13.

2-(Morpholin-4-yl)-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (**3b**) Yield: 28 %; mp: 220–222 °C; IR (cm^{-1}): 3392 (NH), 2922, 2858 (C–H aliphatic), 2208 (CN), 1647 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm 3.66–3.69 (m, 4H, CH$_2$N, J = 4.8 Hz), 3.77–3.78 (m, 4H, CH$_2$O, J = 4.8 Hz), 7.49–7.87 (m, 5H, Ar–H), 11.95 (s, 1H, NH, D$_2$O exchangeable); MS m/z: 282 [M$^+$, 45.2 %], 77 [C$_6$H$_5^+$, 11.3 %], 71 [100 %]; Anal. Calcd. for C$_{15}$H$_{14}$N$_4$O$_2$: C, 63.82; H, 5.00; N, 19.85. Found: C, 63.90; H, 5.04; N, 20.02.

6-Oxo-4-phenyl-2-(4-phenylpiperazin-1-yl)-1,6-dihydropyrimidine-5-carbonitrile (**3c**) Yield: 15 %; mp: 240–242 °C; IR (cm^{-1}): 3363 (NH), 2916, 2989 (C–H aliphatic), 2191 (CN), 1635 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm 3.17 (m, 4H, CH$_2$N), 3.87 (m, 4H, CH$_2$N), 6.77–7.81 (m, 10H, Ar–H), 12.10 (s, 1H, NH, D$_2$O exchangeable); MS m/z: 357 [M$^+$, 19.3 %], 132 [100 %], 77 [C$_6$H$_5^+$, 42.0 %]; Anal. Calcd. for C$_{21}$H$_{19}$N$_5$O: C, 70.57; H, 5.36; N, 19.59. Found: C, 70.74; H, 5.41; N, 19.72.

2-[4-(4-Chlorophenyl)-piperazin-1-yl]-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (**3d**) Yield: 11 %; mp: 230–232 °C; IR (cm^{-1}): 3406 (NH), 2943, 2881(C–H aliphatic), 2214 (CN), 1654 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm 3.12–3.14 (m, 4H, CH$_2$N), 3.91–3.92 (m, 4H, CH$_2$N), 6.97–7.01 (d, 2H, Ar–H, J = 9 Hz), 7.23–7.26 (d, 2H, Ar–H, J = 9 Hz), 7.52–7.88 (m, 5H, Ar–H), 10.14 (s, 1H, NH, D$_2$O exchangeable); Anal. Calcd. for C$_{21}$H$_{18}$ClN$_5$O: C, 64.37; H, 4.63; N, 17.87. Found: C, 64.42; H, 4.68; N, 17.98.

2-[4-(4-Methoxyphenyl)-piperazin-1-yl]-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (**3e**) Yield: 13 %; mp: 270–272 °C; IR (cm^{-1}): 3394 (NH), 2962, 2897 (C–H aliphatic), 2198 (CN), 1631 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm 3.10–3.11 (m, 4H, CH$_2$N, J = 5 Hz), 3.69 (s, 3H, CH$_3$O), 3.94 (m, 4H, CH$_2$N, J = 5 Hz), 6.82–6.86 (d, 2H, Ar–H, J = 9 Hz), 6.92–6.96 (d, 2H, Ar–H, J = 9 Hz), 7.51–7.89 (m, 5H, Ar–H), 12.10 (s, 1H, NH, D$_2$O exchangeable); ^{13}C

NMR (CDCl$_3$) δ ppm 44.8 (CH$_2$N), 49.8 (CH$_2$N), 55.6 (CH$_3$O), 114.7 (CN), 93.5, 117.7, 118.4, 128.7, 128.8, 131.5, 137.0, 145.3, 153.8, 161.0, 166.0 (Ar–C), 170.1 (C=O); MS m/z: 387 [M$^+$, 55.1 %], 231 [100 %]; Anal. Calcd. for C$_{22}$H$_{21}$N$_5$O$_2$: C, 68.20; H, 5.46; N, 18.08. Found: C, 68.28; H, 5.49; N, 18.21.

General procedure for the synthesis of *S*-(5-cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl) (substituted phenylamino) alkanethioates (4a–c)

A mixture of compounds **2a–c** (2 mmol), the appropriate primary aromatic amine (2 mmol) and triethyl amine (0.28 mL, 2 mmol) in dry DMF (20 mL) was heated under reflux until completion of the reaction (24 h for **4c** and 35 h for **4a** and **4b**). The mixture was concentrated under reduced pressure, cooled, poured onto ice-cold water (20 mL), and the separated solid was filtered, dried, and crystallized from ethanol.

S-(5-Cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl) (phenylamino)ethanethioate (**4a**) Yield: 22 %; mp: 120–122 °C; IR (cm^{-1}): 3304, 3269 (NH), 2991, 2953 (C–H aliphatic), 2225 (CN), 1716, 1670 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm 3.15 (s, 2H, CH$_2$CO), 7.24–7.82 (m, 10H, Ar–H), 9.40 (s, 1H, NH, D$_2$O exchangeable), 12.71 (s, 1H, NH, D$_2$O exchangeable); Anal. Calcd. for C$_{19}$H$_{14}$N$_4$O$_2$S: C, 62.97; H, 3.89; N, 15.46. Found: C, 63.04; H, 3.94; N, 15.62.

S-(5-Cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl) 2-[(4-chlorophenyl)amino]propanethioate (**4b**) Yield: 29 %; mp: 252–254 °C; IR (cm^{-1}): 3435, 3246 (NH), 2926, 2954 (C–H aliphatic), 2210 (CN), 1651, 1624 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm 1.12-1.23 (d, 3H, <u>CH$_3$</u>CH), 3.30–3.35 (m, 1H, CH$_3$<u>CH</u>), 7.23–7.52 (m, 5H, Ar–H), 7.61–7.69 (d, 2H, Ar–H),7.81–7.88 (d, 2H, Ar–H), 10.40 (s, 1H, NH, D$_2$O exchangeable), 12.50 (s, 1H, NH, D$_2$O exchangeable); ^{13}C NMR (CDCl$_3$) δ ppm 37.6 (<u>CH$_3$</u>CH), 83.1 (CH$_3$<u>CH</u>), 119.7 (CN), 121.1, 128.3, 128.4, 128.5, 128.5, 128.6, 129.0, 129.2, 130.3, 130.7, 138.1 (Ar–C), 160.2 (C=O), 165.5 (SC=O); Anal. Calcd. for C$_{20}$H$_{15}$ClN$_4$O$_2$S: C, 58.46; H, 3.68; N, 13.64. Found: C, 58.61; H, 3.72; N, 13.81.

S-(5-Cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl) 3-[(4-methylphenyl)amino]propanethioate (**4c**) Yield: 17 %; mp: 172–174 °C; IR (cm^{-1}): 3344, 3298 (NH), 2974, 2935 (C–H aliphatic), 2225 (CN), 1689, 1647 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm 2.27 (s, 3H, CH$_3$), 2.72-2.76 (t, 2H, <u>CH$_2$</u>CH$_2$N, *J* = 7.2 Hz), 3.07–3.11 (t, 2H, CH$_2$<u>CH$_2$</u>N, *J* = 7.2 Hz), 7.16–7.67 (m, 5H, Ar–H), 7.84–7.87 (d, 2H, Ar–H, *J* = 7 Hz), 7.92–7.95 (d, 2H, Ar–H, *J* = 7 Hz), 9.97 (s, 1H, NH, D$_2$O exchangeable), 12.20 (s, 1H, NH, D$_2$O exchangeable); MS m/z: 390 [M$^+$, 0.5 %], 302 [100 %], 77 [C$_6$H$_5^+$, 24.3 %]; Anal. Calcd. for C$_{21}$H$_{18}$N$_4$O$_2$S: C, 64.60; H, 4.65; N, 14.35. Found: C, 64.68; H, 4.73; N, 14.51.

General procedure for the synthesis of 2-(5-cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-ylsulfanyl)-*N*-(substituted phenyl) alkanamides 6a–c and 6-oxo-2-{[2(3)-oxo-2-(substituted amino)alkyl]sulfanyl}-4-phenyl-1,6-dihydropyrimidine-5-carbonitriles (8a–g)

Method A

A mixture of compound **1** (0.46 g, 2 mmol), chloro derivatives **5a, 7c** or **7f** [11, 15, 17] (2 mmol) in 10 % KOH solution (1 g KOH in 10 mL water) was stirred at room temperature for 48 h. The separated solid was filtered, dried, and crystallized from ethanol.

Method B

A mixture of compound **1** (0.46 g, 2 mmol), chloro derivatives **5a–c** [11–13] or **7a–g** [11, 14–17] (2 mmol), and anhydrous K_2CO_3 (0.55 g, 4 mmol) in dry DMF (20 mL) was heated under reflux for 16 h. The mixture was concentrated under reduced pressure, cooled, and poured slowly onto ice-cold water (15 mL). The separated solid was filtered, dried, and crystallized from ethanol.

2-(5-Cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-ylsulfanyl)-*N*-*p*-tolyl-acetamide (**6a**)   Yield: 47 % (method A), 45 % (method B); mp: 232–234 °C; IR (cm^{-1}): 3263, 3180 (NH), 2922, 2858 (C–H aliphatic), 2220 (CN), 1695, 1660 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm 2.27 (s, 3H, CH$_3$), 4.16 (s, 2H, CH$_2$CO), 7.10–7.13 (d, 2H, Ar–H), 7.34–7.55 (m, 5H, Ar–H), 7.85–7.88 (d, 2H, Ar–H), 9.41 (s, 1H, NH, D$_2$O exchangeable), 10.22(s, 1H, NH, D$_2$O exchangeable); MS m/z: 376 [M$^+$, 0.4 %], 107 [100 %], 106 [CH$_3$C$_6$H$_4$NH$^+$, 99.4], 77 [C$_6$H$_5^+$, 54.5 %]; Anal. Calcd. for C$_{20}$H$_{16}$N$_4$O$_2$S: C, 63.81; H, 4.28; N, 14.88. Found: C, 63.90; H, 4.31; N, 15.02.

2-[(5-Cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl)sulfanyl]-*N*-phenylac-etamide (**6b**)   Yield: 25 % (method B); mp: 138–140 °C; IR (cm^{-1}): 3304, 3197 (NH), 2953, 2924 (C–H aliphatic), 2210 (CN), 1668, 1637 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm 1.44–1.46 (d, 3H, C̲H̲$_3$CH, J = 7.2 Hz), 4.43–4.46 (q, 1H, CH$_3$C̲H̲, J = 7.2 Hz), 6.98–7.95 (m, 10H, Ar–H), 10.31 (s, 1H, NH, D$_2$O exchangeable), 11.07 (s, 1H, NH, D$_2$O exchangeable); MS m/z: 376 [M$^+$, 20.6 %], 77 [C$_6$H$_5^+$, 41.5 %], 57 [100 %]; Anal. Calcd. for C$_{20}$H$_{16}$N$_4$O$_2$S: C, 63.81; H, 4.28; N, 14.88. Found: C, 63.94; H, 4.26; N, 15.03.

3-[(5-Cyano-6-oxo-4-phenyl-1,6-dihydropyrimidin-2-yl)sulfanyl]-*N*-phenyl-propi-onamide (**6c**)   Yield: 47 % (method B); mp: 212–214 °C; IR (cm^{-1}): 3298, 3197 (NH), 2954, 2924 (C–H aliphatic), 2210 (CN), 1668, 1654 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm 2.85–2.87 (t, 2H, CH$_2$C̲H̲$_2$CO), 3.48–3.50 (t, 2H, C̲H̲$_2$CH$_2$CO), 7.03–8.03 (m, 10H, Ar–H), 9.98 (s, 1H, NH, D$_2$O exchangeable), 11.72 (s, 1H, NH, D$_2$O exchangeable); MS m/z: 376 [M$^+$, 0.7 %], 229 [100 %], 77 [C$_6$H$_5^+$, 17.3 %]; Anal. Calcd. for C$_{20}$H$_{16}$N$_4$O$_2$S: C, 63.81; H, 4.28; N, 14.88. Found: C, 63.89; H, 4.31; N, 14.98.

6-Oxo-2-{[2-oxo-2-(piperidin-1-yl)ethyl]sulfanyl}-4-phenyl-1,6-dihydropyrim-idine-5-carbonitrile (**8a**)   Yield: 52 % (method B); mp: 290–292 °C; IR (cm^{-1}): 3296, (NH), 2947, 2922 (C–H aliphatic), 2198 (CN), 1675, 1637 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm 1.57–1.59 (m, 6H, C$\underline{H_2CH_2CH_2}$), 3.31 (m, 4H, $\underline{CH_2}$N), 3.76 (s, 2H, CH$_2$CO), 7.48–7.95 (m, 5H, Ar–H), 11.91 (s, 1H, NH, D$_2$O exchangeable); MS m/z: 354 [M$^+$, 0.5 %], 84 [C$_5$H$_{10}$N$^+$, 100 %], 77 [C$_6$H$_5^+$, 49.2 %]; Anal. Calcd. for C$_{18}$H$_{18}$N$_4$O$_2$S: C, 61.00; H, 5.12; N, 15.81. Found: C, 61.12; H, 5.17; N, 16.02.

2-{[2-(Morpholin-4-yl)-2-oxoethyl]sulfanyl}-6-oxo-4-phenyl-1,6-dihydropyrim-idine-5-carbonitrile (**8b**)   Yield: 65 % (method B); mp: 208–210 °C; IR (cm^{-1}): 3292, (NH), 2958, 2927 (C–H aliphatic), 2212 (CN), 1675, 1640 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm 3.30 (s, 2H, CH$_2$CO), 3.66–3.67 (t, 4H, CH$_2$N, $J = 4.5$ Hz), 3.77–3.78 (t, 4H, CH$_2$O, $J = 4.5$ Hz), 7.49–7.97 (m, 5H, Ar–H), 11.80 (s, 1H, NH, D$_2$O exchangeable); Anal. Calcd. for C$_{17}$H$_{16}$N$_4$O$_3$S: C, 57.29; H, 4.52; N, 15.72. Found: C, 57.38; H, 4.59; N, 15.93.

6-Oxo-2-[(2-oxo-2-(4-phenylpiperazin-1-yl)-ethyl)sulfanyl]-4-phenyl-1,6-dihy-dropyrimidine-5-carbonitrile (**8c**)   Yield: 53 % (method A), 46 % (method B); mp: 120–122 °C; IR (cm^{-1}): 3387, (NH), 2953, 2920 (C–H aliphatic), 2222 (CN), 1660, 1647 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm 3.13 (m, 4H, CH$_2$N), 3.61 (m, 4H, CH$_2$N), 4.03 (s, 2H, CH$_2$CO), 6.81–7.97 (m, 10H, Ar–H), 10.26 (s, 1H, NH, D$_2$O exchangeable); ^{13}C NMR (CDCl$_3$) δ ppm 37.5 ($\underline{CH_2}$CO), 41.8 (CH$_2$N), 45.8 (CH$_2$N), 116.3 (CN), 89.5, 119.7, 120.4, 128.5, 128.6, 129.4, 130.2, 138.0, 151.2, 167.4, 167.4 (Ar–C), 170.7 (C=O), 171.3 (SC=O); MS m/z: 431 [M$^+$, 0.3 %], 229 [100 %], 77 [C$_6$H$_5^+$, 23.3 %]; Anal. Calcd. for C$_{23}$H$_{21}$N$_5$O$_2$S: C, 64.02; H, 4.91; N, 16.23. Found: C, 64.17; H, 4.97; N, 16.39.

2-{2-[4-(4-Methoxyphenyl)-piperazin-1-yl]-1-methyl-2-oxo-ethylsulfanyl}-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (**8d**)   Yield: 20 % (method B); mp: 192–194 °C; IR (cm^{-1}): 3292, (NH), 2993, 2929 (C–H aliphatic), 2214 (CN), 1683, 1640 (C=O); ^{1}H NMR (DMSO-d_6-) δ ppm 1.47–1.49 (d, 3H, C$\underline{H_3}$CH, $J = 7.2$ Hz), 3.11–3.13 (m, 4H, CH$_2$N, $J = 4.08$ Hz), 3.69 (s, 3H, CH$_3$O), 4.13–4.15 (m, 4H, CH$_2$N, $J = 4.08$ Hz), 4.37–4.39 (q, 1H, CH$_3$C$\underline{H}$, $J = 7.2$ Hz), 6.83–7.89 (m, 9H, Ar–H), 12.0 (s, 1H, NH, D$_2$O exchangeable); ^{13}C NMR (CDCl$_3$) δ ppm 37.5 ($\underline{CH_3}$CH), 44.8 (CH$_2$N), 49.8 (CH$_2$N), 55.6 (CH$_3$O), 83.8 (CH$_3\underline{CH}$), 117.8 (CN), 114.7, 118.4, 128.7, 129.0, 131.5, 132.5, 137.1, 145.3, 150.5, 153.8, 161.2 (Ar–C), 163.0 (C=O), 170.0 (SC=O); Anal. Calcd. for C$_{25}$H$_{25}$N$_5$O$_3$S: C, 63.14; H, 5.30; N, 14.73. Found: C, 63.24; H, 5.28; N, 14.90.

2-{[3-(Morpholin-4-yl)-3-oxopropyl]sulfanyl}-6-oxo-4-phenyl-1,6-dihydropyrim-idine-5-carbonitrile (**8e**)   Yield: 48 % (method B); mp: 282–284 °C; IR (cm^{-1}): 3265, (NH), 2950, 2924 (C–H aliphatic), 2204 (CN), 1665, 1635 (C=O); ^{1}H NMR (DMSO-d_6-) δ ppm 3.12 (t, 2H, CH$_2$C$\underline{H_2}$CO), 3.23 (t, 2H, C$\underline{H_2}$CH$_2$CO), 3.66–3.67 (m, 4H, CH$_2$N, $J = 4.5$ Hz), 3.77–3.78 (m, 4H, CH$_2$O, $J = 4.5$ Hz), 7.48–7.88 (m, 5H, Ar–H), 11.80 (s, 1H, NH, D$_2$O exchangeable); ^{13}C NMR (CDCl$_3$) δ ppm 38.0 (CH$_2\underline{CH_2}$CO), 45.2 ($\underline{CH_2}$CH$_2$CO), 66.2 (CH$_2$N), 67.1 (CH$_2$O), 117.6 (CN), 128.8, 129.1, 131.4, 131.5, 132.3, 137.1, 155.0 (Ar–C), 163.6 (C=O), 170.0 (SC=O); MS

m/z: 370 [M$^+$, 9.1 %], 240 [100 %], 77 [C$_6$H$_5$$^+$, 50.4 %]; Anal. Calcd. for C$_{18}$H$_{18}$N$_4$O$_3$S: C, 58.36; H, 4.90; N, 15.12. Found: C, 58.44; H, 4.96; N, 15.30.

6-Oxo-2-[3-oxo-3-(4-phenylpiperazin-1-yl)-propylsulfanyl]-4-phenyl-1,6-dihy-dropyrimidine-5-carbonitrile (**8f**) Yield: 59 % (method A), 55 % (method B); mp: 260–262 °C; IR (cm^{-1}): 3365 (NH), 2956, 2926 (C–H aliphatic), 2210 (CN), 1668, 1645 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm 2.85–2.88 (t, 2H, C̲H̲$_2$CH$_2$CO), 3.30 (m, 4H, CH$_2$N), 3.51 (m, 4H, CH$_2$N), 3.59 (t, 2H, CH$_2$C̲H̲$_2$CO), 6.80–7.91 (m, 10H, Ar–H), 11.80 (s, 1H, NH, D$_2$O exchangeable); MS m/z: 445 [M$^+$, 6.3 %], 301 [100 %], 77 [C$_6$H$_5$$^+$, 49.4 %]; Anal. Calcd. for C$_{24}$H$_{23}$N$_5$O$_2$S: C, 64.70; H, 5.20; N, 15.72. Found: C, 64.84; H, 5.24; N, 15.88.

2-{3-[4-(4-Chlorophenyl)-piperazin-1-yl]-3-oxo-propylsulfanyl}-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (**8g**) Yield: 41 % (method B); mp: 148–150 °C; IR (cm^{-1}): 3344, (NH), 2935, 2924 (C–H aliphatic), 2216 (CN), 1689, 1647 (C=O); ^{1}H NMR (DMSO-d_6) δ ppm 3.07–3.09 (t, 2H, C̲H̲$_2$CH$_2$CO, $J = 3.6$ Hz), 3.27–3.28 (m, 4H, CH$_2$N, $J = 4.2$ Hz), 3.59–3.60 (m, 4H, CH$_2$N, $J = 4.2$ Hz), 3.40 (t, 2H, CH$_2$C̲H̲$_2$CO, $J = 3.6$ Hz), 6.91–7.89 (m, 9H, Ar–H), 12.20 (s, 1H, NH, D$_2$O exchangeable); Anal. Calcd. for C$_{24}$H$_{22}$ClN$_5$O$_2$S: C, 60.06; H, 4.62; N, 14.59. Found: C, 60.22; H, 4.59; N, 14.76.

In vitro anticancer testing

Materials and methods

The breast tumor cell line (MCF-7), the colon tumor cell line (HCT-116), and the human lung fibroblast cell line (MRC-5) were obtained frozen in liquid nitrogen (−180 °C) from the American Type Culture Collection (ATCC) and were maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing.

All chemicals used in this study are of high analytical grade. They were obtained from either Sigma-Aldrich or Bio-Rad.

Measurement of potential cytotoxicity

The cytotoxic activity of the newly synthesized compounds was measured in vitro on breast tumor cell line (MCF-7) and colon tumor cell line (HCT-116) using Sulforhodamine-B stain (SRB) assay applying the method of Skehan et al. [18] In addition, compound **8g** was examined for its cytotoxic effect on the human lung fibroblast cell line (MRC-5).

The cells were plated in a 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the test compounds to allow attachment of the cells to the wall of the plate. The test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the test compounds (0, 1, 2.5, 5, and 10 µg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the test compounds for

48 h at 37 °C in atmosphere of 5 % CO_2. After 48 h, the cells were fixed with trichloroacetic acid, washed with water, and stained for 30 min with 0.4 % (wt/vol) Sulforhodamine-B stain dissolved with 1 % acetic acid. Excess stain was removed by four washes with 1 % acetic acid and the attached stain was recovered with Tris EDTA buffer. The colour intensity was measured in ELISA reader. For each compound, the relation between the surviving fraction and the drug concentration was plotted to get the survival curve of each tumor cell line. The IC_{50} values (the concentration required for 50 % inhibition of cell viability) were calculated using sigmodial dose response curve-fitting models (GraphPad, Prizm software incorporated), each concentration was repeated three times. The results are given in Table 1 and represented graphically in Fig. 2.

Kinase inhibitory activity

Materials and methods
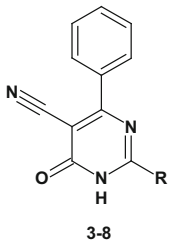
The kinase inhibitory activity of compound **8g** was determined using the Kinexus compound profiling service, Canada. The protein kinases employed in the compound profiling process were GSK-3β, pim1, pim2, EGFR and CDK5/p25 enzymes. All the kinases were cloned, expressed, and purified using proprietary methods. Quality control testing was routinely performed to ensure compliance to acceptable standards. ^{33}P-ATP was purchased from PerkinElmer. All other materials were of standard laboratory grade.

Kinase protein assays

A radioisotope assay format was used for profiling evaluation of protein kinase target, and all assays were performed in a designated radioactive working area. Protein kinase assays were performed at ambient temperature for 20–30 min in a final volume of 25 μL according to the following assay reaction components: Component 1, 5 μL of diluted active protein kinase ($\sim$10–50 nM final concentration in the assay); Component 2, 5 μL of stock solution of substrate; Component 3, 5 μL of kinase assay buffer; Component 4, 5 μL of compound **8g** or 10 % DMSO; Component 5, 5 μL of ^{33}P-ATP (250 μM stock solution, 0.8 μCi).

The assay was initiated by the addition of ^{33}P-ATP and the reaction mixture was incubated at ambient temperature for 30 min. After the incubation period, the assay was terminated by spotting 10 μL of the reaction mixture onto a Multiscreen phosphocellulose P81 plate. The Multiscreen phosphocellulose P81 plate was washed three times for approximately 15 min each in a 1 % phosphoric acid solution. Radioactivity of the P81 plate was counted in the presence of scintillation fluid in a Trilux scintillation counter. A blank control was set up that included all the assay components except the addition of the appropriate substrate (replaced with equal volume of assay dilution buffer). The corrected activity for protein kinase

Table 1 Results of in vitro cytotoxic activity of the synthesized compounds on breast tumor cell line (MCF-7) and colon tumor cell line (HCT-116)

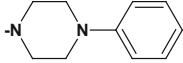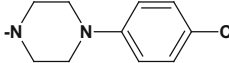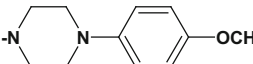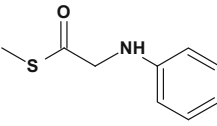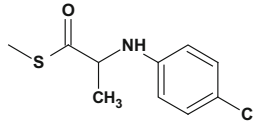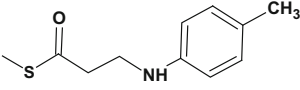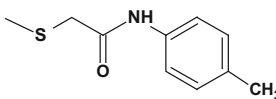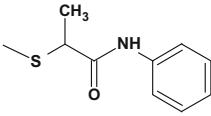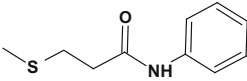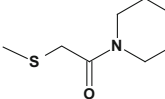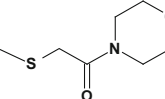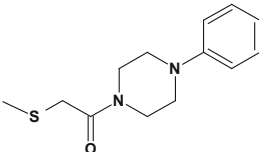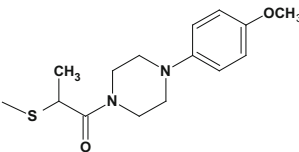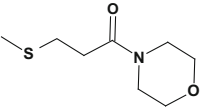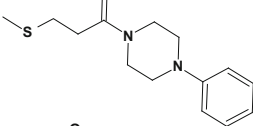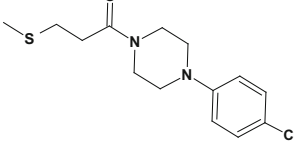| Compound no.	R	IC$_{50}$ in nM/mL[a]	
		MCF-7	HCT-116
3a		52.8	67.8
3b		51.4	74.1
3c		27.9	47.3
3d		34.2	44.9
3e		53.7	52.1
4a		60.2	52.5
4b		32.1	32.3
4c		58.2	57.4
6a		46.5	50.5

Table 1 continued

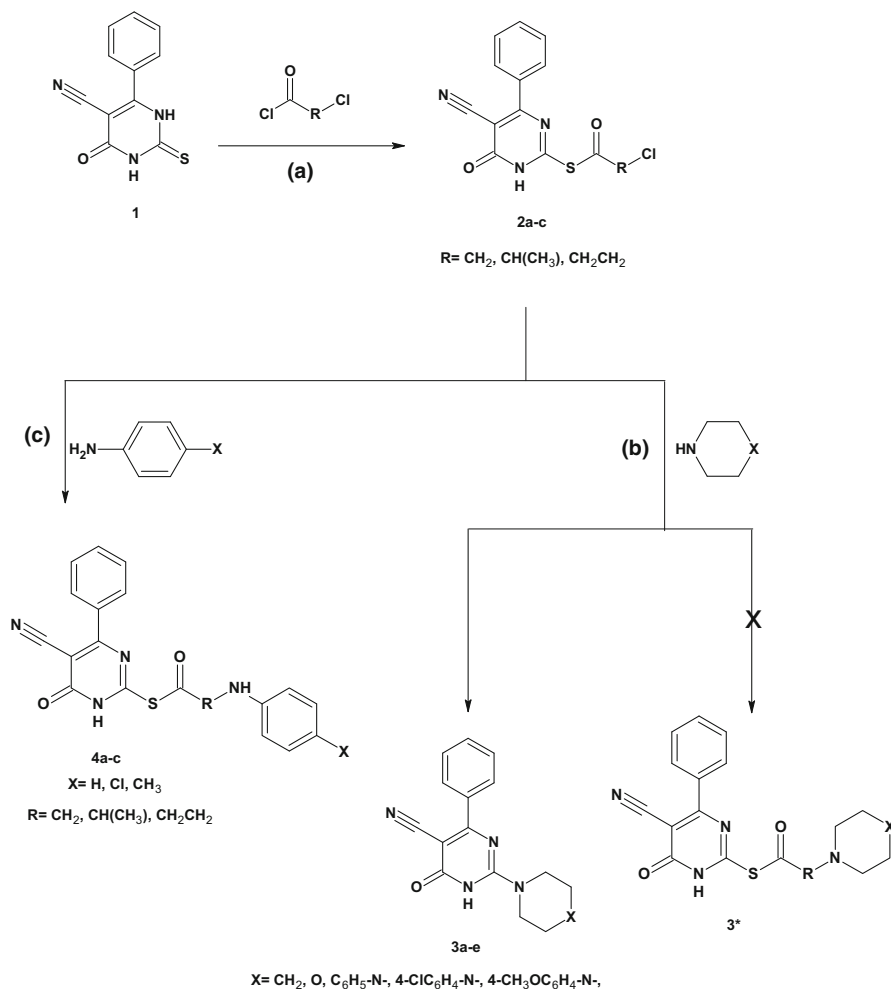| Compound no.	R	IC$_{50}$ in nM/mL[a]	
		MCF-7	HCT-116
6b		40.7	37.2
6c		42.0	53.9
8a		77.3	47.7
8b		41.0	46.8
8c		35.2	63.1
8d		25.9	23.8
8e		47.3	50.7
8f		21.4	51.6
8g		18.3	37.7

[a] The values given are means of three experiments

target was determined by removing the blank control value. The results were displayed in terms of percent inhibition.

Results and discussion

Chemistry

Schemes 1 and 2 outline the synthesis of the target compounds.

R= CH$_2$, CH(CH$_3$), CH$_2$CH$_2$

4a-c
X= H, Cl, CH$_3$
R= CH$_2$, CH(CH$_3$), CH$_2$CH$_2$

3a-e

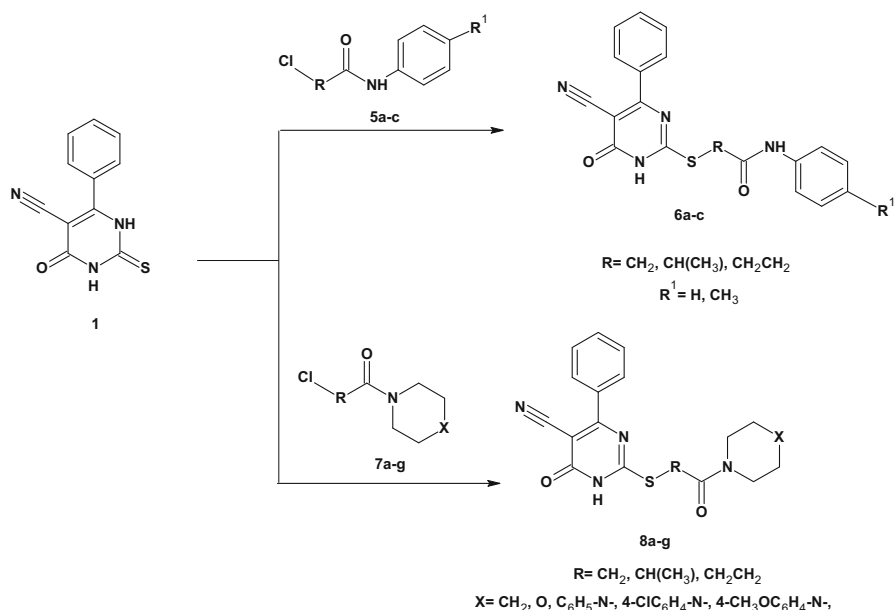**X= CH$_2$, O, C$_6$H$_5$-N-, 4-ClC$_6$H$_4$-N-, 4-CH$_3$OC$_6$H$_4$-N-,**

3*

Reaction conditions: (a) Dry benzene, dry DMF, K$_2$CO$_3$, reflux 10 h;
(b) Dry benzene, dry DMF, TEA, reflux 16 h;
(c) dry DMF, TEA, reflux 24-35 h.

Scheme 1 Preparation of the *S*-acylpyrimidine derivatives **2–4**

Scheme 2 Preparation of the *S*-alkylpyrimidine derivatives **6** and **8**

4-Oxo-6-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile **(1)** [10] was allowed to react with chloroacetyl chloride, 2-chloropropionyl chloride, and 3-chloropropionyl chloride in dry benzene using K_2CO_3 as a base. A small amount of DMF was added to enhance the solubility of the starting compound **1**. The products were identified as *S*-chloroalkanethioates **2a–c** based on their spectral data. Thus, the IR spectra of compounds **2a–c** indicated the appearance of two C=O bands at 1691–1732 cm^{-1} and 1643–1651 cm^{-1} corresponding to the introduced acyl group and the ring carbonyl group, respectively. Also, only one NH band appeared at 3186–3444 cm^{-1} together with CH aliphatic band at 2926–2997 cm^{-1}.

Moreover, ^{1}H NMR spectra of compounds **2a–c** showed an exchangeable singlet signal at δ 12.51–13.10 ppm corresponding to NH proton (which indicated selectivity for *S*-acylation over *N*-acylation). Meanwhile, the introduced aliphatic protons appeared as a singlet signal at δ 3.93 ppm (CH_2Cl protons) in compound **2a**, as doublet and quartet signals at δ 1.55–1.57 ppm and δ 4.47-4.55 ppm (CH_3CHCl protons) in compound **2b**, and as two triplet signals at δ 2.72–2.76 ppm and δ 3.31-3.41 ppm corresponding to CH_2CH_2Cl protons in compound **2c**. On the other hand, ^{13}C NMR spectrum of compound **2c** revealed the appearance of CH_2CH_2Cl carbons at δ 26.2 and δ 33.9 ppm, while two C=O signals appeared at δ 167.7 and δ 173.2 ppm corresponding to the ring C=O and S–C=O carbons, respectively.

The aim of the present work was to prepare compounds **3*** via nucleophilic substitution of the chloroalkylpyrimidines **2a–c** with secondary amines. The reaction was carried out by refluxing equimolar amounts of compounds **2a–c** and

the appropriate secondary aliphatic amine in dry benzene/DMF mixture, to facilitate the work up of the reaction. However, examining the spectral data of the products indicated that the secondary amine replaced the *S*-acyl group to give compounds **3a–e**. The IR spectra of compounds **3a–e** indicated the disappearance of the thioate C=O band and the appearance of only one C=O absorption band at 1626–1654 cm^{-1}. Further evidence was obtained from ^{1}H NMR spectra of compounds **3a–e** that indicated the disappearance of the characteristic aliphatic *S*-acyl protons and the appearance of the secondary amine aliphatic protons. Moreover, ^{13}C NMR spectrum of compound **3a** revealed the disappearance of *S*-acyl carbons [both aliphatic and C=O carbons] and the appearance of the signals of piperidine carbons at δ 24.1 ppm, δ 25.6 ppm, and δ 46.0 ppm and only one C=O signal appeared at δ 170.1 ppm [ring C=O]. Likewise, ^{13}C NMR spectrum of compound **3e** revealed the appearance of piperazinyl carbons at δ 44.8 ppm and δ 49.8 ppm. The signal of the methoxy carbon appeared at δ 55.6 ppm and the C=O signal appeared at δ 170.1 ppm. Moreover, the mass spectra of compounds **3a-c** and **3e** showed their corresponding molecular ion peaks at m/z 280, m/z 282, m/z 357, and m/z 387, respectively.

In order to prove this unexpected replacement, trials were done to prepare compound **3b*** via reacting compound **1** with morpholine under the same reaction conditions (dry benzene/DMF, TEA and reflux for 16 h.). However, the reaction did not give any product and the starting material was obtained unreacted (No replacement took place). Thus, it seemed that the introduced *S*-acyl group afforded good leaving group and facilitated the nucleophilic substitution by amines.

On the other hand, reacting compounds **2a–c** with appropriate primary aromatic amines in dry DMF resulted in the formation of the target compounds **4a–c**. The IR spectra of compounds **4a-c** indicated the appearance of two carbonyl absorption bands at 1651–1716 and 1624–1670 cm^{-1} and two NH absorption bands at 3246–3435 cm^{-1}. In addition, ^{1}H NMR spectra of compounds **4a–c** showed new exchangeable singlet signal at δ 9.40–10.40 ppm corresponding to the introduced phenylamino NH proton. In addition, the alkyl protons were shifted upfield due to replacement of the highly electronegative chlorine atom by the less electronegative phenylamino group. Thus, in compound **4a**, a singlet signal appeared at δ 3.15 ppm assigned to CH$_2$CO protons, while in compound **4b**, doublet and quartet signals appeared at δ 1.12–1.23 ppm and δ 3.30–3.35 ppm assigned to CH$_3$CH protons, respectively. Finally, in compound **4c**, two triplet signals appeared at δ 2.72–2.76 ppm and δ 3.07–3.11 ppm corresponding to COCH$_2$CH$_2$ protons. On the other hand, ^{13}C NMR spectrum of compound **4b** showed two signals at δ 37.6 ppm and δ 83.1 ppm respectively, due to CH$_3$CH carbons, while the two C=O carbons appeared at δ 160.2 ppm and δ 165.5 ppm. The results obtained from this reaction indicated that the less nucleophilic aromatic amines did not replace the alkyl thioate group, but instead they replaced the chlorine atom.

In this work, trials were done to *S*-alkylate pyrimidine derivative **1** with equimolar amounts of compounds **5a–c** [11–13] or **7a–g** [11, 14–17] in 2*N* potassium hydroxide/water system at room temperature. However, only compounds **6a**, **8c,** and **8f** were obtained after stirring for 48 h, while the rest of the compounds failed to be synthetized through the same procedure even after prolonging the

stirring time up to 72 h, and the starting compounds were separated unreacted. Therefore, another base/solvent system was tried, and the reaction was carried out in anhydrous potassium carbonate/dry DMF system under reflux for 16 h. The application of these conditions afforded the target compounds **6a–c** and **8a–g** in poor to moderate yields.

The IR spectra of compounds **6a–c** and **8a–g** indicated the appearance of new amidic C=O absorption band at 1647–1695 cm^{-1}.

Moreover, ^{1}H NMR spectra of compounds **6a–c** showed an exchangeable singlet signal at δ 9.41–10.31 ppm corresponding to phenylamino NH proton. Meanwhile, the introduced aliphatic protons appeared at δ 1–5 ppm. On the other hand, ^{13}C NMR spectra of compounds **8c–e** revealed the presence of two C=O signals at δ 163.6–170.7 ppm and δ 170.0–171.3 ppm corresponding to the ring C=O and SC=O carbons, respectively. In addition, the introduced aliphatic carbons appeared as one or two signals at δ 37.5–85.8 ppm. Also, the mass spectra of compounds **6a–c** showed their corresponding molecular ion peaks at m/z 376, while the mass spectra of compounds **8a, 8c, 8e**, and **8f** showed their corresponding molecular ion peaks at m/z 354, m/z 431, m/z 370, and m/z 445, respectively.

In vitro anticancer testing

The newly synthesized compounds were evaluated for their in vitro cytotoxic activity against human breast carcinoma cell line (MCF-7) and human colon carcinoma cell line (HCT-116) using Sulforhodamine-B stain (SRB) assay applying the method of Skehan et al. [18]

The relation between the surviving fractions and the drug concentrations was plotted to obtain the survival curve of the MCF-7 and HCT-116 tumor cell lines after addition of the specified compounds. The parameter used was IC$_{50}$, which corresponded to the concentration required for 50 % inhibition of the cell viability.

The IC$_{50}$ of the synthesized compounds are shown in Table 1, and the results are represented graphically in Fig. 2.

From the results in Table 1, it was found that all the compounds exhibited considerable response on both cell lines; however, the MCF-7 cell line was found to be more sensitive to the action of the compounds.

Within the 2-substituted-pyrimidines, compound **8g** was the most potent on the MCF-7 cell line with IC$_{50}$ 18.3 nM/mL, while compound **8d** was the most potent compound on the HCT-116 cell line with IC$_{50}$ 23.8 nM/mL.

SAR analysis of the effect of substitution at position 2 of the pyrimidine ring on both cell lines indicated the following:

- For the 2-alkylaminopyrimidines **3a–e**, it was found that increasing the bulkiness of the hydrophobic group led to parallel increase in the cytotoxic activity. Thus, substituted phenyl piperazine derivatives (**3c** and **3d**) afforded better cytotoxic activity than piperidine derivative **3a** or morpholine derivative **3b** on both cell lines.
- For the 2-S-acylpyrimidines **4a–c**, the increase in the length of the alkyl linker from CH$_2$ to CH$_2$CH$_2$ was not accompanied by parallel increase in the cytotoxic
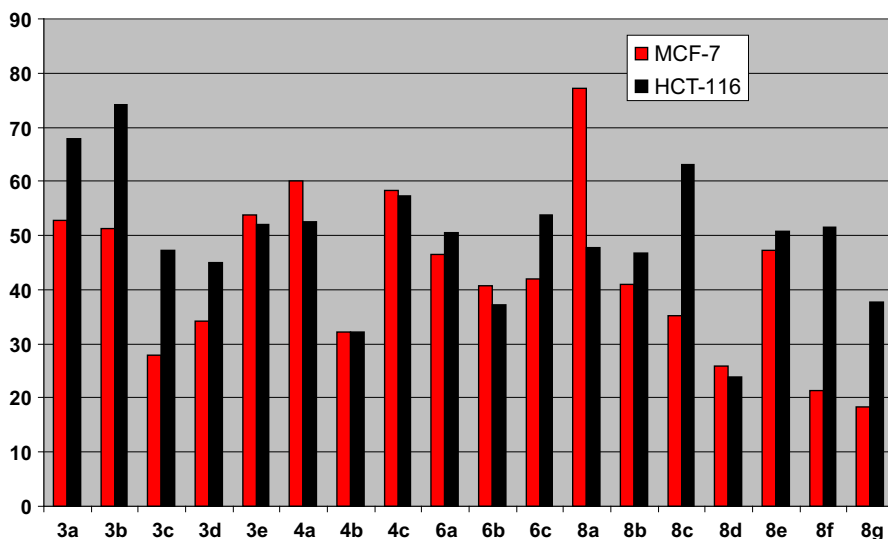
Fig. 2 IC_{50} in nM/mL of the synthesized compounds against breast tumor cell line (MCF-7) and colon tumor cell line (HCT-116)

activity. [Compare compound **4a** and **4c**]. However, the cytotoxic activity was significantly improved by the branching of the linker using $(CH_3)CH$ alkyl linker. [Compare compound **4a, 4b,** and **4c**].

- Regarding the *S*-alkyl derivatives **6a–c** and **8a–g**, it was found that compounds substituted with cyclic aliphatic amines **8a–g** (especially phenyl piperazine derivatives) gave better results on both cell lines (especially MCF-7 cell line) than those substituted with aromatic amines **6a–c**. Here also, increasing both the length and the branching of the alkyl chain of the linker enhanced the cytotoxic activity on both cell lines.

- No significant difference was noticed between the cytotoxic activity of the *S*-alkyl derivatives (**6a–c** and **8a–g)** and the *S*-acyl derivatives **4a–c**.

Finally, compound **8g** (the most potent compound in the present work) was tested for its possible cytotoxicity on the normal cell line MRC-5 (human lung fibroblast cell line). The result indicated that compound **8g** exhibited IC_{50} of 64.38 nM/mL, three to four times its potency on the tumor cell lines.

Kinase inhibitory activity of compound 8g

Searching the literature pointed out that analogous pyrimidine derivatives showed significant inhibitory activity against different kinases such as pim1, pim2 [19], GSK-3β [20], EGFR [21], and CDK5/p25 [22].

Compound **8g** that showed the highest cytotoxic activity among the pyrimidine derivatives was subjected to further testing to explore its mechanism of action. The testing was performed using the Kinexus compound profiling service. Compound **8g**

was screened against five kinases, namely pim1, pim2, GSK-3β, EGFR, and CDK5/p25 at a single dose (50 μM). The results indicated that compound **8g** inhibited all the kinases tested by varying extents ranging from 3 to 87 %. The highest inhibition observed was with pim1 kinase at 87 % followed by GSK-3β (24 %), pim2 (19 %), EGFR (18 %), and finally CDK5/p25 by 3 %.

Thus, the compound might act by selective inhibition of pim1 kinase. The IC_{50} of compound **8g** against pim1 was further estimated as 11.62 μM.

Pim1 kinase is a serine/threonine kinase that is involved in many biological processes such as cell survival, proliferation, differentiation, migration, metabolism, and apoptosis [23–27]. Overexpression of pim1 was reported in hematologic cancers and solid cancers [23–27]. Therefore, targeting pim1 kinase was expected to afford potent and selective anticancer agents. The ATP binding site of pim1 contains proline-123 amino acid in the hinge region. Thus, the enzyme lacks a key amide H-bond donor and thereby is incapable of making H-bonds with ATP. This fact enables the design and the synthesis of selective pim1 inhibitors. Although many pim1 inhibitors were reported in the literature, only a few of them contains a pyrimidine core [25–27]. Thus, the 4,6-diarylpyrimidin-2-one **V** (Fig. 3) was reported as patent CDC7 and pim1 inhibitor by Novartis [26]. Here, the carbonyl
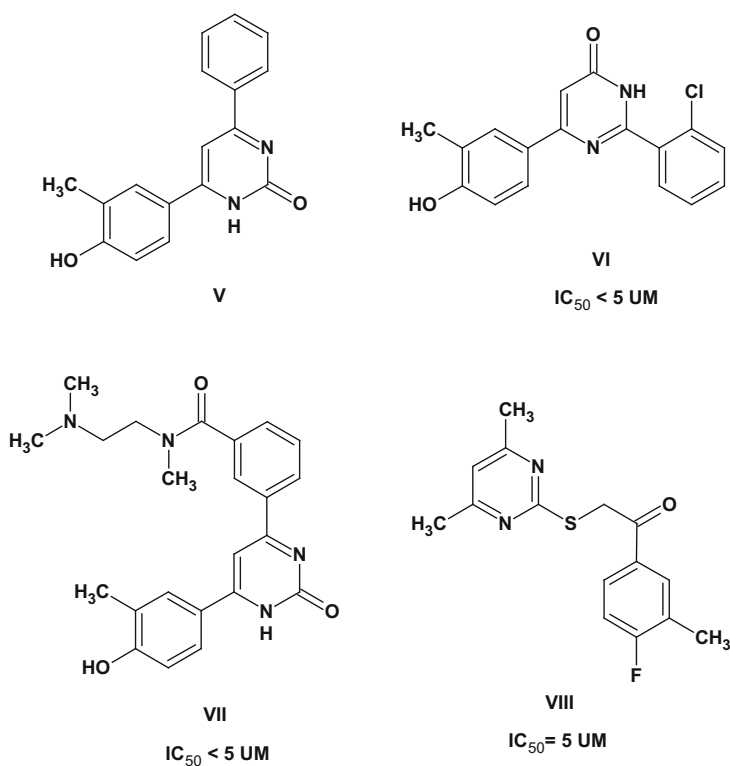

Fig. 3 Structures of pim1 inhibitors containing pyrimidine core

group bind to Lys67, and the hydroxyl group on the phenyl at position 4 made a H-bond with the hinge residue Glu121 [26].

Moreover, a series of 6-phenylpyrimidinones exemplified by compounds **VI** and **VII** were published in 2008 as patented by Exelixis Company. Better pim inhibitory activity was exhibited by 4,6-diarylpyrimidinones than by 2,6-diarylpyrimidinones [26, 28]. In 2009, the *S*-alkylpyrimidine derivative **VIII** was identified through high throughput screening as pim1 inhibitor with IC_{50} of 5 μM [19].

In this work, the introduction of compound **8g** with comparable structural features to compounds **V–VIII** and with potent and selective pim1 kinase inhibition makes this compound a promising lead pim1 inhibitor. Further optimization of its structure needs to be done to improve its pim1 inhibition and cytotoxic activity.

Conclusion

Two series of *S*-alkyl and *S*-acylpyrimidinones were prepared as cytotoxic agents. All the compounds showed significant cytotoxic activity on MCF-7 and HCT-116 cell lines with higher sensitivity towards the MCF-7 cell line. Compound **8g** was the most potent on the MCF-7 cell line with IC_{50} 18.3 nM/mL, whereas its IC_{50} in normal cell line (MRC-5) was 64.38 nM/mL, indicating its safety and selectivity towards MCF-7 cell line, while compound **8d** was the most potent compound on the HCT-116 cell line with IC_{50} 23.8 nM/mL. Compound **8g** was screened against five kinases. The compound showed selective inhibitory activity against pim1 kinase with IC_{50} 11.62 μM. Further studies are still needed to identify the SAR of theses derivatives as pim1 kinase inhibitors and to improve both the kinase inhibitory activity and the cytotoxic activity of these derivatives.

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