European Journal of Medicinal Chemistry 45 (2010) 5286–5291

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and antitumor activity of novel 6-aryl and 6-alkylpyrazolo[3,4-*d*]pyrimidin-4-one derivatives

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ARTICLE INFO

Article history:
Received 13 May 2010
Received in revised form
19 August 2010
Accepted 24 August 2010
Available online 16 September 2010

Keywords:
Pyrazolo[3,4-*d*]pyrimidin-4-one
Antitumor activity
HCT116

ABSTRACT

A series of new 6-arylpyrazolo[3,4-*d*]pyrimidin-4-ones and 6-alkylpyrazolo[3,4-*d*]pyrimidin-4-ones were synthesized. Some of the newly synthesized compounds were tested *in vitro* on human colon tumor cell line (HCT116). Most of the test compounds exploited potent antitumor activity, especially compound **10a** which displayed the highest activity among the test compounds with IC_{50} equal to 0.47 μg/mL

1. Introduction

Cancer describes the malignant growth of cells. Most tumors arise from a combination of genetic mutations in the cell. These genetic changes lead to activation of oncogenes and suppression or deletion of tumor suppressor genes. As a result, there is unregulated cell proliferation and also a delay in programmed cell death [apoptosis] [1]. Most of the clinically used antineoplastic drugs aim to exploit the proliferative process [e.g. DNA replication or chromosome segregation][1]. Pyrazolo[3,4-*d*]pyrimidines are of considerable chemical and pharmacological importance because of their structural similarities with purine and many derivatives of pyrazolo[3,4-*d*]pyrimidines were reported as antitumor agents [2–9]. Different mechanisms account for the cytotoxic effect of this class of compounds, where they have been reported to act as cyclin dependent kinase inhibitors [4,5], tyrosine kinase inhibitors [6] and potent xanthine oxidase inhibitors [7].

Several 1-aryl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ones were reported to possess antiproliferative activity against HCT116 and other cell lines (e.g. compounds **1**–**3**) [2,3,5].

In 2004, Markwalder et al. [5] pointed out that the best results in terms of antiproliferating activity of pyrazolo[3,4-*d*]pyrimidine were obtained with the following: 4-pyrimidinone ring [substitution at 5-NH or removal of the 4-carbonyl group destroyed the activity], substitution at position 1 with 2,4,6-trichlorophenyl group, substitution at position 3 with ethyl or methylsulphanyl group and substitution at position 6 with methyl or substituted benzyl group. The authors noted also that substitution at position 6 of pyrazolo[3,4-*d*]pyrimidin-4-one ring offered opportunities to modulate its physical properties such as solubility.

Encouraged by these findings, we thought of preparing several pyrazolo[3,4-*d*]pyrimidine derivatives taking into account the main requirements for antineoplastic activity [4-pyrimidinone, 3-methylsulphanyl group and N1 phenyl ring] and to examine the effect of different substitutions at position 6 [aryl groups **5a**–**d** and **6a**–**e**, substituted aminomethyl **9a**,**b** or substituted aminoethyl group **10a**–**d**].

2. Results and discussion

2.1. Chemistry

The synthesis of the target compounds is outlined in Schemes 1 and 2. The starting compound, 5-amino-3-methylsulphanyl-1-phenyl-1*H*-pyrazole-4-carboxamide (**4**) [10] was reacted with aromatic aldehydes in acetic acid to give rise to 6-aryl-3-methylsulphanyl-1-phenyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ones **5a**–**d**. Meanwhile, carrying out the same reaction in ethanol in the presence of piperidine afforded 6-aryl-3-methylsulphanyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ones **6a**–**e**. On the other hand, reacting compound **4** with chloroacetyl chloride and 3-chloropropionyl chloride afforded 6-chloromethyl and 6-(2-chloroethyl)pyrazolo[3,4-*d*]pyrimidin-4-one derivatives **7** and **8**, respectively. Reacting the latter compounds with primary amines furnished 6-(substituted amino)methyl and 6-(substituted amino)ethylpyrazolo[3,4-*d*]pyrimidin-4-ones **9a**,**b** and **10a**–**d**, respectively. Finally, fusion of the pyrazole derivative **4** with

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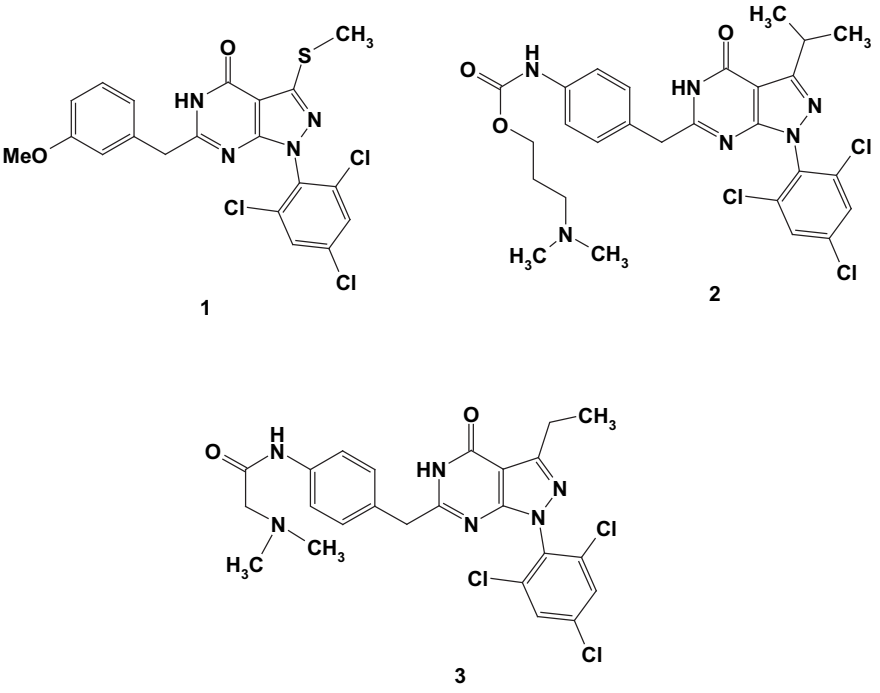
doi:10.1016/j.ejmech.2010.08.048



propionic anhydride furnished 6-ethyl-3-methylsulphanyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (**11**).

3. Conclusion

In summary, a series of new 6-arylpyrazolo[3,4-*d*]pyrimidin-4-ones (**5**–**6e**) and 6-alkylpyrazolo[3,4-*d*]pyrimidin-4-ones (**9**–**11**) were synthesized. Some of the newly synthesized compounds were tested *in vitro* on human colon tumor cell line (HCT116). Most of the test compounds exploited potent antitumor activity, especially compound **10a** which displayed the highest activity among the test compounds with IC_{50} equal to 0.47 μg/mL. These results suggested that substitution at position 6 of pyrazolo[3,4-*d*]pyrimidin-4-one with alkyl groups was preferred to aryl groups. Besides, introduction of substituted amino group on the alkyl group resulted in higher cytotoxic activity.

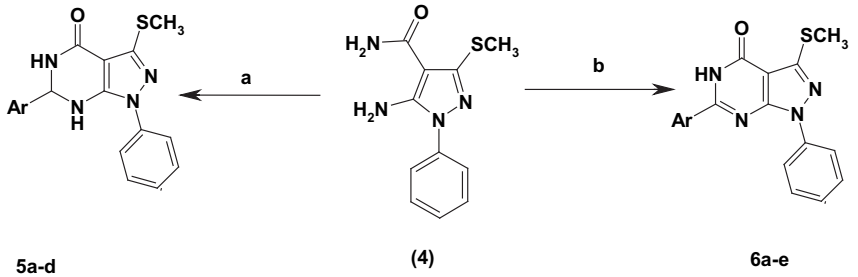
4. Experimental part

4.1. General

Melting points were determined using a Griffin apparatus and were uncorrected. IR spectra were recorded on Shimadzu IR 435 spectrophotometer and Mattson Genesis II FTIR and values were represented in cm^{-1}. ^1H NMR and ^{13}C NMR were carried out on Varian Gemini 200 MHz spectrophotometer, Microanalytical center, Cairo University, Cairo, Egypt, using TMS as an internal standard and chemical shifts were recorded in ppm on δ scale and coupling constants (J) are given in Hz. The electron impact (EI) mass spectra were recorded on Hewlett Packard 5988 spectrometer or Shimadzu QP-2010 plus, Microanalytical center, Cairo University, Cairo, Egypt. Analytical thin layer chromatography (TLC) on silica gel plates containing UV indicator was employed routinely to follow the course of reactions and to check the purity of products. All reagents and solvents were purified and dried by standard techniques. Elemental microanalyses were performed at Microanalytical Center, Cairo University, Cairo, Egypt, and were within ±0.4%.

4.1.1. General procedure for the synthesis of 6-aryl-3-methylsulphanyl-1-phenyl-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-d] pyrimidin-4-ones ***5a–d***

A mixture of 5-amino-3-methylsulphanyl-1-phenyl-1*H*-pyrazole-4-carboxamide (**4**) [10] (0.50 g, 0.002 mol) and the appropriate aromatic aldehyde (0.002 mol) in glacial acetic acid (20 mL)

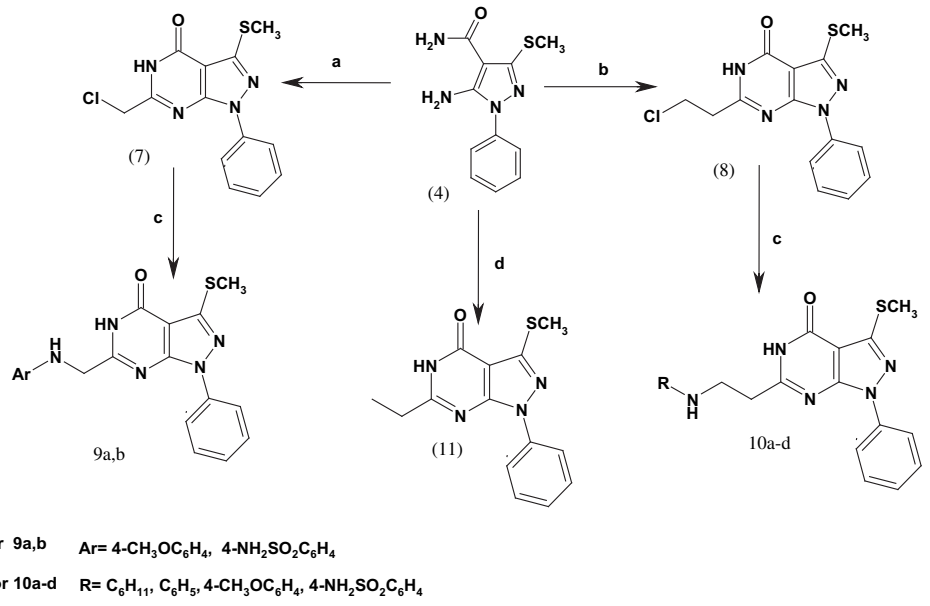


5a-d **(4)** **6a-e**

for 5a-d Ar= C_6H_5, 2-ClC_6H_4, 4-ClC_6H_4, 3-$NO_2C_6H_4$

for 6a-e Ar= 4-ClC_6H_4, 2-OHC_6H_4, 3-$NO_2C_6H_4$, 2-$CH_3OC_6H_4$, 2-thienyl

Scheme 1. Reagents: a) ArCHO, acetic acid; b) ArCHO, piperidine, ethanol.



Scheme 2. Reagents: a) $ClCH_2COCl$, Fusion at 200 °C; b) $ClCH_2CH_2COCl$, Fusion at 200 °C; c) RNH_2 or $ArNH_2$, Triethylamine, ethanol; d) $(CH_3CH_2CO)_2O$, Fusion at 200 °C.

was heated under reflux for 14 h. The solvent was concentrated under reduced pressure, and the solid formed was filtered, dried and crystallized from the suitable solvent (given below).

*4.1.1.1. 3-Methylsulphanyl-1,6-diphenyl-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**5a**).* (Crystallized from ethanol); yield: 16%; mp: 282–283 °C; IR (cm^{-1}): 3450, 3200 (NH), 2920, 2840 (CH-aliphatic), 1680 (CO); ^1H NMR (DMSO-d_6) δ ppm 2.5 (s, 3H, SC***H***$_3$), 6.0 (s, 1H, ***H6***), 7.3 (s, 1H, N***H***, D_2O exchangeable), 7.7 (s, 1H, N***H***, D_2O exchangeable), 7.3–7.6 (m, 10H, Ar–***H***); Anal. Calcd for $C_{18}H_{16}N_4OS$: C, 64.27; H, 4.79; N, 16.65. Found: C, 64.39; H, 4.33; N, 16.54.

*4.1.1.2. 6-(2-Chlorophenyl)-3-methylsulphanyl-1-phenyl-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**5b**).* (Crystallized from methanol); yield: 61%; mp: 184–185 °C; IR (cm^{-1}): 3400, 3200 (NH), 2920, 2840 (CH-aliphatic), 1660 (CO); ^1H NMR ($CDCl_3$) δ ppm 2.5 (s, 3H, SC***H***$_3$), 5.0 (s, 1H, N***H***, D_2O exchangeable), 5.6 (s, 1H, N***H***, D_2O exchangeable), 6.3 (s, 1H, ***H6***), 7.2–8.1 (m, 9H, Ar–***H***); Anal. Calcd for $C_{18}H_{15}ClN_4OS$: C, 58.30; H, 4.08; N, 15.11. Found: C, 57.70; H, 3.80; N, 14.75.

*4.1.1.3. 6-(4-Chlorophenyl)-3-methylsulphanyl-1-phenyl-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**5c**).* (Crystallized from acetic acid); yield: 24%; mp: 272–273 °C; IR (cm^{-1}): 3400, 3200 (NH), 2920, 2850 (CH-aliphatic), 1680 (CO); ^1H NMR (DMSO-d_6) δ ppm 2.4 (s, 3H, SC***H***$_3$), 5.7 (s, 1H, ***H6***), 6.5 (s, 1H, N***H***, D_2O exchangeable), 6.8 (s, 1H, N***H***, D_2O exchangeable), 7.2–8.3 (m, 9H, Ar–***H***). MS *m*/*z*: 368 [$(M-2)^+$, 40.40%], 51 [100%]; Anal. Calcd for $C_{18}H_{15}ClN_4OS$: C, 58.30; H, 4.08; N, 15.11. Found: C, 58.67; H, 4.54; N, 14.96.

*4.1.1.4. 3-Methylsulphanyl-6-(3-nitrophenyl)-1-phenyl-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**5d**).* (Crystallized from ethanol); yield: 58%; mp: 189–190 °C; IR (cm^{-1}): 3350, 3200 (NH), 2920, 2840 (CH-aliphatic), 1660 (CO), 1520, 1350 (NO_2); ^1H NMR (DMSO-d_6) δ ppm 2.4 (s, 3H, SC***H***$_3$), 5.9 (s, 1H, ***H6***), 7.3–8.3 (m, 11H, Ar–***H*** + two N***H***); ^{13}C NMR (DMSO-d_6) δ ppm 12.3 (SCH_3), 66.0 (C-6), 98.4 (C-3_a), 121.3, 121.7, 123.1, 126.9, 129.6, 129.9, 133.0, 137.7, 143.6, 147.2, 147.6, 148.7 (aromatic carbons), 161.2 (C=O); MS *m*/*z*: 381 [M^+, 2.89%], 77 [$C_6H_5^+$, 100%]; Anal. Calcd for $C_{18}H_{15}N_5O_3S$: C, 56.68; H, 3.96; N, 18.36. Found: C, 56.80; H, 3.50; N, 18.06.

*4.1.2. General procedure for the synthesis of 6-aryl-3-methylsulphanyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-ones **6a–e***

A mixture of 5-amino-3-methylsulphanyl-1-phenyl-1*H*-pyrazole-4-carboxamide (**4**) (0.50 g, 0.002 mol), the appropriate aromatic aldehyde (0.002 mol) and piperidine (1 mL) in absolute ethanol (30 mL) was heated under reflux for 8 h. The solvent was concentrated under reduced pressure, and the solid formed was filtered, dried and crystallized from the suitable solvent (given below)

*4.1.2.1. 6-(4-Chlorophenyl)-3-methylsulphanyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**6a**).* (Crystallized from acetic acid); yield: 37%; mp: 292–293 °C; IR (cm^{-1}): 3400 (NH), 2920, 2840 (CH-aliphatic), 1680 (CO); ^1H NMR (DMSO-d_6) δ ppm 2.4 (s, 3H, SC***H***$_3$), 7.3–8.2 (m, 9H, Ar–***H***), 12.3 (br s, 1H, N***H***, D_2O exchangeable); Anal. Calcd for $C_{18}H_{13}ClN_4OS$: C, 58.62; H, 3.55; N, 15.19. Found: C, 58.50; H, 3.40; N, 15.21.

*4.1.2.2. 6-(2-Hydroxyphenyl)-3-methylsulphanyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**6b**).* (Crystallized from DMF); yield: 58%; mp: 304–305 °C; IR (cm^{-1}): 3400(NH/OH), 2920, 2850 (CH-aliphatic), 1690 (CO); ^1H NMR (DMSO-d_6) δ ppm 2.6 (s, 3H, SC***H***$_3$), 6.9–8.1 (m, 9H, Ar–***H***), 11.8 (br s, 1H, O***H***, D_2O exchangeable), 12.1 (br s, 1H, N***H***, D_2O exchangeable); Anal. Calcd for $C_{18}H_{14}N_4O_2S$: C, 61.70; H, 4.03; N, 15.99. Found: C, 61.82; H, 4.42; N, 15.82.

*4.1.2.3. 3-Methylsulphanyl-6-(3-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**6c**).* (Crystallized from acetic acid); yield: 29%; mp: 322–323 °C; IR (cm^{-1}): 3450 (NH), 2900, 2840 (CH-aliphatic), 1680 (CO), 1520, 1340 (NO_2); ^1H NMR (DMSO-d_6) δ ppm 2.6 (s, 3H, SC***H***$_3$), 7.4 (t, 1H, J = 7.4 Hz, Ar–***H***), 7.5 (t, 2H, J = 8 Hz, Ar–***H***), 7.8 (t, 1H, J = 8 Hz, Ar–***H***), 8.0 (d, 2H, J = 8.4 Hz, Ar–***H***), 8.4 (d, 1H, J = 6.6 Hz, Ar–***H***), 8.5 (d, 1H, J = 6.8 Hz, Ar–***H***), 8.9 (s, 1H, Ar–***H***), 13.0 (br s, 1H, N***H***, D_2O exchangeable); MS *m*/*z*: 379 [M^+, 38.98%], 77 [$C_6H_5^+$, 100%]; Anal. Calcd for $C_{18}H_{13}N_5O_3S$: C, 56.98; H, 3.45; N, 18.45. Found: C, 56.74; H, 3.65; N, 18.36.

*4.1.2.4. 6-(2-Methoxyphenyl)-3-methylsulphanyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**6d**).* (Crystallized from toluene); yield: 29%; mp: 235–236 °C; IR (cm^{-1}): 3300 (NH), 2900, 2840 (CH-aliphatic), 1690 (CO); ^{1}H NMR (DMSO-d_6) δ ppm 2.7 (s, 3H, SC***H***$_3$), 4.0 (s, 3H, OC***H***$_3$), 7.0–8.4 (m, 9H, Ar–***H***), 11.0 (br s, 1H, N***H***, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ ppm 12.7 (SCH_3), 55.8 (OCH_3), 109.0 (C-3$_a$), 112.0, 120.4, 121.0, 126.3, 128.8, 130.3, 132.7, 138.1, 145.2, 153.2, 155.8, 156.7, 157.2 (aromatic carbons), 167.9 (C=O); Anal. Calcd for $C_{19}H_{16}N_4O_2S$: C, 62.62; H, 4.43; N, 15.37. Found: C, 63.03; H, 4.61; N, 15.53.

*4.1.2.5. 3-Methylsulphanyl-1-phenyl-6-(2-thienyl)-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**6e**).* (Crystallized from acetic acid); yield: 25%; mp: 314–315 °C; IR (cm^{-1}): 3450 (NH), 2919, 2849 (CH-aliphatic), 1672 (CO); ^{1}H NMR (DMSO-d_6) δ ppm 2.6 (s, 3H, SC***H***$_3$), 7.2 (t, 1H, J = 4 Hz, 4′-***H*** of thienyl), 7.4 (t, 1H, J = 7.8 Hz, Ar–***H***), 7.5 (t, 2H, J = 7.8 Hz, Ar–***H***), 7.9 (d, 1H, J = 5 Hz, 3′-***H*** of thienyl), 8.0 (d, 2H, J = 8.2 Hz, Ar–***H***), 8.2 (d, 1H, J = 3.6 Hz, 5′-***H*** of thienyl), 12.7 (br s, 1H, N***H***, D_2O exchangeable); Anal. Calcd for $C_{16}H_{12}N_4OS_2$: C, 56.45; H, 3.55; N, 16.46. Found: C, 56.01; H, 4.00; N, 16.02.

*4.1.3. General procedure for the synthesis of 6-(substituted)-3-methylsulphanyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d] pyrimidin-4-ones **7** and **8***

A mixture of 5-amino-3-methylsulphanyl-1-phenyl-1*H*-pyrazole-4-carboxamide (**4**) (0.50 g, 0.002 mol) and the appropriate acid chloride (0.002 mol) was heated at 200 °C in an oil bath for 5 h. The solid formed was triturated with ethanol (10 mL), filtered, dried and crystallized from acetic acid.

*4.1.3.1. 6-Chloromethyl-3-methylsulphanyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**7**).* Yield: 96%; mp: 260–261 °C; IR (cm^{-1}): 3450 (NH), 2900, 2840 (CH-aliphatic), 1670 (CO), 760 (C–Cl); ^{1}H NMR (DMSO-d_6) δ ppm 2.7 (s, 3H, SC***H***$_3$), 4.6 (s, 2H, C***H***$_2$Cl), 7.3 (t, 1H, J = 7 Hz, Ar–***H***), 7.5 (t, 2H, J = 7.4 Hz, Ar–***H***), 8.0 (d, 2H, J = 8 Hz, Ar–***H***), 12.7 (s, 1H, N***H***, D_2O exchangeable); Anal. Calcd for $C_{13}H_{11}ClN_4OS$: C, 50.90; H, 3.61; N, 18.26. Found: C, 51.20; H, 3.43; N, 17.78.

*4.1.3.2. 6-(2-Chloroethyl)-3-methylsulphanyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**8**).* Yield: 83%; mp: 270–271 °C; IR (cm^{-1}): 3300 (NH), 2919, 2849 (CH-aliphatic), 1680 (CO), 755 (C–Cl); ^{1}H NMR (200 MHz, DMSO-d_6) δ ppm 2.6 (s, 3H, SC***H***$_3$), 3.1 (t, 2H, $ClCH_2C\boldsymbol{H}_2$, J = 6.6 Hz), 4.0 (t, 2H, $ClC\boldsymbol{H}_2CH_2$, J = 6.6 Hz), 7.3 (t, 1H, J = 7.2 Hz, Ar–***H***), 7.5 (t, 2H, J = 7.4 Hz, Ar–***H***), 8.0 (d, 2H, J = 7.4 Hz, Ar–***H***), 12.4 (s, 1H, N***H***, D_2O exchangeable); Anal. Calcd for $C_{14}H_{13}ClN_4OS$: C, 52.42; H, 4.08; N, 17.46. Found: C, 52.03; H, 4.19; N, 17.50.

*4.1.4. General procedure for the synthesis of 6-(substituted)-3methylsulphanyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d] pyrimidin-4-ones **9** and **10***

A mixture of 6-chloromethylpyrazolo[3,4-*d*]pyrimidine **7** or 6-(2-chloroethyl)pyrazolo[3,4-*d*]pyrimidine **8** (0.001 mol), the appropriate primary amine (0.001 mol) and triethylamine (0.30 mL) in absolute ethanol (10 mL) was heated under reflux for 15 h. The reaction mixture was concentrated under reduced pressure, and the solid formed upon cooling was filtered, dried and crystallized from the suitable solvent.

*4.1.4.1. 6-((4-Methoxyanilino)methyl)-3-methylsulphanyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**9a**).* (Crystallized from ethyl acetate); yield: 43%; mp: 205–206 °C; IR (cm^{-1}): 3419, 3200 (NH), 2985, 2920 (CH-aliphatic), 1664 (CO); ^{1}H NMR (DMSO-d_6) δ ppm 2.6 (s, 3H, SC***H***$_3$), 3.6 (s, 3H, OC***H***$_3$), 4.2 (d, 2H, C***H***$_2$NH), 5.7 (br s, 1H, N***H***, D_2O exchangeable), 6.6 (d, 2H, J = 9 Hz, Ar–***H***), 6.7 (d, 2H, J = 9 Hz, Ar–***H***), 7.3 (t, 1H, J = 7.6 Hz, Ar–***H***), 7.4 (t, 2H, J = 7.7 Hz, Ar–***H***), 7.9 (d, 2H, J = 8.2 Hz, Ar–***H***), 12.2 (s, 1H, N***H***, D_2O exchangeable); Anal. Calcd for $C_{20}H_{19}N_5O_2S$: C, 61.05; H, 4.87; N, 17.80. Found: C, 61.00; H, 4.66; N, 17.89.

*4.1.4.2. 6-((4-Sulphonamidoanilino)methyl)-3-methylsulphanyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**9b**).* (Crystallized from ethanol); yield: 35%; mp: 254–255 °C; IR (cm^{-1}): 3414, 3374 (NH/NH_2), 2920, 2850 (CH-aliphatic), 1680 (CO); ^{1}H NMR (DMSO-d_6) δ ppm 2.6 (s, 3H, SC***H***$_3$), 4.4 (d, 2H, C***H***$_2$NH), 5.8 (br s, 1H, N***H***, D_2O exchangeable), 6.8–6.9 (2 s, 2H, $SO_2N\boldsymbol{H}_2$, D_2O exchangeable), 6.5 (d, 2H, J = 7.8 Hz, Ar–***H***), 6.7 (d, 2H, J = 8.2 Hz, Ar–***H***), 7.3–7.5 (m, 3H, Ar–***H***), 7.8 (d, 2H, J = 7.2 Hz, Ar–***H***), 12.3 (br s, 1H, N***H***, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ ppm 12.7 (SCH_3), 44.6 (CH_2NH), 111.7 (C-3$_a$), 120.6, 121.0, 126.3, 127.0, 128.9, 131.2, 138.0, 151.0, 153.0, 157.4, 159.8 (aromatic carbons), 168.0 (C=O); MS *m*/*z*: 442 [M^+, 9.17%], 77 [$C_6H_5^+$, 100%]; Anal. Calcd for $C_{19}H_{18}N_6O_3S_2$: C, 51.57; H, 4.10; N, 18.99. Found: C, 51.71; H, 4.28; N, 18.55.

*4.1.4.3. 6-(2-(Cyclohexylamino)ethyl)-3-methylsulphanyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**10a**).* (Crystallized from ethyl acetate); yield: 65%; mp: 230–231 °C; IR (cm^{-1}): 3318, 3277 (NH), 2925, 2850 (CH-aliphatic), 1678 (CO); ^{1}H NMR (DMSO-d_6) δ ppm 0.9–1.8 (m, 11H, aliphatic-***H***), 2.5 (s, 3H, SC***H***$_3$), 2.7 (t, 2H, $NHCH_2C\boldsymbol{H}_2$, J = 6.3 Hz), 2.9 (t, 2H, $NHC\boldsymbol{H}_2CH_2$, J = 6.3 Hz), 6.5 (t, 1H, $N\boldsymbol{H}CH_2CH_2$, D_2O exchangeable), 7.3 (t, 1H, J = 7.2 Hz, Ar–***H***), 7.5 (t, 2H, J = 7.8 Hz, Ar–***H***), 8.0 (d, 2H, J = 7.2 Hz, Ar–***H***), 12.3 (s, 1H, N***H***, D_2O exchangeable); Anal. Calcd for $C_{20}H_{25}N_5OS$: C, 62.64; H, 6.57; N, 18.26. Found: C, 62.95; H, 6.66; N, 18.45.

*4.1.4.4. 6-(2-(Anilino)ethyl)-3-methylsulphanyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**10b**).* (Crystallized from ethyl acetate); yield: 77%; mp: 240–241 °C; IR (cm^{-1}): 3401, 3275 (NH), 2924, 2849 (CH-aliphatic), 1682 (CO); ^{1}H NMR (DMSO-d_6) δ ppm 2.6 (s, 3H, SC***H***$_3$), 2.8 (t, 2H, $NHCH_2C\boldsymbol{H}_2$, J = 6.6 Hz), 3.4 (t, 2H, $NHC\boldsymbol{H}_2CH_2$, J = 6.6 Hz), 5.6 (t, 1H, $N\boldsymbol{H}CH_2CH_2$, D_2O exchangeable), 6.5–8.0 (m, 10H, Ar–***H***), 12.3 (s, 1H, N***H***, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ ppm 12.7 (SCH_3), 33.9 ($\boldsymbol{CH}_2CH_2NH$), 38.6 ($CH_2\boldsymbol{CH}_2NH$), 103.7 (C-3$_a$), 112.1, 115.8, 120.6, 126.3, 128.8, 138.2, 145.1, 148.3, 153.4, 157.5, 160.6 (aromatic carbons), 168.5 (C=O); MS *m*/*z*: 377 [M^+, 23.55%], 272 [[(M – $C_6H_5N{=}CH_2)^+$,100%], 106 [$(C_6H_5NHCH_2)^+$, 76.38%]; Anal. Calcd for $C_{20}H_{19}N_5OS$: C, 63.64; H, 5.07; N, 18.55. Found: C, 64.07; H, 4.91; N, 18.33.

*4.1.4.5. 6-(2-(4-Methoxyanilino)ethyl)-3-methylsulphanyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**10c**).* (Crystallized from ethanol); yield: 59%; mp: 144–145 °C; IR (cm^{-1}): 3420, 3374 (NH), 2923, 2849 (CH-aliphatic), 1683 (CO); ^{1}H NMR (DMSO-d_6) δ ppm 2.6 (s, 3H, SC***H***$_3$), 2.9 (t, 2H, $NHCH_2C\boldsymbol{H}_2$, J = 6.4 Hz), 3.4 (t, 2H,

Table 1
Results of *in vitro* cytotoxic activity of some of the synthesized compounds on human colon tumor cell line (HCT116).

Compound no.	IC_{50} in μg/mL[a]
5a	2.4
6c	0.67
9a	2.7
9b	3.09
10a	0.47
10b	1.34
10d	2.82

[a] The values given are means of three experiments.

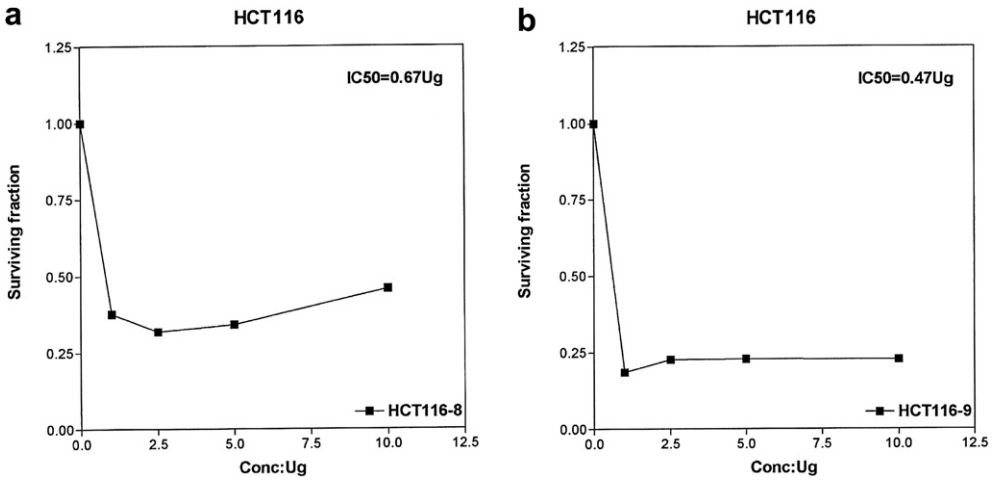


Fig. 1. Effect of different concentrations of (a) compound **6c** and (b) compound **10a** on the viability of HCT116 cell line.

NHC***H***$_2$CH$_2$, J = 6.8 Hz), 3.6 (s, 3H, OC***H***$_3$), 6.6 (d, 2H, J = 8.6 Hz, Ar–***H***), 6.7 (d, 2H, J = 8.6 Hz, Ar–***H***), 7.3 (t, 1H, J = 7.2 Hz, Ar–***H***), 7.5 (t, 2H, J = 7.8 Hz, Ar–***H***), 8.0 (d, 2H, J = 7.2 Hz, Ar–***H***), 9.9 (br s, 1H, N***H***CH$_2$CH$_2$, D$_2$O exchangeable), 12.3 (s, 1H, N***H***, D$_2$O exchangeable); MS m/z: 407 [M$^+$, 24.47%], 272 [(M − $CH_3OC_6H_4N{=}CH_2)^+$, 45.87] 136 [$(CH_3OC_6H_4NHCH_2)^+$, 100%]; Anal. Calcd for $C_{21}H_{21}N_5O_2S$: C, 61.90; H, 5.19; N, 17.19. Found: C, 62.27; H, 5.62; N, 17.08.

*4.1.4.6. 6-(2-(4-Sulphonamidoanilino)ethyl)-3-methylsulphanyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**10d**).* (Crystallized from acetic acid); yield: 70%; mp: 232–233 °C; IR (cm^{-1}): 3370, 3246 (NH/NH$_2$), 2922, 2849 (CH-aliphatic), 1682 (CO); ^{1}H NMR (DMSO-d_6) δ ppm 2.6 (s, 3H, SC***H***$_3$), 2.9 (t, 2H, NHCH$_2$C***H***$_2$), 3.4 (t, 2H, NHC***H***$_2$CH$_2$), 6.5 (br s, 1H, N***H***CH$_2$CH$_2$, D$_2$O exchangeable), 6.9 (s, 2H, SO$_2$N***H***$_2$, D$_2$O exchangeable), 6.6 (d, 2H, J = 8.6 Hz, Ar–***H***), 7.3 (d, 2H, J = 7.8 Hz, Ar–***H***), 7.4–7.5 (m, 3H, Ar–***H***), 8.0 (d, 2H, J = 7.8 Hz, Ar–***H***), 12.4 (s, 1H, N***H***, D$_2$O exchangeable); Anal. Calcd for $C_{20}H_{20}N_6O_3S_2$: C, 52.62; H, 4.42; N, 18.41. Found: C, 52.30; H, 4.10; N, 18.39.

*4.1.5. 6-Ethyl-3-methylsulphanyl-1-phenyl-4,5-dihydro-1H-pyrazolo[3,4-d]pyrimidin-4-one (**11**)*

A mixture of 5-amino-3-methylsulphanyl-1-phenyl-1*H*-pyrazole-4-carboxamide (**4**) (0.50 g, 0.002 mol) and propionic anhydride (0.26 g, 0.26 mL, 0.002 mol) was heated at 200 °C in an oil bath for 5 h. The solid formed was triturated with ethanol (10 mL), filtered, dried and crystallized from ethanol. Yield: 33%; mp: 262–263 °C; IR (cm^{-1}): 3319 (NH), 2938, 2863 (CH-aliphatic), 1686 (CO); ^{1}H NMR (DMSO-d_6) δ ppm 1.2 (t, 3H, C***H***$_3$CH$_2$, J = 7.6 Hz), 2.6 (s, 3H, SC***H***$_3$), 2.6–2.7 (q, 2H, CH$_3$C***H***$_2$, J = 7.6 Hz), 7.3 (t, 1H, J = 7 Hz, Ar–***H***), 7.5 (t, 2H, J = 7 Hz, Ar–***H***), 8.0 (d, 2H, J = 8.2 Hz, Ar–***H***), 12.3 (s, 1H, N***H***, D$_2$O exchangeable); Anal. Calcd for $C_{14}H_{14}N_4OS$: C, 58.72; H, 4.93; N, 19.57. Found: C, 58.67; H, 4.98; N, 19.94.

4.2. Biological testing

4.2.1. Materials and methods

The human colon tumor cell lines (HCT116) were obtained as a gift from NCI, MD, USA.

All chemicals and solvents were purchased from Sigma–Aldrich.

4.2.1.1. Measurement of potential cytotoxicity. The cytotoxic activity of the newly synthesized compounds was measured *in vitro* on human colon tumor cell line (HCT116) using sulforhodamine-B stain (SRB) assay applying the method of Skehan et al. [11].

Cells were plated in 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. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the test compound (0, 1, 2.5, 5 and 10 μg/mL) were added to the cell monolayer. Triplicate wells prepared for each individual dose. Monolayer cells were incubated with the test compound for 48 h at 37 °C in atmosphere of 5% CO_2. After 48 h, 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 attached stain was recovered with Tris EDTA buffer. Colour intensity was measured in ELISA reader. The relation between surviving fraction and compound concentration was plotted and IC_{50} [the concentration required for 50% inhibition of cell viability] was calculated for each compound and results are given in Table 1. Fig 1. showed the effect of different concentrations of (a) compound **6c** and (b) compound **10a** on the viability of HCT116 cell line.

4.2.2. Results and discussion

All of the test compounds exhibited significant antitumor activity against HCT116 with IC_{50} between 0.47 and 3.09 μM. Higher activity was obtained with 4,5-dihydropyrazolopyrimidine derivative **6c** than with 4,5,6,7-tetrahydro derivative **5a**. On the other hand, 6-alkyl derivatives gave better results than 6-aryl derivatives. Furthermore, 6-(substituted amino)ethyl-pyrazolopyrimidin-4-one derivatives **10a**,**b** give better results than 6-(substituted amino)methyl derivatives **9a**,**b**. The best result was obtained with 6-(2-(cyclohexylamino) ethyl)-3-methylsulphanyl-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*] pyrimidin-4-one (**10a**).

Acknowledgments

We are grateful to Prof. Dr. Samia Shouman, Professor of Pharmacology, and all members of the department of Cancer Biology, National Cancer Institute, Cairo, Egypt, for carrying out the cytotoxicity testing.

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