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SYNTHESIS OF NOVEL ARYL SUBSTITUTED PYRAZOLO [3, 4-D] PYRIMIDINES AND THEIR EVALUATION AS CYTOTOXIC AGENTS

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ABSTRACT

A new series of aryl substituted pyrazolo [3, 4-d] pyrimidine with anticipated antitumor activity has been synthesized. A full chemical characterization of the new compounds is provided. The new compounds were tested for their cytotoxic activity on breast cancer and colon cancer cell lines. Some of the compounds were found to have potent antitumor activity with IC50 values ranging from 13.0 to 20.1 µM on HCT-116 cell line (colon cancer) and 9.2 to 15.9 µM on MCF-7 cell line (breast cancer) compared to Cisplatin as a reference compound.

KEYWORDS: Pyrazolopyrimidine, Antitumor, Cytotoxic, MCF-7, HCT-115, breast cancer and colon cancer.

1. Introduction

Pyrazolopyrimidine derivatives constitute an important family of compounds due to their applications as pharmaceuticals and in the field of chemotherapy. pyrazolo[3,4-d]pyrimidine exhibited potent cell growth inhibitory activity against human cancer cell lines[1]. They were found to exhibit there antitumor activity by inhibition of different types of enzymes such as cyclin-dependent Kinases (CDK) [1-3], Src and Abl Tyrosine Kinases [4], Glycogen Synthase Kinase-3 (GSK-3) [5-7], adenosine deaminase (ADA) [8], and Epidermal Growth Factor Receptor Protein Tyrosine Kinase (EGF-RTK) [9].

These compounds exhibit antiproliferative effects in the HCT116 (colon cancer) and other cell lines as on MCF-7 cell line (breast cancer) [10]. The potency of these antiproliferative effects is enhanced in anilide derivatives [2]. Several pyrazolo[3,4-d]pyrimidines were reported to inhibit tumor cell proliferation by the interference with the signalling pathway at the level of Src tyrosine kinase and at the level of the downstream effector signal mitogen activated protein kinases (MAPKs), ERK1-2. [11] Substituted pyrazolo [3, 4-d] pyrimidine scaffold was previously reported and was proved to be active against several tumor cell lines. [12] **fig 1**

novel class of pyrazolopyrimidine derivatives as GSK-3 inhibitors reported to have improved cellular activity.

It was decided to obtain similar heterocyclic compounds and also build up a fused pyrimidine ring with substitutions at both positions **1** and **4.** The compounds synthesized were tested for their capacity to inhibit in-vitro growth of two tumour cell lines, MCF-7 (breast cancer) and HCT-116 (colon cancer).

2. Experimental

2.1. Chemistry

All melting points were determined on Stuart apparatus and the values given are uncorrected. IR spectra were determined on Shimadzu IR 435 spectrophotometer (KBr, cm^{-1}), Faculty of Pharmacy, Cairo University, Egypt. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Varian Gemini 200 MHz

and 300 MHz spectrophotometer using TMS as internal standard. Chemical shift values are recorded in ppm on δ scale, Microanalysis Center, Cairo University, Egypt. Mass spectra were recorded on Hewlett Packard 5988 spectrometer, Microanalysis Center, Cairo University, Egypt. Elemental analyses were carried out at the Microanalysis Center, Cairo University, Egypt; found values were within ±0.35% of the theoretical ones, unless otherwise indicated. Progress of the reactions was monitored using TLC sheets precoated with UV fluorescent silica gel Merck 60F 254 and were visualized using UV lamp.

Compounds **Ia** [13], **Ib** [14], **IIa-c** [15-17], **IIIa** [18], **IVa** [19] and **VIa** [19] were prepared as reported in the literature.

2.2. Procedures for the synthesis of compounds IIIb and IIIc

Method 1: A suspension of the appropriate derivative **Ia,b** (0.01 mol) in formamide (30 ml) was stirred at 145°C for 3 h.; the solution was then cooled poured on ice-cold water, filtered, washed with water, dried and finally crystallized from formic acid.

Method 2: A suspension of the appropriate derivative **IIa-c** (0.01 mol) in 85% formic acid (40 ml), was heated under reflux for 7-9 h.; the reaction mixture was then cooled, filtered, washed with water, dried and crystallized from formic acid.

2.2.1. 3-methyl-1-(4-nitrophenyl)-1H-pyrazolo [3, 4-d] pyrimidin-4(5H)-one (IIIb)

Yield: 2.6 g (96%); M.p.: >300ºC; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.50 (s, 3H, CH_3); 8.20 (s, 1H, H6); 8.34 (d, *J* = 9.3 Hz, 2H, H2',6'); 8.39 (d, *J* = 9.6 Hz, 2H, H3',5');12.51 (s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 1685 (C=O); MS (70 eV): *m/z* 272 (M^+ + 1); 271 (100%). Anal. Calcd for $C_{12}H_9N_5O_3$ (271.23): C, 53.14; H, 3.34; N, 25.82. Found: C, 53.30; H, 3.64; N, 26.36 (N differ than theoretical value by 0.54 %.).

2.2.2. 3-methyl-1-(4-chlorophenyl)-1H-pyrazolo [3, 4-d] pyrimidin-4(5H)-one (IIIc)

Yield: 1.94 g (74%); M.p.: >300ºC; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.52 (s, 3H, CH_3); 7.60 (d, *J* = 9.0 Hz, 2H, H3',5'); 8.08 (d, *J* = 9.0 Hz, 2H, H2',6'); 8.15 (s, 1H, H6); 12.37 (s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 1689 (C=O); MS (70 eV): *m/z* 263 (M^+ + 3); 262 (M^+ + 2); 261 (M^+ + 1). Anal. Calcd for $C_{12}H_9ClN_4O$ (260.68): C, 55.29; H, 3.48; N, 21.49. Found: C, 55.36; H, 3.69; N, 21.41.

2.3. General procedure for the synthesis of compounds IVb and IVc

A suspension of the appropriate derivative **IIIa-c** (0.01 mol) in phosphorus oxychloride (80 ml) was heated under reflux for 7-9 h.; the solution was cooled, poured on ice-cold water. The precipitated product was filtered, dried and crystallized from appropriate solvent.

2.3.1. 4-chloro-3-methyl-(4-nitrophenyl)-1H-pyrazolo [3, 4-d] pyrimidine (IVb)

Yield: 2.03 g (70%), M.p.: 210-212ºC (ethanol/DMF); ^{1}H-NMR (200 MHz, DMSO-d_6): δ = 2.71 (s, 3, CH_3); 8.37 (d, *J* = 9.4 Hz, 2H, H2',6'); 8.41 (d, *J* = 9.4 Hz, 2H, H3',5') ; 8.96 (s, 1H, H6) ppm; IR (cm^{-1}): 1685 (C=O) disappeared; MS (70 eV): *m/z* 291 (M^+ + 2); 289 (100%). Anal. Calcd for $C_{12}H_8ClN_5O_2$ (289.68): C, 49.75; H, 2.78; N, 24.18. Found: C, 49.60; H, 2.90; N, 24.22.

2.3.2 4-chloro-3-methyl-1-(4-chlorophenyl)-1H-pyrazolo [3, 4-d] pyrimidine (IVc)

Yield: 1.48 g (53%), M.p.: 189-190ºC (ethanol); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.73 (s, 3H, CH_3); 7.65 (d, *J* = 9.0 Hz, 2H, H3',5'); 8.16 (d, *J* = 9.0 Hz, 2H, H2',6'); 8.92 (s, 1H, H6) ppm; IR (cm^{-1}): 1689 (C=O) disappeared; MS (70 eV): *m/z* 280 (M^+ + 2); 278 (M^+); 263 (100%). Anal. Calcd for $C_{12}H_8Cl_2N_4$ (279.12): C, 51.64; H, 2.89; N, 20.07. Found: C, 51.43; H, 3.01; N, 19.81.

2.4. General procedure for the synthesis of compounds Va-h

A suspension of appropriate derivative IVa-c (1 mmol) and the appropriate amine (1 mmol) in ethanol (30 ml), triethylamine (0.3 g, 3 mmol) was added, and the reaction mixture was heated under reflux for 2-7 h.; (the reaction was monitored using TLC until the starting materials is consumed in the reaction). The reaction mixture was allowed to cool leading to separation of the product, the crude product was filtered off, dried and crystallized from appropriate solvent.

2.4.1. N-(4-ethylphenyl)-3-methyl-1-phenyl-1H-pyrazolo [3, 4-d] pyrimidin-4-amine (Va)

Yield: 0.18 g (55%), M.p.: 151-154ºC (ethanol/hexane); ^{1}H-NMR (200 MHz, DMSO-d_6): δ = 1.21 (t, *J* = 7.4 Hz, 3H, CH_3"); 2.63 (q, *J* = 7.4 Hz, 2H, CH_2); 2.78 (s, 3H, CH_3); 7.24 (d, *J* = 7.8 Hz, 2H, H3",5"); 7.35 (t, *J* = 7.4 Hz, 1H, H4'); 7.50-7.65 (d, *J* = 7.2 Hz & m, 4H, H2",6", H3',5'); 8.21 (d, *J* = 8.4 Hz, 2H, H2',6'); 8.42 (s, 1H, H6); 8.76 (s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3431 (NH). Anal. Calcd for $C_{20}H_{19}N_5$ (329.40): C, 72.93; H, 5.81; N, 21.26. Found: C, 73.00; H, 5.87; N, 21.11.

2.4.2. 3-methyl-1-phenyl-N-p-tolyl-1H-pyrazolo [3, 4-d] pyrimidin-4-amine (Vb)

Yield: 0.18 g (58%), M.p.: 145-146ºC (ethanol); ^{1}H-NMR (200 MHz, DMSO-d_6): δ = 2.32 (s, 3H, CH_3"); 2.77 (s, 3H, CH_3); 7.20 (d, *J* = 8.0 Hz, 2H, H3",5"); 7.32 (t, *J* = 7.2 Hz, 1H, H4'); 7.45-7.65 (d , *J* = 8.4 Hz & m , 4H, H2",6", H3',5'); 8.19 (d, *J* = 8.2 Hz, 2H, H2',6'); 8.40 (s, 1H, H6); 8.72 (s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3441 (NH). Anal. Calcd for $C_{19}H_{17}N_5$ (315.37): C, 72.36; H, 5.43; N, 22.21. Found: C, 72.31; H, 5.59; N, 22.52.

2.4.3. N-(4-ethylphenyl)-3-methyl-1-(4-nitrophenyl)-1H-pyrazolo[3,4-d] pyrimidin-4-amine (Vc)

Yield: 0.31 g (84%), M.p.: 228-229ºC (ethanol); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.22 (t, *J* = 7.5 Hz, 3H, CH3); 2.63 (q, *J* = 7.5 Hz, 2H, CH2); 2.75 (s, 3H, CH3); 7.23 (d, *J* = 8.4 Hz, 2H, H3",5"); 7.56 (d, *J* = 8.4 Hz, 2H, H2",6"); 8.38 (d, *J* = 6.9 Hz, 2H, H2',6'); 8.46 (s, 1H, H6); 8.57 (d, *J* = 7.0 Hz, 2H, H3',5'); 8.77 (s, 1H, NH, D2O exchangeable) ppm; IR (cm^{-1}): 3433 (NH). Anal. Calcd for $C_{20}H_{18}N_6O_2$ (374.4): C, 64.16; H, 4.85; N, 22.45. Found: C, 64.40; H, 4.57; N, 22.60.

2.4.4. N-(4-methoxyphenyl)-3-methyl-1-(4-nitrophenyl)-1H-pyrazolo [3, 4-d] pyramidin-4-amine (Vd)

Yield: 0.32 g (85%), M.p.: 275-277ºC (ethanol); ^{1}H-NMR (200 MHz, DMSO-d_6): δ = 2.77 (s, 3H, CH_3); 3.78 (s, 3H, OCH_3); 6.98 (d, *J* = 6.6 Hz, 2H, H3",5"); 7.53 (d, *J* = 6.8 Hz, 2H, H2",6"); 8.40 (d, *J* = 9.4 Hz, 2H, H2',6'); 8.44 (s, 1H, H6); 8.58 (d, *J* = 9.4 Hz, 2H, H3',5'); 8.84 (s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3437 (NH). Anal. Calcd for $C_{19}H_{16}N_6O_3$ (376.37): C, 60.63; H, 4.28; N, 22.33. Found: C, 60.86; H, 4.27; N, 22.55.

2.4.5. 3-methyl-1-(4-nitrophenyl)-N-p-tolyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (Ve)

Yield: 0.29 g (80%), M.p.: 246-247ºC (ethanol); ^{1}H-NMR (200 MHz, DMSO-d_6): δ = 2.32 (s, 3H, CH_3"); 2.76 (s, 3H, CH_3); 7.20 (d, *J* = 8.2 Hz, 2H, H3",5"); 7.53 (d , *J* = 8.6 Hz 2H, H2",6"); 8.36 (d ,

J = 9.4 Hz 2H, H2',6'); 8.44 (s, 1H, H6); 8.55 (d, *J* = 9.6 Hz, 2H, H3',5'); 8.80 (s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3433 (NH). Anal. Calcd for $C_{19}H_{16}N_6O_2$ (360.37): C, 63.32; H, 4.48; N, 23.32. Found: C, 63.32; H, 4.38; N, 23.60.

2.4.6. 1-(4-chlorophenyl)-3-methyl-N-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (Vf)

Yield: 0.19 g (54%), M.p.: 201-202ºC (ethanol); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.77 (s, 3H, CH_3); 7.19 (t, *J* = 7.2 Hz, 1H, H4"); 7.40 (t, *J* = 8.1 Hz, 2H, H3",5"); 7.59 (d, *J* = 9.0 Hz, 2H H3',5'); 7.69 (d, *J* = 8.4 Hz, 2H, H2",6"); 8.25 (d, *J* = 9.0 Hz, 2H, H2',6'); 8.44 (s, 1H, H6); 8.81 (s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3431 (NH). Anal. Calcd for $C_{18}H_{14}ClN_5$ (346.34): C, 64.38; H, 4.20; N, 20.86. Found: C, 64.60; H, 4.30; N, 20.61.

2.4.7. 1-(4-chlorophenyl)-N-(4-ethylphenyl)-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine(Vg)

Yield: 0.14 g (38%), M.p.: 165-166ºC (ethanol); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.21 (t, *J* = 7.5 Hz, 3H, CH_3"); 2.63 (q, *J* = 7.5 Hz, 2H, CH_2); 2.76 (s, 3H, CH_3); 7.23 (d, *J* = 8.4 Hz, 2H, H3",5"); 7.47 (d, *J* = 8.4 Hz, 2H, H2",6"); 7.52 (d, *J* = 9.0 Hz, 2H, H3',5'); 8.26 (d, *J* = 9.0 Hz, 2H, H2',6'); 8.41 (s, 1H, H6); 8.77 (s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3444 (NH). Anal. Calcd for $C_{20}H_{18}ClN_5$ (363.84): C, 66.02; H, 4.99; N, 19.25. Found: C, 66.18; H, 5.02; N, 18.92.

2.4.8. 1-(4-chlorophenyl)-3-methyl-N-p-tolyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (Vh)

Yield: 0.24 g (65%), M.p.: 123-125ºC (ethanol); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.32 (s, 3H, CH_3"); 2.76 (s, 3H, CH_3); 7.20 (d, *J* = 7.2 Hz, 2H, H3",5"); 7.55 (d , *J* = 7.8 Hz, 2H, H2",6"); 7.90 (d , *J* = 7.8 Hz, 2H, H3',5'); 8.24 (d, *J* = 8.1 Hz, 2H, H2',6'); 8.40 (s, 1H, H6); 8.75 (s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3441 (NH). Anal. Calcd for $C_{19}H_{16}ClN_5$ (349.82): C, 65.24; H, 4.61; N, 20.02. Found: C, 65.38; H, 4.70; N, 20.16.

2.5. General procedure for the synthesis of compounds VIb and VIc

Hydrazine hydrate (0.1 mol) was added to a suspension of the appropriate derivative **IVb,c** (0.01 mol) in ethanol (35 ml), the reaction mixture was heated under reflux for 2.5 h.; The precipitated product was filtered, washed with ethanol, dried and crystallized from appropriate solvent.

2.5.1. 4-hydrazinyl-3-methyl-1-(4-nitrophenyl)-1H-pyrazolo[3,4-d]pyrimidine (VIb)

Yield: 2.60 g (91%), M.p.: 265-267ºC (ethanol/DMF); ^{1}H-NMR (200 MHz, DMSO-d_6): δ = 2.35 (s, 3H, CH_3); 8.23 (d, *J* = 9.4 Hz, 2H, H2',6'); 8.29 (s, 1H, H6); 8.41 (d, *J* = 9.0 Hz, 2H, H3',5'); 9.05 (broad s, 3H, NH, NH_2, D_2O exchangeable) ppm; IR (cm^{-1}): 3437 (NH), 3325 & 3309 (NH_2) ; MS (70 eV): *m/z* 286 (M^+ + 1); 285 (100%). Anal. Calcd for $C_{12}H_{11}N_7O_2$ (285.26): C, 50.53; H, 3.89; N, 34.37. Found: C, 50.60; H, 3.70; N, 34.10.

2.5.2. 1-(4-chlorophenyl)-4-hydrazinyl-3-methyl-1H-pyrazolo[3,4-d]pyrimidine (VIc)

Yield: 2.55 g (93%), M.p.: 244-246ºC (ethanol); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.62 (s, 3H, CH_3); 3.82 (s, 1H, NH, D_2O exchangeable); 4.95 (broad s, 2H, NH_2, D_2O exchangeable); 7.56 (d, *J* = 9.0 Hz, 2H, H3',5'); 8.22 (d, *J* = 9.0 Hz, 2H, H2',6'); 8.36 (s, 1H, H6) ppm; IR (cm^{-1}): 3294 (NH), 3278 & 3199 (NH_2) ; MS (70 eV): *m/z* 276 (M^+ + 2); 274 (100%) (M^+). Anal. Calcd for $C_{12}H_{11}ClN_6$ (274.71): C, 52.47; H, 4.04; N, 30.59. Found: C, 52.67; H, 4.24; N, 30.59.

2.6. General procedure for the synthesis of compounds VIIa-r

A suspension of **VIa-c** (1 mmol), and the appropriate aldehyde (1 mmol) in ethanol (35 ml), was heated under reflux with stirring for 3-4 h.; the reaction mixture was then cooled and the separated precipitate was filtered, dried and crystallized from appropriate solvent.

2.6.1. benzaldehyde(3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)hydrazone (VIIa)

Yield: 0.18 g (55%), M.p.: 190-192ºC (ethanol); ^{1}H-NMR (200 MHz, $CDCl_3$): δ = 2.85 (s, 3H, CH_3); 7.30-7.50 (m, 3H, H3",4",5"); 7.45-7.65 (m, 3H, H3',4',5'); 7.75-7.85 (m, 2H, H2",6"); 8.00 (s, 1H, HC=N); 8.10 (d, *J* = 8.0 Hz, 2H, H2',6'); 8.30 (s, 1H, H_6); 12.25 (s, 1H. NH) ppm; IR (cm^{-1}): 3207 (NH); MS (70 eV): *m/z* 328 (M^+); 77 (100%). Anal. Calcd for $C_{19}H_{16}N_6$ (328.37): C, 69.50; H, 4.91; N, 25.59. Found: C, 69.62; H, 4.93; N, 25.61.

2.6.2.2-hydroxybenzaldehyde(3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)hydrazone (VIIb)

Yield: 0.20 g (57%), M.p.: 214-215ºC (ethanol/water); ^{1}H-NMR (200 MHz, DMSO-d_6): δ = 2.57 (s, 3H, CH_3); 6.90-7.00 (m, 1H, H5"); 7.20-7.30 (m, 1H, H3"); 7.30-7.43 (m, 3H, H3',4',5'); 7.48-7.58 (m, 1H, H4"); 7.75-7.85 (m, 1H, H6"); 8.00 (d, 2H, H2',6'); 8.25 (s, 1H, HC=N); 8.68 (s, 1H, H6); 10.26 (broad s, 1H, OH, D_2O exchangeable) ; 11.91 (broad s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3396 (NH & OH). Anal. Calcd for $C_{19}H_{16}N_6O$ (344.37): C, 66.27; H, 4.68; N, 24.40. Found: C, 66.10; H, 4.50; N, 24.16.

2.6.3. 3-hydroxybenzaldehyde(3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)hydrazone (VIIc)

Yield: 0.12 g (35%), M.p.: 241-243ºC (ethanol/water); ^{1}H-NMR (200 MHz, DMSO-d_6): δ = 2.56 (s, 3H, CH_3); 6.80-6.90 (m, 1H, H4"); 7.15-7.25 (m, 1H, H5"); 7.30-7.38 (m, 1H, H2"); 7.48-7.63 (m, 3H, H3',4',5'); 8.00-8.10 (m, 1H, H6"); 8.22 (d, *J* = 8.8 Hz, 2H, H2',6'); 8.36 (s, 1H, HC=N); 8.44 (s, 1H, H6); 9.55 (s, 1H, OH, D_2O exchangeable) ; 11.92 (broad s, 1H, NH, D_2O exchangeable) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 14.10 (CH_3); 102.00 (C3a); 116.96 (C2"); 120.90 (C4"); 121.28 (C2',6'); 126.00 (C6"); 126.41 (C4'); 129.02 (C3',5'); 129.38 (C5"); 136.54 (C1"); 138.26 (C1'); 144.93 (C=N); 147.85 (C3); 149.50 (C7a); 153.57 (C6); 157.46 (C3"); 157.71 (C4) ppm; IR (cm^{-1}): 3332 (NH), 3238 (OH). Anal. Calcd for $C_{19}H_{16}N_6O$ (344.37): C, 66.27; H, 4.68; N, 24.40. Found: C, 66.10; H, 4.50; N, 24.14.

2.6.4. 3-nitrobenzaldehyde(3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)hydrazone (VIId)

Yield: 0.20 g (53%), M.p.: 237-239ºC (benzene/DMF); ^{1}H-NMR (200 MHz, DMSO-d_6): δ = 2.56 (s, 3H, CH_3); 7.32-7.42 (m, 3H, H3',4'.5'); 7.45-7.55 (m, 1H, H5"); 7.65-7.75 (m, 1H, H6"); 7.95-8.05 (m, 1H, H4"); 8.19 (d, *J* = 7.6 Hz, 2H, H2',6'); 8.42 (s, 1H, HC=N); 8.50 (s, 1H, H6); 8.77 (s, 1H, H2"); 12.09 (s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3300 (NH). Anal. Calcd for $C_{19}H_{15}N_7O_2$ (373.37): C, 61.12; H, 4.05; N, 26.26. Found: C, 60.93; H, 4.15; N, 26.00.

2.6.5. 3-fluorobenzaldehyde(3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)hydrazone (VIIe)

Yield: 0.16 g (45%), M.p.: 186-190ºC (ethanol/water); ^{1}H-NMR (200 MHz, $CDCl_3$): δ = 2.75 (s, 3H, CH_3); 7.05-7.15 (m, 1H, H2"); 7.25-7.35 (m, 2H, H4",5"); 7.30-7.45 (m, 3H, H3',4',5'); 7.50 (s, 1H, H6"); 7.98 (s, 1H, HC=N); 8.05 (d, *J* = 6.0 Hz, 2H, H2',6'); 8.30 (s, 1H, H6); 10.50 (s, 1H, NH, D_2O

exchangeable) ppm; IR (cm^{-1}): 3218 (NH). Anal. Calcd for $C_{19}H_{15}FN_6$ (346.36): C, 65.89; H, 4.37; N, 24.26. Found: C, 66.00; H, 4.40; N, 23.95.

2.6.6.4-methoxybenzaldehyde(3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)hydrazone (VIIf)

Yield: 0.20 g (55%), M.p.: 238-239ºC (ethyl acetate); ^{1}H-NMR (200 MHz, DMSO-d_6): δ = 2.56 (s, 3H, CH_3); 3.81 (s, 3H, OCH_3); 7.01 (d, *J* = 8.8 Hz, 2H, H3",5"); 7.25-7.40 (m, 3H, H3',4',5'); 7.66 (d, *J* = 8.8 Hz, 2H, H2",6"); 8.10 (d, *J* = 8.0 Hz, 2H, H2',6'); 8.29 (s, 1H, HC=N); 8.38 (s, 1H, H6); 11.87 (broad s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3220 (NH). Anal. Calcd for $C_{20}H_{18}N_6O$ (358.40): C, 67.02; H, 5.06; N, 23.45. Found: C, 67.20; H, 5.00; N, 23.12.

2.6.7.4-hydroxy-3-methoxybenzaldehyde(3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)hydrazone (VIIg)

Yield: 0.15 g (40%), M.p.: 206-208ºC (ethanol/water); ^{1}H-NMR (200 MHz, DMSO-d_6): δ = 2.85 (s, 3H, CH_3); 3.80 (s, 3H, OCH_3); 6.75-7.85 (m, 1H, H2"); 7.00-7.10 (m, 1H, H5"); 7.25-7.35 (m, 1H, H6"); 7.45-7.60 (m, 3H, H3',4'.5'); 8.15 (d, *J* = 6.0 Hz, 2H, H2',6'); 8.30 (s, 1H, HC=N); 8.42 (s, 1H, H6); 9.50 (broad s, 1H, OH); 11.80 (broad s, 1H, NH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 14.10 (CH_3); 55.75 (OCH_3); 102.19 (C3a); 110.76 (C2"); 115.30 (C5"); 121.22 (C2',6'); 122.51 (C6"); 126.31 (C4'); 126.71 (C3',5'); 128.96 (C1"); 138.33 (C1'); 144.82 (C=N); 147.10 (C3); 147.83 (C3"); 148.84 (C7a); 149.30 (C4"); 153.70 (C6); 154.92 (C4) ppm; IR (cm^{-1}): 3527 (NH), 3238 (OH) ; MS (70 eV): *m/z* 374 (M^+), 77 (100%). Anal. Calcd for : C, 55.29; H, 3.48; N, 21.49. Found: C, 55.36; H, 3.69; N, 21.41. Anal. Calcd for $C_{20}H_{18}N_6O_2$ (374.40): C, 64.16; H, 4.85; N, 22.45. Found: C, 64.00; H, 4.70; N, 22.26.

2.6.8.benzaldehyde[3-methyl-1-(4-nitrophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl]hydrazone (VIIh)

Yield: 0.31 g (83%), M.p.: 241-243ºC (ethanol/DMF); ^{1}H-NMR (200 MHz, DMSO-d_6): δ = 2.60 (s, 3H, CH_3); 7.40-7.60 (m, 3H, H3",4",5"); 7.85-7.95 (m, 2H, H2",6"); 7.93 (s, 1H, , HC=N); 8.31 (d, *J* = 9.0 Hz, 2H, H2',6'); 8.40 (s, 1H, H6); 8.48 (d, *J* = 9.0 Hz, 2H, H3',5'); 12.04 (broad s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3211 (NH). Anal. Calcd for $C_{19}H_{15}N_7O_2$ (373.37): C, 61.12; H, 4.05 N; 26.26. Found: C, 61.40; H, 4.02; N, 25.99.

2.6.9. 2-hydroxybenzaldehyde[3-methyl-1-(4-nitrophenyl)-1H-pyrazolo [3, 4-d] pyrimidin-4-yl] hydrazone (VIIi)

Yield: 0.31 g (79%), M.p.: 290-291ºC (ethanol/DMF); ^{1}H-NMR (200 MHz, DMSO-d_6): δ = 2.76 (s, 3H, CH_3); 6.85-7.05 (m, 1H, H5"); 7.25-7.35 (m, 1H, H3"); 7.65-7.75 (m, 1H, H4"); 7.75-7.85 (m, 1H, H6"); 7.95 (s, 1H, HC=N); 8.31 (d, *J* = 9.0 Hz, 2H, H2',6'); 8.51 (d, *J* = 9.0 Hz, 2H, H3',5'); 8.61 (s, 1H, H6); 10.19 (broad s, 1H, OH, D_2O exchangeable) ; 11.98 (broad s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3412 (NH), 3387 (OH). Anal. Calcd for $C_{19}H_{15}N_7O_3$ (389.37): C, 58.61; H, 3.88; N, 25.18. Found: C, 58.91; H, 3.67; N, 25.44.

2.6.10. 3-hydroxybenzaldehyde[3-methyl-1-(4-nitrophenyl)-1H-pyrazolo [3, 4-d] pyrimidin-4-yl] hydrazone (VIIj)

Yield: 0.38 g (97%), M.p.: 281-283ºC (ethanol/DMF); ^{1}H-NMR (200 MHz, DMSO-d_6): δ = 2.73 (s, 3H, CH_3); 6.75-6.85 (m, 1H, H4"); 7.05-7.15 (m, 1H, H5"); 7.25-7.35 (m, 1H, H2"); 7.95-8.05 (m, 1H, H6"); 8.31 (s, HC=N); 8.36 (d, *J* = 9.0 Hz, 2H, H2',6'); 8.40 (s, 1H, H6); 8.50 (d, *J* = 9.0 Hz, 2H, H3',5'); 9.64 (broad s, 1H, OH, D_2O exchangeable) ; 12.04 (broad s, 1H, NH, D_2O exchangeable)

ppm; IR (cm^{-1}): 3340 (NH), 3275 (OH) ; MS (70 eV): *m/z* 390 ($M^+ + 1$); 389 (100%). Anal. Calcd for $C_{19}H_{15}N_7O_3$ (389.37): C, 58.61; H, 3.88; N, 25.18. Found: C, 58.91; H, 3.84; N, 25.35.

2.6.11. 3-nitrobenzaldehyde [3-methyl-1-(4-nitrophenyl)-1H-pyrazolo [3, 4-d] pyrimidin-4-yl] hydrazone (VIIk)

Yield: 0.37 g (88%), M.p.: 295-296ºC (ethanol/DMF); ^{1}H-NMR (200 MHz, DMSO-d_6): δ = 2.73 (s, 3H, CH_3); 7.65-7.75 (m, 2H, H5",6"); 7.95-8.05 (m, 1H, H4"); 8.17 (s, 1H, HC=N); 8.30 (d , *J* = 9.0 Hz, 2H, H2',6'); 8.43 (d, *J* = 9.0 Hz, 2H, H3',5'); 8.50 (s, 1H, H6); 8.70-8.80 (m, 1H, H2"); 12.17 (broad s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3305 (NH). Anal. Calcd for $C_{19}H_{14}N_8O_4$ (418.37): C, 54.55; H, 3.37; N, 26.78. Found: C, 54.67; H, 3.29; N, 26.99.

2.6.12. 3-nitrobenzaldehyde [3-methyl-1-(4-nitrophenyl)-1H-pyrazolo [3, 4-d] pyrimidin-4-yl] hydrazone (VIIl)

Yield: 0.21 g (54%), M.p.: 246-247ºC (ethanol/DMF); ^{1}H-NMR (200 MHz, DMSO-d_6): δ = 2.68 (s, 3H, CH_3); 7.15-7.25 (m, 1H, H2"); 7.35-8.45 (m, 2H, H4",5"); 7.60 (s, 1H, H6"); 7.94 (s, 1H, HC=N); 8.31 (d, *J* = 9.0 Hz, 2H, H2',6'); 8.33 (s, 1H, H6); 8.49 (d, *J* = 9.0 Hz, 2H, H3',5'); 12.01 (broad s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3365 (NH) ; MS (70 eV): *m/z* 392 ($M^+ + 1$); 391 (M^+). Anal. Calcd for $C_{19}H_{14}FN_7O_2$ (391.36): C, 58.31; H, 3.61; N, 25.05. Found: C, 58.59; H, 3.51; N, 24.96.

2.6.13. 4-methoxybenzaldehyde [3-methyl-1-(4-nitrophenyl)-1H-pyrazolo [3, 4-d] pyrimidin-4-yl] hydrazone (VIIm)

Yield: 0.33 g (82%), M.p.: 280-283ºC (ethanol/DMF); ^{1}H-NMR (200 MHz, DMSO-d_6): δ = 2.73 (s, 3H, CH_3); 3.86 (s, 3H, OCH_3); 7.00 (d, *J* = 8.4 Hz, 2H, H3",5"); 7.65 (d, *J* = 8.4 Hz, 2H, H2",6"); 7.95 (s, 1H, HC=N); 8.31 (d, *J* = 9.0 Hz, 2H, H2',6'); 8.35 (s, 1H, H6); 8.49 (d, *J* = 9.0 Hz, 2H, H3',5'); 11.91 (broad s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3313 (NH). Anal. Calcd for $C_{20}H_{17}N_7O_3$ (403.39): C, 59.55; H, 4.25; N, 24.31. Found: C, 59.71; H, 4.08; N, 24.00.

2.6.14. 4-hydroxy-3-methoxybenzaldehyde[3-methyl-1-(4-nitrophenyl)-1H-pyrazolo[3, 4-d] pyrimidin-4-yl] hydrazone (VIIn)

Yield: 0.23 g (56%), M.p.: 265-266ºC (ethanol/DMF); ^{1}H-NMR (200 MHz, DMSO-d_6): δ = 2.82 (s, 3H, CH_3); 3.87 (s, 3H, OCH_3); 6.75-6.85 (m, 1H, H2"); 7.05-7.15 (m, 1H, H5"); 7.25-7.35 (m, 1H, H6"); 8.19 (s, 1H, HC=N); 8.31 (d, *J* = 8.4 Hz, 2H, H2',6'); 8.45 (s, 1H, H6); 8.56 (d, *J* = 9.2 Hz, 2H, H3',5'); 9.49 (broad s, 1H, OH, D_2O exchangeable); 11.90 (broad s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3493 (NH), 3219 (OH). Anal. Calcd for $C_{20}H_{17}N_7O_4$ (419.39): C, 57.28; H, 4.09; N, 23.38. Found: C, 57.30; H, 4.01; N, 23.50.

2.6.15. benzaldehyde [1-(4-chlorophenyl)-3-methyl-1H-pyrazolo [3, 4-d] pyrimidin-4-yl] hydrazone (VIIo)

Yield: 0.23 g (63%), M.p.: 205-206ºC (ethanol); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.80 (s, 3H, CH_3); 7.40-7.50 (m, 3H, H3",4",5"); 7.60 (d, *J* = 8.4 Hz, 2H, H3',5'); 7.70-7.75 (m, 2H, H3",5"); 8.09 (d, *J* = 8.1 Hz, 2H, H2',6'); 8.20 (s, 1H, HC=N); 8.45 (s, 1H, H6); 11.97 (broad s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3217 (NH). Anal. Calcd for $C_{19}H_{15}ClN_6$ (362.82): C, 62.90; H, 4.17; N, 23.16. Found: C, 63.19; H, 4.35; N, 22.82.

2.6.16. 3-hydroxybenzaldehyde[1-(4-chlorophenyl)-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl] hydrazone (VIIp)

Yield: 0.30 g (80%), M.p.: 274-275ºC (ethanol); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.81 (s, 3H, CH_3); 6.78-6.83 (m, 1H, H4"); 7.08-7.13 (m, 1H, H5"); 7.28-7.32 (m, 1H, H2"); 7.6 (d, *J* = 9.0 Hz, 2H, H3',5'); 7.90-7.95 (m, 1H, H6"); 8.09 (d, *J* = 9.0 Hz, 2H, 2',6'); 8.30 (s, HC=N); 8.40 (s, 1H, H6); 9.60 (broad s, 1H, OH, D_2O exchangeable) ; 11.93 (broad s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3332 (NH & OH). Anal. Calcd for $C_{19}H_{15}ClN_6O$ (378.82): C, 60.24; H, 3.99; N, 22.19. Found: C, 60.50; H, 4.10; N, 22.00.

2.6.17. 3-fluorobenzaldehyde [1-(4-chlorophenyl)-3-methyl-1H-pyrazolo [3,4-d]pyrimidin-4-yl] hydrazone (VIIq)

Yield: 0.25 g (65%), M.p.: 213-214ºC (ethanol); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.59 (s, 3H, CH_3); 7.18-7.22 (m, 1H, H2"); 7.35-7.45 (m, 2H, H4",5"); 7.50 (s, 1H, H6"); 7.60 (d, *J* = 7.5 Hz, 2H, H3',5'); 8.10 (d, *J* = 7.8 Hz, 2H, H2',6'); 8.20 (s, 1H, HC=N); 8.41 (s, 1H, H6); 12.00 (broad s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3199 (NH). Anal. Calcd for $C_{19}H_{14}ClFN_6$ (380.81): C, 59.93; H, 3.71; N, 22.07. Found: C, 60.21; H, 3.73; N, 21.95.

2.6.18. 4-hydroxy-3-methoxybenzaldehyde [1-(4-chlorophenyl)-3-methyl-1H-pyrazolo [3, 4-d] pyrimidin-4-yl] hydrazone (VIIr)

Yield: 0.15 g (37%), M.p.: 253-254ºC (ethanol); ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.85 (s, 3H, CH_3); 3.80 (s, 3H, OCH_3); 6.80-6.84 (m, 1H, H2"); 7.06-7.11 (m, 1H, H5"); 7.30-7.34 (m, 1H, H6"); 7.60 (d, *J* = 9.0 Hz, 2H, 3',5'); 8.08 (d, *J* = 9.0 Hz, 2H, 2',6'); 8.30 (s, 1H, HC=N); 8.41 (s, 1H, H6); 9.44 (broad s, 1H, OH, D_2O exchangeable); 11.80 (broad s, 1H, NH, D_2O exchangeable) ppm; IR (cm^{-1}): 3549 (NH), 3217 (OH). Anal. Calcd for $C_{20}H_{17}ClN_6O_2$ (408.84): C, 58.75; H, 4.19; N, 20.56. Found: C, 58.99; H, 4.33; N, 20.81.

2.7. Cytotoxic activity

2.7.1. Mesurement of potential cytotoxicity by SRB assay (at the Egyptian National Cancer Institute)

potential cytotoxicity of compounds were tested using method of Skehan et al. [20]

Cells were plated in 96-multiwell plate (10^4cells/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, 5, 12.5, 25 and m0 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^{o}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. Color intensity was measured in ELISA reader. The relation between surviving fraction and compound concentration was plotted and IC50 [the concentration required for 50% inhibition of cell viability] was calculated for each compound and results are given in Tables 1-2, and figures 2-5.

Table 1

Surviving fraction for four concentrations of each tested compound (Va-Vh), and cisplatin as reference compound and their IC50 values on HCT 116 cell line.

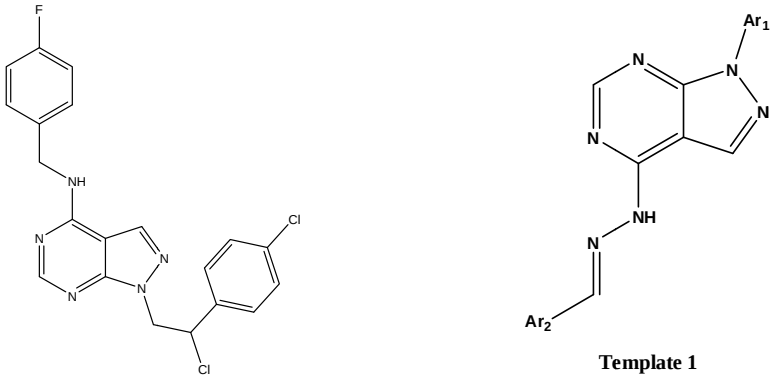
Compound	The surviving fraction at each concentration(µg/ml)				IC50 (µg/ml)	IC50 (µM)
	5	12.5	25	50		
Cisplatin	0.367	0.273	0.253	0.332	3.89	13
Va	0.730632	0.606267	0.306531	0.252164	17	51.6
Vb	0.693075	0.690321	0.377709	0.295085	20.1	63.7
Vc	0.665749	0.583733	0.543315	0.392732	31.9	85.2
Vd	0.572251	0.372702	0.409543	0.278872	7.55	20.1
Ve	0.469991	0.399170	0.342181	0.362937	4.65	12.9
Vf	0.461156	0.429931	0.282567	0.242875	4.5	13
Vg	0.759461	0.629158	0.460584	0.305101	22	60.5
Vh	0.641319	0.641927	0.517204	0.328589	27.1	77.5

Table 2

Surviving fraction for four concentrations of each tested compound (VIIa-VIIr) and cisplatin as reference compound and their IC50 values on MCF7 cell line.

Compound	Cell line	The surviving fraction at each concentration(µg/ml)				IC50 (µg/ml)	IC50 (µM)
		5	12.5	25	50		
Cisplatin	MCF7	0.106	0.128	0.131	0.143	2.82	9.4
VIIa	MCF7	0.579098	0.444910	0.592532	0.592532	9.22	28.1
VIIb	MCF7	0.452403	0.466364	0.193426	0.273280	4.5	13.1
VIIc	MCF7	0.512847	0.387358	0.313465	0.324018	5.87	17
VIId	MCF7	0.671860	0.450186	0.437759	0.470593	10.6	28.4
VIIe	MCF7	0.752924	0.665577	0.660980	0.536701	–	–
VIIf	MCF7	0.600041	0.676304	0.583337	0.382081	35.4	98.8
VIIg	MCF7	0.817286	0.678858	0.381570	0.352475	3.89	54
VIIh	MCF7	0.717526	0.581294	0.310226	0.382250	17	43.1
VIIi	MCF7	0.309716	0.229182	0.239735	0.328600	20.1	9.2
VIIj	MCF7	0.735046	0.360402	0.314144	0.295413	31.9	28.8
VIIk	MCF7	0.640394	0.388211	0.313291	0.346739	7.55	21.7
VIIl	MCF7	0.340706	0.289626	0.329126	0.428564	4.65	9.6
VIIm	MCF7	0.838739	0.639015	0.392128	0.300695	4.5	48.1
VIIn	MCF7	1.006794	0.817796	0.499566	0.309716	22	59.1
VIIo	MCF7	0.670174	0.611943	0.560198	0.344961	27.1	88.5
VIIp	MCF7	0.520509	0.349900	0.346325	0.453083	2.82	15.9
VIIq	MCF7	0.472493	0.418690	0.340706	0.380038	9.22	12.2
VIIr	MCF7	0.893906	0.694182	0.530725	0.391616	4.5	74.8

Fig 1. Representing some pyrazolo[3,4-d]pyrimidine derivatives reported as antitumor agents



Template 1

Fig2. Represents IC50 for cisplatin and compounds Va-Vh on HCT116 cell line.

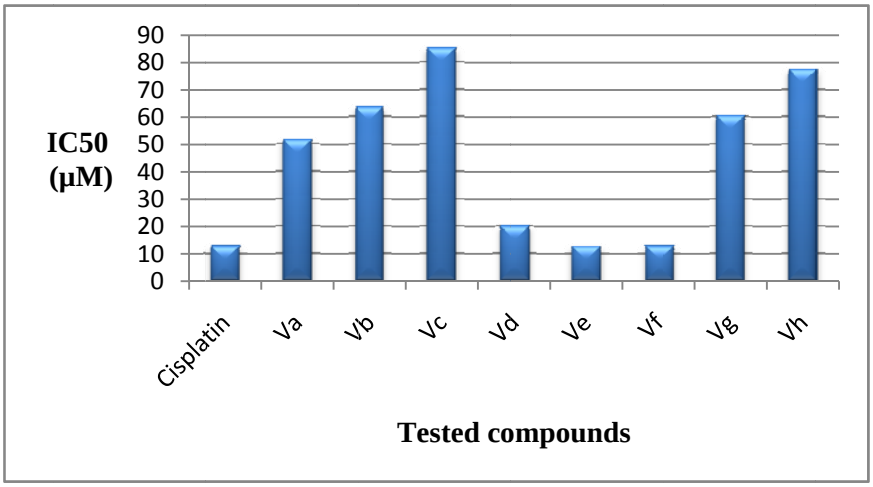


Fig 3. Represents IC50 for cisplatin and compounds VIIa-VIIr on MCF7 cell line.

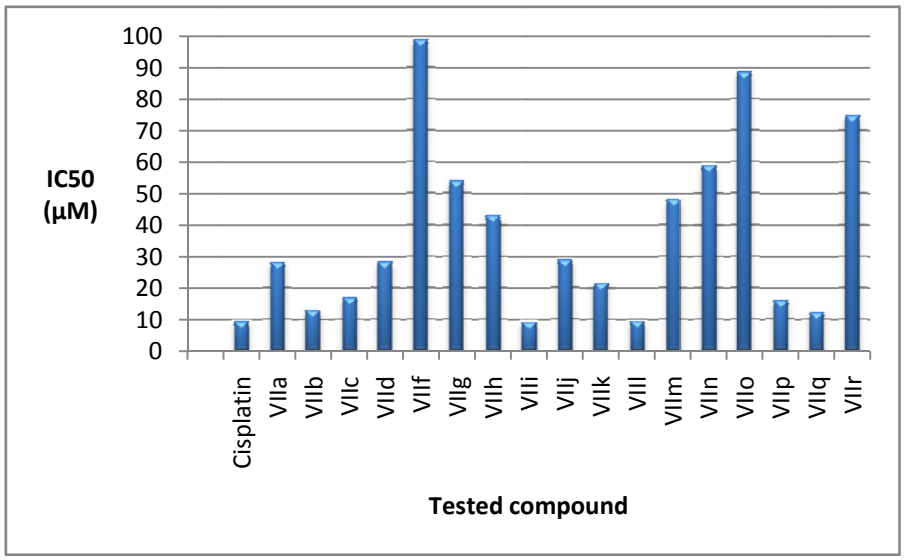


Fig 4. Showing surviving fraction against concentration for Va-Vh on HCT-116 cell line.

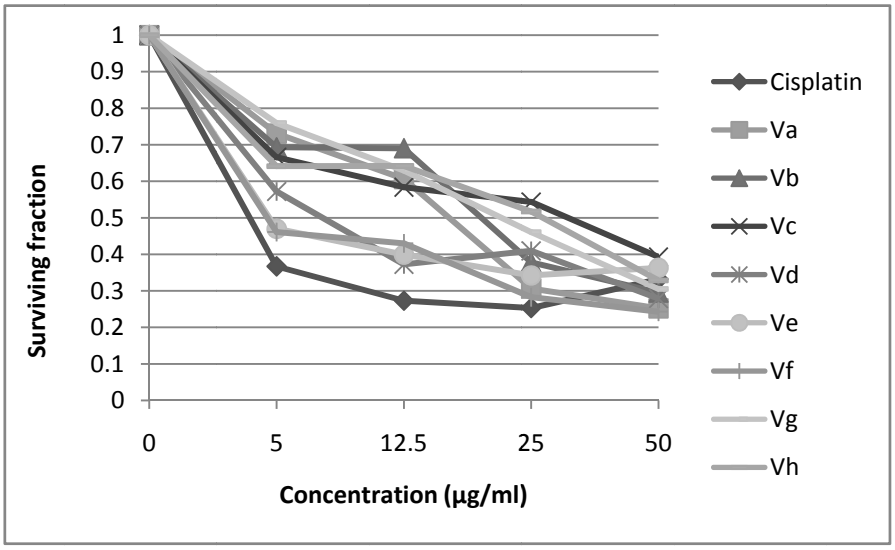


Fig 5. Showing surviving fraction against concentration for VIIa-VIIi on MCF-7 cell line.

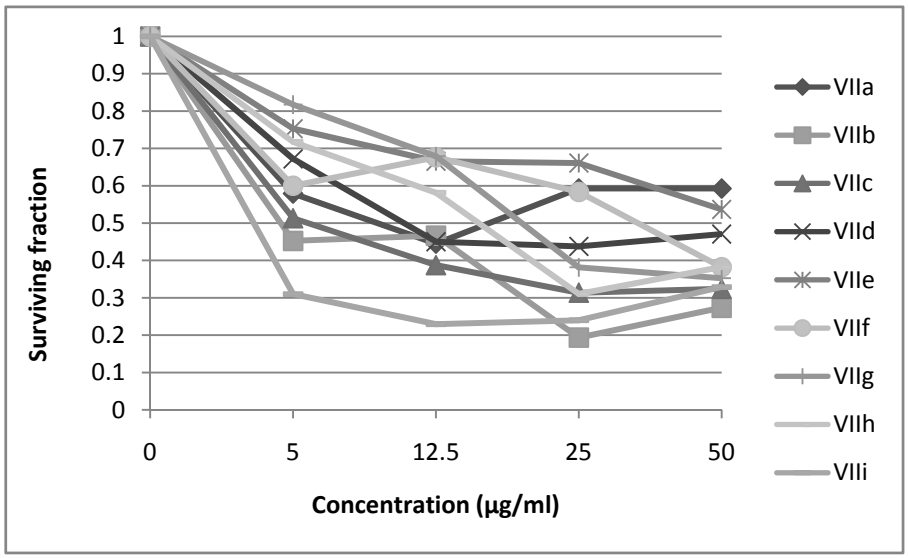
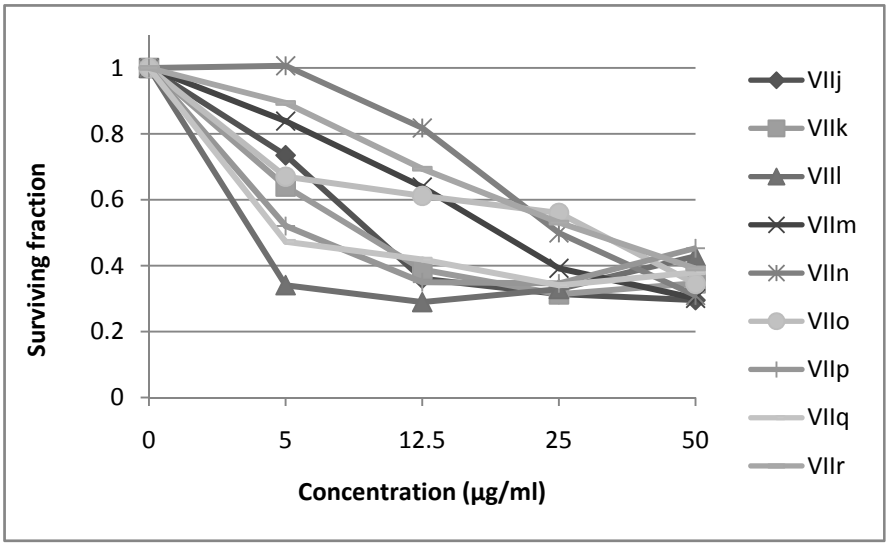
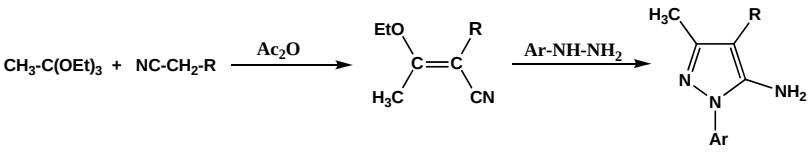


Fig 6. Showing surviving fraction against concentration for VIIj-VIIr on MCF-7 cell line.



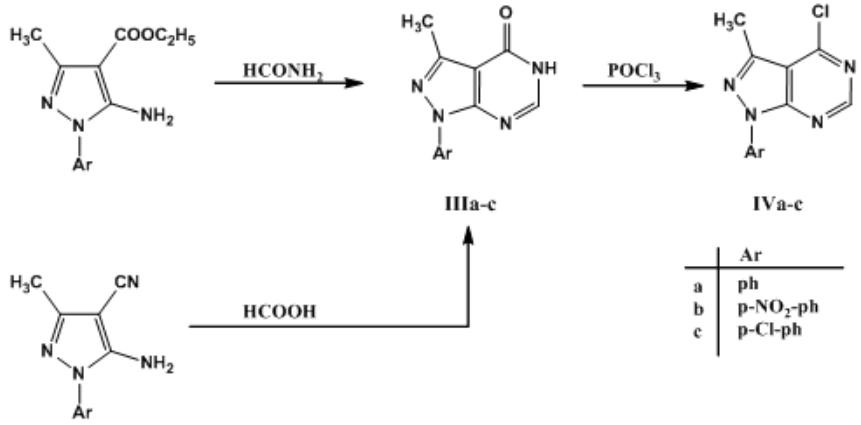
SCHEME-1



Ia,b & IIa-c

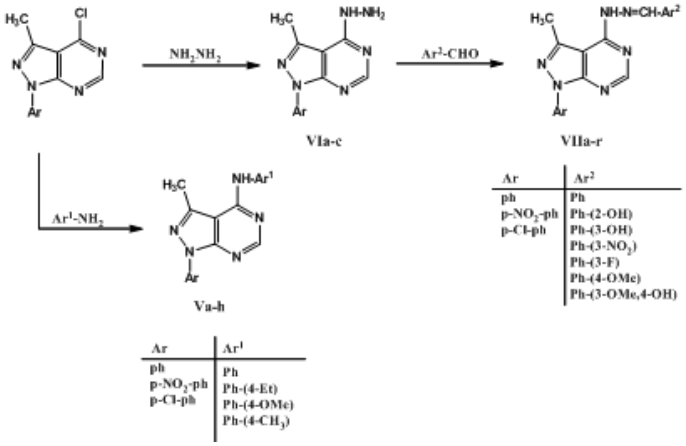
	Ar	R
Ia	ph	$COOC_2H_5$
Ib	p-NO_2-ph	$COOC_2H_5$
IIa	ph	CN
IIb	p-NO_2-ph	CN
IIc	p-Cl-ph	CN

SCHEME-2



	Ar
a	ph
b	p-NO_2-ph
c	p-Cl-ph

SCHEME-3



Ar	Ar^2
ph	Ph
p-NO_2-ph	Ph-(2-OH)
p-Cl-ph	Ph-(3-OH)
	Ph-(3-NO_2)
	Ph-(3-F)
	Ph-(4-OMe)
	Ph-(3-OMe,4-OH)

Ar	Ar^1
ph	Ph
p-NO_2-ph	Ph-(4-Et)
p-Cl-ph	Ph-(4-OMe)
	Ph-(4-CH_3)

3. Results and Discussion

3.1. Chemistry

The synthesis of the target compounds is outlined in Schemes 1-3. Compounds **Ia** [13], **Ib** [14], **IIa-c** [15-17], **IIIa** [18], **IVa** [19] and **VIa** [19] were prepared as reported in the literature.

IIIa,b were prepared by two different methods, either by heating **Ia,b** in formamide or by heating **IIa,b** in formic acid. On the other hand **IIIc** was prepared only by heating **IIc** in formic acid, **IIIb,c** were then confirmed by ^{1}H-NMR which revealed deshielded singlet 1H corresponding to H6, and singlet, 1H, D_2O exchangeable corresponding to NH, by disappearance of CN, NH_2 and appearance of C=O peaks in the IR spectrum, and mass spectrum.

On the other hand **IVa-c** were prepared by reflux of **IIIa-c** in phosphorus oxychloride, new compounds were confirmed by ^{1}H-NMR which revealed disappearance of the singlet D_2O exchangeable signal that was corresponding to NH in **IIIb,c**, and increased deshielding of H6 by the inductive effect of chlorine atom, by disappearance of C=O peak in the IR spectrum, and by microanalysis and mass spectra which gave fragments showing the isotopic pattern due to the presence of chlorine atom.

Derivatives **Va-h** were synthesized by reflux of **IVa-c** with the appropriate amine using triethylamine as a catalyst, the formed derivatives were confirmed by ^{1}H-NMR which revealed appearance of singlet D_2O exchangeable signal corresponding to NH, and appearance of another signals characterizing the introduced group (see section 4), by appearance of NH peak in the IR spectrum. Some of these derivatives were additionally confirmed by mass spectrum, ^{13}C-NMR.

A reaction between **IVa-c** with hydrazine hydrate affords **VIa-c,** new derivatives were confirmed by ^{1}H-NMR which revealed appearance of two singlet D_2O exchangeable signals corresponding to NH_2 and NH, and slight shielding of H6 due to replacement of chlorine atom, by IR spectrum which shows two peaks (single and forked) corresponding to NH and NH_2, and by mass spectra (see section 4). **VIa-c** were consumed in the synthesis of **VIIa-r** by reflux with the appropriate aldehyde, and the produced derivatives were confirmed by ^{1}H-NMR which revealed disappearance of singlet D_2O exchangeable signal corresponding to NH_2, appearance of a deshielded singlet signal corresponding to CH=N and appearance of another signals characterizing the introduced aryl (see section 4), by disappearance of NH_2 peak in the IR spectrum. Some of these derivatives were additionally confirmed by mass spectrum, ^{13}C-NMR.

3.2. Antitumor activity

The antitumor activity was determined for the newly synthesized compounds (**Va-h and VIIa-r**). Compounds were subjected to in-vitro detection of their IC50 values on two cell lines MCF-7 and HCT-116 at pharmacology lab, cancer biology unit at the Egyptian National Cancer Institute, results are summarized in tables 1-2 and figures 1-5.

4. Conclusion

The results of the antitumor activity testing were found to be parallel with the performed docking study.

For compounds tested at on HCT-116 cell line all of the test compounds exhibited significant antitumor activity with IC50 values 12.9 and 85.2 µM, while those tested on MCF-7 cell line all of

them exhibited significant antitumor activity with IC50 between 9.2 and 98.8 except **VIIe** which showed an IC50 value > 50 µM.

Summary, it was found that compounds **Ve**, **Vf**, were found to be approximately as potent as reference drug cisplatin with IC50 value 13.0 µM, while compound **Vd** showed potency closed to cisplatin with IC50 value 20.1 µM. In addition compounds **VIIi** and **VIIl** were found to possess an IC50 values 9.2 and 9.6 µM compared with cisplatin 9.4 µM, while compounds **VIIq, VIIb, VIIp** showed IC50 values 12.2, 13.1, 15.9 µM which were close to cisplatin.

5. References

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