

Synthesis and Antiproliferative Activity of 1-(4-(1*H*-Indol-3-yl)-6-(4-Methoxyphenyl)Pyrimidin-2-yl)Hydrazine and Its Pyrazolo Pyrimidine Derivatives

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Abstract

The target compounds 1-(4-(1*H*-indol-3-yl)-6-(4-methoxyphenyl)pyrimidin-2-yl)hydrazine (**5**) was synthesized by reacting 6-(1*H*-indol-3-yl)-4-(4-methoxyphenyl)pyrimidine-2(1*H*)-thione **4** with hydrazine hydrate. Compound **5** was used as a precursor for the synthesis of new pyrazolo pyrimidine derivatives **6-9**. Moreover, the 5-amino-1*H*-pyrazole-4-carbonitrile derivative **6** was then converted into another set of novel compounds **10-14**. On the other hand a series of transformations were carried out using the newly synthesized 1-(4-(1*H*-indol-3-yl)-6-(4-methoxyphenyl) pyrimidin-2-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**11**) to afford the pyrazolo pyrimidine derivatives **15-18**. Antiproliferative activities for some of the newly synthesized compounds were evaluated.

Keywords: Pyrazolopyrimidine; Indole aldehyde; Chalcone; Antiproliferative activity; Triazin-4-one; 5-aminopyrazole

Introduction

Chalcones constitute an important group of natural products and are the precursors of flavonoids, isoflavonoids and many synthetic heterocycles of different biological activities including pyrimidines, pyrazolines, pyrazolopyrimidines and diazepines [1-5].

Chemically, chalcones are characterized by their easy synthesis by Claisen-Schmidt condensation in good yields and the presence of the α,β unsaturated carbonyl system linked to two aromatic rings which encourage the chalcones cyclization through Michael addition to give interesting heterocyclic compounds which exhibit antitumor [6,7], antimitotic [8], antimutagenic [9] antibacterial [10], antiviral [11], anti-inflammatory [12], antiulcerative [13] and hepatoprotective [14]. In the present work we have been synthesized a series of novel heterocyclic compounds derived from a previous synthesized chalcone compound [1]. The new compounds were screened for their antiproliferative activities.

Experimental

General

Melting points were measured on a digital Electrothermal 9100 apparatus (Kleinfeld, Gehrden, Germany) and are uncorrected. FTIR spectra (KBr) were obtained on a Nicolet 205 spectrophotometer (Nicolet, Madison, WI, USA). The ¹H NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer (Varian inc., Palo Alto, CA, USA). Chemical shifts are expressed in δ values. The mass spectra were recorded on a Shimadzu GCMS-QP-1000EX mass spectrometer (Shimadzu, Kyoto, Japan) at 70 eV. Elemental analysis were carried out at the Micro-analytical Center of Cairo University. All reactions were routinely followed by TLC.

3-(2-hydrazinyl-6-(4-methoxyphenyl)pyrimidin-4-yl)-1*H*-indole (**5**)

The hydrazino-pyrimidine derivative **5** was prepared by reacting compound **4** [1] (10.0 mmol) with hydrazine hydrate (10.0 mmol) catalyzed by acetic acid (5 drops) in refluxing ethanol for 6 h. Evaporation of alcohol and recrystallization with ethanol gave

compound **5** as pale brown crystals mp 160-2°C, yield 80% (element test for the product confirmed the disappearance of sulfur). IR: $\nu_{\max}/\text{cm}^{-1}$ 3212 (NH₂), 3184 (NH), 3109 (NH), 1618 (C=N), 1576 (C=N). ¹H MR (DMSO-*d*₆): δ 3.78 (s, 3H, OCH₃), 7.05-7.3 (m, 4H, Ar H's), 7.52 (d, 2H, *J*=8.8 Hz, Ar-H), 7.94 (s, 1H, pyrimidin), 8.4 (d, 2H, *J*=8.8 Hz, Ar-H's), 8.78 (s, 1H, indole), 8.93-8.95 (brs, 3H, D₂O Exch., NH₂, NH), 11.65 (s, 1H, D₂O Exch., indole NH). MS: *m/z* (%) 331 [M⁺, 5.08]. Anal. Calcd. For C₁₉H₁₇N₅O (331.37): C, 68.87; H, 5.17; N, 21.13. Found: C, 68.76; H, 5.35; N, 21.30.

1-(4-(1*H*-indol-3-yl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-5-amino-1*H*-pyrazole-4-carbonitrile (**6**)

To a solution of compound **5** (10 mmol) in ethanol (30 mL), ethoxymethylene malononitrile (10 mmol), was added. The reaction mixture was heated under reflux condition for 8 h. Then the formed precipitate was filtered and recrystallized from ethanol to give pale brown crystals of compound **6** mp 174-6°C, yield 76% IR: $\nu_{\max}/\text{cm}^{-1}$ 3356 (NH₂), 3164 (NH), 2224 (C≡N). ¹H NMR (DMSO-*d*₆): δ 3.49 (s, 3H, OCH₃), 7.01-7.27 (m, 4H, Ar H's), 7.46 (d, 2H, *J*=8.6 Hz, Ar-H), 7.87 (s, 1H, pyrazole), 8.28 (s, 1H, indole), 8.30 (d, 2H, *J*=8.6 Hz, Ar-H's), 8.56 (s, 1H, pyrimidin), 8.85 (s, 2H, D₂O Exch., NH₂), 11.66 (s, 1H, D₂O Exch., indole NH). MS: *m/z* (%) 407 [M⁺, 0.39]. Anal. Calcd. For C₂₃H₁₇N₇O (407.43): C, 67.80; H, 4.21; N, 24.06. Found: C, 67.66; H, 4.38; N, 24.25.

3-(2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-6-(4-methoxyphenyl)pyrimidin-4-yl)-1*H*-indole (**7**)

A solution of compound **5** (10 mmol) in absolute ethanol and

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acetylacetone (10 mmol) was heated at reflux temperature for 5 h. The mixture was evaporated under reduced pressure, and the obtained product was recrystallized from ethanol to afford pale brown crystals of pyrazolo pyrimidine derivative 7 mp 187-9°C, yield 70% IR: $\nu_{\max}/\text{cm}^{-1}$ 3170 (NH), 3109 (CH-Ar), 1634 (C=N), 1600 (C=N), 1574 (C=N). ¹H NMR (DMSO-*d*₆): δ 2.3 (s, 3H, CH₃), 2.58 (s, 3H, CH₃), 3.83 (s, 3H, OCH₃), 6.99 (s, 1H, pyrazole), 7.17-7.26 (m, 4H, Ar-H's), 7.53 (d, 2H, *J*=8.6 Hz, Ar-H's), 7.94 (s, 1H, indole), 8.38 (d, 2H, *J*=8.6 Hz, Ar-H's), 8.94 (s, 1H, pyrimidine), 11.7 (s, 1H, D₂O Exch., indole NH). MS: *m/z* (%) 395 [M⁺, 2.0]. Anal. Calcd. For C₂₄H₂₁N₅O (395.46): C, 72.89; H, 5.35; N, 17.71. Found: C, 72.70; H, 5.52; N, 17.90.

1-(4-(1H-indol-3-yl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-3-methyl-1H-pyrazol-5(4H)-one (8)

Compound 5 (10 mmol) and ethyl acetoacetate (10 mmol) in acetic acid (30 mL) was heated at reflux temperature for 6 h. The mixture was poured into ice cold water and the obtained product washed with ice cold water, dried and recrystallized from ethanol to afford pale brown crystals of 8 mp 210-2°C, yield 72% IR: $\nu_{\max}/\text{cm}^{-1}$ 3216 (NH), 1718 (C=O). ¹H NMR (DMSO-*d*₆): δ 1.22 (s, 3H, CH₃), 2.24 (s, 2H, CH₂, pyrazol), 3.83 (s, 3H, OCH₃), 7.02-7.23 (m, 4H, Ar H's), 7.52 (d, 2H, *J*=8.6 Hz, Ar-H's), 7.9 (s, 1H, indole), 8.4 (d, 2H, *J*=8.6 Hz, Ar-H's), 8.95 (s, 1H, pyrimidin), 11.72 (s, 1H, D₂O Exch., indole NH). MS: *m/z* (%) 397 [M⁺, 54]. Anal. Calcd. For C₂₃H₁₉N₅O₂ (397.43): C, 69.51; H, 4.82; N, 17.62. Found: C, 69.66; H, 4.64; N, 17.80.

1-(4-(1H-indol-3-yl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-3,4-dimethylpyrano[2,3-*c*]pyrazol-6(1H)-one (9)

Compound 5 (10 mmol) and ethyl acetoacetate in excess (30 mL) was heated at reflux temperature for 6 h. The mixture was poured into ice cold water and the obtained product washed with water, dried and recrystallized from ethanol to give pale brown crystals of the pyrazolone compound 9 mp 188-0°C, yield 72% IR: $\nu_{\max}/\text{cm}^{-1}$ 3367 (NH), 1715 (C=O). ¹H NMR (DMSO-*d*₆): δ 2.25 (s, 3H, CH₃), 2.32 (s, 3H, CH₃), 3.80 (s, 3H, OCH₃), 5.60 (s, 1H, pyranone), 6.99-7.26 (m, 4H, Ar H's), 7.38 (d, 2H, *J*=8.8 Hz, Ar-H's), 7.42 (d, 2H, *J*=8.8 Hz, Ar-H's), 8.11 (s, 1H, indole), 8.70 (s, 1H, pyrimidin), 10.41 (s, 1H, D₂O Exch., indole NH). MS: *m/z* (%) 463 [M⁺, 8.6]. Anal. Calcd. For C₂₇H₂₁N₅O₃ (463.49): C, 69.97; H, 4.57; N, 15.11. Found: C, 69.78; H, 4.72; N, 15.30.

1-(4-(1H-indol-3-yl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-1H-pyrazolo[3,4-*d*]pyrimidin-4-amine (10)

A mixture of compound 6 (10 mmol) and formamide (30 ml) was heated at 150°C for 3 h. The reaction mixture was cooled and poured into water. The formed solid was filtered off, dried and recrystallized from ethanol to afford the pyrazolo pyrimidine derivative 10 as pale brown crystals 152-4°C, yield 68% IR: $\nu_{\max}/\text{cm}^{-1}$ 3389 (NH₂), 3190 (NH). ¹H NMR (DMSO-*d*₆): δ 3.89 (s, 3H, OCH₃), 6.93-7.13 (m, 4H, Ar H's), 7.23 (d, 2H, *J*=8.7 Hz, Ar-H's), 7.35 (d, 2H, *J*=8.7 Hz, Ar-H's), 7.93 (s, 1H, D₂O Exch., NH₂), 8.01 (s, 1H, prazole), 8.07 (s, 1H, pyrimidine), 8.12 (s, 1H, indole), 8.2 (s, 1H, pyrimidin), 10.82 (s, 1H, D₂O Exch., indole NH). MS: *m/z* (%) 434 [M⁺, 24.1]. Anal. Calcd. For C₂₄H₁₈N₈O (434.45): C, 66.35; H, 4.18; N, 25.79. Found: C, 66.50; H, 4.32; N, 25.61.

1-(4-(1H-indol-3-yl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-1H-pyrazolo[3,4-*d*]pyrimidin-4(5H)-one (11)

Synthesis of compound (11):

- **Method A:** Compound 6 (10 mmol) was heated under reflux conditions in formic acid (30 ml, 85%) for 8 h. The reaction mixture was cooled and poured into ice cold water. The formed solid was filtered off, dried, and recrystallized from

dimethylformamide to give 11.

- **Method B:** Compound 12 (10 mmol) was heated under reflux temperature in triethyl orthoformate (30 ml) for 10 h. The product which separated on cooling was filtered off, washed with ethanol, dried and recrystallized from dimethylformamide to give pale brown crystals of compound 11 mp 190-2°C, yield 65% IR: $\nu_{\max}/\text{cm}^{-1}$ 3398 (NH), 3177 (NH), 1665 (C=O). ¹H NMR (DMSO-*d*₆): δ 3.87 (s, 3H, OCH₃), 7.10-7.15 (m, 4H, Ar-H's), 7.35 (d, 2H, *J*=8.6 Hz, Ar-H's), 7.40 (d, 2H, *J*=8.6 Hz, Ar-H's), 7.45 (s, 1H, D₂O Exch., NH), 7.50 (s, 1H, prazole), 8.11 (s, 1H, indole), 8.18 (s, 1H, pyrimidine), 8.21 (s, 1H, pyrimidin), 10.99 (s, 1H, D₂O Exch., indole NH). MS: *m/z* (%) 435 [M⁺, 63]. Anal. Calcd. For C₂₄H₁₇N₇O₂ (435.44): C, 66.20; H, 3.94; N, 22.52. Found: C, 66.38; H, 3.76; N, 22.71.

1-(4-(1H-indol-3-yl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-5-amino-1H-pyrazole-4-carboxamide (12)

To (10 m mol) of compound 6, cold concentrated sulphuric acid (10 ml), was added portion wise with ice bath cooling and stirring. After 10 minutes, the ice bath was removed and the mixture was stirred for an additional 15 minutes. The resulting pale yellow solution was carefully poured onto crushed ice and the resulting precipitate was collected, washed with water, dried and recrystallized from dioxane to give yellow crystals of the pyrazolo carboxamide 12 mp 167-9°C, yield 65% IR: $\nu_{\max}/\text{cm}^{-1}$ 3398 (NH₂), 3267 (NH₂), 3114 (NH), 1640 (C=O). ¹H NMR (DMSO-*d*₆): δ 3.82 (s, 3H, OCH₃), 7.25-7.29 (m, 4H, Ar-H's), 7.49 (d, 2H, *J*=8.8 Hz, Ar-H's), 8.05 (d, 2H, *J*=8.8 Hz, Ar-H's), 8.29 (s, 2H, D₂O Exch., NH₂), 8.41 (s, 1H, prazole), 8.57 (s, 1H, indole), 8.69 (s, 1H, pyrimidin), 8.74 (s, 2H, D₂O Exch., NH), 12.72 (s, 1H, D₂O Exch., indole NH). MS: *m/z* (%) 425 [M⁺, 1.03]. Anal. Calcd. For C₂₃H₁₉N₇O₂ (425.44): C, 64.93; H, 4.50; N, 23.05. Found: C, 64.70; H, 4.70; N, 23.26.

7-(4-(1H-indol-3-yl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-3H-pyrazolo[3,4-*d*][1,2,3]triazin-4(7H)-one (13)

To a suspended solution of compound 12 (10 m mol) in concentrated hydrochloric acid (30 ml) at 0-5°C, a solution of sodium nitrite (3.00 g) in water (5 ml) was added over 20 minutes. After 2 h of stirring at room temperature the foamy mixture was filtered off. The resulting solid was washed with ice cold water, dried and recrystallized from ethanol to give pale brown crystals of compound 13 mp 148-0°C, yield 64% IR: $\nu_{\max}/\text{cm}^{-1}$ 3230 (NH), 3118 (NH), 1685 (C=O). ¹H NMR (DMSO-*d*₆): δ 3.84 (s, 3H, OCH₃), 7.15-7.26 (m, 4H, Ar H's), 7.31 (d, 2H, *J*=8.6 Hz, Ar-H's), 7.65 (d, 2H, *J*=8.6 Hz, Ar-H's), 8.21 (s, 1H, prazole), 8.49 (s, 1H, indole), 8.63 (s, 1H, pyrimidine), 8.99 (s, 1H, D₂O Exch., NH), 11.82 (s, 1H, D₂O Exch., indole NH). MS: *m/z* (%) 436 [M⁺, 0.04]. Anal. Calcd. For C₂₃H₁₆N₈O₂ (436.40): C, 63.30; H, 3.70; N, 25.68. Found: C, 63.45; H, 3.90; N, 25.50.

1-(4-(1H-indol-3-yl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-6-thioxo-6,7-dihydro-1H-pyrazolo[3,4-*d*]pyrimidin-4(5H)-one (14)

To a solution of compound 12 (10 m mol) in dimethylformamide (30 ml), 20% potassium hydroxide solution (potassium hydroxide 1.68 g, water 7 ml) and carbon disulfide (5 ml) were added. The reaction mixture was heated under reflux for 15 h, then poured into water and filtered off. The filtrate was precipitated with HCl (0.1N) and the solid product was collected, washed with water, dried, and recrystallized from ethanol/dimethylformamide to give pale brown crystals of compound 14 mp 198-0°C, yield 68% IR: $\nu_{\max}/\text{cm}^{-1}$ 3431 (NH), 3216 (NH), 3148 (NH), 1727 (C=O). ¹H NMR (DMSO-*d*₆): δ 3.84 (s, 3H, OCH₃), 7.14-7.25 (m, 4H, Ar H's), 7.48 (d, 2H, *J*=8.6 Hz, Ar-H's), 8.26

(s, 1H, prazole), 8.28 (d, 2H, $J=8.6$ Hz, Ar-H's), 8.32 (s, 1H, indole), 8.35 (s, 1H, pyrimidin), 8.90 (s, 1H, D₂O Exch., NH), 9.93 (s, 1H, D₂O Exch., NH), 11.67 (s, 1H, D₂O Exch., indole NH). MS: m/z (%) 467[M⁺, 5]. Anal. Calcd. For C₂₄H₁₇N₇O₂S (467.50): C, 61.66; H, 3.67; N, 20.97. Found: C, 61.48; H, 3.82; N, 20.80.

1-(4-(1H-indol-3-yl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-4-chloro-1H-pyrazolo[3,4-*b*]pyrimidine (15)

A mixture of compound 11 (10 m mol) was heated in phosphorus oxychloride (20 mL) for 10 h. The solution was cooled and poured into ice-water. The solid was filtered off, dried and purified on silica gel using chloroform/n-hexane (1:1) as eluent to give pale brown crystals mp 270-2°C, yield 64% IR: $\nu_{\max}/\text{cm}^{-1}$ 3433 (NH). ¹H NMR (DMSO-d₆): δ 3.81 (s, 3H, OCH₃), 7.09-7.26 (m, 4H, Ar H's), 7.43 (d, 2H, $J=8.7$ Hz, Ar-H's), 8.37 (d, 2H, $J=8.7$ Hz, Ar-H's), 8.41 (s, 1H, prazole), 8.58 (s, 1H, indole), 8.63 (s, 1H, pyrimidin), 8.87 (s, 1H, pyrimidin), 11.67 (s, 1H, D₂O Exch., indole NH). MS: m/z (%) 453 [M⁺, 2.0]. Anal. Calcd. For C₂₄H₁₆ClN₇O (453.88): C, 63.51; H, 3.55; N, 21.60. Found: C, 63.35; H, 3.72; N, 21.40.

1-(4-(1H-indol-3-yl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-1H-pyrazolo[3,4-*d*]pyrimidine-4-thiol (16)

A solution of compound 15 (10 m mol) and thiourea (10 m mol) in dioxane (40 ml) was heated at reflux for 4 h. The formed precipitate was collected and dissolved in NaOH (20 ml, 10%). Then the mixture was filtered off and the filtrate was precipitated with HCl (0.1 N). The solid product was collected and recrystallized from ethanol to give pale brown crystals mp 157-9 °C, yield 60% IR: $\nu_{\max}/\text{cm}^{-1}$ 3285 (NH), 3138 (NH). ¹H NMR (DMSO-d₆): δ 3.80 (s, 3H, OCH₃), 7.29-7.33 (m, 4H, Ar H's), 7.39 (d, 2H, $J=8.8$ Hz, Ar-H's), 7.48 (d, 2H, $J=8.8$ Hz, Ar-H's), 7.99 (s, 1H, prazole), 8.19 (s, 1H, pyrimidin), 8.23 (s, 1H, indole), 8.29 (s, 1H, pyrimidin), 11.34 (s, 1H, D₂O Exch., indole NH), 12.16 (s, 1H, D₂O Exch., indole SH). MS: m/z (%) 451 [M⁺, 18.0]. Anal. Calcd. For C₂₄H₁₇N₇OS (451.50): C, 63.84; H, 3.80; N, 21.72. Found: C, 63.55; H, 3.72; N, 21.56.

1-(4-(1H-indol-3-yl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-4-(methylthio)-1H-pyrazolo[3,4-*d*]pyrimidine (17)

Compound 16 (0.005 mol.) in ethanol (20 ml), and 10% sodium hydroxide solution (5 ml) were treated with methyl iodide (0.005 mol.). The reaction mixture was refluxed for 6 h, then cooled and poured into ice cold water. The separated solid was filtered off, dried and recrystallized from dioxane to give pale brown crystals mp 178-0°C, yield 68% IR: $\nu_{\max}/\text{cm}^{-1}$ 3285 (NH). ¹H NMR (DMSO-d₆): δ 1.23 (s, 3H, SCH₃), 3.89 (s, 3H, OCH₃), 7.03-7.27 (m, 4H, Ar H's), 7.39 (d, 2H, $J=8.6$ Hz, Ar-H's), 7.80 (s, 1H, prazole), 8.11 (d, 2H, $J=8.6$ Hz, Ar-H's), 8.17 (s, 1H, indole), 8.26 (s, 1H, pyrimidin), 8.95 (s, 1H, pyrimidin), 9.90 (s, 1H, D₂O Exch., indole NH). MS: m/z (%) 465 [M⁺, 18.0]. Anal. Calcd. For C₂₅H₁₉N₇OS (465.5): C, 64.50; H, 4.11; N, 21.06. Found: C, 64.65; H, 3.97; N, 21.26.

1-(4-(1H-indol-3-yl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-4-hydrazinyl-1H-pyrazolo[3,4-*d*]pyrimidine (18)

Synthesis of compound (18):

- **Method A:** To a solution of compound 15 (1.67 g, 0.005 mol) in dioxane (30 ml), hydrazine hydrate (2 ml) was added and the mixture was heated at reflux for 4 h. The reaction mixture was cooled and poured into water and the separated solid was filtered off, dried and recrystallized from dioxane.
- **Method B:** Compound 17 (0.005 mol) was heated in hydrazine

hydrate (20 ml) for 2 h, cooled then the reaction mixture was poured into water, neutralized with HCl, filtered, dried and purified with column chromatography (silica gel) using chloroform: methanol (9:1), as eluent to give pale brown crystals mp 165-6°C, yield 60% IR: $\nu_{\max}/\text{cm}^{-1}$ 3456 (NH₂), 3385 (NH), 3224 (NH). ¹H NMR (DMSO-d₆): δ 3.83 (s, 3H, OCH₃), 7.02-7.16 (m, 4H, Ar H's), 7.21 (d, 2H, $J=8.8$ Hz, Ar-H's), 7.32 (d, 2H, $J=8.8$ Hz, Ar-H's), 7.91 (s, 1H, prazole), 7.98 (s, 1H, pyrimidin), 8.24 (s, 1H, indole), 8.29 (s, 1H, pyrimidin), 8.99-9.18 (brs, 3H, D₂O Exch., NH₂, NH), 11.42 (s, 1H, D₂O Exch., indole NH). MS: m/z (%) 449 [M⁺, 1.63]. Anal. Calcd. For C₂₄H₁₉N₉O (449.47): C, 64.13; H, 4.26; N, 28.05. Found: C, 63.95; H, 4.45; N, 28.23.

Results and Discussion

Through several years of research and cross referencing other related journals on the preparation of chalcones and their derivatives, chalcone compound 3 and thiopyrimidinone 4 were prepared as reported in literature [1].

The hydrazino-pyrimidine derivative 5 was synthesized by reacting the thiopyrimidinone 4 with hydrazine hydrate in refluxing alcohol, the structure of compound 5 was confirmed by IR, ¹H NMR, MS spectra and elemental analysis, where its IR revealed the absorption bands at $\nu_{\max}=3212$ for the NH₂ and 3184, 3109 cm⁻¹ for the two NH groups, ¹H NMR spectrum gave the signals at $\delta=8.93-8.95$ as a broad singlet for NH₂, hydrazine NH and at 11.65 ppm., for the indole NH respectively. MS spectrum substantiated its exact molecular weight (cf. Scheme 1 and Experimental section).

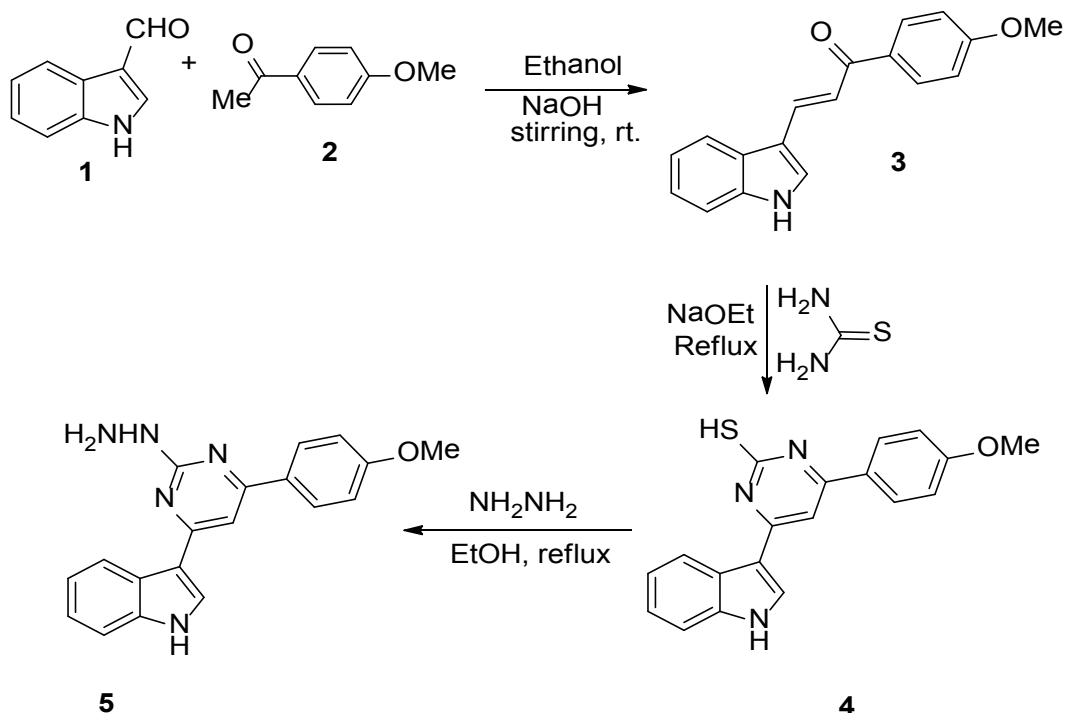
The hydrazino-pyrimidine derivative 5 was used as an assorted precursor for the synthesis of some biologically active heterocycles, where the amino pyrazole carbonitrile compound 6 was synthesized by refluxing a 1:1 mixture of compound 5 in ethanol with ethoxymethylene malonitrile for 4 h. The structure of compound 6 was confirmed by IR, MS, ¹H NMR and elemental analysis, where its IR spectrum gave the absorption bands at $\nu_{\max}=3312$ for NH₂, 3175 for NH Indole and 2226 cm⁻¹ for the CN group respectively, its ¹H NMR substantiated an NH₂ singlet at 8.86 and a singlet at $\delta=11.66$ ppm for NH indole.

The pyrazolo pyrimidine compound 7 was synthesized by heating an alcoholic solution of compound 5 (10.0 mmol.) with acetylacetone (10.0 mmol.) at reflux temperature for 5 h. The FTIR spectrum of compound 7 disclosed the absorption bands at 3169 for NH indole, 1635 and 1575 cm⁻¹ for C=N groups respectively. ¹H NMR revealed NH indole singlet at $\delta=10.41$, singlet pyrimidine H at $\delta=8.95$ ppm and two singlets for the two CH₃ protons respectively.

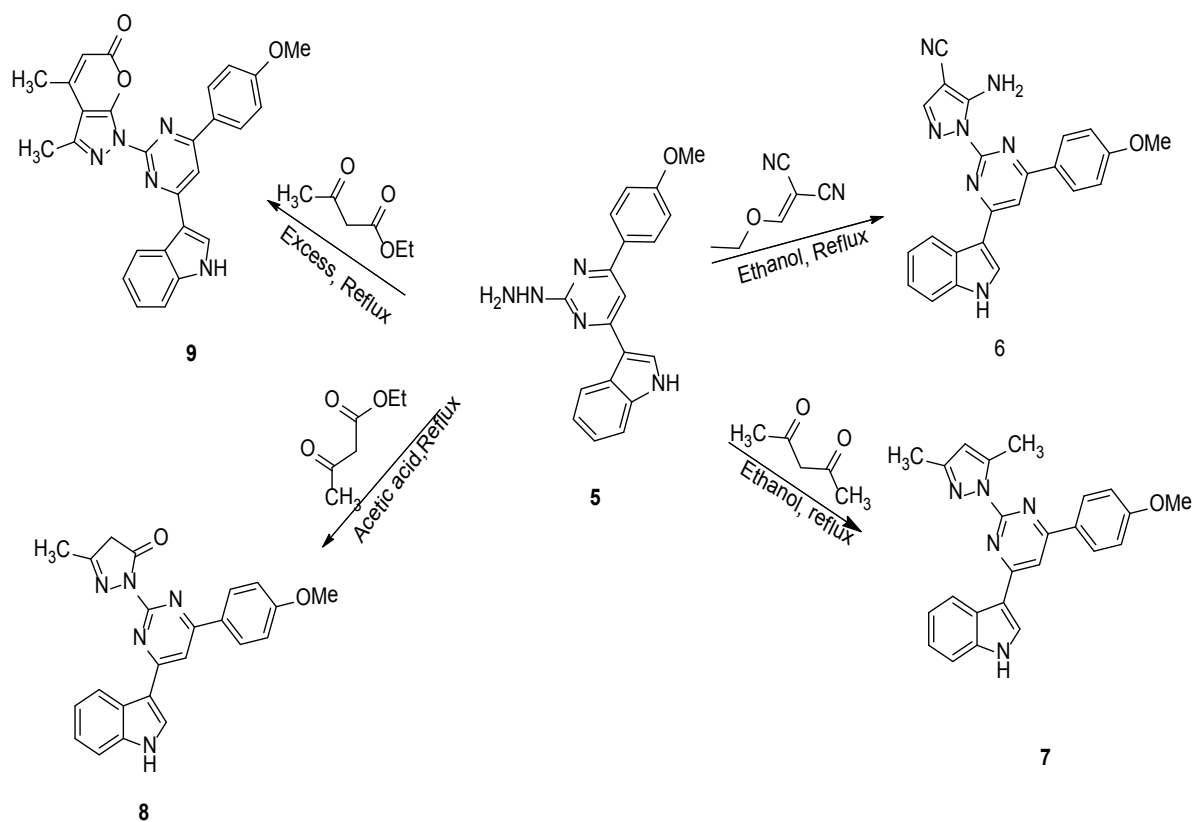
The pyrimidine pyrazolone compound 8 was obtained by refluxing a solution of compound 5 and ethyl acetoacetate in acetic acid for 6 h. The structure of compound 8 was deduced from its analytical and spectral data, which were in full agreement with the proposed structure (cf. Experimental section).

Furthermore, the hydrazino-pyrimidine derivative 5 was reacted with ethyl acetoacetate in excess to afford compound 9. IR spectrum of compound 9 revealed the absorption bands at $\nu_{\max}=3367$ and 1715 cm⁻¹ characteristic for NH indole and C=O groups respectively, ¹H NMR exhibited the NH indole singlet at $\delta=10.82$, two singlets at $\delta=1.23$ and 2.43 for 2 CH₃ protons and a singlet at $\delta=5.6$ ppm for pyranone H respectively (Scheme 2).

The 5-amino-1H-pyrazole-4-carbonitrile compound 6 was found to be an adequate, key starting for the synthesis of some other new heterocyclic compounds, where it was heated in formamide for 3 h., to



Scheme 1



Scheme 2

produce compound **10**. The structure of compound **10** was confirmed by its spectral and analytical data.

The pyrazolo pyrimidinone derivative **11** was obtained on one hand by refluxing compound **6** in excess formic acid and on the other hand by heating compound **12** under reflux condition in triethyl orthoformate. The IR spectrum of compound **11** substantiated the absorption bands of indole NH, pyrimidinone NH and C=O respectively at their prospective values. ¹H NMR, MS and elemental analysis gave the confirmatory data for compound **11** (cf. Scheme 3 and Experimental section).

Treatment of compound **6** with cold concentrated sulphuric acid portion wise and sterilizing on an ice bath for about an hour. afforded the pyrazolo carboxamide derivative **12**. IR spectrum of compound **12** substantiated absorption bands at 3398 for pyrazole NH₂, 3267 for amide NH₂, 3114 for indole NH and 1640 for the amide C=O, it's ¹H NMR displayed three singlets at δ 8.48, 9.14 and 11.42 ppm for (D₂O exchangeable) pyrazole NH₂, amide NH₂ and indole NH respectively.

Continuing a series of synthesis the pyrazolo carboxamide derivative **12** was transformed into the pyrazolo triazinone **13** by the addition of sodium nitrite solution to a suspended solution of **12** in concentrated hydrochloric acid at 0-5°C with stirring at room temperature.

Structure of compound **13** was approved by IR, ¹H NMR, MS measurements and elemental analysis (cf. Scheme 3 and Experimental section).

Refluxing a solution of compound **12** in dimethylformamide with 20% potassium hydroxide solution and carbon disulfide (5 ml) afforded the thioxopyrazolo pyrimidinone derivative **14**, which structure was deduced from its analytical and spectral data, (cf. Experimental section).

The pyrimidinone derivative compound **11** was heated in phosphorus oxychloride for 10 h. to give compound **15** which in turn was transformed by reaction with thiourea into the pyrimidin-4-thiol compound **16**.

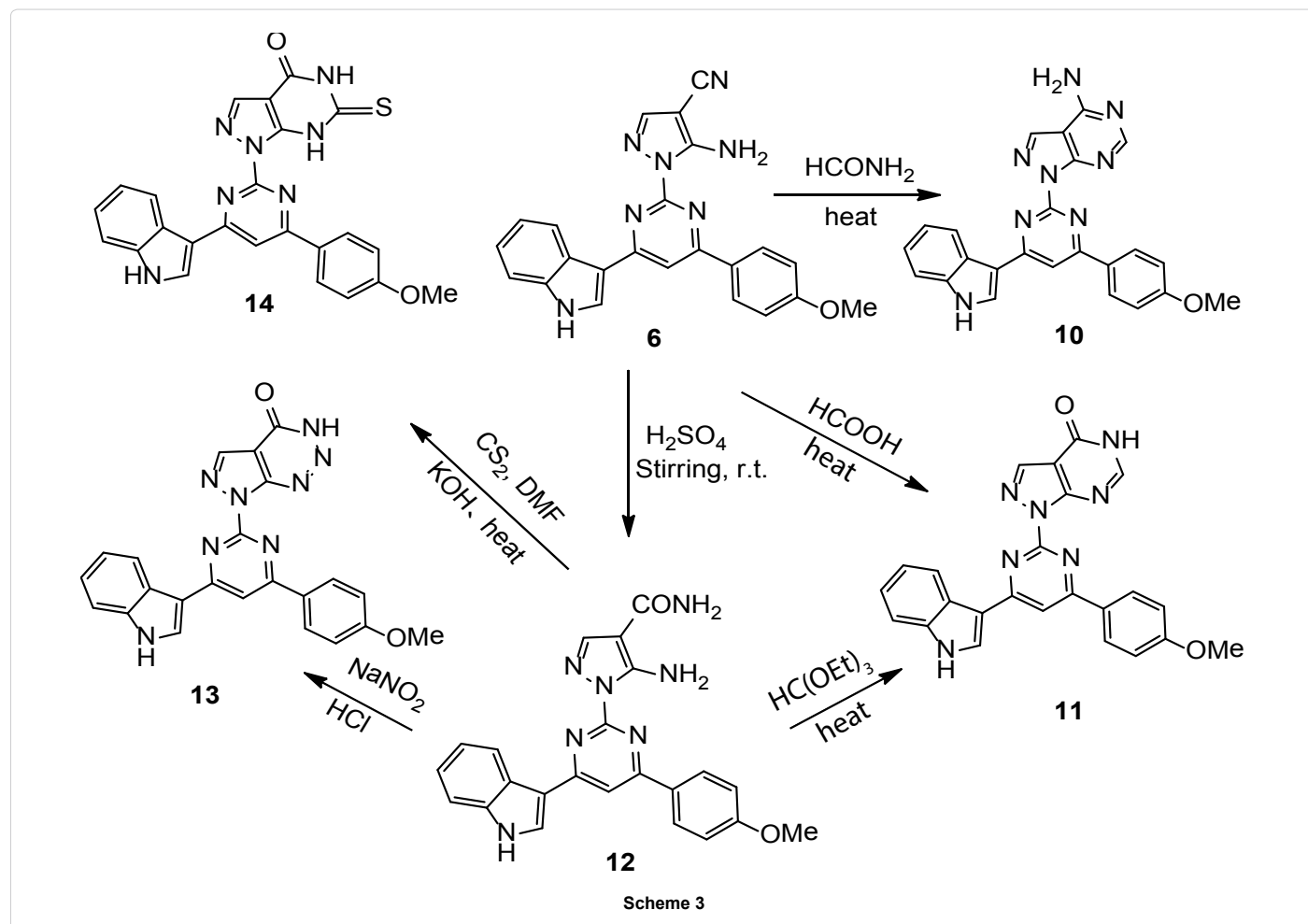
Compound **16** in ethanol and 10% sodium hydroxide solution was treated with methyl iodide then the reaction mixture was subjected to reflux affording the methylthiopyrazolo pyrimidine derivative **17**.

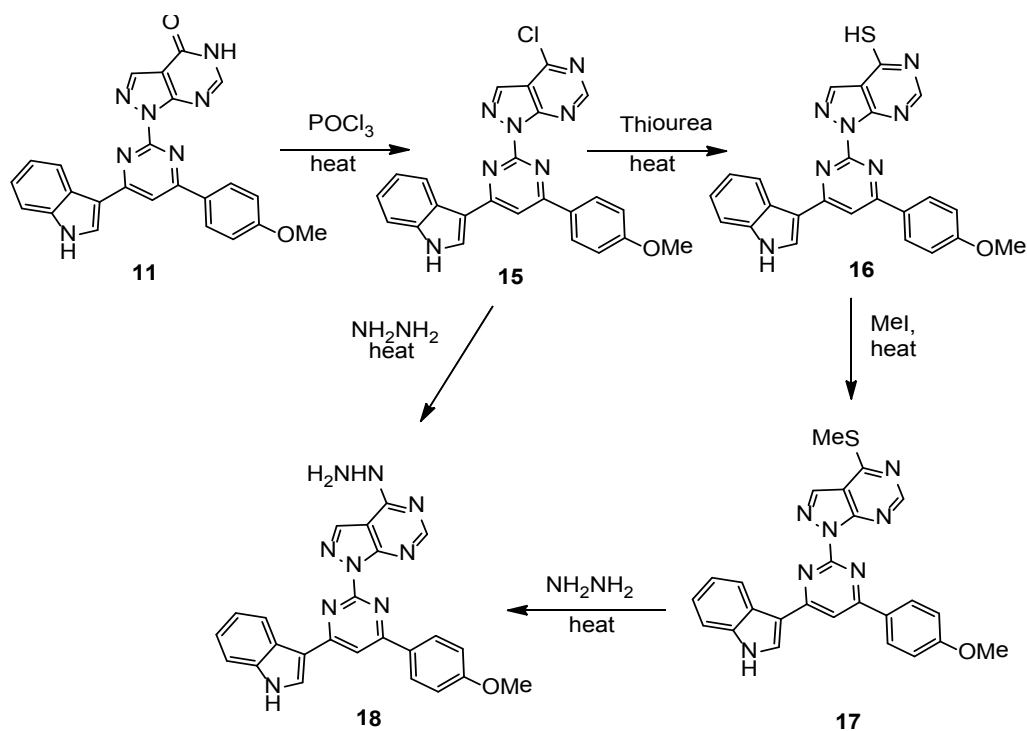
Compound **18** could be synthesized by reacting both of compounds **15** and **17** with hydrazine hydrate. Structures **15**, **16**, **17**, and **18** were based on their correct elemental analysis and spectral data (cf. Scheme 4 and Experimental section).

Anti-proliferative activity

The newly synthesized compounds 5, 7, 8, 9, 11, 12, 14 and 18 were tested for their *in vitro* antiproliferative activities in the National Cancer Institute (NCI), where a single dose (10 μM) of the test compounds was used against 60 cell lines panel assay [15-19].

The data were reported as mean-graph of the percent of growth of the treated cells, and presented as percentage growth inhibition (GI%) caused by the tested compounds (Table 1).





Scheme 4

It was found that all tested compounds displayed significant activities against the tested cell lines using 10 μ M concentration with positive cytotoxic effects (PCE) of 7/58, 23/53, 53/53, 52/53, 149/49, 41/57, 46/57 and 46/58 respectively (Table 2).

Compounds 7, 8, 9, 11, 12, 14 and 18 showed cytotoxic effects on the most of the cancer cell lines with mean growth inhibition percentage (MGI%) of 10%, 35%, 44%, 50%, 19%, 29% and 34% (Table 2) and compound 5 showed slight selective activity against HOP-62, NCI-H226, NCI- H522, SNB-75, OVCAR-4, PC-3 and MCF7 cancer cell lines with GI values of 13%, 20%, 11%, 28%, 11%, 11% and 21% respectively (Table 1).

With respect to broad spectrum antitumor activity, close examination of the data presented in Table 1 revealed that compounds 11, 14, and 18 are the most active derivatives showing effectiveness towards numerous cell lines belong to different tumor subpanels. Consequently, compounds 11, 14, and 18 were selected and tested against a panel of 60 different tumor cell lines at a 5 – log dose range [1-5].

These response parameters GI₅₀, TGI, and LC₅₀ were calculated for each cell line, using the known drug 5 – Fluorouracil (5 – FU) as a positive control.

Compounds 11, 14 and 18 exhibited remarkable growth inhibitory activity pattern against renal cancer (GI₅₀=4.26, 3.43 and 12.30, μ M) non-small cell lung cancer (GI₅₀=5.65, 3.92 and 7.83 μ M) breast cancer (GI₅₀=4.16, 4.16 and 6.16 μ M) ovarian cancer GI₅₀ 9.54, 6.3 μ M and melanoma cancer (GI₅₀=9.58, 4.24 and 3.70 μ M) CNS (GI₅₀=5.43, 2.50 and 3.38 μ M) prostate cancer GI 9.58, 4.24 and 3.7 (GI₅₀=27.22, 4.89 and 18.8 μ M) respectively

It was found also that compounds 11, 14 and 18 are about 1.5-3.0 fold more active than the positive control 5 FU, with GI₅₀ (10.47, 7.24 and 14.12 μ M) TGI (58.8, 36.30 and 60.25 μ M) and LC₅₀ (> 100, 87.09 and 95.49 μ M) value respectively (Table 3).

Comparing the antitumor activities of compounds 11, 14 and 18 with those of Gefitinib and Erlotinib showed that compounds 11, 14 and 18 (Table 4) possess activities almost equal to or higher than those of Gefitinib and Erlotinib against most cell lines except non-small lung (EKVX and NCI – H522), melanoma (SK – MEL – 28), ovarian cancer (IGROVI and SK – OV – 3) renal cancer (ACHN and TK- 10) and breast cancer (MDA-MB468) on the other hand compounds 8 and 12 showed selective activities toward CNS, renal and breast cancer cell lines, compound 9 gave selective activities toward leukemia cell lines, where compound 7 displayed moderate antitumor activities.

Regarding the activity toward individual cell lines, compounds 9, 12 showed selective activity against leukemia cell lines CCRF-CEM, K – 562, MOLT – 4 and PRMI – 8226 with GI values of 26%, 22%, 19%, 23%, 31%, 40% and 44%, 58%, respectively.

Compound 12 revealed selective activities towards SR leukemia cell lines with GI values of 34% whereas compound 18 disclosed weak activity against k-562 and PRMI-8226 cell lines with GI values of 23% and 17%.

Non-small lung A549/ATCC cell line proved to be selectively sensitive to 8, 9, 11, 12, 14 and 18 with GI values of 33% 34%, 72%, 28%, 41 and 58%. In addition compounds 7, 12, and 18 proved to be susceptible to the HOP-62, NCI-H226 and NCI/H522 cell lines with GI values of 33%, 34%, 91%, 45%, 69%, 73%, 30% lethal and 35%,

%Growth inhibition (GI%)								
18	14	12	11	9	8	7	5	<i>Subpanel tumor cell lines</i>
								Leukemia
-	nt	22	nt	26	nt	nt	-	CCRF-CEM
-	nt	-	nt	-	nt	nt	-	HL-60(TB)
23	nt	33	nt	19	nt	nt	-	K-562
-	nt	40	nt	31	nt	nt	-	MOLT-4
17	nt	58	nt	44	nt	nt	-	PRMI-8226
-	nt	34	nt	nt	nt	nt	nt	SR
								Non-small cell lung cancer
58	72	41	34	28	33	13	-	A549/ATCC
nt	25	nt	16	nt	17	11	Nt	EKVX
91	NT	43	55	-	-	75	13	HOP-62
73	55	69	L	nt	54	45	20	NCI-H226
nt	nt	nt	76	46	nt	10	-	HOP-92
-	31	-	26	11	24	-	-	NCI-H23
29	35	17	37	25	18	11	-	NCI-H322M
52	32	19	43	-	36	-	-	NCI-H460
35	nt	L	69	96	43	30	11	NCI-H522
								Colon Cancer
-	-	-	12	-	17	-	-	Colo 205
-	23	-	25	-	10	-	-	HCC-2998
66	48	48	57	17	49	14	-	HCT-116
24	10	28	16	-	12	-	-	HCT-15
22	39	24	30	-	44	-	-	HT29
nt	23	nt	43	13	49	-	-	KM12
16	-	14	19	-	19	-	-	SW-620
								CNS Cancer
60	61	-	31	19	41	14	-	SF-268
41	55	34	21	25	16	-	-	SF-295
67	94	74	89	28	L	46	-	SF-539
27	37	-	11	16	12	-	-	SNB-19
L	L	74	L	45	47	23	28	SNB-75
56	94	30	43	20	45	25	-	U251
								Melanoma
29	32	-	39	18	26	-	-	LOX IMVI
67	55	16	51	19	39	17	-	MALME-3M
13	61	25	35	-	27	22	-	M14
-	24	12	33	11	22	-	-	MDA-MB-435
48	55	27	43	16	23	-	-	SK-MEL-2
27	40	30	25	-	22	-	-	SK-MEL-28
10	33	26	61	13	55	-	-	SK-MEL-5
43	80	39	39	11	31	-	-	UACC-257
15	24	47	47	34	57	-	-	UACC-62
								Ovarian Cancer
-	25	-	26	-	16	-	-	IGROVI
33	31	-	57	12	23	-	-	OVCAR-3
L	52	39	77	37	19	-	11	OVCAR-4
-	-	12	-	-	15	-	-	OVCAR-5
71	L	27	59	21	32	55	-	OVCAR-8
17	nt	16	34	18	33	-	-	NCI/ADR-RES
70	53	19	61	-	28	28	-	SK-OV-3
								Renal Cancer
28	L	-	57	17	42	-	-	786-o
33	L	65	86	36	89	17	-	A498
47	34	55	63	22	43	-	-	ACHN
35	16	37	18	28	24	12	-	CAKI-1
64	64	43	76	-	70	29	-	RXF 393
11	31	21	17	18	12	-	-	SN12C

-	L	-	45	-	28	-	-	TK-10
29	52	26	29	55	38	23	-	UO-31
								Prostate Cancer
17	65	57	26	34	29	-	10	PC-3
17	46	-	20	-	28	-	-	DU-145
								Breast Cancer
23	18	30	30	16	30	-	21	MCF7
48	59	49	65	36	56	15	-	MDA-MB-231/TCC
94	76	60	56	23	53	45	-	HS 578T
-	87	27	47	12	69	28	-	BT-549
65	27	36	88	22	45	-	-	T-47D
32	nt	-	nt	-	nt	nt	-	MDA-MB-468

nt=not tested, GI<10% compound proved to the cancer cell line.

Table 1: Percentage growth inhibitor (GI%) of *in vitro* subpanel tumor cell lines at 10 UM conc.

60 cell lines assay in one dose 10.0UM conc.		
PCE	MGI%	Compd No.
7/58	0	5
23/53	10	7
53/53	35	8
41/57	19	9
52/53	44	11
46/57	29	12
49/49	50	14
46/58	34	18

Table 2: Mean growth inhibition percentage and positive cytotoxic effects of new compounds.

Subpanel tumor cell lines											
MG-MID ^a	Breast cancer	Prostate cancer	Renal cancer	Ovarian cancer	Melanoma	CNS Cancer	Colon cancer	NSC lung cancer	Leukemia	Activity	Compd No.
10.47	4.16	27.22	4.26	9.54	6.56	5.43	51.9	5.65	84.5	GI ₅₀	11
58.8	67.09	b	39.81	60.25	19.85	33.65	b	42.65	b	TGI	
b	b	b	b	b	b	97.72	b	b	b	LC ₅₀	
						2.5					
7.24	4.16	4.89	3.47	6.3	4.24	84.7	36.43	3.92	86.09	GI ₅₀	14
36.30	27.28	b	26.76	26.0	45.47	56.75	72.21	35.89	b	TGI	
87.9	70.79	b	98.0	93.11	b	3.38	93.32	71.03	b	LC ₅₀	
14.12	6.16	18.8	12.3	10.23	3.7	18.28	63.09	7.83	b	GI ₅₀	
60.25	57.32	b	45.49	87.09	79.03	69.50	b	40.36	b	TGI	18
95.49	b	b	97.72	b	b	b	b	94.40	b	LC0	
22.6	76.4	22.7	45.6	61.4	70.6	72.1	8.4	b	15.1	GI ₅₀	
b	b	b	b	b	b	b	b	b	b	TGI	5-FU
b	b	b	b	b	b	b	b	b	B	LC ₅₀	

Full panel mean graph midpoint (μM); Compounds showed values >100 UM

Table 3: Compounds 11, 14 and 18 median growth inhibitory (GI50 UM) total growth inhibitory (TGI, UM) and median lethal (LC50, UM) concentration of *in vitro* subpanel cell lines.

where compounds **8**, **11** and **14** showed activities against NCI-H226 and NCI-H22, NCI-H323M and NCI-H460 cell lines with GIS values of 54%, lethal 55%, 24%, 26%, 31%, 18%, 37%, 35%, 36%, 43%, and 32%.

Compounds **9** and **11** showed strong activities against HOP-92 and NCI-H522 cell lines in 76%, 46%, 69%, and 96%, Meanwhile, compounds **12** and **18** showed certain activity against NCI-H3222-M and NCI-H460 cell lines in 17%, 29%, 19% and 52% respectively, compounds **9**, **11**, and **14** showed activities in 55%, 25% and 25% against HOP-62, EKVX and NCI-H322M cancer cells.

For the different cell lines of colon cancer, compounds **8**, **11**, **12**, **14**

and **18**, showed GI values of 49%, 57%, 48%, 48%, 66% against colon HCT-122 and 44%, 30%, 39%, 24%, 22% with colon HT29 cell lines, while compounds **11** and **14** demonstrated moderate activities against HCC- 2998 cancer cell lines with GI values 25% and 23% respectively, on the other hand compounds **12** and **18** verified sensitivity in 28% and 24% to colon HCT-15 cancer cell, compounds **8**, **11**, and **14** displayed modest activities against KM12 cancer cell with GI value 94%, 43% and 23% respectively.

On screening the activities toward CNS cancer cell lines, compounds **7**, **8**, **9**, **11**, **14**, **12**, **18** showed strong potency against CNS cancers, SF-539, SNB-75 and U251 with GI values of 46%, 89%, 94%,

<i>GI₅₀</i> (UM)					
Gefitinib	Erlotinib	18	14	11	Subpanel tumor cell lines
Non-small cell lung cancer					
7.94	7.94	8.08	5.15	10.5	A549/ATCC
0.05	0.05	nt	5.12	8.59	EKVX
10.00	12.58	2.94	2.03	2.30	HOP-62
15.84	6.30	2.66	2.66	3.20	NCI-H226
15.84	19.95	26.6	4.17	8.30	NCI-H23
6.30	5.01	nt	6.72	7.24	NCI-H460
6.30	1.00	11.10	3.65	4.63	NCI-H522
7.94	6.30	1.9	nt	nt	HOP-92
0.08	0.05	70.4	nt	nt	NCI-H322M
6.30	5.01	5.31	nt	nt	NCI-H460
Colon Cancer					
6.30	31.62	>100	15.3	>100	Colo 205
10.00	79.34	>100	50.6	>100	HCC-2998
7.94	5.01	4.35	6.74	3.38	HCT-116
3.98	50.11	>100	16.6	>100	HT29
7.94	63.09	>100	>100	29.9	KM12
CNS Cancer					
7.94	19.95	4.21	3.57	6.91	SF-268
1.99	15.84	nt	1.94	7.01	SF-295
10.00	12.58	2.5	2.37	3.46	SF-539
12.58	3.98	3.17	2.93	21.4	SNB-19
6.30	12.58	1.9	1.57	1.82	SNB-75
10.00	19.95	7.06	3.18	3.93	U251
Melanoma					
7.94	5.01	6.4	4.71	55.0	LOX IMVI
3.16	5.01	4.73	3.3	4.07	MALME-3M
5.01	6.30	24.4	16.1	8.38	M14
3.16	15.84	>100	4.91	9.55	MDA-MB-435
12.58	12.58	4.40	3.41	3.38	SK-MEL-2
0.31	31.62	6.30	3.02	7.04	SK-MEL-28
3.98	15.84	32.4	-	3.67	SK-MEL-5
6.30	100.00	4.96	3.55	4.86	UACC-257
5.01	1.25	15.7	2.35	2.93	UACC-62
Ovarian Cancer					
0.20	0.25	27.7	7.34	32.5	IGROVI
5.01	3.16	5.05	7.68	9.36	OVCAR-3
7.94	19.95	3.95	2.11	3.41	OVCAR-4
10.00	19.95	>100	>100	58.0	OVCAR-5
10.00	7.94	5.01	3.46	4.42	OVCAR-8
12.58	6.30	9.25	3.30	8.97	NCI/ADR-RES
0.63	0.39	4.75	2.91	3.06	SK-OV-3
Renal Cancer					
7.94	5.01	4.17	3.51	3.2	786-O
0.4	1.58	15.8	1.75	1.01	A498
0.2	0.15	3.72	4.63	3.49	ACHN
0.16	0.10	>100	3.05	5.08	CAKI-1
5.01	6.3	3.48	2.55	2.24	RXF 393
6.3	6.3	78.0	4.70	5.24	SN12C
0.10	0.10	7.43	4.06	5.58	TK-10
1.25	1.99	11.6	1.95	5.97	UO-31
Breast Cancer					
10.0	10.0	32.0	7.93	9.98	MCF7
12.58	1.99	4.2	3.73	3.42	MDA-MB-231/ATCC
10.0	6.3	2.18	2.46	3.23	HS 578T
7.94	39.81	5.02	2.02	2.98	BT-549
6.3	3.16	7.04	6.57	4.01	T-47D
0.01	0.2	5.39	5.72	4.11	MDA-MB-468

Table 4: GI₅₀ values (UM) of compounds 11, 14 and 18 Gefitinib and Erlotinib over the most cell lines of non-small lung cancer, colon cancer, CNS cancer, Melanoma, Ovarian cancer, Renal cancer and Breast cancer.

28%, 74%, 67%, 23%, 47% lethal 74% lethal, 25%, 45%, 43%, 94%, 20%, 30% and 56%, Compounds **9**, **11**, **14** and **18** showed GI values of 31%, 61%, 19%, 60%, 21% 55%, 25% and 41% to CNS cancer SF-268 and SF-295 cell lines, respectively. CNS cancer SNB-19 proved to be sensitive to compounds **14** and **18** with GI values of 37% and 27% while compounds **8** and **12** showed certain potency to SF-268 and SF-295 cancer cells with GI values of 41% and 34% respectively.

On screening of the activities of tested compounds towards melanoma it was found that compounds **8**, **11** and **14** have the values of GI ranging from 22% to 80% against LOX, IMVI, MALME-3M, M14, MDA-MB-435, SK-MEL-2, SK-MEL 28, SK-MEL-5 UACC-257 and UACC-62 cell lines respectively.

Compound **7** showed moderate activities against M14 cell line, whilst compounds **9** and **12** possessed activity against UACC-62 cell line with GI values of 34% and 47%, Melanoma SK-MEL-2, SK-MEL-28 UACC-257 cell lines were found to be sensitive to compounds **12** and **18** in as shown in Table 1, at the same time compound **18** was active towards LOX IMVI and MALME-3M cell lines GI, 29% and 67%, and compound **12** has slight activity against SK-MEL-5 cell line with GI value 26%.

The results obtained toward ovarian cancer cell lines revealed that compounds **7**, **8** and **11** showed moderate activities against ovarian OVCAR-8 and SK-OV-3 cell lines, compounds **8**, **11** and **14** were effective towards OVCAR-3 and OVCAR-4 cell lines while compounds **9** and **12** showed different activities against OVCAR-8 OVCAR-4 cell lines respectively (cf, Table 1).

The reactivity of compounds **8**, **11**, **14** and **18** against Ovarian NCI3/ADR-RES, IGR-OVI, OVCAR-8 and OVCAR-4, OVCAR-3 and SK-OV-3 cell lines were also measured and recorded in Table 1.

In case of renal cancer, compounds **8**, **11**, **14** and **18** substantiated different activities against 786-O, A498, ACHN, CAKI-1, RXF393, SNI2C, TK-10 and UO-31 cell lines with GI values from 24%-lethal. Renal A498, ACHN, CAKI-I, SNI2C and UO-31 cell lines were found to be sensitive to compounds **9** and **12** with GI values of 36%, 65%, 22%, 55%, 28%, 37%, 18%, 21%, 55% and 26% respectively, whereas RXF393 cell line was affected by compounds **7** and **12** with GI values of 29% and 43% respectively.

Prostate PC-3 and DU-145 cell lines were proved to be selectively sensitive to compounds **8**, **11** and **14** with GI values of 29%, 26%, 65%, 28%, 20% and 46% respectively, while compounds **9** and **12** possessed selective activity towards PC-3 cell line with GI values of 34% and 57% respectively.

Regarding the breast cancer; MCF7, MDA-MB-231/ATCC, HS 578T, BT-549, T-47D cell lines were found to possess convincing response to compounds **8**, **9**, **11**, **12** and **18** with GI values ranging from 23% to 94%, compound **7** showed GI effectiveness against breast HS 578T and BT-549 cell lines with values of 45% and 28%, additionally compound **18** gave selective activity against breast MDA-MB-468 cell line with GI values of 32%.

Conclusion

The authors herein endeavored to design and synthesize new pyrazolopyrimidine derivatives and screen some of these compounds for their anti-proliferative activity.

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